Microplastics in the stomach contents of common dolphin (*Delphinus delphis*) stranded on the Galician coasts (NW Spain, 2005-2010)

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ABSTRACT

Plastic debris is currently recognised as one of the major global threats to marine life. However, few data exist on the presence and abundance of microplastics (plastics < 5 mm in size) in marine mammals. This is the first record of the presence of microplastics in the digestive tracts of marine mammals on the Iberian Peninsula. This study made use of 35 samples of common dolphin stomach contents. Potassium hydroxide was used to digest the organic fraction in the stomach contents and to facilitate the retention of solid elements (microplastics and prey remains) from the samples. Microplastics were identified in all the samples analysed but their abundance varied greatly from one stomach to another. Most plastic items were small fibres although some fragments and a bead were also found. Although factors affecting accumulation of microplastics and their effect on common dolphins are unknown, the fact that all stomachs analysed contained microplastics is a cause for concern.

Highlights

- This is the first study that focuses on microplastics in the stomach contents of marine mammals in the NW Iberian Peninsula.
- A specific protocol designed to obtain the microplastics, but also to preserve for diet analysis the hard structures of the dolphin prey, was used.
- On average, 12 microplastics items were identified in each stomach contents analysed and their type, number and size are described in detail.

Keywords: Microplastics; Common dolphin; Marine mammals, NE Atlantic

1. Introduction

Plastics have been reported to constitute between 60% and 80% of debris in the marine environment (Derraik, 2002), and they are currently considered as one of the greatest threats to marine biodiversity (UNEP, 2011), believed by Halpern *et al.* (2008) to be above other environmental threats such as resource overexploitation, other types of pollution, invasive species or climate change..

Every year it is estimated that between 5 and 13 million tonnes of plastic end up in the ocean, mainly as a result of poor waste management (Jambeck *et al.*, 2015). Due to the physical and chemical characteristics of plastics, which make them very resistant to heat, oxidative damage and microbial degradation (Thompson *et al.*, 2009), very long time-periods are necessary for these materials to become fragmented and decomposed (Cole *et al.*, 2011; Vroom *et al.*, 2017). In addition, plastics are easily transported by rivers, winds and ocean currents, accumulating along coastlines and within mid-ocean gyres (see Van Sebille *et al.*, 2015). For all these reasons, plastics have a ubiquitous distribution in the marine environment (Cole *et al.*, 2011).

Plastics can provoke negative effects on marine organisms, e.g. by causing external physical injuries like strangulation, movement restriction or even amputations (see e.g.

Williams *et al.*, 2011; Baulch and Perry, 2014; Sigler, 2014); or internal injuries and starvation by totally or partially blocking the digestive tract (see Gall and Thompson, 2015), sometimes causing the death of the individual (De Stephanis *et al.*, 2013).

Microplastics (*MPs*), defined as "small plastic pieces less than 5 millimetres long" (GESAMP, 2015), can float, be neutrally buoyant or sink depending on their composition, density and shape. Consequently, *MPs* can be found in the whole water column and even as part of sea floor sediments (see review in Cole *et al.*, 2011). *MPs* have also been found in the digestive tracts of a large number of marine species such as zooplankton (Sun *et al.*, 2017), crustaceans (Goldstein, 2013), fish (Lusher *et al.*, 2013), sea turtles (Santos *et al.*, 2015), seabirds (Van Franeker and Law, 2015) and marine mammals (e.g. Besseling *et al.*, 2015; Lusher *et al.*, 2015a).

. Because *MPs* have a large surface-volume ratio, they can adsorb on their surface Persistent Organic Pollutants (*POPs*) or heavy metals from the surrounding water (Cole *et al.*, 2011; Fossi *et al.*, 2014), and consequently, ingestion of small pieces of plastics might contribute to incorporation of pollutants into the tissues (Tanaka *et al.*, 2013). Besides, as noted by Alonso *et al.* (2014), once *POPs* are incorporated into the food chain they can bioaccumulate and biomagnify with each trophic level. So when these pollutants reach high concentrations inside the body, they can interfere with important biological processes (see review in Rochman, 2015).

The short-beaked common dolphin (*Delphinus delphis*), is the most frequently observed cetacean in the waters of the Galician continental shelf (NW Spain) (Saavedra *et al.*, 2018) and it is also the cetacean species most commonly found stranded on the Galician coast (Lopez *et al.*, 2002). In this study, we analysed the presence of *MPs* in a historical collection of 35 stomach contents of common dolphins stranded in Galicia between 2005 and 2010. The methodology was based on those used in previous studies performed on other taxa (e.g. Besseling *et al.*, 2015; Lusher *et al.*, 2015a). The aims of our analyses were to: (a) describe, quantify and measure the number of *MPs* in the stomach contents of common dolphins; (b) examine the

occurrence of these *MPs*; (c) compare the size and number of *MPs* between sexes and size classes of these cetaceans; and (d) determine whether there is any temporal trend in the presence of *MPs* in common dolphin stomachs.

