Microplastic ingestion and diet composition of planktivorous fish

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Scientific Significance Statement

Microplastics (MP) pollution in marine ecosystems is a worldwide problem. Factors that influence their ingestion by different fish species are still not well understood. To our knowledge, this is the first work coupling MP accumulation and a full taxonomic description of planktivorous fish diet. We found that MP accumulation was more associated with prey size consumed by fish than with prey type, and species depending on larger mesozooplankton prey accumulated more MP than those dependent on smaller planktonic prey. We identify horse mackerel as a suitable bioindicator for MP monitoring in the pelagic Iberian ecosystem.

Abstract

Planktivorous pelagic fish are susceptible to accumulating microplastics (MP), which have the same size range as their prey and accumulate in their feeding and spawning grounds. We analyzed stomach contents of pelagic fish (European sardine, horse mackerel, anchovy, chub mackerel, Atlantic mackerel, and bogue) from Atlanto-Iberian waters to investigate the relationship between MP ingestion, their diet composition and select a potential bioindicator. We found significant differences between diet of the studied fish species in terms of prey type and size. MP ingestion was significantly related to diet composition. Species with diets that include smaller prey (European sardine, chub mackerel, and bogue) had lower MP concentration in the stomachs than fish depending on larger mesozooplanktonic prey. Horse mackerel had the highest proportion of larger prey (> 1000 μm) and the highest MP abundance in the stomachs, and thus are a suitable bioindicator for MP monitoring in the pelagic Iberian ecosystem.

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Over 92% of ocean plastic pollution is composed of particles smaller than 5 mm, known as microplastics (MP) (Eriksen et al. 2014). MP enter directly into the aquatic environment (e.g., microbeads used in cosmetics and microfibers from textiles) or result from the breakdown of larger plastic debris (Cole et al. 2011). Being dispersed by currents and wind, MP are globally distributed in different marine environments (Woodall et al. 2014; Waller et al. 2017; Antunes et al. 2018).

MP are difficult to detect and can interact with a wide range of organisms from all trophic levels with unknown effects on biota and human health (Barboza et al. 2018). MP were detected in several marine fish, including commercial value ones (e.g., Neves et al. 2015; Bellas et al. 2016; Bessa et al. 2018). Once ingested, MP may cause physical damage, suffocation, gut blockage, and nutritional depletion (Jovanović et al. 2017). Additionally, additives incorporated during plastic manufacturing and pollutants adsorbed on their surface may increase their toxicity (Avio et al. 2015).

Laboratory observations suggest that MP uptake by fish is influenced by particle properties (shape, size, color, and density) and by trophic ecology of the species (e.g., feeding behavior, diet composition, and habitat) (Ory et al. 2018). MP trophic transfer may occur indirectly by consumption of contaminated prey (Chagnon et al. 2018), or directly through water or sediment (Besseling et al. 2013; Ory et al. 2018). Experimental work is crucial to identify potential risks and pathways but does not provide an accurate representation of natural conditions (Phuong et al. 2016).

Small- and semi-pelagic fish species have high economic importance worldwide and represent the bulk of fish biomass, particularly in upwelling regions (FAO 2016). Their low and middle trophic position is crucial in pelagic food webs, having an important effect on lower trophic levels dominated by plankton organisms and, at the same time, controlling predatory fish (Cury et al. 2000). Pelagic fish are planktivorous during entire their life cycle (such as sardines and anchovies) or at least during the initial stages of development, incorporating piscivory at adult stage (such as horse mackerel and chub mackerel) (Garrido et al. 2015). Planktivorous fish are particularly susceptible to accumulating MP with similar size, shape, and color to their prey, particularly planktonic organisms (Wright et al. 2013). Differences in feeding strategies may affect the MP consumption. For instance, filter-feeders are more susceptible to passively uptake MP from surrounding water (Collard et al. 2017), while particulate-feeders may actively consume MP by confounding them with their prey (de Sá et al. 2015; Ory et al. 2018).

