



Abundance, distribution, and risk assessment of microplastics in freshwater fish, water and sediments in a subtropical river system

By

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ABSTRACT

Plastic pollution has emerged as one of today's most pressing environmental challenges, with a significant portion of it originating from microplastics (less than 5mm). Their buoyancy and endurance in aquatic habitats pose major risks to aquatic species. The fragmentation of weirs in our rivers is another significant issue faced by aquatic fauna, as weirs act as sinks for microplastic pollution, which can result in their consumption by freshwater organisms. The current study assessed sediment and water samples along the Luvuvhu River system to determine the abundance, distribution, and diversity of microplastics, using a combination of diversity indices and multivariate analysis by exploring the impacts of weir-induced alterations on microplastic accumulation, given that weirs can serve as plastic sinks and disrupt natural water flow. Fish species were collected from the Luvuvhu River. In the laboratory, microplastic abundances were quantified both upstream and downstream of weirs. The study's finding generally showed high microplastic abundance during the cool-dry season in the surface water samples upstream (mean 409.4 ± 582.1 particles m^{-3}) and downstream (mean 221.1 ± 363.6 particles m^{-3}) of weirs and high during the hot-wet season upstream (mean 73.3 ± 61.9 particles dwt) and downstream (mean 30.8 ± 17.4 particles dwt) of weirs in the sediment samples. Microplastic types were dominated by fibres, with the colour scheme dominated by transparent in both the upstream and downstream sites. A significant difference was observed among sites for fragments, with film and beads being significant among locations. Low-density polyethylene (LDPE) and polypropylene (PP) were dominant polymers in surface water, while polyethylene terephthalate (PET) and polyethylene (PE) were dominant in sediment samples. Among the 215 fish (27 species) examined, microplastics were detected in 98.8 % of them. Microplastics in fish were dominant downstream of the weir during the cool-dry and hot-wet seasons. Particularly high microplastic abundances were found in *Anguilla marmorata* (range 0–29 particles), with fibres and transparent particles being the most prevalent. The results further showed high microplastic abundances in benthopelagic feeders, highlighting that habitat plays a great role in microplastic ingestion in fish. The most common polymer types found in fish were polypropylene (PP) and polyethylene (PE). Microplastic abundance was found to be higher during the hot-wet season (mean 5.2 particles) compared to the cool-dry season (mean 3.0 particles). This increase may be attributed to a larger number of plastic particles being washed into the river during the hot-wet season. Wastewater treatment systems and laundry washing are a primary source of microplastics pollution, particularly due to the

observed dominance of fibres in the weirs. Microplastic abundances indicated that the Luvuvhu River is polluted, and weirs played a significant role in microplastic retention and accumulation. The present study's findings provide important information for stakeholders to integrate weir design and maintenance practices that minimize microplastic ingestion by fish and accumulation, while enhancing sediment flushing.

Keywords: Microplastics, Luvuvhu River, weirs, sediments, surface water, downstream, upstream, water pollution, freshwater fish

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DECLARATION

I, Samkelisiwe Thobile Themba, student number 201925567, hereby declare that the study on "Abundance, distribution, and risk assessment of microplastics in freshwater fishes in a subtropical river system" has not been submitted by anyone else or to any other institution and is my own research work, and that all sources that I used have been acknowledged by means of references at the end of this dissertation.

Signature

A handwritten signature in blue ink, appearing to read 'S. THEMBA', with a circular mark around the first letter 'S'.

Date 30/11/ 2025

CHAPTER 1: GENERAL BACKGROUND



Some of the fish species (largescale yellowfish *Labeobarbus marequensis* (4 fish); Leaden labeo *Labeo molybdinus* (dark coloured)) caught during the research study along the Luvuvhu River system. Photo by Samkelisiwe T Themba

General Introduction

It is estimated that between 1.15 and 3.8 million tonnes of plastic waste enter the ocean via rivers annually (Lebreton et al., 2017; Ma et al., 2024). Furthermore, it is projected that by 2040, plastic pollution will reach 712 million metric tons in aquatic ecosystems (Kan et al., 2023). Microplastics have been identified in both land and freshwater ecosystems, highlighting river flows as a key transport mechanism (Akdogan and Guven, 2024; Chen et al., 2019). Therefore, microplastics, referred to as plastic particles measuring less than 5 mm, have been recognised as widespread contaminants in freshwater habitats (Rodrigues et al., 2019; Samandra et al., 2022). Primary microplastics are intentionally produced in small sizes for use in industrial and cosmetic products, whereas secondary microplastics form as a result of the biological, physical, and chemical breakdown of larger plastic items (Godoy et al., 2019; Winkler et al., 2019; Sánchez, 2020). According to Ghosh et al. (2025), microplastics are diverse collections of particles that vary in shape, size, polymer types, and chemical composition, all of which may be related to their toxicity. Major microplastics can be categorised as polypropylene (PP), polyethylene (PE), polystyrene (PS), polyamides (PA), and polyvinyl chloride (PVC) based on the monomer structure of the polymer's backbone (Bouwmeester et al., 2015; Li et al., 2016). Research has indicated that aquatic organisms can consume and accumulate microplastics and associated contaminants (Dahms et al., 2020; Parker et al., 2022). However, the specific toxicity effects of the various types of microplastics remain largely unclear (Bakir et al., 2014; Hamlin et al., 2015; Hu et al., 2016; Ammar et al., 2023).

Microplastics, when released into the environment, enter freshwater systems either directly from the primary sources of contamination or indirectly through wind or precipitation-related events that transport plastics from land into surface waters (Zhang et al., 2019; Chen et al., 2020). Additionally, wastewater treatment plant discharges and effluents can disperse microplastics throughout the ecosystem (Chen et al., 2019). Thus, microplastics may be suspended in the water column and/or in the sediments, which serve as transient sinks (Zhang et al., 2019). The physical and temporal detention may also be influenced by other variables such as flow velocity, water depth, human activities, and/or floods (Bondelind et al., 2020; Zhang et al., 2020).

Microplastics are prevalent in all aquatic environments, inhabiting a size range similar to that of natural food, making them easily consumable by aquatic species, including fish (Akhbarizadeh et al., 2020; Missawi et al., 2020; Xu et al., 2022; Ben-Haddad et al., 2022a; Li et al., 2022; Dalu et al., 2023b, 2024). Research on microplastic ingestion suggest that a variety of taxa from different trophic levels consume microplastics due to different feeding habits and environmental conditions (Ribeiro et al., 2019). Human exposure via the ingestion of contaminated species is a similar concern (Oliveira et al., 2019; Saha et al., 2022, 2023a, 2023b). Studies aim to investigate the physiological, ecological, and behavioural effects of microplastic exposure on aquatic species, as well as their population dynamics (Guzzetti et al., 2018; Franzellitti et al., 2019; Prokić et al., 2019). Fish can consume microplastics directly through mistaken prey identities or indirectly by devouring other creatures that have swallowed microplastics (Ory et al., 2017; Mohan et al., 2024; Akinhanmi et al., 2024). A growing body of research suggests that freshwater fish eat microplastics (Silva-Cavalcanti et al., 2017; Dalu et al., 2023b). Some investigations found up to 100 % contamination in examined fish in specific places (Dalu et al., 2023b). Microplastics have a major impact on individuals, communities, and populations at all trophic levels (Ben-Haddad et al., 2022; De la Torre et al., 2023). Microplastics have been shown in laboratory experiments to affect aquatic species' growth, reproduction, immunity, metabolism, survival and other toxicity responses, such as oxidative stress (Wright and Kelly, 2017). Other studies have discovered behavioural changes, such as decreased predation, altered feeding, foraging, and swimming habits (Vazquez and Rahman, 2021).

Microplastics can potentially cause adverse effects to fish, including gastrointestinal congestion, choking, and feeding difficulties (Wright and Kelly, 2017). Additionally, they can cause significant biological consequences such as neurotoxicity, inflammation, genotoxicity, histopathological alterations, and reproductive disruption (Vazquez and Rahman, 2021). Microplastics' physical characteristics, hydrodynamic conditions, and biological fouling can increase the toxicological effects of hazardous substances (Forero-López et al., 2022; Romdhani et al., 2022) and facilitate the harbouring of invasive species (García-Gómez et al., 2021). Due to their buildup in aquatic food webs, the toxicity of these pollutants increases when discovered in combination with microplastics (Andrady, 2011). Once they reach the food chain, they can have an influence on both the environment and human health (Rangel-Buitrago et al., 2024).

Some research conducted in natural aquatic environments have found that fish ingestion of microplastics may be linked to feeding behaviours (Romeo et al., 2015). Microplastics that are the same size and colour as fish food might be mistakenly eaten during feeding, resulting in microplastic contamination in fish tissues (Savoca et al., 2019). Furthermore, some fish can identify microplastics from their diet after eating and spitting them out (Xiong et al., 2019). The consumption of microplastics by fish is similarly linked to the number of plastics in the environment (Carbery et al. 2018). A greater abundance of plastic debris at the bottom of riverbeds results in the presence of greater quantities of microplastics in the guts of freshwater fish in Bangladeshi water bodies' middle and lower levels (Parvin et al., 2021). Similarly, there is a significant link between microplastic intake in demersal fish and microplastic quantity in upper Thames River sediments (Wardlaw et al., 2022). Despite an increasing number of research efforts on freshwater systems, the interaction between diverse species and microplastics in fragmented rivers (in-stream weirs) with high sediment loads remains poorly understood.

Most, if not all, rivers of the world are subjected to one or more major forms of alteration and disturbance (Dallas and Rivers–Moore, 2014). These physical barriers such as dams (Watkins et al., 2019) or weirs (Ramulifho et al., 2019), might temporarily block microplastic transportation downstream. Floods and habitat changes are examples of environmental disruptions that might release previously submerged or sequestered microplastics, allowing them to spread across aquatic ecosystems and potentially impact on organisms (Ockelford et al., 2020; von Friesen et al., 2020). The alteration of stream flow caused by weirs has been increasing at an alarming rate in the past decades due to climatic factors and human disturbances (Rolls et al., 2012; Yang et al., 2018). For example, consecutive weirs were constructed in the Luvuvhu River, mostly in its upstream catchment (Ramulifho et al., 2019), for water storage and to cater to the high demands of irrigation agriculture (Mathivha et al., 2016). Weirs poses a threat to riverine biota and the ecosystem services provided by rivers. They not only disrupt fish movement but can also act as accumulation points for plastic pollution. Although some studies have attempted to link hydrological flow alteration patterns with fish and microplastic pollution (Parvin et al., 2021; Dalu et al., 2023a), no study has been conducted in the subtropical or semi-arid region of the world such as the Luvuvhu River to investigate the ecological consequences of river modifications caused by the presence of weirs and the impacts of plastic pollution. There is a lack of holistic information on water quality and aquatic biodiversity that could be linked to the impact of weirs in the Luvuvhu River in relation

to plastic pollution. This study aims to close this knowledge gap by investigating the magnitude and extent of the consequences of building weirs on plastic pollution dynamics.

Aims

The current study investigated the microplastic abundance, distribution, and dynamics in water, sediment, and fish upstream and downstream of weirs along a subtropical Luvuvhu River system.

Objectives

- To quantify microplastic concentrations in water and sediments upstream and downstream of weirs.
- To assess seasonal differences in retention and potential downstream export.
- To evaluate how particle traits (i.e., type, colour, polymer type) affect retention.
- To assess the abundance of microplastics in fish upstream and downstream of weirs.

Hypotheses

- Microplastic concentrations will be elevated upstream of the weir, with retention efficiency high during the dry season. Large, dense particles are expected to be preferentially deposited, particularly in fine-sediment zones, while high-flow pulses will mobilise stored microplastics, creating downstream flux peaks.
- Fish collected upstream of weirs will have higher concentrations of microplastics compared to those collected downstream, highlighting the potential role of the weirs in microplastics accumulation of fish.

Thesis structure

Chapter one introduces and outlines the study. It also includes background information, study's aims and objectives, hypotheses, and research structure.

Chapter two is a detailed review of the literature pertaining to the subject of research. The literature review covers the theoretical literature relevant to the topic and examines the empirical research, with a focus on recent discoveries on microplastics abundances, distributions and diversity in surface water, sediment and freshwater fish species and how weir

designs contribute to microplastic sinks in sediment and accumulation in surface water, resulting in consumption by freshwater fish.

Chapter three – Microplastics in fragmented rivers: Assessing the retentive role of weirs and how river weirs alter microplastic dynamics. This chapter examined the characteristics and spatial distribution of microplastics in water and sediment samples obtained from the Luvuvhu River system both upstream and downstream of weirs over two seasons (cool-dry and hot-wet). It also looked at how seasonal fluctuations affect microplastic abundance in river systems, as well as how weir structure contributes to microplastic accumulation.

Chapter four – Barriers or burdens? Investigating the role of river weirs in fish microplastic bioaccumulation and contamination. This study quantified and compared the abundance of microplastics in freshwater fish both upstream and downstream of weirs. By identifying and characterising microplastics based on colour, shape, size, as well as polymer type, the study aimed to identify possible pollution sources and understand feeding-related exposure processes. Variations in microplastic ingestion among fish species may reflect differences in eating behaviour, habitat preference, and trophic position, as well as levels of environmental pollution among sites. As a result, the investigation not only quantified contamination levels, but also investigated spatial patterns and ecological variables that influence microplastic accumulation. Finally, measuring microplastic abundance in fish species provides useful information into pollution exposure, ecosystem health, and the potential threats posed by weirs as the main contributors to microplastic pollution and sinks in the river.

Chapter five, the main focus was the summary, conclusions and recommendations. This chapter provides a synthesis of all the chapters in the study. It also provides recommendations, action plans and suggestions for future research

CHAPTER TWO: LITERATURE REVIEW



A weir along the Lutandwa River, a tributary of the Luvuvhu River. Photo by Samkelisiwe T Themba

The long-ignored issue of plastic pollution in aquatic systems is now regarded as a severe threat to the global ecology (Roch et al., 2019; MacLeod et al., 2021). Plastic waste is quickly accumulating in aquatic areas such as lakes, wetlands, rivers, and reservoirs, harming ecosystems (Andrady, 2017; Isangedighi et al., 2020; Belioka et al., 2024). However, there is a lack of understanding about the volume and impact of plastic in rivers, as well as the causes of pollution (Blettler et al., 2018; Al-Zawaidah et al., 2021; Van Emmerik et al., 2022; Dalu et al., 2024). Numerous factors, such as the cheapness of plastics and urban expansion, have resulted in an increased production of plastics for commercial and household use. As a result, plastic pollution in aquatic habitats is one of the rising concerns around the world, and it is one of the most difficult contaminants to remove once they breakdown into smaller particles (Bashir et al., 2020; Thushari and Senevirathna et al., 2020; Hossain et al., 2022).

Microplastics are solid plastic particles with a diameter less than 5 mm (Thompson et al., 2024). They are found in both aquatic and terrestrial environments, as well as in remote and protected environments, raising global concerns about their possible negative effects (Horton et al., 2018; Nimma et al., 2025). Their ubiquitous presence in natural environments has the potential to cause intake by a variety of organisms, including zooplankton (Botterell et al., 2019; Sipps et al., 2022; Thirunavukkarasu et al., 2025), shrimp (Nan et al., 2020; Shan et al., 2025; Bonilla-Aguirre et al., 2026), and fish (Klangnurak and Suwaree, 2020; Parvin et al., 2021; Dalu et al., 2024; Kolandhasamy et al., 2025), which could result in negative effects such as low growth rate, mechanical harm, decreased fertility, and even morbidity (Ma et al., 2020; Wang et al., 2020a).

Microplastics can leach harmful substances used as additives in the plastic manufacturing process and also adsorb pollutants from the surrounding environment (Campanale et al., 2020; Mei et al., 2020; Da Costa et al., 2023; Iftikhar et al., 2024). Thus, microplastics may therefore enhance the transmission of adsorbed pollutants from environmental matrix to biota, resulting in bioaccumulation through the food chain and becoming a hazard to high trophic levels (Carbery et al., 2018; Li et al., 2020b; Saikumaret al., 2024; McHale et al., 2024). These issues are of major public importance, with, most of these research focusing on marine ecosystems (Collard et al., 2019; Kasamesiri et al., 2020; Dalu et al., 2023), and research into freshwater systems remains limited.

