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Co-occurrence of microplastics and endocrine-disrupting chemicals in subantarctic seabirds

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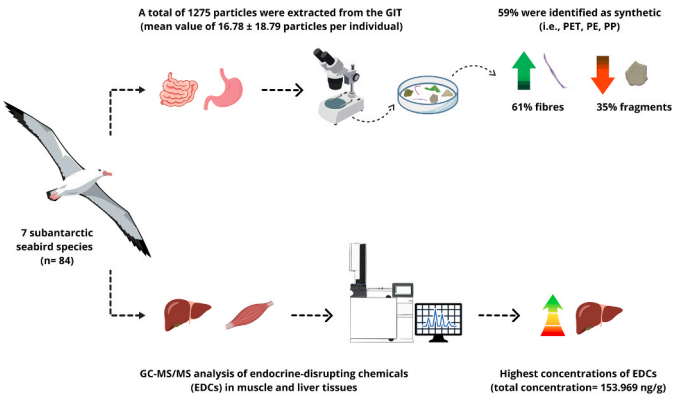
HIGHLIGHTS

- Anthropogenic particles were detected in every seabird species and age classes.
- BFRs were detected in liver and muscle across all seabird species, regardless age.
- 59% of the analysed anthropogenic particles presented a synthetic origin.
- Liver showed higher accumulation of PBDE and MeO-PBDE congeners compared to muscle.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite the remoteness of their breeding sites, subantarctic seabirds are susceptible to anthropogenic pollutants (e.g. microplastics) and other chemical stressors (e.g. plastic additives) that are released from ships and research stations, arrive in ocean currents, are transported in the atmosphere, or are ingested when the birds feed north of the Antarctic Polar Front. In this study, we investigated the presence and levels of microplastics and several

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Gastrointestinal tract
Liver

groups of endocrine-disrupting chemicals (EDCs) in adults or chicks of seven seabird species breeding at the subantarctic islands of South Georgia. A total of 1275 anthropogenic particles were recovered in the gastrointestinal tracts of 76 seabirds, with a frequency of occurrence of 97.4%, a mean value of 16.78 ± 18.79 particles per individual and of 0.03 ± 0.03 particles/g body weight. Ten percent (n = 130 particles) of the particles were identified chemically using microFTIR spectroscopy, of which 59% were synthetic, 18% were natural, 19% were anthropogenic unknown and 4% were anthropogenic cellulosic. Of the EDCs, only polybrominated diphenyl ethers (PBDEs) and methoxylated polybrominated diphenyl ethers (MeO-PBDEs) congeners occurred at levels above the limit of quantification. Liver samples consistently exhibited the highest concentrations of both contaminant groups. The highest concentrations of PBDEs were in adult brown skuas (133.96 ng/g) and of MeO-PBDEs were in wandering albatross chicks (6.50 ng/g). This research provides evidence of plastics and plastic additives in subantarctic seabirds, underscoring the need to strengthen measures aimed at reducing marine pollution.

1. Introduction

Plastic pollution is a widespread environmental issue affecting marine ecosystems worldwide [1, 2]. A large proportion of plastic waste reaches the ocean due to inadequate waste management and disposal practices [3], where it represents a persistent anthropogenic stressor [4]. Once in the marine environment, plastics are subject to physical and chemical processes such as wind, waves, and ultraviolet radiation, which promote their fragmentation into progressively smaller particles, including micro- and nanoplastics [5, 6].

Microplastics are defined as plastic particles between 1 μm and 5 mm [7, 8]. They are widespread and persistent in marine environments [9], and available to a wide range of marine organisms [5, 6]. Recent studies suggest that microplastics are now pervasive in Antarctic and Southern Ocean ecosystems, further reinforcing concerns about their persistence and bioavailability in remote marine environments [10–12]. Their prevalence has been measured in Antarctic marine food webs, from lower trophic levels (e.g., Antarctic krill, *Euphausia superba*, and salps) [13] to top predators (e.g., penguins) [14], and in the environment, including sediments, snow, ice and water [15–18].

Although microplastics can pose a major threat to Antarctic and Southern Ocean biota [19], most studies focus solely on quantifying their abundance and overlook the transfer of chemical additives into organisms [20, 21]. These additives may threaten biota due to their persistence, bioaccumulation potential and toxicity [21–23]. Fragmentation of plastics in marine settings releases compounds that were within the plastic or later adsorbed from the environment [24, 25]. These include endocrine-disrupting chemicals (EDCs) [26], affecting homeostasis and other functions of the endocrine system, with deleterious consequences for physiological status and reproductive performance [27–29]. Among the many endocrine disruptors, our study focuses on man-made plastic additives, including brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and their structural analogues, methoxylated polybrominated diphenyl ethers (MeO-PBDEs) [30, 31]; ultraviolet filters (UV-filters); musk fragrances; and bisphenols (BPs). MeO-PBDEs can be formed through debromination of synthetic PBDEs [32, 33]. However, studies show that some congeners can also occur naturally in the environment; specifically, two of the isomers, 6-MeO-BDE47 and 2'-MeO-BDE68, are produced naturally by algae, marine sponges and cyanobacteria [30, 33]. These EDCs are typically lipophilic and can bioaccumulate through the food web [31, 34–36]. Due to their extensive usage and applications [37, 38] they have been reported in several environmental compartments (e.g. soil, wastewater, water, biota) [29]. They may also leach from ingested plastics, leading to further accumulation in tissues [39, 40] and associated to health risks [41, 42]. For this reason, some PBDEs, specifically tetraBDE (BDE-47), pentaBDE (BDE-99), hexaBDE (BDE-153), heptaBDE (BDE-154), decaBDE (BDE-209), and one UV-filter, UV-328, have been banned or their use has been restricted in some countries, mainly in Europe [43–45].

Seabirds play a central role in marine ecosystems and are amongst the organisms most affected by plastic pollution through ingestion of plastics and exposure to plastic-derived chemicals [39, 46, 47]. In the Antarctic and subantarctic regions, penguins and albatrosses are key sentinels of human pressures, including pollution [48, 49], and have been included in the Commission on the Conservation of Antarctic Living Resources (CCAMLR) monitoring programs since the 1980s [50, 51]. Concerns over declining albatross and large petrel populations also led to the establishment of the multilateral Agreement on the Conservation of Albatrosses and Petrels (ACAP) [52]. Antarctic and subantarctic seabirds have been used to assess the presence of ingested macro-, meso- and microplastic pollution [53–56], and levels of legacy persistent organic pollutants [53], perfluoroalkyl substances (PFAS) [54, 55] and polychlorinated biphenyls (PCBs) [56]. Despite recent progress, key gaps remain in understanding emerging pollutants in the Southern Ocean, particularly the levels of plastic particles and additives, their transfer to organisms, their potential for tissue accumulation, and their biological effects. The aims of this study were therefore to: (i) assess the exposure to meso- and microplastics of a range of subantarctic seabird species, and (ii) determine the presence and concentrations of associated endocrine-disrupting chemicals in internal organs.

