Chapter- 1: Introduction

1.1 Background Study

Microplastics (plastic particles less than 5 mm in size) are a contaminant that ecotoxicologists are becoming more concerned about for both human health and aquatic habitats (Akdogan, Z., & Guven, B. 2019). MPs can be stated as the smaller particles (<5 mm) of synthetic plastic polymers which are very persistent and ubiquitous in all the environmental media (Anderson et al., 2015).

The introduction of more plastic particles into coastal habitats has been facilitated by rapid urbanization and related human activity. According to size, plastic debris can be categorized into macro (>25 mm), meso (25-5 mm), micro (<5 mm), and nano (<1000nm) particles (Cozzolino et al., 2020). Micro plastics (MPs) mostly originate from domestic washing of synthetic clothing, tires, city dust, road markings, and marine coatings, and end up in rivers or directly into the sea (Nematollahi et al., 2020).

These particles come from the direct release of smaller plastics such as pre-production pellets, cosmetics, personal care items and cleaning products into the environment, which are known as primary microplastics, or from the gradual wear and tear of larger objects during use and after they are vanished to the environment which are known as secondary microplastics (Kershaw et al., 2019). Their smaller size and low density contribute their long-range transport (Cozar et al., 2017; Barboza et al., 2019) and global distribution (Cozar et al., 2014; Suaria et al., 2016; Auta et al., 2017). Plastics degrade mechanically and thermally into pieces of various sizes and shapes (Barnes et al., 2009).

MPs are highly persistent in the nature and marine plastic debris assume to hamper not only the organisms but also ecosystem properties and services which includes, fisheries, navigation, tourism which may influence the society and economy adversely (Harding, S. 2016; Guzzetti et al., 2018). Microplastics are easily ingested by marine species because of their smaller size. There is a lot of evidence that commercial species ingest MPs (Fossi et al., 2016; Scopetani et al., 2018; Azevedo-Santos et al., 2019; Barboza et al., 2020). The weathering processes as like: photo-degradation, oxidation, and mechanical abrasion cause plastic trash gradually break down into smaller pieces in the marine environment

(Thompson et al., 2009; Andrady, 2011) and the plastic particles smaller than 5 mm are referred to as microplastics (Anderson et al., 2015).

Microplastics can remain in the marine and other environments for many years (Strungaru et al. 2019; Barboza et al., 2019) some of them are often available to a wide range of organisms, which includes species that are used for the human consumption (Barboza et al., 2018; Gallo et al., 2018). Coastal nations produced approximately 275 million tons of plastic waste, of which it is believed that 2-5% ended up in the oceans (Jambeck et al., 2015).

Microplastics, which can be found in the aquatic ecosystem as fragments, fibers, granules, spheroids, pellets, beads, or flakes with a size of 0.1-5000 m, may be produced by human activity directly (primary MPs) or by the mechanical breakdown, biodegradation, and photodegradation of the larger plastic objects (secondary MPs) (Cozzolino et al., 2020). The majority of MPs found in marine environment were composed by polyethylene (PE; fishing gear), polyvinyl chloride (PVC; plastic coatings for the freight transport and bottle tops), polyethylene terephthalate (PET; water bottles), polypropylene (PP; from the fragmentation of soft plastic bags, and food packaging film) (EFSA, 2016; Avio et al., 2015; Mercogliano et al., 2020). MPs have the potential to move from one trophic level to the next of the marine food chain when they are consumed by marine creatures, which cause direct physical harm and potential toxicological effects. Microplastics can be transferred and accumulate in different tissues of organisms, undergoing biomagnification along the food chain (Barboza et al., 2018; Mercogliano et al., 2020). Several processes are involved in methods for identifying MPs, including chemical identification and optical identification of the plastic polymers (Bessa et al., 2019; Lusher et al., 2017). After completing the removal, cleanup, and isolation (or separation) of plastic particles from biological tissues, visual identification is done (Foekema et al. 2013; Lusher, 2015). The crucial steps are the extraction of microplastics from the whole organism or their tissues (Foekema et al. 2013; Lusher, 2015). Traditionally, MPs which lies between 1–5 mm size range, such as colorful plastic compounds and pre–production resin pellets, are identified by visual sorting with the unaided eye. Based on information about the surface roughness and particle structure, stereo-microscopy may be used to detect particles with size range in the hundreds of micron (Shim et al., 2018). The chemical characteristics of polymer define the composition of the extracted microplastics and it can be carried out through Fourier transform infrared (FTIR) spectroscopy, Thermo-analytical techniques, Raman spectroscopy (Catarino et al., 2017).

1.2 Microplastics as pollutant in Fish

Microplastics (MPs) may be intentionally consumed by fish along with their prey. When the prey adheres to or consumes the microplastic, accidental consumption has additionally been documented (Jovanovic, 2017). Research on fish consumption of microplastic has been done all around the world (Davison and Asch, 2011; Boerger et al., 2010; Neves et al., 2015; Cole et al., 2015; Guven et al., 2017). Recently, it was found that there is no connection between the trophic level of fish and the microplastics they consume (Guven et al., 2017). Fish (Kripa et al., 2014), benthic fauna (Scruthy and Ramasamy, 2016; Naidu et al., 2018), and crustaceans all have been found to consume microplastic in India (James et al., 2020).

Therefore, top predatory animals like marine mammals (Deudero and Alomar, 2015) and humans (Crawford and Quinn, 2016) may accumulate higher quantity of microplastics as active elements of trophic interactions. According to various studies, microplastics harm organisms by causing physical harm and inflammation, altering feeding and reproductive activity, blocking the digestive tract, being toxic to cells and lowering the progeny survival rate (Cole et al., 2015; Savoca et al., 2019; Proki et al., 2019; Strungaru et al., 2019). At the sea surface, low-density objects predominate (Cózar et al., 2015). Fish regularly eat microplastics by foraging in the water column (Boerger et al., 2010). Microplastics may be stored in the digestive tract or may move through the gut after being swallowed by organisms, with possible physicochemical impacts (Browne et al., 2008).

According to research by Lusher et al. (2013), fibers can obstruct feeding appendages or, clog digestive tracts and restrict the passage of food (Gregory 2009; Ryan et al, 2009), and also result in a false sense of satiety that reduces appetite (Ryan, 1988). Ingested microplastics may have physical and chemical effects on aquatic species, with the latter being modified by the presence of additives and adsorbed organic compounds (Barboza et al., 2019). The discovery of microplastics in the stomachs of numerous economically significant fish species raises serious concerns for human health due to the potential effects of the transfer of these microscopic plastic items and associated contaminants to edible fish tissues. (Fossi et al., 2018).

Bombay duck, or *Harpadon nehereus* is one of the most commercially significant species, which made up more over 10% of all marine catches derived from the Bay of Bengal (BOB), Bangladesh, over the past few decades (DoF, 2018). This has a high demand among the people of Bangladesh and also has a strong export value both in fresh and dry conditions (Sarker et al., 2017).

1.3 Significance of the Study

Microplastics have been identified as serious concern pollution for more than 20 years and have been found in the Polar Regions and on every continent. (Auta et al., 2017; Andrady, 2017; Chen et al., 2018). It has been determined that terrestrial human activities such as industrial manufacture, agriculture, and municipal solid waste landfilling are the main causes of the buildup of microplastics in the oceans (Vandermeersch et al., 2015; Driedger et al., 2015).

Some previous studies shown that synthetic fibers are the dominant type of polyester microplastics detected in water, sediments and various organisms (Lourenco et al., 2017; Abbasi et al., 2018; Halstead et al., 2018). Almost 90% of the microplastics that are prevalent in coastal areas around the world are made of synthetic fibers (Barrows et al., 2018). The major sources of synthetic fibers found in the aquatic environment have been identified as wastewater, produced by commercial and home textile laundries (Browne et al., 2011). The procurement of plastic trash in the marine environment may be caused by the increasing usage of plastics and their inappropriate handling and disposal. There are many different types of plastic waste in the environment, mostly derived from industrial effluents (Karlsson et al., 2018), fragmentation of larger plastic items (Mani et al., 2015) domestic discharge (Browne et al., 2011), and they come in a different range of sizes, from meters to micrometers. Research on fish consumption of microplastic has been done all around the world (Boerger et al., 2010; Davison and Asch, 2011; Neves et al., 2015;

Cole et al., 2015; Guven et al., 2017). The presence of microplastics was also recorded in Bangladesh from fish species (Hossain et al., 2019, 2020; Parvin et al., 2021) and sea salt (Parvin et al., 2022).

A coastline community called Patenga is 14 kilometers south of Bangladesh's coastal city of Chattogram. It is close to the Karnaphuli River's mouth. Almost 800 industries are located near to the banks of the Karnaphuli river in different areas, including oil refineries, tannery factories, fish processing plants, chemical industries, textile mills, Triple Super Phosphate (TSP) plant, Karnaphuli Paper Mills (KPM), Chattogram Urea Fertilizer Ltd (CUFL), paint and dye manufacturing units, and the Karnaphuli Fertilizer Company Ltd (KAFCO) (Hossain et al., 2005; Bhuyan, M. S., & Islam, M. S. 2017). This river accumulates huge amount of untreated effluents from industries such as oil refineries, spinning mills, dying, , textile, cotton, steel mills, and others (Ali et al.,2016), these effluent flows to the sea near Potenga beach. Shipping, transportation and tourist activities are also equally responsible for the pollution. Millions of locals and tourists visit Patenga (Bashar, R., & Nandy, A.2019) every year due to its free accessibility and various attractions like sunrises/sunsets, street-side Burmese markets, sea-food restaurants and it is a popular beach area in the country. So microplastic may be very available here. But there was no work done on Potenga beach regardless the plastic pollution, that’s why the area was chosen. As microplastic is a burning issue all over the world, that’s why it is important to identify the microplastic presence in the fishes that are collected from Potenga beach, Chattogram. The thesis aimed to find out the microplastic presence, categorize them and also assessing their chemical composition.

