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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

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The effects of water quality and biotope degradation on macroinvertebrate communities in the Hennops river, South Africa

By

Lara Fangueiro Mariano

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Master's Dissertation

Submitted in fulfilment of requirements for the degree

Master of Science

in

Animal, Plant and Environmental Science

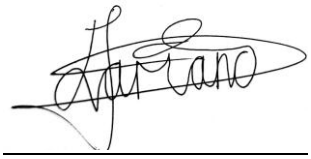
to the Faculty of Science, University of the Witwatersrand, Johannesburg South Africa

Supervised by Ass Prof. Darragh Woodford (School of Animal, Plant and Environmental Sciences) (WITS) and Ass Prof. Heidi Richards (School of Chemistry) (WITS)

2025

DECLARATION

I, Lara Fangueiro Mariano, affirm that this research report is a product of my independent and original efforts. It is presented to the University of the Witwatersrand, Johannesburg, for the Master of Science degree in Animal, Plant and Environmental Sciences, specialising in Freshwater Eco-Hydrology. This body of work has not, in part or whole, been submitted for any other degree or examination at any other institution.



(Signature of Candidate)

18 of November 2025 at the University of the Witwatersrand, Johannesburg.

ABSTRACT:

This project investigated the effects of water quality and biotope degradation on macroinvertebrate communities within the highly urban impacted catchment of the Hennops River (Gauteng, South Africa), with the goal of providing critical data to inform restoration efforts and ensure the river's ecological integrity aligns with national conservation objectives.

Water quality and habitat characteristics were assessed in the urban impacted Hennops River in comparison to the rural Magalies River to understand their impact on macroinvertebrate communities. This study revealed higher pollution levels in the Hennops, contrary to expected elevated phosphates in the agriculturally impacted Magalies. The Hennops River exhibited limited, homogenized habitats and elevated conductivity, nitrogen, and phosphate, correlated with reduced macroinvertebrate diversity, while the Magalies displayed greater biotope diversity and better water quality.

The macroinvertebrate communities in the Hennops and Magalies rivers were compared to assess the impact of urbanization, revealing significantly lower diversity and ecological health in the Hennops, contrary to initial predictions of similarity between the Hennops and lower Magalies reaches. The Hennops River exhibited a concentrated distribution within the ordination analyses, indicative of homogenous, low-diversity assemblages strongly influenced by heavy metal contamination, conductivity and nitrates, mostly likely from raw sewage, signifying severe environmental stress compared to the diverse and abundant communities found in the Magalies River, which maintained higher SASS scores and ecological ratings.

This study urgently calls for enhanced water management and legislative enforcement in Gauteng, South Africa. The results indicate that unchecked urbanisation, poor maintenance of wastewater treatment infrastructure, and weak regulatory enforcement have significantly

degraded water quality and altered biotic communities in both rivers. To align with Sustainability Development Goal 6 and secure future water resources, this study advocates for the enforcement of robust environmental management plans and rehabilitation strategies, grounded in ecological data, to improve river health. Ultimately, this research aims to catalyse actionable changes that ensure sustainable water security and fulfil South Africa's commitment to local and global sustainability goals.

Keywords:

Hennops River, Water Quality, Habitat Degradation, Macroinvertebrate Communities, Pollution, Restoration Efforts

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To all the property owners such as Maloney's Eye Farm - KJA Group, Happy Acres, Parnassus St benedict's farm, Sleepy Rivers Caravan Park, Private property homeowners Jacal Canvas, Kempton Park Golf Club, Irene lodge, Zwartkop Lapa Reserve and Resort, Rock Falls Ranch, Made on Earth Hiking Trails and Willem Snyman, who all welcomed me on their properties and showed their ongoing support to rehabilitate and maintain the Hennops and Magalies rivers.

I extend my sincere appreciation to my team members who assisted in sampling these rivers and bravely faced risks alongside me: Josefina Mariano, Saayim Ori, Joao Paulo Mariano, Jorge Lourenco, Dr Jonathan Levin, Amy Chen, Linda Nyembezi, Jessica Blair, Josh Jansen Van Vuuren, and Anay Coetzee. This project would not have been possible without your continuous support.

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ABREVIATIONS LIST:

African Union Development Agency: AUDA

Aluminium: Al

Ammonium: NH_4^+

Analysis of Variance: ANOVA

American Public Health Association: APHA

Arsenic: As

Cadmium: Cd

Calcium: Ca

Canonical Correspondence Analysis: CCA

Catchment Management Agency: CMA

Catchment Management Strategy: CMS

Chemical Oxygen Demand: COD

Chromium: Cr

Cobalt: Co

Copper: Cu

Correspondence Analysis: CA

Degrees Celsius: °C

Department of Higher Education and Training: DHET

Department of Water Affairs: DWA

Department of Water Affairs and Forestry: DWAF

Department of Water and Sanitation: DWS

Dissolved Oxygen: DO

East Rand Water Care Company: ERWAT

Electrical Conductivity: µS/cm

Figure: Fig.

Freshwater Biodiversity Information System: FBIS

General Linear Models: GLM

Geomorphic Habitat Units: GHUs

Gravel, Sand, and Mud: GSM

Hennops River site 1: H1

Hennops River site 2: H2

Hennops River site 3: H3

Internal Strategic Perspectives: ISPs

Iron: Fe

Lead: Pb

Magalies River site 1: M1

Magalies River site 2: M2

Magalies River site 3: M3

Magnesium: Mg

Manganese: Mn

Member of the Executive Council: MEC

Milligrams per Litre: mg/L

Millivolts: mV

Mud, Sand, and Gravel: GSM

National Environmental Management Act: NEMA

National Environmental Management: Biodiversity Act: NEMBA

National Water Act: NWA

National Water and Sanitation Master Plan: NWSMP

National Water Resources Infrastructure Agency: NWRIA

National Water Resources Strategy: NWRS

Nickel: Ni

National Institute of Standards and Technology: NIST

Nitrate: NO3

Non-Governmental Organizations: NGOs

Oxidation Reduction Potential: ORP

PAST software: PAST

Parts Per Thousand: ppt

Ponds and Dams: PAD

Potassium: K

Principal Component Analysis: PCA

Principal Component Regression: PCR

Quality Assurance and Control: QA/QC

River Health Assessment Method: RHAM

Resource Quality Objectives: RQOs

Selenium: Se

Sodium: Na

South Africa: SA

South African National Accreditation System: SANAS

South African Police Service: SAPS

South African Scoring System version 5: SASS 5

South African Water Quality Guidelines for Aquatic Ecosystems: SAWQG: AE

Stones in Current: SIC

Sustainable Development Goal: SDG

Target Water Quality Ranges: TWQRs

United States Environmental Protection Agency: US EPA

Vanadium: V

Vegetation: Veg

Water Management Area: WMA

Water Research Commission: WRC

Water Use Licences: WUL

Wastewater Treatment Plants: WWTPs

Zinc: Zn

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CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 – Literature Review

River ecosystems possess significant environmental value (Nguyen *et al.*, 2018), and their health and integrity are crucial for both socio-economic well-being (Dickens *et al.*, 2018) and global biogeochemistry cycles (Schlesinger and Bernhardt, 2020). Rivers serve as primary renewable freshwater sources, vital for both aquatic ecosystems and human water supply (Vörösmarty *et al.*, 2010), while also providing numerous essential ecosystem services (Berger *et al.*, 2016; Yeakley *et al.*, 2016; Raquel Calapez *et al.*, 2023). Historically, human concern regarding freshwater focused primarily on its availability for consumption. However, the past 50 years have witnessed a substantial deterioration in freshwater quality, underscoring the critical need for effective freshwater management (Tampo *et al.*, 2021, 2023). Consequently, aquatic ecosystems are experiencing significant pollution and habitat degradation, threatening their sustainability, functionality, and the provision of vital ecosystem services (UN-Water, 2011, 2012).

The assessment of freshwater ecosystems presents a significant challenge due to their inherent vulnerability to a complex interplay of biotic and abiotic stressors, which can profoundly alter their structure and function (Borgwardt *et al.*, 2019) (Fig. 1.1). A consistent finding across numerous studies is the pervasive influence of anthropogenic activities in altering global freshwater availability and quality (Rodell *et al.*, 2018; Hirabayashi *et al.*, 2021). Rivers and streams are particularly vulnerable and highly threatened by these stressors (Allan, 2004; Best and Darby, 2020).

Freshwater ecosystems are disproportionately rich in biodiversity, even though they account for just 0.25% of the world's water and cover less than 1% of the Earth's surface (Dudgeon *et al.*, 2006; Strayer and Dudgeon, 2010). Globally, freshwater biota are

experiencing alarmingly high extinction rates, ranking among the most endangered organisms on Earth; yet, these critical systems are often overlooked in conservation and water management programs (Burkhead, 2012; Cooke, Paukert and Hogan, 2012; Torres-Romero *et al.*, 2024). Anthropogenic climate change, habitat degradation, water pollution, flow modifications, invasive species, and the overexploitation of freshwater sources represent the primary threats to freshwater species (Dudgeon *et al.*, 2006; Hirabayashi *et al.*, 2021). Globally, a third of all freshwater species are threatened and face the risk of extinction (Balian *et al.*, 2008; Vörösmarty *et al.*, 2010; Collen *et al.*, 2014). Sub-Saharan Africa and other tropical regions are currently experiencing severe water shortages. Human activities and rising temperatures are placing significant pressure on rivers in these areas. This multi-faceted threat exacerbates the degradation of water quality and quantity, severely limiting water availability (Traoré *et al.*, 2016; Chetty and Pillay, 2019; Agboola, Downs and O'Brien, 2020; Tampo *et al.*, 2020). Recognizing the critical importance of river ecosystems, many African countries are increasing investments in both the protection and restoration of these systems, as well as understanding the ecological status of their rivers (Darwall *et al.*, 2009; Fayiga, Ipinmoroti and Chirenje, 2018; Wantzen *et al.*, 2019).

The ecological status of a river is a comprehensive assessment of its overall health and condition, derived from the measurement of various integrated ecological aspects (River Health Programme, 2005) (Fig. 1.1). There are several biotic indices and metrics that are used worldwide to evaluate the health and integrity of a river (Barbour *et al.*, 1996; Hering *et al.*, 2006; Agboola, Downs and O'Brien, 2019; He *et al.*, 2020; Ko *et al.*, 2020) (Fig. 1.1). As defined by Kleynhans (2007a), a river's ecological status encompasses the characteristics and features that enable a river and its riparian areas to support biota and flora, including the provision of essential ecosystem services. This status is reflected by both the driving forces (physico-chemistry, hydrology, and geomorphology) and the biological responses (aquatic

invertebrates, diatoms, riparian and aquatic vegetation, and fish) (Fig. 1.1) (Kleynhans and Louw, 2007).

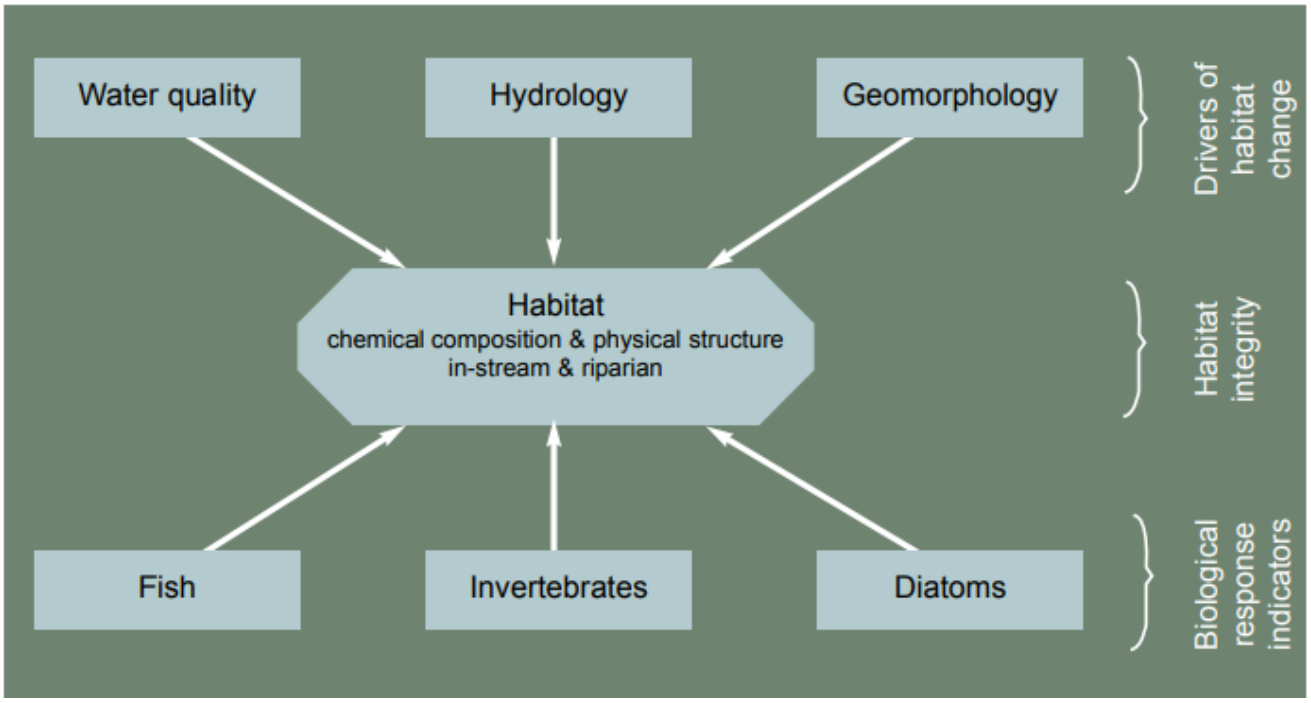


Figure 1.1: A diagram showing the important features and bio-indicators that determine river habitat health (River Health Programme, 2005).

A holistic approach using a combination of biotic indices is widely recognized as providing a more comprehensive evaluation of river health than conventional water quality assessments alone (Gonçalves and Menezes, 2011) (Fig. 1.1). River water samples are limited in their ability to reflect temporal variations in water quality, as they only represent conditions at the specific moment of collection (Metcalfe, 1989), but lacks the ability to understand the biological viability, or the river's capacity to support life. In addition to the environmental components outlined by Kleynhans (2007a)—changing riparian zones, variations in channel profiles, instream habitat modifications, and fluctuating freshwater physicochemical conditions—aquatic macroinvertebrates are particularly vulnerable and susceptible to anthropogenic changes in freshwater environments (Nessimian *et al.*, 2008; Ambelu, 2011; Agboola, Downs and O'Brien, 2020; Ko *et al.*, 2020).

Freshwater macroinvertebrate responses to alterations in aquatic environments is globally recognized, making them an integral component of monitoring indices used to assess freshwater integrity and inform freshwater management (Richter *et al.*, 2003; Agboola, Downs and O'Brien, 2019; Edegbene *et al.*, 2021; Tampo *et al.*, 2021, 2023). Macroinvertebrate species have short life cycles which makes them good bio-indicators of water quality and habitat conditions (River Health Programme, 2005). Their use in bio-monitoring programmes can aid in long-term management of water resources.

The varying response levels of freshwater macroinvertebrate taxa to organic pollution, reflected in their assemblage composition, abundance, and diversity, allowing for inferences and interpretations regarding the pollution load in freshwater aquatic environments (Gresens *et al.*, 2009; Wantzen *et al.*, 2019; Tampo *et al.*, 2023). The widespread use of macroinvertebrates in water quality assessments is attributed to their varying tolerance levels to water chemistry changes, cost-effective sampling, and ubiquitous presence in freshwater environments, making them a suitable standard for comparative studies (Mary, 1999; Dickens and Graham, 2002; Hering *et al.*, 2006; Resh, 2008; Gresens *et al.*, 2009; Li, Zheng and Liu, 2010; Kabore *et al.*, 2015; Agboola, Downs and O'Brien, 2019; Ko *et al.*, 2020). Naturally pristine rivers are characterized by high species diversity and abundance (Barbour *et al.*, 1996, 1999), however, increasing human activities significantly disrupt these characteristics, influencing the assemblage structure, biodiversity, and abundance of freshwater aquatic biota (Wright *et al.*, 1993; Pinel-Alloul *et al.*, 1996).

Macroinvertebrate biotic indices have been extensively applied and developed worldwide, particularly in Europe and America, for water quality and ecosystem health assessments. In Sub-Saharan Africa, there is a growing body of literature and increasing efforts to incorporate macroinvertebrate communities into current freshwater ecosystem monitoring programs and bioassessments (Adams, 1993; Dallas, 1997; Kabore *et al.*, 2015).

This project will focus on the current water quality and river ecological status of the Hennops River in Gauteng, South Africa (SA), and the assessments of the river will include water quality physicochemical assessments, macroinvertebrate indices as well as biotope variability and habitat viability.

The Hennops River originates in Terenure, Kempton Park, and flows northward towards Tembisa township (Petersen, Dabrowski and Forbes, 2017) (Fig. 1.2). Subsequently, the Sesmyl Spruit empties into the Rietvlei Dam and then converges with the Olifantsfontein/KaalSpruit to form the known Hennops River (DHET, 2021) (Fig. 1.2). From there, the river flows northwest through Centurion, ultimately joining the Crocodile River near Hartebeestpoort Dam at the base of the Magaliesburg Mountains (Nawn, 2009; Petersen, Dabrowski and Forbes, 2017; DHET, 2021; Ali, Abiye and Adam, 2022) (Fig. 1.2). Considered one of Gauteng's longest rivers (DHET, 2021) (Fig. 1.2), the Hennops River suffers from severe pollution and contamination. Many sections of the river contain water laden with solid waste, untreated sewage, fertilizers, and industrial effluents, resulting in low dissolved oxygen levels and limited aquatic biota habitation (DHET, 2021).

Sadiki (2020) demonstrated that the enforcement of legislation intended to protect surface water resources on the Hennops River was inadequate and that stream water quality failed to meet established guidelines. These pollutants pose significant risks to both formal and informal settlements, as well as to instream aquatic biota (Sadiki, 2020). As this river is one of Gauteng's longest rivers, the ecological status is important as it could impact other catchments linked to the Hennops River. In contrast to previous studies, such as Sadiki (2020) and those conducted by the Department of Higher Education and Training (DHET), which primarily examined localized sections of the Hennops River in the Centurion area, this study extended its sampling from the river's spring to its northwestern boundary to evaluate the overall ecological health of the Hennops River within the Gauteng Province. Furthermore,

samples from the Hennops River were compared to those from the Magalies River, also within the Crocodile catchment, to provide a comparative analysis of similar rivers with differing land-use contexts and to enhance understanding of the Hennops River's current ecological status.

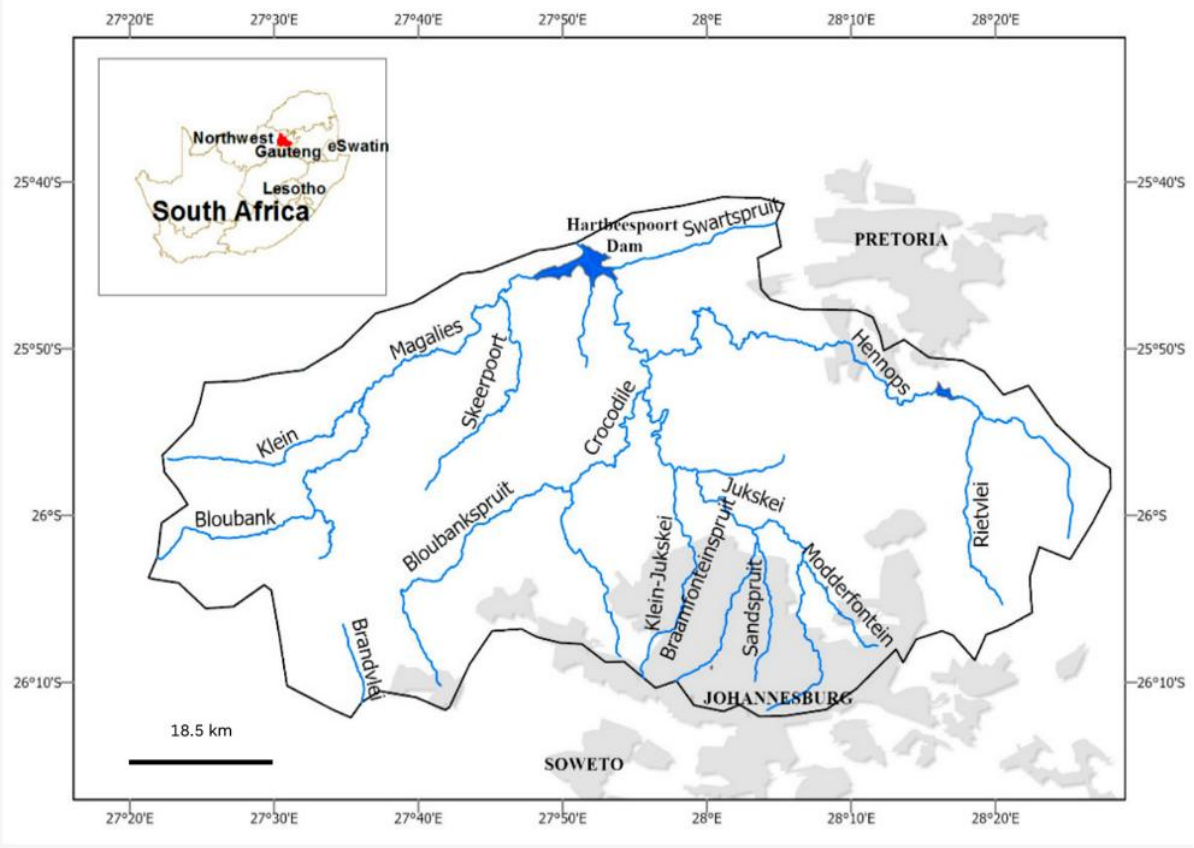


Figure 1.2: A map showing the Upper Crocodile River (West) catchment in Gauteng, South Africa (Ali, Abiye and Adam, 2022).

This study complements a 2022/2023 Water Research Commission (WRC) funded project (Letseka *et al.*, 2023), that analysed the water quality, chemical composition and ecotoxicity of the Hennops River as well as stakeholder involvement. As a social component of the WRC project, the University of Witwatersrand hosted a community awareness campaign on July 15, 2022, in Esiphethweni, Tembisa, to promote the restoration and cleanup of the Hennops River. This campaign involved partnerships with local communities,

NGOs in Tembisa, Envirocare, the Department of Water and Sanitation, ERWAT East Rand Water Care Company, Greenpeace, SAPS-South Thembisa, Esiphethweni youth, 4-room Art Gallery, and community members such as the African Union Development Agency and the Department of Forestry, Fisheries and the Environment (Kempton Express, 2022). Disseminating the findings of this study to local communities and existing partnerships could foster greater community buy-in and facilitate ongoing access for the rehabilitation of the Hennops River.

The rationale of this project will be to contribute to current literature by adding a peer-reviewed study of macroinvertebrate response data to the existing water quality and biodiversity assessments done on the Hennops River. The findings of this study could provide compelling evidence for the restoration of the Hennops River system and contribute to existing water monitoring initiatives.

1.2 - Study Area

Water management for these rivers falls under the authority of Gauteng's metropolitan municipalities which are the City of Tshwane, City of Johannesburg, and Ekurhuleni (Gauteng Province, 2019). The Hennops and Magalies rivers are located within the Limpopo Water Management Area (WMA), which encompasses the former Crocodile West and Marico WMA and the Limpopo WMA. Notably, current water management data for the study areas are still organized under the former Crocodile West and Marico WMA (DWS, 2019; Sadiki, 2020).

This project, conducted over a two-year period (2023-2024), aimed to comprehensively evaluate the impacts of water quality and biotope degradation/availability on aquatic macroinvertebrate communities within the Hennops River. Sampling sites were strategically selected based on ecoregion, position relative to water users

(upstream/downstream), land use patterns, accessibility (private/public), the presence of weirs, dams, and impassable culverts, and the urbanization gradient (Kleynhans and Louw, 2007). Sampling was conducted seasonally, occurring at the end of the dry season (September-November 2023) and at the end of the wet season (March-May 2024).

This research focused on three distinct sections of the studied rivers—upper, middle, and lower—analysing a total of six sampling sites. Along the Hennops River, sites were located at Kempton Park Golf Club, approximately 2 km from the source; Irene Lodge; and near Enoch's Restoration Centre, at the border of the North West province (Fig.1.3) (Images of the sampled sites Appendix 1.A).

The rural Magalies River was utilized as a proxy system and experimental control to facilitate a comparative analysis with the urban impacted Hennops River. Sampling sites along the Magalies River were established at the source in Maloney's Eye Farm, Sleepy Rivers Caravan Park, and Hekpoort, near the North West Province border (Fig. 1.3, Appendix 1.A). The intent was to establish a low-impact control system, but within the Upper Crocodile River (West) catchment in Gauteng, rivers with minimal human influence are scarce. While the Magalies River experiences moderate to high levels of agricultural runoff downstream towards Hartebeestpoort Dam, which compromises its ecological status, it remains a valuable comparative system. Common factors, such as shared catchment (Fig. 1.2), similar dolomite rock aquifers, and comparable water pH (Huizenga, 2004), allowing for meaningful comparisons. Both rivers are groundwater fed year-round (Ali et al., 2022) and should support relatively similar aquatic life, as they drain the same two ecoregions (Highveld vs. Western Bankenveld) in different proportions. Sampling sites along the Magalies River were placed over a shorter range compared to the Hennops River due to its shorter length and concentrated human impacts in its lower reaches. This discrepancy was addressed by

maintaining an equal number of sampling sites on each river, ensuring that sites are located within the same ecoregion.

To ensure a robust comparison between the two rivers, the upper sampling site on the Magalies River (M1) is positioned at the ecotone between the Highveld and Western Bankenveld ecoregions, aligning with the upper site on the Hennops River (H1) (Fig. 1.3). The middle (M2, H2) and lower (M3, H3) sampling sites on both rivers are located within the Western Bankenveld ecoregion (Fig. 1.3). Additionally, the similar latitudes of all six sites further enhance their comparability.

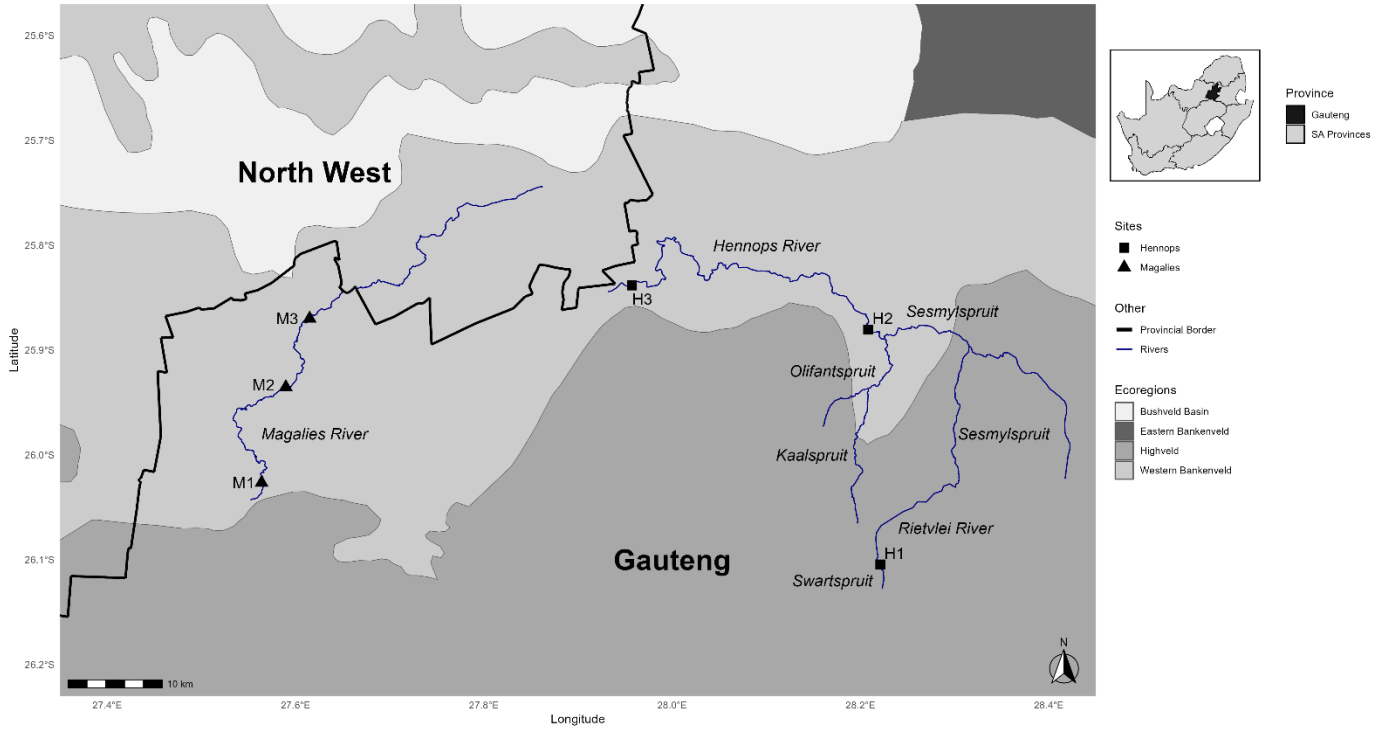


Figure 1.3: A map of the Sampled Sites and their Eco-Regions in Gauteng, South Africa.

Land classification for all sampling sites was determined using multiple indices to accurately document land use (Balasubramanian, 2015; US EPA, 2017; Copernicus EU, 2018; Ritchie and Roser, 2019; Farmonaut, 2024). The upper sampling sites, M1 and H1, were classified as open space with agriculture and urban/industrial, respectively. For the middle sites, H2 was categorized as agriculture, and M2 as an open space. The lower

sampling sites, H3 and M3, were both classified as open spaces, with M3 additionally including an urban and farming component.

During the scouting and in-river sampling phases of this project, the Hennops River's visual appearance was notably unappealing, characterized by intense odours, plastic pollution, chemical refuse, foam, and visible effluent discharges with elevated nutrient content from various Wastewater Treatment Plants (WWTPs) (Appendix 1.A). This overwhelming observational evidence underscores the urgent need for the river's reassessment and increased efforts toward water resource rehabilitation and management in Gauteng, crucial for aligning with Sustainable Development Goal (SDG) 6. The alignment to National imperatives for future water security and aquatic biodiversity is of the highest importance, especially in the face of climate change, and should therefore be adequately monitored and managed, as water is the most essential resource for life.

1.3 - Aims, Objectives and Hypotheses

The study aims to examine the current water quality status of the Hennops River, and how different water quality parameters are affecting the presence, abundance and diversity of macroinvertebrates. Using previous studies coupled with *in situ* water assessments and laboratory analysis of water samples, will give a good understanding of the present water quality status. It was expected that there would be significant water quality differences between the Magalies and Hennops rivers. This would be true temporally and spatially as these two rivers have different water quality drivers. It was expected that there would be increased levels of phosphates in the Magalies River compared to the Hennops River because of the increased agriculture. There would also be higher Chemical Oxygen Demand (COD) concentrations in the Hennops River compared to the Magalies River because of the

increased number of WWTPs. Moreover, lower Dissolved Oxygen (DO) levels in the Hennops River compared to the Magalies River due to the higher organic load.

Another aim was to measure the biotope availability and variation of the Hennops River, and how this would affect the presence, abundance and diversity of macroinvertebrates. These parameters were examined using the Rapid Habitat Assessment Method (RHAM) technique and later compared with South African Scoring System (SASS) 5 data. It was expected that in areas with high anthropogenic influences the river channel would become homogenised, decreasing the number of biotopes available, leading to less abundant and diverse macroinvertebrate communities.

The central aim of this project was to evaluate the current presence, abundance, and diversity of macroinvertebrate communities along the Hennops River in comparison to the Magalies River, so as to understand the effects of intensive urbanisation on a river ecosystem. This assessment was conducted using the SASS 5 procedure methods, coupled with further taxonomical laboratory analysis. It was hypothesized that the freshwater macroinvertebrate communities would exhibit higher abundance and diversity in the upper reaches of the Magalies River but would decline in the lower reaches. Additionally, it was anticipated that these lower Magalies River aquatic macroinvertebrate communities could be similar in diversity to those in the Hennops River.

Lastly, this project aimed to understand the broader implications of this study's findings for water management and legislation in Gauteng, South Africa.

CHAPTER 2: ASSESSING THE CURRENT WATER QUALITY STATUS, BIOTOPE AVAILABILITY AND VARIATION OF THE HENNOPS AND MAGALIES RIVERS

2.1 - Introduction

Water resources in South Africa (SA) are significantly constrained by rainfall and climate conditions, as it ranks among the 30th driest countries globally (DWS, 2024b). This country has a semi-arid climate and known for highly variable rainfall (Vogel, 2000), with an annual average of less than 500 mm, substantially below the world average of 850 mm (Plant *et al.*, 2001; DWS, 2024b); SA is also classified as a negative runoff zone, where yearly evaporation is greater than yearly rainfall, resulting in only 8.6% of annual rainfall contributing to surface water availability (Weaver, Le Roux and Pretorius, 1999; Miller, 2002; Huizenga, 2004).

Population growth, especially in both urban and rural areas, significantly impacts water quality through agricultural practices—which alone account for 61% of SA's available surface freshwater withdrawal (Knight, 2019)—as well as through economic development and rapid changes in land cover and use. The intensification of land use and economic development is driven by the need to accommodate expanding populations (Huizenga, 2004). In 1996, approximately 12 million people lacked access to uncontaminated freshwater, and around 21 million lacked access to waterborne sanitation systems (Walmsley, Walmsley and Silberbauer, 1999). Consequently, surface waters in SA tend to have elevated salinity levels (Plant *et al.*, 2001). However, catchments draining from sandstone aquifers, such as the lower Magalies River near Hartebeestpoort Dam, exhibit low salinity. The closed system observed within dolomitic aquifers suggests carbonate dissolution as the primary source of dissolved carbon and a main pH influencer (Leketa, Abiye and Butler, 2018)(Fig. 1.2).

The Department of Water and Sanitation (DWS) identifies urban pollution, eutrophication, sedimentation, salinisation, and acidification as the primary threats to SA's freshwater systems (Gitau, Chen and Ma, 2016). These stressors pose significant risks to freshwater system integrity and are expected to worsen with ongoing climate change. Disruptions to water availability and supply will have far-reaching consequences within the water-energy-food nexus, potentially leading to the degradation of critical infrastructure. The DWS attributes the decline in water quality to systemic issues, including inadequate cross-sectoral governance and regulatory coordination. Additionally, poor implementation of sustainable land use practices and deficient communication among municipalities contribute to this problem (Gitau et al., 2016).

The latest Blue Drop and Green Drop reports reveal a critical and concerning state of SA's water and sanitation services (DWS, 2023a, 2023b). While the DWS has achieved comprehensive audit coverage, the findings indicate a significant deterioration in both drinking water quality and wastewater treatment performance since 2014 (DWS, 2023a, 2023b). A substantial number of water supply systems are now in a critical state, with a dramatic increase in microbiological contamination posing severe public health risks. Similarly, the majority of WWTPs are at high or critical risk of discharging inadequately treated effluent, leading to widespread environmental degradation (DWS, 2023a, 2023b). These challenges are rooted in systemic issues including aging infrastructure, pervasive skills shortages, financial non-viability, and deficiencies in governance and accountability. The DWS has responded with strengthened regulatory surveillance, collaborative action plans, and technological advancements, yet the sustainability of these interventions remains contingent on fundamental shifts towards municipal self-sufficiency, robust asset management, and a renewed social compact with communities (DWS, 2023a, 2023b). Addressing this crisis demands a holistic, integrated approach that prioritizes sustained

investment, human capital development, and transparent, accountable governance to uphold the constitutional right to safe water and protect environmental integrity (DWS, 2023a, 2023b)

The escalating human population directly increases stress on natural resource systems, making comprehensive water resource management strategies more imperative than ever to ensure future water security. Thorough assessments of riverine ecosystem health are essential to determine the specific factors contributing to the observed decline in aquatic biodiversity within the studied rivers. These assessments must accurately evaluate both water quality parameters and habitat availability to determine their respective contributions to biodiversity loss (Toerien and Walmsley, 1979; Nawn, 2009; Sadiki, 2020). The Hennops River, one of Gauteng's longest rivers and a vital natural water resource (Region 4: Regional Integrated Development Plan, 2014; Letseka *et al.*, 2023), provides essential ecosystem services and contributes substantially to the hydrological balance of numerous surrounding areas. The confluence of the Hennops and Jukskei rivers forms the Crocodile River (Fig. 1.2), which contributes 90% of the inflow into Hartebeestpoort Dam (Leketa, Abiye and Butler, 2018; Leketa *et al.*, 2019; Letseka *et al.*, 2023). While the Magalies River's inflow contribution is minor, it is necessary for diluting the predominantly polluted rivers. Water hyacinth and other invasive species thrive due to the previously stated primary issues (Ali et al., 2022).

