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Trophic and fitness correlates of mercury and organochlorine compound residues in egg-laying Antarctic petrels

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ABSTRACT

Understanding the drivers and effects of exposure to contaminants such as mercury (Hg) and organochlorine compounds (OCs) in Antarctic wildlife is still limited. Yet, Hg and OCs have known physiological and fitness effects in animals, with consequences on their populations. Here we measured total Hg (a proxy of methyl-Hg) in blood cells and feathers, and 12 OCs (seven polychlorinated biphenyls, PCBs, and five organochlorine pesticides, OCPs) in plasma of 30 breeding female Antarctic petrels *Thalassoica antarctica* from one of the largest colonies in Antarctica (Svarthamaren, Dronning Maud Land). This colony is declining and there is poor documentation on the potential role played by contaminants on individual physiology and fitness. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values measured in the females' blood cells and feathers served as proxies of their feeding ecology during the pre-laying (austral spring) and moulting (winter) periods, respectively. We document feather Hg concentrations (mean ± SD, 2.41 ± 0.83 μg g^{-1} dry weight, dw) for the first time in this species. Blood cell Hg concentrations (1.38 ± 0.43 μg g^{-1} dw) were almost twice as high as those reported in a recent study, and increased with pre-laying trophic position (blood cell $\delta^{15}N$). Moulting trophic ecology did not predict blood Hg concentrations. PCB concentrations were very low (Σ_7PCBs, 0.35 ± 0.31 ng g^{-1} wet weight, ww). Among OCPs, HCB (1.02 ± 0.36 ng g^{-1} ww) and *p, p'*-DDE (1.02 ± 1.49 ng g^{-1} ww) residues were comparable to those of ecologically-similar polar seabirds, while Mirex residues (0.72 ± 0.35 ng g^{-1} ww) were higher. PCB and OCP concentrations showed no clear relationship with pre-laying or moulting feeding ecology, indicating that other factors overcome dietary drivers. OC residues were inversely related to body condition, suggesting stronger release of OCs into the circulation of egg-laying females upon depletion of their lipid reserves. Egg volume, hatching success, chick body condition and survival were not related to maternal Hg or OC concentrations. Legacy contaminant exposure does not seem to represent a threat for the breeding fraction of this population over the short term. Yet, exposure to contaminants, especially Mirex, and other concurring environmental stressors should be monitored over the long-term in this declining population.

1. Introduction

Antarctic ecosystems are under growing pressure of environmental change (Meredith et al., 2019), and are not spared by chemical contamination despite their remoteness (Corsolini, 2009; Trathan et al., 2015). Mercury (Hg), a non-essential metal, and organochlorine compounds (OCs), synthetic chemicals widely used until the 1970s for industrial and agricultural applications, are highly toxic and can reach Antarctica *via* long-range transport by atmospheric and oceanic currents (Corsolini, 2009; Fitzgerald et al., 2007). Secondary sources such as re-emissions from Antarctic soil, melting permafrost and sea-ice add to long-range transport contributions (Cabrerizo et al., 2013; Cossa et al., 2011), and have the potential to increase under climate warming (Cabrerizo et al., 2013; Potapowicz et al., 2019). Hg (as its methylated

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form, methyl-Hg) and OCs are readily transferred to marine food webs, where they bioaccumulate in the tissues of organisms and biomagnify at each trophic step (Bargagli et al., 1998; Borgå et al., 2001; Corsolini, 2009). Antarctic predators are thus exposed to Hg and OCs through food ingestion (Bargagli, 2008; Bustnes et al., 2006a). Hg and OCs can affect physiology and behaviour in wildlife (Frye et al., 2012; Tan et al., 2009), with short- and long-term fitness consequences such as decreased reproductive output (both Hg and OCs, Bustnes et al., 2007; Dietz et al., 2019; Roos et al., 2012) and adult survival (OCs, Erikstad et al., 2013; Goutte et al., 2015). Demographic effects of Hg and OCs can be severe in polar species, that can be vulnerable to the synergistic effects of multiple stressors (Bustnes et al., 2006b; Goutte et al, 2015, 2018). While extensive work on the patterns and effects of Hg and OC residues has been done in the Arctic (*e.g.*, Dietz et al., 2019), still very little is known on both the drivers and consequences of Hg and OC exposure in Antarctic wildlife (but see Carravieri et al., 2018; Goutte et al., 2014a, 2014b), which hinders our ability to fully understand the impact of environmental change on Antarctic animal populations.

Seabirds are good bioindicators of chemical contamination in Antarctic and subantarctic environments (Carravieri et al., 2020; Polito et al., 2016; Roscales et al., 2016). Concentrations of most contaminants are overall low in Antarctic seabirds when compared to ecologically-similar species from industrialised regions or the Arctic (Blévin et al., 2013; Bustnes et al., 2006a; Roscales et al., 2016). However, concentrations of Hg and OCs such as hexachlorobenzene (HCB) and Mirex can be comparable to those of Northern Hemisphere seabirds (Bengtson Nash et al., 2007; Bustnes et al., 2006a). Feeding ecology plays an important role in explaining within- and among-species variation in contaminant residues in seabirds, as often shown by their correlation with dietary chemical proxies (Anderson et al., 2009; Leat et al., 2013; Monteiro et al., 1998). Hg and OC concentrations in blood reflect dietary exposure over the short-term (a few weeks to a few months before sampling, Bearhop et al., 2000; Clark et al., 1987; Monteiro and Furness, 2001), while feather residues may represent exposure over the long-term (several months, Anderson et al., 2009; Bearhop et al., 2000; García-Fernández et al., 2013). OCs are lipophilic molecules that can be stored in lipid tissues over long timescales, and be released into the circulation when lipid reserves are depleted (whole-organism half-lives of some OCs can be 300–400 days, Clark et al., 1987a). Contaminant concentrations circulating in blood are thus potentially influenced by diet assimilated at different time scales. Information on both present and past dietary exposure could contribute to explain variation in blood contaminant concentrations.