The most abundant coastal pelagic fish species in the Western Iberia Upwelling Ecosystem (WIUE) are European sardine, horse mackerel, anchovy, and chub mackerel, all of which are planktivorous, at least during the larval and juvenile stages (Garrido et al. 2008, 2015; Garrido and Murta 2011). There are known differences in the feeding behavior and diet composition of these species (Garrido and van der Lingen 2014; Bachiller and Irigoien 2015; Garrido et al. 2015), but the implications on MP accumulation are unknown.

Our three objectives are: (1) investigate the presence of MP in stomach contents of six of the most common pelagic fish species in the WIUE: European sardine (Sardina pilchardus), horse mackerel (Trachurus trachurus), anchovy (Engraulis encrasicholus), chub mackerel (Scomber colias), Atlantic mackerel (Scomber scombrus), and bogue (Boops boops); (2) investigate the relationship between MP ingestion and interspecific differences of diet composition and feeding behavior, as well as habitat; and (3) assess the most susceptible species and the best candidate as bioindicator for MP monitoring in the pelagic marine environment. To our knowledge, this is the first work coupling MP accumulation and a full taxonomic description of planktivorous fish diet.

Methods

We collected a total of 327 pelagic fish from six commercial species (European sardine, horse mackerel, anchovy, chub mackerel, Atlantic mackerel, and bogue) in 16 stations along the west and south Iberian coast (Fig. 1; Table 1) during a research cruise (PELAGO14) carried out from April 3rd to May 12th in 2014. Western and southern parts of Iberian coast were analyzed separately due to known differences in oceanography and productivity (Mason et al. 2005) that were shown to affect diet composition of planktivorous fish populations (Garrido et al. 2008). We used a 20 mm mesh size pelagic trawl at depths between 16 and 50 m. Total length (cm) and weight (g) of fish were determined onboard and stomachs removed and frozen for posterior laboratory analyses (Table 1).

We conducted microscopic analysis of food items in pools of 2–10 stomachs from the same fish species and length class (± 1 cm), and collected in the same trawl haul (Table 1) (Garrido et al. 2015). After identifying prey items, we processed samples to extract potential MP, using a solution of 10% KOH, prepared in ultrapure water, for 24 h at 60°C (Dehaut et al. 2016) and filtered through 20 μm polycarbonate membrane. All potential MP were visualized and photographed using a stereo microscope (LEICA S9i) with an integrated camera (IC80 HD). We categorized particles by color (black, blue, transparent, white, red, green, and other) and size using the ImageJ software. We measured particles at their largest cross section and categorized them according to five size classes (≤ 0.5 mm, 0.5–1 mm, 1–2 mm, 2–3 mm, and > 3 mm). We distinguished fibers from other types of MP shapes.

To account for background contamination, we washed all material with ultrapure water and samples were always kept under a clean air laminar flow hood (HEPA filter, class ISO5) or maintained in covered glass recipients. Procedural blanks were performed simultaneously with real samples. During microscopic analyses for prey and MP identification, we placed two open Petri dishes with filters near to the working zone in order to control airborne fiber contamination.
We selected a subset of 38 potential MP in order to identify their chemical composition by Fourier transform infrared spectrometry (FTIR) using a PerkinElmer Spotlight 200i FTIR Imaging System equipped with a mercury cadmium telluride (MCT) array detector cooled by liquid nitrogen. We collected spectra in attenuated total reflection mode using a germanium crystal. Measurement resolution was set at 4 cm\(^{-1}\) ranging from 4000 to 600 cm\(^{-1}\) with a minimum of four scans. To confirm the polymer type, we compared all spectra to library databases and then compare analysis of the polymer characteristic bands with spectra assignments. Only polymers matching reference spectra for more than 70% were accepted.

