UNIVERSITY OF KWAZULU–NATAL

MICROPLASTIC CONCENTRATIONS ON THE URBAN COASTLINE OF KWAZULU–NATAL, SOUTH AFRICA, AND ITS IMPACT ON JUVENILE FISH

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Microplastic concentrations on the urban coastline of KwaZulu–Natal, South Africa, and its impact on juvenile fish

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Submitted in fulfilment of the academic requirements for the degree of Philosophical Doctorate in the School of Life Sciences, University of KwaZulu–Natal, Durban

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As the candidate's supervisor I have/~~have not~~ approved this thesis/dissertation for submission.

Signed: _____ Name: __D Glassom__ Date: __22 June 2018__

ABSTRACT

The global production of plastics per annum has increased from 1.5 million tonnes in the 1950's to 300 million tonnes today. Following this increasing production trend, plastic concentrations have increased over time in marine environments. Improper sewage treatment, industrial spillages, garbage and fishing activities among many others, have made the marine environment a sink for plastic debris. The main aims of this study were to determine (1) microplastic levels within five estuaries along the Durban coastline and on intervening beaches, (2) the incidence of plastic ingestion by estuarine mullet, (3) the effects of plastic ingestion on long–term fish health and (4) the plastic concentrations along the KwaZulu–Natal coastal shelf. To achieve these aims (1) plastic was isolated from estuarine sediment, beach sediment and the surface water of each estuary, (2) fish from the most polluted estuary were dissected to investigate the incidence of plastic ingestion, (3) small juvenile fish were kept in tanks and fed plastics for three months to monitor their growth and survival and (4) coastal water samples were collected using a manta trawl net to quantify floating debris in the ocean. Overall, an attenuating plastic concentration trend away from the city centre was found, with the Durban Harbour, Isipingo and uMgeni Estuaries having the highest contamination levels. The highest recorded plastic levels were found in the Bayhead area of the harbour, with 745.4 ± 129.7 particles per 500 mL, which mostly consisted of plastic fragments. Fibres dominated other estuaries with proportions ranging from 38% of total plastics in the uMgeni Estuary to 66% in the Mdloti. Plastic particle concentration in estuarine sediment generally increased from larger to smaller size classes. High plastic concentrations were also found on the coastal shelf of KwaZulu–Natal, with sites south of the harbour having the highest plastic concentrations, however no seasonal differences were found. There is also evidence pointing toward long range movement of particles and thus pollution at the source must be dealt with before it reaches the open ocean. Seventy three percent of the mullet sampled at the harbour ingested plastic particles with an average of 3.751 ± 4.667 (S.D.) particles per fish. Particles that were ingested were mainly fibres that are thought to come from sewage inputs to the harbour. Juvenile fish in microplastic feeding experiments had lower growth and survival than control fish. This has possible economic and ecological consequences for future fish stocks that use urban estuaries as nursing areas.

PREFACE

The work described in this dissertation was carried out at the School of Life Science, University of KwaZulu–Natal, Durban, under the supervision of Dr. David Glassom and Prof. Albertus J. Smit. Research findings within this dissertation represent the original work by the author, and have not been submitted in part or in whole for any other degree or diploma to any other tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

I certify the above statement is correct:

Trishan Naidoo, June 2018

Dr. David Glassom, June 2018

DECLARATION 1 – PLAGIARISM

I, **Trishan Naidoo**, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Trishan Naidoo, June 2018

DECLARATION 2 – PUBLICATIONS

Details of contributions to publications that form part of and/or include research presented in this thesis:

Publication 1

Naidoo, T., Glassom, D., Smit, A.J. 2015. Plastic pollution in five urban estuaries of KwaZulu–Natal, South Africa. Marine Pollution Bulletin. 101: 473-480.

Publication 2

Naidoo, T., Smit, A.J., Glassom, D. 2016. Plastic ingestion by estuarine mullet *Mugil cephalus* (Mugilidae) in an urban harbour, KwaZulu–Natal, South Africa. African Journal of Marine Science. 38: (1) 145-149.

Publication 3

Naidoo, T., Goordiyal, K., Glassom, D. 2017. Are nitric acid (HNO$_3$) digestions efficient in isolating microplastics from juvenile fish? Water, Air and Soil Pollution. 228: (12) 1-11.

Author's contributions:

Trishan Naidoo – Undertook sampling activities and write–up of draft manuscripts.

David Glassom – Had original idea of the research and had a major impact on study design and editing the manuscript.

Albertus. J. Smit – Developed both ideas in study design and sampling techniques and was involved in editing the manuscript.

Kimerra Goordiyal – Examined the four species of juvenile fish for microplastics and added this field data to publication 3 for verification of the method (Chapter 6). She was also involved in the write–up of these results.

Signed: _____
 Trishan Naidoo, June 2018

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CHAPTER 1

Introduction

1.1 History of plastic production

At the beginning of the 1900's, the world entered the plastic age (Thompson et al., 2009a). Plastics were initially composed of natural materials including shellac, guttapercha, ebonite and a cellulose material celluloid that made the first photographic film (Brydson, 1966). In 1907, a commercially useful synthetic polymer called bakelite was developed (Brydson, 1966). Bakelite was a resin useful for its insulating properties and thus used in the manufacture of telephones and light switches (PlasticsEurope, 2013).

By the 1940's and 1950's, plastic production had become industrialised with global production approximately 1.5 million tonnes per annum (Barnes et al., 2009; Claessens et al., 2011). Manufacturers began using coal to produce resins, polystyrenes and nylons (Brydson, 1966). By the 1960's, petroleum had become the main raw material for production, which streamlined the manufacturing process (Brydson, 1966). It was at this stage that the presence of plastics and signals of their potential threat to the environment, such as ingestion by fish were becoming apparent (Harris, 1959; Carpenter et al., 1972; Carpenter and Smith, 1972). Today it is estimated that 8% of our oil production is used for plastic production (Thompson et al., 2009b).

In 2006, global production levels were around 245 million tonnes (PlasticsEurope, 2008), increasing to 270 million tonnes by 2010, (PlasticsEurope, 2012) and 280 million tonnes in 2011 (PlasticsEurope, 2013). Production levels currently exceed 300 million tonnes, a level that's considered unsustainable (Thompson et al., 2009a; PlasticsEurope, 2016). Rochman et al. (2013a) predicted that if current trends of production continue, the cumulative total mass of plastics produced by 2050 will be 33 billion tonnes.

1.2 Characteristics and uses

Plastics have been defined as "a wide range of materials that at some stage in manufacture are capable of flow such that they can be extruded, moulded, cast, spun or applied as a coating."(Thompson et al., 2009b). Characteristics that make them desirable to society include high durability, transparency, low

mass, high insulation and high resilience to biological break down (Wabnitz and Nichols, 2010). Manufacturing of plastic is cheap, while its low mass decreases transporting costs and resistance to biodegradation prolongs its use (Brydson, 1966). These characteristics make plastic polymers ideal for many uses in agriculture, the motor industry, clothing and other textiles, equipment in sports and science, transporting water, protecting perishable consumables, disposable utensils, furniture, and building material (Harris, 1959; Brydson, 1966; Malikane et al., 2000; PlasticsEurope, 2008; Browne et al., 2011). Several manufactured plastic products are used only a single time before being discarded, which are termed throw–away or disposable plastics (Rios et al., 2007).

1.3 General constituents, structure and characteristics

Plastics have a hydrocarbon backbone structure. Polyethylene (PE) is made from repeated $-CH_2-$ groups whereas poly vinyl chloride (PVC) has repeated $-CH_2-CHCl-$ units (Brydson, 1966). In the case of nylon, nitrogen is also present in the backbone structure (Morét-Ferguson et al., 2010). Repeated groups are bonded together to form polymers (Brydson, 1966). The structure of each polymer together with the added chemicals as side chains, known as additives, shape the characteristics of plastics to best suit their uses (Harris, 1959). Plasticisers and stabilisers are among these additives (Harris, 1959). Additives include fillers that strengthen the material e.g. silica, plasticisers such as phthalic acid to make plastics easier to mould and flame retardants and biocides to extend the life of plastic material (Gregory, 1978; Thompson et al., 2009a). In addition to chemicals, natural materials such as water, salt and lime are also used in production (Harris, 1959).

There are many types of polymerisation reactions that bond these constituents together but the main three known reactions are addition, condensation and rearrangement (Brydson, 1966). Thermoplastics are the most commercially important class of plastics, usually formed by addition polymerisation where double bonds in individual monomer units are broken and units are then allowed to re–bond together forming a polymer (Brydson, 1966). This type of polymerisation is used for PE, polystyrene (PS) and PVC production. Condensation polymerisation uses ester linkages and mostly produce thermosetting plastics such as phenolics, epoxy resins and various polyesters (Brydson, 1966). The difference between thermoplastics and thermosets is that thermoplastics can be heated and reshaped after use, whereas thermosetting plastics are harder to remould when heated for reuse (Lithner et al., 2011). Around 85 % of plastics that are produced are thermoplastics, which are easier to recycle (Xanthos, 2005).

1.4 Plastic types and sources

Primary sources of microplastics found in the environment originate from precursor material such as pre–production pellets or intentionally made products, such as facial scrubbers, that are termed primary microplastics (Arthur et al., 2009). Most plastics are moulded from pre–production pellets which are also known as nurdles or mermaids tears (Hammer et al., 2012). They are 3 – 5 mm in diameter, may be cylindrical, disk, ovoid or rod shaped and their typical colours are opaque, transparent, white and black (Gregory, 1978). Pellets are transported to manufacturers and remoulded into various products (Mato et al., 2001).

Scrubbers, also known as microbeads, are found in a multitude of products including airblast media used to strip paint from metals, rotomilling, powders, cleaning and cosmetic products such as body scrubs and toothpaste (Gregory, 1996; Fendall and Sewell, 2009; Thompson et al., 2009a). These are usually polyethylene polymers that are < 500 µm in diameter (Gregory, 1996). There are now policies in place to help reduce the use of microbeads in cosmetic products from product manufacturers by using alternatives such as polylactic acid (PLA) polymers, which are primarily made from plant sugars (Geldenhuys, 2014). Many countries, including the United Kingdom, are also now banning the use of microbeads in cosmetics and other disposable products (Xanthos and Walker, 2017).

Secondary sources of microplastics are formed from the fragmentation of larger discarded plastics (Arthur et al., 2009). The most abundant of these are polyester, acrylic or polypropylene fibres which are now prevalent in marine environments (Browne et al., 2011; Dubaish and Liebezeit, 2013; Zhao et al., 2014). They are usually 1 – 5 µm in diameter and around 500 µm in length (Frias et al., 2010). Sources are polyester clothing, polypropylene rope, air filters, diapers and fishing nets (Mohamed Nor and Obbard, 2014). Waste water treatment facilities can offer a pathway for fibres into coastal marine ecosystems they may not effectively remove all fibres after domestic use (Browne et al., 2011; Dubaish and Liebezeit, 2013).

Plastic films that end up in the environment can originate from wrappings, bags and sacks (Brien, 2007). This material is produced from low density polyethylene (LDPE) and is used extensively in agriculture (Ohtake et al., 1998; PlasticsEurope, 2008). Improper disposal of plastic film after use has made them a likely pollutant in many coastal marine ecosystems (Brien, 2007), including South Africa, due to a high market demand for packaging film material (Malikane et al., 2000). This is a cause for concern as estimates predict that a LDPE film of around 60 µm would take around 300 years to degrade (Ohtake et al., 1998).

The absence of effective waste collection an improper disposal is a major issue globally that needs to be addressed (Jambeck et al., 2015). Around 80% of marine litter is plastic and around 80% of plastics are estimated to come from terrestrial inputs (Andrady, 2011; Lebreton et al., 2012). Improper sewage treatment, industrial spillages and garbage all contribute to this input (Barnes et al., 2009; Hoellein et al., 2014; McCormick et al., 2014). Illegal dumping in both domestic and industrial settings is also known to be a large source (Sheavly and Register, 2007). Light plastics such as thin films discarded to waste disposal services, may subsequently enter coastal environments through aeolian transport (Barnes et al., 2009). Industrial plastics may also be lost during transit and the manufacturing process (Mato et al., 2001). Through runoff, these can enter river systems that pass through estuaries into the ocean (Bakir et al., 2014b).

Of the 20% of marine litter that is presumed to be from oceanic inputs, fishing activities are responsible for around 18% (Andrady, 2011). Oceanic plastic may also originate from shipping, military and research activities (Sheavly and Register, 2007). During the 1980's, floating debris from ships was observed contain many plastic items (Azzarello and Van Vleet, 1987). In 1990, the dumping of plastics at sea was therefore prohibited internationally by the Protocol to the International Convention for the Prevention of Pollution from Ships (MARPOL) (Barnes et al., 2009).

In urban settings, rivers and estuaries are major sources of plastic to the ocean (Bakir et al., 2014b; Wagner et al., 2014; Barboza and Gimenez, 2015). Moore et al. (2011) for example, estimated that 2.3 billion particles of plastic, mainly foams, fragments and pre–production pellets, weighing 30 tonnes flows out from both the Californian Los Angeles and the San Gabriel rivers combined, in 72 hours. Also showing the importance of estuarine inputs of plastic, Zhao et al. (2014) found that sediment samples from the Yangtze Estuary, flowing from a highly industrialised and populated area of China, had an average of 4137.3 ± 2461.5 plastic particles/m^3, which was much higher than nearshore seawater samples which contained only 0.167 ± 0.138 plastic particles/m^3. Plastics are also abundant in the Ganges, Mississippi, Nile rivers (Lebreton et al., 2012), Laurentian Great Lakes (Eriksen et al., 2013) and Singapore's coastal systems (Mohamed Nor and Obbard, 2014). In estuaries away from urban areas, plastic concentrations are much lower. For example, water samples that were taken from the Goiana Estuary in Brazil was found to contain 0.2604 plastic particles/m^3 and plastic types were mainly associated with fishing activities (Lima et al., 2014).

Plastics are abundant as beach strandline debris globally, including Norderney (Dekiff et al., 2014), the Bristol channel in the United Kingdom (Williams and Simmons, 1997), Califorinia (Moore et al., 2011),

New Zealand (Gregory, 1978), Brazil (Costa et al., 2010), the Adriatic Sea (Lazar and Gračan, 2011), the Belgian coast (Claessens et al., 2011), the lagoon of Venice, Italy (Vianello et al., 2013), Hawaiian Islands (Corcoran et al., 2009) and South Africa (Ryan and Moloney, 1990). Browne et al. (2011) found that 18 sandy beaches from around the world had microplastic fibres, and beaches in the Northern Pacific were most polluted. Pellets and fragmented particles may follow a similar trend, since the number of microplastics in beach sediments are positively correlated to the number of people living in the area (Costa et al., 2010; Browne et al., 2011). High plastic pollution levels also coincide with areas of high plastic production in industrialised city centres (Claessens et al., 2011). Sediment concentrations typically range from two fibres per 250 mL in Australia to 31 fibres per 250 mL in Portugal (Frias et al., 2010; Browne et al., 2011), however beaches near industrial harbours may have higher concentrations (Mathalon and Hill, 2014). These plastics can be deposited and stored deep within beach sediment (Turra et al., 2014).

If plastics are washed out to sea, hydrodynamic processes can transport them considerable distances, including offshore toward large oceanic gyres (Hammer et al., 2012). Currents within oceanic gyres converge, concentrating plastics (Hammer et al., 2012). The North Pacific Central Gyre (NPCG), now termed the "Great Pacific Garbage Patch" has been said to have an accumulation of floating debris the size of Texas (Hammer et al., 2012). There are five main oceanic gyres in the world, however the gyres in the northern hemisphere such as the NPCG are considered to be the most polluted (Moore et al., 2001). Plastics in this gyre outweigh plankton six times over with an average of 3,34,271 particles/km^2, consisting mostly of films, monofilament line and fragments (Moore et al., 2001). Current estimates are that there are five trillion plastic particles afloat on all oceans with a total mass exceeding 250,000 tonnes (Eriksen et al., 2014), yet there still are relatively low concentrations of plastic pollution in the Southern Ocean, with $1 - 6$ particles/km^2, consisting mostly of fishing associated debris (Ryan et al., 2014). Plastics have also been found at remote islands, deep ocean basins and even in polar regions (Barnes et al., 2009). Deep sea sediment for example, can have 0.5 microplastics/25 cm^2 (van Cauwenberghe et al., 2013b).

1.5 Plastic sizes

Discarded plastics can broadly be divided into mega–debris > 100 mm, macro–debris > 20 mm and meso–debris 20 – 5 mm (Barnes et al., 2009). The distinction of smaller plastics or microplastics however, is far less simple. The suitable upper end size limit to microplastics has been suggested to be 5 mm (Arthur et al. (2009). This definition is now most widely accepted and is therefore the size limit

chosen for this study. Those opposing this definition maintain that microplastics should only include those particles ≤ 1 mm (Costa et al., 2010). This size has been regarded as the upper size limit of 'true microplastics' by Claessens et al. (2011). These authors have noted that few studies have defined microplastics as strictly ≤ 1 mm, disregarding pre–production pellets occurring in samples. The lower limit to microplastics discussed by Arthur et al. (2009) was 333 µm, as this is usually the mesh size of neuston nets deployed in surface waters to sample plastics. However, much smaller plastics, < 20 µm, have been found in coastal sediments (Mohamed Nor and Obbard, 2014) and they may be more numerous than larger particles (Barnes et al., 2009).

1.6 Fragmentation, buoyancy and biofouling

The inability of most microflora to break down synthetic plastics, ensures that plastics have a long life span in the environment compared to natural materials (Billingham et al., 2000; Barnes et al., 2009). Fragmentation however, may occur in the marine environment via mechanical break down, such as by wave action and abrasion with sand and rock particles as they pass through estuaries (Isobe et al., 2014) or through solar degradation (Corcoran et al., 2009). The ultraviolet B component of sunlight facilitates a chemical break down of plastics via photocatalysis, which is an oxidative reaction that breaks the bonds holding polymer chains together (Andrady et al., 1998; Copinet et al., 2004; Fendall and Sewell, 2009). During plastic manufacturing, additives are used to prevent these reactions, lengthening their lifespan especially if they are continually exposed to sunlight, such as outdoor equipment (Claessens et al., 2011). Plastics entering open ocean current systems are further protected from sunlight due the cooling effect that water offers, shielding particles from ultraviolet B (Barnes et al., 2009).

Fragmented plastic material may show pits, grooves and linear fractures which are indicative of break down (Gregory, 1978; Corcoran et al., 2009). The extent of these can provide an indication of the time that the material has been in the environment (Gregory, 1978). Pellets, PVCs and PSs may also discolour over time (Gregory, 1978; Andrady et al., 1998). Rios et al. (2007) found that old pellets found in industrialised areas, showing more discolouring and fractures, had higher organic pollutant loads on their surface than pellets found elsewhere. Upon examination of the pits, grooves and fractures on plastic material under scanning electron microscopy (SEM), Reisser et al. (2014) and Zettler et al. (2013) also noted scraping marks on plastic material that was thought to be from organisms that colonise plastics in the marine environment.

Bacteria, cyanobacteria, fungi, diatoms, dinoflagellates, coccolithophores, corals, bryozoans, hydroids, filamentous algae, coralline algae, worms, barnacles, tunicates, insect eggs and isopods have all been found on plastics in the marine environment (Carpenter and Smith, 1972; O'Brine and Thompson, 2010; Claessens et al., 2011; Baztan et al., 2014; Reisser et al., 2014). These organisms are considered part of an epiplastic community now termed the plastisphere (Zettler et al., 2013; Reisser et al., 2014). Microbial films can quickly develop on LDPE films (Lobelle and Cunliffe, 2011; Harrison et al., 2014). Thereafter, they then assist the attachment of other invertebrates such as barnacles (Zardus et al., 2008). O'Brine and Thompson (2010) found that after four weeks, bioflims were distinguishable on the surface of polyethylene bag strips and macro–invertebrates such as mussels and tunicates could be found only after eight weeks. Barnacles on the other hand, could only be found after six months on plastics by Artham et al. (2009). These authors believe that the surface properties of the plastic material itself may influence the attachment of microbial films. The shading effect that biofouling offers also protects particles from ultraviolet radiation and break down (Barnes et al., 2009). As much as 90% of ultraviolet light can be blocked off via marine biofouling organisms (O'Brine and Thompson, 2010). Therefore, biofouling can affect both the buoyancy and the fragmentation of plastics.

1.7 The effects of high plastic concentrations on biota and the environment

Plastic causes harmful effects on marine biota. Plastic nets, lines, ropes and straps have been known to physically trap marine organisms such as sea turtles, marine mammals and sharks (Cliff et al., 2002; Wabnitz and Nichols, 2010). In the case of marine mammals and turtles this can make movement difficult and decrease their ability to reach the surface for air and tightly wound lines can restrict blood flow and cause the loss of limbs (Wabnitz and Nichols, 2010). Plastic rings around mammals and turtles have been found to directly asphyxiate organisms as they grow (Gregory, 2009). Fishing nets can also entangle and capture fish long after the nets have been discarded, an occurrence known as ghost fishing (Gregory, 2009). In addition to entanglement, plastics can cause obstructions that affect the ability of marine organisms to find food. For example, the foraging activity of the gastropod *Nassarius pullus* (Linné 1758) was found to decrease with increased plastic loads on beaches (Aloy et al., 2011).

Plastic ingestion has been noted in a wide range of taxa ranging from annelids to mammals (Appendix C). Mortality has been noted mainly from macroplastic ingestion, for example in seabirds (Azzarello and Van Vleet, 1987; Rodríguez et al., 2012; Provencher et al., 2014) and turtles (Lazar and Gračan, 2011) after ingesting macroplastic films, fragments and bottle caps that they have mistaken for food. In most cases

mortality is due to gut blockage and starvation (Rodríguez et al., 2012). Microplastic ingestion is mainly associated with sublethal effects such as decreased feeding efficiency and/or weight loss (Besseling et al., 2013; de Sá et al., 2015), transfer to haemolymph/organs (Browne et al., 2008), inability to excrete particles (Murray and Cowie, 2011) and inflammation (Wright et al., 2013a). These, together with all other effects of microplastic ingestion may result in consequences such as decreased growth rates (Wright et al., 2013b; Bakir et al., 2014a), liver toxicity and pathology (Rochman et al., 2013b) and decreased reproductive output (Sussarellu et al., 2016). Since microplastics have a higher surface area to volume ratio than macroplastics, they may carry proportionately higher chemical loads that are responsible for most of these sublethal effects. Being smaller also means that microplastics can become bioavailable to many more consumers such as filter–feeding mussels and barnacles (Thompson et al., 2004; Browne et al., 2008). There is still much more room for research on microplastics and its biological effects that are only now being investigated in depth.

Plastics and their additives can have negative chemical effects on biota. Lithner et al. (2011) for example, looked at 55 different plastic polymers and found that polyurethanes, polyacrylonitriles, PVC, epoxy resins, and styrenes were likely to be among the most hazardous due to possible mutagenic and carcinogenic monomers within the material (see also Rochman et al., 2013b). Adding to this, chemical additives that make plastics malleable such as phthalates, bisphenol A (BPA), polybrominated diphenyl ethers (PBDE) and tetrabromobisphenol A (TTBPA) can leach out from plastics and affect reproduction, cause genetic aberrations and hormonal imbalances (Thompson et al., 2009b; Hammer et al., 2012).

Metals and persistent organic pollutants (POPs) such as polychlorinated biphenyl's (PCBs), dichloro–diphenyl–trichloroethanes (DDTs), polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons (AHs) and hexachlorocyclohexanes (HCHs) have been found to adhere to the surface of plastics (Rios et al., 2007; Ashton et al., 2010; Frias et al., 2010; Rios et al., 2010; Hammer et al., 2012; Khan et al., 2015). Persistent organic pollutants can act as endocrine disruptors or carcinogens in organisms (Rios et al., 2010). Plastics may release these pollutants once ingested by marine organisms, as simulated desorption experiments have shown (Bakir et al., 2014a). Organisms around urban centres may be at a higher risk of exposure to these pollutants (Perra et al., 2010; Claessens et al., 2011; Hirai et al., 2011). Harbours and other estuaries around the world demonstrate elevated levels of metals (Hennig, 1985; Wepener and Vermeulen, 2005; Pillay, 2014) and organic pollutant loads (Bouwman, 2004; Vosloo and Bouwman, 2005) making these areas prime spots to investigate ecotoxicological effects that may be occurring in marine organisms that are exposed to plastics. However, coal and wood can also transport equally high, if not higher, amounts of these organic pollutants to biota than microplastics, therefore

research priorities should be carefully weighed prior to investigations (Beckingham and Ghosh, 2017). Concerns about microplastics in personal care products (Wu et al., 2016) and their influence on the occurance of cancers (Erren et al., 2015) have also been raised.

Plastic pollution can make the environment unattractive, decreasing the value of the environment, in addition to being particularly dangerous to various environmental functions (Sheavly and Register, 2007). The effects range from obstructing maritime activities to the disruption of gaseous exchange between the sediment and water column as plastic sinks and smothers the benthos (Gregory, 2009). Carson et al. (2011) found that plastics can change the physical properties of beaches. These authors found that the water permeability and temperature range of beach sediment was affected by plastic contaminants which may in turn affect various infaunal organisms. They also suggested that the temperature anomaly caused by the plastics could affect the sex distribution of turtle populations and cause desiccation stress on benthic beach fauna. Another major concern is that since plastics carry many organisms they may disperse alien species such as algal species that form red tides, which strip away the oxygen from coastal water bodies leaving many dead fauna in their path (Masó et al., 2003). Pathogenic bacteria can concentrate on plastics from the surrounding water, affecting marine biota that come into contact with it (McCormick et al., 2014).

1.8 Plastic pollution along the South African coastline

The South African coastline is around 3400 km long and has 300 estuaries (Harrison, 2004). Some of these are found within the main industrial hubs around the coast near which plastics can accumulate and concentrate chemical pollutants (Ryan et al., 2012). Ogata et al. (2009) for example found that pre–production pellets collected from South Africa contained high concentrations of HCHs. The city of Durban, located in KwaZulu–Natal, is one of the largest industrialised centres around the county's coast (Ryan et al., 2012) with other major urban centres at Cape Town in the Western Cape or Port Elizabeth and East London in the Eastern Cape. KwaZulu–Natal covers the species rich subtropical section of the country (Forbes et al., 1996). Threats to estuaries in KwaZulu–Natal include freshwater abstraction, habitat loss, sewage outlets or spills, chemical inputs, sedimentation, mouth closures and plastic pollution (Forbes et al., 1996; Forbes and Demetriades, 2008). Little research has been done on the latter threat on the South African coastline, however, Ryan and Moloney (1990) sampled 52 beaches in the Western Cape and found that plastics overall constituted 90% of debris, mainly in the form of pre–production pellets, polystyrene and fragments. Ryan (1988) found an average of 3640 plastic particles/km^2 in waters off the same coast, composed mostly of foams, fragments, pellets and fibres. These were suggested to

mostly be transported by the Agulhas Current running down the east coast of South Africa. Lamprecht (2013) had previously provided the only quantitative data on microplastics found in South African marine systems. Lamprecht (2013) sampled two beaches in Table Bay in the Western Cape and found that plastics could account for 93% of the debris and and found that these were comprised mainly of pellets, fragments and styrofoam. Some non–urban beaches in the country could also have high proportions of plastics. Madzena and Lasiak (1997) for example, found that the Wild Coast, formally called Transkei, in the Eastern Cape could become heavily impacted near recreationally used tourist beaches, with plastics accounting for as much as 83% of the strandline litter on beaches in the area. While conducting the research in this thesis, microplastic pollution research in South Africa has increased substantially. There is now more awareness and many more student projects focusing on microplastics, which has resulted in more beach clean-ups in local areas. In addition to the research covered in this dissertation, there is now also literature on the distribution of microplastic fibres along the South African coastline (Nel and Froneman, 2015) and a reflection of the demographics of the country in relation to the concentration of microplastic (Nel et al., 2017). More recently, a review on microplastic research in both marine and freshwater systems in South Africa has been done by Verster et al. (2017).

1.9   Local distribution around Durban

Durban has a shoreline 80 km long with 16 estuaries along its course (Forbes and Demetriades, 2008). All but four estuaries are classified as temporarily open to the sea (Forbes and Demetriades, 2008). The uMkhomazi, Durban Harbour, Isipingo and uMgeni estuaries have permanently open connections to the sea and plastics may be transported out of these systems (Forbes and Demetriades, 2008). Once out of the estuary, inshore currents have been implicated in controlling whether plastics are deposited as beach strandline debris or moved further out to sea. An important feature that could concentrate the plastics discharging from the estuaries and moving along this coastline is the presence of a semi–permanent cyclonic coastal lee eddy, known as the Durban Eddy. It is formed because of the shape of the coastline that surrounds Durban, which redirects the Agulhas Current flowing southward and turns it back up the coast (Cawthra et al., 2012). Plastics from the Durban metropolitan area may accumulate in this eddy, as found by Moore et al. (2001) for large oceanic gyres.

Arthur et al. (2009) suggested that research focus on microplastic hotspots as there are large gaps in what is known regarding microplastic concentrations in the environment and even less on the harmful effects to marine biota. Durban's population has been recorded at ~ 3.5 million (www.durban.gov.za) and extensive use of plastics within this urban hub is common. Macroplastic pollution has become a common sight on

beaches and estuaries. Annual coastal clean–ups reveal high plastic loads in coastal environments. In 2013, 6123 people collected 34,180 kg of litter from 320 km of KZN beaches including the Durban Harbour, while in 2016, 2407 people collected 17,460 kg of litter from 162 km of the KZN coastline (data from beach cleanups by Ezemvelo KwaZulu-Natal Wildlife). Most of this litter was plastic and packaging material, food containers, plastic bags and cigarette butts were the most common forms. These are macroplastics that can be collected by cleaners, however, there is no known quantitative account of the extent of microplastics on the beaches and in estuaries of Durban. Despite beach clean–ups, light weight plastics may be transported from dump sites back into the ocean via storm water drains or via aeolian transport (Wabnitz and Nichols, 2010).

