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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 from the Iberian Peninsula. This study made use of 35 samples of common dolphin stomach contents. Microplastics were identified in all the samples analysed, an average of 12 items per stomach although abundance varied widely from one stomach to another. Most plastic items were small fibres although some fragments and a bead were also found. Excluding the smallest fibres as possible airborne contamination, the estimated occurrence of microplastics could drop to as low as 94%. 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.

1. Introduction

In number, 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 (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 (e.g. 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 and Goodwin, 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

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bioaccumulate and biomagnify with each trophic level. 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) (Lopez et al., 2004) and it is also the cetacean species most commonly found stranded on the Galician coast (López 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). It is possible that small plastic fibres recorded in the present study represent airborne contamination. Thus, Foekema et al. (2013) found small fibres < 1 mm in length in most samples from fish digestive tracts and concluded that these were likely airborne. We therefore analyse the consequences of excluding such fibres.

The aims of our analysis were to: (a) describe, quantify and measure the number of *MPs* in the stomach contents of common dolphins, taking into account the possibility that small plastic fibres may represent airborne contamination; (b) compare the occurrence, size and number of *MPs* between sexes and size classes of these cetaceans; and (c) determine whether there is any evidence of a 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 27 km on average). The Galician stranding network is operated by the NGO "Coordinadora para o Estudio dos Mamíferos Mariños" (CEMMA). Its trained personnel (equipped with nitrile gloves, rubber boots and waterproof suits, which should help minimize cross-contamination of samples with *MPs*) 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 Garcia-Hartmann, 1993). Due to limited freezer storage capacity at the CEMMA premises, the stomach compartments of cetaceans with decomposition states ranging from 2 (fresh) to 4 (highly decomposed) (see Kuiken and Garcia-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 such contamination was minimal since the stomach contents were directly emptied into the glass jar, which was then immediately sealed. Information on stranding date, location, sex, length and decomposition state were available for all animals. 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 prey remains and parasites found in the dolphin 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 decreasing mesh sizes (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 microfibre filters.
IV. The glass microfibre filters were placed in closed glass Petri dishes and dried in an oven at 50 °C for 4 h.
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). 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.

In the laboratory, to minimize the risk of contamination of the samples with synthetic textile fibres from researchers' clothes, samples processing was carried out at a clean bench, by researchers wearing white laboratory coats (100% cotton) and blue nitrile gloves. We could thus identify and discard any white microfibres 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. However, we cannot rule out airborne contamination and we therefore consider this issue at the data analysis stage.

2.3. Data analysis

The data on number of *MPs* per stomach content were tested for normality and we 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 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 and Wallis (1952) test. All tests were performed using the 'stats' package included in the statistical software R (version 3.4.2, R Core Team, 2017).

Because in our analysis we did not include blank samples (use of which is recommended by Hanke et al., 2013) some of the *MPs* we found in the stomachs could be the results of airbone contamination. To determine the possible extend of this contamination, we carried out an analysis to determine how presence of *MPs* changes in the stomachs if small fibres are excluded. Although Foekema et al. (2013) refers to fibres of length < 1 mm as likely to have been airborne, it is not clear what cut-off size should be used. We therefore recalculated all metrics (occurrence, mean size and number of *MPs*) for fibres in each stomach using cut-off lengths ranging from 0.1 up to 5 mm. Evidently the smallest cut-off size will exclude almost no fibres whereas the largest will exclude almost all of them.

3. Results

Of the common dolphins whose stomach contents were analysed in this study, 14.3% (n = 5) of the stomach contents were collected from individuals that were in an advanced decomposition stage, while the

