STUDIES ON THE BIOACCUMULATION OF MICROPLASTICS IN SELECTED MARINE FISHES ALONG MUMBAI COAST, INDIA

Dissertation submitted in partial fulfilment

of the requirements

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by

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DEDICATED TO

MY BELOVED

MOM, DAD, BROTHER, SISTER, GUIDE,
TEACHERS AND ALL MY NEAR & DEAR ONE'S

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ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION
(A University Established Under Sec. 3 of UGC Act 1956)
Ministry of Agriculture & Farmers Welfare,
Govt. of India.

Dated: 30th June, 2019

CERTIFICATE

Certified that the dissertation entitled **"STUDIES ON THE BIOACCUMULATION OF MICROPLASTICS IN SELECTED MARINE FISHES ALONG MUMBAI COAST, INDIA"** is a bonafide record of independent research work carried out by **Ms. Nely Debbarma** during the period of study from August 2018 to June 2019 under our supervision and guidance for the degree of **Master of Fisheries Science (Fisheries Resource Management)** and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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DECLARATION

I hereby declare that the dissertation entitled "**STUDIES ON THE BIOACCUMULATION OF MICROPLASTICS IN SELECTED MARINE FISHES ALONG MUMBAI COAST, INDIA**" is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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ABSTRACT

The present study was conducted to establish the intensity of microplastic pollution in Mumbai waters, with the objectives to assess the bioaccumulation of microplastics in the tissues of selected marine fishes and to study the temporal variability of microplastics in fish tissues. Fishes were collected from experimental trawling over a period of seven months from October, 2018- April 2019. A total of 188 fishes were analysed. Collected fishes were sorted, measured and weighed. They were then dissected carefully to separate the body parts (gills, guts and muscles). Alkaline digestion with 10% KOH was followed for separating the microplastics from tissue samples. Separated parts were analysed for microplastic accumulation, size, morphology, colour etc. On an average the numbers of microplastics particles in the GI tract, gills and muscles tissues from total fish (n=188) are 6.62±1.68, 6.21±1.67 and 0.81±0.37 in GI tract, gills and muscles respectively. The microplastics having a size of < 100 µm comprising of beads was found to be most predominant in all the tissues. Black and blue were the most predominant colours of microplastics from the tissues of *Johnius* spp. The study result shows that there is a marked monthly/temporal variation of microplastics in the fish tissues. In the present study there were 151 juveniles and 37 adults fish from the total fish sampled .The mean number of microplastics fish^{-1} found in the gills, guts and muscles of juvenile fishes are 5.52, 6.30 and 0.75 respectively. And the microplastics in the adult fishes were 5.58, 5.73 and 0.56 in gills, guts and muscles respectively. The Pearson correlation coefficient shows that there is no significant correlation between the fish body length and microplastics numbers in gills and guts tissue. A positive correlation was found between the fish body length and microplastics numbers in muscle tissue of fish. Therefore the present study shows that there is a potential risk of microplastics bioaccumulation in the fish body and subsequent risk to the consumers and organisms sharing the food chain.

सारांश

मुंबई के जल में सूक्ष्म प्लास्टिक प्रदूषण की तीव्रता को स्थापित करने के लिए वर्तमान अध्ययन का आयोजन किया गया था, जिसमें चयनित समुद्री मछलियों के ऊतकों में माइक्रोप्लास्टिक्स के बायोकैकुम्यूलेशन का मूल्यांकन करने और मछली के ऊतकों में माइक्रोप्लिस्ट की अस्थायी परिवर्तनशीलता का अध्ययन करने के उद्देश्य से किया गया था। अक्टूबर, 2018- अप्रैल 2019 से सात महीनों की अवधि में प्रायोगिक ट्रॉलिंग से मछलियों का संग्रह किया गया। कुल 188 मछलियों का विश्लेषण किया गया। एकत्रित मछलियों को छांटा, मापा और तौला गया। फिर उन्हें शरीर के अंगों (गलफड़ों, हिम्मत और मांसपेशियों) को अलग करने के लिए सावधानी से विच्छेदित किया गया। ऊतक के नमूनों से माइक्रोप्लास्टिक्स को अलग करने के लिए 10% KOH के साथ क्षारीय पाचन का पालन किया गया था। माइक्रोप्लास्टिक संचय, आकार, आकृति विज्ञान, रंग आदि के लिए अलग-अलग हिस्सों का विश्लेषण किया गया था। जीआई ट्रैक्ट में माइक्रोप्लास्टिक कणों की संख्या के आधार पर, कुल मछली (n = 188) से गलफड़े और मांसपेशियों के ऊतक 6.62±1.68, 6.21 ± 1.67 और 0.81±0.37 जीआई ट्रे में GI हैं। मोतियों से युक्त <100 am के आकार वाले माइक्रोप्लास्टिक को सभी ऊतकों में सबसे प्रमुख पाया गया। जॉन्सियस एसपीपी के ऊतकों से काले और नीले माइक्रोप्लास्टिक्स के सबसे प्रमुख रंग थे। अध्ययन के परिणाम से पता चलता है कि मछली के ऊतकों में माइक्रोप्लास्टिक की एक मासिक / अस्थायी भिन्नता है। वर्तमान अध्ययन में कुल मछलियों के 151 किशोर और 37 वयस्क मछलियों के नमूने लिए गए थे। किशोर मछलियों के गलफड़े, हिम्मत और मांसपेशियों में पाए जाने वाले माइक्रोप्लास्टिक मछलियों की औसत संख्या क्रमशः 5.52, 6.30 और 0.75 है। और वयस्क मछलियों में माइक्रोप्लास्टिक्स क्रमशः 5.58, 5.73 और 0.56 गल, हिम्मत और मांसपेशियों में थे। पियर्सन सहसंबंध गुणांक दर्शाता है कि गलफड़ों और कण्ठ के ऊतकों में मछली के शरीर की लंबाई और माइक्रोप्लास्टिक्स संख्या के बीच कोई महत्वपूर्ण संबंध नहीं है। मछली के मांसपेशियों के ऊतकों में मछली के शरीर की लंबाई और माइक्रोप्लास्टिक संख्याओं के बीच एक सकारात्मक संबंध पाया गया। इसलिए वर्तमान अध्ययन से पता चलता है कि मछली के शरीर में माइक्रोप्लास्टिक बायोकैम्कुलेशन का संभावित जोखिम है और बाद में उपभोक्ताओं और जीवों को खाद्य श्रृंखला साझा करने का जोखिम है।

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1. INTRODUCTION

Pollution of the oceans by microplastics (MPs) represents a major environmental threat next to global warming. The alarming threat of microplastics found in the environment and aquatic organisms has unveiled the eyes of researchers, environmentalists, and various stakeholders worldwide as it has the probable chance to enter in the food chain and accumulate in organisms including humans. Microplastics (MPs) are small plastic particles with a size of <5 mm (Arthur *et al*., 2009). In 2014, the estimated number of floating plastic particles in the world's oceans was 5.25 trillion (269,000 MT), out of which 4.85 trillion particles were microplastics of <4.75 mm in size (Eriksen *et al*., 2014). An estimated 4.8 and 12.7 million metric tons (MT) of plastic waste enters into the ocean every year (Jambeck *et al*., 2015), and there have been an emerging environmental and health issues raised with regard to the plastic materials being disposed of in the ocean water around the world.

Plastics have become an indispensable material in many areas of modern life, where it is used for clothing, storage, transportation, packaging, construction and a host of consumer goods in our day to day life. Barnes *et al*. (2009) reported that the mass production of plastic material made a breakthrough post Second World War, in the 1960s and 1970s, shifting from the use of traditional materials. One of the greatest properties of plastics is its durability which is also one of the main reasons that they present a threat to the marine environment. The risk increases as long as plastic continues to enter the ocean. The growing interest in the study of plastic materials arises from it being persistent in the aquatic and terrestrial environment in the form of small plastic particles, known as microplastics (< 5 mm) which are non-biodegradable in the environment.

Due to the huge amount of plastics being consumed worldwide and their irresponsible disposal made microplastics an alarming threat to various marine ecosystems, including deep sea and open Ocean and coastal regions (Claessens *et al*., 2011; Van Cauwenberghe *et al*., 2013). According to an estimate given by Jambeck *et al*. (2015) out of 192 countries, 5 countries contributed more than 50% of mismanaged plastics in the world. It was found to be mostly coming from the developing countries which have not yet devised a proper waste management facility. Hence the microplastics could be a serious threat to the organisms and environment in the coming days. It is therefore very important to have a proper waste management system and facilities to tackle pollution.

Studies have revealed that 80% of marine plastic pollution is sourced directly from inland, particularly from urban areas (Cable *et al*., 2017; Hajbane and Pattiaratchi, 2017; Wang *et al*., 2017). Wide varieties of marine organisms, including zooplankton, bivalves, invertebrates, fish, birds, and cetaceans incidentally take up MPs from sediment or the water column mistaking them for food (Cole *et al*., 2013; Lusher *et al*., 2015; Ferreira *et al*., 2016). MPs of micrometers (µm) in dimension have the propensity to translocate from the gut to the circulatory system in many organisms where they may reside for relatively long periods of time (Browne *et al*., 2008; Van Cauwenberghe *et al*., 2015; Collard *et al*., 2017).

Microplastics generally have been classified as primary and secondary microplastics. Primary microplastics are plastic materials which are designed and produced ranging from a few µm to 5 mm in size and are found in products like toothpaste, face wash, face scrub, body scrub, other personal care products, and pre-production resin pellets. While secondary microplastics are those plastics materials which are generated by the breakdown of larger plastic materials due to mechanical and oxidative processes (Hidalgo-Ruz *et al*., 2012) in the environment.

Several experimental shreds of evidence have shown that microplastics in the marine organisms have the potential to be transferred across the food chain in various trophic levels. Fish meal is an important commodity which is produced in bulk quantity and used as poultry feed, pig rearing and thus as a result may end up in non-marine foods. The transference of microplastic particles through the food web is of great concern as humans consume these marine organisms which have ingested microplastic particles in their body from the marine environment that can pose a serious threat to the health (Duis and Coors, 2016; Santana *et al*., 2017).

The occurrence of microplastics in the marine environment could result in a severe physiological impact on organism's health when these tiny particles are ingested into the body system leading to obstruction in the gut and food depletion (Wright *et al*., 2013). The presence of several harmful additives/monomers used in the production of plastic polymers can possibly leach into the digestive tract reducing survival, feeding, immunity or antioxidant capacity (Browne *et al*., 2013).

Microplastics contamination in the future will be increasing due to the continuous breakdown of the present stocks of plastic materials already present in the marine environment and also with the continuous demand and production of new plastic materials in the coming days. Microplastics have been rising as imminent environmental pollutants in recent years due to mass production of plastic materials worldwide coupled with improper waste management, and thus resulting in a serious threat to the environment and its inhabitants. Evidence from several experiments has indicated that microplastics have the potential to be transferred between various trophic levels (Farrel and Nelson, 2013; Bouwmeester *et al*., 2015).

The work on microplastics in the fish biota of Indian waters has not been reported so far and hence this forms the preliminary work on microplastics in fish tissues, studied on *Johnius* spp. in Mumbai coastal water of India. Mumbai is the most populous city in India. In the world, Mumbai comes as the 4th most populous city and one of the urban agglomeration regions in the world with a population of approx. 22 million as of 2011 Indian census. The city of Mumbai has a coastal length of 149 km where there are many inhabitants in and around the coast.

The aim of the present study is to determine the degree of microplastic pollution in the Mumbai waters through the presence of microplastics in the Croaker (*Johnius* spp.) group of fishes. Its presence in three different tissues such as gut, gills, and muscles were studied. The study is of great importance since the coastal waters of the Mumbai are hugely polluted due to sewage and municipal waste which is not properly treated or is directly let out in the open seas along with plastic litter. The plastic litter and anthropogenic debris which are lying in almost all the beaches in the city lead to marine plastic pollution contributing to microplastic pollution in the system due to their subsequent breakdown. These plastic materials and debris washed ashore from beaches driven by wind and wave action finally end up in the open oceans.

Therefore the present study of microplastic estimation in the tissues of *Johnius* spp., which is one of the important seafood fishes found in the Northwest coast of Indian waters will clearly reflect the status of microplastic pollution in the Mumbai coastal waters. The fish selected for this study, *Johnius* spp. is a demersal fish species with carnivorous feeding nature feeding upon benthic organisms including shrimps which were found in their gut contents. Keeping all the above facts in mind, the present study was conducted to establish the intensity of microplastic pollution in Mumbai waters, with the following objectives

- To assess the bioaccumulation of microplastics in the tissues of selected marine fishes.
- To study the temporal variability of microplastics in fish tissues.

2. REVIEW OF LITERATURE

2.1. Microplastics and its classification

Since the 1940s, when the mass production of plastics began in earnest, the volume of plastic produced has risen rapidly. With legislation to curb the indiscriminate disposal of plastic waste emerging slowly, plastic debris entering the marine environment increased in parallel with rates of production during this time (Moore, 2008; Ryan *et al*., 2009; Barnes *et al*., 2009).

According to the current globally accepted definition (Arthur *et al*., 2009; GESAMP, 2015) Microplastics are any plastic particles smaller than 5 mm (secondary or primary). Microplastics are found to distribute globally all over the world oceans. Microplastics have been classified into different shapes and sizes by different workers in a different way of categorization have been observed in their studies. Basically, they had been categorized into primary microplastics and secondary microplastics (Andrady, 2011; Cole *et al*., 2011).

Primary microplastics are engineered to be produced as the size of micron and are found in daily uses like face wash, face scrub, body wash, and other exfoliating products and as resin pellets. While secondary microplastics are those plastics materials which are generated by the breakdown of larger plastics materials due to mechanical and oxidative processes (Hidalgo-Ruz *et al*., 2012) in the environment. Secondary microplastics are the most predominant group found in most of the studies Beaches are found to be the most likely source of secondary microplastics in marine environments (Andrady, 2011; Kataoka and Hinata, 2015).

The other classification of microplastics is based on its shapes, colours, and sizes. Several authors have classified microplastics based on different shapes, colours and sizes have been observed in their studies and there has not been a fixed classification for microplastics so far. Based on its shapes microplastics had been classified into five types as 1). Fragments, 2). Fibres; 3). Foams, 4). Beads, and 5).Pellets (Lusher *et al*., 2017). Microplastics based on shapes, they have also been classified into four types as 1.Fibres (filaments and lines), 2.Films, 3.Fragments, and 4.Foams (Free *et al.,* 2014; Karami 2017).

The colour and size categorization of microplastics is generally based on the source of study materials and it varies with various workers. Abbasi *et al*. (2018) has classified colours into 5 categories (white-transparent, yellow-orange, red-pink, blue-green and black-grey) and sizes into 5 categories (< 100 µm, 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm) in his study on fish fish tissues microplastics from Musa estuary in Persian Gulf. In the study of microplastics from muscles tissues from some commercial fish species in Northeast of Persian the size has been classified into 6 categories (< 100 µm, 100-250 µm, 250-500 µm, 500-1000 µm, 1000-5000 µm, and > 5000 µm) (Akhbarizadeh *et al*., 2018). Li *et al*. (2018) has classified microplastics colours into 8 colours categories (Red, Black, Brown, Blue, Yellow, Green, White, Transparent) and 5 size classes (20-50 µm, 50-100 µm, 100-500 µm, 500-1000 µm and 1000-5000 µm) of microplastics from oysters in Pearl River Estuary, South China.

2.2. Source of microplastics pollution

Microplastics pollution in the marine environment is contributed mostly from the plastics being disposed into the river system which ultimately make it way to the ocean water and thus degrade within a given time period and contribute to microplastics pollution in the marine environment. It has been estimated that rivers transported 70-80 % of plastic to the marine ecosystems (GESAMP, 2010). The other source is also contributed by the fishing industries from wear and tear of vessels having plastic materials in its construction in some form or other and lost fishing gear materials. In the fishing industries sector abandoned, lost or otherwise discarded fishing gears (ALDFG) are the main source of plastic waste into the marine environment (FAO, 2016). The ingested synthetic fibers are similar to those which are commonly being used in fishing gears (Lusher *et al*., 2013).

A study has reported that fish collected closer to WWTPs had significantly higher concentrations of microplastic in their digestive tissues compared to fish from further locations in the freshwater zone of the Rio de la Plata estuary, Argentina (Pazos *et al*., 2017). A study has shown that there exists a significant relationship between the microplastic concentrations and the population density within the range of 101 to 102 person km^2 and has also been determined for four estuarine rivers that flow into the Chesapeake Bay, United States of America (Yonkos *et al*., 2014). The positive slopes of the regression line for the population density and urban ratio suggest that microplastics are abundant both in count and mass in river basins where the population density and urban ratio are high (Kataoka *et al*., 2019). So it is clear evidence that population density and urbanisation are significantly correlated to microplastics concentrations.

Wang *et al*. (2017) reported that the distribution of microplastics in the Wuhan lakes generally presented a decreasing concentration trend away from the urban center. And almost all the lakes with microplastic concentrations higher than 5000 n/m^3 are located inside or near the area surrounded by the City Third Ring Road (China). Due to high population densities and several anthropogenic activities coupled with the production of a considerable amount of municipal waste and effluent in the urban centres which may contain large quantities of plastics (Browne *et al*., 2011; Hidalgo-Ruz *et al*., 2012).

The type and degree of microplastic pollution in a particular marine ecosystem is possibly correlated to the level of specific anthropogenic activities, like washing of clothes, in the region than to the total production and utilization of plastic materials (Jambeck *et al.,* 2015). Anderson *et al*. (2016) have reported that microplastics inputs are expected to be higher in highly populated and industrialized watersheds in comparison to areas with less human activity.

A study has reported that 80% of all the plastic pollution in the marine water is directly sourced from adjacent inland areas, particularly from urban areas (Cable *et al*., 2017; Wang *et al*., 2017). Hidalgo-ruz *et al*. (2012)

have reported that freshwater systems act as a means of transport systems for microplastics from terrestrial to marine ecosystems. Wagner *et al*. (2014) have reported that microplastics can reach the river and estuary directly through sewage discharge or indirectly through runoff and atmospheric deposition. Eerkes-Medrano *et al*. (2015) have reported that the estuarine environments play an important role in the transport and accumulation of microplastics as it acts as a transitional zone between riverine and marine environments. Andrady (2011) has reported that only 10% of plastics is estimated to be originated from ships and fishing activities in the world oceans, while the remaining 90% comes from land sources.

2.3. Microplastics distribution in the marine environment

Microplastics tend to represent an increasing proportion of marine debris and until this date, various modelling studies have attempted to identify numerous sources, distribution and accumulation areas (Eriksen *et al*., 2014; van Sebille *et al.*, 2015; Clark *et al*., 2016). Microplastics are distributed in every habitat of open oceans and seas, including beaches, surface waters, column water and deep seafloor (Lusher, 2015).

The properties of its being small size and low-density MPs in the oceans are readily transported across a wide area and distance by ocean currents (Cole *et al*., 2011; Eriksen *et al*., 2013). A recent study has provided evidence that the Mediterranean Sea has relatively higher MPs abundance as compared to other water basins (Suaria *et al*., 2016). MPs are known to be found even in deep-sea habitats (Van Cauwenberghe *et al*., 2013).

MPs are found to be accumulated in oceanic gyres and accumulation zones, especially in the North Pacific and North Atlantic Ocean (Eriksen *et al*., 2013). MPs are bioavailable for many marine organisms since their sizes are in the same range of plankton (Wright *et al*., 2013).

Welden and Lusher (2017) have described that the spatial patterns and rates at which plastic debris and associated chemicals entering the aquatic environments are likely to be driven by changes in precipitation patterns and increase in the intensity and frequency of storm events linked to the changing climate.

Sagawa *et al*. (2018) have reported in his study that distributions of microplastics were significantly affected by the size of microplastics regardless of the polymer type. The larger microplastics are likely to be on the beaches and are unlikely to be on the bottoms. It has been found that the amount ratio of larger microplastics (>2 mm) was highest in the beach sediments and it decreased in the order of the surface water and bottom sediments for both foamed polystyrene (FPS) and 'PE and PP'.

2.4. Microplastics in fish tissues

Lusher *et al*. (2013) have reported that the GI tract from pelagic and demersal fishes from the English Channel represented 1.9 particles fish^{-1} on an average, ranging from 130- > 5000 µm in size of the 184 fish with ingested microplastics. The abundance of plastic pieces ranged from 1-15 per fish. The ingested plastics particles primarily consisted of fibres, followed by fragments and beads and black being the most predominant colour.

A study on the variety of commercial fishes examined from Portugal, their stomach contains 1.40 ± 0.66 particles/fish (n= 52), with particles size in the range of 220-4800 µm (Neves *et al*., 2015). Tanaka and Takada (2016) has investigated microplastics sampled from Tokyo Bay in the digestive tract of 64 Japanese anchovies (*Engraulis japonicas*) and has detected plastic in 49 out of 64 fish (77%). Majority of the plastics observed were fragments (86%), and second abundant were beads (7.3%) and some microbeads were found, which are similar to those found in facial cleansers.

Karami *et al*. (2017a) have reported that microplastic loads were higher in the eviscerated flesh in comparison to the excised organs of two species (*Chelon subviridis* and *Johnius belangerii*) of dried fish, and thus the study highlighted that evisceration does not necessarily eliminate the risk of microplastic intake by the consumers. The predominant MPs types in the eviscerated flesh and excised organs of dried fish were fragments followed by films and filaments.

Akhbarizadeh *et al.* (2018) in his study in northeast of Persian Gulf has reported an average MPs abundance in the muscle of the fish *P. indicus, Sphyraena jello* and *Epinephelus voioides,* and the shrimp, *Alepes djedaba*, was 1.85 ± 0.46, 0.57 ± 0.17, 0.78 ± 0.22 and 0.80 ± 0.12 g^{-1}, respectively. The result demonstrated that benthic fish had higher microplastics content in their muscles. And fibrous shapes of microplastics were more prevalent in the study.

Abbasi *et al*. (2018) have reported that the MPs numbers were found to be higher in the skin, muscle, and gills in relation to gut and liver of *S. Sihama* and *P. Indicus* from the Musa estuary in the Persian Gulf. The predominant colour of microplastic was found to be black or grey filamentous fragments (71%), and blue and green fragments comprise of 12% from pooled microplastics.

Herrera *et al*. (2019) have reported his study in the Canary Islands coast that the average number of microplastics ingested by all the sampled fish (*Scomber colias*) was 2.17±2.04 items per fish, (mean ± SD). The study has found that 74.2% of the sampled fish has ingested fibres, and second followed by fragments, paints, lines, and films. And the predominant colour was blue (55%) and black or dark (23.5%).

2.5. Temporal variation of microplastics

In a study conducted by Yukari Matsuguma *et al*. (2017), dated sediment cores from Japan, microplastic pollution started in the 1950s, and their abundance increased markedly toward the surface layer in 2000's. Continuous fragmentation of larger plastic debris and the rising popularity of ''plastic scrubbers'' appears to have increased the volume of microplastic debris in the oceans, resulting in a decrease in the average size of plastic litter over time (Barnes *et al*., 2009). This was highlighted by Thompson *et al*. (2004), who demonstrated that microplastic concentrations in the 1980s and 1990s were significantly greater than those in the 1960s and 1970s in an analysis of CPR samples from the North Sea and Northwest Atlantic.

Furthermore, the incidence of plastic ingestion by Fulmars (ocean-foraging seabirds), washed ashore in the Netherlands, increased from 91% to 98% between the 1980s and 2000, whilst the average consumption doubled from 15 to 30 plastic fragments per bird during this period (van Franeker *et al*., 2011). Concentration trends within the past decade are not overtly apparent, and there is some debate as to whether levels of plastic debris are still increasing or have stabilized. The study by Thompson *et al*. (2004) indicated a minimal change in microplastic contamination between the 1980s and 1990s. Similarly, an evaluation of > 6, 100 surface trawls conducted throughout the Northwest Atlantic Ocean found no significant difference in microplastic abundance over a 22 year period (Law *et al*., 2010). The average number of plastics debris items consumed by Fulmars, beached on the shores of the Netherlands, decreased slightly from the mid-1990s, but has remained relatively stable since the turn of the century, currently averaging 26 plastic fragments per bird (van Franeker *et al*., 2011).

In contrast, Claessens *et al*. (2011) indicate that microplastic concentrations have steadily increased over the past two decades. Analysis of sediment cores taken along the Belgian coast indicates microplastic pollution tripled from 55 microplastics/kg of dry sediment (1993– 2000) to 156 microplastics/kg of dry sediment (2005–2008), in line with global production rates. However, the use of sediment cores is a new technique, and bioturbation from tourism or sediment-dwelling biota might have affected this data. Any further conclusions are hampered by both a lack of studies that have specifically considered trends of microplastic abundance over time. Meta-studies are difficult to develop due to varieties of sampling methodologies, huge spatial variations in microplastic abundance, and lack of standardised size definitions of microplastics (Ryan *et al*., 2009; Barnes *et al*., 2009). Impact of microplastics on the marine environment whilst it is apparent that microplastics have become both widespread and ubiquitous, information on the biological impact of this pollutant on organisms in the marine environment is only just emerging (Barnes *et al*., 2009; Ryan *et al*., 2009). The possibility that microplastics pose a threat to biota, as their small size makes them available to a wide range of marine organisms, is of increasing scientific concern (Barnes *et al.,* 2009; Derraik, 2002; Thompson *et al*., 2004).

Seasonal variation on the occurrence of plastic and plastic ingestion was observed in the tropical estuary of South America, mainly due to the increase of freshwater and rainfall during the rainy season (Lima *et al*., 2014). In the Northern South sea microplastics ingested by brown shrimp shows significant temporal variations with higher MPs uptake in October (2013) in comparison to March (2013 and 2014) (Devriese *et al*., 2015).

High variability in plastic abundance on a spatial and temporal scale was observed between the two studied sites in remote coral islands in the Maldives (Imhof *et al*., 2017).

2.6. Effects of microplastic pollution in the marine environment and biota

A wide variety of marine taxa, such as the birds, sea turtles and marine mammals are known to be affected by entanglement with plastics materials and also ingestion of microplastics particles, which have the consequences like impaired movement, decrease in feeding ability, reduced reproductive output, lacerations, ulcerations and death (Laist, 1997; Derraik, 2002; Moore, 2008; Gregory, 2009). Several studies in the laboratory has confirmed that microplastics have the tendency to be ingested by a wide range of marine biota, including fish and shellfish for human consumption, which has the potential to impair various cellular, metabolic or physiological pathways (Browne *et al*., 2008; Wegner *et al*., 2012; Avio *et al*., 2015).

Microplastics can not only cause physical damage but they have the potential to transfer various chemicals adsorbed on their surface to the organisms encountered (Mato *et al*., 2001). Microplastics particles possess a large surface area to volume ratio, which makes it favourable to come in association with the environmental contaminants including polycyclic aromatic hydrocarbons (PAHs) (Rios *et al*., 2007) and metals (Betts, 2008; Ashton *et al*., 2010).

Besides the physical damage caused, microplastics have potentially toxic effects on the marine organisms when they ingest these microplastics particles because of the chemical additives used in plastic manufacture, and also the persistent organic pollutants and metals which are adsorbed on their surfaces (Teuten *et al*., 2009; Rochman *et al.,* 2014). It has been reported from several studies that the consumption of microplastics associated with hydrophobic toxicants, may lead those chemicals to desorb into biological tissues, which can lead to potential detrimental effects to endocrine and immune systems of an organisms with implications on the reproductive ability (Teuten *et al*., 2009; Murphy *et al*., 2015; Jepson *et al*., 2016).

Hermabessiere *et al*. (2017) has reported that polybrominated diphenyl ethers (PBDE), phthalates, nonylphenols (NP), bisphenol A (BPA) and antioxidants are the most common plastic additives found in the marine environments. And these all the additives are well-known health hazards to organisms including humans. The plasticizers used in making of plastics such as bisphenol-A (BPA) which are used in a wide range of plastics products have the potential to affect the hormonal systems and reproductive output of molluscs, fish, crustaceans and insects (Endo *et al*., 2005; Teuten *et al*., 2007; Oehlmann *et al*., 2009). Sajiki and Yonekubo (2003) have reported that the leaching of well-known plastic additives, BPA in the marine environment can occur from the food and drinking water bottles and materials.

Ryan (1988) described that the accumulation of plastics debris in the digestive tract in an organism may cause a false sense of feeling of fullness and thus lead to decreased in food consumption. An experiment has shown developmental defects of larval development on exposure to nanoplastics and microplastics (Della Torre *et al*., 2014; Nobre *et al*., 2015). Gregory (2009) has described that the plastics discarded in the environment can transport alien/invasive species, also inhibition of gaseous exchange and resulting in smothering of sea beds.

2.7. Microplastics accumulation through trophic transfer

Since all the organisms are linked to one another through a wide array of the food chain there is a possibility that microplastics once ingested by an organism can be transferred to other organisms through the food chain. Microplastics are highly bioavailable to marine organisms, either through direct ingestion or indirectly by trophic transfer from contaminated prey. Microplastics occupy the same size range as plankton and grains of sand, making them accessible to a variety of organisms using different feeding strategies. Organisms may, therefore, ingest unknown quantities in conjunction with natural prey items, particularly non-selective feeders, which filter large quantities of water and sediment for organic nutrients (Browne *et al*., 2008; Cole *et al*., 2013; Farrell and Nelson, 2013).

