The occurrence and distribution of microplastics in surficial sediments and fish in a South African reservoir

By

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ABSTRACT

Microplastics have become a major environmental concern globally due to their potential impacts on ecosystem function. They are known to be ubiquitously present, persistent and bio–accumulative, yet ecological impacts of microplastic remain poorly understood despite their ubiquity across all habitat types globally. The combined effects of seasonality and human population density on the extent of microplastics pollution are not well understood. To understand microplastics along human population gradient, I assessed sediment microplastics along a tropical reservoir shoreline across three seasons and seven sites. Multivariable analysis was used to assess relationships among substrate embeddedness, sediment organic matter, human population density, microplastics particle densities and microplastics characteristics. Subsequently, the functional response approach was developed and applied for quantifications of microplastics uptake by the fish across different environmental densities. Microplastics densities were relatively high during the hot-dry season (mean range 120–6417 particles^{-1} kg^{-1} dwt) while the hot-wet season had the lowest densities (mean range 5–94 particles^{-1} kg^{-1} dwt). Microplastics abundances positively correlated with population density, demonstrating the direct effects of human activity on microplastics contamination. Furthermore, I exposed a key species, the banded *Tilapia sparrmanii* (i.e. Smith 1940) to different concentrations of microplastics particles. Tilapia consumed microplastics even when relatively rare in their environment, and consumption rates related negatively to concentrations supplied, conducive with a saturating type II (i.e. inversely–density–dependent) functional response. Attack rate (i.e. search efficiency), handling time and maximum feeding rate estimates towards microplastic were estimated, providing key information on feeding behavior in relation to exposure concentrations. I propose the utility of functional response approaches for predictive quantifications of microplastic uptake

rates. The sediment microplastics quantification results highlight the need to further explore microplastics distribution patterns in freshwater ecosystems within the global south. Further, my findings suggest particular risk for fauna during low rainfall periods through microplastics concentration effects. In turn, this can better-link laboratory exposure studies to environmental concentrations which are known to cause ecological impact, and provide a means of comparing uptakes among species and across environmental contexts.

Keywords: attack rate; handling time; pollution; consumer–resource, microplastics, Nandoni reservoir, freshwater ecosystem, contamination, freshwater pollution

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PREFACE

Each data chapter in this thesis is a stand–alone and has been published or is under review in international peer reviewed journals. As such, there may be some repetitions in the introductory sections of the chapters. The research reported in this thesis was done in conjunction with other scientists that are listed as co–authors of the mentioned articles:

1. **Mbedzi R**, Dalu T, Wasserman RJ, Murungweni F and Cuthbert RN. 2020. Functional response quantifies microplastic uptake by a widespread African fish species. *Science of the Total Environment*. DOI: **In press**
2. **Mbedzi R**, Dalu T, Wasserman RJ, Murungweni F and Cuthbert RN. Spatiotemporal variation in microplastic contamination along a subtropical reservoir shoreline. *Environmental Science and Pollution Research*. **Under review**

DECLARATION

I declare that "*Occurrence and distribution of microplastic in freshwater sediments and fish: A case of Nandoni Reservoir, South Africa"* is my own work. All other sources, used or quoted, have been indicated and acknowledged by means of complete references. This dissertation has not been submitted for a degree at another university.

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CHAPTER ONE: INTRODUCTION

Plastic has many societal benefits which are undisputable, but there are some serious environmental concerns that are allied with plastics (Andrady and Neal, 2009; Barnes *et al*., 2009; North and Healden, 2013). The increase in plastic production and poor plastic waste management have resulted in increased dumping of plastic in aqueous environments (Panda *et al*., 2010). Microplastics are tiny pieces of plastic, <5mm in size (GESAMP, 2015; Boucher and Friot, 2017; Li *et al*., 2018). They are the smallest form of plastic litter which can be harmful to aquatic life (Van Couvenberghe *et al*., 2013) and can be consumed by many different species from a range of habitats (Arthur *et al*., 2009; Brande–lavridsen, 2017; Halstead *et al*., 2018).

Plastics are divided into macro-, micro- and nanoplastics because it helps to evaluate their potential sources, fate, effects and to identify mitigation measures to reduce their environmental impacts (Koelmans, 2015; Alimi *et al*., 2018). In this thesis, microplastics (MPs) are classified as plastics <5 mm and greater than 63 µm (Andrady, 2011, GESAMP, 2015). Primary microplastics are produced and purposefully made for industrial or domestic use (Auta *et al*., 2017; Kiprop, 2018). They are comprised of tiny plastic bits used in facial cleansers, tooth paste, resin pellets, synthetic fibres, paints, medical products, recycling of tyres and cosmetics (i.e. showering or bathing gels and bathing scrubs) (Sundt *et al*., 2014; Boucher and Froit, 2017). Secondary microplastics are fragmented from large plastic debris and can lose some properties such as color due to weathering processes (Nel and Froneman, 2015; Estahbanti and Fahrenfel, 2016; Wu *et al*., 2018). As plastic debris disintegrate, microplastics spread both on land and aquatic environments, and a variety of organisms can feed on these microplastics (Law and Thompson, 2014; Alomar *et al*., 2016).

Plastics have unique properties that make them relevant and important for our everyday life (Thompson *et al*., 2009; Naidoo *et al*., 2015). Plastics can be used at different temperatures, have low thermal conductivity, durable, versatile, has high strength to weight ratio and very cheap (Klein, 2011; Ivleva *et al*., 2017). This has made societal consumption of plastic products to increase exponentially in recent years (Halstead *et al*., 2018). According to Plastic Europe (2017), the annual global production of plastic products was 335 million tonnes, whereas, in South Africa, the consumption rate was 1.28 million tonnes in 2009 and increased to 1.4 million tonnes in 2014 (Plastics SA, 2015; Van Cauwenberghe *et al*., 2015). The South African plastic application rate varies with plastic type for instance, low-density polyethylene (LDPE) have the greatest application rate at 21.2 % compared to polyethylene (PET) bottles (13.9 %), high density polyethylene (HDPE; 15.1 %) and polyvinyl chloride (PVC; 11.1 %) (Plastic SA, 2015).

The public and government authorities have gained much interest on microplastics over the past recent years (Peng *et al*., 2018). MPs pollution has been observed entirely as a marine problem, but there is an increasing amount of evidence considering the state of MPs pollution in freshwater systems worldwide (Wagner, 2014; Silva–Cavalcanti *et al*., 2016; Nel et al., 2018). There is noticeably limited information on the occurrence or existence of MPs in freshwater ecosystems of Africa (Khan *et al*., 2018). In the past few years, MPs have been detected in freshwater surface waters and sediments (Erikson, 2013; Van Cauwenberghe *et al*., 2015). Reservoirs may also be potential areas for accumulation of microplastics (Zhang *et al*., 2015).

Nandoni reservoir has some of its base flows originating from populated areas, wherein activities in populated areas are linked to the water quality deterioration (Gumbo *et al*., 2016). However, there was no information regarding microplastics pollution for the reservoir. The study focused on the occurrence and distribution of microplastics at Nandoni Reservoir and aimed at filling in gaps by providing new data that will be useful for future management of the reservoir.

1.1 Problem statement

An understanding of the issue of microplastics occurrence and distribution in sediments and fish in freshwater ecosystems is limited. The significance of microplastics impacts on freshwater ecosystems such as accumulation of different dissolved chemicals, continuous slow break down of microplastics into very small plastic pieces in the water column and sediment and physical impact on aquatic organisms due to ingestion of microplastics is crucial. However, causes of differences in seasonal densities of microplastics in fish and sediment in Nandoni reservoir are not known. Since microplastics pollution has detrimental environmental effects, a more comprehensive understanding of the occurrence of microplastics in Nandoni reservoir is therefore necessary.

1.2 Justification of study

Microplastics pollution is a developing problem in many watercourses around the world. Most researches in South Africa describing the occurrence of microplastics as well as quantifying the amount and impact of microplastics pollution have focused on marine environments, mainly on sea birds and fishes dating back to the early years 1980s (Furness, 1983; Ryan *et al*., 1998; Ryan, 2008; Khan *et al*., 2018). Studies focusing on quantification of microplastics abundance in freshwater systems are limited, hence more freshwater studies are required (Erickson *et al*., 2013; Wagner *et al*., 2014; Su *et al*., 2016; Peng, 2017; Nel *et al*., 2017). Most of these quantification studies report more on spatial distribution and less on temporal trends (Di and Wang, 2018).

1.3 Research aims

- Assess the sediment microplastics pollution, in terms of composition, distribution and density across three seasons (hot-dry, hot-wet, cool-dry) in Nandoni reservoir.
- Employ a functional response approach to quantify the density–dependence of microplastics uptake by the banded tilapia *Tilapia sparrmanii* (i.e. Smith, 1940), which is a widespread and key species in warm freshwater habitats of southern Africa.

1.4 Hypothesis

1. Sediment microplastics densities will show strong seasonal (hot–dry, hot–wet, cool–dry) differences due to different precipitation patterns. High microplastics loads will be in highly populated sites during the hot–dry season because human activities, low precipitation rate and substrate embeddedness strongly influence the abundance of microplastics found along the reservoir shorelines.
2. Microplastics uptake by juvenile stages of the banded tilapia fish will relate positively to environmental microplastics concentrations because fish normally mistake microplastics for food. Further, it is expected that tilapia will uptake microplastics even when present at relatively low densities in aquatic environments.

