TEMPORAL PATTERNS AND SEASONAL VARIATION IN MICROPLASTIC LOADS IN THE WATER COLUMN AND IN THE TISSUES OF CONSUMERS ALONG THE SOUTHERN AND SOUTH-EASTERN COASTS OF SOUTH AFRICA

A thesis submitted in fulfilment of the requirements for the degree of

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By

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Abstract

Plastic pollution in the marine environment has become an environmental concern and a subject of ecological research. The field of microplastic pollution in particular has expanded dramatically in the last few years. Though much data exists on the spatial variability of microplastics in the marine environment globally, little is known about temporal variability, especially on short-term time scales in the southern hemisphere. Similarly, virtually nothing is known about the temporal patterns in microplastic ingestion by marine invertebrates, despite the fact that numerous studies have demonstrated that vertebrates and invertebrates routinely ingest microplastics with varied physiological effects. This study aimed to, 1) provide base-line data for microplastic loads in the nearshore environment along the Eastern Cape Province of South Africa over four short-term time scales: daily, weekly, monthly, and yearly; and, 2) assess whether there are any seasonal patterns in microplastics ingested by selected filter-feeding consumers at two sites along the southern and south-eastern Cape coastlines of South Africa. Results for part one of this study demonstrate no temporal patterns over the different time scales considered (ANOVA, $p > 0.05$ in all cases). Microplastic counts ranged on average from 55 ± 289 to 930 ± 462 microplastic particles.m^{-3}. With the exception of two instances, microfibres constituted > 50 % (range: 47 to 97 %) of the total microplastic counts. Part two of this study assessed the size range of, and seasonal and spatial patterns in ingested microplastic. No significant differences were found in the number of microplastics ingested within seasons between the mussels *Perna perna* (Linnaeus, 1758) and *Mytilus galloprovincialis* (Lamarck, 1819), and the barnacles, *Octomeris angulosa* (Sowerby, 1825) and *Tetraclita serrata* (Darwin 1954) (Student's t-test; d.f = 18; $p > 0.05$ in all cases), or between the two sites sampled, Kenton-on-Sea, Eastern Cape, and Wilderness, Western Cape (ANOVA; d.f. = 18; $p > 0.05$ in all cases). The nitric acid digestion technique was used to determine the presence of ingested microplastics. Microplastic loads ranged from 2 ± 1 to 33 ± 19 microplastics.g^{-1} wwt across all consumers, and the size of ingested microplastics ranged from 1 to 16 μm. Though highly variable, the absence of statistically significant

differences in ingestion rates points to a ubiquity in the availability of microplastics within the water column over time and space.

Declaration 1: Publications

The main findings of this thesis described in Chapters 3 and 4, have been submitted for consideration for publication in *Marine Pollution Bulletin*. As such each chapter stands alone. As a consequence, there may be some repetition in the introductory sections of the two chapters. The titles of the two papers submitted for publication are:

Redelinghuys S, Froneman PW (2018) Temporal patterns in microplastic loads in the water column off the Eastern Cape coastline, South Africa. Submitted for peer review to *Marine Pollution Bulletin*.

Redelinghuys S, Froneman PW (2019) Seasonal variation in microplastic loads ingested by selected filter feeders along the south-east Cape coastline of South Africa. Submitted for peer review to *Marine Pollution Bulletin*.

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Research findings contained in this dissertation represent the candidate's original work, except where the work of others has been acknowledged in the text. Research findings have not been submitted in part or in full towards any other degree at any other universities or tertiary institutions. Conclusions arrived are that are those of the candidate and are not necessarily to be attributed to Rhodes University or donors and funders.

Personal

Firstly, I would also like to thank my parents, Christine, Anton and Tanya for your continued love, support and encouragement, and the sacrifices you have made for my education. At times it felt like your prayers were the only thing getting me through this. Mom, you should be graduating with me, I dedicate this thesis to you. To Chantel, you have been a mother, a sister, a friend, and one of the most important people in my life from the very beginning. Without your encouragement and love I would not have had the courage to pursue my dreams and I would certainly not have come this far. To Clint, my love, for all the Tim Tams, the laughs, your endless love and understanding, and for being my rock to limpet on, I could never tell you how thankful I am for all your patient help through this. To Professor Tony Booth, I am endlessly grateful for your guidance, patience, and advice throughout this

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Chapter 1

General introduction

1.1 Plastic: Manufacture, uses, and presence in the marine environment

The term ‘plastic' is used to refer to the malleability and degree to which natural and anthropogenic material can be shaped or moulded, as well as being used as a classification for a type of man-made material (GESAMP 2015). Plastic is a sub-category of polymers (GESAMP 2015) and is defined as ‘synthetic organic polymers, which are derived from the polymerization of monomers extracted from oil or gas' (Derraik 2002, Rios *et al.* 2007, Thompson *et al.* 2009), a definition inclusive of virgin resin pellets, as well as the mixtures of melted down virgin pellets and additives, like fillers, stabilizers and colorants (GESAMP 2015).

Plastics are divided into two broad categories: thermosets and thermoplastics (Derraik 2002, Thompson *et al.* 2009). Thermosets are plastics that once cured, cannot be melted or deformed by heat. Thermoplastic describes those plastics can again become plastic, that is to say malleable or mouldable, when re-exposed to heat (Baeurle *et al.* 2006). Plastic production is dominated by six classes: low- and high-density polyethylene (LDPE, HDPE), polyvinyl chloride (PVC), polyurethane (PUR), polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET) (GESAMP 2015). These plastics were developed from the first plastic product, Bakelite, developed in 1909 (Chandrasekaran 2017). From this product, through optimizing manufacturing techniques, a range of lightweight, cheap, durable, corrosion-resistant, strong, versatile, and bio-inert plastics, were produced (Laist 1987, PlasticsEurope 2010, Andrady 2011, Van Cauwenberghe *et al.* 2015). Since mass production of plastic started in the 1940s, annual global production values have risen exponentially, from approximately 1.5 million tonnes in 1950 to 348 million tonnes in 2017 (Cole *et al.* 2011, PlasticsEurope 2018).

Plastics are used in numerous applications, from industrial packaging and various uses in construction to everyday applications in household and cosmetic products (Andrady 2011). The properties that make plastic such a desirable product are, however, the same properties which classify it as marine litter and make it such a pernicious threat (Laist 1987, Pruter 1987, Barnes *et al.* 2009, Sivan 2011). Galgani *et al.* (2010) describe marine pollution as 'any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment.' While a percentage of global plastic waste is recycled, 8.1% globally in 2016 (PlasticsEurope 2017), the majority of plastic waste is discarded in landfills, where breakdown or decomposition may take place over several centuries (Moore 2008, Barnes *et al.* 2009). It has been suggested that decomposition of plastic is at an order of magnitude such that it may leave traces in the geochemical fossil record (Waters *et al.* 2016). Galloway *et al.* (2017) estimate that 50% of plastic products are used only once before discard, and while emphasis is being placed on recycling plastics where possible, global production of plastic still far exceeds recovery of plastic through recycling (PlasticsEurope 2017).

Plastic pollution has become a global problem, affecting localities that do not possess plastic-producing or -processing facilities, from the poles to the equator, from developed coastlines to oceanic islands and mid-ocean gyres (Gregory *et al.* 1984, Gregory 1999, Errikson and Burton 2003, Moore 2003, Barnes and Milner 2005, Ebbesmeyer *et al.* 2007, Ivar do Sul *et al.* 2009, Cole *et al.* 2011, Anastasopoulou *et al.* 2013). Plastics are dispersed primarily through hydrodynamic processes and ocean currents (Ng and Obbard 2006), resulting in plastic being found throughout the marine environment (Van Cauwenberghe and Janssen 2014), from surface waters to the deep water habitats and benthic environments of the abyssal plain (Browne *et al.* 2007, Gregory 2009, Lozano and Mouat 2009, Morét-Ferguson *et al.* 2010, Kukulka *et al.* 2012, Anastasopoulou *et al.* 2013, Setäla *et al.* 2014). Plastic particles have also been recorded in ice cores taken in the Arctic Ocean (Obbard *et al.* 2014).

1.2 Microplastic pollution: history, classification, and sources

Carpenter and Smith (1972) and Carpenter *et al.* (1972) first reported small plastic particles floating in the surface waters of the open ocean in 1972. However, Harper and Fowler (1987) described the identification of small plastic particles in bird carcasses collected during the 1960s, illustrating that small plastic fragments existed as pollutants as early as 20 years after the onset of mass production of plastics. The majority of present literature separates plastic debris into size classes of either <5 mm, termed microplastics, or >5 mm, termed macroplastics (Arthur *et al.* 2009), though some authors set the upper limit for microplastic particles at < 1mm (Browne *et al.* 2010, Vianello *et al.* 2013, Dekiff *et al.* 2014). GESAMP (2015) suggests that more rigorous definitions should be adopted for scientific purposes, separating plastics into nano- (<1 µm), micro- (<1 mm), meso- (<2.5 cm), macro- (<1 m), and mega-size (>1 m) size classes. This thesis uses the nomenclature of the National Oceanic and Atmospheric Administration (NOAA), Derraik (2002), Betts (2008), Barnes *et al.* (2009), Ryan *et al.* (2009), Cole *et al.* (2011), and Nel and Froneman (2015), defining macroplastics as >5 mm, and microplastics as <5 mm.

Microplastics can, on the basis of their origin, be sub-divided into primary and secondary microplastics (Cole *et al.* 2011). Primary microplastics (Figure 1.1) are smaller than 5 mm by design (e.g., virgin resin pellets/nibs) and are used as precursor materials in the manufacture of larger plastic objects (Derraik 2002, Barnes *et al.* 2009, Ivar do Sul *et al.* 2009, Andrady 2011, Cole *et al.* 2011, Sivan 2011). These pellets are also used in industrial processes as paint and rust strippers, the most common types of which are acrylic plastics and polyester (Gregory 1996, Derraik 2002, Browne *et al.* 2007, Andrady 2011), and as exfoliants in cosmetic products and abrasives in household cleaning products (Andrady 2011, Cole *et al.* 2011). In particular, polyethylene, polypropylene, and polystyrene microplastics have replaced natural scrubbers like pumice and oatmeal (Gregory 1996, Cole *et al.* 2011).

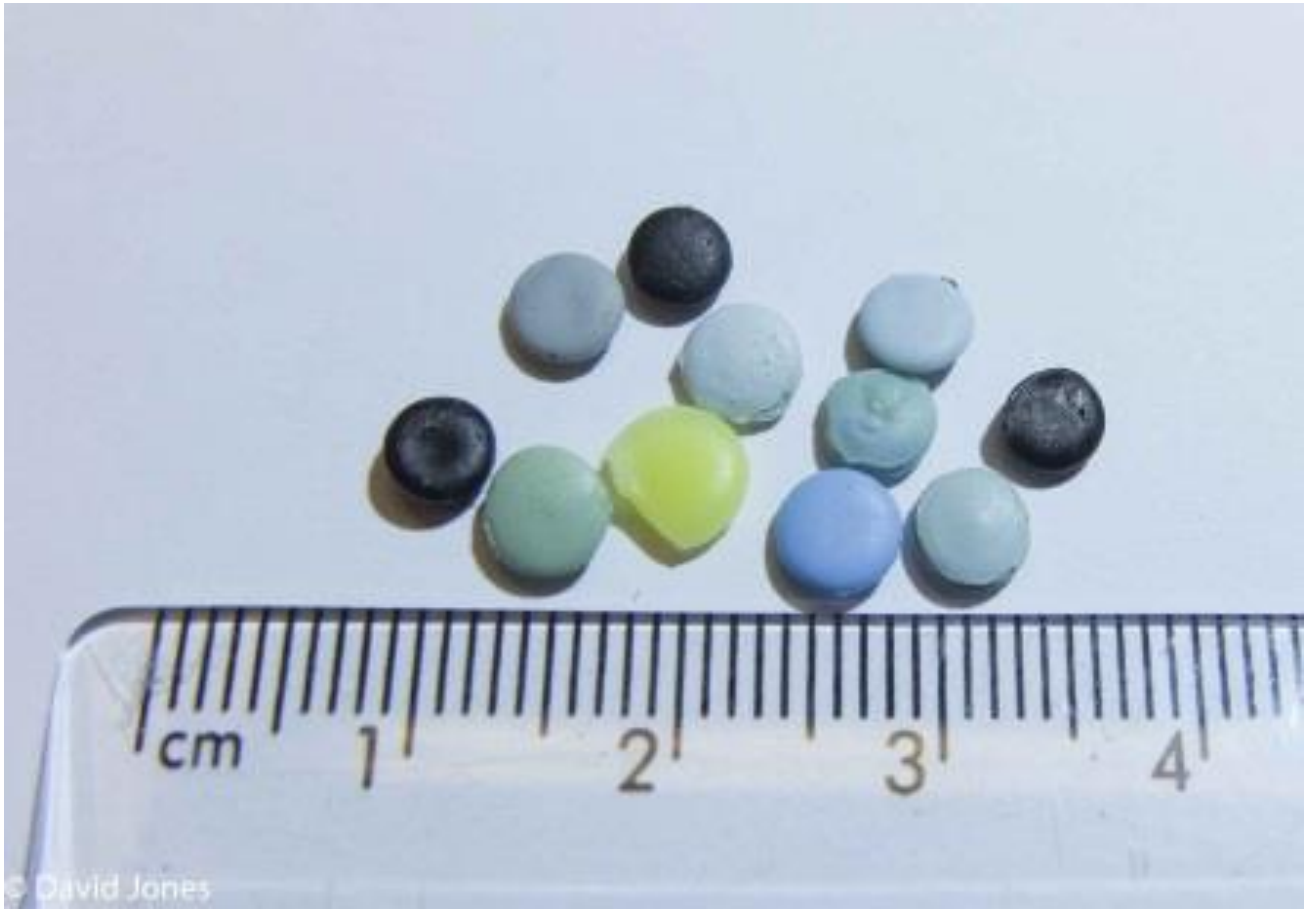

Figure 1.1: Primary microplastic particles, also known as nibs, nurdles, and virgin resin pellets. Credit: David Jones, Just One Ocean 2018.

Secondary, or indirect, microplastics (Figure 1.2) are formed through the degradation of larger plastic objects, often from the brittle, weathered surface layer of the larger plastic object (Derraik 2002, Browne *et al.* 2008, Barnes *et al.* 2009, Andrady 2011, Sivan 2011, Bakir *et al.* 2012, Anastasopoulou *et al.* 2013). Degradation occurs through photo-degradation, thermo-oxidative degradation, biodegradation, and hydrolysis, the latter two of which are considered negligible for studies in the marine environment (Andrady 2011). Photo-degradation refers to the fragmentation of plastics due to long periods of sunlight exposure, resulting in oxidation of the polymer matrix by ultraviolet (UV) radiation, most commonly through UV-B radiation (Browne *et al.* 2007, Rios *et al.* 2007, Moore 2008, Barnes *et al.* 2009, Andrady 2011). This is most commonly seen in plastics found on beaches due to the direct exposure of these plastics to UV radiation, and the higher oxygen availability of the open

air when compared with water, resulting in cracked, fragmented, brittle, and yellowed plastics (Moore 2008, Barnes *et al.* 2009, Andrady 2011). Thermo-oxidative radiation embrittles plastic through heat stress (Andrady 2011). Thermo-oxidative radiation is not often seen in isolation, as temperature is linked to solar exposure on sea surface waters and on beaches, and so, often acts as a catalyst to the oxidation of the polymer matrix induced by photodegradation (Andrady 2011).

Figure 1.2: Primary and secondary microplastic particles (with jagged edges) which have broken off of larger plastic objects due to weathering. Credit: David Jones, Just One Ocean 2018.

Degradation occurs initially and primarily at the surface layers of the plastic object due to the limited penetration of UV-B radiation in plastic, and the low oxygen diffusion rate in plastic particles due to the presence of UV stabilising additives (Blaga and Yamasaki 1976, Blaga 1980, Cunliffe and Davis 1982, Qayyum and White 1993). Plastic particles fracture off of the surface layer when the object is

exposed to a stressor such as abrasion against rocks (George 1995). Microplastic particles may also separate from the larger plastic object inside the body of a consumer through digestion, either mechanical as in gizzards or chemical as in true stomachs, which may release these particles back into the environment through egestion, evacuation, or through the death and eventual decay of the body of the consumer, a phenomenon often seen in seabirds (Gregory 1978).

Plastic derived from land-based sources are transported to the marine environment through waterways such as storm drains, sewage outlets, improperly treated wastewater, rivers, from litter left on beaches, directly from the air, loss of cargo, and discard at sea (Pruter 1987, Gregory 1996, Williams and Simmons 1997, Derraik 2002, Thompson 2006, Barnes *et al.* 2009, Fendall and Sewell 2009, Gregory 2009, Ogata *et al.* 2009, Andrady 2011, Browne *et al.* 2011, Doyle *et al.* 2011, GESAMP 2015). Microplastics may also be lost from point sources. A common source of secondary microplastics is the various fishing gears used in the fishing industry, owing to their proximity to the ocean (Gregory 2009). Watson *et al.* (2006), notes that the whole global fishing fleet has replaced its gear with plastic alternatives, a trend seen in almost all market sectors as natural materials are replaced with cheaper, plastic alternatives (Gregory 2009). Since the 1950s, plastics have replaced virtually all the natural materials previously used by the fishing industry, owing to the desirable properties of buoyancy and increased durability when compared with natural materials like hemp and cotton (Gregory 2009).

On an environmental impact scale, biodegradation has not been observed for the more commonly used plastics of high molecular weight (Andrady 2011), whereas biopolymers like cellulose, chitosan, and chitin, and at least one synthetic polymer (aliphatic polyesters) have been observed to biodegrade in the marine environment (Doi *et al.* 1992, Mayar *et al.* 1996, Leathers *et al.* 2004, Andrady 2011). Polyolefins manufactured using starch similarly undergo biodegradation, but only of the starch constituents (Breslin and Boen 1993, Gonsalves and Patel 2003, Andrady 2011). For this reason,

Andrady (2011) and Thompson *et al.* (2004) do not define these polymers as 'biodegradable' as only the bonds within the plastic are degraded, and mineralisation of the material is not complete.

