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Short Communication

Microplastic Occurrence in the Gastrointestinal Tract of a Risso's Dolphin *Grampus griseus* in the Northeastern Mediterranean Sea

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ABSTRACT

Risso's dolphin *Grampus griseus* was stranded on the coast of Arsuz-Uluçınar, Iskenderun Bay, Turkey in the northeastern Mediterranean on 29 March 2022. This study was conducted to reveal the relationship between *G. griseus* and microplastic debris in the northeastern Mediterranean Sea. The gastrointestinal tract (GIT) of one stranding *G. griseus* was examined, and 454 microplastics particles were extracted. Of all, the majority of them were fibers (96%), black in colour (62%), and 0.5-1 mm in size (38%). This paper represents the first data indicating the microplastic abundance in *G. griseus* from the northeastern Mediterranean Sea. Also, it creates a baseline to understand the relationship between cetaceans and microplastics in this region.

Keywords: Stranding dolphin, microplastic pollution, Iskenderun Bay, marine litter

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INTRODUCTION

As a consequence of unstoppable growth in plastic production, waste plastic materials reaching to marine environments are increasing day by day. The interaction between marine animals and plastic waste materials poses a threat to their well-being and causes many problems including mortality (Sharma, Sharma, & Chatterjee, 2021). For example, marine mammals were entangled in the fishing gear (Frantzis, 2007; Gomeric et al., 2009) or they try to swallow the fishing nets with the aim of feeding on trapped prey in the net (Levy et al., 2009). Similarly, some cases where large quantity of plastic materials mainly plastic bags, plastic bottles and their caps extracted from the stomach of cetaceans were reported (Simmonds, 2012).

Plastic materials found in the marine litter break up into smaller plastic particles as a result of photo degradation, oxidation, and mechanical abrasion (Andrady, 2011). Sometimes, microplastics (MPs) particles reach the marine environments from wastewater treatment plant effluents (Sun, Dai, Wang, van Loosdrect & Ni, 2019), and riverine effluents (Pojar et al., 2021). These small size plastic particles are called as MPs (less than 5 mm in size) (Arthur, Baker & Bamford, 2009), and they endanger the health of marine animals. To date, MPs ingestion has been reported in many marine animals from different trophic levels such as zooplankton (Sun, Liang, Zhu, Zhao & Zhang, 2018), bivalve (Yozukmaz, 2021), crustacea (Wu et al., 2020), fish (Kılıç & Yücel, 2022), sea birds, and cetaceans (Poeta, Staffieri, Acosta & Battisti, 2017; Fossi, Panti, Baini & Lavers, 2018).

The presence of MPs particles in the gastrointestinal tract (GIT) of cetacean species may result from direct ingestion and/or trophic transfer (Nelms et al., 2019; Novillo, Raga & Tomas, 2020). Even though, MPs found in the GIT of cetacean species may not cause congestion in the digestive system, MPs provide sorption sides for chemical contaminants which lead to the entrance of dangerous polluters into their body (Tien, Wang & Chen, 2020) that pose a threat to species well-being.

Risso's dolphin *Grampus griseus* (G. Cuvier, 1812) was considered as a regular cetacean inhabitant of the Mediterranean Sea (Bearzi, Reeves, Remonato, Pierantonio & Airoldi, 2011; Lanfredi et al., 2021). Sighting, bycatch, and stranding records of this species from Turkish waters were reported from the Mediterranean Sea, Aegean Sea, and Marmara Sea (Öztürk, Öztürk & Dede, 2001; Tonay, Dede, Öztürk & Öztürk, 2009; Öztürk, Tonay & Dede , 2011; Altuğ et al., 2011; Dede, Saad, Fakhri & Öztürk, 2012; Dede, Tonay, Bayar & Öztürk, 2013; Kesici et al., 2021). Even though this species were continuously observed in the Mediterranean Sea, a recent study indicated there is a significant decrease in their subpopulations, and they have been under the IUCN Red list of endangered species since 2020 (Lanfredi et al., 2021). In this study, a dead stranded *G. griseus* was analyzed in terms of MPs content in the stomach and intestines to understand the danger of MPs in the feeding behaviour of this species as marine mammals.

MATERIAL AND METHODS

Sampling

Risso's dolphin *Grampus griseus* was stranded on the coast of Arsuz-Uluçınar, Iskenderun Bay, Turkey in the northeastern Mediterranean Sea on 29 March 2022 [Latitude: 36.409264° Longitude: 35.875059°] (Figure 1). The sex of *G. griseus* was identified, and all morphometric characters of *G. griseus* were measured. The weight of the species was estimated with weighbridge, while the body was transported to the faculty. The mouth was checked for the existence of any macroplastic materials before digestion. Then, the specimen was dissected, and all gastrointestinal track (GIT) from the pharynx to anus was removed and transported to the laboratory in a metal bucket. The outer surface of GIT was cleaned with distilled water. The inside of the intestines was first emptied by compression, and then washed with distilled water, and the contents were transferred to jars.

