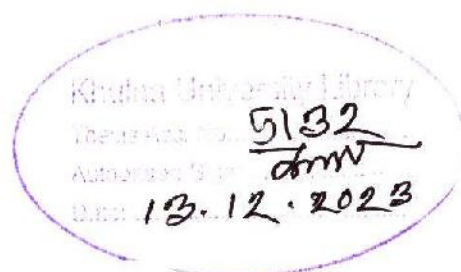


Microplastics in *Mystus gulio*, *Lates calcarifer* and *Hyporhamphus limbatus* from the Sundarbans of Bangladesh

A Thesis

By



Sagarika Singh

Student ID: MS-200610

Session: 2021-2022

The thesis submitted to the Fisheries and Marine Resource Technology Discipline in partial fulfillments of the requirements for the degree of

Master of Science

in

Coastal and Marine Science



Fisheries and Marine Resource Technology Discipline

Khulna University

Khulna-9208, Bangladesh

December, 2022

Microplastics in *Mystus gulio*, *Lates calcarifer* and *Hyporhamphus limbatus* from the Sundarbans of Bangladesh

Approved as to the style and content

by



.....
(Signature of the Head)

Dr. Md. Golam Sarower

Professor and Head

Fisheries and Marine Resource Technology Discipline

Khulna University,

Khulna-9208, Bangladesh



December, 2022

Declaration

Microplastics in *Mystus gulio*, *Lates calcarifer* and *Hyporhamphus limbatus* from the Sundarbans of Bangladesh

The results presented in this thesis with the above-mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

Sagarika Singh 12.12.22
.....

(Signature of the Candidate)

Sagarika Singh

Student ID: MS-200610

Session: 2021-2022



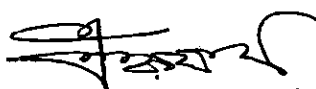
.....
(Signature of the Supervisor)

Muhammad Abdur Rouf, PhD

Professor

Fisheries and Marine Resource Technology Discipline

Khulna University, Khulna



.....
(Signature of the Co-supervisor)

Md. Nazrul Islam, PhD

Professor

Forestry and Wood Technology Discipline

Khulna University, Khulna

Fisheries and Marine Resource Technology Discipline

Khulna University

Khulna-9208, Bangladesh

December, 2022

Dedicated To

My Beloved Parents

Table of Contents

Acknowledgement	i
Abstract.....	ii
List of tables	iii
List of figures.....	iv
List of abbreviations.....	v
Chapter 1.....	1
1. Introduction	1
1.1 Background of the study	1
1.2 Aims and objectives	4
Chapter 2.....	5
2. Literature review.....	5
2.1 MPs definition and types	5
2.2 MPs sources	6
2.3 Researches on MPs	7
2.4 Information of three fish species	10
Chapter 3.....	12
3. Materials and methods	12
3.1 Fish sample collection from sampling stations	12
3.2 Sample preparation	13
3.3 Hydrogen peroxide treatment	13
3.4 Saline (NaCl) solution floatation and filtration	13
3.5 Microscopic observation of MPs.....	15
3.6 FTIR analysis	15
3.7 Data analysis	15
3.8 Contamination control	15
Chapter 4.....	17
4. Results	17
4.1. Abundance of MPs	17
4.2. Shape of MPs	19

4.3. Color of MPs.....	20
4.4. FTIR analysis of polymer	22
Chapter 5.....	24
5. Discussion.....	24
Chapter 6.....	32
6. Conclusion.....	32
Chapter 7.....	33
7. References	33
Chapter 8.....	48
8. Annex	48
8.1 Annex 1	48
8.2 Annex 2	48
8.3 Annex 3	53
8.4 Annex 4	54
8.5 Annex 5	57
8.6 Annex 6	58

Acknowledgement

At first, all my admiration goes to the supreme creator and ruler of the universe, the almighty God for His great mercy to keep me alive and pursue my education in Fisheries and Marine Resource Technology Discipline as well as to complete my thesis work for the degree of Master of Science in Coastal and Marine Science.

I would like to express my sincere gratitude and profound appreciation to my honorable supervisor, **Muhammad Abdur Rouf, PhD**, Professor, Fisheries and Marine Resource Technology Discipline, for his continuous guidance, valuable suggestions, critical observations, and sustained support in writing this thesis.

I would like to give special thanks to my honorable co-supervisor **Md. Nazrul Islam, PhD**, Professor, Forestry and Wood Technology Discipline, for his continuous guidance, valuable suggestions, critical observations, and sustained support in writing this thesis.

I recall with warm appreciation and great indebtedness for support, guidance, and suggestions extended to me by all my respected teachers specially **Shaikh Tareq Arafat**, Assistant Professor in Fisheries and Marine resource Technology Discipline, Khulna University.

Special thanks go to my friends **Lopa Mudra Das** and **Diba Biswas** for their support in completing my thesis work in the laboratory.

I would like to acknowledge the Khulna University Research Cell (KURC) and National Science and Technology (NST) fellowship of Bangladesh for their essential supports.

Finally, I would like to express my very profound gratitude to my parents for all their care, unfailing support, and continuous encouragement throughout my study. This accomplishment would not have been possible without them.

Sagarika Singh

Student ID: MS 200610

Coastal and Marine Science

FMRT Discipline

Khulna University, Khulna.

December, 2022

Abstract

This study investigated the abundance and characteristics of microplastics (MPs) in gastrointestinal tracts (GT), gill and muscle of three commercial fish species (*Mystus gulio*, *Lates calcarifer*, *Hyporhamphus limbatus*) from the Sundarbans of Bangladesh. The fishes were examined for MPs investigation following alkali digestion protocol, microscopic observations and chemical analysis by Fourier Transformed Infrared Spectroscopy (FTIR). MPs were detected in all parts of all fish species except the muscle of *M. gulio*. Positive relationship was found between MPs abundance and body weight of the fish species. A total of 499 MPs were found in the GT, gill and muscle (5g) of three species with an average MPs abundance of 12.20 items/g in GT, 8.03 items/g in gill and 2.37 items/g in muscle, respectively. A remarkable number of MPs was identified in muscle of two fish species. Among the various shapes and colors of MPs, fiber (45-58.82%), and black (30.83-72.41%) respectively, were dominant. The dominant polymer identified by FTIR analysis was latex (17%) and ethylene vinyl acetate (16%) while high-density polyethylene (11%) and low-density polyethylene (11%) were second dominant type. The study results show the presence of MPs in fishes from the Sundarbans of Bangladesh that can be transferred through food chain to human.



List of tables

Table 1: Abundance of MPs in GT, gill and muscle of the three species from the Sundarbans..	17
Table 2: Shape of MPs in GT, gill and muscle of the three species from the Sundarbans.....	19
Table 3: Colors of MPs in GT, gill and muscle of the three species from the Sundarbans.....	20
Table 4: FTIR analysis of the three fish species from the Sundarbans.....	22
Table 5: Abundance of MPs in GT, gill and muscle of different fish species from different regions of the world.....	24
Table 6: Total body weight and MPs abundance data.....	48
Table 7: MPs abundance (items/g) in different body parts.....	49
Table 8: Body weight (g) and total number of MPs.....	49
Table 9: Relationship between body weight (g) and MPs number.....	49
Table 10: Data of MPs colors.....	50
Table 11: Data of MPs shapes.....	51
Table 12: Polymer analysis by FTIR data.....	52

List of figures

Figure 1: Image of <i>Mystus gulio</i>	10
Figure 2: Image of <i>Lates calcarifer</i>	11
Figure 3: Image of <i>Hyporhamphus limbatus</i>	11
Figure 4: Fish sampling stations in the study area of Sundarbans	12
Figure 5: Flow chart of sample preparation, digestion and analytical procedure for MPs detection in fish.....	14
Figure 6: MPs abundance (items/g) in GT, gill and muscle of the three species from the Sundarbans.....	18
Figure 7: Relationship between the body weight and number of MPs in three species from the Sundarbans	18
Figure 8: Comparison of MPs shape in GT, gill and muscle of the three species from the Sundarbans.....	20
Figure 9: Comparison of MPs colors in GT, gill and muscle of the three species from the Sundarbans	21
Figure 10: Percentage (%) of polymer types by FTIR of three species from the Sundarbans..	23
Figure 11: MPs separation and identification procedures.....	53
Figure 12: Some MPs particle (Shapes and colors).....	56
Figure 13: FTIR spectra of representative samples.....	57

List of abbreviations

Abbreviation	Meaning
MPs	Microplastics
GT	Gastrointestinal Tract
μ -FTIR	Micro-Fourier Transformed Infrared
g	Gram
mL	Milliliter
μ m	Micrometer
mm	Millimeter
cm	Centimeter
rpm	Round per minute
h	Hour
HCl	Hydrochloric Acid
H ₂ O ₂	Hydrogen Peroxide
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
EVA	Ethylene vinyl acetate
HDPE	High-density polyethylene
LDPE	Low-density polyethylene
PP	Poly propylene
PMMA	Polymethyl methacrylate
PS	Polystyrene
PES	Polyester
PU	Polyurethane
EDPM	Ethylene propylene diene monomer
SEM	Scanning electron microscope
SBR	Styrene butadiene rubber
PET	Polyethylene terephthalate
CA	Cellulose acetate

Chapter 1

1. Introduction

1.1 Background of the study

Microplastics (MPs) are that portion of plastic waste that has a diameter smaller than 5 mm. The majority of marine waste worldwide is made of plastic, and up to 12.7 million tons of plastic debris are thought to enter the oceans each year (Cheung *et al.*, 2018). Plastic disposal-related environmental pollution is a serious global issue. According to reports, 192 coastal countries created 275 million metric tons of plastic debris in 2010, of which 4.8 to 12.7 million metric tons ended up in the ocean. 280 million tons of plastics were produced worldwide in 2012 and used in numerous commercial and residential uses (Sanchez *et al.*, 2014). Since mass-produced plastics first appeared in the 1930s, production has grown yearly, now totaling more than 322 million tons annually worldwide (Steer *et al.*, 2017).

Relatively big plastics may go through a range of biotic and abiotic processes after being discharged into the environment, resulting in smaller and smaller plastic particles rather than disappearing (Lambert and Wagner, 2016). In the maritime environment, plastic debris eventually breaks down into MPs, or objects with a diameter of less than 5 mm (Hossain *et al.*, 2019), through weathering processes such as photo-degradation, oxidation, and mechanical abrasion (Andrady, 2011; Thompson *et al.*, 2009). Some MPs are produced as tiny plastic particles known as primary MPs, whereas bigger plastic particles degrade when exposed to ultraviolet radiation and other natural weathering agents and transform into secondary MPs (Hidalgo-Ruz *et al.*, 2012).

The widespread use of plastic products is a significant source of MPs, and the majority of MPs pollution in the marine environment originates from the terrestrial environment (Yuan *et al.*, 2019). It is recognized that drainage systems from inland urban areas as well as anthropogenic activities along the coast including harbors, leisure activities, shipping ports, and fishing allow both bigger plastic debris and MPs to enter the marine environment. Large oceanic swaths of plastic marine debris are easily moved by tides and currents before accumulating in coastal regions with constrained water circulation. Additionally, MPs are released into the environment through a variety of personal care and cosmetic goods, including lotion, shampoo, toothpaste, shower gel, lipstick, hair dye, shaving cream, face masks, and eye shadow, as well as through the washing of clothing (Hossain *et al.*, 2019).

MPs have recently been identified as a developing pollutant and have generated considerable worry throughout the world (Yuan *et al.*, 2019). Around 10% of all species impacted by marine debris inhaled MPs, and their abundance has been rising in the oceans. MPs may offer dangers to organisms through ingestion, transmission to humans through the food chain (Neves *et al.*, 2015), bioaccumulation, and bio-magnification through the food chain, among other mechanisms (Cheung *et al.*, 2018). Wildlife include birds, sea turtles, marine mammals, invertebrates, and fish are at risk of asphyxia, entanglement, and ingestion when bigger plastic items are present. The potential biological effects on animals include impairments to their capacity for movement, nutrition, and reproduction, ulcerations (Cheung *et al.*, 2018), internal obstructions, damage to fish's digestive tracts (Jabeen *et al.*, 2017), inflammation, and gastrointestinal system laceration (Pinheiro *et al.*, 2017).

Mangrove habitats always act as buffers in estuaries and are thought to be barriers to the transfer of inorganic or organic contaminants from the land to the sea, which calls for additional research focus (Li *et al.*, 2019). Scientists believe that the MPs pollution of semi-enclosed waters differs greatly from that of other coastal types due to their distinct geographical characteristics, which is why MPs pollution of intertidal mangrove ecosystems is attracting more and more attention. MPs are widespread in marine and estuarine environments, but the data on the abundance and contamination of MPs in mangrove wetlands is relatively unknown (Huang *et al.*, 2020). However, some studies have identified wide distributions of MPs in the worldwide mangrove, such as mangrove sediments from Singapore, Maowei Sea and Qinzhou Bay of China (Nor and Obbard, 2014; Li *et al.*, 2019), which may imply that the mangrove wetland is an effective retaining area for MPs as well as other contaminants (Zhang *et al.*, 2020a).

Sundarban mangrove forest, situated in the southwest at 21°38'10.18" and 22°29'51.65" N and 89°02'22.87" and 89°53'13.93" E from Hariabhanga River to the west of Baleswar River in the east, is one of the largest continuous blocks of mangrove forests in the World. Total geographic area is approximately 6,017 km² which represents 23% of total forest area of Bangladesh. The super confluence of the Ganges/Padma and Meghna rivers, which crosses international boundaries, together with the 15th-longest river in the world, Brahmaputra/Jamuna, creates and modifies this mangrove. According to Lebreton *et al.* (2017) and Meijer *et al.* (2021), rivers are important conduits for the movement of plastics into oceans and bays. The top ten global plastic waste carriers are these rivers, which annually transport more than 72,000 tons of plastic before discharging into the Bay of Bengal (Schmidt *et al.*, 2017). The Sundarbans serves as a

basin for the long-term buildup of plastic garbage since it has 177 rivers that receive water from three major rivers and flow through it to the Bay of Bengal (Banglapedia, 2012).

As plastics can be drifted/washed from the oceans to the coasts (Onink *et al.*, 2021), there is a probability of some of these plastics can be drifted or washed toward the nearby Sundarbans from the Bay of Bengal. Fishing nets and other trans-boundary marine debris that has been dumped within the Exclusive Economic Zones of the Bay of Bengal could possibly wash into the Sundarbans (Nelms *et al.*, 2021). The integrity of these delicate and diverse ecosystems is thus a problem.

According to Mukul *et al.* (2020), the Sundarbans mangroves are home to 334 plant species and 1,136 animal species, making up 35% of Bangladesh's biodiversity. These mangrove forests offer a special home for mammals, fish, turtles, birds, and other exotic species, including some that are threatened, vulnerable, or endangered. Plastics have the capacity to change the genetic expression, tissues, reproduction, population size, and community structure of wildlife populations and fisheries stocks (Rochman *et al.*, 2016). It is concerning that MPs have been discovered in fish and coral species in the Bay of Bengal and nearby areas (Hossain *et al.*, 2019; Ghosh *et al.*, 2021). Plastic garbage is predicted to have an influence on 291 different fish species supported by 177 tidal streams and channels in this forest, endangering the livelihoods of around 200,000 subsistence fishermen (Sen, 2019). Out of the 322 fish species found in Sundarbans, 150 are considered to be commercially significant (Habib *et al.*, 2020).

In recent years, although MPs ingestions by fishes within the Sundarbans is not reported, the scenario is different in the Bay of Bengal. For instance, MPs are found in the marine commercial fishes (Hossain *et al.*, 2019; Ghosh *et al.*, 2021) and shrimps (Hossain *et al.*, 2020) collected from the Northern Bay of Bengal of Bangladesh. Thus, a study has been conducted to determine the abundance of MPs in three commercial fish species (*Mystus gulio*, *Lates calcarifer*, *Hyporhamphus limbatus*) from the coastal mangroves of Bangladesh. Besides, these three Sundarbans fish species contribute as food in daily meal for a large number of population. Therefore, it is an ideal place for an investigation of MPs contamination in the environment and biota in Sundarbans and this study was undertaken to analyze MPs accumulation in fishes collected from Sundarban mangrove forest. The abundance, types, shape and color of MPs were analyzed among different fish species, and the related factors that affected MPs distribution were also discussed. Furthermore, the difference in MPs accumulation in fish bodies such as GT, gills and muscle were compared. The present study will provide baseline data for MPs accumulation in fishes from mangrove forests as the first evidence in Bangladesh. In this study, gastrointestinal tracts of fishes were examined for MPs following alkali digestion

protocol, microscopic observations and chemical analysis by Fourier Transformed Infrared Spectroscopy (FTIR).

1.2 Aims and objectives

The overall objective of the study is to assess MPs in fishes from the Sundarbans of Bangladesh. The specific objectives are as follow:

- To investigate the abundance, types, shape, colors of MPs in GT, gill and muscle of three commercially important fishes (*Mystus gulio*, *Lates calcarifer* and *Hyporhamphus limbatus*) from Sundarbans.



Chapter 2

2. Literature review

2.1 MPs definition and types

The term "MPs" refers to particles with longest lengths ranging from 0.1 μm to 5000 μm that are made up of a heterogeneous mixture of materials with a variety of shapes, including fragments, fibers, beads, spheres, films, and pellets (Lusher *et al.*, 2017). MPs are mostly categorized according to their physical traits, including size, shape, and color. When investigating MPs, size is crucial since it determines the kind of organisms it may effect. The nomenclature utilized differs between research groups, but researchers often employ five primary categories when reporting MPs shape. Color perception is subjective, and since colors are frequently reported throughout a wide spectrum, visual identification of MPs cannot just be dependent on color. Generally, there are two types of MPs- primary and secondary MPs.

2.1.1 Primary MPs

Primary MPs are made plastic particles with a size range of less than 5 mm (GESAMP, 2015). They consist of beads used for abrasive blasting surfaces as well as pre-production resin pellets, which are frequently 3-5 mm in diameter and employed as raw materials in the production of plastic. The method by which primary MPs enter the environment depends on their intended use: MPs from abrasive blasting typically enter through the atmosphere and wastewater, while MPs from cosmetic products typically enter through the wastewater. Primary MPs used for raw materials may also accidentally become lost during transit and transshipment or through runoff from processing plants. Primary MPs may flow directly into the oceans or through freshwater watercourses to the marine environment when they are too tiny to be retained by wastewater treatment facilities.

2.1.2 Secondary MPs

The environment's weathering and disintegration of bigger plastics results in secondary MPs (GESAMP, 2015). Secondary MPs can be created either during the usage of plastic products (such as paints, tires, and textiles) or after plastics have been disposed of in the environment. Multiple routes exist for secondary MPs to enter the environment, which include particles from textiles may enter through wastewater following washing or through air when drying (Browne *et al.*, 2011); weathering of plastics used in agricultural applications may enter the environment through surface runoff from soil; abrasion of tires during use generates MPs that enter the environment through air and surface runoff; fragmentation and weathering of items in landfills

by UV light which may introduce MPs into the atmosphere, rivers and the ocean by wind and surface runoff and weathering of plastic litter in coastal areas and beaches which may remain in coastal sediments or be transported further offshore. UV radiation exposure, temperature, and abrasion are the key environmental factors associated with the production of secondary MPs. Areas in aquatic habitats with low temperatures and little UV exposure, such the deep sea, will inhibit the production of secondary MPs (Andrady, 2011).

