Marine Pollution Bulletin 149 (2019) 110648

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Boops boops as a bioindicator of microplastic pollution along the Spanish Catalan coast

Odei Garcia-Garin[a,*], Morgana Vighi[a], Alex Aguilar[a], Catherine Tsangaris[b], Nikoletta Digka[b], Helen Kaberi[b], Asunción Borrell[a]

[a] *Department of Evolutionary Biology, Ecology and Environmental Sciences, Institute of Biodiversity Research (IRBio). Faculty of Biology. University of Barcelona, Barcelona, Spain*

[b] *Institute of Oceanography, Hellenic Centre for Marine Research, Athens, Greece*

ARTICLE INFO

Keywords:
Bogue
Indicator species
Marine litter
Mediterranean Sea
Fish

ABSTRACT

Microplastic pollution is a growing cause of concern for the marine environment, particularly in the Mediterranean Sea, which is considered to be one of the most polluted seas worldwide. In this study, the gastrointestinal tracts of 102 bogues (*Boops boops*), sampled from three areas off the Catalan coast (Spain) subject to different degrees of industrialization, were analysed to assess microplastic ingestion and thus estimate local levels of microplastic pollution. Microplastics were detected in 46% of samples analysed. As expected, the abundance and frequency of occurrence of ingested microplastics were higher off the most anthropized area of Barcelona. The majority of ingested microplastics were blue fragments ranging 0.1–0.5 mm, and the most common polymer type was polypropylene. The results of this study indicate the area off Barcelona as a possible area of concentration for microplastics, further supporting the use of *B. boops* as a bioindicator to assess microplastic pollution.

1. Introduction

The presence of marine litter has been reported in all marine compartments of seas and oceans worldwide (Cózar et al., 2014; Alomar et al., 2016). The largest component of marine litter is represented by artificial polymers, *i.e.,* plastics (Geyer et al., 2017). Large plastic items that enter the sea are gradually broken into small pieces by the mechanical erosion caused by winds and waves, photodegradation, and biodegradation (Barnes et al., 2009; Thompson et al., 2004), and gradually become microplastics *i.e.,* plastic items smaller than 5 mm in size (Arthur et al., 2009). Apart from these, microplastics can be of primary origin, which include the microbeads used in cosmetics and personal care products, capsules, textile microfibres, or virgin pellets used for manufacturing larger plastic items. Once in the sea, microplastics are driven by oceanic currents, travel long distances due to their buoyancy and durability (Eriksen et al., 2014), and they represent a considerable portion of the litter found in marine waters (de Haan et al., 2019). Recent studies estimated that 5 trillion microplastics are currently floating in the world's oceans and that the concentration of plastic particles floating in the surface waters of the Mediterranean Sea is 890,000 particles km^{-2} (Eriksen et al., 2014).

Microplastics may pose a threat to the marine environment (Rezania et al., 2018). Marine species at all levels of the trophic chain, including zooplankton (*e.g.*, Cole et al., 2014), worms (Wright et al., 2013), shellfish (*e.g.*, Digka et al., 2018), fish (*e.g.*, Bellas et al., 2016), seabirds (Codina-García et al., 2013), sharks (Fossi et al., 2014) and cetaceans (Fossi et al., 2016) have been reported to ingest microplastics. Despite evidence of the translocation of microplastics from the gastrointestinal tract to other tissues, *i.e.,* the presence of microplastics in the hepatic tissue of the mullet (*Mugil cephalus*) under laboratory conditions (Avio et al., 2015) and in eviscerated flesh of four commonly consumed dried fish species (Karami et al., 2017), related adverse effects in wild organisms are still lacking (Avio et al., 2015). Furthermore, although microplastics are chemically inert, the organic compounds used as plasticizers to improve the properties of plastics might produce adverse effects in some marine species, including alterations in the endocrine system and reproductive capacity (Lithner et al., 2011). Moreover, persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and dichlorodiphenyltrichloroethane (DDT) may be adsorbed and accumulated on post-consumed microplastics, increasing their toxic potential effects (Rios et al., 2007).

Different methods are used to assess the extent of microplastic pollution in the sea and thus estimate its potential risk for marine fauna.

* Corresponding author.
E-mail address: odei.garcia@ub.edu (O. Garcia-Garin).

https://doi.org/10.1016/j.marpolbul.2019.110648
Received 19 June 2019; Received in revised form 4 October 2019; Accepted 4 October 2019
Available online 10 October 2019
0025-326X/

Manta trawl nets are employed to assess the density of microplastics floating in the water column (*e.g.*, de Haan et al., 2019), while analyses of sediment samples are used to determine microplastic densities in the ocean floor and beaches (*e.g.*, Van Cauwenberghe et al., 2014; Alomar et al., 2016). Bioindicator species have also been proven particularly effective in assessing the microplastics levels in the biota (Fossi et al., 2018) and thus, potentially, their environmental concentrations. The EU Marine Strategy Framework Directive (MSFD) monitoring guidelines for the Mediterranean Sea indicate the analysis of the fish gastrointestinal tract (GI) as a viable method to assess microplastic pollution (Galgani et al., 2013). Among the possible fish species proposed, the bogue (*Boops boops*; Linneaus, 1758) stands out as a suitable bioindicator due to its ubiquitous distribution in the Mediterranean, the small size of its gut, and the high frequency of occurrence of microplastics in its digestive tract (Bray et al., 2019). In addition, as this species feeds on different types of bottoms including sand, mud, rocks and seagrass beds, performing vertical migrations at depths ranging from 0 to 350 m, it can be representative of several marine compartments (El-Haweet et al., 2005). Finally, its commercial value across the Mediterranean facilitates sample collection in local markets and thus further supports the use of the bogue as a commonly agreed upon bioindicator (Bray et al., 2019).

