Microplastics in *Sepia officinalis* caught on the central Adriatic coast: preliminary results

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Abstract

Microplastics (µPs) represent an emerging problem for the marine environment given their wide bioavailability for all aquatic organisms, from zooplankton to top predators. This work aimed to evaluate a method of extracting microplastics from the gastrointestinal tract of 122 *Sepia officinalis* caught from the Adriatic Sea (along Abruzzo region coasts) to measure its quantity in a poorly investigated species. The extraction method used for gastrointestinal content was performed using 10% potassium hydroxide. In 98/122 (80.32-95% CI=73.27-87.37%) wild animals microplastics were detected with a mean concentration of 6.82±5.52 µPs/subject. Among the fragments, as supported by various authors, those of black color were the most represented ones; however, also blue fibers and transparent spheres were isolated. This study, in agreement with previous ones, poses further attention to the wide microplastic diffusion in the marine environment (surface, columns, sediments, and animals). The obtained results provide the basis for future investigations on this public health concern.

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Introduction

In 2019, 368 million tons of plastics were produced worldwide (PlasticsEurope, 2020), and more specifically, in 2018, 29,1 million tons of plastic waste were collected in European member states, including Norway and Swisse, for processing. However, only a small portion of this material was recycled (32.5%), while the rest ended up in energy recovery (42.6%), landfills (24.9%), or was released directly into the environment (Barnes *et al.*, 2009; Dris *et al.*, 2015; PlasticsEurope, 2020). In marine waters, plastic debris is degraded into macroplastics (25-1000 mm), mesoplastics (5-25 mm), and small particles classified as microplastics (µPs) (<5 mm) (Cole *et al.*, 2011; Galgani *et al.*, 2013; Faure *et al.*, 2015; Pellini *et al.*, 2018). The µPs represent 10% of plastics manufactured and make up 80-85% of marine waste (Thompson, 2006; Auta *et al.*, 2017). They pollute the water column, sediments, and biota of coastal waters, the open ocean, and freshwater environments (Coyle *et al.*, 2020). The predominant µPs in the marine ecosystem, involving fish and birds, are polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene polymers (Białowąs *et al.*, 2022). The majority of µPs have been detected in seafood and results composed of PVC, PE, PET, and PP (Avio *et al.*, 2015; EFSA, 2016; Mercogliano *et al.*, 2021).

A rising concern associated with the presence of µP in marine waters is linked to the potential impact of these particles in the trophic chain, through ingestion by marine organisms ranging from zooplankton to top predators (EFSA, 2020).

Several authors have shown that microplastics can induce an increase in reactive oxygen species at the intracellular level and cause inflammation and oxidative stress (Von Moos *et al.*, 2012; Jeong *et al.*, 2017).

Ingestion of µPs can also be considered a carrier of chemical pollutants (*i.e.*, additives of these particles or persistent organic pollutants) adsorbed on them for multi-cellular organisms through the food chain. This condition poses a risk to the marine species' biological functions with possible implications for consumers (Hanke *et al.*, 2013; Seltenrich, 2015; Koelmans *et al.*, 2016; Barboza *et al.*, 2018).

In the Mediterranean sea, µPs ingestion has been investigated in bivalve mollusks, crustaceans, and fish species (Avio *et al.*, 2015; Romeo *et al.*, 2015; Collard *et al.*, 2017; Digka et al, 2018; Pellini *et al.*, 2018; Giani et al, 2019; Gomiero *et al.*, 2019; Mancuso *et al.*, 2019; Piarulli *et al.*, 2019; Renzi *et al.*, 2019; Savoca *et al.*, 2019a, 2019b; Capillo *et al.*, 2020; Mercogliano *et al.*, 2021).

Scientific works on finding microplastics in cuttlefish (*Sepia officinalis*) are scarce and have involved only few specimens (Abidli *et al.*, 2019; Frey, 2019; Oliveira *et al.*, 2020).

This work aimed to evaluate the presence of microplastics in cuttlefish collected in the Adriatic Sea to monitor this phenomenon in a species in a poorly investigated area.

Materials and Methods

Fresh samples

The survey was conducted on 122 specimens of *Sepia officinalis* normally marketed in the city of Teramo and fished in the central Adriatic Sea, along the Abruzzo coast, between Alba Adriatica and Roseto degli Abruzzi (Figure 1).

