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Interspecific and intraspecific variation in organochlorine pesticides and polychlorinated biphenyls using non-destructive samples from *Pygoscelis* penguins☆

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ABSTRACT

As humans are present in Antarctica only for scientific and tourism-related purposes, it is often described as a pristine region. However, studies have identified measurable levels of Persistent Organic Pollutants (POPs), such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), in the Antarctic region. These are highly toxic anthropogenic compounds with tendency to travel long distances and reach remote environments, where they can bioaccumulate in the biota. Penguins are exposed to POPs mainly through their diet, which they partially eliminate via feathers. Species of the genus *Pygoscelis* occur around Antarctic continent and its surrounding regions, and can act as indicators of contaminants that reach the continent. Here, we report OCP and PCB levels in feathers of male and female penguins of *P. adeliae, P. antarcticus* and *P. papua* from King George Island, South Shetland Islands, Antarctica. Interspecific, sex- and body-size-related differences were investigated in the contamination profiles of PCBs and OCPs. Feather samples were collected from adult penguins (n = 41). Quantification of compounds was performed by gas chromatography-tandem mass spectrometry. The three *Pygocelis* species presented similar contamination profiles, with higher concentrations of dichlorodiphenyltrichloroethane (∑DDT; 1.56–3.82 ng g^{-1} dw), lighter PCB congeners (∑PCB: 11.81–18.65 ng g^{-1} dw) and HCB (hexachlorobenzene: 1.65–4.06 ng g^{-1} dw). Amongst the three penguin species, *P. antarcticus* had lower and *P. papua* higher concentrations of most of the compounds identified. We found interspecific differences in POP accumulation as well as sex differences in POP concentrations. Our data indicate a small but significant positive correlation between body size and the concentrations of some compounds. Despite the overall low concentrations found, this study increases knowledge of the occurrence of POPs in Antarctic penguins, thereby reinforcing concerns that Antarctica, although remote and perceived to be protected, is not free from the impact of anthropogenic pollutants.

1. Introduction

Antarctica is often referred to as one of the last pristine environments on the planet (Bargagli, 2008). This is partly due to its geographical isolation, which is reinforced by the atmospheric circulation and oceanic currents surrounding the continent and partly by the absence of a native human population and industrial/agricultural activities, with human presence restricted to scientific and tourist activities (Bargagli, 2008). Under the Antarctic Treaty, the entire region is designated as a natural reserve devoted to peace and science, protected under the Protocol on Environmental Protection to the Antarctic Treaty (ATS, 1991). However, the increase in global anthropogenic disturbances (e.g., habitat destruction, invasive species, pollution and climate change) also affects the environmental conditions of the continent (Bhardwaj et al., 2018; Convey and Peck, 2019).

Contamination generated by anthropogenic activities is a concern for all ecosystems globally. The Antarctic environment receives contaminants from distant sources, mainly through long-range atmospheric and maritime transport from lower latitudes (Wania, 2003). Furthermore, local sources such as ships, research stations and tourism activities also contribute to contaminant loads (Bargagli, 2008; Choi et al., 2008; Convey and Peck, 2019; Mwangi et al., 2016).

Persistent organic pollutants (POPs) are anthropogenic compounds that are potentially highly toxic and persistent in the environment.

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They tend to bioaccumulate in the tissues of organisms and may biomagnify along the food chain, making them a serious threat for the biota and the environment (Adeola, 2004). Furthermore, POPs have high dispersal capacity due to their volatility and the role of successive evaporation and condensation cycles during movement from warmer to colder regions of the planet (Wania, 2003), enabling them to reach remote regions such as Antarctica. Climate change, especially global warming, can influence the fate of POPs by remobilising previously trapped compounds, for instance from ice caps or permafrost, into the environment (Nadal et al., 2015; Potapowicz et al., 2020).

POPs include organohalogenated compounds, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), which are intensively used for industrial purposes and pest control, respectively. However, due to their toxic effects on different taxa, which include mutagenicity and teratogenicity, the production and use of these chemicals is tightly regulated or banned in many countries by the Stockholm Convention, which aims to protect the environment from the harmful impacts of POPs (UNEP, 2018). Even so, due to their high persistence in the environment and long half-life, many of these compounds are still present in the environment.

Various seabirds have been widely used as environmental sentinels as they have long life cycles, large populations and breeding colonies, and show strong fidelity to their breeding areas (Burger and Gochfeld, 2004). They also typically occupy higher trophic levels, making them suitable for studying the impacts of pollutants on marine ecosystems (Burger and Gochfeld, 2004; Hazen et al., 2019; Pacyna et al., 2019).

The genus *Pygoscelis* includes three species: Adélie penguin (*P. adeliae*), chinstrap penguin (*P. antarcticus*) and gentoo penguin (*P. papua*). They are amongst the dominant avifauna in the Antarctic Peninsula and sub-Antarctic islands (Black, 2016). They represent the largest biomass on the continent, with the global size of Adélie, chinstrap and gentoo penguin populations estimated to be 9.4 million, 6.8 million and 860,000 mature individuals, respectively (Herman et al., 2020; Southwell et al., 2017; Strycker et al., 2020). Due to their wide distributions, they have potential to provide data on the pollutants present in the region and their transfer through the marine trophic web, making them suitable sentinels with which to understand contamination levels and pathways in the Antarctic environment (Metcheva et al., 2006; Souza et al., 2020).

All three species have similar trophic position and feeding habits, with diets primarily consisting of Antarctic krill (*Euphausia superba*) and different proportions of fish. Some studies have suggested that gentoo penguins consume a significantly larger proportion of fish than other pygoscelids (Herman et al., 2017; Polito et al., 2011; Volkman et al., 1980).

Pygoscelis penguins moult their feathers annually after the reproductive period. During this process, they fast for approximately three weeks losing up to 50% of their body mass (Black, 2016). During this process, contaminants present in their fat and protein reserves can be transferred to their bloodstream, and consequently may be deposited in feathers during their formation (Black, 2016; García-Fernández et al., 2013; Groscolas and Cherel, 1992; Jaspers et al., 2019). Contaminants present in preen oil also contribute to POP levels in the feathers (Jaspers et al., 2008). Feathers may also act as passive samplers, as environmental contaminants may be deposited externally on the feather surface (Jaspers et al., 2019).

