

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

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## **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.

**Keywords:** bogue; indicator species; marine litter; Mediterranean Sea; fish

## **Capsule**

The results of this study indicate the area off Barcelona as a possible area of concentration of microplastics and support the use of *Boops boops* as a suitable bioindicator for monitoring microplastic pollution in the Mediterranean Sea.

**Declarations of interest:** none

### **Highlights**

- 1- Levels of microplastic ingestion were assessed in bogues from the Catalan coast.
- 2- The occurrence of ingested microplastics was higher in bogues off Barcelona.
- 3- Bogues from less anthropized areas had lower amounts of microplastics in their guts.
- 4- The bogue is a suitable indicator of microplastic pollution in the Mediterranean Sea.

## 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 microfibrils, 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<sup>-2</sup> (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. 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., 2013; 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*; Linnaeus, 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-Hawet 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).

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

## 10 **2. Materials and methods**

### 11 *2.1. Study area and sampling*

12 A total of 102 bogues were collected during spring 2018 in three different areas of the Spanish  
13 Catalan coast (34 specimens per area), selected according to a gradient of industrialization and  
14 urbanization: 1) a highly anthropized area, located off the city of Barcelona; 2) an intermediate-  
15 anthropized area, near the town of Blanes; 3) an MPA, off Cap de Creus (Fig 1). Fish were caught  
16 by local fishermen using trawling (22 individuals from Cap de Creus and 13 from Barcelona), purse  
17 seine (34 individuals from Blanes and 21 from Barcelona) and trammel nets (12 individuals from  
18 Cap de Creus) in areas located between 3 and 9.5 km from the coastline, at depths ranging between  
19 22 and 90 m. After collection, fish were stored at  $-20\text{ }^{\circ}\text{C}$ . Total length and total wet weight were  
20 measured for each fish (Table S1).

### 21 *2.2. Extraction of microplastics*

22 Fish were defrosted at  $5\text{ }^{\circ}\text{C}$  before dissection. The fish GI were dissected and weighed (wet weight,  
23 GIWW). To eliminate organic matter and enable detection of microplastics, samples were digested  
24 with hydrogen peroxide according to the protocol defined within the MEDSEALITTER project  
25 (MEDSEALITTER consortium, 2019). The GI content of each individual was placed into a glass  
26 beaker in 1:20 (w/v)  $\text{H}_2\text{O}_2$  (15%  $\text{H}_2\text{O}_2$ , Chem-Lab, Germany) and heated on a hot plate at  $55\text{--}65\text{ }^{\circ}\text{C}$   
27 until  $\text{H}_2\text{O}_2$  evaporation. Aliquots of 10 ml  $\text{H}_2\text{O}_2$  were added gradually to the beakers until all the  
28 organic matter was digested (the digestion process taking between 48 and 96 hours). Samples were  
29 then diluted with 50 ml Milli-Q and vacuum-filtered on fibreglass filters (pore size  $1.2\text{ }\mu\text{m}$ ,  
30 Whatman, GE Healthcare, UK), which were dried at room temperature for 24 hours and  
31 subsequently stored in Petri dishes.

### 32 *2.3. Microplastic detection and quantification*

33 Filters were examined under a stereomicroscope (Olympus, SZE and SZX7), and the microplastics  
34 detected were photographed using a digital camera (Luminera) and the INFINITY ANALYZE  
35 software. Items were counted and classified in four categories according to maximum length ( $< 0.1$ ,  
36  $0.1 - 0.5$ ,  $0.5 - 1.0$ ,  $1.0 - 5.0\text{ mm}$ ), colour, and type (fragment, fibre and granule). Average  
37 microplastic abundance was expressed as a) average number of microplastic items per individual  
38 considering the total number of examined individuals, b) average number of microplastic items per  
39 individual considering only individuals containing microplastics and c) average number of  
40 microplastic items per gram GIWW, considering only individuals containing microplastics. The  
41 frequency of occurrence of ingested microplastics was calculated as the percentage of the  
42 individuals containing microplastics out of the total number of sampled individuals.

### 43 *2.4. FT-IR analysis*

1 Fourier-transform infrared spectroscopy (FT-IR) was used in microplastic items larger than 300  $\mu\text{m}$   
2 to identify the type of synthetic polymer. FT-IR analysis was carried out with an Agilent Cary 630  
3 FT-IR spectrometer using a self-generated polymer library. The confidence level for the comparison  
4 of the sample spectrum to that of the self-generated library database was set up to 80% (Digka et al.  
5 2018). A minimum of 10% of the microplastics detected in the bogues GIs were analysed by FT-IR,  
6 as recommended by the marine litter monitoring guidelines provided by the MSFD technical group  
7 on marine litter (Galgani et al., 2013).

