BENTHIC MACROINVERTEBRATES AS ECOLOGICAL HEALTH INDICATORS IN THE LUAPULA BASIN FOR POTENTIAL INTER-BASIN WATER TRANSFER TO THE KAFUE BASIN

By

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A thesis submitted to the University of Zambia, School of Mines, in partial fulfilment of the requirements of the Master of Science Degree in Integrated Water Resources Management

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Lusaka.

February 2025.

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DECLARATION

This thesis was written and submitted in accordance with the rules and regulations governing the award of Master of Science in Integrated Water Resources Management of the University of Zambia. I further declare that the thesis has neither in part nor in whole been presented as substance, for the award of any degree, either to this or any other university. Where other people's work has been drawn upon, acknowledgement has been made.

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ABSTRACT

Water scarcity is a global challenge, affecting sustainable development, human well-being and ecosystem health. In Zambia, the Kafue River Basin, an important economic and ecological resource, faces growing water demand that exceeds its natural supply and supply capacity, leading to resource allocation conflicts. In response, the government is considering inter-basin water transfers from the resource-abundant Luapula River Basin. However, such transfers could pose significant environmental risks to both basins. This study evaluated the ecological health of rivers and streams in the Luapula Basin using benthic macroinvertebrate assemblages and water quality parameters.

The Luapula Basin was divided into three hydro-environmental zones using remote sensing techniques. Macroinvertebrate samples were collected from designated sites across these zones, with in situ measurements of physicochemical variables. Laboratory analyses of water samples followed APHA (1998) standards. Similarity Percentage (SIMPER) was used to assess macroinvertebrate compositional heterogeneity, the Zambia Invertebrate Scoring System (ZISS) and Shannon Diversity Index was used to evaluate river health and biodiversity and Canonical Correspondence Analysis (CCA) was employed to determine how altitude and water quality influence macroinvertebrate distribution. Seasonal and zonal variations in biodiversity, stream health and water quality were tested using multivariate and univariate ANOVA, while Pearson correlation analyses explored relationships between biodiversity attributes and health indicators.

Pollution-tolerant taxa, such as Decapoda, Odonata, Gastropoda and Hemiptera, dominated the macroinvertebrate community, while sensitive Ephemeroptera families were present seasonally, especially during the wet season. SIMPER analysis indicated spatial differentiation among zones, with dry and wet season dissimilarities of 56.67% and 63.33%, respectively. Most sites were classified as moderately impaired based on ZISS scores, with a few showing major seasonal impairments. MANOVA revealed significant inter-zone differences in biodiversity and health metrics ($p < 0.05$), driven by ZISS scores ($p < 0.05$) and taxa richness ($p < 0.01$). Taxa richness, ZISS scores and Shannon diversity were positively correlated, while evenness correlated negatively. Functional feeding group (FFG) analysis indicated a heterotrophic system dominated by gathering collectors, predators and

scrapers, with limited riparian-shredder linkages and a prevalence of polyvoltine prey populations.

Water quality showed significant seasonal variations (MANOVA: $p < 0.001$), with ANOVA identifying turbidity, hardness, calcium, magnesium, chloride, potassium and faecal coliforms as key drivers. The water quality index highlighted iron, turbidity and faecal coliforms as major contaminants, with seasonal improvements varying by zone. CCA linked dry-season macroinvertebrate assemblages to altitude, iron, sodium, chloride, potassium, turbidity, magnesium, and hardness. In contrast, wet-season assemblages were shaped by altitude, faecal coliforms, pH, potassium, conductivity, and dissolved solids.

The study concludes that the Luapula Basin experiences moderate ecological impairment, with seasonal water quality fluctuations driven by natural and anthropogenic factors. Planned water transfers risk further ecological degradation, emphasising the need for comprehensive hydrological and ecological assessments to guide sustainable resource management.

DEDICATION

To my beloved father and mother, Gibson Hachimbi Nyambe and Brenda Mwabafu Inambao.

ACKNOWLEDGEMENTS

I am thankful to the almighty God for his limitless grace and unwavering guidance, which have been the cornerstone of my journey. I owe a profound debt of gratitude to Prof. Imasiku Anayawa Nyambe, Dr. Kawawa Banda and Prof. Henry Sichingabula for their pivotal roles in shaping my academic path. Their invaluable supervision and unwavering support throughout my research endeavours have been a guiding light to my success. I wish to express my deepest gratitude to Mr. Gordon Hangoma Mudenda for his unwavering belief in my academic abilities.

I would like to express my heartfelt gratitude to Mrs. Ingrid Mugamya for her tireless administrative support, which has greatly eased the challenges of navigating the academic landscape. I sincerely appreciate the OR Tambo Africa Research Chairs Initiative (ORTARChI) for the scholarship and financial support that have lightened the burden of pursuing my academic aspirations.

I greatly appreciate Ms. Namakau Muyumbana for her friendship and insightful discussions throughout my research process. Her ideas have been a constant source of suggestion. Special recognition is owed to my PhD partner, Mr. Chalwe Chibwe, whose presence and support during data collection have made this journey both academically enriching and personally fulfilling.

To my sisters, Nasilele Nyambe, Lizzy Nyambe and Lynnet Nyambe, I am profoundly grateful for your unwavering support and constant belief in my abilities. Finally, I want to express my heartfelt gratitude to Ms. Lungowe Kayunga. Her unwavering support and understanding have been a constant source of strength throughout this study. Her encouragement has inspired me to persevere even in the face of challenges.

