Evaluating the presence of microplastics in striped dolphins \textit{(Stenella coeruleoalba)} stranded in the Western Mediterranean Sea

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A B S T R A C T

Litter is a well-known problem for marine species; however, we still know little about the extent to which they're affected by microplastics. In this study, we analyse the presence of this type of debris in Western Mediterranean striped dolphins' intestinal contents over three decades. Results indicated that frequency was high, as 90.5\% of dolphins contained microplastics. Of these microplastics, 73.6\% were fibres, 23.87\% were fragments and 2.53\% were primary pellets. In spite of the high frequency of occurrence, microplastic amount per dolphin was relatively low and highly variable (mean ± SD = 14.9 ± 22.3; 95\% CI: 9.58–23.4). Through FT-IR spectrometry, we found that polyacrylamide, typically found in synthetic clothes, was the most common plastic polymer. Here, we establish a starting point for further research on how microplastics affect this species' health and discuss the use of striped dolphins as indicators of microplastics at sea.

1. Introduction

Plastic is ubiquitous in the marine environment. Its input to seas and oceans has been increasing since the 1970s due to its availability and the ease and cost of production for both industrial and consumer use (Dauvergne, 2018). Jambeck et al. (2015) estimated that between 4.8 and 12.7 million tonnes of plastic were disposed in the oceans in 2010; and Eriksen et al. (2014) estimated that there were more than 5 trillion plastic pieces floating at sea, most of which seem to be microplastics (< 5 mm, Gago et al., 2016; Galloway et al., 2017). On top of that, further increases in microplastic inputs are expected due to the massive consumption, misuse and mismanagement of personal protective equipment (PPE) and single-use plastic in the context of the COVID-19 pandemic (Fadare and Okoffo, 2020; Prata et al., 2020). Knowing if these microplastics are primary or secondary can provide us with information about the pollution source in different areas (Gago et al., 2016). For instance, primary microplastics are often manufactured in the form of microbeads, microfibres or glitter and are usually found in cosmetic products, textiles, air blasting and anti-fouling systems as well as in some drug delivery systems (Galloway et al., 2017; Guerranti et al., 2019; Tagg and Ivar do Sul, 2019). Alternatively, secondary microplastics are generated by the degradation of bigger plastic items that undergo physicochemical weathering when they are released into the environment. Degradation processes include photooxidation, mechanical breakdown and biodegradation (Gewert et al., 2015), and they can take up to years to completely break down plastic debris. For example, a bag made of polyethylene terephthalate (PET) can take more than 50 years to degrade completely under the sea in natural conditions (Webb et al., 2013). Furthermore, the rate at which we generate plastic is quicker than its degradation rate, so this contributes to further accumulation of plastics in the marine environment and, consequently, in marine biota.

Microplastics float and sink through the water column, where they can be ingested by marine organisms, either through direct capture or through feeding on previously exposed organisms. This way, microplastics potentially affect the entire food web (Choy et al., 2019; Nelms et al., 2019). Their presence and effects on small marine species, such as zooplankton, filter feeder invertebrates and some fish, is starting to be well documented (Cole et al., 2013; Karlsson et al., 2017; Messineti et al., 2018). However, studies of microplastics in non-commercial large marine vertebrate species, such as cetaceans, are less common. Cetaceans are regarded as reliable sentinels of marine pollution due to their position at the top of the marine food web, conspicuous nature, and reliance on marine resources (Frias et al., 2010; Schwacke et al., 2013; Bakir et al., 2014; Ivar do Sul and Costa, 2014; Fossi et al., 2018). Up to date, 60\% of cetacean species have been documented to have interacted with plastic items (Fossi et al., 2018) but only a handful of studies have looked for microplastics directly in their digestive tracts (Table 1).

