Title:

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

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Abstract: Plastic pollution in the oceans represents a risk for the marine environment and biota. 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 ingestion of plastic litter by blue sharks (*Prionace glauca*), an opportunistic and widespread species in Mediterranean Sea, caught in the Pelagos Sanctuary SPAMI (North-Western Mediterranean Sea). The analysis of the ingested debris in the stomach contents was performed following the MSFD Descriptor 10 standard protocol developed for sea turtles and implemented with FT-IR spectroscopy technique. The results showed that the 25.26% of samples ingested plastic debris of wide scale of sizes from microplastics (<5 mm) to macroplastics (> 25 mm). The FT-IR analysis showed that ingested plastic debris, mainly transparent sheetlike items, were composed by polyethylene. Considering that in the Mediterranean Sea Blue shark is categorized as "Critically Endangered" by IUCN List and, besides, given that this species is a top predator of the pelagic habitat, the high amount of plastic litter found in this research contributes to highlight the real impact of plastic debris both on the marine environmental in a protected area and also on a species with an important ecological role in the food web.

Keywords:

Marine litter
Plastic ingestion
Mediterranean Sea
Blue sharks

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^2 (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 neurotransmission, genotoxicity and, as extreme consequences, drowning and death (Avio et al., 2015; Coe and Rogers, 1997; Gregory, 1978; Hjelmeland et al., 1998; Jackson, G.D. 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 (Outi 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), *Centroscymnus coelolepis* (Carrassón et al., 1992; Cartes et al., 2016), *Etmopterus spinax* (Aikaterini Anastasopoulou et al., 2013; 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 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 ofalbacore and bluefin tuna (Camhi et al., 2008; De la Serna et al., 2002; Garibaldi, 2015; Garibaldi and Orsi Relini, 2000; Megalofonou et al., 2005a, 2005b). 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.

Figure 1. Sampling area

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 () whereas over this value adults (TL >120 cm) (Tab. 1).

Table 1. Specimens of *P. glauca* sampled subdivided by size class: juveniles (TL≤120 cm) and adults (TL >120 cm).

		Juveniles		Adults	
		Male	**Female**	**Male**	**Female**
n° of specimens		29	31	27	52
Total length (cm)	*Min*	52	66	121	122
	Max	115	115	262	199
	Mean ± S.D.	90.94 ± 39.58	94.95 ± 40.05	157.4 ± 39.81	157.78 ± 39.83

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^2)) 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 more than 80% with reference polymers present in libraries (Agilent Polymer Handheld ATR Library, Agilent Elastomer Oring 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 (Tab.2).

Table 2. Number of specimens of blue sharks analyzed, number of full stomachs and frequency of occurrence of marine litter in the stomach contents of juveniles (TL≤120 cm) and adults (TL >120 cm).

	Juveniles			Adults			All
	M	F	Tot.	M	F	Tot.	
n° individuals analyzed	29	31	60	27	52	79	**139**
Full stomachs	17	26	43	17	35	52	**95**
Frequency of full stomachs with marine litter (%)	41.18%	30.77%	34.88%	17.65%	17.14%	17.31%	**25.26%**

Overall, 109 items of marine litter were found, amounting to a total weigh 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 30693.61 cm^2 (range: 0.019-27.65 cm^2).

Analyzing the presence/absence of marine litter in different size classes, juvenile blue sharks seems more likely to ingest marine litter than adults showing higher percentage of occurrence (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 30615cm^2 (range 0.23-27644.99 cm^2). 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^2 (range 0.01871-18.907 cm^2). 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).

