Environmental microplastic concentrations and uptake in selected rocky shore invertebrate filter-feeders, grazers and predators in False Bay, South Africa

By

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Thesis submitted in fulfilment of the requirements for the degree

MTech: Oceanography

In the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

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Date submitted: 05 December 2022

Declaration

I, Liam Ferguson, declare that the contents of this thesis represent my own work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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Abstract

Plastic pollution in an ever-increasing threat globally and poor waste management in South Africa has caused an increase of plastic pollution in the environment. Microplastics (MPs) are plastic particles smaller than 5 mm in size, and the little to no research has been done on MP pollution within the marine coastal environment and rocky shores in South Africa. Sampling was done in February 2020 at a rocky shore within Simon's Town Marina, Cape Town. Water (n = 5), sediment (n = 5) and biota (n ≤ 30) samples were collected. Biota samples were split between various feeding strategies, with filter feeders, grazers and scavengers/predators sampled to determine whether feeding strategy affects the potential uptake of MP particles.

Water, sediment, and biota samples were digested in 10 % potassium hydroxide (KOH) and then extracted over a 20 µm mesh. MPs were extracted from sediment using the density separation method. Once isolated, MP particles were classified according to type, size and colour, and further classification was done using an FTIR-ATR according to polymer type.

The results show that a higher concentration was found in sediment (38 ± 2 MP/kg) compared water (0.37 ± 0.056 MP/L) as the area has low water energy, allowing MP particles to settle within the sediment. For biota samples, filter feeders had the lowest average MP particle concentrations (0.28 ± 0.04 MP/g) but displayed the highest variation of MP particle colours due to the non-selective feeding strategy, where other feeding strategies ingested mostly black/grey particles. Grazers had the highest MP particle concentration (1.45 ± 0.27 MP/g), and this was due to grazers feeding on contaminated algae closer to the benthic regions where MP particles have settled in the sediment. Scavengers/predators showed lower concentrations of MP particles (1.08 ± 0.21 MP/g) but are more dependent on the trophic transfer of MP particles through detritus and prey instead of feeding on MP particles directly from the water column.

Majority of the MP particles found in environmental and biota samples were between 100 µm and 500 µm in size. The most abundant MP polymer type was nylon (27.27 %), as well as and PET and natural MP particles such as cotton (18.18 %). Antifouling paint particles were also found, and this is due to the boat building and maintenance facility that is in close proximity to the sampling site. A risk assessment was also conducted and indicated that polymer type poses a greater risk of MP pollution than MP concentrations.

An experimental study was also done, exposing organisms to MPs of known sizes. Not much is known about the ingestion rates, preferred size of MP ingestion and the relationship between the soft tissue weight (g) and microplastics per individual (MP/I) on microplastic ingestion by marine organisms in Cape Town. The blue mussel (*Mytilus galloprovincialis*) was sampled in December 2021 acclimatised to environmental conditions within a laboratory setting for 48 hours.

Microplastic filaments (MPFs) were created and filtered using 10 µm-filtered reverse osmosis (RO) water through a vertical multi-tiered filtering system, creating MPFs of two different size classes, between 65 µm and 500 µm (small), and 500 µm and 1000 µm (large). These size classes were chosen to represent realistic sizes found in the environment according to the field study. Mussels were placed in glass beakers, in one litre of 20 µm-FSW with 50 MPFs per beaker. Half of the beakers were exposed mussels to smaller MPFs, and the other half exposed mussels to larger MPFs. At predetermined time intervals after initial exposure, mussels from the beakers for each MPF size class were removed and analysed for MPFs.

The results showed that the highest concentration of MPFs in the soft tissue was observed at 3 hours after initial exposure (7.21 ± 1.69 MP/g) and after 1 hour after the initial exposure for MP/I 23.16 ± 4.16 MP/I). This could be due to plastics needing time to be dispersed throughout the water. The results also showed that mussels preferred MPFs of the larger size (500 µm – 1000 µm). The concentration of MPFs in the size between 500 µm and 1000 µm was also the highest at 180 minutes (8.91 MP/g) and again at 60 minutes for MP/I (27.33 MP/I). For the smaller MPFs (65 µm – 500 µm), the peak ingestion was seen at 180 minutes for the soft tissue weight and at 60 minutes MP/I, reinforcing that peak ingestion rates occur between 60 and 180 minutes after initial exposure.

These studies are useful as it provides insight in the levels of microplastic pollution in a rocky shore in Cape Town, and also the potential sources of pollution, and also the preferred ingested MPF size in mussels in Cape Town, as well in when mussels are at the peak feeding rates after initial exposure to microplastic. The study provides reason to the importance of the combination of field and experimental studies in microplastics research and provides motivation or more studies to be undertaken.

Acknowledgements

I wish to thank:

CPUT for the available resources and facilities;
Dr. Conrad Sparks and Dr. Adetunji Awe as their roles of Supervisors and their guidance;
Danielle Julius for assistance in the field and laboratory and constant support; and Shafiek and Ilona Ferguson always providing encouragement and support.

The financial assistance of National Research Foundation (NRF) towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the National Research Foundation.

Table of Content

List of Figures

List of Tables

List of Equations

Glossary

Biodegradable	The ability for a material or substance to be broken down naturally by the organisms in an ecosystem.
Contamination	The presence of a foreign material or substance having no harmful effects.
Invertebrates	A cold-blooded animal with no backbone
Microplastics	Plastic particles less than 0.5 mm in diameter, varying in type, colour, and shape.
Plastic	Synthetic or semi-synthetic polymers.
Pollution	The presence of a substance or material that may cause harmful effects.
Ubiquitous	Present or found everywhere
Rocky shore	The interface of land and sea, forming a narrow border around the coastline of a country.

Abbreviations/Acronyms

APP	Antifouling paint particles
Natural	Cellulose/protein-based polymer
CF	Contamination factor
FSW	Filtered seawater
FTIR	Fourier-transform infrared spectroscopy
KOH	Potassium Hydroxide
MP	Microplastic
MPA	Marine Protected Area
MPF	Microplastic filaments
MPPLI	Microplastic Pollution Load Index
Nat	Natural
PA6	Polyamide 6
PAA	Polyacrylic acid
PE	Polyethylene
PET	Polyethylene terephthalate
PLI	Pollution Load Index
Polyamide/Nylon	PA6
H	Polymer Risk Index
PRI	Pollution Risk Index
PUR	Polyurethane
RO	Reverse Osmosis
SA	South Africa
SOP	Standard Operating Procedure
SR	Synthetic Rubber
STW	Soft Tissue Weight
TW	Total weight
WWTW	Wastewater Treatment Works

1. Chapter 1

Introduction

Plastic production has increased exponentially since the mass production of plastic started in the 1950's. The low cost, durability and high demand of plastic makes plastic products a fundamental part of everyday consumer life (Bayo et al., 2020). These characteristics, however, in addition to poor waste management of plastic waste makes plastic pollution an ever increasing problem in the environment (Rillig, 2012; Ryan et al., 2009).

Plastic enters the terrestrial and aquatic environment through littering and poor waste management of plastic waste (GESAMP, 2019). The plastic debris in terrestrial environment gets blown around by the wind and lands in rivers, eventually flowing into the marine environment (Paul-Pont et al., 2018). Plastic waste can also enter the marine environment directed from marine-related activities, such as plastic waste from boats, ships, and recreational activities in the coastal region. Plastic waste also travels to the marine environment through marine outfall pipes, which disposes wastewater from wastewater treatment works (WWTW) and stormwater drains to the ocean (Iroegbu et al., 2020). While large plastic debris can easily be disposed of, microscopic plastic particles are difficult to detect and difficult to remove from waste and these microscopic particles eventually enters the marine environment.

Microplastics (MP) are microscopic plastic particles less than five millimeters (<5mm) in size (Mathalon and Hill, 2014; Zhao et al., 2014). A lot of these microscopic particles are manufactured at this size, called primary MPs, as it serves as a basic plastic base and is melted and moulded into common plastic items. The plastic is also easier to transport in pellet form. The microscopic pellets can also be used in abrasives in everyday products such as toothpastes and face washes (Leslie, 2014; Mato and Isobe, 2001), as well as in a lot of industrial activities. Secondary MPs are microscopic plastic particles that have been fragmented into <5mm particles from a larger plastic source (Zhao et al., 2014). The process of mechanical fragmentation is sped up when plastics spend a prolonged period exposed to sunlight, weakening the structural integrity of the plastic (Singh and Sharma, 2008). Plastic debris poses short and long term negative effects to marine organisms, human health, aesthetics and tourism (Derraik, 2002; Oigman-Pszczol and Creed, 2007). The longevity of plastic makes it a significant problem within the marine environment, as the plastic particles will persist in the marine environment for a long time (Ryan et al., 2009).

MPs tend to float in the upper layer of the water column due to their buoyant nature. The buoyant nature of plastic also allows it to be dispersed fairly quickly with waves, wind and ocean currents (Ryan et al., 2009). Some plastics, such as polyvinyl chloride (PVC), are negatively buoyant and do sink (Egbeocha et al., 2018; Mathalon and Hill, 2014; Wright et al., 2013). This makes MPs biologically available to a large variety of organisms across the entire water column. Unlike mobile organisms, sessile invertebrates cannot move away from polluted areas to find a cleaner and healthier habitat (Thushari et al., 2017b). Ingested MPs has negative effects on organisms. These effects can include gut blockage, false satiation, inflammation, depletion of energy reserves, reduction of survival, compromise of growth and fecundity and the retardation of larval development (Wright et al., 2013). Although MPs generally float in the water column or settle in marine sediments, the behaviour of MPs within a rocky shore environment is relatively unknown. Therefore, it is vital to investigate the concentrations of MPs in various marine coastal invertebrates that use different feeding strategies and that feed at different depths. This will not only indicate what organisms are vulnerable to MP ingestion, but it will also indicate where most MPs are within the rocky shore.

Once the concentrations of MPs in various marine invertebrates are known, experimental studies can be done on targeted species in a laboratory setting. These laboratory procedures can determine various mechanisms of MPs uptake in organisms, such as preferred MP size (Graham and Thompson, 2009), shapes (Sundt *et al.*, 2014) and colours, rate of uptake, mechanism of uptake, and the translocation of MPs into various bodily tissues and organs of organisms.

Since rocky shore invertebrates cannot relocate to less polluted water, they are constantly exposed to MPs within the localised region. Therefore, marine coastal invertebrates are ideal candidates to use as bioindicator organisms for MP pollution. This method has already been trialled mussels (Tanabe et al., 2000) and oysters (Thushari et al., 2017b). With bioindicators, the concentrations of the extracted MPs within the monitored species could indicate current MP pollution concentrations, as well as the preferred shape, size, and colour in the region.

2. Chapter 2

Literature review

2.1 Plastics

Plastic production has reportedly grown from 335 million tonnes in 2016 to 348 million tonnes in 2017, with an increase of 3.74% providing jobs to more than 1.5 million people in Europe alone (Bayo et al., 2020). Due to the high durability and poor waste management of plastic debris, plastic has become a widespread problem throughout the world, polluting all habitats due to their longevity and their ability to travel long distances (Rillig, 2012; Ryan et al., 2009).

Plastic debris poses short and long term negative effects to marine organisms, human health, aesthetics and tourism (Oigman-Pszczol and Creed, 2007). Plastic litter ends up in the marine environment from land and sea-based activities. Land-based activities include industrial activities, commercial activities, littering from the public and poor waste management and disposal by governmental sectors (GESAMP, 2019). Sea-based activities include waste from shipping routes and debris from shipping accidents (Derraik, 2002).

Most plastics are buoyant, and they can be easily dispersed by ocean currents, waves and the wind over large distances fairly quickly (Rillig, 2012; Ryan et al., 2009). The longevity, low cost and disposability of plastics make it a large problem to the marine environment (Derraik, 2002), and the longevity of plastics makes it a short and long term problem for marine fauna. As plastics get dispersed through the marine environment through waves, currents and wind, they are exposed to chemicals in the water and UV radiation from sunlight for prolonged periods, weakening the structural integrity of the plastic (Barnes et al., 2009), causing it to increase the concentrations of microscopic pieces of plastic particles in the environment. This phenomenon gives rise to MPs that are microscopic particles of plastic dispersed throughout the water column that resemble food particles to marine fauna.

With effective waste management, plastic litter can be disposed of in an effective manner. However, with poor waste management skills of citizens during everyday life causing plastic debris to enter the environment through littering, the debris eventually enters the marine environment through rivers and wind. Poor waste management from governmental sectors is also a major contributing factor. With landfills being filled to over their respective capacities and poor disposal methods, as well as a lack of recycling methods, plastic waste will inevitably enter the marine environment.

2.2 Waste management of plastics in South Africa

Plastic debris in the marine environment is increasing and is due to poor waste management, especially in developing countries such as South Africa. The global plastic production has risen to 348 million tons in 2017 (Plastics Europe, 2018). Single use plastics are the most common worldwide, most plastics are discarded within a year (Hopewell et al., 2009) in an improper manner and end up in the environment (Barrows et al., 2018). Populated coastal zones are prone to general waste and plastic pollution, due to waste and plastic pollution stemming from anthropogenic sources. In 2010, South Africa ranked 11th highest globally for plastic waste generation, producing 0.63 million tons of plastic waste for the year, of which marine plastic contribute as much as 0.25 tons (Jambeck et al., 2015).

South Africans use up to 50 kg of plastics daily, and the South Africa produced 42 million tons of general waste in 2017, of which 5% (2.2 million tons) was plastic waste (Department of Environmental Affairs, 2018). Of that 5%, 15% was recycled and the remaining plastic stayed in landfill sites around the country (Department of Environmental Affairs, 2018).

According to Meme (2010), South Africa has a poor wastewater infrastructure that cannot be managed effectively to cope with an increasing population. Only a fraction of the wastewater is treated before discharge and the percentage of treated wastewater has decreased with increased population size (Brown, 1987). Therefore, wastewater treatment plants (WWTW) are large contributors of MPs into the coastal environment. Textiles, medicinal, cosmetic and industrial MPs can land in the coastal marine environment through WWTPs (Barrows et al., 2018; Bayo et al., 2020; Belzagui et al., 2020; de Villiers, 2018; Leslie, 2014; Nel et al., 2017a). With 158 WWTPs within the province (DEA, 2018), it is expected that MP counts along the Western Cape coastline is elevated.

Due to South Africa being a developing third world country, effective waste management is not a high priority for the governmental system which prioritises high poverty, unemployment and HIV/AIDS statistics over ecological issues (Verster *et al*, 2017). The issue is that 60 000 people are employed in the plastic production sector in South Africa annually, contributing to job creation. However, the same number of jobs are not being created with effective waste disposal systems (Verster *et al*, 2017). The lack of job creation within the waste management sector indicates that there is not effective waste disposal framework currently in place, and that littering, and the disposal thereof is a major problem within South Africa.

The Western Cape is one of the largest contributors to waste production within South Africa, behind Gauteng and KwaZulu-Natal (Department of Environmental Affairs, 2018). Twenty per cent (20%) of the country's waste can be attributed to the Western Cape (Department of Environmental Affairs, 2018). This translates to 8.4 million tons of general waste, which translates to 0.4 tons (400 000 kg) in 2017 (Department of Environmental Affairs, 2018). The largest metropole within the Western Cape is Cape Town, (StatsSA, 2018), making Cape Town the largest contributor of waste within the province (de Villiers, 2018; Sparks, 2020a).

2.3 Rocky shore zonation and community structure in South Africa

The rocky shore is one of the major crossover ecosystems between the terrestrial and aquatic environments, to the marine environment. An array of plant and animals inhabit this area of the marine environment and are exposed to a diurnal tidal cycle in South Africa, being submerged in water and exposed twice a day (Bustamante, 1994; Bustamante et al., 1997). Rocky shores have three distinct zones which dominate the shore. These being the Littorina, Balanoid and the Infratidal zone (Branch & Branch, 2016).

The lowest zone on the rocky shore is the Infratidal zone and is covered with water for majority of the day (Bustamante, 1994; Branch *et al*., 2016). This zone is dominated by larger seaweeds, such as *Laminaria pallida* and *Ecklonia maxima* dominate with zone, as well as small primary consumer organisms such as sea urchins and some whelk species (Branch *et al*., 2016). They graze on the seaweeds and other pieces of seaweeds floating in with the water column that has been broken up by the strong waves and currents.

The Balanoid zone is dominated by barnacles and other colonial organisms as well as grazers, with smaller bushy seaweeds, such as *Ulva spp*. and *Porphyra capensis*, and other encrusting algae (Branch *et al*., 2016). Other prominent organisms in this zone are mussels such as the blue mussel (*Mytilis galloprovincialis*) and the brown mussel (*Perna perna*) (Branch *et al*., 2016). Sea squirts such as *Pyura stolonifera* and sea anemones also mostly inhabit the Balanoid zone and are filter feeders, filtering floating particles out of the water column.

The Littorina zone is the uppermost region of the rocky shore and named after a marine mollusc found thin this region, *Littorina littorea* (Branch & Branch, 2016). Generally, the zone is only completely covered during spring tides and receive splashes during the normal tidal cycles. This zone is also inhabited by many opportunistic feeders that feed on nutrients washed out by the rising tide and the waves washing out (Chappuis et al., 2014). The rocky shore is also inhabited by predatory organisms that prey on the invertebrates in the rocky shore. Starfish, such as the Spiny Starfish (*Marthasterias glacialis*) and some whelk species prey on the invertebrates that dominate the rocky shore.

2.4 Microplastics

MPs are plastic particles that measure less than five millimeters in size that are considered to be a threat to the marine and coastal environment (Tu et al., 2020; Wright et al., 2013). These plastics particles end up in the marine environment through anthropogenic sources s MPs are present in all spheres of the marine environment, such as the deep ocean basins (Zhang et al., 2020), shallow continental shelf waters (Thiel et al., 2013), marine coastal regions (Sparks, 2020b; Thushari et al., 2017b), sediment (Costa et al., 2010; Piarulli and Airoldi, 2020), catchments from freshwater river flow (Chen et al., 2020) and marine organisms (Wright et al., 2013).

MPs can be divided into two categories, primary and secondary MPs. Primary MPs are plastic particles manufactured smaller than 5mm in size (Mathalon and Hill, 2014; Zhao et al., 2014). These small pellets are mostly used as the base material to be melted down into many every day plastic objects. Specially-made MPs can also be used as abrasives in cosmetics and medical products, such as face washes and toothpastes (Leslie, 2014; Mato and Isobe, 2001). Secondary MPs are plastic particles that have been broken down into smaller pieces by mechanical and chemical fragmentation (Zhao et al., 2014). Larger plastics are bashed around the coastal environment by waves and rocks, which causes the plastics to break down into smaller particles. The process is sped up when plastics are exposed to the UV radiation of sunlight for a prolonged period, softening and weakening the plastic, making them easier to break down (Singh and Sharma, 2008).