Historically, the Hennops River exhibited clean, high-quality water and functioned as a robust freshwater resource. However, the river's ecological integrity has drastically declined in the past decades. Sinclair et al. (1983) documented the Hennops River as having riparian zones with dense vegetative cover, a stark contrast to the current condition, characterized by sewage contamination, foam accumulation, eutrophication, and a significant reduction in riparian vegetation. It was also reported that water temperatures in the Hennops River ranged from 8.1°C to 16.8°C. While rainfall patterns have always been variable, Sinclair's research

indicated the river's water quality was quite good, supporting diverse fungal and faunal species. In contrast, the river is now heavily impacted by urbanization, which significantly influences the presence, variety and abundance of aquatic biota.

The river's geological context, featuring ancient marine sedimentary beds, dolomitic aquifers, and calcium-rich formations, results in naturally elevated pH levels. This geological foundation once supported a complex ecosystem, encompassing diverse fauna, including invertebrates and terrestrial vertebrates, such as baboons and monitor lizards. Historically, the area sustained large megafauna, such as eland and kudu, and the river provided habitat for river otters (Sinclair, Ebersohn and Eicker, 1983; Hennops River Fountain Source Sanctuary Project, 2017). The Hennops River, which previously offered significant recreational opportunities and provided all main ecosystem services, has since degraded (Nawn, 2009; Yeakley *et al.*, 2016; Hennops River Fountain Source Sanctuary Project, 2017). Currently, both formal and informal recreational areas are threatened by the compromised water quality originating from the river (Hennops River Fountain Source Sanctuary Project, 2017; Sinclair et al., 1983). As previously discussed in chapter 1, the geomorphology (sediments and riverbed) and hydrology interact to drive habitat changes within a river, with chemical composition influencing the physical structure of both instream habitat and riparian zones (Fig. 1.2).

This study aimed to investigate the interconnected effects of water quality and biotope availability in the Hennops and Magalies rivers. It was hypothesized that the Magalies River would have higher phosphate levels due to agriculture, while the Hennops River would show elevated COD and lower DO due to its numerous WWTPs and greater organic load.

To assess these relationships, the current water quality status of the rivers were determined using *in situ* measurements and laboratory analyses. Concurrently, biotope

availability and variation were be quantified through the Rapid Habitat Assessment Method Model (RHAMM) (Kleynhans and Louw, 2015).

2.2 - Methods

Physico-Chemical Parameters Methods

In Situ Measurements

Prior to the collection of physical water samples, in situ physicochemical parameters were measured using two handheld multiparameter meters (YSI Professional Plus and YSI ProQuatro). Measurements were replicated every 30 seconds over a five-minute interval to obtain representative mean values for each sampling site during both dry (September – November 2023) and wet (March – May 2024) seasons. The multi-probe YSI's were positioned midstream or slightly lateral to the main flow—avoiding areas of emergent vegetation or stagnant water—to ensure consistent readings of the active water column. Parameters recorded included water temperature (°C), dissolved oxygen (DO; mg/L), electrical conductivity (EC; µS/cm), nitrates (NO_3^-; mg/L), and oxidation–reduction potential (ORP; mV). To ensure data reliability and consistency across all sampling events, strict field quality assurance and control (QA/QC) procedures were implemented. All YSI meters were calibrated prior to each sampling day using certified standards (85% air-saturated water for dissolved oxygen; and Nitrogen sensors were calibrated the day before with varying solutions 1mg/L, 10mg/L and 100mg/L). Calibration checks were repeated at the end of each field day to verify instrument stability, with deviations exceeding ±5% prompting recalibration. Duplicate readings were recorded at a frequency of one in every five sites to assess measurement precision, maintaining a tolerance of ±0.2 pH units, ±0.5 mg/L DO, and ±10 µS/cm EC between replicates. All sensors were rinsed with distilled water and inspected for fouling between measurements to avoid cross-contamination. Field logs were maintained to record

calibration times, environmental observations, and instrument performance, ensuring full traceability in accordance with American Public Health Association (2017) Standard Methods for the Examination of Water and Wastewater (APHA, 2017).

Laboratory Analysis of Water Chemistry

During both the wet and dry seasons, three 250 mL water samples were collected from each sampling site (3 × 250 mL jars × 6 sites × 2 seasons = 36 × 250 mL sample jars). Samples were collected mid-stream at approximately mid-depth to ensure representative capture of the flowing water column. All sampling bottles were sterile prior to use, pre-rinsed with site water, and sealed immediately after collection. Each was labelled and stored in insulated cooler boxes maintained at approximately 4 °C, then transferred to a freezer (–19 °C) until delivery to the analytical laboratories at the end of each sampling season (end of November 2023 and end of May 2024).

Of the three samples collected per site and season, one 250 mL sample was submitted to Waterlab (Pty) Ltd, Pretoria, for further analysis on pH (measured at 25 °C), nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate (PO_4^{3-}), free and saline ammonia (NH_3), and ammonium (NH_4^+) concentrations. A second 250 mL sample was sent to the School of Chemistry, University of the Witwatersrand (Johannesburg) for determination of COD (mg/L) and trace-metal concentrations using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP–OES). All laboratory analyses were performed for both the wet and dry seasons to ensure seasonal comparability. For nutrient and general water chemistry parameters, samples were maintained unacidified but refrigerated to minimise biological transformation. All analyses were conducted following the APHA (2017) Standard Methods for the Examination of Water and Wastewater (APHA, 2017). The Waterlab (Pty) Ltd facility is SANAS-accredited (Accreditation No. T0221) for water-quality testing, while the

University of the Witwatersrand, School of Chemistry performed additional analyses using equivalent ISO- and APHA-compliant protocols under controlled laboratory conditions. A third 250 mL sample from each site (from both seasons) was retained under deep-freeze storage at –19 °C as a backup for potential re-analysis or supplementary testing, ensuring sufficient sample volume in the event of instrument reruns or quality-control discrepancies. Full chain-of-custody documentation was maintained throughout sampling, transport, and analysis to ensure traceability and analytical integrity.

Comprehensive Quality Assurance and Control (QA/QC) procedures were implemented throughout the analytical process to ensure data accuracy, precision, and traceability (Appendix 2.A). All laboratory instruments were calibrated daily using multi-point standards traceable to South African National Accreditation System (SANAS) and National Institute of Standards and Technology (NIST) reference materials. Field and laboratory blanks were included to assess potential contamination, while duplicate samples were analysed at a frequency of one per ten samples to evaluate analytical precision. Certified reference materials were run concurrently with each batch to verify instrument performance and recovery efficiency, maintaining recoveries within ±10% of certified values. Detection limits, linear calibration ranges, and method accuracy were confirmed in accordance with APHA (2017) specifications. All measurements were recorded in duplicate, with results expressed as the mean of replicate determinations. Any outlier data were rechecked against instrument calibration and sample integrity records before inclusion in statistical analyses.

Organic pollutants, typically anions (Zhao *et al.*, 2020), often indicate anthropogenic sources, whereas cations suggest geological influences (Dalmieda and Kruse, 2019) and heavy metals can signify external contaminants (Yan *et al.*, 2013). By identifying specific pollutants, inferences can be made about potential sources and their influence on macroinvertebrate variation and community structure. Among the water quality variables

tested in this study were various cations, including ammonium (NH_4^+), aluminium (Al), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb), zinc (Zn), calcium (Ca), cobalt (Co), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), arsenic (As), and nickel (Ni). The anions analysed comprised nitrate (NO3), chromium (Cr), selenium (Se), and vanadium (V). While physiological assessments are needed to fully understand the links between water quality and biotic responses, this study will draw conclusions using a combination of theoretical, statistical, and physicochemical data.

Physical Habitat Measurements

At each site, two transects were established, or at least one where variations in river depth limited replication, ensuring that the same Geomorphic Habitat Units (GHUs) (riffle or run) was sampled during both dry (September – November 2023) and wet (March – May 2024) seasons. The RHAM protocol provides an effective framework for transect-based sampling, facilitating the identification and quantification of meso-habitats or biotopes available to aquatic biota. Given that this study examined highly polluted areas and rivers, biological safety and security were prioritized. All personnel involved in river sampling wore protective clothing, including full-body waders, shoulder-length gloves, and face masks. After each sampling session, equipment and personnel were rinsed with freshwater and disinfected to prevent site cross-contamination and protect equipment from deterioration.

Sampling commenced with the identification of the three principal geomorphic habitat units (GHUs): pools, riffles, and runs. Only riffle and run habitats were selected for assessment, as these zones represent the dominant habitats for benthic macroinvertebrates rather than fish assemblages (Righi-Cavallaro *et al.*, 2010; Wright *et al.*, 2025). Still-point photos were taken of the upstream and downstream sections at each sampling site. A 100m rope was used at sites with wide channels, high flow and low water clarity. To facilitate

walking along the transect and ensure safety, particularly preventing individuals from being washed downstream due to loose footing and depth, the rope was equipped with hook attachments. This was particularly necessary in the Hennops River, which exhibited wide channels and depths often reaching chest depth, along with poor water clarity, however it was not required for the Magalies River.

A measuring tape was used along the transect of a GHU, in 1 m intervals with substratum categorized within 30 cm quadrats (minimum of six measurements across the river channel). At each interval, the composition of the substratum (gravel, sand and mud (GSM), bedrock, stones-in-current (SIC), etc.), the presence of algae and macrophytes, and in-stream cover (woody debris, rocks, roots, undercut banks, overhanging riparian vegetation, and aquatic vegetation) were recorded (Kleynhans and Louw, 2015). In highly polluted and turbid waters where visual assessment of the river substrate was not possible, a hand grab of sediment using shoulder-length gloves was necessary for substrate assessment. Measuring the GHUs and identifying the biotopes serves as a proxy for available niches for invertebrates, and this can be thoroughly assessed using RHAM.

The calculation for the average wetted depth can be based on the measurements along the transects at each sampling site using the guideline summarised by The Comet Program (The COMET® Program, 2006). A transparent velocity head rod was utilized to measure the water velocity along each interval of the transect, with a minimum of 8 measurement for each transect (GroundTruth LTD, 2024). The combination of water velocity and depth values, results in velocity–depth scores which represent the available flow variations, depths within the GHUs and biotopes at their respective sites (The COMET® Program, 2006; Vatankhah, 2018). In addition, landscape-scale variables such as elevation, surrounding land uses, and upland/lowland areas were recorded as complimentary data. The available habitats indicate

the potential for macroinvertebrate communities in the respective biotopes along the studied rivers.

2.3 –Statistical Methods

Statistical analyses, done in RStudio and using PAST software (Hammer, Harper and Ryan, 2001; RStudio, 2022), involved non-parametric tests including Kruskal Wallis and Several-samples test, Chi-squared test, ordination analyses such as PCA biplots and PCA with convex hulls, correlation analysis, side-by-side comparisons of chemical water parameters and discharge comparisons to evaluate each river and site across seasons. The specific differences analysed were the predicted increased phosphates in the Magalies River and elevated COD and organic loads, alongside reduced DO, in the Hennops River. It was also anticipated that the Magalies River would exhibit greater biotope availability and variation compared to the Hennops River, based on RHAM data. For habitat analysis, a Principal Component Analysis (PCA) was initially conducted to identify significant biotope variations and potential outliers. Subsequently, the Several-Sample analysis was used to determine the statistical significance of site separations observed in the PCA, informing the development of biotic response hypotheses. Furthermore, to quantify inter-river variation in habitat availability, as derived from the numerical data produced by RHAMM (Kleynhans and Louw, 2015), Chi-squared tests were utilized to compare biotope count between sampled sites. It was expected that increased biotope availability would correlate with more complex and diverse macroinvertebrate communities. These statistical approaches collectively aimed to elucidate the influence of both water quality and habitat characteristics on macroinvertebrate communities across the Hennops and Magalies rivers.

2.4 - Results

In situ Water Measurements

The *in situ* water quality analysis, done in RStudio (RStudio, 2022), revealed distinct differences between the Magalies and Hennops rivers, with temperature being relatively constant across sites, though slightly lowered at forested middle site (M2) (Fig. 2.4.1.). The DO was generally higher in Magalies sites, while Hennops sites, particularly H2, exhibited lower levels, with H3 being most similar to the Magalies sites (Fig. 2.4.1.). Nitrate levels were significantly elevated in Hennops sites, especially H2 (Fig. 2.4.1.). Although pH remained relatively stable across sites, M2 showed a notable alkaline peak in the wet season, and site H1, despite expectations of acidity due to illegal industrial dumping (orange-coloured effluents - Appendix 1.A), had a near-neutral (Fig. 2.4.1.). Alarmingly, during the dry season, the Hennops River sites were, on average, 175% more acidic than the Magalies River sites. This disparity in acidity increased significantly in the wet season, with Hennops River sites being, on average, 270% more acidic than those in the Magalies.

The Oxidative Reduction Potential (ORP) showed no negative values, with the Magalies lowest site (M3), showing very high values in the dry season (Fig. 2.4.1.). Conductivity was markedly higher in Hennops sites, particularly the middle site (H2), indicating higher dissolved mineral and salt concentrations (Fig. 2.4.1.). Overall, the Hennops River displays clear signs of greater environmental stress, indicated by elevated nitrate and conductivity, coupled with lower DO and ORP. The Magalies River, conversely, exhibits conditions influenced more by seasonal variations, including dilution effects during the wet season (Fig. 2.4.1.).

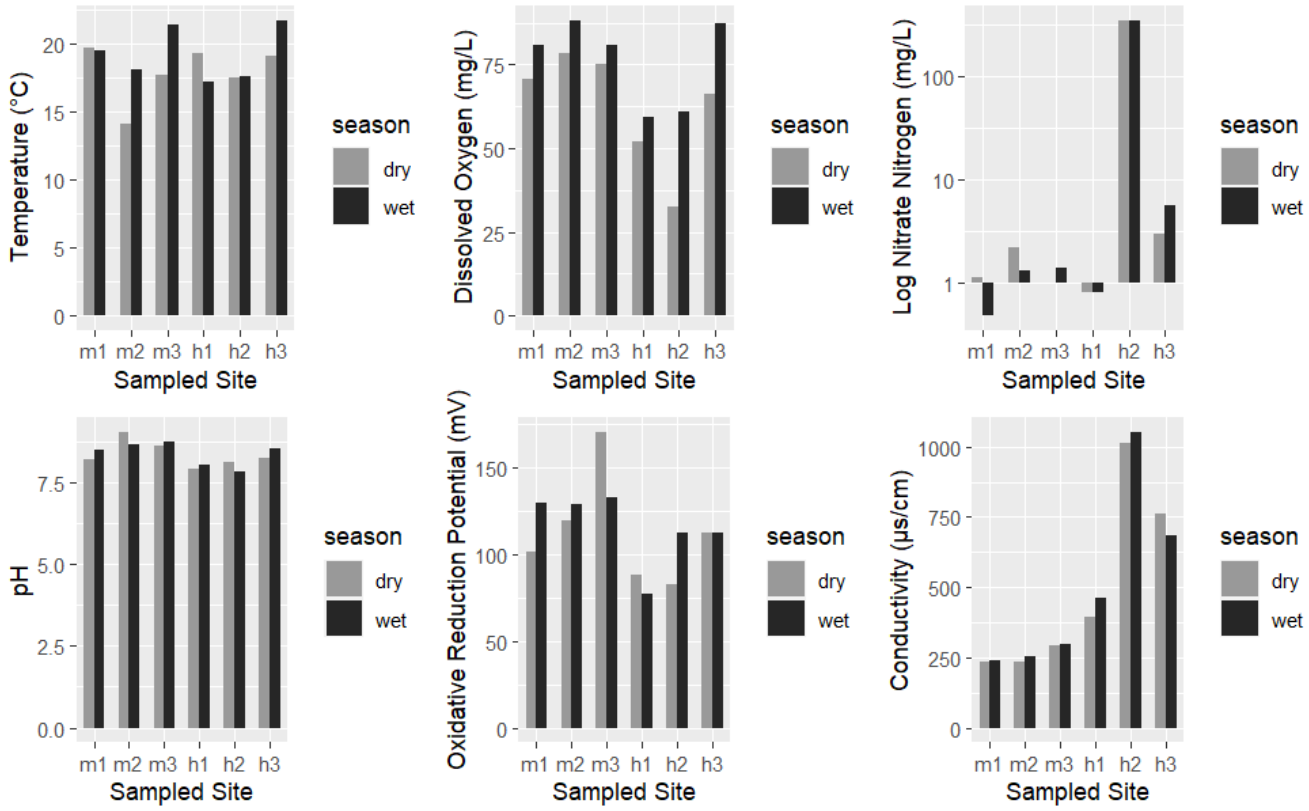


Figure 2.4.1: In situ water data collected from the Magalies and Hennops rivers across seasons.

Laboratory Analyses of Heavy Metals

In both wet and dry season, results from heavy metal analysis were recorded and compared between both rivers (Appendix 2.B). The grouped bar chart reveals distinct seasonal and spatial patterns in metal concentrations between the Magalies River and Hennops River (Appendix 2.B). During the dry season, the Hennops River exhibited higher concentrations for 12 of the 17 metals analysed (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, and V), while the Magalies River showed higher concentrations for only 5 metals (Ca, K, Mg, Na, and Zn) (Appendix 2.B). The most notable differences between rivers during the dry season were observed for Fe, with Hennops recording 0.775 mg/L compared to Magalies at 0.388 mg/L, and Mg, with Magalies recording 10.814 mg/L compared to Hennops at 7.833

mg/L (Appendix 2.B). In the wet season, this pattern shifted, with the Magalies River showing higher concentrations for 8 metals (As, Ca, Cr, Cu, K, Mg, Na, and Zn), while the Hennops River maintained higher levels for 9 metals (Al, Cd, Co, Fe, Mn, Ni, Pb, Se, and V). Seasonal variations differed markedly between the two rivers: the Magalies River exhibited substantial increases from dry to wet season for 12 metals, most prominently Na which increased by 49.2% from 17.926 mg/L to 26.744 mg/L, Se by 52.8% from 0.211 mg/L to 0.323 mg/L, and As by 38.6% from 0.126 mg/L to 0.175 mg/L (Appendix 2.B). Conversely, the Hennops River showed increases for 11 metals during the wet season, with the most pronounced being Se at 61.1% increase from 0.225 mg/L to 0.362 mg/L, Pb at 34.3% increase from 0.345 mg/L to 0.463 mg/L, and Ni at 33.0% increase from 0.100 mg/L to 0.133 mg/L (Appendix 2.B). Notably, the Hennops River exhibited decreases in Na by 7.9%, K by 10.2%, and Fe by 1.3% during the wet season, contrasting with increases observed in the Magalies River for these same metals (Appendix 2.B). Both rivers showed decreases in Al and V concentrations during the wet season, with Magalies decreasing by 18.6% and 14.9% respectively, and Hennops decreasing by 13.9% and 7.4% respectively (Appendix 2.B).

Flow

The discharge patterns between the Hennops and Magalies rivers show distinct differences: the Magalies River exhibits moderate discharge with higher flow rates in the dry season, while the Hennops River displays a substantial downstream increase in discharge, peaking in the wet season (Fig. 2.4.2). Specifically, the upper Magalies site (M1) has low discharge year-round, the middle site (M2) shows the highest Magalies discharge in the dry season, and the lower site (M3) also has low discharge (Fig. 2.4.2). In contrast, the upper Hennops site (H1) has the lowest discharge overall, the middle site (H2) has a moderate flow increase in the wet season, and the lower site (H3) demonstrates the highest discharge, especially during the wet season (Fig. 2.4.2). It is worthy of noting that all the Magalies sites

and H1 have more discharge in the dry season compared to the wet season, this could indicate a connection to surrounding land-uses (Fig. 2.4.2).

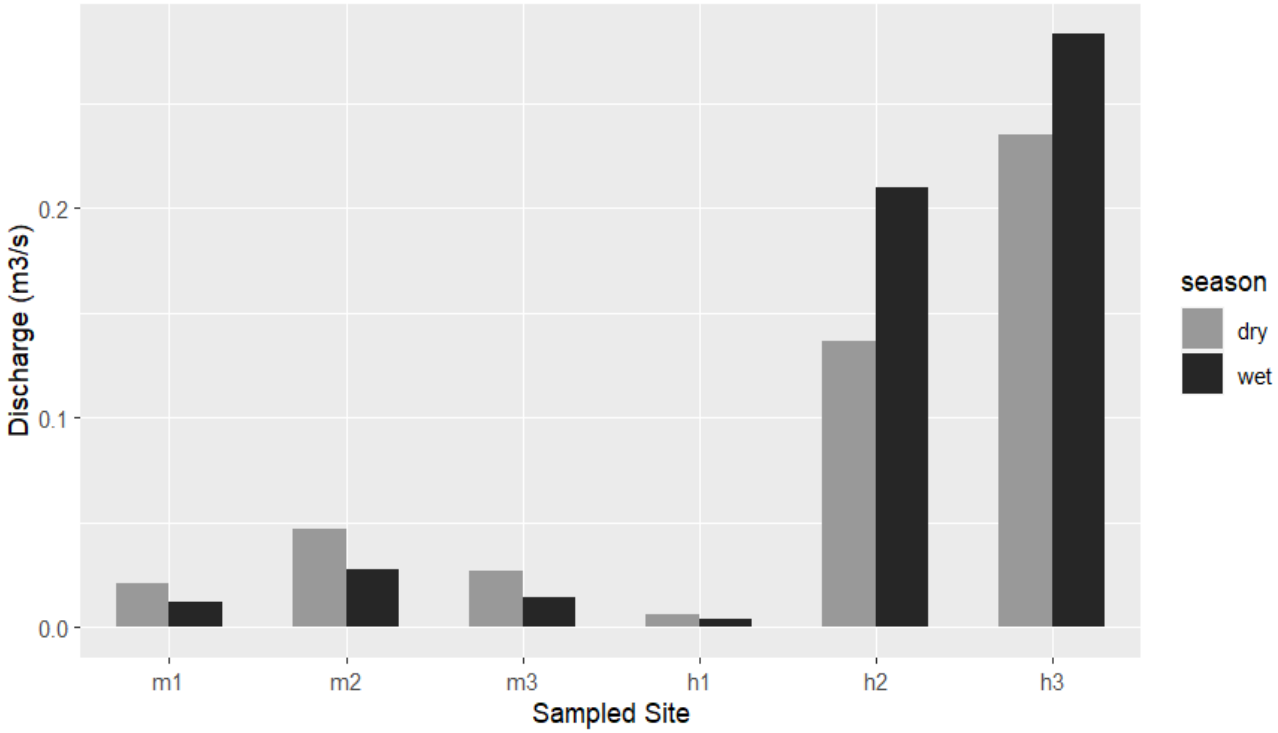


Figure 2.4.2: A double Bar Graph showing the Average Discharge (m^3/s) at each sampled site across seasons

Initial Statistical Analyses

Statistical analyses revealed significant differences in both water quality and habitat characteristics between the Hennops and Magalies rivers. Non-parametric tests, necessitated by non-normal data distributions (Levene's test for water quality: $p < 0.0001$; Levene's test for habitat: $p < 0.01$), including Kruskal-Wallis tests (water quality: X^2=263.1,p<0.0001; habitat: X^2 =263.1,p<0.0001) and a Several-sample test (habitat: $F = 20.65$, $p < 3.082E-32$), strongly indicated unequal medians or means between the rivers for both environmental drivers. Furthermore, Chi-squared tests confirmed a significant variation in the amount of available biotope across the sampled sites and seasons (X^2 =842.07, p<0.000001).

Ordination Analyses

The PCA biplot, representing in situ and laboratory-tested water quality data across all sites and seasons, reveals a clear distinction between the Magalies and Hennops River sites (Fig. 2.4.3). Across both seasons, the Magalies sites are tightly clustered, indicating similar water quality derived from both field measurements and laboratory analyses of major ions and heavy metals, as described in the methods. In contrast, the Hennops River, the upper site (H1) displays the greatest similarity to the Magalies sites across both wet and dry seasons, while the middle site (H2) plots as a distinct outlier, particularly in the wet season, suggesting the strongest anthropogenic influence and the most impaired water quality (Fig. 2.4.3). The lower site (H3) separates from both the Magalies and other Hennops sites, suggesting that it is influenced by additional downstream processes or inputs (Fig. 2.4.3). The primary environmental variables structuring the ordination are conductivity, which strongly influences site separation along PC1 (45.2%), and nitrates, contributing most to variation along PC2 (26.1%) (Fig. 2.4.3). Higher conductivity and nitrate concentrations, coupled with lower DO and more variable pH and ORP, characterize the Hennops sites—particularly the middle reach (H2). In contrast, the Magalies sites exhibit lower conductivity and nitrate levels with higher DO and more stable physico-chemical conditions. Overall, the PCA indicates a pronounced pollution gradient between the two river systems, with the Hennops River showing significant environmental stress and anthropogenic loading, particularly at the middle site (H2) (Fig. 2.4.3). The lower reaches of the Hennops (H2 and H3) also show higher discharge and greater variability in water quality parameters compared to both the Magalies and the upper Hennops site (H1) (Fig. 2.4.3).

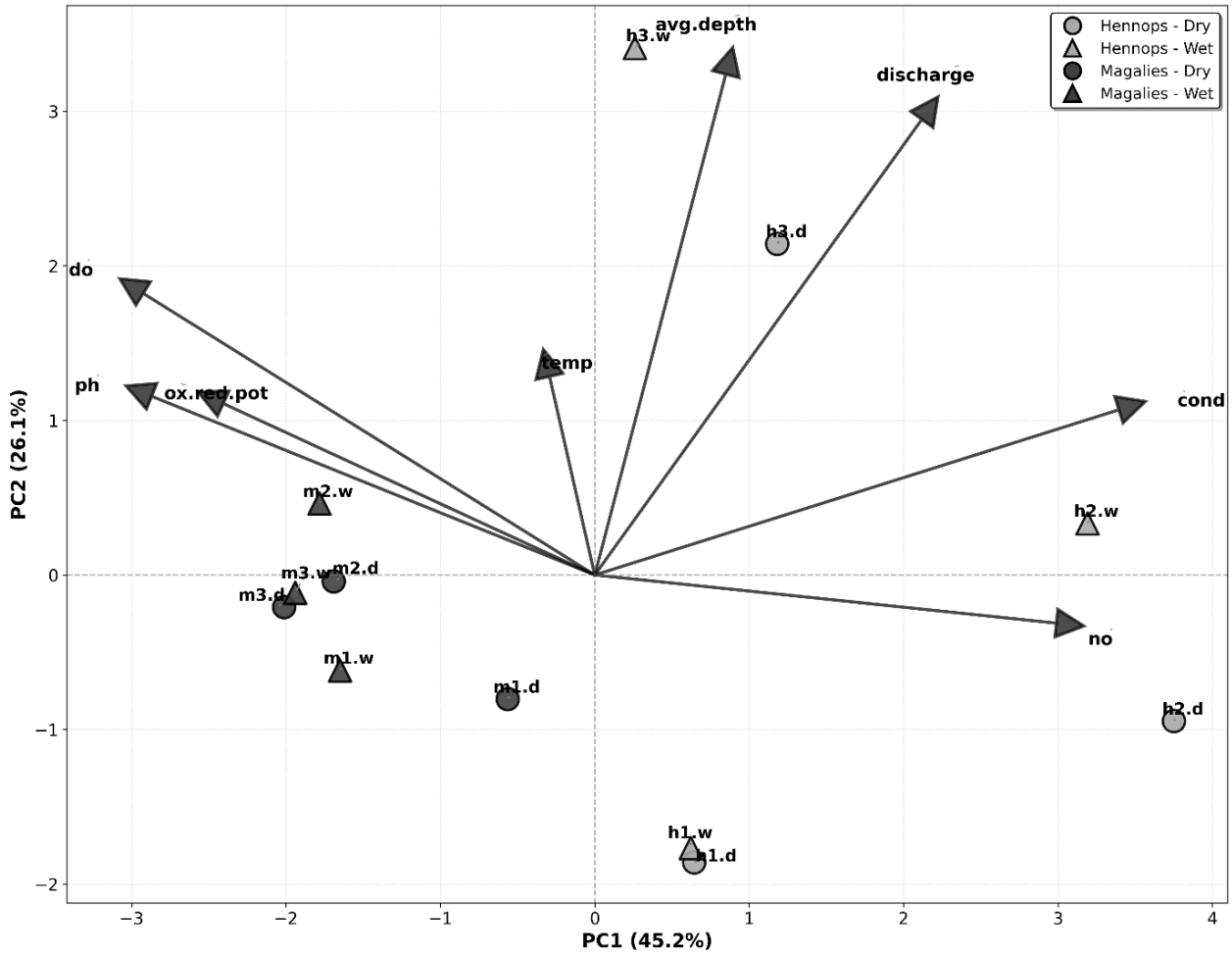


Figure 2.4.3: PCA biplot of all in situ and tested water quality data across all sites and seasons

To clarify relationships that were previously masked by the strong influence of the middle Hennops site (H2), a secondary PCA was conducted excluding H2 (Fig. 2.4.4). This analysis revealed clearer patterns of variation between and within the remaining sites. The Magalies sites exhibited a broader spread along PC2 (32.2%), suggesting increased within-river variability once the dominant H2 outlier was removed. The Hennops sites, in contrast, separated primarily along PC1 (46.4%), driven by differences in conductivity. The lower Hennops site (H3) remained distinct from both the Magalies and the upper Hennops site (H1), while H1 occupied an intermediate position between these two groups. The Magalies sites continued to cluster relatively closely, although with slightly greater dispersion along

PC2 compared to the previous analysis. Conductivity continued to be the strongest driver of variation along PC1, influencing the separation of Hennops and Magalies sites, while ORP and DO became more influential along PC2. This shift indicates that, in the absence of H2's dominant effect, the ordination better captures subtler gradients in oxygen dynamics and redox conditions across the remaining sites. Overall, this analysis confirms that conductivity remains the primary discriminating factor between the two rivers, while DO and ORP emerge as important secondary gradients influencing site variation when the most polluted middle reach (H2) is excluded (Fig. 2.4.4).

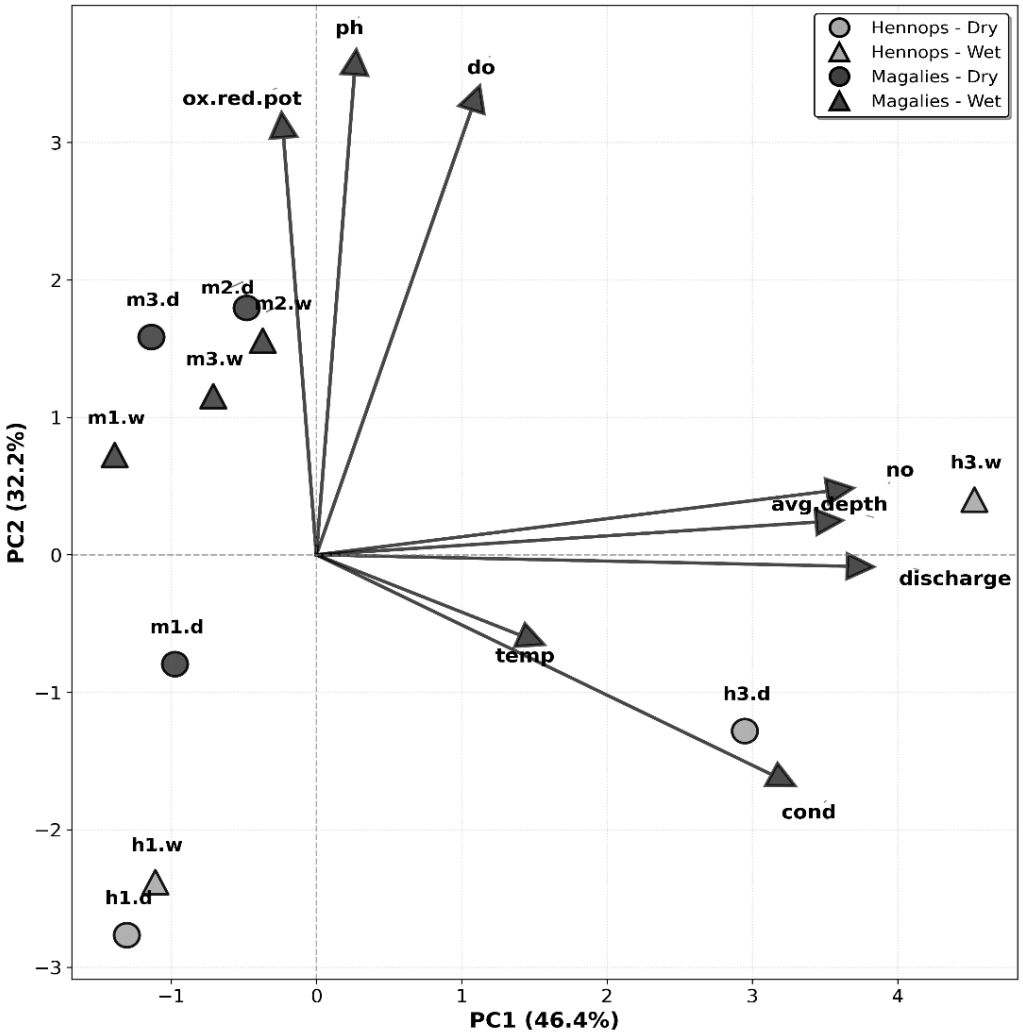


Figure 2.4.4: PCA biplot of all in situ and tested water quality data across all sites and seasons, excluding H2.

The PCA loading analysis revealed that conductivity is the dominant factor influencing the principal component, exhibiting a strong positive loading (~0.90) (Appendix 2.C). The ORP shows a moderate positive loading (~0.35), indicating a less pronounced positive correlation, while sodium has a weak positive loading (~0.10) (Appendix 2.B). Temperature and COD have minor negative loadings (~-0.05) (Appendix 2.C). The remaining parameters and various metals, have negligible loadings, suggesting little influence on this principal component (Appendix 2.C). Overall, conductivity is the primary driver of the variability captured by this component (Appendix 2.C).

The correlation matrix analysis revealed significant relationships between water quality parameters (Appendix 2.C). Conductivity showed a strong positive correlation potassium and magnesium, indicating that these parameters tend to increase or decrease together (Appendix 2.C). Potassium and magnesium were also strongly positively correlated with each other and with manganese (Mn) (Appendix 2.C). In contrast, conductivity exhibited a strong negative correlation with DO, suggesting that higher conductivity is associated with lower DO. Nitrate levels were high in middle Hennops sites (H2) and near the legal limit in Lower Hennops sites (H3) but did not show a strong correlation with conductivity (Appendix 2.C). Several metals (Al, As, Ca, Fe) displayed moderate positive correlations, while COD had a moderate negative correlation with ORP (Appendix 2.C). Temperature and pH showed weak correlations with most other parameters and DO indicating a negative correlation with all Hennops sites (Appendix 2.C).

The PCA of benthic habitat availability, incorporating convex hulls and 95% confidence ellipses, illustrates clear differences in substratum composition and proportional habitat availability between the Magalies and Hennops rivers (Fig. 2.4.5). The Magalies sites form a compact cluster, indicating relatively consistent substratum profiles and habitat conditions, with most variation arising from differences in GHU (riffle versus run). In

contrast, the Hennops sites display greater dispersion, suggesting increased variability in habitat structure likely associated with anthropogenic impacts on channel morphology and substrate stability. The convex hulls and confidence ellipses visually reinforce these differences, with the Hennops River exhibiting a broader ellipse and larger hull area, denoting greater heterogeneity in benthic habitat composition. Within the Hennops River, the middle site (H2) is clearly separated from both upstream (H1) and downstream (H3) sites—particularly in the wet season riffle habitats—indicating altered or unstable substratum conditions. The lower Hennops site (H3) run biotopes appear as extreme outliers, reflecting simplified or degraded habitat structure. Conversely, the upper Hennops site (H1) clusters closer to the Magalies group, suggesting relatively undisturbed or more natural habitat conditions. Among the Magalies sites, M1 and M3 show strong overlap, reflecting similar proportions of riffle and run habitats and consistent benthic substratum composition. M2, while still associated with the Magalies cluster, is positioned slightly apart, indicating subtle differences in biotope availability or substrate distribution compared to the other two Magalies sites. Overall, the PCA highlights the contrasting habitat variability between the two systems, with Magalies River sites exhibiting more stable and uniform benthic habitats, while Hennops River sites display greater heterogeneity and structural alteration, particularly at the middle and lower reaches (Fig. 2.4.5).