Several seabird species have been used as sentinels to study POP contamination in the Antarctic ecosystem. Most studies have analysed tissues rich in lipids, such as fat and liver (Potapowicz et al., 2020). This approach has advantages, as POPs are often lipophilic and can be present at higher concentrations in fat-rich tissues. However, this also means that individuals are usually sacrificed for the purpose of such studies. Therefore, other studies have focussed on non-destructive matrices, such as blood and faeces (Potapowicz et al., 2020). This approach allows work with living animals and their release after sample collection. In this context, feathers are useful matrices to biomonitor environmental contaminants, including legacy POPs (García-Fernández et al., 2013; Jaspers et al., 2019). Feathers are a non-destructive tool for biomonitoring environmental contaminants in the Antarctic species, which fall under the protection of the Environmental Protocol, which strictly regulates the collection or handling of any living animal in the Antarctic Treaty area. However, despite the recognised potential, studies in this field remain scarce. The use of non-destructive methods to increase understanding of the dynamics of POP accumulation and elimination through feathers provides an important new contribution to this research field.

In this study, we report the levels of OCPs and PCBs in the feathers of male and female of *P. adeliae, P. antarcticus* and *P. papua* in Admiralty Bay, King George Island, South Shetland Islands, Antarctica. The main aim of the study is to evaluate interspecific and intraspecific differences in the contamination profiles of PCBs and OCPs, as well as the influence of sex and body size.

2. Materials and methods

2.1. Study area and sample collection

Fieldwork was carried out between November 2013 and March 2014 in Admiralty Bay, King George Island (Fig. 1). Adult penguins (*P. adeliae*: male n = 6, female n = 7; *P. antarcticus*: male n = 7, female n = 7; *P. papua*: male n = 7, female n = 7) were captured during the breeding season using a long-handled net. Weight and morphometric measurements (flipper length, bill height, bill length, bill width, head-bill length, foot length, abdominal circumference and total length) of the captured animals were recorded; each individual was ringed using metal rings provided by the National Center for Research and Conservation of Wild Birds. Breast feathers were cut close to the base with pointless surgical scissors and stored in paper envelopes. Approximately 50 μL of blood was collected for the molecular identification of sex.

2.2. Molecular identification of sex

DNA extraction was performed as described by Sambrook et al. (1989). A fragment of the Chromo Helicase DNA-binding gene was amplified using the polymerase chain reaction (PCR) and the P2/P8 primer (Griffiths et al., 1998). The PCR products were separated on a 2% agarose gel/1x TBE gel at 200 V for 2 h, stained with ethidium bromide and visualised using 300-nm UV light as described by Souza et al. (2020).

2.3. POP measurements in feathers

Determination of OCPs and PCBs was carried out in collaboration with the Department of Analytical Chemistry at the Adam Mickiewicz University (Poznań) and the Gdansk University of Technology (Gdansk), both located in Poland. Feather samples were washed three times with ultrapure water (Milli-Q®), dried at 20 °C and cut with stainless steel scissors that were cleaned with acetone and n-hexane. Extraction was performed as described by Jaspers et al. (2010). About 500 mg of each sample was incubated overnight with 12 mL of hexane:dichloromethane mixture (4:1, v/v), 12 mL of 4M HCl and 10 μL of labelled internal standards (4,4-DDT-d_8, ^{13}C-PCB 28 and ^{13}C-PCB 180 at 0.1 μg mL^{-1}). The samples were vortexed the next day, after which the organic layers were collected. Another 10 mL of hexane: dichloromethane (4:1, v:v) was added to the samples, after which the organic layers were collected twice and combined with the previously collected layers. The extract was evaporated under nitrogen flow until

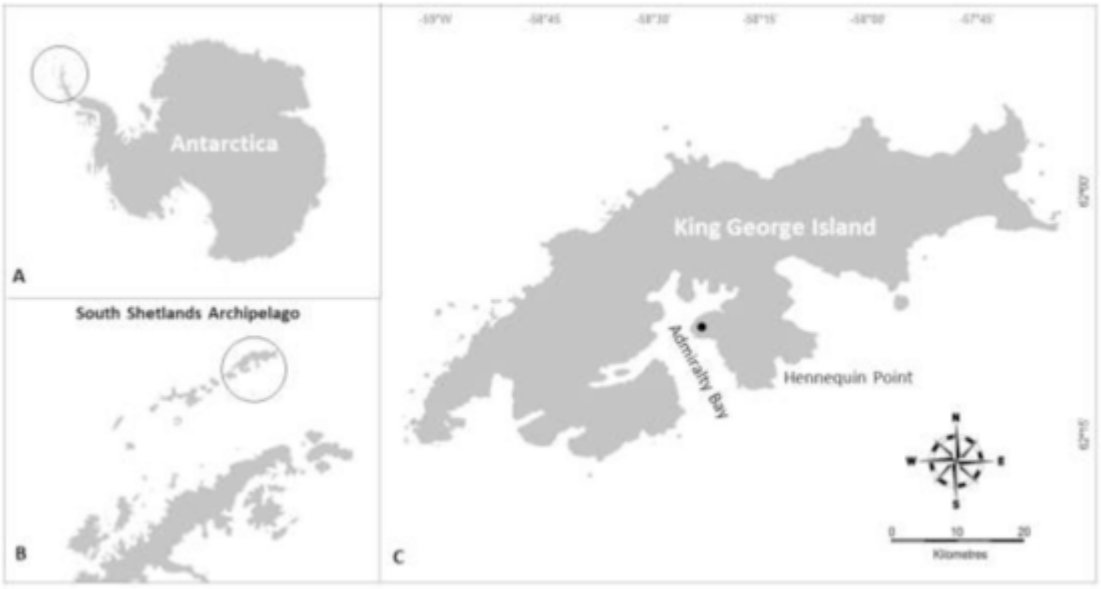

Fig. 1. Study area: A) Antarctic continent with Antarctic Peninsula shown in the circle; B) Antarctic Peninsula with South Shetland Islands and King George Island shown in the circle; C) King George Island. Sampling site at Hennequin point is marked with a black dot.

