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High frequency of plastic-derived contaminants (phthalate acid esters) in the preen gland of dead procellariiform seabirds from the Patagonian Sea

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ABSTRACT

Plastic additives such as phthalate acid esters (PAEs) are an emerging concern due their toxicity and potential impacts on marine fauna. However, the source and extent of PAEs exposure in seabirds remain poorly understood. Procellariiform seabirds frequently ingest plastics and may uptake leached PAEs. Lipophilic PAEs can be excreted via preening oil, providing a minimally invasive option for monitoring exposure. In this study, plasticizers dimethyl phthalate (DMP), dibutyl phthalate (DBP), and di-2-ethylhexyl phthalate (DEHP) were quantified in the preen gland of dead procellariiforms ($n = 31$) from the Patagonian Sea (36 to 54 degrees latitude S). Additionally, plastic items (>1 mm) in the stomach of these birds were evaluated as a possible source of PAEs. PAEs were quantifiable in 71 % of individuals, with median concentrations of 336.0, 256.0, and 33.5 ng/g for DEHP, DBP, and DMP, respectively. While PAEs occurrence frequencies were similar to previous reports, DEHP concentrations were notably higher. Plastics were found in 23 % of seabird stomachs; yet no relationship was detected with PAEs in preen gland, suggesting that stomach contents alone are not a reliable indicator of plastic exposure in these species. These findings support the use the preen gland as an effective matrix for assessing PAEs exposure in seabirds. Further research should identify alternative exposure routes (i.e. prey, seawater, plastics <1 mm), metabolic pathways and sub-lethal effects of chronic exposure. Overall, this study confirms widespread PAE exposure in Patagonian Sea seabirds and underscores the need for global regulatory measures and consumer behavioral changes to reduce ocean pollution.

1. Introduction

In recent years, concern over marine plastic pollution has grown significantly with an estimated 4 to 12 million tons (Mt) of land-based plastic waste entering the oceans annually (Jambeck et al., 2015; Lebreton and Andrady, 2019). Moreover, fishing and other ocean-based activities add substantial amounts of marine debris (Monteiro et al., 2018; Ryan, 2020; Richardson et al., 2022).

Additives such as plasticizers, flame retardants, antioxidants, heat and ultraviolet light stabilizers, and lubricants are often added to plastic polymers to improve the performance, functionality and ageing properties of plastic products (Hermabessiere et al., 2017; Hahladakis et al., 2018). With the increased production and consumption of plastics worldwide, the production of these additives has also increased, both in

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quantity and diversity (UNEP, 2023). Among these, phthalate acid esters (PAEs) are widely used as plasticizers by various industries (Hahladakis et al., 2018). More than 25 different PAEs are produced and marketed, and their industrial application is typically determined by their molecular weight (Lorz et al., 2000; Zhang et al., 2021). Low molecular weight PAEs (e.g. dimethyl phthalate-DMP) are mainly used in the production of cellulose acetate film, lacquers, rubber coating agents, adhesives, insect repellents, and pesticides. High molecular weight PAEs (e.g. di-2-ethylhexyl phthalate-DEHP) are primarily used in plastic pipes, floor tiles, furniture, children's toys, car interiors and medical supplies. Dibutyl phthalate (DBP), of intermediate molecular weight, is mainly used as a solvent or fixative for perfumes, cosmetics, coatings and pesticides and to regulate the release speed of some drugs (Kamrin, 2009). PAEs typically represent 20–30 % of the weight of the final plastic product, but in some cases can be as high as 67 % (Net et al., 2015; Hermabessiere et al., 2017; Da Costa et al., 2023).

As PAEs are not chemically bound to the polymer matrix, they can easily leach into the environment during plastic manufacturing, use and disposal (Cadogan et al., 1994; Net et al., 2015). Thus, they have become ubiquitous in the environment, including in the air, sediment, fresh and sea water, and tissues of marine biota (Net et al., 2015). PAEs can be uptaken by marine animals from water, through prey, and directly by plastic ingestion (Hardesty et al., 2015; Baini et al., 2017; Yamashita et al., 2021; Blasi et al., 2022; Garcia-Garin et al., 2022). Once absorbed by living organisms through the gut, these compounds are metabolized by hydrolysis and subsequent oxidation reactions, potentially leading to the formation of toxic products (Hu et al., 2016). PAE metabolism and tissue accumulation will depend on their molecular weight and solubility in fat (lipophilicity) (Hu et al., 2016; Zhang et al., 2021; Savoca et al., 2023). Unlike persistent organic pollutants, PAEs do not bioaccumulate (Staples et al., 1997; Net et al., 2015; Zhang et al., 2021). Yet, it has been hypothesized that despite their rapid metabolism and elimination, a stable background concentration may be reached (mostly in fatty tissues) through chronic low-level exposure via regular plastic and/or dietary ingestion (Hoppin et al., 2002; Silva et al., 2004; Yamashita et al., 2021). Thus, there is a need to better understand the risks related to exposure to PAEs in long-lived species.

Numerous field and laboratory studies have demonstrated the toxic effects of PAEs on human health and other living organisms, including neurotoxicity and impacts on metabolism, development, and the reproductive, immune, and endocrine systems (Oehlmann et al., 2009; Hlisníková et al., 2020; Zhang et al., 2021; Janjani et al., 2024; Xu et al., 2025). Additionally, PAE exposure has been reported to alter gut microbiota composition, leading to associated adverse health effects like immune system alterations (Chiu et al., 2020; Adamovsky et al., 2020; Almamoun et al., 2023). The severity of these effects depends on factors such as exposure levels, the specific PAE compound, the exposed organism, and the synergistic/antagonistic interaction with other pollutants (Staples et al., 1997; Net et al., 2015; Savoca et al., 2023; Xu et al., 2025). Studies on the effects of PAEs in birds are limited and mostly restricted to domestic species (Abdul-Ghani et al., 2012; Guibert et al., 2013; Li et al., 2018; Zhang et al., 2018a; Luo et al., 2019), but there is evidence that DBP exposure leads to dose-dependent reduction in steroidogenesis (Bello et al., 2014), and DEHP exposure can induce liver, renal and ovarian toxicity (reviewed by Alam et al., 2025).