In expressing my gratitude to these remarkable individuals and entities, I acknowledge that their contributions have played an indispensable role in the realisation of this academic milestone.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
APHA	American Public Health Association
ASPT	Average Score per Taxon
BFPOM	Benthic Fine Particulate Organic Matter
CSO	Central Statistics Office
CPOM	Coarse Particulate Organic Matter
EC	Electrical Conductivity
EIA	Environment Impact Assessment
8NDP	Eighth National Development Plan
FAO	Food and Agriculture Organisation
FPOM	Fine Particulate Organic Matter
FFG	Feeding Functional Group
GDP	Gross Domestic Product
IBTW	Inter Basin Water Transfer
IWRM	Integrated Water Resources Management
JICA	Japan International Cooperation Agency
MANOVA	Multivariate Analysis of Variance
MEA	Millennium Development Goals
NASS	Namibia Scoring System
OKAS	Okavango Assessment System
PCC	Pearson Correlation Coefficient
RBM	Rapid Biomonitoring Methods
7NDP	Seventh National Development Plan
SDGs	Sustainable Development Goals
TDS	Total Dissolved Solids
TFPOM	Transport Fine Particulate Organic Matter
UK	United Kingdom

WARMA	Water Resources Management Authority
WQI	Water Quality Index
WWF	World Wide Fund for Nature
SASS	South African Scoring System
ZABS	Zambia Bureau of Standards
ZIP	Zambia Water Investment Programme
ZISS	Zambia Invertebrate Scoring System

CHAPTER ONE: INTRODUCTION

1.1 Background

Zambia's geographical situation is characterised by an abundant disposition of vast freshwater ecosystems that support an array of socio-economic activities, which include but are not limited to agriculture, fishing, mining, hydropower generation and tourism. The Zambian freshwater estate is estimated at 15 million hectares (Kakwasha et al., 2020) and it provides the basis for extensive freshwater ecosystem networks, which include rivers, lakes and swamps.

According to the Zambia Water Investment Programme (ZIP) of 2022, Zambia has an estimated renewable water resources potential of 144 km^3/annum, comprising 86.6 km^3 of surface water in the form of rivers, lakes and swamps and 57.4 km^3 of groundwater (Ministry of Water Development and Sanitation, 2022). This water estate is the basis for the country's extensive sustainable development agenda which is currently housed in the Eighth National Development Plan (8NDP)(Ministry of Finance and and National Planning, 2022) through its four Strategic Development Areas (SDAs), namely:

i. Economic transformation and job creation;
ii. Human and social development;
iii. Environmental sustainability including climate resilience; and
iv. Good governance environment.

The successful implementation of the 8NDP and subsequent intermediary National Development Plans (NDPs) in tandem with Vision 2030 will most likely cause an upward adjustment in the total water resources utilisation rate, which is currently estimated to be 77.77 km^3/annum according to the ZIP of 2022. Zambia's water resource utilisation capacity currently accounts for 54% of the total renewable water resource and it is apportioned discriminately across a wider social and economic spectrum which includes energy, agriculture, water supply and mining (Ministry of Water Development and Sanitation, 2022). It is against this background that the Zambian Government through the 7^{th} National Development Plan of 2017 and 8^{th} National Development Plan of 2022 recognises the need for water resource infrastructure as being critical in the provision of sustainable water resources management, irrigation, hydropower generation, domestic water supply and sanitation. In the same vein, the Zambian government also identifies inter-basin water transfer as a viable

strategy for achieving improved water resources development and management (Ministry of Water Development and Sanitation, 2022).

According to Bourguignon (2023), inter-basin water transfer (IBWT) involves transferring water from a water-surplus basin to a water-deficient basin, aiming to alleviate water scarcity and promote economic development. Al-Gamal (2017) describes inter-basin water transfer as manmade water conveyance schemes which move water from one river basin where it is available to another river basin where it could be utilised better for human development.

The observed increase in human water demand has laid out a basis that justifies the need to transfer water resources between river basins, especially in places that experience uneven and unpredictable rainfall (Snaddon, 1999). Snaddon (1999) further asserts that the rationale for considering the implementation of IBWT projects to deal with issues of uneven water resource distribution between river basins varies according to climate and social needs, in that countries that comparatively experience favourable rainfall quantities consider IBWTs primarily to enhance hydro-power generation capacity, while drier countries consider IBWTs for portable and irrigation water.

IBWTs have over the years been implemented successfully to meet the growing demand for water in river basins considered to be water-stressed. However, Al-Gamal (2017) notes that the growing need to make water available in water-stressed river basins for both social and economic undertakings does provoke controversy in some places because conveyancing of water resources between natural basins is considered as a subtraction in the donor basin and an addition in the receiving basin (Al-Gamal, 2017).

The concept of transferring water from one river basin to another has evolved over the years in both technique and capacity in terms of transfer quantities (Meador, 1992). However, the implementation of such transfer schemes does have the potential for detrimental ecological impacts, including the introduction of invasive species, changes in water quality and hydrological regimes and alteration of natural habitats (Meador, 1992). Additionally, the implementation of inter-basin water transfer schemes also have the potential to impact and reverse conservation efforts by escalating threats to critically endangered species, Ramsar-listed wetlands and protected areas (Al-Gamal, 2017).

O'Keeffe submits a justification of how inter-basin water transfer schemes have with time become an acceptable engineering solution to addressing the problem of water shortages in

many places around the world, including China, the United States of America (USA), Canada and South Africa. However, it was also noted that the implementation of such transfer schemes was usually justified only in terms of direct cost, whereas the environmental and social costs may also be considerable (O'Keeffe, 1988). Following the revision and reduction in transfer quantities of the original Texas water systems plan in the USA, O'Keeffe (1998) also emphasises the importance of incorporating comprehensive environmental impact assessments (EIA) in the planning stages of IBWT projects. Nevertheless, caution is always to be taken, as such assessments are almost always inadequate to evaluate the complex ecological alterations that may arise from inter-basin water transfer Snaddon (1999).