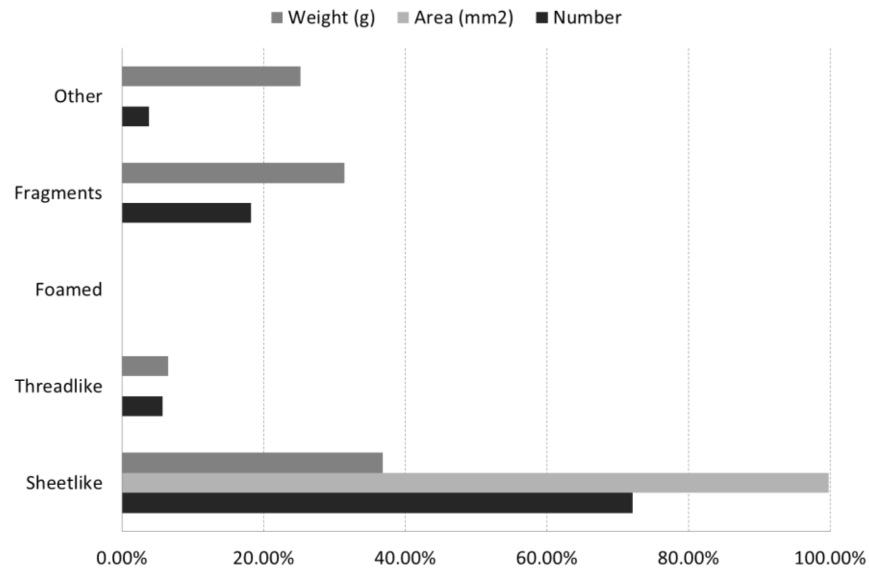

Figure 2. Weight, area and number of different user plastics categories found in the in gastric contents of blue sharks.

Items were also grouped in three size classes following (Galgani et al., 2013b): microplastics (<5mm), mesoplastics (5-25 mm), macroplastics (>25 mm). All size classes were present in sheetlike, fragments and other typologies of plastic. More than 50% of plastic pieces belong to mesoplastics, followed by microplastics (25.71%) and macroplastics (20%). Mesoplastics and macroplastics were present in all category of plastic; in particular mesoplastics accounted for 39.05% and 11.43% of sheetlike and fragments, respectively; the threadlike type was composed of the same quantity of mesoplastics and macroplastics (Fig.3a). A similar proportion of microplastics was present in sheetlike, fragments and other, whereas they were absent in the threadlike type.

