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Baseline

# Plastic ingestion in aquatic-associated bird species in southern Portugal

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ABSTRACT

Excessive use of plastics in daily life and the inappropriate disposal of plastic products are severely affecting wildlife species in both coastal and aquatic environments. Birds are top-predators, exposed to all threats affecting their environments, making them ideal sentinel organisms for monitoring ecosystems change. We set a baseline assessment of the prevalence of marine plastic litter affecting multi-species populations of aquatic birds in southern Portugal. By examining 160 stomach contents from 8 species of aquatic birds, we show that 22.5% were affected by plastic debris. Plastic was found in *Ciconia ciconia*, *Larus fuscus* and L. *michahellis*. *Ciconia ciconia* ingested the highest amount (number of items and total mass) of plastic debris. Polydimethylsiloxane (PDMS, silicones) was the most abundant polymer and was recorded only in *C. ciconia*. Plastic ingestion baseline data are of crucial importance to evaluate changes through time and among regions and to define management and conservation strategies.

Since the mass production of plastics started in the 1950s, pollution of this inexpensive and long-lasting material has rapidly emerged as a global environmental concern (Barnes et al., 2009). The rapid and significant accumulation of plastic debris is pervasive and is affecting marine and terrestrial ecosystems virtually everywhere on the planet, far beyond areas of high human population density (e.g., Browne et al., 2011; Duis and Coors, 2016; Thompson et al., 2009). The drawbacks of plastic waste are not limited to aesthetic values; there is now clear and increasing evidence that it represents a major threat to wildlife (Barnes et al., 2009). The number of potentially detrimental consequences of plastic debris has escalated in terms of effects and taxa affected (Bergmann et al., 2015).

Aquatic birds are especially susceptible to the ubiquitous and increasing presence of plastic contamination (e.g., Acampora et al., 2017; Wilcox et al., 2015). Indeed, some of the earliest reports of plastic litter in the marine environment are of plastic caps, toys and bags ingested by seabirds in the 1960s (Harper and Fowler, 1987; Kenyon and Kridler, 1969).

Plastic pollution has a wide range of negative effects on aquatic birds. These include entanglement in multi-pack beverage rings, plastic bags and other plastic items (Bond et al., 2012; Gregory, 2009; Laist, 1997; Udyawer et al., 2013; Votier et al., 2011); smaller plastic debris can be ingested by mistake or because they resemble natural food items (Cadée, 2002; Jackson et al., 2000) causing internal wounds and ulcers, gastrointestinal obstruction and poisoning from exposure to plastic fragments and the organic pollutants associated with them.

In Europe, the Marine Strategy Framework Directive (MSFD) has proposed ingestion of debris by marine organisms as a marine litter indicator to quantify progress towards a “Good Environmental Status” (GES). In particular, due to their susceptibility to plastic debris ingestion, aquatic birds have been considered as good bioindicators for plastic pollution. Of all the seabird species, the Northern Fulmar (*Fulmarus glacialis*) is probably the most well-known bioindicator. Since 2009, monitoring ingestion of plastic litter in beached specimens of *F. glacialis* has been adopted by the Oslo-Paris Convention (OSPAR, 2010) and MSFD (Directive, 2008) as a marine environment quality indicator in the southern North Sea.

The selection of an individual species as an indicator is crucial for analyses of spatial and temporal trends in plastic pollution (Avery-Gomm et al., 2012; Kühn and van Franeker, 2012; Mallory et al., 2006; Provencher et al., 2009; Van Franeker et al., 2011). At the same time, surveys for a wide array of species (including non-indicator species) are also important to understanding the pervasiveness of plastic ingestion and identifying factors that account for differences in the quantities and qualities of plastic ingested by different species (Avery-Gomm et al., 2013; Provencher et al., 2014; Roman et al., 2016). Additionally,