The freshwater ecosystem has been recognised as a major source and conduit for microplastics (Li et al., 2020), which are connected to various emission sources such as illegal discharge of untreated wastewater, effluent from wastewater treatment plants (WWTPs), human waste disposal, and rainwater runoff (Eckert et al., 2018; Dalu et al., 2023b). According to a recent study by Meijer et al. (2021) and Arnon- (2025), freshwater systems serve as a pathway for microplastic transfer from terrestrial to aquatic environments. Approximately 20 million metric tonnes of microplastics are released into the ocean each year, and rivers are a major source of microplastic-related marine pollution worldwide (Strokal et al., 2023; Goßmann et al., 2025).

Microplastic particles can float in bodies of water owing to their small size after being released into aquatic settings. However, after microorganism growth microplastics on the water surface can be pulled down into the deep-water column and even deposited in sediments (Lagarde et al., 2016; Ballent et al., 2012; Meng et al., 2024). Sedimentary microplastics could be carried along with sediments or resuspended and moved by hydraulic forces (Ballent et al., 2016). Such horizontal and vertical movement imply that sediment particles can serve as microplastics carriers (Horton and Dixon, 2018). As a result, microplastic dispersion patterns vary, as does sediment particle transit behaviour (He et al., 2020a; Soler et al., 2024; Li et al., 2024b; Mancini et al., 2025). However, the combined impacts of catchment parameters and sediment characteristics on the presence of microplastics have not been thoroughly examined to date, which limits the assessment of the environmental risks associated with microplastic pollution in aquatic ecosystems (He et al., 2020a).

Ingestion is the most frequent impact of plastic waste, as it has been recorded in over 600 species across various trophic levels (Pinheiro et al., 2017; Collard et al., 2019; Kasamesiri et al., 2020; Dalu et al., 2023b). Fish are among the most vulnerable taxa, with multiple reports of plastic debris ingestion by numerous species (Pinheiro et al., 2017; Collard et al., 2019; Kasamesiri et al., 2020). For example, microplastics have been detected in the gastrointestinal contents of coastal freshwater fish from the Rio de la Plata Estuary, with all fish collected containing microplastics (Khan et al., 2023a). The concentration of microplastics in the gut contents was considerably higher near effluent discharge points (Khan et al., 2023a; Zhuang et al., 2024; Wang et al., 2024). Dalu et al. (2023b) found that all collected fish had microplastics in their gastrointestinal tracts, with high concentrations of microplastics being found downstream of the wastewater treatment works outflow points. Jabeen et al. (2017) highlighted

microplastics in 95.7 % of freshwater fish, and the amount of microplastics in fish intestines was significantly high than in fish stomachs. It is thought that their consumption of microplastics is intentional, as they mistake microplastics for food floating in the water column, causing their consumption of plastics to be accidental or preferential, as in the case of *Perca fluviatilis* (Peters et al., 2016; Lonnstedt et al., 2016).

Several studies (e.g., Lonnstedt et al., 2016; Kasamesiri et al., 2020) have found that microplastics have a detrimental physiological and physical impact on freshwater fish. Fish that consume microplastics are at risk of physical dangers, including intestinal tract and digestive appendage obstruction, gastrointestinal tissue damage and inflammation, which can impede proper nutrient absorption (Peda et al., 2016; Khan et al., 2023b; Zhuang et al., 2024). Thus, when swallowed, microplastics directly influence the fish's immune system by activating degranulation, altering behaviour, and impairing a predator's capacity to detect physiological interference (Pinheiro et al. 2017; Dalu et al., 2024).

The uptake of microplastics by fish from the water body may also be related to their size and living environment (Parvin et al., 2021; Wang et al., 2024; Nabi et al., 2024). Demersal fish species are more vulnerable to microplastics because bottom sediments serve as a potential sink for microplastic particles (Woodall et al., 2014). Rummel et al. (2016) discovered that pelagic fish species ingested more microplastics than demersal fish. In contrast, two earlier studies found that fish swallow more microplastics as their body weight increases (Hossain et al., 2019). However, a study by Carbery et al. (2018) found that microplastics consumed by fish are mostly related to environmental plastic abundances rather than other variables. As a result, it is critical to understand which element has the most influence on fish ingestion of microplastics.

Weirs are structures on a river that are fitted and operated to produce flow data from which systematic records of water level, discharge, or flow are retrieved (Wessels and Rooseboom, 2009). Streamflow is a key element that influences the ecological status of flowing water habitats and thus the life history characteristics of aquatic organisms (Schneider and Petrin, 2017; Su et al., 2021). Natural flow regimes are critical to rivers' ecological functionality and sustainability (Jumani et al., 2020; Carpenter–Bundhoo et al., 2023). However, the increasing social, economic, and human demand for water resources has led to the transformation of

numerous rivers (Leitner et al., 202; Zhang et al., 2024). Furthermore, while high water quality and ecosystem integrity are critical for fish communities and human development, humans have altered them (Ramulifho et al., 2023). Many weirs were built in the river systems with little or no consideration for their environmental impact and their potential for change (Winemiller et al., 2016). Weir construction alters river flow regimes and disrupts physical conditions, posing one of the most significant risks to the river's functionality, integrity, sustainability, and connectivity (Casserly et al., 2020). Weirs operate as sinks for microplastic deposition in our river system, increasing the consumption of microplastics by freshwater fish. Weirs and other in-stream structural barriers interrupt a river's natural continuity, turning it into a series of disjointed segments. This change impedes organism migration and causes population fragmentation (Brooks et al., 2018).

CHAPTER THREE: MICROPLASTICS IN FRAGMENTED RIVERS: ASSESSING THE RETENTIVE ROLE OF WEIRS AND HOW RIVER WEIRS ALTER MICROPLASTIC DYNAMICS



One the study weirs along the Mutshindudi River, a tributary of the Luvuvhu River system.
Photo by Tatenda Dalu

ABSTRACT

Microplastics are an emerging environmental concern, with significant implications for freshwater ecosystems. Whereas, weirs alter river flow by slowing water velocity and creating zones of sediment deposition, which can trap and concentrate microplastics alongside organic matter. At the same time, turbulence generated by weirs may fragment larger plastics into microplastics, influencing their downstream transport and ecological impacts. The study aimed to investigate the abundance and distribution of microplastics in sediment and water samples along the Luvuvhu River system across two different seasons (i.e., cool–dry, hot–wet) using a combination of diversity indices and multivariate analysis by exploring the impacts of weir–induced alterations on microplastic accumulation, given that weirs can serve as plastic sinks and disrupt natural water flow. Microplastics were more abundant during the cool–dry season in the surface water samples upstream (mean 409.4 ± 582.1 particles m^{-3}) and downstream (mean 221.1 ± 363.6 particles m^{-3}) of weirs and high during the hot–wet season upstream (mean 73.3 ± 61.9 particles dwt) and downstream (mean 30.8 ± 17.4 particles dwt) of weirs in the sediment samples. Microplastic types were dominated by fibres, with the colour scheme dominated by transparent in both the upstream and downstream sites. A significant difference was observed among sites for fragments, with film and beads being significant among locations. For microplastic colours, significant differences were observed among sites for blue coloured microplastics, with locations and seasons highlighting significant differences for transparent and other coloured microplastics. Low–density polyethylene (LDPE) and polypropylene (PP) were dominant polymers in surface water, while polyethylene terephthalate (PET) and polyethylene (PE) were dominant in sediments. The sediment pollution load index ($PLI > 3.5$) showed that most of the sampling sites were polluted with microplastics. High microplastic abundances were observed upstream of weirs during the cool–dry season, due to low water flow and water levels, which trapped microplastics upstream of the weirs. Microplastic abundances indicated that the Luvuvhu River is highly polluted, and weirs played a significant role in microplastic retention and accumulation. Therefore, to address these dynamics, river management strategies should integrate weir design and maintenance practices that minimise microplastic accumulation while enhancing sediment flushing. Overall, recognising weirs as both potential sinks and transformers of microplastics is essential for effective freshwater pollution monitoring and mitigation.

Keywords: Microplastics, weirs, sediments, water, downstream, upstream, pollution

INTRODUCTION

Microplastics, defined as plastic particles smaller than 5 mm, are now widely recognised as pervasive contaminants in freshwater ecosystems (Hartmann et al., 2019; Li et al., 2024). In recent years, microplastic pollution in the environment has received a lot of attention (Dalu et al., 2025). Microplastics can accumulate in large quantities in aquatic ecosystems' water and sediments (Themba et al., 2024). Microplastics are primarily produced through direct production (primary microplastics) (Wang et al., 2019), and weathering and fragmentation of microplastics in the environment (secondary microplastics) (Julienne et al., 2019). Microplastics can be found in synthetic textiles (35 %), automobile tyres (28 %), city dust (24 %), road markings (7 %), marine coatings (4 %), personal care items (2 %), and plastic pellets (0.3 %) (Teggers et al., 2025). Worldwide plastic manufacturing has reached 400 million metric tons annually, with single-used plastics accounting for 36% of total output (Tathavadekar, 2025). Regardless of high advanced technology, the amount of global plastic waste produced is expected to triple by 2060, with half ending up in landfills and the natural environment (Tathavadekar, 2025). The majority of microplastic particles entering the freshwater ecosystem are secondary microplastics produced by the breakdown of microplastic products such as single-use packaging, tyres, and synthetic fibres (Adetuyi et al., 2024). Microplastics can enter freshwater through a variety of channels, including air fallout, effluents from wastewater treatment plants (WWTPs), direct garbage disposal due to poor waste management and runoff from agricultural, recreational, industrial, and urban sectors (Free et al., 2014; Nikiema et al., 2020). Thus, microplastics have emerged as a serious threat to aquatic environments due to their persistence, ubiquity, and inherent hazardous potential (Zhao et al., 2014).

Aquatic ecosystems are one of the primary recipients of microplastics and play a significant role in the transportation and distribution of these plastics (Mendes et al., 2021; Yaranal et al., 2021). In the aquatic environments, sediments act as an important sink for microplastics (Goswami et al., 2021; Eo et al., 2021). Microplastics are difficult to degrade, leading to their accumulation in sediments, and this buildup increases the ecological risks posed by microplastics in sediments (Niu et al., 2021). Therefore, researchers should focus on controlling microplastics pollution in aquatic ecosystems. Assessing the ecological risks and identifying the sources of microplastics pollution is important for managing their contaminants (Nahian et al., 2022; Deme et al., 2022). Understanding the ecological risks of microplastics in

sediments can help managers better mitigate and manage sediments as this knowledge supports the development of targeted control measures in areas where the ecological risk from microplastics is high (Kabir et al., 2022).

Rivers serve as critical conduits for the transport of microplastics from terrestrial sources to marine environments, yet they also act as sinks where particles accumulate in sediments and biota (Wicaksono et al., 2021; Xia et al., 2022). Understanding the dynamics of microplastic retention and movement within river systems is essential for assessing ecological risks and informing management strategies. River fragmentation through the construction of weirs and other low-head barriers is a common feature worldwide, altering hydrodynamics, sediment transport, and pollutant behaviour (Wu et al., 2020; Yu et al., 2025). Weirs create impounded zones upstream that promote sediment deposition, while scour and turbulence downstream enhance resuspension and redistribution (Abdollahpour et al., 2017). Such modifications to flow and sediment regimes suggest that barriers may play a significant role in trapping, retaining, or remobilising microplastics (Wicaksono et al., 2021; Mutshekwa et al., 2023; Themba et al., 2024). Similarly, Wang et al. (2020b) and Dahms et al. (2020) found more microplastics upstream of a dam. However, despite increasing awareness of microplastic pollution in freshwater systems, the influence of small-scale hydraulic structures on their transport remains poorly understood. Seasonal hydrology further complicates these dynamics (Wu et al., 2024). During dry seasons, reduced flows may enhance deposition and retention of microplastics in impounded sediments, whereas high-flow conditions in wet seasons can mobilise and export accumulated particles downstream (Kumar et al., 2021). Additionally, particle-specific properties, including size, shape, density, and polymer type, are likely to influence transport pathways and deposition patterns around weir structures.

Although rivers are one of the primary routes for plastic debris to enter the marine environment and with rivers estimated to discharge 5 to 12 million metric tons of plastic debris into the marine environments each year (Wicaksono et al., 2021). Microplastic pollution research has mostly focused on marine habitats, with little attention being paid to freshwater ecosystems within the global south (Bellasi et al., 2020). In South Africa, there is a limited body of knowledge about microplastics in freshwater bodies but the number of studies in this environment has been steadily increasing over past 5 years (Ramaremsa et al., 2022). Although microplastic pollution in man-made lakes and reservoirs has received considerable attention in the global north, research on the fate of microplastics in small in-stream barriers, such as weirs,

remains limited (Wicaksono et al., 2021). We conducted the study on six weirs located in the Luvuvhu River system in the Limpopo Province of South Africa to assess how river weirs influence microplastic transport and retention across wet and dry seasons. Specifically, we aimed to (i) quantify microplastic concentrations in water and sediments upstream and downstream of weirs, (ii) assess seasonal differences in retention and potential downstream export, (iii) evaluate how particle traits (i.e., type, colour, polymer type) affect retention, and (iv) determine how high-flow events remobilise stored microplastics. We hypothesize that microplastic concentrations will be elevated upstream of the weir, with retention efficiency high during the dry season (Figure 3.1). Large, dense particles are expected to be preferentially deposited, particularly in fine-sediment zones, while high-flow pulses will mobilise stored microplastics, creating downstream flux peaks. By integrating spatial, seasonal, and particle-specific analyses, this research will clarify the role of weirs in regulating microplastic dynamics in fragmented rivers and their implications for contaminant transport.

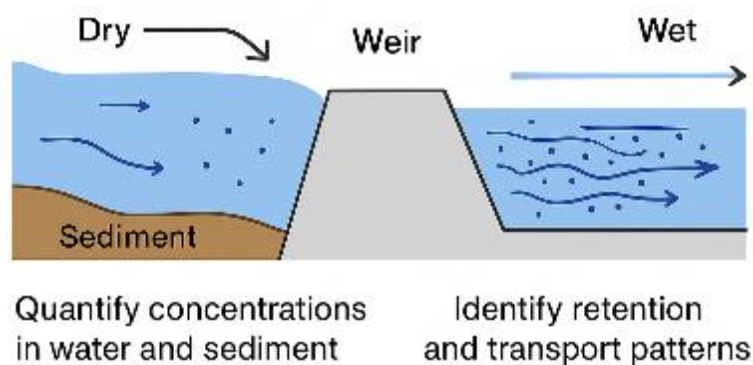


Figure 3.1 An illustration of microplastic dynamics caused by the building of a weir across a river during the wet and dry seasons

MATERIALS AND METHODS

Study area

The Luvuvhu River originates from the Soutpansberg Mountains, together with its other tributaries (Munyai et al., 2025). The Luvuvhu River flows for about 200 km from its source to its confluence with the Limpopo River at Pafuri, within the Kruger National Park. The river is in the savanna biome, with hot summers and dry winters (Kleynhans, 1996). The mean annual precipitation ranges between 610 mm and 800 mm. The local climate in the winter and summer months is characterised by low (i.e., 9 °C) and high (i.e., 40 °C) temperatures,

respectively. Rainfall is largely seasonal and mostly occurs during summer months, between October and April (Ramulifho et al., 2019). Land-use activities along the Luvuvhu River catchment include forestry, agriculture, and settlements.