CHAPTER TWO: LITERATURE REVIEW

2.1 Microplastics in aquatic ecosystems

2.1.1 Marine ecosystems

Knowledge of microplastics distribution in marine environment is prominent (Hidalgo–Ruz *et al*., 2012; Nel and Fronemen, 2015). Marine littering is when plastics waste are misplaced or illegally thrown away and are transferred into seas and oceans from the terrestrial environments (Hopewell *et al*., 2009). According to Andrady (2011), 80 % of plastic pieces found in marine environments are from populated terrestrial coastal regions (Ryan *et al*., 2009). Plastic pieces found in marine environments have been identified as a reason for biodiversity loss (Thompson, 2015) due to the fact that the majority of the marine's plastic particles are from rivers, lakes, reservoirs and estuaries (Ryan *et al*., 2009).

Microplastics are accumulating in marine ecosystems and continue to breakdown into smaller particle sizes (Andrady, 2011; Lusher *et al*., 2012; Gilgani *et al*., 2015). Numerous microplastics studies from researchers have tried to address microplastics sources, fate and consequences in marine environments (Sundt *et al*, 2014; GESAMP, 2015, 2019). Several effects of microplastics in aquatic environments are based on field research studies and experiments conducted, which continue to show microplastics effects on aquatic organisms (Kalcikova *et al.,* 2017; Halstead *et al.,* 2018; Nel *et al.*, 2018). Microplastics concentrations are increasing in aquatic systems close to populated or urban areas due to increased land-based sources of plastics as well as currents and other oceanographic conditions (Barnes, 2002; Ngupula *et al*., 2014; Wang *et al*., 2017;

Jiang *et al*., 2018). This indicates that there are many aspects to take into consideration in order to be able to forecast the future state of microplastics situation.

Microplastics particles moving into the sea are fragments from many specific sources from distinct locations (Browne *et al*., 2007). Ocean currents move seawater by various means such as temperature, breaking waves, wind, Coriolis Effect, salinity and density (White *et al*., 2010). Microplastics properties such as short length and low density make contributions to their ubiquitous delivery across long distances from their point sources particularly through ocean currents whilst heavier debris like nylon, polystyrene, and acryl are commonly found near their sources (Cole *et al*., 2011; Eriksson *et al*., 2013; Eerkes–Medrano, *et al*., 2015). These properties make it possible for microplastics to spread throughout the oceans, from beaches or surface waters down to the sediments. As the currents move, they move organisms and plastic debris to huge slow whirlpools of water and nonnative habitats, while plastic debris pose a likelihood to damage biodiversity and the environment in coastal areas (Barnes 2002; Gregory, 2002). MPs particles last for a longer period than most naturally floating surfaces (Jiang *et al*., 2018).

2.1.2 Freshwater ecosystems

Rivers and reservoirs are sources of drinking water for many communities and a habitat for wildlife (Hoellein, 2016). Lack of microplastics studies in freshwater ecosystems is limiting our understanding of sources and fate of microplastics (Peng *et al*., 2017). Freshwater ecosystems are identified as temporary sinks for microplastics pollution (Nel *et al*., 2018). When plastic waste directly or indirectly enters freshwater ecosystems such as through wastewater effluent points and refuse sites, light weight litter will float in streams and be carried away to the ocean surface while heavier plastic litter will sink down into sediments (Morris, 1980; Barnes *et al*., 2009).

Studies conducted about freshwater systems indicate that most freshwater systems are the main channels which carry microplastics from catchments to oceans (Dekeif *et al* 2014; Naidoo *et al* 2015; Besseling *et al*, 2017). Browne *et al*., (2010) and Moore *et al*., (2002) have shown how the high unidirectional flow of freshwater systems drive the movement of plastic debris into the oceans. Some studies from researchers have highlighted that freshwater ecosystems carry as much microplastics loads as marine ecosystems (Zhang *et al*., 2015). The majority of most microplastics are made up of polyethylene which accumulates in sediments and biota within the freshwater ecosystems (Kalcikova *et al*., 2017).

According to the Department of Trade and Industry (DTI) report in 2014, the South African cosmetic and personal care industry is a vibrant and dynamic market. It was estimated at R25.3 billion retail level, contributing 1 % to the gross domestic product (GDP). Microplastics in personal care consumer products are intentionally produced and have been used for the past 50 years (Sundt *et al*., 2014; Wagner, 2014). They are intended to be washed away and end up in the drain (Li *et al.*, 2018). Improper wastewater treatment is one of the dominant sources of microplastics introduced in freshwater ecosystems, since treated wastewater can contain significant amounts of microbeads and fibres (Naidoo *et al*., 2015; Tagg *et al*., 2015). Incomplete removal of microplastics in treatment water plants results to microplastics pollution. Hence if microplastics are not filtered out in treatment plants, they can be released directly into river systems (Talvitie *et al*., 2017). Microplastics have the potential to rapidly reach the sea through the discharge of treated wastewater from which microplastic particles may be transported through rivers, reservoirs and lakes (Magnusson *et al*., 2016).

The contribution of microbeads to the plastic litter pool is often underestimated (Kalcikova *et al*, 2017). Microbeads released into the receiving freshwater systems can have particle sizes just below 60 mm (Eerkes–Medrano, 2015). According to the study conducted by Talvatie *et al*., (2017) wastewater treatment cannot remove 100% of microplastics in wastewater because the smallest size fraction is <100 mm yet, advanced wastewater treatment technologies can substantially reduce the MPs discharged from wastewater treatment plants into the freshwater aquatic environments. More recently, pharmaceutical personal care products (PPCPs) have been discovered in various surface and ground waters, some of which have been linked to ecological impacts at trace concentrations (Syder *et al*., 2003).

2.2 Microplastic effects

According to Ryan *et al*. (2009) and Eriksson *et al*. (2014), microplastics pieces of all sizes are located in all ocean areas. Microplastics pollutants are destroying marine ecosystems as they act as conduits for species movements from one area to another (e.g. bryozoans attached on buoyant plastic particles; Barnes 2002; Moore, 2008). According to Nel and Froneman (2015), the status of microplastics pollutants alongside the shoreline of South Africa showed that seaside sediment and water samples are seriously polluted with microfibres that are fragmented from synthetic materials.

Plastic has been shown to absorb and concentrate a "cocktail of toxic chemicals" transporting them to aquatic ecosystems and possibly releasing them in organisms after being ingested (Teuten *et al.,* 2009; Rochman, 2013; Jonsson *et al*., 2014; Dris *et al*., 2018). The chemicals may be brought into contact with plastic polymers during manufacturing or may be absorbed within marine surroundings (continual pollution persistent natural pollutants) (Rochman and Browne, 2013, GESAMP, 2015). Different types of plastics have different absorption potential. Microplastics act as a mode of transportation for persistent organic pollutants within the aquatic ecosystems and may lead to increased exposure of aquatic life to toxic pollutants (Koelmans *et al.,* 2013). Such properties causes the persistent organic pollutants (POPs) to travel long distances, being easily and widely distributed as well as building up and increasing intensity through food chain accumulating in fatty tissues (Orris *et al.,* 2000). The effect of POPs in combination with microplastics on organisms is not fully understood and very few of these POPs (pesticides, industrial chemicals and by–products) effects are well–known (Jonnson *et al.,* 2014). For example, a pesticide such as dichlorodiphenyltrichloroethane (DDT) has a very long side-effect history (Beckvar *et al*., 2005). The most well-known side-effect of DDT was seen on the eggshells of birds. The shells were so thin that they broke during incubation which affected chick mortality and brooding behavior (Dirksen *et al.,* 1995). Polychlorinated biphenyl (PCB) is well-known for being toxic to fish (Van den berg *et al*., 1998) and studies have shown correlation between ingested plastics and PCBs in the tissue and eggs of great shearwaters (Moore 2008).

Microplastics may be intentionally or incidentally ingested by organisms (Cole *et al.,* 2011). Incidental ingestion happens when plastic debris is swallowed together with natural food or through food chain (Silva-Cavalcanti *et al*., 2017; Peters and Bratton, 2016). Intentional ingestion happens when plastic are mistaken for food and intentionally captured and ingested by an organism (Sighicelli *et al*., 2018; Wagner *et al*., 2014; Remy *et al*., 2015). Plastics can resemble jellyfish and other types of food (Cliff *et al*., 2002), posing a hazard to larger animals such as cetaceans and turtles (Balazs, 1985; Moore, 2008). Previously conducted microplastic studies in marine surroundings showed that a few plastic particles were ingested by seabirds (Furness 1985; Ryan *et al*., 1988; Mallory 2008). The consequence of marine plastic pollutants by means of feeding and entanglement of marine species, varying from zooplankton to cetaceans, seabirds, marine mammals and reptiles are well documented (Gregory, 2009; Erikson *et al*., 2014). Aquatic organisms, as well as plastic debris, come in all shapes and sizes. Therein lay the risk of plastics blocking or becoming stuck in the gastrointestinal tract (Lusher *et al*., 2012; Cole *et al*., 2013; Dris *et al*., 2018). Some plastic debris are so small in size that they get easily ingested and injure the digestive tract of animals (Derraik, 2002; Farell and Nelson, 2013; Lusher *et al*., 2013).

The size of plastic pieces in relation to the size of the gastrointestinal tract of the organism ingesting them is relevant for the organism's capacity to ingest the plastic (Jonsson *et al*., 2014). Larger pieces of plastic are known to affect many types of species as Cliff *et al*. (2002) found sharks in KwaZulu–Natal entangled and tissues damaged by strapping bands. Cotton textile fibres were found in fishes. Seagoing birds such as albatross, shearwater, petrel and fulmar have been found with plastic debris in their stomachs and 44 % of all seabird species have been known to ingest plastic (Moore, 2008). Microplastics block the digestive system of aquatic animals which results to low degrees of oxygen and consequently animals die (Gosling, 2015). The build-up of microplastics in aquatic environments is the main difficulty due to the fact that plastics are indigestible and hard to remove from the environment (Faizen, 2017). It is important to start limiting the amount of plastic produced and thrown away. A move towards natural packing of materials would be a big step, as it would demand for an even greater effort in the recycling of plastics (Jonson *et al*., 2014).