MPs are present in the marine environment due to anthropogenic input. Poor waste disposal methods of plastic waste are one of the main causes of plastic letter being introduced into the marine environment. Referring to Figure 1, up to 80% of plastic waste disposed of in an improper manner in rivers ends up being washed out into the ocean during normal tidal cycles (Paul-Pont et al., 2018) and is worsened during heavy rainfall. MPs from storm runoff drains, outfall pipes and wastewater treatment plants also run into the marine coastal environment (Bui et al., 2020; Iroegbu et al., 2020).

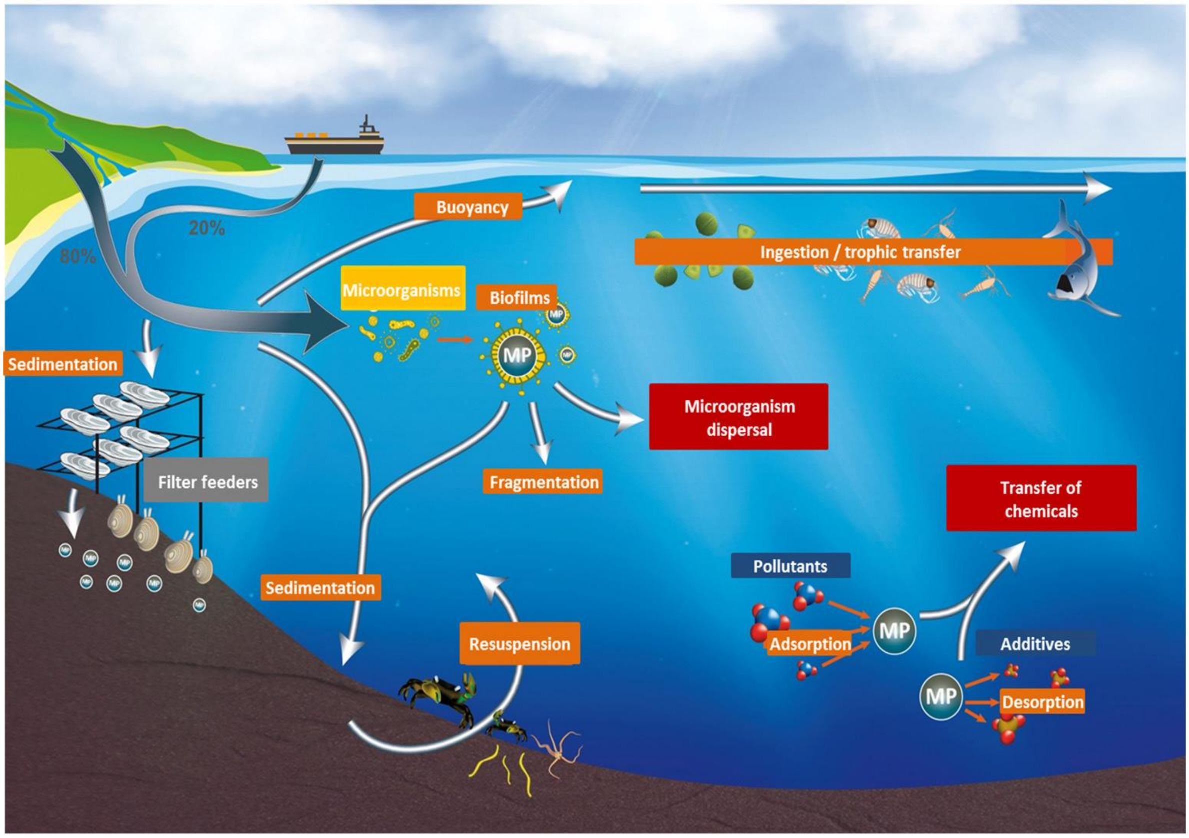


Figure 2.1: The fate of Microplastics in the marine environment (Paul-Pont et al., 2018).

MPs have been found in marine coastal waters, sediments, and marine organisms of South Africa. In marine coastal waters and beach sediments, elevated concentrations of MPs were found to correlate to areas of high population densities close to the coast (de Villiers, 2018; Naidoo and Glassom, 2019; Nel et al., 2017a; Nel and Froneman, 2015; Ryan et al., 2018). MPs are also found in the marine organisms that inhabit the coastal environment. Marine organisms ingest MPs either due to non-selective feeding strategies such as filter feeders like mussels (Sparks, 2020a) and zooplankton (Cole et al., 2013) or through the misidentification of MPs as food particles (Hall et al., 2015; Nelms et al., 2018).

Even though a few studies on MPs have been done in South African marine waters, this is miniscule to the volume of work done on MPs internationally. There are even fewer studies conducted in South Africa on marine coastal environments, such as rocky shores. Therefore, there is a definite need to do more field and laboratory studies on MPs in South Africa.

2.4.1 Microplastics in coastal zones of the marine environment

With approximately half of the world's population living on or near the coast (Barritaud et al., 2021), the coastal areas become a major area of concern for MP contamination (Zhang, 2017).

This is due to direct sources of MP contamination, such as beach littering, sewerage and stormwater input and maritime activities. Studies have suggested that there is a direct correlation between coastal MP contamination and coastal populations (Zhao et al., 2015) with the largest concentrations of MPs observed in industrial and harbour sites (Auta et al., 2017).

Low density MPs tend to float in upper regions of the water column and in low-energy environments, deposit into the benthic region of the water column (Zhang, 2017). High density MPs tend to suspend in the mid-regions of the water columns and over time also settle into the benthic region (Zhang, 2017). The low-density particles have the potential of being transported over long distances at potentially high velocities via surface current and wind forces. Compared to the higher density particles that is suspended in the mid-water region, they are not exposed to surface currents and winds and get transported at a significantly lower velocities for shorter distances. Particles with high surface area to volume ratio's, such as films, fibers and foams, possess a higher rate of biofouling and aggregation which increases the density, causing the particles to sink faster (Ryan, 2015). Generally, the MP particles get deposited onto the shore.

MPs in the coastal regions are not only subjected to wind and surface currents, but also tidal cycles. Stranded plastic particles are easily transported back into the ocean by spring-high tides and reintroduced into the marine environment (Fok et al., 2017), until they are transported into deeper water regions by spring-low tides. Therefore, the distance on the shoreline is directly dependant on wave heights and associated tidal cycles (Fok et al., 2017).

2.4.2 Microplastics in the rocky shores

Generally, rocky shores are environments of high energy and little-to-no sediment within the environment. However, little is known about the behaviour of MPs in the dynamics of the rocky shore, as many studies are targeted to analyse MPs in open ocean and beach sediment scenarios.

The rocky shore can be responsible for the creation of secondary MPs as plastic debris is washed around the rocky substrate causing the mechanical fragmentation of the plastic debris (Thiel et al., 2013; Thushari et al., 2017b). Due to the lack of sediment and the high energy in the rocky shore environment, MPs tend to be washed out to the high tide mark (Thiel et al., 2013). These particles can also be resuspended multiple times within the rocky shore and marine organisms will be exposed to MPs for a prolonged period. Thiel *et al.*, 2013 also found that plastic debris found at the high tide mark is entangled in seaweeds of different sizes, which can lead to the conclusions that plastic debris in the rocky shore can become entangled in seaweeds and get stuck between rocky crevices.

2.4.3 Microplastics in urban harbours and urban rocky shores

Marine environments situated in heavily influenced urban areas and harbours are greatly exposed to MP contamination due to their proximity to MPs sources, such as shipping and transport, industrial and leisure activities (Au et al., 2017; Bayo et al., 2020; Chouchene et al., 2019; Mathalon and Hill, 2014; Rose and Webber, 2019).

Harbours and marinas are generally in protected and calm areas. This is for protection and ease of access for ships moving cargo, as well as or the protection of humans that want to use the marina for other commercial and recreational activities. Both harbours and marinas tend to have a high-energy low tide and a low-energy high tide, concentrating MPs within a certain area of the intertidal zone (Chouchene et al., 2019). Lower densities will still accumulate at the high tide mark, floating in the surface layer and easily influenced by environmental factors such as wind and wave propulsion, but higher density MPs will have a bigger chance of sinking to a deeper depth with calmer waters (Mathalon and Hill, 2014).

Harbours have more industrial activities and these industrial activities do pollute the surrounding area. A study conducted around a plastic production area found as much as 124.7 particles per kilogram of sediment and up to 20.5 particles per litre of surrounding water (Li et al., 2020). Therefore, industrialised areas do contaminate surrounding areas. Dredging activities also resuspend settled MPs and reintroduce the particles back into water column, making it biologically available again (Chouchene et al., 2019; Rose and Webber, 2019). Harbours also provide hard substrates, such as harbour walls, wavebreakers, piers and support pillars, that is ideal attachment areas for marine invertebrates to use. Therefore, marine invertebrates will be exposed to MPs in the artificial rocky shore environment.

Marinas are prone to pollution from recreational users, using the area for activities such as swimming, fishing, and boating. The recreational activities of humans increase the MP concentrations in the intertidal zone, as well as stir up any settled MPs in the environment. Any restaurants, shops and public bathroom facilities will also have wastewater that can pollute the marina.

2.4.4 Factors that affect the uptake of microplastics by rocky shore organisms

The ingestion of MPs can cause adverse effects on marine organisms. These effects can include gut blockage, false satiation, inflammation, depletion of energy reserves, reduction of survival, compromise of growth and fecundity and the retardation of larval development (Wright et al., 2013). This will lead to individual deaths of organisms but can affect local populations of organisms.

Little is known about the behaviour of MPs within the rocky shore environment. Three major factors control the behaviour of MPs in the rocky shore environment, namely the particle size, particle density and predator-prey relationships between organisms.

2.4.4.1 Microplastic particle size

MPs are often ingested by non-selective feeders such as mussels (Scott et al., 2019; Sparks, 2020; Webb et al., 2019), zooplankton (Cole et al., 2013) and oysters (Scanes et al., 2019; Thushari et al., 2017b) which just filter water and ingest particles obtained out of the water column. MP particles can also resemble particle sizes that marine rocky shore organisms normally ingest, meaning that organisms misidentify MPs as food particles that they tend to ingest (Hall et al., 2015; Lusher et al., 2017; Nelms et al., 2018). Aided to organisms mistakenly ingesting MPs as food, the microscopic size of the particles increases then number of particles within the environment, increasing the chances that organisms, although mistakenly, ingest MP particles.

2.4.4.2 Microplastic particle density

Filter and suspension feeders such as mussels and oysters feed in the upper part of the water column, making them more vulnerable to low-density, or positively buoyant MPs floating in the surface layer of the water column such as polyethylene (PE) and polypropylene (Li et al., 2018; Wright et al., 2013; Zhu et al., 2020). MPs with a higher density, or are negatively buoyant, such as polyvinyl chloride (PVC), is a heavier plastic in composition and sinks to the rocky substrate if the sea conditions are calm enough (Wright et al., 2013). This makes the plastic biologically available to any organisms such as detritus feeders/scavengers like polychaete worms and sea cucumbers (Oladimeji Ayo Iwalaye et al., 2020; Thushari et al., 2017b), and grazers that could be found on the rocky floor (Li et al., 2018).

MPs have a strong adsorption effect on pollutants, attracting pollutants in the marine environment (Feng et al., 2020), creating a biofilm around the MP particle (Lobelle and Cunliffe, 2011; Tu et al., 2020). The development of a biofilm will also affect the density of a MP particle. When a particle develops a biofilm, microbial colonisation occurs around the MP particle, making it more dense (Lobelle and Cunliffe, 2011; Tu et al., 2020). This makes the particle heavier, causing it to sink and making it biologically available to organisms inhabiting the deeper regions of the rocky shore (Egbeocha et al., 2018). The rate of biofouling is influenced by physical variables, such as surface energy, season, hardness of the polymer and water conditions (Muthukumar et al., 2011).

2.4.4.3 Predator-prey relationships

MPs can also be transferred up the food chain via predator-prey relationships. Lower trophic-level organisms generally ingest MPs much faster than higher trophic-level organisms via their non-selective feeding strategies and misidentification of MPs as food. The retention time of MPs within the gut of an organism plays a large role in how far up the food chain (Egbeocha et al., 2018). The longer the MPs stays in the organism, the higher the chances of the organism getting preyed on and transferring the MP particle up through the food chain through a predatory species (Au et al., 2017).

Organisms may retain ingested MPs particles for longer periods of time due to low food concentrations. Since food sources may be dwindling, organisms may retain food for longer periods of time and rely on the nutrients gained from the mistakenly ingested MPs particles. The longer the MPs are retained, the higher the chances that the organism will be preyed upon and transferred to another organism and transferring it up the food chain (Ogonowski et al., 2016). Therefore, transfer of MP particles up into higher trophic levels can lead to the bioaccumulation and biomagnification of MPs (Avio et al., 2015; Farrell and Nelson, 2013).

MP ingestion and uptake is important, however, there is a lack of knowledge in South Africa regarding the understanding the uptake of MPs by rocky shore organisms. This mechanism can be understood by running experimental procedures on rocky shore organisms that will ingest MPs in the rocky shore.

Various factors influence MP uptake by rocky shore organisms, factors such as polymer type (Wright et al., 2013), MP shape (Sundt et al., 2014) and MP size (Scott et al., 2019; Wright et al., 2013). The particle density plays a large role, as heavier particles will be able to sink to the sea floor faster and expose itself to deeper lying organisms (Lobelle and Cunliffe, 2011). Biofouling and biofilms developing around the MP also increases the density of the particle, allowing it to sink to deeper depths (Lobelle and Cunliffe, 2011).

Experimental procedures therefore have an important role to play in the understanding of MP uptake.

2.5 Experimental exposure of Microplastics to marine invertebrates

Laboratory studies have documented the effects of MPs on marine organisms, ranging from neurotoxicity and DNA damage in clams (Ribeiro et al., 2017), delayed larval development in oysters (Sussarellu et al., 2016) and induced valve closure in mussels (Wegner *et al.*, 2012). Over the years, bivalves have become ideal organisms to monitor water quality in coastal regions due to their ability to filter large quantities of water, their sessile nature and ability to accumulate a wide range of particulate and dissolved pollutants (Tanabe et al., 2000).

These characteristics can make sessile invertebrates ideal for monitoring MPs pollution in the coastal environment, whether it be constituents or concentrations (Thushari et al., 2017b). Various factors affect the uptake of MPs in the wild, but experimental procedures commonly use unrealistic levels of MPs that organisms are unlikely to encounter in their natural environment to obtain results, prompting a debate to whether laboratory experiments yield realistic results (Paul-Pont et al., 2018; Woods et al., 2018). For any MP experimental procedure, four main aspects of MPs should be considered when exposing organisms to MPs, namely polymer type and density with regards to the target species, MP shape and MP size (Paul-Pont et al., 2018).

2.5.1 Polymer type and density of microplastic particles with regards to the target species

There are many types of plastics and just as many uses, whether day-to-day, industrial, commercial, and medicinal. The industry is dominated by six major types of plastic, namely polyethylene (PE; high-density polyethylene – HDPE; and low-density polyethylene – LDPE), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polystyrene (PS), polyethylene terephthalate (PET) (GESAMP, 2019).

The main manufactured groups of MPs account for nearly 80% of plastics around the world (Paul-Pont et al., 2018) and are the most frequently reported polymer types found in marine environments. Heavier polymer types such as PVC tend to sink (Wright et al., 2013), even more so after being exposed to the elements for an extended period and a biofilm starts to develop, causing there to be an increase in density (Lobelle and Cunliffe, 2011; Tu et al., 2020).

The polymer types should match the type of MPs found in the target animal's natural environment, as mentioned in studies done in China (Zhao et al., 2014) and experimental studies (Paul-Pont et al., 2018; Woods et al., 2018). The effect of this constraint is reduced when doing short term experimental procedures, as biofilms are well developed after approximately a week, as shown by Lobelle and Cunliffe (2011). Therefore, the polymer type should be considered when doing experimental procedures. Organisms that are found in the upper region of the water column are more likely to encounter positively buoyant MP particles, such as polyethylene (Wright et al., 2013), whereas high density plastics such as PVC will sink and become biologically available to benthic organisms (Wright et al., 2013).

The polymer type used in experiments should also consider the geographical location of the sampling site used to obtain the experimental specimen (Paul-Pont et al., 2018). This should be researched and documented before the experiment is undertaken, to show what polymer types are relevant is the targeted species and targeted environment.

The weathering of individual particles and polymers is also a very important aspect to consider but is commonly ignored during experimental procedures (Paul-Pont et al., 2018). This is because the natural weathering of MP particles is difficult to replicate in a laboratory setting and even more difficult to reliably replicate on a consistent basis.

2.5.2 Microplastic origins: Primary MPs VS secondary MPs

A few groups of MPs can be easily identified and be placed in their respective morphological groups. Primary MPs are easily identifiable, and spheres (bead/pellets/granules) can be placed easily. Fibres, films, fragments, and foams can be easily identified, with some exceptions, as secondary MPs.

A comparative study in Norway done by Sundt *et al* (2014) showed that between 84.5% and 96.3% of MPs found in the environment were secondary MPs, compared to between 3.7% and 15.5% of MPs found to be primary MPs in the aquatic environment. Therefore, if extrapolated to environmental scale, secondary MPs are more suitable to be used in experimental studies as more organisms are likely to take up secondary MPs as they are more common in the natural environment (Paul-Pont et al., 2018). However, most experimental MP studies use primary MPs in the form of pellets or nurdles (Cole, 2016). One of the reasons that primary MPs are used is that they are easily sourced for experiments and easily labelled or dyed with fluorescent materials to make them easily identifiable during the extraction phase of the experiment (Yokota et al., 2017). Even though they do produce good results in experimental procedures, the exclusive use in experimental procedures may produce biased results that are unrealistic relative to the wide range of MP shapes found in the natural environment.

The real difficulty of using secondary MPs while conducting experiments is collecting or creating secondary MPs that is suitable enough to produce reliable results during experiments. The shapes of secondary MPs are irregularly shaped and is difficult to design a reliable and repeatable method to create secondary MPs for experimental studies (Cole, 2016). As suggested by Graham and Thompson (2009), the most accurate way to conduct experiments with the focus on secondary MPs is to use MP particles directly sampled out at sea.

Creating secondary MPs can be done via physical or mechanical degradation of commercial pellets or other commercial objects. The mechanical degradation of plastic will yield a fine powder, which will have to be held in a liquid medium (Paul-Pont et al., 2016). Such a powder held in a liquid medium will have particles of different sizes and sieving will be necessary to classify different sizes of secondary MPs according to the experiment protocols (Cole, 2016; Graham and Thompson, 2009; Paul-Pont et al., 2016).

The major drawback of creating or collecting suitable secondary MPs is that it is very time consuming and labour intensive. To collect secondary MPs, one must collect seawater, sediment or biota and extract MPs using proper protocols which is very time consuming.

2.5.3 Microplastic size

Organisms in the marine environment feed on specific organisms with specific sizes to which their feeding apparatus and methods have been adapted. Therefore, the same can be extrapolated about MP ingestion, that particle size dictates the probability of MP particle ingestion (Wright et al., 2013). This resemblance of MP particle size to food particle size means that organisms feed on MPs, either due to mistaken identity or through non-selective feeding methods (Scott et al., 2019; Sparks, 2020a; Webb et al., 2019).

