

Abundance and characteristics of microplastics in commercially important bottom dwelling finfishes and shellfish of the Vembanad Lake, India

Ramachandran Nikki¹, Abdul Jaleel K. U.*¹, Ragesh Saraswathi², Shini Shaji¹, Mahua Saha³ and Dinesh Kumar P. K¹.

¹CSIR- National Institute of Oceanography, Regional Centre, Kochi-682018, Kerala, India.

²Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science & Technology (CUSAT), Cochin-682016, Kerala

³CSIR-National Institute of Oceanography, Dona Paula, Goa

*Corresponding author: jaleel@nio.org

Abstract

This paper characterizes microplastics (MPs) in the heavily urbanised, brackish water Vembanad Lake (India), focussing on some commercially important bottom-feeding fishes and shellfish (*Arius maculatus*, *Etroplus suratensis*, *E. maculatus* and *Villorita* sp.). The average abundance of MPs was higher in the water column (872 ± 573 nos./m³) than in finfishes (15±13 particles per fish) and shellfish (23±20 nos./ind.). Fibre was the most abundant MP type in the water and the organisms examined. The size of MPs obtained from finfishes ranged between 0.04 and 4.73 mm (4±3 mm), with a majority of particles being <4 mm. No correlation was found between biological features (e.g. gut length, mouth size) of fishes and the size of MPs in their gut. In *Villorita* sp., the abundance of MPs was positively correlated with the size of the individuals. Raman spectroscopy confirmed the presence of chlorinated polyvinyl chloride, polyethylene, polypropylene and polyester in the samples.

Keywords: Vembanad Lake; *Arius maculatus*; *Etroplus suratensis*; *Etroplus maculatus*; *Villorita* sp.; microplastics.

1. Introduction

Plastics are synthetic organic polymers that have become an integral part of day-to-day life owing to its highly versatile nature (Derraik, 2002; Rios et al., 2010; Thompson et al., 2009, Crawford and Quinn, 2017). The incessant and indiscriminate use of plastics and improper waste management practices have resulted in their accumulation as a persistent pollutant in aquatic ecosystems across the world (e.g., Costa and Barletta, 2015; Jambeck et al., 2015; Robin et al., 2020). Based on size, plastic litter is categorized into macroplastics (>25 mm), mesoplastics (5-25 mm), microplastics (<5 mm) and nanoplastics (<0.1 μm) (Fendall and Sewell, 2009; Browne et al., 2010; Crawford and Quinn, 2017; Ng et al., 2018). Macroplastics gradually break down into smaller fragments due to oxidation, ultraviolet radiation and mechanical forces and those fragments which are less than 5 mm in diameter are called secondary microplastics (Barnes et al., 2009; Cole et al., 2011; Lippiatt et al., 2013). Microplastics (MPs) are also deliberately manufactured for use in cosmetics, personal care products, dermal exfoliators, cleaning agents, etc. and these are categorized as primary microplastics (Lee et al., 2013; Crawford and Quinn, 2017). Owing to its lightweight and persistent nature (Lusher et al., 2015), MPs are widely dispersed in shorelines, water column, deep-sea sediments and even incorporated in the polar ice caps (Crawford and Quinn, 2017). The widespread distribution of MPs thus make them bioavailable to various organisms inhabiting both pelagic and benthic realm (Moore, 2008; Wright et al., 2013; Carbery et al., 2018). Besides the recognized physical impacts of microplastics when ingested by aquatic organisms (Wright et al., 2013), these particles also serve as a potential source of other toxic chemicals that are added during their manufacturing process and sorbed to their surface (Hermabessiere et al., 2017; Teuten et al., 2009; Bakir et al., 2014). Hence, MPs act as vectors of toxic chemicals which eventually get incorporated into aquatic food chains leading to their accumulation and biomagnification (Crawford and Quinn, 2017). In this context, it is essential to study the quantitative and qualitative aspects of microplastics and their implications on biota and their environment.

Even though numerous studies have reported microplastic ingestion by marine organisms, information regarding the levels of contamination and consumption by organisms from freshwater and estuarine habitats are relatively limited. These habitats are important transport routes of MPs to the ocean (Browne et al., 2011; Possatto et al., 2011; Vendel et al., 2017; Horton et al., 2017). The Vembanad Lake (VL) is among the most vulnerable aquatic ecosystems, providing a variety of goods and services to the coastal population and serving as an important habitat for diverse fauna and flora (Qasim et al., 1969; Haridas et al., 1973; Kurup and Samuel, 1987; Qasim, 2003; Asha et al., 2014). This system acts as a major drainage basin for seven rivers flowing through the most urbanized cities

of the state of Kerala (Srinivas et al., 2003). The VL system receives heavy load of pollutants (Miranda et al., 2008; Madhu et al., 2010; Deepulal et al., 2012; Kripa et al., 2012; Gireeshkumar et al., 2013; Sruthy and Ramasamy, 2017; Shylaja et al., 2018) and thus acts as a carrier of these pollutants to the Arabian Sea via two permanent openings (at Cochin and Azhikode). Hydrodynamic forces (e.g., tides, runoff) and geomorphology of the water body influences the suspension or retention, settling and distribution of MPs (Bessa et al., 2018). The long water residence time (between 115 and 120 days) of the VL (John et al., 2020), due to the seasonal closure of two water barrages (Thanneermukkom and Pathalam), increases the retention of plastics and consequent fragmentation. This is followed by the sorption of organic matter and enhanced microbial fouling over the surface of MPs (Kaiser et al., 2017). Thus, they acquire greater density and consequently sink and accumulate in the sediments. MPs are often similar in size of sediment particles and may resemble settled organic matter. Many organisms (including sedimentary fauna) have been observed to consume these particles, either by selective feeding or by accidental ingestion (Browne et al., 2010; Kripa et al., 2014; Divya et al., 2019; Haegerbaeumer et al., 2019).

In the current study, we examined the abundance of MPs from water and some commercially important bottom dwelling finfishes, *Arius maculatus* (Thunberg, 1792), *Etroplus suratensis* (Bloch, 1790) and *Etroplus maculatus* (Bloch, 1795) and a shellfish, *Villorita* sp. from the VL. The species selected are widely distributed in the brackish water system of peninsular India and their fishery extends throughout the year. About 25,000 tons of *Villorita cyprinoides* (black clam) are harvested per year from VL alone (Suja and Mohammed, 2010). Black clams are bottom dwelling suspension feeders, mainly feeding from the benthic boundary layer (BBL) and thereby they are highly exposed to microplastic contamination. They filter out MPs from the BBL and incorporate into the sediments (Galloway et al., 2017). In India, a few attempts have been made to study the occurrence of MPs in marine and freshwater environments (Sruthy and Ramasamy, 2017; Sarkar et al., 2019; Ganesh et al., 2020; Maharana et al., 2020; Robin et al., 2020) and in aquatic biota (Kripa et al., 2014; Divya et al., 2019; Naidu et al., 2018; Naidu, 2019; Karuppasamy et al., 2020, Devi et al., 2020; Suresh et al., 2020; Saha et al., 2021). The present work aims to study the abundance and characteristics of MPs in the water column and in a few ecologically significant and commercially important (bottom feeding) finfishes and shellfish from the VL. We also examined the relationship between the abundance of microplastic ingested by these organisms and their various morphological features (e.g., mouth size, gut length of fishes etc.).

