Journal of Sustainability Science and Management
Volume 17 Number 5, May 2022: 179-199
eISSN: 2672-7226

MICROPLASTICS IN MARINE BENTHIC FILTER FEEDER: A REVIEW ON THE OCCURRENCE, ROUTES OF INGESTION, METHOD OF EXTRACTION AND EFFECTS TO THE ECOSYSTEM

JEMIMAH SHALOM[1], NURZAFIRAH MAZLAN[2]*, MIFTAHUL JANNAH[2], SAFAA NAJAH SAUD[3] AND ONG MENG CHUAN[1]

[1]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.*
[2]*Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.*
[3]*Faculty of Information Sciences and Engineering, Management and Science University, Seksyen 13, 41000, Shah Alam, Selangor, Malaysia.*

**Corresponding author: nurzafirah@ums.edu.my*
Submitted final draft: 18 May 2021 *Accepted: 23 September 2021* http://doi.org/10.46754/jssm.2022.05.015

Abstract: Plastic pollution has been a global concern as it contaminates the earth. Hence, the purpose of this review discusses the rate of plastic pollution and how it has accumulated in the various benthic feeders in the ocean. This review focuses on the occurrences of microplastics, routes of ingestion, method of extraction and the effects of plastic on marine ecosystems. The marine invertebrates considered in this study include filter and benthic feeders. Hence, a compilation of 54 published studies, reviewed articles, conference materials and books from related topics were reviewed to identify the gaps in the research. Microplastics were identified with a scheme to colour, size, shape and polymer type. Techniques to extract these microplastics were also described. Findings from this review showed that the animals tended to accumulate large amounts of denser forms of microplastics. Therefore, this review will be a fundamental touchstone for the studies regarding microplastics in marine invertebrates.

Keywords: Microplastics, marine invertebrates, benthic feeders, characteristics.

Introduction

Plastic pollution in marine invertebrates is prominent as the concentration of plastics in the ocean has increased tremendously. Plastic pollution can disrupt both the animals and humans' food chains as it moves up the food web through consumption.

A study conducted by Ritchie and Roser (2018) showed how there is an increment of global plastic production from approximately 2 million tonnes in 1950 to 7 billion tonnes in 2015. Hence as the production rate increased, the concentration of macro and microplastics in the ocean has increased. It went from 400 tonnes in 1950 to 1.18 million tonnes in 2020 and from 0 tonnes in 1950 to 594 thousand tonnes in 2020.

These plastic substances have greatly affected the marine environment, especially those organisms that cannot move as quickly as others to migrate to cleaner waters. Plastic pollution does not need to be substantial plastic bags that can cover and choke organisms, but it can be the smallest pieces with dire consequences in the long run.

According to Kubowicz and Booth (2017), the current problem regarding plastic pollution is the stabilisers present in the plastics. The stabilisers cause plastic substances to be more durable and last longer. The plastics in question are known as single use plastics. These plastics include plastic bottles, bags and straws. These products have high production rates due to their convenience and the fact that they are cheap. However, these products have low degradation rates. As time goes by, the plastic products do not degrade but break into smaller pieces. Hence, they continue to harm both in their original sizes and as they fragment into smaller pieces. Therefore, despite the advantages of plastics, we should be aware of its dangers if they are not disposed of properly. Examples of these single-use plastics can be seen in Figure 1.

Figure 1: Single-use plastics
Figure obtained from Calgary (2018)

Furthermore, Chamas *et al.* (2020) had conducted a study to recognise how the different types of plastics in the environment have their unique degradation rates. A specific surface degradation rate (SSDR) was used to determine the half-lives of high-density polyethene (HDPE) in the marine environment.

Figure 2 explains the different degradation rates according to the different shapes of plastics from the HDPE plastics. Each shape of plastics has its own configuration; hence some plastics degrade faster than others. In this situation, film-type plastics degrade faster but beads-type microplastics take a longer time to degrade.

Besides that, Haward (2018) also mentions how plastic pollution in the marine environment has derived from land-based pollution. The data stated that plastic pollution is approximately 4.8 to 12.7 million metric tonnes of plastic annually. These microplastics are continuously being consumed by marine life at an increasing rate. The plastic products can also degrade into smaller sized plastics known as microplastics (Zhang *et al.,* 2017). Microplastics tend to be categorised as plastic substances less than 5 mm in diameter (Law & Thompson, 2014). Examples of these microplastics can be seen in Figure 3. Due to the movement of the ocean currents, the surface distribution of microplastics varies throughout the ocean. Hence, the plastic detection process of the microplastic trend in the ocean is challenging to identify.

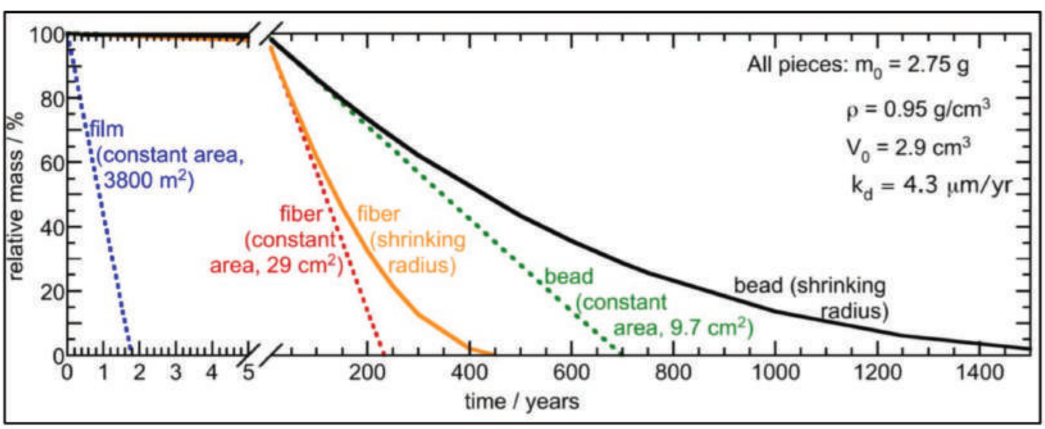

Figure 2: The degradation rate of different forms of plastics
Figure obtained from Chamas *et al.* (2020)

Figure 3: Examples of microplastics
Figure obtained from Law and Thompson (2014)

Mechanism of the Literature Retrieval

A thorough literature review was done for this research paper. The search resulted in the compilation of current research articles and article reviews on related studies regarding microplastics in marine benthic filter feeders, prioritising the occurrence, routes of ingestion, method of extraction and effects of these microplastics to the ecosystem. The search engines that have been used for the literature retrieval include Science Direct, PubMed, ISI. Web of Knowledge and Google Scholar. The general scope of the reviewed publications is focused from 2014 to 2020.

The search terms and the Boolean operator used for the articles' compilation can be seen in Figure 4. The keywords used include microplastics and invertebrates, microplastics and effects and microplastics and marine filter feeders to identify related articles.

Based on the results obtained from the searches conducted, the articles that were filtered and selected based on the topic of interest. Hence, we could exclude papers regarding microplastic concentrations found in terrestrial organisms, microplastics that located in the soil and freshwater organisms and articles that were not in the English language.

