



Environmental flow and habitat requirements of aquatic macroinvertebrates from selected rivers in the Limpopo River Basin

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ABSTRACT

Managing rivers sustainably requires a thorough understanding of the response of aquatic biota to riverine microhabitat variability. The Limpopo River Catchment (LRC) is under extreme pressure from an increase in anthropogenic activities and demands. These pressures have a significant impact on the aquatic biodiversity within the riverine ecosystems within the LRC and is therefore very important to understand the responses of aquatic organisms for conservational and management purposes. Aquatic macroinvertebrates are often used in biomonitoring of rivers and was assessed during this study to evaluate the responses towards environmental flows of main stream rivers and tributaries in the LRC. The main aims were to assess and determine the drivers of macroinvertebrate community structures and preferences towards velocity and depth. Different approaches, taxonomical and functional traits, were used to provide results which will reflect the aims of this study. Sixteen sites in the LRC were sampled during 2021, that included both main stream rivers and tributaries, and two sites in the Olifants River were sampled during 2020 and 2021. Habitats at each site were measured and described in terms of depth, velocity, hydraulic biotope, and substrates. Water quality measurements were taken *in-situ* and samples taken in triplicate for nutrient analysis in the laboratory. Aquatic macroinvertebrates were sampled via a surber-sampler and in duplicate within each biotope present for quantitative data collection purposes. Data analysis consisted of diversity indices, multivariate analysis (RCA and RDA) and habitat suitability curves (HSCs). Taxonomical and functional traits revealed differences in spatial variations towards water quality and habitat preferences although slight variances were shown of the responses towards velocity and depth alterations. The substratum type was uncovered to be the main driver of the macroinvertebrate community structures within the LRC. The Olifants River study revealed that overall macroinvertebrates preferred shallow, slow flowing waters and the stones in current (SIC) biotope. *Tricorythus* sp.(mayfly) was identified as an indicator taxon for faster flowing waters. Further studies should be conducted on the LRC in consecutive years to provide accurate indicator taxa for the main stream rivers and tributaries. Taxonomic and functional approaches complement one another and is used together to provide more detail.

KEYWORDS:

Environmental flow, freshwater rivers, velocity, depth, substrate type, aquatic macroinvertebrates, indicator taxa, functional traits, habitat preference, habitat suitability curves.

Table of contents

ACKNOWLEDGEMENTS	I
ABSTRACT	III
CHAPTER 1 INTRODUCTION.....	1
1.1 Background and Introduction.....	1
1.1.1 Water in South Africa.....	1
1.1.2 Freshwater River ecosystems.....	1
1.1.3 Environmental flows.....	2
1.1.4 Habitat preference and alteration.....	2
1.1.5 Limpopo River Catchment	3
1.1.6 Kruger National Park River systems	3
1.1.7 Bioindicators.....	5
1.1.8 Aquatic Macroinvertebrates	6
1.2 Aims and Objectives	7
1.3 Chapter Breakdown:.....	7
CHAPTER 2	9
MACROINVERTEBRATE COMMUNITY STRUCTURE IN THE LIMPOPO SYSTEM IN RELATION TO HABITAT AND WATER QUALITY DRIVERS.....	9
2.1 Introduction	9
2.2 Methods.....	11
2.2.1 Description of Study Area	11
2.2.2 Field Surveys.....	11

2.2.3	Laboratory Analysis	18
2.2.4	Data Analyses	18
2.3	Results	19
2.3.1	Abiotic analyses.....	19
2.3.2	Diversity results	21
2.3.3	Macroinvertebrate community structures	22
2.3.4	Trait analysis	26
2.4	DISCUSSION	33
2.4.1	Water and sediment quality	34
2.4.2	Macroinvertebrate community structures between biotopes.....	34
2.4.3	Macroinvertebrate responses towards sediment and water quality	36
2.4.4	Functional trait responses towards sediment and water quality	37
2.5	CONCLUSION	37

CHAPTER 3 FLOW AND HABITAT PREFERENCES OF MACROINVERTEBRATE INDICATORS (TAXONOMIC AND TRAITS) WITHIN THE OLIFANTS RIVER, SOUTH AFRICA		39
3.1	Introduction	39
3.2	Methods.....	42
3.2.1	Description of Study Area	42
3.2.2	Field Surveys.....	43
3.2.3	Laboratory Analysis	45
3.2.4	Data Analysis.....	45
3.3	Results	46

3.3.1	Flow and habitat preferences.....	46
3.4	DISCUSSION	62
3.4.1	Flow and Habitat preference	62
3.4.2	Taxonomical:	62
3.4.3	Habitat suitability curves:	63
3.4.4	Functional traits	64
3.5	CONCLUSION	65
CHAPTER 4 GENERAL CONCLUSIONS AND RECOMMENDATIONS		66
4.1	CONCLUSIONS	66
4.2	RECOMMENDATIONS	67
BIBLIOGRAPHY		69
ANNEXURES.....		95
APPENDIX A: MACROINVERTEBRATE DIVERSITY		95
APPENDIX B: WATER QUALITY AND SEDIMENT PARTICLE SIZE		103
APPENDIX C: VELOCITY AND DEPTH.....		104
APPENDIX D: FUNCTIONAL TRAITS		105

List of Tables

<i>Table 2-1: Locality details and general comments of biomonitoring sites on the Limpopo River and some of its major tributaries and the Olifants River within the KNP.</i>	<i>13</i>
<i>Table 3-1: Location details and codes used for Mamba and Balule sites sampled in 2020 and 2021.</i>	<i>43</i>
<i>Table 3-2: Velocity, depth, substrate, hydraulic biotope, and turbulence categories used in determining habitat heterogeneity</i>	<i>44</i>
<i>Table 3-3: Summary of fitted generalized linear models (GLM) fitted for the response variables towards depth (m). The values in bold suggest the significant taxa:</i>	<i>47</i>
<i>Table 3-4: Summary of fitted generalized linear models (GLM) fitted for the response variables towards velocity (cm/s). The values in bold suggest the significant taxa:</i>	<i>50</i>
<i>Table 3-5: Summary of fitted generalized linear models (GLM) fitted for the functional response variables towards depth (m). The values in bold suggest the significant functional traits:</i>	<i>58</i>
<i>Table 3-6: Summary of fitted generalized linear models (GLM) fitted for the functional response variables towards velocity (cm/s). The values in bold suggest the significant functional traits:</i>	<i>60</i>
<i>Table 3-7: The optimal habitats, velocity-, and depth- values for macroinvertebrates based on literature:</i>	<i>64</i>

List of Figures

- Figure 2-1: A map indicating the locations of the sixteen sampling sites in the Limpopo River Catchment, South Africa, sampled during April-May 2021..... 12
- Figure 2-2: PCA biplot of the abiotic environmental variables, water quality variables [(nutrient concentrations (NO_2 , NO_3 , NH_4 , PO_4), anions (sulphate (SO_4), chlorine (Cl)), and physiochemical properties (pH, EC, temperature, dissolved oxygen (DO), turbidity, chemical oxygen demand (COD)) measured at sites in the Limpopo River and tributaries. Axis 1 explains 36.66%, and axis 2 explains 21.86% of the total variation. Site codes are explained in Table 2-1..... 20
- Figure 2-3: PCA biplot of the sediment particle sizes (>0, >53, >212, >500, and >2000) and habitat heterogeneity measured at sites in the Limpopo River and tributaries. Axis 1 explains 36.7%, and axis 2 explains 22.46% of the variation of the data. Site codes are explained in Table 2-1. 21
- Figure 2-4: Diversity index results between sites sampled in the Limpopo River Catchment; a) total number of taxa per biotope sampled, b) Species Richness, c) Pielou's evenness, d) Shannon Wiener diversity. Site codes are explained in Table 2-1..... 22
- Figure 2-5: PCA biplot analysis showing sampling sites sampled and taxa contribution for the Limpopo River Catchment. The green arrow indicates a cluster of taxa, and the red arrow indicates a cluster of sampling sites. Axis 1 explains 35.18% variation of the data, and axis 2 explains 15.83%. Site codes are explained in Table 2-1. Taxa abbreviations are defined in Appendix A..... 23
- Figure 2-6: PCA biplot of the water quality variables [(nutrient concentrations (NH_4 , NO_2 , SO_4 , PO_4 , NO_3 , NO_2), physiochemical properties (pH, EC, temperature, DO, turbidity, Cl, TH, COD), sediment particle sizes (>0, >53, >212, >500, and >2000), habitat heterogeneity and macroinvertebrates. The green arrow indicates a cluster of taxa. Axis 1 explains 27.50% variation, and axis 2 explains 18.37% of the total variation. Site codes are explained in Table 2-1. Taxa abbreviations are defined in Appendix A..... 24
- Figure 2-7: PCA biplot of the forward selected variables summarizing the variation in taxa composition that is explained by all environmental variables. The

variables which provide the significant changes in communities are sediment particle sizes >500 and >53, HabiHet (Habitat heterogeneity), T (Temperature) and COD (Chemical Oxygen Demand). The green arrow indicates a cluster of taxa at the fine sand (>53). Axis 1 explains 30.69% of the data, 2 explains 44.77% of the data. Site codes are explained in Table 2-1. Taxa abbreviations are defined in Appendix A..... 25

Figure 2-8: Compared variable groups are represented by circles in the following Venn diagram as explained by variation partitioning. The first group is represented by the water quality drivers (a), the second group (b) represents the habitat requirements and (c) represents both components together as drivers of community structure distribution. a) explains 12.8% of the data, b) explains 56% of the data and c) explains 31.2% of the data and results obtained. Site codes are explained in Table 2-1..... 26

Figure 2-9: Functional traits of the macroinvertebrate community per site in the Limpopo River Basin (Site codes can be found in table 2-1). (a) Body size, b) body shape, c) attachment mechanism, and d) mobility..... 27

Figure 2-10: Functional traits of the macroinvertebrate community per site. The following graphs represent the following traits: a) respiration, b) functional feeding group, c) habitat preference, and d) flow preference. Site codes are explained in Table 2-1..... 28

Figure 2-11: Functional traits of the macroinvertebrate community per site. a) aquatic life stage, b) hydraulic habitat preference. Site codes are explained in Table 2-1. 29

Figure 2-12: PCA biplots and supplementary variables of a) sites and functional traits (body size, body shape, attachment type, respiration, mobility, functional feeding groups, habitat preference, flow preference, hydraulic habitat preference and aquatic life stage) and b) samples and environmental variables. Axis 1 explains 37.33% variation of the data and axis 2 explains 21.12%. Site codes explained in Table 2-1 31

Figure 2-13: RDA triplot of the forward selected variables summarizing the variation in the composition that is explained by all environmental variables and functional traits. The variables which provide the significant changes in communities

are sediment particle sizes SO_4 , fine sand, coarse silt, and COD. Site codes are explained in Table 2-1.	32
Figure 2-14: Compared groups are represented by circles in the following Venn diagram and explained by variation partitioning. The first group is represented by the water quality drivers (a), the second group (b) represents the functional traits and (c) represents both components together as drivers of community structure. a) explains 51.1% of the data, b) explains 41.3% of the data and c) explains 7.6% of the data and results obtained.....	33
Figure 3-1: A map indicating the locations of the two sampling sites in the Olifants River, KNP.	42
Figure 3-2: a) PCA biplot of the environmental variables (biotopes vegetation (veg), gravel, sand and mud GSM and stones; velocity and depth) and macroinvertebrate taxa in the two sampling sites, Balule (squares) and Mamba (circles), in the Olifants River, KNP. b) Biplot of the sampling site and macroinvertebrate taxa (the following colors represent the following biotopes: green – vegetation, red – stones in current (SIC), and brown – gravel, sand and mud (GSM)). Axis 1 explains 30.44% of the data, and axis 2 explains 16.39% variation of the data. Taxa abbreviations are defined in Appendix A	47
Figure 3-3: Habitat preference curves (HPCs) and habitat utilization curves (HUCs) for depths (a), water flow velocities (b), and substrate categories (c) separately for samples collected in 2020 and 2021. Categories of substrate: GSM—gravel, sand and mud, SIC—stones in current, veg—vegetation.	53
Figure 3-4: Habitat availability curves (HACs) and habitat utilization curves (HUCs) for depths (a), water flow velocities (b), and substrate categories (c) separately for samples collected in 2020 and 2021. Categories of substrate: GSM—gravel, sand and mud, SIC— stones in current, veg—vegetation.	Error! Bookmark not defined.
Figure 3-5: PCA biplot of environmental variables (biotopes vegetation, GSM and stones; velocity and depth) and functional traits ((body size, body shape, attachment type, respiration, mobility, functional feeding groups, habitat preference, flow preference, hydraulic habitat preference and aquatic life	

stage) (the following colors represent the following biotopes: green – vegetation, red – SIC, and brown – GSM). Axis 1 explains 23.69% of the data, and axis 2 explains 8.33% variation of the data..... 57

Chapter 1

Introduction

1.1 Background and Introduction

1.1.1 Water in South Africa

Water is crucial for the survival of all organisms worldwide. Water connects most parts of the natural environment, which includes both biotic and abiotic factors (Li *et al.*, 2009). Freshwater resources in South Africa are rapidly declining and posing a major threat to not only aquatic biodiversity, but also to the surrounding biodiversity depending on water for their existence (Knight & Rogerson, 2019; Molobela & Shinha, 2011). Therefore, the monitoring, management and rehabilitation of freshwater resources are vital for the survival of animals, plants, and humans.

Due to the exponential growth of the human population, many river systems have been polluted through anthropogenic activities, leading to the degradation of water quality (Khatri & Tyagi, 2014; Burri *et al.*, 2019; Malakane *et al.*, 2020). The ecological state of rivers is affected by the effluents of activities such as mining, development, industries, and agriculture that produces pollutants that end up in rivers (Macklin *et al.*, 2006; Castillo *et al.*, 2017; Yao *et al.*, 2021; Malakane *et al.*, 2020; Krodkiewska *et al.*, 2022). Additionally, human water demands must be balanced with the requirements of rivers, but the pressures in water resource allocations are increasing due to both rising human demand and uncertainties of the water requirements of riverine ecosystems (Petts, 2009).

1.1.2 Freshwater River ecosystems

Riverine ecosystems are known to be hotspots for biological richness, with almost 10% of worldwide animal biodiversity connected with freshwater habitats (Dudgeon, 2019). These habitats comprise of <1% of the Earth's surface. Riverine ecosystems are one of the most threatened and vulnerable systems (Dudgeon, 2019; Best & Darby 2020). Rivers are downstream transmitters of material obtained from their catchments and the impacts that follow do not stay at a local place. Rivers naturally need to transport and transfer nutrients, sediments etc. from headwaters to lower reaches, however, with increasing anthropogenic influences now also transports pollutants and human debris. Therefore, the increase of vulnerability of biodiversity throughout the river's network can occur (Nelson & Murray, 2008; Dudgeon, 2019). The vulnerability of biodiversity is therefore also subjected to impacts such as flow regulation, land-

use change and catchment transformation, dams, water abstraction, overexploitation of biological resources, invasive species, and climate change (Dudgeon, 2019). As these ecosystems are already exposed to several anthropogenic stressors, numerous species within habitats have been fragmented and have limited abilities to migrate as the ecosystem is altered (Dallas & Rivers-Moore, 2014). Most species have the abilities to adapt to changes in ecosystems, however, with increasing anthropogenic stressors these changes occur to rapidly to adapt to. The increase of stress on river ecosystems due to anthropogenic activities is of growing concern worldwide due to the increase of demand and degradation of freshwater ecosystems. Understanding how aquatic species respond to such stressors is important to manage ecosystem functioning, conserving species, and the ecosystem services they provide (Adams, 2001; Burnett *et al.*, 2021; Firmiano *et al.*, 2021; Strungaru *et al.*, 2021).

1.1.3 Environmental flows

Environmental flows (or Eflows) are characterised by the quality, quantity, and timing of water flows that are required to sustain riverine ecosystems, as well as the human communities dependent on these ecosystems (Kothari & Mishra, 2015). Each river system has unique flow regimes with specific features, for example seasonal pattern flows, frequency, timing, predictability, the extent of extreme events, and the rate of alterations (Kothari & Mishra, 2015). Changes in the natural flow regime can cause extreme change in the riverine ecosystems. Instream environmental flow is used primarily for maintaining both development and reproduction of target organisms and facilitates optimum habitat areas (Bunn & Arthington, 2002). A suitable environmental flow is crucial for the functional preservation of riverine ecosystems and natural flow regimes play a valuable role as the main driver in numerous biological processes and provides long-term ecological viability of aquatic habitats (Maddock, 2016).

1.1.4 Habitat preference and alteration

Although it is common knowledge that different species inhabit different habitat types within streams (Li *et al.*, 2009), there is a discrepancy between the knowledge and the capability to quantify the habitat characteristics that an organism selects. There are numerous environmental factors that influence the habitats of aquatic organisms that includes water depth, river width, velocity, and physical habitat structure (Maddock, 2016). Within the same river systems there are various zones for aquatic organisms to use for growth, reproduction, and foraging activities. These are important factors determining the abundance and distribution of aquatic organisms (Maddock,

2016). With the increase of stress on aquatic ecosystems due to anthropogenic activities, the changes in the natural flow conditions are probably the primary threat to the integrity of aquatic ecosystems (Ramulifho *et al.*, 2019).

1.1.5 Limpopo River Catchment

The Limpopo River Catchment (LRC) stretches over four countries, including South Africa, Mozambique, Botswana, and Zimbabwe (FAO-SAFR, 2004). The LRC maintains biologically diverse ecosystems and provide important ecosystem services to surrounding communities (UN-HABITAT/UNEP, 2007). The LRC is a semi-arid to arid area that is susceptible to frequent droughts and occasional flooding events (Rapolaki *et al.*, 2020). This area has relatively poor rural communities that are directly dependent on rain-fed sources for activities such as farming and drinking water (El-Mahdy, 2022).

Several authors emphasized the challenges that the Limpopo River riparian countries face to meet the current water demand, as well as the concerns for future water requirements (Scanlon *et al.*, 2006; Cuthbert *et al.*, 2016; Xiao *et al.*, 2018; Saveca *et al.*, 2022). In the past, the Limpopo River was known to be a strong-flowing perennial river but over the years it has been altered to a weakly-flowing perennial river (Nhassengo *et al.*, 2021). During droughts of the river, flows often cease and long sections of the middle and lower regions of the river may have little to no surface water (Nhassengo *et al.*, 2021). The over-use of water and impacts of droughts can negatively affect the downstream ecosystems (Nhassengo *et al.*, 2021). Another challenge the Limpopo River faces is pollution, and it has been reported in some case. For instance, the death of crocodiles within the Olifants River, a tributary of the Limpopo River, resulting from accumulation of toxic elements from the sediments (LBPTC, 2010), metals bioaccumulating in the muscles of fish (Jooste *et al.*, 2014) and the decline in water quality (Saveca *et al.*, 2022). The conservation of rivers is, therefore, a crucial component for the protection of riverine ecosystems. Furthermore, there are important national parks and conservation regions that fall within the LRC (Rapolaki *et al.*, 2020) such as the Kruger National Park that is the premier conservation area in South Africa

1.1.6 Kruger National Park River systems

The South African National Parks (SANParks) is committed to preserve ecological processes and life support systems which contribute to the preservation of both sustainable use of species and ecosystems by humans, as well as protecting genetic diversity (Büchling & Maroun, 2021). The

KNP is situated within the savanna biome, that consists of five large, dynamic, and biologically diverse transboundary river systems and have been the focus of applied river ecosystems research for more than 30 years. The KNP is committed to conserving ecosystems considering their dynamic nature and both biotic and abiotic components that are interdependent, as well as interactive, which is vital for the survival for specialised inhabitants of rivers (Venter & Deacon, 1995; Riddell *et al.*, 2019).

SANParks is responsible for the management of several national parks, including the Kruger National Park (KNP). There are six major River systems flowing through the KNP, all which originate outside of the KNP, which includes the Olifants River. The Olifants River, is exposed to human impacts that include flow regulation, water abstraction and numerous anthropogenic inputs polluting the water sources.

River flow and water quality management have been a rising issue due to anthropogenic activities in the surrounding areas polluting the inflow of water into the river systems causing various effects such as a degradation of water quality. The KNP is situated within only two of the five transboundary river catchments and five perennial rivers flow across the KNP namely the Luvuvhu, Olifants, Letaba Rivers that flow into the Limpopo Catchment, and the Crocodile and Sabie Rivers flowing into the Incomati Basin (Riddell *et al.*, 2019). The KNP is also considered to be the most downstream 'user' of water resources in the country and has continuously been working to guarantee the progressive implementation of the environmental water requirements (EWRs) of each river as directed under South Africa's National Water Act (of 1998). According to Venter & Deacon (1995), the five rivers flowing through the KNP form an integral part of the riverine ecosystems and any management or development actions occurring within the upper catchment regions will finally affect the rivers in the KNP.

The Olifants River is one of the biggest rivers flowing through the KNP (Venter & Deacon, 1995). According to Gerber *et al.* (2015), historically the Olifants River is considered to be one of the most polluted systems in South Africa. This system is one of the main water sources providing for industrial, agricultural, mining, as well as domestic uses. Pollution arises from both intensive and subsistence agricultural activities along the river, as well as industrial and mining activities contributing to the pollution of metals and inorganic nutrients in the Upper Olifants catchment area (Makalane *et al.*, 2020). Due to the mining development, the Olifants River is mostly affected by salt enrichment originating from both phosphate and sulphate through controlled effluent discharge (Ashton *et al.*, 2001; Hobbs *et al.*, 2008; Dabrowski *et al.*, 2014). After 2002 the KNP successfully promoted the implementation of a zero-discharge policy (for the Phalaborwa Mining Complex) that caused a clear improvement of the water quality of the river systems (Riddell *et al.*,

2019). The Olifants River still periodically experiences the impacts of large point source pollution events.

For instance, the Olifants River still experiences intermittent fish kills at the Phalaborwa Barrage resulting from anoxic sediment due to the industrial phosphate plant spill in 2013 (Marr *et al.*, 2017). This serves as a large water resource to the local mines and regional economy of Phalaborwa (Riddell *et al.*, 2019).

It is thus vital to measure and regulate the protection and rehabilitation of water resources on a scientific manner, i.e., studies regarding environmental flow to maintain or restore good ecological status (Elvira *et al.*, 2021). The main concern on all our minds still stands: “To what degree the flow regime of a river can be altered before the degradation of the ecosystem starts?”.

1.1.7 Bioindicators

Bioindicators are living organisms used to monitor alterations within ecosystems and to identify the changes of human impact and natural variability that are used to obtain more information in terms of the impacts of contaminants on the temporal and spatial modification (Sumudumali & Jayawardana, 2021). It uses animals for the past, current, and future assessment of future risks of procedures of the ecosystem and can be recognized through a whole community or single taxon, providing information about anthropogenic and natural variations in ecosystems through the evaluation of their presence/absence, diversity patterns, or abundance (Odountan *et al.*, 2018). There are three classes of bioindicators: compliance indicators, diagnostic indicators and early warning indicators that are constructed on the purpose of their use (Sumudumali & Jayawardana, 2021). Compliance indicators indicate deviations from suitable health boundaries of the aquatic ecosystem, while diagnostic indicators reveal the reasons for these deviations (Sumudumali & Jayawardana, 2021). According to Sumudumali & Jayawardana (2021), the early warning indicators show the impending declining health of the aquatic ecosystem. Good bioindicator organisms comprise of desirable characteristics for instance cosmopolitan distribution, taxonomy, sufficient abundance, and wide distribution for replicate sampling (Sumudumali & Jayawardana, 2021). There is a large variety of aquatic taxa that are used in the biomonitoring process such as fish, macroinvertebrates, plankton and periphyton (Sumudumali & Jayawardana, 2021).

1.1.8 Aquatic Macroinvertebrates

Aquatic macroinvertebrates are organisms that do not have a backbone and can be seen with the naked eye, such as earthworms, shrimps, leeches, clams, snails, and a wide range of aquatic insect forms (Reboredo-Fernández *et al.*, 2014). Macroinvertebrates inhabit various habitats such as on, under, and around rocks, bottom-sediments of rivers, streams and vegetation around the banks of rivers and streams (Reboredo-Fernández *et al.*, 2014). These organisms can live in the same ecosystem for several months or years, depending on the life cycle of the specific organism, thus, long enough to reflect the impact of pollution and short enough to respond to water quality alterations (Reboredo-Fernández *et al.*, 2014). Freshwater macroinvertebrates are more commonly used than any other biological group as indicators for biological integrity of stream ecosystems as they provide a good reflection of the prevailing physio-chemical state and flow regime within a river (Thirion, 2016). Aquatic macroinvertebrates are used to indicate water quality as they are good biological indicators because various taxa tolerate different abiotic conditions and are sensitive to certain concentrations of pollution because of anthropogenic activities and climate change (Rawat *et al.*, 2019). An indication of water quality and ecological status of aquatic ecosystems can be portrayed by the presence or absence of different taxa present within the water system (Rawat *et al.*, 2019). According to Aazami *et al.* (2020), aquatic macroinvertebrates are ecologically considered within an aquatic ecosystem as they comprise of specific characteristics for example having a high species richness, various reactions to environmental factors (such as dissolved oxygen, temperature, conductivity, and salinity) and most of their lifecycles are within the water column, thus, changes in the environment can be measured over time.

