

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

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## Abstract

Microplastics ( $\mu$ Ps) 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  $6.82 \pm 5.52 \mu\text{Ps}/\text{subject}$ . 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 Suisse, 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 ( $\mu$ Ps) (<5 mm) (Cole *et al.*, 2011; Galgani *et al.*, 2013; Faure *et al.*, 2015; Pellini *et al.*, 2018). The  $\mu$ Ps 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  $\mu$ Ps in the marine ecosystem, involving fish and birds, are polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene polymers (Białowas *et al.*, 2022). The majority of  $\mu$ Ps 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  $\mu$ P 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  $\mu$ Ps 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; Seltnerich, 2015; Koelmans *et al.*, 2016; Barboza *et al.*, 2018).

In the Mediterranean sea,  $\mu$ Ps 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).

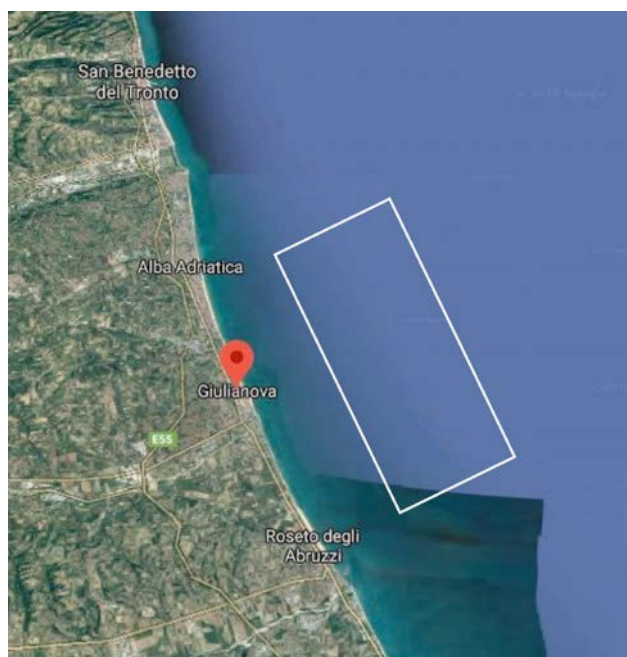
According to Renzi *et al.* (2018), collected samples were measured to determine the total length and the total weight. Furthermore, the sex of each subject was determined, as well as their age by measuring the dorsal length of the coat, following what was described by Ezzedine-Najai and El Abed (2001).