1.5 Objectives of the Study

I. To identify micro plastics from the fish (*Harpadon nehereus)* gut collected from Potenga Sea Beach, Chattogram.

II. To categorize the micro plastics according to their type, shape, size and colour.

Chapter -2: Review of Literature

2.1 Plastics use in the oceanic environment

The majority of studies have their concentration on "ghost fishing" done by abandoned gear in the benthic region (Bullimore et al., 2001; Tschernij and Larsson, 2003) as well as the entanglement of marine mammals (Laist, 1997), cetaceans (Clapham et al., 1999), and other organisms (Erikson and Burton, 2003) in net originated litter. Acording to Andrady, 2011, the micro cracking and embrittlement of plastic compounds are caused by weathering on beaches, which produces microparticles that are dispersed into water by wave or wind action. In contrast to the inorganic fines prevalent in saltwater, microplastics concentrate persistent organic pollutants (POPs- Persistent organic pollutants) by partition. The yearly demand for plastics on a global scale has been rising steadily in the recent years and is currently estimated to be around 245 million tons (Andrady, 2003). Plastics are preferably suited for a range of applications since they are a lightweight, versatile, strong, and potentially colorless material. Traditional materials as like metal, glass, and paper are being replaced with more affordable plastic packaging along with designs that are either similar or better. Due to this, around a third of the plastic resin produced is used to create consumer packaging material, which includes single-use, disposable items that are regularly discovered in beach debris (Andrady, 2003). Polyethyelene (PE), Polystyrene (PS), Polypropylene (PP), Polyvinyl chloride (PVC) and Polyethylene terephthalate (PET) are among the main kinds of polymers that are used in packaging. Their production reflect their high volume of use, and as a result, these in particular have a significant potential of ending up in the oceanic environment (Ribic et al., 2010). The future incorporation of plastic garbage into the oceans will be increased due to extensive fishing, marine uses of the water, recreation and shifting demographics favoring immigration to coastal locations, and other factors. The whole world's fishing now uses plastic equipments, and part of it is recklessly dumped or inevitably lost at ocean while being used (Watson et al., 2006). Mostly fishing gear uses nylon and polyolefin (PE and PP) (Timmers et al., 2005). Around 18% of the marine plastic waste that is present in the ocean environment comes from the fishing industry. Aquaculture is a big contributor to the ocean's plastic waste problem. (Hinojosa and Thiel, 2009).

2.2 MPs in the marine environment

Plastic production has significantly increased in recent decades as a result of poor waste management methods in different regions around the world (Lusher et al., 2017a). Plastics are lightweight, long-lasting, durable and inexpensive synthetic or semi-synthetic organic polymers (Vedolin et al., 2018).

MPs primarily come from synthetic garments washed at home, city dust, tires, road markings, and marine coatings, and they finally ends in rivers or the ocean. Most of the MPs litter that is dumped on land is washed away by the surface runoff, where it eventually degrades into secondary microplastics (Nematollahi et al., 2020). An increased amount of plastic particles have been introduced in the marine habitats as a result of rapid urbanization and related human activity. According to size, plastic debris is typically divided as macro (>25 mm), meso (25-5 mm), micro (5 mm), and nano (1000nm) Particles (Cozzolino et al., 2020). Despite ongoing discussions on their real ecological effects, microplastics are so common that worries about their possible hazards to creatures, including humans, have been raised (Wright and Kelly, 2017; Imhof and Laforsch, 2016; Conkle et al., 2018). As an example, the major microplastics are in fact plastic microbeads produced as exfoliates in the size range mentioned above for insertion in personal care products (Browne et al., 2011; Wang et al., 2016).

Plastics are vast class of synthetic polymers that can be used to create a variety of finished goods, including polyethylene, nylon, polyvinyl chloride, and polystyrene (Leal et al., 2019). It has been found in nearly all aquatic settings, including rivers, oceans, lakes, estuaries, reservoirs and even the arid parts of Antarctica and the Arctic Ocean (Villegas et al., 2021).

Even though it is very hard to remove microplastics from contaminated environments, establishing a waste management system that includes an efficient control of the pollution source is seen as a proper method to limit the danger of microplastic contamination. (Anderson et al., 2015; Estahbanati and Fahrenfeld, 2016).

2.3 Microplastics (MPs) in fish

According to Barboza et al, 2020 the gastrointestinal system contained more microplastics (MP) than the gills. The most often found polymers are polyester and polyethylene. Wild fish that have consumed MP have shown signs of neurotoxicity and oxidative damage.

Because of its great resistant capacity, marine plastic debris is anticipated to significantly affect the economy and society as well as various ecosystem properties and services, such as fisheries, navigation and tourism, (Guzzetti et al., 2018). Fish usually consume microplastics through foraging in the water column (Boerger et al., 2010). As an example, in the North Pacific Subtropical Gyre mesopelagic fishes consumed plastic fibers, films and filaments, (Davison and Asch, 2011). The quantity and variety of plastic waste and other food items discovered in the fish guts taken by local fishermen were evaluated by Silva-Cavalcanti et al. in 2017. The majority of plastic debris found in the intestines of a fish species has been found in 83% of the fish we examined. Microplastics (5 mm) made up the majority of the plastic waste retrieved from fish guts (88.6%), with fibers being the most common form (46.6%). It was discovered that fish swallowed more microplastics in the river's urbanized parts and that this consumption was inversely connected with the variety of other foods found in each fish's gut. Its findings indicate that urbanization is a significant driver in the contamination of freshwater habitats with microplastics and that freshwater biota is susceptible to microplastics pollution.

The physical effect of plastic waste on organisms includes, entanglement, ingestion, and suffocation/asphyxia, has been the subject of the most research (Barnes et al., 2009; Ryan et al., 2009). According to Laist, 1997, 270 taxa from various trophic levels (Cole et al., 2011) have reported ingesting plastic trash, making this the most frequent effect Fish are one of the taxa most impacted, with several accounts of various species ingesting plastic trash. For instance, an investigation of the fish stomach gathered in the North Pacific revealed that between 20% to 35% of the fish had detritus, particularly plastics, inside their guts (Boerger et al., 2010; Choy and Drazen, 2013). According to a different study, the guts of 26% to 52% of the fish that caught in the English Channel, contained plastic waste (Lusher et al., 2013). According to Peters and Bratton (2016), incidental ingestion of MPs happens when objects are swallowed along with natural foods. Trophic transfer also occurs when fish eat prey that has consumed plastic waste (Cedervall et al., 2012; Mattsson et al., 2015). As opposed to accidental ingestion, purposeful ingestion happens when an animal intentionally ingests a plastic particle after mistaking it for food (Ivar do Sul and Costa, 2007). Although little is known about the biological effects on fish after consuming plastic waste, the few research that are now available on the subject which indicate that it may be exceedingly detrimental. According to Peda' et al. (2016), fish that consume plastic waste may experience intestinal damage, which could hinder their capacity to absorb nutrition. Moreover, consuming plastics may alter fish behavior and their capacity to detect predators (Mattsson et al., 2015; Lonnstedt and Eklov, 2016). Moreover, fish immune systems are hampered (Greven et al., 2016) and their metabolic functions, such as fat metabolism (Cedervall et al., 2012; Mattsson et al., 2015) are affected when plastic debris is ingested. Last but not least, pollutants produced from eaten plastic can have hazardous effects including stressing out fish's livers (Rochman et al., 2013; Lu et al., 2016). These pollutants have the potential to reach humans through bioaccumulation and biomagnification in the food chain (Silva-Cavalcanti et al., 2017). Due to several instances of fish species used as food resources by humans ingesting plastic, plastic waste has been identified as a possible danger in commercial fisheries (Rochman et al., 2015) and seafood which derived from the aquaculture (Van Cauwenberghe and Janssen, 2014).

Chapter -3: Materials and Methods

3.1 Study area

The study was conducted on Kathgorh Bazar, Potenga (Latitude. 22.25957°; Longitude. 91.79161°) (Figure: 1). Potenga beach is one of the most popular beach of Chattogram, which is equally important for tourism and shipping. It is also ecologically important as it is connecting place of Karnafuli River and Bay of Bengal. The Potenga beach is affecting daily for thousands of tourist and shipping activities. The area is also important for shipping and many ships anchor in that region. These are alarming sources of plastic pollution in Potenga beach. Plastics are persistent in nature. They breakdown and form microplastics which are ingested by fish and other organisms. As an important tourist and port area of Chattogram Potenga beach is at a tremendous risk of plastic pollution.