Microplastics are being reported in an increasing number of marine organisms from different trophic levels, including zooplankton (Frias *et al*.,2014; Desforges *et al*., 2015), barnacles (Goldstein *et al*., 2013), bivalves (Van Cauwenberghe and Janssen, 2014), decapod crustaceans (Devriese *et al*.,2015), fish (Boerger *et al*., 2010; Lusher *et al*., 2013; Neves *et al*., 2015), marine mammals (Besseling *et al*., 2015; Lusher *et al*., 2015) and seabirds (Avery-Gomm *et al*., 2012). The continuous consumption of high numbers of zooplankton(which ingested microplastics) by predators like juvenile fish, or even large filter feeders like humpback whales, making it likely that microplastics will bioaccumulate in larger animals (Desforges *et al*., 2015). The ingestion of microplastics by organisms throughout the water column, in addition to the benthos, indicates the ease at which microplastics may transfer throughout the food web (Setälä *et al*., 2014). Setälä *et al*. (2013), study shows for the first time the potential of plastic microparticle transfer via planktonic organisms from one trophic level (mesozooplankton) to a higher level (macrozooplankton). To date, empirical studies have demonstrated that trophic transfer occurs under laboratory conditions for low trophic level organisms, such as crabs (Batel *et al*., 2016; Farrell and Nelson, 2013; Setälä *et al*., 2014; Watts *et al*., 2014), but the extent to which this occurs in the wild and in higher trophic level organisms, is yet unclear.

Eriksson and Burton (2003) found that scats (faeces) collected from an Antarctic fur seal (*Arctocephalus tropicalis* and/or *A. gazella*) colony contained plastic particles, ranging from 2 to 5 mm (<0.5 mm were not included in the analysis). The authors suggest that, as the furseals are unlikely to have ingested plastic of this size directly, the observed microplastic presence could be explained by a ‘plastics concentrating stage’, whereby a species of fish (*Electrona subaspera*) consume plastic particles from the water column and are in turn predated upon by the fur seals.

The possibility that microplastics found in scat is a result of direct plastic consumption (either accidentally or through naivety) cannot be excluded. For example, twelve of 32 seal species have been documented to ingest marine debris (Kuhn *et al*., 2015; Ryan *et al*., 2016; Hoarau *et al*., 2014) inferred that small plastic pieces found within marine turtles resulted from fragmentation of larger plastic pieces within the gastrointestinal tract. This indicates that microplastics detected in GITs may have originally been directly ingested as macro-plastics.

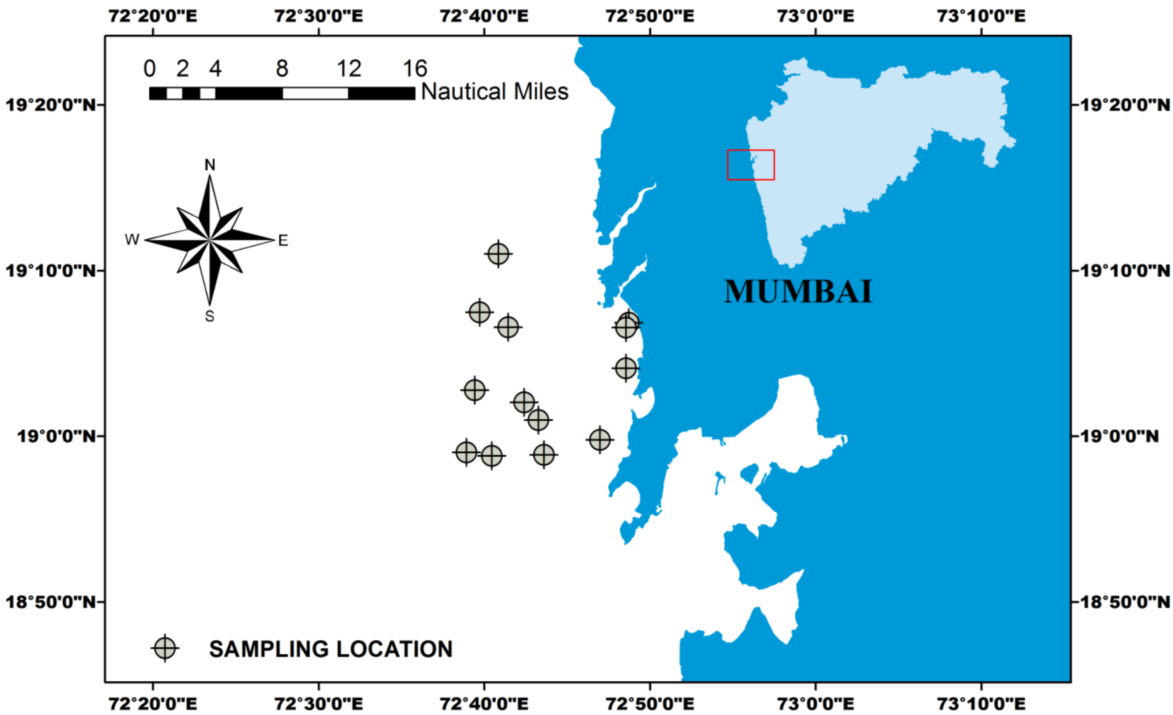
The differences in shape and density of microplastics particles favour it to diversely disperse in different compartments of the aquatic environment (surface and column water and sediment) and making it bioavailable to the aquatic organisms at different trophic levels (Betts, 2008; Thompson *et al*., 2009; Cole *et al*., 2011) Browne *et al*. (2007) reported that the uptake of microplastics in the marine food web depends on the size, shape, and density of the particles, as these features, determine their position in the water column and thus their availability to potential consumers.

Wright *et al*. (2013) have reported that plastics particles are ingested by a wide range of aquatic organisms and thus are subsequently transferred in the food chain. And the ingestion of these plastics particles can cause physical damage to the organism resulting in obstruction and internal abrasions. Nelms *et al*. (2018) have reported from their study on marine top predators that trophic transfer represents an indirect, yet potentially major pathway of microplastic ingestion for any species whose feeding ecology involves the consumption of whole prey, including humans.

3. MATERIALS AND METHODS

3.1. Study areas and sample collection

Fish sample collection was done on a monthly basis by Narmada M.F.V for a period of seven consecutive months starting from October 2018 to April 2019 along Mumbai coastal water. The fish selected for study purpose was *Johnius* spp., which is collected by a shrimp trawler every month. A total of 188 fish samples were collected for a period of seven months. The details of the sampling station for each month were being noted for the GPS location and water depth of fish sampling. Fish samples were later stored in a deep freezer at -20ºC for further processing and analysis.



Map 1: Map showing the sampling locations of the study area

Plate 1: Fish sample collection from MFV Narmada

3.2. Sample processing

In the laboratory, the specimens were thawed and the total length and body weight were recorded. Each fish was dissected and the gills, gut (gastrointestinal tract) and muscle retrieved. The retrieved tissues from each individual specimen were weighed in a weighing balance. For tissue digestion approx. 3 g of muscles are used and whole tissues were taken for gills and gut.

Tissues were subjected to digestion with 10 % KOH in a 60 mL borosilicate glass at 40ºC for a period of 48 to 72 hrs to remove all the organic matter and leaving behind aluminosilicates/silica and plastics (Karami *et al*., 2017). During the digestion period, the samples were occasionally agitated for proper digestion of tissues and any organic matters.

After 48-72 hrs of alkali digestion, the digestates were filtered through a pore size of 1.6 µm glass microfiber filter paper (GF/1F), 47 mm dia, Axiva glass fiber filters) under vacuum filtration. After this, the filtered tissues were transferred into a 50 ml laboratory bottle filled with 10-15 mL 4.4

M NaI solution. The bottles were then sonicated for 5 min at 50 Hz and subsequently agitation for 5 min at 200 rpm on an orbital shaker. The solution was then finally centrifuged at 500 ×g for 2 min and subsequently again filtered through GF/1F Axiva glass fiber filter. The filtered tissues were then subsequently kept in individual Petri dishes and dried at room temperature for further analysis.

Total length and body weight of each fish are recorded

↓

Fish tissues i.e., gills, guts, and muscles are removed from individual fishes

↓

Weight of gills, guts, and muscles are recorded and are now ready for digestion.

↓

Adding of 10 % KOH solution to the fish tissues

↓

Incubating the samples at 40º C for 48 – 72 Hrs

↓

Filtering the digestates through filter membrane by vacuum filtration

↓

Transferring the filter membrane to 10- 15 ml of 4.4 M NaI solution

↓

Sonication at 50 Hz for 5 min + agitation

↓

Centrifugation at 500 × g for 2 min

↓

The supernatant containing microplastics is filtered through a filter membrane

Chart 1: Flow chart of sample processing for isolation of microplastics

3.3. Visual observation of MPs

Visual observation of materials retained in the filter papers was done using an Olympus SZX16 Stereozoom microscope. The visual assessment of MPs was made according to the shape, colour, and size of the material, and the images were captured.

The shapes of MPs were categorized into five types as fragments, films, fibers, pellets, and beads.

The size category was made in five size classes: class 1 (< 100 µm), class 2 (100-250 µm), class 3 (250-500 µm), class 4 (500-1000 µm), class 6 (> 1000 µm).

Based on colours categorization a total of eight colours were found (Translucent, White, Black, Blue, Brown, Red, Green and Yellow).

3.4. Quality assurance and quality control (QA/QC)

To minimize microplastic contamination in the laboratory, nitrile gloves and cotton lab coats were worn during the entire processing of samples. All the chemicals and distilled waters were filtered with 1.6 µm glass fibre filters. All the materials to be used were washed with detergent and were rinsed thoroughly with filtered distilled water and were dried in a hot air oven. A method blank was carried to check any potential MPs contamination throughout the entire process of analysis (Nuelle *et al*., 2014).

3.5. Data analysis

The data from the obtained results were analysed by Microsoft excel office. One way ANOVA was performed to see any significant difference ($p < 0.05$) in the microplastics content in all the tissue samples obtained from different months. Pearson correlation coefficient was performed to see any correlation between the microplastics in the fish tissues and their total body length. The statistical package IBM SPSS statistics version 22 was used to perform the analysis of data obtained in the study.

4. RESULTS

4.1 Sampling details and Incidence of microplastics (MPs) in various body parts of *Johnius* spp.

The results in Table 1 shows that all the sampled fish (n=188) from the selected locations from Mumbai coastal water has some sort of microplastics particles in their tissues studied with 100 % incidence in all the months. The average microplastics particles with its standard deviation in all the months are shown in Table 2. The total numbers of microplastic particles in the GI tract, gills, and muscles of *Johnius* spp. (n=188) are 1245, 1170 and 516 respectively.

The mean number of microplastics particles per individual in GI tract in the month of October, November, December, January, February, March and April are 7.94±4.1, 8.21±1.89, 8.50±2.96, 6.10±2.44, 5.96±1.49, 6.58±2.43 and 7.67±1.68 respectively. The mean numbers of microplastics particles per individual in gills for the months starting from October 2018 to April 2019 are 8.52±3.13, 5.42±2.72, 6.73±1.80, 5.80±1.77, 4.76±1.71, 6.63±2.11 and 5.07±1.67 respectively. And the mean microplastics number per individual in 1 g of muscle for the study period are 1.02±0.74, 0.58±0.34, 0.57±0.31, 0.81±0.37, 0.98 ±0.54, 0.85±0.34 and 1.06±0.37 respectively.

The total average numbers of microplastics particles per individual in 188 *Johnius* spp. are 6.62±1.68, 6.21±1.67 and 0.81±0.37 in GI tract, gills and muscles respectively.

The one way ANOVA performed on the MPs found from the gut of fish from each month shows that microplastics content in the November month has significantly lower numbers in relation to all the other months. The microplastics from the month of January, February and March from the guts has no significant difference among them but have higher microplastics content than November month and lesser than the month of October, December and April. The highest microplastics were obtained in the month of October, December, and April and there are no significant differences in them (Table 2).

The one way ANOVA performed for the gills microplastics numbers from all the month shows that the microplastics numbers in the October month are higher than all the months. February and April's months have the least microplastics content in the gills with no significant difference with the month of November, January and March but lesser than the month of December. But there was no significant difference of microplastics number for the month of November, December, January and March (Table 2).

The one way ANOVA result from muscles samples from all the months shows that the microplastics content g^{-1} muscles in the months of October, November, December, January, February, and March do not have any significant difference among them. But these all the months have lesser microplastics number in the muscle in comparison to the month of April (Table 2).

Table 1: Sampling location and incidence of microplastics in *Johnius* spp. harvested from Mumbai waters using experimental trawling

Sampling date	Sampling location		Sample size	No. individual with plastics	Incidence of microplastics in different parts of fish		
	Longitude	Latitude			GI tract	Gills	Muscles
28-Oct-18	19°02'02.2"N	72°42'24.8"E	31	31	100	100	100
20-Nov-18	19°00'58.0"N 19°06'51.1"N 19°11'00.0"N	72°43'16.2"E 72°48'42.9"E 72°40'51.0"E	38	38	100	100	100
13-Dec-18	19°02'46.7"N	72°39'26.0"E	30	30	100	100	100
10-Jan-19	18°59'00.7"N 19°06'32.34"N	72°38'54.9"E 72°48'33.31"E	30	30	100	100	100
19-Feb-19	18°58'48.3"N	72°40'27.4"E	25	25	100	100	100
23-Mar-19	19°06'34.3"N 19°07'28.7"N	72°41'26.6"E 72°39'42.6"E	19	19	100	100	100
12-Apr-19	18°59'46.50"N 18°58'51.48"N	72°46'59.01"E 72°43'35.77"E	15	15	100	100	100
Total			188	188	100	100	100

Table 2: Length-weight characteristics and incidence of microplastics in different body parts of *Johnius* spp.

Month	Total length (mm)	Body weight (g)	Microplastics in different parts of fish				
			MPs (No/ fish)	Gut (g)	MPs (No/ fish)	Gill (g)	Meat (g)
Oct	113.45±34.91	22.38±21.65	7.94^{c}±4.11	50.95±52.34	8.52^{c}±3.13	43.38±38.30	1.02^{a}±0.74
Nov	136.95±14.68	30.35±9.85	8.21^{a}±1.89	5.52±3.29	5.42^{ab}±2.72	10.73±7.50	0.58^{a}±0.34
Dec	114.43±13.77	17.08± 6.26	8.50^{c}±2.96	17.82±7.23	6.73^{b}±1.80	23.21±10.16	0.57^{a}±0.31
Jan	116.73±12.22	18.39±6.66	6.10^{b}±2.44	15.13±8.30	5.80^{ab}±1.77	20.63±6.43	0.81^{a}±0.37
Feb	128.72 ±19.62	24.62±16.41	5.96^{b}±1.49	29.97 ±18.94	4.76^{a} ±1.71	16.40± 7.33	0.98^{a} ±0.54
Mar	170.11±231.94	17.10±4.97	6.58^{b}±2.43	16.17±7.84	6.63^{ab}±2.11	26.82±9.50	0.85^{a}±0.34
Apr	121.47±10.47	20.21±5.30	7.67^{c}±1.68	16.89±7.47	5.07^{a}±1.67	17.04±8.51	1.06^{b}±0.37
Mean value	127.28±10.47	22.10±5.30	6.62±1.68	21.74±7.47	6.21±1.67	22.57±8.51	0.81±0.37

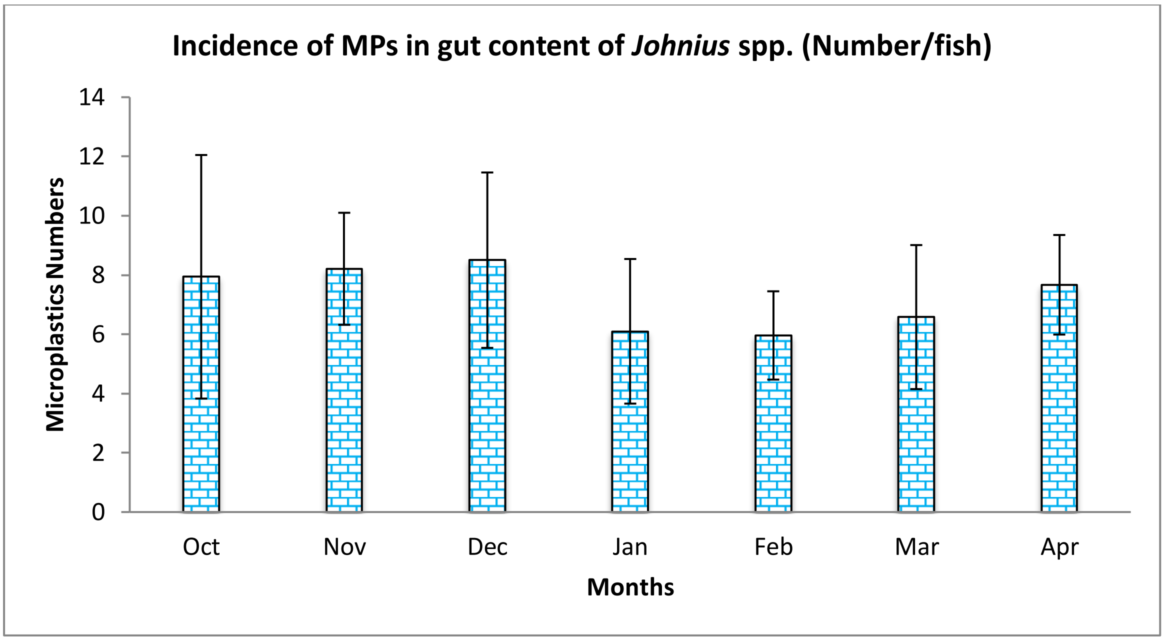


Figure 1: Temporal variations of microplastics in the gut content of *Johnius* spp. harvested from Mumbai waters

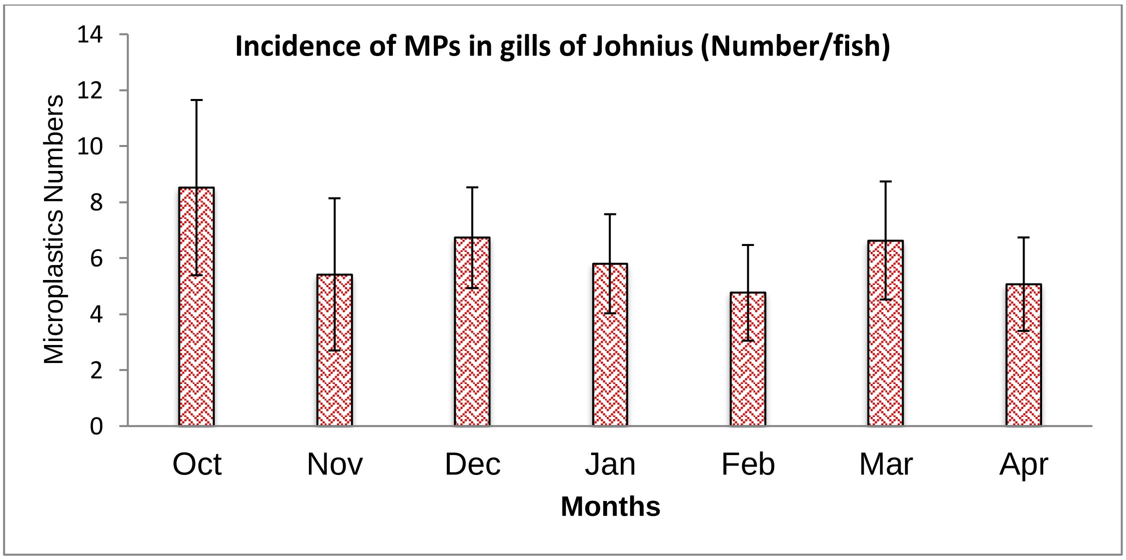


Figure 2: Temporal variations of microplastics in gills parts of *Johnius* spp, harvested from Mumbai water

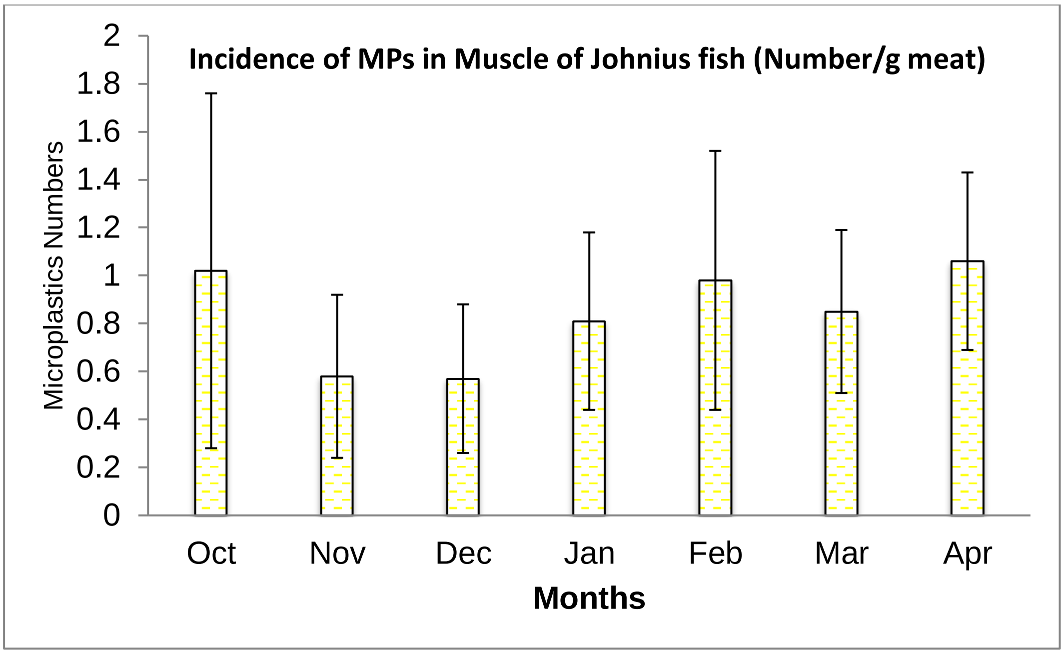


Figure 3: Temporal variations of microplastics in the muscle parts of *Johnius* spp. harvested from Mumbai water

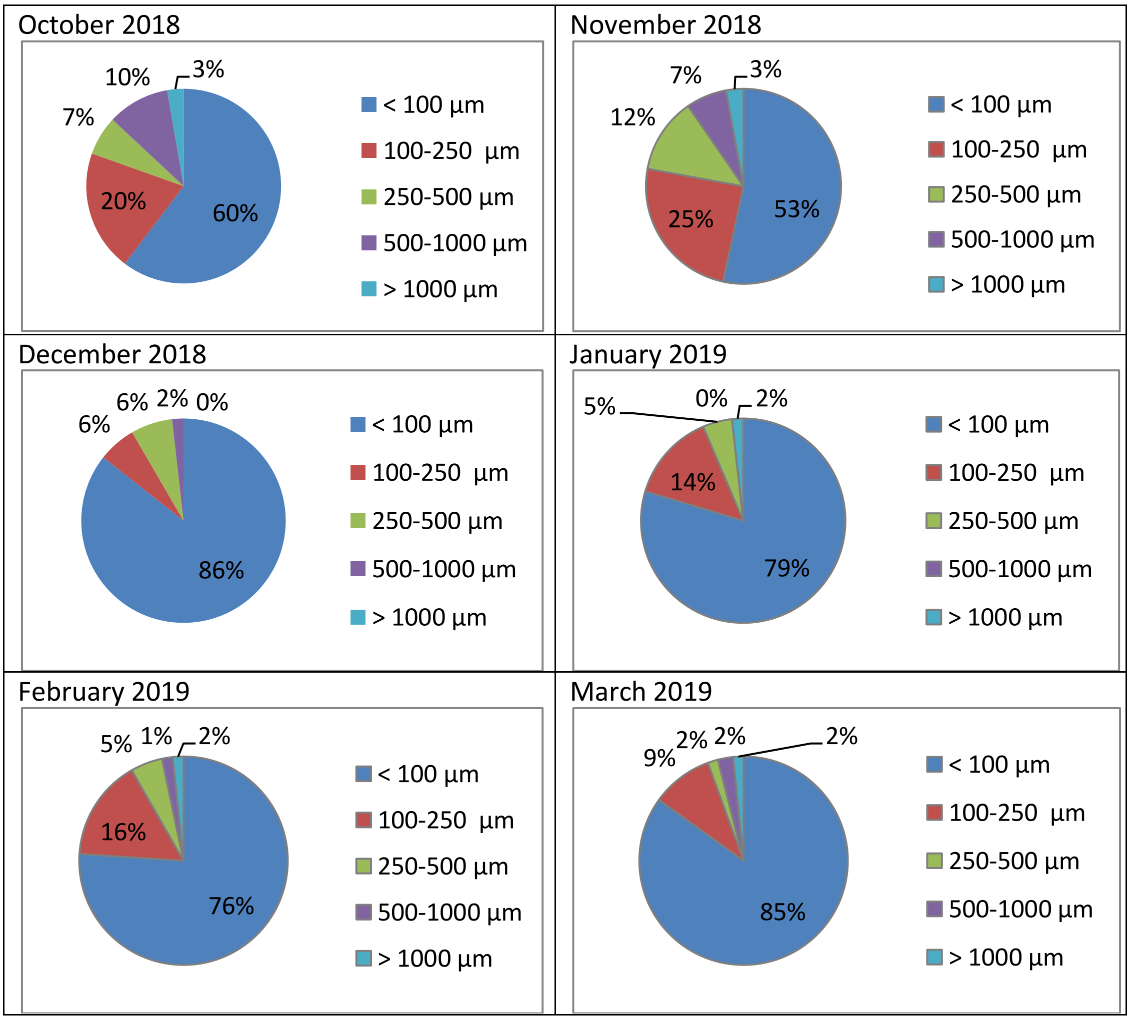
4.2. Microplastics (MPs) size distribution in different body parts of *Johnius* spp. harvested from Mumbai waters through experimental trawling

The microplastics particles size distribution are classified into five size classes starting from < 100 µm, 100-250 µm, 250-500 µm, 500-1000 µm to > 1000 µm (Abbasi *et al*., 2018).

4.2.1 Microplastics (MPs) size distribution in gill parts of *Johnius* spp.

The microplastics size in the gills are most abundantly distributed in the size class of < 100 µm where the percentage composition in the month of October, November, December, January, February, March and April are 60%, 53%, 86%, 80%, 76%, 85% and 91% respectively (Figure 4).

As shown in Fig. 4, the preceding sizes in the gills on all the months are followed by 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm respectively. The highest average frequency of microplastic particles in the gills is represented by size group of < 100 µm in all the months followed by 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm (Fig. 5). The monthly microplastics size distribution in the gills is shown in Fig. 6 and Fig. 7. It shows that the size class < 100 µm is more predominant followed by the subsequent size classes as 100-250 µm, 250-500 µm, 500=1000 µm, and > 1000 µm respectively. The pie chart of the pooled percentage of microplastics size distributed in the gills from all the months is predominated by < 100 µm and followed by 100-250 µm, 250-500 µm, 500-1000 µm and >1000 µm with 73 %, 15 %, 6%, 4 % and 2 % respectively (Fig. 7).