Here we focus on Antarctic petrels *Thalassoica antarctica* from Svarthamaren, Dronning Maud Land, Antarctica, one of the largest known colonies of the species (Schwaller et al., 2018). Antarctic petrels are pagophilic (*i.e.*, highly associated with sea-ice), and feed mainly on crustaceans, but also on fish and squid (Descamps et al., 2016a). They are restricted to Antarctic waters throughout the year, but range widely in the Southern Ocean outside the breeding period (Delord et al., 2020). The number of breeding Antarctic petrels at Svarthamaren has declined in the last decades as a consequence of large-scale climatic and oceanographic changes (Descamps et al, 2015, 2016b). However, additional potential threats, from chemical contamination for example, may have a role in the trajectory of this population. Winter and early breeding exposure to contaminants can be a significant driver of short-term reproductive output in polar seabirds, affecting breeding propensity (Tartu et al., 2013) and egg size (Fort et al., 2014), a fitness indicator (Amundsen et al., 1996). Here we focused specifically on the early-breeding period in female Antarctic petrels sampled over a few days during egg-laying, thus minimizing seasonal effects on circulating contaminant residues (Van den Brink et al., 1998). This study had multiple aims: 1) quantify Hg and OC residues and their inter-relationships, and compare concentrations to those of other polar seabirds; 2) test whether pre-laying (spring) or moulting (winter) feeding strategies (inferred from the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in blood and feathers, respectively) explain variability in Hg and OC concentrations; and 3) quantify the association of Hg and OC residues with egg volume, hatching success, chick survival and female and chick body condition. We expected lower residues of polychlorinated biphenyls (PCBs) in Antarctic petrels than in Arctic species, but higher concentrations of HCB and Mirex, according to the preferential production and use of those compounds in the Northern and Southern Hemispheres, respectively (Bengtson Nash et al., 2007; Bustnes et al., 2006a). Pre-laying trophic position ($\delta^{15}N_{Blood}$) was predicted to drive blood Hg concentrations (Carravieri et al., 2018). Feather and/or blood Hg concentrations in female Antarctic petrels were expected to be related to egg volume, as previously found in little auks *Alle alle* (Fort et al., 2014), an ecologically-close Arctic species with similar Hg concentrations.

2. Material and methods

2.1. Study site and sampling procedure

Fieldwork was carried out at the Svarthamaren Antarctic petrel colony (71°53′S, 51°10′E) in Dronning Maud Land, Antarctica, during the austral summer of 2017–18. Thirty-one females were captured at their nest between 22–27 November, a few hours after egg-laying (females generally leave for the sea within 24 h after laying their single egg, Lorentsen and Røv, 1995). The nests were chosen within an area of approx. 100 × 50 m. We selected nests where the female could be easily identified by examining cloacal characters (Copestake et al., 1988; Lorentsen and Røv, 1995), *i.e.*, which had clear signs of recent egg-laying (dilated cloaca and traces of fresh blood on the cloaca/feathers and on the egg). At capture, the bird was put into a soft bag and the egg gently removed from the nest. Eggs were weighed with a 200 g-Pesola balance (precision ± 2 g), and their length and breadth were measured with a calliper (±0.1 mm). Eggs were kept in a warm insulated box while processing the females. A blood sample (≤2 ml) was taken from the brachial vein of the female with a heparinized syringe, and stored in heparinized microtubes. Ten body feathers (hereafter feathers) were plucked from the lower back region. Females were weighed with a 1000-g Pesola scale (±5 g), their bill height and culmen measured with a calliper (±0.1 mm), and their wing length measured with a ruler (±1.0 mm). Females were immediately released onto their nests after replacing the egg. The whole procedure typically lasted 15–20 min. One egg was lost (broken) during the capture of a female, making the final sample size N = 30. Female blood was centrifuged at 5000 rpm for 10 min, within 4 h from sampling. Red blood cell and plasma fractions were stored separately at approximately −10 °C in a cavity built in, and sealed with, ice for 2 months. Thereafter, they were transferred to a freezer at −20 °C until laboratory analyses. All nests were monitored every other day from incubation to chick-rearing to estimate hatching success and chick survival (at 20 days of age, Carravieri et al., 2018; Descamps et al., 2015). Chick body mass and head-bill length were measured with a 1000-g Pesola scale and a calliper, respectively (see above for precision), during our last nest check of the season, which took place approx. 20 days after hatching.

Egg volume was calculated using the formula: Volume (cm^3) = 0.00051 x length (mm) x breadth $(mm)^2$ (Amundsen et al., 1996). The scaled mass index (SMI, Peig and Green, 2009) was used as a proxy of body condition as presented in Carravieri et al. (2018).

2.2. Stable isotope, Hg and OC determination

Isotopic and total Hg analyses were carried out in red blood cells and feathers at the laboratory Littoral, Environment and Societies (LIENSs), La Rochelle. Red blood cells were lyophilised and homogenised. Feathers were washed to remove surface lipids and contaminants, dried at 50 °C for 48 h, and homogenised by cutting them with stainless scissors into 1–2 mm fragments (Carravieri et al., 2013). Ten body

feathers per individual were pooled and homogenised together in order to get an integrative, averaged measure of isotopic and Hg values within each individual over the moulting period (Carravieri et al., 2014a). Body feathers in Antarctic fulmarine petrels moult gradually over at least four months after the breeding period, *i.e.*, during the austral winter (Beck, 1970; Delord et al., 2020).