In the present study, the GI content of *B. boops* was analysed to assess the levels of microplastic ingestion in three differently urbanized and industrialized areas off the Spanish coast of the Mediterranean Sea: (1) the area off Barcelona, affected by several anthropogenic activities producing marine litter inputs, such as industrial outfalls, beach tourism, fishing, aquaculture and shipping; (2) the area off the small town of Blanes, characterized by local tourism and fishing activities; and (3) the area off Cap de Creus, a marine protected area (MPA), subject to heavy dominant winds and currents, where fishing and tourism are regulated. The aim of the study was to identify any differences in microplastic levels among the three areas and validate the use of the bogue as a bioindicator for microplastic pollution.

2. Materials and methods

2.1. Study area and sampling

A total of 102 bogues were collected during spring 2018 in three different areas of the Spanish Catalan coast (34 specimens per area), selected according to a gradient of industrialization and urbanization: 1) a highly anthropized area, located off the city of Barcelona; 2) an intermediate-anthropized area, near the town of Blanes; 3) an MPA, off Cap de Creus (Fig. 1). Fish were caught by local fishermen using trawling (22 individuals from Cap de Creus and 13 from Barcelona), purse seine (34 individuals from Blanes and 21 from Barcelona) and trammel nets (12 individuals from Cap de Creus) in areas located between 3 and 9.5 km from the coastline, at depths ranging between 22 and 90 m. After collection, fish were stored at −20 °C. Total length and total wet weight were measured for each fish (Table S1).

2.2. Extraction of microplastics

Fish were defrosted at 5 °C before dissection. The fish GI were dissected and weighed (wet weight, GIWW). To eliminate organic matter and enable detection of microplastics, samples were digested with hydrogen peroxide according to the protocol defined within the MEDSEALITTER project (MEDSEALITTER consortium, 2019). The GI content of each individual was placed into a glass beaker in 1:20 (w/v) H_2O_2 (15% H_2O_2, Chem-Lab, Germany) and heated on a hot plate at 55–65 °C until H_2O_2 evaporation. Aliquots of 10 ml H_2O_2 were added gradually to the beakers until all the organic matter was digested (the digestion process taking between 48 and 96 h). Samples were then diluted with 50 ml Milli-Q and vacuum-filtered on fibreglass filters (pore size 1.2 μm, Whatman, GE Healthcare, UK), which were dried at room temperature for 24 h and subsequently stored in Petri dishes.

2.3. Microplastic detection and quantification

Filters were examined under a stereomicroscope (Olympus, SZE and SZX7), and the microplastics detected were photographed using a digital camera (Luminera) and the INFINITY ANALYZE software. Items were counted and classified in four categories according to maximum length (< 0.1, 0.1–0.5, 0.5–1.0, 1.0–5.0 mm), colour, and type (fragment, fibre and granule). Average microplastic abundance was expressed as a) average number of microplastic items per individual considering the total number of examined individuals, b) average number of microplastic items per individual considering only individuals containing microplastics and c) average number of microplastic items per gram GIWW, considering only individuals containing microplastics. The frequency of occurrence of ingested microplastics was calculated as the percentage of the individuals containing microplastics out of the total number of sampled individuals.

2.4. FT-IR analysis

Fourier-transform infrared spectroscopy (FT-IR) was used in microplastic items larger than 300 μm to identify the type of synthetic polymer. FT-IR analysis was carried out with an Agilent Cary 630 FT-IR spectrometer using a self-generated polymer library. The confidence level for the comparison of the sample spectrum to that of the self-generated library database was set up to 80% (Digka et al., 2018). A minimum of 10% of the microplastics detected in the bogues GIs were analysed by FT-IR, as recommended by the marine litter monitoring guidelines provided by the MSFD technical group on marine litter (Galgani et al., 2013).

2.5. Contamination precautions and quality control

To prevent contamination throughout the analysis, the researchers performing the analyses wore white coats, and air currents were reduced to a minimum. All glass beakers were rinsed with purified water and fish samples were covered with aluminium foil during digestion. A glove bag was used for sample rinsing and filtration. Filters were protected with glass lids during stereoscope observation. Procedural blank samples were used during all steps, and items similar to those found in blank samples were excluded from statistical analyses, as they were considered airborne contamination.

2.6. Statistical analysis

Standardized data exploration techniques were used to identify outliers and possible collinearity between the physiological and spatial terms (Zuur et al., 2010). Microplastic abundance (calculated as in a), i.e., number of items per individual) in *B. boops* was modelled using GLMs (generalized linear models) with a negative binomial error distribution to account for overdispersion. Models were fitted with different combinations of the following explanatory variables: the level of anthropogenic impacts, categorized as low (MPA), medium (Blanes), high (Barcelona); the depth of the fishing area; the distance between the fishing area and the coastline, calculated using the measuring tool from Qgis (QGIS Development Team, 2018); the fishing method (trawling, purse seine and trammel nets); and the Fulton's condition factor, calculated as: $K = 100 * (\text{weight/total length}^3)$ (Froese, 2006). The information-theoretic approach was used for model selection (Burnham and Anderson, 2002) and models were compared using the AIC (Akaike's Information Criterion) (Akaike, 1974).

A Tukey HSD test was performed to compare microplastic abundance (a) in the three sampling areas. Correlations between the number and size of the ingested microplastics, and the fish body length, weight and GIWW were tested using Spearman's rank correlations. Types of