The city of Durban has been developed around the harbour and many storm water drains from the city empty into the harbour. Many industries in the area also work with primary microplastics that may enter river systems. High plastic concentrations in areas such as these usually result in more interactions with the biota in the system (Clark et al., 2016). Negative effects of pollutants in the Durban Harbour have resulted in fish kills through sewage spills (Guastella and Smith, 1994) and imposex in gastropods through tributyl tin (TBT) pollution (Marshall and Rajkumar, 2003). The Bayhead mangroves in Durban Harbour have a natural heritage status protecting the remaining mangrove area (Forbes et al., 1996). This is a site that serves as a nursery area for many juvenile fish but is also threatened because of its proximity to the urban centre (Forbes et al., 1996). There was a larger mangrove forest in the past, but with development of the harbour by the 1960's – 1970's, most of the natural habitat was destroyed (Forbes et al., 1996). It is clear that there are high inputs of plastic in the area and that fish frequent it, but the specific plastic concentrations and the level of impact to fish using this nursery area have not been quantitatively described.

1.10 Thesis composition and main aims

This thesis revolves around three sub–topics. The first is a baseline assessment of environmental microplastic concentrations (Chapter 3 and 4), which aimed to quantify microplastic concentrations for five KwaZulu–Natal estuaries, their intervening beaches and corresponding coastal ocean sites. Background data on microplastic concentrations in Africa is scarce, and none existed for these Durban municipal sites, making this quantification important. Subsequently, Nel et al. (2017) presented microplastic data on the sediment and surf zone in the vicinity of the study area. It also paved the way for subsequent chapters that link back to these environmental microplastic concentrations. However, even before the baseline assessment, a method to isolate microplastic particles from estuarine and beach

sediment was refined in a pilot study. The pilot study aimed to determine an efficient microplastic separation technique from each of these sediment types (Chapter 2).

Estuaries represent an important nursery ground for a variety of estuarine and marine breeding fish species. Therefore, the second sub–topic broadly aimed to highlight the ingestion of microplastic particles with estuarine fish. Seventy sub-adult mullet from the most polluted estuary were collected, dissected and examined for microplastic (Chapter 5). The difficulty in isolating these microplastic particles prompted a method chapter, which also formed part of this sub–topic. The aim of this method chapter was to build on and evaluate an existing microplastic isolation technique and tailor it for use on juvenile fish (Chapter 6).

Before the last sub–topic, the background levels of pollution were already acquired and estuarine fish were known to consume microplastic particles. The last task was then to use this knowledge and investigate whether the ingestion of environmental concentrations of microplastic, affect fish physiologically. This was seen as an important research gap and proved difficult to fulfill using field data. Therefore, tank experiments that aimed to investigate whether ingesting microplastic has a negative impact on juvenile fish growth and survival, were used (Chapter 7). This was tested during a 92 day experiment. The plastic types and concentrations given to fish were according to the concentrations found in Durban Harbour. This aided with developing further questions on the population wide effects of fish ingesting microplastics from these systems. All three sub–topics are then placed into context at the conclusion of the thesis (Chapter 8).

CHAPTER 2

Pilot Study

2.1 Determining the quantity of sediment to collect

A pilot study was undertaken prior to main sampling activities to determine: (1) the volume and (2) the number of resuspensions of estuarine and beach sediment that allowed for sufficient (enough to compare between samples) and efficient retrieval (the fastest retrieval of > 80% of plastics in a set number of resuspensions) of plastic particles and plastic types. The objective was to mix and resuspend 250 mL, 500 mL and 750 mL of beach and estuarine sediment for a fixed number of times in hypersaline NaCl solution; and then to compare the number of plastic particles and plastic types found between these volumes and resuspensions. It was hypothesised that (1) the number of plastic particles and plastic types found would be the greatest within 750 mL sediment samples and this volume would also offer the most efficient retrieval; and (2) the number of resuspensions required for an efficient retrieval would be lower in coarser beach sediment compared to finer estuarine sediment.

2.2 Sediment collection, processing and analysis

Sediment samples were collected from the Isipingo Estuary and the adjacent shore strandline (30° 00′S 30° 57′E). Three replicates of 250, 500 and 750 mL sediment volumes for each sediment type were excavated with a hand held corer of 50 mm internal diameter at the highest tidal line. Cores were taken at 10 cm depth. At the laboratory, each sediment sample was mixed in a hypersaturated NaCl solution of 140 g. L^{-1} for three minutes to 'float out' plastics (Frias et al., 2010). Thereafter, the coarse particles were allowed to settle for one minute, before the suspension was poured through a 1000 µm sieve. The filtrate was then collected into a 2 L glass bottle and allowed to further settle. The settling time before the suspension could be passed comfortably through a 100 µm sieve for estuarine sediment was approximately two hours, whilst beach sediment settled well within a few minutes. The remaining solution was then filtered under vacuum through a 20 µm Millipore mesh filter. All filters and sieves were initially analysed using a dissecting microscope for airborne microplastic contaminants, such as polyester fibres, before being used. After sample filtration, filtered material was allowed to dry to constant weight

before microplastics could be picked out, counted and characterised according to type and colour using a dissecting microscope (Bausch + Lomb Inc.) at $15 \times$ magnification.

The process of mixing, filtering and analysing was repeated six times for each sample of estuarine sediment and three times for beach sediment. This number of repeats/resuspensions for the respective sediment type was based on a preliminary analysis of $1 - 2$ kg of sediment from the Isipingo Estuary which revealed only a small number of added fibres after the allotted suspension. Since this study consisted of much less sediment, these were fixed as the maximum number of resuspensions for the respective sediment type.

The number of plastic particles and types found were repeatedly measured over the respective series of resuspensions for each sample. A repeated measures analysis of variance (ANOVA) was performed using the Statistical Package for the Social Sciences (SPSS v.21) to determine if there were significant differences for the number of plastic particles and types within the volumes of sediment taken for each sediment type. The assumption of normality and homoscedasticity of residuals as well as sphericity were all satisfied for both sediment types. TukeyHSD tests were used to look at multiple comparisons between sediment volumes.

2.3   Results of the pilot study

2.3.1   Estuarine sediment

The number of plastic particles found ranged from 0 to 14, with the maximum found in the initial suspension of a 500 mL sediment sample. The number of plastic particles and types retrieved in all three sediment volumes decreased over successive resuspensions in the NaCl solution. There was also an overall difference in the number of plastic particles and types found between successive resuspensions ($F = 14.847$, $df = 6$, $p = <0.0005$ and $F= 13.357$, $df = 12$, $p = 0.070$, respectively). The total number of plastic particles and types found between the three volumes of sediment were similar (Figure 2.1 a, c) throughout successive resuspensions and were not significantly different ($F =1.249$, $df = 2$, $p = 0.352$ and $F = 3.059$, $df = 3$, $p = 0.121$, respectively).

There was a non significant interactive effect between successive resuspensions and sediment volume for the number of plastic types retrieved from estuarine sediment ($F = 1887$, $df = 12$, $p = 0.070$). There was a significant interactive effect between successive resuspensions and volume for the number of plastic particles retrieved ($F = 2.193$, $df = 12$, $p = 0.034$).

Cumulative curves showed that 90% of the plastic particles and 85% of the plastic types were retrieved after three suspensions for 500 mL of sediment, which was much higher than for 750 mL or 250 mL sediment volumes (Figure 2.1 b, d). A sediment volume of 500 mL that is resuspended three times was therefore estimated to be the most efficient for processing of estuarine subtidal sediment collected from the field.
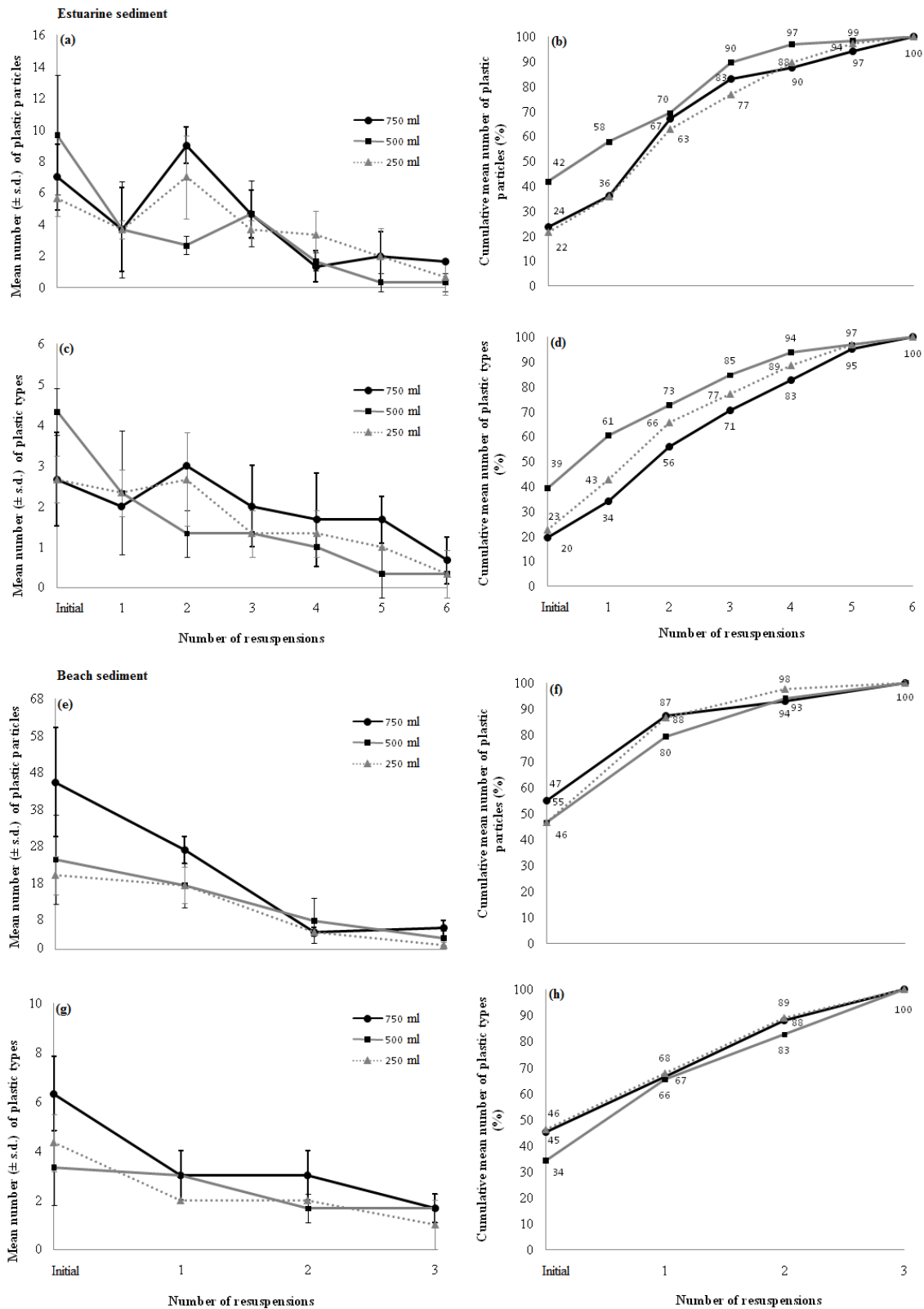
Figure 2.1. The mean number (a, c, e and g) and cumulative mean number (b, d, f and h) of plastic particles and types found in 250, 500 and 750 mL of estuarine and beach sediment respectively, throughout a series of resuspensions. Error bars are standard deviation values.

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2.3.2 Beach sediment

The number of plastic particles and types found in beach sediment decreased with each resuspension (Figure 2.1 e, g) and between the first two resuspensions 80 – 98% of the plastic particles and types were found (Figure 2.1 f, g). Overall, there was a difference in the number of plastic particles ($F = 29.685$, $df = 3$, $p \leq 0.0005$) and plastic types ($F = 17.816$, $df = 3$, $p \leq 0.0005$) that were found throughout subsequent resuspensions. There was no significant difference between 250 mL and 500 mL sediment ($p < 0.05$) but both of these significantly differed ($p < 0.05$) from 750 mL sediment, which displayed higher mean number and type of particles (Figure 2.1 e, g). Neither number of particles ($F = 2.394$, $df = 6$, $p = 0.071$) nor types ($F = 1.680$, $df = 6$, $p = 0.183$) showed interactive effects between the number of resuspensions and volume.

2.4 Discussion and conclusion of pilot study

The first hypothesis that the 750 mL volume of sediment would be the most efficient for the retrieval of plastics was falsified for estuarine sediment because of of the similarity in total plastics found between different sediment volumes and because 500 mL of sediment allowed for a faster retrieval of plastics. Although the hypothesis may be partially accepted for beach sediment because of significantly higher plastic mean values in 750 mL sediment, 500 mL was also the chosen collection quantity for beach sediment in the main study. This volume both allowed for enough plastics to be retrieved to be compared and also standardised the volume quantity allowing for comparison between beach and estuarine sediment in main sampling events. The second hypothesis that the number of resuspensions required for an efficient retrieval was lower for coarser beach sediment was accepted and may be attributed to the smaller sediment particles in estuarine sediment holding back more plastic particles than beach sediment by clumping. Martins and Sobral (2011) and Mohamed Nor and Obbard (2014) also noted that finer sediment would retain particles from freely suspending.

Through the pilot study it was deduced that adequate plastic particles and plastic types would be retrieved from 500 mL of both estuarine and beach sediment, after three and two resuspensions respectively, for comparison among stations in the main study. Two more sieve sizes, 500 µm and 250 µm, were set to be included in the main study which may allow for the solution mix to pass more readily through the 100 µm as well as offering a wider size range of particles to be examined between main stations.

CHAPTER 3

Plastic pollution in five urban estuaries of KwaZulu–Natal, South Africa

3.1 Abstract

Monitoring plastic concentrations in estuaries is vital in assessing the magnitude of terrestrial inputs to oceanic environments. Data on plastics ≤ 5 mm in estuaries are scant. This study determined microplastic levels within five estuaries along the Durban coastline and on intervening beaches. Plastics were isolated from estuarine sediment, beach sediment and the surface water of each estuary and characterised. Sediment at the Bayhead area of Durban Harbour had the highest average plastic concentrations (745.4 ± 129.7 particles per 500 mL) and an attenuating concentration trend away from the city centre was found. Prevailing south to north longshore drift was hypothesised to result in plastic accumulation on the northern shores of beaches with estuarine effluents, however, this was not found. Fragments composed the largest percent of plastics (59%) found in Bayhead, whereas fibres dominated other estuaries with proportions ranging from 38% of total plastics in the uMgeni estuary to 66% in the Mdloti.

3.2 Introduction

The low mass, high durability and low production cost which make plastics ideal for an array of uses, have resulted in a burgeoning plastic use since the end of the second world war (Carpenter and Smith, 1972; Koelmans et al., 2014; Bergmann et al., 2015). Production currently exceeds 280 million tons per annum (PlasticsEurope, 2012), placing strain on marine environments as unwanted plastics enter through improper sewage treatment, industrial spillages and user discards (Barnes et al., 2009; Thompson et al., 2009b; McCormick et al., 2014).

Plastics are now ubiquitous and have been found in oceanic gyres (Moore et al., 2001; Boerger et al., 2010; Rios et al., 2010), on remote islands (Baztan et al., 2014), in deep ocean basins (van Cauwenberghe et al., 2013b) and even in polar regions (Barnes et al., 2009); however the highest pollution levels

coincide with heavily industrialised city centres (Claessens et al., 2011). In particular, microplastics (Thompson et al., 2004; Arthur et al., 2009) have increased in marine systems in recent years in concert with a general decrease in the mean size of plastic particles (Bergmann et al., 2015). This has caused concern about possible wide-ranging effects of microplastic ingestion on marine fauna because smaller particles are bioavailable to a wider consumer range (Thompson et al., 2004; Browne et al., 2008) and they are considered to be an emerging threat to marine habitats (Bergmann et al., 2015). A variety of deleterious effects have already been observed (Browne et al., 2008; von Moos et al., 2012) and trophic transfers may pose a threat to the rest of the food web, including humans indirectly (Farrell and Nelson, 2013). Nanometre-size particles may be able to penetrate cell membranes although this has not yet been observed in the field (Bergmann et al., 2015). Research effort has increased accordingly with over 100 papers on microplastics now published (Bergmann et al., 2015).

Estuaries are major conduits for transporting plastics from catchments to the ocean, especially in urban areas where they may serve as industrial outlets and areas of recreational fishing activities (Dekiff et al., 2014; Sadri and Thompson, 2014). Quantitative records of plastic pollution, including by microplastics, in estuarine and other sheltered coastal environments are under-represented in the literature despite several recent publications (Costa et al., 2011; Ivar do Sul and Costa, 2013; Vianello et al., 2013; Lima et al., 2014; Morritt et al., 2014; Zhao et al., 2014). Some of the factors determining concentrations and distribution of plastics in estuarine habitats include wind speed, wind direction and the size and density of fragments (Browne et al., 2010).

In South Africa research on debris in marine and coastal environments and its ingestion by seabirds began in the mid-1980s (Ryan, 1987, 1988), although incidental reports of plastic ingestion pre-date this (Hughes, 1970, 1974 cited in Bergmann et al. (2015)). An increase in plastic debris on beaches in the southern andwestern part of the country, including particles down to 2 mm in size, was recorded between 1985 and 1989 (Ryan and Moloney, 1990) and contaminants on polyethylene pellets have been used to infer changes in the concentration of persistent organic pollutants (POPs) along the South African coast (Ryan et al., 2012). However, data remain scarce, particularly on the east coast of the country; a rare exception being work on the effects of litter on large sharks (Cliff et al., 2002). There are no data on microplastic pollution or on plastic pollution of estuarine water and sediments despite the presence of 73 estuaries in the KwaZulu–Natal, 16 of which fall within the Durban metropolitan area, including Durban Harbour, which is considered an estuarine bay. These estuaries are nursery areas for millions of fish fry (Wallace et al., 1984), with up to 160 species of fish in South Africa dependent on estuaries at some stage of their life cycle (Lamberth and Turpie, 2003). Durban supports a variety of industries that might

discharge effluents into rivers and estuaries with the harbour, often referred to as Durban Bay, particularly subject to increasing human disturbance (Forbes and Demetriades, 2008). This study therefore aims to characterise and determine the extent of plastic pollution within estuaries close to Durban, including the Durban Harbour. Due to the large number of stormwater outfalls and rivers that drain into the harbour (Rathbone et al., 1998), we hypothesised that the plastic concentration is highest at the Durban Harbour and attenuates at sites further away. Southeasterly winds prevail in KZN causing a dominant south to north longshore drift (Guastella and Smith, 2013). It was therefore further hypothesised that plastics accumulate to a higher extent on beaches north of contaminated systems.

3.3 Material and methods

3.3.1 Site

The Mdloti (29° 38′S, 31° 08′E), uMgeni (29° 48′S, 30° 02′E), Durban Harbour (29° 52′S, 31° 04′E), Isipingo (30° 00′S, 30° 57′E) and iLovu (30° 07′S, 30° 51′E) estuaries located in Durban, KwaZulu-Natal, South Africa were sampled (Fig. 3.1). The Mdloti and uMgeni estuaries are 27 km and 7 km north of Durban Harbour, whereas the Isipingo and iLovu estuaries are 18 km and 34 km south of Durban Harbour, respectively. (Forbes and Demetriades, 2008) classified the uMgeni, Durban Harbour and Isipingo estuaries, closest to the city centre, as industrialised, whilst the Mdloti and iLovu estuaries further away are semi-rural.
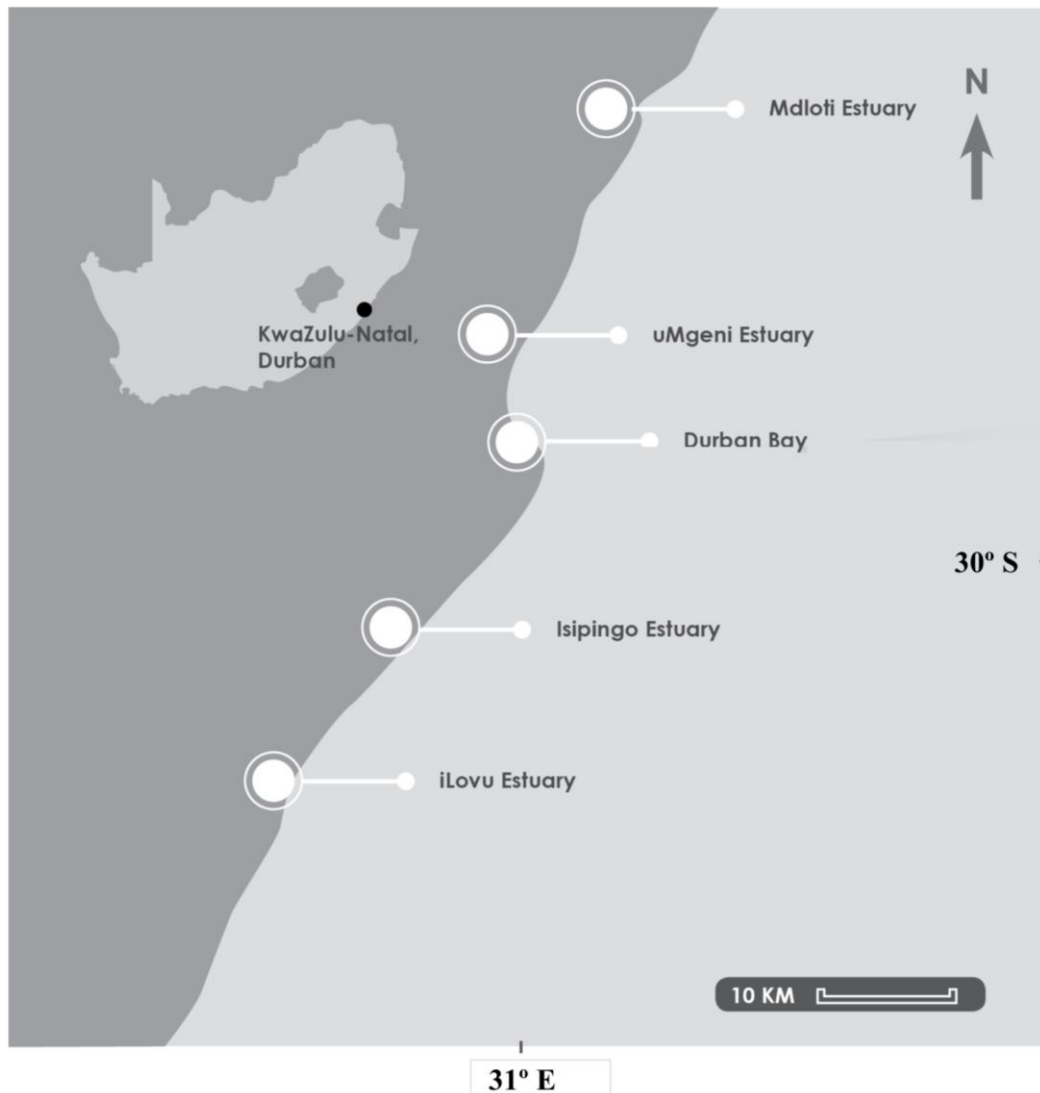
Figure 3.1. The estuarine systems sampled in Durban, KwaZulu–Natal, South Africa.

3.3.2   Field collection

Estuarine and beach sediment samples

Samples were obtained from the head, middle and mouth of each estuary at spring low tides. At each station, subtidal and supratidal sediment samples were retrieved at ankle depth and on the high tide mark, respectively. Adjacent to each estuary, four beach stations were sampled 500 m and 1000 m north and south of each estuary mouth. At each tidal level within estuarine stations and at each beach station, five replicates of 500 mL sediment were collected. Beach and estuarine sediment samples were collected down to 10 cm depth with a corer that has a 50 mm internal diameter. Beach sediment was collected from the shore drift line and if multiple lines were present, the highest was sampled.

Surface water samples were collected at the head, middle and mouth of each estuary. Five replicates were collected at each station by towing a conical zooplankton net with a 300 μm mesh and mouth diameter of 30 cm at constant speed. A General Oceanics flow metre (model 2035 MK4) was fixed to the opening of the net to ensure constant sample volumes of 10000 L. New PVC honey jars were used to carefully transport samples back to the laboratory where they were filtered through 1000, 500, and 250 μm sieves. Filtered material was covered in foil to prevent airborne contamination and allowed to dry in an oven at 60 °C to constant mass before searching for plastics under a dissecting microscope. The jars were rinsed before use with distilled water and 10 jars filled with distilled water were stored for 24 h before they were examined under the microscope for contamination. No contamination was found.

3.3.3 Laboratory processing

Each sediment sample was mixed by hand for three minutes and allowed to settle in a 140 g L^{-1} hypersaturated sodium chloride (NaCl) solution to float out plastics, following (Frias et al., 2010). The suspension was then passed, in order, through 1000, 500, 250, 100 and 20 μm filters. The last filtration was done under vacuum. This process was repeated three times for each estuarine sediment sample and twice for each beach sediment sample following results of a pilot study, which showed that these numbers of suspensions allowed for the most effective retrieval of plastic particles. Filtered material was then analysed as done for water samples. 500 mL of harbour sediment was also dried until constant mass for comparison with studies that used mass instead of volume to quantify the number of plastic particles. Particles were classified (Fig. 3.3) using guidelines from (Hidalgo-Ruz et al., 2012 and references therein), with the exception of twine. We classified twine as a tightly wound rope like material, distinguishable from fibres, which could be singular or bundled haphazardly together, but never woven. Particles that were indistinguishable from detrital material were further analysed with Fourier Transform InfraRed Spectroscopy (FT-IR) using a Perkin Elmer Spectrum 100 Series FT-IR spectrometer. Samples of unknown composition were compared to a Perkin Elmer Attenuated Total Reflectance (ATR) library of plastic polymers.

3.3.4 Statistical anaylsis

Fully nested analyses of variance (ANOVAs) were performed to compare sediment and water column plastic concentrations within and between estuaries using *R* 3.0.3 (R_Development_Core_Team, 2014). Data were log_{10} transformed where necessary to meet the assumptions of normality and variance homogeneity. Tukey's HSD tests were used for all multiple comparisons within and between estuaries at 95% family-wise confidence level. Pearson correlation analyses conducted on SPSS version 21, were

used to correlate plastic concentrations in supratidal sediment and the surface water of each estuary. The assumption of normality was satisfied on $\log_{10}$ transformed data.

3.3.5 Multivariate analyses

Non-Metric Multidimensional scaling was performed using PRIMER (Plymouth Routines In Multivariate Ecological Research version 6), on plastics that were found in samples to determine if the composition of plastics differed among estuaries. Plastic concentration per type across the set of filters were averaged and used to create a matrix of Bray–Curtis similarities, after square root transformation to account for the large number of zero values in the matrix and for 'rare' plastic types. nMDS was then run on the sample matrix to determine if patterns emerged between sites, stations, and tidal levels. Plots that displayed the lowest stress values were used to make conclusions on plastic composition. ANOSIM tests were carried out to further investigate the composition of plastic types shown by nMDS plots. Global *R* statistics were used to decide if there was sufficient evidence to support the null hypotheses.

3.4 Results

3.4.1 Total number of plastic particles

Plastic particles were recorded in all samples. A total number of 13680 particles were found within the five systems. Supratidal estuarine sediment contained 46% of the particles, with sediment at the head station in Durban Harbour, known as Bayhead, alone accounting for 19% of the total. Beach sediment, water samples and subtidal estuarine sediment accounted for 24%, 16% and 14%, respectively. The number of plastic particles per 500 mL varied from five at the middle subtidal station in the iLovu to 896 at the Bayhead supratidal station. Mean plastic concentration differed among estuaries and among stations within estuaries (Table 3.1).

3.4.2 Estuarine sediment

Overall, Durban Harbour had the highest mean concentration of plastic particles (159.9 ± 271.2 particles per 500 mL, tidal zones combined). This was followed by the uMgeni and Isipingo estuaries (41.7 ± 23.0 and 47.6 ± 22.8, respectively, Fig. 3.2 a). The Mdloti and iLovu estuaries, situated furthest north and south from the city centre, had the lowest concentration of plastic particles overall (19.9 ± 16.2 and 13.7 ± 5.6, Fig. 3.2 a). All estuaries except the uMgeni and Isipingo differed significantly from each other in terms of plastic concentrations (Fig. 3.2 a).

There was no overall difference in the number of plastics found among sampling stations, with tidal levels combined, within any estuary, except Durban Harbour (Fig. 3.2 a).Within Durban Harbour, Bayhead had the highest concentration of plastic particles (745.4 ± 129.7 particles per 500 mL). Most particles were found in supratidal sediment (Fig. 3.2 a, b). Conversely, supratidal sediment at the Mdloti head station had a lower mean plastic concentration than middle and mouth stations (Fig. 3.2 b). The plastic concentration in subtidal sediment did not differ among stations at any estuary (Fig. 3.2 c).

Table 3.1. Results of nested ANOVAs comparing mean plastic concentrations within estuarine sediment, beach sediment and the surface water of sampled estuaries. Factors are nested in a hierarchical manner and data for all plastic types are combined. Significance values are ** at $0.001 < p \leq 0.01$ and *** at $p \leq 0.001$.

	df	SS	MS	F	Pr(>F)
Estuarine sediment					
Estuary	4	10.401	2.6002	104.38	***
Station (Estuary)	11	3.887	0.3534	14.19	***
Tidal level (Estuary, Station)	15	5.428	0.3619	14.53	***
Residuals	116	2.89	0.0249		
Beach sediment					
Beach	4	2.1668	0.5417	20.399	***
Direction (Beach)	5	0.5131	0.1026	3.864	**
Distance (Beach, Direction)	10	0.6992	0.0699	2.633	**
Residuals	80	2.1244	0.0266		
Surface water					
Estuary	4	6.859	1.7147	30.826	***
Station (Estuary)	10	4.633	0.4633	8.329	***
Residuals	59	3.282	0.0556		

3.4.3   Beach sediment

Mean plastic concentrations differed between beaches (Table 3.1). The number of plastic particles per 500 mL varied from eight at 1 km south of iLovu mouth, to 99 at 500 m north of Durban Harbour. The plastic concentration did not differ among beaches adjoining Durban Harbour (38.6 ± 20.9 particles per 500 mL), Isipingo (46.0 ± 23.0 particles per 500 mL) and uMgeni (38.5 ± 12.3 particles per 500 mL) (Fig. 3.2 e). Plastic concentrations at these beaches were significantly higher than both the iLovu (20.4 ± 10.0 particles per 500 mL) and Mdloti (20.0 ± 7.5 particles per 500 mL) beaches (Fig. 3.2 e).