According to Renzi *et al.* (2018), collected samples were measured to determine the total length and the total weight. Furthermore, the sex of each subject was determined, as well as their age by measuring the dorsal length of the coat, following what was described by Ezzedine-Najai and El Abed (2001).

Furthermore, an accurate anatomical-pathological examination of all samples was carried out and the gastrointestinal tract was taken to evaluate the presence of μPs.

Extraction method

According to Dehaut *et al.* (2016), 10% potassium hydroxide (KOH) was used for the digestion of the gastrointestinal tract. Accidental contamination was avoided during sample handling and procedural blanks were completely free of contamination. Before carrying out the extraction protocol, to exclude false positives, a negative control was carried out.

The gastrointestinal tract was placed in a container containing 250 mL of a 10% KOH solution and incubated in a water bath at

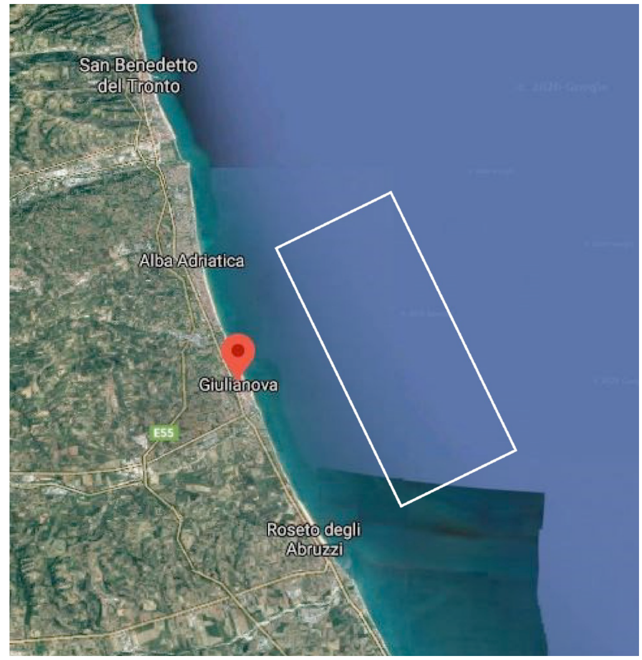

Figure 1. Fishing area of analyzed cuttlefish samples (modified from Google Earth).