Following the opening of the Orange River/Great Fish River inter-basin water transfer in 1977 in South Africa, O'Keeffe (1988) conducted a comparative study to assess the changes in hydrology, chemistry and benthic invertebrate communities of the donor and recipient basins pre and post the inter-basin transfer scheme. The outcome of this study did indicate that because of the transfer scheme, the Great Fish River which was previously characterised by an irregular seasonal flow changed into a perennial flow with an increase in runoff of over 500% in the upper river. O'Keeffe (1988) further reported that an insignificant change was also reported in discharge mainly owing to abstractions for irrigation purposes. The low salinity inflow from the Orange River is reported to have also caused a reduction in the concentration of sodium, magnesium, chloride and sulphates in the Great River. A substantial change in benthic invertebrate communities was also reported, especially in riffle biotopes. Only 33 taxa identified were common to both pre and post-transfer surveys. Most notable in fauna alterations was the replacement of the previously dominant *Simulium adersi* and *Simulium nigritarse* by *Simulium chutteri,* a blood-feeding pest of livestock, which now causes problems for farmers (O'Keeffe, 1988).

Arising from the many lessons and experiences drawn from around the world, ecological considerations have become a key input factor in both water governance and development. As stated previously, water development technologies such as inter-basin water transfer when implemented without serious consideration of the potential ecological impacts may lead to serious impairment of overall ecosystem function and biodiversity loss.

The composition of benthic macroinvertebrate communities serves as an important indicator of ecosystem health, reflecting the overall ecological balance and function of aquatic freshwater ecosystems. By extension, this means that knowledge of the health status of

freshwater ecosystems can be leveraged to inform decisions regarding inter-basin water transfer by assessing the potential impacts on biodiversity and ecological integrity across interconnected water systems. This study investigated the ecological health of selected sites of the Luapula River Basin using benthic macroinvertebrate community structure and water quality parameters. This was in line with the proposed inter-basin water transfer scheme to the Kafue River Basin. The study employed taxonomic and functional attributes of macroinvertebrate communities and water quality assessment to determine the ecosystem health of the Luapula River Basin in the broader context of catchment protection and water conservation.

1.2 Statement of the Problem

Water supply and availability is a major issue for Zambia's sustainable economic development especially in the Kafue River Basin. The current water supply and storage infrastructure cannot meet the increasing water demand for economic, social and ecological consumption (WWF, 2016 and 2018). The Kafue River Basin is responsible for more than K48 billion of the country's Gross Domestic Product (GDP) and supports about 50% of the national population through various sectors such as agriculture, fishing, tourism, industry and hydropower generation (WWF, 2016 and 2018). To address this issue, inter-basin water transfer schemes have been proposed in the Seventh National Development Plan of 2017, the Eighth National Development Plan of 2022 and the Zambia Water Investment Programme of 2022. Inter-basin water transfer schemes present a promising solution for addressing water scarcity and supporting sustainable development. These schemes involve the redistribution of water from catchments with surplus water resources such as the Luapula River Basin to those facing deficits like the Kafue River Basin to secure a sustained water supply for various purposes including social, economic and ecological consumption. However, despite their potential benefits, inter-basin water transfer schemes can also bring about significant ecological challenges. These schemes may disrupt natural ecosystem functions and processes, leading to habitat degradation, the introduction of invasive species and alterations in water quality and hydrological regimes. Therefore, it is important to strike a balance between meeting immediate water demands and safeguarding the ecological health of freshwater ecosystems.

In light of these considerations, this study assessed the ecological health of selected sites in the Luapula River Basin, which serves as a potential donor basin for inter-basin water transfer to the Kafue River Basin. The study involved the use of benthic macroinvertebrate community structure and water quality parameters as indicators of ecological health. The study is part of a

larger study entitled “The Water Conservation: Ehancing Catchment Potection and Management in the Zambezi and Luapula River Basins”.

1.3 Objectives

The general objective of this research was to investigate the ecological health of selected sites of the Luapula River Basin using benthic macroinvertebrate assemblage and water quality parameters, and the following are the specific objectives:

i. To delineate the Luapula River Basin into hydro-environmental zones;
ii. To assess the ecosystem health and function of selected sites based on benthic macroinvertebrate assemblage; and
iii. To investigate the interactions between water quality parameters and benthic macroinvertebrate taxa.

1.4 Research Questions

The study responded to the following research questions:

i. What is the extent of spatially separated ecological habitats based on hydro-environmental attributes in the Luapula River Basin?
ii. Based on benthic macroinvertebrate assemblage, what is the status of ecosystem health and function in selected sites of the Luapula River Basin?
iii. Do water quality parameters interact with benthic macroinvertebrate taxa and associated ecosystem attributes?

The study forms part of a PhD study focusing on the assessment of potential ecological impacts of inter-basin water transfer between the Luapula River Basin and Kafue River Basin.

1.5 Significance of the Study

Water being a fundamental and finite resource plays a centric role in society, the economy and the environment. However, the Kafue River Basin is currently experiencing escalating water demand that surpasses existing storage infrastructure capacity. The resultant supply disruptions have significantly challenged various socio-economic activities including hydro-power generation, agriculture, industry, mining, tourism and domestic water supply. This situation is further compounded by a growing population residing within the river catchment, making immediate and sustainable measures necessary to ensure long-term water availability.