## 8 2.5. Contamination precautions and quality control

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

## 16 2.6. Statistical analysis

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

29 A Tukey HSD test was performed to compare microplastic abundance (a) in the three sampling  
30 areas. Correlations between the number and size of the ingested microplastics, and the fish body  
31 length, weight and GIWW were tested using Spearman's rank correlations. Types of ingested  
32 microplastics (shapes, class sizes and colours) were compared using the Pearson's Chi-squared test.  
33 The significance level was set at  $p < 0.05$ . Calculations were carried out within the programming  
34 environment R (R Core Team, 2014).

## 35 3. Results

### 36 3.1. Microplastic quantification for each area

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

41 A total of 32 different GLMs were fitted from the combination of the 6 variables plus the  
42 Depth\*Coast interaction (Table 2). The model with the lowest AIC score was that including the  
43 level of anthropogenic impacts and the distance to the coastline (M19, AIC = 243; Table 2),  
44

1 suggesting that higher ingestion rates of microplastics occur in locations near the coastline and with  
2 high anthropogenic impacts (Table 3). Accordingly, results from the Tuckey HSD test highlighted  
3 significant differences in microplastic abundance between the area off Barcelona and the other two  
4 areas (Table 4), while the difference in microplastic abundance between the area off Blanes and that  
5 off Cap de Creus was very small ( $0.50 \pm 0.14$  and  $0.53 \pm 0.14$ , respectively; Table 1). GLMs taking  
6 into account depth, fishing method and condition factor were not significant (Table 2).

7 In the bogues sampled off Barcelona and Blanes, the number of ingested microplastics showed a  
8 significant negative correlation with the fish body length (Spearman's  $r$ ,  $S = 10397$ ,  $\rho = -0.59$ ,  $p <$   
9  $0.001$  and  $S = 8901$ ,  $\rho = -0.36$ ,  $p < 0.05$ ; respectively) and the fish weight (Spearman's  $r$ ,  $S = 88724$ ,  
10  $\rho = -0.62$ ,  $p < 0.001$  and  $S = 14842$ ,  $\rho = -0.50$ ,  $p = 0.001$ ; respectively). Conversely, none of these  
11 correlations were significant in samples from the Cap de Creus MPA (Spearman's  $r$ ,  $S = 6309$ ,  $\rho =$   
12  $0.04$ ,  $p = 0.84$  and  $S = 8979$ ,  $\rho = 0.09$ ,  $p = 0.58$ ) (Fig 2).

13 No correlation was found between the number of ingested microplastics and GIWW in samples  
14 from Blanes and the Cap de Creus MPA (Spearman's  $r$ ,  $S = 7911$ ,  $\rho = -0.21$ ,  $p = 0.24$ , and  $S = 6774$ ,  
15  $\rho = -0.36$ ,  $p = 0.84$ ; respectively), while the number of ingested microplastics showed a negative  
16 correlation with GIWW in samples from Barcelona (Spearman's  $r$ ,  $S = 10377$ ,  $\rho = -0.59$ ,  $p < 0.001$ ).  
17 Finally, no correlations were found between the microplastic size and the fish body length, weight  
18 or GIWW (Spearman's  $r$ ,  $p > 0.05$ ).

### 21 3.2. Microplastic characterization (shape, size, colour and polymer type)

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

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

## 32 4. Discussion

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

### 44 4.1. Microplastic quantification

45 Significant differences were detected in the levels of microplastics ingested by *B. boops* in the three  
46 areas. As expected, the results of microplastic quantification indicated that bogues sampled from the  
47 most anthropized area off Barcelona presented the highest abundance and frequency of occurrence  
48 of ingested microplastics. Our results are consistent with those obtained by Bellas et al. (2016), who  
49 analysed microplastic ingestion by the demersal fish species *Mullus barbatus* in three areas off the  
50

1 Spanish Mediterranean coast and found the highest microplastic occurrence (33.3%) in the samples  
2 from the area off Barcelona.

3  
4 Barcelona is located between two rivers, the Besòs and the Llobregat, and hosts a population of 1.6  
5 million people (Instituto Nacional de Estadística, <http://www.ine.es/welcome.shtml>), a number of  
6 large industries, one of the most important commercial and tourist ports of the Mediterranean coast,  
7 and a large airport. Liubartseva et al. (2018) identify Barcelona as the second city of the  
8 Mediterranean Sea in terms of estimated inputs of plastic marine debris, with a total contribution of  
9 1,800 tons per year. Dominant marine currents along the Catalan coast follow a pattern from north  
10 to south parallel to the coast. They originate from the 30-km wide mesoscale Northern Current,  
11 which flows cyclonically along the continental slope from the Gulf of Genova to the southern Gulf  
12 of Valencia (Font et al., 1995). Indeed, urbanization has been reported to have a major influence on  
13 microplastic ingestion by fish (Peters and Bratton, 2016), and locations where currents converge  
14 accumulate marine litter and therefore marine biota more frequently ingest microplastics (Moore et  
15 al., 2001). Due to all these factors, bogues sampled in the marine area off Barcelona are exposed to  
16 higher microplastic concentrations than those occurring in other areas along the Catalan coast.

