Evaluating the potential effects of microplastics at environmentally realistic concentrations in South African freshwater systems.

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ABSTRACT

Microplastic pollution is spatially broad, microplastics can be found in various habitats including freshwater systems. Microplastic exposure to aquatic organisms has been associated with several physical impacts on aquatic organisms from multiple trophic levels such as; increased immune response, a decrease in food intake, excessive loss of weight, reduced growth rate, reduced energy and adverse effects on successive generations. However, these significant effects of microplastics exposure have been observed mostly in studies that use concentrations of microplastics that exceed environmental concentrations. Therefore, there is an overall lack of research on the effects of microplastics on freshwater organisms using environmentally realistic concentrations, especially in the Southern Hemisphere. A series of toxicity tests were used to expose a range of taxa including *Tilapia sparrmanii*, *Caridina nilotica*, and *Melanoides tuberculata* to different environmentally realistic concentrations of microplastics of different polymers. The study results show that the environmentally realistic concentrations had no statistically significant effects on most of the chosen test organisms and selected study endpoints, except for *T. sparrmanii* microplastic particle egestion and growth in polyethylene exposures which produced significant results. Although this study showed that at environmentally realistic concentrations and 21 days of exposure, minute effects on the test taxa were detected, various studies have proven that with longer exposure to microplastics, significant effects on freshwater organisms can be detected. Additionally, studies using concentrations higher than the current environmental concentrations have recorded significant effects on organisms and therefore, with increasing concentrations in the environment, more significant effects may be observed. Therefore, plastic pollution in the environment should be reduced as microplastics are in continuous production and circulation, and microplastic concentrations in freshwater environments are predicted to increase.

Key words: Microplastics, Pollution, Freshwater, Environmentally-realistic, Organisms

UMXHOLO

Ungcoliseko lweMicroplastic lubanzi ngokwesithuba, ii-microplastics zinokufumaneka kwiindawo ezahlukeneyo zokuhlala kubandakanya neenkqubo zamanzi acocekileyo. Ukubonakaliswa kweMicroplastic kwizinto eziphilayo zasemanzini kuye kwanxulunyaniswa neempembelelo ezininzi zomzimba kwizinto eziphilayo zasemanzini ukusuka kumanqanaba amaninzi etrophic afana; ukwanda kwe-immune response, ukunciphisa ukutya, ukunciphisa umzimba, ukunciphisa izinga lokukhula, ukunciphisa amandla kunye nemiphumo emibi kwizizukulwana ezilandelelanayo. Nangona kunjalo, ezi ziphumo zibalulekileyo zokuvezwa kwe-microplastics ziye zabonwa ikakhulu kwizifundo ezisebenzisa ugxininiso lwee-microplastics ezidlula ukugxila kokusingqongileyo. Ke ngoko, kukho ukunqongophala kophando ngokubanzi kwiziphumo ze-microplastics kwizinto eziphilayo zamanzi ahlaziyekileyo zisebenzisa ukugxila okubonakalayo kokusingqongileyo, ngakumbi kwi-Hemisphere yaseMazantsi. Uthotho lovavanyo lwetyhefu lusetyenziselwe ukuveza uluhlu lwetaxa equka iTilapia sparrmanii, iCaridina nilotica, kunye neMelanoides tuberculata kugxininiso oluyinyani lokusingqongileyo lwee-microplastics zeepolima ezahlukeneyo. Iziphumo zophononongo zibonisa ukuba ugxininiso lwendalo olungqongileyo aluzange lube neziphumo ezibalulekileyo zezibalo kwizinto ezininzi ezikhethiweyo zovavanyo kunye neendawo ezikhethiweyo zokufunda, ngaphandle kwe-T. sparrmanii microplastic egestion ye-particle egestion kunye nokukhula kwi-polyethylene exposures. Nangona olu phononongo lubonise ukuba kugxininiso lwendalo esingqongileyo kunye neentsuku ezingama-21 zokuvezwa, iziphumo zemizuzu kwirhafu yovavanyo zichongiwe, izifundo ezahlukeneyo ziye zangqina ukuba ngokuvezwa ixesha elide kwi-microplastics, iziphumo ezibalulekileyo kwizinto eziphilayo zamanzi acocekileyo zinokubonwa. Ukongeza, amaphononongo asebenzisa ugxininiso oluphezulu kunogxininiso lwangoku lokusingqongileyo lubhale iziphumo ezibalulekileyo kwizinto eziphilayo kwaye ngenxa yoko ngokunyuka kokugxila kokusingqongileyo, iziphumo ezibaluleke ngakumbi zinokujongwa. Ke ngoko, ungcoliseko lweplastiki kwindawo kufuneka luncitshiswe njengoko i-microplastics ikwimveliso eqhubekayo kunye nokujikeleza, kwaye ukugxilwa kwe-microplastic kwindawo yamanzi acocekileyo kuqikelelwa ukuba kwanda.

Amagama angundoqo: Microplastics, Ungcoliseko, Amanzi acocekileyo, Indalo-eyinyani, Izilwanyana zase manzini

DECLARATION

I, Zintle Mtintsilana, declare that the thesis titled: *Evaluating the potential effects of microplastics at environmentally realistic concentrations in South African freshwater systems* is my work and that all sources that have been used or quoted have been indicated and acknowledged by complete references. This thesis has not been submitted for a degree from any other higher institution.

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This thesis is dedicated to my son Lulo-uthando Lutho Mtintsilana.

"God is within her, she will not fail" **Psalm 46:5**

TABLE OF CONTENTS

ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of variance
AWARMN	African Water Resources Mobility Network
COVID-19	Coronavirus disease 2019
DO	Dissolved Oxygen
EC	Electric Conductivity
EC_x	Effective Concentration x %
EC_{10}	Effect Concentration 10 %
EC_{50}	Effect Concentration 50 %
ESKOM	Electricity Supply Commission
GDP	Gross Domestic Product
HIV	Human Immunodeficiency Virus
IWR	Institute for Water Research
NOEC	No Observed Effect
NRF	National Research Foundation
PE	Polyethylene
PET	Polyethylene terephthalate
PL	Polyester
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PVC	Polyvinyl chloride
OECD	Organisation for Economic Co-operation and Development
SA	South Africa
SAIAB	South African Institute for Aquatic Biodiversity
WRC	Water Research Commission

List of Figures

List of Tables

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

The first chapter introduces the general background, the importance of the study, the study justification, the study aim, the research question, and the objectives to achieve the research.

1.2 Background

Water is important for sustaining life on earth, both humans and organisms are dependent on water, not just water but clean freshwater. In South Africa (SA), everyone has a right to access to clean drinking water (Humby & Grandbois, 2011). However, SA is a water-scarce country, and the protection of freshwater resources is a priority, however, in light of other socio-economic issues such as; Human Immunodeficiency Virus and Acquired immune deficiency syndrome (HIV and AIDS), unemployment and poverty, it receives inadequate attention (Verster et al., 2017). The significant increase in urbanization and changes in land use have caused large amounts of wastewater discharge from households, agriculture and industries, causing an increase in point and non-point source pollution risk (Zhang et al., 2021). Due to poor management of wastewater in South Africa, wastewater is not always treated effectively (Green Drop report, 2022).

Poor water quality caused by the introduction of pollutants into freshwater sources adversely affects the provision of freshwater ecosystem services and the livelihoods of people and organisms (both terrestrial and freshwater) who rely on freshwater systems (Dodds et al., 2013). Aryani et al. (2021), in their study, discovered that microplastics affected ammonia concentration in the water, therefore, affected the water quality. However, their study also indicated that microplastics did not affect water temperature, pH and dissolved oxygen. When freshwater resources are contaminated by microplastics, access to good quality freshwater and food security (for example access to good quality fish and shrimps) are both negatively impacted, as microplastic pollution reduces water quality and the quality of food (Verster et al., 2017). Since SA is a water-scarce country, the quality of its freshwater resources is even at a higher risk, therefore, there is a need for further research (Verster et al., 2017).

Globally microplastics have emerged as a concern to water quality, particularly due to their potential to cause physical harm and their ability to carry harmful pollutants from the surrounding environment, thus acting as vectors, and at the same time leach out additives such as endocrine-disrupting

chemicals (for example bisphenol A and phthalates) (Issac & Kandasubramanian, 2021). Microplastic pollution is spatially broad and is distributed in a wide variety of habitats such as terrestrial environments, aquatic environments, alpine environments, in the air and both in the Arctic and Antarctica (Gasperi et al., 2018; Morgana et al., 2018). In SA, microplastics have also been widely reported in soil, groundwater, surface water and drinking tap water in Johannesburg and the North West province (Bouwman et al., 2018; Saad et al., 2022). However, there has been little emphasis on their toxicity to freshwater systems. The widespread of microplastic pollution is exacerbated by the lack of recycling, unsecured open landfills and mismanagement of plastics which eventually break down into microplastics (Aragaw, 2021; Rochman et al., 2013).

Literature has shown that microplastic pollution has increased significantly over the last four decades in aquatic (marine and freshwater) systems, imitating the growth in global plastic manufacturing and usage around the world (Au et al., 2015; Pereao et al., 2020). Exposure to microplastics has been linked with numerous physical effects on freshwater organisms from multiple trophic levels such as increased immune response, reduced food consumption, decrease in reproduction, excessive weight loss, mortality, reduction in the growth rate, reduced energy and negative effects on successive generations (Castro-Castellon et al., 2022; Lusher et al., 2017; Saad et al., 2022). However, Burns & Boxall (2018), Lusher et al. (2017) and Mbedzi et al. (2020), all report that their studies proved that at current environmentally realistic concentrations, there is limited proof to suggest that microplastics cause severe significant effects on freshwater environments, however, there is still need for further research as only a few studies have used environmentally relevant concentrations.

1.3 Study justification

The toxicity of microplastics is usually not assessed using multiple species and environmentally realistic concentrations as most studies use concentrations higher than those recorded in aquatic environments (Guimarães et al., 2021; Wang et al., 2019). Guimarães et al. (2021), reported that in ecotoxicology studies on microplastics, only 17 % of published studies used environmentally realistic concentrations. The environmental concentrations identified in the reviewed studies ranged between 1770 particles/L to 930 trillion particles/L, these figures represent more than 5 000 000 000 % difference. It is important to use environmentally realistic concentrations as they mimic the actual concentrations in the environment. It is also important to use multiple species as different species are affected differently by the same stressor, therefore, will have different responses to the same stressor. This also helps to see the impacts of microplastics on taxa in different parts of the food web.

Some authors do not disclose the methods used to quantify microplastic concentrations during their experiments, such as Wang et al. (2019), de Sá et al. (2018) and Ory et al. (2018), making it difficult to replicate their experiments. Additionally, there is a lack of standardization and consistency of test methods (Aragaw, 2021; De Ruijter et al., 2020), resulting in a lack of adequate ecotoxicological methods, for example, Aryani et al. (2021), used g/L to quantify the concentrations of microplastics while Au et al. (2015), used number of particles/L.

The outbreak of the COVID-19 pandemic has induced a large increase in personal protective equipment (PPE) (gloves and masks) where rubber and also plastics are the main components used during the manufacturing process (Wu et al., 2020). Klemeš et al. (2020), reported that the accumulated medical waste including PPE in China from only the 20th of January to the 31st of March 2020 was approximately 207 kilo tonnes. Therefore, plastic and microplastic pollution in the environment is predicted to increase drastically with continuous usage of PPE and plastic products.

The endpoints used during ecotoxicity studies are not always suitable for the time frame of the study or the chosen study taxa (Marty et al., 2017). It is, therefore, important to research and identify suitable and ecologically relevant endpoints for studies, select the appropriate test taxa and choose the suitable time frame for the study depending on the chosen study endpoints and test taxa.

1.4 Study aim, research question and objectives

1.4.1 Aim

To assess the potential toxicity caused by microplastics in freshwater systems.

1.4.2 Research Question

What are the effects of microplastic exposure to freshwater organisms at environmentally realistic concentrations?

1.4.3 Objectives

1. To identify suitable ecologically relevant endpoints for evaluating the potential effects of microplastics at environmentally realistic concentrations.
2. To contribute to the development of methods for assessing the potential toxicity of microplastics in freshwater ecosystems.
3. To determine the potential effects of microplastics on freshwater organisms from multiple trophic levels.

1.5 Thesis outline

This thesis is divided into 6 chapters, chapter one is the introduction, which gives a background on microplastic pollution, the study justification and importance, followed by the aim of the study, research question and study objectives. Chapter two summarises key literature in order to illustrate how plastics are produced and identify common plastic polymers, illustrate how different microplastics are formed, show how ubiquitous microplastic pollution is, and identify key sources of microplastics and their transport. Highlight the effects of microplastics on different freshwater organisms and humans and discuss and identify ecologically relevant endpoints suitable for this study. Chapter three is the methodology chapter; it highlights the different data collection and data analysis methods used in this study. Chapter four reports the results obtainedduring this study. Chapter five discusses the results obtained in chapter four and states the study's limitations. Chapter six is the last chapter and concludes the study, it also provides recommendations for future studies and to reduce microplastic pollution in the environment.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter reviews the key literature that is foundational to this study. It illustrates how plastics are produced, and identifies the most common plastic polymers, their properties, and their uses. It illustrates how different microplastics are formed through different processes, it shows how ubiquitous microplastic pollution is and identifies key sources of microplastics and their transport. This chapter also highlights the effects of microplastics on different freshwater organisms and humans and discusses and identifies ecologically relevant endpoints.

2.2 Plastics

Plastic is defined as a synthetic or semi-synthetic organic polymer of high molecular mass that is malleable and can be shaped into different solid objects (da Costa et al., 2016). The manufacturing of plastics has increased significantly globally over the past five decades, increasing from half a million tonnes per year in 1960 to approximately 300 million tonnes in 2013 (World Economic Forum, 2016). Current predictions estimate that roughly 12 billion tonnes of plastic waste will be produced by the year 2050 (Zhao et al., 2022). However, approximately 20 % of plastic waste in the environment is recycled or burned (Zhao et al., 2022). The United States, Europe and Asia conjointly account for 85 % of plastic production in the world (World Economic Forum, 2016). The European plastic industry employs approximately 1.45 million people, generating 26.3 billion euros for public finance and welfare (Plastic Europe, 2015). In South Africa, the plastic industry contributes roughly 1.6 % to the gross domestic product (GDP) and 14.2 % to the manufacturing sector, and local plastics consumption has increased yearly by 9 % (PlasticsSA, 2020). Plastics are composed of one or more polymers, plasticizers, dyes, colourants and fillers (PlasticsSA, 2020)

2.3 Common plastic polymers

In 1907 the first major plastic polymer was discovered, and new polymers have been developed and discovered since then (PlasticsSA, 2020). The first plastic to be produced was Bakelite, which was manufactured in 1907 by Leo Hendrik Baekeland (Crespy et al., 2008). Its production aimed to produce a material that could be used in different applications for daily life use, and it had a massive positive impact on the economy and society (Crespy et al., 2008). At the time, Bakelite was also largely used in the growing automobile and radio industries, it has since created a legacy in which the plastic age now

prevails (Crespy et al., 2008). Plastics are mostly manufactured from crude oil, additionally, they also can be manufactured from renewable raw materials and the production of plastics accounts for roughly 5 % of global oil consumption (Chalmin, 2019; Millet et al., 2019). There are two categories of plastic materials, these are thermoplastics and thermoset plastics. Thermoplastics are those plastic materials that can be heated to produce products such as; plastic bottles and cups, these end products can be re-heated to melt or soften the plastic (Millet et al., 2019). While thermoset plastics are plastic materials that will no longer melt after the setting process and are used in the manufacturing of electronic chips, (Millet et al., 2019).

Plastic production from crude oil starts in the distillation process of an oil refinery, it involves the separation of heavy crude oil into lighter fractions (Shaw & Sahni, 2014). Each of these fractions is a mixture of hydrocarbon chains, and these vary in the structure and size of their molecules (Shaw & Sahni, 2014). Naphtha is one of these fractions, it is a vital raw material for plastic production and is used to produce different plastic monomers such as ethylene, propylene, and styrene, through the process of cracking (Millet et al., 2019). These monomers are the building blocks used to produce plastics, through the process of polymerisation and depending on the various types of monomers used each plastic polymer will have its own different properties (Millet et al., 2019).

Numerous types of plastic polymers form from plastic monomers, as a result of their differences in, chemical and crystalline structures, density, hardness, production process, conductivity, design, degradability, capacity to absorb water, and additives (Bouwman et al., 2018). Additives added to plastics include dyes, plasticisers, accelerants, cross-linking additives, flame retardants, antioxidants, UV stabilizers, surfactants, inorganic fillers and photosensitizers (Bouwman et al., 2018). The purpose of all these additives is to produce plastics of desired properties, for example, UV stabilizers protect plastics from damage from UV rays and sunlight, while dyes give the desired coloured to the plastics. However, these additives pose their own pollution problems, for example, plasticisers can leach from the plastic into the environment (Bouwman et al., 2018).

It has been estimated that of the 8.3 billion metric tonnes of plastic manufactured since the 1950s, approximately 5.8 billion metric tonnes were disposed of and of that amount, roughly 500 000 metric tonnes were recycled and approximately 700 metric tonnes were burned (Chalmin, 2019). That leaves roughly 4.6 billion metric tonnes currently in both terrestrial (landfills) and aquatic environments (Chalmin, 2019). In 1980, 60 million metric tonnes of plastics were produced worldwide, by 2000 production surpassed 187 million metric tonnes, and by 2010 production surpassed 265 million and 348 million in 2017. This is an average growth rate of 8.5 % annually since 1950 when it was 1.5 million

metric tonnes. Plastic polymers inherently have low toxicity as a result of their insolubility in water and because they are biochemically inert (Worm et al., 2017).

The most commonly detected plastic polymers in aquatic systems and most commonly manufactured are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET) and polyurethane (PU) (Gewert et al., 2015; Worm et al., 2017; Xu et al., 2020; Miloloža et al., 2022).

2.3.1 Polyethylene

This plastic polymer was first produced in the United Kingdom in the 1930s and is the most popular polymer, especially in modern packaging (PlasticsSA, 2020). Two types of polyethylene have been produced, namely, high-density polyethylene which has a high density and low-density polyethylene which has a lower density. The recycling rate of low-density polyethylene is around 31 % while for high-density polyethylene in 2018, it was reported to be around 29.3 %. According to Eagan et al. (2017), roughly 70 million metric tonnes of polyethylene are produced per annum and represent 40 % of the total demand for plastic products. The service life of polyethylene is dependent on the plasticisers used and thus the estimates vary depending on the plasticiser (Gewert et al., 2015). In moderate climates, Gewert et al. (2015), report that polyethylene has a service life of about 15–20 years. Furthermore, in aquatic environments, the degradation processes are even slower, as the conditions are not optimum for polymer degradation (Cruz, 2013; Gewert et al., 2015). Nonetheless, both abiotic and biodegradation are still possible but on a longer time scale (Gewert et al., 2015). Polyethylene is commonly used for plastic bags, bottles and plastic films (Gilbert & Patrick, 2017). In South Africa, polyethylene is the most recycled plastic polymer, however, the exact figures have not been published (PlasticsSA, 2020). Density also plays an important role in the distribution of plastics in both terrestrial and aquatic environments. Therefore, the density of polyethylene ranges from 0.890-0.970 g/cm^3 (McKeen, 2016).

2.3.2 Polyethylene terephthalate

In water, polyethylene terephthalate (PET) is susceptible to hydrolytic degradation (Wypych & Faulkner, 1999). Additionally, abiotic weathering of this polymer in freshwater environments is likely to occur mainly through hydrolytic degradation processes and photo-induced oxidation (Gewert et al., 2015). It is used for the manufacturing of bottles, packaging and construction materials (Raheem et al., 2019). According to Rahimi et al. (2016), roughly 3.1 million metric tonnes of polyethylene terephthalate are produced annually in North America alone.

2.3.2.1 Polyester

Polyester (PL) is a type of polyethylene terephthalate and falls under the category of polymers that have an ester functional group in every repeat unit of their main chain (Jaffe et al., 2020). Polyester is used in the manufacturing of apparel, towels, bed sheets, pillows and home furniture such as couches (Jaffe et al., 2020). It can easily be blended with other fabrics such as cotton and other natural fibres (Jaffe et al., 2020).

According to Al-Sabagh et al. (2016), the first recycling effort for polyethylene terephthalate, specifically polyethylene terephthalate bottles in the world was in 1977, however, the main factor affecting the suitability of polyethylene terephthalate recycling is the nature of contaminants present once the polyethylene terephthalate is exposed to the environment such as; acids, colouring contaminants and acetaldehyde. Polyethylene terephthalate's recyclability makes it the first choice for various applications and its waste accounts for 12 % by volume of the world's total plastic waste. In South Africa in 2018, 98 649 tonnes of polyethylene terephthalate bottles were recycled (PlasticsSA, 2020). The amount of recyclable polyethylene terephthalate in the US was about 2.65 million tonnes in 2014, however, the actual recycled quantity of polyethylene terephthalate was approximately 0.82 million tonnes and the recycling rate was 31 % (Rahimi et al., 2016). During the recycling process no plastic waste is produced, however there is carbon dioxide emission during the transportation, heating and moulding processes. Vo & Pham (2021), reported that the density of polyethylene terephthalate is 0.96-1.45 g/cm^3.

2.3.3 Polypropylene

Polypropylene (PP) was first manufactured in the 1950s, it is also quite versatile and is used to produce a variety of plastic products (PlasticsSA, 2020). According to Maddah (2016), it is a petrochemical product that is derived from the monomer propylene. Roughly 50 million metric tonnes of polypropylene are produced per annum (Eagan et al., 2017). Additionally, it is very cheap and flexible for moulding (Eagan et al., 2017). Polypropylene has lower stability compared to polyethylene, therefore, is more susceptible to degradation (Gewert et al., 2015). Amongst the plasticpolymers, polypropylene has the lowest density and has high chemical and temperature resistance, making it suitable for the manufacturing of trays, funnels and pails. Vo & Pham (2021), report that thedensity of polypropylene is 0.83-0.92 g/cm^3.

2.3.4 Polystyrene

Polystyrene (PS) is manufactured from styrene and is highly prone to outdoor weathering such as UV light (Gewert et al., 2015). In the United States alone, approximately 3 million metric tonnes of

polystyrene are produced annually (Ma & Huang, 2007). According to Ma & Huang (2007), it has high resistance to numerous chemicals, acids, alcohol, bases, water, and detergents. It is commonly used for flower pots, packaging, toys, and disposable cutlery (Gilbert & Patrick, 2017; Ma & Huang, 2007).

Recycling of polystyrene requires the manufacturers to take full responsibility for the recycling and disposal of any of the polystyrene material they sell. Various countries around the world, such as Zimbabwe and China have banned the use of polystyrene disposable cutlery. Vo & Pham (2021), report that the density of PS is 1.04-1.10 g/cm^3. While expanded polystyrene which is a less dense and lightweight form of polystyrene has a density of 0.016 - 0.36 g/cm^3 (Bouwman et al., 2018).

2.3.5 Polyvinyl chloride

Polyvinyl chloride (PVC) was first produced in 1920 to replace natural rubber, it has become known as one of the most versatile plastics as it can be both rigid and flexible making it popular in various industries (PlasticsSA, 2020). The rate of degradation of polyvinyl chloride is enhanced by humidity, the presence of other chemicals and mechanical stress (Gewert et al., 2015). Polyvinyl chloride is commonly used for clothing, plumbing and construction materials. Furthermore, it is one of the world's most commonly produced plastic polymers, and roughly 40 million tonnes are manufactured per annum (Gilbert & Patrick, 2017). Vo & Pham (2021), report that the density of polyvinyl chloride is 1.16-1.58 g/cm^3.

2.3.6 Polyurethane

According to Akindoyo et al. (2016), polyurethane is different from most plastic polymers, it was first produced in 1937 during World War II as a replacement for rubber. Today, polyurethane is one of the most used, versatile and researched materials worldwide (Akindoyo et al., 2016). This is because of the plastic polymer's durability and toughness combined with the elasticity of rubber (Akindoyo et al., 2016). This makes this plastic polymer suitable for replacing metal, plastics, and rubber.

Polyurethane is used in the manufacturing of paints, liquid coatings, elastomers, insulators, elastic fibres and foams (Akindoyo et al., 2016). Polyurethane is susceptible to biodegradation (Gewert et al., 2015). The recycling process of polyurethane is done usually under four classes; advanced chemicaland thermochemical recycling, mechanical recycling, energy recovery and product recycling (Gama etal., 2018). The material recycling process can generate feedstock chemicals for industry, additionally,the energy recovery includes the partial or complete oxidation of waste materials, which results in the production of gaseous fuels and electricity (Gama et al., 2018). The by-products from the recycling process are non-hazardous, therefore, are disposable in the environment (Gama et al., 2018). Polyurethane is more economical compared to other plastic polymers due to its recycling process and

it is environmentally non-hazardous (Gama et al., 2018). It has a density of 1.2 g/cm^3 (Bouwman et al., 2018).

2.3.7 Polycarbonate

Polycarbonate (PC) was first discovered in 1953 by Dr Herman Schell (Youssef, 2018). It is a naturally transparent thermoplastic, it is heat and impact-resistant and lightweight (Tjong & Meng, 2000). Due to these properties, it is used in the manufacturing of bullet-proof glass, protective gear, eye lenses, CDs, medical supplies, construction materials and electronics (Bouwman et al., 2018; Youssef, 2018). Polycarbonate is not recycled in South Africa because of economic implications and difficulties in separating the layers of the plastic (Sadan & De Kock, 2020), therefore, no recycling figures are published. It has a density of 1.2 g/cm^3 (Youssef, 2018).

2.4 Microplastics

Large plastic particles can break down over time due to different chemical and physical processes into smaller plastic particles called microplastics (Ling et al., 2017). Microplastics can form from large plastic particles through processes such as; mechanical abrasion, biological degradation, chemical interaction and photodegradation (Xu et al., 2020; Zhao et al., 2022). In literature there are conflicting definitions of microplastics, for instance, Gasperi et al. (2018), define microplastics as plastic particles that are < 5 mm in diameter. However, Coppock et al. (2017), argue that microplastics are plastic particles that range only between 1 µm - 5 mm in diameter. Particles smaller than 1 µm are referred to as nanoplastics, due to their size they cannot be seen through the naked eye. Nanoplastics can form from the breakdown of both macro- and microplastics (Boyle & Örmeci, 2020). The different characteristics of plastic polymers such as brittleness influence the shapes and sizes of the microplastics produced through the processes mentioned above.