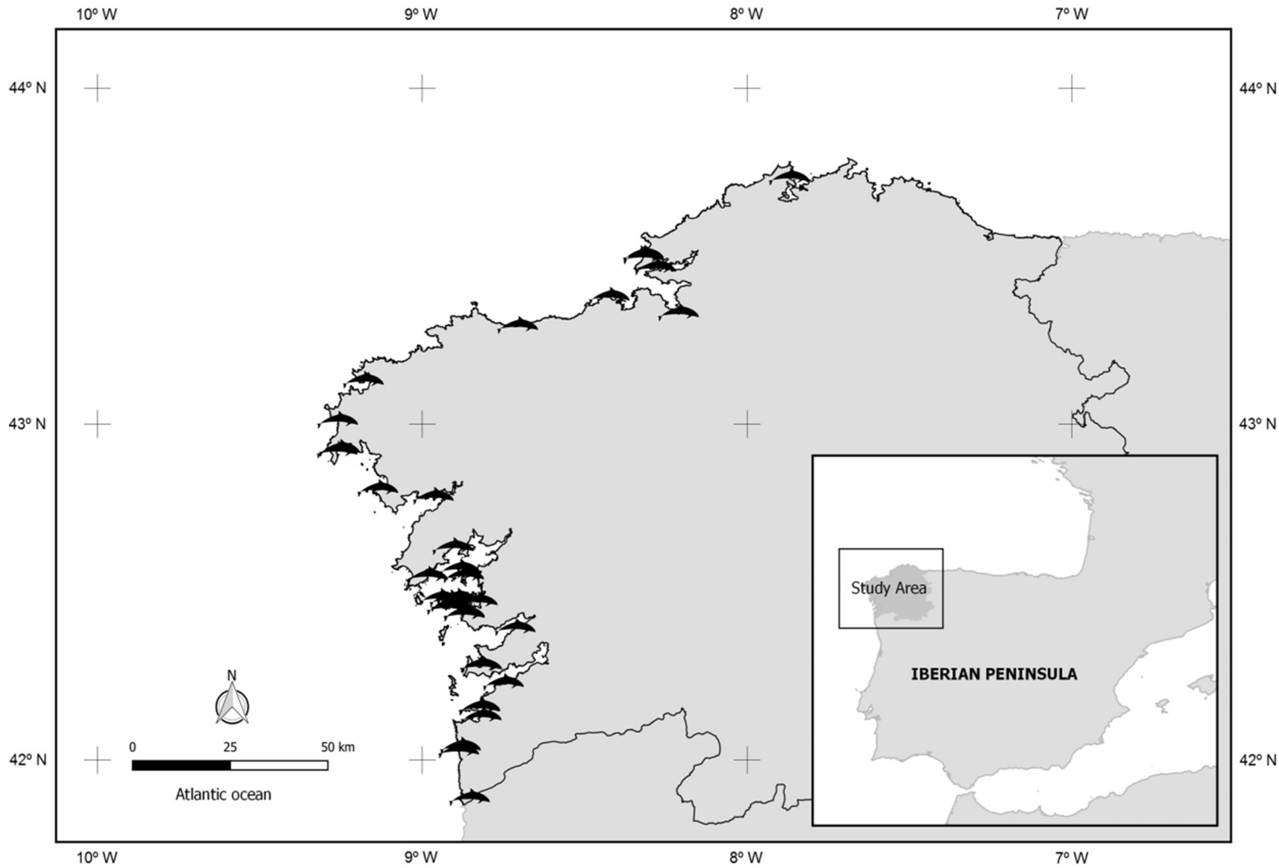

Fig. 1. Map showing the location of the 35 common dolphins stranded on the Galician coast between 2005 and 2010 used in this study.

remainder (85.7%, n = 30) were obtained from moderately or slightly decomposed animals (after Kuiken and Garcia-Hartmann, 1993). *MPs* were found in all samples analysed (n = 35), although it is notable that no macroplastics were detected in any of the samples, except for 1 fibre with a length of 5.88 mm, i.e. slightly above the cut-off size for microplastics. However, since no other macroplastics were found, we included it in the analysis together with the remaining items. 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.

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 over the years 2005 to 2010 (Fig. 3). However there was no correlation between the total length of dolphins and the number of *MPs* in their stomachs ($r_s = -0.28$, $p = 0.10$), nor of dolphin length with the mean size of *MPs* ($r_s = -0.18$, $p = 0.28$). A Kruskal-Wallis test revealed that the sex of the dolphins had no significant influence on the mean number of *MPs* present ($p = 0.07$); or in the mean size of *MPs* ($p = 0.37$).