Different methods have been applied to determine the impacts of MP particle size through experimental procedures. However, three main methods dominate the literature in terms of testing different sizes of MP ingestion. These are (i) exposure to a specific size class (Farrell and Nelson, 2013; Setälä et al., 2014), (ii) exposure to several size classes separately (Cole and Galloway, 2015) and (iii) exposure to different size classes of MP particle at the same time (Avio et al., 2015; Green, 2016). Even though all methodologies provide advantages, using a broad range of MP particle sizes provides the basic knowledge of what that specific organism will interact with.

The preferred size of MPs can also be dependent on the life stage the selected specimen is in. Cole & Galloway (2015) reported that juvenile oysters have a small mouth and therefore, any MP particle larger than an estimated 80µm would be too large to ingest. Most studies choose to use the MP size in the same category as the studied specimen's prey (Paul-Pont et al., 2018).

2.6 Conclusion and dissertation aim/objectives

Plastic contamination in the marine environment has risen to explicably high levels, with marine water, marine sediments and marine organisms all polluted with MPs. Plastic waste, and MPs land up in the environment via anthropogenic input (GESAMP, 2019). Plastic debris has a negative effect on the environment, human health, aesthetics and tourism (Oigman-Pszczol and Creed, 2007). Due to most plastics being buoyant, they are easily dispersed by ocean currents, wind and waves (Rillig, 2012; Ryan et al., 2009).

The plastic waste volume can be reduced by designing plastic products from a re-se and recycle view instead of single use disposable plastics (Hopewell et al., 2009). The environmental impact of plastics can also be lessened by recovering plastics from landfills with proper waste management protocols implemented by governmental sectors and used for recycling (Hopewell et al., 2009). Plastics can also be incinerated but this does not satisfy the demand for plastic to manufacture new plastic products. It will also add to the already abundant carbon dioxide in the atmosphere. Environmental education to reduce littering can also stem the volume of MPs being put into the environment (Andrady and Neal, 2009).

The aim of the dissertation is to investigate the levels of MP pollution within a rocky shore environment via a field study, and to understand which organisms are most vulnerable to MP contamination within a rocky shore using organisms with different feeding strategies. Organisms with different feeding strategies do occupy different areas within the rocky shore and this will indicate where MPs are aggregating within rocky shores. Experimental studies, which involves select organisms taken into an experimental setting, is vital in understanding MP ingestion. With experimental studies, the size preference of MP uptake can be determined with experimental procedures, controlling the temporal and size scale that the organisms are exposed to.

The experimental procedures are not only vital to further understand the information gathered from field studies, but to ultimately determine if there is an effective bioindicator species that inhabits the rocky shore. This will be used to monitor MP concentrations within the rocky shore environment.

3. Chapter 3 - Material and Methods

3.1 Field study

3.1.1 Study site

False Bay is a calm and southward facing Bay on the southern point of the Cape Peninsula. The area extends from Cape Point in the west to Cape Hangklip in the east, and strong southerly winds are responsible for the clockwise cyclonic circulation within the bay.

The water circulation is driven by the dynamics of the winds within the bay. In Summer, the circulation is controlled by predominantly south-easterly winds and by north-westerly winds in the Winter (Pfaff et al., 2019). The Summer months produce high air temperatures that increase sea surface temperatures. The southerly winds in move surface waters in a westerly direction, aiding to the cyclonic movement of water throughout the bay. There are various potential sources of MP contamination throughout the Cape Peninsula coastline. These include seasonal rainfall, water circulation, waste management, harbour-related activities, waste-water treatment works (WWTW's), outfall and stormwater pipes.

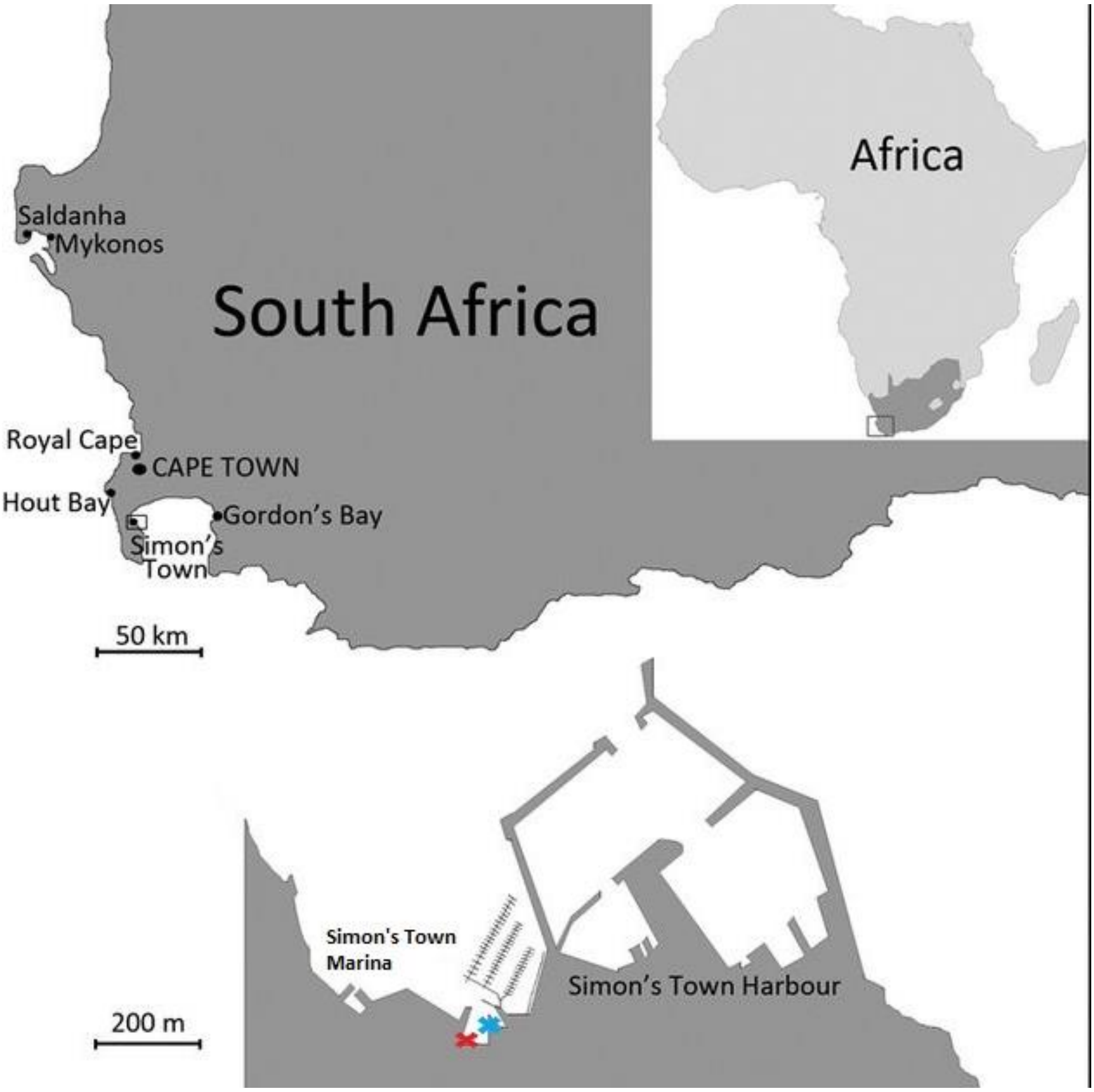


Figure 3.1: Map of sampling site, Simon's Town Marina, Cape Town (The red cross indicates the location of the sampling site, and the blue cross indicates the location of the boat building/maintenance facility) (Peters and Robinson, 2017).

Simon's Town is a is approximately 40 km from the City of Cape Town and is one of the last working fishing villages in the Cape Peninsula (Ojemaye and Petrik, 2019). The fishing harbour does not just provide safe storage for fishing vessels and fishing-related economic activities, but also does fishing vessel maintenance. Simon's Town is a semi-enclosed bay that is generally shallow throughout the water column. The general wind pattern that occurs offshore in the mouth of the greater False Bay causes increased wave height and storm surges. With the general flow pattern being clockwise throughout the greater False Bay area, cold water flows past Simon's Town from the south-west regions of False Bay, bringing potential pollutants from more southern regions of the bay.

3.1.2 Field sampling and laboratory processing of water and sediment samples

Water and sediment samples were collected during February 2020 from Simon's Town Marina (34°11'32.8"S 18°25'59.4"E). All sampling equipment was rinsed with site water before use. All glassware and metalware were autoclaved before use. All equipment was also rinsed with reverse osmosis (RO) water that was filtered through a 10 µm mesh before use.

Water samples were collected on the surface of the water column where the water was at least 50 cm deep. Bulk sampling of five separate replicates were taken of 20 ℓ per replicate, filtering each though a 250 µm metal sieve (equates to 100 ℓ of water sampled at the site). The particles on the sieve were rinsed into a clean falcon tube and taken to the laboratory for further analysis. Once in the laboratory, water was transferred from the falcon tubes into glass jar. The water was extracted from the falcon tubes three times using 10 µm filtered RO water to rinse out the falcon tube. The water was then digested in 10 % KOH (100 g KOH pellets added to 900 ml of filtered RO water, and then filtered through a 10 µm mesh after the pellets were fully dissolved to remove any MPs within the 10 % KOH solution) in a drying oven at 50 °C for 24 hours (model DHG 9070A) to remove any biological material. Once the sample had been digested, the contents was filtered through a 20 µm mesh and extracted three times. The mesh was placed in a clean petri dish and left to dry for analysis.

Marine sediment was collected at the strandline (high-tide mark). Five replicates at five-meter intervals (parallel to the water) were collected from a 0.25 m x 0.25 m quadrat to a depth of 5 cm with a metal spoon. The sediment was collected in a container and taken to the laboratory for further analysis. Once in the laboratory, at least 200 g (weighed to the nearest 0.01 g with a Highland® HCB 302 portable precision balance) of sediment was transferred into an aluminium container with a metal spoon and covered with aluminium foil. The sediment was then placed in a drying oven to dry completely or until a constant weight. Once dried, 100 g of sediment was taken for MP analysis.

The sediment in the jar for MP analysis was digested with 10 % KOH (Same preparation method as before) in a drying oven at 50 °C for 24 hours to remove any biological material within the sediment. Once digested, the sample was submersed in hypersaline solution (360 g of salt per 1ℓ of 10 µm filtered RO water, and then filtered through a 10 µm mesh once the salt is fully dissolved to remove any MPs within the hypersaline solution). The sediment was stirred for two minutes with a metal spoon and left to settle for 15 minutes. Once the settling period was completed, the supernatant was filtered through a 20 µm mesh. The hypersaline extraction process was done three times per sample. After the sample was extracted three times, the mesh was placed in a clean petri dish and left to dry for analysis.

3.1.3 Biota laboratory processing

Marine biota was collected at the site. At least 20 specimens and as much as 30 specimens (20 ≤ n ≤ 30) were collected per species at the site. Specimens were put into plastics Ziplock bags and kept on ice in a cooler box for transportation back to the laboratory.

Table 3.1: Organisms sampled at Simon's Town Marina

Feeding strategy	Organism	Common name (referred to in text)	Number of samples (n)
Filter feeder	*Mytilus galloprovincialis*	Mussel	30
	Pyura stolonifera	Redbait	27
Grazer	*Parvulastra exigua*	Cushion star	23
	Scutellastra longicosta	Limpet	30
	Oxystele tigrana	Winkle	30
Scavenger/predator	*Marthasterias glacialis*	Starfish	25
	Burnupena lagenaria	Whelk	30

Once in the laboratory, the total whole weight (shell and wet soft tissue - WW) was taken in grams (g) and the soft tissue carefully removed from the shell and weighed (tissue weight – TW). Where applicable, the total shell length (TL) was also measured in millimeters (mm) using Vernier callipers. The soft tissue was placed in a glass jar and digested in an 10 % KOH solution (Same preparation method as before) in a 1:2 ratio (one-part tissue to two-parts KOH) in a drying oven for 24 hours at 50 °C. Once digested, the contents in the jar were filtered over a 20 µm mesh. The contents of the jar were extracted three times over the 20 µm mesh using 10 µm filtered RO water to rinse out the jar, and the mesh was placed in a clean petri dish and left to dry for analysis.

3.1.4 Sampling and laboratory processing for microplastic particle analysis

Once isolated, MP particles were analysed under microscope. The particles were classified according to MP type (fiber/filament, fragment, sphere, or film), colour (white, transparent, yellow/orange, red/pink, blue/green or black/grey) and size in millimeters (< 0.1, 0.1 – 0.5, 0.5 – 1, 1 – 2, 2 – 5 and > 5). Once isolated, 10 % of total MP particles are scanned by an FTIR for plastic composition analysis.

Once the MPs were isolated and identified, 10 % of the total number of MPs (selected particles also had to be bigger that 500 µm in size) were scanned using an FTIR (Perkin Elmer Two ATR-FTIR Spectrometer) scanner in order to identify polymer types following the method of Sparks and Awe (2021). The spectral wave was set to range between and 4000 and 450 cm^{-1}, the resolution was set to 4 cm^{-1}, the data interval set to 1 cm^{-1} and the number of scans was set to ten. A background scan was done before starting any scan in order to calibrate the FTIR and the ATR crystal was cleaned with ethanol between each scan. The polymers were identified by comparing spectral scans with the ST Japan Library and a Perkin Spectral Library provided by the supplier (Perkin Elmer).

3.1.5 Statistical analysis

All statistical analysis was done using IBM SPSS Statistics V28 software package. MP data for water was expressed as MP/L and as MP/kg for sediment samples. MP count for biota data was expressed as MP/g of soft tissue weight (STW). The data was tested for normality using the Kolmogorov-Smirnov test, using data skewness and histograms. It was found that data was not normal and non-parametric tests for significance was undertaken using Mann-Whitney tests U tests (tested for significant difference between two groups) and Kruskal-Wallis H tests (tested for significance between 2 or more groups; H = Degrees of freedom, number of groups - 1) The variance of data was presented as the standard error around the mean (SE) and the significance level was $p < 0.05$.

A Spearman-rank correlation was conducted in within grazers in order to determine the relationship between the total number of microplastics, the soft tissue wight (g) and the total weight (g).

3.1.6 Quality control

The colour and type of clothing worn when field sampling was noted to be able to identify any contamination in field samples. In order to minimise plastic contamination from foreign sources with field and laboratory work, glass and metal equipment were used where possible. Within the processing lab, doors and windows were closed at all times to eliminate airborne contamination and the use of air conditioning was strictly prohibited. The metal and glass equipment were autoclaved and then rinsed with 20 µm-filtered MilliQ ultra-pure water before every use. The water, 10 % KOH and hypersaline solution were filtered through 20 µm mesh before use. All samples and solutions were covered with foil where possible minimise airborne contamination.

Extraction efficiencies were performed, where a known number of MP particles (particles were prepared to be between 65 µm and 500 µm in size) were put into a liter of 20 µm-filtered MilliQ ultra-pure water and was treated as a water sample and MPs were isolated according to the water processing SOP. MPs were then counted, and the yield was calculated (90 % yield was calculated).

Negative controls (blanks) were used by filtering empty filtering meshes to determine any contamination from the filtering system used. Positive controls were used in the lab where any processing of samples was done by using a petri dish and a damp filter paper. The petri dish with damp filter paper was left open when any processing was done in the lab in order to capture any airborne MP contaminants. The contamination controls were recorded at the end of each day of processing and subtracted from the analysed results. Petri dished were kept closed at all times and only open for any processing or analysing procedures.

3.1.7 Risk assessment calculations

Risk assessment indices were applied to all samples of MPs to provide comparative assessments of the potential risk MPs could pose to the environment. The concentration of MPs (C_{MP}) is assessed against the background concentrations by using the MP contamination factor (MPCF).

Equation 3.1

$$MPCF_i = \left(\frac{C_{microplastic}}{C_{baseline}}\right)$$

$C_{baseline}$ value is the lowest average concentration of MPs for the given site (Simon's Town) for water, sediment, and the various feeding strategies (filter feeders, grazers and scavengers/predators). The MP pollution index (MPPLI) was also calculated:

Equation 3.2

$$MPPLI_{site} = \sqrt[2]{MPCFil \times MPCFrag}$$

The MPCFil and MPCFrag were MPCFs for filaments and fragments. Filament and fragments were the most abundant MP particles found across all sample types. The chemical toxicity of the various polymers found was assessed based of the methodology used by Lithner *et al* (2011). Hazard scores are assigned to various polymer types the assess the risk of various polymer types poses to the environment.

Equation 3.3

$$H_i = \sum P_n \times S_n$$

H_i is the polymer risk index , and P_n is the ratio of a polymer type recorded at the site and S_n is the polymer hazard score assigned by Lithner *et al* (2011). The pollution risk index was (PLI) was calculated:

Equation 3.4

$$PRI = \sum H_i \times MPPLI_{site}$$

The PRI is the ecological hazard of polymers in relation to the polymer risk index (H_i).

To calculate the microplastic pollution load index (MPPI), a baseline value was required in the same area to compare against current microplastic pollution loads for this study. Where applicable, current microplastic loads were compared against Julius (2022) (Unpublished data) and results were compared with water, sediment, filter feeders, grazers and predatory invertebrates that were sampled at the same site.

3.2 Experimental study

3.2.1 Microplastic filaments creation and identification

Microplastic filaments (MPF) were created at different size classes. MPFs were shaved off from a neon yellow high-visibility jacket with a steel scissors. The bright colour of the high-visibility jacket provided an easily identifiable MPF source, as it is not typically found in the natural coastal marine environment (Woods et al., 2018).

Experimental procedures commonly use concentrations several times greater than that reported in field studies. This is because of the difficulty in accurately replicating microplastic concentrations found in natural environments in experimental studies, and allows the control of factors that otherwise would not have been controlled in field studies (Gerber, 2017). MPF's were separated into different size classes by filtering the cut MPFs with 10 µm-filtered reverse osmosis (RO) water through a multi-tiered filtering system with various mesh sizes vertically stacked on top of each other. An emphasis was placed on smaller MPFs measuring between 65 µm and 500 µm, as well as longer MPFs that range between 500 µm and 1000 µm. The size categories and concentrations were chosen to be consistent with MPF sizes found in marine water, sediment and biota samples consistent with environmental data (Woods et al., 2018). MPFs were soaked in filtered seawater (FSW) for 2 days before use to mimic conditions found in the environment (Ward et al., 2019).

3.2.2 Specimen depuration and acclimatisation

Mussels were collected from Maiden's Cove (-33.945049, 18.373579) and taken to the laboratory. In total, 30 mussels were collected for the MPF size experiment. The mussels were left in depurating tank for a period of two days (48 hours) to depurate in 25 ℓ of 20 µm filtered seawater (FSW) and each mussel was provided with 0.1 g of food (food was sourced from a local pet store). The FSW was kept constant with conditions found at the sampling site (salinity was kept at 31 ppt and temperature was kept at 13 °C). The water was aerated with a submersible pump. This period was selected to allow mussels to adjust to experimental conditions (Webb et al., 2020) and to clear their gut of any MPs they took up from the environment.