Plastic pollution is expected to double from 2020 to 2030 (UNEP, 2021), further expanding PAEs spread and their potential impacts on wildlife. Therefore, systematic monitoring in sentinel species is essential to detect early signs of ecological harm, track pollution trends over time, and inform targeted conservation and policy measures. Such monitoring could help assess the transfer of PAEs in marine food webs and evaluate the effectiveness of mitigation efforts. Procellariiform birds (albatrosses, petrels and shearwaters) are long-lived seabirds known to frequently ingest plastic debris, as they feed preferably on small prey on the waters' surface where plastics tend to float and accumulate (Ryan, 2008; Avery-Gomm et al., 2018; Roman et al., 2019, 2020; Kühn et al., 2021). As a result, these species may be particularly vulnerable to chronic PAE exposure. Moreover, some species (e.g., petrels) often retain ingested plastics due to limited regurgitation ability (Ryan, 2015). Stomach oils, which are present in the digestive tract of procellariiform birds, may act as an organic solvent, facilitating the leaching of hydrophobic additives from plastics and their transfer to tissues (Tanaka et al., 2015, 2020; Kühn et al., 2020). Some studies in procellariiform birds from the North Atlantic Ocean suggest that food and water intake are relevant sources of phthalate exposure, due to high PAE content in their prey and environment (Provencher et al., 2020; Collard et al., 2024). However, although procellariiform birds are more abundant and diverse in the southern hemisphere (Onley and Scofield, 2020), studies on PAEs in abiotic and biotic compartments of marine ecosystems of this region remain scarce and are limited to the coastal areas off Brazil and Australia (Hardesty et al., 2015; Neves et al., 2023; Vanstreels et al., 2023; de Lima et al., 2024).

The uropygial (preen) gland is the most conspicuous integumentary gland in birds and is histologically classified as a sebaceous gland (Salibian and Montalti, 2009). Its secretion has been proposed as a route of depuration/excretion of lipophilic contaminants in birds, including PAEs (Jaspers et al., 2008; Hardesty et al., 2015; Yamashita et al., 2021; Matos et al., 2024). Although the specific mechanisms by which PAEs reach the preen gland remain unknown, the physiological and lipid synthesis properties of the gland may facilitate capture and deposition of lipophilic compounds (Yamashita et al., 2007; Salibian and Montalti, 2009). Moreover, in seabirds the gland is more complex than in terrestrial birds, with functional advantages such as continuous secretion (Chiale et al., 2014; Stangier et al., 2023). This renders the preen gland as a potential minimally invasive and accessible matrix for contaminant monitoring, even in living birds, in contrast to traditionally assessed tissues such as liver or muscle (Yamashita et al., 2007). Additionally, its potential as a proxy for plastic ingestion monitoring is under discussion (Hardesty et al., 2015; Provencher et al., 2020).

We hypothesize that the preen gland, due to its lipid-rich nature and excretory function, accumulates lipophilic PAEs at detectable levels and may represent a relevant matrix for assessing exposure to plastic-derived contaminants in Procellariiform seabirds. In this study, we focused on three representative phthalates (DBP, DMP, and DEHP), selected for their representation of low, intermediate, and high molecular weight and lipophilicity, their documented toxicological effects, and their comparability with previous studies in similar species. Accordingly, the aims of this study were to 1) detect and quantify three phthalate compounds (DBP, DMP and DEHP) in the preen gland of procellariiform species collected dead along the coastline and from offshore fisheries in the southern Patagonian Sea; 2) assess the presence, mass and number of plastic items in the stomach of individual birds as a potential source of PAEs exposure; 3) evaluate the relationship between PAEs in preen glad and the occurrence of plastic in the stomach at the time of death, in order to assess the applicability of the preen gland as a proxy for plastic ingestion.

2. Methods

2.1. Sample collection

Carcasses of 31 procellariiform birds of seven species (Table 1) were collected between 36°S to 54°S along the coastline of Buenos Aires ("northern region") and Chubut ("central region") provinces in Argentina and from offshore fisheries ("southern region") operating on the southern Patagonian Sea (Fig. S1). The data in this study represents a subset of fresh carcasses (<48 h postmortem) of procellariiform birds from Gallo et al. (2024) that were moderately-preserved and with intact digestive tract and preen gland. Carcasses were examined following standardized protocols (Gallo et al., 2021). The upper digestive tract (proximal esophagus to pyloric sphincter) was removed and stored frozen at −20 °C. The entire preen gland was dissected, and the capsule

Table 1
Summary of the sample size (N = individuals examined, N_p = individuals examined that had plastics in the upper digestive tract) and number of individuals with quantifiable levels (higher than LOQ) of phthalate compounds (DMP = dimethyl phthalate, DBP = dibutyl phthalate, DEHP = di-2- ethylhexyl phthalate) in the preen gland for seabirds collected along the coastline and from offshore fisheries in the Patagonian Sea. Numbers within brackets represent the number of samples with trace levels (detected at concentrations below the LOQ).

Species	English name	Code	N	N_p	DMP	DBP	DEHP
Albatrosses							
Thalassarche chrysostoma	Grey-headed albatross	GHA	5	0	5	5	1[2]
Thalassarche melanophris	Black-browed albatross	BBA	7	0	7	7	7
Petrels							
Macronectes giganteus	Southern giant petrel	SGP	6	4	6	5[1]	6
Pterodroma mollis	Soft-plumaged petrel	SPP	2	1	2	2	2
Procellaria aequinoctialis	White-chinned petrel	WCP	5	0	4[1]	5	5
Shearwaters							
Calonectris borealis	Cory's shearwater	CS	2	1	2	2	2
Puffinus puffinus	Manx shearwater	MS	4	1	4	3[1]	4
Total			31	7	30 [1]	29 [2]	27[2]

along with the base of the papilla were collected (Fig. S2), wrapped in heat-treated aluminum foil (450 °C for six hours), and then frozen at −20 °C. The anatomical location and structure of this gland —being an internal organ surrounded by dermal tissues— provide protection against external contamination, making it a reliable matrix for assessing in vivo exposure (Yamashita et al., 2007; Eulaers et al., 2011; Pacyna-Kuchta, 2023). The gland was handled exclusively with clean stainless steel instruments, disposable scalpel blades, and nitrile gloves, and was never touched by plastic items. Instruments were thoroughly rinsed between individuals using detergent and MilliQ water, followed by sequential washes with solvents (methanol, dichloromethane, and hexane; Uhart et al., 2020).

2.2. Standards and reagents

Three PAE were analyzed: dimethyl phthalate (DMP, CAS 131–11-3), dibutyl phthalate (DBP, CAS 84–74-2), and di-2-ethylhexyl phthalate (DEHP, CAS 117–81-7). Surrogate-internal standards were: dimethyl phthalate-3,4,5,6-d4 (DMP-d4, CAS 93951–89-4, 99.2 %), dibutyl phthalate-3,4,5,6-d4 (DBP-d4, CAS 93952–11-5, 99.3 %), and di-2-ethylhexyl phthalate-3,4,5,6-d4 (DEHP-d4, CAS 93951–87-2, 99.4 %). All standards were obtained from Sigma-Aldrich (St. Louis, USA) and mixed with methanol (UPLC Optima®; Fisher Chemical, Geel, Belgium) to obtain working standard solutions (1 mg/L). HPLC-grade dichloromethane (Dorwil, Buenos Aires, Argentina) and acetonitrile (J.T. Baker, D.F., Mexico) were used for sample extraction. Octadecylsilane (Selectra®; United Chemical Technologies, Bristol, USA) was used for solid-phase extraction.