2. Material and methods

2.1. Study site and target species

The subantarctic island group of South Georgia is located at 53°S to 55°S, 34°W to 42°W and supports globally important breeding populations of several seabird species [57, 58]. This study focused on the following species and age classes for which samples were available from opportunistic collection of birds found freshly dead at colonies or elsewhere at South Georgia, or following vessels or building strikes or mortality in fishing gear in surrounding waters: wandering albatross *Diomedea exulans* chicks, black-browed albatross *Thalassarche melanophris* chicks and adults, grey-headed albatross *Thalassarche chrysostoma* chicks, white-chinned petrel *Procellaria aequinoctialis* adults, common diving petrel *Pelecanoides urinatrix* adults, Antarctic prion *Pachyptila desolata* adults and brown skua *Stercorarius antarcticus* adults. All these species have circumpolar breeding distributions on subantarctic islands and differ in diets and at-sea distributions [59–61] (Table S1).

2.2. Sample collection

A total of 84 individuals from 7 seabird species, including adults and chicks, were collected opportunistically during the breeding season from 2004 to 2022 (Table S2). Annual sample sizes were highly variable, reflecting the opportunistic nature of carcass collection over the years. Necropsies were conducted at South Georgia and in Cambridge (UK), and selected tissues (gastrointestinal tract, liver, muscle) were wrapped in aluminium foil and preserved at - 20℃ until further analysis. Due to differences in sample availability associated with carcass preservation and tissue degradation conditions, gastrointestinal tract, liver and muscle samples were obtained from 76, 82 and 77 individuals,

respectively (Table S3) as not all organs could be recovered from every individual.

2.3. Sampling and analysis of anthropogenic particles

2.3.1. Gastrointestinal tract preparation and digestion

Plastic ingestion was assessed using the gastrointestinal tract of all individuals, following protocols adapted from Kim, Bessa and colleagues [62, 63]. The exterior of each stomach and intestine was rinsed with distilled water to minimize potential plastic contamination. During dissection, mesoparticles and visually identifiable particles from the stomachs and intestines were also collected for visual and chemical characterization. This procedure was conducted in the shortest possible time in a closed laboratory to reduce contamination. Petri dishes with clean filters were placed on the bench as contamination controls.

After analyses of contents, the stomach and intestines were transferred to clean glass beakers and, a 10% potassium hydroxide (KOH) filtered solution (3x volume/ratio of biological material) was added to digest the organic content. The samples were left at room temperature for two weeks and then sieved using a 63 μm stainless steel sieve. To improve the digestion of the organic material, without compromising the retrieval of plastics, a 10% hydrogen peroxide (H_2O_2) solution was then added and the samples left for a further 24 h at room temperature. The samples were sieved again, and the floating phase was vacuum filtered through a 1.2 μm glass microfibre filter.

2.3.2. Observation, characterization and identification of anthropogenic particles

All filters were observed under a stereomicroscope LEICA M80 (Leica Mycrosystems GmbH, Wetzlar, Germany) to detect potential microplastics > 50 μm, which were then photographed using an image analysis system IC80 HD Camera with Leica Application Suite (LAS) software. The recovered items were considered to be anthropogenic particles (i.e., particles that are created or processed by humans, including synthetic and dyed cellulosic fibres). These particles were characterized according to their shape (e.g. filaments, fragments, films, foams, pellets/granules/beads), colour (e.g. black, purple, blue, multicolour, colourless and others) and size classes (50–99 μm, 100–299 μm, 300–999 μm and 1000–4999 μm). Mesoplastic particles (size class: 5–25 mm) [64] were also observed under the stereomicroscope and characterized.

A sub-sample of the extracted particles (130 particles; 10% of the total following the minimum recommendations for environmental sample monitoring, proposed in the strategy for the Marine Strategy Framework Directive [64]), was selected at random to assess chemical composition using infrared spectroscopy. Large opaque particles were analysed in attenuated total reflectance (ATR) mode in the mid-infrared interval (400–4000 cm^{-1}), in a Bruker Optics Vertex 70 FTIR spectrometer purged by CO_2-free dry air and coupled to a Bruker Platinum ATR single reflection diamond accessory. A liquid-nitrogen-cooled wide band mercury cadmium telluride (MCT) detector and a germanium (Ge) on potassium bromide (KBr) substrate beamsplitter were used. The remaining particles were analysed by micro-Fourier Transform Infrared Spectroscopy (microFTIR), on a Bruker Optics Vertex 70 FTIR spectrometer coupled to a Bruker Hyperion 2000 microscope (purged by CO_2-free dry air), in transmission mode, with a 15x Cassegrain condenser and objective, and a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector. The samples were analysed on a calcium fluoride (CaF_2) disc, allowing to detect bands above 900 cm^{-1}. For both FTIR-ATR and microFTIR each spectrum was an average of 128 scans (for background and sample) at a 4 cm^{-1} resolution, applying the 3-term Blackman–Harris apodization function. The FTIR-ATR spectra were corrected regarding the wavelength dependence of the penetration depth of the electric field in ATR (for a mean refractive index of 1.25).

FTIR-ATR and microFITR data were analysed by comparison with a spectral library database using the open-source platform Open Specy 1.0 [65]. Only particles that presented a match ≥ 70% were accepted [66, 67]; particles with matches < 70% were classified as unidentified or 'anthropogenic unknown' if the three highest-scoring matches corresponded to synthetic materials.

2.3.3. Quality control

To prevent and monitor airborne cross-contamination during sample processing, procedures followed those in Bessa, Galgani and colleagues [63,64]. A closed laboratory room with restricted access was allocated for sample processing, and nitrile gloves and cotton coats were used. All laboratory materials and equipment were cleaned with distilled water and ethanol. As far as possible, the equipment was glass and metal in order to minimize the use of plastic. Several strategies were adopted during sample processing to minimize contamination by airborne particles. Distilled water and all reagents (KOH and H_2O_2) were previously filtered using a 1.2 μm glass microfibre filter. Glass beakers were covered with aluminium foil. Open Petri dishes containing new filters were placed on the workbench as contamination controls and at the end analysed for possible contamination. Fibres found in the blank laboratory samples were analysed visually and counted under a stereomicroscope LEICA M80 (Leica Mycrosystems GmbH, Wetzlar, Germany) to determine the mean (±SD) per filter. To minimise the risk of misidentification, filters obtained from sample digestion and procedural controls were examined independently by two observers. Any particles categorised differently by the two observers were excluded from further analysis. In order to characterize their chemical composition, a sub-sample of randomly selected particles was analysed using microFTIR.

