Ecological role of macroplastics as habitats for aquatic macroinvertebrates in the Crocodile River, Mpumalanga

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A research proposal submitted in partial fulfilment of the requirements for the BSc Honours in Environmental Science

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ABSTRACT

Plastic pollution has become a global ecological concern, whereby macroplastics (>5mm) are disrupting freshwater ecosystems. The study aimed colonisation patterns of aquatic macroinvertebrates on macroplastic substrates along the Crocodile River in Mpumalanga. The objectives were to assess the colonisation patterns of macroinvertebrates on macroplastic substrates along the Crocodile River and to compare the diversity, abundance, and composition of macroinvertebrate communities between macroplastic substrates and natural substrates. It was hypothesised that there will be a presence of macroinvertebrate communities on macroplastic debris within the Crocodile River across different seasons and that macroinvertebrate communities and diversity will significantly differ between macroplastic and natural substrates. The hypotheses were tested by computing diversity metrics including the abundance, species richness, evenness, Shannon-Weiner and Simpson diversity indices. They were compared between natural and plastic substrates across cool-dry (winter) and wet-cool (spring) seasons. Two Multivariate techniques were used: PERMANOVA to test for significant differences in biodiversity and SIMPER to identify which taxa contribute most to dissimilarity between substrates. A Shapiro-Wilk test was used to test the normality of Environmental variables, a paired t-test was used for normally distributed data and a Wilcoxon signed-rank test for non-normal data. Thereafter, a Principal Component Analysis (PCA) was used to visualise the seasonal patterns. The major findings revealed that macroinvertebrate communities were present on macroplastic debris; however, the differences in diversity and community structure between macroplastics and natural substrates were not pronounced. Significant variations were observed between seasons, with abundance, richness and Shannon-Weiner being significantly higher in the cool-dry season compared to the wet-cool season. The implications of these findings are significant; the macroplastic may act as a habitat to aquatic macroinvertebrates, just like other natural substrates and therefore, will indirectly perform an ecological role in the polluted rivers unexpectedly. The research will add to the knowledge of the ecological hazards of macroplastic pollution of riverine systems and will guide the monitoring strategies of bioindicators and ecological policies on environmental management to protect aquatic biodiversity.

Keywords: Macroplastic pollution, aquatic macroinvertebrates, freshwater ecosystems, diversity and community structure

TABLE OF CONTENTS

Contents

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DECLARATION

I, Venerate Mdaka, student number 220432627, declare that this dissertation titled "Colonisation of Macroplastics by Aquatic Macroinvertebrates along the Crocodile River, Mpumalanga" submitted in fulfilment of the requirements for the degree of Bachelor of Science Honours in Environmental Science at the University of Mpumalanga, is my own original work. I declare that this work has not been submitted previously for any degree or examination at this or any other university and all sources used are acknowledged and referenced.

Signature:  Date: 16 / 11 / 2025

CHAPTER ONE: INTRODUCTION

Background

Macroplastic pollution, characterised as plastic particles with a diameter exceeding 5mm has become a global concern due to its widespread distribution and abundance (Arthur et al., 2009; Lechthaler et al., 2020; Morales-Caselles et al., 2021; Petersen & Hubbart, 2021; Nyberg et al., 2023; Rakib et al., 2023; Tang et al., 2023; Gallitelli and Scalici, 2024). Macroplastic in aquatic ecosystems are largely because of anthropogenic activities such as disposal of food wrappers, take-away containers, improper waste management, industrial and commercial activities (Kershaw and Rochman, 2015; Oswald et al., 2025). Ultimately, their presence affects water quality and impact on aquatic ecosystems (Blettler et al., 2018; Azevedo-Santos et al., 2021). Freshwater ecosystems, including the Crocodile River in Mpumalanga, South Africa, are affected by macroplastic pollution (Mashamba., 2023; Dalu et al., 2025).

The interactions between macroplastics and aquatic macroinvertebrates are not well understood in river ecosystems, including how different types and sizes of plastics affect macroinvertebrate colonisation (Hoellein et al., 2024), as well as the potential impact of plastic pollution on ecosystem processes (Lamb et al., 2020). The study will assist in understanding the relationship between macroplastic and aquatic macroinvertebrates.

Problem Statement

Macroplastic pollution is a growing concern in the Crocodile River due to the detrimental effects it poses to aquatic macroinvertebrates (Dalu et al., 2025). However, there are limited studies about the influence of macroplastics pollution on aquatic macroinvertebrates colonisation in freshwater ecosystems (Ali et al., 2025) including the Crocodile River. Research has highlighted potential lethal effects of macroplastics pollution within the Crocodile River, including contamination of aquatic ecosystems by toxic chemicals derived from macroplastics (Dalu et al., 2025).

Their findings revealed notably higher microplastic abundances during the autumn season, with counts reaching 338 items, contrasting with the unexpectedly lower counts recorded during spring and summer, which ranged from 243 to 263 items. Furthermore, their research highlighted a consistent dominance of specific plastic types across all surveyed sites and seasons. Plastic bags and films were identified as the most prevalent category by proportion, while polypropylene was the most abundant polymer type identified analytically. This pattern suggests persistent sources and the widespread use of these materials in the local environment.

Macroplastic colonisation by macroinvertebrates results in the development of biofilm (algae, protozoa, and fungi) because the surface of the plastic becomes rougher providing safer refuge, and there's high food availability (Gallitelli et al., 2023; Ferreira, 2024).

Macroplastic contamination in water bodies is associated with several ecological hazards to macroinvertebrates and aquatic biodiversity (Azevedo-Santos et al., 2021). Among the documented effects, there are ingestion of plastic particles, obstructed digestive tracts and reduced feeding efficacy, entanglement in larger plastic debris, and consequent impeded movement or death, leaching of toxic additives (Obuzor et al., 2023) and absorbed pollutants (Dey et al., 2024) in degrading plastics, which leads to bioaccumulation and hampered physiological functions (Gall & Thompson, 2015; Van Emmerik, 2020. In the context of the Crocodile River, such impacts are particularly concerning for benthic macroinvertebrates, which play a central role in nutrient cycling, organic matter breakdown, and as prey for higher trophic levels (Ozkan., 2024). Reduction in the diversity and abundance of macroinvertebrates under the influence of exposure to macroplastic may thus have an adverse effect on ecosystem functioning, decrease water quality regulation, and disrupt food webs, eventually leading to a decrease in the ecological integrity of the river system (Green., 2020).

Justification of study

Aquatic macroinvertebrates play a significant role ecologically through nutrient cycling, material transport, and decomposition (Wallace & Webster., 1996; Nieto et al., 2017), processing organic matter, and serving as a food source to numerous organisms

(Nieto et al., 2017). It is important to study macroinvertebrates because they are used to determine the biotic integrity of aquatic ecosystems (Kerans & Karr, 1994).

This study intends to fill this gap by examining the colonisation relationship and interdependence between macroplastics and macroinvertebrates in the Crocodile River. It will analyse the colonisation trends of macroinvertebrates on macroplastics and natural substrates to better understand how macroplastic pollution affects macroinvertebrate's colonisation patterns, and thus their diversity.

Aim and Objectives

The study aimed to assess colonisation patterns of aquatic macroinvertebrates on macroplastic substrates along the Crocodile River in Mpumalanga. The objectives were to assess the colonisation patterns of macroinvertebrates on macroplastic substrates along the Crocodile River and to compare the diversity, abundance, and composition of macroinvertebrate communities between macroplastic substrates and natural substrates.

Hypothesis

The study hypothesised that there will be a presence of macroinvertebrate communities on macroplastic debris within the Crocodile River across different seasons and that macroinvertebrate communities and diversity will significantly differ between macroplastic and natural substrates.

CHAPTER TWO: LITERATURE REVIEW

Macroplastic pollution

The single-use plastics is the most frequently encountered form of waste in rivers, outnumbering other waste types (Crosti et al., 2018; Castro-Jiménez et al., 2019; González-Fernández et al., 2021; Cesarini et al., 2023; Gallitelli et al., 2023). Plastics function like sponges, absorbing and carrying contaminants, heavy metals, invasive species, and antibiotics (Zettler et al., 2013; Naik et al., 2019; Joel et al., 2021; Joo et al., 2021; Liu et al., 2021; Gallitelli et al., 2023).

Recent findings indicate that freshwater ecosystems not only convey macroplastics to marine settings but also retain some of these plastics, stopping them from entering the oceans (Horton et al., 2017; Lebreton et al., 2017; Schmidt et al., 2017). The existence of macroplastics in river systems shows that they serve as macroplastic reservoirs, since merely 1% of macroplastics are found in oceanic environments (Corcoran et al., 2015; van Sebille et al., 2015; Wang et al., 2018; Schwarz et al., 2019).