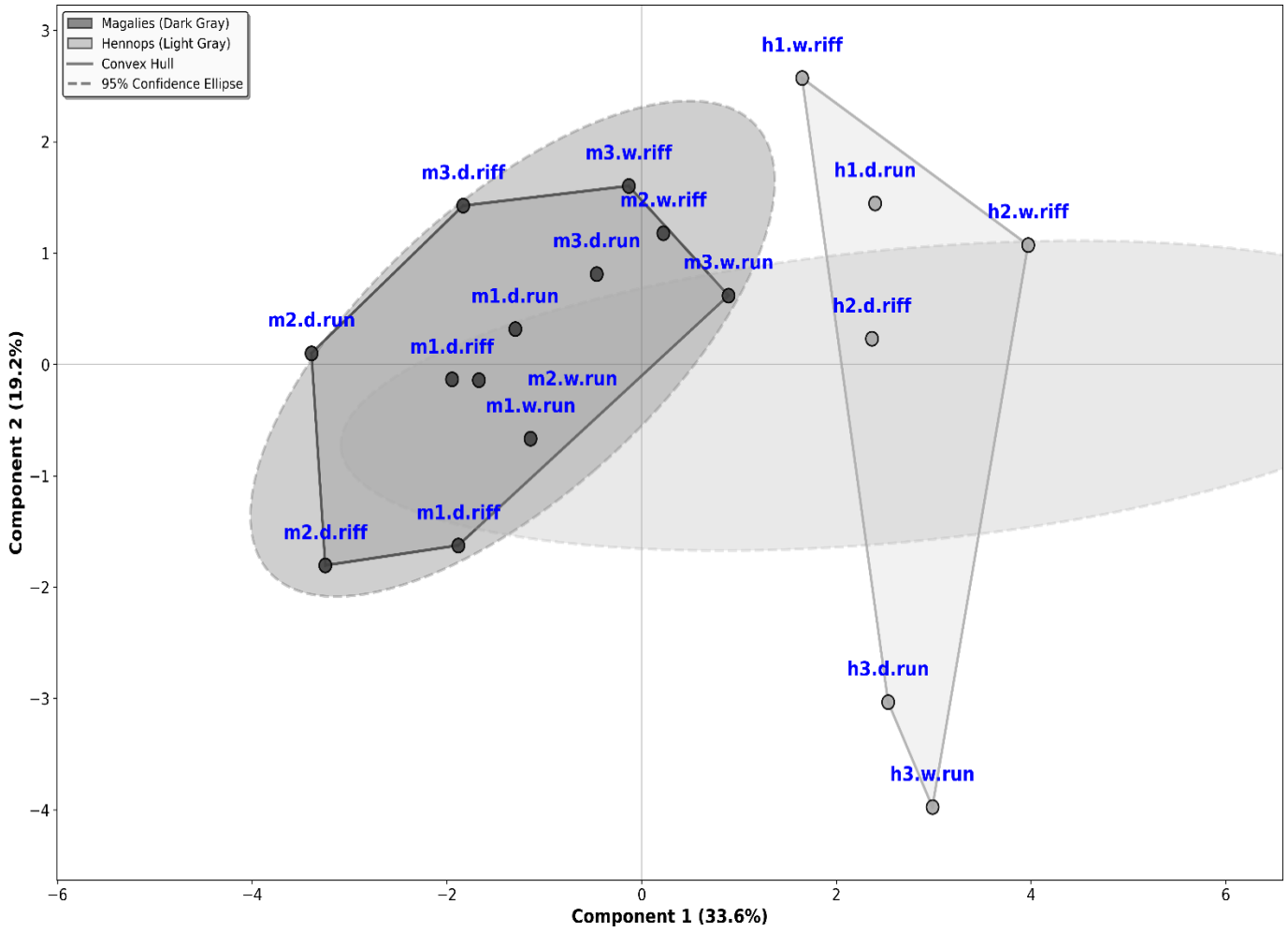


Figure 2.4.5: PCA output with convex hulls and 95% ellipses, showing the proportional benthic habitat availability and variation across all sites and seasons

To further examine the key habitat variables driving site-level variation, a PCA of biotope availability across all sampled sites and seasons was conducted (Fig. 2.4.6). The ordination reveals a distinct separation in habitat characteristics between the Magalies and Hennops rivers (Fig. 2.4.6). The Magalies sites are closely associated with features indicative of higher habitat complexity—such as embedded substrates, overhanging vegetation, woody debris, and finer sediment fractions—reflecting greater habitat stability and structural diversity (Fig. 2.4.6). In contrast, the Hennops sites are positioned along vectors representing simpler, more physically dominated habitats characterized by coarser substrata, including cobble, gravel, bedrock, and boulders (Fig. 2.4.6). Variation along PC1 (33.6%) primarily

differentiates sites based on substrate composition, ranging from fine, vegetated, and organic-rich habitats on the negative side (associated with Magalies sites) to coarse, mineral-dominated habitats on the positive side (typical of Hennops sites) (Fig. 2.4.6). PC2 (19.2%) further distinguishes sites according to substrate type and surface cover, separating sand- and algae-dominated microhabitats from those characterized by bedrock and boulder dominance (Fig. 2.4.6). Within the Hennops River, the lower sites (H3 and H6) are associated with bedrock and boulder substrata, suggesting reduced habitat diversity and channel homogenization (Fig. 2.4.6). The upper sites (H1 and H2) correspond more closely with cobble- and gravel-dominated habitats, reflecting moderate flow and sediment conditions (Fig. 2.4.6). In contrast, Magalies sites show tighter clustering and overlap between riffle and run habitats, indicative of consistent habitat structure and substratum composition across seasons (Fig. 2.4.6). Among the Magalies sites, the middle reach (M2) is slightly offset from the others, suggesting localized habitat modification relative to the more rural upper (M1) and lower (M3) sites. Overall, this analysis highlights that the Magalies River supports more complex and stable benthic habitats, while the Hennops River exhibits simplified, coarser substratum structures and reduced riparian vegetation, indicative of greater hydrological alteration and disturbance (Fig. 2.4.6).

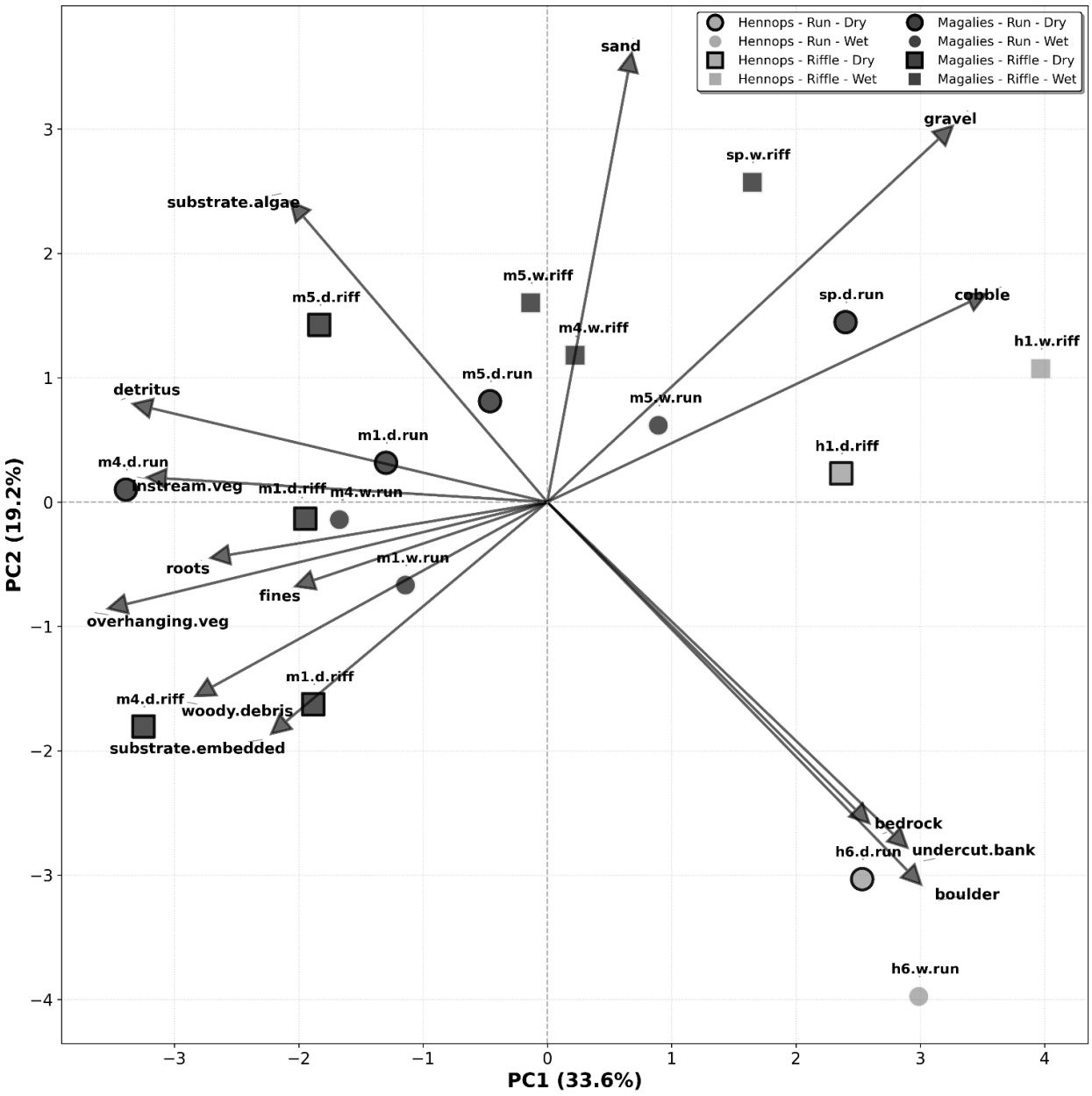


Figure 2.4.6: A PCA biplot output of the variation of biotope availability across all sampled sites and seasons.

2.5 - Discussion

This section clearly demonstrates that urbanization significantly impacts river ecosystems, creating distinct differences in water quality and habitat between the Hennops and Magalies rivers. The Magalies generally exhibits healthier conditions due to fewer stressors and greater resilience. In contrast, the Hennops consistently shows severe

degradation across most water quality parameters and simplified habitats, reflecting intense urban and industrial impacts – particularly affect the Hennops middle to lower reaches (H2, H3) whereas H1 showed the most similarity to the Magalies sites. These findings emphasize the scale and nature of urbanization that critically influence a river's ecological state (Allan, 2004b, 2004a).

The study's hypotheses regarding water quality and habitat were largely supported, revealing significant degradation in the Hennops River. *In situ* measurements showed that DO levels were generally lower at Hennops sites, particularly H2, compared to Magalies sites. Across both seasons, DO levels in both rivers were mostly below the recommended 80–120% saturation for aquatic ecosystems (DWAF, 1996), though a 13% average increase across all sites was observed, likely due to rainfall, which even doubled DO levels at H2 in the wet season. During the wet season, only Magalies sites and the lower Hennops site (H3) met the legal DO limit, suggesting some downstream recovery in the Hennops River (DWS, 2019).

The contrasting seasonal patterns of heavy metals between the Magalies and Hennops rivers suggest distinct hydrological regimes and contamination sources. The Magalies River exhibited substantial wet season increases in Na (49.2%), Se (52.8%), and As (38.6%), indicating non-point source pollution mobilization through surface runoff and erosion of accumulated pollutants from agricultural lands, urban surfaces, and mining-affected areas during rainfall events (Li *et al.*, 2014; Jin *et al.*, 2025). This pattern is characteristic of catchments where wet season precipitation facilitates the transport of metals through agricultural runoff containing fertilizers and pesticides (Popov *et al.*, 2012; Yao *et al.*, 2014), consistent with observations in other river systems where metal concentrations increased during rainy seasons due to surface runoff (Edokpayi *et al.*, 2017). Conversely, the Hennops River showed wet season decreases in Na (7.9%), K (10.2%), and Fe (1.3%), suggesting a

dilution effect from higher discharge volumes (Huang *et al.*, 2005; Giri and Singh, 2014). This dilution mechanism occurs when increased water volume during wet seasons effectively reduces metal concentrations, as documented in multiple river systems globally (Connor *et al.*, 1998; Resongles *et al.*, 2014). However, concurrent increases in Se (61.1%), Pb (34.3%), and Ni (33.0%) in the Hennops River point to specific point sources or contaminated sediment remobilization during high flow conditions (Ciszewski, 2001; Žák, Rohovec and Navrátil, 2009), potentially from industrial discharges or legacy mining activities. The consistently higher Fe concentrations in Hennops (0.775 mg/L dry, 0.765 mg/L wet) compared to Magalies (0.388 mg/L dry, 0.405 mg/L wet) suggest ferruginous mineral presence or iron-rich industrial effluents, while higher Mg levels in Magalies (10.814 mg/L dry) versus Hennops (7.833 mg/L dry) may reflect dolomitic geological substrate differences contributing to natural weathering inputs. The wet season decreases in Al and V observed in both rivers (Magalies: -18.6% and -14.9%; Hennops: -13.9% and -7.4%) are consistent with pH-dependent precipitation mechanisms, where aluminium and vanadium hydroxides precipitate or sorb onto suspended particulate matter at elevated pH values (Cappuyns and Swennen, 2014; Gustafsson, 2019). These findings indicate that the Magalies River is more susceptible to diffuse pollution during rainfall events, while the Hennops River is more influenced by point source inputs that are diluted during increased flow, necessitating tailored management strategies focusing on non-point source control in Magalies and point source remediation in Hennops (Jin *et al.*, 2025).

The Hennops River wet season orthophosphate levels (0.1−1.2 mg/L) were significantly elevated, vastly exceeding the ≤ 0.125 mg/L legal limit for aquatic ecosystems (DWS, 2019), contrary to predictions for the Magalies River (< 0.1 mg/L). This points to substantial phosphate pollution in the Hennops, likely from detergent-rich greywater and WWTPs. The COD was also considerably higher in the Hennops in the wet season (21 mg/L

at H1 and augmented to 111 mg/L at H2 downstream from a WWTP) than in the Magalies (all sites were mostly below readable limits, even M2 which is downstream from a WWTP), confirming greater WWTP effluent leakage and organic pollution load in the Hennops and its contribution to lowering DO.

Wet season samples revealed severely high ammonia levels in the Hennops River (e.g., 7.9 mg/L at H2), and even the Magalies River slightly exceeded the ≤ 0.0725 mg/L aquatic legal limit, indicating faecal contamination in both rivers. Conductivity and nitrate were primary drivers of site differentiation; Hennops sites, particularly H2, showed strong associations with high conductivity, high nitrate, and low DO, signalling severe stress. The Hennops River's significantly higher acidity, worsening in the wet season, also points to substantial mobilization of acidic pollutants, threatening its ecological integrity. Similarly, habitat analyses showed significant differences, with Magalies sites possessing more complex, natural structures versus the Hennops' simpler, often homogenized habitats.

Water temperature measurements revealed relatively narrow variation between the Magalies and Hennops Rivers (18.0-22.0°C). Notably, the current temperature ranges recorded in both the Hennops and Magalies rivers exceed historical thermal baselines for the Upper Crocodile (West) catchment, where natural perennial river temperatures typically ranged between 8–17 °C (DWAF, 2005; DWA, 2012; DWS, 2016), showing a 18-35 % increase in temperatures. These historical values reflect pre-urbanisation, moderately modified conditions, highlighting a meaningful thermal shift consistent with increased urban heat inputs, reduced riparian shading, and altered hydrological regimes. The Magalies River exhibited a 1.0°C seasonal increase (19.7°C dry to 20.7°C wet), while the Hennops River showed minimal variation (19.3°C dry to 19.7°C wet, +0.3°C), indicating stronger thermal buffering capacity in the Hennops system (Zhi *et al.*, 2023). This thermal stability in Hennops likely reflects higher wet season discharge volumes that enhance mixing and dilution (Van

Vliet *et al.*, 2013), or stronger groundwater contributions that provide thermal inertia through subsurface water inputs (Webb, Clack and Walling, 2003). Temperature functions as a master variable governing multiple water quality parameters in both systems. The inverse relationship between temperature and DO means that warmer wet season conditions in Magalies (up to 22°C) reduce oxygen solubility compared to the more thermally stable Hennops River (USGS, 2019; Atlas Scientific, 2021). Furthermore, elevated temperatures accelerate biogeochemical reaction rates including organic matter decomposition, microbial metabolism, and metal speciation (Ficklin, Stewart and Maurer, 2013; Rajesh and Rehana, 2022), potentially explaining the observed seasonal increases in dissolved metals in the Magalies River where warmer conditions enhance bacterial activity and pollutant breakdown. The minimal inter-river temperature differences (0.3-1.0°C) suggest similar regional climatic forcing, while site-specific variations (18-21°C within each river) reflect local controls such as riparian vegetation shading, channel morphology, water depth, and flow velocity (Stefan and Sinokrot, 1993). These coupled thermal-chemical dynamics underscore how temperature regulates oxygen availability, metal bioavailability, and overall ecosystem health across both river systems.

Magalies River

The Magalies River historically maintained moderate to good ecological condition, supported by relatively intact riparian vegetation, heterogeneous riffle-pool sequences, and lower anthropogenic nutrient inputs (DWAF, 2005; WRC, 2012). Earlier surveys reported median pH around 7.9, EC ≤ 50 mS m^{-1}, and nitrate concentrations < 1.0 mg L^{-1}—typical of lightly impacted sub-catchments draining natural and agricultural landscapes (DWAF, 2005). This studies wet-season medians reaffirm this comparatively healthy state: pH 6.5–9.0, DO > 120 % saturation, EC ≈ 240–298 µS cm^{-1} (~240–300 µS cm^{-1}), and COD ≤ 8 mg L^{-1}, all aligning with the legal limits and guideline thresholds for a Category B–C system (DWS,

2019; WRC, 2024). However, isolated exceedances—Zn 0.48 mg L^{-1}, Cu 0.17 mg L^{-1}, Pb 0.09 mg L^{-1}—exceed legal limits by >2 000–4 000 %, suggesting localised contamination likely linked to runoff or minor industrial effluent in lower reaches. The overall stability in DO and low COD values, coupled with vegetated banks and variable substrate composition observed in the field, indicates that the Magalies still retains functional habitat diversity and ecological resilience, despite increasing urbanisation in its downstream reaches. The comparison between historic and current data underscores diverging trajectories: the Hennops continues to deteriorate under urban effluent stress, while the Magalies, though under emerging pressure, largely sustains its historic moderate ecological condition.

The Magalies River is primarily influenced by a single WWTP, the Magaliesburg Percy Stewart WWTW, situated between M1 and M2. This facility is relatively small, with minimal backup systems and vulnerable to failure, serving domestic wastewater from Magaliesburg and Ga-Mohale (Mogale City Local Municipality, 2012; Goba, 2024). With no other WWTPs along its course within Gauteng, site M3 benefits from sufficient longitudinal distance for water quality recovery and biotope restoration.

The secluded spring site, M1, within an open farm area, boasts excellent habitat conditions due to its intact riparian zone and minimal anthropogenic disturbances. This site exhibits the widest habitat diversity, including various stony habitats, abundant aquatic vegetation, organic matter, and woody debris (Appendix 1.A). With minimal invasive plants and wide biotope availability, M1 is poised to host a diverse macroinvertebrate community (Hasselquist *et al.*, 2018). Crucially, M1 receives no direct WWTP discharges, resulting in near-pristine water quality. While dry season DO was 70%, it rose to 80% in the wet season, complying with the legal limit (DWS, 2019). This, coupled with higher ORP, below-limit nitrogen, stable pH, very low COD (<5 mg/L), minimal heavy metals, and low salinity, indicates better oxygenation and less organic pollution compared to the Hennops River.

These factors collectively suggest M1's clear, cool waters should support a thriving macroinvertebrate community and overall river ecosystem.

The middle Magalies site, M2, is located 29 km downstream from Magaliesburg and its Percy Stewart WWTW, reveals urban impacts on water quality and habitat. While diverse instream habitats persist, a clear water quality decline from M1 is evident, marked by excessive filamentous algae indicating increased nutrients (Oberholster *et al.*, 2017; Yang *et al.*, 2024), with M2 having the highest Magalies' nitrogen levels, though still within legal limits (DWS, 2019). Significantly, the presence of dense, invasive Poplar trees (*Populus adenopoda*, Salicaceae) in the riparian zone may contribute to lower wet season discharge, as these invasive species can considerably reduce stream flow (Mapuru, Xulu and Gebreslasie, 2024). Invasive trees in SA can take up to 7 % of annual rainfall, meaning less water in streams for aquatic habitats (Le Maitre *et al.*, 2002), hence the removal of these trees is crucial to not lessen the discharge further. Murkier water and the highest COD among Magalies sites strongly suggest the upstream WWTP and Magaliesburg town are primary stressors. Despite these pressures, the Magalies' middle reaches show signs of dilution or natural recovery. However, unusual patterns like lower wet season discharge, potentially influenced by water abstraction or irrigation return flows affecting dry season discharge and water quality, warrant further investigation.

The lower Magalies River, particularly around M3, features extensive agriculture, including commercial farming, small-scale cultivation (maize, vegetables, sorghum, groundnuts), and livestock production (cattle, sheep, poultry) (DALRRD, 2019). This area also supports sustainable agriculture initiatives like shade-net greenhouses and community gardens (DALRRD, 2019). Despite its proximity to a road, M3, classified as open space/urban, benefits from substantial vegetation cover and diverse biotopes, including increased GSM, likely due to a weir and agricultural sedimentation (Schmidt *et al.*, 2018). A

very dense forest buffer zone, especially between M2 and M3, significantly improves water quality by filtering pollutants, stabilizing banks, regulating temperature, and providing habitat (Anbumozhi, Radhakrishnan and Yamaji, 2005; Dudgeon *et al.*, 2006; Nessimian *et al.*, 2008; Hasselquist *et al.*, 2018; Borgwardt *et al.*, 2019; Graziano, Deguire and Surasinghe, 2022). Located downstream from a farm and below a weir, and with no further upstream WWTPs beyond M2, M3 has sufficient distance for water quality to recover, evidenced by a lack of algal growth. M3 consistently showed elevated ORP, the highest across both seasons for the Magalies, indicating better oxygenation, effective breakdown of organic matter, enhanced bacterial performance, and a strong capacity for self-purification and contaminant elimination (Goncharuk *et al.*, 2010; Song *et al.*, 2022), reflecting good overall ecological health as also shown in the PCA biplot.

Hennops River

Over four decades after Toerien and Walmsley (1979) identified headwater WWTPs as major contributors to mineral loading, faecal contamination, and flow disruptions, the Hennops River remains impacted by two city municipal WWTPs: ERWAT Olifantsfontein (affecting H2) and Sunderland Ridge (impacting H3). The H1 spring site, however, receives no direct WWTP discharges. Historical records consistently depict the Hennops River as one of the most degraded tributaries within the Upper Crocodile (West) catchment, with chronic wastewater inputs, organic enrichment, and loss of instream habitat heterogeneity (DWAF, 2005; Silberbauer, 2006; DWS, 2015). Mid-2000s monitoring at DWS station (A2H014Q01) reported median conductivity of approximately 710 µS/cm, pH 8.15, nitrate + nitrite-N 4.7 mg/L, and orthophosphate ~0.23 mg/L (Silberbauer, 2006), reflecting early eutrophic conditions linked to municipal wastewater discharges and storm-water inflows from Centurion and Olifantsfontein. By comparison, the collected dataset reveals a marked deterioration: conductivity values exceeding 1 000 µS/cm (~1050 µS/cm), orthophosphate

levels between 0.7–1.2 mg/L, and ammonia concentrations up to 9.2 mg/L, representing >800 % and >12 000 % increases above their respective legal thresholds (DWS, 2019). The COD concentrations (82–111 mg/L) likewise surpass SA's general discharge limit of 75 mg/L (Makuwa *et al.*, 2020), confirming persistent organic overloading. Dissolved oxygen fell to 59 % saturation at polluted sites (H2–H3), and high metals—Pb ≈ 0.45 mg/L, Cu ≈ 0.11 mg/L, Zn ≈ 0.58 mg/L —exceeded legal limits for aquatic-life limits by several thousand percent (DWS, 2019). These exceedances coincide with field evidence of algal growth, dark water colouration, and reduced substrate diversity, consistent with prior river health programme assessments categorising the Hennops as Ecological Category E/F, with degraded riparian vegetation and limited refuge habitat for aquatic macroinvertebrates (DWAF, 2005; DWS, 2019).

The Hennops River's headwaters in Kempton Park are immediately impacted by urbanization, emerging near the D2 Isando Business Park, O.R. Tambo International Airport, and Kelvin Coal Power Station. The H1 sampling site, situated within a golf course 2 km from the spring, shows significant disturbance, with unusually high banks (3 m) indicating soil erosion and flood susceptibility, alongside concrete blocks in the stream (Appendix 1.A). This starkly contrasts with the Magalies' pristine M1 spring site. Despite its industrial surroundings, H1 recorded the Hennops' lowest COD at 21 mg/L, still considerably higher than the Magalies' average of less than 5 mg/L. Furthermore, laboratory analyses revealed severely high ammonia levels (1.8 mg/L) at the spring, drastically exceeding the ≤ 0.0725 mg/L legal limit for aquatic ecosystems (DWS, 2019). This, coupled with illegal industrial dumping causing orange discoloration of the stream (Appendix 1.A), underscores the profound impact of industrial discharges and urban influences from the river's very origin, leading to immediate water quality degradation and habitat impairment. Consequently, the

macroinvertebrate communities at this site are expected to reflect these poor initial conditions, despite some limited biotope variation.

The Hennops middle site, H2, is located 2 km downstream from the highly polluted Olifantsfontein/KaalSpruit confluence, showed no opportunity for water quality recovery or biotope rehabilitation. This site, receiving significant nitrogen, chemical waste, and raw sewage, had distinct water and biotope conditions, especially in the dry season. The water was notably grey with extensive white foam. H2 consistently exhibited the lowest DO, low ORP, high acidity, exceptionally high COD (111 mg/L, nearly double the legal limit), and the highest recorded nitrates. Laboratory analyses confirmed alarming ammonia levels (7.9 mg/L), indicating severe faecal contamination and dominant WWTP runoff, leading to severe organic pollution and reduced oxygen. The habitat at H2 was homogenized due to urbanization, increased discharge, and altered flow, primarily offering GSM with occasional SIC and no accessible marginal vegetation except invasive elderberry (*Sambucus canadensis*) (NEMBA, 2021). These conditions suggest that aquatic biota would consist mainly of highly tolerant species, with potential for massive ecological disruption from severe water contamination and heavy metal toxicity.

Despite its remote location and restored riparian zone, H3 is downstream from the Sunderland Ridge WWTP, known for poor discharge (Sadiki, 2020; Moatshe, 2025). Similar to H2, it showed extensive foam and recorded a COD of 82 mg/L, exceeding recommended guidelines. Although H3 had the highest ORP, suggesting relative improvement, it still signifies severe organic pollution and reduced oxygen, and its persistently lower values compared to M3 underscore chronic environmental stress. The combined effects of wet season flooding and increased upstream discharges regularly scour H3's riverbed, as shown in the habitat PCA, resulting in exposed bedrock and limited instream biotopes (Death, 2008; López-López and Sedeño-Díaz, 2015), despite a robust riparian zone with dense vegetation.

Globally, forested buffer zones are crucial for reducing water quality impairment, especially in low-gradient, higher-order streams with slow groundwater movement (Glandon *et al.*, 1981; Brüsch and Nilsson, 1993; Daniels and Gilliam, 1996; Nakamura, 2001; Anbumozhi, Radhakrishnan and Yamaji, 2005). These zones also lower stream temperatures through shading, effectively mitigating agricultural nutrient and chemical runoff (Anbumozhi, Radhakrishnan and Yamaji, 2005). In the Magalies River, the extensive forested riparian zones between M2 and M3 contribute to M3's increased recovery potential, as evidenced by its highest ORP readings. A similar, though slight, water quality improvement due to a vast section of restoring riparian zones buffering water toxicity from H2 is also observed at H3 in the Hennops River.

A strong pollution gradient exists between the Hennops and Magalies rivers, with the Hennops experiencing severe water quality degradation that profoundly impacts macroinvertebrate communities. Elevated conductivity disrupts osmoregulation, high nitrate leads to eutrophication and oxygen depletion, and low DO directly limits sensitive taxa survival (Nacke, Kirkpatrick and Wormington, 2015; Zhang, Zhao and Ding, 2019; Mkulo *et al.*, 2025; Čerba *et al.*, 2025). Consequently, the Hennops' macroinvertebrate communities are likely dominated by pollution-tolerant species. A strong positive correlation of potassium in the lower Hennops (H2 and H3) indicates potential eutrophication risks from excessive algal growth, soil salinity concerns for irrigation, and broader environmental impacts, including nutrient imbalances and harm to aquatic biodiversity. These elevated potassium levels are likely influenced by chemical discharges from upstream businesses, including potential pesticide runoff from an informal farm irrigating with contaminated water, contributing to poor water quality despite available rocky habitat.

Historical assessments of the Hennops River and Magalies River similarly reflect their contrasting instream and riparian habitat conditions. The 2005 State-of-Rivers Report

for the Crocodile (West)-Marico WMA described the Magalies River as retaining “good to intermediate” riparian vegetation and a comparatively intact riffle–pool substrate structure, with limited channel modification (DWAF, 2005). In contrast, the Hennops was mapped as heavily modified—shortened channels, reduced substrate diversity, alien-plant encroachment and sedimentation were dominant features (DWAF, 2005). The collected field data observations align with this historic divide: in the Hennops, it recorded frequent fine-sediment accumulation, sparse marginal vegetation, uniform stoneware substrate and visible effluent signs, indicating a simplified biotope. Conversely the Magalies sites exhibited greater substrate variability (cobbles, gravel, patches of macrophytes), dense riparian buffer strips and clearer flow morphology, consistent with a more resilient habitat framework. This continuity of habitat condition over time (from the collected data from 2023-2024) reinforces the linkage between habitat heterogeneity, substrate complexity and ecological integrity in river systems under stress.

While habitat analyses revealed significant differences—Magalies sites having more complex structures versus the Hennops' simpler habitats—ordination analyses (PCAs and Biplots) indicate water quality, specifically conductivity and nitrates, is the dominant influence in this study. The pronounced pollution gradient, with the Hennops experiencing severe water quality degradation, appears to be the primary driver of observed differences. The Hennops' year-round urban impacts, including constant urban runoff, WWTP discharge, and informal township influences, prevent the recovery observed in the Magalies, which benefits from periods of respite from its single point source impact. Despite the evident habitat degradation in the Hennops River, it is chemical pollution that predominantly determines its ecological health. Recommended actions for resolving this water resource management crisis in Gauteng SA can be found in Chapter 5.

CHAPTER 3: THE DIVERSITY AND ABUNDANCE OF MACROINVERTEBRATE COMMUNITIES ALONG THE HENNOPS AND MAGALIES RIVERS

3.1 - Introduction

Human activities compromise the habitat value of water resources in two key ways: by functionally degrading water quality through waste discharges and nutrient enrichment, and by structurally modifying habitats with channelization, damming, and sedimentation, along with altering flow regimes through diversion and extraction (López-López and Sedeño-Díaz, 2015). Freshwater ecosystems consequently represent some of the most globally endangered and threatened habitats (Dudgeon *et al.*, 2006; Vörösmarty *et al.*, 2010; Reid *et al.*, 2019; Tickner *et al.*, 2020; Vári *et al.*, 2022; Birnie-Gauvin *et al.*, 2023; Sayer *et al.*, 2025). Newly industrialized countries experience heightened environmental pressures on freshwater ecosystems due to rapid population growth, industrialization, urbanization, and constant rapid land-use changes (Thorne and Williams, 1997; Mustow, 2002). Freshwater organisms, representing approximately 9.5% of global species diversity (Balian *et al.*, 2024), necessitates improved conservation efforts. Developing robust biomonitoring methods is crucial for assessing aquatic ecosystem health and safeguarding their essential goods and services, complementing traditional chemical and physical monitoring techniques (Sedeño-Díaz and López-López, 2007; Li, Zheng and Liu, 2010; Springer, 2010; Espinal Carreón, Sedeño Díaz and López López, 2013).

Bioindicators offer insights into ecosystem states, past trends, and human impacts by revealing biological responses to environmental changes, such as water quality, contaminants, and sedimentation - most commonly through observing shifts in macroinvertebrate communities (Reece and Richardson, 2000; Oertel and Salánki, 2003; López-López and Sedeño-Díaz, 2015). Macroinvertebrates are invertebrates visible to the

unaided eye or with a magnifying glass. Freshwater macroinvertebrates occupy a relatively small niche/area within rivers and generally inhabit localized areas in freshwater habitat environments (Bilton, Freeland and Okamura, 2001; Brederveld *et al.*, 2011; Hussain, P and it, 2012; Mellanby, 2012; Washko and Bogan, 2019; Li *et al.*, 2021). Aquatic macroinvertebrates include insects, mites, molluscs, worms and crustaceans (Chessman, 2003; Strayer, 2011; Qazi A. Hussain, 2012; Walker *et al.*, 2018).

Macroinvertebrate communities, with their diverse functional groups, are crucial to freshwater ecosystems because they facilitate the flow of energy through food webs (Cummins and Klug, 1979; Hanson, Springer and Ramirez, 2010). In headwater streams, macroinvertebrates are essential for maintaining heterotrophic balance by processing terrestrial organic matter. Macroinvertebrates regulate autotrophy by influencing the primary production of periphyton, and attached aquatic community of algae, bacteria, fungi, and other microbes found on submerged surfaces like river stones. In the downstream reaches, macrophytes (aquatic plants) enhance habitat complexity and oxygen availability through photosynthesis, both of which support diverse macroinvertebrate communities (Sand-Jensen, 1998; Thomaz and Cunha, 2010; López-López and Sedeño-Díaz, 2015).

Macroinvertebrates serve as effective bioindicators, offering a comprehensive understanding of river ecological health, through their direct contact with contaminants and their varying tolerances to water quality conditions (from sensitive individuals in pristine areas to others surviving in hypoxic conditions owed to their diverse respiratory adaptations and pigments) (Chapman *et al.*, 2004; Jesus, 2008). Aquatic macroinvertebrates exhibit high diversity and abundance across a wide range of stream habitats, often thriving even in small streams where fish communities are limited or may not be supported (López-López and Sedeño-Díaz, 2015). Macroinvertebrates, many of which have life spans of around one year or longer, are valuable in short-term environmental assessments because they respond

sensitively to contaminant exposure, offering insights that inform long-term monitoring programmes (Mathuriau *et al.*, 2011; Sumudumali and Jayawardana, 2021; Tampo *et al.*, 2021; Orozco-González and Ocasio-Torres, 2023). Macroinvertebrate assemblage structures predictably reflect local environmental conditions, with impacted areas showing declines in species richness and abundance, forming a diversity gradient that serves as the basis for macroinvertebrate biomonitoring assessments (Barbour *et al.*, 1999; Birk and Hering, 2006; Bonada *et al.*, 2006; Ligeiro *et al.*, 2013; Heino *et al.*, 2015; López-López and Sedeño-Díaz, 2015; Poikane *et al.*, 2016; Dalu *et al.*, 2018; Karaouzas *et al.*, 2019; Li *et al.*, 2019; Vitecek, Johnson and Poikane, 2021; Bae *et al.*, 2023; Feio *et al.*, 2023). Sampling macroinvertebrates requires relatively inexpensive equipment, minimal human resources, and is easily performed, with family-level identification sufficient to represent their environmental tolerances (Wrona, Culp and Davies, 1982; Loke *et al.*, 2010; López-López and Sedeño-Díaz, 2015; Turley *et al.*, 2016; Tampo *et al.*, 2021). In addition to external pressures, the composition of macroinvertebrate communities at a monitoring site can be influenced by internal factors like life stage, seasonality, and drift dispersal. The substratum, or benthic riverbed, forms the principal habitat for most aquatic macroinvertebrates, which display a range of substrate preferences linked to flow, sediment, and organic content (Allan and Castillo, 2007; López-López and Sedeño-Díaz, 2015).