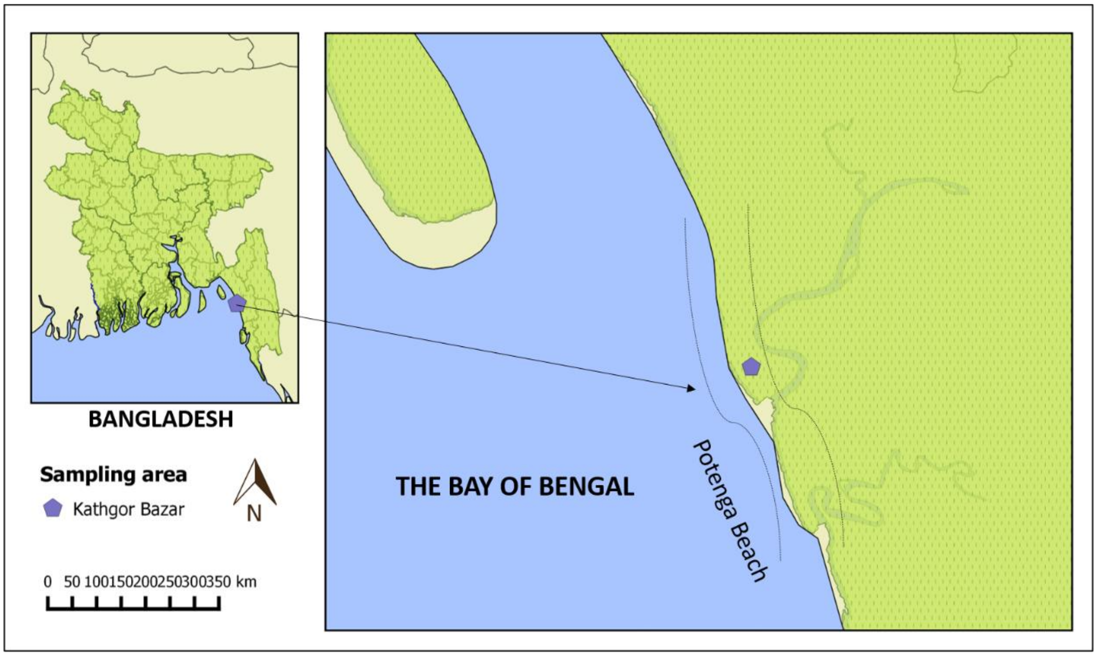

Figure 1: Map of the study area

3.2 Sampling methods

The sampling of *Harpadon nehereus* was done from the Kathgorh Bazar (Latitude. 22.25957°; Longitude. 91.79161°), Potenga beach. The fish were collected in November 2022. The fish were mainly caught by the local fisherman. They bring the fish directly to the beach and sell them to the local fish vendors. These fish directly goes to the Kathgorh

Bazar, Potenga. For the study 96 specimens were collected. The fishes were chosen randomly. Fish were bought and preserved in ice box and immediately fetched in the Aquatic Ecology laboratory of Chattogram Veterinary and Animal Sciences University (CVASU). Then the fishes were preserved in -20^{o}C refrigeration for Micro plastic (MPs) assessment.

3.3 Laboratory analysis

3.3.1 Sample preparation

Weighting: Then the preserved samples were thawed in a steel tray with distilled water for easy gutting of the fishes. Total length, total weight of the fish were measured and recorded at first. Weight measurement was done through electric balance (Model: PS 1200.R2).

Gutting: The gastrointestinal tract (GT) of the fishes was removed. For removal sharp scissors, scalpel and forceps were used. The whole GT from esophagus to anus was removed carefully and kept in a petridish.

Gut weight: Then the sample weight was taken with electric balance and data were recorded. After measuring the weight, the GT were transferred into 800ml beaker for further analysis.

Digestion: Then the digestion of the gut samples was done with 200ml 30% hydrogen peroxide ((Li et al., 2015; Su et al., 2016). Many studies has shownthat, hydrogen peroxide (H_2O_2) solution is more effective to digest organic material (Nuelle et al., 2014; Mathalon and Hill, 2014; Avio et al., 2015) than sodium hydroxide (NaOH) and hydrochloric acid (HCl) (Cole et al., 2015).With some modifications from Avio et al.,2015, 200 ml of H_2O_2 were added into the beaker and the beakers were covered with foil papers and placed in shaking incubator (Model: S1500) at 38 ^{o}C and 80 rpm for 7 days according to the amount of gut content. More gut content require more time to digest.

Density separation: The density separation was done to separate the digested organic portion from the dissolved microplastics. The process was followed from Coppock et al., 2017 with some changes.

- After digestion the gut were totally dissolved and it would look like whitish water and no fragments were remain.
- Then separation was done. For this, 250 ml zinc chloride ($ZnCl_2$) (0.5gm/L) solution (Table: 1) was added into each beaker and kept 2-3 days or one week if settlement does not occur.
- The micro plastics floated upwards, whereas undissolved organic residues and inorganic matters sink at the bottom of the beaker (Lusher et al., 2017b).

Table 1: Zinc chloride ($ZnCl_2$) solution preparation: 1.5gm/Cm^3 density solution

$ZnCl_2$	Distilled water
500gm of $ZnCl_2$	1 Liter of Distilled water

Filtration and visualization: The density separator supernatant was filtered by vacuum pump filter machine (Rocker 300) using cellulose nitrite filter paper (pore size of 0.45 µm; diameter of 47 mm). Then the filter paper was taken into a clean Petridish for visualization. The filter papers were examined according to Masura et al., 2015, under electron microscope (Model: OPTIKA, B-192, Italy) at 40X magnification.

3.3.2 Microplastic type, color, shape, size identification:

- Microplastics less than 2mm were identified under the electron microscope and were measured according to Virsek et al., 2016 with some modifications.
- The separation of microplastics or to identify MPs from organic matters and debris were followed from Hidalgo-Ruz et al., 2012.
- After identifying the MPs they were measured, categorized according to colour and shape and noted for further analysis. Then they were measured using the PROVIEW software.
- Image of MPs were taken by using a digital camera (Model: OPTIKA CB3) which was connected with the microscope.
- By following the procedure of virsek et al., 2016. Mps were divided into 6 types: Fragments, Filament, Film, Foam, Pellets and Granules.
- The colors were divided into Black, Blue, Green, Red, Brown, White and Transparent and MPs were divided in these forms.

- The size classes were divided into 3 forms: <500 µm, 500 µm to < 1mm and 1mm to 5mm. MPs were classified according to these classes.
- Microplastics larger then 2mm were identified visually by using millimeter scale.
- The items were categorized visually (Hidalgo-Ruz et al., 2012) and narrated according to colour, maximum length and shape (Lusher et al., 2013). The morphology of the microplastics were classified into fiber, fragment and particle (Li et al., 2015) and verified according to their physical characteristics (Kolandhasamy et al., 2018). According to Li et al. (2015), microplastics were classified into three sizes in this study, they are: <500 µm, 500 µm to 1 mm, 1–5 mm. MPs were categorized into fragment, filament, angular, round, and irregular shapes as suggested by Lusher et al. (2017a, 2017b) and Hidalgo-Ruz et al. (2012). Each particle's longest or widest dimensions were calculated to the nearest micrometer. (Choy and Drazen, 2013; Phillips and Bonner, 2015). Microplastics were categorized into five different colours of blue, red, white/transparent, green, black.

3.4 Statistical analysis

The quantity of MPs was indicated as the number of particles per gram (g) of gut weight (MPs/g). Statistical analysis were performed by using Microsoft Excel and IBM SPSS (version 26) to assess if there were significant variation in MP abundance with total weight and gut weight. A correlation test and Tukey post hoc Tukey B^a analysis was employed to estimate the correlation between MPs abundance, total MPs item, total weight and gut weight.

3.5 Contamination control

- Fishes were collected, handled, transported, refrigerated and thawed carefully to prevent any contamination or damage.
- All the petri dish, scissors, scalpel and forceps were rinsed with filtered water to prevent risk of contamination.
- The working space was cleaned before work. Dissection of fish specimen and removal of GT was done properly. Avoid taking other organs and the GT was

placed in a clean petri-dish and immediately covered to prevent contamination by air- borne fibers (De Witte et al., 2014; Devriese et al., 2015).

- All the glassware were handled carefully and rinsed with filtered water (Hossain et al., 2019).

Chapter -4: Results

4.1 Fish weight, length and microplastic abundance

A total 96 fishes were sampled from the selected research stations and the mean weight (MW), mean gut weight (MGW), microplastics abundance (MPA) and mean plastic items were determined and documented in the Table 1. The MW, MGW were 129.75 ± 34.77 g and 6.16 ± 3.46 g, respectively (Table: 2). The recovered mean MPA and mean plastic items were 18.31 ± 7.17 item/g and 98.34 ± 53.11 items.Total weight (g) and length (cm) of the sampled fishes ranged from 72.01 to 188.45 and 23.40 to 31.80, respectively. The gut weight of the examined fishes was ranged between 0.98 to 9.91g. The mean plastic items of MPs were ranged between 25 to 198 items, again the mean abundance was 7.82 to 28.09 items/g respectively (Table: 2).

Table 2: Growth parameters of the studied fishes

Growth Parameter	Mean	Range
Mean weight (MW)	129.75 ± 34.77 g	72.01 - 188.45 g
Mean gut weight (MGW)	6.16 ± 3.46 g	0.98 - 9.91g
Microplastics abundance (MPA)	18.31 ± 7.17 item/g	7.82 - 28.09 items/g
Mean plastic item	98.34 ± 53.11 item	25 - 198 item

4.2 Variations of MPs based on total weight

The highest numbers of MPs were found in the weight class 170 to 190g (186.06 ± 11.96 items) (Figure: 2), while the lowest total MPs were found in the lowest weight class 70 to 90g fish weight (38.38 ± 9.09 items). The 130 to 150g total weight size class had the least abundance of MPs (12.36 ± 3.09 items/g) and 70 to 90g size class had the highest abundance of MPs (29.31 ± 6.73 items/g) (Figure: 2). The proportionate of the MPs item were increased when the weight was also increased but the MPs abundance didn’t show positive relation with the increasing weight, which denotes that, the abundance of the MPs didn’t increase as much as the weight of the sampled fishes.