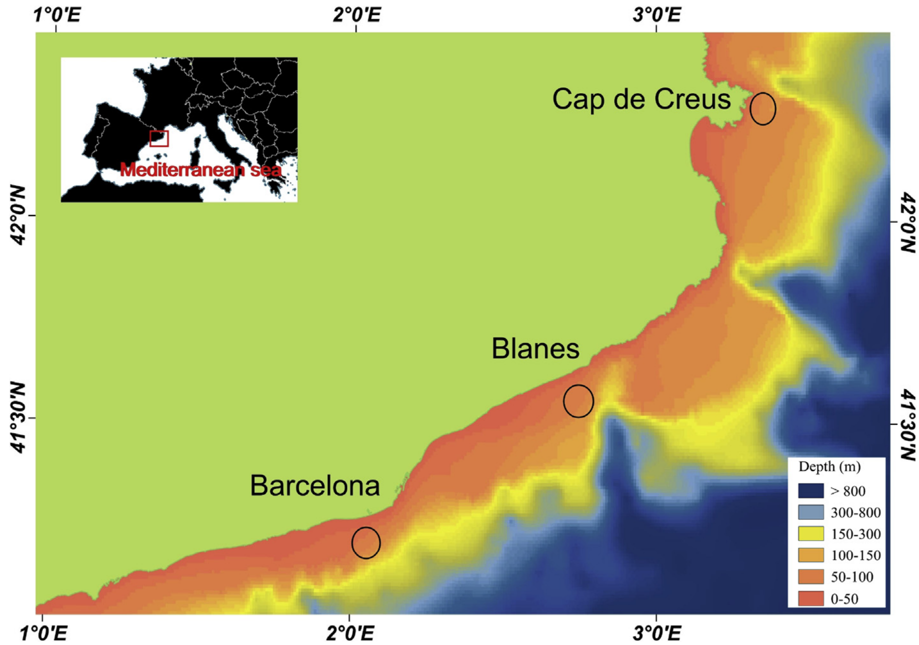

Fig. 1. Study area showing the three sampling areas: Barcelona, Blanes and Cap de Creus MPA.

ingested microplastics (shapes, class sizes and colours) were compared using the Pearson's Chi-squared test. The significance level was set at $p < 0.05$. Calculations were carried out within the programming environment R (R Core Team, 2014).

3. Results

3.1. Microplastic quantification for each area

In total, 46% of the fish had microplastics in their GI tracts. Microplastic abundance (a) ranged from 0 to 6 items per individual and the frequency of occurrence of ingested microplastics was higher in samples from the area off Barcelona (65%) than in those from the areas off Blanes and Cap de Creus (35% and 38%, respectively) (Table 1).

A total of 32 different GLMs were fitted from the combination of the 6 variables plus the Depth*Coast interaction (Table 2). The model with the lowest AIC score was that including the level of anthropogenic impacts and the distance to the coastline (M19, AIC = 243; Table 2), suggesting that higher ingestion rates of microplastics occur in locations near the coastline and with high anthropogenic impacts (Table 3). Accordingly, results from the Tuckey HSD test highlighted significant differences in microplastic abundance between the area off Barcelona and the other two areas (Table 4), while the difference in microplastic abundance between the area off Blanes and that off Cap de Creus was very small (0.50 ± 0.14 and 0.53 ± 0.14, respectively; Table 1). GLMs taking into account depth, fishing method and condition factor were not significant (Table 2).

In the bogues sampled off Barcelona and Blanes, the number of ingested microplastics showed a significant negative correlation with the fish body length (Spearman's r, S = 10,397, $\rho = -0.59$, $p < 0.001$ and S = 8,901, $\rho = -0.36$, $p < 0.05$; respectively) and the fish weight (Spearman's r, S = 88,724, $\rho = -0.62$, $p < 0.001$ and S = 14,842, $\rho = -0.50$, $p = 0.001$; respectively). Conversely, none of these correlations were significant in samples from the Cap de Creus MPA (Spearman's r, S = 6,309, $\rho = 0.04$, $p = 0.84$ and S = 8,979, $\rho = 0.09$, $p = 0.58$) (Fig. 2).

No correlation was found between the number of ingested microplastics and GIWW in samples from Blanes and the Cap de Creus MPA (Spearman's r, S = 7,911, $\rho = -0.21$, $p = 0.24$, and S = 6,774, $\rho = -0.36$, $p = 0.84$; respectively), while the number of ingested microplastics showed a negative correlation with GIWW in samples from Barcelona (Spearman's r, S = 10,377, $\rho = -0.59$, $p < 0.001$). Finally, no correlations were found between the microplastic size and the fish body length, weight or GIWW (Spearman's r, $p > 0.05$).

Table 1
Biological parameters, frequency of occurrence and abundance of ingested microplastics (MP) in *B. boops* from the three sampling areas.

Area	Barcelona	Blanes	Cap de Creus MPA
Number of individuals examined	34	34	34
Mean fish length (cm)	19.41 ± 2.81	19.86 ± 1.11	23.97 ± 3.93
Mean fish weight (g)	74.43 ± 28.69	103.92 ± 18.05	178.10 ± 111.65
Fulton's condition factor (K)	0.99 ± 0.11	1.32 ± 0.12	1.17 ± 0.17
Mean GIWW (g)	4.98 ± 2.26	8.17 ± 2.04	9.81 ± 3.66
Number of individuals containing MP	22	12	13
MP frequency of occurrence (%)	64.71	35.29	38.24
MP number	57	17	18
MP longest dimension length range (μm)	50–2960	66–3300	88–4700
MP abundance (mean ± SD)			
a) Number of items per individual in all individuals examined	1.68 ± 0.31[a]	0.50 ± 0.14[b]	0.53 ± 0.14[c]
b) Number of items per individual in individuals containing MP	2.59 ± 0.35	1.42 ± 0.23	1.38 ± 0.18
c) Number of items per gram weight in individuals containing MP	0.83 ± 0.15	0.20 ± 0.05	0.16 ± 0.02

[a, b, c] Indicate significant differences between fish sampling areas (Tuckey HSD test).

Table 2
Results from the GLMs fitted with a negative binomial error distribution and ranked by Akaike Information Criterion (AIC) for microplastic abundance (a) in *B. boops*. Explanatory variables included in the models: level of anthropogenic impacts (low, medium and high), depth (m), coastline distance (km), fishing method (trawling, purse seine and trammel nets) and condition factor (Fulton's K). The best-fit model is shown in bold.