Microplastic extraction

At the beginning of all processes, the equipment and tools used in laboratory as well as laboratory surfaces were cleaned with filtered ethanol and distilled water twice. The stomach and intestine content was filtered through steel sieves with different mesh sizes (2000 µm, 1000 µm, 750 µm, 200 µm) and mesh filters with 50 µm in size. The filters were covered with tin foil and kept closed to avoid contamination until the microscopic examination. In this study, chemical digestion was not applied ,but stomach and intestine content was directly filtered due to the high organic load in the GIT.

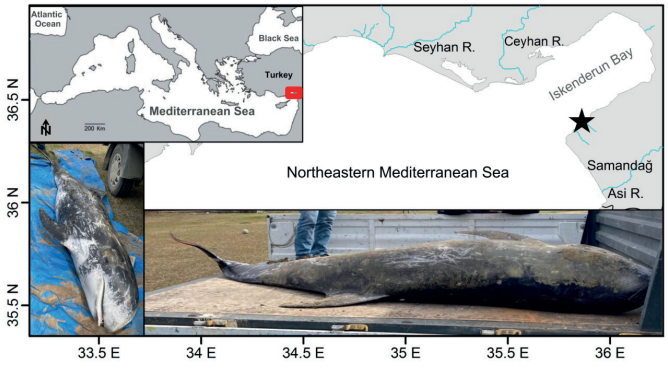

Figure 1. Pictures of stranded Risso's dolphin *Grampus griseus* with the stranding location.

Microscopic examination

The filters were investigated for the presence of MPs under Olympus SZX7 microscope with an attached Olympus DP 20 digital camera. Particles with no cellular or organic structures, fibers with the equally thick end, coloured particles and twisted flat ribbons were considered as MPs like particles (Nor & Obbard, 2014). Observed particles were exposed to a hot needle to check whether they are plastic in nature (Hanke et al., 2013). The number of MPs, size, colour and MP type (fiber, pellet and fragment) were recorded.

It is important to underline that during microscopic examination, each identified MPs like particles was checked with hot needle to validate the plastic nature. Also, organic remaining was carefully examined to reveal the trapped MPs inside of organic structures.

Contamination prevention

All steps of MPs extraction were carried out in restricted laboratories (Bessa et al., 2019), and all doors and windows were shut down (Torre, Digka, Anastasopoulou, Tsangaris, & Mytilineou, 2016). To eliminate contamination, personnel always wore cotton aprons and nitrile gloves throughout the procedure. Finally, triplicate wet filters were inserted into petri dishes for quality check. There is not any MP particle detected in the blank filters.

RESULTS & DISCUSSION

Plastics are intensely preferred in many industries due to their benefits over metal and wooden materials. However, they have become an alarming pollutant in marine environments since MP particles may easily be ingested by marine animals due to their intense and widespread presence. Also, its small size allows them to transfer to upper trophic levels via the food chain. MPs act like pollution vectors and create a gate for chemical pollutants to enter the body of marine animals (Koelmans, Bakir, Burton, & Janssen, 2016; Tien et al., 2020; Koelmans, Diepens & Mohamed Nor, 2021), which leads to health problems and even death.

Top predators like marine mammals are at higher risk in terms of MPs toxicity since as the size of the animal increases, the amount of MPs increases due to the connection with upper trophic levels (Rebelein, Int-Veen, Kammann, & Scharsack, 2021; Müller, 2021; Moore et al., 2022). Lusher, Hernandez-Milian, Berrow, Rogan & O'Connor (2018) examined the 528 stranded and caught cetacean species from Ireland, and MP and/or macroplastic particles were detected at the 8.5% of examined species. Among them, they examined the digestive tract of 8(+1) *G. griseus* specimens, and they reported the presence of macro debris in 2 specimens. However, the presence of micro debris was not reported. Nelms et al. (2019) examined the MPs existence of 5 different marine mammals (n=50), including 1 *G. griseus* individual, and MPs were detected in all examined animals. Novillo et al. (2020) examined the MPs existence in the stomach of striped dolphins (*Stenella coeruleoalba*) (n=30) from the Western Mediterranean Sea, and MPs were detected in the 90% of examined cetacean species. Al-

though there are some previously published articles reporting plastic ingestions by Risso's dolphin (Shoham-Frider, Amiel, Roditi-Elesar & Kress, 2002; Baini et al., 2017; Lusher et al.,2018; Alexiadou, Foskolos, & Frantzis, 2019; Nelms et al., 2019), this is the first report presenting the MPs existence in the GIT of a Risso's dolphin *Grampus griseus,* a top predator from the Mediterranean Sea. Also, this incident is the most eastern stranding records of this species from Turkish waters.

