VARIATION IN MICROPLASTICS CONTENT DETECTED IN COMMERCIALLY IMPORTANT MARINE SPECIES OF THE EASTERN AEGEAN SEA.

E. FARAGGIANA[1], G. PIETROLUONGO[1], B. QUINTANA[1], K.G. DEXTER[2], H. MOORE[3] AND A. MILOU[1]

[1] *Archipelagos Institute of Marine Conservation, P.O. Box 42 Pythagorio 83 103 Samos (Greece)*

[2] *The University of Edinburgh, Alexander Crum Brown Road, Edinburgh EH9 3FF (UK)*

[3] *Nottingham Trent University, 50 Shakespeare St, Nottingham NG1 4FQ (UK)*

SUMMARY: The increasing abundance of marine plastic debris is a global threat to biodiversity and it represents a concerning issue in areas such as the Mediterranean Sea (Deudero and Alomar, 2015). This study aims to provide an evaluation of microplastics distribution throughout the trophic chain, by analysing a range of edible marine species inhabiting different water columns levels across the Eastern coast of Samos Island, in the Eastern Aegean Sea (Greece). The gastrointestinal tract of 27 specimens were analysed; including four commercial fish species (*Sarda sarda*, *Sphyraena viridensis*, *Boops boops*, *Diplodus annularis*) and two edible invertebrate species (*Paracentrotus lividus*, *Ostrea edulis*). All individuals showed signs of plastic contamination, with a total of 1302 microplastic items recorded and identified between all 27 examined samples. A significant variation in microplastic abundance across four different habitats (water column levels) was recorded, with semi-pelagic, omnivorous fish species reporting a consistently higher number of microplastic items than both demersal fish and benthic invertebrate species (ANOVA: $F(3,22) = 4.970$, $p = 0.010$). Plastic fibres were ubiquitous in all samples analysed and represented the most abundant typology of plastics detected. The results of this study prove the undeniable persistence of microplastics across the water column and throughout the marine trophic chain. It is not only the serious implications on all marine life that requires prompt safety actions to be taken, but also the potential effects on the health of seafood consumers that represents a crucial priority for future investigations.

1. INTRODUCTION

Plastic pollution is widespread throughout the marine environment, with estimates reporting 5.25 trillion particles of plastics floating in the oceans in 2014 (Eriksen *et al*., 2014). Marine plastic debris represents a global threat to biodiversity and it might seriously affect the ecosystems functions and services of areas such as the Mediterranean Sea, defined as one of the most polluted seas worldwide (Deudero and Alomar, 2015). Yet, detailed documentations on the distribution and extent of plastic pollution in the Mediterranean basin and its effects on marine life are currently lacking. A wide range of marine organisms is negatively affected by plastic entanglement and ingestion, including marine mammals, sea turtles, seabirds, fish and invertebrates. Microplastics, in particular, are small plastic detritus (of size < 0.5 mm) that are widely ingested by all marine biota, including fish, mussels, worms, seabirds and zooplankton (Cole *et al*., 2013). Investigating the microplastics content in organisms inhabiting different levels of the water column represents a priority for current research aiming to explore the transfer of microplastics throughout the marine trophic chain. Despite plastic debris has already been found in some Mediterranean fish species (Anastasopoulou *et al.*, 2013), it is crucial to conduct additional research on commercially important marine species, in order to examine the possible consequences and impacts on the health of seafood consumers.

This study aims to investigate how the abundance and distribution of microplastics varies across marine species living in different habitat types and with different feeding strategies. Both fish and invertebrates have been analysed, all being native, edible species, including highly commercial fish consumed locally on Samos Island and in the surrounding areas. The species selected for this study include epipelagic predator fish species, feeding on smaller fish, invertebrates, cephalopods; epipelagic and demersal omnivorous fish species feeding both on animal preys and algae (IUCN Red List, 2014); as well as benthic, invertebrate species living on the sea bottom and feeding on algae, seagrass and phytoplankton (Jonsson *et al*. 1999).