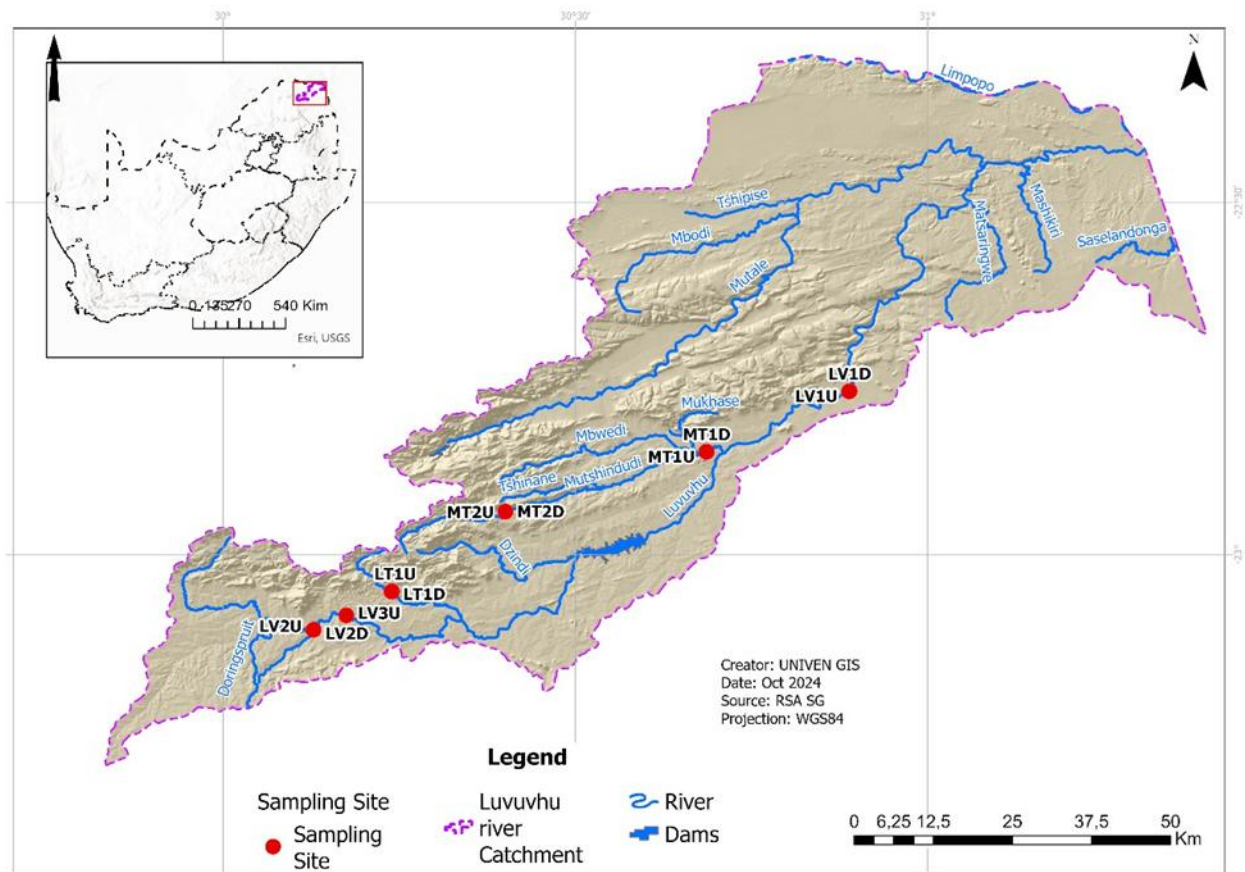


Figure 3.2 Map highlighting the study sites within the Luvuvhu River catchment in the Limpopo Province, South Africa.

Six study weirs were randomly selected along the Luvuvhu River systems for water and sediment sample collection upstream and downstream of weirs across two seasons (i.e., hot-wet, cool-dry) (Figure 3.2). The land use activities around the study sites include washing clothes, bathing, fishing, and agricultural activities (Figure 3.1). This study considered weirs located in three rivers, namely the Lutanandwa ($n = 1$ weir), Mutshundudi ($n = 2$ weirs), and Luvuvhu ($n = 3$ weirs) as primary study sites. Sampling sites were located upstream and downstream of weirs across two different seasons: cool-dry (May 2024) and hot-wet (December 2024).



Figure 3.3 Land use activities happening in the Luvuvhu River system

Sampling points and measurement of physicochemical parameters

At each sampling site, a portable HANNA handheld multi-parameter was used for measuring basic water parameters such as conductivity ($\mu\text{S cm}^{-1}$), water temperature ($^{\circ}\text{C}$), total dissolved solids (TDS) (mg L^{-1}), and pH in the upstream and downstream sections of the weirs and across seasons. The measurements were taken before collecting water and sediment samples to avoid disturbance in readings.

Sampling of water and sediments for microplastic determination

Water

Water samples for microplastic determination were collected from six sampling sites upstream and downstream of weirs ($n = 2$ subsamples per site) over two seasons (i.e., cool-dry and hot-wet). A bulk method (Hung et al., 2021) was used to collect 100 L of surface water using a stainless-steel bucket (field duplicates, $n = 2$) and filtered through a $63\ \mu\text{m}$ mesh size sieve. Following each collection, the sieve was rinsed with river water to avoid cross-contamination between sites. The strained particles were then rinsed with water into a clean pre-labelled 250-mL sample jar and transported to the University of Mpumalanga Aquatic Systems Research Group Laboratory for further analysis.

Sediment

A clean metal shovel was used to collect 2 kg of sediment ($n = 2$) samples from random spots within a $30 \times 30\ \text{cm}$ quadrat (from the upper 5 cm sediment layer along the river's shoreline). Samples were collected from both sides of the river shoreline, upstream and downstream of the weirs, and placed into labelled Ziplock bags. Visible organic matter was removed from the

samples *in situ*, as organic materials have been shown to influence the recovery of microplastics (Nel et al., 2019; Mbedzi et al., 2020). The sediment samples were then stored in Ziplock bags and transported to the laboratory for further analysis. Upon arrival at the laboratory, the samples were dried at 60 °C for 72 hours before analysis.

Microplastic extraction from water and sediment samples

Water

To prevent contamination, all surfaces and equipment were cleaned with milliQ distilled water before laboratory analysis (Cuthbert et al., 2024). Furthermore, the air-conditioning was turned off during sample processing to prevent airborne microplastic contamination (Nkosi et al., 2023). Cotton lab coats and gloves were used throughout the laboratory procedures to maintain sterility and minimise the risk of microplastic contamination in both water and sediments. The surface water samples were filtered onto 2 µm mesh size cellulose membrane filter paper (47 mm in diameter) and immediately placed in labelled Petri dishes. The samples were left to dry, covered, at room temperature for 3 to 4 days before being identified and quantified for microplastics.

Sediment

The same procedure to prevent microplastic contamination in water was also used for sediment samples. The dried sediment samples were crushed and properly mixed before a subsample of 0.2 kg was sieved through a 2-mm mesh steel sieve to remove large debris and pebbles. The sieved residual sediment was weighed to be used to determine the number of microplastic particles per kilogram dry weight (dwt). Large microplastics (2–5 mm) that were found in the material left on the sieve were counted as part of the overall microplastic count. After sieving, the samples were placed in labelled 750 mL clean glass containers, and 70 mL of 30 % stabilised hydrogen peroxide was added to facilitate digestion of the sediment organic matter over a 24 h period. This process was followed by a microplastic density separation process using 500 mL of ZnCl₂ solution (density 1.68 g cm⁻³). Samples were shaken for 1 hour at 1500 rpm using an automated orbital shaker, allowing the trapped particles between the sediment particles to be released and suspended before the mixture was left to settle for 24 h. The mixture was then carefully decanted and filtered through a 2 µm cellulose membrane filter (Nel et al., 2018; Mbedzi et al., 2020), and the resulting solution was placed in a Petri dish for microplastic identification and quantification.

Microplastic identification and quantification from water and sediment samples

This study considered microplastics between 2 μm and 5 mm (Nkosi et al., 2023). After extraction, the samples were identified and quantified using an Olympus dissecting microscope at a magnification of $\times 200$. All suspected microplastics were examined and enumerated based on their colour (i.e., black, blue, red, yellow, green, white, transparent, other) and shape (fibres, fragments, film, beads, foam). The particles that possessed an unnatural colouration (e.g., multi-coloured, bright colourations) and an unnatural shape (e.g., perfectly spherical and sharp edges) were counted as microplastics (Mbedzi et al., 2020). A compact Fourier Transform Infrared Spectrometer (FTIR) ALPHA II (Bruker Nano GmbH, Berlin, Germany) was used for the polymer characterisation of potential microplastics identified under the microscope. The identified microplastics were quantified as the number of particles m^{-3} in water samples and as particles per kilogram of dry weight (dwt) in sediment samples.

Quality control

Quality assurance measures were applied during both field sampling and laboratory analysis to minimise contamination from external microplastic sources. In the field, samples were collected downwind using glass and metal equipment, with all tools rinsed in Milli-Q ultrapure water. Procedural blanks, including site blanks in glass jars, confirmed negligible airborne contamination, though minor contamination from sieve adhesives were identified and corrected during spectral analysis ($\geq 95\%$ match threshold). In the laboratory, strict contamination control was maintained by wearing cotton laboratory coats and gloves, covering samples with aluminium foil, minimising plastic use, and cleaning workspaces with ethanol. All solutions were prepared with filtered Milli-Q water, and procedural blanks indicated no detectable contamination. Extraction efficiency tests, using spiked neon fibres demonstrated 100 % recovery for water and 90 % for sediment, validating the reliability of the applied extraction protocols.

Sediment microplastic risk assessment

The microplastic indices proposed by Kabir et al. (2021) were utilised to evaluate the potential effects of microplastics in the natural aquatic environment. The microplastic contamination factor determines microplastic concentrations in relation to background levels (i.e., 20 particles kg^{-1} for our study), categorized as follows: low (<1), moderate (1–3), considerable (3–6), and very high (>6).

$$MpPLI_{river} = \sqrt[N]{SD_1 \times SD_2 \times \dots \times SD_N}$$

where $MpPLI_{river}$ is the microplastic pollution index for the river, which is the square root of site numbers for a river (nth root) of the total SD indices multiplied together. The SD allows for the standardisation, with a high degree of accuracy, of microplastic concentrations found in the river sediment for each system. Any site is polluted or in poor condition when $SD > 1$ and no pollution when < 1 .

Data analysis

The data was analysed using IBM SPSS Statistical software (version 30). A non-parametric test was employed to analyse the significant differences in microplastic abundance across seasons (i.e., cool-dry, hot-wet) and sites. Tukey's post hoc analysis was performed for all variables found to be significantly different ($p < 0.05$) across seasons and sites. ANOVA was used to calculate the differences in environmental variables. The PAST statistical software, version 4.03, was also utilized to calculate diversity indices (i.e., species richness, Shannon–Wiener diversity, Whittaker β -diversity, and evenness). where (i) species richness represents the total count of microplastic types from surface water and sediments at each site, (ii) Shannon–Wiener diversity reflects the types/species of microplastics and their frequency of occurrence, (iii) Whittaker β -diversity indicates the heterogeneity of microplastic types at a site during each season, and (iv) evenness represents the diversity of microplastics at a site based on their relative frequency (Dalu et al., 2021).

RESULTS

Microplastics in water

High microplastic abundances were observed in water during the cool-dry season, while the hot-wet season showed low total microplastic abundances (Table 3.1). A high microplastic abundance (mean 409.4 ± 582.11 particles m^{-3}) was observed upstream of weirs for the cool-dry season compared to the hot-wet season (mean 291.7 ± 316.1 particles m^{-3}). During the cool-dry season, fibres were the most dominant microplastic shape (mean 82.3 ± 154.5 particles m^{-3}), while fragments were the least dominant microplastic shape (mean 18.8 ± 34.9 particles m^{-3}) downstream. For upstream, a high fibre microplastic abundance (mean 185.8 ± 344.6 particles m^{-3}) was also observed, with fragments being the least dominant (mean 30.5 ± 55.7 particles m^{-3}) microplastic shape (Table 3.1). In the hot-wet season, fibres were the most

prevalent microplastic type for both upstream and downstream, with foam (mean 11.7 ± 23.5 particles m^{-3}) being the least common microplastic type downstream. Fragments (mean 14.1 ± 31.4 particles m^{-3}) was the least dominant microplastic types upstream. The colours of microplastics were predominantly transparent for both upstream and downstream (Table 3.1). Using an FTIR for verification, we observed that among the microplastics found in water samples, the most common polymers were low-density polyethylene (LDPE) and polypropylene (PP).

We observed significant differences for water among sites for fibres ($p = 0.007$), foam ($p > 0.001$), and film ($p = 0.002$), whereas for locations only film ($p = 0.050$) was found to be significantly different (Table 3.2). For microplastic colours in water samples, we observed significant differences among sites for black ($p = 0.010$), red ($p = 0.001$), yellow ($p = 0.009$), white ($p = 0.029$), and transparent ($p = 0.004$) colours, with locations and seasons having significant differences for black ($p = 0.010$) and other ($p = 0.010$), respectively (Table 3.2). Using Tukey's posthoc analysis we observed significant ($p < 0.05$) differences for fibres (Luvuvhu 2 vs Luvuvhu 3), foam (Luvuvhu 2 vs Mutshundudi 2, Mutshundudi 2 vs Luvuvhu 3), film (Luvuvhu 2 vs Lutanandwa, Luvuvhu 2 vs Mutshundudi 2), black (Luvuvhu 2 vs Lutanandwa), red (Luvuvhu 1 vs Luvuvhu 3, Luvuvhu 2 vs Luvuvhu 3, Luvuvhu 3 vs Mutshundudi 1), yellow (Luvuvhu 2 vs Mutshundudi 2, Mutshundudi 2 vs Luvuvhu 3), white (Luvuvhu 2 vs Mutshundudi 2), transparent (Lutanandwa vs Luvuvhu 2, Luvuvhu 2 vs Mutshundudi 2) and total (Lutanandwa vs Luvuvhu 2, Luvuvhu 2 vs Mutshundudi 2, Luvuvhu 2 vs Luvuvhu 3) microplastics in water across different locations (Figure 3.4).

Table 3.1 Mean ($\pm$ standard deviation) of microplastic abundance in water and sediment during the cool–dry and hot–wet season, upstream and downstream of weirs along the Luvuvhu River

Variable	Water (particles m ⁻³)				Sediment (particles kg ⁻¹ dwt)			
	Cool–dry		Hot–wet		Cool–dry		Hot–wet	
	Down	Up	Down	Up	Down	Up	Down	Up
<i>Types</i>								
Fibre	82.3 $\pm$ 154.5	185.8 $\pm$ 344.6	96.4 $\pm$ 139.3	148.2 $\pm$ 184.2	18.7 $\pm$ 14.3	36.6 $\pm$ 36.5	18.7 $\pm$ 15.2	34.1 $\pm$ 39.7
Foam	28.2 $\pm$ 70.3	30.5 $\pm$ 59.2	11.7 $\pm$ 23.5	16.4 $\pm$ 31.8	2 $\pm$ 4.5	3.7 $\pm$ 4.3	3.3 $\pm$ 11.5	2.9 $\pm$ 5.4
Fragment	18.8 $\pm$ 34.9	30.5 $\pm$ 55.7	28.2 $\pm$ 67.4	14.1 $\pm$ 31.4	5.8 $\pm$ 5.1	5.8 $\pm$ 5.9	3.3 $\pm$ 4.4	8.7 $\pm$ 12
Film	54.1 $\pm$ 82.3	115.2 $\pm$ 156.7	32.9 $\pm$ 53.3	94.1 $\pm$ 134.1	2 $\pm$ 3.3	6.6 $\pm$ 8.3	4.5 $\pm$ 5.4	22 $\pm$ 24.1
Beads	37.6 $\pm$ 69.9	47 $\pm$ 73.8	28.2 $\pm$ 65.9	18.8 $\pm$ 31.9	1.2 $\pm$ 3.1	4.5 $\pm$ 10.1	0.8 $\pm$ 2.8	5.4 $\pm$ 7.8
<i>Colours</i>								
Black	37.6 $\pm$ 92.1	94.1 $\pm$ 155.5	32.9 $\pm$ 58.7	51.7 $\pm$ 64.4	2.5 $\pm$ 4.5	5.8 $\pm$ 6.3	7 $\pm$ 8.3	6.6 $\pm$ 9.3
Blue	11.7 $\pm$ 18.7	35.2 $\pm$ 73.3	14.1 $\pm$ 31.4	9.4 $\pm$ 17.4	2 $\pm$ 3.3	5.8 $\pm$ 7.3	3.7 $\pm$ 6.7	5.8 $\pm$ 10.6
Red	9.4 $\pm$ 22.4	23.5 $\pm$ 60	44.7 $\pm$ 106.6	51.7 $\pm$ 103.6	2 $\pm$ 4.5	0.8 $\pm$ 1.9	1.6 $\pm$ 3.8	6.2 $\pm$ 11.7
Yellow	30.5 $\pm$ 60.8	23.5 $\pm$ 40.1	4.7 $\pm$ 19.4	9.4 $\pm$ 22.4	2.9 $\pm$ 3.9	2.5 $\pm$ 7.2	1.2 $\pm$ 3.1	13.7 $\pm$ 22
Green	18.8 $\pm$ 44.9	25.8 $\pm$ 78.6	7 $\pm$ 21.1	9.4 $\pm$ 17.4	1.2 $\pm$ 2.2	2 $\pm$ 2.5	0.4 $\pm$ 1.4	5.8 $\pm$ 11
White	40 $\pm$ 93.8	35.2 $\pm$ 67.6	16.4 $\pm$ 24.7	14.1 $\pm$ 28	3.3 $\pm$ 5.7	7.9 $\pm$ 9.4	3.3 $\pm$ 11.5	3.3 $\pm$ 4.9
Transparent	70.5 $\pm$ 117	141.1 $\pm$ 205.4	77.6 $\pm$ 121.2	145.8 $\pm$ 188.1	13.3 $\pm$ 13.3	30 $\pm$ 29.8	13.3 $\pm$ 12.3	31.6 $\pm$ 37.9
Other	2.3 $\pm$ 9.7	30.5 $\pm$ 60.8			2.5 $\pm$ 2.6	2.5 $\pm$ 3.3		
Total	221.1 $\pm$ 363.6	409.4 $\pm$ 582.1	197.6 $\pm$ 260.1	291.7 $\pm$ 316.1	30 $\pm$ 19.4	57.5 $\pm$ 43.9	30.8 $\pm$ 17.4	73.3 $\pm$ 61.9