According to the first visual examination of *G. griseus,* the specimen was freshly dead, and there was no deformation or injury in the external appearance, which may explain the cause of death. The specimen was female, and the length and the weight were measured as 294 cm and 370 kg, respectively. Detailed information regarding the morphological measurements of studied specimen was given in Table 1.

During the visual examination of stomach content in the laboratory, nine different sized squid beaks (undigested or partially digested tissue were found) were found in the fore stomach ,but other stomachs were empty. While the first part of the intestines contained greenish fluid, the remaining part was brown.

Table 1. External measurement of Risso's dolphin *Grampus griseus*.

Character	Length (cm)
Body length	294
Distance between tip of rostrum to mid-point of anus	240
Distance between tip of rostrum to mid-point of genital slit	195
Distance between tip of rostrum to mid-point of umbilicus	161
Distance between tip of rostrum to tip of dorsal fin	180
Distance between tip of rostrum to font of dorsal fin, at anterior insertion of dorsal fin	127
Distance between tip of rostrum to centre of blowhole	31
Distance between tip of rostrum to end of gape	27
Distance between tip of rostrum to centre of eye	33
Distance between tip of rostrum to front of pectoral fin	51
Length of left fluke	38
Length of right fluke	38
Total fluke span, tip to tip	76
Basal length of dorsal fin	37
Height of dorsal fin	31
Maximum width of pectoral fin	25
Outer length of pectoral fin, from anterior insertion to tip	60
Inner length of pectoral fin, from posterior insertion to tip	43
Girth behind pectoral fin	60
Girth in front of dorsal fin,	70
Girth behind the dorsal fin,	59
Girth at the genital slit	45
Girth at anus	25

Cephalopods are the major prey of *G. griseus* (Clarke, 1996), which explains the existence of squids in the stomach content. For example, Öztürk, Salman, Öztürk & Tonay (2007) extracted cephalopod remains from the *G. griseus* in the eastern Mediterranean Sea. Also, similar to findings of this study, Blanco, Raduan & Raga (2006) extracted squid species in the stomach of *G. griseus* sampled from the Mediterranean Sea.

A total of 484 MPs particles were extracted from the GIT of *G. griseus* (Figure 2). Eastern Mediterranean Sea, especially the region between Turkey and Cyprus, was categorized as the hotspot of the plastic debris (Liubartseva, Coppini, Lecci, Clementi, 2018). When the stranding location of *G. griseus* was taken into consideration, this high MPs amount is most probably related with the contamination status of the surrounding environment.

In terms of plastic type, majority of the identified particles were fibers in shaped (96%). Minor portion of identified MPs were pellets and fragments from unidentifiable larger objects (Figure 3). In the literature, the percentage of fibers among extracted MPs was reported as 73.6% in the Western Mediterranean Sea (Novillo et al., 2020), 84% in the British coast (Nelms et al., 2018), and 83.6% in Ireland (Lusher et al., 2018).

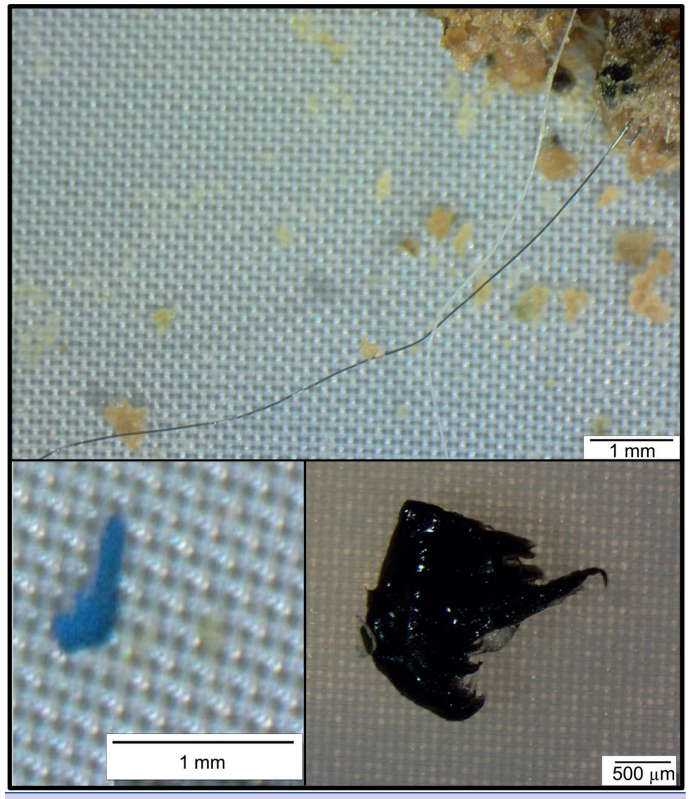

Figure 2. Some examples of extracted MPs from gastrointestinal track of Risso's dolphin *Grampus griseus*.

