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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).

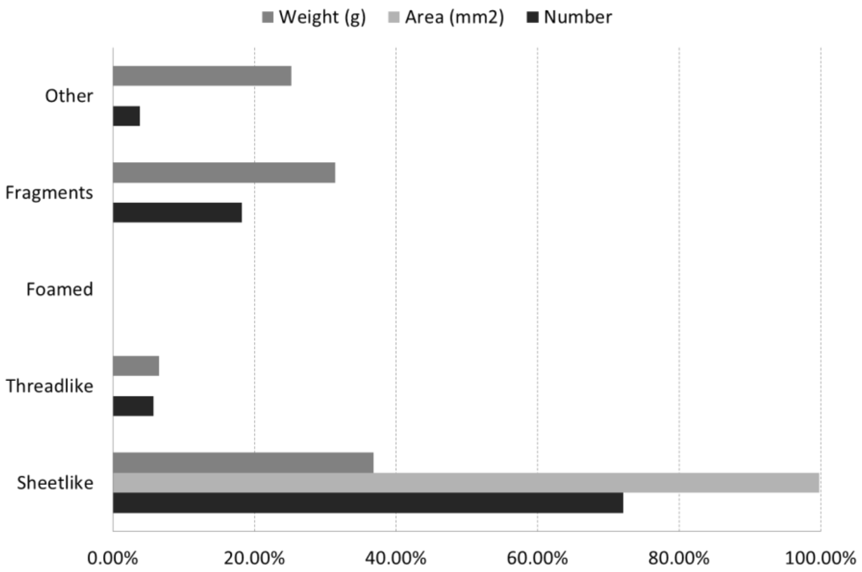

Figure 2. Weight, area and number of different user plastics categories found in the in gastric contents of blue sharks.

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.