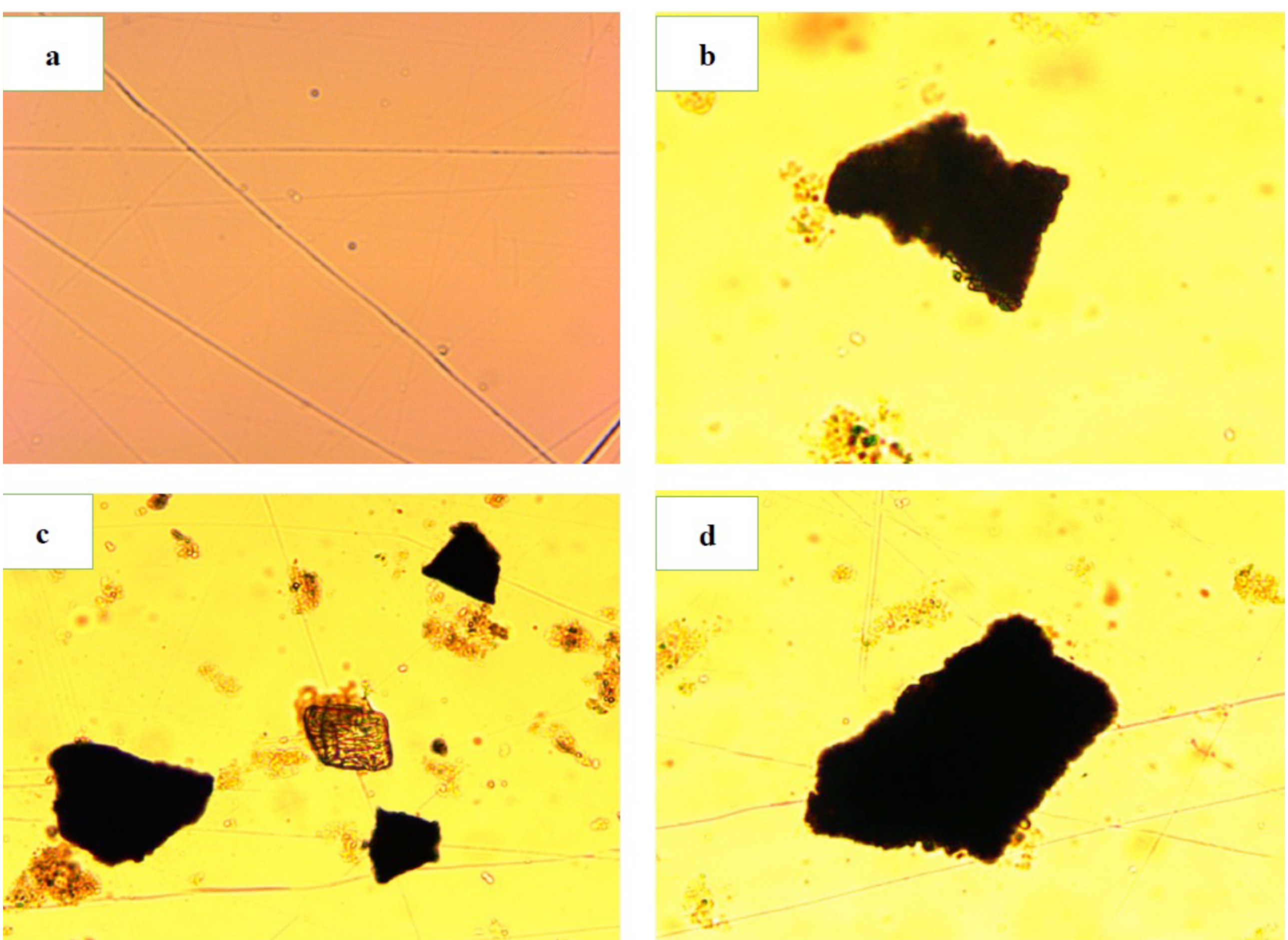

Figure 2. a) Blank sample; b-d) Black fragments isolated from various samples (10× magnification).

60°C for 24 hours. After 24 hours, with the aid of a vacuum pump and paper filters, the digested material was purified. The filter was then cleaned with ultra-pure water from the retained material which was subsequently placed in a test tube and centrifuged at 1800 rpm for 10 minutes in a refrigerated centrifuge (Heraeus mod. Megafuge 1.0R).

The sediment obtained was then observed with a stereomicroscope (Zeiss mod. Steam SV 11), the material designated as microplastic was collected, mounted on a microscope slide, clarified with lactophenol, and covered with a coverslip. Finally, an analysis was carried out with an optical microscope (Zeiss mod. Axio Lab. A1) equipped with a video camera (Zeiss mod. Axiocam 105 color) for determining the color and size.

Statistical analysis

The presence and number of microplastics in relation to the sex group, age, and size of the animal were analyzed by one-factor analysis of variance with XL Stat (2001) software package. The significance level was set at $P<0.05$.

Results and Discussion

The samples examined were 60 females and 62 males. Seventy were younger than 12 months and 52 were between 12 and 18 months. Males had a mean total length of 39.36±4.99 cm and a mean total weight of 189.25±46.26 g. On the other hand, females had an average total length of 39.34±4.75 cm and an average total weight of 198.45±77.34 g.

This study is the first to identify microplastics in the digestive system of wild-caught cuttlefish from the Adriatic Sea.

Microplastics were observed in 98 of the 122 samples tested (80%), with a mean concentration of 6.82±5.52 µPs per subject.

No statistically significant differences were recorded between the presence of µPs and the calculated biometric ratios, sex, and age

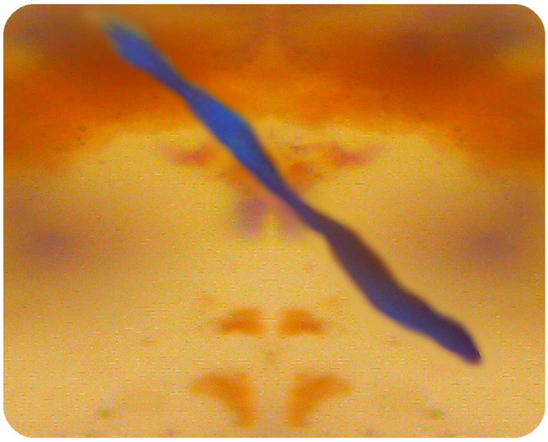

Figure 3. Blue fiber from sample number 16 (10× magnification).