2. Materials and methods

2.1 Study area and Sampling strategy

The Vembanad Lake (VL) in the southwest coast of India runs almost parallel to the coast from Alappuzha (Lat. 9°30' N) in the south to Munambam (Lat. 10°10' N) with an area of 151,250 ha (Mohan et al., 2014). Samples for quantitative and qualitative analysis of microplastics were collected from the VL in February 2019. Surface water samples (50 L) were collected using stainless steel bucket and filtered through 20 µm mesh, from five locations namely, Cochin barmouth (site 1), Aroor (site 3), Murinjapuzha (site 6), Thanneermukkom (site 7) and Kumarakom (site 8). The collected samples in the mesh were backwashed (using distilled water) into 250 mL capacity clean glass jars with metallic lid and preserved in 5% formalin. A total of 30 fin-fishes, 10 each of *A. maculatus*, *E. suratensis* and *E. maculatus* were collected from Vallarpadam (site 2), Aroor (site 3) and Vaduthala (site 4) respectively from locally operated stake nets and gill nets. Samples of *Villorita* sp. (30 numbers) were collected from Perumbalam (site 5) with the help of local fishermen engaged in clam harvesting (Fig 1). All the collected samples were immediately frozen in ice and later stored at -20°C till further analysis.

2.2 Sample processing

2.2.1 Water

In the laboratory, pre-treatment of the water samples was carried out using 30% H₂O₂ (wet peroxide oxidation, WPO) in order to digest organic matter present in it. The WPO mixture was then subjected to saltwater density separation using ZnCl₂ to separate MPs through floatation (Masura et al., 2015). The supernatant was then filtered using 1µm Whatman GF/C filter paper. Filters were dried, examined and enumerated under a high resolution stereo zoom microscope (Olympus SZX16). The abundance of MPs was expressed in nos./m³.

2.2.2 Fish and shellfish samples

In the laboratory, the preserved specimens were thawed and washed with distilled water to remove the associated debris. All the collected specimens were identified up to species level using standard identification keys and recent publications (e.g. Jayaram, 1981; Fischer and Bianchi, 1984; Nandan, 2012). Prior to dissection, routine morphological and meristic measurements like standard length, weight, mouth gape/width of the fishes were taken (Table 1). The mouth size of each fish was calculated using the following formula considering that the conceptual size of the maximum width of food (in this case, plastic particle) corresponds to 50% of mouth gape size (D).

Accordingly,

Mouth gape size of fish: $D = \sqrt{2AB}$

Where, D = Mouth gape size, AB= Measurement of upper-maxilla length; (Shirota, 1970)

The entire digestive tract from all the fish samples were dissected out following the standard procedure (Claessens et al., 2013; Lusher et al., 2013; Rocha-Santos and Duarte, 2015) and subsequently placed in a petri dish, loosely covered with aluminium foil to prevent contamination. The gut contents were examined under a stereo zoom microscope (Olympus SZX16) and observations were noted (e.g. status of fullness, major contents). Following the examination, the gut contents were subjected to wet digestion using 10% KOH (3:1) at 60°C for 24 hours with continuous agitation at 125 rpm to remove the organic matter (Rochman et al., 2015; Dehaut et al., 2016). The digestion process was continued until the solution turned transparent or white in color (at this point all the biological materials or organic matter would be completely removed from the sample) and left to cool before further processing. The digested tissues were then diluted with distilled water and filtered through 20 µm mesh (1 µm cellulose nitrate membrane filter paper got clogged when solution was directly passed through it) and the contents retained in the mesh was back washed into 1 µm cellulose nitrate membrane filter paper (Whatman GF/C). The filter papers were then dried at 40°C for 24 hours in a petridish. An advanced model of stereo zoom microscope (Olympus SZX16) was used to ascertain the presence of MPs in the filter paper, their images and morphometrics were taken (using Cell Sens software) simultaneously. After enumeration, the filter paper containing MPs were kept in a labelled petri dish and stored in dry conditions. Proper measures have been taken to avoid contamination by air-borne MPs. Throughout the analysis, all work surfaces and instruments were thoroughly cleaned with 70% ethanol and glass wares were rinsed with distilled water. Laboratory blanks were used during extraction and enumeration of MPs in order to test for cross contamination by plastic materials.

In the case of shellfishes, after the complete removal of the surface debris, their morphometric measurements (length and breadth) were taken and based on their shell length they were categorized into 3 size classes (size class I: 1-1.9 cm, size class II: 2-2.9 cm and size class III: 3-3.9 cm) each containing 10 individuals. The specimens were dissected out and based on their function, the organs were divided into digestive (gills, intestine, stomach) and non-digestive (mantle, gonad, adductor, visceral tissue) parts. Each part was further digested separately following the same procedure used for the fish samples (given above), except that the digested samples were directly filtered through 1 µm filter paper. MPs were then enumerated and their morphometrics were taken (values expressed in nos./ind.).

2.2.3 Contamination control

For controlling the contamination by airborne MPs, the extraction procedures were carried out in a clean fume hood. Throughout the analysis, all work surfaces and instruments were thoroughly cleaned with 70% ethanol and glasswares were rinsed with deionised distilled water. Laboratory blanks were employed during the extraction and identification process to test for cross contamination of plastic components, with no MPs being found in the blanks. All the chemicals used for preservation and processing were filtered through 1 µm cellulose nitrate membrane filter paper (Whatman GF/C). Microplastic contamination was regulated and evaluated in all the materials and solutions used during analysis. Standard non-plastic equipment made of metal and glass were used to the best extent possible, and they were thoroughly cleaned with deionised distilled water. It was also ensured that the researchers only wore 100% cotton laboratory coats and headscarf while handling the sample.

2.3 Characterization and identification of microplastics

Characteristics of MPs such as colour (black, white, blue, red, green, yellow and transparent), type (fibres, fragments film, microbead and foam) and size range (0.01-0.5 mm, 0.5-1 mm, 1-1.5 mm, 1.5-2 mm, 2-2.5 mm, 2.5-3 mm, 3-3.5 mm, 3.5-4 mm, 4-4.5 mm and 4.5-5 mm) were quantified for each fish and shellfish.

Representative of MPs (5%) from water, fish and clams were analysed for their qualitative composition by using Confocal Raman spectrometer with AFM (WITeeAlpha 300RA AFM, SNOM& RAMAN, Germany), 532 nm or 633 nm excitation laser and a grating of 600 groves/mm using 20x/0.4 objective with integration time of 10 seconds. The resulting spectra were compared with the Wiley's KnowItAll Raman spectral library for polymers.

2.4 Statistical analysis

The variation in total abundance and characteristics of MPs in the three species of fishes and in the three size classes of shellfish were tested using one-way ANOVA. Statistical tests were considered significant at p values < 0.05 . We also performed correlation analyses between morphological features (e.g., mouth size, gut length, etc.) of fishes and abundance of microplastics ingested. All analyses were performed using IBM SPSS 20.

3. Results

In the present study, MPs were recovered from all samples (water and biological) analysed. Abundance of MPs in the water column collected using 20 µm mesh ranged from 460 (site 7) to 1880 nos./m³ (site 1) with a mean value of 872 ± 573 nos./m³. Fibre (83%) was the most dominant

microplastic type followed by fragment (16%) and film (1%). We examined the colour of MPs extracted from the water samples in order to understand the degree to which they are contaminated with chemical pollutants and also the degree to which they are confused with natural food sources by aquatic organisms. Transparent MPs (57%) were dominant, followed by green (13%) and red (11%) (Fig 2).

3.1 Occurrence of microplastics in the gastrointestinal tract of finfishes

The gut of all the fish samples analysed was found to be almost full. Detritus (includes partially digested phytoplankton and animal parts, sand grains, microplastics etc.) constituted the bulk of the gut content (> 99%) of *Etroplus suratensis* and *E. maculatus*. In *Arius maculatus*, prawns formed the major component (77%) followed by detritus (22%) and partially digested fishes (1%).

