Marine litter in stomach content of small pelagic fishes from the Adriatic Sea: sardines (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*)

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Received: 1 June 2018 / Accepted: 13 November 2018 / Published online: 27 November 2018

Abstract
Marine litter impacts oceans and affects marine organisms, representing a potential threat for natural stocks of pelagic fish species located at the first levels of the marine food webs. In 2013–2014, on a seasonal basis, marine litter and microplastics in stomach contents from *Sardinia pilchardus* and *Engraulis encrasicolus* were evaluated. Selected species are plankitivores of great ecological and commercial importance in the Adriatic Sea. Collected data were correlated to possible factors able to affect ingested levels as well as species, season of sampling, biometry and sex of animals. Almost all tested samples (80 organisms for each species) contained marine litter (over 90% of samples from both species) and also microplastics; while any meso- or macroplastics were recorded. On average, recorded items were as follows: 4.63 (*S. plichardus*) and 1.25 (*E. encrasicolus*) per individual. Sardines evidenced a higher number of microplastics characterised by a smaller size than those recorded in anchovies. For sardines, sex, Gastro Somatic Index and sampling season showed negligible effects on the number of ingested litter; conversely, anchovies showed differences related with both sex of animals and dominant colour of ingested materials with prevalence for black and blue colours.

Keywords Marine litter · Microplastic · Plastic ingestion · Stomach content · Edible fish species · Human consumption

Introduction

Global production of plastics has been growing for more than 50 years, reaching 300 million tonnes in 2013, with a 3.9% increase compared to that in 2012 (Plastic Europe 2014). By 2014, the rate of global production had reached 311 million tonnes per year and the long-term forecasts suggest that by 2050 annual global production shall be between 850 million tonnes and 1124 million tonnes (Crawford and Quinn 2016). Due to its large use, scarce biodegradability and growing inputs, a huge quantity of plastic is accumulating in marine environments (Thompson et al. 2009; Law et al. 2010; UNEP 2014), leading to a growing concern for the conservation of marine ecosystem (Barnes et al. 2009). Indeed, "Marine Litter" was introduced by the Marine Strategy Framework Directive (MSFD - Directive 2008/56/EC) as one of the 11 descriptors to define marine ecosystems' environmental status and to target the "Good Environmental Status" in 2020 (Galgani et al. 2013). The greatest alarm is raised by the accumulation of large quantities of very small pieces of plastic litter (< 5 mm in diameter), known as microplastics (MPs), in marine organisms (Collard et al. 2017a and references therein). MPs could represent a threat for the integrity of marine ecosystems as well as for their conservation, due to their ability to absorb chemicals from water (Browne et al. 2013), to release harmful chemicals in the environment (Lee et al. 2018) and to be a vector for allochthonous microorganism diffusion in high-value marine ecosystems (Zettler et al. 2013). Recent studies have shown that MPs are ingested by a large number of marine organisms (Avio et al. 2015; Collard et al. 2017b; Dehaut et al. 2016; Fossi et al. 2016), penetrating the marine

Responsible editor: Philippe Garrigues

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trophic web (Ivar do Sul and Costa 2014; Setala et al. 2014). Cole et al. (2014) demonstrated that MPs could affect the feeding habits, reproduction and breathing of copepods. Microplastics have been reported in the digestive system of various fish species from the Pacific Ocean (Choy and Drazen 2013; Davision and Asch 2011) and other Oceans or Seas (Sanchez et al. 2014; Neves et al. 2015; Romeo et al. 2015), using a direct visual sorting methodology. In Italian coastal water, a baseline assessment on levels of microplastics in commercial organisms highlighted the MP presence in 45% of biota from the Adriatic Sea (Dehaut et al. 2016), and particularly in 95% of the benthic flatfish *Solea solea* (Pellini et al. 2018). For Adriatic fishery, small pelagic planktivores fishes, such as sardines and anchovies, are the most important commercial species with high economic and social value (Kraus et al. 2015). *Sardina pilchardus* is a marine, freshwater, brackish, pelagic-neritic, oceanodromous species (Riede 2004), living within a depth range of 10–100 m (Whitehead 1990) and feeding mainly on phytoplankton (Nikolioudakis et al. 2011). It spawns in batches in the open sea or near the coast (Murua and Saborido-Rey 2003; Muus and Nielsen 1999). *Engraulis encrasicolus* is a pelagic–neritic species (Riede 2004) that occurs in a wide depth range of 0–400 m (Schneider 1990). It is a coastal marine species able to tolerate wide salinity ranges from 5 to 41 PSU, entering estuaries, lagoons and lakes especially during the spawning period. During summer, this species tends to move towards surface water feeding mainly on meso-zooplankton (Borme et al. 2009). According to an in vitro experiment, MPs showed to be efficiently transferred from the water environment to 19% of sardine specimens through feeding (Avio et al. 2015), although the transfer mechanism and the proportion of microplastic content in organisms and environment still remain unclear (Qu et al. 2018). Sardines and anchovies play a key ecological role in coastal ecosystems, transferring energy from plankton to higher trophic levels (Cury et al. 2000). Indeed, they are among the most important commercial fishing resources in the Mediterranean Sea, composing the main diet for pelagic predators (Fossi et al. 2017), and representing the basic diet of harbour porpoises (*Phocoena phocoena*) in the Black Sea (Tonay et al. 2007). Due to their relevance for human consumption, a complete study targeting levels of litter and MPs in natural stocks is essential. Furthermore, a better focus on seasonal fluctuations of marine litter and MPs in stomach contents of selected species could allow filling knowledge gaps on transfer towards the marine trophic web.

This study investigates marine litter and microplastics in stomach contents of the two most important commercial species in the Adriatic Sea, sardines (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*), collected between June 2013 and May 2014. Main characteristics of marine litter and plastic items (i.e. number, size, shape and colour) were determined. In particular, we aimed to (1) evaluate differences in content, shape and colour between considered species related to their feeding strategy; (2) assess relationships between biometric data, sex and litter recorded features according to the species; (3) assess the influence of seasonality on the marine litter and microplastic uptakes in sardines and anchovies.

Material and methods

Sampling strategy and laboratory processing

Commercial fish specimens of *S. pilchardus* and *E. encrasicolus* were sampled between 2013 (in June, July, September, October and December) and 2014 (in January, April and June) in an area of the central Adriatic Sea (Italy, Fig. 1). Light attraction purse seines (*lampara*) was the fishing gear used to catch sardines and anchovies. A random sample of commercial sardines and anchovies (about 2 kg for each species) was taken from the fishing vessels in the Ortona fishing area. The specimens (10 for each sampling month) were stored on ice until laboratory processing. Here, fish samples were put on aluminium foil, and the total length (TL, nearest 0.1 cm) and the total weight (TW, nearest 0.1 g) of each fish were recorded with calibre and with a digital balance, respectively. Specimens from each species were dissected for sex determination and a subsample of individuals (half of them were female) was subsequently processed. Fishes were rinsed with milli-Q water and then transferred into a container under a flow laminar hood for stomach extraction. Stomach samples were collected, weighed, washed with milli-Q water and immediately preserved in 4% solution of buffered formaldehyde, in order to prevent degradation processes and then stored in a 70% solution of ethanol until the marine litter extraction.