Search Results and Description of Studies

All the studies that have been found were filtered through the specific and related topics before it has been reviewed for this article. For this article review, a full 54 articles were chosen. From the chosen articles, 88% were of published research articles. Based on the Scopus data that has been accessed on 24th September 2020, extensive research was carried out on the topic of plastic and/or microplastics according to the statistical analysis provided in Figure 5 (a-d).

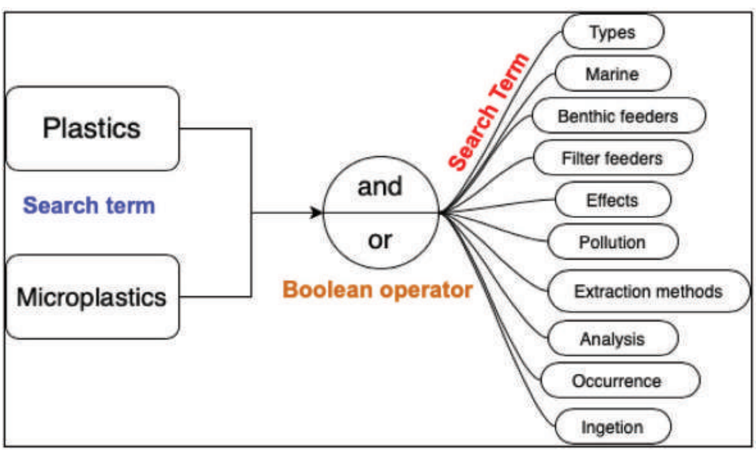

Figure 4: The search terms and the Boolean operator used for the compilation of the articles

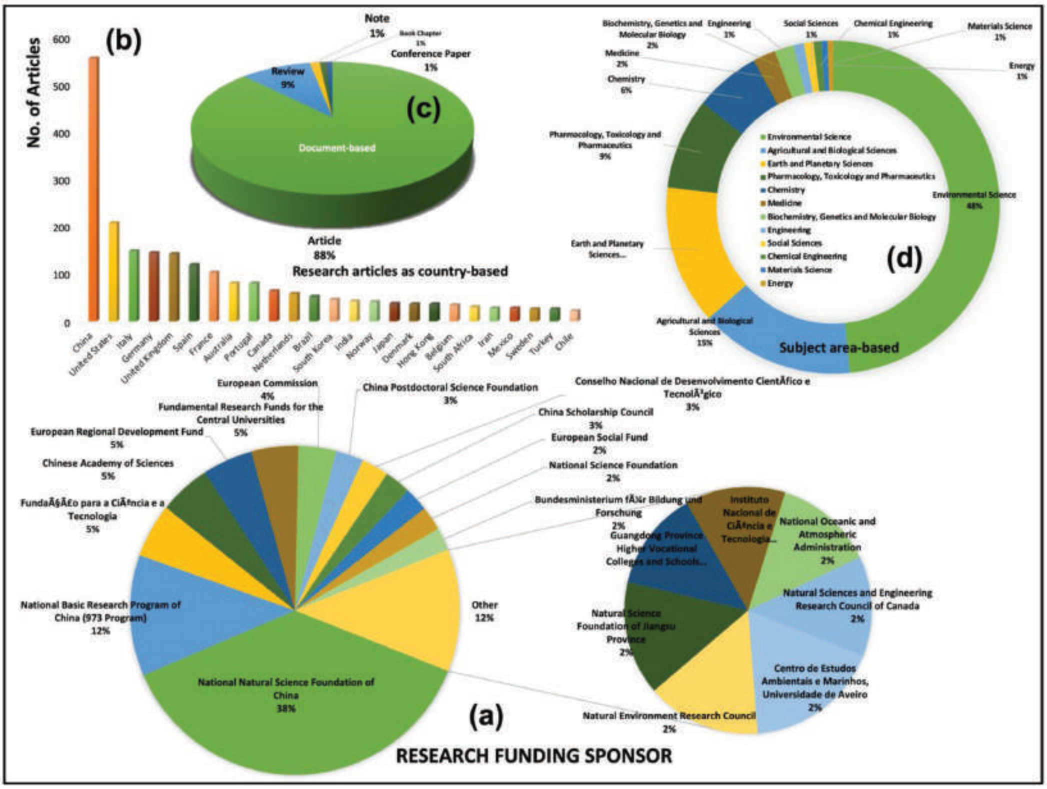

Figure 5: Plastic and microplastics statistical analysis on (a) research funds, (b) research article: Country-based, (c) document-based and (d) subject area-based

Occurrences of Microplastics

Plastics can be distinguished into four forms: The sizes, colour, shape and polymer types ingested by both the marine filter organisms and benthic feeders.

Size of Microplastic

From the 54 papers reviewed, most of the articles stated that the papers' microplastic considered plastics with sizes less than 5 mm. Besides that, the studies show that larger particles of plastics tend to accumulate in the organisms (Ryan, 2016). The differences in microplastics sizes are dependent on the sources of these plastics. Some forms of plastic polymers degrade faster than others. The degradation rates are dependent on the conditions of the environment and the type of plastic polymers itself.

The size of plastics predominantly affects the rate and accumulation of said plastics in marine organisms. Depending on the organisms, some organisms tend to take up larger sized microplastics compared to other organisms. Gonçalves *et al.* (2019) studied the consumption and excretion rate of microplastics by the Mediterranean mussel (*Mytilus galloprovincialis*). In this study, the organisms used were identified to commonly have 2 µm, 5 µm, 6 µm and 10 µm size bits of various microplastics. However, this study focused on two microplastic concentrations: 10 Microplastic mL^{-1} and 1000 Microplastic mL^{-1}.

The experiment shows that the organisms could reduce the concentration of 6 and 10 µm sized of microplastics from the water column. Larger sized microplastics were located in the digestive tract of the organisms within five minutes of exposure. The concentration of microplastics tends to accumulate as the duration of exposure increased. This study has also shown that there has been no visible size preference for removing the microplastics from the water column.

Furthermore, a study by Van Cauwenberghe *et al.* (2015) shows how the blue mussel (*Mytilus edulis*) acts as a selective feeder as the microplastics sizes that have been ingested are in a range of between 10-30 µm. The exposure experiment that was conducted used ten replicates of the blue mussels. The mussels were exposed to polystyrene microspheres of three different sizes: 10 mm, 30 m and 90 mm. Plastic retention in the blue mussel differed according to the particle selection process of the organisms. Plastic retention is dependent on the standard filtration rate of mussels. The higher the filtration rate, the higher the presence of microplastics accumulated in the mussels. The blue mussel's general filtration rates are approximately 0.55 µg/g/L/h (Zhang *et al.,* 2019).

Colour of Microplastic

We have identified that there are various colours of microplastic obtained from the compiled journals. The colours ranged from transparent, red, blue, yellow and other colourful microplastics. The colours of the microplastics can be seen in Figure 6. The colour of the microplastics is a common method to distinguish the samples using a standard dissecting microscope.

A study by Nelms *et al.* (2018) also determines the microplastic samples' colour using the naked eye. This study also used FT-IR to reconfirm the microplastic samples. Samples with a higher confidence level than 70% and dependable spectra matches were accepted for the colour analysis. Due to the colour pigment of these microplastics, it is presumed to be ingested by marine organisms. As the colour may make the organisms assume it to be food or other organic matter (Xiong *et al.,* 2019). A study by Davidson and Dudas (2016) also explains how microplastics tend to appear in colours that are not usual in the environment. Hence the colours bright colours like red and blue tend to be standard colours of the collected microplastics.