2.4. Chemical analyses

2.4.1. Polybrominated diphenyl ethers (PBDEs) and methoxylated polybrominated diphenyl ethers (MeO-PBDEs)

PBDEs and MeO-PBDEs concentrations were analysed in liver and muscle tissues from the seven study species. The liver was selected due its role in detoxification of xenobiotic compounds [68, 69] and muscle for its importance in locomotion, including flight and swimming [70]. Previous studies of PBDEs and MeO-PBDEs in seabirds also focused on liver or muscle [39, 71]. Following Matos and colleagues [72], liver and muscle samples were lyophilised for 72 h and stored at 4 °C if not immediately analysed. The target compounds included six PBDEs (BDE28, BDE47, BDE99, BDE154, BDE153, BDE183) and seven MeO-PBDEs (6-MeO-BDE-47, 4-MeO-BDE-49, 5-MeO-BDE-47, 5-MeO-BDE-100, 4'-MeO-BDE-103, 5'-MeO-BDE-99, 4'-MeO-BDE-101) (Table S4).

Muscle samples (~500 mg DW) were extracted overnight with a water: acetonitrile:toluene mixture, with internal standards (BDE37, BDE77, FBDE-126, 2 ´-MeO-BDE-68) added prior to extraction. After addition of salts (magnesium sulphate plus sodium chloride) and centrifugation, the supernatant was split; half was stored for later analysis, and half was cleaned with EMR-Lipid, evaporated, and reconstituted in 70 μL of trichloroethylene. The final extract was prepared in a 2 mL vial with an inserter for GC-MS/MS analysis [72]. The same protocol was used on liver samples, with an additional cleaning step due to the high fat content [73]. After evaporation, extracts were treated with n-hexane, sulfuric acid, and ultra-pure water, followed by purification in an aluminium oxide column. The final extract was evaporated, reconstituted in trichloroethylene, and transferred to a 2 mL vial with a glass insert for injection into the GC-MS/MS system for analysis [72].

2.4.2. Ultraviolet (UV) filters, musk fragrances and bisphenols

A total of 23 compounds (eight bisphenols, five musk fragrances, and ten UV filters) were targeted (Table S5) using a DLLME approach [74]. The muscle and liver extracts (~0.9 mL) [72], were spiked with 50 μl of Internal Standard 1 (IS1) (Benzophenone-d10, 8 ppm, Bisphenol A-d,

8 ppm, Bisphenol D-d, 8 ppm), 120 µl of acetic anhydride (derivatization solvent), and 80 µl of tetrachloroethylene (extraction solvent), transferred to 3 mL of ultrapure water (pH adjusted to ≥10 with potassium carbonate), and centrifuged (2 min, 2100 rpm). An 80 µL aliquot of the tetrachloroethylene layer was collected. Before GC-MS analysis, 5 µL of IS2 (Tonalide-d5, 2.5 ppm) was added to the tetrachloroethylene layer in a glass insert placed in a 2 mL vial for injection into the GC-MS system [74].

2.4.3. Quality controls

Following Cruz, Menezes-Sousa and colleagues [73, 75], all glassware was previously baked for 1 h at 300 °C and then rinsed with acetone to avoid possible background contamination. All vials were closed with polytetrafluoroethylene-based caps. An internal validation was conducted. Due to the limited availability of certified reference materials for the studied matrices, the method was validated using certified reference materials for fish muscle. Method validation followed previously published protocols [75]. Procedural blanks were measured with each batch of samples and were prepared simultaneously using the same chemical reagents and volumes as for samples. The blank samples were analysed for both matrices (liver and muscle) to verify the existence of targeted substances in the matrices under study and thus used to correct the value obtained if the contribution was higher than 5% of the estimated amount. The limit of detection (LOD) was defined as the lowest concentration in a spiked blank sample that gave a signal:noise ratio of 3, and the limit of quantification (LOQ) was set as the lowest concentration in the sample that could be quantified with precision. The linearity of the method was evaluated by preparing calibration curves in matrix-matching (muscle and liver), with seven calibration levels with well-distributed concentrations throughout the linear range. Calibration curves were generated by least squares linear regression, plotting the peak area ratios of target compounds and their respective internal standard against the concentration of each target substance. For all targeted compounds a good linearity was obtained throughout the studied range (muscle: 0.25 ng/g to 20 ng/g DW; liver: 0.5 ng/g to 80 ng/g DW). All analytes provided a coefficient of determination (R2), higher than 0.90.

2.5. Statistical analyses

The following metrics were calculated for all species: (1) frequency of occurrence (F.O.) of anthropogenic particles, (2) total number of anthropogenic particles, (3) mean and standard deviation in abundance of anthropogenic particles, (4) mean number of anthropogenic particles per gut weight (g), (5) mean number of anthropogenic particles per body weight, (6) frequency of occurrence of anthropogenic particle types, (7) frequency of occurrence of anthropogenic particle colours, (8) frequency of occurrence of anthropogenic particle size, and (9) frequency of occurrence of anthropogenic particles by chemical composition. We were not able to record the body weight of one white-chinned petrel, and therefore this individual was excluded from the relevant statistical analyses. The limited sample size per year and the uneven temporal distribution of carcasses in this study precluded annual comparisons. As a result, long-term temporal trends could not be assessed, and samples were combined across years to maximise statistical power. The temporal heterogeneity in sample collection also introduces uncertainty in some of our comparisons among species and age classes, given the long-term changes in plastic production, additive use and regulation. Nevertheless, this approach is justified given the opportunistic nature of sample collection and the expectation that variability within and among species is more strongly influenced by ecological and individual traits than by year.

The seven species collected were adults or chicks (Table 1), and so comparisons among species were of particle abundance per body weight. Data were tested for normality using Shapiro-Wilk test and for homogeneity using Levene's test. Parametric tests were used, including T-tests to compare values for adults and chicks in black-browed albatrosses and Welch ANOVA to compare values for chicks among species, followed by pairwise comparisons using Games-Howell tests. Non-parametric Wilcoxon tests were used to compare values between chicks and adults across all sampled species to evaluate general patterns of particle ingestion between life stages. To analyse the effect size, Wilcoxon effect size was used. This did not control for species and so results should be interpreted with caution. Kruskal-Wallis tests were used to compare values for adults among species, and a Dunn's test with Holm correction was used to identify significant differences, taking into account the number of statistical tests. A Tweedie Gam model was used to investigate differences among species in particle abundance per body weight of chicks, using data from black-browed albatrosses as the reference as it was the only species for which adult and chick samples were obtained. Another Tweedie Gam model was used for the comparison of samples from adults. Before fitting the Tweedie models, the DHARMa package was used to check model assumptions, including normality, homogeneity and zero inflation.