The plastic concentration combined and compared between north and south stations among beaches were not significantly different (Durban Harbour — $p = 1.000$, iLovu — $p = 0.562$, Mdloti — $p = 0.998$ and uMgeni — $p = 0.999$) except for Isipingo ($p = 0.013$), at which combined south stations had significantly higher mean plastic concentrations (58.2 ± 22.3 particles per 500 mL) than north stations (33.7 ± 16.9 particles per 500 mL). Overall, no significant differences were observed between distances within each site (Fig. 3.2 e).
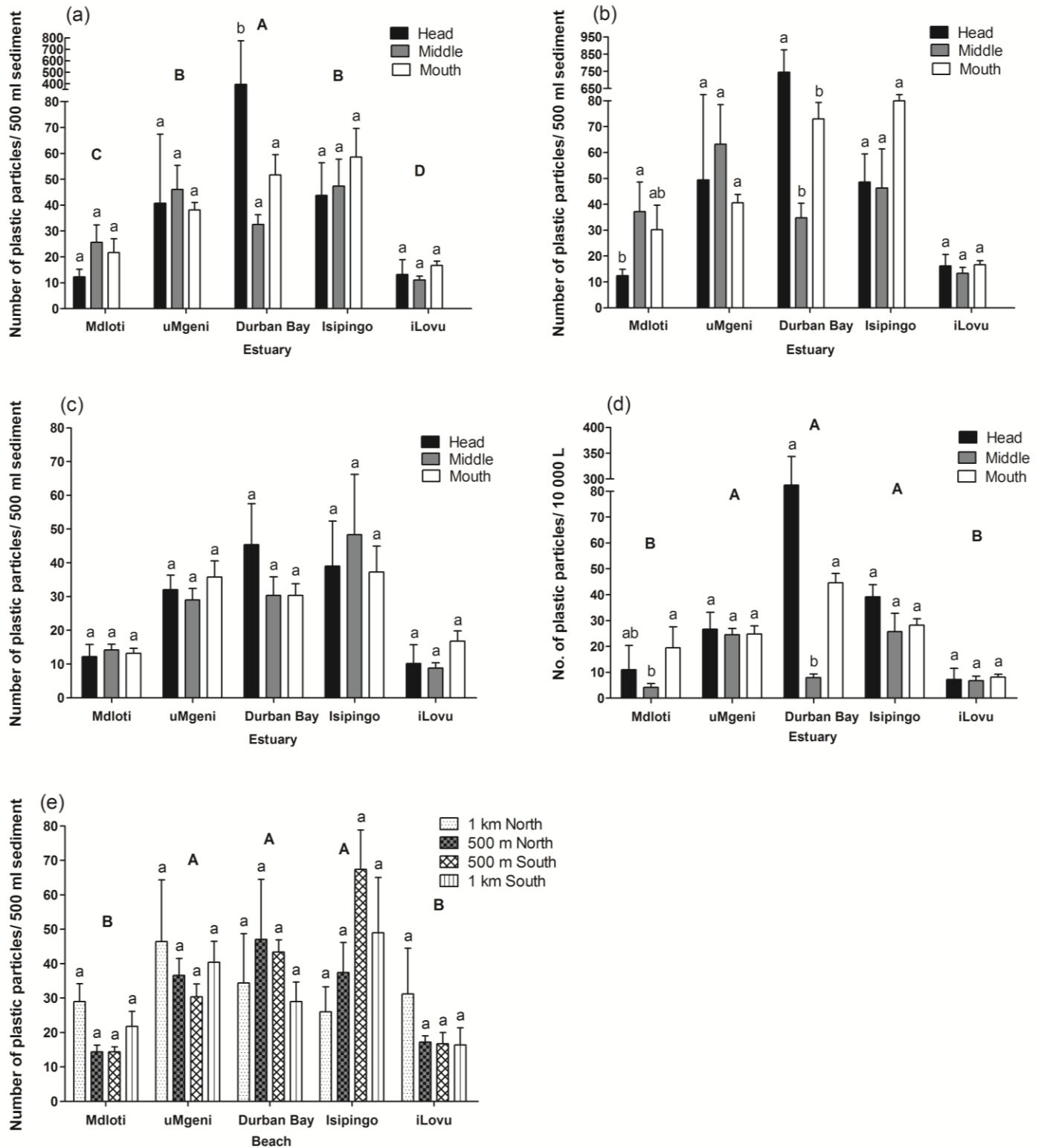
Figure 3.2. Number of plastic particles (mean ± S.D.) found within (a) estuarine sediment in which data from tidal zones are combined, (b) estuarine supratidal sediment, (c) estuarine subtidal sediment, (d) estuarine surface water and (e) beach supratidal sediment. Letters in uppercase denote Tukey's post-hoc differences between estuaries, whereas letters in lowercase indicate differences between stations within each estuary.

3.4.4 Surface water

The mean plastic concentration in surface water samples among estuaries differed overall (Table 3.1). The number of plastic particles per 10000 L varied from two at the iLovu head station to 487 at Bayhead. Durban Harbour had the highest mean plastic concentration (70.3 ± 119.3 particles per 10000 L). This did not differ significantly from the Isipingo and the uMgeni estuaries (31.1 ± 11.1, 25.3 ± 6.0 particles per 10000 L, respectively). Surface water at these estuaries had significantly higher plastic concentrations than both iLovu (10.2 ± 11.3 particles per 10000 L) and Mdloti (11.0 ± 11.5 particles per 10000 L; Fig. 3.2 d) estuaries.

Within Durban Harbour, most plastic particles were found at Bayhead (158.2 ± 185.4 particles per 10000 L; Fig. 3.2 d), whereas this was not the case for the other estuaries. The middle stations at both the Mdloti and Durban Harbour had significantly lower plastic concentrations than other stations within each estuary (Fig. 3.2 d). The mean plastic concentration among stations within the uMgeni, Isipingo and iLovu estuaries did not significantly differ from each other (Fig. 3.2 d).

3.4.5 Sediment and surface water correlations

Although sediment and surface water column plastic concentrations showed a significant positive relationship when data were combined across all sites ($r = 0.706$, $n = 71$, $p < 0.0005$ and $r = 0.679$, $n = 71$, $p < 0.0005$, respectively), this relationship held true only for Durban Harbour when sites were assessed individually ($r = 0.776$, $n = 15$, $p = 0.001$).

3.4.6 Plastics composition

nMDS plots and ANOSIM indicated a large overlap and no difference in plastic type composition between sites (stations nested in sites, $R = 0.399$, $p = 0.2\%$; nMDS plots not shown), tidal levels ($R = 0.012$, $p = 10.1\%$) and size categories ($R = 0.277$, $p = 0.1\%$) for estuarine or beach sediment (direction nested in beaches, $R = 0.360$, $p = 5.2\%$, distance, $R = -0.009$, $p = 73.5\%$ and size categories, $R = 0.321$, $p = 0.1\%$). A large overlap was also noted in plastic composition between stations nested in sites ($R = 0.247$, $p = 0.3\%$) and size categories ($R = 0.089$, $p = 2.5\%$) for water samples.
.

The main types of plastics found were pellets, fragmented material from the disintegration of larger plastic items, polystyrene, films, scrubbers, monofilament line, twine and fibres (Fig. 3.3). Fragments comprised the largest proportion of all plastics found in the Durban Harbour, whereas fibres dominated at the other estuaries (Fig. 3.3 a). Durban Harbour also contained a higher proportion of scrubbers than other estuaries (Fig. 3.3 a). A total of 299 and 67 pellets were collected from five replicate surface tows and

five sediment cores at the Bayhead station alone. A higher proportion of polystyrene particles was found at the uMgeni and Isipingo than other estuaries (Fig. 3.3 a).
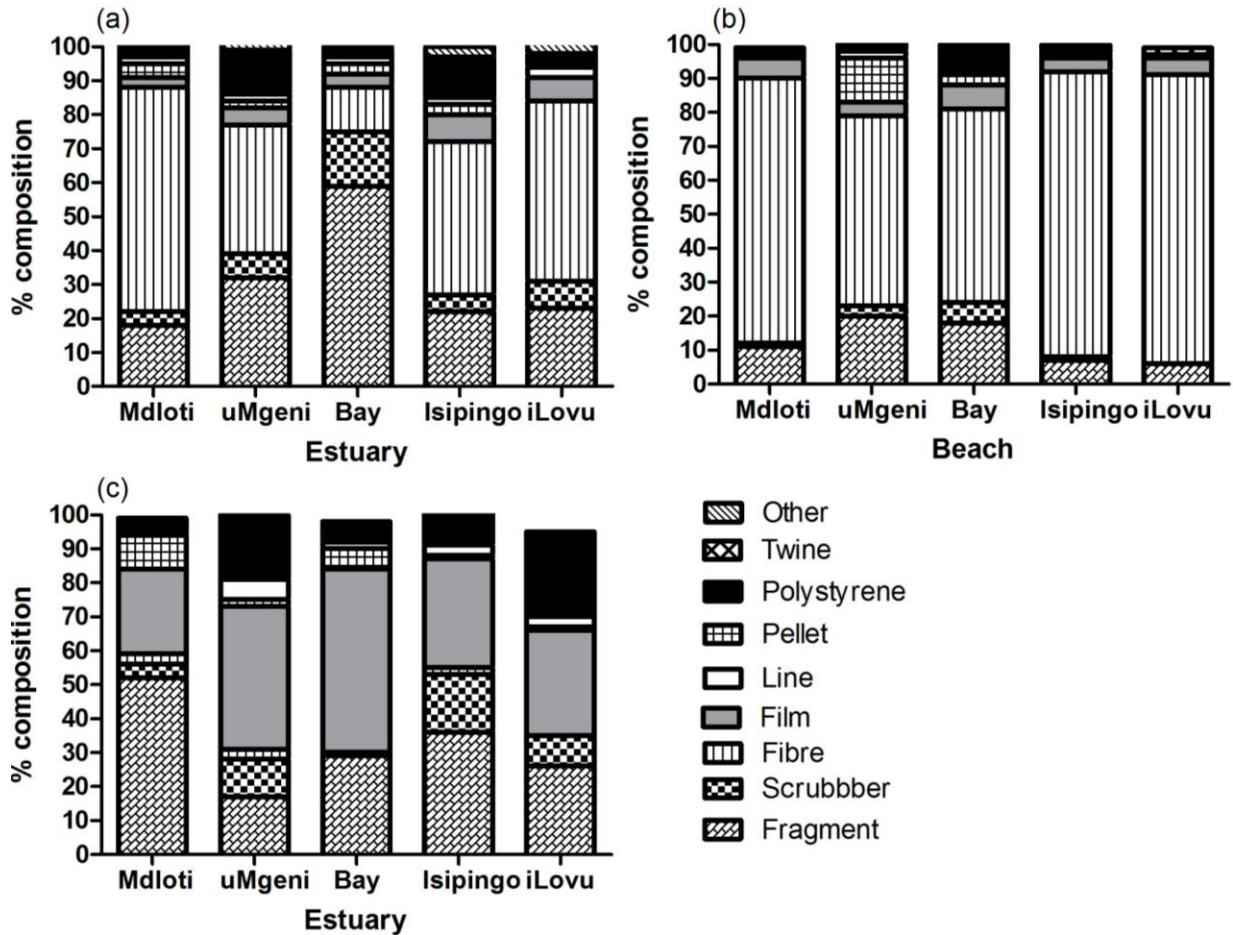
Figure 3.3. Relative proportion of plastic types found within (a) estuarine sediment, (b) beach sediment and (c) the surface water of estuaries sampled. In the legend, 'line' refers to monofilament fishing line.

Estuarine and beach sediment had similar plastic compositions but all samples of beach sediment had higher fibre proportions (Fig. 3.3 a, b). In addition, uMgeni beach samples contained a large proportion of virgin pre-production pellets which accounted for 13% of the plastics compared to < 3% at other beaches (Fig. 3.3 b). Plastic fragments at the uMgeni and Durban Harbour beaches accounted for 20% and 18% of plastic items whilst fragments accounted for < 11% at other sites (Fig. 3.3 b).

Fibres were not generally collected in surface water samples (Fig. 3.3 c) owing to the mesh size of the sampling net. Plastic films and fragments contributed a high proportion to plastics in surface water samples of all estuaries. Polystyrene, most noticeably at the uMgeni and iLovu estuaries, and scrubbers at the Isipingo also contributed a large portion of the total plastic pool (Fig. 3.3 c).

3.4.7 Size distribution of plastics

The number of particles in estuarine sediment generally increased in concentration from large to small size classes (Fig. 3.4). This however, was not the case for the 20 – 100 μm size class (Fig. 3.4). Beach sediment samples displayed a similar trend, with the exception of the uMgeni estuary in which particle size was more evenly distributed (Fig. 3.4). Water samples of the Mdloti and uMgeni estuaries had a higher proportion of > 1000 μm particles than other estuaries. The Durban Harbour, Isipingo and iLovu estuaries had a higher percentage of particles in the 250 – 500 μm size class (Fig. 3.4).
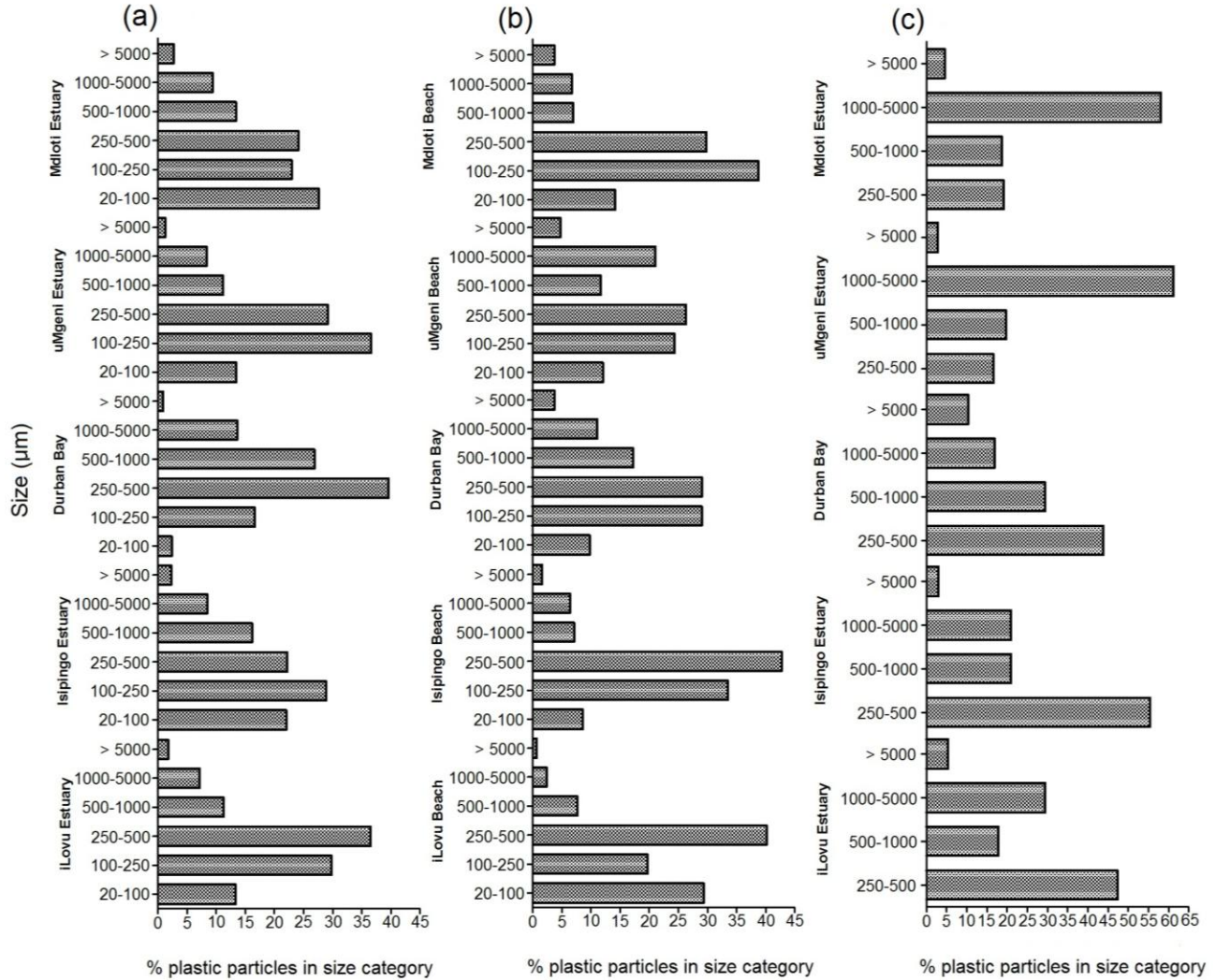
Figure 3.4. The plastic particle size distribution between (a) estuarine sediment, (b) beach sediment and (c) the surface water of estuaries sampled. The size range for water samples differs, with no class < 250 μm due to the mesh size of the net used for collection.

3.5 Discussion

3.5.1 Levels of pollution within and among estuaries

These data demonstrate high plastic concentration in urban estuaries of Durban, peaking in the centrally located Durban Harbour and diminishing in estuaries located away from the urban centre (Fig. 3.2). These results are supported by previous observations near harbours within urban hubs (Claessens et al., 2011; Mathalon and Hill, 2014) but studies use disparate samples and units, making comparisons difficult (Mohamed Nor and Obbard, 2014). Nonetheless, plastic concentrations at Bayhead, exceeded the plastic loads found at the Belgian harbours surveyed by Claessens et al. (2011), although fibre concentrations were lower than those found by Mathalon and Hill (2014) at the Halifax harbour in Novia Scotia. Durban is prone to pollution due to its high population density and industrial wastes, typical of harbour regions (Forbes et al., 1996; Armitage and Rooseboom, 2000; Mohamed Nor and Obbard, 2014).

Plastic can enter the harbour via a number of routes. Several rivers flow through industrialised suburbs of Durban, and enter the harbour through canals at Bayhead (Harris and Cyrus, 1999; Williams et al., 2004) and storm water drains throughout the harbour (Forbes and Demetriades, 2008). Many industries trading with plastic powders, pellets and other raw materials also surround the harbour. Lightweight films could be transported into the harbour by wind (Wabnitz and Nichols, 2010; Ivar do Sul et al., 2014). Discarded and weathered angling gear, particularly monofilament line, is also a plastic source (Guastella and Smith, 1994). Another possible source could be ship repairs at the dry docks near Bayhead, which can be a direct source of polyurethane, nylon, polystyrene and polyester (Reddy et al., 2006). Microbeads used in cosmetic products and fibres also made up a large contribution to the total particles found in Durban Harbour and other estuaries, possibly bypassing wastewater works situated on rivers passing through the city (Fig. 3.3; Dickens and Graham, 1998; Fendall and Sewell, 2009).

Although information on the movement of microplastic particles within estuaries is scarce (Ivar do Sul et al., 2014), macroplastics may be retained in estuaries for months to years (Ivar do Sul et al., 2014) and enclosed areas may trap the most microplastics (Claessens et al., 2011). Bayhead is considered a partially enclosed lagoon and low flow rates could favour deposition, restricting the movement of water and plastics that enter (Marshall and Rajkumar, 2003; Ballent et al., 2012). This may explain the strong correlation between the particles in the surface water and in the sediment, which was found only at the harbour. The freshwater input from the canals may also cause stratification that could further increase the residence time of plastic debris (Lima et al., 2014). Substantial amounts of plastics may get flushed out

only during flooding events or dredging (Harris and Cyrus, 1999; Bakir et al., 2014b). The nearshore environment may therefore receive higher plastic loads soon after these events.

The Durban Harbour, Isipingo and uMgeni estuaries had higher plastic concentrations than the Mdloti and iLovu (Fig. 3.2). Their intervening beaches also had higher plastic concentrations (Fig. 3.2) showing the impact of a high terrestrial input of plastics on the nearshore environment, which has also been shown by (Silva-Cavalcanti et al., 2009). Plastic movement along beaches was assumed to follow a generalised northerly alongshore current (Guastella and Smith, 2013). However plastic concentrations north and south directions of estuary mouths did not significantly differ (Fig. 3.2). Movement may also be complicated by deposition and re-emergence of plastics from beach sediment during heavy winds and large swells (Williams and Tudor, 2001). Transport models for plastics in the nearshore environment are still being developed due to the large number of factors that could influence particle movement (Isobe et al., 2014). With the large storms and swells along the KwaZulu-Natal coast (Guastella and Smith, 2013), these items may move further out to sea, become re-deposited higher up on shore or may even re-enter estuarine environments (Bakir et al., 2014b).

3.5.2 Plastics composition among estuaries

No site in this study had a distinctive suite of plastics. Common types that accounted for large proportions were fibres, films and fragments. Of these, fragments are found in most microplastic studies (Costa et al., 2010) and form the major component of plastics found in the open ocean (Morét-Ferguson et al., 2010). Fibres are also ubiquitous (Browne et al., 2011; Dubaish and Liebezeit, 2013; Wright et al., 2013b; Dekiff et al., 2014; Mohamed Nor and Obbard, 2014; Zhao et al., 2014) and a common source is polyester clothing from washing machine effluent, because wastewater treatment facilities may not effectively remove them (Browne et al., 2011; Dubaish and Liebezeit, 2013). Other sources of fibres include discarded and weathered polypropylene rope material used in maritime activities (Zhao et al., 2014), air filters, diapers and fishing nets (Mohamed Nor and Obbard, 2014). If ingested in high quantities, these fibres may intertwine forming larger balls that may restrict gastrointestinal canals (Murray and Cowie, 2011).

Films comprised a large proportion of plastics in water samples (Fig. 3.3) which is not surprising since packaging is in high demand in South Africa (Malikane et al., 2000). Films were also highly concentrated in water samples taken around the North Pacific Subtropical Gyre (Moore et al., 2001) and near mangrove habitats in Singapore (Mohamed Nor and Obbard, 2014). Films have a high surface area to volume ratio and a low density, and are thus a dominant type of plastic in the water column (Claessens et al., 2011). A

large number of pre–production pellets was found in sediment and surface water samples taken at Bayhead, although they did not form a large proportion of the total. Pellets commonly contaminate beaches and estuaries, with beaches adjoining heavily industrialised areas of New Zealand typically containing > 10,000 pellets per linear metre (Gregory, 1978) and an estimated 762 million pellets in a 50 × 78 m area of beach in Santos Bay, Brazil (Turra et al., 2014). This beach is near Santos harbour, a large industrial port (Fisner et al., 2013).

Most plastics in our samples were < 5 mm (Fig. 3.4), a pattern similar to that reported by Mohamed Nor and Obbard (2014) for mangrove ecosystems and (Moore et al., 2011) in two Californian river systems. Smaller plastics have different risks to organisms than larger plastics, since they have a higher surface area to volume ratio, allowing them to collectively carry higher contaminant loads (Martins and Sobral, 2011) and be encountered more often than larger particles (Zhao et al., 2014), posing a particular threat to filter feeding organisms (Moore et al., 2001). Plastics may accumulate pollutants such as PCBs (polychlorinated biphenyls) and PAHs (polyaliphatic hydrocarbons) (Hirai et al., 2011). PCB concentrations of around 41 ng/g, four times higher than background levels have previously been measured on plastic pellets on beaches near Durban (Ryan et al., 2012). Combined with levels of plastic pollution reported here, this implies a high risk to biota in the area that ingests plastic particles. The harbour in particular is an important nursery ground for 144 species of fish larvae (Guastella and Smith, 1994; Forbes et al., 1996; Harris and Cyrus, 1999) which may ingest these plastic particles (Wright et al., 2013b). However, the environmental hazards associated with plastics may also be high at the Isipingo and uMgeni estuaries as they are subjected to industrial effluents and impacts from informal settlements along their catchments (Kalicharran and Diab, 1993; Shozi, 2011). High heavy metal concentrations and high coliform counts were reported for both these systems and the Mdloti (Shozi, 2011; Olaniran et al., 2014).

3.6 Conclusion

In conclusion, I present the first quantitative information on microplastic pollution of South African estuarine habitats. Pollution levels are highest around metropolitan areas such as the Durban Harbour and may be retained in the area of the Bayhead in particular, due to low levels of water exchange with the ocean. Diminishing levels of pollution in estuaries away from the city centre and on their associated beaches highlight the local sources of plastics in these systems. Fibres, fragments and films that are generally < 5mm constitute the main plastic effluents being discharged from Durban's estuaries, although plastic fragments are the main concern at the harbour whilst fibre loads were more concerning at the other estuaries.

CHAPTER 4

Coastal shelf plastic concentrations at five sites along KwaZulu-Natal, South Africa

4.1 Abstract

Ocean pollution is a global issue; yet limited quantitative data on microplastic concentrations are available for the South African coastal shelf. Estuarine outlets within industrial areas that are found along the coastline serve as conduits for plastics and other pollutants to the ocean. This study aimed to investigate coastal plastic concentrations around KwaZulu-Natal. Fifty–eight manta trawl samples were collected and analysed over a period of one year. An average of 40063 ± 32841 plastic particles/km^2 was found in surface trawls. The highest concentrations of plastics were predominantly located towards the South of the Durban coastal area in summer and winter. The maximum concentration was found at the Isipingo site in winter with 122727 particles/km^2. Winter plastic concentrations were significantly higher than those in summer (46103 ± 29005 and 25757 ± 28205 particles/km^2 respectively). The main plastic types were fragments, films and fibres that were commonly white, clear, opaque, blue and black in colour.

4.2 Introduction

From the onset of production, 8300 million metric tonnes of plastic has been produced with $\approx 59\%$ being discarded (Geyer et al., 2017). The trillion's of plastic particles now afloat in our oceans is a global issue that cannot be overlooked (Eriksen et al., 2014). Rivers and estuaries are the major conduits, especially in urban settings (Bakir et al., 2014b; Wagner et al., 2014) and collectively account for $1.15 - 2.41$ million tonnes of plastics entering the oceans annually (Lebreton et al., 2017). Moore et al. (2011) estimated that 2.3 billion plastic particles, consisting mainly of foams, fragments and pre–production pellets, weighing 30 tonnes, flows out from both the Californian, Los Angeles and San Gabriel rivers over a period of 72 hours. Estuarine sediments are also inundated with plastic, much like in the Yangtze Estuary, which is considered one of the largest plastic dischargers, and holds an average of 4137 ± 2461 plastic particles/m^3 of sediment (Zhao et al., 2014; Lebreton et al., 2017). Other 'hotspots' are the Ganges, Mississippi and

Nile rivers (Lebreton et al., 2012), the Laurentian Great Lakes (Eriksen et al., 2013) and Singapore's coastal systems (Mohamed Nor and Obbard, 2014). South African urban estuaries are no exception (Naidoo et al., 2015), but the quantity and fate of plastics that are discharged from them, and are subsequently afloat along our local currents, are not well known. Quantifying this is important, since high plastic concentrations can bring about frequent interactions with marine life, especially when plastics closely resemble the size and colour of prey items (Clark et al., 2016; Di Mauro et al., 2017; Ory et al., 2017). These interactions can result in negative effects leading up from the cellular level (von Moos et al., 2012) to affecting an organisms overall health (Rochman et al., 2013b).

The South African coastline is 3400 km long and has 300 estuaries which are located in areas that vary in their level of urbanisation, making it ideal to investigate how urban development impacts ocean plastic concentrations (Harrison, 2004; Nel et al., 2017). The country discharges 0.09 – 0.25 Million Metric Tonnes (MMT) of plastics to the ocean annually and is ranked as one of the top 20 countries of mismanaged waste (Jambeck et al., 2015). Sixteen estuaries are found in close proximity to the city of Durban, located in KwaZulu–Natal (KZN), which is one of the largest industrialised centers along the South African coastline (Forbes and Demetriades, 2008; Ryan et al., 2012). Quantifying the coastal plastic levels in this area is important since plastics can accumulate and concentrate chemical pollutants. Ogata et al. (2009) for example found that pre–production pellets collected from South Africa contained high concentrations of hexachlorocyclohexanes (HCHs). These and other chemical pollutants can be detrimental to marine organisms (Rochman et al., 2013b).

Quantitative data on plastic concentrations in South Africa has been limited to estuaries (Naidoo et al., 2015), surf–zones, (Nel and Froneman, 2015) beaches (Ryan and Moloney, 1990; Madzena and Lasiak, 1997; Lamprecht, 2013) and beach clean–ups, whilst data on coastal and oceanic plastic concentrations are dated (Ryan, 1988) or from the African sector of the Southern Ocean (Ryan et al., 2014). Plastic concentrations, therefore, need to be quantified on the east coast to determine how, and in what concentration, plastic travels along local currents. For instance, an iconic feature of the KZN coastline is a semi–permanent cyclonic eddy (Guastella and Roberts, 2016), which may influence particle concentrations. The shelf around Durban is narrow and can slope down to 100 meters within 7 km from the coastline in some areas (Roberts et al., 2010). South-westward currents are usual within this part of the shelf (Schumann, 1986), but the shape of the coastline can create north-eastward currents with the formation of the cyclonic eddy (Roberts et al., 2010). There is also a seasonal difference in rainfall, with more rain and run-off generally in summer, possibly influencing coastal plastic concentrations (Forbes and Demetriades, 2008). The city of Durban has been developed around an industrialised harbour and

many storm water channels run into it (Forbes and Demetriades, 2008). These channels may act as vectors for pollutants, including plastic, as industries in the area use primary microplastics that may inadvertently enter the ocean, via the estuary. I therefore aimed to (1) investigate ambient plastic concentrations along the KwaZulu–Natal coastal shelf and to (2) determine if there are seasonal differences in plastic concentrations. The objectives were to collect and analyse water samples from five sites along the KwaZulu–Natal coastal shelf, using a manta trawl. It was hypothesised that plastic concentrations are higher in the Durban area and at coastal sites further south, since southward movement may be aided by the Agulhus current. Since there is higher rainfall in summer, it was further hypothsised that would cause greater run–off and therefore higher plastic concentrations in the ocean.