2.3 Microplastics types

Microplastics have different characteristics such as shapes which can be used to indicate their origin such as line/fibre which usually originates from fishing lines, clothing, or other textiles (Wu *et al*., 2018). Microplastics can be classified according to polymer types such as polyethylene (HD/LD–PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyamide fibers (nylon) (Andrady, 2011). Featured surface textures on microplastics such as grooves, fractures, mechanical pits flakes, granules and solution pits can be used to indicate the processes of mechanical and oxidative weathering (Zbyszewski *et al*., 2014). A study conducted by Klein (2015) suggested that microplastics can act as a sink for hydrophobic contaminants, they act as a pathway for transferring environmental contaminants from freshwaters to biota (Wagner *et al*., 2014) and concentrate harmful persistent organic pollutants (POPs) onto their surface (Nel *et al*., 2018).

2.4 Policy and legislations regarding water quality in South Africa

South Africa's freshwater resources including rivers, lakes and groundwater, are under an escalating strain from a growing population and an expanding economy (Oberhoster and Ashton, 2008). The freshwater quality has been declining due to increased pollution resulting from industries, urbanization, sewage waste, afforestation, mining and agriculture (Ashton *et al*., 2008). Projections stipulate that South Africa will be using absolutely all exploitable freshwater resources and will be unable to satisfy the desires of people and industries through the year 2020 (Schlacher and Wooldridge, 1996). Without a radical improvement in freshwater management and water treatment technologies, microplastics pollutants will reduce the benefits of freshwater resources and increase the costs related to treatment of water from these resources (Taylor *et al*., 2007).

The Water Act no.36 of (1998) has the purpose to assure that the nation's freshwater resources are protected from contamination and MPs pollution. Freshwater resources must be used, developed, conserved, controlled and managed in methods which include the assembly of basic human needs of present and future generations. This act encourages equitable right of access to quality freshwater. It promotes efficient, sustainable and useful use of water inside the public interest; enabling social and monetary development; safety of aquatic and allied ecosystems in addition to their organic range and ensures that all international responsibilities are met.

The National Management Waste Act no.59 of (2008) is to improve the regulation regulating waste management to protect health and the environment by imparting possible measures for the prevention of pollution and ecological degradation. It is for securing ecologically sustainable management. It also promotes the goals of recycling which are to preserve resources as well as reduce the environmental effects of waste with the aid of reducing the amount of waste disposed at landfills. In most African nations, even within the presence of reusing and recycling practices, effective plastic waste management frequently lacks a legal foundation (Leerar *et al*., 2015). This results in urban and industrial waste in developing nations being dispatched to disposal sites or dumped as combined bulks. This type refuse dumping is a major motive of pollutants in African waters and is a known and recognized source for MPs pollutants (Sharholy, 2008). To enhance water and waste management practices, sustainable methods must be a concern (Li, 2018). Some of these approaches could include establishing permanent recycling stations or working with communities to promote recycling and change their perception of plastic from disposable single–use items. There is little progress in reduction of releasing plastic to the surroundings due to environmental regulations including National Environmental Management Act (107, 1998) but our sustained call for plastic causes contamination to the environment through plastic pieces to grow (Matete and Trois, 2008).

CHAPTER THREE: SPATIOTEMPORAL VARIATION IN MICROPLASTIC CONTAMINATION ALONG A SUBTROPICAL RESERVOIR SHORELINE

3.1. Introduction

Human activity has resulted in pollution of aquatic ecosystems with synthetic polymers, i.e. plastics, which may be harmful to ecosystem function (Lambert Waters et al., 2016; Wagner, 2018). The light weight, high durability and low production costs of plastics makes them ideal for different purposes, and has resulted in an increase in plastic use since the 1950's (GESAMP, 2015). Microplastics (< 5 mm) that contaminate aquatic ecosystems are diverse in shape, size and origin (GESAMP 2015, 2019). Microplastics manufactured intentionally, either as resin pellets to produce larger items or indirectly in cosmetic products such as facial scrubs and toothpastes, are called primary microplastics, while secondary microplastics disintegrate from larger plastic debris (Horton et al., 2017). Microbeads, fragments, foam and fibre are common categories used when identifying microplastics (Eriksen et al., 2013). Such characterization can potentially be used to indicate microplastics origin, such as line/fibre which usually originates from fishing lines, clothing, and/or other textiles (Wu et al., 2018). Microplastics can also be classified according to polymer types such as polyethylene (HD/LD−PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyamide fibers (nylon) (Andrady, 2011; Zbyszewski et al., 2014).

The South African government categorised the plastic industry as an important sector for economic growth in enhancing export, innovation and recycling (DTI, 2016). However, the country is eleventh globally as a main contributor of marine plastic debris, despite waste disposal protocols and legislation to promote recycling being in place (Verster et al., 2017). Excessive plastic production has placed a strain on aquatic ecosystems as unwanted microplastics enter aquatic ecosystems through wastewater discharge, degradation of larger plastic items and user discards (Barnes et al., 2009). Thus, the widespread abundance of microplastics in the environment is directly due to human activities (Rilig, 2012), yet there is currently a paucity of understanding into how microplastics pollution relates to human population densities. Furthermore, whilst much research has focused on investigating the source, fate, abundance and impact of microplastics in marine systems, only a few studies have been conducted within freshwater ecosystems (e.g. rivers, lakes, reservoirs) (Biginagwa et al., 2016; Horton et al., 2017; Nel et al., 2018; Hurley et al., 2018; Tibbetts et al., 2018). Accordingly, this emphasises the need for further studies in these ecosystems to close knowledge gaps.

Freshwater systems are an important conduit for microplastics between inland terrestrial inputs and marine environments (Mani et al., 2015). Microplastics enter water sources through various routes such as storm water (Silva-Cavalcanti et al., 2017; GESAMP, 2019), wastewater discharge (Nel et al., 2018; GESAMP, 2019) and littering (Dris et al., 2017; GESAMP, 2019). Micro−beads, for example, are typically buoyant in waterbodies and can be desorbed upon entering gastrointestinal tract, thereby affecting the pH and ion balance in organisms (Tanaka et al., 2013). Moreover, microplastics with sorbed co-contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) can also be deposited in sediment surfaces. The aggregation of microplastics particles with organic matter in sediments can increase particle size and density, resulting in increased microplastics sedimentation rates (Long et al., 2015; Nel et al., 2019). Of greatest concern is the potentially wide range of effects microplastics pose to ecosystems and public health due to microplastics ingestion by aquatic organisms and trophic transference through entire food webs (Teuten et al., 2007; Browne et al., 2008; Farrell and Nelson, 2013; Nel et al., 2018; Cuthbert et al., 2019).

Studies focusing on microplastics occurrence and distribution in freshwater sediments are lacking within Southern Hemisphere tropical regions. Since microplastics pollution may have detrimental environmental effects, a comprehensive examination of the occurrence, characteristics and distribution of microplastics in a model subtropical reservoir was carried out. Such systems can serve as regional models for ecosystem types, potentially highlighting the sources, nature and extent of microplastics pollutants. Thus, the current study aimed to contribute to the limited body of knowledge on microplastics that currently exists for freshwater ecosystems in the Southern Hemisphere. More specifically, the study assessed the sediment microplastics pollution, in terms of composition, distribution and density, across three seasons. We hypothesized that sediment microplastics densities would show strong seasonal (hot-dry, hot-wet, cool-dry) and site (low and high population density) differences, with high microplastics loads in highly populated sites and during the hot-dry season, with human population activities and substrate embeddedness strongly influencing the abundance of microplastics found along the reservoir shorelines.

3.2. Materials and methods

3.2.1. Study area

Nandoni Reservoir (22°59'11"S, 30°36'16.19"E) is located between Mutoti and Budeli villages, Thulamela Municipality, approximately 10 km from the town of Thohoyandou, Limpopo province, South Africa and is mainly used for domestic water supply and irrigation. The reservoir is 2215 m long, has a catchment area of 1380 km^2 and a total capacity of 16.4 million m^3. The region is generally characterised by warm, humid summers and cool−dry winters. The average temperatures in summer and winter are 23 °C and 17 °C, respectively. The average annual precipitation for the entire catchment varies between 610 to 800 mm, with a mean annual runoff of 519 million m^3 (Heath and Classen, 1990). The prevailing wind direction is from the east to southeast in both the summer and the winter months. The topography of the reservoir area falls under the Soutpansberg Group. It is comprised of low−lying, undulating terrain which is underlain by a gneiss sequence. The soil in most parts has been eroded due to continuous cultivation. Erosion in the reservoir basin occurs generally in areas of dissipative topography where erodible material is available.

The study was carried out over three seasons, i.e. hot−dry (September 2018), hot−wet (March 2019) and cool−dry (June 2019). Site selection was based on human population density along the reservoir shorelines, where seven sites were selected around the reservoir: four sites (site 1 – Mulenzhe village (population density (PD) 2566, area – 4.37 km^2), site 4 – Budeli village (PD 2362, area – 3.56 km^2), site 5 – Muledane village (PD 1428, area – 1.57 km^2), site 6 – Dididi village (PD 2312, area – 2.66 km^2) were categorized as high PD sites and three sites (site 2 – PD 6, site 3 – PD 0, site 7 – PD 4) were categorized as low PD sites (Fig. 1). Most of these sites were also areas of high deposition as highlighted by the substrate content, i.e. high amounts clay/silt to sandy soil being observed and no samples were collected during the hot-dry season for sites 5 to 6.

