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Invited paper

Evidence of microplastic ingestion in the shark *Galeus melastomus* Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea☆

Carme Alomar*, Salud Deudero

Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Muelle de Poniente s/n, 07015 Palma de Mallorca, Spain

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A B S T R A C T

Microplastic (<5 mm) ingestion has been recorded in *Galeus melastomus*, the blackmouth catshark, around the Balearic Islands. In total 125 individuals were analyzed for microplastic ingestion. Results have shown that 16.80% of the specimens had ingested a mean value of 0.34 ± 0.07 microplastics/individual. Stomach fullness index ranged from 0.86 to 38.89% and regression analyses showed that fuller stomachs contained more microplastics. A higher quantity of filament type microplastics were identified compared to granular or hard plastic type. No significant differences were given between ingestion values of two locations over the continental shelf providing further evidence of the ubiquitous distribution of microplastics. The findings in this study reflect the availability of this man made contaminant to marine species in seafloor habitats. Based on results from this study, data on microplastic ingestion could be used to study trends in the amount and composition of litter ingested by marine animals in accordance with descriptor 10 of the Marine Strategy Framework Directive.

1. Introduction

Plastic pollution in the Mediterranean Sea has been documented (Pham et al., 2014) and recent studies have demonstrated that plastic is ingested by both pelagic and benthic fish species (Neves et al., 2015; Romeo et al., 2015; Lusher et al., 2013). The Balearic Islands, in the western Mediterranean Sea, are highly exposed to plastic pollution (Deudero and Alomar, 2015) and microplastic (<5 mm) concentrations have already been detected in coastal shallow waters (Alomar et al., 2016), deep sea areas (Woodall et al., 2014) and surface waters (Faure et al., 2015).

In addition, semipelagic fish from the Balearic Islands ingest microplastic filaments (Nadal et al., 2016) providing further evidence of the transfer of microplastics from the marine environment to biota. Microplastics have the potential to cause mortality, reduction of feeding activity, inhibited growth and development, endocrine disruption, energy disturbance, oxidative stress, immunity, neurotransmission dysfunction and even genotoxicity (Avio et al., 2015; Rochman et al., 2014; Wright et al., 2013). Physiological effects produced by microplastic ingestion, such as inflammation and lipid accumulations in liver as well as induction of oxidative stress have already been detected in zebrafish (Lu et al., 2016). Therefore, concerning scientific observations of physiological effects derived from microplastic ingestion, consequences for the marine food web are expected.

Amongst benthic fish species, the blackmouth catshark *Galeus melastomus* Rafinesque, 1810 has been recorded to ingest plastic debris in deep-waters from the Ionian Sea (Anastasopoulou et al., 2013). This species is considered a trophic generalist, preying on shrimps, cephalopods, fishes and euphausiids (Carrasson et al., 1992; Olaso et al., 1998; Fanelli et al., 2009; Valls et al., 2011). *Galeus melastomus* is an important bycatch species in demersal and longline fisheries and is usually discarded in the traditional deep trawl fishery for rose shrimp, *Aristeus antennatus* (Tursi et al., 1993; Torres et al., 2001). Research surveys indicate that it is an abundant demersal species (Valls et al., 2011) and the most abundant shark on the upper and middle slopes to depths of about 1400 m living in muddy bottoms (Baino et al., 2001; Rey et al., 2004). Therefore, assessing vulnerability of this elasmobranch to marine litter is essential and data on ingestion of microplastics could provide a snapshot of the availability of this contaminant in the marine environment at a wide spatial scale. Consequently, this data could

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* Corresponding author.
E-mail address: c.alomar@ba.ieo.es (C. Alomar).

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be useful to assess “Trends in the amount and composition of litter ingested by marine animals” giving response to indicator 10.2.1 of descriptor 10 in the European Marine Strategy Framework Directive (MSFD Directive, 2008/56/EC).

Given that the Mediterranean Sea is highly exposed to plastic concentrations (Deudero and Alomar, 2015) and given the scarcity on data on microplastic ingestion in elasmobranchs but the pivotal role which chondrichthyans play in the structure of marine food webs worldwide (Albo-Puigserver et al., 2015) this research aims at:

1) Quantify microplastic ingested by the elasmobranch *Galeus melastomus* at two locations over the continental shelf with expected loads of microplastic inputs
2) Explore trends of microplastic ingestion according to the percentage stomach fullness index of *Galeus melastomus*

2. Materials and methods

2.1. Sampling and visual sorting of stomach contents

A total of 125 *Galeus melastomus* were sampled in two locations around Mallorca Island to study microplastic ingestion by elasmobranchs (Fig. 1.). A total of 81 individuals were collected in Palma and 44 individuals in Soller over the continental shelf at approximately 600 m depth. Samples were provided from the bycatch of commercial trawling fishing vessels which had a 40 mm minimum mesh size net designed to capture demersal species (DO L, n°1967, 21 December 2006). After ships arrived at seaports, samples were frozen and stored in bags at −18 °C until further laboratory procedures. In addition, individuals of other chondrichthyes species: *Scyliorhinus canicula* (5 individuals), *Chimaera monstrosa* (2 individuals) and *Etmopterus spinax* (8 individuals) were also sampled upon availability.