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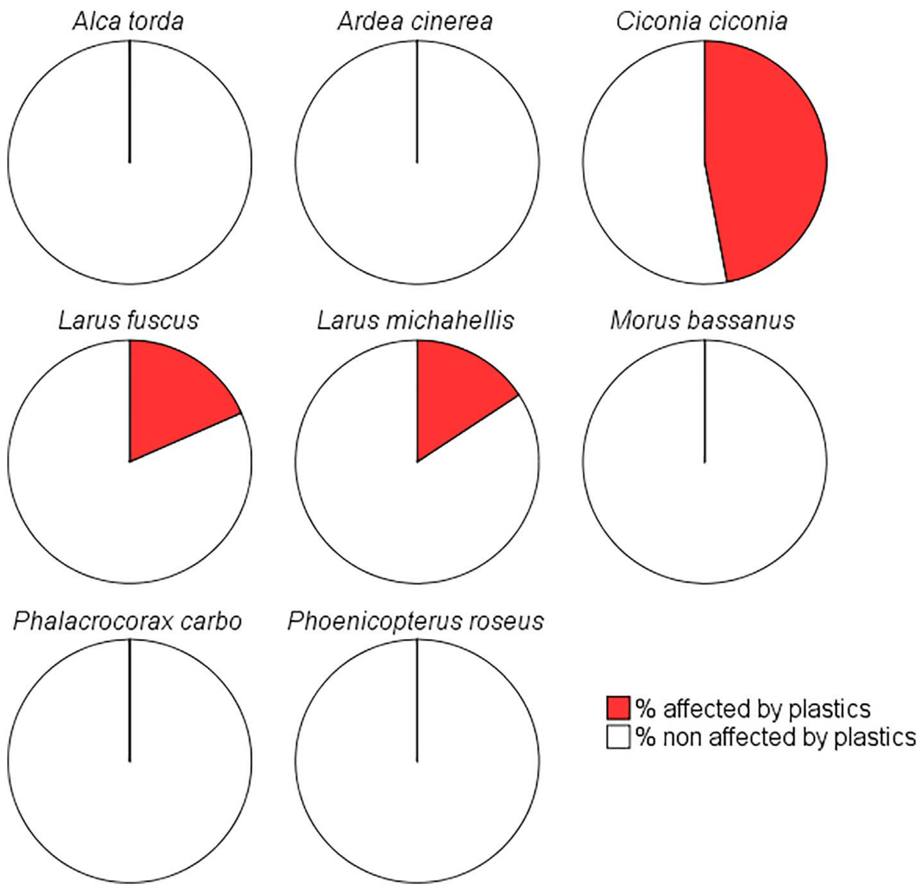


**Fig. 1.** Plastic litter occurrence (%) in the stomach of eight aquatic species in southern Portugal: *Alca torda* ($n = 2$), *Ardea cinerea* ($n = 1$), *Larus michahellis* ($n = 75$), *Ciconia ciconia* ($n = 9$) *Larus fuscus* ($n = 62$), *Morus bassanus* ($n = 8$), *Phalacrocorax carbo* ($n = 1$) *Phoenicopterus roseus* ($n = 2$).

comprehensive multi-species investigations may also be valuable in detecting alternative species for use in monitoring programmes (e.g. Acampora et al., 2016).

Plastic ingestion data are of particular value in regions where baseline studies are not yet available; not only they are important for assessing changes through time and differences among regions, they are also fundamental to a functional definition of management and conservation efforts (Avery-Gomm et al., 2013). While there is little information on the abundance, distribution and fluctuations (spatial and temporal) of plastic litter in Portuguese waters and shores (e.g., Antunes et al., 2013; Martins and Sobral, 2011; Oliveira et al., 2015), there is no published information concerning marine litter in aquatic birds in Portugal. A quantitative assessment that includes both numerical and mass trends is critical. In fact, number and mass of plastic items do not always match and plastic abundance evaluated in terms of mass is considered to be ecologically more relevant (Provencher et al., 2017; van Franeker and Law, 2015). Additionally, recent studies have stressed the importance of spectroscopic techniques in plastic monitoring schemes; these are critical to avoid misidentification of natural items for synthetic polymers (e.g., Wesch et al., 2016). Moreover, knowledge of the composition of plastic debris could lead to more effective mitigation measures (Ryan et al., 2009).

The south of Portugal is characterized by several lagoons near the coastline some of which are referred as areas of high diversity of wildlife including the presence of more than a 100 aquatic bird species. Here, we provide a first quantitative (number of items and total mass of litter) and qualitative (visual and spectroscopic assessment) baseline data on plastic ingestion by multi-species populations of aquatic birds in southern Portugal.