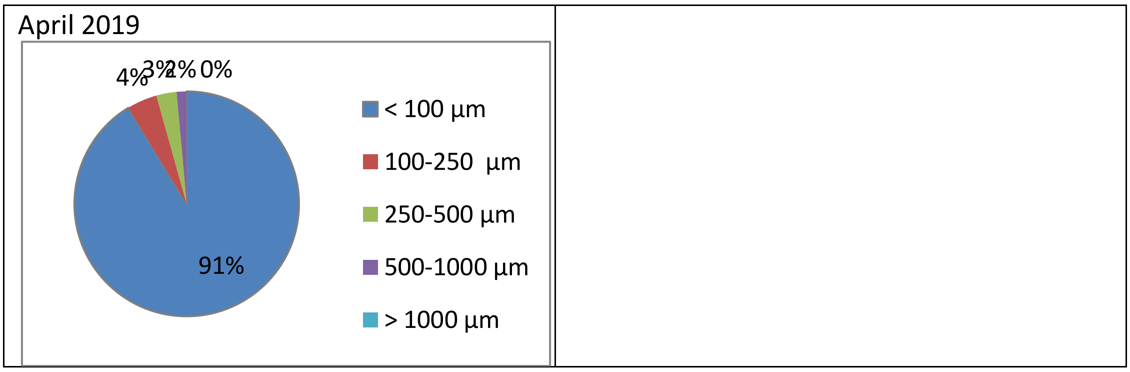
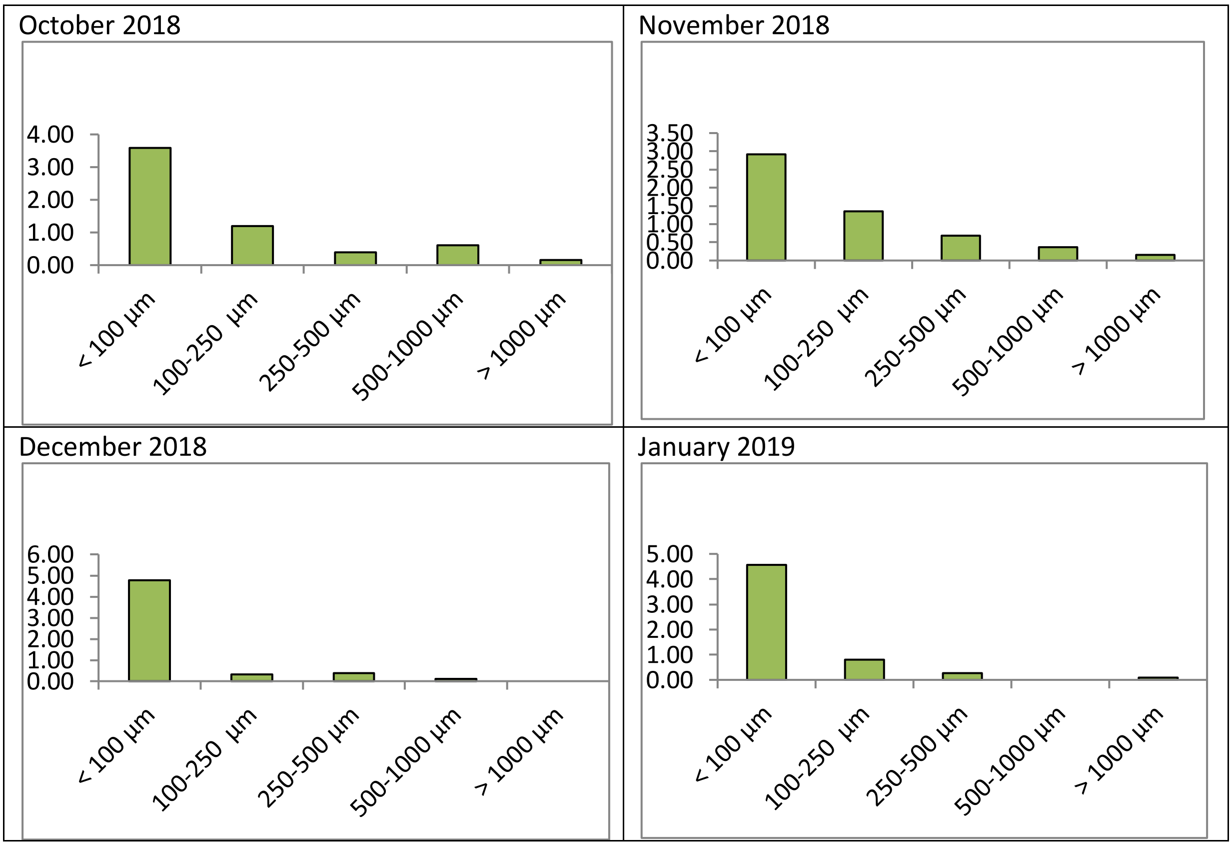


Figure 4: Percentage-wise size distribution of microplastics extracted from the gills of *Johnius* spp. during experimental periods



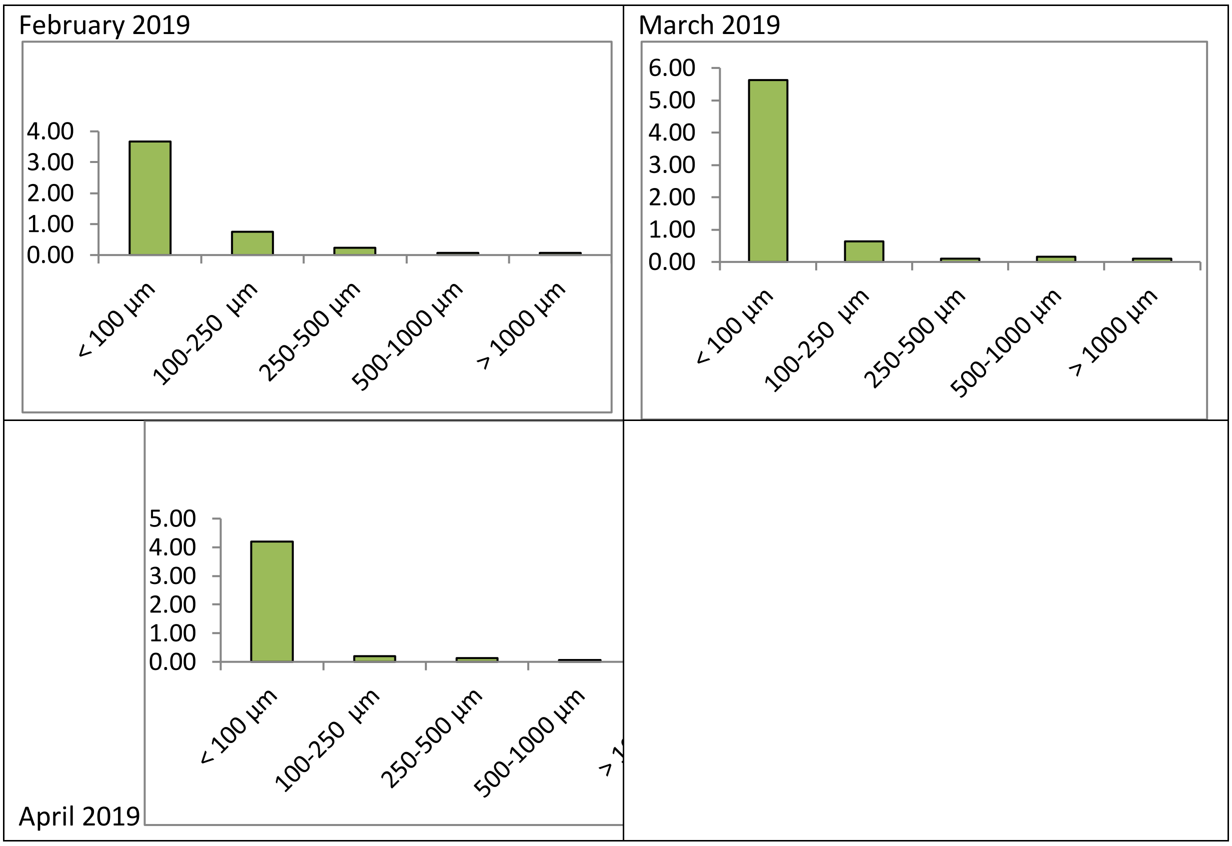


Figure 5: Average frequency of microplastics in the gills based on the size distribution

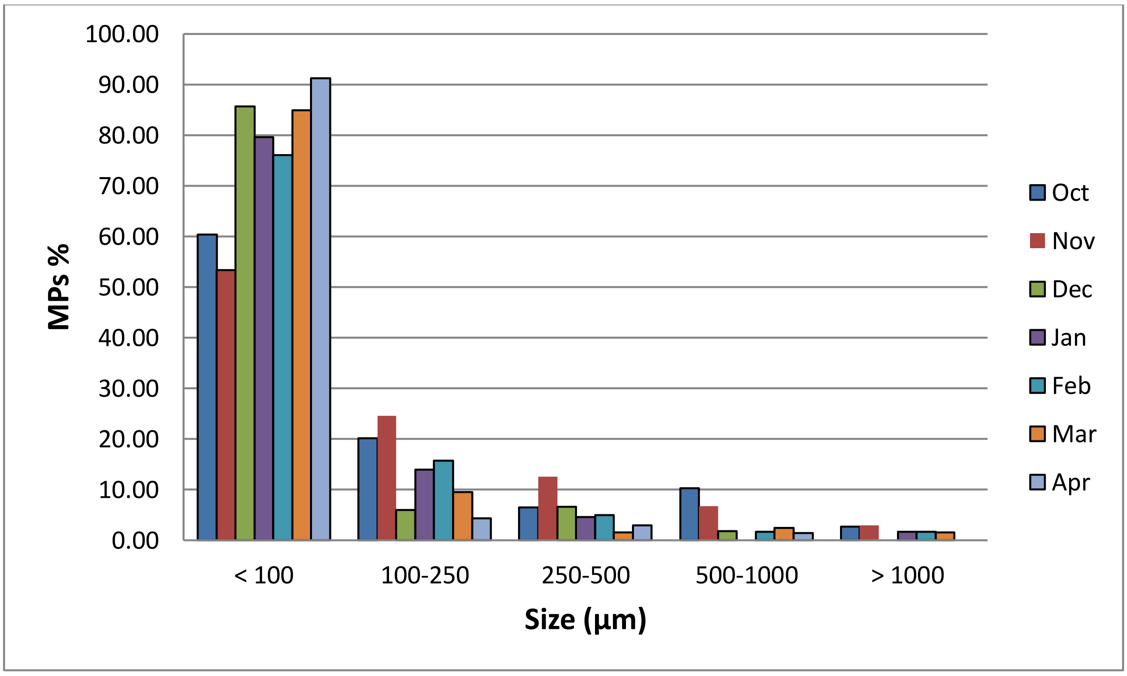


Figure 6: Temporal variations in MPs size distribution in the gills (%)

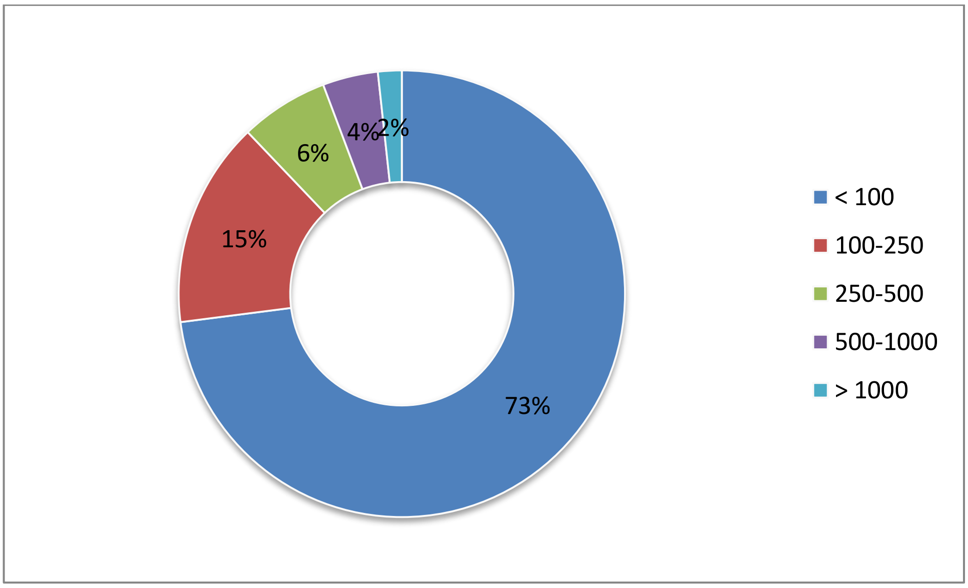


Figure 7: Percentage size distribution of MPs in the gills pooled from all the months

4.2.2 Microplastics (MPs) size distribution in the GI tract of *Johnius* spp.

In the GI tract as shown in Fig. 8 the most abundant size group of microplastics particles are < 100 µm represented with 62%, 47%, 75%, 77%, 69%, 77% and 72%, for the months of October, November, December, January, February, March and April respectively.

And the next abundant sizes in the GI tract are represented by 100-250 µm, 250-500 µm, followed by 500-1000 µm and > 1000 µm for all the months (Fig. 8). The average highest frequency of microplastic particles in the GI tract are also represented by size class of < 100 µm, followed by 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm respectively in all the months (Fig. 9). The monthly microplastics size distribution in the GI tract is shown in Fig. 10. The pie chart of pooled percentage of microplastics size distributed in the GI tract from all the months is predominated by < 100 µm and followed by 100-250 µm, 250-500 µm, 500-1000 µm and >1000 µm with 68 %, 15 %, 9%, 5 % and 3 % respectively (Fig. 11).

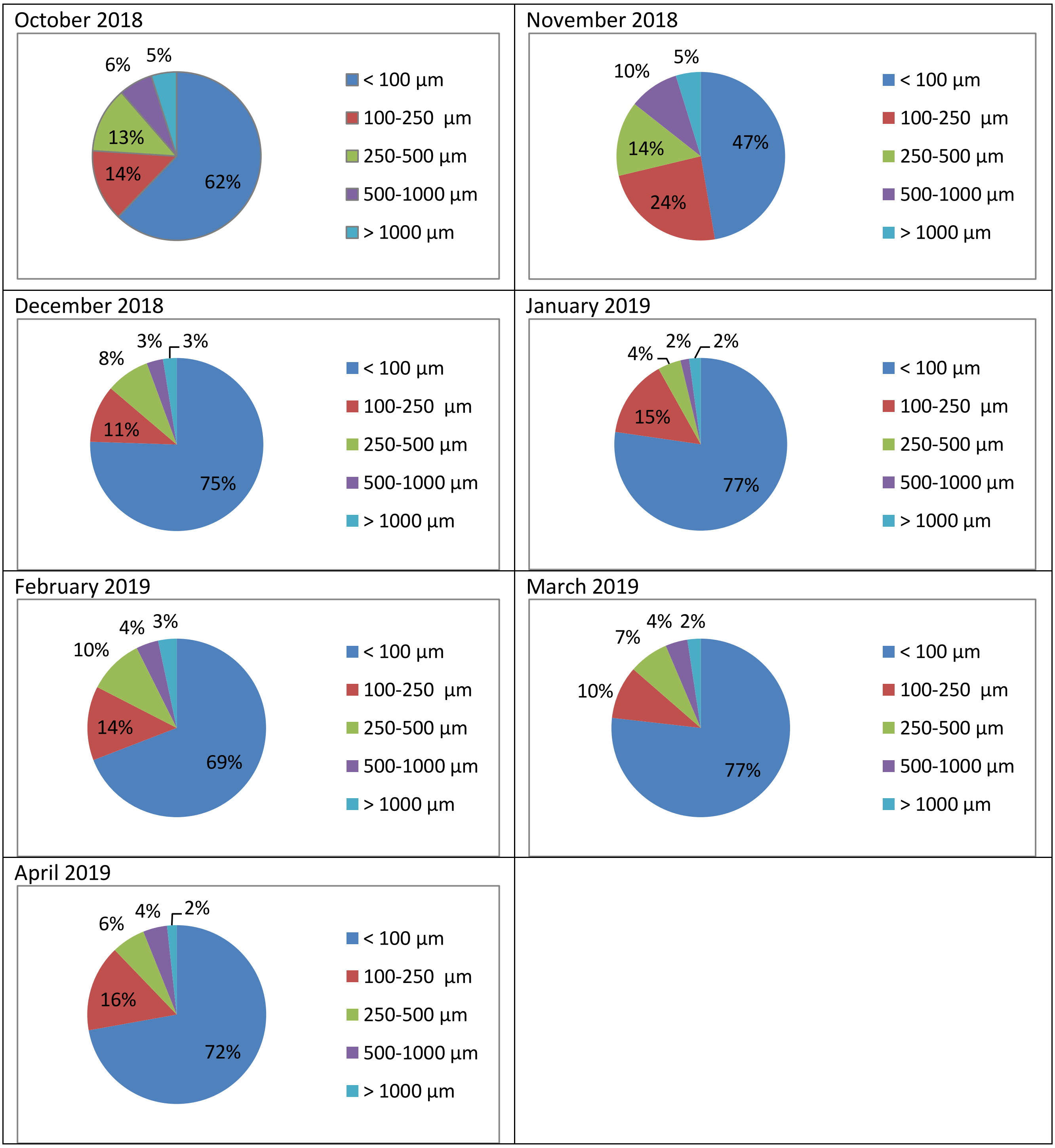


Figure 8: Percentage-wise size distribution of microplastics extracted from the GI tract of *Johnius* spp.

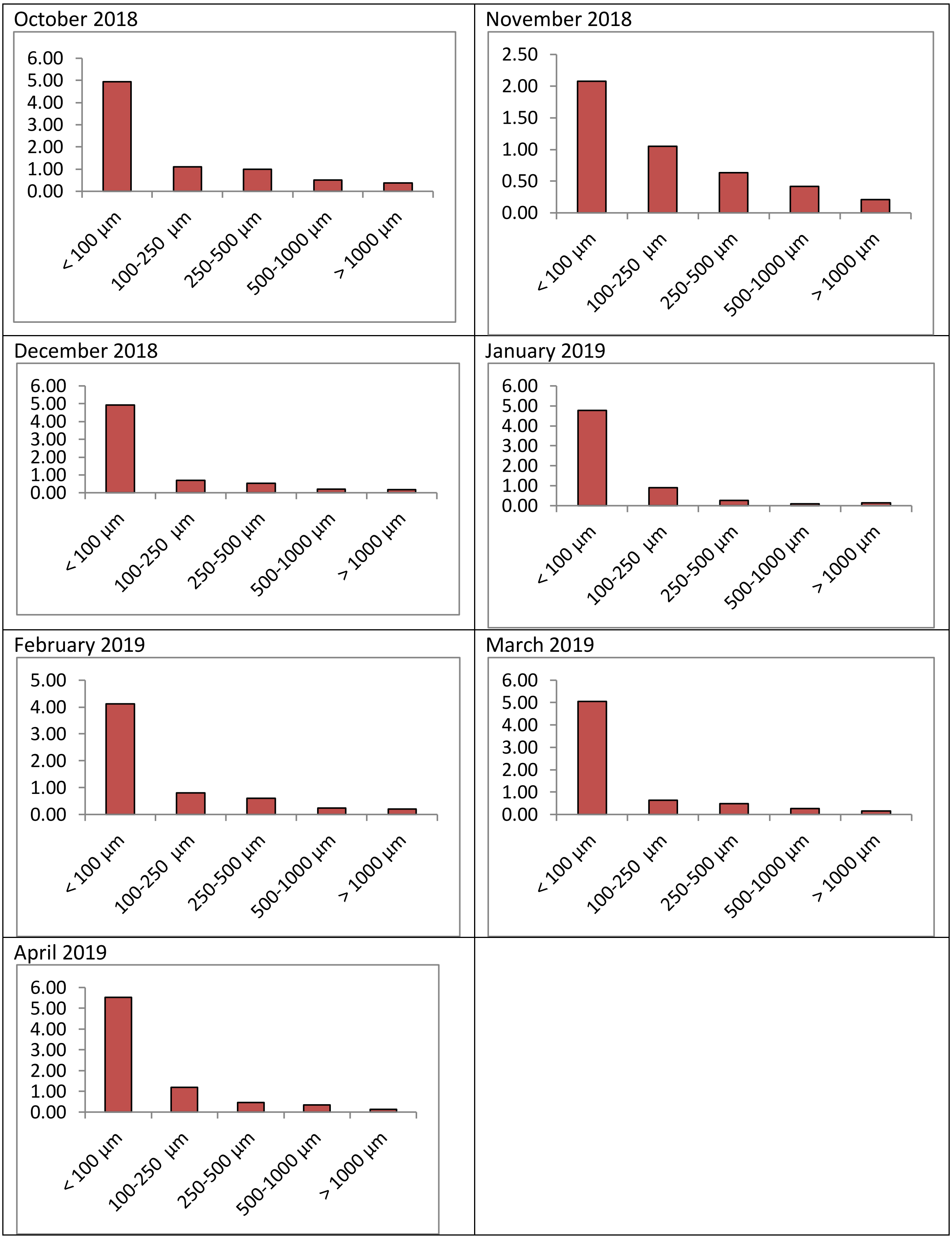


Figure 9: Average frequency of microplastics in the GI tract based on the size distribution

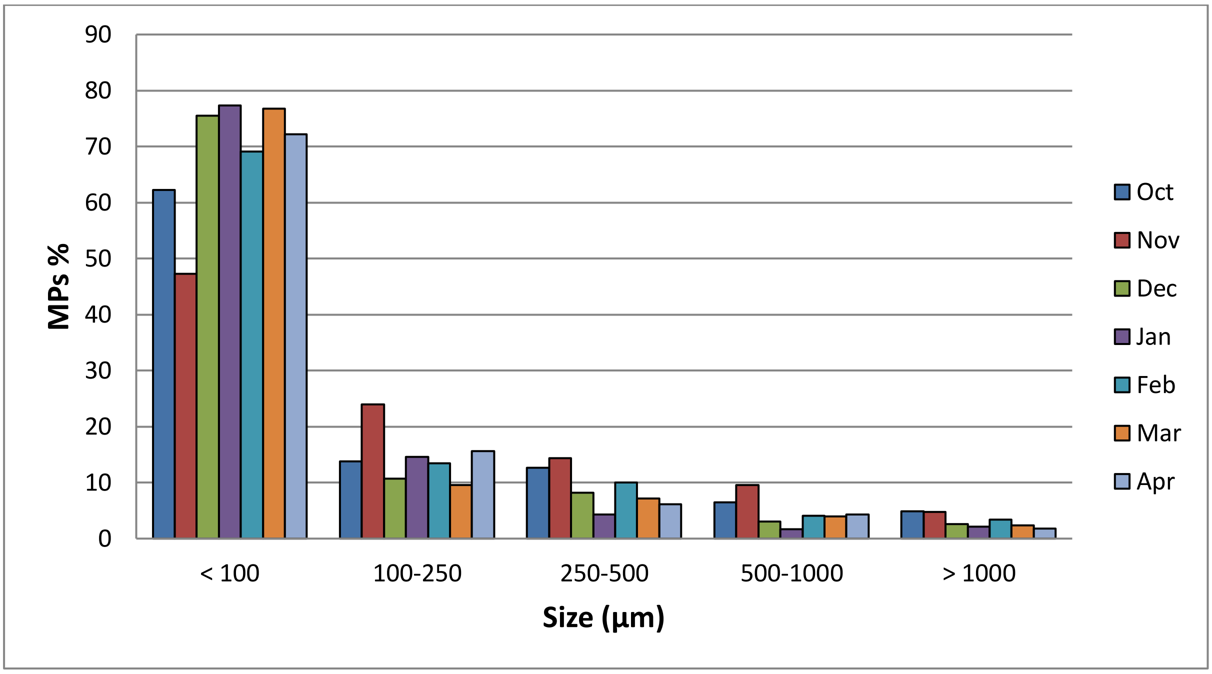


Figure 10: Temporal variations in MPs size distribution in the GI tract (%)

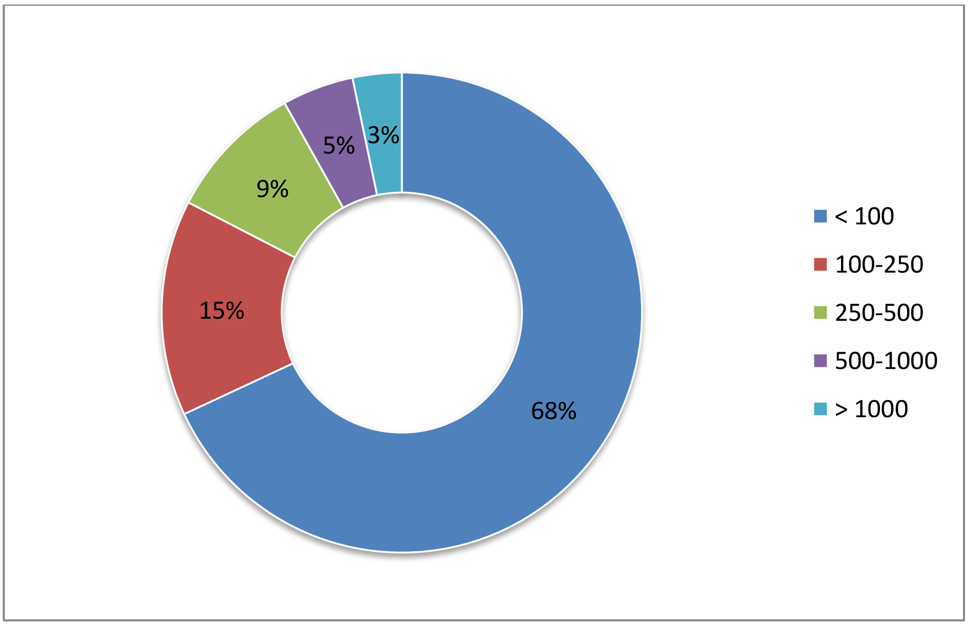
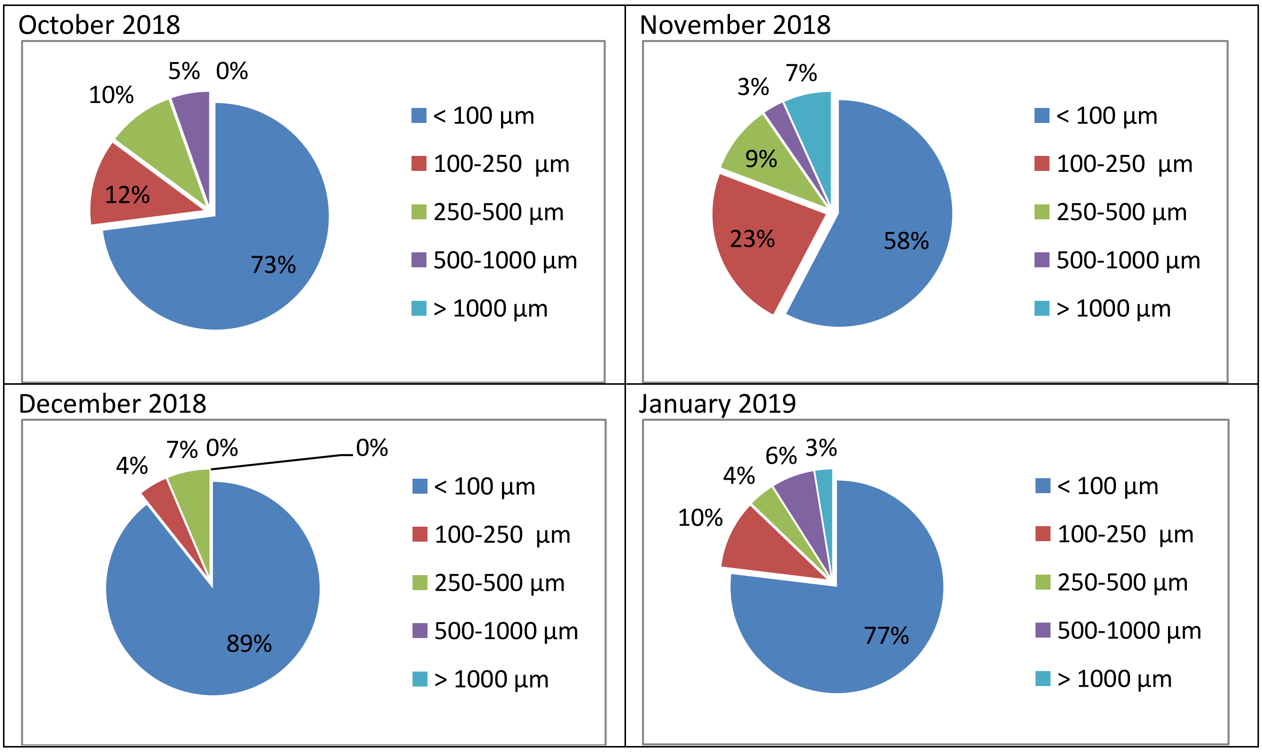


Figure 11: Percentage size distribution of MPs in the GI tract pooled from all the months

4.2.3 Microplastics (MPs) size distribution in edible muscle portion of *Johnius* spp. harvested from Mumbai waters

The most abundant size class of microplastics distributed in the muscle is < 100 µm in all the months with percentage composition of 73%, 58%, 89%, 77%, 86%, 89% and 88% in October, November, December, January, February, March and April respectively (Fig. 12). And these are followed in abundance by the size class of 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm (Fig. 12).

And the average highest frequency of microplastic particles in the muscles is also represented by the size group of < 100 µm, followed by 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm respectively in all the months as shown in Fig. 13. The monthly microplastics size distribution in the muscles is shown in a bar diagram shown in Fig. 14. In all the above-given figures the size distribution is abundant in the size class of < 100 µm followed by the subsequent size classes. The % pooled microplastics size class distribution from all the months are 77 %, 12 %, 6 %, 3 % and 2 % for < 100 µm, 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm respectively (Fig. 15).



