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Microplastics ingestion and chemical pollutants in seabirds of Gran Canaria (Canary Islands, Spain)

Alberto Navarro ^{a,*}, Octavio Pérez Luzardo ^b, May Gómez ^a, Andrea Acosta-Dacal ^b, Ico Martínez ^a, Jorge Felipe de la Rosa ^c, Ana Macías-Montes ^b, Alejandro Suárez-Pérez ^d, Alicia Herrera ^a

- a Marine Ecophysiology Group (EOMAR), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain
- b Toxicology Unit, Research Institute of Biomedical and Health Sciences (IUIBS), Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain
- ^c Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain
- ^d "La Tahonilla" Wildlife Recovery Center, 38111 Santa Cruz de Tenerife, Spain

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ABSTRACT

Plastic pollution constitutes an environmental problem in the Canary Islands nowadays. Nevertheless, studies evaluating the impact of plastics on its avifauna are still scarce. Gastrointestinal tracts of 88 birds belonging to 14 species were studied for the presence of plastics. Moreover, their livers were analyzed for the determination of bromodiphenyl ethers (BDEs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs). Among Cory's shearwaters (n=45), the frequency of occurrence of plastic ingestion was considerably high (88.89 %). This species had the highest mean value of items (7.22 \pm 5.66) and most of them were compatible with lines derived from fishing gear. PCBs and PAHs were detected in all of the samples and OCPs in the great majority of them (98.86 %). Our results highlight the problems that plastic debris (mainly for seabirds) and organic pollutants pose to these species.

1. Introduction

Global ecosystems have been experiencing severe negative impacts since the industrial revolution, with estimates that approximately 75 % of the world's ice-free land area has been significantly altered, >85 % of wetlands have been lost, and the oceans face major threats such as pollution and overfishing (WWF, 2020). Among birds, seabirds populations have been greatly affected in the last decades, with a decrease of about 70 % of monitored populations between 1950 and 2010 (Paleczny et al., 2015). Around 28 % of species are threatened globally due to diverse issues such as habitat degradation, hunting, introduction of invasive species that prey on birds or eggs, interaction with fishing activities, as well as plastic pollution (Croxall et al., 2012; Roman et al., 2020; Žydelis et al., 2013). This last problem has raised a growing concern in the scientific community and society during the last years. The impact of plastic debris on marine fauna was first reported in the scientific literature in the late 1960s, when the ingestion of plastic by seabirds was described for the first time (Kenyon and Kridler, 1969). Since then, the impact of this material on >1500 species, both

vertebrates and invertebrates, has been described (Santos et al., 2021).

There are two main ways by which plastic debris directly affects marine fauna: entanglement and ingestion (Laist, 1987). On the one hand, entanglement can result in the direct death of the animal by asphyxia or drowning, or trigger other factors that will limit its survival by causing injuries, loss of limbs, growth difficulties, weakness, difficulty feeding and/or fleeing from predators (Derraik, 2002; Gall and Thompson, 2015; Gregory, 1991; Laist, 1997). On the other hand, plastic ingestion has been reported throughout the food chain, from small invertebrates (Devriese et al., 2015) and planktivorous fish (Boerger et al., 2010), to large predators such as cetaceans (Fossi et al., 2012), pinnipeds (Rebolledo et al., 2013) or sharks (Bernardini et al., 2018). Plastic uptake can occur directly by mistaking it for food (Ryan, 2016), although it can also take place indirectly through other pathways, such as by preying on individuals that have previously ingested plastic (Farrell and Nelson, 2013; Furtado et al., 2016; Hammer et al., 2016) or even during the ventilation process by accidentally acquiring particles present in the surrounding water (Watts et al., 2014). Plastic ingestion can cause serious injuries along the gastrointestinal tract such as

E-mail address: alberto.navarro@ulpgc.es (A. Navarro).

^{*} Corresponding author.

obstructions, ulcers or perforations, as well as progressively weaken the animal by creating a false sensation of satiety (Gregory, 1991). Furthermore, the ingestion of this material has been related to other alterations such as inflammation and lipid accumulation in the liver (Lu et al., 2016), changes in the intestinal microbiota (Montero et al., 2022), reduction of acetylcholinesterase enzyme activity (Oliveira et al., 2013) and oxidative stress (Lei et al., 2018). In addition, a great deal of scientific literature reports the ingestion of small plastic particles known as microplastics (<5 mm). These, in turn, can be classified as primary (those manufactured specifically for their abrasive qualities), secondary (originating from the degradation of larger plastics) or tertiary (preproduction pellets used to mold plastic articles) (Carbery et al., 2018). Despite their small size, ingestion of these particles can also generate physical damage (Auta et al., 2017). Besides, these debris are potential vehicles for transferring chemical pollutants to wildlife, including both substances used as additives during the manufacturing process of plastics (e.g., phthalates) as well as other compounds (e.g., organochlorine pesticides) that can be adsorbed and concentrated due to the hydrophobic character and high surface-to-volume ratio of microplastics (Guo et al., 2020; Oehlmann et al., 2009; Rochman et al., 2013; Scopetani et al., 2018; Teuten et al., 2009). Leaching of hydrophobic chemicals present in plastic is enhanced when the plastic is in an oily environment, as for example would occur when plastics ingested by seabirds mix with stomach oils (Tanaka et al., 2015).

Seabirds are one of the vertebrate groups most affected by plastic ingestion, being reported in 44 % of all seabird species (Kühn and van Francker, 2020) and all seabird families being affected by the ingestion of this type of debris (Santos et al., 2021). It is estimated that, at this rate, 99 % of species will be affected by 2050 (Wilcox et al., 2015). The Canary Islands are a key point for the migration and breeding of several species of seabirds, such as the Cory's shearwater (Calonectris borealis), the yellow-legged gull (Larus michahellis) or the Madeiran storm-petrel (Oceanodroma castro). Recently, plastic pollution and its effects have begun to be studied in this archipelago, where the action of the Canary Current deposits large amounts of debris on the coasts every year (Baztan et al., 2014; Herrera et al., 2018). To this, we must add the waste that is generated in the islands and for various reasons can end up in the sea (Baztan et al., 2014; Rapp et al., 2020). However, studies on wildlife are scarce, especially in birds where only one species has been studied, the Cory's shearwater (Rodríguez et al., 2012). Moreover, we should highlight other studies conducted in the islands demonstrating plastic ingestion in other species such as cetaceans (Puig-Lozano et al., 2018), fish (Herrera et al., 2019), seaturtles (Orós et al., 2016) and jellyfish (Rapp et al., 2021). In addition, several pollutants, such as organochlorine pesticides (OCPs), bromodiphenyl ethers (BDEs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organophosphorus flame retardants (OPFRs), ultraviolet filters and the extensively used pesticide chlorpyrifos, have been reported to be associated with plastics collected from beaches in the Canary Islands (Camacho et al., 2019). Recently, an experiment conducted with European seabass demonstrated the transfer of organic contaminants (such as BDEs, PCBs and DDE) into the liver of fish fed with 10 % microplastics collected from beaches of the Canary Islands (Herrera et al., 2022). Therefore, plastic pollution should continue to be monitored in this region.