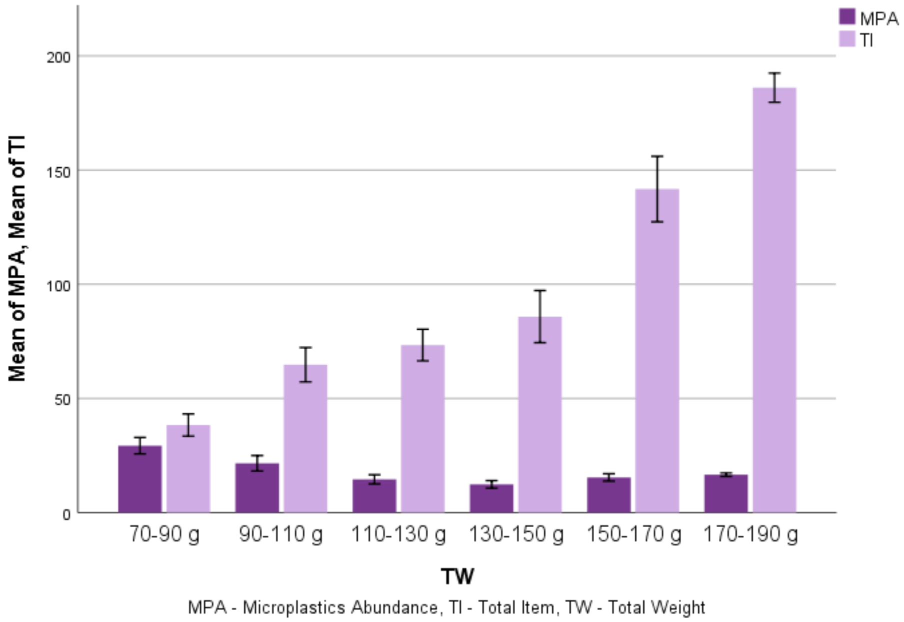

Figure 2: Variations of MPs abundance and total items with the total weight. (MPA-Microplastics abundance, TI - Total item, TW- Total weight)

4.3 Microplastics abundance based on the gut weight

The total items and microplastics abundance show relation with the Gut Weight (Figure: 3). The highest total items were found in highest gut weight 10 to 12g (186.06 ± 11.96 items) and the lowest items were found in the lowest gut weight 0 to 2g (38.38 ± 9.09), which reveals that the items number increased with the gut weight and showed positive relation. The highest mean abundance of MPs was found in 0 to 2g gut weight (29.31 ± 6.73 item/g) and the lowest abundance was found in 6 to 8g gut weight (12.36 ± 3.10 item/g) respectively. The proportionate of the MPs item were increased when the gut weight was also increased but the MPs abundance didn't show positive relation with the increasing gut weight, which denotes that, the abundance of the MPs didn't increase as much as the gut weight of the sampled fishes.

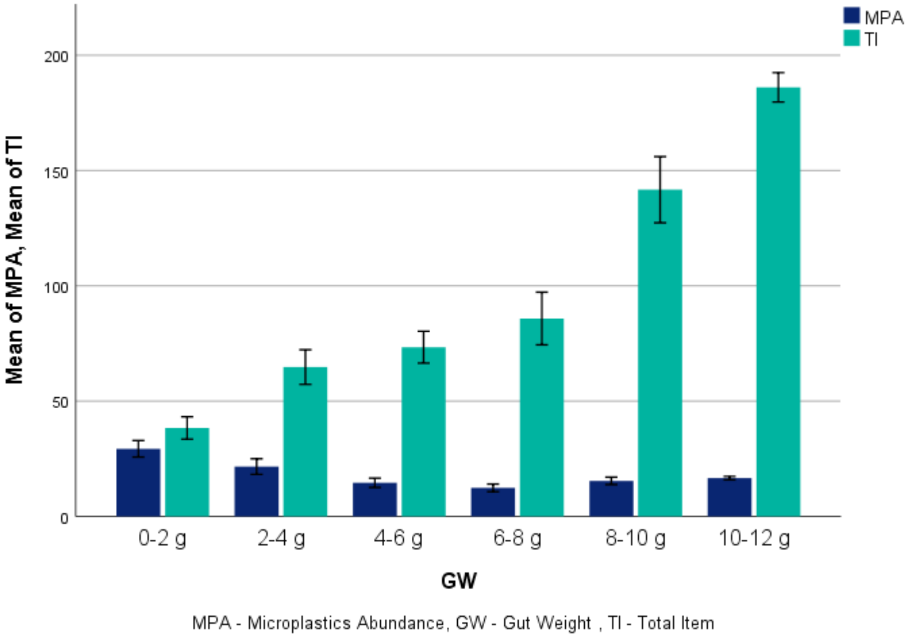

Figure 3: Variations of MPs abundance and total items with the gut weight**.** (MPA- Microplastics abundance, TI - Total item, GW- Gut weight)

4.4 Relationship between gut weight and total MPs item and MPA

A One-way ANOVA was conducted to identify the significant variation between the mean plastic abundance and gut weight and mean item and gut weight, respectively. The test statistic between gut weight and MPA ($F = 30.72$, $p < 0.001$), gut weight and total items ($F = 162.78$, $p<0.001$). There was a significant variation observed in the MPA and item in the fish gut weight and total weight.

The Tukey Tukey B[a] analysis also proved that; the total MPs numbers are significantly different among the gut weight (Table: 3). There is significant variation between the size class 0 to 2g and 2 to 4g. The size class 4 to 6g didn’t show any differences with the size class 2 to 4g and 6 to 8g, but the 8 to 10g size class also showed variation with the size class 6 to 8g and 10 to 12g (Table: 3). So, the study can conclude that there is a significant difference in total items of MPs according to their gut weight except for the size class 4 to 6g.

Table 3. Comparison of MPs total item (TI) based on their gut weight (GW)

Size class of gut weight (SGWT)	Total items (TI)
0-2 g	38.38 ± 0.98^{a}
2-4 g	64.75 ± 2.60^{b}
4-6 g	73.38 ± 0.50^{bc}
6-8 g	85.81 ± 0.66^{c}
8-10 g	141.69 ± 1.21^{d}
10-12 g	186.06 ± 0.47^{e}

Tukey post hoc Tukey B[a] analysis also revealed that there is a significant difference in mean MPs abundance (MPA) among the gut weight size class 0 to 2g and 2 to 4g, but there is no significant difference in MPA among the size classes 6 to 8g, 4 to 6g, 8 to 10g and 10 to 12g (Table: 4). The study also showed the MPs abundance are different among these 4 similar classes and 0 to 2g and 2 to 4g gut weight size classes (Table: 4).

Table 4. Comparison of MPA (Microplastics abundance) based on their gut weight (GW)

Size class of gut weight (SWGT)	Microplastics abundance (MPA)
6-8 g	12.364 ± 0.03^{a}
4-6 g	14.5728 ± 0.38^{a}
8-10 g	15.4025 ± 2.01^{a}
10-12 g	16.6007 ± 1.5^{a}
2-4 g	21.6128 ± 3.3^{b}
0-2 g	29.3075 ± 0.6^{c}

4.5 Correlation between total weight (TW), gut weight (GW) and total items (TI)

The Pearson Correlation Test exert that there was positive correlation between total items (TI) of MPs with the total weight (TW) and gut weight (GW) (Figure:4,5), suggesting that the fishes with relatively high body weight and gut weight contained higher number of MPs ($r^2 = 0.763$, $r = 0.874$, $p = 0.00001$;$r^2 = 0.819$, $r = 0.905$, $p = 0.00001$). The findings revealed that, the total items (TI) of MPs increase positively with the total weight (TW) (Figure: 4) and the total items (TI) of MPs also positively correlated with gut weight (GW) (Figure: 5).

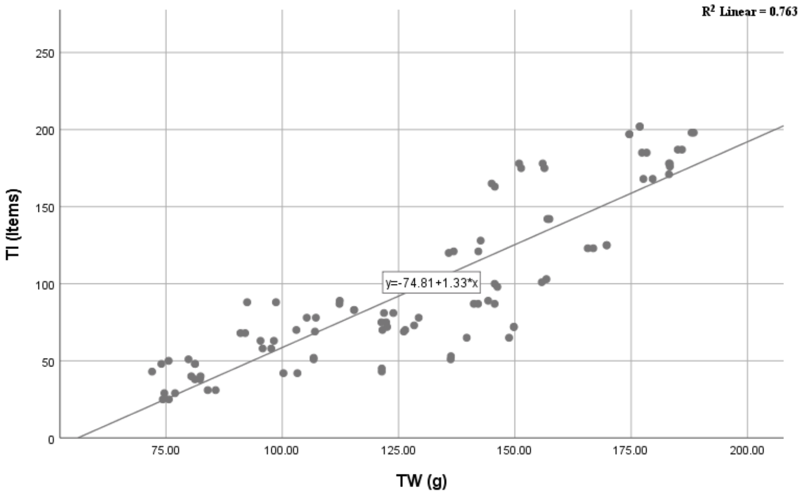

Figure 4: Correlation between total items (TI) and total weight (TW). The regression line showed the Total item (TI) is positively increasing with the increasing Total weight (TW) of the fishes.

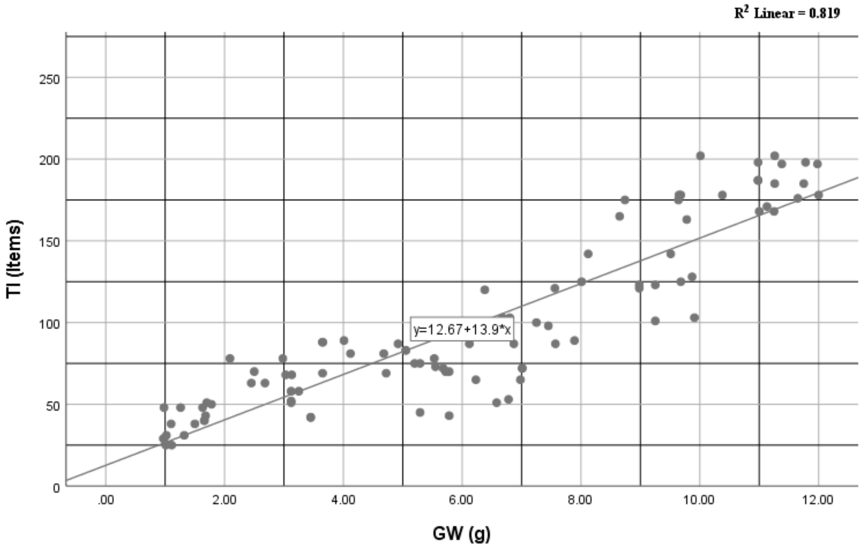

Figure 5: Correlations between Total items (TI) and Gut weight (GW). The regression line showed the Total item (TI) is positively increasing with the increasing Gut Weight (GW).