reduced to approximately 2 mL. Clean-up was performed using solid phase extraction columns that were filled with acidified silica gel and anhydrous Na_2SO_4. Columns were conditioned with 4 mL of hexane:dichloromethane (4:1, v/v); the sample extract was then added to the column and eluted using hexane: dichloromethane (4:1 v:v; 2 × 4 mL). The sample was evaporated under nitrogen flow until almost dry and, then, reconstituted to 150 μL using isooctane. The determination and quantification of the OCPs dichlorodiphenyldichloroethylene (-DDE), dichlorodiphenyltrichloroethane (-DDT), dichlorodiphenyldichloroethane (-DDD), α-HCH, β-HCH, hexachlorobenzene (HCB), heptachlor, mirex and PCBs (PCB-28, -52, −77, −101, −118, −126, −138, −153, −169 and −180) were performed using a gas chromatograph (Agilent 7890B) coupled to a tandem mass spectrometer (Agilent 7000D) (GC-MS/MS) operated under multiple reaction monitoring (MRM) mode. Full measurements conditions and parameters were reported in a previous study (Pacyna-Kuchta et al., 2020). Briefly, column HP-5MS 5% Phenyl Methyl Silox 30 m × 250 μm × 0.25 μm (Agilent Technologies, Inc) was used. The column temperature was initially held at 70 °C for 2 min, raised to 150 °C at the rate of 25 °C/min, then to 200 °C at the rate of 3 °C/min, and to 280 at a rate of 8 C, held for 10 min, finally to 300 °C at a rate of 100 °C/min, and held at the final temperature for 5 min. Total analysis time was 47.067 min. Helium (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min. A solvent delay of 6 min was used to prevent filament damage. Splitless mode was used. Injection volume was 5 μL, N_2 was used as Collision Gas (1.5 mL/min), and He as Quench Gas (2.5 mL/min). Parameters used in equipment and MRM analysis are available in the Supplementary Table S1 and S2.

2.4. Quality assurance and control (QA/QC)

Analytical quality was guaranteed using analytical blanks (n = 3). The average recovery for internal standards was 69% for 4,4-DDT-d_8 (Sigma-Aldrich, USA), 72% for ^{13}C-PCB28 and 83% for ^{13}C-PCB180 (Cambridge Isotope Laboratories, USA). The limit of detection (LOD) was calculated as the concentration corresponding to the signal equal to three times the standard deviation of the blank signal. For compounds not detected in the blanks, LODs were calculated based on the standard deviation of the response (s) and the slope of the calibration curve (b). This was according to the formula LOD 3.3(s/b). LODs for OCPs and PCBs ranged between 0.011 ng g^{-1} and 2.67 ng g^{-1} dry weight. All results were corrected using the blank concentrations and are expressed as ng.g^{-1} dry weight.

2.5. Statistical analyses

Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software Inc®); the graphics were created with the computer program R (3.2.3 - http://www.r-project.org). The normality of the concentration distribution ($P < 0.05$) was verified using the Shapiro-Wilk test (Test-W); however, when the number of quantified samples was <7, non-parametric tests were directly applied. Interspecific differences were tested using the Kruskal-Wallis test, with *post-hoc* Dunn's Multiple Comparisons Test used to identify significant differences among the three studied species. Intraspecific sex differences were tested using the Student' s *t*-test or the Mann-Whitney *U* test. Relationships between the concentrations of each compound and the morphometric measurements were investigated using Pearson or Spearman correlation tests. A significance level of 5% was adopted for all tests.

3. Results

OCPs and PCBs were detected in all the feather samples of the three penguin species studied. The compounds detected were PCB congeners (PCB-28, -52, −101, −118, −138, −153 and −180), mirex, HCB, α-HCH, β-HCH and isomers DDT and DDE. The PCB and OCP concentrations are detailed in Table 1 and the concentration distributions in males, females and the three species of penguins are shown in Fig. 2 and Figure S2, respectively.

3.1. Distribution of organochlorine compounds (OCs)

PCBs constituted the main group of organochlorine pollutants in the feathers of *Pygoscelis* penguins and were detected in all the samples that were analysed. The predominant congeners were PCB-52 and PCB-28, constituting more than 70% of the total PCBs. The predominant OCPs were HCB and DDE. We detected α-HCH, mirex and PCB-180 in only 15%, 32% and 54% of the samples, respectively. PCB-153 and PCB-138 were detected in 100% of the samples. The compounds -DDD, PCB-77,

Table 1
Mean ± standard deviation, median, range (ng.g $^{-1}$ dry weight) of OCP and PCB concentrations with sample number (n) in feathers of *Pygoscelis adeliae, P. antarcticus* and *P. papua* from King George Island, Antarctica.