Marine debris, particularly macroplastics, affect cetaceans mainly by entanglement and/or ingestion, the later sometimes in massive

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amounts (e.g. Unger et al., 2016). When ingested, macroplastics may cause severe negative effects such as intestinal blockages or malnutrition, among others (Moore et al., 2013). Nevertheless, it is very unlikely that microplastics will cause severe damage or the death of these animals, as their size in relation to the organism’s size is negligible. Notwithstanding, microplastics may hide other potential threats, such as their association with Persistent Organic Pollutants (POPs). Microplastics can adsorb high concentrations of POPs already present in the environment that eventually leak out into the organism’s body; disrupting the function of their organs due to chronic exposure (Bakir et al., 2014). In addition, additives in their composition, such as phthalates, are suspected of endocrine disruption, and having a significant impact during the juvenile cetaceans’ development as well as on the reproductive success in their adult stage (European Commission DG ENV, 2000). Due to all these reasons microplastic ingestion could represent an additional factor hindering cetacean populations’ stability and survival, decreasing their fitness and adding to other stressors such as food depletion and infections (Fossi et al., 2018). Hence, large amounts of microplastics ingested over long periods of time may decrease the resilience and adaptability of cetacean populations. It is therefore of great importance to scan the occurrence, categories, and origin of microplastics in long-living marine vertebrates such as cetaceans (Schwacke et al., 2013; Fossi et al., 2018) in order to lay the foundations for future monitoring and experimental studies focusing directly on risk, environmental and health assessments.

The present study arises from the lack of microplastic quantification in resident or transient cetaceans in the Mediterranean Sea, and from the need of comparing results from this region with other studies elsewhere. We study *Stenella coeruleoalba* (family Delphinidae), commonly known as the striped dolphin, because it is the most abundant cetacean species along the western Mediterranean waters (Gómez de Segura et al., 2006; Aguilar and Gaspari, 2012). It inhabits temperate and subtropical waters of all oceans (Aguilar and Gaspari, 2012). In the Mediterranean, it is found mainly as resident species in open waters beyond the continental shelf (Notarbartolo di Sciarra et al., 1993; Forcada et al., 1994; Gannier, 2005; in Aguilar and Gaspari, 2012), although more neritic incursions have been recorded during the last two decades (Aznar et al., 2017). The IUCN (International Union for the Conservation of Nature; Aguilar and Gaspari, 2012) classifies the Mediterranean subpopulation as vulnerable on the Red List of Threatened Species. Mediterranean *Stenella coeruleoalba* seemed to feed mainly on oceanic cephalopod species (Blanco et al., 1995; Meissner et al., 2012) but the diet may have shifted significantly in the last decades towards more neritic species such as juvenile hake (*Merluccius merluccius*) and southern shortfin squid (*Illex coindetii*) (Aznar et al., 2017). Like other marine organisms, they can ingest microplastics directly from the water column, via trophic transfer, or during the inhalation at the air-water interface (Lusher et al., 2015; Fossi et al., 2018).

We here quantify and characterise by direct observation the microplastics in the digestive tract of a representative species from the Western Mediterranean Sea, the striped dolphin. We take advantage of the existence of a well-established stranding network in East Spain (see Material and Methods section), and the availability of fresh carcasses, to increase the knowledge about the interactions between microplastics and this pelagic – oceanic cetacean species.

### 2. Materials and methods

#### 2.1. Study area

The Valencian Community is located in the East of Spain (from 40°31’N – 0°31’E to 37° 51’N – 0° 45’W, Fig. 1). Its coast is 518 km long and it is divided into three provinces: Castellón (139 km long), Valencia (135 km long), and Alicante (244 km long), north to south. In total, it has 4,935,010 inhabitants. Including their metropolitan areas, Valencia is the most populated area (1,550,885 inhabitants), followed by Alicante (452,462 inhabitants) and Castellón (144,446 inhabitants, INE, 2019). All three cities are located on the coast. Tourism is the most important economic activity of the region, especially during the summer months. In 2019, 27.8 million tourists visited the Valencian Community (Turisme Comunitat Valenciana, 2019).

The main coastal surface sea current flows from north to south and several rivers outflow in the area. The most important one is the Ebro River, which outflows just above the northern limit of the Valencian Community (Fig. 1).