In the present study, MPs were recovered from all the 30 fish samples analyzed (belonging to 3 species). Overall, 438 MPs particles were identified from these samples with a mean abundance of 15 ± 13 particles per fish. Relatively high abundance of MPs was recovered from *Arius maculatus* (21 ± 20 nos./ind.) followed by *Etroplus suratensis* (13 ± 5 nos./ind.) and while their abundance was lowest in *E. maculatus* (10 ± 8 nos./ind.) (Fig 3). No significant variation was found in the abundance of MPs in the three species analyzed (ANOVA $F=1.914$, $p=0.167$).

Among the MPs recovered from the gut of all the three species, fibre (71%) formed the most abundant type followed by fragment (24%). Other types of MPs such as film and microbead were obtained only from *A. maculatus*. Foam was obtained only from a single specimen of *E. maculatus*. Fig 4 shows the species selected for the study and MPs recovered from these species. There was a significant variation in the abundance of film ($F=4.366$ $p=0.023$) in the three species analysed, while no significant variation was observed for the rest of the MPs.

Microplastics extracted from the gut were also classified based on their colour, viz red, black, green, blue, yellow, white and transparent. Irrespective of the number of MPs recovered, the colour wise distribution of ingested MPs showed more or less similar pattern in all the selected fish species with black being the dominant colour, followed by red, green, transparent and blue. White (obtained from *A. maculatus* and *E. maculatus*) and yellow (only from *E. maculatus*) coloured MPs were less frequent in the guts of fishes. A significantly higher quantity of green ($F=3.494$, $p=0.045$) and blue ($F=6.337$, $p=0.006$) coloured MPs were noted in *A. maculatus* while there was no significant variation for the other colours.

To check whether there is any relation between the biological features of the fish (standard length, mouth gape) and size of microplastics, we further examined the size of microplastics extracted

from each species. The standard length and mouth gape of *A. maculatus* ranged between 19 and 22 cm and 1.7 and 2 cm respectively. In *E. suratensis* the standard length ranged between 7 and 11 cm and mouth gape between 1 and 1.1 cm. In the case of *E. maculatus* standard length ranged between 5.3 and 6.4 cm and mouth gape between 0.9 and 1 cm. The present study revealed no comprehensive correlation between biological features of the fish and the size of MPs in their gut. The size of MPs obtained from finfish samples ranged between 0.04 and 4.73mm. Percentage contribution of different size classes to the total abundance of plastic particles in fishes are given in Fig 5. In *A. maculatus*, high abundance of plastic particles were obtained in the 0.5-1 mm size class (27%) followed by 0.01-0.5 mm (18%), 1-1.5 mm (16%), 2.5-3 mm (11%), > 3 mm (10%), 1.5-2 mm (9%) and 2-2.5 mm (8%) size classes. In *E. suratensis*, high abundance of MPs were recovered in the 0.01-0.5 mm (42%) followed by 0.5-1 mm (37%), >4.5 mm size range (9%) 1.5-2 mm (8%) and 3.5-4 mm (4%). In *E. maculatus*, highest abundance of particles were obtained from 1-1.5mm size range (40%) followed by the 0.5-1mm (25%), 0.01-0.5mm (20%) 1.5-2mm (10%) and >4.5mm (5%) size classes. No significant variation in the occurrence of MPs was found among the size classes except 3-3.5 mm size class ($F=3.578$, $p=0.042$).

3.2 Occurrence of microplastics in shellfish

Overall, 682 MPs particles were obtained from both digestive and non-digestive parts of all 30 individuals of *Villorita* sp., with a mean abundance of 23 ± 20 nos./ind. The total number of MPs particles recovered from digestive and non-digestive parts were 351 (mean \pm SD: 12 ± 15 nos./ind.) and 331 (11 ± 10 nos./ind.) respectively. To evaluate the relationship between microplastic intake and the size of the organism, the samples were categorized into 3 major size classes (size class I: 1-2 cm, size class II: 2-3 cm and size class III: 3-4 cm) each containing 10 individuals. Comparatively, higher abundance of MPs was recovered from size class III in digestive (225 nos.) and non-digestive parts (196 nos.) with a mean abundance of 23 ± 22 nos./ind. and 20 ± 14 nos./ind. respectively, followed by size class II digestive (79 nos.) and non-digestive parts (91 nos.) with a mean abundance of 8 ± 5 nos./ind. and 9 ± 6 nos./ind. respectively. Relatively low abundance of MPs was recovered from size class I (47 nos. in digestive and 44 nos. in non-digestive part) with a mean abundance of 5 ± 3 nos./ind. and 4 ± 1 nos./ind. respectively (Fig 6). In *Villorita* sp. the abundance of MPs showed an increasing trend with increasing size of the individuals and this was found statistically significant ($F=13.000$, $p=0.00002$).

The most common type of MPs obtained from *Villorita* sp. was fibre (81%) followed by fragment (15%), film (4%) in both digestive and non-digestive parts. Interestingly, no microbeads and

foam were recovered from *Villorita* sp. (Fig 7). Fibre showed a significant variation in the three size classes of *Villorita* sp. ($F=14.959$, $p=0.00001$).

Colour of MPs recovered from *Villorita* sp. were black, blue, red, transparent, green and yellow, of which black (27%) was the most dominant followed by blue (26%) red (21%) and transparent (15%) in both digestive and non-digestive parts. A significant variation was found in the abundance of these dominant colours in the three size classes of *Villorita* sp. (Black: $F=15.775$, $p=0.000004$; blue: $F=10.047$, $p=0.0001$; transparent: $F=9.776$, $p=0.0002$ and red: $F=8.849$, $p=0.0004$).

The size of different MPs recovered from digestive part and non-digestive part of clams ranged 0.012-2.85 mm and 0.013-4.35 mm respectively with majority of them falling under ≤ 0.5 mm (39%) followed by 0.5-1 mm (25%), 1-1.5 mm (13%), 1.5-2 mm (13%), and >2 mm (10%). (Fig 8).

In this study the tentative MPs (especially fragments resembling organic debris) were taken for qualitative confirmation. The polymer type of microplastic samples was confirmed using Raman spectroscopy. Chlorinated Polyvinyl chloride (CPVC), Polyethylene (PE), Polypropylene (PP) and Polyester (PES) were the different types of MPs obtained from the present study (Fig 9).

4. Discussion

Rivers and estuaries play a key role in the transportation of plastic debris from the land to the sea (Browne et al., 2010; Sadri and Thompson, 2014). Depending on the geographic location, topography and physical settings of these ecosystems, plastics can possibly remain trapped in them for extended periods of time. Based on river runoff, the seasonal cycle in the VL system is distinguished as high runoff during south west monsoon (June–September), moderate runoff during north-east monsoon (October–December) and low runoff months or dry period between January and May (Shivaprasad et al., 2013). These seasonal variations in runoff may have influence in abundance and settling of MPs in the lake. The present study was conducted during the north east monsoon and recorded 872 ± 573 nos./m³ MPs in the water column with high spatial variation in its abundance. Studies conducted by Zhao et al (2014, 2015) reported much higher values of MPs from the Yangtze Estuary (4137.3 ± 2461.5 nos./m³,) and three other urban estuaries in China (>680 nos./m³). Wu et al. (2019) reported 1485.7 ± 819.9 nos./m³ and 788.0 ± 464.2 nos./m³ from Haihe and Yondingxinhe Estuary. Recently, Mao et al. (2020) recovered 3.12 – 11.25 nos./L of MPs from Wuliangshuai Lake, northern China. In another study by Naidoo et al. (2015) from five urban estuaries of KwaZulu-Natal, South Africa reported high abundance of MPs (>14 particles per 500 ml). Several factors including hydrological conditions (run off, flushing rate etc.) the discharge of untreated effluent and the sedimentation rate (Eriksen et al., 2013; Free et al., 2014; Vaughan et al., 2017) might have played

significant role in the distribution and accumulation of land based MPs to the aquatic ecosystem. The high water residence time of the VL (John et al., 2020) should be considered as one of the main reasons for high abundance of MPs and their bioavailability (Ivar do Sul and Costa, 2014; Vermeiren et al., 2016). The first work on MPs in the VL was carried out by Sruthy and Ramasamy (2017) and they reported 252.80 ± 25.76 particles per m^2 from surface sediments.