		P. adeliae			P. antarcticus			P. papua		
		Adult	Female	Male	Adult	Female	Male	Adult	Female	Male
α-HCH	Mean	0.51	0.51	<LOD	0.14 ± 0.03	0.14 ± 0.03	<LOD	0.18 ± 0.12	0.22 ± 0.18	0.10
	Median	–	–	–	0.14	0.14	<LOD	0.10	0.22	–
	Min-max	–	–	–	0.12–0.17	0.12–0.17	<LOD	0.09–0.34	0.09–0.34	–
	n	1	1	0	2	2	0	3	2	1
β- HCH	Mean	1.09 ± 0.61	1.18 ± 0.84	1.00 ± 0.35	0.92 ± 0.31	1.20 ± 0.07	0.76 ± 0.28	1.43 ± 0.81	1.40 ± 0.91	1.46 ± 0.85
	Median	0.95	0.97	0.92	0.94	1.23	0.64	1.18	1.18	1.22
	Min-max	0.51–2.59	0.51–2.59	0.62–1.58	0.54–1.26	1.12–1.26	0.54–1.23	0.17–3.01	0.17–2.97	0.75–3.01
	n	10	5	5	8	3	5	13	7	6
∑HCH	Mean	1.14 ± 0.76	1.28 ± 1.06	1.00 ± 0.35	0.96 ± 0.36	1.30 ± 0.08	0.76 ± 0.28	1.47 ± 0.84	1.46 ± 0.96	1.48 ± 0.84
	Median	0.95	0.97	0.92	1.00	1.28	0.64	1.18	1.18	1.27
	Min-max	0.51–3.10	0.51–3.10	0.62–1.58	0.54–1.38	1.23–1.38	0.54–1.23	0.17–3.01	0.17–2.97	0.75–3.01
HCB	Mean	3.34 ± 1.71	3.54 ± 2.24	3.09 ± 0.94	4.06 ± 5.91	2.94 ± 1.98	5.40 ± 8.85	1.65 ± 2.65	1.07 ± 0.79	2.14 ± 3.60
	Median	3.32	2.77	3.32	1.80	2.85	1.80	0.84	0.78	0.91
	Min-max	1.53–7.49	1.76–7.49	1.53–4.05	0.19–21.18	0.78–5.13	0.19–21.18	0.0710.12	0.29–2.39	0.07–10.12
	n	12	6	6	11	6	5	13	6	7
Mirex	Mean	0.29 ± 0.24	0.10 ± 0.01	0.49 ± 0.12	0.14 ± 0.08	0.08	0.19	0.25 ± 0.16	0.28 ± 0.16	0.06
	Median	0.25	10	0.49	0.14	–	–	0.26	0.29	–
	Min-max	0.09–0.10	0.09–0.10	0.40–0.58	0.08–0.19	–	–	0.06–0.48	0.08–0.48	–
	n	4	2	2	2	1	1	7	6	1
DDE	Mean	2.32 ± 1.54	1.38 ± 0.89	3.26 ± 1.50	1.47 ± 0.65	1.60 ± 0.61	1.32 ± 0.79	3.61 ± 2.55	3.73 ± 1.68	3.49 ± 3.51
	Median	2.07	1.17	2.95	1.19	1.40	1.06	2.49	3.47	2.05
	Min-max	0.48–6.07	0.48–3.09	1.72–6.07	0.76–2.70	1.13–2.70	0.76–2.67	0.7110.35	1.96–6.83	0.7110.35
	n	12	6	6	11	6	5	14	7	7
DDT	Mean	0.15 ± 0.16	0.14 ± 0.14	0.15 ± 0.20	0.21 ± 0.12	0.24 ± 0.13	0.15 ± 0.09	0.21 ± 0.12	0.27 ± 0.13	0.15 ± 0.09
	Median	0.09	0.10	0.02	0.18	0.21	0.12	0.21	0.25	0.11
	Min-max	0.01–0.46	0.07–0.46	0.01–0.45	0.06–0.46	0.11–0.46	0.06–0.29	0.01–0.53	0.13–0.53	0.01–0.27
	n	12	7	6	12	7	5	14	7	7
∑DDT	Mean	2.28 ± 1.69	1.32 ± 1.10	3.39 ± 1.64	1.56 ± 0.72	1.61 ± 0.86	1.47 ± 0.88	3.82 ± 2.60	4.01 ± 1.77	3.64 ± 3.55
	Median	1.74	1.24	2.96	1.34	1.34	1.18	2.73	3.67	2.07
	Min-max	0.07–6.52	0.07–3.55	1.74–6.52	0.31–3.03	0.31–3.03	0.84–2.96	0.8310.61	2.23–7.36	0.83–10.61
PCB-28	Mean	3.52 ± 1.32	2.52 ± 0.36	4.52 ± 1.13	3.26 ± 0.91	3.44 ± 1.04	3.05 ± 0.77	4.96 ± 1.66	5.69 ± 1.94	4.22 ± 0.95
	Median	2.97	2.64	4.79	3.35	3.42	3.17	4.85	5.02	4.31
	Min-max	1.87–5.60	1.87–2.89	3.05–5.60	2.00–4.85	2.00–4.85	2.02–4.01	3.01–9.76	3.63–9.76	3.01–5.64
	n	12	6	6	13	7	6	14	7	7
PCB-52	Mean	8.46 ± 4.77	4.57 ± 0.99	12.35 ± 3.58	6.84 ± 2.52	7.54 ± 2.77	6.02 ± 2.14	9.36 ± 3.12	11.16 ± 2.91	7.57 ± 2.26
	Median	7.16	4.53	11.08	7.03	7.03	6.11	8.71	10.53	8.08
	Min-max	3.27–17.89	3.27–5.92	8.41–17.89	2.94–11.50	4.12–11.50	2.94–8.75	4.41–15.82	8.25–15.82	4.41–11.10
	n	12	6	6	13	7	6	14	7	7
PCB-101	Mean	2.76 ± 1.45	1.75 ± 0.54	3.77 ± 1.36	1.66 ± 0.66	1.83 ± 0.69	1.46 ± 0.62	2.61 ± 1.25	3.33 ± 1.40	1.90 ± 0.45
	Median	2.41	1.65	3.56	1.40	1.57	1.39	2.09	3.15	1.77
	Min-max	1.26–5.70	1.26–2.70	2.41–5.70	0.71–2.86	1.22–2.86	0.71–2.59	1.36–4.91	1.82–4.91	1.36–2.72
	n	12	6	6	13	7	6	14	7	7
PCB-118	Mean	0.82 ± 0.46	0.52 ± 0.13	1.12 ± 0.49	0.48 ± 0.17	0.54 ± 0.19	0.41 ± 0.11	0.82 ± 0.37	1.06 ± 0.36	0.59 ± 0.19
	Median	0.65	0.47	1.00	0.42	0.42	0.41	0.64	1.00	0.51
	Min-max	0.37–1.83	0.37–0.72	0.64–1.83	0.26–0.83	0.34–0.83	0.26–0.58	0.39–1.53	0.61–1.53	0.39–0.98
	n	12	6	6	13	7	6	14	7	7