#### 2.2. Gut content analysis

Digestive tracts (*N* = 43) were obtained from necropies of fresh striped dolphins’ carcasses. Strandings and necropies in the region are managed by a coordinated network formed by private and public institutions (Tomás et al., 2008). All dolphins were found stranded dead on beaches from the Valencian Community between 1988 and 2017, with the exception of one that died at the rescue center. All the animals used for analyses were in apparent good condition according to body shape, except one that was reported as thin during the examination previous to the necropsy. We classified the state of the carcasses by following the criteria proposed by Geraci and Lounsbury’s (2005) stranding field guide. In this scale, 1 means “alive”; 2, “freshly dead”; 3, “starting decomposition but organs basically intact”; 4, “advanced decomposition”; and 5, “mummified or skeletal remains only”. Only individuals belonging to states 2 (75.6%) or 3 (24.4%) were taken into account for the analyses. Choosing states 2 and 3 guarantees that the carcass was not floating in the sea or laying in the sand for a long time before the examination and necropsy; reducing potential post-mortem contamination. Furthermore, none of the dolphins presented a meaningful amount of sand in mouth or upper digestive tract; which further confirms that sand-borne contamination was residual. We used sterile and stainless steel material in all the necropsies. Digestive tracts prior to

### Table 1

Published research articles reporting microplastics by directly looking at digestive tracts of cetaceans in Europe.

| Species                  | Location                                | Scientific articles                                                                 |
|--------------------------|-----------------------------------------|--------------------------------------------------------------------------------------|
| *Delphinus delphis*     | Ireland, United Kingdom and NW Spain    | Curran et al., 2014; Hernández-González et al., 2018; Lusher et al., 2018; Nelms et al., 2019 |
| *Phocoena phocoena*     | Ireland and United Kingdom              | Lusher et al., 2018; Nelms et al., 2019                                              |
| *Stenella coeruleoalba* | Ireland and United Kingdom              | Lusher et al., 2018; Nelms et al., 2019                                              |
| *Tursiops truncatus*    | Ireland and United Kingdom              | Lusher et al., 2018; Nelms et al., 2019                                              |
| *Grampus griseus*       | United Kingdom                          | Nelms et al., 2019                                                                  |
| *Kogia breviceps*       | United Kingdom                          | Nelms et al., 2019                                                                  |
| *Lagenorhynchus acutus* | United Kingdom                          | Nelms et al., 2019                                                                  |
| *Lagenorhynchus albirostris* |                                              | Nelms et al., 2019                                                                  |
| *Orcinus arca*          | Ireland                                 | Lusher et al., 2018                                                                  |
| *Euphius cavroisi*      | Ireland                                 | Lusher et al., 2018                                                                  |
| *Megaptera novangiae*   | The Netherlands                         | Besseling et al., 2015                                                               |
| *Mesoplodon mirus*      | Ireland                                 | Lusher et al., 2015                                                                  |
2017 ($N = 30$) were closed with natural-fibre strands and frozen immediately at $-20 \, ^\circ C$ after necropsy and thawed at room temperature for the study; while digestive tracts from 2017 ($N = 11$) were dissected during the necropsies.

We analysed digestive contents for microplastic detection following Lusher et al. (2015) and Foekema et al. (2013) protocols with few adaptations, but always took care to avoid contamination. We washed the contents through 200 $\mu$m and 100 $\mu$m nested sieves. Prior to microplastic analysis, parasites and diet items were rinsed over the sieve and then separated for further studies. After this step, contents were introduced into glass bottles with a filtered 10% KOH solution, 3 times the volume of the contents during three weeks. After digestion, we filtered the resulting solutions under vacuum using a Büchner filter and GF/C microfiber filters in a type I laminar flow cabinet with positive pressure to guarantee that external air-borne particles could not contaminate the samples. When remains were too complex and dense to accurately separate microplastics, we resuspended the digestion product with a supersaturated solution of NaCl (140 g/L) and let it set for 24 h. Afterwards, the supernatant was re-filtered under vacuum as mentioned above. We identified the particles retained on the filters with a dissecting microscope (Leica MZ APO, 8–80×). When doubts arose concerning the nature of some particles, each particle was exposed to a hot needle to see whether it changed its shape, hence confirming its plastic composition (Hanke et al., 2013).

We classified plastic items into categories according to their shape and colour, following the protocol used by the Harbour and Coasts Study Centre (Spain, Ministry of Agriculture and Fishing, Nourishment and Environment, 2017). Only microplastics, i.e. plastic items smaller than 5 mm (GESAMP, 2015; Gago et al., 2016), were considered for the analyses.