In the Southern Ocean, $\delta^{15}N$ values of marine organisms increase with trophic level (Cherel et al., 2010), and $\delta^{13}C$ values indicate their latitudinal feeding habitats (Carpenter-Kling et al., 2020; Jaeger et al., 2010). Specifically, $\delta^{13}C$ isoscapes indicate that values $< -21.2‰$ (feathers) or $< -22.9‰$ (blood) in seabirds correspond to feeding in Antarctic waters (Jaeger et al., 2010). Isotopic values in red blood cells and feathers are representative of a bird's feeding ecology during the 3–4 weeks preceding sampling (here the pre-laying period, in spring) (Bearhop et al., 2002; Carleton and Del Rio, 2005) and during the moult, respectively. A continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112) was used to measure $\delta^{13}C$ and $\delta^{15}N$ values in sample aliquots of ~0.3 mg dw, carefully packed and folded into tin containers. Results are in δ notation relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Internal laboratory standards (acetanilide) were used to check accuracy. Measurement errors were <0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Blood is a good proxy of Hg and OC burdens in seabird internal tissues, and typically represents recent exposure (Fromant et al., 2016; Henriksen et al., 1998). Hg is mainly found in association with red blood cell proteins (Tavares et al., 2013), and is efficiently excreted in feathers (Braune and Gaskin, 1987; Renedo et al., 2021). In most seabirds, $Hg_{Feather}$ residues are thought to be indicative of Hg accumulated over the inter-moulting period (~a year in Antarctic petrels, *i.e.*, the breeding and wintering seasons preceding sampling, Carravieri et al., 2014a). Hg in red blood cells (Hg_{Blood}) and feathers ($Hg_{Feather}$) was quantified with an Altec AMA 254 spectrophotometer (aliquots mass: ~5 mg dry weight, dw) as described in Carravieri et al. (2014b) and Blévin et al. (2013). All analyses were carried out in duplicate (relative standard deviation < 10%). The certified reference material (CRM) DOLT-5 (certified Hg concentration: 0.44 ± 0.18 μg g^{-1} dw) was measured at the beginning and end of each set of analysis, and every 10 samples. Measured values were 0.44 ± 0.01 μg g^{-1} dw, N = 7. CRM mass (and thus Hg mass introduced in the analyser) was adjusted to mirror the Hg mass present in red blood cell samples. Blanks were analysed at the beginning of each set of samples and the limit of detection (LOD) of the AMA was 0.05 ng.

OCs were measured in plasma at the Norwegian Institute for Air Research (NILU), Tromsø. Twelve OCs were targeted, including seven PCBs (congeners CB-52, -99, −101, −118, −138, −153, and −180) and five organochlorine pesticides (OCPs: HCB, Mirex, *trans*-nonachlor, *p, p'*-DDE and *p, p'*-DDT). OCs were extracted from plasma using liquid-liquid extraction in a biphasic mixture of ethanol saturated with ammonium sulphate and hexane. Each sample was spiked with mass-labelled (^{13}C) internal standards prior to extraction to correct for losses during processing and for ion suppression/enhancement. Each sample extraction batch included three blanks and a standard reference material of fortified human serum (National Institute of Standards & Technology (NIST) material 1958). Lipid content (%) was determined gravimetrically by evaporating the collected hexane phase to dryness and weighed to determine the amount of extracted organic content from initial sample amount. Sample extract underwent clean-up with acidified silica (5%, H_2SO_4) using automated solid phase extraction. Collected sample fraction was evaporated to approximately 50 μL followed by the addition of ^{13}C-PCB-159 recovery/syringe standard to each sample. OCs were quantified by gas chromatography high resolution Orbitrap mass spectrometry (GC-HRMS) using methodology described by Warner and Cojocariu (2018), and summarized in the Supplementary Information. Quantification was performed using internal standard calibration with isotopic dilution. Data processing was performed using Tracefinder software v. 4.1 EFS. Concentrations reported for OCs were blank corrected based on the average concentration detected within blank samples. LOD and limit of quantification (LOQ) were compound dependent, and calculated as three and ten times the standard variation within blank samples, respectively. LODs ranged between 1 and 177 pg on column (1.72 and 305 pg g^{-1}), and LOQs between 3 and 589 pg on column (5.17 and 1016 pg g^{-1}). Analytes that had a quantification/qualifier ion ratio >20% of the ratio determined within the quantification standard were not reported. Recoveries of certified reference material to assess method performance ranged between 45% (*p, p'*-DDT) and 104% (*p, p'*-DDE). LODs, LOQs and recoveries of each OC are reported in the Supporting Information (Table S1).

Results are given as mean ± SD in μg g^{-1} dw for Hg, in pg g^{-1} wet weight (ww) and ng g^{-1} lipid weight (lw) for OCs, and in ‰ for stable isotope values. "Blood" within the text refers either to red blood cells for Hg and stable isotope values, or plasma for OC values. For our comparison of Hg and OC concentrations with the literature, we focused on seabird species that are ecologically similar to Antarctic petrels, *i.e.* mid-trophic species feeding on a mixture of fish and crustaceans. Comparisons of OC residues are made on a lw basis in plasma samples only. When possible, we converted OC concentrations from ww to lw by using the mean plasma lipid content (%).

2.3. Statistical analyses

Statistical analyses were carried out in R version 3.5.2 (R Core Team, 2018). Preliminary analyses showed that plasma lipid content and OCs were not correlated (Fig. S1a). Therefore, we used absolute OC concentrations for statistical analyses.

Aim 1: Hg_{Blood} and OC concentrations with quantification frequencies (QF) above 80% were used in a principal component analysis (PCA, R package *ade4*, Dray and Dufour, 2007). For the selected OCs, concentrations below the LOQ were substituted with randomly generated values between zero and the LOQ. Hg_{Blood} and OC values were log-transformed and scaled (centred on the mean and then divided by the standard deviation) prior to the PCA. As OC residues were strongly associated with each other and had high loadings on principal component 1 (PC1, see Results and Table S2), scores were extracted from this axis as a continuous variable representing OC concentrations (hereafter $PC1_{OCs}$) and used in further statistical analyses. $Hg_{Feather}$ were not included in the PCA (since measured in a different tissue), but their correlation with Hg_{Blood} was tested in multifactorial models (see Aim 2 below). $PC1_{OCs}$ were not related to plasma lipid content (Fig. S1b).

Aim 2: we tested the relationship between contaminant concentrations (Hg_{Blood} and $PC1_{OCs}$) and feeding ecology over spring ($\delta^{13}C_{Blood}$ and $\delta^{15}N_{Blood}$ values) and winter ($\delta^{13}C_{Feather}$ and $\delta^{15}N_{Feather}$ values). $Hg_{Feather}$ were also included as an explanatory variable of Hg_{Blood}. The initial models were thus: $Hg_{Blood} \sim Hg_{Feather} + \delta^{13}C_{Blood} + \delta^{15}N_{Blood} + \delta^{13}C_{Blood} + \delta^{15}N_{Blood}$ and $PC1_{OCs} \sim \delta^{13}C_{Blood} + \delta^{15}N_{Blood} + \delta^{13}C_{Blood} + \delta^{15}N_{Blood}$.