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

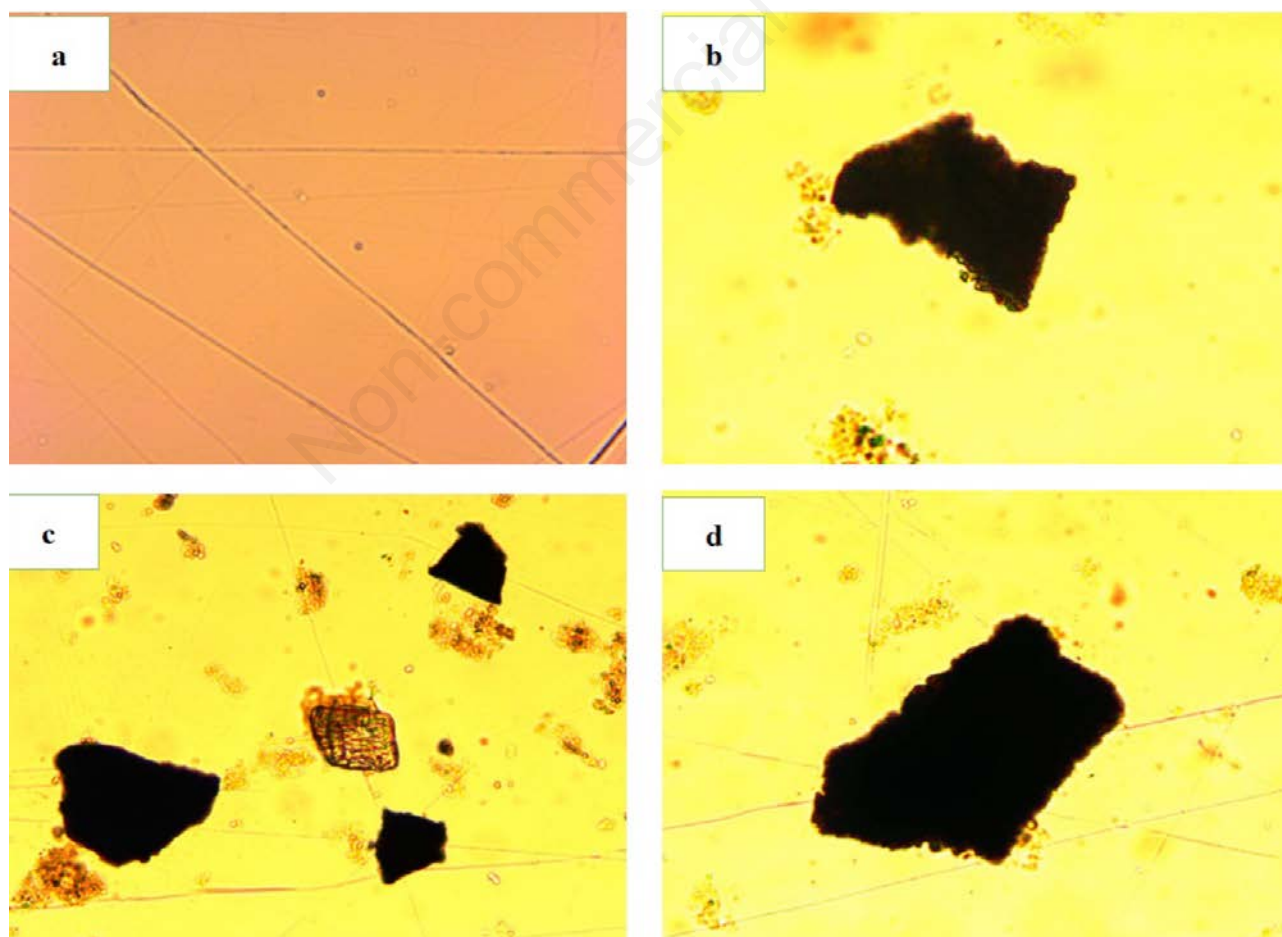
### Extraction method

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 $\times$  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  $39.36 \pm 4.99$  cm and a mean total weight of  $189.25 \pm 46.26$  g. On the other hand, females had an average total length of  $39.34 \pm 4.75$  cm and an average total weight of  $198.45 \pm 77.34$  g.

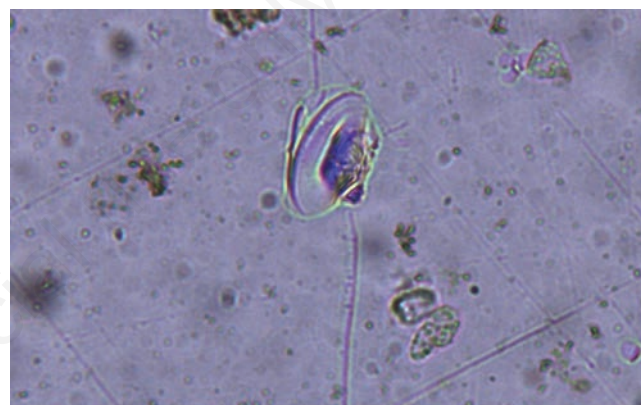
This study is the first to identify microplastics in the digestive system of wild-caught cuttlefish from the Adriatic Sea.

Microplastics were observed in 98 of the 122 samples tested (80%), with a mean concentration of  $6.82 \pm 5.52$   $\mu$ P/s per subject.