2.3. Sample preparation

A robust and reproducible analytical method for the determination of PAEs was developed by adapting the procedure described by Michlig et al. (2018) to the matrix and target analytes under investigation. The method involved acetonitrile extraction, followed by clean-up through dispersive solid-phase extraction (dSPE) and freeze-out techniques, with final analysis performed using gas chromatography coupled with tandem mass spectrometry (GC–MS/MS). Whole preen glands were weighed (wet weight), finely chopped with a clean metal scalpel blade, and lyophilized for 72 h. The dried samples were reweighed (dry weight) and stored at room temperature until further analysis. Approximately 0.4 g of the dried sample was spiked with 100 μL of internal standard solution (DEHP/DMP/DBP-d4, 1 mg/L) in a 10 mL glass centrifuge tube. Subsequently, 4 mL of acetonitrile was added, and the mixture was vortexed for 10 s, shaken at 150 rpm for 30 min at 30 °C, and centrifuged at 2500 rpm for 5 min. From the resulting supernatant, 1 mL was transferred to a glass tube containing 20 mg of octadecylsilane, vortexed for 30 s, and centrifuged at 2500 rpm for 3 min. The extract was then frozen at −20 °C overnight. A 0.5 mL aliquot of the final supernatant was subjected to GC–MS/MS analysis.

Glassware used in sample processing was decontaminated by soaking for 30 min in hot purified water with Extran detergent (Merck Millipore, Burlington, USA). Following mechanical washing, the glassware was rinsed with petroleum ether 30–60 °C pesticide grade (Sintorgan, Buenos Aires, Argentina). The materials were then dried in an oven at 250 °C for 4 h and wrapped in heat-treated aluminum foil (450 °C for 6 h) to prevent potential air contamination.

2.4. Instrumental and analytical conditions

All analyses were performed using an Agilent 7890B gas chromatograph coupled with an Agilent 7000C Triple Quadrupole GC–MS/MS system (Agilent, Santa Clara, CA, USA). Chromatographic separation was achieved on an Rtx-5 ms column (15 m × 0.53 mm, 1 μm; Restek, Bellefonte, PA, USA), pre-connected to an uncoated guard/restrictor capillary and an integrated transfer line capillary. High-purity helium (99.999 %) served as the carrier gas, with an initial flow rate of 2.25 mL/min maintained for 3 min, followed by a decrease to 1.5 mL/min for the remainder of the run. Sample injections (3 μL) were performed with the injector set at 280 °C, applying a pressure pulse of 40 psi for 0.75 min before activating the split vent. The oven temperature program started at 80 °C, held for 1 min, then ramped at 45 °C/min to 320 °C, held for 1.7 min, resulting in a total run time of 8 min. The triple quadrupole mass spectrometer operated in electron impact (EI) ionization mode at 70 eV. The transfer line, ion source, and quadrupoles (Q1 and Q2) were maintained at 280 °C, 320 °C, and 150 °C, respectively.

2.5. Quality assurance and calibration

Quantification was performed using 6-point matrix-matched calibration curves for each analyte, ranging from 1.6 to 100 μg/L. The calibration curves exhibited correlation coefficients (R^2) above 0.9986 and relative standard deviations (RSD) below 15 %. Each analytical batch included spiked and replicate samples for quality control. Surrogate and internal recovery standards were added to all samples, and PAEs concentrations were corrected accordingly. Recoveries ranged from 86 % to 108 %. The limits of detection (LOD) and quantification (LOQ) were calculated using the standard deviation (SD) of recoveries of isotopically labeled standards, following the equations LOD = 3 × SD / slope and LOQ = 10 × SD / slope, where the slope corresponds to that of the matrix-matched calibration curve (ICH Harmonised Tripartite Guideline, 2005). Since matrix blanks were not available in all cases, both the estimation of LOD/LOQ and the calibration curves were based on isotopically labeled standards. The LOQ was further validated using the lowest spike levels meeting identification and performance criteria for recovery and precision, following the SANTE/11312/2021v2 Guideline (European Commission, 2024). Final method LOQs were established at 30 ng/g dry weight (dw) for DMP and DBP, and 50 ng/g dw for DEHP. All recovery experiments were conducted in triplicate to ensure methodological robustness. Procedural (reactive) blanks were processed and analyzed alongside the preen gland samples within each batch to monitor potential contamination.

2.6. Detection and quantification of plastic items in the digestive tract

Methods employed to quantify plastic ingestion were described in detail by Gallo et al. (2024). Briefly, frozen upper digestive tracts were thawed, and their contents were thoroughly washed with running water through a 1 mm mesh sieve. Retained material was transferred to Petri dishes, air dried for at least 24 h at room temperature, and examined under stereomicroscope. Isolated items were counted, and total mass was measured with a precision scale (± 0.0001 g). The type of plastic items was classified into industrial pellets ("nurdles") or user plastics (all non-industrial remains of plastic items) (Provencher et al., 2017). User plastics were further differentiated in the following subcategories (GESAMP, 2019): (a) hard fragments (rigid pieces of larger objects), (b) sheet (flat, flexible pieces of plastic bags and packaging), (c) thread (long fibrous material that has a length substantially longer than its width), (d) foam (polystyrene and/or polyurethane; near-spherical or granular particle, which deforms readily under pressure and can be partly elastic, depending on weathering state), and (e) other plastic items (plastic material that does not fit in the previous categories, such as cigarette filters, rubber bands, balloons, etc.).

2.7. Statistical analyses

Samples in which PAEs were detected at concentrations below the limit of quantification (<LOQ) were classified as "trace levels". For statistical analyses, these values were treated as left-censored data and imputed using a concentration equal to 50 % of the LOQ for the corresponding analytical run, following established substitution methods for censored data in environmental and biomonitoring studies (Hornung and Reed, 1990; Helsel, 2005). The arithmetic mean, standard deviation (SD) and median of the PAE concentration were calculated for all individuals with detectable levels. Additionally, these statistical measures were calculated for the subset of individuals with quantifiable levels (i.e. excluding those with trace levels). Frequency of quantifiable levels (FQ = no. individuals with detectable non-trace levels / total no. individuals evaluated) and frequency of trace levels (FT = no. individuals with trace levels / no. individuals evaluated) were calculated for each PAE compound.