Additionally, they play a vital role in the riverine ecosystem because they are an essential component of the system. Aquatic macroinvertebrates of freshwater ecosystems process transported organic matter in rivers, therefore contributing to the purification of water, and function as a valuable food source for prey animals in- and outside of the system (Thirion 2016). Macroinvertebrates are influenced by various factors such as food, flow speed, substratum, vegetation, riffle depth, shade, water temperature and conductivity, effects of droughts, floods, altitude and season, and competition between species and zoogeography (Sumudumali & Jayawardana, 2021). According to Malakane *et al.* (2020), their study confirms that combining both physio-chemical and biological measures in water quality assessment offer a favourable and constructive result by indicating sensitivity to changes in the environment.

According to Thirion (2016), there have been no all-inclusive studies done in South Africa on the distribution of macroinvertebrates and the only available information is selected families found

within museums and literature records. The main aim of this work is focussing on the Ecological Classification Process that includes the pattern of macroinvertebrates' sensitivity towards environmental factors. (Thirion, 2016). This approach provides a better understanding of the alterations within a river system's natural state. The habitat features within a riverine system are important when determining the habitat- requirements, preferences and -suitability of aquatic macroinvertebrates (Buffagni, 2020). The methods in assessing the macroinvertebrate response to driver attributes is established on a qualitative combination of information obtained from field surveys, the characteristics of the present macroinvertebrate species, and the habitat available arising from driver conditions (Thirion, 2016; Szalkiewicz *et al.*, 2022).

1.2 Aims and Objectives

This study mainly focussed on selected mainstream rivers and tributaries within the Limpopo River Catchment (LRC). The aims of this study were to 1) determine the preferred flow and associated habitats required by macroinvertebrates and 2) to identify suitable macroinvertebrate indicator species in relation to specific flow, habitat preferences or functional traits.

To achieve these aims the following objectives were set:

- Conduct surveys during low flow at selected sites in the Limpopo River Catchment and some of the major tributaries;
- Conduct surveys during low flow at selected sites in lower Olifants River (KNP);
- Measuring water quality and flow parameters at each site;
- Collect macroinvertebrate samples from all available biotopes;
- Relate macroinvertebrate community structures to water quality and/or flow changes;
- Relate macroinvertebrate community structures to habitat preferences;
- Relate flow and habitat preferences to functional traits of the macroinvertebrate community; and
- Identify indicator species of environmental flow conditions.

1.3 Chapter Breakdown:

Chapter 1: General introduction: This chapter contains the introduction to the importance of riverine ecosystems, an overview of the Limpopo River and Olifants River and preliminary comments on the important components of this study. The aims and objectives are presented for the project.

Chapter 2: Macroinvertebrate community structure in the Limpopo system: In this chapter, the aquatic macroinvertebrate community at 16 sites in the Limpopo River Catchment were determined. The influence of the main drivers (i.e. habitat, flow and water quality) on the macroinvertebrate community structures at the selected sites were determined.

Chapter 3: Flow and habitat preferences of macroinvertebrates in the Olifants River: This chapter presents the results on the relationship between macroinvertebrate community attributes (i.e. diversity and traits) and physico-chemical attributes (water quality, flow and habitat) at two sites in the one of the main tributaries of the Limpopo catchment, i.e. the Olifants River that were sampled over two consecutive years.

Chapter 4: General conclusion and recommendations: provides some concluding remarks and recommendations that emanated from the study.

Chapter 5: References: A list of all the references used throughout this dissertation is presented.

Appendices: Raw data of the aquatic macroinvertebrate diversity results, water quality measurements, sediment particle size, flow and depth measurements, and functional trait results of the Limpopo River system and Olifants River system.

Chapter 2

Macroinvertebrate Community Structure in the Limpopo System in Relation to Habitat and Water Quality Drivers

2.1 Introduction

In South Africa, almost 71% of the main rivers are known to be either endangered or critically endangered due to exploitation (DWAF, 1996). The Limpopo River Catchment (LRC) is situated within an arid and semi-arid region which makes water a valuable commodity in this area. There are large parts within the LRC that are comprised of protected areas, such as the Great Limpopo Transfrontier Park, that includes the Kruger National Park, and contain unique biota with numerous threatened species (LBPTC, 2010). Shongwe *et al.* (2009) states that the LRC is affected by climate change and numerous studies have indicated that there is an increase in uncontrolled abstraction in the upstream region of the LRC and that there is a decrease in discharge of the river during the dry period. There are reports that the Limpopo River is no longer perennial (FAO-SAFR, 2004; LBTC, 2010).

Aquatic macroinvertebrates are often used for biomonitoring of riverine ecosystems as they are responsive indicators of different environmental flow conditions by responding to alterations in natural flow conditions (Masese *et al.*, 2009). Changes within the riverine ecosystems have various drivers, such as hydrology and water quality, which exert their impact on instream ecosystems. Aquatic organisms within riverine ecosystems have irregular spatial distribution patterns (Schröder *et al.*, 2013) because of the physical, hydraulic and/or hydrological state of the river (Bo *et al.*, 2017; Serpa *et al.*, 2019) that shape them as they adapt to their habitats which leads to highly specialised species (Parsons & Norris, 1996). An ecosystem is much more complex than just an assemblage of species. Each species provides a contribution to the ecosystem function through their functional traits, which create a relationship between environmental conditions and the characteristics of the species inhabiting different habitats (Violle *et al.*, 2007; Hevia *et al.*, 2017; Paz *et al.*, 2022). As a result, organisms that possess the appropriate combination of traits can resist or adapt to external pressures (de la Feunte *et al.*, 2017). A few studies confirm that the functional approach provides an understanding of the relationship between community structure and aquatic ecosystem functioning when under external pressures (Statzner *et al.*, 2001; Dolédec *et al.*, 2007; Verberk *et al.*, 2013; Mondy *et al.*, 2013; Hering *et al.*, 2015; Navarro-Ortega *et al.*, 2015). Based on a few studies (Pallottini *et al.*, 2016; Edegbene, 2020a; 2020b; Odume, 2020) that identified certain traits and ecological preferences relating to pollution, the following traits were used in this study: body size, body

shape, attachment type, respiration, mobility, functional feeding groups, habitat preference, flow preference, hydraulic habitat preference and aquatic life stage.

Hydraulic (water depth and flow velocity) and habitat (substrate type) features, as well as their combined impact, are assessed as key drivers of the spatial distribution of benthic aquatic biota at the microhabitat scale (Vimos-Losjano *et al.*, 2017). Biotopes are defined mostly on hydraulic (water depth and flow velocity) and substrate (vegetation, stones, or sand) features (Bonada *et al.*, 2006; Dallas, 2007a; 2007b). Availability and suitability of biotopes within a river affect macroinvertebrate community structure that comprise of numerous taxa with different requirements and preferences (Demars *et al.*, 2012). For example, the vegetation and stone biotopes often have a higher diversity of macroinvertebrates (Chutter, 1970; Dallas, 2007a; 2007b), while sandy biotopes have a lower diversity of macroinvertebrates (Quinn & Hickey, 1990; Brewin *et al.*, 1995). Sediment also plays a role in the habitat preference of macroinvertebrates. Sediment particle size mostly rely on the responses of sediment stability towards the hydrological stressors that occur within the ecosystem (Ran *et al.*, 2019). Finer particles within sediment can easily be moved through flow and therefore can result in the formation of solid particles within the riparian zone's surface layer (Knighton, 1980; Rhoads, 1989). These finer particles can also be released into the water column via hydrodynamic disturbances which occur under variable sedimentary conditions, which in turn can cause risk to aquatic biota (Tang *et al.*, 2016) for instance clogging of gills, reducing visibility to find food or prey.

There is a lack of information regarding the drivers of macroinvertebrate community structures and identifying suitable taxa that represents the environmental flow and habitat requirements of rivers in South Africa (Moore, 1991; Palmer, 2000; Brown, 2001; Nunkumar, 2002; Madikizela & Dye, 2003; Schael & King 2005; Bonada *et al.*, 2006; Bonada *et al.*, 2007; Dallas, 2007a; 2007b; Griffiths *et al.*, 2015; Picker & Griffiths 2019). Therefore, this chapter provides more information on the changes in the natural flow of the Limpopo River and selected tributaries and aimed to use macroinvertebrates as bioindicators. The aims of this chapter were to (1) determine whether the macroinvertebrate structures (through community composition and traits) were driven by habitat requirements and/or water quality and (2) to identify a suitable indicator species for the main drivers of community structures in the Limpopo catchment.

2.2 Methods

2.2.1 Description of Study Area

General

The LRC is known to be one of the largest catchments within Southern Africa, stretching around approximately 412 000 km², located 250 to 2300 m above sea level and crosses over four countries, starting in South Africa and flowing east through Botswana, Zimbabwe, and through Mozambique where it enters the Indian Ocean (Elmi Mohamed, 2014). The LRC is also the second most populated catchment in Southern Africa after the Orange River catchment and home to almost 17 million people (Turton & Funke, 2008 LBPTC 2010; Mosase *et al.*, 2019). The Limpopo River flows an entire distance of 1750 km through the basin in South Africa (Boroto, 2001; Sullivan & Sibanda, 2010).

Sampling sites

A total of sixteen sites were surveyed for this study during September/October 2020 and April/May 2021 that included the following rivers: Crocodile, Matlabas, Lephale, Mogalokwena, Sand, Luvuvhu, Letaba, Shingwedzi, two sites on the Olifants (within the KNP and sampled in both 2020 and 2021) and three sites on the Limpopo (Table 2-1 and Figure 2-1).

2.2.2 Field Surveys

Water quality and flow determination

In-situ water quality (i.e., temperature, pH, electrical conductivity (EC), and dissolved oxygen (DO)) was recorded at each site using calibrated water quality meters (ExStik II DO600 and EC500 ExTech Instruments). Water samples for further chemical analyses were collected in triplicate in pre-cleaned polypropylene containers and frozen until analyses.

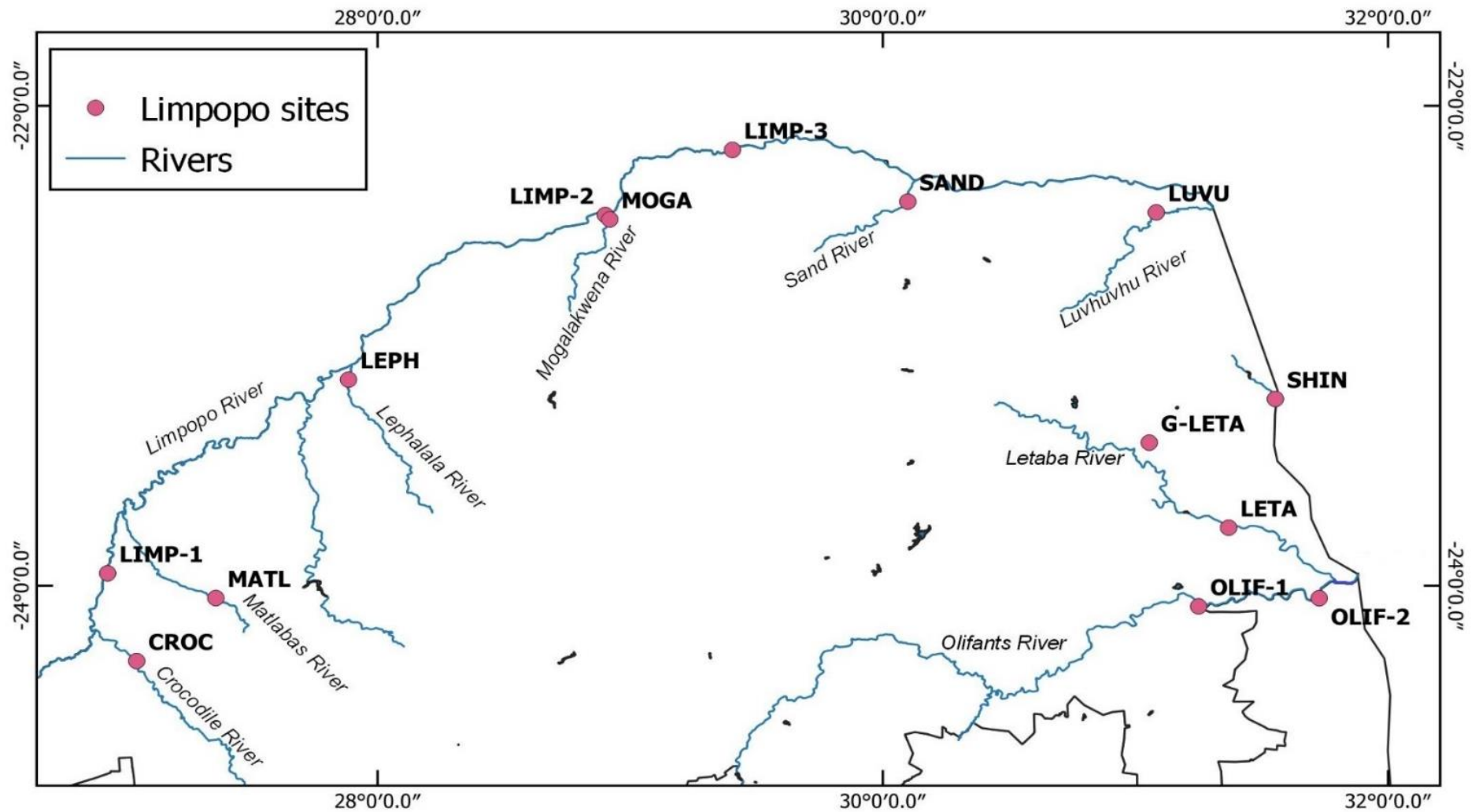


Figure 2-1: A map indicating the locations of the sixteen sampling sites in the Limpopo River Catchment, South Africa, sampled during September/October 2020 and @April-May 2021.

Table 2-1: Temporo-spatial details and general comments on biomonitoring sites on the Limpopo River and some of its major tributaries, and the Olifants River within the KNP.

Code	River	Latitude (S)	Longitude (E)	Date	Comments	Site photos
20_MAMBA (OLIF-1 in Figure 2-1)	Olifants	-24.08642	31.25094	29-Sep-20	Low flow, water, algae very dominant on substrates in flowing portions. Habitat heterogeneity high. Biotopes sampled: three SIC replicates, four vegetation replicates, two bedrock replicates and two GSM replicates.	
20_BALUL (OLIF-2 in Figure 2-1)	Olifants	-24.05214	31.72878	1-4 Oct 20	High rainfall in the upper catchment and on-site resulted in dramatic changes, e.g., turbidity, inundated substrates, accessibility, and more. Water turned from clear to red brown. Biotopes sampled: three SIC replicates, three vegetation replicates, one bedrock and one GSM sample.	
21_CROC	Crocodile	-24.31417	27.04614	21-Apr-21	High flow, water turbid, habitat heterogeneity moderate to low. Time at the site was limited, but so was habitat. Biotopes sampled: three GSM replicates and two vegetation replicates.	
21_SPANW (LIMP-1 in Figure 2-1)	Limpopo	-23.9485	26.93123	22-Apr-21	High flow, water clearer, habitat heterogeneity very high. Time at the site was limited. Biotopes sampled: two SIC replicates, two vegetation replicates and two GSM replicates.	

21_MATL	Matlabas	-24.05186	27.35964	23-Apr-21	Moderate to low flow, crystal clear water, low to moderate habitat heterogeneity. Biotopes sampled: two SIC replicates and two vegetation replicates.	
21_LEPH	Lephalale	-23.14128	27.88503	24-Apr-21	High flow, water slightly discolored, downstream from a weir with some good habitat, but reduced habitat heterogeneity further downstream. Biotopes sampled: two SIC replicates and two vegetation replicates.	
21_LEPHA (LIMP-2 in Figure 2-1)	Limpopo	-22.45519	28.90175	25-Apr-21	High flow, water light to red brown, with high habitat heterogeneity. Biotopes sampled: one SIC sample, two GSM replicates and two vegetation replicates.	
21_MOGA	Mogalakwena	-22.47344	28.91950	26-Apr-21	Flow limited to a trickle at one location downstream from a dam wall. Rest of stream made up of isolated pools. Water clear, with potentially high habitat heterogeneity. Sampling time was limited but so was habitat diversity. Biotopes sampled: three GSM replicates.	

21_LIMP (LIMP-3 in Figure 2-1)	Limpopo	-22.18383	29.40519	27-Apr-21	High flow, water slightly clear with greenish tint. Biotopes sampled: three SIC replicates, two vegetation replicates and two GSM replicates	
21_SAND	Sand	-22.39928	30.09942	28-Apr-21	Very low flow, water clear with substrate dominated with sand. Habitat heterogeneity was low, and sampling time was limited. Biotopes sampled: two SIC replicates, two vegetation replicates and two GSM replicates.	
21_LUVU	Luvuvhu	-22.44444	31.08344	29-Apr-21	Very high flow, with deeper fast flowing sections wadable during low flow (Sep 2018) inaccessible). Habitat heterogeneity was high with cobble-boulder substrates dominant and different velocity-depth classes. Matumi root wads were inaccessible due to high flow. Sampling time limited. Biotopes sampled: two SIC replicates, two vegetation replicates and two GSM replicates.	
21_SHIN	Shingwedzi	-23.22194	31.55492	1-May-21	Moderate to low flow, with pool habitats and flow over dominant. Stones biotopes sampled in the vicinity of the bridge, dominated by bedrock. Biotopes sampled: one SIC sample, one Bedrock sample, two GSM replicates, and two vegetation replicates.	

21_BALUL	Olifants	-24.05214	31.72878	3-May-21	High flow, with high habitat heterogeneity. Deposition occurs above the crossing during high flows, with gravel-cobble-sand bed several layers deep. Biotopes sampled: two SIC replicates, two vegetation replicates and two GSM replicates.	
21_LETA	Letaba	-23.75833	31.36997	4-May-21	Moderate to low flow, water clear with high habitat heterogeneity. Biotopes sampled: two SIC replicates, two vegetation replicates and two GSM replicates.	
21_MAMBA	Olifants	-24.08642	31.25094	5-May-21	High flow, slightly turbid waters with high habitat heterogeneity. Biotopes sampled: two SIC replicates, two vegetation replicates and two GSM replicates.	
21_G-LET	Letaba	-23.67708	31.09833	6-May-21	Moderate to low flow over sand-gravel-bedrock bed, with moderate habitat heterogeneity. Biotopes sampled: two SIC replicates, two vegetation replicates and two GSM replicates.	

Instream habitat

Data were collected quantitatively through sampling specific designated areas within different hydraulic biotopes, depths, velocity, and substrates. The habitat present in each area were measured and then described regarding depth, velocity, hydraulic biotope, and substrates. At each site, biotopes present were identified and sampled randomly in different areas along the site. Depth and velocity measurements were documented at each site using a handheld unit OTT MF Pro (Bandini *et al.*, 2022).

Within the stone biotope, three components of individual substrates were measured, namely, coarse gravel (between 15 – 64 mm), cobbles (64 – 254 mm) and boulders (>254 mm) with a standard 30 cm ruler. Only the substrates that were loosely composed on the riverbed was measured. The measurements were taken to quantify the substrate samples and was expressed as an area and as a percentage of the square (40 x 40 cm) of the Surber-sampler, which was used to sample macroinvertebrates (see below). Sediment samples were collected at each site in triplicate using a sediment scoop and placed in a sterile container. Excess water was removed from samples, and it was stored at -20 °C for transport to the laboratory.

Aquatic macroinvertebrates

Using a Surber-sampler, aquatic macroinvertebrates were collected quantitatively between the different biotopes (Surber, 1937) and have been sampled twice per biotope per site at different flow conditions. This Surber-sampler is comprised of a squared net, which can differ in size, this study used a 40 x 40 cm net with a mesh size of 500 μm^2 , with a metal frame which outlines a zone which can be used on the river bottom, providing quantitative collection of macroinvertebrates (Doretto *et al.*, 2019). The contents collected within the 40 x 40cm square were transferred into the net whereafter contents were stored in a sterilized container, filled with 96% ethanol to preserve the organisms until laboratory analysis. The contents provided a quantitative data representation of macroinvertebrate community structures from present biotopes from two sites in the Olifants River, KNP.

The three main biotopes, vegetation, stones and gravel, sand, and mud (GSM), were sampled following set methods per biotope. Vegetation sampling followed a reproducible protocol using sweep nets for identical size sampling areas as the Surber-sampler (40 x 40 cm) which is used for both stones and GSM biotopes. Contents of the nets were emptied and drained of river water then transferred into sterilized, plastic container which was filled with 96% ethanol to preserve

organisms for transportation to the laboratory for further analyses. All biotope samples per site were analysed separately and pooled post laboratory analyses to provide a total.

2.2.3 Laboratory Analysis

Water samples were defrosted and tested for nutrients using a Merck Spectroquant (Spectroquant® Pharo 300, Merck) and appropriate test kits. These include the following: Nitrate (NO_3), Nitrite (NO_2), Ammonium (NH_4), Sulphate (SO_4), Chloride (Cl); Phosphate (PO_4); Magnesium (Mg; mg / L), Calcium (Ca; mg / L), Alkalinity (mmol / l HCO_3), Chemical Oxygen Demand (COD), and turbidity.

Sediment samples were allowed to defrost whereafter they were transferred to 50 mL Falcon tubes and dried in a freeze-dryer (Labronco FreeZone 6 Freeze dryer) for a total of 96 h. The sediment particle size was determined using a mechanical shaker comprising of a set of sieves of various sizes from course to fine particles; 4000 μm (medium gravel), 2000 μm (fine gravel), 500 μm (medium sand), 212 μm (medium sand), 53 μm (fine sand), and <53 μm (coarse silt to clay)) (Blott & Pye, 2012). Samples were weighed (50 g) and then added on top of the sieve system, and samples were sieved for 5 min. Each sieve retained sediment that was weighed, and the percentage composition of each particle size was calculated.

Macroinvertebrate samples were counted and identified under a Zeiss stereo microscope from samples using identification guides for freshwater invertebrates of Southern Africa (Day, 1999; Day, 2001; Day & Day, 2002; Day *et al.*, 2003; Harrison, 2009). Identification of organisms was down to the lowest taxonomic level possible. Macroinvertebrate functional traits used trait by taxon matrix comprising of 10 traits: body size, body shape, attachment type, respiration, mobility, functional feeding groups, habitat preference, flow preference, hydraulic habitat preference and aquatic life stage.

2.2.4 Data Analyses

Diversity indices (Shannon-Wiener diversity, species richness, and Pielou's evenness) were used for quantitative measures of the various taxa within the dataset (such as a community), and it simultaneously considered how equally the taxa were distributed among the environmental variables (Odum and Barrett, 2005) for the determination of both constancy and diversity of various biotopes and sites. Analyses were conducted using the Primer v. 6 software.

Constrained multivariate analysis is a common tool for linking ecological communities to environmental variables (Sîrbu *et al.*, 2021). According to Shen *et al.* (2016), Principal Component Analysis (PCA) is an important visual aid and dimension tool that identifies impertinent directions reflecting the maximal variation within the data. Therefore, this can be used to project data onto these directions, providing a low dimensional representation of data. Redundancy analysis (RDA) is a widely used technique for determining the relationship between environmental factors and ecological indicators (Shi *et al.*, 2022). A PCA and RDA were used to determine the environmental factors that have the highest contribution towards the distribution of macroinvertebrate communities. The results of the PCA and RDA will provide data to identify suitable indicator taxa for environmental flows.

Ten functional trait categories were selected: body size, body shape, attachment type, respiration, mobility, functional feeding groups, habitat preference, flow preference, hydraulic habitat preference and aquatic life stage. These traits are well documented as good indicators for determining environmental changes (Forio *et al.*, 2017; Reynaga *et al.*, 2020; Sotomayor *et al.*, 2021). Using these traits, diversity indices were employed to determine the functional diversity indicating the taxa dominance and abundance (Pla *et al.*, 2012). Furthermore, the traits were used to determine the influence of water quality, habitat – and flow-preference of the macroinvertebrate community using PCA and RDA multivariate statistical analysis.

In this study, for each set of explanatory variables, forward selection was used to identify the explanatory variables that is the most suitable to model the response variable, where ceasing occurred when a significance level of 0.05 was reached (Dray *et al.*, 2007; Blanchet *et al.*, 2008). Each species data set was analysed through two methods of variation partitioning which included a set of environmental variables and a set of spatial variables (Legendre *et al.*, 2005). Forward selection of predictors, RDA, PCA, and their attribute graphs, variation partitioning with environmental and spatial variables were in accordance with Sîrbu *et al.*, (2021).

