Marine Pollution Bulletin 173 (2021) 113105

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Microplastics, bisphenols, phthalates and pesticides in odontocete species in the Macaronesian Region (Eastern North Atlantic)

Tania Montoto-Martínez [a,*], Jesús De la Fuente [b], Raquel Puig-Lozano [b], Nuno Marques [c], Manuel Arbelo [b], José Joaquín Hernández-Brito [a,d], Antonio Fernández [b], María Dolores Gelado-Caballero [a]

[a] *Grupo de Investigación en Tecnologías, Gestión y Biogeoquímica Ambiental (TGBA), Departamento de Química, Universidad de Las Palmas de Gran Canaria (ULPGC), Edificio de Ciencias Básicas, Campus Universitario de Tafira, 35017, Las Palmas, Spain*

[b] *Veterinary Histology and Pathology, Institute of Animal Health and Food Safety (IUSA), Centro Atlántico de Investigación de Cetáceos, Universidad de Las Palmas de Gran Canaria (ULPGC), Campus Universitario de Montaña de Cardones, 35415, Las Palmas, Spain*

[c] *Museu da Baleia da Madeira, Canical, Madeira, Portugal*

[d] *Plataforma Oceánica de Canarias (PLOCAN), Carretera de Taliarte s/n, 35200, Telde, Gran Canaria, Spain*

ARTICLE INFO

Keywords:
Microplastics
Organic persistent contaminants (OPCs)
MicroRaman
Odontocete
Protocol
Macaronesia

ABSTRACT

The gastrointestinal contents of twelve individuals from six odontocete species that stranded between 2018 and 2019 in the Macaronesian Region (Eastern North Atlantic) were examined for the presence of marine debris. In addition, concentrations of eleven organic persistent contaminants (nonylphenols, bisphenols, phthalates and pesticides) were analysed in muscle samples by liquid chromatography. No particles larger than 5 mm were found, except for two plastic labels that were found on the same dolphin. On the contrary, all animals contained microplastics of diverse sizes, most of them being fibres (98.06%, n = 708). The predominant detected pollutants were bisphenols (4–984 ng/g) and DEHP (102–1533 ng/g). Also, except for two individuals, all animals had pesticide levels in their tissues. This work has allowed the establishment of a protocol for the study of microplastic ingestion in cetaceans, and tests the potential of microRaman to improve the understanding of microplastic alteration processes.

1. Introduction

Finding its way to the environment through littering and terrestrial runoff, the vast quantity of discarded plastic that is increasingly entering the oceans on an unprecedented scale emerges as an important human environmental perturbation. As such, marine debris has been identified as a global problem alongside with other key issues of our time. It can impact biodiversity in a number of ways, and its effects may vary depending on the type and size of the debris and the organisms that encounter it.

1.1. Marine mammals and plastic ingestion

Marine mammals are considered important indicators for marine ecosystem health, particularly in relation to pollution. Given their long life expectancy, their tendency to feed at high trophic levels and their unique fat stores that can serve as depots for anthropogenic toxins, these animals are considered important sentinels for marine pollution (Bossart, 2011).

The impact of marine debris on these animals has been widely documented and has grown rapidly over the last decade (Provencher et al., 2017). In all cases, ingestion and entanglement are highlighted as the main deleterious interactions, with plastic being the major material involved. Previous studies have documented very similar frequency of occurrence for ingestion of debris by marine mammals, with, in particular, percentages for ingestion by mysticeti and odontoceti of 53.8% and 61.5% respectively, according to Kühn et al. (2015).

Knowledge on plastic ingestion by cetaceans is difficult to obtain (Burkhardt-Holm and N'Guyen, 2019). On top of that, evaluating the severity of impacts that may be physical and/or toxicological poses an

---

* Corresponding author.

E-mail addresses: tania.montoto@ulpgc.es (T. Montoto-Martínez), jesus.delafuente@ulpgc.es (J. De la Fuente), raquel.puig101@alu.ulpgc.es (R. Puig-Lozano), nunomarques@museudabaleia.org (N. Marques), manuel.arbelo@ulpgc.es (M. Arbelo), joaquin.brito@plocan.eu (J.J. Hernández-Brito), antonio.fernandez@ulpgc.es (A. Fernández), maria.gelado@ulpgc.es (M.D. Gelado-Caballero).

https://doi.org/10.1016/j.marpolbul.2021.113105
Received 12 July 2021; Received in revised form 21 October 2021; Accepted 23 October 2021

additional challenge (Puig-Lozano et al., 2018). Moreover, there is a wide range of sub-lethal effects such as injuries within the gastrointestinal tract, malnutrition, reduced reproduction, growth and/or longevity, etc.(Panti et al., 2019), that add up to the more evident ones.

Stranded cetaceans represent a significant opportunity to study the interaction of marine megafauna with plastic debris and, therefore, several studies have addressed this issue from this opportunistic access to deceased stranded animals. Plastic ingestion reports from necropsies have been performed on several cetacean species up to date. Yet, very little is known about the presence of microplastics in higher trophic level species such as cetaceans (Moore et al., 2020). Until recently, studies focused on particles larger than 2.5 cm, and were not able to assess the microlitter presence. This still remains a challenging task due to large gastrointestinal content volumes and the difficulties of sampling.

Now, a few studies have tackled microplastic ingestion in particular, including reports of microplastics in single species, mysticete (Besseling et al., 2015; Garcia-Garin et al., 2021; Lusher et al., 2015) and odontocete (Xiong et al., 2018; Zhu et al., 2019), a number of a particular odontocete species (Hernández-González et al., 2018; Moore et al., 2020; Novillo et al., 2020), or a set of different stranded species over a period of time, as is the case of Lusher et al. (2018), Nelms et al. (2019) and the present study.

1.2. *Organic persistent contaminants*

Plastic debris may be a source of chemical contaminants into pelagic and benthic marine habitats (Teuten et al., 2007). Plastics accumulate a complex mixture of these contaminants present in the surrounding seawater, that adds to the cocktail of chemicals already present from manufacturing. These include different additives such as plasticizers, antioxidants, flame-retardants and UV-stabilizers and, in some cases make up a large proportion of the plastic product (Rochman, 2015). These substances have a potential for unwanted geophysical and/or biological effects.

The rapidly increasing global production of chemicals, and the expanding worldwide distribution as chemical products or in consumer goods is generating a "chemical intensification" where plastic polymers that degrade to microplastics represent an important source among the so called novel entities by Steffen et al. (2015). Within the chemical production sectors, the chemicals used in textile production represent a high percentage expected to grow at a compound annual growth rate of 4.5% from 2019 to 2025 (Grand View Research, 2019). These might come in the way as pesticides (used in natural fibre production and dyes), flame retardants or performance-enhancing coatings such as water repellents or fire retardants (UNEP, 2011). Phthalates, for example, may constitute up to 50% of the total weight of PVC plastics (Rochman, 2015). Di-2-ethylhexyl phthalate (DEHP) is commonly used as plastic coating such as water proof or anti-pilling, whereas nonyl phenol ethoxylates (NPEs) are frequently used in detergents and auxiliaries, with consequent release to water (UNEP, 2011).

A search of the literature revealed several studies with evidences suggesting chemical contaminants transfer from plastic debris to marine animals. Field studies and monitoring indicates that interactions between marine litter and a mixture of chemical compounds are of significance (Panti et al., 2019) and research has identified plastics as a vector for toxic trace elements in the environment, posing chemical toxicity concerns (Bradney et al., 2019).

1.3. *Research needs in the field*

Concerning plastic ingestion by marine megafauna, standardized techniques have been proposed by Provencher et al. (2017) in order to facilitate spatial and temporal comparisons between and among species, and to walk towards a more cohesive approach by the scientific community. Panti et al. (2019) also considered that a standardized, simple and cost-effective protocol should be implemented to allow the analysis of samples for the presence of microlitter to be performed in a comparable and transparent way. Moreover, there is still very little scientific understanding of the potential impacts of microplastics on cetaceans (IWC, 2020), and even less about the extent of biomagnification of plastic-related chemicals in higher trophic level animals, or how these contaminants might be impacting marine organisms (compared to other sources of chemical contamination in the environment).

Being aware of the predicted increase for the flux of plastics in the oceans (Jambeck et al., 2015) and that 24% of the 90 cetacean species recognized by the International Union for Conservation of Nature are assigned to a threatened category (i.e. Critically Endangered, Endangered or Vulnerable) (IUCN, 2020), we consider that delving into the effects of plastic ingestion on cetaceans is a crucial issue. Furthermore, this study was carried out in an area of great ecological importance for marine mammals, with high rates of cetacean diversity reported in numerous studies, as Correia et al. (2020) compile in their recent publication. In particular, the Canary Islands represent a major hotspot in European waters with 30 cetacean species (7 mysticetes and 23 odontocetes) out of the 90 described worldwide recorded in the archipelago (Arbelo et al., 2013; Díaz-Delgado et al., 2018; Herrera et al., 2021). The area has ideal oceanographical characteristics for both tropical and temperate-water cetaceans, as the oligotrophic waters of the open ocean coexist with nutrient-rich waters of the coastal upwelling caused by the Canary Current (Fernández et al., 2009).

Taking advantage of the opportunity provided by cetacean stranding nets to study the interaction of marine litter with cetaceans, and the previous multidisciplinary experience of the teams involved in this work, this study investigates (1) the level of plastic ingestion in stranded cetaceans (with a especial focus on microplastics, sieving the gastrointestinal contents down to 200 μm), (2) the concentrations of organic persistent contaminants in the skeletal muscle of these same cetaceans. Thus, this study aims to contribute to these research gaps by implementing a protocol that facilitates large-scale comparisons of plastic ingestion and provides baseline data for future research on the potential ecotoxicological effects of the ingestion of these particles.

2. Material and methods

2.1. *Specimens and sampling sites*

We examined the entire gastrointestinal tract (GIT), from oesophagus to anus, of 12 individuals from 6 odontocetes species that stranded between 2018 and 2019 along the coasts of Madeira and the Canary Islands, in the Macaronesian Region (Eastern North Atlantic). The list of specimens is reported in Table 1, and their distribution is shown in Fig. 1. All of the species studied are included in the Spanish Catalogue of Threatened Species, with two listed in the "vulnerable" category (BOE (Official Bulletin of the Spanish State), 2011).