Given the non-normal distribution of data (tested with the Shapiro-Wilk test), univariate non-parametric analyses (Kruskal-Wallis test, followed by Dunn pairwise test, with p-values adjusted with the Bonferroni method) were performed to explore differences in PAEs concentration among taxonomic groups (albatrosses, petrels, and shearwaters). Non-parametric permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was employed to explore variation in phthalate composition (i.e. relative proportions of each PAE compound) among species. As data distribution departed from normality (Shapiro-Wilk test), it was normalized applying a square root transformation. The species bias between sampling regions, resulting from opportunistic sample collection, was considered in the analysis, and "region" was included as a covariable (northern coast, central coast, southern offshore; Fig. S1). Each term was tested by 999 random permutations, and model selection was based on the Akaike's Information Criterion (Burnham and Anderson, 2002).

The frequency of occurrence of plastic items in the upper digestive tract was also calculated (FO = no. individuals with plastic items / no. individuals evaluated). Wilcox tests were used to compare the concentrations of phthalate compounds between individuals with and without plastic items in their upper digestive tract. Linear regression was used to evaluate whether the concentrations of PAEs were correlated to the number or total mass of plastic items in the upper digestive tract. Statistical analyses were performed using R v4.1.2 (R Core Team, 2022) with the packages "*vegan*" v2.6.4 (Oksanen et al., 2022) and *ggplot2* v3.4.2 (Wickham, 2023). Significance level of 0.05 was used for all tests.

3. Results

Phthalate compounds were detected in the preen gland of all 31 seabirds evaluated (Tables 1 and 2; individual metadata and quantification results in Supplementary File 1). All samples had at least two PAE compounds above the LOQ, and their combined concentration ranged from LOQ to 9653 ng/g dw per individual (Table 2). The simultaneous occurrence of all three PAEs at quantifiable levels (≥LOQ) was observed in 24 samples (71 %). We detected quantifiable levels of DMP in 97 % of seabirds, with median concentrations at 33.5 ng/g dw (range = LOQ – 104 ng/g dw); additionally, one bird (3 %) had trace levels. DBP was quantifiable in 94 % of seabirds, with a median of 256.0 ng/g dw (range: LOQ – 1012 ng/g dw); additionally, two birds (6 %) had trace levels. DEHP was quantifiable in 87 % of seabirds, with a median of 336.0 ng/g dw (range: LOQ – 9653 ng/g dw); additionally, two birds (6 %) birds had trace levels, and two birds (6 %) did not have detectable levels.

The concentrations of DEHP differed significantly among taxonomic groups (K-W = 13.98, df = 2, $P = 0.001$), with higher levels in petrels and shearwaters, whereas differences in DMP and DBP concentrations were not statistically significant (Fig. 1). We also examined the variation in PAEs composition by species (Fig. 2), and PERMANOVA analysis revealed significant differences between sampling regions ($P = 0.005$), but not between species ($P = 0.075$, Table 3). In accordance with the results of the univariate tests, seabirds collected from offshore fisheries (80 % albatrosses) showed a higher relative contribution of DBP (57 %), whereas those from coastal regions (Chubut and Buenos Aires provinces, shearwaters and petrels) had higher contribution of DEHP (73 % and 90 % respectively; Fig. 2).

Plastic debris (>1 mm) were detected in the upper digestive tract of 23 % of seabirds, with an average (mean ± SD) of 1.2 ± 2.9 items per bird and a total mass of 0.0078 ± 0.0207 g per bird (Table 2). Overall, the plastic items found were primarily user plastics from the sheet subcategory (62 %), followed by other types (19 %), hard fragments (14 %) and foam (5 %). No significant associations were found between the concentration of PAEs and the presence, number and total mass of plastic items in the upper digestive tract at the time of death (all $P > 0.05$; Fig. 3).

4. Discussion

This study is the first to detect and quantify PAEs in the preen gland of seven procellariiform species in the Patagonian Sea, including the three species known to have the highest frequencies of plastic ingestion in the region, namely Southern giant petrels (*Macronectes giganteus*), White-chinned petrels (*Procellaria aequinoctialis*) and Cory's shearwaters (*Calonectris borealis*) (Gallo et al., 2024). Our results revealed that most seabirds presented simultaneous occurrence of three PAEs at quantifiable levels in their preen gland, indicating pervasive exposure to these plastic-derived contaminants. Nonetheless, no significant relationship was found with the plastic content in the gut at the time of death, suggesting additional sources of exposure (e.g. prey, seawater, plastics <1 mm) or a time-lag in the leaching and/or deposition of PAEs acquired from ingested plastic items (Tanaka et al., 2020; Van Hassel et al., 2024). This study provides evidence of the accumulation and elimination of potentially harmful compounds through the preen gland of seabirds. This reflects a potential detoxification pathway through the preen gland, as previously observed for other lipophilic anthropogenic contaminants (Tanaka et al., 2020; Yamashita et al., 2021; Matos et al., 2024). Importantly, unlike internal tissues such as liver or muscle that can only be obtained from dead individuals, the preen gland represents a matrix that could be sampled in living birds, offering a minimally invasive and accessible option to assess exposure to PAEs in wild populations. However, further studies are needed to confirm the generality and mechanisms of this process across bird taxa.

Table 2
Results for the frequency of occurrence of plastic items in the upper digestive tract (FO%), detection and quantification of PAEs (ng/g dry weight) in the preen gland of procellariiform birds collected along the coastline and from offshore fisheries in the Patagonian Sea. Frequency of quantifiable levels (FQ = no. individuals with levels higher than LOQ / no. individuals evaluated), and trace levels (FT = no. individuals with levels higher than LOD but lower than LOQ / no. individuals evaluated) were calculated for each phthalate compound. Mean ± SD and median were calculated separately for all individuals with detectable levels ("all"; i.e. including individuals with trace levels) and/or for the subset of individuals with quantifiable levels (">LOQ"; i.e. excluding individuals with trace levels). Maximum phthalate concentrations are also provided for each compound. Species names are abbreviated as per Table 1.