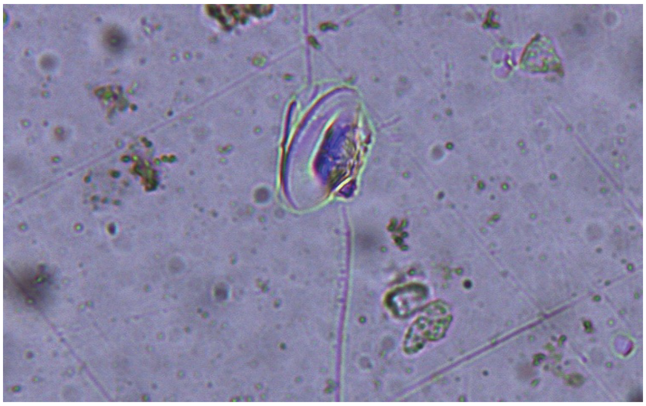

Figure 4. Sphere from sample number 4 (10× magnification).

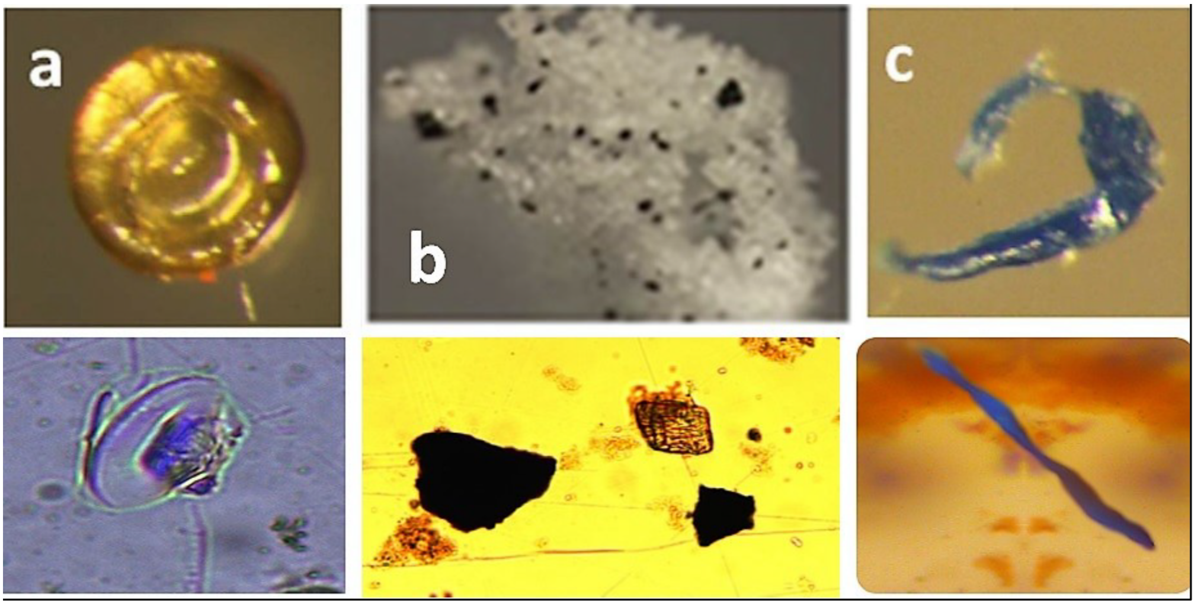

Figure 5. Imaging comparisons between the present findings and the captured ones by Cole *et al*., (2014). The first column represents polyamide sphere particles, the second polyethylene fragments, and the third one is polyvinyl chloride fibers. The first line of images (a-c) is composed by optical microscopy performed by Cole *et al*. (2014); the second line is comprised images belonging to the present investigation.

of the cephalopods examined. The data can be overlapped with what was found in the bibliography, although the previous analyses carried out on *Sepia officinalis* concerned a few subjects. Frey (2019) analyzed 4 cuttlefish, in Viareggio, and isolated µPs in 65% of the samples. Abidli *et al.* (2019) and Oliveira *et al.* (2020), on the other hand, isolated µPs in 100% in 3 samples from the Bizerte Lagoon (northern Tunisia) and 12 samples from Portugal, respectively.

However, Vandermeersch *et al.* (2015) remind us that comparing the abundance of µPs in aquatic species from different regions of the world is difficult due to the lack of standardization of digestion methods and methods for quantifying µPs (microplastics per individual or per gram of tissue).