Alteration or loss of suitable substrata—through flooding, sedimentation, or land-cover change—can distort biomonitoring outcomes by excluding substrate-specific taxa and reducing observed diversity (Death & Winterbourn, 1995; Descloux et al., 2014). Alteration or loss of suitable substrata—through flooding, sedimentation, or land-cover change—can distort biomonitoring outcomes by excluding substrate-specific taxa and reducing observed diversity (Death and Winterbourn, 1995; Descloux, Datry and Usseglio-Polatera, 2014). Consequently, standardized sampling procedures and consistent substrate selection were

essential to ensure reliable and comparable assessments (Barbour *et al.*, 1999; Carter and Resh, 2001).

Based on the contrasting environmental conditions between the Hennops and Magalies rivers, several outcomes were predicted. Macroinvertebrate communities within the Hennops River were expected to exhibit lower SASS scores compared to those in the Magalies River, indicative of poorer ecological health. Consistent with this, the Magalies River was predicted to demonstrate higher macroinvertebrate diversity, evenness, and species richness than the Hennops River, as evidenced by kick sample count data. Conversely, the Hennops River communities were likely to be characterized by lower diversity but higher abundance of a small number of pollution-tolerant species, reflecting a skewed community structure. Furthermore, while Correspondence Analysis (CA) was expected to reveal similarities in species composition between the rivers, Canonical Correspondence Analysis (CCA) was predicted to illustrate distinct community segregation, with water quality identified as the primary driver of these differences.

The primary goal of this chapter was to assess the current presence, abundance and diversity of the macroinvertebrate communities along the urban-impacted Hennops River, in comparison to the rural Magalies River. This was assessed using the SASS 5 procedure (Dickens and Graham, 2002). It was expected that the freshwater macroinvertebrate communities would have a higher abundance and diversity in the upper reaches of the Magalies River but then would decline in the lower reaches of the Magalies River, and these downstream communities would be similar to the macroinvertebrate communities in the Hennops River.

3.2 - Macroinvertebrate Sampling Method

In Field Sampling

To sample the macroinvertebrate communities, SASS 5 was used, which is a rapid bioassessment method specifically designed for riverine macroinvertebrate biota (Dallas, 1995; Dickens and Graham, 2002). A total of 36 SASS kick techniques were performed over the two rivers and seasons (3 biotopes x 6 sites x 2 seasons). The sampling required collection and evaluation efforts to be focussed on wadeable streams. The three biotopes that were sampled at each site included stones (in and out of current), vegetation in-stream and marginal vegetation, and GSM (Dickens and Graham, 2002), for each site across dry (September-November 2023) and wet (March-May 2024) seasons. To sample the various biotopes a soft-mesh square net (30 cm x 1mm mesh) was used to capture the specimens and examined in flat-white bottom trays using forceps, pipettes and a magnifying glass (Dickens and Graham, 2002). The data were documented on the SASS scoring sheets. The collected macroinvertebrates were assigned a family-level classification, which each taxon has an assigned sensitivity/tolerance score, strictly according to Dickens and Graham procedure (2002). This score reflects the organism's ability to resist or persist under different levels of pollution and fluctuations in environmental and water quality (Dickens and Graham, 2002). The total score per taxon is calculated and then divided by the total taxa present in that sample which gave an Average Score per Taxon (ASPT) (Dickens and Graham, 2002). This numerical value is important to understand the ecological status of the selected rivers. Upon completion, after sample identification and scoring in the field, the collected macroinvertebrates were preserved in 90% Ethanol, for laboratory analyses for further taxonomic identification, count and diversity analyses.

The SASS 5 procedure and data is most meaningful when assessed in conjunction with habitat quality, quantity and diversity (Kleynhans and Louw, 2015). The SASS procedure is based on the premise that poor habitat diversity causes a decline in the diversity of macroinvertebrate communities, which is then indicated by a low SASS score (Dallas, 1997; Chutter, 1998). The SASS method is most effective for polluted waters and the SASS score is more meaningful for comparison between basins (Chutter, 1998). This allows for an appropriate comparison between the Hennops River and the Magalies River, as well as aids in understanding the abundance, occurrence, and diversity of macroinvertebrate assemblages along the Hennops and Magalies rivers.

Laboratory Analysis On Macroinvertebrate Data

The collected and preserved 36 SASS kick samples (3 biotopes x 6 sites x 2 seasons) were retained for further macroinvertebrate abundance and diversity analyses. Sampling represented three biotopes (GSM, VEG, Stones) sampled during both wet and dry seasons across the six study sites. The separation process of macroinvertebrates from sediment and organic matter was very time intensive. Fixed-fraction subsampling was implemented as a time-efficient approach to reduce sorting effort (Petkovska and Urbanič, 2010). As the paper suggested, sorting through a quarter of a sample was sufficient for analysis. However, this method involves trade-offs: by analysing only a proportion of the total sample, rare or low-abundance taxa may be under-represented, potentially leading to an underestimation of total species richness or diversity (Courtemanch, 1996; Barbour *et al.*, 1999). Despite this limitation, fixed-fraction subsampling is widely used in biomonitoring programmes because it provides consistent, repeatable results when applied uniformly across sites and allows greater spatial and temporal coverage within practical time constraints (Bailey, Norris and Reynoldson, 2001; Doberstein, 2003). While subsampling inevitably reduces the probability of capturing all rare taxa, the approach remains effective for detecting broader community

responses to environmental gradients when applied consistently and with adequate replication.

To ensure consistency and minimize error, a standardized screening process was implemented, with each assistant provided with a 'working jar' for initial sorting and then an 'identified sample jar' for final specimen placement. Specimens were counted and identified to the nearest species level or assigned a morphospecies designation, where precise identification was not possible. Identified specimens were then transferred to long-term storage into sorted and labelled sample jars.

The identification process was facilitated by both a physical reference collection and an online image library developed for the project, with identifications based on a macroinvertebrate field guide (Fry, 2021) and dichotomous keys such as the "Guides To The Freshwater Invertebrates of Southern Africa", volumes 1 through 10 (WRC, 1999) (Appendix 3.A – reference collection of specimens). These guides were also used for assigning feeding functional groups to each recorded taxa, as supplementary data (WRC, 1999; Fry, 2021) (Appendix 3.A). Supervision was maintained throughout the invertebrate sorting and identification process to ensure comparable standards and minimize errors. The functional groups of the collected macroinvertebrates were broadly categorized into two main trophic levels based on their feeding strategies (WRC, 1999; Fry, 2021) (Appendix 3.A). Primary consumers, which feed on plant matter, algae, or detritus, include collector/gatherers, filterers, scrapers/grazers, shredders, detritivores, phytophagous and saprophagous groups (WRC, 1999; Fry, 2021) (Appendix 3.A). Conversely, secondary consumers primarily feed on other invertebrates, encompassing predators, piercers, parasites, and scavengers (WRC, 1999; Fry, 2021) (Appendix 3.A).

3.3 – Statistical Methods

To analyse the collected macroinvertebrate sampling data, comparisons were conducted within biotopes. A non-parametric Kruskal-Wallis test was chosen as the most suitable model for comparing multiple samples, due to the data not conforming to assumptions for parametric statistical tests. The test facilitated the comparison of three or more groups (the sampled sites) based on a dependent variable with at least an ordinal scale of measurement. Given the standardized sampling effort, over both wet and dry seasons within each biotope (GSM, Veg and Stones) (two replicates at each site), the Kruskal-Wallis test effectively compared SASS count diversity data (continuous numerical) against sampled sites (categorical data). To assess community structure, biodiversity indices, including Shannon-Wiener, Simpson's Diversity, and Inverse Simpson's, were calculated and then compared across river and season using the Analysis of Variance (ANOVA), as the data conformed to normality. While Simpson's Diversity Index quantifies the probability of selecting two individuals of the same species (where higher values denote lower diversity and greater dominance), the Inverse Simpson's Index offers a more intuitive metric, with higher values directly indicating greater diversity and representing the "effective number of species" in a community.

To visualize the relationship between SASS (South African Scoring System) and ASPT (Average Score Per Taxon) scores, a biplot was generated. These metrics, key to river health assessment and biodiversity evaluation (Fig. 3.4.1, Appendix 3.B), were subsequently used to calculate EcoStatus utilizing aquatic ecoregion-specific biological bands (Dallas, 2007b). Furthermore, CA was used to investigate trends in individual species occurrences. Finally, CCA was performed to examine the relationship between macroinvertebrate community composition, water and habitat measurements, providing a robust analysis of environmental drivers.

3.4 - Results

River health data

The distribution of Hennops River sites reveals a cluster towards the bottom left of the biplot, indicating macroinvertebrate communities with a low proportion of sensitive taxa (Fig. 3.4.1). Specifically, the upper Hennops site (H1) consistently displayed an 'E' ecological rating across both dry and wet seasons, suggesting a stable but low level of species diversity, with a minor increase in diversity observed during the wet season (Fig. 3.4.1). In contrast, the middle Hennops site (H2) showed a marked seasonal fluctuation, exhibiting a poor 'F' rating in the dry season but improving significantly from an 'F' to an 'E' rating in the wet season. Despite these seasonal variations, the overall ecological status and composition of macroinvertebrate communities across the upper, middle, and lower Hennops sites remained relatively similar.

The Magalies River sites present PES Classifications are largely contrasted to the Hennops River (Fig. 3.4.1, Appendix 3.B) (Dallas, 2007b). The upper Magalies site (M1) demonstrated stability, maintaining a high 'A' ecological rating for macroinvertebrate diversity in both dry and wet seasons, with a slightly higher diversity suggested during the dry period (Fig. 3.4.1, Appendix 3.B). Notably, the middle Magalies site (M2), located downstream from a WWTP and agricultural areas, exhibited signs of environmental stress. This site showed a low 'D/E' ecological rating during the dry season, indicative of poor diversity, but experienced a modest improvement to a lower 'C' rating in the wet season, potentially due to dilution or improved water quality associated with rainfall (Fig. 3.4.1, Appendix 3.B). The lower Magalies site (M3) displayed a macroinvertebrate community structure closely aligned with the upper Magalies site, suggesting a potential recovery or regeneration of the community downstream. This site achieved a moderate 'C' rating for

macroinvertebrate diversity during the dry season, which further improved to a ‘B’ rating during the wet season, indicating a positive response to increased water availability or other favourable conditions (Fig. 3.4.1, Appendix 3.B).

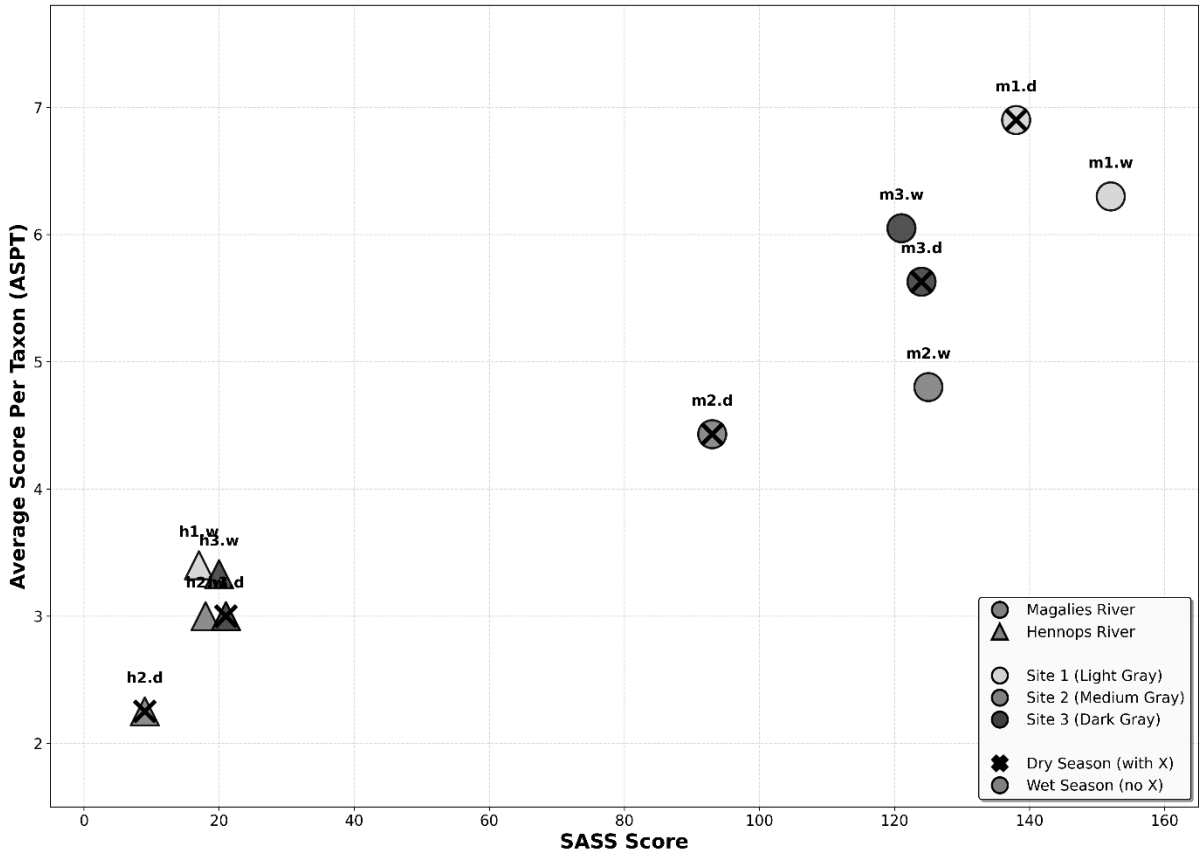


Figure 3.4.1: Scatterplot correlation between SASS and ASPT scores which were calculated in the field.

Diversity Indices

Following the rigorous sorting and identification process, the samples yielded significant biodiversity. In total, 16 orders, comprising of 58 families and 144 morphospecies, were documented, with a grand total of 46,720 individual macroinvertebrates collected.

The Magalies River exhibits high diversity within its river macroinvertebrate communities, characterized by the presence of a substantial number of taxonomic families and orders. The Magalies River sites, on average, exhibit a higher number of unique

macroinvertebrate families during the dry season (26 families) compared to the wet season (22 families). This suggests that, across its sampled sections, family richness in the Magalies River may be slightly reduced during wet periods, possibly due to water abstraction and other contributing factors discussed in both Chapter 2 and later in this chapter. This rich diversity includes several high-quality indicator species (with a quality value between 8–12), such as *Afrobrianx ferdyi* (Coleoptera), Ptilodactylidae (Coleoptera), *Suragina sp.* (Anthericidae, Diptera), *Machadorythus maculatus* (Machadorythidae, Ephemeroptera), *Tricoythus sp.* (Tricorythidae, Ephemeroptera), Torrenticolidae (Hydracarina), *Platycypha sp.* (Chlorocyphidae, Odonata), *Neoperla spio* (Perlidae, Plecoptera), *Chlorolestes faciatus* (Synlestidae, Odonata) and *Chimara sp.* (Philopotamidae, Trichoptera), representing a wide functional diversity. Furthermore, the river boasted significant richness within key groups, with 17 morphospecies in Ephemeroptera and 33 in Diptera, 14 in Trichoptera, 9 in the Odonata, 18 in the Coleoptera, 5 in Annelida, 10 Hemiptera and 10 in the Hydracarina - collectively suggesting optimal ecological conditions, and diverse macroinvertebrate communities, particularly evident at the spring site (M1).

In contrast, the Hennops River sites consistently displayed a lower number of unique families, averaging 7 in the dry season, which significantly increased to 10 families during the wet season. This pattern indicates that the Hennops River's macroinvertebrate family richness generally improves during wetter conditions. However, this low diversity, especially when compared to the Magalies River, is coupled with widespread habitat degradation. This degradation is characterized by prevalent 4-meter-high erosion walls along the riverbanks, a general lack of surrounding vegetation, and sparse aquatic vegetation amidst numerous stones. Across all Hennops River sites, stones and GSM were consistently available primary biotopes for macroinvertebrates (see Chapter 2). The middle site (H2), in particular, is highly impacted, evidenced by the unusual presence of Daphniidae (Brachiopoda, Pancrustacea), a

lentic species indicative of still-water habitats like ponds or dams. Furthermore, the only available vegetation biotope at H2 (which was an invasive shrub) contained invasive moth flies such as *Psychoda sp.* (Psychodidae, Diptera) and native opportunistic *Clogmia sp.* (Psychodidae, Diptera) (Kvifte, 2023). In the dry season, H2 recorded the lowest abundance and diversity scores across the entire study. While overall diversity was low across Hennops sites, abundance was remarkably high for certain pollution-tolerant groups. Aquatic worms, Tubificidae (Annelida), were found in the thousands with a peak count of 15098 individuals at H3 in the wet season, compared to a peak count of 367 individuals at M2, in the dry season. Similarly, Chironominae (Chironomidae, Diptera) were notably larger (2-3 cm instead of the typical 2-3 mm) and significantly more abundant in the Hennops (peaking at 4003 individuals at H3) than in the Magalies (peaking at 94 individuals at M3), underscoring the severe ecological impairment and a community dominated by tolerant species in the Hennops River.

The variation in overall macroinvertebrate abundance by site and season reveals distinct temporal and spatial patterns in macroinvertebrate populations across the sampled river sections (Fig. 3.4.2). A prevailing trend of elevated macroinvertebrate abundance was observed during the wet season, particularly pronounced at the Hennops River sites, most notably the lower reach (H3) and upper reach (H1). Specifically, the lower Hennops site (H3) exhibited the highest overall abundance, with counts of 21,697 individuals in the wet season samples (Fig. 3.4.2). Conversely, the middle (M2) and lower (M3) reaches of the Magalies River presented an atypical inverse seasonal pattern, with higher abundances recorded in the dry season (M3 at 3,223 individuals versus 1,441 in the wet season) (Fig. 3.4.2).

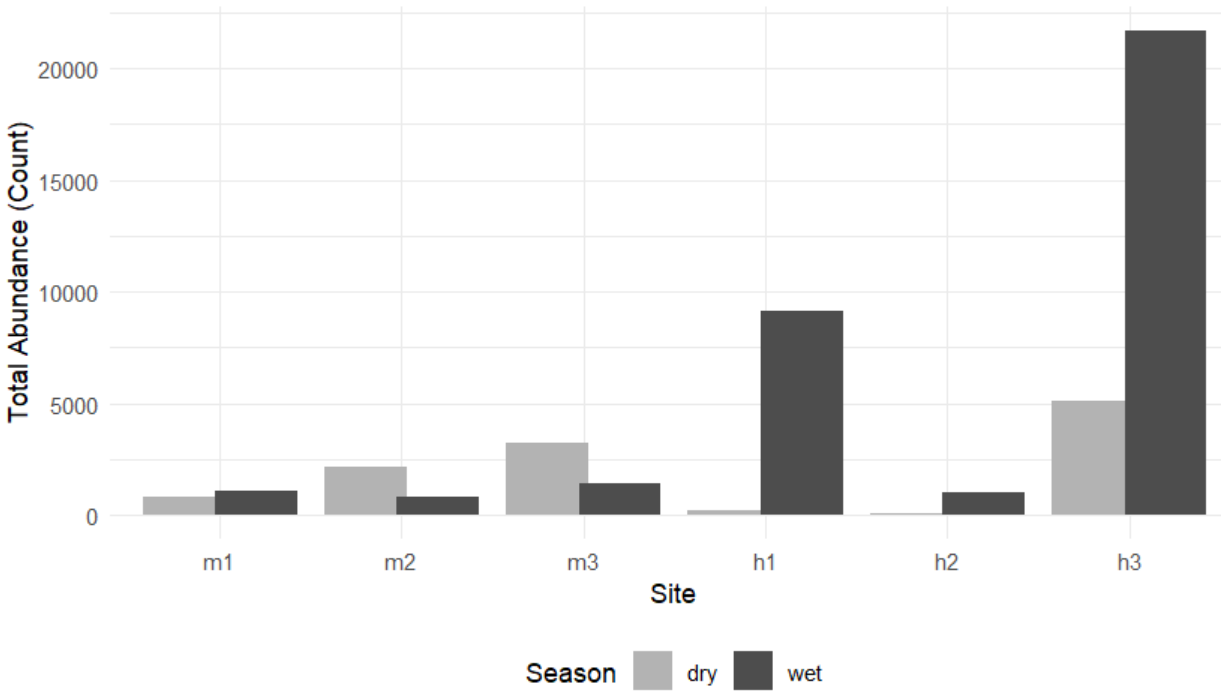


Figure 3.4.2: Total Abundance of Counted Macroinvertebrates in each site across seasons.

A first analysis to understand the observed patterns is by looking at the Shannon-Wiener and Simpson's indices on the morphospecies level (Fig. 3.4.3). As predicted, the Magalies River consistently displayed substantially higher Shannon-Wiener and Inverse Simpson index values compared to the Hennops River, clearly indicating a considerably greater species diversity in the Magalies. Interestingly, a consistent seasonal pattern in diversity was not evident across all sites; some locations exhibited slightly higher diversity during the dry season, while others showed the reverse, suggesting that seasonal effects on macroinvertebrate diversity are likely site-specific and influenced by local environmental factors (Fig. 3.4.4).

The diversity indices reveal a stark contrast between the two river systems: Magalies River sites consistently maintain substantially higher macroinvertebrate diversity compared to Hennops River sites (Fig. 3.4.3). While Magalies River sites generally exhibit either stable or slightly reduced diversity during the wet season, the Hennops River sites typically show an

increase in diversity from dry to wet seasons, with H1 and H2 in particular displaying a positive seasonal response (Fig. 3.4.4). This suggests differing ecological conditions and responses to seasonal changes, with Magalies sites generally supporting richer and potentially more stable communities, while Hennops sites experience diversity gains under wet conditions, albeit from a lower baseline.

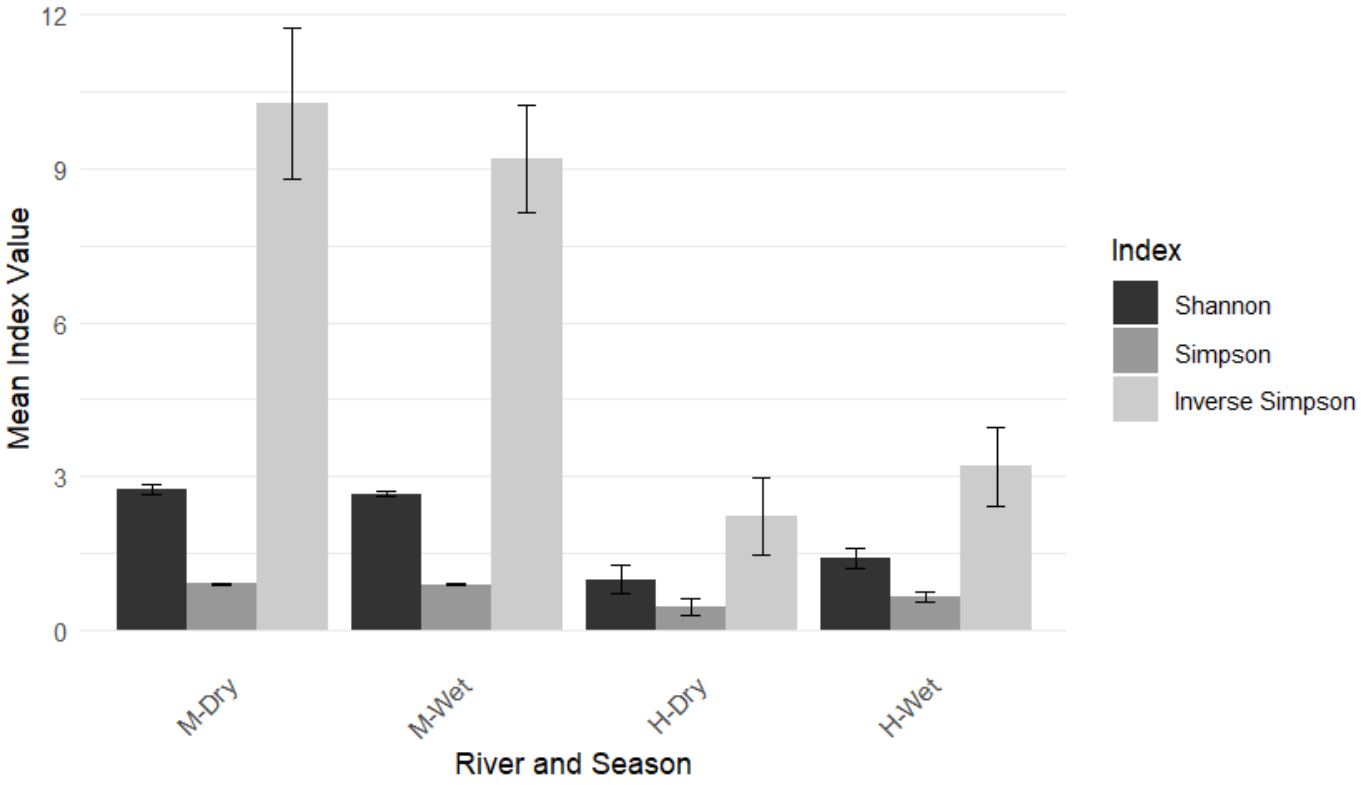


Figure 3.4.3: Shannon-Wiener and Inverse Simpson's diversity indices (at morphospecies level), including standard error bars, to show the ecological diversity across and season for both Hennops and Magalies rivers.

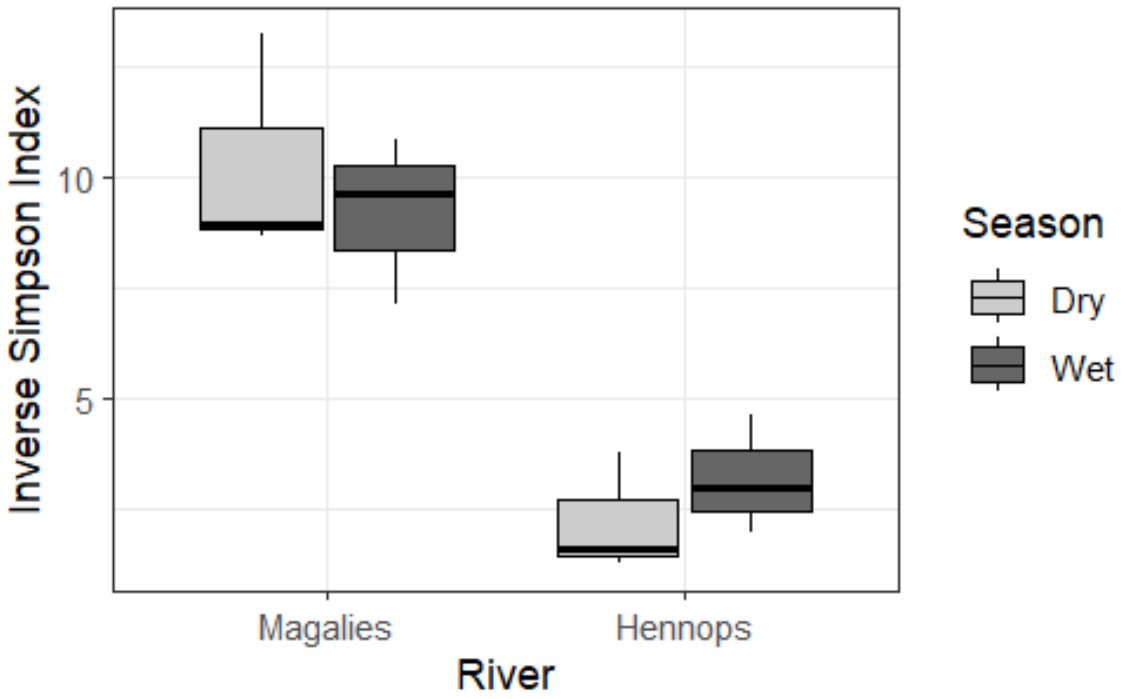


Figure 3.4.4: Inverse Simpson's index box plot, with standard deviation bars, separated per river and season

To further understand these scores statistical modelling was performed. The significant difference in macroinvertebrate diversity between the Magalies and Hennops rivers was strongly supported by the results of the general linear models (GLM) and the ANOVA conducted on both the Shannon-Wiener and Inverse Simpson indices. The GLMs revealed a strong significant spatial effect on both diversity indices. For the Shannon-Wiener index, the ANOVA showed a significant effect of River ($F(1, 8) = 71.30$, $p < 0.001$). The coefficient for the Magalies River in the linear model output was 1.75 ($SE = 0.25$, $p < 0.001$), indicating a substantial and significant increase in Shannon diversity in the Magalies River compared to the Hennops River during the dry season. For the Inverse Simpson index, the ANOVA also showed a significant effect of River ($F(1, 8) = 42.73$, $p < 0.001$). The coefficient for the Magalies River was 8.06 ($SE = 1.52$, $p < 0.001$), again demonstrating significantly higher diversity in the Magalies River during the dry season. In contrast, the

effects of Season and the 'River:Season' interaction were not statistically significant ($p > 0.05$) for either diversity index in the linear models.

Patterns of diversity

A Kruskal-Wallis test was performed on the raw richness diversity data, which represents the actual number of species found in each sample, due to the data's non-normal distribution. The resulting p-value ($p < 0.001$) was exceptionally low, indicating an extremely high level of statistical significance and a significant difference between sample medians. The highly significant results suggested that there were substantial differences in the abundance or distribution of macroinvertebrates across the sampled sites. The presence of many ties, in the Kruskal-Wallis test, indicated that multiple sites had the same number of species.

Following the highly significant Kruskal-Wallis test, Dunn's post-hoc test was performed to identify which specific site comparisons exhibited significant differences in macroinvertebrate diversity. The results revealed significant differences ($p < 0.001$) for several comparisons between the Hennops River sites during the dry season and the Magalies River sites during the dry season, specifically: H1- M1 ($p < 0.001$), H2 - M1 ($p < 0.001$), H1 - M3 ($p < 0.001$), and H2 - M3 ($p < 0.001$). These low p-values indicate substantial and statistically significant differences in macroinvertebrate communities between the Hennops River, particularly in the dry season, and the Magalies River during the dry season. While numerous significant differences were observed between sites of the two rivers, fewer significant differences were found when comparing sites within the same river.

The CA separated sampling sites and associated macroinvertebrate taxa along the X-axis primarily based on habitat and water-flow variations, representing a gradient from more lentic (still water) to more lotic (flowing water) conditions (Appendix 3.C). Along the Y-axis,

the top right quadrant shows a dense cluster containing numerous species groupings, including most of the Magalies River sites as well as the upper (H1) and lower (H3) Hennops River sites, with the presence of the high-quality indicator species *Afrobrianx ferdyi* (Coleoptera) and *Ptilodactylidae* (Coleoptera), alongside *Suragina sp.* (Anthericidae, Diptera), *Neoperla spio* (Perlidae, Plecoptera), and *Platycypha sp.* (Chlorocyphidae, Odonata) suggesting better ecological conditions in this cluster (Appendix 3.C). The middle Hennops site (H2) in the dry season remains distinct from the Magalies sites and the upper and lower Hennops sites (Appendix 3.C). However, during the wet season, the stones biotope at H2 is an outlier, exhibiting the presence of lentic species from the Daphniidae family (Brachiopoda, Pancrustacea), which are atypical for riverine environments and indicative of still water habitats like ponds or dams (Appendix 3.C). This unusual occurrence likely reflects the influence of surrounding land uses, potentially related to the discharge from upstream WWTP distilling ponds. Additionally, the vegetation biotope at H2 was characterized by a dominant presence of the urban opportunist moth flies, *Psychoda sp.* and *Clogmia sp.* (Psychodidae, Diptera) (Appendix 3.C).

Due to issues encountered while running the CCA, a collinearity reduction strategy was implemented to address multicollinearity among environmental variables (water and habitat variables). A correlation matrix of the water quality variables was generated to identify highly correlated parameters (Appendix 3.D). This analysis revealed strong intercorrelations among both water quality and habitat variables (Appendix 3.D). Consequently, to mitigate collinearity and ensure a more stable and interpretable CCA, these highly correlated variables were excluded from the ordination; they nonetheless remained part of the broader overall analysis.

The CCA biplot clearly separates the Hennops River sites from the Magalies River sites along the horizontal CCA Axis 1, which is strongly influenced by water quality

variables (Fig 3.4.5). The Hennops River sites, encompassing both dry and wet season samples, are generally situated on the right side of the plot (Fig 3.4.5), aligning with vectors indicative of elevated concentrations of heavy metals, increased conductivity, and higher nitrogen levels. This trend is illustrated by site H2, which recorded the highest concentration at '350+++' mg/L on the sampling day (Fig 3.4.5).

The macroinvertebrate communities at the upper (H1) and lower (H3) Hennops sites demonstrated notable similarities in both diversity and abundance, with an average presence of approximately 6-9 families per sample. In contrast, the middle site (H2) consistently exhibited lower diversity, averaging only 5 families. This collective pattern, particularly the increase in diversity observed at H1 and H3 during the wet season, suggests a modest ecological recovery of the river's communities during periods of higher flow. However, this recovery remains constrained by the prevailing habitat characteristics across all Hennops sites, which, despite presenting varied substrata in GSM and SIC, are notably deficient in both instream and riparian vegetation, factors that significantly influence associated water quality variables (Appendix 3.D). In contrast, the Magalies River sites are predominantly situated on the left side of the plot, away from these pollutant vectors, advocating they experience lower levels of these water quality stressors (Fig 3.4.5). This spatial segregation indicates heavy metal contamination, high conductivity, faecal waste and nitrogen to be the key environmental drivers distinguishing the water quality, and consequently the macroinvertebrate communities, between the two rivers. The positive ORP values in the Magalies are indicative of recovering communities (Fig 3.4.5, Appendix 3.D).

Along the vertical CCA Axis 2, which shows an association with vegetation variables like overhanging vegetation, a pattern emerges primarily within the Magalies River sites (Fig 3.4.5). Sites positioned in the upper portion of the biplot, such as some of the dry season Magalies samples, are more closely aligned with the overhanging vegetation vector,

indicating a potential positive influence of this habitat feature on their macroinvertebrate assemblages (Fig 3.4.5). Conversely, the Hennops River sites, generally located further down on the CCA2 axis and associated with higher pollutant loadings on CCA1, exhibit a biotic response indicative of a stressed environment (Fig 3.4.5).

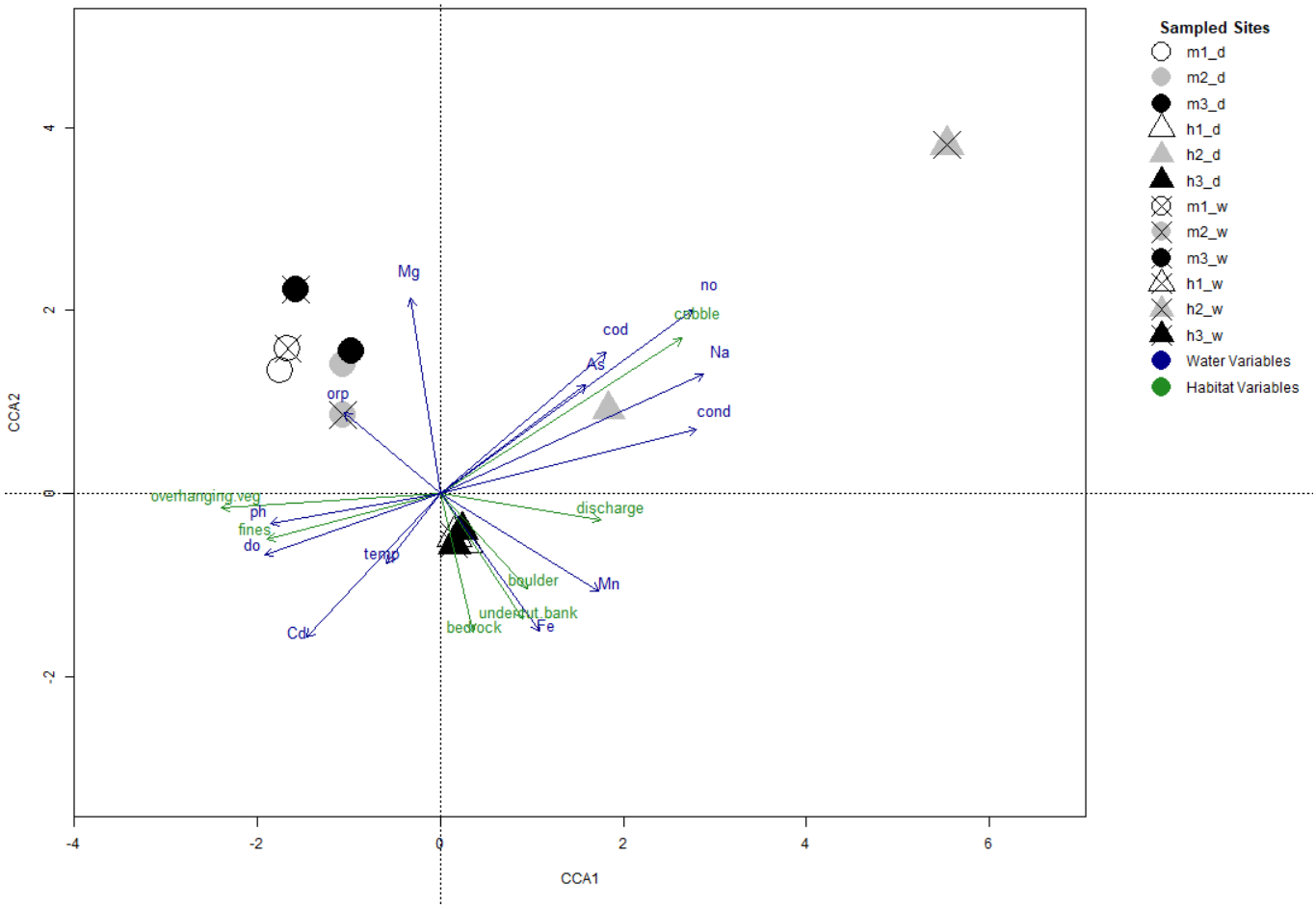


Figure 3.4.5: A CCA biplot with original variance, examining the biotic responses (macroinvertebrate presence) to environmental drivers (water and habitat variables).