Microplastics can be found in different habitats all over the world. The distribution of microplastics is a result of characteristics of plastics such as their corrosion resistance, durability and lightweight (Xu et al., 2020). In aquatic environments, microplastics are ubiquitous in all sections from the surface of the water to benthic sediment, allowing them to be easily accessible to numerous aquatic biota occupying different habitats in the water column (Castro-Castellon et al., 2022; Guo & Wang, 2019). There has been an increase in the number of microplastics in aquatic systems due to plastic production outweighing the capacity for recycling and proper disposal (Rochman et al., 2013). Horton et al. (2017), report that rivers transport 70–80 % of plastic debris including microplastics, resulting in their extensive deposition of plastic debris into the world's oceans. While for aerial transport, Gasperi et al. (2018), discovered 0.3-60 fibres/m^3 being actively transported.

Plastic debris including microplastics for decades have been identified to be one of the major components of pollution in freshwater systems (Klein et al., 2018; Weber et al., 2021). Microplastics have been identified by Horton et al. (2017), as one of the rapidly increasing sources of pollution in aquatic systems, consequently becoming a high-priority environmental concern. Several studies have reported the potentially toxic impacts of microplastics on freshwater systems (de Sá et al., 2018; Klein et al., 2018). This is why microplastics are now considered to be emerging pollutants and are even recognized as an emerging threat to the whole environment (Avio et al., 2017; Miloloža et al., 2021; Kumar et al., 2022).

The amount of microplastics in the environment has been a subject of increasing environmental concern although the toxicological effects of these microplastics are still unclear (Cheung & Fok, 2017; Kumar et al., 2022). According to Pereao et al. (2020), dams are likely to trap some plastics and microplastics, especially those made up of polymers with high densities. The difference in plastic density causes microplastics to become accessible to organisms at different levels of the water column (Pereao et al., 2020). In their study, Zhang et al. (2015), noted that microplastics accumulated behind the wall of the Three Gorges Dam. Conversely, Weideman et al. (2019), from their research reported that dams do not have the ability to trap floating microplastics, and that the trapping of microplastics is dependent on the size of the dam, water velocity flow and the characteristics of microplastics particles.

Microplastics degrade slowly and over the years have accumulated in freshwater systems, in some locations even more than larger items of debris such as rocks, (Ivleva et al., 2017; Napper & Thompson, 2016; Wagner et al., 2014; Zhao et al., 2022), and there is evidence that microplastics continue to increase. This accumulation of microplastics in freshwater systems is a worldwide ecological problem and is of increasing scientific concern, however, for a long time the contamination of freshwater systems by microplastics has been overlooked, the first microplastics study was published in 2011 and we have a limited understanding of the abundance in freshwater ecosystems, transport pathways and their impacts on freshwater ecosystems and humans (Eerkes-Medrano et al., 2015; Ivleva et al., 2017). In water and sediments, these microplastics have reached densities of up to 100 000 items per m^3 (Eerkes-Medrano et al., 2015).

According to Akindele et al. (2019) and Zhao et al. (2022), freshwater systems such as rivers and dams are the route through which microplastics from terrestrial environments are transported into marine systems. Regardless of the poor recycling and high plastic pollution, in numerous African countries, microplastics have been barely studied in African freshwater systems, with only a few countries with published studies, such as Tanzania, Nigeria and South Africa (Aragaw, 2021; Akindele et al., 2019;

Bouwman et al., 2018; Biginagwa et al., 2016; Nel & Froneman, 2015). Akindele et al. (2019), confirmed that African freshwater systems were under threat from microplastic contamination. For instance, in Lake Victoria in Tanzania, microplastics have been found in the guts of two different fish species (Biginagwa et al., 2016). Additionally, in a study by Akindele et al. (2019), their research showed that snails when feeding cannot differentiate between algae with microplastics and algae without microplastics. Nevertheless, freshwater microplastic reports are scarce in Africa with no studies from West African freshwater systems so far (Akindele et al., 2019).

According to Aragaw (2021) and Nel & Froneman (2015), the degree of microplastic pollution in aquatic systems in the Southern hemisphere is largely unknown, particularly in Africa, furthermore, in South Africa the data on microplastic pollution is limited (Saad et al., 2022). In South Africa, studies by; Lamprecht (2013), Naidoo et al. (2015), and Nel & Froneman (2015), have detected microplastics in marine systems around South Africa as attention has been on marine systems while a few authors such as Bouwman et al. (2018), have focused on freshwater systems. Jambeck et al. (2015), caution that it is crucial to increase the understanding of microplastic pollution in aquatic systems in South Africa because it was ranked in the top 20 countries with the highest mass of poorly managed plastic waste in the world.

2.4.1 Types of microplastics and shapes

Microplastics can be categorized as i) primary microplastics that are manufactured specifically in this form for cosmetic goods such as microbeads or as pre-production pellets, and, ii) secondary microplastics that are produced through mechanical action, photo-degradation and biological degradation of larger plastics (Gupta et al., 2022; Lahens et al., 2018; Napper & Thompson, 2016). Examples of these include; fibres from synthetic clothing that are released through washing and released into freshwater systems through wastewater treatment plants (Ivleva et al., 2017). Due to the decrease in the use of microbeads in cosmetic products, in aquatic environments, secondary microplastics are reported to be the leading source of microplastic pollution (Horton et al., 2017). Furthermore, their abundance is predicted to increase immensely with the continued input of plastic particles from various sources (Gupta et al., 2022; Horton et al., 2017).

According to (Kooi & Koelmans, 2019), there are different shapes of microplastics, the most common shape that is found in both sediment and water is fibres which make up roughly 48.5 %, then fragments which comprise 31 %, beads 6.5 %, films 5.5%, and lastly foam 3.5 %. The shape of a microplastic can influence the intake of microplastics by aquatic organisms (Xu et al., 2020).

2.4.2 Sources and transport of microplastics

Major sources of environmental plastics and microplastics have been reported to be clothing, household products, plastic packaging and customer goods since they are the most encountered in freshwater systems, these come from mostly terrestrial landfills and wastewater treatment plants (Schwarz et al., 2019). Additionally, Miloloža et al. (2021), state that other major microplastic sources identified are synthetic textiles (34 %), tire wear (29 %), city dust (24 %), road markings/dust (7 %), marine coatings (4 %), microbeads (2 %), and plastic pellets (0.3 %). Polyethylene and polypropylene are reported to contribute to the most pollution not only in freshwater systems but across different environments (Schwarz et al., 2019).

Microplastics of different types can become airborne through the wind from landfills and can be deposited into aquatic systems, additionally, effluents from wastewater treatment plants, urban storm-water flows, runoff from informal settlements, runoff from terrestrial landfills and industries have also been identified as a source of microplastics in freshwater systems (Chen et al., 2020; Zhao et al., 2022). This is all illustrated in Figure 1 below.

The washing of clothing can release microplastic fibres, these fibres have been reported as the most commonly detected microplastic shape in freshwater environments. (Napper & Thompson, 2016; Stafford, 2016; Zhao et al., 2022). This occurs when waste effluent from washing machines containing these fibres travels through wastewater to water treatment plants and is then released into freshwater ecosystems such as rivers which transport these microplastics and deposit them in marine environments (Aragaw, 2021; Dris et al., 2015; Napper & Thompson, 2016). In their study, Dris et al. (2015), discovered large quantities of fibres that entered directly from a wastewater treatment plant into the River Seine. Browne et al. (2013), sampled wastewater from domestic washing machines and discovered that a single piece of clothing produced roughly 1900 fibres per wash. In Denmark in 10 of the biggest wastewater treatment plants, the concentrations of microplastics in the effluent ranged from 19 to 447 particles/L with a median of 54 particles/L, with the most abundant type of microplastics reported to be polyethylene and polyester (Xu et al., 2020). Figure 2 below illustrates the sources, transport, distribution, fate, and transformation of microplastics in aquatic environments.

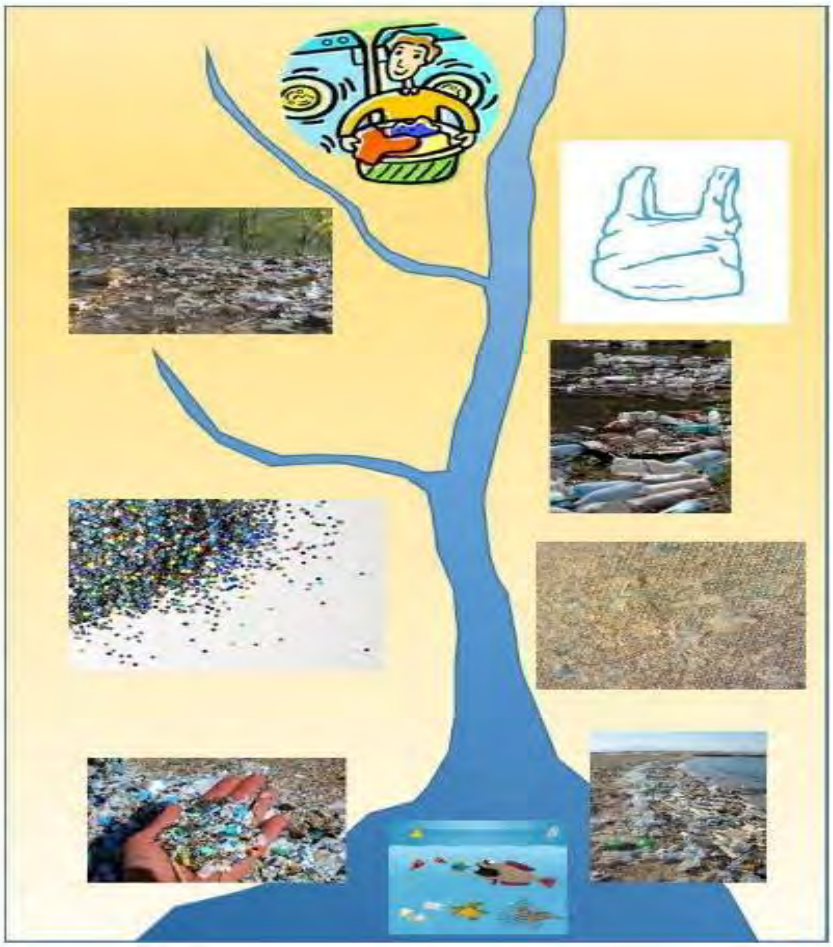

Figure 1 General representation of how plastics which breakdown into microplastics along the way are transported by rivers from landfills and wastewater treatment plants to the ocean (source: Bouwman et al., (2018))

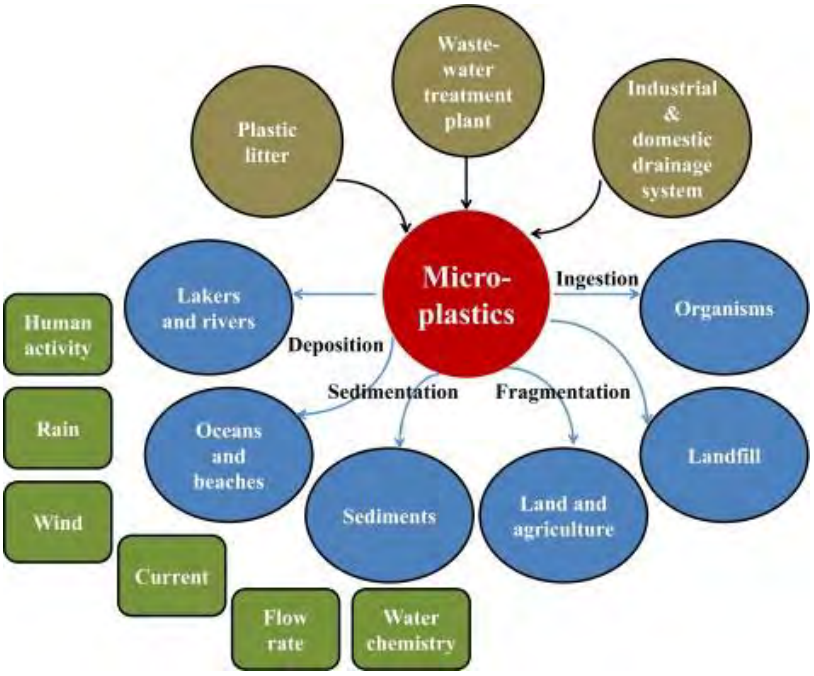

Figure 2 Sources, transport, distribution, fate, and transformation of microplastics in aquatic environments. The black arrows represent the source of microplastics. The blue arrows indicate the transport, distribution, fate or transformation of microplastics which can be affected by human activity, hydrological and meteorological conditions (source: Xu et al., (2020)).

2.4.3 Distribution and detected concentrations of microplastics in freshwater systems

The distribution, abundance, and occurrence of microplastics in aquatic environments are determined by several factors: the nature of the environment, microplastics properties (type, density, size, and shape), climate conditions (air turbulence, waves), industrialization, the standard of living, waste management, development, and urbanization (Miloloža et al., 2021).

Density also plays an important role in the distribution of microplastics in freshwater systems and within the water column. The different polymer densities of microplastics allow their vertical distribution in aquatic environments from the water surface to the benthic sediment, consequently, influencing the bioavailability of these microplastics to different aquatic creatures (Cole et al., 2011; Guo & Wang, 2019). High-density microplastics are likely to sink to the bottom of the water column where they are easily accessible to benthic feeders whereas the low-density microplastics float and therefore, can be ingested by filter feeders (Pereao et al., 2020). The uptake of microplastics by

organisms from various feeding groups is influenced by the particle; size, colour, density and shape (Pereao et al., 2020).

Microplastic concentrations are higher in freshwater systems next to urban areas compared to rural areas, furthermore, their distribution and abundance is affected by population density, and both hydrological conditions and climate (Di & Wang, 2018; Xu et al., 2020). For instance, in two rivers located in Switzerland (Vuachère and Venoge rivers), microplastic concentrations were reported to increase significantly after a rainfall event, particularly in urban areas, while in Lakes Zurich and Constance both also located in Switzerland, strong winds triggered vertical mixing and thus reducing microplastic abundance (Faure et al., 2015).

Seasons play a significant part in the distribution and concentrations of microplastics in freshwater systems (Alimi et al., 2021). For example, in a study by Weideman et al. (2019), in the Orange-Vaal river, South Africa, the authors discovered that microplastic abundance was higher in surface waters during the rainy season compared to the dry season. Table 1 below summarises the distribution (location and polymer type) and concentrations of microplastics in freshwater systems around the world.

Table 1 The location, polymer type and concentrations of microplastics in freshwater systems around the world.

Location	Polymer type	Concentration	References
Lake Hovsgol, Mongolia	Not disclosed	20 264 particles/km^2	(Free et al., 2014)
River Rhine, Europe	PS, PP, acrylate, PET, PMMA and PVC	892 777 particles/km^2	(Mani et al., 2015)
Great Lakes tributaries, USA	Not disclosed	0.00005-0.032 particles/L	(Baldwin et al., 2016)
Taihu Lake, China	PP, PP and PET	3.4–25.8 particles/L	(Paul-Pont et al., 2016)
Lake Ziway, Ethiopia	Not disclosed	6.3–115.9 kg	(Merga et al., 2020)
Oxbow lake in Yenogoa, Nigeria	Not disclosed	8.369 particles/L	(Alimi et al., 2021)
Naivasha lake, Kenya	PP, PE, and Polyester	0.000407±0.000135 particles/L	(Alimi et al., 2021)
Johannesburg (stream), South Africa	Not disclosed	0.705 particles/L	(Dahms et al., 2020)
Orange-Vaal River, South Africa	Not disclosed	2.3 ± 7.2 particles/L (wet season); 1.4 ± 2.6 particles/L (dry season)	(Weideman et al., 2019)
Gauteng and Northwest Province	Not disclosed	1.9-5.12 particles/L	(Bouwman et al., 2018)

Microplastic investigation in aquatic systems is growing, however, both Au et al. (2015) and Pereao et al. (2020), state that even less is understood about the toxicological implications of microplastic exposure to freshwater organisms. Au et al. (2015) and Bouwman et al. (2018), stress the importance

of investigating the presence of microplastics in freshwater systems as microplastics are transported by rivers into dams and marine environments.

Ecotoxicological studies for microplastics have been conducted mainly using marine (77 %) and not freshwater (23 %) organisms (de Sá et al., 2018). Furthermore, the main focus of many microplastic studies is to quantify the abundance of microplastics in aquatic environments (Bouwman et al., 2018), resulting in a lack of ecotoxicological studies. This lack of ecotoxicological data on the behaviour of microplastics in freshwater ecosystems has been mentioned by various writers (de Sá et al., 2018; Eerkes-Medrano et al., 2015; Vijayaraghavan et al., 2022). This lack of knowledge is highly concerning since freshwater organisms are directly affected by wastewater, terrestrial runoff and discharges potentially containing high levels of microplastics and contaminants (de Sá et al., 2018; Gupta et al., 2022). Gray & Weinstein (2017), state that the literature is even more limited on the potential adverse effects of invert exposure on invertebrates compared to vertebrates in freshwater systems.

Even though only 23 % of ecotoxicological studies are based in freshwater environments, most of those studies use acute or high concentrations of microplastics that are not environmentally realistic, consequently, leaving significant gaps in our understanding of the effects of microplastics at environmentally realistic concentrations (de Sá et al., 2018; Horn et al., 2020). Furthermore, these high concentrations used in many ecotoxicology studies do not mimic the concentrations in the environment, making it difficult to predict the actual effects of microplastics.

2.5 Ecologically relevant endpoints

The term endpoint refers to the predicted adverse effects the test organisms may experience during an experiment, Stokes & Marsman (2013), state that endpoints are important as they can indicate when a test organism is in distress during an experiment. Furthermore, endpoints can be used to evaluate or predict the effects of toxic agents in natural environments. Endpoints become ecologically relevant when they are suitable for the chosen test taxa, test type and the period of the toxicity testing. The most common study endpoints in ecotoxicological studies are growth, hatching rate, ingestion (for example microplastics and sediments), mobility, reproduction, behaviour, respiration and mortality because it is unavoidable and common in toxicity tests (Dong et al., 2018; Duan et al., 2008; IACUC, 2019; Song et al., 2014). Although these endpoints are the most common, some are suitable only for acute tests (short-term) such as mortality (OECD, 2019), while some are suitable for chronic tests (long-term) such as reproduction and growth. However, mortality and behaviour are suitable for both test types. Since some of these endpoints are suitable for specific types of toxicity tests, it is important to choose the ones that are best suitable for the chosen study test.

Although the test organisms for chronic and acute endpoints should be dependent on the organism's life span, some studies use test organisms that are not appropriate for the chosen test (Marty et al., 2017). For example, testing reproduction should be based on the organism's life cycle, some organisms reproduce everyday which would be suitable for acute tests, while some reproduce after seven days or after 21 days, therefore the test organism should be chosen based on the life cycle and type of toxicity tests.

Study endpoints should also be dependent on the age of the chosen test taxa. Marty et al. (2017), recommend that for early life (embryo and juvenile), hatching rate, development and growth should be used as endpoints, while for adults reproductive success, parental body weight and offspring survival are the recommended endpoints.

It is also important to choose taxa from multiple tropic levels, as organisms are affected differently by the same pollutant due to their different sensitivities. Furthermore, no species is sensitive to all pollutants. Species sensitivity is dependent on the mode of action of the chosen pollutant, chemical properties, exposure route and time, and the physiology of the organism (Palma et al., 2016; Van Gestel et al., 2018). Therefore, it is crucial to constantly test several different species, with different life traits, functions, feeding strategies and most importantly from different positions in the food web. Testing multiple different test taxa also helps to understand what is happening in reality in freshwater systems at an ecosystem level. Furthermore, different organisms have different sensitivities to the same pollutant. For example, snails are described by Weber et al. (2021), as being resilient and therefore, not very sensitive to pollutants and changing environmental conditions. This is because snails exhibit an avoidance behaviour (Araújo et al., 2016), in which they shield themselves or limit interaction with pollutants by retreating into their shells.

2.6 Toxicity of microplastics

Several authors state that the effects of microplastics on freshwater ecosystems are not fully understood yet (de Sá et al., 2018; Eerkes-Medrano et al., 2015; Ivleva et al., 2017; Vijayaraghavan et al., 2022). Previous ecotoxicology studies have proven that the effects of microplastics have been recorded in diverse groups of aquatic organisms such as vertebrates, invertebrates and planktonic (Ribeiro et al., 2019). Additionally, filtration, direct ingestion and respiration have been reported as the primary pathways for microplastic intake. However, predator species can also ingest microplastics through direct ingestion or food transfer, and humans can also ingest microplastics in this way. The toxicity of microplastics is dependent on the size, plastic polymer, additives and shape of the microplastic (Miloloža et al., 2021). Microplastic exposure is associated with several physical impacts

on organisms from multiple trophic levels (Lusher et al., 2017). These physical effects on individual organisms are further discussed below.

2.6.1 Amphipods

Au et al. (2015), noted in their study, that exposure of amphipods to polyethylene and polypropylene microplastics reduced growth and reproduction. Specifically, exposures of 5 and 10 microplastics/L of polyethylene microplastics resulted in a decrease in reproduction, this occurred until day 28 of the experiment, thereafter reproduction stabilized (Au et al., 2015). Their study also showed that the number of microplastics ingested per test organism decreased throughout the long-term exposure to polyethylene microplastics. Au et al. (2015), caution that in many invertebrates due to exposure to microplastics, minimal lipid reserves may decrease, and a decrease in feeding may occur resulting in a decrease in growth, reproduction, and even mortality.

2.6.2 Fish

Wertz (2015), reports that microplastics can enter the body of organisms with gills such as through respiration direct and indirect ingestion. A study on the effects of polyethylene on fish at environmentally relevant concentrations showed that after two months of dietary exposure to microplastics, fish suffered from liver toxicity, tumour promotion**,** and displayed signs of endocrine disruption through abnormal growth of germ cells in the gonads (Rochman et al., 2013).

2.6.2.1 Egestion of microplastic particles

There are several laboratory studies on the ingestion of microplastics but few on egestion, particularly at concentrations resembling those in the environment (Weis, 2019). Organisms may ingest microplastics directly, due to confusion with potential food, or passively, for example during particle filtration or grazing (Foley et al., 2018). In a study by Ory et al. (2018), the authors discovered that planktivorous juvenile fish (*Seriolella violacea*) ingested microplastics that were similar in colour to the food they were being fed, at times microplastics can be ingested accidentally. In their study, Foley et al. (2018), discovered that juvenile fish food consumption and ingestion were significantly reduced during exposure to microplastics. Mbedzi et al. (2020), found microplastics inside all the *Tilapia* that were exposed to the microplastics, the number of microplastics inside the *Tilapia* increased slightly with increasing concentrations. However, there were no statistically clear differences in microplastic consumption in the concentrations selected, indicating that the fish consumed the plastics even when they were sparse in the environment at lower concentrations (Mbedzi et al., 2020). This suggests that *Tilapia sparrmanii* ingest microplastics even in environments where there are low concentrations of microplastics (Mbedzi et al., 2020).

Once consumed, microplastics remain in the digestive system of aquatic organisms for periods of hours, days and weeks before they are egested depending on the type of organism and microplastic (Foley et al., 2018). However, the residence time of microplastics in different freshwater organisms is poorly known (Ory et al., 2018). Vijayaraghavan et al. (2022), discovered large amounts of polyvinyl chloride microplastics that were ejected through faecal matter after they exposed juvenile *Etroplus suratensis* to different concentrations of polyvinyl chloride microplastics, indicating that indeed the test taxa ingested the microplastics. However, the authors highlighted that the retention time of polyvinyl chloride microplastics in the test taxa was not known.

2.6.2.2 Growth

Pannetier et al. (2020), exposed juvenile *Oryzias latipes* to a mixture of microplastics and discovered that the microplastic exposure did not affect the body length and body mass of the fish. Huang et al. (2020), discovered similar results when they exposed *Poecilia reticulata* to polystyrene microplastics with two concentrations (100 and 1000 µg/L) for 28 days. Mbedzi et al. (2020), also discovered that the length of *T. sparrmanii* exposed to 39-772 particles/L of polyethylene microplastics was not significantly affected by the exposure to the microplastics.

Contrastingly, Naidoo & Glassom (2019), discovered that there was a statistically significant decrease in the growth of juvenile *Ambassis dussumieri* exposed to a mixture of polyethylene, polyvinyl chloride and polystyrene microplastics. Even though their study used environmentally relevant concentrations (0.051 g/290L), a statistically significant effect might have been observed because their experiments ran for 95 days, meaning that the longer the exposure time to microplastics, the higher the potential effects. Likewise, Xia et al. (2020), discovered that polyvinyl chloride microplastics had a significant effect on the body mass and body length growth of *Cyprinus carpio*. It is important to note that the authors ran the experiment for 60 days, perhaps this is why these effects were observed. Similarly, Jabeen et al. (2018), discovered that a mixture of microplastic particles (polyethylene and polystyrene), resulted in a decrease in the mass of *Carassius auratus* that were exposed to the microplastics. Furthermore, Vijayaraghavan et al. (2022), also discovered similar results when they exposed juvenile *E. suratensis* to different concentrations of polyvinyl chloride microplastics. The authors stated that the significant decrease in the body mass of the test taxa was caused by false satiation, reduced feeding, intestinal inflammation, decreased energy budget and a decrease in food conversion.

2.6.2.3 Inflammation

Microplastic exposure has been reported to cause inflammation responses in organisms, such as inflammation of the intestines and jaws (Blackburn & Green, 2022; Jabeen et al., 2018; Vijayaraghavan et al., 2022). However, there is limited literature on the effects of microplastics on inflammation and damage of fish and *Tilapia* jaws, however, it has been observed before as a physical effect of microplastics on freshwater organisms. In a study by Lu et al. (2016), on the toxicological effects of polystyrene on *Danio rerio*, in which the microplastics sizes ranged between 5-20 µm, in a concentration of 20 mg/L, the authors discovered that the exposure to the microplastics caused inflammation, disturbed lipid and energy metabolism. Additionally, Jabeen et al. (2018), reported during their study, injury and inflammation of the jaws were caused by chewing of microplastic particles that were of the same size as the food fed to the *D. rerio*, inflammation and injury of jaws in freshwater organisms can result in a decrease in food ingestion and eventually reduce growth and death.

2.6.2.4 Gill accumulation

Ding et al. (2018), in their study in which they investigated the effects of polystyrene microplastics on the freshwater fish *Oreochromis niloticus,* using concentrations of 1, 10, and 100 µg/ L, discovered that microplastic accumulation occurred in the gills of the fish, and this accumulation had increased with increasing experimentation time. Furthermore, in a study by Guerrera et al. (2021), the authors proved that microplastic accumulation was higher in gills compared to the gut, Huang et al. (2020), also discovered similar results. Ding et al. (2018), report that gills are the most likely organ in which microplastic accumulation can occur. Guerrera et al. (2021), state that microplastic accumulation in gills is dependent on the microplastics' shape and increases with exposure increasing time. Furthermore, Ding et al. (2018), report that the accumulation of microplastic is also dependent on the concentration of microplastics and the organisms exposed.