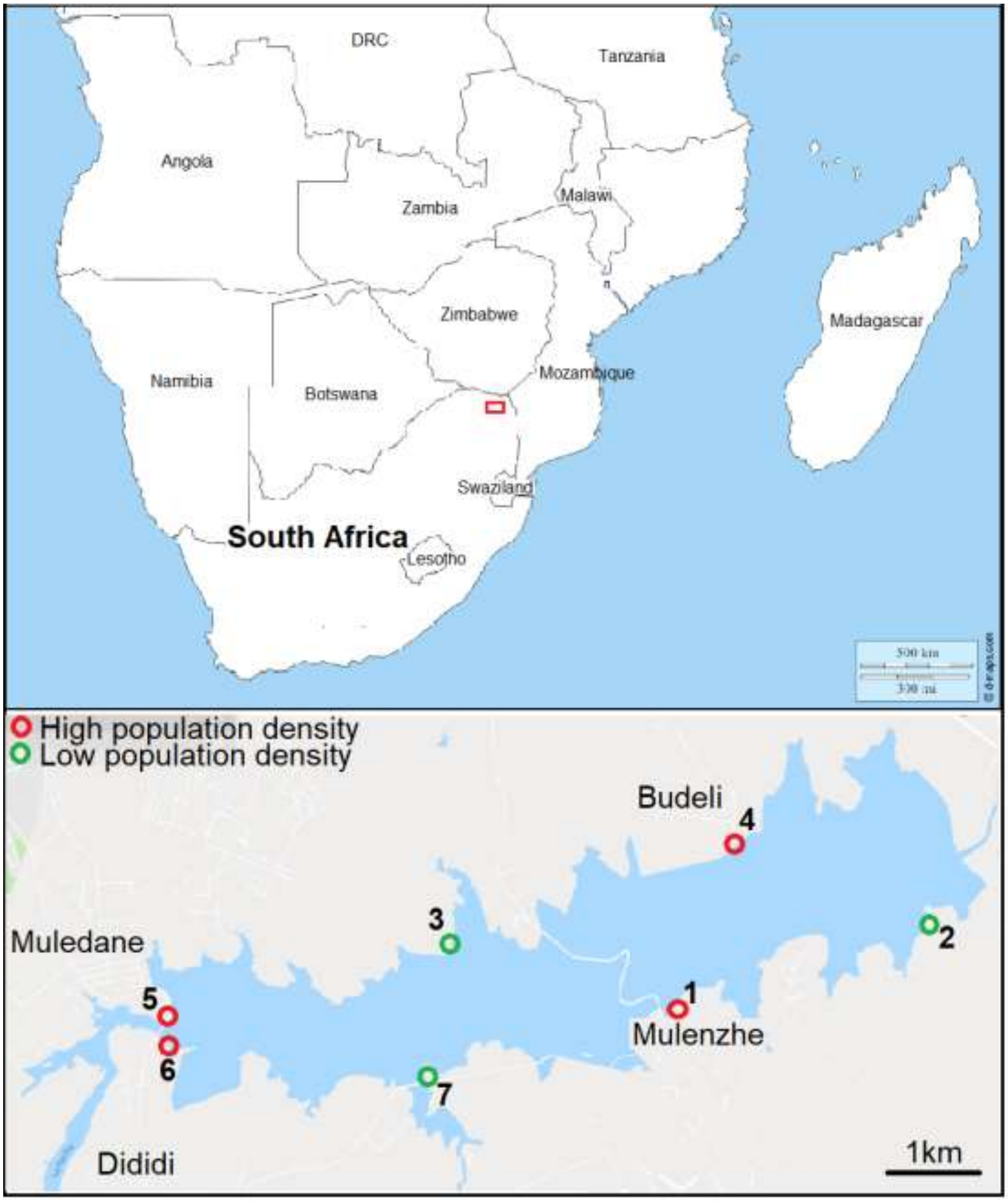

Fig. 3. 1. Location of the study sites within Nandoni reservoir, Limpopo Province, South Africa

3.2.2. Sediment characteristics

Substrate embeddedness was determined according to Platts et al. (1983) through the assessment of the surface covered by fine sediment: 1 (> 75 %), 2 (50–75 %), 3 (25–50 %), 4 (5–25 %) and 5 (< 5 %). The sediment organic matter (SOM) was determined using the Chan et al. (2001) modified Walkley–Black method.

3.2.3. Extraction and enumeration of sediment microplastics

Microplastics considered for this study were <5 mm (GESAMP, 2016, 2019), but >63 µm (mesh size utilised). Two sediment samples (~1.5–2 kg) were collected per site on the littoral zone (i.e. one sample was made up of three sediment subsamples collected from three random spots, approximately 10 m apart) from the upper 5 cm sediment layer along the reservoir shoreline and stored in labelled clear-plastic ziplock bags. In the laboratory, the sediment samples were dried in an oven at 60 ºC for 72 h until a constant weight was reached. After drying, each sediment sample was homogenized using a riffle splitter, and thereafter a sediment subsample of 0.5 kg was separated from the riffle splitter and sieved through a 500 µm mesh steel sieve to remove large organic matter particles and rocks. The sediment material retained on the sieve was analysed for large microplastics (500 µm −5 mm), for inclusion in the total microplastics count.

To prevent contamination, prior to all analyses the entire laboratory was cleaned with all surfaces and equipment cleaned with milliQ distilled water. No air-conditioners or fans were utilized in the lab during the study to minimise the risk of potential air-borne microplastics particle transport. Each sieved 0.5 kg subsample was placed into a clean 5 L beaker and a 63 µm mesh filtered hyper–saturated saline solution (100 g coarse salt L^{-1}) was added. The mixture was stirred vigorously to allow the release and suspension of trapped plastic particles, before allowing the denser sediment to settle out for 3–24 h, depending on the soil type. After this time, the supernatant was filtered through a 63 µm mesh and the entire process was repeated five times so that all microplastics could be quantified (Nel *et al*., 2018). To further reduce potential air−borne microplastics particle contamination, all samples were covered with a small tray. The microplastics on the 63 µm mesh sieve were carefully rinsed with distilled water into 50 mL polystyrene jars, before the samples were visually sorted under an Olympus dissecting microscope at ×50 magnification, whereby all possible microplastics particles were enumerated according to colour (i.e. colours: red/pink, white, black/blue, yellow/orange). Particles were deemed to be microplastics if they possessed unnatural colouration (e.g. bright colouration, multi−coloured) and/or unnatural shape (e.g. sharp edges, perfectly spherical; Hidalgo−Ruz *et al*., 2012). As visual inspection alone was not adequate to characterize and exhaustively quantify microplastics, further physical analysis was utilised (Mintenig *et al*., 2017; GESAMP, 2019). Therefore, a vibrational Platinum-ATR Fourier−transform infrared spectroscopy (FT−IR) (Bruker Alpha model, Germany) was employed on selected microplastics particles for confirmation. This technique offers available libraries for microplastics polymer identification and is more efficient for dense samples, as in the present study (Picó and Barceló, 2019). The number of microplastics particles was estimated as number of microplastics particles kg^{-1} of dry weight (dwt).

To test microplastics recovery rates, soil samples (n = 3, 0.5 kg) were collected from non-impacted terrestrial sites about 30-50 cm underground. The samples were each spiked with 0.1 g (~309 particles or 77 particles L^{-1}) ultra-high molecular weight, surface-modified multi-coloured polyethylene powder, 125 µm particle size (Sigma-Aldrich, UK) and homogenized (well-mixed) before being separated similar to field samples using a hypersaturated saline solution. The recovery rates ranged between 88 % and 95 % (mean 92 %) of the microplastics particles.

3.3. Data analysis

All microplastics particle data were log (x + 1) transformed to homogenize variances. Correlation analysis was used to assess for relationships of the environmental variables (substrate embeddedness, SOM), population densities and microplastics abundances using SPSS v16.0 (SPSS Inc. 2007).

We tested whether combined microplastics particle types (i.e. colours: red/pink, white, black/blue, yellow/orange) abundances differed among seasons and sites. Distance−based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001) based on Euclidean distance dissimilarities was carried in PRIMER v6 add-on package PERMANOVA+ (Anderson et al., 2008) to determine the differences in microplastics particles types and abundances among study sites and/or seasons. Each term in the analysis was tested using 9999 permutations of the correct relevant permutable units (Anderson and ter Braak, 2003), with significant terms investigated using posteriori pairwise comparisons with the PERMANOVA t statistic (Anderson et al., 2008). Spatiotemporal variation in microplastics particles was analysed using non-metric multi-dimensional scaling (n-MDS) based on Bray-Curtis similarity measures.

3.4. Results

3.4.1. Sediment characteristics

Shoreline substrate embeddedness, determined according to Platts et al. (1983), was rated 1 (> 75 % fine sediment) for sites 1, 3 and 6), with sites 2 and 5 exhibiting a rating of 2 (50–75 % fine sediment), site 4 a rating of 3 (25–50 % fine sediment) and site 5 a rating of 5 (< 5 % fine sediment). Sediment organic matter was generally high in sites with low fine sediment content (range 10.6–26.7 %) compared to high fine sediment sites (1.5–7.6 %). A significant interaction between *sites* and *seasons* on SOM was detected (F = 4.488, p = 0.004). No significant relationships were observed for *microplastics loads* and *substrate embeddedness* (r = −0.23, p = 0.174) and *population density* (r = 0.10, p = 0.098) but microplastics load was significant positively correlated (r = 0.63, p = 0.021) with SOM suggesting that sites with high SOM (e.g. sites 1, 3, 6) had greater microplastics loads.

3.4.2. Sediment microplastics

Control samples contained no microplastics. As such, microplastics encountered in samples were considered from the collection site and not an aspect of laboratory contamination. Overall, up to four different microplastics colour types (i.e. red/pink, white, black/blue and yellow/orange) were observed, with white microplastics being the dominant colour at all sites (Fig. S1). Generally, high microplastics numbers were observed during the hot−dry season (mean range 120–6417 particles kg^{-1} dwt) compared to low numbers observed during the hot−wet season (mean range 5–94 particles kg^{-1} dwt) (Fig. 2). Overall, the high population density sites had high microplastics densities (mean 833.9±634.9 particles kg^{-1} dwt) compared to the low population density sites (mean 77.5±27.4 particles kg^{-1} dwt). High population density sites 1 (mean

6417±4407 particles kg^{-1} dwt) and 4 (1414 particles kg^{-1} dwt) during the hot−dry season recorded high microplastics abundances (Fig. 2).