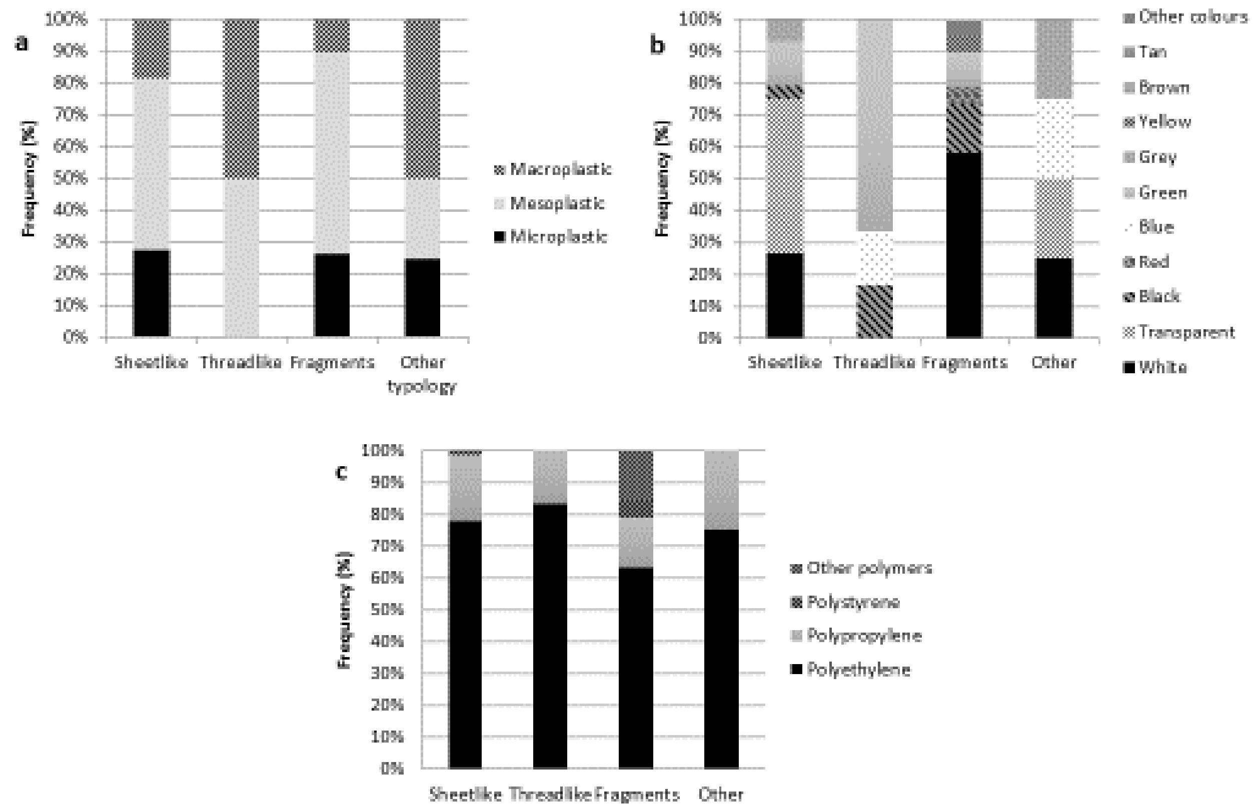

Figure 3. Categories of plastics (sheetlike, threadlike, fragments and other) in relation to a) sizes (macroplastic:>25 mm, mesoplastic: 5-25 mm, microplastic:<5 mm) b) colors and c) type of polymers.

Sheetlike plastics showed all colors, except red, which is present just in fragment category, and blue, present in threadlike and other categories. However sheetlike fragments were represented mainly by transparent and white pieces (47.37 and 28.95%, respectively); fragments were mostly white, black and green. Threadlike pieces were composed of 4 green pieces, one blue and one black. Other class was composed of white, transparent, blue and grey colors in the same proportion (Fig.3b).

With regards to the polymer type, FT-IR analysis revealed that the majority of plastic pieces (75.2%) were made of polyethylene (PE), both low density and high density followed by of Polypropylene (PP) (19.1%), and a small proportion of polystyrene (PS) (1.90%) and other polymers (3.8%).

PE and PP were present in all plastic items independent of shape (Fig. 3c).

On the contrary, other polymers were present only in 4 fragments made of Polyacrylate polyester, Ehylene propylene diene, Polyester and Polyvinyl chloride (PVC).

With regards to the relationship between the polymer type and plastic item size, PE and PP represented the main polymers in all size classes, in particular in Mesoplastics. PS and other polymers were much less represented and only in meso- and macroplastics, respectively (Fig. 10).

All types of polymers were present in mesoplastics, with 41% represented by PE.

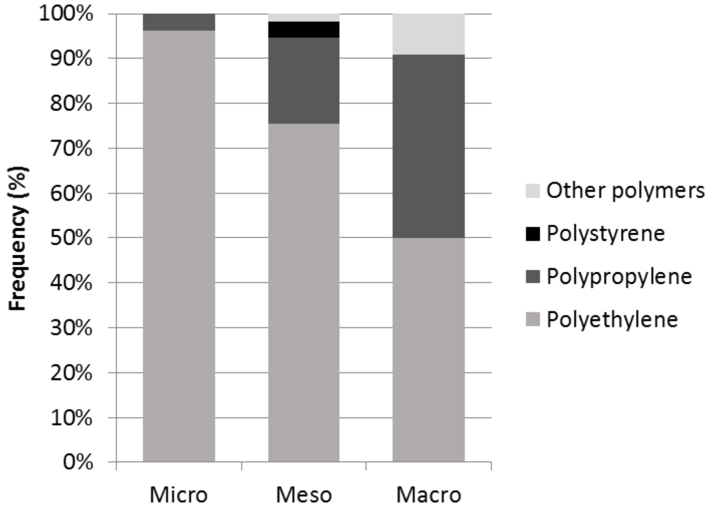

Figure 4. Frequency of plastic polymers in relation to size.

3.3 Characteristics of plastic in different sizeclasses

Mesoplastics represented the majority of plastic ingested in both juveniles and adults. The percentage of microplastics was higher in juveniles than in adults (Fig.5a).