While particles of low-density float at the sea surface (Suaria and Aliani 2014), microplastics are not confined to the sea surface (Ballent *et al.* 2012). A negative relationship has been observed between wind speed and the number of particles at the surface (Lattin *et al.* 2004, Thompson *et al.* 2004). Particles floating at the surface may be colonised by epibionts, progressing from a biofilm of microbial and bacterial communities to an algal covering, and finally a community of invertebrates (Muthukumar *et al.* 2011). Microplastics have been observed to sink due to decreasing buoyancy as fouling progresses (Stefatos and Charalampakis 1999, Backhurst and Cole 2000, Katsanevakis *et al.* 2010, Andrady 2011). Chemical contaminants like DDT (dichlorodiphenyltrichloroethane) can also change the density of contaminated plastic particles, resulting in the sinking of the plastic particle from the surface to the surface microlayer, and increasing the availability of the plastic for colonisation by epibionts (Bakir *et al.* 2014b). Organisms of higher trophic positions have been observed to feed on these epibiont encrustations, often accidentally ingesting the microplastic particle, which returns the particle to its original density, allowing the plastic particle to return to the water surface (Andrady 2011) in a slow cyclic process first observed and described by Stevens (1992) and Stevens and Gregory (1996). This cycle may repeat a number of times before the plastic particle ultimately settles permanently on the seafloor, after which it is thought to be indefinitely buried (Ye and Andrady 1991, Gregory 2009). Natural events like storms and anthropogenic activities like dredging and bottom-trawling can re-suspend plastic particles buried in sediment, which then re-enter the water column (Browne *et al.* 2010, Browne *et al.* 2011). Denser plastics (e.g., nylons) have been observed to submerge in the water column and have been found in coastal sediment samples (Andrady 2011). Where a decrease in the abundance of virgin plastic pellets in the marine environment is observed it could be due to a drop in production of these pellets, but could also be due to an increase in ingestion rates of the particles by various organisms, which effectively removes the pellet from the

environment until such time as it is excreted, or until the organism dies and the pellet is returned to the environment upon decay of the organism (Gregory 2009).

Microplastic counts in the marine environment vary depending on the environment sampled (e.g. surface layer, sediment). Microplastic counts in the North Atlantic Subtropical Gyre are in excess of 100 000 pieces.km^{-2} (Law *et al.* 2010, Eriksen *et al.* 2014), and Goldstein *et al.* (2013) found a maximum concentration of 32.76 particles.m^{-3} in the North Pacific Subtropical Gyre. De Jesus *et al.* (2018) found between 16 ± 4 and 312 ± 145 particles.kg^{-1} dry weight in sediments along the Baja California Peninsula of Mexico, as compared with Wessel *et al.* (2016) who found 50.6 ± 9.96 particles.m^{-2} in beach sediment in the Northern Gulf of Mexico. Along the USA coastline 413.8 ± 76.7 microplastic particles.m^{-2} were found at Charleston Harbor and 221.0 ± 25.6 microplastic particles.m^{-2} were found at Winyah Bay in beach sediment (Gray *et al.* 2018). In the Persian Gulf, 1258 ± 291 microplastic particles.kg^{-1} were found in beach sediment at Bostanu as compared with 122 ± 23 microplastic particles.kg^{-1} at Gorsozan (Naji *et al.* 2016). In South Korea, Lee *et al.* (2013) and Heo *et al.* (2013), found such variable values as 8205 plastic particles.m^{-2} beach sediment and 976 ± 405 plastic particles.m^{-2}, respectively.

1.3 Ecological impacts of plastics in the marine environment

The environmental and ecological impacts of macroplastic pollution have been extensively studied in the marine environment, but less so for microplastics. Macroplastics pose a threat to wildlife primarily through ingestion and entanglement, whereas microplastics pose different threats depending on the size of the consumer, but primarily through the uptake and release of persistent organic pollutants (POPs) into the tissues of the consumer once ingested (Laist 1987, Laist 1997, Andrady 2011, Besseling *et al.* 2013). Entanglement has a debilitating effect on all organisms resulting in an overall lower quality of life (Laist 1987). Entanglement does not only occur with

plastic debris like plastic bags, but also with fishing line, nets, and fish traps, which may result in a phenomenon known as ghost fishing (Gregory 2009). Entanglement may impair an organism's ability to feed, reproduce, fly, swim, and otherwise avoid predators by creating drag on the body of the organism, or creating open wounds and lesions (Gregory 2009). Turtles, marine mammals, seabirds, invertebrates, crustaceans, and fish (teleosts and Chondrichthyes) have been listed as particularly affected by entanglement and ingestion of plastics (Derraik 2002, Errikson and Burton 2003, Gregory 2009, Katesanevakis and Issaris 2010, Lazar and Graçan 2011, Murray and Cowie 2011, Possato *et al.* 2011).

Anastasopoulou *et al.* (2013) found that particular categories of plastic were related to feeding behaviours of particular species. For example, the nektobenthic opportunistic feeder the blackmouth catshark (*Galeus melastomus* (Rafinesque, 1810) ingested plastic of all categories; the bathybenthic feeders the velvet belly lanternshark (*Etmopterus spinax* (Linnaeus, 1758)) and the blackspot seabream (*Pagellus bogaraveo* (Brünnich, 1768)) ingested primarily hard plastics, and the pelagic and bathypelagic feeders the pelagic stingray (*Pteroplatytrygon violacea* (Bonaparte, 1832)) and the longnose spurdog (*Squalus blainville* (Risso, 1827)) ingested plastic bags only (Madurell 2003). Carson (2013) found that species of various sizes and trophic niches not only ingest but also attack macroplastics, mistaking these objects for prey items, which has been identified as another source of microplastic fragmentation.

A further threat is that macroplastic debris may act as a vector for the transport of marine species, introducing a non-native species, and thereby extending the range of non-native species (Derraik 2002). In areas of accumulation in and on the sediment, macroplastics have been found to create an ‘artificial hardground’, where gas-exchange is impaired (Uneputty and Evans 1997, Moore 2008, Gregory 2009) due to a decrease in the permeability of the sediment (Derraik 2002, Cole *et al.* 2011), potentially creating hypoxic or anoxic conditions (Goldberg 1997, Gregory and Andrady 2003).

Owing to their small size and presence throughout the water column, microplastics mix with the prey items of all trophic levels (Teuten *et al.* 2009, Boerger *et al.* 2010, Cole *et al.* 2011), and are thus available to all consumers for ingestion, accidental or otherwise (Browne *et al.* 2008, Thompson *et al.* 2009). Microplastic pellets and fragments have been found in the digestive tracts of detritivores, planktivores, herbivores and carnivores (Thompson *et al.* 2004, Anastasopoulou *et al.* 2013, Farell and Nelson 2013). Predators mistake microplastics for prey items and so they are actively targeted, (Azzarello and Van-Vleet 1987, Shaw and Day 1994, Cole *et al.* 2011, Anastasopoulou *et al.* 2013), but are also ingested indirectly through foraging, and the ingestion of lower trophic prey items that have ingested microplastics, a process known as trophic transfer (Anastasopoulou *et al.* 2013). Of importance to this study is the observation that deposit- and filter-feeding invertebrates are encountering and ingesting microplastics at a higher rate when compared to other invertebrates and organisms of higher trophic levels. This is due to their selection of prey items in the same size range as microplastic particles (Thompson *et al.* 2004, Ward and Shumway 2004, Besseling *et al.* 2015). Microplastic ingestion has been found to block feeding appendages of plankton and decapod crustaceans, for example the Norway lobster (*Nephrops norvegicus*), resulting in a decrease in the feeding rate and amount of food ingested (Murray and Cowie 2011, Simmonds 2012, Cole *et al.* 2013). In teleosts, two separate studies found that excessive amounts of plastic in the digestive tract of fish affected their buoyancy control (Boerger *et al.* 2010, Carson 2013). This would presumably also affect the larvae of various species of fish which have been observed to ingest microplastics (e.g., Carpenter 1972, Possato *et al.* 2011 and Ramos *et al.* 2012) owing to a larger particle size to body size ratio. Since the various life stages of many species, vertebrates and invertebrates alike, are not able to egest the bioinert plastic particles (Andrady 2011, Cole *et al.* 2011, Murray and Cowie 2011, Simmonds 2012), they accumulate in the digestive tract, which can lead to pseudosatiation, resulting in malnutrition and eventual starvation (Browne *et al.* 2008, Moore 2008, Boerger *et al.* 2010, Tourinho *et al.* 2010).

It has been hypothesised that passive feeders such as filter feeders experience adverse effects from microplastic ingestion more acutely than active predators as a result of a higher chance of encounter between the passive feeders and microplastic particles and therefore, a higher chance of ingestion (Di Beneditto and Awabdi 2014). Furthermore, in filter feeders frequently encountering microplastics, larger consumers like baleen whales are less affected than smaller consumers like mussels (Andrady 2011), due to the size of the consumer relative to the plastic particle. Species observed ingesting microplastic particles include various zooplankton species, echinoderms (holothurians and *Cucumaria* and *Thyonella* species), molluscs (*Mytilus edulis* (Linnaeus, 1758)), lugworms (*Arenicola marina* (Linnaeus, 1758)), bivalves, crustaceans, seabirds (*Puffinus gravis* (O'Reilly, 1818), *Fulmarus glacialis* (Linnaeus, 1761)), fish such as king mackerel (*Scomberomorus cavalla* (Cuvier, 1829)), and cetaceans such as baleen whales (*Megaptera novaeangliae* (Borowski, 1781)), Franciscana dolphins (*Pontoporia blainvillei* (Gervais and d'Orbigny, 1844)) and True's beaked whale (*Mesoplodon mirus* (True, 1913)) (Azzarello and Van-Vleet 1987, Ryan 1988, Berk *et al.* 1991, Thompson *et al.* 2004, Voparil *et al.* 2004, Leys and Eerkes-Medrano 2006, Teuten *et al.* 2007, Browne *et al.* 2008, Graham and Thompson 2009, Boerger *et al.* 2010, Denuncio *et al.* 2011, Simmonds 2012, Foekema *et al.* 2013, De Witte *et al.* 2014, Van Cauwenberghe and Janssen 2014, Besseling *et al.* 2015, Lusher *et al.* 2015).

Microplastic particles are considered biochemically inert due to their large molecular size which prevents passage through cell membranes and hence interaction with the endocrine system (Teuten *et al.* 2009). However, microplastics pose an additional threat to organisms through the leaching of contaminants into the tissues of consumers from the surface of the ingested microplastic particle onto which they are sorbed (Ryan 1988, Mato *et al.* 2001, Thompson *et al.* 2004, Teuten *et al.* 2007, Teuten *et al.* 2009, Andrady 2011, Hirai *et al.* 2011, Bakir *et al.* 2012, Besseling *et al.* 2015). The toxicity danger posed by microplastics arises from one of three pathways. Firstly, microplastics adsorb and concentrate harmful chemicals, heavy metals, and organic contaminants (Teuten *et al.* 2009, Bakir *et*

al. 2014b) from seawater due to the hydrophobic nature of many of these persistent organic pollutants (Teuten *et al.* 2009, Andrady 2011). The contaminants occur at differing concentrations in the world's oceans and have been found to have higher sorption capacity for plastics than for naturally occurring sediments (Teuten *et al.* 2007, Teuten *et al.* 2009). Moreover, microplastics used in industrial processes are often contaminated with heavy metals during the process (Cole *et al.* 2011). Examples of heavy metals that have been found sorbed onto microplastics include mercury, cadmium, silver, nickel, selenium, chromium, zinc, arsenic, lead, and copper, which have been found to be carcinogenic, teratogenic, and/or mutagenic (Davies 1978).

Various relationships govern the relative toxicity of leached contaminants. Fries and Zarfl (2012) suggest that there is an inverse relationship between the size of the microplastic particle and the sorption rate of contaminants onto the particle. The study found that larger microplastic particles (1 – 5 mm) reached sorption equilibrium more slowly than smaller microplastic particles (200 – 250 µm) did (Fries and Zarfl 2012). Desorption rates of sorbed contaminants from plastic vectors vary in accordance with the physiological environments to which they are exposed in different consumers (e.g., temperature and pH) (Bakir *et al.* 2014a). Findings suggest that contaminants are more easily leached into the bodies of warm-blooded organisms like birds than in cold-blooded animals like fish (Bakir *et al.* 2014a).

Takada (2006), Bakir *et al.* (2012), and Heskett *et al.* (2012) found that the sorption rates of contaminants onto microplastics is specific to the polymer and pollutant in question, and underscores the need to accurately measure the distribution coefficients for each plastic type and contaminant type. This is supported by Hirai *et al.* (2011) and Rochman *et al.* (2012) who found that polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) had higher sorption rates for high- and low-density polyethylene (HDPE and LDPE) and polypropylene (PP) than polyethylene terephthalate (PET) and polyvinyl chloride (PVC), supporting the hypothesis of Fries and Zarfl (2012) that lower

density polymers sorb contaminants at a higher rate than high density polymers. Polybrominated diphenyl ethers (PBDEs) have a higher affinity for polyethylene (PE) than for polypropylene, but polypropylene sorbed higher concentrations of PCBs, PAHs, and dichlorodiphenyltrichloroethane (DDT) (Hirai *et al.* 2011). Overall, C-phenanthrene (Phe) and polyethylene bear the highest potential for transport of contaminants to organisms (Teuten *et al.* 2009, Bakir *et al.* 2014a, b). As Bakir *et al.* (2014a, b) explain, this is cause for concern because polyethylene is one of the most common polymers found in marine pollution (Teuten *et al.* 2007). Polyvinyl chloride and polyethylene sorbed DDT, Phe, and diethylhexyl diphthalate (DEHP) at different rates according to the varying affinities between contaminant and plastic type, with contaminants desorbing at a faster rate from polyethylene than PVC (Bakir *et al.* 2014a). Bakir *et al.* (2014a, b) found the following combinations of contaminant-plastic vector to be of highest concern: Phe-PE > Phe-PVC ≥ DDT-PVC = DDT-PE. Bakir *et al.* (2014a,b) thus propose that the potential amount of contaminant that can be sorbed onto a plastic particle is dependent more on the affinity for sorption of the particular plastic-pollutant combination, and less on the concentration of the pollutant in the surrounding water, a conclusion supported by Yu *et al.* (2006) and Teuten *et al.* (2007). Teuten *et al.* (2009) found the most harmful of the organic contaminants to be alkylphenols, bisphenol A, the organochlorine pesticides DDT and hexachlorocyclohexanes, petroleum hydrocarbons, polybrominated diphenylethers (PBDEs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons.

Of further consequence is the interaction between contaminants, as they do not occur in isolation in the marine environment. This may impact on rates of sorption onto the plastic vector through possible competitive behaviour by the contaminants for binding sites on the microplastic vector (Bakir *et al.* 2012). Bakir *et al.* (2012) found that contaminant sorption rate was linear in experimental systems containing only one contaminant. This is in contrast with experimental systems with two contaminants (Phe and DDT), where the sorption rate of Phe was non-linear as affinity of the microplastic vector decreased due to a reduction in the number of binding sites for Phe on the vector

as a result of DDT preferentially binding to the vector (Bakir *et al.* 2012). A higher concentration of DDT binds when compared to Phe owing to its higher hydrophobicity (Bakir *et al.* 2012). Further consideration needs to be given to the class of plastic as polymers can be categorised as either glassy or rubbery which vary in the density of their structures (George and Thomas 2001, Bakir *et al.* 2012). Glassy polymers like unplasticised PVC are dense in structure with limited free volume, whereas Ultra-High Molecular Weight (UHMW) polyethylene is categorised as a rubbery polymer, with multiple voids between molecules (George and Thomas 2001). The nature of the pollutant (i.e., hydrophobic or hydrophilic) further affects the rate of binding and therefore, the magnitude of competition between pollutants in sorption onto plastic vectors (Bakir *et al.* 2012).

The physiological effects of contaminants are varied, depending on the properties and behaviour of the contaminant once in the tissues of the consumer. Acevedo *et al.* (2013) found that alkylphenol additives and the monomer bisphenol A (BPA) behaved in a similar fashion to oestrogen in the bodies of male and female consumers of multiple species. Biologically active conjugated BPA, though a synthetic material, is considered to be of more concern if present in the bloodstream as compared to unconjugated BPA since it also acts as an oestrogen (Watson *et al.* 2005, Thomas and Dong 2006, Acevedo *et al.* 2013), with effects such as the development of mammary gland adenocarcinomas in rats (Acevedo *et al.* 2013). Furthermore, when considering the effects of plastics on hormones in the body of a consumer, Foster (2001) found an inverse relationship between phthalate plasticizers and testosterone production, which may result in hypospadias and the improper formation of the vas deferens and epididymis seen in male rats. It is clear that ingesting plastic affects the hormonal system in all animals. In seabirds, leaching of halogenated hydrocarbons into body tissues has been linked to delayed ovulation, a decrease in steroid hormone levels, and failure to reproduce (Azzarello and Van-Vleet 1987). A similar transfer process of contaminants has been suggested by Tanaka *et al.* (2013) in short-tailed shearwaters (*Puffinus tenuirostris* (Temminck, 1835)).

Due to the wide range of organisms that routinely ingest plastic particles of all sizes, it is likely that trophic transfer of these particles occurs, with the result of also transferring the contaminants into the body of the secondary consumer (Andrady 2011, Farell and Nelson 2013). Eriksson and Burton (2003) found what is hypothesised to be secondary microplastic particles in seal scat from the remote Macquarie Island, approximately half-way between New Zealand and Antarctica which the authors believed were ingested by organisms in lower trophic levels which the seals then fed on. It has been observed in the stingray, *Dasyatis guttata* (Bloch and Schneider, 1801), that plastic particles present in its digestive tract were ingested by its prey and trophically transferred to the stingray (Eriksson and Burton 2003, Possato *et al.* 2011). Of importance to human consumers is the possibility that the contaminants sorbed by the plastic particles may be trophically transferred to human bodies through ingestion of seafood, even if the plastic particles themselves are not transferred (Browne *et al.* 2013, Rochman *et al.* 2013), owing to the contaminants leaching into the tissues of consumers.