Shape of Microplastic

The microplastics' shape can be used to identify where they are derived from (Chen *et al*., 2020). According to Chen *et al.* (2018), it was stated that pellets and beads, which are known to be primary microplastics, are originally produced industrially from care products. Nevertheless, secondary microplastics such as fibres and filaments are derived from fishing nets. Fragments and films are then fragmented from plastic bags and wrapping materials.

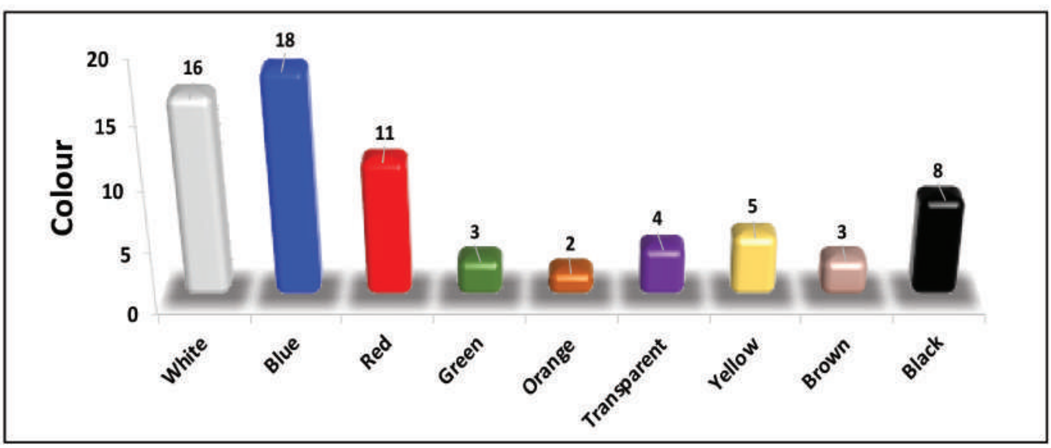

Figure 6: Occurrence of extracted microplastic colours from compiled articles

A study by Davidson and Dudas (2016) also mentions how sand and glass particles can be differentiated from the microplastic samples. Whereby, sand and glass are shaped geometrically in appearance. They often hold edges and corners that are squared to be easily distinguished from microplastics. Hence the shape is vital to determine the type of materials in the samples.

Because fibres and filaments are considered dense microplastics, there are higher concentrations of fibres and filaments in marine invertebrates (Karlsson *et al.,* 2018). There is only a small sum of transparent films, beads and other substances found in these marine invertebrates. The shapes of these microplastics also act as a component to determine the relationships between the microplastics and the marine communities. Microplastics that have irregular or needle-like fragments attach more quickly to marine organisms causing increased levels of both internal and external damages compared with the smoother and the round-shaped microplastics. The compiled occurrences of the different microplastic shapes can be seen in Figure 7.

Polymer Types

Based on a study by Gewert *et al.* (2015), it has been stated that 60% of all debris located on the ocean is plastics. The plastics polymers dumped in the ocean exhibited physical stress, weather degradation, sunlight and oxidation. The degradation process of each of the plastics is vital to understand all the environmental threats. The degradation rates and the longevity of the plastics are dependent on the additives added to it. Some of these plastics that degrade into microplastics include Polyethylene terephthalate (PET), polyvinyl chloride (PVC) and Polystyrene (PS).

Polyethylene Terephthalate (PET)

Polyethene terephthalate (PET) is a plastic polymer with substantial and stiff synthetic fibres. It has a chemical formula of ($C_{10}H_8O_4$) (Qureshi *et al.,* 2020). According to Weber *et al.* (2018), Polyethylene terephthalate (PET) was discovered in the amphipods (*Gammarus pulex*). An example of the plastic found can be seen in Figure 8. There were both juvenile and adult amphipods exposed to irregularly shaped, fluorescent PET fragments for 24 hours. The results showed that the juveniles ingested more microplastics compared to the adults.

The amphipod (*Gammarus pulex*) is known to be a detritus feeder, so it usually feeds on non-digestible substances (Vander Vorste *et al.,* 2017). Therefore, they ingest the PET like feed in the water. The easy consumption of PET by

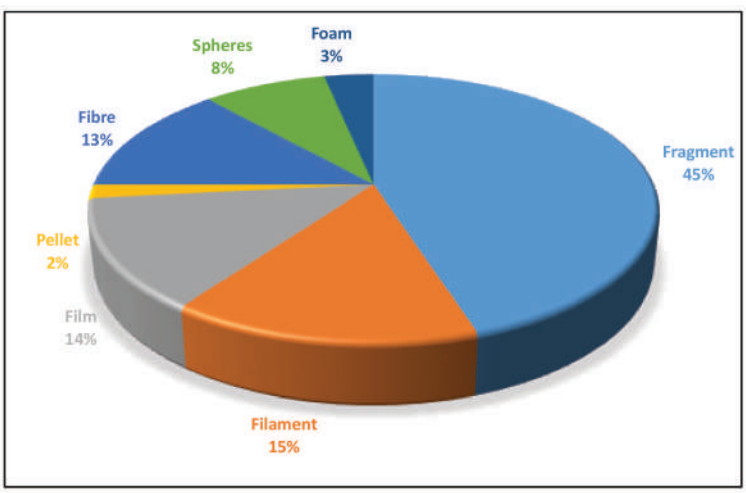

Figure 7: Occurrence of the different microplastics shapes from compiled articles

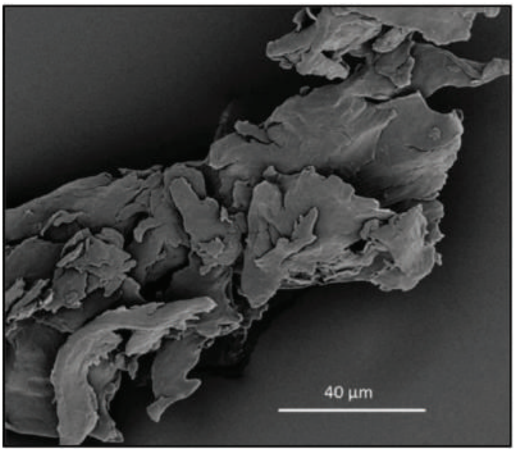

Figure 8: PET plastic in an amphipod (*Gammarus pulex*)
Figure obtained from Weber *et al.* (2018)

these organisms is because they might have a lower sensitivity to synthetic particles. Another study by Karlsson *et al.* (2017) shows that the blue mussel (*Mytilus edulis*) had ingested PET. This study compares the concentration of microplastics in the water, sediment and the mussels. The concentration comparison between these three showed that due to the mussels' filter-feeding habits, the microplastics concentration in the mussels were higher compared with the surrounding sediment and water.