2.3. Polymer analyses

After the classification and quantification of items, we kept 30 microplastic items for polymer identification. These items were selected randomly. We identified polymers by ATR FT-IR (Attenuated Total Reflection Fourier Transformed Infrared Spectroscopy) with an Agilent Technology Cary 630 spectrometer. We thoroughly cleaned the ATR diamond and its base with ethanol before and after procedure, between every sample and in between measurements of the same sample. Before every sample scan, the spectrometer scanned the background 8 times. Microplastics are solid samples that do not need to be included in any particular material; hence, the background was the air filling the working space. The experimental nominal working resolution was 4 and the apodization function used was Happ-Genzel. We analysed each sample three times in order to assure accuracy; and in each measurement, 8 scans were performed. We used ATR as the measurement mode and set the wavelength range to 4000–650 cm$^{-1}$. We compared the resulting spectra with those from our custom polymer library and only accepted matches above 70% as valid. All samples scoring under 70% were classified as “Unidentified”. We analysed all the spectra using the spectrometer’s native software Agilent Microlab.

2.4. Contamination control

Blank samples (GF/C filters) were exposed to open air in the laboratory at the same time digestive contents were filtered through nested sieves in the necropsies’ facilities in order to assure that airborne contamination was produced. Procedural blanks were subjected to the same process as the digestive tract samples. Tap water was tested for the presence of microplastics by previously filtering 5 L following the same procedure than with the samples and the procedural blanks. KOH solutions were prepared with Milli-Q water and filtered before use. Only glass and stainless steel material was used to manipulate and...
store the samples. White cotton laboratory coats and blue nitrile gloves were used during the whole process, and single-use gowns were used on top of them during necropsies according to health and safety protocols. Sponges used for surface cleaning were always made of the same material and colour (bright yellow) so as to identify potential contamination quickly. Any plastic item found in samples with the same characteristics of the tools used in the working space was discarded to prevent false positives. All materials were thoroughly cleaned with filtered deionized water, soap and ethanol 70%; and then checked under a dissecting microscope for any microplastic presence prior to analysis.

2.5. Statistical analyses

Microplastic content information and biometric data were registered in a Microsoft Excel (version 16.4.1) spreadsheet. We calculated 95% confidence intervals for the mean microplastic content, excluding cases without microplastics, by bootstrapping 10,000 replications in the free software QPweb v1.0.13. Likewise, the confidence interval for prevalence was obtained by Sterne’s method. We calculated the rest of the analyses using R Studio version 1.0.143. Specifically, we explored data with descriptive statistics and checked the normality of these data using both graphical tools and the Shapiro-Wilk test. To explore temporal changes, we separated microplastic content per individual into two groups of dolphins collected in two separated periods, 1989–2007 and 2010–2017, since there were no dolphins collected in the period 1989–2007. We used the Mann-Whitney-U test to check for differences between these groups. Finally, we used the Kruskal-Wallis test to see whether location, sex and state of the carcass had any effects on the amount of microplastics ingested by dolphins.

3. Results

3.1. Dolphin biometrics and microplastic counts

Mean total length (± SD) of dolphins was 160.31 ± 66.38 cm. According to their body length, they were all adults or late juveniles. Also, 55.8% were males (N = 24) and 44.2% females (N = 19), Location, biometric data and other information are provided in the Supplementary material.

Supplementary material.

3.2. Microplastic colours and polymers

In total, the predominant microplastic colour found in the dolphins was black (50.1%), followed by red (21.2%), translucent (10.9%), white (3.8%), and other less frequent colours, such as yellow (Fig. 3). Specifically, most of the fibres were black (68.4%), followed by red (23.2%), translucent (5.1%), green (2.9%), white and yellow (0.2%) each, see Fig. 3). On the other hand, colour of fragments was mostly translucent (46.8%), followed by white (15.8%), black (15.2%), red (2.5%), green and others (1.3% each, Fig. 3). Table 2 shows total mean and median items per colour, together with their confidence interval.