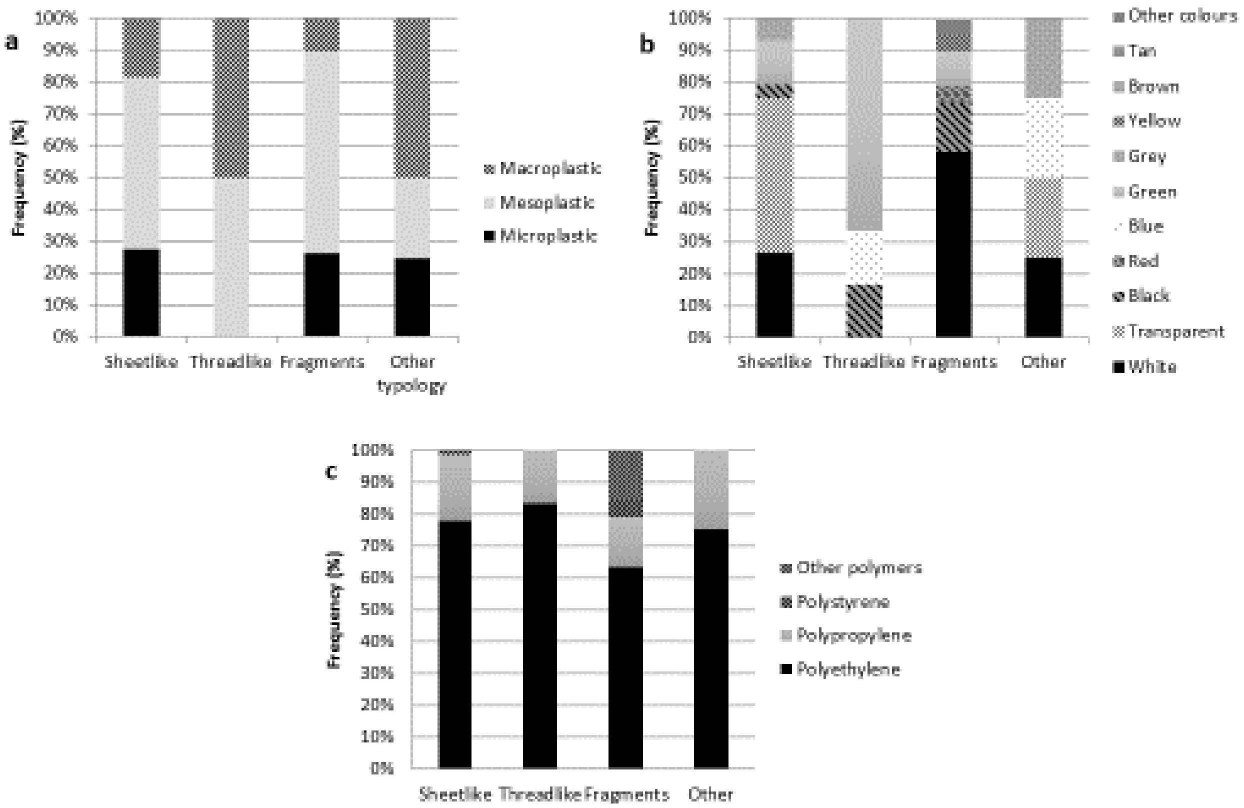

Figure 3. Categories of plastics (sheetlike, threadlike, fragments and other) in relation to a) sizes (macroplastic:>25 mm, mesoplastic: 5-25 mm, microplastic:<5 mm) b) colors and c) type of polymers.

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.

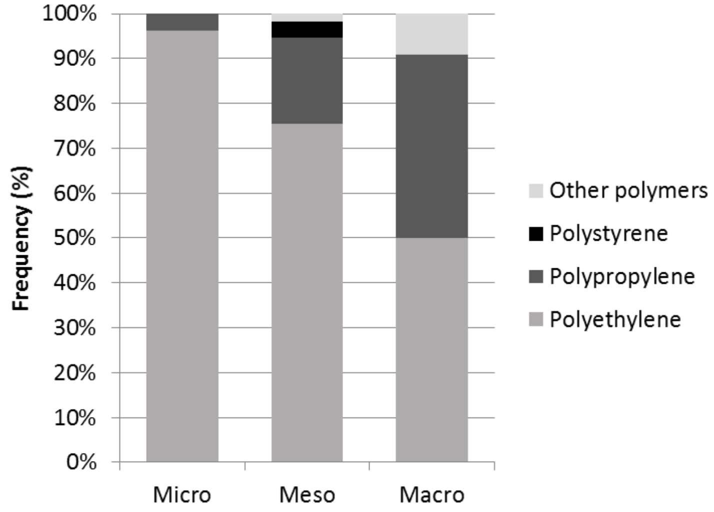

Figure 4. Frequency of plastic polymers in relation to size.

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).