17  
18 The amounts of microplastics found in the GI tracts of the bogues sampled in the area off Blanes  
19 and in the Cap de Creus MPA were similar, and the average frequency of occurrence in both areas  
20 was consistent with the value of 37.5% found by Rios-Fuster et al. (2019) in *B. boops* from  
21 southern Spain. The same authors reported similar values of microplastic occurrence ( $\approx 30\%$ ) also  
22 in samples of *Sardina pilchardus* from Blanes and *Trachurus mediterraneus* and *Engraulis*  
23 *encrasicolos* from Cap de Creus MPA. Although lower abundance and frequency of occurrence  
24 might be expected in the marine protected area, consistently with our results, Nadal et al. (2016)  
25 also found high frequencies of microplastics occurrence in bogues sampled from Espardell, an  
26 island inside the MPA Ses Salines (Eivissa, Spain). These discrepancies indicate that microplastic  
27 presence in the sea must be interpreted from a wider perspective, evaluating levels of  
28 industrialization and urbanization in the proximity, but also the influence of seasonal currents, river  
29 discharges, wastewater treatments, rainfall, and tourism fluxes. The Cap de Creus MPA is very  
30 popular among international tourists due to its high natural and cultural values, and despite its high  
31 level of protection and preservation, high amounts of litter are generated on the land that may  
32 accidentally enter the sea. Furthermore, the dominant pattern of winds and currents may also  
33 generate local areas of microplastic accumulation during certain periods of the year.

34  
35 Results obtained from the best-fit model showed that bogues ingest higher rates of microplastics  
36 closer to the coastline. This result is consistent with those obtained by Rios-Fuster et al. (2019), and  
37 confirms the hypothesis that the greatest overlap between microplastics and marine fauna occurs in  
38 coastal waters (Clark et al., 2016), as higher concentrations of litter are often found in proximity of  
39 densely populated urban centres, touristic areas and shipping routes (Suaria et al., 2014).

40  
41 The abundance of ingested microplastics was inversely correlated with body length and weight in  
42 the bogues from Barcelona and Blanes but not in those from Cap de Creus MPA. Although similar  
43 studies show no effect of body length on microplastic ingestion occurrence in other fish species  
44 (*e.g.*, Foekema et al., 2013, Digka et al., 2018), some authors suggest that larger individuals are less  
45 likely to ingest microplastics (*e.g.*, Compa et al., 2018; Bessa et al., 2018), which may explain the  
46 higher abundance of microplastics in the GIs of the smaller individuals from Barcelona and Blanes.  
47 However, explanations for the discrepancy of the relationship between microplastics and body  
48 length between areas remain unknown, and it should be highlighted that the bogues from Cap de  
49 Creus were, on average, larger in size and weight, which likely had an effect on that relationship  
50 (Fig 2). In addition, no correlation with the Fulton's condition factor (K) was found in the bogues  
51 sampled for this study, despite Compa et al. (2018) reported that individuals of *S. pilchardus* with  
52 lower condition factor ingested more microplastics than those in individuals in better conditions.

1 Although Compa et al. (2018) did not find any difference in the abundance of ingested  
2 microplastics between mature and immature individuals, microplastic ingestion rates could be also  
3 related with the fish developmental stages, as mature and immature individuals often show  
4 behavioural and feeding habits dissimilarities.  
5

#### 6 4.2. Microplastic characterization

7 Microplastics ingested by *B. boops* from the Catalan coast were primarily fragments (~60%) and  
8 secondly fibres (~40%) (Fig 3A). Fragments are the result of the degradation of larger plastic items,  
9 while fibres are the most abundant component of primary microplastics in seas and oceans  
10 worldwide (Bessa et al., 2018). Our results revealed, proportionally, a smaller contribution of  
11 fragments and a larger contribution of fibres than those detected in fish of the Northern Ionian Sea  
12 by Digka et al. (2018), who reported approximately 80% fragments and 20% fibres, respectively,  
13 showing a similar order of prevalence. Conversely, other studies (e.g., Lusher et al., 2013; Bellas et  
14 al., 2016; Güven et al., 2017; Compa et al., 2018; Bessa et al., 2018) found a higher percentage of  
15 fibres than fragments in fish GIs. These contrasting results may be related to different sources and  
16 waste management strategies in the sampling areas, which could prevent or reduce the amounts of  
17 plastic items that reach the sea from land, brought by rivers or wind (Digka et al., 2018; Boucher  
18 and Friot, 2017).  
19