Though much work has been conducted on microplastic pollution in the Northern Hemisphere, the Southern Hemisphere is less well-studied. The first study in South Africa by Ryan (1988) sampled plastic particles at the sea surface in the waters off the Cape Province using neuston trawls and found an average of 3640 particles.km^{-2}. A subsequent study by Naidoo *et al.* (2015) sampled surface waters along the Durban coastline (KwaZulu-Natal) with a conical zooplankton net, and found the highest mean plastic concentration at Durban Harbour: 70.3 ± 119.3 particles per 10 000 L. A study conducted in the same year by Nel and Froneman (2015) along the south-eastern coastline of South Africa found that microplastic densities in the water column ranged between 257.9 ± 53.36 and 1215 ± 276.7 particles.m^{-3} when collected using a WP-2 net. Nel *et al.* (2017) conducted sampling along the whole length of the South African coastline using a 10 L bucket and 63 µm mesh sieve, and found significantly higher microplastic counts at Richard's Bay Harbour and Durban Harbour (413.3 ± 77.53 particles.m^{-3} and 1200 ± 133.2 particles.m^{-3} respectively), explaining that these finding support the hypothesis that harbours are sources or distributors of microplastic particles to the marine

environment. Since the equipment used to collect samples differed between studies, the values are not comparable, underscoring the need for a standardised method of sampling. Sediment was also sampled by Naidoo *et al.* (2015), Nel and Froneman (2015), Nel *et al.* (2017), de Villiers (2018), and Ryan *et al.* (2018). No studies in the southern hemisphere are known to have sampled marine organisms in order to examine ingested microplastic loads.

1.4 Aims and objectives

To better understand the potential ecological impacts of plastic on marine ecosystem functioning, the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP 2015) suggests the need for research programmes to assess the temporal variations of microplastic particle abundances in the water column (GESAMP 2015). No published data exists on the temporal variation of microplastics in the water column, or the ingestion of microplastics by marine invertebrates along the southern African coastline. The aim of this study therefore, was to provide baseline data on the temporal variation in microplastic loads in the water column, and to provide baseline data on the number of ingested microplastics in consumers and assess whether any temporal patterns exist in the consumed microplastic loads. The main objectives of this study were therefore, to 1) sample microplastic loads in the water column over four different time scales, and 2) determine the spatial and temporal variation in loads of microplastics ingested by filter feeders. To that end, water samples were collected from the surf-zone at Kariega Beach, Kenton-on-Sea, Eastern Cape, South Africa, over one day, one week, one month, and one year. For part two, four species of filter-feeders (*Perna perna* (Linnaeus 1758) and *Mytilus galloprovincialis* (Lamarck 1819) mussels, and *Octomeris angulosa* (Sowerby 1825) and *Tetraclita serrata* (Darwin 1854) barnacles were sampled at Kariega Beach, Kenton-on-Sea, Eastern Cape, and Wilderness Beach, Wilderness, Western Cape, during the austral winter of 2017 and summer of 2018.

1.5 Thesis structure

This thesis comprises six chapters. Chapter 1 has provided a general discussion on plastic, its history, its applications and its role in marine pollution, the study species, sampling site, aims and objectives, and the essential concepts of the study. Chapter 2 describes the study areas and sites common to Chapters 3 and 4. Chapter 3 discusses temporal variation of microplastic loads in the marine environment in the water column over four short-term time scales, while Chapter 4 examines seasonal and spatial variation in the number of ingested microplastic particles in four species of filter-feeders. To conclude, Chapter 5 is a general discussion that provides a synopsis of the study, recommendations, shortcomings, and conclusions.

Chapter 2

Study area and site

2.1 Area and site description

2.1.1 General area and current description

South Africa is divided into nine provinces, four of which are on the 2954 km coastline (Figure 2.1) (Branch *et al.* 2010). Branch *et al.* (2010) divided the coastline into three broad coastal regions – the east, south, and west coasts. The Agulhas Current is the primary current flowing along the south-eastern seaboard of the region and forms part of the anticyclonic circulation system found in the Indian Ocean which forms as a result of the wind patterns found at these latitudes (Lutjeharms 2006). The Agulhas Current is the largest Western Boundary Current in the world (Lutjeharms 2006) and is found from 27°S to 40°S in the Indian Ocean (Gordon 1985, Lutjeharms and Van Ballegooyen 1988, Lutjeharms and de Ruijter 1994, Lutjeharms 2006). It is a fast-flowing current and is approximately 100 km wide (Lutjeharms 2006), carrying warm tropical and subtropical water of, on average, 27°C in summer, and 22°C in winter, with a $2°C$ change in temperature downstream along its length (Lutjeharms and de Ruijter *et al.* 1996, Lutjeharms *et al.* 2001) from Mozambique in the north-east, downward along the South African coastline in a south-westerly direction, moving off shore along the coastline stretching from Port Alfred to Port Elizabeth (Coetzee 1988, Lubke 1988). The behaviour of the current changes from Port Elizabeth downstream due to the widening of the continental shelf forming the Agulhas Bank (Lutjeharms 2006). This study was restricted to a single station as a previous study indicated that there were no significant spatial patterns in microplastic counts along the South African coastline (Nel *et al.* 2017).

Figure 2.1: The South African map, indicating sampling areas: Wilderness, Western Cape Province, and Kenton-on-Sea, Eastern Cape Province. (GoogleEarth 2018).

2.1.2 Kenton-on-Sea, Eastern Cape Province

Kenton-on-Sea is a small coastal town in the Eastern Cape Province of South Africa, situated between the Bushmans- and Kariega River-Estuaries (Figure 2.2) (Coetzee 1988, Hill *et al.* 2008). These estuaries are said to lie in the warm-temperate region which extends from Cape Point to the Mendu Estuary in the Eastern Cape (Whitfield 1998). Upwelling events during summer often coincide with a drop in water temperature along the coastline (Whitfield 1998). The Kowie River lies approximately 25 km east of Kenton-on-Sea, and together with the Bushmans and Kariega Rivers provide little input of freshwater into the coastal environment due to water abstraction upriver (Hill *et al.* 2008). The primary source of freshwater for the area is the Great Fish River which lies 55 km east of Kenton-on-Sea, providing comparatively large volumes of freshwater to the coastal environment (Hill *et al.* 2008).

The rocky shore at Kenton-on-Sea comprises of Aeolian dune rock (Lubke 1988, Marker 1988, Hill *et al.* 2008). This rock type has eroded in the area through wave action to form sheer cliffs and such geological formations as blowholes (Lubke 1988). The area also has sandy shores and dunes, which are areas of sediment deposition (Marker 1988). The sandy shore is formed through wave action, usually lying on a low rocky platform which may be exposed at low tide (Marker 1988). Due to the gentle sloping of the beach, there is a large expanse between the wave break and the dunes against which winds blow, drying and transporting sand inland, which then accumulates against the first object encountered, usually plants (Marker 1988). Kenton-on-Sea has a rich marine fauna, all of which are potential consumers of microplastics. Beal and Bryden (1997) have identified various phyla present at this site: Porifera, Cnidaria, Ctenophora, Annelida, Arthropoda, Bryozoa, Brachiopoda, Mollusca, Echinodermata, Chordata, Platyhelminthes, Nematoda, Sipuncula, and Aves (Branch *et al.* 2010).

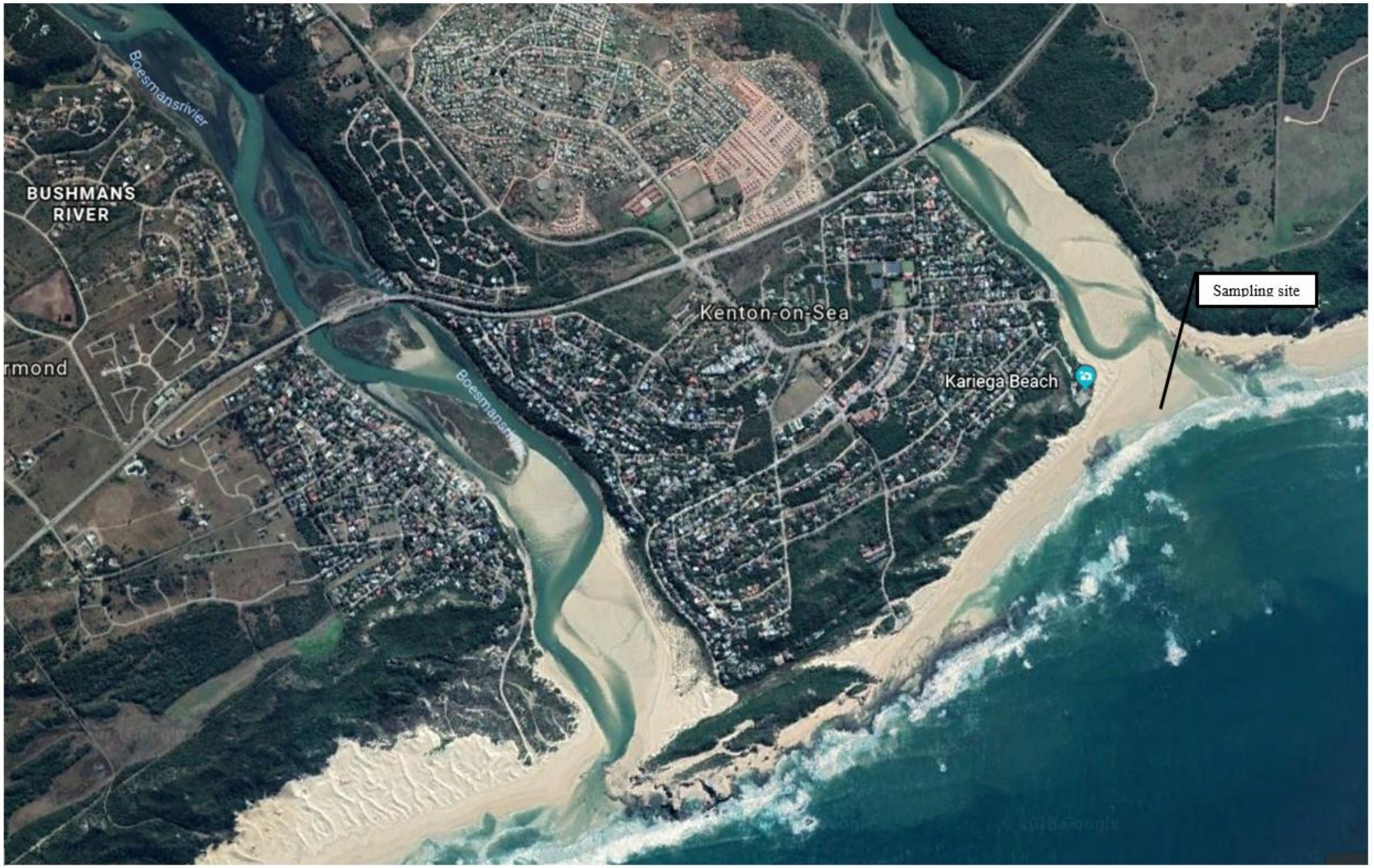

Figure 2.2: The Eastern Cape Province sampling area, Kenton-on-Sea, indicating sampling site, Kariega Beach. (GoogleEarth).

2.1.3 Wilderness, Western Cape Province

Wilderness is a small coastal town between the Kaaiman's River and the Touws River, in a small bay in which the Wilderness sandy beach is found (Figure 2.3). McLachlan *et al.* (1981) rated Wilderness Beach as 15.0 on a 20-point exposure scale. The permanently open Knysna River-Estuary is the largest source of freshwater output in the area (Allanson *et al.* 2016, Human *et al.* 2016). The estuary lies in the same warm-temperate region as Kenton-on-Sea (Whitfield 1998). The area lies immediately downstream of the Knysna River-Estuary into which run-off flows from the surrounding cattle-farms (Switzer 2008). Grey water from households is also discharged into the Knysna River-Estuary (Switzer 2008) and so it may be a likely source of microplastic pollution. Phyla found in the area include Porifera, Cnidaria, Ctenophora, Platyhelminthes, Echiura, Sipunculida, Annelida, Arthopoda, Nemertea, Nematoda, Hexapoda, Chelicerata, Crustacea, Bryozoa, Mollusca, Brachiopoda, Echinodermata, Chordata, Reptilia, Aves, and Mammalia (Branch *et al.* 2010).

Figure 2.3: The Western Cape Province sampling area, Wilderness, indicating sampling site, Wilderness Beach. (GoogleEarth).

Chapter 3

Temporal patterns in microplastic loads in the water column off the Eastern Cape coastline of South Africa

3.1 Introduction

Many studies have reported on the presence of microplastics in the water column. Environments sampled include surface waters, subsurface waters, coastal, offshore, and pelagic environments (Andrady 2017, Anderson *et al.* 2016, Abayomi *et al.* 2017, La Daana *et al.* 2018, Morgana *et al.* 2018, Zhu *et al.* 2018). The most commonly occurring plastics in marine environments are polyethylene (PE), polypropylene (PP), and polystyrene (PS) (Zhang 2017). Microplastic counts vary depending on the environment sampled and sampling equipment used, with counts consisting of microfragments, microfibres and microfilms (Anderson *et al.* 2016, Abayomi *et al.* 2017, Andrady 2017, Gewert *et al.* 2017, Morgana *et al.* 2018), though many studies have found that microfibres dominate microplastic counts (Anderson *et al.* 2016, La Daana *et al.* 2018, Obbard 2018, among others). Of these, black and blue fibres constitute the majority of the colour classes (Lusher *et al.* 2014, Abayomi *et al.* 2017, Gewert *et al.* 2017, La Daana *et al.* 2018), which Browne *et al.* (2011) and Napper and Thompson (2016) attribute to the release of fibres when washing clothing and other textiles. Up to 700 000 fibres can be released in a single wash by an average 6 kg washing load (Napper and Thompson 2016).

In the marine environment, coastal waters may have higher numbers of microplastics owing to their proximity to land-based sources of microplastics. These waters are continuously influenced by the strong hydrodynamic processes of wind, tides, wave action, and thermohaline gradients which may favour the settling and accumulation of microplastics in the sediment at the freshwater-seawater interface (Zhang 2017). Storm water discharge during the rainy season and the associated changes in

wind direction and speed also results in the accumulation of microplastics at the interface, but more pertinently, may result in vertical mixing in the water column which re-suspends buried and settled microplastics (Zhang 2017). This cycle, along with other processes, may keep microplastics 'trapped' in the nearshore environment, with transport into the open ocean relying primarily on surface currents (Zhang 2017).

Few studies have assessed temporal fluctuations in microplastic counts. The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP 2015) suggests the need for research programmes to assess the temporal variations of microplastic particle abundances in the water column (GESAMP 2015). Little data exists on the temporal behaviour of microplastics in the nearshore environment on short-term time scales, with most studies conducted on a seasonal scale. The primary factors dictating seasonal availability of microplastics in the nearshore environment include up- and down-welling events, weather disturbances, and vertical stratification (Zhang 2017). Other oceanographic features that affect dispersal and distribution of microplastics include the geostrophic factors of oceanic fronts and eddies, wind-driven Ekman transport, and wave-propagated Stokes drift (Zhang 2017). Abayomi *et al.* (2017), sampled surface waters in Doha Bay and did not find a difference in microplastic loads between 7 January 2015 and 29 March 2015. Antunes *et al.* (2018) sampling off the Portuguese coast over two years (2011-2013) found that microplastic concentrations were higher at most sampling sites during winter/autumn. To correct the deficit of knowledge regarding microplastic particle behaviour over short-term time scales, this chapter assessed the temporal variation in microplastic counts and composition on two-hourly, daily, weekly, and monthly time scales along the south-eastern coastline of South Africa.

3.2 Materials and methods

3.2.1 Sampling area and site description

See Chapter 2 for details.

3.2.2 Sample collection

The methodology employed during this study has been adapted from Hidalgo-Ruz *et al.* (2012). Water column samples were collected from the surf-zone at a depth of approximately 50 cm over four different time scales: two-hourly over a period of 24 hours (1 – 2 March 2017), daily over one week (1 – 7 March 2017), weekly over a period of one month (1 – 29 March 2017), and finally, monthly over the period of one year (1 March 2017 – 1 February 2018). Independent triplicate water samples were collected using a 50 L plastic drum. Prior to collection, the drum was washed with distilled water to minimise contamination. In each case the bulk water sample was gravimetrically passed through a 20 μm sieve, and retained debris rinsed into a prewashed polycarbonate jar using deionised water and transported to the laboratory. The reduced water sample was then gently filtered (vacuum <5 Hg) through a 5 μm (47 mm diameter) cellulose nitrate filter (Sartorius). Filter papers were then examined for microplastic particles using an Olympus dissecting microscope operated at between 40 and 400 X magnification according to the methods outlined in Nel *et al.* (2017). Microplastics were identified according to the criteria of Norén (2007): (1) no structures of organic origin should be visible in the plastic particle or fibre, (2) fibres were equally thick and had three-dimensional bending to exclude a biological origin, (3) particles were clear and homogeneously coloured, (4) transparent particles were examined under high magnification to exclude a biological origin. To ensure consistency in counts, a second counter randomly recounted samples. Identified plastic particles were separated into two classes, fragments and microfibres according to the definition used by GESAMP (2015). Post-sampling contamination was controlled for by eliminating major sources of in-laboratory

contamination. Distilled water was used to clean the equipment between each sample extraction. Additionally the equipment used was glass where possible. Samples were covered when they were not in use to minimise contamination from the surrounding air. Although samples were uncovered during filtration and during counting, control experiments were run to account for possible contamination during these procedures. Particles >5 mm in diameter were not considered during the investigation.

3.3 Statistical analyses

Statistical analyses were conducted using *R* (R Core Team 2018) and XLSTAT (2019). Visual representation of data analysed was performed using Microsoft Excel (2013). For purposes of statistical testing, no differentiation was made between microplastic fragments and microplastic fibres, and density is expressed as microplastic particles.m^{-3}. One-way ANOVA tests were performed for each time scale using time as the independent variable. Assumptions were tested using a Shapiro-Wilk normality test and a Bartlett test of homogeneity of variances, both of which were satisfied for all time scales ($p > 0.05$ in all cases).

3.4 Results

Average microplastic density of samples collected every two hours over a period of one day ranged from 141 ± 133 to 265 ± 48 microplastic particles.m^{-3} (Figure 3.1). There were no significant temporal differences in the microplastic counts observed over the 24-hour period (ANOVA; $F(2,33) = 0.77$, $p = 0.47$). Daily counts over a period of one week also demonstrated no statistically significant patterns between days, (ANOVA; $F(2,18) = 0.51$, $p = 0.61$), with average abundances ranging from 152 ± 92 to 293 ± 125 microplastic particles.m^{-3} (Figure 3.2). Weekly abundances fell within a similar range, between 198 ± 4 and 238 ± 92 microplastic particles.m^{-3}, and also did not show a statistically significant difference in microplastic counts (ANOVA; $F(2,9) = 0.05$, $p = 0.95$) (Figure 3.3). Finally,

mean monthly microplastic densities values ranged from 85 ± 203 to 269 ± 154 microplastic particles.m^{-3}, and also did not show statistically significant temporal differences (ANOVA; $F(2,33) = 0.35$, $p = 0.70$) (Figure 3.4). With two exceptions, microfibres numerically dominated the microplastic counts on all temporal scales considered, contributing between 47 and 97 % of the total counts (Figures 3.5 to 3.8).