The macroinvertebrate PCA biplot illustrates distinct differences in species dominance across sites and seasons within the Magalies and Hennops rivers (Fig. 3.4.6). The ordination clearly separates the two river systems along PC1 (34.7%), which reflects a gradient from pollution-sensitive taxa in the Magalies River to pollution-tolerant taxa in the Hennops River. The Magalies sites, represented by circles, cluster on the left side of the

ordination and are closely associated with *Baetis harrisoni* and *Hydropsyche.sp*—both taxa indicative of good water quality and higher dissolved oxygen levels. This clustering suggests stable and well-oxygenated habitats supporting more sensitive macroinvertebrate assemblages, consistent across both dry (indicated by "X") and wet seasons. In contrast, the Hennops sites, shown as triangles, plot toward the right-hand side of the ordination and are associated with *Tubifex.sp* (Tubificidae) and Chironominae—taxa known for their tolerance to organic pollution, low oxygen concentrations, and enriched sediments (Fig. 3.4.6). The strong alignment of Tubifex along PC1 highlights its dominance in more degraded, low-quality conditions, particularly at sites H2 and H3 across both seasons (Fig. 3.4.6). Seasonal variation is evident within each river, but the overall community structure remains primarily driven by site-level differences. Magalies sites remain tightly grouped across seasons, suggesting ecological stability, while Hennops sites exhibit greater dispersion, reflecting temporal fluctuations in habitat quality and pollution levels (Fig. 3.4.6). Overall, this analysis demonstrates that macroinvertebrate community composition clearly differentiates the two rivers, with the Magalies River dominated by sensitive taxa and the Hennops River characterized by pollution-tolerant assemblages, confirming the gradient of environmental stress previously observed in the water quality analyses (Fig. 3.4.6).

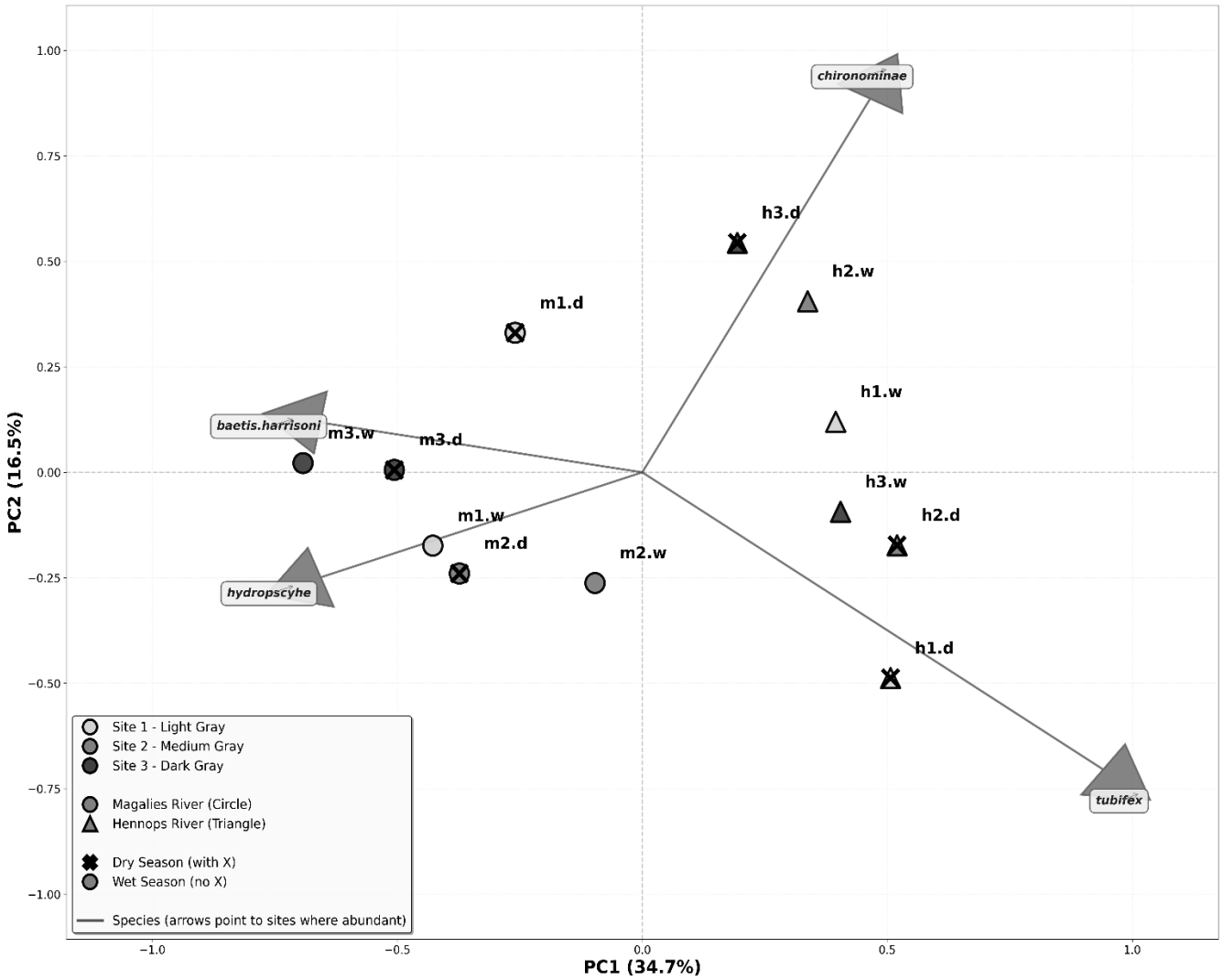


Figure 3.4.6: A biplot showing what species were most dominant at each site across each season.

CCA and PCR loadings indicate that pollution (Appendix 3.E), specifically heavy metal contamination and high conductivity (represented by PC1), is the primary factor distinguishing Hennops from Magalies sites. The greater habitat diversity (gravel/cobble, organic matter) in Magalies sites likely contributes to their higher biodiversity.

3.5 - Discussion

Using the SASS 5 protocol and detailed taxonomic identification, this study primarily aimed to assess the presence, abundance, and diversity of macroinvertebrate communities in

the Hennops and Magalies rivers, and to link these patterns to the variation in environmental drivers established in Chapter 2. The overall findings demonstrate a clear contrast: Hennops sites are under significant pollution-induced environmental stress, whereas Magalies sites are relatively less impacted. These contrasting environmental factors largely dictate the significant differences in macroinvertebrate communities observed between the two river systems.

It was hypothesized that freshwater macroinvertebrate communities would exhibit higher abundance and diversity in the upper reaches of the Magalies River but would subsequently decline in its lower reaches, potentially becoming similar in diversity to those found in the Hennops River. However, this initial prediction was not entirely supported; instead of a continuous decline through the urbanized middle sites, the Magalies River exhibited a distinct ecological pattern: high diversity at its spring, a decrease in the impacted middle reaches by the town of Magaliesburg, and a subsequent recovery in the lower site. This resilience can be attributed to natural buffers, including expansive open lands and a restored riparian zone, leading to improved ecological integrity. Furthermore, the findings revealed a clear divergence in the overall ecological health of the two river systems. As partially hypothesized, the Magalies River generally exhibited higher SASS scores and greater species diversity, as indicated by both the SASS ratings, Shannon-Wiener, and Inverse Simpson indices, collectively suggesting an environment with relatively low anthropogenic modification. This was further supported by the CA and CCA biplots, which showed significant clusters of high sensitivity indicator species (e.g., *Afrobrianx ferdyi*, *Neoperla spio*), better ecological conditions, and similar environmental characteristics among sites along the Magalies River.

Magalies River

Historic reports characterise many Magalies reaches as moderately modified to intact, with MIRAI ≈ Category C and stable trends where riparian structure and riffle–run complexity persist (DWS, 2015, 2016, 2024c). The SASS protocol showed SASS–ASPT medians of resilience: SASS ~125 (wet) / 124 (dry); ASPT ~6.05 (wet) / 5.63 (dry)—values consistent with B–C condition for many SA rivers and close to site-specific River Eco-status Monitoring Programme (REMP) target bands (example targets in the Crocodile West–Marico WMA include minimum SASS 140 and ASPT 5.4–5.5 at selected benchmark sites) (DWS, 2015, 2016, 2024c). This study's community metrics show substantially higher richness and balanced abundance in Magalies (e.g., M1 dry = 51 taxa), consistent with REMP/River Health Programme interpretations that structural habitat heterogeneity and better water quality support richer assemblages and the persistence of more sensitive families (including Ephemeroptera, Plecoptera, and Trichoptera) even where localised pressures occur downstream (Dallas, 2000; DWS, 2015, 2016, 2024c).

The Magalies River demonstrates substantially greater ecological integrity, with wet- and dry-season median SASS values of 125–124 (only ≈ 11 % below target) and ASPT values of 6.05–5.63 (slightly above target), corresponding to Ecological Category B–C conditions. These results closely align with earlier REMP findings describing the Magalies as moderately modified but functionally resilient, supported by stable riffle–run habitat and intact riparian vegetation (DWS, 2017a, 2017b). The historic comparative analysis thus indicates that while the Magalies River retains near-reference assemblages, the Hennops River remains ecologically impaired, reflecting persistent urban and effluent pressures.

The numerical analyses indicated a pronounced difference in macroinvertebrate abundance and diversity, as well as their seasonal responses, between the two river systems.

Specifically, while the Hennops River might exhibit higher total macroinvertebrate abundance, its diversity count is notably lower compared to the Magalies River. This observed pattern generally indicates pollution, which was evident in this study. Such patterns of high abundance for a few taxa, are indicative of an imbalanced community structure under contamination, and this trend has also been documented in microbial communities (Godfrey, 1978; Lin and Yo, 2008; Paruch *et al.*, 2019).

Site M1 consistently exhibited the highest family richness across all sampled locations, with 29 unique families recorded in the dry season and a slight reduction to 26 in the wet season, indicating robust and relatively stable biodiversity. Site M1 maintained a high 'A' ecological rating, clearly indicates robust ecological integrity and stable, healthy conditions. This excellent state is attributed to its secluded location within an open farm, characterized by an intact riparian zone, minimal surrounding anthropogenic disturbances, and no major land degradation upstream. The management of riparian buffer zones proved effective in reducing water pollution from adjacent agricultural fields (Anbumozhi and Yamaji, 2001; Anbumozhi, Radhakrishnan and Yamaji, 2005). Site M1 also boasts a more diverse habitat, with features like varied stone types, extensive aquatic vegetation, abundant organic matter, and woody debris, all of which contribute to its higher observed biodiversity (Hasselquist *et al.*, 2018). This was reflected in the great diversity with high quality value macroinvertebrates groups found at M1. Some of these include various Ephemeroptera, Trichoptera, Coleoptera, Odonata, Diptera and Hydracarina. The statistical test results provided by the Kruskal-Wallis CA, CCA biplot all confirm that the biodiversity is very high at M1, which shares very similar families and species, with M3. M2's separation from other Magalies sites and its statistical proximity in the CCA to the Hennops sites indicate it shares common stressors, though not with the same intensity as observed in the Hennops River.

The middle Magalies site (M2), despite its classification as an open area, the upstream land cover at this site is primarily a caravan park featuring a very dense riparian zone. The presence of numerous invasive trees, like the Poplar (*Populus adenopoda*, Salicaceae), immediately adjacent to the river, may have contributed to the lower discharge observed during the wet season. Coupled with influences from the upstream town, WWTP, and intensive farming, this site exhibited the highest heavy metal contaminants, nitrogen, and COD levels among the Magalies sites. This clearly indicated significant urban impacts, further reflected by less varied instream biotopes available at this location. A clear pattern emerged showing higher family richness during the dry season, with 24 unique families, compared to only 21 in the wet season. This may indicate a reduction in diversity during periods of higher flow, increased temperatures or other seasonal influences. Such observations imply environmental stress due to pollution stemming from both point and non-point sources (Gitau, Chen and Ma, 2016; Almaaofi, Etemad-Shahidi and Stewart, 2017; Aalami, Abbasi and Nourani, 2018). The Magaliesburg Percy Stewart WWTW located 29 km upstream, heavily affected M2, which exhibited a poor 'D/E' ecological rating during the dry season, improving to a 'C' rating in the wet season. A notable decline from M1 was observed, markedly associated with the proliferation of excessive long filamentous algae (Appendix 1A). This condition was coincident with this site recording the highest nitrogen levels (2.23 mg/L) among the Magalies sites. Furthermore, M2 and lower M3 sections of the Magalies River exhibited higher macroinvertebrate abundance during the dry season according to the results. In the Magaliesberg Region, macroinvertebrate populations in the Magalies River typically exhibit greater abundance during winter and early spring (dry season) compared to summer (wet season) (Gabbud, Robinson and Lane, 2019), a trend consistently observed in this study. While the precise timing may vary slightly across specific investigations, the general pattern indicates that the river is less populated with macroinvertebrates during the

summer months (Gabbud, Robinson and Lane, 2019). This observation, coupled with the relatively better water quality, points to other restorative factors, such as an excellent riparian zone and instream habitat, which collectively support the higher abundance and diversity found in these sections during the dry season. Conversely, the summer months can typically bring increased temperatures and potentially lower oxygen levels in the river, creating stressful conditions that can deplete macroinvertebrate populations (Gabbud, Robinson and Lane, 2019). The flushing of accumulated sediment from intakes diminishes in early autumn, macroinvertebrate populations often rebound, with their numbers increasing during the cooler winter and early spring periods (Gabbud, Robinson and Lane, 2019).

The lower Magalies site (M3) demonstrates a significant recovery of its macroinvertebrate community in the wet season, aligning with the healthy upper site and improving its rating from 'C' to 'B'. Even with its proximity to a road bridge, this site benefits from extensive vegetation cover, being located about 30 meters downstream from a farm and below a weir. Site M3, lacking an upstream WWTP, is influenced only by the Magaliesburg Percy Stewart WWTW located in the town of Magaliesburg, upstream from the middle reaches of the river (M2). This extended longitudinal space allows the lower reaches (M3) sufficient time for its macroinvertebrate communities to recover. The inverse seasonal trend observed at M2 was reinforced by the findings at M3. At this site, family richness was greater in the dry season (23 unique families) than in the wet season (19 unique families). The river's lower reaches exhibit extensive agriculture, from large-scale commercial and small-scale crop farming (maize, vegetables, sorghum, groundnuts) to livestock production (cattle, sheep, poultry), also supporting sustainable practices like shade-net greenhouses and community gardening (DALRRD, 2019). While the Magalies River did exhibit some signs of stress, evidenced by lower ecological ratings and diversity in certain areas, it consistently outperformed the Hennops sites. This relatively higher ecological resilience indicates the

river's capacity to overcome upstream impacts and restore ecological function. This recovery is strongly linked to the extensive restored riparian zones both upstream and at the site, as other studies have shown that well-maintained riparian zones effectively buffer anthropogenic impacts like agricultural runoff and contribute to sedimentation control, thereby preventing soil erosion (Anbumozhi, Radhakrishnan and Yamaji, 2005). Riparian zones are vital, highly biodiverse areas crucial for river health. Protecting them effectively means moving from simple fixed-width buffers to more complex, multi-layered designs that are part of larger watershed conservation plans to boost biodiversity and ecosystem strength (Graziano, Deguire and Surasinghe, 2022). The strategies, informed by our observations on the Magalies River, offer a deeper understanding of the findings.

The primary impact of urbanization is evident in the vastly different recovery capacities of the Hennops and Magalies rivers. The Hennops River's catchment experiences significantly larger and more frequent WWTP discharges, stemming from large municipal populations—for example, comparing a town of 6,500 residents influencing the Magalies to a municipality with over 3 million residents affecting the Hennops (Africa, 2011). Specifically, the Hennops River is impacted by two major facilities: ERWAT Olifantsfontein affecting H2, and Sunderland Ridge WWTP impacting H3, resulting in pervasive, constant urban runoff and WWTP pollution that fundamentally limits its recovery potential throughout the year, even in the wet season. In stark contrast, the Magalies River, influenced by a single, smaller WWTP (the Magaliesburg Percy Stewart WWTW) along its sampled sites, demonstrates greater ecological resilience. This resilience is evident in M2's modest improvement from a 'D' to a 'C' rating in the wet season, and M3's significantly better and quicker recovery compared to Hennops site H3, attributed to extensive forested riparian zones and more longitudinal space for water quality restoration after the WWTP.

Hennops River

Historic biomonitoring in the Crocodile (West)–Marico WMA repeatedly classed Hennops reaches as degraded with water-quality stress and simplified instream habitat; MIRAI outputs in DWS technical reporting highlight water quality as the dominant impairment driver and the loss of sensitive taxa (e.g., Perlidae, Heptageniidae, >2 spp. Hydropsychidae) from impacted reaches (REMP Tech Reports) (DWS, 2015, 2016, 2024c). Comparing the historical recorded data with collected data the SASS–ASPT medians are extremely low: SASS 18–21; ASPT 3.0–3.33 (wet vs dry, river medians), firmly indicating E/F-grade bio-condition and corroborating chronic nutrient/metal/COD exceedances reported in your chemistry section (and consistent with REMP/River Health Programme narratives of long-term degradation) (Dallas, 2000; DWS, 2015, 2016, 2024c). At the community level, the identified taxa richness per site-season in Hennops is modest (e.g., H1 wet = 14 taxa; H6 wet = 14 taxa), with very high total abundances dominated by tolerant groups at the worst sites—patterns typical of organically enriched, low-oxygen systems in the WMA's urban core (DWS, 2024c). These findings align with REMP diagnostics where water-quality metrics were the most impaired, followed by instream habitat modification, and where taxa requiring unmodified physico-chemical conditions were absent or rare (e.g., Ephemeroptera, Plecoptera, and Trichoptera families) (DWS, 2024c).

Comparison of the current biomonitoring results against historical and regulatory benchmarks reveals a stark ecological contrast between the Hennops and Magalies Rivers. Median SASS and ASPT values in the Hennops River (SASS = 18–21; ASPT = 3.0–3.33) fall 85–87 % below the regional REMP benchmark of 140 and 38–44 % below the expected ASPT target of 5.4 (DWS, 2016, 2024c). These scores correspond to Ecological Category E/F under legal limits (DWS, 2019), denoting severely modified conditions dominated by pollution-tolerant taxa such as Chironomidae and Oligochaeta. Such results are consistent

with historic DWS and River Health Programme assessments that identify water-quality deterioration, organic enrichment, and habitat simplification as chronic stressors in the Hennops system (DWS, 2019; WRC, 2024).

The Hennops River exhibits significantly lower diversity, with a range of 1 to 9 families present, compared to an average of 20 in the Magalies River, alongside extensive habitat degradation characterized by eroded riverbanks, sparse vegetation, and homogenized riverbeds. Its macroinvertebrate communities are consequently dominated by pollution-tolerant groups, notably the abundant aquatic worms Tubificidae (Annelida) and non-biting midges Chironominae (Chironomidae, Diptera) – which same species comparisons on the Chironominae showed to be 10 times larger in the Hennops River than in the Magalies River (average 2-3 cm vs 2-3 mm). Both taxa are collector gathers, representing essential primary consumers (Mermillod-Blondin, Gérino and Degrange, 2002; Antczak-Orlewska *et al.*, 2021; Fry, 2021). River communities dominated by these two families are often associated with highly polluted streams exhibiting severe ecological impairment. These species, being exceptionally tolerant, can account for the majority of the richness and abundance, thriving on ample organic matter for feeding and benefiting from a lack of predators (Saether, 1979; Henriques-Oliveira, Nessimian and Dorvillé, 2003; Odume, 2012; Ding, Rong and Shan, 2016; Odume *et al.*, 2016; Serra *et al.*, 2017; Edegbene *et al.*, 2021). In some cases, macroinvertebrates found were extremophiles, such as the *Tubifex sp*. and the Chironominae, adapted to survive even in highly toxic conditions, ranging from extremely saline to minimal DO and very high nitrates (Lin and Yo, 2008; Ding, Rong and Shan, 2016; Odume *et al.*, 2016; Glasby, Erséus and Martin, 2021). Studies revealed that highly tolerant taxa exhibited a competitive advantage, enabling their success under conditions of minimal DO (Justus *et al.*, 2014). Opportunistic moth flies, specifically *Psychoda* and *Clogmia* (Psychodidae, Diptera), were present at all Hennops sampled sites. These taxa are well-known synanthropic

(urban exploiter) species, closely associated with wastewater and sewage infrastructure worldwide (Kvifte, 2023). Interestingly, another non-biting midge subfamily, Tanypodinae (Chironomidae, Diptera), was present across all Hennops sites in the wet season, though only at H3 in the dry. This group typically consists of predators, indicating some basic variation in the feeding functional groups within these communities (WRC, 1999; Fry, 2021), especially since the majority of other highly tolerant species found are herbivorous. Nonetheless, the complete absence of Trichoptera from all three Hennops sample sites signifies a critical void in the ecosystem, as these insects represent a major functional group capable of fulfilling diverse ecological roles, including collecting, gathering, filtering, shredding, scraping, or predation, depending on the species (WRC, 1999; Dohet, 2002; Schmera and Eros, 2004; Houghton, 2007; Fry, 2021). The Hennops River displayed a consistently poor ecological status (E/F) across all its sampled sites. The species composition of the Hennops sites in the CCA Biplot, inferred from their position relative to the strong water quality gradients, suggests a higher tolerance to pollution compared to the communities found in the less impacted, and in all cases more vegetated, Magalies River sites.

The Hennops River's headwaters emerge in Kempton Park, immediately experiencing urban impact due to their proximity to the industrial sector. The spring site (H1) recorded the lowest COD levels for the Hennops at 21 mg/L, which, while within legal limits, was notably higher than the Magalies River's average of 6 mg/L. Furthermore, H1 also showed ammonia levels above the legal limit, indicative of industrial runoff influencing the water quality. The spring site (H1) is a shallow, narrow river with high banks, making it susceptible to flooding, industrial effluents, and significant instream disturbance. Despite its location within a golf course offering a more restored riparian zone, instream habitats remain somewhat limited, with all three biotopes exhibiting restricted availability. Site H1 demonstrated a significant increase in family richness from the dry season (7 unique families) to the wet season (14

unique families), indicating a positive response of biodiversity to wet season conditions. This contrasts sharply with the Magalies River, which features a more varied and diverse, albeit lower-abundance, species assemblages. Consistent with its overall ecological state, H1 exhibited a low proportion of sensitive taxa and maintained a poor 'E' ecological rating. The close clustering of H1 and H3 in the CA and CCA biplots further indicated shared macroinvertebrate assemblage structures between these sites, and showing a recovery of macroinvertebrate diversity in the lower reaches. Interestingly, in the wet season, H1 introduced other functional families beyond the typically dominant Chironomidae and Tubificidae such as snails like the *Lymnaea truncatula* (Lymnaeidae, Mollusca) and *Stenophysa marmorata* (Physidae, Mollusca)—both scrapers/grazers, as well as the predatory groups such as the flatworms Dugesiidae (Platyhelminthes) and beetles in the *Rhantus* and *Hydaticus* genera (Dytiscidae, Coleoptera) (WRC, 1999; Fry, 2021). This represented a moderate improvement in the functional diversity given the immediate upstream stressors on the stream. However, this diversity drastically declines with increasing pollution downstream, particularly evident in the middle reaches.

The middle site (H2), located within a farm 2 km downstream from the Olifantsfontein/KaalSpruit confluence with the Hennops River. The site appears to lack sufficient longitudinal distance from WWTP and nutrient-rich greywater discharge from Tembisa township, for macroinvertebrate community recovery. This results in high levels of pollution, including nitrogen, chemical waste, and raw sewage. Consequently, H2 was completely distinct from other sites, particularly in the dry season, both in water quality and limited available biotopes. This site notably showed evidence of urban impacts as three lentic morpho-species of Daphniidae (Brachiopoda, Pancrustacea) were found. Inflows from the underperforming ERWAT Olifantsfontein WWTP upstream from H2, characterised by the rare and unusual settling pond community in a lotic biotope (Sharma, 2001), shows how a

highly tolerant lentic community replace the more sensitive lotic communities observed at H1 and H3. Consistently the least diverse site, H2 recorded only 5 unique families in the dry season and a modest increase to 9 in the wet season, suggesting persistently challenging environmental conditions for macroinvertebrate family establishment. This site also experienced the study's highest conductivity of 1052 µS/cm, almost 3 times more conductive than the Magalies River sites, directly affecting macroinvertebrate establishment (Armstead, Bitzer-Creathers and Wilson, 2016). This distinctiveness in species composition strongly indicates severe local environmental degradation at H2, positioning it as a highly impacted outlier likely due to upstream WWTP influence. This strongly suggests that WWTP runoff comprised the majority of the surface water at the site, rather than natural river water. Supporting this, laboratory analyses confirmed the ammonia concentration to be an alarming 1130 times over the legal limit (DWAF, 1996). Furthermore, H2 recorded the highest COD levels at 111 mg/L, nearly double the legal limit (DWAF, 1996), coupled with the highest recorded nitrates from both *in situ* and laboratory analyses. The land cover mosaics within the Hennops River's watershed directly dictate the primary sources of dissolved nitrogen observed in the river, which include wastewater, fertilizers, atmospheric deposition, and cleaning detergents, all pointing to intense point source pollution (Toerien and Walmsley, 1979; Valiela and Bowen, 2002; Ocón *et al.*, 2013; Ding, Rong and Shan, 2016; Oberholster *et al.*, 2017; Sadiki, 2020; Ouyang *et al.*, 2025).

This is further reflected as the middle Hennops site (H2), consistently records the lowest macroinvertebrate abundance across both seasons and experiences marked ecological fluctuations between 'F' and 'E' ratings. These observations collectively imply persistent environmental challenges and generally stressed conditions throughout the Hennops River, despite some minor wet season improvements. However, the seasonal increases brought the appearance of indigenous water boatmen *Corixidae* (Hemiptera) and water beetles from the

Limnichidae (Coleoptera), with the latter possessing a high-quality value of 10. Their presence suggests initial signs of ecological recovery, even within this highly stressed and polluted environment. Crucially, the mayfly *Baetis harrisoni* (Baetidae, Ephemeroptera), an important indicator species, also returned to the community, primarily found within vegetation. This localized observation at H2 supports a broader theme of abundance demonstrating a pattern of high counts at the river's source, very minimal counts in the impacted middle sections, and subsequent recovery in its lower reaches. Overall, H2 consistently exhibited the lowest SASS scores and reduced diversity, indicative of a highly stressed aquatic ecosystem, even with minor signs of recovery observed in the wet season, still maintains a poor 'E/F'.

The Hennops lower site (H3) is located in an open, remote area, far from roads and industrial sectors, and boasts a largely restored riparian zone. High water discharges limit the instream habitat, leaving the riverbed mostly bedrock with GSM and SIC primarily along the riparian edges. It is situated downstream from the Sunderland Ridge WWTP, a facility frequently cited in studies and newspaper articles for its poor water quality discharge (Sadiki, 2020; Moatshe, 2025). Site H3 recorded a COD of 82 mg/L, exceeding the recommended limit by 26%. However, this site consistently recorded the highest DO levels compared to the other Hennops sites across both seasons, notably increasing from 66.1% to 87.4% in the wet season. Uniquely among the Hennops sites, H3 displayed a slight decrease in family richness from the dry season (9 unique families) to the wet season (7 unique families), contrasting with the general increase observed at H1 and H2 during the wet period and drawing similarities with patterns seen in the Magalies sites. This suggests downstream recovery from H2, where the sewage effluent was likely diluted to some extent by the time it reached H3. The limited instream habitat for macroinvertebrates, likely compels communities to gather along the banks near the riparian zones, an observation consistently made during this study's

sampling. Furthermore, the Hennops' higher discharge and increased river order resulting from upstream convergence contributes to these challenging conditions.

Despite the highly impacted middle section, the river shows signs of recovery towards the lower reaches. This improvement could be positively influenced by the Hennops Hiking Trails upstream, 16 hectares of open and protected area with robust riparian zones, which aid in restoring macroinvertebrate communities. The lower reaches improved with the return of various functional groups: aquatic *Nematoda sp.* (predator/shredders), leeches such as *Marsupiobdella africana* (Hirudinae, Annelida), and four genera of Blackflies, including *Simulium adersi* and *Paracnephia* (Simuliidae, Diptera), which act as filters, helping to clarify the water (WRC, 1999; Fry, 2021). Primary consumer groups are also re-establishing, mayflies from three genera (*Baetis harrisoni*, *Labiobaetis*, and *Cloeodes* (Baetidae, Ephemeroptera)), along with water boatmen (*Corixidae*, Hemiptera), and two snail species (*Stenophysa marmorata* (Physidae, Mollusca) and *Melanoides tuberculata* (Thiaridae, Mollusca)) (WRC, 1999; Fry, 2021). Observations also included monitors (Nile Monitor – *Varanus niloticus*) and vervet monkeys (*Chlorocebus pygerythrus*), mirroring findings from previous reports (Sinclair, Ebersohn and Eicker, 1983; Hennops River Fountain Source Sanctuary Project, 2017). Sites H1 and H3 cluster closely in the CA and CCA biplots, indicating that they share similar macroinvertebrate assemblage structures. They also demonstrate a significant increase in abundance during the wet season, with H3 supporting the highest overall counts. This overall pattern contributes to the river's ecological health being characterized by higher diversity at its spring, declining in its impacted middle reaches, and subsequent recovering in the lower reaches—a resilience fostered by natural buffers like expansive open lands and a restoring riparian zone. The analysis of the CCA biplot strongly implies a fundamental ecological divergence between the Hennops and Magalies rivers,

driven by contrasting water quality and habitat characteristics, due to much higher urban impacts in the Hennops River catchment.

The Hennops River's macroinvertebrate communities are consistently associated with higher concentrations of heavy metals, elevated conductivity, and increased nitrogen, suggesting a highly polluted and stressed environment where only more tolerant species can thrive (Mermillod-Blondin, Gérino and Degrange, 2002; Lin and Yo, 2008; Odume, 2012; Odume *et al.*, 2016; Serra *et al.*, 2017). The analysis of SASS scores, diversity indices, and further supported by the CCA biplot and PCR loadings strongly implicates water quality as a primary driver of these observed differences. The Magalies River's communities are distinctly separated from these pollutant indicators, implying generally lower levels of contamination. This underscores that the distinct environmental conditions and chronic stressors differentiating the rivers create significantly distinct biological communities (Azrina *et al.*, 2006), with the Hennops River consistently harbouring much less diverse macroinvertebrate populations compared to the Magalies. This distinction must be viewed within the context of urban impact: the Hennops River experiences year-round urban stressors all along the river from WWTPs to informal effluent. Whereas the Magalies River has one primary source of impact—the town of Magaliesburg and its WWTP located between M1 and M2—with no other major water stressors before M3, aside from external runoff from different land-cover uses. Also, the volume of WWTP effluent is on a different magnitude of scale, affecting the Hennops River much more, compared to the Magalies river.

The implications of these findings are significant for the ecological health and management of these vital freshwater resources. The consistently poor EcoStatus of the macroinvertebrate communities in the Hennops River underscores the severe environmental stress it faces, significantly impacting its ability to provide essential ecosystem services. These include all main categories—provisioning, supporting, cultural and regulating

services—with macroinvertebrate communities specifically contributing vital provisioning and regulating services essential for urban streams (Yeakley *et al.*, 2016; Raquel Calapez *et al.*, 2023). The relatively better condition of the Magalies River, although not without localized stressors, highlights the importance of maintaining good water quality and habitat integrity. Further investigations into the specific sources of heavy metal pollution and contamination in the Hennops are crucial, and the implementation of targeted remediation measures are necessary to improve its ecological health and safeguard the water source and biodiversity of its aquatic macroinvertebrate communities.

CHAPTER 4: ASSESSING THE FINDINGS OF THIS STUDY IN CONTEXT OF WATER MANAGEMENT IN GAUTENG, SOUTH AFRICA.

4.1 – Introduction

The management of natural resources, particularly water, is a global imperative driven by concerns over availability. Given that less than 0.025% of the world's water is accessible for drinking (Li, Zhang and Liao, 2019), this vital resource demands careful management, treatment, and allocation at both global and local scales – processes largely governed by legislation and its enforcement. Recognizing the critical need for effective water resource management, several nations, including Australia, parts of Europe, and the United States, have incorporated biological assessments into their governmental mandates. Furthermore, countries like Colombia and Costa Rica have explicitly legislated the use of such assessments for monitoring and safeguarding their aquatic ecosystems (Couceiro *et al.*, 2012; López-López and Sedeño-Díaz, 2015).

Effective legislation is paramount in ensuring accountability among water users, including businesses, for their impact on surface water resources. Within SA, the responsibility for the management of these resources, including setting and enforcing water quality standards, primarily falls under the purview of the national Department of Water and Sanitation (DWS). The link between surface water quality and the provision of safe drinking water is undeniable, and indicates that while technological advancements can aid in safeguarding water sources, the consistent distribution of safe drinking water ultimately hinges on the robust enforcement of existing legislation (Kayser *et al.*, 2015).

Surface water resources that yield freshwater suitable for human consumption are designated as drinking water catchments (Almaarofi, 2017). However, despite their critical importance, numerous studies consistently demonstrate the vulnerability of surface waters to

various forms of pollution, originating from both point sources (identifiable discharges) and non-point sources (diffuse contamination across landscapes). This vulnerability highlights the need for strong laws and strict enforcement to protect these vital catchments. This will ensure that communities have a long-term supply of safe drinking water (Almaaofi, Etemad-Shahidi and Stewart, 2017; Aalami, Abbasi and Nourani, 2018; Meissner *et al.*, 2018; Tang *et al.*, 2019). Previous research highlights various anthropogenic stressors, including urbanization, deforestation, agriculture, industries, mining, and wetland destruction, as negatively impacting surface water quality (Dasgupta, Ranjan and Ramalingam, 2017; Szalinska, 2018; Sadiki, 2020). Urbanization increases fertilizer use, leading to nutrient pollution (nitrogen and phosphorus, including pharmaceuticals) and eutrophication. Industrial, mining, and WWTP discharges introduce chemically contaminated water (point source pollution), altering river pH and nutrient levels. Additionally, atmospheric pollution like acid rain from nitrogen and sulphur emissions further degrades surface water quality, compounding the effects of authorized and unauthorized water use.

This chapter aims to provide a concise overview of current legislation relevant to the well-being of the Hennops River, specifically focusing on future water security in relation to water management, habitat, and macroinvertebrate communities. By presenting the findings of this study, the intention is to inform ongoing monitoring programs and advocate for potential revisions to existing legislation. These revisions should push for more stringent 'liveable' limits, rather than simply adhering to current legal limits and guidelines that include macroinvertebrate indices as part of the law. The core principle is that the health of macroinvertebrate communities directly reflects water potability and usability: a diverse, thriving community indicates healthy, usable water, while the absence or overabundance of certain species signals impairment. This approach emphasizes the link between environmental stressors, such as faecal and chemical waste (proxy-measured by COD,

ammonia, and nitrates), and their impact on macroinvertebrate communities, as reflected in SASS scores. Ultimately, this chapter serves as holistic practical understanding of present water resource conditions and to provide insight for proactive legal engagement to safeguard these resources for the continual sustenance of life – encompassing people, the environment, animals, and plants. The findings of this chapter can hold broader implications for water management and the development of effective water resource legislation within Gauteng, SA.

