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PHTHALATE ESTERS (PLASTICIZERS) IN THE UROPYGIAL GLAND AND THEIR RELATIONSHIP TO PLASTICS INGESTION IN SEABIRDS ALONG THE COAST OF ESPÍRITO SANTO, EASTERN BRAZIL

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Abstract: Plastic ingestion is a problem for seabirds worldwide. In addition to direct health effects such as obstruction or perforation of the gastrointestinal tract, plastic ingestion can also lead to indirect health effects through the release of chemicals that may be absorbed and cause systemic and chronic toxicity. Among chemicals that can be released by plastics are phthalate esters, a group of chemicals widely used as plasticizers or additives to change the physical characteristics of plastics. In this study, three phthalate esters, dimethyl phthalate (DMP), dibuthyl phthalate (DBP), and diethylhexyl phthalate (DEHP), were quantified in the uropygial gland of 48 seabirds from 16 species collected ashore in a tropical region, the coast of Espírito Santo, Eastern Brazil. Including trace levels, DMP was detected in 16 birds (33%) from 10 species, with an average concentration of 0.014 ± 0.005 ng/µl (mean ± SD for individuals with concentrations above the practical level of detection of 0.01 ng/µl). DBP was detected in 15 birds (31%) from 11 species, with an average concentration of 0.049 ± 0.032 ng/µl. DEHP was detected in 21 birds (44%) from 11 species, with an average concentration of 0.115 ± 0.105 ng/µl. DMP concentration in the uropygial gland was positively associated with the presence, number, and mass of plastic items in the upper digestive tract. However, no such relationship was noted for DBP nor DEHP, suggesting the concentration of phthalate compounds in the uropygial gland might not always serve as a reliable proxy for plastic ingestion. In spite of relatively high frequencies of detection, the low concentrations of phthalates detected in this study suggest levels of exposure below known toxicity thresholds. Further studies on the potential adverse effects of phthalate exposure in seabirds are necessary, especially on the reproductive development of embryos and chicks.

INTRODUCTION

Plastic pollution is one of the most pressing challenges for marine conservation in the 21st century, and it has been estimated that as many as 90% of all individual seabirds will ingest plastics at some point in their life.[42] In addition to the direct effects that ingested plastics may have by causing obstruction or perforation of the gastrointestinal tract,[31,36,37] plastics can also release a plethora of chemicals such as plasticizers, flame retardants, polycyclic aromatic hydrocarbons and organochlorine pesticides that may be absorbed by the digestive tract, potentially leading to systemic and chronic toxic effects.[6,15,34,35,38]

Phthalates, also known as phthalate esters or dialkyl phthalates, are a group of chemicals widely used as plasticizers, i.e. additives to increase the

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flexibility, workability, and longevity of plastic products. More than 3 million tons of phthalates are used on an annual basis by various industries.[13] Phthalates are predominantly used in the production of flexible polyvinyl chloride (PVC) products,[2,24] and phthalates may make up to 70% of the PVC polymer mass.[16] Pure phthalates are colorless viscous liquids that are soluble in oil; they are practically odorless and have a bitter taste.[2,24] More than 25 different phthalate compounds are produced and marketed.[24,43] Diethylhexyl phthalate (DEHP) is the most widely employed plasticizer in PVC, commonly used in building materials (e.g. flooring tiles, carpets, caulks, sealants), medical devices (e.g. tubing, drainage bags), paints, adhesives, adhesive removers, and toys.[21,24,26] Dibuthyl phthalate (DBP) is mainly used as an additive in latex adhesives, as a solvent in dyes, as a plasticizer in cellulose plastics, in coating of medications, and in a variety of cosmetics.[21,27] Dimethyl phthalate (DMP) is used in solid rocket propellants, lacquers, safety glasses, rubber coating agents, molding powders, insect repellants, and pesticides.[21,28] DEHP and DBP are also widely used as fragrance solvents.[18,19,24]

Once absorbed by living organisms, phthalates are metabolized through hydrolysis, and therefore are not biomagnified in food chains; as a result, their detection in higher trophic-level organisms is a reliable indication of direct exposure to plastics or other sources.[38,39] Concerns about the possible adverse effects of phthalate exposure in humans have been raised since the 1980s, and are particularly focused on the potential leaching of these compounds from medical devices (e.g. PVC containers and tubes used in fluid therapy, hemotherapy, dialysis, etc.), toys for infants and toddlers, childcare and cosmetic products, and plastics used in food processing and packaging.[18,19,21,24] Direct contact with phthalates may cause skin and ocular irritation, but in most cases the acute toxicity incidence of these compounds is considered very low.[24,28] In contrast, there is evidence of significant chronic toxicity of these compounds, especially DEHP, in mammals, including reproductive effects in males (testicular atrophy, decreased testosterone levels and fertility), carcinogenicity (liver and testicular cancer), and embryotoxicity (abnormal sexual development, especially in males).[21,24] There is also concern about the potential adverse effects that phthalates can have on aquatic animals, especially when there is contamination of waterbodies by industrial and commercial wastewater and plastic waste products.[43] In addition to the effects documented in mammals, there is evidence that phthalates can cause a variety of adverse effects such as oxidative stress, immunotoxicity, thyroid toxicity, and endocrine disruption in fishes and aquatic invertebrates.[43]

Because seabirds can often ingest plastic items and retain them for extended periods in their digestive tract, they could be vulnerable to the adverse effects of chronic exposure to chemicals leaching from plastics, including phthalates.[15,30,39] Furthermore, because phthalates have relatively short half-lives and generally do not bioaccumulate in the long term,[9,43] it has been suggested that the concentration of these lipophilic compounds in the uropygial or preen gland oil of seabirds could serve as a proxy to the presence of plastics in the gastrointestinal tract.[15] If confirmed, this would make quantifying phthalates in uropygial gland an invaluable and minimally invasive method to study plastic ingestion in living wild seabirds. In addition, it could also be useful for clinical veterinary purposes to diagnose the presence of plastics in the gastrointestinal tract that could be missed through imaging in radiography, ultrasonography, or endoscopy.