20 In the present study, microplastics were classified into 4 size categories according to their largest  
21 dimensions. The main microplastic size class was 0.1 - 0.5 mm (Fig 3B), supporting the role of  
22 indirect intake from microplastics ingested by prey (i.e., zooplankton) as an important mechanism  
23 of microplastic ingestion in fish (Avio et al., 2017; Neves et al., 2015). However, future research is  
24 needed to improve knowledge regarding the mechanisms of microplastic ingestion by boggles  
25 (Nadal et al., 2016). In addition, Digka et al. (2018) also found that microplastics between 0.1 - 0.5  
26 mm were the most prevalent in mussels and fish from the Adriatic Sea. However, microplastics <  
27 0.1 mm may have been underestimated due to the reduced recovery rates for smaller particles (Avio  
28 et al., 2015).  
29

30 The predominant colour of the microplastics ingested by boggles was blue (Fig 3C), a result  
31 consistent with other studies (e.g., Romeo et al., 2015; Güven et al., 2017; Peters et al., 2017;  
32 Compa et al., 2018; Digka et al. 2018). The prevalence of this colour may suggest that fish ingest  
33 microplastics regardless of their colour, as blue microplastics are not distinctively visible to fish  
34 (Peters and Bratton, 2016).  
35

36 Finally, the most common polymer types detected in the litter ingested by *B. boops* were  
37 polypropylene, polyethylene and polystyrene. These results were expected because these three  
38 polymers are present in most plastic litter found in the water column worldwide (Suaria et al., 2016;  
39 Cózar et al., 2017). Polyethylene is used to manufacture plastic bags and bottles (Suaria et al., 2016;  
40 Cózar et al., 2017), which makes it the most abundant plastic in the world; polypropylene is highly  
41 abundant in bottle caps and packages (Suaria et al., 2016); and polystyrene is used widely for  
42 fishing boxes and other common containers. Consistently with our findings, polypropylene and  
43 polyethylene were also predominant in other studies of microplastic ingestion in fish from the  
44 Mediterranean Sea (Avio et al., 2017; Digka et al., 2018) and other European seas (Collard et al.,  
45 2017).  
46

#### 47 4.3. The use of bioindicators for marine litter monitoring in the international legislative framework

48 New international and EU directives are focusing on the reduction of waste and on the  
49 implementation of monitoring programs to assess the extent of marine litter pollution and its  
50 impacts in order to plan adequate mitigation measures. Among others, the Waste Directive  
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55



1 (amending 2008/98/EC), the Packaging Directive (94/62/EC), the Plastic Carrier Bags Directive  
2 (2015/720/UE amending 94/62/EC), the Single Use Plastic Directive (2018/0172/EC) and the  
3 Directive on Port reception facilities for the delivery of waste from ships (directive COM(2018) 33)  
4 are addressing these issues. In addition, the UNEP/MAP Regional Plan for Marine litter  
5 Management in the Mediterranean (UNEP/MAP IG.21/9) highlights the urgent need to act against  
6 marine litter. From the UN Environment Integrated Monitoring and Assessment Programme of the  
7 Mediterranean Sea and Coast and Related Assessment Criteria (IMAP), adopted in 2016, the use of  
8 bioindicator species for marine litter monitoring is clearly recommended by the Candidate Indicator  
9 24: *Trends in the amount of litter ingested by or entangling marine organisms, focusing on selected*  
10 *mammals, marine birds, and marine turtles*, under Ecological Objective 10 (EO10). Moreover, the  
11 UNEP/MAP (Galgani, 2017) reported recently that bioindicator species are highly needed to  
12 monitor microplastics and marine litter in general. To comply with legal requirements and the  
13 urgent need to address the issues posed by marine litter, several studies focusing on microplastic  
14 ingestion are investigating suitable bioindicator species (Bray et al., 2019; Fossi et al., 2018). In this  
15 framework, furthermore, MSFD (Commission Decision 2017/848) aims to achieve the Good  
16 Environmental Status, and it will be possible when we achieve the D10 criteria, which states:  
17 *Properties and quantities of marine litter do not cause harm to the coastal and marine environment.*  
18 Results from the present article provide a further support for the adoption of *B. boops* as a  
19 bioindicator species for marine litter (*i.e.* the ever-increasing microplastics) monitoring.

## 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65

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.

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## 5144 **Figures and Tables**

5145  
5146 Table 1. Biological parameters, frequency of occurrence and abundance of ingested microplastics (MP) in *B. boops*  
5147 from the three sampling areas.