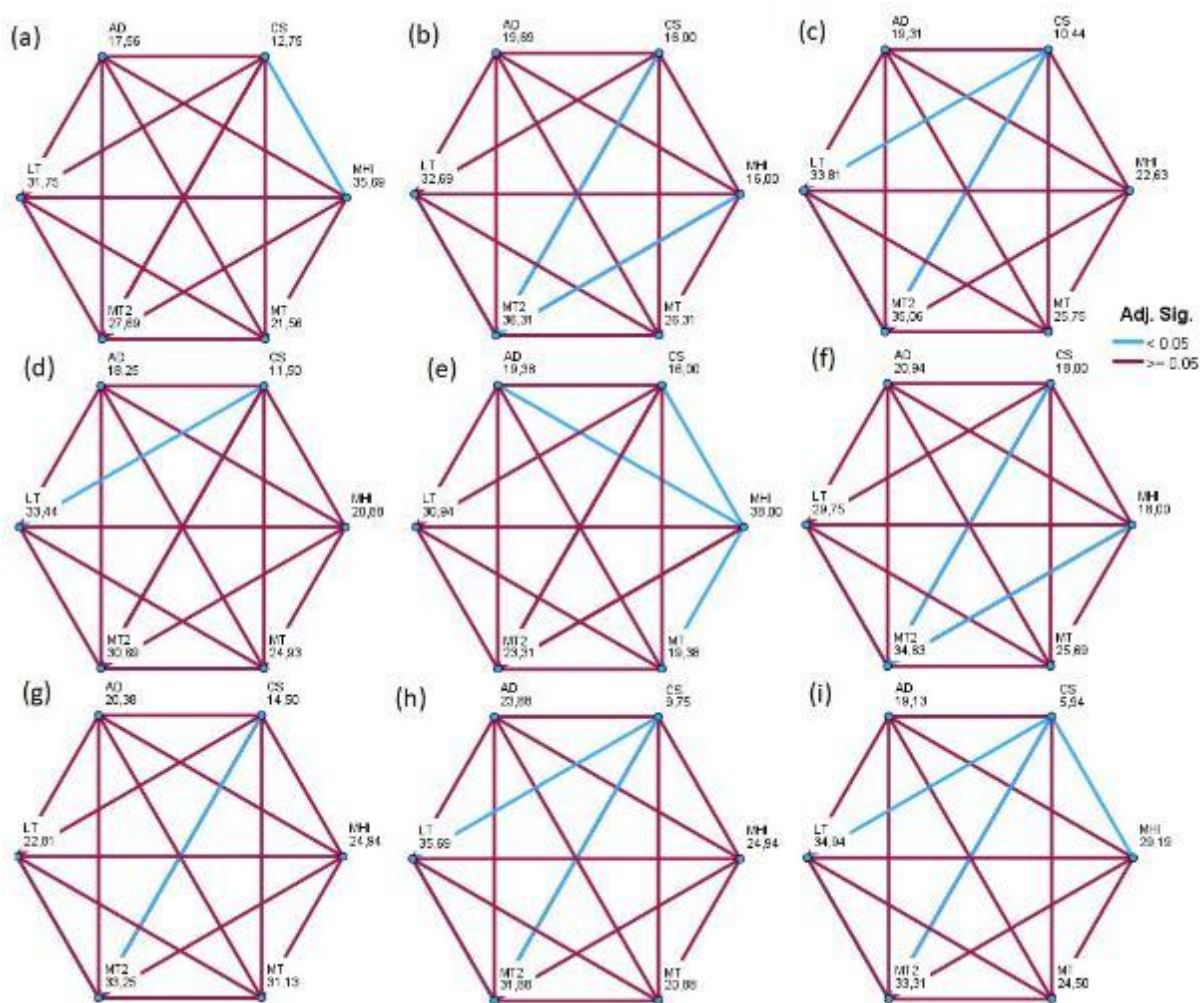


Figure 3.4 Pairwise comparisons of location in microplastic types and microplastic colours in water: (a) fibre, (b) foam, (c) film; (d) black, (e) red; (f) yellow, (g) white, (h) transparent and (i) total microplastics. Abbreviations: AD – Luvuvhu 1, CS – Luvuvhu 2, MHI – Luvuvhu 3, MT – Mutshundudi 1, MT2 – Mutshundudi 2, LT – Lutanandwa

Sediment microplastic abundances

High microplastic abundances were observed during the hot–wet season compared to low microplastic abundances observed during the cool–dry season for sediment (Table 3.1). During the cool–dry season, upstream had high microplastic abundances (mean 57.5 ± 43.9 particles kg^{-1} dwt), with low microplastic abundances (mean 30 ± 19.4 particles kg^{-1} dwt) being observed at downstream sites (Table 3.1). Fibres were the most prevalent shape downstream (mean 18.7 ± 14.3 particles kg^{-1} dwt) and upstream (mean 36.6 ± 36.5 particles kg^{-1} dwt). Beads were the least common microplastic type downstream, whereas foam was the least common microplastic type upstream (Table 3.1). Microplastic colours were dominated by transparent coloured microplastics both for upstream and downstream sites (Table 3.1).

During the hot–wet season, upstream of the weir had high microplastic abundances compared to downstream (Table 3.1). Fibres were the most prevalent microplastic type downstream (mean 18.7 ± 15.2 particles kg^{-1} dwt) and upstream (mean 34.1 ± 39.7 particles kg^{-1} dwt). Beads (mean 0.8 ± 2.8 particles kg^{-1} dwt) and fragments (mean 3.3 ± 4.4 particles kg^{-1} dwt) were the least common microplastic type downstream, whereas beads (mean 5.4 ± 7.8 particles kg^{-1} dwt) and foam (mean 2.9 ± 5.4 particles kg^{-1} dwt) were the least common microplastic type upstream. Transparent microplastics dominated the colour scheme for both downstream and upstream of the weirs (Table 3.1). A significant difference was observed among sites for fragments ($p = 0.008$), with locations having differences for film ($p = 0.009$) and beads ($p = 0.026$) (Table 3.2). For microplastic colours, significant relationships were observed among sites for blue ($p = 0.038$), with locations and seasons highlighting differences for transparent ($p = 0.045$) and other coloured microplastics ($p < 0.001$) (Table 3.2). Using an FTIR for verification, we observed that among the microplastics found in sediment samples, the most common polymers were polyethylene terephthalate (PEP) and polypropylene (PP).

Table 3.2 Variation among microplastic type, colours, and diversity indices with sites, location, and seasons in the Luvuvhu River system. Bold values indicate significance level at $p < 0.05$

Variables	Water			Sediment		
	Sites	Location	Season	Sites	Location	Season
<i>Microplastic type</i>						
Fibre	0.007	0.276	0.250	0.100	0.227	0.617
Foam	<0.001	0.637	0.529	0.554	0.129	0.210
Fragment	0.057	0.853	0.538	0.008	0.662	0.314
Film	0.002	0.050	0.478	0.143	0.009	0.059
Beads	0.140	0.788	0.149	0.760	0.026	0.722
<i>Microplastic Colours</i>						
Black	0.010	0.010	0.811	0.147	0.387	0.451
Blue	0.052	0.755	0.407	0.038	0.260	0.614
Red	0.001	0.390	0.116	0.517	0.715	0.556
Yellow	0.009	0.544	0.061	0.573	0.337	0.395
Green	0.159	0.654	0.724	0.567	0.080	0.524
White	0.029	0.954	0.626	0.521	0.087	0.920
Transparent	0.004	0.125	0.770	0.157	0.045	0.678
Other	0.826	0.076	0.010	0.164	0.799	<0.001
<i>Diversity indices</i>						
Microplastic richness	<0.001	0.737	0.801	0.055	0.027	0.219
Shannon–Wiener	<0.001	0.933	0.558	0.097	0.106	0.193
Evenness	0.773	0.491	0.081	0.157	0.078	0.125
Whittaker	0.004	0.726	0.641	0.147	0.320	0.177

Microplastic diversity indices

In water, we observed significant differences among sites for microplastic richness ($p < 0.001$), Shannon–Wiener diversity index ($p < 0.001$), and Whittaker β –diversity ($p = 0.004$) (Table 3.2). However, no significant differences ($p > 0.05$) were observed for evenness among sites, location, and season (Table 3.2). No significant differences in water microplastics were observed between sites, location, and season for the Shannon–Wiener diversity index, evenness, and Whittaker β –diversity. In sediment, a significant difference was observed between location and microplastic richness ($p = 0.027$) (Table 3.2). Using Tukey’s posthoc analysis, we observed significant ($p < 0.05$) differences for fragments (Lutanandwa vs Luvuvhu 3) and total (Luvuvhu 1 vs Luvuvhu 2) microplastics in sediments across different locations (Figure 3.5).

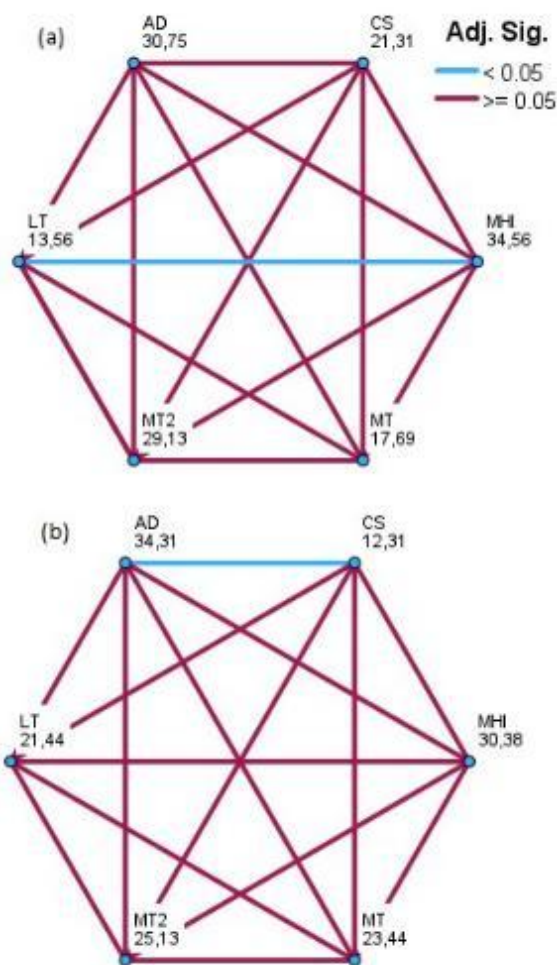


Figure 3.5 Pairwise comparisons of location in microplastic types and microplastic colours in sediment: (a) fragment; (b) total microplastics. Abbreviations: AD – Luvuvhu 1, CS – Luvuvhu 2, MHI – Luvuvhu 3, MT – Mutshundudi 1, MT2 – Mutshundudi 2, LT – Lutanandwa

Relationship between microplastics and selected physicochemical variables

In water, with an increase in ORP ($r = 0.37$, $p = 0.010$), there was an increase in yellow microplastic (Table 3.3). A decrease in temperature ($r = -0.35$, $p = 0.014$) led to an increase in other coloured microplastics. In sediments, an increase in pH ($r = 0.29$, $p = 0.043$) resulted in an increase in foam, whereas a decrease in ORP ($r = -0.30$, $p = 0.040$) led to an increase in film (Table 3.1). For total microplastics, decreases in TDS ($r = -0.54$, $p < 0.001$), salinity ($r = -0.55$, $p < 0.001$), conductivity ($r = -0.48$, $p = 0.001$), temperature ($r = -0.66$, $p < 0.001$), and resistivity ($r = -0.43$, $p = 0.002$) resulted in an increase in total microplastic abundances, whereas an increase in ORP ($r = 0.61$, $p < 0.001$) led to an increase in total microplastics. Additionally, an increase in TDS ($r = 0.38$, $p = 0.007$), salinity ($r = 0.40$, $p = 0.004$), temperature ($r = 0.52$, $p < 0.001$), and resistivity ($r = 0.29$, $p = 0.049$) resulted in an increase in black

microplastic colours, while a decrease in ORP ($r = -0.54, p < 0.001$) led to an increase in black microplastic colour. A decrease in TDS ($r = -0.42, p = 0.003$), salinity ($r = -0.39, p = 0.006$), conductivity ($r = -0.45, p = 0.001$), and temperature ($r = -0.46, p = 0.001$), and Resistivity ($r = 0.33, p = 0.024$) resulted in an increase in transparent coloured microplastics, whereas an increase in ORP ($r = 0.43, p = 0.003$) led to an increase in transparent coloured microplastics. Lastly, with an increase in salinity ($r = 0.29, p = 0.046$) and temperature ($r = 0.42, p = 0.003$), there was an increase in other coloured microplastics, whereas a decrease in ORP ($r = -0.43, p = 0.002$) resulted in an increase in other coloured microplastics (Table 3.3).

Table 3.3 Correlation analysis between microplastics and selected physicochemical variables.

The values in parenthesis are p -values next to the r -values

Microplastic	TDS	Salinity	Conductivity	pH	Temperature	Resistivity	ORP
<i>Water</i>							
Yellow	0.44 (0.250)	0.54 (0.234)	-0.30 (0.132)	0.35 (0.117)	0.29 (0.149)	0.77 (0.55)	0.37 (0.010)
Other	-0.40 (0.529)	0.22 (0.721)	0.35 (0.213)	0.43 (0.132)	-0.35 (0.014)	0.65 (0.097)	0.27 (0.461)
<i>Sediment</i>							
Foam	-0.29 (0.247)	0.54 (0.751)	0.41 (0.123)	0.29 (0.043)	-0.72 (0.147)	0.54 (0.157)	0.40 (0.125)
Film	0.37 (0.538)	0.27 (0.112)	0.28 (0.059)	-0.33 (0.722)	0.62 (0.421)	0.55 (0.244)	-0.30 (0.040)
Total	-0.54 (<0.001)	-0.55 (<0.001)	-0.48 (0.001)	0.45 (0.123)	-0.66 (<0.001)	-0.43 (0.002)	0.61 (<0.001)
Black	0.38 (0.007)	0.40 (0.004)	-0.42 (0.314)	0.35 (0.321)	0.52 (<0.001)	0.29 (0.149)	-0.54 (<0.001)
Transparent	-0.42 (0.003)	-0.39 (0.006)	-0.45 (0.001)	-0.55 (0.134)	-0.46 (0.001)	-0.33 (0.024)	0.43 (0.003)
Other	-0.29 (0.321)	0.29 (0.046)	0.42 (0.210)	0.42 (0.245)	0.42 (0.003)	0.33 (0.245)	-0.43 (0.002)

Microplastic pollution load index

In the cool-dry season, upstream sites showed high contamination factor values (range 6.6 to 26.9), with the exception of Lutanandwa which demonstrated a low contamination factor value. Downstream sites, including Lutanandwa, Luvuvhu 1, 2 and 3 showed high contamination factor values, while Mutshundudi 2 exhibited considerable contamination and Mutshundudi 1 showing low contamination. During the hot-wet season, the upstream sites: Mutshundudi 1 and 2, Luvuvhu 1 and 3 had high contamination factor values, whereas Lutanandwa and Luvuvhu 2 had low contamination levels. Luvuvhu 1 and 3 downstream sites recorded high contamination factors, while Lutanandwa, Mutshundudi 2, and Luvuvhu 2 had low contamination and Mutshundudi 1 showed moderate contamination. Additionally, the microplastic pollution load index values were consistently high in both the cool-dry and hot-wet seasons, highlighting significant microplastic pollution across all periods (Table 3.4).