Fig. 4 illustrates occurrence, mean number and mean size of fibres using different cut-off sizes to exclude potential airborne particles. Fibres > 1 mm have a 100% frequency of occurrence, with occurrence decreasing progressively from cut-off values of 1.9 mm upwards. The mean number of microfibres per stomach content decreases rapidly with increasing cut-off sizes until fibres a cut-off size of 2.3 mm, above which the mean number declines more slowly. However, the average of the mean size of microfibres in stomachs increases with increasing cut-off size, but with a possible point of inflection in the curve around 2.3 mm. While we cannot say with certainly which fibres were airborne the change in the trajectories three metrics at a cut-off size between 2 mm and 2.5 mm could indicate that fibres under this size are usually airborne. Applying this threshold, a common dolphin has a 94% probability of having microfibres in its stomach contents with an average number of 3.60 fibres with an average size of 3.73 mm.

4. Discussion

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

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

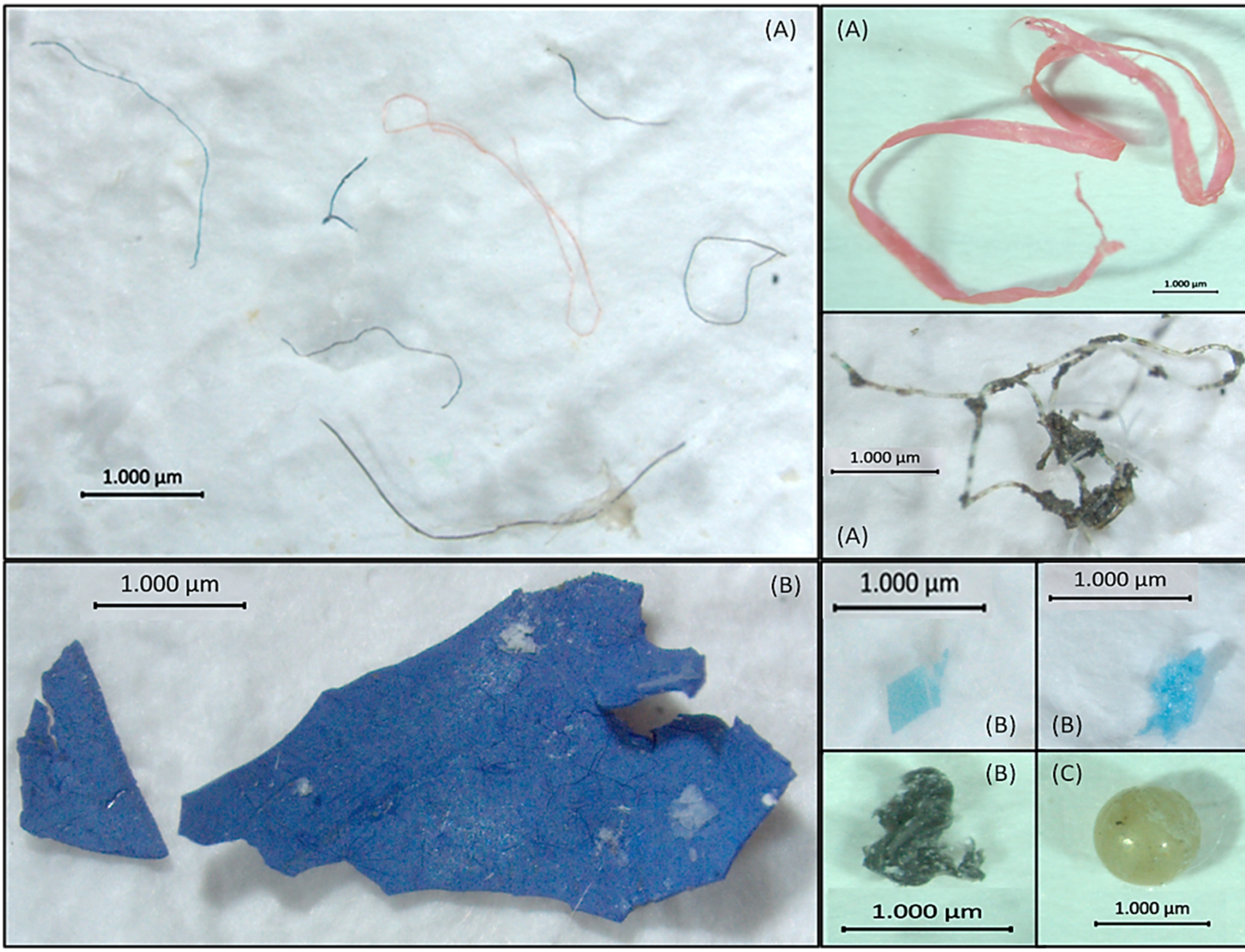

Fig. 2. Examples of different types of microplastics found in common dolphins stomach contents: (A) fibres, (B) fragments and (C) bead.