We performed all statistical analysis using the open source software R version 3.5.0 (R Development Core Team, 2011; www.r-project.org). Stomach content weight was compared between species using a nonparametric Kruskal-Wallis test, to investigate if the potential difference in the number of MP found in the stomachs per species was related to stomach fullness. We used analysis of covariance to test for differences of MP ingestion between fish species and areas. Since Atlantic mackerel and horse mackerel were not represented in all areas, the study of the combined effect of species and area on MP accumulation were restricted to European sardine, anchovy, bogue, and chub mackerel. Statistical tests were considered significant at \(p\) values < 0.05.

Differences in the diet composition between species and areas were investigated using Adonis (permutation multivariate ANOVA; Anderson 2001) in “vegan,” using the Bray-Curtis distance and 999 permutations (Oksanen et al. 2010). We analyzed data dispersions of the prey communities among groups using the Betadisper test (permutational analysis of multivariate dispersions) in “vegan” and performed multiple comparisons using the “pairwise.adonis” function (Martinez Arbizu 2019) to identify the differences between each species pair.

We applied the principal component analysis (PCA, using the “prcomp” from the package “stats” in R) to inspect the relationship between prey type or prey size in the diet and MP accumulation. Prey size classes 0–200, 200–500, 500–1000, 1000–2000, and > 2000 \(\mu\)m were used in the analysis. The variables were scaled before the analysis to have unit variance. In Table 1.

**Table 1.** Fish species, sample size (n), area, size class (cm), total number of MP, occurrence (%) of pool of stomachs with MP, and MP per fish in the stomach contents of *B. boops*, *E. encrasicolus*, *Sardina pilchardus*, *Scomber colias*, *Scomber scombrus*, and *T. trachurus* from the Portuguese coast.

| Species            | Sample size (n) | Area  | Size class (cm) | Total number of MP | Occurrence (%) | Median MP per fish | Interquartile range |
|--------------------|----------------|-------|-----------------|---------------------|----------------|--------------------|---------------------|
| *B. boops*         | 19             | W, S  | 20–24           | 9                   | 60             | 0.25               | 0.00–0.75           |
| *E. encrasicolus*  | 131            | W, S  | 12–17           | 77                  | 79             | 0.48               | 0.10–0.90           |
| *Sardina pilchardus* | 76            | W, S  | 14–22           | 22                  | 58             | 0.16               | 0.00–0.53           |
| *Scomber colias*   | 58             | W, S  | 19–27           | 27                  | 64             | 0.23               | 0.00–0.67           |
| *Scomber scombrus* | 19             | W     | 22–36           | 17                  | 100            | 1.00               | 0.29–2.50           |
| *T. trachurus*     | 24             | W     | 13–21           | 41                  | 100            | 1.75               | 1.20–2.00           |
order to identify the significant principal components to explain the variance in the data, the Broken Stick criterion was followed, using the “evplot” function (package “lmom”).

**Results**

We found MP in stomach contents of all analyzed fish species, identifying a total of 193 potential MP (Supporting Information Fig. S1). No MP were observed in procedural blanks. Fibers were the primarily MP found in all fish species representing more than 80% of particles, except for horse mackerel (76%) and Atlantic mackerel (65%) that also accumulated other forms of MP (Fig. 2A). The most prevalent MP colors were blue (44%) and black (29%) (Fig. 2B). MP size ranged from 21 μm to 5 mm (except one fiber at 20 mm), being the most common size class < 500 μm (34%) (Fig. 2C).

We selected randomly a subset of 38 potential MP (20% of total particles) between all species in order to verify their polymeric identity. Within these particles, 23 were fibers and 15 belong to other shape categories (fragments, films, and filaments). FTIR analyses revealed that the predominant polymeric form was polypropylene with an overall frequency of 21%, followed by polyethylene (16%), cellulose (16%), rayon (13%), styrene/acrylic copolymer (11%), polyacrylate (8%),

![Fig. 2.](image.png)

**Fig. 2.** Microplastics frequency (%) found in *B. boops, E. encrasicolus, Sardina pilchardus, Scomber colias, Scomber scombrus,* and *T. trachurus* caught in the Atlanto-Iberian ecosystem characterized by shape (A), color (B), and size class (C).
nylon-6 (4%), polyethylene terephthalate (4%), and polymeric epoxy plasticizer (4%). A total of 13% of all particles analyzed did not reach the pre-established threshold of 70% match with any of the materials within the FTIR spectra libraries, being excluded from analysis.