2. Materials and methods

2.1 Study area and samples collection

Galicia is situated in the North-west corner of the Iberian Peninsula (41°52' – 43°47' N, 7°02' – 9°17' W) and has almost 1500 km of coastline with a relative narrow continental shelf (with width of about 27km on average). The Galician stranding network has been operated by the NGO "Coordinadora para o Estudio dos Mamíferos Mariños" (CEMMA). Its trained personnel (equipped with nitrile gloves, rubber boots and waterproof suits to minimize the *MPs* cross-contamination) attend the strandings and collect *in situ* both biometric measurements and biological samples from the dolphin carcasses following the standardized protocol of the European Cetacean Society (Kuiken and Hartmann, 1993). Given this NGO does not have the necessary equipment nor have enough storage capacity to store frozen all the cetaceans stranded in Galicia over time, the stomach compartments of cetaceans with decomposition states ranging from 2 (fresh) to 4 (highly decomposed) (see Kuiken and Hartmann, 1993) were carefully opened in the field and their contents collected and stored in glass jars with 70% ethanol. Although the protocol followed does not prevent aerial contamination of the samples with microplastic fibres, it is expected that this was minimal since the stomach contents were directly emptied into the glass jar, which was then immediately sealed. **Fig. 1 shows** the stranding locations of the 35 common dolphins analysed in this study.

2.2 Laboratory procedure

We adapted protocols previously used for other taxa (e.g. Besseling *et al.*, 2015; Lusher *et al.*, 2015a, 2018) to the characteristics of our samples. These adaptations allowed us to preserve the dolphins' prey remains and parasites found in their stomach contents. The protocol used followed the steps described below:

- I. Stomach contents (stored in ethanol) were rinsed through a set of four metal sieves with a decreasing mesh size (5 mm, 1 mm, 0.5 mm and 0.355 mm) to facilitate cleaning of prey remains and to prevent the clogging of the sieves. To allow the possibility of later studies on diet and parasites, both prey remains (fish bones and otoliths, cephalopod beaks, eye lenses and crustacean remains) and parasites found in the samples were rinsed, removed, sorted and stored. Fish bones and otoliths were first sterilized with 70% ethanol and then dried and stored while all other remains were stored in 70% ethanol (following Santos *et al.*, 2013).
- II. The remaining material retained in the sieves was placed and sealed in sterilized glass containers to which a solution of 10% KOH was added (using a volume of approximately three times the volume of the retained material).
- III. After approximately three weeks (once the organic material was dissolved) the remaining solution was filtered under a vacuum pump using a Buchner Filter and glass microfibrefilters.
- IV. The glass microfibrefilters were placed in closed glass Petri dishes and dried in an oven at 50°C for 4 hours.
- V. Finally, *MPs* were visually identified under a Leica S8 APO stereoscopic microscope (Leica Microsystems GmbH, Wetzlar, Germany) fitted with a camera (Carl Zeiss Axiocam ERc5s) and measured using an image analysis software program (ZEN, Blue edition).

In the laboratory, to minimize the risk of contamination of the samples with synthetic textile fibres from researchers clothes, all the procedure was carried out at a clean

bench, by researchers wearing white laboratory coats (100% cotton) and blue nitrile gloves in order to be able to identify and discard the white microfibrils originating from laboratory coats (potential contamination) while manipulating the samples. In addition, all the stainless steel tools used to work with the samples were washed with distilled water before being used and metal sieves were covered while they were not in use to prevent airborne fibres affecting the samples.

2.3 Data analysis

The *MPs* identified were categorized according to their physical characteristics into 3 main groups: fibres, fragments and beads. Their colour and size were also recorded.

Because of blank samples were not performed following EU published guidelines (see Hanke *et al.*, 2013). Apart from regular analysis with all *MPs* items, to reduce the likelihood of add microfibers' airborne contamination into results obtaining more conservative conclusions, we also decided to do an extra analysis following the Foekema *et al.*, 2013 test about discard smallest microfibrils which could increase the background levels of *MPs* to get more conservative results.

The data on number of *MPs* per stomach content were tested for normality, they were found to be non-normally distributed and therefore non-parametric tests were used. The correlations between dolphin lengths and the size and number of *MPs* found in their stomach contents were tested using the Spearman rank correlation test. The existence of a trend in the number of *MPs* identified over the data series was also tested using Spearman's correlation between number of *MPs* and years. Differences in the size and number of *MPs* between the sexes of common dolphins were tested using the Kruskal-Wallis test. All tests were performed using the 'stats' package included in the statistical software R (version 3.4.2, R Core Team, 2017).