		P. adeliae			P. antarcticus			P. papua		
		Adult	Female	Male	Adult	Female	Male	Adult	Female	Male
PCB-138	Mean	0.43 ± 0.31	0.29 ± 0.10	0.60 ± 0.40	0.21 ± 0.14	0.26 ± 0.17	0.16 ± 0.10	0.48 ± 0.27	0.60 ± 0.31	0.35 ± 0.13
	Median	0.34	0.30	0.46	0.20	0.22	0.16	0.43	0.46	0.35
	Min-max	0.14–1.17	0.14–0.45	0.16–1.17	0.01–0.54	0.07–0.54	0.01–0.29	0.19–1.03	0.27–1.03	0.19–0.49
	n	13	7	6	14	7	7	14	7	7
PCB-153	Mean	0.30 ± 0.19	0.22 ± 0.08	0.39 ± 0.25	0.17 ± 0.09	0.20 ± 0.09	0.14 ± 0.08	0.33 ± 0.15	0.41 ± 0.17	0.24 ± 0.07
	Median	0.22	0.21	0.30	0.17	0.19	0.16	0.26	0.43	0.24
	Min-max	0.13–0.72	0.13–0.36	0.15–0.72	0.01–0.33	0.08–0.33	0.01–0.22	0.14–0.66	0.20–0.66	0.14–0.36
	n	13	7	6	–14	7	7	14	7	7
PCB-180	Mean	0.18 ± 0.05	0.15 ± 0.03	0.24 ± 0.04	0.12 ± 0.05	0.13 ± 0.05	0.11 ± 0.04	0.18 ± 0.09	0.22 ± 0.08	0.10 ± 0.01
	Median	0.17	0.15	0.24	0.10	0.11	0.09	0.16	0.19	0.11
	Min-max	0.11–0.27	0.11–0.18	0.22–0.27	0.09–0.21	0.09–0.21	0.09–0.15	0.09–0.32	0.16–0.32	0.09–0.11
	n	6	4	2	7	4	3	9	6	3
∑PCB	Mean	15.18 ± 9.04	8.62 ± 4.09	22.84 ± 6.75	11.81 ± 4.43	13.89 ± 4.10	9.72 ± 4.81	18.65 ± 5.62	22.44 ± 5.26	14.86 ± 3.54
	Median	12.97	10.16	20.03	12.32	14.14	10.67	16.94	22.85	14.31
	Min-max	0.30–12.97	0.30–12.97	15.94–32.93	0.14–18.98	8.98–18.98	0.14–14.21	9.41–30.00	16.07–30.00	9.41–21.11

PCB-126, PCB-169 and heptachlor had concentrations below the LOD in all the samples.

3.2. Interspecific differences

Concentrations of PCB-28 varied between penguin species (KW = 11.13; p = 0.0038), with gentoo penguins showing significantly higher levels than Adélie and chinstrap penguins (Dunn's Test, both $p < 0.05$). Similarly for PCB-153 (KW = 9.364; p = 0.0093) and -DDE (KW = 7.319; p = 0.0257), where gentoo penguins showed higher levels compared to chinstrap penguins (Dunn's Test, $p < 0.05$). In contrast, gentoo penguins showed HCB concentrations (KW = 6.763; p = 0.0340) that were lower than those of Adélie penguins (Dunn's Test, $p < 0.05$). Also, chinstrap penguins had lower concentrations of PCB-101 (KW = 7.513; p = 0.0234), PCB-118 (KW = 10.01, p = 0.0067) and PCB-138 (KW = 11.37; p = 0.0034) compared to Adélie and gentoo penguins (Dunn's Test, all $p < 0.05$). There were no significant interspecific differences among the other compounds.

3.3. Sex-related differences

Males and females gentoo and Adélie penguins showed significant intraspecific differences with respect to several compounds. Female gentoo penguins had higher concentrations of PCB-28 (U = 8.000; p = 0.0379), PCB-52 (t = 2.582; df = 12; p = 0.0240), PCB-101 (t = 2.570; df = 12; p = 0.0245), PCB-118 (U = 5.000; p = 0.0111), PCB-153 (t = 2.421; df = 12; p = 0.0323) and PCB-180 (U = 0.0000; p = 0.0238) compared to males. In contrast, male Adélie penguins had higher concentrations of PCB-28 (U = 0.0000; p = 0.0022), PCB-52 (U = 0.0000; p = 0.0022), PCB-101 (U = 2.000; p = 0.0087), PCB-118 (U = 2.000; p = 0.0087) and -DDE (U = 3.000; p = 0.0152) than the females. No significant sex differences were found with respect to other compounds or in chinstrap penguins.

3.4. Body size-related differences

Concentrations of OCP and PCB compounds were individually correlated with the morphometric measurements and weights of the specimens sampled. The morphometric parameters of each species are shown in Supplementary Table S3.

Gentoo penguin weight (KW = 28.23; $p < 0.0001$), foot size (KW = 17.70; $p < 0.0001$) and abdominal circumference (KW = 32.08; $p < 0.0001$) were significantly greater than those of Adélie and chinstrap penguins (Dunn's Test, $p < 0.05$). Gentoo penguins also demonstrated longer bill length (KW = 23.93; $p < 0.0001$) and greater total body length (KW = 22.68; p = 0.001) than chinstrap penguins (Dunn's Test, $p < 0.05$). The total body length of Adélie penguins (KW = 22.68; p = 0.001) was greater than that of chinstrap penguins (Dunn's Test, $p < 0.05$); moreover, their bill height (KW = 6.559; p = 0.0376) was greater than those of chinstrap and gentoo penguins (Dunn's Test, both $p < 0.05$).

Males and females showed no significant differences in body measurements, except in the case of chinstrap penguins, where the body mass of males was approximately 8% greater than that of females (t = 2.423; df = 12; p = 0.0322).

There were small but significant positive correlations between the morphometric measurements and POP concentrations in the three penguin species. Concentrations of most compounds were weakly correlated with foot length (r values between 0.31 and 0.36), weakly to moderately correlated with total length (r values between 0.34 and 0.45) and weight (r values between 0.38 and 0.54), and moderately correlated with abdominal circumference (r values between 0.40 and 0.59) (Table 2).