No statistical differences were found between the number of ingested MP and the sampling area ($F = 0.066, p = 0.798$). We found significant differences between the number of MP accumulated among species ($F = 6.022, p = 0.001$). Particularly, horse mackerel presented higher number of MP per stomach than European sardine, anchovy, and chub mackerel (Table 1, Fig. 4B). The number of fibers and particles accumulated was also similar between the two areas ($F = 0.434, p = 0.51$ and $F = 1.245, p = 0.28$, respectively) and significantly different between species ($F = 3.586, p = 0.022$ and $F = 6.022, p = 0.001$, respectively), being higher for horse mackerel and anchovy comparing to European sardines and chub mackerel.

Stomach content weight was similar between all species (Chi-squared = 10.40, $p = 0.065$). All pelagic species analyzed in this study were planktivorous but the diet composition was different between them (Fig. 3, Supporting Information Table S1). Calanoid copepods were a major prey group identified for all studied species, namely *Calanus helgolandicus*. European sardine, chub mackerel, and bogue consumed smaller prey items, including phytoplankton (mostly dinoflagellates). This contrasts with Atlantic mackerel and horse mackerel, for which the contribution of small prey such as phytoplankton was lower. Anchovy diet was intermediate between these two groups,
ingesting small microplankton and large mesozooplankton, such as decapods and euphausiids. Permutational multivariate analysis of variance (PERMANOVA) revealed that diet of pelagic fish tested here did not vary significantly between areas (Adonis test $F = 2.49$, $p = 0.07$) but varied significantly between species (Adonis test $F = 7.86$, $R^2 = 0.44$, $p < 0.01$) in combination with the Betadisper test ($F = 5.46$, $p = 0.001$) (Fig. 4A). Multiple comparisons revealed significant differences of diet between horse mackerel and several species namely anchovy ($F = 15.19$, $p$-adjusted $= 0.01$), chub mackerel ($F = 36.22$, $p$-adjusted $= 0.015$), and European sardine ($F = 18.15$, $p$-adjusted $= 0.01$). Anchovy diet was significantly different from chub mackerel ($F = 8.25$, $p$-adjusted $= 0.01$). Diet of Atlantic mackerel was different from that of European sardine and chub mackerel ($F = 10.56$, $p$-adjusted $= 0.01$ and $F = 5.96$, $p$-adjusted $= 0.06$, respectively).

The first principal components (PCs) of the analysis of MP and prey type in stomachs accounted for a small percentage of the variance. The Broken stick model identified seven significant PCs explaining 77% of the variance. PCA results of MP accumulation and prey size identified three significant

**Fig. 4.** Biplot of distances to the group centroid of multivariate dispersion analysis of prey composition (A) and boxplot of number of MP (B) by fish species (*Sardina pilchardus* [pil], *E. encrasicolus* [ane], *Scomber colias* [mas], *B. boops* [bog], *T. trachurus* [hom], and *Scomber scombrus* [mac]) collected in the Portuguese coast during the PELAGO14 survey. PcoA1 and PcoA2 in pannel A refer to principal coordinates of the multivariate dispersion.
components for prey size explaining 83% of variance. Small prey vs. large prey defined the first component and MP accumulation was mainly associated with prey > 1000 μm. The second and third components showed preferential association to prey of size class 1000–2000 μm. Species whose diet depends mostly on prey of that size range accumulated a higher percentage of MP, particularly horse mackerel and Atlantic mackerel, followed by anchovy (Supporting Information Fig. S2).

