Microplastics in marine biota of North Aegean Sea: Summarizing and comparing preliminary findings

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Abstract Rising scientific literature and legislation measures have highlighted the concern on plastic pollution and the issue of microplastics. In the context of this study, 3 marine species of commercial interest (bogues, Mediterranean mussels, blue crabs) were examined, concerning microplastics' presence and characteristics. The abundance of the ingested items ranged between 1.22 and 3.30 items/individual. In total, 172 items were extracted from the samples, while only three items were categorised as mesoplastics. The most frequent size for all three species was particles smaller than 200 μm, while fragment was the most frequent type of microplastic (58.48%). In total, 12 colours were recorded, with blue being the most frequent one. Within the sampling areas, significant differences were determined ($p < 0.05$, Kruskal-Wallis test). Specifically, samples collected at Lesvos were significantly different from those collected at Thermaikos Gulf ($p < 0.05$, Dunn's Multiple Comparison test), while no difference existed among the samples of the different species originating from Thermaikos Gulf. This study contributes to fill the gap of knowledge regarding microplastic contamination of the North Aegean marine biota. In addition, this effort records for the first time the presence of microplastics in blue crabs at the North Aegean region.

Keywords: microplastics, Aegean Sea, marine biota

1. Introduction

Microplastics (MPs), *i.e.* plastic particles with size smaller than 5 mm, most commonly result by the fragmentation of largest plastic pieces and/or are produced in that size for industrial and commercial uses (such as for the cosmetics, textiles etc) (Crawford & Quinn, 2017). The combination of last decades increasing plastics production and use through anthropogenic activities and the failure of sufficient waste management application, have caused the ubiquitous presence of plastics and microplastics in marine environments (Alimi et al., 2018; UNEP, 2016). In addition, the effect of wind, waves, currents and solar radiation contribute to their properties' degradation and wide spreading through various marine habitats (Andrady et al., 2015).

Microplastics ingestion by marine biota has been repeatably described in scientific literature, as well its possible negative impact on organisms, such as the alteration on their energy efficiency, the disruption of their endocrine system (e.g., Lusher et al., 2017; Rochman et al., 2015; Oliveira et al., 2013).

Although microplastics have also been a topic of discussion in seafood consumption and its possible negative impact on human health, no specific analytical procedures have been validated concerning their identification and quantification (EFSA CONTAM Panel, 2016).

In recent years, legislation and measures taken on national and European level, as well as international agreements have been focusing on the mitigation of plastic pollution. Indicatively, Marine Strategy Framework Directive (Directive 2008/56/EC, MSFD) include the Descriptor 10 that is devoted to marine litter, while the criterion D10C3 depicts the urgency of highlighting micro-litter ingestion by marine organisms and maintaining it at a level that does not negatively impact them (Commission Decision EU 2017/848).

The focus of the present study has been reporting of microplastics' ingestion and characteristics on three marine species of commercial interest of North Aegean, in line with related guidelines and protocols.

2. Materials and methods

Specimen of three marine species were analysed in the context of a comparison study, *Boops boops* (Linnaeus, 1758, bogue), *Mytilus galloprovincialis* (Lamarck, 1819, Mediterranean mussel) and *Callinectes sapidus* (Rathbun, 1896, blue crab). Bogue samples were bought from the marketplace of Mytilene in February 2019, mussels were provided from a mariculture unit operating in Thermaikos Gulf and blue crabs were caught in Thermaikos Gulf and were supplied by a local fisher, both in August 2019. Samples stored at −20 °C until further processing.

In the laboratory, appropriate morphometric measurements related to each species, such as total body length, Standard Length (SL), Shell Length (ShL), total weight (W), Carapace Width (CW), were performed. Each specimen was dissected and the gastrointestinal track of bogues, the soft tissue of mussels and the stomach of blue crabs were extracted-and weighted. To degrade organic matter and enable detection of microplastic particles, the above-mentioned tissues of each individual were treated with hydrogen peroxide (30% H_2O_2, 1:20 w/v) and heated at 55-65ºC on hot plate up to the point the tissue degraded. After digestion, the remaining material was diluted with 100 mL of ultra clean water (Ultra-clear™ TP, TWF device, EVOQUA®) and filtered on fiberglass filters (Whatman® GF/C pore size 1.2 μm). Each filter was visually inspected under stereomicroscope Nikon SMZ800 and the potential microplastic particles were photographed with the attached Tucsen True Chrome HD camera and measured with ISCapture program. Maximum length, area, shape and colour of the items found were recorded.

The recorded data were organized into excel sheets and categorized to different types (such as fiber, fragment etc) according to the guidelines of Hanke et al. (2013). The statistical analyses implemented in R version 3.6.3, 2020, The R Foundation for Statistical Computing Platform.