4. Discussion

Contaminant studies in the Antarctic region began in the 1960s, with the initial studies detecting low pesticide concentrations in biotic and abiotic samples (George and Frear, 1966; Sladen et al., 1966). Since then, various studies have focussed on monitoring the presence of halogenated compounds in this area (Bustnes et al., 2007; Colabuono et al., 2014, 2016; Corsolini et al., 2002; Goerke et al., 2004; Montone et al., 2016; Potapowicz et al., 2020; Roosens et al., 2007; Schiavone et al., 2009). Sladen et al. (1966) analysed a fat sample from an emperor penguin (*Aptenodytes forsteri*) that had remained frozen since its collection in 1911, 30 years before the global use of DDT commenced, and detected no residue of halogenated com-

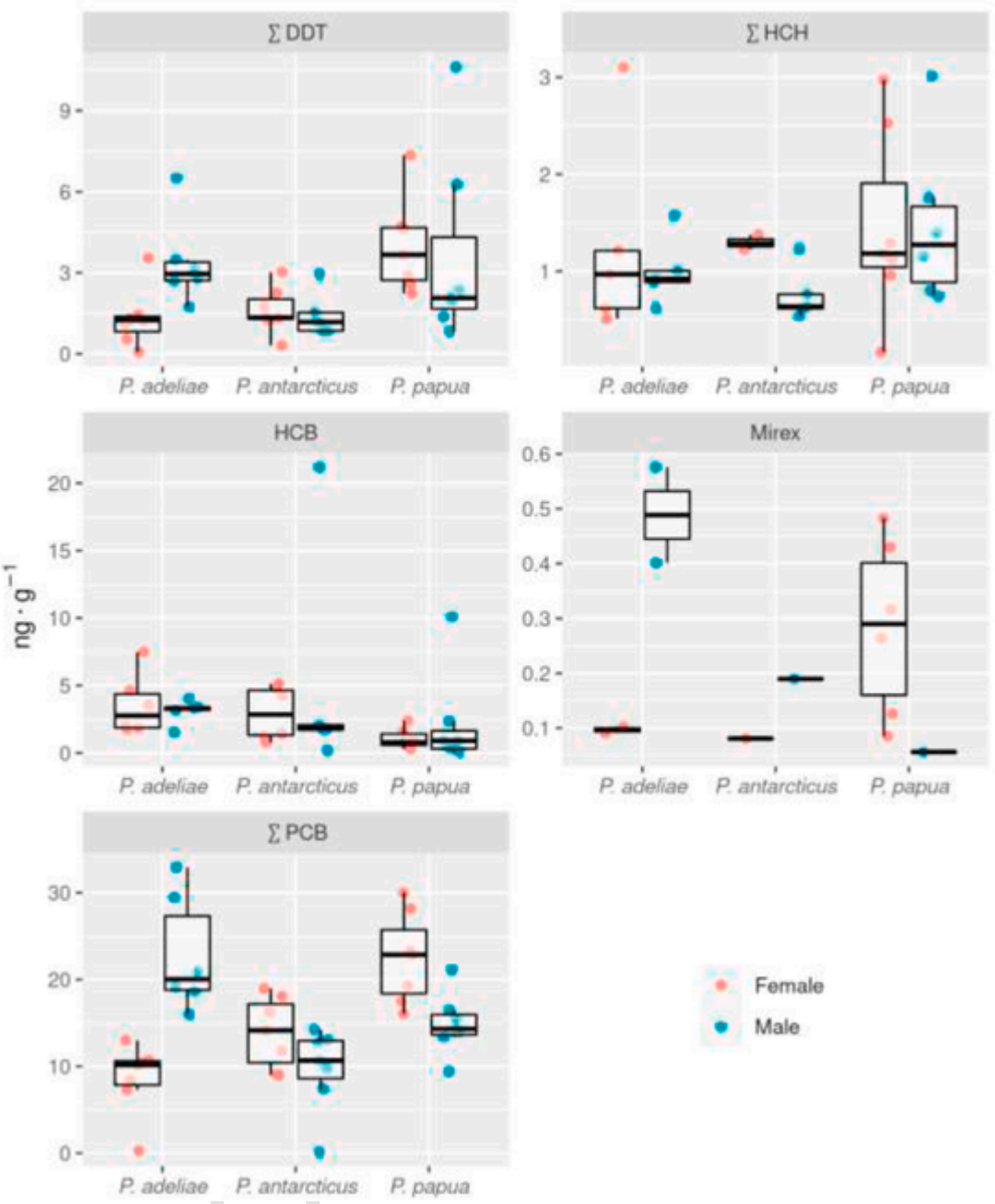

Fig. 2. Boxplot of the concentrations (ng g^{-1} dry weight) of OCPs and PCBs in the feathers of females and males of Pygoscelis adeliae, P. antarcticus, and P. papua from King George Island, Antarctica. The horizontal bar inside the boxplot represent the median and the dots represent the quantified samples.

pounds or their metabolites. Other studies used samples from *Pygoscelis* penguins to study OCPs, including invasively-collected samples, such as fat, liver and muscle (de Boer and Wester, 1991; Inomata et al., 1996; Kim et al., 2010, 2015; Lukowski, 1983) and less invasive samples, such as eggs and blood (e.g. Inomata et al., 1996; Jara-Carrasco et al., 2014; Kim et al., 2010; Mello et al., 2016). However, only a single study has reported concentrations of OCPs in feathers (Metcheva et al., 2017).