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.

Despite the precautions taken, there is a relative high risk of contamination with synthetic fibres carried by air as found in previous studies such as Foekema et al. (2013), who commented that fibres under 1 mm in length found in fish digestive tract samples were likely airborne. If we took this as a cut-off point for exclusion of the smallest fibres, we would still have 100% occurrence of *MPs* in the dolphin stomachs, albeit with a modest reduction in the average number of microfibres present and occurrence increase in their average size. Having investigated the consequences of using range of different possible cut-off points, we suggest a more conservative cut-off value of 2.3 mm. Under this scenario the occurrence of microfibres is reduce to 94% of common dolphins, with fibre abundance reduced to around one third of the "all fibres" value. It is unlikely that the fragments and bead < 1 mm find length 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

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%

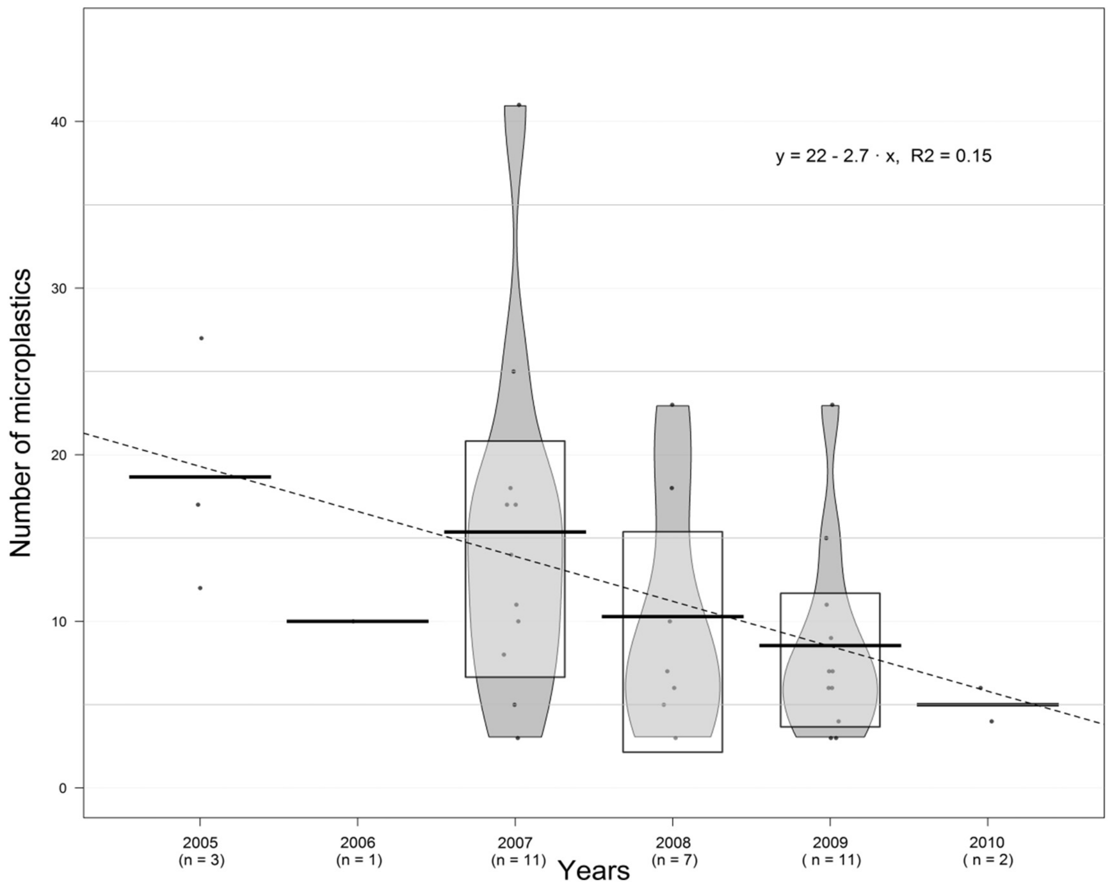

Fig. 3. Interannual trend in the mean number of microplastics found in the stomach contents of common dolphins stranded in Galicia (2005–2010). The points represent the raw data, the horizontal bars represent the central tendencies (means), the beans represent the smoothed densities (estimated density 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).

