Microplastic sampling in fish, crustacean, squid, and bivalve species

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March 2019

Introduction

Synthetic polymers (plastics) account for up to 80% of all marine litter (Derraik 2002). Most types of plastics do not biodegrade and therefore endure in the environment for decades, even centuries (Hopewell et al. 2009). Plastic waste floats in the oceans, is carried by ocean currents, accumulates in ocean gyres, sinks to the ocean floor and can be found on beaches where it is washed up from the ocean or disposed of directly.

80% of marine plastic debris is suggested to be land-based and finds its way via untreated wastewater, wind, rivers and directly from the beach to the oceans (Ramirez-Llodra et al. 2011). The remaining 20% of marine plastic debris originates from sea-based activities such as fisheries and shipping (UNEP 2005).

A recent study has shown that 233,500 tonnes of macro- and mesoplastic and 35,500 tonnes of microplastic debris floats in the world's oceans (Eriksen et al. 2014). Whilst macroplastic accounts for the highest amount of floating plastics by mass, microplastic (plastic particles < 5 mm) is by far more abundant in terms of plastic particle counts (Eriksen et al. 2014).

The Mediterranean Sea has been identified as an accumulation zone for marine plastic debris. Thereby, plastic debris is accumulating at a comparable scale to that in oceanic subtropical gyres (Cózar et al. 2015). Moreover, the mass of plastics afloat in the Mediterranean accounts for almost one tenth of the total mass of 269,000 tonnes of plastics floating at sea worldwide (Eriksen et al. 2014).

Due to its persistence and ubiquitous occurrence in various sizes and forms, marine plastic pollution impacts a wide range of marine invertebrate and vertebrate species (Deudero & Alomar 2015; Kühn et al. 2015). The impact of plastic on marine life is manifold and occurs throughout the food web (Fossi et al. 2018; Law 2017; Barboza et al 2019). Main impacts of plastic debris on cetaceans are entanglement, ingestion, and vector for toxic chemicals. Harmful encounters with marine debris include ingestion and entanglement incidences (Gall 2015). The uptake of microplastics can occur in a wide range of species due to their small dimensions and takes place during food uptake, through respiration across gills, or via trophic transfer through prey (Avio et al. 2015; Kühn et al. 2015).

In June 2018 a random sample of different marine vertebrate and invertebrate species from an Italian coastal area in the Ligurian Sea have been collected in order to determine their load with microplastics. This report summarizes the respective results of this analysis.

Methods

In collaboration with a local fisherman a total of 29 individuals belonging to 6 species have been collected during regular fishing activities in the coastal waters of Viareggio, Italy, in the Ligurian Sea (see Fig. 1). All species considered in this study are listed in Table 1.

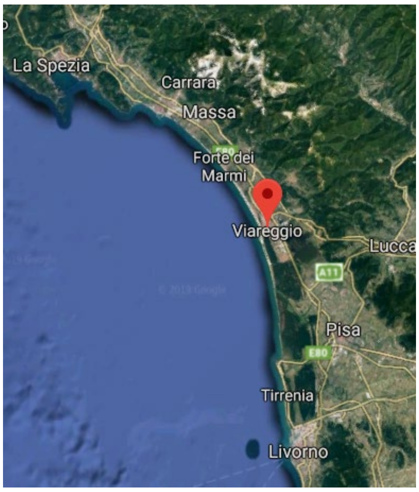

Figure 1: Localization of the sampling site (© Google Maps)

Fig. 2: Fishing boat on which samples have been taken.

The gastrointestinal tract of fishes and the invertebrate specimens have been packed up (Fig. 3), were put on ice and sent by courier to a specialized laboratory in Germany[1] for analysis.

The analysis of the gastrointestinal tract of fishes and the tissues of invertebrates was carried out according to a validated method developed by Roch and Brinker for the detection of microplastics in the gastrointestinal tract of fish (Roch & Brinker 2017).

Figure 3: Packed samples ready for dispatch to the laboratory in Germany.

[1] Fisheries Research Station Baden-Württemberg, Langenargen, Germany

Table 1: Overview of collected species and number of individuals

* *The size of the fishes was determined by the so-called fork length, those of the squid by the length of the mantle, and for crustaceans by the total body length.*

	Species *(scientific name)*	***Species*** *(common name)*	***Size of individuals*** *(in cm)**	***Number of sampled individuals***	
Vertebrate	*Sarpa salpa*	Salema	28; 26.5; 25.5; 24; 26	5	 commons.wikimedia.org
	Mugil cephalus	Flathead grey mullet	36.5; 40.5; 42.5; 44; 38	5	 commons.wikimedia.org
Invertebrate	*Sepia officinalis*	Common cuttlefish	13; 11.5; 10; 9	4	 commons.wikimedia.org
	Donax trunculus	Wedge clam	n.a.	5	 en.wikipedia.org
	Squilla mantis	Mantis shrimp	13; 14; 11; 12; 11	5	 www.uniprot.org
	Penaeus kerathurus	Striped prawn	16; 16.5; 14; 15; 13	5	 www.naturamediterraneo.com

Results

Of 29 samples sent to the laboratory, 28 turned out to be viable for analysis. The tissue of one specimen of the common cuttlefish was too small and thus excluded from the analysis.