Aim 3: the relationships between contaminants and (i) egg volume, (ii) hatching success, and (iii) female and chick SMI, were tested by setting the initial model as follows: *physiological/fitness parameter* ~ $Hg_{Blood} + Hg_{Feather} + PC1_{OCs}$. These models had a total sample size of 29, because one female had a very small egg volume (three SD from the mean), and was removed from all models. Outputs were similar when including this outlier in the analysis, but we decided to present results and estimated effects without it, as they are expected to be closer to the mean effects in the population.

Generalised linear models with different link functions (see Results) were used to address aims 2 and 3. We adopted an information-theoretic approach through the use of Akaike's information criterion corrected for small sample sizes (AICc, R package MuMIn, Bartón, 2019; Burnham and Anderson, 2002). Model assumptions (*e.g.*, residuals' homoscedasticity) were assessed *via* visual inspection of residuals of the initial models (Zuur et al., 2009). For binomial models, model fit was checked through the overdispersion term value. Explanatory variables were not

significantly collinear (variance inflation factors < 3, Dormann et al., 2013), and were standardised (mean = 0, SD = 1) to facilitate comparison of effect sizes. Interactions among the explanatory variables were not included to avoid overfitting and because they were not considered biologically essential. For each model, the AICc, the difference between AICc of the specific model and of the best model (ΔAICc), and the AICc weight (normalized weight of evidence in favour of the specific model, relative to all candidate models, Burnham and Anderson, 2002) were calculated. If the null model performed better than all other candidates, or was within AICc <2 from other models, the effects of all explanatory variables were considered nonsignificant. When multiple models had similarly high support in explaining the response (ΔAICc < 2), we used model averaging to make multi-model inference (Burnham and Anderson, 2002). Averaged parameter estimates (β_{avg}) of all predictor variables contained in the most supported models, weighted using AICc weights, were therefore produced.

3. Results

3.1. Hg and OC values and their inter-relationships

Hg was present at quantifiable concentrations in all blood (1.38 ± 0.43 μg g^{-1} dw) and feather samples (2.41 ± 0.83 μg g^{-1} dw, Table 1). Among the 12 targeted OCs, only CB-180 and *p, p'*-DDE were detected above LOQs in all females (Table 2). The OC pattern was largely dominated by OCPs (89% of Σ_{12}OCs, Fig. 1), in particular HCB (40%), which had the highest absolute mean concentration, even though it was not detected in a few individuals (Table 2). Mean concentration of *p, p'*-DDE (1017 ± 1485 pg g^{-1} ww) was also high, with large between-individual variation (Table 2). Residues of *p, p'*-DDT were detected in two individuals only, with DDT/DDE ratios of 0.06 and 0.07. OCs retained for statistical analyses were CB-138, -153 and −180, HCB, Mirex, and *p, p'*-DDE which all had high QFs (>80%, Table 2). The two first axes of the PCA explained 67% of the total variation in blood contaminant concentrations (Fig. 2). OC residues had high loadings on PC1. Since OC concentrations were negatively associated with PC1 (Fig. 2), increasing $PC1_{OCs}$ represent decreasing OC concentrations. In contrast, PC2 was mainly associated with Hg_{Blood} (high PC2 scores indicating high Hg_{Blood} concentrations). Hg_{Blood} and OC concentrations were thus not related to each other. In addition, concentrations of Hg_{Blood} were not related to $Hg_{Feather}$ (Table S3).

3.2. Effect of winter and spring feeding ecology on Hg and OC concentrations

Descriptive statistics of $\delta^{13}C_{Blood}$, $\delta^{15}N_{Blood}$ and $\delta^{13}C_{Feather}$, $\delta^{15}N_{Feather}$ values are reported in Table 1. Hg_{Blood} concentrations in egg-laying females were positively associated to their trophic level during spring as indicated by a statistically significant effect of $\delta^{15}N_{Blood}$ on Hg_{Blood} residues (β_{avg} ± standard error (SE), 0.17 ± 0.05, Fig. 3). We did not detect any relationship between Hg_{Blood} and other trophic tracers ($\delta^{13}C_{Blood}$, $\delta^{15}N_{Blood}$, $\delta^{13}C_{Feather}$, Table S3). $PC1_{OCs}$ were not predicted by any trophic tracer (the null model was the most supported one, Table S3).

Table 1
Hg concentrations and stable isotope values in red blood cells and feathers of egg-laying Antarctic petrels (N = 30) from Svarthamaren, Antarctica. The first line indicates the mean ± SD, while the second line shows the median and range [min; max].

	Hg (μg g^{-1} dw)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Blood	1.38 ± 0.43	−26.5 ± 0.1	9.2 ± 0.3
	1.34 [0.66; 3.05]	−26.5 [-26.8; −26.3]	9.1 [8.7; 9.9]
Feathers	2.41 ± 0.83	−24.4 ± 0.7	9.2 ± 0.7
	2.33 [1.22; 4.11]	−24.5 [-25.6; −22.6]	9.1 [7.7; 10.9]

Table 2
Lipid content, and absolute and lipid-corrected concentrations of organochlorine compounds (OCs) in plasma of egg-laying Antarctic petrels (N = 30) from Svarthamaren, Antarctica. The first line indicates the mean ± SD, while the second line shows the median and range [min; max]. QF represents the quantification frequency; LOD and LOQ represent the limits of detection and quantification, respectively.