Sampling took place between June 2014 and June 2016 and comprised aquatic birds that had been brought to the wildlife recovery center RIAS in Olhão, southern Portugal. Birds were collected by volunteers along southern Portugal by locals and therefore sampling was irregular over time, space and species. The birds used in this study were either dead when they were admitted to the recovery facility or died during their stay. A total of 160 individuals belonging to 8 species were investigated: two razorbills (*Alca torda*), one grey heron (*Ardea cinerea*), nine white storks (*Ciconia ciconia*), 62 lesser black-backed (*Larus fuscus*) and 75 yellow-legged gulls (*L. michahellis*), eight northern gannets (*Morus bassanus*), one great cormorant (*Phalacrocorax carbo*) and two greater flamingos (*Phoenicopterus roseus*). Birds were labelled and frozen at $-20\,^{\circ}C$ for later necropsy.

Dissections were performed following van Franeker (2004). For each sample and when available, data on age (juvenile or adult), gender, probable cause of death and body condition were recorded. Gender and age were derived from development stage of sexual organs and plumage evaluation. Body condition scoring (0–4) was evaluated following (Pinilla and Català, 2000). The gastrointestinal tract (esophagus, stomach and intestines) was collected and stored at $-20\,^{\circ}C$. Stomach contents were rinsed and sieved through a 1 mm mesh, retained in a petri dish and air dried for at least 2 days (Van Franeker et al., 2011).

Contents were examined under a stereomicroscope (SteREO Discovery V8 1x-8x). Plastic items were counted and individually weighted (Sartorius advantage AW-224 Balance) to the nearest 0.0001 g. These items were then classified in three different ways: (a) by categories as industrial or user and, within user, in sheetlike, fragment, threadlike, foamed or other (as in Van Franeker et al., 2011); (b) by colour, as dark (i.e., black, dark brown and dark blue), light (i.e., white and yellow), warm (i.e., orange, red and pink) or cold colors (i.e., pale blue and green; as in Codina-García et al., 2013) and (c) by polymer, as polyacrylamide (PAM), polydimethylsiloxane (PDMS), polyethylene (PE), polystyrene (PS), polytetrafluoroethylene (PTFE). To obtain information on their resin or polymer composition, Raman spectroscopy analysis was performed (JASCO NRS-4100). A laser beam (532 or 785 nm) was focused on the sample surface using a 5 × or 20 × objective, resulting in a spot size of ~30 or ~5 μm, respectively; the laser power was in the 0.5–5.0 mW range depending on the specific sample, and it was kept low enough to prevent sample damage. Given the high spatial resolution of the Raman spectrometer, for each sample at least three spectra at three different points of the sample surface were acquired. In order to identify the polymer composition the spectra were then compared with those of the most common polymers included in a home-made spectral database. When identification through Raman analysis was ambiguous or not possible, usually due to intense photoluminescence background, Fourier-Transform Infrared Spectroscopy (FTIR) was used as an additional technique (JASCO FT/IR-4700), performing both transmission and attenuated total reflectance (ATR) measurements.\

Results show that a total of 135 plastic items with an average mass of 3.84 g were recorded in 160 birds comprising 19 females and 32 males (note that in 92 samples gender was not recorded). Overall, 2 was the most common condition index (CI) recorded (note that in 29 samples CI could not be recorded). Plastic was found in three species (*Larus fuscus*, *L. michahellis*, *Ciconia ciconia*) out of the eight species processed and with an average incidence rate of 43.4% (Fig. 1 and Tables 1, 2, 3).

This study provides baseline data on plastic ingestion in eight aquatic bird species in southern Portugal. We report evidence of plastic ingestion in three species. Overall, our results show that the prevalence of plastic ingestion by *Laridae* in southern Portugal is at similar levels to other parts of southern and northern Europe (e.g., Acampora et al., 2016; Codina-García et al., 2013). Interestingly, although other works have reported relatively high occurrences, mass and numbers of plastic items in *Morus bassanus* (Codina-García et al., 2013), the eight individuals we necropsied in our study did not show any evidence of plastic consumption. In contrast, we found a remarkably high percentage of *Ciconia ciconia* individuals with ingested plastic, compared to other studies in Iberia (e.g., Peris, 2003).