A variety of elements affect the movement, decomposition, and durability of macroplastics in freshwater environments, including the shape and dimension of macroplastics, kind of macroplastics, biofouling present on macroplastics, and hydrological characteristics (Chen et al., 2019; Schwarz et al., 2019). The characteristics of macroplastics, such as polymer type, dimensions, form, and density (Ryberg et al., 2019), determine their durability in river systems (Chen et al., 2019; Schwarz et al., 2019).

Macroplastics are non-polar, which enables them to readily facilitate the proliferation of micro- and macro-organisms (Amaral-Zettler et al., 2021; Gallitelli et al., 2023). Despite existing studies, there is still scarce knowledge regarding the colonisation of macroplastics in freshwater environments, which could be harmful to aquatic organisms (Blettler et al., 2019; Blettler and Mitchell, 2021) apart from the relationships between plastics and microorganisms (Zettler et al., 2013; Oberbeckmann et al., 2018; Wang et al., 2021). Integrating solutions to macroplastic pollution

Plastic pollution in freshwater habitats constitutes a serious issue as it has accumulated over time within riverine environments (Williams and Simmons, 1996), while data on plastic monitoring in freshwater systems is scarce. Human activities, such as discarding plastic waste on land and adjacent to river locations, are the main drivers of plastic contamination in freshwater ecosystems (Horton et al., 2017).

Inadequate waste management, analogous to coastal regions, has led to plastic pollution in rivers (van der Wal et al., 2015; González et al., 2016). The elevated levels of macroplastics result in animals consuming macroplastics, a fact corroborated by multiple studies (Sanchez et al., 2014; Faure et al., 2015; Biginagwa et al., 2016; Pazos et al., 2017). This shows the necessity for increased scientific focus to address the problem of macroplastics in freshwater ecosystems (Eerkes-Medrano et al., 2015; Lebreton et al., 2017; Li et al., 2017).

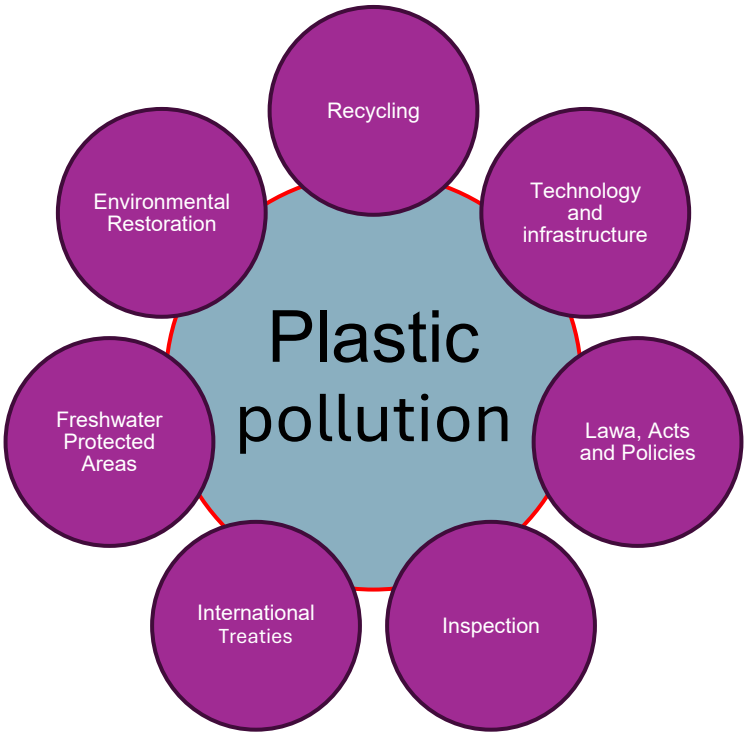

Diagram 1: The flow chart shows interconnected and interdependent actions that are needed to address plastic pollution in aquatic ecosystems (Azevedo-Santos et al., 2021)

The above diagram 1 highlights the mitigation measures and strategies that can be adopted by societies, companies and project managers supported by the study of (Azevedo-Santos et al., 2021). Societies need to integrate mitigation measures to remove macroplastics and avoid incorrect disposal of waste (Table 1). Mitigation strategies will include ecological rehabilitation, correct disposal, and strict regulation (Azevedo-Santos et al., 2021)

Table 1: Laboratory report of macroinvertebrates affected by macroplastics ingestion (Azevedo-Santos et al., 2021)

Freshwater macroinvertebrates that were negatively affected by plastic ingestion

Group	Species	Effects	Reference
Crustacean	*Daphnia magna Straus, 1820*	Sublethal & Lethal	Jemec et al. (2016) and Rehse et al. (2016)
Crustacean	*Daphnia pulex Leydig, 1860*	Sublethal	Liu et al. (2018)
Crustacean	*Hyalella azteca (Saussure, 1858)*	Sublethal & Lethal	Au et al. (2015)
Mollusk	*Corbicula fluminea (Mu¨ller, 1774)*	Sublethal	Guilhermino et al. (2018)
Mollusk	*Dreissena polymorpha Pallas, 1771*	Sublethal	Magni et al. (2018)
Cnidarian	Hydra attenuata Pallas, 1766	Sublethal	Murphy and Quinn (2018)

Azevedo-Santos et al., (2021) research indicates laboratory evidence indicating that aquatic macroorganisms like freshwater crustaceans are susceptible to interaction with diverse types of plastics which includes 206 freshwater organisms that were affected through entanglement and ingestion of macroplastics. The detrimental effects of macroplastics on crustaceans can be both lethal and sublethal and ghost nets affect freshwater decapod crustaceans through entanglement (Azevedo-Santos et al., 2021). Table 1 show six groups of macroinvertebrates were found ingesting macroplastics including introduced invasive species (Choelho et al., 2018). The negative effects of plastic material on macroinvertebrates may be lethal or sublethal (Table 1). Macroinvertebrates can uptake plastics (Hurley et al., 2017) and the individuals may contain an effective of plastic material into aquatic food webs

(Azevedo-Santos et al., 2021) through bioaccumulation and biomagnification (Tanaka et al., 2013; Mellink et al., 2022).

According to the research report of Dalu et al., (2025) conducted in the Crocodile River, they collected macroplastic waste that was 1134 with a high number of macroplastics collected in autumn and a smaller number of macroplastics in spring. The most occurring plastic waste that was collected in the Crocodile River were food wrappers, plastic bags, and plastic bottles (Dalu et al., 2025). The least macroplastic waste that was collected included containers of peanut butter and yoghurt, cigarettes butts and boxes, red tape, and bubble pipes (Dalu et al., 2025). The most common plastic polymer found in the Crocodile River was Polystyrene ranging between 5.3% and 53.3% (Dalu et al., 2025).

CHAPTER THREE: MATERIALS AND METHODS

Ethical clearance

The University of Mpumalanga School of Biology and Environmental Sciences Research Ethics Committee granted ethical approval for the study (Reference number: UMP/Mdaka/220432627/BIO/BScHons/2025/1).

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Creating Opportunities

Ms E Kola

School of Biology and Environmental Sciences

Mbombela Campus.

Dear **Venraate Tsuxeko Mdaka**

Protocol Reference Number: UMP/Mdaka/220432627/BIO/BScHons/2025/1

Project Title: Colonisation of macroplastics by aquatic macroinvertebrates along the Crocodile River

Approval Notification: In response to your application received on **13/05/2025** The Research Ethics Committee-Animal Sciences has considered the above mentioned application and the protocol has been granted **FULL APPROVAL**.

Any alteration/s to the approved research protocol i.e. Questionnaire/Interviews Schedule, Informed Consent form, Title of the project, Location of the study, Research Approach and methods must be reviewed and approved through the amendment/ modification prior to its implementation. In case you have further queries, please quote the above reference number.

PLEASE NOTE: Research data should be stored securely in the School/ division for a period of 5 years.

The Ethical Clearance certificate is only valid for a period of 3 years from date of issue. Thereafter, Recertification must be applied for on an annual basis.

Wishing you the best with your study.

Yours faithfully,

Ms E. Kola: SEC (Chair)

Cc: Research Office Administrator: .....

Cc: Faculty Research Committee Chair: .....

DECLARATION OF INVESTGATOR(S)

I/We fully understand the conditions under which I am/we are authorised to carry out the abovementioned research and guarantee to ensure compliance with these conditions. I agree to completion of a yearly progress report.

..... 15/05/2025

Signature Date

Study area

The study was conducted in the Mbombela region, in selected tributaries of the Crocodile River that runs through Mpumalanga province in South Africa (Figure 1). The Crocodile River is a vital waterway in South Africa, supporting diverse river ecosystems and serving as a dependable water source for Nelspruit (Soko, 2021; Nkosi et al., 2023). The Crocodile River in Nelspruit is known as Lower Crocodile River which is a sub-catchment of the Inkomati River Basin which incorporates Mpumalanga, Swaziland, and Mozambique. Mpumalanga encompasses three sub-catchments which are Komati, Crocodile and Sabie (DWA e al., 2013). The river originates from the Steenkamp mountains and flow through the region before merging with Komati River at Komatipoort (Waalewijn et al., 2005; DWA et al., 2013) and covers a total catchment area of 10,446 km² and a length of 326 km consisting large and smaller tributaries with Elands and Kaap River as the main tributaries (DWA et al., 2013).