Table 3.4 Sediment contamination factor values for the Luvuvhu River system, indicating the pollution load index. Contamination factor (CF) colour scale: low = CF <1 (green), moderate = CF 1–3 (yellow), considerable = CF 3–6 (orange), high = CF >6 (red)

Sites	Coo–dry		Hot–wet	
	Up	Down	Up	Down
Mutshundudi 1	6.6	0	6.1	2.5
Mutshundudi 2	20	5	7.9	0
Lutanandwa	0	6.5	0	0
Luvuvhu 1	10.6	18	48	7.5
Luvuvhu 2	15	9.6	0	0
Luvuvhu 3	26.9	15	30.6	11.1
Pollution load index	13.1	8.8	15.4	3.5

DISCUSSION

Our study described the characteristics and the spatial distribution of microplastics in water and sediment samples collected from the Luvuvhu River system. Compared to other freshwater systems worldwide, microplastic pollution in this area exhibits varying levels in both water and sediment. Generally, we observed high microplastic abundances in water during the cool–dry season. This could be caused by low water flow and water levels, which can cause microplastics to accumulate in the water surface. We observed high microplastic abundance upstream of weirs for both the cool–dry and hot–wet seasons. These observations were similar to Wicaksono et al. (2020) who found high microplastic abundances upstream of three weirs compared to the downstream sites. The microplastic abundances upstream of weirs may be caused by the trapping and blocking of microplastics by the weirs, resulting in the accumulation of microplastics.

The retention of microplastics upstream of the weirs can also be influenced by hydrology; for example, slower–moving areas, such as backwaters and pools, are frequently associated with lower turbulence and shear stress, which encourages the deposition of non–buoyant microplastics (Dahms et al., 2020). On the other hand, due to their higher velocities and turbulence, riffles and fast–flowing courses encourage the resuspension of previously settled microplastics (de Carvalho et al., 2021; Lu et al., 2023). We observed high microplastic abundances during the hot–wet season compared to low microplastic abundances observed during the cool–dry season for sediment and this was consistent with findings by Dalu et al. (2023) who observed high microplastic abundances during the hot–wet season for sediment along the Luvuvhu River system. The findings of the study are in contrast with findings from

Themba et al. (2024) who reported high microplastic densities in sediments during the cool–dry season in the Nandoni Reservoir. Rainfall events may have caused the deposition and resuspension of microplastics, leading to increased pollution during the hot–wet season (Zhang et al., 2020; Themba et al., 2024). According to Guo et al. (2021), sediments are more likely to be sinks for microplastics derived from terrestrial environments during the hot–wet season, due to increased rainfall and runoff, potentially resulting to their redistribution throughout the river and downstream environments. However, anthropogenic activities can result in an increase in microplastics diversity by introducing new plastic debris. We observed high microplastic abundances upstream, with lower microplastic abundances observed downstream for both the cool–dry and hot–wet seasons. This is consistent with a study by Wicaksono et al. (2020). Dahms et al. (2020) also observed high abundances upstream of the weir and a decreased abundance below it. Microplastic abundances at these two sites, coupled with stream characteristics such as an increase in general water velocity after the weir, suggest that stream characteristics can have a significant impact on microplastic abundances in different matrices (Dahms et al., 2020). Furthermore, water velocity and depth may influence the distribution of microplastics, suggesting that locations with reduced flow and high depth may allow microplastics to accumulate in the benthic sediments (Dahms et al., 2020). The abundance of microplastics in the upstream of the weirs is evidence that these systems play a significant role in microplastic retention, acting as microplastic sinks and accumulation zones.

Fibres were the most dominant microplastic shape, upstream and downstream of the weirs across seasons. Fragments were the least common type of microplastic during the cool–dry season for both upstream and downstream locations. In the hot–wet season, foam was the least common microplastic type downstream, while fragments were the least common microplastic types upstream. The findings of the study are consistent with findings from Themba et al. (2024) who reported high microplastic fibres in the Nandoni Reservoir found along the Luvuvhu River system. This is in contrast to a study by Dalu et al. (2023b), which highlighted that fibres and fragments were the most dominant microplastic types in the Luvuvhu River. Most fibres come from synthetic textiles (Boucher and Friot, 2017), and they are the most common and prevalent microplastic shape observed in aquatic environments (Wicaksono et al., 2020). We observed community members doing laundry in some river sites, and this was also a major contributor to the dominance of fibres in the weirs, as it bypasses wastewater treatment systems and becomes a direct source of pollution (Stanton et al., 2022). For example, synthetic fibres such as polyester, nylon, and acrylic are particularly problematic as they are made of

plastic, and the friction and agitation of washing cause these fibres to shed and accumulate in river systems. However, although community members were not using washing machines, they were scrubbing the clothes against bedrock, which can cause friction and release fibres into the water. Furthermore, the abundance of these fibres within the natural environment could be attributed to airborne deposition, fishing, and agricultural activities occurring in and/or near the Luvuvhu River (Dalu et al., 2024). According to Themba et al. (2024), the dominance of fibres is caused by fishing nets, synthetic fibres, and improper waste management, including illegal dumping of waste within the river system. Transparent microplastics dominated the colour scheme for both water and sediments (i.e., upstream and downstream) for both seasons which was consistent with the findings of Dalu et al. (2023a), Nkosi et al. (2023), and Themba et al. (2024). The microplastic colours help us identify their origin (Nkosi et al., 2023; Mutshekwa et al., 2025). It may also be that microplastics aged and lost their original colouration over time (Themba et al., 2024; Bhat et al., 2024). Studies (e.g., Zhao et al., 2022; Martinho et al., 2023; Junaid et al., 2024) have shown that microplastics age and change colour due to photoaging from ultraviolet light and oxidative processes, which initially cause whitening or discolouration but can progress to yellowing and/or tanning as pigments and antioxidants degrade and new chromophores form due to weathering. The original microplastic colour significantly influences its aging rate and ultimate colour change, with dark colours, such as black, being more resistant to aging due to pigments acting as ultraviolet shields, while lighter colours may show more pronounced degradation.

Low DPE and PP were the main polymers found in surface water, both of which are commonly used in fishing equipment, household items and packaging materials. Microplastic shape, colour, and polymer composition were discovered across seasons and sites, with fibres and transparent colours dominating. The investigation confirmed the presence of PET and PE in sediment, as well as LDPE and PP in water samples. The pollution load index found a high concentration of microplastics in the sediment of the Luvuvhu River system. The presence of PP and LDPE aligns with findings from other freshwater studies (e.g., Chen et al., 2024; Mutshekwa et al., 2025; Ranjani et al., 2021), where these polymers are frequently detected due to their low density, widespread use, and resistance to degradation. We also observed PET and PE in sediment samples. Most of the raw materials used in textile products, such as blankets and clothes, are PET in the form of polyester fibres (Chen et al., 2024). In the Luvuvhu River, communities wash their blankets and clothes in the river, generating a large number of PET fibres, which cause these fibres to accumulate in the river. Furthermore, the diversity index

showed significant differences among sites for microplastic richness, Shannon–Wiener diversity index, and Whittaker β –diversity, which suggests that the sources of microplastics for the Luvuvhu River varied across sites. These findings were consistent with those of Nkosi et al. (2023), who also found significant differences across seasons for the Shannon–Wiener index. In our study, agriculture, sewage effluences, anthropogenic activities, and illegal dumping were noted in the study sites, which may be another potential contributor for the high microplastic abundances.

Sediment microplastic densities also exhibited seasonal variation, with higher abundances during the hot–wet season. This variation could be attributed to high precipitation, which results in increased run–off and water flow, allowing microplastics to be transported into the rivers (Nkosi et al., 2023). This might explain the observed high microplastic loads in upstream sites of the weirs during the hot–wet seasons, as also indicated by the high sediment contamination factor values. We also observed high sediment contamination factor values in the cool–dry season, both upstream and downstream. This could be attributed to reduced water flows due to low rainfall, which allows microplastics and disturbed loose sediments to be deposited into the riverbed sediment (Li et al 2018; Dalu et al., 2021; Nkosi et al., 2023). suggesting that the Luvuvhu River is highly polluted by microplastics across seasons and sites. Our observations are consistent with a study by Dalu et al. (2023a) who reported high microplastic contamination factor risk for both seasons’ upstream sites (mean, hot–wet PLI 16.3 ± 6.0 ; cool–dry PLI 9.6 ± 6.1). Similarly, a high contamination factor risk was also observed downstream sites for both seasons (mean, hot–wet PLI 10.9 ± 4.2 ; cool–dry PLI 6.4 ± 0.2). Mbedzi et al. (2020) highlighted that spatiotemporal variations can be influenced by context, for example, areas with high population density might have a large microplastic abundances compared to areas with low population density. Furthermore, the dynamics of river flow can affect the seasonal spatiotemporal distribution of microplastics (He et al., 2020a).

CONCLUSION

There has been widespread attention in recent years regarding microplastic pollution in the environment. The study found that water and sediment samples from both upstream and downstream of the Luvuvhu River systems contained microplastics, demonstrating the extent of microplastic transportation and distribution. This was supported by the high microplastic in the risk assessments, which indicate that, in addition to the presence of blocking microplastics

by weirs, other sources of microplastic pollution include bathing and washing, illegal dumping sites, water abstraction, and riparian brick making. The upstream sites had high microplastic abundance compared to the downstream sites, suggesting that weirs play a role in the retention and transportation of microplastics. Fibres were the most dominant microplastic shape, upstream and downstream of the weirs, suggesting that Most fibres come from synthetic textiles. We observed community members doing laundry on some of the sites; this can also be a major contributor to the dominance of fibres in the weirs. The results of this study will be useful in informing water managers and other stakeholders on the management of plastic pollution and how to manage barriers such as weirs and their role in microplastic abundances in inland water.

CHAPTER FOUR: BARRIERS OR BURDENS?

INVESTIGATING THE ROLE OF RIVER WEIRS IN FISH MICROPLASTIC BIOACCUMULATION AND CONTAMINATION



Microplastic polymer identification at the University of Venda using an FTIR spectrometer.
Photo by Ayanda Xozmuti

ABSTRACT

Microplastic pollution in aquatic environments is a significant global issue that might interact with other stressors. River fragmentation is pervasive worldwide through anthropogenic barriers such as dams and weirs. These barriers may concomitantly contribute to the entrainment and concentration of pollutants, such as microplastics, which can be released following barrier removed initiatives. This study quantified microplastic abundances in fish species within the Luvuvhu River, South Africa, across two seasons (i.e., hot–wet, cool–dry), both upstream and downstream of weirs. Among the 215 fish (27 species) examined, microplastics were detected in 98.8 % of them. Microplastics in fish were dominant downstream of the weir during the cool–dry and hot–wet seasons. Particularly high microplastic abundances were found in *Anguilla marmorata* (range 0–29 particles), with fibres and transparent particles being the most prevalent. The results further showed high microplastic abundances in benthopelagic feeders, highlighting that habitat plays a great role in microplastic ingestion in fish. The most common polymer types found in fish were polypropylene (PP) and polyethylene (PE). Significant differences were observed across seasons, with concentrations highest in the hot–wet season. Multiple regression analysis revealed a significant positive relationship between fish standard length and total microplastic levels, which suggests fish consume more microplastics due to increased food demand as a result of growth or due to accumulations across their ontogeny. These results suggest that microplastics accumulate around riverine barriers, but that their concentrations are similar both upstream and downstream and are primarily driven by seasonality and biological differences.

Keywords: Microplastics, Fish, Injection, Weirs, accumulation, distribution, Luvuvhu River

INTRODUCTION

Plastic pollution is a widespread issue affecting both land and water. The production of plastic is accelerating as both developed and developing countries embrace a culture of use and disposal (Chen et al., 2022). The global consumption of plastics is rising steadily, due to their many desirable characteristics, including low weight, durability, formability, recyclability, and resistance to corrosion (Zhang et al., 2020). In 2020, global plastic production reached 367 million tons (Plastics Europe, 2021) and is projected to double within the next 20 years (Lebreton and Andrady, 2019). While some plastic waste is recyclable, an estimated 5 to 12 million tons of plastic waste are released into aquatic environments worldwide each year (Baalkhuyur et al., 2020). Consequently, plastic pollution has become a significant environmental issue.

Microplastics are tiny plastic particles measuring less than 5mm, which come from both primary and secondary sources (Thompson et al., 2004; Frias and Nash, 2019). Primary microplastics are intentionally manufactured for specific uses, such as microbeads, while secondary microplastics are formed when larger pieces of plastic break down into smaller fragments. This breakdown can occur due to various processes, including exposure to ultraviolet light, mechanical abrasion, and increases in temperature (Corcoran et al., 2009; Song et al., 2017). Microplastics are distributed throughout the environment by water, wind, and fauna, causing broad contamination of natural environments (Hale et al., 2020). An emerging environmental concern is the accidental consumption of microplastics by freshwater fish (Parvin et al., 2021; Parker et al., 2022; Dalu et al., 2023), with multiple reports documenting the ingestion of plastic debris across various fish species.

The small size of microplastics allows for their ingestion by a wide range of organisms, including shrimp (Cuthbert et al., 2024), bivalves (Zhu et al., 2021), zooplankton (Themba et al., 2024), and fish (Dalu et al., 2024) in freshwater environments. However, once ingested, microplastics can have numerous adverse effects on aquatic organisms. These effects may include gastrointestinal obstruction, impaired reproduction, growth, and asphyxia (Ramos et al., 2012; Li et al., 2020). Such ingestion poses serious dangers due to physiological disruptions and the potential for biomagnification within the food chain (Atugoda et al., 2021; Li et al., 2021). Fish that consume plastic debris may suffer from intestinal injuries (Pedà et al., 2016), which can reduce their ability to absorb nutrients. Furthermore, ingesting plastics can alter fish

behavior and hinder their ability to detect predators (Mattsson et al., 2015). Plastic ingestion also affects fish metabolic processes, including fat metabolism (Cedervall et al., 2012; Mattsson et al., 2015), and can interfere with their immune systems (Greven et al., 2016). Recent research (e.g., Atamanalp et al., 2023; Solomando et al., 2021) indicates that chronic exposure to microplastics can inhibit antioxidant enzyme activities in critical tissues, such as the brain, gills, and liver, in both marine and freshwater fish. Additionally, contaminants released from ingested plastics can lead to toxic effects, including hepatic stress in fish (Lu et al., 2016; Rochman et al., 2013). Given these potential dangers, microplastic pollution poses a significant threat to environmental health. However, there is still limited knowledge about the biological consequences of plastic debris ingestion in fish. The few available studies suggest that consuming plastic can be extremely harmful (Parvin et al., 2021; Dalu et al., 2023, 2024).

The accumulation of pollutants in aquatic environments may be aggravated by other anthropogenic stressors, such as riverine fragmentation. For centuries, barriers such as dams and weirs have been constructed in waterbodies worldwide, with substantial implications for ecological connectivity (Dolan et al., 2025). These barriers are increasingly viewed as a conservation concern given that they can impede dispersal of migratory fish species (Zarri et al., 2022). However, they also potentially act as zones for the accumulation of problematic entities such as pollutants and invasive species. Indeed, various studies (e.g., Dahms et al., 2020; Wang et al., 2020) have found that microplastics are more prevalent in areas upstream of the dams within water and biotic compartments. In recent years, particularly in North America and Europe, there has been a substantial increase in barrier removal rates, which perversely risks the release and dispersal of such problematic entities, such as microplastics, as barrier removal efforts rise (Dolan et al., 2025).

The Luvuvhu River is a significant water resource in Limpopo Province of South Africa, serving multiple purposes such as agriculture, forestry, domestic use and supporting the ecosystem (Ramulifho et al., 2019). Furthermore, the river has undergone riverbank construction and features several in-stream barriers, which influence the fate of pollutants in the system. Microplastic ingestion by freshwater fish in the river is also affected by the existence of significant in-stream barriers, such as dams and weirs (Ramulifho et al., 2019). However, research on the effects of small in-stream barriers, such as weirs, on microplastic ingestion by fish is limited. This study aims to determine the influence of weirs on microplastics ingestion by freshwater fish along the Luvuvhu River system within the cool–

dry and hot–wet seasons by comparing microplastic abundances and characteristics in fishes from above and below the weirs. We hypothesized that fish collected upstream of weirs will have higher concentrations of microplastics compared to those collected downstream, highlighting the potential role of the weirs in microplastics accumulation of fish.