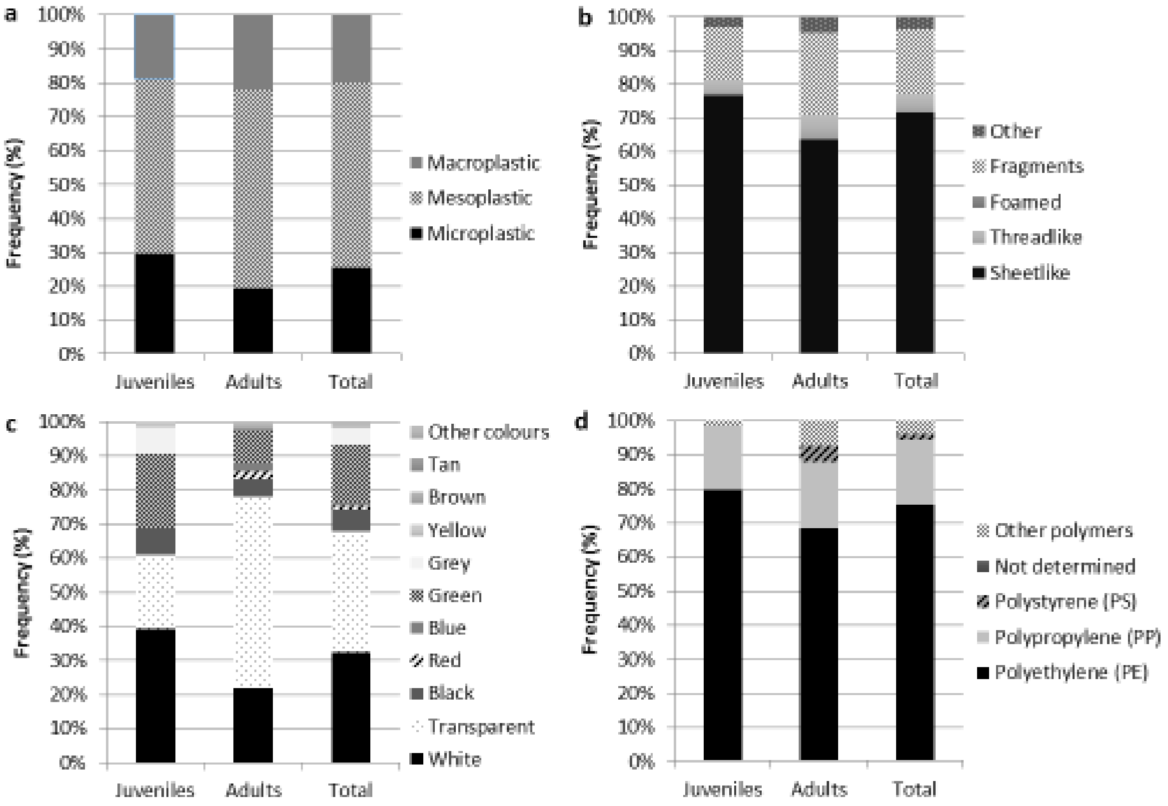

Figure 5. Analysis of plastic ingested by the different sizeclasses, juveniles (n=43) and adults (n=52) in relation to a) size, b) shape, c) color and d) type of polymer

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	Study area	Habitat	N° of stomaches analyzed	Plastic occurence (%)	Bibliography
Carcharhiniformes	***Prioncace glauca*** **(Linnaeus, 1758)**	**GSA09**	**WMS**	**Pelagic**	**95**	**25.3%**	**Present study**
Carcharhiniformes	*Galeus melastomus* (Rafinesque,	GSA20	IS, CMS	Demersal	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	Demersal	9	11.1%	Cartes et al.2016
Squaliformes	*Squalus acanthias (*Linnaeus, 1758)	GSA 17	AS	Demersal	9	44.4%	Avio et al. 2015
Squaliformes	*Squalus acanthias (*Linnaeus, 1758)	GSA20	IS, CMS	Demersal	10	0%	Anastasopoulou et al. 2013
Squaliformes	*Squalus blainville* (Risso, 1827)	GSA20	IS, CMS	Demersal	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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5 References

Acampora., H., Schuyler, Q.A., Townsend, K.A., Hardesty, B.D., 2014. Comparing plastic ingestion in juvenile and adult stranded short-tailed shearwaters (Puffinus tenuirostris) in eastern Australia. Mar. Pollut. Bull. 78, 63–68.

Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark Galeus melastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environ. Pollut. https://doi.org/10.1016/j.envpol.2017.01.015

Alves, L.M.F., Nunes, M., Marchand, P., Le Bizec, B., Mendes, S., Correia, J.P.S., Lemos, M.F.L., Novais, S.C., 2016. Blue sharks (Prionace glauca) as bioindicators of pollution and health in the Atlantic Ocean: Contamination levels and biochemical stress responses. Sci. Total Environ. 563–564, 282–292. https://doi.org/10.1016/j.scitotenv.2016.04.085

Anastasopoulou, A., Mytilineou, C., Lefkaditou, E., Dokos, J., Smith, C.J., Siapatis, A., Bekas, P., Papadopoulou, K.-N., 2013. Diet and feeding strategy of blackmouth catshark Galeus melastomus. J. Fish Biol. 83, 1637–1655. https://doi.org/doi:10.1111/jfb.12269,

Anastasopoulou, Aikaterini, Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep Sea Res. Part Oceanogr. Res. Pap. 74, 11–13. https://doi.org/10.1016/j.dsr.2012.12.008

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea 111, 18–26.