Animals were stored at −20 °C until processing, and dissections were carried out in a necropsy facility at the Institute for Animal Health and Food Safety, at Las Palmas de Gran Canaria University ($n = 10$), and the Museu da Baleia facilities ($n = 2$). In all of them, the gastrointestinal tracts were extracted for further investigation concerning microplastic (MP) contents at the laboratories of the *Environmental Technologies, Management and Biogeochemistry research group* from the University of Las Palmas de Gran Canaria (ULPGC). Skeletal muscle samples were sent to the Department of Analytical Chemistry at the Faculty of Science of the National Distance Education University (UNED), in Madrid, for the determination of organic persistent contaminants.

2.2. *Gastrointestinal content extraction*

All the animals included in the present study were examined and necropsied according to standard procedures (Ijsseldijk et al., 2019). Gastrointestinal tracts were transferred to a clean stainless steel necropsy table, where they were washed externally with tap water and cut

Table 1

List of specimens analysed in the present study. All of the species are included in the Spanish Catalogue of Threatened Species, two of them, marked with an asterisk, under the "vulnerable" category. Location Stranded: Madeira (MA), Canary Islands (CI). Sexual development (SD): I, immature; M, mature. Body condition (BC): G, good; M, moderate; P, poor; VP, very poor. Decomposition condition categories (DCC): 1, extremely fresh carcass, just dead; 2, fresh carcass; 3, moderate decomposition; 4, advanced decomposition; 5, mummified or skeletal remains.

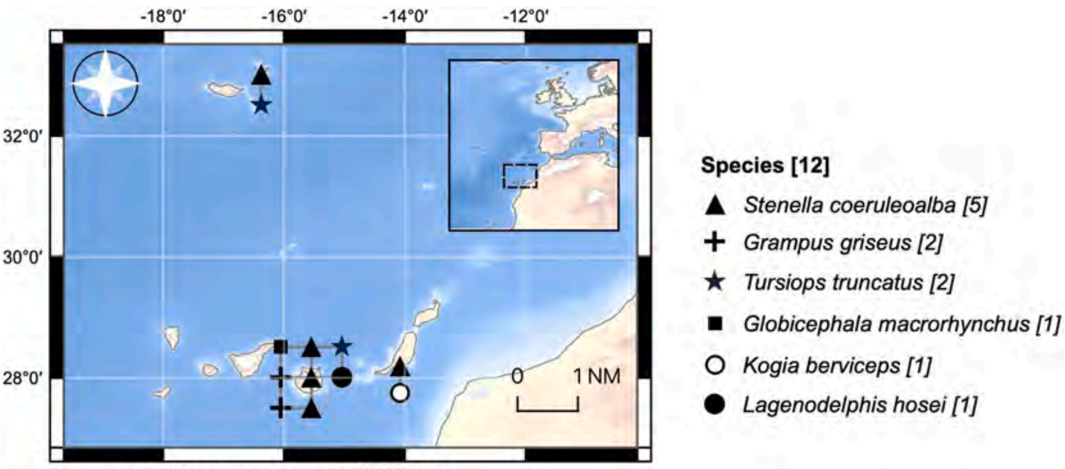
Id	Date	Location stranded	Species	Sex	Length (cm)	SD	BC	DCC	Stomach content	Histopathological findings	Pathological entity
42	3/8/18	Porto Santo (MA)	**T. truncatus*	♂	243	I	P	2	No	Severe parasitic bronchopneumonia; parasitic gastritis; lymphoplasmacytic enteritis; multiorgan parasitic disease; leukocytosis and intravascular coagulation.	Associated with significant loss of nutritional status.
43	6/6/18	Porto Santo (MA)	*S. coeruleoalba*	♀	215	M	P	2	No	Nonsupurative meningoencephalitis and radiculitis in cranial nerves; lymph node hyperplasia; leukocytosis and intravascular coagulation; hyaline casts in renal tubules.	Associated with significant loss of nutritional status.
920	4/7/18	Playa de Guayedra (CI)	**G. macrorhynchus*	♂	390	M	P	1	No	Active stranding pathology; severe pyogranulomatous laryngitis; multiorgan parasitic disease.	Associated with significant loss of nutritional status.
933	22/9/18	Playa del Águila (CI)	*S. coeruleoalba*	♂	184	I	M	1	No	Nonsuppurative meningoencephalitis; multiorgan epithelial hyperplasia and necrosis; lymphoid depletion and necrosis; leukocytosis and intravascular coagulation; active stranding pathology.	Associated with significant loss of nutritional status.
934	25/9/18	Pozo Izquierdo (CI)	**T. truncatus*	♀	161	I	VP	4	Sand and small pieces of plastic debris.	Emaciation; mild interstitial lymphoplasmacytic bronchopneumonia.	Associated with significant loss of nutritional status.
939	20/10/18	Playa de Bocabarranco (CI)	*G. griseus*	♀	146	I	P	3	No	Leukocytosis and intravascular coagulation.	Associated with significant loss of nutritional status.
950	21/1/19	El Rincón (CI)	*S. coeruleoalba*	♀	195	M	M	3	No	Nonsuppurative encephalitis; multiorgan parasitic disease.	Associated with good nutritional status.
956	14/2/19	Costa Calma (CI)	*S. coeruleoalba*	♀	170	M	NA	4	Abundant squid lens and spines of fish.	Degenerative acute changes in skeletal muscle.	Not determined.
957	16/2/19	Playa del Roque (CI)	*K. berviceps*	♂	164	I	P	3	Moderate presence of fish bones.	Cervico-thoracic trauma; degenerative acute changes in skeletal muscle; leukocytosis and intravascular coagulation.	Associated with significant loss of nutritional status.
963	5/3/19	Punta Gaviota (CI)	*L. hosei*	♂	160	I	M	3	Moderate presence of otoliths and squid beaks.	Leukocytosis and intravascular coagulation.	Associated with good nutritional status.
977	5/4/19	Playa del Berriel (CI)	*G. griseus*	♀	198,5	I	VP	2	Moderate presence of otoliths, lens, and squid beaks.	Multiorgan epithelial hyperplasia and necrosis; lymphoid depletion and necrosis; leukocytosis and intravascular coagulation.	Associated with significant loss of nutritional status.
983	20/4/19	Playa de Bocabarranco (CI)	*S. coeruleoalba*	♂	164	I	M	3	Scarce presence of squid beaks.	Fractured rostrum; submandibular skin lineal lesions compatible with net impressions; degenerative acute changes in skeletal muscle; enfisema and alveolar edema; fishery devices attached to the carcass.	Interaction with fishing activities.

into different sections: oesophagus, stomach compartments, duodenal ampulla and intestines. A custom-made adaptation to the necropsy table was made so that it would be more functional for microplastic research. The drainage was connected to a set of three stacked stainless steel sieves (1000, 500 and 200 μm) where the washed stomach contents were retained after thorough GIT rinses (Fig. 2).

Each of the GIT sections was examined separately to their diagnostic evaluation. Fish otoliths and squid beaks were picked out for diet studies and the diagnosis for marine debris ingestion was in line with the recommendations of the latest International Whaling Commission Workshop on Marine Debris (2020). As described in detail in the protocol published by Montoto-Martínez et al. (2020b), once the full section was carefully examined, it was rinsed with plenty of water, scrubbing the inside of the epithelial walls, and trapping all material below 200 μm in the sieves attached to the necropsy table. The retained material was transferred to jars and transported to the laboratory for the digestion procedure. In between the different GIT sections inspections, the table was thoroughly cleaned and a new air control filter was placed next to the operation area.

2.3. Laboratory analysis

The retained material was digested in 10% potassium hydroxide (KOH) solution following Foekema et al. (2013) protocol to remove organic material. Once the material was degraded, the content was passed through Whatman® glass microfibre filters (Grade GF/F, 47



Source map: EMODnet Bathymetry WMS 1.3.0

Fig. 1. Study area in the Macaronesian Region (Eastern North Atlantic), indicating the islands on which each of the stranded cetaceans were found, and their species.

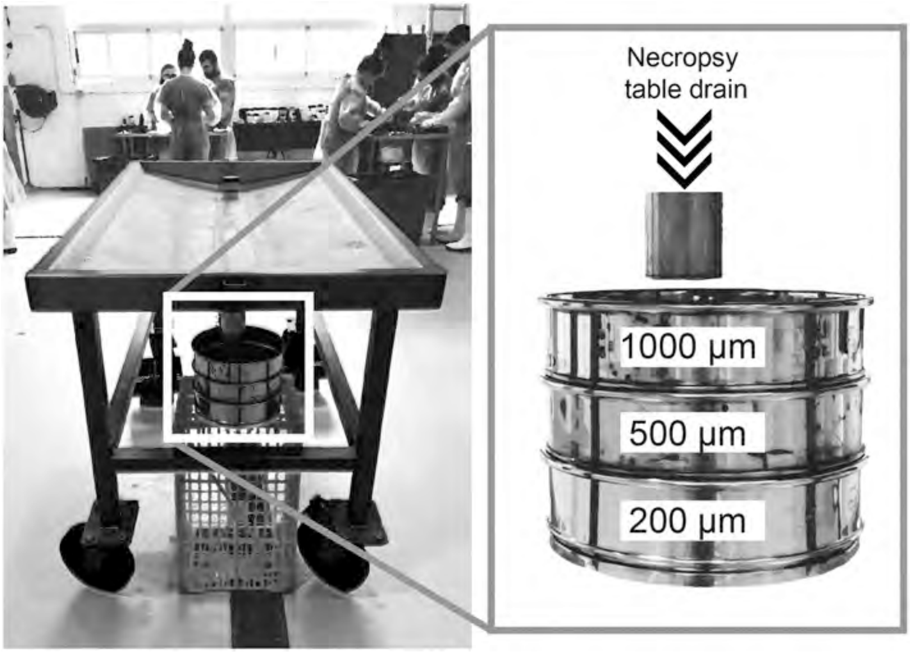


Fig. 2. Arrangement of sieves for filtering gastrointestinal contents, directly coupled to the drainage system of the necropsy table. This system was proofed advantageous and applicable by any research group that already counts with the necessary facilities to perform cetaceans autopsy analysis.

mm), dried overnight at 60 °C and inspected under a stereomicroscope (Leica S9i). Following Lusher et al. (2014), number, size, colour and shape of microplastic particles identified per individual were recorded. When classifying the particles, several categories were considered: fibres, fragments, films, pellets, beads and foams. However, all the microplastics found could be considered as fibres, fragments or films, thus discarding the rest of the categories in the analysis of the results.

2.4. Contamination controls

Common measures to limit the risk of sample contamination were implemented throughout: (1) Cotton lab clothes were worn during the analysis; (2) all equipment was cleaned and rinsed with Milli-Q water and checked under a microscope for airborne contamination before use; (3) a damp filter paper in a petri dish was placed within the necropsy table to catch any airborne particles that could be settling on the samples; (4) procedural blanks (250 ml of Milli-Q water run through the vacuum filtration system) were carried out, undergoing the same treatment as samples (exposure to air, digestion, vacuum filtration, etc.); (5) all samples were covered with aluminium foil after each step of the procedure. No microplastics were found in the procedural blanks and all controls were clear.