In terms of the size, 4% of extracted MPs belong to mesoplastics size class (5-20 mm); whereas the rest belong to microplastic size glass (<5 mm). Majority of the extracted particles were within the range of 0.5-1.0 mm size class (Figure 3), and the mean size of extracted fibers was 1.3±1.6 mm.

In terms of colour, white, black, blue, green, and red particles were identified. In this context, transparent and white MPs were categorized as white; blue and purple MPs were categorized as blue; red and pink MPs were categorized as red. Majority of the extracted MPs were black in colour, which is consistent with the previous studies (Lusher et al., 2018; Nelms et al., 2018; Novillo et al., 2020) (Figure 3).

The amounf of MPs in the stomach of common dolphin *Delphinus delphis* was found as 12 average ind^{-1} from the Spanish coast (Hernández-González et al., 2018), striped dolphins (*Stenella coeruleoalba)* was found as 14.9 average ind^{-1} from the Western Mediterranean Sea (Navillo et al. 2020). The amount of MPs extracted from Risso's dolphin *Grampus griseus* was significantly higher than that of the previous reports. There are many possible reasons for these variations. Firstly, previous studies focused on the stomach content whereas in this study, both intestine and stomach content was examined. Secondly, the amount of MPs was found to be variable between species (Nelms et al., 2020) and location (Novillo et al., 2018). Thirdly, the straightening of the MPs particles was checked with the hot needle method, advanced validation methods Fourier transform infrared spectroscopy could not be used. This may cause an overestimation of the examined MPs particles.

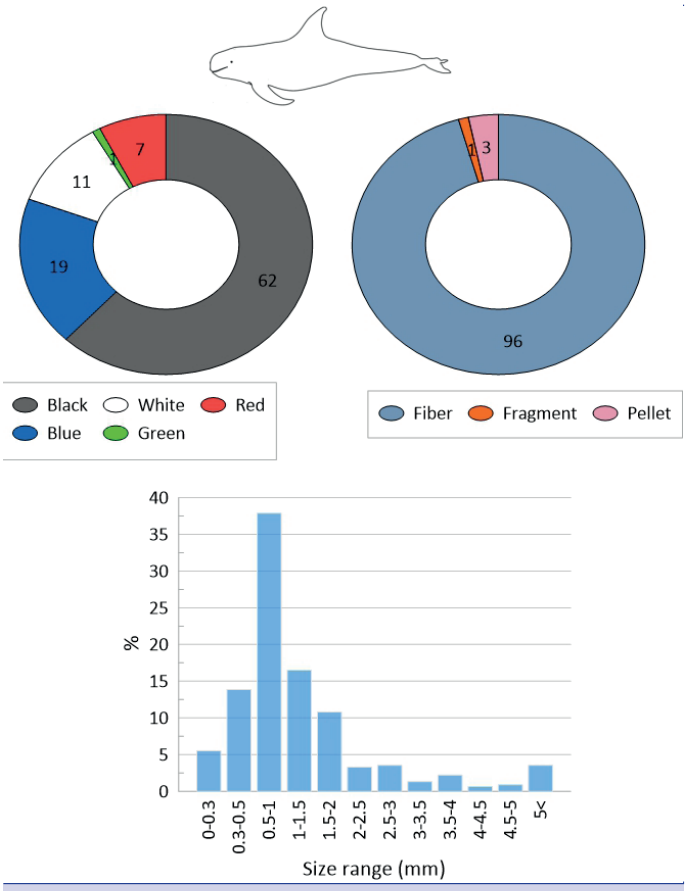

Figure 3. Characterization of extracted microplastics by colour (%), shape (%) and size (mm).

CONCLUSION

In this study, 454 MPs particles were extracted from the gastrointestinal tract of Risso's dolphin *Grampus griseus* from the northeastern Mediterranean Sea. Majority of the extracted particles were fibers in shape, black in colour, and in the 0.5-1 mm size range. The high amount of MPs particles in the GIT of Risso's dolphin *Grampus griseus* shows the intensity of MPs contamination risk of marine mammals considering their role as pollutant vectors. Also, this high presence proves the significant MPs occurrence in the Mediterranean Sea pelagic waters. More comprehensive studies need to be conducted to evaluate the threat of MPs on marine mammals.

Compliance with Ethical Standard: Authors declare that ethical approval is not require for this type of study.

Conflict of Interests: Authors declare that there is no conflict of interest.

Financial disclosure: The authors declare that no funds, grants, or other support were received in this study.

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