	Model	AIC
M1	Level of anthropogenic impacts + Coast * Depth + K + Method	251
M2	Level of anthropogenic impacts + Coast + Depth + K + Method	251
M3	Level of anthropogenic impacts + Coast + Depth + K	247
M4	Level of anthropogenic impacts + Coast + Depth + Method	249
M5	Level of anthropogenic impacts + Coast + K + Method	249
M6	Level of anthropogenic impacts + Depth + K + Method	259
M7	Coast + Depth + K + Method	276
M8	Level of anthropogenic impacts + Coast + Depth	245
M9	Level of anthropogenic impacts + Coast + K	245
M10	Level of anthropogenic impacts + Depth + K	260
M11	Level of anthropogenic impacts + K + Method	257
M12	Level of anthropogenic impacts + Depth + Method	257
M13	Level of anthropogenic impacts + Coast + Method	247
M14	Depth + K + Method	275
M15	Coast + K + Method	274
M16	Coast + Depth + Method	274
M17	Coast + Depth + K	274
M18	Level of anthropogenic impacts + Depth	260
M19	**Level of anthropogenic impacts + Coast**	**243**
M20	Level of anthropogenic impacts + K	259
M21	Depth + Method	276
M22	K + Method	274
M23	Coast + Method	273
M24	Level of anthropogenic impacts + Method	255
M25	K + Depth	272
M26	Coast + K	273
M27	Coast + Depth	273
M28	Level of anthropogenic impacts	259
M29	Method	274
M30	Coast	274
M31	K	271
M32	Depth	272

3.2. Microplastic characterization (shape, size, colour and polymer type)

The proportion of shape, size class and colour categories did not differ among areas (Pearson's Chi-squared test, $p > 0.05$). The majority of ingested microplastics in the three areas were fragments of different colours and sizes (Fig. 3). The most common size class was 0.1–0.5 mm, found in the samples from all areas (Fig. 3 B), and the most common colour was blue in the samples from Barcelona and Blanes and black in the samples from Cap de Creus MPA (Fig. 3C).

Considering the microplastics analysed by FT-IR ($n = 9$), polypropylene was the most common polymer type (56%), followed by polyethylene (33%) and polystyrene (11%). Examples of microplastics found in the fish GI with the corresponding FT-IR spectra are shown in Fig. 4.

4. Discussion

In this study, the ingestion of microplastics was investigated in bogue samples to assess the levels of microplastic pollution in three areas off the Catalan coast and validate the use of this species as a bioindicator for microplastic pollution. The use of bioindicator species is strongly recommended by the MSFD and other monitoring programmes (e.g. UNEP/MAP) to increase the knowledge on the extent of marine litter pollution and its impacts on marine species. Previous studies made using the same species as a bioindicator detected similar microplastic occurrence levels in the Balearic Islands of Mallorca and Ibiza (Mediterranean Sea) (Nadal et al., 2016). The occurrence of microplastic found by these authors in the full stomach and intestine of the 337 bogues analysed was 68%. However, only 9% of the 32 bogues sampled by Neves et al. (2015) in the North Atlantic, off the Portuguese coast, had microplastics in their digestive tracts, indicating a spatial variability in the levels of microplastic ingested by the bogues that reflects local levels of microplastics in the sea.

Table 4
Summary of the results from the Tuckey HSD multiple comparisons of means for the factor “level of anthropogenic impacts” (categorized in: Low (“L”), Medium (“M), High (“H”)).

Linear hypotheses	Coefficient estimate	Standard error	Z value	Pr(> |z|)
L − H = = 0	−3.36	0.61	−5.48	< 0.001
M − H = = 0	−1.87	0.33	−5.69	< 0.001
M − L = = 0	1.49	0.54	2.75	0.02

4.1. Microplastic quantification

Significant differences were detected in the levels of microplastics ingested by *B. boops* in the three areas. As expected, the results of microplastic quantification indicated that bogues sampled from the most anthropized area off Barcelona presented the highest abundance and frequency of occurrence of ingested microplastics. Our results are consistent with those obtained by Bellas et al. (2016), who analysed microplastic ingestion by the demersal fish species *Mullus barbatus* in three areas off the Spanish Mediterranean coast and found the highest microplastic occurrence (33.3%) in the samples from the area off Barcelona.

Barcelona is located between two rivers, the Besòs and the Llobregat, and hosts a population of 1.6 million people (Instituto Nacional de Estadística, http://www.ine.es/welcome.shtml), a number of large industries, one of the most important commercial and tourist ports of the Mediterranean coast, and a large airport. Liubartseva et al. (2018) identify Barcelona as the second city of the Mediterranean Sea in terms of estimated inputs of plastic marine debris, with a total contribution of 1800 tons per year. Dominant marine currents along the Catalan coast follow a pattern from north to south parallel to the coast. They originate from the 30-km wide mesoscale Northern Current, which flows cyclonically along the continental slope from the Gulf of Genova to the southern Gulf of Valencia (Font et al., 1995). Indeed, urbanization has been reported to have a major influence on microplastic ingestion by fish (Peters and Bratton, 2016), and locations where currents converge accumulate marine litter and therefore marine biota more frequently ingest microplastics (Moore et al., 2001). Due to all these factors, bogues sampled in the marine area off Barcelona are exposed to higher microplastic concentrations than those occurring in other areas along the Catalan coast.

The amounts of microplastics found in the GI tracts of the bogues

Table 3
Summary of the results from the best-fit GLM, fitted with the variables “level of anthropogenic impacts” and “distance to the coastline” (M19).

Term	Coefficient estimate	Standard error	Z value	Pr(> |z|)
Intercept	5.20	1.10	4.73	< 0.001
Level of anthropogenic impacts (Low)	−3.36	0.61	−5.48	< 0.001
Level of anthropogenic impacts (Medium)	−1.87	0.33	−5.69	< 0.001
Coast	−0.62	0.15	−4.20	< 0.001