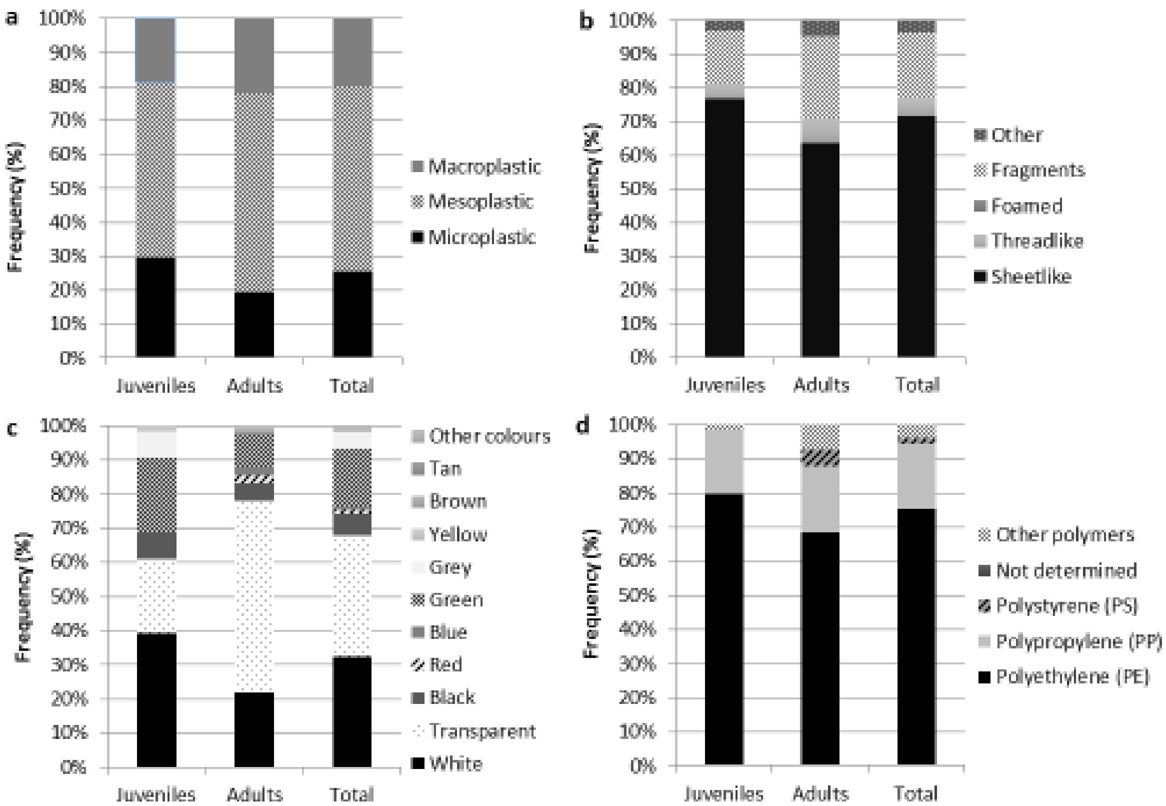

Figure 5. Analysis of plastic ingested by the different sizeclasses, juveniles (n=43) and adults (n=52) in relation to a) size, b) shape, c) color and d) type of polymer

With regards to size, in both classes the most widespread typology was sheetlike,54.69% and 60.98%, respectively in juveniles and adults (Fig.5b). Transparent and white were the most common colors in adults (56.15%) and in juveniles (39.1%) respectively (Fig. 5c).
In juveniles, transparent and green are present with the same percentage (21.9%) and together constitute quite 45% of plastics (Fig. 5c). Adults showed more differences among color categories: white (22%) and green (9.8%) are the most signified colors after transparent (Fig. 5c).
Both in juveniles and in adults PE was the most abundant polymer (79.69% in juveniles, 68.29% in adults), followed by PP, however present in much smaller quantities than PE in both classes (<20%) (Fig.5d).

4 Discussions and conclusions

This paper represent the first study on occurrence and characterization of marine plastic litter in stomachs of blue sharks in the Mediterranean Sea and worldwide scale, given that data available so far regarding marine litter ingestion these species came from feeding ecology studies.
It is difficult to compare among different studies on amounts, types of ingested debris because the methods used for marine litter quantification and characterization are not standardized (Fossi et al., 2018), however comparing the occurrence of plastic ingested by sharks species sampled in different areas of Mediterranean Sea, with data obtaining in the present study (Tab. 3), *P. glauca* shows high occurrence of plastic items (25% of specimens with full stomachs), second only to *Squalus acanthias*, caught in the Adriatic Sea where the authors isolated, from 9 speciments, plastics particles smaller than 1 mm (Avio et al., 2015).