The aims of this paper are: (1) to study the prevalence of plastic ingestion in different bird species in Gran Canaria, as well as the characteristics of the ingested material (shape, color and polymer) and (2) to identify the different chemical pollutants (BDEs, OCPs, PCBs and PAHs) present at the hepatic level.

2. Materials and methods

For this study, carcasses of wild birds admitted to the Tafira Wildlife Rehabilitation Center, Gran Canaria, during 2020 and January 2021 were analyzed. Some birds died during the recovery process, while others were euthanized due to the impossibility of their rehabilitation because of their clinical condition. On the other hand, some of the animals had already died at the time of admission. The carcasses were kept frozen ($-20~^\circ\text{C}$) until dissection. A total of 88 animals belonging to 14 different species were sampled (Table 1). Most of the animals were seabirds. However, carcasses of some freshwater birds were also included. The species had different feeding habits and some nested in the Canary Islands while others were migratory (see Table S1). From each animal, the liver was separated for subsequent determination of chemical pollutants and the digestive tract (from the beginning of the esophagus to the cloaca) for the study of plastic ingestion. Both samples were kept in clean glass jars frozen at $-20~^\circ\text{C}$ until their corresponding analysis.

2.1. Microplastics analysis

The digestive tracts, previously separated, were individually subjected to chemical digestion with 10 % KOH for 24–120 h at 60 °C in order to degrade as much organic matter as possible. The resulting contents where then filtered through stainless steel filters with a mesh size of 25 μm with a kitasate-vacuum pump system. Subsequently, the filter of each sample was placed on a Petri dish to proceed to the counting of plastic residues (>1 mm) with the help of a Leica S9i stereo microscope with camera. The visualized plastics were classified according to their shape (fragment, line, film or pellet) (Fig. 1) and color (red, brown, orange, yellow, green, blue, purple, pink, silver, gray, black, semitransparent, transparent or white). Additionally, 34 % of the plastics found were separated for polymer type determination by Fourier transform infrared spectrometer (FTIR) using the Perkin Elmer FT-IR C106269 instrument.

2.2. Analysis of chemical pollutants

2.2.1. Sample preparation and extraction

The QuEChERS method (Anastassiades et al., 2003) is a matrix dispersion extraction method, which was initially developed for the analysis of pesticides in fruits and vegetables, but has proven to be versatile, allowing the analysis of many other compounds in complex matrices such as blood, milk, meat, eggs and even soil (Acosta-Dacal et al., 2021; Perestrelo et al., 2019). Recently, a QuEChERS-based method has been developed for the simultaneous analysis of POPs and

Table 1Classification of the species sampled.

Order	Family	Species	Common name	n =
				88
Procellariiformes	Procellariidae	Calonectris borealis	Cory's shearwater	45
		Bulweria bulwerii		3
	Hydrobatidae	осеапоdroma	Bulwer's petrel Madeiran storm-	5 5
	пуагоранаце	castro	petrel	3
		Oceanodroma	Leach's storm-	1
		leucorhoa	petrel	
Charadriiformes	Laridae	Larus michahellis	Yellow-legged gull	20
		Larus	Mediterranean	1
		melanocephalus	gull	
		Chroicocephalus	Black-headed	2
		ridibundus	gull	
	Scolopacidae	Actitis hypoleucos	Common sandpiper	2
		Calidris alba	Sanderling	1
		Arenaria interpres	Ruddy turnstone	1
		Limosa lapponica	Bar-tailed godwit	1
Pelecaniformes	Ardeidae	Egretta garzetta	Little egret	1
		Bubulcus ibis	Cattle egret	1
Gruiformes	Rallidae	Gallinula chloropus	Common moorhen	4
			moornen	

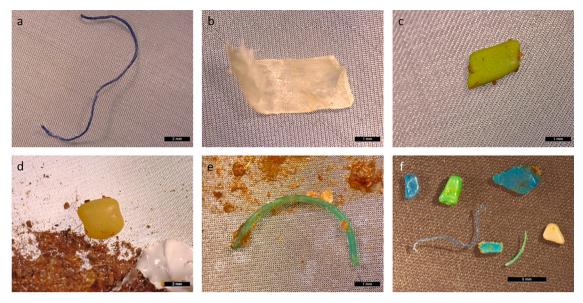


Fig. 1. Different types of plastic debris found in the gastrointestinal contents of the birds analyzed. a) Line. b) Film. c) Fragment. d) Pellet (center) and film (right). e) Line. f) Multiple fragments and lines.

other organic compounds in liver (Rial-Berriel et al., 2021). We applied it to birds liver samples. Briefly, after the liver homogenization, 1 g of liver homogenate was weighed into a tube suitable for homogenization with a Precellys Evolution homogenizer (Bertin Technologies, Rockville, Washington D.C., USA) (Acosta-Dacal et al., 2021; Anastassiades et al., 2003; Perestrelo et al., 2019), operated at 6500 rpm, 2×30 s. After that, when necessary, fortification was performed, either for calibration curves or for the preparation of quality controls (QC). The homogenate was then diluted with 4 ml ultrapure water, and 1 ml of the diluted homogenate was placed in a 5 ml centrifuge tube for processing. At this point, 10 µl of the ISP mixture (acenaphthalene-d10, atrazine-d5, carbendazim-d3, chlorpyrifos-d10, chrysene-d12, cyromazine-d4, diazinon-d10, linuron-d3, PCB 200, phenanthrene-d10, and pirimicarb-d6) was added to all tubes (fortified or not) to reach a final concentration of 10 ng/ml. Then, anhydrous magnesium sulfate (480 mg) and sodium acetate (120 mg) were added to each tube, followed by 30 s of vortexing and 1 min of vertical-manual shaking. Finally, the centrifuge tubes were centrifuged for 5 min at 4500 rpm and 2 °C. The supernatant was filtered through a 0.2 µm Chromafil PET-20/15 syringe filter (polyester, certified for HPLC, Macherey-Nagel, Düren, Germany) into an amber vial directly, for sequential GC-MS/MS analysis.