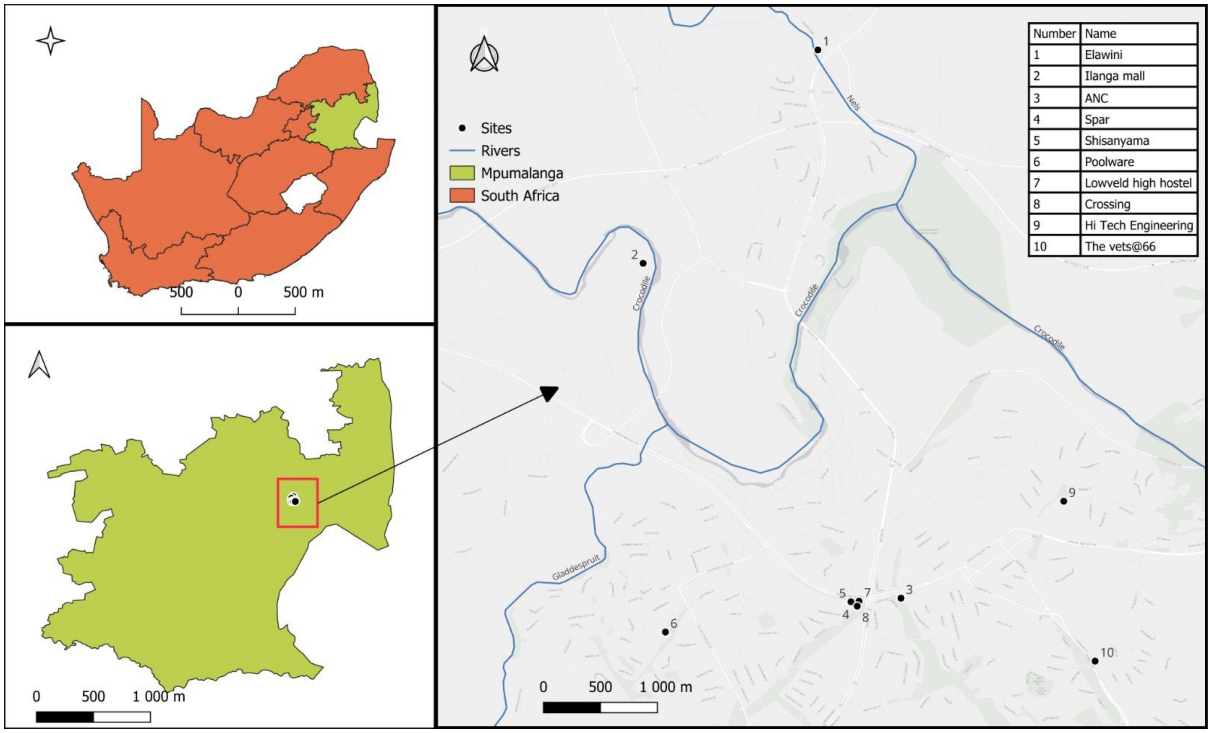

Figure 1. Geographic distribution of sampling sites along the Crocodile River in Nelspruit.

In the upper sections of the Crocodile River, human activities have compromised the surface water quality in recent decades (Che et al.,2021), as a result, plastics are frequently irresponsibly discarded into aquatic ecosystems (Dalu et al., 2020). The main anthropogenic activities impacting the Crocodile River include Sappi Ngodwana and its association with wood processing and paper making. The Ngodwana Dam wall has an influence on the water quality and flow in the lower Ngodwana River. Treated sewage is discharged to the river, which results in a high nutrient load, and agricultural activities (tobacco farming, citrus farming, and tea plantation) (Ferreira et al., 2008; Soko et al., 2014).

For example, the packaging materials from industries and agricultural plastic film, fertilizer bags, and irrigation pipes can be broken down and find their way into the river system. Similarly, wastewater release usually releases plastic debris from household and urban waste. These inputs generate environments that facilitate the introduction and accumulation of macroplastics and thereby influencing the colonisation of macroinvertebrates.

Data collection

This study was conducted in cool–dry season, winter (early June) and in wet-cool season, spring (Mid-September) of 2025. Macroplastics were collected from the river to evaluate colonisation patterns by macroinvertebrates. The collected macroplastic were classified based on type such as plastic bottles, bags and food wrappers. Additionally, natural substrates like vegetation, and rocks were collected and sampled from areas adjacent to the macroplastics. This allowed for a comparative analysis of macroinvertebrate colonisation between macroplastic &natural substrates, providing Insights about the ecological implications of plastic pollution.

Physicochemical sampling

Environmental variables were measured using a multiparameter probe (Hanna H19829 Combo meter model) to capture the physical properties of the stream, including total dissolved oxygen (mg L^{-1}), electrical conductivity (µS cm^{-1}), oxidation-reduction potential (mV), temperature (°C) & pH. In addition, water samples were collected in situ during sampling into 500ml polyethene bottles and stored at -20°C

before analysis. At each site, water samples were collected from two different points of the river so that we could use the average as a final reading or variable. The collected water samples were used to determine the concentration of available nutrients. The nutrients were analysed within 24 hours. A Hanna multiparameter photometer (HI83300) was employed to determine the nutrient levels in the water. The nutrients examined in the water included phosphate, ammonia, and nitrate. The unit of measurement that was used to express the concentration of the nutrients in the water was milligrams per litre (mg/l).

Macroinvertebrates sampling

During fieldwork, macroinvertebrate samples were collected from both natural substrates (such as rocks and plants) and macroplastic substrates (including bottles, plastics, and food wrappers). The sampling approach was dependent on the substrates found at each river site. At each site, A random sampling approach was employed with a 20-minute timed protocol to dislodge macroinvertebrates from substrates into a net. Macroinvertebrates colonising these substrates were collected and placed in labelled jars. Macroinvertebrates were scrapped and rinsed off from specific macroplastic substrates into a net and then transferred into the labelled jars. The same processes were followed for dislodging macroinvertebrates from natural substrates. Once all samples from respective substrates were collected in a tray the content were preserved in a 250ml bottle with 70% ethanol and labelled accordingly (e.g., Site, macroplastics) for later identification in the lab.

Data analysis

Environmental variables

A Normality of variance was tested using the Shapiro-Wilk test to determine whether the data followed a normal distribution (parametric) or were non-parametric (not normally distributed) with unequal variances. A paired t-test was used for normally distributed data and a Wilcoxon signed-rank test for non-normal data. A Principal Component Analysis (PCA) was used to visualise the seasonal patterns. Therefore, PERMANOVA was conducted to compare environmental variables between seasons.

Macroinvertebrates diversity

Macroinvertebrates were counted and identified in the lab using microscope and a macroinvertebrate guide. Macroinvertebrate structure and community were described using five biodiversity metrics, abundance, richness, evenness, Shannon-Weiner diversity index and Simpson's Diversity Index. The five metrics were then compared between the natural and plastic substrates and across the seasons using Permutational Multivariate Analysis of Variance, PERMANOVA. Additionally, SIMPER analysis was applied to identify the taxa that contribute the most to this seasonal difference (Balotelli et al., 2023).

CHAPTER FOUR: RESULTS

Physiochemical parameters

The results of PERMANOVA indicated significant differences PERMANOVA *F*=2.343, *df*=1, p<0.05 in some of the environmental variables between season 1 (cool-dry, winter) and season 2 (wet-cool, spring), comparing natural and plastic substrates. Subsequent analyses based on Kruskal-Wallis tests revealed that ORP, pH and %DO were the environmental variables that were significantly different (p<0.05) between seasons 1 and 2 (Table 2).

Table 2. Physiochemical variables measured in the Crocodile River, Mpumalanga across two seasons (mean ± standard deviation). Also shown are the results of the Kruskal-Wallis test performed independently for each environmental variables between the two seasons. Abbreviations: Temp= water temperature, ORP= oxygen reduction potential, pH the potential of hydrogen, %DO=percent dissolved oxygen, electrical conductivity, TDS= total dissolved solids, P Pent= phosphorus pentoxide.