Analysis of ingested microplastics

The amount of marine litter and microplastics ingested was evaluated by the analysis of stomach contents. Stomachs were excised and extracted in a glass beaker with 20 mL of 30% H_2O_2 per gramme of tissue at 50 °C for 48 h (Nuelle et al. 2014; Avio et al. 2015). Supernatant solution was filtered through 0.45-μm filters and accurate rinsing of all glassware with artificial seawater was performed to improve the recovery of micro-items. Activities were performed under air laminar flow hood to reduce contamination by air. Filters were dried using oven (temperature < 40 °C) in glass Petri capsules up to constant weight. Items were quantified and measured by stereomicroscopy (Nikon SMZ-800 N); identified microplastic items were divided in classes by shape, colour and size (Galgani et al. 2013; Alomar et al. 2016; Blašković et al. 2017; Fastelli et al. 2016). The Nikon's software for the imaging analysis was applied for litter dimensional measurements (Nikon ACT-1). As visual identification alone is

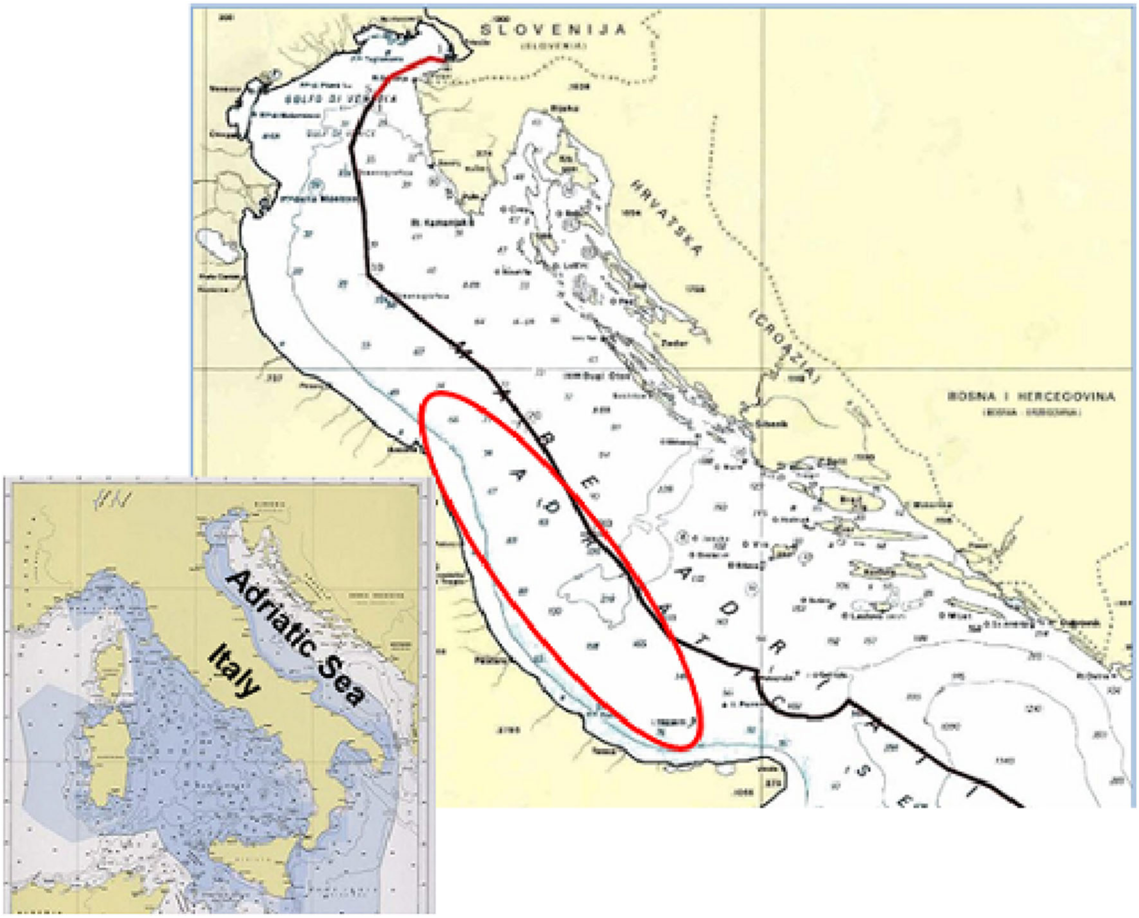


Fig. 1 Localization of fishing area of studied species. Fishing area (red circle) associated to collected sardine and anchovy samples is represented within the Adriatic Sea

inappropriate for studies on small microplastics < 100 μm (Lenz et al. 2015), we reported data on both total marine litter and microplastics (100–5000 μm size) as mean ± standard deviation (SD); ingestions are reported as items/specimens. Items collected in anchovies and sardines were analysed by μFT-IR (Nicolet i-10 MX infrared imaging microscope, Thermo Fisher Scientific) to evaluate prevalent types of collected items. The instrument was equipped with standard detector for microscopy optimised to work under room temperature (DTGS) operating in the spectral range 7600–450 cm^{-1} and with the liquid nitrogen cooled MCT-A operating within the spectral range 7800–650 cm^{-1}. Collected data are elaborated by Thermo Scientific™ OMNIC™ Picta™ user interface. The major and minor dimensions of the identified MP particles were measured as the longest continuous axis in the centre of the particle, and the minor was the longest axis perpendicular to the major axis. To ensure the quality of collected data, periodical inter-calibration exercises were performed to evaluate differences between operators. Scientists used cotton dresses during activities to reduce accidental contaminations and worked under air laminar flow hood. Furthermore, procedural blanks were used to evaluate external contamination during the experimental procedure. In particular, wet filters ($n = 10$) were left overnight exposed to air, in an opened glass Petri dish. Then, filters were extracted as tissue samples and checked by the four-eye approach following the same procedure performed on the samples. Data were corrected by the subtraction of blanks (< 0.8 average fibre items per filter disk).

Data analysis

Gastro Somatic Index (GaSI), in per cent, GaSI = stomach weight/(body weight − stomach weight) × 100 according to Desai (1970), was calculated in order to compare the feeding intensity during the observation period and to assess the environmental and physiological effects on the recorded feeding intensity (Mohammadizadeh et al. 2010). Firstly, a Pearson's correlation analysis ($P < 0.01$) was performed on the biometric data matrix for each species, in order to observe relationships among biometric and feeding intensity descriptors and only variables not correlated with each other were tested as continuous explanatory variables in subsequent statistical analyses (i.e. GaSI and TL; see "Results"). Non-metric Multidimensional Scaling (*nm*-MDS) and ANOSIM test were performed on a Euclidean distance dissimilarity matrix, created using standardised data in order to highlight potential differences between the two fish species in relation to litter contents. Statistical analyses were performed to quantify the contribution of each species to inter-specific differences and to test the significance level of this dissimilarity. In addition, for each species, *nm*-MDS and ANOSIM test were used to test intra-specific differences in litter contents as affected by biometric features, sampling month and dominant colour of items. Univariate and multivariate analyses were performed with GraphPad Prism v.5.0 (GraphPad Software, San Diego, CA, USA, www.graphpad.com) and Primer v6.0 (Primer-E Ltd., Plymouth Marine Laboratory, UK) packages, following methods reported by Clarke and Warwick (2001).