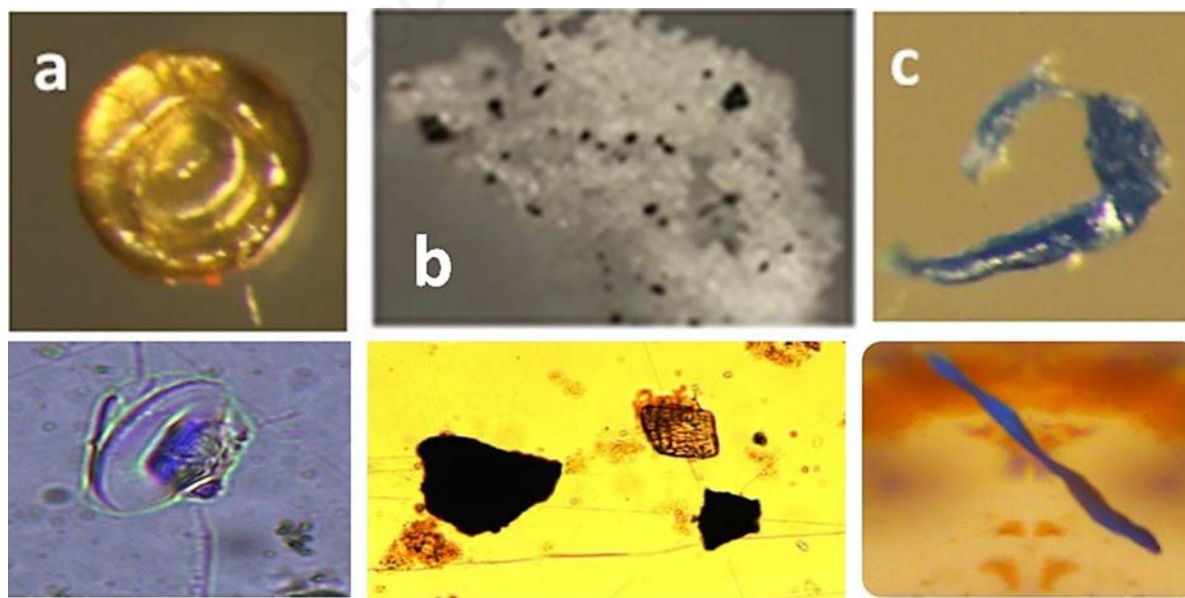
No statistically significant differences were recorded between the presence of  $\mu$ Ps and the calculated biometric ratios, sex, and age



**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  $\mu$ Ps in 65% of the samples. Abidli *et al.* (2019) and Oliveira *et al.* (2020), on the other hand, isolated  $\mu$ Ps 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  $\mu$ Ps in aquatic species from different regions of the world is difficult due to the lack of standardization of digestion methods and methods for quantifying  $\mu$ Ps (microplastics per individual or per gram of tissue).

### Types, colors, and sizes of isolated microplastics

Three types of  $\mu$ Ps 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  $\mu$ m in length and from 3.59 to 95.28  $\mu$ m in width; the mean length was 25.18  $\mu$ m ( $\pm$ 24.67  $\mu$ m) and the mean width was 17.12  $\mu$ m ( $\pm$ 16.73  $\mu$ m).

In one subject only, sample 16, a filament with a length of 70.6  $\mu$ m and a width of 4.86  $\mu$ m was isolated (Figure 3). On the other hand, in sample 4, a transparent sphere with a diameter of 13.55  $\mu$ m 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  $\mu$ Ps belonged. In the absence of appropriate equipment, the iconography produced by Cole *et al.* (2014) was used; however, we can hypothesize that the  $\mu$ Ps 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, Schirizzi *et al.* (2017) demonstrated that exposure to 10  $\mu$ m-sized plastic microspheres could induce elevated reactive oxygen production, especially in the human brain and epithelial cells. The risks associated with the ingestion of  $\mu$ Ps 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  $\mu$ Ps on fish health, and the health and hygiene implications for the consumers.

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