2.6.2.5 Effects of microplastics on the healing

The effects of microplastics on the healing of freshwater organisms such as fish are poorly studied. In a study conducted by Gu et al. (2020), where they investigated the effects of polystyrene microplastics on caudal fin regeneration in *D. rerio*. The authors discovered that exposure to polystyrene microplastics significantly disturbed the regeneration of the caudal fin in *D.* rerio, this was because the exposure to the microplastics altered the signalling networks that are vital for repairing tissue and regulating fin regeneration. This microplastic exposure in fish could disturb the ability of fish to regenerate and might ultimately impair their overall fitness in the environment (Gu et al., 2020).

2.6.2.6 Mortality

Lei et al. (2018), exposed *D. rerio* to polyamides, polypropylene, polyvinyl chloride and polystyrene microplastics of the following concentrations, 0.001–10 mg/L for 10 days, mortality was observed but it was not statistically significant, intestinal damage was also observed. Vijayaraghavan et al. (2022), also discovered similar results when they exposed juvenile *E. suratensis* to different concentrations of polyvinyl chloride microplastics. Likewise, several studies such as Lu et al. (2016) and Karami et al. (2017b), also reported that there was no statistically significant effect on mortality for the duration of their respective studies.

Zhang et al. (2021), have identified filter-feeding fishes such as *Thryssa kammalensis* and *Hypophthalmichthys molitrix* as being the most vulnerable to microplastic pollution due to their feeding strategy. This is because the respiratory process cofunctions with the feeding mechanism when filtering plankton from the water. These characteristics make *T. kammalensis* and *H. molitrix* more susceptible to microplastic pollution effects than other fishes with feeding strategies.

2.6.3 Snails

Gastropods have been described by Veiga et al. (2016) and Weber et al. (2021), as being highly stress tolerant, this high tolerance of gastropods is because they have the ability to move into their shells when they face adverse environmental changes such as pollution causing total isolation from the environment. According to Weber et al. (2021), the effects of microplastics on gastropods are rarely studied. Furthermore, the knowledge of microplastic toxicity on freshwater gastropods is even more limited causing a scarcity of data on the effects of microplastics on gastropods, especially freshwater gastropods (Weber et al., 2021). Previous microplastic toxicity studies have primarily concentrated on the effects of microplastics on fish, bivalves and crustaceans (de Sá et al., 2018).

2.6.3.1 Ingestion and egestion

In a study conducted by Gutow et al. (2016), the authors stated macrozoobenthos such as snails have an opportunistic feeding behaviour, they feed on algae and can also ingest microplastics attached to the algae. The study also showed that snails cannot tell the difference between algae and microplastics (Gutow et al., 2016). Additionally, Akindele et al. (2019), discovered polyethylene microplastics in the digestive tracts of *Melanoides tuberculata*.

Weber et al. (2021), in their study on the freshwater gastropod *(Lymnaea stagnalis),* using concentrations ranging between 6400-100 000 000 particles/L, confirmed that the test organisms did indeed ingest polystyrene microplastics of different shapes and sizes. Additionally, they discovered

that ingestion did not significantly affect the study endpoints in both environmentally relevant concentrations and very high microplastic concentrations used in the study.

In a study conducted by Imhof et al. (2013), they discovered microplastics in the faecal matter of freshwater snails indicating that they ingest microplastics. Ingesting microplastics can cause physical disturbances in the digestive system of organisms, reducing feeding due to false satiation (Gray & Weinstein, 2017). However, several studies such as Graham & Thompson (2009) and Van Cauwenberghe et al. (2015), have reported that sometimes microplastics that have been ingested can pass through the gut of organisms, with minimal or no observed adverse effects.

2.6.3.2 Growth

Au et al. (2015), state that exposure to microplastics can result in slowing down of growth of gastropods. Au et al. (2015), explain that the slowing down or decrease in growth is caused by the reduced natural food intake caused by microplastic ingestion. In a study by Imhof & Laforsch (2016), on the effects of microplastics on the freshwater mud snail (*Potamopyrgus antipodarum*), the authors discovered that the exposure to a mixture of microplastics which included polyethylene and polyvinyl chloride had no significant effect on the growth rate of the test organism. Akindele et al. (2019), discovered polyethylene in the digestive tracts of *M. tuberculata*, the authors explained that the ingestion of microplastics can affect growth in *M. tuberculata* as it causes false satiation, this is in agreement with the findings of Au et al. (2015).

2.6.3.3 Reproduction

Weber et al. (2021), exposed a freshwater gastropod (*L. stagnalis*), for 28 days to polystyrene concentrations of 6400, 160 000, 4 000 000 and 100 000 000 particles/L, the authors discovered that the exposure had no significant effect on the reproduction of the test taxa. Imhof & Laforsch (2016), also discovered similar results, when they exposed *P. antipodarum* to a mixture of microplastics. Although both Weber et al. (2021) and Imhof & Laforsch (2016), recorded no significant effect of microplastics on the test taxa, Au et al. (2015), caution that the exposure and ingestion of microplastics can result in a decrease in reproduction.

2.6.3.4 Mortality

Weber et al. (2021), discovered that exposure of freshwater gastropods (*L. stagnalis*) to polystyrene microplastics had no significant effect on the mortality of the test taxa. However, they observed slight effects on immune cell phagocytosis. Furthermore, the authors justified the lack of mortality as the high resilience of the test taxa (Weber et al., 2021). Imhof & Laforsch (2016) discovered similar results.

These findings can be explained by the fact that gastropods have generally a high tolerance to pollutants due to their ability to isolate themselves inside their shells (Veiga et al., 2016).

2.6.4 Shrimps

There is limited literature on the effects of microplastics on freshwater shrimps. Gray & Weinstein (2017), emphasise the importance of considering the potential chronic effects of prolonged exposures of microplastics to grass shrimps (*Palaemonetes pugio*), such as changes in the energetics, reduced growth, reproduction, movement and fitness. Since the concentrations of microplastic particles in aquatic systems are predicted to increase over time, therefore, it is important to understand the threat that they pose to invertebrates, such as shrimps.

2.6.4.1 Ingestion and egestion

Gray & Weinstein (2017), reported that when ingested, fibres were more toxic to *P. pugio*, this toxicity may be due to the inability to completely egest this type of microplastic, especially those small in size. Furthermore, in a 10-day chronic toxicity test conducted by Au et al. (2015), the authors discovered that polypropylene fibres ingestion was more toxic to the test organism than the polyethylene spheres, this could have been a result of differential toxicities of the different microplastics. Au et al. (2015), also discovered that fibres had longer clearance times than spheres, which is a result of the shape of the plastics. Additionally, Gray & Weinstein (2017), discovered that the shape of the microplastic had a significant influence on the number of particles ingested and egested by the shrimps.

In an acute study by Wang et al. (2021), the authors exposed three shrimp species *(Penaeus monodon*, *Marsupenaeus japonicas* and *Litopenaeus vannamei*) to the following microplastic concentrations 0 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 200 mg/L and 300 mg/L. However, the authors did not disclose the types of microplastics used during the study. They discovered that there were microplastics present in the excreta of all the test taxa, with *P. monodon* having the highest, followed by *M. japonicus* and *L. vannamei*, this was observed because P. monodon has a higher metabolic rate compared to the other two test taxa (Wang et al., 2021).

Klein et al. (2021), exposed *Neocaridina palmata* to environmentally realistic concentrations ranging between 20-20,000 particles/L of polyethylene, polystyrene, and polyvinyl chloride microplastics, they discovered that a higher number of small irregular shaped polyethylene microplastics were egested by the test organisms compared to the other microplastic polymers. The authors also discovered that ingestion and egestion increased with increasing concentrations. Furthermore, Klein et al. (2021),

highlighted that ingestion and accumulation of microplastics in the guts of freshwater organisms determined the types of effects induced in endpoints such as mortality, growth, and behaviour.

2.6.4.2 Growth

Li et al. (2021), conducted a study on the effects of polyethylene and polystyrene microplastics using concentrations of 1260 particles/L and 1100 particles/L respectively, they exposed these microplastics to brine shrimps (*Artemia parthenogenetica*) for 45 days. They discovered that both polyethylene and polystyrene microplastics significantly reduced the growth rate of brine shrimps and the average body length of adult brine shrimp. The authors deduced that the decrease in growth was caused by the accumulation of microplastics in the intestine which cause intestinal blocking which may reduce the feeding rate, and as a result growth (Li et al., 2021). They also discovered that the remaining microplastics in the guts changed the diversity of gut microbiota and caused epithelial cell and digestive tract damage (Li et al., 2021).

Blarer & Burkhardt-Holm (2016), in their study where they exposed *Gammarus fossarum* to polyamide microplastics, using concentrations of 138.7 particles/L for 28 days. They discovered that the exposure reduced food assimilation, which resulted in a decrease in the weight of the test organisms. Wang et al. (2021), exposed mysid shrimp (*Neomysis japonica*) to polystyrene microplastics of 10 000 mg/L for four days, and they discovered that the exposure caused a decrease in the growth of the test organisms. On the contrary Peixoto et al. (2019), found different results when they exposed brine shrimp (*Artemia franciscana*) to concentrations of 0.4, 0.8 and 1.6 mg/L of microplastics of undisclosed plastic polymer for 44 days. The authors discovered that the microplastic exposure did not have any significant effect on the growth of the test organisms.

2.6.4.3 Reproduction

Peixoto et al. (2019), discovered that microplastic exposure significantly affected the reproduction of *A. franciscana* when they exposed them to concentrations of 0.4, 0.8 and 1.6 mg/L of undisclosed microplastic polymer.

2.6.4.4 Mortality

Gray & Weinstein (2017), conducted an acute study on the impacts of polystyrene, polypropylene, and polyethylene microplastics on adult *P. pugio*. The authors exposed the shrimps to different microplastic shapes, which included: spheres, fragments and fibres at a concentration of 50 000 particles/L of water for 3 hours. The microplastic exposures had a significant effect on mortality and ingestion. Furthermore, the spheres and fragments were not acutely toxic and mortality rates were higher in the exposure containing fibres (Gray & Weinstein, 2017).

Wang et al. (2021), discovered similar results in their study, where mortality increased with increasing concentrations, their results indicated that the mortality of *P. monodon*, *M. japonicus* and *L. vannamei* were 47 %, 53 % and 20 % respectively after 48 h of 300 mg/L microplastic exposure. Theirresults indicate that *L. vannamei* has a higher tolerance to microplastics compared to the other two species used in their study, furthermore, this species had been discovered to have consumed fewer microplastics compared to the other two species, perhaps this is the reason why mortality was lower. Peixoto et al. (2019), also reported a significant effect on mortality when they exposed *A. franciscana* to concentrations of 0.4, 0.8 and 1.6 mg/L of undisclosed microplastic polymers.

2.6.5 Size and shape implications

The small size of microplastics allows them to be consumed by different freshwater organisms including vertebrates and invertebrates with different feeding strategies and from different trophic levels (Imhof et al., 2013). Additionally, the size of the microplastics ingested is important as microplastics smaller than 25 µm can be translocated into the tissue of the organisms (Ivleva et al., 2017). Wright et al. (2013), stated that the shape of microplastics plays an important role in the toxicity of the microplastics to the exposed test organisms. Fibres, due to their sharp shape are stated to be the most dangerous shape of microplastics compared to spherical and irregular shaped microplastics. This is because they can be easily embedded in the tissue of organisms (Au et al., 2015).

2.6.6 Toxicity to humans

2.6.6.1 Exposure routes

Although microplastics have been detected in different ecosystems, the risks associated with exposure to humans have not been adequately studied. Microplastics have been reported to enter human bodies through drinking water, food or inhalation of the particles in the air (Cox et al., 2019). In a study by Cox et al. (2019), the authors estimated that an average human per year consumes between 39 000 to 52 000 microplastic particles, from water, other sources and inhalation only. In South Africa microplastics have been detected in drinking tap water in two different provinces, the concentrations in the tap water ranged between 1.9-5.12 particles/L (Bouwman et al., 2018). Furthermore, Karbalaei et al. (2018), in their study detected microplastic concentrations ranging between 14-118 particles/L in bottled drinking water.

Microplastics can become airborne through transfer by the wind from clothes that have been out to dry or after a dry period (Liebezeit & Liebezeit, 2014). Once the microplastics are in the atmosphere they are easily inhaled by humans. Prata et al. (2020), state that roughly 26-139 microplastics are inhaled a day by an individual. Once inhaled, microplastic properties such as size and density

determine where in the respiratory system they are deposited, with the smaller and less dense particles being deposited deeper in the lungs (Prata et al., 2020).

In terms of food, humans can ingest microplastics through secondary transfer, when they ingest aquatic and terrestrial organisms that contain microplastics (Karbalaei et al., 2018). Microplastics have been reported in the tissues, gills and guts of aquatic animals such as bivalves, crustaceans, and fishes (Ding et al., 2018; Karbalaei et al., 2018; Lei et al., 2018). However, it is very difficult to precisely determine the number of microplastics transferred to humans through secondary transfer as the retention time of microplastics in the guts of aquatic organisms is poorly understood (Vijayaraghavan et al., 2022). Besides aquatic organisms, microplastics have also been identified in different types of salt (Karbalaei et al., 2018). Karami, et al. (2017a), in their study on 17 brands of salts from eight different countries discovered that the microplastic concentrations ranged between 0–10 particles/kg. Additionally, microplastics have been identified in other foods such as beer (Liebezeit & Liebezeit, 2014), honey and sugar (Liebezeit & Liebezeit, 2013).

2.6.6.2 Potential effects

The toxicity of microplastics to humans is primarily related to the leaching of chemicals that are used to manufacture plastics and those absorbed in the environment and also the physical effects of microplastics (Karbalaei et al., 2018). The effects of microplastics on human health are divided into three, firstly, physical effects caused by inhalation or ingestion, these are an increase in respiratory diseases such as asthma, increased risk of cancer and chronic inflammation (Blackburn & Green, 2022; Prata et al., 2020). Secondly, biological effects which include an increased risk of infections and antimicrobial resistance (Blackburn & Green, 2022). Lastly, chemical effects caused by the leaching of plastic additives and chemicals carried by microplastics from the environment, these effects include a decrease in reproduction and increased risk of cytotoxicity and oxidative stress, increased incidences of immune and neurodegenerative diseases and increased allergic reactions (Blackburn & Green, 2022; Prata et al., 2020).

2.7 Experimental design in Ecotoxicology

2.7.1 Experiments

The purpose of an experiment is to investigate a hypothesis that is tested scientifically (McLeod, 2012). In an experiment, an independent variable which is the cause is manipulated, while the dependent variable which is the effect is measured; other variables are controlled (McLeod, 2012). Experiments should always be objective, McLeod (2012), emphasises that the opinions and views of the researcher must not affect the results of a study. This is important as it validates the data and makes it less biased.

Ecotoxicology is a subdivision of toxicology that investigates the toxic effects of both synthetic and natural substances on living organisms (Selck et al., 2016). In ecotoxicology, hazardous substances are studied either by using carefully controlled laboratory experiments or observation of responses to such substances in uncontrolled settings such as the natural environment.

2.7.1.1 Field observational studies

Green et al. (2018), define field observation studies as studies conducted in uncontrolled settings such as the environment. According to Colinese (2007), field studies are important for determining the behaviour and fate of substances after they have been released into the environment. Data are collected in observational studies through surveys or monitoring to provide information on the toxicological responses under natural environmental conditions of the test organisms (Colinese, 2007). The advantage of this type of experiment is that the behaviour of the test organisms in a field experiment is likely to reflect real life because of its natural environment, therefore, there will be a higher ecological validity than in a laboratory experiment (Colinese, 2007). However, Green et al. (2018), highlights two major disadvantages of observational studies concerning the exposure of organisms to hazardous substances in the natural environment, these include that the responses to these toxic substances can be affected by numerous uncontrolled factors, and often the exposure concentrations are not accurately estimated. Furthermore, field studies require multiple sites, frequent visits and monitoring, require time, and might be far away from the laboratory, making this type of study expensive (Colinese, 2007).

2.7.1.2 Laboratory designed experiments

Colinese (2007), and Green et al. (2018), refer to a laboratory experiment as an experiment that is conducted under very controlled conditions, where measurements can be highly accurate. In this type of experiment, the researcher makes a decision about the location where the experiment will be conducted, determines the test period, picks the specimens and the number and decides on the conditions of the experiment (Aust & Kranz, 1988). Designed laboratory experiments make it possible to model the relationship between exposure and test organisms because most factors are controlled, such as temperature and constant exposure to a test substance (Green et al., 2018).

2.7.1.3 Advantages of laboratory experiments

Firstly, it is easier to replicate a laboratory experiment compared to other types of experiments, this is due to a standardized procedure used during laboratory experiments. Secondly, the exposure concentrations can be measured accurately (Green et al., 2018). Lastly, the substance being exposed

and other experimental factors allow for the modelling of the relationship between exposure and response (Green et al., 2018).

2.7.1.4 Disadvantages of laboratory experiments

Firstly, laboratory experiments cannot always accurately simulate the real-life behaviour of the test organisms, therefore, there will be low ecological validity, however, they are useful for testing relationships between different variables (Aust & Kranz, 1988). Lastly, laboratory experiments cannot always accurately simulate the real-life behaviour of the substance being tested in the environment.

2.7.2 Test types

Laboratory ecotoxicological experiments are typically classified according to the life span of the organisms involved. If the exposure duration is short compared to the test organism's life span and uses lethal concentrations, then the experiments are classified as short-term or acute (Hammer & Hammer, 2001). While, if the exposure duration is longer relative to the organism's life span and uses repeated sublethal concentrations, it is classified as long-term or chronic (Green et al., 2018).

Chronic tests are important as they may represent effects at environmentally relevant concentrations. However, there are numerous studies on the acute effects of microplastics such as; Au et al. (2015), Gray & Weinstein (2017), Vijayaraghavan et al. (2022), Wang et al. (2021), Weber et al. (2021), and Zhang et al., (2021), but there are even fewer studies on the chronic effects as compared to acute studies.

2.7.3 Considerations for experimental design in microplastics ecotoxicology

2.7.3.1 Controls

Different types of controls are used in ecotoxicological experiments. For the negative control, the test organisms are not exposed to the test substance. Whereas a positive control involves exposing a group of organisms to a substance where a test is run against a known quantity, besides the test substance known to produce an effect, for example, if the substance being investigated had to be dissolved in another substance (Harris et al., 2014).

2.7.3.2 Treatments

Generally, ecotoxicological experiments involve one or more treatment groups and a control. The treatment groups vary only in the quantity of the test substance that the test organisms are exposed to, while all other conditions are kept the same (Harris et al., 2014). Thus, besides the amount of test substance, other factors such as test species, size, age, sex, experimental conditions (such as light and

temperature) and diets should be kept the same in all treatment groups including the control (Harris et al., 2014). These principles were employed during this study.

The number of concentrations and the spacing between the concentration levels are important factors to consider when developing exposure experimental designs to adequate power to detect magnitude effects that are of biological importance. The reason for choosing test substance concentrations is to identify the concentration at which biologically important effects appear while spacing the levels of the test concentrations as closely as practical. A literature review can be used to find exposure concentrations expected to cause an effect of interest or small range-finding tests can be conducted before a larger definitive one.

2.7.3.3 Choice and spacing of test Concentrations

The idea of an "environmentally relevant concentration" is essential in the field of ecotoxicology, as it allows us to determine whether a substance is not simply a hazard (has the potential to cause harm) but poses a risk (likelihood of harm taking place) (Harris et al., 2014). It is defined as the amount of a substance or chemical that is present in the environment (Weltje & Sumpter, 2017). The purpose of environmentally relevant concentrations is for researchers to conduct research on chemicals that harm wildlife using concentrations of test chemicals that are representative of those experienced by wildlife in nature (Weltje & Sumpter, 2017).

2.7.3.4 Replication

Replication is expected in ecotoxicological experiments due to the inherent variety of measurements of living organisms (Harris et al., 2014). A replicate is the basic unit of organisation of test subjects that are in identical surrounding conditions and exposure to the test substance and are of the same taxon (Green et al., 2018). Each replicate receives a different treatment randomly to make sure that each replicate captures all the sources of variability in the experiment besides the amount of substance exposure.

CHAPTER 3: METHODOLOGY

3.1 Introduction

This chapter presents the methods and materials used in order to achieve the aims and objectives of this study. These include the microplastic polymers used and their sourcing, their preparation, the test taxa used, study endpoints, experimental design, and statistical considerations. This chapter aims to fulfil the second objective of the study ii) To contribute to the development and adaptation of methods for assessing the potential toxicity caused by microplastics on selected freshwater organisms.

3.2 Microplastic polymers

The microplastic polymers that were tested during this study are: polyvinyl chloride, polyethylene and polypropylene, pictures of these are below in Figure 3. These microplastic polymers were chosen because they are widely used, chemically different, have a range of anticipated toxicities, are commonly found in freshwater systems, have different concentrations in the environment and have different physical and chemical modes of action (Campanale et al., 2020).

Figure 3 Polypropylene, polyvinyl chloride and polyethylene microplastics.

3.3 Sourcing of microplastics for laboratory experiments

For experimental purposes, it is advised to source microplastics that have not been exposed to environmental contaminants. Microplastics that have not been exposed to environmental contaminants are called virgin microplastics. However, the use of virgin microplastics does not mirror

the actual environmental conditions as in the environment microplastics interact with other environmental factors. Virgin plastics are common and can be easily obtained from any plastic production factory or wholesale shop in South Africa. The polyethylene microplastics were sourced from unused bottle tops while the polyvinyl chloride microplastics were sourced from an unused polyvinyl chloride pipe. Lastly, polypropylene microplastics were sourced from unused polypropylene laboratory specimen plastic containers.

3.4 Preparation of microplastics for laboratory experiments

3.4.1 Granulation/shearing:

Kefer et al. (2022), state that the granulation process produces microplastics similar in shape and texture to those found in aquatic environments that have degraded naturally. To obtain appropriately sized microplastics, the first process is either granulation or shearing of the chosen plastic polymers. The process of granulation allows the whole plastic to be broken-down producing microplastics of different sizes and shapes. An instrument called a granulator can be used for this process. The size and shape of the microplastics produced during this process are determined by the blade size and speed of the granulator. This process is also commonly used during plastic recycling in South Africa. When granulators are used, it is important to follow manufacturer instructions when operating the machine and during cleaning to avoid cross-contamination. The polyethylene microplastics used during the study were produced using a granulator in which polyethylene bottle tops were granulated, these were provided for the study by the Grahamstown recycling facility.

However, granulators can be expensive, especially for small-scale production of microplastics for laboratory experiment purposes, therefore, a hand-held saw with 2 mm sized blades can be used to manually granulate microplastics, as the gradual and gentle sawing process produces small amounts of microplastics of different shapes and sizes. When a hand-held saw is used, it is important to use a clean surface and to make sure that the blades are cleaned thoroughly to avoid cross-contamination. This process was used to produce the polyvinyl chloride and polypropylene microplastics used in the study. The polyethylene bottle caps, polyvinyl chloride pipe and polypropylene containers were all separately washed thoroughly before the granulation process.

3.4.2 Sieving

For this study, a stainless-steel sieve with a mesh size of 2 mm was used to filter microplastics to attain microplastic particles less than 2 mm. The purpose of choosing a metal sieve was to prevent cross-

contamination (Mai et al., 2018). Furthermore, the sieve was regularly and thoroughly cleaned after every use to prevent cross-contamination.

3.4.3 Estimating microplastic concentrations and units of measurement

According to Harford et al. (2014), standard ecotoxicological concentrations should be reported in mg/L. However, microplastics do not dissolve in water, therefore, some laboratory studies on the toxicity of microplastics have reported concentrations in items/L or particles/L (Duis & Coors, 2016; S. Klein et al., 2018). In this study, particles/L was used to report concentrations as both Duis & Coors, (2016) and Klein et al. (2018), have stated that this way of reporting microplastic concentrations is acceptable.

It is important to precisely estimate the number of microplastics for exposure experiments. Thus, it is crucial to develop an estimation method that is not time-consuming, especially when undertaking toxicity testing. In this study, a standardised rapid estimation method was developed. Microplastics were first sieved then placed into a calibrated tube (in ml) and filled to a known volume (0.5 ml). The 0.5 ml of microplastics were then spread evenly onto a clean microscope slide, and the slide was then divided into 8 equal fields with evenly distributed microplastics across the fields. Under a compound microscope, the number of microplastic particles in four randomly selected microscope fields were then counted. The average of the number of particles in the four fields was then used to multiply the number of fields to obtain the estimated number of microplastic particles as follows:

No. of microplastics = average particles in counted microscope fields x number of microscope fields – Eq. 1.

The concentration of the microplastics can be increased or decreased by using relevant factors, e.g. 2, 4, or 5. For instance, if a volume of 1 ml of microplastics yields 2000 particles, then 2 ml (a factor of 2) should then yield 4000 particles, provided the size ranges are kept constant. Exposure concentrations are reported in particles/L through this simple estimation method. This process is illustrated in Figure 4 below.

3.4.4 Fractionation of microplastics by size and shape

Besides the polymer and size, the microplastic shape has been stated as an important factor that can affect organisms exposed to them (Ašmonaitė & Almroth, 2018; Espinosa et al., 2016). Thus, it is important that microplastics are also characterised in terms of their shape in toxicity testing. During the study, two rapid and simple shape estimation methods were developed.

The first method, which was extremely time-consuming is to count the entire particles per shape through the slide. The second method, which was straightforward was randomly picking microplastic

particles from the slides and observing their shapes under a compound microscope. When this method was used, a minimum of 50 particles were observed under a compound microscope, and thenumbers per shape were multiplied as a fraction of the total number of microplastics. The different shape types were then expressed in relative proportion (%). The shape proportions and average sizes of the microplastics are listed in Table 2 below. While the concentrations are listed in Table 3 below.

Table 2 The shape proportions and average sizes of microplastic polymers.