MATERIALS AND METHODS

Study area

The study was conducted at six weirs located along the Luvuvhu River Catchment with an area of approximately 5,941 km² (Figure 1.1). The Luvuvhu River Catchment is located in the Vhembe District Municipality of the Limpopo Water Management area in the northeastern part of South Africa. The mean annual rainfall is 608 mm, while topography varies from 200 m to 1,500 m, which greatly influences rainfall and runoff distribution patterns within the catchment (Odiyo et al., 2015). The river is characterised by a hot and wet summer climate, with mild and dry winters. High rainfall occurs in the upper reaches where the Soutpansberg Mountains are located, with less rainfall moving east, in the lower reaches around the Kruger National Park. The Luvuvhu River and its tributaries include the Mutshundudi, Lutanandwa, Livhungwa, and Mutale rivers that rise in the Soutpansberg Mountains (Fouche, 2009; Nkuna and Odiyo, 2011). Land–use activities in the Luvuvhu River Catchment include forestry, agriculture, and settlements. The local climate throughout the summer and winter months are characterised by high (i.e., 40 °C) and low (i.e., 9 °C) temperatures, respectively (Munyai et al., 2025). The Luvuvhu River Catchment hosts a variety of aquatic and riparian ecosystems, supporting diverse fauna and endemic plant species (Brown, 2014).

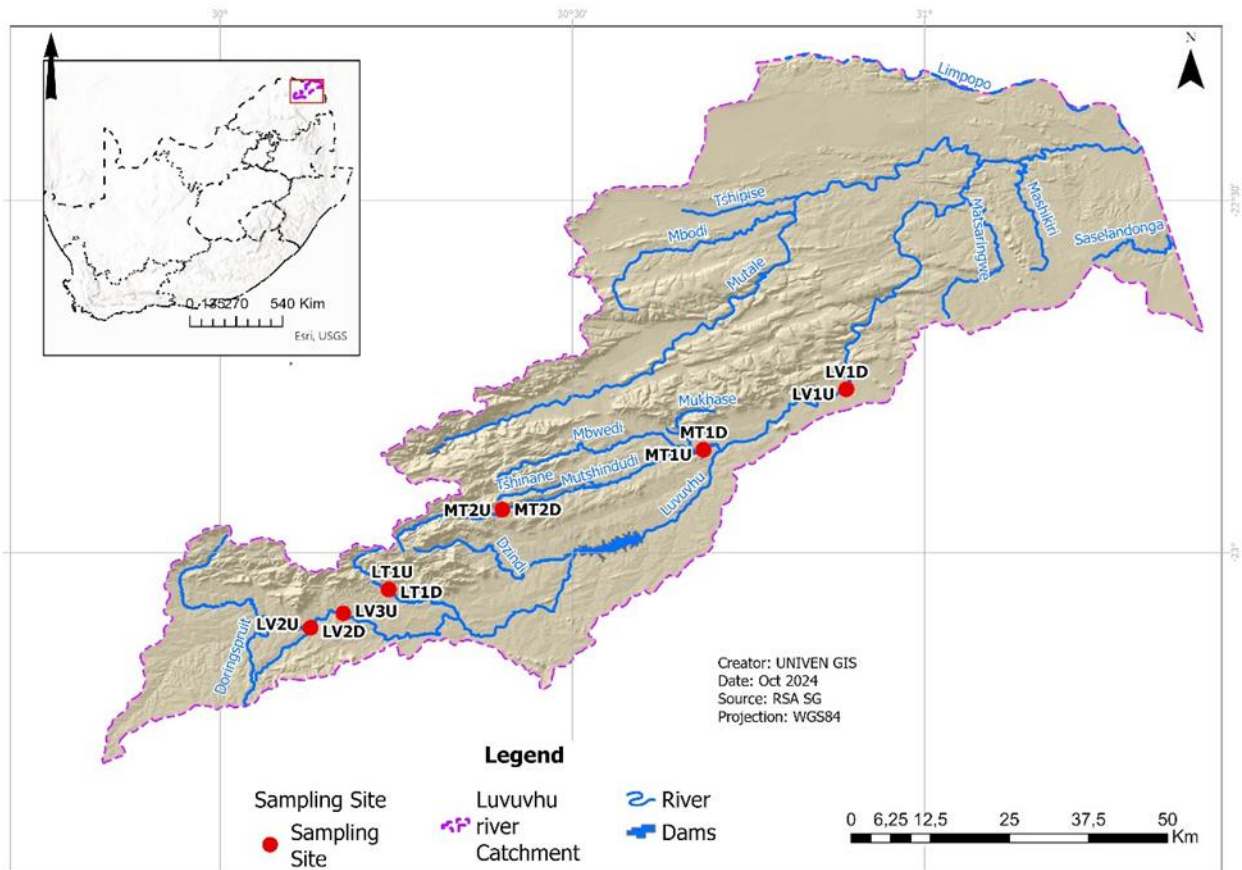


Figure 4.1 An area map showing selected study weirs within the Luvuvhu River catchment in the Limpopo Province, South Africa.

Water variables

Conductivity ($\mu\text{S cm}^{-1}$), pH, water temperature ($^{\circ}\text{C}$), and total dissolved solids (TDS) (mg L^{-1}) were measured *in-situ* at 6 sampling sites ($n=4$ per site) along the Luvuvhu River. Measurements were taken upstream ($n=2$) and downstream ($n=2$) of weirs across the two sample seasons (i.e., cool–dry, hot–wet), using a portable HANNA handheld multi–parameter. The parameters were taken before fish sampling to avoid disturbances.

Fish collection

Fish were sampled from each site, upstream and downstream of weirs across two different seasons: cool–dry (May 2024) and hot–wet (December 2024). Fish were sampled using a standardised backpack electrofisher (model: SAMUS725G, SAMUS, Poland) in the wadeable parts of each site. A pulsed DC current (300–600 V, 30–80 Hz, pulse width 30 μs –2.5 ms) was applied, stunning fish momentarily. The stunned individuals were netted, and upon netting/collection, the fish were immediately euthanised using an overdose of 40 mg L^{-1} of

clove oil that was added to the collection buckets containing sampled fish and river water (Mofu et al., 2021). This prevented the fish from regurgitating anything that they had consumed due to stress or being confined in a small area. Once euthanised, collected fish were preserved in 70 % ethanol in buckets, awaiting further analyses in the laboratory where they were identified and separated into their taxa using keys by Skelton (2024), before standard length was measured to the nearest cm.

Extraction and enumeration of microplastics in fish

Collected and identified fish were dissected to remove the guts, which were placed in separate labelled glass test tubes. The test tubes were covered with aluminium foil to avoid external contaminants. All equipment was thoroughly cleaned using milliQ distilled water, and care (i.e., wearing gloves, laboratory coats) was taken to prevent contamination. Nitric acid (55 %) was added to each test tube until the gastrointestinal tract and gills were fully submerged or covered, before being allowed to react for 6 h. Thereafter, samples were heated to 100 °C to break down any remaining tissue for 1 h, following Claessens et al. (2013) methodology. Each gut sample was then vacuum filtered onto a mixed cellulose ester hydrophilic sterile membrane filter (diameter 47 mm, pore size 0.45 µm), which was then placed into a clean petri dish, waiting for further microscopic analysis. Each filter was analysed under an Olympus compound light microscope at ×100 magnification, whereby microplastic particles were visually identified using colour and morphology before being enumerated (see Hidalgo–Ruz et al., 2012).

Microplastic identification and quantification from fish samples

The microplastics were classified into the following commonly used morphological shapes: film, fibres, foam, and fragments (Free et al., 2014). Colours were also classified as follows: black, blue, red, white, green, black, transparent, and other, which are representative of commonly occurring colours in most aquatic microplastic studies (Barrows et al., 2018). Particles were deemed to be microplastics if they possessed unnatural colouration (e.g., bright colouration, multi-coloured) and/or unnatural shape (e.g., sharp edges, perfectly spherical; Hidalgo–Ruz et al., 2012). As visual inspection alone was not adequate to characterise and exhaustively quantify microplastics, further physical analysis was utilised (Mintenig et al., 2017). A compact Fourier Transform Infrared Spectrometer (FTIR) ALPHA II (Bruker Nano GmbH, Berlin, Germany) was used for the polymer characterisation of potential microplastics

identified under the microscope. The identified microplastics were quantified as the number of particles per fish.

Data analysis

Analysis of deviance was used to compute main effect coefficients (Fox and Weisberg, 2019). Post-hoc comparisons were implemented as needed using Tukey-adjusted estimated marginal means (Lenth, 2024). Generalised linear mixed models were used to test for differences in fish richness and abundance in response to seasonality and barrier location, with individual barriers included as a random effect. Generalised linear mixed models were further used to analyse the effects of seasonality (i.e., hot-wet, cool-dry) and barrier location (i.e., upstream, downstream) on microplastic abundances in sampled fish, as well as their two-way interaction. The models on microplastics additionally included standard length to account for potential variation from the fish sample source and body size. Individual weirs were included as a random effect to account for repeated measures through space (above and below) and time (between seasons). Individual fish were further included as a random effect in microplastics models to control for repeated measures from different body parts within each fish. A Poisson family was used in the models (Brooks et al., 2017), with residual overdispersion and zero-inflation tested using simulation analyses (Hartig, 2024). In the case of overdispersion or zero-inflation, a negative binomial family was used instead, and residuals were rechecked. Separate models were fit for total microplastics, as well as for each particle type and colour.

RESULTS

Fish abundances

During the two study seasons, 215 fish taxa belonging to 27 species were collected from the Luvuvhu River systems, belonging to three groups of demersal (6 species), pelagic (1 species) and benthopelagic (20 species), with *Pseudocrenilabrus philander* being the most abundant (Table 4.1). The highest number of fish was caught during the cool-dry season (i.e., abundance 145), with only 70 fish being caught during the hot-wet season. Most of the fish sampled were juveniles, as indicated by their standard lengths (Table 4.1).

Table 4.1 Microplastic abundances observed in fishes across study seasons (cool–dry, hot–wet), downstream and upstream of weirs within the Luvuvhu River system, South Africa. FFG–functional feeding group, *N*–taxon abundance, SL– standard length.

Fish species	FFG	Seasons	<i>N</i>	SL	Fibre	Film	Foam	Fragment	Beads
Downstream									
<i>Pseudocrenilabrus Philander</i>	Benthopelagic	Cool–dry	6	5.4 ± 1.5	2.3 ± 1.5	0.3 ± 0.8	0.1 ± 0.4	0.5 ± 1.2	0.5 ± 1.2
<i>Tilapia sparrmanii</i>	Benthopelagic	Cool–dry	5	4 ± 2.4	3.6 ± 2.9	1.2 ± 1.6		1 ± 1.4	1.2 ± 2.6
<i>Labeobarbus marequensis</i>	Benthopelagic	Cool–dry	13	5.7 ± 1.3	1.5 ± 1.6	1.1 ± 1.5	0.3 ± 1.1	0.4 ± 0.9	0.9 ± 1.8
<i>Chiloglanis pretoriae</i>	Benthopelagic	Cool–dry	9	4.6 ± 0.9	2.4 ± 2.7	0.6 ± 1.1	0.5 ± 1.6	0.3 ± 0.7	3.1 ± 6.1
<i>Opsaridium peringueyi</i>	Benthopelagic	Cool–dry	5	4.9 ± 1.2	4.6 ± 5.1	2 ± 2.8	0.6 ± 1.3	0.4 ± 0.8	
<i>Enteromius eutaenia</i>	Benthopelagic	Cool–dry	3	4.7 ± 0.2	2 ± 2	0.6 ± 1.1	0.6 ± 1.1		
<i>Engraulicypris brevianalis</i>	Benthopelagic	Cool–dry	4	2.8 ± 0.8	2.5 ± 0.5	0.7 ± 0.9	1 ± 2	0.7 ± 1.5	0.5 ± 0.5
<i>Enteromius neefi</i>	Benthopelagic	Cool–dry	11	3.5 ± 0.3	1.2 ± 0	0.5 ± 0			0.4 ± 0
<i>Chiloglanis swierstrai</i>	Benthopelagic	Cool–dry	1	4.5 ± 0					
<i>Oreochromis mossambicus</i>	Benthopelagic	Cool–dry	3	4.8 ± 0.8	0.6 ± 1.1				0.3 ± 0.5
<i>Chiloglanis paratus</i>	Benthopelagic	Cool–dry	2	4.2 ± 0.3					
<i>Petrocephalus wesselsi</i>	Pelagic	Cool–dry	1	7 ± 0		4 ± 0		1 ± 0	
<i>Coptadon rendalli</i>	Benthopelagic	Cool–dry	7	5.4 ± 1.1	1.2 ± 2.5	1.1 ± 2	0.2 ± 0.7	0.1 ± 0.3	0.7 ± 1.2
<i>Enteromius anoplus</i>	Benthopelagic	Cool–dry	2	3.2 ± 0.3	0.5 ± 0.7				
<i>Enteromius trimaculatus</i>	Benthopelagic	Cool–dry	1	2.5 ± 0	6 ± 0	6 ± 0		4 ± 0	
<i>Labeo molybdinus</i>	Benthopelagic	Cool–dry	12	6 ± 2.8	1.7 ± 2.5	1 ± 1.5	0.5 ± 0.7	0 ± 0.2	1.2 ± 1.5
Upstream									
<i>Coptadon rendalli</i>	Benthopelagic	Cool–dry	8	3.6 ± 0.9	3.3 ± 3.4	1 ± 1.7	0.2 ± 0.4	0.1 ± 0.3	0.2 ± 0.4
<i>Oreochromis niloticus</i>	Benthopelagic	Cool–dry	1	9 ± 0	2 ± 0	1 ± 0		2 ± 0	
<i>Tilapia sparrmanii</i>	Benthopelagic	Cool–dry	6	3.5 ± 1.7	2.6 ± 3	1.3 ± 1.9	0.1 ± 0.4	0.3 ± 0.8	0.6 ± 1.2
<i>Enteromius anoplus</i>	Benthopelagic	Cool–dry	5	4.3 ± 0.9	4.8 ± 2.3	1.4 ± 1.3	0.4 ± 0.5	0.8 ± 0.8	0.2 ± 0.4
<i>Pseudocrenilabrus Philander</i>	Benthopelagic	Cool–dry	14	3.9 ± 1.2	5.1 ± 4.3	0.6 ± 0.7	0.3 ± 0.4	0.5 ± 1	1.1 ± 1.4
<i>Enteromius brevipinnis</i>	Benthopelagic	Cool–dry	2	4.1 ± 0.2	1 ± 0	1.5 ± 2.1	1.5 ± 0.7	0 ± 0	1.5 ± 2.1
<i>Enteromius unitaeniatus</i>	Benthopelagic	Cool–dry	8	3.3 ± 0.9	2.1 ± 1.8	1.4 ± 1.5	0.2 ± 0.7	0.1 ± 0.3	2 ± 3.3
<i>Marcusenius pongolensis</i>	Demersal	Cool–dry	1	7 ± 0		2 ± 0			
<i>Labeo molybdinus</i>	Benthopelagic	Cool–dry	5	8.8 ± 0.8	1.8 ± 1.3	0.6 ± 0.8	0.4 ± 0.5	0.6 ± 1.3	
<i>Glossogobius callidus</i>	Benthopelagic	Cool–dry	1	4 ± 0	4 ± 0				3 ± 0
<i>Enteromius viviparus</i>	Benthopelagic	Cool–dry	1	5.3 ± 0	1 ± 0	5 ± 0			
<i>Petrocephalus wesselsi</i>	Pelagic	Cool–dry	2	6.6 ± 0.9	0.5 ± 0.7	1 ± 1.4	2.5 ± 2.1		5.5 ± 6.3
<i>Tilapia sparrmanii</i>	Benthopelagic	Cool–dry	4	3.7 ± 0.2	2.7 ± 2.7	1.5 ± 1.7	0.2 ± 0.5	0.5 ± 0.5	0.5 ± 1
<i>Oreochromis mossambicus</i>	Benthopelagic	Cool–dry	2	5.9 ± 1.4	1.5 ± 0.7		0.5 ± 0.7		1.5 ± 2.1
Downstream									
<i>Pseudocrenilabrus Philander</i>	Benthopelagic	Hot–wet	4	6.9 ± 1	4.2 ± 1.2	1 ± 1.1	0.2 ± 0.5	0.7 ± 0.9	1 ± 2
<i>Labeobarbus marequensis</i>	Benthopelagic	Hot–wet	4	7.6 ± 1.6	3.5 ± 5.6	2.2 ± 2.8	0.2 ± 0.5	1 ± 1.1	2.5 ± 2.5
<i>Chiloglanis pretoriae</i>	Benthopelagic	Hot–wet	4	5.6 ± 1.1	2.2 ± 2	1 ± 0.8		1.5 ± 1.2	0.2 ± 0.5
<i>Chiloglanis paratus</i>	Benthopelagic	Hot–wet	3	6 ± 0.6	5 ± 7	1.3 ± 2.3	1 ± 1.7	0.6 ± 1.1	
<i>Labeo molybdinus</i>	Benthopelagic	Hot–wet	7	9.9 ± 2.5	5 ± 4.3	3.8 ± 1.6	0.7 ± 1.1	0.4 ± 0.7	1.7 ± 2.4
<i>Enteromius manicensis</i>	Benthopelagic	Hot–wet	3	7.5 ± 0.7	2 ± 2	0.6 ± 1.1	0.6 ± 1.1		0.3 ± 0.5
<i>Engraulicypris brevianalis</i>	Benthopelagic	Hot–wet	4	4.9 ± 0.6	3.2 ± 2.9	3.7 ± 2.7	0.5 ± 0.5	1 ± 1.4	0.5 ± 0.5
<i>Tilapia sparrmanii</i>	Benthopelagic	Hot–wet	4	7.1 ± 4.3	7.2 ± 5.6	3.7 ± 2.5	2.2 ± 2.8	2.7 ± 2.6	0.7 ± 0.9
<i>Micropterus nigricans</i>	Demersal	Hot–wet	2	4.8 ± 0.2	0.5 ± 0.7				
<i>Labeo cylindricus</i>	Benthopelagic	Hot–wet	4	10.7 ± 2	6.2 ± 3.8	1 ± 0.8	1 ± 0.8	1.2 ± 2.5	2 ± 0.8
<i>Micropterus punctulatus</i>	Demersal	Hot–wet	5	5.2 ± 1.7	0.8 ± 1	0.4 ± 0.8			0.2 ± 0.4
<i>Coptodon rendalli</i>	Benthopelagic	Hot–wet	3	8.8 ± 1	5.3 ± 3	1 ± 1	1.3 ± 1.1	1.3 ± 1.5	2.6 ± 0.5
<i>Anguilla marmorata</i>	Demersal	Hot–wet	2	19 ± 1.4	11.5 ± 7.7	3 ± 1.4	0.5 ± 0.7	4.5 ± 2.1	0.5 ± 0.7