In a recent study, the authors evaluated the esophagus and stomach contents of seabirds collected along the coast of Espírito Santo, eastern Brazil, and found debris items in 30% of the examined birds, the majority of which were plastics (97%).[41] In this study, the concentration of phthalate compounds (DBP, DMP, and DEHP) in the uropygial gland of a subset of the seabirds examined previously was determined, and their relationship to plastic ingestion was investigated.

MATERIALS AND METHODS

Sample collection

The authors examined a subset of the individuals examined in a previous study,[41] comprising coastal and pelagic birds collected over a 26-mon period (20 April 2019 to 20 June 2021) along the coast of Espírito Santo (southeastern Brazil). The coastline of Espírito Santo state extends approximately 392 km from Riacho Doce stream (18.35S, 39.67W) to Itabapoana River (21.31S, 40.96W).

Individuals were classified according to their stranding code:[12] 1 (live animal), 2 (fresh carcass), 3 (carcass in moderate decomposition, but organs basically intact), 4 (carcass in advanced decomposition), and 5 (mummified or skeletal remains). Only live birds that died within 5 d of admission

and moderately preserved carcasses (codes 2 and 3) whose digestive tract and uropygial gland were intact were further evaluated. Carcasses were necropsied following standard protocols, and relevant metadata were recorded (species, age group, sex, body condition).[20] The upper digestive tract (from the proximal esophagus to the pyloric sphincter) was removed intact and stored frozen at −20°C. The entire uropygial gland was dissected, wrapped in heat-treated aluminum foil, and then frozen at −20°C. The gland was handled exclusively with stainless steel instruments, clean disposable scalpel blades, and nitrile gloves, and was never touched by plastic or latex items.

Detection and quantification of plastic ingestion

Methods employed to quantify plastic ingestion were detailed in a previous study.[41] The upper digestive tract was thoroughly washed through a 0.1-mm mesh sieve and retained material was examined under a stereomicroscope (×7.5 to 35 magnification). Debris items were counted and total mass of debris for each individual was measured with a scale (precision ± 0.01 g); when the total mass was lower than 0.01 g, total mass was inferred as 0.01 divided by the square root of 2 for calculations of mean and standard deviation.[40]

Detection and quantification of phthalate esters

Phthalate extraction and quantification were conducted at the Plant Morphogenesis and Biochemistry Laboratory (Federal University of Santa Catarina, Florianópolis, Brazil) by gas chromatography with flame ionization detection, using previously described protocols,[15] with modifications. All glassware used in sample extraction and analysis was previously washed thrice extensively and sequentially with hexane, dichloromethane (DCM), and methanol (Fisher Chemical, Leicestershire, UK, LE11 5RG and Honeywell–Riedel-de Haën, Seelze, Germany, 30926) and dried in a flow cabinet to remove eventual traces of organic contaminants. Further, the glassware was capped with aluminum foil previously heat-treated at 450°C, overnight, and stored in paper boxes to avoid contact with plastic materials used in laboratory routine practices. Whole uropygial glands were weighed, cut into small pieces using a new and clean metal scalpel blade, and lyophilized over 72 h. The dried samples were stored at −80°C until phthalate extraction.

Lyophilized samples (approximately 100 mg, dry weight) were solvent-extracted (DCM, 1:3, v/v) using an Ultraturrax apparatus (Omni tip™ homogenizing kit, Omni International, Kennesaw, GA 30144, USA), followed by ultrasound exposure (2.4 GHz, 30 min). The extracts were collected, vacuum filtered, and concentrated in a rotary evaporator. Next, minimum amounts of DCM were added to the dried extract, which was then transferred to a septum-equipped Teflon-lined vial (1.5 ml). The organosolvent was removed by nitrogen streaming and the extracts were stored at −80°C.

The dried extract was solubilized in 1 ml DCM, and aliquots (1 µl) were injected into a liquid-gas chromatograph (Shimadzu GC 2014, Kyoto, 604-8511, Japan) equipped with a manual injector (225°C), a TG-5MS capillary column (30 m × 0.25 mm × 0.25 µm, Thermo Fisher Scientific, Waltham, MA 02451, USA), and a flame ionization detector (320°C, 40 ms sampling rate). The GC column temperature program for phthalate separation was set as follows: initial oven temperature 70°C (1 min), increasing at 30°C/min to 120°C, then at 10°C/min to 285°C (5 min), and finally increasing at 10°C/min to 310°C (10 min). Nitrogen at 30.1 ml/min was used as carrier gas, with a 1:40 split rate. Prior to sample injection, for the purpose of phthalate concentration calculations, standard curves were built (0.05 to 10 ng/µl) for each compound (DMP, DBP, and DEHP) using analytical chemical standards (Sigma-Aldrich, St. Louis, MO 68178, USA). Compound identification was performed according to their retention times (min) and confirmed by spiking samples with the chemical standards of interest (i.e., co-injections).

The concentrations of phthalates were determined by averaging the peak area of the analytes, following three consecutive injections, using GC Solution 2.31 (Shimadzu) software for processing chromatograms (baseline correction, peak detection, and integral area calculation). Considering the signal-to-noise ratio (S:N = 10) obtained with the DEHP, DMP, and DBP standard curves, a practical limit of detection (LOD) threshold at 0.01 ng/µl was calculated, well above the limit of detection of the instrument, allowing confidence in the results. Samples where phthalates were detected, but their levels were below the practical limit of detection, were classified as "trace levels."

Because of the eventual presence of phthalates in the laboratory glassware and environment, three environmental controls were collected for analysis. For that, a clean swab was exposed to the

laboratory ambient conditions (30 s) and transferred to a capped vial. Environmental controls were then processed identically to samples and analyzed in tandem with uropygial gland samples. No detectable levels of plasticizers were found.