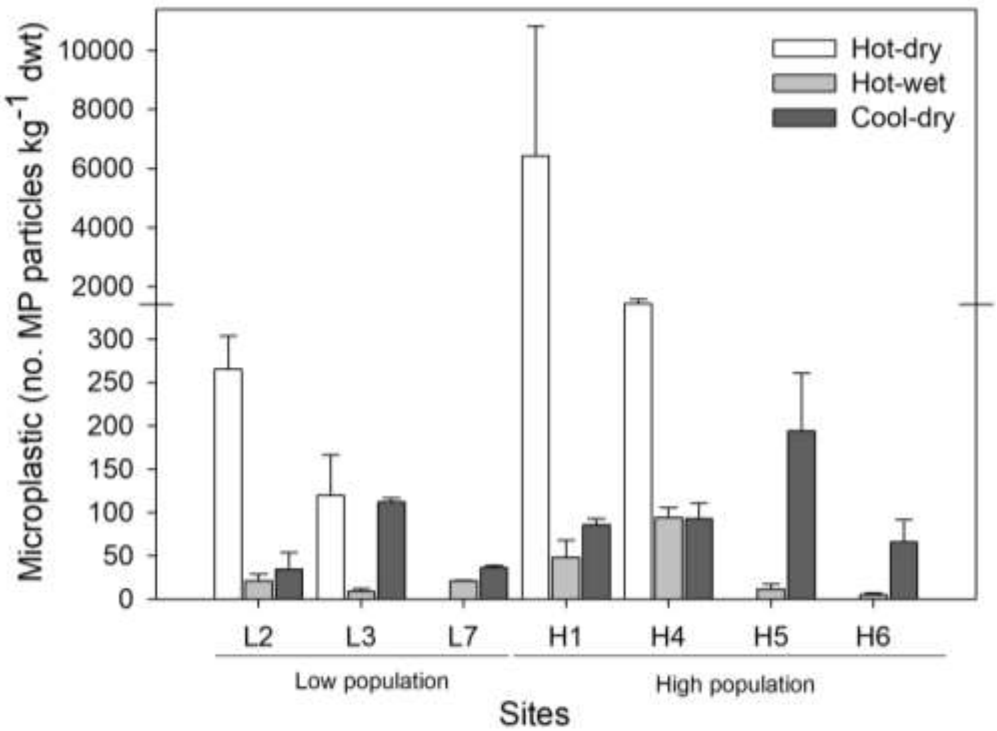

Fig. 3. 2. Distribution among sites and seasons of microplastic concentrations in shoreline sediment of Nandoni reservoir, Limpopo province, South Africa

Using PERMANOVA, significant differences in microplastics abundances were observed among *sites* (Pseudo−F = 2.469, *p*(MC) = 0.006), *seasons* (Pseudo−F = 7.453, *p*(MC) < 0.001) and *site* × *season* (Pseudo−F = 2.564, *p*(MC) = 0.001). The significant '*site* × *season*' interaction indicated greater microplastics density differences among sites as seasons changed, with higher microplastics abundance being observed during the hot-dry season (Fig. 2). Pairwise comparisons highlighted significant differences in microplastics colour abundances for *sites 1 vs 4* (t = 2.477, p = 0.025), *2 vs 4* (t = 3.208, p = 0.006) and *3 vs 4* (t = 3.040, p = 0.007). Furthermore, pairwise significant seasonal differences in microplastics colour abundances were also observed for the *hot−dry vs hot−wet* (t = 3.255, p = 0.002), *hot−dry vs cool−dry* (t = 2.710,

p = 0.009) and *hot−wet vs cool−dry* (t = 1.981, p = 0.024) with high microplastics densities being observed for the *hot-dry* season.

Low human population density category sites had generally reduced microplastics numbers (mean range 17–193 particles kg^{-1} dwt) compared to sites categorised as highly populated density sites (mean range 40–3915 particles kg^{-1} dwt) (Fig. 2). No significant differences were observed in microplastics densities within the two *population groups* (i.e. low, high) areas (F = 1.330, p = 0.365) and *seasons* (F = 1.140, p = 0.467). However, a significant interaction effect (F = 4.156, p = 0.026) was observed between *population groups* and *season* indicating that changes in population group activities across seasons resulted in a change in microplastics abundances.

The *n*−MDS ordination based on microplastics numbers for all sites discriminated slightly among seasons (stress values of 0.07 indicated a useful two−dimensional representation of the groups; Fig. 3). The overlap observed among seasons, especially during hot−wet and cool−dry seasons, could be attributed to reduced activity (i.e. reduced laundry washing) along the reservoir shoreline (Fig. 3). Selected polymers of all microplastics types were identified using vibrational FT-IR technique, resulting in eight polymer types: 20.2 % polypropylene, 22.7 % polyethylene, 30.9 % polystyrene, 9.7 % polyvinyl chloride, 5.8 % polyester, 4.5 % high-density polyethylene, 3.9 % polydimethylsiloxane and 2.3 % poly(lauryl acrylate).

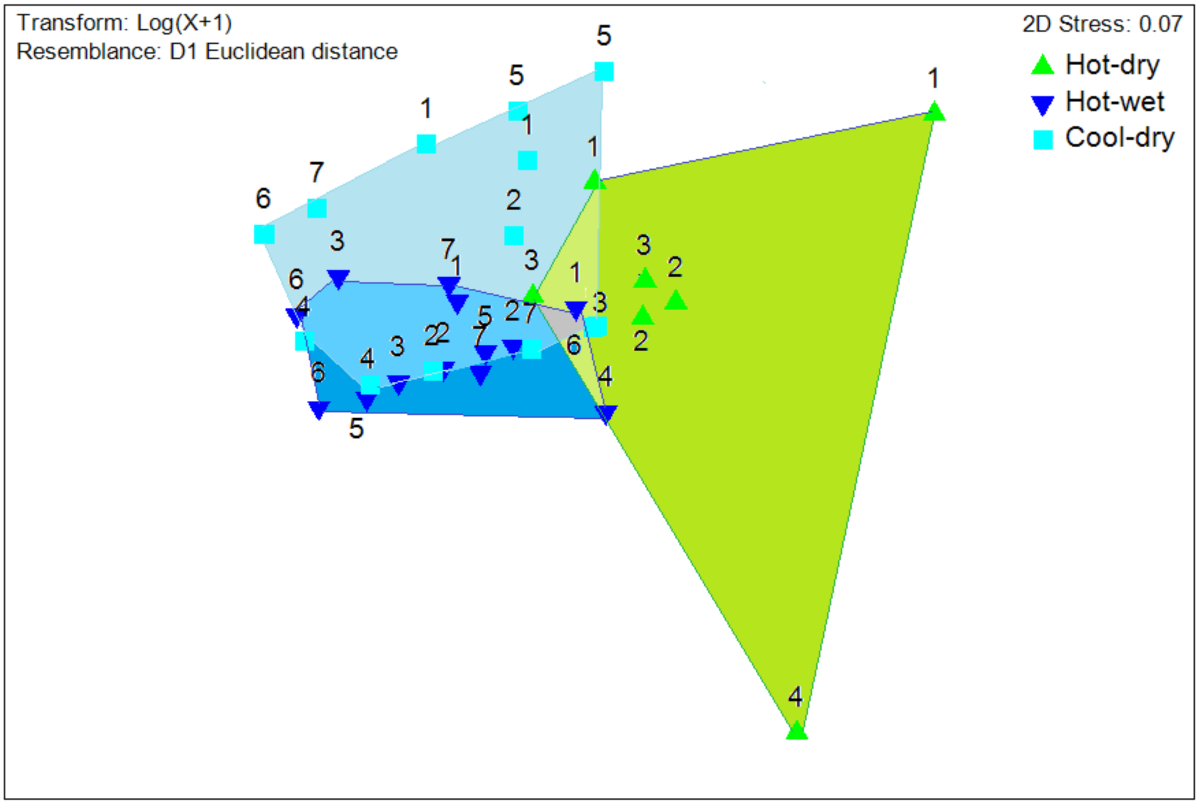

Fig. 3. 3. *n*-MDS ordination highlighting variation of microplastics densities across sites and seasons. Polygons indicate the three seasons: light blue – cool-dry, dark blue – hot-wet and green – hot-dry

3.5. Discussion

Whilst microplastics continue to accrue across all habitat types globally, there is currently little information on microplastics pollution in freshwaters, and particularly in subtropical regions. In many African remote areas, rural population collect water from freshwater resources. It therefore becomes a problem when water sources for domestic and agricultural use are polluted with contaminants such as microplastics, owing to negative effects on water quality, food security and population well−being (Verster *et al*., 2017). We anticipated microplastics to be present at our study sites owing to their widespread distribution within freshwater and marine environments (Nel *et al*., 2018; Ngupula *et al*., 2014; Silva−Cavalcanti *et al*., 2016).

The present study highlights that human population density with regards to activities interacted with seasonal variation to influence the abundance and distribution of microplastics in reservoir sediments. In particular, the study highlights that microplastics abundances along the focal shoreline were significantly higher in areas with high human population density under certain seasons. The study further attributes the possible microplastics sources might be from laundry washing (Fig. 4), cosmetic and cleaning products, as well as discarded litter which breaks down into microplastics, since no significant differences were observed for population density and microplastics loads. Thus, the lack of significant relationship highlight that the pollution likely mixes within the lake and results in more homogenous distribution of microplastics.