2.3 Results

2.3.1 Abiotic analyses

The spatial variation in the water and sediment quality variables influenced the ordination of sites more than the temporal variation. Considering water quality, between the sites there were clear spatial variation in sediment and water quality variables (Figure 2-2). Sites affected by different

environmental variables grouped together and were characterised by the same variables. The PCA analysis revealed the significant spatial separation of Matlabas (21_MATL), Mogalakwena (21_MOGA) and Sand (21_SAND) sites from the rest of the sites (Figure 2-2). Matlabas was associated with TDS and EC whereas Mogalakwena was associated with nitrites and phosphates. The Sand River site was associated with high levels of salts (Cl^- , SO_4^{2-}). The Crocodile River site was characterised by high levels of turbidity. The mainstream sites grouped together on the left side of the x – axis, whereas the tributaries were widespread on the right side of the x – axis.

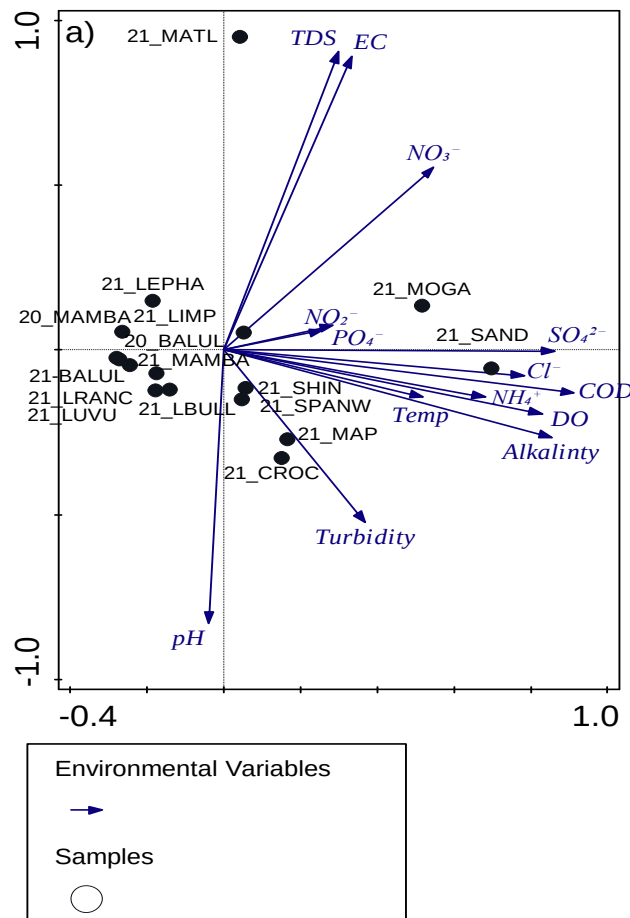


Figure 2-2: PCA biplot of the abiotic environmental variables, water quality variables [(nutrient concentrations (NO_2 , NO_3 , NH_4 , PO_4), anions (sulphate (SO_4), chlorine (Cl)), and physiochemical properties (pH, EC, temperature, dissolved oxygen (DO), turbidity, chemical oxygen demand (COD)) measured at sites in the Limpopo River and tributaries. Axis 1 explains 36.66%, and axis 2 explains 21.86% of the total variation. Site codes are explained in Table 2-1.

Considering only sediment particle size, spatial variation was noted on the PCA biplot. Limpokwena (21_LIMP) was highly associated with very fine sand, whereas both Mamba (20_MAMBA) and Balule (20_BALUL) was characterised by medium sand (Figure 2-3). The cluster of sites on the left of the x – axis was associated with medium sand although Mogalakwena (21_MOGA) was more characterised with coarse sand. Habitat heterogeneity was mostly associated with Balule (21_BALU) and Limpokwena.

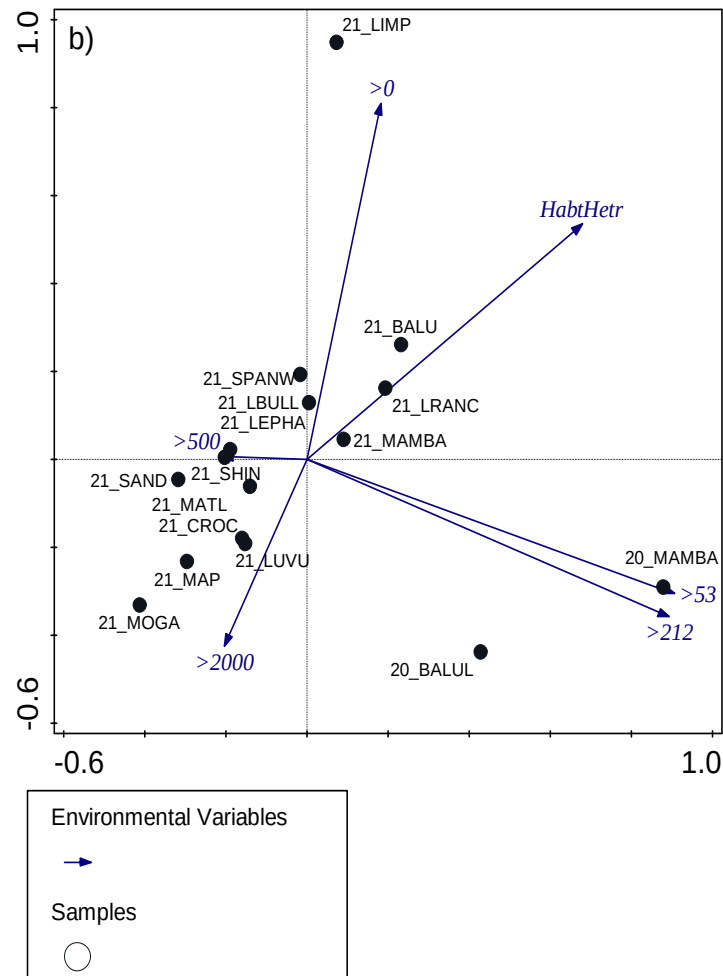


Figure 2-3: PCA biplot of the sediment particle sizes (>0, >53, >212, >500, and >2000) and habitat heterogeneity measured at sites in the Limpopo River and tributaries. Axis 1 explains 36.7%, and axis 2 explains 22.46% of the variation of the data. Site codes are explained in Table 2-1.

2.3.2 Diversity results

Diversity indices were used to portray the diversity of the macroinvertebrate community structures between sampling sites that are color-coded in the graphs below (Figure 2-4). The data obtained explained the diversity between sites sampled. The number of taxa, Margalef's Species Richness, Pielou's evenness, and Shannon Wiener diversity indicated the diversity of the macroinvertebrate community structures per sampling site.

The highest abundance of taxa was found at the Mapungubwe (21_Map) site and Mamba (20_MAMBA) showed the second highest number of species. The Margalef's species richness indicated that Mapungubwe (21_MAP) also had the highest species richness between sites and a significant low richness can be noted at the Mogalakwena site (21_MOGA). Figure 2-4 indicated

that the highest evenness was at Lephalale (21_LEPH) and Mapungubwe (21_MAP) sites. A significantly low evenness was noted at Balule (20_BALU) due to the dominance of *Tarebia granifera*. The Shannon Wiener diversity revealed that Balule (20_BALU) had the lowest diversity although the number of taxa present at the site was higher.

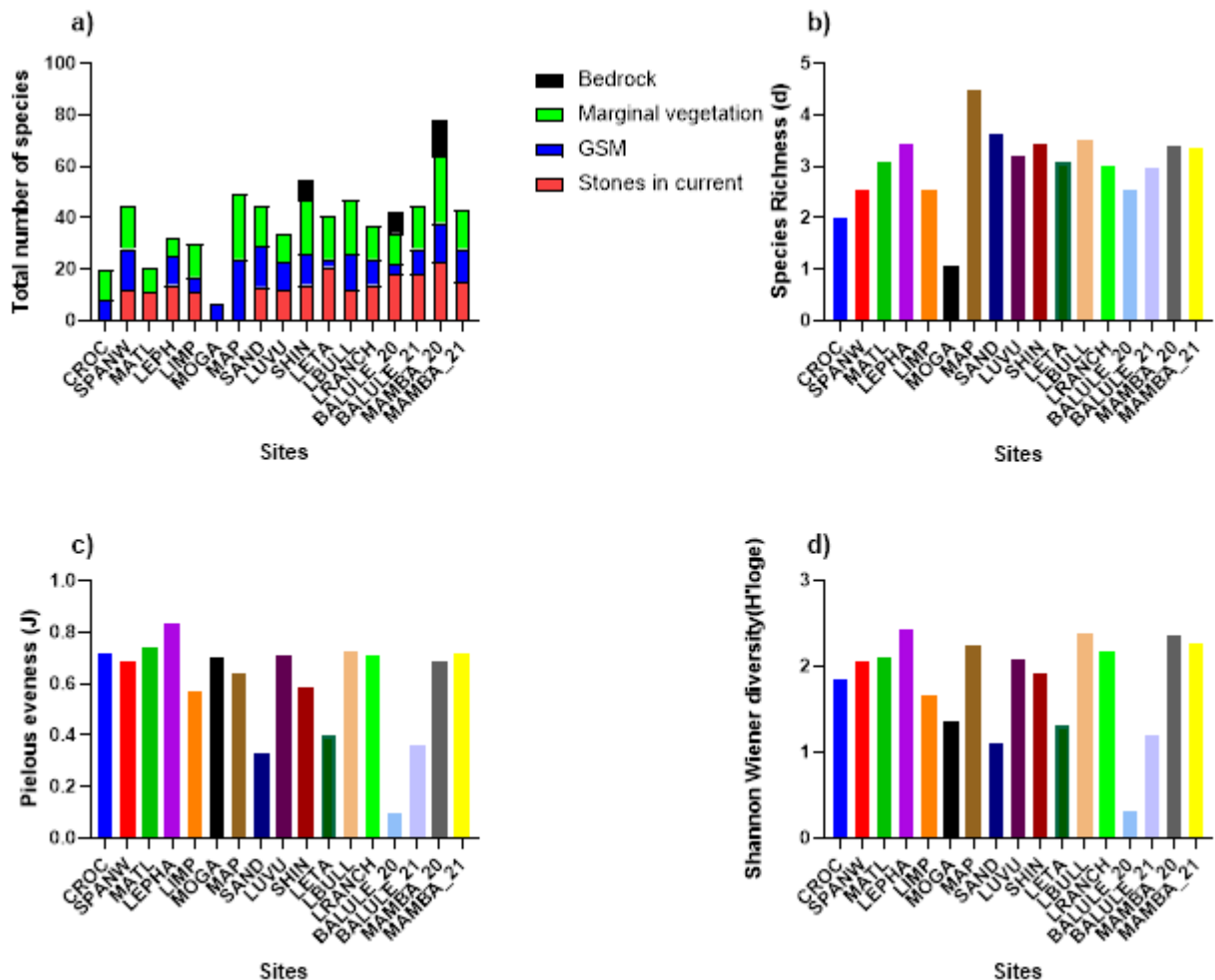


Figure 2-4: Diversity index results between sites sampled in the Limpopo River Catchment; a) total number of taxa per biotope sampled, b) Species Richness, c) Pielou's evenness, d) Shannon Wiener diversity. Site codes are explained in Table 2-1.

2.3.3 Macroinvertebrate community structures

The PCA analysis (Figure 2-5) revealed the spatial variation within the macroinvertebrate community structures between sampling sites. Three large clusters were noted with the largest grouping of taxa (bottom left) and the largest assemblage of sites grouped together, while Mamba (20_MAMBA) was associated with a high abundance of taxa. Mapungubwe (21_MAP) was associated with the smallest grouping of taxa. Chironomidae, Leptophlebiidae and Baetidae was distinctively the outliers of this biplot.

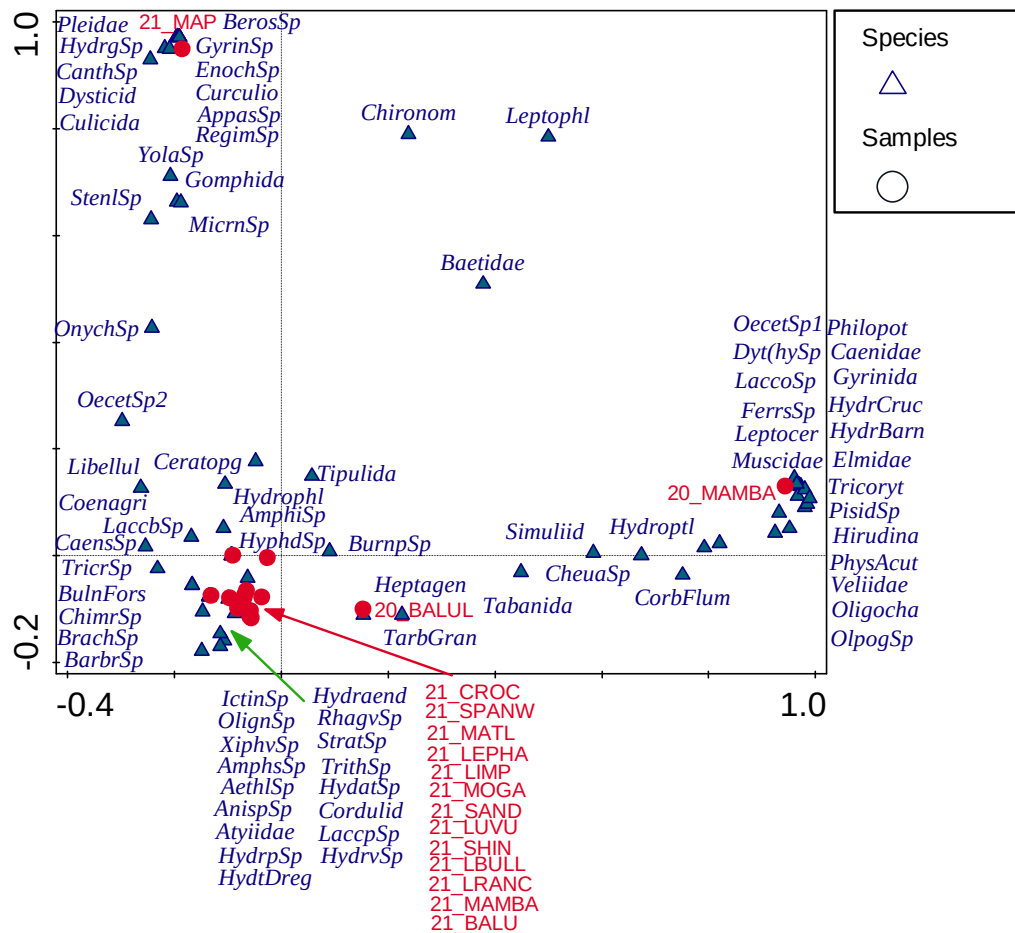
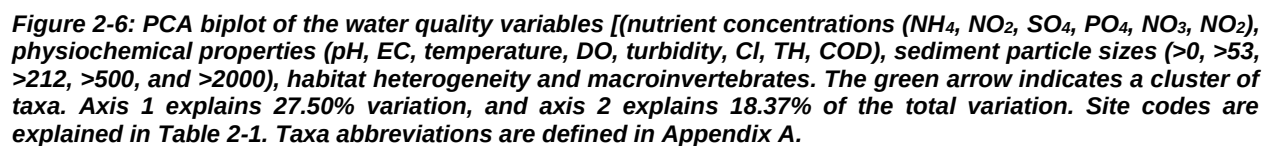


Figure 2-5: PCA biplot analysis showing sampling sites sampled and taxa contribution for the Limpopo River Catchment. The green arrow indicates a cluster of taxa, and the red arrow indicates a cluster of sampling sites. Axis 1 explains 35.18% variation of the data, and axis 2 explains 15.83%. Site codes are explained in Table 2-1. Taxa abbreviations are defined in Appendix A

Considering the macroinvertebrate taxa, water quality and habitat preference variables, clear spatial variation between variables were present in Figure 2-6. Similar to the PCA biplot of samples and taxa (Figure 2-5), three clear groupings of taxa were noted. The largest group of taxa were associated with various water quality variables (NO_2 , SO_4 , PO_4 , NO_3 , Cl , EC , TDS , DO and alkalinity) and very fine sand and silt substrate type, the second largest grouping of taxa was associated with fine sand substrate type and the smallest grouping of taxa was slightly associated with ammonium and to a lesser extent pH and COD . Similar to the PCA mentioned above (Figure 2-5), three outlier taxa occurred: Chironomidae, Leptophlebiidae and Baetidae.

A very strong association was made between Heptageniidae and *Tarebia granifera* with Balule (20_BALUL) (Figure 2-5). These results are most likely due to the lower flow conditions during



24

heterogeneity, although it should be noted that Elmidae was also slightly associated with habit heterogeneity. *Rhagovelia* sp. (water strider), *Tricorythus* sp. (mayfly), *Aethloptera* sp. (caddisflies), *Amphisyche* sp. (caddisflies) and *Oecetis* sp. 2 (long-horned caddisflies) grouped together having no significant associations with any of the variables in the forward variation selection process.

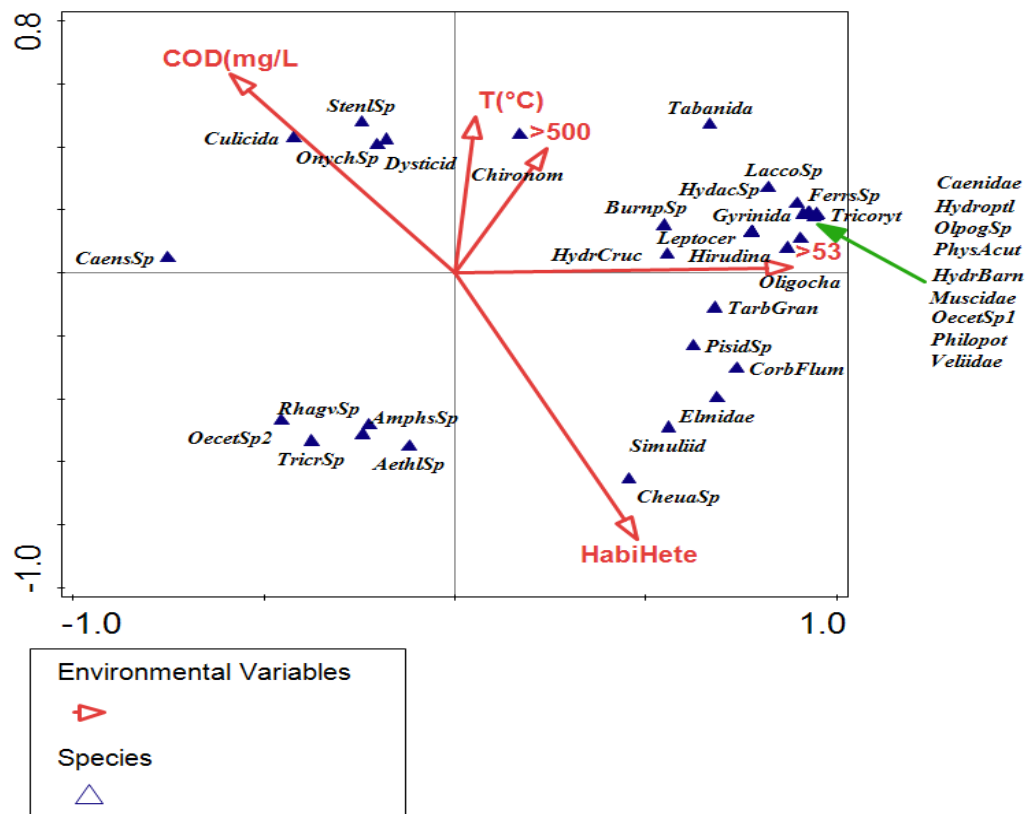


Figure 2-7: PCA biplot of the forward selected variables summarizing the variation in taxa composition that is explained by all environmental variables. The variables which provide the significant changes in communities are sediment particle sizes >500 and >53, HabiHet (Habitat heterogeneity), T (Temperature) and COD (Chemical Oxygen Demand). The green arrow indicates a cluster of taxa at the fine sand (>53). Axis 1 explains 30.69% of the data, 2 explains 44.77% of the data. Site codes are explained in Table 2-1. Taxa abbreviations are defined in Appendix A.

In Figure 2-8, the variation partitioning analysis indicated the drivers that influenced the community structures of macroinvertebrates between sites and explained the percentage the different drivers had on the macroinvertebrate communities. Habitat variables had the highest influence of 56% and the combined drivers of both habitat variables and water quality variables explained 31.2% of the influence.

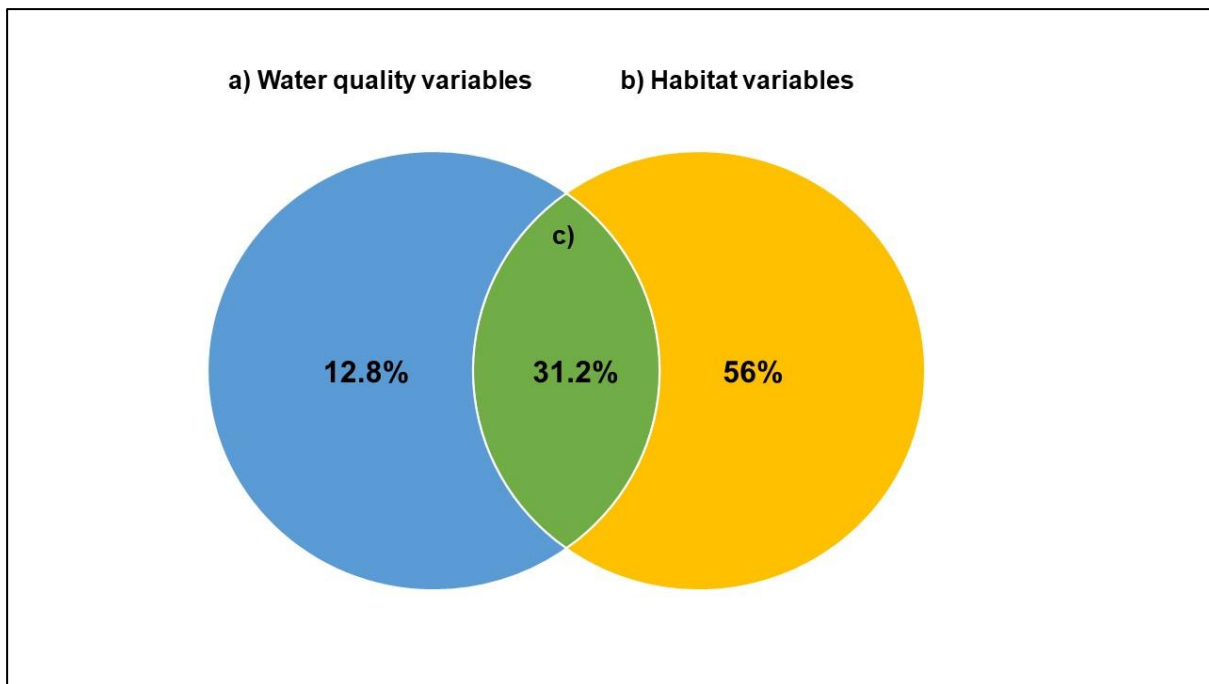


Figure 2-8: Compared variable groups are represented by circles in the following Venn diagram as explained by variation partitioning. The first group is represented by the water quality drivers (a), the second group (b) represents the habitat requirements and (c) represents both components together as drivers of community structure distribution. a) explains 12.8% of the data, b) explains 56% of the data and c) explains 31.2% of the data and results obtained. Site codes are explained in Table 2-1.

2.3.4 Trait analysis

Figure 2-9 presents functional traits of the macroinvertebrate community structure per site. Balule (20_BALU) was dominated by large taxa whereas the very small to medium body sizes were evenly distributed among the other sites. (Figure 2-9a). Figure 2-9b provided the body shape of the taxa and the cylindrical/tubular body shape dominated almost all the sites except for Limpokwena (21_LIMP) that was dominated by other and flattened body shapes, while Sand (21_SAND) was dominated by streamlined body shape. Figure 2-9c indicated a clear dominance of taxa being free living as to having attachment mechanisms and Figure 2-9d clearly indicated a dominance of crawlers over all the sites.

The respiration, functional feeding group, habitat preference and velocity preference are represented in the figures below (Figure 2-10). It is evident that the most taxa present per site have gills for respiration (Figure 2-10a) and at Limpokwena (21_LIMP) there was a strong presence of spiracles used as respiration. Figure 2-10b indicates the functional feeding groups between sites where it is evident that deposit feeders dominated overall except for Balule (21_BALU & 20_BALU) which was dominated by filter feeders. Shredders and collectors were the groups having the lowest abundance among taxa.