In the present study, MPs recorded from the digestive tracts of all the bottom dwelling finfishes (15 ± 13 particles per fish), were examined. Similarly, Mizraji et al (2017) reported the occurrence of MPs in about 99% of the fishes examined in upper tidal pools from Las Cruces with high anthropogenic pressure. Silva-Cavalcanti et al. (2017) assessed the ingestion of MPs by *Hoplosternum littorale*, a common freshwater fish in Pajeu River and found 83% of the fish had plastic debris inside the gut.

Interestingly, the present findings indicated that the fishes consumed plastics intentionally or inadvertently when they cannot differentiate plastics from prey or organic matter. The gut content of *Etroplus* was found to be dominated by settled organic matter (>99%) along with sand grains. These imply their omnivorous feeding (Bindu and Padmakumar, 2008; Sahadevan, 2017) and frequent contact with sedimentary habitat (Keshava and Joseph, 1988). They prefer smaller sized particles, ranging from decayed organic matter to fresh filamentous algae, as they have a smaller mouth gape (1-1.1 cm for *E. suratensis* and 0.9-1 cm for *E. maculatus*). These fishes are active swimmers and forage on wider areas and thus the chances of getting contaminated with MPs are higher. Similarly, bottom dwelling herbivorous fishes (active foragers) are in particular at high risk of ingestion of MPs (Talley et al., 2020). *Arius maculatus* or other catfishes are nocturnal benthic feeders and can locate their prey efficiently in the muddy waters through their well-developed maxillary barbels (Mazlan et al., 2008; Ambily, 2016). They are generalist feeders which feed mainly on filamentous algae, detritus, crustaceans, small fishes etc. and the composition varies according to season and availability (Mazlan et al., 2008; Ambily, 2016). In the present study the mouth gape observed in *A. maculatus* ranged from 1.7 to 2 cm and the consumed prey included prawn (77%) followed by detritus (22%) and partially digested fishes (1%) and compared to the other 2 species studied, highest abundance of MPs was recorded in this *A. maculatus*. Moreover, *A. maculatus* might have encountered more plastic particles through trophic transfer from the smaller prey organisms (Brillant and MacDonald 2000; Browne et al., 2008). MPs are more frequently ingested by omnivores than herbivores and carnivores (Romeo et al., 2015; Bessa et al., 2018). The present findings also corroborated the results of Silva-Cavalcanti et al. (2017) where higher the plastic debris intake greater the dominance of other food items in the gut. This implies the cascading effect of trophic transfer of MPs through the process of biomagnification (Bellas et al., 2016) when the predators (fishes and crustaceans) target the bottom dwelling prey. The

present result affirm that the ingestion of MPs depends on feeding strategies or habitat of an organism (Jabeen et al., 2017; Mizraji et al., 2017; Baalkhuyur et al., 2018; Kumar et al., 2018, Saha et al., 2021). Formation of biofilms on MPs may play a major role in mimicking plastic as a nutritious food source (Kooi et al., 2017) especially in the case of organisms relying on chemoreceptors to select prey. This might be another reason for getting a greater number of ingested MPs from the gut of *A. maculatus*.

Villorita sp. is a sedentary filter feeding organism and one of the major shellfish resources of the VL. In the present study MPs were recovered from almost all the samples analysed. Similar observation was noted by Su et al. (2018) in Asian clams. Filter-feeding is a strategy less selective than predation (Rummel et al., 2016) and the ingestion solely depends on the availability of MPs in the benthic boundary layer. The abundance of MPs shows a proportionate increase with size of the individual which is directly related to food intake (Dowarah et al., 2020; Saha et al., 2021) and this is well evident in the present study. Moreover the incorporation of MPs during the process of filter feeding depends on the availability of concentration of particles in their ambient environment. In general, uptake of MPs in the water column is mediated by inhalant siphon and is transported to the gills. Followed by the filtration the trapped particles are then transported by ciliary movement to the stomach, intestine and primary as well as secondary digestive tubules (von Moos et al. 2012). Non-digestive parts have a great chance to incorporate MPs by direct contact through adherence instead of ingestion (Beninger et al., 1999; Kolandhasamy et al., 2018). This might be the reason for the more or less similar occurrence of MPs in both digestive and non-digestive parts of *Villorita* sp.

Fibres were the dominant morphotypes of MPs ingested by both finfishes and shellfish of the VL. Microfibres of synthetic origin comprise an overwhelming fraction (>81%) of MPs found in marine environments and ingested by biota (UNEP, 2016; Naidu et al., 2018; Divya et al., 2019; Naidu, 2019). Rivers and estuaries are transitional systems which are potentially more prone to fibre contamination than the marine environment because of close proximity to point sources (Jabeen et al., 2017). Mizraji et al. (2017) reported the high abundance of fibres in the digestive tract of omnivorous fishes as observed in the present study. Many of the studies suggested that a major portion of MPs entered the aquatic system from domestic laundry discharges and also from the fragmentation of macroplastics including fishing gears (Browne et al., 2011; Welden and Cowie, 2016). Similar observations were also made from coastal and the brackish environment from India (Veerasingam et al., 2016; Sruthy and Ramasamy, 2017; Saha et al., 2021). Majority of the fibres might have entered the estuary from domestic laundry discharges and also from the fragmentation of fishing gears (Browne et al., 2011; Welden and Cowie, 2016).

During the present study transparent plastic particles were the dominant component in the water samples while black, red and blue coloured particles dominated in fish and shellfish samples. Many studies have reported high abundance of yellow and black coloured MPs from highly polluted aquatic environments (Endo et al., 2005; Ogata et al., 2009; Frias et al., 2010; Karapanagiotti et al., 2011). It is well known that most of the fishes are discriminate feeders that select their food based on preferential feeding factors (colour, size etc.). However, selected fishes are omnivores and mainly feed on detritus. The ingestion of dark coloured plastic particles, thus, might be co-captured along with food (Lusher et al., 2017; Ory et al., 2018). Clams on the other hand are indiscriminate feeders, collecting particulate matter generally in a non-selective manner. Recent study by Ward et al. (2019) also described the selective ingestion of microplastics based on physical characteristics in bivalves. The analysis of the relationship between the size of the organism and the size of the ingested MPs revealed that lower size group (size class I: 1-2 cm) preferentially consumed plastic particles of less than 0.5mm (> 65%) while ~ 75% of the ingested particles in the larger size group (size class III) falls under > 0.5mm in the larger size group. The size of mouth, feeding habit, and the structure of the intestines of organisms are the main factors that affect the ability to take up and accumulate MPs. Organisms are capable of ingesting more MPs as they grow. With growth, the mouth size, the structure of intestine, demand of energy etc. likely to increase the amount of water they filter, and hence, more MPs (Dowarah et al., 2020). For these organisms, MP uptake increases as particle size decreases, showing size-related effects (Jemec et al., 2016; Lei et al., 2018; Ziajahromi et al., 2018). Chlorinated Polyvinyl chloride (CPVC), Polyethylene (PE), Polypropylene (PP) and Polyester (PES) were the major types of MPs observed in the study. These are the major types of polymer reported in the aquatic environment due to their durability and wide range of application in fishing activities; packing and textile industries (Erni-Cassola et al., 2019). Veerasingam et al. (2016) reported the presence of PE and PP on Goa beaches, India. An earlier study conducted on the VL reported the occurrence of polyethylene, polypropylene and polystyrene in the sediment of the lake (Sruthy and Ramasamy, 2017). Naidu et al. (2018) reported the presence of polystyrene MPs in the benthic invertebrates from the coastal waters of Kochi. Devi et al. (2020) reported the occurrence of PP, Nylon 6, PET and PBT in the gut content of alien fish *Piaractus brachypomus* collected from the VL. Further studies are required to quantify the degree of pollution and its effect on the biota.