Statistical analyses

Frequency of detection (FD = no. individuals with detectable nontrace levels ÷ no. individuals evaluated) and frequency of trace levels (FT = no. individuals with trace levels ÷ no. individuals evaluated) were calculated for each phthalate compound. The arithmetic and geometric mean and standard deviation (SD) of the phthalate concentration was calculated for all individuals and also for the subset of individuals with detectable levels (not including individuals with trace levels). For individuals with trace levels of phthalates, the concentration was inferred as the LOD divided by the square root of 2.[40] Linear regression was used to determine whether there was a correlation between the number of plastic items and the total mass of plastics ingested by an individual, and to evaluate whether concentrations of different phthalate compounds were correlated to one another. Wilcoxon 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 phthalate compounds was correlated to the number or total mass of plastic debris.

RESULTS

Phthalates in the uropygial gland of 48 seabirds from 16 species were quantified (Table 1). Individual details of the sampled specimens and phthalate quantification results are provided in Supplementary File S1. Plastics were found in the upper digestive tract of 12 individuals (25%) (Table 2); the correlation between an individual's number of ingested plastic items and the total mass of ingested plastics was significant but weak ($P = 0.005$; $R^2 = 0.138$; Fig. 1A).

Phthalates were detected in 30 samples (63%) from 13 species (81%), with concentrations greater than 0.01 ng/µl in 25 samples (FD = 52%) from 12 species (75%), and trace levels in 5 samples (FT = 10%) from 4 species (25%) (Tables 1, 2). DMP was detected in 16 samples (33%) from 10 species (63%), with concentrations greater than 0.01 ng/µl in 8 samples (17%) from 6 species (38%), and trace levels in 7 samples (15%) from 6 species (38%). DBP was detected in 15 samples (31%) from 11 species (69%), with concentrations greater than 0.01 ng/µl in 8 samples (17%) from 6 species (33%), and trace levels in 7 samples (15%) from 6 species (33%). DEHP was detected at concentrations greater than 0.01 ng/µl in 21 samples (44%) from 11 species (69%); no samples had trace levels of DEHP. No significant linear correlation was observed between DMP and DBP ($P = 0.778$; Fig. 1B), DMP and DEHP ($P = 0.751$; Fig. 1C), or DBP and DEHP ($P = 0.430$; Fig. 1D).

There was a significant difference in the DMP concentration between seabirds with and without ingested plastics ($W = 93.5$, $P < 0.001$; Fig. 2A), but no difference was detected for DBP ($W = 157$, $P = 0.089$; Fig. 2D) or DEHP concentration ($W = 183$, $P = 0.393$; Fig. 2G). DMP concentration had a positive linear correlation with the number of plastic items ($R^2 = 0.096$, $P = 0.018$; Fig. 2B) and the total mass of plastics ($R^2 = 0.367$, $P < 0.001$; Fig. 2C). It should be noted, however, that only three birds examined had an ingested mass of plastics greater than 0.01 g, hence this linear correlation is essentially driven by only three data points: a kelp gull (*Larus dominicanus*), a great egret (*Ardea alba*), and a Cory's shearwater (*Calonectris borealis*). No significant correlations were found between the number or mass of ingested plastics and the concentration of DBP or DEHP (all $P > 0.5$; Figs. 2E, 2F, 2H, 2I).

DISCUSSION

This is the first study to quantify phthalate levels in seabirds in a tropical region, and it is the first to evaluate the presence of these compounds in the uropygial gland of nonprocellariiform species. In fact, only two studies have previously attempted to quantify phthalate compounds in the uropygial gland oil of seabirds. A study in Australia[15] found detectable levels (quantifiable + trace) of DBP and DEHP in the uropygial gland oil from all short-tailed shearwaters (*Ardenna tenuirostris*; live and dead, $n = 16$) and all wedge-tailed shearwaters (*Ardenna pacifica*; live, $n = 8$), with average concentrations of 0.04 ± 0.05 ng/µl for DBP (maximum = 0.20 ng/µl) and 0.06 ± 0.06 ng/µl for DEHP (maximum = 0.22 ng/µl). In contrast, a study in Canada[32] did not detect DMP, DBP, DEHP, nor three other phthalate compounds in the uropygial gland oil from northern fulmars (*Fulmarus glacialis*; dead, $n = 10$). Compared with the findings of the Australian study,[15] DBP was detected in a much smaller proportion of samples (17% at quantifiable levels and an additional 15% at trace levels) and with lower

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 detectable levels of phthalate compounds in the uropygial gland for seabirds collected along the coast of Espírito Santo, Eastern Brazil. Numbers within brackets represent the number of samples with "trace levels." DMP, dimethyl phthalate; DBP, dibuthyl phthalate; DEHP, diethylhexyl phthalate.

Family	Species	English name	Code	N	N_p	DMP	DBP	DEHP	Phthalates (Σ)
Haematopodidae	*Haematopus palliatus*	American oystercatcher	AMOY	1	0	0 [1]	1	1	1
Laridae	*Anous stolidus*	Brown noddy	BRNO	4	0	0	0	3	3
	Larus dominicanus	Kelp gull	KEGU	1	1	1	0 [1]	0	1
	Sterna hirundinacea	South American tern	SATE	1	0	0	1	1	1
	Sterna hirundo	Common tern	COTE	8	1	0 [1]	3 [1]	3	4
	Thalasseus acuflavidus	Cabot's tern	CATE	10	3	0 [3]	0 [2]	5	5 [2]
Scolopacidae	*Arenaria interpres*	Ruddy turnstone	RUTU	1	0	0	0	0	0
Ardeidae	*Ardea alba*	Great egret	GREG	2	1	1	1	1	1
	Nycticorax nycticorax	Black-crowned night heron	BCNH	1	0	0	0	0	0
Phaethontidae	*Phaethon aethereus*	Red-billed tropicbird	RBTR	1	0	0 [1]	0	0	0 [1]
Procellariidae	*Calonectris borealis*	Cory's shearwater	COSH	2	2	1	0 [1]	1	2
	Procellaria aequinoctialis	White-chinned petrel	WCPE	3	2	0 [3]	0 [1]	3	3
	Puffinus puffinus	Manx shearwater	MASH	7	1	0 [2]	1	1	2 [1]
Fregatidae	*Fregata magnificens*	Magnificent frigatebird	MAFR	1	0	0	0	0	0
Phalacrocoracidae	*Nannopterum brasilianus*	Neotropic cormorant	NECO	1	0	0	1	1	1
Sulidae	*Sula leucogaster*	Brown booby	BRBO	4	1	0 [2]	0 [1]	1	1 [1]
Total				48	12	3 [13]	8 [7]	21	25 [5]