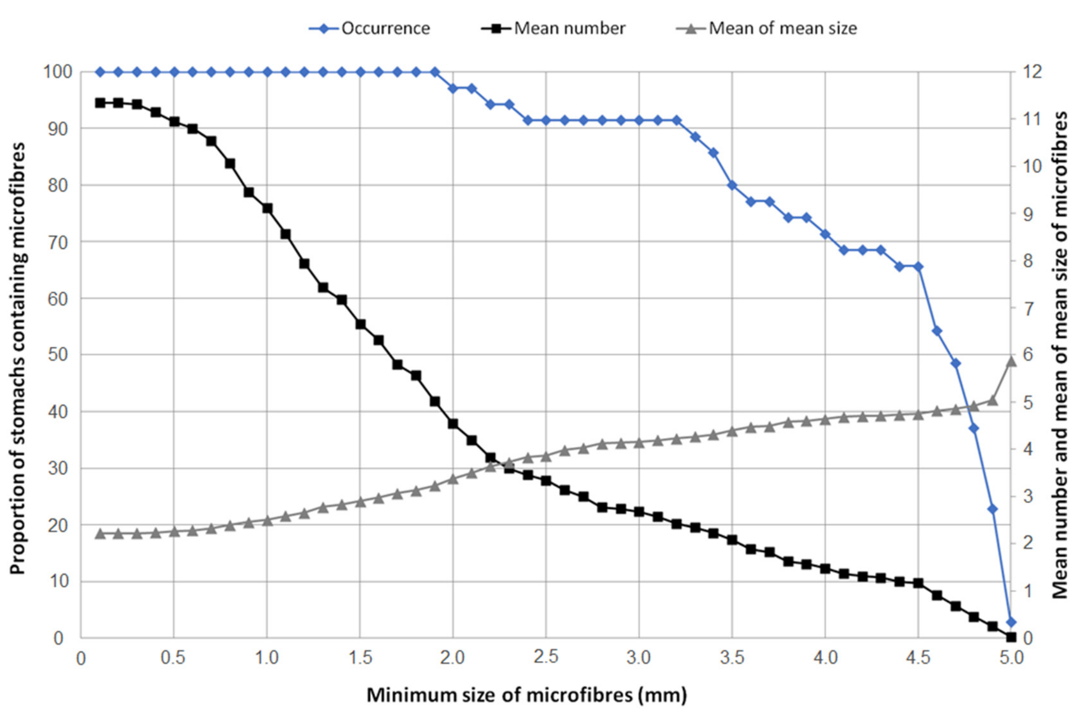

Fig. 4. Microfibres' frequency of occurrence (%), mean number and mean of mean size in each of 35 stomach contents of common dolphins.

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.

In 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), the number of *MPs* ranged from 0.03 (SD = 0.18) to 4.89 (SD = 0.45) per gastrointestinal tract, rather lower than the 12 *MPs* found on average in our 35 common dolphin stomach contents, although the differences is reduced if we exclude fibres < 1 mm in length and almost vanishes if we exclude fibres < 2.3 mm in length. Our results may imply that biomagnification occurs, and that *MPs* 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 on fish also reported that two of the most prevalent colours in their samples were blue (see Nadal et al., 2016; Rummel et al., 2016), and black (Lusher et al., 2013, 2015a); in agreement 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 ~463 million microplastics items annually through the consumption of contaminated prey. If we allow that dolphins feed several times a day and that we recovered only a proportion of those eaten, even our highest figures seem to imply an ingestion rate of microplastics several orders of magnitude lower than that suggested by Lusher et al. (2015b). At least, the number and size of *MPs* identified in this study in the stomach contents do not seem to be enough to cause a physical obstruction of the digestive tract. 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 bioaccumulation of pollutants in some cetaceans (see Fossi et al., 2012). Hence, the role that the ingestion of *MPs* might have in increasing pollutant loads in marine mammals needs to be further investigated.

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