Results

Biometric data and feeding intensity

Concerning TL, *Sardina pilchardus* generally showed no difference between female and male individuals, with lower mean values in September (13.5 cm) for both sexes and higher mean values in July 2013 for both female (14.9 cm) and male (15.1 cm). Conversely, a marked variability in the male pool of July 2013 was highlighted (Table 1). Female individuals had a higher TW than that of the male specimens in July (26.9 vs.23.8 g) and October 2013 (23.1 vs. 19.3 g) and in June 2014 (21.5 vs. 17.1 g) (Table 1). The highest GaSI indices were found in December 2013 for both female (5.6) and male (5.0), while the lowest indices were obtained in June 2013 for both sexes (0.2). Total length and total weight were positively correlated ($r = 0.81$), while no relationship was found between GaSI-TL and GaSI-TW couples.

As for sardines, female and male specimens of *E. encrasicolus* showed negligible differences in TL (Table 1). Minimum lengths were measured in October 2013 for female (11.5 cm) and in September 2013 (11.9 cm) for male, while maxima in January 2014 for both female (14.6 cm) and male (14.9 cm). Mean total weight was lower in September 2013 for both female (10.4 g) and male individuals (11.4 g), while higher values were measured in July 2013 (20.0 g) for female and in October 2013 (19.7 g) for male organisms. The highest GaSI index was obtained in April and June 2014 for both sexes (3.7 for female and 3.1 for male), while the lowest values were calculated in December 2013 for female (1.6) and in July and October 2013 for male specimens (1.5). TL was positively correlated to TW ($r = 0.86$), while both of the biometric data were negatively related to GaSI (female, $r = -0.53$; male, $r = -0.52$).

Given these preliminary results, GaSI and TL were used as continuous explanatory variables in further multivariate analyses.

Litter in stomach contents

In general, almost all analysed specimens (80 per species) contained marine litter (96% of *S. pilchardus* and 91% of *E. encrasicolus*). Some representative images of particles recovered in stomach contents of the two species are reported in Fig. 2.

Table 1 Biometric data associated to studied species. Mean values and standard deviation (SD) of total length (TL; cm), total weight (TW; g) and Gastro Somatic Index (GaSI; %) are reported for each sampling month after grouping data according to animals' sex

	TL				TW				GaSI			
	Female		Male		Female		Male		Female		Male	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S. pilchardus												
June 2013	14.3	0.5	14.1	0.4	21.5	2.8	20.6	0.8	0.2	0.1	0.2	0.1
July 2013	14.9	0.3	15.1	2.1	26.9	3.3	23.8	3.4	1.1	0.5	1.1	0.4
Sept 2013	13.5	0.9	13.5	1.8	16.5	3.4	15.2	2.4	1.0	0.2	0.7	0.3
Oct 2013	14.7	1.0	13.9	1.0	23.1	4.0	19.3	4.1	1.0	0.2	0.6	0.2
Dec 2013	14.1	1.1	14.1	0.7	20.6	5.3	20.0	3.6	5.6	2.1	5.0	2.2
Jan 2014	14.8	0.8	15.1	0.7	21.5	3.1	21.2	2.6	2.2	0.8	2.1	0.4
April 2014	14.1	0.5	13.6	0.9	18.7	2.2	16.0	3.2	2.8	0.6	2.2	0.4
June 2014	14.3	0.8	13.6	0.6	21.5	2.8	17.1	2.5	2.5	0.3	2.3	0.4
E. encrasicolus												
June 2013	13.9	0.9	13.3	0.4	17.8	3.8	15.7	2.3	2.1	0.4	2.8	1.5
July 2013	14.3	0.7	14.3	0.3	20.0	1.6	18.8	1.1	1.7	0.3	1.5	0.1
Sept 2013	12.4	0.3	11.9	1.3	10.4	0.5	11.4	2.8	2.3	1.0	2.4	1.1
Oct 2013	11.5	6.3	14.8	0.8	14.4	7.5	19.7	3.9	1.7	0.2	1.5	0.2
Dec 2013	14.1	0.3	14.2	0.7	17.9	1.5	18.1	2.9	1.6	0.3	1.8	0.4
Jan 2014	14.6	0.5	14.9	0.6	18.3	2.0	19.3	2.6	2.5	0.5	2.1	0.4
April 2014	13.8	0.7	13.0	0.4	16.8	2.5	12.9	1.2	3.7	2.8	3.1	1.3
June 2014	14.4	0.9	13.8	0.6	19.6	3.5	17.2	3.2	3.7	1.9	3.1	2.8

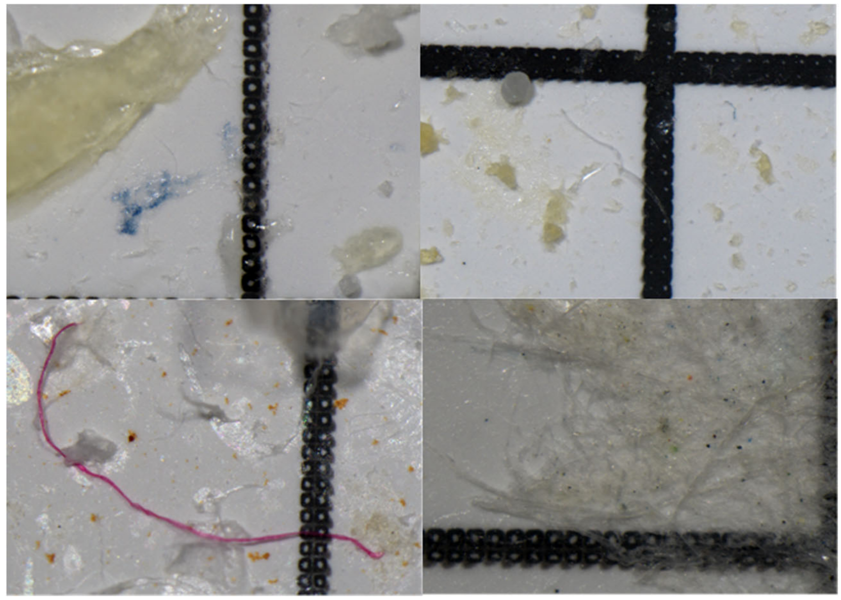


Fig. 2 Plastic litter recovered in the stomach content of analysed species. Pictures of some collected marine litter are reported as representative of the general features of marine litter and microplastics collected in this study

Pellet was recorded only once in *S. pilchardus* (Fig. 2, top right), while fibres and fragments were the most represented item shapes in both species (Fig. 2, pink fibre).