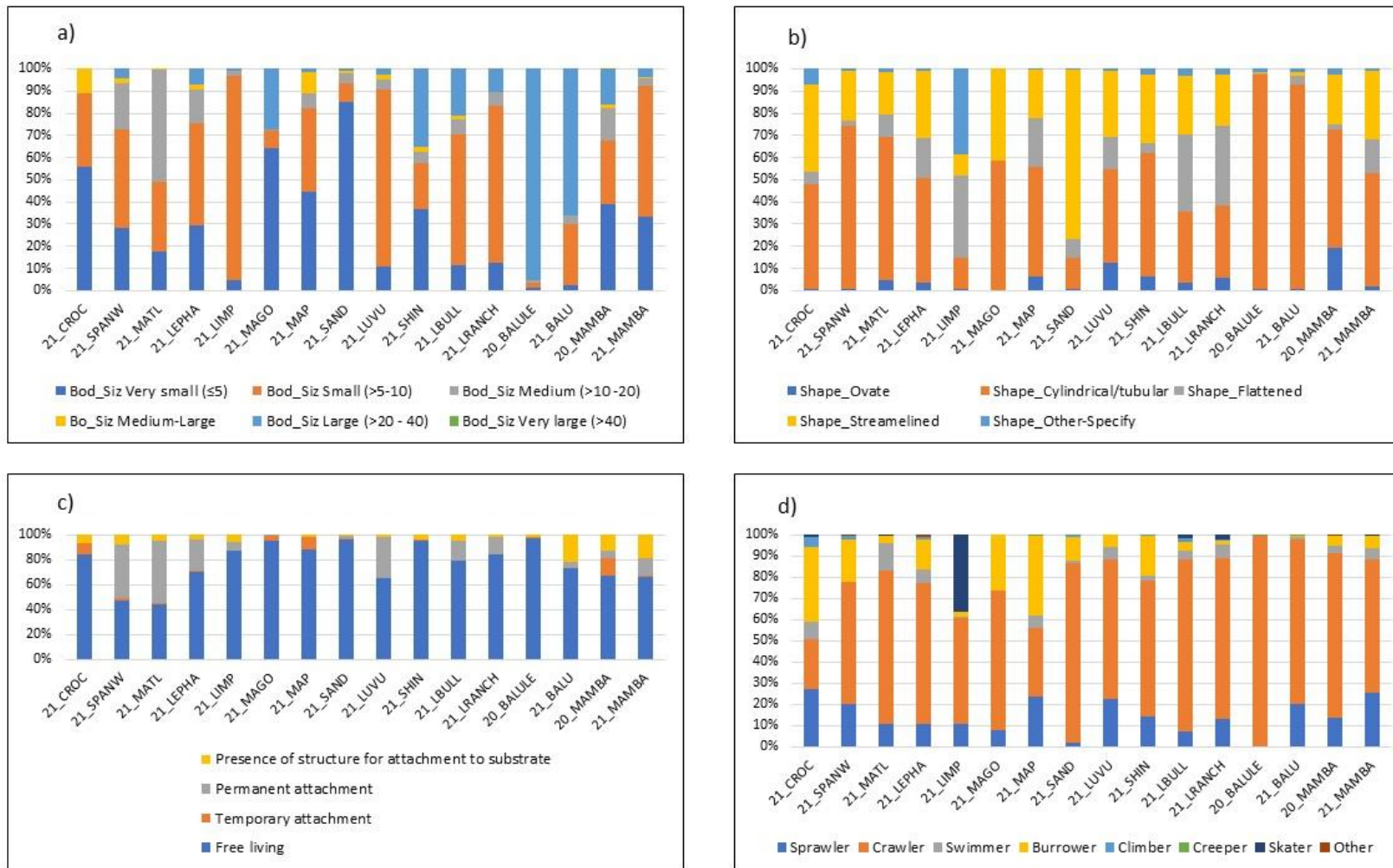


Figure 2-9: Functional traits of the macroinvertebrate community per site in the Limpopo River Basin (Site codes can be found in table 2-1). (a) Body size, b) body shape, c) attachment mechanism, and d) mobility.

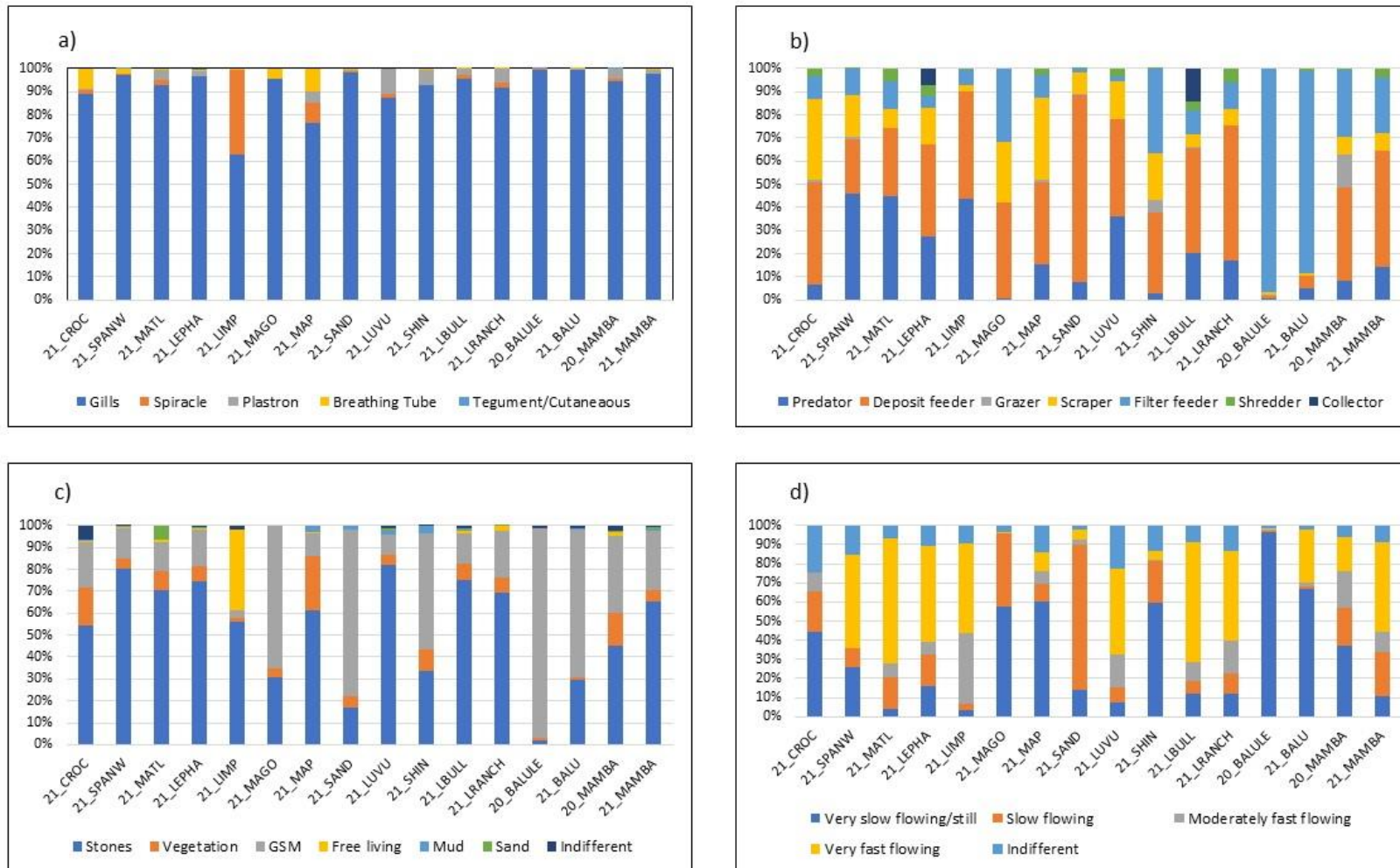


Figure 2-10: Functional traits of the macroinvertebrate community per site. The following graphs represent the following traits: a) respiration, b) functional feeding group, c) habitat preference, and d) flow preference. Site codes are explained in Table 2-1

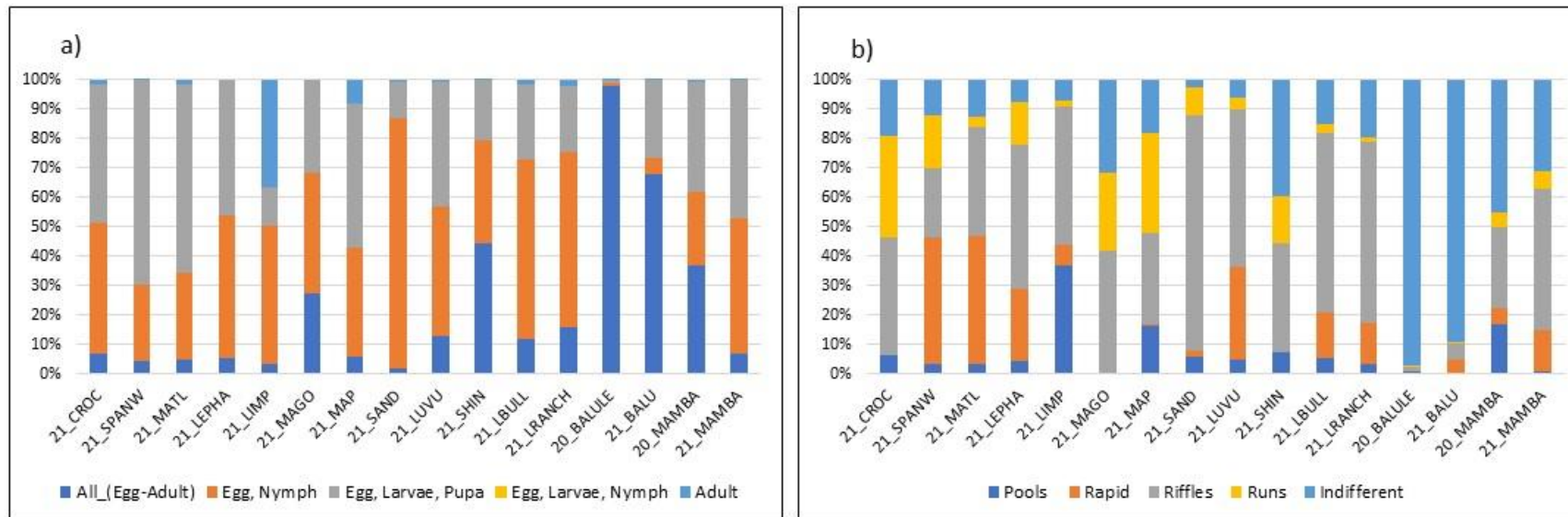


Figure 2-11: Functional traits of the macroinvertebrate community per site. a) aquatic life stage, b) hydraulic habitat preference. Site codes are explained in Table 2-1.

Figure 2-10 c represents the habitat preference of macroinvertebrates and stones dominate over most sites although Balule 2020 (20_BALU) and 2021 (21_BALU), as well as Sand (21_SAND) sites had the lowest score of stones due to taxa that prefers a certain habitat. Velocity preference between sites indicated an evenness between very slow/still and very fast flowing water but Sand (21_SAND) had a dominance of slow flowing velocity preference taxa.

Balule (20_BALU) was dominated by taxa living their entire life cycles within water whereas the other sites were dominated by aquatic stages; egg and nymphs and egg, larvae, and pupa (Figure 2-11a). This could be due to *Tarebia granifera* which dominated this site. Riffles and rapids were the most preferred hydraulic habitat for macroinvertebrates between sites although Balule (20_BALU) and 2021 (21_BALU) indicated the most preferred hydraulic habitat was the indifferent habitat i.e. generalists (Figure 2-11b).

The PCA biplot (Figure 2-12) revealed the combined data of functional traits of macroinvertebrates, water quality variables and habitat variables between sites. Figure 2-12a and 2-12b presented the spatial variation of the communities between sampling sites. Balule (21_BALU) and Mamba (20_MAMBA) grouped together and was associated with filter feeders, large body size, indifferent hydraulic preference, and all aquatic life stages. Most of the functional traits grouped together in the top left of the graph (Figure 2-12a) where the association with the following water quality variables were made (Figure 2-12b): COD, turbidity, NH₄ alkalinity and PO₄. Most of the functional traits was widespread along the left of the x – axis where most of the sampling sites grouped together. Associations were made that most functional traits were grouped with water quality variables although very slow flowing water, GSM and high tolerance was slightly associated with medium to coarse gravel.

The 21_CROC, 21_MAP, 21_SAND, 21_MOGA and 21_SHIN sites grouped together, being associated with various water quality variables, 21_BALUL and 20_MAMBA group together, being associated with nitrates, indifferent hydraulic preferences, all aquatic life stages, filter feeders and large body size, and 21_SPANW, 21_LEPHA, 21_LUVU, 21_MAMBA, 21_MATL, 21_LRANCH, 21_LBULL, 21_LIMP, and 20_BALUL grouped together, being mostly associated with TDS, EC, very fine sand, nitrites, small body size, predators, flattened body shape, and moderately fast flowing water (Figure 2-12a & 2-12b).

The RDA analysis revealed the forward selection of variables which summarizes the variation in taxa composition in terms of environmental variables and functional traits (Figure 2-13). The SO₄ fine sand and coarse silt was associated with the significant spatial variance of the macroinvertebrate community structure. The >0 particle size (very fine sand) was associated with oval body shape, moderately fast flowing water, and very fast flowing water. Filter feeders, large to very large body size, siphon, gills as respiration and have aquatic stages which include egg, larvae, and nymph, and all aquatic life stages grouped together and was associated with fine sand (> 53).

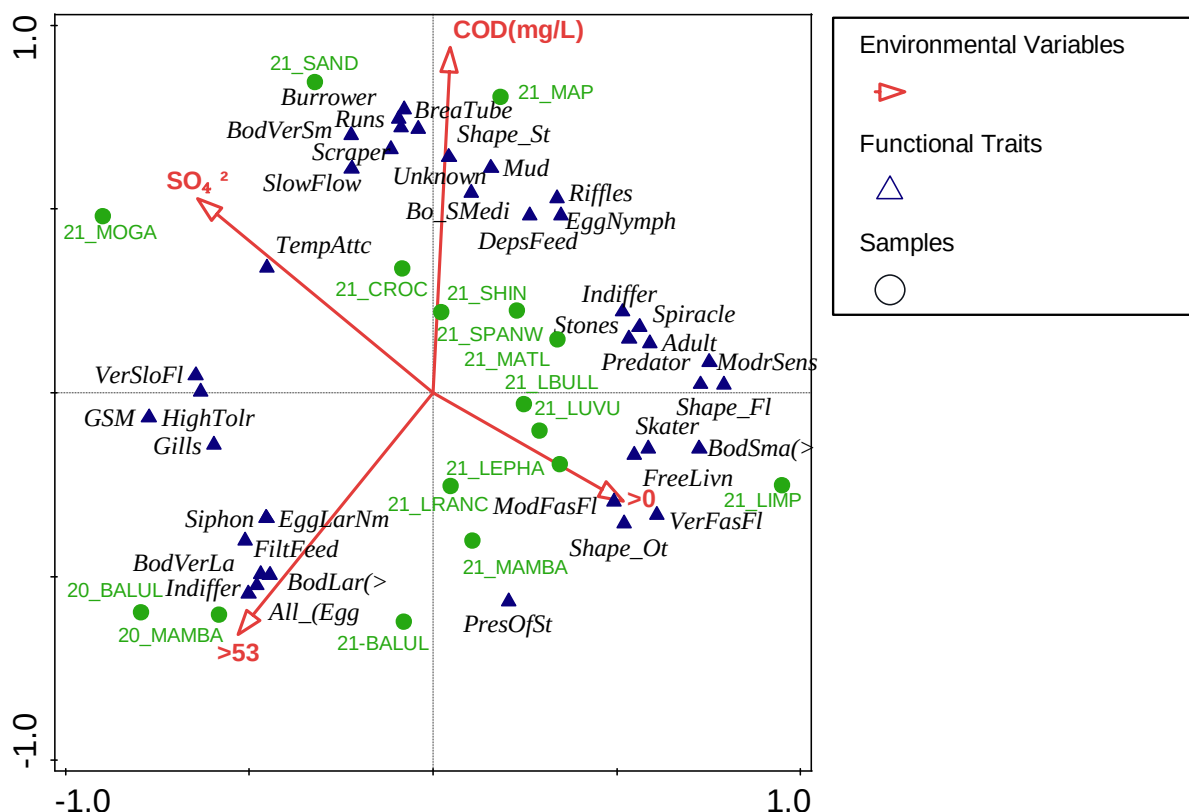


Figure 2-13: RDA triplot of the forward selected variables summarizing the variation in the composition that is explained by all environmental variables and functional traits. The variables which provide the significant changes in communities are sediment particle sizes SO₄, fine sand, coarse silt, and COD. Site codes are explained in Table 2-1.

The drivers that influence the functional traits of macroinvertebrates between sites and the explanation of the different drivers of macroinvertebrate community structure can be seen below (Figure 2-14). Water quality variables had the highest contribution of 51.1% and the sediment particle size explain 41.3% while the combination of the two variables explain 7.6% of the influence on community structures.

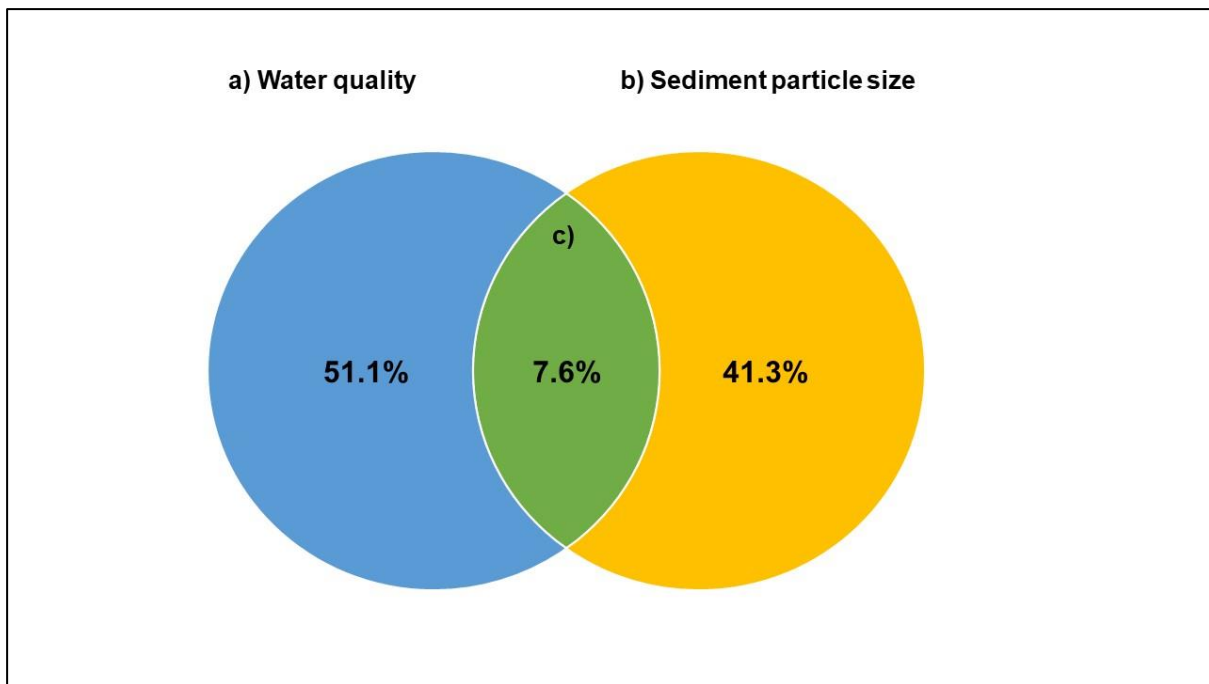


Figure 2-14: Compared groups are represented by circles in the following Venn diagram and explained by variation partitioning. The first group is represented by the water quality drivers (a), the second group (b) represents the functional traits and (c) represents both components together as drivers of community structure. a) explains 51.1% of the data, b) explains 41.3% of the data and c) explains 7.6% of the data and results obtained.

2.4 DISCUSSION

The primary aims of this study was to (1) determine the macroinvertebrate community responses towards water quality and habitat quality within the LRC by assessing both taxonomic and functional trait data and (2) identifying a suitable indicator species reflecting the main driving force of the community structure. In this study site-specific information was gathered and used. The community responses were influenced by both water– and habitat- quality and clear indications were noted that the taxonomic responses were driven by habitat quality and the functional trait responses were driven by water quality.

Taxa should be identified to a sufficient level to allow the detection of changes within macroinvertebrate communities (Terilizzi *et al.*, 2003). Therefore, macroinvertebrates have been identified to the lowest taxonomic level possible in this study and it ranged from family, genus, and species level identifications. Not all organisms could be identified to species level because of the following: damaged organisms and juvenile individuals that could only be identified to genus or species level when fully developed. Identification guides used during this study dated between 199 and 2008 which was also a limiting factor to identifying certain taxa only up to family level. No specific species were identified as indicators for main drivers of the community structures

although a significant dominance of an invasive taxa, *Tarebia granifera*, was present. although the main driver of the macroinvertebrate responses was relating to habitat variables.

2.4.1 Water and sediment quality

The sampling occurred during low-flow periods where the sites in 2020 were dry and below normal compared to 2021. The increase in nutrient concentrations can be due to nutrients accumulating because of lower water volumes (Rolls *et al.*, 2012; King *et al.*, 2015). In 2021, sampling of sites occurred after the first rainfall of the season, although it was still considered to be the low-flow period. The increase of nutrient concentrations can be attributed to runoff from surrounding land use which is transported to rivers through the rain (Patience *et al.*, 2021). The tributaries had significantly higher nutrient concentrations than mainstream rivers sampled within the KNP border, such as the Crocodile River, corroborating with a study conducted by Windisch *et al.*, (2022). This can be attributed to high agricultural land use surrounding the tributaries whereas within the KNP, rivers close to the borders are highly influenced. There were differences between the mainstream and tributaries in terms of traits, habitat, and water quality drivers.

In the PCA with sediment and water quality variables, clear spatial variation was evident. The tributaries mostly grouped together with associations with high levels of the various nutrients and the mainstream river sites within the KNP grouped on the opposite side of the x – axis. The Matlabas River was associated with high EC and TDS that can possibly be attributed to groundwater moving through the rocks and sediment into the river, carrying some of the associated minerals into the river. The Mogalakwena River was associated with high levels of phosphates and nitrates which can be attributed to the very low volume of water present at the site that led to an increase in the concentration levels. The Sand River was a single alluvial channel, and the main drivers of the river was flow related and was associated with high salt concentrations (Cl, SO₄) due to the lower water volumes resulting in an increase in nutrient concentrations. During low flow periods sediment would be less likely to be transported throughout the river and create various substrate types at different points within the river.

2.4.2 Macroinvertebrate community structures between biotopes

Environmental changes can influence the structure of macroinvertebrate assemblages in response to different stressors (Feld & Hering, 2007; Kuzmanovic *et al.*, 2017; Mathers *et al.*, 2019). Such changes include changes in taxa composition, as well as abundance within affected

areas, where the majority is tolerant taxa, while sensitive taxa are present in areas that are minimally disturbed (Oertel & Salanki 2003). Diversity indices were used to determine the abundance, dominance, and distribution of macroinvertebrate taxa among the sites selected for this study.

The Margalef's Species Richness index indicates a similar abundance of species among the stones and vegetation biotopes between sites. The GSM biotope presented a range of Species Richness where the sites Balule (20_BALU) and Letaba (20_LETA) both had very low abundances of species present that could be positively correlated to the low flow conditions during sampling (Appendix C) and similar higher species richness among other sites. The bedrock biotope had a gradual decrease in species richness between 2020 and 2021, although it should also be noted that the bedrock biotope was only present and sampled once at three different sites. In the Balule (20_BALU) site the highest abundance of taxa, that can be explained by the dominance of invasive taxa *Tarebia granifera* and no natural predators are present to keep the numbers low. This taxon is the only invasive taxa present in this study.

The Shannon Diversity Index indicated that all biotopes were rather similar in diversity and can be due to optimal habitat availability and flow conditions. Although low diversities were noticed from the stones and vegetation biotope at the Balule site during 2020 and the GSM biotope at the Balule site from both 2020 and 2021. This provides an indication that the habitat at the Balule site was not optimal for the diversity of organisms, whereas similar diversity among the other sites indicates optimal habitat. The Shannon Wiener Diversity Index indicated that most biotopes between sites were similar in diversity although the vegetation biotope had the highest number of taxa and higher diversity present due to vegetation being optimal habitat for macroinvertebrate communities (Dallas, 2007a; 2007b).

The higher evenness at the bedrock biotope was due to lower flow conditions that created a dominance of bedrock habitat available to inhabit. Among the other sites, the evenness is similar, which relates to more habitat availability. The Balule site (20_BALU) has the overall lowest evenness of all biotopes combined over all sites indicating strong dominance of one taxon namely *Tarebia granifera*, also known to be an invasive species (Appleton *et al.*, 2009; Nwoko *et al.*, 2022). The high abundance of this invasive taxon can be explained by the lower flow conditions in 2020 than 2021 and gives an indication that *Tarebia granifera* prefers slower flowing water (Tolley-Jordan & Owen, 2007).