4.6 Correlation between Gut weight (GW) and micro plastics abundance (MPA)

The mean MPs abundance didn't show the positive correlation with the gut weight ($r^2 = 0.349$, $r = 0.597$, $p < 0.05$) (Figure: 6), which means the mean abundance of MPs were not positively correlated with the gut weight and there is no chance of getting more abundance of MPs in high gut weight fishes. It is more likely that the small gut weight fish showed more abundance of MPs in their relatively small gut (Figure: 6).

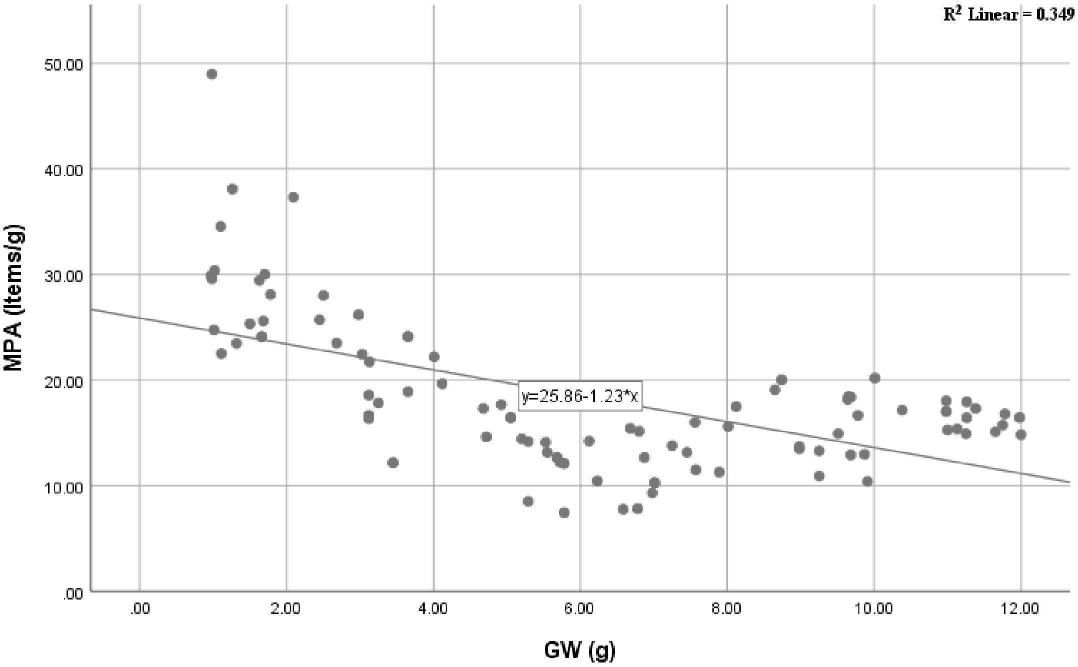

Figure 6: Correlations between Gut weight (GW) and Microplastics abundance (MPA). The regression line showed that, the MPA (microplastics abundance) is not positively increasing with the increasing gut weight of the fishes.

4.7 Types of microplastics

Fragment and filament types of MPs were recovered from the samples. The amount of fragments and filaments showed variation with the gut weight (Figure: 7). The gut weight class 0 to 2g contained the least amount of filament and fragments, which were 6.6% and 6.5% and the gut weight size class 10 to 12g contained the highest percentage of filaments and fragments and they were 35% and 29.1% respectively. The result also revealed that, the filament type of MPs were predominant in each gut weight size classes except size class 0 to 2g (6.5%) and the dominant size classes with filament type MPs were 2 to 4g (11.6%),4 to 6g (13.1%),6 to 8g (15.9%), 8 to 10g (23.8%), and 10 to 12g (29.1%). The findings also stated that filament type of MPs were dominant (59.31%) over fragment type of MPs (40.69%) of the total fish samples.

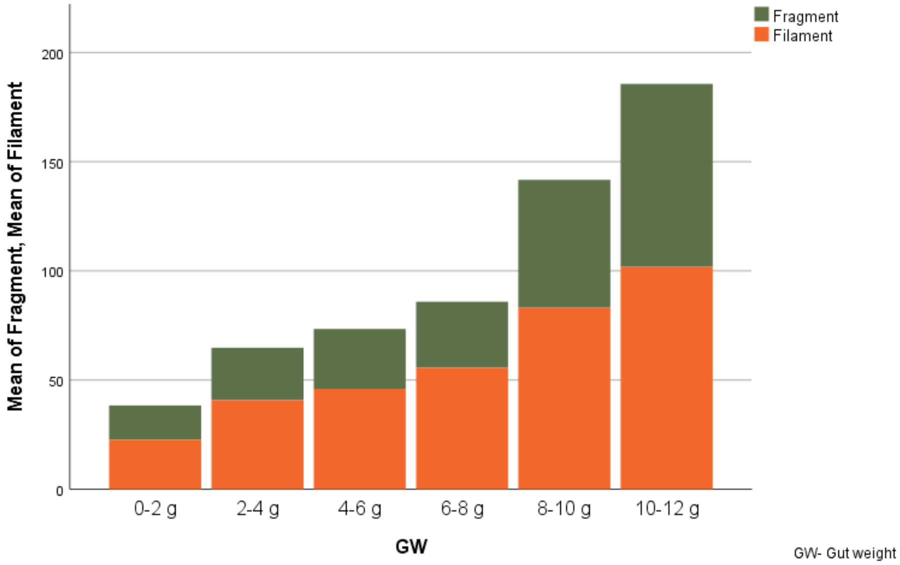

Figure 7: Type variations of microplastics

4.8 Shapes of Microplastics

The shapes of microplastics that were observed from the fish samples were irregular, angular and elongated, with elongated (59.31%) being the most dominant (Figure: 8) along with irregular (29.71%). The least found shapes were angular (10.98%). The highest percentage of irregular, angular and elongated shape MPs were found in gut size class 10 to 12g and they were 37.7%, 44% and 29.1% respectively. The lowest percentages of all shapes of MPs were found in the initial gut size class 0 to 2g, they were irregular (8%), angular (2.7%) and elongated (6.5%) respectively.

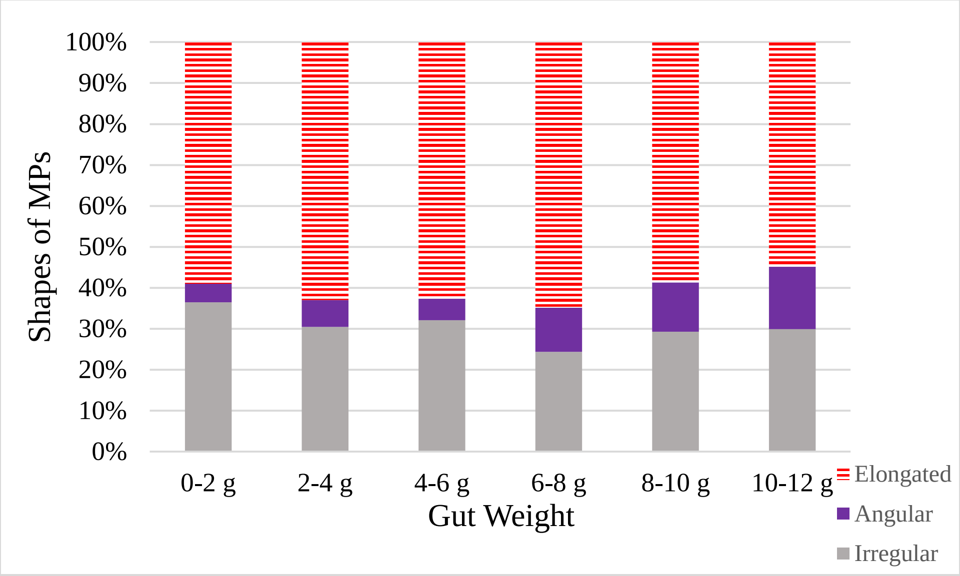

Figure 8: Shape variation of Microplastics

4.9 Colors of microplastics

The MPs that were observed in the fish samples, were classified into 5 categories they are: blue (37.57%), black (30.63%), green (7.45%), red (8.42%) and white (15.92%) (Figure: 9). The blue colored MPs were most dominant (37.57%) and the green colored (7.45%) were least found among the samples (Figure: 9).