In *Sardina pilchardus*, the lowest number of particles was observed in April 2014 (8 items/ind.), with a prevalence of fragments (83%) and litter size ranging between 5.27 and 1310 μm (Table 2). The highest values were obtained in July 2013 (23 items/ind.), with a litter size ranging between 6.67 and 889 μm. Fragments were the most representative litter shape, even if fibres were also found in large quantities (Table 2). Percentages of items > 100 μm ranged within 17.5% (April 2014) and 44.5% (June 2013). Noticeably, black and orange tiny fragments were observed, yet their nature remained undetermined, due to their very low size dimension (Fig. 2d). A large number of black (14.3–70.0%), tan (3.3–34.4%), blue (10.0–37.1%) and multicolour (3.1–40.8%) fragments (10–20 μm) were found in the stomach contents of this species (Fig. 3a).

In contrast to *S. pilchardus*, *E. encrasicolus* showed the lowest number of items in July 2013 (3), with litter size ranging from 40.1 to 2220.6 μm and a prevalence of litter fibre shape (Table 2).

Table 2 Litter features (< 5000 μm) in stomach contents of the two studied species. Marine litter (total items/ind.) is reported as mean value (SD) grouping data per species and per sampling month. Items > 100 μm of size are, also, reported (mean, range) as the microplastic fraction within the total marine litter recorded. Furthermore, litter size (mean, SD) and shape (mean percentage of items/ind.) are reported

Species	Total items/ind.		> 100 μm items/ind.		Size (μm)		Shape % items/ind.			
	Mean	SD	Mean	Range	Mean	SD	Fibres	Fragment	Film	Pellet
S. pilchardus										
June 2013	11	6.1	4.9	0–7.1	591.5	694.6	91	9	0	0
July 2013	23	20.2	7.9	0.6–8.5	206.1	266.4	43	57	0	0
Sept 2013	10	7.6	3.3	0–5.2	349.1	572.9	40	60	0	0
Oct 2013	16	13.8	5.1	0.9–7.8	428.4	716.6	35	65	0	0
Dec 2013	11	8.1	2.7	0–5.0	448.4	634.8	55	45	0	0
Jan 2014	16	13.9	5.8	1.4–7.3	119.8	193.0	27	73	0	0
April 2014	8	2.7	1.4	0–4.0	254.5	389.8	17	83	0	0
June 2014	15	16.8	5.9	2.1–7.0	328.8	579.9	29	71	0	0
E. encrasicolus										
June 2013	5	2.3	0.9	0–1.2	1462.8	1011.5	83	0	17	0
July 2013	3	3.5	0.5	0–0.9	1044.2	858.7	67	33	0	0
Sept 2013	5	3.1	0.9	0–1.3	1108.5	1275.1	60	40	0	0
Oct 2013	15	6.3	2.8	0.2–3.2	1862.5	6905.3	87	13	0	0
Dec 2013	6	5.1	1.3	0.5–2.1	850.6	788.0	83	17	0	0
Jan 2014	8	9.0	1.5	0.7–2.5	866.6	895.8	50	38	13	0
April 2014	6	4.4	1.1	0.3–1.9	814.7	772.6	67	33	0	0
June 2014	5	3.1	1.0	0–2.2	1747.7	3353.9	80	20	0	0

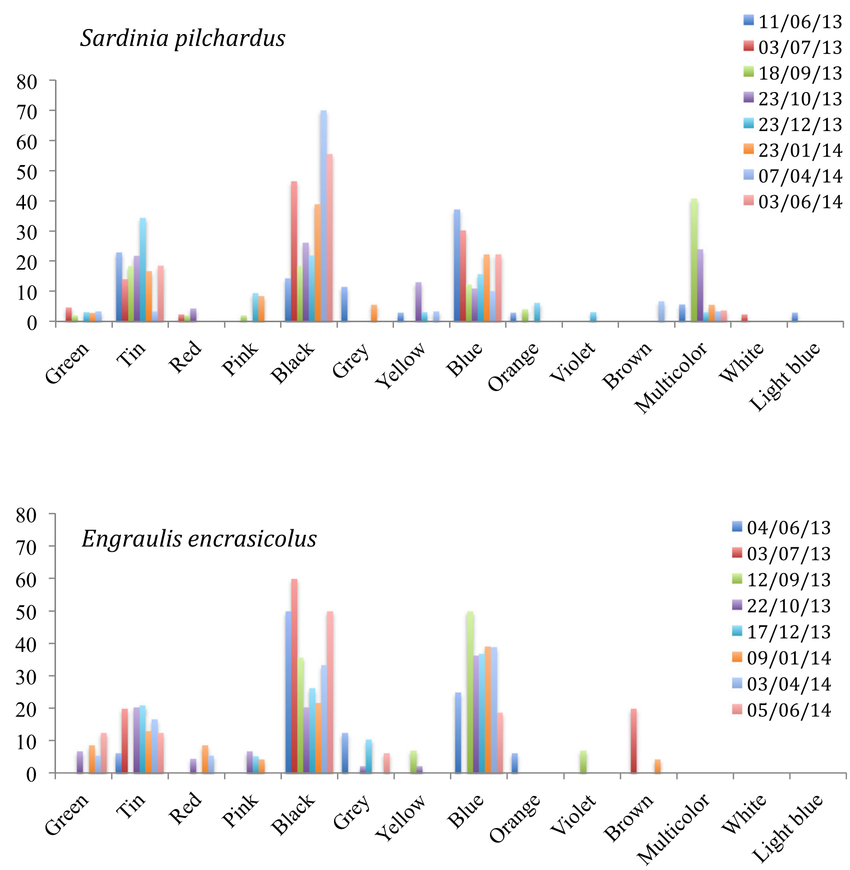


Fig. 3 Average colour fingerprint of recovered microplastic litter in the stomach content of considered species. Data are represented as percentage related to the total amount recovered and grouped according to the species and the month of sampling

Maximum values of items per individual (15), mean size (1862.5 μm) and fibre shape (87%) were all observed in October 2013 (Table 1). Films were also recorded in June 2013 (17%) and in January 2014 (13%).

A small-sized and unidentifiable litter < 100 μm was observed, with percentages ranging between 17% (July 2013) and 22% (December 2013) (Table 2). Blue (18.8–50.0%) and black (20.5–60.0%) microplastics were the most abundant in almost all sampling months, while other colours were only occasionally recorded (Fig. 3b). Based on the chemical composition of MPs, five polymers were recorded at high frequency including polypropylene (PP), polyvinylchloride (PVC), polyacrylic (PA), polytetrafluoroethylene (PTFE), polyethylene (PE) and polyethylene terephthalate (PET). The μ-FT-IR spectrum for each species is shown in Fig. 4. In sardines, the largest abundance of polymer type was identified as PP, accounting for 50%, while PVC, PTFE and PA accounted for 30%, 10% and 10%, respectively. On the contrary, the most abundant plastic type in anchovies was PVC (93%) while PET contributed the most with 7%.