Upstream									
<i>Coptadon rendalli</i>	Benthopelagic	Hot-wet	6	6.5 ± 1	4.1 ± 2.9	1.3 ± 1.9	1 ± 1.5	1.1 ± 2.4	0.5 ± 0.5
<i>Enteromius radiatus</i>	Benthopelagic	Hot-wet	1	5.5 ± 0	5 ± 0				1 ± 0
<i>Enteromius unitaeniatus</i>	Benthopelagic	Hot-wet	2	7.3 ± 0.1	11 ± 7	5 ± 1.4		0.5 ± 0.7	0.5 ± 0.7
<i>Enteromius brevipinnis</i>	Benthopelagic	Hot-wet	1	5 ± 0	4 ± 0	4 ± 0	1 ± 0	2 ± 0	3 ± 0
<i>Marcusenius pongolensis</i>	Demersal	Hot-wet	1	7 ± 0	1 ± 0	2 ± 0			
<i>Clarias gariepinus</i>	Demersal	Hot-wet	2	21 ± 12.7	11.5 ± 4.9	7 ± 5.6	2 ± 2.8	3.5 ± 2.1	0.5 ± 0.7
<i>Pseudocrenilabrus philander</i>	Benthopelagic	Hot-wet	6	8.1 ± 1.8	7.6 ± 5.5	0.8 ± 0.7	1 ± 1	0.8 ± 1.3	2 ± 1.6
<i>Tilapia sparrmanii</i>	Benthopelagic	Hot-wet	2	6.5 ± 3.5	4 ± 2.8	2.5 ± 2.1	1 ± 1.4	0.5 ± 0.7	

Microplastic ingestion by fish

A total of 215 fish were examined for the presence of microplastics in their digestive systems. In total, 1 423 microplastic particles were detected, with abundance ranging from 0 to 29 particles per fish. Microplastic abundance was higher during the hot-wet season (mean 5.2) compared to the cool-dry season (mean 3.0). Of the 145 fish caught during the cool-dry season, 98.8% contained microplastics, whereas in the hot-wet season, all fish sampled had microplastics in them (Table 4.1). Microplastics were not detected in two fish species: *Chiloglanis swierstrai* and *Chiloglanis paratus* (Table 4.1). High microplastic abundance was observed in *Anguilla marmorata*, with a range of 0 to 29 particles. During the cool-dry season, fibres were the most abundant type of microplastic, while foam and fragments were the least abundant. Similarly, in the hot-wet season, fibres were the dominant type of microplastic, with foam and beads being the least common.

During the cool-dry season, *Pseudocrenilabrus philander*, in the upstream site, had higher microplastic abundance (range 0–23 particles) compared to the downstream site (range 0–8 particles). Fibres were the most dominant microplastic type found upstream (mean 5.1 ± 4.3 particles), whereas foam was the least dominant (mean 0.3 ± 0.4 particles). Similarly, fibres were the most prevalent type downstream (mean 2.3 ± 1.5 particles), with foam again being the least dominant (mean 0.1 ± 0.4 particles) (Table 4.1). Transparent microplastics were the most frequently observed colour in *P. philander*, with white and yellow being the least dominant.

Labeo molybdinus showed higher microplastic abundances downstream of the weir (range 0–13 particles) compared to upstream (range 0–7 particles). Downstream, fibres were the most dominant microplastic type (mean 1.7 ± 2.5 particles), whereas fragments were the least dominant (mean 0 ± 0.2 particles). Upstream, fibres were also dominant (mean: 1.8 ± 1.3 particles), with foam being the least dominant (mean 0.4 ± 0 particles) (Table 4.1).

During the hot–wet season, *A. marmorata* had the highest microplastic abundance (range 0–29 particles), while *Micropterus nigricans* had the lowest (range 0–5 particles). Both species were only found downstream of the weir. In *A. marmorata*, fibres were the most dominant microplastic type (mean 11.5 ± 7.7 particles), whereas beads and fragments were the least dominant, each with a mean of (0.5 ± 0.7 particles). In *M. nigricans*, fibres were the only type of microplastic detected. Transparent particles were most common in *A. marmorata*, with white being the least dominant. In *M. nigricans*, black was the dominant colour. *Tilapia sparrmanii* downstream had higher microplastic abundances (range 0–26 particles) compared to upstream (range 0–9 particles). Fibres were the most dominant type in both locations, downstream (mean 7.2 ± 5.6 particles) and upstream (mean 4 ± 2.8 particles) (Table 4.1).

In the cool–dry season, all species observed were benthopelagic feeders, with the exception of *Petrocephalus wesselsi*, which is a pelagic feeder, and *Marcusenius pongolensis*, which is a demersal feeder (Table 4.1). Pelagic feeders were located both upstream and downstream, while demersal feeders were found only upstream. Among the benthopelagic feeders, there was a higher abundance of microplastics (range 0 – 28 particles). Pelagic feeders had a lower range of microplastics (range 0 – 5 particles), while demersal feeders had an average of 2 particles. In the hot–wet season, only benthopelagic and demersal feeders were present. Despite the higher abundance of benthopelagic species, demersal feeders had a greater number of microplastics (range 0 – 29 particles), compared to the benthopelagic feeders (range 0 – 24 particles).

Throughout both seasons, the majority of microplastics observed were transparent fibres and black particles (Table 4.1). Transparent microplastics were dominant in most fish species, except for *Oreochromis niloticus*, which was predominantly associated with yellow particles, while black particles were the most common in *L. molybdinus*. Using an FTIR for verification, we observed that among the microplastics found in fish, the most common polymers were polyethylene (PE) and polypropylene (PP).

Fish species–variation in microplastic ingestion

Fibres were the most abundant microplastic type overall (mean = 1.9 particles), whereas other types were half as abundant or lower on average. The effects of seasonality, location, body part, and length showed subtle difference among plastic types. While effects were mostly not significant (Table 4.2), seasonal effects were significant for fibres and fragments, with greater

abundances of these types in the hot–wet season. Numbers of fibres and beads were also affected by the fish body part sampled, with numbers found in gills significantly high than GIT for fibres ($p = 0.002$), but low for beads ($p = 0.001$).

Transparent microplastics were the most dominate overall (mean = 2.0 particles), followed by black coloured microplastics (mean = 0.5 particles). Seasonality significantly affected red, blue, and yellow microplastics, with more particle colours again detected during the hot–wet period. Location had only a significant effect on green particles, with more detected downstream than upstream. Body part had a significant effect on blue and red particles, with more microplastics detected in gills as compared to GIT ($p < 0.05$). Fish length significantly positively affected black particle abundances only.

The total number of microplastics collected from fish differed significantly between the seasons, with a mean total of 5.2 microplastics being sampled from fish during the hot–wet season, compared to 3.0 in the cool–dry season. There were no significant effects of barrier location and the seasonality–location interaction.

Overall, fish species richness was significantly affected by seasonality ($\chi^2 = 4.192$, $df = 1$, $p = 0.041$), with a high diversity detected in the cool–dry season (mean = 4.0 particles) compared to the hot–wet season (mean = 2.5 particles). There was no significant effect of barrier location ($\chi^2 = 1.941$, $df = 1$, $p = 0.163$) or their interaction ($\chi^2 = 0.107$, $df = 1$, $p = 0.743$) on fish diversity. Fish abundance was also affected by seasonality ($\chi^2 = 9.944$, $df = 1$, $p = 0.002$), with a significantly higher number sampled in the cool–dry (mean = 11.8) compared to the hot–wet (mean = 5.9) season. There was no significant effect of barrier location ($\chi^2 = 3.414$, $df = 1$, $p = 0.065$) or their interaction ($\chi^2 = 0.986$, $df = 1$, $p = 0.321$) on abundance.

Table 4.2 Generalised linear mixed model coefficients assessing microplastic abundances according to seasonality, weir location, body part, and standard length. Models were fit with either a Poisson or negative binomial error distribution, with a random effect structure to account for repeated measures over space and time, as well as of individual fish. Significant terms are shown in bold.

Microplastics variable	Term	χ^2	df	p-value
Total	Seasonality	7.781	1	0.005
	Location	0.526	1	0.468
	Body part	1.797	2	0.407
	Length	4.466	1	0.035
	Seasonality $\times$ Location	0.379	1	0.537
<i>Microplastic type</i>				
Fibre	Seasonality	5.811	1	0.016
	Location	1.419	1	0.233
	Body part	12.019	2	0.002
	Length	2.313	1	0.128
	Seasonality $\times$ Location	0.508	1	0.476
Film	Seasonality	3.160	1	0.075
	Location	0.442	1	0.506
	Body part	0.048	2	0.976
	Length	0.396	1	0.529
	Seasonality $\times$ Location	0.962	1	0.327
Foam	Seasonality	2.003	1	0.157
	Location	0.367	1	0.547
	Body part	5.738	2	0.057
	Length	2.695	1	0.101
	Seasonality $\times$ Location	0.057	1	0.811
Fragment	Seasonality	5.436	1	0.020
	Location	0.131	1	0.718
	Body part	3.143	2	0.208
	Length	4.013	1	0.045
	Seasonality $\times$ Location	0.116	1	0.733
Beads	Seasonality	0.417	1	0.518
	Location	0.412	1	0.521
	Body part	13.896	2	0.001
	Length	1.950	1	0.163
	Seasonality $\times$ Location	0.245	1	0.621
<i>Microplastic colour</i>				
Black	Seasonality	0.149	1	0.699
	Location	2.780	1	0.941
	Body part	4.891	2	0.087
	Length	4.538	1	0.033
	Seasonality $\times$ Location	0.556	1	0.456
Blue	Seasonality	8.310	1	0.004
	Location	1.111	1	0.292
	Body part	7.791	2	0.020
	Length	2.965	1	0.851
	Seasonality $\times$ Location	0.061	1	0.804
Red	Seasonality	5.037	1	0.025
	Location	0.041	1	0.840
	Body part	19.965	2	<0.001
	Length	1.817	1	0.178
	Seasonality $\times$ Location	0.381	1	0.537
Yellow	Seasonality	4.260	1	0.039
	Location	0.101	1	0.751
	Body part	0.052	2	0.974
	Length	0.814	1	0.367
	Seasonality $\times$ Location	0.005	1	0.944
Green	Seasonality	0.83	1	0.347

	Location	6.655	1	0.010
	Body part	0.529	2	0.063
	Length	0.039	1	0.843
	Seasonality × Location	0.698	1	0.403
White	Seasonality	1.045	1	0.306
	Location	0.179	1	0.673
	Body part	0.492	2	0.782
	Length	2.670	1	0.100
	Seasonality × Location	0.167	1	0.683
Transparent	Seasonality	2.639	1	0.104
	Location	1.791	1	0.181
	Body part	0.060	2	0.971
	Length	1.350	1	0.245
	Seasonality × Location	0.236	1	0.627

There was a strong, positive, and significant correlation between microplastic abundances and standard length for *Coptodon rendalli* ($r^2 = 2.73$, $p < 0.05$), *Enteromius neefi* ($r^2 = 0.54$, $p < 0.05$), *Enteromius trimaculatus* ($r^2 = 0.612$, $p < 0.05$), *Engraulicypris brevianalis* ($r^2 = 0.12$, $p < 0.05$), *Labeo molybidinus* ($r^2 = 0.65$, $p < 0.05$), *Labeobarbus marequensis* ($r^2 = 0.61$, $p < 0.05$), *Pseudocrenilabrus Philander* ($r^2 = 0.62$, $p < 0.05$) and *Tilapia sparrmanii* ($r^2 = 0.69$, $p < 0.05$), however no significant positive correlation was observed in microplastic abundance and standard length for *Chiloglanis pretoriae* ($r^2 = 0.04$, $p > 0.05$) (Figure 4.2).

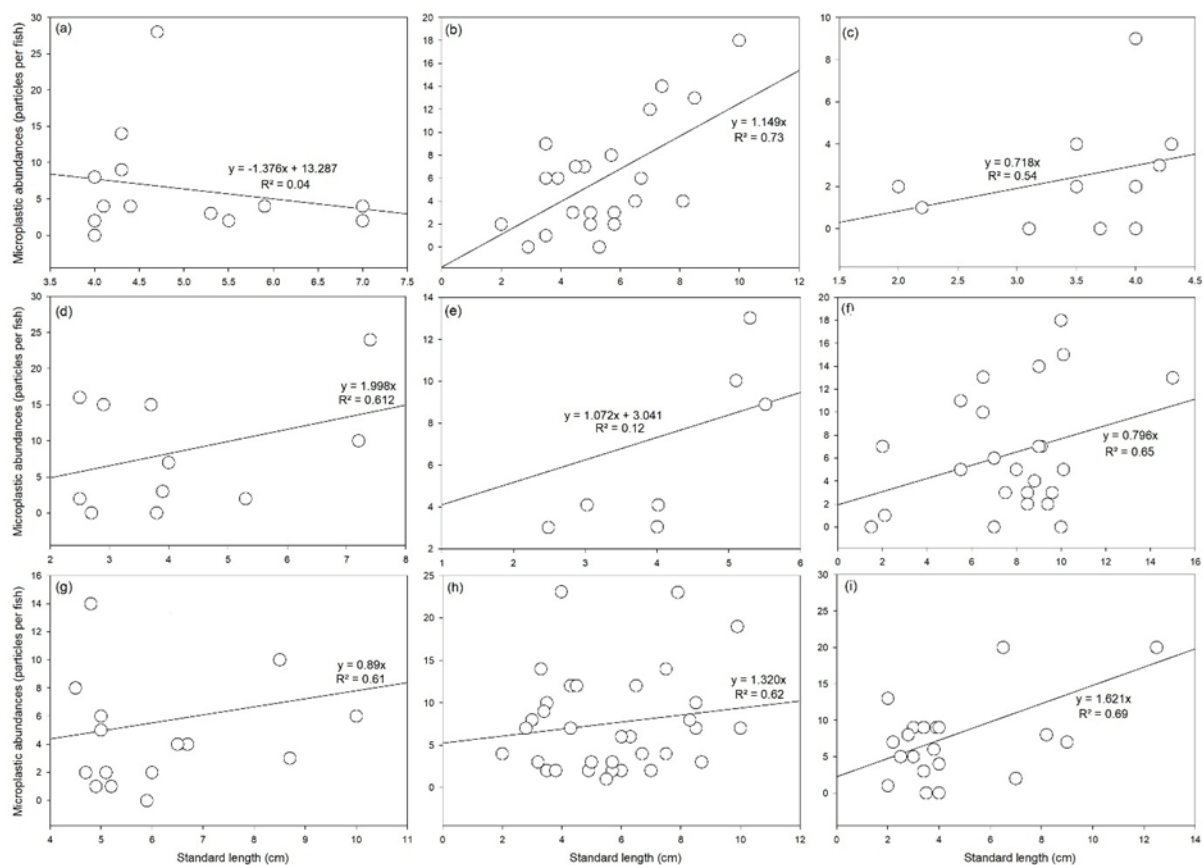


Figure 4.2 Relationship between microplastic abundance and standard length on (a) *Chiloglanis pretoriae*, (b) *Coptodon rendalli*, (c) *Enteromius neefi*, (d) *Enteromius trimaculatus*, (e) *Engraulicypris brevianalis*, (f) *Labeo molybidinus*, (g) *Labeobarbus marequensis*, (h) *Pseudocrenilabrus Philander*, and (i) *Tilapia sparrmanii*.