2.2 MPs sources

According to a widely used estimation, up to 80% of plastic debris comes from land-based sources and 20% comes from maritime activities (Faris and Hart, 1994).

2.2.1 Land inputs

Waste from landfills in coastal areas, watersheds and rivers, industrial outfalls, beach littering, tourism, and recreational use of the coasts are the main land-based sources of plastic debris (UNEP, 2009). Storms, tidal waves, and tsunamis are other extreme phenomena that constitute a major immediate source of land-based plastic waste (NAS, 1975).

Waste generated by coastal populations

Globally, coastal communities produce about 4.8–12.7 million tons (metric tons) of plastic waste that could end up in the ocean as marine debris (Jambeck *et al.*, 2015). A study's structure combines information on 192 coastal nations' solid waste production, population size, and economic standing (Eriksen *et al.*, 2016) and according to estimates, populations within 50 kilometers of the coast produce 31.9 million metric tons of improperly managed plastic waste annually, with individual country totals ranging from 1.1 to 8.8 million metric tons/year and a conversion rate from improperly managed plastic waste to potential plastic debris ranging from 15 to 40%.

Waste introduced by rivers

Rivers may become contaminated with plastic trash via surface runoff from urban environments, dumpsites (Gasperi *et al.*, 2014), and intentional or accidental dumping on river banks (Rech *et al.*, 2015). The plastic could sink, end up on riverbanks downstream, or get into the ocean. Runoff from manufactured plastic micro- and nanoparticles found in consumer goods has the potential to introduce these materials to the marine environment (Maynard, 2006). These include of the industrial abrasives (Andrady, 2011) and micro-particles utilized as exfoliants in the cosmetic sector (Eriksen *et al.*, 2013; Fendal and Sewell, 2009; Gregory, 1996). River sediments and coastal sediments are replete with synthetic nanoparticles in the form of microfibers from the washing of synthetic textiles (Browne *et al.*, 2011).

2.2.2 Maritime inputs

The dumping of waste from ships, though a common practice historically, has sufficiently been reduced since the 1990 international shipping regulation MARPOL Annex V, indicated by waste management procedures on commercial, private, and military vessels.

Shipping, fishing, fish farming, offshore mining, illegal dumping at sea, and natural disasters are the current maritime sources of plastic waste (UNEP, 2009). Marine litter from shipping sources include merchant, public transportation, leisure, naval and research vessels. Initially, it was believed that 20% of marine debris came from maritime activities (Faris and Hart, 1994). According to estimates by O'Hara *et al.* (1988), the only component of commercial fishing gear that makes up all of the debris found in the ocean is 5%. Overall, the fishing industry is suspected to be responsible for 18 % of the marine plastic debris found in the marine environment (Andrady, 2011). In areas with limited input from other anthropogenic sources, fishing gear may contribute significantly higher proportions of trash (Eriksson and Burton, 2003; Merrell, 1984; Slip and Burton, 1991).

2.2.3 Catastrophic events

While the majority of trash is regularly dumped into the oceans, catastrophic occurrences cause spikes in the amount of debris. Large volumes of trash could end up in the oceans as a result of floods, cyclones, and tsunamis (Thiel and Haye, 2006). This mainly happens locally and infrequently. According to several studies, river floods send a lot of marine debris into the coastal waters (Carson *et al.*, 2013; Moore *et al.*, 2011; Morritt *et al.*, 2014). The recent tsunami in Japan in 2011 is the first incident to prompt organized study efforts in estimating and monitoring plastic waste dumped into the oceans (Lebroton and Borrero, 2013; Bagulayan *et al.*, 2012; Calder *et al.*, 2014). Areas that typically receive relatively minor amounts of debris may sporadically receive enormous amounts of plastic debris as a result of catastrophic catastrophes. Large numbers of artificial structures (floats, buoys, ropes, boats, etc.) that have previously been inhabited by coastal species may also be lost as a result of their actions. This separation of overgrown structures raises concerns in particular since, if positively buoyant, these rafts could be transported across considerable distances.

2.3 Researches on MPs

There are several studies that investigated the presence of MPs in GT, gill and muscle of fishes from different regions of the world. Abundances, shape, color and types of MPs in different parts of fish bodies were reported by many researchers. Though they mainly focused on GT analysis but there are also some reports on gill analysis from different regions of the world

whereas very few reports were found on muscle analysis. It is a matter of regret that there are no reports on the presence of MPs in muscle from the Sundarbans of Bangladesh.

MPs were found in fish from the Zhanjiang mangrove wetland in South China (Huang *et al.*, 2020), in deep-sea fish from the northern continental slope of the South China Sea (Zhu *et al.*, 2019), in fish from the Rio de la Plata estuary's coastal freshwater (Pazos *et al.*, 2017), in commercial fish off the Portuguese coast (Neves *et al.*, 2015), in pelagic and demersal fish from the English Channel (Lusher *et al.*, 2013), in fish from coastal and fresh waters of China (Jabeen *et al.*, 2017), in three commercial fish from the Mondego estuary, Portugal (Bessa *et al.*, 2018), in demersal fish species from the Spanish Atlantic and Mediterranean coasts (Bella *et al.*, 2016), in multiple environmental compartments in Poyang Lake, China (Yuan *et al.*, 2019), in captured and wild mullets from eastern coast of Hong Kong (Cheung *et al.*, 2018), in striped red mullet (*Mullus surmuletus*) (Alomar *et al.*, 2017), in wild fish larvae from the western English Channel (Steer *et al.*, 2017), in fishes from coastal areas of China (Su *et al.*, 2019), from freshwater fish in the Chi River, Thailand (Kasamesiri and Thaimuangphol, 2020), in digestive tract of gudgeons (*Gobio gobio*) from French rivers (Sanchez *et al.*, 2014), fishes from the Northern Bay of Bengal at Bangladesh (Hossain *et al.*, 2019), in brown shrimp (*Metapenaeus monoceros*) and tiger shrimp (*Penaeus monodon*) from the Northern Bay of Bengal, Bangladesh (Hossain *et al.*, 2020), in two commercially important dried fish from the Bay of Bengal (Hassan *et al.*, 2022), in different commercial freshwater fish species from Bangladesh (Parvin *et al.*, 2021), in commercial fish species from the Bay of Bengal, Bangladesh (Ghosh *et al.*, 2021). MPs were also found in mangrove sediment of Maowei Sea of the South China Sea (Li *et al.*, 2019), the northern coast of the Persian Gulf (Naji *et al.*, 2019) and coastal mangrove ecosystem of Singapore (Nor and Obbard, 2014).

Fish have been exposed to marine MPs, which are extensively found throughout the water column and come in a range of sizes, shapes, types, and colors (Romeo *et al.*, 2015). Numerous researches have reported on the nature and morphology of MPs from various regions (e.g. Hossain *et al.*, 2019; Teng *et al.*, 2019; Frias and Nash, 2019; Kolandhasamy *et al.*, 2018; Li *et al.*, 2015, 2016). According to Hossain *et al.* (2019), the bulk of the plastic particles were fibers (50–55%) among the types of MPs that were discovered, such as fiber and fragment. The most common types of MPs seen in fish and marine habitats are fibers and films (Browne *et al.*, 2011; Gago *et al.*, 2018; Hossain *et al.*, 2019; Kane and Clare, 2019). Synthetic fibers were discovered in fish by Lusher *et al.* (2013) at a rate of 68.3%, and similar findings were made by Rochman *et al.* (2015). According to earlier studies, 96% of fibers were discovered by Pazos

et al. (2017), 83% by Compa *et al.* (2018), 70% by Güven *et al.* (2017), 66% by Neves *et al.* (2015), and 71% by Bellas *et al.* (2016). Due to their one-dimensional nature and ease of fragmentation, the fibers predominate over other shapes (fragments) (Wang *et al.*, 2021).

Coloration is a common sign of increased demand and the use of a large variety of plastic products in our daily life and resulting in large quantities of plastic wastes (Zhang *et al.*, 2020a). The abundance of black color MPs in the fish intestine was reported in many studies (Hossain *et al.*, 2019, 2020; Ghosh *et al.*, 2021; Huang *et al.*, 2020; Pazos *et al.*, 2017; Bessa *et al.*, 2018), blue colors MPs obtained by (Hossain *et al.*, 2019, 2020; Ghosh *et al.*, 2021; Huang *et al.*, 2020; Parvin *et al.*, 2021; Pazos *et al.*, 2017; Bessa *et al.*, 2018); purple/violet by (Ghosh *et al.*, 2021; Parvin *et al.*, 2021), transparent by (Ghosh *et al.*, 2021; Huang *et al.*, 2020; Hossain *et al.*, 2019, 2020; Jabeen *et al.*, 2017; McNeish *et al.*, 2018; Parvin *et al.*, 2021; Pazos *et al.*, 2017; Bessa *et al.*, 2018), green by (Ghosh *et al.*, 2021; Huang *et al.*, 2020; Hossain *et al.*, 2019, 2020; Parvin *et al.*, 2021; Pazos *et al.*, 2017; Bessa *et al.*, 2018), and red by (Hossain *et al.*, 2019, 2020; Ghosh *et al.*, 2021; Huang *et al.*, 2020; Parvin *et al.*, 2021; Pazos *et al.*, 2017; Bessa *et al.*, 2018; Bellas *et al.*, 2016).

Several researchers also looked into chemical properties of MPs. A huge molecule or macromolecule called a polymer is mostly made up of a multitude of smaller components. Using the FTIR technique, the types and properties of MPs polymers that are present in marine fish and aquatic fish were identified, and the scientific literature pertaining to MPs research using the FTIR technique was reviewed. Because FTIR may be used with any light source frequency, analysis can be completed more quickly. In addition, FTIR sensitivity is 80–200 times greater than that of conventional dispersing instruments (Rianda *et al.*, 2020). Aquatic environments contain a variety of plastic polymers, although the most prevalent MPs in the marine environment and among fish are PP, PE, PES, and PS (Gago *et al.*, 2018; Halstead *et al.*, 2018; Andrady, 2011), as well as HDPE, LDPE, PET, and PA (Silva *et al.*, 2018).

Mangrove ecosystems are an important component of both aquatic and terrestrial environments with unique floral and faunal compositions, and thus their preservation is of critical importance (Naji *et al.*, 2019). Despite the greater knowledge on marine MPs, to date, only a few studies have investigated the occurrence of MPs in fishes from Bangladesh and there is no study in the fishes from Sundarbans of Bangladesh. Therefore, the purpose of this study was to investigate, for the first time, the abundance and distribution of MPs in GT, gill and muscle of fishes from

the Sundarban mangrove ecosystem of Bangladesh, with emphasis on an area which is adjacent to intensive anthropogenic activities.

2.4 Information of three fish species

This study investigated the most three common commercial species of fishes that are found in the Sundarban regions. They are *Mystus gulio*, *Lates calcarifer* and *Hyporhamphus limbatus*.

M. gulio (Hamilton, 1822) is commonly known as long whiskers catfish and locally known as Nuna tengra in Bangladesh. It is a brackish water fish that lives in the Sundarbans (Huda *et al.*, 2003) and the Bay of Bengal and also enters tidal rivers of Bangladesh (Rahman, 1989). It is also found in Meghna river (Rahman, 2005, 1989) canals, beels, haors, oxbow lakes and estuaries (Shafi and Quddus, 2001). It is a demersal species and Juveniles and adult of this species feed on debris, zooplanktons, zoobenthos, other benthic invertebrates, fish eggs and larvae (Siddique, 2007). The species is used as food fish in Bangladesh and controls water pollution by consuming aquatic detritus (Siddique, 2007). Tengra is caught by cast, drag, seine nets and hooks (Shafi and Quddus, 2001).



Figure 1: *Mystus gulio* (Fishbase)

L. calcarifer (Bloch, 1790) is commonly known as barramundi and locally known as vetki in Bangladesh. It is a eurihaline and catadromous species (Kungvankij *et al.*, 1986) that lives in marine, freshwater and brackish water ranged between 10-40 m depth. They are found in the Bay of Bengal, estuaries and tidal rivers, sometimes in freshwater. They are very common in estuaries of Barisal, Patnuakhali and Khulna (Rahman, 1989). Adults barramundi are voracious

carnivore and juvenile are omnivore (Kungvankij *et al.*, 1986). They are used as food fish in Bangladesh (Yadav, 1999). It is a very popular and sought-after fish of very considerable economic importance.



Figure 2: *Lates calcarifer*

H. limbatus (Valenciennes, 1847) is commonly known as Congaturi halfbeak and locally known as Ek Thota in Bangladesh. This fish is mainly riverine and found mostly in Southern-Eastern parts of Bangladesh (Rahman, 1989; IUCN Bangladesh, 2000) i.e. available in the Sundarbans (Huda *et al.*, 2003) and Passur river in Khulna. They live in pelagic-neritic zone and feed mainly on insects, zooplanktons (Ariyaratne *et al.*, 2008). They are marketed as fresh and dried salted.

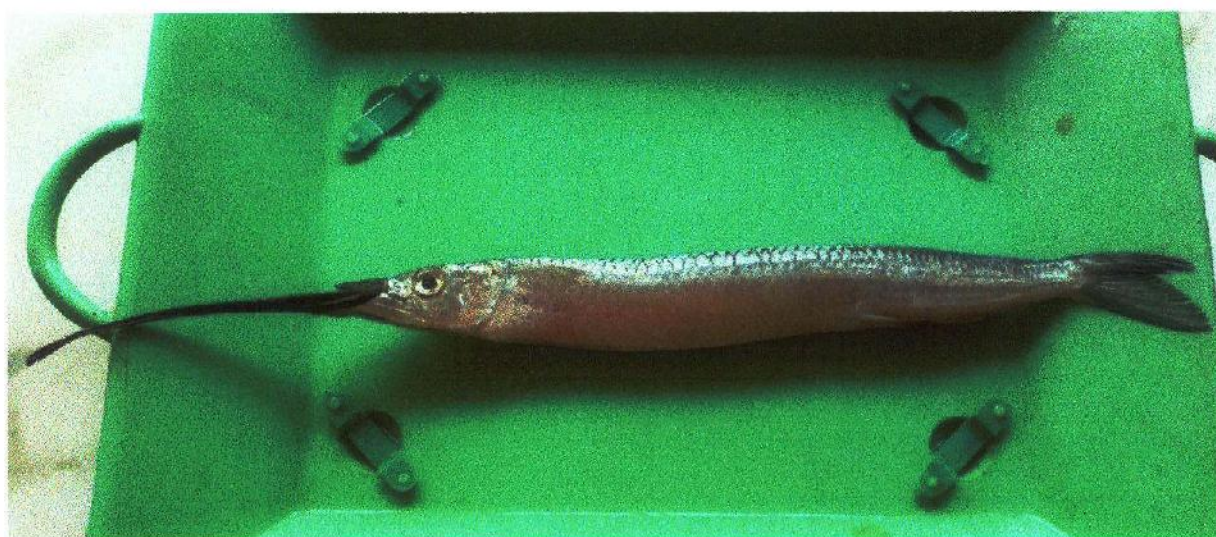


Figure 3: *Hyporhamphus limbatus*

Chapter 3

3. Materials and methods

3.1 Fish sample collection from sampling stations

Long whiskers catfish (*M. gulio*), barramundi (*L. calcarifer*) and Congaturi halfbeak (*H. limbatus*) were collected from three landing stations (Joymuni, Sarankhola and Munshiganj) located near the Dhumkoli, Bhola and Baleshwar rivers adjacent to the Sundarbans in Bangladesh (Figure 4) and only fresh samples were used for this study. One sampling was done from each sampling station. Total of 9 individual consist of three species were collected from study stations. Each sample was collected prior to the identification of the target species following species identifying guideline.

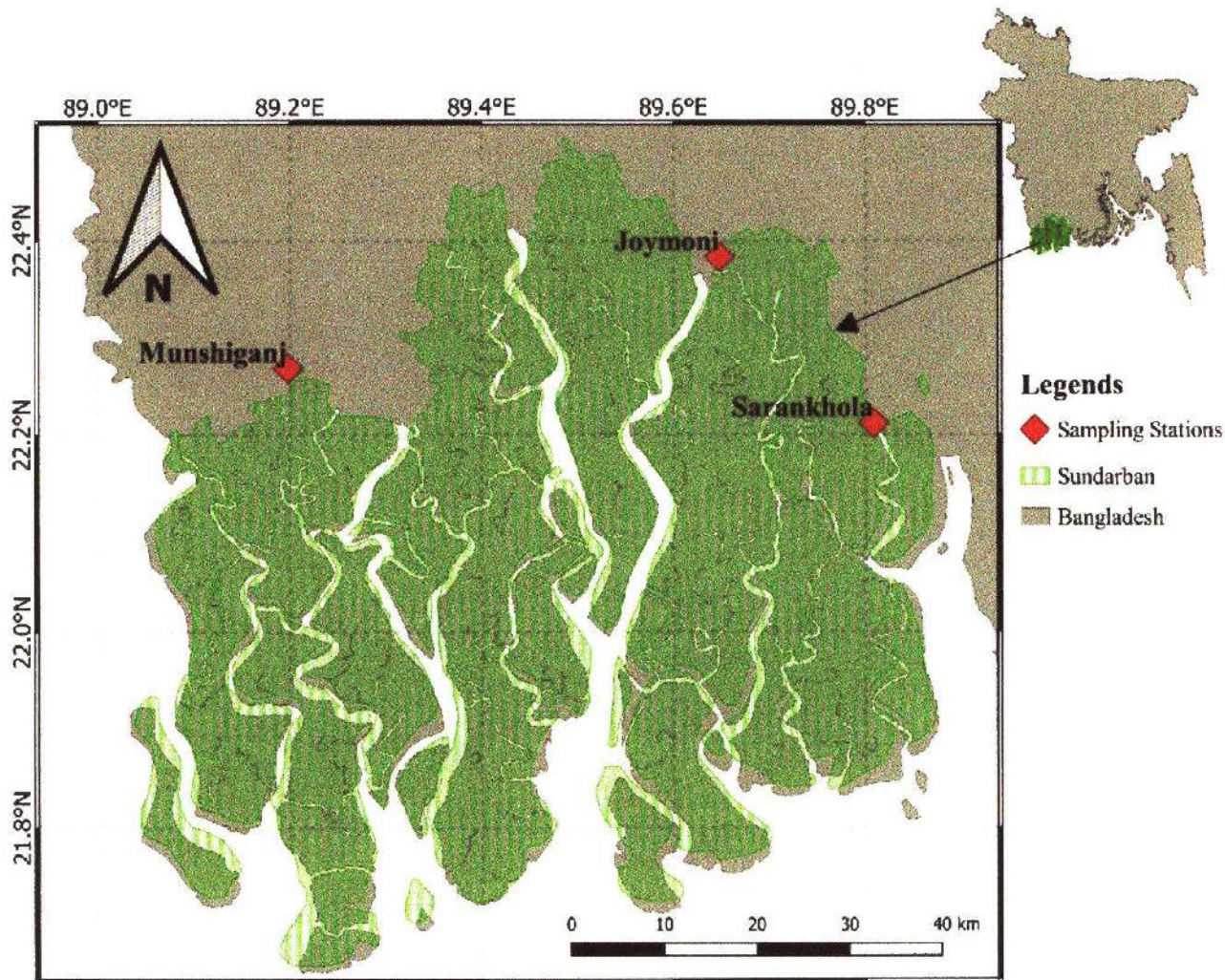


Figure 4: Fish sampling stations in the study area of the Sundarbans

3.2 Sample preparation

Collected fishes were preserved in icebox and transferred to the laboratory of Fisheries and Marine Resource Technology Discipline at Khulna University and kept in -20°C refrigerator for MPs analysis. The preserved fish specimens were thawed in a metal tray and washed in the laboratory by using double distilled water. Basic measurements, such as total length, fork length and body weight of each specimen were recorded. Then GT, gill and muscle were dissected separately in a metal tray by using scissors, scalpel and forceps and transferred to a petri-dish, weighed and transferred into a 500 mL glass beaker. Thus, the total number of samples from nine individual of each species were 27 (9×3). While removing GT, from the top of the esophagus, keeping the whole stomach intact and the gut would be cut some millimeter before the anus to avoid the contamination from outside through anus (Hermesen *et al.*, 2017). The protocol is summarized in Figure 5.

