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First record of plastic debris in the stomach of Mediterranean lanternfishes

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This study highlights for the first time the presence of plastic debris in the stomachs of Mediterranean lanternfishes (Myctophidae): Electrona risso, Diaphus metopoclampus, Hygophum benoiti *and* Myctophum punctatum. *Samples were collected in the central Mediterranean Sea between 2010 and 2014. Plastics ingested belonged to small microplastics (0.2 - 2 mm), large microplastics (2 - 5 mm) and mesoplastics (5 - 25 mm), having mainly clear colors. Their frequency of occurrence in stomachs was equal to 2.7%, but it increases to 5.8% if only migratory species are considered. The higher number of plastics was found in* E. risso *and* H. benoiti *(5 in both species). The plastic ingestion may represent a risk for vertical migrant lanternfishes due to the increase in buoyancy. Ecotoxicological aspects linked to the potential effects of contaminants on lanternfish biology and to the transfer of pollutants throughout the marine trophic web up to top predators should be deepened.*

Key words: Micro-plastics ingestion, mesopelagic fish, Myctophidae, stomach content, trophic web, Mediterranean

INTRODUCTION

An emerging problem in marine environment is represented by the plastic presence in the water column (THOMPSON *et al.*, 2004) and the risk of their input and accumulation in the trophic web (TEUTEN *et al.*, 2009; GALGANI *et al.*, 2013a). Plastics are worldwide distributed, from surface to deep waters, include particles less than 20 mm of several polymers such as polyester, nylon, polyethylene, polypropylene, etc. (BARNES *et al.*, 2009) and stay in marine waters for long periods (GALGANI *et al.*, 2013a). Plastic pollution is moved throughout the world's oceans by the prevailing winds and surface currents, converging in accumulation zones of oceanic gyres (ERIKSEN *et al.*, 2014). According to CÓZAR *et al.* (2014) data on the presence of plastics in the water coloumn of Mediterranean Seawaters can be defined scarce, but recently FOSSI *et al.*

(2014) concluded that the mean abundance of microplastics in the Pelagos Sanctuary (western Mediterranean) is of the same order of magnitude as that found for the North Pacific Gyre, one of the most impacted areas in the world. The monitoring of this phenomenon in European waters is one of the objectives of the European Marine Strategy Framework Directive (MFSD) (GALGANI *et al*., 2013a, b).

A growing concern linked to the presence of plastics in sea water is related to the potential impact of these particles in the marine trophic web through ingestion by several marine organisms, ranging from zooplankton to top predators (FOSSI *et al*., 2012, 2014; COLE *et al*., 2013; COLLIGNON *et al*., 2014; IVAR DO SUL & COSTA, 2014; ROMEO *et al*., 2015), in particular in those species which use filter feeding, such as baleen whales (FOSSI *et al.,* 2012). The ingestion of plastics may represent a vehicle of chemical pollutants (additives of these particles or POPs adsorbed on them) towards the organisms and the food chain, representing a risk for biological functions of marine species and having implications also for their consumers as humans (TEUTEN *et al*., 2009; GALGANI *et al*., 2013a; SELTENRICH, 2015).

On the Mediterranean scale, macro-plastic ingestion is generally reported for cetaceans and turtles (LAZAR & GRAČAN 2011; CAMPANI *et al*., 2013; DEUDERO & ALOMAR, 2014), while few data are available on fish (MASSUTÍ *et al.,* 1998; DEUDERO, 2001; KARAKULAK *et al*., 2009; ANASTASOPOULOU *et al*., 2013; ROMEO *et al*., 2015; BATTAGLIA *et al*., 2016b), mainly from some dietary studies reporting just a list of ingested debris. However, information of plastic ingestion by pelagic fish is instead poorly investigated (ROMEO *et al.*, 2015), representing a lack of knowledge which needs an in-depth analysis.

The present paper reports for the first time the occurrence of plastic debris in the stomach content of four Mediterranean mesopelagic fish belonging to the family of Myctophidae (lanternfishes): the Benoit's lanternfish *Hygophum benoiti* (Cocco, 1838), spotted lanternfish *Myctophum punctatum* Rafinesque, 1810, spothead lanternfish *Diaphus metopoclampus* (Cocco, 1829), electric lanternfish *Electrona risso* (Cocco, 1829).