Discussion

Most MP found in this study were fibers and the predominant color was blue, similarly to other studies (Neves et al. 2015; Bessa et al. 2018; Herrera et al. 2019). Polypropylene and polyethylene were the main polymer forms found, agreeing with previous studies carried out at the Portuguese coastal area (Frias et al. 2014; Neves et al. 2015; Antunes et al. 2018). These polymers were the most produced by plastic industry being frequently found in different marine environments around the world (GESAMP 2015). Most colored fibers were made of cellulose (cotton), semisynthetic cellulose (rayon), and polyethylene terephthalate (polyester), from textiles and entering marine environment via wastewater treatment (Browne et al. 2011; Rochman et al. 2015). Nylon-6 fibers are widely used in fishing industry, while styrene/acrylic copolymer and polyacrylate are waterproof polymers frequently used in paints and coating products for nautical activities (GESAMP 2015). MP ranged between 21 and 5000 μm, overlapping prey size range of pelagic fish studied. However, the methodology used does not allow detection of particles < 20 μm, which would be preferentially retained by European sardine and chub mackerel. Moreover, small particles may easily pass through the digestive tract and be rapidly eliminated with faeces (Karakolis et al. 2018).

Atlantic mackerel and horse mackerel ingested more MP of various colors and shapes that the other pelagics. This can be related to their feeding behavior, selecting larger prey and generally found deeper in the water column than the other investigated species. Some studies point to demersal fish as being more prone to accumulating MP than pelagic fish (e.g., Bellas et al. 2016; Bessa et al. 2018), this is probably associated with plastic/MP sink and accumulation in the bottom sediments (Woodall et al. 2014). Other studies found opposite results or no relation between MP ingestion and fish distribution in water column (e.g., Anastasopoulou et al. 2018). Most fish were collected from mixed shoals, pointing that feeding behavior is also important for differential ingestion of MP.

Fish species depending on smaller prey, such as European sardines and chub mackerel, had the lowest concentration of MP in stomachs. These species are the most efficient in retaining small particles (Garrido et al. 2007, 2015). European sardines can alternate their feeding behavior between particulate-feeding and filter-feeding, depending on the light intensity, size, and available prey concentration (Garrido and van der Lingen 2014). Laboratory studies suggest that particles > 890 μm (the size of most of the MP found in this work) elicit a particulate-feeding mode, which is a visual and selective feeding mode (Garrido et al. 2007). Chub mackerel presented the highest diet similarity with European sardine. Particularly, both ingest high numbers of pelagic fish eggs, suggesting that they mostly feed in the upper water column, according to Zwolinski et al. (2006). These two species are distributed upper in the water column than Atlantic mackerel and horse mackerel off the Atlanto-Iberian waters. Since MP tend to accumulate in the neuston layer as buoyant pelagic fish eggs, one could expect higher accumulation of MP for these species.

Horse mackerel, a particulate-feeding species, accumulated a high number of MP but mostly < 1000 μm (63%), while the modal prey size class was 1000–2000 μm. Although this species is frequently found in mixed shoals with other small pelagics off the Iberia, it is known to distribute and feed deeper than other small pelagics in this area (V. Marques pers. comm.). On the contrary, Atlantic mackerel was one of the species that accumulated the highest concentration of MP with similar size range to its prey (1000–2000 μm), suggesting that MP might be confounded with prey. However, the data presented here do not allow us to determine if there is selection of a given MP size, for which the MP availability in the water would have to be known.

MP abundance found in the coastal pelagic fish species caught off Atlanto-Iberian was in agreement with concentrations found in digestive tract of other pelagic fish species from the Portuguese coastal and estuarine waters (Neves et al. 2015; Bessa et al. 2018) and from western Spanish Mediterranean coast (Compa et al. 2018). Conversely, Herrera et al. (2019) reported high numbers of MP per stomach for chub mackerel in the Canary Islands coast while Neves et al. (2015) found lower numbers of MP in stomachs of horse mackerel and Atlantic mackerel. Differences among studies can be related with environmental and ecological factors, given that vertical migration and trophic ecology of populations of the same fish species are known to vary seasonally (Garrido et al. 2008) and spatially (Costalago et al. 2015).