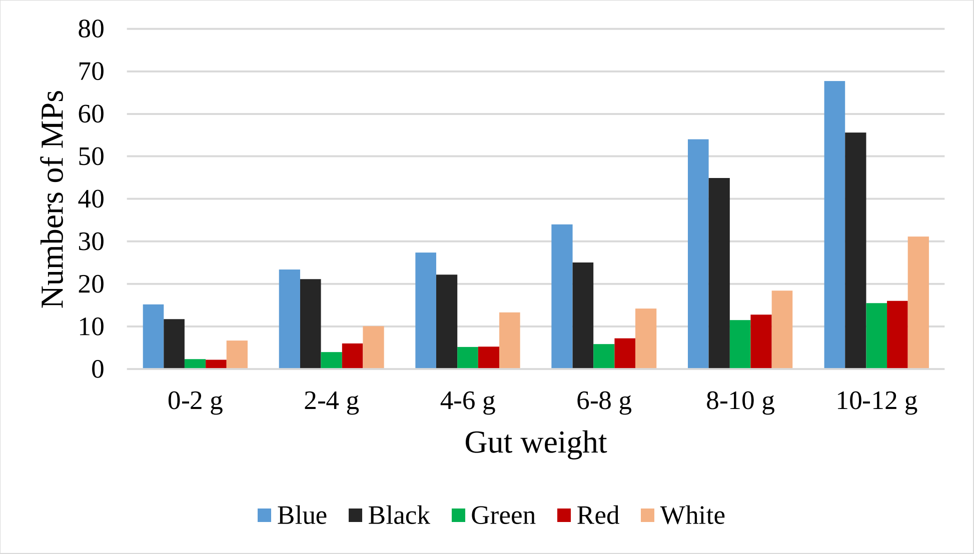

Figure 9: Color variations of Microplastics

The dominant colors of MPs were blue (37.57%) and black (30.63%) colors, followed by white 15.92%, red (8.42%) and green (7.45%). The MPs were dominant in 10 to 12g size classes with the percentages of blue 30.5%, black 30.8%, green 34.9%, red 32.3%, and white 33.2% (Figure: 10). The least dominant MPs were found in 0 to 2g size classes, they were blue 6.9%, black 6.5%, green 5.3%, red 4.4%, and white 7.1% (Figure: 10).

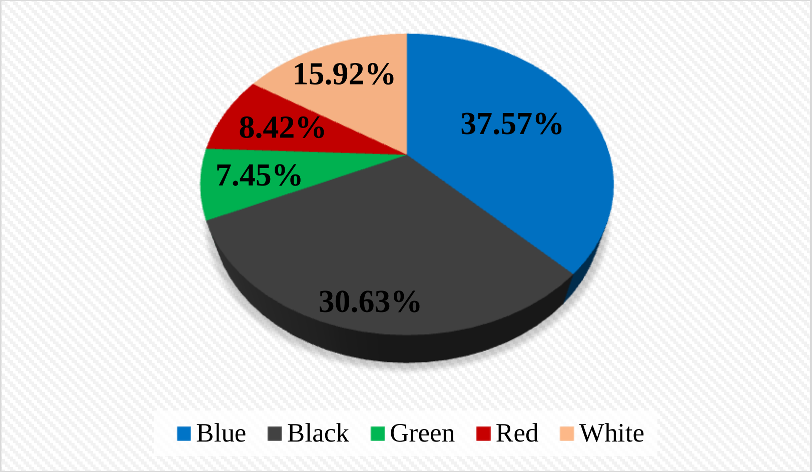

Figure 10: Percentage of different colors of microplastics

4.10 Sizes of microplastics:

The identified microplastics were classified into 3 different size classes: <500 µm, 500 µm to < 1mm and 1mm to <2mm. The highest proportion of microplastics was found in the class 500 µm to < 1mm accounting for 50.68% (Figure: 11). The second highest proportion of microplastics was observed in the <500 µm class, at 29.63%. The class 1mm to 5mm included the least quantity of microplastics, contributing 19.69% of the total MPs (Figure:11). The gut size class 10 to 12g seemed to have the highest percentage of the MPs sizes and the lowest percentage of MPs was present in size class 0 to 2g (Figure: 12)

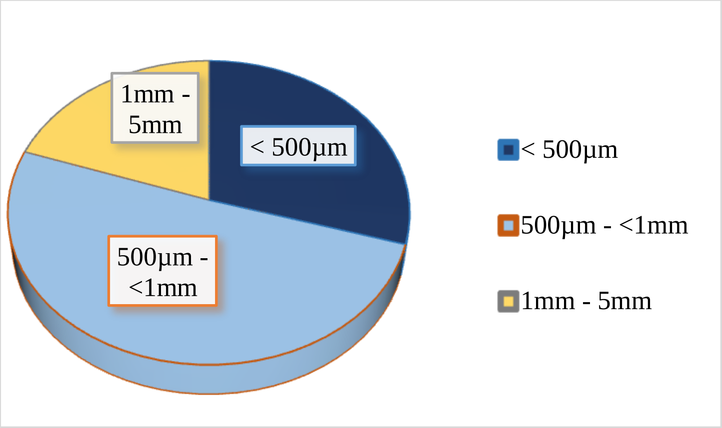

Figure 11: Size variations of microplastics

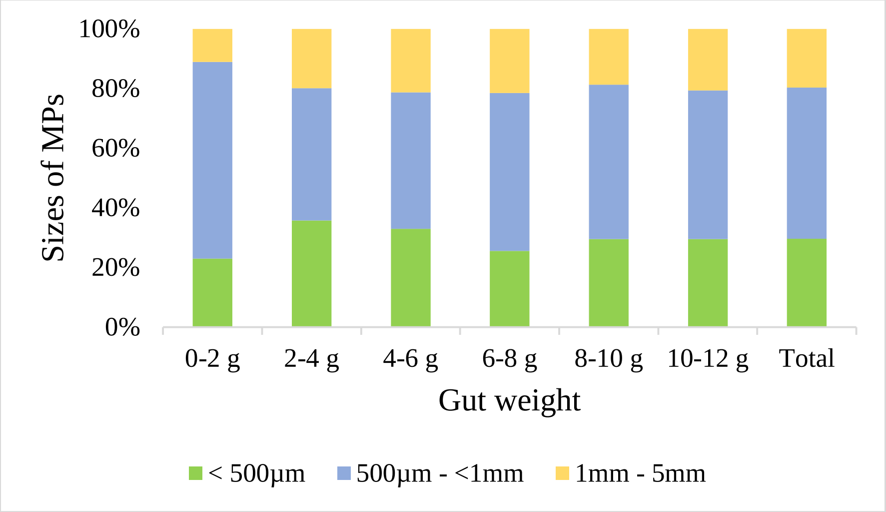

Figure 12: Percentage of different sizes of microplastics

Chapter- 5: Discussion

5.1 Microplastics in fish

The Bay of Bengal is highly contaminated with MPs, where Microplastics are present in the surface waters of the Bay of Bengal at a rate of 500–20,000 items/km^2, with a larger abundance around the Nicobar Island, exceeding 100,000 items/km^2 (Eriksen et al., 2018).

In this study MPs were found in the 96 samples of *H. nehereus* from the Potenga sea beach, Chattogram. All the fish individuals contained MPs in their gut. This may be due to the cannibalistic behavior of the fish. The mean weight (MW) and mean gut weight (MGW) were, 129.75 ± 34.77g and 6.16 ± 3.46g. The MPs abundance (MPA) and Total item (TI) were 18.31 ± 7.17 (item/gut weight) and 98.34 ± 53.11 items. With the growth in the total weight, the number of MPs increased significantly (Figure: 2). The highest total weight size class among the analyzed samples, 170 to 190g, had the highest number of MPs (186.06 ± 11.96 items), while the lowest total MPs were discovered in the lowest total weight size class, 70 to 90g fish weight (38.38 ± 9.09 items) (Figure: 2). The mean of total number of MPs from total weight 90 to 110, 110 to 130, 130 to 150 and 150 to 170g were, 64.75 ± 14.10, 73.38 ± 12.99, 85.81 ± 21.45, and 141.69 ± 26.93 items respectively. The mean abundance of the MPs did not show specific correlation with the increasing total weight (Figure: 2) where the mean abundance of MPs were 29.31 ± 6.73, 21.61 ± 6.34, 14.57 ± 3.77, 12.36 ± 3.09, 15.40 ± 3.00 and 16.60 ± 1.41 item/g respectively. This revealed that, the abundance of MPs didn't increase as it should be according to their weight.

The total items and microplastics abundance also showed relation with the Gut Weight Size class (Figure: 3). The highest total items were found in highest gut weight 10 to 12g (186.06 ± 11.96) (Figure: 3). The highest mean abundance of MPs were showed in 0 to 2g size class (29.31 ± 6.73), along with 2 to 4g (21.61 ± 6.34) and the lowest abundance was seen 6 to 8g size class (12.36 ± 3.10) (Figure: 3).

As suggested in earlier studies related with fish (Lusher et al., 2013; de Sá et al., 2015; Ory et al., 2017,2018a), microplastics may have been taken up directly by fish actively (i.e. ingested by confusion with prey), from the seawater passively (e.g. gill water

filtration) and as well as through the ingestion of contaminated prey. Barboza et al, 2020 found that 150 specimens yielded a total of 368 microplastic items: 175 came from the digestive system (48%); 112 came from the gills (30%); and 81 came from the muscle (22%).
According to Ryan (2013), 95.5% floating plastic waste (non-plastic materials 4.5% such as glass, tin, wood, and paper), of which the composition of packaging items were 54.6%, plastic fragments 30.5%, fishing/boating 6.3% and user items were 4.1% respectively. Plastics are found everywhere in the ecosystem and have been linked to interactions with 700 marine species (Gall and Thompson, 2015; Proki et al., 2019). They are also detected in fish digestive systems, which are causing more concern (Ory et al., 2018b; Strungaru et al., 2019).

In order to comprehend the health of the marine ecosystem, the danger of exposure to creatures, and to foresee potential adverse impacts on the environment and public health, microplastics must be monitored (Crawford and Quinn, 2017; Savoca et al., 2019). As previously noted (Lusher et al., 2013), fish can also ingest microplastics from the nets used to catch them.

This study documented by Hossain et al, 2019, the presence of microplastic in three marine fish species from Bangladesh's northern Bay of Bengal, including *H. translucens, H. nehereus, and S. gibbosa*. The findings indicate that microplastics were present in all fish species living in shallow coastal waters, nearshore locations, and offshore areas (Hossain et al., 2019). These findings show that microplastic pollution of fish is also present in the northern part of Bay of Bengal.