Considering the importance of lanternfish in the marine trophic web as prey of large pelagic fish, this study provides an important contribute to the knowledge of plastic occurrence in lanternfish and discusses the potential hazard associated to the debris ingestion for these species and for its transfer troughout the trophic web.

MATERIAL AND METHODS

Overall 296 of *D. metopoclampus* were caught during two trawl surveys (October 2010 and May 2011) in the Strait of Sicily (central Mediterranean Sea) by bottom trawl on board commercial vessels targeting shrimps (details of sampling are given in BATTAGLIA *et al*., 2014). Specimens were washed and preserved in aluminum foils to avoid external contamination. Moreover, a total of 73 *H. benoiti,* 71 *M. punctatum* and 82 *E. risso* were collected from 2014; they were found stranded on the shore of the Sicilian coast of the Strait of Messina (central Mediterranean Sea). Specimens were preserved frozen in filtered sea water at -20°C. Each individual was measured (standard length L_S to the nearest 0.1 millimeter) and weighed (total weight W in g). Stomachs were removed and examined in laboratory. To prevent airborne contamination (fibres affecting the sample) the dissection was carried out under controlled conditions. According to LUSHER *et al.* (2015), all manipulation instruments and equipment were cleaned and checked under a microscope for contamination with airborne fibres before use. All gut sections were put in a Petri plate, covered prior to analysis by a larger glass plate and were exposed to air for the minimum time possible. The glass plates were always cleaned using filtered water before re-use.

Plastic debris were counted, weighed and measured (length, width, thickness) and characterized by the stereomicroscope Zeiss Discovery V.8 coupled with Axiovision digital image processing software and their colour was recorded.

According to GALGANI *et al.* (2013b) plastics ingested were grouped into microplastics (< 5 mm) and mesoplastics (5 - 25 mm). Moreover, the microplastics were in turn categorized as small microplastics (0.2 - 2 mm) and large microplastics (2 - 5 mm), as reported by COLLIGNON *et al.*, (2014). The frequency of microplastic occurrence (*%F*) in fish was estimated as the proportion of the individuals examined that contained plastics. These values were also calculated for two categories of lanternfish, vertical migratory (including highly migrant *H. benoiti and M. punctatum* and weakly migrant *E. risso*) and non-migratory (*D. metopoclampus*) species, according to their ecology (SCOTTO DI CARLO *et al.*, 1982; BERDAR *et al.*, 1983; BATTAGLIA *et al.*, 2014, 2016a). Chi-squared test (χ^2) was performed in order to check potential significant differences between the occurrence of plastics in stomachs of lanternfishes. Yates correction was applied to the χ^2 values.

RESULTS

In all, 14 plastic fragments were found in the stomach content of lanternfishes, partitioned as follows: 5 in both *H. benoiti* and *E. risso*, 3 in *M. punctatum*, 1 in *D. metopoclampus* (Table 1). Table I shows the mean values and range of fish size (L_S, mm) and weight (*W*, g) as well as the number of plastics found for each species. The frequency of microplastic occurrence (*%F*) recorded a higher value for vertical migratory lanternfish (5.8%) than for non-migratory species (0.3%).

Shape of plastics varied from irregular to rectangular, round or elongated and the most

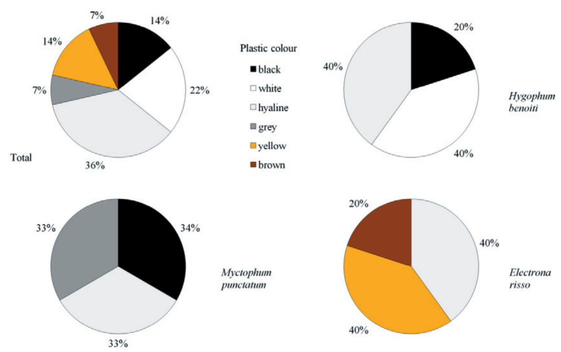

Fig. 1. Percentage of color categories of plastic fragments in the stomach contents of lanternfishes caught in central Mediterranean.

part of them was flat. Different colors of plastic fragments were observed and light colors were dominant; hyaline and white plastics were the most abundant (36% and 22% respectively), as shown in Fig. 1. Only *E. risso* ingested yellow and brown plastics, whereas debris particles found in the stomach of *H. benoiti* and *M. punctatum* were quite similar in colour. The unique plastic ingested by *D. metopoclampus* was white (Fig. 1).