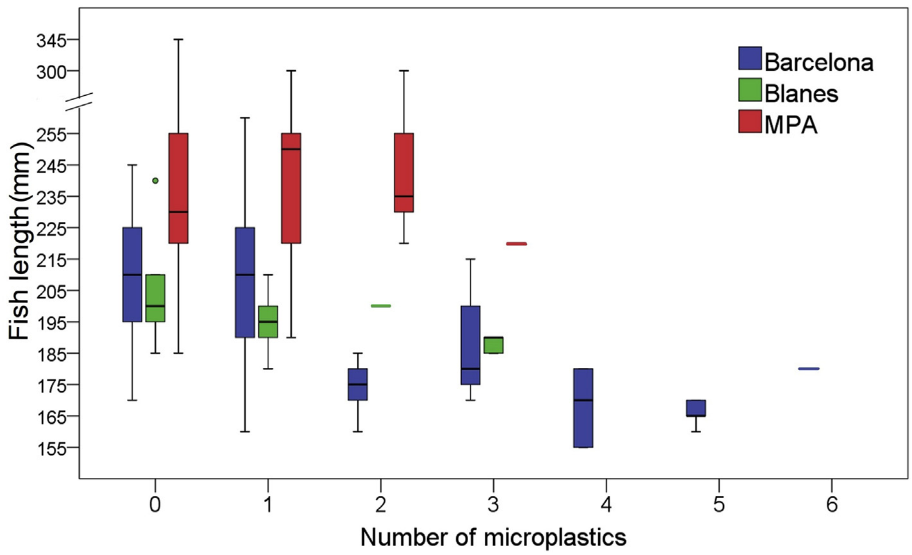

Fig. 2. Box plot showing the relationship between the bogues body length and the number of microplastics ingested. The central line indicates the median fish length for each area and number of microplastics; the edges of the box indicate the 25th and 75th percentiles; whiskers extend to extreme data points not considered outliers, and outliers are plotted individually as circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sampled in the area off Blanes and in the Cap de Creus MPA were similar, and the average frequency of occurrence in both areas was consistent with the value of 37.5% found by Rios-fuster et al. (2019) in *B. boops* from southern Spain. The same authors reported similar values of microplastic occurrence (≈30%) also in samples of *Sardina pilchardus* from Blanes and *Trachurus mediterraneus* and *Engraulis encrasicolos* from Cap de Creus MPA. Although lower abundance and frequency of occurrence might be expected in the marine protected area, consistently with our results, Nadal et al. (2016) also found high frequencies of microplastic occurrence in bogues sampled from Espardell, an island inside the MPA Ses Salines (Eivissa, Spain). These discrepancies indicate that microplastic presence in the sea must be interpreted from a wider perspective, evaluating levels of industrialization and urbanization in the proximity, but also the influence of seasonal currents, river discharges, wastewater treatments, rainfall, and tourism fluxes. The Cap de Creus MPA is very popular among international tourists due to its high natural and cultural values, and despite its high level of protection and preservation, high amounts of litter are generated on the land that may accidentally enter the sea. Furthermore, the dominant pattern of winds and currents may also generate local areas of microplastic accumulation during certain periods of the year.

Results obtained from the best-fit model showed that bogues ingest higher rates of microplastics closer to the coastline. This result is

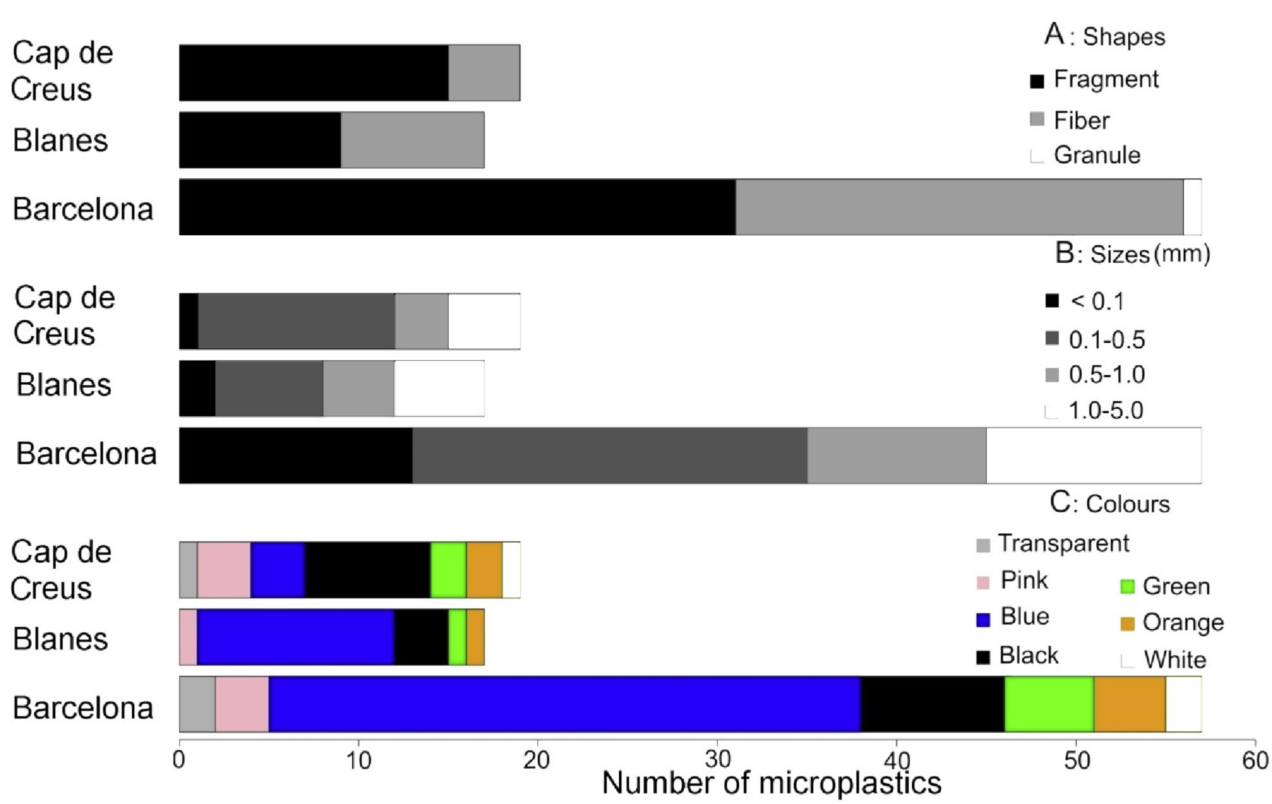

Fig. 3. Shape (A), size (B) and colour (C) of microplastics detected in *B. boops* from the three sampling areas. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