To prevent airborne transferred microplastics onto the samples, all the necessary precautions were applied. Specifically, white coats and nitrile gloves were worn at every step and the necessary laboratory utensils used were made of glass (such as petri dishes, vials etc). Further, the dissection of the specimens and the rinsing and filtration of the samples took place inside a Captair pyramid glove bag (Erlab®). Moreover, blank samples (i.e., glass petri dish containing a clean GF/C filter) were present throughout the different steps of the procedure in order to detect potential air contamination.

Results

A grand total of 93 specimens were examined for this study, 27 *Boops boops* (average SL: 16.7 ± 1.3 SD cm), 36 *Mytilus galloprovincialis* (average ShL): 6.20 ± 0.45 SD cm) and 30 *Callinectes sapidus* (average CW: 10.6 ± 0.9 SD cm) (Table 1).

Table 1. Summary results of the three examined species.

	Bogues	Mussels	Blue crabs
No of samples	27	36	30
Average Standard Length (cm)	16.7 ± 1.3 SD	-	-
Average Shell Length (cm)	-	6.20 ± 0.45 SD	-
Average Carapace Width (cm)	-	-	10.6 ± 0.9 SD
Weight (g)	85.4 ± 30.8 SD	14.11 ± 4.2 SD	60 ± 8.4 SD
Ind. with MPs	74.07%	75%	96.67%
Total found items	84	44	44
Fiber	33	14	20
Fragment	51	29	20
Film	0	1	3

In total 172 items were found in the analysed tissues of the three species. The maximum number of ingested items was found in bogues (84 items), followed by blue crabs (44 items) and mussels (44 items). Concerning the shape type of found items, the majority in total were fragments (58.48%), followed by fibers (39.18%) and films (2.34%). Within species, the most abundant shape type were fragments in bogues and blue crabs, while fragments and fibers had the same occurrence in mussels. Films were detected only in mussels and blue crabs (Table 1).

The average number of microplastics per examined specimen was higher for bogues recording 3.30 ± 2.74 SD items/individual, followed by mussels (1.22 ± 1.17 SD items/individual) and blue crabs (1.48 ± 1.21 SD items/individual) (Figure 1).

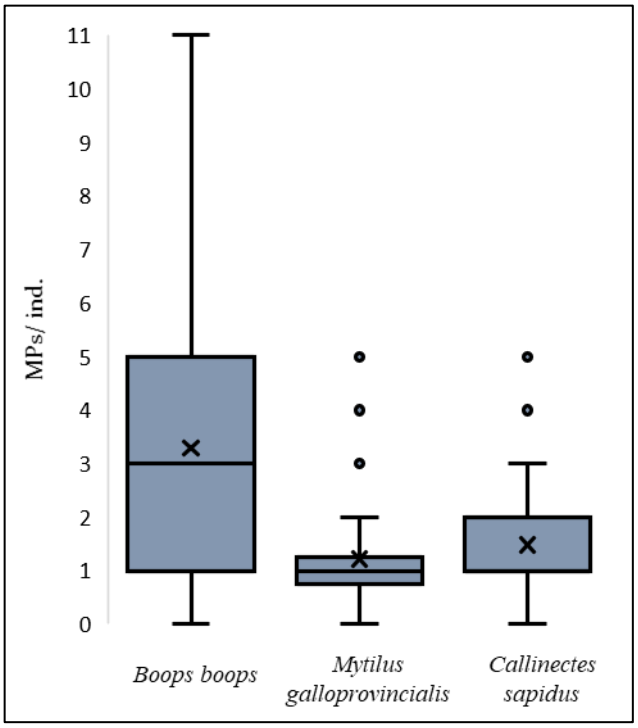

Figure 1. Items found per individuals for the 3 marine species.

Concerning the size, the maximum dimension of each item was considered as the most representative measurement to facilitate comparison. İn all three species tested, the most frequent size recorded was MPs items with a length smaller than 200 μm. In more details, the items found in bogues had the smaller size range, followed by blue crabs and mussels. All the findings in bogues and blue crabs were categorised as MPs, while three items retrieved from the mussels had length greater than 5 mm and characterized as mesoplastics (Figure 2).