4.3 Material and Methods

Five sites along the KwaZulu–Natal coastal shelf were sampled during the spring/summer season (02/09/2016 to 30/11/2016) (Fig. 4.2). The following year, samples were collected at the same sites starting from the winter season (03/08/2017), with the exception of Sodwana. Although winter conditions were still present, the sampling encroached into spring at the last site of iLovu (15/09/2017). All samples were collected with a manta trawl typically used to collect surface plastics (Fig. 4.1). A buoy was fitted to the top of the net to keep the net afloat and for recovery in the case of rope or knot failure. The trawl net was a 333 µm mesh, which conformed to most studies (Clark et al., 2016), and the cod end was fitted with a stainless steel collecting jar. The width of the net opening was 45 cm. The net was trawled for six minutes against the direction of the current and GPS co–ordinates were recorded. Trawls were generally around 500 m and were done at the side of, and 25 meters behind a research vessel traveling at 2–3 knots. Plastic concentrations were calculated as the number of plastic particles/km^2 according to Brunner et al. (2015) and Viršek et al. (2016). Five replicate tows were done along a single transect at each site during each season, except during the winter sampling for Isipingo where only three replicates were collected due to equipment failure. Water depth at each site was 35–40 m, which was within 5 km from the shore. Sub-surface samples were collected only in summer at Sodwana and at winter in Durban and Amanzimtoti, since sub-surface sampling was only done in very calm conditions. I was unsure if the trawl design was adequate to collect sub–surface samples due to the wings of the trawl providing lift to the net rather than a design suited for sinking. During these tows, the bouy was removed and attached to a 10 m rope and the net was allowed to sink while towing speeds were reduced.

Each sample was decanted into hard plastic 1 L polyethylene bottles and kept out of direct sunlight, for transport to the laboratory. At the laboratory, 1000, 500 and 250 µm stacked sieves were rinsed and

examined under the microscope for contamination. Water samples were then sieved into these and left to dry after being covered with foil, to prevent airborne contamination. Once dry, the contents of each sieve were analysed under a dissecting microscope to isolate and enumerate any microplastics present. Plastic particles were classed into morphotypes such as plastic fragments, fibres, films, line, polystyrene and pellets, according to Hidalgo-Ruz et al. (2012), The class 'other' was plastic that did not fall within the common categories. Plastics that were present in the sieves were removed using a pair of forceps and placed in zip sealed bags. A close eye was kept when analysing samples for any particles that may resemble fragments of the new green rope that was used to pull the net. Spectroscopy was not used to further identify plastic particles since particles captured by the trawl mesh were large enough to be confirmed plastic under the microscope.

A nested anova was run on *R* to compare the average plastic concentrations among seasons. Sites and the depth of sample were nested in seasons. The plastic concentrations from all three sieves were combined for each replicate. The residuals of the ANOVA resembled that of a normal distribution ($W = 0.966$, $p = 0.109$), using a Shapiro–Wilk normality test and they were plotted against the fitted values to observe for equality of variance.

Figure 4.1. Manta trawl used for sampling coastal plastics on the KwaZulu–Natal shelf.
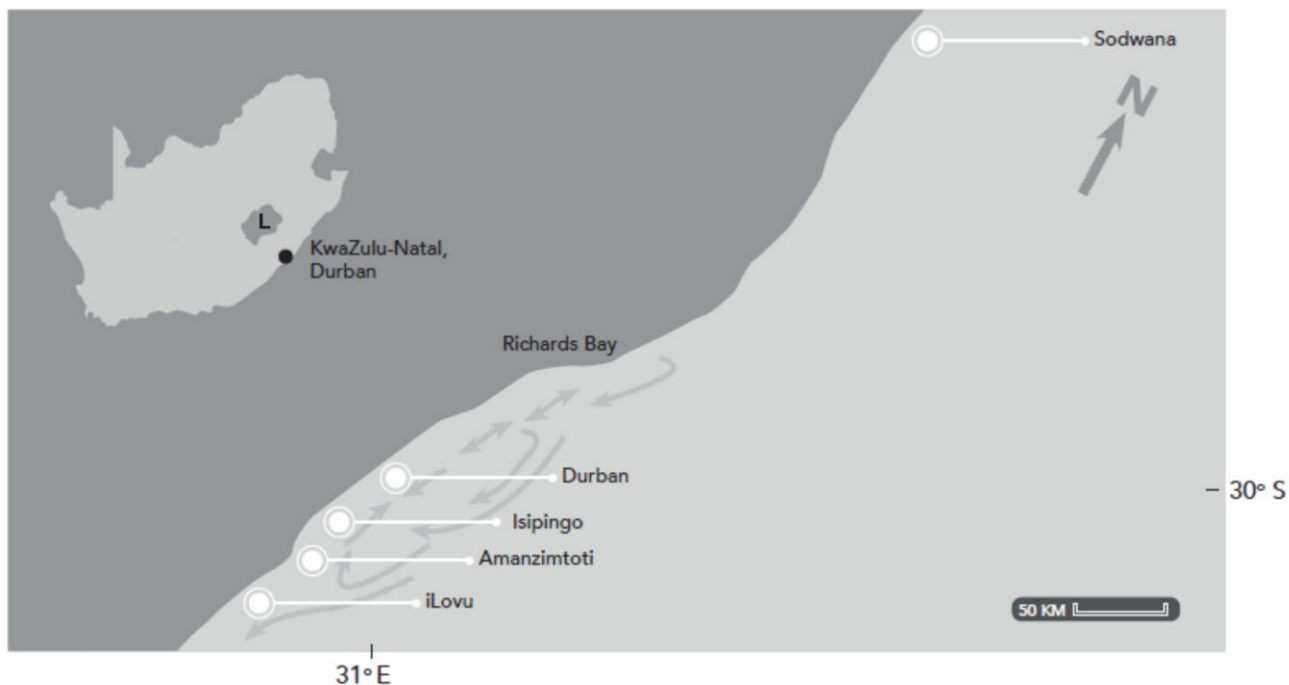
Figure 4.2. Sampling sites along the KwaZulu–Natal coastline, South Africa. Current lines were adapted from deLecea (2012) and Roberts et al. (2016). The letter 'L' denotes Lesotho.

4.4 Results

4.4.1 Plastic concentrations

Plastic particles were found at all sites except a single replicate each from Isipingo and Sodwana, in summer. Plastic concentrations varied and an overall average of 35579 ± 30141 particles/km^2 were found for all samples, whilst an average of 40063 ± 32842 particles/km^2 were found for surface trawls only. Winter plastic concentrations were significantly higher than those in summer (46104 ± 29005 and 25758 ± 28206 particles/km^2 respectively, $F = 21.632$, $df = 1$, $p = <0.001$). There was also a significant interaction between season and sites ($F = 16.753$, $df = 7$, $p = <0.001$) but no interaction among seasons, sites and depth ($F = 0.644$, $df = 3$, $p = 0.591$). The highest concentration of plastic was found at Amanzimtoti in summer (95455 particles/km^2) and at Isipingo in winter (122727 particles/km^2, Fig. 4.3). These were the only two sites that had significantly different plastic concentrations within the respective season (Fig 4.3). Sodwana had the lowest surface plastic concentrations (9091 ± 5567), yet this did not significantly differ from other sites, although Sodwana was not sampled in winter. Surface and sub-surface concentrations did not differ within sites (Sodwana summer, $p = 1.000$, Durban winter, $p = 0.999$ and Amanzimtoti winter, $p = 0.999$).
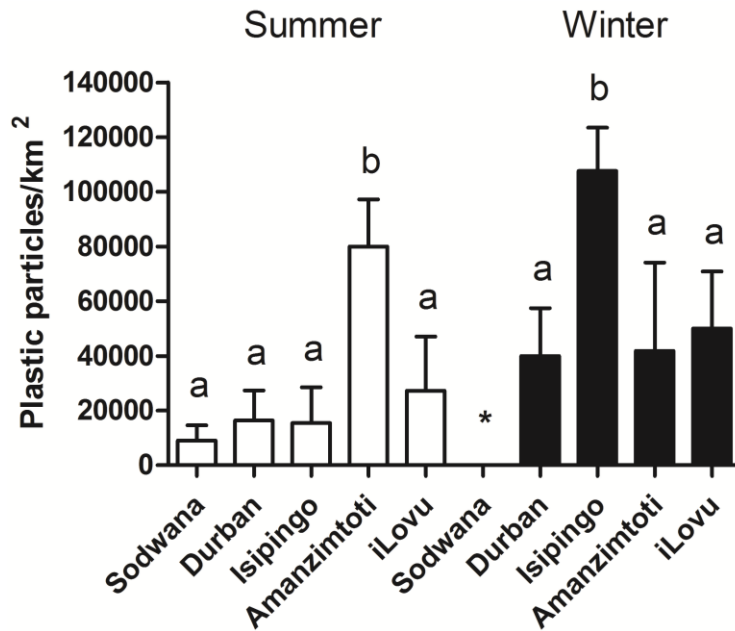
Figure 4.3. The average number of plastic particles/km^2 at five sites along the KwaZulu–Natal coastline. Bars represent the standard deviation (+ S.D.) and letters in lower case represent post–hoc tests within each season.* – Not sampled.

4.4.2 Plastic types, colour and sizes

Overall, fragments, fibres and film contributed the largest portions to the total plastic pool (Fig. 4.4). This ranged from 23.3 – 72.7% for fragments, 2.3 – 43.3% for fibres and 10.8 – 33.3% for film. In summer, Amanzimtoti had the highest proportion of pellets and fragments, while Isipingo had a higher proportion of line compared to the other sites (Fig. 4.4). Polystyrene was more prominent during winter and all sites displayed similar ratios of film. The main plastic colours were white, clear, opaque, blue and black. Higher proportions of clear, green and pink particles were found in summer, whilst white particles were dominant in winter (Fig. 4.4). Red and yellow particles also featured during winter (Fig. 4.4). Plastics at iLovu were composed of a larger variety of colours in winter compared to mainly clear particles during summer (Fig. 4.4).
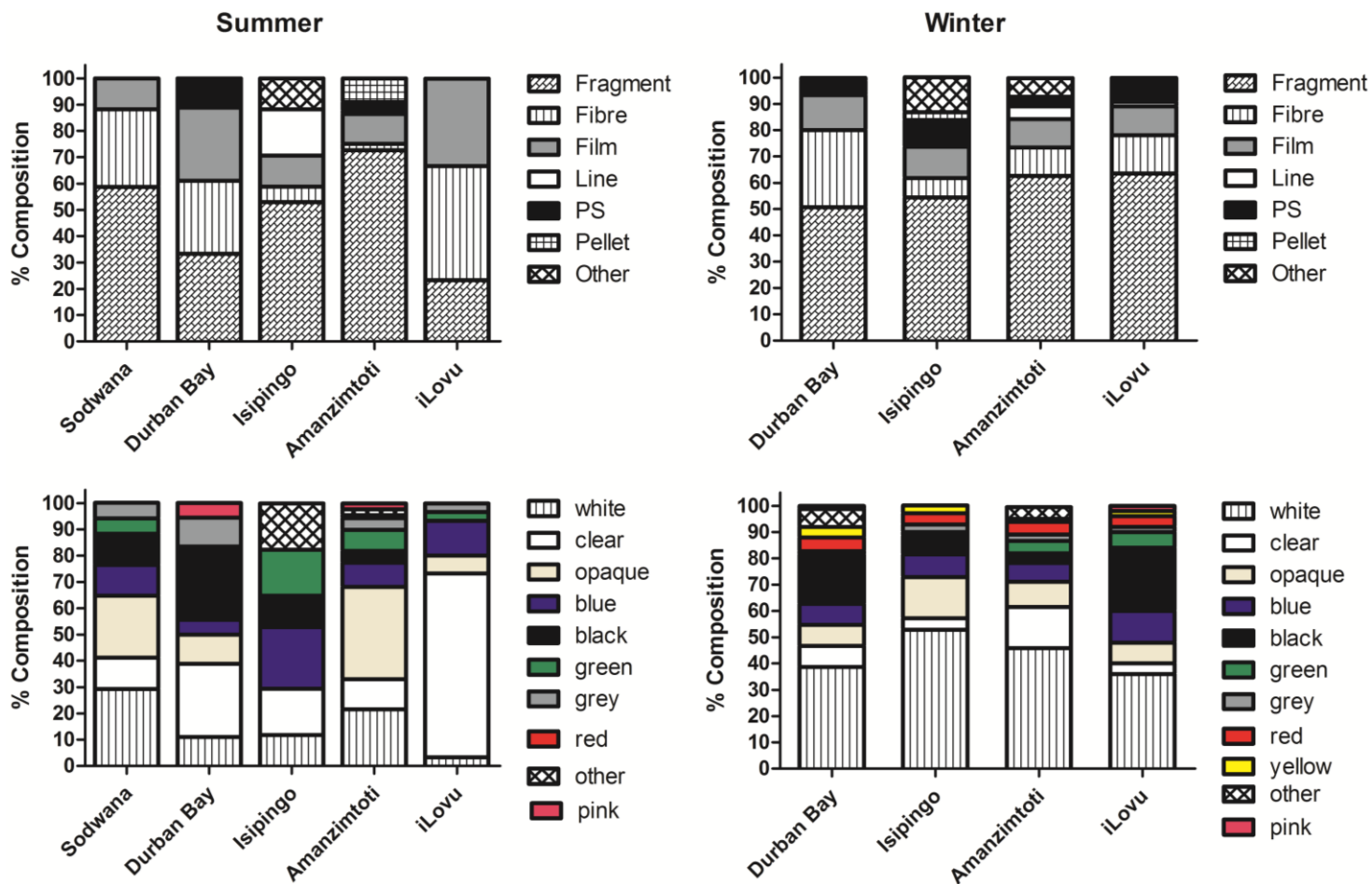
Figure 4.4. The proportion of plastic types and colours found in coastal samples along KwaZulu–Natal. PS – Polystyrene.

During summer, Sodwana and iLovu had higher proportions of smaller particles < 1000 μm in length, which accounted for 71% and 77%, respectively; while Durban, Isipingo and Amanzimtoti, had mainly larger particles > 1000 μm, accounting for 56%, 63% and 49%, respectively (Fig. 4.5). Particles that were > 5000 μm were found only at Durban, Isipingo and Amanzimtoti, with the largest proportion occurring at Isipingo and accounting for 25% of the particle size range (Fig. 4.5). During winter, plastic particles > 1000 μm contributed > 40% to each site (Fig. 4.5). Particles > 5000 μm were found only at Amanzimtoti and Isipingo during winter, although at low proportions of 1.2 and 1.4%, respectively (Fig. 4.5).
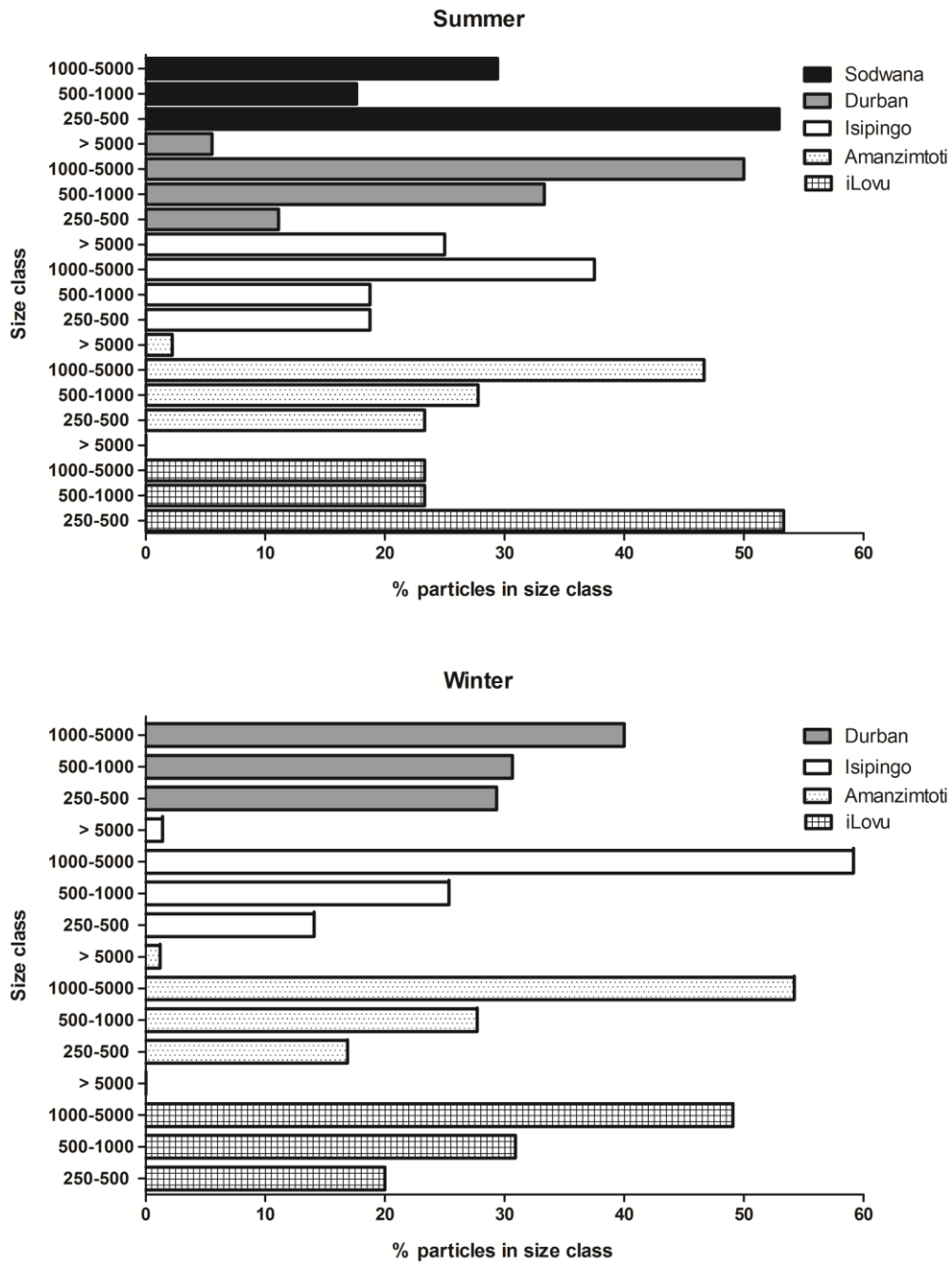
Figure 4.5. The size distribution of plastic particles collected in manta trawls along the KwaZulu–Natal coastline. Summer and winter data are presented.

4.5 Discussion

Plastic particles were found at all sites, with Amanzimtoti and Isipingo having the highest concentrations in summer and winter, respectively. These sites have high levels of urbanisation. In addition, plastic particles moving in from the nearby Durban urban area may account for these high concentrations. Sodwana was expected to have the lowest plastic concentrations, which it did, but did not significantly differ from the other urban sites further south. This implies that some degree of long range movement of microplastics may occur into the Sodwana area, either via the Agulhas Current possibly from along Mozambique or an inshore counter–current from further south, since this site is relatively free from major industries and major urbanisation. Ryan (1988) noted that the Agulhas Current is a major conduit for plastics transport toward the Western Cape, but just how far north of the coastline it accumulates most particles is not well known. Possible pollution sources could be Richards Bay, Kosi Bay and Maputo. Plastics have also been shown to move into this area from the Durban area via a northward moving inshore counter–current, even though most may get stranded on the nearby Durban coastline or move south via the Agulhas Current (Steinke and Ward, 2003). This movement was shown by Steinke and Ward (2003) who used plastic drift cards to assess mangrove dispersal patterns and found that 38% of the cards dropped inshore at Durban were recovered within 2–3 km, whilst offshore cards could travel as far north as Sodwana or even south and to the west coast of the country. As a result, plastics have the possibility to move far from their source rapidly, since the northward flowing counter–current can reach speeds of 1 m/s (Guastella and Roberts, 2016). More evidence for movement of particles in both directions was found by Guastella and Roberts (2016), who used satellite drifters to track currents along the coast. Two of the five drifters deployed in the Durban Eddy moved northward with the counter–current while three followed the southwestward flowing Agulhas Current. Plastic particles may also spend some time in the cyclonic rotating Durban Eddy, as surface drifters have done (Guastella and Roberts, 2016). Complicating particle movement even further is the presence of another rotating current just north the Durban Eddy, the Durban Swirl, which may also help move plastic particles northwards, and even back into the Agulhas Current (Roberts et al., 2016). This intricate pattern of currents along the coastline could be a possible reason as to why Nel et al. (2017) did not find clear trends of plastic pollution and population densities in the area, and proposed that long range transport of plastics is dominant in the area.

Ryan (1988) also noted that the Agulhas Current was responsible for distributing plastics towards the south western Cape of South Africa and found it to hold an average of 3640 plastic particles/km^2, 30 years ago. On the KwaZulu-Natal coastline, I found eight times the amount of plastics, composed of similar types as found by Ryan (1988), which included foams, fragments, pellets and fibres. Film material now

features more prominently than in the study by Ryan (1988), which could suggest that proportion of packaging material now used and discarded in South Africa is higher (Malikane et al., 2000).

Table 4.1. Plastic concentrations in decreasing order from similar studies.

Area of study	Average	Maximum	Reference
	Plastic particles/km^2		
Arabian Gulf		1460000	Abayomi et al. (2017)
Baltic Sea – Near Stockholm	421000		Gewert et al. (2017)
North Pacific Central Gyre	334271		Moore et al. (2001)
North Atlantic Subtropical Gyre		>200000	Law et al. (2010)
Mediterranean	116000	890000	Collignon et al. (2012) & Eriksen et al. (2014)
Baltic Sea – Offshore	46500		Gewert et al. (2017)
Laurentian Great Lakes	43000	466000	Eriksen et al. (2013)
KwaZulu–Natal Coastal Shelf	**40063**	**122727**	**This study**
Belgian Continental Shelf	4250		van Cauwenberghe et al. (2013a)
Tasman Sea – Offshore	685		Rudduck et al. (2017)

Compared to other parts of the world, including major oceanic gyres, the plastic concentrations found on the KwaZulu-Natal coastline were relatively low (Table 4.1). For example, Moore et al. (2001) found eight times more plastic in the North Pacific Central Gyre on average, and Abayomi et al. (2017) found eleven times more plastic in the Arabian gulf by maximum, than the KwaZulu-Natal coastline. Lower plastic concentrations were also found at the urbanised Stockholm coastal shelf (Gewert et al., 2017). For comparison, the plastic concentrations found on the KwaZulu-Natal coastline were instead, similar to those offshore in the Baltic Sea (Gewert et al., 2017) and the Laurentian Great Lakes (Eriksen et al., 2013). The plastic morphotypes in these studies were similar to those found here, which included fragments, fibres, polystyrene and films as dominant types. Plastics were mainly white, clear, opaque, blue and black. Blue and black particles were also the most common colours found on the south-eastern coastline of South Africa (Nel and Froneman, 2015). The colours of these particles are of importance as it

could affect the likelihood of marine organisms ingesting them (Ryan, 1987; Ory et al., 2017). The size of particles also determines which marine organisms can ingest them. For example, the particles found here were relatively large and therefore may not be available to small filter feeding organisms but may be consumed by fish and other vertebrates (see Appendix C).

Unexpectedly, plastic concentrations in winter were higher than in summer. Possible reasons for this could be that the samples were taken on different days at different sites and, therefore, not well standardised to pick up on the differences in rainfall. It is also likely that prevailing wind and currents in winter accumulated particles within a particular sampling area at the time of sampling. Since seasonal sampling was done during different years, it could also be that inter-annual variation 'hides' these seasonal differences. Inter–annual variation is common and can be quite large in some areas (see Rudduck et al., 2017). Particle movement in this zone is also difficult to track since it is a transitional zone, with particles moving toward the beach and also being washed back offshore (Lebreton et al., 2012; Isobe et al., 2014).

4.5.1   Caveats, future work and recommendations

One caveat of this study is that it fails to capture the lower end of the microplastic size spectrum and thus underestimates microplastic concentrations (Conkle et al., 2017). Collecting plastic particles using a finer mesh and with alternate methods should therefore be done in future. In addition, including more sub-surface and sediment samples is also important since estimates are that only $< 10\%$ of ocean plastics are found on the surface (Clark et al., 2016). The use of a Tucker trawl seems to be more appropriate for sub–surface samples (Brunner et al., 2015). There is also a need to better track these particles on the coast since we have an intricate eddy current system. This is important since clean–up operations would also be more effective (Sherman and Van Sebille, 2016). I also recommend that samples be collected at closer intervals and dates within the same year, if logistically possible, to better investigate if there are any seasonal differences in plastic counts caused by the increased rainfall and run-off during summer months.

CHAPTER 5

Plastic ingestion by estuarine mullet *Mugil cephalus* (Mugilidae) in an urban harbour, KwaZulu–Natal, South Africa

5.1 Abstract

Coastal urban environments have high plastic pollution levels, and hence interactions between plastic debris and marine life are frequent. We report on plastic particles found in the guts of mullet *Mugil cephalus* in Durban Harbour, KwaZulu-Natal, South Africa. Of 70 mullet (13.0 – 19.5 cm total length), 73% had plastic particles in their guts, with a mean of 3.8 particles per fish (S.D. 4.7). The number of plastic particles consumed showed no relation to digestive tract content or fish length. White and clear plastic fibres were ingested most commonly. This urban population of *M. cephalus* had a higher incidence of plastic in their gut than has been reported in fish from other coastal areas with similar levels of pollution.

5.2 Introduction

Plastic has become essential to many daily activities due to its low mass, high durability and low production cost (Koelmans et al., 2014); as a result, global plastic production has increased steadily and currently exceeds 299 million tonnes per year (PlasticsEurope, 2015). Single use of plastics by modern society has added to the contamination of the global marine environment (Browne et al., 2011). The presence of plastic in the environment facilitates the interaction with plastic by organisms, and the potential for its ingestion. For example, fish were found to ingest plastics in coastal areas (Dantas et al., 2012), the open ocean (Boerger et al., 2010) and even at depths of 850 m in the Mediterranean Sea (Anastasopoulou et al., 2013). Increasing plastic production and discards may make these encounters more frequent (Thompson et al., 2009b). Ingestion rates in some habitats may be high. For example, Davison and Asch (2011) estimated that planktivorous fish in the North Pacific Central Gyre could consume between 12000 and 24000 tonnes of plastic per year. Fish that ingest plastics show signs of a

false sense of satiation (Ramos et al., 2012), slowed digestion rates (Jackson et al., 2000) and liver toxicity (Rochman et al., 2013b), and there is a possibility that they could have increased buoyancy, which may affect vertical migration (Boerger et al. 2010).

Estuarine biota, especially those near industrial hubs, may encounter higher water column and sediment plastic loads than those in the open ocean (Bakir et al., 2014b). Given that estuaries are important nursery areas for juvenile fish (Cyrus and Forbes, 1996; Forbes and Demetriades, 2008), plastic debris may pose a severe threat to this life-history stage. Durban Harbour on South Africa's east coast has been reported to contain 144 species of juvenile fish and the threat of plastic pollution in this environment may potentially affect fish stocks (Harris and Cyrus, 1999; Markic and Nicol, 2014). The most commonly caught species in Durban Harbour is the flathead mullet *Mugil cephalus* (Linnaeus, 1758) (Pradervand et al., 2003). Juvenile and subadult mullet usually use estuaries as nursery areas (Lamberth and Turpie, 2003). Under laboratory conditions, a single individual of this species (measuring just 25 mm standard length) was found to ingest as many as 45 particles within 19 hours (Hoss and Settle, 1990). However, the incidence of *in situ* plastic ingestion by *M. cephalus* needs to be quantified.

Mugil cephalus was therefore used as a sentinel species to investigate the ingestion of plastics in the heavily industrialised Durban Harbour. The number of plastic particles found in mullet guts are referred to as ingested plastics in this chapter. Mullet are considered to be a good indicator species, being cosmopolitan in coastal ecosystems globally, with the exception of the polar regions, and found in fresh water, brackish water and marine environments (Whitfield et al., 2012). Mullet are consumed by carnivorous fish and birds entering coastal systems, providing a potential pathway for the transfer of plastics to high trophic levels (Farrell and Nelson, 2013). I hypothesised that *M. cephalus* from Durban Harbour would ingest plastic particles, and that the amount of plastic ingested would be influenced by the total length and digestive tract contents of the fish. If larger fish ingested more plastic particles than smaller fish, this would imply that by ingesting more food, larger fish may encounter more plastics. If smaller fish ingested similar amounts of plastic to larger fish, this would imply that proportionally more plastic is retained in the digestive tract of small than large fish when other waste material is egested.

5.3 Material and Methods

A total of 70 late-juvenile and subadult mullet were collected using a castnet on 11 November 2014 at the Bayhead mangroves of Durban Harbour, KwaZulu–Natal, South Africa (29°53′20.44″ S, 31°00′35.35″ E). Mullet were euthanised by a concussion method, which was approved by the Ethics Committee of the

University of KwaZulu–Natal (Reference: 113/14/Animal), then placed on ice and transported back to the laboratory. The total length (TL; cm) of each fish was recorded, the digestive tract removed and the total mass (g) noted. Digestive tracts were dissected and their contents, consisting mainly of benthic microalgae and sediment, were placed into glass Petri dishes. All Petri dishes containing digestive tract content were rinsed, dried with compressed air and checked under a microscope for any plastic contaminants. Each dish was covered with a second dish and placed in an oven at 60 °C for 24 h before being examined. Those containing digestive tract contents were examined under a dissecting microscope for microplastics, following the methods of Boerger et al. (2010). Metal forceps used for examining digestive tract contents were checked first for plastic contamination under a dissecting microscope. In addition, a cotton laboratory coat was worn to ensure that synthetic fibres from clothing did not contaminate samples. To determine whether contamination was a confounding factor under laboratory conditions (see Davison and Asch, 2011; Foekema et al., 2013), three replicates of empty Petri dishes were situated as follows: (i) open on the bench top; (ii) open in the oven; and (iii) covered with another dish, in the desiccator. On examining these dishes after 24 h, fibre contaminants were present in one of the open bench-top dishes and in two of the open dishes left in the oven, but none were found in the covered dishes. Therefore, it was assumed that this investigation was at minimal risk of airborne contamination.