Bravo Rebolledo, E.L., Van Franeker, J.A., Jansen, O.E., Brasseur, S.M., 2013. Plastic ingestion by harbour seals (Phoca vitulina) in the Netherlands. Mar. Pollut. Bull. 67, 200–202.

Bres, M., 1993. The behaviour of sharks. Rev. Fish Biol. Fish. 3, 133–159.

Camedda, A., Marra, S., Matiddi, M., Massaro, G., Coppa, S., Perilli, A., Ruiu, A., Briguglio, P., de Lucia, G.A., 2014. Interaction between loggerhead sea turtles (Caretta caretta) and marine litter in Sardinia (Western Mediterranean Sea). Mar. Environ. Res. 100, 25–32. https://doi.org/10.1016/j.marenvres.2013.12.004

Camhi, M.D., Pikitch, E.K., Babcock, E.A., 2008. Sharks of the Open Ocean. Biology, Fisheries and Conservation. Blackwell Science. Fish Aquat. Resour., 13.

Campana, S.E., Dorey, A., Fowler, M., Joyce, W., Wang, Z., Wright, D., Yashayaev, I., 2011. Migration pathways, behavioural thermoregulation and overwintering grounds of blue sharks in the Northwest Atlantic. PLoS ONE 6e16854.

Campani, T., Baini, M., Giannetti, M., Cancelli, F., Mancusi, C., Serena, F., Marsili, L., Casini, S., Fossi, M.C., 2013. Presence of plastic debris in loggerhead turtle stranded along the Tuscany coasts of the Pelagos Sanctuary for Mediterranean Marine Mammals (Italy). Mar. Pollut. Bull. 74, 225–230. https://doi.org/10.1016/j.marpolbul.2013.06.053

Carrassón, M., Stefanescu, C., Cartes, J.E., 1992. Diets and bathymetric distributions of two bathyal sharks of the Catalan deep sea (western Mediterranean. Mar. Ecol. Prog. Ser., 1 82, 21–30.

Cartes, J.E., Soler-Membrives, A., Stefanescu, C., Lombarte, A., Carrassón, M., 2016. Contributions of allochthonous inputs of food to the diets of benthopelagic fish over the northwest Mediterranean slope (to 2300m). 123–136.

Carvalho, F.C., Murie, D.J., Hazin, F.H.V., Hazin, H.G., Leite-Mourato, B., Burgess, G.H., 2011. Spatial predictions of blue shark (Prionace glauca) catch rate and catch probability of juveniles in the Southwest Atlantic 68, 890–900. https://doi.org/10.1093/icesjms/fsr047

Cliff, G., Dudley, S.F.J., Ryan, P.G., Singleton, N., 2002. Large sharks and plastic debris in KwaZulu-Natal, South Africa. Mar. Freshw. Res. 53, 575–581.

Coe, J.M., Rogers, D.B. (Eds.), 1997. Marine debris: sources, impacts, and solutions, Springer series on environmental management. Springer, New York.

Cortés, E., 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes 726–738.

Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, J.Á., Irigoien, X., Duarte, C.M., 2015. Plastic Accumulation in the Mediterranean Sea. PLOS ONE 10, e0121762. https://doi.org/10.1371/journal.pone.0121762

Day, R.H., Wehle, D.H.S., Coleman, F.C., 1985. Ingestion of plastic pollutants by marine birds. In R. S. Shomura & H. O. Yoshida (Eds.), Proceedings of the Workshop on the Fate and Impact of Marine Debris (pp. 344–386). Honolulu, Hawaii: U.S. Dep. Commer., NOAA Tech. Memo. NMFS.