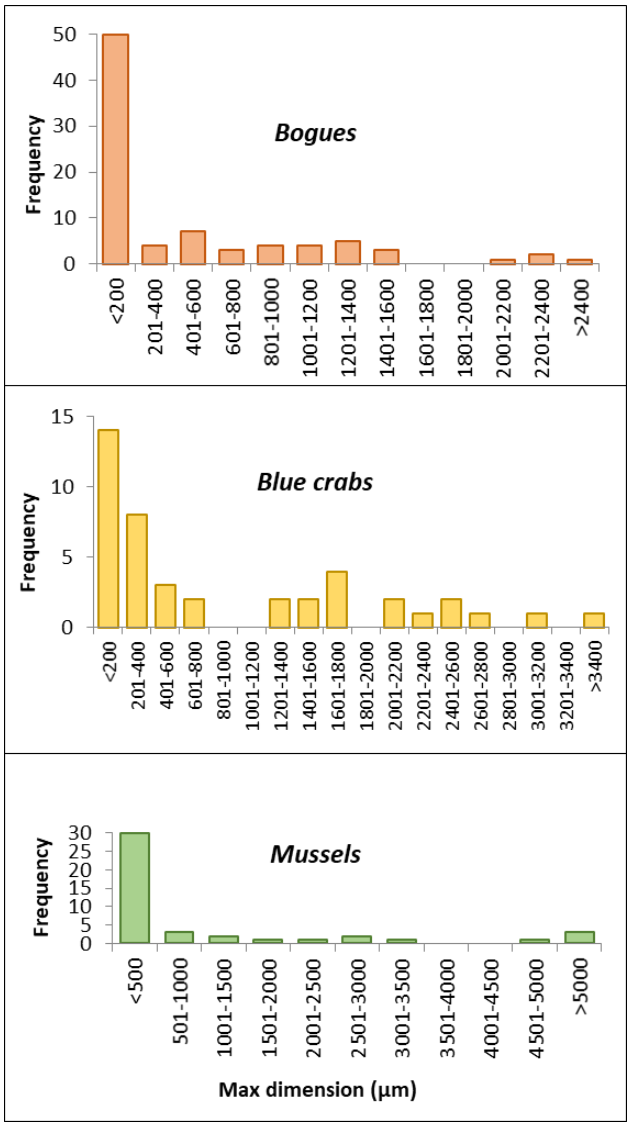

Figure 2. Size distribution of found items for (a) Bogues (b) Blue crabs, and (c) Mussels.

Regarding the colour of the MPs, in total 12 different colours were identified. The most common colour was blue (30.41%), followed by brown (19.30%), red (11.70%) and black (9.94%). Among species, blue was the most frequent colour in mussels and blue crabs (56.82% and 31.21% respectively), and brown in bogues (30.95%) (Figure 3).

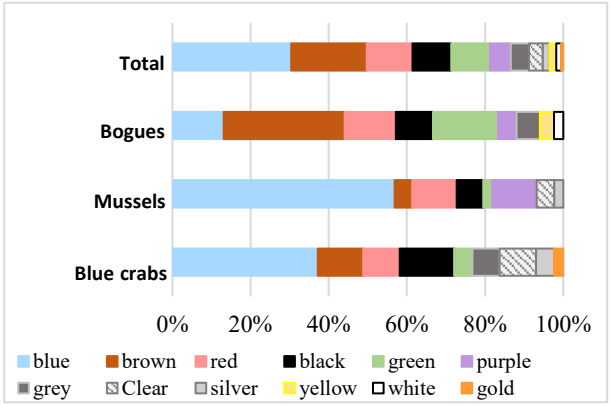

Figure 3. Colour distribution of found items for each species.

3. Discussion

This study highlighted the microplastics ingestion in three commercially popular marine species of North Aegean, *Boops boops*, *Mytilus galloprovincialis* and *Callinectes sapidus*. To summarise, the highest MPs abundance recorded in bogues, although they record the smaller size range of the found items. Examining the sampling area as a factor, significant differences highlighted ($p < 0.05$, Kruskal-Wallis test). Samples from Lesvos found as significant different than those from Thermaikos Gulf ($p < 0.05$, Dunn's Multiple Comparison test). Within mussels and blue crabs that collected from Thermaikos Gulf, no significant differences were recorded, thus the similar MPs abundances among those two species could be justified.

Comparing the present findings with previous studies, similar results have been reported for bogues in the Western Mediterranean Sea (3.75 items/individual; Nadal et al., 2016). In mussels, higher values have been recorded in Ionian Sea (1.7-2 items/individual; Digka et al., 2018). Concerning blue crabs, under our knowledge, this is the first study recording microplastics ingestion in Greek waters, while a recent study for blue crabs from Lesina lagoon in Italy reported an average micro-litter occurrence of 2.5±1.6 SD items/animal (Renzi et al., 2020).

Regarding the potential impact on human health after consumption of these species, it is worthwhile to mention that only mussels are usually consumed without the removal of their digestive tract. On the basis of our results, the consumption of a portion of mussels would lead to a maximum intake of ~250 MPs, much less than the estimated by the European Food Safety Authority maximum ingestion of 900 particles per portion (EFSA CONTAM Panel, 2016).

4. Conclusion

Our findings provide information on the amount and types of microplastics ingested by three marine commercial species of the Aegean region. Further systematic research across a broader range of species and habitats is needed in order to document the magnitude of microplastic pollution in the Aegean and its potential ecological and biological consequences.

Acknowledgment

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 1331).

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