Sediment microplastics densities showed a seasonal difference along the shoreline, with high microplastics abundances being observed during the hot-dry season and this was most likely due to reduced river water flow as a result of low precipitation and increased pressure to do laundry by the lakeside. Whereas, during the hot-wet season people normally capture rainwater and wash at their houses away from the lake shore. The high abundance of microplastics during the hot-dry season suggests that microplastics were temporarily stored in sediments before being redistributed in other seasons. Indeed, Nel *et al*. (2018) suggested that freshwater sediments are temporary sinks for microplastics. In contrast to the current study (mean 616 particles kg^{-1} dwt), Fischer et al. (2016) recorded low average sediment microplastics values of 112 and 234 particles kg^{-1} dwt in two Italian lakes: Bolsena and Chiusi, respectively. Thus, one can assume that Nandoni reservoir is heavily polluted during hot-dry season, with the hot-wet and cool-dry seasons showing low to moderate microplastics contamination. Nandoni reservoir can be considered a microplastics exporter as it acts as a sink during the hot-dry season and when flows increase during the hot-wet season, it may become a microplastics source to other areas thereby reducing microplastics in the reservoir, hence lower overlap with the hot-dry season.

We also suspect little input from catchment wastewater discharges as the area is mostly rural-based and uses pit latrines; hence, most microplastics do not reach aquatic ecosystems or water treatment works, as indicated by low microplastics densities at the reservoir mouth sites i.e. sites 5 and 6. However, some may reach the reservoir from the breakdown of macroplastics (Dalu *et al*., 2019). Whilst hydrodynamics are known to significantly influence the deposition and distribution of plastics on marine shorelines (GESAMP, 2019), the influence of these factors is likely to be less pronounced in smaller freshwater environments which are subject to reduced hydrodynamics from, for example, wave action. Results showed that highly populated areas had greater plastic numbers, reaching 6417 particles kg^{-1} dwt, whilst sites with low population densities had low plastic numbers ranging between 9 and 265 particles kg^{-1} dwt. Therefore, it is likely that microplastics contamination levels in the absence of proper waste management infrastructures can have non-localised effects in such impounded freshwater ecosystems (Lambert and Wagner, 2017; Verster *et al*., 2017; Tibbets *et al*., 2018). Studies by Andrady (2011) have highlighted that densely populated areas are considered a major land-based source of microplastics pollution through the breaking down of directly or indirectly discarded plastic debris and poorly regulated discharge of domestic effluent. Thus, it is assumed that more plastics will enter from densely human populated areas which suggest that human density is strong determinant of the amount of microplastics input while residence time is the determining factor of microplastics distribution (Mahoney, 2017).

Microplastics colour helped to indicate their potential origin in the present study. Recovered microplastics were found in a variety of colours with white being the dominant colour. In some studies (e.g. Su *et al*., 2016), microplastics colours such as blue, white and black were identified, with white also being the dominant colour. Microplastics colours indicate the parent plastic product, and some colours change to white due to degradation process, making white the dominant colour; in turn, these particles may be ingested by aquatic biota (Lambert and Wagner, 2017). Polystyrene foam microplastics were also identified which could have originated from disposable food containers and cups. Zhang *et al*. (2018) and Nel *et al*. (2018, 2019) implied that the higher organic matter in the sediment soil samples strongly influenced the microplastics recovery. The results showed that sites with high SOM generally had higher microplastics as compared to sites with low SOM content.

Whilst the present study demonstrates high prevalence of microplastics in a subtropical reservoir, further in-depth studies in Austral freshwaters are required to understand the presence of microplastics, and other key drivers of differences. In particular, meteorological and hydrodynamic effects on pollutant concentrations and distributions require further examination, owing to the effects on other shoreline systems (GESAMP, 2019). Nonetheless, our findings suggest key spatiotemporal context-dependencies are important drivers of differences in microplastics abundances, with differences emergent across seasons according to human population densities close to reservoir shorelines. In turn, our empirical results serve to inform lab-based exposure studies, as these often use unrealistic microplastics concentrations to quantify ecological impacts.

CHAPTER FOUR: FUNCTIONAL RESPONSES QUANTIFY MICROPLASTIC UPTAKE BY A WIDESPREAD AFRICAN FISH SPECIES

4.1. Introduction

Microplastic (< 5 mm in size) pollution continues to proliferate across all habitat types and regions globally (Mason *et al*., 2018; Wagner and Lambert, 2018). Principally driven by the production of artificial plastic materials for human use (Cole *et al*., 2011), primary (i.e., *via* direct microplastic release) and secondary (i.e., *via* macroplastic degradation) origin microplastics enters the environment in a variety of shapes, sizes and densities (Rocha–Santos and Duarte, 2014), with ecosystem ramifications mostly unknown. Enormous quantities of microplastic are released into the environment through household wastewaters (Mason *et al*., 2016; Wagner and Lambert, 2018), with many particles subsequently transported *via* riverine systems into seas or lakes, where high concentrations accrue (Erieksen *et al*., 2013; Fischer *et al*., 2016; Su *et al.*, 2016). Whilst microplastics have exerted negative ecological impacts on marine ecosystem biota, such as increased mortality (Reichert *et al*., 2018) and decreased reproduction (Cole *et al*., 2015), knowledge concerning the impacts of microplastics on freshwater biota remains rudimentary, despite the particular vulnerability of freshwaters to environmental change (Al–Jaibachi *et al*., 2018; Blettler *et al*., 2018; Provencher *et al*., 2018; Cuthbert *et al*., 2019a; Windsor *et al*., 2019).

Laboratory–based exposure trials are routinely used to examine the effects of microplastic on flora and fauna, although such studies have recently come under scrutiny for using excessive, environmentally–unrealistic concentrations (Cunningham and Sigwart, 2019). Nevertheless, concentrations of microplastics in aquatic environments are known to be highly variable spatiotemporally (Hurley *et al.*, 2018; Tibbetts *et al.*, 2018; Nel *et al.*, 2018), and therefore uptake of microplastic by biota may vary rapidly over their range in aquatic ecosystems. In turn, impacts of microplastic uptake on organisms may vary substantially owing to potential density–dependent effects (Cuthbert *et al*., 2019a). Yet, there is currently a lack of understanding of how uptake rates by biota respond to differential environmental microplastic concentrations, hampering predictive quantifications of how uptake rates, and thus impact, relate to environmental heterogeneity in microplastics pollution.

Functional responses quantify resource use as a function of resource density (Holling, 1959). Whilst functional responses have been widely used to quantify the nature of consumer–resource interactions in many ecological fields (e.g., Abrams 1982; Cuthbert *et al*., 2019b), they have yet to be applied to quantify direct microplastics uptake by organisms. Three forms of functional responses are commonly described: the density–independent linear Type I response; inversely density–dependent hyperbolic Type II response, where consumption rates are high at low densities, and; positively density–dependent Type III response, which is sigmoidal due to low consumption rates at low densities (Hassell, 1978). In theory, Type II functional responses are destabilising for resources (e.g., prey), owing to a lack of low density refuge. By extension, in a microplastic uptake context, a Type II functional response may be indicative of high consumption rates even under low environmental concentrations. However, despite the utility of functional responses in other ecological fields, there has been a distinct lack of its application to microplastic uptake quantifications.

In the present study, we therefore employ functional response approach to quantify the density–dependence of microplastics uptake by the banded tilapia *Tilapia sparrmanii* Smith, 1940, which is a widespread and key species in warm freshwater habitats of southern Africa. This species has been introduced to many freshwater basins, and is distributed extensively outside of its native range (Ellender and Weyl, 2014). Banded tilapia readily consumes animal prey, however its diet is also known to include plant material (Zengeya and Marshall 2007; Marshall 2011). Given its broad diet and extensive distribution, banded tilapia represent a suitable model species for examination of microplastics uptake. We hypothesise that microplastics uptake by juvenile stages of the fish will relate positively to environmental microplastics concentrations. Further, we expect that tilapia will uptake microplastics even when present at relatively low densities in aquatic environments.

4.2. Materials and methods

Collection of captive–bred fish and all experiments were carried out in compliance with the ethical clearance approved by the University of Venda Research Committee (no. SES/18/ERM/10/1009).

4.2.1. Experimental design and analysis

Banded tilapia, *Tilapia sparrmanii* Smith, 1840 were supplied from Netshituni Fishery Project Phiphidi, Vhembe District, South Africa. All fish were transported to the Department of Ecology and Resource Management, University of Venda, Thohoyandou and were housed in 30 L buckets with continuously aerated water (23.2 ± 0.3 ºC). Fish were allowed to acclimate to the system for at least 48 hours prior to use in microplastics trials. Experiments were conducted in individual 10 L buckets filled with 4 L filtered (63 µm mesh sieve) matured tap water. Banded tilapias were size matched with respect to standard length (SL) (size ± SD: 32.4 ± 2.1 mm). Fish were randomly selected two hours prior to use and placed individually in 10 L buckets to acclimate. After the acclimatisation period, fish were presented with microplastic (i.e., ultra–high molecular weight, surface–modified polyethylene powder, 125 µm particle size (Sigma–Aldrich, UK) at four densities (0.05 g (~155 particles/39 particles L^{-1}), 0.1 g (~309 particles/77 particles L^{-1}), 0.5 g (~1545 particles/386 particles L^{-1}), 1 g (~3090 particles/773 particles L^{-1})), each mixed with 2 g tilapia fish food (Rainbow Kingdom, Louis Trichardt) and with five replicates per density. Whilst recent studies have been criticised for using unrealistic microplastic concentrations (Cunningham and Sigwart, 2019), quantities used in the present study span both 'low' (i.e. < 100 particles L^{-1}) and 'high' (i.e. > 100 particles L^{-1}) dosages, with 100 particles L^{-1} deemed as the highest measured environmental concentration (Burns and Boxall, 2018). Microplastics treatment presentations were fully crossed and randomised so that all combinations were trialed simultaneously. Feeding trials were run for 4–hours, after which microplastics consumption was examined. Controls were run simultaneously and consisted of five replicates in the absence of microplastics, with only fish food.