The objective of this research project is therefore to assess and quantify the microplastics content in commercially important, native marine species of the Aegean Sea. In specific, the study addresses the following research questions: 1) How does microplastics abundance and distribution vary across species and between marine organisms living in different habitats and with different feeding strategies? It is expected that species inhabiting all level of the water column show some extent of microplastic contamination, since transfer through the trophic chain has already been documented (Wang et al., 2016); 2) Is there any correlation between microplastics abundance and the body weight, body length or the age of individuals analysed? A logical assumption would be that larger, predator species are more contaminated with microplastics since they rely on the consumption of preys belonging to lower trophic levels, which might have already ingested plastics (Miliou *et al*., 2016); 3) Do organisms living in different habitats and with different feeding strategies show specific preferences for different microplastics colours, size ranges, and typology? A significant difference in the distribution of different microplastics types is expected since previous studies document a prevalence of plastic fibres (among other categories of plastics) in native species of the Aegean Sea (Miliou *et al*., 2016).

2. METHODS

2.1. Sample species and study site

Twenty-seven individuals including fish and invertebrate species were caught from the Eastern waters of Samos Island (Greece) throughout the months of January and February 2018. Most fish samples have been acquired from the local fish market in Pyhtagoreio, and only a few individuals were obtained from Vathy's market. The two invertebrate species used in this research study (*Paracentrotus lividus* and *Ostrea edulis*) were caught in the shallow waters facing Archipelagos Institute of Marine Conservation, in Mesokampos. The sites from which all samples have been collected are shown on the map of Samos Island, created with ArcGIS v. 10.6 (Figure 1). These locations can be considered the habitat in which fish were originally caught since local fishing activities mostly occur in the surrounding waters of the aforementioned harbours (Pyhtagoreio and Vathy). The fish samples analysed in this study were therefore all native, edible species inhabiting different levels of the water column: two epipelagic, predator species *(Sarda sarda*, *Sphyraena* *viridensis*); a semipelagic, omnivorous species (*Boops boops*); and a demersal species (*Diplodus annularis)*. The invertebrates collected were also native, edible, locally caught species, inhabiting the benthic zone of the water column. Those were the European flat oyster (*Ostrea edulis*), a filter feeder; and the purple sea urchin (*Paracentrotus lividus*), a browser mostly feeding on seagrass. This sampling methodology allowed to obtain a range of samples from different levels of the water column and therefore to investigate the transfer of microplastics across the marine trophic chain.

Figure 1. Map of Samos Island, showing the locations where the samples analysed in this study were collected (Pythagoreio, Mesokampos, Vathy).

2.2. Data collection

Relevant body measurements and information were noted for each sample collected: body weight, total length (from head to tip of the tail), species name, sex, habitat range (level of water column inhabited), and feeding strategy. The approximate age of each individual was estimated using body length as a growth parameter and calculated using von Bertalanffy's inverse growth rate equation (Siegfried and Sansó, 2006). Fish were then dissected and their full gastrointestinal tract (GIT) was extracted and preserved in hypersaline solution, following the guidelines indicated in Avio *et al*. (2015). With regards to invertebrate species, the full organic content inside the shell of *P. lividus* was collected, whereas the motile organ (foot) of *O. edulis* was removed and only its gastrointestinal contents were used for later analyses. Once the GIT of each individual settled for at least five hours in 250 ml of hypersaline solution, it was ready to be filtered through filter paper (Glass Fiber Filter Frisenette, nominal pore size 1.2 μm, diameter 47 mm), with the aid of a vacuum pump (KNF LABOPORT series) attached to a glass conical flask (ENDO glassware, capacity 1000 ml). Each time, 20 ml of supernatant solution were filtered together with 20 ml of 15% H_2O_2, a chemical compound which favours the breakdown of organic material (Bissey *et al*., 2006).

This procedure was repeated ten times for each sample, in order to obtain a total of 200 ml filtered solution for each individual (Avio *et al*., 2015). Once the filter papers dried after at least 24 hours, the samples were analysed under a dissecting microscope (using x2; x4 magnification) for microplastics detection and classification. All the non-organic, suspected plastic items with size < 5 mm, were subjected to a hot needle test, as described in MERI (2015). All items identified as microplastics were then counted and classified according to the following parameters: colour (black, transparent, coloured microplastics); category (fibres, other types of microplastics); size ranges (< 0.5 mm, 0.5 – 2.5 mm, 2.5 – 5 mm). During the phase of microscope analyses, a total of 11 macro fibres (plastic items with size > 5 mm) were detected among all individuals. However, since this project focused on microplastics only, all plastic items > 5 mm have not been included in statistical analyses.