3.3 Hydrogen peroxide treatment

Each sample was placed in one glass beaker and approximately 50 mL of 30% H_2O_2 was added to each beaker to digest the organic matter depending on the weight of the soft tissue in each beaker (Li *et al.*, 2015; Su *et al.*, 2016). Several studies identified hydrogen peroxide (H_2O_2) solution to be more effective to digest biogenic material (Nuelle *et al.*, 2014; Avio *et al.*, 2015; Mathalon and Hill, 2014) than hydrochloric acid (HCl) and sodium hydroxide (NaOH) (Cole *et al.*, 2014). The beakers were covered with foil paper and placed in an oscillation incubator at 65°C at 80 rpm for 24 h and then kept at room temperature for 24–48 h depending on the digestion effect of the soft tissue (Avio *et al.*, 2015).

3.4 Saline (NaCl) solution floatation and filtration

Saline solution (1.2 g/mL NaCl) was used to separate MPs from the dissolved liquid of soft tissues through floatation (Hidalgo-Ruz *et al.*, 2012; Li *et al.*, 2015). Approximately 200 mL of filtered NaCl solution was added to each beaker. The samples were left at room temperature for one week. Saturated salt solution allows the separation of less dense particles, i.e. a layer of MPs floated upwards, whereas undissolved organic residues and inorganic matters subsided at the bottom of the beaker (Lusher *et al.*, 2017b). The samples were sieved (Hermesen *et al.*, 2017) through a $50\text{ }\mu\text{m}$ stainless steel sieve (CIM, Japan). The residue on the sieve was backwashed into a clean petri-dish with double distilled water and covered for further analysis (Kolandhasamy *et al.*, 2018).

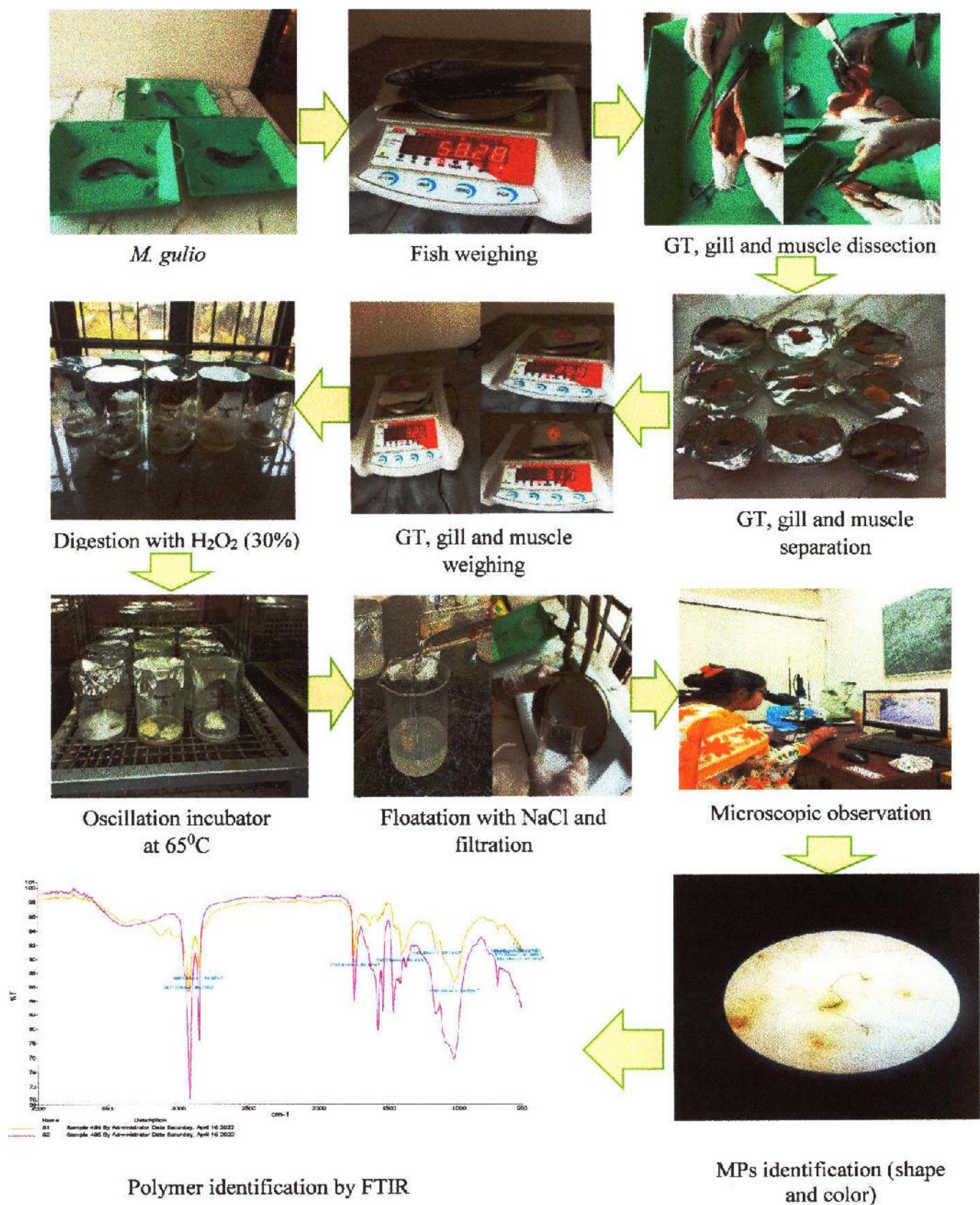


Figure 5: Flow chart of sample preparation, digestion and analytical procedure for MPs detection in fish

3.5 Microscopic observation of MPs

The samples were visually inspected under a trinocular microscope (AmScope, model T120C, USA) and images of plastic items were taken with a Realme Nazro 20 Pro camera at different magnifications. The items were assed visually (Hidalgo-Ruz *et al.*, 2012) and described according to color and shape (Lusher *et al.*, 2013). The morphotypes of MPs were classified into fiber, filament, and fragment (Li *et al.*, 2016; Hidalgo-Ruz *et al.*, 2012) and confirmed according to their physical characteristics (Kolandhasamy *et al.*, 2018). MPs were categorized into six different colors of black, blue, transparent/white, purple, green and red.

3.6 FTIR analysis

The FTIR analysis of the samples at 0% and 60% RH was analyzed with a Perkin Elmer FTIR Spectrometer (Waltham, USA), using the attenuated total reflection (ATR) mode (4000-650 cm^{-1}). A representative number (21) of samples were randomly selected for analysis of polymer types. The selected MPs represented the most common types of visually inspected particles from all samples. Absorption bands of each polymer were compared to the absorption bands of each polymer reported in the literature (Jung *et al.*, 2018).

3.7 Data analysis

The shape, color and polymer type of MPs were measured for each fish species. The relationships between fish traits (i. e. body weight, GT weight, gill weight and mussel weight) and MPs abundance was carried out with linear regression analysis. All the analysis were conducted at 5% significance level. In addition, MPs shape, color pattern, type were analyzed following descriptive statistical analysis. All statistical tests were performed using IBM SPSS software (version 22.0).

3.8 Contamination control

During fish sample collection, transportation to the laboratory, preservation, thawing and washing, dissection and removal of gastrointestinal tract, muscle, gill, alkali digestion, NaCl floatation and MP identification, care was taken throughout the process in order to avoid cross contamination. The working table was cleaned before use and covers were placed over samples wherever necessary. The removal of GT, gill, muscle were done swiftly and were placed in a petri- dish and immediately covered to prevent possible contamination by airborne fibers (Devriese *et al.*, 2015; DeWitte *et al.*, 2014). Exclusive cotton coat and clothing, latex gloves, glass and metal ware were used at the laboratory (Neves *et al.*, 2015). All of the glassware were

rinsed three times with filtered water. The samples were immediately covered if they were not in use. All of the experimental procedures were completed at the earliest convenience.

Chapter 4

4. Results

4.1. Abundance of MPs

The range of the total abundance of MPs in three individual fishes were 2.49-40.35 items/g and the average abundance of MPs in GT, gill and muscle of the fishes were 12.20 items/g, 8.03 items/g and 2.37 items/g, respectively (Table 1). The highest abundance of MPs was found in GT, second highest was in gill and the lowest was found in muscle of the three fish species from the Sundarbans of Bangladesh.

Table 1: MPs abundance in gastrointestinal tract (GT), gill and muscle of the three species from the Sundarbans

Species Name	Sample No	Body Parts			Items/g/individual
		GT (items/g)	Gill (items/g)	Muscle (items/g)	
<i>M. gulio</i>	S 1	3.99±0.03	0.00±3.08	0.00	7.53±3.07
	S2	4.00±0.03	5.17±3.08	0.00	
	S3	3.95±0.03	5.48±3.08	0.00	
<i>L. calcarifer</i>	S 1	4.21±0.86	7.99±2.57	2.72±0.54	11.62±2.97
	S2	2.49±0.86	3.49±2.57	3.19±0.54	
	S3	3.38±0.86	3.59±2.57	3.79±0.54	
<i>H. limbatus</i>	S 1	16.00±12.32	0.00±13.51	6.00±3.37	48.66±23.80
	S2	31.45±12.32	24.78±13.51	0.00±3.37	
	S3	40.35±12.32	21.74±13.51	5.66±3.37	
Total		109.82	72.24	21.35	67.80
Average (items/g/individual)		12.20±14.21	8.03±9.03	2.37±2.48	22.60±23.05

All samples of the collected fish species had MPs except the muscle of *M. gulio*. The highest abundance of MPs was found in all parts (GT, gill and muscle) of *H. limbatus* (Table 1 and Figure 6).

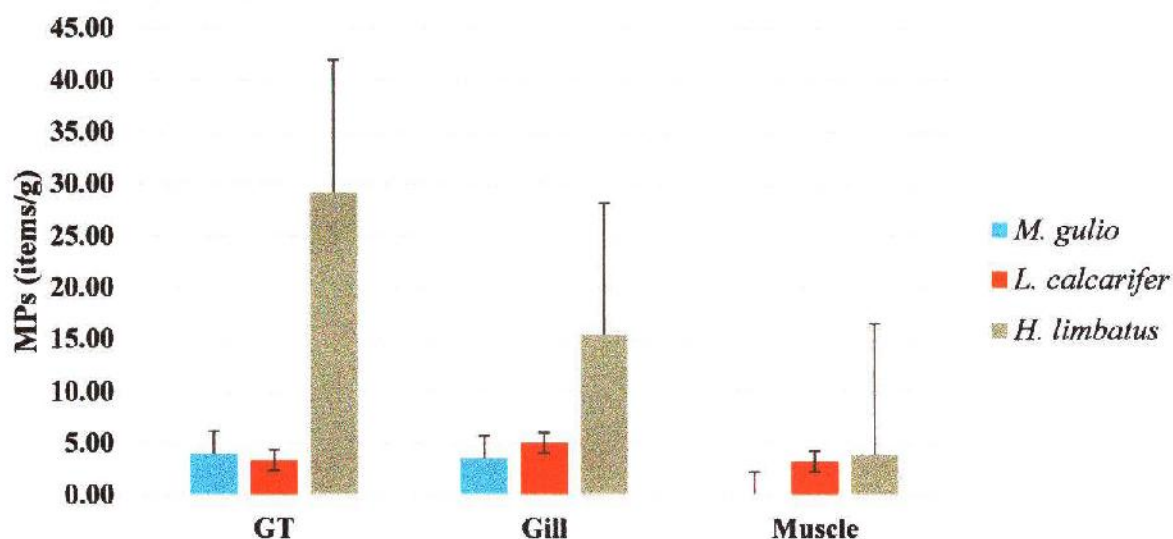


Figure 6: MPs abundance (items/g) in GT, gill and muscle of the three species from the Sundarbans

The total numbers of MPs were found 50, 3467 and 897 in *M. gulio*, *L. calcarifer* and *H. limbatus*, respectively. A positive significant ($p < 0.0001$) correlation ($r=0.95$) was found between the body weight and total numbers of MPs, suggesting that species with relatively more weight is likely to contain higher numbers of MPs (Figure 7).

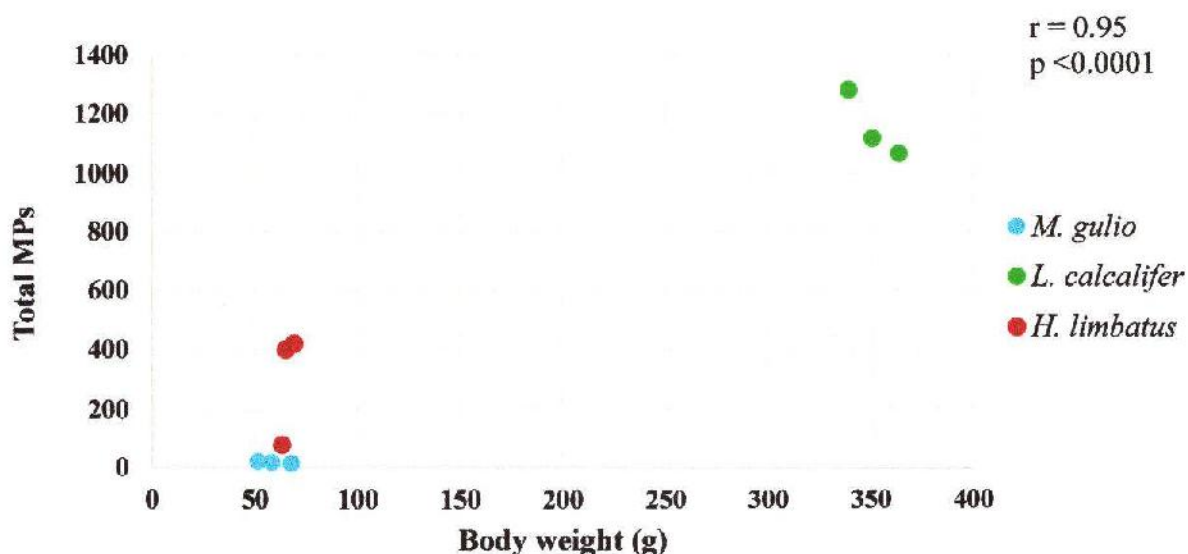


Figure 7: Relationship between the body weight (g) and number of MPs in three species from the Sundarbans

4.2. Shape of MPs

Fiber, filament and fragment shapes of MPs were found in the fishes of this study where highest amount of MPs was found in *L. calcarifer* and the lowest amount was found in *M. gulio*. Fiber shape (8.66-51.67 items/g) MPs was dominant in all the three fish species. The highest number of fiber shape (51.67 items/g) MPs was found in *L. calcarifer*, the second highest number (40.00 items/g) was found in *H. limbatus*, and the lowest number (8.66 items/g) MPs was found in *M. gulio*. The highest number of filament shape (28.33 items/g) MPs was found in *L. calcarifer*, the second highest number (28.00 items/g) was in *H. limbatus* and the lowest number (5.66 items/g) found in *M. gulio*. The highest number of fragment shape (11.67 items/g) MPs was found *L. calcarifer*, the second highest number (10.66 items/g) was found in *H. limbatus*, and lowest number (2.33 items/g) was found in *M. gulio* (Table 2).

Table 2: Shape of MPs in GT, gill and muscle of three fish species from the Sundarbans

Name of Species	Body parts	Shape (items/g & %)		
		Fiber	Filament	Fragment
<i>M. gulio</i>	GT	5.33±1.53 (48.48%)	3.33±1.15 (30.30%)	2.33±1.53 (21.21%)
	Gill	3.33±3.05 (58.82%)	2.33±2.08 (41.18%)	0.00
	Muscle	0.00	0.00	0.00
	Subtotal	8.66	5.66	2.33
<i>L. calcarifer</i>	GT	24.67±7.57 (56.06%)	13±4.58 (29.55%)	6.33±5.51 (14.39%)
	Gill	17.67±6.03 (56.38%)	10±4.36 (31.91%)	3.67±3.51 (11.7%)
	Muscle	9.33±1.15 (57.14%)	5.33±1.15 (32.65%)	1.67±1.53 (10.20%)
	Subtotal	51.67	28.33	11.67
<i>H. limbatus</i>	GT	18±5.29 (45%)	14.67±5.77 (36.67%)	7.33±3.05 (18.33%)
	Gill	10.67±10.06 (55.17%)	7.33±6.43 (37.93%)	1.33± 2.31(6.90%)
	Muscle	11.33±10.26 (58.62%)	6±5.29 (31.03%)	2±3.46 (10.34%)
	Subtotal	40	28	10.66

The highest percentage of fiber shape (58.82%) was found in gill of *M. gulio* and the lowest percentage (45%) was found in muscle of *H. limbatus*. The highest percentage of filament shape (41.18%) was found in gill of *M. gulio* and lowest percentage (29.55%) was found in GT of *L. calcarifer*. The highest percentage of fragments shape (21.21%) was found in GT of *M. gulio* and the lowest percentage (6.90%) was found in gill of *H. limbatus* (Table 3). Relative comparison of MPs shapes in GT, gill and muscle are shown in Figure 8.