Polyvinyl Chloride (PVC)

Polyvinyl chloride (PVC) is a versatile thermoplastic polymer which has a chemical formula of $(C_3H_3Cl)n$ (Li *et al.,* 2020). Based on Rist *et al.* (2016), it can be seen that the Asian green mussel (*Perna Viridis*) has taken up PVC from the sediment as it was exposed to the plastic in a laboratory experiment. The samples were exposed to four different concentrations (0 mg/l, 21.6 mg/l, 216 mg/l, 2160 mg/l) of PVC plastic for periods of two hours a day. It was confirmed that this species takes up this plastic when PVC was identified in this species' faeces. The Asian green mussel (*Perna Viridis*) are suspension feeders. They are commonly known to ingest PVC as it is a high-density plastic and is often found on the seafloor (Wright *et al.,* 2013).

Polystyrene (PS)

Polystyrene (PS) is a synthetic hydrocarbon polymer. It has a chemical formula of $(C_8H_8)_n$. According to Sussarellu *et al.* (2016), it was confirmed that the pacific oyster (*Crassostrea Gigas*) had ingested microspheres which were confirmed to be polystyrene microplastics.

The ingestion is because the pacific oyster (*Crassostrea Gigas*) is a filter feeder (Cole & Galloway, 2015); hence they ingest the microplastics in the course of feeding. In this experiment, the oyster was exposed to two sizes of polystyrene microplastics, which are 2 µm and 6-µm. However, there is higher ingestion of the 6-um compared to the 2- µm microplastics. Hence, it could be due to the oysters' particle selection mechanism, which has the highest efficiency for particles that are 5 to 6-µm in size (Ward & Shumway, 2004). Figure 9 shows examples of these polystyrene beads in the pacific oyster (*Crassostrea Gigas*).

A study by Thushari *et al.* (2017) also shows that there is an ingestion of PVC by the striped barnacles (*Balanus amphitrite*), the periwinkle (*Littoraria sp.*) and the rock oyster (*Saccostrea forskalii*). The results describe

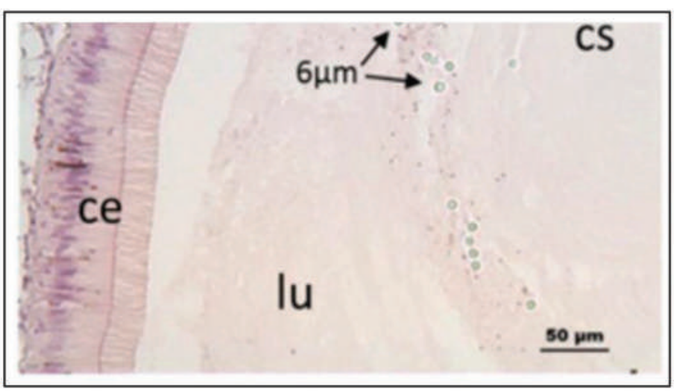

Figure 9: Polystyrene beads in the pacific oyster (*Crassostrea gigas*).
ce: ciliated epithelium, cs: crystalline style, lu: lumen
Figure obtained from Sussarellu *et al.* (2016)

that the microplastic accumulation in filter feeders is the highest. The highest average microplastic accumulation was seen in the rock oyster samples, which is 0.57 particles/g, while the lowest was observed in the periwinkles, which are 0.17 particles/g. There were large concentrations of PET and Polyamide (PA) plastic; there were low concentrations of PS in the samples.

Method of Microplastic Extraction

Microplastic extraction is a very delicate process. Hence, a specific solvent must be used to ensure that the microplastic in an organism does not dissolve (Lusher *et al.,* 2017), but only the organisms tissue will undergo digestion. The standard method of microplastic extraction includes filtration.

Filtration

According to Thushari *et al.* (2017), the method used to extract the samples is filtration. Before the filtration process, the samples of striped barnacles (*Balanus amphitrite*), the periwinkle (*Littoraria sp.*) and the rock oyster (*Saccostrea forskalii*) were placed in 20 ml of 69% of concentrated nitric acid for 24 hours. The samples were then boiled for two hours and it was dissolved in 200 ml of filtered deionised water. The solution with the diluted samples was then vacuum filtered by a pre-weighed five µm cellulose nitrate membrane. There were also blank extractions performed. This was done to remove contaminants that may be present in the chemical reagents used.

Furthermore, according to Xu *et al.* (2020), a microplastic extraction from four barnacle species was also conducted using the filtration method. The samples were first cleaned with deionised water and were measured. The tissue of the barnacles was separated and the dissecting procedure was carried out. The chemical digestion was aligned with Rochman *et al.* (2015), whereby five tissues of barnacles were first placed together as one replicate in a 100 ml beaker. As forceps were used, deionised water was used to clean it. This was done to prevent microplastics from sticking to the forceps. The samples were then digested with 60 ml 10% potassium hydroxide. Moreover, left in an incubator at 40°C for 48 hours, it was then filtered.

Besides that, a study done by Karlsson *et al.* (2017) collected 23 samples of mussels. The tissue from the mussel was extracted and kept in a glass container. 15 ml of a homogenised buffer was added. The samples' incubation duration is for 60 minutes at 60°C for the proteins in the tissue to denature. The samples were then grounded using a mortar. Then 500 µg/mL of proteinase K was added, with $CaCl_2$ to activate the enzymes. The samples were then left for incubation for more than 2 hours at 50°C and shaken for 20 minutes. The samples were incubated again but at room temperature with 30 ml of 30% H_2O_2 to degrade the remaining chitin. The samples were then vacuum-filtered until dry and then freeze-dried and grounded with a mortar for microplastic analysis. The microplastic filter extraction set-up for plastic density separation can be seen in Figure 10. In these three examples, after the samples were

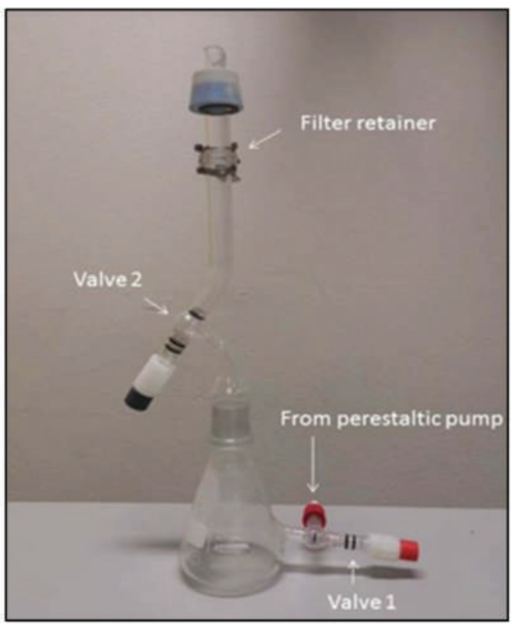

Figure 10: The microplastic filter extraction set-up for plastic density separation
Figure obtained from Karlsson *et al.* (2017)

filtered, the filter paper's microplastics were collected for further analysis.

Routes of Microplastic Uptake

The routes of microplastic uptake by these marine organisms are different. This is because some marine organisms are more exposed than others with their unique feeding habits. Standard uptake methods by marine invertebrates include filter-feeding and deposit-feeding (Setälä *et al.,* 2016). The potential routes and biological interactions of microplastics can be seen in Figure 11.