Metcheva et al. (2017) investigated the presence of the OCPs, such as α-HCH, β-HCH, *p,p'*-DDE and *o,p'*-DDE, in the feathers of chinstrap and gentoo penguins from Livingston, South Shetland Islands, and Petermann Island, off the Antarctic Peninsula. However, they did not quantify concentrations of PCBs. No study has evaluated the concentration of any of these compounds in Adélie penguins' feathers or in feathers of any species from King George Island. Metcheva et al. (2017) reported higher values of OCPs (in some cases, greater than three orders of magnitude) than we measured in the present study for chinstrap penguins (Table 3). The difference between studies may be explained by the interpretation of the sum of ΣHCHs and ΣDDTs, as more HCH and DDT isomers were identified by Metcheva et al. (2017) than in the present study. But despite this, as in the present study, they identified higher concentrations of β-HCH in the ΣHCHs. This result was expected owing to the fact that β-HCH is the dominant HCH isomer in seabirds, and appears to be more recalcitrant than other isomers (Moisey et al., 2001). In contrast to our study, the DDT isomer was the dominant component in ΣDDTs in Metcheva et al. (2017) study. Higher concentrations of DDT (compared to DDE) generally indicate recent exposure or local sources (Hellou et al., 2013). Since the Stock-

Table 2
- Correlations of concentrations of pesticides and chlorinated biphenyls (r value; p value) with morphometric parameters of adult *Pygoscelis adeliae, P. antarcticus,* and *P. papua* from King George Island, Antarctica.

	Weight	Flipper length	Foot length	Bill height	Bill length	Bill width	Head-bill length	Abdominal circumference	Total length
α-HCH	**	**	**	**	**	**	**	**	**
β-HCH	**	**	**	r = −0.4002; p = 0.0284	**	**	**	**	**
Mirex	**	**	**	**	**	**	**	**	**
HCB	r = −0.4668; p = 0.0047	r = −0.3332; p = 0.0470	r = −0.3631; p = 0.0295	**	r = −0.3980; p = 0.0179	**	**	r = −0.3966; p = 0.0183	r = −0.3326; p = 0.0475
DDE	r = 0.4817; p = 0.0029	**	**	**	**	**	**	r = 0.5661; p = 0.0003	r = 0.3957; p = 0.0153
DDT	**	**	**	**	**	**	**	**	**
PCB-28	r = 0.4117; p = 0.0102	**	r = 0.3443; p = 0.0318	**	**	r = −0.3236; p = 0.0475	**	r = 0.5193; p = 0.0008	r = 0.3356; p = 0.0367
PCB-52	**	**	**	**	**	**	**	**	**
PCB-101	r = 0.3841; p = 0.0173	**	r = 0.3322; p = 0.0388	**	**	**	**	r = 0.4778; p = 0.0024	r = 0.3407; p = 0.0338
PCB-118	r = 0.4288; p = 0.0072	**	**	**	**	**	**	r = 0.5303; p = 0.0006	r = 0.3754; p = 0.0185
PCB-138	r = 0.5329; p = 0.0005	r = 0.3837; p = 0.0145	r = 0.3189; p = 0.0449	**	**	**	**	r = 0.5900; p = <0.0001	r = 0.3961; p = 0.0114
PCB-153	r = 0.5378; p = 0.0003	r = 0.4336; p = 0.0046	**	**	**	**	**	r = 0.5346; p = 0.0004	r = 0.4549; p = 0.0028
PCB-180	**	**	**	**	**	**	**	r = 0.4690; p = 0.0320	**

Table 3
Comparison of the concentrations of organochlorine pesticides detected in Pygoscelis antarcticus and P. papua feathers. Results are presented as ng. g^{-1} dry weight.

	P. antarcticus		*P. papua*		
	Present study	Metcheva et al. (2017)	Present study	Metcheva et al. (2017)	Metcheva et al. (2017)
Region	King George Island	Livingston Island	King George Island	Livingston Island	Peterman Island
N	14	24	14	15	3
α-HCH	0.14 ± 0.03	43 ± 13	0.18	58 ± 6	44 ± 4
β- HCH	0.92 ± 0.31	285 ± 145	1.43 ± 0.85	154 ± 54	238 ± 4
ΣHCHs	0.77 ± 0.43	419 ± 178	1.19 ± 0.91	356 ± 179	437 ± 12
DDE	1.47 ± 0.68	326 ± 57	3.61 ± 2.65	313 ± 71	227 ± 4
DDT	0.21 ± 0.12	1856 ± 263	0.21 ± 0.13	1783 ± 172	2125 ± 6
∑DDTs	0.97 ± 0.53	2596 ± 312	2.48 ± 1.78	2507 ± 254	2628 ± 13

holm Convention came into force, the production and global use of DDT have declined (Van Den Berg et al., 2017). Some studies have indicated that melting glacial ice is a potential source introducing DDT and other POP compounds to the Antarctic food web as these compounds trapped in the icebergs in Antarctica are released and can partially be made available for the base of the food chain (Geisz et al., 2008; Potapowicz et al., 2020).

DDE is the most persistent metabolite and its predominance has been reported in other Antarctic studies (Corsolini, 2009; Corsolini et al., 2006; Kim et al., 2015). DDE isomers are obtained almost entirely from the degradation of DDT (ATSDR, 2019). The prevalence of this isomer indicates that DDT residues are formed exclusively due to past contamination, as they are easily redistributed globally and persist in the environment long enough to reach Antarctica (Goerke et al., 2004). Corsolini et al. (2006) also suggested that the high percentage of this metabolite in penguins is due to its high degree of biomagnification in the Antarctic food web.

Feathers have the potential to act as a passive sampler collecting contaminants present in the environment, with dust or airborne particles passively deposited on the feathers contributing to the total POP concentrations detected (Jaspers et al., 2007, 2019). However, as already noted by Jaspers et al. (2008), water is an ideal medium to remove external contamination, while it does not remove preen oil. Contaminant presence in this oil indicates an internal source and it is an important element that integrates the concentrations of POPs in penguin feathers. Therefore, in this study, we opted to wash feathers using only Milli-Q ultrapure water preserving the preen oil for its future extraction during POP extraction from the whole feather.

DDEs and PCBs are among the most common anthropogenic compounds present in Antarctic seabirds (Corsolini, 2009). The concentrations of these compounds measured in the present study were relatively low, as might be expected for this remote region (Table 1). Gentoo penguins showed the highest concentrations while chinstrap penguins showed the lowest concentrations for most compounds (eight and 11 of the 13 quantified, respectively). Gentoo penguins also included fewer samples below the LOD. Several inter- and intra-specific biological factors, such as diet, body size, age, sex, life cycle, habitat use and migration, can affect the bioaccumulation of POPs as well as the

species' physiology and the chemical characteristics of the compounds under consideration (Borgå et al., 2004).