2.2.2. GC-MS/MS

Gas chromatography was employed for the separation of BDEs (8 congeners), OCPs (12 compounds), PAHs (16 compounds) and PCBs (18 congeners) using an Agilent 7890B gas chromatograph (Agilent Technologies, Palo Alto, USA). Two Agilent J&W HP-5MS (5 % phenylmethyl-polysiloxane, Agilent Technologies) ultra-inert fused silica capillary columns with a total length of 30 m (two 15 + 15 m columns), a film thickness of $0.25~\mu m$ and 0.25~mm in diameter were used for the separations. The columns were joined by means of a purged joint to allow the application of the back-flushing technique that reduces background noise and extends the lifetime of the column. An ultra-inert glass wool inlet liner was used at the injection port at 250 °C, and injection (1.5 µl) was performed in pulsed mode without splitting. The gases used were supplied by Linde (Dublin, Ireland), with the carrier gas being helium 5.0 (99.999 % purity) at a constant flow rate of 1.5 ml/ min, and the collision gas nitrogen 6.0 (99.9999 % purity). The initial oven temperature of 80 °C was held for 1.8 min, then increased at a rate of 40 °C/min to 170 °C, then increased at a rate of 10 °C/min to 310 °C, and finally held for 3 min at 310 °C. The post-run back-off to clean the

column was set at 315 $^{\circ}$ C for 5 min at 5.8 ml/min for the first column, and the final run time at 21.05 min. An Agilent 7010 mass spectrometer (Agilent Technologies, Palo Alto, USA) was used for the identification and quantification of the compounds. This equipment was operated in multiple reaction monitoring (MRM) mode, with 24-time segments, cycle time between 300 and 600 ms and dwell time between 15 and 40 ms. Electron impact (EI) and transfer line ionization source temperatures were set at 280 $^{\circ}$ C, with a solvent delay of 3.7 min.

2.3. Quality assurance/quality control

2.3.1. Microplastics analysis

Strict measures were taken to avoid microplastic contamination of samples. Air circulation in the laboratory was minimized during sample preparation for KOH digestion, filtration and microplastic counting. White cotton lab coats and latex gloves were used during sample analysis. KOH-Solution was filtered through a stainless steel filter (mesh size: $25~\mu m$) prior to its use. Instruments were washed with ethanol and rinsed 3 times with pure water prior to use. Procedural blanks were run to determine background contamination during all the microplastics analysis (digestion, filtration and counting). Petri dishes remained sealed during sample storage to avoid any external contamination. However, we were not able to perform such stringent measures to completely guarantee the absence of airborne microfiber contamination during bird dissection. For this reason, in this study we did not consider microfibers in the counting of the residues and focused on the analysis of particles larger than 1 mm.

2.3.2. Analysis of chemical pollutants

Quality Control samples (QCs), blanks and calibration curve were prepared in chicken liver matrix free of the target analytes. This matrix had been used in the validation of the extraction method. A nine-point calibration curve covering the range 50–0.195 ng/ml and was prepared by spiking with the appropriate volume of working mix solutions 1 g of chicken liver homogenate and extracting it using the same procedure as in the samples. Similarly, QCs were prepared at a single concentration of 2.5 ng/ml in the same matrix and were injected every 25 samples. All samples, QCs, calibration points, and blanks were added $10~\mu l$ of P-IS mix solution at 100~ng/ml and were left to stand for 1~h in dark prior to extraction. Chromatographic and mass spectrometric conditions of the compounds and limits of detection (LODs) and

quantification (LOQs) in ng/ml of the extraction method have been added as supplementary material (Tables S2 & S3).

2.4. Statistical analyses

Normality and homoscedasticity of data were checked by Shapiro-Wilk and Levene's test, respectively. A statistical analysis was carried out to compare the liver concentrations of BDEs, OCPs, PCBs and PAHs with the plastic loads found in the digestive tract of the birds. Three groups were considered according to the load of plastics: "no", "low" (1–8 items) and "high" (9–23 items). The analyses were performed only on the two species with the largest sample size: Cory's shearwaters and yellow-legged gulls. Statistical analysis was performed using the R Version 4.0.5 with RStudio Version 1.4.1106. One-way ANOVA Test was applied to determine if there were significant differences (*P*-value < 0.05), and Tukey's post hoc for multiple comparisons when ANOVA Test indicated significant differences.

3. Results

3.1. Plastic ingestion

Plastic debris was found in 53 of the 88 birds analyzed, affecting 5 of the 14 species included in this study. The results were variable among the different species (Table 2). No microplastic contamination was found in the procedural blanks.

Among the Cory's shearwaters analyzed, plastic was found in 40 of 45 individuals (88.89 %). The mean number of items per individual of 7.22 (± 5.66) was the highest among the species studied. Likewise, the individual with the most plastic items reported among all birds was also a Cory's shearwater with 23 items. The majority of plastics reported in this species consisted of lines (75.08 %), although fragments also accounted for a notable proportion (21.23 %). Films and pellets were found less frequently (3.08 % and 0.62 % respectively). The predominant color was green (19.38 %), followed by blue (16.92 %) and white (14.77 %) (Fig. 2).

All five Madeiran storm-petrels examined contained plastic in their digestive tract (mean 5.60 ± 2.88 items per bird). In this case, most of the plastics were fragments (78.57 %), the rest being lines (14.29 %) and films (7.14 %). The most observed colors were white (25 %) and yellow (21.43 %) (Fig. 3).

With respect to the yellow-legged gulls, out of a total of 20 individuals, 6 of them (30 %) contained plastic. The mean number of items per individual was 0.55 \pm 1.05. In this species, equal proportions of lines and films were found (36.36 %), with the remainder being fragments (27.27 %) (Fig. 4). The predominant color was green (54.55 %). Moreover, only 1 of the 2 black-headed gulls studied contained plastic inside. In this case, it was a single red film. Finally, 6 transparent lines were found in the digestive tract of the cattle egret.

In contrast, no plastic was found in the other species: Leach's storm-

Table 2Frequencies of observation (FO), mean number of items, standard deviation (sd) and maximum number of items (max) detected between the different species.

Common name	Species	n	FO%	$\begin{array}{c} \text{Mean} \\ \pm \text{ sd} \end{array}$	$\begin{array}{l} \text{Mean} \\ \pm \ \text{sd}^{\text{a}} \end{array}$	Max
Cory's shearwater	Calonectris borealis	45	88.89	$\begin{array}{c} \textbf{7.22} \pm \\ \textbf{5.66} \end{array}$	$\begin{array}{c} 8.13 \pm \\ 5.36 \end{array}$	23
Madeiran storm- petrel	Oceanodroma castro	5	100	5.60 ± 2.88	5.60 ± 2.88	10
Yellow- legged gull	Larus michahellis	20	30.00	$\begin{array}{c} \textbf{0.55} \pm \\ \textbf{1.05} \end{array}$	$\begin{array}{c} 1.83\ \pm\\ 1.17\end{array}$	4
Black-headed gull	Chroicocephalus ridibundus	2	50.00	$\begin{array}{c} 0.50 \; \pm \\ 0.71 \end{array}$	1	1
Cattle egret	Bubulcus ibis	1	100	6.00	6.00	6

^a Includes only birds that ingested plastic.

petrel (n=1), Bulwer's petrel (n=3), Mediterranean gull (n=1), little egret (n=1), bar-tailed godwit (n=1), common sandpiper (n=2), sanderling (n=1), ruddy turnstone (n=1) and common moorhen (n=4).

Among all the birds sampled, 371 items were found. Thirty-four percent of the items were analyzed by FTIR to determine the type of polymer (Fig. 5). The predominant plastic type was polyethylene (PE) (70.87 %), followed by polypropylene (PP) (15.75 %) and ethylene-methacrylic acid copolymer (10.24 %). At lower levels, propylene-acrylic acid copolymer (2.36 %) and Nova-thene polyolefine (0.79 %) were detected.

3.2. Chemical pollutants

BDEs (7 congeners), PCBs (15 congeners), OCPs (8 substances) and PAHs (7 substances) were found. Detection frequencies and concentrations were variable among species (Table 3) (Figs. 6 to 9). Only one pollutant was detected in 100 % of the birds, PCB 153. However, other compounds were also detected in the vast majority of the animals: p,p'-DDE (95.45 %), hexachlorobenzene and PCB 138 (94.31 %), naphthalene (93.18 %), fluorene (92.04 %) and PCB 180 (82.95 %).

3.3. Statistical analyses

In the case of Cory's shearwaters, 24 specimens showed a "low" and 16 "high" plastic load, while no plastics were found in 5 specimens. In the yellow-legged gulls, 6 individuals presented a "low" load and the rest (14 birds) had no plastics in their digestive tract. The median concentrations of contaminants in shearwaters in which no plastic was detected ("no") were 0 ng/g (BDEs), 11.16 ng/g (OCPs), 1.93 ng/g (PCBs) and 18.83 ng/g (PAHs). In those showing a "low" load were 0 ng/g (BDEs), 1.66 ng/g (OCPs), 1.81 ng/g (PCBs) and 22.04 ng/g (PAHs). Finally, in "high" loaded shearwaters the values were 0 ng/g, 0.91 ng/g, 1.03 ng/g and 24.42 ng/g for BDEs, OCPs, PCBs and PAHs, respectively. Among the yellow-legged gulls, those with zero plastic load ("no") presented median values of 2.23 ng/g (BDEs), 93.84 ng/g (OCPs), 51.80 ng/g (PCBs) and 10.90 ng/g (PAHs), while those with "low" loadings showed median values of 5.87 ng/g, 29.12 ng/g, 55.03 ng/g and 12.94 ng/g for BDEs, OCPs, PCBs and PAHs, respectively. Statistical analyses did not show in any case significant differences between the concentrations of the different groups of contaminants and the plastic loads found in either species (see Figs. S1 & S2).

4. Discussion

4.1. Plastic ingestion

Plastic has become a ubiquitous pollutant in the marine environment in the last decades, estimating that between 4.8 and 12.7 million tons end up in the oceans every year (Jambeck et al., 2015) and becoming an evolutionary trap for wildlife (Santos et al., 2021). Globally, one of the groups most affected by plastic ingestion is the order *Procellariiformes*, where 91 of the 144 species within this taxon have been reported to have ingested plastic (Kühn and van Franeker, 2020).

In our study, the high frequency observed in Cory's shearwaters is similar to that reported by Rodríguez et al. (2012), which was 83.5 %, on the island of Tenerife (Canary Islands). The same applies to the mean number of items per bird (8.0 \pm 7.9). In addition, in both studies, the majority of the items found in this species were compatible with fishing-related debris (high proportion of lines). In fact, a good proportion of the macro-debris found on beaches in the Canary Islands comes from the fishing sector, such as nets and ropes (Gil Gamundi and Martínez-Gil Pardo de Vera, 2020; Herrera et al., 2022a), which tend to degrade generating microplastics (Welden and Cowie, 2017). On the other hand, there is quite a difference with respect to the predominant color, where in the samples analyzed by Rodríguez et al. (2012) >50 % of the items

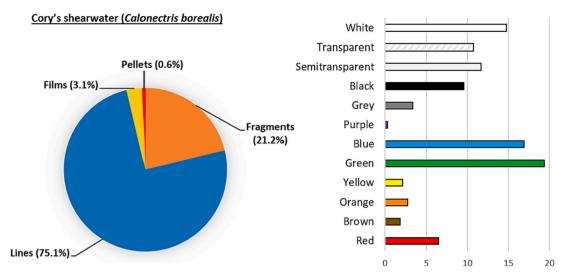


Fig. 2. Classification of plastics found in Cory's shearwaters according to their shape (left) and color (right) expressed in percentage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

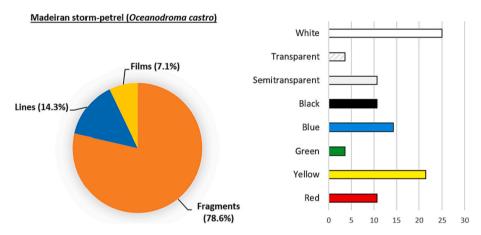


Fig. 3. Classification of plastics found in Madeiran storm-petrels according to their shape (left) and color (right) expressed in percentage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

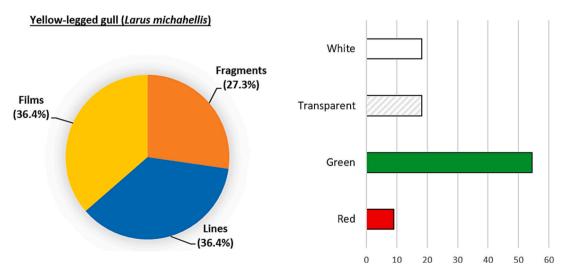


Fig. 4. Classification of plastics found in yellow-legged gulls according to their shape (left) and color (right) expressed in percentage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

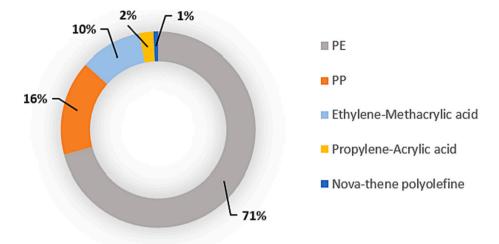


Fig. 5. Proportions of the different polymers determined by FTIR.

were white. Conversely, another study conducted on this species in Brazil reports a lower prevalence (23.7 %) and mentions hard plastics as the most frequent (Tavares et al., 2017).