Filter Feeding

Filter feeding is a method whereby marine invertebrates' strain and filter the water while consuming small organisms such as plankton and other food particles. This feeding method commonly occurs with organisms such as oysters and sea cucumbers (Cunha *et al.,* 2019).

A study by Zhu *et al.* (2020) was conducted on the bioaccumulation of microplastic in the Pacific oyster (*Crassostrea Gigas*). From the samples collected, it was seen that the microplastic tend to accumulate in the gills and the mantle of the oyster. There was also a smaller amount of accumulation in the digestive glands of the oysters. The results showed that the type of microplastics that were recognised in gills and the mantle were mainly fibres while the digestive glands had bits of fragment-type microplastics. The examples and the collection of these microplastics can be seen in Figure 12.

Another study also determined accumulation of microplastics in oysters but it compared and calculated the microplastic samples' frequency in the sediments and the soil at the six major seaports. Knowing that oysters are filter feeders, the results showed a higher accumulation of microplastics in the oysters than the sediment itself. Similar to the previous research, it was concluded that the fibre-like microplastics were found in the oysters and sphere-like microplastics were recognised in the sediments (Jahan *et al.,* 2019).

In this study, it was determined that the ports with higher rates of activity with a lot more ship traffic have higher concentrations of plastics in both the sediment and the oysters. These activities include tourism activities, recreational boating and also urban activities. With the data collected, a correlation test was carried out.

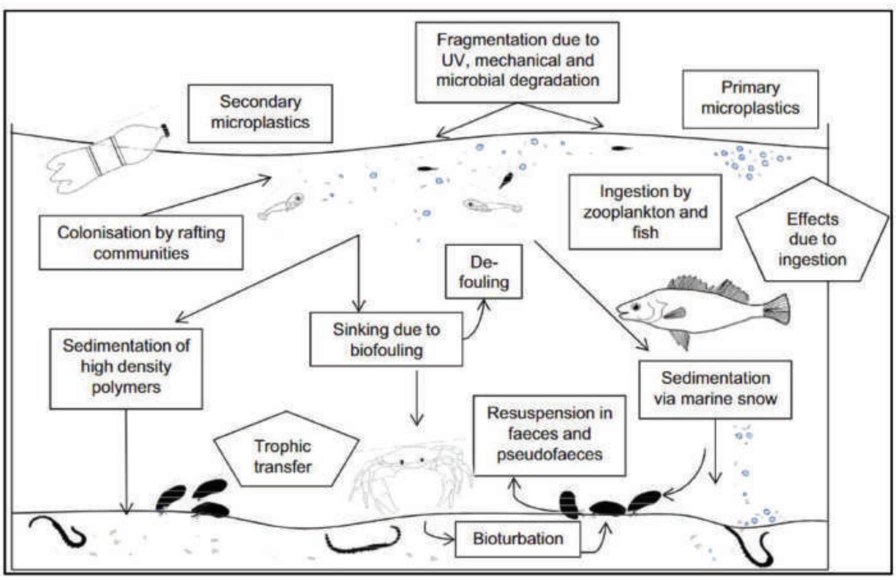

Figure 11: Potential routes of microplastic in the ocean
Figure obtained from Wright *et al.* (2013)

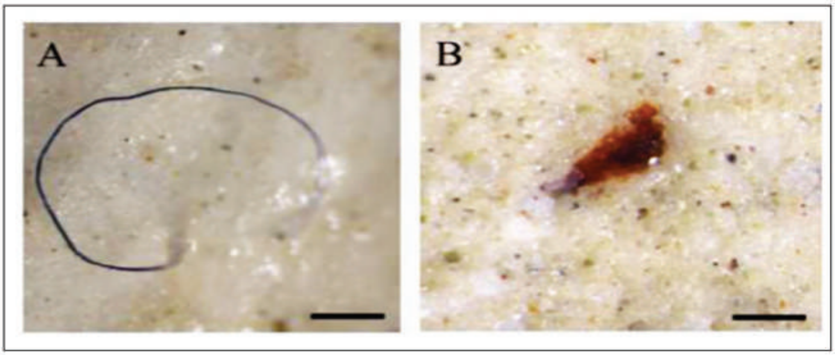

Figure 12: Shows the forms of plastic collected (A) Thread-type microplastic, (B) Fragment-type microplastic
Figure obtained from Zhu *et al.* (2020)

With that, a correlation was identified between the abundance of plastics and the size of the sediments. The correlation is related to the frequency of microplastics identified in the oysters (Figure 13).

Another organism that is also exposed to the uptake of microplastics is the mussels. These organisms are also filter feeders. In a study by Li *et al.* (2020), microplastics were exposed to the blue mussel (*Mytilus edulis*) to determine how these microplastic get taken up by their filter-feeding and harm these organisms. As all these studies have shown, these organisms trap the microplastics in their gills and guts through filter-feeding. Hence, the microplastics can quickly accumulate and affect these organisms.

Based on a study by Setälä *et al.* (2016), the filtering capacity of the blue mussel (*Mytilus edulis*) was examined by identifying the concentrations of algae in the mussel. The method used was adapted from the study done by Riisgård *et al.* (2011). It was noted that the blue mussel could filter large amounts of water when an algal concentration is present. Based on their study, the blue mussel was observed to have ingested an average of 635 beads each.

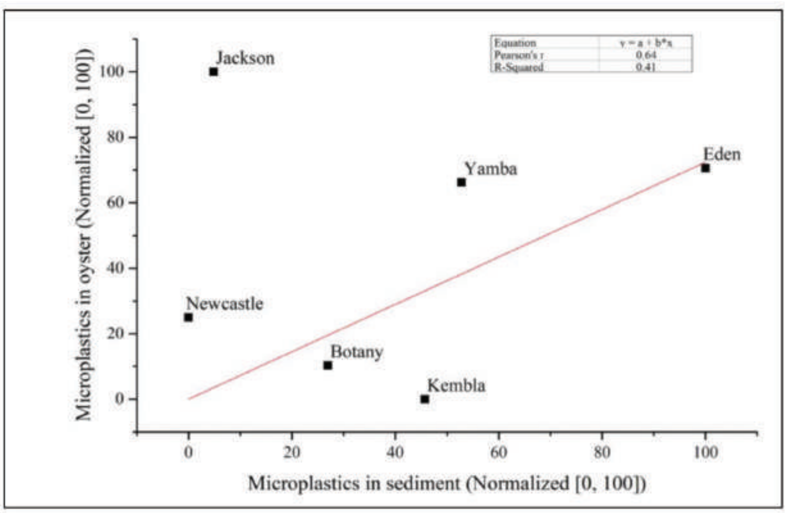

Figure 13: Correlation between the frequency of microplastics in the sediments and the oysters in the selected seaports
Figure obtained from Jahan *et al.* (2019)

Setälä *et al.* (2016) concluded that the ingested beads increased as the concentration of beads increased. In the highest concentration, 250 beads ml^{-1}, beads were present in both bivalves and mysids, but less than half of the deposit-feeding Amphipods (*Monoporeia affine*) had ingested the beads. The study summarised that the plastic-type beads were quickly taken up by filter-feeding organisms found at the lower columns in the sea. At the lowest concentration of beads, the variability of microplastic concentrations between the different feeding process was the highest. In the bivalves, 90% of the organisms have shown to have ingested beads. Therefore, this means that the bivalves are highly effective at filtering capacity in comparison to other organisms. Hence the bivalves showed that despite low concentrations of the plastic particle, there is still an uptake of the microplastic beads. The examples of these plastics can be seen in Figure 14.