Variable	S1 Winter (cool-dry)	S2 Spring (Wet-cool)	Degree of freedom	x^2	*p*-value
Temp[a]	15.25 ± 2.03	21.75 ± 2.08	1	89.07	**P<0.05***
ORP[b]	151.85 (45.83-195.50)	69.35 (26.95-124.10)	1	3.17	p>0.05
pH[b]	7.125 (0.09-7.32)	8.1 (7.14-9.17)	1	7.10	**P<0.05***
%Do[b]	80.95 (Range)	102.78 (Range)	1	3.18	p>0.05
EC[a]	378.75 (158.10)	433.30(207.88)	1	3.70	p>0.05
TDS[a]	198.05 (99.30)	307.7 (147.52)	1	36.53	**P<0.05***
Phosphorus[a]	1.165 (0.53)	1.43 (0.46)	1	1.40	p>0.05
P pent[a]	2.64 (1.22)	3.31 (1.02)	1	1.84	p>0.05
Phosphate[a]	3.474 (1.59)	4.47 (1.27)	1	2.76	p>0.05

The first two axes of the PCA biplot explained 68.1% of the total variance in the physiochemical variables (PC1=27.5% and PC2=40.6%) therefore these two axes were used to interpret the results (figure 2). This PCA provides a clear distinction between the two seasons (i.e., S1, cool-dry and S2, wet-cool). The physiochemical variables between seasons varied significantly with season 1 (cool-dry) positioned with

negative scores on PC1, forming a distinct cluster. Conversely, samples from the Wet-Cool season (Season 2) were associated with positive scores on PC1, creating a separate, non-overlapping cluster (Figure 2). Season 1 (winter) exhibited greater variability in physio-chemical conditions while Season 2 (Spring) maintained more stable conditions.

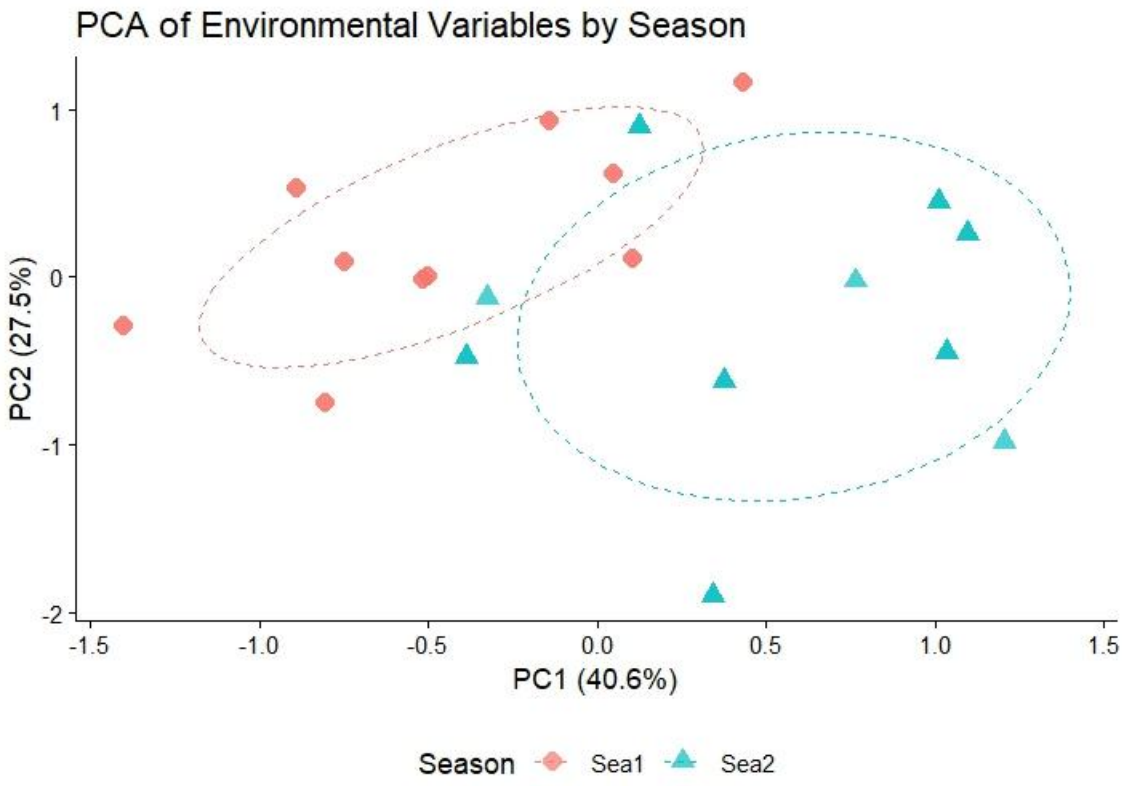

Figure 2: PCA biplot of environmental variables showing differences between season1 (cool-dry) and season 2 (wet-cool) in Crocodile River, Mpumalanga

Species Composition

A total of 4157 species were collected during Season 1(Cool-dry) in early June, while 604 were collected in Season 2 (wet-cool) in mid-September. Natural Substrates supported higher species abundance in season 1 (2231) compared to season 2 (181). Collected macroinvertebrates were categorized into 21 orders, 70 families and 136 species.

SIMPER (Similarity Percentages Analysis)

The SIMPER analysis shows which taxa contribute most to the difference in macroinvertebrates between macroplastic and natural substrates. *Chirinominae* was the main contributor, accounting 21,4% of the dissimilarity and was more abundant on plastic substrates (52.6%) than on natural substrates (32.3%). *Tanypodinae* was the second main contributor with 15,4% of dissimilarity and more abundant on natural substrates (52%) compared to plastics (16,9%). Minor contributors were *Marsupiobdella Africana*, *Stenophysa marmota*, and *Culicine* accounting for less than 1% of the dissimilarity.

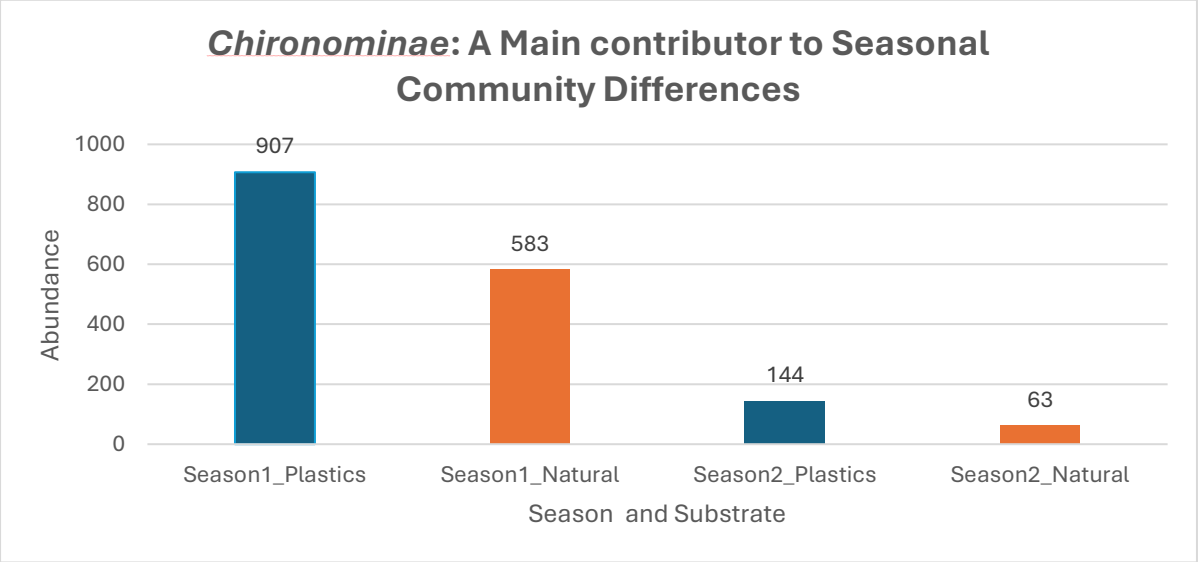

Figure 3: *Chironominae* Abundance: Driving Seasonal Community Difference

The graph visualises SIMPER results, highlighting that *Chironominae* (figure 3) was the primary contributor to the seasonal difference. In season 1 (cool-dry), the total abundance was 1490, which drastically dropped to 207 in season 2 (wet-cool). This sharp decline emphasises their significant variation between seasons, making them the dominant factor influencing seasonal differences. A similar trend was observed with *Tanypodinae* as the second main contributor (Figure 4). *Tanypodinae* had a total abundance of 1377 in S1, which dropped sharply to zero in S2. The significant decrease highlights their major role in driving the differences between seasons.