concentrations (samples with detectable levels = 0.049 ± 0.032 ng/μl, maximum = 0.099 ng/μl). Of note, the LOD of 0.01 ng/μl in this study was lower than the LOD of 0.02 ng/μl in the study by Hardesty and colleagues.[15] On the other hand, although DEHP was detected in a smaller proportion of samples (44% at quantifiable levels, no samples at trace levels), concentrations (0.115 ± 0.105 ng/μl, maximum = 0.345 ng/μl) were higher than those reported in the Australian study.[15] In this study, DMP was detected with a low frequency (6% at quantifiable levels and 27% at trace levels), and concentrations were low (samples with detectable levels = 0.014 ± 0.005 ng/μl, maximum = 0.020 ng/μl). The relative abundance of these compounds in the samples is therefore consistent with previous studies, corroborating that they are relatively widespread throughout marine ecosystems, especially DEHP.

A few other studies have attempted to detect phthalate compounds in other tissues of seabirds. A study on the Aleutian Islands, Alaska,[30] evaluated the pectoral muscle of 115 seabirds from 10 species and detected DMP in 87% of samples (6.3 ± 15.7 ng/g, maximum = 140.8 ng/g), DBP in 84% of samples (20.7 ± 62.1 ng/g, maximum = 509.6 ng/g), and DEHP in 64% of samples (20.5 ± 52.6 ng/g, maximum = 398.7 ng/g). A study in Norway[17] evaluated 16 eggs from three species of seabirds, and found detectable levels of DEHP in 81% of eggs (9.7 ± 11.0 ng/g, maximum = 42.0 ng/g). Conversely, a study in England[1] evaluated the yolk of 13 eggs from European herring gulls (*Larus argentatus*), and detected DBP and DEHP in respectively 23% and 8% of egg yolks. The results cannot be directly compared with these studies due to the different types of samples evaluated, but they confirm that seabirds worldwide are routinely exposed to phthalate compounds, especially DBP and DEHP.

Plastic ingestion is a widespread problem for seabirds,[42] and it has been proposed that plastic items retained in the digestive tract could be a source of exposure to phthalates.[15,30,39] There is some evidence that the stomach of seabirds provides adequate conditions for the leaching of phthalate compounds from ingested plastics.[8,22] However, ecological models for seabirds and other marine organisms suggest that other routes of exposure such as food and water intake may play a more significant role in determining phthalate exposure than the ingestion of plastics.[7]

Table 2. Results for plastic ingestion and for phthalate detection and concentration (ng/μl) in the uropygial gland of seabirds collected along the coast of Espírito Santo, Eastern Brazil. Arithmetic-geometric means and standard deviation were calculated separately for all individuals examined ("all") and/or for the subset of individuals with levels above the practical limit of detection of 0.01 ng/μl ("detected"); these values are omitted when the compound was detected in all individuals examined, or when the compound was detected in only one individual.

Variable	Total $N = 48$	BRNO[a] $N = 4$	COTE[a] $N = 8$	CATE[a] $N = 10$	COSH[a] $N = 2$	WCPE[a] $N = 3$	MASH[a] $N = 7$	BRBO[a] $N = 4$	GREG[a] $N = 2$
Plastics ingestion (%)									
Prevalence	25	0	13	30	100	67	14	25	50
Dimethyl phthalate (DMP) (%)									
FD[b]	6	0	0	0	50	0	0	0	50
FT[c]	27	0	13	30	0	100	29	50	0
Mean ± SD (all)	0.003 ± 0.004	—	—	—	0.010 ± 0.014	—	—	—	0.005 ± 0.007
Mean ± SD (detected)	0.014 ± 0.005	—	—	—	—	—	—	—	—
GM[d] (detected)	0.013	—	—	—	—	—	—	—	—
Maximum	0.020	—	—	—	0.020	—	—	—	0.011
Dibuthyl phthalate (DBP)									
FD (%)[b]	17	0	38	0	0	0	14	0	50
FT (%)[c]	15	0	13	20	50	33	0	25	0
Mean ± SD (all)	0.009 ± 0.022	—	0.032 ± 0.044	—	—	—	0.005 ± 0.012	—	0.010 ± 0.014
Mean ± SD (detected)	0.049 ± 0.032	—	0.084 ± 0.021	—	—	—	—	—	—
GM[d] (detected)	0.040	—	—	—	—	—	—	—	—
Maximum	0.099	—	0.099	—	—	—	0.033	—	0.019
Diethylhexyl phthalate (DEHP)									
FD (%)[b]	44	75	38	50	50	100	14	25	50
FT (%)[c]	0	0	0	0	0	0	0	0	0
Mean ± SD (all)	0.050 ± 0.089	0.019 ± 0.017	0.066 ± 0.114	0.028 ± 0.041	0.027 ± 0.039	0.209 ± 0.043	0.049 ± 0.130	0.024 ± 0.049	0.006 ± 0.009
Mean ± SD (detected)	0.115 ± 0.105	0.025 ± 0.014	0.176 ± 0.127	0.057 ± 0.041	—	0.209 ± 0.043	—	—	—
GM[d] (detected)									
Maximum	0.345	0.040	0.316	0.126	0.055	0.254	0.345	0.097	0.012
All phthalates combined									
FD (%)[b]	52	75	50	50	100	100	29	25	50
FT (%)[c]	10	0	0	20	0	0	14	25	0
Mean ± SD (all)	0.061 ± 0.094	0.019 ± 0.017	0.098 ± 0.121	0.031 ± 0.041	0.041 ± 0.030	0.217 ± 0.045	0.055 ± 0.128	0.029 ± 0.055	0.021 ± 0.03
Mean ± SD (detected)	0.114 ± 0.103	0.025 ± 0.014	0.195 ± 0.091	0.057 ± 0.041	0.037 ± 0.025	0.209 ± 0.043	0.189 ± 0.220	—	—
GM[d] (detected)	0.040	0.022	0.145	0.048	0.055	0.206	0.345	—	—
Maximum	0.345	0.040	0.316	0.126	0.055	0.254	0.345	0.097	0.042

[a] Species codes are the same as provided in Table 1.
[b] Frequency of detection (FD) = no. individuals with detectable nontrace levels ÷ no. individuals evaluated.
[c] Frequency of trace levels (FT) = no. individuals with trace levels ÷ / no. individuals evaluated.
[d] Geometric mean (GM).

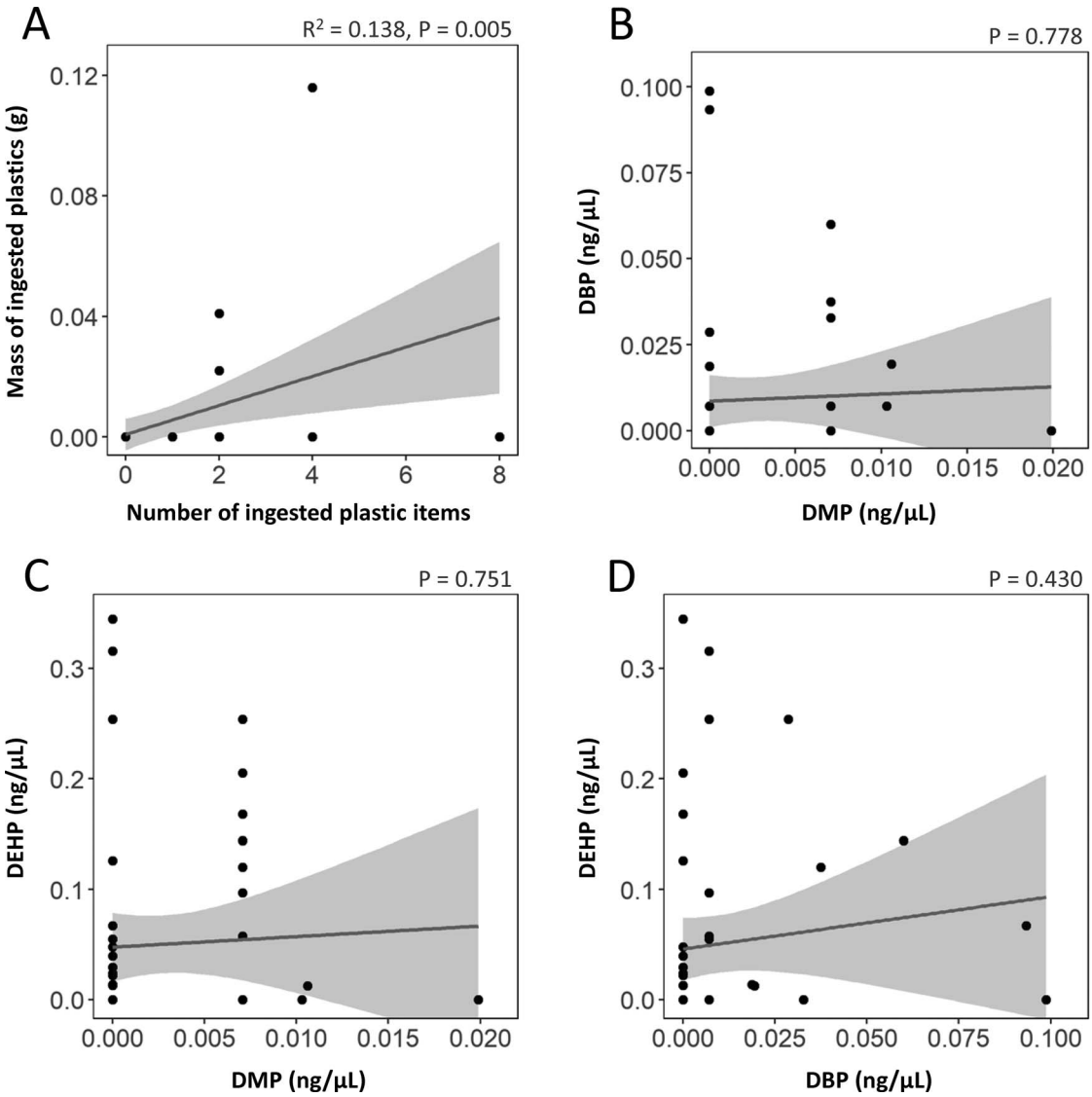

Figure 1. Relationship between the number and mass of ingested plastic items (A) and among the concentration of different phthalate compounds in the uropygial gland (B, C and D) of seabirds collected along the coast of Espírito Santo, Eastern Brazil. Shaded areas represent 95% confidence intervals for the regression line. DMP, dimethyl phthalate; DBP, dibuthyl phthalate; DEHP, diethylhexyl phthalate.

The presence and concentration of phthalates also varies considerably among different types of plastic, being highest in flexible PVC products.[16,24] However, PVC products are usually denser than water and will sink[25,38] whereas seabird taxa that frequently ingest plastics (e.g. Procellariiformes) are more prone to ingest floating debris.[42] As a result, phthalates concentrations might not accurately reflect the ingestion of other plastics that might not be meaningful sources of phthalates, such as nylon, polystyrene foam, and polyethylene bags that are often ingested by seabirds.[3,4,33,41] Furthermore, virtually nothing is known about the species-specific differences in the rates of leaching, absorption, and metabolization of phthalates from plastics ingested by seabirds.

Field studies examining the correlation between phthalate compounds in the uropygial gland of seabirds and the presence of plastics in their digestive tract have found mixed results. A study in Australia[15] found a strong correlation between the concentration of DBP and DEHP in

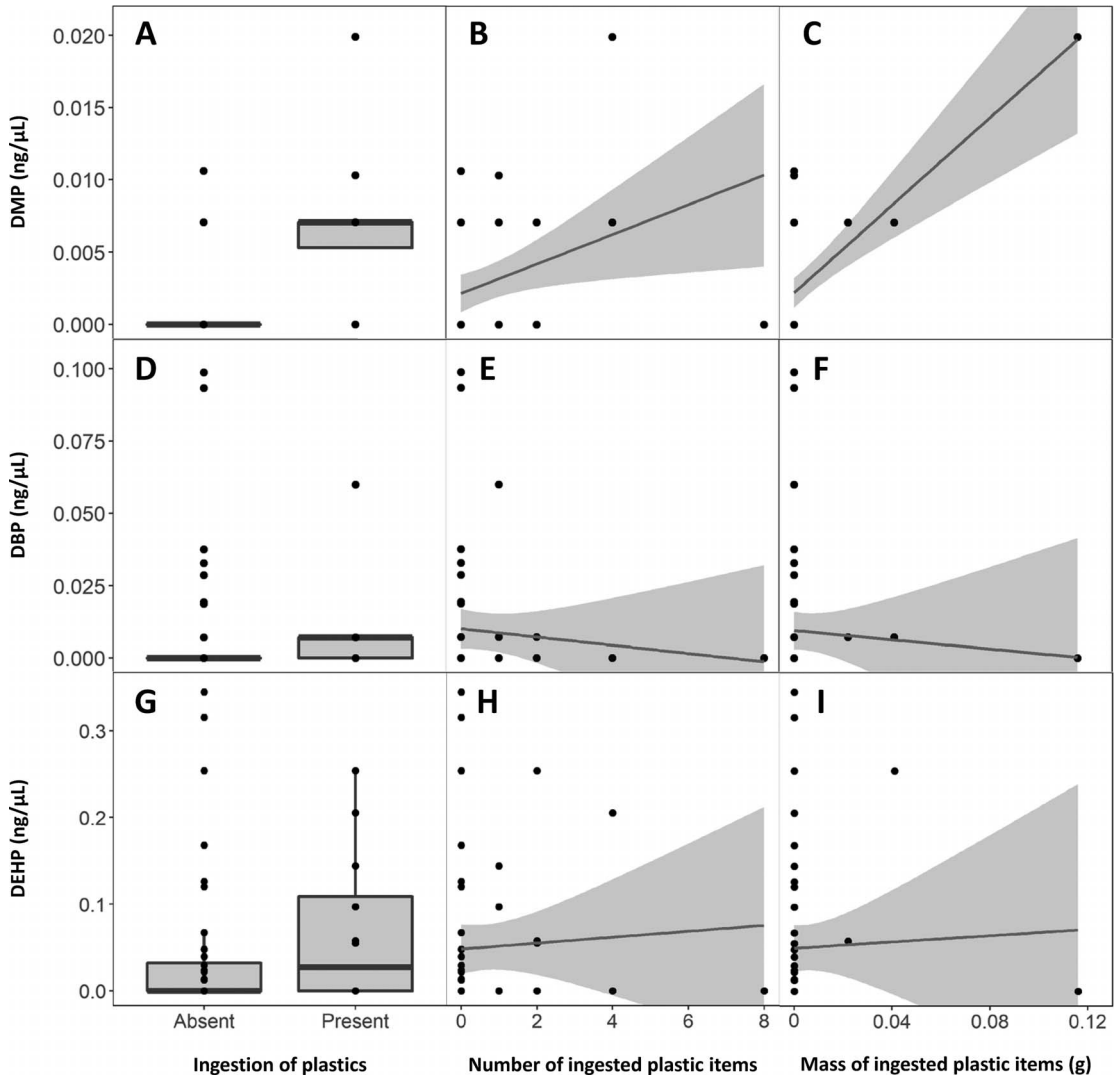

Figure 2. Relationship between the presence (A, D, G), number (B, E, H) and mass (C, F, I) of ingested plastic items and the concentration of phthalate compounds in the uropygial gland of seabirds collected along the coast of Espírito Santo, Eastern Brazil. Shaded areas represent 95% confidence intervals for the regression line. DMP, dimethyl phthalate; DBP, dibuthyl phthalate; DEHP, diethylhexyl phthalate.

uropygial gland oil and the number of plastic items found in the stomach of dead shearwaters; however, this result was based on a very small sample size (n = 8). In contrast, a study in Canada[32] did not detect significant levels of six phthalate compounds (including DBP and DEHP) in the uropygial gland oil of dead northern fulmars, despite some individuals having up to 100 plastic items in the stomach; yet their study was also based on a small sample size (n = 10). In this study (n = 48, of which 12 birds had ingested plastics), the presence, number, and mass of plastic items in the upper digestive tract of seabirds was correlated with the uropygial gland concentration of DMP, but not of DBP nor DEHP. Notwithstanding, the correlation between the concentration of DMP in the uropygial gland and the number or mass of plastic items was relatively weak (respectively, $R^2 = 0.096$ and $R^2 = 0.367$) and was essentially driven by three data points, and should thus be considered with caution. Additional studies are therefore needed to further explore the relationship between the concentration of phthalates in the uropygial gland and the quantity and type of plastics ingested by seabirds. Until this relationship and the routes of

exposure of seabirds to phthalates are better understood, the findings of this study and a previous study[32] suggest that interpretation of the concentration of phthalate compounds in the uropygial gland as a proxy for plastic ingestion in seabirds is unwarranted.