De la Serna, J.M., Valeiras, J., Ortiz, J.M., Macías, D., 2002. Large pelagic Sharks as By–catch in the Mediterranean Swordfish Longline Fishery: Some Biological Aspects. Elasmobranch Fisheries –. NAFO Scr Doc 02137, N4759.

Denuncio, P., Bastida, R., Dassis, M., Giardino, G., Gerpe, M., Rodriguez, D., 2011. Plastic ingestion in franciscana dolphins, Pontoporia blainvillei (Gervais and D'Orbigny, 1844), from Argentina. Mar. Pollut. Bull. 62, 1836–1841.

Deudero, S., Alomar, C., 2015. Mediterranean marine biodiversity under threat: Reviewing influence of marine litter on species. Mar. Pollut. Bull. 98, 58–68. https://doi.org/10.1016/j.marpolbul.2015.07.012

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLoS ONE 9, e111913. https://doi.org/10.1371/journal.pone.0111913

Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019

Fossi, M.C., Romeo, T., Baini, M., Panti, C., Marsili, L., Campani, T., Canese, S., Galgani, F., Druon, J.-N., Airoldi, S., Taddei, S., Fattorini, M., Brandini, C., Lapucci, C., 2017. Plastic Debris Occurrence, Convergence Areas and Fin Whales Feeding Ground in the Mediterranean Marine Protected Area Pelagos Sanctuary: A Modeling Approach. Front. Mar. Sci. 4. https://doi.org/10.3389/fmars.2017.00167

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Thompson, R.C., VanFraneker, J., Vlachogianni, T., Scoullos, M., Mira Veiga, J., Palatinus, J., Matiddi, M., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, J., 2013b. Monitoring Guidance for Marine Litter in European Seas. JRC scientific and policy reports.

Galgani, F., Souplet, A., Cadiou, Y., 1996. Accumulation of debris on the deep sea floor off the French Mediterranean coast. Mar. Ecol. Prog. Ser. 142, 225–234. https://doi.org/10.3354/meps142225

Galloway, S.T., 2015. Micro- and nano-plastics and human health. In M. Bergmann, L. Gutow & M. Klages (Eds.), Marine anthropogenic litter (pp. 347–370). Berlin: Springer.

Garibaldi, F., 2015. By-catch in the mesopelagic swordfish longline fishery in the Ligurian Sea (western Mediterranean). ICCAT, Collect. Vol. Sci. Pap., 3 71, 1495–1498.

Garibaldi, F., Orsi Relini, L., 2000. Abbondanza estiva, struttura di taglia e nicchia alimentare della verdesca, Prionace glauca, nel Santuario pelagico del Mar Ligure. Biol. Mar. Medit. 7, 324–333.

Gramentz, D., 1988. Involvement of loggerhead turtle with the plastic, metal, and hydrocarbon pollution in the central Mediterranean. Mar. Pollut. Bull. 19, 11–13. https://doi.org/10.1016/0025-326X(88)90746-1

Gregory, M.R., 2009. Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364, 2013–2025. https://doi.org/10.1098/rstb.2008.0265

Gregory, M.R., 1978. Accumulation and distribution of virgin plastic granules on New Zealand beaches.

Hjelmeland, K., Pedersen, H.B., Nilssen, E.M., 1998. Trypsin content in intestines of herring larvae, Clupea harengus, ingesting inert polystyrene spheres or live crustacea prey.

Hummel, D.O., 2002. Atlas of plastics additives: analysis by spectrometric methods. Springer, Berlin ; New York.

Hutton, I., Carlile, N., Priddel, D., 2008. Plastic ingestion by flesh-footed shearwaters, Puffinus carneipes, and wedge-tailed shearwaters, Puffinus pacificus. Pap. Proc. R. Soc. Tasman. 142, 67–72.

Jackson, G.D., Buxton, N.G., George. M.J., 2000. Diet of the southern opah Lampris immaculatus on the Patagonia Shelf; the significance of the squid Moroteuthis ingens and antropogenic plastic.

Koch, H.M., Calafat, A.M., 2009. Human body burdens of chemicals used in plastic manufacture. Philosophical transactions of the Royal Society of London B, 364, 2063–2078.

Kohler, N.E., Turner, P.A., Hoey, J.J., Natanson, L.J., Briggs, R., 2002. Tag and recapture data for three pelagic shark species: blue shark (Prionace glauca), shortfin mako (Isurus oxyrinchus), and porbeagle (Lamna nasus) in the North Atlantic Ocean. Collect. Vol. Sci. Pap. ICCAT 54, 1231–1260.

Kühn, S., Rebolledo, E.L.B., Franeker, J.A. van, 2015. Deleterious Effects of Litter on Marine Life, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, pp. 75–116.

Laist, D.W., 1997. Impacts of Marine Debris: Entanglement of Marine Life in Marine Debris Including a Comprehensive List of Species with Entanglement and Ingestion Records, in: Coe, J.M., Rogers, D.B. (Eds.), Marine Debris. Springer New York, pp. 99–139.

Lazar, B., Gračan, R., 2011. Ingestion of marine debris by loggerhead sea turtles, Caretta caretta, in the Adriatic Sea. Mar. Pollut. Bull. 62, 43–47. https://doi.org/10.1016/j.marpolbul.2010.09.013

Leone, A., Urso, I., Damalas, D., Martinshon, J., Zanzi, A., Mariani, S., Sperone, E., Micarelli, P., Garibaldi, F., Megalofonou, P., Bargelloni, L., Franchi, R., Macias, D., Prodohl, P., Fitzpatrick, S., Stagioni, M., Tinti, F., Cariani, A., 2017. Genetic differentiation and phylogeography of Mediterranean-North Eastern Atlantic blue shark (Prionace glauca, L. 1758) using mitochondrial DNA: panmixia or complex stock structure? PeerJ 5e4112. https://doi.org/10.7717/peerj.4112

Lopez, S., Meléndez, R., Barría, P., 2010. Preliminary diet analysis of the blue shark Prionace glauca in the eastern South Pacific 745–749.

Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028

Madurell, T., 2003. Feeding Strategies and Trophodynamic Requirements of Deep- Sea Demersal Fish in the Eastern Mediterranean. University of the Balearic Islands.

Marcovecchio, J.E., Moreno, V.J., Pérez, A., 1991. Metal accumulation in tissues of sharks from the Bahía Blanca estuary, Argentina.

Matiddi, M., Hochsheid, S., Camedda, A., Baini, M., Cocumelli, C., Serena, F., Tomassetti, P., Travaglini, A., Marra, S., Campani, T., Scholl, F., Mancusi, C., Amato, E., Briguglio, P., Maffucci, F., Fossi, M.C., Bentivegna, F., de Lucia, G.A., 2017. Loggerhead sea turtles (Caretta caretta): a target species for monitoring litter ingested by marine organisms in the Mediterranean Sea. Environ. Pollut. 230, 199–209. https://doi.org/10.1016/j.envpol.2017.06.054

McCord, M.E., Campana, S.E., 2003. A quantitative assessment of diet of the Blue Shark (Prionace glauca) off Nova Scotia, Canada. J.Northw.Atl.Fish.Sci., Vol 32: 57-63.

Megalofonou, P., Damalas, D., de Metrio, G., 2009. Biological characteristics of blue shark, Prionace glauca, in the Mediterranean Sea 1233–1242.

Megalofonou, P., Damalas, D., Yannopoulos, C., 2005a. Composition and abundance of pelagic shark by-catch in the eastern Mediterranean Sea. Cybium 29, 135–140.

Megalofonou, P., Yannopoulos, C., Damalas, D., De Metrio, G., Deflorio, M., de la Serna, J.M., Macias, D., 2005b. Incidental catch and estimated discards of pelagic sharks from the swordfish and tuna fisheries in the Mediterranean Sea. Fishery Bulletin 103, 620–634.