Types, colors, and sizes of isolated microplastics

Three types of µPs were observed in the samples examined: fragments, fibers, and spheres (Figures 2 a-d, 3, and 4).

The fragments represented the most abundant type, with sizes ranging from 4.72 to 146.98 µm in length and from 3.59 to 95.28 µm in width; the mean length was 25.18 µm (±24.67 µm) and the mean width was 17.12 µm (±16.73 µm).

In one subject only, sample 16, a filament with a length of 70.6 µm and a width of 4.86 µm was isolated (Figure 3). On the other hand, in sample 4, a transparent sphere with a diameter of 13.55 µm was isolated (Figure 4).

In literature, the predominant color observed for all the species of mollusks studied is represented by black (56.18%) followed by blue (18.45%), red (10.79%), light gray (9.54%), green (2.58%), white (1.50%) and yellow (0.96%) (Abidli *et al.*, 2019). The colors detected in the present study were similar to those recorded by Abidli *et al.* (2018) in sediments of the littoral zone of the northern Tunisian coast, by Li *et al.* (2015), and by Renzi *et al.* (2018).

Most of the papers consulted for the drafting of this paper selected and analyzed microplastics using Fourier spectroscopy to identify the class of polymers to which the µPs belonged. In the absence of appropriate equipment, the iconography produced by Cole *et al.* (2014) was used; however, we can hypothesize that the µPs isolates could belong to three types of polymers: polyester, PVC and polyamide (Figure 5).

Conclusions

The data presented in this paper, in line with what has already been published, appear critical, especially if we imagine that about 8 million tons of plastics are dumped into the oceans annually.

The ubiquity of microplastics on the sea surface, in the water column, in sediments, and animals highlights the prevalence of this contaminant in our seas. Microplastics can be ingested by marine organisms, including shellfish and fish suitable for human consumption, posing a potential health risk to consumers (Wright *et al.*, 2013; Seltenrich, 2015).

Indeed, Schirinzi *et al.* (2017) demonstrated that exposure to 10 µm-sized plastic microspheres could induce elevated reactive oxygen production, especially in the human brain and epithelial cells. The risks associated with the ingestion of µPs are not only due to the material itself but also to the chemical additives incorporated during production, such as bisphenol A and phthalates that are known endocrine disruptors, and their ability to absorb and concentrate environmental contaminants, including persistent organic pollutants and metals, and subsequently transfer them through food chains after ingestion (Hugo *et al.*, 2008; Oehlmann *et al.*, 2009; Koelmans *et al.*, 2013; Germanov *et al.*, 2018).

Indeed, very small plastic particles can cross cell membranes, resulting in the bioavailability of plastic-derived pollutants in human systems (Vethaak and Leslie, 2016).

The monitoring of this phenomenon in European waters is one of the objectives of the European marine strategy framework directive (European Commission, 2008); the trend in the abundance and distribution of microplastics will be continuously monitored in European waters. Therefore, further studies are needed to determine the involvement of other species that have never been examined, the implications of ingesting µPs on fish health, and the health and hygiene implications for the consumers.

References

Abidli S, Antunes JC, Ferreira JL, Lahbib Y, Sobral P, Trigui El Menif N, 2018. Microplastics in sediments from the littoral zone of the north Tunisian coast (Mediterranean sea). Estuar Coast Shelf Sci 205:1-9.

Abidli S, Lahbib Y, Trigui El Menif N, 2019. Microplastics in commercial mollusks from the lagoon of Bizerte (northern Tunisia). Mar Pollut Bull 142:243-52.

Auta HS, Emenike CU, Fauziah SH, 2017. Distribution and importance of microplastics in the marine environment: a review of the sources, fates, effects and potential solutions. Environ Int 102:165-76.

Avio CG, 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. Mar Environ Res 111:18-26.

Barboza LGA, Vieira LR, Branco V, Figueiredo N, Carvalho F, Carvalho C, Guilhermino L, 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, dicentrarchus labrax (Linnaeus, 1758). Aquat Toxicol 195:49-57.

Barnes DK, Galgani F, Thompson RC, Barlaz M, 2009. Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc B Biol Sci 364:1985-98.

Białowąs M, Jonko-Sobuś K, Pawlak J, Polak-Juszczak L, Dąbrowska A, Urban-Malinga B, 2022. Plastic in digestive tracts and gills of cod and herring from the Baltic sea. Sci Total Environ 822:153333.