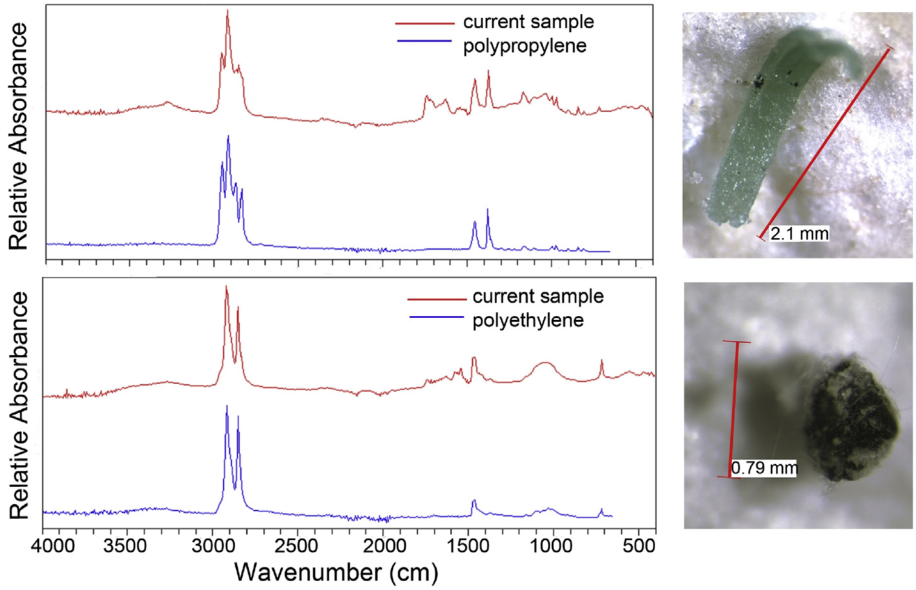

Fig. 4. Examples of microplastics found in fish gastrointestinal tract with relative Fourier-transform infrared spectroscopy spectra (level of certainty of 82 and 95% for the first and second microplastic item spectra, respectively).

consistent with those obtained by Rios-fuster et al. (2019), and confirms the hypothesis that the greatest overlap between microplastics and marine fauna occurs in coastal waters (Clark et al., 2016), as higher concentrations of litter are often found in proximity of densely populated urban centres, touristic areas and shipping routes (Suaria et al., 2014).

The abundance of ingested microplastics was inversely correlated with body length and weight in the bogues from Barcelona and Blanes but not in those from Cap de Creus MPA. Although similar studies show no effect of body length on microplastic ingestion occurrence in other fish species (*e.g.*, Foekema et al., 2013; Digka et al., 2018), some authors suggest that larger individuals are less likely to ingest microplastics (*e.g.*, Compa et al., 2018; Bessa et al., 2018), which may explain the higher abundance of microplastics in the GIs of the smaller individuals from Barcelona and Blanes. However, explanations for the discrepancy of the relationship between microplastics and body length between areas remain unknown, and it should be highlighted that the bogues from Cap de Creus were, on average, larger in size and weight, which likely had an effect on that relationship (Fig. 2). In addition, no correlation with the Fulton's condition factor (K) was found in the bogues sampled for this study, despite Compa et al. (2018) reported that individuals of *S. pilchardus* with lower condition factor ingested more microplastics than those individuals in better conditions. Although Compa et al. (2018) did not find any difference in the abundance of ingested microplastics between mature and immature individuals, microplastic ingestion rates could be also related with the fish developmental stages, as mature and immature individuals often show behavioural and feeding habits dissimilarities.

4.2. Microplastic characterization

Microplastics ingested by *B. boops* from the Catalan coast were primarily fragments (~60%) and secondly fibres (~40%) (Fig. 3A). Fragments are the result of the degradation of larger plastic items, while fibres are the most abundant component of primary microplastics in seas and oceans worldwide (Bessa et al., 2018). Our results revealed, proportionally, a smaller contribution of fragments and a larger contribution of fibres than those detected in fish of the Northern Ionian Sea by Digka et al. (2018), who reported approximately 80% fragments and 20% fibres, respectively, showing a similar order of prevalence. Conversely, other studies (e.g., Lusher et al., 2013; Bellas et al., 2016; Güven et al., 2017; Compa et al., 2018; Bessa et al., 2018) found a higher percentage of fibres than fragments in fish GIs. These contrasting results may be related to different sources and waste management strategies in the sampling areas, which could prevent or reduce the amounts of plastic items that reach the sea from land, brought by rivers or wind (Digka et al., 2018; Boucher and Friot, 2017).

In the present study, microplastics were classified into 4 size categories according to their largest dimensions. The main microplastic size class was 0.1–0.5 mm (Fig. 3B), supporting the role of indirect intake from microplastics ingested by prey (*i.e.,* zooplankton) as an important mechanism of microplastic ingestion in fish (Avio et al., 2017; Neves et al., 2015). However, future research is needed to improve knowledge regarding the mechanisms of microplastic ingestion by bogues (Nadal et al., 2016). In addition, Digka et al. (2018) also found that microplastics between 0.1 and 0.5 mm were the most prevalent in mussels and fish from the Adriatic Sea. However, microplastics < 0.1 mm may have been underestimated due to the reduced recovery rates for smaller particles (Avio et al., 2015).

The predominant colour of the microplastics ingested by bogues was blue (Fig. 3C), a result consistent with other studies (*e.g.*, Romeo et al., 2015; Güven et al., 2017; Peters et al., 2017; Compa et al., 2018; Digka et al., 2018). The prevalence of this colour may suggest that fish ingest microplastics regardless of their colour, as blue microplastics are not distinctively visible to fish (Peters and Bratton, 2016).

Finally, the most common polymer types detected in the litter ingested by *B. boops* were polypropylene, polyethylene and polystyrene. These results were expected because these three polymers are present in most plastic litter found in the water column worldwide (Suaria et al., 2016; Cózar et al., 2017). Polyethylene is used to manufacture plastic bags and bottles (Suaria et al., 2016; Cózar et al., 2017), which makes it the most abundant plastic in the world; polypropylene is highly abundant in bottle caps and packages (Suaria et al., 2016); and polystyrene is used widely for fishing boxes and other common containers. Consistently with our findings, polypropylene and polyethylene were also predominant in other studies of microplastic ingestion in fish from the Mediterranean Sea (Avio et al., 2017; Digka et al., 2018) and other European seas (Collard et al., 2017).