4.2.1– Overview of Current Water Legislation and Policy Context

Recognizing SA's water scarcity and unequal distribution, the National Water Act (NWA) (Act No. 36 of 1998) establishes the legislative foundation for sustainable water-resource management to ensure long-term water security and public health. A key regulatory instrument under this Act is the Water Use Licence (WUL) (Appendix 4.A), which authorises and governs activities that may impact water resources, including abstraction, effluent discharge, and land-based operations near watercourses (DWS, 2023). WULs are designed to ensure that all significant water users comply with Section 21 of the NWA, aligning individual and industrial use with Resource Quality Objectives (RQOs) and Target Water Quality Ranges (TWQRs). However, compliance monitoring and enforcement have been inconsistent, particularly at the municipal and industrial levels, where many facilities continue to operate with expired or non-compliant licences (Ntombela *et al.*, 2016; DWS, 2023). Complementary legislation, including the National Environmental Management Act (NEMA) and the National Environmental Management: Biodiversity Act (NEMBA), reinforces this framework by integrating environmental protection into development planning and mandating the conservation of aquatic biota (Kidd, 1999; Adom and Simatele, 2021). The National Water Resources Strategy (NWRS) situates the Hennops River within the jurisdiction of the Limpopo North Catchment Management Agency (CMA) (Nepfumbada and Muller, 2012). While the DWS remains accountable for safeguarding national water

resources and has introduced initiatives such as the Blue Drop and Green Drop programmes, enforcement—particularly at the municipal level—has been insufficient (Ntombela *et al.*, 2016). Persistent wastewater discharges from treatment plants into the Hennops River highlight the gap between legislative intent and implementation.

The Internal Strategic Perspectives (ISPs) provide an internal DWS planning framework for managing South Africa's catchments before full stakeholder consultation. These documents describe each catchment's hydrological balance, water use, challenges, and strategic priorities, supporting sustainable supply, ecological protection, and coordinated management across the 19 Water Management Areas (DWAF, 2004; Pollard *et al.*, 2023). The subsequent National Water and Sanitation Master Plan (NWSMP) and the establishment of the National Water Resources Infrastructure Agency (NWRIA) aimed to stimulate infrastructure investment to meet economic and ecological demands (Nepfumbada and Seetal, 2020).

Despite the decentralisation model introduced by the NWA, governance challenges persist. The Upper Crocodile (West) Catchment, which includes the Hennops and Magalies rivers, has an inactive CMA and inconsistent DWS monitoring, impeding adherence to the South African Water Quality Guidelines for Aquatic Ecosystems (SAWQG: AE) (DWAF, 1996). Chapter 4 of the Gauteng Nature Conservation Bill (GDARD, 2014) acknowledges the need to protect aquatic biota and their habitats; however, implementation remains limited by the absence of measurable criteria for maintaining habitat integrity at an ecoregional scale. Research emphasises that riparian zones—biodiversity hotspots essential to river health—require adaptive, variable-width buffer designs integrated into watershed management policies to enhance long-term resilience (Graziano, Deguire and Surasinghe, 2022). Yet, the current regulatory framework lacks explicit biological benchmarks, such as community- or

family-level bioassessment requirements, which are essential for safeguarding macroinvertebrate assemblages and ecological integrity.

The SAWQG: AE provide the scientific basis for establishing Target Water Quality Ranges (TWQRs)—thresholds defining acceptable physical, chemical, and biological conditions necessary to preserve ecosystem function (DWAF, 1996; WRC, 2024). Provincial Members of the Executive Council (MECs) are responsible for implementing these standards through regulation and permitting, including managing protected species lists, controlling alien species, and authorising conservation activities (GDARD, 2014).

The DWS formalised legally binding Resource Quality Objectives (RQOs) for the Upper Crocodile (West) catchment under Government Gazette No. 42775, Notice 562 of 2019 (DWS, 2019). These objectives specify the ecological categories, flow regimes, and water-quality conditions required to maintain aquatic integrity across the Crocodile (West), Marico, Mokolo, and Matlabas WMAs. Within Gauteng, the Upper Hennops and Rietvlei Rivers form part of Integrated Unit of Analysis 1 (Upper Crocodile, Hennops, Hartbeespoort system), while the Magalies River falls under Integrated Unit of Analysis 2 (Magalies system). These serve as benchmarks for evaluating ecological compliance in one of South Africa's most urbanised catchments (DWS, 2017a, 2017b, 2019; WRC, 2024).

RQOs function as adaptive diagnostic tools that inform effluent control, stormwater attenuation, and riparian rehabilitation strategies (Barbour et al., 1999). As no updated gazette has been issued since 2019, the present study contributes empirical evidence that may guide the refinement of biotic and chemical targets in future revisions of both the TWQRs and RQOs (DWS, 2019; WRC, 2024).

For consistency with national biomonitoring frameworks, only wet-season data were used for comparison against TWQRs and RQOs (Appendix 4.B). Wet-season conditions

represent high-flow states commonly used in national monitoring due to improved sampling efficiency and dilution-driven stability (Dickens and Graham, 2002; Dallas, 2007a).While dry-season data can reveal base-flow contamination, focusing on wet-season data ensures comparability with REMP and SASS protocols (Dallas, 2000, 2007b; Dickens and Graham, 2002; DWS, 2016, 2024c) .

Comparison of measured wet-season data with RQOs and TWQRs revealed extensive exceedances across key parameters, particularly in the Hennops River (Appendix 4.B). Orthophosphate concentrations (0.7–1.2 mg/L) exceeded the RQO limit (≤ 0.125 mg/L) by ~860%, while ammonia levels (1.8–9.2 mg/L) surpassed the legal threshold (≤ 0.0725 mg/L) by > 12 000%, indicating extreme nutrient enrichment driven by wastewater effluent and urban runoff (DWAF, 1996; DWS, 2019). Trace metals were similarly elevated, with lead (0.45 mg L^{-1}) exceeding its limit (≤ 0.007 mg/L) by ~6300%, copper (0.17 mg/L) by 2300%, iron (0.76 mg/L) by 660%, and aluminium (0.64 mg/L) by 327% (Appendix 4.B). These results reflect chronic effluent loading from the ERWAT Olifantsfontein and Sunderland Ridge WWTPs and insufficient community and institutional oversight.

The Magalies River displayed comparatively better but still concerning conditions. Although pH (6.5–9.0) and DO (80.7 – 88.3 %) remained within optimal ranges, zinc concentrations (0.48 mg/L) exceeded the RQO threshold (≤ 0.002 mg/L) by > 23 000%, while copper and lead surpassed limits by ~2100% and 4200%, respectively (Appendix 4.B). These patterns confirm spatial differentiation within the Upper Crocodile (West) catchment, where the Magalies retains partial ecological integrity (Categories A–C) and the Hennops remains heavily degraded (Categories E/F) (Appendix 4.B).

The chemical exceedances are mirrored in the biological data. In the Hennops River, average SASS (66) and ASPT (4.58) values fell below the RQO targets (SASS ≥ 55; ASPT ≥

4.2), indicating a Category E/F condition consistent with degraded water quality and poor habitat integrity (Appendix 4.B) (DWS, 2019). Dominance by tolerant taxa such as Chironomidae and Tubificidae, typical of organically enriched systems, corroborates sustained eutrophication and metal stress that suppress sensitive Ephemeroptera, Plecoptera, and Trichoptera assemblages (Death and Winterbourn, 1995; Barbour *et al.*, 1999). Conversely, the Magalies River achieved higher SASS (132) and ASPT (5.71) values, corresponding to Category B conditions and partial resilience (Appendix 4.B). Nevertheless, persistently high zinc (> 23 000%) and copper (> 2100%) levels suggest latent toxicity that could hinder long-term recovery (Appendix 4.B). These findings demonstrate how chemical pollution gradients translate into predictable biotic impoverishment and reaffirm macroinvertebrate-based bioassessment as a reliable diagnostic tool for RQO and TWQR compliance (Appendix 4.B) (DWS, 2019; WRC, 2024).

Certain critical parameters—such as vanadium, calcium, and potassium—are omitted from both the RQOs and TWQRs, despite their known ecological relevance. Similarly, the absence of a standardised COD limit within these frameworks represents a regulatory gap, particularly given consistently elevated COD across both rivers. The legal discharge limit for COD in South Africa is 75 mg/L, applicable to industrial and domestic effluents (Makuwa *et al.*, 2020). Yet, this threshold proved ecologically insufficient: at sites H2 and H3, COD reached 111 mg/L and 82 mg/L, correlating with Category E/F biotic ratings (Appendix 4.B). Even during the dry season, when COD declined below the legal threshold (44–69 mg/L), aquatic communities remained stressed, and the water exhibited a dark coloration indicative of persistent organic pollution. These results suggest that current legal limits inadequately protect biological diversity and that more stringent, ecologically informed COD standards are required to sustain aquatic life rather than merely regulate effluent discharge.

The findings underscore that uncontrolled urban development and weak regulatory enforcement have severely impacted water quality and biotic communities in both rivers. While the Magalies River shows moderate ecological modification, the Hennops River exhibits extensive degradation driven by township runoff, failing WWTPs, and illegal riverbank activities which are in violation of their WULs. Documented incidents include direct discharge of untreated effluent, widespread faecal contamination, paint dumping near the Hennops spring (H1), and a chemical spill downstream. Similarly, the Magalies River experiences reduced discharge due to unregulated abstraction and invasive riparian vegetation (M2–M3). Reports of toxic algal blooms in the connected Rietvlei Dam, producing microcystin—a known neurotoxin and carcinogen—highlight the human health risks of these conditions (Boikhutso, 2025).

In summary, persistent exceedances of nutrient and metal thresholds, weak enforcement of national legislation, and the absence of critical regulatory parameters underscore a pressing ecological crisis within the Upper Crocodile (West) catchment. Both the Hennops and Magalies rivers exhibit evidence of environmentally unsustainable activity and non-compliance with existing frameworks, though the impacts are more severe in the urbanised Hennops system. Given South Africa's chronic water scarcity and reliance on these rivers as critical freshwater sources, urgent intervention is required to strengthen monitoring capacity, improve wastewater treatment performance, and enforce effluent discharge standards. These measures are essential to restoring ecological integrity, safeguarding public health, and sustaining Gauteng's role as a vital economic hub for the nation.

4.3 - Existing Proactive Rehabilitation of the Hennops

This pervasive pollution is largely attributed to systemic failures in municipal wastewater treatment infrastructure, inadequate sanitation, and poor waste management,

issues that have persisted for decades (Toerien and Walmsley, 1979; Bega, 2018, 2024; Hennops Revival, 2019; SWPN, 2019; DHET, 2021; SAHRC, 2021; Moatshe, 2025). In the face of this governmental deficit, non-governmental organizations (NGOs) have emerged as crucial actors in mitigation and rehabilitation efforts. Two prominent examples, FRESH (Fountain River Environmental Sanctuary Hennops) led by Willem Snyman, and Hennops Revival founded by Tarryn Johnston, demonstrate distinct yet complementary approaches to addressing the crisis along the Hennops River.

FRESH focuses on long-term ecological restoration and strategic upstream litter interception. Their initiatives include the deployment of large litter traps, often integrated with biofilters as floating wetlands, designed to capture substantial volumes of plastic waste near the river's source by the KaalSpruit. FRESH has successfully removed approximately 500,000 m^3 of plastic bags, and Snyman has observed "visible improvements in water quality due to the biofilters" according to a BusinessTech article (Thorne, 2025). A key component of their model is the employment of dozens of unemployed local residents for waste collection, providing both environmental benefit and socio-economic support (Bega, 2024; Thorne, 2025). FRESH also advocates for improvements in wastewater treatment and the creation of natural wetlands and reed-beds to act as filters to biologically remove toxic substances.

Hennops Revival emphasizes broad community mobilization and direct, large-scale clean-up campaigns. Since its inception, the organization has removed over 5 million kilograms of waste from the river through more than 700 clean-up events (Lindeque, 2025). Their efforts have engaged thousands of community volunteers, logging an impressive 39,000 volunteer hours (Tshivhenga, 2025). Critically, Hennops Revival has created 8,000 job opportunities, primarily for Centurion's homeless and unemployed populations, thereby linking environmental care with community empowerment and providing vital income as

well as purpose (Lindeque, 2025). The organization also pursues innovative waste management solutions, such as experimenting with collected plastic to produce waterproof, fireproof, and rustproof paint, aiming to create a self-sustaining revenue stream for future clean-ups.

Despite their significant, quantifiable achievements, both NGOs face formidable, shared challenges. The overwhelming and continuous influx of pollution, particularly raw sewage from informal settlements and run-down WWTPs, means that their efforts, while indispensable, primarily address the symptoms rather than the root causes of the crisis. Faecal contamination levels remain dangerously high throughout, particularly in the middle reaches, indicating persistent, largely unabated microbiological contamination, compounded by toxic chemical greywater. Both organizations consistently encounter municipal inaction, poor management, and a lack of cooperation, with FRESH even reporting direct sabotage of their infrastructure by municipal contractors (Bega, 2024; Thorne, 2025). Furthermore, securing consistent funding and resources remains a perpetual obstacle, limiting their capacity to scale operations to meet the immense challenge.

The dedicated efforts of NGOs like FRESH and Hennops Revival are vital for mitigating the immediate impacts of pollution, fostering community engagement, and raising public awareness. They effectively fill a critical governance void, demonstrating the immense capacity of civil society to act where the state has failed. However, the long-term, sustainable rehabilitation of the Hennops River is contingent upon a fundamental shift in governmental responsibility. This necessitates substantial investment in upgrading and maintaining wastewater treatment infrastructure, rigorous enforcement of environmental regulations, and genuine, sustained collaboration between all levels of government, civil society organizations, and the private sector. Without addressing the systemic failures at the source

of pollution, the ongoing efforts of these NGOs will remain a continuous, uphill battle against an overwhelming levels of contamination.

KleenHealth, spearheaded by the EcoLogic Award-winning Kyle Odgers, employs cutting-edge nano-bubble technology as a powerful tool for bioremediation in the Hennops water systems. This innovative process involves saturating polluted water with trillions of microscopic gas bubbles, typically oxygen (to increase DO), which significantly increases the surface area for gas transfer and creates a highly oxidative environment (Evans, Bowman and Odgers, 2020). This enhanced oxygenation and the unique properties of nano-bubbles accelerate the natural breakdown process of contaminants by stimulating beneficial microbial activity, thereby effectively cleaning the water and restoring the ecological health of rivers like the Hennops.

In conclusion, SA's water resources face a critical management challenge stemming from legislative enforcement deficiencies, lack of polluter accountability and widespread water quality degradation. Although the NWA and related legislation provide a foundation for sustainability. The severely polluted Hennops River, burdened by multiple pollution sources and an ineffective CMA, starkly illustrates these systemic problems. In light of this disparity, NGOs' efforts to manage ongoing pollution, without robust governmental cooperation, can only address immediate effects and serve as short-term options, ultimately lacking long-term solutions. To safeguard the long-term health of SA's aquatic ecosystems and ensure the availability of safe water, urgent action is required. This includes stricter enforcement and proactive pollution mitigation, but also the clear identification of polluters and rigorous enforcement of their accountability.

CHAPTER 5 : CONCLUSION AND RECOMMENDATIONS

Water Quality and Biotope Availability

The provided analysis in Chapter 2 reveals significant differences in water quality and habitat between the urbanized Hennops River and the more agriculturally influenced Magalies River, underscoring the detrimental impact of urbanization on river ecosystems. The Hennops River consistently exhibits higher levels of pollution indicators, including elevated orthophosphate (exceeding legal limits), increased conductivity, higher sodium concentrations, and severely high ammonia levels, all pointing to substantial faecal contamination from WWTPs and detergent-rich greywater from informal settlements. Furthermore, the Hennops River shows considerably higher COD and greater acidity, which intensifies during the wet season and increases the toxicity of heavy metals. Conversely, the Magalies River generally maintains higher DO levels and much lower COD. In terms of habitat and biotope availability, the Magalies River has more stable discharge patterns and consistent substratum, supporting diverse macroinvertebrate communities, while the Hennops River's fluctuating flows and simplified habitats (characterized minimal SIC, GSM, bedrock, and a major lack of riparian vegetation) reduces biotope availability and favour pollution-tolerant species: Chironomidae and Tubificidae. Overall, the chapter concludes that while both water quality and habitat degradation contribute to the impaired ecological condition of the Hennops River, water pollution—particularly high conductivity and nitrates—is the dominant factor driving the observed differences in macroinvertebrate communities, making pollution source control paramount for restoration.

This necessitates a shift from expensive membrane filters to more affordable biological methods like activated charcoal or phytoremediation, integrating reed beds or floating plants within WWTPs to absorb metals from treated water before river discharge.

An excellent strategy to start implementing would be sustainable drainage systems with greater retention capacity and longer hydraulic residence times offers a practical means to enhance stormwater treatment and improve runoff quality in Johannesburg (van der Merwe *et al.*, 2025). Additionally according to research, the addition of the phytofiltration process (specifically rhizofiltration) at WWTPs is more effective at removing heavy metals when free-floating plants are used, as opposed to submerged or emergent plants (Rezania *et al.*, 2016). This is because of their unique morphology and faster growth rate (Rezania *et al.*, 2016). Innovative solutions, such as Chitosan/lanthanum hydrogel beads, offer an effective and rapid method for phosphate removal, achieving completion within six hours (Koh *et al.*, 2022). These beads also possess the added benefit of removing cationic heavy metals: Cu, Pb, and Zn (Koh *et al.*, 2022). Additionally, nitrogen removal can be achieved when combined with technologies like reverse osmosis, ion exchange, or biological denitrification (Choudhary, Muduli and Ray, 2022).

For saline water, new studies highlight the potential of "affordable and easily producible" graphene oxide-polymer support composite membranes for desalination and purification (Elzubair, Uchôa and Prado da Silva, 2024). While graphene oxide–polymer composites offer a cost-effective and efficient approach to desalination, potential environmental and health risks remain. Studies have shown that degradation or abrasion of these materials can release graphene oxide fragments capable of inducing oxidative stress and cellular damage in aquatic organisms (Zhang et al., 2016; Fadeel et al., 2018). However, the graphene oxide-polymer is still a foreseeable option if maintained properly. Additionally, manufacturing and disposal may contribute to microplastic pollution or incomplete recovery of spent membranes (Walsh et al., 2024). Therefore, large-scale application should be supported by life-cycle and toxicological assessments to ensure that the advantages of graphene-based materials do not compromise long-term environmental safety.

Beyond technological solutions, restoring and protecting natural ecosystems is paramount. Riparian zones are vital for river health, stabilizing banks, filtering pollutants, regulating temperature, and providing crucial habitats, all while benefiting human well-being (Sweeney *et al.*, 2004; Anbumozhi, Radhakrishnan and Yamaji, 2005; Singh, Tiwari and Singh, 2021; Graziano, Deguire and Surasinghe, 2022). To effectively conserve riparian zones and enhance biodiversity and ecosystem resilience, a strategic shift is needed towards integrating variable-width, multi-tiered buffer designs into watershed-scale conservation policies, moving away from fixed-width approaches (Graziano, Deguire and Surasinghe, 2022). For subsequent research, integrating an ArcGIS land-use categorical map is recommended to facilitate a more comprehensive analysis of land use variations and their impacts. Additionally, the incorporation of the Vegetation Response Assessment Index (VEGRAI) alongside the RHAM would enhance the robustness of the habitat analysis and improve alignment with RQO requirements, thereby strengthening the overall validity and comprehensiveness of the study.

The adoption of vertical farming systems presents a transformative opportunity within SA's water–food–energy nexus, offering a sustainable pathway to reduce water abstraction while maintaining agricultural productivity. Studies have demonstrated that vertical farms can use up to 98% less water and produce 200–300% higher crop yields per unit area compared to traditional agriculture, while occupying as little as one-tenth of the land area (Benke and Tomkins, 2017; Al-Kodmany, 2018). The "less drop, more crop" approach exemplifies how technological innovation can decouple food production from freshwater depletion. Moreover, these systems require little to no pesticide application and generate minimal nutrient runoff, directly mitigating eutrophication and nutrient loading in downstream aquatic environments. Given that approximately 61% of South Africa's available freshwater is currently allocated to agriculture (Knight, 2019; DWS, 2024a), the gradual

integration of vertical farming could substantially reduce surface-water withdrawals, allowing greater flow retention and natural hydrological renewal within rivers such as the Hennops and Magalies rivers. While infrastructural investment remains a barrier, privately operated or public–private partnership models could deliver both economic and ecological benefits, aligning agricultural efficiency with national water-conservation and river-restoration objectives.

Macroinvertebrate Diversity

This study, using the SASS 5 protocol and further taxonomical analysis, compared macroinvertebrate communities in the urbanised Hennops River and the more agricultural Magalies River, revealing a clear contrast in ecological health. The Magalies River generally exhibited higher diversity and SASS scores, showing resilience with high diversity at its spring, a decline in its middle reaches due to a WWTP influence, and subsequent recovery downstream, attributed to natural buffers and restored riparian zones. In stark contrast, the Hennops River consistently displayed significantly lower macroinvertebrate diversity, dominated by pollution-tolerant species like Chironomidae and Tubificidae, and lacked sensitive groups such as caddisflies, Trichoptera. Its sites showed consistently poor ecological status, with the middle section (H2) being severely impacted by extremely high COD, conductivity, ammonia and raw sewage from WWTPs and informal settlements, even leading to unusual still-water macroinvertebrate communities. While the lower Hennops River showed some signs of recovery due to dilution and riparian restoration, the study concluded that water pollution, particularly heavy metals, high conductivity, and nitrogen, is the primary driver of the severe ecological degradation and distinct macroinvertebrate communities observed in the urban river system. Overall, both rivers' ecological health follows a pattern of higher diversity at their springs, declining in their urban impacted middle reaches, and subsequently recovering in the lower reaches. This resilience is attributed to

natural buffers, such as expansive open lands and restoring riparian zones, coupled with sufficient longitudinal distance from WWTPs for ecosystem recovery.

For future research, it is advised that the collected m diversity dataset be further explored through advanced diversity and functional trait analyses to enhance understanding of macroinvertebrate assemblage composition and ecological dynamics. As well as to include The Fish Response Assessment Index (FRAI) and Macroinvertebrate Response Assessment Index (MIRAI) to provide a more comprehensive interpretation of the collected biological data and to ensure greater alignment with RQO standards. Further research should incorporate comparative analyses between the Freshwater Biodiversity Information System (FBIS), iNaturalist records, and the collected macroinvertebrate data to deepen understanding of biotic community composition and spatial variability along the Hennops and Magalies Rivers. For further research into contamination analysis, the incorporation of stable-isotope sampling of key aquatic organisms (e.g., tissue $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ from macroinvertebrates or fish) could be paramount for identifying pollutant sources and strengthening enforcement of existing legislation (Levin *et al.*, 2023; Levin, 2024). These isotopic tracers can be effectively integrated into monitoring programmes to trace contamination pathways and attribute responsibility at the user level, thereby enhancing regulatory compliance and catchment-scale accountability.

Legislation

This study reveals severe degradation of the Hennops River and moderate impairment of the Magalies River, despite SA's robust legislative framework under the National Water Act (1998) and associated water-quality guidelines. Wet-season data exceeded the 2019 RQOs and updated TWQRs by up to 12 000%, with extreme nutrient and metal pollution linked to wastewater discharges and urban runoff. These chemical exceedances corresponded

with low SASS and ASPT scores, confirming ecological categories of ‘E/F’ in the Hennops and ‘B’ in the Magalies. The findings underscore weak regulatory enforcement, critical gaps in current standards—such as the absence of COD and certain metal thresholds—and the urgent need for integrated management and stricter compliance to restore aquatic ecosystem integrity. In the face of these systemic governmental failures, NGOs like FRESH and Hennops Revival have emerged as vital actors, demonstrating significant, quantifiable achievements in pollution mitigation, community engagement, and awareness, effectively bridging critical governance gaps, although their efforts remain largely short-term and symptomatic without sustained governmental cooperation and investment to address root causes.

Addressing the current water crisis in SA demands a multi-faceted approach that acknowledges the impracticality of reducing industrialization and urban activities. The focus must instead shift to ensuring existing infrastructure functions correctly to meet demand, thereby safeguarding both economic growth and future environmental provisions. As seen in this study that the main contributor of nitrogen and ammonia are the WWTPs (H2, H3). This involves also involves implementing practical solutions, from advanced treatment technologies and ecological restoration to robust regulatory oversight, to mitigate various stressors on water systems. Strengthening polluter accountability requires not only the accurate identification of pollution sources but also rigorous enforcement of compliance, which could be supported through stable isotope analyses—specifically $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotopes—from macroinvertebrate and fish tissues to trace nutrient and contaminant pathways and identify polluters (Levin *et al.*, 2023; Levin, 2024). Moreover, future monitoring frameworks should prioritise the integration of existing ecological datasets—including FBIS records, iNaturalist records, REMP data, TWQRs guidelines and national RQO benchmarks—into a unified digital platform that supports real-time reporting, spatial

analysis, and transparent compliance tracking, thereby transforming biomonitoring outcomes into actionable management interventions.

The effectiveness of the nation’s foundational environmental legislation is compromised by inadequate enforcement mechanisms and insufficient maintenance of treatment and monitoring infrastructure, resulting in widespread non-compliance. The NGOs efforts expose governmental failures, underscoring the need for sustained investment to achieve long-term ecological, freshwater, and human sustainability.

These proposed solutions, in this chapter, can locally manage various pollution levels, complementing the vital maintenance of existing WWTPs. A major source of effluent discharge problems is failing WWTPs that improperly filter and treat wastewater. The Green Drop Certification Programme Report (DWS, 2023b) alarmingly found 64% of 995 audited WWTPs are at high or critical risk of discharging inadequately treated water, a significant performance decline since 2013. Only 23 WWTPs received the prestigious Green Drop Certificate for good quality discharge. Critically, 102 out of 115 (89%) of Department of Public Works systems were classified as "critical," scoring below 31% (DWS, 2023a, 2023b). Rectifying the current water crisis demands immediate substantial intervention and rehabilitation of the nation's critically impaired WWTP infrastructure.

In response to these challenges, the DWS is implementing a multi-faceted strategy to address SA's water crisis. This includes enhanced regulatory surveillance through tools like the Green Drop Watch Report (DWS, 2023b), which flags critical wastewater systems for immediate intervention and continuous monitoring (Hennops Revival, 2025). The DWS has also initiated targeted action plans for 30 municipalities with severe performance issues, requiring corrective action plans for critical WWTPs within 60 days (Hennops Revival, 2025). Crucially, they are fostering inter-departmental collaboration with entities such as

COGTA, MISA, and the National Treasury to provide comprehensive support (DWS, 2023a, 2023b; Hennops Revival, 2025). Furthermore, the DWS is leveraging technological integration with online systems like IRIS for auditing and the "Very Rough Order of Measurement" (VROOM) model for infrastructure assessment, alongside pursuing legislative reforms to strengthen drinking water regulation and building internal regulatory capacity (Hennops Revival, 2025). The comprehensive implementation of these initiatives remains to be observed. Sustained and genuine partnerships among the private sector, government, and civil society are essential for effectively complementing the DWS response strategies. Such collaborations are critical for bringing about a positive change in the water quality of these vital systems.

Although the Hennops and Magalies rivers reflect severe ecological degradation, this study shows that recovery is within reach. After just one rainy season, the water quality improved—chemically, biologically, and visually—revealing nature's remarkable capacity for self-natural remediation processes when pressures are reduced. With SA's legislative framework already established, progress now depends on effective enforcement, operational WWTPs, and collective stewardship. If immediate and sustained action is taken, the Hennops could realistically return to a Category C ecological state within five to eight years, signalling a restored balance between human use and ecosystem health. The rivers' revival is not only possible—it is a powerful reminder that when governance and nature work together, restoration follows.

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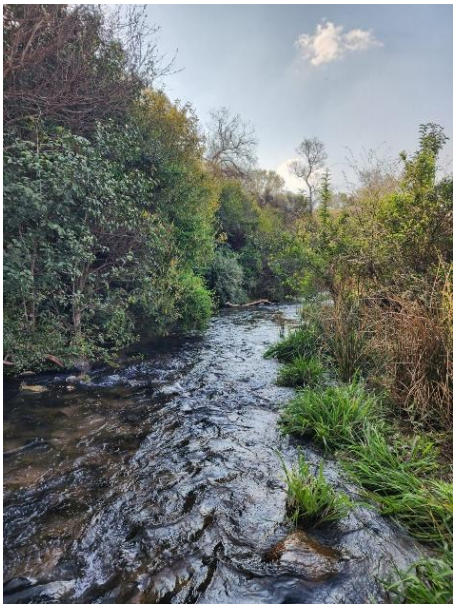
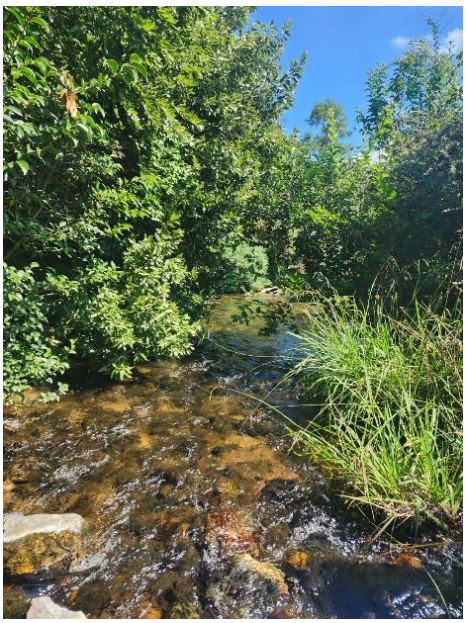
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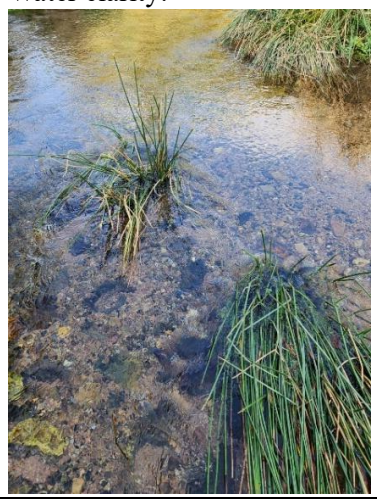
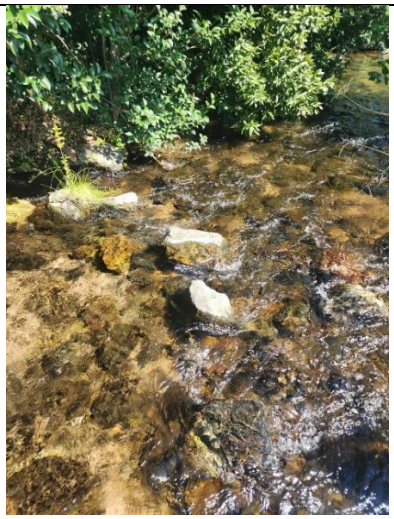
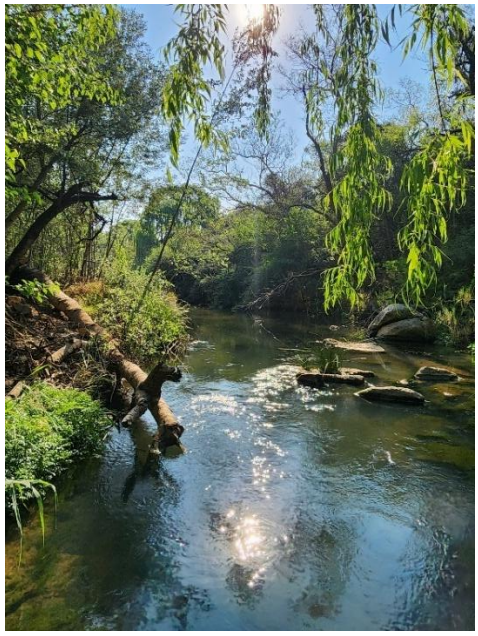
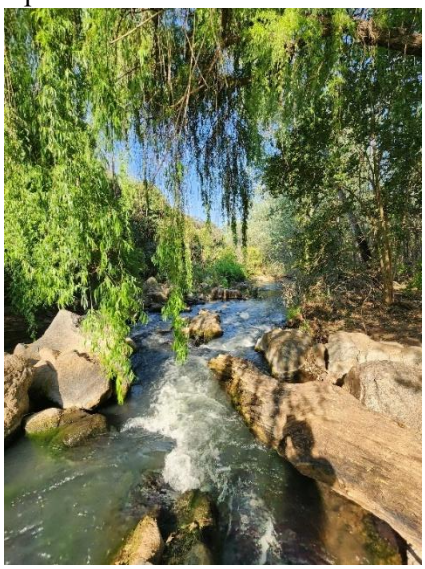
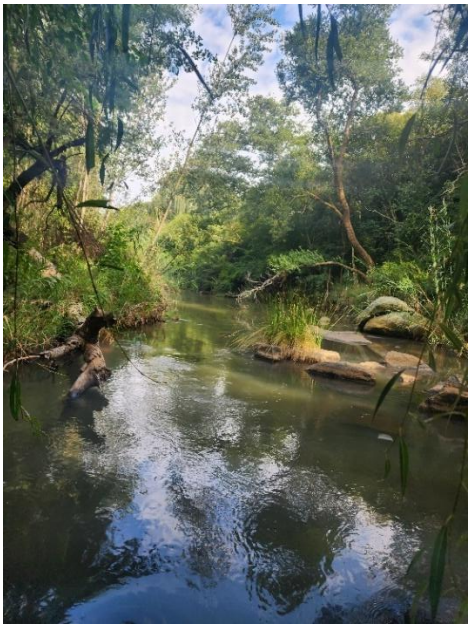
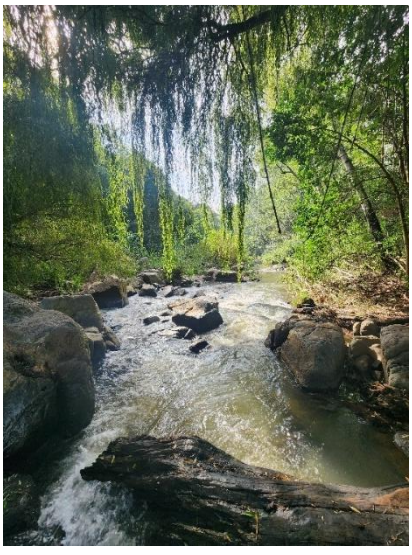
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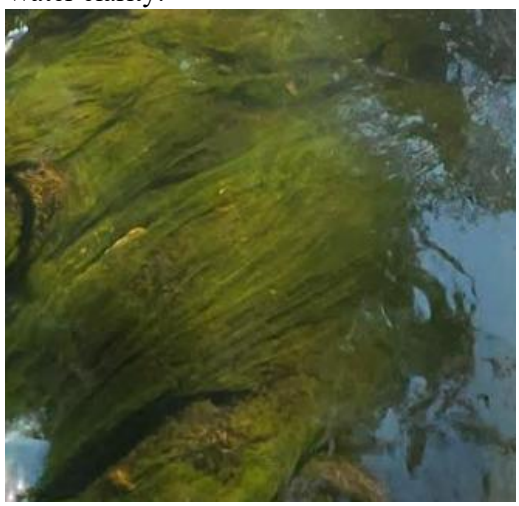
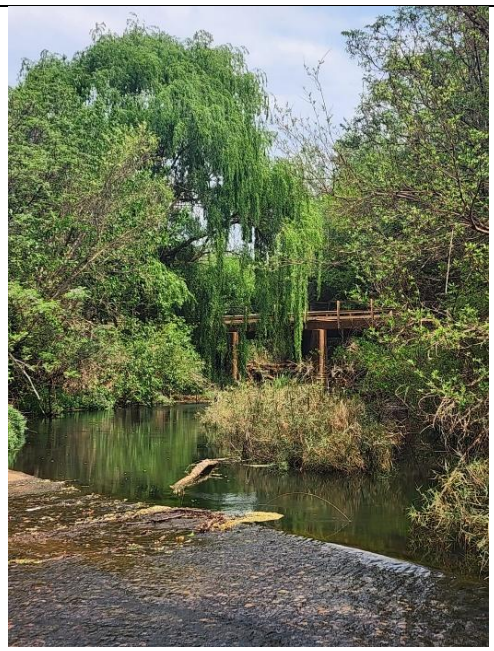
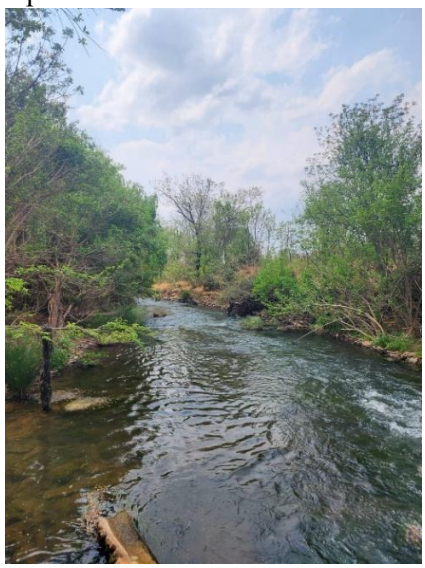
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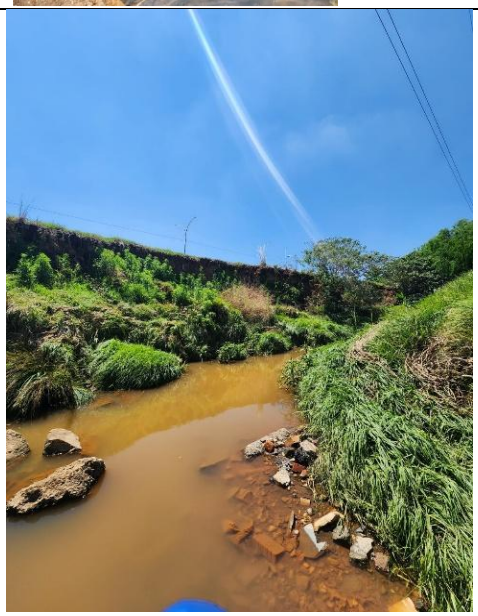
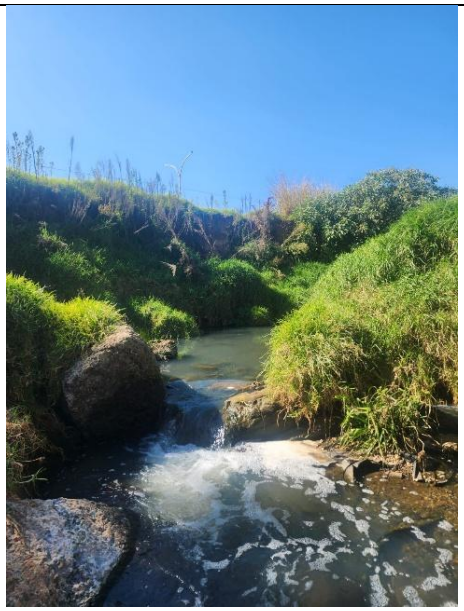
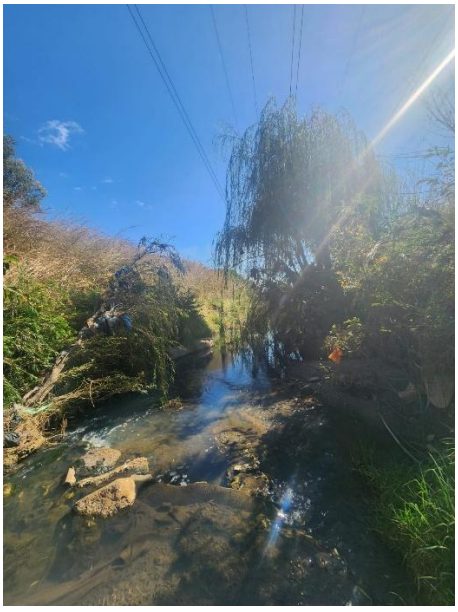
Chapter 1:

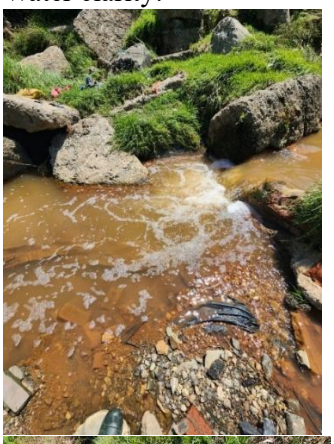
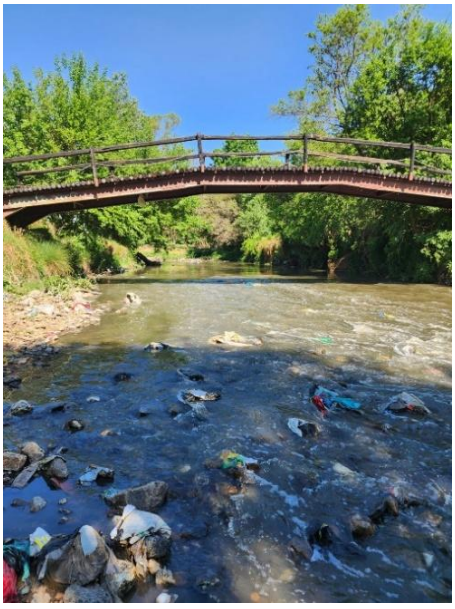
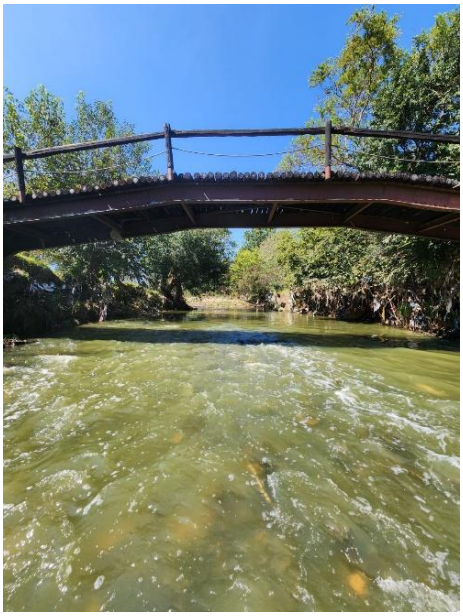
Appendix 1.A: A table showing the upstream and downstream still-point photographs of each site in the dry and wet season.

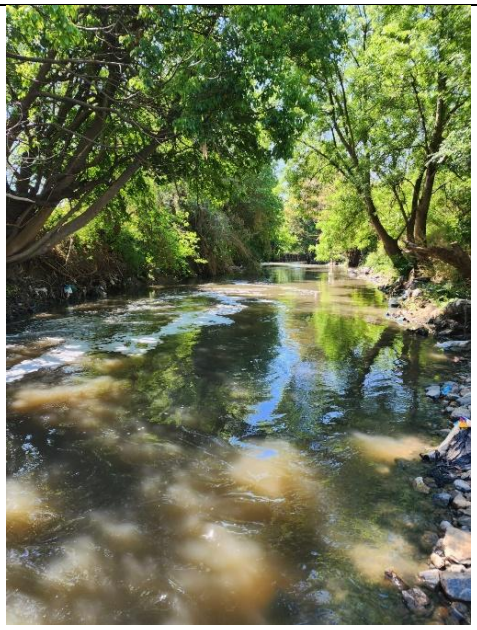
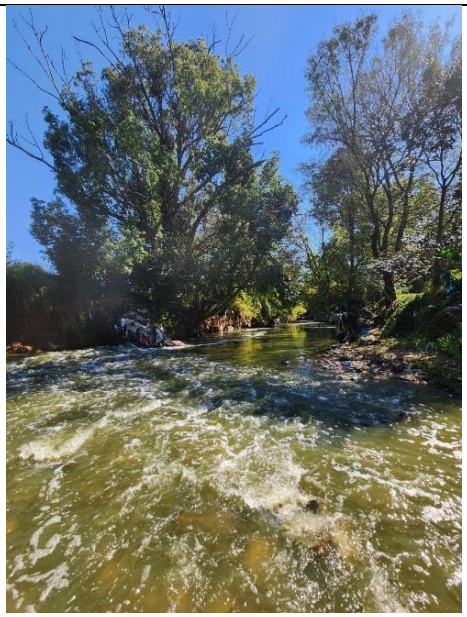
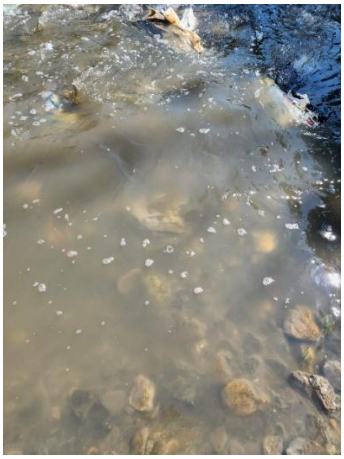
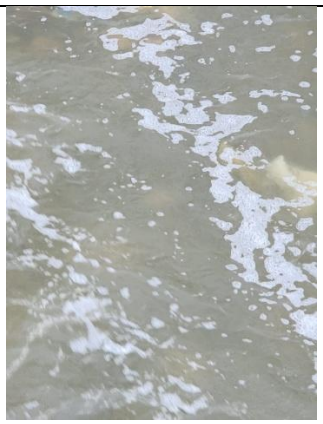
River Site	Dry	Wet
M1	 Upstream	 Upstream
	 Downstream	 Downstream

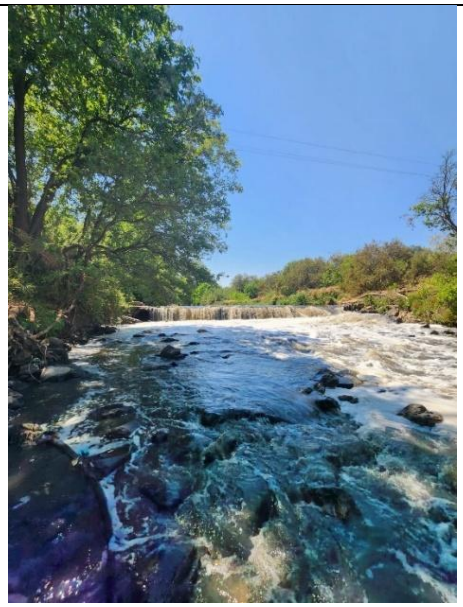
M1	Water clarity: 	
M2	 Upstream  Downstream	 Upstream  Downstream

M2	Water clarity: 	
M3	 Upstream  Downstream	 Upstream  Downstream

M3	Water clarity: 	
H1	 Upstream  Downstream	 Upstream  Downstream

H1	Water clarity:  	 
H2	 Upstream	 Upstream

	 Downstream	 Downstream
H2	Water clarity:  	 

H3	 Upstream	 Upstream
	 Downstream	 Downstream

H3	Water clarity:  	 

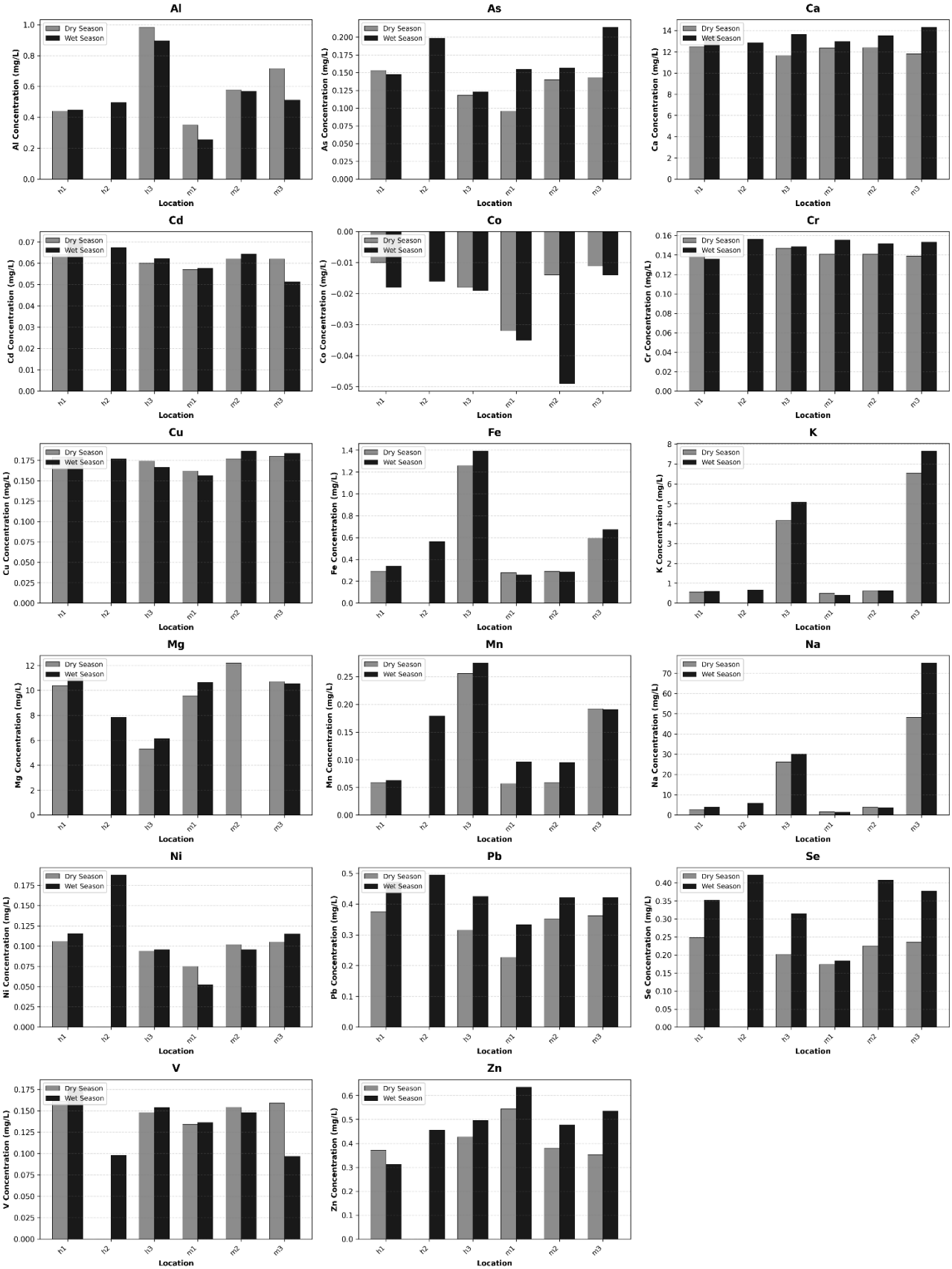
Chapter 2:

Appendix 2.A: A Table Summary of analytical QA/QC procedures and detection limits for in situ and laboratory analyses.

Parameter/ Group	**Analytical Method**	**Instrument / Technique**	**Detection Limit (mg/L)**	**Recovery Range (% of CRM)**	**Precision (% RSD)**	**QA/QC Measure Applied**
pH	APHA 4500-H^+ B	pH meter (calibrated daily at 25 °C)	—	—	±0.1 units	In-field calibration, duplicate readings.
Nitrate (NO_3^-)	APHA 4500-NO_3^- H	UV–Vis spectrophotometry	0.01	95–105	<5	CRMs, duplicates, field blanks. *In situ* YSI measurements were calibrate day before with varying solutions (1mg/L, 10mg/L and 100mg/L).
Nitrite (NO_2^-)	APHA 4500-NO_2^- B	UV–Vis spectrophotometry	0.01	95–105	<5	CRMs, duplicates.
Orthophosphate (PO_4^{3-})	APHA 4500-PE	UV–Vis spectrophotometry (ascorbic acid method)	0.005	90–110	<10	CRMs, blanks, duplicates.
Ammonia (NH_3) / Ammonium (NH_4^+)	APHA 4500-NH_3 F	Spectrophotometric (phenate method)	0.02	90–110	<10	Calibration standards, duplicates.
Chemical Oxygen Demand (COD)	APHA 5220 D	Dichromate reflux, colorimetric	5	85–110	<10	Duplicate samples, reagent blanks.

Metals (Al, Cu, Fe, Mn, Zn, Pb, etc.)	APHA 3120 B	Inductively Coupled Plasma–Optical Emission Spectrometry (ICP–OES)	0.001–0.01	90–110	<5	Calibration curves, CRMs, duplicates, blanks.
Conductivity (EC)	APHA 2510 B	Conductivity meter	1 µS cm^{-1}	—	<3	In-field calibration.
Dissolved Oxygen (DO)	APHA 4500-O G	Portable DO meter (membrane electrode)	0.01	—	<5	Calibrated to 85% air saturation before each use.
Oxidation–Reduction Potential (ORP)	APHA 2580 B	ORP electrode (Ag/AgCl reference)	—	—	<3	Calibrated before sampling.

Appendix 2.B: (A) Laboratory analysis of heavy metals at each sampled site across seasons.



Appendix 2.C: Water PCA Loadings and PCA Correlations

Parameter	Loading	Correlation
Temperature	~-0.05	~-0.05
DO	~0.00	~-0.60
pH	~0.00	~-0.05
COND	~0.92	~1.00
ORP	~0.35	~0.80
COD	~-0.05	~-0.40
Average depth	~0.02	~0.55
pH-test	~0.00	~-0.15
Nitrate-test	~0.00	~-0.10
Nitrite-test	~0.00	~-0.05
Orthophosphate-test	~0.00	~0.00
Ammonia + Ammonium-test	~0.00	~0.15
Al	~0.00	~0.55
As	~0.00	~0.55
Ca	~0.00	~0.55
Cd	~0.00	~-0.45
Co	~0.00	~0.25
Cr	~0.00	~0.10
Cu	~0.00	~0.30
Fe	~0.00	~0.55
K	~0.00	~0.95
Mg	~0.00	~0.70
Mn	~0.00	~0.80
Na	~0.02	~0.15
Ni	~0.00	~0.10
Pb	~0.00	~-0.20
Se	~0.00	~0.15
V	~0.00	~0.00
Zn	~0.00	~-0.05

Chapter 3:

Appendix 3.A: A Table showing the Reference Collection of specimens (WRC, 1999; Fry, 2021).

Digital collection with images:

https://docs.google.com/spreadsheets/d/16PsINJfLcDc8aELdyMig1vbjRojOSE9PL0GrXbNn694/edit?gid=0#gid=0

No.	genus.species	site - date- biotope	phylum/order- family- tribe- morph	functional.grp-page-key notes- additional notes
1	spongillidae.a	m1 - 14/09/2023-stones	porifera-spongillidae-n/a-a	filter-14-this is a gemmule. often yellow and very coiled to increase surface area for filter feeding. varies in size. -specimen is 2 mm---
2	dugesiidae.a	m1 - 14/09/2023-stones	platyhelminthes-dugesiidae-tricladida-a	predator-16-distinctive white eyes, with small black spot. flat oval body. -<20 mm---
3	corbicula.fluminalis	m5-25/9/2023-stones	mollusca-cyrenidae-n/a-a	filterer-24-shell is robust with concentric ribs. shell is yellow, brown to olive green. the interior is purplish. umbo located centrally. -5 - 20 mm ---
4	pisidium.costulosum	m2-20/9/2023-veg	mollusca-pisidiidae-n/a-a	filterer-26-very light concentric ribs on shell's surface. cream in colour -6-9 mm---
5	pisidium.b	m5-25/09/2023-stones	mollusca-pisidiidae-n/a-b	filterer-26-concentric ribs on shell's surface. darker in colour with spots on shell.-6-9 mm.---
6	lymnaeidae.a	sp-25/4/2024-veg	mollusca-lymnaeidae-n/a-a	scraper-30-open to the right. brown -<22 mm---
7	lymnaea.truncatula	h6-01/10/2023-stones	mollusca-lymnaeidae-n/a-a	scraper-30-open to the right. light brown/yellow -<22 mm---
8	galba.truncatula	sp-25/04/2024-stones	mollusca-lymnaeidae-n/a-a	scraper-30-open to the right. light brown /yellow with small bubble patterns -<22 mm---
9	stenophysa.marmorata	sp-25/4/2024-veg	mollusca-physidae-n/a-a	scraper/grazer-31-opens to the left. shell is highly spotted and is generally yellow and brown. sharply pointed shell. -15 mm---
10	physella.acuta	h6-01/10/2023-stones	mollusca-physidae-n/a-a	scraper/grazer-31-opens to the left. shell dark brown/black. sharply pointed shell. -16 mm---
11	helisoma.duryi	m2-20/9/2023-veg	mollusca-planorbidae-n/a-a	scraper/grazer-32-discoid shape (spirals) and generally yellowish brown. -2 - 20 mm---

12	planorbella.b	m5-25/09/2023-stones	mollusca-planorbidae-n/a-b	scraper/grazer-32-discoid shape (spirals). light and dark brown. -2 - 20 mm---
13	pettancylus.a	m5-25/9/2023-stones	mollusca-ancylinae-n/a-a	scraper-33-tip of shell is at the bottom central and has concentric radial lines on shell. -< 10 mm---
14	burnupia.a	m5-25/09/2023-gsm	mollusca-ancylinae-n/a-a	scraper-33-tip of shell is heavily leveled to the right side. dark brown-< 10 mm---
15	melanoides.tuberculata	h6-01/10/2023-gsm	mollusca-thiaridae-n/a-a	scrapers-34-well developed spiraled cone shaped shell. tip often eroded. white shell with brown speckles. -5 - 20 mm ---
16	marsupiobdella.africana	sp-25/04/2024-gsm	annelida-hirudinae-n/a-a	predator-36-body highly segmented . two small black eyes. caudal sucker. no particular pattern on body, besides one black dot close anterior end (closer to the eyes)-< 20 mm---
17	helobdella.a	h6-01/10/2023-gsm	annelida-hirudinae-n/a-a	predator-36-body highly segmented . two small black eyes. caudal sucker. body highly patterned longitudinal along body. -< 20 mm---
18	salifidae.a	sp-25/04/2024-veg	annelida-hirudinae-n/a-a	predator-36-very hard to see body segments. has very prominent caudal sucker. light brown- < 40 mm---
19	salifidae.b	m1-14/9/2023-gsm	annelida-hirudinae-n/a-b	predator-36-very hard to see body segments. has very prominent caudal sucker. dark brown- < 40 mm---
20	tubifex.a	m1-14/09/2023-stones	oligochaeta-tubificidae-microdrilli-a	collector/gatherer-37-small thin and globular internal organs.-< 100 mm---
21	tubifex.b	m5-25/4/2023-gsm	oligochaeta-tubificidae-microdrilli-b	collector/gatherer-37-much wider and larger than morpho species A. - < 150 mm---
22	nematoda.a	h6-23/03/2024-stones	nematoda-nematoda-n/a-a	predator/shredder-38-unsegmented body. sometimes translucent and light-yellow colour.-< 10 mm---
23	hydrachna.a	m1-14/9/2023-stones	arachnida-hydracarina-n/a-a	predator-40-dark red. 8 legs. -< 5 mm---
24	hydrachna.b	m1-14/9/2023-stones	arachnida-hydracarina-n/a-b	predator-40-spiky - 8 legs. -< 5 mm---
25	torrenticolidae.c	m1-14/9/2023-stones	arachnida-torrenticolidae-n/a-c	predator-40-green -8 legs. -< 5 mm---
26	torrenticolidae.d	m1-14/9/2023-stones	arachnida-torrenticolidae-n/a-d	predator-40-green and red - 8 legs. - < 5 mm---

27	hydrachna.e	m1-14/9/2023-gsm	arachnida-hydracarina-n/a-e	predator-40-yellow green - 8 legs. -< 5 mm---
28	hydrachna.f	m1-14/9/2023-gsm	arachnida-hydracarina-n/a-f	predator-40-blue- 8 legs.-< 5 mm---
29	hydrachna.g	m1-14/9/2023-gsm	arachnida-hydracarina-n/a-g	predator-40-brown - 8 legs . -< 5 mm---
30	hydrachna.h	m1-27/03/2024-veg	arachnida-hydracarina-n/a-h	predator-40-grey - 8 legs. -< 5 mm---
31	torrenticolidae.i	m1-14/9/2023-gsm	arachnida-torrenticolidae-n/a-i	predator-40-black and red - 8 legs. -< 5 mm---
32	hydrachna.j	m2-20/9/2023-gsm	arachnida-hydracarina-n/a-j	predator-40-round yellow - 8 legs. -< 5 mm---
33	isotomurus.maculatus	m5-25/09/2023-veg	collembola-isotomidae-n/a-a	collector/gatherer-41-hairy body. highly patterned segmented body and large conspicuous antennae. -< 8 mm---
34	cloeodes.a	m1-14/9/2023-veg	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-subproximal arc of setae on tibiae and tarsi. edentate tarsal claws with minute striations subapically. medial caudal filament well developed. long setae along all three ceri. double lamellae-3 - 14 mm - vol 7 dichotomous key pg.65 and 96--
35	labiobaetis.a	m1-14/9/2023-veg	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-antennae have notched shape/arrowed. femur has villopore. medial caudal filament well-developed.-3 - 14 mm -vol 7 dichotomous key pg. 60 and 102--
36	acanthiops.a	m1-14/9/2023-veg	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-two tails. dorsal abdominal tubercles. pronotum. -body 7 mm. ceri 4 mm -vol 7 dichotomous key pg.44 and page 92-4 - 14 mm-
37	acanthiops.b	m1-14/9/2023-stones	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-three tail. dorsal abdominal tubercles. pronotum. -body 7 mm. ceri 4 mm -vol 7 dichotomous key --
38	acanthiops.erepens	m1-14/9/2023-stones	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-round head. three tails. dorsal abdominal tubercles. pronotum. -body 7 mm. ceri 4 mm -vol 7 dichotomous key --

39	afroptilum.sudafricanum	m1-14/9/2023-veg	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-simple setae. two rows of denticles on tarsal claws. dorsoventrally-flattened abdomen. medial caudal filament is well developed. light brown, yellow coloration. -3 - 14 mm -vol 7 dichotomous key --
40	baetis.harrisoni	m1-14/9/2023-veg	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-most common type. ceri have little setae and medial filament shorter than others. posterior margin of abdominal tergite shows small spines-3 - 14 mm -vol 7 dichotomous key pg.62 (fig. 2.48 D) and page 94--
41	demoreptus.capensis	m1-14/9/2023-gsm	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-two tails. villiopore present. two rows of denticles and a pair of subapical setae.-3 - 14 mm -vol 7 dichotomous key pg. 41 and 98--
42	dicentroptilum.spinulosum	m5-18/03/2024-stones	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-larger size. ceri 6 mm. robust body. pair of ventral papillae om pro-coaxe. presence of dorsal row of long setae on tibia and tarsi. two rows of denticles. present/absent dorsal abdominal tubercles. medial caudal filament well developed. -8.5 mm -vol 7 dichotomous key pg 48 and 99 --
43	dabulamanzia.a	m5-18/03/2024-stones	ephemeroptera-baetidae-n/a-a	collector/gatherer/scraper-46/47-present sub proximal arc of setae on tibia. one row of denticles. medial caudal filament well developed. ceri range between 2.5 - 3.5 mm. -4.8 - 7.2 mm -vol 7 dichotomous key pg 63 and 98--
44	caenis.a	m1-14/9/2023-gsm	ephemeroptera-caenidae-n/a-a	collector/gatherer-48/49-conspicuous skirt on lower abdomen gill 2. with black/brown and yellow patterns. 3 tails -2 - 9 mm-vol 7 dichotomous key pg.34 and 120--
45	caenis.b	m1-27/03/2024-veg	ephemeroptera-caenidae-n/a-b	collector/gatherer-48/50-conspicuous skirt on lower abdomen gill 2.. almost all yellow/brown with no patterns. 3 tails-3 - 9 mm-vol 7 dichotomous key pg.34 and 121--
46	machadorythus.maculatus	m1-14/9/2023-stones	ephemeroptera-machadorythidae-n/a-a	collector/gatherer/predator-51-protruding eyes and gill chamber.-7 mm -vol 7 dichotomous key --

47	castanophlebia.a	m1-14/9/2023-stones	ephemeroptera-leptophlebiidae-n/a-a	collector/filterer-54-gills end in long filaments. ceri widely spread with medial filament marginally longer than cerci. -4 - 15 mm-vol 7 dichotomous key --
48	aprionyx.a	m5-18/03/2024-gsm	ephemeroptera-leptophlebiidae-n/a-a	collector/filterer-54-much larger eyes than the castanophlebia sp. yellow/brown in colour.-5 - 15 mm-vol 7 dichotomous key --
49	tricoythus.a	m1-14/9/2023-stones	ephemeroptera-tricorythidae-n/a-a	collector/gatherer-59-robust mandibles. long setae fringe like structures by mandibles -6 - 10 mm -vol 7 dichotomous key --
50	tricoythus.b	m1-14/9/2023-stones	ephemeroptera-tricorythidae-n/a-b	collector/gatherer-59-bigger eyes than morpho A -6 - 10 mm -vol 7 dichotomous key --
51	pinheyscha.a	m1-14/9/2023-gsm	odonata-aeshnidae-n/a-a	predator-64-large eyes and fatter bodies. has spots on middle thorax-< 70 mm-vol 7 dichotomous key --
52	zosteraeschna.a	m1-14/9/2023-stones	odonata-aeshnidae-n/a-a	predator-64-angled eyes not round. highly patterned -< 70 mm-vol 7 dichotomous key --
53	crenigomphus.a	m1-14/9/2023-stones	odonata-gomphidae-n/a-a	predator-66-segmented antennae. mouth and head structure is different from the aeshnids. -< 35 mm-vol 7 dichotomous key --
54	paragomphus.a	m1-14/9/2023-gsm	odonata-gomphidae-n/a-a	predator-66-dots on either side of abdomen. slight green colour -< 35 mm-vol 7 dichotomous key --
55	notogomphus.a	m1-14/9/2023-stones	odonata-gomphidae-n/a-a	predator-66-very small eyes. no visible patterns all one colour.-< 35 mm-vol 7 dichotomous key --
56	tetrathemis.polleni	m4-18/09/2023-veg	odonata-libellulidae-n/a-a	predator-68-labial palp has distinct teeth on inner margins. very small lateral spines on segments 8 and 9. ->18mm -vol 7 dichotomous key. pg 190 --
57	platycypha.a	m1-14/9/2023-veg	odonata-chlorocyphidae-n/a-a	predator-78-two serrated caudal lamellae-< 30 mm -vol 7 dichotomous key --
58	pseudagrion.a	m4-03/04/2024-veg	odonata-coenagrionidae-n/a-a	predator-80/81-slender body. three caudal filaments (3 spotted pointed tails) and vary in shape and size. light brown and yellow colouring with patterns. antennae same length-< 35 mm-vol 7 dichotomous key. pg 178--
59	chlorolestes.faciatus	m1-14/9/2023-veg	odonata-synlestidae-n/a-a	predator-88-rounded caudal lamellae with no tip/point-< 45 mm -vol 7 dichotomous key . pg 177--
60	ptilodactylidae.a	m1-14/9/2023-stones	coleoptera-ptilodactylidae-n/a-a	detritivorous-96-toe-winged beetle. resembles elimidae larva but has long setal filaments. has tracheal

				gills at end of abdomen. -mature larvae 4- 15 mm ---
61	hydaticus.a	sp-09/11/2023-veg	coleoptera-dytiscidae-n/a-a	predator-97-has yellow and black longitudinal patterns on back wings -adults 1 - 45 mm ---
62	rhantus.a	sp-09/11/2023-veg	coleoptera-dytiscidae-n/a-a	predator-97-light brown yellowish section on meta thorax.-adults 1 - 45 mm ---
63	rhantus.b	sp-09/11/2023-veg	coleoptera-dytiscidae-n/a-b	predator-97-brown textured colour all over body-adults 1 - 45 mm ---
64	elmidae.a	m1-14/9/2023-gsm	coleoptera-elmidae-n/a-a	scraper-99/100-larva. larger head than body. tapering posteriorly. anal gills present. -1 - 8 mm ---
65	elmidae.b	h6-23/03/2024-stones	coleoptera-elmidae-n/a-b	scraper-99/100-larva. head and body same width however still tapering posteriorly. anal gills present -1 - 8 mm---
66	elpidelmis.c	m5-25/9/2023-stones	coleoptera-elmidae-n/a-c	shredder-99/100- smooth texture on exoskeleton. round pronotum and long antennae-adults 1 - 8 mm---
67	elpidelmis.d	m5-25/9/2023-stones	coleoptera-elmidae-n/a-d	shredder-99/100- two indentation on pronotum and dimples along abdomen.-adults 1 - 8 mm---
68	aulonogyrus.a	m1-14/9/2023-veg	coleoptera-gyrinidae-n/a-a	predator-101-has yellow rims along the outer edges of the wing covers. -4 - 17 mm ---
69	gyrinidae.a	m5-25/09/2023-stones	coleoptera-gyrinidae-n/a-a	predator-101-larvae are segmented. anal hooks present -4 - 17 mm ---
70	helophorus.a	sp-25/4/2024-stones	coleoptera-helophoridae-n/a-a	phytophagous/saprophagous-103-longitudinal grooves on middle thorax as well as dimples along wing covers. -3.5 mm ---
71	hydraenidae.a	m5-25/9/2023-stones	coleoptera-hydraenidae-n/a-a	scraper-105-larvae has urogomphi present (3 anal spikelets). 10 visible abdominal segments.-0.5 - 3 mm ---
72	hydraenidae.b	m5-25/09/2023-stones	coleoptera-hydraenidae-n/a-b	scraper-105-identified by abdomen - 6/7 sternites and presence of an intercostal sternite. antennae are clubbed with 5 segments. dark brown/black with dimples along abdomen.-0.5 - 3 mm ---
73	pterosthetops.brincki	m5-25/9/2024-stones	coleoptera-hydraenidae-n/a-c	scraper-105-6 - 7 sternites and presence of intercostal sternite. antennae are clubbed with 5 segments. yellow and brownish colour with dimples on exoskeleton.-0.5 - 3 mm -- dichotomous key 10, pg 125-
74	anacaena.b	sp-25/4/2024-veg	coleoptera-hydrophilidae-n/a-b	predators -106-larva. light brown segmented body. 6 legs. well-developed mouth parts. -1 - 50 mm ---

75	limnichidae.a	m1-14/9/2023-stones	coleoptera-limnichidae-n/a-b	collector/gatherer-109-undulating junction between pronotum and elytra. exoskeleton is very textured and rough-adults 1 - 3 mm ---
76	afrobrianax.ferdyi	m1-14/9/2023-stones	coleoptera-psephenidae-n/a-a	scraper-111-flattened oval bodies. yellowish-brown or grey (early instar). the margins of body are fringed with setae. legs and head are hidden under body.gills are on abdominal sternites and are under body. -4 - 17 mm ---
77	scirtidae.a	m1-14/9/2023-gsm	coleoptera-scirtidae-n/a-a	phytophagous/detritivorous-112-well developed legs, antennae, and flattened body. body tapers posteriorly. long segmented antenna -larvae 2 - 8 mm ---
78	suragina.a	m1-14/9/2023-stones	diptera-anthericidae-n/a-a	predator-120- two posterior lobes with fringed fine hairs. segmented body has ringed welts. tapering anteriorly. 7 pairs of prologs, 8th pair has a single proleg. -10 - 18 mm -vol 9 dichotomous key --
79	ceratopogoninae.a	m1-14/9/2023-stones	diptera-ceratopogonidae-ceratopogoninae-a	predator-122-Larva. sclerotised head with thin needle-like body. prolegs absent -2 - 15 mm -vol 9 dichotomous key --
80	ceratopogoninae.b	m4-03/04/2024-veg	diptera-ceratopogonidae-ceratopogoninae-b	predator-122-pupa. abdominal spikes present. two distinctive antennae. two anal spikes present.-2 - 15 mm -vol 9 dichotomous key --
81	tanypodinae.a	m1-14/9/2023-stones	diptera-chironomidae-tanypodinae-a	predator-124-sclerotised head and prolegs as long as head. procercal anal setae are pointed backwards. large anal claws. body segments t2 and t3 can be enlarged. Absent ventral tubules -2 - 20 mm -vol 9 dichotomous key page 115--
82	chironominae.a	sp-25/04/2024-veg	diptera-chironomidae-chironominae-a	collector/gatherer/scraper-124-smaller sclerotised head and short prolegs. ventral tubules present. small anal tubules. lateral tubules present. -2 - 20 mm -vol 9 dichotomous key page 115--
83	orthocladiinae.a	sp-25/04/2024-n/a	diptera-chironomidae-orthocladiinae-a	collector/gatherer/scraper-124-small, sclerotized head capsule. Absent ventral and anal tubules. absent procercal setae-2 - 20 mm-vol 9 dichotomous key page 115--
84	chironominae.b	sp - 25/4/2024-veg	diptera-chironomidae-chironominae-b	collector/gatherer/scraper-124-pupae has fluffy feature on head and many setae on the anal feature.-2 -