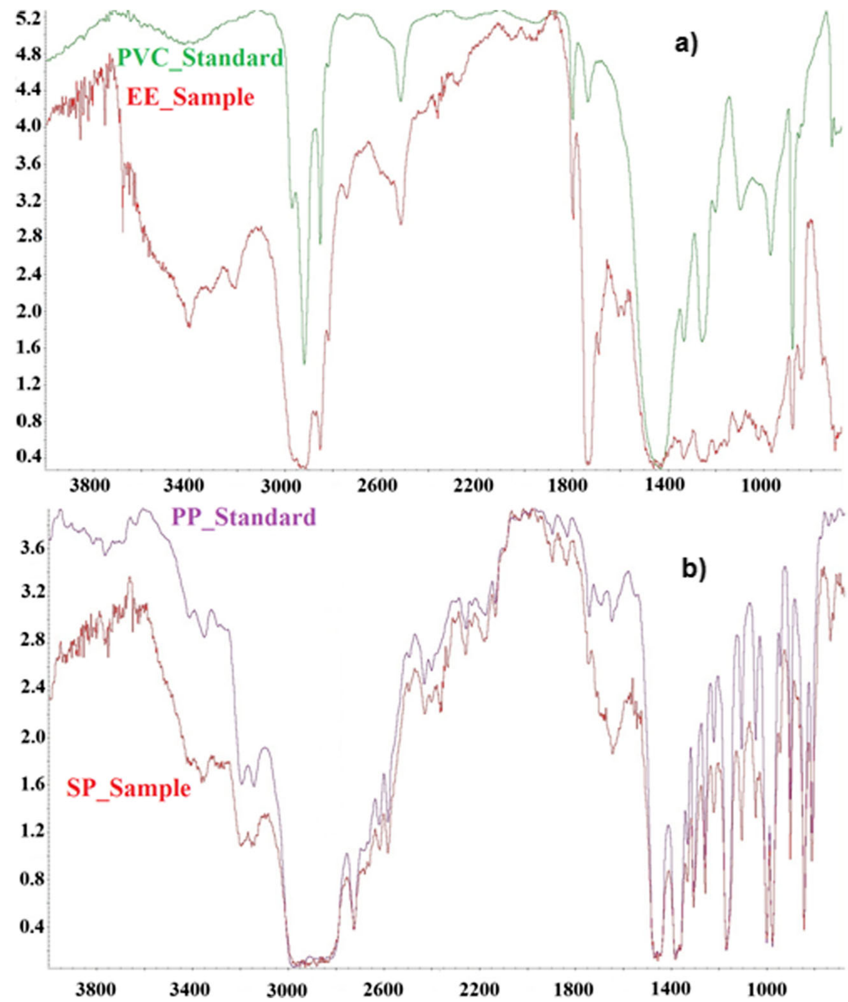
Inter- versus intra-specific features

Non-metric multidimensional scaling performed on the entire litter dataset showed a clear difference between the two species (Fig. 5). Particle number and size were the litter features most responsible for this dissimilarity. Sardines ingested a higher number of items, with smaller sizes, compared to anchovies, as confirmed by the ANOSIM test (Global R, 0.169; significance level of 0.01%). Multivariate analysis was repeated on each species separately, in order to evaluate the influence of biometric data, seasonality and dominant colour of ingested items. The resulting uncorrelated biometric data (GaSI and TL concerning *S. pilchardus* and TL concerning *E. encrasicolus*) were chosen as discriminating factor in the statistical analyses of the litter uptake. No significant difference was recorded related to sex, TL, dominant colour of litter ingested and seasonality in *S. pilchardus*. On the contrary, *E. encrasicolus* showed significant differences according to sex (Global R, 0.140; significance level of 1.2%) and dominant colour of litter ingested (Global R, 0.285; significance level of 0.9%).

Discussion

Microplastics can be ingested by a wide range of marine species, representing a treat for metabolic, physiological or cellular paths (Browne et al. 2008; Wegner et al. 2012). For this reason, commercial fishes for human consumption represent one of the most controversial target species concerning marine litter pollution. Recent studies on microplastics in commercial fishes have reported a wide range of percentages of individuals containing plastic fragments in stomach, intestine and

Fig. 4 μFT-IR spectra for the most abundant polymer in anchovies (**a**) and sardines (**b**). Ordinates reported transmittance (%), while abscisses reported wavenumbers (cm^{-1})



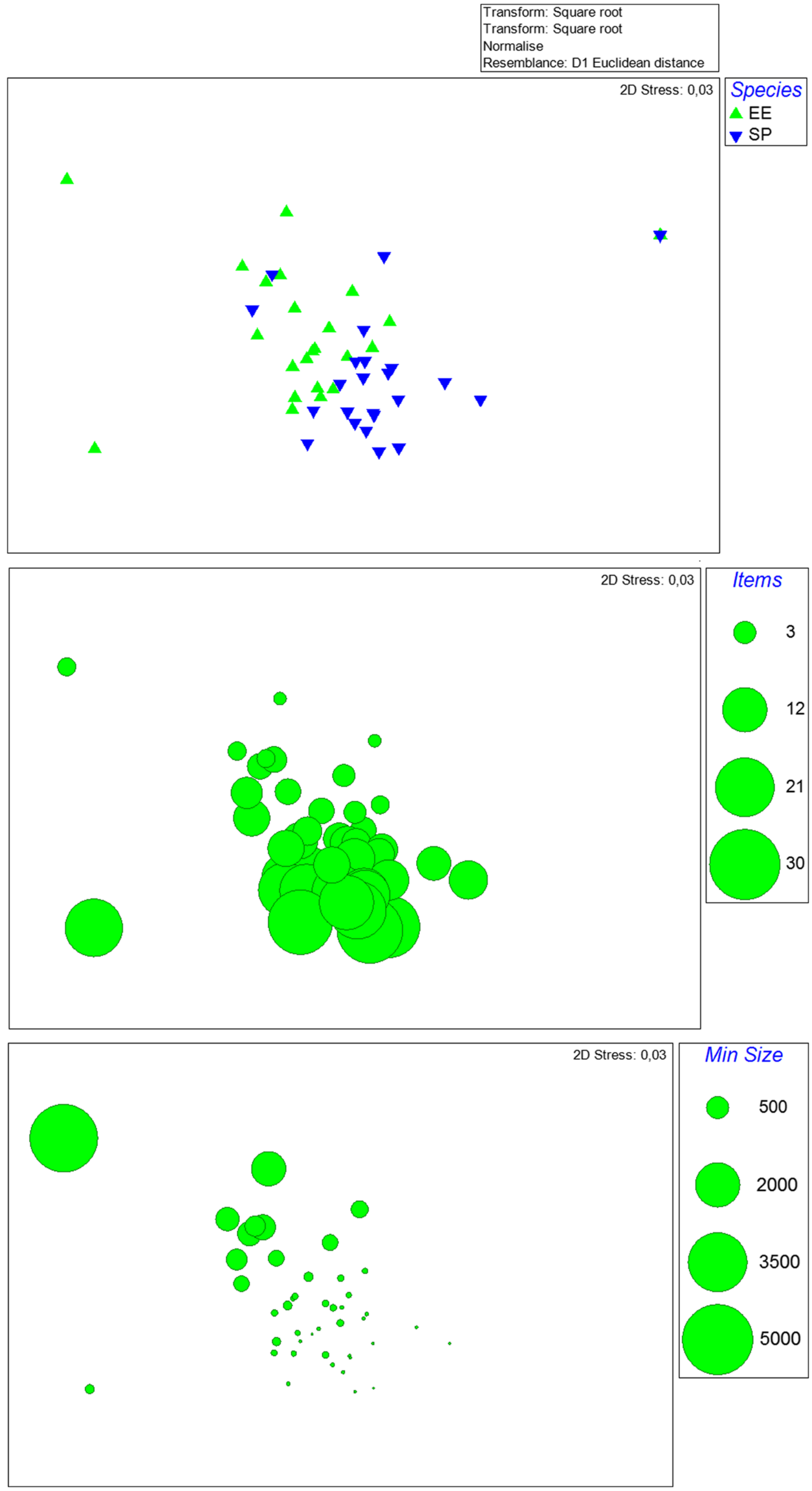
digestive organs, from 2.6–5.5% from North Sea and Baltic Sea (Lusher et al. 2017; Rummel et al. 2016) to 68% from the Swedish coasts (Karlsson et al. 2017). Microplastic pollution has also been found in marketed fish species from the Portuguese coast (20%, Neves et al. 2015) and pelagic fishes from the Central Mediterranean Sea–Aeolian Islands (32%, Romeo et al. 2015).