Capillo G, Savoca S, Panarello G, Mancuso M, Branca C, Romano V, Spanò N, 2020. Quali-quantitative analysis of plastics and synthetic microfibers found in demersal species from southern Tyrrhenian sea (central Mediterranean). Mar Pollut Bull 150:110596.

Cole M, Lindeque P, Halsband C, Galloway TS, 2011. Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull 62:2588-97.

Cole M, Webb H, Lindeque P, Fileman E, Halsband C, Galloway TS, 2014. Isolation of microplastics in biota-rich samples and marine biota. Sci Rep 4:4528.

Collard F, Gilbert B, Compère P, Eppe G, Das K, Jauniaux T, Parmentier E, 2017. Microplastics in livers of European anchovies (Engraulis encrasicolus, L.). Environ Pollut 229:1000-5.

Coyle R, Hardiman G, O'Driscoll K, 2020. Microplastics in the marine environment: a review of their sources, distribution processes, uptake and exchange in ecosystems. Case Studies Chem Environ Eng 2:100010.

Dehaut A, Cassone AL, Frère L, Hermabessiere L, Himber C, Rinnert E, Rivière G, Lambert C, Soudant P, Huvet A, Duflos G, Paul-Pont I, 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. Environ Pollut 215:223-33.

Digka N, Tsangaris C, Torre M, Anastasopoulou A, Zeri C, 2018. Microplastics in mussels and fish from the northern Ionian sea. Mar Pollut Bull 135:30-40.

Dris R, Imhof H, Sanchez W, Gasperi J, Galgani F, Tassin B, Laforsch C, 2015. Beyond the ocean: contamination of freshwater ecosystems with (micro-)plastic particles. Environ Chem 12:539–50.

EFSA, 2020. Risk assessment and toxicological research on micro- and nanoplastics after oral exposure via food products. EFSA J 18:e181102.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA J 14:e04501.

European Commission, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy. In: Official Journal, L 164/19, 25/06/2008.

Ezzeddine-Najai S, El Abed A, 2001. Etude de la croissance de la seiche Sepia officinalis Linné, 1758 (Cephalopoda, decapoda) de la region nord de Tunisie. Rapp Comm Int Mer Medit 36:263.

Faure F, Saini C, Potter G, Galgani F, de Alencastro LF, Hagmann P, 2015. An evaluation of surface micro- and mesoplastic pollution in pelagic ecosystems of the western Mediterranean Sea. Environ Sci Pollut Res 22:12190-7.

Frey S, 2019. Microplastic sampling in fish, crustacean, squid, and bivalve species. Report for Ocean Care.

Galgani F, Hanke G, Werner S, De Vrees L, 2013. Marine litter within the European marine strategy framework directive. ICES J Mar Sci 70:1055-64.

Germanov ES, Marshall AD, Bejder L, Fossi MC, Loneragan NR, 2018. Microplastics: no small problem for filter- feeding megafauna. Trends Ecol Evol 33:227-32.

Giani D, Baini M, Galli M, Casini S, Fossi MC, 2019. Microplastics occurrence in edible fish species (Mullus barbatus and Merluccius merluccius) collected in three different geographical sub-areas of the Mediterranean Sea. Mar Pollut Bull 140:129-37.

Gomiero A, Strafella P, Øysæd KB, Fabi G, 2019. First occurrence and composition assessment of microplastics in native mussels collected from coastal and offshore areas of the northern and central Adriatic Sea. Environ Sci Pollut Res Int 26:24407-16.

Hanke G, Galgani F, Werner S, Oosterbaan L, Nilsson P, Fleet D, Kinsey S, Thompson R, Van Franeker J, Vlachogianni T, Palatinus A, Scoullos M, Veiga J, Matiddi M, Alcaro L, Maes T, Korpinen S, Budziak A, Leslie H, Gago J, Liebezeit G, 2013. Guidance on monitoring of marine litter in european seas: a guidance document within the common implementation strategy for the marine strategy framework directive. Luxembourg: Publication Office of the European Union, Joint Research Centre.

Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N, 2008. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. Environ Health Perspect 116:1642-7.

Jeong CB, Kang HM, Lee MC, Kim DH, Han J, Hwang DS, Soussi S, Lee SJ, Shin KH, Park HG, Lee JS, 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclopina nana. Sci Rep 7:41323.

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 50:3315-26.