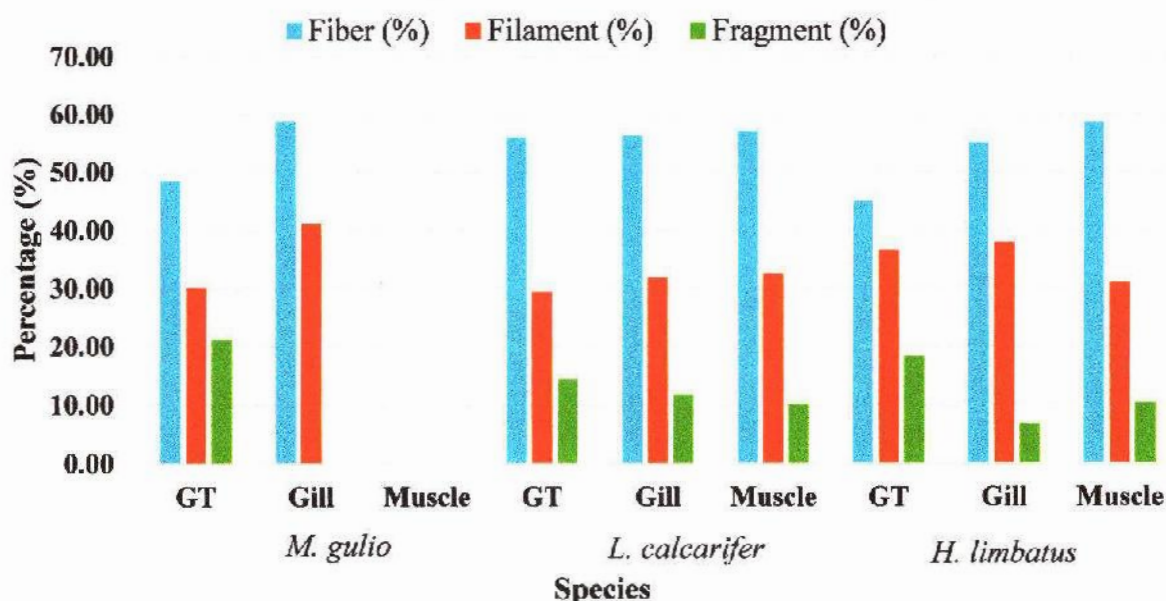


Figure 8: Comparison of MPs shape in GT, gill and muscle of the three species from Sundarbans

4.3. Color of MPs

The dominant color of MPs was black (10.67-58.34 items/g), followed by blue, purple, transparent, green and red in three fish species from the Sundarbans. The highest number of black color (58.34 items/g) MPs was found in *L. calcarifer*, while lowest number (10.67 items/g) was found in *M. gulo*. The highest number of blue color (18 items/g) MPs was found in *H. limbatus*, while lowest number (3 items/g) was found in *M. gulo*. The highest number of transparent color (6.33 items/g) MPs was found in *L. calcarifer*, while lowest number (0.67 items/g) was found in *M. gulo*. The highest number of green color (4 items/g) MPs was found in *L. calcarifer*, while lowest number (1 items/g) was found in *M. gulo*. Purple color MPs was not found in *M. gulo* but was found in other two fish species in relatively higher abundance than transparent, green and red color MPs. The GT of *L. calcarifer* and *H. limbatus* have all the six colors of detected MPs from Sundarbans (Table 3).

Table 3: Colors of MPs in GT, gill and muscle of the three species from the Sundarbans

Name of Species	Body Parts	Colors (items/g & %)					
		Black	Blue	Green	Purple	Red	Transparent
<i>M. gulo</i>	GT	7±3.60 (63.64%)	1.67±1.53 (15.15%)	0.67±1.15 (6.06%)	0	1±1.73 (9.09%)	0.67±1.15 (6.06%)
	Gill	3.67±3.51 (64.71%)	1.33±2.31 (23.53%)	0.33±0.58 (5.89%)	0	0.33±0.58 (5.89%)	0

	Muscle	0	0	0	0	0	0
	Subtotal	10.67	3	1	0	1.33	0.67
<i>L. calcarifer</i>	GT	28±15.87 (63.64%)	4.33±2.52 (9.85%)	2±2 (4.55%)	4.33±2.52 (9.85%)	1.33±2.31 (3.03%)	4±6.93 (9.09%)
	Gill	20.67±8.08 (65.96%)	8±8 (25.53%)	0	1.33±2.31 (4.25%)	1.33±2.31 (4.25%)	0
	Muscle	9.67±3.21 (59.18%)	0	2±2 (12.24%)	2.33±1.53 (14.29%)	0	2.33±2.52 (14.29%)
	Subtotal	58.34	12.33	4	8	2.67	6.33
<i>H. limbatus</i>	GT	12.33±5.13 (30.83%)	15.33±11.01 (38.33%)	2.67±2.31 (6.67%)	3.33±3.05 (8.33%)	4±4 (10%)	2.33±4.04 (5.83%)
	Gill	13.67±12.67 (70.69%)	1.33±2.31 (6.9%)	0	3±2.64 (15.52%)	0	1.33±2.31 (6.9%)
	Muscle	14±12.49 (72.41%)	1.33±1.15 (6.9%)	0.67±1.15 (3.45%)	2±2 (10.34%)	0	1.33±2.31 (6.9%)
	Subtotal	40	18	3.34	8.33	4	5

The highest percentage of black color (72.41%) MPs was found in muscle of *H. limbatus* and the lowest percentage (30.83%) was found in GT of *H. limbatus* (Table 3). Relative comparison of MPs colors in GT, gill and muscle are shown in Figure 9.

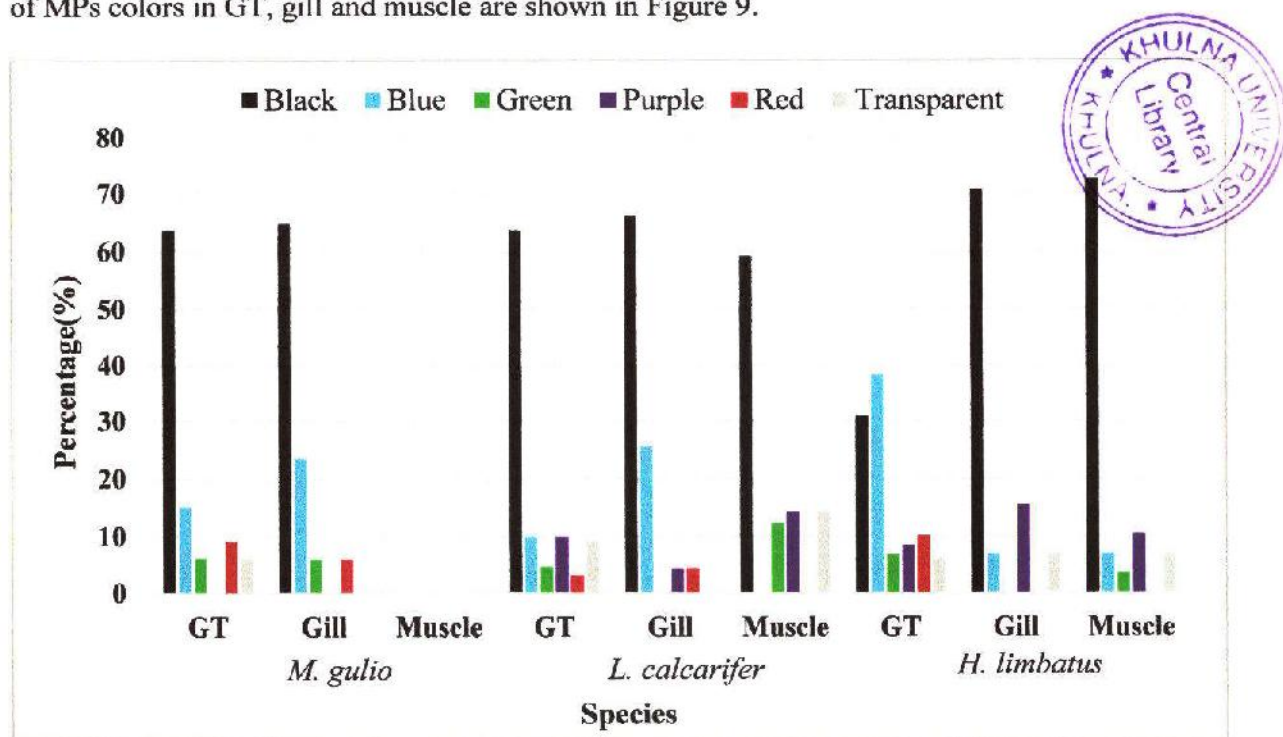


Figure 9: Comparison of MPs colors in GT, gill and muscle of the three species from the Sundarbans

4.4. FTIR analysis of polymer

A total of 21 samples were taken for FTIR analysis and MPs polymers were identified among 15 samples (Table 4). Considering all samples of three species, the most abundant analyzed polymers was latex (17%) and EVA (16%), followed by HDPE (11%), LDPE (11%), PU (8%), PET (8%), CA (8%), PVC (6%), PP (6%), PMMA (5%), nylon (3%) and nitrile (1%) as illustrated in Figure 10.

Table 4: FTIR analysis of the three fish species from the Sundarbans

Polymer types	Number/sample and %			Total (Number/sample and %)
	<i>M. gulio</i>	<i>L. calcarifer</i>	<i>H. limbatus</i>	
Ethylene vinyl acetate (EVA)	0.50 (21.43%)	1.00 (16.67%)	0.43 (12.50%)	1.93 (16.38%)
Latex	0.50 (21.43%)	0.88 (14.58%)	0.57 (16.67%)	1.95 (16.55%)
High-density polyethylene (HDPE)	0.33 (14.29%)	0.50 (8.33%)	0.43 (12.50%)	1.26 (10.70%)
Low-density polyethylene (LDPE)	0.33 (14.29%)	0.50 (8.33%)	0.43 (12.50%)	1.26 (10.70%)
Polyvinyl chloride (PVC)	0.17 (7.14%)	0.38 (6.25%)	0.14 (4.17%)	0.69 (5.86%)
Cellulose acetate (CA)	0.33 (14.29)	0.50 (8.33%)	0.14 (4.17%)	0.97 (8.23%)
Polyurethane (PU)	0.17 (7.14)	0.50 (8.33%)	0.29 (8.33%)	0.96 (8.15%)
Polypropylene (PP)	0.00	0.63 (10.42%)	0.14 (4.17%)	0.77 (6.54%)
Polyethylene terephthalate (PET)	0.00	0.50 (8.33%)	0.43 (12.50%)	0.93 (7.89%)
Polymethyl methacrylate (PMMA)	0.00	0.25 (4.17%)	0.29 (8.33%)	0.54 (4.58%)
Nylon	0.00	0.25 (4.17%)	0.14 (4.17%)	0.39 (3.31%)
Nitrile	0.00	0.13 (2.08%)	0.00	0.13 (1.10%)
Total	2.33 (100%)	6 (100%)	3.43 (100%)	11.78 (100%)

Considering the samples of *M. gulio*, the most abundant analyzed polymers was EVA (21.43%) and latex (21.43%), followed by HDPE (14.29%), LDPE (14.29%), CA (14.29%), PU (7.14%)

and PVC (7.14%) whereas, in case of *L. calcarifer*, the most abundant analyzed polymers was EVA (16.67%) and latex (14.58%), followed by PP (10.42%), HDPE (8.33%), LDPE (8.33%), PU (8.33%), PET (8.33%), CA (8.33%), PVC (6.25%), PMMA (4.17%), nylon (4.17%), nitrile (2%) and in case of *H. limbatus*, the most abundant analyzed polymers was latex (16.67%), followed by EVA (12.50%), HDPE (12.50%), LDPE (12.50%), PET (12.50%), PU (8.33%), PMMA (8.33%), PP (4.17%), CA (4.17%), PVC (4.17%), nylon (4.17%). PP, PET, PMMA, nylon, and nitrile types of polymer were absent in *M. gulio* (Table 4).

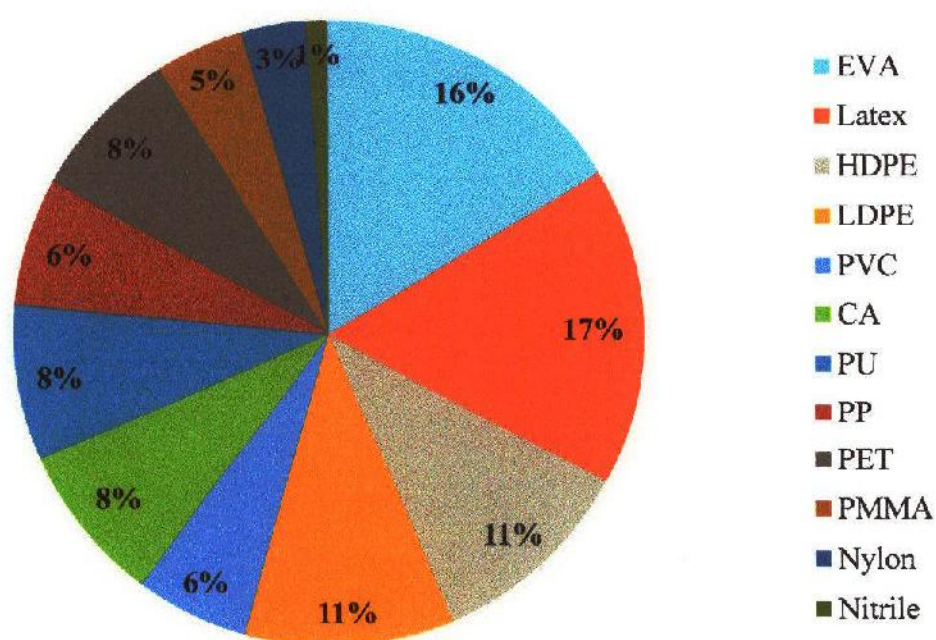


Figure 10: Percentage (%) of polymer types by FTIR of three species from the Sundarbans

Chapter 5

5. Discussion

This study has reported the presence of MPs in all body parts (GT, gill and muscle) of three fish species (*M. gulio*, *L. calcarifer* and *H. limbatus*) except the muscle of *M. gulio* from the Sundarbans of Bangladesh. These results are comparable to other studies from different regions of the world.

Table 5: Abundance of MPs in GT, gill and muscle of different fish species from different regions of the world

Study area	MPs (items/g)			Species name	References
	GT	Gill	Muscle		
Northern Bay of Bengal	0.07-0.08	×	×	<i>H. nehereus</i> , <i>H. translucens</i> , <i>S. gibbosa</i>	Hossain <i>et al.</i> , 2019
Bay of Bengal	3.40-3.87	×	×	<i>M. monoceros</i> , <i>P. monodon</i>	Hossain <i>et al.</i> , 2020
Bay of Bengal	0.45-6.45	×	×	<i>P. hamrur</i> , <i>S. tenuifilis</i> , <i>S. sona</i> , <i>C. chrysophrys</i> , <i>S. brachysoma</i> , <i>H. nehereus</i> , <i>O. pama</i> , <i>C. neglecta</i> , <i>A. chacunda</i> , <i>M. cordyla</i>	Ghosh <i>et al.</i> , 2021
Coastline of Belgian	×	×	0.26-0.51	<i>Mytilus edulis</i>	De Witte <i>et al.</i> , 2014
Coastline of China	×	×	0.9-4.6	<i>M. edulis</i>	Li <i>et al.</i> , 2016
Atlantic ocean	×	×	0.36±0.07 0.47±0.16	<i>M. edulis</i> , <i>C. gigas</i>	Van Cauwenberghe and Janssen, 2014
Black Sea	1.1-6.6	0.7-2.8	1.1-1.2	<i>M. barbatus</i> , <i>A. immaculata</i>	Atamanalp <i>et al.</i> , 2021
Sundarbans of Bangladesh	2.49-40.35	3.49-24.78	2.72- 6.0	<i>M. gulio</i> <i>L. calcarifer</i> <i>H. limbatus</i>	Present study

The abundance of MPs in GT, gill and muscle observed in the present studies which are much higher than the previous studies showed in Table 5. Other studies also reported the abundance of MPs in items/individual, for example, 1.0-3.8 items/individual by Ghosh *et al.* (2021); 0.5-9 items/individual by Parvin *et al.* (2021); 1.1-2 items/individual by Cannon *et al.* (2016); 1.1-7.2 items/individual by Jabeen *et al.* (2017) and average 2.60 items/individual by Guven *et al.* (2017); 1.5-7.6 items/individual by Li *et al.* (2016); 0.6-8.0 items/individual by Huang *et al.* (2020).

Until recently, evidence of fish MPs ingestion mostly comes from the GT (Hossain *et al.*, 2019; Zhu *et al.*, 2019; Huang *et al.*, 2020; Pazos *et al.*, 2017; Su *et al.*, 2019; Parvin *et al.*, 2021; Bessa *et al.*, 2018; Bellas *et al.*, 2016; Atamanalp *et al.*, 2021) and gill (Huang *et al.*, 2020; Atamanalp *et al.*, 2021; Su *et al.*, 2019) analysis. There are also some reports on investigating MPs abundance in muscle and liver (Huang *et al.*, 2020; Su *et al.*, 2019) but no MPs was found in those studies. Atamanalp *et al.* (2021) reported the occurrence of MPs in fish muscle from Black Sea while there are no report on MPs in fish muscle from Bangladesh. Surprisingly, we found MPs in muscle of *L. calcarifer* and *H. limbatus* in present study, which is the first reports of MPs in fish muscle from the Sundarbans of Bangladesh.

The abundance of MPs in waters allows fish to easily take these particles and store them in their tissues. Recent researches had been showed that most of fish species are susceptible to MPs ingestion. After ingestion by fish, these pollutants can accumulate in the fish GT and transport to other fish organs (Wang *et al.*, 2020). But MPs smaller than 20 μm can be transported from the GT to the edible muscle (Devriese *et al.*, 2015). Currently there are several scientific data regarding MPs in humans. MPs were detected in human breast milk with a size ranging from 2 to 12 μm (Ragusa *et al.*, 2022). Ibrahim *et al.* (2021) described the presence of MPs in human colectomy samples as an additional measure, demonstrating that MPs partially traverse the intestinal barrier. Schwabl *et al.* (2019) reported the discovery of MPs in human feces. Recently MPs were in the human placenta, which serves as the point of contact between the mother and the fetus when they are exposed to the outside world (Ragusa *et al.*, 2021; Braun *et al.*, 2021). Amato-Lourenço *et al.* (2021) discovered 8.12 to 16.8 m fibers and 5.5 m polymeric MPs in human lung tissue as proof of inhalation exposure. Though size estimation was out of our scope so we couldn't be sure but this might be the reason for the presence of MPs in the muscle of three fish species. Several literatures have described a number of MPs exposure pathways, including inhalation, cutaneous contact, and ingestion (Prata, 2018; Cox *et al.*, 2019) both in fish and human body. MPs can become internalized in human tissues after being consumed or breathed (Prata *et al.*, 2020). The MPs in GT may enter the body tissue by

endocytosis mechanisms or para-cellular diffusion, pass through the epithelium, and then be transferred by dendritic cells through the lymphatic circulation to the circulatory system (Prata *et al.*, 2020). Regarding the respiratory system, it is likely that inhaled MPs pass via the lower respiratory tract, which is characterized by a thin mucus layer, and then diffuse into the bloodstream as a result of cellular absorption or para-cellular diffusion (Mowat, 2003). The discovery of plastic particles in human blood only recently provided conclusive evidence that MPs were transported throughout the body by the bloodstream (Leslie *et al.*, 2022).