In the case of the Madeiran storm-petrel, all the specimens analyzed presented plastic. Although there are reports of plastic ingestion in other species of the same genus (Oceanodroma) (e.g., Bond and Lavers, 2013; Youngren et al., 2018), to our knowledge, this would be the first report of plastic ingestion in this species. However, we consider it necessary to analyze more specimens of this species in the future, since the sample size available for this study was very limited. Both Madeiran stormpetrels and Cory's shearwaters feed in pelagic ecosystems, so the plastics found in their digestive tracts come from these environments. Plastic debris can remain in the gastrointestinal tract of birds for several months (Herzke et al., 2016; Ryan, 2015), and could have been ingested by birds at various points during their migration. While it is true, the Canary Current represents an important feeding ground for Procellariiformes species nesting on the islands (Ramos et al., 2019; Rodríguez et al., 2013). This wind-driven surface current, associated with the North Atlantic gyre, drags marine debris toward the Canary Islands (Baztan et al., 2014; Herrera et al., 2018). A study by Herrera et al. (2020) shows high concentrations of neustonic microplastics in the Canary Islands, reporting values of 1,007,872 items/km² and recording twice as many microplastics as zooplankton in dry weight in the waters north of Gran Canaria.

On the other hand, although no plastic residues were found in the Leach's storm-petrel specimen, nor in the three dissected Bulwer's petrel specimens, there is evidence of plastic ingestion in these species in other regions of the planet (Bond and Lavers, 2013; Krug et al., 2021; Laist, 1997).

In the case of the Laridae family, the yellow-legged gull was the second species with the largest sample size in this study. This species has been studied previously in other regions such as the Iberian Peninsula. Nicastro et al. (2018) reported a prevalence of 18.67 % in this species from the analysis of 75 specimens, with fragments and films being the most prevalent categories and light colors the most predominant. A lower prevalence was obtained by Basto et al. (2019), where the frequency was 10.48 %. The most prevalent category in that case was films and light-transparent colors the most predominant. Meanwhile, Lopes et al. (2021) analyzed the anthropogenic residues present in pellets regurgitated by this species to compare populations in natural and anthropized environments throughout their reproductive cycle. In their study, they show high prevalences in some anthropized areas (e.g. about 94 % in samples taken in a landfill) and lower prevalences in natural areas (e.g. in one of the two areas studied the prevalence was <10 %). In the Canary Islands, yellow-legged gulls are widely distributed along the coast, especially concentrated around fishing docks and frequently

visiting landfills. Most of the individuals that enter the Wildlife Recovery Center usually present symptoms compatible with botulism, probably after having been intoxicated in landfill areas (Montesdeoca et al., 2017). Therefore, the generalist nature of this species in terms of its diet and its relationship with landfills, give the yellow-legged gull a high risk of ingesting plastic in Gran Canaria.

Very few samples of other gull species (2 black-headed gulls and 1 Mediterranean gull) were available, since they are considerably less abundant in the Canary archipelago and generally limit their stay to the winter season. Like the yellow-legged gull, these species also frequent fishing docks and landfills, abounding in these places plastic debris that can potentially be ingested by the birds. In fact, although no plastics were found in the digestive tract of the Mediterranean gull analyzed, it is noteworthy that this animal was admitted to the Wildlife Rehabilitation Center with a hook stuck inside its upper digestive tract attached to a nylon. The animal underwent surgery, but died. Moreover, plastic ingestion has been reported in both species in the literature (Codina-García et al., 2013; Franco et al., 2019).

In this study, no plastic debris was found in any of the four wader species analyzed (common sandpiper, sanderling, ruddy turnstone and bar-tailed godwit). Although it is true that in this study there were very few specimens of these four species, since their admissions to the Wildlife Rehabilitation Center are very low. Likewise, these species have hardly been studied worldwide, with very few studies on microplastic ingestion in these species (Laist, 1997; Lourenço et al., 2017; Zhao et al., 2016). Taking into account that the tide line represents a crucial feeding area in these species and that it is also an area where considerable amounts of plastics that reach the coast are deposited (Herrera et al., 2018), they are birds quite exposed to these residues. Therefore, they should be included in future works.

In the case of herons (*Ardeidae*), 2 specimens were studied: 1 little egret and 1 cattle egret. Only in the latter plastic was visualized in its digestive tract, consisting of 6 transparent lines type items. Plastic ingestion in little egret has been reported by Toda et al. (1994) with a rather low prevalence (2/27 animals analyzed contained plastic). Likewise, there are also reports in cattle egret (Zhao et al., 2016). This last species, in general, does not frequent the aquatic environment as much as the little egret, but it tends to concentrate and feed around landfills and urban areas (Garrido et al., 2012).

Finally, plastics were not observed in any of the 4 dissected common moorhen specimens. Despite the small sample size, it should be noted that plastic ingestion in this species has not been reported in the scientific literature to date. Although, if it has been reported in other species of the family *Rallidae* such as in the common coot (*Fulica atra*) (Gil-Delgado et al., 2017) or in the clapper rail (*Rallus crepitans*) (Weitzel et al., 2021).

(continued on next page)

 Table 3

 Median concentrations (in ng/g) and frequencies (between parentheses) of organic pollutants detected in birds of Gran Canaria. Pollutant concentrations are calculated per gram of liver sample.