56 Area	Barcelona	Blanes	Cap de Creus MPA
57 Number of individuals examined	34	34	34
58 Mean fish length (cm)	19.41 ± 2.81	19.86 ± 1.11	23.97 ± 3.93
59 Mean fish weight (g)	74.43 ± 28.69	103.92 ± 18.05	178.10 ± 111.65

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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 <sup>a</sup>	0.50 ± 0.14 <sup>b</sup>	0.53 ± 0.14 <sup>c</sup>
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

<sup>a, b, c</sup> 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 corrected (AICc) 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
<b>M19</b>	<b>Level of anthropogenic impacts + Coast</b>	<b>243</b>
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

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

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

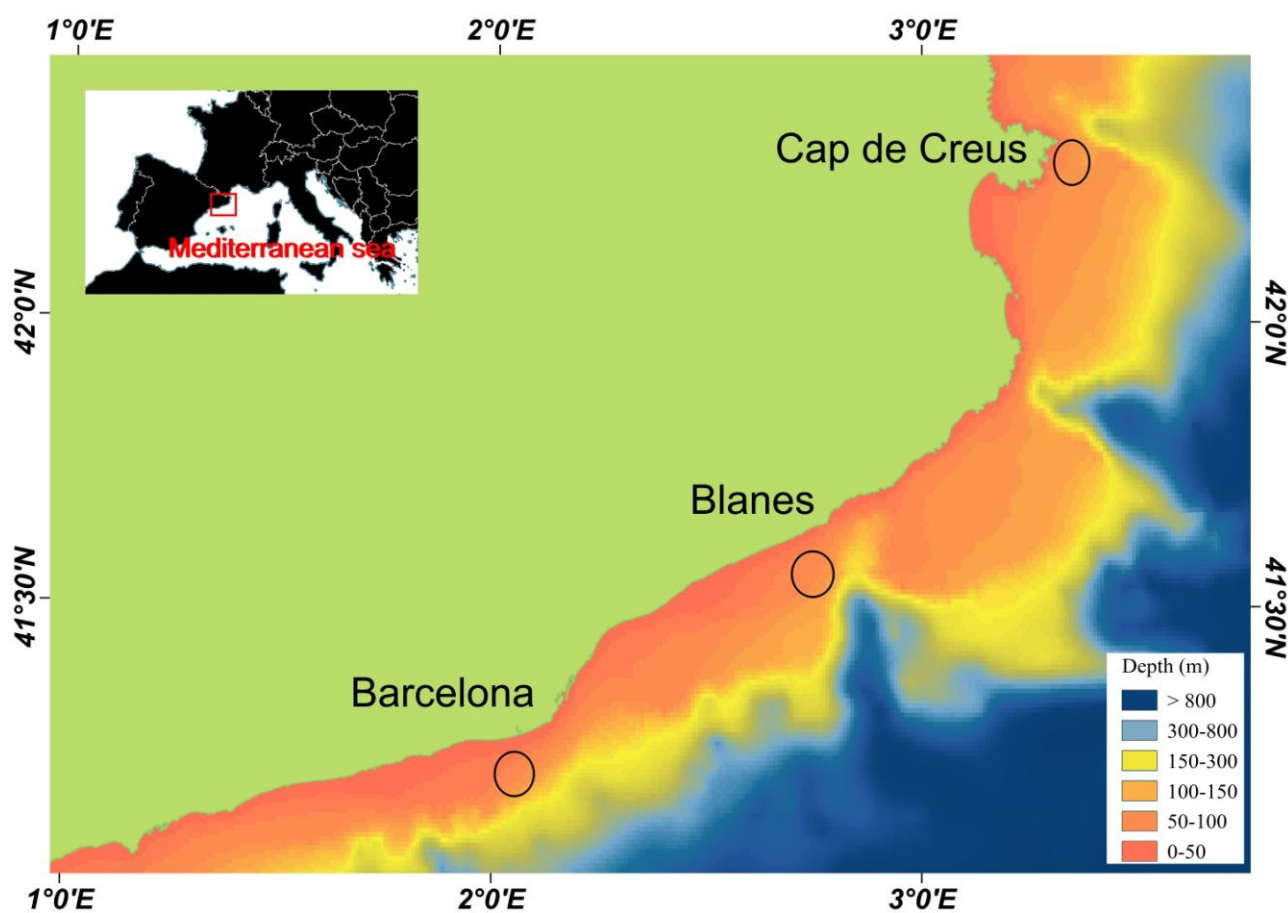


Figure 1. Study area showing the three sampling areas: Barcelona, Blanes and Cap de Creus MPA.