	QF (%)	Absolute concentrations (pg g^{-1} ww)	Lipid-corrected concentrations (ng g^{-1} lw)
Lipid (%)	90	0.60 ± 0.30 (27)	
		0.64 [0.17; 1.37]	
CB-52	0	<LOD	<LOD
CB-99	13	36.8 ± 19.0 (4)	12.7 ± 12.1 (4)
		42.5 [<LOQ; 52]	10.1 [<LOQ; 28.5]
CB-101	0	<LOD	<LOD
CB-118	67	45.3 ± 31.5 (20)	13.4 ± 16.5 (18)
		35 [<LOQ; 147]	7.19 [<LOQ; 63.6]
CB-138	93	72.0 ± 78.0 (28)	21.7 ± 38.4 (25)
		40.7 [<LOQ; 309.8]	6.84 [<LOQ; 184]
CB-153	97	150 ± 121 (29)	42.0 ± 64.6 (26)
		111 [<LOQ; 541]	16.8 [<LOQ; 320]
CB-180	100	103 ± 76.9 (30)	27.6 ± 41.3 (27)
		77.3 [26.4; 361]	12.1 [5.48; 214]
HCB	83	1019 ± 361 (25)	264 ± 228 (22)
		947 [<LOQ; 1908]	162 [<LOQ; 831]
p, p'-DDE	100	1017 ± 1485 (30)	298 ± 579 (27)
		444 [205; 5675]	88.5 [20.9; 2615]
p, p'-DDT	7	319 ± 81.3 (2)	113 (1)
		319 [<LOQ; 376]	
Mirex	97	718 ± 345 (29)	175 ± 212 (27)
		566 [<LOQ; 1806]	103 [0.001; 1070]
trans-nonachlor	7	195 ± 138 (2)	32.9 ± 5.04 (2)
		195 [<LOQ; 292]	32.9 [<LOQ; 36.5]
Σ_7PCBs	100	350 ± 312 (30)	99.0 ± 162 (27)
		248 [90.3; 1366]	37.4 [17.6; 810]
Σ_5OCPs	100	2594 ± 2012 (30)	700 ± 983 (27)
		1932 [864; 9391]	344 [82.9; 4609]
Σ_{12}OCs	100	2944 ± 2307 (30)	799 ± 1142 (27)
		2216 [1076; 10548]	377 [109; 5418]

3.3. Correlation of Hg and OC residues with physiological and fitness parameters

Egg volume (mean ± SD, 85.6 ± 5.97 cm^3) was not affected by Hg_{Blood}, $Hg_{Feather}$ or OC residues (Table S4). Female SMI (mean ± SD, 607 ± 32 g) was not related to Hg_{Blood} or $Hg_{Feather}$ concentrations (Table S4), nor to plasma lipid content (Fig. S1c). In contrast, $PC1_{OCs}$ had a statistically significant effect on female SMI (β ± SE, 16.9 ± 5.6, Fig. 4, Table S4), indicating that increasing OC concentrations were associated with decreasing female SMI. Hatching success (67%) and chick SMI (mean ± SD, 493 ± 65 g) were not related to the maternal Hg_{Blood}, $Hg_{Feather}$, or $PC1_{OCs}$ values (Table S4). At our last nest check, all chicks were alive except one. This indicates that within the range of concentrations observed, there was no effect of maternal Hg or OCs on short-term chick survival.

4. Discussion

This is the first study to document concentrations of Hg in feathers and OCs in plasma of the Antarctic petrel, and to investigate correlations of OCs and fitness parameters in this species. Hg and OC concentrations were not related to short-term reproductive output. However, OC residues were inversely related to the females' body condition.

Both $\delta^{13}C_{Blood}$ and $\delta^{13}C_{Feather}$ values were highly negative, indicating that female Antarctic petrels fed in Antarctic waters both during the pre-laying (spring) and moulting (winter) periods. The range of $\delta^{13}C_{Blood}$ was small, consistent with all females feeding at high Antarctic latitudes during the pre-laying period in spring, as previously shown for the breeding season (Carravieri et al., 2018; Tarroux et al., 2020). The range of both $\delta^{13}C$ and $\delta^{15}N$ values in feathers was larger than in blood,

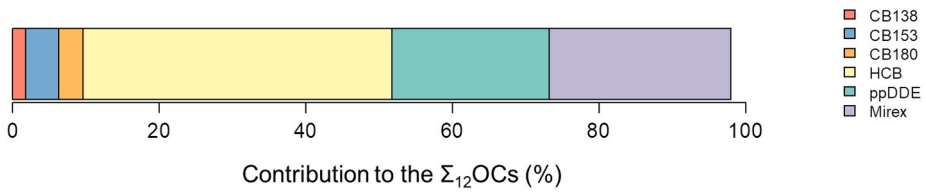

Fig. 1. Stacked bar plot of plasma OC residues in egg-laying Antarctic petrels from Svarthamaren, Antarctica. Values correspond to mean contribution to the Σ_{12}OCs. Contributions of CB-52, -99, -101, -118, *p, p'*-DDT, and *trans*-nonachlor were all <1% and are not represented.

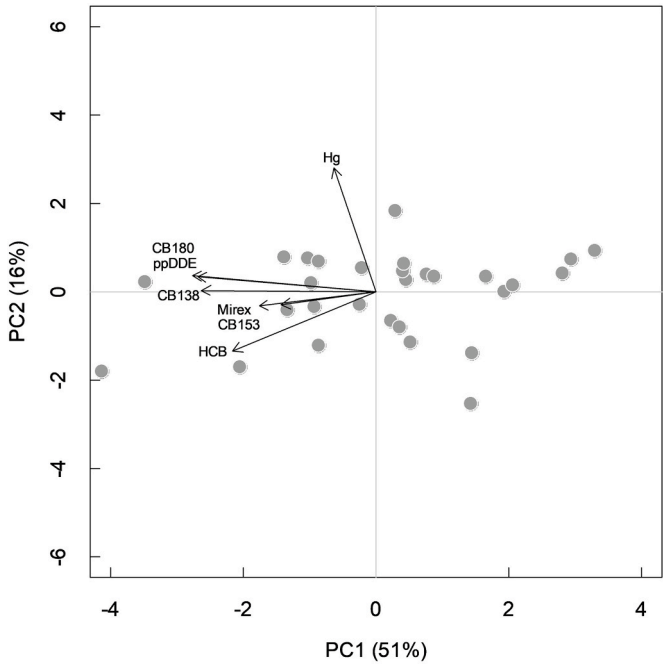

Fig. 2. Biplot of individual scores and contaminant loadings on the two principal axes (PC1 and PC2), obtained from a principal component analysis (PCA) on scaled red blood cell trace elements and plasma organochlorine compound concentrations in egg-laying Antarctic petrels from Svarthamaren, Antarctica.