2.3. Data analysis

The raw data collected during the experiment was organised and formatted in Microsoft Excel v. 16.10. All the parameters recorded for each individual (listed in paragraph 2.2) were entered in different spread sheets and manipulated according to the type of statistical test to be carried out. Species that have been sampled only once were excluded from the analyses due to lack of statistical relevance, thus slightly reducing the total sample size. Once the data was formatted and ready to be analysed, all statistical analyses were conducted in RStudio v. 1.0.136. Each variable has been tested in order to check whether it followed a normal distribution. If the data was highly skewed, variables were transformed using a common logarithm conversion (log10). In order to explore the distribution of total microplastics across different species, different habitats (water column levels), different feeding strategies, and different organism classifications (fish or invertebrates), separate ANOVAs (analysis of variance) were conducted. When the data did not follow a normal distribution, the correspondent non-parametric test for ANOVA was performed (Kruskal-Wallis test). Secondly, potential correlations between microplastics abundance and body length, body weight and age of organisms were investigated by carrying out Pearson product-moment correlation analysis or the equivalent non-parametric version (Spearman's rank-order correlation). Ultimately, to investigate possible trends in terms of specific preferences for different microplastics colours, shapes, and size shown by organisms belonging to different species, habitats, and with different feeding strategies, an ANOVA framework was performed by carrying out multiple ANOVAs tests.

3. RESULTS

3.1. Variation in microplastics distribution across habitat ranges

The distribution of microplastics significantly differed between levels of the water column, referred to as habitat ranges (one-way ANOVA: $F(3,22) = 4.970$, $p = 0.010$). Semipelagic organisms were significantly more contaminated with microplastics than both benthic species ($p = 0.011$) and demersal species ($p = 0.042$), as reported by Tukey's HSD test (Figure 2).

The abundance of total microplastics, however, did not significantly vary between different species, nor between organisms with different feeding strategies.

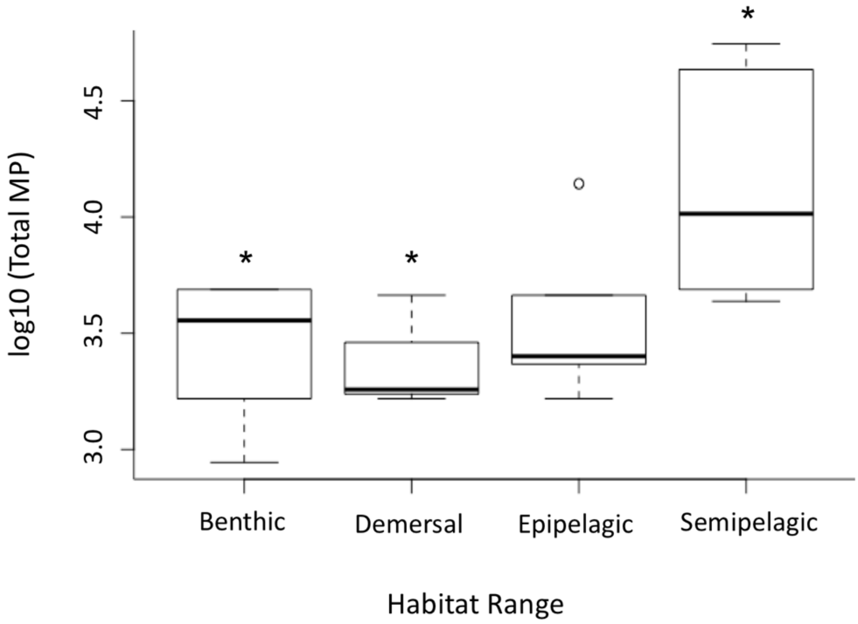

Figure 2. Differences in the amount of total microplastics (total MP) across different habitat ranges. Asterisks on boxplots indicate where the levels of significance lie. The number of total microplastics has been logged using a common logarithm conversion (log10) in order to normalise the data.

3.2. Inter-specific preferences for microplastics of different size ranges

A significant variation in the distribution of microplastics with size range 0.5 – 2.5 mm can be detected between organisms living in different habitats (one-way ANOVA: $F(3,22) = 3.324$, $p = 0.038$), individuals with different feeding strategies (one-way ANOVA: $F(3,21) = 3.295$, $p = 0.04$), and between fish and invertebrate species (one-way ANOVA: $F(3,23) = 4.374$, $p = 0.047$). Tukey's HSD test reported a higher proportion of microplastics with size range 0.5 – 2.5 mm in semipelagic organisms compared to benthic species ($p = 0.034$), and in omnivorous fish species compared to invertebrate, filter feeders organisms ($p = 0.047$), as respectively shown in Figure 3a), 3b) and 3c).