MSFD Technical Subgroup on Marine Litter, 2013. Guidance on monitoring of marine litter in European seas. Publications Office, Luxembourg.

Net, S., Sempéré, R., Delmont, A., Paluselli, A., Ouddane, B., 2015. Occurrence, Fate, Behavior and Ecotoxicological State of Phthalates in Different Environmental Matrices. Environ. Sci. Technol. 49, 4019–4035. https://doi.org/10.1021/es505233b

Outi Setälä, Joanna Norkko, Maiju Lehtiniemi, 2015. Feeding type affects microplastic ingestion in a coastal invertebrate community.

Peacock Andrew J., 2000. Handbook of polyethylene. Structures, properties and applications.Marcel Dekker, Inc.

Plotkin, P.T., Amos, A.F., 1990. Effects of anthropogenic debris on sea turtles in the northwestern Gulf of Mexico. In Proceedings of the Workshop on the Fate and Impact of Marine Debris 736–743.

Rochman, C.M., Kurobe, T., Flores, I., Teh, S.J., 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment.

Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., Fossi, M.C., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. 95, 358–361. https://doi.org/10.1016/j.marpolbul.2015.04.048

Rondinini, C., Battistoni, A., Peronace, V., Teo li, C., 2013. Lista Rossa IUCN dei Vertebrati Italiani. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma.

Schuyler, Q., Hardesty, B.D., Wilcox, C., Townsend, K., 2014. Global Analysis of Anthropogenic Debris Ingestion by Sea Turtles. Conserv. Biol. 28, 129–139. https://doi.org/10.1111/cobi.12126

Serrano, R., Fernández, M., Rabanal, R., Hernández, M., Gonzales, M.J., 2000. Congener-specific determination of polychlorinated biphenyls I shark and grou- per livers from the Northwest African Atlantic Ocean.

Sims, D., Fowler, S.L., Ferretti, F., Stevens, J., 2016. Prionace glauca (Regional Assessment: Mediterranean): The IUCN Red List of Threatened Species.

Stevens, J., 2009. Prionace glauca: The IUCN Red List of Threatened Species.

Stevens, J.D., 1973. STOMACH CONTENTS OF THE BLUE SHARK (PRIONACE GLAUCA L.) OFF SOUTH-WEST ENGLAND.

Strid, A., Jörundsdóttir, H., Päpke, O., Svavarsson, J., Bergman, Å., 2007. Dioxins and PCBs in Greenland shark (Somniosus microcephalus) from the North-East Atlantic.

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Scientific Reports.

Teodoro Vaske Júnior, Rosangela Paula Lessa, Otto Bismarck Fazzano Gadig, 2009. Feeding habits of the blue shark (Prionace glauca) off the coast of Brazil.

Tomás, J., Guitart, R., Mateo, R., Raga, J.A., 2002. Marine debris ingestion in loggerhead sea turtles, Caretta caretta, from the Western Mediterranean. Mar. Pollut. Bull. 44, 211–216. https://doi.org/10.1016/S0025-326X(01)00236-3

UNEP, 2011. UNEP year book: Emerging issues in our global environment (79 p). Nairobi: United Nations Environmental Programme.

van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.-L., Heubeck, M., Jensen, J.-K., Le Guillou, G., Olsen, B., Olsen, K.-O., Pedersen, J., Stienen, E.W.M., Turner, D.M., 2011. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. Environ. Pollut. 159, 2609–2615. https://doi.org/10.1016/j.envpol.2011.06.008

Vanadia, A., Zuffa, M., Storai, T., 2004. Osservazioni su di una femmina gravida di Prionace glauca (Linnaeus, 1758) catturata nelle acque siciliane. ESE 27. https://doi.org/10.1285/i15910725v27p13

Vas, P., 1991. Trace metal levels in sharks from British and Atlantic waters.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031