Uncertainty and variability in the MP data represent two of the main limiting factors for an appropriate assessment of their content in the environment and in organisms. Different methods were tested during the last decade showing some advantages and disadvantages of both. Cole et al. (2014) compared different extraction methods of microplastics from gut contents, pointing out limits and advantages of each of the tested methods. The extraction method selected in this study allows a better comparison of results with the existing literature, even though it was considered to be quite aggressive by some authors. In particular, degradation/bleaching of some polymer shapes was reported in some cases (Enders et al. 2017).

In the present study, the results demonstrate that more than 90% of the analysed sardines and anchovies ingested marine litter. Microplastic fraction is represented by percentages ranging within 18% (anchovies) to 33% (sardines) compared to the ingested litter. Maximum microplastic uptake observed in our study is higher compared to that recorded in recent studies focused on sardines (19%: Avio et al. 2015) as well as on top predators (20%: Romeo et al. 2015). It is worth mentioning that a potential source of bias related to the "net feeding" is not considered in this study. The ingestion by sardines and anchovies of plastic debris trapped in the end of the net could lead to overestimation of natural ingestion, as also observed in a study performed in the North Pacific zone (Davison and Asch 2011), where the occurrence of net feeding in 4 fishes out of 71 was noted. This phenomenon is certain to be explored in future investigations. Environmental conditions, human impacts, flooding events and current pattern in marine and coastal areas could certainly be responsible for different microplastic densities (Desforges et al. 2014) and consequently could affect their ingestion by commercial fishes. Nevertheless, it has also been demonstrated that the different feeding behaviours between organisms explain their susceptibility to MPs (Thevenon et al. 2014).

Feeding strategy seemed to be also the key factor responsible for the clear difference observed in this study between

Fig. 5 Non-metric multidimensional scaling (*nm*-MDS) of collected data on microplastics. Data are represented grouped by the factor species (top), and as bubble superimposed with the average item numbers (middle image) and average minimum size (µm) of recovered marine litter (bottom). EE (*E. encrasicolus*), SP (*S. plichardus*)



the two species in relation to litter items, size and shape. Our results indicate that, compared to *Engraulis encrasicolus*, *Sardinia pilchardus* was characterised by the highest ingestion of marine litter of the smaller sizes (Fig. 4). This means that a greater retention of small plastic particles and a greater filtration apparatus characterise the ingestion system of sardines, compared to anchovies, as also reported in Collard et al. (2017b). Indeed, phytoplankton accounted for 79% of the sardines' diet especially in winter (Nikolioudakis et al. 2011), when they were found to be able to filter over a broad prey size and exhibit high stomach fullness (Table 2). On the contrary, the anchovies' diet is mostly composed of zooplankton (Plounevez and Champalbert 1999), especially copepods and crustacean larvae, justifying the greater number of higher sizes of items observed in their stomach contents.

Generally, pelagic species ingest more particles while feeding, while benthic organisms mostly ingested fibres (Neves et al. 2015) even if a recent study performed on holothurians recorded a possible selective uptake of fragments by this species (Renzi et al. 2018). On the contrary, in the present study, fibres and fragments represented the dominant litter items, with pellets recorded only occasionally in *S. pilchardus*. These results are probably due to the intake route of litter. Greater microplastic content is assumed to derive from fish's prey and not directly from the water column filtration, whose percentage of MPs has been demonstrated to be lower than 19% (Avio et al. 2015).

The colour of microplastics affects their bioavailability because of their resemblance to prey (Wright et al. 2013). In our study, MP dominant colours in *S. pilchardus* (tin, black and blue) were not actively selected by this species and did not represent a key factor for discriminating litter and microplastic content and shapes. On the contrary, *E. encrasicolus* showed significant differences in item levels, size and shape of microplastics in the stomach contents, due to sex and colours. In this species, the dominant black and blue colours (more in female individuals) were actively selected through the feeding mechanism. Indeed, it has been shown that the darkest preys (with ephippia) are preferred over those without ephippia (Plounevez and Champalbert 1999). Although no difference due to sex has been found in feeding strategy of anchovies (Tudela and Palomera 1997), our study highlighted that females ingested on average more items (8 items/ind.) than males (5 items/ind.) with greater size (1657 μm of females against 1161 μm of males), especially during the spring–summer months, recognised as the spawning period (Carpi et al. 2015). During this period, in addition to zooplankton, the female individuals of anchovies probably filter indiscriminately small planktonic organisms in order to obtain more energy, as also supposed by Borme et al. (2009). This feeding mechanism led anchovies to migrate towards surface water, where an immediate ingestion of floating MPs, mistaken as prey, occurs (Wieczorek et al. 2018). Moreover, the colour could play an important role in the selective consumption of microplastics by pelagic fish. Wieczorek et al. (2018) observed that microplastics identified in the surface water (65% black) were very similar to those identified from fish guts (67% black). In our study, a higher occurrence of black items in stomach content could indicate their dominance in coastal environments they inhabit, consistent with the results of other studies (Karami et al. 2017; Wieczorek et al. 2018). This observation may justify the dominant black and blue colours found in this study in the stomach contents of anchovies. Another possible explanation of observed differences could be related to zooplankton's contents. It could be possible that fish MP intake is affected by the presence of MPs in zooplankton as supported by the size range of detected MPs. Further studies are needed to better clarify these hypotheses.

Following the μFT-IR analysis, recorded plastic items could be due to common plastic objects (PP); soft plastic including pieces of plastic bags, food packaging, large pieces of PVC plastic sheeting used to package goods during transport aboard ships (PVC); bottle cap, fishing nets (PE) and plastic beverage bottles (PET).

Considered species are of large commercial interest for human consumption. Although there is an increased scientific interest on the presence of microplastics in both marine environment and trophic web, as well as in food products for human consumption, the knowledge of adverse effects of MPs on human health is still very limited (Barboza et al. 2018; Rist et al. 2018; Santillo et al. 2017; Wright and Kelly 2017). Moreover, microplastics can absorb on their surface environmental pollutants, representing a potential vehicle for the transfer of chemicals, microbes and viruses in fishes and seafood (Koelmans et al. 2016). This could lead to an increased risk of exposure to pollutants and disease for humans (Barboza et al. 2018 and references therein; de Lucia et al. 2014; Teuten et al. 2009). Both fresh and dried small pelagic fishes are often consumed as a whole and a possible suggestion to reduce litter intakes by humans is to eviscerate them before consumption. However, the evisceration process does not ensure a complete reduction of the risk for human health, as reported in Karami et al. (2017), and it is a time-consuming process, labour intensive and uneconomical on a commercial scale (Lakshmanan et al. 1999).