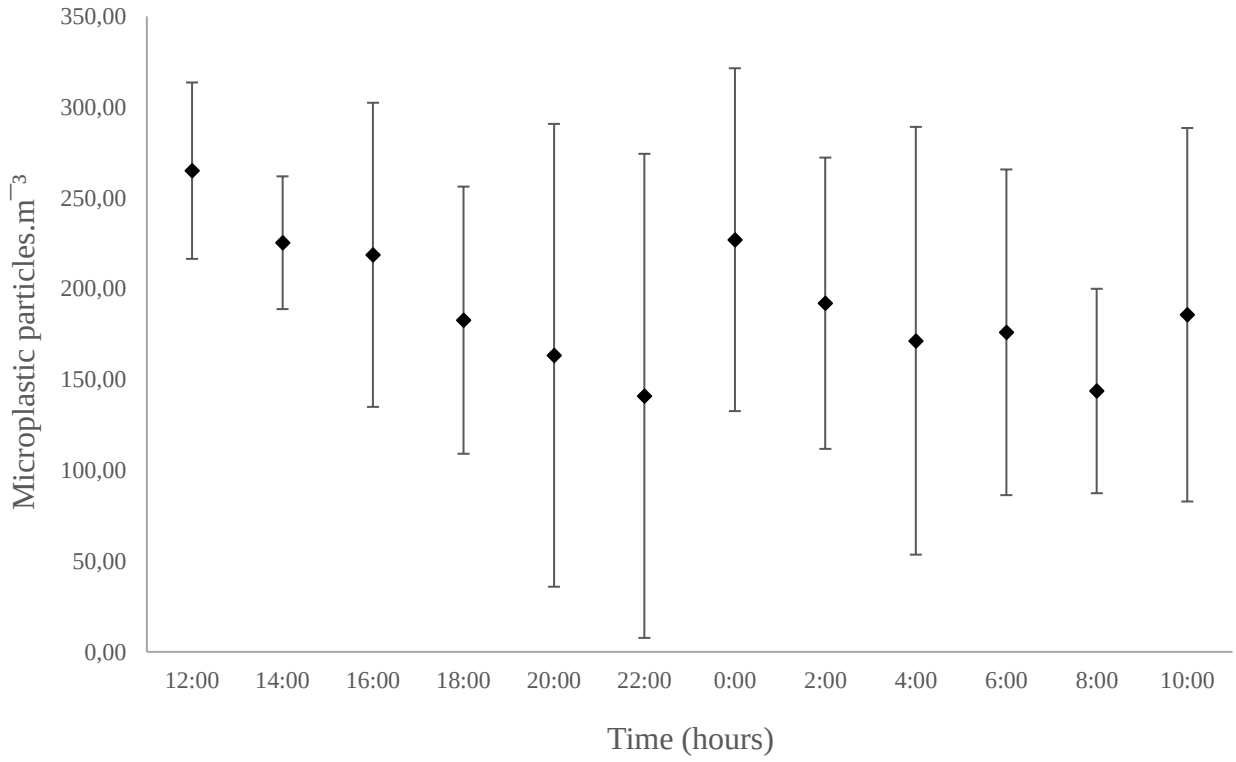

Figure 3.1: Mean (± standard deviation) microplastic counts (microplastic particles.m^{-3}) in the water column (n = 3) taken from the nearshore environment along the Eastern Cape coastline of South Africa at two-hourly intervals over 24 hours at Kariega Beach, Kenton-on-Sea, Eastern Cape.

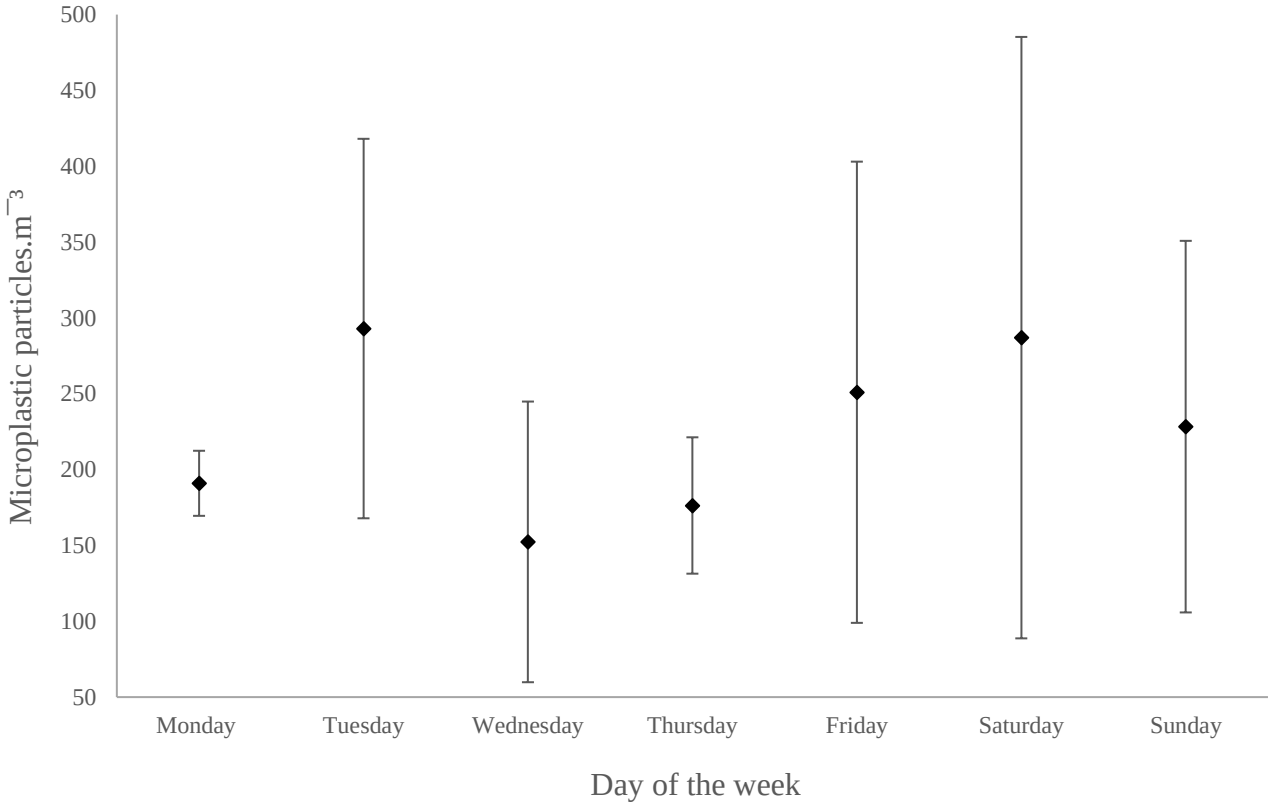

Figure 3.2: Mean (± standard deviation) microplastic counts (microplastic particles.m^{-3}) in the water column (n = 3) taken from the nearshore environment along the Eastern Cape coastline of South Africa daily over one week at Kariega Beach, Kenton-on-Sea, Eastern Cape.

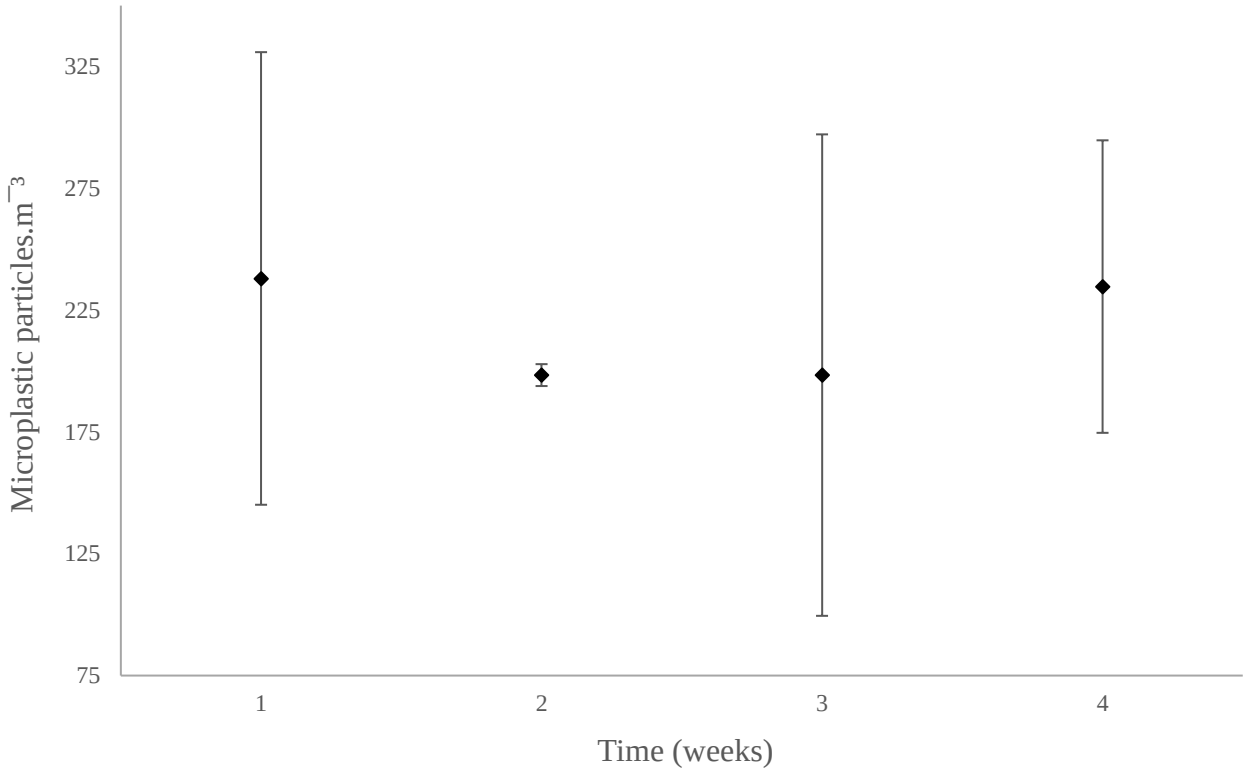

Figure 3.3: Mean (± standard deviation) microplastic counts (microplastic particles.m^{-3}) in the water column (n = 3) taken from the nearshore environment along the Eastern Cape coastline of South Africa weekly over one month at Kariega Beach, Kenton-on-Sea, Eastern Cape.

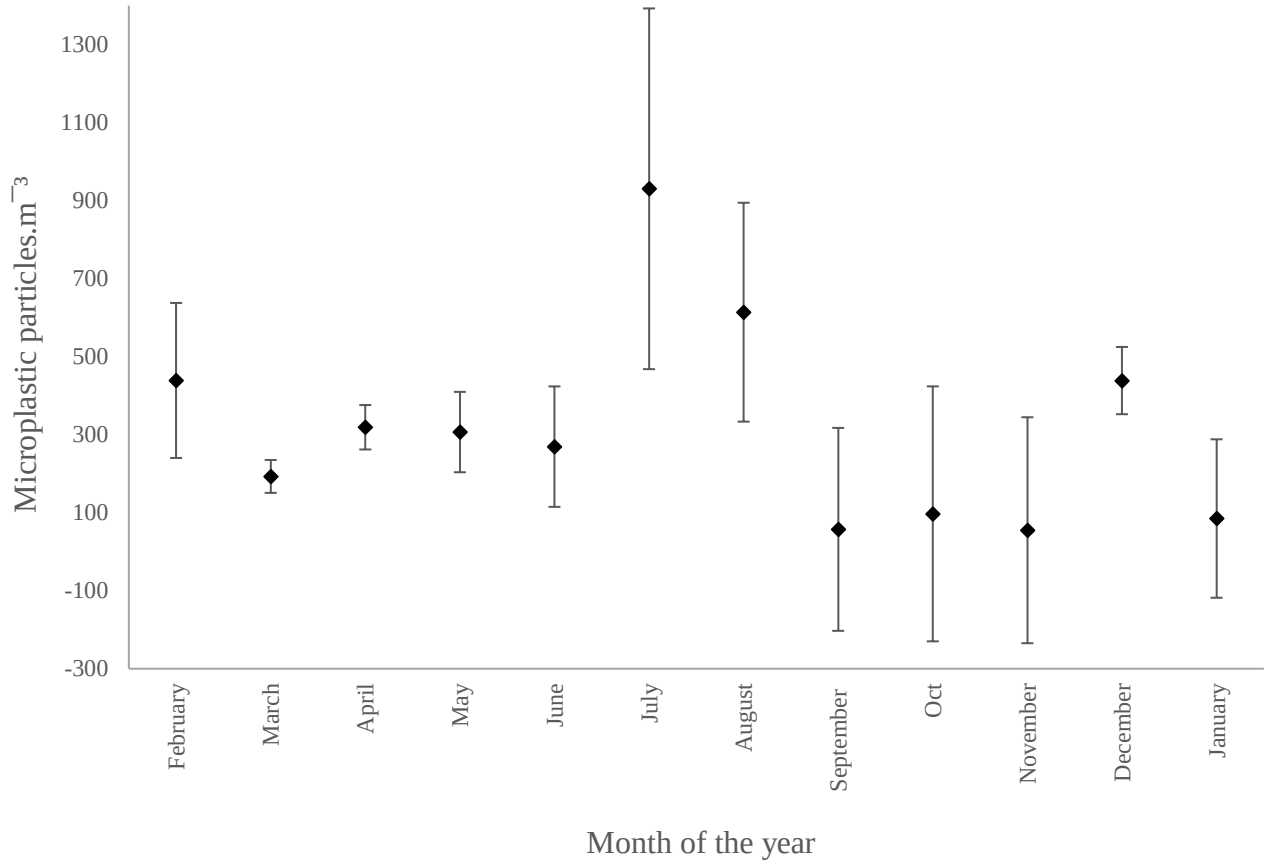

Figure 3.4: Mean (± standard deviation) microplastic counts (microplastic particles.m^{-3}) in the water column (n = 3) taken from the nearshore environment along the Eastern Cape coastline of South Africa monthly over one year at Kariega Beach, Kenton-on-Sea, Eastern Cape.

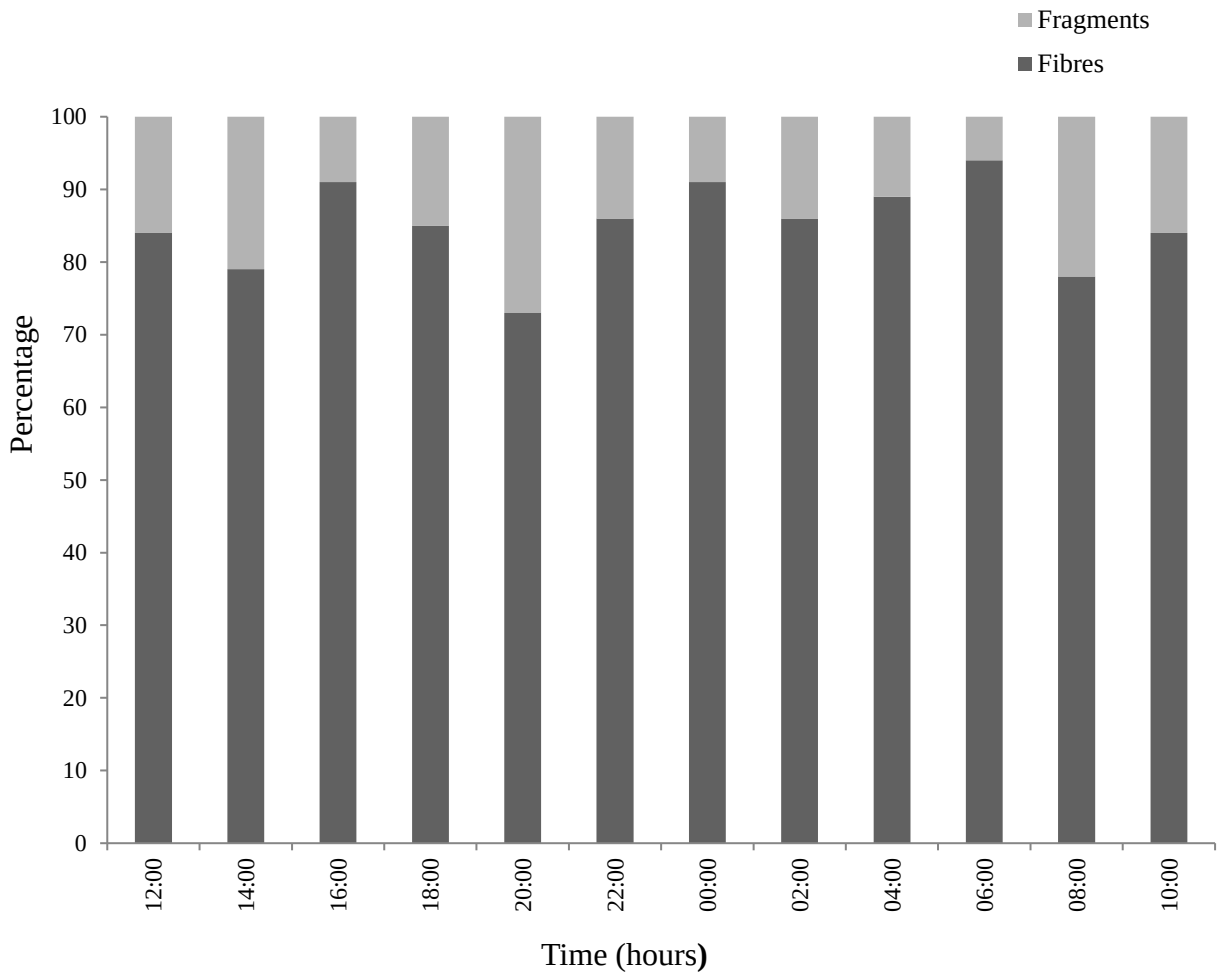

Figure 3.5: Proportional contribution of microplastic fragments and fibres to total microplastic counts sampled every two hours over a period of 24 hours at Kariega Beach in the nearshore environment along the Eastern Cape coastline of South Africa.