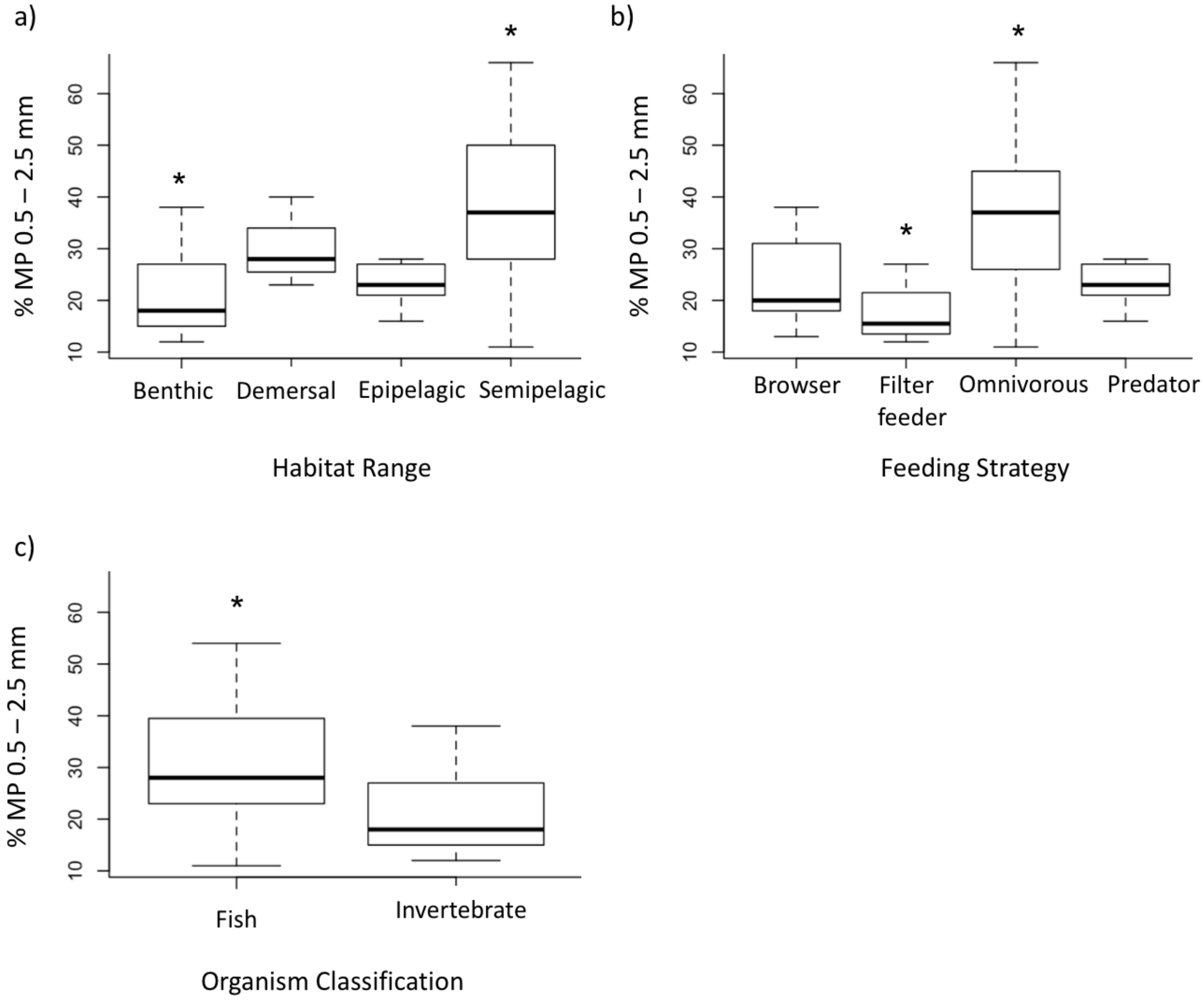

Figure 3. Distribution of microplastics with size range 0.5 – 2.5 mm detected between different categories of organisms. As consistently shown in these boxplots, between 40% and 50% of microplastics with size range 0.5 – 2.5 mm is found in semipelagic, omnivorous fish species.

3.3. Prevalence of microplastics fibres across all categories

Microplastics fibres are ubiquitous among all samples analysed and represent the most abundant typology of plastics recorded overall (Figure 4). Since fibres appear to be homogeneously distributed between all samples, no significant differences in fibres abundance have been detected between different species, habitat ranges, nor between organisms with different feeding strategies.