Current knowledge about the presence of MPs in the environments and related cycling through the trophic web, and consequently on human health, need to be implemented in order to better evaluate the feeding habits of marine species and better manage the coastal environments. Further evaluations are therefore required to understand how microplastic contamination of fishes might affect human health through transference of toxins (Santillo et al. 2017).

Finally, results presented in this study could be considered as a preliminary analysis on levels recorded in these species and on some factors that could be related to the observed levels. Even if some aspects could be of some health concern, as well as these two species often consumed without being degutted, presented results are geographically limited and could not realistically represent human intakes by the Italian population. Further studies should be performed with this specific aim to collect samples that could be considered more representative for the evaluation of the human exposure by diet.

Acknowledgments We thank Sergio Pelosi and Gianfranco Pazienza for field assistance and support to the laboratory biometric measurements.

References

Alomar C, Estarellas F, Deudero S (2016) Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar Environ Res 115:1–10

Avio CG, Gorbi S, Regoli F (2015) Experimental development of a new protocol for extraction and characterization of microplastics in fish tissue: first observation in commercial species from Adriatic Sea. Mar Environ Res 111:18–26

Barboza LGA, Vethaak AD, Lavorante BRBO, Lundebye AK, Guilhermino L (2018) Marine microplastic debris: an emerging issue for food security, food safety and human health. Mar Pollut Bull 133:336–348

Barnes DKA, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc Lond Ser B Biol Sci 364:1985–1998

Blašković A, Fastelli P, Čižmek H, Guerranti C, Renzi M (2017) Plastic litter in sediments from the Croatian marine protected area of the natural park of Telaščica Bay (Adriatic Sea). Mar Pollut Bull 114: 583–586

Borme D, Tirelli V, Brandt SB, Fonda Umani S, Arneri E (2009) Diet of *Engraulis encrasicolus* in the northern Adriatic Sea (Mediterranean): ontogenetic changes and feeding selectivity. Mar Ecol Prog Ser 392:193–209

Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC (2008) Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ Sci Technol 42: 5026–5031

Browne MA, Niven SJ, Galloway TS, Rowland SJ, Thompson RC (2013) Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr Biol 23: 2388–2392

Carpi P, Martinelli M, Belardinelli A, Russo A, Arneri E, Coluccelli A, Santojanni A (2015) Coupling an oceanographic model to a Fishery Observing System through mixed models: the importance of fronts for anchovy in the Adriatic Sea. Fish Oceanogr 24(6):521–532

Choy CA, Drazen JC (2013) Plastic for dinner? Observation of frequent debris ingestion by pelagic predatory fishes from the central North Pacific. Mar Ecol Prog Ser 485:155–163

Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. Primer-E, Plymouth

Cole M, Webb H, Lindeque PK, Fileman SE, Halsband C, Galloway TS (2014) Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci Rep 4:4528

Collard F, Gilbert B, Compère P, Eppe G, Das K, Jauniaux T, Parmentier E (2017a) Microplastics in livers of European anchovies (*Engraulis encrasicolus*, L.). EnvironPollut 229:1000–1005

Collard F, Gilbert B, Eppe G, Roos L, Compère P, Das K, Parmentier E (2017b) Morphology of the filtration apparatus of three planktivorous fishes and relation with ingested anthropogenic particles. Mar Pollut Bull 116:182–191

Crawford CB, Quinn B (2016) Plastic production, waste and legislation, Microplastic pollutants. Elsevier Inc., pp 39–56

Cury P, Bakun A, Crawford R, Jarre A, Quiñones R, Shannon L, Verheye H (2000) Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES J Mar Sci 57(3):603–618

Davision P, Asch R (2011) Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. Mar Ecol Prog Ser 432:173–180

Dehaut A, Cassone AL, Frere L, Hermabessiere L, Himber C, Rinnert E, Riviere G, Lambert C, Soudant P, Huvert A, Deflos G, Paul-Pont I (2016) Microplastic in seafood: benchmark protocol for their extraction and characterization. Environ Pollut 215:223–233

Desai VR (1970) Studies on the fishery and biology of tortor (Hamilton) from river Narmada. J Inland Fish Soc India 2:101–112

Desforges JPW, Galbraith M, Dangerfield N, Ross PS (2014) Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. Mar Pollut Bull 79:94–99

Enders K, Lenz R, Beer S, Stademan CA (2017) Extraction of microplastic from biota: recommended acidic digestion destroys common plastic polymers. ICES J Mar Sci 74(1):326–331

Fastelli P, Blăsković A, Bernardi G, Romeo T, Čižmek H, Andaloro F, Russo GF, Guerranti C, Renzi M (2016) Plastic litter in sediments from a marine area likely to become protected (Aeolian Archipelago's islands, Tyrrhenian Sea). Mar Pollut Bull 113:526–529

Fossi MC, Marsili L, Baini M, Giannetti M, Coppola D, Guerranti C, Caliani I, Minutoli R, Lauriano G, Finoia MG, Rubegni F (2016) Fin whales and microplastics: the Mediterranean Sea and the Sea of Cortez scenarios. EnvironPollut 209:68–78

Fossi MC, 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 (2017) Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ Pollut 237:1023–1040

Galgani F, Hanke G, Werner S, De Vrees L (2013) Marine litter within the European Marine Strategy Framework Directive. ICES J Mar Sci 70:1055–1064

Ivar do Sul JA, Costa MF (2014) The present and future of microplastic pollution in the marine environment. Environ Pollut 185:352–364

Karami A, Golieskardi A, Ho YB, Larat V, Salamatinia B (2017) Microplastics in eviscerated flesh and excised organs of dried fish. Sci Rep 7:5473

Karlsson TM, Vethaak AD, Almorth BC, Ariese F, van Velzen M, Hassellov M, Leslie HA (2017) Screening for microplastic in sediment, water, marine invertebrates and fish: method development and microplastic accumulation. Mar Pollut Bull 122:403–408

Koelmans AA, Bakir A, Burton GA, Janssen CR (2016) Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. Environ Sci Technol 50:3315–3326. https://doi.org/10.1021/acs.est.5b06069

Kraus R, Supić N, Lučić D, Njire J (2015) Impact of winter oceanographic conditions on zooplankton abundance in northern Adriatic with implications on Adriatic anchovy stock prognosis. Estuar Coast Shelf Sci 167:56–66

Lakshmanan R, Venugopal V, Rao BYK, Bongirwar DR (1999) Stability of lipids of Indian mackerel to gamma irradiation. J Food Lipids 6: 277–285

Law KL, Moret-Ferguson S, Maximenko NA, Proskurowsk G, Peacock EE, Hafner J, Reddy CM (2010) Plastic accumulation in the North Atlantic Subtropical Gyre. Science 329:1185–1188