In conclusion, MP ingestion was significantly influenced by diet of planktivorous fish, with species feeding on smaller prey (such as European sardine, chub mackerel, and bogue) accumulating less MP in their stomachs when compared to species feeding on larger mesozooplankton organisms. Species that ingested more MP were Atlantic mackerel and horse mackerel, having a greater susceptibility suffering adverse effects posed by MP when compared to other species from this study.

The fish species studied in this work were assessed considering the quality criteria defined by Fossi et al. (2018) to select appropriated sentinel species for monitoring marine litter ingestion (background, habitat and trophic information, feeding behavior, spatial distribution, commercial importance,
and sensitivity to litter ingestion). Our study evidences horse mackerel as a suitable bioindicator since it is widely distributed in the WlUE and has high abundance in the pelagic environment and a widespread distribution in the Mediterranean Sea and eastern Atlantic Ocean (Abanuza et al. 2008 and references within). The commercial interest of horse mackerel has attracted a considerable number of studies providing information on its biology and ecology (e.g., Abanuza et al. 2008), including its feeding ecology (e.g., Garrido and Murta 2011). European horse mackerel stocks are annually assessed by ICES in Atlantic-European waters; therefore, the species is included in regular monitoring programs, with specimens routinely collected and analyzed from scientific surveys and fishery samples. This allows the access to a large number of specimens, which is required for a bioindicator. Most of the species studied here would fulfill the requirements identified in Fossi et al. (2018); however, horse mackerel ingested the largest number of MP.

Additional studies are needed, incorporating other regions, and focusing on the differential accumulation of MP throughout ontogeny, particularly for those species that change their diet dramatically as they grow, such as horse mackerel, chub mackerel and Atlantic mackerel, becoming piscivory as adults, contrary to anchovy and European sardine that remain planktivorous throughout the life cycle.

References

Abanuza, P., et al. 2008. Stock identity of horse mackerel (Trachurus trachurus) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. Fish. Res. 89: 196–209. doi:10.1016/j.fishres.2007.09.022

Anastasopoulou, A., and others. 2018. Assessment on marine litter ingested by fish in the Adriatic and NE Ionian Sea macro-region (Mediterranean). Mar. Pollut. Bull. 133: 841–851. doi:10.1016/j.marpolbul.2018.06.050

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26: 32–46. doi:10.1111/j.1442-9993.2001.001070.x

Antunes, J., J. Frias, and P. Sobral. 2018. Microplastics on the Portuguese coast. Mar. Pollut. Bull. 131: 294–302. doi:10.1016/j.marpolbul.2018.04.025

Avio, C. G., and others. 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198: 211–222. doi:10.1016/j.envpol.2014.12.021

Bachiller, E., and X. Irigoien. 2015. Trophodynamics and diet overlap of small pelagic fish species in the Bay of Biscay. Mar. Ecol. Prog. Ser. 534: 179–198. doi:10.3354/meps11375

Barboza, L. G. A., A. Dick Vethaak, B. R. B. O. Lavorante, A. K. Lundebye, and L. Guilhermino. 2018. Marine microplastic debris: An emerging issue for food security, food safety and human health. Mar. Pollut. Bull. 133: 336–348. doi:10.1016/j.marpolbul.2018.05.047

Bellas, J., J. Martinez-Armental, A. Martinez-Cámara, V. Besada, and C. Martinez-Gómez. 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109: 55–60. doi:10.1016/j.marpolbul.2016.06.026

Bessa, F., P. Barria, J. M. Neto, J. P. G. L. Frias, V. Otero, P. Sobral, and J. C. Marques. 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128: 575–584. doi:10.1016/j.marpolbul.2018.01.044

Besseling, B., A. Wegner, E. M. Foekema, M. J. Heuvel-Greve, and A. A. Koelmans. 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm Arenicola marina (L.). Environ. Sci. Technol. 47: 593–600. doi:10.1021/es302763x