All correlation analyses performed to explore possible associations between microplastics abundance and the body weight, body length, and age of individuals were not statistically significant.

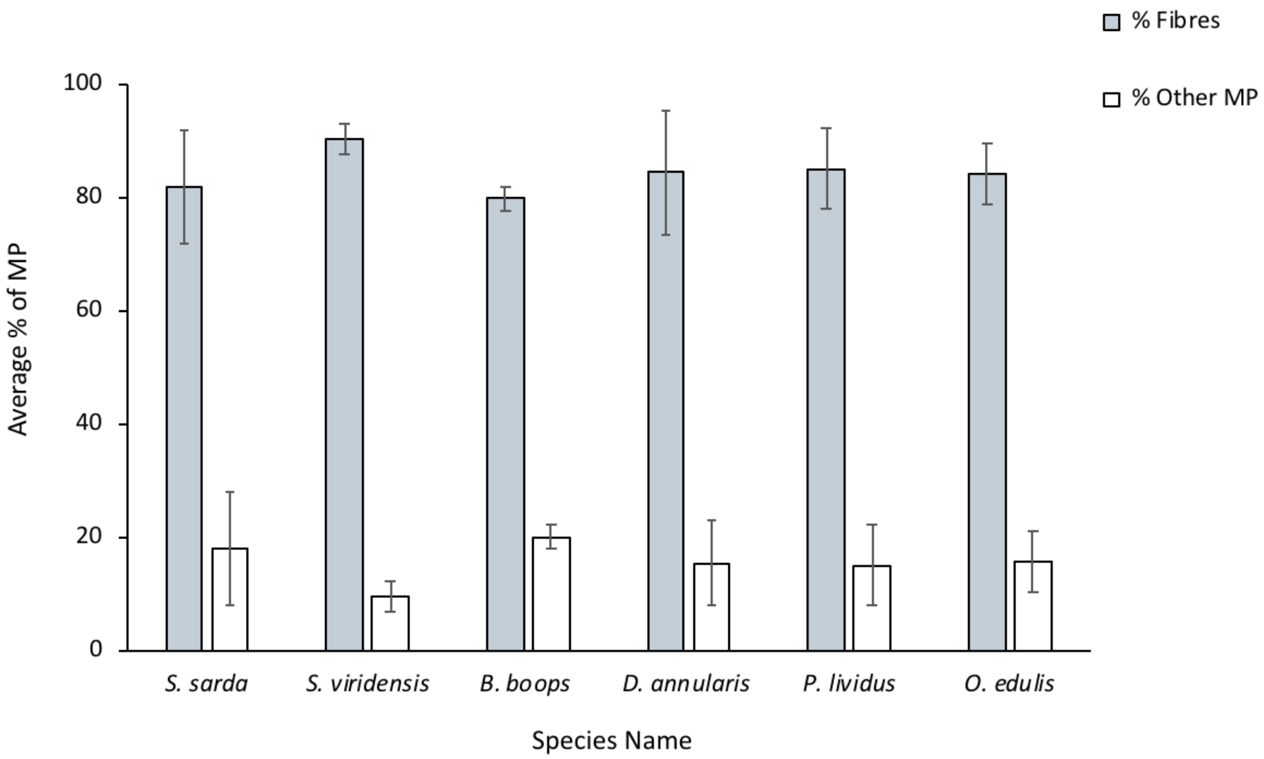

Figure 4. Between 80% and 90% of microplastics detected in all samples were plastic fibres. Other types of microplastics, such as hard fragments, pellets, and plastic sheets (indicated as % other MP) were found in minor quantities. Error bars represent the standard error of the mean calculated for the average percentage of microplastics detected in each species.

4. DISCUSSION

4.1. Persistence of microplastics across different levels of the water column

This study shows that microplastics is present across all the examined habitat ranges, confirming that there is a persistent occurrence of microplastics throughout the marine trophic chain (Farrell and Nelson, 2013). As shown in Figure 2, semipelagic species report a significantly higher concentration of microplastics than organisms inhabiting other levels of the water column. Semipelagic organisms analysed in this study include fish species such as *B. boops*, *D. annularis*, and *S. cabrilla*, all inhabiting a broad marine habitat range, living both in the open water and near the sea bottom. These are omnivorous species feeding on smaller fish, invertebrates, as well as algae and seagrass (Derbal *et al*. 2007). The implications of such feeding ecology and habitat preferences might result in higher chances of ingesting microplastics. The high frequency of microplastics occurrence in small semipelagic fish such as *B. boops* has already been detected in the Mediterranean Sea, where plastic contamination was ubiquitous among individuals inhabiting a wide range of locations (Nadal *et al*., 2016). Highly commercial species such as *B. boops* are particularly exposed to intense fishing activities and the use of fishing lines and nets might increase the chances of microplastic ingestion. Previous research showed that many microplastics components are in fact made of polyethylene and polypropylene, contained in many packaging and fishing items (Reisser *et al*., 2013).