Deposit Feeding

Deposit feeders are organisms that prey on smaller organisms such as zooplankton and phytoplankton present on the surface of the water or organic matter that settles on the seafloor (Rühl *et al.,* 2020). Organisms that commonly use this method of feeding include flounder fish, crabs and sea cucumbers.

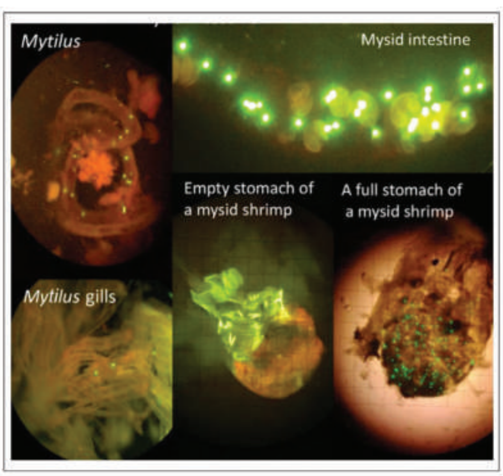

Figure 14: The microscopy images of the ingested microplastics
Figure obtained from Setälä *et al.* (2016)

A study by Brennecke *et al.* (2015) investigated microplastics' retention in the Fiddler crab (*Uca rapax*). The results obtained also corresponded with other studies regarding deposit feeders and how these organisms ingest and retain microplastics. Microplastics tends to contaminate the body and tissue, as the fiddler crab respires and feeds in the water. The study explains that the retention of microplastics is mostly accumulated in the hepatopancreas and the gills. Hence, microplastics were more often found in this organ of the fiddler crab.

Figure 15 shows the movement of these microplastics in the Fiddler crab (*Uca rapax*). It begins as these microplastics are ingested and move along and the oesophagus. As it reaches the stomach, it gets transported through the midgut and moves into the hepatopancreas. There the hepatopancreas can be an area where the microplastics can start to accumulate if they are ingested.

Hence during the respiration process, the microplastic can be entangled in the gills. However, what is unclear in this study is why

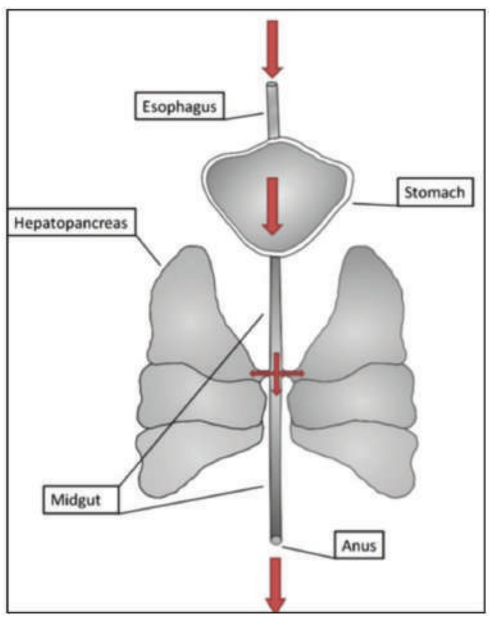

Figure 15: The elementary anatomy of the fiddler crab's digestive system. The red arrows on the figure describe the assumed movement of the microplastics
Figure obtained from Brennecke *et al.* (2015)

the microplastics are being accumulated and other substances such as sands and grains are not entangled or gathered in the organs.

The results also corresponded with other studies regarding deposit feeders and how these organisms retain the microplastics and how microplastics' presence correlates with the accumulation of polychlorinated biphenyls (PCBs). A study by Graham and Thompson (2009) involves four species of both deposit and suspension feeders, which include the Florida sea cucumber (*Holothuria floridana*), grey sea cucumber (*Holothuria grisea*), Orange-footed Sea Cucumber (*Cucumaria frondosa*) and stripped sea cucumber (*Hyonella gemmate*). This study is significant as most studies before were focused on marine vertebrates, but marine invertebrates have not been studied as much.

Based on Graham and Thompson (2009) study, fragments have been commonly found in the benthos. Therefore, organisms that are on and in the seafloor are susceptible to ingesting said plastics. Hence, this study of species is essential because the benthic holothurians rake, shovel and scoop large amounts of sediment to extract nutrients and food particles in their mouths. Hence, we can quickly identify the number of microplastics ingested according to the region's rate of plastic pollution.

According to Van Cauwenberghe *et al.* (2015), another deposit feeder was studied, which was the lugworm (*Arenicola marina*). Their study considers the parameters which will facilitate the uptake of microplastics by the organisms.

These parameters include the shape, colour, size and density of the plastic substances. These factors identify the location of these plastic particles in the water. Hence organisms in different levels of the water column will have different levels of accumulation of the different microplastic types.

Lower density particles float and are more available to organisms like fish. However, high-density substances sink and accumulate in the sediments to be ingested by deposit feeders.

Method of Microplastics Analysis

There are many methods of microplastic analysis. The methods that are commonly used in the various microplastic analysis include Fourier transform infrared (FT-IR) spectroscopy, Raman Spectroscopy, ImageJ Software, MicroCamLab (Microsoft Word) and a Scanning electron microscope.

Fourier Transform Infrared (FT-IR) Spectroscopy

The Fourier transform infrared (FT-IR) spectroscopy is a prevalent technique used to determine the polymeric composition of microplastics from any samples. According to Chen *et al.* (2020). Each sample of microplastic collected has a unique composition, which in turn produces unique spectral images accordingly. By comparing the libraries of spectral images already in hand, the plastic polymer of the microplastic samples collected can be determined (Wang & Wang, 2018).

A study by Zhu *et al.* (2020) also used FT-IR to identify the microplastics' composition. The spectrum range for the FT-IR was calibrated for both transparent and semi-transparent particles. An attenuation total reflection mode was coordinated for coloured particles. The collected samples were also compared with a library to identify and determine the polymer type. Examples of the component analysis using FT-IR can be seen in Figure 16.

RAMAN Spectroscopy

A study by Thushari *et al.* (2017) used RAMAN spectroscopy to detect and analyse the microplastic samples. The Raman spectrometer was attached to the Olympus 1 × 71 microscopes and attached to the thermo-electrical cooled charge-coupled device detector. The Nova software controls the spectrometer.

The sample spectra observed for each sample were compared to a reference spectrum of the significant plastic types in the present library. The example of the polystyrene sample using Raman spectroscopy can be seen in Figure 17.