3. Results

The information on common dolphins stranded in Galicia, the stomach contents of which were analysed in this study shows that 14.3% ($n = 5$) of the stomach contents were collected from individuals that were in an advanced decomposed stage, while the remainder (85.7%, $n = 30$) were obtained from moderately or slightly decomposed animals (after Kuiken and Hartmann, 1993). *MPs* were found in all samples analysed ($n = 35$), although it is notable that no macroplastics were detected in any of the digestive tract of these common dolphins. A total of 411 *MPs* items were recovered (see **Fig. 2** for examples) of which 397 were fibres (96.59%), 13 were fragments (3.16%) and 1 was a bead (0.24%). The number of *MPs* ranged from 3 to 41 items per stomach, with an average of 12 (Standard Deviation (SD) = 8). Fibres appeared in all the stomach contents, fragments appeared in 11 out of 35 stomach contents and the bead appeared in only 1 stomach content.

Only one fiber whose length exceeded the stipulated size to be microplastic (5.88 mm in length) was found, however, since no more macroplastics were found and this does not produce a great bias to the final result, it was decided to include it in the analysis.

The size of the rest fibres ranged from 0.29 mm to 4.92 mm and their mean length was 2.11 mm (SD = 1.26). The size of the fragments ranged from 0.49 mm to 4.07 mm and their mean length at the longest point was 1.29 mm (SD = 0.93). The diameter of the bead was 0.95 mm. The percentage of occurrence of each type of *MPs* in the stomach contents was 100% in the case of fibres, 28.6% for fragments and 2.9% for beads. The most frequent colour of *MPs* was blue ($n = 186$; 45.26%), followed by black ($n = 101$; 24.57%), green ($n = 64$; 15.58%), red ($n = 59$; 14.36%) and yellow ($n = 1$; 0.24%) (**Table 1**).

Spearman correlation analysis showed a relatively strong negative trend ($r_s = -0.47$, $p = 0.004$) in the number of *MPs* in the common dolphin stomach contents between years 2005 and 2010 (**Fig. 3**). The Spearman rank correlation test indicated that there was no correlation between the total length of dolphins and the number of *MPs* in their

189 stomachs ($r_s = -0.28$, $p = 0.10$), nor of dolphin length with the mean size of *MPs* ($r_s = -$
190 0.18 , $p = 0.28$). A Kruskal-Wallis test revealed that the sex of the dolphins had no
191 significant influence on the mean number of *MPs* present ($p = 0.07$); or in the mean
192 size of *MPs* ($p = 0.37$).

193 On the other hand, assuming that fibres lesser than 1 mm observed in the samples are
194 airborne contamination (Foekema *et al.*, 2013). **Fig. 4** shows that fibres greater than 1
195 mm have also a 100% frequency of occurrence, decreasing progressively from 1.9 mm
196 onwards. The mean number of microfibrils per stomach content decreases rapidly until
197 the size of 2.3 mm, after which the mean number is more stable. However, the mean of
198 mean size of microfibrils in stomachs increases rapidly. In all three metrics there is a
199 change apparent around the size of 2.3 mm, which could represent the end of influence
200 of airborne particles. This would leave a common dolphin having a 94% probability of
201 having microfibrils in its stomach content and an average number of 3.60 fibres with an
202 average size of 3.73 mm.

204 4. Discussion

205 While many previous studies on macroplastic ingestion have been carried out in marine
206 mammals (e.g. Denuncio *et al.*, 2011; De Stephanis *et al.*, 2013; Bravo Rebolledo *et*
207 *al.*, 2013), only a few recent studies have been conducted on the presence of
208 microplastics in the digestive tract of cetaceans (e.g. Besseling *et al.*, 2015; Lusher *et*
209 *al.*, 2015a).

210 This study constitutes the first analysis of occurrence of *MPs* in the stomach contents
211 of common dolphins in the Iberian Peninsula and provides similar results to those
212 obtained by Lusher *et al.* (2018) for different species of cetaceans in Irish waters (e.g.
213 bottlenose dolphin *Tursiops truncatus*; striped dolphin *Stenella coeruleoalba*; harbour
214 porpoise *Phocoena phocoena*; and common dolphin *Delphinus delphis*). These authors
215 also found that all the cetaceans stomach contents examined for *MPs*, contained them,
216 and they reported *MPs* of a similar length ranges to the ones we found in our study.

One of the main concerns in this type of study is the contamination of the samples during collection, processing and analysis (see Wesch et al., 2017). As we have used a historical collection of stomach contents, we cannot guarantee that the collecting protocols implemented have avoided all possible *MP* contamination although, as we have explained in the methods section, if there was contamination it should have been minimal.