MPs particles, that are too small, can be misidentified by fish or accidentally ingested as prey, and also fish can ingest other contaminated (with these MPs particles) organisms (Zakeri *et al.*, 2020). In a previous study, Barboza *et al.* (2020) concluded that MPs taken up by fish can occur by passive (e.g., gill filtration) or by active (i.e., ingestion by confusion with prey) mechanisms and it can be divided into smaller particles by internalizing MPs in the fish digestive system. The MPs abundance in fish GT is closely related to the habitat of the fish (Hossain *et al.*, 2019; Ghosh *et al.*, 2021; Zhang *et al.*, 2020a, 2020b), the environmental pollution level of plastics and the feeding strategy of fish (Romeo *et al.*, 2015; Battaglia *et al.*, 2016) and it may differ according to the nutritional status of the fish (Li *et al.* 2020). In this study, highest (48.66 items/g/individual) MPs was observed in *H. limbatus* and comparatively very low MPs observed in both the *M. gulio* (7.53 items/g/individual) and *L. calcarifer* (11.62 items/g/individual) (Table 1). *M. gulio* and *L. calcarifer* are demersal and *H. limbatus* is a pelagic fish species. In the marine environment, some studies have reported a relatively higher abundance of MPs in pelagic than demersal fishes (Lusher *et al.*, 2013; Bessa *et al.*, 2018), which is in line with the present results. However, opposite results are also present where higher levels of MPs are in demersal fish species (Bellas *et al.*, 2016; Jabeen *et al.*, 2017; Neves *et al.*, 2015). So the result of the present study showed that the ingestion of MPs had no relation with habitat of fish species and feeding behavior. There were no relation because may be the sample size was too small to determine the relations.

Positive relationship between the fish body weight and ingested MPs abundance observed in present study suggests that the larger fish have more MPs accumulation in their bodies than those smaller fish. This result supports the result of Hossain *et al.* (2019, 2020), Ghosh *et al.* (2021), Atamanalp *et al.* (2021), Huang *et al.* (2020) and doesn't supports the result of Pazos *et al.* (2017), Bessa *et al.* (2018). MPs ingested by fish may come from two origins. Firstly, fish could ingest MPs together with prey during feeding (primary ingestion) (Romeo *et al.*, 2015). Alternatively fish may ingest preys that have already ingested MPs (secondary

ingestion) (Romeo *et al.*, 2015). An increase in the abundance of MPs in the marine environment will also increase the bioavailability of MPs to fish (Wright *et al.*, 2013).

The shape of MPs might be an important indicator for their parent materials as different shapes of MPs could be present. MPs found in Bangladesh can be categorized as fibers, filaments, pieces, foam, beads, granules, bars, ovals, elongated, and irregular shapes based on their morphological properties (Hossain *et al.*, 2021). Fish are known to ingest MPs with different shapes, and colors as these plastics have been widely spread throughout water column (Romeo *et al.*, 2015).

Many researchers reported shape and type of MPs in fish species from different regions (e.g. Hossain *et al.*, 2019; Teng *et al.*, 2019; Frias and Nash, 2019; Kolandhasamy *et al.*, 2018; Li *et al.*, 2016; Li *et al.*, 2015). Among the different shapes of MPs (e.g. fiber, filament, and fragment), the majority of the detected MPs were fibers (64–94%), which is higher than the fibers (50–55%) reported by Hossain *et al.* (2019). This result is also in agreement with the studies by Huang *et al.* (2020), Boerger *et al.* (2010), Wright *et al.* (2013), Claessens *et al.* (2011) who reported a majority of fibers (59%) in the samples. Lusher *et al.* (2013) found 68.3% of synthetic fibers in fish from the English Channel, the same results were also recorded in commercial fish from California (Rochman *et al.*, 2015). The previous records also reported 96% fiber in coastal freshwater fish from Río de la Plata estuary (Pazos *et al.*, 2017), 83% in fishes from Spanish coast (Compa *et al.*, 2018), 70% in Mediterranean Sea fishes (Güven *et al.*, 2017), 66% in fishes off the Portuguese coast (Neves *et al.*, 2015), and 71% in fishes from the Spanish coast (Bellas *et al.*, 2016).

The fibers are dominant than other shapes (filaments, fragments), because they are one-dimensional materials and break into smaller pieces easily (Wang *et al.*, 2021). Fishing nets, ropes, laundry and urban wastes may be the possible sources of these fibers in the coastal and marine waters of Bangladesh (Browne *et al.*, 2011; Claessens *et al.*, 2011). Domestic laundry discharges, wastewater treatment plants effluents are also a major source of fibers in the marine environment followed by fragmentation of fishing gear (e.g., ropes and nets) and recreational sailing gear (Browne *et al.*, 2011). Filamentous MPs originate mainly from packaging materials and plastic bags, whereas fragments shape MPs are derived primarily from the breakdown of plastic wastes and plastic products (Zhang *et al.*, 2018). The high abundance of fibers, filaments, and fragment shapes of MPs in Bangladesh indicates that they came mostly from textiles and plastic wastes.

The shape of MPs provide evidential information about the source of plastic particles i.e., primary or secondary MPs (Park *et al.*, 2020). According to Lehtiniemi *et al.* (2018), the

primary shapes of MPs are quite symmetrical, while the secondary MPs are more asymmetrical. The quantity of secondary MPs in the aquatic environment is higher than primary MPs (Galgani *et al.*, 2015; Lehtiniemi *et al.*, 2018). The occurrence of fibers provides evidence of secondary MPs as these are generally produced from clothes and other textile products/by-products (Park *et al.*, 2020). Further, the occurrence of fragments can provide another evidence of secondary MPs as these fragments are typically produced via the breakdown or fragmentation of large plastic materials (Cole *et al.*, 2011). Based on the MPs identified from this study, it can be implicated that most of the MPs extracted were secondary MPs. This result is consistent with the study of fish and shrimp from the Bay of Bengal, Bangladesh (Hossain *et al.*, 2020). Further study is needed to identify the source of MPs in fishes. Fiber types of MPs are more toxic than other types of MPs particle (Ziajahromi *et al.*, 2017), as well as prolong the duration of fiber retention in the intestinal tracts (Au *et al.*, 2015; Lei *et al.*, 2018). The ingestion of fragments, and sharp-edge MPs might cause the blockages of the digestive tract which can results in satiation, starvation, abrasion, damages in stomach wall and physical deterioration in fishes (Gregory, 2009; Wright *et al.*, 2013; Jabeen *et al.*, 2017).

Coloration is a common sign of increased demand and the use of a large variety of plastic products in our daily life and resulting in large quantities of plastic wastes (Zhang *et al.*, 2020). Six different colors (i.e. black, blue, purple/violet, white/transparent, green and red) of MPs were found in the examined GT, gill and muscle samples. The dominant color of the MPs is black, followed by blue, purple/violet, transparent, green and red (Table 3 and Figure 9). The abundance of black color MPs in the fish intestine was also reported in many other studies (Hossain *et al.*, 2019, 2020; Ghosh *et al.*, 2021; Huang *et al.*, 2020; Pazos *et al.*, 2017; Bessa *et al.*, 2018), blue colors MPs obtained by (Hossain *et al.*, 2019, 2020; Ghosh *et al.*, 2021; Huang *et al.*, 2020; Parvin *et al.*, 2021; Pazos *et al.*, 2017; Bessa *et al.*, 2018), purple/violet by (Ghosh *et al.*, 2021; Parvin *et al.*, 2021), transparent by (Ghosh *et al.*, 2021; Huang *et al.*, 2020; Hossain *et al.*, 2019, 2020; Jabeen *et al.*, 2017; McNeish *et al.*, 2018; Parvin *et al.*, 2021; Pazos *et al.*, 2017; Bessa *et al.*, 2018), green by (Ghosh *et al.*, 2021; Huang *et al.*, 2020; Hossain *et al.*, 2019, 2020; Parvin *et al.*, 2021; Pazos *et al.*, 2017; Bessa *et al.*, 2018), and red by (Hossain *et al.*, 2019, 2020; Ghosh *et al.*, 2021; Huang *et al.*, 2020; Parvin *et al.*, 2021; Pazos *et al.*, 2017; Bessa *et al.*, 2018; Bellas *et al.*, 2016). This study result also synchronized with the coloration of MPs found in shrimps and fish species of the Bay of Banges (Hossain *et al.*, 2019).

The dominance of black color MPs is related to the fish age because young fish prefer the black color more. The preference of this black color MPs is due to its similarity with their food color (McNeish *et al.*, 2018; Ory *et al.*, 2018; Ferreira *et al.* 2020). Although phyto-planktons can be transparent, white, or yellow color, but most of them have black patches. Plastic products are usually brightly colored but black plastic refracts light. For this reason, black plastic products are preferred more (Wang *et al.*, 2020). The presence of blue color MPs is thought to be because there are large amounts of blue MPs in sea water and these are actively ingested (Barboza *et al.*, 2020). It is known that black, blue and red MPs are the dominant colors in fish specimens (Lin *et al.*, 2020). Although the color of MPs is determined by the primary source, transformations by UV radiation, weathering and microbial degradation may change the color (Zhang *et al.* 2020b). In Bangladesh, the plastics used in shopping bags, packaging bags, textile fabrics and fishing nets are mainly transparent, which might be ingested by fishes. As for the colored MPs, it is inferred that fishes ingested these MPs by confusing them with their preferential prey (Barboza *et al.*, 2020). However, these transparent and colored MPs might be ingested by fishes accidentally during regular feeding activity (Lusher *et al.*, 2013).

FTIR analysis was conducted to identify the main polymer types of MPs from mangrove fishes. Majority of polymers found in the present study were latex (17%) and EVA (16%), while others were HDPE (11%), LDPE (11%), PU (8%), PET (8%), CA (8%), PVC (6%), PP (6%), PMMA (5%), nylon (3%) and nitrile (1%). Almost all types of polymers (9) were found in muscle (EVA, latex, CA, PU, PMMA, nylon, PET, HDPE, LDPE). The most common MPs reported in the marine environment and fish species are PP, PE, and PES (Gago *et al.*, 2018; Halstead *et al.*, 2018) which were also encountered in higher proportion in this study. The two most common polymers (PE and PP) in the fishes of this study is also accorded by Neves *et al.* (2015). There is a dissimilarity of the presence of polymer types in different studies. This study observed 12 types of polymer whereas Hossain *et al.* (2019, 2020) reported 3 types (PET, polyamide, rayon) and Ghosh *et al.* (2021) reported 6 types (PE, PP, PES, PU, EPDM, SBR) of polymers in fishes and shrimps from the Bay of Bengal of Bangladesh. Trash bags, milk jugs, shopping bags, food wrap, plastic film etc. are the main source of HDPE and LDPE in the environment (Hossain *et al.*, 2021). Single use plastics, mostly PP, together with rope and netting, are also the most abundant types of MPs found in marine environment (Nelms *et al.*, 2019). It is also suspected that these MPs might have originated mainly from weathering or degradation of plastics discarded by the population living in the Bay of Bengal's basin and aquaculture or fishing industry (ropes, fishing nets)(Browne *et al.*, 2011; Neves *et al.*, 2015;

Rochman *et al.*, 2015). PET particles may arise from coastal tourism activities. Latex comes from natural source but personal and health care products (e.g. gloves, mattresses, swim caps, balloons), clothing, coatings, glues might be another source of latex in the aquatic environments (Wikipedia). The possible source of EVA in the water may be EVA foam used for bags to package frozen foods (Buonocore *et al.*, 2014), slippers and sandals, water ski boots, fishing rods, fishing reel handles, floats for fishing gears (i.e. purse seine, gillnets) (Wikipedia, 2022). Due to the versatility of PU, it is found in mattresses, couches, insulation, liquid coatings and paints, tough elastomers (e.g. roller blade wheels, soft flexible foam toys), shoes, fibers, automotive, furniture (Matmach.com, 2022) which may be the source of PU in the water.

From this study, the presence of MPs in commercial fishes from Sundarbans have been identified which is a major concern for the biota in Sundarban region as well as the people who consume fishes for their daily meal. Because of plastic pollution, Ministry of Environment and Forests initiated the National 3R (reduce, reuse and recycle) strategy for waste Management to decrease the amount of waste material which may reduce the plastic waste by increasing the reuse and recyclability of used plastic (Liang *et al.*, 2018). Also some possible management strategy might be of help to overcome this threats including educating the general public about the negative impacts of plastic use through media advertisements and other initiatives by governmental and non-governmental organizations, introducing a program that rewards people for collecting plastic debris to discourage people from throwing it out in random places, enhancing inter-university and inter-institutional research collaboration to evaluate the effects of plastic trash on several environmental compartments, expanding research prospects and financing for the search of biodegradable polymer and commercially viable polymers, particularly for packaging, utilizing the nation's enormous jute production capacity to produce affordable biodegradable plastic substitutes and provide incentives for these kinds of firms, giving financial support to plastic recycling rather than plastic manufacturing sectors, levying substantial taxes on industries involving plastic, from importing raw materials to marketing finished goods thus people will stop utilizing plastic products because of their high cost, inclusion of plastic waste collection locations at all potential sources of plastic pollution, including institutions, offices, shopping malls, and river and road transportation systems, increasing the capacity of local governments to collect the most plastic waste possible, use of current regulatory laws strictly to end the usage of plastic bags, creating a national action plan for the management and monitoring of plastic trash at the source (Hossain *et al.*, 2021).

The occurrence of MPs in three fish species indicates that future research across a wider range of species and habitats should be considered in order to understand the potential effects of MPs in the Sundarbans ecosystem of Bangladesh and the Bay of Bengal region.

Chapter 6

6. Conclusion

In this study, all the investigated fish species were found to ingest MPs which indicates the extensive distribution of MPs in the Sundarbans of Bangladesh. MPs abundance was positively related to the body weight of the mangrove fish and a majority of ingested MPs were fibers in type, filament in shape and black in color. The polymer types of MPs are diverse (12 types) with latex, EVA and PE being dominant. Shape-size-color- morphology indicated the synergistic hazards having implications to fish biota and environments of the Sundarbans. Special concern from this study is to get MPs in muscle of fish species that may cause health risk for human being. These exposed risks need to be assessed through further investigation for better explain the factors influencing the occurrence of MPs in fish, their sources and future impact on fisheries and coastal population in the Sundarbans. Additionally, the rise of public awareness is necessary to reduce single-use plastics, consumption of plastics, and minimize their input into the aquatic environment. There requires stimulating a new management approach toward the collection and reuse of plastics materials, particularly in the Bay of Bengal's basin towards the sustainable use of the ocean resources.

Chapter 7

7. References

- Alomar, C., Sureda, A., Capó, X., Guijarro, B., Tejada, S., & Deudero, S. (2017). Microplastic ingestion by *Mullus surmuletus* Linnaeus, 1758 fish and its potential for causing oxidative stress. *Environmental research*, 159, 135-142.
- Amato-Lourenço, L. F., Carvalho-Oliveira, R., Júnior, G. R., dos Santos Galvão, L., Ando, R. A. and Mauad, T., 2021. Presence of airborne microplastics in human lung tissue. *Journal of Hazardous Materials*, 416, 126124.
- Andrady, A. L. (2011). Microplastics in the marine environment. *Marine pollution bulletin*, 62(8), 1596-1605.
- Ariyaratne, T. M. G., Amarasinghe, P. B., Lopez, N. C., Kakkaeo, M., & Vijverberg, J. (2008). Selective feeding of small zooplanktivorous pelagic fish species in tropical Asian reservoirs (Sri Lanka, Thailand) and Lake Taal (Philippines). *Aquatic ecosystems and development: comparative Asian perspectives*, 235-248.
- Atamanalp, M., Köktürk, M., Uçar, A., Duyar, H.A., Özdemir, S., Parlak, V., Esenbuğa, N. and Alak, G. (2021). Microplastics in tissues (brain, gill, muscle and gastrointestinal) of *Mullus barbatus* and *Alosa immaculata*. *Archives of Environmental Contamination and Toxicology*, 81(3): 460-469.
- Au, S. Y., Bruce, T. F., Bridges, W. C., Klaine, S. J. (2015). Responses of *Hyaella azteca* to acute and chronic microplastic exposures. *Environmental Toxicology and Chemistry*, 34(11): 2564–2572.
- Avio, C. G., Gorbi, S., & Regoli, F. (2015). Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. *Marine environmental research*, 111, 18-26.
- Bagulayan, A., Bartlett-Roa, J. N., Carter, A. L., Inman, B. G., Keen, E. M., Orenstein, E. C., & Franks, P. J. (2012). Journey to the center of the gyre: The fate of the Tohoku Tsunami debris field. *Oceanography*, 25(2), 200-207.

- Banglapedia (2012) National Encyclopedia of Bangladesh, 2nd edn. Bangladesh: Asiatic Society of Bangladesh. Available online at: <https://en.banglapedia.org/index.php/River> (accessed August 30, 2021).
- Barboza, L. G. A., Lopes, C., Oliveira, P., Bessa, F., Otero, V., Henriques, B., Raimundo, J., Caetano, M., Vale, C., & Guilhermino, L. (2020). Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. *Science of the Total Environment*, 717, 134625.
- Battaglia, P., Pedà, C., Musolino, S., Esposito, V., Andaloro, F., & Romeo, T. (2016). Diet and first documented data on plastic ingestion of *Trachinotus ovatus* L. 1758 (Pisces: Carangidae) from the Strait of Messina (central Mediterranean Sea). *Italian journal of zoology*, 83(1), 121-129.
- Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., & Martínez-Gómez, C. (2016). Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. *Marine pollution bulletin*, 109(1), 55-60.
- Bessa, F., Barría, P., Neto, J. M., Frias, J. P., Otero, V., Sobral, P., & Marques, J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. *Marine pollution bulletin*, 128, 575-584.
- Boerger, C. M., Lattin, G. L., Moore, S. L., & Moore, C. J. (2010). Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Marine pollution bulletin*, 60(12), 2275-2278.
- Braun, T., Ehrlich, L., Henrich, W., Koeppel, S., Lomako, I., Schwabl, P. and Liebmann, B., 2021. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. *Pharmaceutics*, 13, 921.
- Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environmental science & technology*, 45(21), 9175-9179.
- Buonocore, G., Sico, G., & Mensitieri, G. (2014). Safety of Food and Beverages: Packaging Material and Auxiliary Items.

- Calder, D. R., Choong, H. H., Carlton, J. T., Chapman, J. W., Miller, J. A., & Geller, J. (2014). Hydroids (Cnidaria: Hydrozoa) from Japanese tsunami marine debris washing ashore in the northwestern United States. *Aquatic Invasions*, 9(4).
- Cannon, S. M., Lavers, J. L., & Figueiredo, B. (2016). Plastic ingestion by fish in the Southern Hemisphere: A baseline study and review of methods. *Marine pollution bulletin*, 107(1), 286-291.
- Carson, H. S., Lamson, M. R., Nakashima, D., Toloumu, D., Hafner, J., Maximenko, N., & McDermid, K. J. (2013). Tracking the sources and sinks of local marine debris in Hawaii 'i. *Marine environmental research*, 84, 76-83.
- Cheung, L. T., Lui, C. Y., & Fok, L. (2018). Microplastic contamination of wild and captive flathead grey mullet (*Mugil cephalus*). *International journal of environmental research and public health*, 15(4), 597.
- Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K., & Janssen, C. R. (2011). Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine pollution bulletin*, 62(10), 2199-2204.
- Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin*, 62(12), 2588-2597.
- Cole, M., Webb, H., Lindeque, P. K., Fileman, E. S., Halsband, C., & Galloway, T. S. (2014). Isolation of microplastics in biota-rich seawater samples and marine organisms. *Scientific reports*, 4(1), 1-8.
- Compa, M., Ventero, A., Iglesias, M., & Deudero, S. (2018). Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. *Marine pollution bulletin*, 128, 89-96.
- Cox, K. D., Covernton, G. A., Davies, H. L., Dower, J. F., Juanes, F. and Dudas, S. E., 2019. Human Consumption of Microplastics. *Environmental Science and Technology*, 53, 7068–7074.