5. Conclusions

The VL, an ecologically and economically significant system, is vulnerable to pollution by various anthropogenic inputs, particularly by plastics. This transition ecosystem often serves as pathways for the transport of these inputs and MPs to the oceans. Studies on MPs in estuarine environments are

meagre in India and so, the current study revealed the occurrence of high concentration of microplastics in the water column and also indicated widespread ingestion of MPs by bottom dwelling finfishes and shellfish in the VL. As sediments are the ultimate sink for all pollutants present in the water column, deposit and filter feeders are highly vulnerable to ingest the MPs present in the benthic boundary layer because of their non-selective feeding behaviour. Fibres and fragments with size less than 1.5 mm were the dominant morphotype of MPs obtained from water, finfish and shellfish samples. This implies the high bioavailability of MPs to the bottom dwelling finfishes and shellfishes of the VL. In the present study *A. maculatus* and *Villorita* sp. ingested more MPs, making them candidate species for monitoring MPs in the VL and similar habitats. The occurrence of MPs in the digestive and non-digestive parts of shellfish further underlines the risk and high levels of plastic pollution in the VL and the possibility of MPs getting incorporated in them via adherence over and above ingestion. Consequently, the predation on these organisms by the higher trophic organisms may be a pathway for the transfer of MPs up the food chain. As benthic invertebrates are a major component of some fish diets, effects of microplastic on benthic species could influence higher trophic levels. Moreover, the degree of MP pollution in the non-digestive tissue in *Villorita* sp. has direct implications for human populations in the region, as it is commercially harvested for food in and around the VL. Further detailed studies on spatio - temporal aspects of MPs in water, sediment and biota are required to make sure about, their sources, distribution and rate of accumulation in these ingested organisms and the transfer of MPs across aquatic food webs, as they have a potentially cascading effect on the ecosystem and on human health.

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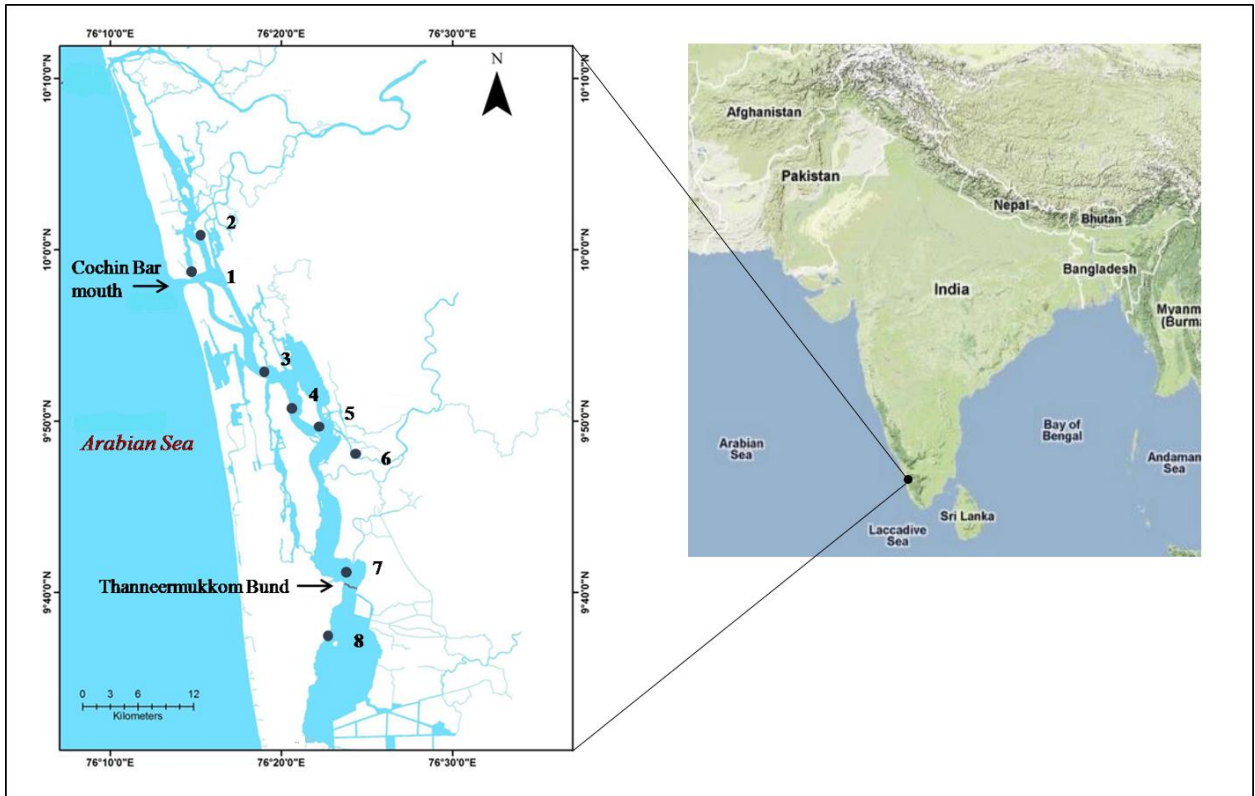


Fig 1: Map showing the study area and sampling sites.