4.3. The use of bioindicators for marine litter monitoring in the international legislative framework

New international and EU directives are focusing on the reduction of waste and on the implementation of monitoring programs to assess the extent of marine litter pollution and its impacts in order to plan adequate mitigation measures. Among others, the Waste Directive (amending 2008/98/EC), the Packaging Directive (94/62/EC), the Plastic Carrier Bags Directive (2015/720/UE amending 94/62/EC), the Single Use Plastic Directive (2018/0172/EC) and the Directive on Port reception facilities for the delivery of waste from ships (directive COM (2018) 33) are addressing these issues. In addition, the UNEP/MAP Regional Plan for Marine litter Management in the Mediterranean (UNEP/MAP IG.21/9) highlights the urgent need to act against marine litter. From the UN Environment Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP), adopted in 2016, the use of bioindicator species for marine litter monitoring is clearly recommended by the Candidate Indicator 24: *Trends in the amount of litter ingested by or entangling marine organisms, focusing on selected mammals, marine birds, and marine turtles,* under Ecological Objective 10 (EO10). Moreover, the UNEP/MAP (Galgani, 2017) reported recently that bioindicator species are highly needed to monitor microplastics and marine litter in general. To comply with legal requirements and the urgent need to address the issues posed by marine litter, several studies focusing on microplastic ingestion are investigating suitable bioindicator species (Bray et al., 2019; Fossi et al., 2018). In this framework, furthermore, MSFD (Commission Decision, 2017/848) aims to achieve the Good Environmental Status, and it will be possible when we achieve the D10 criteria, which states: *Properties and quantities of marine litter do not cause harm to the coastal and marine environment*. Results from the present article provide a further support for the adoption of *B. boops* as a bioindicator species for marine litter (*i.e.,* the ever-increasing microplastics) monitoring.

5. Conclusions

Our results identify the area off Barcelona as a possible area of concentration for microplastics and further support the use of *B. boops* as bioindicator of microplastic pollution in the Mediterranean Sea, potentially reflecting both environmental microplastic loads and their main characteristics. In addition, the results from this study contribute to increasing the knowledge about levels of microplastic pollution in the Mediterranean, highlighting that highly anthropized areas can be potential hotspots for microplastic accumulation and thus ingestion by marine fauna. The assessment of microplastic levels and the identification of potential hotspots of microplastic accumulation and/or higher risk for marine fauna is a necessary requirement for planning targeted measures to reduce the potential risks related to marine litter.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to the *Confradia de pescadors de Barcelona*, the *Confradia de pescadors de Blanes*, the *Confradia de pescadors de Roses* and the *Confradia de pescadors de Cadaqués*, which provided *B. boops* samples. This study was supported by the project MEDSEALITTER (1 MED15_3.2_M12_334; European Union - European Regional Development Fund- Interreg MED). OGG's Ph.D. was funded through an FPU scholarship granted by the Spanish Government. Constructive feedback from two anonymous reviewers contributed to improve the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.110648.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19, 716–723. https://doi.org/10.1109/TAC.1974.1100705.
Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1–10. https://doi.org/10.1016/j.marenvres.2016.01.005.
Arthur, C., Baker, J., Bamford, H., 2009. Proceedings of the International Research Workshop on the Occurrence. Effects and Fate of Microplastic Marine Debris. Tacoma. Washington. National Oceanic and Atmospheric Administration. EUA.
Avio, C.G., 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.
Avio, C.G., Cardelli, L.R., Gorbi, S., Pellegrini, D., Regoli, F., 2017. Microplastics pollution after the removal of the Costa Concordia wreck: first evidences from a biomonitoring case study. Environ. Pollut. 227, 207–214.
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. Biol. Sci. 364 (1526), 1985–1998. https://doi.org/10.1098/rstb.2008.0205.
Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109 (1), 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026.
Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128, 575–584. https://doi.org/10.1016/j.marpolbul.2018.01.044.
Bray, L., Digka, N., Tsangaris, C., Camedda, A., Gambaiani, D., de Lucia, G.A., et al., 2019. Determining suitable fish to monitor plastic ingestion trends in the Mediterranean Sea. Environ. Pollut. 247, 1071–1077. https://doi.org/10.1016/j.envpol.2019.01.100.
Boucher, J., Friot, D., 2017. Primary Microplastics in the Oceans: A Global Evaluation of Sources 43pp IUCN, Gland, Switzerland (en).
Burnham, K.P., Anderson, D.R., 2002. Model selection and multimodel inference: a practical information-theoretic approach. In: Ecological Modelling, second ed. https://doi.org/10.1016/j.ecolmodel.2003.11.004.
Clark, J.R., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T.M., Galloway, T.S., 2016. Marine microplastic debris: a targeted plan for understanding and quantifying interactions with marine life. Front. Ecol. Environ. 14, 317–324. https://doi.org/10.1002/fee.1297.
Codina-García, M., Militão, T., Moreno, J., González-Solís, J., 2013. Plastic debris in Mediterranean seabirds. Mar. Pollut. Bull. 77 (1–2), 220–226. https://doi.org/10.1016/j.marpolbul.2013.10.002.
Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 1–8. https://doi.org/10.1038/srep04528.
Collard, F., Gilbert, B., Eppe, G., Roos, L., Compère, P., Das, K., Parmentier, E., 2017. Morphology of the filtration apparatus of three planktivorous fishes and relation with ingested anthropogenic particles. Mar. Pollut. Bull. 116 (1–2), 182–191. https://doi.org/10.1016/j.marpolbul.2016.12.067.
Compa, M., Ventero, A., Iglesias, M., Deudero, S., 2018. Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. Mar. Pollut. Bull. 128, 89–96. https://doi.org/10.1016/j.marpolbul.2018.01.009.
Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Ubeda, B., Hernández-León, S., et al., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. U.S.A. 111 (28), 10239–10244. https://doi.org/10.1073/pnas.1314705111.
Cózar, A., Martí, E., Duarte, C.M., García-de-Lomas, J., van Sebille, E., Ballatore, T.J., Eguíluz, V.M., Gonzalez-Gordillo, J.I., Pedrotti, M.L., Echevarría, F., Trouble, R., Irigoien, X., 2017. The Arctic Ocean as a dead end for floating plastics in the North Atlantic branch of the thermohaline circulation. Sci. Adv. 3 (4), e1600582.
de Haan, W.P., Sanchez-Vidal, A., Canals, M., 2019. Floating microplastics and aggregate formation in the western Mediterranean Sea. Mar. Pollut. Bull. 140, 523–535. https://doi.org/10.1016/j.marpolbul.2019.01.053.
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. https://doi.org/10.1016/j.marpolbul.2018.06.063.
El-Haweet, A., Hegazy, M., Abu-Hatab, H., Sabry, E., 2005. Validation of length frequency analysis for *Boops boops* (bogue) growth estimation. Egypt. J. Aquat. Res. 31 (1), 399e408.
Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., et al., 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 (12), 1–15. https://doi.org/10.1371/journal.pone.0111913.
Foekema, E.M., De Gruijter, C., Mergia, M.T., Van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in north sea fish. Environ. Sci. Technol. 47, 8818–8824. https://doi.org/10.1021/es400931b.