		Albatrosses		Petrels			Shearwaters	
	Total	GHA	BBA	SPG	SPP	WCP	CS	MS
Sample size	31	5	7	6	2	5	2	4
Plastics items								
FO%	23 %	0 %	0 %	67 %	50 %	67 %	50 %	25 %
DMP								
FQ%	97 %	100 %	100 %	100 %	100 %	80 %	100 %	100 %
FT%	3 %	0 %	0 %	0 %	0 %	20 %	0 %	0 %
Mean ± SD (all)	39.7 ± 18.5	–	–	–	–	29.6 ± 8.2	–	–
Median (all)	33.0	–	–	–	–	33.0	–	–
Mean ± SD (>LOQ)	40.6 ± 18.2	37.2 ± 6.6	35.7 ± 5.1	31.3 ± 4.0	57.5 ± 12.0	33.3 ± 1.3	29.5 ± 3.5	71.5 ± 33.9
Median (>LOQ)	33.5	36.0	33.0	31.0	57.5	33.0	29.5	74.0
Maximum	104.0	48.0	46.0	38.0	66.0	35.0	32.0	104.0
DBP								
FQ%	94 %	100 %	100 %	83 %	100 %	100 %	100 %	75 %
FT%	6 %	0 %	0 %	17 %	0 %	0 %	0 %	25 %
Mean ± SD (all)	247.6 ± 199.0	–	–	58.7 ± 46.5	–	–	–	366.8 ± 466.1
Median (all)	254.0	–	–	43.0	–	–	–	220.0
Mean ± SD (>LOQ)	263.6 ± 195.6	266.0 ± 50.8	331.8 ± 116.8	67.4 ± 46.2	330.0 ± 69.3	259.8 ± 114.4	121.5 ± 85.6	484.0 ± 493.3
Median (>LOQ)	256.0	256.0	302.0	47.0	330.0	293.0	121.5	405.0
Maximum	1012.0	325.0	464.0	148.0	379.0	392.0	182.0	1012.0
DEHP								
FQ%	87 %	20 %	100 %	100 %	100 %	100 %	100 %	100 %
FT%	6 %	40 %	0 %	0 %	0 %	0 %	0 %	0 %
Mean ± SD (all)	1262.3 ± 2379.7	44.0 ± 32.9	–	–	–	–	–	–
Median (all)	289.0	25.0	–	–	–	–	–	–
Mean ± SD (>LOQ)	1354.0 ± 2443.8	–	193.0 ± 139.5	1813.7 ± 3843.5	1752.5 ± 2271.9	1765.2 ± 2915.2	299.0 ± 195.2	2828.5 ± 2428.7
Median (>LOQ)	336.0	–	152.0	270.0	1752.5	542.0	299.0	2536.5
Maximum	9653.0	82.0	490.0	9653.0	3359.0	6973.0	437.0	5544.0

The frequency of occurrence of PAEs in preen gland in this study was similar to previous reports (Table S1); however, the concentrations were higher than those reported in both preen gland oil (Hardesty et al., 2015) and muscle tissue (Padula et al., 2020) of procellariiform birds collected in Australia and Alaska, respectively. Likewise, when comparing our results with a study in Brazil that included Cory's shearwaters and White-chinned petrels (Vanstreels et al., 2023), the overall frequency of occurrence of PAEs was higher in our study (see comparison in Table S1). Conversely, Provencher et al. (2020) failed to detect multiple phthalates (including DMP, DBP and DEHP) in preen gland oil of dead Northern fulmars (*Fulmarus glacialis*) from the Canadian High Arctic, with the exception of di-n-octyl phthalate (DnOP) which was found in 50 % of birds (Sühring et al., 2022). Notwithstanding, later studies have detected primary metabolites of PAEs, known as monoalkyl phthalate esters (MPEs), in the plasma of Northern fulmars, albeit at relatively low frequencies (Collard et al., 2024). Direct comparisons between studies are challenging due to differences in the specimens analyzed, the analytical methods employed, and how the results are reported; nevertheless, all studies are in agreement that PAEs (and MPEs) are present at detectable levels in seabirds nearly worldwide (i.e. Southwest Atlantic Ocean, Australia, Aleutian Archipelago, Canadian Arctic; Hardesty et al., 2015; Padula et al., 2020; Sühring et al., 2022; Vanstreels et al., 2023; Collard et al., 2024; this study).

Overall, DEHP showed the highest relative contribution to total PAEs found, followed by DBP and DMP in descending order (Table 2). The properties of a specific chemical compound will determine the partitioning between uptake/bioaccumulation and rate of excretion (Ribeiro et al., 2019; Tourinho et al., 2019; Savoca et al., 2023). Previous studies showed that PAE metabolism and tissue accumulation within organisms depend on their molecular weight and solubility in fat (lipophilicity) (Hu et al., 2016; Zhang et al., 2021; Savoca et al., 2023), with longer-chain PAEs being more lipophilic (Ghosh and Sahu, 2022; Khoshmanesh et al., 2024). Lipophilicity can be quantified by the octanol-water partition coefficient (K_{OW}), which among the studied PAEs is highest in DEHP, intermediate in DBP and lowest in DMP (Net et al., 2015; Khoshmanesh et al., 2024). This may explain the overall pattern of PAEs observed in this study, with DEHP occurring at the highest concentrations, followed by DBP and DMP. This agrees with previous studies showing that DEHP exposure is widespread in marine wildlife (Hermabessiere et al., 2017). Although the health impacts of PAE exposure in the studied seabird species are currently unknown, the detected levels of these compounds were below known acute and chronic toxicity thresholds established for mammals (David and Gans, 2002, EFSA Panel on Food Contact Materials et al., 2019). For instance, long-term PAE exposure can cause toxicity in aquatic organisms even at concentrations as low as μg/L (Xu et al., 2025). Further research is warranted to further understand the metabolic pathways and toxic thresholds of PAEs in seabirds. Moreover, analyzing PAEs and their primary metabolites (MPEs) across multiple biological matrices (e.g., guano, blood, liver) could provide a more thorough understanding of their absorption, distribution, metabolism, and elimination. Importantly, due to the mounting evidence that PAEs, particularly DEHP and DBP, produce adverse endocrine effects in humans and wildlife, the use of alternative chemicals has increased worldwide, as companies are seeking replacements to satisfy consumers and comply with stricter governmental regulations (CPSC, 2014). Thus, the inclusion of new, alternative plasticizers (e.g., DEHPT, ATBC) and their metabolites in future assessments is merited.