The laboratory results show that 57% of all analysed samples (individuals) contained microplastics (see Fig. 4).

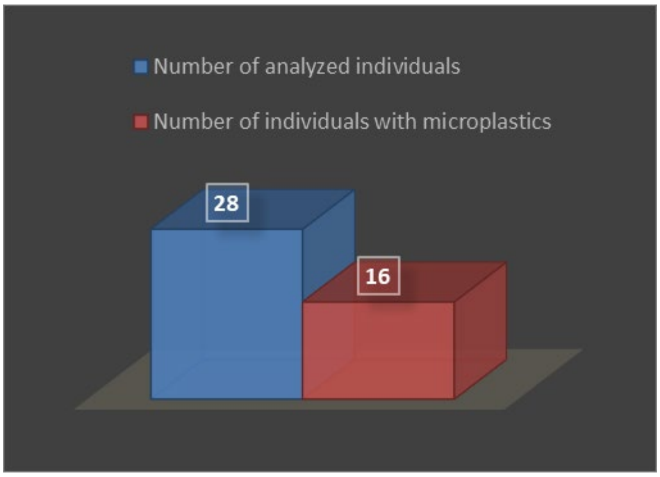

Fig. 4: Total number of individuals positive to microplastic uptake.

It is striking that more than half of the sampled individuals contained microplastics in four of the six sampled species (see Fig. 5). All studied individuals of Sarpa salpa were positive to microplastic uptake.

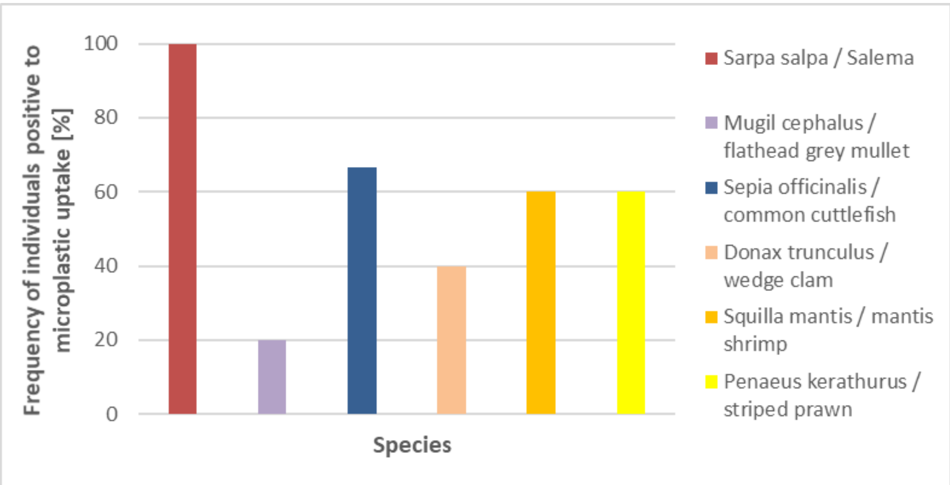

Fig. 5: Portion of individuals positive to microplastic ingestion per sampled species.

The magnitude of the microplastic load per species (= mean number of microplastic particles / species) is shown in Figure 6.