Plastics found in samples were characterised according to colour and type following (Lusher et al., 2013). Small particles were removed carefully and placed into glass cavity slides to be photographed, identified and measured under a Nikon ECLIPSE Ti Series inverted microscope, fitted with a DS–US camera powered by NIS–Elements BR software. After plastics had been removed from samples, the dry masses of digestive tract contents were recorded.

5.3.1 Statistical methods

A general linear model (GLM) was used to assess the relationship between the number of plastic particles found in mullet and their total length and the dry mass of their digestive tract contents, using *R* 3.2.1 (R_Development_Core_Team, 2015). In all, four rows with missing data were deleted and a Hosmer–Lemeshow goodness-of-fit test was used to assess the fit of the model using the ResourceSelection package for R. There was no evidence that the model did not fit well (χ_2 = -7.716, *df* = 8, *p* = 1.000).

5.4 Results

Plastic particles were found in 51 fish, comprising 72.8% of the 70 mullet sampled. A total of 260 plastic particles at a mean of 3.8 particles per fish (S.D. 4.7) were found and a maximum of 23 particles were

found within a single fish of 16.7 cm TL. Plastic particles ranged from 0.2 to 15 mm; however, only a subset of particles were measured at random which included the smallest and largest particles. Mullet length ranged from 11.0 to 19.5 cm, with the majority within the 13.0 – 17.9 cm size range. The GLM suggested that neither fish length ($t = 0.803$, $df = 62$, $p = 0.425$), nor the dry mass of digestive tract contents ($t = 0.501$, $df = 62$, $p = 0.618$), nor the interaction between them ($t = -0.683$, $df = 62$, $p = 0.497$), exhibited a significant relationship with the number of plastic particles ingested per fish (Fig. 5.1).

Fibres were the most common plastics found in the digestive tracts under study, comprising 51.2% of the total. Fragments, polystyrene, films, monofilament line and twine contributed 34.6%, 7.3%, 5.0%, 1.5% and 0.4%, respectively, to the overall composition of plastics found. Fragments were the most colourful with white (41.8%), clear (22.0%), opaque (13.2%) and black (5.5%) the most abundant, whereas other types of plastics were mainly clear and white, except twine that was red only. Of the total, 4.8% of fragments could not be identified conclusively as plastic due to their small size and fouling from other gut contents (Fig. 5.2). These fragments were, however, included in all analyses due to their strong resemblance to plastic fragments.
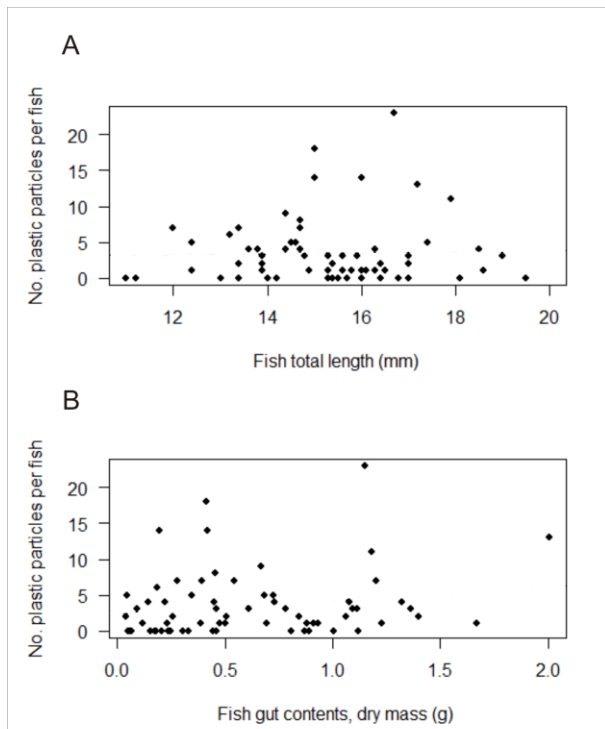
Figure 5.1. Relationship between number of plastics ingested by *Mugil cephalus* and (a) fish total length and (b) fish gut content dry mass.
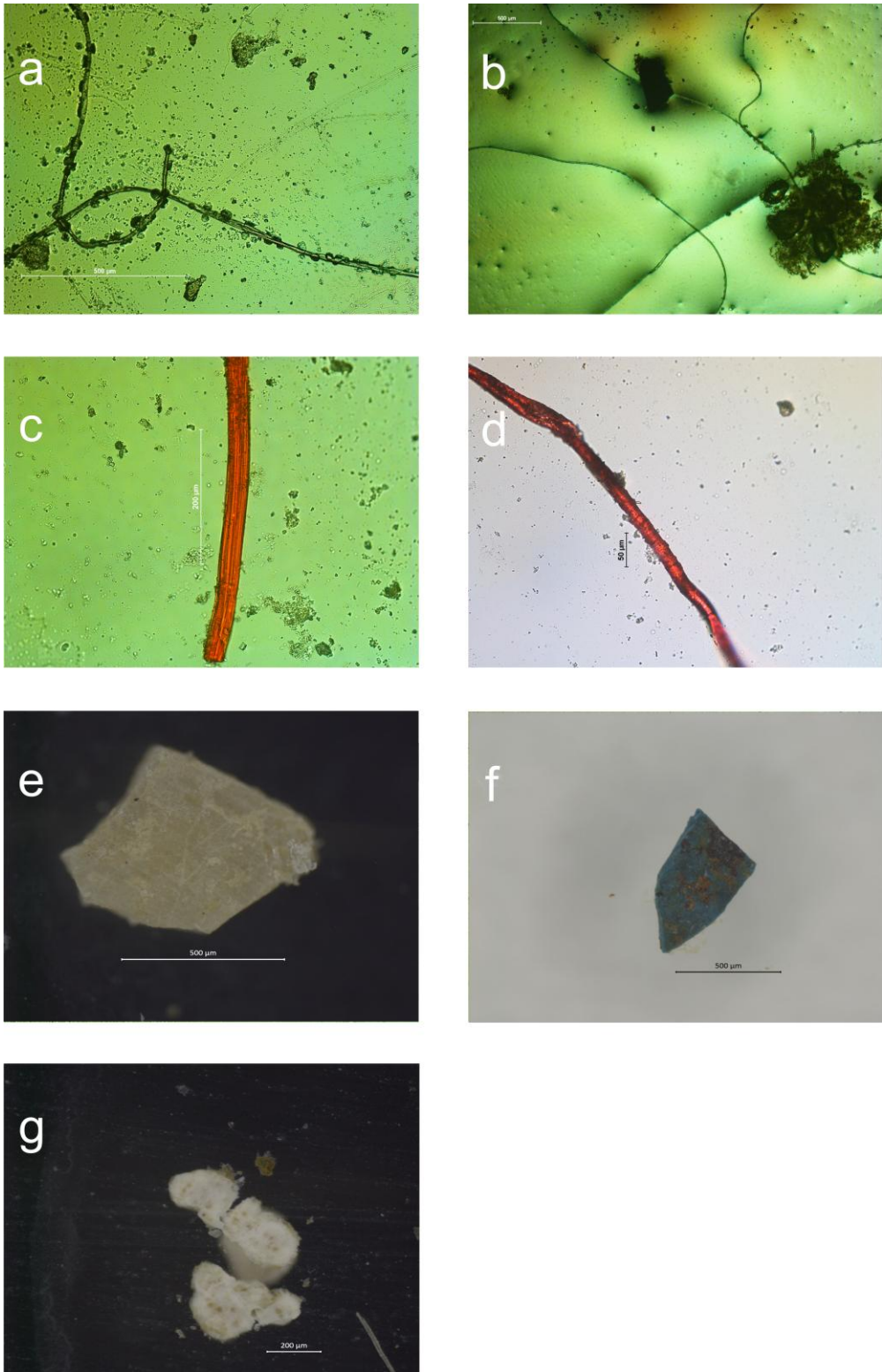
Figure 5.2. (a–d) Examples of fibres, (e) opaque fragment with fracture lines, (f) blue fragment with fouling on its surface, and (g) polystyrene particles found in the digestive tracts of *Mugil cephalus* specimens collected in Durban Harbour.

5.5 Discussion

In all, 73% of *M. cephalus* examined contained plastic particles, validating the hypothesis that this species in Durban Harbour is prone to plastic ingestion. This proportion is higher than that found in other *in situ* studies of plastic ingestion by fish (Appendix C), with the next highest being ingestion rates of 51 – 52% by red gurnard *Aspitrigla cuculus* and blue whiting *Micromesistius poutassou* collected in the English Channel (Lusher et al. 2013). Compared to fish examined by Boerger et al. (2010), the maximum number of plastic particles ingested by a single *M. cephalus* from Durban Harbour was four times lower, yet the average number of particles ingested by *M. cephalus* was considerably higher. Among marine environments, including the harbour in this investigation, the lowest incidence of plastic ingestion was found in fish from deep waters (Anastasopoulou et al. 2013).

The high incidence of plastic ingestion by *M. cephalus* in this study may be due to their mode of indiscriminate benthic feeding (Whitfield et al. 2012), because plastics accumulate in intertidal sediments of Durban Harbour (Naidoo et al., 2015). In a study by Hoss and Settle (1990), experimentally fed early juvenile *M. cephalus* ingested five times more plastic than did five other fish species, with up to 75% of fish consuming polystyrene spheres 210 – 350 μm in diameter. These experimentally fed plastics were at the lower end of the size range found in our samples.

Mugil cephalus may change their diet as they grow, with increasing amounts of detritus and sediment in the guts of larger fish (De Silva and Wijeyaratne, 1977), which may affect the type and quantity of plastic ingested. Boerger et al. (2010) recorded a higher number of plastic particles on average in larger fish. However, we did not find differences in plastic ingestion relative to fish size (Fig. 5.1), implying either that: (i) smaller mullet may be able to access areas that have higher sediment plastic concentrations; (ii) smaller mullet may retain more plastic particles per gram of digestive tract because their digestive tracts could be less developed and take longer to egest the particles; or (iii) the total size range of fish in our samples was not wide enough to show differences. Nevertheless, the possibility of plastic being retained in the digestive tract and any associated negative effects on fish warrants further experimental investigation. Smaller juvenile mullet (2.1 – 2.5 cm) than those investigated here were found to consume plastic particles (Hoss and Settle 1990) and could be included in further investigations.

Most plastic types ingested by *M. cephalus* were consistent with other studies on ingestion of microplastics by fish (e.g. Boerger et al., 2010; Ramos et al., 2012; Foekema et al., 2013; Lusher et al., 2013) (Appendix C). However, habitat use may affect the type of plastic most likely to be ingested. For example, fibres formed the lowest proportion of the plastic ingested by pelagic–feeding fish in the

Mediterranean Sea (Anastasopoulou et al. 2013), but the highest proportion in benthic– or demersal–feeding fish, such as *M. cephalus* collected in Durban Harbour and gudgeons *Gobio gobio* from an urban French river (Sanchez et al., 2014).

Although there are no comparable studies of plastic ingestion by benthic fish from deep seas, Woodall et al. (2014) reported that the deep sea has become 'a major sink' for fibres, with concentrations of up to 15 fibres per 50 mL in the Mediterranean deep-sea sediment, whereas up to 10 fibres per 50 mL were found in Durban Harbour (Naidoo et al. 2015). Potentially, therefore, rates of ingestion may be similar to those found in urban environments. Fibres are probably derived from clothing and other synthetic textiles, because fibres found in marine environments around the world closely resemble those found in washing machine effluent and have the potential to pass through sewage treatment works (Browne et al., 2011). Other sources of fibres found in the harbour may include weathered fishing and boat–mooring equipment (Guastella and Smith, 1994; Murray and Cowie, 2011).

Most of the plastics ingested by *M. cephalus* were clear or white. However, clear and white plastic particles together constituted the majority of all plastic particle colours in nearby sediments (Naidoo et al. 2015) and in offshore waters (Ryan, 1988), implying that ingestion of particles of these colours is not selective. This concurs with studies of fish in the North Pacific Central Gyre (Boerger et al. 2010) and the English Channel (Lusher et al. 2013), and of estuarine fish of the Goiana Estuary, in north–east Brazil (Dantas et al., 2012; Ramos et al., 2012). However, possible selective feeding on clear and white plastic particles by longnosed lancetfish *Alepisaurus ferox* was observed by Choy and Drazen (2013).

The high incidence of plastic ingestion by *M. cephalus* found in this study may have negative impacts as reported for other fish species (Jackson et al., 2000; Ramos et al., 2012; Rochman et al., 2013b), which could ultimately lead to population effects.

5.6 Conclusion

This is the first *in situ* investigation of ingestion of microplastics by fish in South African waters. *Mugil cephalus* from Durban Harbour had a high incidence of plastic ingestion, with fibres being the primary type reported. Fibres may originate from domestic and industrial sources which pass through water treatment works and enter the sediment where the fish forage while feeding on benthic algae. Future work to investigate a range of species, coupled with laboratory experiments, is required to gain more information on the extent of plastic ingestion in urban settings and possible biological effects.

CHAPTER 6

Are nitric acid (HNO₃) digestions efficient in isolating microplastics from juvenile fish?

6.1 Abstract

A standard method for the detection and isolation of microplastics is required to adequately investigate plastic ingestion by juvenile fish. Dissections of juvenile fish guts require precise handling, which can affect processing time if sample numbers are high. To investigate the efficacy of nitric acid (HNO_3) in aiding the isolation of microplastics using whole fish, we digested juvenile glassfish, *Ambassis dussumieri* (Cuvier, 1828) at room temperature and at 80°C. For a complete digestion, overnight incubation in 10 mL of 55% AR HNO_3 was sufficient for a whole fish of 1 g at room temperature. When coupled with elevated temperature the digestion time is shortened to a few minutes and larger fish of 3 g can be digested in 30 minutes. Four of the five types of plastic survived the process, with nylon being the exception. This is a shortfall to the method; however, until a better method replaces it, we still value the use of HNO_3 for its simple, inexpensive, swift and complete digestions of whole fish. Four fish species from benthic and planktonic feeding guilds were digested using this method to validate its use. The number of plastic particles ingested did not differ between benthic and pelagic species and microplastic fibres comprised the majority of the plastic types found.

6.2 Introduction

Microplastic ingestion by larval and early juvenile fish (hereby termed early stages) has not been as well documented as their adult counterparts. Research has mainly been limited to experimental studies, such as de Sá et al. (2015). This is concerning as these early stages are vulnerable to environmental perturbations (Whitfield, 1990) and may suffer a more pronounced effect of microplastic ingestion due to the size of juvenile fish relative to microplastic particles. Juveniles may encounter plastics more often due to their sheltering and feeding under plastic debris within current lines in the ocean and using polluted estuaries,

which may also have high organic pollutant loads associated with plastic particles, as nurseries (de Sá et al., 2015).

Efficiently isolating microplastics from these early life stages of fish is a key step toward identifying how microplastic ingestion ranks among the many other mortality risks to fish populations and may thus guide future stock management (Markic and Nicol, 2014). Information on the size or stage at which a fish would be at risk of ingestion of a specific suite or size of microplastics is vital, as smaller organisms may not be able to consume all particles within the general microplastic size range (Cole and Galloway, 2015). In part, the reason for the lack of such information is the difficulty in isolating plastics from these small fish and the lack of a standard method to do so, making comparisons difficult (Avio et al., 2015). Unlike adult fish, examining the gut contents of juveniles may require high precision dissections, primarily due to their small size, making it difficult to process large sample numbers under time constraints (Khan et al., 2015). In addition, organic matter and other foreign particles consumed by fish may mask microplastics, complicating the isolation process (Lusher et al., 2017).

The use of nitric acid (HNO_3) to digest tissue for microplastic quantification Claessens et al. (2013), could aid the isolation process. The method has been considered as one of the more destructive plastic isolation techniques, since it may degrade plastics (Lusher et al., 2017), yet derivatives of it have been prescribed by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) as a monitoring protocol (Dehaut et al., 2016). It has also been used to isolate microplastics from clams (Davidson and Dudas, 2016), shrimp (Devriese et al., 2015) and lugworms (van Cauwenberghe et al., 2015). Desforges et al. (2015) suggested that more polymers should be tested using nitric acid isolation techniques to determine if they survive the digestion protocol. Despite the drawbacks, nitric acid may be the simplest, most inexpensive way of processing large sample numbers quickly, which is useful for routine bio–monitoring projects (Desforges et al., 2015).

Existing isolation methods include using other acids, strong bases, other oxidizing agents and enzymes to digest biological tissue (see Lusher et al. (2017) for a review). However, these have limitations. For example, Collard et al. (2015) found that using sodium hypochlorite did not degrade polyamides but reduced the mass of PVC, while other studies suffer from shortcomings such as long incubation times (Foekema et al., 2013), discolouration of plastics (Dehaut et al., 2016), using expensive digestion agents (Cole et al., 2014), staining tissues, rendering particles the same colour as the stain less visible (Davison and Asch, 2011), using many components and digestion steps making the process slower and at the end

still using HNO_3 to clear up membrane filters further (Collard et al., 2015), or grinding gut samples before extraction which could potentially invalidate counts if plastics are also broken down (Avio et al., 2015).

In my case, I aimed to adapt the methodology of Claessens et al. (2013) for use on juvenile fish. The proposed method would need to be quick, use realistic acid volumes and concentrations and be applicable to a range of plastic types. It would also need to have some degree of sample storage practicality to avoid plastic deterioration if samples are to be stored after the digestion preceding filtration. We used juvenile glassfish *Ambassis dussumieri* (Cuvier, 1828) as a sentinel species to investigate the method. This species can spend its entire life cycle in estuaries (van der Elst, 1993) and may therefore be influenced by the high particle concentrations generally found in estuaries, especially near urban areas (Naidoo et al., 2015). I aimed to expand on previous studies investigating the efficiency of the HNO_3 digestion method by examining the acid volume use, the time taken for digestions and the plastic types that survive the HNO_3 digestions. The objectives were to (1) digest juvenile fish of similar sizes in different volumes of acid to check for acid use efficiency (2) digest fish at an elevated temperature to examine if this increases efficiency (3) immerse different plastics in acid, either directly or within fish spiked with plastics, to assess if any plastic degradation occurs. Juvenile fish of four species from two feeding guilds were digested using the protocol to validate it and to assess the frequency of microplastic ingestion by juvenile fish in the harbour. I predicted that (1) a low acid volume would be sufficient to digest whole juvenile fish, without clogging up membrane filters (2) higher temperatures would speed up the reaction (3) that all plastic types would be resistant to acid digestion for at least 24 hours and that (4) microplastic concentrations and types from field collected fish will differ between species that belong to different feeding guilds.

6.3 Materials and methods

6.3.1 General procedures

Glassfish were collected from the Durban harbour, KwaZulu–Natal, South Africa (29° 52'S, 31° 04'E) using seine nets. Fish were rinsed with distilled water and fish mass (g) and standard length (mm) were recorded. All acid digestions were performed within a fume cupboard, using 55% AR HNO_3. Glass honey jars (350 mL) set in water baths and covered with watch glasses were used to contain the reactions and prevent contamination. All glassware and handling equipment were rinsed in distilled water and checked under the microscope for microplastic contamination before use. At the end of each reaction, the products were added to 100 mL of distilled water to dilute the digested products before vacuum filtration, for both handling and equipment safety. In pilot digestions 2 µm, 5 µm and 10 µm filters clogged up easily and

therefore 20 μm filters were used in this study. After filtration, filters were placed under a microscope and microplastics were counted and photographed. Forceps were then used to carefully transfer plastics to a mass balance. All statistical analyses were done on *R* 3.3.2 (R_Development_Core_Team, 2014).

6.3.2 Acid volume

This was the primary variable to determine since using too little acid may leave incomplete digestions that clog up membrane filters, making them difficult to examine and using too much would be wasteful. Twelve juvenile fish of similar size, 1.290 g ± 0.120 (S.D.) were placed into individual 350 mL glass honey jars containing three replicates of either 5 mL, 10 mL, 20 mL or 30 mL of acid and digested for twelve hours at room temperature. The digested tissue was vacuum filtered and filters were examined visually to determine the optimal volume for tissue degradation.

6.3.3 Tissue digestion

Fish of masses ranging from 0.124 to 3.027 g were digested in 10 mL HNO_3 at either room temperature or at 80°C in a water bath. For each fish, the length (mm) and mass (g) were recorded before acid addition. Once completely disintegrated, the time elapsed was recorded (min). An analysis of covariance (ANCOVA) was first run to determine if there were significant differences between the temperatures while controlling for fish mass. However, the assumption of homogeneity of regression slopes was violated (significant interaction between the independent variable and the co–variate). Therefore, mass, which is the co-variate was converted into categories of small (≤ 1.000 g), medium (>1.000 g ≤ 1.900 g) or larger fish (> 1.900 g) and a factorial analysis of variance (ANOVA) was then used to analyse the data. The elapsed time was log transformed to satisfy the assumptions of normality and homoscedasticity of the residuals. These assumptions were tested using a Lilliefors Kolmogorov–Smirnov normality test ($D = 0.078$, $p = 0.845$) and Levene's test for homogeneity of variance ($F = 2.460$, $df = 5$, $p = 0.056$), respectively. Multiple comparisons of the mass categories were performed with a Tukey's HSD test.

6.3.4 Plastics immersed directly in acid and spiked in fish

Plastic materials used for the digestions were obtained from previous sampling work and clean–ups done in Durban Harbour. Five polymers (plastic types) were used in the experiment. Polyethylene was duplicated to include thin film and microscrubbers with a high surface area to volume ratio (Table 6.2). Digestions were run by immersing three replicates of each of the plastic types directly in HNO_3 for one month at room temperature. To test if being enclosed within fish could influence the digestion outcome, plastics were also spiked into *Ambassis dussumieri* by opening up the gastrointestinal cavity and using a large syringe to insert plastics within. For larger plastic types, fine forceps were used to insert plastics into the cavity. These were then digested at 80°C. Experiments were run according to the general procedures

described above, with 10 mL HNO_3 placed in pre-washed glass honey jars. The different types of plastics that were used were in accordance with the common types recorded in field surveys (Naidoo et al., 2015) and those that had been ingested by fish in an urban harbour (Naidoo et al., 2016). Samples of all plastic types were run on Fourier Transform Infrared Spectrometry (FTIR) to determine the polymers that were used. The fixed mass of plastics in each replicate was recorded before being used. The mass of each plastic type immersed directly in acid was measured at various times during the one month trial period. Foam material was resuspended in distilled water after the digestion and dried to constant mass to get more accurate mass readings. During these measurements a metal sieve was used to capture the plastics for mass measurements before they were placed back into the acid. Larger plastic types were counted before being placed back into the acid and particles were measured at random to check for any difference in size. All fish used for the spiked digestions were of similar weight (1.091 ± 0.118 g) to keep mass standard.

Single paired sample t–tests were performed on each of the plastic types immersed directly in acid wherever possible to determine if the mass after one month had changed. Before paired t–tests were run, the assumption of normality was tested using a Shapiro–Wilk's test with $W = 0.964$, $p = 0.637$, $W = 0.964$, $p = 0.637$, $W = 0.964$, $p = 0.637$ and $W = 1.000$, $p = 1.000$ for film, polyester, PVC and scrubbers, respectively. The assumption of normality was not satisfied for foam data and therefore a Wilcoxon signed–rank test was performed. For experiments digesting fish spiked with plastics, a One–Way ANOVA was used to compare the loss of plastic mass during the digestion for the various plastic types. Data were log transformed. The residuals of the ANOVA were plotted to test if they resembled that of a normal distribution and a Levene's Test was used to test for homogeneity of variance ($p = 1.028$). Finally, a Tukey HSD test was used for comparison among plastic types.

6.3.5 Ingestion by juvenile fish

Microplastic abundance in four fish species, collected from the Durban Harbour was investigated (Table 6.1). Nine fish of each species were placed into individual 350 mL glass vials containing 1 mL of acid per 1 g of fish and digested overnight at room temperature. The digested tissue was then filtered and examined visually using a Zeis ™ DV4 dissecting microscope.

Table 6.1 Juvenile fish from two feeding guilds collected from Durban Harbour.

Feeding mode	Species name	Common name	Mass (g)
Zooplanktivorous	*Ambassis dussumieri* (Cuvier, 1828)	Glassfish	0.43-1.31
	Hilsa kelee (Cuvier, 1829)	Razorbelly	0.23-1.82
Benthivorous	*Silago sihama* (Forsskål, 1775)	Silversilago	0.66-1.92
	Gerres filamentosus (Cuvier, 1829)	Pursemouth	0.29-0.58

A One–Way ANOVA was used to compare the mean abundance of ingested microplastics among the four fish species. Data were log_{10} transformed and the assumption of normality was satisfied using a Shapiro–Wilk's test. A Tukey HSD test was used to compare the number of ingested plastics among the fish.

6.4 Results

6.4.1 Acid volume

Figure 6.1. Filter membranes after *A. dussumieri* digestions in 5 mL, 10 mL, 20 mL and 30 mL HNO_3.

A volume of 10 mL of HNO_3 filtered through a 20 µm membrane filter was found to be the ideal volume to use for the rest of the experiments. Filters with a smaller mesh sizes and a lower acid volume clogged up the membrane when digesting a fish (Fig. 6.1), while using a higher volume than 10 mL of acid was wasteful.

6.4.2 Tissue digestion
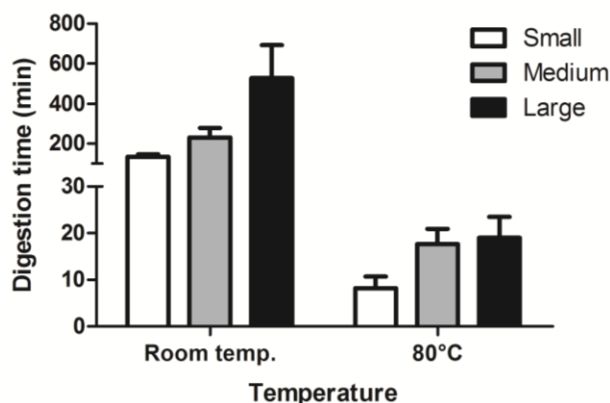
Figure 6.2. The mean digestion time (min) of fish placed in size categories based on their initial mass. HNO_3 digestions occurred either at room temperature or an elevated temperature of 80°C. Bars represent the standard deviation.

Fish digested more rapidly at the elevated temperature than those at room temperature (Fig. 6.2). The largest fish from the 80°C treatment digested 26 times faster than the largest fish from the room temp treatment (Fig. 6.2). Although the main effects of temperature and mass categories were significant ($F = 1334.3$, $df = 1$, $p = <0.001$ and $F = 65.143$, $df = 2$, $p = <0.001$ respectively), there was a significant interaction between the them ($F = 7.394$, $df = 2$, $p = 0.002$). Comparisons between combinations of temperature settings and mass categories were all significantly different at the 0.05 level, with the exception of the medium and large size categories digested at 80°C ($p = 0.996$).

6.4.3 Plastics digestion and fish spiking

6.4.3.1 *Plastics immersed directly in acid*

Overall, plastics used in each replicate had a mean of 0.050 ± 0.002 g (n = 42). The number of particles in each replicate ranged from just two pellet particles to numerous microscrubbers. Most plastic types tested survived the exposure to HNO_3 for the experimental period of one month, with the exception of nylon (Table 6.2). During the first 24 hours of exposure, all of the nylon particles were disintegrated completely by the HNO_3 (Table 6.2). No significant change in mass could be detected after film, foam, PVC and scrubber particles were immersed in acid for one month (Table 6.2). There was not enough variability to test the difference for pellets however there was very little mass change after one month (Table 6.2).

There was a small change in mass of polyester after one month; however this was not significant (Table 6.2).

6.4.3.2. Plastics spiked in fish

The change in mass differed overall among plastic types for spiked digestions (F = 57.650, df = 6, p = <0.0005). However, this difference was found only between nylon and all other plastic types (Table 6.2). The nylon completely disintegrated during the digestion, while other plastic types survived the digestion with no major loss of mass (Fig. 6.3). Apart from nylon, the most mass change was observed in foam material (0.010 ± 0.010 g) and the least change was observed in pellets and PVC, neither of which showed any change in mass. Other plastic types showed very limited change in mass, for example foam, film and polyester had slightly positive changes in mass of 0.010 ± 0.010, 0.002 ± 0.000 and 0.003 ± 0.000 g respectively, owing to the digestion debris that adhered to their surfaces (Fig. 6.3 C, I, L). Mass of scrubbers had a slightly negative change -0.002 ± 0.000 but none of these were significantly different from each other. Partial whitening occurred of the PVC particles immersed in acid which did not occur as much when spiked in fish (Fig. 6.3 N, O).

Table 6.2. Plastic polymers and size measurements (mm) used in the digestion experiments. Mean mass measurements (g) ± S.D. are shown for plastics immersed directly HNO_3 for one month and for those that were spiked in fish. Paired sample t–test results are given for plastics immersed directly in acid and for spiked experiments the different letters in smaller case denote significantly different change in mass among plastic types.