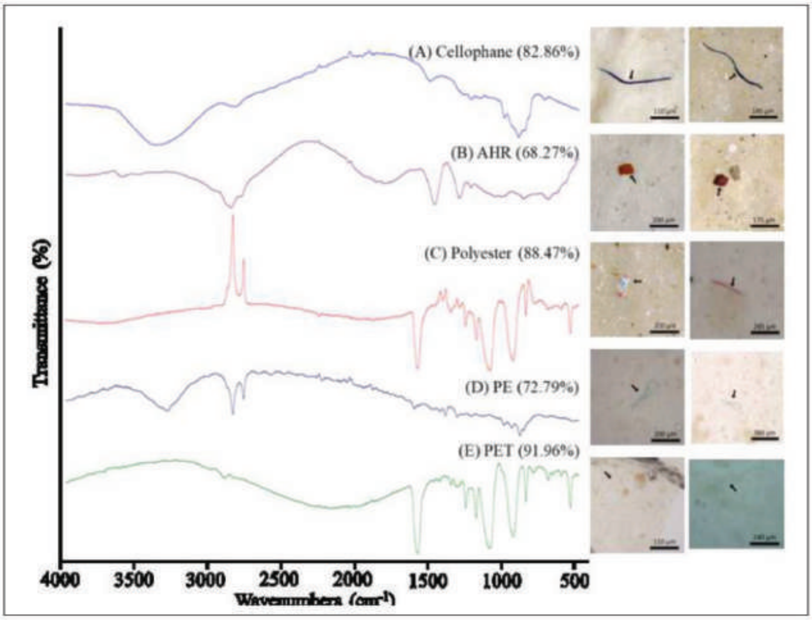

Figure 16: Component analysis of microplastics using μ-FTIR
Figure obtained from Zhu *et al.* (2020)

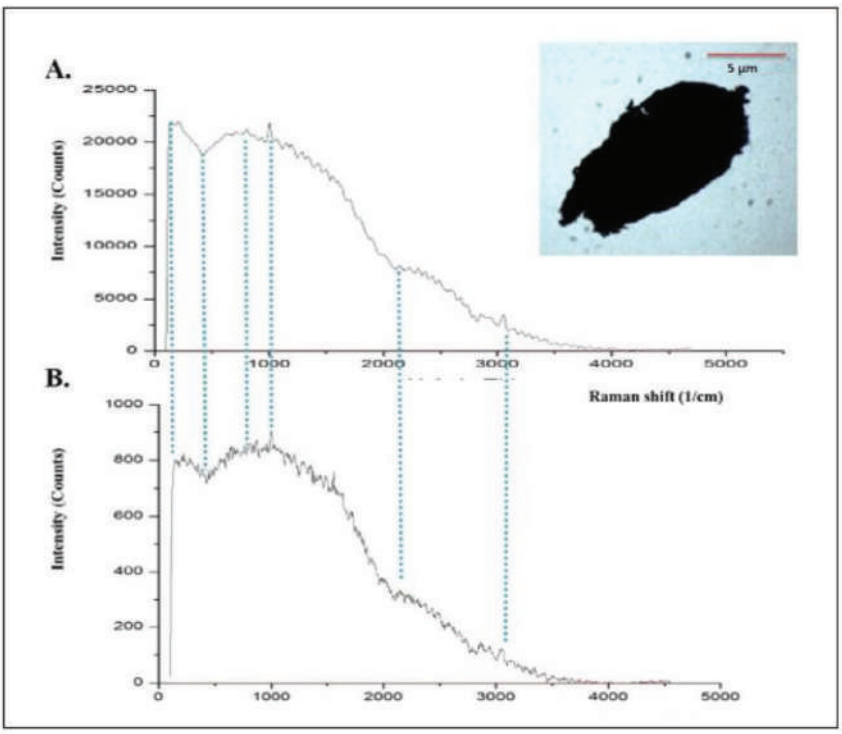

Figure 17: Polystyrene microplastic in rock oyster (*Saccostrea forskalii*). (A): From the rock oyster, (B) from the reference library
Figure obtained from Thushari *et al.* (2017)

Another study by Chen *et al.* (2020) also used Raman Spectroscopy to detect and analyze microplastics. After a wavelength of the laser hits on a sample of microplastic, unique excitations were made. The excitations are determined from the absorption, reflection and scattering by the sample.

The different frequencies observed are due to the different molecular structures and chemical properties of the sample. Therefore, it enables the Raman Spectroscopy to identify the microplastic polymers (Li *et al.,* 2018).

Raman spectroscopy only requires a small amount of sample to produce accurate results. The micro-Raman Spectroscopy is very accurate as it can detect microplastics as small as 1 mm and other methods cannot measure this accurately.

ImageJ

ImageJ is known to be a Java image processing and analysing program. It originated from NIH Image, which was made for Macintosh. The ImageJ program can read various image formats that range from TIFF, GIF, DICOM., FITS, BMP and 'raw.' It is able to calculate the area of a sample image by the statistical values of the pixel. It can manipulate, sharpen and filter the image to get optimum results (Ferreira & Rasband, 2012). Based on a study by Foley *et al.* (2018), the ImageJ software was used to physically measure and analyse the collected microplastic samples.

Another study that used the ImageJ software for microplastic analysis was the study conducted by Reisser *et al.* (2014). The plastic samples obtained from the organisms were analysed using ImageJ and classified according to its morphological groups and the plastic's occurrence frequency abundance. For each plastic that was observed, an image was taken at 50 x Magnification. The images were then uploaded to ImageJ to determine the parameters of the plastic particles. Parameters like length, area and perimeter were measured using this software.

MicroCam Lab (Microsoft Windows)

MicroCam Lab is a windows microcomputer program that can measure small pieces of samples such as microplastics. According to Karlsson *et al.* (2017), the samples left on the filter paper were analysed under a microscope after the filtration process. The particle size and the microplastic surface area were measured using the MicroCam Lab for Microsoft windows. Samples were differentiated from non-plastics using the reference images of the different types of fibers and this was used to analyse the microplastic samples before the Raman spectroscopy was used.

Scanning Electron Microscope (SEM)

The Scanning electron microscope (SEM) is known to be a frequent instrument that is used in the identification of microplastics. This method of analysis is used by Chen *et al.* (2020). A high-intensity electron beam is generated and it scans the samples, producing high-resolution images.

As the microplastic samples are being analysed, they can be identified by comparing their surface features using the SEM. Surface structures such as pits and fractures on the microplastic can be identified using the SEM. An example of how the microplastics samples is under the SEM can be observed in Figure 18. Another study by Fries *et al.* (2013) also equips a SEM with a microanalyser with an energy-dispersive X-ray.

The microanalyser was used to identify inorganic plastic substances. In this study, ten plastic samples were collected and identified as potential microplastic samples. The samples were extracted using the density separation in sodium chloride.

Using the SEM, the polymer types of samples were identified by comparing the already present plastic polymers' standards.