The biotopes and taxa present within various biotopes provided a clear picture of the habitat preference of the macroinvertebrate communities. The vegetation biotope had the highest

number of taxa among all the sites, as well as the highest diversity of taxa among all the sites. Therefore, it can be concluded that the vegetation habitat is the most preferred biotope for macroinvertebrate communities in terms of the data obtained during this study. The diversity between sites did not give a clear indication that there was a definite decline or increase in inverts from upstream to downstream. The result from this study creates an overall picture that diversity is directly connected to the habitat availability.

2.4.3 Macroinvertebrate responses towards sediment and water quality

The PCA and RDA analysis revealed four main environmental factors influenced the community structure the most between sites namely: fine sand and medium sand, habitat heterogeneity and temperature. Chironomidae, which are not sensitive and are a generalist taxa, were present in GSM, cobbles, and vegetation which is a confirmed preference from previous studies (Heath *et al.*, 2010; Ashton & Dabrowski, 2011; Williams *et al.*, 2014) although not in high abundances and not accompanied by other generalist taxa more tolerant to pollution such as Hirudinae and Oligochaeta (Thirion, 2007). This gives an indication that the water is not polluted although further studies should be explored to determine the indicator organisms of pollution within these river systems. Being a more tolerant family, Chironomidae should be regarded as a suitable indicator organisms for ecological effects in aquatic ecosystems rather than sensitive families (Bervoets *et al.*, 2016). The COD influenced taxa that prefer oxygen rich environments although the results do not indicate a specific taxa that had been highly influenced by the COD in particular. *Tricorythus* sp.(mayfly), *Aetheloptera* sp.(caddisflies), *Amphisyche* sp. (caddisflies) and *Oecetis* sp. 2 (long-horned caddisflies) preferred higher flow conditions (de Moor *et al.*, 2003), was associated with higher concentrations of nitrates and nitrites, and a high preference for coarse silt sediment. The limited habitat available created an association with less desirable water quality. These species could be used as an indicator of flow modification as they had a high preference for flow (Fry, 2021) and the result of this study confirmed this preference.

The PCA results indicated that most of the sites (eight in total) had been influenced more by water quality whereas six of the sites were driven by both water quality and habitat variables, similarly. Both Mamba (20_MAMB) and Balule (20_BALU) were mostly driven by sediment substrate type which can be attributed by the very low velocities during the 2020 sampling survey (Appendix B and C). Velocities increased during 2021 sampling because of the first rainfall before sampling occurred.

There was a grouping of taxa at the Mamba site (20_MAMB), and it had a very high diversity and abundance of taxa presented by the diversity indices. The main drivers of this variation could be attributed to the preference of fine sand and medium sand as this substrate type was dominant within the site. The Mamba and Balule sites of 2020 were definite outlier sites due to the lower flow conditions during the sampling period in 2020 which had high associations with habitat type than flow preferences, which was limited. *Cheumatopsyche* sp, Simuliidae and Elmidae was mostly influenced by habitat heterogeneity, associated with stones and vegetation biotopes, which relates to previous studies (Dallas, 2007a; 2007b; Bloechl *et al.*, 2010; Masese *et al.*, 2020) due to high habitat availability. These species can be an indicator for the stones and vegetation habitat preference.

2.4.4 Functional trait responses towards sediment and water quality

Macroinvertebrate traits could be used to refine the macroinvertebrate taxa's preference in terms of habitat variables and water quality. The body sizes very small and small dominated the overall trend of this study which can be attributed to sampling after the first rain that provided for the creation of more habitat and larger organisms to be washed downstream. The body size large dominated the site Balule in both 2020 (20_BALU) and 2021 (21_BALU) and could be used as an indicator trait for that specific site which is associated with variables including nitrate, fine and medium sand. The Limpokwena site (21_LIMP) provided several traits which could be used as indicator traits. This site is highly associated with coarse silt to clay type of sediment and the following functional traits are prominent at this site: flattened body shape, skaters, and spiracle respiration. Among the other sites, cylindrical/tubular body shape, gills and crawlers was the dominating functional traits overall. The Sand (21_SAND) site was highly influenced by water quality variables and a streamlined body type dominated this site, giving indication to a possible indicator trait towards water quality. Although there is a small difference in the percentage of influence that water quality and sediment particle size have, it remains evident that water quality is the main driving force of macroinvertebrate functional trait distribution.

2.5 CONCLUSION

There was a difference in the responses between the taxonomical approach as to the functional traits approach. The taxonomical approach towards the distribution of macroinvertebrates and the influence of water quality compared to habitat variables indicated clearly that habitat variables was the main driver of the macroinvertebrate community structure within this study. Whereas the

functional trait approach revealed that water quality and sediment substrate type had a similar influence on the functional trait spatial variation, although water quality had a 10% higher contribution to the variation.

Diversity results indicated that there was a similar diversity pattern between sites although one site, 20_BALU, had the lowest overall diversity and the highest abundance of *Tarebia granifera* which is considered to be an invasive species and was explained by the limited habitat availability and very low flowing conditions during sampling. The traits revealed a higher possibility of the identification of indicators relating to sites associated with habitat variables, especially regarding sediment substrate type, than the taxonomical approach results indicated. Fine sand was the most preferable substrate type among all the sites and follow up studies will allow the confirmation of this finding.

Strong associations of functional traits and sediment substrate types were revealed as macroinvertebrates with a flattened body, have spiracles to breathe, and are skaters for mobility was associated closely with coarse silt to clay substrate type which was the less dominant sediment substrate type among sites. Further studies on significant functional traits, habitat variables and water quality variables identified could support the findings of the data in this study. Further in depth studies can focus on not only spatial variation but also temporal variation to provide a more accurate representation of data.

Chapter 3

Flow and Habitat Preferences of Macroinvertebrate Indicators (Taxonomic and Traits) within the Olifants River, South Africa

3.1 Introduction

Freshwater ecosystems, particularly rivers (Tickner *et al.*, 2020; Albert *et al.*, 2021), are among the most vulnerable ecosystems worldwide due to various anthropogenic activities (Vörösmarty *et al.*, 2010; Ashton & Dabrowski, 2011; Bunn, 2016). The Olifants River is a tributary of the Limpopo River located in eastern part of South Africa and is known as one of the largest rivers traversing the Kruger National Park (KNP). According to Machete & Shadung, (2019), the Olifants River system is one of the main providers of industrial, mining, agricultural and domestic uses of water. As a result, pollution arises from the intensive anthropogenic activities along the river having a major impact on aquatic biodiversity (Batchelor, 1992; Engelbrecht, 1992; Ballance *et al.*, 2001; Van Vuuren, 2009; Rashleigh *et al.*, 2009; De Villiers & Mkwelo, 2009; Ashton, 2010; Heath *et al.*, 2010; LBPTC, 2010; CSIR, 2012; Malakane *et al.*, 2020). The water quality varies from moderate to poor (de Lange *et al.*, 2005; Chapman, 2006; Beumer, 2010). Mining is one of the primary contributors to the negative effects on river health (Moore *et al.*, 1991; Ballance *et al.*, 2001; O'Keeffe & Le Quesne, 2009; Ashton & Dabrowski, 2011). Furthermore, deterioration of the water quality, that includes the instream habitat, as a result of anthropogenic activities along the river must be contained. South Africa is known to be a semi-arid country where rainfall is low, irregular, and seasonal and as a result a wide range of aquatic ecosystems have adapted to various water quality regimes and flow patterns (DWAF, 1996). According to Wolmarans *et al.* (2014), a controversy exists around the health of the Olifants River, which could be due to lack of detailed studies of macroinvertebrate diversity of this river, especially identifying macroinvertebrates to lower taxonomic levels (Marr *et al.*, 2017).

Biological monitoring is used for assessment and indication purposes in terms of ecological degradation within an environment (Bredenhand, 2005) and is a common tool used to assess water quality as it provides information about past and present conditions of the water. The organisms present within the river have adapted to survive environmental changes which the river has been exposed to previously (Davies & Day, 1998). Therefore, this information can be used to determine past and present changes within the river. Aquatic macroinvertebrates have often been used in the biomonitoring of river and stream ecosystems for numerous environmental stressors for example organic pollution, nutrient enrichment, metals, and acidification (Li *et al.*, 2010). The tolerance levels differ between species and the information obtained from

biomonitoring assessments are used to provide a reflection of the water quality in the river and can be used as a warning system for detecting water pollution.

Rivers are some of the most threatened ecosystems on a global scale (Tickner *et al.*, 2020; Albert *et al.*, 2021) with their natural flows being altered and as a result, an increase in fragmentation occurs due to weirs, impoundments and floodplain development (Grill *et al.*, 2019; Belletti *et al.*, 2020). Natural flows within rivers are one of the main drivers in riverine ecosystems (Rolls *et al.*, 2017) with variances in streamflow affecting the in-stream aquatic ecosystems (Tockner *et al.*, 2010). Macroinvertebrates inhabit rivers and is a major component to biodiversity worldwide and provide various ecosystem services (Dudgeon *et al.*, 2005; Macadam & Stockan, 2015; Raitif *et al.*, 2019). They are also very sensitive to alterations within rivers (Dewson *et al.*, 2007; Hart & Finelli, 1999; Kakouei *et al.*, 2017). Studying the macroinvertebrate responses to flow can provide valuable information for the management of riverine ecosystems.

Riverine organisms respond differently to a range of environmental variables that include natural habitat conditions and stressors (Kakouei *et al.*, 2018). The influence of such stressors, i.e., land-use, climate, and substrate conditions, on riverine species are well understood (Miserendino *et al.*, 2011; Schröder *et al.*, 2013), although the relationship between the abundance of riverine species and the flow of the river is poorly understood (Poff *et al.*, 1997; Kuemmerlen *et al.*, 2014; Pyne & Poff, 2016). Flow (i.e., discharge) is one of the main environmental variables within a river ecosystem (Arthington *et al.*, 2006; Dewson *et al.*, 2007; Domisch *et al.*, 2017;). Over the past years, the detrimental effects of alterations in flow regimes have been recognized. The triggers of detrimental effects include flow reductions due to surface water abstractions, abstractions of groundwater impacting the baseflow, alterations in runoff because of inappropriate catchment land use, as well as alterations to downstream river flow regimes due to upstream impoundments (Mittal *et al.*, 2014; Ramulifho *et al.*, 2019). The changes in the natural flow conditions are most likely the biggest threat to the integrity of aquatic ecosystems (Bunn & Arthington, 2002; Ahn *et al.*, 2018; Al-Jawad *et al.*, 2019). The change and decline in stream flow can result in rivers changing in perennality for instance from permanent to non-perennial (Ramulifho *et al.*, 2019). These alterations have devastating effects as rivers provide habitat structures based on their flow (Strayer & Dudgeon, 2010; Vörösmarty *et al.*, 2010; Rivers-Moore *et al.*, 2013). Flow has been predicted to decrease by up to 35% by 2050 in rivers within the Limpopo catchment due to ongoing climate change (Tharme, 2003; Nilsson & Renöfält, 2008).

Both hydrological and ecological processes functioning on a large scale, provide room to create smaller-scale habitats which result in a natural, interconnected hierarchy where species become more specialised by adapting to habitat conditions that are site-specific (Bedoya *et al.*, 2011).

Alterations in flow are among the most important environmental factors and often reflect in biological responses as flow patterns have a vital influence on the presence and distribution of different types of aquatic species within riverine systems (De Moor, 2002; Bunn & Arthington, 2002; Lloyd *et al.*, 2003; Jackson *et al.*, 2007; Poff & Zimmerman, 2010; White *et al.*, 2016; Kuemmerlen *et al.*, 2015; Pyne & Poff, 2016; Tonkin *et al.*, 2017). Recently, discharge information has been used to analytically derive quantitative flow preferences of macroinvertebrates (Kakouei *et al.*, 2017) and indicate species response along various flow conditions. Macroinvertebrate species are known to have different sensitivities to pollution levels and habitat alterations and thus very useful bioindicators (Dallas & Day, 2004). Suitable and healthy water quality is crucial to maintain healthy communities of aquatic organisms (Dickens and Graham, 2002).

The ecological traits of species have been reported to be informative and the data provides clues to the poorly understood components that threaten species' abundances in their environment (Matthews & Marsh-Matthews, 2003). Macroinvertebrates adapt to the ever-changing ecosystems and therefore there is a high importance of trait and taxonomical based studies. Traits refer to any morphological, behaviour, or physiological characteristic of an organisms and ecological preferences are ecological products of the interactions between organisms and their external environment (Edegbene *et al.*, 2020a; 2020b; Edegbene *et al.*, 2021). Both traits and ecological preferences can be used to determine the ecological integrity of riverine ecosystems, especially the macroinvertebrate taxa which possess traits and ecological preferences enabling them to respond differently to habitat alterations (Odume *et al.*, 2018; Edegbene, 2020b).

Macroinvertebrate community structures can provide a picture of the biotic integrity or ecological health of a river ecosystem (Oberholster *et al.*, 2005; Masese *et al.*, 2009; Malherbe *et al.*, 2010; Arimoro *et al.*, 2011; Marr *et al.*, 2017) and therefore macroinvertebrates will be used in this quantitative study to determine indicator organisms reflecting flow-and habitat-preferences from preselected sampling sites in the Olifants River. The present study explored the major drivers on the macroinvertebrate community structures within the Olifants River during low flow periods and will contribute to a better understanding of the distribution and patterns of macroinvertebrate preference and response to flow and habitat preferences. The main aim of the study was to identify suitable indicator taxa for flow and habitat preferences using taxonomic classification and selected macroinvertebrate traits.

3.2 Methods

3.2.1 Description of Study Area

General

The Olifants River Catchment is threatened due to the high demand for natural resources, pollution and related land alteration, specifically within the upper reaches (Ballance *et al.*, 2001; Wolmarans *et al.*, 2014). The whole catchment area is further distinguished by the widespread invasion of alien vegetation and, less significantly, by alien fauna (Ballance *et al.*, 2001). The Olifants River is one of the main river systems in South Africa (Oberholster *et al.*, 2005) that traverses across the Mpumalanga and Limpopo Provinces. The Olifants River is a tributary of the Limpopo River and one of the largest rivers flowing through the Kruger National Park (Marr *et al.*, 2017) although it is considered to be one of the most polluted river systems in South Africa (Heath *et al.*, 2010; Ashton and Dabrowski 2011; Marr *et al.*, 2017).

Two sites, Mamba and Balule, were sampled on the Olifants River during the low flow periods of 2020 and 2021 (Figure 3-1). Biotopes were sampled in duplicate, where biotope habitat was present.

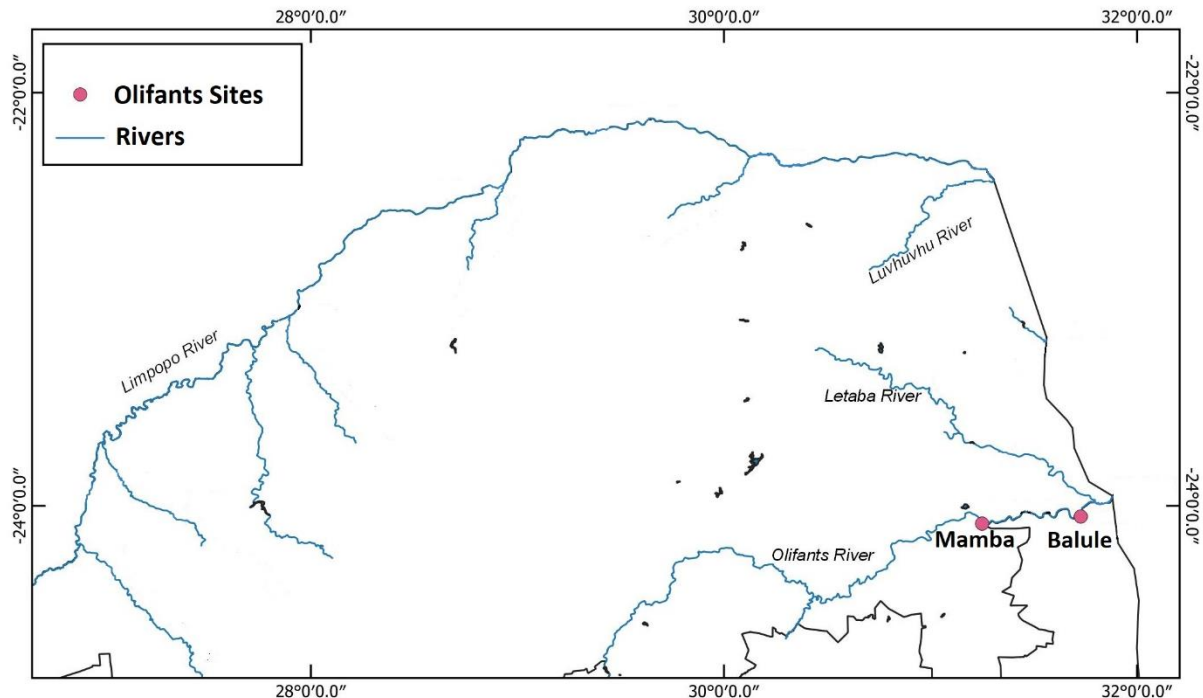


Figure 3-1: A map indicating the locations of the two sampling sites in the Olifants River, KNP.

Sampling sites

The two sampling sites selected for this study are described below in terms of name, site code, locality and general comments of the river condition (Table 3-1).

Table 3-1: Temporo-spatial details and codes used for Mamba and Balule sites sampled in 2020 and 2021.

Code	River	Latitude (S)	Longitude (E)	Date	Comments	Site photos
20_MA MBA	Olifants	-24.08642	31.25094	29-Sep-20	Low flow, water, algae very dominant on substrates in flowing portions. Habitat heterogeneity high. Biotopes sampled: three SIC, four vegetation, 2 bedrock, and two GSM.	
20_BA LUL	Olifants	-24.05214	31.72878	1-4 Oct 20	High rainfall in the upper catchment and on-site resulted in dramatic changes, e.g., turbidity, inundated substrates, accessibility, and more. Water turned from clear to red brown. Biotopes sampled: three SIC, three vegetation, one bedrock and one GSM.	
21_BA LUL	Olifants	-24.05214	31.72878	3-May-21	High flow, with high habitat heterogeneity. Deposition occurs above the crossing during high flows, with gravel-cobble-sand bed several layers deep. Biotopes sampled: two SIC, two vegetation and two GSM.	
21_MA MBA	Olifants	-24.08642	31.25094	5-May-21	High flow, slightly turbid waters with high habitat heterogeneity. Biotopes sampled: two SIC, two vegetation and two GSM.	

3.2.2 Field Surveys

Various equipment was used during the field surveys and samples have been collected using quantitative methods of sampling. Aquatic macroinvertebrates were sampled from different biotopes and at different flows present per site in 2020 and 2021. Water and macroinvertebrate

samples are stored separately for further analyses in the laboratory. Surveys were conducted during low flow periods of the Olifants River.

Flow determination

Depth (m) and velocity (m/s) measurements were completed using an OTT MF Pro which is a handheld unit comprising of a sensor that uses a magnetic-inductive current for accurate determination of stream velocity (Pizarro *et al.*, 2020; Bandini *et al.*, 2022). Depth measurements were taken in increments of percentage, starting with 20%, then 60% and lastly 80% below the water's surface (Table 3-2). The categories used to determine habitat heterogeneity are represented in Table 3-2. below.

Table 3-2: Velocity, depth, substrate, hydraulic biotope, and turbulence categories used in determining habitat heterogeneity

Velocity (m ³ /s)	Fast >0.6	Moderate 0.6-0.3	Slow 0.3-0.1	Stagnant <0.1			
Depth (m)	Very shallow <0.1	Shallow 0.1-0.2	Shallow Intermediate 0.2-0.3	Intermediate 0.3-0.4	Deep intermediate	Deep >0.5	Not wadable >1.5
Substrate	Bedrock	Boulder	Cobble	Gravel	Sand	Mud	Slit
Vegetation	Grass	Herbs	Sedges	Reeds	Branches	Root wads	Algae
Hydraulic Biotope	Cascade	Chute	Rapid	Riffle	Run	Glide	Pool

Habitat variables

Quantitative sampling was used to collect data through specifically sampling selected areas within various depths, velocities, hydraulic biotopes, substrates, and vegetation types. The selected areas are evaluated in terms of the habitat present and described regarding the above-mentioned variables.

Aquatic macroinvertebrates

Refer to chapter 2 for methods used for sampling aquatic macroinvertebrates.

3.2.3 Laboratory Analysis

Macroinvertebrate samples were analysed using a Zeiss stereo microscope by identifying the organisms collected to the lowest taxonomic level possible. Identification was conducted by using identification books (Day *et al.*, 1999; Day *et al.*, 2001; Day *et al.*, 2002; Day *et al.*, 2003; Harrison, 2009). Abundances of species was recorded specifically in terms of biotopes and flow conditions present per site. Functional traits of aquatic macroinvertebrates were analysed using trait by taxon matrix comprising of 10 traits: body size, body shape, attachment type, respiration, mobility, functional feeding groups, habitat preference, flow preference, hydraulic habitat preference and aquatic life stage.

3.2.4 Data Analysis

The PCA and RDA analyses were done based on the methods described in Chapter 2. According to Naman *et al.* (2020), instream flow management trade-off can be evaluated using Habitat Suitability Curves (HSCs) (e.g., the physical habitat simulation model). The HSCs are the most generally used methods for describing environmental requirements of aquatic organisms (Bovee, 1986; Vismara *et al.*, 2001; Tharme, 2003; Conallin, 2010; Poff *et al.*, 2017; Yi *et al.*, 2017; Szalkiewicz *et al.*, 2022). Methods described by Bovee (1986) and Jowett *et al.* (2008) were used to determine HSCs in terms of flow velocities, depth and substrate types. The suitability is frequently expressed by a suitability index (SI), that is determined based on the abundance of the organism within specific habitat parameter values (Szalkiewicz *et al.*, 2022). There are three broad curve types: Category 1 curves, referring to the use of both professional and literature opinions, usually from negotiated definitions derived through the Delphi technique. Category 2 curves, which are based on the frequency distributions of habitat characteristics that are measured at locations utilized by target organisms. Lastly, Category 3 curves, which are created to reduce the bias related with habitat availability by factoring out the impact of limited habitat choice (Thirion, 2016). Category 2 curves, i.e. habitat preference curves (HPC's), were applied in this study. HPC's were determined based on habitat availability curves (HAC's) and habitat utilization curves (HUC's). The HAC's regulate the number of habitats available in the river at the time of sampling and HUC's show the use of habitats by organisms at the time the measurements were taken and is the ratio between habitat availability and habitat use (Szalkiewicz *et al.*, 2022).

Taking both curves into account, HPC's predict the likelihood that a habitat will be selected if all other habitats are equal.

3.3 Results

3.3.1 Flow and habitat preferences

The PCA biplot (Figure 3-2) explained 56.78% of the total variation within the macroinvertebrate community structure and overlays the combined data of velocity and depth preferences of macroinvertebrate taxa between biotopes. The biplot (Figure 3-2a) revealed a widespread spatial distribution of taxa among the environmental variables. The SIC biotope was associated with Simuliidae and *Cheumatopsyche* sp. whereas GSM was mostly associated with Oligochaeta, and Vegetation had a wide range of taxa. Depth had a higher association with a higher abundance of taxa than velocity, which had only a small cluster of taxa of which *Tricorythus* sp. was highly associated with fast flowing water

The biplot revealed a clear spatial separation of the Mamba sampling site between the different years (20_Mamba and 21_Mamba). All the samples from Mamba site of 2021 and the one GSM biotope sampled at Mamba during 2020 are on the left axis whereas, the remaining biotopes sampled at Mamba during 2020 fall on the right axis (Figure 3-2b). The biplot distinctly indicates the sites associated with the different environmental variables. Two Mamba (2020) sites are associated with shallow depth, whereas two 2021 Mamba sites were accompanied by two 2021 Balule sites, all within the SIC biotope, strongly associated with faster flowing water and the other two 2021 Mamba sites associated with vegetation, one was sampled within the vegetation biotope and the other GSM.