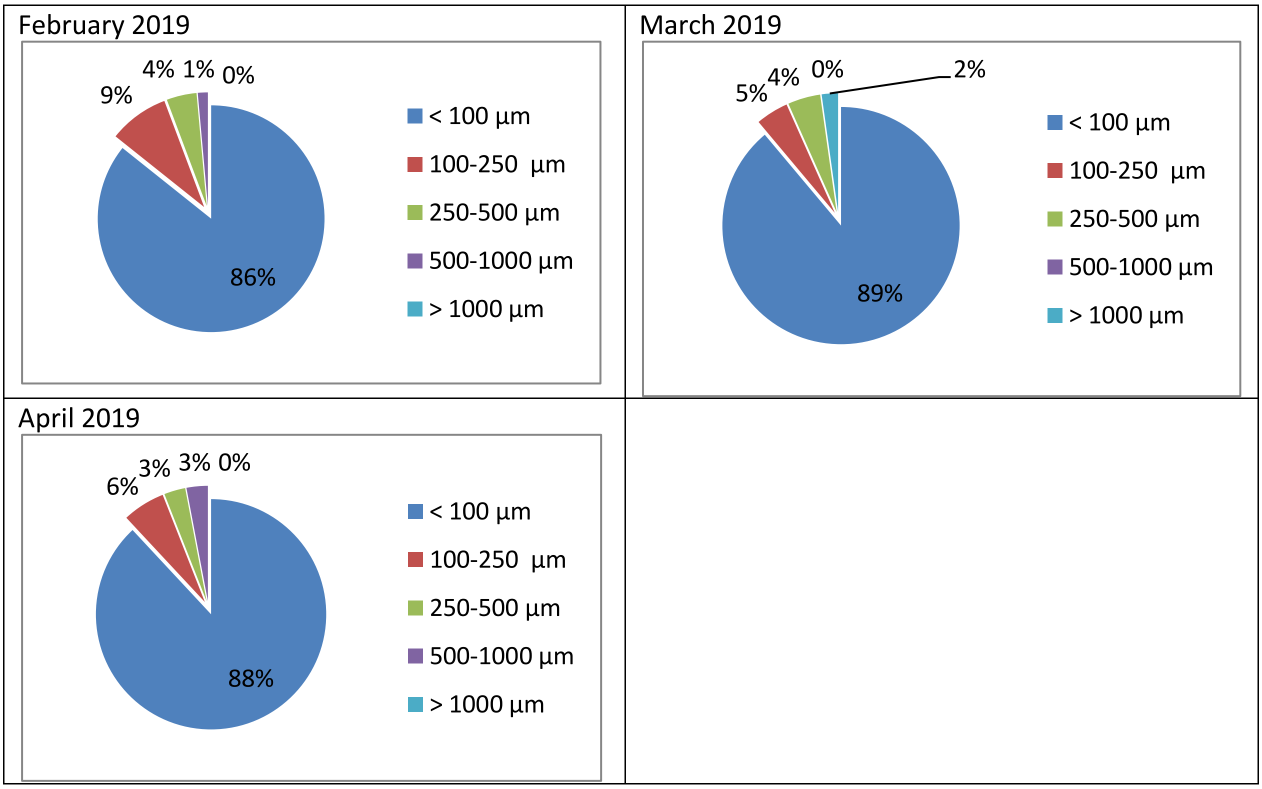
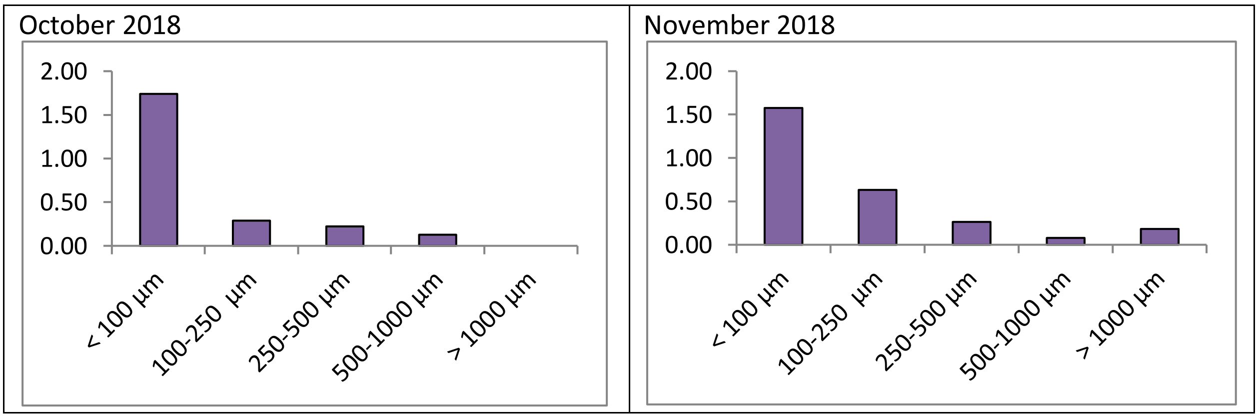


Figure 12: Percentage-wise size distribution of microplastics extracted from the muscle of *Johnius* spp.



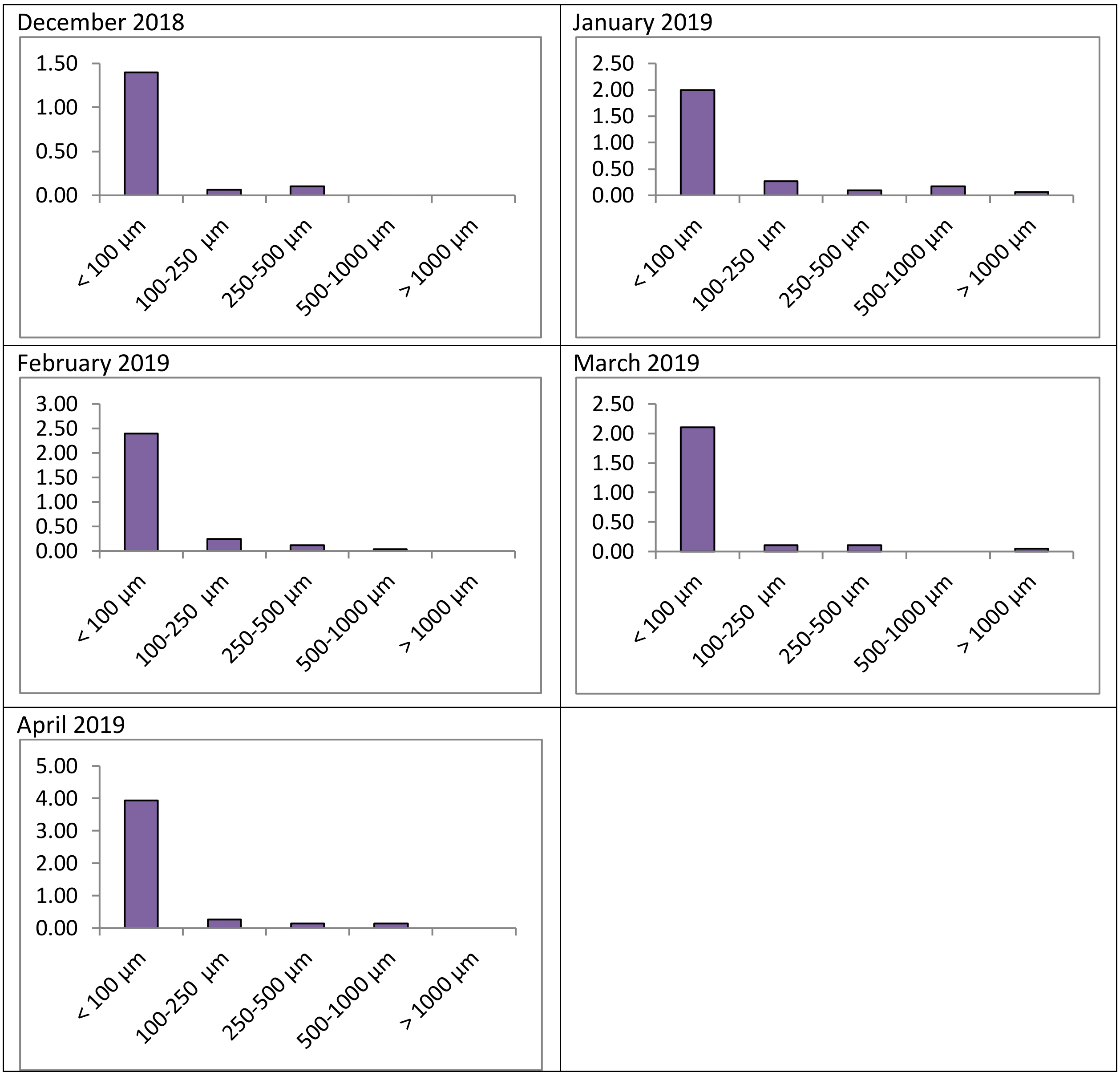


Figure 13: Average frequency of microplastics in the muscle based on size distribution.

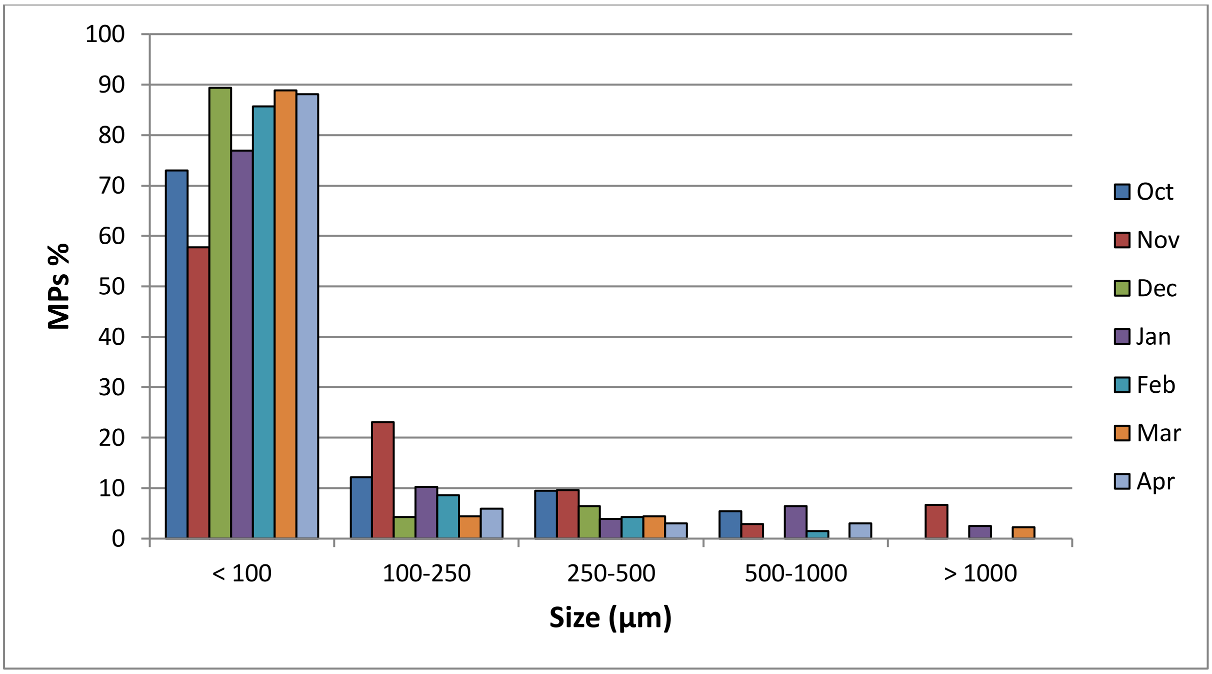


Figure 14: Temporal variation in MPs size distribution in the muscle (%)

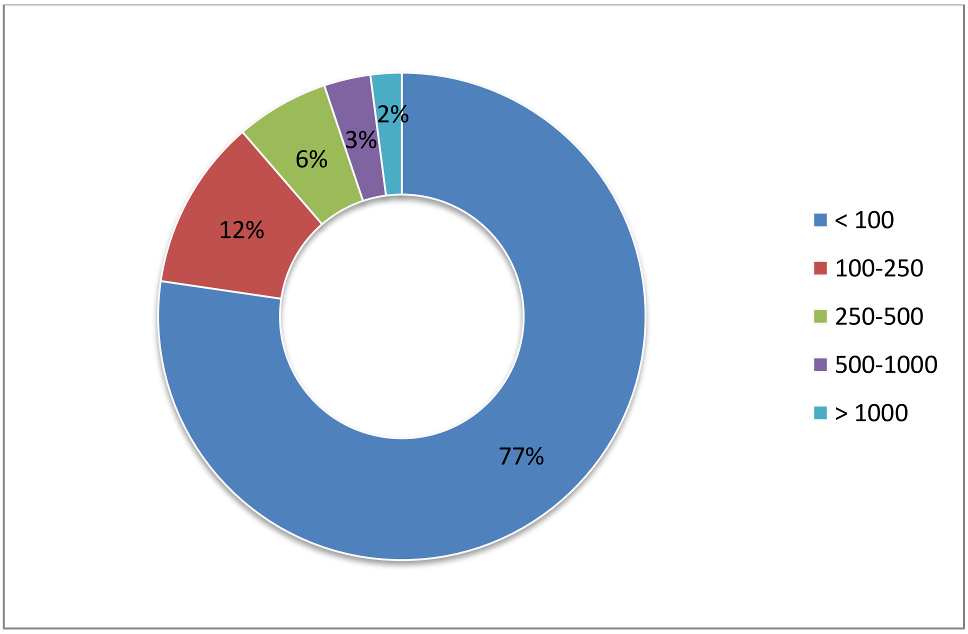


Figure 15: Percentage size distribution of MPs in the muscle pooled from all the months

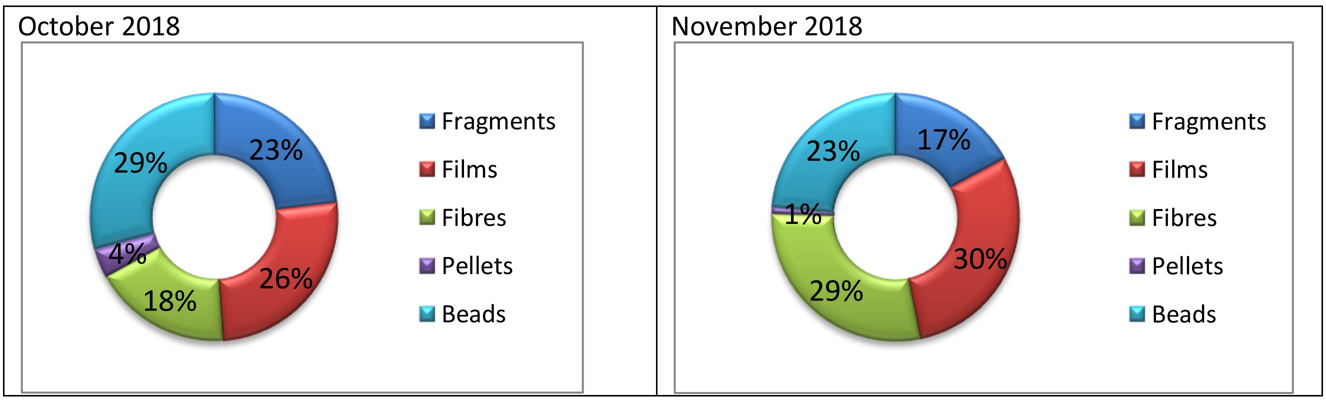
4.3 Shapes of microplastics (MPs) extracted from different body parts of *Johnius* spp. harvested from Mumbai waters based on experimental trawling

The microplastics shapes observed in the study falls in five shape categories, and they are fragments, films, fibres, pellets, and beads.

4.3.1 Shapes of microplastics (MPs) extracted from the gill parts

The proportion of microplastics shapes in the gills are largely contributed by beads except for the month of November 2018 shown in Fig. 16. The percentage contribution of beads in the gills is 29%, 23%, 36%, 44%, 58%, 69% and 72% for the months of October, November, December, January, February, March, and April respectively (Fig. 16).

The percentage proportion of fragments, films, fibres, and pellets are shown in Fig. 16 with varying degree of abundance in all the months with the exception of pellets which is the least shapes of microplastics in all the months. The bar diagram of shapes distribution in the gills is given in Fig. 17. The % pooled microplastics shapes distribution from all the months are 41 %, 20 %, 19 %, 18 % and 2 % for beads, fibres, fragments, films, and pellets respectively (Fig. 18). The stereomicroscopic image of microplastics is shown in Plate 2.



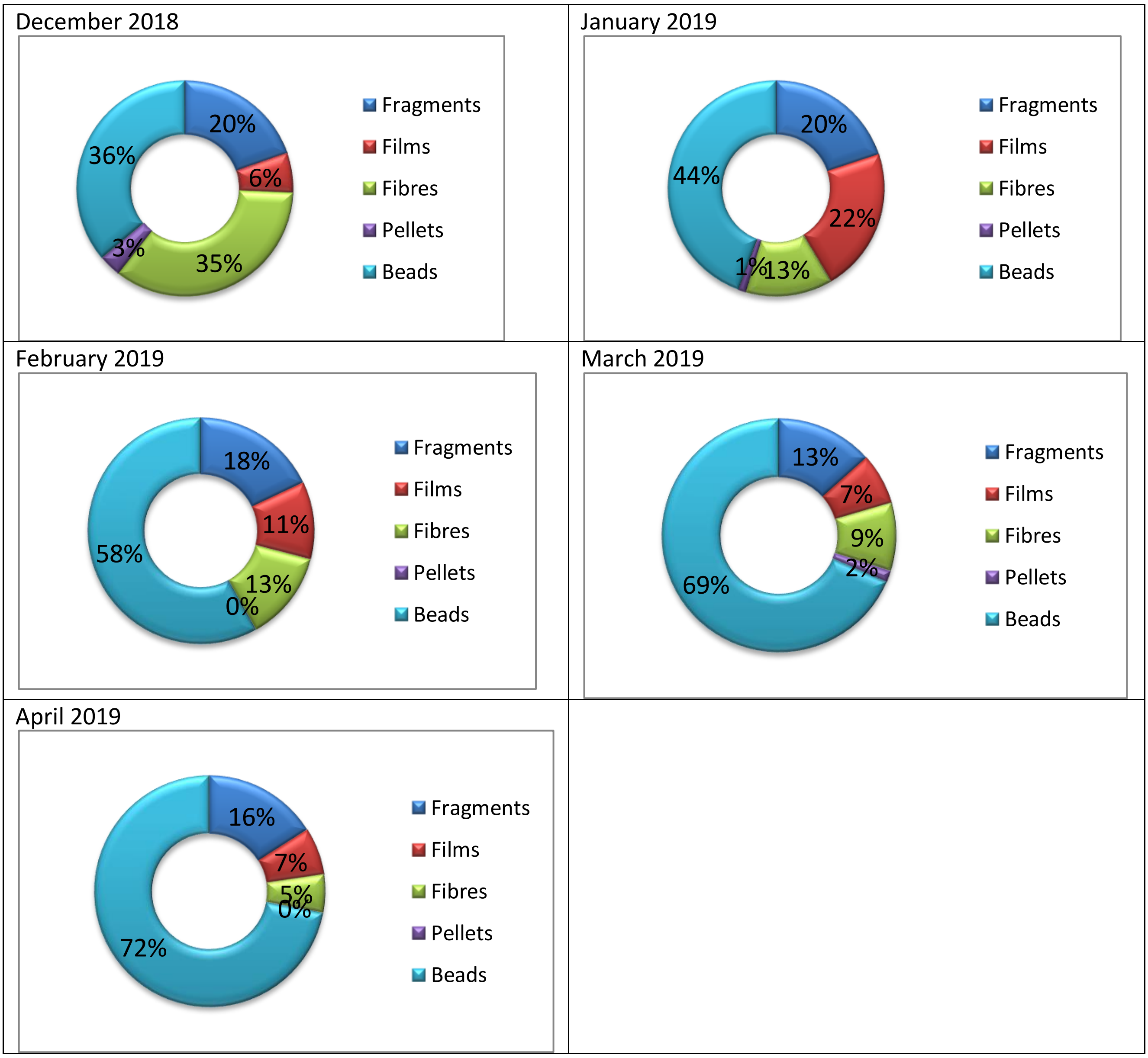


Figure 16: Percentage-wise distribution of shape of microplastics extracted from the gills of *Johnius* spp.

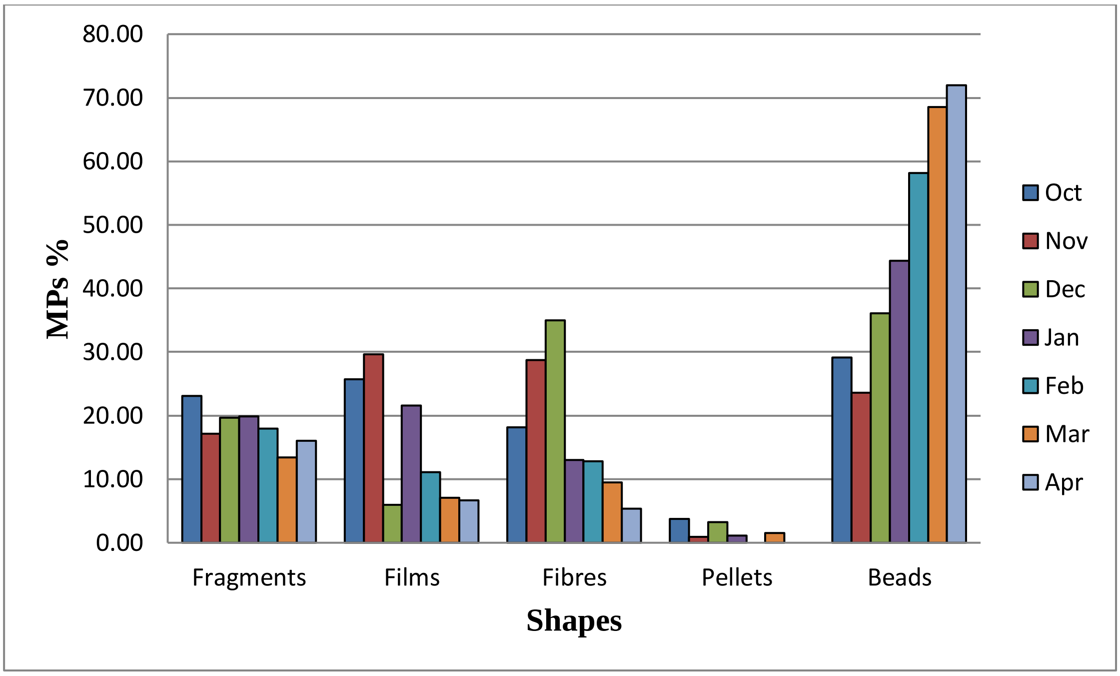


Figure 17: Temporal variations in MPs shapes distribution in the gills (%)

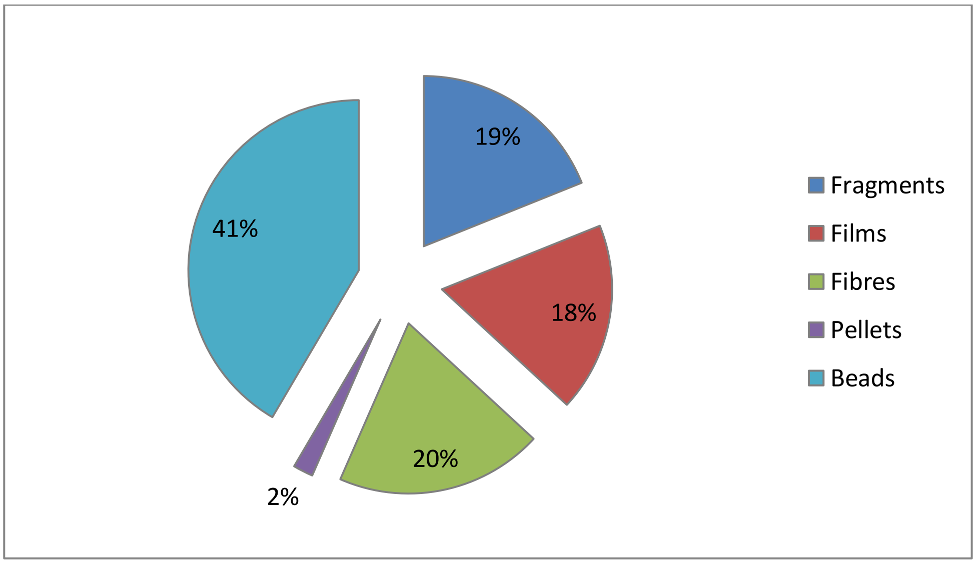


Figure 18: Percentage distribution of MPs shapes in the gills pooled from all the months

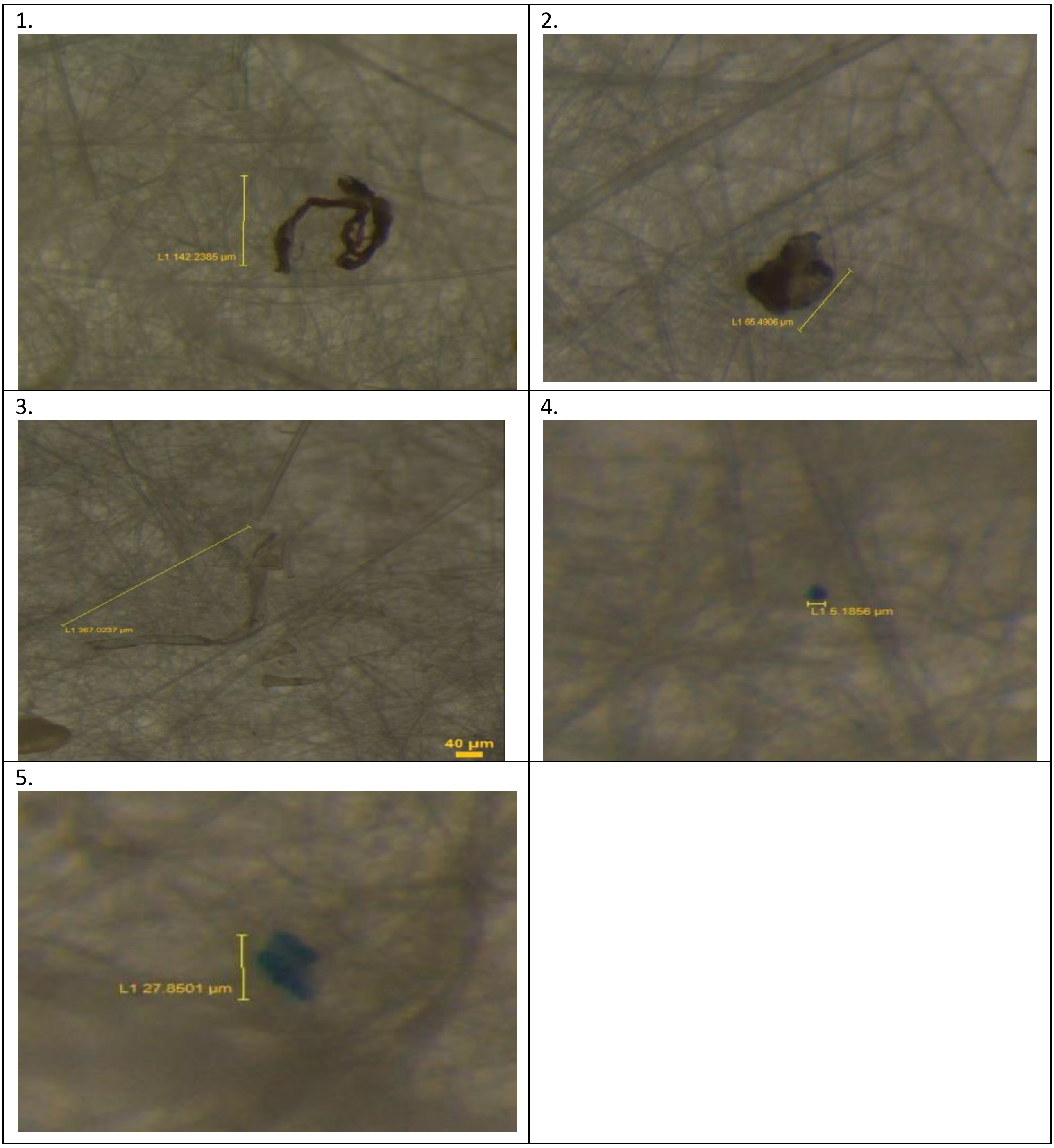
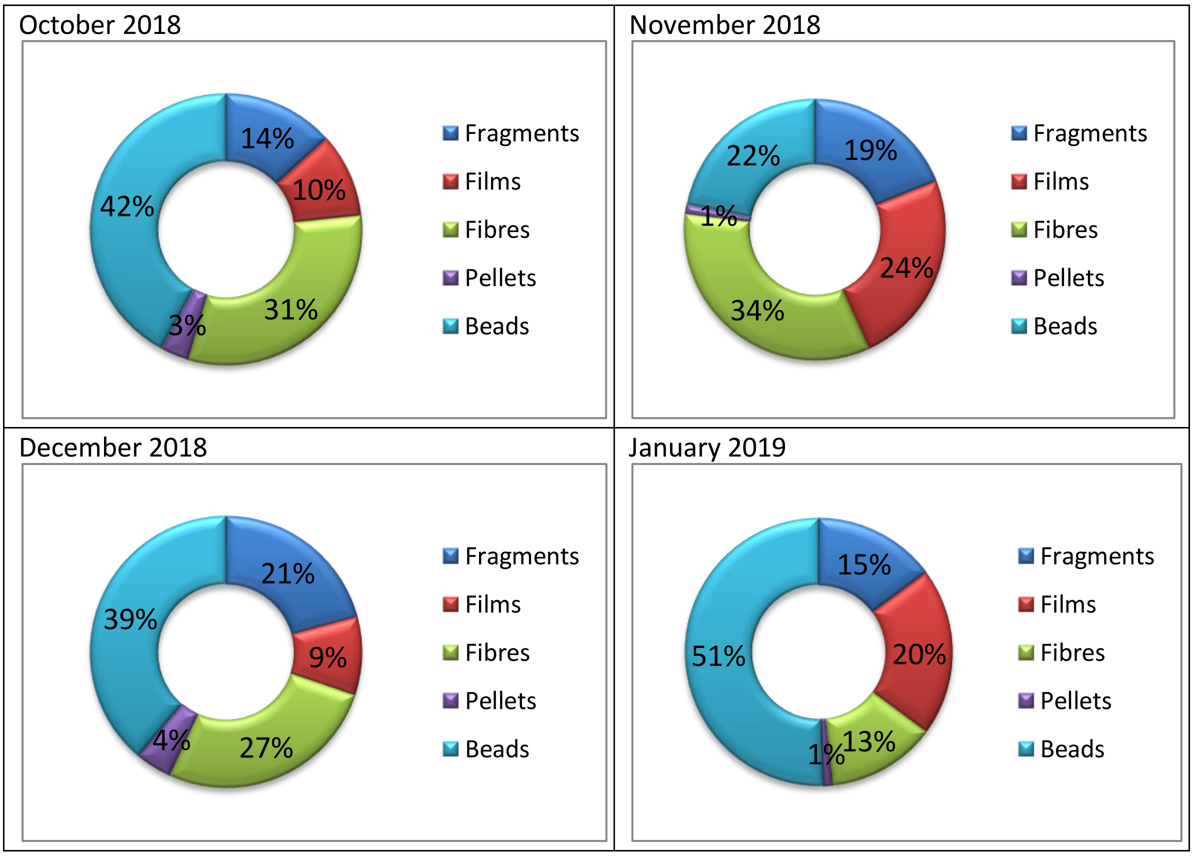


Plate 2: Microplastics shapes observation in the gills (1.Fibre, 2.Fragment, 3.Film, 4.Bead, and 5. Pellet)

4.3.2 Shapes of microplastic (MPs) extracted from the GI tract of *Johnius* spp.

The percentage shapes of microplastics which is abundant in the GI tract was beads for all the months except for the month of November, where fibre was found to be more abundant (Fig. 19).