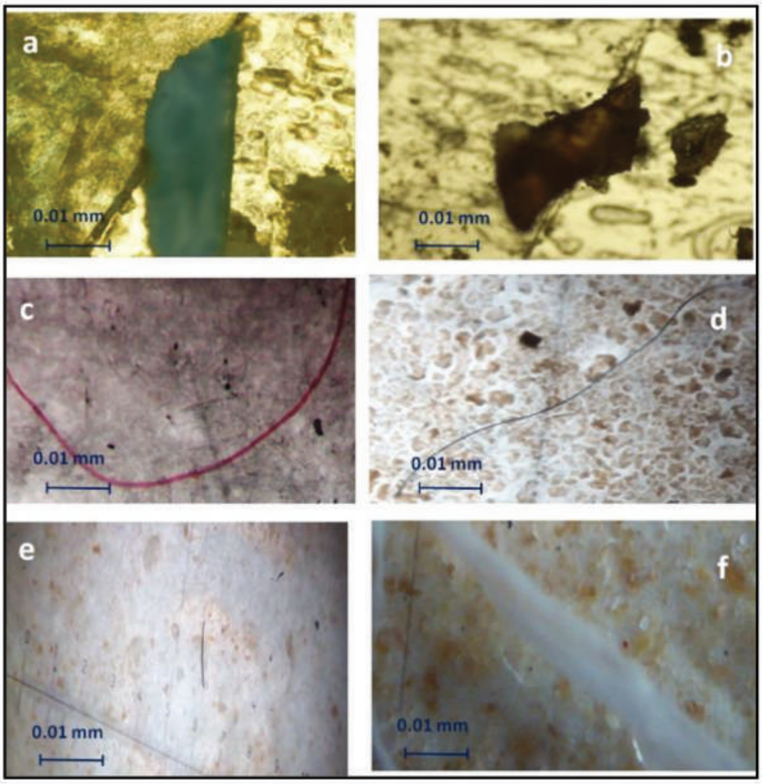

Figure 18: Examples of microplastic found in Rock Oyster (*Saccostrea forskalii*), Striped Barnacle (*Balanus amphitrite*), Periwinkle (*Littoraria sp*). Polystyrene (a,b), polyamide nylon (PA) (c, d, e), polyethylene (PET) (f)
Figure obtained from Thushari *et al.* (2017)

Effects of Microplastic Ingestion on Humans and Marine Organism

Plastics tend to have a low rate of degradation (Gewert *et al.,* 2015). It does not disappear but it breaks into smaller pieces until it finally degrades. These small plastics are known as microplastics. Microplastic has been an emerging problem because it has polluted both the oceans and land, but it has been causing various problems for the organisms that have been consuming it.

The effects of microplastic ingestion are commonly witnessed in marine organisms. According to Ryan (2016), since these plastics are so tiny, they are commonly mistaken for food and they are commonly ingested by a range of marine organisms from the plankton to larger organisms such as whales.

For corals, they survive on a relationship between and algae on its surface, known as zooxanthellae. This zooxanthella provides the coral food and nutrients from photosynthesis, while the corals provide zooxanthellae with a protected environment. Corals also have a feeding mechanism that ingests microplastics (Lusher *et al.,* 2017); hence, as the microplastics get ingested, the retention of these plastic fragments occurs. The retention of these plastics in the coral's mesenterial tissue reduces the capability of feeding by the coral. It also decreases the reserves of energy in the organism.

Besides that, the presence of microplastics also affects plankton. A study done by Nerland *et al.* (2014) shows how the penetration of microplastics on the phytoplankton wall has caused a decrease in the absorption of chlorophyll. However, heterotrophic plankton undergoes phagocytosis as they retain the tiny plastic fragments. On the other hand, zooplankton ingests unknown particles like plastic microspheres as they are exposed to microplastics, according to a study by Setälä (2014). In another study, it was found that zooplankton could ingest polystyrene and this has affected the feeding ability of the zooplankton (Cole & Galloway, 2015).

Furthermore, some microplastics with lower densities tend to float on the surface of the ocean. Hence, it is more readily accessible to marine organisms such as fish. Based on a study by Critchell and Hoogenboom (2018), microplastics were identified in the Spotty-Tailed Damselfish (*Acanthochromis polyacanthus*). The study showed that the plastic fragments present in these fish caused changes in the intestine. Therefore, it results in the detachment of mucosa epithelial lining, which affects physiological functions such as nutrient transport. Aside from that, a study by Lönnstedt and Eklöv (2016), observed the effect of polystyrene on the eggs and larvae of the European fish (*Perca fluviatilis*). Four concentrations of microplastics were exposed to eggs and larvae of the European fish. It was shown that eggs that have been exposed to high concentrations revealed slower hatching rates. The larvae of the European fish exposed to microplastics were smaller and slower than the larvae that were not exposed to microplastics. Hence, this decreases the survival rate of the species.

Moreover, larger marine organisms such as whales, dolphins and seals are also vulnerable to microplastics. In the study by Rebolledo *et al.* (2013), microplastics were detected in the stomach and intestine of harbor seals (*Phoca vitulina*). The presence of microplastics affects the digestive tracts of the marine mammal, which in turn disrupts and affects its feeding habits.

Another class of marine mammals are the filter feeders that filter the water and ingest the microplastics present in the water as a result. The microplastics tend to accumulate in that body cavity. A study by Germanov *et al.* (2018) described how microplastics impact filter-feeding organisms. The effects of microplastic digestion caused blockages and decreases in nutrient absorption. The toxins digested can be passed on to the offspring. The toxins affect the mortality and reproduction of the organisms.

There are also a lot of harmful effects of microplastic ingestion by the sea birds. This is due to the toxic effects of the plastic fragments that disrupt feeding behaviour, increase mortality rates and decrease reproduction rate of animals upon ingestion and retention (Wilcox *et al.,* 2015).

In this study, six species of sea birds were taken into consideration which includes Guanay Cormorant (*Phalacrocorax bougainvillii*), Diving-petrel (*Pelecanoides urinatrix*), Common Diving-petrel (*Pelecanoides urinatrix*), Peruvian pelican (*Pelecanus thagus*), Humboldt Penguin (*Spheniscus humboldti*) and Kelp Gull (*Larus dominicanus*). The most substantial consumption of these six species is from the kelp gull, as it commonly feeds on nets and rubbish.

The ingestion of microplastics by humans however is indirect and occurs as a result of bioaccumulation. There were not many studies regarding the effects of microplastic ingestion by humans. However, the studies conducted showed that the ingestion of microplastics occurs as the organisms at the lower trophic levels consume the microplastics. This organism then gets consumed and moves up the trophic level until it reaches humans as humans consume ocean food sources. Hence the microplastics travel up the food chain (*Ziccardi et al.,* 2016). Hence, when humans consume food from the sea such as fish, clams and oysters, the microplastic is ingested. Based on a study conducted by Wright and Kelly (2017), the human body can eliminate up to 90% of ingested plastics. The shape and size of the microplastics affect the clearance and retention rates by humans. The research shows that the few impacts caused by the ingestion of microplastics include inflammation in the tissues, disruption to the immune cells and cellular proliferation.

Concluding Remarks and Areas of Future Research

In conclusion, the study of microplastics has gained much attention as humans have been consuming and have been using the ocean as a site for dumping rubbish. There are still multiple gaps in this study, where more knowledge and research can be done.

Research on a comparison between invertebrates, effects on human consumption of these plastic filled invertebrates, effects to the food chain has not been sufficiently researched. Since these invertebrates are at the foot of the food chain, they play a huge role in affecting the organisms on higher trophic levels.

The dominant shapes of plastics that have been compiled showed to be fragment, film, fiber and filament. More studies should be conducted to improve the existing knowledge of these invertebrates' ability to accumulate and retain these microplastics.

Acknowledgements

Author would like to thank the reviewers for their constructive comments that improved the manuscript. This work was supported by MSU Seed Research Grant (SG-010-012020-FHLS) from Management and Science University.

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