In the context of this urgency, this study assessed the ecological health of selected sites in the Luapula River Basin, with a particular focus on potential inter-basin water transfer to the Kafue

River Basin. This study feeds into a PhD focusing on the potential ecological impacts of inter-basin water transfer between the Luapula River Basin and the Kafue River Basin. The study employed biomonitoring assessments of benthic macroinvertebrate assemblages and water quality assessment to inform the ecological health of the Luapula freshwater ecosystem. The research findings underscore the efficacy of biomonitoring in assessing freshwater ecosystem health and function, thereby advocating for the integration of ecological considerations into water resources management and development. The study findings further advocate for the widespread adoption of biomonitoring techniques in the conservation and monitoring of freshwater ecosystems to enhance informed and ecologically sound water resource planning and management practices.

1.6 Scope of the Study

This study was confined to assessing the ecological health of selected sites within the Luapula River Basin, a potential donor basin for inter-basin water transfer to the water-stressed Kafue River Basin. The study focused on evaluating freshwater ecosystem health using two primary sets of indicators:

i. **Benthic Macroinvertebrate Assemblages:**
   The study examined the community structure of benthic macroinvertebrates as biological indicators of ecosystem integrity and function. This involved identifying the taxa present, assessing their diversity and functional feeding groups, and interpreting these patterns in relation to local ecological conditions.

ii. **Water Quality Parameters:**
   Physical and chemical water quality parameters were measured and analysed to determine their influence on the interactions with the benthic macroinvertebrate communities. This approach provided insight into the dynamics between abiotic factors and biological responses within the ecosystem.

Additionally, the study involved delineating the Luapula River Basin into distinct hydro-environmental zones. This zoning enabled a spatially explicit assessment of ecological habitats, thereby clarifying how different areas within the basin contribute to overall ecosystem health.

It is important to note that this study did not conduct any comparative assessments of macroinvertebrate taxa or water quality parameters between the Luapula River Basin and the Kafue River Basin. The investigation was limited to the Luapula River Basin, and it did not

include a comprehensive socio-economic or hydrological impact analysis of inter-basin water transfers. Instead, the study provides a focused ecological assessment intended to inform the health status of freshwater ecosystems of the Luapula River Basin.

1.7 Thesis Organisation

The thesis consists of six chapters, which are outlined below:

Chapter 1: This chapter discusses Zambia's freshwater ecosystems, highlighting rising water demand in the Kafue River Basin and the potential of inter-basin water transfer. It highlights the increasing water demand in the Kafue River Basin, which exceeds existing supply capacities, necessitating the consideration of inter-basin water transfer (IBWT) as a potential solution.

Chapter 2. This chapter examines the integration of benthic macroinvertebrate assessments and water quality analyses to evaluate freshwater ecosystem health. It discusses IWRM and IBWT as responses to water crises and highlights the importance of biomonitoring, ecological diversity indices and functional feeding groups in sustainable water resource management.

Chapter 3: This chapter provides an overview of the Luapula River Basin, detailing its geographical, climatic, hydrological and ecological characteristics, including transboundary issues, land use, soil composition, demographics, cultural heritage and economic contributions.

Chapter 4: This chapter details the systematic approach to assessing the ecological health of the Luapula River Basin. It covers site selection using geospatial tools, sampling protocols for benthic macroinvertebrates (using ZISS) and water quality, and the application of statistical analyses (MANOVA, ANOVA, CCA) to evaluate biodiversity and biotic–abiotic relationships.

Chapter 5. This chapter presents findings on the ecological health, biodiversity and water quality of the Luapula River Basin. It identifies distinct hydro-environmental zones, documents ecosystem deterioration via ZISS and reports spatial variations in water quality, highlighting key factors influencing macroinvertebrate distribution through Canonical Correspondence Analysis.

Chapter 6: This chapter discusses the study's limitations, key findings and proposed recommendations for sustainable water resource management in the context of the proposed inter-basin water transfer from the Luapula River Basin to the Kafue River Basin.

1.8 Ethical Consideration

This research was approved by the University of Zambia Natural and Applied Sciences Research Ethics Committee (NASREC).

CHAPTER TWO: LITERATURE REVIEW

The integration of benthic macroinvertebrate taxonomic and functional community structure and physicochemical water quality analysis has emerged as an indispensable tool for evaluating the ecological health and water quality conditions of freshwater ecosystems (Karr, 1981; Cummins, 2016; Banda et al., 2023).

Integrated Water Resources Management (IWRM) emphasises the integration of ecological considerations into water resource management, acknowledging their pivotal role in ensuring the sustainable utilisation of water resources. This review highlights the significance of the integrated approach to ecosystem health assessment as framed by the general and specific objectives in the context of a prospective inter-basin water transfer of the Luapula River Basin with the Kafue River Basin.

2.1 Water Crisis and IWRM Implementation

The global water crisis has consistently ranked among the top global risks identified by the World Economic Forum (WEF), posing significant threats to social, economic and environmental systems. Related risks include extreme weather events, failure of climate adaptation, resource crises and the rapid spread of diseases. With growing global populations and increasing demand for water in agriculture, industry, domestic use and hydropower, Integrated Water Resource Management (IWRM) is critical (World Economic Forum, 2015, 2023, 2024).

The 2015 WEF Global Risks Report emphasises the interconnectedness of water, food, energy and climate change, projecting that effective integrated water resource management will be essential to meet the needs of a global population expected to reach 9 billion by 2050. Addressing this nexus requires systematic approaches to ensure sufficient water for energy generation and food production (World Economic Forum, 2015).