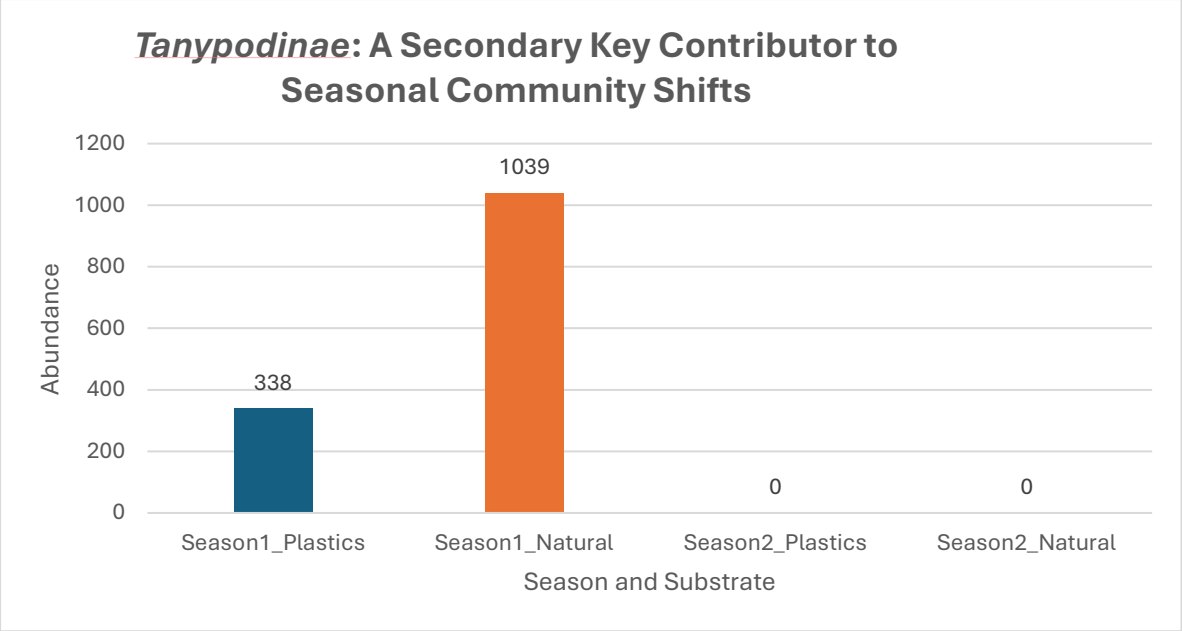

Figure 4: *Tanypodinae* abundance, the second main contributor to community difference

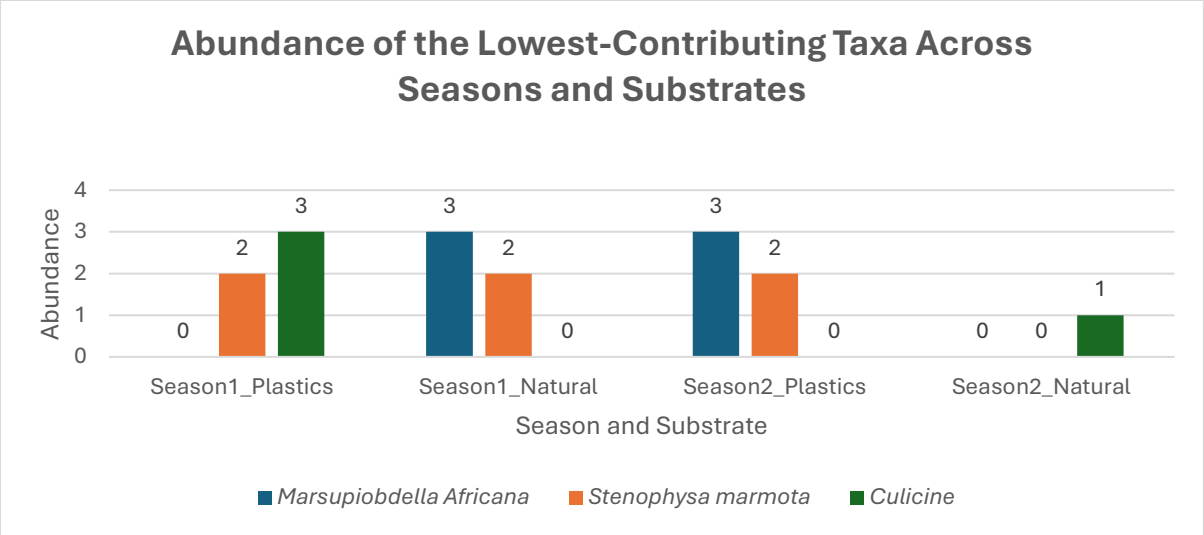

Figure 5: Lowest taxa contributing to the seasonal difference in community composition

This graph represents the minor contributors (Figure 5), compromising three taxa with very low abundance: *Marsupiobdella Africana, Stenophysa marmota,* and *Culicine*. The total abundance of these taxa was 12 individuals in S1 and 5 in S2, indicating that their small number had little to no significant impact on the seasonal difference in community composition.

Taxonomic diversity

There were significant differences in abundance, richness, and Shannon-Wiener diversity index between S1 (cool-dry, winter) and S2 (wet-cool, spring) ($p<0.05$). These three-diversity metrics were significantly higher in S1 (cool-dry) compared to S2 (wet-cool) (figures 6, 7, and 10). When comparing substrate types, macroinvertebrates diversity and abundance differed between natural and plastic substrates across the two seasons, however the difference were not pronounced. Although the differences between substrates were not pronounced, significant variations were observed between the two seasons (tables 3 and 4). In contrast, evenness and Simpson diversity did not differ significantly between the two seasons (Table 3); however, variations in evenness were observed between the natural and plastic substrates (Figure 6), while Simpson diversity showed significant variation in natural substrates but no pronounced difference in plastics (figure 7)

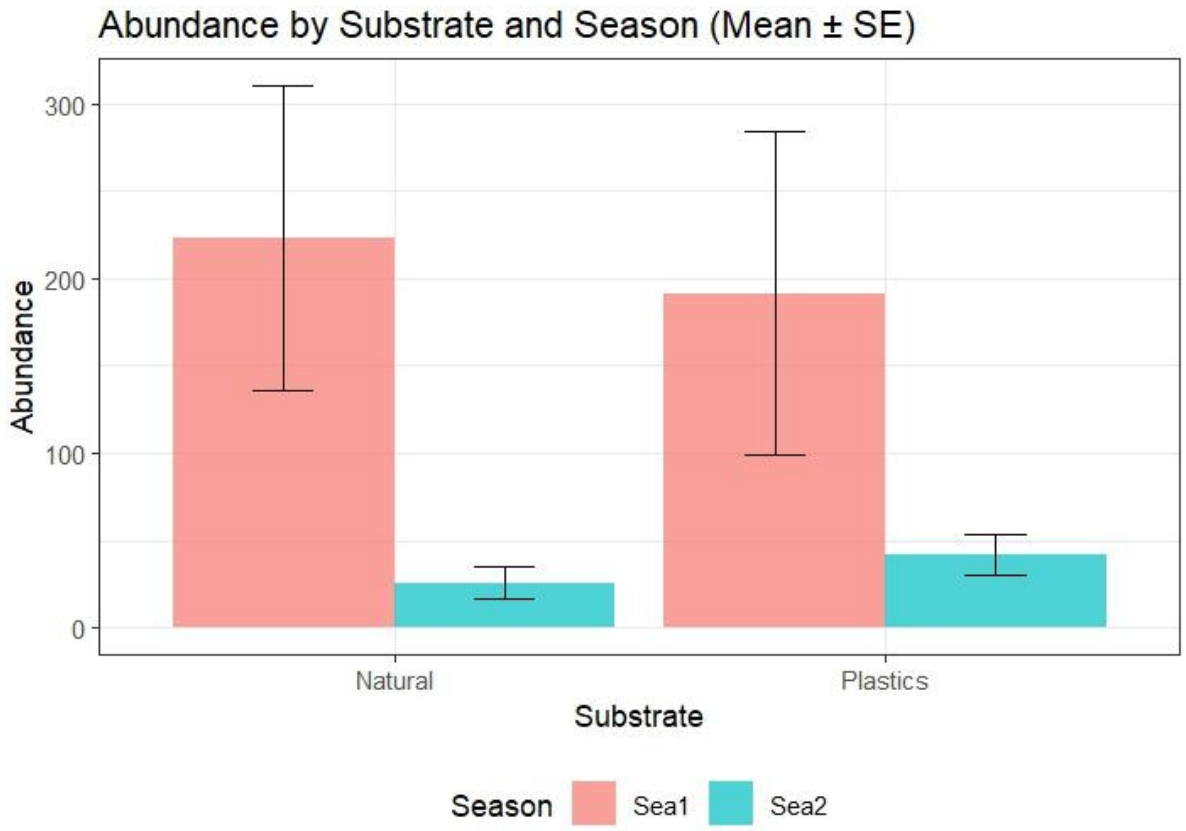

Figure 6: Mean community diversity metric for macroinvertebrates abundance across S1 (cool-dry) and S2 (wet-cool) comparing natural and plastic substrates. The diversity

metrics were computed based on square-root transformed abundance data. Only positive error bars are shown on the plots.