- De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., & Robbens, J. (2014). Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. *Marine pollution bulletin*, 85(1), 146-155.
- Devriese, L. I., Van der Meulen, M. D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., & Vethaak, A. D. (2015). Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Marine pollution bulletin*, 98(1-2), 179-187.
- Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., & Amato, S. (2013). Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Marine pollution bulletin*, 77(1-2), 177-182.
- Eriksen, M., Thiel, M., & Lebreton, L. (2016). Nature of plastic marine pollution in the subtropical gyres. *Hazardous chemicals associated with plastics in the marine environment*, 135-162.
- Eriksson, C., & Burton, H. (2003). Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. *AMBIO: A Journal of the Human Environment*, 32(6), 380-384.
- Faris, J., & Hart, K. (1994). Seas of debris: a summary of the third international conference on marine debris.
- Fendall, L. S., & Sewell, M. A. (2009). Contributing to marine pollution by washing your face: microplastics in facial cleansers. *Marine pollution bulletin*, 58(8), 1225-1228.
- Ferreira, M., Thompson, J., Paris, A., Rohindra, D., & Rico, C. (2020). Presence of microplastics in water, sediments and fish species in an urban coastal environment of Fiji, a Pacific small island developing state. *Marine pollution bulletin*, 153, 110991.
- Frias, J. P., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. *Marine pollution bulletin*, 138, 145-147.
- Gago, J., Carretero, O., Filgueiras, A. V., & Viñas, L. (2018). Synthetic microfibers in the marine environment: A review on their occurrence in seawater and sediments. *Marine pollution bulletin*, 127, 365-376.

- Galgani, F., Hanke, G., & Maes, T. (2015). Global distribution, composition and abundance of marine litter. In *Marine anthropogenic litter* (pp. 29-56). Springer, Cham.
- Gasperi, J., Dris, R., Bonin, T., Rocher, V., & Tassin, B. (2014). Assessment of floating plastic debris in surface water along the Seine River. *Environmental pollution*, 195, 163-166.
- GESAMP (2015). Sources, fate and effects of microplastics in the marine environment: A global assessment. Rep stud GESAMP 90:1-96
- Ghosh, G. C., Akter, S. M., Islam, R. M., Habib, A., Chakraborty, T. K., Zaman, S., Kabir, AHM. E., Shipin, O. V., & Wahid, M. A. (2021). Microplastics contamination in commercial marine fish from the Bay of Bengal. *Regional Studies in Marine Science*, 44, 101728.
- Gregory, M. R. (1996). Plastic 'scrubbers' in hand cleansers: a further (and minor) source for marine pollution identified. *Marine pollution bulletin*, 32(12), 867-871.
- Gregory, M. R. (2009). Environmental implications of plastic debris in marine settings-entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philosophical Transactions of The Royal Society B*, 364, 2013-2025.
- Güven, O., Gökdağ, K., Jovanović, B., & Kıdeys, A. E. (2017). Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. *Environmental pollution*, 223, 286-294.
- Habib, K. A., Neogi, A. K., Nahar, N., Oh, J., Lee, Y. H., & Kim, C. G. (2020). An overview of fishes of the Sundarbans, Bangladesh and their present conservation status. *Journal of Threatened Taxa*, 12(1), 15154-15172.
- Halstead, J. E., Smith, J. A., Carter, E. A., Lay, P. A., & Johnston, E. L. (2018). Assessment tools for microplastics and natural fibres ingested by fish in an urbanised estuary. *Environmental Pollution*, 234, 552-561.
- Hasan, J., Islam, S. M., Alam, M. S., Johnson, D., Belton, B., Hossain, M. A. R., & Shahjahan, M. (2022). Presence of microplastics in two common dried marine fish species from Bangladesh. *Marine Pollution Bulletin*, 176, 113430.

Hermesen, E., Pompe, R., Besseling, E., & Koelmans, A. A. (2017). Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. *Marine pollution bulletin*, 122(1-2), 253-258.

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental science & technology*, 46(6), 3060-3075.

Hossain, M. S., Rahman, M. S., Uddin, M. N., Sharifuzzaman, S. M., Chowdhury, S. R., Sarker, S., & Chowdhury, M. S. N. (2020). Microplastic contamination in Penaeid shrimp from the Northern Bay of Bengal. *Chemosphere*, 238, 124688.

Hossain, M. S., Sobhan, F., Uddin, M. N., Sharifuzzaman, S. M., Chowdhury, S. R., Sarker, S., & Chowdhury, M. S. N. (2019). Microplastics in fishes from the Northern Bay of Bengal. *Science of the Total Environment*, 690, 821-830.

Hossain, S., Rahman, M. A., Chowdhury, M. A., & Mohonta, S. K. (2021). Plastic pollution in Bangladesh: A review on current status emphasizing the impacts on environment and public health. *Environmental Engineering Research*, 26(6).

<https://en.bdfish.org/2011/04/barramundi-lates-calcarifer-bloch-1790/>

<https://en.bdfish.org/2011/08/congaturi-halfbeak-hyporhamphus-limbatus/>

<https://en.bdfish.org/2011/08/long-whiskers-catfish-mystus-gulio-hamilton-1822/>

https://en.wikipedia.org/wiki/Ethylene-vinyl_acetate, viewed in 15/08/2022

<https://en.wikipedia.org/wiki/Latex>, viewed in 15/08/2022.

<https://matmatch.com/learn/material/polyurethane>, viewed in 15/08/2022

<https://www.americanchemistry.com/industry-groups/center-for-the-polyurethanes-industry-cpi/applications-benefits>, viewed in 15/08/2022

<https://www.essentialchemicalindustry.org/polymers/polyurethane.html>, viewed in 15/08/2022

<https://www.fishbase.in/summary/Hyporhamphus-limbatus.html>

<https://www.fishbase.se/summary/5139>

<https://www.fishbase.se/summary/Lates-calcarifer.html>

- Huang, J. S., Koongolla, J. B., Li, H. X., Lin, L., Pan, Y. F., Liu, S., He, W. H., Maharana, D., & Xu, X. R. (2020). Microplastic accumulation in fish from Zhanjiang mangrove wetland, South China. *Science of The Total Environment*, 708, 134839.
- Huda, M. S., Haque, M. E., Babul, A. S., & Shil, N. C. (2003). Field guide to finfishes of Sundarban. *Aquatic Resources Division, Sundarban. Sundarban Biodiversity Conservation Project, Bangladesh Forest Department, Boyra, Khulna, Bangladesh.*
- Ibrahim, Y. S., Tuan Anuar, S., Azmi, A. A., Wan Mohd Khalik, W. M. A., Lehata, S., Hamzah, S. R., Ismail, D., Ma, Z. F., Dzulkarnaen, A., Zakaria, Z. *et al.*, 2021. Detection of microplastics in human colectomy specimens. *JGH Open*, 5, 116–121.
- IUCN, B. (2000). Red book of threatened fishes of Bangladesh. *IUCN-The World Conservation Union*. xii+116 pp.
- Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., & Shi, H. (2017). Microplastics and mesoplastics in fish from coastal and fresh waters of China. *Environmental Pollution*, 221, 141-149.
- Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, 347(6223), 768-771.
- Jung, M. R., Horgen, F. D., Orski, S. V., Rodriguez, C. V., Beers, K. L., Balazs, G. H., Jones, T. T., Work, T. M., Brignac, K. C., Royer, S.-J., Hyrenbach, K. D., Jensen, B. A., Lynch, J. M. (2018). Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine Pollution Bulletin*, 127, 704–716.
- Kane, I. A., Clare, M. A., 2019. Dispersion, accumulation, and the ultimate fate of microplastics in deep-marine environments: A review and future directions. *Frontiers in Earth Science*, 7, 80.
- Kasamesiri, P., & Thaimuangphol, W. (2020). Microplastics ingestion by freshwater fish in the Chi River, Thailand. *GEOMATE Journal*, 18(67), 114-119.

- Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., & Shi, H. (2018). Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. *Science of the total environment*, 610, 635-640.
- Kungvankij, P., Tiro Jr, L. B., Pudadera Jr, B. J., & Potestas, I. O. (1986). *Biology and culture of sea bass (Lates calcarifer)*. Network of Aquaculture Centres in Asia. 70 pp
- Lambert, S., & Wagner, M. (2016). Characterization of nanoplastics during the degradation of polystyrene. *Chemosphere*, 145, 265-268.
- Lebreton, L. C. M., & Borrero, J. C. (2013). Modeling the transport and accumulation floating debris generated by the 11 March 2011 Tohoku tsunami. *Marine pollution bulletin*, 66(1-2), 53-58.
- Lebreton, L., Van Der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. *Nature communications*, 8(1), 1-10.
- Lehtiniemi, M., Hartikainen, S., Näkki, P., Engström-Öst, J., Koistinen, A., & Setälä, O. (2018). Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. *Food webs*, 17, e00097.
- Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., RaleySusman, K. M., He, D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. *Science of The Total Environment*, 619-620, 1-8.
- Leslie, H. A., van Velzen, M. J. M., Brandsma, S. H., Vethaak, A. D., Garcia-Vallejo, J. J. and Lamoree, M. H., 2022. Discovery and quantification of plastic particle pollution in human blood. *Environmental International*, 163, 107199.
- Li, B., Su, L., Zhang, H., Deng, H., Chen, Q., & Shi, H. (2020). Microplastics in fishes and their living environments surrounding a plastic production area. *Science of the Total Environment*, 727, 138662.
- Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., & Shi, H. (2016). Microplastics in mussels along the coastal waters of China. *Environmental pollution*, 214, 177-184.

- Li, J., Yang, D., Li, L., Jabeen, K., & Shi, H. (2015). Microplastics in commercial bivalves from China. *Environmental pollution*, 207, 190-195.
- Li, R., Zhang, L., Xue, B., & Wang, Y. (2019). Abundance and characteristics of microplastics in the mangrove sediment of the semi-enclosed Maowei Sea of the South China Sea: New implications for location, rhizosphere, and sediment compositions. *Environmental pollution*, 244, 685-692.
- Lin, L., Ma, L. S., Li, H. X., Pan, Y. F., Liu, S., Zhang, L., Peng, J.P., Fok, L., Xu, X.R., & He, W. H. (2020). Low level of microplastic contamination in wild fish from an urban estuary. *Marine Pollution Bulletin*, 160, 111650.
- Lusher, A. L., Mchugh, M., & Thompson, R. C. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine pollution bulletin*, 67(1-2), 94-99.
- Lusher, A. L., Welden, N. A., Sobral, P., & Cole, M. (2017b). Sampling, isolating and identifying microplastics ingested by fish and invertebrates. In *Analysis of nanoplastics and microplastics in food* (pp. 119-148). CRC Press.
- Lusher, A., Hollman, P., & Mendoza-Hill, J. (2017a). *Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety*. FAO.
- Mathalon, A., & Hill, P. (2014). Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Marine pollution bulletin*, 81(1), 69-79.
- McNeish, R. E., Kim, L. H., Barrett, H. A., Mason, S. A., Kelly, J. J., & Hoellein, T. J. (2018). Microplastic in riverine fish is connected to species traits. *Scientific reports*, 8(1), 1-12.
- Meijer, L. J., van Emmerik, T., van der Ent, R., Schmidt, C., & Lebreton, L. (2021). More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. *Science Advances*, 7(18), eaaz5803.
- Merrell Jr, T. R. (1984). A decade of change in nets and plastic litter from fisheries off Alaska. *Marine pollution bulletin*, 15(10), 378-384.
- Moore, C. J., Lattin, G. L., & Zellers, A. F. (2011). Quantity and type of plastic debris flowing from two urban rivers to coastal waters and beaches of Southern California. *Revista de*

Gestão Costeira Integrada-Journal of Integrated Coastal Zone Management, 11(1), 65-73. ,

- Morritt, D., Stefanoudis, P. V., Pearce, D., Crimmen, O. A., & Clark, P. F. (2014). Plastic in the Thames: a river runs through it. *Marine pollution bulletin*, 78(1-2), 196-200.
- Mowat, A. M., 2003. Anatomical basis of tolerance and immunity to intestinal antigens. *Natural Reviews Immunology*, 3, 331–341.
- Mukul, S. A., Huq, S., Herbohn, J., Seddon, N., & Laurance, W. F. (2020). Saving the Sundarbans from development. *Science*, 368(6496), 1198-1198.
- Naji, A., Nuri, M., Amiri, P., & Niyogi, S. (2019). Small microplastic particles (S-MPPs) in sediments of mangrove ecosystem on the northern coast of the Persian Gulf. *Marine pollution bulletin*, 146, 305-311.
- NAS. (1975). *Assessing Potential Ocean Pollutants: A Report of the Study Panel on Assessing Potential Ocean Pollutants to the Ocean Affairs Board, Commission on Natural Resources, National Research Council* (No. 2325). National Academies of Sciences.
- Nelms, S. E., Barnett, J., Brownlow, A., Davison, N. J., Deaville, R. *et al.* (2019). Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? *Scientific Reports*, 9, 1075.
- Nelms, S. E., Duncan, E. M., Patel, S., Badola, R., Bhola, S., Chakma, S., Chowdhury, G.W., Godley, B.J., Haque, A.B., Johnson, J.A., & Koldewey, H. (2021). Riverine plastic pollution from fisheries: Insights from the Ganges River system. *Science of the Total Environment*, 756, 143305.
- Neves, D., Sobral, P., Ferreira, J. L., & Pereira, T. (2015). Ingestion of microplastics by commercial fish off the Portuguese coast. *Marine pollution bulletin*, 101(1), 119-126.
- Nor, N. H. M., & Obbard, J. P. (2014). Microplastics in Singapore's coastal mangrove ecosystems. *Marine pollution bulletin*, 79(1-2), 278-283.
- Nuelle, M. T., Dekiff, J. H., Remy, D., & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. *Environmental pollution*, 184, 161-169.

- O'hara, K. J. (1988). *A citizen's guide to plastics in the ocean: more than a litter problem*. Center for Marine Conservation, 1725 DeSales Street, NW, # 500, Washington, DC 20036.
- Onink, V., Jongedijk, C. E., Hoffman, M. J., van Sebille, E., & Laufkötter, C. (2021). Global simulations of marine plastic transport show plastic trapping in coastal zones. *Environmental Research Letters*, 16(6), 064053.
- Ory, N., Chagnon, C., Felix, F., Fernández, C., Ferreira, J. L., Gallardo, C., Ordóñez, O.G., Henostroza, A., Laaz, E., Mizraji, R., Mojica, H., Haro, V.M., Medina, L.O., Preciado, M., Sobral, P., Urbina, M. A., & Thiel, M. (2018). Low prevalence of microplastic contamination in planktivorous fish species from the southeast Pacific Ocean. *Marine Pollution Bulletin*, 127, 211-216.
- Park, T. J., Lee, S. H., Lee, M. S., Lee, J. K., Lee, S. H., & Zoh, K. D. (2020). Occurrence of microplastics in the Han River and riverine fish in South Korea. *Science of the Total Environment*, 708, 134535.
- Parvin, F., Jannat, S., & Tareq, S. M. (2021). Abundance, characteristics and variation of microplastics in different freshwater fish species from Bangladesh. *Science of the Total Environment*, 784, 147137.
- Pazos, R. S., Maiztegui, T., Colautti, D. C., Paracampo, A. H., & Gómez, N. (2017). Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. *Marine pollution bulletin*, 122(1-2), 85-90.
- Pinheiro, C., Oliveira, U., & Vieira, M. (2017). Occurrence and impacts of microplastics in freshwater fish. *J. Aquac. Mar. Biol*, 5(6), 00138.
- Prata, J. C., 2018. Airborne microplastics: Consequences to human health? *Environmental Pollution*, 234, 115–126.
- Prata, J. C., da Costa, J. P., Lopes, I., Duarte, A. C. and Rocha-Santos, T., 2020. Environmental exposure to microplastics: An overview on possible human health effects. *Science of the Total Environment*, 702, 134455.

- Ragusa, A., Notarstefano, V., Svelato, A., Belloni, A., Gioacchini, G., Blondeel, C., Zucchelli, E., De Luca, C., D'Avino, S., Gulotta, A., 2022. Raman Microspectroscopy Detection and Characterization of Microplastics in Human Breastmilk. *Polymers*, 14, 2700.
- Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M. C. A., Baiocco, F., Draghi, S. *et al.*, 2021. Plasticenta: First evidence of microplastics in human placenta. *Environmental International*, 146, 106274.
- Rahman AKA (1989). *Freshwater Fishes of Bangladesh*, 1st edition, Zoological Society of Bangladesh, Department of Zoology, University of Dhaka, Dhaka-1000.
- Rahman AKA (2005) *Freshwater Fishes of Bangladesh*, 2nd edition, Zoological Society of Bangladesh, Department of Zoology, University of Dhaka, Dhaka-1000.
- Rech, S., Macaya-Caquilpán, V., Pantoja, J. F., Rivadeneira, M. M., Campodónico, C. K., & Thiel, M. (2015). Sampling of riverine litter with citizen scientists—findings and recommendations. *Environmental monitoring and assessment*, 187(6), 1-18.
- Rianda, N., Armin, F. and Djamaan, A., 2020. Macroplastic and Microplastic Analysis of Marine Fish and Aquatic Fish Using the Fourier Transform Infrared Spectrophotometry (FTIR) Method. *IOSR Journal of Pharmacy and Biological Sciences*, 15(3): 15-22.
- Rochman, C. M., Browne, M. A., Underwood, A. J., Van Franeker, J. A., Thompson, R. C., & Amaral-Zettler, L. A. (2016). The ecological impacts of marine debris: unraveling the demonstrated evidence from what is perceived. *Ecology*, 97(2), 302-312.
- Rochman, C. M., Tahir, A., Williams, S. L., Baxa, D. V., Lam, R., Miller, J. T., Teh, F. C., Werorilangi, S., & Teh, S. J. (2015). Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Scientific reports*, 5(1), 1-10.
- Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., & Fossi, M. C. (2015). First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. *Marine pollution bulletin*, 95(1), 358-361.
- Sanchez, W., Bender, C., & Porcher, J. M. (2014). Wild gudgeons (*Gobio gobio*) from French rivers are contaminated by microplastics: preliminary study and first evidence. *Environmental research*, 128, 98-100.