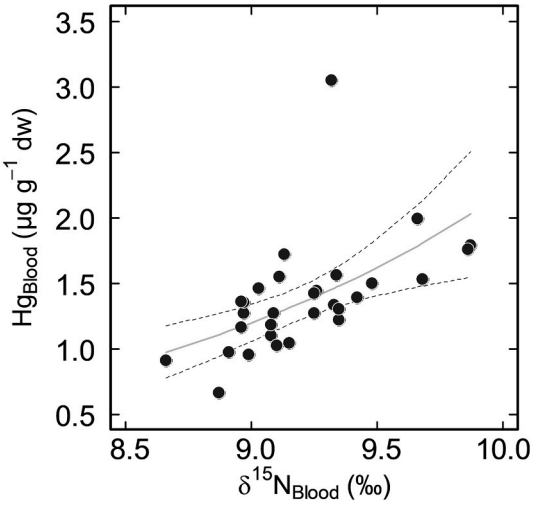

Fig. 3. Relationship between red blood cell Hg concentrations (Hg_{Blood}) and red blood cell $\delta^{15}N$ values ($\delta^{15}N_{Blood}$) in egg-laying Antarctic petrels from Svarthamaren, Antarctica. Coefficient estimates and confidence intervals of the represented model are given in Table S3.

corresponding to a wider foraging distribution that includes low

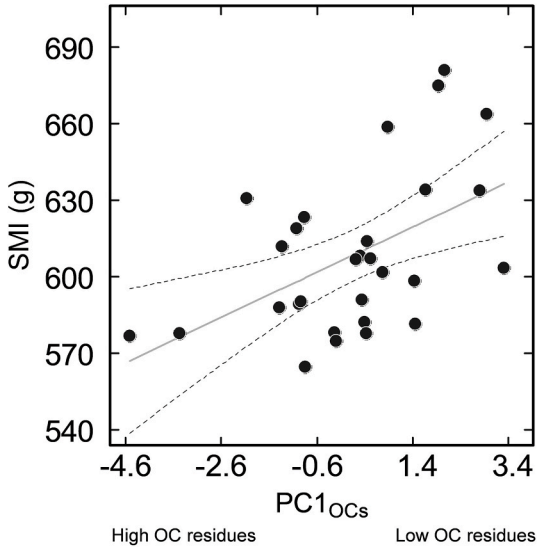

Fig. 4. Relationship between scaled mass index (SMI) and $PC1_{OCs}$ (plasma OC concentrations) in egg-laying Antarctic petrels from Svarthamaren, Antarctica. Coefficient estimates and confidence intervals of the represented model are given in Table S4.

Antarctic latitudes during moult in winter, in accordance with previous isotopic and tracking evidence (Delord et al., 2020; Descamps et al., 2016a). $\delta^{15}N_{Feather}$ values encompassed a whole trophic level (~3‰ range), indicating that prey included crustaceans and fish/squid, most likely Antarctic krill *Euphausia superba* and the myctophid fish *Electrona antarctica* and/or the squid *Psychroteuthis glacialis*, as previously shown (Delord et al., 2020; Lorentsen et al., 1998).

4.1. Hg_{Blood} and $Hg_{Feather}$ concentrations and trophic drivers

Antarctic petrel Hg_{Blood} concentrations were close to those previously reported in ecologically-similar Arctic and Antarctic seabirds, such as little auks (Albert et al., 2019; Fort et al., 2014) and snow petrels *Pagodroma nivea* (Tartu et al., 2015). Hg_{Blood} concentrations in Antarctic petrels were also comparable to those previously found in their internal tissues (muscle, liver, and kidneys, Nygård et al., 2001). However, the present egg-laying Hg_{Blood} concentrations (2017) were almost twice as high as previously reported in late incubating and chick-rearing individuals from the same population (approx. 0.83 µg g^{-1} dw in the breeding seasons of 2013 and 2014, Carravieri et al., 2018). Although rarely considered, breeding stage or timing of sampling can affect Hg_{Blood} residues in seabirds (Carravieri et al., 2018; Hipfner et al., 2011; Tartu et al., 2016; Lerma et al., 2016). In our previous study, Hg_{Blood} concentrations were lowest during late incubation, and then increased slightly, but significantly, across the breeding season (Carravieri et al., 2018). Hence, breeding stage is unlikely to be a key factor in explaining the difference in Hg_{Blood} concentrations between 2013–14 and 2017. At remote sites far from Hg point sources, inter-annual variability in Hg concentrations in seabird blood and feathers is usually very low (Brasso

et al., 2014; Carravieri et al., 2016). An increase in Antarctic petrel Hg_{Blood} concentrations over such a short period is unlikely, and contrasts with recent declines in Hg concentrations in myctophid fish and squid, although from a different Antarctic region (South Georgia and South Orkneys Islands, Seco et al., 2020b; 2020a). Antarctic petrel $\delta^{15}N_{Blood}$ values were very similar in 2013–14 and 2017 samples (approx. 9.2‰), thus suggesting that the difference in Hg_{Blood} concentrations was not driven by a shift of Antarctic petrel's diet to a higher trophic level (Braune et al., 2014). More likely, intra-annual fluctuations in environmental factors (*e.g.*, vertical ocean dynamics, light irradiance, sea-ice melt, atmospheric deposition) that influence Hg transport, methyl-Hg production, and bioavailability to marine predators and their prey (Cossa et al., 2011; Driscoll et al., 2013; Renedo et al., 2020) could explain this temporal difference. Identifying specific mechanisms that explain this result is beyond the scope of our study. The doubling of Hg_{Blood} concentrations in this population highlights that future monitoring is needed to assess whether this trend continues upward and why.