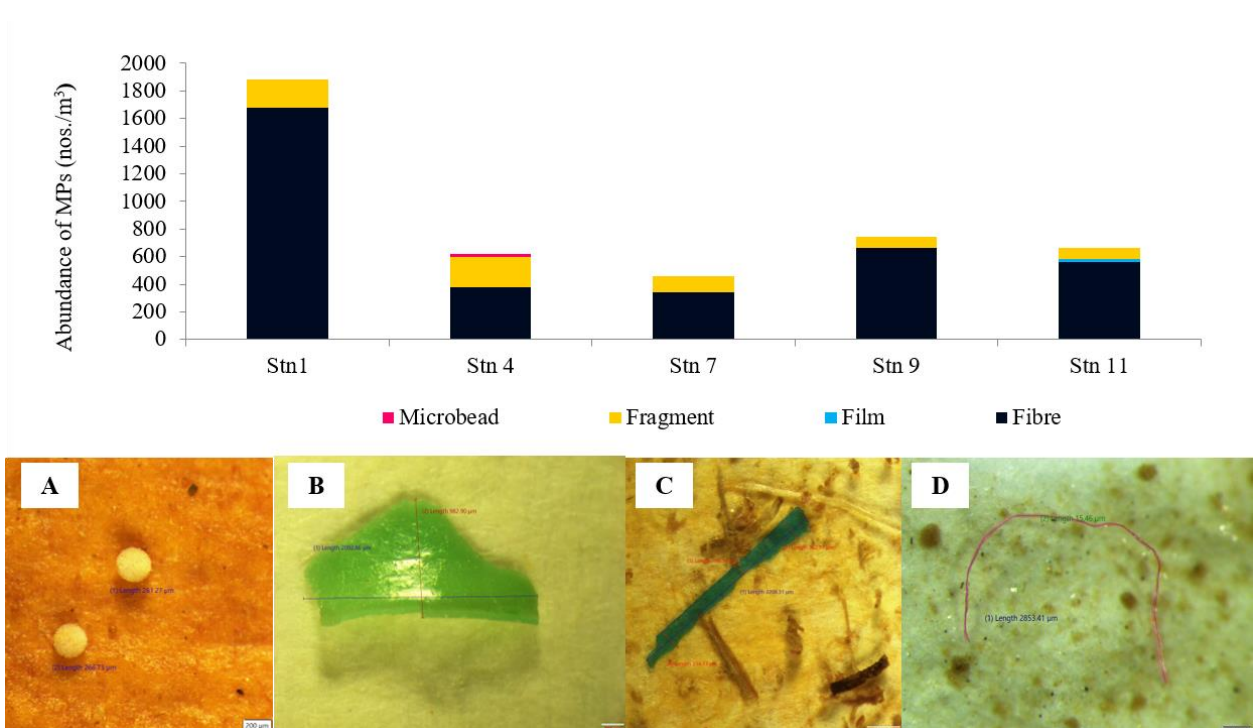


Fig 2: Abundance of microplastics (nos./m³) and morphotypes in water samples. A: white microbead, B: green fragment, C: blue film, D: red fibre.

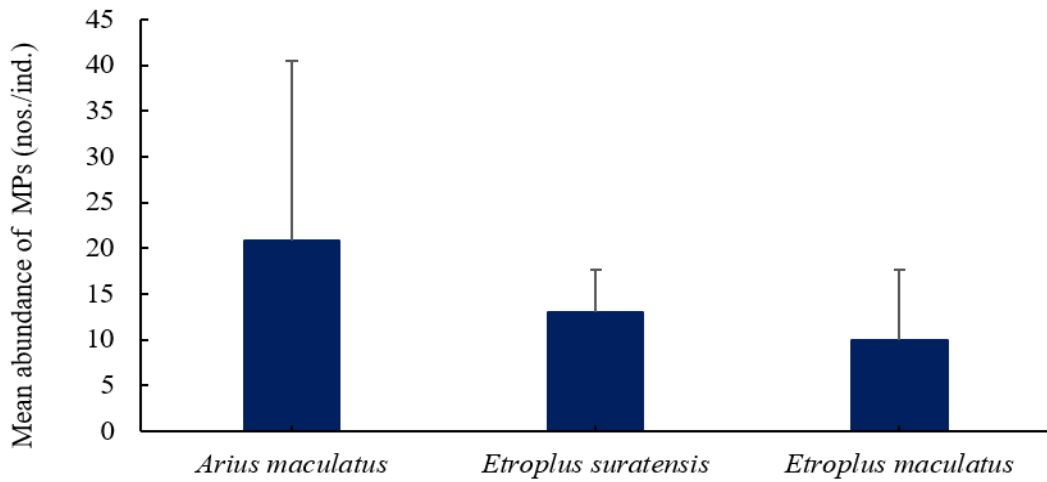


Fig 3: Mean abundance of microplastics in fish samples.

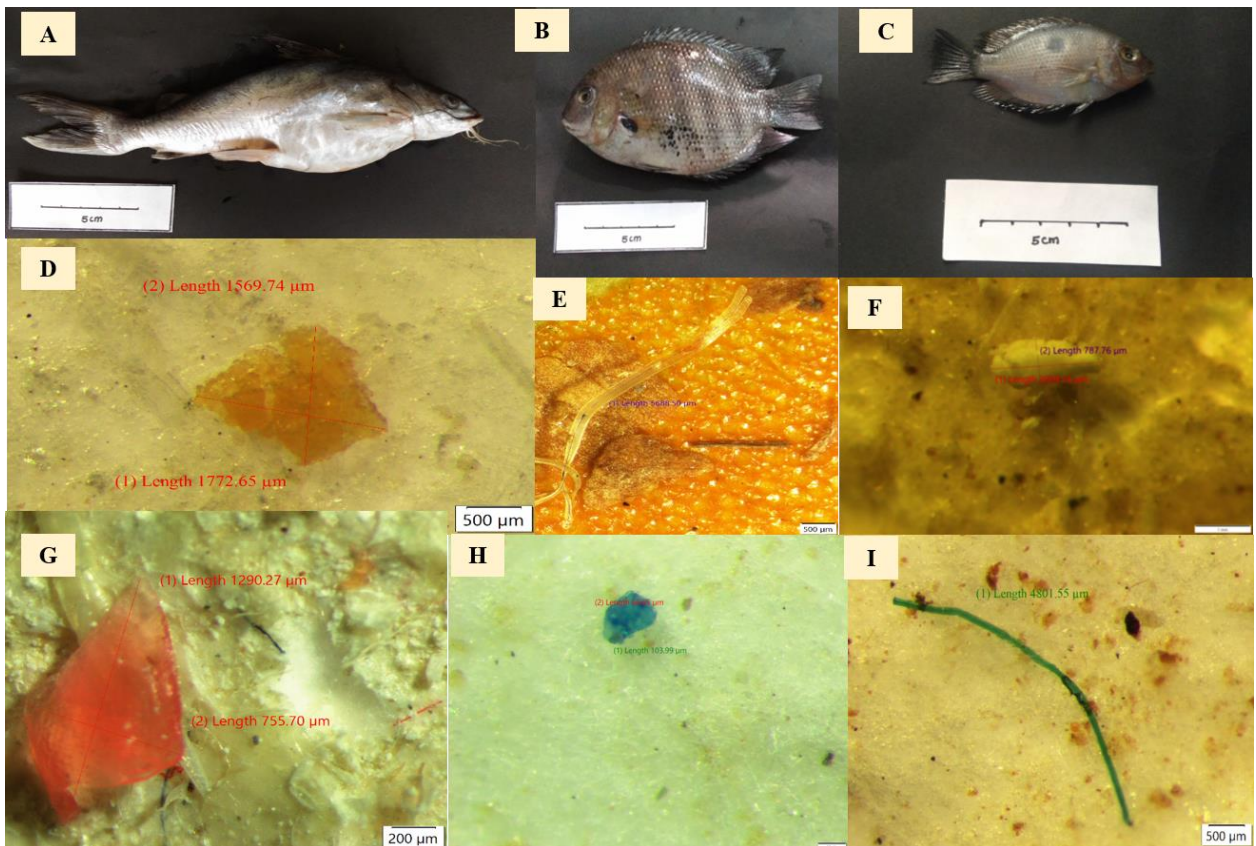


Fig 4: Three species of finfishes selected for the study and microplastics recovered from these species. A: *Arius maculatus*, B: *Etroplus maculatus*, C: *Etroplus suratensis*, D: yellow fragment, E: transparent film, F: white foam, G: red fragment, H: blue microbead, I: green fibre.