	Nylon	Pellet	Film	Foam	Polyester	PVC	Scrubber
Polymer	Polyhexamethylene nonanediamide	Polyethylene high density	Polyethylene high density	Polystyrene	Poly 1, 4 - Butylene terephthalate	Polyvinyl chloride	Polyethylene high density
Shape	Cylindrical	Round	Square	Round	Square	Irregular	Round
	L - 5 mm	L - 4 mm^2	L - 5 mm^2	L - 5 mm^2	L - 5 mm^2	L - 1.940 ± 0.610	D - 0.267 ± 0.050
	D - 0.434 ± 0.030		H - 0.128			W - 0.608 ± 0.139	n = 5
	n = 5					n = 5	
Directly immersed in acid							
before	0.050 ± 0.000	0.050 ± 0.002	0.050 ± 0.001	0.052 ± 0.002	0.049 ± 0.001	0.050 ± 0.001	0.050 ± 0.000
after 1 month	0	0.049 ± 0.003	0.048 ± 0.001	0.052 ± 0.002	0.044 ± 0.003	0.049 ± 0.002	0.049 ± 0.001
	All digested	*	$t = 2.6458$	$V = 0$	$t = 3.0237$	$t = 0.75593$	$t = 1.7321$
			$df = 2$	$p= 0.180$	$df = 2$	$df = 2$	$df = 2$
			$p = 0.118$		$p = 0.094$	$p = 0.529$	$p = 0.225$
Spiked in fish then digested in acid							
before	0.050 ± 0.000	0.045 ± 0.001	0.049 ± 0.001	0.050 ± 0.000	0.050 ± 0.002	0.050 ± 0.000	0.050 ± 0.000
after	0	0.045 ± 0.002	0.051 ± 0.002	0.060 ± 0.012	0.053 ± 0.002	0.050 ± 0.001	0.048 ± 0.002
Δ mass	-0.050 ± 0.000	0.000 ± 0.001	0.002 ± 0.001	0.010 ± 0.012	0.003 ± 0.003	0.000 ± 0.001	-0.002 ± 0.002
Tukey's group	*a*	*b*	*b*	*b*	*b*	*b*	*b*

D – Diameter, W – Width, L –Length, H – Height, * – test was not performed.
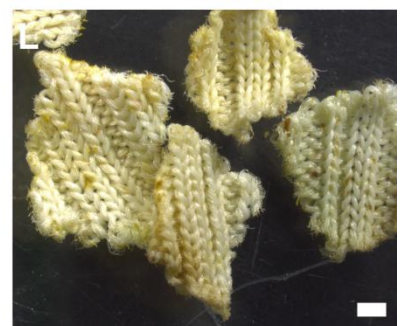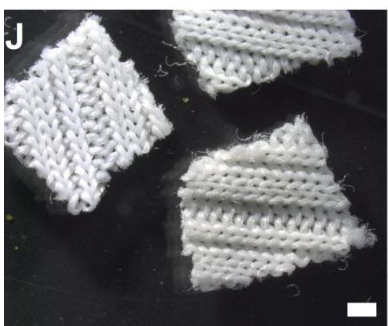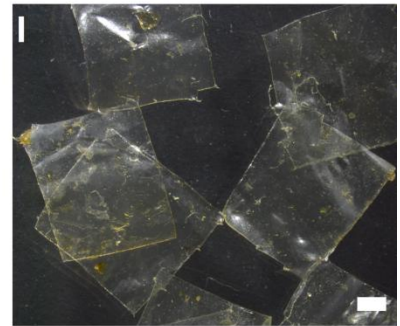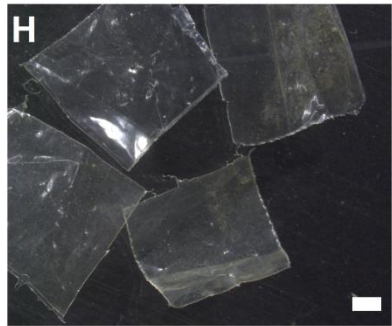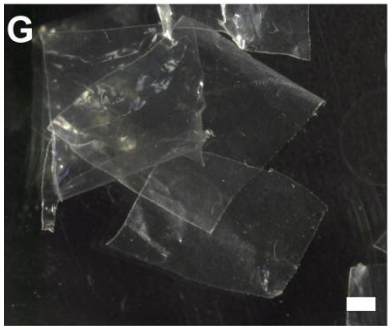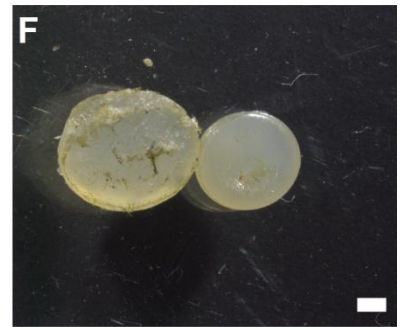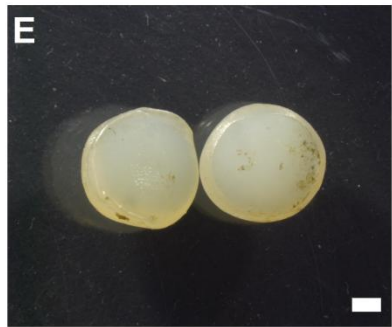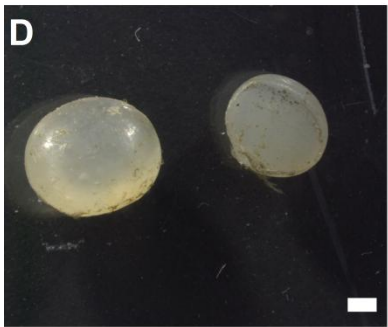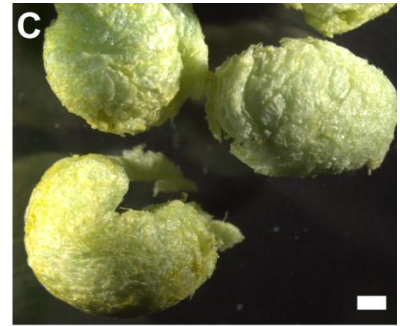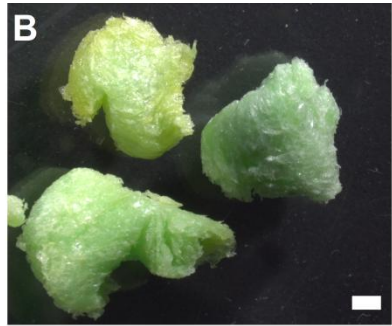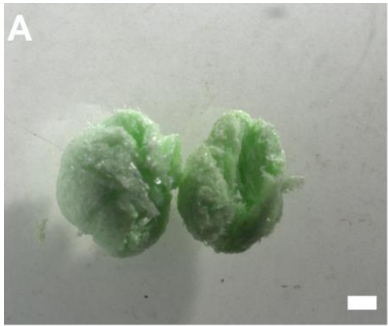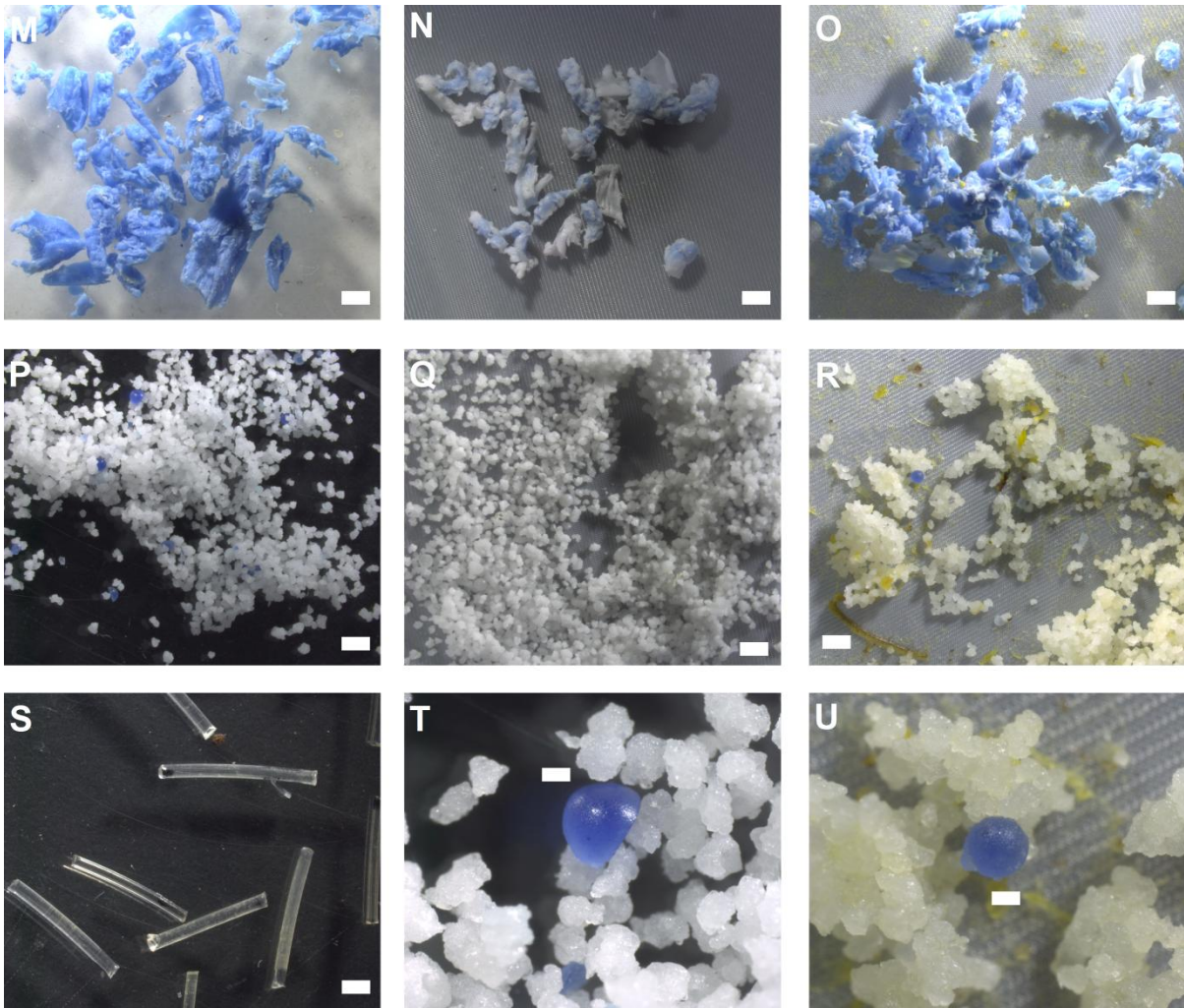
Figure 6.3. Raw plastics on left, plastics immersed in acid in centre and plastics spiked in fish before digestion on right. A-C - Foam, D-F - Pellets, G-H - Film, J-L - Polyester, M-O - PVC, P-R - Microscrubbers, S - Nylon, T-U – closer view of Microscrubbers. All scale bars represent 1mm, except T and U which are 0.2 mm.

6.4.4 Ingestion by juvenile fish
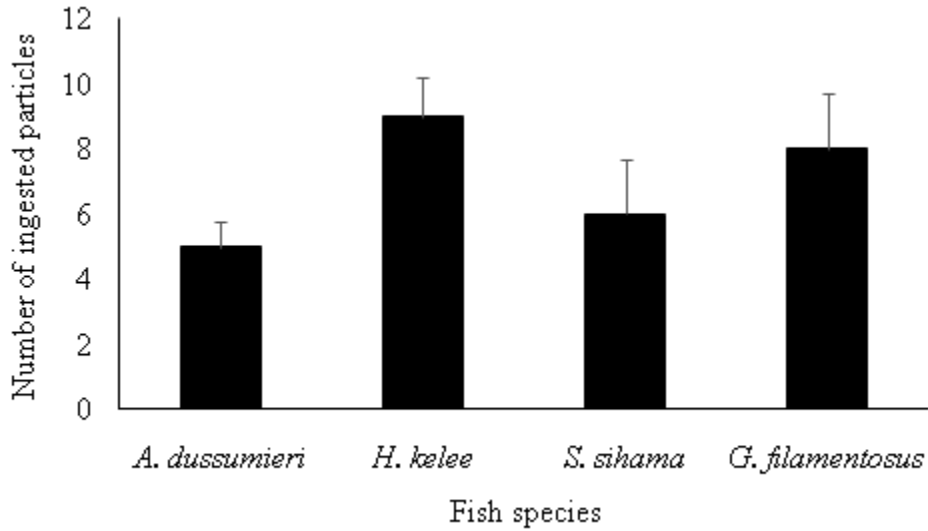
Figure 6.4. Number of microplastics ingested by four species of juvenile fish (mean + S.E.).
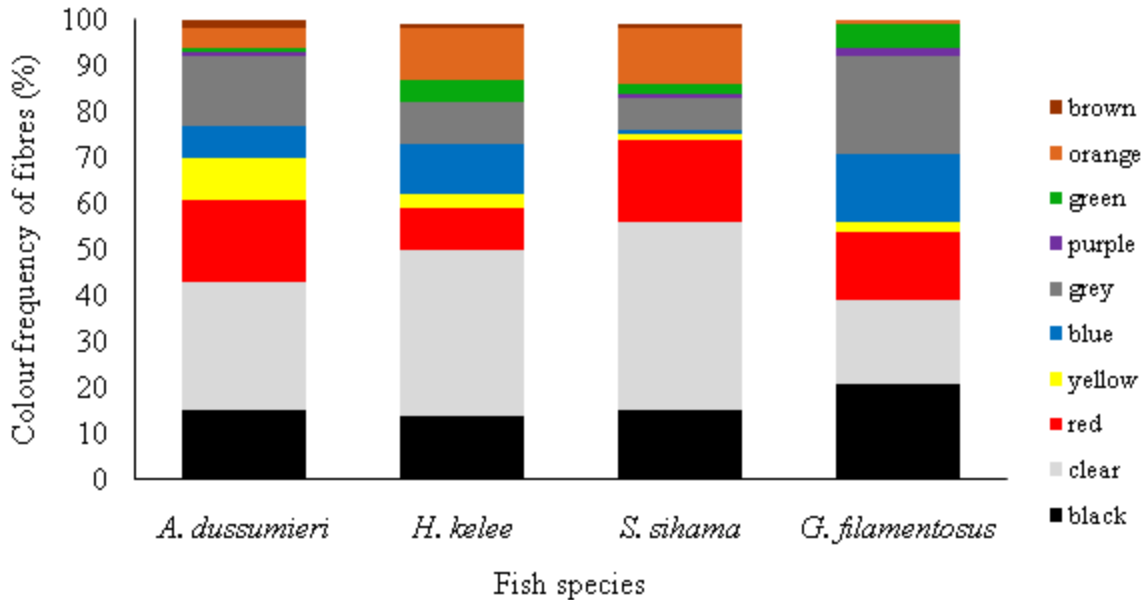
Figure 6.5. The proportion of fibre colours found in four species of juvenile fish.

Microplastic particles were ingested by all juvenile fish from both feeding guilds and the mean abundance of these particles were not significantly different amongst the four species ($F = 2.442$, $df = 35$, $p = 0.082$) (Fig. 6.4). Microplastic fibres contributed the highest proportion for all species (*A. dussumieri*, 98%; *H. kelee,* 94%; *S. sihama,* 98% and *G. filamentosus,* 94%). The colour composition of fibres varied considerably, but were predominantly clear, black and red (Fig. 6.5).

6.5 Discussion

I aimed to test if the use of an HNO_3 method would be suitable for isolating plastics from juvenile fish. For the digestion of a fish of 1 gram, 10 mL of 55% AR HNO_3 was found to be the optimal acid volume. This was half of the volume used by Claessens et al. (2013) who used 69% HNO_3 to digest mussels. This difference could be due to the fish being much smaller with softer tissue than mussels or due to the higher fat content of mussels that may require a higher volume of acid to dissolve. Initially, whole juvenile fish of around 1 g digested in 5 mL of acid clogged membranes making material inspection difficult. Increasing the volume to 10 mL solved this. A similar fish mass to acid volume ratio may therefore prove useful for future digestion experiments on juvenile fish using the same acid concentration.

At elevated temperatures, the digestion times decreased dramatically, lending clear support for the initial hypothesis that the reaction would be sped up. The juvenile fish digested in a matter of minutes when heated, and this is beneficial for processing large sample numbers. Smaller fish digested faster. Additionally, this method eliminates the need for prior dissection of the fish, in contrast to studies that have generally removed gastrointestinal tracts first, rather than using whole animals in their isolation protocols (Lusher et al., 2017). These dissections are difficult to perform on fish in their early stages and some protocols do a separate digestion for fish tissue to assess the whole body burden of plastics afterward (Lusher et al., 2017), which is more relevant for human consumption monitoring (Dehaut et al., 2016).

Most plastics showed miniscule to no mass change for either the direct immersion or spiked experiments; however nylon immersed in acid digested away within the first 24 hours. Nylon was also completely disintegrated during digestions following the fish spiking. This was in contrast to Claessens et al. (2013) who found that 98% of nylon fishing fibres (100 x 400 μm), that were much smaller than those used here, could be recovered when using HNO_3. Therefore, studies using this method for counts should state that these counts are conservative (Desforges et al., 2015). Modulating the concentration of acid does not seem to work, since a decrease in concentration could result in lower digestion efficiency that could block up filter membranes (Karami et al., 2017). It may be the case that one has to determine the common plastics in the environment and visually in a small sample of fish guts first before acid use. The proportion of nylon relative to other microplastics can then be factored in after the digestion. If there is a high concentration of nylon material, as could be the case for estuaries that are heavily impacted by fishing gear (e.g. Possatto et al., 2011), then this method would not be suitable as the plastics would be severely under-sampled. However, in Europe for example, less than one million tonnes per annum of nylon including other polyamides is produced, compared to an overall plastic production of 49 million

tonnes per annum (PlasticsEurope, 2015), therefore nylon is unlikely comprise the largest proportion of plastics in many areas.

Although the nylon disintegrated, all other polymers tested seemed to show little degradation when exposed to the protocol and this is considering that the plastics were immersed in acid for one month. Desforges et al. (2015) noted that using HNO_3 at 80 °C caused a yellowing of the filters after the digestion process. There was some degree of yellow material present on the filters here from the digested tissue, but this did not hamper visual inspection (Fig. 6.3, O, R and U). This could mean that samples can be stored a little while after digestion, if the filtration step can't be done all in a single day.

Using this protocol on field collected juvenile fish; extracted similar quantities and types of microplastic particles from two zooplanktivorous and two benthivorous fish. This is supported by previous work showing that microplastics can be ingested by fish from different feeding guilds (Lusher et al., 2013; Neves et al., 2015; Campbell et al., 2017). There was no significant difference in the average number of ingested microplastics between the fish from the two feeding modes (Fig. 6.4). Therefore, the hypothesis that the average number of ingested microplastics will differ between fish species with different modes of feeding was rejected. A similar pattern of microplastic ingestion between pelagic and benthic fish was reported by Lusher et al. (2013), who sampled five fish per habitat. This trend was also reported by Neves et al. (2015), who assessed the presence of microplastics in four pelagic and five benthic fish.

The high percentage of fibres over other types of microplastic detected in this study (94–98%) was consistent with the findings of Lusher et al. (2013) (68%), Neves et al. (2015) (66%) and Mizraji et al. (2017) (99%). A similar colour range of ingested fibres was identified across all four sampled fish species, suggesting that both the zooplanktivorous and benthivorous fish were feeding indiscriminately (Fig. 6.5). The high prevalence of fibres was expected because the fish were collected from the Durban harbour, where there is a lot of activity such as fishing, boat and ship mooring, and discharge of industrial and wastewater effluents (Rathbone et al., 1998; Forbes and Demetriades, 2008; Browne et al., 2011; Naidoo et al., 2015; Nel et al., 2017). The excessive consumption of fibres by fish, especially juveniles, maybe potentially hazardous because of the additives and pollutants associated with plastics that have been implicated in a variety of harmful effects (reviewed by Wright et al. (2013a) and Jovanović (2017)).

In conclusion, we recommend the nitric acid digestion for use on juvenile fish, provided that researchers are working with high sample numbers, on a tight deadline and that nylon is not largely prevalent in the environment that is being considered. The lengthy, expensive and partially complete digestions of some

of the other methods will inevitable impact sampling efficiency during large monitoring programs, while the advantages of using nitric acid are that it is readily available, cheap and quick under the right conditions. The method was successfully used to document the presence of microplastics in two pelagic (*A. dussumieri* and *H. kelee*) and benthic (*G. filamentosus* and *S. sihama*) fish species from the Durban Harbour.

CHAPTER 7

Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic

7.1 Abstract

Glassfish, *Ambassis dussumieri* (Cuvier, 1828), was used as a sentinel species to investigate the effects of microplastic ingestion on juvenile fish growth and survival. Both virgin plastic and plastic collected from an urban harbour were fed to small juvenile fish daily for 95 days. Control fish were fed fish flakes without plastic. Fish length, width and mass were recorded at intervals of 20 days, while survival was continuously recorded. All fish were fed tropical flakes, measured at 1.7% of the body mass per tank. Plastic fed fish were given one part plastic, to every five parts of food by mass, within environmental levels of contamination in their habitat. Overall, fish in control treatments grew more in body length and body depth compared to those in plastic treatments. Growth in length and depth of fish in control replicates averaged from 3.60 – 3.84 mm and 0.86 – 1.56 mm, while that of fish in plastic treatments averaged from 1.68 – 2.27 mm and -0.80 – 0.01 mm, respectively. Fish mass was also higher in control fish than fish in the virgin plastic treatment; however, the growth in mass was not significantly more than fish in the harbour plastic treatment. The survival probability of fish in controls was also higher than fish in both plastic fed treatments, which became pronounced toward the latter half of the experiment, > 50 days. However, pairwise comparisons between survival curves only showed significant differences between fish survival in the control and the polluted plastic treatment. These fish showed a short gut retention time of plastics, of < 24 hours, which may limit their interaction with the plastic particles and delay the onset of negative responses. However, continuous feeding and exposure, as may be the case of fish that inhabit urban harbours, may pose potential risks to juvenile recruits.

7.2 Introduction

The ingestion of microplastics, ≤ 5 mm in length, by fish has been recorded from the early 1970's (Carpenter et al., 1972), when scientists speculated on its negative influence on fish health (Hoss and Settle, 1990). Recently, fish have been documented ingesting microplastics in a variety of water bodies including rivers (Sanchez et al., 2014), shallow coastal estuarine systems (Naidoo et al., 2016), the ocean surface (Choy and Drazen, 2013) and even the deep ocean (Anastasopoulou et al., 2013). Plastic ingestion has been found in both demsersal and pelagic feeding guilds (Lusher et al., 2013). Field evidence of any negative effects of this ingestion, for example gut lesions or tissue damage, is challenging to observe and may be limited because of destructive plastic isolation methods, such as acid digestion, or separating any observed effects from other field contaminants (Steer et al., 2017). Manipulative feeding experiments are therefore used to determine the biological (Rochman et al., 2013b; Pedà et al., 2016) and ecosystem effects (Bergami et al., 2016) of microplastic ingestion.

Experiments have mainly revealed the negative effects of microplastic ingestion at the tissue, organ and organism levels (Jovanović, 2017). For example, Rochman et al. (2013b) showed that discarded low density polyethylene (LDPE) pellets caused changes to the liver tissue of the Japanese medaka, *Oryzias latipes* (Temminck and Schlegel, 1846); and Pedà et al. (2016) observed that polyvinyl chloride (PVC) pellets affected the intestinal structure of the sea bass, *Dicentrarchus labrax* (L. 1758). Such alterations could result in organism changes that include decreased feeding and decreased body mass (Welden and Cowie, 2016). Higher level effects include impaired development and decreased reproductive potential, even by virgin plastic, as shown for the sea urchin *Lytechinus variegate* (Lamarck, 1816) and the oyster *Crassostrea gigas* (Thunberg) (Nobre et al., 2015; Sussarellu et al., 2016).

Assessing these threats using manipulation experiments, on small juvenile fish is both needed and is ecologically important, as it increases our understanding of the effect that microplastics can have on recruitment (Mazurais et al., 2015). Juvenile fish are already vulnerable to environmental perturbations that can affect their survival at early stages and may be particularly vulnerable to microplastic ingestion (Whitfield, 1990; Lima et al., 2015). They use polluted urban estuaries as nurseries, bringing them in contact with plastic particles at a higher frequency (Lima et al., 2015; Naidoo et al., 2015) and their relative size compared to microplastic particles may make any ingested particles more dangerous or even harder to pass compared to adult fish. Juveniles of commercially important species that use estuaries as nursery areas could also be affected and thus affect fisheries in the long term (Markic and Nicol, 2014) especially since there can be a similar number of plastic particles as juvenile fish in estuaries (Lima et al., 2015). Furthermore, it is predicted that the ocean plastic mass will outweigh fish mass by 2050, outlining

the necessity to evaluate potential impacts (Jovanović, 2017). This study has set out to fill this important research gap, studies on the effects of microplastics on small juvenile fish are scarce and those targeting the chronic long term effects of exposure in an environmentally relevant situation are even more so (Steer et al., 2017).

I therefore aimed to assess the long term impact of microplastic ingestion on the growth and survival of juvenile fish. To test this, *Ambassis dussumieri* (Cuvier, 1828) was used as a sentinel species. These glassfish or glassies are common coastal fish that are translucent and cosmopolitan (Anderson and Heemstra, 2003). They are an integral part of the food chain, consuming at low trophic levels and in turn being preyed on by larger fish and birds from higher trophic levels (Forbes and Demetriades, 2008). They usually feed in the water column on zooplankton (Dyer et al., 2015) and are thus likely to interact with the high microplastic concentrations found in urban estuaries (Naidoo et al., 2015; Clark et al., 2016). It was hypothesised that that growth and survival would decrease in fish exposed to environmentally realistic concentrations of microplastic. The objectives were to feed fish four different plastic types of both virgin plastic and plastic stranded in a polluted harbour and measure their growth and survival over three months. Furthermore, while photographing the plastic and faeces from the fish under the microscope, active nematodes were found. I therefore hypothesised that the presence of microplastics in the fish gut has an effect on the gut nematode abundance.

7.3 Materials and methods

7.3.1 Tank setup

In total, 450 juvenile fish were collected from Durban Harbour (29° 52'S, 31° 04'E), using a fine mesh dip net. The fish had an initial mean standard length and standard deviation of 21.36 ± 4.05 mm. They were tagged and acclimated for a month before being fed virgin plastic, plastic collected from the industrialised Durban Harbour termed 'harbour plastic' or no plastic. These treatments were all replicated three times. Ten fish per 20 L tank were maintained in filtered seawater at a salinity of 35, a constant temperature of 25 ° C and a 12 day: 12 hour night light regime (Fig. 7.1). A set of five tanks connected to a single sump and protein skimmer constituted a single recirculation system. This set of five tanks formed a single replicate for each treatment. The flow rate from each sump was 2500 L/h, which was split and equally distributed using valves between adjacent tanks. A complete water change was done every two weeks, while fish faeces and leftover food particles, including plastic particles, were siphoned out daily after a 30 minute feeding event. This study done was under ethical clearance by the animal ethics research committee of the university (AREC/011/016D).

Figure 7.1. Two thirds of the experimental setup used for the feeding experiments, showing intake and gravity fed drainage to sump.

7.3.2 Tagging

Nine fish per tank were tagged using a green, red or blue fluorescent elastomere (Northwest Marine Technologies, Inc.) at one of three positions (Fig. 7.2), in order to track individual growth. The 10th fish in each tank was identified by the absence of a tag.
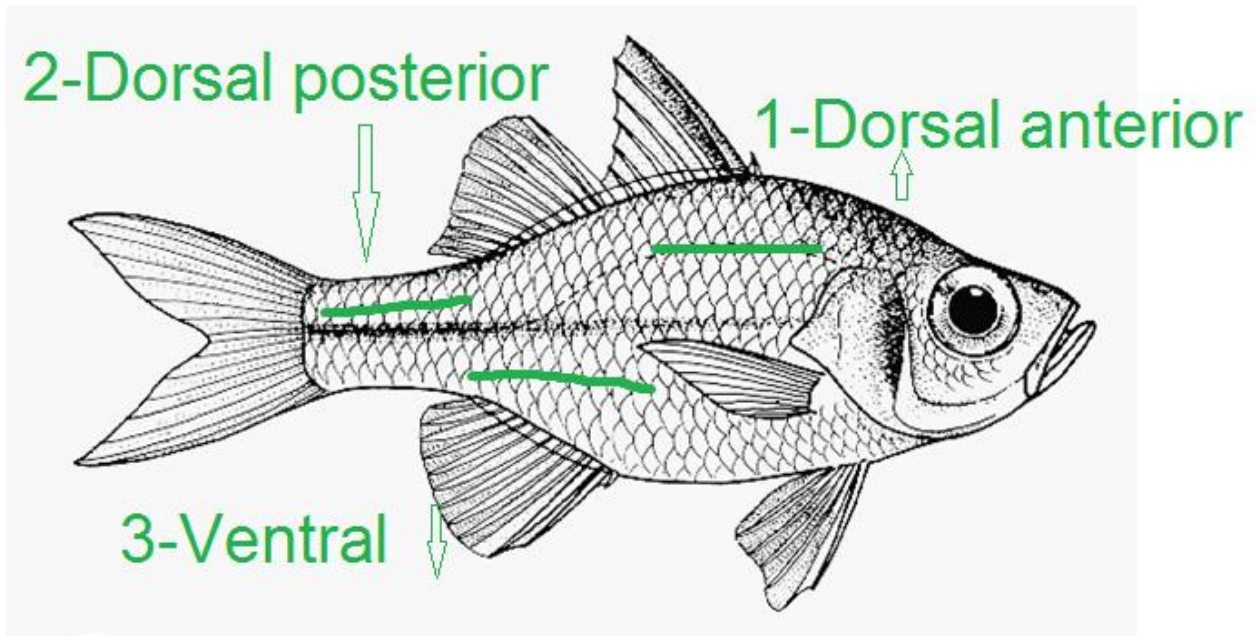
Figure 7.2. Tagging locations on glassfish. A single horizontal line was placed in one of the three positions outlined, using either a green, red or blue tag (image adapted from www.fishbase.org).

7.3.3 Plastic preparation and feeding

A mixture of the plastic types found previously in an urban harbour was used (Naidoo et al., 2015). None were treated or cleaned before use and plastics were mixed in proportions of 54% film, 29% fragments, 6% pellets and 6% polystyrene, representing the same proportions and 95% of the plastic types found in water samples from the harbour. The same proportions of virgin plastic types were used. Particles were ground with a coffee bean grinder and only particles between 1000 μm and 250 μm, which accounted for 73% of plastics size range found in the surface water tows within the harbour, were used in the experiment. Since the glassfish mainly feed on zooplankton within the water column (Dyer et al., 2015), I assumed that these proportions would be relevant to the feeding behaviour of these fish.