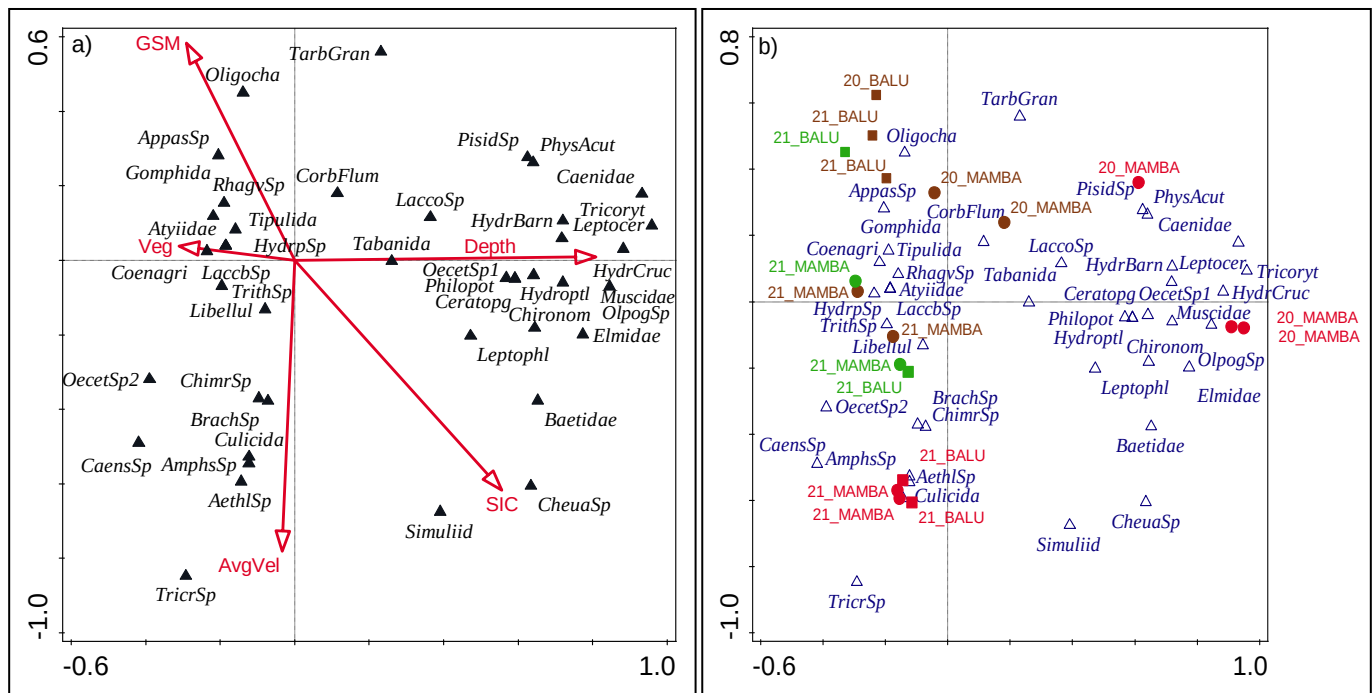


Figure 3-2: a) PCA biplot of the environmental variables (biotopes vegetation (veg), gravel, sand and mud GSM and stones; velocity and depth) and macroinvertebrate taxa in the two sampling sites, Balule (squares) and Mamba (circles), in the Olifants River, KNP. b) Biplot of the sampling site and macroinvertebrate taxa (the following colors represent the following biotopes: green – vegetation, red – stones in current (SIC), and brown – gravel, sand and mud (GSM)). Axis 1 explains 30.44% of the data, and axis 2 explains 16.39% variation of the data. Taxa abbreviations are defined in Appendix A

The table below (Table 3-3) summarizes the response of the macroinvertebrate communities towards depth. The bold values suggest taxa of significant value that responded in a significant manner towards depth. The following taxa was revealed: Baetidae, Ceratopogonidae, *Cheumatopsyche* sp. (caddisflies), Chironomidae, Elmidae, Hydroptilidae, *Hydroptila cruciate* (caddisflies), *Hydroptila barnadi* (caddisflies), Muscidae, *Oecetis* sp. 1 (long-horned caddisflies), *Olpogastra* sp. (dragonfly larvae), and Tricorythidae.

Table 3-3: Summary of fitted generalized linear models (GLM) fitted for the response variables towards depth (m). The values in bold suggest the significant taxa:

Order	Family	Taxa	Nr of occurrences (/18)	R2 (%)	F	p
Trichoptera	Hydropsychidae	<i>Aethaloptera</i> sp.	5	1.3	0.22	0.65
Trichoptera	Hydropsychidae	<i>Amphisysche</i> sp.	2	2.8	0.45	0.51

		<i>Appasus</i> sp.	1	1.9	0.32	0.58
Decapoda	Atyiidae		1	0.4	0.07	0.80
Ephemeroptera	Baetidae		13	22.7	4.7	0.05
Odonata	Libellulidae	<i>Brachythemis</i> sp.	1	1.2	0.19	0.67
Ephemeroptera	Caenidae		5	20.3	4.1	0.06
Ephemeroptera	Caenidae	<i>Caenis</i> sp.	10	2.9	0.47	0.50
Diptera	Ceratopogonidae		5	58.5	22.6	0.01
Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i> sp.	11	21.9	4.5	0.05
Trichoptera	Philopotomidae	<i>Chimara</i> sp.	1	1.4	0.23	0.64
Diptera	Chironomidae		15	72.2	41.6	<0.00001
Odonata	Coenagrionidae		3	0.2	0.03	0.87
Venerida	Cyrenidae	<i>Corbicula fluminalis</i>	9	6.2	1	0.32
Diptera	Culicidae		2	2.4	0.39	0.54
Coleoptera	Elmidae		11	40.9	11.1	0.004
Odonata	Gomphidae		3	6.5	1.1	0.31
Coleoptera	Hydrophilidae	<i>Hydrophylis</i> sp.	1	0.4	0.07	0.80
Trichoptera	Hydroptilidae	<i>Hydroptila barnadi</i>	2	70.1	37.4	0.00001
Trichoptera	Hydroptilidae	<i>Hydroptila cruciata</i>	8	80.2	65	<0.00001
Trichoptera	Hydroptilidae		1	69.3	65	<0.00001
Coleoptera	Hydrophilidae	<i>Laccobius</i> sp.	1	0.4	0.07	0.80
Hemiptera	Naucoridae	<i>Laccocoris</i> sp.	2	4.2	0.71	0.59
Trichoptera	Leptoceridae		3	2.4	0.14	0.14
Ephemeroptera	Leptophlebiidae		2	10.7	1.9	0.18
Odonata	Libellulidae		1	0.5	0.08	0.79

Diptera	Muscidae		2	73.2	43.8	<0.00001
Trichoptera	Leptoceridae	<i>Oecetis</i> sp. 1	1	69.3	36.1	0.00002
Trichoptera	Leptoceridae	<i>Oecetis</i> sp. 2	11	3.8	0.63	0.56
Haplotaxida, Lumbriculida and Moniligastrida	Oligochaeta		2	1.6	0.26	0.62
Odonata	Libellulidae	<i>Olpogastra</i> sp.	2	74.1	45.7	<0.00001
Trichoptera	Philopotomidae		1	12.1	2.2	0.16
Hygrophila	Physidae	<i>Physella acuta</i>	4	45.4	13.3	0.002
Sphaeriida	Sphaeriidae	<i>Pisidium</i> sp.	9	12	2.2	0.16
Hemiptera	Rhagovelia		2	0.0	0.003	0.96
Diptera	Simuliidae		13	0.1	0.01	0.92
Diptera	Tabanidae		3	0.1	0.01	0.93
Clade – Caenogastropoda Clade- Sorbeoconcha	Thiaridae	<i>Tarebia granifera</i>	17	3.5	0.58	0.54
Diptera	Tipulidae		2	9.3	1.6	0.22
Ephemeroptera	Tricorythidae		4	27.6	6.1	0.03
Ephemeroptera	Trichorythidae	<i>Tricorythus</i> sp.	7	7.4	1.3	0.27
Odonata	Libellulidae	<i>Trithemis</i> sp.	2	1.5	0.24	0.63

The table below (Table 3-4) summarizes the response of the macroinvertebrate communities towards velocity. The bold values suggest taxa of significant value that responded in a significant manner towards velocity. The following taxa was revealed: *Aethaloptera* sp. (caddisflies), *Amphisysche* sp. (caddisflies), *Brachythemis* sp., Simuliidae, and *Tricorythus* sp. (mayfly).

Table 3-4: Summary of fitted generalized linear models (GLM) fitted for the response variables towards velocity (cm/s). The values in bold suggest the significant taxa:

Order	Family	Taxa	Nr of occurrences (/18)	R2 (%)	F	P
Trichoptera	Hydropsychidae	<i>Aethaloptera</i> sp.	5	26.8	5.9	0.03
Trichoptera	Hydropsychidae	<i>Amphisyche</i> sp.	2	25	5.3	0.03
		<i>Appasus</i> sp.	1	5	0.86	0.63
Decapoda	Atyiidae		1	5	0.84	0.63
Ephemeroptera	Baetidae		13	0.2	0.04	0.85
Odonata	Libellulidae	<i>Brachythemis</i> sp.	1	24.4	5.2	0.04
Ephemeroptera	Caenidae		5	0.4	0.07	0.79
Ephemeroptera	Caenidae	<i>Caenis</i> sp.	10	4.1	0.68	0.58
Diptera	Ceratopogonidae		5	4.5	0.76	0.60
Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i> sp.	11	0.8	0.13	0.73
Trichoptera	Philopotomidae	<i>Chimara</i> sp.	1	4.7	0.78	0.61
Diptera	Chironomidae		15	1.2	0.20	0.66
Odonata	Coenagrionidae		3	3.9	0.64	0.57
Venerida	Cyrenidae	<i>Corbicula fluminalis</i>	9	0.1	0.02	0.88
Diptera	Culicidae		2	15.4	2.9	0.11
Coleoptera	Elmidae		11	0.8	0.13	0.72
Odonata	Gomphidae		3	1.5	0.25	0.62
Coleoptera	Hydrophilidae	<i>Hydrophylis</i> sp.	1	0.5	0.84	0.63
Trichoptera	Hydroptilidae	<i>Hydroptila barnadi</i>	2	0.4	0.66	0.80
Trichoptera	Hydroptilidae	<i>Hydroptila cruciata</i>	8	0.8	0.14	0.72
Trichoptera	Hydroptilidae		1	0.4	0.06	0.81

Coleoptera	Hydrophilidae	<i>Laccobius</i> sp.	1	5	0.84	0.63
Hemiptera	Naucoridae	<i>Laccocoris</i> sp.	2	1.7	0.28	0.60
Trichoptera	Leptoceridae		3	0.5	0.08	0.79
Ephemeroptera	Leptophlebiidae		2	0	7.76	0.99
Odonata	Libellulidae		1	0.4	0.06	0.81
Diptera	Muscidae		2	0.4	0.06	0.80
Trichoptera	Leptoceridae	<i>Oecetis</i> sp. 1	1	0.4	0.06	0.81
Trichoptera	Leptoceridae	<i>Oecetis</i> sp. 2	11	2	0.33	0.57
Haplotaxida, Lumbriculida and Moniligastrida	Oligochaeta		2	4.2	0.70	0.59
Odonata		<i>Olpogastra</i> sp.	2	0.7	0.11	0.75
Trichoptera	Philopotomidae		1	0.3	0.44	0.84
Hygrophila	Physidae	<i>Physella acuta</i>	4	3	0.50	0.51
Sphaeriida	Sphaeriidae	<i>Pisidium</i> sp.	9	1.1	0.18	0.67
Hemiptera	Rhagovelia		2	9.7	1.7	0.21
Diptera	Simuliidae		13	26.3	5.7	0.03
Diptera	Tabanidae		3	4.6	0.77	0.61
Clade – Caenogastropoda Clade- Sorbeoconcha	Thiaridae	<i>Tarebia granifera</i>	17	10.4	1.8	0.19
Diptera	Tipulidae		2	3.3	0.55	0.53
Ephemeroptera	Tricorythidae		4	0.6	0.09	0.77
Ephemeroptera	Trichorythidae	<i>Tricorythus</i> sp.	7	46.8	14.1	0.001
Odonata	Libellulidae	<i>Trithemis</i> sp.	2	4.5	0.76	0.60

Habitat suitability curves were determined using the flow velocity, depth, and substrate present at the sampling sites. The HPCs and HUCs provided important information for determining the final HSCs and indicated that macroinvertebrates tolerate velocity ranges from 0.072 – 1.164 m/s and depths ranging between 0.002 – 1.155 m. The comparison between HPCs and HUCs (Figure 3-3) revealed the preferences of velocity and depth and according to Jowett *et al.*, (2008) the final habitat suitability criteria must be determined based on both curves. The HPC (Figure 3-3a)

showed a high preference for the depth range of 0.6 - 1.5 m, although the HUC revealed that the depth range 0 - 0.3 m was most utilized by macroinvertebrates. The HPC dipped below the HUC in shallow water, (depth range between 0 – 0.3). The velocity HPC and HUC revealed a similar trend where the velocity range 0 – 0.3 m/s was most utilized and most preferred (Figure 3-3b). Figure 3-3c also showed a similar trend between the HUC and HPC where SIC was the most utilized and most preferred habitat.

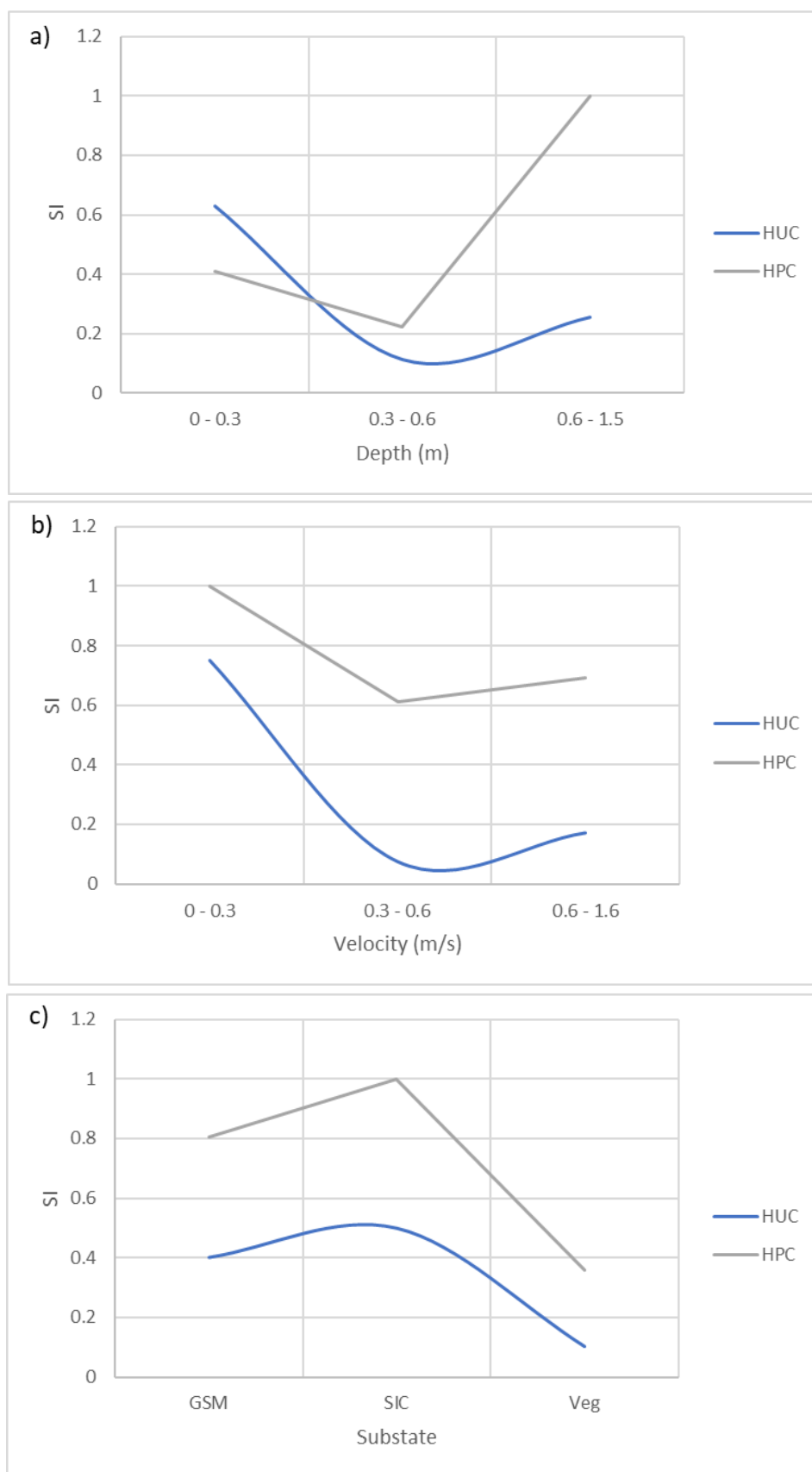


Figure 3-3: Habitat preference curves (HPCs) and habitat utilization curves (HUCs) for depths (a), water flow velocities (b), and substrate categories (c) separately for samples collected in 2020 and 2021 (suitability index (SI)). Categories of substrate: GSM—gravel, sand and mud, SIC—stones in current, veg—vegetation.

Figure 3-4 displays the HUCs and HACs for depth, flow velocity, and substrate types for the samples from 2020 and 2021. The HUC and HAC graph (Figure 3-4a) revealed that 0 - 0.3 m depth was dominant among the sampling sites and also most utilized by macroinvertebrates. The HAC dropped below the HPC in deeper water depth, ranging between 0.6 – 1.5 m. The HUC and HAC followed a similar pattern indicating that the 0 – 0.3 m/s velocity was the most utilized flow velocity range and the most available velocity range (Figure 3-4b). The substrate HAC and HUC had a similar pattern although the HAC had a significantly higher SI value. The HAC showed that both SIC, and GSM was evenly available, and vegetation had the lowest availability. The HUC revealed that SIC was the most utilized substrate type.

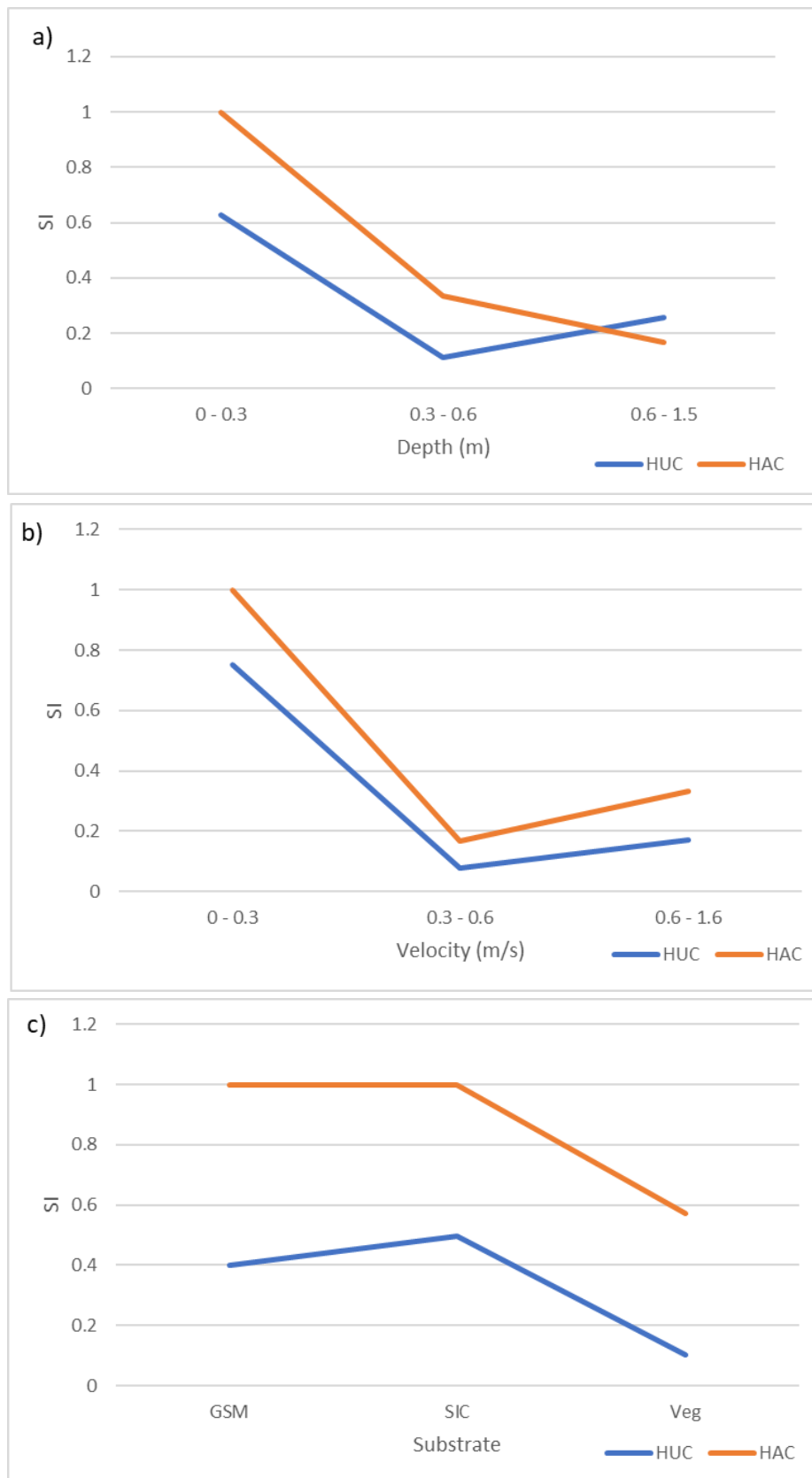


Figure 3-4: Habitat availability curves (HACs) and habitat utilization curves (HUCs) for depths (a), water flow velocities (b), and substrate categories (c) separately for samples collected in 2020 and 2021 (suitability index (SI)). Categories of substrate: GSM—gravel, sand and mud, SIC—stones in current, veg—vegetation.

The PCA biplot (Figure 3-5) overlays the combined data of velocity and depth preferences of macroinvertebrates between biotopes and their functional traits. The PCA analysis revealed spatial distribution of functional traits and environmental variables. Most of the functional traits can be seen as clustering largely together having various environmental variables influencing their ordination. A small cluster of functional traits, very fast flowing, permanent attachment, and rapid hydraulic habitat was associated with velocity and SIC. Siphon, spiracle, egg, larvae, nymph, body shape very large, high sensitivity, temporary attachment style, indifferent, adult aquatic stage, shape; other, and creeper clusters were associated with shallow depths (Figure 3-5a). A large cluster of traits was associated with vegetation, particularly medium body size, pools, mud, grazers, climbers, shredders, and ovate body shape, whereas GSM was associated with crawlers, high tolerance for pollution, and very slow flowing water preference. The SIC and depth indicated a more distributed cluster of functional traits, having a similar influence. Filter feeders, cylindrical body shape, and gills were outliers being slightly associated by depth and SIC. Figure 3-5b revealed the spatial separation of macroinvertebrate functional traits and macroinvertebrate traits. The distribution of traits between sites were very widespread although three distinct outliers was noted: cylindrical body shape, filter feeders and gills. A small cluster, high tolerance for pollution, very slow flowing water and GSM, on the left axis at the top were also recognized as outliers, although not as significant.

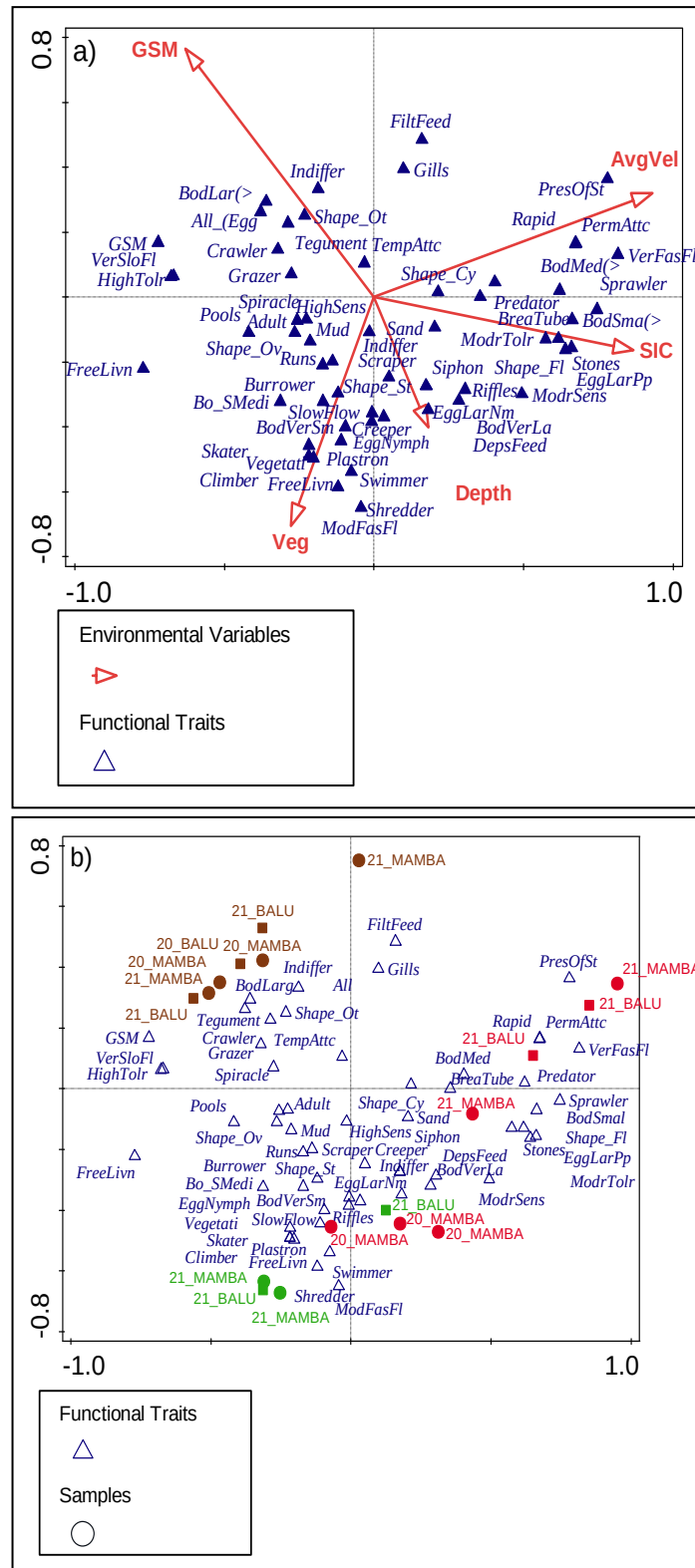


Figure 3-5: PCA biplot of environmental variables (biotopes vegetation, GSM and stones; velocity and depth) and functional traits ((body size, body shape, attachment type, respiration, mobility, functional feeding groups, habitat preference, flow preference, hydraulic habitat preference and aquatic life stage) (the following colors represent the following biotopes: green – vegetation, red – SIC, and brown – GSM). Axis 1 explains 23.69% of the data, and axis 2 explains 8.33% variation of the data.