Diet and trophic level are the main factors that lead to exposure to OCPs (Borgå et al., 2004), and species with higher trophic positions are generally likely to be exposed to higher concentrations of contaminants. While their diets are similar overall, the different species of *Pygoscelis* typically consume different proportions of krill, fish and amphipods. Gentoo penguins consume a higher percentage of fish compared to chinstrap penguins (Polito et al., 2015), with the latter being the largest consumer of krill in the South Shetland Islands (Croll and Tershy, 1998). Gentoo and Adélie penguins also have broader dietary and isotopic niches than chinstrap penguins (Herman et al., 2017; Polito et al., 2015), which could explain why gentoo penguins had higher concentrations of most OCPs and PCBs than chinstrap penguins, except for HCB.

Bengtson Nash et al. (2008) analysed more than 100 halogenated compounds, including OCPs and PCBs, in krill (*E. superba*), with the HCB being the most abundant compound detected, which is consistent with the highest concentrations of this compound being found in chinstrap penguins in the current study.

Gentoo and chinstrap penguins have the largest and smallest body sizes of the genus, respectively. Body size can influence the bioaccumulation of OCPs (Borgå et al., 2004). Concentrations of PCBs, HCBs and DDTs show moderate correlations with abdominal circumference and weight of the *Pygoscelis* penguins, indicating that size impacts the OCP concentrations in the species examined. However, more studies with larger sample sizes are needed to examine this pattern in more detail.

Lighter congeners of PCBs were predominantly detected in the present study. The accumulation profile of feathers and preen gland oil is known to be relatively richer in di-, tri-, and tetra-PCBs (García-Fernández et al., 2013; Yamashita et al., 2007). This may be associated with the fact that less chlorinated congeners are more easily metabolized and remobilized when compared to heavier congeners that in general are strongly bound to fat tissues (García-Fernández et al., 2013; Maervoet et al., 2004; Van Den Brink, 1997). Consistent with this, Montone et al. (2016) detected a predominance of heavier congeners, such as PCB-138, PCB-153 and PCB-180, in fat samples from adult *Pygoscelis* penguins.

Lighter PCB congeners, tri-PCBs and tetra-PCBs, such as PCB-28 and PCB-52, also have a higher dispersion capacity being transported more efficiently to polar regions by the global distillation process (Wania and Mackay, 1993). Consistent with our results, the predominance of low chlorinated (i.e. lighter) PCBs appears to be a specific feature of PCB congeners present in various matrices and samples from Antarctica. For example, higher concentrations of low chlorinated PCBs have been detected in the Antarctic atmosphere (Montone et al., 2003, 2005), at the base of the Antarctic food chain in krill (*E. superba*) (Cipro et al., 2010; Corsolini et al., 2002) and silverfish (*Pleuragramma antarcticum*) (Corsolini et al., 2002), and also in eggs (Cipro et al., 2010) and fat samples from chicks of pygoscelids (Montone et al., 2016).

Differences in the physiology and behaviour of birds can lead to differences in patterns of contaminant accumulation (Taniguchi et al., 2009). In general POP levels in Antarctic birds are lower compared to those of birds at lower latitudes, especially those from more industrialized and agricultural areas. This is because the polar regions have only a few local sources of those compounds, resulting in at least one order of magnitude lower concentrations of PCBs and DDTs (Bhardwaj et al., 2018; Wania and Mackay, 1993). Even so, Van den Brink et al. (1997) reported high levels of HCB in preen oil from some Antarctic seabirds, indicating that the volatility of this compound was sufficient to support its migration to cold regions by global distillation (Wania and Mackay, 1993).

Female gentoo penguins and male Adélie penguins showed higher concentrations of PCBs, indicating that males and females of each species may be differently exposed to POPs. Polito et al. (2015) and Pilcher et al. (2020) discussed sexual differences in the foraging behaviour and diet of gentoo and Adélie penguins. They hypothesised that males, being larger, would dive deeper and consume a larger proportion of fish (Polito et al., 2015) or target older and/or different prey (Pilcher et al., 2020) than females. However, both these and the current study measured no differences in body size of male and female gentoo or Adélie penguins. However, a higher number of fish otoliths was present in female stomach samples, suggesting sexual differences in the diet of gentoo penguins (Polito et al., 2015). Based on values of δ15N, Pilcher et al. (2020) suggested sexual intraspecific differences in prey selection and consumption, with male Adélie penguins feeding preferentially on more fish than females.

A review of the use of feathers as a biomonitoring tool for organochlorine compounds showed that most studies on OCs in feathers from different bird species and locations found measurable concentrations of ∑DDT, HCB, ∑HCH and ∑PCB (García-Fernández et al., 2013), supporting the use of feathers in POP monitoring studies. Other studies have validated the use of feathers as a useful non-destructive matrix for the measurement of POP concentrations (Dauwe et al., 2005; Eulaers et al., 2011; García-Fernández et al., 2013; Jaspers et al., 2019), emphasising their utility and relevance in environmental pollution studies.

5. Conclusions

This study documented the contamination profiles of POPs in the feathers of three members of the genus *Pygoscelis,* confirming that penguin feathers are a useful and a non-destructive tool to biomonitor organohalogenated compounds in Antarctica. The predominant compounds in feathers were DDTs, PCBs and HCB. Despite the overall low concentrations reported, this study increases the available information on POPs in Antarctic penguins, re-emphasising that Antarctica, although remote and protected, is not free from the impact of anthropogenic pollutants. The sex-related differences found emphasise the importance of studying and understanding the differences between males and females that may lead to variation in exposure, accumulation and elimination of pollutants. Body size is another variable that may influence the concentration of POPs, albeit with low correlation. It is important to highlight that this matrix may favour the detection of some compounds compared to others, as is the case for PCB congeners with a lower number of chlorine atoms. Studies that quantify POPs in Antarctic penguin feathers are still scarce. Further studies focusing on other compounds of toxicological interest (e.g. organobromine and organofluorine compounds) are required to provide clearer information on the exposure of penguins to these and other contaminants.

Credit author statement

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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.envpol.2021.116590.

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