Koelmans AA, Besseling E, Wegner A, Foekema EM, 2013. Plastic as a carrier of POPs to aquatic organisms: a model analysis. Environ Sci Technol 47:7812-20.

Li J, Yang D, Li L, Jabeen K, Shi H, 2015. Microplastics in commercial bivalves from China. Environ Pollut 207:190-5.

Mancuso M, Savoca S, Bottari T, 2019. First record of microplastics ingestion by european hake Merluccius merluccius from the Tyrrhenian Sicilian coast (central Mediterranean Sea). J Fish Biol 94:517-9.

Mercogliano R, Santonicola S, Raimo G, Gasperi M, Colavita G, 2021. Extraction and identification of microplastics from mussels: Method development and preliminary results. Ital J Food Saf 10:9264.

Oehlmann J, Schulte-Oehlmann U, Kloas W, Jagnytsch O, Lutz I, Kusk KO, Wollenberger L, Santos EM, Paull GC, Vanlook KJW, Tyler CR, 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philos Trans R Soc B Biol Sci 364:2047-62.

Oliveira AR, Sardinha-Silva A, Andrews PLR, Green D, Cooke GM, Hall S, Blackburn K, Sykes AV, 2020. Microplastics presence in cultured and wild-caught cuttlefish, Sepia officinalis. Mar Pollut Bullet 160:111553.

Pellini G, Gomiero A, Fortibuoni T, Ferrà C, Grati F, Tassetti AN, Polidori P, Fabi G, Scarcella G, 2018. Characterization of microplastic litter in the gastrointestinal tract of Solea solea from the Adriatic Sea. Environ Pollut 234:943-52.

Piarulli S, Scapinello S, Comandini P, Magnusson K, Granberg M, Wong JX, Sciutto G, Prati S, Mazzeo R, Booth AM, Airoldi L, 2019. Microplastic in wild populations of the omnivorous crab Carcinus aestuarii: a review and a regional-scale test of extraction methods, including microfibres. Environ Pollut 251:117-27.

PlasticsEurope, 2020. Plastics - the facts 2020. An analysis of European latest plastics production, demand and waste data. Available from: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2020/.

Renzi M, Guerranti C, Blašković A, 2018. Microplastic contents from maricultured and natural mussels. Mar Pollut Bull 131:248-51.

Renzi M, Specchiulli A, Blašković A, Manzo C, Mancinelli G, Cilenti L, 2019. Marine litter in stomach content of small pelagic fishes from the Adriatic sea: sardines (Sardina pilchardus) and anchovies (Engraulis encrasicolus). Environ Sci Pollut Res 26:2771-81.

Romeo T, Pietro B, 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 95:358-61.

Savoca S, Capillo G, Mancuso M, Bottari T, Crupi R, Branca C, Romano V, Faggio C, D'Angelo G, Spanò N, 2019a. Microplastics occurrence in the Tyrrhenian waters and the gastrointestinal tract of two congener species of seabreams. Environ Toxicol Pharmacol 67:35-41.

Savoca S, Capillo G, Mancuso M, Faggio C, Panarello G, Crupi R, Bonsignore M, D'Urso L, Compagnini G, Neri F, Fazio E, Romeo T, Bottari T, Spanò N, 2019b. Detection of artificial cellulose microfibers in Boops boops from the northern coasts of Sicily (Central Mediterranean). Sci Total Environ 691:455-65.

Schirinzi GF, Pérez-Pomeda I, Sanchís J, Rossini C, Farré M, Barceló D, 2017. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ Res 159:579-87.

Seltenrich N, 2015. New link in the food chain? Marine plastic pollution and seafood safety. Environ Health Perspect 123:34-41.

Thompson RC, 2006. Plastic debris in the marine environment: consequences and solutions. Mar Nat Conserv Eur 193:107-15.

Vandermeersch G, Van Cauwenberghe L, Janssen CR, Marques A, Granby K, Fait G, Kotterman MJ, Diogène J, Bekaert K, Robbens J, Devriese L, 2015. A critical view on microplastic quantification in aquatic organisms. Environ Res 143:46-55.

Vethaak AD, Leslie HA, 2016. Plastic debris is a human health issue. Environ Sci Technol 50: 6825-6.

Von Moos N, Burkhardt-Holm P, Köhler A, 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. Environ Sci Technol 46:11327-35.

Wright SL, Thompson RC, Galloway TS, 2013. The physical impacts of microplastics on marine organisms: a review. Environ Pollut 178:483-92.