The table below (Table 3-5) summarizes the response of the macroinvertebrate functional traits towards depth. The bold values suggest traits of significant value that responded in a significant manner towards depth. The following traits was revealed: very large body, creeper, siphon for breathing, egg and larvae and nymph aquatic life stages.

Table 3-5: Summary of fitted generalized linear models (GLM) fitted for the functional response variables towards depth (m). The values in bold suggest the significant functional traits:

Response	R2[%]	Nr. Of occurrences	F	p
Bod_Siz Very small (≤ 5)	12.3	17	2.2	0.15
Bod_Siz Small (>5-10)	0.4	18	0.07	0.80
Bod_Siz Medium (>10 -20)	1.0	13	0.16	0.70
Bo_Siz Medium-Large	0.4	8	0.07	0.80
Bod_Siz Large (>20 - 40)	3.2	17	0.54	0.53
Bod_Siz Very large (>40)	66.9	2	32.4	0.00003
Shape_Ovate	8.1	16	1.4	0.25
Shape_Cylindrical/tubular	0.4	18	0.06	0.81
Shape_Flattened	1.7	14	0.27	0.61
Shape_Streamlined	0.3	16	0.05	0.83
Shape_Other	8.5	12	1.5	0.24
Sprawler	1.0	16	0.16	0.69
Crawler	1.7	18	0.28	0.60
Swimmer	2.8	15	0.46	0.51
Burrower	0.2	16	0.035	0.85
Climber	0.3	3	0.052	0.82
Creeper	44.9	4	13.0	0.002
Skater	0.3	2	0.05	0.82
Free living	0.6	18	0.10	0.76
Temporary attachment	1.4	7	0.23	0.64
Permanent attachment	0.1	11	0.01	0.91
Presence of structure for attachment to substrate	1.5	18	0.24	0.63
Gills	23.9	18	5.0	0.04
Spiracle	0.4	7	0.06	0.81
Plastron	19.7	12	3.9	0.07
Breathing Tube	1.9	2	0.32	0.58

Tegument/Cutaneous	1.4	2	0.22	0.64
Siphon	74.1	2	45.8	<0.00001
Predator	0.2	17	0.04	0.85
Deposit feeder	10.4	17	1.9	0.19
Grazer	0.3	8	0.05	0.83
Scraper	0.4	15	0.06	0.81
Filter feeder	6.8	17	1.2	0.30
Shredder	2.7	15	0.44	0.52
Stones	6.7	16	1.2	0.30
Vegetation	2.3	16	0.37	0.55
GSM	4.0	18	0.67	0.57
Free living	0.3	2	0.05	0.82
Mud	0.2	7	0.04	0.85
Sand	0.0	4	0.01	0.94
Indifferent	8.2	10	1.4	0.25
Very slow flowing/still	3.7	18	0.62	0.56
Slow flowing	0.1	17	0.02	0.89
Moderately fast flowing	49.7	17	15.8	0.001
Very fast flowing	0.7	14	0.12	0.74
Indifferent	1.4	16	0.22	0.64
Pools	0.2	16	0.04	0.85
Rapid	0.1	11	0.02	0.88
Riffles	0.2	16	0.04	0.85
Runs	0.2	15	0.03	0.87
Highly sensitive	11.8	3	2.1	0.16
Moderately sensitive	0.0	14	0.0005	0.98
Moderately tolerant	4.9	18	0.83	0.63
Highly tolerant	3.3	18	0.55	0.53
All_(Egg-Adult)	4.4	18	0.74	0.60
Egg, Nymph	0.2	17	0.03	0.86
Egg, Larvae, Pupa	7.3	17	1.3	0.28
Egg, Larvae, Nymph	74.1	2	45.8	<0.00001
Adult	1.2	4	0.20	0.66

The table below (Table 3-6) summarizes the response of the macroinvertebrate functional traits towards velocity. The bold values suggest traits of significant value that responded in a significant manner towards velocity. The following traits was revealed: small and medium body sizes, flattened body shape, sprawler, free living, presence of structure for attachment to substrate, predator, stones, GSM, very fast flowing waters, rapid hydraulic habitat preference, moderately to high tolerance of pollution, and egg, larvae and pupa aquatic life stages.

Table 3-6: Summary of fitted generalized linear models (GLM) fitted for the functional response variables towards velocity (cm/s). The values in bold suggest the significant functional traits:

Response	R2[%]	Nr. Of occurrences	F	p
Bod_Siz Very small (≤ 5)	17.0	17	3.3	0.09
Bod_Siz Small (>5-10)	58.0	18	22.1	0.0002
Bod_Siz Medium (>10 -20)	24.0	13	5.1	0.04
Bo_Siz Medium-Large	9.3	8	1.6	0.22
Bod_Siz Large (>20 - 40)	17.8	17	3.5	0.08
Bod_Siz Very large (>40)	0.7	2	0.11	0.75
Shape_Ovate	10.3	16	1.8	0.20
Shape_Cylindrical/tubular	2.1	18	0.34	0.57
Shape_Flattened	41.8	14	11.5	0.004
Shape_Streamlined	8.6	16	1.5	0.24
Shape_Other	0.5	12	0.08	0.78
Sprawler	53.3	16	18.3	0.0005
Crawler	19.6	18	3.9	0.07
Swimmer	7.6	15	1.3	0.27
Burrower	10.1	16	1.8	0.20
Climber	7.1	3	1.2	0.29
Creeper	2.6	4	0.44	0.52
Skater	9.0	2	1.6	0.23
Free living	82.2	18	73.8	<0.00001
Temporary attachment	1.9	7	0.30	0.59
Permanent attachment	26.9	11	5.9	0.03
Presence of structure for attachment to substrate	60.4	18	24.4	0.0001
Gills	8.7	18	1.5	0.23
Spiracle	4.3	7	0.73	0.59

Plastron	7.9	12	1.4	0.26
Breathing Tube	9.4	2	1.7	0.22
Tegument/Cutaneous	4.0	2	0.66	0.57
Siphon	0.4	2	0.07	0.80
Predator	25.8	17	5.6	0.03
Deposit feeder	2.2	17	0.36	0.56
Grazer	6.5	8	1.1	0.31
Scraper	11.9	15	2.2	0.16
Filter feeder	0.5	17	0.08	0.78
Shredder	8.9	15	1.6	0.23
Stones	48.4	16	15.0	0.001
Vegetation	11.9	16	2.2	0.16
GSM	36.9	18	9.3	0.01
Free living	9.0	2	1.6	0.23
Mud	5.9	7	1.00	0.67
Sand	3.1	4	0.52	0.52
Indifferent	0.3	10	0.05	0.83
Very slow flowing/still	29.3	18	6.6	0.02
Slow flowing	9.6	17	1.7	0.21
Moderately fast flowing	9.3	17	1.6	0.22
Very fast flowing	85.3	14	92.9	<0.00001
Indifferent	2.6	16	0.43	0.52
Pools	12.8	16	2.4	0.14
Rapid	28.0	11	6.2	0.02
Riffles	0.4	16	0.06	0.81
Runs	9.6	15	1.7	0.21
Highly sensitive	1.6	3	0.26	0.62
Moderately sensitive	9.8	14	1.7	0.21
Moderately tolerant	28.9	18	6.5	0.02
Highly tolerant	30.0	18	6.9	0.02
All_(Egg-Adult)	13.0	18	2.4	0.14
Egg, Nymph	9.1	17	1.6	0.22
Egg, Larvae, Pupa	42.6	17	11.9	0.003
Egg, Larvae, Nymph	0.4	2	0.07	0.80
Adult	5.4	4	0.91	0.64

3.4 DISCUSSION

Aquatic macroinvertebrates are adapted to specific habitats and therefore a commonly used tool for biomonitoring indicating habitat preferences within an aquatic ecosystem (de Klerk & Wepener, 2013). Flow is one of the main environmental driver variables within river ecosystems and evaluating macroinvertebrates can assist to assess flow preferences, which in turn can provide a good indication of the current condition of the river system (Arthington *et al.*, 2006; Dewson *et al.*, 2007; Domisch *et al.*, 2017). Clear differences between the taxonomical and functional trait approach were noted. Indicator traits and taxa have been identified during this study.

3.4.1 Flow and Habitat preference

The multivariate analyses indicated that there was not a distinct spatial ordination of macroinvertebrates, and that depth had the main influence on the distribution of macroinvertebrates. Depth ranging between 0 – 0.3 m was the most available habitat for utilization and also the most preferred during this study, which was similar to the findings of other international studies (Jowett *et al.*, 1990; Degani *et al.*, 1993; Gore *et al.*, 1998; Jowett, 2003; Li *et al.*, 2009; Shearer *et al.*, 2015; Leszczyńska *et al.*, 2017). These studies investigated the following depth ranges: <0.75 m, 0.2 – 0.3 m, 0.27 m, 0.05 – 0.2 m, 0.1 – 1 m, <0.6 m, 0.3 m, 0.07 – 0.6 m. *Tricorythus* sp. (mayfly) responded to increased water velocity, and this is supported by the findings of this study conducted in South Africa and of Akamagwuna *et al.*, (2019). Simuliidae and *Cheumatopsyche* sp. had a high preference for the SIC habitat and corroborates with international findings by Dolédec *et al.*, (2007), Srisuka *et al.*, (2017), and Olkeba *et al.*, (2022). The highest taxa diversity was found in the marginal vegetation habitat type. According to literature (Tessier *et al.*, 2004; Sun *et al.*, 2010), this is due to the food and shelter this habitat provides.

3.4.2 Taxonomical:

A total of 4847 individuals were sampled during 2020 and a total of 5625 individuals were sampled during 2021, where the most abundant taxa were *Tarebia granifera*, Simuliidae and *Hydroptila cruciate* (Appendix A). The high abundance of *Tarebia granifera* could be explained by the low flow conditions and habitat availability during 2020 and that it is an invasive taxon (Appleton *et al.*, 2009; Jones *et al.*, 2017; Whitfield *et al.*, 2021) that dominated most of the available habitats.

There is a significant interaction between flow velocity and habitat because of the association between current flows and macroinvertebrate communities that can differ within individual habitat types. For instance, habitats with low flow within riffles were more likely to be inhabited by organisms drifting from upstream habitats with higher flow and could reflect higher abundances than low flow habitats (White *et al.*, 2019, Karaouzas *et al.*, 2019). Although the results from this study indicate a contrast because of the high abundance and preference for low flow habitats because of low flow habitats being the most abundant within the Olifants River during this study. This could also be explained by the limited habitat availability that was associated with lower flow velocities.

3.4.3 Habitat suitability curves:

Habitat suitability curves provide valuable information related to habitat preferences of macroinvertebrates (Shearer *et al.*, 2015; Vázquez *et al.*, 2020). Based on the HPCs, HUCs and HACs macroinvertebrates at the two sites in the Olifants River prefer a habitat comprised of a combination of shallow depths, SIC and low water flow velocity. It should be noted that sampling during high flow periods macroinvertebrates could present different preferences towards flow. This is due to the provision of substrate stability and food availability (Cook & Sullivan, 2018). Habitat provided by marginal vegetation could provide additional sources of organic matter and protection from predators (Bell *et al.*, 2013, Wolters *et al.*, 2018) although the SIC habitat was favoured during this study. Higher flow conditions provide an increase in oxygen availability and more food for filter feeders and therefore predators will also be present (Wallace & Webster, 1996, Cummins, 1974), which was not the case with lower flows recorded in this study. There was a significant number of predators present at higher flow ranges although filter feeders seem to be less than expected. Based on the HPCs and HUCs, the suitability index's highest values on the HCS for the communities of macroinvertebrates were allocated for the velocity values ranging between 0 – 0.3 m/s and depths ranging between 0 – 0.3 m. The most macroinvertebrates utilised these velocity and depth ranges within this study because it was the most available biotopes. These ranges also overlap with results from previous studies that are summarized in Table 3-7 for comparison. Regarding the substrate type, fine sand to silt dominated among most of the sites and the SIC habitat was assigned the highest SI values which revealed it was the most preferred biotope for macroinvertebrates.

Table 3-7: The optimal habitats, velocity-, and depth- values for macroinvertebrates based on literature:

Habitat	Velocity values (m/s)	Depth ranges (m)	Reference
All habitats	<0.60	<0.75	Jowett <i>et al.</i> , 1990
All habitats	<0.60	0.2 – 0.3	Degani <i>et al.</i> , 1993
Riffles	0.65	0.27	Gore <i>et al.</i> , 1998
All habitats	0.05 – 0.4	0.05 – 0.2	Jowett, 2003
All habitats	0.1 – 0.6	0.1 – 1	Li <i>et al.</i> , 2009
All habitats	<0.75	<0.6	Shearer <i>et al.</i> , 2015
-	<0.60	-	Leszczyńska <i>et al.</i> , 2017
Riffles	0.3 - - 0.7	0.3	Theodoropoulus <i>et al.</i> , 2018
-	0.06 – 0.6	0.07 – 0.6	Szałkiewicz <i>et al.</i> , 2022

3.4.4 Functional traits

Analysing macroinvertebrate's functional traits provides advantages because it could provide connections between pattern and process (Verberk *et al.*, 2008). The variation of different functional traits at the two sampling sites can be clarified by habitat, flow and depth preference and the environmental variability at these sites. There was no significant spatial variation between the functional traits although it is confirmed that most of the functional traits grouped together and associated with specific environmental variables, have been similar to previous international and local studies (Akamagwuna *et al.*, 2019; Erasmus *et al.*, 2021; Paz *et al.*, 2022).

Macroinvertebrates with the following traits: presence of a structure for attachment, permanent attachment, have a high preference for higher velocity and SIC. Individuals having a flattened body shape were reported to comprise of adaptations to attachment, as well as reducing drag effects resulting by the water flow (Statzner & Bêche, 2010), therefore their association with fast flowing waters and stones habitat.

Both depth and the vegetation habitat had a high preference of numerous functional traits. The GSM habitat was dominated by crawlers, which preferred very slow flowing waters. Crawling individuals are sedentary and have a lower ability to move from habitats with increased sedimentation (Wilkes *et al.*, 2017) that increases their vulnerability to changes (Akamagwuna *et al.*, 2019). Moreover, most crawling individuals were collector-feeders that are adapted to soft-bottom sediments and could benefit from the organic particles, as food, present in sediment (Benfield *et al.*, 2006, Murphy *et al.*, 2017), hence their association with the GSM habitat type. Additionally, the preference of a taxon cannot be allocated to a trait or attribute alone but instead

the result of how a trait was related to environmental conditions (Verberk *et al.*, 2013). According to Lancaster & Downes (2009), the term “flow velocity preference” have already been explained in ecohydrology proposing that it should only be referred to as “association”. Considering this, velocity preference was analysed accompanied by depth-, and habitat preferences. Shallow depths and slower water flow was mostly preferred by macroinvertebrates during this study due to the prevalence of these conditions. Depth revealed less significant traits than velocity. Depth was associated with very large body size, creepers, siphon for breathing and egg, larvae and nymph aquatic life stages. Whereas velocity was associated with small to medium body sizes, flattened body shape, sprawlers, free living, presence of an attachment to substrate, predator, stones, GSM, very fast flowing waters, rapid hydraulic habitat preference, moderately to high tolerance for pollution and egg, larvae and pupa aquatic life stage.

3.5 CONCLUSION

The macroinvertebrate community structures and responses towards specific environmental variables, velocity, and depth, were explored within the Olifants River, in terms of functional traits and a taxonomical approach and to possibly identify indicator taxa representing flow and depth preferences. Similarities between this study and previous studies have been found although further studies could be done to confirm the trend occurring in this study.

There were major differences between the functional traits approach and the taxonomical approach. From the taxonomical approach, the PCA revealed that Mamba (20_MAMBA_SIC) differed significantly from the other SIC surveys for Mamba and Balule, although there was no differentiation between the other sites and biotopes. The functional traits did not reveal any distinct difference with the Mamba (20_MAMBA_SIC) because there was good grouping of traits with biotopes.

Creepers and siphon for breathing was concluded to be the most significant indicator traits for depth, whereas flattened body shape, small to medium body size, sprawlers, free living, predators, very fast flowing waters, and rapid hydraulic habitat preference was identified as indicator traits for velocity.

Tricorythus sp. (mayfly) was confirmed to be associated with higher flow velocities and can thus be a positive indicator species for faster flowing waters. There were other taxa that had significant associations with depth and velocity, although none were significant enough to be used as an indicator taxon. More in detail studies should be conducted and it is recommended to include

temporal variation to identify indicator taxa more accurately for flow and depth within the Olifants River.

Chapter 4

General Conclusions and Recommendations

4.1 CONCLUSIONS

The Limpopo River Catchment (LRC) is an important catchment that provides valuable water resources (surface and groundwater) and is under increasing pressure arising from mining, agriculture, domestic, and environmental demands (Ashton *et al.*, 2001; FAO-SAFR, 2004; Nhassengo *et al.*, 2021). These pressures negatively impact the riverine ecosystems within the LRC, and the conservation and management of these systems are vital for the survival of freshwater biodiversity (UN-HABITAT/UNEP, 2007). The aims of the current study were to determine the main drivers of the macroinvertebrate community structures and possibly identify suitable indicator taxa representing preferences towards environmental variables. The main drivers in the taxonomical approach were found to be habitat quality and the functional approach revealed to be driven by water quality. Both of these approaches indicated differences and therefore is used in unison as they complement each other and provide more detail.

In South Africa there is a lack of information on the macroinvertebrate responses to environmental flow conditions (Agboola *et al.*, 2019). Multiple studies have been done in South Africa using macroinvertebrates as bioindicators to monitor the health of rivers (Dickens & Graham, 2002; Dallas, 2004; Dallas, 2007a; 2007b; Dallas & River-Moore, 2014; Barber-James & Pereira-da-Conceicao, 2016) or to assess the functional traits (Kefford *et al.*, 2004; Ochieng *et al.*, 2019) although all of these studies only identify macroinvertebrates up to family level. This creates a major limitation as South Africa is way behind on taxonomy and ecology at genus and species level, resulting in implications for the search for an indicator species. In this study, very little organisms could be identified up to species level and many of the species identified could indeed be several species. This could impact the use of bioindicators as biomonitoring tools.

The multivariate analysis revealed that habitat variables, which consisted of substratum type, were the main driving force of the macroinvertebrate distribution patterns within the LRC study, whereas both water quality and habitat variables had similar influences on the variation in the functional traits. This study completed in the LRC provided additional information regarding studies in the LRC and studies of macroinvertebrate responses to various environmental variables within freshwater riverine ecosystems. Macroinvertebrates were identified to the lowest taxonomic

level possible that could provide additional information regarding macroinvertebrate studies in South African rivers. Responses of macroinvertebrates towards environmental flows are also poorly understood and this study provides some information of how macroinvertebrates respond to alterations in different depth and flow ranges. Water quality parameters, that included *in-situ* measurements and nutrient analysis, did not reveal significant spatial variation within the taxonomical approach to the macroinvertebrate community structure.

The multivariate analysis from the Olifants River study revealed similar spatial variation between the taxonomical and functional trait approaches to responses of macroinvertebrates to velocity and depth. Shallow habitats with slower flowing water were the main preference in the Olifants River. *Tricorythus* sp. (mayfly) was identified as an indicator taxon for faster flowing water as it has been confirmed in literature (Arimoro & Muller, 2010). More studies focussing on this genus at different sites along rivers within the LRC can provide a conclusive link that this genus can be used as an indicator taxon for faster flowing water. Fry (2021) indicated that *Tricorythus* sp. (mayfly) is moderately sensitive and can live in moderate water quality which makes this taxon a good indicator in rivers that are prone to be affected by pollution. Simuliidae and *Cheumatopsyche* sp. was highly associated with the SIC biotope and this was also evident in previous studies (Olkeba *et al.*, 2022). The significance of other taxa was not enough to form a conclusive decision to identify other taxa as indicator taxa for environmental flow. Conclusively, the macroinvertebrate responses within the LRC revealed important information into understanding the riverine ecosystem functionality.

In conclusion, the main drivers of macroinvertebrate community structures were determined in mainstream rivers and tributaries in the LRC and the Olifants River presented clear spatial variation in the macroinvertebrate preferences towards flow, although there were no indicator taxa identified which was significant enough to consider. The more detailed study on the Olifants River provided more information to assist in identifying suitable indicator taxa, although further studies are recommended. Taxonomical and functional approaches indicated different trends and therefore should be used in unison as they complement one another and provide more detailed information.

4.2 RECOMMENDATIONS

For future studies on rivers within the LRC, the following additional work should be conducted:

- Specific studies focussing on Simuliidae, *Tricorythus* sp. (mayfly), and *Cheumatopsyche* sp. could provide more insight into environmental preferences.
- This study found that functional traits can provide insight on the diversity and community structure of aquatic macroinvertebrates when accompanied by additional components such as environmental variables. Further research over consecutive years could additionally build on the results obtained from the current study and will be valuable to determine significant traits determining the preferences for environmental variables.
- Where possible, South African invertebrate identification guides need to be updated as this study relied on books published between 1999 and 2008, where many of the identification keys only go up to family level. For many macroinvertebrate groups, (e.g. Baetidae) there are identification keys available although they are not necessarily compiled within a single guide.

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Annexures

Appendix A: Macroinvertebrate Diversity

Abbreviations					
HydrogSp	<i>Hydroglyphus</i> sp.	Libellul	Libellulidae	CheuaSp	<i>Cheumatopsyche</i> sp.
CanthSp	<i>Canthydrus</i> sp.	Ceratopg	Ceratopogonidae	Hydroptl	Hydroptilidae
Dytiscid	Dytiscidae	Coenagn	Coenagrionidae	CorbFlum	<i>Corbicula fluminalis</i>
Culicida	Culicidae	LaccbSp	<i>Laccobius</i> sp.	IctinSp	<i>Ictinogomphus</i> sp.
BerosSp	<i>Berosus</i> sp.	CeansSp	<i>Caenis</i> sp.	OlighSp	<i>Oligoneuriopsis</i> sp.
GyrinSp	<i>Gyrinus</i> sp.	TricrSp	<i>Tricoythus</i> sp.	XiphvSp	<i>Xiphoveloidea</i> sp.
EnochSp	<i>Enochrus</i> sp.	BulnFors	<i>Bulinus forskali</i>	AmphsSp	<i>Amphisysche</i> sp.
Curculio	Curculionidae	ChimrSp	Chimara sp.	AethlSp	<i>Aethaloptera</i> sp.
AppasSp	<i>Appasus</i> sp.	BrachSp	Brachythemis sp.	AnispSp	<i>Anisops</i> sp.
RegimSp	<i>Reginbartia</i> sp.	BarbrSp	<i>Barbarochton</i> sp.	HydrpSp	<i>Hydrophylus</i> sp.
YolaSp	<i>Yola</i> so.	Tipulida	Tipulidae	Hydraend	Hydraenidae
MicrnSp	<i>Micronecta</i> sp.	Hydrophl	Hydrophilidae	RhagvSp	<i>Rhagovelia</i> sp.
OnychSp	<i>Onychogomphus</i> sp.	AmphiSp	<i>Amphiops</i> sp.	StratSp	<i>Stratiomyidae</i> sp.
Tabanida	Tabanidae	Simuliid	Simuliidae	TrithSp	<i>Tricorythus</i> sp.
OecetSp2	<i>Oecetis</i> sp. 1	HyphSp	<i>Hyphydrus</i> sp.	HydatSp	<i>Hydaticus</i> sp.
BurnpSp	<i>Burnupia</i> sp.	Heptagen	Heptageniidae	Cordulid	Cordulidae

LaccpSp	<i>Laccophilus</i> sp.	OecetSp1	<i>Oecetis</i> sp. 1	FerrsSp	<i>Ferrisia</i> sp.
HydrvSp	<i>Hydrovatus</i> sp.	Dyt(HySp)	Dytiscidae (<i>Hydactis</i> sp.)	Leptocer	Leptoceridae
HydtDreg	<i>Hydaticus Dregei</i>	LaccoSp	<i>Laccocoris</i> sp.	Philopot	Philopotomidae
Gyrinida	Gyrinidae	HydrBarn	<i>Hydroptila barnardi</i>	PisidSp	<i>Pisidium</i> sp.
HydrCruc	<i>Hydroptila cruciata</i>	Tricoryt	Tricorythidae	Hirudina	Hirudinae
PhysAcut	<i>Physella acuta</i>	Oligocha	Oligochaeta	OlpoSp	<i>Olpogastra</i> sp.