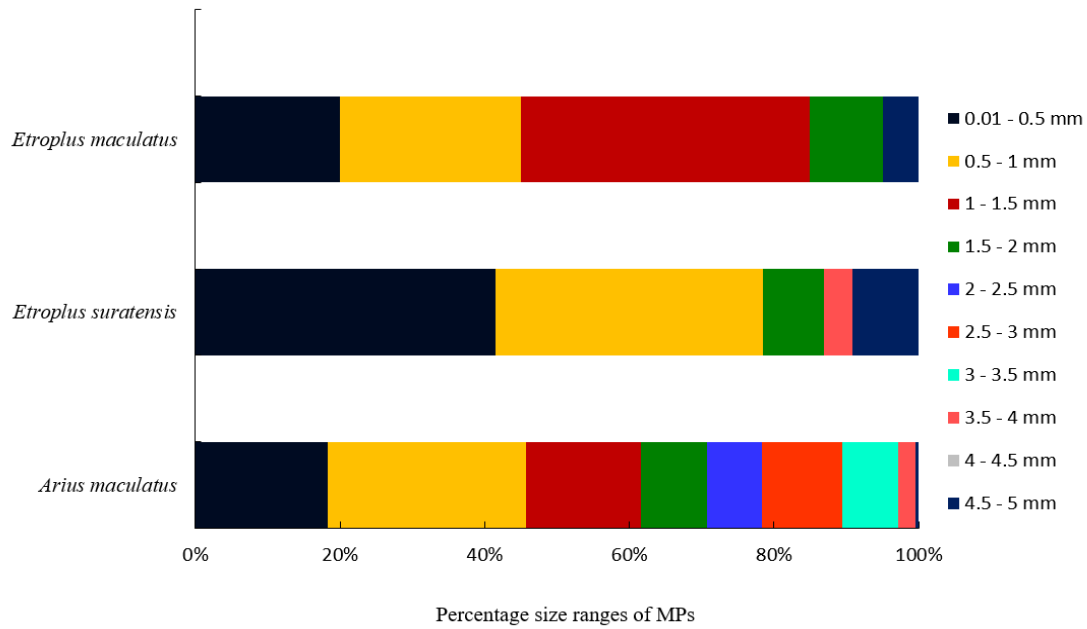


Fig 5: Percentage of different size range of microplastics in fishes.

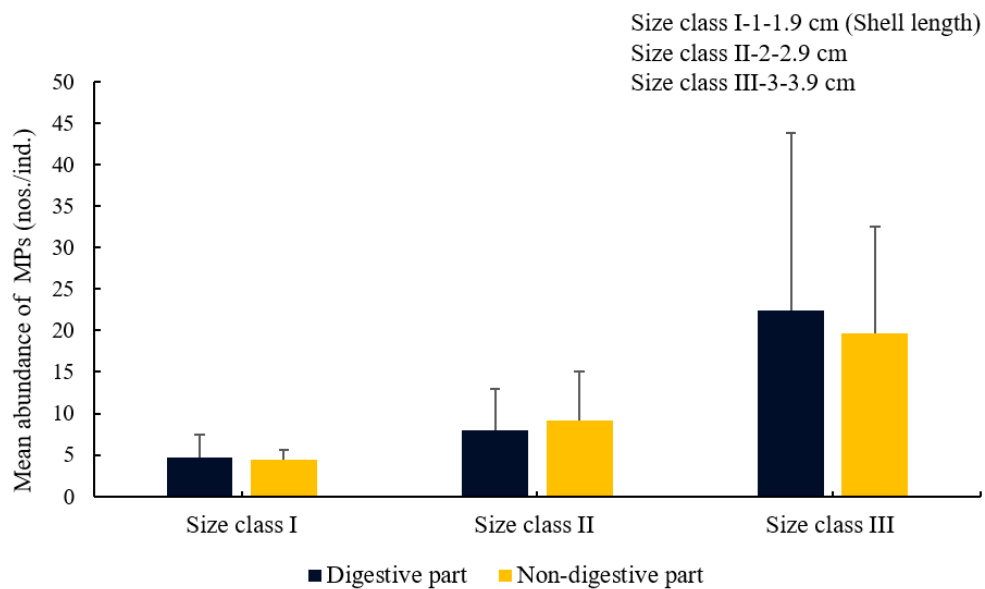


Fig 6: Abundance of microplastics in all size classes of shellfish (*Villorita* sp.). Based on shell length: Size class I - 1 to 1.9 cm. Size class II- 2 to 2.9 cm. Size class III - 3 to 3.9 cm.

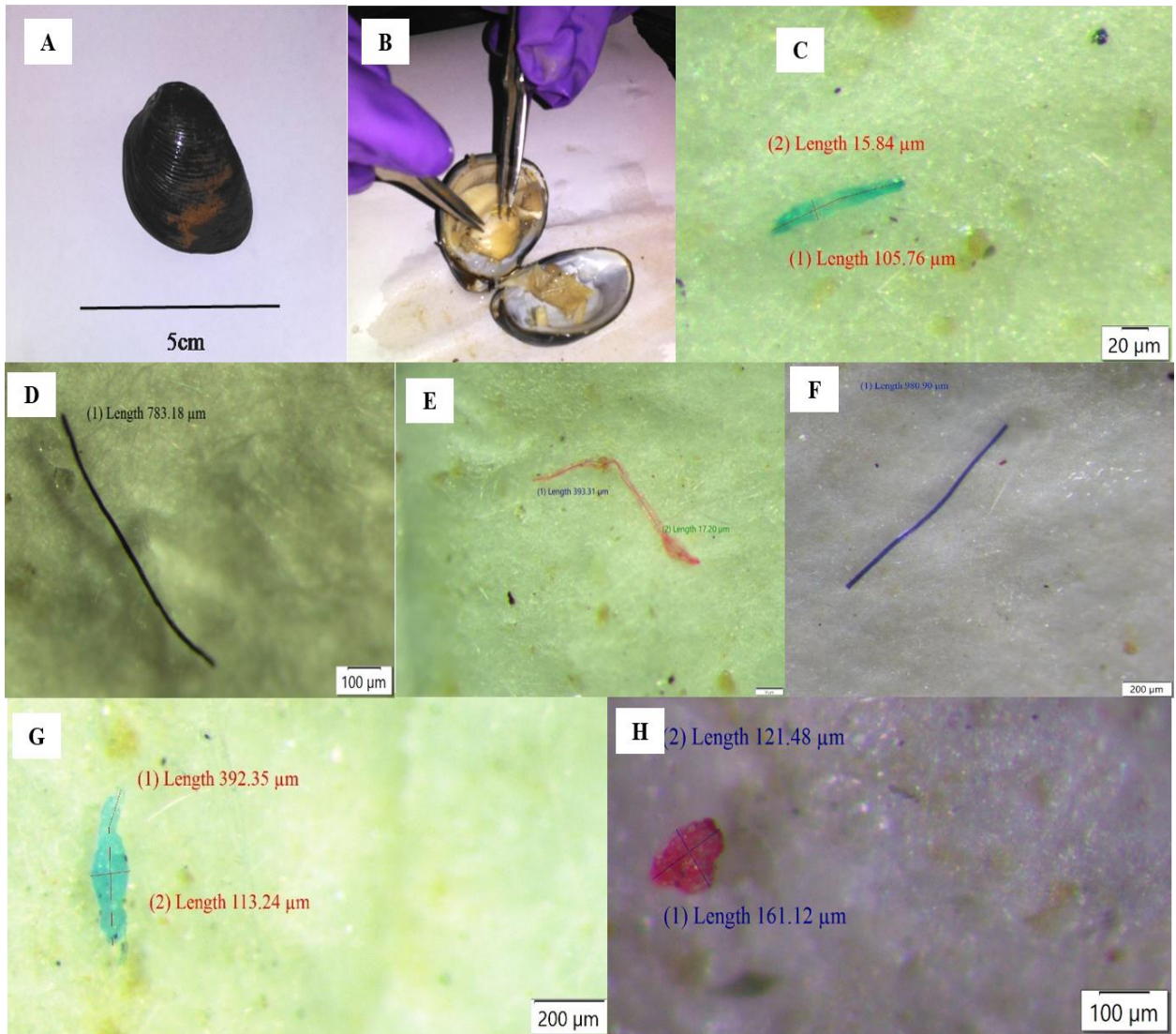


Fig 7: Microplastics extracted from the shellfish: *Villorita* sp. A: *Villorita* sp., B: dissection of *Villorita* sp. C: green fragment, D: black fibre, E: red film, F: blue fibre, G: blue fragment, H: red foam.