Hanjra and Qureshi (2010) highlight the link between food security, water scarcity, energy crises and climate change. They argue that eradicating poverty and hunger necessitates targeted investments in climate change mitigation, land and water conservation and infrastructure development. Similarly, Gleick (2003) notes that while 20th-century water infrastructure projects provided widespread benefits, they also caused significant social, economic and ecological challenges. A lack of access to safe drinking water remains a critical issue, affecting over 1 million people and contributing to waterborne diseases and deaths. Gleick (2003)

advocates for supplementing centralised infrastructure with decentralised, cost-effective solutions, equitable pricing and environmental protections. Although the United Nations' 2003 International Freshwater Year raised awareness about freshwater management, progress has been impeded by insufficient funding, water conflicts, unsustainable groundwater use and limited monitoring capacity.

In Africa, water is central to development in agriculture, health, education and the economy. Hanjra et al. (2009) argue that integrated approaches to agricultural water, education and market interventions can reduce poverty and hunger while advancing Millennium Development Goals (MDGs). Naik (2017) defines a water crisis as a scarcity severe enough to threaten communities or societies, attributing Africa's economic water crisis to inadequate management structures rather than absolute scarcity. Islam and Susskind (2015) warn that water stress, driven by population growth, poor allocation practices and infrastructure underinvestment, could exacerbate conflicts in regions like Africa and the Middle East.

IWRM, a target under Sustainable Development Goal (SDG) 6, aims to ensure sustainable water management. However, implementation in developing countries like Zambia faces challenges, including inadequate governance structures, financing, management instruments and community participation (Uhlendahl et al., 2011).

The World Wide Fund for Nature (WWF) underscores the critical role of sustainable water management in Zambia's development. For example, the Kafue Flats alone contribute over K48 billion to the national GDP and support more than half of the population through agriculture, hydropower, and livelihoods. However, growing water demand from mining, agriculture, industry and domestic use has strained the Kafue Basin's resources, highlighting the need for collaborative governance and ecosystem protection measures (WWF, 2016, 2018).

The reported deficit in water supply quantities for social, economic and ecological consumption in the Kafue River Basin is the basis on which this study investigated the ecological health of the Luapula River Basin in light of the potential inter-basin water transfer scheme.

2.2 Inter-basin Water Transfer

The Zambian Government through the 2017 Seventh National Development Plan (7NDP) and 2022 Eighth National Development Plan (8NDP) notes the existing relationships between economic growth, industrial growth and water consumption. Collectively, Zambia is said to have a renewable water resource per capita of 8700 m^3. The 7NDP and 8NDP also acknowledge

the significant role that water supply and storage infrastructure plays in achieving sustainable water resources management and development. This is key to achieving food, water and energy security through sustained water supply for agriculture, fishing, tourism, mining, hydropower generation and domestic water supply. Through the 7NDP and 8NDP, the Zambian Government identifies inter-basin water transfer between water resource-rich catchments and water resource-stressed catchments as a strategy to alleviate the water supply crisis. As outlined in the two national development plans, inter-basin water transfer schemes should be implemented through catchment water potential assessments and water transfer infrastructure development programs. However, the implementation of inter-basin water transfer schemes is accompanied by heightened socioeconomic and ecological costs. Snaddon et al. (1998) notes that the growing need to reconcile water supply disparities is one of the driving factors to the increasing implementation of inter-basin water transfer schemes in southern Africa. Snaddon et al. (1998) also notes that the implementation of inter-basin water transfer schemes usually follows complex engineering protocols in terms of financing and technical expertise. However, it has also been observed that such implementations usually lack consideration of social and ecological implications (Snaddon et al., 1998).

Pittock et al. (2009) argued that although the implementation of inter-basin water transfer schemes potentially addresses the problem of water supply scarcity in regions with water shortages, there are potential social and ecological impacts, especially on local communities in both the donor and receiving basins that cannot be downplayed. Pittock et al. (2009) noted that from an environmental perspective, the implementation of large-scale inter-basin water transfers interrupts the connectivity of river systems which disrupts fish spawning and migration. It modifies the flow regime and river morphology, and enables the introduction of invasive species in both donor and receiving basins. Additionally, Pittock et al. (2009) notes that apart from hydropower generation, another major driver of inter-basin water transfer is the growing need to promote irrigation agriculture in areas with poor water availability. Furthermore, Pittock et al. (2009) highlights some of the governance inadequacies that have generally characterised the implementation of inter-basin water transfer schemes, including the failure to examine alternatives to inter-basin water transfer, poor consultation with affected people and the general failure to give sufficient consideration to ecological, social and cultural impacts of the inter basin-water transfer in both the donor and receiving basin.

2.3 Biological Monitoring of Freshwater Ecosystems

Tansley (1935) describes an ecosystem as "the whole system, including not only the organism-complex but also the whole complex of physical factors forming what is called the environment of the biome". Tansley (1935) further asserts that although our primary interest is biased towards the biotic aspect of this "whole complex", our fundamental conception of the complex ecosystem interactions makes it difficult to separate it from the abiotic aspect, "with which they form one physical system".

In line with Tansley (1935), an ecosystem refers to a community of living plants and animals, as well as non-living components such as water, land and air. All these components are collectively known as the ecosystem composition. Ecosystems rely heavily on natural cycles such as the hydrological cycle, carbon cycle, nutrient cycle and primary production, which are collectively referred to as the ecosystem function (Mooney, 2005). In addition, an ecosystem also has a structure, which incorporates the many complex trophic interactions between biotic components and abiotic components.

The Millennium Ecosystem Assessment (MEA) (Mooney, 2005) reports that the loss of biodiversity and ecosystem change over the past 50 years has been substantial and irreversible on many fronts. This loss of biodiversity and ecosystem change has mainly been driven by escalated demands for food, water and other nature-based resources. The observed ecosystem change has over time contributed substantially to human well-being and economic advancement through the utilisation of various ecosystem services. However, the increasing unsustainable demand for ecosystem services has also greatly contributed to the observed degradation of natural ecosystems, the growing risk of non-linear changes and the reversal of targeted efforts to reduce global poverty and hunger, especially in developing countries (Mooney, 2005).