Pollutant	Actitis hypoleucos $(n=2)$	Arenaria interpres $(n=1)$	Bubulcus ibis $(n=1)$	Bulweria bulwerii (n = 3)	Calidris alba (n = 1)	Calonectris borealis $(n = 45)$	Chroicocephalus $ridibundus$ $(n = 2)$		Gallinula chloropus (n = 4)	$\begin{array}{l} \textit{Larus} \\ \textit{melanocephalus} \\ (n=1) \end{array}$	Larus $michahellis$ $(n=20)$	$\begin{array}{c} \textit{Limosa} \\ \textit{lapponica} \\ (n=1) \end{array}$	Oceanodroma castro $(n = 5)$	Oceanodroma leucorhoa (n = 1)
Acenaphthalene						0.29 (4.44)								
Acenaphthene		0.31 (100)				0.29 (4.44)								
Anthracene		(===)				0.59 (4.44)					0.29 (5)			
BDE 100	0.82 (100)		0.02 (100)		0.09 (100)	(1.11)	0.66 (100)	0.84 (100)		4.53 (100)	0.42 (80)		0.57 (80)	
BDE 153	2.12 (50)		1.07 (100)		(100)		1.15 (100)	0.26 (100)		4.57 (100)	0.78 (55)		0.71 (20)	
BDE 154	0.38 (100)		(100)				0.57 (50)	0.39 (100)		6.05 (100)	0.42 (40)		0.75 (80)	
BDE 183	(100)						(30)	(100)		(100)	2.89		(80)	
BDE 47	1.51 (100)				0.22 (100)	0.20 (4.44)	2.78 (100)	0.55 (100)		12.32 (100)	1.05 (80)		0.22 (40)	
BDE 85	(100)				(100)	(4.44)	(100)	(100)		(100)	0.10 (5)		(40)	
BDE 99	1.81 (100)				0.58 (100)	0.04 (2.22)	1.77 (100)	0.36 (100)	0.26 (25)	3.13 (100)	1.31 (85)		0.24 (80)	
o,p'-DDD	(100)			0.94 (66.67)	0.48 (100)	0.82 (2.22)	7.49 (50)	(100)	0.36 (25)	(100)	(65)	0.19 (100)	3.30 (100)	0.47 (100)
p,p'-DDE	148.50 (100)	0.12 (100)	353.56 (100)	6.95 (100)	14.87 (100)	0.90 (91.11)	413.59 (100)	67.63 (100)	49.67 (100)	670.58 (100)	52.46 (100)	22.00 (100)	85.49 (100)	35.27 (100)
p,p'-DDT	(100)	(100)	(100)	0.17 (33.33)	(100)	(91.11)	1.28 (50)	(100)	(100)	(100)	(100)	(100)	1.09	(100)
Dieldrin	2.32 (50)	10.68 (100)	265.44 (100)	(55.55)		5.39 (4.44)	236.99 (50)	4.00 (100)		50.05 (100)	19.93 (55)		44.47 (80)	
Fluoranthene	(00)	(100)	(100)			1.48 (13.33)	(00)	(100)		(100)	1.46		(00)	
Fluorene	1.03 (100)	0.64 (100)	0.54 (100)	0.93 (100)	0.77 (100)	0.67 (95.56)	0.76 (100)	0.27 (100)	0.76 (75)		0.60 (95)		0.58 (80)	0.53 (100)
Hexachlorobenzene	5.54 (100)	3.33 (100)	(===)	0.68 (100)	(===)	0.38 (95.56)	28.34 (100)	2.68 (100)	0.31 (75)	131.99 (100)	1.17 (100)	2.62 (100)	16.95 (100)	1.85 (100)
Hexaclorociclohexano- beta	(===,	(===)		(===)		(5.70 (50)	(===)	(, -)	(223)	(===,	()	3.82 (20)	(200)
Methoxychlor	5.59 (50)	7.10 (100)		5.37 (66.67)	1.32 (100)	4.77 (20)	18.39 (50)				9.84 (50)		3.44 (60)	
Mirex	1.84 (50)	, ,		(**************************************	,	2.12 (8.89)	15.71 (50)			22.35 (100)	2.39 (45)		5.60 (80)	
Naphthalene	10.88 (100)	13.68 (100)	39.43 (100)	15.47 (100)	30.71 (100)	21.83 (95.56)	23.05 (50)	15.56 (100)	13.76 (100)	2.63 (100)	11.92	24.90 (100)	28.87 (80)	13.66 (100)
PCB 101	6.91 (100)	/	,	0.57 (33.33)	/	0.78 (13.33)	24.88 (50)	5.78 (100)	/	90.45 (100)	2.27 (60)	1.52 (100)	5.79 (100)	1.92
PCB 105	5.74 (100)		0.17 (100)	0.62 (33.33)		0.28 (11.11)	7.54 (100)	8.35 (100)	0.77 (75)	26.57 (100)	1.40 (60)	1.04 (100)	2.95 (100)	0.99 (100)
PCB 118	11.59 (100)		(100)	1.90 (33.33)		0.95 (13.33)	24.63 (100)	19.10 (100)	0.56 (75)	99.33 (100)	4.37 (55)	2.25 (100)	9.07 (100)	3.16 (100)
PCB 126	0.70 (100)			(55.55)		0.05 (2.22)	0.33 (50)	0.38 (100)	(70)	3.32 (100)	0.53	(100)	0.49 (80)	0.93
PCB 138	119.18		4.97 (100)	1.62 (100)	1.79	0.53	85.27	128.15 (100)	2.94 (100)	467.44	15.89 (100)	10.74 (100)	50.58 (100)	19.67

Table 3 (continued)														
Pollutant	Actitis	Arenaria	Bubulcus ibis	Bulweria	Calidris	Calonectris bore alis	Chroicocephalus ridibundus	Egretta	Gallinula	Larus melanocenhalus	Larus michahellis	Limosa Japponica	Oceanodroma	Oceanodroma
	(n = 2)	(n = 1)	(n = 1)	(n = 3)	(n = 1)	(n = 45)	(n=2)	(n = 1)	(n = 4)	(n = 1)	(n = 20)	(n = 1)	(n = 5)	(n = 1)
PCB 153	225.05	0.29	3.33	3.05	2.42	0.58	78.46	57.94	2.53	450.98	15.28	3.96	28.16	10.91
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
PCB 156	13.69			0.18		0.71	4.01	9.78	69.0	20.06	1.51	0.51	1.63	0.34
	(100)			(33.33)		(6.67)	(100)	(100)	(25)	(100)	(09)	(100)	(80)	(100)
PCB 157	2.55					0.32	1.15	1.94		3.63	0.49	0.25	0.59	
	(100)					(4.44)	(100)	(100)		(100)	(20)	(100)	(80)	
PCB 167	8.42			0.65		0.91	9.14	11.29	0.56	46.41	1.99	0.50	2.97	1.49
	(100)			(33.33)		(13.33)	(100)	(100)	(75)	(100)	(75)	(100)	(100)	(100)
PCB 169										0.21	0.64		0.76	0.14
										(100)	(10)		(20)	(100)
PCB 180	295.25		9.82	1.65	1.27	0.48	40.22	96.40	2.44	266.67	14.29	2.25	16.23	89.9
	(100)		(100)	(100)	(100)	(68.89)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
PCB 189	6.32						0.64	1.87	0.21	3.69	0.51	0.56	0.38	
	(100)						(100)	(100)	(25)	(100)	(20)	(100)	(09)	
PCB 28	0.32						1.32	1.07		7.37	0.29		0.78	0.07
	(100)						(100)	(100)		(100)	(55)		(80)	(100)
PCB 52	0.63	0.34	0.32	0.36	0.23	0.33	2.78	1.09	0.23	12.77	0.57	0.48	0.42	0.20
	(100)	(100)	(100)	(66.67)	(100)	(33.33)	(100)	(100)	(25)	(100)	(09)	(100)	(100)	(100)
PCB 77											2.49			
											(5)			
Phenanthrene							0.11				0.14			
							(20)				(15)			

