Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast

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**ABSTRACT**

The ingestion of microplastics and natural fibres (< 5 mm) was assessed for two commercial fish species in the western Mediterranean Sea: *Sardina pilchardus* and *Engraulis encrasicolus*. Gastrointestinal tracts from 210 individuals from 14 stations were examined with 14.28–15.24% of the small pelagic fish *S. pilchardus* and *E. encrasicolus* having ingested microplastics and natural fibres. A latitudinal increase in condition index (Fulton's K) of *S. pilchardus* gave an indication that larger individuals with better physical condition are less likely to ingest microplastics and natural fibres. Fibres were the most frequent particle type (83%) and Fourier Transform Infrared spectroscopy (FT-IR) analysis indicated polyethylene terephthalate was the most common microplastics material (30%). Results from this study show that both microplastics and natural fibres of anthropogenic origin are common throughout the pelagic environment along the Spanish Mediterranean coast.

1. **Introduction**

Marine litter is ubiquitous and abundant throughout the marine environment giving rise to global concern of the sources, sinks and impacts surrounding marine litter. It has been found floating on the surface, suspended in the water column and deposited on the seafloor with its unlimited presence ranging from the equator to the polar regions (Enders et al., 2015; Eriksen et al., 2014; Pham et al., 2014). Marine litter enters ecosystems from either land-based sources, for instance inadequate waste management, or maritime activities, such as shipping and derelict fishing gear (Gregory, 2009; NOAA Marine Debris Program, 2015). In 2010, plastic waste worldwide was estimated at 275 million metric tons (MT) generated in 192 coastal countries of which an estimated 4.8 to 12.7 million MT entering the ocean (Jambeck et al., 2015).

One of the most common marine litter types is plastic pollution (Mifsud et al., 2013). Plastic marine litter of less than < 5 mm has been defined as microplastics and enter the marine environment either as primary sources, such as plastic pellets or microspheres, or as secondary sources, through the fragmentation and degradation of larger plastic litter into fibres (filaments), fragments, granules and films (Barnes et al., 2009; Derraik, 2002). However, sources of marine litter is diverse and therefore particles of anthropogenic origin were differentiated into two classifications: microplastics (e.g. polyethylene, polyamide) and natural fibres (e.g. cotton, wool) (Ladewig et al., 2015). Microplastics have recently been widely studied and are made of a variety of materials which can leach harmful contaminants into the marine environment (Burgess-cassler et al., 1991). Natural fibres on the other hand, are composed of natural materials primarily chemically modified viscose from the textile industry (Remy et al., 2015). Recent estimates put the global plastic concentration floating in the oceans as 51.2 × 10¹² particles with an estimated mass of 236 thousand metric tons with the Mediterranean estimated at having over half of the particles (28.2 × 10¹² particles) (van Sebille et al., 2015).

The alarming concentration of microplastics in the Mediterranean Sea brings into question the potential impact on the marine biodiversity. A recent review on the threat of marine litter primarily plastic on the marine biodiversity in the Mediterranean indicated 134 species so far having been impacted by plastic pollution through ingestion and/or entanglement with species ranging from invertebrate communities to large mammals (Deudero and Alomar, 2015). The physical characteristics of marine litter, especially microplastics, does not only have the potential to cause physical harm to marine biota but also contaminants such as plastic additives, heavy metals and persistent organic pollutants can sorb to the surface of the particle and if ingested, can accumulate within the organism (Fossi et al., 2014).

Over the past few years, there has been an increase in microplastic ingestion studies on an array of marine biota worldwide using a variety of techniques: microplastics (e.g. polyethylene, polyamide) and natural fibres (e.g. cotton, wool) (Ladewig et al., 2015). Microplastics have recently been widely studied and are made of a variety of materials which can leach harmful contaminants into the marine environment (Burgess-cassler et al., 1991). Natural fibres on the other hand, are composed of natural materials primarily chemically modified viscose from the textile industry (Remy et al., 2015). Recent estimates put the global plastic concentration floating in the oceans as 51.2 × 10¹² particles with an estimated mass of 236 thousand metric tons with the Mediterranean estimated at having over half of the particles (28.2 × 10¹² particles) (van Sebille et al., 2015).

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of sampling techniques (Lusher et al., 2017). However, only a fraction of the studies have documented the ingestion in the stomach contents of pelagic and demersal fish species in the Mediterranean Sea (Battaglia et al., 2016; Bellas et al., 2016; Nadal et al., 2016). With the majority of microplastics found being fragments and filaments, the presence of microplastics in the stomach contents raises questions to the sources and fates in the marine environment. A recent IUCN report has identified some of the primary sources of microplastics originating from the fragmentation of synthetic textiles and tires entering the environment primarily through abrasion and road runoff (Boucher and Friot, 2017). The deep sea has been found to be a sink for microplastics with several sea-floor species having ingested microfibres between 334 and 1783 m depth, while sediments studies in the Balearic Islands indicate marine areas closer to urbanized areas are larger sinks for microfibres compared to marine protected areas, being sinks of anthropogenic fragments (Alomar and Deudero, 2017; Taylor et al., 2016; Woodall et al., 2014). Microplastics in the marine environment and their bioavailability at different trophic levels on the seafloor, water column and sea surface makes it necessary to analyze species with similar feeding strategies.