The second most abundant shape of microplastics from all the samples in all the months being the fibres, followed by fragments and films forming an equal proportion and least being pellets in all the months (Fig. 19). The bar diagram shows that the Beads and fibres are more in number in all the months which are followed by the other shapes group (Fig. 20). The percentage pooled microplastics shapes from all the months are 44 %, 24 %, 15 %, 15 % and 2 % for beads, fibres, fragments, films and pellets respectively (Fig. 21). The images of microplastics from the GI tracts are shown in Plate 3.



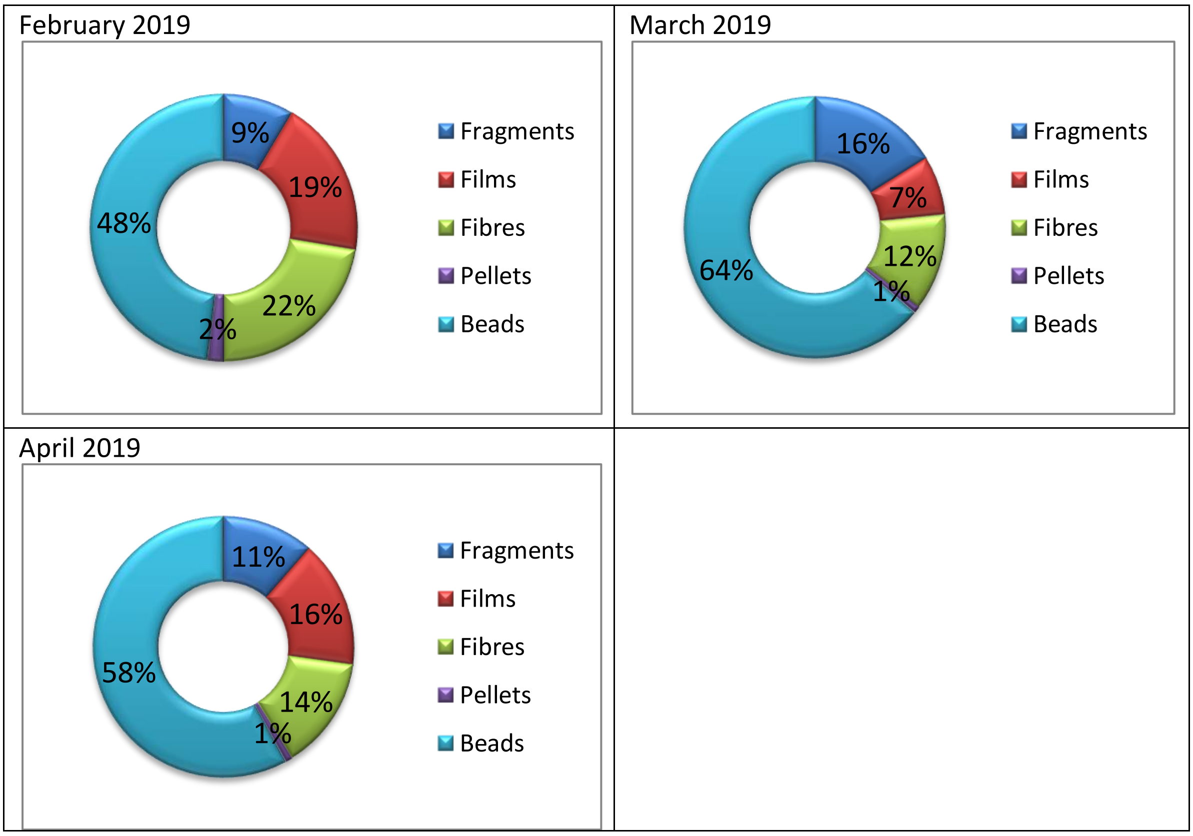


Figure 19: Percentage-wise shapes the distribution of microplastics extracted from the GI tract of *Johnius* spp.

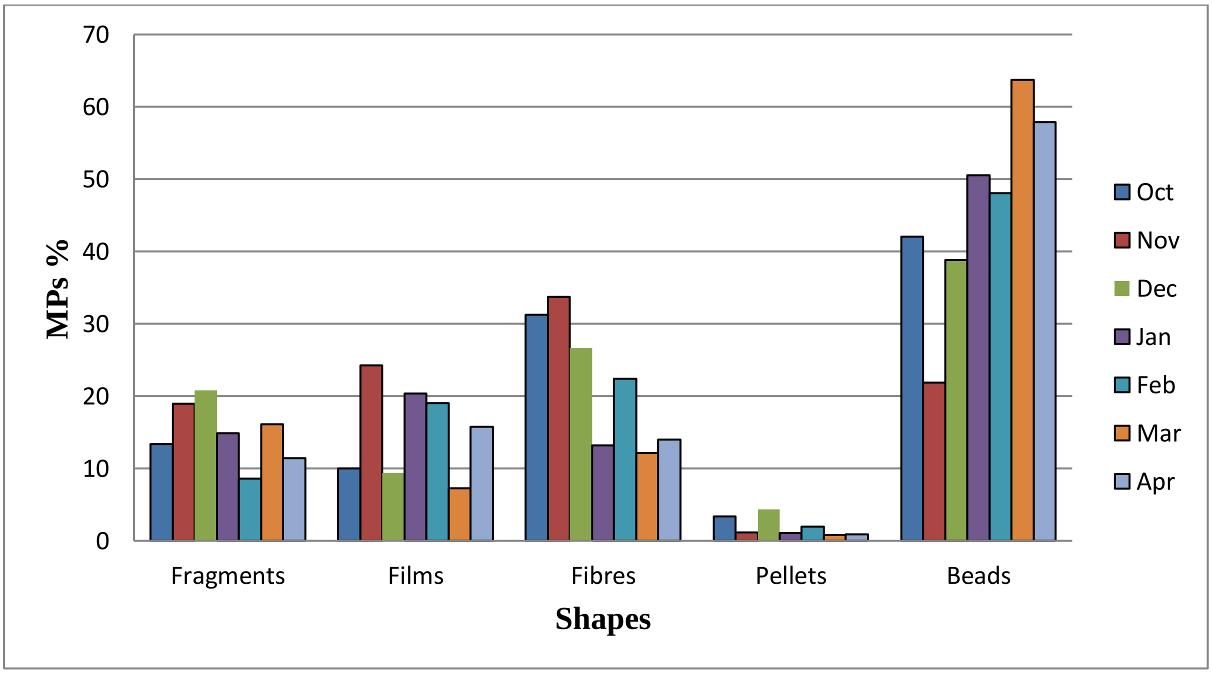


Figure 20: Temporal variations in MPs shapes distribution in the GI tract (%)

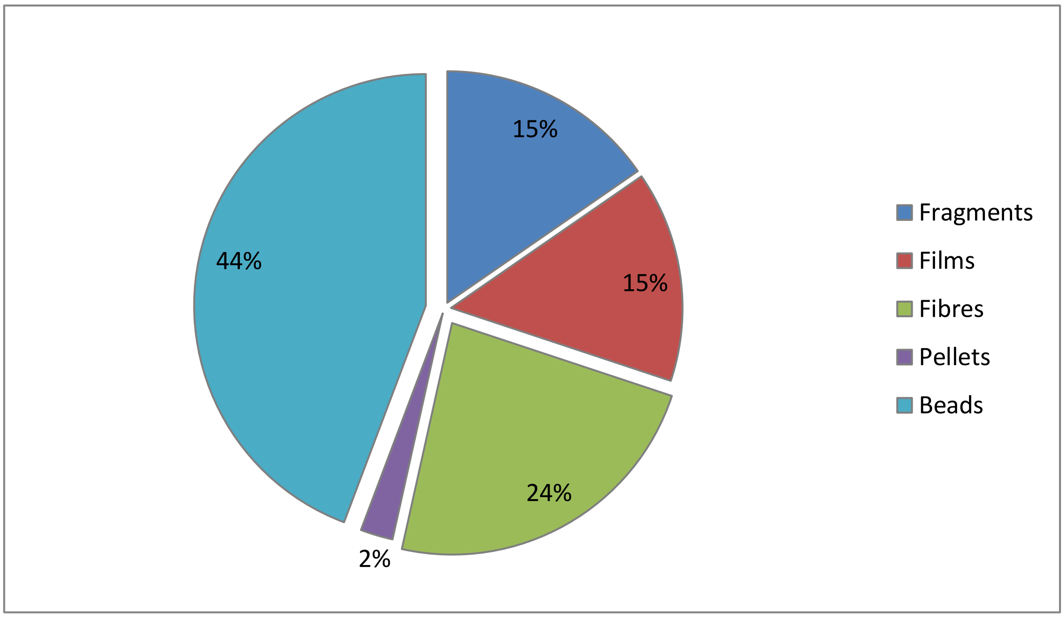
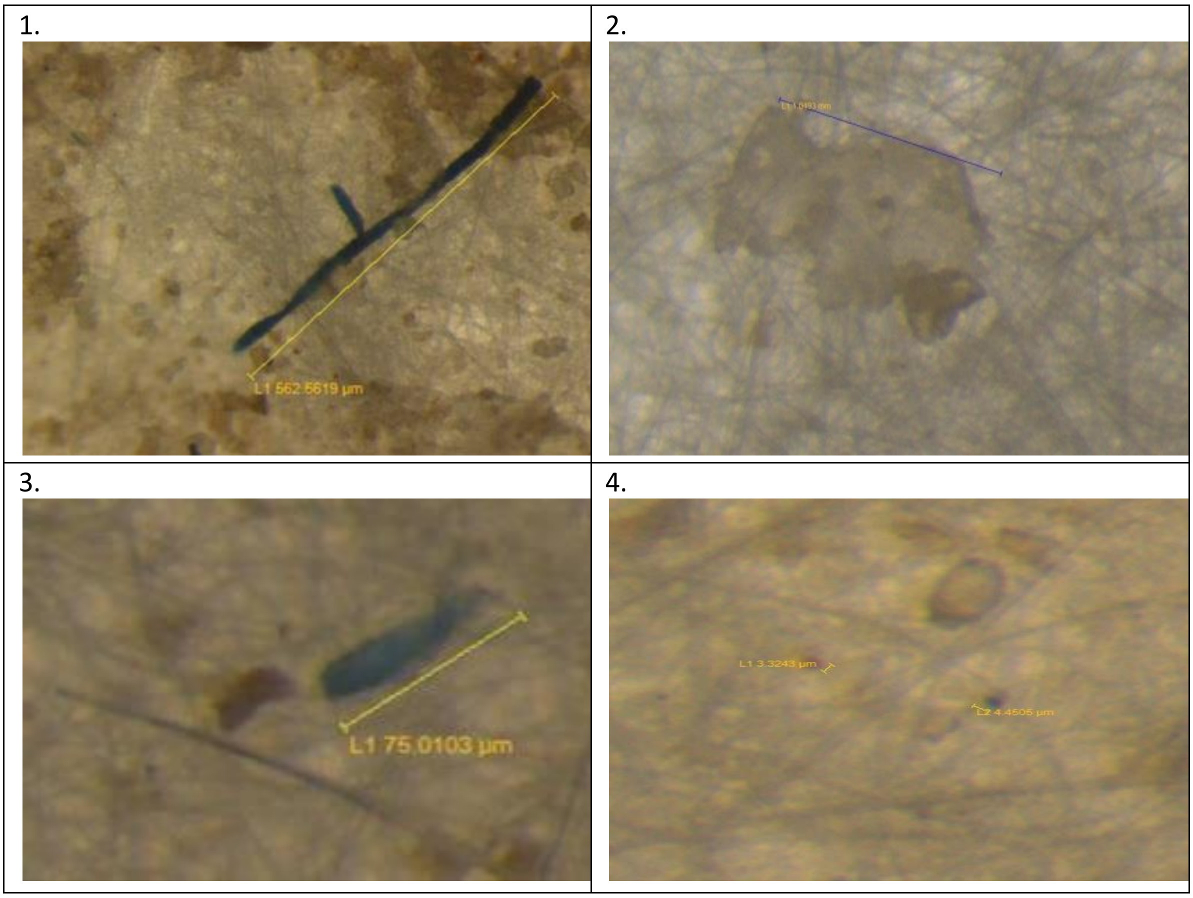


Figure 21: Percentage distribution of MPs shapes in GI tract pooled from all the months



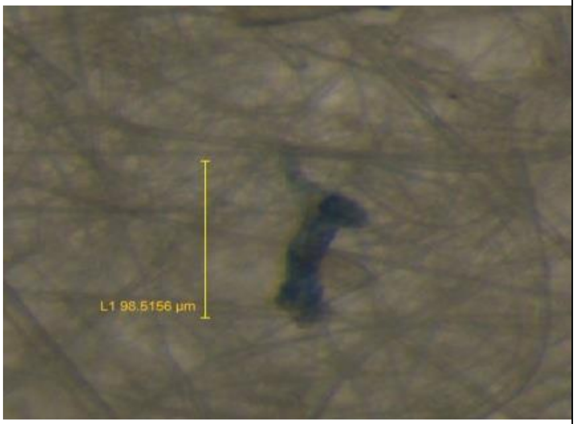
5. 	

Plate 3: Microplastics shapes observation in GI tract (1.Fibre, 2.Fragment, 3.Film, 4.Bead, and 5. Pellet)

4.3.3 Shapes of microplastics (MPs) extracted from the edible muscle

The percentage shapes of microplastics which is predominant in the muscles tissue were beads and are followed by fibres in all the months and the percentage of the other shapes vary in all the months (Fig. 22).

In the muscle tissue, the bar diagram shows that the beads and fibres are more in number in all the months which are followed by the other shapes group (Fig. 23). The percentage pooled microplastics shapes from all the months are 55 %, 24 %, 10 %, 10 % and 1 % for beads, fibres, fragments, films and pellets respectively (Fig. 24). The images of microplastics from the muscle is shown in Plate 4.

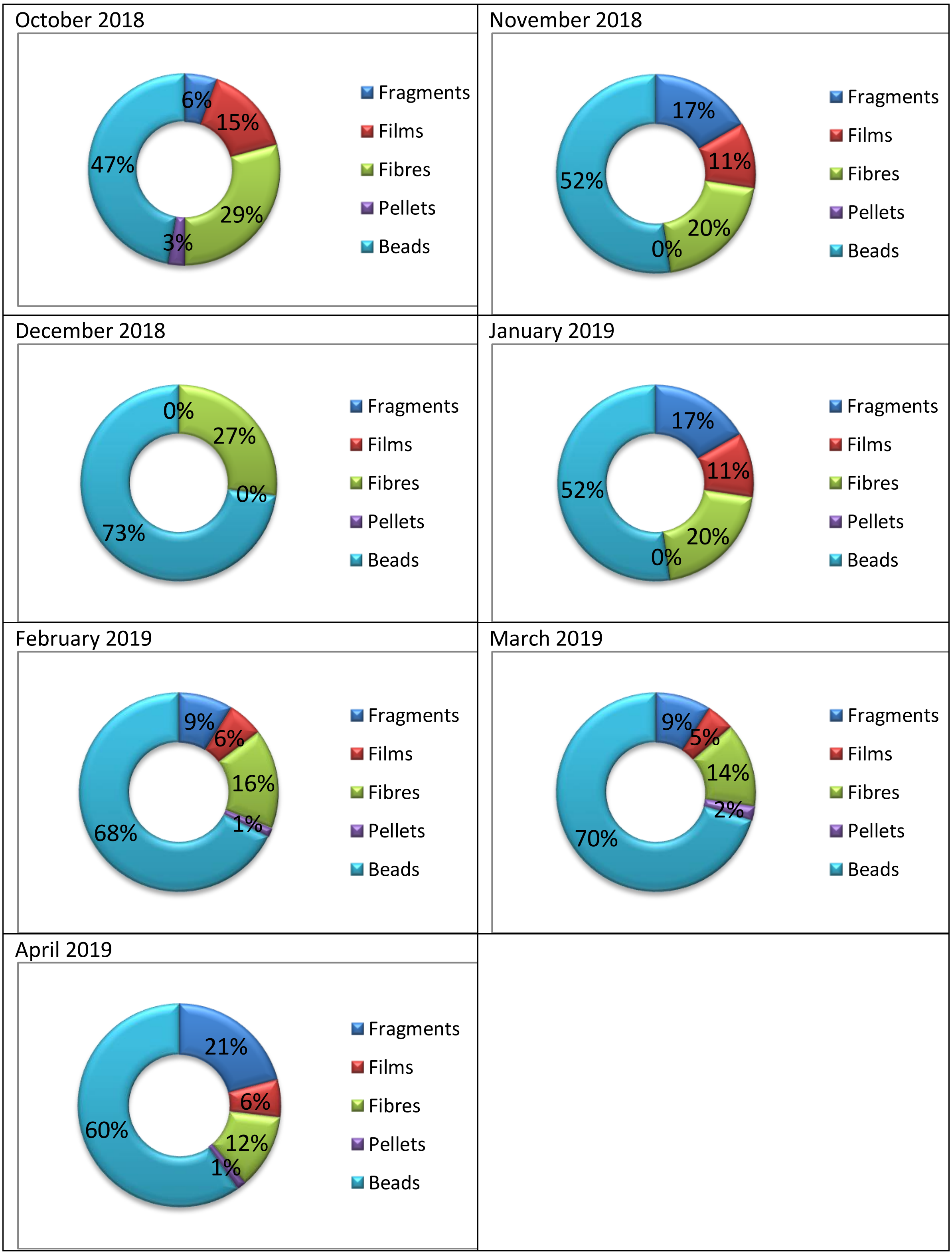


Figure 22: Percentage-wise shapes distribution of microplastics extracted from muscle of *Johnius* spp.

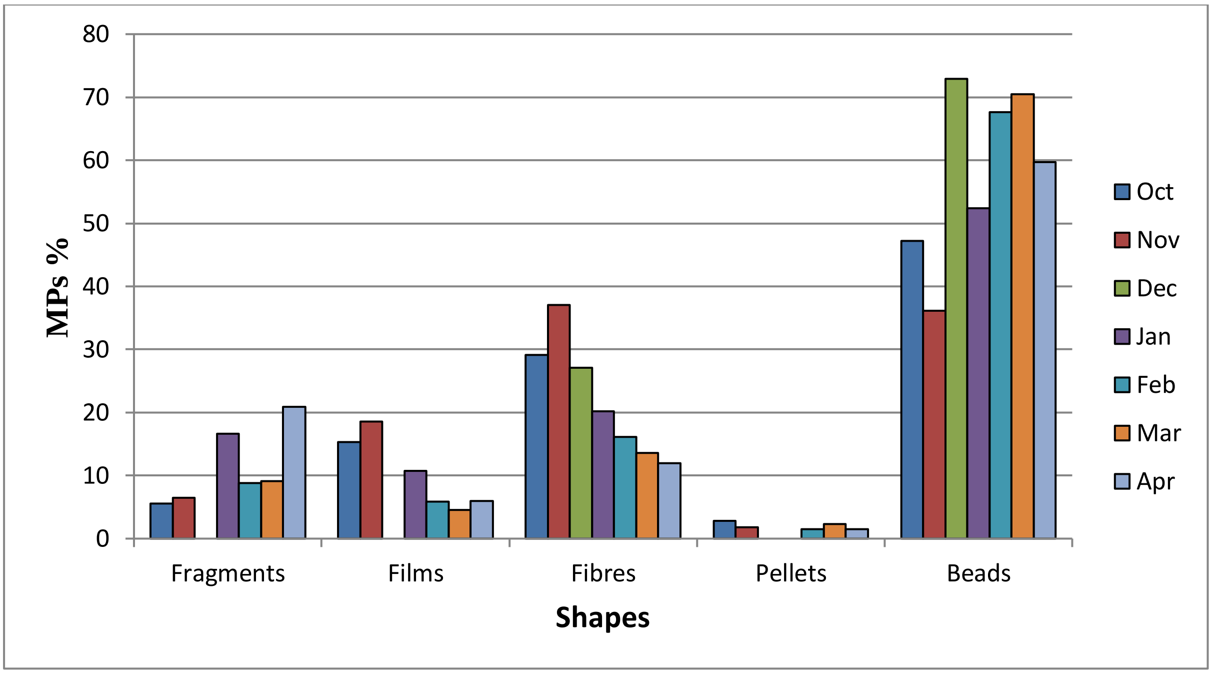


Figure 23: Temporal varations in MPs shapes distribution in muscle (%)

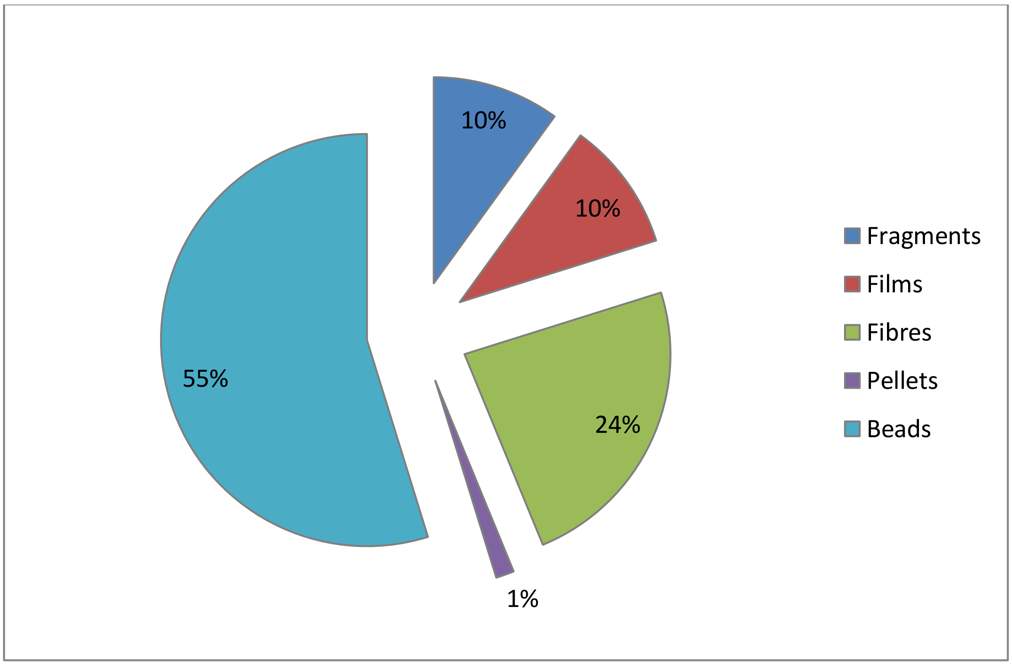


Figure 24: Percentage distribution of MPs shapes in muscle pooled from all the months

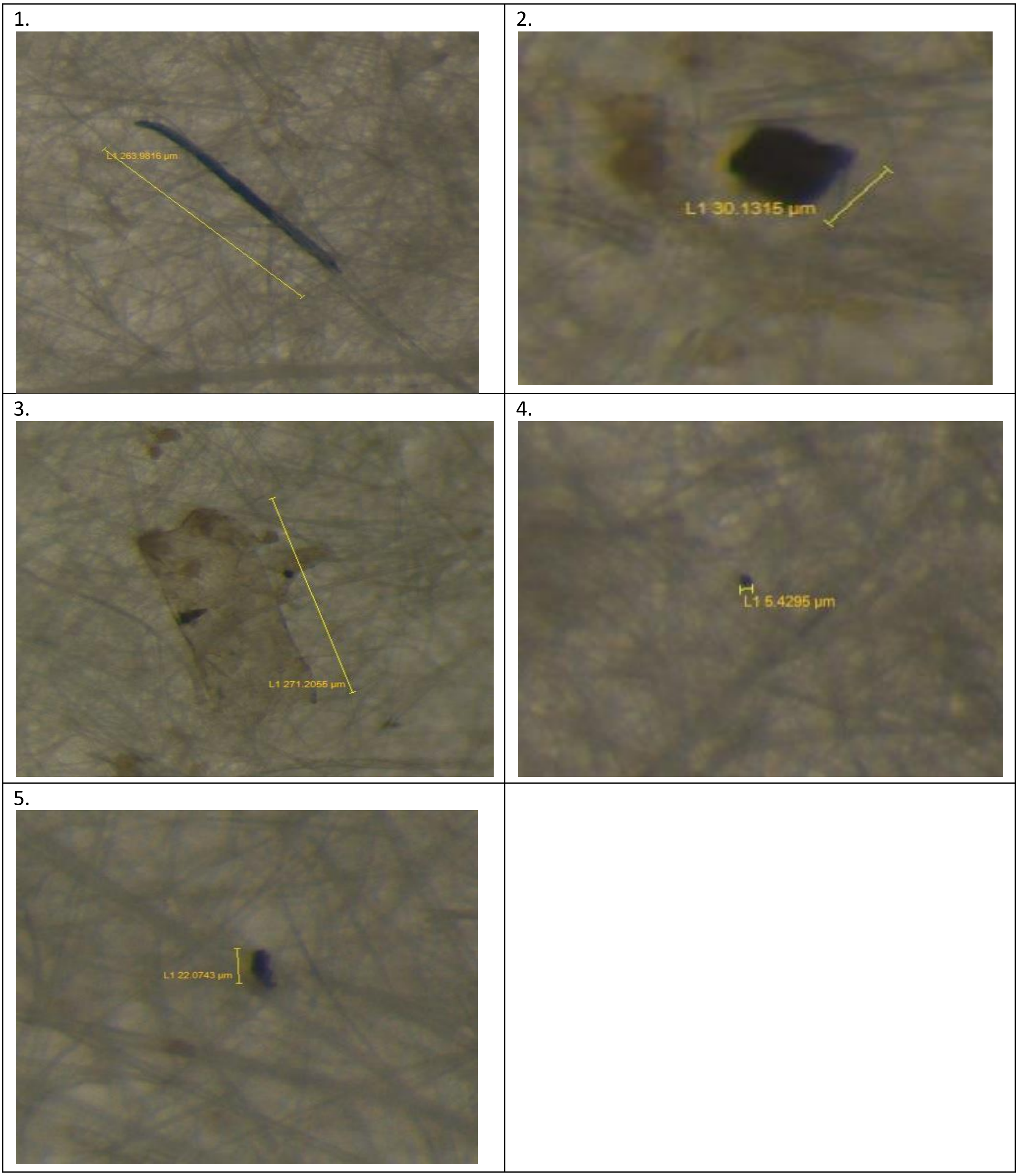


Plate 4: Microplastics shapes observation in the muscle (1.Fibre, 2.Fragment, 3. Film, 4.Bead, and 5. Pellet)

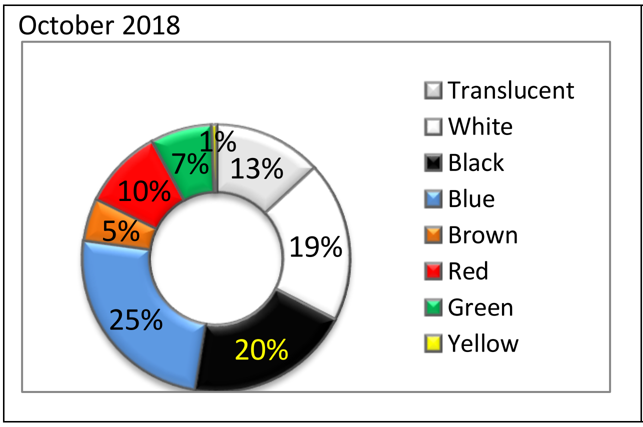
4.4 Microplastics (MPs) colours distribution in different body parts of *Johnius* spp. harvested from Mumbai waters through experimental trawling

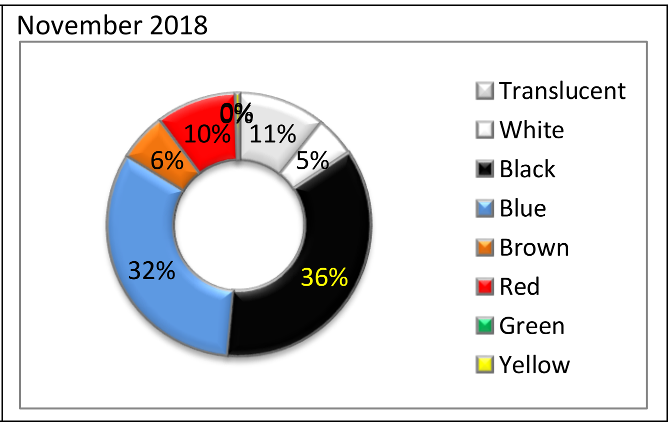
The types of microplastics colours observed in the study comprise of 8 colours and they are translucent, white, black, blue, brown, red, green and yellow.

4.4.1 Colour of microplastics (MPs) extracted from the gill parts of *Johnius* spp.

In the study the predominant colours in the gills were found to be black (28%), followed by blue (24%), red (15%), translucent (11%), brown (10%), white (8%), green (3%) and yellow (1%) respectively (Fig. 27).

The percentage distribution of MPs colours in the gills for specific months is shown in Fig. 25 and Fig. 26.