$Hg_{Feather}$ concentrations were intermediate when compared to results available in other Antarctic seabirds. Antarctic petrel $Hg_{Feather}$ concentrations were higher than those of penguin species (Brasso et al., 2014; Polito et al., 2016), comparable to those of skuas (*Catharacta* sp, Bargagli et al., 1998; Calle et al., 2015), and lower than those of black-bellied storm petrels *Fregetta tropica* (Pacyna et al., 2019). $Hg_{Feather}$ concentrations were also intermediate when compared to Arctic seabirds, being higher than those of Alcidae, and lower than those of Laridae species (reviewed in Albert et al., 2019). These inter-specific comparisons are made to put results in a polar context, and cannot be interpreted solely in terms of trophic position. Instead, pole-specific Hg dynamics and transfer to food webs, as well as species-specific moulting patterns and wintering distribution come into play to explain them. Concentrations of $Hg_{Feather}$ were twice as high as Hg_{Blood}, and there was no correlation between the two. $Hg_{Feather}$ concentrations reflect the Hg burden accumulated in internal tissues over the inter-moult period, thus covering a longer time period than Hg accumulated in blood. In addition, exposure to Hg over wintering grounds could be higher than around the high latitude Antarctic breeding sites. In winter, Antarctic petrels migrate north of the marginal sea-ice zone, up to the northern limits of Antarctic waters (Delord et al., 2020; Descamps et al., 2016b), where ecologically-similar seabird species were shown to have higher Hg_{Blood} and $Hg_{Feather}$ concentrations than "true" Antarctic species (Becker et al., 2016; Carravieri et al, 2014c, 2020).

As expected, Hg_{Blood} concentrations were positively related to the pre-laying female trophic position ($\delta^{15}N_{Blood}$ values), indicating that individuals feeding higher in the food web (*i.e.*, larger proportions of fish/squid in their diet) accumulated more Hg than those relying mainly on krill (Carravieri et al., 2018). This is consistent with myctophid fish and squid having higher tissue Hg concentrations than krill in Antarctic waters (Anderson et al., 2009; Seco et al, 2019, 2020a, 2020b), and confirms the biomagnification of Hg in high latitude Antarctic food webs during the austral spring. Feeding habitat ($\delta^{13}C_{Blood}$ values) did not drive Hg_{Blood} concentrations, likely as a result of the spatial homogeneity of Hg transfer to food webs around Antarctica (Brasso et al., 2015; Carravieri et al., 2018). Feeding ecology over winter ($\delta^{13}C_{Feather}$ and $\delta^{15}N_{Feather}$ values) had a weak explanatory power of Hg_{Blood} concentrations, confirming that Hg acquired during winter is efficiently and rapidly excreted from the organism though feather and egg deposition, and/or stored long-term in internal tissues (Bearhop et al., 2000; Cherel et al., 2018).

4.2. Plasma OC concentrations and trophic drivers

Exposure of seabirds to OCs can happen in Antarctica (Bustnes et al., 2006a; Mello et al., 2016; Roscales et al., 2016). As expected from biomagnification mechanisms (Borgå et al., 2001), plasma OC concentrations of Antarctic petrels were up to 10 times lower than those of their predator at Svarthamaren, the south polar skua *Catharacta maccormicki* (Bustnes et al., 2007). Antarctic petrels and skuas had similar OC patterns, with notably HCB, Mirex and *p, p'*-DDE being the strongest contributors (Bengtson Nash et al., 2007; Bustnes et al., 2006a). As expected, PCB concentrations were 15–124 times lower in Antarctic petrels than in ecologically similar Arctic seabird species (Table 3). Antarctic petrels also had lower PCB residues than female snow petrels from Adélie Land, Antarctica (Tartu et al., 2015, Table 3). HCB residues were comparable to those of Arctic and Antarctic species, while Mirex concentrations were six times higher in Antarctic petrels (Table 3). This is in agreement with previous results in south polar skuas from the same site (Bustnes et al., 2006a). Plasma *p, p'*-DDE were comparable to those of Arctic-breeding Mandt's black guillemots *Cepphus grylle mandtii* (Eckbo et al., 2019), and were three times higher than those of female snow petrels (Tartu et al., 2015, Table 3). Plasma *p, p'*-DDT residues were quantified in two individuals here, while they were under detection limits in ecologically similar Arctic species (Eckbo et al., 2019; Haarr et al., 2018). Overall these results indicate very low exposure to PCBs, intermediate exposure to HCB and *p, p'*-DDE, and relatively high exposure to Mirex in Antarctic petrels. This calls for further investigations on potential toxic effects in Antarctic petrels, as Mirex can impact fitness in Antarctic predators (Goutte et al., 2018).

Plasma OC residues were not influenced by either pre-laying (spring) or moulting (winter) feeding ecology, despite relatively strong variation in blood $\delta^{15}N$ values between individuals. This contrasts with previous studies on polar seabirds (Carravieri et al., 2014b; Mello et al., 2016; Roscales et al., 2016), and could have two non-mutually exclusive explanations: 1) physiological factors (differential OC metabolism and excretion, and transfer of OCs to the eggs, Borgå et al., 2001; Bustnes et al., 2010; Dehnhard et al., 2017) overcame trophic factors in driving plasma OC variation in females upon egg-laying; 2) there is little spatial and trophic variation in OC residues among prey of Antarctic petrels. The latter explanation is consistent with previous studies showing low OC biomagnification factors between crustaceans and fish in the Arctic (Borgå et al., 2001). In addition, Antarctic marine invertebrates and fish species were shown to have comparable OC residues in their tissues (Ko et al., 2018). Finally, other Antarctic and subantarctic krill-eating seabirds were shown to accumulate more OCs than fish-eating species (Carravieri et al., 2020).

4.3. Correlation of Hg and OC with fitness components

Reproduction is very sensitive to Hg toxicity in birds. Parental Hg concentrations can be associated with altered breeding behaviours (Evers et al., 2008; Heath and Frederick, 2005; Tartu et al., 2015), reduced egg size (Evers et al., 2003; Fort et al., 2014), and hatching success (Heinz et al., 2009; Yu et al., 2016). Egg-laying maternal Hg_{Blood} concentrations are correlated to *in ovo* Hg values (reviewed in Ackerman et al., 2020). *In ovo* Hg concentrations can also affect growth, behaviour, and survival of chicks in experimental settings (Heinz, 1976a, 1976b, 1979), with contrasting evidence from the wild (*e.g.*, Ackerman et al., 2008; Herring et al., 2012, 2010; Kenow et al., 2003). Hg effects can be species-specific, dependent on the level of exposure, and/or on concurring physiological, environmental and ecological factors (Heinz et al., 2009; Hill et al., 2008). Here, maternal Hg_{Blood} or $Hg_{Feather}$ concentrations were not related to egg volume, contrary to our prediction based on results in Arctic breeding little auks, which had similar blood Hg concentrations (Fort et al., 2014). In addition, we did not detect an effect of maternal Hg_{Blood} nor $Hg_{Feather}$ concentrations on hatching success, chick survival and the SMI of 20-day-old chicks. The females' SMI was also unrelated to Hg_{Blood} or $Hg_{Feather}$ concentrations. Previous studies on the relationship between body condition and Hg concentrations in birds have reported negative (Ackerman et al., 2019; Fort et al., 2015), positive (Kalisińska et al., 2010), or no relationship (Heath and Frederick, 2005; Herring et al., 2014; Tartu et al., 2015). These contrasting results suggest that this relationship could be modulated by concurring environmental perturbations, or by the birds' physiological