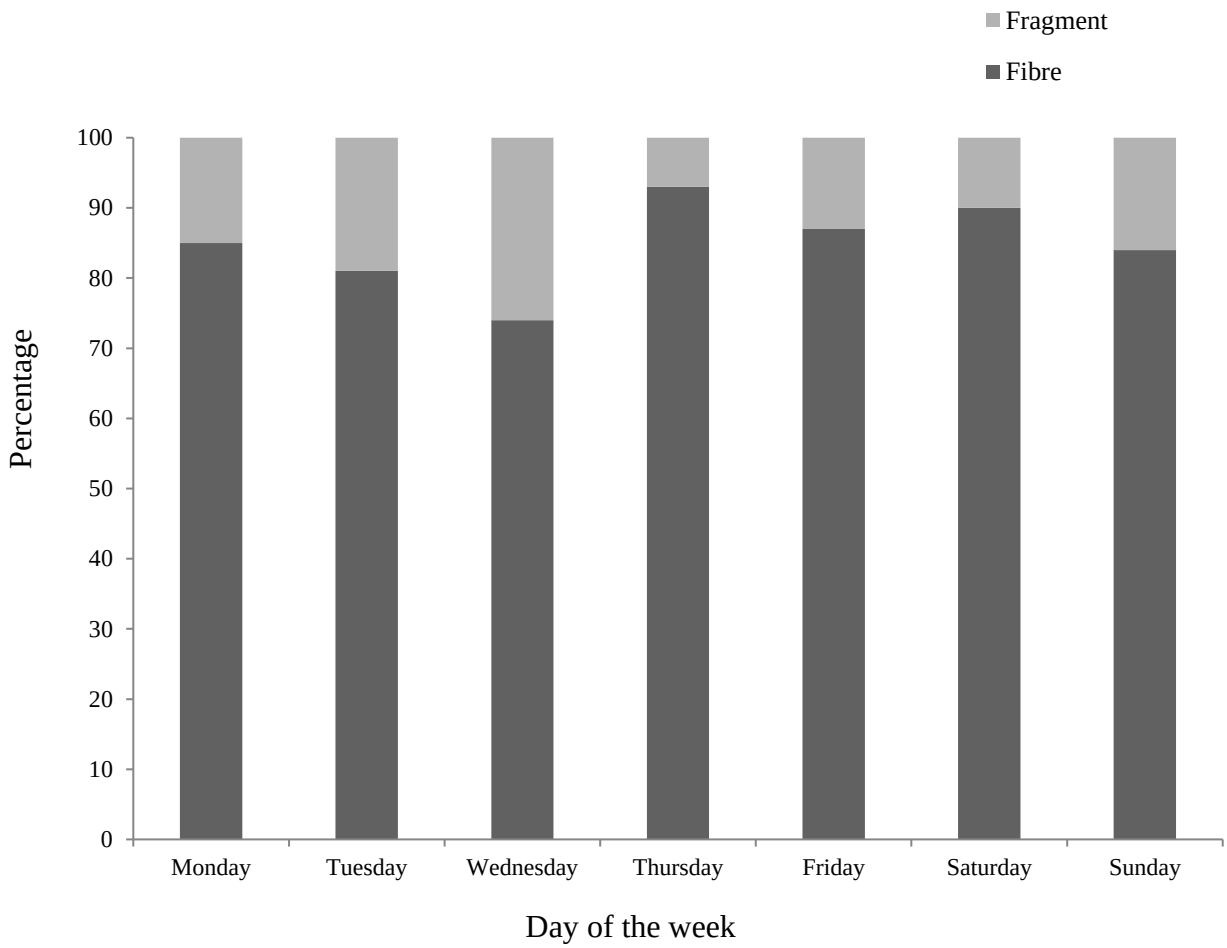

Figure 3.6: Proportional contribution of microplastic fragments and fibres to total microplastic counts sampled daily over a period of one week at Kariega Beach in the nearshore environment along the Eastern Cape coastline of South Africa.

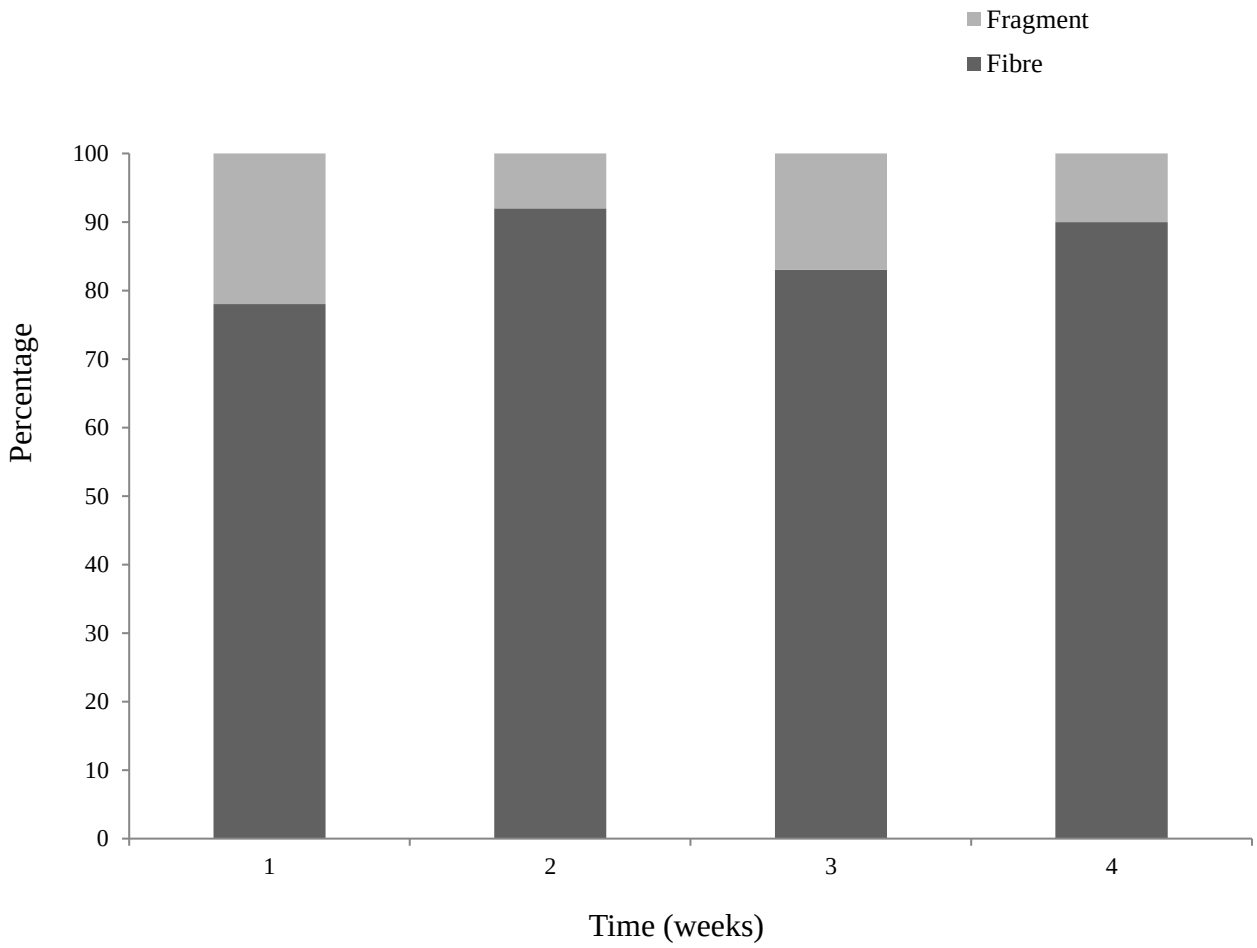

Figure 3.7: Proportional contribution of microplastic fragments and fibres to total microplastic counts sampled weekly over a period of one month at Kariega Beach in the nearshore environment along the Eastern Cape coastline of South Africa.

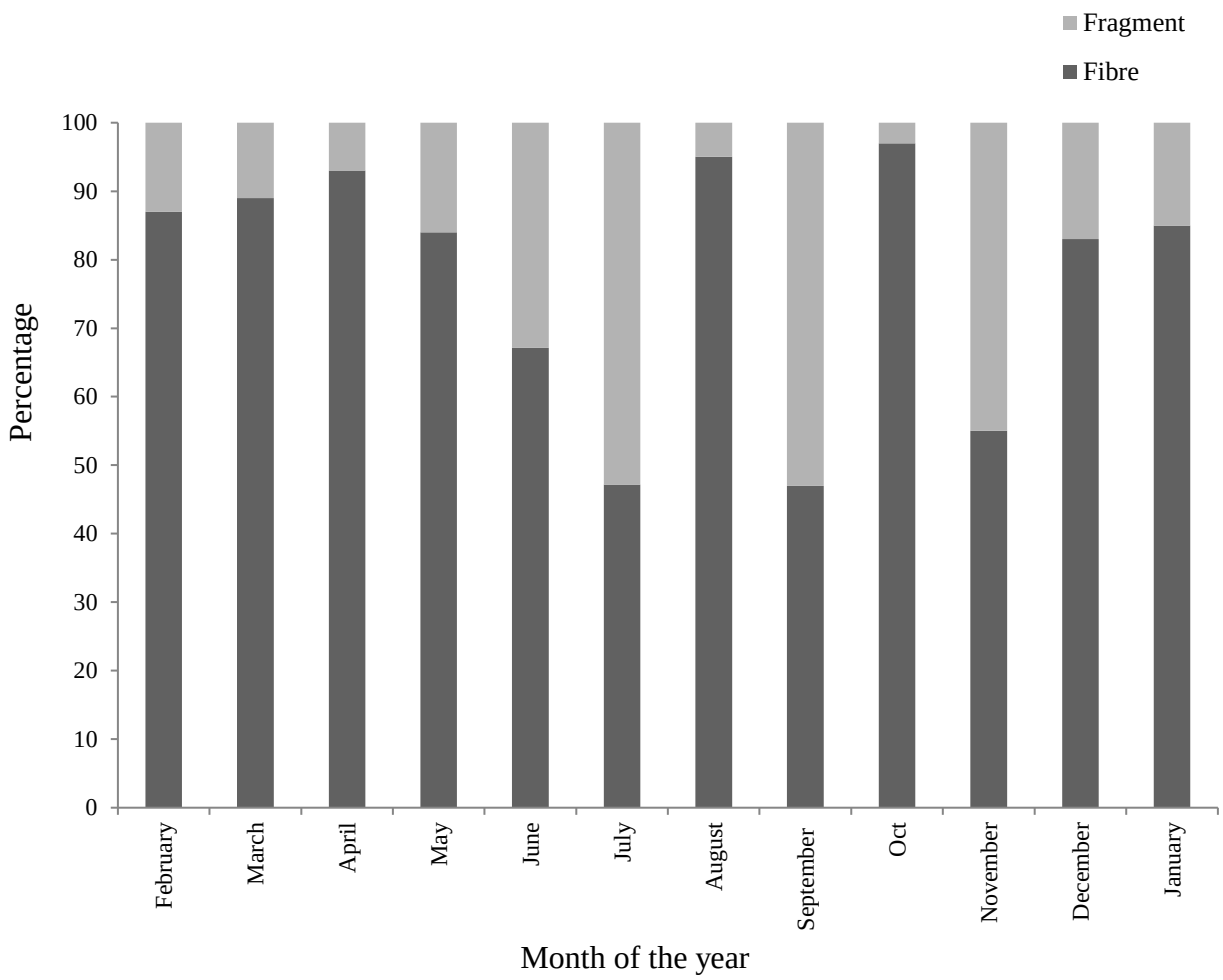

Figure 3.8: Proportional contribution of microplastic fragments and fibres to total microplastic counts sampled monthly over a period of one year at Kariega Beach in the nearshore environment along the Eastern Cape coastline of South Africa.

3.5 Discussion

Considerable research effort has been dedicated to investigating the spatial distribution and composition of microplastics in both the water column and sediments in a variety of marine environments (Browne *et al.* 2007, Gregory 2009, Lozano and Mouat 2009, Morét-Ferguson *et al.* 2010, Kukulka *et al.* 2012, Anastasopoulou *et al.* 2013, Van Cauwenberghe *et al.* 2015, Setäla *et al.* 2014, Van Cauwenberghe and Janssen 2014). This study failed to find any significant temporal patterns in microplastic density over the various time scales considered (Student's t-test; $p > 0.05$ in

all cases), a finding supported by that of Abayomi *et al.* (2017) in the Arabian Gulf. During the course of this investigation, mean microplastic density ranged from 55.00 ± 289.63 to 930.33 ± 432.39 microplastic particles.m^{-3}. These estimates are in the range reported by Nel and Froneman (2015) in the water column along the south and east coasts of South Africa, (257.9 ± 53.36 to 1215 ± 276.7 particles.m^{-3}, and indeed, for selected Northern Hemisphere coastal environments (Desforges *et al.* 2014, Zhao *et al.* 2014) suggesting that microplastic contamination in the coastal regions of the world's ocean is a global phenomenon. It should be noted that this study did not consider plastic particles (<20 μm) or >5 mm in size suggesting that the overall plastic counts are likely to be substantially higher than those presented in the current investigation.

A key finding of this study was that microplastic counts were highly variable contributing to the absence of any discernible temporal patterns in counts at the different time scales considered (p > 0.05 in all cases). A recent review by Zhang (2017) indicated that the transport of microplastics in coastal waters is dependent on the physical properties (size and density) of the particles, ocean dynamics (waves and water column characteristics) and shoreline typography. These factors together with source location contribute to variations in microplastic densities in the coastal environment (Zhang 2017). Pulses in microplastic density in nearshore waters can likely be attributed to a substantially higher loss of microplastic particles than normal from a point source (e.g. storm water outflow, plastic processing plants, sewage plants, and harbour spills) (GESAMP 2015). Pulses in microplastic density can also be associated with river discharge (GESAMP 2015). Browne *et al.* (2011), Kusui and Noda (2003), Eriksen *et al.* (2013), Depledge *et al.* (2013), Desforges *et al.* (2014), Yonkos *et al.* (2014) and de Sa *et al.* (2015) found a positive correlation between human population density and microplastic particle loads. By contrast, Nel and Froneman (2015) found no significant correlation between human population density and microplastic loads in the nearshore coastal waters along the South African coastline. The absence of any pattern was thought to reflect general

hydrology of the region which contributed to the distribution of microplastics along the coastline, a similar conclusion to that of Nel *et al.* (2017).

Microfibres numerically dominated the microplastic counts in all but two cases, contributing between 47 % and 97 % of the total counts (Figures 3.5 to 3.8). While no global estimates for proportional contribution of fibres and fragments exist for microplastic particles in the water column, Laglbauer *et al.* (2014), Naji *et al.* (2016) and Zobkov & Esiukova (2017) similarly found a higher proportional contribution of fibres to total microplastic counts in sediment samples in the Persian Gulf, Baltic Sea, and Slovenia, respectively. Although several possible sources of microfibres have been identified (e.g. fishing industry), the most likely source of microfibres is land-based activities. Browne *et al.* (2011) suggested that a single item of clothing may release >1500 microfibres per washing cycle, while Napper and Thompson estimate that up to 700 000 fibres can be released per washing cycle by an average 6 kg washing load. Due to the inability of water treatment plants to remove these fibres from wastewater, fibres are transported via rivers or sewage outflow sites into the marine environment (Zhang 2017).

In conclusion, this research has shown that microplastics are ubiquitous in the nearshore marine environment off the south-eastern seaboard of South Africa, and have no statistically significant patterns in density over short-term temporal scales (<1 year). The pervasive presence of microplastics in the water column likely poses an ecological threat, particularly to filter feeding invertebrates since these particles are often in the same size range of food particles routinely ingested by these organisms (Thompson *et al.* 2004, Ward and Shumway 2004, Di Beneditto and Awabdi 2014, Besseling *et al.* 2015). Future investigations should assess the extent of microplastic consumption by filter feeders and determine the likely consequence of the ingestion of these particles on the fitness of these organisms.

Chapter 4

Seasonal variation in microplastic loads ingested by selected filter feeders along the south-east Cape coast of South Africa

4.1 Introduction

Microplastic ingestion by marine organisms and its physiological effects have been the topic of many investigations (e.g., Wegner *et al.* 2012, Avio *et al.* 2015, Woods *et al.* 2018). To date over 220 species of vertebrates and invertebrates have been found to routinely ingest microplastics (Woods *et al.* 2018). In particular, the ingestion of microplastics appears to be widespread in filter feeders such as mussels and barnacles (Table 1). Among a host of other detrimental effects, the presence microplastics in the immediate environment of mussels and/or the ingestion of microplastics in mussels have been observed to: decrease filtration rate or stop filtration altogether at high concentrations (Woods *et al.* 2018); accumulate on the gills and the digestive gland (Woods *et al.* 2018); disrupt homeostasis resulting in the production of stress and immune-related proteins; cause weight loss and a reduction in growth through disturbing nutrient uptake (Detree and Gallardo-Escarate 2018); reduce the number of byssal threads produced and reduce strength of byssal attachment by half (Green *et al.* 2018); and cause changes on a cellular level, including changes in immunological responses and in the gene expression profile (Avio *et al.* 2015). Wegner *et al.* (2012) observed an increase in the volume of pseudofaeces produced in *Mytilus edulis*, which they hypothesised to be a response to recognition of plastic particles as being non-nutritive.

Table 1: Concentrations, type, and size of microplastics ingested by marine organisms.