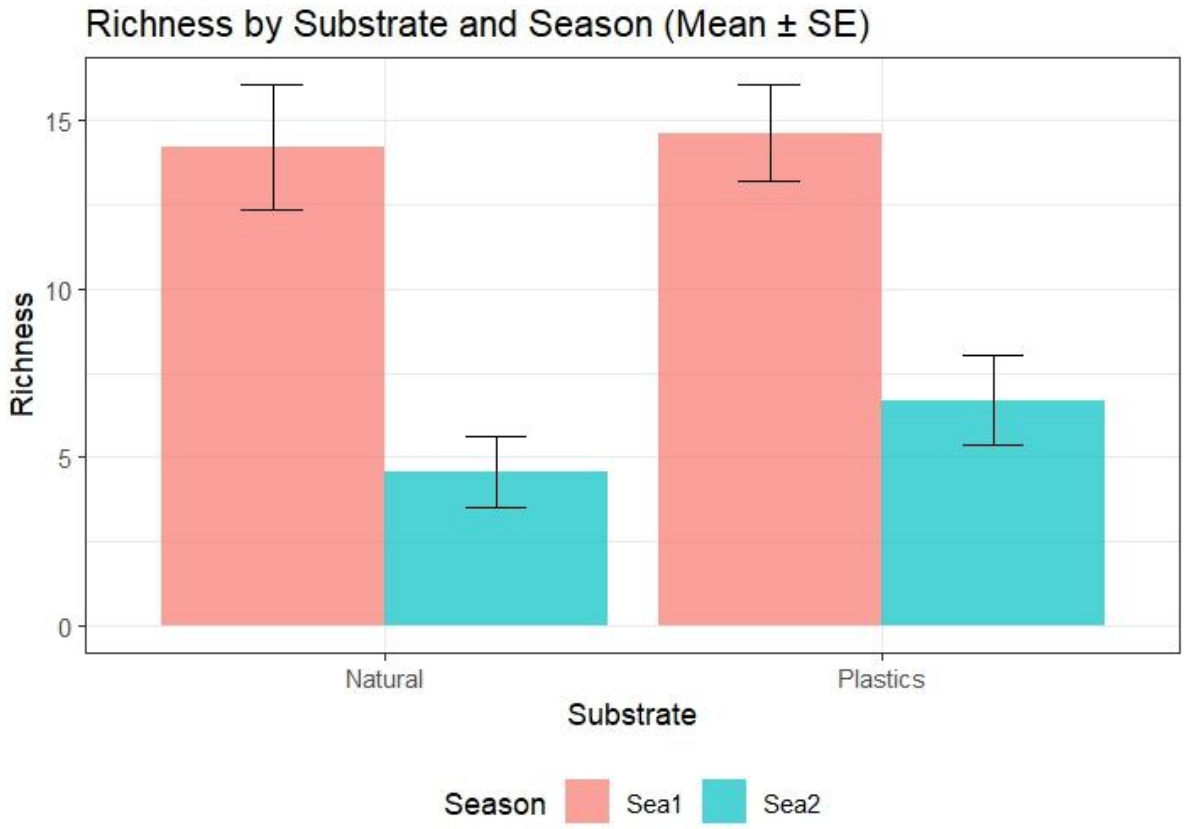

Figure 7: Mean community diversity metrics for species richness across S1 (cool-dry) and S2 (wet-cool) comparing natural and plastic substrates. The diversity metrics were computed based on square-root transformed abundance data. Only positive error bars are shown on the plots.

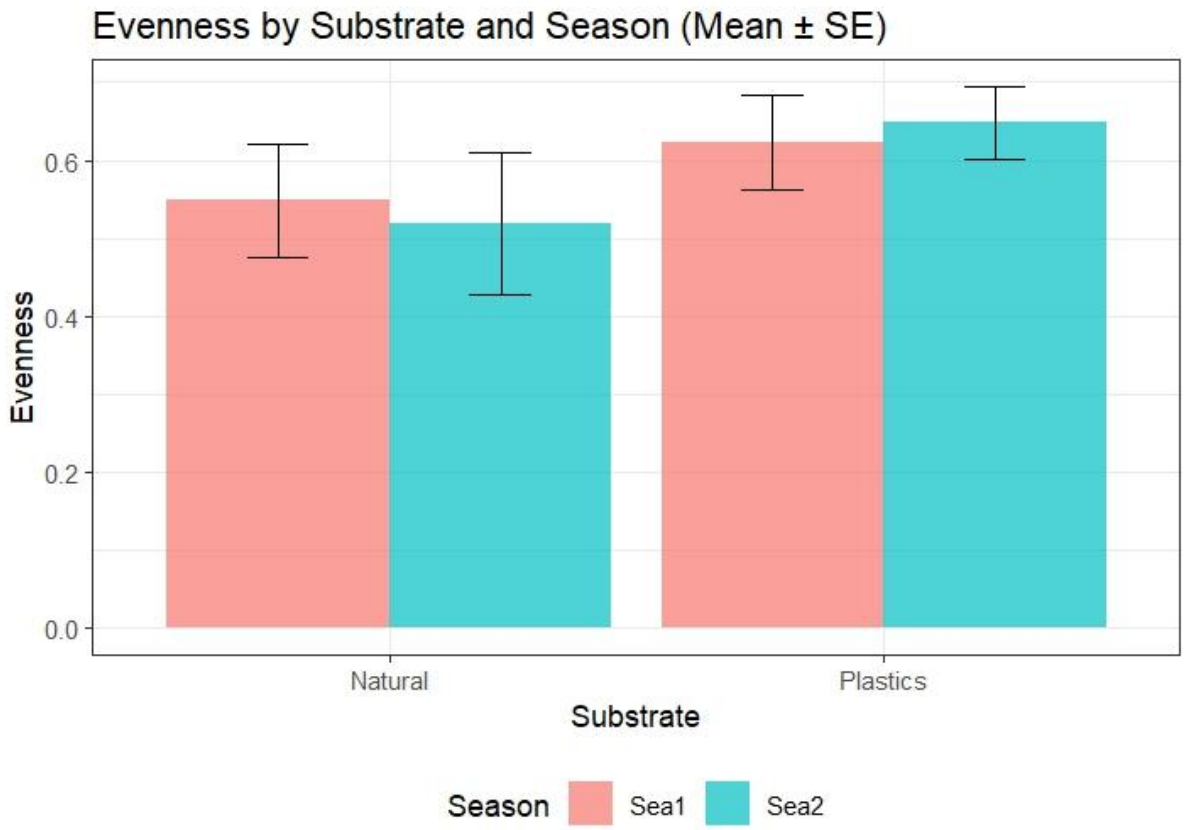

Figure 8: Mean community diversity metric for macroinvertebrates evenness across S1 (cool-dry) and S2 (wet-cool) comparing natural and plastic substrates. Positive errors bars represent estimated standard deviations.

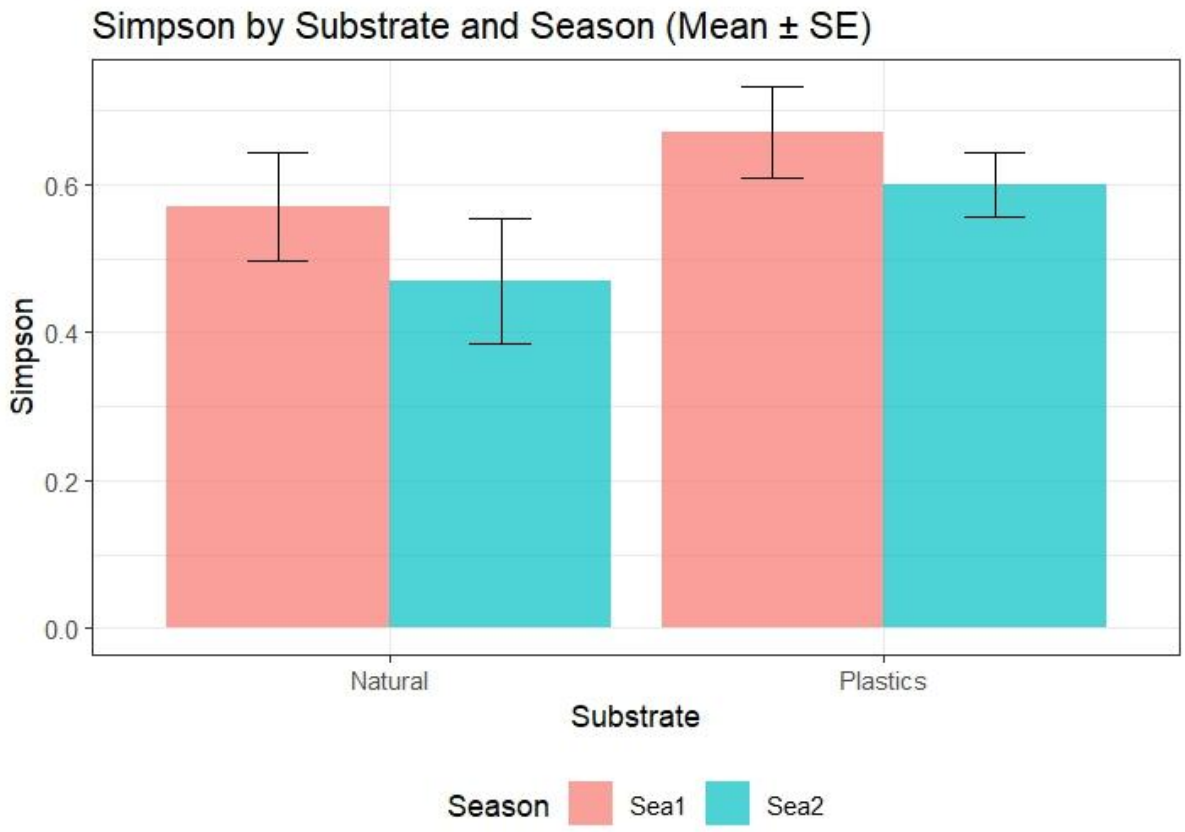

Figure 9: Mean community diversity Simpson across S1 (cool-dry) and S2 (wet-cool) comparing natural and plastic substrates. Positive errors bars represent estimated standard deviation.