As Foekema *et al.*, 2013 tested in their work, despite the precautions taken, there is a relative high risk of contamination with synthetic fibres carried by air. However, following their proceeding we obtained that the frequency of occurrence of fibres in our results is not very sensitive to small particles, so microfibres are in 94% of common dolphins even excluding small particles that could be airborne. Moreover, it is unlikely that the fragments and bead < 1 mm found could have been due to airborne contamination. Therefore, it is worth noting that these two types of *MPs* were found in 11 out of the 35 samples, approximately 1/3 of the total (Frequency of occurrence: 31.43%).

Although our sample size is small and therefore caution should be applied when interpreting the results, we found a negative trend in the number of *MPs* in the dolphin's stomachs contents over the study period (from 2005 to 2010). Several factors could explain these differences among years, including differences in dolphin feeding areas (with different marine debris concentrations/sources) and/or changes in diet (see Santos *et al.*, 2013 for common dolphin), with different prey having different *MPs* concentrations (see Van Franeker *et al.*, 2018 for harbour porpoises).

Nelms *et al.* (2018) argue that *MPs* could be transferred from fish to marine mammals via their food (i.e. secondary ingestion) generating a biomagnification effect. Common dolphins feed on a wide variety of prey species in the Atlantic waters of the Iberian Peninsula (i.e. Santos *et al.*, 2013), albeit the species has been reported as having an apparent preference for "fatty" fish species in other areas (e.g. in the Bay of Biscay, Meynier *et al.*, 2008; Spitz *et al.*, 2010). Therefore, the amount of *MPs* in a dolphin

stomach will be the result of the combination of the *MPs* present in each of its prey and the proportion of each prey type in the dolphin diet.

When contrasting our hypothesis with the results of earlier studies carried out on several demersal and pelagic fish species (see Boerger *et al.*, 2010; Lusher *et al.*, 2013; Nadal *et al.*, 2016; Rummel *et al.*, 2016), we can see that in these fish on average, the number of *MPs* range from 0.03 (SD = 0.18) to 4.89 (SD = 0.45) per gastrointestinal tract, much lower than the 12 *MPs* found on average in our 35 common dolphin stomach contents. This suggests that biomagnification occurs, even assuming a possible slight contamination of the samples and suggests that *MP* are retained in the digestive tracts of fish and cetaceans, in the latter case for a longer period than the food remains. Moreover, these studies found that the size of *MPs* in fish ranged from 0.15 mm to 3 mm, similar to the ones identified on the common dolphins of this study (from 0.29 mm to 4.92 mm). Regarding the colour of the *MPs*, these studies have also reported that two of the most prevalent colours in their analyzed samples were blue (see Nadal *et al.*, 2016; Rummel *et al.*, 2016), and black (Lusher *et al.*, 2013, 2015a); which correspond with the most frequent colours observed in our 35 samples from common dolphin stomachs.

Lusher *et al.* (2015b) calculated that one single striped dolphin, in Irish waters, could consumed annually ~463 million microplastics through the consumption of contaminated prey. However, the number and size of *MPs* identified in this study in the stomach contents do not seem to be enough to compromise the normal functioning of the digestive tracts of these dolphins in the sense of causing a physical obstruction. Even so, it is worth noting that *MPs* might also have a role as vectors of toxic pollutants (Teuten *et al.*, 2009) which could contribute to reach high concentrations of pollutants in some cetaceans (see Fossi *et al.*, 2012). Hence, the role that the ingestion of *MPs* could have in increasing pollutant loads in marine mammals would need to be further investigated.

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Table 1. Description of *MPS* identified in common dolphin stomachs according to: number (N (and %N)), size range (in millimetres), and frequency of occurrence (%) by category and colour.

Category	N (%)	Size (mm)	Occurrence (%)	Colour (%)				
				Black	Blue	Green	Red	Yellow
Fibres	397 (96.6%)	0.29 - 4.92	100%	23.4%	45.6%	16.1%	14.9%	0%
Fragments	13 (3.2%)	0.49 - 4.07	28.6%	61.5%	38.5%	0%	0%	0%
Beads	1 (0.2%)	0.95	2.9%	0%	0%	0%	0%	100%

Fig. 1. Map showing the location of the 35 common dolphins stranded on the Galician coast between 2005 and 2010 used in this study.

Fig. 2. Examples of different types of microplastics found in common dolphins stomach contents: (A) fibers, (B) fragments and (C) bead.

Fig. 3. Interannual trend in the mean number of microplastics found in the stomach contents of common dolphins stranded in Galicia (2005 – 2010). The points represent the raw data, the horizontal bars represent the central tendencies (means), the beans represent the smoothed densities (estimated density of the distribution of the individual observations), the bands/boxes represent the inference intervals 95% (Bayesian Highest Density Intervals, HDI) and the dashed line represent the linear regression (the formula is showed in the plot).

Fig. 4. Microfibres' frequency of occurrence (%), mean number and mean of mean size in each of 35 stomach contents of common dolphins.