3.2.3 MPF size uptake exposure experiment

Eleven 2 ℓ glass beakers were prepared and cleaned with 10 µm RO water. Two mussels were placed in each glass beaker with 0.5 ℓ of water per mussel (1 ℓ per beaker) and 50 MPF's and 0.1 g of food in order to entice them to feed on particles . Each beaker was aerated with a single aeration tube and the aeration tube was placed not more than three centimeters from the bottom of the glass beaker. The FSW was kept constant with conditions found at the sampling site (salinity was kept at 31 ppt and temperature was kept at 13 °C). A beaker was labelled as the control beaker with no MPFs being inserted into the control beaker. The remaining ten beakers were divided into two groups of five each, with one set of beakers being exposed to a smaller size of MPFs of less than 500 µm (65 µm – 500 µm) and the other set of five being exposed to a larger size of MPFs in each beaker of more than 500 µm (500 µm – 1000 µm) (Ward et al., 2019).

Once the MPFs were inserted into the beakers and the time elapsed, two mussels were removed from a small MPF size beaker and another two was removed from a large MPF size beaker at five different timing events (T_1 – T_5). Timing events were T_1 = 15 minutes, T_2 = 30 minutes, T_3 = 1 hour, T_4 = 3 hours and T_5 = 6 hours after initial exposure. Once removed, the pair of mussels was combined and placed in a glass jar and kept for digestion and analysis. Three replicates of the experiment were done. Once the time had elapsed, the last remaining two mussels from the control was removed.

The total shell length was recorded using Vernier callipers (mm) and the total whole weight (shell and soft tissue was recorded (g) (weighed to the nearest 0.01 g with a Highland® HCB 302 portable precision balance). The soft tissue was removed, and the soft tissue weight was taken in grams (g) and digested in 10 % potassium hydroxide (KOH) to remove any organic material and placed in an oven at 60 °C for 24 hours to digest. Once 24 hours elapsed, the contents of each jar were extracted over a 20 µm mesh three times, using 10 µm filtered RO water to rinse out the jar, and the mesh was placed in a clean petri dish and left to dry for analysis.

3.2.4 Sample analysis for microplastics

Under a dissecting microscope, samples were analysed for MPFs. Only MPFs that was created from the neon yellow high-visibility jacket was counted and MPFs were either in the size class of between 65 µm and 500µm or 500 µm and 1000 µm.

3.2.5 Statistical analysis

All statistical analysis was done using IBM SPSS Statistics V28 software package. MPF count was expressed as MP/I and MP/g of soft tissue weight. The data was tested for normality using the Kolmogorov-Smirnov test, using data skewness and histograms. It was found that data was not normal and non-parametric tests was done for significance was undertaken using Kruskal-Wallis H tests (tested for significance between 2 or more groups; H = Degrees of freedom, number of groups - 1). The variance of data was presented as the standard error around the mean (SE) and the significance level was $p < 0.05$.

3.2.6 Quality control

In order to minimise plastic contamination from foreign sources with field and laboratory work, glass and metal equipment were used where possible. Within the processing lab, doors and windows were closed at all times to eliminate airborne contamination and the use of air conditioning was strictly prohibited. The metal and glass equipment were autoclaved and then rinsed with 20 µm-filtered MilliQ ultra-pure water before every use. The water and 10 % KOH solution were filtered through 20 µm mesh before use. All samples and solutions were covered with foil where possible minimise airborne contamination.

Extraction efficiencies were performed in practice runs of the experiment, where a known number of MP particles (the same size classes of MPFs that were to be used in the experiment were used to perform the extraction efficiencies) were put into a liter of 20 µm-FSW and was run for the duration of the experiment and extracted according to the SOP. MPFs were then counted, and the yield was calculated (100 % yield was calculated).

Negative controls (blanks) were used by filtering empty filtering meshes to determine any contamination from the experimental and filtering system used. Positive controls were used in the lab where any processing of samples was done by using a petri dish and a damp filter paper. The petri dish with damp filter paper was left open when any processing was done in the lab in order to capture any airborne MP contaminants. The contamination controls were recorded at the end of each day of processing and subtracted from the analysed results. Petri dished were kept closed at all times and only open for any processing or analysing procedures. During the experiment, the top of the tanks was kept closed with foil in order to minimise any airborne contamination.

4. Chapter 4 - Results

4.1 Field Study

4.1.1 Environmental parameters (Water and sediment)

Water (MP/L) and sediment (MP/kg) concentrations

Microplastic concentration was analysed in five water (MPs/L) and five sediment (MPs/kg) samples collected in Simon's Town Marina. Sediment samples had the highest mean concentration (38 ± 2.00 MPs/kg) compared to water samples (0.37 ± 0.06 MPs/L) (Figure 4.1). The post hoc test showed a significant difference between water and sediment samples (U = 0.00, p = 0.007).

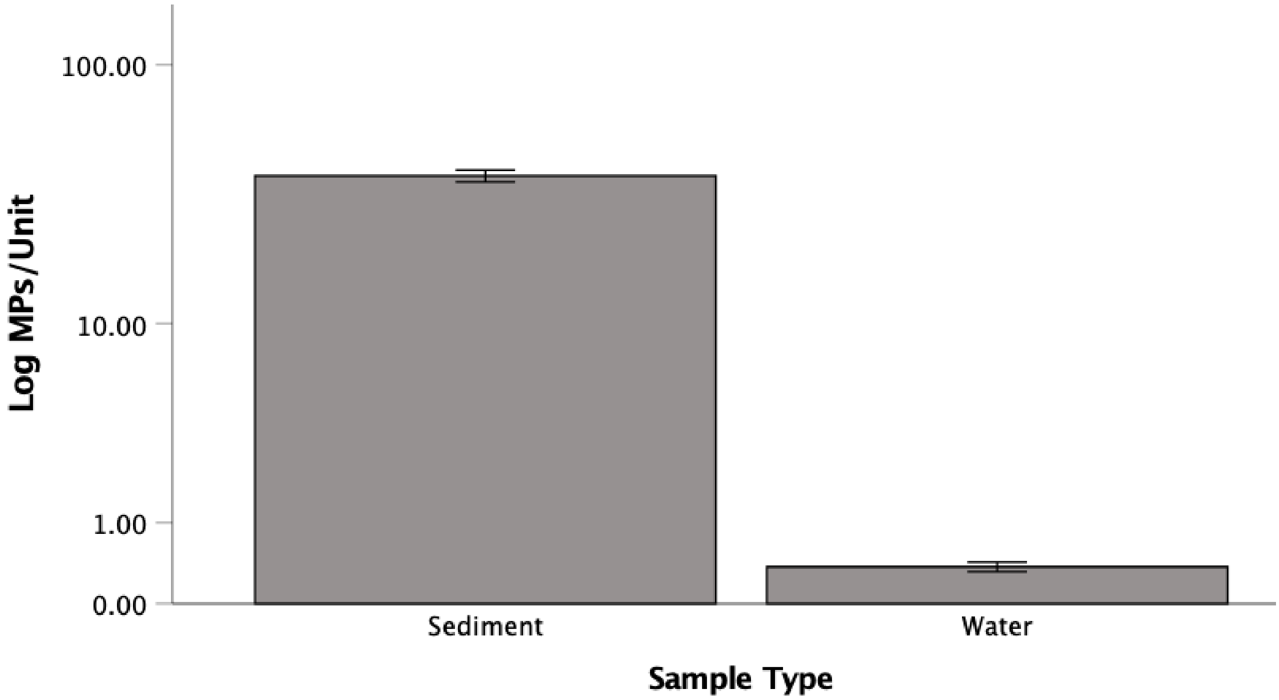


Figure 4.1: Mean MP concentration found in water (MPs/L) and sediment (MPs/kg) samples in Simon's Town.

Microplastic types in water and sediment

MP type was analysed in five sediment and five water samples collected in Simon's Town Marina (). Filaments were the most dominant MP type in both sediment and water samples (86.67% and 97.50% respectively). Fragments were only recorded in sediment samples (6.67%) and there were no spheres recorded for both sample types.

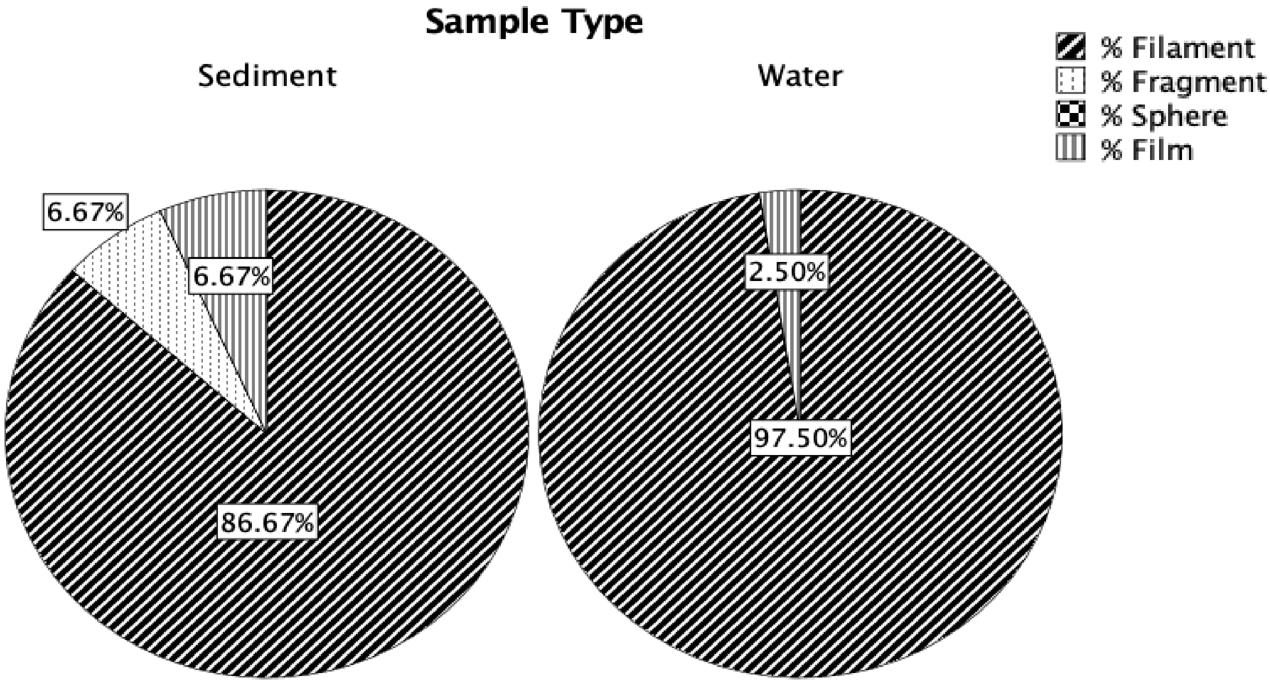


Figure 4.2: MP type in water and sediment samples collected in Simon's Town Marina.

Microplastic colours in water and sediment

Referring to Figure 4.3, MP particle colour varied more in sediment samples than that in water samples. Black particles dominated water samples with 53.23 % compared to only 11.67 % in sediment samples. Green particles dominated sediment samples. Yellow particles respresented the least in both water and sediment samples.

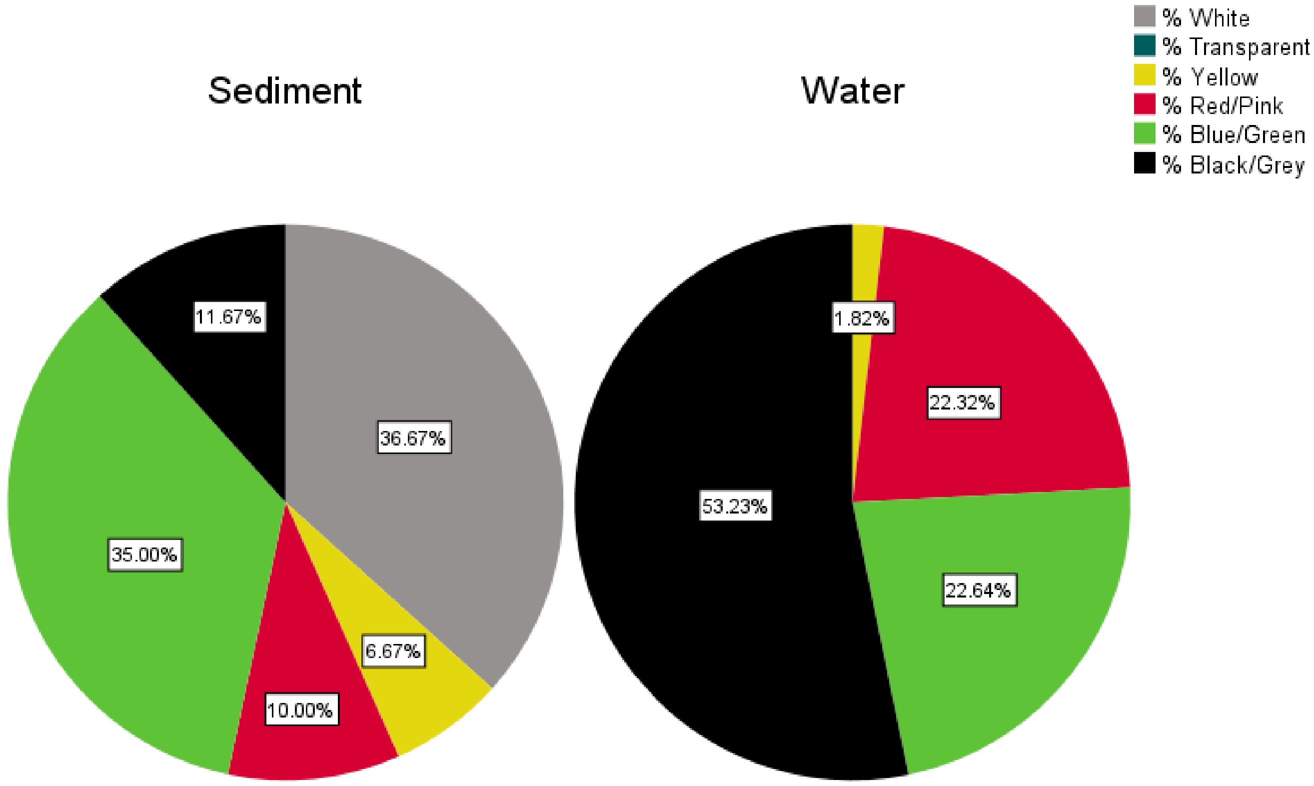


Figure 4.3: Percentage (%) of MP colours identified in sediment and water samples.

Microplastic sizes (µm) in water and sediment

MP size (µm) varied in sediment and water samples. The most dominant MP size in sediment and water samples was between 100 – 500 µm (75% and 95% respectively) followed by MPs between 500 – 1000 µm (25% and 5% respectively) (*Figure 4.2: MP type in water and sediment samples collected in Simon's Town Marina.*).

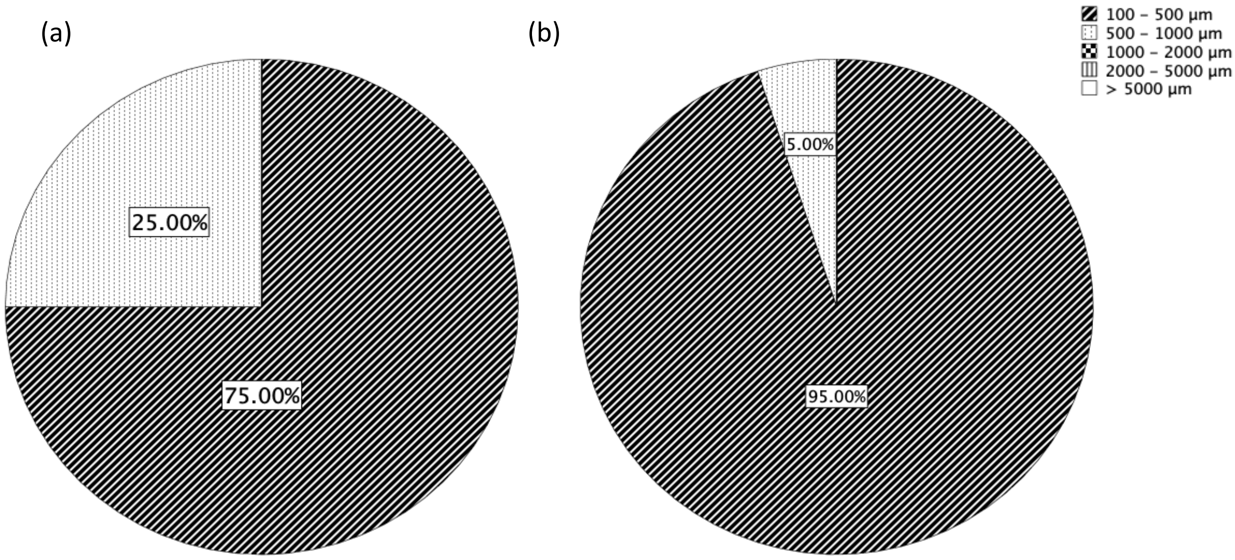


Figure 4.4: Percentage (%) of MP size µm in a) sediment and b) water samples collected in Simon's Town Marina.

4.1.2 Biota

Microplastic concentrations (MP/g) and abundance (MP/I) in organisms using different feeding strategies

Microplastic abundance (MPs/I) and concentration (MPs/g) was analyzed in various feeding strategies sampled in Simon's Town Marina. Scavengers/predators showed the highest abundance (4.47 ± 0.51 MPs/I) (Figure 4.5: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in various feeding strategies sampled in Simon's Town Marina). The post hoc test showed a significant difference between all feeding types, except between filter-feeders and grazers (H(2) = -12.091, p = 0.208). Grazers showed the highest concentration (1.45 ± 0.27 MPs/g) (Figure 4.5: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in various feeding strategies sampled in Simon's Town Marina). Filter-feeders had the lowest abundance and concentration for all feeding types (1.54 ± 0.28 MPs/I and 0.28 ± 0.44 MPs/g respectively). The post hoc test showed a significant difference between all feeding types, except between grazers and scavenger/predator species (H(2) = -5.304, p = 0.586).