The MEA also reports on the observed rapid change in ecosystem structure and function especially in the second half of the 20th century. The change in structure and function has mainly been characterised by the conversion of more terrestrial landscapes to cropland, with about a quarter of the earth's terrestrial systems now covered by cultivated systems. Water resources management is another aspect that has been called to scrutiny since the 1960s. The MEA reports an increase in water abstraction mainly for agricultural use and accompanying this is an increase in water withdrawal from natural systems and impoundments behind dam systems. The continued increase in the demand for ecosystem services mainly driven by

expanding human demographics has discriminately subjected ecosystem services such as water supply and capture fisheries to unsustainable exploitation and degradation. The prevailing unmitigated degradation of the earth's natural ecosystems presents a challenge of biodiversity loss and ecosystem impairment. It is important to note that ecosystems are both structural and functional units held together by a delicate balance between biotic and abiotic entities along with many functional processes that regulate the whole system. The loss of such a balance due to either anthropogenic or natural forces would therefore affect an ecosystem's integrity/health by incapacitating its ability to resist impairment (Mooney, 2005). This study considers ecosystem health and ecosystem integrity as interchangeable expressions.

Ecological integrity refers to an ecosystem's adaptive capacity to resist both anthropogenic and natural impairment while maintaining a balance around its composition, structure and function. Karr (1993) defines ecological integrity as "the sum of the elements, biodiversity and processes". Karr (1993) further asserts that the degradation of this integrity leads to "biotic impoverishments and the systematic reduction in the earth's capacity to support living systems". A biological system is considered to be healthy and of sound ecological integrity when its "inherent potential is realised, its condition is stable, its capacity for self-repair is maintained and external support for maintenance is minimal" (Karr, 1993).

Karr (1993) further notes a focal transition in environmental protection priorities in that primary attention has with time shifted from a human-centric approach to understanding ecological health to a more balanced ecologically inclusive approach. Water chemistry has for a long time been used as a surrogate measure of the ecological health of water resources globally. However, the understanding of the existing linkage between the ecological health, biological diversity and biological integrity of natural systems is now a critical aspect of environmental protection criteria, especially when applied in concert with water chemistry methods. This means that any efforts to build systematic criteria of ecological assessment for an ecological system should be based on its biology, seeing that the degradation of such a system would imply the system's failure to support the resident biological forms (Karr, 1993). Hart (1994) defines biological monitoring as "any activity in environmental monitoring and assessment in which inferences about the status or quality of the environment are drawn from structural and the functional attributes of individuals, populations, communities or ecosystems".

The use of biological indicators in the assessment of freshwater ecosystem condition and health has in recent years grown in popularity and application over the traditional physicochemical water quality assessments. Barbour et al. (2000) argue that the use of physicochemical water parameters to measure the ecological conditions of freshwater systems falls short because of its inability to capture the prevailing biogeochemical changes taking place within a particular system over a varied temporal and spatial scale. In reaffirming the preceding assertion, Elias et al. (2014) provide a descriptive concept of assessing the conditions of aquatic systems using a much more efficient, effective and lower-cost method known as biomonitoring. Hart (1994) defines biomonitoring as an exercise in which an inferential position is made about the condition of an environment based on the structural and functional attributes of individuals, populations, communities or ecosystems.

Karr (1981) also disputes the use of physicochemical water parameters as a surrogate for more comprehensive biotic assessments in determining the impact of anthropogenic activities on aquatic life. In reaffirming the above assertion, Karr (1998) proposed a biotic assessment system that employs a series of fish community attributes related to species composition and ecological structure to evaluate the quality of aquatic biota.

Amid growing environmental concerns, Hart (1994) highlights the growing need for ecological approaches in environmental assessment and problem-solving. Hart (1994) further advocates for biomonitoring as a practical tool for assessing environmental quality based on individual, population, community and ecosystem attributes.

Elias et al. (2014) note that despite the remarkable progress that has so far been accomplished in the application of biomonitoring indices for ecological and environmental quality assessments, the same cannot be said about the development and application of such biomonitoring indices in tropical rivers of Africa. Elias et al. (2014) also note that the development of biomonitoring indices is usually regional-specific to account for regional variations that may arise from the tolerance to environmental stressors of resident biotic communities. These variations can significantly alter the reliability and sensitivity of such tools when recklessly applied to tropical systems without proper consideration of existing variations. Attempts to modify non-tropical biomonitoring indices for application in ecological assessments of tropical systems are usually marred by a challenge of incomplete taxonomical resolutions (Elias et al., (2014).

2.4 Ecological Diversity Indices

In their attempt to comprehend the conceptual aspects that underlie the quantification of ecological diversity, Daly et al. (2018) argue that understanding the concept of ecological diversity is crucial to comprehend the function and health of an ecosystem. However, the development of a quantification criterion for such a multi-dimensional concept is often problematic (Brown, 2001).

Magurran (1998) describes diversity as an optical illusion whose complexities only become more vivid with our increasing efforts at its comprehension. Additionally, Magurran (1998) postulates that the concept of diversity remains the central theme of ecological studies today because it provides a basis upon which ecosystem wellbeing can be assessed. Furthermore, interesting debate still surrounds the quantification aspect of diversity, which is still mainly considered "a straightforward concept which can be quickly and painlessly measured."