Once at the laboratory, the samples were individually thawed at room temperature and biological parameters were recorded: total length (TL mm), fresh weight (FW), sex was recorded and classified into female, male and immature (i.e. individuals smaller than 140 mm with no defined sex). A percentage stomach fullness index was assigned to each individual from 0% (empty stomachs) to 100% (full stomachs). For microplastic identification, stomachs were removed and directly dissected following previous procedures (Nadal et al., 2016). Stomach contents were analysed in laboratory using a stereomicroscope (Euromex NZ 1903-S) with a CMEX 3.0 MP camera attached to it which included a special calibration software, ImageFocus® 4.0. (Euromex software). Optical enhancement from 6.7x to 40.5x was applied and microplastics were identified and stored in glass vials with deionized water. Glass vials were then send to the University of Eastern Finland (SIB Labs) for polymer identification. Particles in each glass vial were photographed with Leica EZ4 stereomicroscope with a HD camera and analyzed with Imaging Fourier Transform Infrared (FTIR) spectroscopy (30 scans, 4000-700 cm-1 PerkinElmer Spectrum Spotlight 300). Commercial and custom-made spectral databases were utilized for microplastic identification. Type of microplastics identified was classified into fragments, filaments and films following the Marine Strategy Framework Directive technical subgroup on marine litter (Galgani et al., 2013) and colour of microplastics was also recorded. For each studied specimen the number of microplastics ingested per gram of fresh weight of *Galeus melastomus* (MPs/g FW) considering all stomachs (full and empty) was provided. The approximate extraction and sorting time for microplastic identification was of twenty minutes per sample and depending on the fullness of the stomach it could increase to one hour per individual.

Measures to avoid (airborne) contamination were adopted while handling and processing samples (Alomar et al., 2016). During the sorting procedure at the laboratory, two glass Petri dishes were placed at both side of the stereomicroscope and checked for microplastics before and after each sample. If a filament, granular or film man made structure was detected, type and colour of these

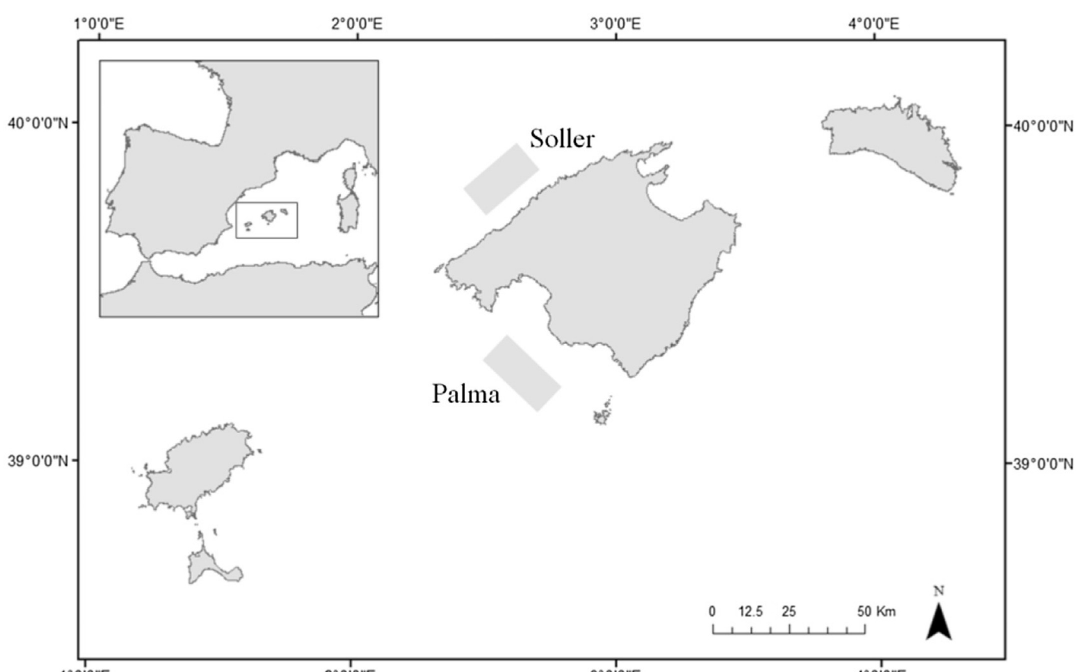

Fig. 1. Sampling locations (grey boxes: Soller and Palma) of elasmobranchs around Mallorca Island (Balearic Islands, Western Mediterranean) for assessment of microplastic ingestion.