Our study is based on highly mobile bird species, for which collection sites (i.e. coastal, offshore) may not accurately represent areas of exposure (Law et al., 2010; Yamashita et al., 2021). We found that albatrosses (Grey-headed and Black-browed albatross) exhibited higher

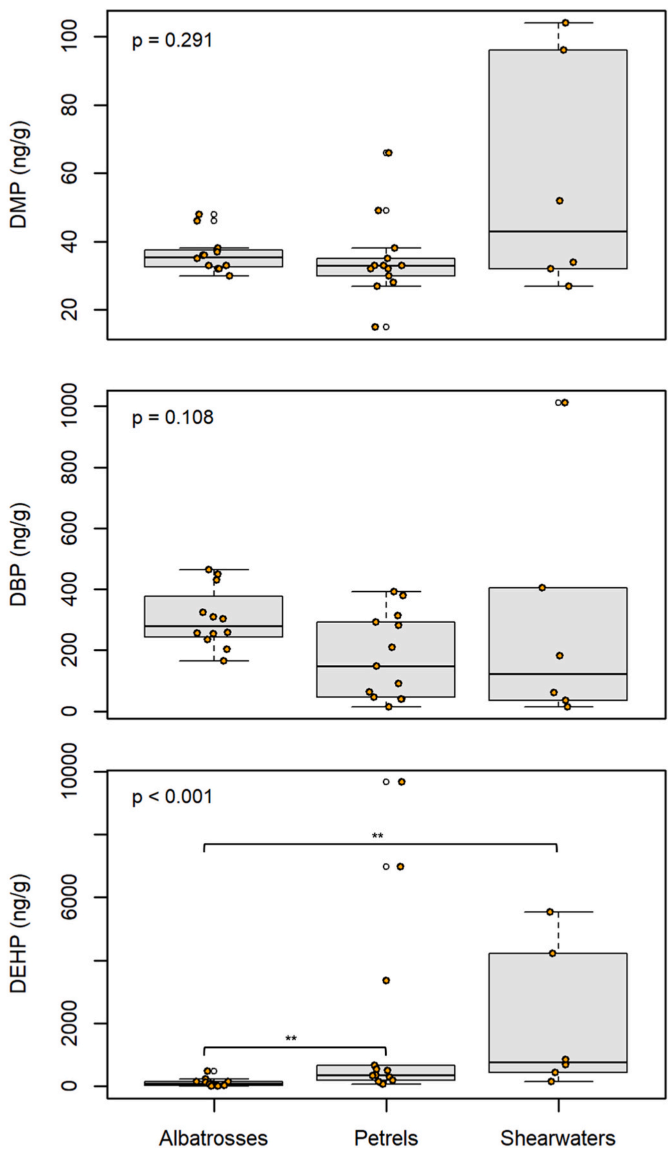

Fig. 1. Boxplots showing concentrations of PAEs (ng/g, dry weight) in the preen gland of procellariiform birds according to their taxonomic group. Horizontal black lines represent the medians, boxes indicate the interquartile range (IQR), and whiskers extend to 1.5 X IQR. P-values were calculated with Kruskal-Wallis test, and bars with asterisks represent significant differences between taxonomic groups from pairwise comparison (Dunn's test with Bonferroni correction, P-value <0.01).

relative contribution of DBP, while shearwaters (Manx and Cory's shearwater) and petrels (White-chinned, Southern giant, and Soft-plumaged petrel) showed a greater relative contribution of DEHP. These differences, along with the wide range of PAEs concentrations found in this study, may be related to numerous factors, such as species-specific foraging behavior, trophic position, prey choice (Padula et al., 2020; Sühring et al., 2022; Matos et al., 2024), feeding locations (Blasi et al., 2022; Sambolino et al., 2024), expression of genes associated to biotransformation of pollutants (Kreitsberg et al., 2023; Serafini et al., 2024), the quantity, size and types of ingested plastics (Hardesty et al., 2015), and variations in metabolism and/or excretion rates (Vanstreels et al., 2023; Collard et al., 2024). Given the small and unbalanced sample sizes across species, our findings should be interpreted with caution. Further research including larger and more representative sample sizes will be valuable to improve our understanding of PAEs exposure and excretion patterns, enabling more accurate risk assessments for different seabird species. Moreover, since PAEs do not biomagnify in marine food webs (Staples et al., 1997; Net et al., 2015; Zhang et al., 2021), measuring metabolites (e.g., MEHP) would provide a reliable indicator of trophic exposure and help elucidate the pathways of PAE accumulation in seabirds.

The broader implications for seabird populations globally stem from impacts of phthalate exposure on development and reproduction, which have been documented across a range of taxa in experimental settings, including in domestic birds (reviewed by Alam et al., 2025). Additionally, there is evidence of PAE maternal transfer to eggs, and while this mechanism may help reduce the contaminant burden in females, it could adversely affect hatching success and chick health (Huber et al., 2015; Allen et al., 2021; Sühring et al., 2022; Veríssimo et al., 2024). These findings are particularly concerning in the case of long-lived species such as procellariiform birds, which typically exhibit low reproductive rates and are already subject to both natural and anthropogenic stressors which may act additively or synergistically with plastic pollution (Phillips et al., 2023). In this context, even low concentrations of toxic chemicals could be harmful to populations experiencing high levels of natural stress (e.g. starvation) and/or dominated by weakened/unhealthy individuals (Bustnes et al., 2015).

PAEs are not chemically bound to plastic polymers and are therefore prone to leaching in the presence of stomach oils. This raises the possibility that PAE concentrations in the tissues of marine organisms could be positively correlated with the ingestion of plastic items (Hardesty et al., 2015; Provencher et al., 2020; Sühring et al., 2022; Vanstreels et al., 2023; Collard et al., 2024). However, evidence for this correlation has been controversial, with earlier studies finding a strong correlation (Hardesty et al., 2015) but later studies finding none or only weak correlation (Provencher et al., 2020; Vanstreels et al., 2023). Our results align with the studies that did not find a significant correlation, suggesting that the concentration of PAEs in the preen gland is not a reliable proxy for plastic ingestion, or more specifically, for the amount or type of plastics in the gut at the time of death. Several factors may contribute to this lack of correlation. For instance, this and previous studies have focused primarily on plastic items >1 mm (1–5 mm 36 %, 5–25 mm 46 %, and > 25 mm 19 %; Gallo et al., 2024); therefore, further research is needed to evaluate the potential contribution of nano- and microplastics (<1 mm) as sources of PAEs. In addition, the presence and concentration of PAEs in plastics is polymer dependent, with polyvinyl chloride (PVC) products standing out as having the highest PAE levels (up to 70 % by mass) (Hermabessiere et al., 2017). However, the absence of PVC in our samples (Gallo et al., 2024; Table S2 for subset included in this study) suggests that the phthalates detected in this study may have originated from the leaching of other polymers, or from alternative sources such as contaminated prey and/or water (Baini et al., 2017; Provencher et al., 2020; Collard et al., 2024; López-Vázquez et al., 2024; Sambolino et al., 2024). Furthermore, because the stomachs of birds contained a combination of different polymers (Table S2), it was difficult to directly associate the PAEs detected in the preen gland with a specific plastic types in the gut at the time of death. Assessing the relative importance of different sources of PAEs is difficult and represents a key challenge that should be addressed in future studies (Yamashita et al., 2021). To our knowledge, no studies have assessed the presence of PAEs in procellariiform seabird prey items or seawater in the Patagonian Sea, in contrast to other regions such as the North Atlantic or Mediterranean Sea, where such information is available (Net et al., 2015; Hidalgo-Serrano et al., 2022; Savoca et al., 2023). Notwithstanding, studies from nearby or broader regions in the southern hemisphere have reported PAEs in sediments, water, and krill (e.g. Neves et al., 2023 in northern Brazil; Cincinelli et al., 2005; Han and Liu, 2017; Zhang et al., 2018b; Motas et al., 2025 in Antarctica). Additionally, other plastic-associated chemicals (e.g. BPA, PBDEs, UV filters) have been documented in fish and seawater from the South Atlantic (Megill et al., 2024). In conclusion, our results complement these findings and offer baseline data for the Patagonian Sea.