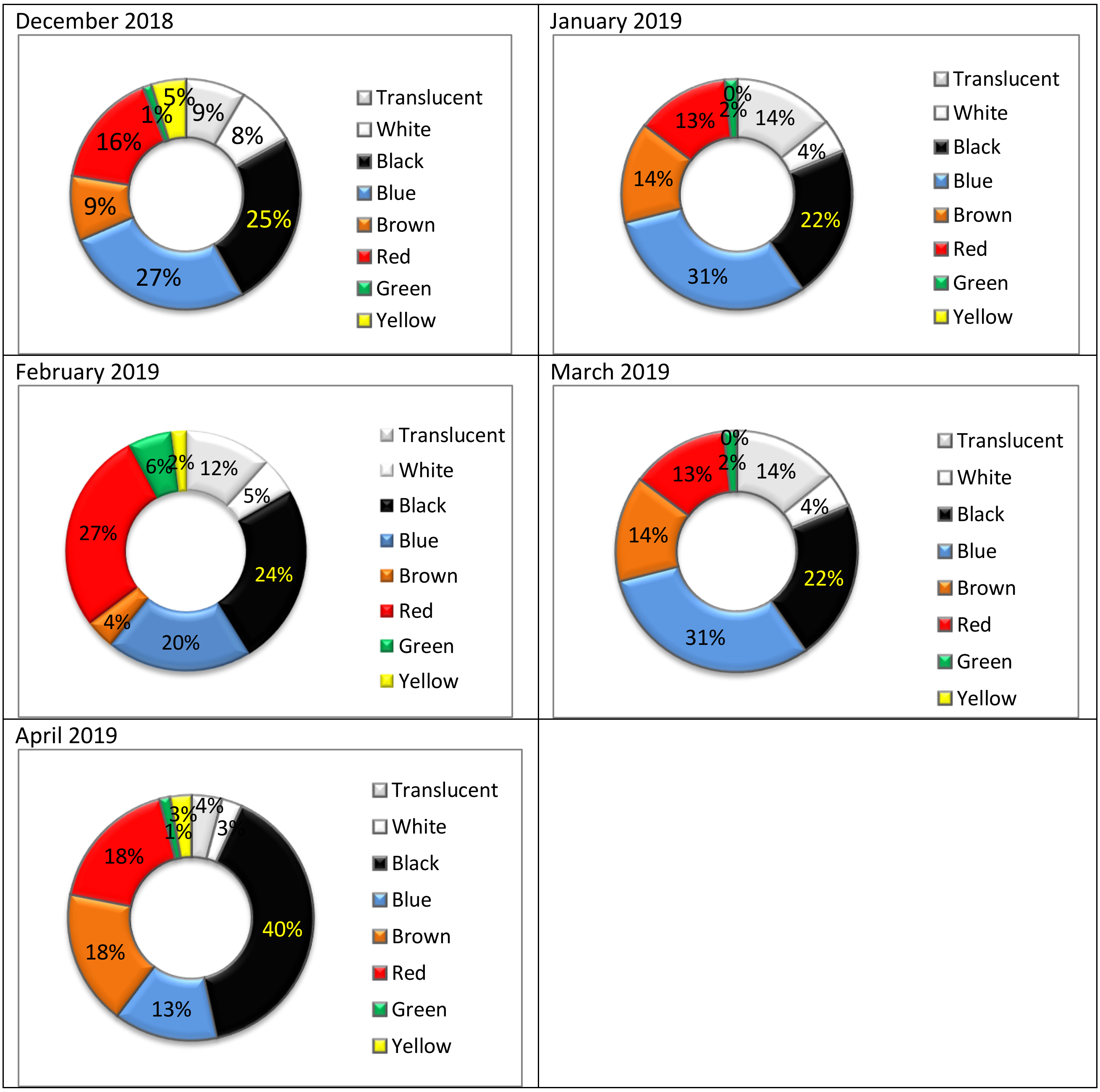


Figure 25: Percentage-wise distribution of colour of microplastics from gills of *Johnius* spp.

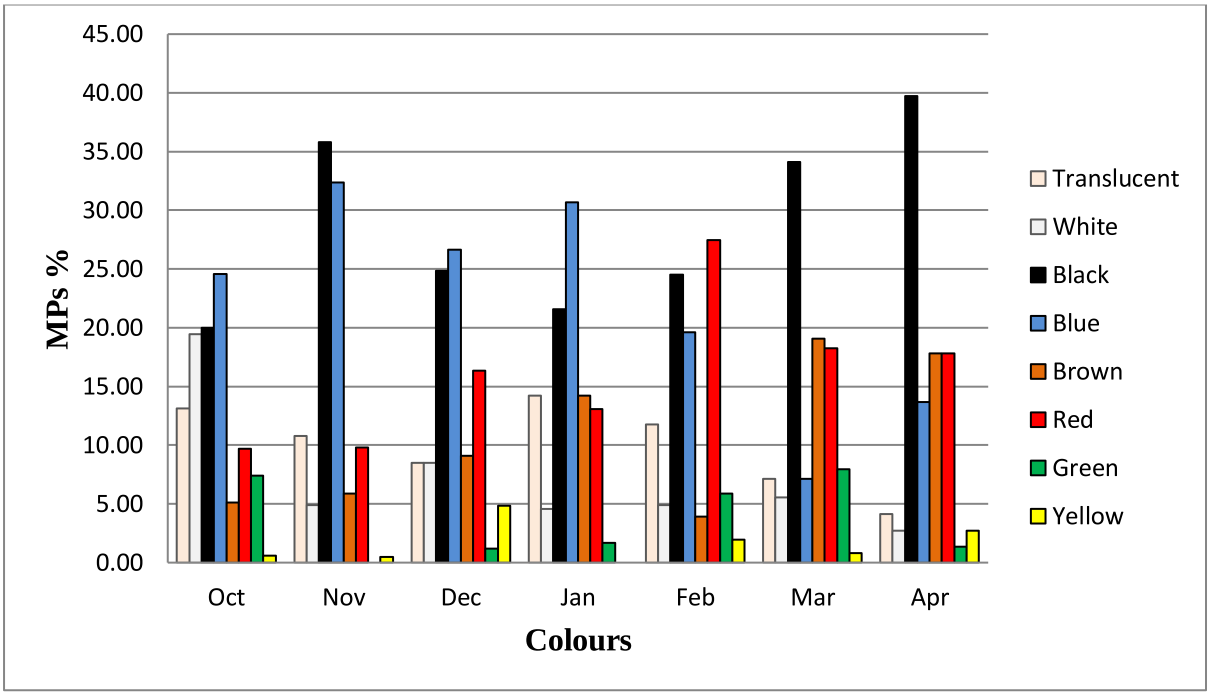


Figure 26: Temporal variations in MPs colours distribution in gills (%)

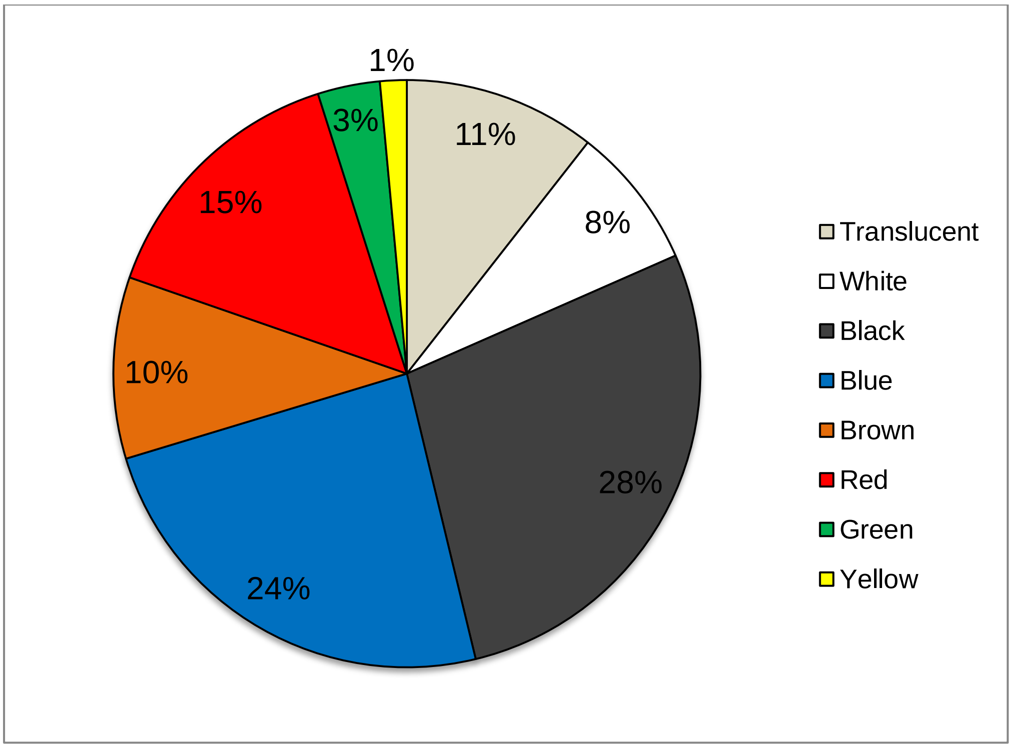
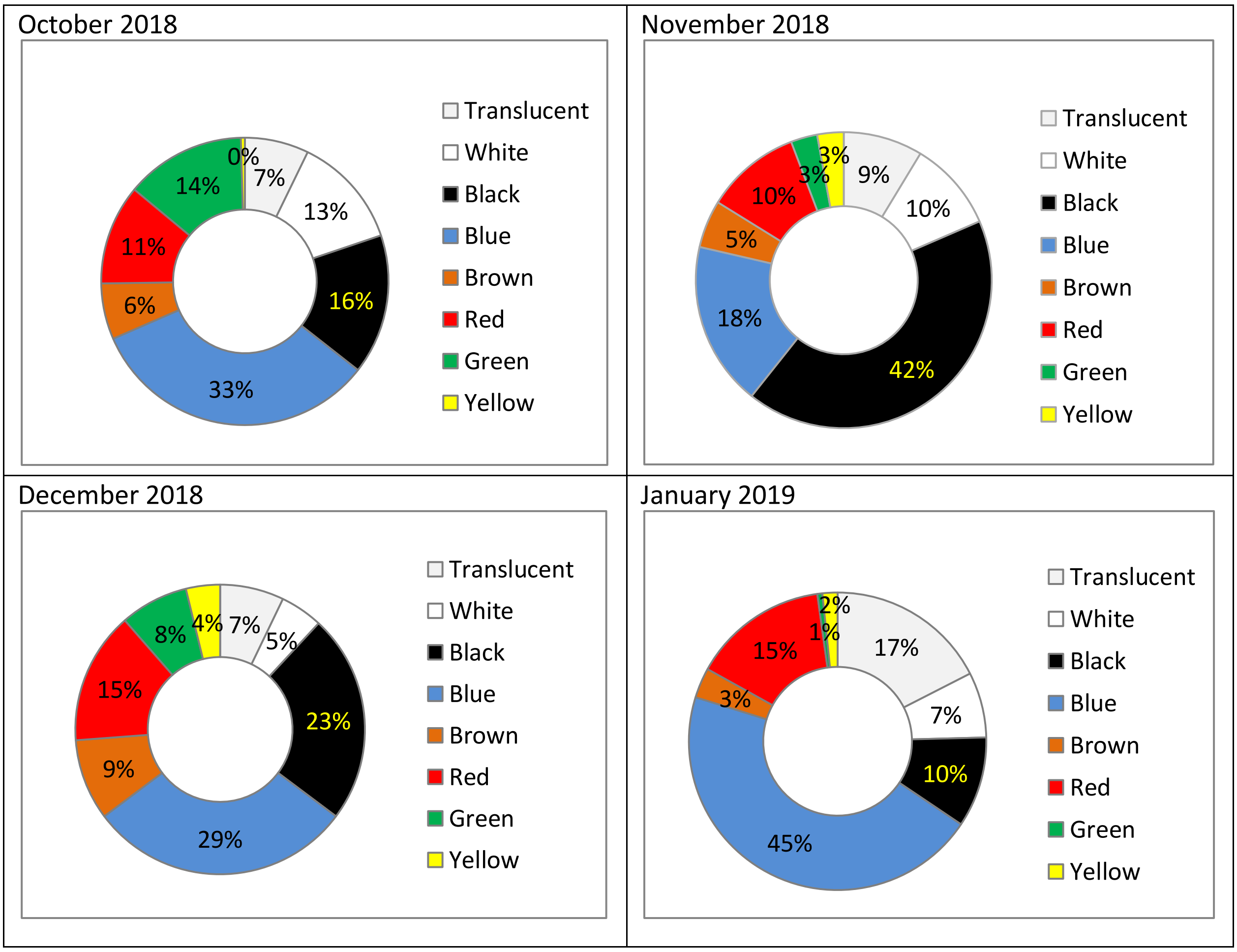


Figure 27: Percentage distribution of MPs colours in gills pooled from all the months

4.4.2 Colour of microplastics (MPs) extracted from the G.I tract of *Johnius* spp.

The predominant colours in the GI tract was blue (27%), followed by black (23%), red (15%), translucent (11%), white (8%), brown and green of same proportion (7% each) and yellow (2%) respectively (Fig. 30).

The microplastics colours in the GI tract for each particular months are shown in a pie chart (Fig. 28) and bar diagram (Fig. 29).



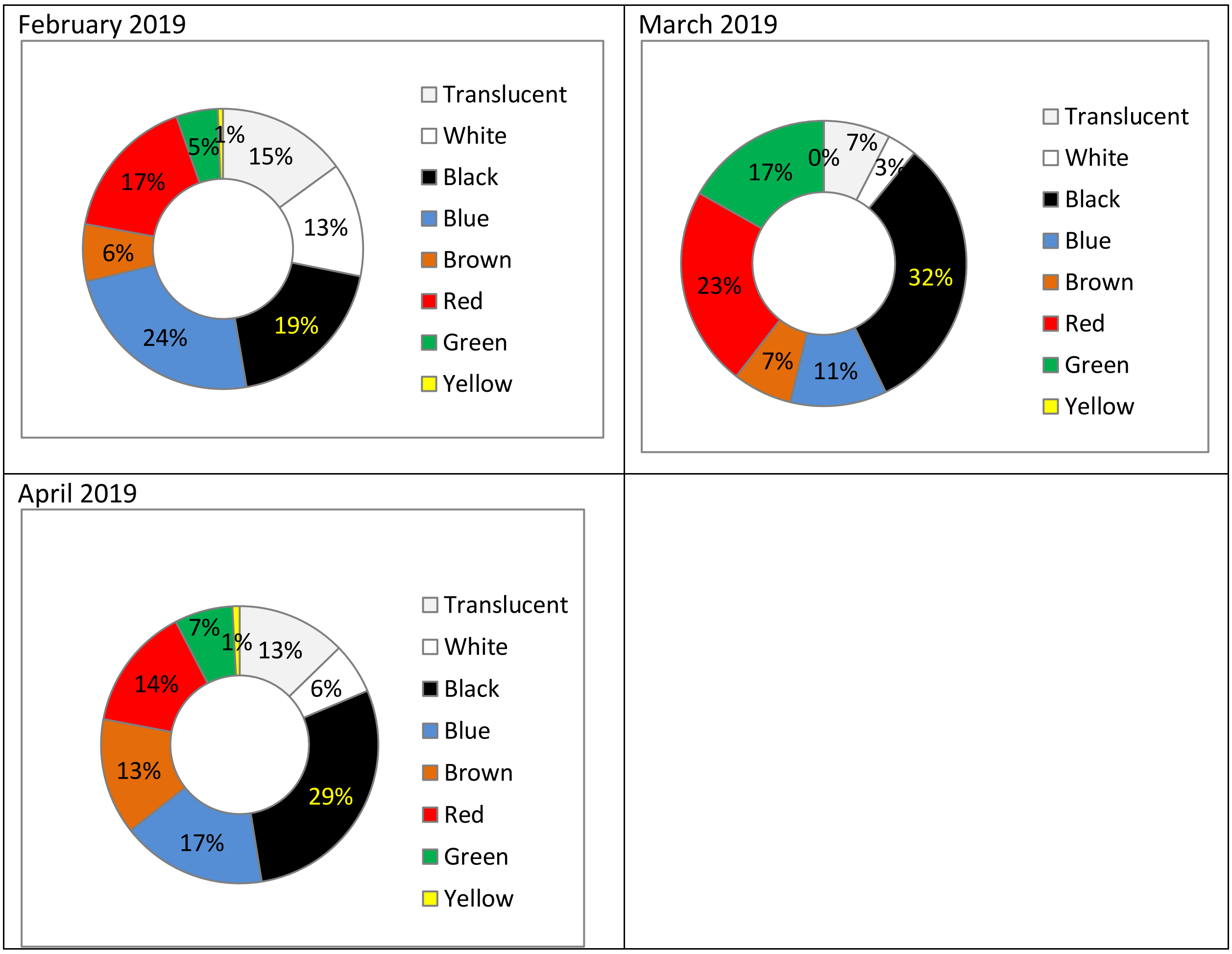


Figure 28: Percentage-wise distribution of microplastics colours found in the GI tract of *Johnius* spp.

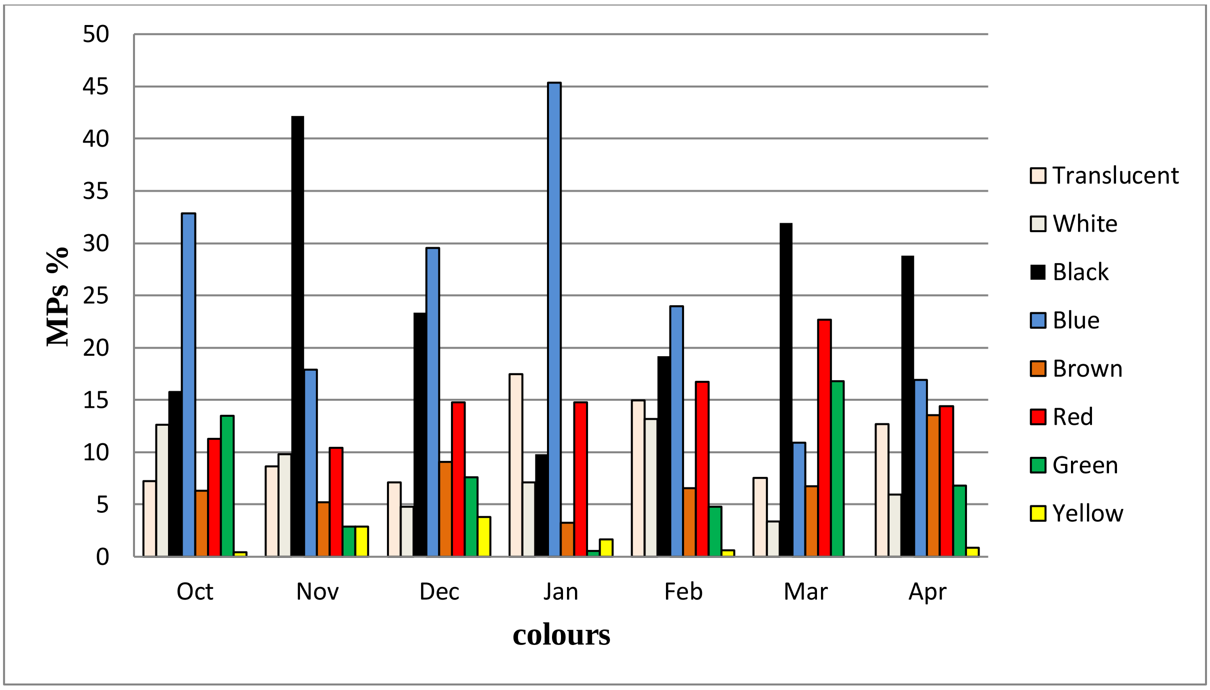


Figure 29: Temporal variations in MPs colours distribution in GI tract (%)

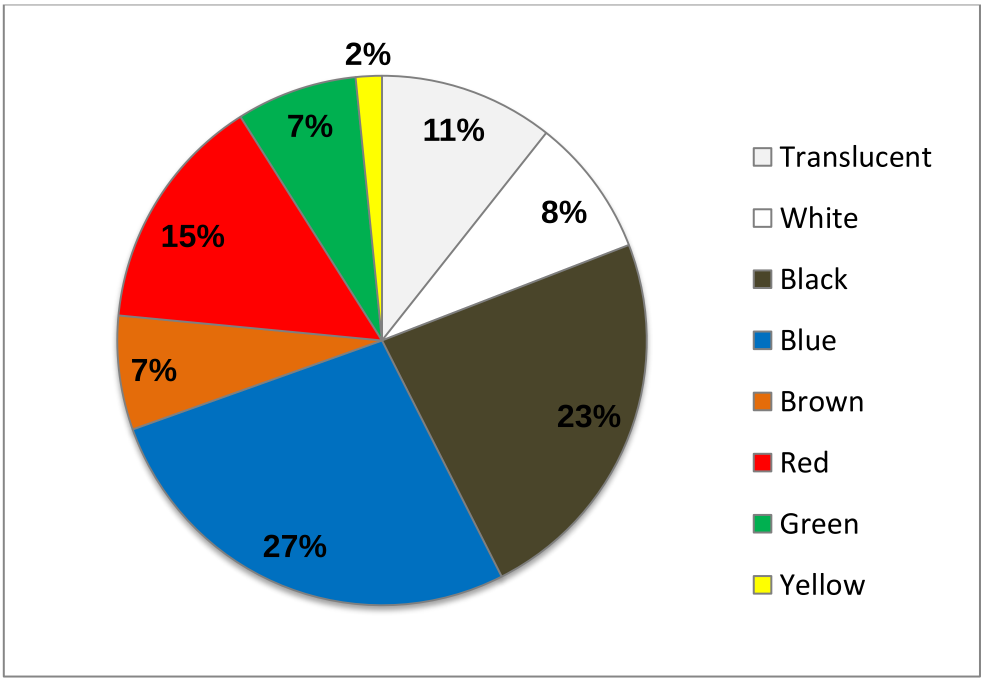
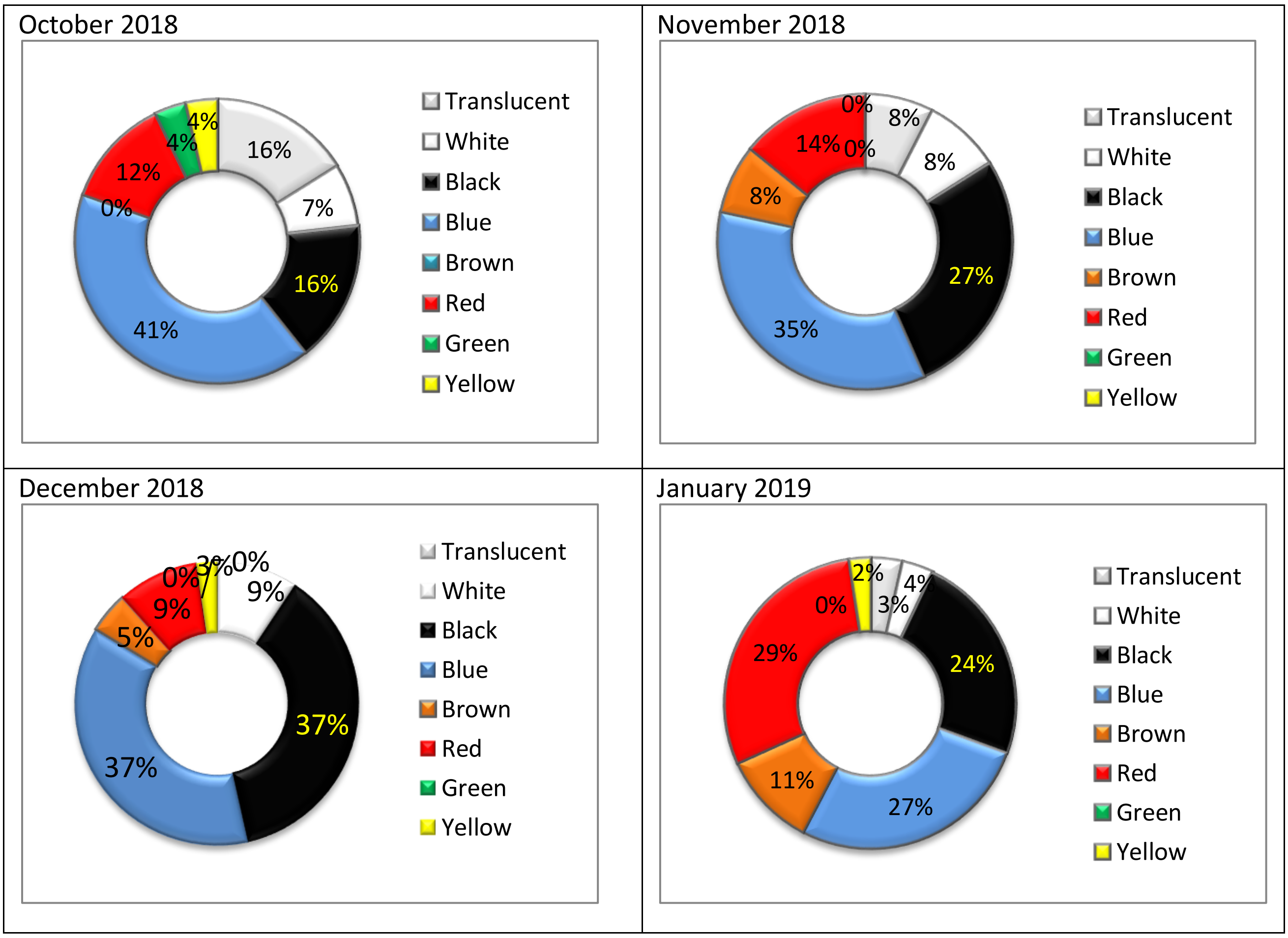


Figure 30: Percentage distribution of MPs colours in GI tract pooled from all the months

4.4.3 Colour of microplastics (MPs) extracted from the edible muscle portion of *Johnius* spp.

The predominant colours in muscle pooled from all the months are black and blue which accounted 28 % each and is followed by red (18%) and all the rest colours in a small percentage (Fig. 33). The percentage distribution of microplastics colours from the edible portion that is the muscle is shown in a pie chart (Fig. 31) and in a bar diagram (Fig. 32).



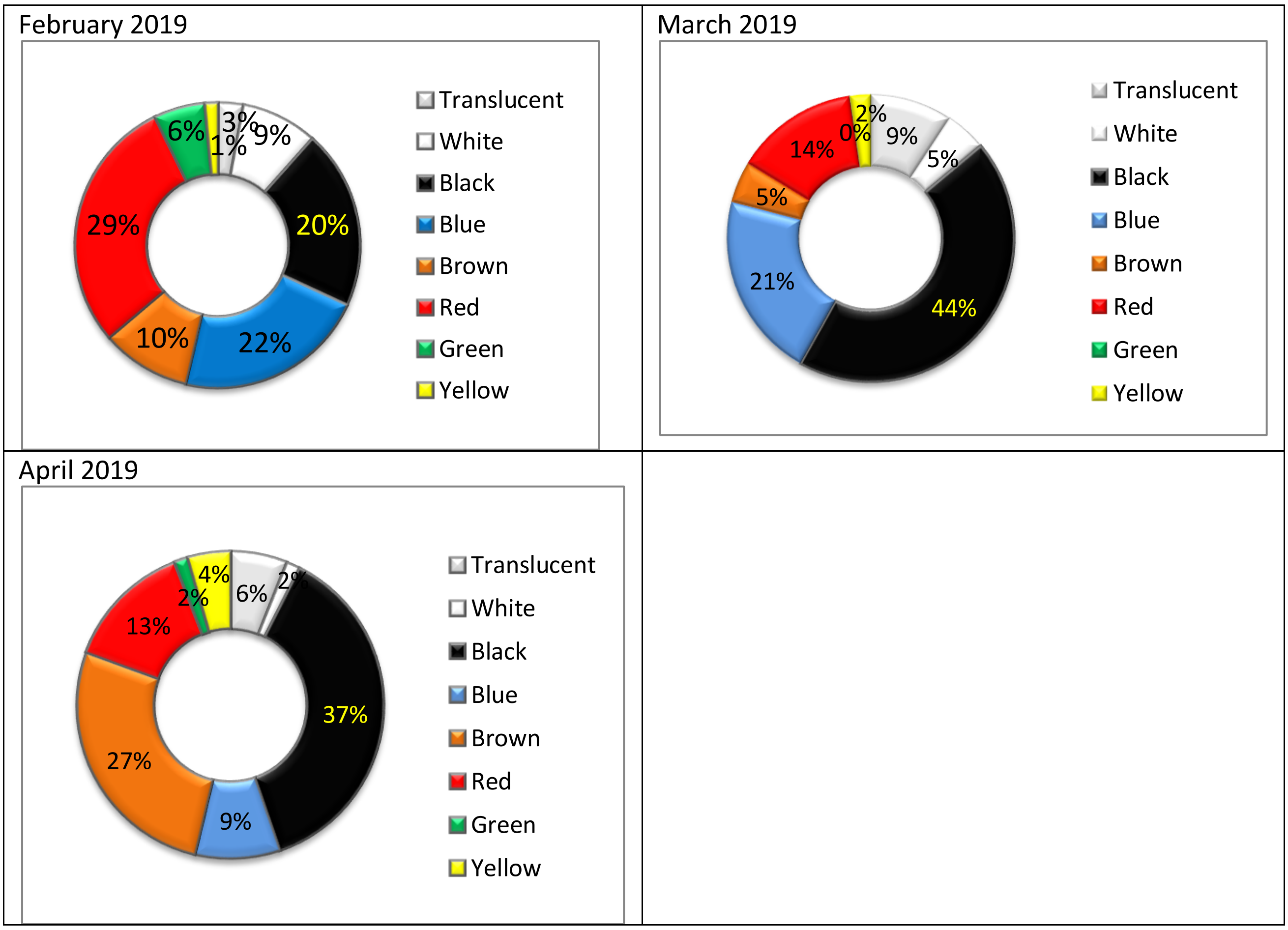


Figure 31: Percentage-wise distribution colours of microplastics extracted from the muscle of *Johnius* spp.