PERMANOVA tests were used to assess whether the overall composition of particle types, colours and sizes differed between age classes (chicks and adults), and among species. To determine whether PERMANOVA results reflected the differences in particle composition or differences within species, a test for homogeneity of multivariate dispersion (betadisper test in vegan), was performed. In the cases that

Table 1 –
Number of samples (N), frequency of occurrence (F.O. %), total count, mean abundance (± SD), mean gut weight (g), mean particle abundance per gut weight (± SD), mean body weight (g) and mean particle abundance per body weight (± SD) in adults and chicks of seven species of seabirds sampled at South Georgia.

Species	N	Frequency of occurrence (%)	N anthropogenic particles	Mean abundance± SD	Mean gut weight (g)	Mean number of particles/ gut weight (g) ± SD	Mean body weight (g)	Mean number of particles/ body weight (g) ± SD
Wandering albatross chicks	10	100	151	15.1 ± 10.4	447.1	0.04 ± 0.02	3941.7	0.004 ± 0.002
Grey-headed albatross chicks	11	91	99	9.0 ± 6.2	136.1	0.07 ± 0.04	1447	0.007 ± 0.004
Black-browed albatross chicks	14	100	219	15.6 ± 11.9	102.3	0.16 ± 0.14	1612.7	0.010 ± 0.008
Black-browed albatross adults	3	100	96	32.0 ± 29.5	408.3	0.07 ± 0.05	3656.7	0.008 ± 0.007
White-chinned petrel adults	9	89	214	23.1 ± 19.4	152.7	0.19 ± 0.16	1206.5	0.021 ± 0.019
Antarctic prion adults	8	100	80	10.0 ± 2.9	18.4	0.55 ± 0.17	136.8	0.074 ± 0.025
Common diving petrel adults	15	100	136	9.1 ± 2.9	18.7	0.50 ± 0.19	150.8	0.061 ± 0.021
Brown skua adults	6	100	280	46.7 ± 44.5	148.0	0.32 ± 0.32	1426.7	0.031 ± 0.027

PERMANOVA showed differences among species for adults or chicks, a pairwise PERMANOVA with Holm correction was performed. A non-metric multidimensional scaling (NMDS) was used to obtain graphical distributions of the parameters for chicks and adults and to determine the effects of type, colour and size of anthropogenic particles per body weight.

For PBDEs and MeO-PBDEs, sample concentrations were reported as not detected (n.d.), above the method detection limit and below the method quantification limit (<LOQ) and above the method quantification limit (>LOQ). The value of LOQ was defined as the lowest concentration sample in the calibration curve of each compound that could be quantified with precision. The value of LOD was defined as LOQ/3.3 (Table S6). A Wilcoxon rank sum test followed by a Cliff's Delta was used to test for significant differences in the average concentrations of contaminants between tissues (liver and muscle) and to understand the effect size, respectively. Kendall's rank correlations were used to assess whether particle abundance per body weight was related to the ∑BFRs concentrations in muscle, liver and combined tissues from each individual. All statistical analyses were performed using RStudio (Version 4.5.0). NMDS were built using the "vegan" package and the betadisper test used the same package. Figures with error bars and jittered points were made using the "ggplot2" package [76].

3. Results

3.1. Characterization of anthropogenic particles in seabirds

A total of 1275 particles were recovered from the 76 individuals of seven seabird species, with a frequency of occurrence of 97.4%, a mean value of 16.78 ± 18.79 particles per individual and of 0.03 ± 0.03 particles/g body weight.

Frequency of occurrence of particles was 100% for five of the seven species studied (Table 1). The highest mean abundance of particles ranged from 0.004 ± 0.002 particles/g in Antarctic prion adults to 0.074 ± 0.025 particles/g in wandering albatross chicks (Table 1). Fibres were more common than fragments or other types of particles in the