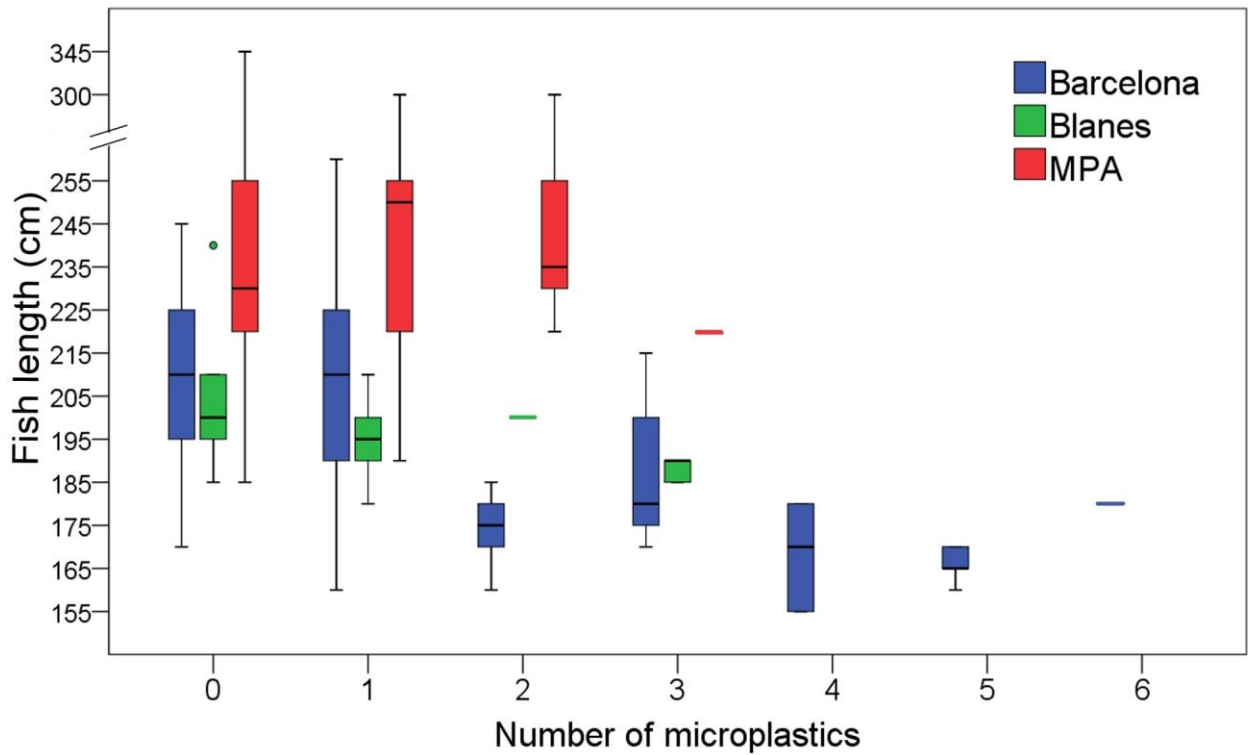


Figure 2. Box plot showing the relationship between the bogues body length and the number of microplastics ingested. The central line indicates the median fish length for each area and number of microplastics; the edges of the box indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers extend to extreme data points not considered outliers, and outliers are plotted individually as circles.