Lee H, Byun DE, Kim JM, Kwon JH (2018) Desorption modeling of hydrophobic organic chemicals from plastic sheets using experimentally determined diffusion coefficients in plastics. Mar Pollut Bull 126:312–317

Lenz R, Enders K, Stedmon CA, Mackenzie DMA, Nielsen TG (2015) A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. Mar Pollut Bull 100(1):82–91

de Lucia GA, Caliani I, Marra S, Camedda A, Coppa S, Alcaro L, Campani T, Giannetti M, Coppola D, Cicero AM, Panti C, Baini M, Guerranti C, Marsili L, Massaro G, Fossi MC, Matiddi M (2014) Amount and distribution of neustonic micro-plastic off the western Sardinian coast (Central-Western Mediterranean Sea). Mar Environ Res 100:10–16

Lusher AL, Hollman PCH, Mendoza-Hill JJ (2017) Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fisheries and Aquaculture Technical Paper. No. 615, Rome 147p

Mohammadizadeh F, Valinassab T, Jamili S, Marinfar A, Bahri-Shabanipour AH, Mohammadizadeh M (2010) A study on diet composition and feeding habitats of sawtooth barracuda (Sphyraenaputnamae) in Bandar-Abbas (north of Persian Gulf). J Fish Aquat Sci 5(3):179–190

Murua H, Saborido-Rey F (2003) Female reproductive strategies of marine fish species of the North Atlantic. J Northwest At Fis Sc 33:23–31

Muus BJ, Nielsen JG (1999) Sea fish. Scandinavian fishing year book, Hedehusene 340 pp

Neves D, Sobral P, Ferreira JL, Pereira T (2015) Ingestion of microplastics by commercial fish off the Portuguese coast. Mar Pollut Bull 101:119–126

Nikolioudakis N, Palomera I, Machias A, Somarakis S (2011) Diel feeding intensity and daily ration of the sardine *Sardina plchardus*. Mar Ecol Prog Ser 437:215–228

Nuelle MT, Dekiff JH, Remy D, Friies E (2014) A new analytical approach for monitoring microplastics in marine sediments. Environ Pollut 184:161–169

Pellini G, Gomiero A, Fortibuoni T, Ferrà C, Grati F, Tassetti N, Polidori P, Fabi G, Scarcella G (2018) Characterization of microplastic litter in the gastrointestinal tract of *Solea solea* from the Adriatic Sea. Environ Pollut 234:943–952

Plastic Europe (2014). Plastics—the fact 2014/2015. https://www.plasticseurope.org/application/files/5515/1689/9220/2014plastics_the_facts_PubFeb2015.pdf

Plounevez S, Champalbert G (1999) Feeding behaviour and trophic environment of *Engraulis encrasicolus* (L.) in the bay of Biscay. Estuar Coast Shelf Sci 49:177–191

Qu X, Su L, Li H, Liang M, Shi H (2018) Assessing the relationship between the abundance and properties of microplastics in water and in mussels. Sci Total Environ 621:679–686

Renzi M, Blašković A, Bernardi G, Russo GF (2018) Plastic litter transfer from sediments towards marine trophic webs: a case study on holothurians. Mar Pollut Bull 135:376–385

Riede K (2004) Global register of migratory species—from global to regional scales. Final report of the R&D-Projekt 808 05 081. Federal Agency for Nature Conservation, Bonn 329 pp

Rist S, Almroth BC, Hartmann NB, Karlsson TM (2018) A critical perspective on early communications concerning human health aspects of microplastics. Sci Total Environ 626:720–726

Romeo T, Battaglia P, Pedà C, Consoli P, Andaloro F, Fossi MC (2015) First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar Pollut Bull 95(1):358–361

Rummel CD, Loder MG, Fricke NF, Lang T, Griebeler EM, Janke M, Gerdts G (2016) Plastic ingestion by pelagic and demersal fish from the North Sea and Balthic Sea. Mar Pollut Bull 102(1):134–141

Sanchez W, Bender C, Porcher JM (2014) Wild gudgeons (Gobio gobio) from French rivers are contaminated by microplastic: preliminary study and first evidence. Environ Res 128:98–100

Santillo D, Miller K, Johnston P (2017) Microplastics as contaminants in commercially important seafood species. Integr Environ Assess Manag 13:516–521. https://doi.org/10.1002/ieam.1909

Schneider W (1990) FAO species identification sheets for fishery purposes. Field guide to the commercial marine resources of the Gulf of Guinea. Prepared and published with the support of the FAO Regional Office for Africa. FAO, Rome 268 pp

Setala O, Flemig-Lehtinen V, Lehtinen M (2014) Ingestion and transfer of microplastic in the planktonik food web. Environ Pollut 185:77–83

Teuten EL, Saquing JM, Knappe DRU, Barlaz MA, Jonsson S, Björn A, Rowland SJ et al (2009) Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc B 364:2027–2045

Thevenon F, Carroll C, Sousa J (2014) Plastic debris in the ocean: the characterization of marine plastics and their environmental impacts. In: Situation analysis report. 52 IUCN, Gland. https://doi.org/10.2305/IUCN.CH.2014.03.en

Thompson RC, Moore C, vom Saal FS, Swan SH (2009) Plastics, the environment and human health: current consensus and future trends. Philos Trans R SocLond B 364:2153–2166

Tonay AM, Dede A, Oztürk AA, Oztürk B (2007) Stomach content of harbour porpoises (*Phocoena phocoena*) from the Turkish western Black Sea in spring and early summer. Rapp Commint Mer Mdit 38: 616

Tudela S, Palomera I (1997) Trophic ecology of the European anchovy Engraulis encrasicolus in the Catalan Sea (northwest Mediterranean). Mar Ecol Prog Ser 160:121–134

UNEP (2014) UNEP year book 2014. Emerging issues update. Chapter 8: plastic debris in the ocean. http://www.unep.org/yearbook/2014/PDF/chapt8.pdf. Accessed May 2018

Wegner A, Besseling E, Foekema EM, Kamermans P, Koelmans AA (2012) Effects of nanopolystyrene on the feeding behaviour of the blue mussel (*Mytylus edulis* L.). Environ Toxicol Chem 31:2490–3297

Whitehead PJP (1990) Clupeidae. In: Quero JC, Hureau JC, Karrer C, Post A, Saldanha L (eds) Check-list of the fishes of the eastern tropical Atlantic (CLOFETA), vol Vol. 1. JNICT, Lisbon; SEI, Paris; and UNESCO, Paris, pp 208–227

Wieczorek AM, Morrison L, Croot PL, Allock AL, MacLoughlin E, Savard O, Brownlow H, Doyle TK (2018) Frequency of microplastics in mesopelagic fishes from the Northwest Atlantic. Front Mar Sci 5 article 39. https://doi.org/10.3389/fmars.2018.00039

Wright SL, Kelly FJ (2017) Plastic and human health: a micro issue? Environ Sci Technol 51(12):6634–6647

Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine organisms: a review. Environ Pollut 178: 483–492

Zettler ER, Mincer TJ, Amaral-Zettler LA (2013) Life in the "Plastisphere": microbial communities on plastic marine debris. Environ Sci Tecnol 47:7137–7146