2.5. Organic persistent contaminants (OPCs) detection

The chromatographic separation of the analytes was carried out in Agilent Technologies' liquid chromatography equipment (model 1260) with automatic injection with diode array detector (HPLC-DAD) and column ACE-1210-1546 (150 × 4.6 mm) coupled to a liquid chromatography equipment with mass spectrometry Agilent Technologies (model 6100).

The mobile phase was a mixture of acetonitrile (Solvent A) and ultrapure Milli-Q water (Solvent B) both with a concentration of 0.1% acetic acid. The programmed gradient used was: 0–30 min, 45% A and 55% B, 30–31 min, 80% A and 20% B and finally 100% B for 9 min. The column is then reconditioned to 45% A and 55% B. The mobile phase flow was set at 0.8 ml $\min^{-1}$, with the sample volume injection being 20 μl and the detection wavelength 210 nm, for all analytes.

The conditions for obtaining the mass spectra of each of the organic contaminants vary depending on their nature. Thus, they were divided into three main groups: (1) Bisphenols: bisphenol S (BPS), bisphenol F (BPF) and bisphenol A (BPA); (2) phthalates: di (2-ethylhexyl) phthalate (DEHP), dibutylphthalate (DBP) and diethylphthalate (DEP); (3) pesticides: dichrolophenyl dichloroethane (DDD), dichlorodiphenyl dichlorethylene (DDE) and dichlorodiphenyl trichloroethane (DDT). In this way, it was possible to optimize and develop three different mass detection methods for each group of contaminants.

2.6. *Acquisition and treatment of microRaman spectra*

A subsample of particles (n = 12) was selected for analysis and chemical determination by Raman microspectroscopy. The measurements were carried out on a Horiba Jobin-Yvon Labram HR800-UV equipped with 4 lasers covering a wide range of wavelengths from UV to NIR at the facilities of the Scientific and Technological Centres of the University of Barcelona (Spain). According to the abundance ratio and the greater difficulty of identification of fibres with respect to fragments and films, 10 out of the 12 particles selected for analysis with the equipment were fibres, representing 1.4% of the total. The most frequent colours found in the study were also taken into account for the selection.

For each of the particles, several measurements were made, adjusting in each case the acquisition time and wavelength, among other parameters. Given the size of the fibres, the use of a microscope to focus the light beam was essential. The work routine involved finding the most optimal areas for measurement by microscopy. For each particle, at least two zones were selected, and the acquisition time was increased and the laser was changed from 532 nm to 785 nm depending on the results obtained in the first scans. To avoid destruction of the samples and an increase in noise, the laser power applied and the acquisition time were gradually increased depending on the nature and state of each particle, with a maximum of 100 s.

Subsequently, the obtained spectra were treated with the Spectragryph software (Menges, 2016): the peaks were normalized and baselines were applied with the adaptive method. The spectra were compared with the SLoPP library, which consists of 148 reference spectra, including a diversity of polymer types, colours and morphologies, and the SLoPP-e library, that accounts for the effects of environmental aging on microplastics, including 113 spectra (Munno et al., 2020). The fifth updated version of the Pigment Checker Library (Caggiani et al., 2016) was also used, together with other self-prepared reference spectra.

2.7. *Statistical analysis*

Descriptive statistics and analysis were performed with RStudio (R Core Team, 2019). Non-parametric statistics were used, as data lacked normality. Spearman rank correlation coefficients were calculated to evaluate the strength and direction of correlations between contaminants and microplastic abundance, among other variables that tested negative. The mean numbers of foreign particles per GIT section (oesophagus, stomach, duodenal ampulla and intestines) were compared with non-parametric tests (Kruskal-Wallis and Wilcoxon).

3. Results and discussion

This study is the first to evaluate and quantify microplastic ingestion and organic persistent contaminants concentrations in tissues of Macaronesian stranded cetaceans. We examined the entire gastrointestinal tract of 12 individuals from 6 odontocetes species that stranded along the coasts of Madeira and the Canary Islands between 2018 and 2019 (Fig. 1). Sex and morphometrics, as well as body and decomposition categories among other relevant pathological data, are reported in Table 1. There were five *S. coeruleoalba*, two *T. truncatus*, two *G. griseus*, one *G. macrorhynchus*, one *K. berviceps* and one *L. hosei*; six females and six males, of which eight were immature animals.

Existing research recognizes the importance of the issue addressed in this article: (1) These animals are considered important sentinels for marine pollution (Bossart, 2011); (2) despite the fact that the impacts of marine debris on fauna is a vast and increasingly studied research topic, knowledge on the incidence and levels of microplastics in large marine vertebrates is lacking (Duncan et al., 2019); (3) as expressed in the *Outcomes from the European Cetacean Society workshop* (Panti et al., 2019), “the development of protocols which allow harmonised approach to monitor marine litter impact on marine mammals, including microplastics, has become essential for future research”; (4) marine mammals are often used as flagship species, convenient to communicate awareness and stimulating community action (Bowen-Jones and Entwistle, 2002).

In this sense, before going into detail and discussing the more quantitative research results, we highlight two key qualitative achievements:

1. The validation of a protocol for microplastic sampling that serves to obtain samples from different multidisciplinary teams (i.e. veterinary and marine sciences schools), without interfering in the work of any of the parties (Montoto-Martínez et al., 2020b).
2. The successful table set up (Fig. 2) used for the extraction of microplastic particles from the GIT contents that proved advantageous and applicable by any research group that already has the necessary facilities to perform cetaceans autopsy analysis, fulfilling the harmonisation needs as explicated by Panti et al. (2019).

3.1. *Plastic ingestion*

3.1.1. *Quantity and type of particles*

Microplastics were found in all animals investigated, as was also the case in other studies that specifically addressed these particles (Hernández-González et al., 2018; Lusher et al., 2018; Moore et al., 2020). Of the 722 particles found, the majority (n = 708, 98.06%) were fibres. The fragments were found in a third of the animals analysed, two bottlenose dolphins and two striped dolphins, in different sections of their digestive tracts. Also, two plastic films were found in a female bottlenose dolphin.

With the exception of these two plastic films and a fragment barely exceeding 5 mm in size, no macroplastics were found in the analysed GITs. We consider it important to highlight this result because, although there are studies that have demonstrated the impact of ingestion of large quantities of marine debris on some whales (Unger et al., 2016), the percentage of occurrence in marine mammals in particular is relatively low (4.4%) according to a study by Kühn and van Franeker (2020). Moreover, reports of the absence of marine debris in such studies are not so frequent, to the point of sometimes exaggerating the presence of these materials, however minor, as discussed by Völker et al. (2020). In this regard, the most recent workshop on marine debris of the International Whaling Commission recommended that zero values for marine debris ingestion or entanglement should be recorded in necropsy reports (IWC, 2020).

On the other hand, all animals contained microplastics of diverse sizes, being most of particles classified as fibres (98.06%, n = 708). Fragments (1.66%, n = 12) appeared in 4 out of 12 animals analysed, which corresponds to two *Tursiops truncatus* and two *Stenella coeruleoalba*. Two films, corresponding to the plastic labels mentioned above and identified as polypropylene, were found in the oesophagus and the stomach of a female *Tursiops truncatus* (Cet ID = 934). An average of 59.08 fibres (SD = 40.52, n = 12) and 3.00 fragments (SD = 1.15, n = 4) were found per animal.

Focusing on particles smaller than 5 mm, we found some studies to compare our results with, although not without difficulties, due to the different methodological approaches. Lusher et al. (2018) investigated 21 stranded cetaceans and found microplastics in the GIT of all of them, from 1 to 88 particles per animal and mostly fibres (83.6%). Also, Moore et al. (2020) and Hernández-González et al. (2018) detected microplastics within every animal sampled: mean 11.6 ± SD 6.6 per beluga, and mean 12 ± SD 8 per common dolphin. Novillo et al. (2020) found microplastic particles in 90.5% of the dolphins studied, accounting for a mean of 14.9 ± SD 22.3 microplastic items per animal. In this study, the average of particles found is up to five times higher (59.08 ± SD 40.52 fibres and 3.00 ± SD 1.15 fragments) than the previous reported values. However, it is important to note that the size range addressed in this research work, using a sieving system that allowed the retention of particles down to 200 μm, is larger than the addressed in other studies previously mentioned. In this sense, other authors agree that comparisons are not easy, since the results depend to a large extent on various factors such as the methodology used or the geographical region studied (Moore et al., 2020). Moreover, different cetacean species from the same geographic area might have species-specific prey preferences and feeding strategies that imply differences in their potential microplastic particles uptake (Burkhardt-Holm and N'Guyen, 2019). There is little published data to evaluate this key issue, and implicit practical limitations in working with stranded animals makes it difficult to address it with the depth it deserves.

Nonetheless, the percentage of fibres (98.06%) with respect to fragments found corroborates the growing evidence that microfibres make up a significant part of microplastic pollution in the marine environment (Dris et al., 2016). Also, as Moore et al. (2020) observed, our results show that microplastics abundance can be unevenly distributed throughout the gastrointestinal tract. These results may indicate that particles of this size are transitory, as suggested also by other authors (Lusher et al., 2018; Nelms et al., 2019).

3.1.2. Sizes

The size of the fibres ranged from 0.1 to 34 mm (mean: 2.66; SD: 2.51 mm). As for fragments, sizes ranged from $4.42 \cdot 10^{-3}$ to 5.6 mm^2, and their mean area was of 1.11 mm^2 (SD: 1.94 mm^2) (Fig. 3). Our data are in consonance with other reports, presenting the highest percentage of fibres among 1 and 3 mm in length. This fraction was also the most predominant on coastal Macaronesian waters, the region where the present study takes place (Montoto-Martínez et al., 2020a). At a global scale, Suaria et al. (2020) accounted for fibres in oceanic surface waters ranging from 0.09 to 27.06 mm (median: 1.07 mm), with only three fibres over 10 mm and only three over 15 mm. In our case, fifteen fibres are over 10 mm, and six over 15 mm. Following the same trend within other analysed GITs, Moore et al. (2020) did not find particles >5 mm in length, being most of them smaller than 2 mm. The mean length of the fibres found in our study was very similar (mean: 2.66; SD: 2.51 mm) to the range found by Hernández-González et al. (2018) (mean: 2.1; SD: 1.26 mm) and by Nelms et al. (2019) (mean: 2; SD: 2.3 mm).