Daly et al. (2018) define diversity indices as mathematical functions designed to provide a quantification form of ecological biodiversity. The application of such mathematical constructions in ecological studies provides a basis for comparing "different spatial regions, temporal periods, taxa (species), functional groups or trophic levels" (Daly et al., 2018). Magurran (1998) pays recognition to the many efforts that have characterised the development of a wide range of diversity indices and mathematical models to come up with a quantification criterion of biodiversity. Despite its knack for eluding definition, biodiversity can easily be conceptualised into two forms, which include species richness and abundance (Magurran, 1998). In its broadest sense, biodiversity has been defined as a concept of variation at every level of biological and ecological organisation (Daly et al., 2018). Daly et al. (2018) also assert that the construction of mathematical functions that quantify both ecological and biological attributes into meaningful outcomes is now an essential aspect of ecological monitoring and conservation. However, this development has in itself presented a challenge of a lack of consensus in the suitability and applicability of the various formulated diversity indices in biodiversity assessments.

According to DeJong (1975), The Shannon Diversity Index is the most widely used derivative of the Shannon's information theory of communication. It's application in ecological studies has allowed for the characterisation of biological communities and ecosystems from a diversity stand point. DeJong (1975) also classifies the Simpson diversity index as a measure of concentration of species and that its value increases with decreasing diversity. The Simpson

diversity index represents the probability that two randomly sampled species belong to the same species. The Simpson index is considered to be a dominance index in that it tends to be more affected by the more dominant species within a randomly sampled community, while belittling the contribution of rare species within the same community (DeJong, 1975). The Simpson diversity is however considered to be counter intuitive in that higher scores from this index represent low diversity. It against this background that the most applied formulation of this index is the Gini-Simpson index, which represents the probability that two randomly sampled species belong to two different species (Daly et al., 2018).

2.5 Benthic Macroinvertebrates and Bio-assessment Indices

The use of macroinvertebrate rapid bio-assessment indices in environmental assessments based on taxon abundances has grown in prominence over the years (Brown, 2001). Brown (2001) also notes that the shift in approach towards the use of community-based taxon abundances has been driven by the understanding that "changes in community structure may result from behavioural responses or from shifts in the competitive ability or fecundity of organisms making up that community and are not necessarily dependent on the deaths of organisms." This is coupled with the understanding that the reported increase in environmental perturbations has the potential to subtly affect organism reproductive behaviour.

Ollis et al. (2006) submits that river catchments are consistently faced with issues of environmental perturbations mainly arising from the prevailing unsustainable ecological activities. The conditions of freshwater ecosystems within river catchments are a direct reflection of the prevailing anthropogenic activities. To ensure the protection and effective management of these systems, Ollis et al. (2006) submits that there is a need for deliberate policy support for more comprehensive ecological assessments of freshwater water systems as that is the starting point of appropriate conservation strategies.

Karr (1993) also notes a shift of focus in the overall concept of environmental protection from a solely human-centric approach to a much more encompassing ecological health approach. Karr (1993) also submits that the success in our efforts to champion the protection of the ecological health of river systems demands that we complement traditional water chemistry and toxicology testing with more ecologically robust methods. The methods should include "evaluations relative to regional expectations, use of multimetric indices that reflect the multivariate nature of biological systems and index metrics that evaluate conditions from individual, population, assemblage and landscape perspective" (Karr (1993).

Ollis et al. (2006) also notes that several biological entities have in the past been used in bio-assessment of water quality and the ecological integrity of aquatic ecosystems and these include bacteria, protozoans, diatoms, algae, macrophytes, macroinvertebrates and fish. However, Macroinvertebrates are usually a preferred choice of biological indicator mainly because of the following reasons (Ollis et al., 2006):

i. Benthic macroinvertebrates are largely non-mobile, ubiquitous and relatively abundant inhabitants of rivers, occupying most habitats;
ii. There are often many species within a community with varying sensitivities to stresses and relatively quick reaction times, resulting in a spectrum of graded, recognisable responses to environmental perturbation;
iii. The responses to different types of pollution have been established for many common species;
iv. Macroinvertebrates have life cycles that are long enough for temporal changes caused by perturbations to be detected, but their life spans are also short enough to enable the observation of recolonization patterns following perturbation; and
v. They are relatively easy and inexpensive to collect.

The United Kingdom (UK) is reported to have been the first country that officially introduced the use of Rapid Biomonitoring Methods (RBMs) for assessing river pollution in the 1970s. Since 1970 the use of improved RBMs has gained widespread recognition as a quick response to the need for cheap and effective water quality assessment (Elias et al., 2014). Elias et al. (2014) also highlight the development of similar RBMs in the Southern Region of Africa, which have mostly been adapted from the South African Scoring System version 5 (SASS5), "which has been extensively tested in South Africa, and its capability and reliability proven for assessment of water quality and general river condition." Some of the RBMs currently in use in the Southern region of Africa include (Elias et al., 2014):

i. The Zambia Invertebrate Scoring System (ZISS) in Zambia;
ii. The Namibia Scoring System (NASS) in Namibia; and
iii. The Okavango Assessment System (OKAS) in Okavango Delta.

The Zambia Invertebrate Scoring System (ZISS) is a product of an extensive survey of tropical rivers within Zambia conducted from the year 2009 to 2012. The RBM is based on 151 collected samples of benthic macroinvertebrates, located on 95 rivers in six of the nine freshwater ecoregions (Dallas et al., 2018). According to Dallas et al. (2018), the ZISS protocol "which is based on the South African Scoring System (SASS), includes a total of 85 taxa, of

which 79 are shared with SASS." Dallas et al. (2018) further add that the "assignment of sensitivity weightings to new ZISS taxa was based on sensitivity weightings of closely related SASS families, known life-history modes and anatomical adaptations and correlation of occurrence to impact ratings".