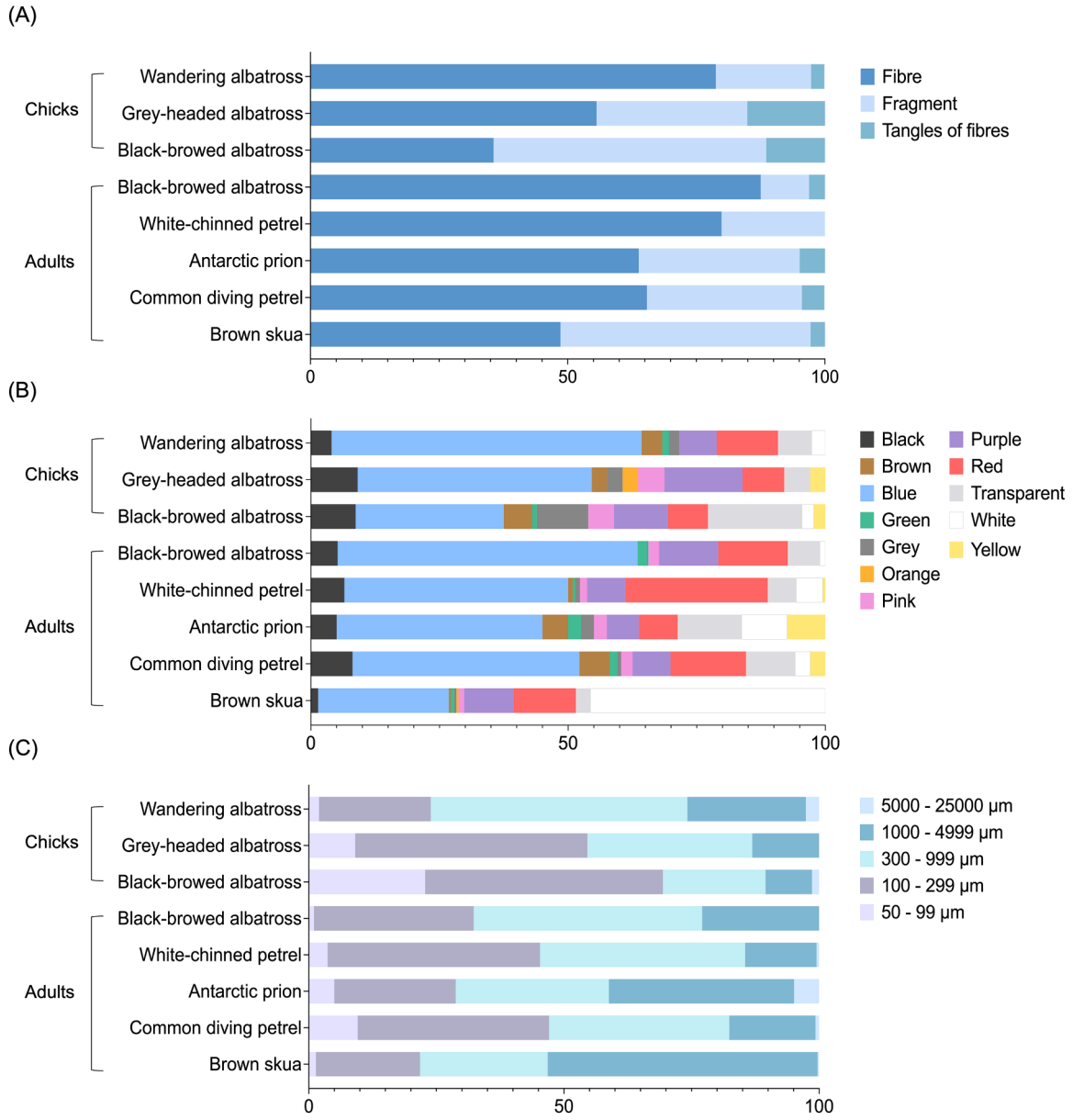

Fig. 1. Frequency of occurrence of (A) type, (B) colour and (C) size composition of anthropogenic particles in adults and chicks of seven species of seabirds sampled at South Georgia.

gut content of all species except black-browed albatross chicks and brown skua adults. Tangles of fibres were found in all species except white-chinned petrel adults (Fig. 1). The colours of ingested particles were broadly similar across species; blue was predominant in most species, and white in brown skua adults. Mesoplastics (5000–25000 μm) were found in the majority of the species and life stages but with low frequency of occurrence (≤ 5%) and were not detected in grey-headed albatross chicks or black-browed albatrosses. Smaller particles (50–4999 μm) were found in all the species and life stages, with a frequency of occurrence of > 90% (Fig. 1, Table S7). A mean of 4.90 ± 1.35 particles was recovered from the filters used as procedural blanks throughout the sampling process.

3.2. Anthropogenic particle ingestion

No differences in particle abundance were found between chicks and adults of black-browed albatrosses (T-test: t(15) = -0.337, p = 0.741, *d*= -0.214) with mean values of 0.008 ± 0.007 for adults and 0.009 ± 0.008 for chicks. Significant differences in particle abundance were found between sample groups (Wilcoxon rank sum test: p < 0.001) (Figure S1), with a large effect size (Wilcoxon effect size: rank-biserial correlation= 0.702, n = 40 adults, n = 35 chicks). Particle concentrations were significantly higher in adults than chicks (median of particles ingested by adults = 0.046; median chicks = 0.005).

There was a significant effect of species on particle abundance in chicks (Welch's ANOVA: W (2, 19.32)= 5.775, p = 0.011, ω^2= 0.30), with higher abundance in black-browed albatrosses than wandering albatrosses (Games-Howell test: p = 0.031; Hedges' *g*= 1.03) (Figure S2). The predicted abundance was 0.0099 particles/g body weight in black-browed albatross chicks. Predicted abundance was 0.0038 particles/g (61.2% lower, p < 0.05) in wandering albatross chicks, and 0.0070 particles/g (29.2% lower) in grey-headed albatross chicks, although this last difference was not statistically significant (p = 0.230) (Tweedie Gam: F= 4.791, p = 0.015).

There was also a significant effect of species on particle abundance in adults (Kruskal-Wallis: χ^2= 22.424, df= 4, p < 0.001, ε^2= 0.526) (Figure S3). Results of pairwise comparisons, Dunn's test with Holm correction, are shown in Table S8. The predicted abundance was 0.0080 particles/g body weight in black-browed albatrosses. Predicted abundance was 0.0314 particles/g (2.82 times higher, p < 0.05) in brown skua adults, 0.0607 particles/g (6.37 times higher, p < 0.001) in common diving petrel adults, 0.0745 particles/g (8.05 times higher, p < 0.001) in Antarctic prion adults and 0.0205 particles/g (similar to the reference level) in white-chinned petrel adults, although this last difference was not statistically significant (p = 0.0794) (Tweedie Gam: F= 9.975, p < 0.001).

3.3. Composition of ingested particles

Significant differences in particle type (PERMANOVA: R^2= 0.368, F= 41.271, p = 0.001), colour (PERMANOVA: R^2= 0.247, F= 23.334, p = 0.001) and size composition (PERMANOVA: R^2= 0.360, F= 39.923, p = 0.001) were found between sample groups (Figure S4). There were no significant variability within -age classes for type (betadisper test: F= 0.046, p = 0.830), colour (betadisper test: F= 0.016, p = 0.901) and size composition (betadisper test: F= 0.694, p = 0.408).

Of the samples from chicks, there were significant differences among species in particle type (PERMANOVA: R^2= 0.207, F= 4.046, p = 0.001), colour (PERMANOVA: R^2= 0.110, F= 1.917, p = 0.028) and size composition (PERMANOVA: R^2= 0.219, F= 4.357, p = 0.005) (Figure S5). Results of pairwise comparison, pairwise PERMANOVA with Holm correction, are shown in Table S9 and Figure S5. There was no significant variability within species in type (betadisper test: F= 2.308, p = 0.116), colour (betadisper test: F= 1.549, p = 0.229) or size composition of plastics (betadisper test: F= 2.640, p = 0.087).

Significant differences among species in particle type (PERMANOVA: R^2= 0.445, F= 6.824, p = 0.001), colour (PERMANOVA: R^2= 0.343, F= 4.445, p = 0.001) and size composition (PERMANOVA: R^2= 0.474, F= 7.675, p = 0.001) were also found in the samples from adults (Figure S6). Results of pairwise comparison, pairwise PERMANOVA with Holm correction, are shown in Table S10 and Figure S6. There was no significant variability within species for type (betadisper test: F= 0.744, p = 0.569), colour (betadisper test: F= 0.148, p = 0.963) or size composition (betadisper test: F= 0.463, p = 0.763).

3.4. Chemical characterization of anthropogenic particles

Of the 1275 particles recovered from the 76 gut contents, 130 (~10% from each species) were randomly selected for chemical composition analysis. A wide diversity of polymers (Figure S7) was identified in three main categories: i) synthetic (i.e., polyethylene, polystyrene), ii) anthropogenic cellulose, and iii) anthropogenic unknown. Additionally, some particles were identified as of natural origin (cotton, resin or linen). The category "anthropogenic unknown" was assigned to particles with a specific polymer composition that could not be determined due to inconclusive database matches. Although their spectral similarity to synthetic polymer references was below 70% the three highest-scoring matches corresponded to synthetic materials. Given this uncertainty, these particles were classified as having a likely synthetic origin.

Among chicks, the most common particles were synthetic (frequency of occurrence of 51%). Anthropogenic unknown particles (frequency of occurrence of 27%) had a similar frequency of occurrence across species. Natural origin particles (frequency of occurrence of 22%) had lower prevalence in wandering albatross when compared to the other species (Figure S8).