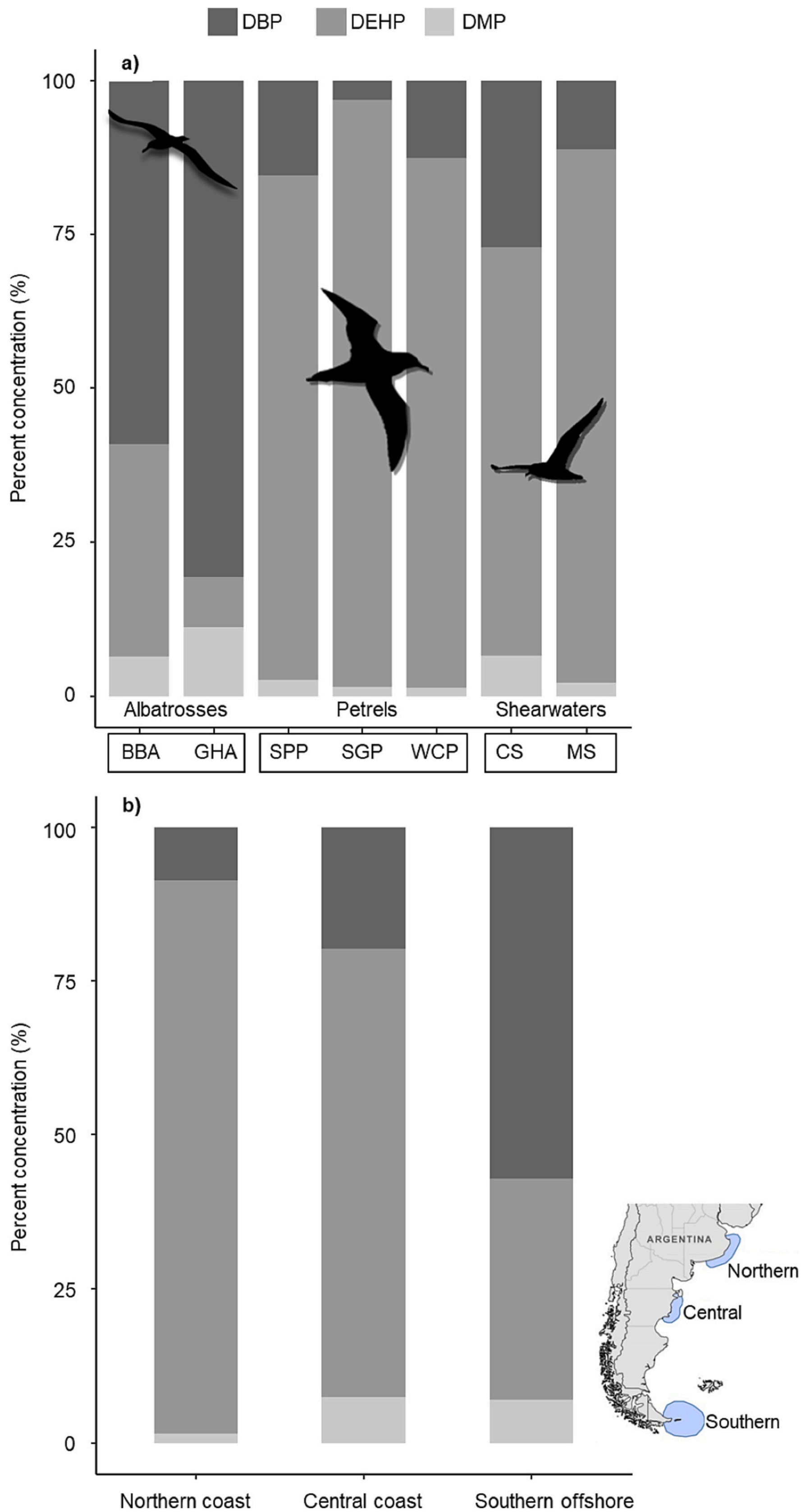

Fig. 2. Composition of phthalate compounds by (a) taxonomic groups and procellariiform species (codes correspond to those in Table 1), (b) sampling region. Percent concentrations were calculated as the percentage of each individual compound out of the total phthalate concentration (ng/g) for each group.

Table 3
PERMANOVA results for PAEs composition (proportion of DMP, DBP and DEHP) in preen gland from procellariiform birds collected along the coastline and from offshore fisheries in the Patagonian Sea. Models are ranked by the Akaike Information Criterion (AIC), and the best-fit model is shown in bold.

Model	AIC	Explained deviance (%)	Degrees of freedom
Region	**300.13**	**32.69**	**28**
Region+Species	305.52	45.62	22
Species	307.31	34.45	24

Additional complexity stems from the fact that oral intake of contaminants does not always reflect their bioaccessibility within an organism (López-Vázquez et al., 2024) and the transfer and accumulation of specific chemical additives into particular organs or tissues is influenced by various factors (Kühn et al., 2020; Tanaka et al., 2020). For example, the leaching of additives from plastics may require time (2–5 % of additives were leached from the matrix within 100 h in an in vitro procellariiform model; Van Hassel et al., 2024), and depends on medium conditions (e.g. pH, temperature, presence of food, gastric secretions), polymer characteristics, and the specific chemical involved (Coffin et al.,