5.2 Abundance of MPs (items/g)

The total MPs items showed positive correlations with the total weight and gut weight of the fishes, which means that the total items increases with the increasing numbers of total weight and gut weight. The highest MPs (186.06 ± 11.96 items) were found in the highest total weight size class 170 to 190g. The lowest total weight size class 70 to 90g fish weight contained lowest numbers of total MPs (38.38 ± 9.09 items). Similarly the highest gut weight class (10 to 12g) and lowest gut weight class (0 to 2 g) contained highest MPs (186.06 ± 11.96) and lowest MPs numbers (38.38 ± 9.09). The mean abundance of the

MPs identified from the fish samples did not show positive relationship with the Total Weight (TW) and gut weight (GW) which clarified that the highest total weight and gut weight did not contain the highest mean abundance, rather than the relatively small fish contain more mean abundance of MPs.

The abundance of MP in a fish species is positively correlated with the total weight (TW) and gut weight (GW), suggesting that species with relatively more weight is likely to contain higher number of MPs (r = 0.82, p = 0.0007; r = 0.78, p = 0.0009) (Hossain et al., 2019). The findings didn't show similarities, which might be due to the feeding behavior, species variation etc. On the contrary One way ANOVA showed that total MPs and the MPs abundance were significantly different among the gut weight size groups (p = 0.00001) (Hossain et al., 2019).

5.3 Correlation of MPs

The Pearson Correlation Test was done to reveal the relations between gut weight, total weight and total items. The Pearson Correlation Test exert that there was positive correlation between total MPs numbers with the total weight (TW) and gut weight (GW), suggesting that the fishes with relatively high gut weight contained higher number of MPs ($r^2 = 0.82$, r = 0.905, p = 0.00001; $r^2 = 0.76$, r = 0.874, p = 0.00001) (Figure: 4,5). The greater total weight and gut weight fishes contained the higher numbers of total MPs. This may due to their large guts which contain more food along with more MPs. Again the Tukey B^a analysis also proved that, the total MPs numbers are significantly different among the gut weight size classes. There is significant variation between the size class 0 to 2g and 2 to 4g (Table: 3).

Tukey B^a analysis revealed that there is significant difference of mean MPs abundance (MPA) among the gut weight size class 0 to 2g and 2 to 4g, but there is no significant difference of MPA among the size classes 6 to 8g, 4 to 6g, 8 to 10g and 10 to 12g (Table: 4).

On the other hand the mean MPs abundance didn't show the positive correlation with the gut weight (Figure: 6). The highest mean abundance of MPs was showed in 0 to 2g size class (29.31 ± 6.73 item/g) and the lowest abundance of MPs was found in 6 to 8g size class (12.36 ± 3.10 item/g). Which means the mean abundance of MPs was not positively correlated with the gut weight and there is no chance of getting more abundance of MPs in high gut weight fishes. It is more likely that the small gut weight fish showed more abundance of MPs in their relatively small gut.

5.4 Types of MPs

Two types of MPs were found from that study, filament and fragment. The study showed that the filaments were mostly abundant among the recovered MPs which was 59.31% and fragment type of MPs were 40.69% of the total fish samples. The lowest gut weight contained the lowest percentage of filament (6.6%) and fragment (6.5%) again the highest gut weight contained the highest filament (35%) and fragment (29.1%) (Figure: 7) these may be due to the feeding behavior of Bombay duck fish. The results showed similarities with the other previous studies.

In fish fibers were more abundant (54 %) than fragments, in agreement with other studies, such as: 66 % in fish from Portuguese coastal waters (Neves et al., 2015), 97 % in fish from the Mondego River estuary, central coast of Portugal (Bessa et al., 2018), 68 % in fish from the English Channel (Lusher et al., 2013), 70 % in fish from the Mediterranean Sea (Guven et al., 2017) and 74 % in fish from Canary Islands coast (Herrera et al., 2019).

The maximum amount of fiber consumed in the current study was 50–55%, which is less than the previous records of 83% in fishes from the Spanish coast (Compa et al., 2018), 70% fishes from the Mediterranean Sea (Güven et al., 2017), 66% fishes off from the Portuguese coast (Neves et al., 2015), 68% fishes from the English Channel (Lusher et al.2013).

Fibers up taken by the investigated fish may have come from nets, ropes and other materials related to fishery, which directly input into marine waters, and also from continental sources (e.g. washing machines, harbor industry, textile industry, river/estuarine fishery). The predominance of fibers over fragments in gills of all the species suggests that fibers are more available in seawater of fish habitat because microplastics present in gills were taken through passive water filtration. However, the relative percentage of fibers and fragments in the gastrointestinal tract reveal the differences among the species and suggests contribution of active and preferential ingestion of microplastics with particular shape by fish. Moreover, colour and shape are also important to prey-perception by visual predator fish (Blaxter, 1980).

5.5 Shapes of MPs

The shapes of microplastics that were observed from the fish samples were irregular, angular and elongated, where elongated shaped MPs were mostly found in the study (59.31%). The irregular shaped MPs were 29.71% and the least found shapes were angular (10.98%) (Figure: 8). Elongated shaped MPs were highly available because most of the MPs were filament and their shape is elongated. Oceanic water contains more filamentous MPs which float and move easily and are less likely to deposit in the bottom, so fish consume them more. For this reason elongated MPs were dominant. On the contrary fragments can be irregular or angular, that's why they are divided into two categories, where irregular shapes are more prominent to occur due to breakdown of fragments. The previous studies showed the dominance of both fibers or filament type and irregular fragment type MPs.

The forms of the identified MPs were irregular filamentary, angular, and rounded. According to Choi et al. (2018), the majority of the MPs had irregular forms and was found in sheepshead minnows (Cyprinodon variegatus) from the Republic of Korea (Choi et al., 2018). According to Jabeen et al. (2017), one of the potential impacts of irregular and sharp-edged MPs is damage to the stomach wall. According to other studies (Desforges et al., 2015; Sun et al., 2017; Steer et al., 2017), fiber was the most prevalent kind of MP across the three fish species that were studied.

5.6 Colors of MPs

In that study 5 different colors of MPs were identified which were blue, black, green, red and white. Blue MPs were the most dominant among them (37.57%) and the least dominant were green (7.45%). The other colors of MPs were black (30.63%), white (15.92%) and red (8.42%) (Figure: 9). Blue MPs were mostly dominant in fish gut because fish might confused them as prey in the oceanic environment. Other studies also showed the most abundance of blue MPs in fish gut. The second and third dominant MPs were black colored MPs (30.63%) and white MPs (15.92%) (Figure: 9, 10). Previous studies showed the dominance of whitish MPs in fish gut as ingesting them as whitish prey. Red and green were most unlikely found in oceanic environment thus less found in fish gut (Figure: 10).

The microplastics identified in D. labrax, T. trachurus, and S. colias were primarily blue in color, which is compatible with earlier research with fish (Neves et al., 2015) and animals (Hernández-González et al., 2018) from the NE Atlantic Ocean. The present study found that blue microplastics were more prevalent than other colors. This may be because blue microplastics are more common in seawater, fish preys are more likely to be contaminated by blue microplastics, and/or fish preferentially actively eat blue microplastics over microplastics of other colors. In both sediment and NE Atlantic seawater samples, blue microplastics were the most frequent (Lusher et al., 2014; Woodall et al., 2014). Blue microplastics are more likely than other hues to be ingested by fish and their prey because they are more prevalent. All of the researched species are visual predators, and this type of predators use color as a key indicator when assessing potential prey. As a result, microplastics may be consumed by these predators when they mistake them for potential prey, with color undoubtedly having a significant role in this.

Additionally, in deep waters, fish prey may seem blue when illuminated by light from the water surface because the light blue component of the ocean entirely dominates at depths of 100 meters or more (Blaxter, 1980; Archer, 1995).

Whitish was the second most common hue of microplastics found in the fish under study. This might be because whitish microplastics are more prevalent in the seawater of the NE Atlantic Ocean, they contaminate prey at higher rates, and fish actively eat them because they mistake them for whitish prey (McNeish et al., 2018; Ferreira et al., 2019).

The species that spend more time in areas near the coast are likely to be exposed to a wider variety of microplastic colors (due to recent inputs), whereas species that prefer to stay farther from the coast are likely exposed primarily to aged microplastics, which frequently have lost their original color during their permanence in seawater. (Murta et al., 1993; Olaso et al., 1999).

The observation is similar to the findings of Ory et al. (2018a) and includes the detection of MPs in five different colors, including white/transparent, black, red, blue, and green.

5.7 Sizes of MPs

MPs of different sizes may be uptaken by the fishes through active predation, like thinking as food particles or may be ingested accidentally with the prey. In that study, the highest proportion of microplastics (50.68%) were found in the size between 500 µm to < 1mm (Figure: 12). the MPs of size class <500 µm and 1mm to 5mm contained 29.63% and 19.69% respectively, which clearly denoted that the highest amount of MPs were identified in between 500µm to < 1mm (Figure: 11). these size class covered both fragments and filaments. These different sizes of mps may be ingested through active predation as we worked with potentially predatory fish. Medium size ranges denoted that the mps may be actively ingested which are easily entered into the mouth. The species examined are visual predators, so it's possible that they intentionally consumed rather big microplastics that were about the size of some of their food. According to several research such as de Sá et al. (2015) and Ory et al. (2018 a, b,) fish may actively absorb some microplastics they consume because they mistakenly perceive them to be food. Because of their greater size, carnivorous feeding habits, and higher body weight (129.64 ± 23.92 g), the larger size MPs predominated in pink Bombay-duck (Hossain et al., 2019).