Microplastic Polymer	**Average size (µm)**	**Shape proportion (%)**		
		Fibres	**Spherical**	**Irregular** (non-spherical and non-uniform)
Polyethylene	29.26	65	15	20
Polyvinyl chloride	24.83	20	30	50
Polypropylene	18.43	30	20	50

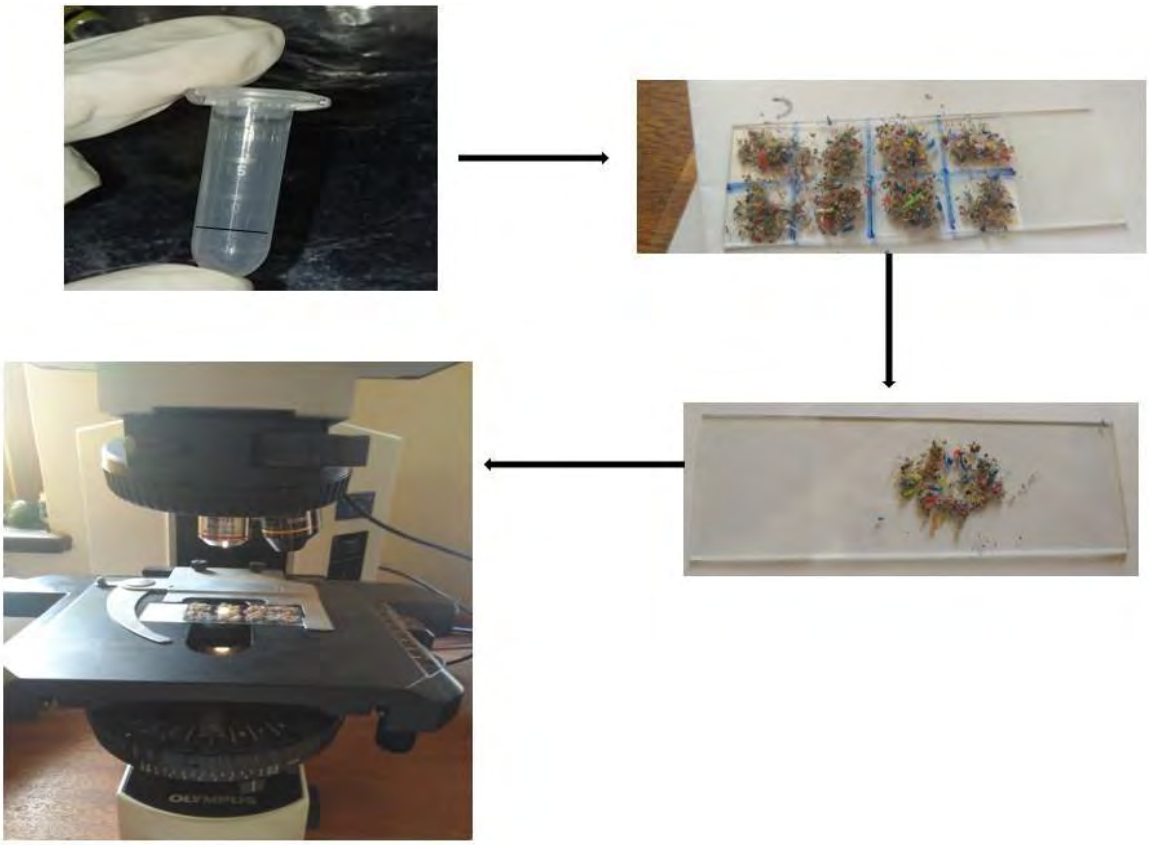

Figure 4 Schematic diagram of the process of counting, size determination and shape proportions of microplastics using a compound microscope.

3.5 Experimental design

3.5.1 Experiments

The study at hand employed laboratory-based ecotoxicological experiments to determine the potential effects of microplastics on the selectedtest organisms. The general guidelines that were employed during this study were the Organisationfor Economic Co-operation and Development (OECD) Guidelines for testing chemicals in ChronicToxicity Studies (OECD, 2018). These are referred to as a collection of about 150 of the most relevant internationally agreed testing methods used by government, industry and independent laboratories to identify and characterise potential hazards of chemicals (OECD, 2018).

3.5.2 Test types

Chromic tests were performed in this study. Chronic tests are important as they may represent effects at environmentally relevant concentrations. However, there are numerous studies on the acute effects of microplastics such as; Au et al. (2015), Gray & Weinstein (2017), Vijayaraghavan et al. (2022), Wang et al. (2021), Weber et al. (2021), and Zhang et al., (2021), but there are even fewer studies on the chronic effects as compared to acute studies. For this study only chronic tests were employed, the tests ran for 21 days, this is because thegoal was to evaluate the effects of microplastics, during prolong exposure, using environmentally relevant concentrations. 21 days was chosen for the study as the microplastic exposure period to keep the study uniform, standardized and accommodate all the test taxa as they had different life spans and growth rates. This also allowed direct comparison of the results of the different test taxa at the end of the study as they were exposed to the same microplastic polymers and concentrations for thesame amount of time.

3.5.3 Considerations for experimental design in microplastics ecotoxicology

3.5.3.1 Controls

During the study study, there was no positive control only a negative control which was dechlorinated water as no solvent was used for the tests.

3.5.3.2 Choice and spacing of test Concentrations

In this study, the test organisms were exposed to a series of widely spaced exposure concentrations of microplastics that were environmentally relevant and have been detected in the environment in accordance with studies by Au et al. (2015), Karami et al. (2017b), Klein et al. (2021), Li et al. (2021) and lastly, Li et al. (2020), who specifically focused on South African freshwater systems. The literature above was chosen as it represented the number or amount of microplastics detected in the environment that have been reported in the literature, making them environmentally relevant, therefore suitable for this study. The study concentrations were estimated using the schematic diagram in Figure 4 and are listed in Table 3 below.

Table 3 Microplastic concentrations used in the study.

Plastic Polymer	Number of microplastic particles /L
Polyethylene concentrations	0
	922
	1844
	3699
Polyvinyl chloride concentrations	0
	721
	1442
	2884
Polypropylene concentrations	0
	1566
	3132
	6264

3.5.3.3 Replication

For the duration of the study, each treatment group and control were replicated to ensure that multiple subjects are exposed to each group (Harris et al., 2014). Each concentration had three replicates , in accordance with the general laboratory guidelines employed in this study (OECD, 2018). Each replicate had two organisms, this model was selected based on the number of organisms that were available inthe IWR laboratory during the experimentation and data collection phases of this research. It should be noted that WRC report deadlines, lockdown restrictions, the selected test taxa's physiology, seasons and availability of test taxa in the field and Hatchery all had a significantly negative effect on the number of test taxa available for the experimentation process.

3.5.4 Test organisms

Microplastics are found in different components of the water column and sediments, causing organisms that inhabit these spaces to become vulnerable to the toxic effects of microplastics. For this study, the chosen test taxa were *Tilapia sparrmanii* (freshwater *Tilapia*), *Melanoides tuberculata* (freshwater snails) and *Caridina nilotica* (freshwater shrimps) as they are present in the freshwater systems around the country. *Melanoides tuberculata* were selected as they are cultured at the Institute for Water Research (IWR) laboratories, at Rhodes University, while *C. nilotica* of various sizes and ages were collected at Bushmans River and cultured at the IWR laboratories. Lastly, the *T. sparrmanii* were bought as fries at Revendall Hatchery. The test organisms were also selected because

they have different sensitivities to stress and have different feeding strategies therefore, the exposure routes will be different.

The details of the selected test taxa follow.

- *Melanoides tuberculata*. The Red-Rimmed Melania is a freshwater snail native to northern Africa and southern Asia and invasive in many parts of the world including Southern Africa. It is highly polymorphic and tall-spired and can tolerate a wide range of environmental conditions. According to Raw et al. (2016), these snails are grazers and are primary consumers. Adults and their offspring produced during the exposure period were chosen for the study. The size of the adults ranged between 3-5 mm in shell length with an average of 4.1 mm. The shell width ranged between 2.4-4 mm with an average of 2.9 mm.
- *Caridina nilotica*. This Atyid shrimp is native to freshwater from northern to southern Africa. It is of importance in several freshwater fisheries in the region. Atyid shrimps are omnivores and detritivores scavenging on carcases of dead animals such as fish and other shrimps (Ignatow et al., 1996; Mensah, 2012). Juveniles were chosen for the study, the size of the juveniles ranged between 10-12 mm in body length with an average of 10.8 mm. The body width ranged between 1-2 mm with an average of 1.6 mm.
- *Tilapia sparrmanii.* According to Froese & Pauly (2019), *T. sparrmanii* is native to Southern Africa. Genner et al. (2018), state that they are omnivorous, feeding on both animal and plant material. Additionally, they are predominantly macrophagous, nonetheless, they feed on small invertebrates and small fish (Zengeya & Marshall, 2007). Juveniles were chosen for the study, the size of the juveniles ranged between 36-60 mm in body length with an average of 46 mm. The body width ranged between 10-20 mm with an average of 15 mm, the mass ranged between 1.4-4.4 g with an average of 2.4 g.

Offspring (offspring born during the experimentation period) were chosen for *M. tuberculata* and juveniles for *C. nilotica* and *T. sparrmanii* because Bindhumol et al. (2003) and Dong et al. (2018), report that toxins are more toxic to embryos, hatchlings and juveniles and have been discovered to cause genetic default, affect growth and development as they are more susceptible than adults to the adverse effects of toxins.

3.5.5 Experimental setup

Before the beginning of the experiments, the test taxa were kept in the culture laboratory in the IWR in dechlorinated water at a laboratory temperature set to 24°C. All the test taxa were kept in 20 litre water tanks and fed twice everyday. Since *C. nilotica* were collected from Bushmans River and the *T. sparrmanii* were bought, they were depurated in the culture laboratory for two weeks, for the first week they were kept in 50 % of the water they were collected in and 50 % of dechlorinated tap water, after that the tanks were only refilled with dechlorinated water whenever the water in the tanks evaporated, gradually filling the tanks with only dechlorinated tap water. All the test organisms were then acclimated in the experiments laboratory also in the IWR for one week in separate tanks filled with dechlorinated water at a room temperature set also at 24°C. The dechlorinated water originated from the tap, it then went through dechlorination, filtration and oxygenation systems and left to settle out for a few hours. During the depuration and acclimation processes no mortalities occurred in the *T. sparrmanii*, however, mortality occurred in *C. nilotica*, with a high number of mortalities during the depuration process.

For *M. tuberculata* and *C. nilotica*, the experiments were run in 600 ml beakers filled with 350 ml of dechlorinated water with the microplastic concentrations, two of the same organisms were introduced randomly into each beaker. While for the larger juvenile *T. sparrmanii*, the experiments were run in fish tanks filled with 9 litres of dechlorinated water, a heater (set to 24°C), an aerator and the concentrations of microplastics were also placed in the tanks, the experimental design for the test taxa is shown in Figures 5 and 6 below. There were three concentrations for each microplastic polymer and a control, each concentration had three replicates and each replicate had two test organisms, two organisms instead of one were placed in each replicate to mimic the natural environment, because in the environment organisms do not live in isolation. All the test organisms were randomly selected for the different concentrations. Using a water quality multi-parameter probe, Dissolved oxygen (DO), pH, electrical conductivity (EC) and temperature were measured every five days only for monitoring purposes, the water quality variables recorded in the beginning of the study experiments and at the end of the experiments are shown in Appendix A below. The exposures were changed every five days,

the water in the tanks and beakers were removed and placed in designated container for proper disposal. The tanks, heaters, aerators and beakers were rinsed to prevent cross contamination. Fresh dechlorinated and filtered water was then poured in the tanks and beakers, followed by checking of any presence of impurities in the water such as algae, sediment or microplastics. The organisms were then placed back in their designated beaker or tanks. The counted microplastic exposures, then food were placed on the surface of the water followed by the observation of test taxa's behaviour.

T. sparrmanii and *C. nilotica* were fed TetraMin tropical flakes (batch number: 0445736305819) twice a day in the morning and late afternoon, the flakes were predominately irregular in shape and brown, yellow, orange and green in colour and are composed of different things such as fish meal, dried yeast, brown rice, shrimp meal and wheat gluten. While the *M. tuberculata* were fed Spirulina powder (batch number: 6009679325862) also twice a day in the morning and late afternoon during the exposure period, the powder also had an irregular shape and was green in colour. The powder was made from dried green algae that was cultivated in freshwater ponds. Before feeding the food was inspected for any presence of impurities such as microplastics and the food was stored in an airtight glass container.

Lights in the laboratory were switched off for 12 hours during the night (6 pm-6 am) and switched on for 12 hours during the day (6 am-6 pm).

Figure 5 Experimental design for *T. sparrmanii*.

Figure 6 Experimental design for *M. tuberculata* and *C. nilotica.*

3.5.6 Study endpoints

Objective one of this study aimed to identify ecologically relevant endpoints for microplastic exposure. These have already been discussed in chapter 2 above, based on that review, these were the endpoints chosen for this study. Table 4 below illustrates the endpoints chosen for this study for each test taxa and the frequency of monitoring of those endpoints. Mortality, reproduction, and exoskeleton shredding were monitored every day, for *Tilapia* egestion and growth were measured every five days. For *C. nilotica* and *M. tuberculata* growth was measured on the last day of the experiments. In addition, general observations during the experimentation period were recorded everyday, these can be found in Appendix B below.

Table 4 The test taxa, study endpoints monitored and frequency of monitoring on the different microplastic exposures.

Organisms	**Endpoints**	**Frequency of monitory**
T. sparrmanii: juveniles	• Growth • Egestion	Every five days
M. tuberculata: adults	• Reproduction • Growth	Every day and after 21 days
C. nilotica: juveniles	• Growth • Exoskeleton shredding	Every day and after 21 days

3.5.6.1 Reproduction

Monitoring the reproduction of *M. tuberculata* was done visually as they give birth to live offspring (Ellis-Tabanor & Hyslop, 2005), making it easy to count the number of offspring, the counting occurred while the offspring were in the beaker. Offspring were classified as those born during the exposure period. In the results section below, reproduction is represented as a cumulative number, that is the total number of offspring produced per two adults in each beaker, after 21 days of exposure and shown as number of offspring per two adult snails.

3.5.6.2 Growth

Growth was monitored by measuring the length and width of the different organisms, using a microscope for the smaller organisms such as *M. tuberculata*, measuring was from the apex of the shell to the lower tip of the aperture (length) and across for the width and recorded in mm (Figure 7) (Dickens et al., 2018; Ellis-Tabanor & Hyslop, 2005). The offspring of *M. tuberculata* were also measured at the end of the experiments, however since they appeared on different days of the exposure period their lengths and widths were not comparable.

A ruler was used to measure the growth of *T. sparrmanii* and *C. nilotica*. For *C. nilotica*, measuring started from the tip of the carapace to the end of the tail fan (length) and across for the width and recorded in mm (Figure 8), in addition to measuring, exoskeleton shredding was also monitored as it also indicates growth (Brillon et al., 2005; Gao et al., 2017). The *T. sparrmanii* was measured from the mouth to the caudal fin (whole body length) and across for the width and recorded in mm (Figure 9), additionally, they were also weighed using a scale and recorded in grams (Frimpong et al., 2014), during this process one specimen was removed from a container at a time and the measuring process was fast and accurate to prevent excessive stress to the test taxa. To differentiate between the two *T. sparrmanii* placed in each tank during the measuring process, the distinct features of the *T. sparrmanii* such as size, colour, body shape, shape of the dorsal, pectoral and caudal fins, injuries and spots on the body were noted at the beginning and during the exposures. For *T. sparrmanii* growth is presented as final size- initial size. For additional stress, the *T. sparrmanii* were handled with care and measuring was not done until they had settled down and measuring was done quickly and effectively.

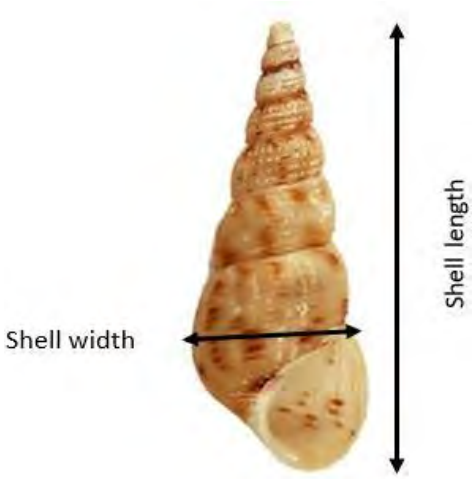

Figure 7 Measuring the width and length of *M. tuberculata*. (Source:(Pointier & Compendium, 2013))

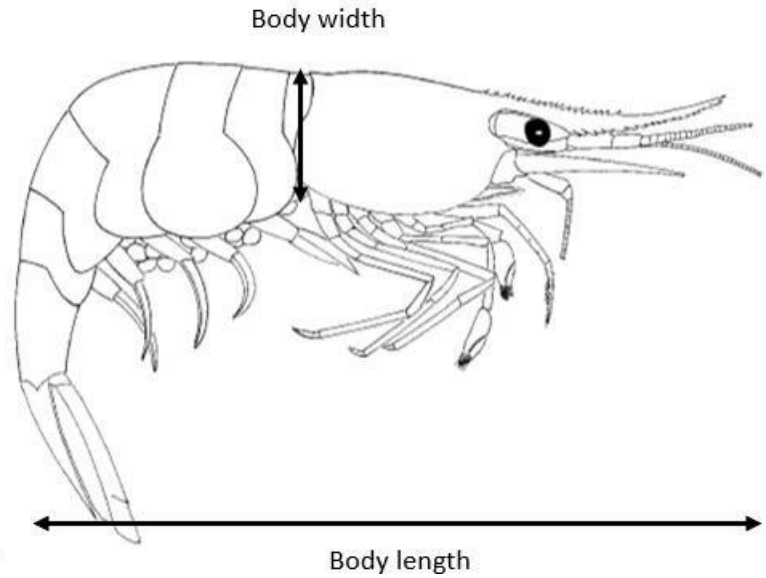

Figure 8 Measuring the width and length of *C. nilotica*. (Source:(Richard & Clark, 2005))

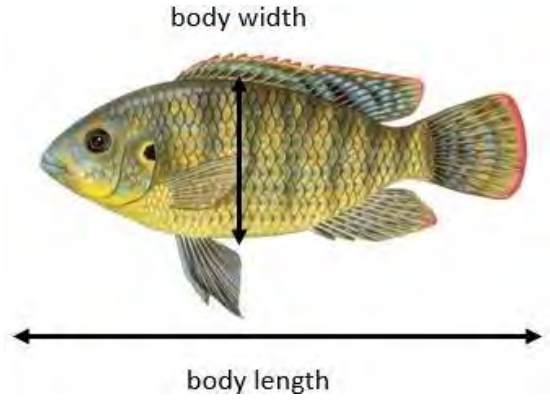

Figure 9 Measuring the width and length of *T. sparrmanii*. (Source: Susanne Weitemeyer)

3.5.6.3 Egestion

For monitoring the egestion of microplastics, faecal analysis was used to determine microplastic consumption. Faecal analysis has been used to determine microplastic consumption in different taxa, including copepods, amphipods, isopods and molluscs (Klein et al., 2021; Lusher et al., 2017). During this process faecal matter was assessed visually to quantify egested microplastics, this also assisted in indicating changes in the faecal matter such as an increase or decrease (Lusher et al., 2017). This process was used for *T. sparrmanii* because they feed immediately when being fed. They were fed TetraMin tropical flakes (twice everyday) in the presence of different microplastic concentrations, then after 15 minutes, they were rinsed thoroughly with dechlorinated water then transferred into a clean tank filled with only clean dechlorinated water, and the fish were then left in the tank for approximately two hours, after two hours the faecal matter was analysed for microplastic presence. Furthermore, the egested microplastics were only counted, the morphology and size of the egested microplastics were not relooked.

It is important to note that initially mortality was selected as a study endpoint as it is unavoidable during ecotoxicological studies (Scharmann, 2000), however during the exposure period in this study no mortalities occurred therefore, this endpoint did not form part of the results chapter.

3.6 Data analysis

Firstly, the study endpoints data were compared using linear model analysis. A One-way (Analysis Of Variance) ANOVA test was used to compare the generated statistical model with a null model to assess the model's significance. A null model is a patten-generating model which is generated with random ecological data or samples of a specific distribution. During this process some elements of the data remain constant while others are allowed to differ stochastically, this process helps to specify statistical distribution and randomization of the observed data and predict a random process without specifying all of its parameters. A p-value is the probability under a specified statistical model that a statistical summary of the data would be equal to or more extreme than its observed value in light of the whole null model, which is built around the null hypothesis, where if $p < 0.05$ indicates a significant difference between the null and the generated statistical model, while $p > 0.05$ suggests no significant difference. Where statistically significant effects were observed a Tukey Post Hoc test was run to assess the significance of differences between the group means. Boxplots were also plotted to calculate the mean, minimum, maximum, first and third quantiles, see the distribution of the data and to visualize the effects of the microplastic exposures on the different test taxa. R 4.3.0, was used for the statistics and plotting.

3.7 Ethical Consideration

Conducting research on animals requires ethical clearance. Ethical considerations include (not exclusively) the minimisation of physical, psychological, and social risks and the fulfilment of moral and legal standards. Therefore, this project was granted ethical clearance by Rhodes University Animal Research Ethics Committee (RUAREC). Ethics clearance number: 2021-0684-6375.

CHAPTER 4: RESULTS

4.1 Introduction

This chapter represents the results that were found for the study objective, specifically objective 3 which aimed to determine the potential effects of microplastics on organisms from multiple trophic levels. The data from each toxicity test was used to plot boxplots to illustrate the response of the test taxa (*Melanoides tuberculata*, *Tilapia sparrmanii* and *Caridina nilotica*) to the different microplastic polymers (polypropylene, polyethylene, and polyvinyl chloride) exposures tested during this study. The boxplots were primarily used to visualize the data, determine trends and patterns that were not always statistically indistinguishable or significant. However, to determine whether the observed trends, patterns, responses and effects were significant, statistical significance tests were conducted.

4.2 Adult *M. tuberculata* Reproduction

The boxplot in Figure 10 shows the number of offspring produced per two adult *M. tuberculata* after 21 days of exposure to a range of polypropylene concentrations. The highest average number of produced offspring, with a median of 2 was recorded in 1566 particles/L. While the lowest median was 1, which was observed in the control, 3132 particles/L and 6264 particles/L concentrations. The boxplot illustrates no clear trend of *M. tuberculata* reproduction with increasing concentrations of polypropylene.

In the polyvinyl chloride exposures presented in Figure 10, in the following concentrations: 1442 particles/L, 2884 particles/L and the control, the recorded median was 1 offspring per two adult snails. Furthermore, the median, maximum value and minimum value were all equal for the concentration of 1442 particles/L. The lowest median of 0 was recorded in the concentration of 721 particles/L. These results do not indicate any clear response of adult *M. tuberculata* after 21 days of exposure concerning reproduction. These findings are similar to the reproduction results of polypropylene above in Figure 12A.

The reproductive success of adult *M. tuberculata* exposed to various polyethylene concentrations for 21 days is presented in Figure 10. Just like the results for the reproduction response in polypropylene and polyvinyl chloride exposures, the boxplot once again shows no clear response of adult *M. tuberculata* to the polyethylene exposures in the reproduction endpoint. In the control and 1844 particles/L the highest average number of offspring per two adults, was determined to be 1, while in concentrations 922 particles/L and 3699 particles/L, the median was equal to 0, furthermore in the

concentration 3699 particles/L the median, minimum value and maximum value were all equal to 0 as there was no recorded reproduction for the duration of the study.

Even though the results in Figure 10 illustrate differences in the microplastic exposures, all the boxplots did not indicate any clear responses. Furthermore, significance testing showed that for the adult *M. tuberculata* reproduction test, the assigned model was not different to the null model (p=0.901), consequently, no statistically significant changes in adult *M. tuberculata* reproduction could be linked to the differing microplastic exposures after 21 days of exposure.

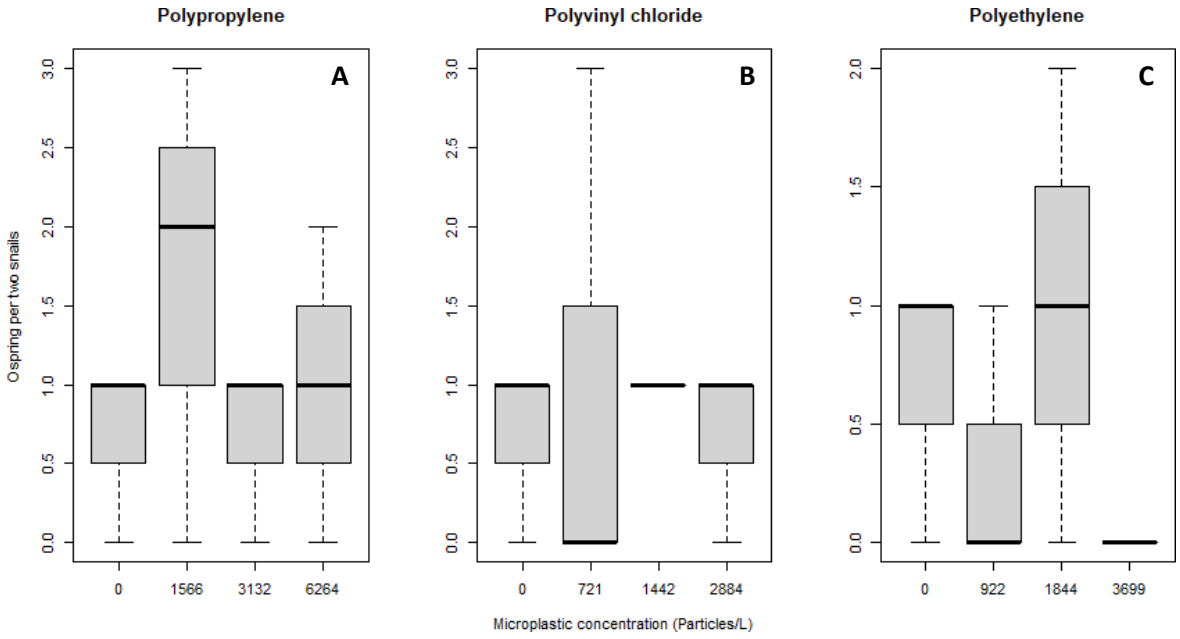

Figure 10 Boxplot illustrating offspring produced per two adult *M. tuberculata* after 21 days of exposure to different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.3 Adult *M. tuberculata* growth-shell length

After a 21-day exposure to polypropylene microplastics, the adult *M. tuberculata* shell lengths are presented in Figure 11A. In 1566 particles/L the highest median of 5 mm was recorded, followed by 6264 particles/L with 4.6 mm, lastly in the control and 3132 particles/L concentrations the median was 4.5 mm. All the test concentrations for polypropylene had an average shell length that was higherthan the initial average length of 4.1 mm indicating that on average growth did occur, however, no clear response to the varying concentrations of polypropylene was detected in the boxplot,

For the test assessing the response of adult *M. tuberculata* growth (body length) to different concentrations of polyvinyl chloride. No clear growth response of adult *M. tuberculata* to the various microplastic concentrations was observed in the boxplot in Figure 11B. The highest median of 4.8 mm was recorded in 721 particles/L., while in the rest of the polyvinyl chloride, the median was equal to 4.5 mm. importantly, all the medians for the polyvinyl chloride exposures were higher than the initial average shell length, which was 4.1 mm, showing that growth did occur during the 21-day exposure period.