3.3. Polymer analysis

From items analysed by FT-IR, 40.9% were of polyacrylamide, 27.3% were PET (polyethylene terephthalate), 13.6% were alginic acid and 9.1% were HDPE (high density polyethylene) (Figs. 4, 5, Table 3). Of these polymers, 73% were synthetic polymers, 15.4% were not plastic polymers (alginic acid and 1 sample identified as cellulose filter paper) and 11.5% were unmatched (under 70% of match). Polymers identified as non-plastics were excluded from the analyses. Concerning the cellulose filter paper, it could not be considered as contamination during the laboratory procedure since we did not use cellulose filter papers at any time. Notably, all of the items identified as PET were specifically linked as belonging to polyester fibres (see Table 3).

3.4. Temporal and sex effects on microplastic amount

Concerning changes over time, no significant differences in microplastic contents, colours or shape were found between dolphins collected during the period 1989–2007 and dolphins collected during the period 2010–2017, nor between males and females or state or carcass (U-Mann-Whitney, p > 0.005; Kruskal-Wallis, p > 0.005, respectively).
3.5. Blanks and contamination control

Procedural and air-borne contamination control blanks were all clean. Nevertheless, two items were discarded because they were very similar to working materials (nitrile gloves and yellow sponge). Finally, three microplastics were found from the filtered tap water, so we subtracted them from the total counts.

4. Discussion

4.1. Microplastic contents

The number of microplastics found per dolphin was relatively low for their body size. Approximately 50% of the individuals had less than 5 items, and if we look closely, 25% of the dolphins had just one microplastic item or no microplastics at all. Dolphins with high microplastic abundance were not frequent and presented secondary microplastics made of the same colour and consistency. An explanation for this could be that all of those items were generated from a single and bigger piece of plastic, although we cannot prove nor assure this statement. Although low, average microplastic content is similar to the amounts found in other cetacean species (Table 4) and higher than in fish species from the area. For instance, Nadal et al. (2016) found an average of 3.75 microplastics per individual in *Boops boops*, Alomar and Deudero (2017) found 0.34 microplastics per individual in *Galeus melastomus* and Compa et al. (2018) found 0.21 microplastics per individual in *Sardina pilchardus* and 0.18 ± 0.20 in *Engraulis encrasicolus*.

Regarding microplastics in Mediterranean waters, de Haan et al. (2019)

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**Table 2**  
Microplastics prevalence by colour in striped dolphins from the Valencian Community waters. Mean ± SD, median, and confidence intervals were calculated excluding individuals with no microplastics (N = 38). In “Others”, CI (95%) for the mean could not be calculated due to the constant nature of the variability.

|        | Black | Red       | White      | Translucent | Green | Others  |
|--------|-------|-----------|------------|-------------|-------|---------|
| Prevalence (%) | 76.7  | 46.5      | 11.9       | 30.2        | 20.9  | 6.9     |
| Mean ± SD  | 10.72 ± 11.53 | 5.85 ± 9.79 | 11 ± 5.79  | 7.62 ± 12.34 | 1.78 ± 0.83 | 1 ± 0 |
| Median    | 5     | 3         | 11         | 3           | 2     | 1       |
| Range     | 1–37  | 1–45      | 2–17       | 1–45        | 1–3   | 1       |
| CI (95%) for the mean | 7.15–15.2 | 3.25–14.3 | 5–14.4     | 3.15–18.7 | 1.22–2.22 | –      |
| CI (95%) for prevalence | 61.7–87.6 | 32.5–61.7 | 4.8–52.9   | 18.4–45.3  | 11.1–35.9 | 0–0.12 |
found 0.18 ± 0.16 items/m² on average at the sea surface. Due to these low numbers, and also to the small size of microplastics compared to dolphin size and digestive tract length, these results have to be taken with caution when considering potential impacts on dolphins’ health. It is not likely that microplastics themselves pose a physical threat to this species. However, a chemical threat cannot be ignored. Substances present on plastics, such as POPs and endocrine disruptors, are of concern. They can be either part of or adsorbed to microplastics and contribute to the total burden of chemicals to which animals are exposed at sea (Gallo et al., 2018). Endocrine disruptors can be a potential threat for population stability, causing low reproduction rate and hormonal dysfunction (Gallo et al., 2018).