Based on microplastic size, all species had more fibers in the gastrointestinal tract (36%) and gills (50%) of the size range 151–500 mm and 501–1500 mm, respectively, than fibers in other size ranges. *D. labrax, S. colias, and T. trachurus* had more fibers in the size range 151-500 mm (39%) than other size ranges in the dorsal muscle (58%) (Barboza et al., 2020).

According to laboratory research, fish eating behavior may be more strongly stimulated chemically than visually by particles smaller than 1230 mm (van der Lingen, 1994).

Chapter- 6: Conclusions

The study showed evidence of microplastic contamination in fish species *(H. nehereus*) intended for human food consumption that were caught in Potenga Sea Beach, Chattogram, Bangladesh coastal waters. Microplastics were found in the gastrointestinal tract of every species analyzed, proving that fish have been contaminated by microplastics. The study focused into the extent and impact of microplastic pollution in fish from Bangladesh's Potenga Sea Beach. Due to its high level of urbanization, tourism, industry, ecological significance, and population density, this research location was chosen. The findings showed that the fish (*H. neherius*) contained microplastics, indicating serious dangers to aquatic life, the environment, and the ecosystem as a whole. These results provide a crucial framework for determining the degree of microplastic pollution on the Potenga Sea beach. The information this study has generated will be valuable for Policymakers, scientists, ecologists, environmental activists, hydrologists, and non-governmental groups. It can help them with their strategic planning and make it easier to put conservation and management practices for the water body into action. It should be noted, nonetheless, that more investigation and ongoing monitoring efforts are necessary to fully comprehend the long-term effects of microplastic contamination and to create long-lasting solutions to this pressing worldwide issue. These projections could help to find out the consumption rate of MPs sourced from the fish of the people of the Chattogram region and the health issues related with that. It will also help to create daily microplastic consumption limits and strengthen the foundation for microplastic risk assessments for humans. Furthermore, the results of this study and numerous others found in the literature demonstrate the necessity for additional research on microplastics and their impacts in line with the WHO's "One Health" strategy.

Chapter- 7: Recommendations and Future Perspectives

The following recommendations may be made based on the findings of the current study:

- It is necessary to work on minimizing microplastic pollution right now as it is a serious issue around the world. Controlling microplastic pollution at its source is the most efficient way to reduce it in the aquatic environment.
- Policymakers will play a crucial role in developing the essential regulatory framework to encourage mitigation efforts that contribute to reduce plastic waste from the source as well as encourage cleaning of plastic pollution before it causes the most substantial damage.
- It is necessary to monitor pollution at the source and conduct public awareness programs, which will reduce consumption patterns and littering. Mass media should be used to aware people.
- Increasing demand for MPs pollution monitoring at national and global levels requires the improvement of existing methods and the development of effective methodologies to reduce the effort and identification time.
- Future research should be done to determine the concentration of microplastics in fish, the way of human ingestion, and how they impact aquatic organisms, humans and the environment.

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Photo gallery

Plate 1: Fish collection

Plate 2: Thawing of fish

Plate 3: Measuring total length

Plate 4: Weighting

Plate 5: Disection of fish

Plate 6: Removal of gut

Plate 7: Weighting of gut

Plate 8: Measuring of H_2O_2

Plate 9: Pouring H_2O_2

Plate 10: H_2O_2 treatment

Plate 11: Shaking incubator

Plate 12: Pouring $Zncl_2$

Plate 13: Density seperator

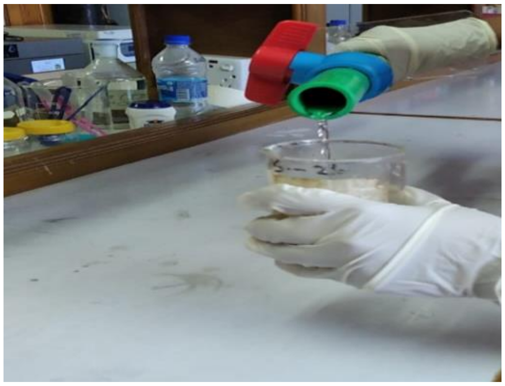


Plate 14: Seperation of supernatant

Plate 15: Filtration

Plate 16: Filtrate sample

Plate 17: Visualization

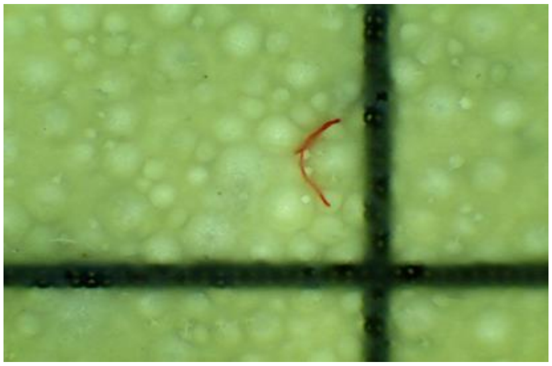


Plate 18: Red elongated filament

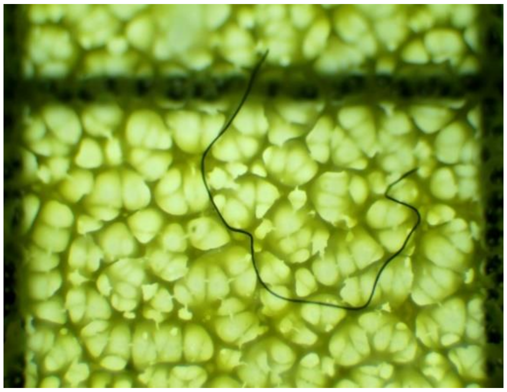


Plate 19: Black elongated filament

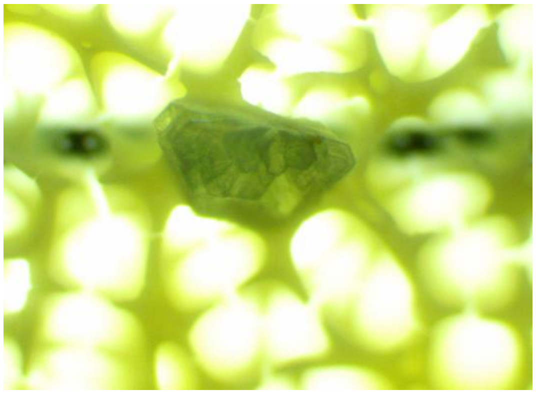
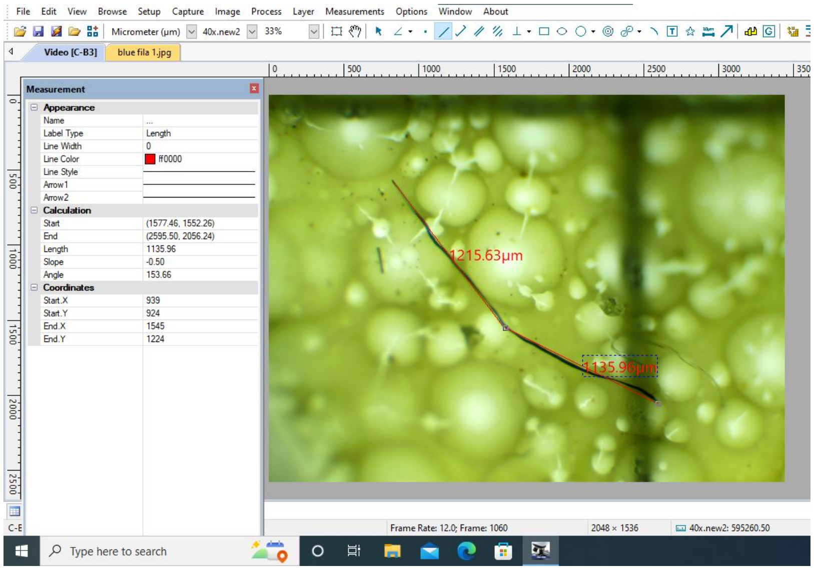


Plate 20: Transparent irregular fragment



Measurement

Appearance	
Name	...
Label Type	Length
Line Width	0
Line Color	🟥 ff0000
Line Style	
Arrow1	
Arrow2	
Calculation	
Start	(1577.46, 1552.26)
End	(2595.50, 2056.24)
Length	1135.96
Slope	-0.50
Angle	153.66
Coordinates	
Start.X	939
Start.Y	924
End.X	1545
End.Y	1224

Plate 21: Measurements of MPs

Appendix

Appendix 1: One way Anova of T_Item (Total Items) and MPA (Microplastics Abundance) of between size groups, within size groups

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
T_Item	Between Groups	241256.344	5	48251.269	162.783	.000
	Within Groups	26677.313	90	296.415		
	Total	267933.656	95			
MPA	Between Groups	3080.666	5	616.133	30.720	.000
	Within Groups	1805.053	90	20.056		
	Total	4885.719	95			

Appendix 2: Correlations between TW (Total Weight), FGT (Fish Gut Weight), MPA (Micro plastics Abundance) and T_Item (Total Item)

Correlations

		TW	FGT	MPA	T_Item
TW	Pearson Correlation	1	.973**	-.597**	.874**
	Sig. (2-tailed)		.000	.000	.000
	N	96	96	96	96
FGT	Pearson Correlation	.973**	1	-.591**	.905**
	Sig. (2-tailed)	.000		.000	.000
	N	96	96	96	96
MPA	Pearson Correlation	-.597**	-.591**	1	-.268**
	Sig. (2-tailed)	.000	.000		.008
	N	96	96	96	96
T_Item	Pearson Correlation	.874**	.905**	-.268**	1

	Sig. (2-tailed)	.000	.000	.008	
	N	96	96	96	96

**. Correlation is significant at the 0.01 level (2-tailed).