In Figure 11C is the boxplot illustrating the growth response of *M. tuberculata* after a 21-day exposure to four concentrations of polyethylene microplastics. The plot doesn't suggest any apparent response to the differing polyethylene concentrations. In the control, the lowest median of 4.5 mm was recorded, followed by 1844 particles/L with 4.6 mm, then 922 particles/L with 4.8 mm, and lastly, in the highest polyethylene concentration of 3699 particles/L, the median was 4.9 mm, which was the highest. It is important to note that all the medians were greater than the initial average shell length.

For the test that assessed the relation between microplastic exposures and *M. tuberculata* growth measured by shell length after a 21-day exposure, all the boxplots results showed no apparent trend with increasing microplastic exposures, it is therefore not surprising that no statistically significant effects were observed as the statistical model employed was not distinct from the null model ($p=0.246$).

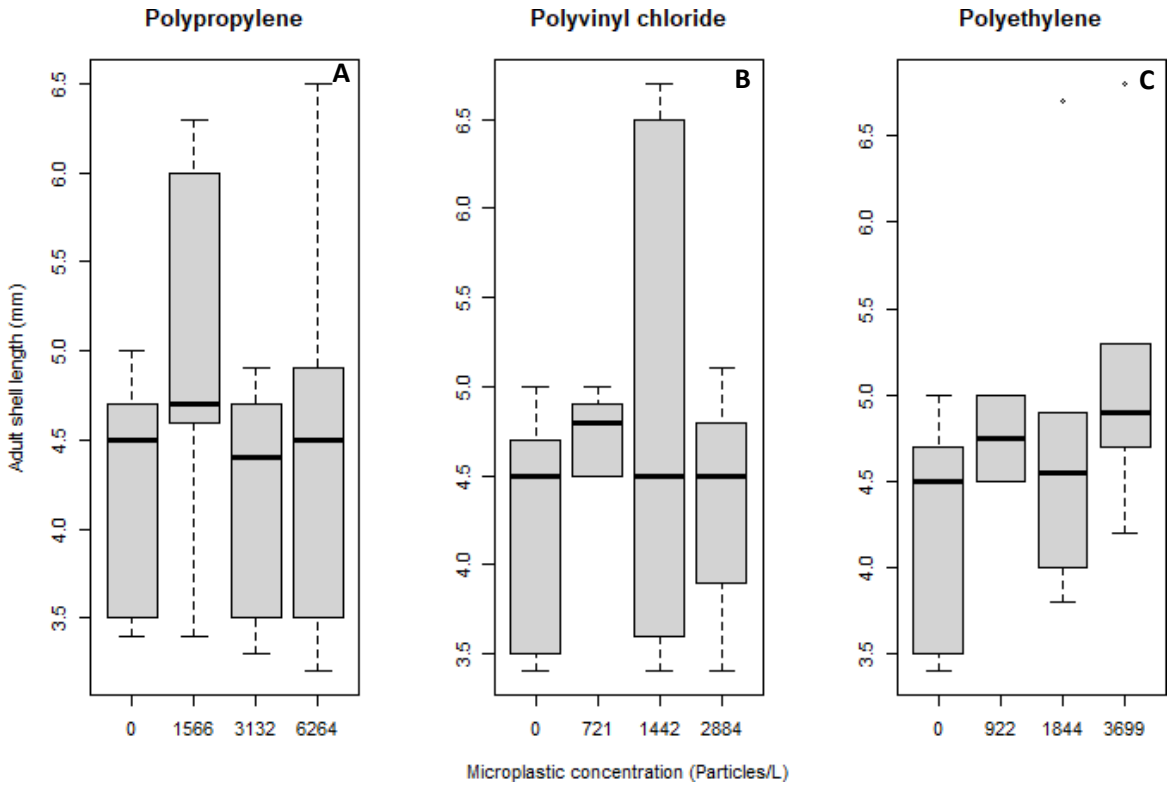

Figure 11 Boxplot illustrating growth as shell length in adult *M. tuberculata* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.4 Adult *M. tuberculata* growth-shell width

The growth response of adult *M. tuberculata* measured by shell width after 21 days of exposure to increasing concentration polypropylene is presented in Figure 12A. In 6264 particles/L the lowest median of 3 mm was recorded, followed by the control and 3132 particles/L concentrations where the median was equal to 3.1 mm, and lastly, 1566 particles/L with the highest median of 3.5 mm. These results indicate that no clear results were detected on adult *M. tuberculata* growth after 21 days in different polyethylene concentrations even though all the medians were above the initial average shell width of 2.9 mm. These findings were similar to those of the growth response measured by shell length in Figure 11A above.

The growth response measured by the shell width of juvenile *M. tuberculata* in relation to the different concentrations of polyvinyl chloride is shown in Figure 12B. Just like most of the results in this chapter, no clear response to the varying microplastic concentrations was observed in this figure. In the highest polyvinyl chloride concentration (2884 particles/L) the lowest median of 3 mm was recorded, followed by the control with a median of 3.1 mm, then 1442 particles/L with 3.2 mm, and lastly, in the 721

particles/L concentration, the median was 3.3 mm which was the highest. It is important to note that all these figures were above the initial average shell width of 2.9 mm and these results are like those of growth measured by shell length in Figure 11B above.

For the test measuring the growth of *M. tuberculata* measured by shell width in Figure 12C. The figure suggests no clear response to the polyethylene microplastic exposures. These findings were similar to those in Figures 12A and 12B showing growth measured as shell width. The lowest media (3.1 mm) was recorded in the control, followed by 1844 particles/L with 3.2 mm and then 922 particles/L with 3.3 mm and 3699 particles/ L which was the highest polyethylene concentration, the highest median of 3.5 mm was observed.

As in the test on *M. tuberculata* measuring growth by shell length above, the overall statistical model was not different from the null model (p=0.369), as a result, no statistically significant effects of microplastics on *M. tuberculata* growth measured by shell width were recorded after 21 days of exposure to various microplastic concentrations. Furthermore, the variation in the data was high, therefore, in the boxplots no clear patterns with increasing microplastic exposures were seen.

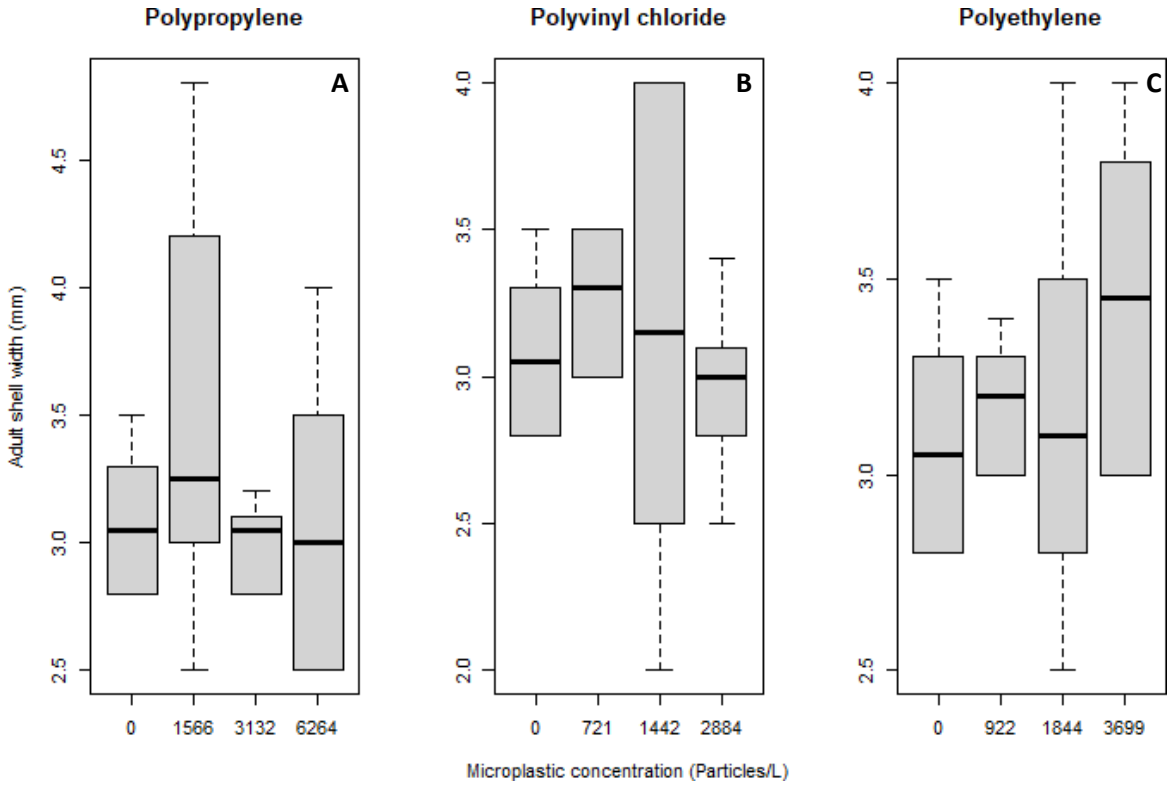

Figure 12 Boxplot illustrating growth as shell width in adult *M. tuberculata* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.5 Juvenile *T. sparrmanii* growth-length

The body lengths of juvenile *T. sparrmani* exposed for 21 days to polypropylene microplastics are displayed in Figure 13A. The figure shows that in the control the lowest average growth was recorded at 6 mm after. The figure also suggests that the average growth increased with increasing concentrations of polypropylene, with both 1566 particles/L and 3132 particles/L concentrations recording an average growth of 7 mm, while in the highest concentration (6264 particles/L) the highest median of 8 mm was recorded.

Figure 13B, shows the growth response of juvenile *T. sparrmanii* measured by body length after 21 days of immersion in varying polyvinyl chloride concentrations. According to the boxplot, in the concentration of 721 particles/L, the median was the highest at 7.8 mm, followed by the control with a median of 6 mm, in both 1442 particles/L and 2884 particles/L concentrations the median values were almost equal at 5.5 mm and 5.4 mm respectively. From the boxplot, there isn't a clear trend found of juvenile *T. sparrmanii* growth to the different polyvinyl chloride exposures.

The boxplot showing the growth of juvenile *T. sparrmanii* after 21 days of exposure to various concentrations of polyethylene measured by body length is in Figure 13C. 1844 particles/L had the highest average growth of 8 mm, followed by the control at 6 mm, then 922 particles/L at 5 mm and lastly 3699 particles/L at 4.5 mm**,** it is important to note that this concentration also had the lowest minimum value of 0 mm, due to an injured *T. sparrmanii*. Just like in the polyvinyl chloride concentrations, the boxplot did not indicate any apparent response to the different polyethylene concentrations.

In the *T. sparrmanii* growth test measured by body length, the test model generated was significantly different to the null model (p=0.00099), therefore, statistically significant impacts of microplastics on *T. sparrmanii* growth by body length were detected. Upon further inspection, polypropylene yielded a p-value of 0.0702 and polyvinyl chloride p=0.215 (showed no clear response), however, statistical significance was observed in the polyethylene exposure where the p-value was 0.00287. Perhaps with greater replication a significant growth response could have been observed also in the polypropylene and polyvinyl chloride exposures.

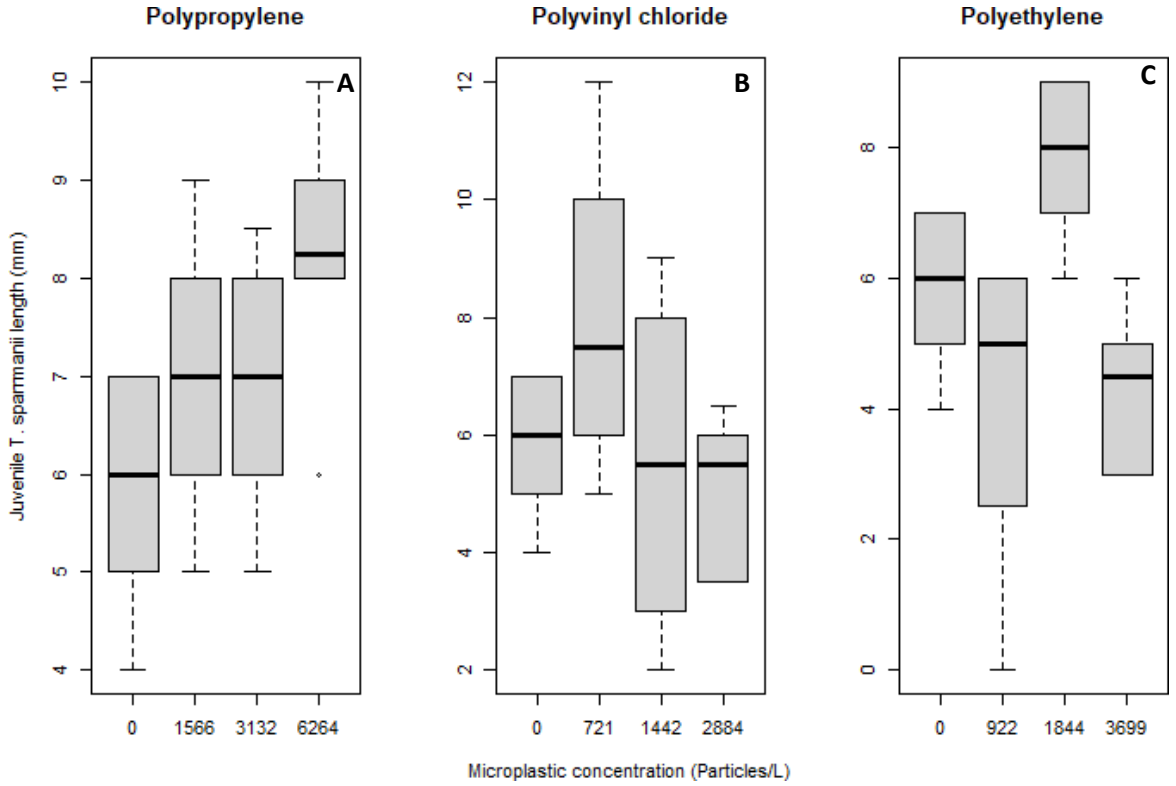

Figure 13 Boxplot presenting growth as body length in juvenile *T. sparrmanii* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.6 Juvenile *T. sparrmanii* growth-width

The boxplot in Figure 14A displays the growth response measured by the body width of *T. sparrmanii* after 21 days of exposure to a range of polypropylene microplastic concentrations. The 1566 particles/L concentration displayed the highest average growth of 3.5 mm, followed by 3132 particles/L with a median of 3.3 mm, then 6264 particles/L with a median of 2.8 mm, and lastly, the control with a median of 2.8 mm. Furthermore, the figure suggests no apparent response to the different polypropylene concentrations, unlike the results in Figure 13A above showing growth by body length which suggested an increase in average growth with increasing concentrations of polypropylene.

The body widths of juvenile *T. sparrmanii* after 21 days of exposure to different polyvinyl chloride solutions are presented in Figure 14B. In the control the lowest median of 2.8 mm was observed, while in the following three concentrations: 721 particles/L, 1442 particles/L and 2884 particles/L, the medians were almost equal; 3 mm, 3 mm and 3.1 mm respectively. Importantly, in the 721 particles/L and 2884 particles/L concentrations, the maximum, minimum and median values were all equal and

in each concentration were two outliers. However, just like the previously reported results, the figure displays no trend with increasing polyvinyl chloride microplastic exposures, these findings are similar to those observed in Figure 13B above which showed growth measured by body length.

The boxplot showing the growth of juvenile *T. sparrmanii* after 21 days of exposure to various concentrations of polyethylene measured by body width is in Figure 14C. In the control and 1844 particles/L concentrations, the median was equal to 2.8 mm which was the highest, followed by 3699 particles/L with 2.1 mm and lastly 922 particles/L with the lowest median of 1.5 mm and one outlier. These findings show no clear response to the different concentrations of polyethylene, these observations are similar to those in Figure 13C above which measured growth by body length.

Similar to the test on *T. sparrmanii* growth measured by body length above, the overall statistical model generated was significantly different to the null model ($p=0.00921$), indicating that there were statistically significant impacts of microplastics detected on *T. sparrmanii* growth measured by body width caused by the varying concentrations of microplastics. Further analysis showed that polypropylene ($p=0.592$) and polyvinyl chloride ($p=0.553$) exposures yielded no significant response, however, polyethylene ($p=0.0483$) indicated that the plastic polymer exposures had statistically significant effects on *T. sparrmanii* growth measured by body width. Possibly, with more test taxa in the exposures and greater replication a significant growth response might be observed also in the polypropylene and polyvinyl chloride exposures.

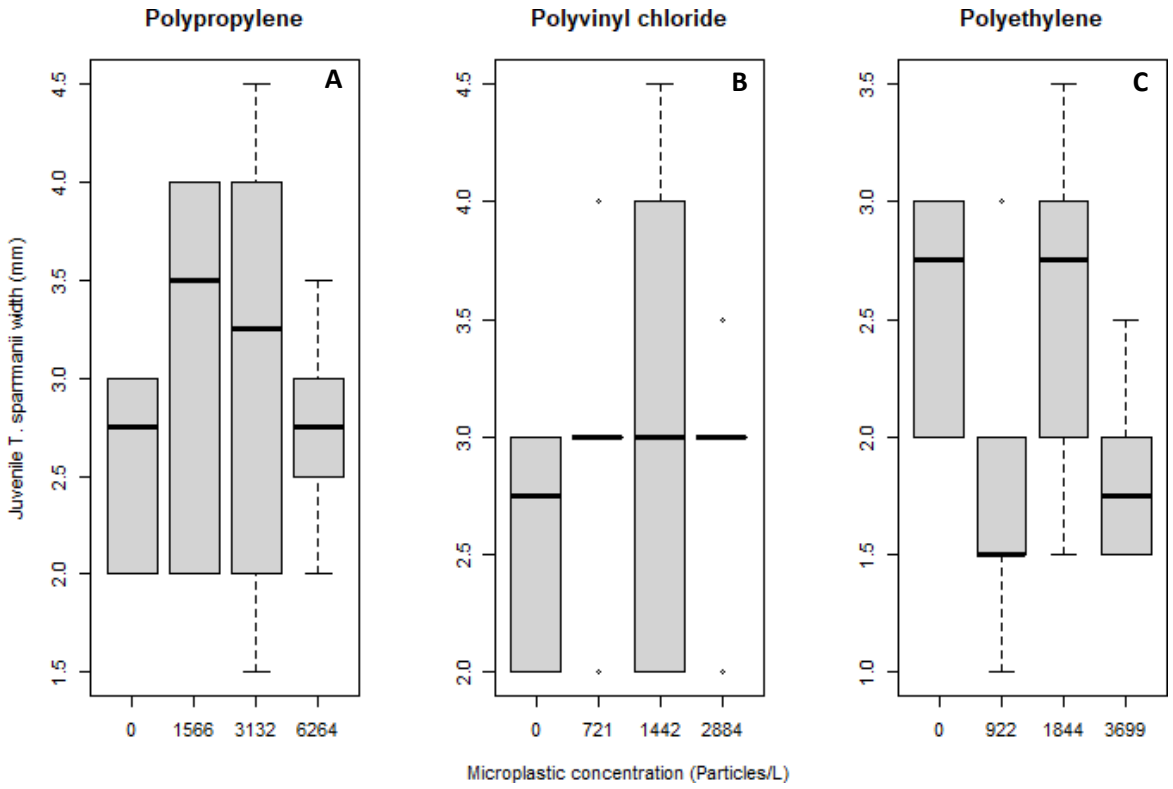

Figure 14 Boxplot showing growth as body width in juvenile *T. sparrmanii* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.7 Juvenile *T. sparrmanii* growth-mass

In Figure 15A is the growth (mass) response of juvenile *T. sparrmanii* in differing concentrations of polypropylene after an exposure period of 21 days. The highest median (1 g) was recorded in the highest polypropylene concentration (6264 particles/L), followed by 1566 particles/L with 0.9 g, then 3132 particles/L with 0.6 g and lastly the control with the lowest, which was 0.5 g. The results in the boxplot indicate no apparent trends with increasing concentrations of polypropylene, these findings are similar to those of body width growth in Figure 14A above.

For the test assessing the response of juvenile *T. sparrmanii* growth (mass) to increasing concentrations of polyvinyl chloride. The Figure in 15B demonstrates no apparent response of *T. sparrmanii* to the various concentrations of polyvinyl chloride however, this is no surprise as both the body length and width in Figures 13A and 14A the polypropylene results in Figure 15A above which also displayed no clear response to the varying concentrations of microplastics. In 721 particles/L concentration, the highest median of 0.7 g was displayed, followed by 1442 particles/L with 0.56 g and lastly, in both the control and 2884 particles/L concentrations the median of 0.5 g was recorded.

After 21 days of exposure to polyethylene microplastics, juvenile *T. sparrmanii* body masses are presented in Figure 15C. The highest median of 0.85 g was at 1844 particles/L, followed by 922 particles/L with a median of 0.6 g, in the control and the highest polyethylene concentration (3699 particles/L) the lowest medians were recorded at 0.5 g and 0.55 g respectively. Just like most of this study's results, the boxplot displayed no clear response of *T. sparrmanii* growth to the differing concentrations of polyethylene.

Although the chosen microplastic exposures had a significant effect on growth evaluated by both body length and width in juvenile *Tilapia*, it is surprising that growth measured by body mass yielded different results. Additionally, the statistical model was not distinguishable from the null model (p=0.0694), indicating that the varying microplastic exposures had no statistically significant effect on growth measured by body mass in juvenile *Tilapia*. However, with wider test concentration ranges of microplastics and more replicates, significant effects could have been observed.

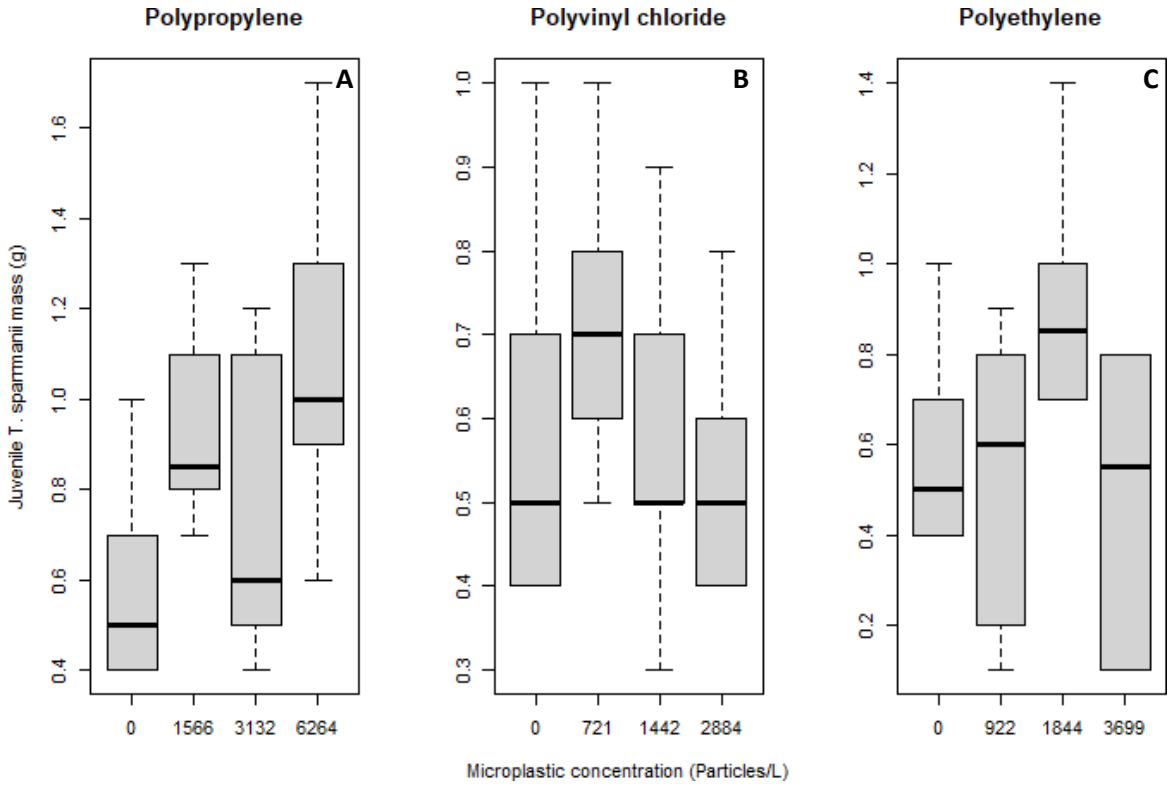

Figure 15 Boxplot displays growth as body mass in juvenile *T. sparrmanii* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.8 Particles egested by juvenile *T. sparrmanii*

In Figure 16A is the boxplot displaying the total number of egested polypropylene microplastics by juvenile *T. sparrmanii* after exposure to polypropylene microplastics for 21 days. In 6264 particles/L which was the highest concentration, the highest average number of egested microplastics was recorded with a median of 4 microplastics with the minimum and the maximum values also equal to 4. Followed by the 3132 particles/L and 1566 particles/L concentrations where the median was equal to 2 egested microplastics, however, 1566 particles/L had a lower minimum value. The boxplot suggests that the number of egested microplastics increased with increasing concentrations of polypropylene.

The boxplot in Figure 16B displays the changes in *T. sparrmanii* microplastic egestion after a 21-day to different concentrations of polyvinyl chloride microplastics. Just like the polypropylene results above, the boxplot suggests that the total number of egested microplastics increased with increasing concentrations of polyvinyl chloride, with the highest polyvinyl chloride concentration (2884 particles/L) displaying the highest median of 4, followed by 1442 particles/L with 3 and then lastly 721 particles/L with the lowest median of 2.

Figure 16C presents the accumulated microplastic particle egestion by juvenile *T. sparrmanii* in varying concentrations of polyethylene after 21 days of immersion. Just like in the egestion results for polypropylene and polyvinyl chloride above, the boxplot also suggests that the average number of egested polyethylene microplastics increased with increasing concentrations, furthermore, the raw data and the boxplot both illustrate that egestion of microplastics was higher in polyethylene exposures compared to polypropylene and polyvinyl chloride exposures. In the highest concentration of 3699 particles/L, the highest median of 7 was recorded, followed by 1844 particles/L with 5, and lastly 922 particles/L with the lowest average number of egested microplastics of 4.

The final *T. sparrmanii* test was concerning the number of microplastic particles egested by juvenile *T. sparrmanii* after a 21-day exposure to different microplastic exposures. The boxplots suggested an increase in the number of egested particles with increasing microplastic concentrations for all the chosen plastic polymers, furthermore, the statical model employed was distinct from the null model ($p=0.0421$), indicating statistical significance in this study endpoint. The number of egested microplastic particles was higher in polyethylene (0.0104), while for polypropylene ($p=0.189$) and polyvinyl chloride ($p=0.158$) the observed effects were not statistically significant. Perchance with greater replication and higher microplastic concentrations significant effects for polypropylene and polyvinyl chloride could have been detected.