Author	Location	Taxonomic order	Species		Laboratory/ Field/ Market/ Aquaculture Farm	Microplastic size (µm)	Concentration	Plastic type	Beads, fragments, fibres
Ayukai 1987	N/A	Calanoidia	Copepod	*Acartia clausi*	L	15.7	1140 beads mL^{-1}	Polystyrene	Beads
Catarino *et al.* 2017	UK	Mytiloida	Mussel	*Mytilus edulis*	A	-	2.5 n/g ww	-	Fibre
Catarino *et al.* 2018	UK	Mytiloida	Mussel	*Modiolus modiolus*	F	-	0.09 ± 0.03 n/g ww	-	Fibre
		Mytiloida	Mussel	*Mytilus edulis*		-	3.0 ± 0.9 n/g ww	-	
Cho *et al.* 2019	South Korea	Ostreioda	Oyster	*Crassostrea gigas*	M	100-200	0.07 ± 0.06 n/g ww	-	Fragment
		Mytiloida	Mussel	*Mytilus edulis*			0.12 ± 0.11 n/g ww		
		Veneroidea	Clam	*Venerupis philippinarum*			0.34 ± 0.31 n/g ww		
		Pectinida	Scallop	*Patinopecten yessoensis*			0.08 ± 0.08 n/g ww		
Choi *et al.* 2018	N/A	Cypriondonti-formes	Fish	*Cyprinodon variegatus*	L	6-350	50 & 250 mg L^{-1}	Polyethylene	Beads, irregular shapes
Christaki *et al.* 1998	N/A	Oligotrichida	Ciliate	*Strombidium sulcatum*	L	0.49-1	-	-	Beads
Cole and Galloway 2015	N/A	Ostreioda	Oyster	*Crassostrea gigas*	L	1 & 10	1, 10, 100 & 1000 microplastics mL^{-1}	Polystyrene	Beads
Cole *et al.* 2013	N/A	Calanoidia	Copepod	*Centropages typicius*	L	1.7-30.6	3000 beads mL^{-1}	Polystyrene	Beads
		Calanoidia	Copepod	*Calanus helgolandicus*			2240 beads mL^{-1}		
		Calanoidia	Copepod	*Acartia clausi*			635 beads mL^{-1}		
		Calanoidia	Copepod	*Temora longicornis*			-		
Cole *et al.* 2015	N/A	Calanoidia	Copepod	*Calanus helgolandicus*	L	20	75 beads mL^{-1}	Polystyrene	Beads
Courtene-Jones *et al.* 2017	UK	Mytiloida	Mussel	*Mytilus edulis*	F	1220	1.05-4.44 n/g ww	-	Fibre

Author	Location	Taxonomic order	Species		Laboratory/ Field/ Market/ Aquaculture Farm	Microplastic size (µm)	Concentration	Plastic type	Beads, fragments, fibres
Davidson and Dudas 2016	Canada	Veneroidea	Clam	*Venerupis philippinarum*	A	-	1.7 ± 1.2 n/g ww	-	Fibre
					F	-	0.9 ± 0.9 (0.07-5.47) n/g ww	-	
Desforges *et al.* 2015	Northeast Pacific Ocean	Calanoidia	Copepod	*Neocalanus cristatus*	F	-	8-9180 particles m^{-3}	-	Unidentified fibres and fragments
		Euphausiacea	Krill	*Euphausia pacifica*		816mm	-	-	
De Witte *et al.* 2014	Belgium	Mytiloida	Mussel	*Mytilus edulis*	M	1000-1500	0.35 n/g ww	-	Fibre
					F		0.04-0.81 n/g ww		
Digka *et al.* 2018	Greece	Mytiloida	Mussel	*Mytilus galloprovincialis*	A	100-500	2.5 ± 0.3 n/g ww	-	Fragment
					F		5.3 ± 0.5 n/g ww	-	
Fernandez 1979	N/A	Calanoidia	Copepod	*Calanus pacificus*	L	8-32	10^5-10^6 mL^{-1}	Polystyrene	Beads
Fernandez *et al.* 2004	N/A	Copelata	Tunicate	*Oikopleura dioica*	L	0.2, 0.5, 0.75, 1, 2, 3 & 6		Polystyrene	Beads
Frost 1977	N/A	Calanoidia	Copepod	*Calanus pacificus*	L	6.4, 10.3, 20 & 32	500 mL^{-1} sphere suspension	Polystyrene	Beads
Hammer *et al.* 1999	N/A	Oxyrrhinales	Dinoflag-ellate	*Oxyrrhis marina*	L	1 &4	10^6 mL^{-1}	Polystyrene	Beads
Huntley *et al.* 1983	N/A	Calanoidia	Copepod	*Calanus pacificus*	L	11.1, 15, 16.5, 20 & 25	<100 particles mL^{-1}	Polystyrene	Beads
Kaposi *et al.* 2014	N/A	Temnopleuro-ida	Sea urchin	*Tripneustes gratilla*	L	10-45	1, 10, 100, 300 spheres mL^{-1}	Polystyrene	Beads
Karlsson *et al.* 2017	Netherla-nds	Mytiloida	Mussel	*Mytilus edulis*	F	200	37 (6-107) n/g ww	-	Beads
Lee *et al.* 2013	N/A	Harpacticoida	Copepod	*Tigriopus japonicus*	L	0.05, 0.5 & 6	0.125, 1.25, 12.5 &25 µg.mL^{-1}	Polystyrene	Beads
Leslie *et al.* 2017	Netherla-nds	Mytiloida	Mussel	*Mytilus edulis*	F	10-300	13.2 n/g ww	-	Fibre
Li *et al.* 2015	China	Mytiloida	Mussel	*Mytilus galloprovincialis*	M	5-250	2.4 n/g ww	-	Fibre
		Pectinida	Scallop	*Patinopecten yessoensis*				-	

Author	Location	Taxonomic order	Species		Laboratory/ Field/ Market/ Aquaculture Farm	Microplastic size (µm)	Concentration	Plastic type	Beads, fragments, fibres
Li *et al.* 2016	China	Mytiloida	Mussel	*Mytilus edulis*	F	5-250	2.7 (0.9-4.6) n/g ww	-	Fibre
					A		1.6 (0.9-4.6) n/g ww	-	
Li *et al.* 2018	UK	Mytiloida	Mussel	*Mytilus edulis*	M	5-250	0.9 n/g ww	-	Fibre
					F		0.7-2.9 n/g ww	-	
Lo and Chan 2018	N/A	Littorinimorpha	Sea snail	*Crepidula onyx*	L	2-5	10, 6 X 10^4,1.4 X 10^5 particles mL^{-1}	Polystyrene	Beads
Mathalon and Hill. 2014	Canada	Mytiloida	Mussel	*Mytilus edulis*	M	-	7.42 n/g ww	-	Fibre
					F	-	2.79-3.00 n/g ww	-	
Messinetti *et al.* 2017	N/A	Camarodonta	Sea urchin	*Paracentrotus lividus*	L	10	0.125, 1.25, 12.5 $\mu g.mL^{-1}$	Polystyrene	Beads
Moore *et al.* 2001	North Pacific Central Gyre	Salpida	Salp	*Thetys vagina*	F	0.355- > 4.760 (mm)	2.23 particles m^{-1}	Polypropylene	Fragments
Phuong *et al.* 2018	France	Ostreioda	Oyster	*Crassostrea gigas*	F	50-100	0.23 ± 0.20 n/g ww	-	Fragment
		Mytiloida	Mussel	*Mytilus edulis*			0.18± 0.16 n/g ww	-	
Qu *et al.* 2018	China	Mytiloida	Mussel	*Mytilus edulis*	F	250-1000	1.52-5.36 n/g ww	-	Fibre
		Mytiloida	Mussel	*Perna viridis*				-	
Renzi *et al.* 2018	Italy	Mytiloida	Mussel	*Mytilus galloprovincialis*	M	1700-1900	8.33 ± 3.58 (4.4 - 11.4) n/g ww		Fibre
					F	1890	7.2 n/g ww	-	
Steer *et al.* 2017	English Channel	Perciformes	Fish	*Callionymus lyra*	F	100- >5000	0.26-3.79m^{-3}	Nylon, rayon, polyethylene and acrylic	Fibres and fragments
		Anguilliformes	Eel	*Anguilla anguilla*					
		Gadiformes	Fish	*Trisopterus minutus*					
		Pleuronectifo-rmes	Fish	*Microchirus variegatus*					
		Gadiformes	Fish	*Merlangius merlangus*					

Author	Location	Taxonomic order	Species		Laboratory/ Field/ Market/ Aquaculture Farm	Microplastic size (µm)	Concentration	Plastic type	Beads, fragments, fibres
Thushari *et al.* 2017	Thailand	Ostreioda	Oyster	*Saccostrea forskalii*	F		0.57 ± 0.22 (0.2-0.6) n/g ww	-	Fibre
Van Cauwenberghe and Janssen 2014	France	Ostreioda	Oyster	*Crassostrea gigas*	M	16-20	0.47 ± 0.16 n/g ww	-	-
	Germany	Mytiloida	Mussel	*Mytilus edulis*	A	5-10	0.36 ± 0.37 n/g ww	-	-
Van Cauwenberghe *et al.* 2015	Belgium	Mytiloida	Mussel	*Mytilus edulis*	F	20-90	0.2 ± 0.3 n/g ww	-	-
Vanderm-eersch *et al.* 2015	Netherla-nds	Mytiloida	Mussel	*Mytilus edulis*	A	-	0.32 ± 0.22 n/g ww	-	Fibre
	France	Mytiloida	Mussel	*Mytilus edulis*	M		0.06 ± 0.13 n/g ww		Fibre
	Italy	Mytiloida	Mussel	*Mytilus galloprovincialis*	F		0.05 ± 0.11 n/g ww		Fragment
					F		0.16 ± 0.11 n/g ww		
					A		0.25 ± 0.26 n/g ww		
	Portugal	Mytiloida	Mussel		F		0.34 ± 0.33 n/g ww		Fibre
							0.08 ± 0.09 n/g ww		Fragment
	Spain	Mytiloida	Mussel		F		0.15 ± 0.33 n/g ww		Fibre
					M		0.04 ± 0.09n/g ww		
Vroom *et al.* 2017	N/A	Calanoidia	Copepod	*Acartia longiremis*	L	15 & 30	50-200 beads/fragments mL^{-1}	Polystyrene	Beads
		Calanoidia	Copepod	*Calanus finmarchicus*					Beads and fragments
Wilson 1973	N/A	Calanoidia	Copepod	*Acartia tonsa*	L	7-70	3000-4000 beads mL^{-1}	-	Beads

*N/A: not applicable.

The physiological effects on barnacles are less well documented. Bhargava *et al.* (2018) found that barnacle nauplii ingest plastics even at low concentrations, regardless of whether exposure was chronic or acute. The microplastics bioaccumulated as nauplii proceeded through successive larval phases (Bhargava *et al.* 2018). In a study on *Lepas* spp. in the North Pacific Subtropical Gyre, Goldstein and Goodwin (2013) found that larger individuals contained more microplastics than smaller individuals, with a significant relationship between the number of ingested microplastics and capitulum length (Goldstein and Goodwin 2013). The study of harmful effects are limited to studies like those of Thushari *et al.* (2017), who compared concentrations of harmful chemicals in the tissues of the rock oyster, *Saccostrea forskalii* (Gmelin, 1791), the striped barnacle, *Balanus amphitrite* (Darwin, 1854), and the periwinkle, *Littoraria* sp., that had ingested chemical-laden microplastics. They recorded higher levels of chemical contaminants in filter feeders that had ingested chemical laden microplastics when compared to filter feeders which had not ingested microplastics. As Bakir *et al.* (2014a, b) and Morgana *et al.* (2018) found in water and sediment samples, polyethylene was the most commonly occurring plastic type in gooseneck barnacles (*Lepas* spp.) (Goldstein and Goodwin 2013).

This study assessed the spatial and temporal patterns in microplastic ingestion by four commonly found filter feeders along the south-eastern coastline of South Africa, *Mytilus galloprovincialis* and *Perna perna* mussels, and *Tetraclita serrata* and *Octomeris angulosa* barnacles. These species were selected for the fundamental reason that, though both mussels and barnacles are filter feeders, their feeding modes differ, allowing for comparisons. Mussels are passive feeders, feeding on organic material that adhere to the mucus-lined gills (Gosling 2003), whereas barnacles can alternate between active and passive filter feeding (Riisgard 2015). When actively feeding, barnacles extend cirri from between their shell plates which comb the water for food (Branch *et al.* 2010). Feeding type affects the number of microplastics that are ingested (Setälа *et al.* 2016). Setala *et al.* (2016) found that *Mytilus trossulus* (Gould, 1850) mussels and *Macoma balthica* (Linnaeus, 1758) clams, both

bivalves, contained significantly higher numbers of microbeads when compared to amphipods (*Monoporeia affinis* (Lundström, 1855) and *Gammarus* species) and polychaetes (*Marenzelleria* spp.). Furthermore, Thushari *et al.* (2017) found that the filter feeders *Saccostrea forskalii* and *Balanus amphrite* ingested a higher percentage of microplastics when compared to the periwinkle (*Littoraria* spp.), a benthic grazer. The authors also found that ingested particles consisted only of microfibres. Though these filter feeders ingested only fibres, Woods *et al.* (2018) found 71 % of available microfibres in the pseudofaeces of the blue mussel, *Mytilus edulis*. Bivalves and other filter feeders selectively separate nutritive and non-nutritive particles, rejecting the latter in pseudofaeces. Woods *et al.* (2018) also found that the microfibres found in the pseudofaeces were significantly longer than those found in the digestive gland and the gills, which may be as a result of *Mytilus edulis* not being able to discern between shorter fibres, other plastic particles, and nutritive particles.

Few studies, if any, have examined whether there are temporal trends in the amounts of microplastics ingested by marine organisms. Woods *et al.* (2018) note that acclimation periods to differing concentrations of microplastic fragments should be allowed for based on evidence that other filter feeders (e.g., *Calanus pacificus*) display seasonal acclimation to seasonal changes in the population levels of prey items (Runge 1980). This study aimed to determine whether there are significant spatial and temporal, particularly seasonal, differences in the number of microplastics ingested by selected filter feeders at two sites along the southern and south-eastern coastlines of South Africa. Objectives include examining ingested plastic loads for any seasonal trends or patterns. It is hypothesised that there will be no significant difference in the microplastic loads ingested by the four species of filter feeders between the two sampling sites, and so no spatial trends or patterns will be observed in the amounts of ingested microplastics. Studies have found no significant spatial differences in microplastic loads in the water column along the south-eastern coastline of South Africa (Nel and Froneman 2015), which would indicate that individuals are being exposed to the same concentrations of microplastic in the water column regardless of location. It is hypothesised that there will be no

effect of site or season on the number of microplastics ingested by *T. serrata*, *O. angulosa*, *M. galloprovincialis*, and *P. perna*.

4.2 Materials and methods

4.2.1 Sampling area and site description

The sampling area is described in Chapter 2.

4.2.2 Sample collection

4.2.2.1 Organisms investigated

The four species of filter feeders investigated are *Tetraclita serrata, Octomeris angulosa, Mytilus galloprovincialis*, and *Perna perna*. *Tetraclita serrata*, commonly known as the grey volcano barnacle, is found in the intertidal zone from KwaZulu-Natal to Namibia. It inhabits the same intertidal zone as *O. angulosa*, the eight-shell barnacle, and is the dominant species between the two in sheltered areas, but is replaced by *O. angulosa* in areas with high wave exposure (Branch *et al.* 2010). *Mytilus galloprovincialis*, the Mediterranean mussel, is a non-native species introduced from Europe and partly overlaps in distribution with *P. perna*. Currently its distribution runs from the coastline of the Eastern Province of South Africa up to and along the Namibian coastline (Branch *et al.* 2010). The brown mussel, *P. perna*, is endemic to southern Africa, found on the west coast along Namibia and along the east and south coasts from Tanzania past the tip of South Africa, from the intertidal zone to a few meters in depth (Branch *et al.* 2010). Both mussel species form dense beds, overlapping at the intertidal zone.

4.2.2.2 Field sampling

A total of 10 organisms were sampled per species from both the Kariega Beach at Kenton-on-Sea, Eastern Cape, South Africa (33°41'00.43''S, 26°40'57.36''E) (Figure 2.2) and the Wilderness Beach, Wilderness, Western Cape, South Africa (33°59'48.20''S, 22°35'01.06''E) (Figure 2.3) at low tide in July 2017 (winter samples) and January 2018 (summer samples). Barnacles sampled ranged in size from 11 to 26 mm, and mussels between 31 and 65 mm. The animals collected were whole and intact and were transported to the laboratory immediately for further processing. All equipment employed in sample collection was rinsed using deionised water prior to use. Water samples were taken at each site at each sampling event to establish average expected microplastic loads for each location and processed in the same manner as described in Chapter 3.

4.2.3 Laboratory processing

Given concerns raised by reviewers in previous studies, animals collected were placed on ice and transported back to the laboratory where they were immediately processed. Animals were sacrificed by freezing them at -20°C for 20 hours. Once thawed, the shells of the animals were removed and discarded, and a section of the soft tissue was taken. These were then weighed (g) on a Satorius microbalance and then dissolved in 20 ml nitric acid (3M) over 24 hours in a sealed fume hood. Thereafter, the solution was heated to a constant temperature of 100°C in a water bath for two hours and then diluted with deionised water to obtain a nine-fold dilution. The solution was left to cool at room temperature in a covered glass beaker and then gently filtered through a 5 µm (47 mm diameter) glass cellulose filter using a vacuum pump (<5 Hg). The cellulose filters were then examined under a Nikon binocular microscope at 40 X magnification and the number of microplastic particles counted and recorded. The size of the particles was then measured using a calibrated eyepiece micrometer. Control samples comprising distilled water passed through the filters as described above were run to

account for any potential background contamination. A second counter randomly selected samples for recounting to eliminate counter bias. Results were expressed as the number of microplastics found per gram of wet weight tissue (microplastics.g^{-1} wwt).

4.3 Statistical analysis

Statistical analyses were conducted using *R* (R Core Team 2018) and XLSTAT (2019). Visual representation of analysed data was done through Microsoft Excel 2013. Assumptions of normality and homogeneity of variances were tested using the Shapiro-Wilk test and Levene's test respectively. Both assumptions were satisfied for each site-season data set. A two-way ANOVA was used to test whether site and season significantly impacted on the number of ingested microplastics found in each of the four species. Size of plastic particles could not be included as a co-variate since data sets were uneven. A *t*-test was performed on water samples collected at each site to determine whether or not there was a significant difference in the number of microplastics in the water column between the two sites or between the two seasons in order to establish the microplastic concentrations the consumers were exposed to at each site.