Based on the relatively small size of particles found, we believe that it is unlikely that they would cause any physical injuries. However, physical harm is not the only adverse effect that may be considered when microplastic exposure is addressed. Although more studies are needed to truly understand whether the origin of chemical contamination in the organisms studied comes from plastics or from other environmental sources, several lines of scientific evidence suggest that plastic waste carries an additional chemical hazard associated with the organic persistent contaminants it accumulates (Rochman, 2015; Teuten

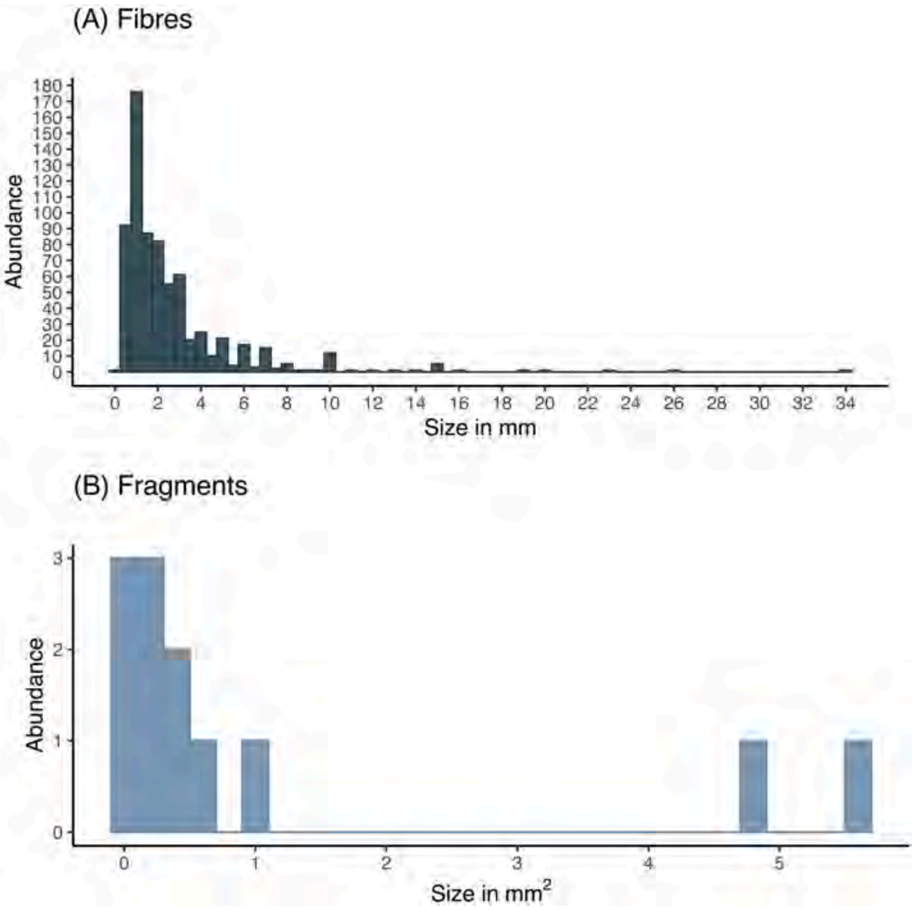


Fig. 3. Size distribution of the fibres and fragments found.

et al., 2009).

3.1.3. Colours

Green was the most frequent colour (n = 274, 37.9%), followed by red (n = 153, 21.19%) and blue (n = 101, 13.99%) (Fig. 4). The predominance of green (37.9%) stands out compared to other colours such as blue, black or red, which are the most frequently found in other similar studies carried out on GIT contents in cetaceans (Hernández-González et al., 2018; Lusher et al., 2018; Nelms et al., 2019). Somewhat surprisingly, among the seawater samples analysed by Montoto-Martínez et al. (2020a) in a previous study in the same region, green did not reach majority percentages either. Mistaken ingestion of oceanic debris due to its resemblance to preferred prey species is unlikely because of odontocete cetacean feeding behaviour and echolocation capabilities (Walker and Coe, 1989), so this may indicate that the uptake of these particles may be indirect. The diet of the odontocete species analysed in this study varies slightly, with the most common prey being cephalopods and mesopelagic fish (Culik and Wurtz, 2011), as confirmed by the stomach content registers shown in Table 1. Several studies reveal the ingestion of microplastics by species that are potential prey for the animals studied (McGoran et al., 2021), such as lanternfishes (Romeo et al., 2016) or cuttlefish (Oliveira et al., 2020). However, it is beyond the scope of this study to determine whether microplastic ingestion by prey may have selective behaviour when ingesting particles of certain colours. Although we do not have enough information or a sufficiently robust sample to be able to draw very specific conclusions, in our case, precisely the animals in which the highest numbers of microplastics were found do not coincide with those that, at the time of their death, had the most prey remains in their stomachs (Fig. 5). With the information provided by the veterinary analysis of the stomach contents for the diet study in the necropsy room, it was found that the only animal with macroplastics (Cet ID = 934) also had a high number of microplastics that were revealed after the chemical digestion of the stomach contents in the laboratory.

3.1.4. Distribution among GIT compartments

The distribution of ingested particles showed no significant differences in their occurrence within the different compartments of the gastrointestinal tract (oesophagus, stomach, duodenal ampulla and intestines) (Kruskal-Wallis test, p = 0.063). Wilcoxon test revealed differences in the means among groups (p = 0.0034) only in the case of the stomach and the duodenal ampulla (Fig. 6), which may be due to the difference in size of these two chambers. Spearman correlation analysis showed a relative significant negative correlation in the number of MPs in the GIT contents and the total length of the animals (R = −0,78, p = 0.007), showing fewer items with increasing length of the animal. However, it should be kept in mind that the sample of animals is small and comprises different species, so further studies are needed to establish more reliable relationships.

3.2. Organic persistent contaminants (OPCs)

Bisphenols and phthalates were found in all the skeletal muscle samples, while 10 out of 12 animals analysed also presented pesticides,

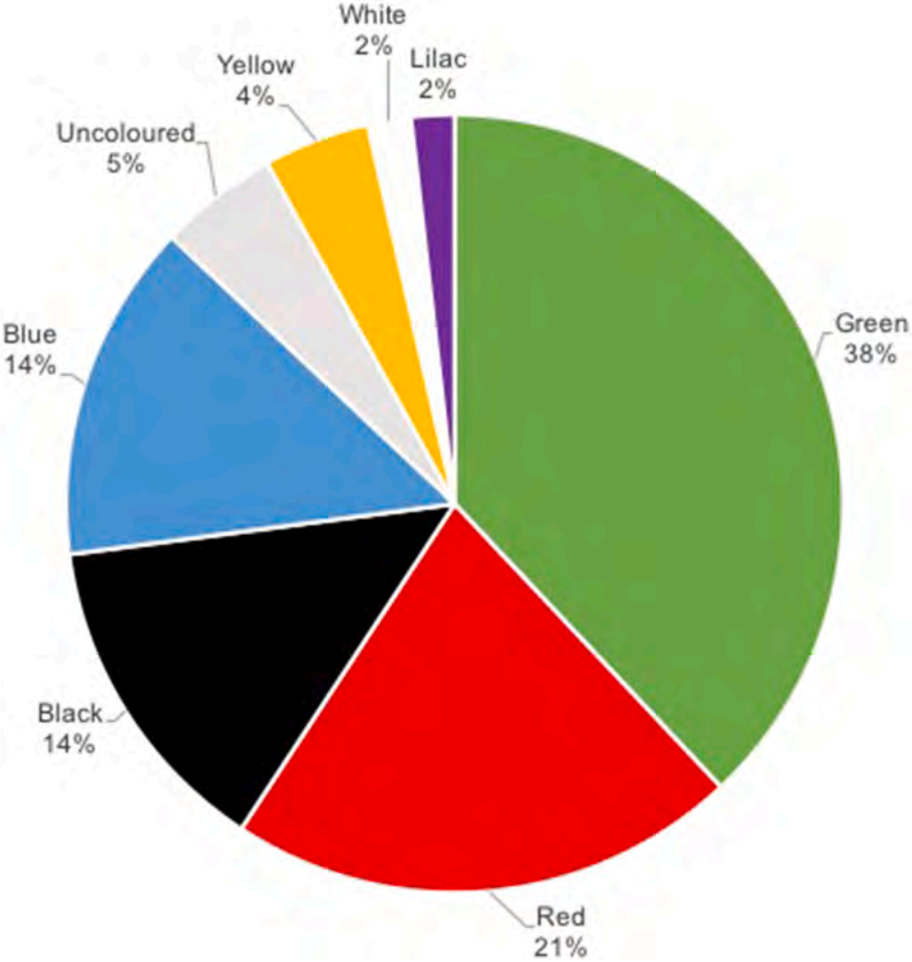


Fig. 4. Colour distribution of the microplastics found. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

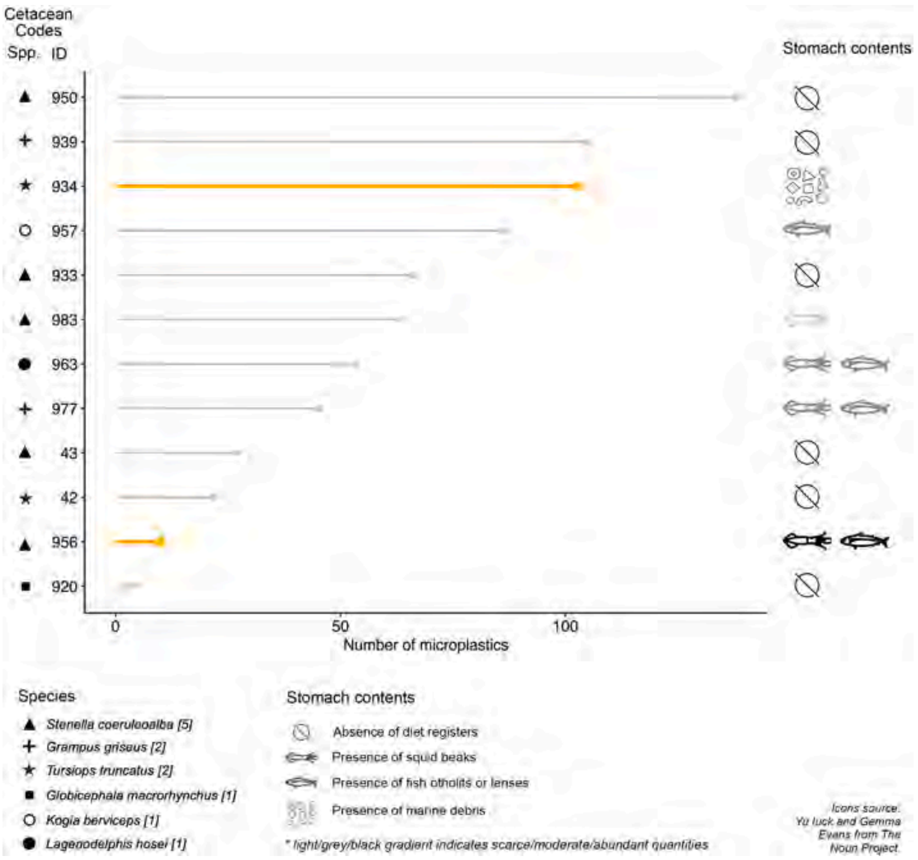


Fig. 5. Relationship between stomach contents analysed for dietary records and the study of microplastic abundance. Cet 934 was the only animal with macroplastics in its stomach contents, which corresponds to a high record of microplastics. It is also noted that the animal that contained the most prey remains in its stomach (Cet ID = 956) had the second lowest microplastic concentrations.