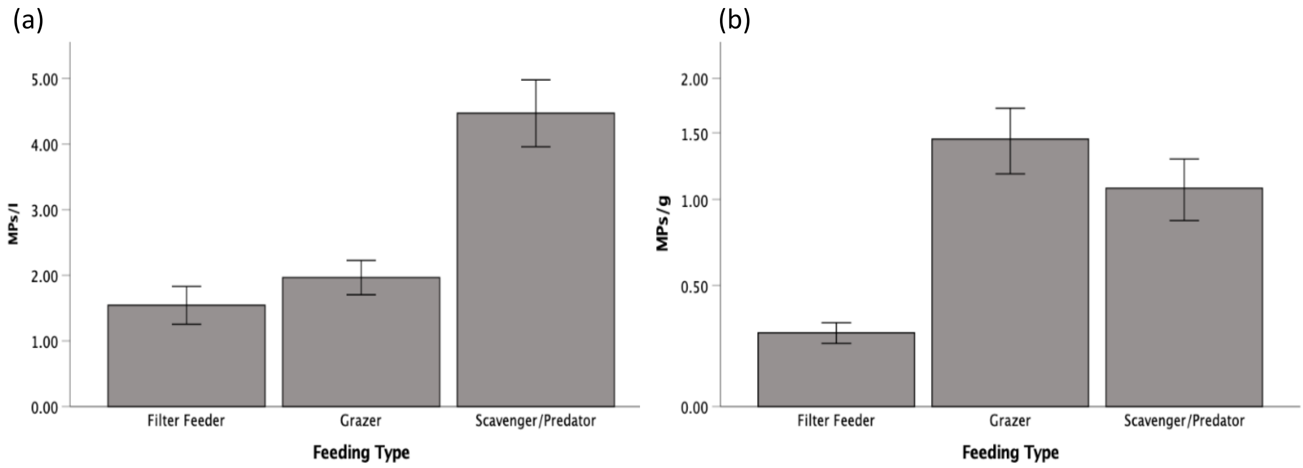


Figure 4.5: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in various feeding strategies sampled in Simon's Town Marina

Microplastic concentrations (MP/g) and abundance (MP/I) in organisms of different feeding strategies

Microplastic abundance (MPs/I) and concentration (MPs/g) was analyzed in various filter-feeding species sampled in Simon's Town Marina. Mussels had the highest abundance (1.68 ± 0.25 MPs/I) (Figure 4.6: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) per filter-feeding type sampled within Simon's Town Marina) and concentration (0.40 ± 0.06 MPs/g) (Figure 4.6: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) per filter-feeding type sampled within Simon's Town Marina) of the filter feeder species.

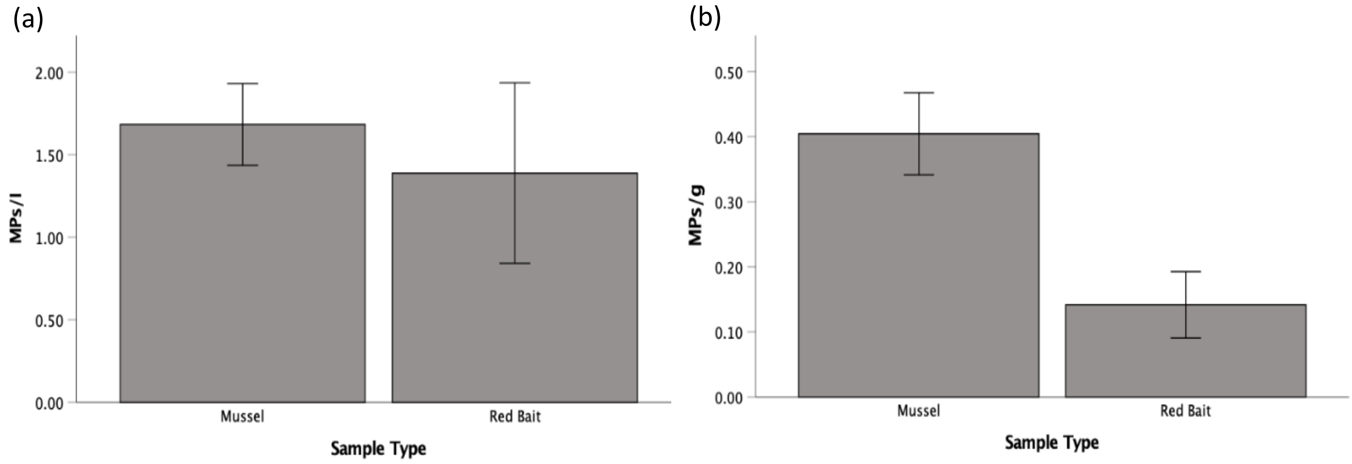


Figure 4.6: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) per filter-feeding type sampled within Simon's Town Marina

Microplastic abundance (MPs/I) and concentration (MPs/g) was analyzed in various grazer species sampled in Simon's Town Marina. Cushion stars had the highest abundance (2.52 ± 0.33 MPs/I) (Figure 4.7: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) per grazer type sampled within Simon's Town Marina) and concentration (3.47 ± 0.57 MPs/g) (Figure 4.7: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) per grazer type sampled within Simon's Town Marina) of the grazer species. Limpets had the lowest abundance (1.24 ± 0.27 MPs/I) and concentration (0.11 ± 0.03 MPs/g) of the grazer species.

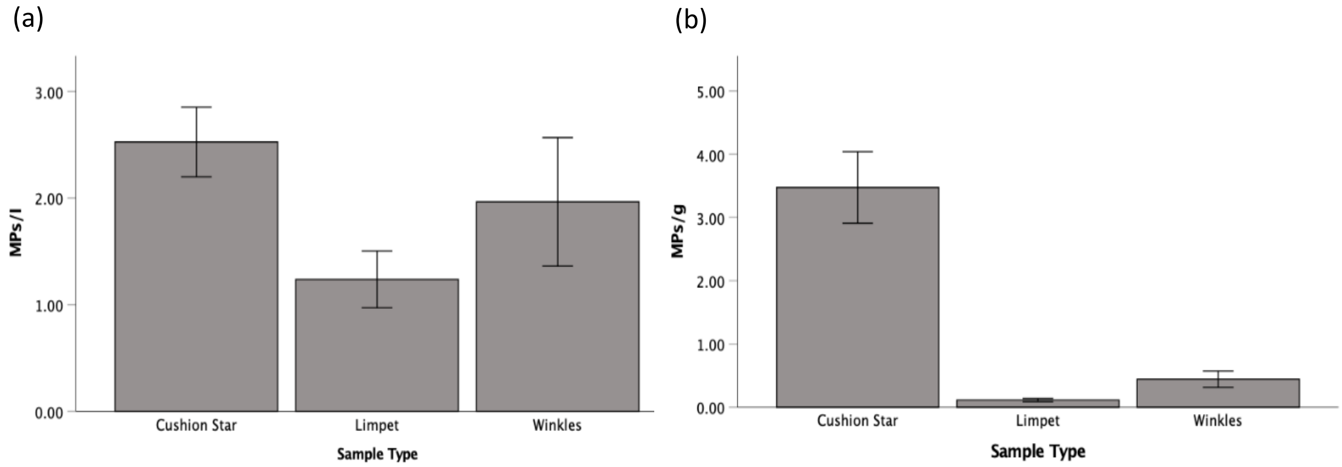


Figure 4.7: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) per grazer type sampled within Simon's Town Marina

Microplastic abundance (MPs/I) and concentration (MPs/g) was analyzed was in various scavenger/predator species sampled in Simon's Town Marina. Starfish species had the highest abundance (6.70 ± 0.86 MPs/I) (figure # a) and whelks had the highest concentration (1.84 ± 0.33 MPs/g) (figure # b).

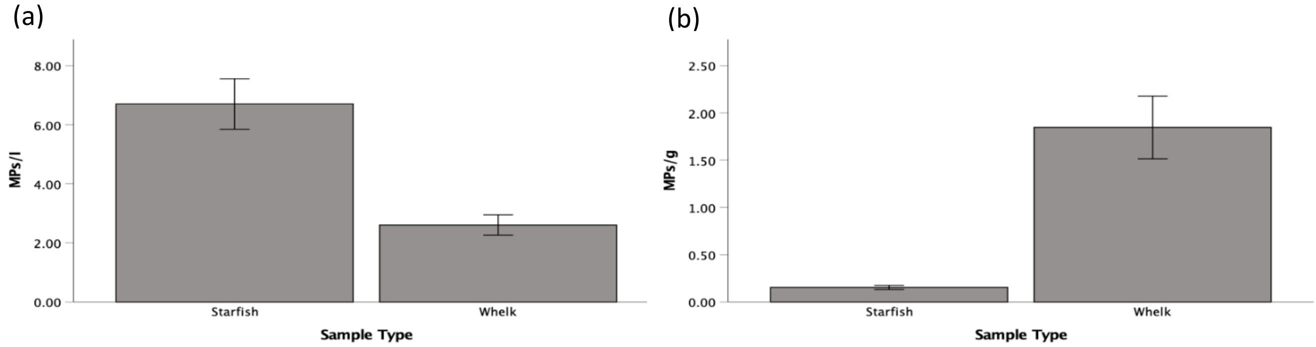


Figure 4.8: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) per scavenger/predator type sampled in Simon's Town Marina

Microplastic types in organisms of different feeding strategies

MP type was analyzed and varied across various feeding types (Figure 4.9: MP type in various feeding types sampled in Simon's Town Marina). Filaments were the dominant MP type with grazers having the highest percentage (99.66%). Fragments was highest in scavenger/predator samples (17.73%). There were no spheres recorded in any biological samples.

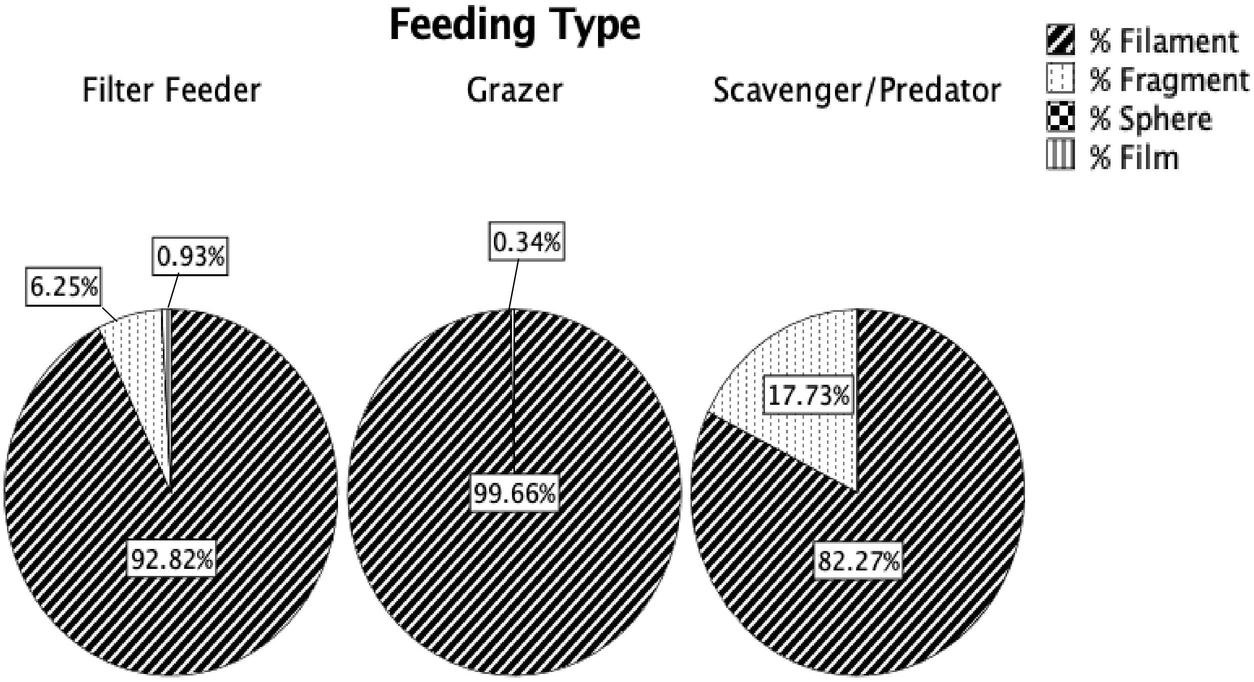


Figure 4.9: MP type in various feeding types sampled in Simon's Town Marina

Filaments was found in majority of the samples obtained (Figure 4.10). In scavengers/predators, 75 % of MP particles found in *B. laganaria* was filaments, while the remaining particles found were fragments of paint particles. In *M. glacialis*, 89 % was filaments with the remining also being fragments or paint particles. For filter feeders, all MP particles found in *P. stolonifera* were filaments, as well as 89.24 % of particles found in *M. galloprovincialis*. The remaining was also paint particle fragments. For grazers, 100 % for particles found in *O. tigrana* and *S. longicosta* were filaments, as well as 99.23 % of particles found in *P. exigua*.

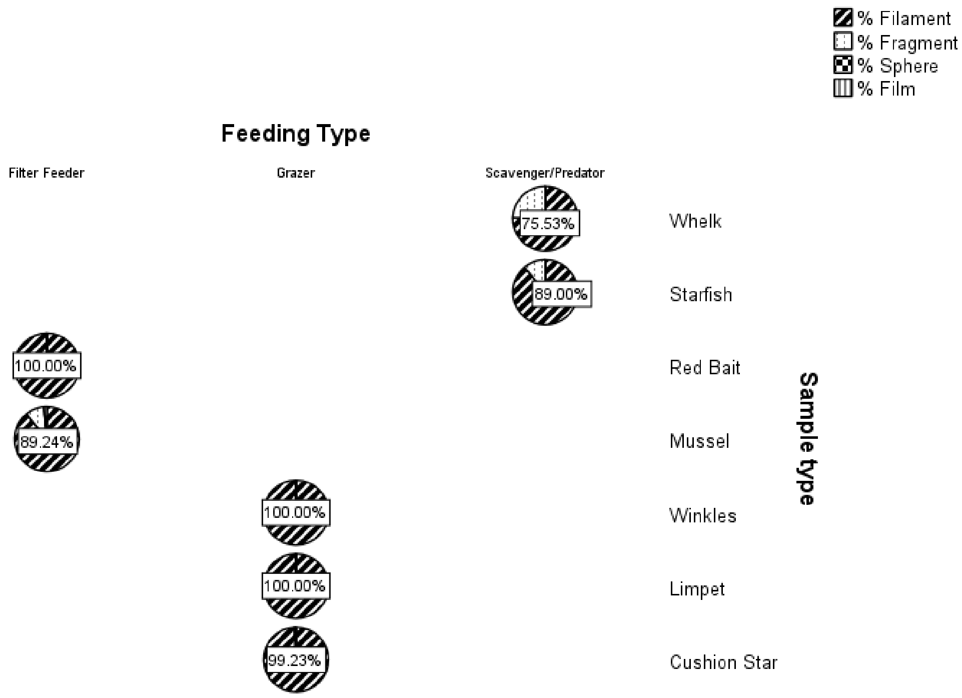


Figure 4.10: Percentage (%) of MP type per feeding strategy and individual organism

Microplastic colours in organisms of different feeding strategies

Black MPs dominated across grazers (60.14 %) and scavengers/predators (51.16 %). Green was the colour found mostly in filter feeders but is marginally higher than black MP particles. Filter feeders had a considerably higher percentage of transparent MP particles grazers and scavengers/predators.

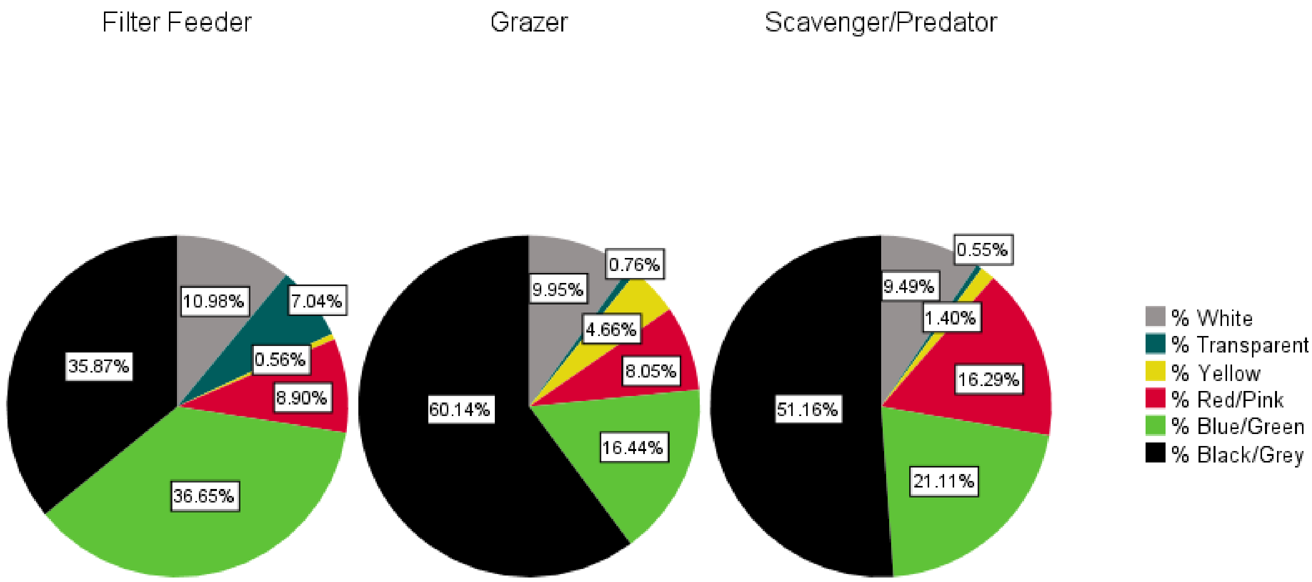


Figure 4.11: Colour composition of MP particles found in organisms using various feeding strategies

Microplastic size in organisms of different feeding strategies

MP size (µm) was analyzed in filter-feeder samples (Figure 4.12). MPs between 100 – 500 µm was the most dominant size (83.33%) followed by MP between 500 – 1000 µm (11.90%) for all filter-feeder species combine (Figure 4.12). Redbait had the highest percentage of MPs between 100 – 500 µm (93.93%) and mussels had the highest percentage of MPs between 500 – 1000 µm and 1000 – 2000 µm (16.60% and 4.17% respectively) (Figure 4.12).