There is abundant evidence that *Ciconiidae* are increasingly reliant on terrestrial anthropogenic resources for foraging. Although it is not possible to establish if the large amount of plastic items found in the *C. ciconia* stomachs we analysed originated from human-related habitats, several recent studies have reported increasing use of agricultural areas and rubbish dumps by European white storks with significant behavioural consequences (Gilbert et al., 2016). For example, Spanish landfill sites provide nearly 70% of white stork diets, affecting their population dynamics and fitness and behavioural traits (Peris, 2003). The pronounced increase in the breeding population of the white stork

**Table 1**
Data on plastics ingested by *Larus michahellis* ($n$ = 75) based on (a) category, (b) colour and (c) polymer type. Values were calculated per individual and included all individuals sampled (affected and non-affected by plastics). 95% CI: Jeffreys' nominal 95% confidence intervals.

| A. | Prevalence (95% CI) | Number | | | Mass | | |
|---|---|---|---|---|---|---|---|
| | | Mean (SD; SE) | Median | Range | Mean (SD; SE) | Median | Range |
| All plastics | 0.1867 (0.1146–0.2893) | 0.2667 (0.859499; 0.099246) | 0 | 5 | 0.0009 (0.0054; 0.00063) | 0 | 0.0448 |
| Industrial plastics | 0 (0–0) | 0.0133 (0.1155; 0.0133) | 0 | 1 | 0.0003 (0.0024; 0.0001) | 0 | 0.0216 |
| User plastics | | | | | | | |
| Sheetlike | 0.1067 (0.0551–0.1967) | 0.2133 (0.1155; 0.0133) | 0 | 5 | 0.0003 (0.0012; 0.0001) | 0 | 0.0076 |
| Threadlike | 0.0267 (0.0074–0.0922) | 0.04 (0.2568; 0.0296) | 0 | 2 | 4.66667E-05 (0.0003; 3.31164E-05) | 0 | 0.002 |
| Foamed | 0.04 (0.0137–0.1111) | 0.05333 (0.2796; 0.0323) | 0 | 2 | 9.86667E-05 (0.0006; 7.09395E-05) | 0 | 0.0045 |
| Fragment | 0.12 (0.0644–0.2126) | 0.26667 (0.8595; 0.0992) | 0 | 5 | 0.00093 (0.00054; 0.0006) | 0 | 0.0448 |
| Other | 0 (0–0) | 0 (0; 0) | 0 | 0 | 0 (0; 0) | 0 | 0 |

| B. | Prevalence (95% CI) | Number | | |
|---|---|---|---|---|
| | | Mean (SD; SE) | Median | Range |
| Dark | 0.08 (0.0372–0.1637) | 0.12 (0.4638; 0.0535) | 0 | 3 |
| Light | 0.1733 (0.1042–0.2743) | 0.44 (1.2108; 0.1398) | 0 | 7 |
| Cold | 0.0267 (0.0074–0.0922) | 0.04 (0.2568;0.0296) | 0 | 2 |
| Warm | 0 (0–0) | 0 (0; 0) | 0 | 0 |
| C. | | | | |
| PAM | 0.04 (0.0137–0.1111) | 0.0533 (0.2796; 0.03229) | 0 | 2 |
| PDMS | 0 (0–0) | 0 (0; 0) | 0 | 0 |
| PE | 0.0933 (0.0459–0.1803) | 0.2533 (1.0013; 0.1156) | 0 | 7 |
| PS | 0.1467 (0.0839–0.2439) | 0.293333 (0.8183; 0.0945) | 0 | 5 |
| PTFE | 0 (0–0) | 0 (0; 0) | 0 | 0 |

**Table 2**
Data on plastics ingested by *Larus fuscus* (n = 62) based on (a) category, (b) colour and (c) polymer type. Values were calculated per individual and included all individuals sampled (affected and non-affected by plastics). 95% CI: Jeffreys' nominal 95% confidence intervals.

| A. | Prevalence (95% CI) | Number | | | Mass | | |
|---|---|---|---|---|---|---|---|
| | | Mean (SD; SE) | Median | Range | Mean (SD; SE) | Median | Range |
| All plastics | 0.2258 (0.1395–0.344) | 0.4677 (1.956; 0.2484) | 0 | 6 | 0.0056 (0.0358; 0.0045) | 0 | 0.2793 |
| Industrial plastics | 0 (0; 0) | 0 (0; 0) | 0 | 0 | 0 (0; 0) | 0 | 0 |
| User plastics | | | | | | | |
| Sheetlike | 0.0968 (0.0451–0.1955) | 0.1452 | 0 | 2 | 0.0006 (0.0025; 0.0003) | 0 | 0.0131 |
| Threadlike | 0.0806 (0.0349–0.1752) | 0.0968 | 0 | 3 | 0.0163 (0.127; 0.0161) | 0 | 1 |
| Foamed | 0.0484 (0.0166–0.1329) | 0.1129 | 0 | 5 | 0.0002 (0.001; 0.0001) | 0 | 0.0077 |
| Fragment | 0.129 (0.0668–0.2345) | 0.4677 | 0 | 14 | 0.0056 (0.036; 0.0045) | 0 | 0.2793 |
| Other | 0 (0–0) | 0 (0; 0) | 0 | 0 | 0 (0; 0) | 0 | 0 |

| | Prevalence (95% CI) | Number | | |
|---|---|---|---|---|
| | | Mean (SD; SE) | Median | Range |
| B. | | | | |
| Dark | 0.0968 (0.0451–0.1955) | 0.129 (0.424; 0.0538) | 0 | 2 |
| Light | 0.1774 (0.102–0.2904) | 0.56451613 (1.7332; 0.2201) | 0 | 11 |
| Cold | 0.0484 (0.0166–0.1329) | 0.129 (0.7784; 0.0989) | 0 | 6 |
| Warm | 0 (0–0) | 0 (0; 0) | 0 | 0 |
| C. | | | | |
| PAM | 0.0161 (0.0028–0.0858) | 0.01612903 (0.127;0.0161) | 0 | 1 |
| PDMS | 0 (0; 0) | 0 (0; 0) | 0 | 0 |
| PE | 0.1452 (0.0783–0.2535) | 0.258064516 (0.7228; 0.0918) | 0 | 4 |
| PS | 0.1452 (0.0783–0.2535) | 0.548387097 (2.0137; 0.25574) | 0 | 13 |
| PTFE | 0 (0–0) | 0 (0; 0) | 0 | 0 |

in Iberia over the last forty years has been attributed to the year-round availability of artificial food from landfill sites, which buffers seasonal declines in food availability and reduces mortality in first-year birds (Gilbert et al., 2016; Tortosa et al., 2002). In addition, a recent study of *C. ciconia* breeding colonies in northern Algeria shows that access to extra food from dumps increases egg volume and hatching mass (Djerdali et al., 2016). Feeding on rubbish dumps also affects home ranges and migratory patterns of white Storks (Blanco, 1996; Tortosa et al., 2002); continuous and abundant food resources from landfill sites have promoted the establishment of resident individuals in a previously wholly migratory species (Gilbert et al., 2016). Specifically, in Iberia, the number of overwintering white storks has increased by an order of magnitude over the last twenty years (Rosa et al., 2009). Foraging on rubbish dumps has a critical influence on resident populations, as the majority of resident white storks congregate near landfills (Tortosa et al., 2002); in general, long distance foraging trips are performed specifically to visit landfills while non-landfill foraging occurs in the proximity of the nest (Gilbert et al., 2016).

The European Union Landfill Directive (1993/31/EC) aims at closing landfill sites or replacing them with covered waste management facilities; this policy will likely have important consequences on the abundance and behaviour of white stork populations. Despite providing spatially and temporally stable food resources, rubbish dumps may be deleterious since white storks are exposed to large amounts of non-edible, anthropogenic debris that mimics food (e.g., Henry et al., 2011). In general, earthworms form the most abundant part of their diet (Antczak et al., 2002); several studies have provided evidence for massive ingestion of rubber bands by white storks, presumably because their colour and shape mimic prey such as *Lumbricidae* (e.g., Sazima and D'angelo, 2015). Rubber bands can be detrimental not only when foraging (i.e. gastrointestinal obstruction, internal wounds). In fact, it has been shown that *C. ciconia* adults can suffer wing and/or bill entanglement while entanglement in plastic bands used to improve nest structure can cause broken wings and/or legs in juveniles (Kwieciński et al., 2006; Sazima and D'angelo, 2015). Moreover, the handling of plastic strings and the efforts to swallow and regurgitate them may be considered as a waste of foraging time and energy (Sazima and D'angelo, 2015).

Our findings support previous work by highlighting a particularly high occurrence of worm-like debris in *C. ciconia*. Silicones (PDMS) belonging to the category others (i.e. rubber bands, elastics) and warm coloured (i.e. orange, red and pink) debris were found only in *C.*

**Table 3**
Data on plastics ingested by *Ciconia ciconia* ($n = 9$) based on (a) category, (b) colour and (c) polymer type. Values were calculated per individual and included all individuals sampled (affected and non-affected by plastics). 95% CI: Jeffreys' nominal 95% confidence intervals.

| A. | Prevalence (95% CI) | Number | | | Mass | | |
|---|---|---|---|---|---|---|---|
| | | Mean (SD; SE) | Median | Range | Mean (SD; SE) | Median | Range |
| All plastics | 0.8889 (0.565–0.9801) | 1.2222 (1.1877; 0.4006) | 1 | 4 | 0.19073 (0.331; 0.1103) | 0.0243 | 0.9474 |
| Industrial plastics | 0 (0–0) | 0 (0; 0) | 0 | 0 | 0 (0; 0) | 0 | 0 |
| User plastics | | | | | | | |
| Sheetlike | 0.3333 (0.1206–0.6458) | 0.6667 (1.3229; 0.441) | 0 | 4 | 0.0040 (0.01; 0.0033) | 0 | 0.0304 |
| Threadlike | 0.2222 (0.0632–0.5474) | 0.2222 (0.441; 0.147) | 0 | 1 | 0.0678 (0.1778; 0.0593) | 0 | 0.5375 |
| Foamed | 0 (0–0) | 0 (0; 0) | 0 | 0 | 0 | 0 | 0 |
| Fragment | 0.7778 (0.4526–0.9368) | 1.2222 (1.2018; 0.4006) | 1 | 4 | 0.1907 (0.331; 0.1103) | 0.0294 | 0.9474 |
| Other | 0.4444 (0.1887–0.7333) | 2.3333 (4; 1.3333) | 0 | 12 | 2.3926 (3.8898; 1.2966) | 0 | 10.6415 |

| | Prevalence (95% CI) | Number | | |
|---|---|---|---|---|
| | | Mean (SD; SE) | Median | Range |
| B. | | | | |
| Dark | 0.3333 (0.1206–0.6458) | 0.8889 (1.5366; 0.5122) | 0 | 4 |
| Light | 0.5556 (0.2667–0.8113) | 2.1111 (2.8038; 0.9346) | 1 | 7 |
| Cold | 0.3333 (0.1206–0.6458) | 0.3333 (0.5; 0.1667) | 0 | 1 |
| Warm | 0.3333 (0.1206–0.6458) | 1.1111 (1.965; 0.655) | 0 | 5 |
| C. | | | | |
| PAM | 0.1111 (0.0199–0.435) | 0.1111 (0.3333; 0.1111) | 0 | 1 |
| PDMS | 0.3333 (0.1206–0.6458) | 2.2222 (4.0551; 1.3517) | 0 | 12 |
| PE | 0.6667 (0.3542–0.8794) | 1.4444 (1.6667; 0.5557) | 1 | 5 |
| PS | 0.3333 (0.1206–0.6458) | 0.4444 (0.7265; 0.2422) | 0 | 2 |
| PTFE | 0.2222 (0.0632–0.5474) | 0.2222 (0.441; 0.147) | 0 | 1 |

*ciconia*.

In conclusion, the assessment of ingested litter in the stomach of beached birds is not a proxy for quantitative abundance of plastic litter in the environment, however, it reflects spatio–temporal differences and fluctuations of plastic litter abundance (Van Franeker et al., 2011; van Franeker and Law, 2015). As global plastic contamination continues to rise, multi-species plastic ingestion surveys and baseline data for new regions, such as ours, are fundamental for larger syntheses aimed at assessing current differences among areas and changes through time. We highlight the importance of implementing accepted, widely recognized protocols (e. g., Avery-Gomm et al., 2013; Van Franeker et al., 2011) to allow comparison with other studies. Finally, we stress the importance of categorizing plastic types according to polymer type. The application of Raman and FTIR spectroscopy allows discrimination between plastic and natural items, and can offer a potential indication of plastic particle sources and impacts (Desforges et al., 2014; Provencher et al., 2017).

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