Phthalate compounds usually have low acute toxicity, with acute lethal doses (LD_{50}) for mammals of phthalate compounds higher than 5,000 mg/kg (oral) or 3,100 mg/kg (dermal).[10] Chronic toxicity can occur at substantially lower concentrations, with no-adverse-effect levels of 20 mg/kg/day for DBP and 5.8 mg/kg/day for DEHP.[5] The European Food and Safety Authority considers the tolerable daily intake (i.e. the amount of a substance that people can ingest daily during their whole life without any appreciable risk to health) for DBP and DEHP to be 50 μg/kg.[11] The concentrations detected in the uropygial gland oil of seabirds in this study and in previous studies is generally low,[15,32] suggesting levels of exposure below known acute and chronic toxicity thresholds, and at present there is no evidence of DMP, DBP, or DEHP having a measurable effect on the health of seabirds. However, existing experimental toxicological studies of phthalate compounds have focused on mammals, and much less is known about their effects on birds.[14,23,29,44] Further studies are therefore warranted on the potential adverse effects of phthalate exposure in seabirds, especially on the reproductive development of embryos and chicks.[1]

Plastic pollution is a pervasive threat to marine organisms and a serious environmental problem at a global scale. The recent endorsement of an international, legally binding agreement by 2024 through the United Nations Environment Programme (UNEP) to stop this planetary hazard, is timely (draft resolution UNEP/EA.5/L.23/Rev.1). As the world works towards prevention, reduction, and elimination of plastic pollution, seabirds will remain key sentinels to assess the effectiveness of actions.

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LITERATURE CITED

1. Allen SF, Ellis F, Mitchell C, Wang X, Boogert NJ, Lin CY, Clokey J, Thomas KV, Blount JD. Phthalate diversity in eggs and associations with oxidative stress in the European herring gull (*Larus argentatus*). Mar Pollut Bull. 2021;169:112564.

2. Api AM. Toxicological profile of diethyl phthalate: a vehicle for fragrance and cosmetic ingredients. Food Chem Toxicol. 2001;39(2):97–108.

3. Avery-Gomm S, Provencher JF, Liboiron M, Poon FE, Smith PA. Plastic pollution in the Labrador Sea: an assessment using the seabird northern fulmar *Fulmarus glacialis* as a biological monitoring species. Mar Pollut Bull. 2018;127:817–822.

4. Baak JE, Linnebjerg JF, Barry T, Gavrilo MV, Mallory ML, Price C, Provencher JF. Plastic ingestion by seabirds in the circumpolar Arctic: a review. Environ Rev. 2020;28(4):506–516.

5. Babich MA. Overview of dialkyl ortho-phthalates. Bethesda (MD): Consumer Product Safety Commission; 2010. https://www.cpsc.gov/s3fs-public/phthalover.pdf

6. Baini M, Martellini T, Cincinelli A, Campani T, Minutoli R, Panti C, Finoia MG, Fossi MC. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. Anal Methods. 2017;9:1512–1520.

7. Bakir A, O'Connor IA, Rowland SJ, Hendriks AJ, Thompson RC. Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environ Pollut. 2016;219:56–65.

8. Bakir A, Rowland SJ, Thompson RC. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ Pollut. 2014;185:16–23.

9. Cousins IT, Mackay D, Parkerton TF. Physical-chemical properties and evaluative fate modelling of phthalate esters. In: Staples CA (ed.). Series anthropogenic compounds, the handbook of environmental chemistry. Berlin (Germany): Springer; 2003. p. 57–84.

10. David RM, Gans G. Summary of mammalian toxicology and health effects of phthalate esters. In: Staples CA (ed.). Series anthropogenic compounds, the

handbook of environmental chemistry. Berlin (Germany): Springer; 2013. p. 299–316.

11. European Food Safety Authority, Panel on Food Contact Materials, Enzymes and Processing Aids. Update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis (2-ethylhexyl) phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials. EFSA J. 2019;17:e05838.

12. Geraci JR, Lounsbury VJ. Marine mammals ashore: a field guide for strandings. Baltimore (MD): National Aquarium in Baltimore; 2005.

13. Golestanzadeh M, Riahi R, Kelishadi R. Association of exposure to phthalates with cardiometabolic risk factors in children and adolescents: a systematic review and meta-analysis. Environ Sci Pollut Res Int. 2019;26(35):35670–35686.

14. Guibert E, Prieur B, Cariou R, Courant F, Antignac JP, Pain B, Brillard JP, Froment P. Effects of mono-(2-ethylhexyl) phthalate (MEHP) on chicken germ cells cultured in vitro. Environ Sci Pollut Res Int. 2013;20(5):2771–2783.

15. Hardesty BD, Holdsworth D, Revill AT, Wilcox C. A biochemical approach for identifying plastics exposure in live wildlife. Methods Ecol Evol. 2015; 6(1):92–98.

16. Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, Duflos G. Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere. 2017;182:781–793.

17. Huber S, Warner NA, Nygård T, Remberger M, Harju M, Uggerud HT, Kaj L, Hanssen L. A broad cocktail of environmental pollutants found in eggs of three seabird species from remote colonies in Norway. Environ Toxicol Chem. 2015;34(6):1296–1308.

18. Hubinger JC. A survey of phthalate esters in consumer cosmetic products. J Cosmet Sci. 2010;61(6): 457–465.

19. Hubinger JC, Havery DC. Analysis of consumer cosmetic products for phthalate esters. J Cosmet Sci. 2006;57(2):127–137.

20. Hurtado R, Saviolli JY, Vanstreels RET (eds.). Reabilitação de Procellariiformes (albatrozes, petréis e pardelas) [Rehabilitation of Procellariiformes (albatrosses, petrels and shearwaters)]. Santos: Comunnicar; 2020.