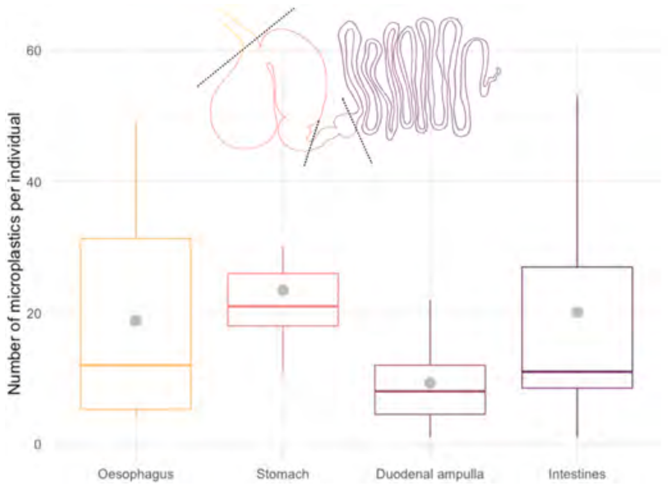


Fig. 6. Microplastic distribution within the gastrointestinal sections. No significant differences were found in the abundance of microplastics within the different gastrointestinal sections except in the case of the duodenal ampulla and the stomach.

although in lower concentration. Compounds NP (nonylphenol) and NP-9 (nonylphenol-9) were not found in any of the samples. In Table S1, mean concentrations of nine detected compounds (in ng/g), are reported together with the standard deviation corresponding to the triple replicate performed. Mean values are plotted in Fig. 7, where maximum concentrations can be observed in red tiles. No correlations were found among the analysed organic persistent contaminants and microplastic abundance (Spearman Test, R = 0.43, p = 0.16), nor among the size of the animal and the total contaminants present in tissue (Spearman Test, R = −0.22, p = 0.48).

3.2.1. *Bisphenols*

The compounds corresponding to the bisphenols family have been found in 94.44% of the samples and their concentrations range from 4 ng/g to 984 ng/g with standard deviation values below the ±100 ng/g in all measurements made. In particular, BPA ranged from 48.67 ng/g (*G. griseus*, Cet ID = 977) to 731.67 ng/g (*G. macrorhynchus*, Cet ID = 920). The concentrations of BPA observed by Page-Karjian et al. (2020) in blubber samples taken from stranded *T. truncatus* in the southeastern United States during 2012–2018 were much higher than those of the present study, reaching up to 258.3 μg/g against 0.6 μg/g for the same species. A global assessment of BPA in the environment performed by Corrales et al. (2015) revealed concentrations in wildlife, mostly for fishes, ranging from 0.2 to 13,000 ng/g.

3.2.2. *Phthalates*

The phthalates studied are present in a lower proportion compared to bisphenols. The most frequently detected compound was DEHP (di-2-ethylhexyl phthalate), which was found in 88% of the samples. This is not surprising, as it is one of the most used ones, representing in 2015 a 37.1% of the global plasticizers market (Hermabessiere et al., 2017). As a fact, reported DEHP concentration in skin biopsy samples from Mediterranean striped dolphins (Baini et al., 2017) is almost eight times higher than the highest concentration found in our study, which also corresponds to a striped dolphin. The least present phthalate was DEP (Diethyl phthalate) with 38.88% of occurrence and a concentration range of 13 to 225 ng/g with a SD less than ±20 ng/g. Median

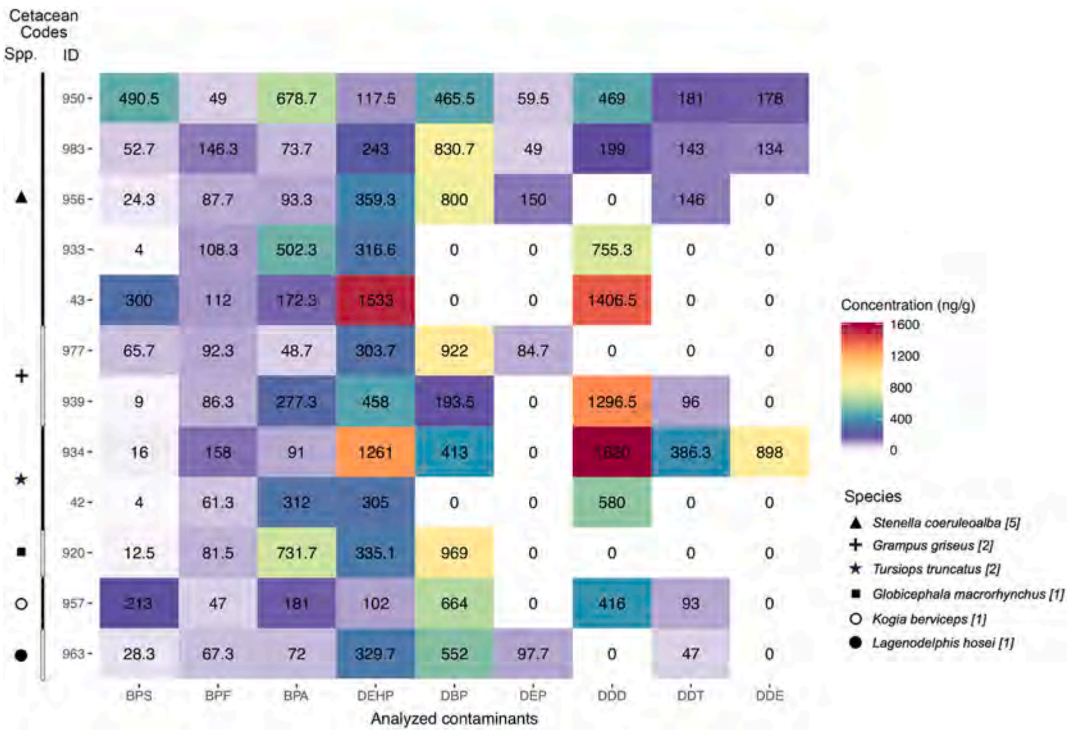


Fig. 7. Heatmap of the concentrations of the different organic persistent contaminants analysed: bisphenol S (BPS), bisphenol F (BPF) and bisphenol A (BPA), di (2-ethylhexyl) phthalate (DEHP), dibutylphthalate (DBP) and diethylphthalate (DEP), dichrolophenyl dichloroethane (DDD), dichlorodiphenyl dichlorethylene (DDE) and dichlorodiphenyl trichloroethane (DDT).

concentrations in our study (74 ng/g) were very similar to those of Page-Karjian et al. (2020) in the southeastern United States (70 ng/g) for the specific case of *Stenella* spp.

3.2.3. *Pesticides*

Finally, it should be noted that the analysed pesticides were found in 50% of the samples in the case of DDD (dichrolophenyl dichloroethane) with concentrations between 199 ng/g to 2204 ng/g; 40% DDT (dichlorodiphenyl trichloroethane) with a minimum concentration of 100 ng/g and a maximum of 465 ng/g, and 8.33% in the case of DDE (dichlorodiphenyl dichlorethylene), whose concentration varies from 898 ng/g to 178 ng/g (SD < 26 ng/g). As shown in Fig. 8, except for two individuals, all animals have pesticide levels in their tissues, which gives an idea of how widespread these contaminants are.

The highest level of tissue contamination found corresponds to the DDD concentration in a *T. truncatus* stranded off the coast of Gran Canaria (Fig. 7, Cet ID = 934). This animal presented a concentration above 1 ppm (1620 ng/g), what Letcher et al. (2010) considered a toxic concentration for organohalogenated compounds in marine mammals' tissues. This may be linked to the intrinsic characteristics of this dolphin species, such as being a nearshore species that feeds in coastal waters, which places them among the marine mammals with the highest accumulation of persistent organochlorines (Carballo et al., 2008).

In a study performed by García-Álvarez et al. (2014) that measured levels of persistent organic pollutants in free-ranging *T. truncatus* of the Canary Islands, the group of pesticides that contributed more to the organochlorine pesticides was that of DDTs, and in particular p,p′-DDE, which accounted for a mean average of 87.6% of ΣDDTs. In addition, in a previous study of the same group, conducted on stranded *T. truncatus* in the same region, ΣDDTs were present in the highest concentrations, ranging from 147 to 21,050 ng/g in blubber (Carballo et al., 2008). According to the authors, these levels of pollution are close to the concentrations that could produce immunosuppression and antiandrogenic effects in marine mammals and can therefore pose a certain risk to the health of these animals.

DDT levels in the Canary Islands in different environmental samples reach quite high concentrations compared to those in other areas of the world. Probably due to the intensive use of this product in the region in the past, high levels of DDT were found in microplastics sampled in Gran Canaria, with a median of 993.5 ng/g in the case of pellets and 32.4 ng/g in the case of fragments in Las Canteras beach, and 76.5 ng/g and 241.6 ng/g, respectively, in Cuervitos beach (Camacho et al., 2019).

3.3. *Chemical fingerprint – microRaman spectra*

Analysis with microRaman spectroscopy allowed us to obtain more information about the types of plastics found in the study and their possible sources. Obtaining the spectra was a process that required successive replications and repetitions, adjusting the parameters of the equipment to obtain the best combination that would return a sufficiently clean spectrum without damaging the particle and without introducing too much noise or producing too much fluorescence. Complementary measurements in the near-infrared region (at 785 nm) and the use of the SLOPP-e spectra library (Munno et al., 2020), which includes reference spectra of degraded polymers, made it possible to identify particles with match rates above 80%. All microplastics selected for spectroscopic analysis, representing 1.6% of the total, were confirmed as being anthropogenic particles. Polyethylene was the major polymer among the fibres, followed by polypropylene and polyethylene terephthalate.