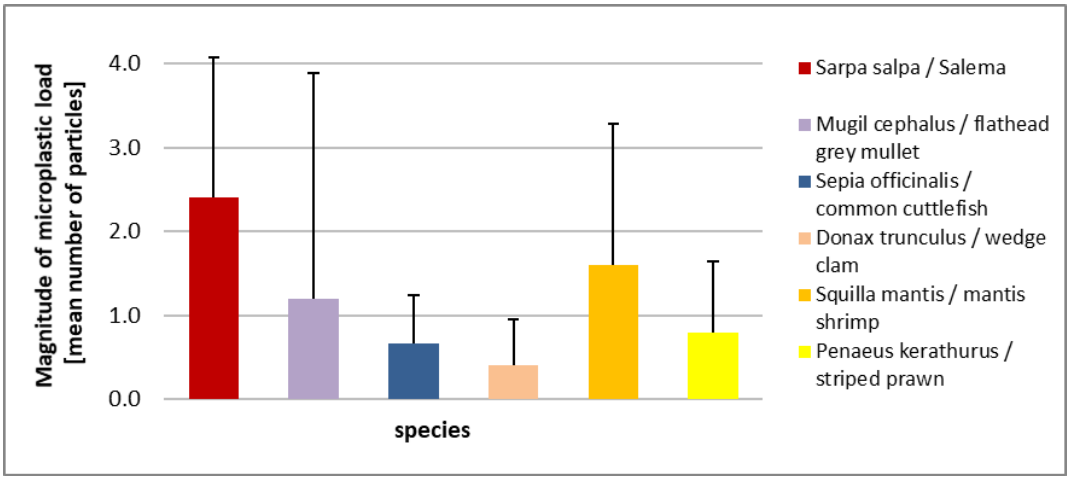

Fig. 6: Strength of the microplastic load per species (mean number of microplastic particles/species)

A total of 35 microplastic particles were found in 16 individuals. Four different microplastic categories have been identified in the samples: fragments, fibres, films, and spheres. Fragments were most abundant in the samples (see Fig. 7).

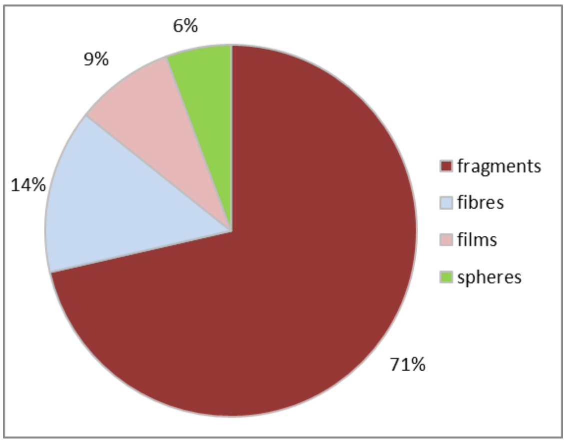

Fig. 7: Microplastic categories contained in the samples and their relative frequency %

Figures 8-13 below show some of the detected microplastic particles.

Fig. 8: Black fragment (Sarpa salpa)

Fig. 9: Green fragment (Mugil cephalus)

Fig. 10: Red fibre (Sepia officinalis)

Fig. 11: Yellow sphere (Squilla mantis)

Fig. 12: Black film (Penaeus kerathurus)

Fig. 13: Pink fragment (Donax trunculus)

Conclusive remarks

Over 50% of the studied individuals in the study area in Viareggio in the Ligurian Sea have been found to be positive to microplastic uptake. This is a remarkable higher portion as compared to the results of a study on microplastics in fish and invertebrates in Genova (Ligurian Sea), Talamone (Thyrrhenian Sea), and Naples (Thyrrhenian Sea), where the overall frequency of specimens positive to microplastic uptake was 25%, 29% and 28%, respectively (Gorbi et al. 2018) (Tab. 2). It is worthy to note that the study from Gorbi et al. considered in general more fish species and overall individuals per sampling area than included in the present study and didn't consider squids. Thus, a direct comparison with overall numbers is biased. However, both studies considered the same crustacean species with a similar number of sampled individuals as well as one same fish species (see Tab. 2). It is striking that the frequency of the microplastic uptake for Squilla mantis seems to be threefold in the study area in Viareggio as compared to Genova and Naples while in all three areas the microplastic frequencies for Penaeus kerathurus were comparably high. Table 2 shows also that all sampled Sarpa salpa in Viareggio had microplastics in their gastrointestinal tract whereas in Talamone none of the sampled individuals was positive to microplastic uptake.

Table 2: Comparison of microplastic uptake frequencies

Data from the present study in Viareggio are compared to the results from Gorbi et al. (2018) for Genova and Naples. Frequency in % indicates the portion of individuals positive to microplastic ingestion. The number in parenthesis indicates the total of individuals sampled.

	Viareggio (Ligurian Sea)	**Genova (Ligurian Sea)**	**Naples (Thyrrhenian Sea)**	**Talamone (Thyrrhenian Sea)**
All collected species in the sampling area	57 % (28)	25 % (59)	28 % (71)	29 % (24)
Squilla mantis	60 % (5)	17 % (6)	20 % (5)	not sampled
Penaeus kerathurus	60 % (5)	50 % (6)	60 % (5)	not sampled
Sarpa salpa	100 % (5)	not sampled	not sampled	0 % (4)

Although comparisons of different studies are impeded (e.g. methodological differences), the results of the present study are concerning. Microplastics were detected in all sampled species. In some species, not only the number of impacted individuals is high, but also the magnitude of the microplastic load per individual (Sarpa salpa). Even though the considered numbers of species and individuals in this study is rather small, the results indicate that microplastics in the Ligurian Sea are of ecological concern and should be further investigated.

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