Table 3. Comparison of plastic occurrence in different species of shark caught in the Mediterranean Sea. The specific areas of sampling are: AS (Adriatic Sea), IS (Ionian Sea), Easten Mediterranean Sea (EMS), CMS (central Mediterranean Sea), WMS (Western Mediterranean Sea), Aegian-Levantine Sea (ALS) and the habitat (demersal and pelagic)

Order	Species	GSA	Stud y area	Habi tat	N° of stomach es analyzed	Plastic occuren ce (%)	Bibliography
Carcharhiniformes	***Prioncace glauca*** **(Linnaeus, 1758)**	**GSA09**	**WMS**	**Pela gic**	**95**	**25.3%**	**Present study**
Carcharhiniformes	*Galeus melastomus* (Rafinesque,	GSA20	IS, CMS	Dem ersal	741	3.2%	Anastasopoulou et al. 2013

	1810)						
Carcharhiniformes	*Galeus melastomus* (Rafinesque, 1810)	Eastern Mediterranean	ALS	Demersal	1350	3.0%	Madurell 2003; Deudero & Alomar 2015
Carcharhiniformes	*Galeus melastomus* (Rafinesque, 1810)	Eastern Mediterranean	ALS	Demersal	125	16.8%	Alomar & Deudero 2017
Carcharhiniformes	*Galeus melastomus* (Rafinesque, 1810)	GSA05	WMS	Demersal	37	10.8%	Carrassón et al.1992
Carcharhiniformes	*Galeus melastomus* (Rafinesque, 1810)	GSA05	WMS	Demersal	125	15.2%	Cartes et al.2016
Carcharhiniformes	*Galeus melastomus* (Rafinesque, 1810)	GSA05	WMS	Demersal	95	6.3%	Cartes et al.2016
Carcharhiniformes	*Galeus melastomus* (Rafinesque, 1810)	GSA05	WMS	Demersal	125	16.8%	Alomar & Deudero 2017
Carcharhiniformes	*Scyliorhinus canicula* (Linnaeus, 1758)	GSA20	IS, CMS	Demersal	1	0%	Anastasopoulou et al. 2013
Squaliformes	*Centrophorus granulosus* (Bloch & Schneider, 1801)	GSA20	IS, CMS	Demersal	5	0%	Anastasopoulou et al. 2013
Squaliformes	*Centroscymnus coelolepis* (Barbosa du Bocage & de Brito Capello, 1866)	GSA05	WMS	Demersal	69	2.9%	Carrassón et al.1992
Squaliformes	*Centroscymnus coelolepis* (Barbosa du Bocage & de Brito Capello, 1866)	GSA05	WMS	Demersal	11	9.1%	Cartes et al.2016
Squaliformes	*Centroscymnus coelolepis* (Barbosa du Bocage & de Brito Capello, 1866)	GSA05	WMS	Demersal	54	1.8%	Cartes et al.2016
Squaliformes	*Etmopterus spinax* (Linnaeus, 1758)	GSA20	IS, CMS	Demersal	16	6.2%	Anastasopoulou et al. 2013
Squaliformes	*Etmopterus spinax* (Linnaeus,	Eastern Mediter	ALS	Demersal	323	6.0%	Madurell 2003; Deudero &

	1758)	ranean					Alomar 2015
Squaliformes	*Etmopterus spinax* (Linnaeus, 1758)	GSA 05	WMS	Dem ersal	9	11.1%	Cartes et al.2016
Squaliformes	*Squalus acanthias (*Linnaeus, 1758)	GSA 17	AS	Dem ersal	9	44.4%	Avio et al. 2015
Squaliformes	*Squalus acanthias (*Linnaeus, 1758)	GSA20	IS, CMS	Dem ersal	10	0%	Anastasopoulou et al. 2013
Squaliformes	*Squalus blainville* (Risso, 1827)	GSA20	IS, CMS	Dem ersal	75	1.3%	Anastasopoulou et al. 2013

Blackmouth catshark (*Galeus melastomus)* is the most studied species of shark in Mediterranean Sea for plastic ingestion (Table 3), with an occurrence between 3% and 17%, much lower than those obtained in the present study.