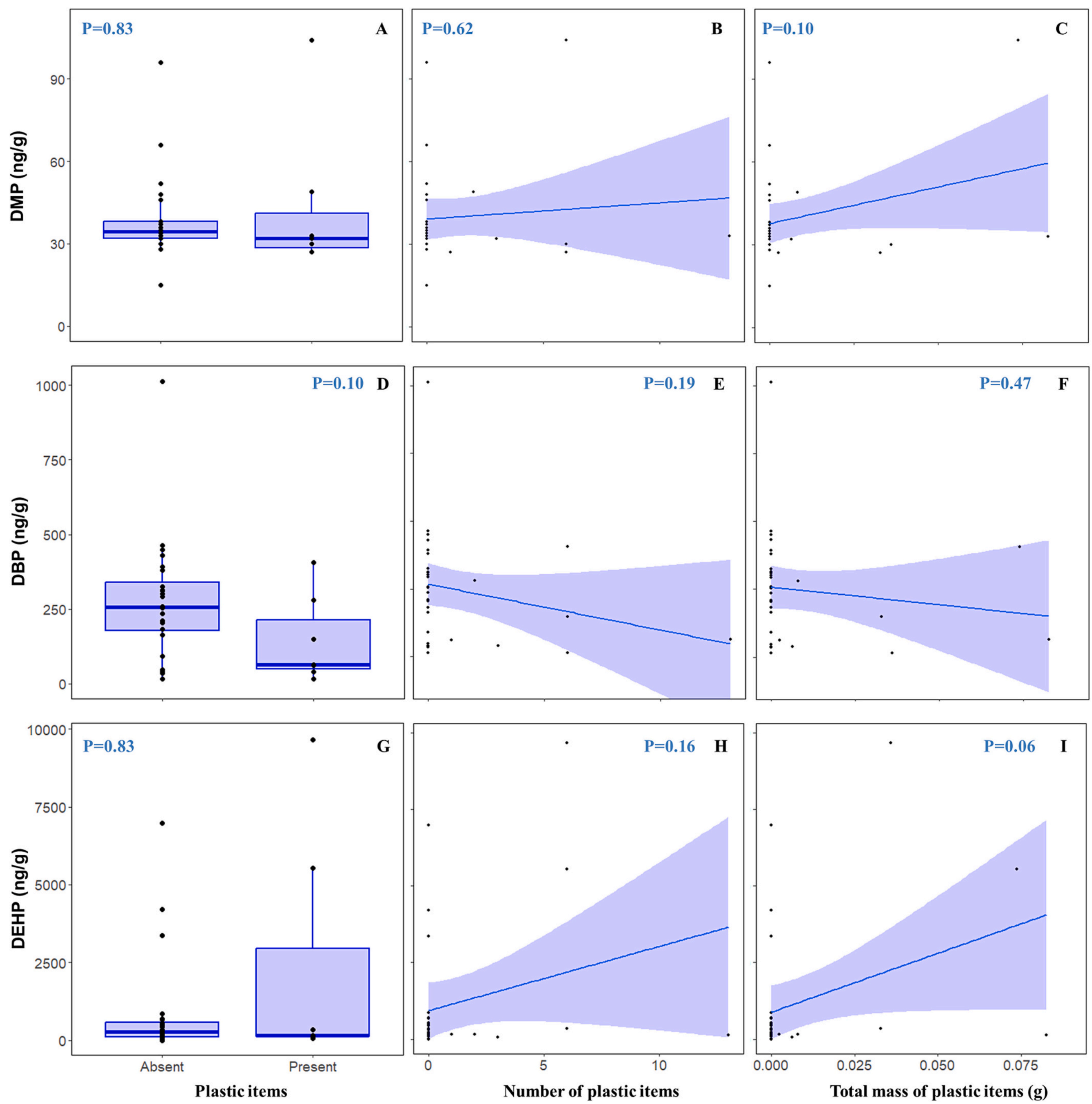

Fig. 3. Relationship between the presence (A, D, G), number (B, E, H) and total mass (C, F, I) of plastic items in the upper digestive tract at time of death and the concentration (ng/g dry weight) of PAEs in the preen gland of procellariiform birds collected along the coastline and from offshore fisheries in the Patagonian Sea. Blue shaded areas represent 95 % confidence intervals for the regression line. In boxplots (A, D, G), the horizontal blue line represents the median, boxes represent the interquartile range (IQR), whisker extend to 1.5 X IQR. *P*-values are shown in each plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2019; Tanaka et al., 2015, 2020; Kühn et al., 2020; Li et al., 2024; López-Vázquez et al., 2024). Petrels may be particularly sensitive to leaching because they retain plastic in the ventricular stomach for several months (Ryan, 2015). This may result in differences in absorbed PAE composition such as was seen in our study, where petrels exhibited DEHP concentrations an order of magnitude greater than those of albatrosses. Experimental studies have demonstrated significant long-term leaching of DEHP from marine debris-derived microplastics into the stomach oil of fulmars, suggesting that ingested plastics can act as a source of DEHP during extended gut residence times (Kühn et al., 2020). Given the increasing influx of plastics in marine environments, it is essential to assess the health effects of chronic exposure to these pollutants in long-lived seabirds.

In conclusion, our results show that exposure to PAEs is common in procellariiform birds in the Patagonian Sea and lend further evidence to the ubiquity of plastic additives in marine ecosystems. Importantly, the use of preen gland oil as testing matrix may render it suitable for monitoring purposes not only in deceased birds, but also in living seabirds with relatively minimal disturbance (Yamashita et al., 2007, 2018, 2021; Hardesty et al., 2015). However, given the ubiquitous nature of plasticizers, stringent laboratory and field sampling protocols are essential to avoid sample contamination and ensure data integrity. Future research should expand sample sizes and include analysis of prey, seawater, and smaller plastic items (<1 mm) to better elucidate sources of PAE exposure, as well as investigate metabolic pathways and potential adverse effects of chronic, low-level exposure in seabirds. Finally, given the classification of PAEs as priority pollutants due to their toxicity by international agencies (e.g., American Institute for Environment and Health, International Agency for Research on Cancer, REACH Regulation of the European Union, United States Environmental Protection Agency), numerous countries have implemented strict regulations or bans on their use. Considering these measures and the widespread exposure detected in seabirds, we recommend that Argentina and other countries in the region align their regulatory frameworks with international standards by adopting legally-binding restrictions on the use of phthalates in the plastics industry. Moreover, we emphasize the role of consumer awareness of the health hazards associated with broad and unrestricted use of plastics as a means to significantly reduce ocean pollution at its source.

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CRediT authorship contribution statement

Luciana Gallo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **María Rosa Repetti:** Writing – review & editing, Formal analysis. **Melina Paola Michlig:** Writing – review & editing, Formal analysis. **Leandro L. Tamini:** Writing – review & editing, Data curation. **Leandro N. Chavez:** Writing – review & editing, Data curation. **Ruben F. Dellacasa:** Writing – review & editing, Data curation. **C. Karina Alvarez:** Writing – review & editing, Data curation. **Sergio A. Rodriguez Heredia:** Writing – review & editing, Data curation. **Ralph Eric Thijl Vanstreels:** Writing – review & editing. **Patricia Pereira Serafini:** Writing – original draft, Funding acquisition. **Marcela M. Uhart:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Permits

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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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Data availability

All data supporting the findings of this study are included within the article and its supplementary materials.

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