- Schmidt, C., Krauth, T., & Wagner, S. (2017). Export of plastic debris by rivers into the sea. *Environmental science & technology*, 51(21), 12246-12253.
- Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T. and Liebmann, B., 2019. Detection of Various Microplastics in Human Stool. *Annals of Internal Medicine*, 171, 453.
- Sen, H. S. (2019). The Sundarbans: A Disaster-Prone Eco-Region. *Berlin: Springer*. doi, 10, 978-3.
- Shafi, M., & Quddus, MMA. (2001). Bangladesher Matsho Shampad (Fisheries of Bangladesh)(in Bengali), Kabir publication. *Dhaka, Bangladesh*, pp. 186-187.
- Siddique, K. U. (2007). Encyclopaedia of flora and fauna of Bangladesh freshwater fishes vol. 23. *Asiatic society of Bangladesh, Dhaka, Bangladesh*. 300 pp.
- Silva, A. B., Bastos, A. S., Justino, C. I. L., da Costa, J. P., Duarte, A. C. and Rocha-Santos, T. A. P., 2018. Microplastics in the environment: Challenges in analytical chemistry - A review. *Analytica Chimica Acta*, 1017, 1-19.
- Slip, D. J., & Burton, H. R. (1991). Accumulation of fishing debris, plastic litter, and other artefacts, on Heard and Macquarie Islands in the Southern Ocean. *Environmental Conservation*, 18(3), 249-254.
- Steer, M., Cole, M., Thompson, R. C., & Lindeque, P. K. (2017). Microplastic ingestion in fish larvae in the western English Channel. *Environmental pollution*, 226, 250-259.
- Su, L., Deng, H., Li, B., Chen, Q., Pettigrove, V., Wu, C., & Shi, H. (2019). The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. *Journal of hazardous materials*, 365, 716-724.
- Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., & Shi, H. (2016). Microplastics in taihu lake, China. *Environmental Pollution*, 216, 711-719.
- Teng, J., Wang, Q., Ran, W., Wu, D., Liu, Y., Sun, S., Liu, H., Cao, R., & Zhao, J. (2019). Microplastic in cultured oysters from different coastal areas of China. *Science of the total environment*, 653, 1282-1292.

- Thiel M, Haye P (2006). The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. *Oceanogr Mar Biol Ann Rev* 44:323–429
- Thompson, R., Swan, S. H., Moore, C. J., & vom Saal, F. S. (2009). Our Plastic Age, *Philosophical Transactions of the Royal Society B, Biological Sciences*.
- UNEP (2009). Marine litter: A global challenge report by United Nations Environmental Programme
- Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental pollution*, 193, 65-70.
- Wang, Q., Zhu, X., Hou, C., Wu, Y., Teng, J., Zhang, C., Tan, H., Shan, E., Zhang, W. & Zhao, J. (2021). Microplastic uptake in commercial fishes from the Bohai Sea, China. *Chemosphere*, 263, 127962.
- Wang, S., Zhang, C., Pan, Z., Sun, D., Zhou, A., Xie, S., Wang, J., & Zou, J. (2020). Microplastics in wild freshwater fish of different feeding habits from Beijiang and Pearl River Delta regions, south China. *Chemosphere*, 258, 127345.
- Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. *Environmental pollution*, 178, 483-492.
- Yadav BN. 1999. Fish and Fisheries, Daya Publishing House, Delhi, India, p. 323.
- Yuan, W., Liu, X., Wang, W., Di, M., & Wang, J. (2019). Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. *Ecotoxicology and environmental safety*, 170, 180-187.
- Zakeri, M., Naji, A., Akbarzadeh, A. and Uddin, S. (2020). Microplastic ingestion in important commercial fish in the southern Caspian Sea. *Marine Pollution Bulletin*, 160, 111598.
- Zhang, C., Wang, S., Pan, Z., Sun, D., Xie, S., Zhou, A., Wang, J., & Zou, J. (2020a). Occurrence and distribution of microplastics in commercial fishes from estuarine areas of Guangdong, South China. *Chemosphere*, 260, 127656.
- Zhang, D., Cui, Y., Zhou, H., Jin, C., Yu, X., Xu, Y., Li, Y., & Zhang, C. (2020b). Microplastic pollution in water, sediment, and fish from artificial reefs around the Ma'an Archipelago, Shengsi, China. *Science of the Total Environment*, 703, 134768.

- Zhang, K., Shi, H., Peng, J., Wang, Y., Xiong, X., Wu, C. and Lam, P. K. S., 2018. Microplastic pollution in China's inland water systems: a review of findings, methods, characteristics, effects, and management. *Science of the Total Environment*, 630, 1641–1653.
- Zhu, L., Wang, H., Chen, B., Sun, X., Qu, K., & Xia, B. (2019). Microplastic ingestion in deep-sea fish from the South China Sea. *Science of the Total Environment*, 677, 493-501.
- Ziajahromi, S., Kumar, A., Neale, P. A., Leusch, F. D. L. (2017). Impact of microplastic beads and fibers on waterflea (*Ceriodaphnia dubia*) survival, growth, and reproduction: implications of single and mixture exposures. *Environmental Science and Technology*, 51(22): 13397–13406.

Chapter 8

8. Annex

8.1 Annex 1

Special apparatus and equipment

- Weight machine
- Icebox
- Aluminum foil paper
- Metal tray
- Scissors, scalpel and forceps
- Petri dishes
- Exclusive cotton coat, latex gloves, glass, metal ware
- 500 mL glass beaker
- Gold coated glass microscopic slide
- 50 μ m stainless steel sieve
- Refrigerator (-20°C)
- Oscillation incubator
- μ -FTIR spectroscope

Reagents

- Double distilled water
- 30% Hydrogen peroxide solution (H₂O₂)
- Saline solution (1.2 g/mL NaCl)

8.2 Annex 2

Table 6: Total body weight and MPs abundance data

Name of Species	No. of Individual	Total body weight (g)	Total GT weight (g)	MPs in total GT (%)	Total gill weight (g)	MPs in total Gill (%)	MPs in 5g muscle & percentage %
<i>M. gulio</i>	3	177.54	8.29	33 (11.58%)	5.78	17 (10.06%)	0
<i>L. calcarifer</i>	3	1054.59	38.54	132 (46.32%)	19.32	94 (55.62%)	16(35.56%)

<i>H. limbatus</i>	3	197.95	4.23	120 (42.11%)	3.71	58 (34.32%)	29(64.44%)
Total				285		169	45

Table 7: MPs abundance (items/g) in different body parts

Sample	Name of species	Average no of MPs items/g
GT	<i>M. gulio</i>	3.98
	<i>L. calcarifer</i>	3.36
	<i>H. limbatus</i>	29.27
Gill	<i>M. gulio</i>	3.55
	<i>L. calcarifer</i>	5.02
	<i>H. limbatus</i>	15.51
Muscle	<i>M. gulio</i>	0.00
	<i>L. calcarifer</i>	3.23
	<i>H. limbatus</i>	3.88

Table 8: Body weight (g) and total number of MPs

Species name	Samples	Body weight (g)	Total number of MPs
<i>M. gulio</i>	S1	67.74	13
	S2	51.52	20
	S3	58.28	17
<i>L. calcalifer</i>	S4	363.94	1066.35
	S5	351	1118.37
	S6	339.65	1282.32
<i>H. limbatus</i>	S7	65.32	399.72
	S8	63.32	78
	S9	69.31	419.67

Table 9: Relationship between body weight (g) and MPs number

Correlations			
		Body Weight (g)	Total no. of MPs
Body Weight (g)	Pearson Correlation	1	.950**
	Sig. (2-tailed)		.000
	N	9	9

Total no. of MPs	Pearson Correlation	.950**	1
	Sig. (2-tailed)	.000	
	N	9	9

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

Table 10: Data of MPs colors

Name of Species	Samples No	Body Parts	Colors					
			Black	Blue	Green	Purple	Red	Transparent
<i>M. gulio</i>	S1	GT	11	0	0	0	0	2
		Gill	0	0	0	0	0	0
		Muscle (3.37g)	0	0	0	0	0	0
	S2	GT	6	3	2	0	0	0
		Gill	4	4	0	0	1	0
		Muscle (3.17g)	0	0	0	0	0	0
	S3	GT	4	2	0	0	3	0
		Gill	7	0	1	0	0	0
		Muscle (3.39g)	0	0	0	0	0	0
<i>L. calcarifer</i>	S1	GT	22	7	4	7	0	12
		Gill	30	8	0	4	4	0
		Muscle (5g)	6	0	4	4	0	0
	S2	GT	16	4	0	4	0	0
		Gill	16	8	0	0	0	0
		Muscle (5g)	11	0	2	2	0	2
	S3	GT	46	2	2	2	4	0
		Gill	16	8	0	0	0	0
		Muscle (5g)	12	0	0	1	0	5
<i>H. limbatus</i>	S1	GT	8	8	0	4	4	0
		Gill	0	0	0	0	0	0
		Muscle (5g)	24	2	0	4	0	0
	S2	GT	11	28	4	0	0	7
		Gill	16	4	0	4	0	4
		Muscle (5g)	0	0	0	0	0	0
	S3	GT	18	10	4	6	8	0

		Gill	25	0	0	5	0	0
		Muscle (5g)	18	2	2	2	0	4

Table 11: Data of MPs shapes

Name of Species	Samples No	Body Parts	Shape		Type		Total
			Filament	Irregular	Fiber	Fragment	
<i>M. gulio</i>	S1	GT	11	2	7	6	13
		Gill	0	0	0	0	0
		Muscle (3.37g)	0	0	0	0	0
	S2	GT	11	0	8	3	11
		Gill	9	0	9	0	9
		Muscle (3.17g)	0	0	0	0	0
	S3	GT	9	0	8	1	9
		Gill	8	0	7	1	8
		Muscle (3.39g)	0	0	0	0	0
<i>L. calcarifer</i>	S1	GT	52	0	40	12	52
		Gill	38	8	33	13	46
		Muscle (5g)	14	0	9	5	14
	S2	GT	24	0	24	0	24
		Gill	24	0	12	12	24
		Muscle (5g)	16	0	12	4	16
	S3	GT	54	2	50	6	56
		Gill	20	4	16	8	24
		Muscle (5g)	19	0	14	5	19
<i>H. limbatus</i>	S1	GT	24	0	20	4	24
		Gill	0	0	0	0	0
		Muscle (5g)	30	0	24	6	30
	S2	GT	50	0	34	16	50
		Gill	28	0	20	8	28
		Muscle (5g)	0	0	0	0	0
	S3	GT	46	0	30	16	46
		Gill	30	0	30	0	30

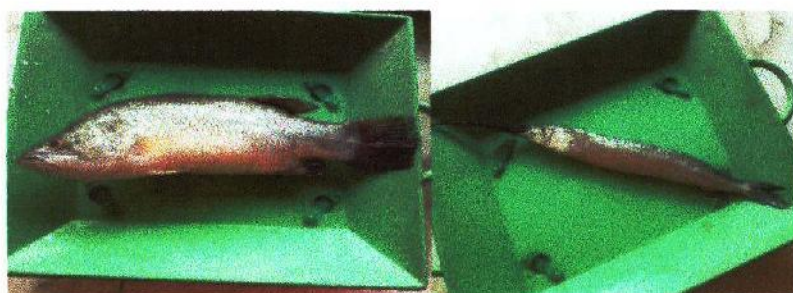


		Muscle (5g)	24	4	22	6	28
--	--	-------------	----	---	----	---	----

Table 12: Polymer analysis by FTIR data

Polymer types	Name of the species			Number	
	<i>M. gulio</i>	<i>L. calcarifer</i>	<i>H. limbatus</i>	Total	Percentage (%)
Ethylene vinyl acetate (EVA)	3	8	3	14	16.28
Latex	3	7	4	14	16.28
High-density polyethylene (HDPE)	2	4	3	9	10.47
Low-density polyethylene (LDPE)	2	4	3	9	10.47
Polyvinyl chloride (PVC)	1	3	1	5	5.81
Cellulose acetate (CA)	2	4	1	7	8.14
Polyurethane (PU)	1	4	2	7	8.14
Polypropylene (PP)	0	5	1	6	6.98
Polyethylene terephthalate (PET)	0	4	3	7	8.14
Polymethyl methacrylate (PMMA)	0	2	2	4	4.65
Nylon	0	2	1	3	3.49
Nitrile	0	1	0	1	1.16
Total	14	48	24	86	100

8.3 Annex 3



Fish species (*L. calcarifer* and *H. limbatus*)



Weighing of fish



Dissection of GT, gill and muscle



Weighing of GT, gill and muscle



H₂O₂ digestion of GT, gill and muscle



Oscillation incubator at 65° at 80rpm



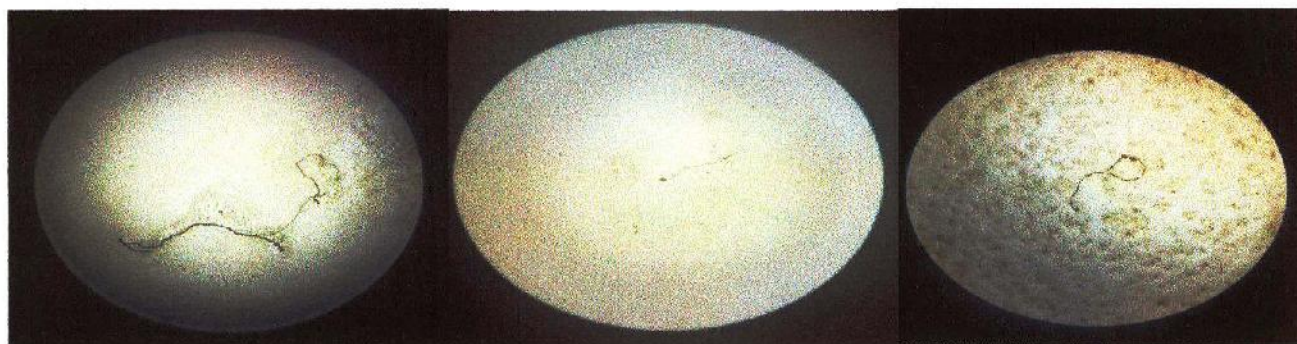
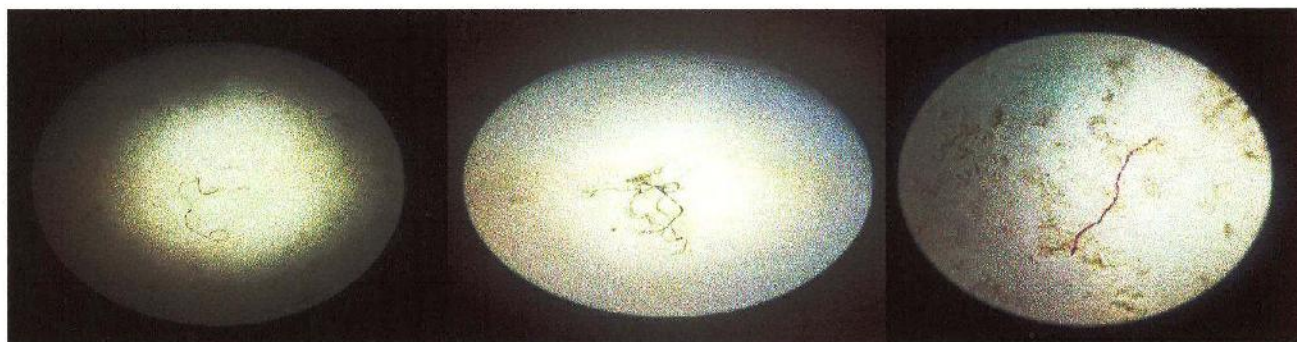
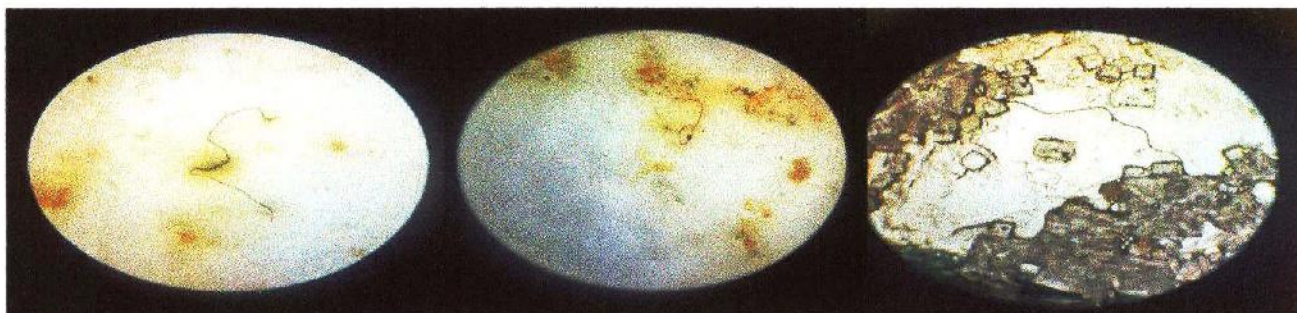
NaCl floatation and filtration

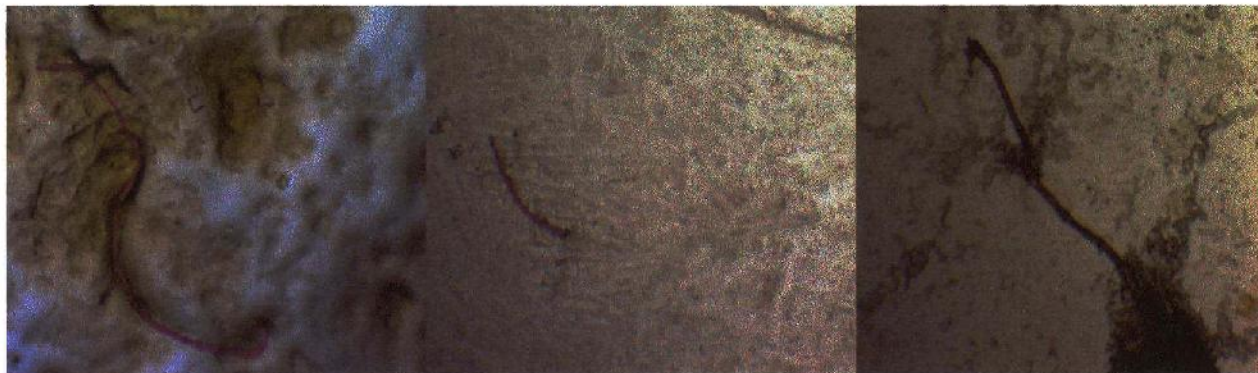
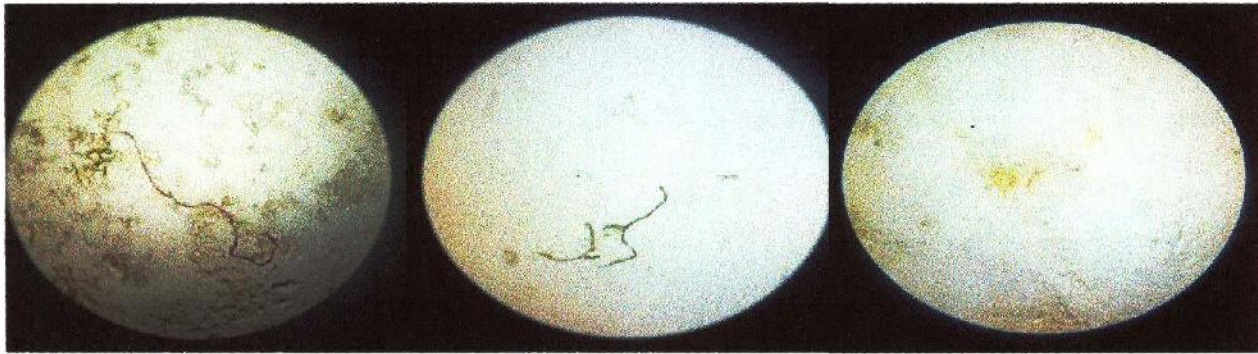