Frequency of occurrence, colour and size (mean values, range and standard deviation values) of plastic fragments found in the stomach contents of lanternfishes are summarized in Table 2. Results of Chi-squared test showed significant differences between the occurrence of plastics in stomachs of *D. metopoclampus* versus the other lanternfishes (*D. metopoclampus vs H. benoiti*: χ^2 = 11.72, p = 0.0006; *D.*

Table 1. Mean values and range of fish length (LS) and weight (W) for each lanternfishes. The number of stomach containing plastics is also reported

Species	Number of stomachs examined	Mean fish lenght ± SD	Length range	Mean fish weight ± SD	Weight range	Number of stomachs with micro- and meso-plastics
		(mm)	*(mm)*	*(g)*	*(g)*	
H. benoiti	73	41.8 ± 6.0	26.1 - 55.0	1.43 ± 0.57	0.31 - 2.85	5
M. punctatum	71	53.2 ± 8.5	26.3 - 68.2	2.67 ± 1.06	0.32 - 5.32	3
E. risso	82	40.4 ± 4.8	19.3 - 53.5	2.27 ± 0.79	0.25 - 5.10	5
D. metopoclampus	296	72.9 ± 8.0	50.8 - 92.8	8.83 ± 2.83	2.84 - 17.52	1
Total	522					14

Table 2. Frequency of occurrence, colour, size of plastic fragments found in the stomach contents of lanternfishes caught in C. Mediterranean. Mean values, range and standard deviation (SD) are reported.

Plastic debris	*Hygophum benoiti*	*Myctophum punctatum*	*Electrona risso*	*Diaphus metopoclampus*
Frequency (%)	6.8	4.2	6.1	0.3
Colour	black, hyaline, white	black, hyaline, grey	hyaline, yellow, brown	white
Length range (mm)	1.59 - 7.55	1.37 - 2.47	0.83 - 1.61	2.88
Mean length and SD	4.10 ± 3.08	1.91 ± 0.55	1.09 ± 0.30	-
Width range (mm)	1.27 - 3.69	0.90 - 1.81	0.50 - 0.86	1.68
Mean width and SD	2.12 ± 0.97	1.22 ± 0.51	0.63 ± 0.14	-
Thickness range (mm)	0.01 - 0.36	0.05 - 1.36	0.02 - 0.20	0.25
Mean thickness and SD	0.10 ± 0.15	0.50 ± 0.74	0.09 ± 0.10	-
Weight range (g)	<0.0001 - 0.0021	<0.0001 - 0.0022	<0.0001	0.0020

metopoclampus vs M. punctatum: $\chi^2 = 4.83$, p = 0.0280; *D. metopoclampus vs E. risso*: $\chi^2 = 10.20$, p = 0.0014). The other comparisons resulted not significant.

Small microplastics resulted the most abundant debris found in stomach contents and represented the totality of plastics ingested by *E. risso* (Fig. 2). Large microplastics were observed in equal proportion in the stomachs of *H. benoiti*, *M. punctatum* and *D. metopoclampus*, whereas mesoplastics were only recovered from the stomachs of *H. benoiti*. Images of plastic debris found in the stomach content of lanternfishes are shown in Figure 3.

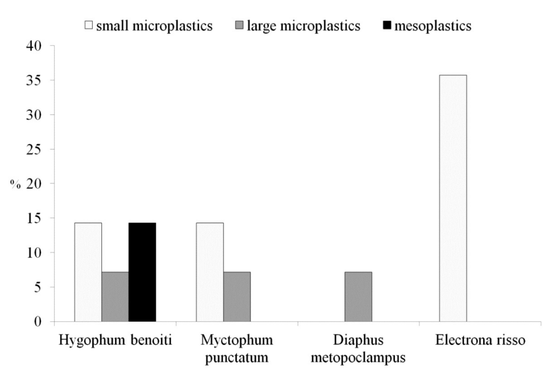

Fig. 2. Total percentage of size categories of plastic fragments in the stomach contents of lanternfishes examined. Mesoplastics: 5 - 25 mm; large microplastics: 2 - 5 mm; small microplastics: 0.2 - 2 mm.