21. Kamrin MA. Phthalate risks, phthalate regulation, and public health: a review. J Toxicol Environ Health B Crit Rev. 2009;12(2):157–174.

22. Kühn S, Booth AM, Sørensen L, van Oyen A, van Franeker JA. Transfer of additive chemicals from marine plastic debris to the stomach oil of northern fulmars. Front Environ Sci. 2020;8:138.

23. Li PC, Li XN, Du ZH, Wang H, Yu ZR, Li JL. Di (2-ethyl hexyl) phthalate (DEHP)-induced kidney injury in quail (*Coturnix japonica*) via inhibiting HSF1/HSF3-dependent heat shock response. Chemosphere. 2018;209:981–988.

24. Lorz PM, Towae FK, Enke W, Jäckh R, Bhargava N, Hillesheim W. Phthalic acid and derivatives. In: Ullman F (ed.). Ullmann's encyclopedia of industrial chemistry. Weinheim (Germany): Wiley, 2007. p. 131–180.

25. Matsuguma Y, Takada H, Kumata H, Kanke H, Sakurai S, Suzuki T, Itoh M, Okazaki Y, Boonyatumanond R, Zakaria MP, Weerts S, Newman B. Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. Arch Environ Contam Toxicol. 2017;73(2):230–239.

26. National Center for Biotechnology Information. PubChem compound summary: bis(2-ethylhexyl) phthalate; 2022. https://pubchem.ncbi.nlm.nih.gov/compound/Bis_2-ethylhexyl_-phthalate

27. NCBI. PubChem compound summary: dibutyl phthalate; 2022. https://pubchem.ncbi.nlm.nih.gov/compound/Dibutyl-phthalate

28. NCBI. PubChem compound summary: dimethyl phthalate; 2022. https://pubchem.ncbi.nlm.nih.gov/compound/Dimethyl-phthalate

29. O'Shea TJ, Stafford CJ. Phthalate plasticizers: accumulation and effects on weight and food consumption in captive starlings. Bull Environ Contam Toxicol. 1980;25(1):345–352.

30. Padula V, Beaudreau AH, Hagedorn B, Causey D. Plastic-derived contaminants in Aleutian Archipelago seabirds with varied foraging strategies. Mar Pollut Bull. 2020;158:111435.

31. Phillips RA, Ridley C, Reid K, Pugh PJ, Tuck GN, Harrison N. Ingestion of fishing gear and entanglements of seabirds: monitoring and implications for management. Biol Conserv. 2010;143(2):501–512.

32. Provencher JF, Avery-Gomm S, Braune BM, Letcher RJ, Dey CJ, Mallory ML. Are phthalate ester contaminants in northern fulmar preen oil higher in birds that have ingested more plastic? Mar Pollut Bull. 2020;150:110679.

33. Provencher JF, Gaston AJ, Mallory ML, O'Hara PD, Gilchrist HG. Ingested plastic in a diving seabird, the thick-billed murre (*Uria lomvia*), in the eastern Canadian Arctic. Mar Pollut Bull. 2010;60:1406–1411.

34. Puskic PS, Lavers JL, Adams LR, Bond AL. Ingested plastic and trace element concentrations in short-tailed shearwaters (*Ardenna tenuirostris*). Mar Pollut Bull. 2020;155:111143.

35. Rios LM, Jones PR, Moore C, Narayan UV. Quantitation of persistent organic pollutants adsorbed on plastic debris from the Northern Pacific Gyre's "eastern garbage patch." J Environ Monit. 2010;12: 2226–2236.

36. Ryan P. Ingestion of plastics by marine organisms. In: Takada H, Karapanagioti H (eds.). Hazardous chemicals associated with plastics in the marine environment, the handbook of environmental chemistry. Cham (Switzerland): Springer; 2016. p. 1–32.

37. Senko JF, Nelms SE, Reavis JL, Witherington B, Godley BJ, Wallace BP. Understanding individual and

population-level effects of plastic pollution on marine megafauna. Endanger Species Res. 2020;43:234–252.

38. Tanaka K, Takada H, Yamashita R, Mizukawa K, Fukuwaka M, Watanuki Y. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Mar Pollut Bull. 2013;69:219–222.

39. Tanaka K, Yamashita R, Takada H. Transfer of hazardous chemicals from ingested plastics to higher-trophic-level organisms. In: Takada H, Karapanagioti H (eds.). Hazardous chemicals associated with plastics in the marine environment, the handbook of environmental chemistry. Cham (Switzerland): Springer; 2018. p. 267–280.

40. Tekindal MA, Erdoğan BD, Yavuz Y. Evaluating left-censored data through substitution, parametric, semi-parametric, and nonparametric methods: a simulation study. Interdiscip Sci. 2017;9(2):153–172.

41. Vanstreels RET, Gallo L, Serafini PP, Santos AP, Egert L, Uhart MM. Ingestion of plastics and other debris by coastal and pelagic birds along the coast of Espírito Santo, Eastern Brazil. Mar Pollut Bull. 2021; 173:113046.

42. Wilcox C, Van Sebille E, Hardesty BD. Threat of plastic pollution to seabirds is global, pervasive, and increasing. Proc Natl Acad Sci. 2015;112(38):11899–11904.

43. Zhang Y, Jiao Y, Li Z, Tao Y, Yang Y. Hazards of phthalates (PAEs) exposure: a review of aquatic animal toxicology studies. Sci Total Environ. 2021;771:145418.

44. Zhang YZ, Zuo YZ, Du ZH, Xia J, Zhang C, Wang H, Li XN, Li JL. Di (2-ethylhexyl) phthalate (DEHP)-induced hepatotoxicity in quails (*Coturnix japonica*) via triggering nuclear xenobiotic receptors and modulating cytochrome P450 systems. Food Chem Toxicol. 2018;120:287–293.

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