Table 3
Comparison of plasma concentrations of major OCs ([OC]) between egg-laying Antarctic petrels (ANPE) from Svarthamaren, Antarctica, and selected polar species.

OC	Reference	Species[b]	[OC] ng g^{-1} ww[c]	Lipid content (%)	[OC] ng g^{-1} lw	$[OC]_{ANPE}$ ng g^{-1} lw	Ratio $[OC]/[OC]_{ANPE}$
ΣPCBs[a]	Eckbo et al. (2019)	Black guillemot	18.1	1.2	1508	99	15
	Haar et al. (2018)	Black guillemot, F	14	0.7	2000		20
	Haar et al. (2018)	Black-legged kittiwake, F	16	0.13	12308		124
	Tartu et al. (2015)	Snow petrel, F			1976		20
HCB	Eckbo et al. (2019)	Black guillemot	2.2	1.2	186	264	0.7
	Tartu et al. (2015)	Snow petrel, F			193		0.7
Mirex	Eckbo et al. (2019)	Black guillemot	0.352	1.2	29	175	0.2
	Tartu et al. (2015)	Snow petrel, F			27		0.2
p, p'-DDE	Eckbo et al. (2019)	Black guillemot	3.188	1.2	266	298	0.9
	Tartu et al. (2015)	Snow petrel, F			93		0.3

[a] Eckbo et al., (2019): Σ_7PCBs = CB-52, -99, -101, -118, -138, -153, -180 (same as this study); Haar et al., (2018): Σ_{12}PCBs = CB-28, -52, -99, -101, -105, -118, -138, -153, -170, -180, -183, and -187; Tartu et al., (2015): Σ_4PCB = CB-101, -138, -153, -180.
[b] When available, we considered values measured in females (F).
[c] When [OC] were given in wet weight (ww), they were converted to lipid weight (lw) by using the mean plasma lipid content.

status. For instance, energetically challenged individuals (starving or migrating, Ackerman et al., 2019; Fort et al., 2015), or those experiencing strong environmental change (Fort et al., 2014), could be more vulnerable to the effect of Hg on their health, and thus show a negative relationship. Here, the lack of association between Hg, body condition and short-term reproductive output suggests that, under current environmental conditions, Hg exposure appears to be of little concern in this population.

In seabirds, OC residues in plasma are correlated with OC concentrations in internal organs (brain, liver, *e.g.*, Henriksen et al., 1998), and in eggs (Verreault et al., 2006). Previous studies on the correlates of OCs and fitness parameters in wild birds have shown negative relationships with egg size, phenology, hatching and breeding success, over the short and long terms (Bustnes et al., 2006b; Goutte et al., 2014b; Helberg et al., 2005), including in Svarthamaren south polar skuas (Bustnes et al., 2007). Here, maternal OC concentrations did not predict egg size, hatching success and chick SMI at 20 days of age. However, female Antarctic petrels having higher concentrations of OCs had lower SMI. This result is consistent with previous findings in Mandt's black guillemots and great black-backed gulls *Larus marinus* (Eckbo et al., 2019; Helberg et al., 2005), but not in the similarly OC-contaminated snow petrel (Tartu et al., 2015). Females in poorer body conditions may have experienced a stronger depletion of their lipid reserves, and thus released larger quantities of OCs in blood (Bustnes et al., 2017; Van den Brink et al., 1998). Alternatively, females with higher blood OC concentrations may have suffered toxic effects that ultimately impacted their SMI. Egg-laying is an energetically costly behaviour, and female Antarctic petrels leave the nest shortly thereafter to feed at sea (Lorentsen and Røv, 1995). In seabirds, low SMI are often the consequence of poor environmental conditions and/or food deprivation (energetic stress, Wanless et al., 2005). Strong release of OC concentrations could thus constitute an additional challenge for individuals in poor body condition, and have long-term fitness consequences (Goutte et al, 2014a, 2018), despite no apparent OC effects on short-term fitness. Further experimental evidence is needed to confirm this hypothesis on potential toxic effects of OCs in egg-laying Antarctic petrels.

5. Conclusion

Antarctic petrels showed low exposure to PCBs, low to intermediate exposure to Hg and OCs, and relatively high Mirex residues. Hg and OC exposure does not seem to be of toxicological concern in the breeding fraction of this population over the short term. However, the clear correlation between female plasma OC residues and SMI could result in long-term fitness effects. In addition, birds with higher OC concentrations and lower SMI may have skipped breeding. Further monitoring of this sensitive population is a priority, especially in the context of climate warming that could increase the quantities of contaminants available to predators, *e.g.*, through enhanced methyl-Hg production in the subsurface ocean (Cossa, 2013), or food-web reorganisation (Braune et al., 2014). Future work in Antarctic petrels should also establish (i) the link between environmental contaminants and breeding propensity; (ii) the exposure and effects of emerging contaminants such as perfluoroalkyl substances (Roscales et al., 2019) and chlorinated paraffins (Li et al., 2016); and (iii) the combined effects of environmental contaminants and other changing biological and environmental factors in Antarctica (*e.g.*, primary productivity, krill abundance, sea-ice dynamics, Meredith et al., 2019).

Credit author statement

AC: conceptualization, methodology, formal analysis, writing - original draft, visualisation; NW: validation; DH: validation; MB-F: investigation; AT: investigation, methodology; JF: methodology; PB: resources, validation; SD: methodology, funding acquisition, resources, project administration. All authors contributed to writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.110518.

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