The finding that plastic fibres are predominant amongst other typologies of microplastics (hard fragments, rubber, plastic sheet, etc.), is also in line with previous studies. Research conducted in the past years on Samos Island both on fish species and invertebrates (holothurians) reports a high concentration of microplastic fibres, both in the benthic sediments and in the open water of the Eastern

Aegean Sea (Miliou *et al*., 2016). The outlined results represent a further contribution to the hypothesis that most fibres found in the marine environment derive from sewage-discharges, as a consequence of washing clothes containing polyester and acrylic fibres (Browne *et al*., 2011).

4.2. Potential inter-specific preferences reported for different typologies of microplastics

This study also identified a significant variation in the distribution of different size ranges of microplastics between organisms. Figure 3 shows that semipelagic, omnivorous fish ingested the largest proportion of microplastics with size range 0.5 – 2.5 mm, compared to invertebrates or other fish species living in different levels of the water column. This finding partly confirms the previously outlined theory that semipelagic species might be more likely to ingest higher concentrations of microplastics. Free-swimming, semipelagic fish were already found with higher concentrations of microplastics if compared to benthic organisms feeding on the sediment surface (Setälä *et al*., 2016). However, the underlying reason of why microplastics with size range 0.5 – 2.5 mm is more abundant than microplastics with different sizes in semipelagic species is hardly supported by the existing literature. Further studies are required to determine whether any inter-specific preferences exist for the ingestion of different microplastics colour, size range and typology. For instance, small invertebrates are expected to ingest smaller microplastics particles, of 1 mm or less in size (Scherer *et al*. 2017), whereas larger predator fish might be found with a greater range of different microplastics sizes.

4.3. Limitations of the study and directions for future research

Any factor that might have affected the outcome of this research study should be considered. Firstly, the lack of significant correlations between microplastics abundance and the body size or age of individuals might be a consequence of a relatively small sample size (n = 27). According to previous studies, the number of plastics fibres would be expected to increase as a function of body weight, since larger individuals are more subject to the accumulation of toxic, polluting substances such as microplastics (Miliou *et al*., 2016). The number of samples utilised in this study, however, has also been limited due to conservation purposes and ethical reasons. The use of live specimens, such as oysters and sea urchins, has been reduced to minimum requirements since those species were not available from local fish markets and had to be caught in the open sea.

Alternative laboratory protocols might be also considered for future improvements of similar studies. For instance, microscope-based methods may under- or overestimate microplastics abundance, whereas techniques such as infrared spectroscopy allow a more precise identification (Song *et al*., 2015). Another important requirement to exclude possible risks of contamination is the complete exclusion of plastic materials, items, and equipment from the laboratory in which experiments take place.

5. CONCLUSION

This study has provided additional insights on the global issue of marine plastic pollution. Firstly, it proved that microplastics is ubiquitous and persistent throughout the marine trophic chain, since 100% of individuals analysed resulted contaminated. The fact that high rates of plastics can be found despite of spatial variation suggests multiple sources from which microplastics can generate, and its property of migrating through the environment. Secondly, it is shown that microfibres, in specific, are predominant in the marine environment. Since they mostly derive from sewage-discharges, washing clothes and fishing items, it is evident that these human activities are causing irreversible damage and more sustainable alternatives should be promptly adopted. Ultimately, this study should spur future research to investigate the unknown consequences of marine plastic pollution on human health. Plastics components such as Bisphenol A are suspect endocrine disruptors, besides being persistent and bio-accumulative in the environment (Seltenrich, 2015). It is crucial to understand whether ingestion of plastic debris by marine organisms implies toxic exposure and potential detrimental health effects on seafood consumers. It is both for the preservation of marine life and its biodiversity, and for the wellbeing of future generations, that is crucial to start changing our way of living in order to reduce the global usage of plastics.

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