Among adults, the most common particles were also of synthetic origin (frequency of occurrence of 64%). Anthropogenic unknown particles (frequency of occurrence of 15%) were only observed in common diving petrels, white-chinned petrels and black-browed albatrosses. Anthropogenic cellulose particles (frequency of occurrence 6%) were only detected in adults, specifically in brown skuas, Antarctic prions, and common diving petrels, and were less frequent than the other categories. Natural origin particles were present in all species (frequency of occurrence 15%) except in the Antarctic prion and had the same frequency as anthropogenic unknown (Figure S8).

Of the 34 particles recovered from the procedural blank (filters), a sub-sample of 3 particles (~10%) were analysed for chemical composition. Two particles were of natural origin and were identified as cotton (81% and 84% match respectively). The remaining particle was synthetic and identified as high-density polyethylene (HDPE) (76% match).

3.5. Endocrine-disrupting chemicals

The total concentrations of PBDEs and MeO-PBDEs congeners detected in muscle and liver are presented in Table S11. Quantifiable concentrations were verified in a small fraction of the samples, ranging from 0% to 16.7% of liver samples and from 0% to 33.3% of muscle samples, depending on the species and compounds. Nevertheless, all the target congeners were detected (above LOD) in both tissues in all age classes and in the seven seabird species (Table S12, Table S13).

Considering samples with concentrations that exceeded LOQ values, there were significant differences in PBDEs and MeO-PBDE congeners among species and tissues (Fig. 2). The highest concentrations of PBDEs and MeO-PBDE congeners were detected in the liver of brown skuas adults (133.96 ng/g) and wandering albatross chicks (6.50 ng/g), respectively (Table S11, Fig. 2).

Differences in congener prevalence were observed; BDE47, BDE153 and 6-MeO-BDE-47 congeners were quantified more frequently across species (Table S11, Fig. 3). The highest diversity of PBDE congeners was detected in brown skua adults, whereas the highest MeO-PBDEs congener diversity was in white-chinned petrel adults (Table S11,

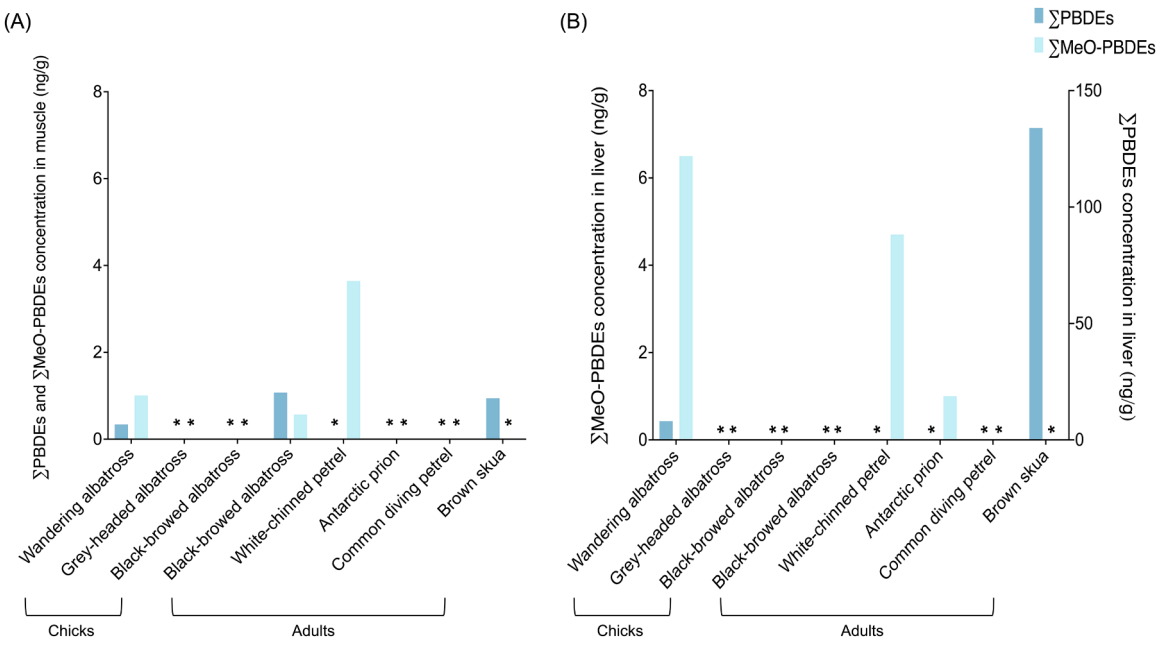

Fig. 2. – Sum of concentrations (ng/g) of PBDEs and MeO-PBDEs in (A) muscle and (B) liver of adults and chicks of seven species of seabirds sampled at South Georgia. Samples under the limit of quantification (< LOQ) are marked with *.