Order	Family	Specie	21_ CROC	21_ SPANW	21_ MATL	21_ LEPA	21_ LIMP	21_ MAGO	21_ MAP	21_ SAND	21_ LUVU	21_ SHIN	20_ LETABA	21_ LRANCH	21_ LBULL	20_ MAMBA	21_ MAMBA	21_ BALU	20_ BALULE
Trichoptera	Hydropsychidae	<i>Aethaloptera</i> sp.	0	0	3	6	21	0	4	0	1	0	0	8	27	0	3	147	0
Coleoptera	Hydrophilidae	<i>Amphiops</i> sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Trichoptera	Hydropsychidae	<i>Amphisycha</i> sp.	0	386	72	13	1	1	0	0	0	0	0	17	32	0	14	12	0
Hemiptera	Notonectidae	<i>Anisops</i> sp.	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Hemiptera	Belastomidae	<i>Appasus</i> sp.	3	1	0	0	2	0	130	1	2	1	0	0	0	0	0	1	0
Decapoda	Atyidae		21	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0
Ephemeroptera	Baetidae		78	245	10	12	81	9	212	23	62	261	61	100	68	257	72	9	21

Trichoptera	Barbachtonidae	<i>Barbarochton</i> sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
Coleoptera	Hydrophilidae	<i>Berosus</i> sp.	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
Odonata	Libellulidae	<i>Brachythemis</i> sp.	0	0	0	0	0	0	0	0	4	0	0	0	5	0	0	1	0
Hygrophila	Planorbidae	<i>Bulinus forskali</i>	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Hygrophila	Planorbidae	<i>Burnupia</i> sp.	0	0	0	0	0	0	0	0	0	42	3	0	0	7	0	0	6
Ephemeroptera	Caenidae		0	0	0	0	0	0	0	0	0	0	80	0	0	137 7	0	0	52
Ephemeroptera	Caenidae	<i>Caenis</i> sp.	86	175	24	21	34	96	139	125 2	19	351	0	87	84	0	215	72	0
Coleoptera	Noteridae	<i>Canthydrus</i> sp.	0	0	0	0	0	0	34	0	0	1	0	0	0	0	0	0	0
Diptera	Ceratopogonidae		3	10	0	0	0	0	11	2	0	60	3	1	2	6	1	2	1
Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i> sp.	0	405	4	15	58	0	1	30	86	0	28	85	128	421	111	47	27
Trichoptera	Philopotomidae	<i>Chimara</i> sp.	0	0	12	1	0	0	0	0	3	0	0	1	1	0	9	0	0
Diptera	Chironomidae		144	338	6	20	25	67	533	152	11	318	63	11	37	346	56	27	50

Odonata	Coenagrionidae		20	29	0	2	2	0	7	12	0	3	0	4	21	2	1	4	0
Venerida	Cyrenidae	<i>Corbicula fluminalis</i>	5	8	0	1	25	0	0	0	1	4	52	0	19	156	8	71	180
Odonata	Cordulidae		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Diptera	Culicidae		36	40	1	1	7	11	152	14	0	15	0	2	1	0	5	2	6
Coleoptera	Curculionidae		0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Coleoptera	Dysticidae		0	0	0	0	0	0	8	1	0	0	0	0	1	0	0	0	0
Coleoptera	Elmidae		3	0	0	2	3	0	0	1	28	0	86	46	34	204	13	23	30
Coleoptera	Hydrophilidae	<i>Enochrus sp.</i>	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0
Gastropoda	Lymnaeidae	<i>Ferrisia sp.</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
Odonata	Gomphidae		0	17	0	0	1	0	18	0	5	0	0	2	0	0	0	8	0
Coleoptera	Gyrinidae		0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Coleoptera	Gyrinidae	<i>Gyrinus sp.</i>	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0	0
Ephemeroptera	Heptageniidae		0	0	0	0	0	0	0	0	3	0	5	0	0	0	0	0	44

Arhyncho bdellida	Hirudinae		0	1	0	1	0	0	0	0	0	0	8	0	0	5	0	0	3
Coleopter a	Dytiscidae	<i>Hydaticus dregei</i>	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleopter a	Dytiscidae	Dytiscidae (hydactis sp)	0	0	0	0	0	0	0	0	0	0	24	0	0	110	0	0	3
Coleopter a	Dytiscidae	<i>Hydaticus sp.</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Coleopter a	Hydraenida e		0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Coleopter a	Dytiscidae	<i>Hydroglyhpu s sp.</i>	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0
Coleopter a	Hydrophili dae		0	0	4	0	0	0	1	0	0	0	3	0	0	0	0	0	1
Coleopter a	Hydrophili dae	<i>Hydrophylis sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Trichopter a	Hydroptilid ae	<i>Hydroptila barnardi</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	0	0	0
Trichopter a	Hydroptilid ae	<i>Hydroptila cruciata</i>	0	1	0	0	0	0	19	9	0	2	0	0	16	101 4	42	11	0
Trichopter a	Hydroptilid ae		0	0	0	0	0	0	0	0	0	0	16	0	0	40	0	0	44
Coleopter a	Dytiscidae	<i>Hydrovatus sp.</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Coleoptera	Dytiscidae	<i>Hyphydrus</i> sp.	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0
Odonata	Gomphidae	<i>Ictinogomphus</i> sp.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Coleoptera	Hydrophilidae	<i>Laccobius</i> sp.	0	0	0	0	0	0	5	1	2	87	0	0	0	0	1	0	0
Hemiptera	Naucoridae	<i>Laccocoris</i> sp.	0	0	0	0	0	0	0	5	0	2	32	0	4	55	0	0	5
Coleoptera	Dytiscidae	<i>Laccophilus</i> sp.	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Trichoptera	Leptoceridae		0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0
Ephemeroptera	Leptophlebiidae		0	0	0	0	0	0	43	0	0	0	0	19	0	40	0	6	0
Odonata	Libellulidae		0	0	0	0	0	0	1	2	0	1	0	2	1	0	0	2	0
Hemiptera	Corixidae	<i>Micronecta</i> sp.	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Diptera	Muscidae		0	0	0	0	0	0	0	0	0	0	1	0	0	6	0	0	0
Trichoptera	Leptoceridae	<i>Oecetis</i> sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Trichoptera	Leptoceridae	<i>Oecetis</i> sp. 2	13	4	10	7	5	0	41	2	9	3	0	50	46	0	38	47	0
Haplotaenidae,	Oligochaetidae		0	1	0	0	0	0	0	0	0	0	5	0	0	8	0	0	3

Lumbriculida and Moniligastrida																			
Ephemeroptera	Oligoneuridae	<i>Oligoneuriopsis</i> sp.	0	0	0	10	0	0	0	0	0	0	0	0	176	0	0	0	0
Odonata	Libellulidae	<i>Olpogastra</i> sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	1
Odonata	Gomphidae	<i>Onychogomphus</i> sp.	0	0	0	0	0	0	24	39	0	0	0	0	12	0	0	0	0
Trichoptera	Philopotomidae		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Hygrophila	Physidae	<i>Physella acuta</i>	0	2	0	0	0	0	0	3	0	3	670	0	6	990	0	0	86
Sphaeriida	Sphariidae	<i>Pisidium</i> sp.	0	0	0	2	3	0	0	0	0	0	0	0	0	15	2	2	0
Hemiptera	Pleidae		0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Coleoptera	Hydrophiliidae	<i>Regimbartia</i> sp.	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Hemiptera	Veliidae	<i>Rhagovelia</i> sp.	4	4	1	0	431	0	0	0	0	0	0	17	17	0	1	1	0
Diptera	Simuliidae		0	90	9	2	40	0	6	0	0	4	11	6	19	750	161	949	18
Coleoptera	Elmidae	<i>Stenelmis</i> sp.	0	0	3	0	0	0	3	1	0	0	0	0	0	0	0	0	0

Diptera	Stratiomyidae	<i>Stratiomyidae</i> sp.	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Diptera	Tabanidae		1	0	0	0	0	1	1	4	2	1	5	0	0	5	0	1	12
Clade-Caenogastropoda Clade-Sorbeconcha	Thiaridae	<i>Tarebia granifera</i>	0	66	0	0	8	69	3	15	2	698	2014	80	84	1144	36	3087	12761
Diptera	Tipulidae		0	0	0	0	0	0	1	0	0	0	11	0	0	0	1	1	3
Ephemeroptera	Tricorythidae		0	0	0	0	0	0	0	0	0	0	7	0	0	128	0	0	31
Ephemeroptera	Trichorythidae	<i>Tricorythus</i> sp.	0	10	19	23	440	0	141	61	32	80	0	263	389	0	139	156	0
Odonatan	Libellulidae	<i>Trithemis</i> sp.	0	0	0	0	0	0	0	27	0	6	2	0	0	0	3	2	3
Hemiptera	Veliidae		0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Hemiptera	Veliidae	<i>Xiphoveloidea</i> sp.	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Coleoptera	Dytiscidae	<i>Yola</i> sp.	0	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0	0

Appendix B: Water Quality and Sediment Particle Size

Environmental Variable	21_ CROC	21_ SPANW	21_ MATL	21_ LEPHA	21_ LIMP	21_ MOGA	21_ MAP	21_ SAND	21_ LUVU	21_ SHIN	21_ LRANCH	21_ LBULL	20_ MAMBA	21- BALULE	20_ BALULE	21_ MAMBA
Turbidity	105.00	60.00	14.00	22.00	29.00	24.00	46.00	41.00	24.00	53.00	35.00	38.00	5.00	12.00	22.67	13.00
NH ₄ ⁺	0.25	0.13	0.22	0.04	0.18	0.41	0.53	0.38	0.29	0.54	0.16	0.05	0.09	0.12	0.10	0.01
NO ₃ ⁻	3.90	4.00	7.20	2.70	5.30	2.80	2.50	4.90	1.22	2.60	0.61	0.81	1.23	1.97	1.57	1.00
NO ₂ ⁻	0.04	0.05	0.04	0.02	0.24	0.01	0.02	0.07	0.01	0.02	0.03	0.03	0.00	0.01	0.00	0.01
PO ₄ ⁻	0.46	0.12	0.16	1.38	0.71	0.48	0.29	0.70	0.31	0.15	0.09	0.05	0.23	0.11	0.26	0.01
Cl ⁻	278.00	244.00	31.20	32.00	159.00	1809.00	144.00	1332.00	40.10	262.00	58.00	85.00	37.00	32.67	36.00	26.00
SO ₄ ²⁻	183.00	174.00	4.00	14.00	42.00	650.00	100.00	399.00	12.00	36.00	44.00	39.00	91.67	62.67	97.33	69.67
COD	38.90	29.70	26.00	15.60	34.80	44.30	51.80	55.80	16.00	29.60	17.90	21.10	5.43	4.27	7.00	5.60
Alkalinity	652.91	619.35	103.73	115.94	274.59	982.42	387.48	1418.72	213.57	1049.54	286.79	439.34	2.20	168.82	2.10	164.75
pH	9.22	8.73	7.14	7.64	8.46	7.53	8.78	8.65	8.34	8.18	8.26	8.42	8.25	8.58	8.44	8.79
T	22.40	22.05	22.30	21.60	22.50	23.60	26.25	27.70	24.90	21.45	22.25	22.70	26.10	20.25	20.80	22.70
EC	781.50	837.00	6855.00	857.00	936.00	2415.00	422.00	2580.00	381.00	900.00	1007.00	804.50	860.00	547.50	742.00	1285.50
TDS	542.00	554.50	4760.00	591.50	653.00	1655.00	288.00	1775.00	262.00	621.50	619.50	448.50	994.00	642.00	520.00	899.50
DO	10.76	11.34	9.28	9.36	10.42	12.77	11.39	16.22	9.99	8.97	10.17	9.71	7.79	10.15	9.30	10.98
>2000	22.38	1.44	2.23	29.77	15.07	30.82	21.83	3.65	53.35	18.80	1.33	1.83	6.11	0.28	43.85	1.41
>500	29.40	58.89	46.50	52.90	58.44	51.81	49.33	74.73	26.72	66.93	24.02	45.08	37.08	34.68	72.72	25.05
>212	31.75	36.82	46.10	9.94	18.96	14.54	23.46	20.91	15.48	6.47	47.96	44.50	194.89	35.08	133.18	66.04
>53	14.51	2.53	4.40	5.55	6.24	2.49	4.94	0.28	3.98	7.41	23.66	7.16	60.88	26.90	49.08	6.75
>0	1.59	0.08	0.34	1.57	12.32	0.07	0.04	0.17	0.09	0.27	2.59	0.97	1.04	2.54	1.17	0.19
Habitat Heterogeneity	46.00	68.00	51.00	59.00	70.00	41.00	46.00	49.00	62.00	59.00	62.00	62.00	68.00	68.00	62.00	62.00

Appendix C: Velocity and Depth

	20_MAMBA_SIC1	20_MAMBA_SIC2	20_MAMBA_SIC3	20_MAMBA_GSM1	20_MAMBA_GSM2	20_BALULE	21_BALU_SIC1	21_BALU_SIC2	21_BALU_G	21_BALU_G	21_BALU_V	21_BALU_V	21_MAMBA	21_MAMBA	21_MAMBA_GSM1	21_MAMBA_GSM2	21_MAMBA_VEG1	21_MAMBA_VEG2
Depth (m)	1.164	0.381	0.680	0.429	0.227	0.246	0.224	0.198	0.072	0.142	0.471	0.263	0.268	0.212	0.318	0.237	0.174	0.268
Avg	0.267	0.321	0.279	0.173	0.074	0.149	1.155	0.947	0.289	0.002	0.004	0.267	1.247	0.709	0.579	0.029	0.038	0.006
Veg	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1
GSM	0	0	0	1	1	1	0	0	1	1	0	0	0	0	1	1	0	0
SIC	1	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0
BEDROCK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix D: Functional Traits

Functional Traits	21_ CROC	21_ SPANW	21_ MATL	21_ LEPHA	21_ LIMP	21_ MOGA	21_ MAP	21_ SAND	21_ LUVU	21_ SHIN	20_ MAMBA	21_ BALU	20_ BALU	21_ MAMBA	21_ LRANCH	21_ LBULL
Bod_Siz Very small (≤5)	55.88	28.59	17.58	29.29	4.97	64.17	44.72	85.04	10.91	36.86	1.11	2.39	39.18	33.62	12.31	11.29
Bod_Siz Small (>5-10)	33.33	43.97	31.32	46.43	92.00	7.87	37.66	8.29	79.64	20.83	2.45	27.88	28.57	58.99	71.02	58.98
Bod_Siz Medium (>10 -20)	0.00	21.22	50.55	15.00	1.94	0.39	6.36	4.45	4.73	4.94	1.02	3.62	14.56	3.32	5.97	6.82
Bo_Siz Medium-Large	10.79	1.69	0.55	2.14	0.34	0.39	9.92	1.20	2.18	2.12	0.13	0.13	1.74	0.21	0.50	1.79
Bod_Siz Large (>20 - 40)	0.00	4.53	0.00	7.14	0.76	27.17	1.34	1.02	2.55	35.25	95.29	65.98	15.94	3.85	10.20	21.12
Bod_Siz Very large (>40)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.00	0.00
Shape_Ovate	0.72	1.04	4.95	3.57	0.67	0.00	6.49	0.96	12.73	6.66	0.93	0.70	19.19	1.82	5.97	3.66

Shape_Cylindrical/tubular	47.24	73.21	64.29	47.14	13.89	58.66	49.11	13.82	41.82	55.52	96.54	92.37	53.31	51.07	32.46	32.01
Shape_Flattened	5.52	2.18	10.44	17.86	37.37	0.00	22.01	8.53	14.91	4.59	0.59	3.67	2.41	15.31	35.95	34.77
Shape_Streamlined	39.33	22.91	18.68	30.71	9.68	41.34	22.33	76.62	29.45	30.86	0.55	1.73	22.77	30.73	23.26	26.65
Shape_Other-Specify	7.19	0.65	1.65	0.71	38.38	0.00	0.06	0.06	1.09	2.37	1.40	1.53	2.31	1.07	2.36	2.92
Sprawler	27.34	20.46	10.99	10.71	10.77	7.87	23.54	2.28	22.55	14.12	0.34	20.46	14.03	25.48	13.43	7.15
Crawler	23.50	57.50	71.98	66.43	50.08	65.75	32.32	84.74	66.18	64.45	99.16	77.62	77.66	62.96	75.62	81.32
Swimmer	8.15	0.22	13.19	6.43	0.51	0.00	6.36	0.66	5.45	2.22	0.06	1.00	3.08	5.14	6.34	4.31
Burrower	35.25	19.97	3.30	14.29	2.19	26.38	37.28	11.60	5.82	19.06	0.40	0.79	5.02	6.10	1.74	4.14
Climber	4.80	1.58	0.00	1.43	0.17	0.00	0.45	0.72	0.00	0.15	0.00	0.09	0.03	0.11	0.50	1.71
Creeper	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.02	0.02	0.08	0.11	0.00	0.00
Skater	0.96	0.22	0.55	0.00	36.28	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.11	2.36	1.38
Other	0.00	0.05	0.00	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.07	0.00	0.00	0.00
Free living	84.65	47.03	44.51	70.00	87.04	95.28	88.49	96.33	65.82	95.51	97.27	73.69	67.41	66.49	84.58	79.20

Temporary attachment	8.63	2.35	0.55	1.43	0.59	4.33	9.67	1.02	0.00	0.91	0.72	0.04	13.97	0.54	0.25	0.57
Permanent attachment	0.00	43.15	50.00	25.00	6.73	0.39	0.32	1.80	32.73	0.00	0.20	4.39	5.88	14.67	13.81	15.27
Presence of structure attachment to substrate	6.71	7.47	4.95	3.57	5.64	0.00	1.53	0.84	1.45	3.58	1.81	21.87	12.74	18.31	1.37	4.96
Gills	88.73	97.44	92.86	96.43	62.63	95.28	76.53	98.02	87.64	92.64	99.51	99.38	94.45	97.64	91.67	95.37
Spiracle	1.92	0.27	2.20	0.00	36.53	0.39	8.84	0.78	1.45	0.25	0.15	0.09	0.91	0.21	2.36	1.71
Plastron	0.72	0.00	4.40	2.14	0.25	0.00	4.96	0.36	10.91	6.35	0.25	0.49	4.38	1.61	5.72	2.84
Breathing Tube	8.63	2.18	0.55	0.71	0.59	4.33	9.67	0.84	0.00	0.76	0.04	0.04	0.00	0.54	0.25	0.08
Tegument/Cutaneous	0.00	0.11	0.00	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.18	0.00	0.00	0.00
Siphon	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
Predator	6.71	45.99	45.05	27.14	43.52	0.79	15.65	7.51	36.36	2.67	0.43	4.84	8.53	14.35	17.04	20.15
Deposit feeder	44.36	23.57	29.12	40.00	46.72	41.34	35.24	80.95	41.82	35.00	1.13	5.41	40.29	50.21	58.33	45.25

Grazer	0.72	0.65	0.00	0.00	0.00	0.00	0.76	0.36	0.00	5.35	0.69	0.04	13.98	0.11	0.12	0.65
Scraper	35.25	18.44	8.24	15.71	2.36	26.38	35.50	9.31	16.00	20.47	0.94	1.07	7.68	7.60	7.09	5.77
Filter feeder	9.83	11.13	12.09	5.00	6.99	31.50	10.24	1.74	2.18	36.36	96.81	87.64	28.79	23.66	11.07	10.07
Shredder	3.12	0.22	5.49	5.00	0.42	0.00	2.61	0.12	3.64	0.15	0.00	1.00	0.72	4.07	6.22	3.82
Collector	0.00	0.00	0.00	7.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.30
Stones	54.20	80.52	70.33	74.29	56.31	30.71	61.32	17.01	81.82	33.64	2.09	29.63	45.13	65.42	69.28	75.30
Vegetation	17.27	4.15	8.79	7.14	1.35	4.33	24.87	4.93	4.73	9.78	0.79	1.15	14.82	4.93	6.97	7.31
GSM	20.62	14.13	13.19	16.43	3.87	64.96	10.24	76.14	9.45	52.90	95.72	67.58	35.54	27.19	21.02	13.65
Free living	0.96	0.22	1.10	0.71	36.36	0.00	0.70	0.12	0.00	0.10	0.02	0.02	1.56	0.11	2.36	1.46
Mud	0.72	0.55	0.00	0.00	0.00	0.00	2.86	1.80	1.45	3.38	0.03	0.11	0.08	0.43	0.25	0.57
Sand	0.00	0.00	6.59	0.71	0.00	0.00	0.00	0.00	1.45	0.00	0.00	0.00	0.70	0.96	0.12	0.16
Indifferent	6.24	0.44	0.00	0.71	2.10	0.00	0.00	0.00	1.09	0.20	1.34	1.51	2.17	0.96	0.00	1.54
Very slow flowing/still	44.60	25.91	4.40	15.71	3.70	57.87	59.92	14.30	7.27	59.40	96.49	66.66	37.25	10.49	12.06	11.94
Slow flowing	20.86	9.60	15.93	17.14	3.11	38.19	9.61	75.60	8.36	22.24	0.51	1.60	19.58	23.45	10.82	6.82

Moderately fast flowing	9.83	0.49	7.69	6.43	36.95	0.00	6.74	2.40	17.09	0.55	0.90	1.94	19.22	10.49	16.67	9.67
Very fast flowing	0.00	48.61	65.38	50.00	47.14	0.39	9.73	5.47	44.36	4.24	0.59	27.97	18.12	46.90	47.26	62.71
Indifferent	24.70	15.38	6.59	10.71	9.09	3.54	13.99	2.22	22.91	13.57	1.51	1.83	5.83	8.67	13.18	8.85
Pools	6.47	3.00	3.30	4.29	36.95	0.00	16.22	5.83	4.73	7.21	0.78	0.45	16.59	0.96	3.48	5.44
Rapid	0.00	43.15	43.41	24.29	6.73	0.39	0.32	1.80	31.64	0.00	0.20	4.39	5.87	13.70	13.68	15.19
Riffles	40.05	23.46	37.36	49.29	46.97	41.34	31.49	80.47	53.45	37.07	1.37	5.54	27.60	47.97	61.82	61.09
Runs	34.53	18.44	3.30	14.29	2.10	26.38	33.91	9.13	4.00	16.04	0.37	0.58	4.82	6.00	1.37	3.01
Indifferent	18.94	11.95	12.64	7.86	7.24	31.89	18.07	2.76	6.18	39.79	97.27	89.04	45.13	31.37	19.65	15.27
Highly sensitive	0.00	0.00	0.00	7.14	0.00	0.00	0.00	0.00	1.45	0.00	0.33	0.00	0.70	0.00	0.00	14.38
Moderately sensitive	24.46	13.91	25.27	27.14	44.11	3.54	25.38	5.47	46.18	17.30	0.65	4.14	9.55	25.05	53.48	40.29
Moderately tolerant	31.65	61.59	70.88	48.57	52.02	38.58	22.46	83.23	46.91	28.34	2.58	29.38	54.61	64.35	34.95	34.93
Highly tolerant	43.88	24.50	3.85	17.14	3.87	57.87	52.10	11.18	5.45	54.36	96.45	66.49	35.15	10.60	11.57	10.40
Unknown	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
All (Egg-Adult)	6.95	4.26	4.95	5.00	3.28	27.17	5.53	1.50	12.73	44.07	97.63	67.85	36.84	6.64	15.67	11.70

Egg, Nymph	44.12	25.97	29.12	48.57	46.97	41.34	37.21	85.16	44.00	35.40	1.14	5.52	25.18	46.04	59.45	61.01
Egg, Larvae, Pupa	47.24	69.50	64.29	46.43	13.22	31.50	48.92	12.92	42.55	20.32	1.20	26.58	37.10	47.22	22.51	25.59
Egg, Larvae, Nymph	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00
Adult	1.68	0.27	1.65	0.00	36.53	0.00	8.33	0.42	0.73	0.20	0.04	0.04	0.79	0.11	2.36	1.71