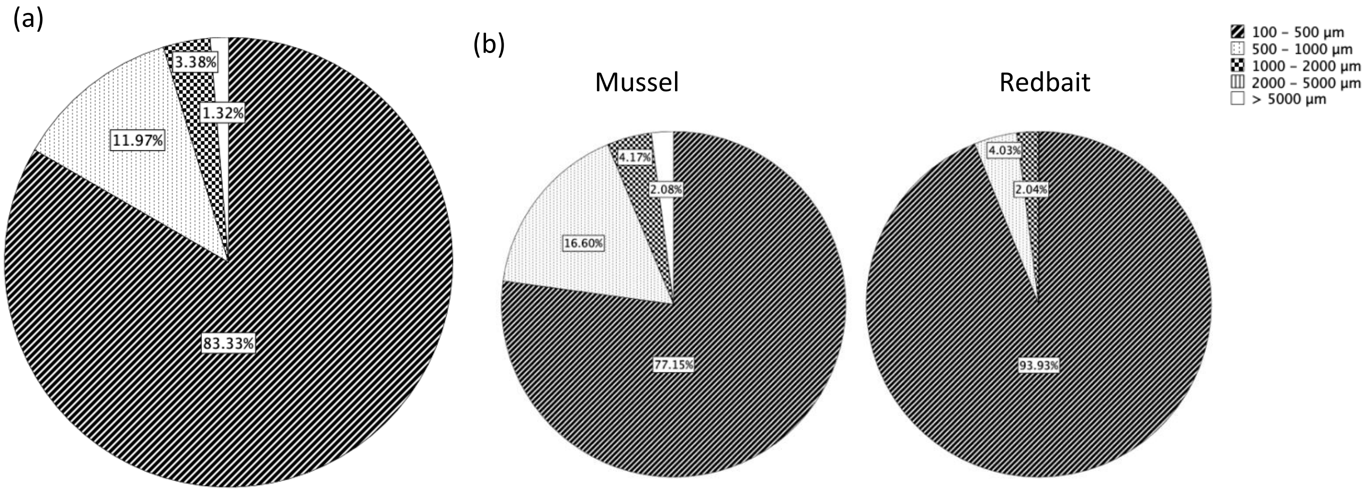


Figure 4.12: MP size (µm) in a) all filter-feeders combined and b) filter-feeder species sampled in Simon's Town Marina

MP size (µm) was analysed in grazer samples (Figure 4.13). MPs between 100 – 500 µm was the most dominant MP size for all grazer samples combine (82.27%) followed by MPs between 500 – 1000 µm (15.11%) (Figure 4.13). Winkles had the highest percentage of MPs between 100 – 500 µm (86.18%), whereas Limpets had the highest percentage of MPs between 500 – 1000 µm (19.23%) (Figure 4.13). Cushion stars had the highest percentage of MPs between 1000 – 2000 µm (3.85%).

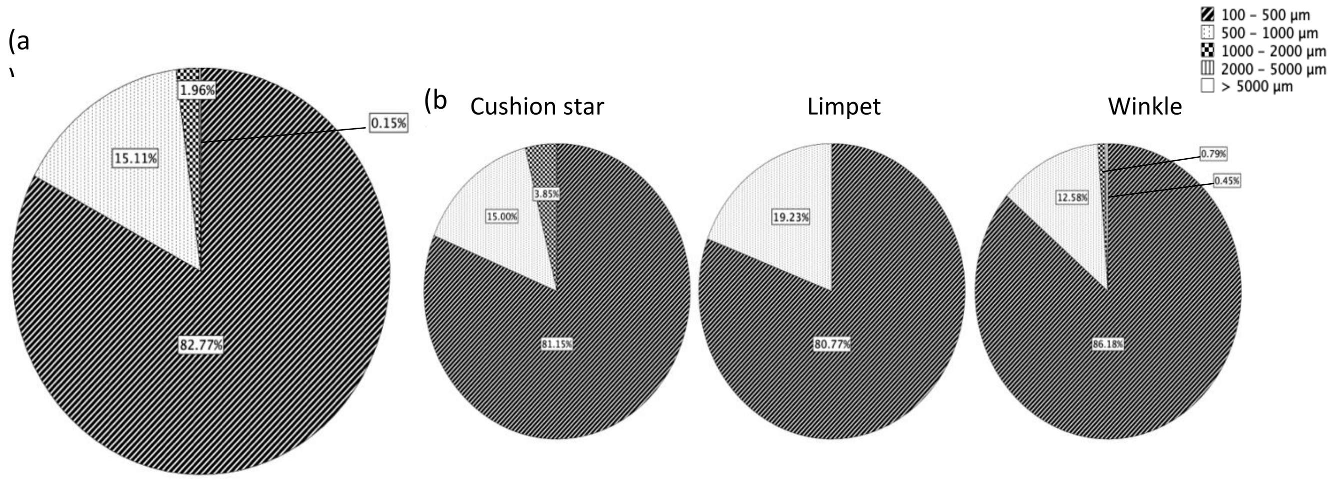


Figure 4.13: MP size (µm) in a) all grazers combined and b) grazer species sampled in Simon's Town Marina

MP size (µm) was analysed in scavenger/predator samples (Figure 4.14). MPs between 100 – 500 µm was the dominant MP size (96.12%) followed by MPs between 500 – 1000 µm (1.98%) (Figure 4.14). Whelks and starfish displayed similar results for MPs between 100 – 500 µm (95.87% and 96.37% respectively) (Figure 4.14). Starfish had the percentage of MPs between 500 – 1000 µm (2.63%) and whelks had the highest percentage of MPs > 5000 µm (2.80%).

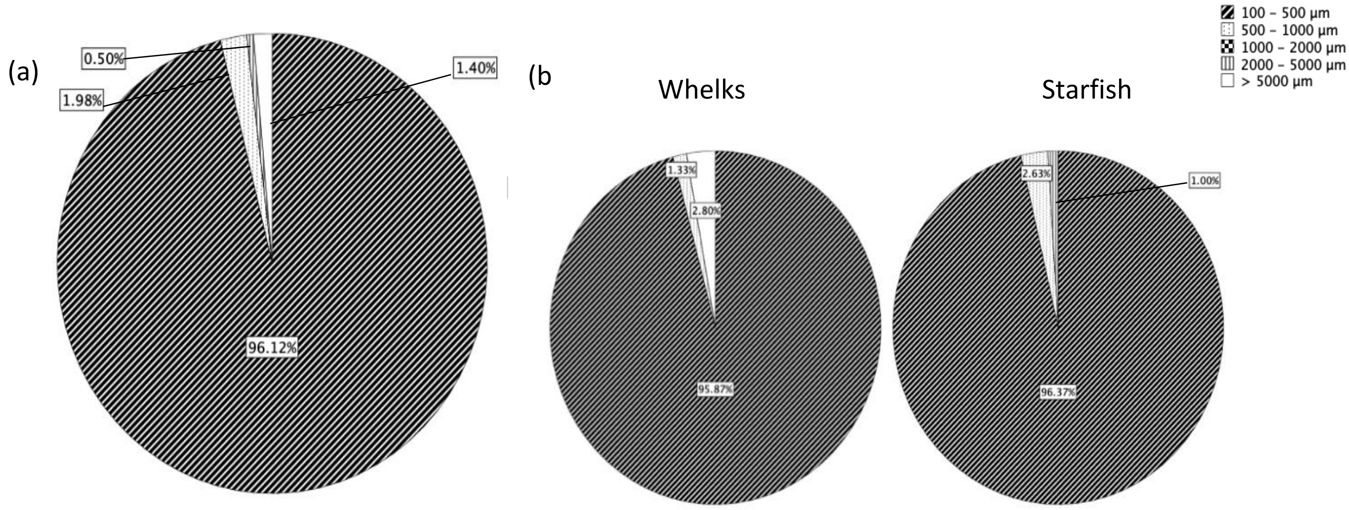


Figure 4.14: MP size (µm) in a) all scavenger/predator combine and b) scavenger/predator species sampled in Simon's Town Marina

Correlation between total number of microplastics, soft tissue weight in grams (g) and total weight (g) in grazers

A Spearman rank correlation was conducted between the total number MPs and total weight (g) of grazer species and the total number of MPs and soft tissue weight (g). The correlation between the total number of MPs and total weight (g) showed a weak (negative) correlation ($r = -0.229$) (Figure 4.15a). The correlation between total number of MPs and soft tissue weight (g) showed a weak (negative) correlation ($r = -0.205$) (Figure 4.15b). The correlation between grazer types showed, although a weak relationship, limpets have a greater correlation between total number of MPs and soft tissue weight ($r=0.275$) compared to other grazer types (Figure 4.15c).

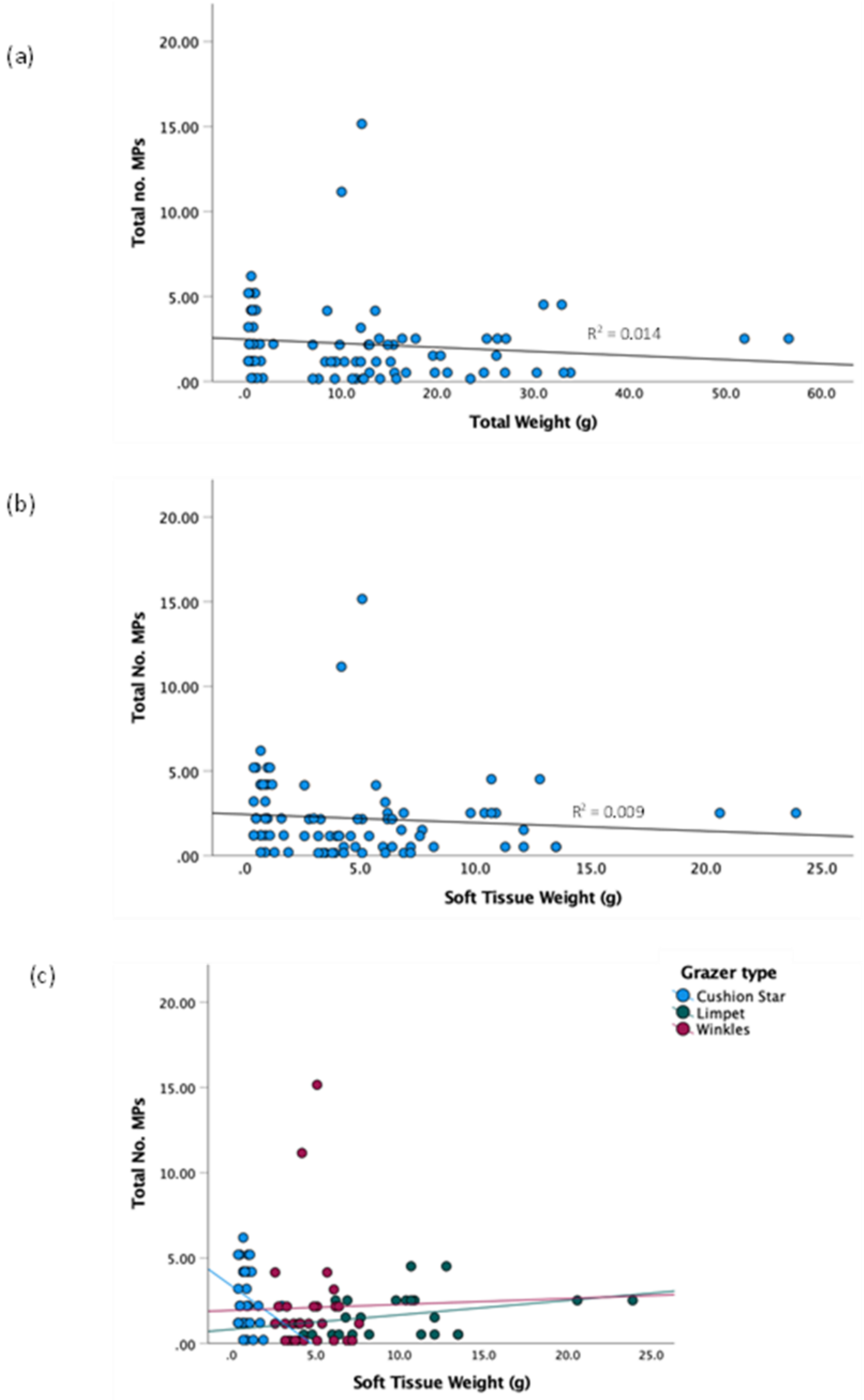


Figure 4.15: (a) Correlation between the total number of MPs in grazers and the total weight (MP/g), (b) Correlation between the total number of MPs in grazers and the soft tissue weight (g), and (c) Correlation between total number of MPs and the soft tissue weight (g), according to species

Polymer identification using FTIR

Majority of the microplastics found within samples were nylon-based (PA6) polymers at 27.27 % of particles scanned by the FTIR (Figure 4.16). The second highest was polyethylene terephthalate (PET) with 18.18 % recorded. Natural (NAT) particles were also recorded and was found to be cotton were also recorded at 18.18 % of samples. The lowest number of samples recorded was polyurethane (PUR) at 4.55 %.

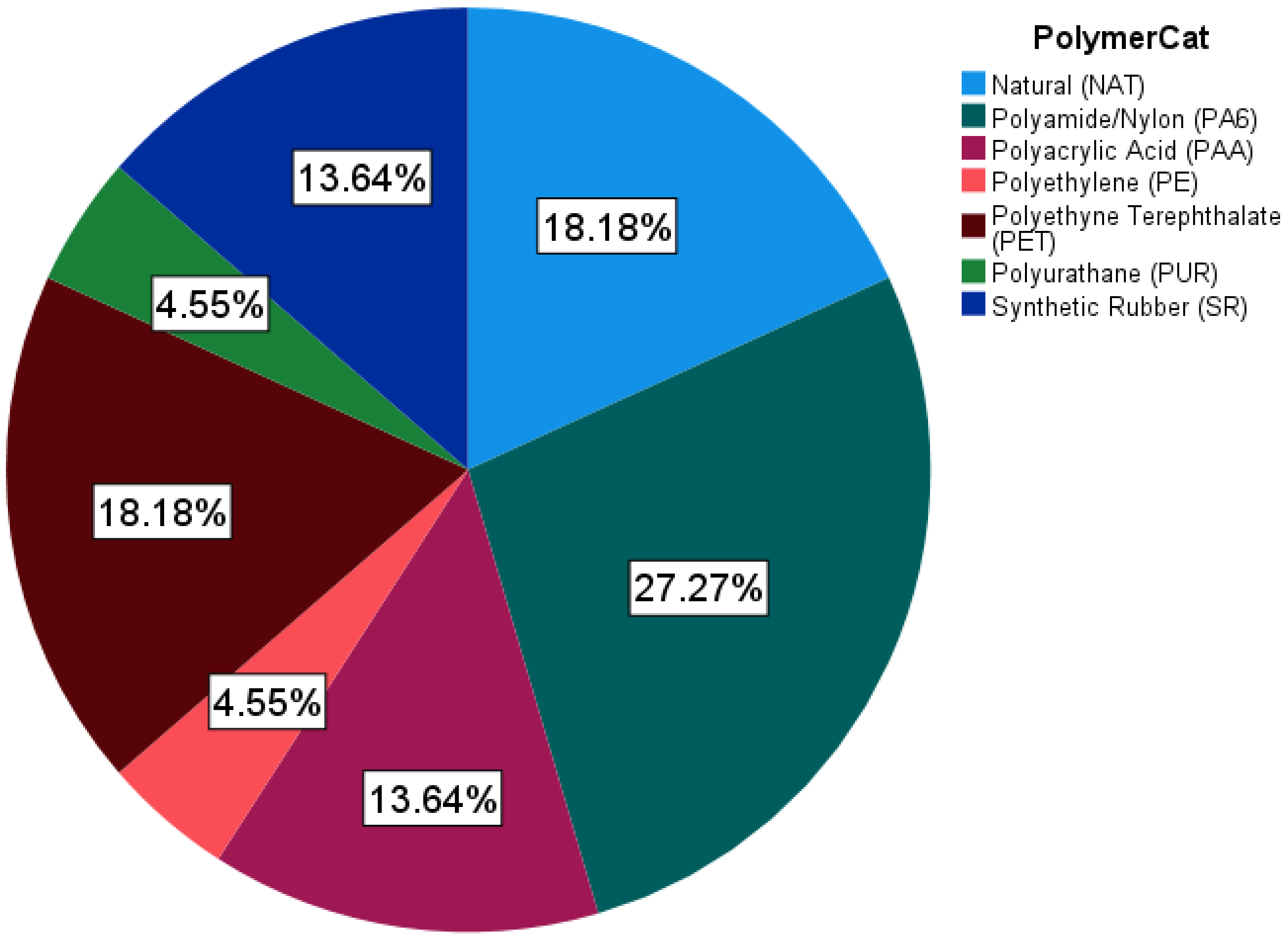


Figure 4.16: Percentage polymer types found in microplastics samples in Simon's Town Marina

Risk assessment of microplastics in water, sediment and biota

A risk assessment was conducted to determine the risk posed by MP samples collected in the Simon's Town Marina. The risk indices are displayed in Table 4.1: Risk assessment indices and categories for microplastic contamination in Simon's Town Marina).

Table 4.1: Risk assessment indices and categories for microplastic contamination in Simon's Town Marina

Risk category	**Low (I)**	**Moderate (II)**	**High (III)**	**Very high (IV)**	**Dangerous (V)**
Contamination factor (CF)	< 1	1 – 3	3 – 6	> 6	
Pollution load index (PLI)	< 1	1 – 3	3 - 5	4 - 5	>5
Polymer risk index (H)	< 10	10 – 100	101 – 1000	1000 – 10000	>10000
Pollution risk index (PRI)	< 150	150 - 300	300 – 600	600 – 1200	>1200

The average Pollution Load Index (PLI) for all sample types combined showed dangerous (V) contamination levels (Figure 4.17). Sediment, grazers and scavenger/predator samples displayed dangerous levels of MP contamination (55.31, 24.06 and 11.48 respectively), whereas water samples (0.32) and filter-feeding organisms (4.48) was had as low and very high levels, respectively (Figure 4.17).

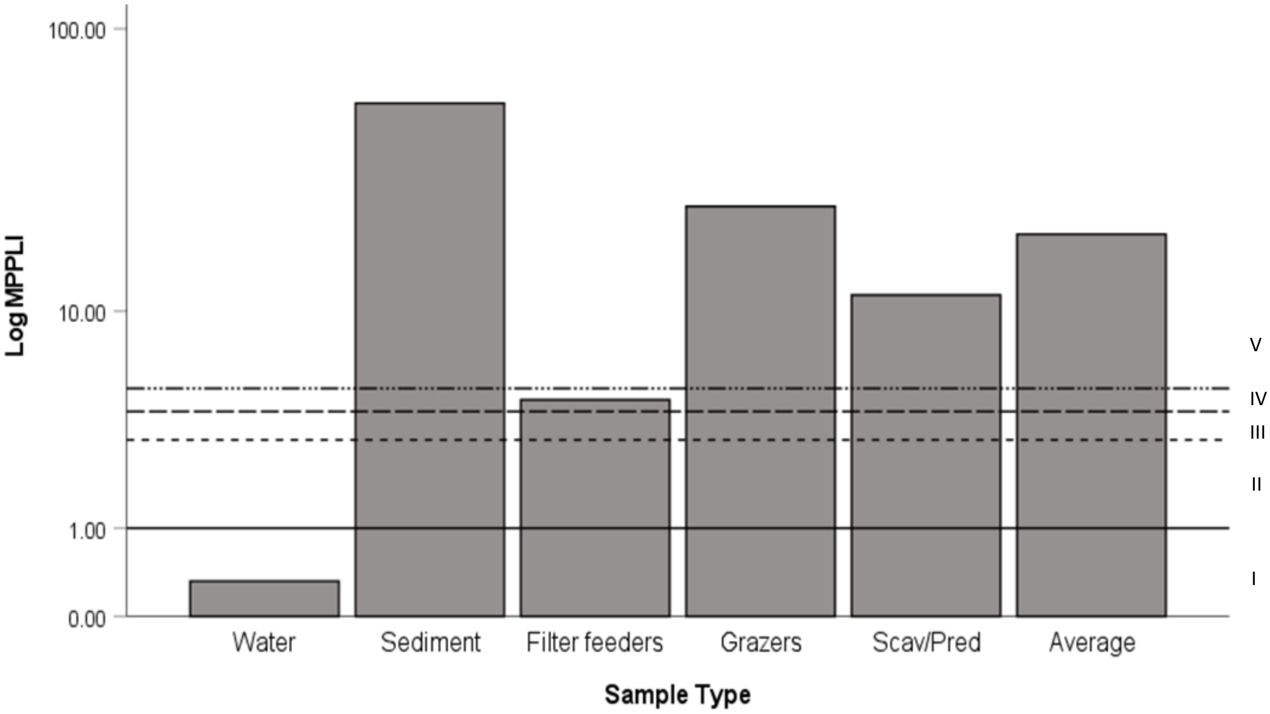


Figure 4.17: Pollution Load Index (MPPLI) in Simon's Town Marina between water, sediment, and various feeding strategies

The average Polymer Risk Index (H) showed a high risk (551.77) associated with polymers identified in Simon's Town (Figure 4.18). Sediment and scavenger/predators had no risk associated with polymers identified. Water and grazer species displayed moderate (50) and high risks (260) associated with polymers identified, respectively. Filter-feeding species showed a very high risk associated with polymers identified (2448.83).