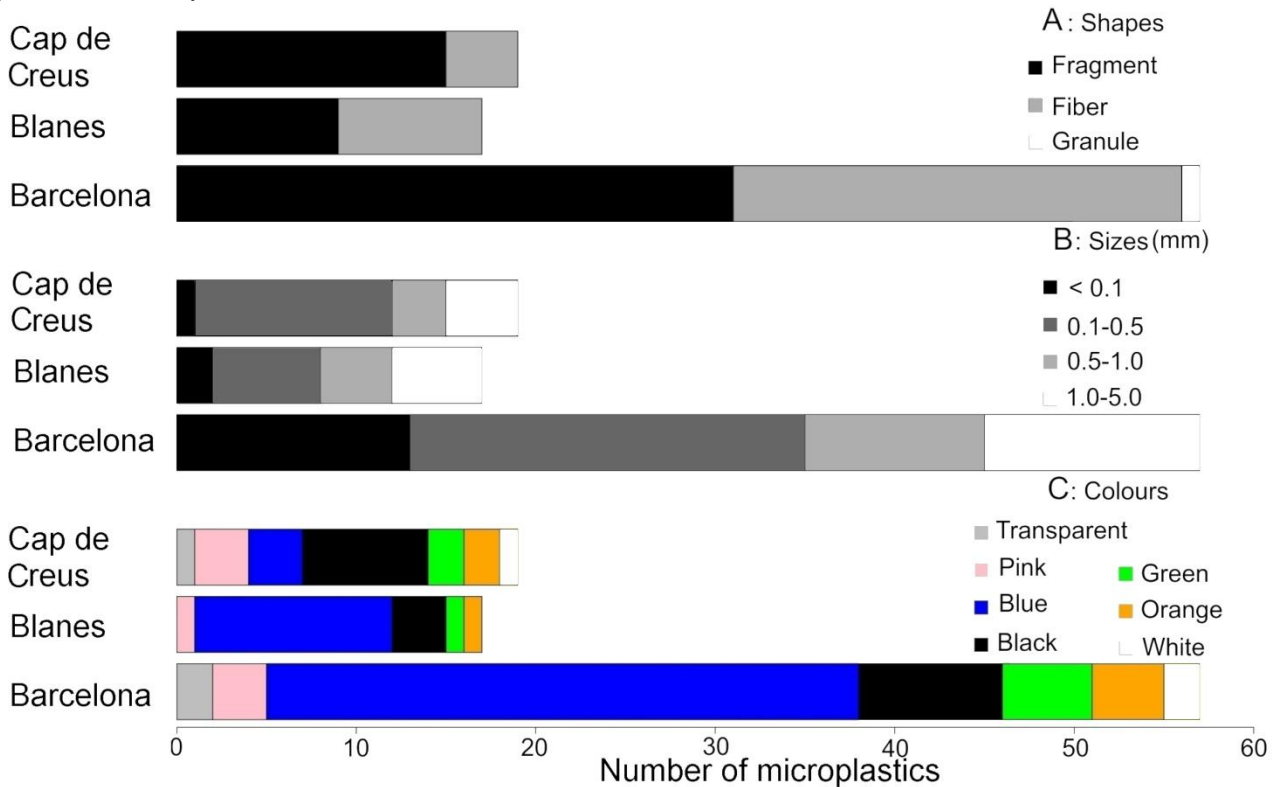


Figure 3. Shape (A), size (B) and colour (C) of microplastics detected in *B. boops* from the three sampling areas.

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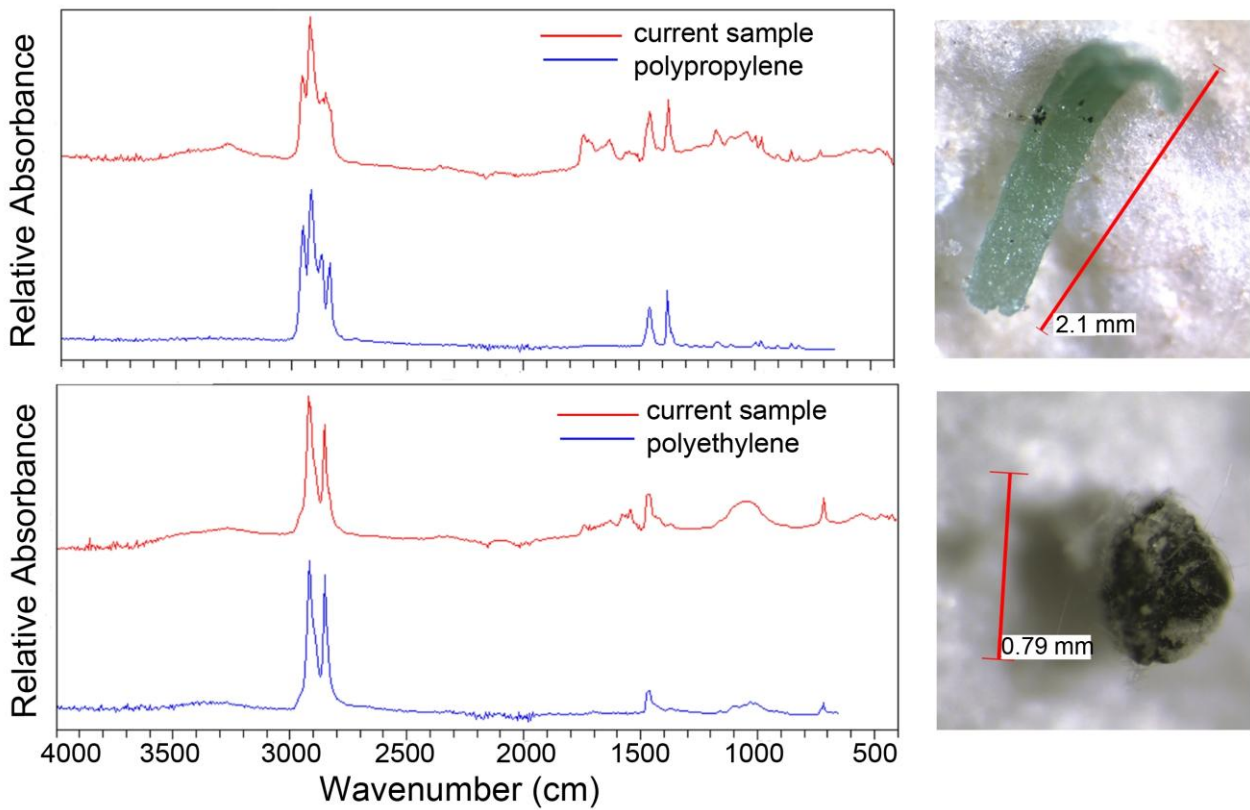


Figure 4. Examples of microplastics found in fish gastrointestinal tract with relative Fourier-transform infrared spectroscopy spectra (level of certainty of 82 and 95% for the first and second microplastic item spectra, respectively).