A factor that could explain the differences observed between the blue shark and blackmouth catshark is the feeding habitat: while *P.glauca* is a pelagic shark which feeds from the surface to more than 600 m depth (Campana et al., 2011; Rondinini et al., 2013) following the prey distribution in mesopelagic waters (Bres, 1993; Garibaldi and Orsi Relini, 2000), blackmouth catshark is demersal and epibatial species, which lives from 150 m up to 1400 m depth and therefore they feed in deep water and on the seafloor (A. Anastasopoulou et al., 2013; Bres, 1993). Although plastics litter is ubiquity in the water column, the mean concentrations of plastic floating on the surface are higher than on the seafloor (Eriksen et al., 2014; Galgani et al., 1996).

In addition, the high occurrence of plastic in the stomach contents of the blue sharks is also related to their opportunistic feeding strategy, playing the role of scavenger. Their position at the top of the Mediterranean food web could also increase the probability of exposure to secondary plastic ingestion as described in other Mediterranean top predator (Romeo et al., 2015).

Concerning the size of the specimens, juvenile blue sharks seem more likely to ingest marine litter than adults. These findings are in accordance with other studies on the occurrence of plastic ingestion in adults and juveniles of different marine species (Acampora. et al., 2014; Bravo Rebolledo et al., 2013; Day et al., 1985; Denuncio et al., 2011; Hutton et al., 2008; Kühn et al., 2015; Plotkin and Amos, 1990; Schuyler et al., 2014; van Franeker et al., 2011). Such differences are probably related to their foraging strategy; in fact, larger (older) specimens are more skilled of predation having a major foraging efficiency, whereas young individuals may have a more opportunistic strategy (Bres, 1993; Kühn et al., 2015).

User plastics constitutes the principal category of marine litter found in blue sharks confirming the composition of marine litter observed in marine turtles (Campani et al., 2013; Gramentz, 1988;

Lazar and Gračan, 2011; Matiddi et al., 2017; Tomás et al., 2002), in large pelagic fishes (Karakulak et al., 2009; Romeo et al., 2015) and in sharks (Alomar and Deudero, 2017; Aikaterini Anastasopoulou et al., 2013; Deudero and Alomar, 2015; Garibaldi and Orsi Relini, 2000). Another analogy with the Loggerhead turtle (*Caretta caretta)*, a widespread species in the Mediterranean Sea with high opportunistic behavior and selected as bioindicator species for the marine litter by the MSFD Technical Subgroup on Marine Litter (2013), is the predominance of sheetlike and fragments among shape categories (Camedda et al., 2014; Campani et al., 2013; Tomás et al., 2002).

Mesoplastics are the predominant size class ingested, both in juveniles and in adults, followed by microplastics and macroplastics. The little amount of macroplastics may be related to the predominant smaller size of the shark's preys.

Furthermore, another factor often considered to influence the ingestion of plastic debris is their color, probably in relation to those of their usual preys, which could trick the predators (Kühn et al., 2015; Wright et al., 2013). The variable amount of plastic items of different colors detected in the present work could be also in relation to the opportunistic feeding strategy of the blue shark (Kühn et al., 2015). With regards to the possible impact of plastic items ingested, independent of the amount, size and shape the chemical composition may play a major role (Wright et al., 2013).

Polyethylene (PE), both low density and high density followed by of Polypropylene were the most abundant plastic types identified in the present study. These are in fact widespread used in packaging, grocery sacks, stretch-wrap, balloons, cables or pipe insulations (Peacock Andrew J., 2000). These low density polymers (Andrady, 2011) represent about 70% of floating plastics in the Western of Mediterranean Sea (Suaria et al., 2016). The plastic debris isolated from the stomach of the blue sharks analyzed in this study, reflect the composition and characteristics of floating plastic litter found in the same study area (Suaria et al., 2016; Suaria and Aliani, 2014).

All things considered, blue shark could give information on the environmental status of the area, not only for pollutant contamination (Alves et al., 2016), but also for plastic pollution.

This study adds important information to assess the source of marine litter that impact the Mediterranean biodiversity suitable for futures mitigation actions. The high occurrence of plastic litter in blue sharks raises a warning alarm on the impact that marine litter could have on the Mediterranean population, which is already declining and listed as Critically Endangered due to the impact of longline fisheries targeting other pelagic species. For this reason, in the future, specific studies aimed at evaluating this impact for the blue shark in the entire Mediterranean basin are needed.

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