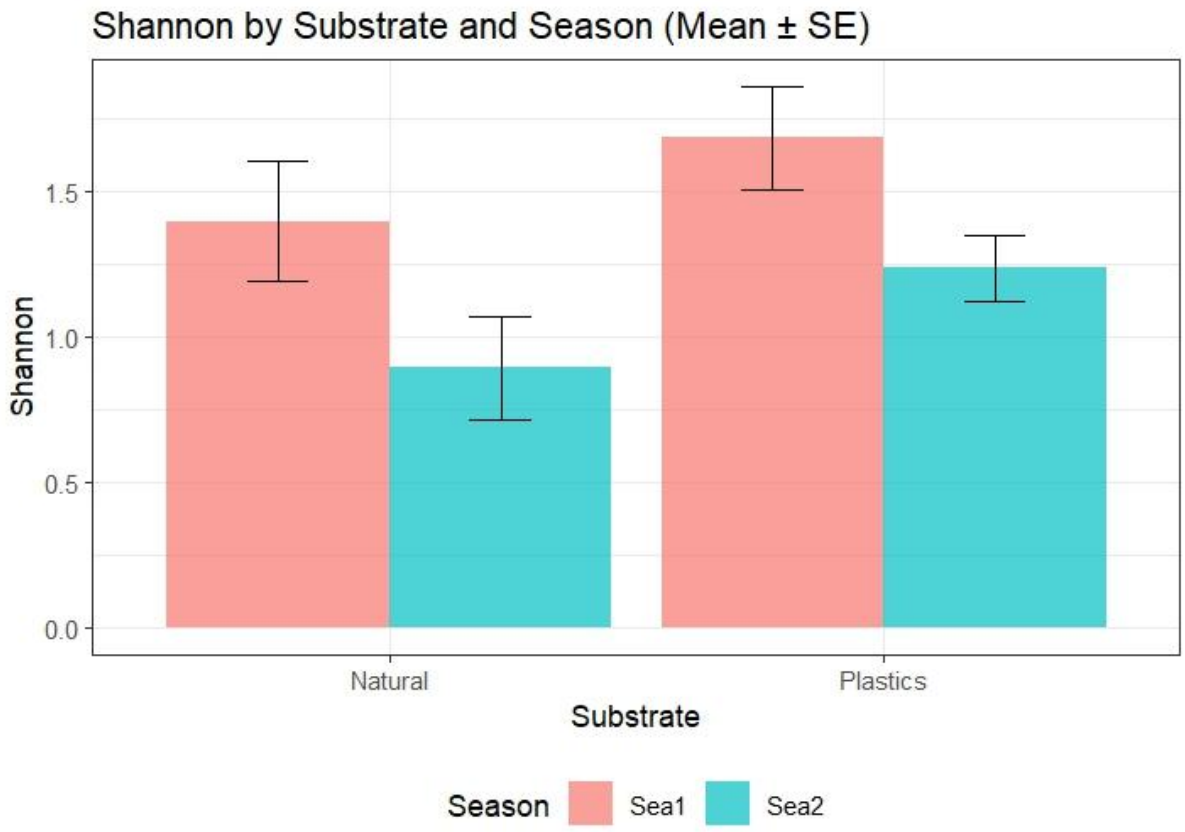

Figure 10: Mean community diversity Shannon-Weiner across S1 (cool-dry) and S2 (wet-cool) comparing natural and plastic substrates. Positive errors bars represent estimated standard deviation.

Table 3: Macroinvertebrates metrics across S1 (cool-dry) and S2 (wet-cool) (mean± deviation). The results of Shapiro-Wilk test based on square root transformed data are also presented.

Diversity indices	S1 Winter (cool-dry)	S2 Spring (Wet-cool)	Degree of freedom	x^2	*p*-value
Abundance	105 (3-996)	17.5 (2-89)	1	8.92	p<0.05*
Species richness	15 (3-23)	4.5 (0-15)	1	12.20	P<0.05*
Evenness	0.42167 ±0	0.56533 ±0.49	1	2.34	p>0.05
Simpson diversity	0.6205 ±	0.46427 (SD)	1	3.87	p>0.05
Shannon	1.5429 (0.56-2.52)	1.043 (0.69-1.69)	1	8.97	p<0.05*

Table 4: Macroinvertebrate metrics of natural and plastic substrates (mean ± standard deviation). The results of the Shapiro–Wilk test based on square-root transformed data are also presented.

Diversity indices	Natutal	Plastics	Degree of freedom	x^2	p-value
Abundance	30.5 (0-926)	65(0-996)	1	0.803	p>0.05
Species richness	8.7(0-20)	11(3-23)	1	1.882	p>0.05
Evenness	0.45	0.53	1	0.096	p>0.05
Simpson diversity	0.45	0.64	1	0.00019	P<0.05*
Shannon	1.0134	1.4631	1	0.000107	P<0.05*

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS

Macroplastic pollution in freshwater ecosystem systems is associated with several ecological hazards to macroinvertebrates and aquatic biodiversity (Azevedo-Santos et al., 2021). This includes the leaching of toxic additives derived from the degradation of macroplastics (Obuzor et al., 2023), which leads to bioaccumulation (Gall & Thompson, 2015; Van Emmerik, 2020. This study employed field sampling, laboratory analysis, and statistical analysis to analyse the ecological role of macroplastics as habitats for aquatic macroinvertebrates in the Crocodile River across different seasons. The results of this study revealed that although macroplastic pollution releases toxic chemicals into the river, macroinvertebrates are still able to use macroplastics as their functional habitat. This is supported by the results of the diversity indices, including Shannon-Weiner, Margalef's, abundance, richness and evenness.

The results supported my first hypothesis, which stated that macroinvertebrate communities would be present on macroplastic debris within the Crocodile River across different seasons. However, it did not support my second hypothesis because there was no significant difference between substrates; the difference was only across seasons. The findings of Hoellein et al. (2014) revealed that microbial biofilm abundance and activity were significantly higher on anthropogenic litter in freshwater environments, indicating that such litter provides a suitable surface for biological colonisation. This aligns with the observation of the current study, where the colonisation patterns of macroinvertebrates on macroplastics were comparable to those on natural substrates. These results demonstrate that macroplastics can play an ecological role as functional habitats, supporting biological communities and contributing to habitat heterogeneity within aquatic ecosystems. Similar observations have been reported by McCormick et al. (2016), who found that anthropogenic material in rivers can host diverse biofilm and invertebrate assemblages, and by Windsor et al. (2019), who showed that plastics in freshwater environments support macroinvertebrate colonisation similar to that in natural habitats.

In this study, environmental variables did not show a significant impact on the availability of macroinvertebrates. The colonisation patterns were observed to be more

strongly influenced by seasonal changes rather than physio-chemical factors. However, these variables still provided important ecological context, as macroinvertebrate composition was higher in Season 1 (cool-dry) than in Season 2 (wet-cool), potentially due to more dynamic environmental conditions during that period (S1). This pattern is supported by the findings of Dallas (2007), who reported that seasonal variability, particularly in temperature and flow, significantly influences macroinvertebrate assemblages in South African rivers. Similarly, Kemp et al. (2014) observed that seasonal hydrological changes have a stronger effect on macroinvertebrate community structure than short-term variations in water quality parameters. Dalu et al. (2025) further confirmed that flow regime and seasonality are major determinants of macroinvertebrates distribution in Southern African rivers, often overriding the influence of physio-chemical variables.

Variations in temperature and pH may also influence the composition and diversity of macroinvertebrate communities in freshwater ecosystems. These physio-chemical parameters affect metabolic rates, reproductive cycles, and overall biological productivity, thereby shaping the structure community structure (Duo et al., 2022). In this present study, the average water temperature in Season 2 (wet-cool) was higher (21.75°C) than Season 1; cool-dry (15.25°C), similarly pH was slightly more alkaline in Season 2 98.12) compared to Season 1 (7.15) (Appendix 3). The observed shifts in macroinvertebrates assemblages could therefore be partly attributed to these environmental variations, although seasonal flow patterns remain the dominant driver of colonisation dynamics. This supports findings by Doe et al. (2022), who demonstrated that fluctuations in temperature and pH significantly influence macroinvertebrate composition and community structure in freshwater systems.