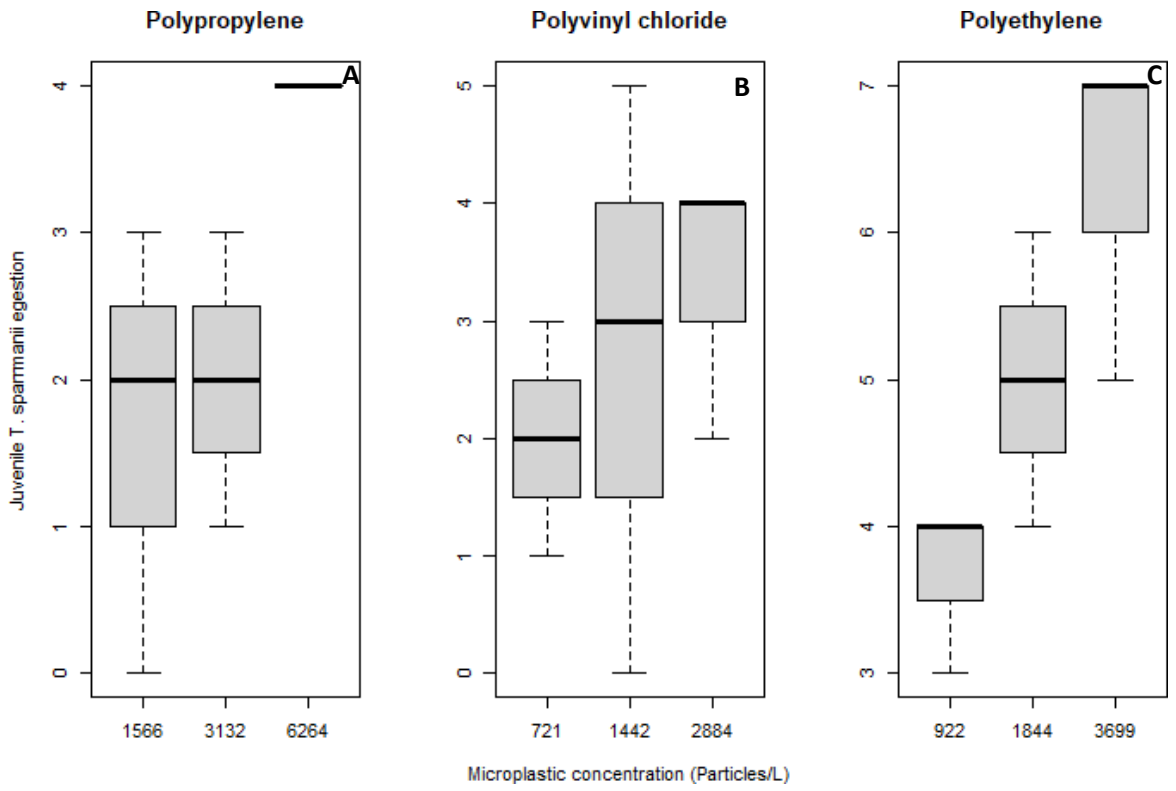

Figure 16 Boxplot illustrating the number of egested microplastic particles by juvenile *T. sparrmanii* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.9 Juvenile *C. nilotica* growth-exoskeletons shredded

In the test concerning juvenile *C. nilotica* growth measured by exoskeletons shredded in Figure 17A. The results suggest that exoskeleton shredding decreased with increasing concentrations of polypropylene, with the control recording the highest average number of shredded exoskeletons after 21 days of exposure (3), in the rest of the polypropylene concentrations the median was equal to 2.

Figure 17B displays the growth response of juvenile *C. nilotica* measured by exoskeleton shredding after a 21-day exposure in varying polyvinyl chloride concentrations. The results are similar to those of polypropylene above as the boxplot suggests a decrease in the average number of exoskeletons shredded with increasing concentrations of polyvinyl chloride. Once again, in the control the highest average number of exoskeletons shredded (3) was recorded, followed by 721 particles/L with a median of 2.3, furthermore, the lowest median of 1.7 was recorded at the concentrations 1442 particles/L and 2884 particles/L.

Figure 17C presents the boxplot illustrating the growth of juvenile *C. nilotica* measured by exoskeletons shredded after 21 days of exposure to a range of polyethylene concentrations. The highest median (3) was at the control, followed by 922 particles/L with a median of 2.9, then both 1844 particles/L and 3699 particles/L with 2. These results indicate no apparent response of juvenile *C. nilotica* growth to the increasing polyethylene concentrations, unlike the results for polypropylene and polyvinyl chloride above in Figures 17A and 17B which suggested a decrease in the number of shredded exoskeletons with increasing microplastic concentrations.

In the *C. nilotica* growth test measured by exoskeleton shredding, just like *M. tuberculata* growth test results, no statistically significant impacts were detected after the exposure period of 21 days. Even though at times the results in the boxplots suggested an effect and at times showed no clear response, the overall statistical model was not different from the null model (p=0.241).

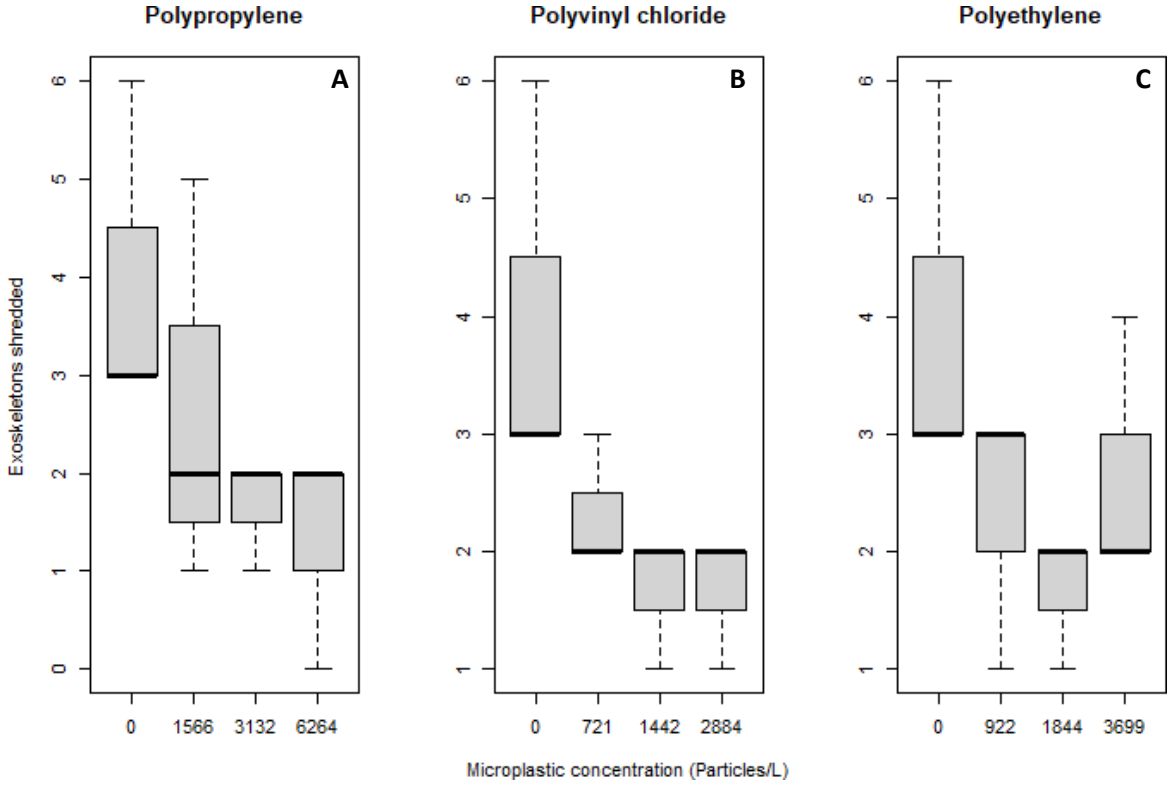

Figure 17 Boxplot presenting the number of exoskeletons shredded by *C. nilotica* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.10 Juvenile *C. nilotica* growth-length

The boxplot in Figure 18A displays the body lengths of *C. nilotica* after 21 days of exposure to varying concentrations of polypropylene. The figure shows that in the concentration of 3132 particles/L, the lowest median of 11.1 mm was recorded, followed by 1566 particles/L with 11.7 mm, then the control with a median of 11.8 mm, while in the highest polypropylene concentration (6264 particles/L) the highest median of 11.9 mm was displayed. Even though there was no clear pattern shown by the C. nilotica to the varying concentrations, it is important to note that the average initial body length of juvenile *C. nilotica* was 10.8 mm, therefore all the values above indicate that there was an average increase in body length after 21 days of exposure.

In Figure 18B is the boxplot illustrating the body lengths of *C. nilotica* immersed for 21 days in multiple concentrations of polyvinyl chloride. The average initial body length of juvenile *C. nilotica* was 10.8 mm, for all the polyvinyl chloride concentrations after 21 days the average body lengths (median) were higher than the initially recorded average body length. In the highest polyvinyl chloride (2884 particles/L) the highest average body length of 12.8 mm was recorded, followed by 721 particles/L with 12.5 mm, while the control and 1442 particles/L concentrations both displayed the lowest median of 11.8 mm. However, just like the results in Figure 17B, the figure demonstrated no apparent response of *C. nilotica* body lengths to the range of polyvinyl chloride microplastic concentrations.

The body length of *C. nilotica* after 21 days of exposure to a range of polyethylene microplastic concentrations is plotted in Figure 18C below. Just like most results in this study, the figure shows no clear response of *C. nilotica* body lengths in varying concentrations of polyethylene. However, the medians for all the concentrations were higher than the initial average growth recorded at the beginning of the study. The concentration of 922 particles/L displayed the lowest median of 11.3 mm, followed by the control with 11.8 mm, then 1844 particles/L with 12.5 mm, and lastly, in 3699 particles/L the highest median (12.6 mm) was recorded.

Microplastic exposure had no statistically significant effect on *C. nilotica* growth (measured by body length), unsurprisingly the boxplots showed no clear responses to the varying microplastic exposures and growth by body length yielded the same results. Furthermore, after the 21-day exposure, the statistical model was not different from the null model ($p=0.354$), indicating that none of the observations in *C. nilotica* body lengths were statistically significant.

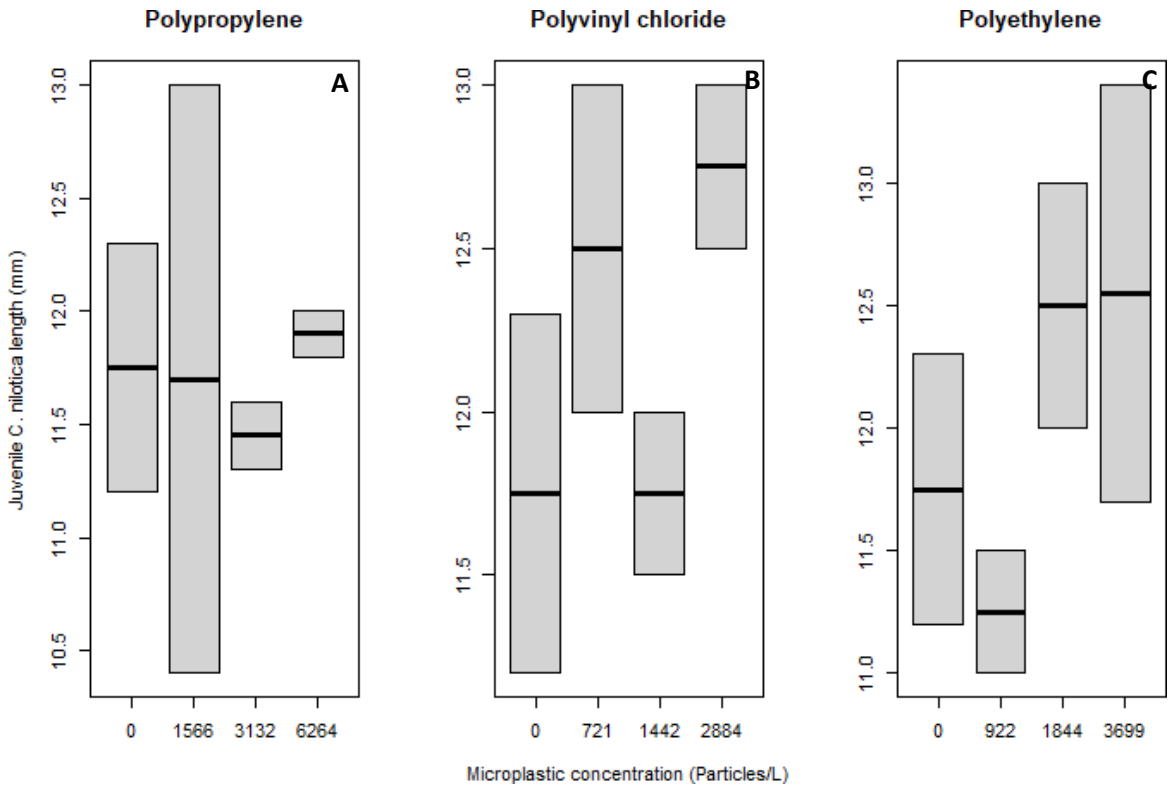

Figure 18 Boxplot illustrating growth as body length in *C. nilotica* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.11 Juvenile *C. nilotica* growth-width

In Figure 19A, the results of *C. nilotica* growth presented by body width after 21 days of exposure to various polypropylene concentrations are displayed. The results illustrate that the lowest median of 1.8 mm was at the control, while in all the other polypropylene concentrations the median was equal to 2 mm. It is important to note that for 3132 particles/L the minimum value, maximum value and median were all equal to 2 mm. The average width at the beginning of the study was 1.6 mm, therefore this indicates that growth did occur after 21 days of exposure, however, no clear response in the boxplot was detected to the varying concentrations. The findings were similar to those of body length above.

For the polyvinyl chloride body width growth test, the results are plotted in Figure 19B. However, just like with the results of polypropylene above and those of growth measured by body length (Figure 18A), no clear pattern of *C. nilotica* to the varying polyvinyl chloride concentrations was observed. The results illustrate that after 21 days of experimentation, The highest average width (2.5 mm) was at the concentrations 721 particles/L and 2884 particles/L, followed by 1442 particles/L with 2 mm and lastly

the control concentration displayed the lowest median of 1.8 mm. For the concentrations 721 particles/L and 1442 particles/L, the medians were equal to both the minimum and maximum values. All the medians above were greater than the initial average body width of 1.6 mm.

In the test relating to polyethylene microplastic exposures and growth of juvenile *C. nilotica*, measured by body width after 21 days of exposure. The boxplot in Figure 19C displayed no clear trend to the increasing levels of polyethylene concentrations, these findings are similar to those of growth measured by body length in Figure 20C above. The lowest median of 1.3 mm was at 922 particles/L which was even lower than the initial average body width of 1.6 mm, while in the control the median was 1.8 mm, then in 1844 particles/L a median of 2.3 mm was recorded and lastly, the highest median (2.6 mm) was recorded at the highest polyethylene concentration (3699 particles/L).

The final test assessed growth (body width) in juvenile *C. nilotica* after a 21-day exposure period. Predictably, given the results from tests using other measures of growth above (body length and exoskeleton shredding) detected no clear responses to the various microplastic exposures, the results for body width were similar. Likewise, once again the statistical model was not distinct from the null model ($p=0.563$), indicating no statistically significant impacts of the microplastic exposures *to C. nilotica* growth (body width) could be detected.

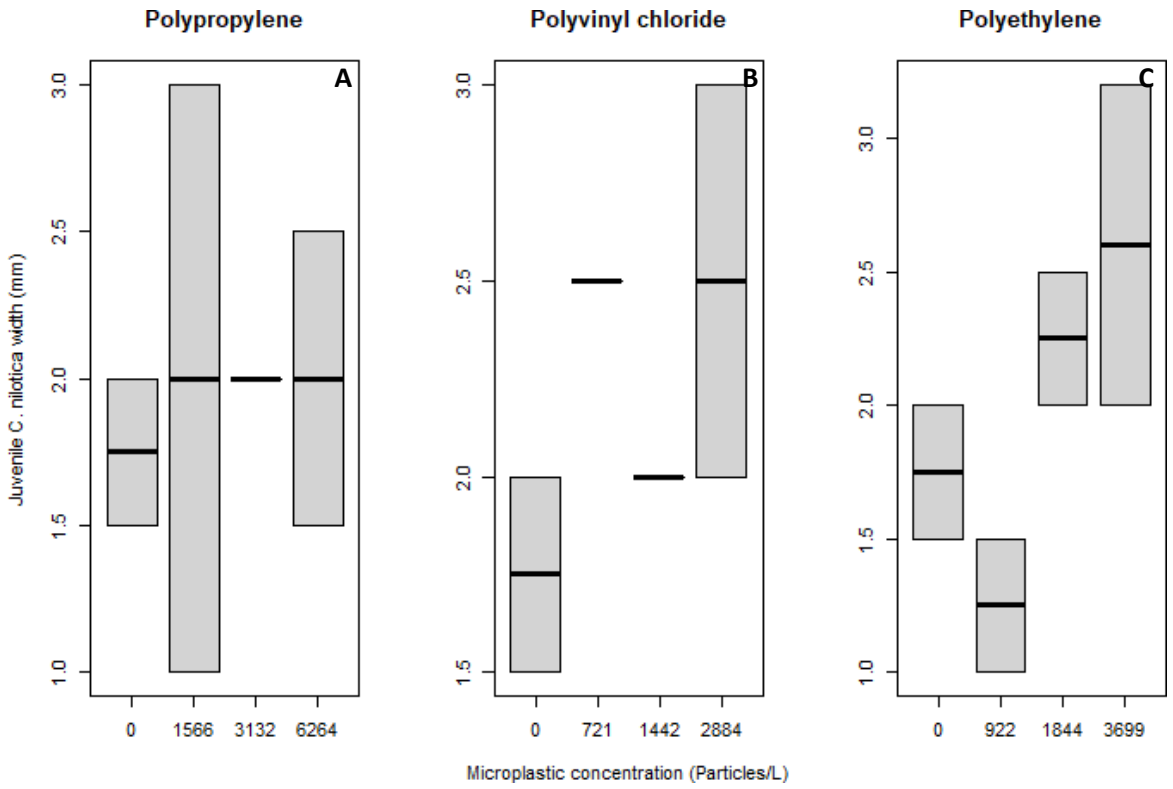

Figure 19 Boxplot illustrating growth measured by body width in *C. nilotica* after 21 days of exposure to the different concentrations of polypropylene, polyvinyl chloride and polyethylene microplastics.

4.12 Other observed effects

4.12.1 Mouth Inflammation

Although inflammation was not a study endpoint, it is important to note that it was observed during the duration of the study. Jaw damage and inflammation were observed in the study in two *Tilapias* in two separate exposures as seen in Figure 20 below.

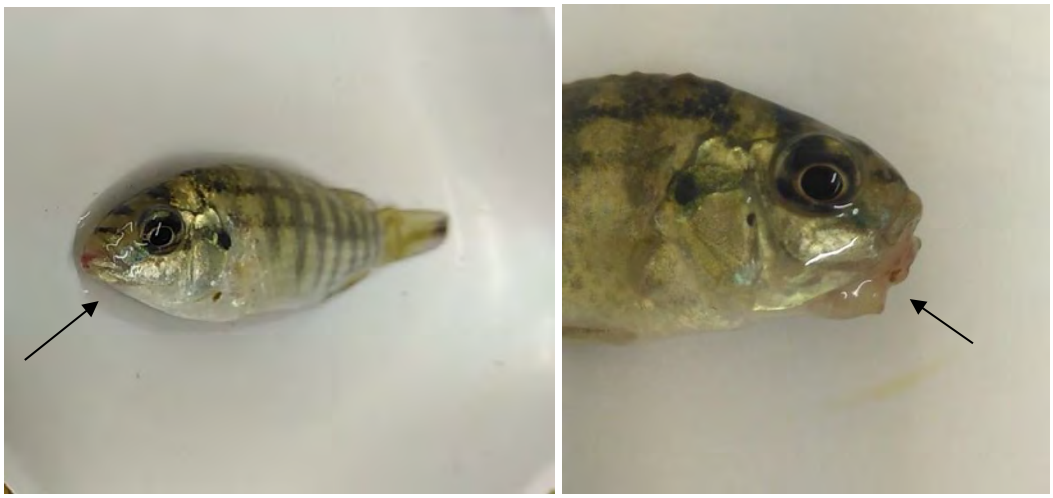

Figure 20 Juvenile *T. sparrmanii* mouth inflammation.

4.12.2 Aggression observations

Although aggression was not a study endpoint. In the current study, *T. sparrmanii* were observed (Appendix B) being aggressive, territorial and chasing each other resulting in a caudal fin injury in a *T. sparrmanii* as seen in Figure 21 below. However, the study did not further investigate how aggression and this injury might have affected the study endpoints such as growth.

Figure 21 Juvenile *T. sparrmanii* caudal fin injury.

CHAPTER 5: DISCUSSION

5.1 Introduction

This chapter discusses and evaluates the objectives of the study and the results that were presented in the chapter above and the study's limitations. It also explores the toxicity of the selected microplastic polymers on freshwater systems using the three selected test taxa.

5.2 *M. tuberculata* Endpoints

5.2.1 Reproduction

In the current study, in all the microplastic exposures no statistically significant effects on *M. tuberculata* reproduction were observed. These findings were similar to those of Imhof & Laforsch (2016), who also recorded no significant effects on reproduction when they exposed *Potamopyrgus antipodarum* to a mixture of microplastics which included polyethylene and polyvinyl chloride. However, it is difficult to make a direct comparison to the study by Imhof & Laforsch (2016), as the authors did not quantify the number of microplastics used during the study and the microplastics were mixed with the food of the gastropods which was not done for this study.

Weber et al. (2021), in their study on the freshwater gastropod *Lymnaea stagnalis*, also observed similar findings in both environmentally realistic concentrations and very high concentrations of microplastics. They also confirmed that the gastropods ingested microplastics (polystyrene) of different shapes and sizes. Weber et al. (2021), stated that in their study ingestion of microplastics had no effect on the reproduction rate and survival of young *L. stagnalis* and gastropods in general. Consequently, Weber et al. (2021), discovered that microplastic ingestion did not significantly affect the reproduction and survival of the test taxa.

Additionally, in toxicity studies, the lack of observed effects of microplastics on gastropods even at very high concentrations may indicate a potentially high-stress tolerance of gastropods, as they can retreat into their shells when exposed to microplastics and other environmental disturbances (Veiga et al., 2016; Weber et al., 2021). Although behaviour was not one of the study endpoints, this phenomenon was also observed in this study in *M. tuberculata* during the initial stages of the experiments. The findings by Veiga et al. (2016), and Weber et al. (2021), mentioned above could be the reason why no significant effects on *M. tuberculata* reproduction were observed during the current study.

Although, this study and the studies by Imhof & Laforsch (2016) and Weber et al. (2021), showed no significant effect on different freshwater gastropods' reproduction after exposure to different microplastic suspensions, Au et al. (2015), caution that exposure and ingestion of microplastics can result in a decrease in the reproduction of the exposed organisms.

5.2.2 Growth-adults

The study results showed no statistically significant effects owing to the different microplastic exposures on the growth of adult *M. tuberculata*. The study findings are similar to a study conducted by Imhof & Laforsch (2016), on the effect of a mixture of microplastics which included polyethylene and polyvinyl chloride on *P. antipodarum,* the adults were exposed for eight weeks while the offspring were exposed for 20 weeks. The authors discovered that the growth (both length and width) of the shells of the adult gastropods and their offspring were not significantly affected by the microplastic exposures. Although adult taxa are sometimes not affected by microplastic exposure due to high tolerance built over time, several authors have warned that exposure to microplastics during the critical early life stages of organisms can cause harm, stunt development and growth, cause impairment and eventually may harm wildlife populations (Imhof & Laforsch, 2016; Rochman et al., 2014).

Although ingestion of microplastics by *M. tuberculata* was not investigated in this study, Au et al. (2015), stated that the growth of organisms during exposure to microplastics is controlled by ingestion, for example, reduced natural food intake caused by microplastic ingestion can result in decreased growth in organisms. Additionally, Akindele et al. (2019), discovered polyethylene microplastics in the digestive tracts of *M. tuberculata*, the authors stated that ingestion of microplastics can affect growth in *M. tuberculata* as it causes false satiation, which is in agreement with the findings of Au et al. (2015). Furthermore, Gray & Weinstein (2017), discovered that ingesting microplastics causes physical disturbances in the digestive system of organisms reducing feeding. However, several studies such as Graham & Thompson (2009) and Van Cauwenberghe et al. (2015), have reported that sometimes microplastics that have been ingested can pass through the gut of organisms, with minimal or no observed adverse effects. This might explain the study results as the different microplastic exposures had no significant effect on the growth of adult *M. tuberculata*. Furthermore, the lack of significant effect on growth observed during the study could also be due to the high tolerance of *M. tuberculata* as stated by Veiga et al. (2016).

5.3 *T. sparrmanii* Endpoints

5.3.1 Growth

Similar to the results for *M. tuberculata*, the study results showed no significant effect on the growth (body length) of *T. sparrmanii* caused by in the polypropylene and polyvinyl chloride microplastic exposures. These results are similar to the findings by Pannetier et al. (2020), who exposed *Oryzias latipes* to a mixture of microplastics and discovered that the microplastic exposures did not affect the body length of the fish. The study findings are also in agreement with the work conducted by Mbedzi et al. (2020), who discovered that the length of *T. sparrmanii* exposed to environmentally realistic concentrations of polyethylene microplastics was not affected by the microplastic exposures. The study results were also similar to those discovered by Huang et al. (2020), where the authors discovered that polystyrene microplastic exposures had no significant effect on the body length of *P. reticulata.*

On the contrary, the growth results (body length) of polyethylene exposures indicated that polyethylene microplastics had a statistically significant effect of *T. sparrmanii* growth. Several studies have also discovered significant effects of microplastic exposure on different freshwater organisms. For example, in a study by Naidoo & Glassom (2019), where they exposed a mixture of polyethylene, polyvinyl chloride and polystyrene to juvenile *Ambassis dussumieri*, using environmentally realistic concentrations. Although environmentally realistic concentrations were used just like in the current study, the authors discovered that there was a significant decrease in the length of the fish in all plastic polymers, this might be because their experiment ran for 95 days while this study only ran for 21 days, Silva (2005), stated that the annual growth rate of *T. sparrmanii* is 15 cm per year, meaning that the expected growth rate in the normal environment after 21 days in 0.86 cm, therefore, 8.6 mm, however, this varies with age. Given the natural variation in fish size, 21 days may have not been long enough to observe detectable results in growth. The longer the exposure time to microplastics the more potential effects may be observed as seen in the study by Naidoo & Glassom (2019). Furthermore, the authors used a combination of microplastics which could have also played a role in their significant effect on fish length. Xia et al. (2020), findings were similar to those of Naidoo & Glassom (2019), they discovered that polyvinyl chloride microplastics had a significant effect on the body length growth of *Cyprinus carpio*. This effect may have been observed because the authors ran the experiments for 60 days compared to the 21 days that this study's experiment ran for. Although the study results were not in agreement (polypropylene and polyvinyl exposures) with the findings by Naidoo & Glassom (2019) and Xia et al. (2020), their studies illustrated that long-term exposure to microplastics even at environmentally realistic concentrations can have severe effects on freshwater

organisms, which is the reality in freshwater systems as microplastics are always present and in circulation.