Microscopic observation

Figure 11: MPs separation and identification procedures

8.4 Annex 4





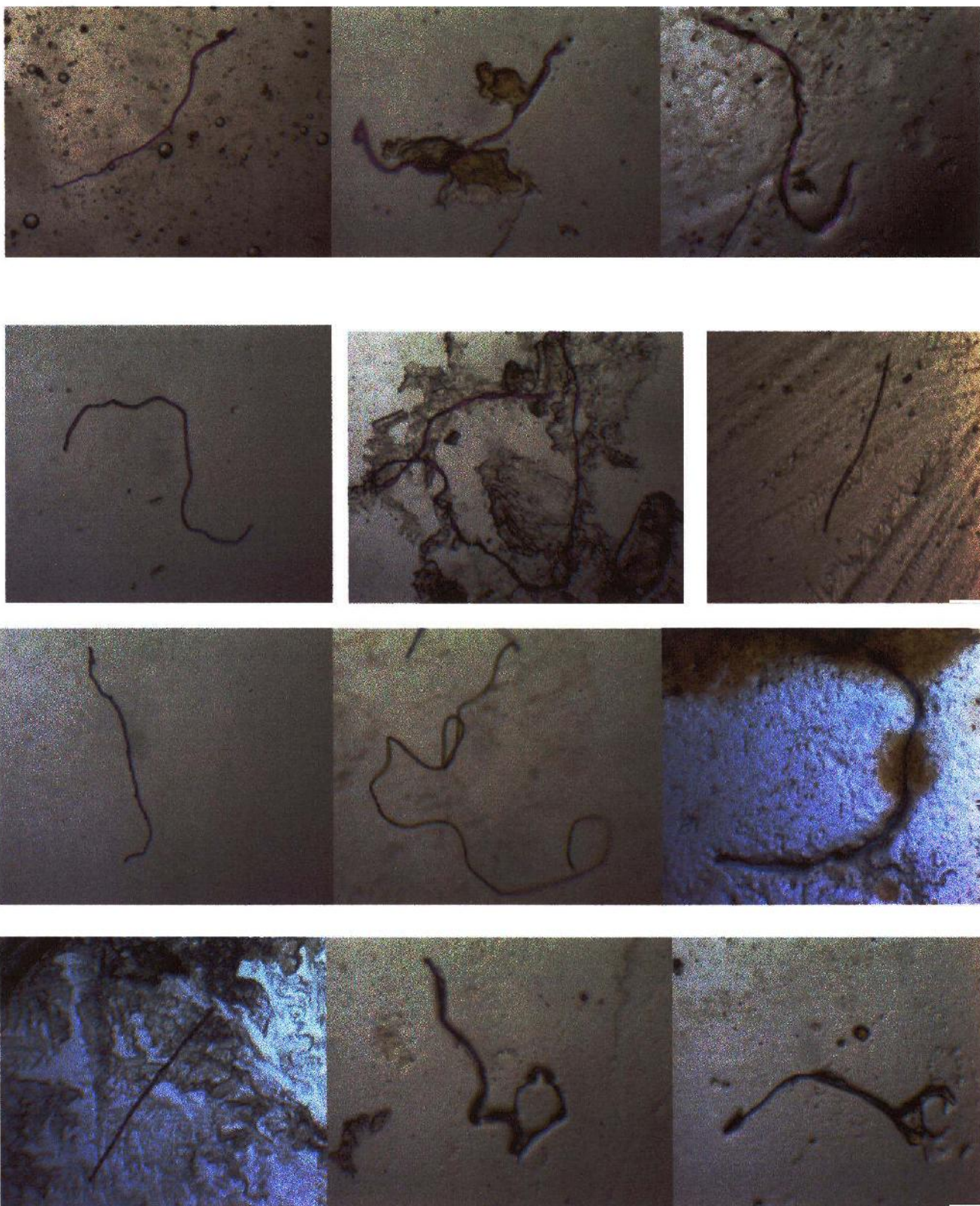


Figure 12: Some MPs particles (Shapes and colors)

8.5 Annex 5

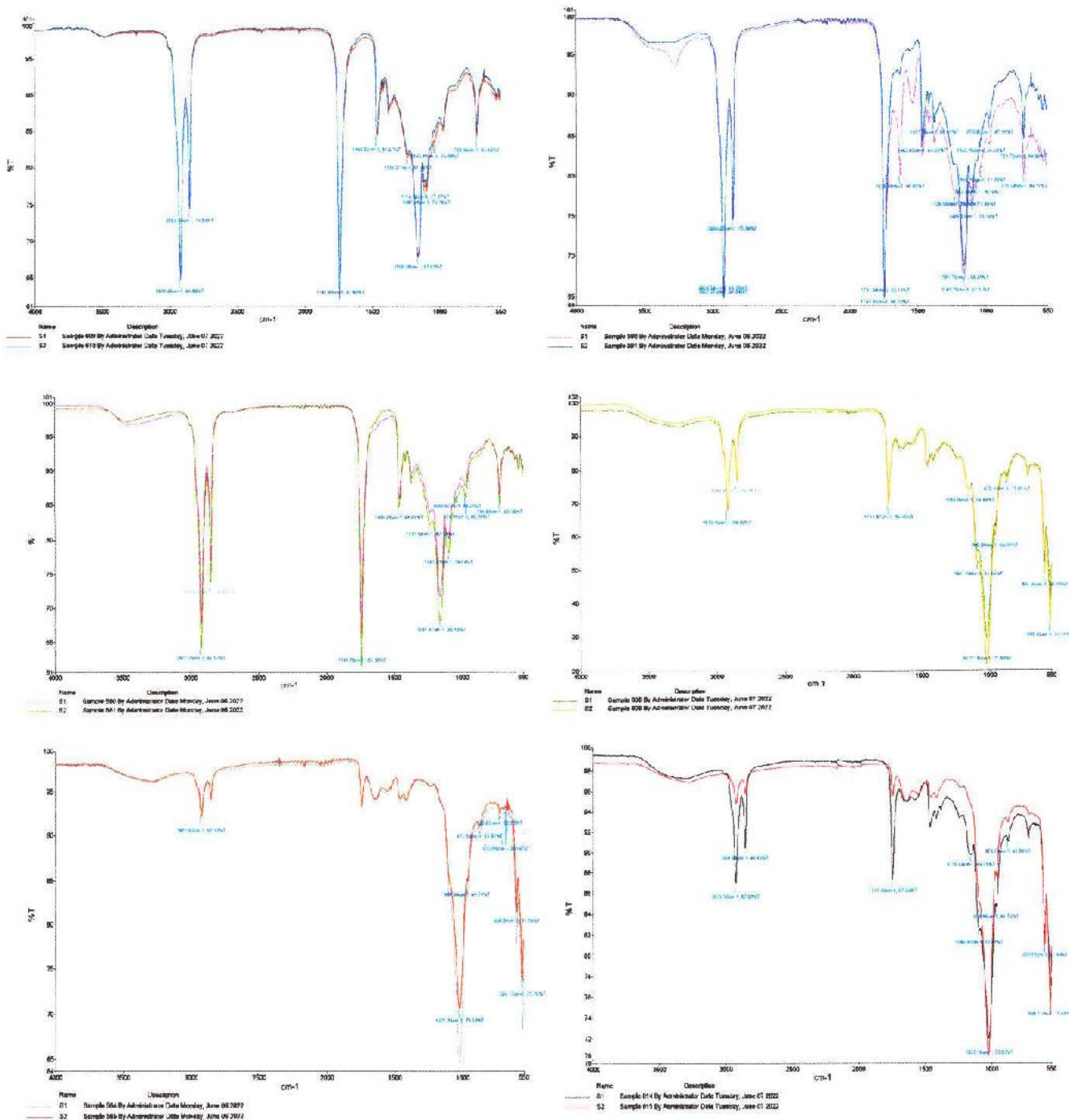


Figure 13: FTIR spectra of representative samples

8.6 Annex 6

FTIR data analysis

A total of 21 samples were taken for analysis by FTIR and polymers were found among 15 samples.

Fish species- *M. gilio*

1. Sample-1 (GT)

2921 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2851 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1742 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1402 = 1472 (LDPE), 1427 (PVC), 1462 + 1467 (LDPE), 1455 (PP), 1451 + 1492 (PS), 1452 + 1494 (ABS), 1469 (EVA), 1447 (Latex), 1440 (Nitrile), 1767 (Nylon), 1490 (PC), 1433 (PMMA), 1451 (PU)

1162 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1031 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

571+563+595+580 = 509 + 554 (PTFE), 537 (PS)

2. Sample-1 (Gill)

1653 = 1605 (Nitrile), 1634 (Nylon), 1601 (PS), 1660 (ABS)

1026 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

712 = 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)

667 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

589+597+576+560+565 = 509 + 554 (PTFE), 537 (PS)

3. Sample-1 (Muscle)

1653 = 1605 (Nitrile), 1634 (Nylon), 1601 (PS), 1660 (ABS)

1559+1549+1538 = 1538 (Nylon), 1503 (PC), 1531 (PU)

620+643+609 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

566+595+580 = 509 + 554 (PTFE), 537 (PS)

4. Sample-2 (GT)

2919 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2851 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1741 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1464 = 1472 (HDPE), 1427 (PVC), 1462 + 1467 (LDPE), 1455 (PP), 1451 + 1492 (PS), 1452 + 1494 (ABS), 1469 (EVA), 1447 (Latex), 1440 (Nitrile), 1767 (Nylon), 1490 (PC), 1433 (PMMA), 1451 (PU)

1172 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1096+1031 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

720 = 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)

602 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

502 = 509 + 554 (PTFE), 537 (PS)

5. Sample-2 (Gill)

2921 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2852 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1742 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1159 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1092+1026 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

962 = 966 (PVC), 997 + 972 (PP), 985 + 964 (PMMA), 967 (Nitrile), 966 (ABS), 904 (CA)

719 = 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)

602 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

560 = 509 + 554 (PTFE), 537 (PS)

6. Sample-2 (Muscle)

1653 = 1605 (Nitrile), 1634 (Nylon), 1601 (PS), 1660 (ABS)

1559 = 1538 (Nylon), 1503 (PC), 1531 (PU)

604+639+667+648+620 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

589+565+579 = 509 + 554 (PTFE), 537 (PS)

Fish species- *L. calcarifer*

7. Sample- 1 (GT)

2922 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2852 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1741 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1464 = 1472 (HDPE), 1427 (PVC), 1462 + 1467 (LDPE), 1455 (PP), 1451 + 1492 (PS), 1452 + 1494 (ABS), 1469 (EVA), 1447 (Latex), 1440 (Nitrile), 1767 (Nylon), 1490 (PC), 1433 (PMMA), 1451 (PU)

1237 = 1241 (PET), 1255 (PVC), 1241 (EVA), 1274 (Nylon), 1238 (PMMA), 1201 (PTFE), 1223 (PU)

1161 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1033+1097 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

972 = 966 (PVC), 997 + 972 (PP), 985 + 964 (PMMA), 967 (Nitrile), 966 (ABS), 904 (CA)

721 = 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)

8. Sample-1 (Gill)

2921 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2851 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1741 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1155 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1090+1022 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

960 = 966 (PVC), 997 + 972 (PP), 985 + 964 (PMMA), 967 (Nitrile), 966 (ABS), 904 (CA)

872 = 808 + 840 (PP), 828 (PC)

600 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

559 = 509 + 554 (PTFE), 537 (PS)

9. Sample- 1 (Muscle)

2922 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2852 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1742 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1161 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1098+1054+1033 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

608 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

576+556 = 509 + 554 (PTFE), 537 (PS)

10. Sample- 2 (GT)

2922 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2853 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1741 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1625 = 1605 (Nitrile), 1634 (Nylon), 1601 (PS), 1660 (ABS)

1464 = 1472 (HDPE), 1427 (PVC), 1462 + 1467 (LDPE), 1455 (PP), 1451 + 1492 (PS), 1452 + 1494 (ABS), 1469 (EVA), 1447 (Latex), 1440 (Nitrile), 1767 (Nylon), 1490 (PC), 1433 (PMMA), 1451 (PU)

1377 = 1376 (Latex), 1360 (Nitrile), 1372 (Nylon), 1364 (PC), 1386 (PMMA), 1331 (PVC), 1377 (LDPE), 1377 (PP), 1368 (CA)

1227 = 1241 (PET), 1255 (PVC), 1241 (EVA), 1274 (Nylon), 1238 (PMMA), 1201 (PTFE), 1223 (PU)

1161 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1033+1062+1096 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

972= 966 (PVC), 997 + 972 (PP), 985 + 964 (PMMA), 967 (Nitrile), 966 (ABS), 904 (CA)

721= 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)

11. Sample-2 (Gill)

2922 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2852 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1741 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1145 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA),
1147 (PTFE), 1166 (PP)

1091+1022 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

960 = 966 (PVC), 997 + 972 (PP), 985 + 964 (PMMA), 967 (Nitrile), 966 (ABS), 904 (CA)

873 = 808 + 840 (PP), 828 (PC)

601 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

559 = 509 + 554 (PTFE), 537 (PS)

12. Sample- 2 (Muscle)

2916+2954 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC),
2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2849 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE +
HDPE), 2838 (PP), 2847 (PS)

1701 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1432+1464 = 1472 (HDPE), 1427 (PVC), 1462 + 1467 (LDPE), 1455 (PP), 1451 + 1492 (PS),
1452 + 1494 (ABS), 1469 (EVA), 1447 (Latex), 1440 (Nitrile), 1767 (Nylon), 1490
(PC), 1433 (PMMA), 1451 (PU)

1310 = 1376 (Latex), 1360 (Nitrile), 1372 (Nylon), 1364 (PC), 1386 (PMMA), 1331 (PVC),
1377 (LDPE), 1377 (PP), 1368 (CA)

1294+1227 = 1241 (PET), 1255 (PVC), 1241 (EVA), 1274 (Nylon), 1238 (PMMA), 1201
(PTFE), 1223 (PU)

720 = 720 (EVA), 750 (PMMA), 720 (PETE), 730 + 717 (LDPE + HDPE), 759 (ABS)

13. Sample- 3 (GT)

2922 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 +
2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2853 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE +
HDPE), 2838 (PP), 2847 (PS)

1742 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1463 = 1472 (HDPE), 1427 (PVC), 1462 + 1467 (LDPE), 1455 (PP), 1451 + 1492 (PS), 1452
+ 1494 (ABS), 1469 (EVA), 1447 (Latex), 1440 (Nitrile), 1767 (Nylon), 1490 (PC),
1433 (PMMA), 1451 (PU)

1236 = 1241 (PET), 1255 (PVC), 1241 (EVA), 1274 (Nylon), 1238 (PMMA), 1201 (PTFE),
1223 (PU)

1114+1160 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189
(PMMA), 1147 (PTFE), 1166 (PP)

1033+1097 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)
722 = 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)

14. Sample- 3 (Gill)

2922 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 +
2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)
1021 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)
960 = 966 (PVC), 997 + 972 (PP), 985 + 964 (PMMA), 967 (Nitrile), 966 (ABS), 904 (CA)
872 = 808 + 840 (PP), 828 (PC)
600+673 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)
559 = 509 + 554 (PTFE), 537 (PS)

Fish species- *H. limbatus*

15. Sample- 1 (GT)

2922 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 +
2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)
2852 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE +
HDPE), 2838 (PP), 2847 (PS)
1741 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)
1162 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA),
1147 (PTFE), 1166 (PP)
1095 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)
971 = 966 (PVC), 997 + 972 (PP), 985 + 964 (PMMA), 967 (Nitrile), 966 (ABS), 904 (CA)
721 = 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)
611 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)
583+552 = 509 + 554 (PTFE), 537 (PS)

16. Sample- 1 (Gill)

1024 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)
961 = 966 (PVC), 997 + 972 (PP), 985 + 964 (PMMA), 967 (Nitrile), 966 (ABS), 904 (CA)
872 = 808 + 840 (PP), 828 (PC)
747+721 = 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)
661+673+695+602 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)
559 = 509 + 554 (PTFE), 537 (PS)

17. Sample- 1(Muscle)

2922 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 +
2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2852 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1742 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1623 = 1605 (Nitrile), 1634 (Nylon), 1601 (PS), 1660 (ABS)

1163 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1060+1097 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

557+577+567 = 509 + 554 (PTFE), 537 (PS)

18. Sample- 2 (GT)

2916+2954 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2848 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1700 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PET), 1743 (CA)

1432+1464 = 1472 (HDPE), 1427 (PVC), 1462 + 1467 (LDPE), 1455 (PP), 1451 + 1492 (PS), 1452 + 1494 (ABS), 1469 (EVA), 1447 (Latex), 1440 (Nitrile), 1767 (Nylon), 1490 (PC), 1433 (PMMA), 1451 (PU)

1227+1294+1207 = 1241 (PET), 1255 (PVC), 1241 (EVA), 1274 (Nylon), 1238 (PMMA), 1201 (PTFE), 1223 (PU)

720 = 720 (EVA), 750 (PMMA), 720 (PET), 730 + 717 (LDPE + HDPE), 759 (ABS)

19. Sample-2 (Gill)

2920 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

1625 = 1605 (Nitrile), 1634 (Nylon), 1601 (PS), 1660 (ABS)

1541 = 1538 (Nylon), 1503 (PC), 1531 (PU)

1162 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1090 = 1094 (PET), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

587+597+578+564+556 = 509 + 554 (PTFE), 537 (PS)

20. Sample- 2 (Muscle)

3360+3366 =

2921 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

1625 = 1605 (Nitrile), 1634 (Nylon), 1601 (PS), 1660 (ABS)

1598 = 1538 (Nylon), 1503 (PC), 1531 (PU)

1097+1033 = 1094 (PETE), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

630+606+614+648+660 = 687 (Nylon), 638 (PTFE), 616 (PVC), 694 (PS), 698 (ABS), 600 (CA)

562+598+555+597+506+552+550 = 509 + 554 (PTFE), 537 (PS)

21. Sample- 3 (GT)

2919 = 2917 (EVA), 2920 + 2960 (Latex), 2917 (Nitrile), 2932 (Nylon), 2936 (PC), 2992 + 2949 (PMMA), 2915 (HDPE + LDPE), 2950 + 2915 (PP), 2922 (ABS)

2851 = 2848 (EVA), 2855 (Latex), 2849 (Nitrile), 2858 (Nylon), 2865 (PU), 2845 (LDPE + HDPE), 2838 (PP), 2847 (PS)

1711 = 1740 (EVA), 1768 (PC), 1721 (PMMA), 1731 (PU), 1713 (PETE), 1743 (CA)

1435+1411+1464 = 1472 (HDPE), 1427 (PVC), 1462 + 1467 (LDPE), 1455 (PP), 1451 + 1492 (PS), 1452 + 1494 (ABS), 1469 (EVA), 1447 (Latex), 1440 (Nitrile), 1767 (Nylon), 1490 (PC), 1433 (PMMA), 1451 (PU)

1226 = 1241 (PETE), 1255 (PVC), 1241 (EVA), 1274 (Nylon), 1238 (PMMA), 1201 (PTFE), 1223 (PU)

1170 = 1167 (Latex), 1197 (Nitrile), 1199 (Nylon), 1186 + 1158 (PC), 1141 + 1189 (PMMA), 1147 (PTFE), 1166 (PP)

1098 = 1094 (PETE), 1020 (EVA), 1013 (PC), 1099 (PVC), 1027 (PS)

720 = 720 (EVA), 750 (PMMA), 720 (PETE), 730 + 717 (LDPE + HDPE), 759 (ABS)