Browne, M. A., P. Crump, S. J. Niven, E. Teuten, A. Tonkin, T. Galloway, and R. Thompson. 2011. Accumulation of microplastic on shorelines worldwide: Sources and sinks. Environ. Sci. Technol. 45: 9175–9179. doi:10.1021/es201811s

Chagnon, C., M. Thiel, J. Antunes, J. L. Ferreira, P. Sobral, and N. C. Ory. 2018. Plastic ingestion and trophic transfer between Easter Island flying fish (Cheilopogon rapaonaiensis) and yellowfin tuna (Thunnus albacares) from Rapa Nui (Easter Island). Environ. Pollut. 243: 127–133. doi:10.1016/j.envpol.2018.08.042

Cole, M., P. Lindeque, C. Halsband, and T. S. Galloway. 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull. 62: 2588–2597. doi:10.1016/j.marpolbul.2011.09.025

Collard, F., B. Gilbert, G. Eppe, L. Roos, P. Compère, K. Das, and E. Parmentier. 2017. Morphology of the filtration apparatus of three planktivorous fishes and relation with ingested anthropogenic particles. Mar. Pollut. Bull. 116: 182–191. doi:10.1016/j.marpolbul.2016.12.067

Compa, M., A. Ventero, M. Iglesias, and S. Deudero. 2018. Ingestion of microplastics and natural fibers in Sardinia pilchardus (Walbaum, 1792) and Engraulis encrasicolus (Linnaeus, 1758) along the Spanish Mediterranean coast. Mar. Pollut. Bull. 128: 89–96. doi:10.1016/j.marpolbul.2018.01.009

Costalago, D., S. Garrido, and I. Palomera. 2015. Comparison of the feeding apparatus and diet of European sardines Sardinia pilchardus of Atlantic and Mediterranean waters: Ecological implications. J. Fish Biol. 86: 1348–1362. doi:10.1111/jfb.12645

Cury, P., A. Bakun, R. J. M. Crawford, A. Jarre, R. A. Quinones, L. J. Shannon, and H. M. Verheye. 2000. Small pelagics in upwelling systems: Patterns of interaction and structural changes in ‘waspwaist’ ecosystems. ICES J. Mar. Sci. 57: 603–618. doi:10.1006/jnsc.2000.0712

de Sá, L. C., L. G. Luis, and L. Guilhermino. 2015. Effects of microplastics on juveniles of the common goby Pomatoschistus microps: Confusion with prey, reduction of predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196: 359–362. doi:10.1016/j.envpol.2014.10.026

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Dehaut, A., et al. 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ. Pollut. 215: 223–233. doi:10.1016/j.envpol.2016.05.018

Eriksen, M., and others. 2014. Plastic pollution in the world’s oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9: 1–15. doi:10.1371/journal.pone.0111913

FAO. 2016. The state of world fisheries and aquaculture 2016: Contributing to food security and nutrition for all. FAO. p. 200. ISBN 978-92-5-109185-2

Fossi, M. C., et al. 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237: 1023–1040. doi:10.1016/j.envpol.2017.11.019

Frias, J. P. G. L., V. Otero, and P. Sobral. 2014. Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. Mar. Environ. Res. 95: 89–95. doi:10.1016/j.marenvres.2014.01.001

Garrido, S., A. Marçalo, J. Zwolinski, and C. D. van der Lingen. 2007. Laboratory investigations on the effect of prey size and concentration on the feeding behaviour of Sardina pilchardus. Mar. Ecol. Prog. Ser. 330: 189–199. doi:10.3354/meps330189

Garrido, S., R. Ben-Hamadou, P. B. Oliveira, M. E. Cunha, M. A. Chicharo, and C. D. Van Der Lingen. 2008. Diet and feeding intensity of sardine Sardina pilchardus: Correlation with satellite-derived chlorophyll data. Mar. Ecol. Prog. Ser. 354: 245–256. doi:10.3354/meps07201

Garrido, S., and A. G. Murta. 2011. Horse mackerel (Trachurus trachurus) feeding off Portugal: Interdecadal and spatial variations of diet composition. J. Fish Biol. 79: 2034–2042. doi:10.1111/j.1095-8649.2011.03148.x

Garrido, S., and C. van der Lingen. 2014. Feeding biology and ecology, p. 122–189. In K. Ganias [ed.], Biology and ecology of sardines and anchovies. CRC Press.