At the end of the experiment, all fish were euthanised by immersion in 40 mg L^{-1} of clove oil and fixed in 99 % ethanol for stomach contents analysis to determine the numbers of microplastics eaten. All fish were separated according to treatments. As the microplastics are relatively resistant to digestion, individual consumption within fish was inferred by quantifying numbers of microplastics in stomach contents under a Carl Zeiss Stemi stereo microscope (Carl Zeiss MicroImaging GmbH, Göttingen).

4.3. Data analysis

Differences in fish lengths (mm integers) among microplastics treatments (5 levels) were examined using generalised linear models (GLMs) assuming Poisson distributions of error with log links. A nested likelihood ratio test was used to infer the treatment effect size and significance level. Similarly, raw microplastics consumption (counts) was analysed using Poisson GLMs according to microplastics treatment (4 levels, excluding microplastics–free control). Residuals were examined in each model to ensure there was no significant evidence for overdispersion, allowing a Poisson rather than quasi–Poisson error family to be used.

Functional response analyses followed two stages. First, logistic regression was used to analyse proportional microplastics consumption (counts) as a function of initial microplastics abundance (i.e., density; continuous predictor) in order to categorise functional responses (i.e., into Types I, III, III). From this regression, a significantly negative first order term indicates a Type II functional response (Juliano, 2001). Second, functional responses were modelled using Rogers' random predator equation owing to the non–replacement of microplastic particles during the experiment (Rogers, 1972):

$$N_e = N_0\left(1 - \exp\left(a(N_e h - T)\right)\right)$$

(1)

where N_e is the number of microplastics particles consumed, N_0 is the initial density of particles, a is the attack rate, h is the handling time and T is the experiment duration (fixed at 1, i.e., 4–hours). Attack rates corresponds to the functional response curve slope (i.e., area or volume cleared per unit time by fish), whilst the handling time, inversely, corresponds to the functional response asymptote (i.e., fish maximum feeding rate). The Lambert W function was used to allow for model fitting, owing to the recursive nature of the random predator equation (Bolker, 2008). A non–parametric bootstrapping procedure (n = 2000) was followed to generate 95 % confidence intervals (CIs) around the functional response curve (Pritchard *et al.*, 2017). In all analyses, significance was inferred at a threshold of $p < 0.05$.

4.4. Results

The length of fish used in the experiment did not differ significantly depending on microplastic exposure group (GLM: $\chi2 = 0.29$, df = 4, $p = 0.99$). Therefore, size–related differences likely did not significantly alter propensities for microplastic consumption by fish. No microplastic particles were consumed in control fish, and it was thus unnecessary to adjust consumption to account for any background levels of plastics. Microplastic was found in 100 % of exposed tilapia, and counts tended to increase slightly with greater exposure concentrations (Fig. 4.1). Nevertheless, there were no statistically clear differences in microplastic consumption among exposure concentrations (GLM: $\chi2 = 4.57$, df = 3, $p = 0.21$), with fish consistently consuming particles even when relatively sparse in the environment.

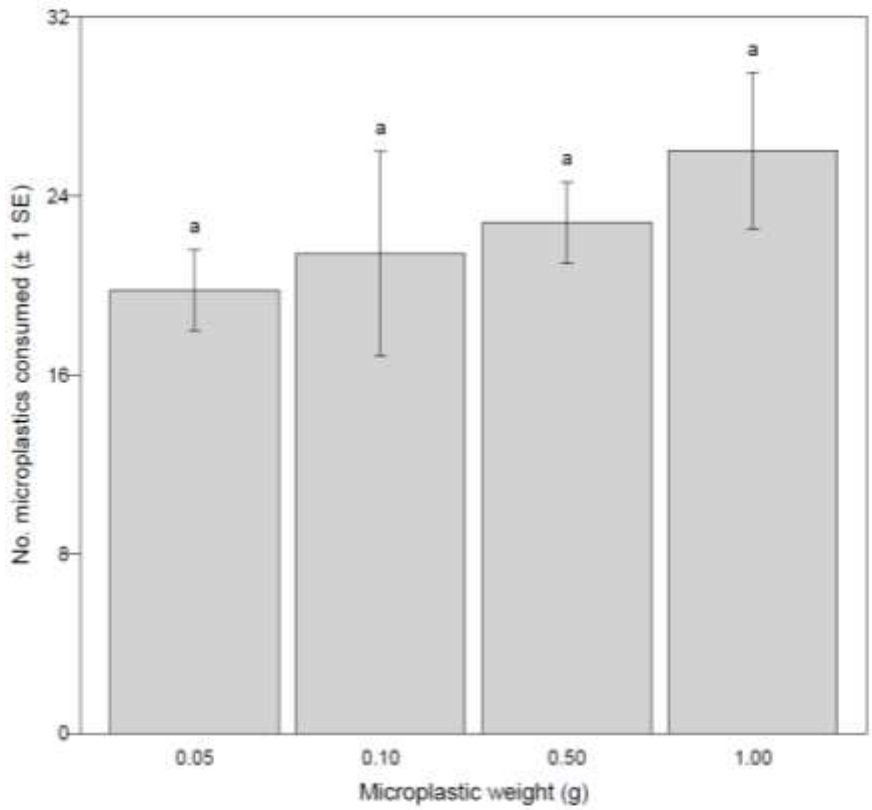

Fig. 4. 1:.Mean (± 1 SE) numbers of microplastics consumed (i.e., counted in gut) by individual fish among different initial exposure weights (g L^{-1}) following 4 hour experimental period. Letters above indicate a lack of significant differences.

The proportion of microplastics consumed (i.e., numbers eaten relative to numbers remaining) was significantly negatively related to the initial experimental particle density (GLM: linear coefficient = 0.0009, z = 19.55, p < 0.001). Therefore, fish exhibited significant evidence for a hyperbolic Type II functional response, characterised by high proportional consumption rates at low densities (Fig. 4.2). This enabled functional response attack rate (a) and handling time (h) parameter estimations to be estimated (random predator equation: a = 0.63, z = 1.88, p = 0.06; h = 0.04, z = 14.52, p < 0.001). Accordingly, fish exhibited maximum consumption rates (1/h; i.e., functional response asymptote) of approximately 25 particles over the 4–hour experimental period (Fig. 4.2).

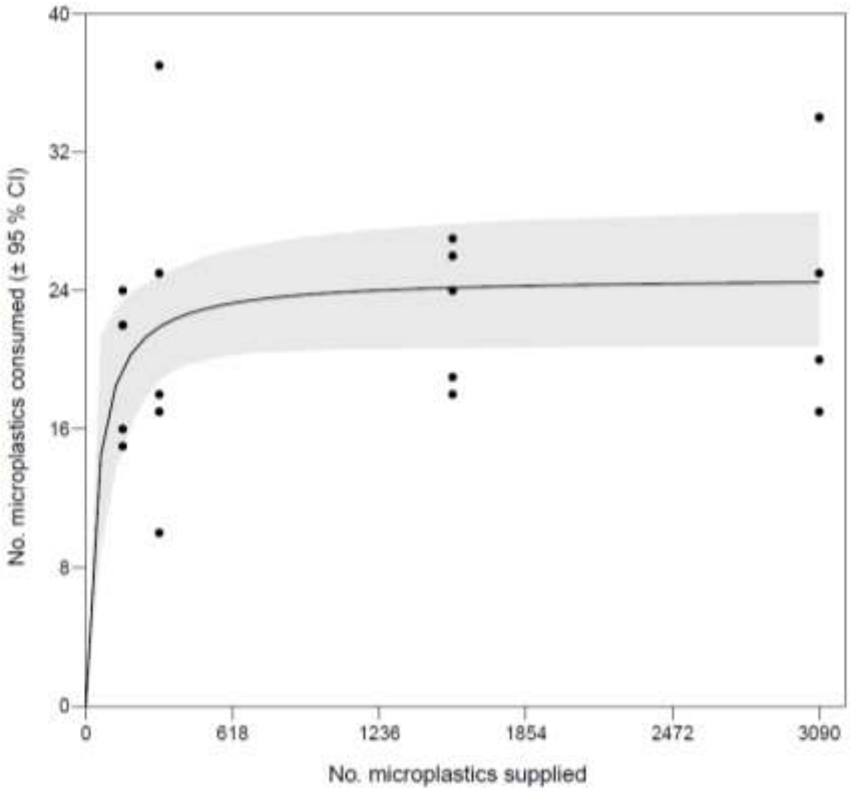

Fig. 4. 2. Type II functional response of fish towards microplastics at different exposures (no. particles 4–L^{-1}) following 4–hour experimental period. Grey areas are bootstrapped 95 % CIs and points are underlying raw consumption data.

4.5. Discussion

The present study demonstrates the potential for *Tilapia sparrmanii* to uptake microplastics across a gradient of environmental concentrations. Tilapias were found to uptake microplastics even when relatively rare in their aquatic environment, with increasing concentrations having a positive but statistically unclear effect on uptake levels. Under higher concentrations than tested in the present study, microplastics particles have been shown to accrue across all tissues of freshwater tilapia following consumption (Ding *et al.*, 2018). In turn, adverse impacts on juvenile fish stages have been reported following exposure to concentrations aligned with the lowest levels in this study, such as anemia (e.g., Hamed *et al*., 2019). Further, uptake of microplastics has been shown to interact with, and potentially alter, toxicity levels of other pollutants upon tilapia (Zhang *et al*., 2019). Therefore, the quantified propensity for *T. sparrmanii* to uptake microplastics in the present study may drive biotic impacts even under relatively low environmental concentrations, with the present study spanning both low and high dosages from empirical measurements (Cunningham and Sigwart, 2019).

Microplastics uptake by *T. sparrmanii* exhibited significant non–linear density–dependence in the present study, with uptake rates falling asymptotically with increasing environmental concentrations. This aligns with a Type II functional response, where proportional uptake rates are highest under low environmental densities (i.e., concentrations) (Holling, 1959). This suggests that *T. sparrmanii* actively uptakes microplastics even where relatively rare in the environment. Functional responses offer great utility in quantifications of resource uptake under different densities (e.g. Cuthbert *et al*., 2019b), and the approach thus lends itself to microplastics studies where density–dependences of uptake may be deciphered. In turn, feeding behaviours may be related against microplastics concentrations documented to causes impact. Here, the functional response approach returned attack rate (i.e., search efficiency) and handling time parameters towards microplastics by *T. sparrmanii*. In this context, the attack rate corresponds to the volume cleared per unit time by fish (i.e., curve initial slope), whilst the handling time is the time taken to capture, consume and digest microplastics particles. Inversely, the handling time estimate enabled maximum feeding rates (i.e., curve asymptote) of microplastics to be returned for *T. sparrmanii*, providing important quantitative information relating to the propensity for this species to uptake microplastics over time. However, further studies are required to examine excretion rates of microplastics, and how these may influence uptake and retainment in organisms.

Just as functional responses can be used to quantify interaction strengths in consumer–resource (e.g., predator–prey) systems (Kalinoski and DeLong, 2016), we propose, for the first time, the use of functional responses in quantifications of microplastics uptake rates. Functional responses can be subsequently compared among species or across environmental contexts to better understand key drivers which may alter uptake rates (Cuthbert *et al*., 2019b). Further, differences in uptake rates across microplastics polymer types and sizes could be examined using a functional response approach. Ultimately, understandings of species–level feeding responses to different environmental concentrations will assist in our understanding of microplastics impacts, and help to better–link laboratory exposure studies to real–world concentrations.

CHAPTER FIVE: GENERAL DISCUSSION

5.1. General discussion

Microplastics are newly emerging contaminants of freshwater sediments and may pose risk to microbes, invertebrates, fish and other animals such as birds. Reviewed published evidence Ngupula *et al*. (2014) and Nel *et al*. (2018), has already indicated the presence of microplastics in African freshwaters. As initially hypothesized, there was a seasonal difference in sediment microplastics densities as microplastics abundances were significantly higher in hot-dry season as compared to hot wet season (Chapter 3). This was because microplastics settled at the reservoir bed due to low water velocity in hot-dry season. Generally, there were slight differences in microplastics abundances between high density populated areas and low density populated areas. This was due to anthropogenic impact on the environment because of some of the human activities in high populated areas. In addition, the density accumulation rates by the dominant fish species were negatively related to environmental microplastics concentrations suggesting that the fish was limited in its capacity to process microplastics (Chapter 4).

The full knowledge of spatial and temporal distribution of microplastics is often limited by the inability to detect microplastics from benthic habitats. Collecting sediment samples, extracting microplastics using NaCl, quantifying them under a microscope and analyzing concentrations was an appropriate method to detect and measure microplastics. This is because the method was simple, accessible at low cost, safe and quick. Using NaCl method for density separation had its benefits, yet more methods need to be developed for complete microplastics extraction efficiency. Since microplastics are present and significantly distributed along the reservoir, monitoring their presence and minimizing their abundance is important.

Nel *et al*. (2018) suggested that freshwater systems may be considered a temporary sink for microplastics pollution highlighting the role of the Bloukrans River system with results ranging from 13.3–563.8 particles kg^{-1} dwt during winter and lower microplastics densities in summer. In contrast, in this study results showed that during hot-dry season microplastics abundances were high ranging from 120–6417 particles kg^{-1} dwt suggesting that Nandoni reservoir is considered a temporary sink in hot-dry season (Chapter 3). Furthermore, residence time of a reservoir might be related to greater microplastics numbers (Free *et al*., 2014). Mani (2015) highlighted that microplastics movement and deposition largely depend on reduced incoming water flow from tributaries, release of water from reservoirs and as a result microplastics concentrations will be greater in abundance in areas with low water velocity with deposited and suspended sediment. Mani (2015) highlighted that populated density, proximity of wastewater treatment plants (WWTPs), turbulence and geomorphological characteristics influence microplastics abundance. In this study, high microplastics numbers were observed in sampling sites with high populated densities and areas of high sediment deposition characterised by areas of high silt/clay. Chemicals in microplastics themselves and adsorbed chemicals onto microplastics are of greatest concern because they can leach and transfer contaminants to aquatic animals. Thus it is recommended to assess the distribution of toxic chemicals in freshwaters such as PCBs.

The uptake of microplastics by juvenile fish helped to determine the behavior of fish towards microplastics, and attack rate of fish to microplastics through functional response experiments (Chapter 4). Freshwater ecosystems have some of the terrestrial vertebrates and invertebrates developing in them, thus future research should focus on microplastics transfer rates from freshwater larvae to terrestrial adults and also assess how growth, survival and reproductivity are affected. It will also be important to assess the uptake of microplastics by fish at different life stages (fry to adults) and how these impact fish growth and development.

5.2. General conclusion

More microplastics studies in African freshwaters are needed to determine the abundance of microplastics polymers, sizes and shapes as this is an emerging contaminant. Since most microplastics float and denser plastic particles sink, it was therefore ideal to sample reservoir sediments to get a representative sample of different types of microplastics polymers present in the reservoir. The abundance and distribution of microplastics in Nandoni reservoir calls for an urgent need for all stakeholders to come together and help with the minimization of microplastics in the reservoir. Investigating fish uptake rates to microplastics was vital thus further field studies are required to help in the understanding of whether the observed laboratory values are similar to the field values. The effects of microplastics ingestion as well as egestion rates should be quantified. Furthermore, the examination and documentation of trophic transfer rates between aquatic prey and predator is therefore recommended. The utilitisation of functional response approaches for predictive quantifications of microplastics uptake rates is recommended. In turn, this can better link laboratory exposure studies to environmental concentrations which are known to cause ecological impact, and provide means of comparing uptakes among species and across environmental contexts.

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SUPPLEMENTARY FILES

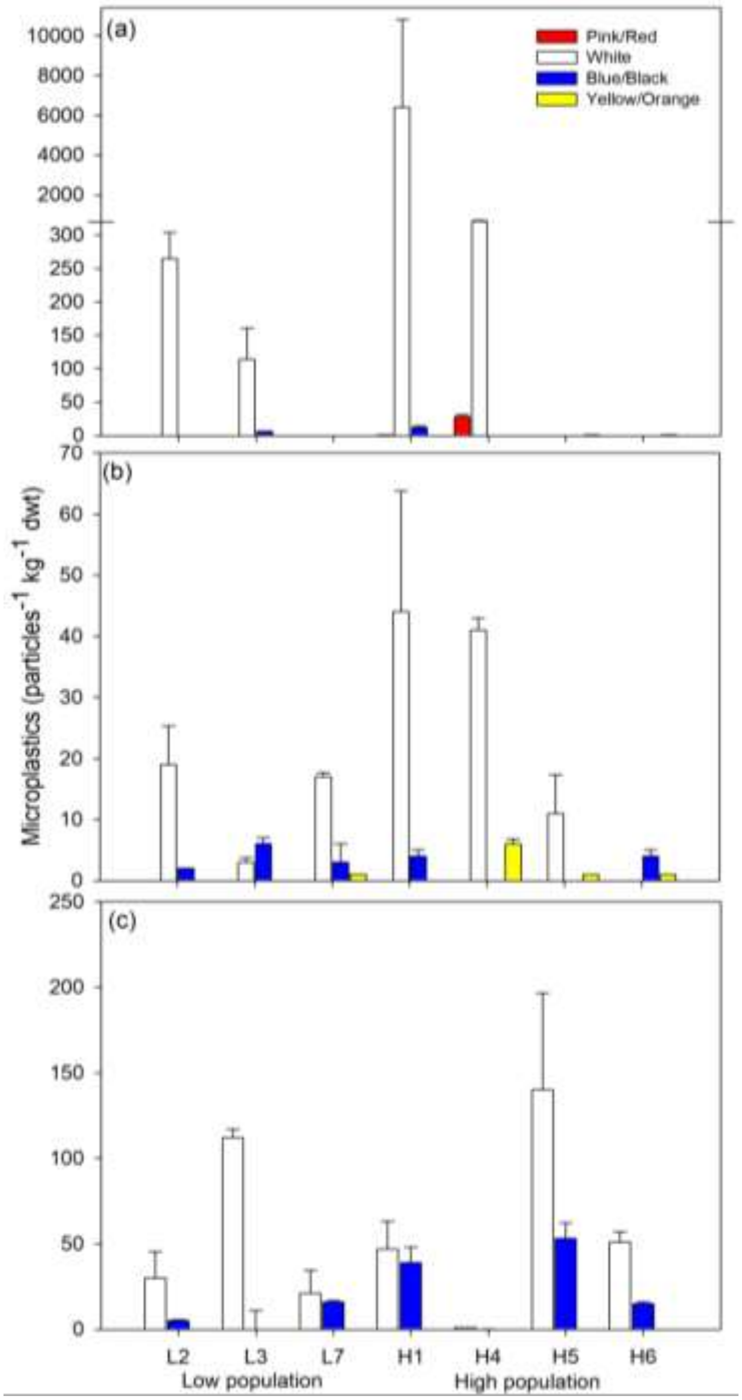

Fig. S. 1: Variation in microplastics particle types among seasons: (a) hot–dry, (b) hot–wet, and (c) cool–dry in Nandoni reservoir, South Africa