				20 mm -vol 9 dichotomous key page 139--
85	tanypodinae.b	m5-25/9/2023-gsm	diptera-chironomidae-tanypodinae-b	predator-124-pupae has a single feather structure on head and very sclerotised anal features -2 - 20 mm -vol 9 dichotomous key page 139--
86	orthocladiinae.b	sp-25/04/2024-veg	diptera-chironomidae-orthocladiinae-b	collector/gatherer/scraper-124-pupae no noticeable head feature and posteriorly has few setae-2 - 20 mm -vol 9 dichotomous key page 139--
87	culicine.a	sp-25/04/2024-veg	diptera-culicidae-culicine-a	filtering collector/scraper/predator-125-elongated siphon and antenna with short setae-4 - 18 mm -vol 9 dichotomous key --
88	anopheline.a	m4-03/04/2024-veg	diptera-culicidae-anopheline-a	filtering collector/scraper/predator-126-short siphon and small hairs protruding from abdominal segments -4 - 18 mm -vol 9 dichotomous key --
89	culicine.b	sp-25/4/2024-veg	diptera-culicidae-culicine-b	filtering collector/scraper/predator-126-pupal respiratory organ situated on head. larger head to body ratio. -4 - 18 mm -vol 9 dichotomous key --
90	hemerodroma.b	h2-11/10/2023-veg	diptera-empididae-n/a-a	predator-129-pupae have long extensions from abdomen. light brown, yellow and orange in colour. -2 - 20 mm -vol 9 dichotomous key --
91	ochthera.a	m1-14/9/2023-stones	diptera-ephydridae-n/a-a	collector/gatherer/shredder/predator-130/131-larva. tail tapering, divided into two long breathing tubes. dark brown-2 - 20 mm -vol 9 dichotomous key --
92	brachydeutera.a	m1-14/9/2023-veg	diptera-ephydridae-n/a-a	collector/gatherer/shredder/predator-130/131-larva. tail tapering, divided into two short breathing tubes. Light brown/yellow-2 - 20 mm -vol 9 dichotomous key --
93	brachydeutera.b	h6-23/03/2024-gsm	diptera-ephydridae-n/a-b	collector/gatherer/shredder/predator-130/131-pupa. two breathing tubes with fluffy features at end. very ovoid body with dark colouration. -2 - 20 mm -vol 9 dichotomous key --
94	brachydeutera.c	h6-23/03/2024-gsm	diptera-ephydridae-n/a-c	collector/gatherer/shredder/predator-130/131-pupa. two breathing tubes with yellow and brown colouration, small setae protruding from appendage. yellowish spots along abdomen. two hooks present at posterior end. -2 - 20 mm -vol 9 dichotomous key --

95	brachydeutera.d	sp-25/4/2024-veg	diptera-ephydridae-n/a-d	collector/gatherer/shredder/predator-130/131-pupa. brown reddish colour. two breathing tubes present and largely segmented casing, with protruding points at each segment. ----
96	limnophora.a	m5-25/09/2023-stones	diptera-muscidae-limnophorinae-a	predator-132-maggot-like, cylindrical and appears white to yellowish. body tapered anteriorly with retractable mouth hooks. posterior abdomen ends with respiratory siphons. two pairs of lobes (4).-5 - 20 mm -vol 9 dichotomous key --
97	limnophora.b	m5-25/09/2023-stones	diptera-muscidae-limnophorinae-b	predator-132-pupae are ovoid with four lobe structures .-5 - 20 mm-vol 9 dichotomous key --
98	psychoda.a	sp-25/04/2024-veg	diptera-psychodidae-n/a-a	collector/gatherer-133-no sclerosed plates on body, only head and posterior area are sclerosed. -1 - 4 mm -vol 9 dichotomous key --
99	psychoda.b	sp-25/4/2024-veg	diptera-psychodidae-n/a-b	collector/gatherer-133-pupae. no sclerotised plates on pupae. antennae are robust.-1 - 4 mm-vol 9 dichotomous key --
100	clogmia.a	sp-09/11/2023-gsm	diptera-psychodidae-n/a-a	collector/gatherer-133-sclerosed plates on each segment of body. also has short hairs protruding from body segments-1 - 4 mm -vol 9 dichotomous key --
101	clogmia.b	h6-23/03/2024-veg	diptera-psychodidae-n/a-b	collector/gatherer-133-segmented sclerotised body with large head and two small antennae on head -1 - 4 mm -vol 9 dichotomous key --
102	paracnephia.a	h6-23/03/2024-veg	diptera-simuliidae-n/a-a	filterer-135-very defined proleg on prothorax. sclerotised head. swollen posterior abdomen-< 7 mm -vol 9 dichotomous key --
103	simulium.a	m3-20/9/2024-veg	diptera-simuliidae-n/a-a	filterer-135-sclerotised head with a pair of cephalic fans. small prolegs present on thorax. swollen posterior abdomen -< 7 mm -vol 9 dichotomous key --
104	simulium.adersi	m4-18/09/2023-stones	diptera-simuliidae-n/a-b	filterer-135-silk casing/cocoon with pupae. visible gills -< 7 mm -vol 9 dichotomous key --
105	simulium.b	h6-23/03/2024-gsm	diptera-simuliidae-n/a-b	filterer-135-torso is shorter than other morpho species A. casing is larger and yellow in colour.-< 7 mm -vol 9 dichotomous key --
106	simulium.c	h6-23/03/2024-gsm	diptera-simuliidae-n/a-c	filterer-135-no casing around pupae. anterior gills present on head. -< 7 mm -vol 9 dichotomous key --

107	tabanus.a	m1-14/9/2023-stones	diptera-tabanidae-n/a-a	predator-138-abdomen ringed with welts. brown colouration. body tapers anteriorly and posteriorly.-10 - 50 mm -vol 9 dichotomous key --
108	chrysops.a	m1-14/9/2023-stones	diptera-tabanidae-n/a-a	predator-138-abdomen ringed by welts. no distinct patterns or colour, pale yellow/white all over.-10 - 50 mm-vol 9 dichotomous key --
109	tipula.a	m4-18/09/2023-veg	diptera-tipulidae-n/a-a	collector/shredder/predator-139-missing feature of the retractable head capsule, terminal lobes and setae (specimen was damaged). ventral welts present. terminal segments pose two spiracles. -1.5 - 60 mm -vol 9 dichotomous key --
110	antocha.a	m1-27/03/2024-veg	diptera-limoniidae-n/a-a	collector/shredder/predator-141-round retractable head capsule. tapering posteriorly -1.5 - 60 mm - dichotomous key vol. 9-page 172 fig. 7.2 G--
111	agapetus.a	h1-14/04/2024-stones	trichoptera-glossosomatidae-n/a-a	scraper/grazer-150-larvae construct cases from sand granules. head and pronotum are sclerotised.-cased caddis flies-3 - 4 mm -ref dichotomous key volume 8, page 93 table 5.2 -
112	hydroptila.a	m1-14/9/2023-stones	trichoptera-hydroptilidae-n/a-a	collector/scraper/grazer/piercers/parasite-151/152-silk case. flattened purse-shaped - made with filamentous algae-cased caddis flies-2 - 6 mm -ref dichotomous key volume 8, page 93 table 5.3-
113	hydroptila.b	m1-14/9/2023-stones	trichoptera-hydroptilidae-n/a-b	collector/scraper/grazer/piercer/parasite-151/152-silk case. flattened purse-shaped - sandy cover-cased caddis flies-2 - 6 mm -ref dichotomous key volume 8, page 93 table 5.4-
114	catoxythira.a	m1-14/9/2023-veg	trichoptera-hydroptilidae-n/a-a	collector/scraper/grazer/piercer/parasite-151/152-silk case. flattened purse-shaped - long strand fluffy algae cover-cased caddis flies-2 - 6 mm -ref dichotomous key volume 8, page 98 fig.5.6 D-
115	setodes.a	m1-14/9/2023-stones	trichoptera-leptoceridae-n/a-a	shredder/collector/scraper/grazer/predator-155/156-cylindrical tapering tusk - shaped of coarse grains - cased caddis flies-2 - 6 mm -pg 155, vol 8 dichotomous key. fig 5.43-
116	triaenodes.a	m1-14/9/2023-veg	trichoptera-leptoceridae-n/a-a	shredder/collector/scraper/grazer/predator-155/156-larval case cut and spirally-arranged pieces of grass or sedge stems. -cased caddis flies-5 -

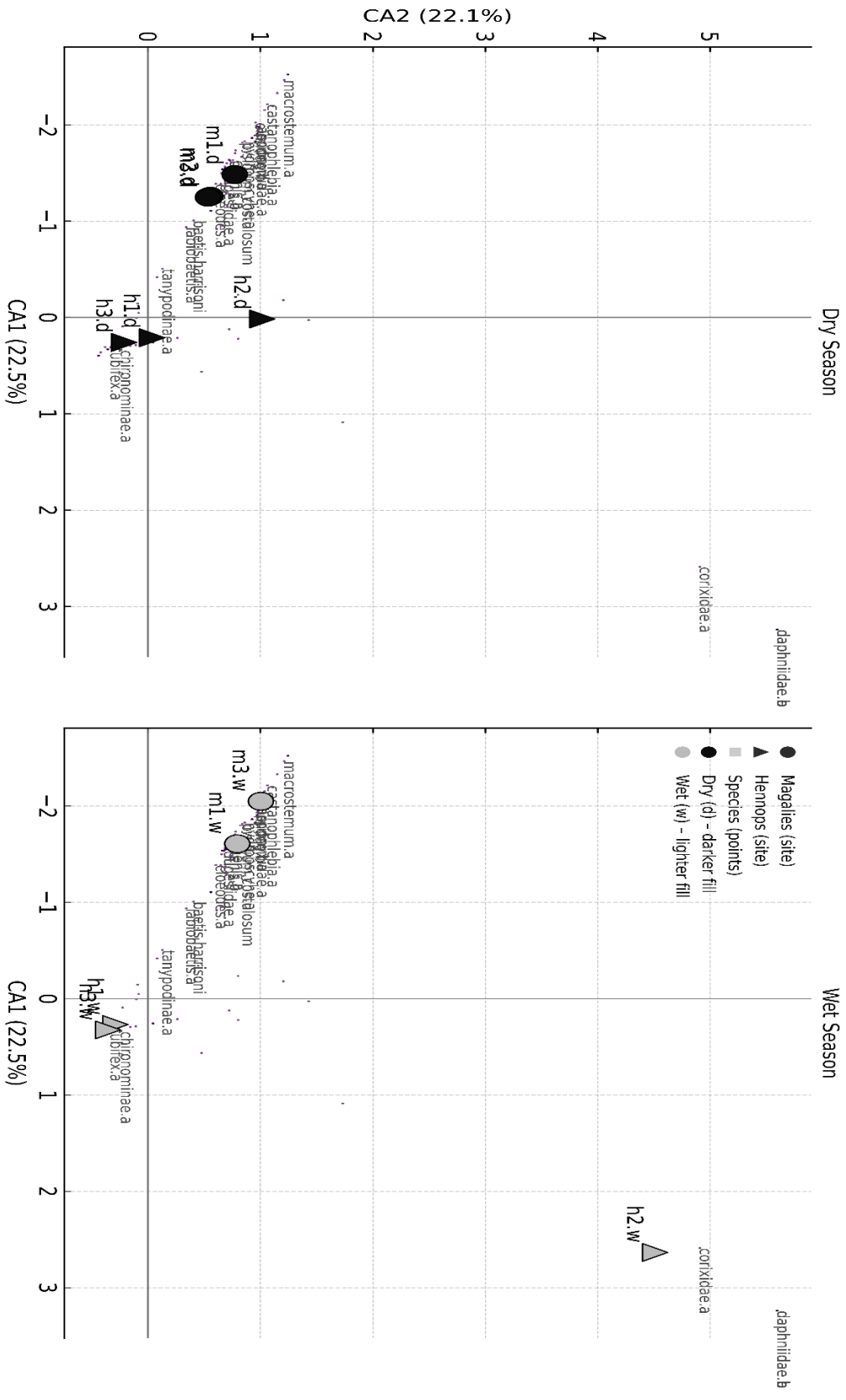
				20 mm-pg 156, vol 8 dichotomous key. Fig 5.45-
117	amphipsyche.a	m1-14/9/2023-stones	trichoptera-hydropsychidae-n/a-a	collector/predator-163/164-large sclerosed head, with two white spots. prominent paired tufted abdominal gills and two anal prolegs with dense tufted setae-caseless -< 42 mm --
118	cheumatopsyche.a	m1-14/9/2023-stones	trichoptera-hydropsychidae-n/a-a	collector/predator-163/164-green and dark brown segmented body. prominent paired tufted abdominal gills and two anal prolegs with dense tufted setae-caseless -< 42 mm --
119	macrostemum.a	m1-14/9/2023-stones	trichoptera-hydropsychidae-n/a-a	collector/predator-163/164-large, segmented body. body is lighter in colour. eyes wider apart than other specimen .prominent paired tufted abdominal gills and two anal prolegs with dense tufted setae-caseless -< 42 mm --
120	sciadorus.a	h1-14/4/2024-stones	trichoptera-hydropsychidae-n/a-a	collector/predator-163/164-very dark brown/black body. prominent paired tufted abdominal gills and two anal prolegs with dense tufted setae-caseless -< 42 mm --
121	hydropscyhe.a	m5-25/09/2023-stones	trichoptera-hydropsychidae-n/a-a	collector/predator-163/164-flat face compared to other specimen in family. prominent paired tuffed abdominal gills and two anal prolegs with dense tuffed setae-caseless -< 42 mm --
122	hydropsychidae.b	m1-27/03/2024-stones	trichoptera-hydropsychidae-n/a-b	collector/predator-163/164-pupae are encased in small silk-lined chamber made of gravel and sand. has wings present -caseless -< 42 mm --
123	leptonema.a	m5-18/03/2024-stones	trichoptera-hydropsychidae-n/a-a	collector/predator-163/164-pro/meso and meta-thorax sclerotised. branched abdominal gills. no hairs/setae on anal hooks/prolegs -caseless -< 42 mm --
124	chimara.a	m1-14/9/2023-stones	trichoptera-philopotamidae-n/a-a	collector/predator-165-commonly orange in colour. no abdominal gills. well-developed prolegs and terminal anal claws. very flat elongated head-caseless -8 15 mm - caseless -
125	appasus.a	m2-20/9/2023-veg	hemiptera-belostomatidae-n/a-a	predator-177-two flattened posterior respiratory siphons. males can be seen carrying eggs on back.-10 - 80 mm---

126	corixidae.a	m4-03/04/2024-stones	hemiptera-corixidae-n/a-a	predator/herbivore-178-early instar. three distinctive dots in centre of abdomen. two anal setae-<10 mm ---
127	sigara.a	sp-09/11/2023-gsm	hemiptera-corixidae-n/a-a	predator/herbivore-178-adult. fore tarsus with dense setae. mottled or striated forewings-20 mm ---
128	micronecta.a	m2-20/9/2023-n/a	hemiptera-micronectidae-n/a-a	predator/herbivore-178-has an exposed scutellum. rounder and more ovate body compared to sigara species. -20 mm ---
129	eurymetra.a	m1-14/9/2023-veg	hemiptera-gerridae-n/a-a	predator-180-early instar of eurymetra natalensis-< 20 mm ---
130	eurymetra.natalensis	m1-14/9/2023-veg	hemiptera-gerridae-n/a-b	predator-180-adult. yellow and black markings on body-< 20 mm ---
131	mesovelia.a	m1-27/03/2024-veg	hemiptera-mesoveliidae-n/a-a	predator-182-pronotum short, long legs with spines present on mid and hind legs. -2 - 3 mm---
132	enithares.a	m2-20/9/2023-veg	hemiptera-notonectidae-n/a-a	predator-186-oar-like hind legs. -4 - 12 mm ---
133	paraplea.a	m2-20/9/2023-n/a	hemiptera-pleidae-n/a-a	predator-187-body convex and punctate-1.5 - 2 mm ---
134	rhagovelia.a	m5-18/03/2024-veg	hemiptera-veliidae-n/a-a	predator/scavenger-188-hind leg femur short and does not extend - can't extend past abdomen. large eyes. yellow colouration on body part closer to body. -2 - 9 mm---
135	neoperla.spio	m1-14/9/2023-stones	plecoptera-perlidae-n/a-a	predator-192/193-yellow and brown. two sets of fluffy gills on thoracic region. swimming hairs on legs. 1 pair of long ceri on posterior. -< 20 mm ---
136	daphniidae.a	h4-22/10/2023-gsm	pancrustacea-brachiopoda-cladocera-a	filterer/collector-200-chitinous exoskeleton. leaf like limbs - which are used for locomotion and respiration. two pairs of bigamous antennae. -1 mm ---
137	daphniidae.b	h1-14/04/2024-stones	pancrustacea-brachiopoda-cladocera-b	filterer/collector-200-chitinous exoskeleton. leaf like limbs - which are used for locomotion and respiration. two pairs of bigamous antennae. -1 mm---
138	daphniidae.c	h1-14/04/2024-stones	pancrustacea-brachiopoda-cladocera-c	filterer/collector-200-chitinous exoskeleton. leaf like limbs - which are used for locomotion and respiration. two pairs of bigamous antennae. -1 mm---
139	diaptomidae.a	m1-14/9/2023-n/a	pancrustacea-copepoda-calanoida-a	filterer/collector-201-cylindrical shape. well-developed antennae. single cephalic eye and abdomen

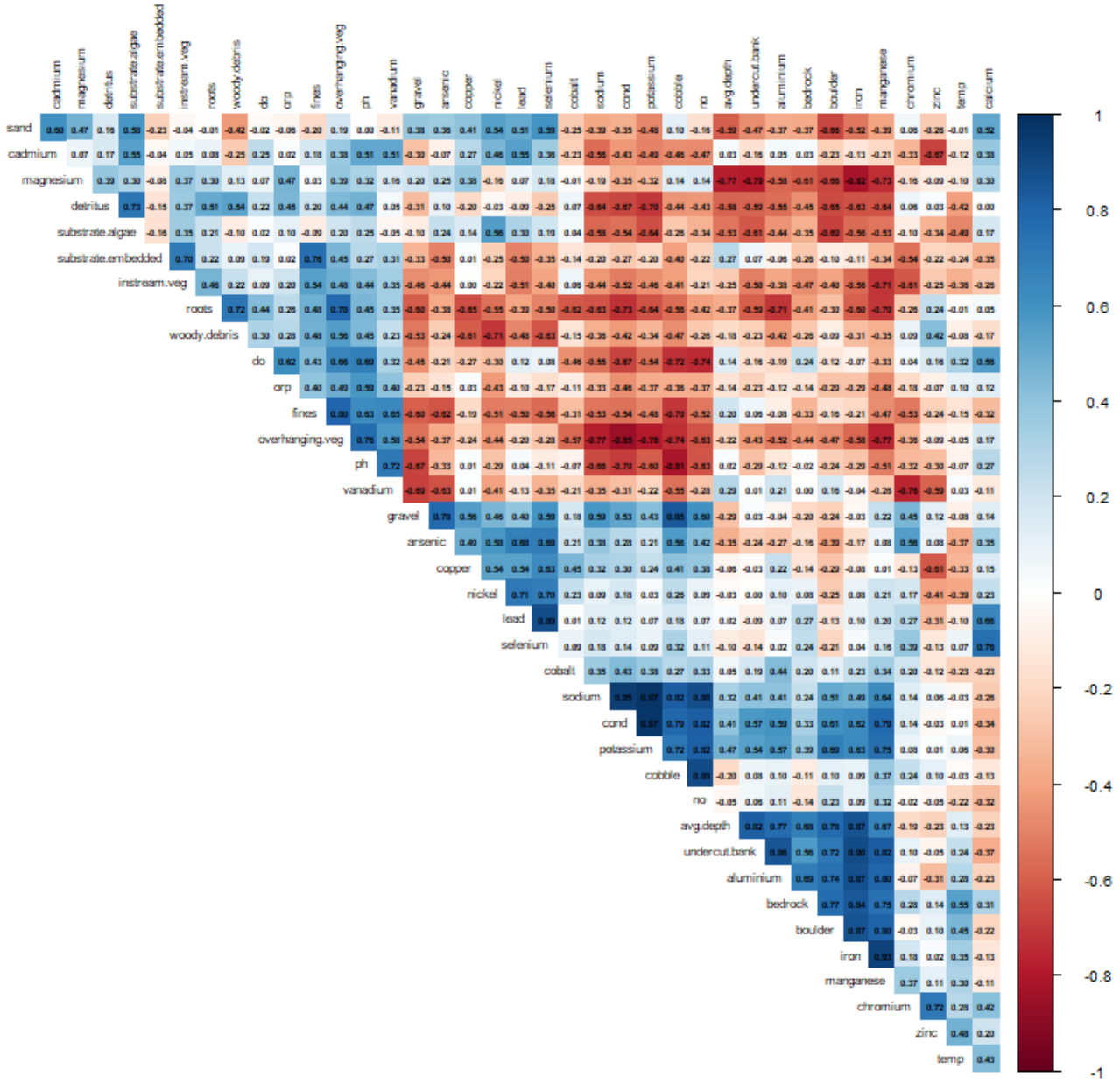
				has five segments. caudal setae. females often seen with egg sacks.-2 mm---
140	cyclopoida.a	m1-14/9/2023-n/a	pancrustacea-copepoda-cyclopoida-a	filterer/collector-201-cylindrical shape. well-developed antennae. single cephalic eye and abdomen has five segments. females often seen with egg sacks.-1 mm---
141	ostracoda.a	m1-14/9/2023-gsm	pancrustacea-oligostraca-ostracoda-a	filterer/collector-202-lateral bivalve carapace which encloses the entire body. Antennae and setae often protrude-1 - 2 mm ---
142	potamonautes.sidneyi	m1-14/9/2023-stones	pancrustacea-potamonautidae-n/a-a	collector/gatherer-204-carapace shape and width, slim pereopods, inflated propodi of the chelipeds, and the shape and terminal segment length-> 3 cm. ---
143	specimen.x	m1-27/03/2024-gsm	x-x-x-x	x-x-aquatic pupa that has lost it casing. has some sclerotised segments on posterior dorsal abdomen. -3 mm ---

Appendix 3.B: A table showing the Eco-Status ratings generated from SASS procedure.

SITE	ECO.STATUS
M1.D	A
M1.W	A
M2.D	E/D
M2.W	C
M3.D	C
M3.W	B
H1.D	E
H1.W	E
H2.D	F
H2.W	E
H3.D	E
H3.W	E/D

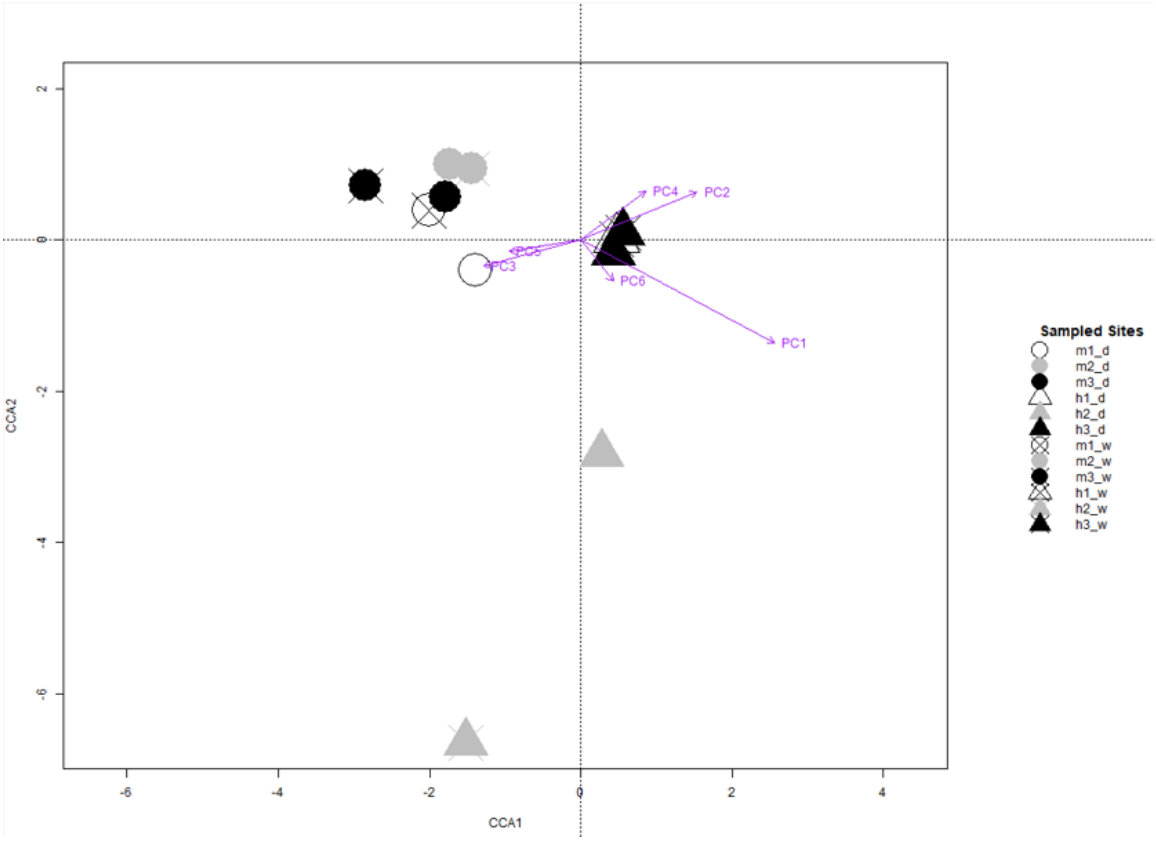


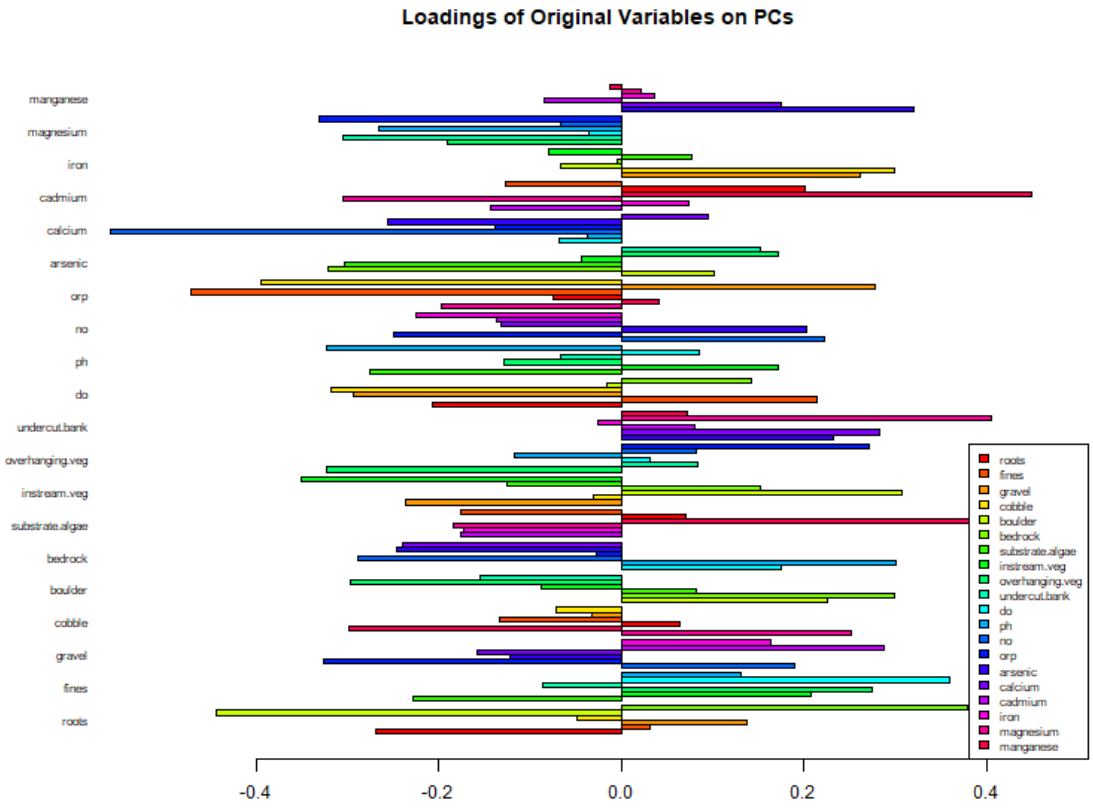
Appendix 3.C : A Correspondence Analysis of the top 20 present macroinvertebrate species along the Hennops and Magalies rivers, across both wet and dry seasons.



Appendix 3.D :Correlation matrix of water quality parameters and habitat variables

Habitat autocorrelated variables: (1) Overhanging vegetation: Cd, ORP, and pH. (2) Cobble (3) Fines: Cd, ORP, and pH. (4) Boulder: Bedrock. (5) Bedrock: Boulder, As, ORP, and pH. (6) Undercut-bank: Discharge. (7) Discharge: Undercut-bank, Fe, Mn, As, ORP, and pH. Water autocorrelated Variables: (1) Magnesium (2) Nitrogen (3) Sodium: Conductivity and Temperature. (4) Conductivity: Sodium and Temperature. (5) Arsenic: Discharge and Bedrock. (6) Manganese: Iron and Discharge. (7) Iron: Manganese and Discharge. (8) Cadmium: ORP, Fines, and Overhanging vegetation. (9) ORP: Cadmium, pH, Discharge, Bedrock, Fines, and Overhanging vegetation (10) pH: ORP, Discharge, Bedrock, Fines, and Overhanging vegetation. (11) Temperature: Conductivity and Sodium. (12) COD (13) DO.





Appendix 3.E: CCA and PCR loadings of water quality parameters and habitat variables

Chapter 4:

Appendix 4.A: A table with sampled rivers and their Facilities for WUL (DWS, 2023c)

River	Facility name / location	Likely water-use type
Hennops River	City of Ekurhuleni – Olifantsfontein WWTP / Ivory Park area	Section 21(f): discharge of treated effluent into river
Hennops River	City of Tshwane – Sunderland Ridge WWTP (Tshwane)	Section 21(f) discharge; possible stream-flow reduction activities
Magalies River	Magalies Water – Bulk water/irrigation abstractor	Section 21 (f) discharge, (i) altering riverbed/flow abstraction
Magalies River	Percy Jackson WWTW	Section 21(f) discharge; possible stream-flow reduction activities

Appendix 4.B: A table showing the average wet season measurement for various parameters based on legal limits set in the TQWRs and ROQs (DWAF, 1996; DWS, 2019; WRC, 2024).

ROQs for Magalies River (A21D–A21E, Resource Unit (RU) 2_2) and for Upper Hennops & Rietvlei Rivers (A21A, RU 1_1) (upper) and Downstream of Rietvlei Dam (A21B) (lower reaches).

Parameter	Sampled River	SAWQG: AE (TWQR) for Aquatic Ecosystems (mg/L) -	Gazetted Legal Limit – RQO 2019 (mg/L)	Average Wet Season Measurement (mg/L)
Ecological Category	Magalies	A – D	B (Upper) → C (Lower)	A (Upper) → C/B (Lower)
	Hennops	A – D	D	E (Upper) - E/F (Lower)
Habitat Integrity	Magalies	N/A	≥ C	A-B (Upper); C-D (Lower)
	Hennops	High ≥80% (A/B); Moderate ≥62% (C); Degraded ≤42% (D)	≥ C (Upper); ≥ D (Lower)	≥ E (Upper); ≥ E/F (Lower)

Macroinvertebrates (SASS/ASPT)	Magalies	N/A	SASS ≥ 60; ASPT ≥ 4.0	SASS - 132; ASPT - 5.71
	Hennops	Good: SASS ≥ 80 & ASPT ≥ 5; Fair: SASS ≥ 60 & ASPT ≥ 4	SASS ≥ 80, ASPT ≥ 4.8 (upper); SASS ≥ 55, ASPT ≥ 4.2 (lower)	SASS - 17; ASPT - 3.4 (upper) SASS - 19, ASPT - 3.1 (lower)
Flow targets ($m^3 s^{-1}$)	Magalies	N/A	Seasonal maintenance per RU 2_2	0.018194747
	Hennops	N/A	(Oct 0.041/0.007 (drought) -Feb 0.100/0.015 - Sep 0.048/0.007)(upper); Seasonal flow maintenance per RU 1_1 (lower)	0.1 (upper); 0.7 - 1.2(lower)
pH	Magalies	6.5 – 9.0	6.5 – 8.5	8.29 - 8.75
	Hennops	6.5 – 9.0	6.5 – 9.0 (upper); 7.5 – 9.2 (lower)	8.05 (upper); 7.83 - 8.55 (lower)
COD (mg L^{-1})	Magalies	N/A	N/A	6
	Hennops	N/A	N/A	21 (upper) - 111 (lower)
DO (%)	Magalies	80-120	66.1	80.7 - 88.3
	Hennops	80-121	66 - 79 (upper); ≥ 66 (lower)	59.9 (upper); 61 - 87.4(lower)
EC (µS/cm)	Magalies	≤ 700 (Ideal); ≤ 1500 (Tolerable)	450	263.66
	Hennops	≤ 700 (Ideal); ≤ 1500 (Tolerable)	850	462.8 (upper); 1052 - 685 (lower)
Orthophosphate(as PO4-) (mg L^{-1})	Magalies	≤ 0.025	≤ 0.125	0.1
	Hennops	≤ 0.025	≤ 0.125	0.1 (upper); 0.7 - 1.2(lower)
NH3 + NH4 (mg L^{-1})	Magalies	≤ 0.2 + ≤ 0.3	≤ 0.0725	0.1
	Hennops	≤ 0.2 + ≤ 0.3	≤ 0.0725	1.8 (upper); 7.9 - 9.2 (lower)
NO3 + NO4 (mg L^{-1})	Magalies	≤ 2.5	≤ 1.0 (50th)	1.68
	Hennops	≤ 2.5	≤ 3.0 (50th)	1.85
Al (mg L^{-1})	Magalies	≤ 0.005	≤ 0.1	0.42
	Hennops	≤ 0.005	≤ 0.15	0.64
As (mg L^{-1})	Magalies	≤ 0.01	0.13	0.15
	Hennops	≤ 0.01	N/A	0.18
Ca (mg L^{-1})	Magalies	N/A	N/A	13.39
	Hennops	N/A	N/A	13.56
Cd (mg L^{-1})	Magalies	≤ 0.00015	N/A	0.06
	Hennops	≤ 0.00015	N/A	0.06
Co (mg L^{-1})	Magalies	≤ 0.0003	N/A	0.02
	Hennops	≤ 0.0003	N/A	0.03
Cr (mg L^{-1})	Magalies	≤ 0.007	N/A	0.15
	Hennops	≤ 0.007	N/A	0.15
Cu (mg L^{-1})	Magalies	≤ 0.0003	≤ 0.0075	0.17
	Hennops	≤ 0.0003	≤ 0.0075	0.18

Fe (mg L^{-1})	Magalies	N/A	≤ 0.3	0.41
	Hennops	N/A	≤ 0.1	0.76
K (mg L^{-1})	Magalies	N/A	N/A	2.89
	Hennops	N/A	N/A	2.12
Mg (mg L^{-1})	Magalies	N/A	N/A	11.75
	Hennops	N/A	N/A	8.34
Mn (mg L^{-1})	Magalies	≤ 0.18	0.15	0.09
	Hennops	≤ 0.18	0.15	0.21
Na (mg L^{-1})	Magalies	N/A	70	26.74
	Hennops	N/A	70	13.29
Ni (mg L^{-1})	Magalies	N/A	≤ 0.07	0.09
	Hennops	N/A	≤ 0.07	0.13
Pb (mg L^{-1})	Magalies	≤ 0.0002	≤ 0.0095	0.41
	Hennops	≤ 0.0002	≤ 0.007	0.45
Se (mg L^{-1})	Magalies	0.002	N/A	0.31
	Hennops	0.002	N/A	0.37
V (mg L^{-1})	Magalies	N/A	N/A	0.13
	Hennops	N/A	N/A	0.14
Zn (mg L^{-1})	Magalies	0.002	≤ 0.002	0.48
	Hennops	0.002	N/A	0.5