4.4 Results

Microplastic loads in the water column were tested between seasons and between sites using a Student's *t*-test (Figure 4.1). Results did not reveal any significant differences between sites: microplastic loads in the water column collected during the winter ranged from 189 to 365 ± 90 microplastic particles.m^{-3} at Kariega Beach, and from 254 to 318 ± 32 microplastic particles.m^{-3} at Wilderness Beach (p = 0.75). During the summer the values ranged from 282 to 420 ± 140 microplastic particles.m^{-3} at Kariega Beach, and from 264 to 406 ± 71 microplastic particles.m^{-3} at

Wilderness Beach (p = 0.35). Nor were there any significant differences found between seasons at Kariega Beach (p = 0.36) or at Wilderness Beach (p = 0.70)

The number of ingested microplastics collected during winter and summer fell into the same range across all species (Figures 4.2 and 4.3). The number of ingested microplastics found in *T. serrata* (n = 10), ranged from 0 to 12.5 microplastics.g^{-1} wwt at Kariega Beach (mean = 6 ± 3 microplastics.g^{-1} wwt), and from 0 to 21 microplastics.g^{-1} wwt at Wilderness Beach (mean = 6 ± 7 microplastics.g^{-1} wwt). In *O. angulosa* (n = 10) ingested microplastics ranged from 0 to 10 microplastics.g^{-1} wwt at Kariega Beach (mean = 4 ± 4 microplastics.g^{-1} wwt), and from 0 to 10 microplastics.g^{-1} wwt of muscle tissue at Wilderness Beach (mean = 4 ± 3 microplastics.g^{-1} wwt). Ingested microplastic loads in *M. galloprovincialis* (n = 10) ranged from 1 to 6 microplastics.g^{-1} wwt at Kariega Beach (mean = 3 ± 1 microplastics.g^{-1} wwt), and from 1 to 5 microplastics.g^{-1} wwt at Wilderness Beach (mean = 2 ± 1 microplastics.g^{-1} wwt). Lastly, ingested microplastic loads in *P. perna* (n = 10) ranged from 0 to 5.08 microplastics.g^{-1} wwt at Kariega Beach (mean = 2 ± 1 microplastics.g^{-1} wwt), and from 1 to 6 microplastics.g^{-1} wwt (mean = 3 ± 1 microplastics.g^{-1} wwt) at Wilderness Beach.

During summer, the number of ingested microplastics found in *T. serrata* (n = 10) ranged from 1 to 25 microplastics.g^{-1} wwt at Kariega Beach (mean = 8 ± 7), and from 0 to 19 microplastics.g^{-1} wwt at Wilderness Beach (mean = 8 ± 5 microplastics.g^{-1} wwt). Ingested microplastic loads in *O. angulosa* (n = 10) ranged from from 0 to 14 microplastics.g^{-1} wwt at Kariega Beach (mean = 8 ± 6 microplastics.g^{-1} wwt), and from 0 to 13 microplastics.g^{-1} wwt at Wilderness Beach (mean = 7 ± 5 microplastics.g^{-1} wwt). In *M. galloprovincialis* (n = 10) the number of ingested microplastic particles ranged from 0 to 20 microplastics.g^{-1} wwt at Kariega Beach (mean = 4 ± 6 microplastics.g^{-1} wwt), and from 0 to 7 microplastics.g^{-1} wwt at Wilderness Beach (mean = 2 ± 2 microplastics.g^{-1} wwt). Finally, ingested microplastics loads in *P. perna* (n = 10) ranged from 0 to 6 microplastics.g^{-1}

wwt, (mean = 2 ± 1 microplastics.g^{-1} wwt) at Kariega Beach, and from 0 to 6 microplastics.g^{-1} wwt, (mean = 3 ± 2 microplastics.g^{-1} wwt) at Wilderness Beach.

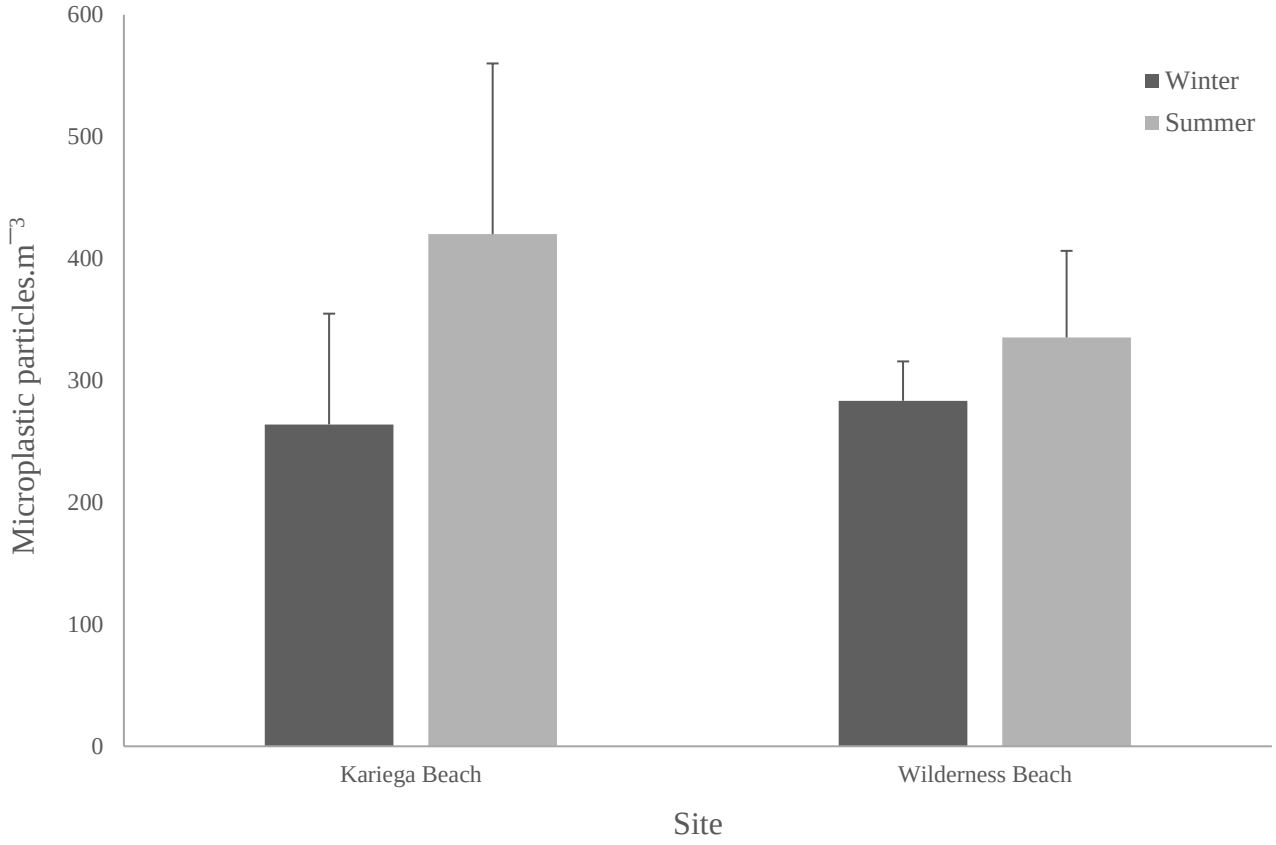

Figure 4.1: Mean microplastic (± standard deviation) counts in the water column (n = 3) taken from the nearshore environment at Kariega Beach, Kenton-on-Sea , Eastern Cape, and Wilderness Beach, Wilderness, Western Cape, South Africa during the austral winter (July 2017) and summer (January 2018).

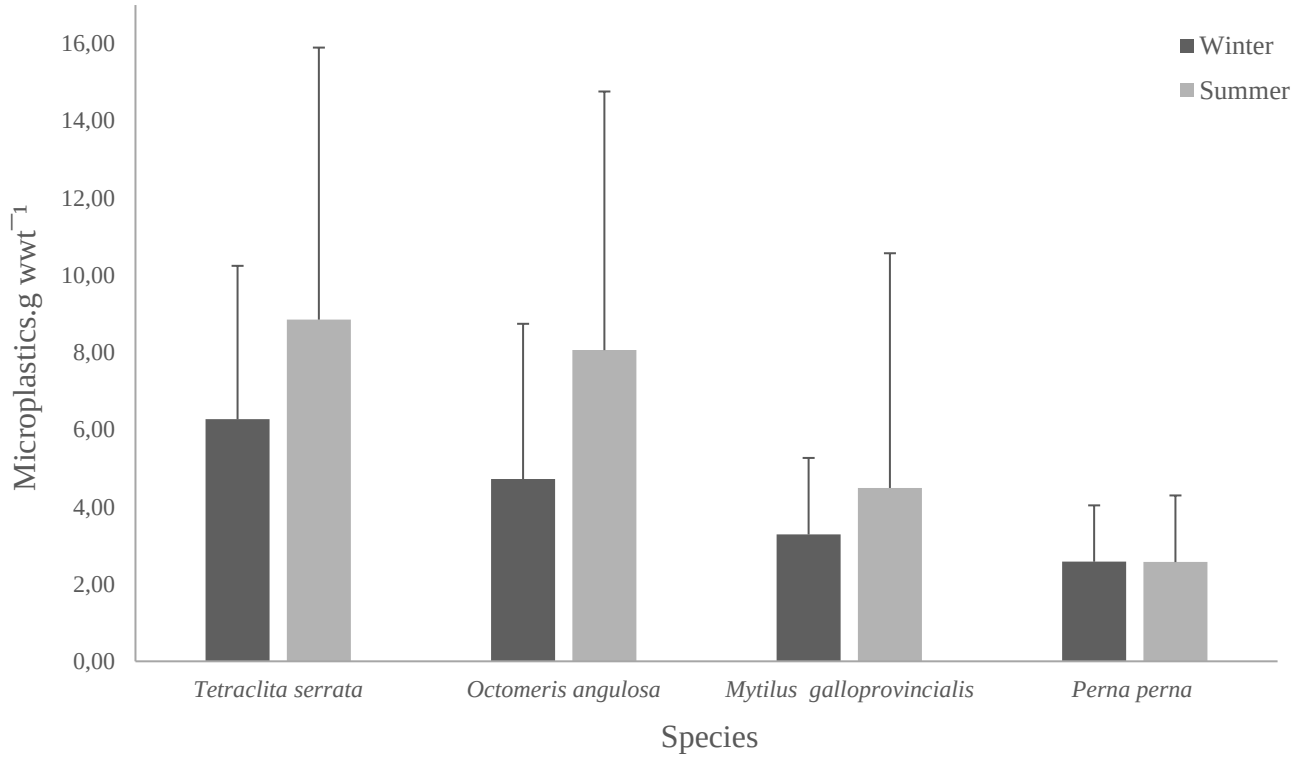

Figure 4.2: A seasonal comparison of mean ingested microplastic loads (± standard deviation) in *Tetraclita serrata*, *Octomeris angulosa*, *Mytilus galloprovincialis* and *Perna perna* (n = 10) at Kariega Beach, Kenton-on-Sea, Eastern Cape, South Africa.

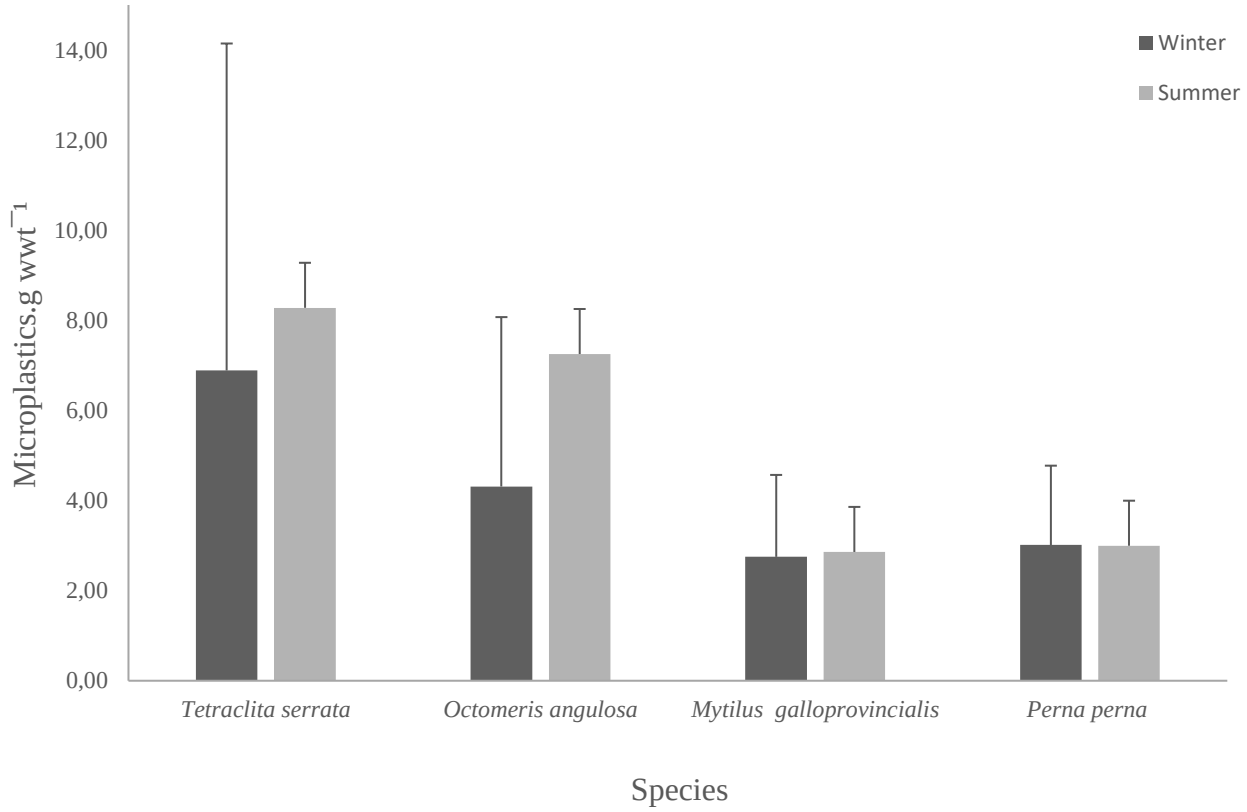

Figure 4.3: A seasonal comparison of mean ingested microplastic loads (± standard deviation) in *Tetraclita serrata*, *Octomeris angulosa*, *Mytilus galloprovincialis*, and *Perna perna* (n = 10) at Wilderness Beach, Western Cape, South Africa.

A two-way ANOVA examined the effect of site (Kariega Beach vs Wilderness Beach) and season (winter vs summer) on the mean number of plastic particles ingested in each of four species of filter feeders. For *T. serrata*, the main effect of season on the number of microplastics ingested was not significant (ANOVA; $F(1,36) = 1.02$, $p = 0.32$), and nor was the effect of site (ANOVA; $F(1,36) = 0.0001$, $p = 0.99$). These main effects were qualified by a non-significant interaction between season and site (ANOVA; $F(1,36) = 0.09$, $p = 0.76$). In mean ingested microplastic loads obtained from *O. angulosa*, neither season (ANOVA; $F(1,36)=3.91$, $p = 0.06$) nor site (ANOVA; $F(1,36) = 0.15$, $p = 0.70$) were found to have a significant effect, and neither was the interaction between the effects (ANOVA; $F(1,36) = 0.02$, $p = 0.90$). The same was seen in *M. galloprovincialis* with a lack significance of the effects of season (ANOVA; $F(1,36) = 0.31$, $p = 0.58$) and site (ANOVA; $F(1,36) = 1.17$, $p = 0.31$) on the number of microplastic particles ingested. These main effects are supported

by a non-significant interaction between season and site (ANOVA; $F(1,36) = 0.21$, $p = 0.65$). Lastly, the findings of the analyses on *P. perna* were no different. Neither the effect of season (ANOVA; $F(1,36) = 0.0004$, $p = 0.98$) or site, ANOVA; ($F(1,36) = 0.53$, $p = 0.47$) or the interaction between the main effects (ANOVA; $F(1,36) = 5.29 \times 10^{-5}$, $p = 0.99$) on the number of microplastics ingested were found to be significant.

Ingested particle sizes ranged from 1 to 16 µm in *T. serrata*, 1 to 15 µm in *O. angulosa* and *M. galloprovincialis*, and 1 to 17 µm in *P. perna*. It is clear that particle sizes overlap, but this data could unfortunately not be statistically analysed due to unequal sample sizes (Figures 4.4 and 4.5).

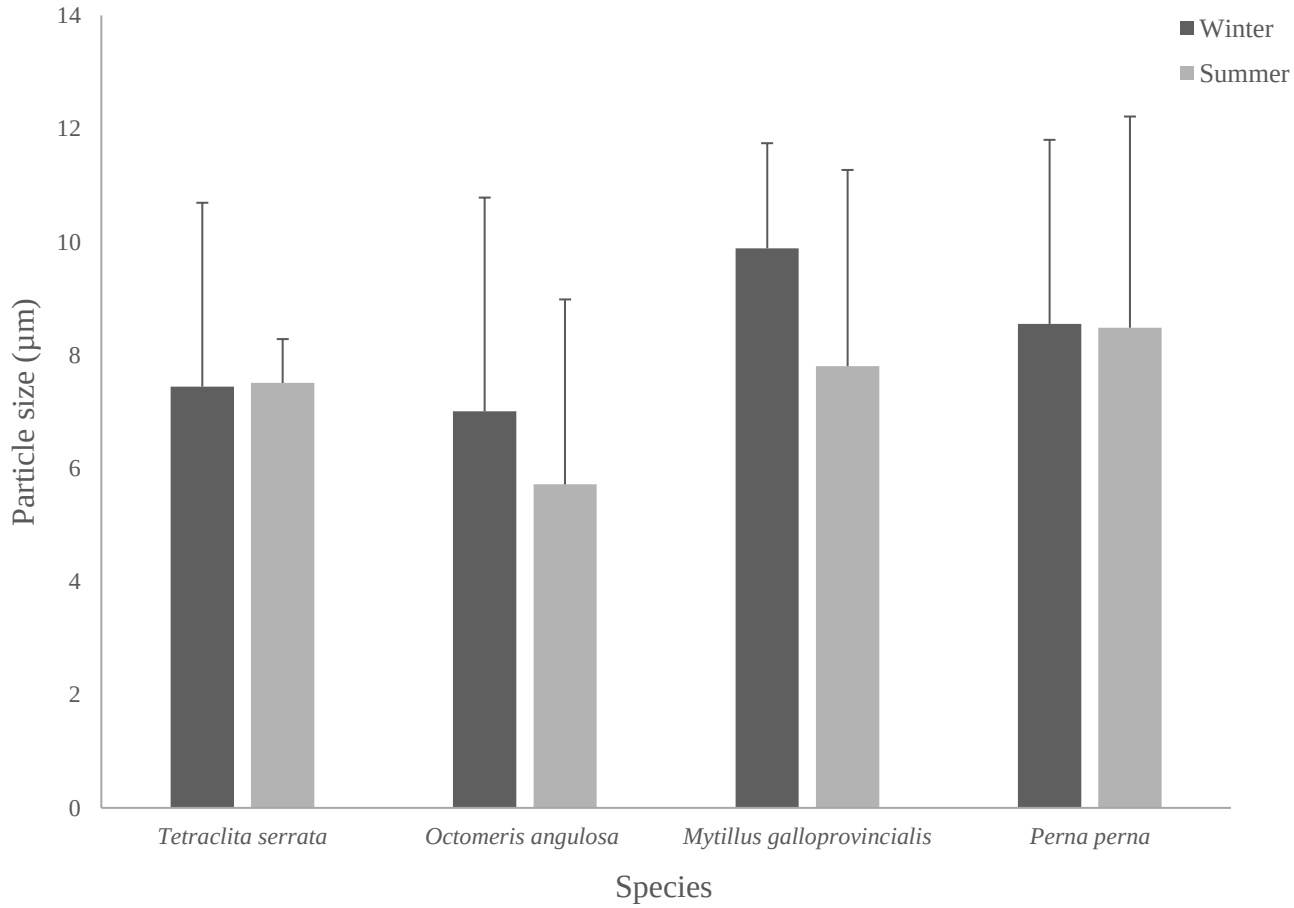

Figure 4.4: Mean (± standard deviation) particle sizes (µ*m*) of microplastics ingested by *Tetraclita serrata, Octomeris angulosa, Mytilus galloprovincialis* and *Perna perna* during the austral winter (July 2017) and summer (January 2018) at Kariega Beach, Kenton-on-Sea, Eastern Cape, South Africa.