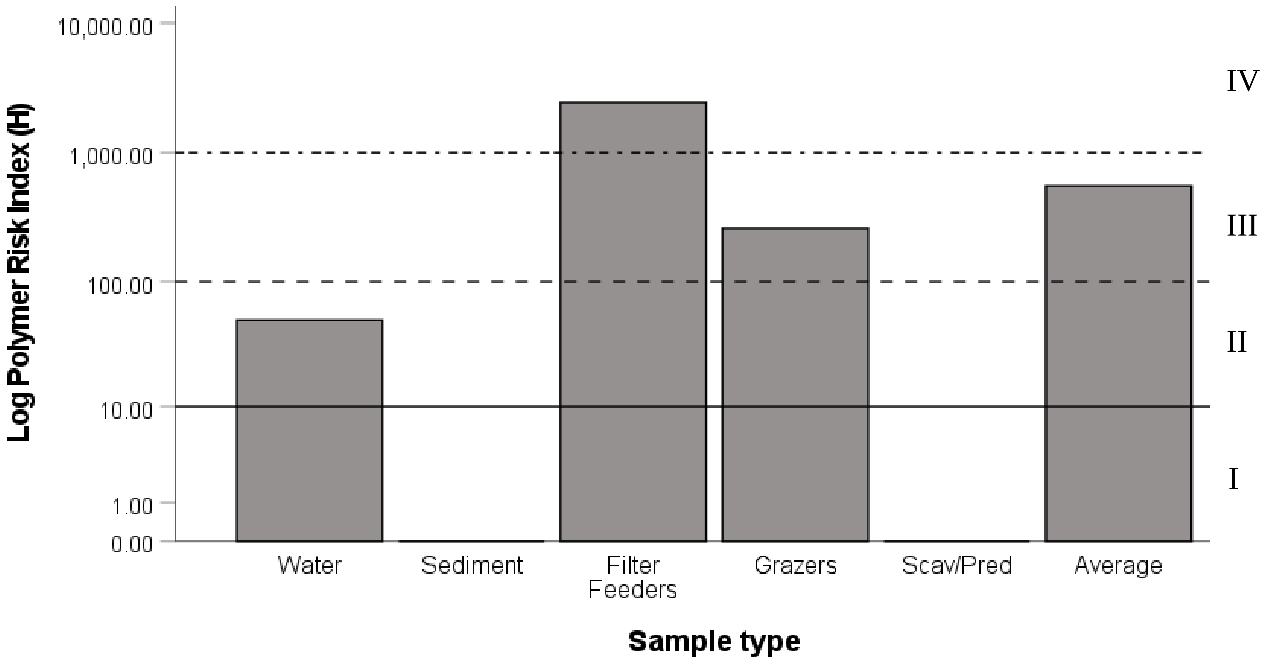


Figure 4.18: Polymer Risk Index (H) in Simon's Town Marina between water, sediment, and various feeding strategies

The average Pollution Load Index (PRI) for all samples combined showed a dangerous risk associated with pollutants identified in Simon's Town (Figure 4.19). Sediment and scavenger/predator species showed no risk posed by pollutants. Water samples displayed a low risk associated with pollutants (15.97), whereas filter-feeders and grazers had a dangerous risk associated with pollutants identified (10968.8 and 6256.7 respectively).

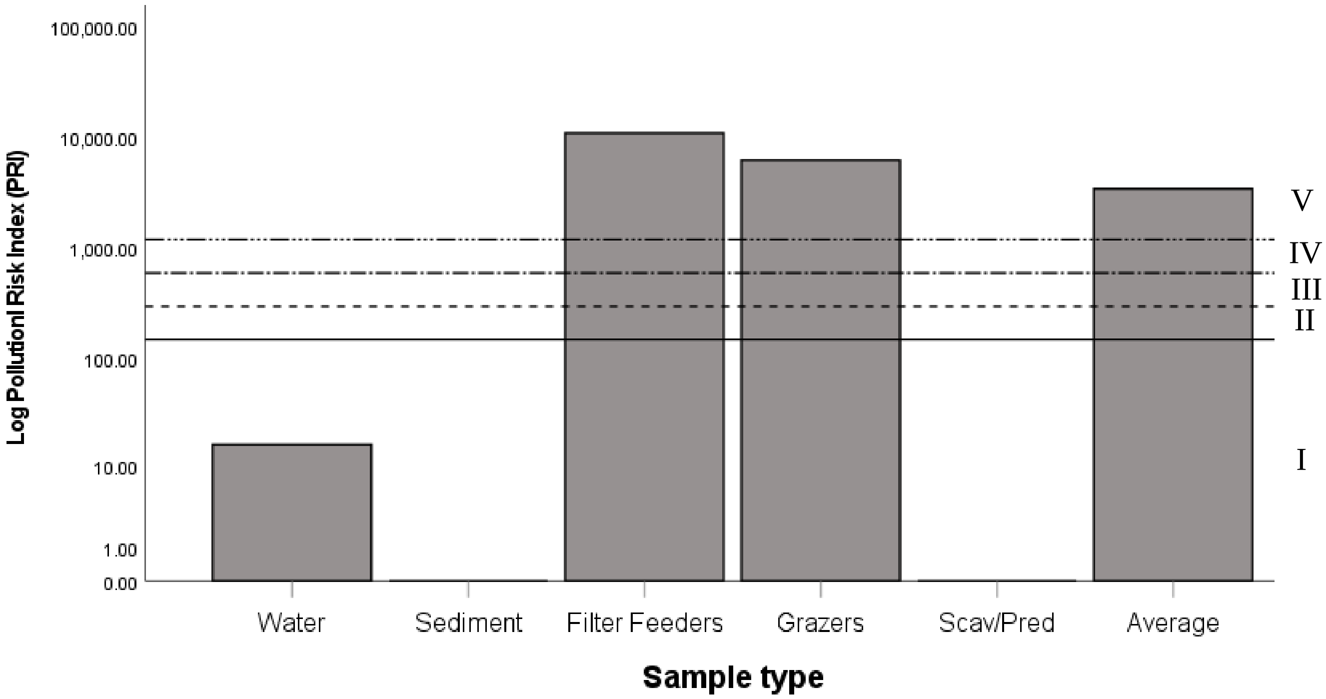


Figure 4.19: Pollution Risk Index (PRI) in Simon's Town Marina between water, sediment, and various feeding strategies

4.2 Experimental Study

4.2.1 Concentrations of microplastic uptake at different time intervals

Mussels were exposed to a set concentration of 50 MPs/L for various periods. MP abundance was highest at 60 minutes (23.16 ± 4.16 MPs/I) (Figure 4.20: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in exposed mussels at various time (minutes) intervals.). MP concentration was highest at 180 minutes (7.21 ± 1.70 MPs/g) (Figure 4.20: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in exposed mussels at various time (minutes) intervals.). MP abundance and concentration was lowest in mussels at 15 minutes (14.00 ± 1.00 MPs/I) and 360 minutes (3.60 ± 0.74 MPs/g).

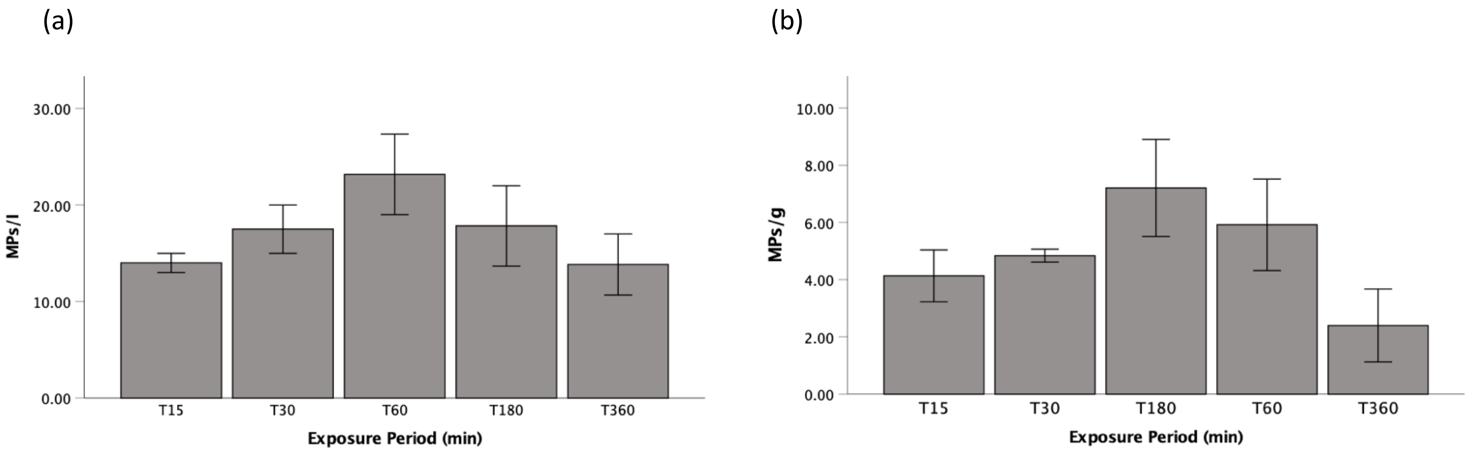
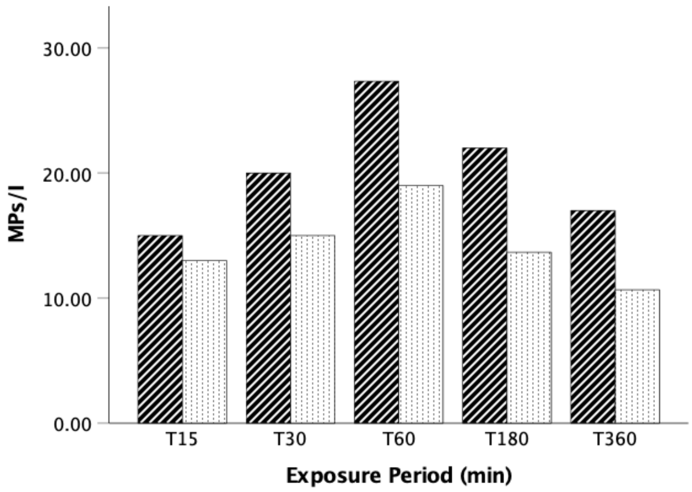


Figure 4.20: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in exposed mussels at various time (minutes) intervals.

Mussels were exposed to different sizes (μm) of MPs at various time intervals. Mussels preferred ingesing MPs between 500 – 1000 μm over MPs between 65 – 500 μm (Figure 4.21: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in exposed mussels at various time (minutes) intervals.). The abundance of MPs between 500 – 1000 μm was highest in mussels exposed for 60 minutes (27.33 MPs/I) (Figure 4.21: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in exposed mussels at various time (minutes) intervals.). The concentration of MPs between 500 – 1000 μm was highest in mussels exposed for 180 minute (8.91 MPs/g) (Figure 4.21: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in exposed mussels at various time (minutes) intervals.).

(a)



(b)

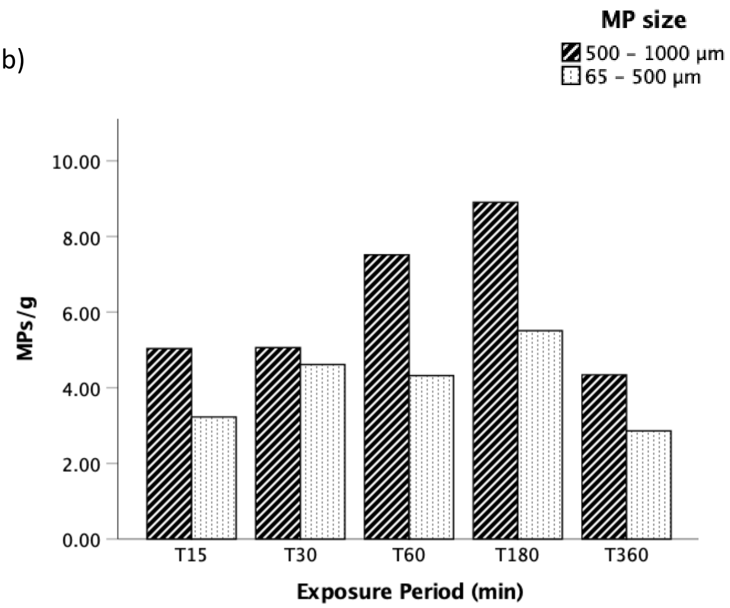


Figure 4.21: Microplastic a) abundance (MPs/I) and b) concentration (MPs/g) in exposed mussels at various time (minutes) intervals.

5. Chapter 5 - Discussion

5.1 Field Study

5.1.1 Environmental parameters (Water and sediment)

Water (MPs/L) and sediment (MPs/kg) concentration

Since Simon's Town Marina sits within the greater Simon's Town Bay within False Bay itself, the water in and around Simon's Town Marina is very calm and the water retention in the area is very high (Pfaff et al., 2019). Marina's are also built for the storage and protection of boats from rough seas and conditions, further making the waters in and surrounding marina's very calm and with low energy and wave action. This low energy environment allows heavier MP particles, such as fragments, to sink to the bottom of the water column. This is why there are more fragments in sediment samples than in water samples. The environment is calm enough to allow the lighter particles to settle in the sediment as well, as concentrations of fibres and film is nearly identical in water and sediment samples. This is confirmed by a significant difference between water and sediment results ($U = 0.000$, $p = 0.007$).

The average MP concentration was found to be 0.37 MP / L (± 0.06 SE) (Figure 4.1). These results are similar to concentrations observed in water samples from Durban Harbour (0.41 MP / L) in 2017 (Nel et al., 2017b). Nel (2017) also found an average of 1.2 MPs / L in Richards Bay Harbour. The mean MP concentration in sediment was 38 MPs/kg (± 2.00 SE) (Figure 4.1). Many harbours and marina's provide place for boating maintenance. It was reported by Sparks and Awe (2022) that at the same site, which is opposite the boating maintenance facility in Simon's Town Marina, the fragments found in samples were mostly antifouling paint particles (APPs). MP sizes reported was less than 1 mm in size (Sparks and Awe, 2022), which was similar for this study (Figure 4.4). A higher percentage of larger MP particles was found in the sediment than in water samples, further adding to the speculation of the low-energy environments allowing heavier MP particles to sink and accumulate in sediment. Harbours tend to be MP sinks due the industrial nature of operations. This, added to the way marinas are built in order to provide shelter to vessels creating calmer, high-retention areas or water, can naturally increase the concentrations of MPs that are found in harbours and marina's (Mathalon and Hill, 2014; Naidoo et al., 2015). The MP particles floating within the water column can be deposited along the sediment of the shoreline and is a representative sample of MP contaminants in the sediment (Thushari et al., 2017a).

Microplastic classification

The type of MPs found in environmental samples (water and sediment) of Simon's Town Marina were that of filaments, fragments, and film (Figure 4.2). The most abundant type of MPs found were filaments overall, with potentially heavier fragments found in the sediment as well. Film was also found in water samples. The fragments found were mostly of antifouling paint particles, which is likely from the boat maintenance facility in close proximity to the sampling site. Sparks and Awe (2022) also found that blue/green and red/pink fibres and fragments dominated sediment samples, in addition to black/grey fragments. This is very similar results found in this study, with black/grey, blue/green and red/pink fibres and fragments found. Due to the boat maintenance facilities as well as many recreational activities occurring in the sheltered bay such as recreational boating, bathing, fishing and diving and many restaurants in the area, as well as a stormwater pipe (Sparks and Awe, 2022), one can expect a variety of MP colours found. The size of MPs varied in water and sediment samples. This is due to environmental conditions (water circulation, photodegradation, bioturbation, wave and current action, wind etc.) facilitating in the production of MPs. The most dominant MP size in water and sediment was 1000 µm (1 mm) and smaller) (Figure 4.4).

5.1.2 Biota

Filter feeders

The main types of MP particles found worldwide is that of filaments (Barrows et al., 2018), indicating that filaments are most likely to be taken up by any marine organism. Sparks (2020) found the highest concentrations of MPs per individual at Simon's Town of all sites sampled. Sparks (2020) also found that the most common type of MP found in Simon's Town was that of fragments, which was reportedly mostly antifouling paint particles (APPs) due to boating maintenance and repairs. The most common colours of MPs found were that of black/grey and blue/green, which is very similar results found in the area by Sparks.

The sampling site, as with this study, was in close proximity to a boating repair and maintenance site, which could be a major contributing factor to higher concentrations of MPs. With the site being a semi-enclosed marina, the water within the marina is retained for a longer period of time, being exposed to buoyant MPs for a longer period of time. The boat maintenance facilities in Simon's Town Marina does contribute to higher concentrations of paint particles. Paint particles that are able to sink due to the heavier particle and calm waters settle in the sediments and pose health risks to the benthic environment (Soroldoni et al., 2017).

Iwalaye et al (2020) also found MPs in one hundred percent of redbait samples off the east coast of South Africa. Being sampled in Winter and in Summer, it was found that more MPs was found in *P. stolonifera* in Summer than in Winter, with 138 particles found in Summer across 30 samples compared to 107 particles found in Winter (O. A. Iwalaye et al., 2020). Comparatively, MPs was only found in 45 % of redbait samples in this study. In Summer, Iwalaye *et al* (2020) found that filaments were the most dominant MP type which is the same found in Simon's Town for the Summer of 2020. It was expected that filter feeders ingested the highest variability of MPs within their soft tissue due to their non-selective method of feeding (Stuart et al., 2016; Thevenon and Carroll, 2015). Non-selective invertebrates such as filter feeders generally ingest more MP particles than organisms that utilise other feeding strategies to obtain food (Iwalaye et al., 2020; Sparks, 2020a).