Font, J., Garcia-Ladona, E., Gorriz, E.G., 1995. The seasonality of mesoscale motion in the Northern Current of the western Mediterranean: severa years of evidence. Oceanol. Acta 18 (2), 207–219.
Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., et al., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). Mar. Environ. Res. 100, 17–24. https://doi.org/10.1016/j.marenvres.2014.02.002.
Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., et al., 2016. Fin whales and microplastics: the Mediterranean Sea and the sea of cortez scenarios. Environ. Pollut. 209, 68–78. https://doi.org/10.1016/j.envpol.2015.11.022.
Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., et al., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.
Froese, B.R., 2006. Cube Law, Condition Factor and Weight – Length Relationships: History, Meta-Analysis and Recommendations, vol. 22. pp. 241–253.
Galgani, F., 2017. Specially Protected Areas Protocol Regional Activity Centre (Barcelona Convention), 2017, Defining the Most Representative Species for IMPA Common Indicator 24. SPA/RAC, Tunis.
Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., et al., 2013. Guidance on monitoring of marine litter in European seas. MSFD technical subgroup on marine litter (TSG-ML). .
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 25–29.
Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş, A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ. Pollut. 223, 286–294. https://doi.org/10.1016/j.envpol.2017.01.025.
Karami, A., Golieskardi, A., Ho, Y.B., Larat, V., Salamatinia, B., 2017. Microplastics in eviscerated flesh and excised organs of dried fish. Sci. Rep. 7, 5473. https://doi.org/10.1038/s41598-017-05828-6.
Lithner, D., Larsson, A., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409 (18), 3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038.
Liubartseva, S., Coppini, G., Lecci, R., Clementi, E., 2018. Tracking plastics in the Mediterranean: 2D Lagrangian model. Mar. Pollut. Bull. 129 (1), 151–162. https://doi.org/10.1016/j.marpolbul.2018.02.019.
Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67 (1–2), 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.
MEDSEALITTER consortium, 2019. Common monitoring protocol for marine litter. Deliverable 4.6.1. https://medsealitter.interreg-med.eu/what-we-achieve/deliverable-database/.
Moore, C.J., Moore, S.L., Leecaster, M.K., Weisberg, S.B., 2001. A comparison of plastic and plankton in the North Pacific central Gyre. Mar. Pollut. Bull. 42 (12). https://doi.org/10.1155/2014/758679.
Nadal, M.A., Alomar, C., Deudero, S., 2016. High levels of microplastic ingestion by the semipelagic fish bogue *Boops boops* (L.) around the Balearic Islands. Environ. Pollut. 214, 517–523. https://doi.org/10.1016/j.envpol.2016.04.054.
Neves, D., Sobral, P., Ferreira, J.L., Pereira, T., 2015. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar. Pollut. Bull. 101 (1), 119–126. https://doi.org/10.1016/j.marpolbul.2015.11.008.
Peters, C.A., Bratton, S.P., 2016. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos river basin, central Texas, USA. Environ. Pollut. 210, 380–387.
Peters, C.A., Thomas, P.A., Rieper, K.B., Bratton, S.P., 2017. Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast. Mar. Pollut. Bull. 124 (1), 82–88. https://doi.org/10.1016/j.marpolbul.2017.06.080.
QGIS Development Team, 2018. QGIS geographic information system. Open Source Geospatial Foundation Project. http://qgis.osgeo.org.
R Core Team, 2014. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Rezania, S., Park, J., Md Din, M.F., Mat Taib, S., Talaiekhozani, A., Kumar Yadav, K., Kamyab, H., 2018. Microplastics pollution in different aquatic environments and biota: a review of recent studies. Mar. Pollut. Bull. 133, 191–208. https://doi.org/10.1016/j.marpolbul.2018.05.022.
Rios-fuster, B., Alomar, C., Guijarro, B., Deudero, S., 2019. Anthropogenic particles ingestion in fish species from two areas of the western Mediterranean Sea. Mar. Pollut. Bull. 144, 325–333. https://doi.org/10.1016/j.marpolbul.2019.04.064.
Rios, L.M., Moore, C., Jones, P.R., 2007. Persistent organic pollutants carried by synthetic polymers in the ocean environment. Mar. Pollut. Bull. 54 (8), 1230–1237. https://doi.org/10.1016/j.marpolbul.2007.03.022.
Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., Fossi, M.C., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. 95 (1), 358–361. https://doi.org/10.1016/j.marpolbul.2015.04.048.
Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., et al., 2016. The mediterranean plastic soup: synthetic polymers in mediterranean surface waters. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep37551.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., et al., 2004. Lost at sea: where is all the plastic? Science 304 (5672) 838 LP-838.
Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut 193, 65–70.
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033.
Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. http://dx.doi.org/10.1111/j.2041-210X.2009.00001.x.