Studies investigating the effect of microplastic on the body width growth of *T. sparrmanii* are limited. In the current only polyethylene exposures had statistically significant effects on *T. sparrmanii* body width. However, these findings are not surprising as they are similar to those recorded during this study in growth measured by body length. While just like the body length results, the body width results for polypropylene and polyvinyl chloride exposures indicated no significant effect of the microplastic exposures on *T. sparrmanii* body width growth.

There was no significant response observed in *T. sparrmanii* in terms of growth measured by body mass caused by all the different microplastic exposures used during this study. These findings are similar to those of growth measured by body length and width in polypropylene and polyvinyl chloride exposures in which similar results were recorded. Contrarily, the result for growth measured by mass in polyethylene exposures produced surprising results as no statistically significant effects caused by polyethylene microplastics were recorded. These findings are astonishing as growth by body length and width both indicated statistically significant effects.

The study findings are in agreement with the results in a study by Pannetier et al. (2020), the microplastic exposures had no significant effect on *O. latipes* body mass after 30 days of exposure. The study results are also similar to the findings by Huang et al. (2020), no significant effects on the body mass of *P. reticulata* were reported by the authors after 28 days of microplastic exposure.

On the contrary there are studies whose findings were not in agreement with the current study's findings, which have discovered that microplastic exposure does indeed have a significant effect on the mass of freshwater fish. For example, Jabeen et al. (2018), discovered that a mixture of microplastics (polyethylene and polystyrene), caused a decrease in the mass of the *Carassius auratus* exposed to the microplastics. Xia et al. (2020) also discovered similar findings, when they exposed *C. carpio* to polyvinyl chloride microplastics. It is important to note that this significant effect could have been observed because their experiments ran for 60 days while this study's experiments only ran for 21 days.

As mentioned above Au et al. (2015), stated that the growth of organisms during exposure to microplastics is controlled by ingestion. Furthermore, Huang et al. (2020), speculated that the lack of significant effects observed during the study on the growth of *Poecilia reticulata* was because the microplastics did not restrict ingestion and nutrient uptake in the fish as it has probably developed an adaptive response to water with microplastics. Naidoo & Glassom (2019), also speculated that the

decrease in length observed in their study might have been caused by the fact that microplastic ingestion caused decreased feeding and false satiation which reduced energy for optimum growth. In addition to ingestion, long-term exposure to microplastics can also result in a significant effect on growth (Naidoo & Glassom, 2019).

5.3.2 Egested particles

For this study endpoint, all the microplastic polymer exposures produced boxplots that suggested an increase in the number of microplastics egested with increasing concentrations, but only in polyethylene exposures was a statistically significant increase in microplastic egestion with increasing concentrations observed. There are limited studies in the literature that investigate the egestion of microplastics, making it very difficult to compare the study results with those of other findings.

According to Jabeen et al. (2018), not all ingested microplastics are egested, some particles depending on microplastic size, shape, concentration and species' ecology can remain in the digestive tract and cause a blockage. Although the intestinal epithelial barrier protects the intestine and body against foreign particles, bacteria and pathogens, the barrier can breakdown (Schwarzfischer & Rogler, 2022). Allowing particles that are <10 µm, can translocate across the intestinal barrier, reach the bloodstream and eventually to the rest of the body (Parker et al., 2021).

Parker et al. (2021), caution about the potential effects of ingesting microplastics, which include: blocking the digestive tract, false satiation, leaching of plasticizers and other additives, and desorption of harmful pollutants that microplastics carry**.** For polypropylene and polyvinyl chloride exposures, the current study findings suggest that ingestion and thereafter egestion of microplastics had no significant effect on *T. sparrmanii* growth as no correlation was found between microplastic egestion and growth. However, for polyethylene exposures the results showed a significant effect on growth (length and width) and also statically proved that the number egested microplastic particles increased with increasing polyethylene concentrations. These observations suggest that there was a correlation between growth (length and width) and the number of particles egested in polyethylene exposures. Furthermore, egestion is beneficial as it is the route in which microplastics exit the body, reducing chances of bioaccumulation, blockage, false satiation and reduces the plastic load in the body. Egestion also reduces the probability of microplastic additives leaching into the body of the test taxa as they are ejected out of the body.

5.4 *C. nilotica* Endpoints

There is a lack of studies that investigate the effects of microplastics on shrimps, especially freshwater shrimps.

5.4.1 Growth

The current study found no significant effect of the different microplastic exposures on juvenile *C. nilotica* growth measured by body length. It is not surprising that the same results were discovered also for growth measured by body width as no significant effect was also observed.

The current study also found no significant effect of the three polymer microplastic exposures on juvenile *C. nilotica* growth measured by exoskeleton shredding. These results were observed also for growth measured by body width and length as no significant effects were observed.

The study results for growth (width, length, and exoskeleton shredding) of *C. nilotica* were all not statistically significant. Therefore, they were not in agreement with the results by Peixoto et al. (2019), who exposed *Artemia franciscana* to different concentrations of undisclosed microplastic polymers for 44 days, the authors observed a significant decrease in growth of the brine shrimps. It is also important to note that it is difficult to directly compare the study findings by Peixoto et al. (2019), to the current study, as the authors did not disclose the plastic polymers tested, concentrations and shapes which all could have played a role in the significant effects observed during their study. Furthermore, their study ran for 44 days while this study only ran for 21.

The study results for growth (width, length, and exoskeleton shredding) of *C. nilotica* were also not in agreement with the findings of Li et al. (2021), who discovered that exposure to polyethylene and polystyrene microplastics separately significantly reduced the growth rate of *A. franciscana*.

Once again this study's findings for growth (width, length, and exoskeleton shredding) of *C. nilotica* were in disagreement with the results published by Blarer & Burkhardt-Holm (2016), they discovered that exposure to polyamide microplastics significantly reduced the growth rate of *G. fossarum*. The authors determined that the decrease in growth was caused by the accumulation of microplastics in the intestines and which caused intestinal blocking which may reduce the feeding rate, and as a result growth (Li et al., 2021).

5.5 Other observed effects

5.5.1 Jaw inflammation and damage

There is limited literature on the effects of microplastics on inflammation and damage of fish jaws. Yet, jaw damage and inflammation were observed in the study in polyethylene exposures. In a study by Lu et al. (2016), the authors discovered that microplastic exposure caused inflammatory responses in *Danio rerio*. Furthermore, in a study by Jabeen et al. (2018), the authors discovered that exposure of *C. auratus* to polyethylene and polystyrene microplastics caused severe damage and inflammation to the jaws of the fish. The authors deduced that the inflammation of the jaws was a result of chewing microplastics when they were mistaken for food, they also discovered that it was microplastic fibres that caused most of the damage to the jaws. In the long term, damage or inflammation of the jaw will result in a decrease in feeding, weight and eventually death.

5.5.2 Aggression observations

In the current study, aggressive behaviour of *T. sparrmanii* was observed which resulted in a caudal fin injury in one *T. sparrmanii*. This injury might have been initiated by another *T. sparrmanii* in the tank, as they have been reported by Golan & Levavi-Sivan (2013), to be aggressive and territorial animals especially males, that are capable of causing extreme body injury. The territorial and aggressive behaviour of *T. sparrmanii* such as chasing and fighting especially during feeding time was observed in this study and is recorded in the general observations in Appendix B.

5.6 General Discussion

Although laboratory experiments were selected for this study, they cannot always accurately simulate real-life conditions and the behaviour of the test taxa and microplastics in the environment. Furthermore, only the effect of selected microplastics on the selected different test taxa were tested, whereas in the environment organisms encounter different situations and other pollutants that might exacerbate the effect of microplastics on the organisms.

The study results show that the microplastic exposures used had no statistically significant effect on *M. tuberculata, T. sparrmanii* and *C. nilotica* for most of the chosen study endpoints except for *T, sparrmanii* growth (length and width) and egestion for polyethylene. This study showed there was a significant increase in microplastic egested in polyethylene concentrations and that there seems to be a relationship between growth and egestion as statistically significant effects on *T. sparrmanii* growth (length and width) tests were also observed. A significant decrease in growth is usually caused by microplastic accumulation in the stomach of the organism resulting in false satiation and reduced feeding which causes a decrease in growth. Additionally, although a significant effect was observed in egestion, little is known about the retention time of the microplastic particles inside the organisms. Ingestion and therefore, egestion in polyethylene exposures was perhaps significant as the polyethylene particles were different colours compared to polypropylene and polyvinyl chloride microplastics which were white in colour. Due to the colourful nature of the polyethylene microplastics they might have been mistaken for food (TetraMin tropical flakes) which was also colourful in nature.

There were also other observed effects that were not study endpoints, these were damage to the jaw and caudal fin injury of a *T. sparrmanii* caused by the aggressive behaviour of this test taxa. These non-significant results were not expected for *C. nilotica* as they have been identified as being very sensitive to pollutants due to their soft bodies and feedingstrategy (Veiga et al., 2016; Weber et al., 2021).

The lack of significant effect observations is common in ecotoxicological studies. For example, Burns & Boxall (2018), Hwang et al. (2020), Pannetier et al. (2020) and Vijayaraghavan et al. (2022), all discovered that there was limited evidence to suggest that microplastics are causing significant harmful impacts on the environment. Furthermore, Mbedzi et al. (2020), and Lusher et al. (2017), reported that this is common in ecotoxicological studies using environmentally realistic concentrations, as effects on test taxa are most likely to be observed in studies using very high microplastic concentrations that exceed environmentally realistic concentrations by several orders of magnitude.

Although the study findings illustrate that the environmentally realistic concentrations of microplastics used in the study had no statistically significant effect on most of the study endpoints. Microplastic pollution in the environment should not be ignored, as plastic and microplastic pollution are increasing in the environment, and the concentrations in freshwater systems are predicted to also increase. As these environmental concentrations increase, microplastics will have significant effects on freshwater organisms as seen in studies that exceed environmentally realistic concentrations (Lusher et al., 2017). Therefore, it is important to reduce plastic and microplastic quantities in the

environment. Furthermore, studies by authors such as Naidoo & Glassom (2019), have proven that long exposure to microplastics even at environmentally realistic concentrations, can result in significant effects on the organisms exposed. It is important to note that in freshwater systems organisms are constantly exposed to microplastics as they are continuously in circulation.

The non-significant response reported in the study only accounts for the physical effects of microplastics which were tested during the study, and not the chemical effects of microplastics caused by leaching plastic additives such as dyes, plasticisers, accelerants, cross-linking additives, flame retardants, antioxidants, UV stabilizers, surfactants, inorganic fillers and photosensitizers as stated by Bouwman et al., (2018). These additives have been reported by different studies to have significant chemical effects on aquatic organisms such as liver damage, altered metabolism, altered protein levels and changes in gene expression (Gunaalan et al., 2020).

Even though in their study Au et al. (2015), discovered that fibres had a greater toxicological impact compared to other microplastic shapes. This study indicated no significant response owing to different microplastic polymers, sizes, and shapes, as the chosen study plastic polymers were composed of different proportions of sizes ranging from 1.7 µm to 500 µm and shapes (fibres, irregular and spherical).

Though mortality is common and unavoidable in ecotoxicology tests (Scharmann, 2000), no mortality was recorded for the duration of this study. Several authors have reported similar findings, for example, Imhof & Laforsch (2016), during their study on the effect of a mixture of microplastics on *P. antipodarum* which ran for over 141 days reported no mortality. Similarly, Weber et al. (2021), discovered that exposure of freshwater gastropods (*L. stagnalis*) to polystyrene microplastics for 28 days had no significant effect on the mortality of the test taxa. However, they observed slight effects on immune cell phagocytosis. These findings can be explained by the fact that gastropods have generally a high tolerance to pollutants due to their ability to isolate themselves inside their shells (Veiga et al., 2016; Weber et al., 2021). Likewise, Pannetier et al. (2020) exposed *O. latipes* juveniles to microplastics for 30 days and observed no mortality. Lei et al. (2018), exposed *D. rerio* to varying concentrations of polyamide, polypropylene, polyvinyl chloride and polystyrene for 10 days, no lethality was recorded but intestinal damage was observed. Vijayaraghavan et al. (2022), also reported similar results, when they exposed juvenile *E. suratensis* to different concentrations of polyvinyl chloride microplastics for 10 days. Additionally, Karami et al. (2017), also recorded no mortality after exposing *D. rerio* for 10 and 20 days to polyethylene microplastics ranging from environmentally realistic concentrations to high concentrations.

Although the study used different freshwater organisms with different feeding strategies, filter feeders were not included, which according to Ribeiro et al. (2019), are most sensitive to microplastics due to their feeding strategy which is filtration, which has been reported to be a primary pathway for microplastic ingestion. Additionally, as reported by various studies by Au et al. (2015), Naidoo & Glassom (2019) and Parker et al. (2021), the uptake or ingestion of microplastics is the main route in which organisms are affected by microplastics. Including these filter-feeders could have potentially produced significant results.

5.7 Study Limitations

The results reported in this study should be considered in light of some limitations. The first is the issue of periodic power cuts by the Electricity Supply Commission (ESKOM), which disturbed all the exposure experiments including the study control concentrations. The power cuts affected the laboratory lights, heaters and aerators would all be off during the period of load shedding. The switching off of aerators reduced the oxygen supply in the *T. sparrmanii* tanks, reduced DO has been reported by Abdel-Tawwab et al. (2015), to reduce growth in fish and feeding rates, therefore, loadshedding could have affected the results by reducing growth in *T. sparrmanii*. The lights were kept on for 12 hours in the laboratory to represent day-time, however during loadshedding during the day the lights were off, this might have caused confusion to the test taxa. For example *T. sparrmanii* only feed during the day and sleep during the night, the lights that were off would confuse the test taxa and instead of feeding the test taxa would sleep thinking it's night time. This would have resulted in reduced feeding rates and overtime a decrease in growth and reproduction, this would have negatively affected the study results. However, *C. nilotica* and *M. tuberculata* might have greatlybenefited as they are nocturnal and are mostly active and feed at night, this increased feeding might have increased their growth rate and also reproduction for *M. tuberculata.* Once again this would havenegatively affected the study results as under normal circumstances (no loadshedding) and in the natural environment different results would have been observed. During loadshedding the laboratory air conditioner which regulated the laboratory temperature at 24°C was off, this caused a decrease inthe room temperature, a decrease in temperature during ecotoxicology studies has been reported byVelki & Ečimović (2015), to act as a stressor and disturb the physiology and functioning of the test organisms, therefore, growth and reproduction of the test taxa would be reduced.

Secondly, level five of the lockdown accompanied by the regulations prevented the sharing of equipment such as the respirometer which is available at the South African Institute for Aquatic Biodiversity (SAIAB). Thirdly, the lack of previous studies on the effect of microplastics on species such as *M. tuberculata* and *C. nilotica* makes it difficult to compare the findings of the study to previous

studies. Thirdly, time was also a limitation, if the experiments could have possibly ran longer, more effects would have been observed. Fourthly, background microplastics were not accounted for. However, it is important to account for background microplastics as they may increase the concentrations of microplastic exposures and introduce alien microplastic polymers in the exposure concentrations. Fifthly, a pH of 5.11 was recorded once during the study. It is important to note that this value was below the OECD guideline ranges. Furthermore, a low water pH has been reported in literature to cause decreased growth and reproduction and even death, therefore, might have affected growth and reproduction during the study which were both endpoints. However, this was not further investigated during the study. Lastly, the study did not focus on functional ecology and ecological traitswhen selecting test taxa, for example, filter feeders were not included in the study, which are the mostvulnerable freshwater taxa due to their feeding strategy.

Chapter 6: CONCLUSION

The study aimed to assess the potential toxicity occasioned by microplastics in freshwater systems using methods that were developed or adapted during the study. The aim was met by three objectives; firstly, to identify suitable ecologically relevant endpoints for evaluating the potential effects of microplastics at environmentally realistic concentrations as literature has shown that authors do not always choose suitable study endpoints. Secondly, to contribute to the development and adaptation of methods for assessing the potential ecotoxicity caused by microplastics on selected freshwater organisms as there is a lack of standardization of methods in the ecotoxicology field. Lastly, to determine the potential effects of microplastics on freshwater organisms from multiple trophic levels. It is important to evaluate the potential effects of microplastics at environmentally realistic concentrations as most ecotoxicology studies employ concentrations that are not environmentally realistic and do not mimic environmental conditions and do not expose multiple organisms.

The study findings illustrate that the environmentally realistic microplastic concentrations tested during this 21-day study had minimal effect on the selected freshwater organisms. The clear conclusion that can be drawn from the statistics and data illustrated in this study is that microplastic particles had minute detectable effects on *M. tuberculata* endpoints which included growth and reproduction. For all of the *C. nilotica* growth endpoints, once again, no statistically significant effects were observed in the growth tests. For *T. sparrmanii* tests, most of the results were similar to those of *M. tuberculata* and *C. nilotica*, however, a statistically significant effect was observed in growth (length and width) and in number of egested particles in polyethylene exposures, suggesting a relationship between the two endpoints. In addition to the selected study endpoints other unexpected effects were observed in *T. sparrmanii* such as inflammation and damage of the jaws andaggressive behaviour that resulted in a caudal fin injury in one *T. sparrmanii*, however, these observations were also not significant.

Therefore, the study results suggest that at the microplastic concentrations tested, the physical effects of microplastic particles on freshwater organisms are minimal. Inspection of the boxplots and raw data showed high variation in the data, therefore, with more samples, replication, higher concentrations of microplastics and exposing filter-feeders, more significant results could be observed.

6.1 Study recommendations

6.1.1 For future studies

There should be further studies investigating the effect of microplastics on snails and shrimps as this is lacking compared to studies on fish. Future studies investigating the ingestion of microplastics

should not use spherical microplastics because they are rare in freshwater environments. Instead, studies should use shapes that are predominant in the environment such as microfibers. Future studies should investigate microplastic egestion as there are numerous laboratory studies in the literature on ingestion but fewer on egestion, particularly at environmentally relevant concentrations. Future studies should also investigate the retention time of microplastics as these are less examined. Researchers should investigate what attracts freshwater organisms to microplastics, and which colours and shapes of microplastics are favoured by organisms. Weis (2019), states that "Every week new articles on microplastics are published in scientific journals, but not all of them are original or important." The author suggests that researchers should focus on producing quality work over quantity and use methods that are realistic and mimic the environment (Weis, 2019). The units to quantify microplastics should be consistent or easily convertible, to facilitate the comparison of microplastics. Since there is evidence of microplastic presence in drinking tap water and consumption by humans, the accumulation of microplastics in humans and its effects should be investigated. Further investigation of policies and governance frameworks aimed at addressing microplastic pollution in freshwater systems holistically. Future studies should focus on the effect of microplastics on filter feeders which have been reported to be the most vulnerable freshwater taxa to microplastic pollution due to their feeding strategy.

6.1.2 To reduce microplastics in the environment

Since washing clothes releases microfibers that end up in the environment, there is a need for textile scientists that can improve the textiles to prevent microfiber shedding during washing. Washing machines require attachments that can trap microplastics preventing them from reaching water treatment plants and eventually freshwater systems. There should be an increase in funding for universities in developing countries to purchase instruments that will assist in furthering studies on the effects of microplastics. Avoid using products with microbeads (tiny plastic particles) such as facial scrubs and body washes, as these microbeads slip through water treatment plants due to their size and end up in freshwater systems. In the United Kingdom on 9 January 2018, microbeads use in personal care products and cosmetics manufactured in the UK was banned (Rhodes, 2018). Perhaps other countries should follow suit. In the UK many supermarkets have banned plastic bags and plastic bottles and instead have introduced public water fountains and bags made of biodegradable material (Rhodes, 2018). Other countries should follow suit. It is important to recycle plastic products properly by taking plastic waste to local recycling centres. There should be frequent landfill and river clean-up initiatives such as River Rescue, as plastics in the landfill, end up in rivers and other freshwater systems (Ivleva et al., 2017), it is, therefore, important to clean up landfills to prevent this. Educate and raise

awareness of how ubiquitous plastics are and their effects and educate others on alternative materials rather than plastics.

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APPENDICES

Appendix A: Water quality variables

Table 5 Physicochemical parameters at the beginning of the polypropylene exposures: *M. tuberculata*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	7.32	7.22	7.21	893	861	860	7.29	7.21	7.15	23.7	23.8	23.0
1566	7.40	7.23	7.32	838	823	893	7.43	7.24	7.65	23.9	23.6	23.7
3132	7.29	7.22	7.34	887	872	877	7.97	7.33	7.52	23.0	23.1	23.7
6264	7.30	7.49	7.20	843	866	867	6.90	6.98	6.99	23.3	23.5	23.8

Table 6 Physicochemical parameters at the end of the polypropylene exposures: *M. tuberculata*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	5.22	5.35	5.25	912	975	990	6.12	6.19	6.69	23.9	23.9	23.6
1566	5.20	5.17	5.16	950	919	899	6.33	6.96	6.15	23.9	22.3	23.1
3132	5.11	5.15	6.17	936	944	920	5.96	6.27	6.40	23.9	23.9	23.1
6264	5.27	5.24	5.30	953	1927	830	6.34	5.88	6.75	23.6	24.0	23.3

Table 7 Physicochemical parameters at the beginning of the polyethylene exposures: *M. tuberculata*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	7.43	7.30	7.31	867	833	863	7.23	6.96	6.85	24.1	23.2	23.3
922	7.31	7.13	7.27	882	845	831	7.11	6.99	7.15	24.0	23.3	23.5
1844	7.32	7.28	7.16	828	861	823	7.21	6.97	7.40	23.5	23.5	23.9
3699	7.28	7.29	7.29	861	823	867	7.33	7.18	5.75	23.7	23.7	23.2

Table 8 Physicochemical parameters at end of polyethylene exposures: *M. tuberculata*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	5.81	5.51	6.12	982	985	973	6.11	6.23	6.18	23.2	23.3	23.1
922	6.33	5.93	6.13	960	933	922	6.05	6.91	6.06	23.3	22.9	23.2
1844	6.02	6.06	6.04	1015	930	929	6.36	7.03	6.12	23.6	23.0	23.1
3699	6.06	6.22	6.02	939	936	934	7.05	7.04	6.40	23.0	22.8	22.8

Table 9 Physicochemical parameters at the beginning of the polyvinyl chloride exposures: *M. tuberculata*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	7.38	7.43	7.24	872	878	848	7.63	7.56	7.35	23.1	23.7	23.3
721	7.22	7.21	7.26	869	884	860	7.83	7.83	6.98	23.2	23.6	23.7
1442	7.31	7.30	7.25	892	851	886	7.22	7.12	7.01	23.6	23.7	23.3
2884	7.23	7.33	7.35	825	849	874	7.40	7.49	7.25	23.5	24.0	23.2

Table 10 Physicochemical parameters at end of the polyvinyl chloride exposures: *M. tuberculata*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Control	**7.07**	**7.03**	**6.40**	**970**	**948**	**8028**	**7.09**	**7.16**	**7.24**	**22.9**	**23.7**	**23.1**
721	6.33	6.12	6.56	936	934	933	6.09	6.78	6.60	22.7	23.3	23.5
1442	6.48	6.78	7.04	940	942	946	6.99	6.45	6.49	23.1	23.1	23.1
2884	6.24	6.35	6.19	967	960	957	7.01	6.95	6.59	23.6	23.5	23.1

Table 11 Physicochemical parameters at the beginning of the polypropylene exposures: *T. sparrmanii.*

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Control	7.17	7.14	7.15	875	875	877	7.09	7.68	7.54	23.1	23.3	23.7
1566	7.14	7.16	7.25	898	905	889	7.49	7.36	6.99	22.99	23.3	23.7
3132	7.15	7.29	7.32	808	880	839	7.03	7.46	7.25	23.7	23.2	23.5
6264	7.25	7.35	7.23	821	834	821	7.99	7.89	6.99	23.5	23.5	23.7

Table 12 Physicochemical parameters at the end of the polypropylene exposures: *T. sparrmanii.*

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	6.21	6.25	6.19	984	976	987	6.21	6.15	6.40	23.0	23.2	23.5
1566	6.24	6.23	6.50	939	961	1001	6.03	6.98	7.18	23.4	23.1	23.4
3132	6.39	6.12	6.27	941	930	951	6.13	6.16	6.24	23.2	23.3	23.2
6264	6.23	6.26	6.21	912	929	922	6.23	6.64	6.14	23.1	23.6	23.0

Table 13 Physicochemical parameters at the beginning of the polyethylene exposures: *T. sparrmanii*.

	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	7.17	7.14	7.15	875	875	877	7.09	7.68	7.54	23.1	23.3	23.7
922	7.15	7.19	7.28	898	885	865	7.31	7.26	7.35	23.3	23.0	23.1
1844	7.14	7.32	7.18	885	898	895	7.47	7.34	7.37	23.2	23.2	23.6
3699	7.16	7.24	7.24	875	895	850	7.11	7.44	7.28	23.1	23.5	23.7

Table 14 Physicochemical parameters at end of the polyethylene exposures: *T. sparrmanii*.

	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	6.21	6.25	6.19	984	976	987	6.21	6.15	6.40	23.0	23.2	23.5
922	7.21	6.17	6.16	1003	1009	979	6.23	6.96	6.15	23	22.9	23
1844	6.15	6.15	6.17	936	944	960	6.56	6.12	6.47	22.9	23	23.1
3699	6.29	6.24	6.39	953	927	928	6.34	6.88	5.95	23.8	24.2	24

Table 15 Physicochemical parameters at the beginning of the polyvinyl chloride exposures: *T. sparrmanii*.