DISCUSSION

The study assessed the abundance and distribution of microplastics in freshwater fishes occurring upstream and downstream of weirs across two seasons (i.e., hot–wet and cool–dry). The results showed that 98.8 % of the fish were contaminated with microplastics, and this was consistent with Dalu et al. (2024), who reported 86.6 % of fish caught having microplastics in their digestive tract in the Nandoni reservoir south Africa. Similarly, Parvin et al. (2021) reported that 73.3 % of fish examined were polluted with microplastics in the gastrointestinal tracts (GIT) in Bangladesh. These findings can be further compared with previous studies by Hossain et al. (2019), Park et al. (2022), and Dalu et al. (2023), who reported the presence of microplastics in all fish sampled. The consistent detection of microplastics in fish from the Luvuvhu River system highlights the pervasive nature of plastic pollution in this aquatic

environment around weirs. The widespread occurrence of microplastics across various fish species further indicates the extent and spread of microplastic contamination in the river.

Microplastic abundance was higher during the hot–wet season compared to the cool–dry season. This increase may be attributed to a larger number of plastic particles being washed into the river during the hot–wet season. This strongly suggests that the deposition and/or resuspension of microplastics due to rainfall events or disturbances likely contributed to the elevated microplastic pollution in the river (Brahney et al., 2020; Zhang et al., 2020). However, other factors, such as anthropogenic activities, can also increase the diversity of microplastics, as these activities introduce a wider variety of plastic waste inputs (Zhang, 2017). In contrast with our findings, Dalu et al. (2024) reported that microplastic abundance in fish was generally higher during the cool–dry season compared to the hot–wet season in the Nandoni Reservoir, south Africa, suggesting that in the cool–dry season due to reduced water flows entering the reservoir, which led to increased concentrations of microplastic to be suspended or to sink which were then ingested by fish.

Fish habitat, feeding behaviour, and the prevalence of plastic litter in aquatic environments may significantly influence microplastic ingestion in fish (Woodall et al., 2014; Jabeen et al., 2017; Park et al., 2020a). In the current study, most of the collected fish were benthopelagic feeders, while six species were demersal feeders and one species was pelagic. The abundance of microplastics was notably higher in benthopelagic species compared to demersal and pelagic species. Benthopelagic fish inhabit and forage in waters just above the sediment, primarily feeding on zooplankton and benthic organisms (Reboa et al., 2022). This habitat offers benthopelagic fish a broad feeding ground and diverse dietary sources, which increases the likelihood of microplastic ingestion (Dalu et al., 2024). These findings align with a study by Bessa et al. (2018), which reported greater quantities of microplastics in benthopelagic fish compared to demersal fish. In contrast, Jabeen et al. (2017) and Parvin et al. (2021) suggested that demersal fish contained more microplastics than benthopelagic species, as demersal species live and feed on or near the bottom of the water column of the aquatic ecosystem. Additionally, studies by Rummel et al. (2016), Park et al. (2022) and Dalu et al. (2023) indicated that pelagic fish species had a high prevalence of microplastics in their stomachs than demersal feeders.

During the two study seasons, we found that fibres were the most dominant type of microplastic both upstream and downstream. The prevalence of fibres in all fish samples aligns with other studies that have reported fibres as the primary shape of microplastics in fish (Jabeen et al., 2017; Hossain et al., 2020; Dalu et al., 2023, 2024). Similarly, Neves et al. (2015) identified fibre microplastics as the dominant type in commercial fish from the Mendoza River and the Portuguese coast in Argentina. The abundance of these fibres in the natural environment may be attributed to several factors, including fishing nets, airborne deposition, and agricultural activities occurring in and around the Luvuvhu River system. One significant source of fibres is laundry washing; synthetic clothing is known to release tiny fibres into aquatic systems during washing (De Falco et al., 2019; Gaylarde et al., 2021). Fish in the Luvuvhu River may mistakenly identify these fibres as food and inadvertently ingest them while feeding, breathing, moving around, or filtering water (Markic et al., 2018; Capone et al., 2020) or by consuming contaminated prey (de Sá et al., 2015; Barboza et al., 2020; Dalu et al., 2023, 2024).

The shape of microplastics provides essential information about their source, indicating whether they are primary or secondary microplastics (Park et al., 2020b). Research shows that the quantity of secondary microplastics in aquatic environments exceeds that of primary microplastics (Lehtiniemi et al., 2018). Based on the microplastics identified in this study, it can be inferred that most of the extracted microplastics were secondary microplastics.

The largest share of microplastics was transparent, which is consistent with findings from numerous studies (e.g., Jabeen et al., 2017; Hossain et al., 2020; Parvin et al., 2021; Dalu et al., 2024). Around the Luvuvhu River, the surrounding communities practice fishing using monofilament fishing lines, and there is also a high incidence of littering of shopping and packaging bags. These plastic particles are mostly transparent, as colour degrades to transparency over time (Themba et al., 2024). While these microplastics are in the water column, they break down into smaller particles that fish might ingest. However, in contrast to our study, de Sa et al. (2015) reported that white microplastics were the most ingested plastic type by common goby *Pomatoschistus microps* juveniles from the Lima and Minho rivers in northern Portugal. Ory et al. (2017) found blue PE microplastics that are similar in colour to blue-pigmented copepod species that they commonly prey upon, which suggests that fish mistakenly ingested microplastics resembling their natural prey.

The FTIR spectrometry confirmed the presence of polyethylene and polypropylene among the 1 423 microplastic particles. The PE and PP are derived from plastic bags, packaging films,

and snack wrappers and may arise from coastal tourism activities (Hossain et al., 2019) and littering. Similarly, Yuan et al. (2019) discovered that PP and PE were the predominant polymer types in selected plastic particles found in fish in Poyang Lake, China.

Multiple regression analysis revealed a significant positive correlation between fish standard length and total microplastics, indicating that with an increase in standard length, there was an increase in the uptake of microplastics by the fish. This finding was consistent with previous research by Schmid et al. (2018), Su et al. (2018), and Dalu et al. (2023), which reported a positive correlation between standard length and high microplastic uptake in fish. The results of the present study suggest that larger fish have higher energy demands and, therefore, higher food intake, which increases the likelihood of direct and indirect ingestion of microplastics. However, this result contradicts the findings by Chen et al. (2022), who observed no correlation between microplastic abundance and fish standard length.

CONCLUSIONS

We examined the abundance of microplastics in 215 fish with 27 species within the Luvuvhu River, South Africa. Microplastics were detected in 98.8 % of freshwater fishes, with fibres being the most abundant microplastic type and transparent being the most dominant colour. The largest abundance of microplastics was identified from downstream of the weir compared to upstream. The most abundant polymer types were composed of PP and PE polymers, illustrating that municipal sewage, littering, agricultural activities, and waste fishing tools are the main sources of microplastics in the river. Benthopelagic fishes generally had higher microplastic abundance than pelagic and demersal fishes. The widespread microplastic abundance and ingestion by fish point to large-scale and widespread pollution in the Luvuvhu River. Furthermore, a significant correlation was observed between microplastic abundance and standard length, which indicates that with an increase in standard length, there was an increase in microplastic abundance.

CHAPTER FIVE: GENERAL SYNTHESIS



Potential sources of microfibres along the Luvuvhu River - women doing laundry on the bedrock. Picture by Tatenda Dalu

GENERAL DISCUSSION

The current study's findings revealed that microplastic contamination was ubiquitous throughout the Luvuvhu River system, indicating a continuous increase of these contaminants into aquatic habitats. This finding is consistent with prior research on microplastic contamination (e.g., Dahms et al., 2020; Mbedzi et al., 2020; Dalu et al., 2023, 2024) in aquatic ecosystems. Microplastic abundances from water, sediments and fish varied significantly between seasons. The cool–dry and hot–wet seasons showed high abundances of microplastics in water and sediments, respectively. This could be attributed to low water flow and water levels during the dry season, which can allow microplastics to concentrate on the water's surface and deposit in sediments (Chapter 3). Furthermore, heavy rainfall causes runoff in nearby areas, resulting in the movement of microplastics from land to river (Lima et al., 2014). Runoff transports microplastics, which might alter their abundance in rivers during wet seasons.

Freshwater fish are constantly at risk of eating microplastic particles due to their feeding habits (Jabeen et al., 2017; McNeish et al., 2018; Park et al., 2020a; Woodall et al., 2014). Thus, freshwater fish may be vulnerable to the detrimental effects of microplastics, including decreased nutrition, mortality, and impaired reproductive success (Chae and An, 2017; Green et al., 2017). The continuous identification of microplastics in fish from the Luvuvhu River system demonstrates the extent of plastic pollution in this aquatic environment near weirs, with 98.8% of fish containing microplastics (Chapter 4). The prevalence of microplastics across different fish species demonstrates the extent and distribution of microplastic contamination in the river. Microplastic abundances were greater during the hot–wet season than in the cool–dry season. The increase could be related to a greater amount of plastic particles being washed into the river during the hot–wet season. This strongly shows that microplastic deposition and/or resuspension caused by rainfall events or disturbances contributed to the river's high microplastic contamination (Brahney et al., 2020; Zhang et al., 2020). Other variables, such as anthropogenic activities (e.g., littering), might increase the diversity of microplastics by introducing a broader range of plastic waste inputs (Zhang, 2017).

Feeding behaviours are an important factor in microplastics ingestion (McNeish et al., 2018; Park et al., 2020a; Khan and Setu, 2022). with our study highlighting the prevalence of microplastics in benthopelagic feeders (Chapter 4). Other studies have discovered that

demersal fish had higher microplastic abundances than pelagic fish (Koongolla et al., 2020; Zhu et al., 2019b; Jabeen et al., 2017). Khan and Setu, 2022, and Parvin et al. (2021) found the dominance of microplastics distribution higher in demersal feeders, followed by benthopelagic and pelagic feeders. This variety could be caused by differences in fish species, different locations (sediments or surface water), different types of pollutants, or the migration nature of fish in different regions.

Anthropogenic influences have a significant impact on microplastic footprints in the environment. The morphology of microplastics reveals important information about their origin, indicating whether they are primary or secondary (Su et al., 2020; Auta et al., 2017; Park et al., 2020b). This study fundings observed fibres as the most common microplastic shapes found in surface water, sediment, and fish species and the current study suggested that they may have come from wastewater treatment plants and laundry washing in the Luvuvhu River system. The dominance of fibres in water and sediment might have led to fish ingesting them while feeding, breathing, moving around, or filtering water (Markic et al., 2018; Capone et al., 2020), resulting in their dominance in fish species. This was consistent with other research findings by Tien et al. (2020) and Liu et al. (2021) who reported the dominance of microplastics fibres in water, sediments and fish of the Fengshan and Dafeng rivers.

The predominant polymers identified in surface water were LDPE and PP, both of which are widely utilised in fishing equipment, household products, and packaging materials. The presence of PP and LDPE aligns with findings from other freshwater studies (e.g., Chen et al., 2024; Mutshekwa et al., 2025; Ranjani et al., 2021), where these polymers are frequently detected due to their low density, widespread use, and resistance to degradation. We also observed PET and PE in sediment samples. Most of the raw materials used in textile products, such as blankets and clothes, are PET in the form of polyester fibres (Chen et al., 2024). In the Luvuvhu River, communities wash their blankets and clothes along the riverbanks, generating a large number of PET fibres, which cause these fibres to accumulate in the river system. Furthermore, the predominance of PP and PE identified during fish study can be linked to the high demand, manufacturing, and subsequent disposal of these polymers in surface water and sediments. This study findings are consistent with a study conducted in freshwater fish (*Rutilus rutilus*) in the non-tidal region of the Thames River, where PE, PP, and polyester were found (Horton et al., 2018). Furthermore, in Japanese Anchovies *Engraulis japonicus* captured in

Tokyo Bay, PP and PE were the most prevalent synthetic polymer forms in the gastrointestinal tract (Tanaka and Takada, 2016).

The colour of microplastics may also reveal their source (Chinfak et al., 2021). It is observed that microplastics coloured green, yellow, red, and orange fade significantly after some time, however blue and transparent microplastics remain stable and retain their original colours (Su et al., 2023; Li et al., 2024). After 1 day and 3 days of exposure to the sun, yellow and red microplastics became transparent (Li et al., 2024). Multiple microplastic colours were observed in our study, with transparent particles being the most abundant in water, sediment, and fish. The neighbouring villages along the Luvuvhu River use single-use plastics such as textile materials, packaged bags, and fish baits, which are usually transparent or brightly coloured and appear to be food for zooplankton and other aquatic organisms (Zhao et al., 2020; Li et al., 2024). These characteristics of microplastics mislead aquatic creatures, which mistakenly consume them as food (Li et al., 2024). This process results in the introduction of microplastics into our food chain, which puts water, sediment, and fish at risk. The sun's impact also discolours the plastic substance, resulting in transparency (Haque et al., 2023). Our finding was similar to that of Hossain et al. (2021), who reported a 43% prevalence of transparent microplastics in Cox's Bazar. However, Hidalgo-Ruz et al. (2012) observed that white microplastics were the most common in the maritime environment, possibly due to weathering. Blue plastics are thought to be widely utilised in synthetic clothing around the world (Gago et al., 2018), as well as in mussel farms (Digka et al., 2018). In conclusion, microplastic pollution in the Luvuvhu River was strongly linked to residential activities (e.g., washing clothes, fishing, littering) and wastewater treatment plants.

The Luvuvhu River system has a long history of human alterations (Ramulifho et al., 2019), with weirs constructed along the system, endangering aquatic species (Marzin et al., 2012). Our study found that microplastics were more abundant in water and sediments upstream of weirs than downstream, indicating that weirs serve as sinks for microplastic pollution in the Luvuvhu River's sediment and surface water. These weirs are constructed on the Luvuvhu River, mostly in its upstream catchment, to create reservoirs to accommodate the ever-increasing needs of a growing human population for various water reasons (e.g., domestic, irrigation) (Mathivha et al., 2016; Ramulifho et al., 2019). However, many weirs in the Luvuvhu River lack fishways that allow for migration in both upstream and downstream sections (Heath et al., 2005). Thus, the dominance of microplastics in fish species upstream

and downstream of the weirs may be linked to the weir design, which traps microplastics in sediment and water, causing fish species to consume them.

GENERAL CONCLUSIONS

The current study provides new insights into microplastic pollution of surface water and sediments in the Luvuvhu River system, as well as indications of microplastic contamination in fish species, both upstream and downstream of weirs. The spatial distribution of microplastics in water and sediments was found to be influenced by flow velocity, tidal exchanges and discharges due to anthropogenic activities to which aquatic organisms may be exposed. The prevalence of low-density PE and PP in water, as well as the presence of PET only in sediments, demonstrated the importance of microplastic density in their environmental dispersal. The weir structure contributed to the amount of microplastics in the river, with microplastics being more prevalent at the upstream site for water and sediment, as well as in both sites for fish. This study is an important starting point for investigating the threat of microplastics to freshwater ecosystems, emphasising the need for researchers, environmentalists, and other stakeholders to collaborate and develop solutions to reduce current pollution levels in South African aquatic ecosystems and around the world. There is a need to promote new weir designs in our river systems that will allow for proper flow movement to avoid microplastic retention and sinks, facilitate fish migration, and prevent microplastic consumption by freshwater organisms around the weirs.

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