No significant difference was found between filter feeders and water samples, which goes against what was expected and against current literature worldwide. This could be because of the fact that due to the low-energy environment, what MPs settle faster than that of other environments of higher energy, making them biologically available for a shorter period of time. With MPs settling faster than expected, they become part of the benthic zone and the sediment, exposing the organisms inhabiting the lower water column to MPs. This could explain the higher

Grazers

Grazers feed by scraping food from hard substrate within the rocky shore. As mentioned by Thushari *et al* (2017), grazers generally have a selective feeding pattern with ingesting food, which can include most algae found growing on rocks within the rocky shore. While *O. tigrana* had the lowest MP concentrations within the soft tissue (0.044 MP/g) amongst grazers for the study, *S. longicosta* and *P. exigua* are arguably more sessile than the periwinkle which could contribute to higher concentrations of MPs in their soft tissue.

Although all grazer species showed a weak correlation between tissue weight (g), total weight (g) and total number of MPs, limpets showed a greater correlation (r = - 0.275) between soft tissue and total number of microplastics (: (a) Correlation between the total number of MPs in grazers and the total weight (MP/g), (b) Correlation between the total number of MPs in grazers and the soft tissue weight (g), and (c) Correlation between total number of MPs and the soft tissue weight (g), according to species). This suggests that MPs could be accumulating and/or trapped within the shell and is accounted for during processing of samples. This is reinforced by Doyle *et al* (2019), where microplastics per gram of wet weight and dry weight differed by 33 %.

Although grazers are more selective feeders than filter feeders, they are forced to feed in a more localised area, due to the fact that they do not rely on suspended particles that float around the water column like filter feeders do. Rather, grazers settle in an area and feed on algae. If there it is an area of high MP contamination, grazers are forced to ingest whatever particles found, which could contribute to higher concentrations of MPs within the soft tissue. In Thailand, *Littoraria spp.* was found to have between on average 0.17 MP/g and 0.23 MP/g per organism between three sites (Thushari et al., 2017b). This, compared to Leslie *et* al (2013), *Littorina littorea* had on average 20 MP/g. Majority of the MP particles was that between the sizes of 300 µm and 5 mm in size (Leslie et al., 2013). The variation of data was deduced to be due to the variation of pollution in rocky shores and the relative distances to anthropogenic sources of MP pollution, which could be recreational activities and the boat maintenance repair in close proximity to the sampling site (Desforges et al., 2014; Thushari et al., 2017b), which is similar to Sparks (2020) and this study..

Doyle *et al* (2019) also found that majority of MPs found in *L. littorea* were that of filaments (97 %), with the highest site having on average 2.40 MP/I. This, compared to Leslie *et al* (2013), is a lot lower than the 20 MP/g found in the Dutch environment compared to the 0.044 MP/g in this study and 2.14 MP/g in Ireland (Doyle et al., 2019).

Limpets sampled for this study. *S. longicosta* reported 0.113 MP/g (0.02 SE) in the Simon's Town Marina. Ojeda *et al* (2021) reported on limpets (*Nacella magellanica*) in Argentina which was the first field study for limpets. Three sites were sampled, and filaments, fragments and film were reported within limpet samples. Of the three sites samples, the highest MP count per individual was 10.00 MP/I (± 6.69 MP/I) and this was the site closest to many anthropogenic activities such as a harbour, and industrial area and fish processing factories (Commendatore et al., 2012). Filaments ranged between 5.7 and 1.6 MP/I (± 1.26 MP/I) and 5.7 MP/I (± 2.36 MP/I) between the three sites (Ojeda et al., 2021). As with this study, filaments were also the most abundant type of MP found, contributing between 55.2 % and 60.7 % of total particles, followed by fragments which contributed between 28.6 % and 41.4 % of particles and films contributing between 3.4 % and 10.7 % (Ojeda et al., 2021). Simon's Town Marina is also home to boating and other industrialised activities, as well as fish processing activities such as Ushuaia Bay in Ojeda *et al* (2021). Blue, black and grey MPs dominated the site as well.

Scavengers/Predators

Whelks were collected as scavengers within the rocky shore as it is known that they feed on dead and decaying matter within the rocky shore. In scavengers/predators, majority of the MPs were that of filaments (82.27 %). A similar study conducted by Ehlers (2022) found that majority of the particles found in three different snail species from three different sites had majority fragments found (52 %) with filaments the second highest MP shape (40 %). The blue and black colours also dominated (Ehlers et al., 2022). MP concentrations in Ehlers (2020) was on average 3.19 MP/g (± 1.67 MP/g) which is slightly higher than this study of 1.84 MP/g (± 0.33 SE).

The starfish, *M. glacialis*, had lower MP concentrations per gram than whelks of just 0.15 MP/g (± 0.02 MP/g), but had a much larger MP count per individual organism of 6.68 MP/I (± 0.854 MP/g). Scavengers and predators do not directly take up MPs from the sediment or water, but studies suggest that they feed on organisms or detritus that is already contaminated with MPs. This gives potential for MPs not only to be passed on through various trophic levels, but to bioaccumulate and bio-magnify (Farrell and Nelson, 2013; Rowland et al., 2007).

5.1.3 Polymer identification

10% of identified MPs was processed for FTIR analysis and identified Polyamide-nylon (PA6) as the dominant polymer type (27.27%) followed by natural based polymer and PET (18.18% and 18.18% respectively) (Figure 4.16). These results are reflective of what was found in a study conducted by Sparks and Awe (2022). Polymamide/Nylon (PA6) is used to produce fishing nets. This could explain for the high percentage found within the marina. Applications of PET include plastic bottles which are prone to ultra-violet degradation (Andrady and Neal, 2009). This makes PET based products prone to forming secondary MPs. In addition, PET has a higher density than other polymer types and with the facilitating weak circulation (Pfaff et al., 2019; Sparks and Awe, 2022) within the marina increases this polymer type’s settling rate. Thus, also making these polymers susceptible to being ingested (directly or indirectly) by organisms residing and feeding from benthic environments. Applications of natural based products include clothes and ropes used on boats, could explain the high percentage of natural particles. These applications in combination with marina related activities and pedestrian access could potentially be linked to sources for MP contamination. The remaining polymers identified could be linked to other applications, sources and environmental conditions within Simon's Town and False Bay.

5.1.4 Risk Assessment of Microplastics and polymers

The Pollution Load Index (PLI) showed generally high to dangerous levels of MP pollution within Simon's Town (Figure 4.17) particularly in sediment and all biological samples. Whereas water samples displayed low levels. This is concerning for species residing and feeding in benthic areas as they could potentially be consuming (directly or indirectly) MPs that have accumulated in sediment. The dangerous levels observed in sediment reinforces sediment as a MP sink. MPs that have settled in sediment have the ability to be resuspended in the water column, making these MPs available to be consumed by organisms feeding from the water, particularly filter-feeders. MPs are mistakenly identified as food particles and are consumed by various organisms depending on feeding strategy. This could explain why filter-feeding species displayed a very high-risk level as they have a non-selective feeding strategy ingesting particles of various type, colour, and size. The Polymer Risk Index (H) (Figure 4.18) showed generally a high risk associated with polymers identified in Simon's Town. It is important to note that even though filter-feeding species did not display the highest mean MP concentration, it had a noticeably very high risk associated with polymers. This indicates that the risk is not dependent on concentration, but the polymers identified and their hazard score. Poly Urethane (PUR) has a hazard score of 13844 (Lithner et al., 2011) and was only identified in filter-feeding species. Polymers with high assigned hazard scores have the potential to pose a threat to organisms ingesting the particle. The Pollution Load Index (PRI) is dependent on the PLI and H calculated. For this study, levels showed a dangerous risk associated with pollutants, particularly in filter-feeder and grazer species (Figure 4.19). This is concerning as filter-feeders are non-selective in their feeding strategy and grazers have the potential to indirectly consume pollutants by scrapping contaminated algae off rocks.

5.2 Experimental Study

In this MPF exposure experiment, 100 % of mussels ingested MPFs, as with Qu *et al* (2018) and Kolandhasamy *et al* (2018). It was shown that higher concentrations of MPFs were taken up by mussels with increasing the concentrations that the mussels were exposed to (Qu et al., 2018), as well with increasing mussel weight (Kolandhasamy et al., 2018). However, in this study, post hoc tests showed no significant differences between MP/g and MP/I. However, previous studies that showed a significant difference between mussel weight (g) and MP/g were studies that exposed the mussels to MPs for longer periods of time that the current study. This suggests that the size of mussels play a role in their MP uptake over longer periods of time and not in acute settings.

The biofouling of MPFs over exposure time could also contribute to elevated levels of MPs ingested later on in the experiment. For MP/g, the highest concentration was observed at mussels removed at three hours (180 minutes) (7.21 ± 1.69 MP/g) after the initial exposure (Figure 4.20) and after one hour (60 minutes) (23.16 ± 4.16 MP/I) for MP/I (Figure 4.20). This was also noticed by Webb *et al* (2020) as MPs would first float on the surface of the water and overtime would be distributed lower in the tanks. This would also explain why the shorter exposure period (15 minutes) had the lowest MP/I.

The peak feeding duration after initial exposure for mussels also seems to be between one and three hours after initial exposure. At the final time period (six hours after initial exposure), the mussels results displayed the lowest concentrations of MP/g (3.6 ± 0.74 MP/g).

The preferred size of mussels for this study were MPFs between 500 µm and 1000 µm. Kolandhasamy *et al* (2018) exposed mussels to particles of 100 µm, but it was shown in a previous study that mussels feed in the environment on particles larger than 60 µm. Therefore, lower ingestion concentrations of particles between 65 µm and 500 µm as in this study was found not unrealistic. Once again, a peak was found for MP/g at 180 minutes after initial exposure for the size class of 500 µm to 1000 µm , as mussels removed at 60 minutes had 27.33 MP/I and 180 minutes had an average of 8.91 MP/g. For the smaller size category (65 µm to 500 µm), the MP count, the MP/g and MP/I was also highest at 60 minutes and 180 minutes. This reinforces that mussels have a peak ingestion period between one and three hours after initial exposure.

6. Chapter 6

Conclusion and recommendations

The result of this study suggests that MPs concentration and abundance is heavily linked to anthropogenic activities. The area surrounding Simon's Town Marina has restaurants and other recreational activities, commercial fishing, and boating activities as well as boat maintenance and boat building activities (Pfaff et al., 2019). These factors could influence MP types, sizes and colours found within the environment (Sparks and Awe, 2022).

The MP type, size and density are main factors that determine where within the water column the MPs are found. Heavier MPs, such as fragments, are generally known to settle into the sediment quicker than lighter MP particles, such as filaments. Filaments are therefore more commonly found in organisms with non-selective feeding strategies, such as filter feeders, whereas fragments are more commonly found in organisms that appear in the benthic region of the water column, such as grazers.

Majority of the MP particles isolated from organisms were dark in colour, indicating that organisms are mistaking darker MP particles for food when feeding. Scavenging and predatory organisms are unlikely to take up MP particles directly from the water column, and it is thought that MPs found within scavenging and predatory organisms provide evidence for MP particle transfer through various trophic levels and could lead to bioaccumulation and biomagnification, although more research should be done to confirm this (Farrell and Nelson, 2013).

With Simon's Town Marina being an environment of low water energy, MPs are more susceptible to sinking and settling in the sediment, as the sediment is thought to be a microplastic sink (Alomar et al., 2016; GESAMP, 2019). The most common MP polymer types were found to be nylon (27.27 %), natural MP such as cotton filaments (18.18 %), PET (18.18 %), synthetic rubber particles in the form of antifouling paint particles (13.64 %) and acrylic particles (13.64 %). These are all forms of plastics expected from recreational activities, but also expected from the boat maintenance and building facility that is within close proximity to the sampling site (Sparks, 2020b). A risk assessment was also conducted, and it was found that the high risks that MP particles pose is due to the MP polymer type and not MP concentrations.

Microplastic uptake experiments are important in understanding the physiological pathways of pollutants in organisms. Furthermore, these experiments are ideal and effective methods to understand the ingestion and accumulation of MP particles in marine invertebrates. However, the drawback of MP exposure experiments is that many studies expose organisms to unrealistic MP particle concentrations in the laboratory setting in order to ensure results. This does not give results of a true and realistic nature, and it is recommended that laboratory exposure concentrations and conditions mimic that of results found in field studies. In this study, a major peak ingestion period by mussels was found between one and three hours after initial exposure. This was seen in MP/g and MP/I (Figure 4.20) and for MP size results for MP uptake in different sizes of MPs (Figure 4.21).

It is recommended that seasonal sampling is undertaken for MPs to gain more knowledge and understanding, as well as to form a historical record of MP pollution in the area. These studies serve as a baseline study for future research and proves that further research is required in order to understand the full extent of MP pollution in the area, and in rocky shores in South Africa. It is also recommended that more experimental studies are done to reinforce the findings and for more acute-exposure studies, the focus of research should be done on hours one to three after initial exposure. This study displays the need for more research to be done on the topic and can add to historical data on the topic. The combination of field and experimental studies a more wholistic approach and understanding to microplastic pollution.

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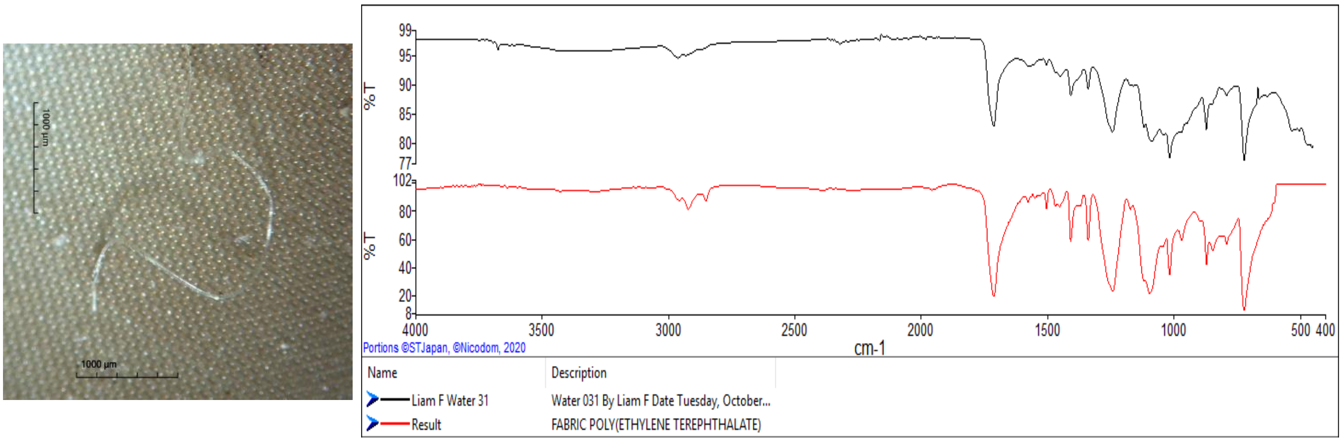
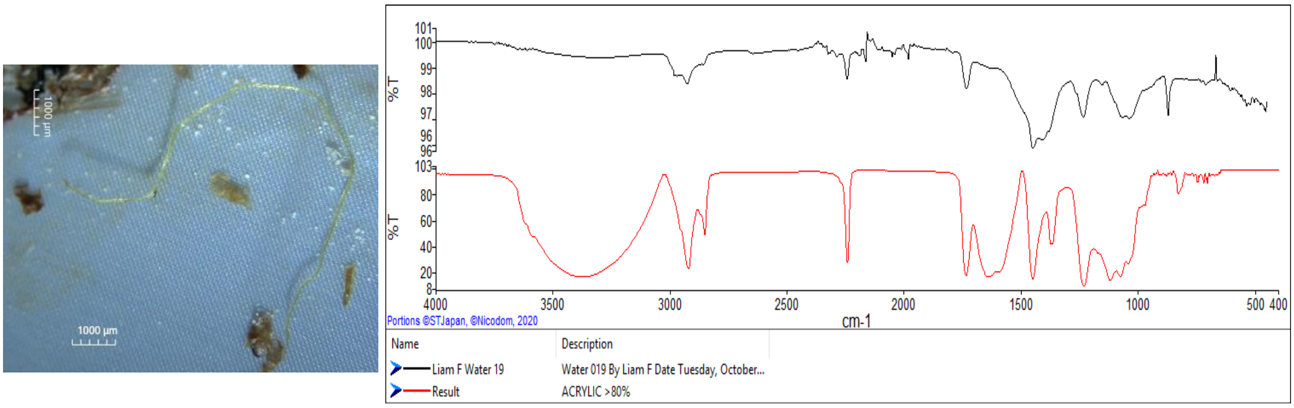
Appendix

(a)



(b)



(c)

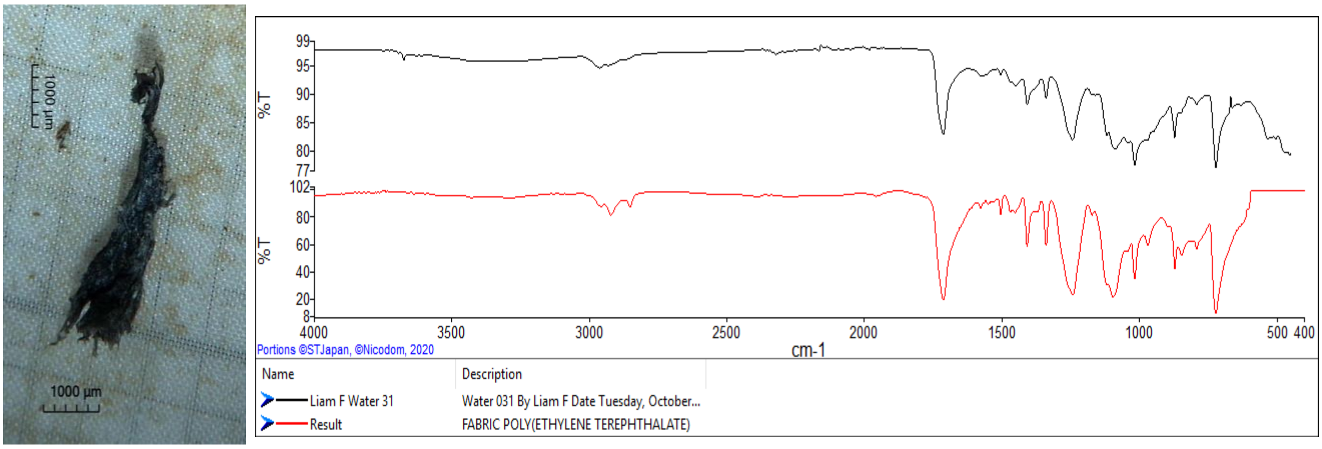


Figure 1: Selected example of visual identification of MPs using a microscope and FTIR analysis a) transparent Polyamide/Nylon filament, b) yellow Polyacrylic acid filament and c) black/grey Polyethylene film.