DISCUSSION

The present paper reports for the first time ingestion of plastic debris in Mediterranean mesopelagic fish, belonging to the family of Myctophidae. Although these species lack of commercial interest for fishery, they have a huge biomass and are significant components in the marine food web (GJØSAETER & KAWAGUCHI, 1980). Indeed they are involved in the energy transfer from zooplankton to higher trophic levels and from epipelagic to deep waters (several lanternfish usually perform diel vertical migrations) (GJØSAETER & KAWAGUCHI, 1980). Lanternfish species are primary source of food for several pelagic top predators (e.g. SPRINGER *et al*., 1999; MOTEKI *et al*., 2001) and are an important prey item of the bluefin tuna in Mediterranean waters (KARAKULAK *et al*., 2009; BATTAGLIA *et al*., 2013). The ingestion of plastic by lanternfish may represent a hazard for their predators and a source of potential pollutants which may determine bioaccumulation phenomena. Indeed, although the frequency of plastics ingested is not elevated, lanternfish are frequently eaten by tunas (KARAKULAK *et al*., 2009; BATTAGLIA *et al*., 2013), which prey on their schoolings, ingesting a large number of individuals (BATTAGLIA *et al*., 2013). This kind of predation may magnify the occurrence of microplastic in this top predator. A recent study (ROMEO *et al*., 2015) reported the occurrence of plastics in the stomach content of some fish top predators (albacore, bluefin tuna, swordfish) in the same study area. The occurrence of plastic in the stomach contents of the bluefin tuna may be in part due to secondary ingestion, consequent to predation on lanternfish. There is increasing evidence that small mesopelagic fish ingest microplastics (BOERGER *et al.,* 2010; DAVISON & ASCH, 2011). In addition, they may play a relevant role in capturing plastic at millimeter scale and at microplastics trophic transfer scale (COZAR *et al*., 2014).

The plastic ingestion by mesopelagic fish have been already reported in the Pacific Ocean (BOERGER *et al*., 2010; DAVISON & ASCH, 2011), with significantly higher percentages of occurrence, likely due to the elevated concentration of debris in the North Pacific Subtropical Gyre (MOORE *et al.*, 2001). The scale of magnitude of the plastic occurrence phenomenon in the Ocean Gyres is higher and not comparable with Mediterranean ones; however the strong upwelling currents of the Strait of Messina can determine small vortexes and areas in which floating plastic debris may accumulate. BOERGER *et al.* (2010) reported a 35% incidence of plastic in fish stomachs, but DAVISON AND ASCH (2011) considered this finding an overestimate (a value 3.3 times greater than the next highest rate of plastic ingestion reported from fishes in the wild), attributable to the influence of net feeding, being samples collected by manta trawl. In the case of *D. metopoclampus* collected by bottom trawl, we can exclude net feeding because of the large size of net mesh, that did not retain the microplastic particles, as confirmed by the low occurrence of plastics in the stomach of this species.

In our study the frequency of occurrence of plastics in lanternfish was equal to 2.7%, but it increases to 5.8% if only migratory species (both highly and weakly migrant) are considered. Indeed, the different feeding and migrating behaviors of lanternfish presumably determine the level of plastic assumption. For example, the larger number (56.7%) of examined specimens belonged to *D. metopoclampus*, but only 7.1% of total plastics were found in its stomachs. According to BATTAGLIA *et al.* (2014), adult *D. metopoclampus* does not carry out large migrations, but prefers stay in deep waters, feeding when prey are available. On the contrary, *H. benoiti, M. puctatum* and *E. risso* are daily vertical migrants (SCOTTO DI CARLO *et al*., 1982, BERDAR *et al*., 1983), even if *E. risso* carries out less extensive depth excursions (BATTAGLIA *et al*., 2016a) and the higher occurrence of plastics in their stomach may be explained by the higher abundance of debris in the epipelagic layer, due to their buoyancy (ALIANI *et al*., 2003).