Studies should use environmentally relevant plastic concentrations of the common types and shapes encountered *in situ* (Huvet et al., 2016; Lenz et al., 2016; Lusher et al., 2017), as many studies have used concentration of plastics that are generally not encountered in the environment e.g. 1000 particles per mL (Cole and Galloway, 2015). Therefore, the highest concentration of plastic found in Durban Harbour was used in this study. This equated to 1.769 g per 10000 L of surface water which was 0.051 g per each 290 L replicate setup or 0.010 g of plastic per tank. During feeding, the pump in each sump was switched off. Fish in each tank were fed 1.7% of their total body weight daily with tropical flake food (Qualipet®). Fish in plastic treatment tanks were given one part of the plastics mixture in addition to every five parts of fish food, by mass. Since fish mass averaged 0.336 ± 0.172 g it meant that a concentration of around 0.011g of

plastic per tank would be added initially. Fish food and ground plastics were sprinkled on the surface of each tank and the fish were allowed to feed *ad libitum*. As fish numbers decreased through mortality, the food proportions were adjusted accordingly for that tank. After 30 minutes, faeces and plastic debris were siphoned out and the pumps where switched on again for floating debris to pass through the tank outlet and get sieved out. Plastics were clearly visible embedded in many of the faeces in the plastic treatments, indicating that plastics were being consumed and passed out.

7.3.4 Retention

Since plastic retention data are still scarce for fish and may play a pivotal role in the magnitude of any negative effects (Jovanović, 2017), a pilot study was conducted to determine the retention of particles in these glassfish. Five fish, with an average total length of 28.52 ± 2.14 mm and a mass of 0.183 ± 0.042 g, were kept in each of five 20 L tanks. An initial exposure of 0.05 g ground PVC fragments was added to the surface of the water in each tank. Each particle weighed approximately 0.001 g. Fish were not fed for the four day exposure and 10 minutes after the initial exposure, one fish from each tank was euthanised in 99% ethanol and stored. Thereafter fish were euthanised daily. On each day, 95% of the water from each tank was siphoned out and the plastics remaining in the water column were isolated and weighed. At the end of the experiment, the euthanised fish were digested in 55% AR nitric acid to isolate the plastics from each individual (Chapter 6).

7.3.5 Determining growth, condition and survival

Each fish was measured to the nearest 0.1 mm with a pair of calipers at time intervals of 19 (n = 412), 38 (n = 288), 68 (n = 192) and 92 days (n = 82). Their mass (g) was also recorded with a mass balance. Any dead fish were taken out, daily recorded for survival data and stored in 10% formalin for analysis of their gut plastic content. This was done by digesting fish in nitric acid to isolate and enumerate consumed plastic particles. Two fish from each tank were also culled before measurements were taken, at each time interval, to determine if plastics accumulated in them as the experiment progressed. This was changed to one fish if fewer fish were present in the tank as time progressed, to even out densities, as different stocking densities could affect the water quality that the fish experience. Culled fish were stored in 10% formalin and also digested to determine their plastic content.

7.3.6 Comparing length, body depth and mass

The fish that survived throughout the experiment were used to compare growth of fish among treatments. Initially boxplots were created in SPSS version 24, to find and remove outliers. Thereafter, data were imported to *R* and nested ANOVA's were run for each of the growth parameters. Aquaria were nested within replicates which were all nested within treatments with an error term built into the model. For

length, four outlier values were removed and for body depth one outlier was removed. For all tests, equality of variance was checked by plotting the residuals against the fitted values; while Shapiro−Wilk normality tests were run to meet the assumption that the residuals approximate that of a normal distribution. Length and body depth data satisfied this assumption ($W = 0.990$, $p = 0.857$ and $W = 0.9821$, $p = 0.352$, respectively) while mass data was log transformed to conform ($W = 0.975$, $p = 0.128$). Tukey's HSD tests were run to compare differences between treatments and graphs produced on GraphPad Prism 5 were used to display these (Fig. 7.4).

7.3.7 Fish survival and plastic ingestion

For each of the plastic treatments and the control, fish were scored with 'zero' for those that survived during growth measurement intervals and 'one' for each mortality incident throughout the experiment. Kaplan−Meier survival curves were then plotted using R to compare survival probabilities of the glassfish among treatments during the course of the experiment. Plots were produced using the survminer package for R. A log−rank test was also used to determine if overall survivorship differed between treatments. Pairwise comparisons were made using a log−rank test with Benjamini−Hochberg (BH) p−value adjustment. Fish that were culled were excluded from the analysis.

Microplastic abundance from fish that died 'naturally', or that were culled, were each correlated with the number of exposure days, to determine if plastics were being accumulated in fish over time. Positive correlations in each case would mean that as the number of experimental days increased, the number of plastic particles found in a fish would increase, giving some indication of an accumulation of particles. For fish that were culled, five individuals from each treatment and five individuals from the control were digested during each measuring interval. Data did not satisfy the assumption of normality and transforming the data did not rectify this. Therefore, Kendall's Tau tests were run on SPSS. The treatments were then split and the correlations were run again. A t−test was run to determine if the number of ingested particles differed between the plastic treatments for culled fish. Data were $\log_{10}+1$ transformed and to satisfy the assumptions of normality ($W = 0.976$, $p = 0.532$) and homoscedasticity (*Bartlett's* $K^2 = 2.937$, $df = 1$, $p = 0.086$). Control fish were digested in the same way as with the treatments, to observe for any contamination. No particles used in the microplastic treatments were found in any of the control fish that were digested from culled fish and 'naturally' dead fish.

7.3.8 Nematodes

Faeces were siphoned off from each treatment into separate buckets for photographing. A pipette was used to transfer the faeces from each replicate into a glass vial. The glass vials were then sealed and stored in the lab for the photographing of microplastics. While observing the microplastics under the

microscope, live nematodes were observed in the sample. These were enumerated to determine if there was a difference between the treatments that contained microplastic and controls. Distilled water was added to the faeces in each vial to bring each volume up to 20 mL before subsamples of standard volume could be taken. A graduated pipette was then used to homogenize the sample before five subsamples of 0.5 mL from each vial were transferred to a glass slide. A cover slip was then placed on the top film of water on each slide and viewed under $40 \times$ magnification. To determine if there was a difference in the number of nematodes per 0.5 mL sub−sample between treatments and controls, an analysis of variance (ANOVA) was run on R. Assumptions of the ANOVA were satisfied using a Shapiro−Wilk's normality test ($W = 0.953$, $p = 0.07$) and a Levene's test for homogeneity of variance ($F = 2.13$, $p = 0.131$). To verify that nematodes were not present in the water source, 60 mL of seawater from each replicate and the water standing stock were settled in Utermoehl chambers and checked under the microscope.

7.4 Results

7.4.1 Pilot study of retention

Between the initial dose and 72 hours, plastics in the water column during successive days could either come from being egested by fish that consumed them, or from the remaining 5% of the water left over during the water change. At the start of the retention experiment, all fish were observed to actively ingest plastic particles. The highest concentration of plastic consumed was found during the first ten minutes of feeding and was quite variable among the first five fish that were culled (0.002 ± 0.002 g per fish, mean $\pm$ S.D.). From the initial dose of 0.05 g, the first five fish consumed a range of $0.06 - 9.46\%$ of the plastic in each tank ($3.6 \pm 3.7\%$). Thereafter, the mass of plastic found in the fish and the water column decreased considerably, with only a few particles of negligible mass present after 24 hours and 48 hours, respectively. At the end of 72 hours there was no plastic observed either in the water column or any fish (Fig. 7.3).
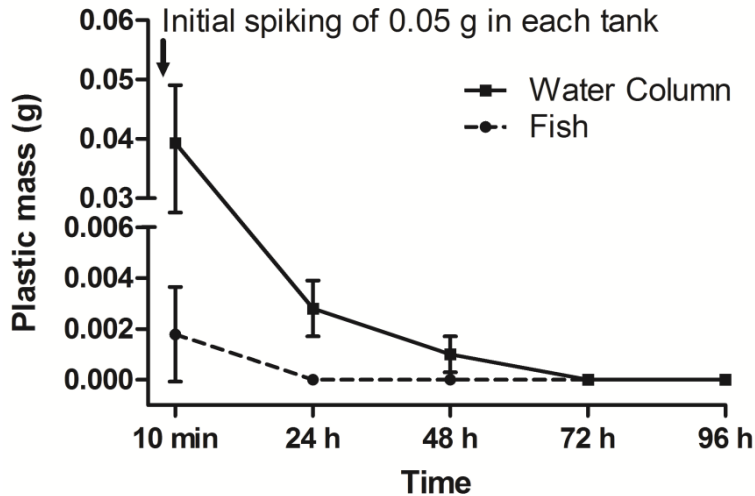
Figure 7.3. Particle retention time of PVC in the gut of *Ambassis dussumieri*, from an initial dose of 0.05 g. Bars represent mean ± S.D.

7.4.2 Length, body depth and mass

Fish length increased significantly more in the control than both the virgin and harbour plastic treatments ($F = 18.61$, *df*= 2, $p < 0.001$), while the growth in length of fish in plastic treatments did not differ from each other ($p = 0.679$, Fig. 7.4 a.). Similarly, the control fish showed significantly higher body depth growth compared to those in both plastic treatments ($F = 24.81$, *df* = 2, $p < 0.001$, Fig 7.4 b.). Fish from the virgin plastic and the harbour plastic treatments showed either minimal change or decreased body depth and were not significantly different between each other ($p = 0.147$, Fig. 7.4 b.).

Although the fish in control tanks showed higher mean mass gains than fish in both plastic treatments (Fig. 7.4 c.) and the overall ANOVA was significant ($F = 3.417$, *df*= 2, $p = 0.038$), control fish only differed from the virgin plastic treatment ($p = 0.030$) and not the harbour plastic treatment ($p = 0.391$). Growth in fish mass from the harbour plastic treatment was also not significantly different from the virgin plastic treatment ($p = 0.537$). For all growth measurements, the replicates within treatments did not differ among each other (Fig. 7.4).
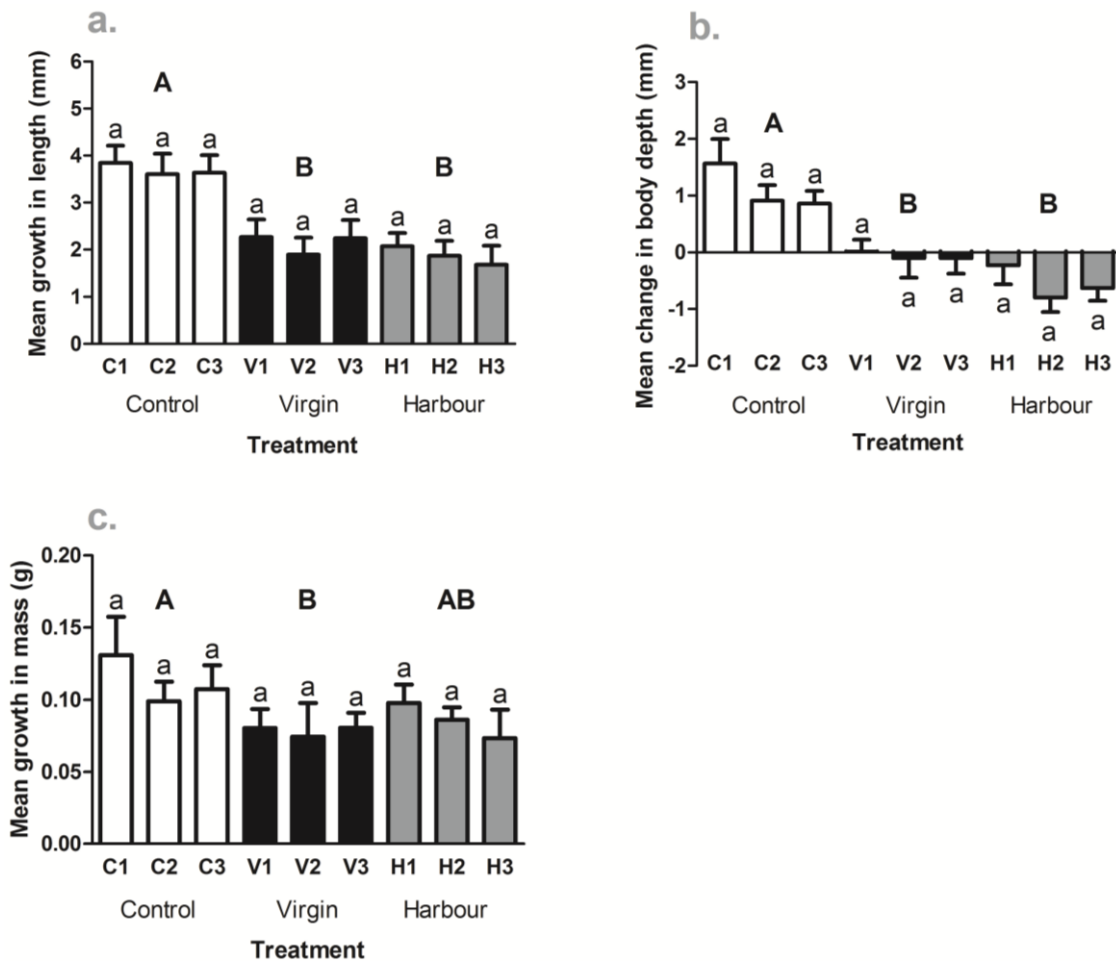
Figure 7.4. Mean change in (a.) fish length (mm), (b.) body depth (mm) and (c.) mass (g) for treatments after a three month exposure to microplastics. Capital letters denote differences between treatments, while letters in smaller case, above the standard error (S.E.) bars, show differences within each treatment.

7.4.3 Fish survival and their microplastic load over time

The survival curves for fish from the control and plastic treatments plotted for the course of the experiment, were significantly different overall (χ^2 = 7.3, df = 2, p = 0.027). At the start of the experiment, all survival curves were similar, however after 50 days the plastic fed treatments showed lower survival probabilities than the control (Fig. 7.5). However, pairwise comparisons indicated that the survival curve of the control was significantly different from the harbour plastic treatment (p = 0.026) but not from the virgin plastic treatment (p = 0.085). Fish in the harbour plastic treatments also showed lower survival probability than those in the virgin plastic fed treatments toward the end of the experiment, but there was no significant difference between the curves (p = 0.490).

Microplastics were found in 31% of the fish that died during the experiment, i.e. not intentionally culled, from both plastic treatments combined. The average number of particles observed per fish were 0.5 ±

0.86, *n* = 30 and 0.65 ± 0.18, *n* = 37, for virgin and harbour plastic treatments, respectively. No evidence of a significant correlation between the number of plastic particles and the number of exposure days was found. This held true whether zero values were left out of the correlation (*coefficient* = -0.064, *n* = 21, *p* = 0.719) or included (*coefficient*= -0.087, *n* = 67, p = 0.375). There was also no correlation found when data were split between harbour plastic (*coefficient* = -0.104, *n* = 37, *p* = 0.436) and virgin plastic (*coefficient* = -0.093, *n* = 30, *p* = 0.534) treatments.

Of the 40 culled fish from the plastic treatments, 93% contained microplastics. The number of microplastics consumed for the culled fish varied considerably. The average number of particles ingested were 29.35 ± 37.59, *n* = 20 and 11.55 ± 11.14, *n* = 20, for fish from virgin and harbour plastic treatments respectively. These were not significantly different (*t* = -1.144, *df* = 33.14, *p* = 0.261). There was a significant albeit weak positive correlation between the number of plastics and the culling date (*coefficient* = 0.333, *n* = 40, *p* = 0.007). When treatments were split and correlations run, there was a significant correlation between these variables for virgin plastic (*coefficient* = 0.409, *n* = 20, *p* = 0.023) and no significant correlation for harbour plastic treatments (*coefficient* = 0.237, *n* = 20, *p* = 0.187).
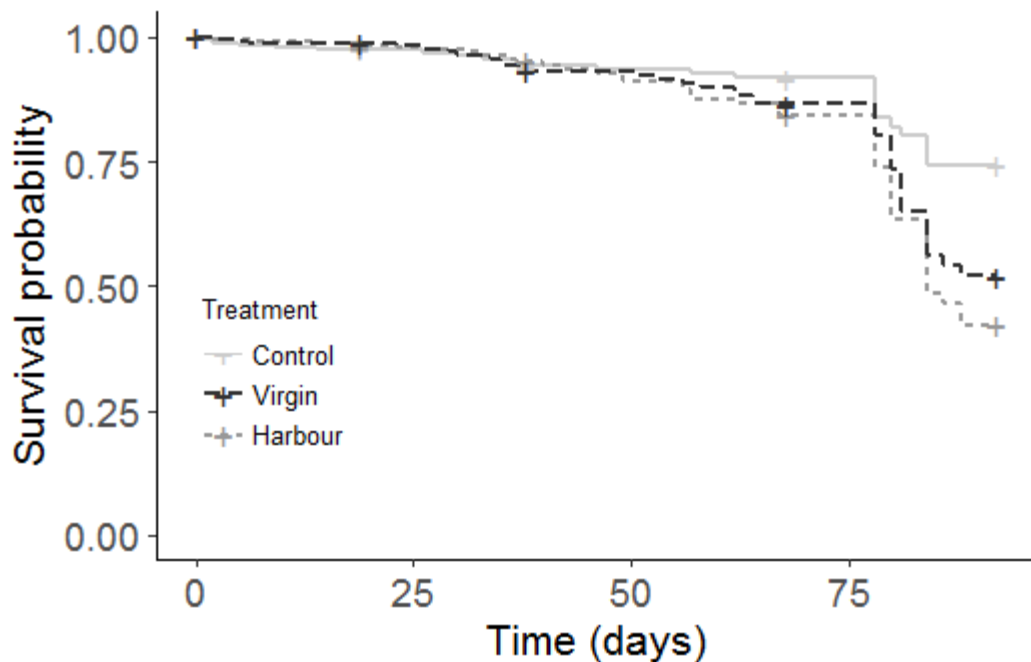
Figure 7.5. Kaplan−Meier survival curves for glassfish within virgin plastic and harbour plastic treatments; and a control without plastics.

7.4.4 Nematodes

Overall, there was a significantly lower number of nematodes, in total, in the faeces of control fish compared to those in the plastic treatments ($F = 11.44$, $df = 2$, $p = 0.0001$, Fig. 7.6). Differences were significant between the control and the virgin plastic ($p = 0.005$), and the control and the harbour plastic treatments ($p < 0.001$), while no difference was found between the two plastic treatments ($p = 0.303$). The range of nematode abundance values are presented in Appendix E1 and E2.
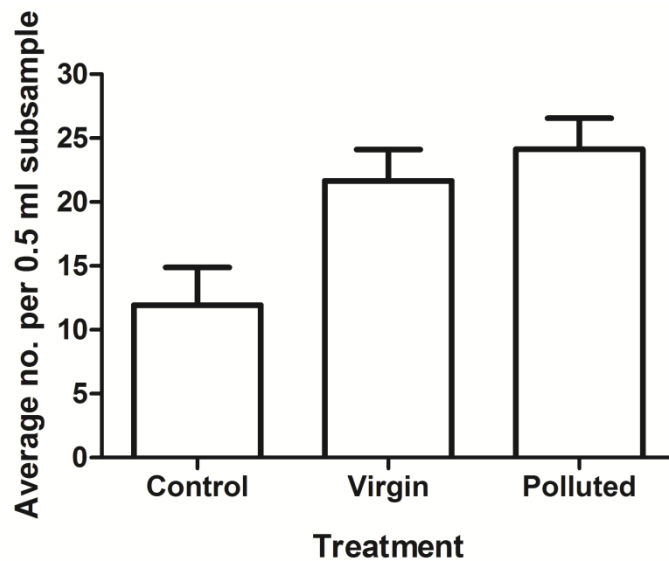
Figure 7.6. The average number of nematodes per 0.5 mL subsample across three replicates pooled for each of the treatments. Bars represent the standard error (S.E.).
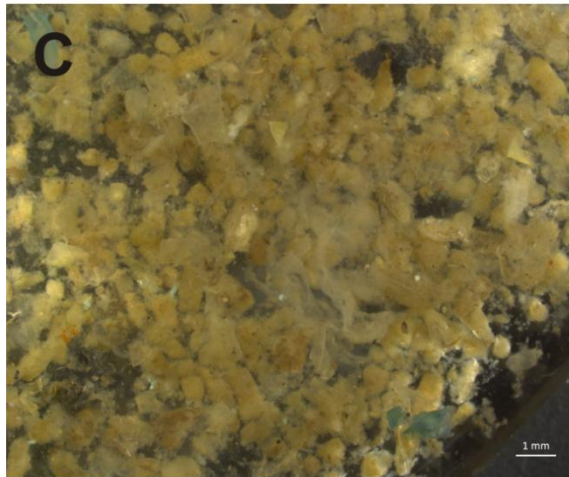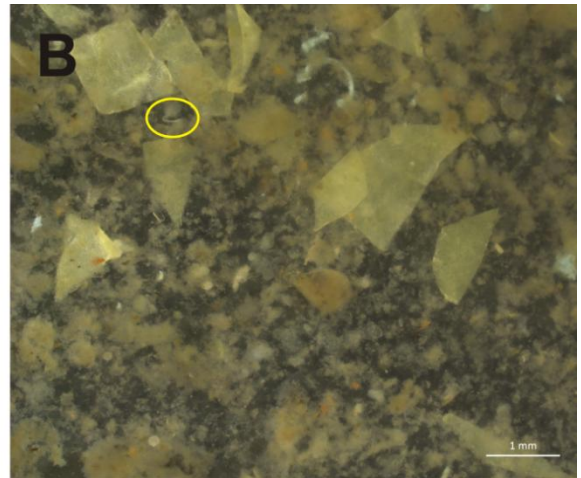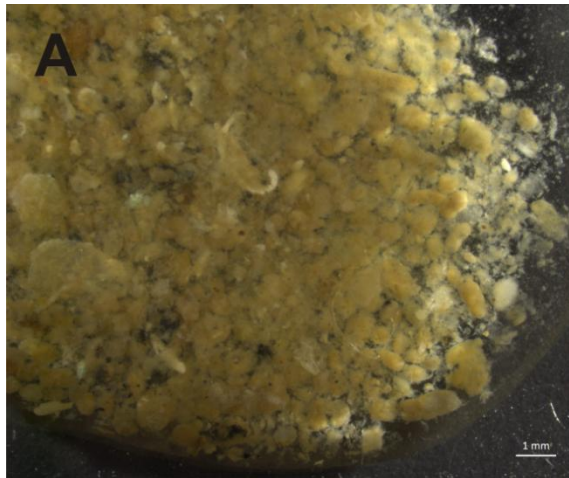
Figure 7.7. Faeces of glassfish showing microplastic particles and nematodes observed. A – Control faeces, B – Virgin plastic treatment, with yellow film and blue fragments, C – Harbour plastic treatment, with blue film. D and E are isolated nematodes. Scale bars are 1 mm in A, B and C. In B the yellow oval encircles a curved nematode in motion.

7.5 Discussion

7.5.1 Retention and accumulation

Plastic retention and accumulation is an important consideration when investigating the health effects of microplastic ingestion (Mazurais et al., 2015). The glassfish used in the pilot experiment consumed and egested plastic particles rapidly with limited evidence for long term plastic particle accumulation in fish, even from correlations in the main experiment. This result is common in the literature for fish (Batel et al., 2016; Jovanović, 2017) and oysters (Nobre et al., 2015) and suggests that minimal impact would be caused if isolated particles are incidentally ingested. Particles would have little time to interact with and bring about changes within the fish. This was observed by Mazurais et al. (2015) and Batel et al. (2016) who found no ill effects on European perch larvae and zebra fish *Danio rerio* (Hamilton, 1822), respectively, during short ingestion experiments, when smooth microspherical particles were egested in a similar amount of time as the glassfish. Retention was also probably influenced by the size and shape of the particles ingested in relation to the test organism, since plastic fibres have been shown to intertwine and be retained for months in the lobster *Nephrops norvegicus*, causing physiological changes, such as decreased feeding rates and lowered body mass (Murray and Cowie, 2011; Welden and Cowie, 2016). In addition, increased retention may favour the dissociation and leaching of plastic associated pollutants in the gut, leading to negative health effects (Nobre et al., 2015; Khan et al., 2017).

Although the fish showed low retention, the daily plastic feed ensured a continuous microplastic−fish interaction. This is similar to what may occur in urban harbours, where new microplastics are introduced to the system via storm water drains and river outlets continuously, together with new prey items (Browne et al., 2010). In this way, longer term studies show organismal changes similar to instances of when particles are retained. For instance, Pedà et al. (2016) found that European sea bass exposed to polluted PVC pellets for three months, had severe changes to their intestinal structure; and after two months Rochman et al. (2013b) observed the detrimental effects of microplastic ingestion on liver function. This may help explain why limited changes in growth and survival of glassfish were found during the beginning of this experiment, whilst in the longer term changes were observed.

7.5.2 Growth

The hypothesis that growth would be adversely affected by the addition of microplastics to the fish's diet was accepted. After three months, the control fish showed significantly more growth in length and body depth. They also had a larger growth in mass, although not significantly more than the harbour plastics treatment. The negative effect that the microplastics had on the growth of the glassfish has also been

shown for freshwater fish (Cedervall et al., 2012), invertebrates such as *Daphnia magna* (Besseling et al., 2014) and earthworms (Huerta Lwanga et al., 2016) and was attributed to a compromised energy budget (Lu et al., 2016). The ingestion of microplastic particles has been shown to place an added energy burden on organisms and a decrease in energy reserves through the catalysis of lipids (Cedervall et al., 2012; Wright et al., 2013a). In this way, the fish may have had to redirect energy usually used for growth, toward other vital maintenance functions such as ridding the body of plastics and their additives. Coping with other stresses such as inflammation (von Moos et al., 2012) and compromised endocrine system (Rochman et al., 2014), liver function and food absorption (Rochman et al., 2013b; Lu et al., 2016), also requires added energy (Wright et al., 2013a). With energy used for targeting these sub−lethal effects, decreased feeding (de Sá et al., 2015; Bergami et al., 2016) and a possible false sense of satiation (Cole et al., 2015) can further reduce the energy available for optimal growth. One interesting result was also that fish body depth in plastic treatments remained stagnant and even showed some decrease over the experimental period. A decreasing length is rare but has been shown in juvenile salmoids that have their nutrition affected under harsh winter conditions (Huusko et al., 2011). A loss in osmoregulatory function may also cause length and body depth shrinkage (Theilacker, 1980). There was also evidence of fish not feeding during the onset of mortality, since there was a much lower percentage of a plastic in 'naturally' dead fish compared to those that were culled. The introduced microplastics were found in fish even toward the end of the experiment, indicating that fish did not avoid it, even after being in the treatment for three months.

It was initially hypothesised that the harbour plastic treatment would be more detrimental to fish, as they may have accumulated organic pollutants (Velzeboer et al., 2014). However, growth measurements for fish did not differ between the virgin plastic and harbour plastic treatments. Increasing evidence has also shown that these pollutants may not be as bioavailable to organisms from plastics as previously thought (see Beckingham and Ghosh, 2017). Intrinsic leachates from the plastics themselves may therefore be of more concern to organisms than pollutants carried over (Nobre et al., 2015). Fish in the harbour plastic treatments were also found to have less difference in mass from fish in the controls, compared to fish in the virgin plastic treatment. One possible reason for this may have been that the negative impacts on mass were offset by the additional nutrition provided by biofilms present on harbour plastics, since they were not cleaned before use.

7.5.3 Fish survival

Control survival curves were significantly different from the harbour plastic treatment but not for the virgin plastic treatment, yet both curves fell sharply towards the latter half of the experiment. This showed

that with continued plastic supply over an extended period, the probability of fish survival feeding on plastic decreases. In addition to microplastics and their chemical additives being potentially toxic (Nobre et al., 2015), they have also been shown to cause DNA damage (Ribeiro et al., 2017) and can also make fish more susceptible to diseases through a reduced immune system function, which can all impact survival (Greven et al., 2016).

The time of exposure and the concentration of plastic particles seem to govern mortality rates (Mazurais et al., 2015). An example of this is shown in earth worms, *Lumbricus terrestris* (L. 1758), when mortality was higher in 60 day experiments of plastic exposure, compared to shorter two week experiments (Huerta Lwanga et al., 2016). Mortality was delayed when plastic concentrations were lower. However, this is not always the case since Pedà et al. (2016) found intestinal alterations in European sea bass exposed to polluted PVC pellets, in a 90 day treatment yet no mortality was found. Size may have an influence here, since the fish used by Pedà et al. (2016) were much larger than fish used for this study or fish used by Mazurais et al., (2015). This suggests that small juvenile fish could be more susceptible to mortality from microplastic ingestion than larger fish. Since the survival of glassfish in this study was affected by microplastic ingestion, it suggests that microplastic ingestion can a potential negative effect on their population.

7.5.4 Nematodes

Faecal casts observed on the bottom of tanks with plastics within them were collected for photographing and nematodes were observed. I then questioned whether the presence of microplastics in fish digestive tracts alters the nematode abundance within it and observed a difference between the controls and plastic treatments. However, the direction of this difference was found to be different depending on the units that one considers. The average number of nematodes was lower in the plastic treatments compared to the control, when considering the average number per dry gram of faeces. The amount of fish faeces was also quite different between treatments and more faeces were collected from the plastic treatments compared to the control, which may influence results (Appendix E1 and E2). More replicates targeting questions on the whole gut infaunal community needs to be performed rather than chance collections on a single day. This can have major consequences especially since parasitic species can also be present, which affects the host fish negatively. In the case of parasitic nematodes of fish such as *Anisakis* sp. the nematode usually completes its life cycle in marine mammals (Audicana and Kennedy, 2008). In some ways, human guts are similar to that of these mammals and, therefore, by eating raw or undercooked fish, the nematodes can be transferred to human digestive systems (Audicana and Kennedy, 2008). The infection in humans in called anisakiasis (Jackson et al., 1978) and can cause allergic reactions such as rashes and anaphylaxis

when raw fish is consumed (Audicana and Kennedy, 2008). Fish can be final or intermediate hosts for various nematode species and they can be found in various organs, such as the swim bladder or even muscle tissue, in both healthy and unhealthy fish (Yanong, 2002).