Garrido, S., A. Silva, J. Pastor, R. Domínguez, A. V. Silva, and A. M. Santos. 2015. Trophic ecology of pelagic fish species off the Iberian coast: Diet overlap, cannibalism and intraguild predation. Mar. Ecol. Prog. Ser. 539: 271–286. doi:10.3354/meps11506

GESAMP. 2015. Sources, fate and effects of microplastics in the marine environment: A global assessment, p. 96. IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Rep. Stud. GESAMP No. 90.

Herrera, A., and others. 2019. Microplastic ingestion by Atlantic chub mackerel (Scromber colias) in the Canary Islands coast. Mar. Pollut. Bull. 139: 127–135. doi:10.1016/j.marpolbul.2018.12.022

Jovanović, B. 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integr. Environ. Assess. Manag. 13: 510–515. doi:10.1002/ieam.1913

Karakolis, E. G., B. Nguyen, J. B. You, P. J. Graham, C. M. Rochman, and D. Sinton. 2018. Digestible fluorescent coatings for cumulative quantification of microplastic ingestion. Environ. Sci. Technol. Lett. 5: 62–67. doi:10.1021/acs.estlett.7b00545

Martinez Abizú, P. 2019. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.3.

Mason, E., S. Coombs, and P. B. Oliveira. 2005. An overview of the literature concerning the oceanography of the eastern North Atlantic region, p. 33. Ralt. Cient. Téc. IPIMAR, Série digital. Available from https://www.ipima.pt/resources/www/docs/publicacoes.site/docweb/2006/Reln33final.pdf. Accessed January 2017.

Neves, D., P. Sobral, J. L. Ferreira, and T. Pereira. 2015. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar. Pollut. Bull. 101: 119–126. doi:10.1016/j.marpolbul.2015.11.008

Oksanen, J. B. G., and others. 2010. Package ‘vegan’ community ecology package. R package version 1.17-2.

Ory, N. C., C. Gallardo, M. Lenz, and M. Thiel. 2018. Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish. Environ. Pollut. 240: 566–573. doi:10.1016/j.envpol.2018.04.093

Phuong, N. N., A. Zalouk-Vergnoux, L. Poirier, A. Kamari, A. Châtel, C. Mouneyrac, and F. Lagarde. 2016. Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ. Pollut. 211: 111–123. doi:10.1016/j.marpolbul.2015.12.035

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. Available from http://www.R-project.org

Rochman, C. M., A. Tahir, S. L. Williams, D. V. Baxa, R. Lam, J. T. Miller, S. Werorilangi, and S. J. Teh. 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5: 14340. doi:10.1038/srep14340

Waller, C. L., H. J. Griffiths, C. M. Waluda, S. E. Thorpe, I. Loaiza, B. Moreno, C. O. Pacherres, and K. A. Hughes. 2017. Microplastics in the Antarctic marine system: A new emerging area of research. Sci. Total Environ. 598: 220–227. doi:10.1016/j.scitotenv.2017.03.283

Woodall, L. C., and others. 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1: 140317. doi:10.1098/rsos.140317

Wright, S. L., R. C. Thompson, and T. S. Galloway. 2013. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut. 178: 483–492. doi:10.1016/j.envpol.2013.02.031

Zwolinski, J., E. Mason, P. B. Oliveira, and Y. Stratoudakis. 2006. Fine-scale distribution of sardine (Sardina pilchardus) eggs and adults during a spawning event. J. Sea Res. 56: 294–304. doi:10.1016/j.seares.2006.05.004
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