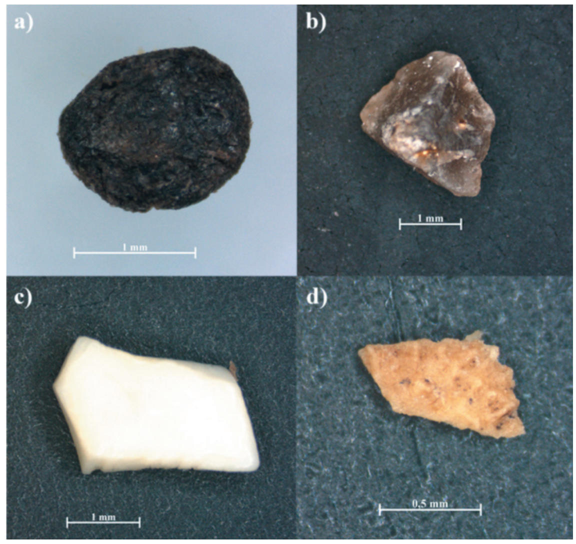

Fig. 3. Images of plastic debris found in the stomach content of H. benoiti *(a),* M. punctatum *(b),* D. metopoclampus *(c),* E. risso *(d)*

In the present study, the colors of plastic debris found in the stomach of lanternfishes were mainly clear (white, hyaline, grey and yellow), similar to those of many zooplankton species, which represent a primary energy source for these fish in the study areas (SCOTTO DI CARLO *et al*., 1982; BATTAGLIA *et al*., 2014, 2016a). The observed plastics had often a similar size to copepods or other small zooplankton prey, thus explaining the tendency of ingesting these particles. Moreover, a dark plastic ingested by *H. benoiti* shown in Fig. 3 had similar shape and color to the eyes of some euphausiid prey found in the same stomach. This may indicate an active and selective feeding on these plastic particles, probably due to the similar shape and color to their natural prey.

Impacts of ingested plastic on lanternfish have not been fully evaluated, but involve two main concerns: physical and toxicological effects. The plastic material may accumulate in digestive tracts and also retained: smallest plastic particles could be evacuated by fish, whereas larger plastic (mesoplastics) may not pass through digestive tracts and lead fish to malnutrition and eventual starvation (BOERGER *et al.*, 2010), also considered the small size of lanternfishes. The ingestion of plastic represents for vertically migrant lanternfish a serious hazard due to the buoyancy of this debris material. Most species of lanternfish carry out extensive daily vertical migrations to epipelagic layers during night to feed on zooplankton and come back to deeper waters during daytime (OLIVAR *et al.*, 2012). An increase of buoyancy caused by plastic ingestion may prevent their return to deep waters, representing a potential source of mortality.

The direct (plastic additives) and indirect (PBT) ecotoxicological effects of microplastics exposure on the lanternfish biology and to the transfer of pollutants throughout the marine trophic web up to top predators should be deepened.

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Prvi zapis o plastičnim krhotinama u želucu mediteranskih riba žaboglavki (Myctophidae)

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SAŽETAK

Ova studija ističe po prvi put prisutnost plastičnih otpadaka u želucima mediteranskih žaboglavki. (Myctophidae): *Electrona risso, Diaphus metopoclampus*, *Hygophum benoiti* i *Myctophum punctatum.* Uzorci su prikupljeni u središnjem Mediteranu u periodu između 2010. i 2014. godine. Progutane čestice plastike pripadaju maloj mikroplastici (0,2-2 mm), velikoj mikroplastici (2-5 mm) i mezoplastici (5-25 mm), te su uglavnom jasnih boja. Njihova učestalost pojavljivanja u probavilima iznosila je 2.7%, dok je kod migratornih vrsta iznosila 5.8%. Veći broj čestica plastike pronađen je kod vrsta *E. risso* i *H. benoiti* (5 po vrsti). Gutanje plastike može predstavljati rizik za vertikalne migratore iz porodice žaboglavki zbog povećanja uzgona. Potrebno je produbiti istraživanja ekotoksikoloških aspekata povezanih s mogućim učincima zagađivala na biologiju žaboglavki, kao i onih povezanih s prijenosom zagađivala kroz trofičku mrežu u moru sve do glavnih grabežljivaca.

Ključne riječi: gutanje mikroplastike, mezopelagične ribe, Myctophidae, sadržaj želuca, trofička mreža, Mediteran