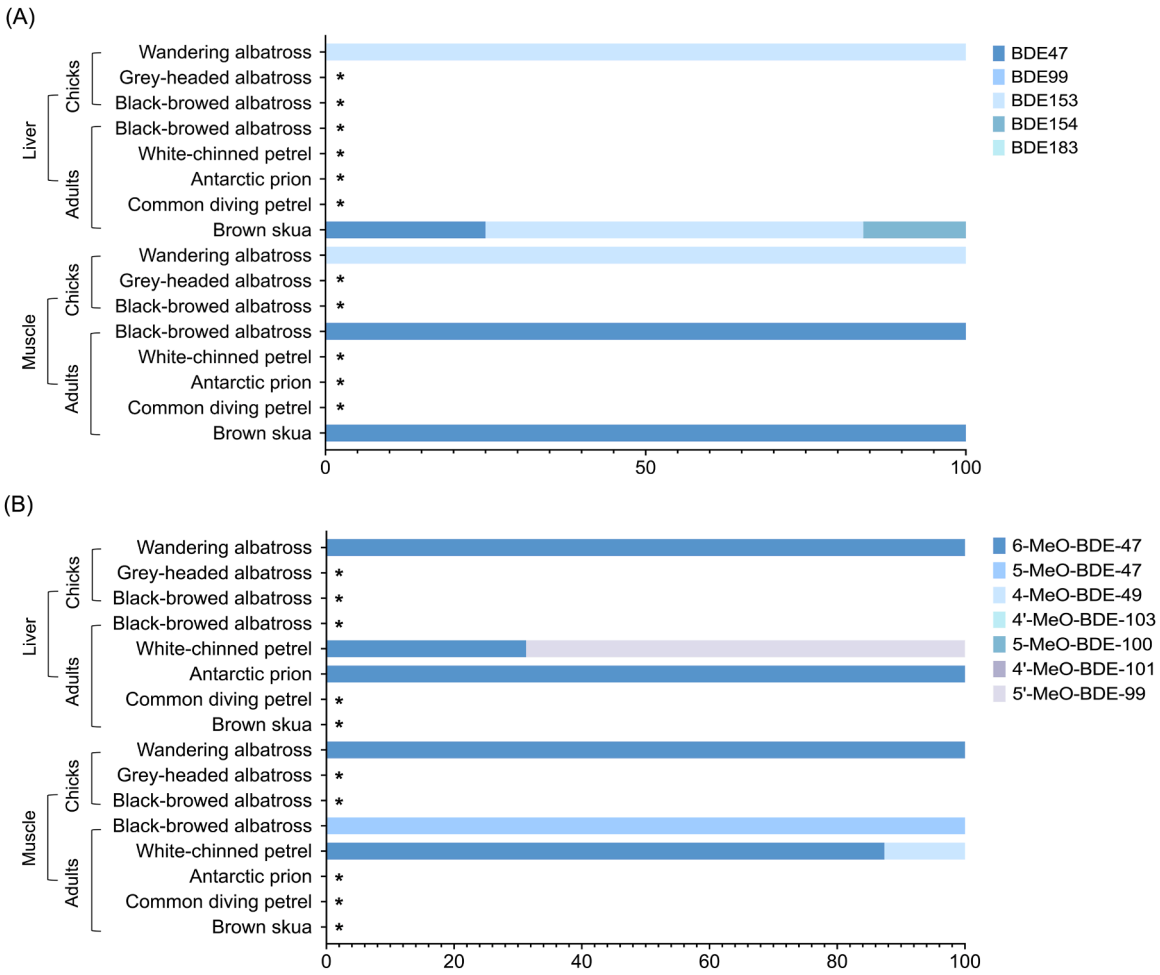

Fig. 3. – Frequency of occurrence (%) of (A) PBDEs and (B) MeO-PBDEs congeners in muscle and liver of adults and chicks of seven species of seabirds sampled at South Georgia. Samples under the limit of quantification (< LOQ) are marked with *.

Fig. 3).

For bisphenols, musk fragrances and UV-filters, concentrations of all congeners were either below the limit of quantification (LOQ) or not detected. None of the species had concentration values above LOQ values (Table S14, Table S15, Table S16, Table S17).

3.6. Brominated flame retardants

Concentrations of brominated flame retardants in the liver were substantially higher than those in the muscle (Wilcoxon rank sum test: W= 68, $p < 0.001$). A large effect size (Cliff's delta: d= 0.889, 95%, CI: 0.53 – 0.98) was verified, suggesting that the differences among tissues were potentially meaningful. The confidence interval revealed that the differences were strong and robust between groups.

3.7. Anthropogenic particles ingestion and ∑BFRs concentration

No monotonic association was found between the abundance of particles and the concentrations of ∑BFRs in liver (Kendall's correlation: $\tau = 0$, $p = 1$), muscle (Kendall's correlation: $\tau = -0.467$, $p = 0.189$), or if results from the two tissues were included in the same analysis (Kendall's correlation: $\tau = -0.357$, $p = 0.216$).

4. Discussion

This study reveals clear inter- and intraspecific differences in the accumulation of ingested anthropogenic particles among subantarctic seabirds. Variation was evident not only in particle abundance relative to body mass, but also in particle characteristics including type, colour and size composition, as well as their chemical composition. Among the endocrine-disrupting chemicals analysed (BFRs, UV-filters, musk fragrances and BPs), only PBDEs and MeO-PBDEs congeners occurred at levels above the limit of quantification (Table S11). The inability to detect some of these EDCs allied with the low proportion of quantified compounds reflects compound-specific analytical sensitivity, rather than their absence or lack of biological relevance. Nevertheless, the detection of several endocrine disruptors in the individuals, even at concentrations below the limit of quantification, may indicate potential exposure to these compounds, which could be of concern in the context of cumulative or mixture effects [27], although the biological relevance of such low levels remains uncertain.

There were no significant relationships between particle abundance and the EDCs in the liver, muscle or when results from the two tissues were combined. Such patterns suggest that particle abundance may not be the main driver of EDCs and that these compounds may have different sources or uptake pathways linked to species-specific ecological traits or environmental availability. Although our results demonstrate the occurrence of microplastics and associated chemicals in these seabirds, the absence of dose-response data or established toxicity thresholds for these species precludes an assessment of toxicological risk. Nonetheless, consideration of our findings within the broader toxicological literature such as known endocrine-disrupting effects of BFRs and UV-filters in avian species provides useful context for interpreting potential physiological implications without overstating causality.

The variation in anthropogenic particles and EDCs among species might be explained by differences in diet (prey species and prey size), scavenging behaviour (on land or behind fishing vessels) and at-sea distribution (in breeding and non-breeding periods). During the incubation and chick rearing periods some of these subantarctic seabird species are limited to foraging predominantly on land (brown skuas) or in Antarctic waters south of the Antarctic Polar Front (Antarctic prion, common diving petrel) [77–79], whereas others (black-browed albatross, grey-headed albatross, wandering albatross and white-chinned petrel) also forage in areas located north of the Antarctic Polar Front (e.g., subantarctic oceanic waters, shelf areas off South America) [77, 80, 81], and hence may be exposed to a wider range of contamination sources. These species also show a wide diversity in diets, feeding on different taxa in different proportions, size ranges and from multiple trophic levels [57, 82]. This might also affect relative exposure to different particles and chemical additives, particularly as some can bioaccumulate and biomagnify in the food web [83, 84]. For instance, the common diving petrels and Antarctic prions are zooplanktivorous, feeding predominantly on small crustaceans [79, 82, 85], whereas white-chinned petrels, grey-headed albatrosses, black-browed albatrosses and wandering albatrosses consume a greater diversity of prey species and sizes, including Antarctic krill, fish and cephalopods [61, 82, 86, 87]. In contrast, brown skuas are opportunistic predators and scavengers, and in the breeding season feed predominantly on land on carrion and seabirds [60, 88]. Diets of grey-headed, black-browed and wandering albatrosses, as well as Antarctic prion can shift across years, depending on prey availability [89–92]. Allied to their diverse diets some of these species (white-chinned petrels, black-browed albatrosses and wandering albatrosses) can also feed on discards from commercial fishing vessels [81, 92], and are therefore potentially more exposed to anthropogenic particles and chemical contamination.