To evaluate the occurrence of microplastics along the Spanish Mediterranean coast, the following commercially important small pelagic fish species were studied: Sardina pilchardus (European Pilchard, Walbaum, 1792) and Engraulis encrasicolus (European Anchovy, Linnaeus, 1758). According to the Food and Agriculture Organization (FAO) of the United Nations, global captures in 2014 for S. pilchardus were 1,207,764 tonnes and E. encrasicolus 271,488 tonnes. In the Mediterranean Sea, average landings between 2000 and 2013 for S. pilchardus were 186,100 tonnes while E. encrasicolus 393,500 tonnes making up between 12.4% and 26.21% of the total landings of the species (FAO, 2016). Both species are small coastal pelagic fish with similar life history traits characterized by a relatively short life cycle and fast growth of the early stages where changes in habitat conditions (environmental hazards) may influence survival at early life stages (Sonanno et al., 2016).

This study aims to determine the presence of anthropogenic particles, microplastics and natural fibres, in the gastrointestinal tracts of two commercial fish in the western Mediterranean Sea by evaluating: 1) the ingestion of anthropogenic particles in commercial fish species along the Spanish Mediterranean coast, 2) which physiological and spatial factors influence anthropogenic particles ingestion and 3) what are the principal characteristics of the ingested anthropogenic particles. Due to the commercial value and potential public health concerns of microplastic ingestion and their presence along the entire Spanish Iberian continental shelf, S. pilchardus and E. encrasicolus are key candidates to evaluate for microplastic exposure.

2. Materials and methods

2.1. Study area

The western Spanish Mediterranean coast extends from the Gulf of Lions in the NW Mediterranean to the Alboran Sea ending at the Strait of Gibraltar with a total shelf length of 1245 km (Lobo et al., 2015). Fish samples were collected along the entire extent of the continental shelf (42°10′26.1″N 3°13.5″E–36°26′23.0″N 4°55′33.2″W) during the 2015 fisheries research survey MEDIAS (MEDiterranean International Acoustic Survey) which lies within the European Data Collection Regulation framework to estimate abundance and biomass of fish stocks. Samples were collected from semi-pelagic trawls at depths ranging from 52 to 150 m at 14 sampling stations (Fig. 1). The sampling of each species was dependent on the availability of a minimum of 15 individuals from each haul resulting in seven sampling stations for each species.

2.2. Laboratory analysis

For each individual, the following biological parameters were recorded: total length (cm), body weight (g) and sexual maturity (immature = 1, maturing = 2, pre-laying = 3) (Vallo, 2008). The gastrointestinal tract from the upper oesophagus to the anus was removed by dissection and immediately frozen at −18°C. Once in the laboratory, samples were thawed at room temperature and inspected using a dissection microscope following previously established protocols (Alomar and Deudero, 2017; Lusher et al., 2013; Nadal et al., 2016). Contents of the gastrointestinal tract were visually sorted 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 6.7× to 40.5× was applied and anthropogenic particles were identified. To prevent and minimize airborne and cross contamination, extreme precaution was taken into account whenever samples were handled and preventative contamination protocols were adapted from previous studies (Lusher et al., 2013; Nadal et al., 2016; Woodall et al., 2014). A white 100% cotton laboratory coat was worn at all times and work areas and materials were swiped clean with 96% alcohol between samples and samples were stored in glass vials with deionized and micro-filtrated water Milli-Q.

2.3. Particle characterization

The identified anthropogenic particles were classified into fragments and fibres and colour of anthropogenic particles was also recorded. The polymer composition for each particle was completed at the SIB Labs at the University of Finland (Alomar and Deudero, 2017). Samples in each glass vial were photographed with a Leica EZ4 stereomicroscope with a HD camera and analyzed with transmission Fourier Transform Infrared (FT-IR) spectroscopy (30 scans, 4000–700 cm−1 PerkinElmer Spectrum Spotlight 300) using commercial and custom-made spectral databases for microplastic identification (Alomar and Deudero, 2017).

2.4. Data analysis

The abundance of microplastics and natural fibres at each location was calculated by the following: number of particles/number of individuals × 100. We analyzed the relationship between the number of ingested particles for the subsequent variables: depth, distance from the coastline (distance) and Fulton’s condition factor (K) calculated using the following formula: K = 100 × (weight / total length3) (Froese, 2006). The shortest distance from each trawl station to the coastline was calculated using the near tool from the Geographic Information Systems ArcGIS 10.4.

Standardized data exploration techniques were used to identify any outliers and possible collinearity between the physiological and spatial terms (Zuur et al., 2010). For this study, each species was modelled separately using GLMMs (generalized linear mixed models) with a negative binomial error distribution to account for the overdispersion. The fixed terms were condition factor, distance from the coastline and the depth while the study station was included as a nested random term. The information-theoretic approach was used for model selection (Table 2) (Burnham and Anderson, 2002) and the models were compared using the lowest AICc (Akaike’s Information Criterion corrected). Due to the high percentage of zeros (85%), a simulation data set was run to rule out zero inflation for both species. Finally, a one-way ANOVA was performed to determine effects between the ingestion of microplastics and natural fibres and sexual maturity. The statistical analysis was done using the MASS and MuMIn packages in RStudio 3.3.1.