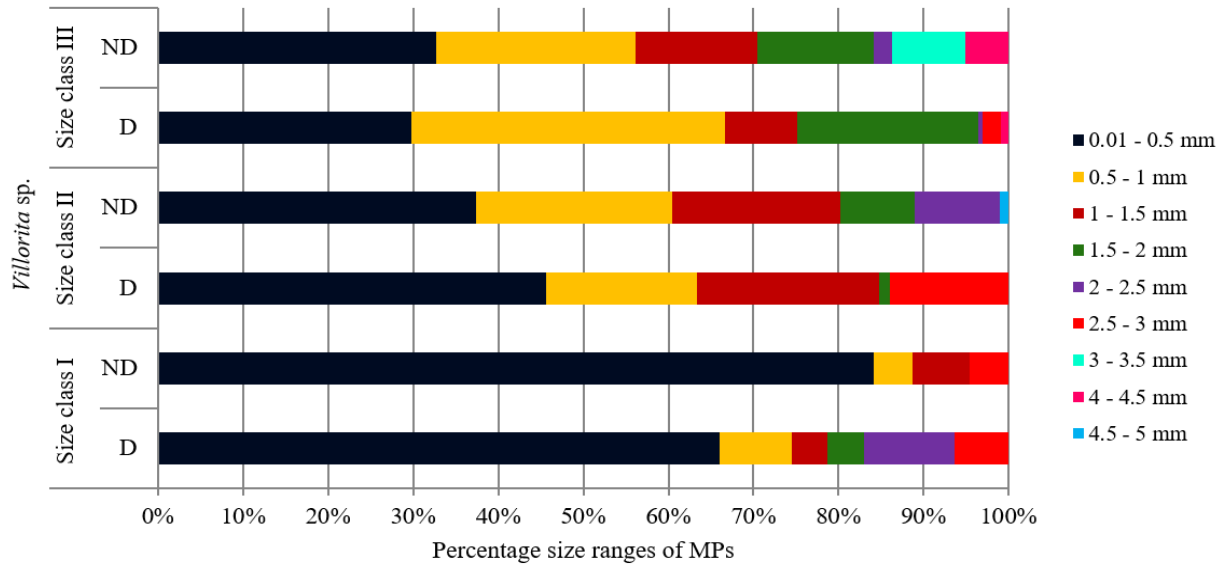


Fig 8: Percentage of different size range of MPs in *Villorita* sp. D: Digestive, ND: Non-digestive parts.

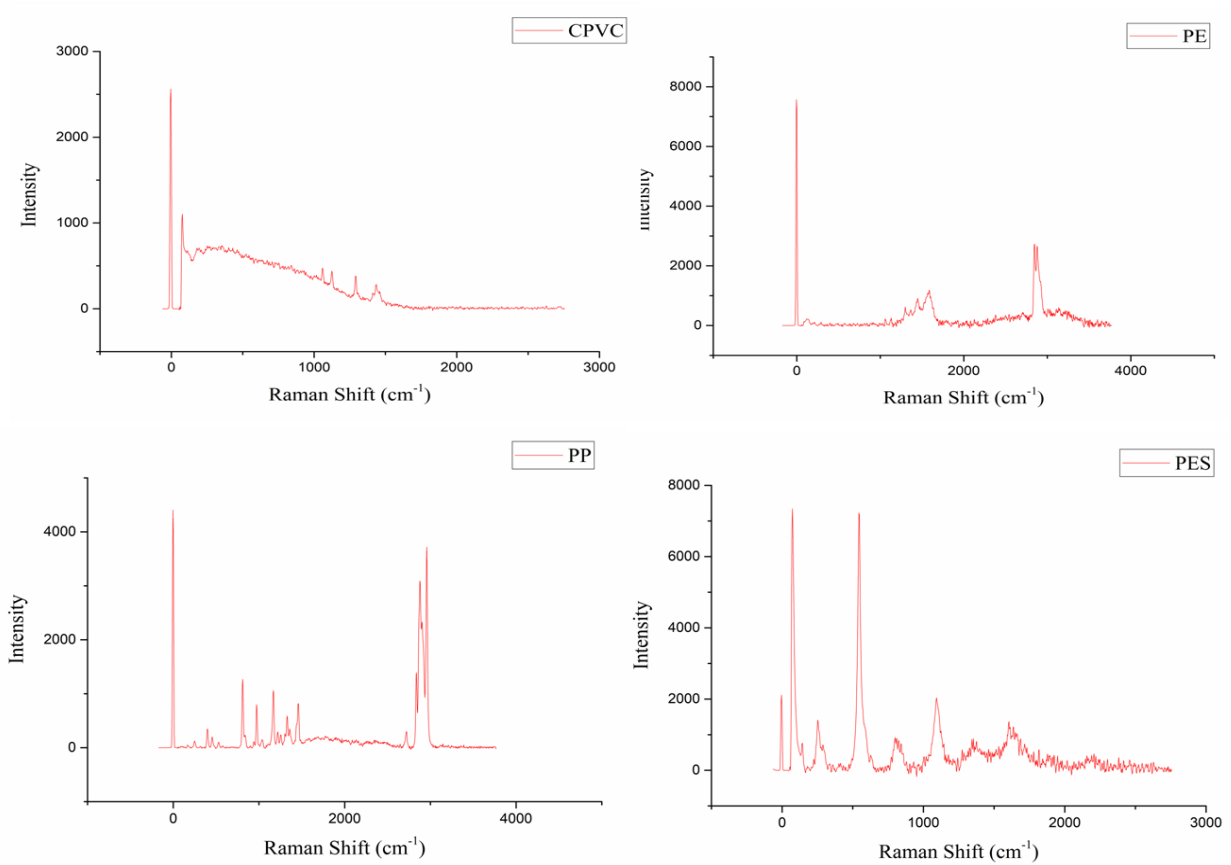


Fig 9: Raman spectra of selected MPs: Chlorinated Polyvinyl chloride (CPVC), Polyethylene (PE), Polypropylene (PP) and Polyester (PES).

	Standard length (cm)	Weight (g)	Upper-maxilla length (UML) (cm)	Mouth gape (cm)	Total length of gut (cm)
<i>Arius maculatus</i>	18.7 - 22.4	102.1 - 197.6	1.4 - 2.4	1.67 - 2.19	45 - 80
<i>Etroplus suratensis</i>	7 - 11.9	40 - 100	0.5 - 0.6	1 - 1.09	34.1 - 71
<i>Etroplus maculatus</i>	5.3 - 6.4	6.49 - 10.37	0.4 - 0.5	0.89 - 1.0	11.8 - 18.3

Table 1: Measured morphological characteristics of the fish.