As PBDEs can have a slow turnover, concentrations in seabird tissues might also reflect exposure in the non-breeding period. During austral autumn and winter (non-breeding season) these seabirds extend their foraging ranges to much further north of the Antarctic Polar Front into areas of high productivity (e.g. coastal upwelling, frontal zones, continental shelf) [59, 77]. For instance, common diving petrels, white-chinned petrels and Antarctic prions migrate to subantarctic or subtropical waters and are associated with productive frontal zones or upwelling systems [81, 93–95]. Albatross species tend to exhibit a more wide-ranging pelagic migrations, with black-browed and wandering albatrosses often using productive continental shelf and shelf-slope waters [93, 94, 96], whereas grey-headed albatrosses primarily exploit oceanic waters and, like wandering albatrosses, may make circumpolar movements [93, 94, 97]. Brown skuas extend their range to oceanic waters between the Antarctic Polar Front and the northern sub-tropical front (southwest Atlantic Ocean) [98]. Since these non-breeding areas are closer to contamination sources, the risk of exposure to flame retardants, as well as plastic particles, may increases greatly.

All these differences in ecology and behaviour among species might influence their exposure to different sources of pollution, and result in the ingestion of particles with different physical and chemical composition [99, 100]. This may expose seabirds to a wide range of contaminants which could have different effects depending on biochemical properties and accumulation patterns [46, 99].

Anthropogenic particles can have different characteristics, including colour and shape. Blue particles were the most frequent in most study species, and white particles in brown skua adults. This is in line with previous studies, as macro- and microplastics ingested by albatrosses and petrels are often blue [101], as are those ingested by other seabird and marine mammal species in the Southern Ocean [14, 102, 103]. Environmental studies show these are the most common colours of floating particles (macro- and micro-) in the Southern Ocean [104, 105]. Fibres and fragments were the most common type of particles in our study species, which is also in accordance with previous research [47, 106, 107].

The most common category of particles that were characterized chemically were synthetic, including various polymers such as polyethylene, polyethylene terephthalate and polystyrene, as in other studies of seabirds in Antarctica [62, 103, 106] and in the Arctic [108–110]. We were unable to identify 19% of particles, probably due to alteration of the chemical composition when plastic particles degrade after release in the environment [111] or during digestion [112]. The natural particles were cotton, linen, resin and lignin. Anthropogenic cellulose fibres were also identified. Although derived from natural sources, these particles are not necessarily benign as they contain chemical additives introduced during manufacturing or adsorb contaminants from seawater [113–116]. Different polymers have distinct

physical and chemical properties [117], which can influence how they interact with contaminants within organisms [118, 119]. These properties are specifically related to the type of additives (e.g., brominated flame retardants, UV-filters, musks) that are incorporated during manufacturing [20].

Brominated flame retardants, including PBDEs and MeO-PBDEs, are legacy contaminants that have been measured in different constituents of the Antarctic environment (e.g. air, sea ice, water, snow, and soil) [120–122], and in organisms on land (e.g., lichen, moss and hair grass) [123] and at sea (e.g., algae, amphipods, limpets, starfishes, fish, and seabirds) [83, 124, 125]. The mean concentrations of PBDEs in our study were higher than those reported previously in the eggs of Adélie penguins *Pygoscelis adeliae* (0.05 ± 0.11 ng/g lw), chinstrap penguins *P. antarcticus* (0.52 ± 1.80 ng/g lw) and gentoo penguins *P. papua* (0.13 ± 0.50 ng/g lw) at the South Shetland Islands [124]. This difference may be attributable to tissue-specific effects, shorter migratory range of penguins and their lower trophic level [126]. In our study, brown skua adults exhibited the highest concentration of PBDE congeners (BDE47, BDE153 and BDE183) in the liver (133.96 ng/g). This presumably relates to their scavenging behaviour, which promotes biomagnification of these compounds in the food web [127, 128]. Our results are therefore in line with other studies in the Antarctic region which have found higher concentrations of persistent contaminants in skuas than other seabirds [124, 125].

The congener 6-MeO-BDE-47 was detected in chicks and adults of several species, which may be attributed to its natural production by marine biota (e.g., algae and sponges), increasing its availability to seabirds [30, 129]. Despite that, several studies have also reported the presence of this congener at variable concentrations across different trophic levels [33, 130–132]. The heterogeneity observed in additive concentrations, PBDEs and MeO-PBDEs congeners was consistently higher in liver than muscle, aligning with previous studies of these compounds and other organic contaminants [133, 134]. This pattern is presumably because the liver is directly involved in the metabolism of xenobiotics (e.g., pharmaceuticals, chemicals) [68] and has a high lipid content, combined with the lipophilic nature of these contaminants [135–137].

5. Conclusion

As long-lived, colonial top predators with wide foraging ranges, seabirds provide powerful sentinels of marine pollution, integrating contaminant exposure across space and time through measurable changes in behaviour, breeding success, body condition and population trends. Our study reinforces the potential of subantarctic seabird species as sentinels of plastic pollution by highlighting the exposure of seven species to microplastics and other anthropogenic particles, as well as EDCs. While plastics are recognised as potential vectors of EDCs, we did not detect a significant association. Nonetheless, our results add new knowledge on man-made plastic additives in subantarctic biota, and baselines for future monitoring programs. Further research is needed to improve our knowledge of exposure to anthropogenic pollutants and chemical stressors, and to better understand the risks and possible transference pathways. There is also a need to strengthen policies seeking to address plastic pollution in Antarctica and the Southern Ocean. This should include systematic long-term monitoring programs for plastic pollution to support evidence-based conservation efforts in this vulnerable region.

Environmental Implication

This study provides new evidence of microplastics, other anthropogenic particles and endocrine-disrupting chemicals (BFRs, UV-filters, musk fragrances and BPs) in tissues of seven subantarctic seabird species. By jointly assessing particle types and chemical additives, it improves on previous studies which typically evaluated these pressures separately. Our findings highlight the value of subantarctic seabirds as sentinels of contaminant loads and polymer types, revealing the presence of priority hazardous substances for monitoring initiatives encouraged by the Scientific Committee on Antarctic Research (SCAR) and environmental assessments within Antarctic Treaty System.

CRediT authorship contribution statement

José C. Xavier: Writing – review & editing, Visualization, Supervision, Resources, Funding acquisition, Conceptualization. **Filipa Bessa:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Richard A. Phillips:** Writing – review & editing, Resources, Formal analysis. **Clara Manno:** Writing – review & editing, Visualization, Supervision, Resources, Funding acquisition, Conceptualization. **Joana Fragão:** Writing – original draft, Visualization, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Maria Paula M. Marques:** Writing – review & editing, Resources, Methodology, Formal analysis. **Luís A.E. Batista de Carvalho:** Writing – review & editing, Resources, Methodology, Formal analysis. **José O. Fernandes:** Writing – review & editing, Resources, Formal analysis. **Sara C. Cunha:** Writing – review & editing, Resources, Methodology, Formal analysis.

Ethical approval

The sampling methods used were under the recommendations from the Scientific Committee for Antarctic Research (SCAR). Sample collection was carried out under permits issued by the Government of South Georgia and the South Sandwich Islands (GSGSSI).

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2026.142018.

Data availability

Data will be made available on request.

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