The MSFD Technical Subgroup on Marine Litter does not consider identification by FTIR or Raman spectroscopy of particles larger than 500 μm to be critical (Galgani et al., 2013). Taking into account that only 2.5% of the total microplastics found in this study are below this threshold, the selection of the particles to be analysed was carefully made by choosing those that could be doubtful for identification and taking into account all the possible materials observed.

The SLoPP-e library was proofed a very useful tool for the identification of microplastics from environmental samples. Through our experience, we have seen how the identification of polymers increased

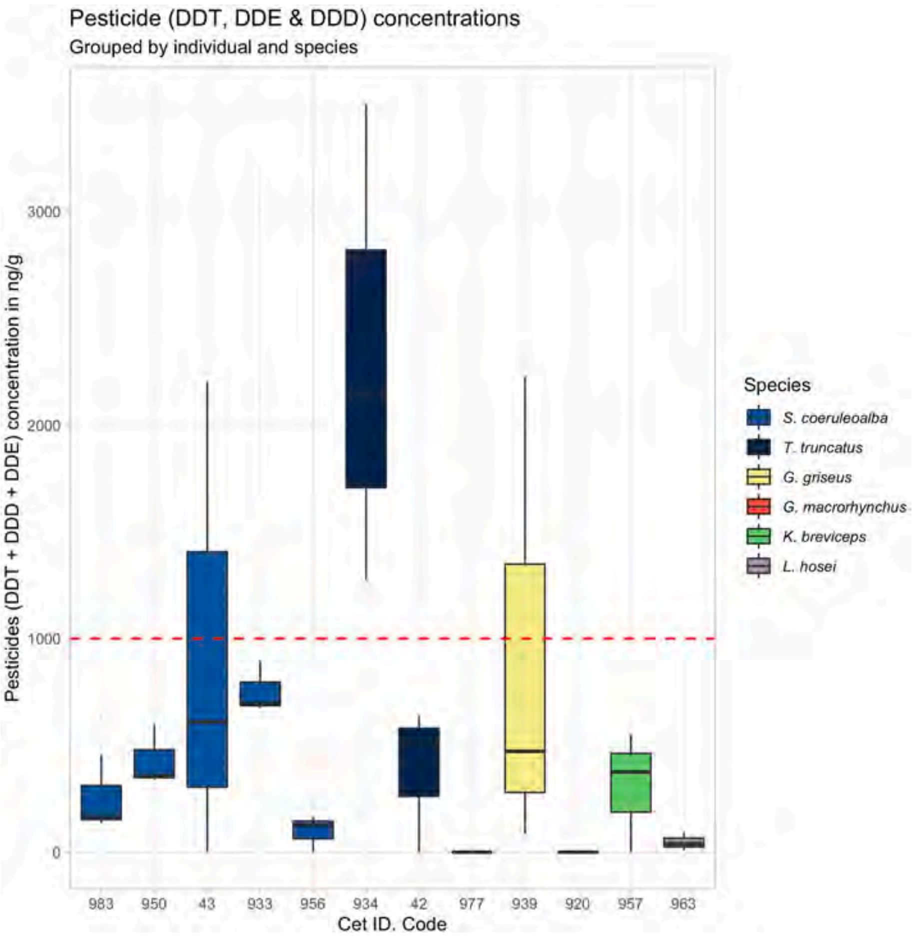


Fig. 8. Concentration of pesticides (DDT, DDD and DDE) in the cetaceans analysed. The red line indicates the concentration at which organohalogenated compounds are considered toxic in marine mammal tissues (Letcher et al., 2010). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by 6 points compared to the identification using only the SLoPP library, which contains spectra of polymers from products that have not undergone any deterioration treatment. Thus, the match to polyethylene returned by the spectra identification software for a blue fibre was 74% with polyethylene from the SLoPP library, and 80% with polyethylene from the SLoPP-e library. Weathering promotes the generation of different functional groups, mainly C = O, O – H and C – O on the surface of MPs (Duan et al., 2021) and therefore generates a series of alterations that make the spectra significantly different and, in general, less clear and more difficult to identify. Among the different factors that affect environmental weathering of plastics, such as oxygen, temperature or the content of water, the presence of organic matter and the biofilm formation, which are both conditions that occur within the gastrointestinal tracts, also have a significant influence.

Another key issue that emerged from the microRaman measures is that, for a correct determination of the particles, it is crucial to examine

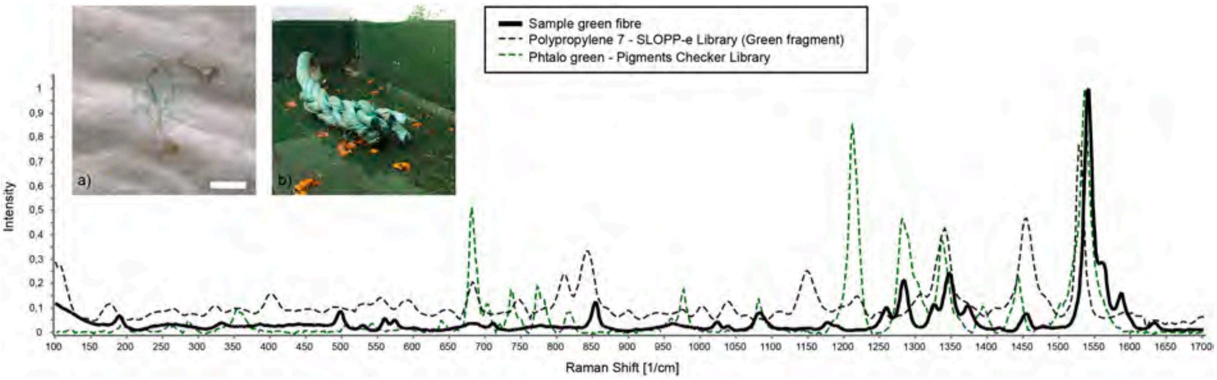


Fig. 9. Spectrum of a green fibre particle (picture a, inset with scale bar 0.5 mm) matching with the Polypropylene 7 reference (SLOPP-e Library, corresponding to a green fragment, 99.62% hit) and the Phtalogreen pigment (Pigments Checker Library, 99.85% hit). Picture b) shows the filaments of a rope on the deck of a ship, one of the possible sources of the green fibres according to the chemical fingerprint revealed by microRaman spectroscopy. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the spectra with particular attention to the alterations that the pigments produce on the chemical fingerprint of the polymers themselves. Given the predominance of the signal corresponding to the pigments contained in the particles, the Pigment Checker database was used to subtract the spectrum corresponding to the colouring and to identify the polymer in question. This was the case for the identification of one of the characteristic green fibres, which was identified as degraded polypropylene green fibre (Fig. 9). In this way, the determination of the chemical fingerprint of these predominant green fibres in the GIT analysed could be associated with the mooring ropes used on ships, which are a common item within marine debris and are generally made of this material.

To further investigate these two aspects, the effect of environmental deterioration of the particles on the spectra, and the relevance of colour, two fibres of the same colour and from two different matrices were compared: the intestinal tract of one of the cetaceans in the present study and a saltwater sample from a previous study carried out by Montoto-Martínez et al. (2020a). Although we do not know the residence time of each of the fibres in the matrices, we venture to indicate that the passage through the digestive tract generates greater degradation of the particles, thus causing a less clear spectrum than that of the fibre extracted from seawater (Fig. 10). The greater presence of noise in the spectrum of the fibre extracted from the cetacean may also be due to residual organic matter, which may not have been completely removed in the sample treatment. Although further tests would be necessary to establish a clear comparison, for the time being, it can be said that both residual organic matter and the deterioration of the microplastic starting materials have a significant influence on the returned spectrum and therefore have to be taken into account for the correct identification of their chemical fingerprint. In this sense, Nauendorf et al. (2016) carried out a study of the effect of microbial colonization and degradation that clearly shows the increase in background noise in the spectra generated by these two factors on samples of pristine plastic bags incubated with organic matter. On the other hand, it is also worth noting with this example how the spectra corresponding to the pigments predominate and often overlap over the spectra of the polymers, making identification impossible in some cases (Ribeiro-Claro et al., 2017). Fig. 10 shows a remarkable coincidence (above 98%) between the spectra of the samples (two red fibres) and the pigments (alizarin and raw sienna). It is therefore very important to bear in mind, when analysing the spectra of microplastics, that they generally contain other components in addition to the polymeric matrix, such as chemical additives or dyes, and therefore we must be cautious when interpreting them. The presence of pigments warns us that we are dealing with a particle of anthropogenic origin, but this does not necessarily mean that it is plastic, as it may be cellulose fibres, as revealed by the authors (Remy et al., 2015). In this case, however, the correspondence with the spectra of polyethylene (PE7 and PE6 from the SLoPP library) allows us to affirm that it is a microplastic.

4. Conclusions

This study provides information on microplastic ingestion in cetaceans and the occurrence of organic persistent contaminants in tissue samples in the Macaronesian Region (Eastern North Atlantic). Except for the case of two plastic labels that were found in the oesophagus and stomach of one dolphin, no plastic particles larger than 5 mm were observed. By contrast, microplastic fibres were present in all animals analysed, though, we believe, in numbers too low to block or compromise the functioning of the digestive tract.

Exposure of cetaceans to organic persistent contaminants is evidenced by the results, since the predominant pollutants bisphenols (BPS, BPF and BPA) and DEHP were detected in 94.44% and 88% of the tissue samples respectively. Also, except for two individuals, all animals had pesticide levels in their tissues. Many questions remain regarding the potential for long-term damage associated with chronic exposure to these organic persistent contaminants.

We include a first approach to the potential of microRaman analysis to improve the understanding of microplastic alteration processes, which must be further investigated. In addition to chemical fingerprinting, organic matter or environmental aging are factors that may be studied by this spectroscopic analyse technique.

Supplementary data to this article can be found online at https://doi.

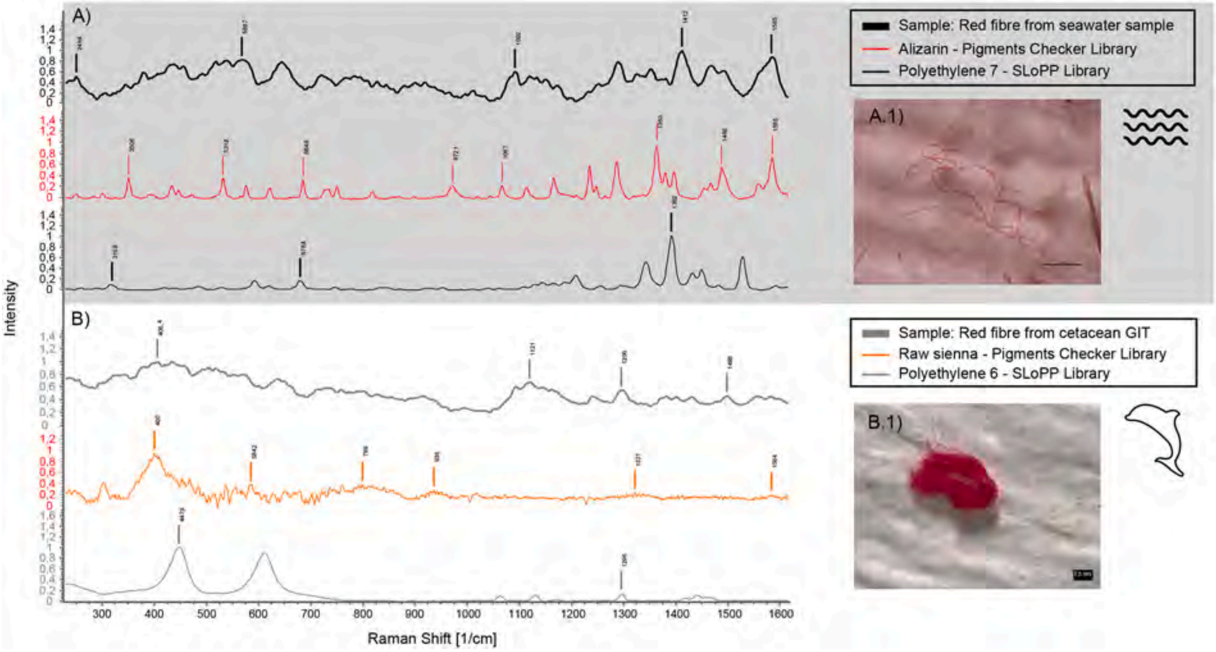


Fig. 10. Figure showing the match of the spectra returned by the Spectragryph software of two red fibres found in two different matrices: (A) In water, in a previous study by the same author (Montoto-Martínez et al., 2020a), and (B) in the gastrointestinal contents of one of the cetaceans analysed in the present study. Both spectra were measured under the same measurement parameters. The percentage of coincidence between the sample fibres and the pigments spectra was very high (above 98%). This warns us how important it is to work with a pigment database in order to be able to carry out a correct identification of microplastics, which are often very colourful. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

org/10.1016/j.marpolbul.2021.113105.

CRediT authorship contribution statement

Tania Montoto-Martínez: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Jesús De la Fuente:** Conceptualization, Methodology, Investigation, Resources, Data curation, Project administration. **Raquel Puig-Lozano:** Methodology, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Nuno Marques:** Methodology, Validation, Investigation, Resources, Writing – review & editing. **Manuel Arbelo Hernández:** Methodology, Validation, Investigation, Resources, Writing – review & editing, Visualization, Supervision. **José Joaquín Hernández-Brito:** Conceptualization, Resources, Supervision, Funding acquisition. **Antonio Fernández:** Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Mª. Dolores Gelado-Caballero:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

Acknowledgements

This work has been supported by a postgraduate research scholarship from the University of Las Palmas de Gran Canaria and carried out with funding from the European Regional Development Fund through the Madeira-Açores-Canarias Territorial Cooperation Operational Program (POMAC) 2014-2020 through the MARCET Project (MAC/1.1b/149).

References

Arbelo, M., Monteros, A.E.de los, Herráez, P., Andrada, M., Sierra, E., Rodríguez, F., Jepson, P.D., Fernández, A., 2013. Pathology and causes of death of stranded cetaceans in the Canary Islands (1999−2005), 103, 87–99. https://doi.org/10.3354/dao02558.

Baini, M., Martellini, T., Cincinelli, A., Campani, T., Minutoli, R., Panti, C., Finoia, M.G., Fossi, M.C., 2017. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. Anal. Methods 9, 1512–1520. https://doi.org/10.1039/C6AY02674E.

Besseling, E., Foekema, E.M., Van Franeker, J.A., Leopold, M.F., Kühn, S., Bravo Rebolledo, E.L., Heße, E., Mielke, L., IJzer, J., Kamminga, P., Koelmans, A.A., 2015. Microplastic in a macro filter feeder: Humpback whale Megaptera novaeangliae. Marine Pollution Bulletin 95, 248–252. https://doi.org/10.1016/j.marpolbul.2015.04.007.

BOE (Official Bulletin of the Spanish State), 2011. Royal Decree 139/201, of 4 february, for the development of the List of Wild Species under Special Protection Regime and the Spanish Catalogue of Threatened Species [Spain].

Bossart, G.D., 2011. Marine mammals as sentinel species for oceans and human health. Vet. Pathol. 48, 676–690. https://doi.org/10.1177/0300985810388525.

Bowen-Jones, E., Entwistle, A., 2002. Identifying appropriate flagship species: the importance of culture and local contexts. Oryx 36, 189–195. https://doi.org/10.1017/S0030605302000261.

Bradney, L., Wijesekara, H., Palansooriya, K.N., Obadamudalige, N., Bolan, N.S., Ok, Y.S., Rinklebe, J., Kim, K.-H., Kirkham, M.B., 2019. Particulate plastics as a vector for toxic trace-element uptake by aquatic and terrestrial organisms and human health risk. Environ. Int. 131, 104937 https://doi.org/10.1016/j.envint.2019.104937.

Burkhardt-Holm, P., N'Guyen, A., 2019. Ingestion of microplastics by fish and other prey organisms of cetaceans, exemplified for two large baleen whale species. Mar. Pollut. Bull. 144, 224–234. https://doi.org/10.1016/j.marpolbul.2019.04.068.

Caggiani, M.C., Cosentino, A., Mangone, A., 2016. Pigments checker version 3.0, a handy set for conservation scientists: a free online raman spectra database. Microchem. J. 129, 123–132. https://doi.org/10.1016/j.microc.2016.06.020.

Camacho, M., Herrera, A., Gómez, M., Acosta-Dacal, A., Martínez, I., Henríquez-Hernández, L.A., Luzardo, O.P., 2019. Organic pollutants in marine plastic debris from Canary Islands beaches. Sci. Total Environ. 662, 22–31. https://doi.org/10.1016/j.scitotenv.2018.12.422.

Carballo, M., Arbelo, M., Esperón, F., Mendez, M., De, L.T., Muñoz, M.J., 2008. Organochlorine residues in the blubber and liver of bottlenose dolphins (Tursiops truncatus) stranded in the Canary Islands, North Atlantic Ocean. Environ. Toxicol. 23, 200–210. https://doi.org/10.1002/tox.20322.

Corrales, J., Kristofco, L.A., Steele, W.B., Yates, B.S., Breed, C.S., Williams, E.S., Brooks, B.W., 2015. Global assessment of bisphenol a in the environment. Dose Response 13. https://doi.org/10.1177/1559325815598308.

Correia, A.M., Gil, Á., Valente, R.F., Rosso, M., Sousa-Pinto, I., Pierce, G.J., 2020. Distribution of cetacean species at a large scale - connecting continents with the macaronesian archipelagos in the eastern North Atlantic. Divers. Distrib. 26, 1234–1247. https://doi.org/10.1111/ddi.13127.

Culik, B.M., Wurtz, M., 2011. Odontocetes: the toothed whales, CMS technical series. UNEP / CMS / ASCOBANS Secretariat, Bonn.

Díaz-Delgado, J., Fernández, A., Sierra, E., Sacchini, S., Andrada, M., Vela, A.I., Quesada-Canales, Ó., Paz, Y., Zucca, D., Groch, K., Arbelo, M., 2018. Pathologic findings and causes of death of stranded cetaceans in the Canary Islands (2006–2012). PLOS ONE 13, e0204444. https://doi.org/10.1371/journal.pone.0204444.

Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? Mar. Pollut. Bull. 104, 290–293. https://doi.org/10.1016/j.marpolbul.2016.01.006.

Duan, J., Bolan, N., Li, Y., Ding, S., Atugoda, T., Vithanage, M., Sarkar, B., Tsang, D.C.W., Kirkham, M.B., 2021. Weathering of microplastics and interaction with other coexisting constituents in terrestrial and aquatic environments. Water Res. 196, 117011 https://doi.org/10.1016/j.watres.2021.117011.

Duncan, E.M., Broderick, A.C., Fuller, W.J., Galloway, T.S., Godfrey, M.H., Hamann, M., Limpus, C.J., Lindeque, P.K., Mayes, A.G., Omeyer, L.C.M., Santillo, D., Snape, R.T.E., Godley, B.J., 2019. Microplastic ingestion ubiquitous in marine turtles. Glob. Chang. Biol. 25, 744–752. https://doi.org/10.1111/gcb.14519.

Fernández, R., Santos, M.B., Carrillo, M., Tejedor, M., Pierce, G.J., 2009. Stomach contents of cetaceans stranded in the Canary Islands 1996–2006. J. Mar. Biol. Ass. 89, 873–883. https://doi.org/10.1017/S0025315409000290.

Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in North Sea fish. Environ. Sci. Technol. 47, 8818–8824. https://doi.org/10.1021/es400931b.

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R.C., Van Franeker, J., Vlachogianni, T., Scoullos, M., Veiga, J.M., Palatinus, A., Matiddi, M., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013. Guidance on monitoring of marine litter in European seas. In: Publications Office of the European Union, Luxembourg.

García-Álvarez, N., Martín, V., Fernández, A., Almunia, J., Xuriach, A., Arbelo, M., Tejedor, M., Boada, L.D., Zumbado, M., Luzardo, O.P., 2014. Levels and profiles of POPs (organochlorine pesticides, PCBs, and PAHs) in free-ranging common bottlenose dolphins of the Canary Islands, Spain. Sci. Total Environ. 493, 22–31. https://doi.org/10.1016/j.scitotenv.2014.05.125.

Garcia-Garin, O., Aguilar, A., Vighi, M., Víkingsson, G.A., Chosson, V., Borrell, A., 2021. Ingestion of synthetic particles by fin whales feeding off Western Iceland in summer. Chemosphere 130564. https://doi.org/10.1016/j.chemosphere.2021.130564.

Grand View Research, 2019. Textile Chemicals Market Size, Share & Trends Analysis Report By Product (Colorants & Auxiliaries, Finishing Agents, Coating & Sizing, Surfactants), By Application (Apparel, Home Furnishing), And Segment Forecasts, 2019 - 2025. Grand View Research.

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere 182, 781–793. https://doi.org/10.1016/j.chemosphere.2017.05.096.

Hernández-González, A., Saavedra, C., Gago, J., Covelo, P., Santos, M.B., Pierce, G.J., 2018. Microplastics in the stomach contents of common dolphin (Delphinus delphis) stranded on the galician coasts (NW Spain, 2005–2010). Mar. Pollut. Bull. 137, 526–532. https://doi.org/10.1016/j.marpolbul.2018.10.026.

Herrera, I., Carrillo, M., Cosme de Esteban, M., Haroun, R., 2021. Distribution of cetaceans in the Canary Islands (Northeast Atlantic Ocean): implications for the natura 2000 network and future conservation measures. Front. Mar. Sci. 8, 669790 https://doi.org/10.3389/fmars.2021.669790.

Ijsseldijk, L.L., Brownlow, A.C., Mazzariol, S. (Eds.), 2019. European Best Practice on Cetacean Post-Mortem Investigation and Tissue Sampling. Joint ACCOBAMS and ASCOBANS Document.

IUCN, 2020. The IUCN Red List of Threatened Species.

IWC, 2020. Report of the IWC Workshop on Marine Debris: The Way Forward, 3-5 December, 2019. La Garriga, Catalonia, Spain. International Whaling Commission, La Garriga, Catalonia, Spain.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771. https://doi.org/10.1126/science.1260352.

Kühn, S., van Franeker, J.A., 2020. Quantitative overview of marine debris ingested by marine megafauna. Mar. Pollut. Bull. 151, 110858 https://doi.org/10.1016/j.marpolbul.2019.110858.

Kühn, S., Rebolledo, E.L.B., Franeker, J.A.van, 2015. Deleterious Effects of Litter on Marine Life. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, pp. 75–116. https://doi.org/10.1007/978-3-319-16510-3_4.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. In: Science of The Total Environment, Levels, trends and effects of legacy and new persistent organic pollutants in the Arctic: An AMAP Assessment, 408, pp. 2995–3043. https://doi.org/10.1016/j.scitotenv.2009.10.038.

Lusher, A.L., Burke, A., O'Connor, I., Officer, R., 2014. Microplastic pollution in the Northeast Atlantic Ocean: validated and opportunistic sampling. Mar. Pollut. Bull. 88, 325–333. https://doi.org/10.1016/j.marpolbul.2014.08.023.

Lusher, A.L., Hernandez-Milian, G., Berrow, S., Rogan, E., O'Connor, I., 2018. Incidence of marine debris in cetaceans stranded and bycaught in Ireland: recent findings and a review of historical knowledge. Environ. Pollut. 232, 467–476. https://doi.org/10.1016/j.envpol.2017.09.070.

Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale Mesoplodon mirus. Environ. Pollut. 199, 185–191. https://doi.org/10.1016/j.envpol.2015.01.023.

McGoran, A.R., Maclaine, J.S., Clark, P.F., Morritt, D., 2021. Synthetic and semi-synthetic microplastic ingestion by mesopelagic fishes from Tristan da Cunha and st HelenaSouth Atlantic, 8. https://doi.org/10.3389/fmars.2021.633478.

Menges, F., 2016. Spectragryph - optical spectroscopy software.

Montoto-Martínez, T., Hernández-Brito, J.J., Gelado-Caballero, M.D., 2020a. Pump-underway ship intake: an unexploited opportunity for marine strategy framework directive (MSFD) microplastic monitoring needs on coastal and oceanic waters. PLOS ONE 15, e0232744. https://doi.org/10.1371/journal.pone.0232744.

Montoto-Martínez, T., Puig-Lozano, R., Marques, N., Fernández, A., De la Fuente, J., Gelado-Caballero, M.D., 2020b. A protocol to address the study of microplastic intake in stranded cetaceans. https://doi.org/10.17504/protocols.io.bcfxitpn.

Moore, R.C., Loseto, L., Noel, M., Etemadifar, A., Brewster, J.D., MacPhee, S., Bendell, L., Ross, P.S., 2020. Microplastics in beluga whales (Delphinapterus leucas) from the eastern Beaufort Sea. Mar. Pollut. Bull. 150, 110723 https://doi.org/10.1016/j.marpolbul.2019.110723.

Munno, K., De Frond, H., O'Donnell, B., Rochman, C.M., 2020. Increasing the accessibility for characterizing microplastics: introducing new application-based and spectral libraries of plastic particles (SLoPP and SLoPP-E). Anal. Chem. 92, 2443–2451. https://doi.org/10.1021/acs.analchem.9b03626.

Nauendorf, A., Krause, S., Bigalke, N.K., Gorb, E.V., Gorb, S.N., Haeckel, M., Wahl, M., Treude, T., 2016. Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments. Mar. Pollut. Bull. 103, 168–178. https://doi.org/10.1016/j.marpolbul.2015.12.024.

Nelms, S.E., Barnett, J., Brownlow, A., Davison, N.J., Deaville, R., Galloway, T.S., Lindeque, P.K., Santillo, D., Godley, B.J., 2019. Microplastics in marine mammals stranded around the british coast: ubiquitous but transitory? Sci. Rep. 9, 1075. https://doi.org/10.1038/s41598-018-37428-3.

Novillo, O., Raga, J.A., Tomás, J., 2020. Evaluating the presence of microplastics in striped dolphins (Stenella coeruleoalba) stranded in the Western Mediterranean Sea. Mar. Pollut. Bull. 160, 111557 https://doi.org/10.1016/j.marpolbul.2020.111557.

Oliveira, A.R., Sardinha-Silva, A., Andrews, P.L.R., Green, D., Cooke, G.M., Hall, S., Blackburn, K., Sykes, A.V., 2020. Microplastics presence in cultured and wild-caught cuttlefishSepia officinalis, 160, 111553. https://doi.org/10.1016/j.marpolbul.2020.111553.

Page-Karjian, A., Lo, C.F., Ritchie, B., Harms, C.A., Rotstein, D.S., Han, S., Hassan, S.M., Lehner, A.F., Buchweitz, J.P., Thayer, V.G., Sullivan, J.M., Christiansen, E.F., Perrault, J.R., 2020. Anthropogenic contaminants and histopathological findings in stranded cetaceans in the southeastern United States, 2012–2018. Front. Mar. Sci. 7 https://doi.org/10.3389/fmars.2020.00630.

Panti, C., Baini, M., Lusher, A., Hernandez-Milan, G., Bravo Rebolledo, E.L., Unger, B., Syberg, K., Simmonds, M.P., Fossi, M.C., 2019. Marine litter: one of the major threats for marine mammals. Outcomes from the european cetacean society workshop. Environ. Pollut. 247, 72–79. https://doi.org/10.1016/j.envpol.2019.01.029.

Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Rebolledo, E.L.B., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevail, A., van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. Anal. Methods 9, 1454–1469. https://doi.org/10.1039/C6AY02419J.

Puig-Lozano, R., Bernaldo de Quirós, Y., Díaz-Delgado, J., García-Álvarez, N., Sierra, E., De la Fuente, J., Sacchini, S., Suárez-Santana, C.M., Zucca, D., Câmara, N., Saavedra, P., Almunia, J., Rivero, M.A., Fernández, A., Arbelo, M., 2018. Retrospective study of foreign body-associated pathology in stranded cetaceans, Canary Islands (2000–2015). Environ. Pollut. 243, 519–527. https://doi.org/10.1016/j.envpol.2018.09.012.

Team, R.Core, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G., 2015. When microplastic is not plastic: the ingestion of artificial cellulose fibers by macrofauna living in seagrass macrophytodetritus. Environ. Sci. Technol. 49, 11158–11166. https://doi.org/10.1021/acs.est.5b02005.

Ribeiro-Claro, P., Nolasco, M.M., Araújo, C., 2017. Characterization of microplastics by Raman spectroscopy. In: Comprehensive Analytical Chemistry, Characterization and Analysis of Microplastics, 75, pp. 119–151. https://doi.org/10.1016/bs.coac.2016.10.001.

Rochman, C.M., 2015. The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 117–140. https://doi.org/10.1007/978-3-319-16510-3_5.

Romeo, T., Pedà, C., Fossi, M.C., Andaloro, F., Battaglia, P., 2016. First record of plastic debris in the stomach of Mediterranean lanternfishes 10.

Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., de Vries, W., de Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B., Sörlin, S., 2015. Planetary boundaries: guiding human development on a changing planet. Science 347. https://doi.org/10.1126/science.1259855.

Suaria, G., Achtypi, A., Perold, V., Lee, J.R., Pierucci, A., Bornman, T.G., Aliani, S., Ryan, P.G., 2020. Microfibers in oceanic surface waters: a global characterization. Science Advances 6, eaay8493. https://doi.org/10.1126/sciadv.aay8493.

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Philos. Trans. R. Soc. Lond., BBiol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284.

UNEP, 2011. The Chemicals in Products Project: Case Study of the Textiles Sector. United Nations Environment Programme DTIE/Chemicals branch.

Unger, B., Rebolledo, E.L.B., Deaville, R., Gröne, A., IJsseldijk, L.L., Leopold, M.F., Siebert, U., Spitz, J., Wohlsein, P., Herr, H., 2016. Large amounts of marine debris found in sperm whales stranded along the North Sea coast in early 2016. Mar. Pollut. Bull. 112, 134–141. https://doi.org/10.1016/j.marpolbul.2016.08.027.

Völker, C., Kramm, J., Wagner, M., 2020. On the creation of risk: framing of microplastics risks in science and media. Global Chall. 4, 1900010. https://doi.org/10.1002/gch2.201900010.

Walker, W.A., Coe, J.M., 1989. Survey of marine debris ingestion by odontocete cetaceans. In: Proceedings of the 2nd International Conference on Marine Debris. R. S. Shomura and M.L. Godfrey, Honolulu, Hawaii, pp. 747–774.

Xiong, X., Chen, X., Zhang, K., Mei, Z., Hao, Y., Zheng, J., Wu, C., Wang, K., Ruan, Y., Lam, P.K.S., Wang, D., 2018. Microplastics in the intestinal tracts of east asian finless porpoises (Neophocaena asiaeorientalis sunameri) from Yellow Sea and Bohai Sea of China. Mar. Pollut. Bull. 136, 55–60. https://doi.org/10.1016/j.marpolbul.2018.09.006.

Zhu, J., Yu, X., Zhang, Q., Li, Y., Tan, S., Li, D., Yang, Z., Wang, J., 2019. Cetaceans and microplastics: first report of microplastic ingestion by a coastal delphinid, Sousa chinensis. Sci. Total Environ. 659, 649–654. https://doi.org/10.1016/j.scitotenv.2018.12.389.