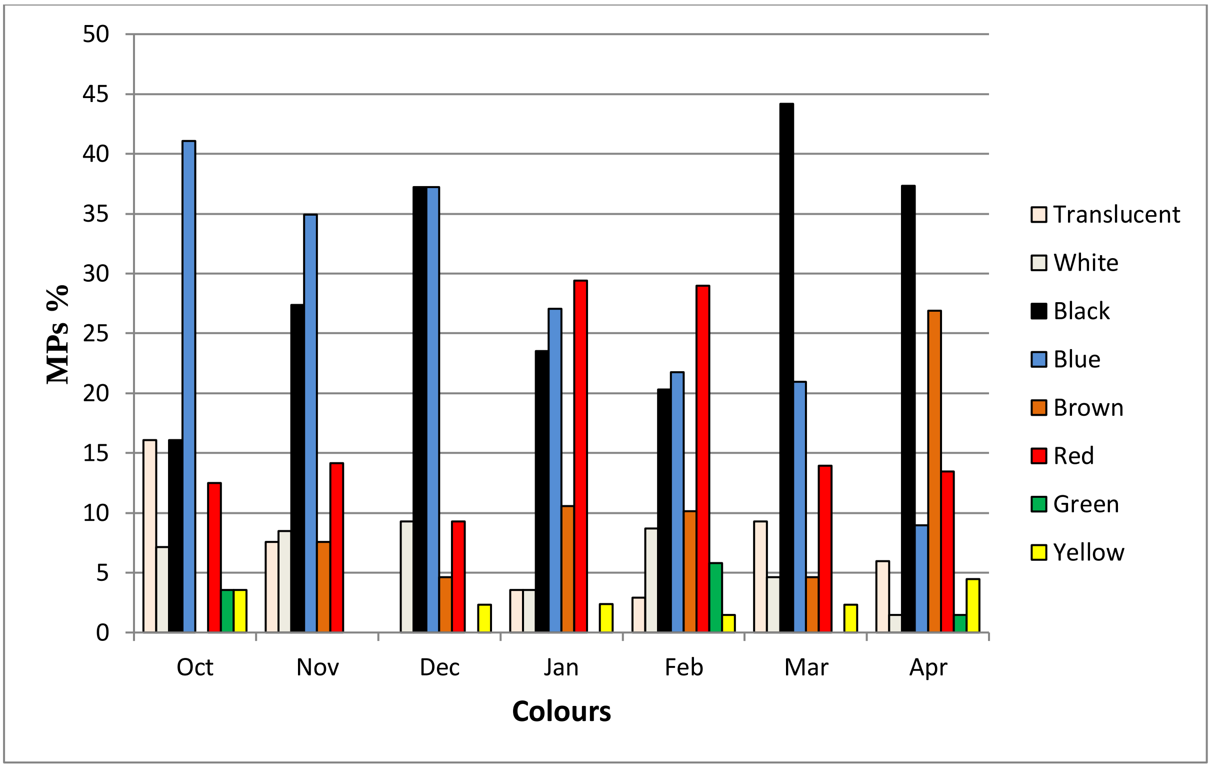


Figure 32: Temporal variations in MPs colours distribution in muscle (%)

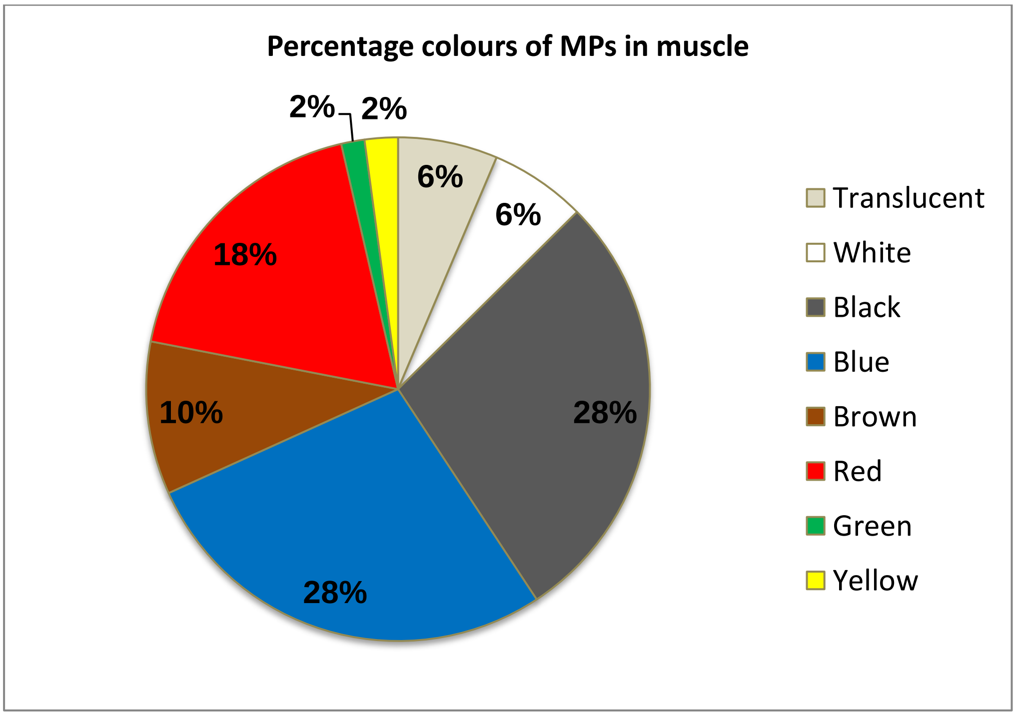


Figure 33: Percentage distribution of MPs colours in muscle pooled from all the months

4.5 Microplastics (MPs) distribution pattern in juvenile and adult *Johnius* spp. harvested from Mumbai waters based on experimental trawling

The total sampled fish in the present study was 188 of which juvenile fish represented 151 in sample size and adult fish accounted with 37 numbers. The microplastics particles observed were specifically studied for their characteristics features distribution like size, shapes, and colours respectively in the juvenile and adult fishes.

4.5.1 <u>Microplastics size and abundance in Juveniles and Adult fish</u>

4.5.1.1 Incidence of microplastics abundance and size in gills

The incidence of MPs size in gills is predominated by < 100 µm in both juvenile and adult fish representing 4.21 and 3.62 nos fish^{-1} respectively followed 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm (Table 3).

The average number of microplastics g^{-1} was 18.10 in juvenile *Johnius* spp. and 8.01 in adult *Johnius* spp. (Table 4). The average specific size group of MPs and their frequency distribution in the gills of juvenile and adult fish are shown in Table-4. And the average size of MPs in the gills of juvenile and adult fish for < 100 µm, 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm are represented in Table 4.

Table 3: Incidence of microplastics size in gill parts of juvenile and adult fish (*Johnius* spp.)

MPs Size (µm)	Average MPs (number/fish)		Avg. MPs (Number/g)	
	Juvenile	Adult	Juvenile	Adult
< 100 µm	4.21	3.62		
100-250 µm	0.73	1.27		
250-500 µm	0.29	0.59		
500-1000 µm	0.20	0.32		
> 1000 µm	0.09	0.08		
Total	5.52	5.88	18.10	8.01

Table 4: Frequency distribution of microplastics sizes extracted from the gill parts of juvenile and adult fish (*Johnius* spp.)

MPs Size range (µm)	Juvenile (n =151)			Adult (n =37)		
	Frequency	%	Average size (µm)	Frequency	%	Average size (µm)
< 100	635	76.23	27.09	134	61.47	29.20
100-250	110	13.21	151.32	47	21.56	148.14
250-500	44	5.28	329.53	22	10.09	348.10
500-1000	30	3.60	662.98	12	5.50	642.08
> 1000	14	1.68	1431.96	3	1.38	2005.77
Total	833	100		218	100	

4.5.1.2 Incidence of microplastics abundance and size in the GI tract

The highest incidence of MPs size in the GI tract is was also represented by < 100 µm followed by the second highest group 100-250 µm and subsequently by 250-500 µm, 500-1000 µm and > 1000 µm in both the juvenile and adult fishes (Table 5).

The frequency number size distribution and the average size of specific size classes of microplastics in the GI tract are given in Table 6.

Table 5: Incidence of microplastic size in GI tracts of juvenile and adult fish (*Johnius* spp.)

MPs Size (µm)	Average MPs (No./fish)		Avg. MPs (No./g)	
	Juvenile	Adult	Juvenile	Adult
< 100 µm	4.36	3.14		
100-250 µm	0.99	1.00		
250-500 µm	0.50	0.86		
500-1000 µm	0.25	0.51		
> 1000 µm	0.20	0.22		
Total	6.30	5.73	13.54	5.26

Table 6: Frequency distribution of microplastics sizes extracted from the GI tract of juvenile and adult fish (*Johnius* spp.)

MPs Size (µm)	Juvenile (n =151)			Adult (n =37)		
	Frequency	%	Average Size (µm)	Frequency	%	Average Size (µm)
< 100	659.00	69.30	22.89	116.00	54.72	25.95
100-250	150.00	15.77	158.91	37.00	17.45	154.00
250-500	75.00	7.89	345.91	32.00	15.09	361.71
500-1000	37.00	3.89	712.82	19.00	8.96	656.58
> 1000	30.00	3.15	1822.56	8.00	3.77	1883.24
	951.00	100.00		212.00	100.00	

4.5.1.3 Incidence of microplastics abundance and size in the muscles

The average microplastics number g^{-1} of muscle in the juvenile and adult fishes are 0.75 and 0.56 respectively (Table 7). The incidence of microplastics in the juvenile and adult fish is highest in < 100 µm followed by 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm (Table 7).

The frequency number size distribution and the average size of specific size classes of microplastics in the meat are given in Table 8.

Table 7: Incidence of microplastic size in the meat of juvenile and adult fish (*Johnius* spp.)

MPs Size (µm)	MPs (No./sample weight)		MPs (No./g meat)	
	Juvenile	Adult	Juvenile	Adult
< 100 µm	2.06	1.89		
100-250 µm	0.24	0.62		
250-500 µm	0.15	0.24		
500-1000 µm	0.09	0.08		
> 1000 µm	0.05	0.08		
Total	2.58	2.92	0.75	0.56

Table 8: Frequency distribution of microplastics sizes extracted from the muscles parts of juvenile and adult fish (*Johnius* spp.)

MPs Size range (µm)	Juvenile fish (n =151)			Adult fish (n =37)		
	Frequency	%	Average Size (µm)	Frequency	%	Average Size (µm)
< 100	311	79.95	24.31	70	64.81	24.37
100-250	36	9.25	151.15	23	21.30	147.49
250-500	22	5.66	313.15	9	8.33	334.76
500-1000	13	3.34	664.41	3	2.78	708.33
> 1000	7	1.80	1615.01	3	2.78	1541.50
	389	100.00		108	100	

4.5.2 Microplastics shapes in Juvenile and Adult fish

4.5.2.1 Microplastics shapes in the GI tract

The incidence of different MPs shapes in the GI tract is predominated by beads, followed by fibres, fragments, films, and pellet both in the juvenile and adult fishes where the pellet is absent in adult fish (Fig. 34). The percentage shapes distribution of MPs particles is shown in Fig. 35.

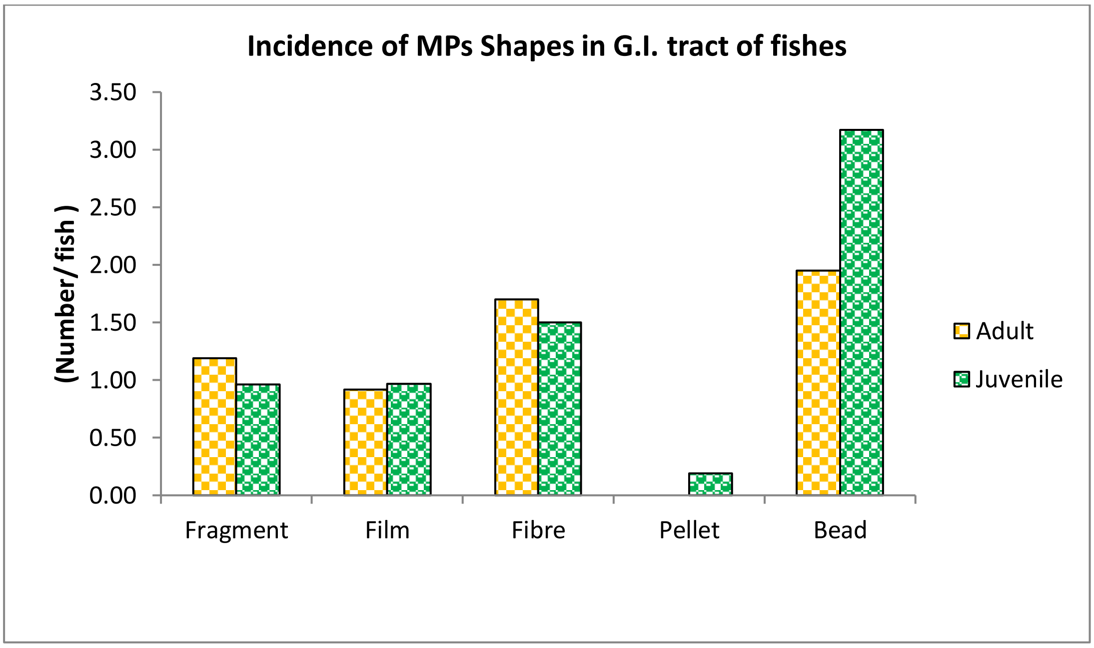


Figure 34: Incidence of different shapes of microplastics in the GI tract of juvenile and adult fish (*Johnius* spp.)

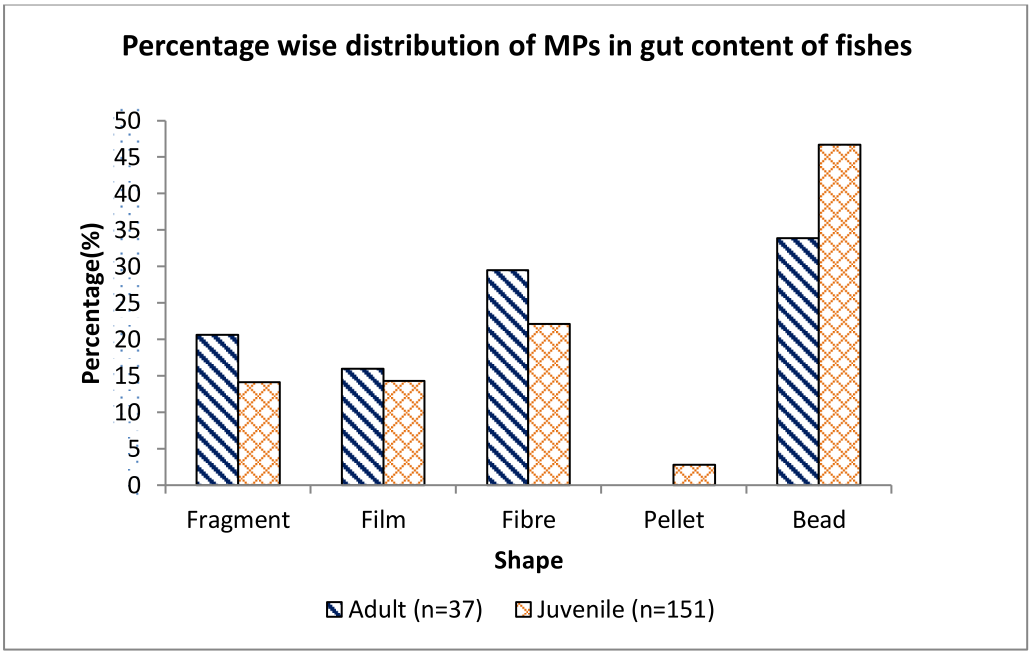


Figure 35: Percentage-wise distribution of different shapes of microplastics in the gut of juvenile and adult fish (*Johnius* spp.)

4.5.2.2 Microplastics shapes in Gills

The incident of MPs shapes in the gills of juvenile and adult fish is also predominated by beads, followed by fibres, fragments, films and beads respectively (Fig. 36). The percentage distribution of MPs shapes in the gill was higher for beads and is followed by fibres, fragments, films, and pellets respectively both in the juvenile and adult fish group (Fig. 37).

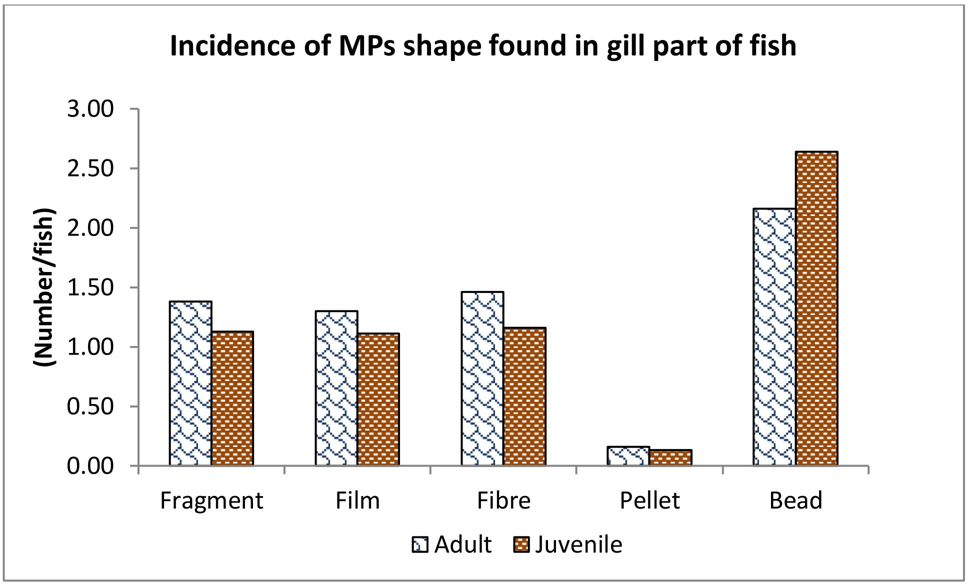


Figure 36: Incidence of different shapes of microplastics in gill part of juvenile and adult fish (*Johnius* spp.)

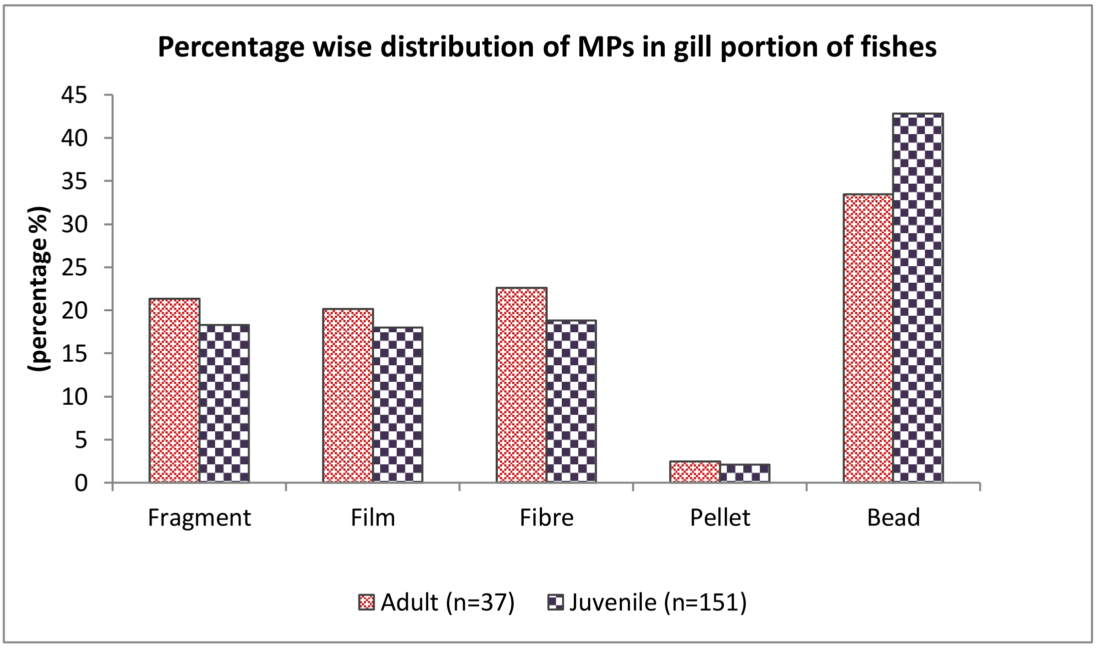


Figure 37: Percentage-wise distribution of different shapes of microplastics in gill parts of juvenile and adult fish (*Johnius* spp.)

4.5.2.3 Microplastics shapes in muscles

The incidence and percentage distribution of MPs shapes in the muscle is also represented by beads as the highest and followed by fibres, fragments, films and pellets in both juvenile and adult fish group of *Johnius* spp. (Fig. 38 and Fig. 39).

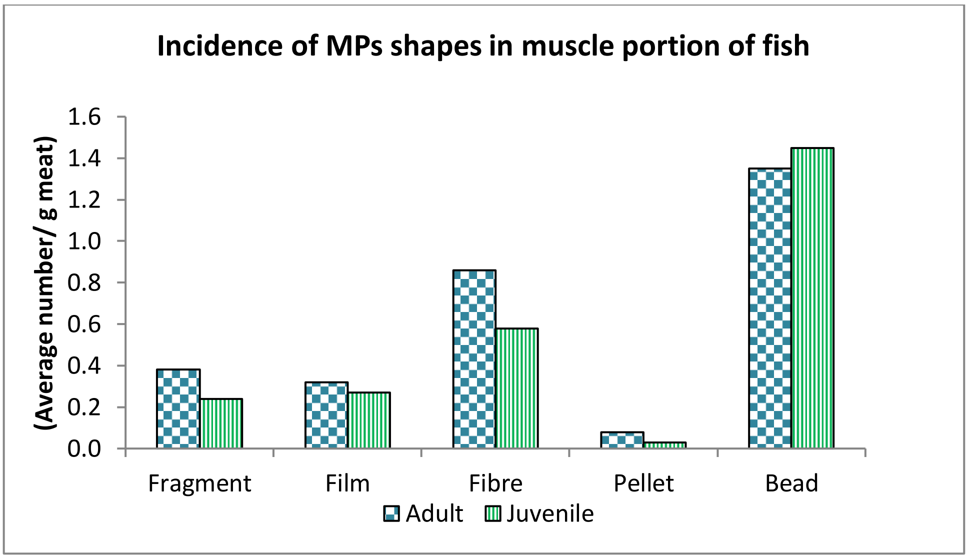


Figure 38: Incidence of different shapes of microplastics in the muscle part of juvenile and adult fish (*Johnius* spp.)

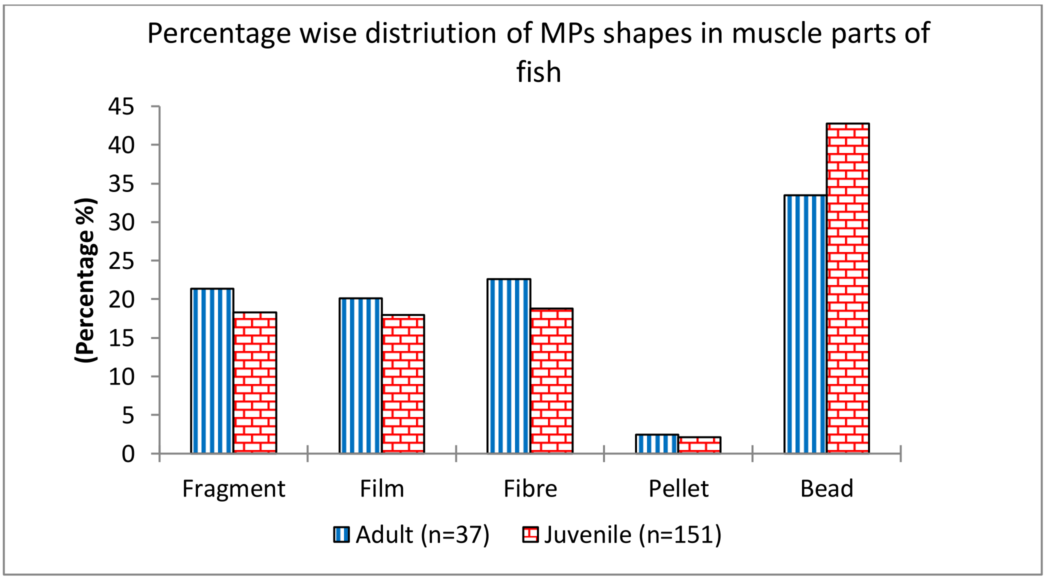


Figure 39: Percentage-wise distribution of different shapes of microplastics in the muscle part of juvenile and adult fish (*Johnius* spp.)

4.5.3 <u>Colours of Microplastics extracted from different body parts of fish</u>

4.5.3.1 Microplastics colours in the gut

The incidence of microplastics colours in the gut of juvenile and adult fish are predominated by black and blue while blue was found to be highest in juvenile fish, and black being the highest in the adult fish group. And these colours are followed by the other colours in small numbers with varying numbers in the adult and juvenile *Johnius* spp. (Fig. 40). The percentage distribution of MPs colours in the gut (GI tract) of juvenile and adult fishes are shown in Fig. 41.

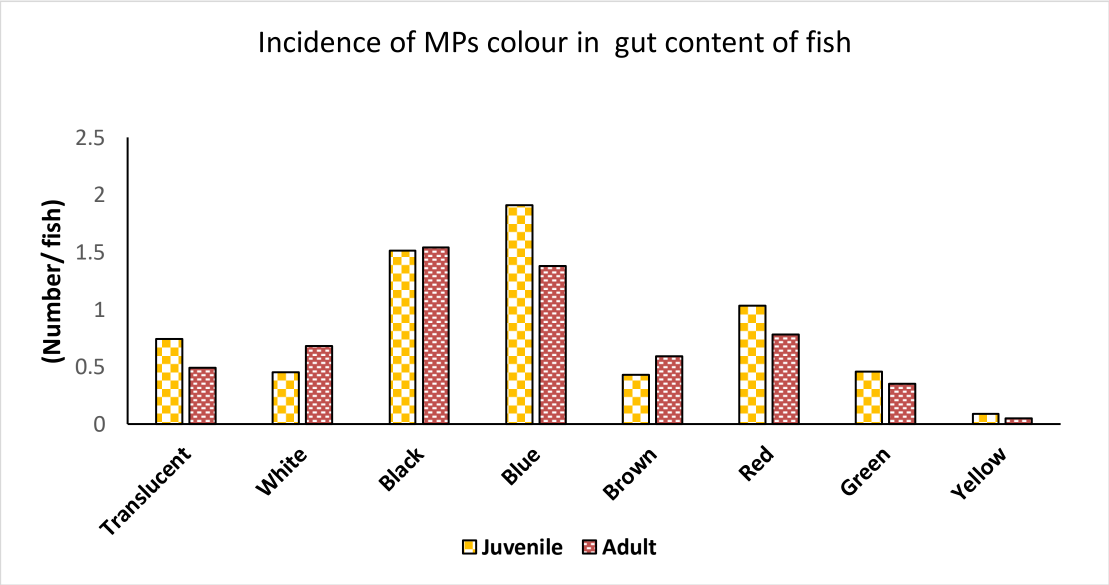


Figure 40: Incidence of microplastics colour in the gut content of juvenile and adult fish

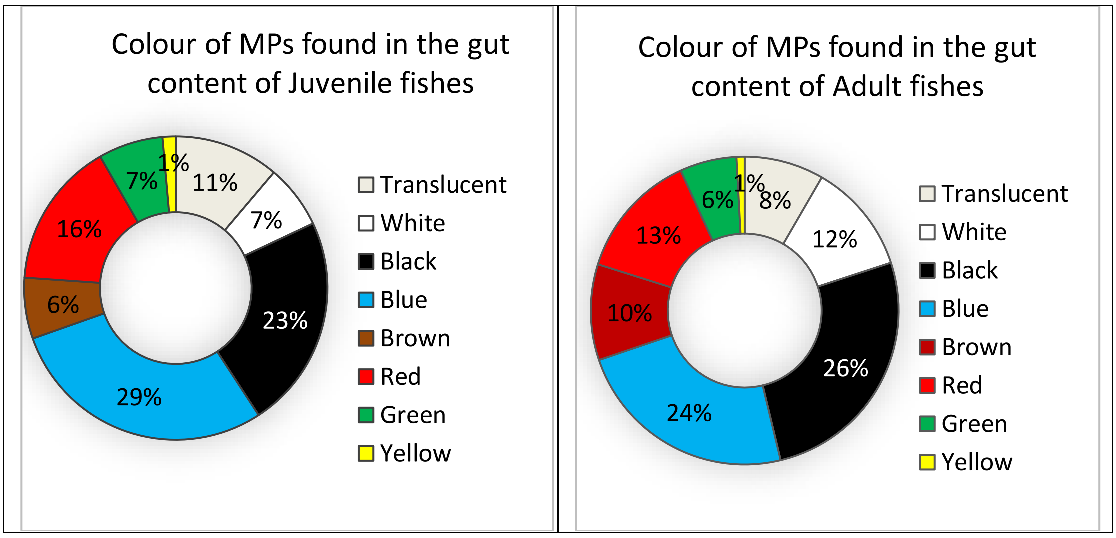


Figure 41: Percentage-wise distribution of colour of microplastics in the gut portion of juvenile and adult fish (*Johnius* spp.)

4.5.3.2 Microplastics colours in the gills

The predominant colours of MPs in the gills of both juvenile and adult fish were found to be black, and the second by blue colour which is followed by the rest colours in varying proportion (Fig. 42 and Fig. 43).