In the present study fibres were more abundant than particles, and both categories were more abundant than primary microplastics. Primary microplastics were found just in two dolphins and all were white industrial pellets. Neither glitter particles nor cosmetic microbeads were found. This is in line with the results of other studies performed in marine mammals and top predator fishes in the European waters of the Atlantic Ocean (Lusher et al., 2015; Bellas et al., 2016; Lusher et al., 2018; Nelms et al., 2018; Nelms et al., 2019), as well as with the results obtained in analyses of waters and sediments of the Ebro river (East Spain, Simón-Sánchez et al. 2019). In fact, after extensive literature research, we found that prevalence of pellets and primary microplastics seems to be low in all studies. This may indicate that sources of industrial pollution are less important than pollution by citizens that consume plastics, or that they are less accessible to marine fauna, not entering in the food webs as other types of microplastics.

As for microplastic colours, the most abundant and frequent was black, followed by red, translucent, white and others (Table 2). Other studies performed in the Atlantic Ocean and other areas of the Mediterranean Sea got similar results to ours (Bellas et al., 2016; Nelms et al., 2018), with dominance of black, red, translucent and white; although the most extensive study performed up to date (Lusher et al., 2018) reports black in the third place, with its ranking being headed by blue and grey. Unlike in the present study, none of these previous studies distinguished in between the colour of fibres and particles. We believe that this is something to consider in such type of studies, since colours of the plastics found may help in identifying their origin and to study whether there is a link between the colour and the different type of polymers. However, this approach has limitations because colour is eventually washed out due to weathering in the environment and also during the digestion process in the digestive tracts.

In 2015, Spain was the destination with the highest number of foreign tourists in Europe, and it is always among the top four destinations receiving the most tourists in the continent (Eurostat, 2017). Valencia is the most populated province of the Valencia Community, although all three provinces multiply their population during spring and summer months, as mentioned above. These visitors add to the

### Table 3

| Type of microplastic analysed by FT-IR and reliability of the match with the corresponding polymer. Microplastics with a reliability lower than 0.7 were discarded from the analysis. PET: polyethylene terephthalate, HDPE: high density polyethylene. |
|---|
| **Type** | **Polymer** | **Reliability** |
| Fibre | Polyacrylamide | 0.8509 |
| Fibre | PET | 0.7969 |
| Fibre | Polyacrylamide polymer | 0.7347 |
| Fibre | Polyacrylamide polymer | 0.7347 |
| Fibre | Polyacrylamide | 0.85609 |
| Fibre | PET | 0.79609 |
| Fibre | PET | 0.79609 |
| Fibre | Polyacrylamide | 0.85609 |
| Fibre | PET | 0.79609 |
| Fibre | PET | 0.79609 |
| Fibre | HDPE | 0.82155 |
| Fibre | Polyacrylamide | 0.7347 |
| Fibre | Latex | 0.600 |
| Fibre | Alginic acid | 0.86094 |
| Fibre | Polyacrylamide | 0.78268 |
| Fragment | Alginic acid | 0.79238 |
| Fragment | Polyacrylamide | 0.83886 |
| Fragment | Natural latex | 0.6 |
| Fibre | Alginic acid | 0.8508 |
| Fibre | Polyacrylamide | 0.77633 |
| Fibre | Polyacrylamide | 0.77633 |
| Fragment | HDPE | 0.76802 |
| Fragment | Polycrylamide | 0.86652 |
| Fragment | Cellulose filter paper | 0.82174 |
| Fragment | Natural latex | 0.6 |
| Fragment | HDPE | 0.82155 |
| Fibre | Unidentified | Fail |
| Fibre | Unidentified | Fail |
| Fibre | Unidentified | Fail |

![Debris resulting from KOH (10%) digestion of gut contents of one striped dolphin stranded in the Valencian Community coast on a GF/C microfiber filter. Red arrow indicates the presence of a blue microplastic. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image)
basal population, potentially increasing debris input to the sea, either directly at the beaches or through consumption elsewhere. Moreover, the Ebro River, which is the river carrying the largest water flow in the Spanish Mediterranean, outflows just above the northern limit of the Valencian Community (Fig. 1). Several studies have pointed out that this river carries important amounts of microplastics in its waters, being most of them fibres (Simon-Sánchez et al., 2019 and references therein). Ebro River influence on Valencian Community waters is very important due to the dominant coastal current that flows from north to south along the coast (André et al., 2005), potentially carrying contaminant and debris discharged by the Ebro River along with it. According to the model designed by Liubartseva et al. (2018), Catalanian waters (northeast Spain) are the second biggest hotspot for microplastic accumulation in the whole Mediterranean Sea, just headed up by the Sicilian sub-basin and followed by the Po River Delta in Italy. Additionally, the coast of the Valencia province (Fig. 1) also appears as one of the areas having the highest densities of plastic debris at the sea surface in the Mediterranean Sea (Liubartseva et al., 2018). In fact, plastic debris have been reported as highly frequent in marine species from the same area, such as the loggerhead sea turtle (Domènech et al., 2019 and references therein). Knowing riverine input dynamics into the sea and sea currents’ flow direction is essential in order to identify pollution sources and the movement of contaminants. Nevertheless, there are not many studies available in this aspect and there are a lot of knowledge gaps (Guerranti et al., 2020).

The most abundant polymer in the analysed dolphins was polyacrylamide, a water-soluble polymer. It is used as a thickening and absorbing agent, serving applications in soil conditioning, cosmetics, flocculation operations, and oil recovery tasks. It can polymerize in the presence of free radicals (European Chemicals Agency, 2017). It may be found at sea due to the high amount of cosmetics used by population. Or it could appear as a subproduct of oil extractions, such as the one existing just above the northern limit of the Valencian Community, in the surrounding waters of the Ebro River delta. Nowadays, there is the active Casablancan oil platform in Tarragona waters (south Catalonia), which has had at least one known spill of 8000 L of crude oil in the area, (De la Torre and Albàigés, 2016; BOE 301, 2018). However in our study, items containing polyacrylamide (all of them fibres, see Table 3) probably came from clothes and other textiles. Acrylic polymeric fibres were also detected in two samples. Alginic acid is a polyacrylamide and polyester were also abundant. Even more different results were found in the study of Nelms et al. (2018) who analysed grey seal scats (Halichoerus grypus), and found that PET constituted only 28% of the samples, while PP and ethylene propylene were more abundant. In another study on fish species in Portugal (Nevés et al., 2015), PP was also the most abundant polymer found, followed by PET, resin, rayon, polyester, nylon and acrylic. Sample sizes in all of these studies, as in ours, are too small to draw conclusions about differences in polymer composition among species and locations. Analysing 10% of the items found is important to get a minimum reliability. This was a limitation in our study, but we highly encourage the standardization of this protocol to determine the origin of the microplastics found and to carefully watch out for items that result to not be plastics.

Analysing the amount and composition of plastics in striped dolphins helps us to understand the extent to which microplastics are present in marine organisms and to assess whether they pose a risk to the dolphin population or not. Furthermore, they are at the top of the trophic chain, which can give us an idea about the accumulation of plastics in predators and in higher trophic levels than other organisms normally used, such as filter feeders. Together with data about other species, we would be able to perform detailed studies about microplastics’ distribution in the trophic chain. However, the use of striped dolphins as bioindicators of environmental health is not ideal. Firstly, this is a protected species and getting the carcasses depends on the presence of a well-established stranding network, and fresh carcasses are not easy to obtain. Secondly, processing their long digestive system is very time consuming and it is sometimes difficult to control air-borne and tap water contamination. Moreover, most of the rescue centres that also gather data do not have the proper facilities to do so. And finally, the digestion process can make the separation process difficult in dolphins that have recently eaten; and microplastics weathering can make polymer identification a real challenge. We think that it is of interest to monitor microplastics in cetaceans, but smaller species such as fish and molluscs seem more appropriate for routine checks on marine pollution, environmental status and water quality due to their size and ease of sampling and processing. Nonetheless, below we further discuss the role of striped dolphins as indicators of microplastics at sea.

According to studies performed by Plastics Europe (2018), recycling of plastics has increased 79% in a 10-year period, plastics used as energy recovery have increased 61% and landfill disposal of plastics has decreased 43% (2006–2016) in Europe. However, despite these figures, the proportion of recycled plastics worldwide is still small, and Spain is trailing behind countries from northern and central Europe. On top of that, the harm to the environment caused by synthetic fibres, the most common type of microplastic found in the present study, has yet to be

Table 4

| Study                  | Microplastics | Fibres (%) | Fragments (%) | Polymer analysis (RAMAN or FTIR) |
|------------------------|---------------|------------|---------------|----------------------------------|
| Present study          | 14.9 average/individual | 73.6      | 23.87         | Yes                              |
| Nelms et al., 2019     | 5.5 average/individual  | 84         | 16            | Yes                              |
| Hernández-González et al., 2018 | 12 average individual      | 96.59     | 3.16          | No                               |
| Lusher et al., 2018    | 1–88 (range)   | 83.6       | 16.4          | Yes                              |
| Lusher et al., 2015    | 29 mps in one individual | 58        | 42            | Yes                              |
| Besseing et al., 2015  | 16 items in one individual (mp amount not specified) | 25        | 75            | Yes                              |
| Curran et al., 2014    | 1 in one individual   | Not specified | Not specified | No                               |
completely understood and, therefore complicates the search for alternatives that would allow dealing with this specific item. Long-term monitoring is necessary in order to see temporal trends of microplastic presence in biota and create appropriate models that would allow describing debris distribution and, therefore, propose appropriate mitigation plans.

4.2. Microplastic analyses' advantages and limitations

Data collected through opportunistic sampling by strandning networks can be biased due to seasonal and spatial variation in natural factors and collection effort. Nevertheless, having constant protocols over years and discarding animals in bad condition gives us some consistency in the observations (Hart et al., 2006; Witt and Godley, 2007; Tomás et al., 2008). As a result of this, we were able to analyse a relatively large sample size of this dolphin species (N = 43), which is unusual when studying a wild protected species. In opposition to our relatively large sample size of this dolphin species (N = 43), it is unusual when studying a wild protected species. In opposition to our study, studies on detection of plastic in cetaceans in the Mediterranean have focused on biomarker studies so far (Fossi et al., 2012, 2014, 2016). However, biomarker methods, such as testing the presence of phthalates in tissues, cannot assure whether the origin of these phthalates are microplastics, as they can be leaked from different materials like waterproof membranes or macroplastics. Likewise, biomarker studies using the oxidative response of organisms cannot fully link biochemical stress to specific factors such as interaction with plastics, given that these responses are general defensive reactions and can be triggered by a variety of reasons, including stress caused by predators (Lasher et al., 2018). Taking into consideration the advantages and limitations that each approach has, we do think that the combination of these two types of studies is necessary, as they may help in elaborating more accurate, complete and sophisticated environmental and population health assessments.

On the other hand, visual sorting of microplastics is sometimes biased and quite difficult to perform when the product of the digestion is complex; since sometimes microplastics and natural small items can be very similar to the eyes even under a microscope. Moreover, eyesight becomes tired at some point during observation, which makes reliability even lower. For this reason, it is important to try and use complementary methods such as polymer analyses. These challenges could be solved by using automated methods such as the one proposed by Primpke et al. (2017), consisting of performing an automated Focal Plane Array (FPA) which eliminates the observer bias, as well as factors such as tired eyesight or poor vision performance. However, as in our case, many laboratories do not have access to this technique. In spite of these handicaps, FT-IR is an easy-to-use, clean and cheap method for polymer analysis if we are willing to build our own reference library.

5. Conclusions

To summarize, the striped dolphin is frequently exposed to microplastics, mostly fibres. However, microplastic burden in their bodies seems relatively low for their body size. Hence, the concern is more about the chemical substances that can potentially damage dolphins’ health and impact their population than about the quantity of microplastics physically present in their digestive systems. The use of striped dolphins as indicators of microplastic presence in pelagic biota at sea in the western Mediterranean is limited due to the difficulties in sampling and long processing time. Nevertheless, monitoring them is interesting and important in order to assess threats to their population. Both techniques used here revealed the importance of fibres probably coming from clothes and textiles. Increasing the amount of data about polymer composition is fundamental in order to precisely target the origin of most complicated residues and decrease their input to the sea. Despite having samples from a long period, most of the dolphins analysed here were from recent years, therefore limiting the detection of potential changes in long term periods. Nonetheless, in this study we establish a good baseline for future studies on this topic.

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CRediT authorship contribution statement

Olga Novillo: conceptualization, sample collection, methodology, software, data analysis, writing of the manuscript.
Juan Antonio Raga: conceptualization, supervision, reviewing of the manuscript.
Jesús Tomás: conceptualization, supervision, reviewing of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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