	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	7.17	7.14	7.15	875	875	877	7.09	7.68	7.54	23.1	23.3	23.7
721	7.32	7.33	7.26	832	822	853	7.31	7.39	7.02	22.6	22.4	22.6
1442	7.26	7.19	7.44	857	853	824	6.78	6.75	6.89	22.8	22.6	22.1
2884	7.23	7.07	7.27	838	861	830	6.84	6.96	6.79	22.9	22.8	23.1

Table 16 Physicochemical parameters at the end of the polyvinyl chloride exposures: *T. sparrmanii.*

	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	6.21	6.25	6.19	984	976	987	6.21	6.15	6.40	23.0	23.2	23.5
721	6.36	6.80	6.51	895	898	863	6.13	6.73	6.45	24.0	24.1	24.1
1442	6.38	6.23	6.28	979	953	899	6.21	6.18	6.24	22.9	24.1	24.1
2884	6.52	7.01	6.47	946	944	916	6.20	5.99	5.95	24.8	25.1	23.3

Table 17 Physicochemical parameters at the beginning of the polypropylene exposures: *C. nilotica*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	7.15	7.12	7.26	861	822	863	7.08	7.12	7.24	23.2	23.3	23.0
1566	7.21	7.32	7.33	845	820	896	7.34	7.26	7.61	23.4	23.3	23.2
3132	7.23	7.27	7.30	823	870	873	7.67	7.37	7.55	23.1	23.0	23.4
6264	7.26	7.42	7.27	856	863	866	6.98	6.98	6.93	23.2	23.4	23.6

Table 18 Physicochemical parameters at the end of the polypropylene exposures: *C. nilotica*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	6.33	6.24	6.27	923	967	986	6.22	6.23	6.45	23.6	23.2	23.2
1566	6.27	6.18	6.31	945	947	976	6.34	6.81	6.32	23.2	22.9	23.4
3132	6.21	6.34	6.27	939	987	897	5.99	6.75	6.47	23.4	23.1	23.4
6264	6.22	6.25	6.32	938	998	934	6.18	5.93	6.34	23.3	23.0	23.3

Table 19 Physicochemical parameters at the beginning of the polyethylene exposures: *C. nilotica*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	7.15	7.12	7.26	823	822	863	7.08	7.12	7.24	23.2	23.3	23.0
922	7.21	7.37	7.24	829	848	842	7.02	7.19	7.37	23.4	23.6	23.1
844	7.33	7.26	7.19	867	866	837	7.14	7.23	7.42	23.8	23.2	23.0
3699	7.42	7.21	7.22	855	861	829	7.21	7.09	6.95	23.2	23.4	23.3

Table 20 Physicochemical parameters at end of polyethylene exposures: *C. nilotica*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	6.33	6.24	6.27	923	967	986	6.22	6.23	6.45	23.6	23.2	23.2
922	6.27	5.97	6.19	1002	978	928	6.18	6.81	6.75	23.1	22.8	23.1
1844	6.34	6.09	6.24	1010	956	948	6.38	7.00	6.14	23.0	23.3	23.6
3699	6.17	6.32	6.10	968	957	933	7.01	7.02	6.46	23.4	22.9	22.3

Table 21 Physicochemical parameters at the beginning of the polyvinyl chloride exposures: *C. nilotica*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	7.15	7.12	7.26	823	822	863	7.08	7.12	7.24	23.2	23.3	23.0
721	7.36	7.37	7.23	867	876	868	7.20	7.74	7.35	23.1	23.5	23.5
1442	7.20	7.22	7.10	883	878	880	7.39	7.32	6.91	23.0	23.5	23.3
2884	7.24	7.15	7.29	892	858	893	7.21	7.44	7.17	23.2	23.9	23.2

Table 22 Physicochemical parameters at end of the polyvinyl chloride exposures: *C. nilotica*.

Particles/L	pH			Electrical conductivity (µs.cm^{-1})			Dissolved oxygen (mg.l^{-1})			Temperature (°C)		
	A	B	C	A	B	C	A	B	C	A	B	C
Control	6.33	6.24	6.27	923	967	986	6.22	6.23	6.45	23.6	23.2	23.2
721	6.21	6.23	6.34	969	998	973	6.10	6.73	6.63	22.9	23.4	23.3
1442	6.32	6.43	7.01	909	985	968	6.87	6.54	6.44	23.1	23.3	23.0
2884	6.41	6.31	6.87	910	988	966	6.94	6.57	6.52	23.0	23.2	23.3

Appendix B: General Observations

Day 1

M. tuberculata

- Upon introduction into the microplastic exposures, the *M. tuberculata* retreated into their shells.
- The *M. tuberculata* did not show any indication of feeding upon introduction.
- Reproduction was monitored throughout the day.

T. sparrmanii

- When the *T. sparrmanii* were introduced into the tanks with microplastics, the *T. sparrmanii* in the Polyethylene exposures (colourful microplastics) ate the microplastics, when food was placed into the tank, they seemed like they couldn't differentiate between the food and the microplastics due to the colour of the Polyethylene microplastics. For Polypropylene and Polyvinyl chloride, however, which were white in colour the *T. sparrmanii* seemed to differentiate between the white microplastics and the food.
- All the *T. sparrmanii* were actively swimming and feeding when food was introduced into the tanks.
- The *T. sparrmanii* were all measured before they were introduced into their respective tanks.

C. nilotica

- No notable behaviour was observed in the *C. nilotica* during the initial introduction into the microplastic exposures.
- The *C. nilotica* swam around as usual after being introduced into the beaker.
- The Water quality variables of all the exposures were measured.

Day 2

- No notable behaviour was observed in the test taxa.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 3

- After feeding, the *T. sparrmanii* in exposure 1442 particles/L of Polyvinyl chloride, the bigger *Tilapia* was observed chasing the smaller one and attempting to injure it.
- The reproduction of *M. tuberculata* was monitored.

- The *M. tuberculata* got out of their shells and started moving around the beakers.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 4

- The reproduction of *M. tuberculata* was monitored.
- The *C. nilotica* became lighter in colour.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 5

- The length, width, and mass of *T. sparrmanii* were measured.
- The number of microplastics egested was also investigated.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.
- The microplastic exposures were all changed, fresh dechlorinated water, food and microplastics were placed into the respective tanks and beakers.
- Water quality variables were measured.

Day 6

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 7

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 8

- The reproduction of *M. tuberculata* was monitored.
- After feeding, a *Tilapia* in 3132 particles/L Polypropylene exposure, the bigger *Tilapia* was observed chasing the smaller one and attempting to injure it.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 9

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

- In the 922 particles/L Polyethylene exposure, one *T. sparrmanii* was observed to have an injury on the caudal fin.

Day 10

- The length, width, and mass of *T. sparrmanii* were measured.
- The number of microplastics egested was also investigated.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.
- The microplastic exposures were all changed, freshwater, food and microplastics were placed into the respective tanks and beakers.
- Water quality variables were measured.

Day 11

- In the 922 particles/L Polyethylene exposure, one *Tilapia* was observed to have a swollen or damaged jaw.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 12

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 13

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 14

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 15

- The length, width, and mass of *T. sparrmanii* were measured.
- The number of microplastics egested was also investigated.
- The reproduction of *M. tuberculata* was monitored.

- Exoskeleton shredding of *C. nilotica* was monitored.
- In the 3699 particles/L Polyethylene exposure, one *T. sparrmanii* was observed to have a swollen or damaged jaw.
- The microplastic exposures were all changed, freshwater, food and microplastics were placed into the respective tanks and beakers.
- Water quality variables were measured.

Day 16

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 17

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.
- After feeding, a *Tilapia* in 3132 particles/L Polyethylene exposure, a *Tilapia* was observed chasing another one and attempting to injure it.

Day 18

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 19

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 20

- No notable behaviour was observed.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.

Day 21

- The caudal fin injury on the *T. sparrmanii* in the 922 particles/L Polyethylene exposure had gotten significantly worse.

- The length, width, and mass of *T. sparrmanii* were measured.
- The number of microplastics egested was also investigated.
- The reproduction of *M. tuberculata* was monitored.
- Exoskeleton shredding of *C. nilotica* was monitored.
- *C. nilotica* and *M. tuberculata* were also measured.
- In the 3699 particles/L Polyethylene exposure one *T. sparrmanii* was observed to have a swollen or damaged jaw.
- Water quality variables were measured.

Appendix C: Adult *M. tuberculata* reproduction raw data

Table 22 Number of offspring per adult *M. tuberculata* in the varying concentrations of polypropylene after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	0	0	0	0	0	0	0	0.5	0	0.5	0.5	0	0.5	0.5	0
1566	0	0	0	0.5	0	0.5	1	0	0.5	1.5	0	1	1.5	0	1
3132	0	0	0	0	0	0.5	0	0	0.5	0	0.5	0.5	0	0.5	0.5
6264	0	0	0	0	0	0	0	0.5	0.5	0	0.5	0.5	0	0.5	1

Table 23 Number of offspring per adult *M. tuberculata* in the varying concentrations of polyethylene after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	0	0	0	0	0	0	0	0.5	0	0.5	0.5	0	0.5	0.5	0
922	0	0	0	0	0	0	0	0.5	0	0	0.5	0	0	0.5	0
1844	0	0	0	0.5	0	0	0.5	0	0	0.5	0	0	1	0.5	0
3699	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 24 Number of offspring per adult *M. tuberculata* in the varying concentrations of polyvinyl chloride after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	0	0	0	0	0	0	0	0.5	0	0.5	0.5	0	0.5	0.5	0
721	0	0	0	0.5	0	0	0.5	0	0	1	0	0	1.5	0	0
1442	0	0	0	0	0	0	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2884	0	0	0	0	0	0	0	0.5	0	0	0.5	0.5	0	0.5	0.5

Appendix D: *M. tuberculata* growth raw data

Table 25 *M. tuberculata* adult shell length (mm) in the varying concentrations of polypropylene after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	4.5, 3.5	4.7, 3.4	5, 4.5
1566	4.6, 4.6	4.8, 3.4	6.3, 6
3132	4.7, 3.5	4.9, 4.3	3.3, 4.5
6264	4.5, 3.2	4.9, 4.5	6.5, 3.5

Table 26 Adult *M. tuberculata* shell length (mm) in the varying concentrations of polyethylene after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	4.5, 3.5	4.7, 3.4	5, 4.5
922	4.5, 5	4.9, 4.5	4.6, 5
1844	4.6,3.8	4.9, 4.5	6.7, 4
3699	6.8, 4.2	4.8, 4.7	5.3, 5

Table 27 Adult *M. tuberculata* shell length (mm) in the varying concentrations of polyvinyl chloride after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	4.5, 3.5	4.7, 3.4	5, 4.5
721	4.5, 4.9	5, 4.9	4.7, 4.5
1442	6.5, 3.6	6.7, 3.4	4.6, 4.4
2884	3.9, 4.8	5.1, 3.4	4.6, 4.4

Table 28 Adult *M. tuberculata* shell width (mm) in the different polypropylene concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	3, 2.8	3.3, 2.8	3.5, 3.1
1566	3, 3.1	3.4, 2.5	4.8, 4.2
3132	3.1, 2.8	3.2, 3	2.8, 3.1
6264	3, 2.5	3.5, 3	4, 2.5

Table 29 Adult *M. tuberculata* shell width (mm) in the different polyethylene concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	3, 2.8	3.3, 2.8	3.5, 3.1
922	3.3, 3.2	3.2, 3	3, 3.4
1844	3.2, 2.5	3.5, 3	4, 2.8
3699	4, 3	3.4, 3	3.8. 3.5

Table 30 Adult *M. tuberculata* shell width (mm) in the different polyvinyl chloride concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	3, 2.8	3.3, 2.8	3.5, 3.1
721	3, 3.5	3.5, 3.5	3, 3.1
1442	4, 2.5	4, 2	3.3, 3
2884	2.8, 3.1	3.4, 2.5	3, 3

Table 31 Offspring *M. tuberculata* shell length (mm) in the different polypropylene concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	2.2	1.2	
1566	1,1,2		1.5, 1.8
3132		2.2	1
6264		2.5	2.3, 1.4

Table 32 Offspring *M. tuberculata* shell length (mm) in the different polyethylene concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	2.2	1.2	
922		2.2	
1844	1.1, 1.3	2.5	
3699			

Table 33 Offspring *M. tuberculata* shell length (mm) in the different polyvinyl chloride concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	2.2	1.2	
721	2.1, 1.1, 2.3		
1442	2	2.8	2.4
2884		1.2	1.5

Table 34 Offspring *M. tuberculata* shell width (mm) in the different polypropylene concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	1.5	0.6	
1566	0.5, 0.3, 0.8		0.6, 0.8
3132		0.8	0.5
6264		1	1, 0.7

Table 35 Offspring *M. tuberculata* shell width (mm) in the varying polyethylene concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	1.5	0.6	
922		1	
1844	0.4, 0.5	1.3	
3699			

Table 36 Offspring *M. tuberculata* shell width (mm) in the different polyvinyl chloride concentrations after 21 days of exposure.

Day	**21**		
Replicate	**1**	**2**	**3**
Concentration (particles/L)			
Control	1.5	0.6	
721	0.8, 0.4, 1		
1442	0.7	0.8	1
2884	0.4	0.4	

Appendix E: Juvenile *T. sparrmanii* growth raw data

Table 37 Juvenile *T. sparrmanii* body length (mm) in the varying concentrations of polypropylene after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (parti-cles/L)															
Control	51, 46	48, 45	60, 43	52, 47	50, 45	61,44.5	53, 48.5	53,49	63, 46	54, 49	54, 50	64, 48	56, 50	55, 52	65,50
1566	43, 43	49, 46	47, 45	44, 45	50, 48	49,46	46, 47	51, 49	50, 48	48, 49	52, 50	52, 51	50, 52	54, 53	53,53
3132	52, 41	42, 40	48, 43	53, 42	44, 41	49,45	54,44	46, 43	50,45	56,46	48, 46	52,48	57, 48	50.5, 48	54, 50
6264	43, 54	42, 48	36, 43	44,56	43.5, 50	37, 45	46, 57	45,51	40,47	47.5, 60	47,54	43, 50	49, 62	50.5, 56	46,52

Table 38 Juvenile *T. sparrmanii* body length (mm) in the varying concentrations of polyethylene after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	51, 46	48, 45	60, 43	52, 47	50, 45	61,44.5	53, 48.5	53,49	63, 46	54, 49	54, 50	64, 48	56, 50	55, 52	65,50
922	53, 47	59, 42	52, 45	54, 47	60, 45	56, 47	56, 44	60,34	56.5, 48	57, 46	61, 36	57,49	59, 47	61.5, 47	58, 50
1844	37, 42	40,46	55, 56	39, 45	43, 48	57,58	40, 47	45, 49	58,60	43, 49	46, 52	60,61	45, 51	49, 54	61, 63
3699	44, 44	41,55	40,45	44.5, 45	43, 56	41, 47	45, 46	43, 57	42, 48	46, 47	44,58	44, 49	47, 49	45, 59	45, 51

Table 39 Juvenile *T. sparrmanii* body length (mm) in the varying concentrations of polyvinyl chloride after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	51, 46	48, 45	60, 43	52, 47	50, 45	61,44.5	53, 48.5	53,49	63, 46	54, 49	54, 50	64, 48	56, 50	55, 52	65,50
721	48, 62	39,45	43,39	50, 63	43, 47	45, 42	52, 64	45, 49	48, 44	53, 66	46, 50	50,44	54, 67	49, 52	55, 47
1442	60,63	57,45	43,45	61, 63	59, 46	45, 43	63, 64	60, 47	49, 46	65, 64	62, 48	50, 47	67, 65	65, 49	52, 47
2884	60,53	42,45	46,45	61, 54	43, 47	47,46	62, 55	45, 49	49, 46.5	64, 56	47, 50	50.5, 48	65, 56.5	48, 51.5	52, 48.5

Table 40 Juvenile *T. sparrmanii* body width (mm) in the varying concentrations of polypropylene after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	16, 12	15, 13	20, 12	16.5, 13	15, 14	20.5, 12.5	17, 14	15.5, 14.5	21, 13	18, 14.5	16,15	22, 13.5	19,15	17,16	22.5, 14
1566	11, 11	14, 13	13, 14	12, 11.5	14.4, 13	14, 15	13, 12	15, 13.5	15, 15.5	14, 13.5	15.5, 14	16, 16.5	15,15	16,15	17, 17
3132	15, 13	11, 10.5	11, 10	15.5, 13	12, 11	12, 11	15.5, 13.5	13, 12	13, 12	16,14	14, 13	15, 13	17,14.5	15,13.5	15.5, 13.5
6264	13, 17	12, 15	10, 14	13.5, 17	12, 15	11, 14.5	14, 17.4	13, 16	12, 15	15, 18	14, 17	13, 16	16,19	15,17.5	13.5, 16.5

Table 41 Juvenile *T. sparrmanii* body width (mm) in the varying concentrations of polyethylene after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	16, 12	15, 13	20, 12	16.5, 13	15, 14	20.5, 12.5	17, 14	15.5, 14.5	21, 13	18, 14.5	16, 15	22, 13.5	19, 15	17, 16	22.5, 14
922	17, 13	18, 11	16, 13	18.5, 14	17.5, 11.5	15.5, 13.5	18, 14	18.5, 12	16, 14	18, 14	18.5, 13	17, 14.5	18.5, 14.5	19, 14	17.5, 15
1844	10, 13	11, 13	18, 20	11, 13.5	11.5, 13.5	19, 20	11.5, 14	12, 14	19.5, 21	12, 15	13, 14.5	20, 21	12.5, 16	14.5, 15	21, 21.5
3699	12, 14	13, 18	10, 13.5	12, 14	13.5, 19	11, 14	13, 15	14,19.5	11.5, 14	14, 15	14, 19.5	12.5, 14.5	14, 15.5	14.5, 20	12.5, 15

Table 42 Juvenile *T. sparrmanii* body width (mm) in the varying concentrations of polyvinyl chloride after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (parti-cles/L)															
Control	16, 12	15, 13	20, 12	16.5, 13	15, 14	20.5, 12.5	17, 14	15.5, 14.5	21, 13	18, 14.5	16, 15	22, 13.5	19, 15	17, 16	22.5, 14
721	12, 16	14, 11	13.5, 11	13.5, 17	13, 12	14.5, 12	14, 18	15, 13	15, 12.5	15, 18.5	14, 13.5	15, 13	16, 19	16, 14	16.5, 14
1442	18, 17	15, 11	11, 12	18.5, 18	15.5, 12	12, 12.5	19.5, 20	16, 13	12.5, 13	20, 20.5	17, 14	12.5, 13.5	21, 21	18, 15.5	13, 14
2884	16, 16	12, 13	12, 13	16, 16	12.5, 13.5	12, 13.5	17, 16.5	13, 14	13, 14	18, 17	14, 15	14.5, 15	19, 18	15.5, 16	15, 16

Table 43 Juvenile *T. sparrmanii* body mass (g) in the varying concentrations of polypropylene after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	3.1, 1.9	1.7, 1.8	4.5, 1.8	3.2, 2	2, 1.9	4.6, 1.9	3.3, 2.2	2.3, 2	4.7, 2	3.4, 2.3	2.5, 2.3	4.8, 2.1	3.5, 2.4	2.7, 2.5	4.9, 2.3
1566	1.8, 1.7	2.1, 1.7	2.3, 1.8	2, 1.8	2.2, 2	2.5, 2	2.1, 2	2.3, 2.3	2.7, 2.3	2.3, 2.2	2.5, 2.7	2.9, 2.5	2.5, 2.5	2.8, 3	3.2, 2.9
3132	3, 1.4	2.1, 1.5	2.5, 2.2	3, 1.5	2.3, 1.6	2.6, 2.3	3.1, 1.6	2.5, 1.9	2.7, 2.4	3.2, 1.7	2.9, 2.2	2.8, 2.7	3.5, 2	3.2, 2.7	2.9, 2.8
6264	1.8, 3.7	2.1, 2.5	1.3, 1.9	1.9, 4	2.2, 2.7	1.3, 2.1	2.1, 4.3	2.5, 3.1	1.4, 2.4	2.3, 4.7	2.8, 3.7	1.6, 2.6	2.7, 5.4	3.1, 3.8	1.9, 2.9

Table 44 Juvenile *T. sparrmanii* body mass (g) in the varying concentrations of polyethylene after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	3.1, 1.9	1.7, 1.8	4.5, 1.8	3.2, 2	2, 1.9	4.6, 1.9	3.3, 2.2	2.3, 2	4.7, 2	3.4, 2.3	2.5, 2.3	4.8, 2.1	3.5, 2.4	2.7, 2.5	4.9, 2.3
922	2.7, 2	4, 1.5	3.7, 1.6	3.1, 1.9	4.1, 1.8	3.8, 1.9	3.3, 2	4.2, 1.9	3.6, 2	3.4, 2.1,	4.3, 2	3.7, 2.2	3.6, 2.2	4.5, 2.2	3.8, 2.4
1844	1.1, 1.8	1.5, 1.7	4.1, 3	1.4, 1.7	1.7, 1.9	4.4, 3.7	1.5, 1.9	1.9, 2.1	4.6, 4	1.6, 2.2	2.1, 2.4	4.8, 4.2	1.8, 2.5	2.4, 2.7	4.9, 4.4
3699	1.7, 1.7	2.4, 3.8	1.4, 1.9	1.7, 1.8	2.3, 3.8	1.3, 2.1	1.8, 1.9	2.1, 3.9	1.4, 2.4	1.7, 2.2	2, 4	1.4, 2.6	1.8, 2.5	1.7, 4.2	1.5, 2.7

Table 45 Juvenile *T. sparrmanii* body mass (g) in the varying concentrations of polyvinyl chloride after 21 days of exposure.

Day	**1**			**5**			**10**			**15**			**21**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Concentration (particles/L)															
Control	3.1, 1.9	1.7, 1.8	4.5, 1.8	3.2, 2	2, 1.9	4.6, 1.9	3.3, 2.2	2.3, 2	4.7, 2	3.4, 2.3	2.5, 2.3	4.8, 2.1	3.5, 2.4	2.7, 2.5	4.9, 2.3
721	2.8, 4.4	1.6, 1.4	2.3, 1.4	2.8, 4.6	1.8, 1.6	2.5, 1.5	2.9, 4.8	2.1, 1.9	2.9, 1.7	3.1, 5	2.2, 2	3, 1.8	3.3, 5.1	2.4, 2.1	3.3, 2
1442	4, 4	2.9, 1.4	1.9, 1.5	4.3, 4	3.2, 1.5	2, 1.6	4.6, 4.1	3.6, 1.6	2.1, 1.7	4.6, 4.2	3.8, 1.7	2.2, 1.9	4.7, 4.3	4.1, 1.9	2.4, 2
2884	2.8, 1.7	1.7, 1.9	1.4, 1.9	2.9, 1.8	1.7, 2	1.6, 1.9	3, 2	1.8, 2.1	1.9, 2.1	3.1, 2.3	1.9, 2.2	2, 2.2	3.3, 2.5	2.1, 2.3	2, 2.4

Appendix F: Number of microplastic particles egested by Juvenile *T. sparrmanii* raw data

Table 46 Microplastic particles egested by juvenile *T. sparrmanii* in different polypropylene concentrations at various times of the 21-day experiments.

Day	**1**	**5**	**10**	**15**	**20**	**21**
Concentration (particles/L)						
1566	1	0	3	1	0	0
3132	0	2	1	1	0	0
6264	3	0	1	1	1	0

Table 47 Microplastic particles egested by juvenile *T. sparrmanii* in different polyethylene concentrations at various times of the 21-day experiments.

Day	**1**	**5**	**10**	**15**	**20**	**21**
Concentration (particles/L)						
922	3	4	2	1	0	1
1844	4	2	4	2	2	1
3699	3	6	5	2	1	2

Table 48 Microplastic particles egested by juvenile *T. sparrmanii* in different polyvinyl chloride concentrations at various times of the 21-day experiments.

Day	**1**	**5**	**10**	**15**	**20**	**21**
Concentration (particles/L)						
721	3	2	1	0	0	0
1442	4	0	3	1	0	0
2884	3	3	1	1	1	1

Appendix G: *C. nilotica* Juvenile body growth raw data

Table 49 Juvenile *C. nilotica* body length (mm) in the different polypropylene concentrations after 21 days of exposure.

Day	**21**	
Replicate	**1**	**2**
Concentration (particles/L)		
Control	11.2	12.3
1566	10.4	13
3132	11.3	11.6
6264	12	11.8

Table 50 Juvenile *C. nilotica* body length (mm) in the different polyethylene concentrations after 21 days of exposure.

Day	**21**	
Replicate	**1**	**2**
Concentration (particles/L)		
Control	11.2	12.3
922	11.5	11
1844	12	13
3699	11.7	13.4

Table 51 Juvenile *C. nilotica* body length (mm) in the various polyvinyl chloride concentrations after 21 days of exposure.

Day	**21**	
Replicate	**1**	**2**
Concentration (particles/L)		
Control	11.2	12.3
721	12	13
1442	11.5	12
2884	12.5	13

Table 52 Juvenile *C. nilotica* body width (mm) in the various polypropylene concentrations after 21 days of exposure.

Day	**21**	
Replicate	**1**	**2**
Concentration (particles/L)		
Control	1.5	2
1566	1	3
3132	2	2
6264	2.5	1.5

Table 53 Juvenile *C. nilotica* body width (mm) in the different polyethylene concentrations after 21 days of exposure.

Day	**21**	
Replicate	**1**	**2**
Concentration (particles/L)		
Control	1.5	2
922	1.5	1
1844	2	2.5
3699	2	3.2

Table 54 Juvenile *C. nilotica* body width (mm) in the different polyvinyl chloride concentrations after 21 days of exposure.

Day	**21**	
Replicate	**1**	**2**
Concentration (particles/L)		
Control	1.5	2
721	2.5	2.5
1442	2	2
2884	2	3

Table 55 Number of exoskeletons shredded per juvenile *C. nilotica* in the different polypropylene concentrations at various times of the 21-day experiments.

Day	**1**	**5**	**10**	**15**	**21**
Concentration (particles/L)					
Control	0	4	1.5	3	3.5
1566	0	2	1	2	3
3132	0	2.5	0.5	0	2
6264	0	1	1	2	0

Table 56 Number of exoskeletons shredded per juvenile *C. nilotica* in the different polyethylene concentrations at various times of the 21-day experiments.

Day	**1**	**5**	**10**	**15**	**21**
Concentration (particles/L)					
Control	0	4	1.5	3	3.5
922	0	2	1	1	3
1844	0	1	2	1	1
3699	0	0	3	3	2

Table 57 Number of exoskeletons shredded per juvenile *C. nilotica* in the different polyvinyl chloride concentrations at various times of the 21-day experiments.

Day	**1**	**5**	**10**	**15**	**21**
Concentration (particles/L)					
Control	1	3	1.5	3	3.5
721	0	2	0	2	3
1442	0	0	2	0	3
2884	0	1	1	1	2