4.2. Chemical pollutants

In our study, we focused on the analysis of four groups of chemical pollutants commonly associated with plastic debris present in marine environments (PCBs, PAHs, BDEs and OCPs) (Camacho et al., 2019). Fifteen congeners were detected in the group of PCBs among all samples. PCB 153 was detected in all the birds studied and very high detection frequencies were obtained for PCB 138 and PCB 180, present in 94.31 % and 82.95 % of the samples, respectively. The highest levels of PCBs were detected in one yellow-legged gull specimen (2708.16 ng/g). However, the median value among individuals of this species was considerably lower (51.80 ng/g). Also noteworthy were the rather high concentrations in the only Mediterranean gull specimen (1498.89 ng/g) and in one of the two common sandpiper specimens (1251.06 ng/g). Regarding PAHs, seven compounds were detected. Among these, naphthalene and fluorene presented very high detection frequencies (93.18 % and 92.04 % respectively). In contrast, the detection frequencies of the other pollutants (anthracene, acenaphthalene, acenaphthene, fluoranthene and phenanthrene) were lower than 10 %. However, the detection of PAHs was positive in all birds. The animal with the highest concentration of PAHs was a Cory's shearwater (50.05 ng/g). In this species, PAHs were the group of contaminants with the highest proportion, with a median value of 22.48 ng/g. PAHs were also the group of pollutants with the highest concentration detected in the sanderling and the bar-tailed godwit analyzed and also the group with the highest median concentration in Bulwer's petrels. With respect to BDEs, seven congeners were detected within this category (#47, 85, 99, 100, 153, 154, 183). BDE 99 was the one with the highest frequency of detection (34.09 %). Within the 10 % of the animals with the highest concentrations of BDEs, we found specimens of yellow-legged gull, Mediterranean gull and black-headed gull, with concentrations between 40.93 and 10.07 ng/g. On the other hand, no BDEs were detected in four of the species studied (ruddy turnstone, Bulwer's petrel, bar-tailed godwit and Leach's storm-petrel). Eight contaminants of the OCPs group (p,p'-DDE, p,p'-DDT, p,p'-DDD, dieldrin, hexachlorobenzene, hexachlorocyclohexane beta, methoxychlor and mirex) were detected. All animals sampled, with the exception of one Cory's shearwater, showed detectable levels of OCPs. The metabolite p,p'-DDE had a frequency of detection of 95.45 % between all samples and it resulted to be the component with the highest concentrations among the OCPs and one of the highest among all pollutants (Table 3). The maximum p,p'-DDE value detected corresponded to a vellow-legged gull, corresponding to 1177.21 ng/g, being this specimen the bird with the highest concentration of OCPs (1252.86 ng/g). It is worth mentioning that recently, very high levels of DDT metabolites (mainly p,p'-DDE) have been reported in soils of the Canary Islands archipelago, being even considerably higher than those reported in other European countries (Acosta-Dacal et al., 2022). Another organochlorine compound detected in birds with a high frequency was hexachlorobenzene (94.31 %). It should also be noted that, in our study, OCPs constituted the group with the highest proportion among the common moorhens, black-headed gulls and Madeiran storm-petrels analyzed, as well as being the predominant group in the cattle egret specimen studied.

Several studies have reported associations between plastic ingestion by seabirds and the presence of chemical pollutants such as PCBs (Ryan et al., 1988; Yamashita et al., 2011), PBDEs (Neumann et al., 2021; Tanaka et al., 2013) and phthalates (Padula et al., 2020). Other works have described the presence of contaminants associated with plastics ingested by seabirds, such as PCBs, OCPs, UV stabilizers, brominated flame retardants and styrene oligomers (Colabuono et al., 2010; Tanaka et al., 2019). Also, the presence of plastic additives has been found in seabirds from different parts of the world (Yamashita et al., 2021). Finally, the transfer of additives from plastics to seabirds has been experimentally demonstrated (Tanaka et al., 2020). In our samples, we found numerous contaminants that have also been detected with a high frequency associated with microplastics in the Canary Islands (Camacho

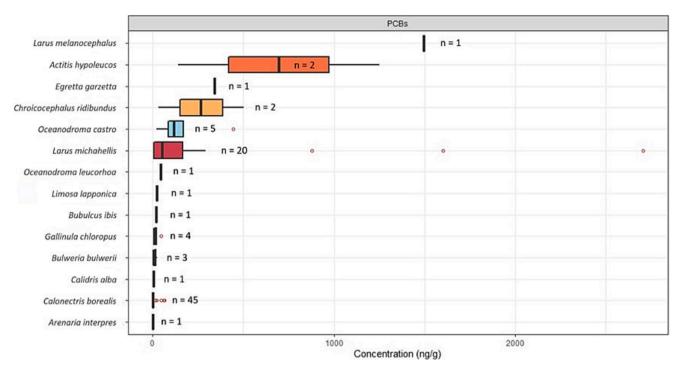


Fig. 6. Concentrations of the sum of PCBs (in ng/g of liver) in the different species analyzed. The central line of each box indicates the median, the width of the box shows the interquartile range, and the extreme lines show the highest and lowest values, excluding outliers (red dots). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

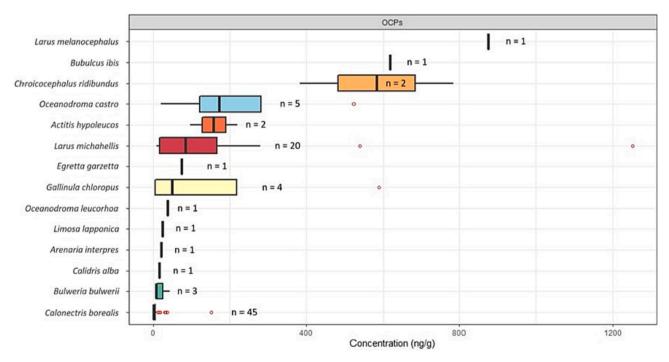


Fig. 7. Concentrations of the sum of OCPs (in ng/g of liver) in the different species analyzed. The central line of each box indicates the median, the width of the box shows the interquartile range, and the extreme lines show the highest and lowest values, excluding outliers (red dots). All animals were considered for this graph, including those with zero value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

et al., 2019). For example, 10 of the 15 PCB congeners found in birds (#52, 77, 101, 105, 118, 138, 153, 156, 167 and 180) have also been found associated with microplastics from the Canary Islands. Besides, in plastics collected from a beach in Gran Canaria, Camacho et al. (2019) found high concentrations of PCB 153 (42.59 ng/g in pellets and 15.92 ng/g in fragments) and PCB 180 (52.45 ng/g in pellets and 14.03 ng/g in

fragments). All PAHs found in birds have also been found in microplastics from beaches in the Canary Islands, being also naphthalene and fluorene detected with very high frequency (96.8 % and 100 %, respectively). Finally, 3/7 BDEs congeners (#47, 99 and 100) and 4/8 OCPs (dieldrin, hexachlorobenzene, mirex and p,p'-DDE) found among our samples have also been reported by Camacho et al. (2019), with