This study revealed lower taxonomic diversity in Season 2 (wet-cool) compared to Season 1 (cool-dry). Season 1 had higher species abundance, evenness, Shannon-Weiner and Margalef's diversity indeces, indicating greater macroinvertebrate composition during the cool-dry period. By contrast, Season 2 which followed rainfall events and showed altered flow conditions exhibited reduced macroinvertebrate composition, suggesting that community structure in the Crocodile River is strongly influenced by seasonal changes in flow. These findings are consistent with recent biomonitoring research showing temporal variability in flow and related seasonal processes (e.g. dilution, flushing, and habitat disturbance during wet season) can

cause significant shift in macroinvertebrate assemblages and reduce diversity indices during high-flow periods (Masese et al., 2023). Local water-quality monitoring in the Crocodile River also documents substantial seasonal and spatial fluctuations in physiochemical parameters that can interact with flow to affect biological communities, corroborating the role of seasonal dynamics in shaping macroinvertebrate patterns in this system (Madonsela et al., 2024).

Conclusion

This research study has some insightful information as regards to the relationship between the macroplastic pollution and the aquatic invertebrate communities in the Crocodile River in Mpumalanga. The study indicates that macroplastic debris can be used as a functional habitat for macoinvertebrates colonisation that allows a diverse biological community comparable to macroinvertebrate communities occupying natural substrates. This colonisation illustrates that macroplastics have become a part of riverine systems, where they serve as novel and functional environments to aquatic macroinvertebrates.

The key finding of this research reveals that seasonal dynamics are the driving factor of macroinvertebrate communities than substrate type. The higher abundance, richness and diversity found in the cool-dry season show that seasonal factors such as water flow are the main drivers of macroinvertebrate community changes, highlighting the crucial role of hydrological patterns in shaping community dynamics.

The major implication of the study is the dual nature of macroplastic pollution. Although it is acknowledged that plastics are an environmental hazard, this study reveals that they can be a habitat of aquatic macroinvertebrates. Nonetheless, this does not dismiss the detrimental effects of plastic pollution but provides a complexity to the ecological effects of plastic pollution. For instance, the removal of these established plastic habitats will possibly impact the communities of macroinvertebrates inhabiting them.

Overall, this research will be an important contribution to the growing global plastic pollution literature that embodies the intricate environmental effects that need to be carefully considered to ensure the integrity of freshwater ecosystems, including the Crocodile River.

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APPENDICES

Appendix 1: Mean values of physicochemical variables for season 1 measured at different sites along the Crocodile River, Mpumalanga

Water quality parameters	*Temp (°C)*	*ORP (Mv)*	*pH*	*%DO (mg/l)*	*DO (mg/l)*	*EC (mS/m)*	*TDS (mg/l)*	P *(mg/l)*	P_2O_5 *(mg/l)*	PO_4^{3-} *(mg/l)*
Site 1	11,61	49,4	7,2	80	8,18	105	52,5	0,4	1	1,3
Site 2	12,51	151,15	7,07	81,9	8,29	116,5	58	1,1	2,5	3,35
Site 3	15,505	162,15	7,095	85,1	7,98	434,5	217,5	2,2	5	6,65
Site 4	17,875	180,65	7,135	86,55	7,74	459,5	229	0,9	2,05	2,75
Site 5	17,6	152,55	7,08	82,15	7,335	427,5	214,5	1,6	3,75	5
Site 6	17,065	195,5	7,275	38,1	3,44	366,5	149,5	1,65	3,7	4,49
Site7	14,35	53,45	7,21	55,3	5,26	616,5	392,5	1,45	3,25	4,35
Site8	14,33	45,8333	7,06	70,2	6,685	566,5	319,5	0,45	1	1,3
Site 9	16,955	67,3	7,105	60,1	5,375	361	180,5	1	2,3	3,05
Site10	14,72	190,95	7,315	110,15	10,335	334	167	0,9	1,85	2,5

Appendix 2: Mean values of physicochemical variables for season 2 measured at different sites along the Crocodile River, Mpumalanga

Water quality parameters	*Temp (°C)*	*ORP (Mv)*	*pH*	*%DO (mg/l)*	*DO (mg/l)*	*EC (mS/m)*	*TDS (mg/l)*	P *(mg/l)*	P_2O_5 *(mg/l)*	PO_4^{3-} *(mg/l)*
Site 1	18,85	98,65	8,67	46,9	3,35	142	100,85	1,7	3,9	5,2
Site 2	19,6	68,75	8,175	68,75	66,4	202	143,65	0,95	2,25	3
Site 3	21,85	49,8	7,84	49,8	97,9	488,5	346,5	2,1	4,85	6,45
Site 4	24	69,95	7,135	305	305	449	319	0,85	1,9	2,55
Site 5	20	124,1	9,17	152,55	152,95	397	282	1,65	3,75	5,05
Site 6	20,3	40,4	7,675	62,4	62,4	309,5	219,5	0,65	1,7	2,95
Site7	21,8	58,95	8,02	184,35	184,35	826,5	587	1,55	3,55	4,7
Site8	23,5	26,95	7,45	89,4	89,4	767,5	544,5	1,65	3,85	5,1
Site 9	25,85	111,15	8,81	116,15	116,15	396,5	282	1,95	4,45	5,95
Site10	21,7	73,8	8,26	170,05	170,05	354,5	252	1,25	2,9	3,75

Appendix 3: Mean values of physicochemical variables for season 1 (cool-dry) and season 2 (wet-cool) measured at different sites along the Crocodile River, Mpumalanga

Water quality parameters	*Temp (°C)*	*ORP (Mv)*	*pH*	*%DO (mg/l)*	*DO (mg/l)*	*EC (mS/m)*	*TDS (mg/l)*	P *(mg/l)*	P_2O_5 *(mg/l)*	PO_4^{3-} *(mg/l)*
Season 1	15.25	124.89	7.15	74.96	7.06	378.75	198.05	1.16	2.64	3.52
Season 2	21.75	72.25	8.12	124.54	124.79	433.5	307.7	1.43	3.31	4.47

Appendix 4: Normality test: Shapiro-Wilk test (* Not normally distributed).

Variable	W	p-value	Test use
Temp	0.9739	0.87	**Paired t-test**
ORP	0.871	0.01*	**Wilcoxon signed-rank test**
pH	0.7794	0.00*	**Wilcoxon signed-rank test**
%DO	0.7126	0.00*	**Wilcoxon signed-rank test**
DO (mg/l)	0.7031	0.00*	**Wilcoxon signed-rank test**
EC	0.9545	0.50	**Paired t-test**
TDS	0.9254	0.16	**Paired t-test**
Phosphorus	0.945	0.35	**Paired t-test**
P pent	0.9411	0.30	**Paired t-test**

Appendix 5: Environmental variables between the two seasons. Values are expressed as mean ± SD for normally distributed variables[a] or median for non-normally distributed variables[b].

Variable	S1 Winter (cool-dry)	S2 Spring (Wet-cool)	Test statistics	*p*-value
Temp[a]	15.25 ± 2.03	21.75 ± 2.08	t_9 = -9.44	0.001953
ORP[b]	151.85 (45.83-195.50)	69.35 (26.95-124.10)	Z = 1.78	0.083984
pH[b]	7.125 (0.09-7.32)	8.1 (7.14-9.17)	Z = 2.6656	0.0039063
%Do[b]	80.95 (Range)	102.78 (Range)	Z = 17838	0.083984
EC[a]	378.75 (158.10)	433.30(207.88)	t = -1.9245	0.0855938
TDS[a]	198.05 (99.30)	307.7 (147.52)	t = -6.044	0.0019531
Phosphorus[a]	1.165 (0.53)	1.43 (0.46)	t =-1.1851	0.249
P pent[a]	2.64 (1.22)	3.31 (1.02)	t = -1.356	0.19922
Phosphate[a]	3.474 (1.59)	4.47 (1.27)	t = -1.66	0.166

Appendix 6: Diversity indices across S1 (cool-dry) and S2 (wet-cool)

Diversity indices	S1 Winter (cool-dry)	S2 Spring (Wet-cool)	Test statistics	*p*-value
Abundance	105 (3-996)	17.5 (2-89)	Z = 2.9866	0.0016899
Species richness	15 (3-23)	4.5 (0-15)	Z = 3.4933	0.00011253
Evenness	0.42167 ±0	0.56533 ±0.49	t = -1.5308	0.14237
Simpson diversity	0.6205 ±	0.46427 (SD)	t = 1.9679	0.064322
Shannon	1.5429 (0.56-2.52)	1.043 (0.69-1.69)	t = 2.9959	0.0068626

Appendix 7: Diversity indices between natural and plastic substrates

Diversity indices	**Natural**	**Plastics**	**Test statistics**	***p*-value**
Abundance	30.5 (0-926)	65(0-996)	Z=0.89622	0.38318
Species richness	8.7(0-20)	11(3-23)	Z=1.3717	0.17626
Evenness	0.45±0.5	0.53±	t =0.30899	0.30899
Simpson diversity	0.45±0	0.64 ±	t = 0.013786	0.0136
Shannon	1.0134 ±0.70	1.4631±	t = 0.010357	0.0106