7.6 Conclusion

It is concluded if these glassfish encountered and ingested isolated plastics particles in the field, then it should be rapidly expelled with minimal harm done to the organism. However, when fish are exposed to a continuous supply over longer periods, as in urban harbours, this can have negative effects on growth parameters and survival. This has serious consequences for juvenile fish species of commercial importance that use urban estuaries as nursery areas, since ecosystem models based on decreasing fish body size predict negative effects on fish biomass and yields; and this can also affect food webs by influencing predation (Audzijonyte et al., 2013). Impacts on survival will also directly affect yields and thus have both economic and ecological consequences. An important part of this research is questioning the consequence of microplastics to the gut infaunal community of organisms. This effect of microplastics has not been considered before and further studies are required on this aspect, especially if it has the potential to affect human health.

CHAPTER 8

8.1   General conclusion

Microplastic pollution has been in the environment for decades, but it is relatively recently that major attention has been paid to it (Arthur et al., 2009). Researchers became aware that (1) large expanses of plastics and microplastics were accumulating in large oceanic gyres (Moore et al., 2001), (2) that fish and other marine biota incidentally consume plastics (Lusher et al., 2013), and that (3) there would be negative health consequences to organisms ingesting microplastics (Rochman et al., 2013b). These observations were the motivation to assess what levels of microplastic pollution is present on the KwaZulu-Natal coast, which fish are likely to consume these microplastics and then to investigate how microplastic ingestion could potentially impact fish.

This dissertation thus formed three sub–topics. The first was an assessment of baseline environmental concentrations of microplastic (Chapter 3), while the second revealed that high environmental microplastic concentrations can result in a high incidence of ingestion by juvenile fish, *in situ* (Chapter 5). The final sub–topic (Chapter 7) showed how environmental concentrations of microplastic had an influence on fish growth and survival, during chronic exposure. Hence this forms a direct link from a pollution source in the environment to a negative impact on fish, which is rare in the literature and fits with the suggestions made by Andrady (2011). The dissertation also covered additional questions that add new information to the field of microplastic research. These included the assessment of the quantity and type of particles that are transported by local coastal currents (Chapter 4) and those ingested by various species of juvenile fish (Chapter 6). It also evaluated efficient methods for microplastic extraction from two sediment types (Chapter 2) and from juvenile fish (Chapter 6). In this general conclusion, I would like to highlight the main aspects of each sub-topic individually and exhibit some of the bridges between them.

For the environmental sub-topic (Chapters 3 and 4), estuarine data showed high standing stock of plastics that attenuate away from the main coastal industrial hub. This trend is largely due to improper handling and high pollution rates in this urban centre and throughout its catchment. This study is novel since microplastic pollution within them have not been quantified before. In South Africa, researchers were

quantifying plastics including microplastics from the 1980's (Ryan, 1988) and their ecological impacts on marine fauna were also being investigated (Ryan, 1987). While research effort in the southern hemisphere was mainly by this author and collegues, most of it was focused on the south-west coast of the country and not much, if any, has been done on the east coast before this thesis. The Durban Harbour, Isipingo and uMgeni systems, as well as their intervening beaches, are faced with the highest standing stock of plastics, compared to estuaries further away from the central hub and this is clearly indicative of a strong influence of terrestrially derived plastic. The rivers and estuaries in the area are therefore important conduits for plastic input to the inshore marine environment. However, inshore currents form intricate eddies that are also semi-permanent making it difficult to track this trend for coastal water. These currents are capable of dispersing plastic particles over long distances. It is therefore important to implement measures to contain the pollution in the metropolitan before it has the opportunity to reach remote areas. Placing more plastic−collecting booms at river outlets is one such measure that can reduce the amount of plastic reaching the open ocean.

The high environmental microplastic concentrations found at the central Durban Harbour was also found to be integrated into the food−web. Chapters 5 and 6 show that fish from Durban Harbour demonstrate a high incidence of ingestion, with 73% of the 70 mullet examined containing microplastics. An important observation here was that the plastic types ingested by these microbenthic feeding fish overlapped with the local types of plastic that were numerically dominant in the harbour sediment found in chapter 3. We can therefore assume that plastic fibres and fragments, originating from terrestrial sources, are being transferred to foraging fish in this environment.

There are over 144 species of juvenile fish that utilise Durban Harbour as a nursery area in their life cycle (Harris and Cyrus, 1999). Since these fish are subjected to the same plastic concentrations, they may similarly ingest plastic particles, particularly if they are non−selective feeders like mullet. A digestion protocol to isolate microplastics from such a wide range of fish, and for constant monitoring programs, was therefore developed in chapter 6. The protocol allowed for the rapid isolation of four of the five plastic polymers tested, nylon being the exception. The method is inexpensive and can be used on an array of fish species. This was shown for the two pelagic and two benthic feeding fish that were effectively digested within the same chapter. In this case all fish contained an abundance of microplastic fibres, but feeding guilds did not differ in microplastic concentration. In these previous studies, it had been clear that fish were ingesting microplastic particles, but the effects it had on fish physiology was still questionable, and is the reason that the tank experiments of chapter 7 were constructed.

Environmentally relevant plastic types and concentrations from the Durban Harbour were used in microplastic feeding experiments, using juvenile harbour glassfish. The experiments of chapter 7 were novel since similar experiments in the literature do not quantify field microplastic concentrations before experiments and therefore tend to expose organisms to unrealistic microplastic levels. These glassfish showed a short retention time of plastic particles, however a continuous supply at these concentrations caused decreased growth and survival, in 95 day exposure periods. This replicated what may occur in the field when plastic particles continuously flow into harbours via channels and storm-water drains. This has serious consequences for populations of economically important fish species that utilise similar polluted harbours as a nursery area. The precise mechanism that retarded growth was not investigated here, but other literature has shown that plastic additives have the potential to negatively affect an organisms energy reserves (Besseling et al., 2013; Wright et al., 2013a) and hence the energy available for optimal growth. This is especially concerning since the abundance of microplastics and larval fish can be the same in estuaries (Lima et al., 2015). Harmful effects of plastic particles on the fish health can therefore have population wide effects and affect the economic value of fish in these systems. The digestion method in Chapter 6 was also used to isolate plastic particles from fish that did not survive the experiment. This was done to test for microplastic accumulation in juvenile glassfish during the long term exposure, however no evidence of this was found. Instead, another interesting finding was that the microplastic particles may also influence the abundance of gut infaunal nematodes in juvenile fish. This is a question that has not been considered yet and this finding thus lends itself to future work.

8.2 Future work

Microplastic research still a largely understudied field, especially in Africa and therefore, future research to obtain background levels of pollution from various inland systems is still needed. Inshore and offshore particle tracking is another needed area of research, to highlight how plastic particles travel once they are ejected from the local systems that were studied here. This will allow us to determine which areas of the coastline are affected by particles originating from urban hubs, which is important for managing waste.

In terms of biological sampling, the future examination of larval fish may also allow us to finally answer questions on whether gut blockage can occur due to microplastics, since the effects may be more pronounced in smaller individuals due to the difference in relative to body size (Foekema et al., 2013). This is an environmentally important question to answer since estuaries are nursery areas for juvenile fish and therefore plastic ingestion poses a threat to the subsequent generations of economically important fish. There are many fish that are consumed which use these estuaries as nursery areas. These include

species such as Rockcod (*Epinephelus andersoni*), Dusky kob (*Argyrosomus thorpei*), Grunter (*Pomadasys commersonnii*), Kingfish (*Caranx sexfasciatus*), Springer (*Elops machnata*), Natal stumpnose (*Rhabdosargus sarba*) and Perch/River bream (*Acanthopagrus vagus*) (Guastella and Smith, 1994), which may be similarly or more exposed to microplastics. Performing a broad scale investigation on various juvenile fish may indicate which are more affected due to their feeding behaviour. In addition, future work could also investigate if such negative effects as shown by the tank experiments in Chapter 7 could be reversed when plastic concentrations are decreased or not present anymore. If this is the case it would inspire hope for the future and more action toward plastic removal from the environment.

For future work, I would especially like to explore the impact that microplastic ingestion has on gut flora and fauna. A variation from the norm would have major implications for food digestion and may explain some of the trends of decreased body growth seen in this thesis and other literature. This interaction needs to be documented in much more detail, as if gut fauna such as parasitic nematodes are affected by plastic particles, then this may then have an indirect impact on human health, especially when raw fish is consumed.

8.3 Concluding remarks

Research on microplastics is still growing rapidly (Cole et al., 2011; Barboza and Gimenez, 2015) and awareness is being raised globally. Recently the number publications investigating microplastic as a topic has increased drastically (Barboza and Gimenez, 2015). For example, searching microplastics as a topic on the ISI Web of Science shows that more than 12 times the the number of articles are now being published compared to the publication rate at the beginning of this decade. This may be attributed to microplastics being previously ignored by research and cleanup operations, since isolating microplastics from sediment, water column and organisms is a difficult process (Hidalgo-Ruz et al., 2012) of which standardised methods with proper contamination control are still needed (Nuelle et al., 2014; Twiss, 2016). To the best of my knowledge, this study gave the first account of microplastic pollution in Durban, but much more interest around the topic is blooming in South Africa. Academics and citizens alike are eager to help reverse the effects of mishandled plastic in this area. If we can come up with innovative ways to monitor and manage plastic waste then their impacts on the environment will surely decrease. This thesis also adds much needed information to microplastic research as a whole by linking a pollution source to an environmental impact, as shown by the slower growth in glassfish exposed to microplastics from Durban Harbour.

In our local area there are a number of beach cleanups occurring in Durban from students at the University of KwaZulu–Natal, while bins near frequented fishing spots have also been installed by the municipality to reduce the amount of nylon line waste near some of the estuaries sampled. There are also buy back centers around Durban that pay money for plastic debris brought to them from the environment. It is therefore now a common site to see local people wheeling loads of plastic waste to local recyclers and hopefully these will reduce the waste observed and photographed at some of the sampling sites within this thesis.

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APPENDICES

Appendix A

Plastic pollution at Bayhead; a – a northwards view of Bayhead, b – an eastwards view of the lagoon, c – red square area of b enlarged, d – pre-production pellets seen as tiny white dots within red circle of c, e – monofilament nylon line found at mouth station, Durban Harbour, f – Plastic bottle fouled with tubeworms and barnacles at the Isipingo estuary, g – fibre under SEM with other unidentified particles on surface and h – scrubber under SEM.

Appendix B

Fish kills witnessed at the uMgeni Estuary during sampling period. Fish kills were also noticed at the Durban Harbour and Isipingo estuaries during sampling events.

Appendix C: Organisms that have ingested plastics in experiments and *in situ*.

Taxon	Species	Feeding type	Plastic type	Plastic size	Location	Enviro.	% Plastic	Source
Annelida								
Polychaeta								
Lugworm	*Arenicola marina*	Deposit	F, PS **(1)**	400 - 1300 µm	‡	B	0 % > 28 d.	Besseling et al., 2013
Arthropoda								
Crustacea								
Cirripedia								
Barnacle	*Semibalanus balamoides*	Filter	F	20 - 2000 µm	‡	O		Thompson et al., 2004
Barnacle	*Lepas sp.*	Suspension	PE, PP, PS	0.609 - 6.770 mm	NPCG	O	33.5 % of 385	Goldstein and Goodwin, 2013
Malacostraca								
Amphipoda								
Sandhopper	*Talitrus saltator*	Detritivorous	PE, PP, F	10 - 45 µm	Italy (Pisa)	B		Ugolini et al., 2013
Amphipod	*Orchetia gammarelluss*	Detritivorous	F	20 - 2000 µm	‡	B - EST		Thompson et al., 2004
Isopoda	*Idotea emarginata*	Detritivorous	PS spherule	1−100 µm	‡	B		Hämer et al., 2014
Decapoda								
Crab	*Carcinus maenas*	Detritivorous	PS spherule **(2)**	0.5 mm	‡		100 % of 24	Farrell and Nelson 2013
Lobster	*Nephrops norvegicus*	Detritivorous	PP - Fil **(3)**	< 5 mm	Clyde Sea		83 % of 120	Murray and Cowie, 2011
Mollusca								
Bivalvia								
Mussel	*Mytilus edulis*	Filter	PS spherule **(2)**	0.3 - 9.6 µm	‡		all in ‡	Brown et al., 2008
Cephalopoda								
Humboldt squid	*Dosidicus gigas*	Carnivorous	P, N		Pacific	O	27 % of 30	Braid et al., 2012
Echinodermata								
Holothuroidea								
Sea cucumber	*Holothuria floridana*	Deposit						
Sea cucumber	*H. grisea*	Deposit	N, PVC	0.25 - 15 mm	‡	BEN	all in ‡	Graham and Thompson, 2009
Sea cucumber	*Cucumaria frondosa*	Suspension						
Sea cucumber	*Thyonella gemmata*	Suspension						
Chaetognatha								
Arrow worm	*Sagitta elegans*	Carnivorous	1 PS spherule	0.6 mm	Niantic Bay	C	1 collected	Carpenter et al., 1972
Chordata								
Vertibrata								
Osteichthyes								
Estuarine Catfish	*Cathorops spixii*	Zoobenthos	PA N - fil **(4)**	1 - 2 mm			18 % of 60	Possatto et al., 2011
Estuarine Catfish	*Cathorops agassizii*	Zoobenthos	PA N - fil (blue)	1 - 2 mm			33 % of 60	Possatto et al., 2011
Estuarine Catfish	*Sciades herzbergii*	Zoobenthos	PA N - fil (blue)	1 - 2 mm			18 % of 62	Possatto et al., 2011
Estuarine drum	*Stellifer brasiliensis*	Hyperbenthos	PA N - fil (blue)	0.047 ± 0.010 mg ○ ◊	Goiana estuary	EST	6.9 % of 330	Dantas et al., 2012
Estuarine drum	*Stellifer stellifer*	Hyperbenthos	PA N - fil (blue)				9.2 % of 239	Dantas et al., 2012
Brazilian Mojarra	*Eugerres brasilianus*	Carnivorous	N- fil (blue) **(1)**	1 - 5 mm			33.4 % of 27 (adults)	Ramos et al., 2012
Flagfin Mojarra	*Eucinostomus sp.*	Hyperbenthos	N- fil (blue) **(1)**	1 - 5 mm			13.6 % of 44 (adults)	Ramos et al., 2012
Caitipa Mojarra	*Diapterus rhombeus*	Benthophagous	N- fil (blue) **(1)**	1 - 5 mm			12.1 % of 33 (adults)	Ramos et al., 2012
Grubby	*Myoxocephalus aenaeus*	Zoobenthos	PS spherule	< 5 mm			4.2 % of 'atleast 5'	
Winter flounder	*Pseudopleuronectes sp.*	Zoobenthos	PS spherule	< 5 mm	Niantic Bay	C	2.1 % of 'atleast 5'	Carpenter et al., 1972
White perch	*Roccus americanus*	Zooplankton	PS spherule	< 5 mm			33 % of 'atleast 5'	
Silversides	*Menidia menidia*	Zooplankton	PS spherule	< 5 mm			33 % of 'atleast 5'	

Table continued

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Taxon	Species	Feeding type	Plastic type	Plastic size	Location	Enviro.	% Plastics	Source
Gudgeons	*Gobio gobio*	Benthophagous	Fb	250 - > 1000 µm	French rivers	FW	12 % of 186	Sanches et al., 2014
Rice fish	*Oryzias latipes*	Zooplankton	P (grinded) (5)	< 500 µm	‡	FW		Rochman et al., 2013 b
Goby	*Pomatoschistus microps*	Carnivorous	PS spherule (6)	420 - 500 µm	‡	EST		de Sá et al., 2015
Southern opah	*Lampris immaculatus*	Carnivorous	N, F, Fl	> 5 mm	Patagonian Shelf	O	14 % of 69	Jackson et al., 2000
Longnosed lancetfish	*Alepisaurus ferox*	Carnivorous					30 % of 144	
Common dolphinfish	*Coryphaena hippurus*	Carnivorous					2 % of 42	
Snake mackerel	*Gempylus serpens*	Carnivorous					< 1 % of 104	
Smith's escolar	*Lepidocybium flavobrunneum*	Carnivorous	N, F, Fl	> 10 mm	NPCG	O	0 % of 45	Choy and Drazen, 2013
Skipjack tuna	*Katsuwonus pelamis*	Carnivorous					0 % of 29	
Yellowfin tuna	*Thunnus albacores*	Carnivorous					0 % of 26	
Bigeye tuna	*Thunnus obesus*	Carnivorous					3 % of 35	
Broadbill swordfish	*Xiphias gladius*	Carnivorous					3 % of 31	
Anchovy	*Engraulis sp.*	Zooplankton	Fb	1.14 to 2.5 mm	Kerala	O	38 % of 16	Kripa et al., 2014
Herring	*Clupea sp.*	Planktivorous					1.4 % of 566	
Gray gurnard	*Eutrigla sp.*	Zoobenthos					< 1% of 171	
Whiting	*Merlangius sp.*	Nektonic feeding					5.7 % of 105	
Horse mackerel	*Trachurus sp.*	Planktivorous		0.04 to 4.8 mm	North Sea	O	1.0 % of 100	Foekema et al., 2013
Haddock	*Melanogrammus sp.*	Zoobenthos					6.2 % of 97	
Mackerel	*Scomber sp.*	Planktivorous					< 1 % of 84	
Cod	*Gadus sp.*	Nektonic feeding					13 % of 80	
Seabream	*Pagellus bogaraveo*	Nektonic feeding	F	5 - 60 mm	Eastern Ionian Sea	D	0.2 % of 640	Anastasopoulou et al., 2013
Lanternfish	*Myctophum sp.*							
Bigfin lanternfish	*Symbolophorus sp.*							
Lanternfish	*Loweina interrupta*							
Lantern fish	*Hygophum reinhardtii*	Planktivorous	F, Fl, N	1 - 2.79 mm	NPCG	O	35 % of 670	Boerger et al., 2010
Snaggletooth	*Astronesthes sp.*							
Pacific saury	*Cololabis saira*							
Chondrichthyes								
Catshark	*Galeus melastomus*	Nektonic feeding	F, N, Fl, Fb					
Pelagic stingray	*Pteroplatytrygon violacea*	Zoobenthos	F					
Longnose spurdog	*Squalus blainville*	Nektonic feeding	F	5 - 60 mm	Eastern Ionian Sea	D	3.1 % of 862	Anastasopoulou et al., 2013
Velvet belly	*Etmopterus spinax*	Nektonic feeding	F					
Basking shark	*Cetorhinus maximus*	Zooplankton and fish	Phthalates		Mediterranean	O		Fossi et al., 2014
Reptilia								
Sea turtle	*Caretta caretta*	Jellyfish	F, Fl, N, P	1–16 cm	Adriatic Sea	O	35.2% of 54	Lazar and Gračan., 2011
Aves								
Petrel	*Pelagodroma marina*	Small fish	P, F, Fl	> 5 mm	South Atlantic & Western Indian Ocean	O		Ryan, 2008
Petrel	*Fregetta grallaria*	Crustaceans, squid	P, F, Fl	> 5 mm		O		
Shearwater	*Fulmarus glacialis*	Fish, squid	P, F, Fil P	1 - 16 mm	North Atlantic	O	71 % of 17	Provincher et al., 2014
Shearwater	*Calonectris diomedea*	Fish	N, F, Fl	12.7 ± 8.0 ●	North Atlantic	O	83.5 % of 85	Rodríguez et al., 2012
Fulmar	*Puffinus gravis*	Fish, squid	F	1 - 16 mm	North Atlantic	O	51 % of 35	Provincher et al., 2014
Gull	*Larus glaucescens*	Oppurtunistic	Fl	< 10 mm	United States	O	12 % of 589 boluses	Lindborg et al., 2012
Albatross	*Phoebastria immutabilis*	Fish, squid	P, F, N, Fm	1.162 ± 2.436 g ◊	North Pacific	O	83.3 % of 18	Gray et al., 2012
Albatross	*Phoebastria nigripes*	Fish, squid	P, F, N, Fm	0.186 ± 0.293 g ◊	North Pacific	O	52 % of 29	Gray et al., 2012
Mammalia								
Fur seal	*Arctocephalus sp.*	Fish	P and F	2 - 5 mm	Macquarie Island	C	ave of ~ 1 particle / scat	Eriksson and Burton., 2003
Harbour seal	*Phoca vitulina*	Fish and squid	F, Fm, Fil, Fl	1.9667 g ꝇ	Netherlands	C	11 % of 107	Robolledo et al., 2013
Harbour porpoise	*Phocoena phocoena*	Fish and crustaceans	Fl	> 5mm	Black sea	C	12 % of 42	Tonay et al., 2007
Fin whales	*Balaenoptera physalus*	Zooplankton and fish	Phthalates		Mediterranean	O		Fossi et al., 2012

Notes: PVC - Poly vinyl chloride, Fm - Foam, Fb - Fibres, Fl - Film, PS - Polystyrene, PP - Polypropylene, P - Pellets, F - Fragments, N - Nylon, Fil - Filaments, PA - polyamide, ꝇ - Fil total mass, ● - For N, ○ - Largest mass, ◊ - mean mass ± std. dev., FW - Fresh water, EST - Estuarine, B - Beach, O - Oceanic, BEN - Benthic, C - Coastal, D - Deep sea, ‡ - Lab Experiment, NPCG - North Pacific Central Gyre, 1 - Feeding decrease and/or weight loss, 2 - Transfer to haemolymph/organs, 3 - Unable to excrete particles, 4 - Entanglement, 5 - Liver toxicity and pathology, 6 - predatory efficiency decreased

Appendix C: References

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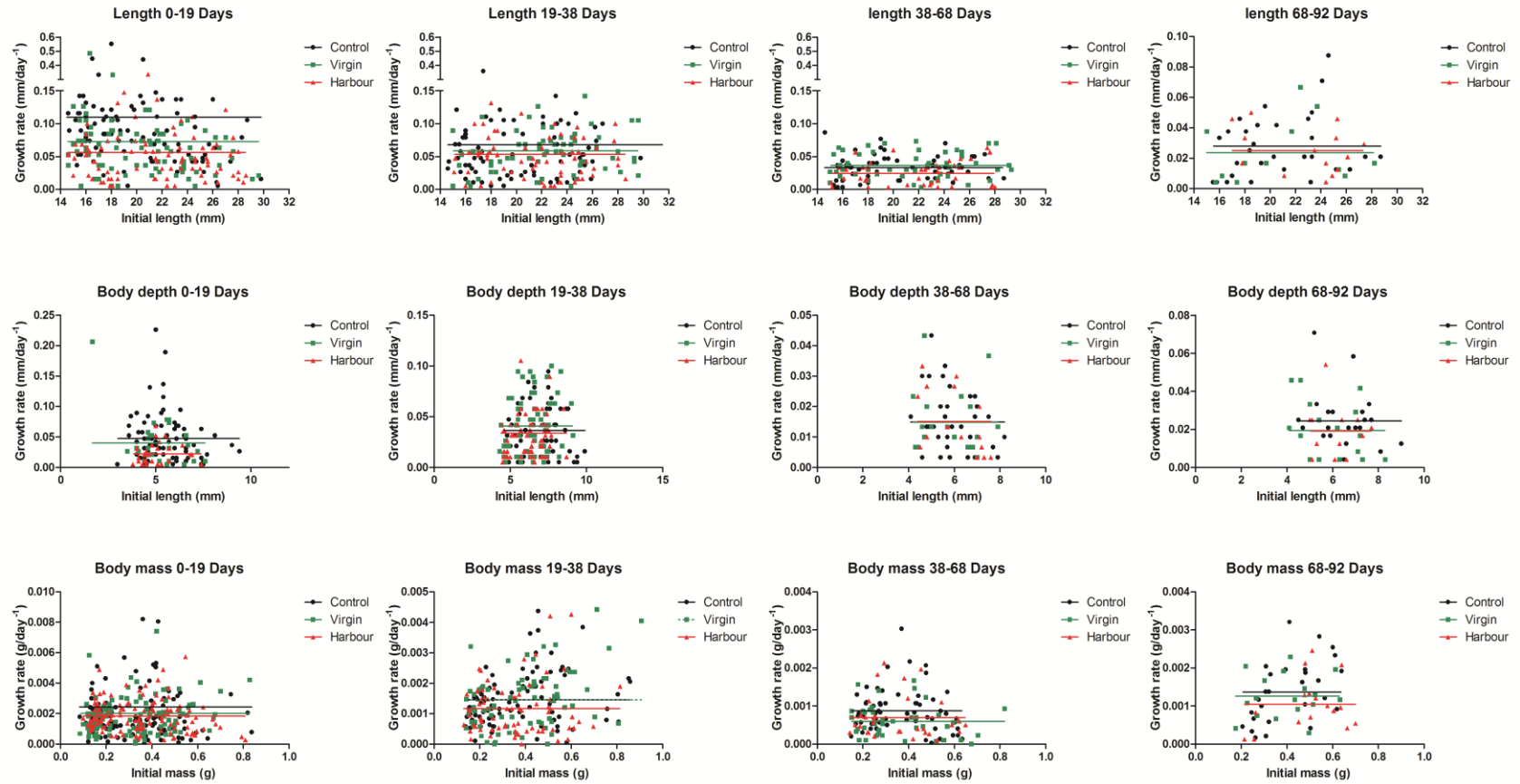
Appendix E1: Mean nematode abundance, per 20 mL (top), per mg. total faeces dry^{-1} (middle) and per mg.faeces dry^{-1} after controlled for plastic mass (bottom). Graphs beside each other are the same, except for a single zero value in the control. The bars represent standard error of the mean.
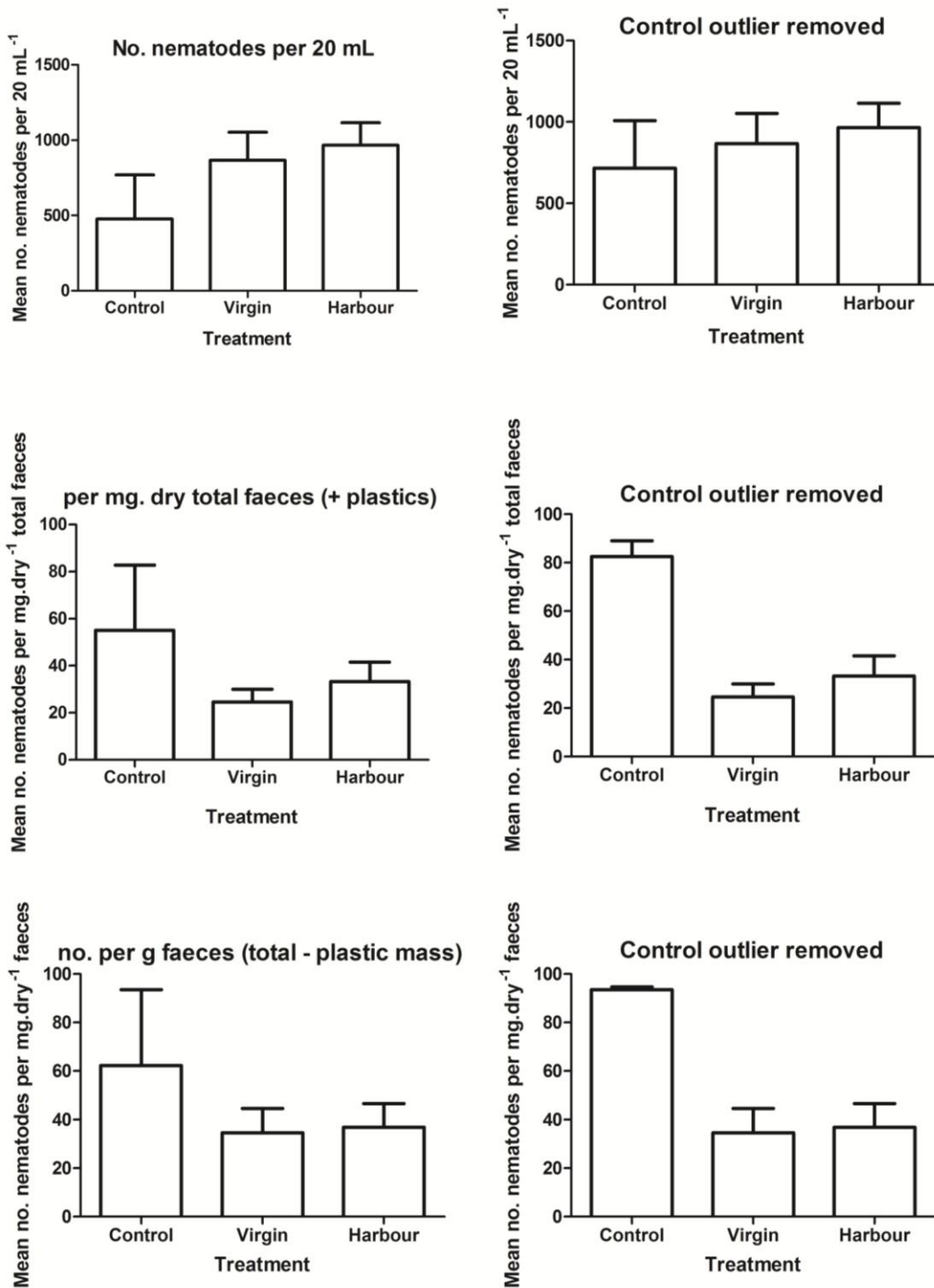
Appendix E2: Nematode counts

The five subsample counts were combined to bring the total to counts per 2.5 mL (0.5 mL x 5). Nematode counts were then multiplied by eight to standardise for the total volume of 20 mL in each vial (2.5 mL x 8 = 20 mL). The total faeces (plastics + faeces) in each vial was then dried; nematode counts were then expressed as per mg dry total faeces. Plastics in each vial/replicate were then isolated from the dried total faeces by digestion. Plastics were weighed and the mass of faeces (only) was obtained by subtracting the plastic mass from the total faeces mass. Nematode counts were then expressed as per mg dry faeces. It first looked like there were more nematodes on the control treatment before controlling for mass. However, once the mass was taken into account the nematode numbers seemed to be much higher in the controls. It could be that the plastics affected their survival while they had been contained in the vials.

Table 1 C: Raw data of nematode counts. These are per mg of faeces dry mass (faeces by subtraction of plastic mass)

Treatment	Nematodes	Mass of faeces (mg)
Control	0	10.47
Control	95	10.66
Control	92	4.59
Virgin	51	11.18
Virgin	37	32.81
Virgin	16	51.3
Polluted	46	27.25
Polluted	47	15.51
Polluted	17	52.44