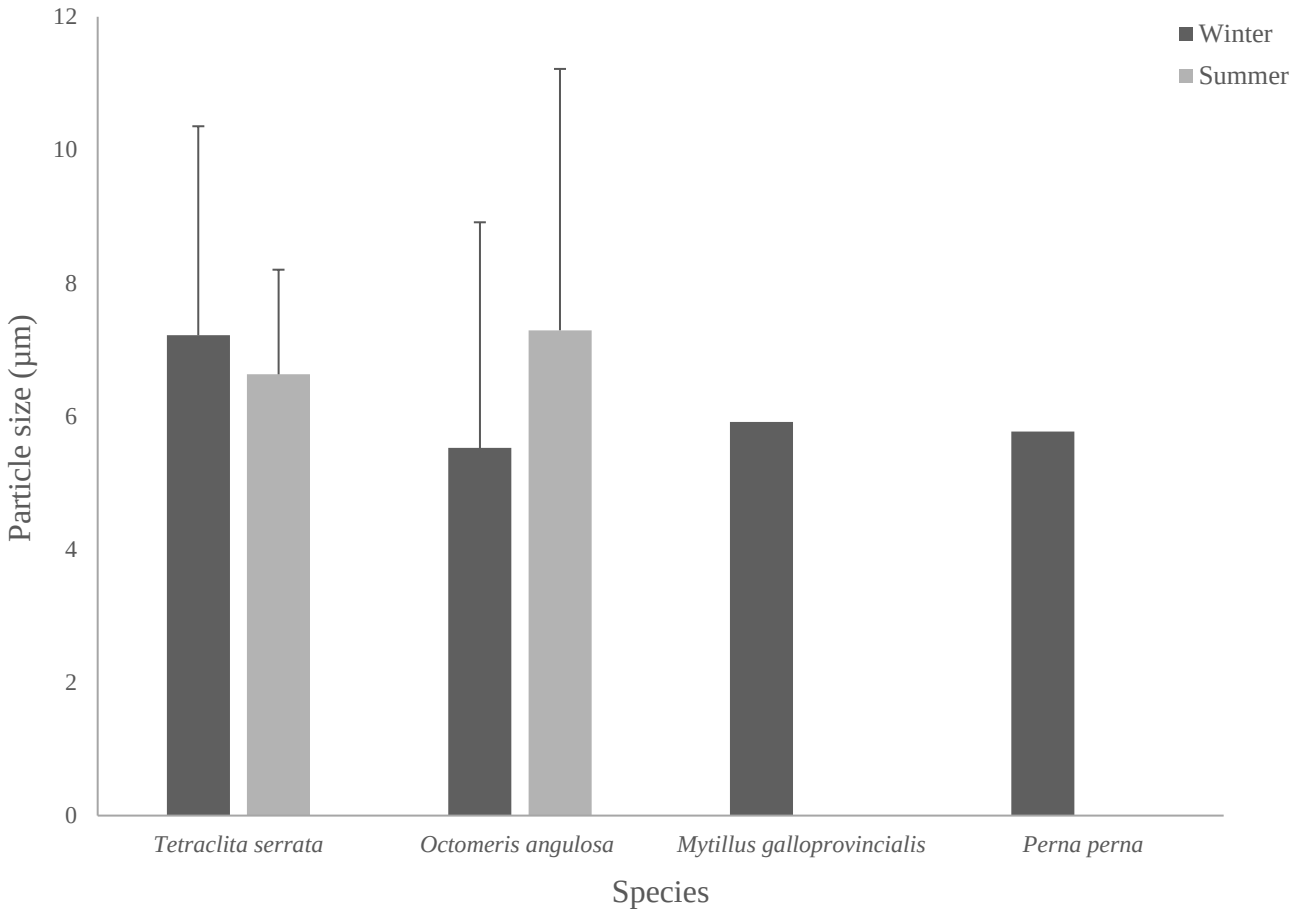

Figure 4.5: Mean (± standard deviation) particle sizes (µm) of microplastics ingested by *Tetraclita serrata, Octomeris angulosa, Mytilus galloprovincialis* and *Perna perna* during the austral winter (July 2017) and summer (January 2018) at Wilderness Beach, Wilderness, Western Cape, South Africa.

4.5 Discussion

The results of the study indicated the absence of any seasonal or spatial patterns in the number of microplastic particles ingested by the study species. The lack of spatial patterns can be explained when considering that previous studies have found no significant spatial differences in the water column or sediment along the south-eastern coastline of South African (Nel and Froneman 2015, Nel *et al.* 2017). The consumers therefore, would be exposed to similar concentrations of microplastic particles in the water column regardless of site or season.

Water column samples were collected at each site in both seasons and did not reveal any temporal trends or patterns in the nearshore environment sampled. In agreement with the findings presented in Chapter 3 and those of Nel *et al.* (2017), there were no apparent spatial (site) or seasonal patterns in microplastic counts in the water column which may explain the absence of seasonal trends in ingested microplastics by the selected filter feeders considered during this investigation. However, since microplastics are retained in the bodies of consumers for a period of time, retention time may be masking any temporal patterns present. Watts *et al.* (2014) found that shore crabs (*Carcinus maenas* (Linnaeus, 1758)) retain microplastics in their body tissues for up to 21 days. Similarly, Wegner *et al.* (2012) found translocated polystyrene particles in the haemolymph of blue mussels (*Mytilus edulis*) up to 48 days after exposure. Finally, Von Moos *et al.* (2012) observed retention time of microplastics in the gut of the blue mussel in a laboratory experiment at up to 96 hours post-exposure. Though retention times are not available for the study species, they will inevitably vary between species and tissue type sampled. Such findings demonstrate that the masking effects of retention time on any temporal trends present in ingested microplastics in each species cannot be dismissed.

The results obtained from this study demonstrated that all four species of filter feeder routinely ingested microplastic particles at the two discrete sites along the south-eastern Cape coastline of South Africa. This result is in agreement with a number of studies conducted elsewhere, both in the northern and southern hemisphere. *Mytilus galloprovincialis* has been found to ingest microplastics along the Norwegian coast (Brate *et al.* 2018), on the Scottish coast (Catarino *et al.* 2018) and in laboratory-based experiments (Capopulo *et al.* 2018). Similarly, *Perna perna* also ingested microplastics on the Brazilian coast (Santana *et al.* 2016) and in laboratory-based experiments (Silva *et al.* 2016). Other documented mussel species which have ingested microplastics include *Mytilus edulis* in Norway (Brate *et al.* 2018), the United Kingdom and Scotland (Catarino *et al.* 2018, Li *et al.* 2018), South Korea (Cho *et al.* 2019), China (Li *et al.* 2016, Qu *et al.* 2018) and in laboratory-based experiments (Woods *et al.* 2018), *Mytilus trossulus* in Scotland (Catarino *et al.* 2018) and

Norway (Brate *et al.* 2018), and *Perna viridis* (Linnaeus, 1758) in China (Qu *et al.* 2018). Microplastic ingestion in barnacles has been less well-studied, but examples include laboratory-based studies on *Lepas anatifera* (Linnaeus, 1758), *Lepas pacifica* (Henry, 1940), and *Megabalanus azoricus* (Pilsbry, 1916) (Goldstein 2012, Goldstein and Goodwin 2013, Hentschel 2015). Other consumers include oysters (Cole and Galloway 2015, Cho *et al.* 2019), tunicates (Fernandez *et al.* 2004), salps (Moore *et al.* 2001), and sea urchins (Messinetti *et al.* 2017) (Table 1).

The selected filter feeders considered during the present study ingested microplastic particles in the same size range (1 to 17 µm), a finding which is consistent with the recently published literature (see, for example, Table 1). The overlap in the size of microplastics ingested is not unexpected when considering the size of particles normally captured on the cilia of the mussels and the cirri of the barnacles. Gosling (2003) and Brate *et al.* (2018) found that mussels (various spp.) are able to ingest particles of maximum size 1 mm and barnacles 6.77 mm (Goldstein and Goodwin 2013), a finding somewhat larger than that of Southward (1955) who found that barnacles typically consume particles in the size range 2 to 1000 µm. It is worth noting that, given that the dissolved tissue samples were passed through a 5 µm filter, it is possible that the microplastic ingestion counts reported for both species could be higher than that reported here. This would also result in the underestimation of the average size of ingested microplastics, since larger particles would be retained in the filter. Finally, it is also possible that the extraction method employed during the present study would also have contributed to the underestimation of microfibres ingested by the selected filter feeders considered during the present investigation.

In conclusion, this research has shown that microplastics are regularly ingested by filter feeders in the near-shore environment along the south-east coast of South Africa, and that ingested microplastic loads follow no seasonal patterns. It also showed that microplastic concentration in the water column does not vary seasonally either, supporting the findings of Chapter 3. Many studies have examined

the harmful physiological effects that microplastic ingestion has on species of all trophic levels (e.g. Paul-Pont *et al.* 2016, Martinez-Gomez *et al.* 2017, Rodriguez-Seijo *et al.* 2017), creating concern by illustrating that marine species are constantly being exposed to microplastics. Eventually these relatively small harmful effects will start to impact on the ecological health of marine systems, repeatedly impacting on populations by impacting on an individual level. Such impacts on individual and population levels should be further assessed.

Chapter 5

Conclusions and remarks

5.1 Main findings

The study of microplastic pollution in the marine environment is gaining momentum as the urgency to find methods of mitigation and regulation of production increases. While microplastic pollution in the water column is well-understood on a spatial scale, this study (Chapter 3) is one of few to assess microplastic pollution on a temporal scale, specifically over four short-term temporal scales in the nearshore marine environment: two-hourly, daily, weekly, and monthly. The lack of significant differences across the four timescales ($p > 0.05$ in all cases), leads to the conclusion that microplastics in the nearshore environment of southern Africa are ubiquitous in time as well as space. The estimates of microplastic loads and composition (microfibres/microfragments) reported during the present study are in the range reported in the published literature both locally (Nel and Froneman 2015, Nel *et al.* 2017) and internationally (Heo *et al.* 2013, Desforges *et al.* 2014, Zhao *et al.* 2014, Naji *et al.* 2016, Gray *et al.* 2018). Since microplastics have been shown to be generally harmful when ingested by both vertebrates and invertebrates, their pervasiveness is cause for concern. It can now be assumed that individuals of many populations are being adversely affected by their presence, which may ultimately impact on ecosystem health and functioning. This is due to the fact that microplastic concentrations in the water column are at levels such that they are detectable at any time, at any place, emphasizing the magnitude of the threat microplastic pollution poses to ecosystem health and functioning. It is clear the impact of microplastic pollution on the marine ecosystem needs to be determined in order to develop the most effective solutions and management stratagems.

This study further assessed temporal and spatial patterns in microplastic loads ingested by four common filter feeders, *M. galloprovincialis* and *P. perna* mussels, and *T. serrata* and *O. angulosa*

barnacles, which dominate the rocky shore communities along the south-eastern coastline of South Africa numerically and by biomass. Analysis of ingested microplastic loads in these species sampled during winter (July 2017) and summer (January 2018) indicated that the selected filter feeders considered during the study routinely ingested microplastic at both sites with the absence of any seasonal patterns, adding weight to the earlier findings. It is hypothesised that passive filter feeders such as mussels experience adverse effects from microplastic ingestion more acutely than active feeders such as barnacles, as a result of a higher chance of encounter between the passive feeders and microplastic particles and therefore, a higher chance of ingestion (Di Beneditto and Awabdi 2014). Data from the present study did not identify any significant differences in the ingestion of microplastics between mussels and barnacles across season or site (ANOVA; $p > 0.05$). The role of feeding mode in determining the vulnerability of filter feeders to microplastic contamination therefore, requires further study.

Additionally, in contrast to the study described in Chapter 3 which found that microfibres contributed a larger proportion to microplastic particle counts than microfragments, this study found an absence of microfibres in the dissolved body tissues of the consumers. Studies sampling the water column have consistently found a higher proportion of microfibres in microplastic counts (Nel and Froneman 2015, Nel *et al.* 2017). The discrepancy may point to the ability of these organisms to identify and reject a microfibre as non-nutritive due to its irregular shape when compared to microfragments, but it is more likely as a result of the nitric acid solvent used to dissolve the soft tissues of the consumers destroying the microfibres. This baseline information paves the way for studies examining adverse impacts on a population level by further studying the deleterious physiological effects of microplastic ingestion on individuals, since, based on these results, organisms can be sampled at any time, at any location and the number of ingested microplastics will be representative of the norm. It is, however, recommended that retention times

be determined for a species under study to conclude if temporal trends are being masked by retention of microplastics in the body of the consumer.

The prevalence of microplastics in the tissues of consumers can largely be attributed to the fact that the microplastic particles are in the same size class of the food particles generally consumed by marine filter feeders (Table 1). The impact of the consumption of microplastics on the fitness of invertebrates is now well studied for a variety of species and includes reduced respiration and growth rates with a subsequent decline in their fecundity (Johnson *et al.* 2011).

5.2 Shortcomings and recommendations

A primary limitation with this and other studies on microplastics in the marine environment, is the wide variety of methodologies used in assessing the scale of the microplastic pollution threat. Results from the various studies are therefore, not directly comparable, hampering the assessment of microplastic loads in the marine environment on a regional scale. As GESAMP (2015) have suggested, a standardised methodology for global use should be developed to allow for direct comparisons in microplastic loads regardless of location.

Additionally, to develop management strategies towards mitigating the microplastic pollution problem in the marine environment by reducing the number of particles introduced into the marine environment, the sources of the particles needs to be determined (GESAMP 2015, Nel *et al.* 2017). Analyses which allow for the identification and quantification of microplastics include Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance (FTIR-ATR) and Raman spectroscopy. FTIR-ATR allows for such identification through using an infrared light source to ‘map’ the surface of the microplastic particle. Raman spectroscopy relies on the scattering of monochromatic light, but achieves the same purpose. Identification of the type of plastic may then

allow identification of the source. Finally, it is suggested that future investigations should undertake/consider a more rigorous analyses of the size range of the ingested microplastics as this may be critically important in determining their uptake by filter feeders.

With respect to the ingestion of microplastics by filter feeding organisms, four limitations were noted. Firstly, all the microplastics ingested are assumed to have deleterious effects on the filter-feeding consumer. Microplastics in the alimentary canal may in fact not pose a threat to the health of the organism since these are likely excreted. However, since only a section of the soft tissue was dissolved, it is not possible to determine which of the microplastics did pose a threat (e.g. translocated microplastics). It is therefore, recommended that future studies should attempt to gain better insight into the eventual fate of ingested microplastic particles once in the body of the consumer.

Secondly, the nitric acid solvent used to dissolve body tissues most likely destroyed microfibres that may have been present, resulting in an underestimation of the total number of microplastics ingested (Desforges *et al.* 2015, Vandermeersch *et al.* 2015). Despite this, ingested microplastic counts fell in the same range as studies across the globe (Table 1), which may point to a general underestimation based on sampling methods, processing methods or sampling equipment.

Thirdly, considering the possible masking effects of retention time of microplastics in the bodies of consumers, retention time has to be taken into account. A laboratory-based study could have been conducted to determine species-specific retention times for the study species which would then be taken into account when assessing the ecological impact of microplastic ingestion by the particular filter feeders. Lastly, retention rates or percentages are lacking which could be determined through the method used by Van Cauwenberghe *et al.* (2015). These authors, however, determined the retention rate over an individual mussel's lifetime as opposed to the retention rate on a yearly or other short-term scale.

The type of tissue collected from each animal was not kept uniform which would have resulted in a misrepresentation of the true number of microplastics ingested. It would have been preferable for whole animals to have been weighed, dissolved and the microplastic particles then counted. Weighing whole animals would allow regression analysis between body size and number of particles ingested. Failing to collect whole animal samples, at least two tissue types should be collected, for example, muscle tissue and the gastrointestinal tract. Additionally, since it is suspected that the nitric acid solvent used in this experiment may have dissolved any microfibres present, a second experiment using a different solvent to dissolve body tissues could have been run alongside the existing experiment, as well as a control.

It is suggested therefore, that mussels of 40 to 60 mm and barnacles of 10 to 25 mm be sampled and that sample sizes ideally be n = 30 individuals to reduce the margin of error, and to avoid under-sampling a large population. Failing this, the experiment should be repeated three times to refine experimental observations. For laboratory procedure, it is recommended that two different solvents be used to dissolve whole animals, to reduce the risk of inadvertently destroying microfibres and thus under-representing the total number of microplastics ingested. Once dissolved, the number of microplastics in the solution should be counted and calculated as number of microplastics per gram wet weight (g wwt^{-1}) to allow for direct comparison. A statistical comparison of the number of ingested microplastics found using different solvents should be performed to assess the suitability of each solvent, followed by the tests described above to determine the effect site, season, and the interaction of site and season has on each of the species.

Chapter 6

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