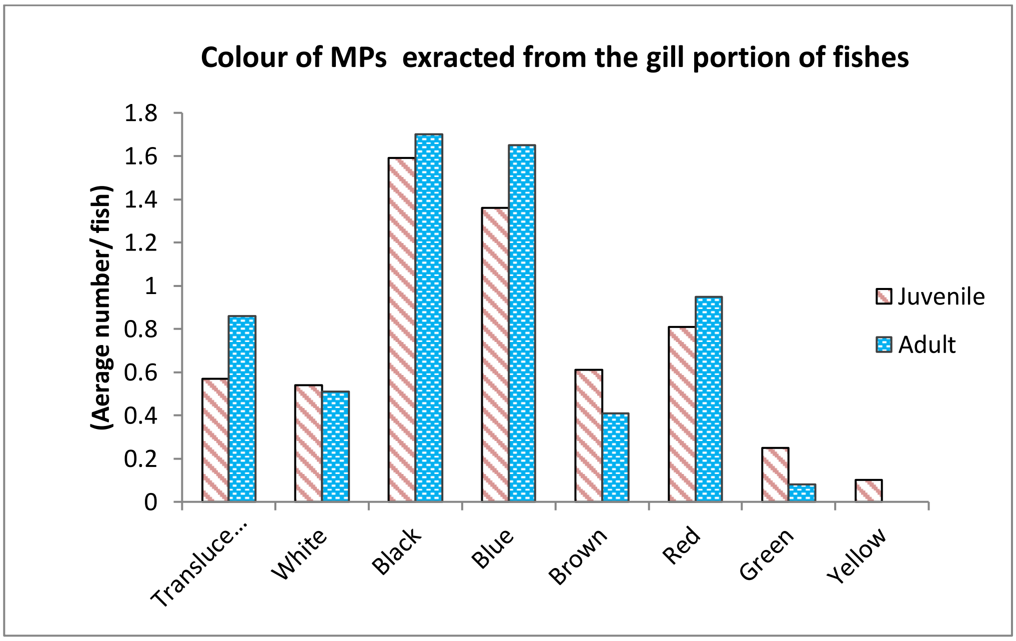


Figure 42: Incidence of microplastics colour in gill parts of juvenile and adult fish

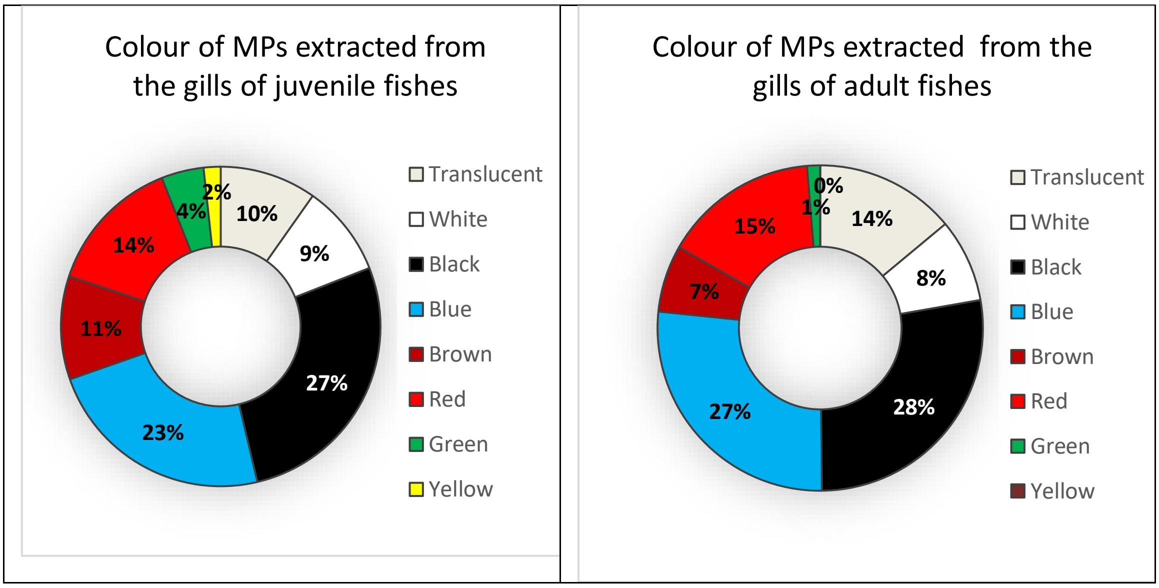


Figure 43: Percentage-wise distribution of colour of microplastics in gill part of juvenile and adult fish

4.5.3.3 Microplastics colours in the muscle

In the muscle portion of juvenile fish the predominant colour was black which is followed by blue, red, brown, translucent, white, green and yellow respectively (Fig. 44 and Fig. 45). And in the adult fish, the colours are predominated by blue, which is followed by black, red, brown and other colours in a small proportion (Fig. 44 and Fig. 45).

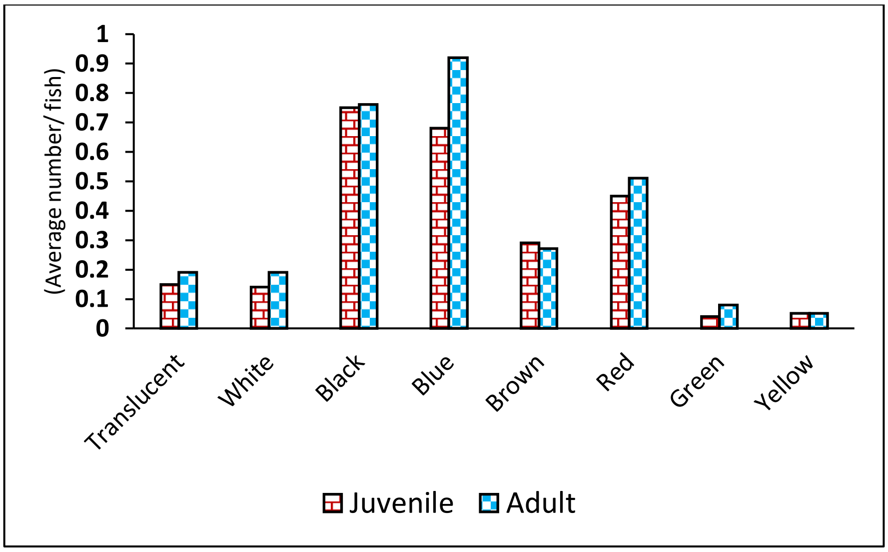


Figure 44: Incidence of microplastics colour in the muscle portion of juvenile and adult fish

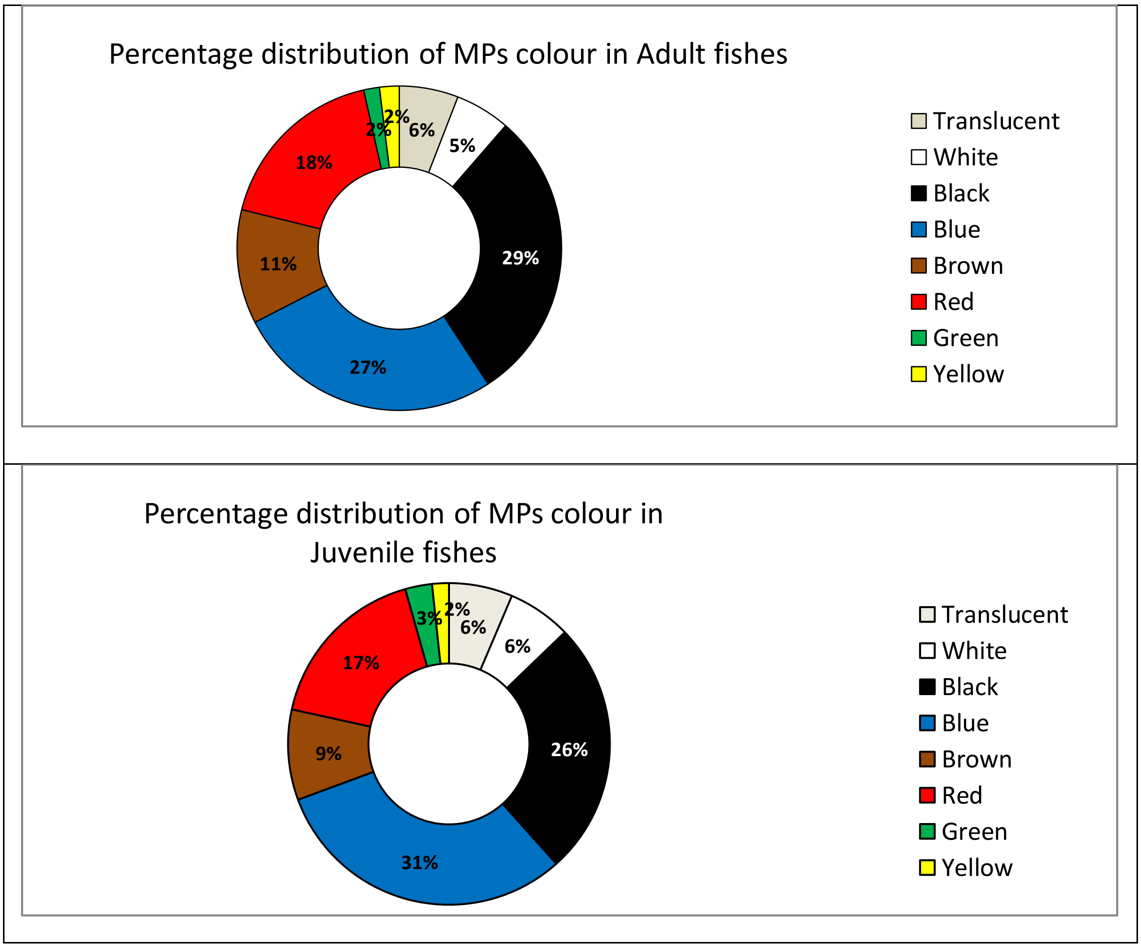


Figure 45: Percentage-wise distribution of colour of microplastics in muscle part of juvenile and adult fish

5. DISCUSSION

5.1. Abundance and temporal variation of microplastics

The present study on the" bioaccumulation of microplastics in the selected fishes along Mumbai coast, India" is carried out in *Johnius* spp. which is sampled by the MFV Narmada. The sampling of the fish was done for a period of seven months, starting from October 2018 to April 2019 in each month. As the objective of the study is to see any microplastics present in the selected fish and whether there is any temporal variation or monthly variation of microplastics the fish tissues gills, guts and muscles of the sampled (n=188) individual fish were taken as a study material. The fish tissues taken were subjected to preservation, dissection, digestion, filtration, and observation of the isolated filtered tissues to see the presence of microplastics.

The present study focuses on the presence of microplastics in the fish tissues where *Johnius* spp. was taken as a model for microplastic pollution indicator from Mumbai waters. The study has shown a 100% occurrence of microplastics in all the tissues examined from fish samples (n=188) in all the studied periods. This is the first study done on fish tissues (GI tract, gills and muscle) in the country from Mumbai coastal water and has reported 100% presence of MPs particles in all the tissue samples in comparison to recent studies on fishes (Foekema *et al*., 2013; Lusher *et al*., 2013; Avio *et al*., 2015; Beer *et al*., 2018; Akhbarizadeh *et al*., 2018; Abbasi *et al*., 2018). The presence of microplastics in all the samples tissues depict a high concentration of microplastics in the Mumbai waters which is an urban city of the country and is inhabited by very high population in and around the coastal areas. The total numbers of microplastic particles in the GI tract, gills, and muscles of *Johnius* spp. (n=188) are 1245, 1170 and 516 respectively. This shows that the number of microplastics is more abundant in the GI tract, followed by gills and muscle.

The total average numbers of microplastics particles per individual in 188 *Johnius* spp. are 6.62±1.68, 6.21±1.67 and 0.81±0.37 in GI tract, gills and muscles respectively. The presence of these many numbers of microplastics is comparatively much higher than almost all the previous studies. Thus it gives a sign of serious alarming threat of microplastics in the Mumbai coastal waters.

The one way ANOVA performed on the MPs found from the gut of fish from each month shows that microplastics content in the November month has significantly lower numbers in relation to all the other months. The microplastics from the month of January, February and March from the guts has no significant difference among them but have higher microplastics content than November month and lesser than the month of October, December and April. The highest microplastics were obtained in the month of October, December, and April and there are no significant differences in them (Table 2). The one way ANOVA performed for the gills microplastics numbers from all the month shows that the microplastics numbers in the October month are higher than all the months. February and April's months have the least microplastics content in the gills with no significant difference with the month of November, January and March but lesser than the month of December. But there was no significant difference of microplastics number for the month of November, December, January, and march (Table 2). The one way ANOVA result from muscles samples from all the months shows that the microplastics content g^{-1} muscles in the months of October, November, December, January, February, and march do not have any significant difference among them. But these all the months have lesser microplastics number in the muscle in comparison to the month of April (Table 2).

5.2. Microplastics distribution in the guts

The total mean microplastics number in the GI tract from our present study was found to be 6.62±1.68 particles per fish, which is comparatively very much higher than most of the previous studies. A study from Musa estuary in the Persian Gulf by Abbasi *et al.* (2018) has reported a mean MPs particles of 1.5, 2.3, 2.9, and 2.8 per fish in the gut of *Sillago sihama, Platycephalus indicus, Cynoglossus abbreviatus,* and *Saurida tumbil* respectively. A study by Lusher *et al*. (2013) reported 1.90± 0.10 particles out of 184 plastics ingested fish from the English Channel. Beer *et al*. (2018) in the Baltic Sea has reported 0.21± 0.47 particles in sprats (n=515) and 0.25± 0.52 particles per fish in herrings (n=299).

In the GI tract, the most abundant size group of microplastics particles are < 100 µm represented with 62%, 47%, 75%, 77%, 69%, 77%, and 72%, for the months of October, November, December, January, February, March and April respectively. And the next abundant sizes in the GI tract are represented by 100-250 µm, 250-500 µm, followed by 500-1000 µm and > 1000 µm for all the months. The lower size group of microplastics found in the gut may be accounted to the microplastics available in the local environment and to the mouth size of the fish, as out of 188 fishes sampled there were 137 numbers of fishes which are juveniles. Zhang *et al*. (2015) have described that the high proportion of small-sized particles are probably due to the fact that large plastic debris could breakdown into several small pieces. The smaller size of the microplastics in the environment is a major threat as its bioavailability to the fishes and other organisms, may come with a more serious threat of their translocation into the other body tissues and bioaccumulate. As these smaller microplastics has the potential to be further degraded to nano-materials and it becomes much easier for it to be translocated to the various organisms sharing the common food web. The average highest frequency of microplastic particles in the GI tract are also represented by size class of < 100 µm, followed by 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm respectively in all the months (Fig. 9).

The percentage different shapes of microplastics which is abundant in the GI tract was beads for all the months except for the month of November, where fibre was found to be more abundant (Fig. 19). The percentage pooled microplastics shapes from all the months are 44 %, 24 %, 15 %, 15 % and 2 % for beads, fibres, fragments, films and pellets respectively (Fig. 21). In the study reported by Avio *et al*. (2015) from Adriatic Sea in some commercial fish species (*S. pilchardus, S. acanthias, M. merluccius, M. barbatus and C. lucernus*), the shape of plastic particles isolated in the GI tract was mainly dominated by fragments (57%), followed by line (23%), film (11%) and pellet (9%).

The predominant colours in the GI tract was blue (27%), followed by black (23%), red (15%), translucent (11%), white (8%), brown and green of same proportion (7% each) and yellow (2%) respectively (Fig. 30). Ory *et al*. (2017) have described that some of the planktivorous fish seem to select microplastics that are visually similar to their diet (blue fragments).

The stomach content of *Johnius* spp. consisted mainly of shrimps and sediment in the present study and undoubtedly it is a demersal fish species. There is an assumption that the accumulation of plastics by fish and other organisms proceeds mainly through ingestion and is, therefore, dependent on the factors like feeding strategy and gut structure as well as the extent of local plastic pollution (Romeo *et al*., 2015; Jabeen *et al*., 2017). The microplastics thus may be accumulated directly and incidentally or deliberately while feeding on the water column or shifting through contaminated sediment, or indirectly through the consumption of contaminated prey (Cannon *et al*., 2016; Jovanović, 2017).

5.3. Microplastics distribution in gills

The mean MPs particles per gills from all the individual fish samples (n=188) was found to be 6.21± 1.67 particles which are less in comparison to gut MPs particles. The microplastics size in the gills are most abundantly distributed in the size class of < 100 µm where the percentage composition in the month of October, November, December, January, February, March and April are 60%, 53%, 86%, 80%, 76%, 85%, and 91% respectively. The preceding sizes in the gills on all the months are followed by 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm respectively. Sagawa *et al.* (2018) have reported from Hiroshima Bay that the ratio of smaller microplastics (< 1 mm) was found to be more highest in the bottom sediment in contrast to the larger microplastics (> 2 mm) which is highest in the beach sediments. And he has found that the smaller microplastics which is dominant in the bottom sediments, decreased in the order of the surface water and beach sediments for both FPS, PE and PP. So the predominance of smaller microplastics and the smaller size groups of microplastics in the tissues from *Johnius* spp. in the Mumbai waters can be accounted to it being a demersal fish species which are dwelling in the bottom water and subsequently their food items consisted mainly of benthic dwelling organisms.

The highest average frequency of microplastic particles in the gills is represented by size group of < 100 µm in all the months followed by 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm (Fig. 5). The proportion of microplastics shapes in the gills are largely contributed by beads except for the month of November 2018. The percentage contribution of beads in the gills is 29%, 23%, 36%, 44%, 58%, 69% and 72% for the months of October, November, December, January, February, March, and April respectively (Fig. 16). The % pooled microplastics shapes distribution from all the months are 41 %, 20 %, 19 %, 18 % and 2 % for beads, fibres, fragments, films, and pellets respectively (Fig. 18).

In the study the predominant colours in the gills were found to be black (28%), followed by blue (24%), red (15%), translucent (11%), brown (10%), white (8%), green (3%) and yellow (1%) respectively (Fig. 27).

5.4. Microplastics distribution in the muscles

The most abundant size class of microplastics distributed in the muscle is < 100 µm in all the months with percentage composition of 73%, 58%, 89%, 77%, 86%, 89% and 88% in October, November, December, January, February, March and April respectively. And these are followed in abundance by the size class of 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm (Fig. 12). The present study result is in consistent with the study reported by Li *et al*. (2018) where the proportion of microplastics in the oyster from Pearl River Estuary in China accounted mostly of microplastics < 100 µm (75.6% - 89.7%) of the total microplastics in each site. And the mean MPs particles in the muscle of *Johnius* spp. from all the samples (n=188) was 0.81±0.37 g^{-1} in our study. While from the recent study from the northeast of Persian Gulf has found the MPs abundance in the muscle of *P.indicus, Sphyraena jello,* and *Epinephelus voioides,* and the shrimp, *Alepes djedaba*, was of 1.85± 0.46, 0.57±0.17, 0.78± 0.22 and 0.80±0.12 g^{-1} respectively (Akhbarizadeh *et al*., 2018). So from the result, it is seen that the MPs g^{-1} in the muscle of *Johnius* spp. (0.81±0.37 g^{-1}) was comparatively much lesser than *P. Indicus* (1.85± 0.46 g^{-1}), but is higher than the other three species studied by Akhbarizadeh *et al*. (2018). Akhbarizadeh *et al*. (2018) have also reported that the microplastics numbers in the fish muscles were more in the benthic fishes which may be the case in our study as *Johnius* spp. which are commonly known as croakers are demersal fishes. The average highest frequency of microplastic particles in the muscles is also represented by the size group of < 100 µm, followed by 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm respectively in all the months as shown in Fig. 13. The monthly microplastics size distribution in the muscles is shown in a bar diagram shown in Fig. 14. In all the above-given figures the size distribution is abundant in the size class of < 100 µm followed by the subsequent size classes. The % pooled microplastics size class distribution from all the months are 77 %, 12 %, 6 %, 3 % and 2 % for < 100 µm, 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm respectively.

The percentage shapes of microplastics which is predominant in the muscles tissue were beads and are followed by fibres in all the months and the percentage of the other shapes vary in all the months (Fig. 22). In the muscle tissue, the bar diagram shows that the beads and fibres are more in number in all the months which are followed by the other shapes group (Fig. 23). The percentage of pooled microplastics shapes from all the months are 55 %, 24 %, 10 %, 10 % and 1 % for beads, fibres, fragments, films, and pellets respectively. While Karami *et al*. (2017) have found that the predominant types of shapes in the eviscerated flesh and excise organ of dried fish was fragments (85.7%) followed by films (10.0%), and filaments (4.08%) and no beads and foams were found in their samples.

The predominant colours in muscle pooled from all the months are black and blue which accounted 28 % each and is followed by red (18%) and all the rest colours in a small percentage (Fig. 33). Whereas the study in the oysters from Pearl River Estuary in China has a higher proportion of light microplastic colours (transparent, white, green and yellow) with 83% followed by deep colours (blue, brown, black and red) with 17% (Li *et al*., 2018). The study reported by Abbasi *et al*. (2018) has found that the overall colour was predominated by black or grey (71%) filamentous fragments, and blue and green fragments comprising about 12% of the pool microplastics in the fish tissues. The value of microplastics particles found in the edible portion of seafood is a serious potential risk to human health once they enter the food web (Farrell and Nelson, 2013). The study source on the distribution of MPs particles in the muscle of fish is very scarce that it is not so much known whether there are still contaminations in the edible portion of the fish from other waters. There need to more studies in the edible portion of the seafood as these can let us know the harmful risk of bioaccumulation of microplastics in the organisms. There need to be subsequent safety limits fixed for microplastics in the body of organisms and humans consuming this seafood, like all the toxic metals whose safety levels are already fixed and standardized. But it is not possible until a number of works are not done in the bioaccumulation of microplastics in the fishes and all other seafood.

5.5. Microplastics in adult and juvenile fish

The total sampled fish in the present study was 188 of which juvenile fish represented 151 in sample size and adult fish accounted with 37 numbers. The microplastics particles observed were specifically studied for their characteristics features distribution like size, shapes, and colours respectively in the juvenile and adult fishes.

The incidence of MPs size in gills, guts, and muscles of both the juvenile and adult fishes were predominated by < 100 µm, followed 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm.

The frequency of microplastics numbers in the gills and guts are considered more appropriate as numbers per fish since the whole GI tract and gills of the tissue are taken for the study of an individual fish. And the microplastics in the muscles tissue is considered to be more appropriate as g fish^{-1}. The mean numbers of microplastics found in the gills of juvenile and adult fishes were 5.52 and 5.58 numbers fish^{-1} respectively. From the result, it shows that the adult fish has higher microplastics numbers in their gills. The Pearson correlation coefficient of total fish length and gills microplastics from sampled fish (n=188) has shown that there is a correlation between the same. So in this regard, it can be concluded that microplastics numbers accumulation in the gills mainly depended on the local environment condition of the habitat of fish and its physiological activity.

The mean microplastics numbers in the guts of juvenile and adult fish are 6.30 and 5.73 numbers fish^{-1} respectively. The results show the microplastics numbers in the juvenile fishes were more in relation to the adult fishes. But the Pearson Correlation coefficient shows that there is no correlation between the number of microplastics in the guts and the fish length (n=188). The present study thus is in consistent with the study by Foekema *et al*. (2013) where it has been reported in their study that there are no relationships between the size of fish and microplastics ingestion. The mean microplastics g^{-1} of muscle in the juvenile and adult fishes are 0.75 and 0.56 respectively which, shows that the juvenile *Johnius* spp. has more microplastics numbers in the muscles in comparison to the adult fishes. But the Pearson correlation coefficient has shown that there is a positive correlation between the microplastics in the muscles and the fish length (n=188). The present study result of correlation relationship between muscles microplastics and fish length is thus in consistent with the study by Akhbarizadeh *et al*. (2018), in which he reported that there is a significant positive correlation ($p < 0.01$) between fish length and collected microplastics from fish muscles.

The most serious is that the Pearson correlation coefficient test performed on the total body length of the fish and the microplastics from each tissue show that there is no significant correlation between microplastics numbers in the guts and gills to the body length. Whereas the correlation result by Pearson correlation coefficient shows that there is a positive correlation between the body length and microplastics numbers in the muscles tissues from *Johnius* spp. The present study thus proved the potential of microplastics to be bioaccumulated in the fish tissues with times mainly the edible portion. The guts and gills do not show any positive correlation of microplastics numbers and fish body length may be concluded to the fact that the microplastics retention and attachment in the guts and gills respectively will depend upon the local environment intensity of pollution. As microplastics can be excreted from the gut and also can be adhered or detached subsequently from the gills to the environment they are exposed to.

The microplastics in the shapes and colours found in the juveniles and adult fishes tissues were of the same nature where the predominant shapes were beads and the colours are predominated mainly by the black and blue colour in all the tissues.

6. SUMMARY

Microplastics were present in 100% of the sampled *Johnius* spp. (n=188) in all the three tissues (gills, guts, and muscles) which gives the evident that the coastal water of Mumbai is heavily polluted by microplastics, and plastics debris going to the waters being the source. The microplastics were smaller in size that is < 100 µm mostly comprising of beads were the predominant size group in all the tissues which is very much critical from the seafood and environment health point of view. Black and blue microplastics colours were found to be more predominant than all the other colours observed in this study from all the fishes.

The total average numbers of microplastics particles per individual in 188 Johnius spp. are 6.62±1.68, 6.21±1.67 and 0.81±0.37 in GI tract, gills and muscles respectively. In the present study, the number of microplastics in the gut was more followed by gills and muscles tissues respectively. The one way ANOVA result at the significance level ($p< 0.05$) shows that there is a marked monthly/temporal variation of microplastics in all the fish tissues sampled in every month. The overall percentage of microplastics size distributed in the gills from all the months is predominated by < 100 µm and followed by 100-250 µm, 250-500 µm, 500-1000 µm and >1000 µm with 73 %, 15 %, 6%, 4 %, and 2 % respectively. The overall percentage of microplastics size distributed in the GI tract from all the months is predominated by < 100 µm and followed by 100-250 µm, 250-500 µm, 500-1000 µm and >1000 µm with 68 %, 15 %, 9%, 5 %, and 3 % respectively. In the muscles the total % of microplastics size class distributed in all the months are 77 %, 12 %, 6 %, 3 % and 2 % for < 100 µm, 100-250 µm, 250-500 µm, 500-1000 µm and > 1000 µm respectively.

The total percentage of microplastics shapes distribution from all the months in the gills are 41 %, 20 %, 19 %, 18 %, and 2 % for beads, fibres, fragments, films, and pellets respectively. In the GI tract, the total percentage of microplastics shapes from all the months are 44 %, 24 %, 15 %, 15 % and 2 % for beads, fibres, fragments, films, and pellets respectively. And the total percentage of microplastics shapes from all the months in the muscles are 55 %, 24 %, 10 %, 10 % and 1 % for beads, fibres, fragments, films, and pellets respectively.

In the present study the predominant colours in the gills were found to be black (28%), followed by blue (24%), red (15%), translucent (11%), brown (10%), white (8%), green (3%) and yellow (1%) respectively. The predominant colours in the GI tract were blue (27%), followed by black (23%), red (15%), translucent (11%), white (8%), brown and green of same proportion (7% each) and yellow (2%) respectively. And the total percentage of microplastics colours in the muscles from all the months are black and blue which accounted 28 % each and is followed by red (18%), brown (10%), translucent (6%), white (6%), yellow (2%) and green (2%).

From the total sampled fish (n=188) in the present study, the juvenile fish represented 151 in numbers and adult fish accounted with 37 numbers. The incidence of MPs size in all the three tissues was predominated by < 100 µm in both juvenile and adult fish and is followed by 100-250 µm, 250-500 µm, 500-1000 µm, and > 1000 µm). The total number of microplastics in the gills of juveniles and adults are 5.52 and 5.88 nos. fish $^{-1}$ respectively. Whereas in the GI tract the frequency was 6.30 and 5.73 nos fish^{-1} for the juveniles and adult fish respectively. And the average microplastics number per g^{-1} of muscle in the juvenile and adult fishes are 0.75 and 0.56 respectively. Both in the juveniles and adult fishes the beads represented more in percentage with black and blue being the most dominant colours. No significant correlation was observed between the microplastics numbers in the gills and guts respectively to the total body length of fish. But a significant positive correlation was there for the microplastics numbers in the muscles tissue to the total fish length.

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ABBREVIATIONS

MPs	:	Microplastics
g	:	Gram
µm	:	Micro meter
mm	:	Millimetre
m	:	Meter
mL	:	Millilitre
g^{-1}	:	per gram
km^2	:	Kilometre square
n/m^3	:	number per cubic meter
MT	:	Metric tonnes
M	:	Molar
%	:	Percentage
^oC	:	Degree Celsius
min.	:	Minutes
hrs	:	Hours
dia.	:	Diameter

Hz	:	Hertz
Rpm	:	Revolutions per minute
KOH	:	Potassium Hydroxide
NaI	:	Sodium Iodide
GF	:	Glass filter
MFV	:	Marine fishing vessel
OAL	:	Overall length
GPS	:	Global Positioning System
GI tract	:	Gastrointestinal tract
QA	:	Quality assurance
QC	:	Quality control
ALDFG	:	Abandoned lost or otherwise discarded fishing gears
WWTPs	:	Wastewater Treatment Plants
FPS	:	Foamed Polystyrene
PE	:	Polyethylene
PP	:	Polypropylene
PAHs	:	Polycyclic Aromatic Hydrocarbons

PBDE	:	Polybrominated Diphenyl Ether
NP	:	Nonylphenol
BPA	:	Bisphenol-A
GESAMP	:	The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection