



# First data on plastic ingestion by blue sharks (*Prionace glauca*) from the Ligurian Sea (North-Western Mediterranean Sea)

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## ABSTRACT

Few studies have focused so far on plastic ingestion by sharks in the Mediterranean Sea. The aim of this paper was to determine, for the first time, the plastic litter ingested by blue sharks (*Prionace glauca*), categorized as “Critically Endangered” in the Mediterranean Sea by IUCN, caught in the Pelagos Sanctuary SPAMI (North-Western Mediterranean Sea). The analysis of the stomach contents was performed following the MSFD Descriptor 10 standard protocol implemented with FT-IR spectroscopy technique. The results showed that 25.26% of sharks ingested plastic debris of wide scale of sizes from microplastics (< 5 mm) to macroplastics (> 25 mm). The polyethylene sheetlike user plastics, widely used as packaging material, are the most ingested debris. This research raises a warning alarm on the impact of plastic debris on a threatened species, with a key role in the food web, and adds important information for futures mitigation actions.

## 1. Introduction

Plastic pollution is present in all the oceans and seas of the world, including the Mediterranean Sea, which is considered one of the most impacted areas by marine litter in the world, with an average concentration calculated at 0.243 items/m<sup>2</sup> (Cózar et al., 2015). Plastic waste can cause physical damages to marine organisms like entanglement and smothering; moreover, plastic ingestion can induce lacerations and ulcerating wounds in the digestive tract, leading to general debilitation (Gregory, 2009; Kühn et al., 2015). Plastics ingestion is the most commonly studied phenomenon, since it could lead to more serious consequences, including changes in satiety and hunger, decrease of the power and capacity of predation, energy disturbance, impairment of reproduction, endocrine disruption, as well as more specific effects such as oxidative stress, dysfunctions in immune defences and neuro-transmission, genotoxicity and, as extreme consequences, drowning and death (Avio et al., 2015; Coe and Rogers, 1997; Gregory, 1978; Hjelmeland et al., 1998; Jackson et al., 2000; Net et al., 2015; Rochman et al., 2014; Wright et al., 2013).

Neutrally buoyant plastic items are the most suitable to be ingested (Setälä et al., 2015), both intentionally and accidentally (Cliff et al., 2002; Laist, 1997). Moreover, plastic debris can be eaten either directly from the water column (primary ingestion), or indirectly (secondary ingestion) from plastic-contaminated food, also in large pelagic species

(Romeo et al., 2015). The potential deleterious effects of ingestion underline the urgency to evaluate the impact of plastics on the whole marine food web and the related consequences for end consumers (Galloway, 2015; Koch and Calafat, 2009; UNEP, 2011), especially in hot spot area of plastic pollution such as the Mediterranean Sea.

Although plastic ingestion by marine organisms has been investigated in several Mediterranean species (Deudero and Alomar, 2015; Fossi et al., 2018), only few data are available on cartilaginous fish from the Mediterranean Sea; these are mainly focused on demersal species such as *Galeus melastomus* (Alomar and Deudero, 2017; Carrassón et al., 1992; Cartes et al., 2016; Deudero and Alomar, 2015; Madurell, 2003; Anastasopoulou et al., 2013a), *Centroscyllium coelelepis* (Carrassón et al., 1992; Cartes et al., 2016), *Etmopterus spinax* (Anastasopoulou et al., 2013b; Cartes et al., 2016; Deudero and Alomar, 2015; Madurell, 2003). Due to their role as apex predators and their wide distribution, sharks could be exposed to plastic ingestion and to other environmental contaminants, through the food web with bioaccumulation and biomagnification processes (Alves et al., 2016; Serrano et al., 2000; Strid et al., 2007). Therefore, they are considered as sentinel organisms for marine pollution biomonitoring studies (Alves et al., 2016; Marcovecchio et al., 1991; Vas, 1991).

The blue shark (*Prionace glauca*) is one of the most wide ranging shark in the Mediterranean Sea (Garibaldi and Orsi Relini, 2000) and worldwide (Stevens, 2009). It is an oceanic and pelagic species with a

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highly migratory behaviour, for reproductive and feeding purposes; it is also able of huge vertical movements, from the surface to over 600 m depth (Camhi et al., 2008; Campana et al., 2011; Garibaldi and Orsi Relini, 2000; Rondinini et al., 2013; Sims et al., 2016). Blue sharks have an opportunistic feeding strategy (Camhi et al., 2008; Carvalho et al., 2011; Garibaldi and Orsi Relini, 2000) with a non-specific diet (Cortés, 1997; Lopez et al., 2010; Vanadia et al., 2004), and are commonly considered “sea shelters” playing a key role in the Mediterranean food web. Most of their preys are pelagic, but bottom fishes and floating elements are also present in their diet (Camhi et al., 2008; Garibaldi and Orsi Relini, 2000). The IUCN Red List assessed the blue shark globally conservation status as “Near Threatened” (Stevens, 2009) however, in the Mediterranean basin, whose population is separated and independent from the North Atlantic one (Kohler et al., 2002; Leone et al., 2017; Megalofonou et al., 2009), is categorized as “Critically Endangered” (Sims et al., 2016). In this area, blue shark is one of the most incidental by-catch species of the long line fisheries targeting swordfish of albacore and bluefin tuna (Camhi et al., 2008; De la Serna et al., 2002; Garibaldi, 2015; Garibaldi and Orsi Relini, 2000; Megalofonou et al., 2005a, b). The Mediterranean population was estimated to face a 90% decline over 30 years and it is increasingly closer to overfishing (Sims et al., 2016). Although the presence of various types of debris (metals, plastic) in *P. glauca* stomachs has been occasionally detected, both in the Mediterranean Sea (Garibaldi and Orsi Relini, 2000) and worldwide (McCord and Campana, 2003; Teodoro Vaske Júnior et al., 2009) scale, no specific analysis and detailed data were carried out.

Thus, the aim of this work was to investigate, for the first time, plastic ingestion in samples of blue sharks from the North Western Mediterranean (Ligurian Sea), in the Specially Protected Area of Mediterranean Importance (SPAMI), Pelagos Sanctuary. To achieve this goal standardized protocols, developed for the analysis of other marine species, were applied to analyze the stomach contents in order to quantify and characterize the litter ingested.

## 2. Materials and methods

### 2.1. Study area and sampling

From 1999 to 2015 a total of 139 blue sharks (*P. glauca*) were sampled in the Western Ligurian Sea, in an offshore area in front of the coast of Sanremo, Imperia and Nice (Fig. 1). This area is part of the Pelagos Sanctuary, a Specially Protected Area of Mediterranean Importance (SPAMI) established in the North-Western Mediterranean Sea for the conservation of cetaceans.

The blue sharks were caught by longlines, deployed both at surface during the night and to a maximum depth of 600 m during the day. Samples were taken directly on board of fishing vessels or at landing, where total length measurement (TL in cm), total weight (TW in g) and sex data were recorded. Specimens were grouped into two size classes on the basis of their total length: TL ≤ 120 cm and TL > 120 cm.

According to Megalofonou et al. (2009), below the threshold of 120 cm samples were considered juveniles (J) whereas over this value adults (TL > 120 cm) (Table 1).

During the necropsy, the stomach of blue sharks were isolated, by means of clamps, to prevent spillage of the contents and removed. The stomachs section was opened and the contents collected. The contents were inspected for the presence of any tar, oil, and preserved in 70% alcohol before the subsequent laboratory analysis. The liquid portion, mucus and digested unidentifiable matter were removed by washing the contents through a 1 mm metal sieve with pre-filtered water. The remaining portion was placed in a petri dish and examined under the microscope. Marine litter items were identified from other ingested material, isolated and placed in closed glass jars, for subsequent counts and characterization.

### 2.2. Marine litter count and characterization

Marine litter was separated from other ingested residue and categorized according to the “Litter in Biota” protocol included in the “Monitoring Guidance for Marine Litter in European Seas” (MSFD Technical Subgroup on Marine Litter, 2013) following the “Guidance of monitoring of Marine Litter in European Seas” protocol developed for sea birds and sea turtles. All items were identified through direct visual sorting of the stomach content using the microscope (Wild Herrtbrugc M5A), isolated and dried at room temperature. The dried items were counted, weighed (Mettler AE 260 DeltaRenge) and scanned with a printer-scanner (Canon MP280). Different measurements (length (cm), width (cm) and area (cm<sup>2</sup>)) of each item were obtained processing the scanned images with ImageJ program. Items were also classified based on their colors. All plastic items were analyzed by Fourier transform infrared (FT-IR) spectroscopy technique (Agilent Cary 630 spectrophotometer) to identify the plastics polymer composition (Hummel, 2002). For each plastic fragment found, depending on its heterogeneity (including degradation status and fouling presence), three measurements were carried out. Only spectra matching > 80% with reference polymers present in libraries (Agilent Polymer Handheld ATR Library, Agilent Elastomer O-ring and Seal Handheld ATR Library and Agilent ATR General Library) were accepted (Fossi et al., 2017; Lusher et al., 2013). In order to avoid the risk of contamination, stringent laboratory and sampling procedures were carried out to ensure the quality of the results.

## 3. Results

### 3.1. Stomach content of plastic items

Of all the 139 blue shark stomachs examined, 44 (31.4%) were found completely empty, due to the fact that some specimens could vomit up food during capture (Stevens, 1973). As a consequence, in order to determine the frequency of marine litter in gastric contents, only full contents (95) were considered (Table 2).

Overall, 109 items of marine litter were found, amounting to a total weight of 6.14 g; the majority (107 items) were represented by user plastic items and only 2 debris were categorized as rubbish.

In 24 out 95 specimens analyzed, the presence of plastic litter was recorded (25.26%) with a range from 1 to 30 items per sample. The total mass of plastics ingested was 3.37 g (range: 0.0001–0.977 g), with a total area of 30,693.61 cm<sup>2</sup> (range: 0.019–27.65 cm<sup>2</sup>).

Analyzing the presence/absence of marine litter in different size classes, juvenile blue sharks are more likely to ingest marine litter than adults showing significant different percentage of occurrence ( $\chi^2 = 3.858$ ,  $p < 0.05$ ) (Table 2). The greater quantity of plastics was found into the stomach of juveniles (65 items), amounting to a total weight of 2.836 g (range: 0.0001–0.977 g) and total area of 30615 cm<sup>2</sup> (range 0.23–27,644.99 cm<sup>2</sup>). Adults ingested 42 plastic pieces, with a total weight 0.5302 g (range: 0.0001–0.5718 g) and a total area 7860.18 mm<sup>2</sup> (range 0.01871–18.907 cm<sup>2</sup>). In addition, no relevant differences were observed between sex (Table 2).

### 3.2. Characteristics of total plastic items

Ingested plastic items were classified based on their shape: sheetlike, threadlike, fragments, foamed and other typologies (other). The majority of plastic items were sheetlike (72.38%), followed by fragments (18.10%), threadlike (5.71%), others (3.81%). No plastic foams were detected.

Total sheetlike items not only had greater external area, but also accounted for the highest weight; the area of threadlike, fragments and other was irrelevant (< 1%) (Fig. 2).

Items were also grouped in three size classes following (Galgani et al., 2013): microplastics (< 5 mm), mesoplastics (5–25 mm),

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