### Annex

Table S1: Fishing methods, spatial parameters of the fishing location, biometric parameters, and ingested microplastics for the 102 *B. boops* sampled.

Area	Fishing method	Distance to the coastline (m)	Depth (m)	Biometric parameters			Ingested microplastics	
				Total fish length (mm)	Total fish weight (g)	GIWW (g)	Occurrence (0/1)	Number of MP
Barcelona	Trawling	7.0	90	170	43.4	2.2	1	5
Barcelona	Trawling	7.0	90	195	75.9	4.9	0	0
Barcelona	Trawling	7.0	90	185	65.3	4.0	0	0
Barcelona	Trawling	7.0	90	175	63.4	4.1	1	2
Barcelona	Trawling	7.0	90	185	61.3	4.5	1	2
Barcelona	Trawling	7.0	90	160	43.9	2.0	1	1
Barcelona	Trawling	7.0	90	175	59.2	2.8	1	2
Barcelona	Trawling	7.0	90	170	45.8	2.9	0	0
Barcelona	Trawling	7.0	90	180	59.1	4.1	1	4
Barcelona	Trawling	7.0	90	165	50.4	2.4	1	5
Barcelona	Trawling	7.0	90	160	47.9	2.8	1	5
Barcelona	Trawling	7.0	90	170	48.9	2.6	1	3
Barcelona	Trawling	7.0	90	155	39.3	1.9	1	4
Barcelona	Purse seine	9.5	25	160	47.9	2.4	1	2





1	Blanes	Purse seine	6.5	25	190	97.4	7.4	1	3
2	Blanes	Purse seine	6.5	25	205	109.1	8.5	0	0
3	Blanes	Purse seine	6.5	25	210	130.3	11.4	0	0
4	Blanes	Purse seine	6.5	25	185	87.1	6.9	1	1
5	Blanes	Purse seine	6.5	25	185	85.8	5.0	1	3
6	Blanes	Purse seine	6.5	25	200	115.5	7.0	0	0
7	Blanes	Purse seine	6.5	25	190	102.9	8.1	1	1
8	MPA	Trammel nets	3.0	20	220	128.0	9.3	1	1
9	MPA	Trammel nets	3.0	20	240	162.9	10.0	0	0
10	MPA	Trammel nets	3.0	20	220	124.7	6.5	1	2
11	MPA	Trammel nets	3.0	20	340	520.0	22.2	0	0
12	MPA	Trammel nets	3.0	20	300	411.7	15.0	1	1
13	MPA	Trammel nets	3.0	50	280	327.0	8.9	0	0
14	MPA	Trammel nets	3.0	50	345	474.6	12.4	0	0
15	MPA	Trammel nets	3.0	50	300	336.2	15.4	1	2
16	MPA	Trammel nets	3.0	50	270	263.9	15.0	0	0
17	MPA	Trammel nets	3.0	50	250	203.7	16.1	1	1
18	MPA	Trammel nets	3.0	50	220	146.6	8.5	1	3
19	MPA	Trammel nets	3.0	50	190	98.7	4.8	1	1
20	MPA	Trawling	5.0	90	230	137.2	11.6	0	0
21	MPA	Trawling	5.0	90	230	141.5	9.2	0	0
22	MPA	Trawling	5.0	90	220	166.5	11.4	0	0
23	MPA	Trawling	5.0	90	225	125.7	9.1	0	0
24	MPA	Trawling	5.0	90	235	127.2	10.2	1	2
25	MPA	Trawling	5.0	90	220	99.4	8.7	0	0
26	MPA	Trawling	5.0	90	215	105.2	8.6	0	0
27	MPA	Trawling	5.0	90	195	86.0	4.9	1	1
28	MPA	Trawling	5.0	90	185	61.8	8.5	0	0
29	MPA	Trawling	5.0	90	195	72.3	8.2	0	0
30	MPA	Trawling	5.0	90	185	70.6	5.8	0	0
31	MPA	Trawling	5.0	90	190	78.7	8.6	0	0
32	MPA	Trawling	5.0	90	245	126.9	7.4	0	0
33	MPA	Trawling	5.0	90	245	141.6	5.6	0	0
34	MPA	Trawling	5.0	90	220	126.3	5.3	0	0
35	MPA	Trawling	5.0	90	225	143.0	11.0	1	1
36	MPA	Trawling	5.0	90	240	152.4	8.5	0	0
37	MPA	Trawling	5.0	90	260	191.9	12.2	0	0
38	MPA	Trawling	5.0	90	255	192.0	10.0	0	0
39	MPA	Trawling	5.0	90	255	175.2	7.7	1	1
40	MPA	Trawling	5.0	90	255	159.2	8.0	1	1
41	MPA	Trawling	5.0	90	250	176.9	8.6	1	1

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