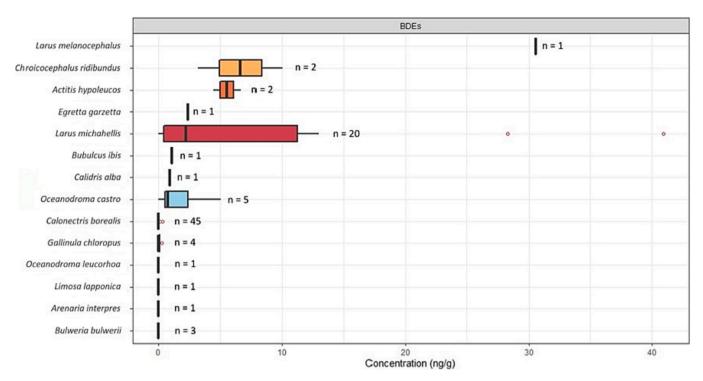


Fig. 8. Concentrations of the sum of BDEs (in ng/g of liver) in the different species analyzed. The central line of each box indicates the median, the width of the box shows the interquartile range, and the extreme lines show the highest and lowest values, excluding outliers (red dots). All animals were considered for this graph, including those with zero value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

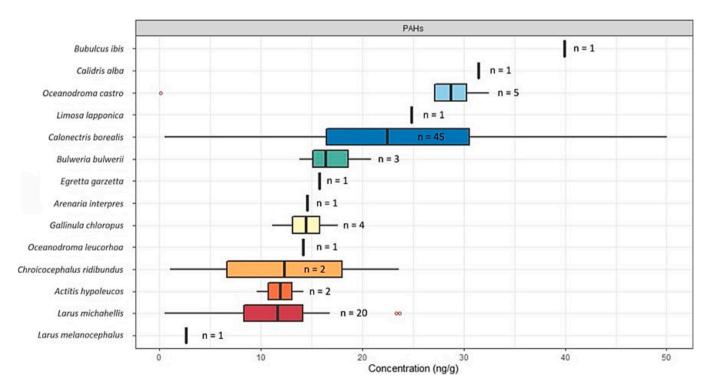


Fig. 9. Concentrations of the sum of PAHs (in ng/g of liver) in the different species analyzed. The central line of each box indicates the median, the width of the box shows the interquartile range, and the extreme lines show the highest and lowest values, excluding outliers (red dots). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

hexachlorobenzene being detected in $100\,\%$ of the plastic samples and p, p'-DDE being the contaminant with the highest concentrations (reaching median levels of up to $56.0\,$ ng/g on a beach in Gran Canaria). However, our statistical analyses did not show significant differences between the

liver concentrations of the different groups of pollutants (POPs and PAHs) and the plastic loads found in the digestive tract of the birds (see Figs. S1 & S2). This seems to indicate that the main route of entry of these contaminants would be directly through their diet. Other authors

have also found no significant correlations between tissue concentrations of POPs and plastic loads (Herzke et al., 2016; Trevail et al., 2014). In the case of Herzke et al. (2016), they showed with their analyses that it is more likely that plastic does not act as a vector of POPs to birds, but as a "passive sampler" reflecting the POPs profiles of ingested prey.

Previous studies have reported and compared the levels of PCBs and DDTs present in Mediterranean and Atlantic Procellariiformes, including samples taken in the Canary Islands (Roscales et al., 2010, 2011b). In general terms, the authors observed spatial variations (higher levels of contamination in Mediterranean vs. Atlantic birds), as well as higher levels of contamination in petrels vs. shearwaters, suggesting that diet could explain these differences (higher accumulation of contaminants in the mesopelagic vs. epipelagic zone). Likewise, dietary differences could also explain the variations in PAHs profiles between shearwaters and petrels in these areas, although the rapid metabolism of these compounds by seabirds could mask geographical differences, being these species poor indicators of pelagic PAHs (Roscales et al., 2011a). In our samples, liver PAHs levels found in Cory's shearwaters appear to be higher than those reported by Roscales et al. (2011a) in the Canary Islands, where the range was between 1.02 and 17.1 ng/g from the analysis of 12 specimens. Although the PAHs burdens in bulwer's petrels reported by Roscales et al. (2011a) appear to be higher than ours, these data should be treated with caution since we only analyzed 3 specimens. In our results, if we compare the loads of the different pollutants between the two species with the largest sample size (Cory's shearwater and yellow-legged gull), it seems that gulls reflect higher loads of BDEs, PCBs and OCPs. This finding could be explained by differences in feeding strategy between the two species. BDEs are widely used as additives to plastics and seabirds could be exposed to these substances by feeding in urban environments and landfills or by feeding in areas of plastic waste accumulation (Gentes et al., 2015; Sühring et al., 2022; Verreault et al., 2018). Particularly, bird feeding in waste management facilities has been associated with exposure to highly brominated congeners such as deca-BDE (Gentes et al., 2015; Verreault et al., 2018). Although the methodology applied in our study is not able to detect the latter compound, higher brominated compounds were detected in gulls (e.g., BDE #153, 154 and 183) compared to shearwaters. In the latter species only BDE-47 and BDE-99 were detected, both with low detection frequency. Considering that gulls are frequent visitors to landfills and other anthropogenic areas, as well as the low plastic ingestion rate found in the analyzed gulls with respect to shearwaters, it is most likely that the differences in concentrations in both species are due to differences in feeding ecology. These differences in feeding strategies could also explain the higher PCBs and OCPs levels in gulls, since landfills are potential sources of POPs discharges to the environment (Weber et al., 2011). Levels of PAHs, however, are apparently higher in shearwaters. This could indicate increased pollution in shearwater feeding areas. Fuel spills are important sources of PAHs release into the marine environment (Pérez et al., 2008), although, no major spills have been detected in the islands since the Oleg Naydenov incident in 2015, which caught fire in the south of Gran Canaria after refueling 1400 tons of fuel. Therefore, the possible pathways of entry of these pollutants into the marine food chains of the archipelago should be further analyzed and monitored.

Finally, we consider that it is necessary to continue monitoring the impacts of plastic pollution and chemical pollutants on seabirds. Likewise, the transfer of chemical pollutants mediated by the ingestion of plastics along the marine food chain should continue to be studied, since the ingestion of plastics occurs practically from the beginning of the marine food chain (Cole et al., 2013; Desforges et al., 2015) and this could contribute to the biomagnification phenomena of certain pollutants. Thus, taking into account the declines in seabird populations worldwide, the conservation of these species could be better managed.

CRediT authorship contribution statement

Alberto Navarro: experimental investigation, formal analysis and writing. Alicia Herrera: supervision, conceptualization, experimental investigation and formal analysis. Andrea Acosta-Dacal, Octavio Pérez Luzardo and Ana Macías-Montes: analysis of chemical pollutants, experimental investigation, methodology. Ico Martínez and May Gómez: resources, review & editing, supervision. Alejandro Suárez-Pérez: resources. Jorge Felipe de la Rosa: experimental investigation. All authors contributed to the acquisition of the data, review the manuscript and approved the final version.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.marpolbul.2022.114434.

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