2.6 Benthic Macroinvertebrates Feeding Functional Groups

According to Cummins et al. (2005), the concept of feeding functional groups categorises macroinvertebrate communities based on their behavioural adaptations for food acquisition. Biological assessment of the ecological health of water systems is generally conducted using two approaches: taxonomic approach; and functional approach. The former is primarily applied to understand macroinvertebrate communities in the context of species richness and diversity in the face of anthropogenic and natural perturbations. The use of the functional approach in biological assessments on the other hand is intended to characterise the ecosystem status of a river system based on macroinvertebrate food acquisition strategies (Cummins et al., 2005). Cummins et al. (2005) describe five feeding functional groups, namely shredders, gathering collectors, filtering collectors, scrappers and predators.

Shredders and gathering collectors actively participate in detritus processing. In terms of resource acquisition, gathering collectors are sediment feeders and they specialise in feeding on fine particulate organic matter (FPOM), which is resident on benthic surfaces of aquatic ecosystems. On the other hand, shredders are more adapted to feeding on coarse particulate organic matter (CPOM), which is in the form of living or decomposing plant material. The high abundance of gathering collectors is indirectly suggestive of a stable supply of FPOM in the ecosystem. The utilisation of FPOM by gathering collectors as a food resource is key in nutrient recycling processes, sediment stabilisation and also prevents the build-up of detritus in aquatic ecosystems, all of which are important for maintaining the overall health and balance of the ecosystem. On the other hand, a suppressed presence of shredders may indicate an unbalanced shredder association linked to an ineffective riparian estate. Shredders have an important role in breaking down large organic particles into smaller ones, thereby releasing nutrients within the systems they inhabit. They are primary decomposers and mainly rely on the riparian section of aquatic systems for their food supply. A consistently low number of shredders in aquatic systems could indicate an existing degradation of the riparian estate, which may affect the availability of essential nutrients for other organisms in the ecosystem (Cummins and Klug, 1979; Cummins et al., 2005; Merritt et al., 2017).

Scrapers are herbivorous aquatic organisms that primarily feed on algae and periphyton while filtering collectors feed on suspended organic including detrital particles, bacteria and faeces (Cummins et al., 2005). Together they are essential for maintaining a balanced ecosystem in freshwater environments by controlling excessive algal growth and the accumulation of suspended organic matter which may negatively impact water quality. A stable population of scrapers and filtering collectors facilitates water clarity, which allows for light penetration, leading to primary productivity. The control of excessive algal growth and accumulation of suspended particles also helps to maintain good habitat quality by mitigating possible alterations in habitat structure. Scrapers and filtering collectors also prevent the smothering of other aquatic organisms by excessive algae growth and accumulated organic loads, thereby enhancing the diversity of the ecosystem (Cummins and Klug, 1979; Cummins et al., 2005; Merritt et al., 2017).

A significant presence of predators in a stream ecosystem can help maintain a stable and multilevel food web. Considering their food acquisition strategies which involve the engulfing of living prey and ingestion of bodily tissue fluids, predators can regulate the population of other macroinvertebrate functional guilds thereby maintaining a balance within the ecosystem (Cummins et al., 2005). However, if this feeding functional group becomes too dominant, it can lead to a reduction in the abundance of other functional guilds and a decrease in the amount of organic matter transported downstream, thereby affecting the nutrient cycling and productivity of the ecosystem (Cummins and Klug, 1979; Cummins et al., 2005; Merritt et al., 2017).

The technique of using behavioural adaptations for food acquisition to classify aquatic invertebrates into functional feeding groups has over the years morphed into a knowledge opportunity to further understand the trophic dynamics within aquatic systems (Gholizadeh and Heydarzadeh, 2020). Gholizadeh and Heydarzadeh (2020) detail a study of how macroinvertebrate feeding groups can be used as surrogates for biological assessment of ecosystem health and function. The outcome of this study speaks to the important role that macroinvertebrate feeding functional groups play in providing information regarding the balance of feeding strategies within macroinvertebrate communities. Which in turn is used as an indirect measure of ecosystem condition based on the abundances of functional categories whose distribution can be linked to prevailing anthropogenic perturbations. Cummins & Klug (1979) further emphasises that this concept of biological categorization is according to behavioural adaptations for food acquisition and not the type of food eaten.

2.7 Water Quality Index

Brown et al. (1970) describe the lack of an explicit form of communication about the quality of water between professionals and the public as an apotheosis of irony. In addressing the growing demands for enhanced expenditure in water pollution control in the United States of America (USA), Brown et al. (1970) and Brown et al. (1972) detail an overview of methodologies that characterised the earlier development of a water quality index. The water quality index is specific to both temporal and spatial extents. The measure of the water quality status of a particular system at any given effort of assessment is informed by a singular value that reflects a flux or lack thereof of physicochemical parameters within the context of the prevailing temporal and spatial scales (Sivaranjani, 2015).

Five years before the works of Brown et al. (1970), Horton (1965) is recognised as having pioneered preliminary works into the subsequent development of a water quality index in the USA. The works of Horton (1965) were centred on ten common water quality parameters, including sewage treatment, dissolved oxygen, pH, coliform density, specific conductance, carbon chloroform extract, alkalinity, chlorides, temperature and pollen. Each parameter was assigned a weight that reflected the significance of a particular parameter and its impact on the index (Brown et al., 1970; Sivaranjani et al., 2015). However, Brown et al. (1970) also submits that despite the significant impact of Horton (1965) in calling for attention to the usefulness of using a water quality index in water quality assessments, Horton's work does not in any way provide the validity of the assigned individual weights and goals. The water quality index developed by Brown et al. (1970) provides a weighted singular expression that reflects the water quality status of a particular system based on various prevailing water quality variables. "This index is so widely used and uniquely rated by the scientific community" (Pullanikkatil et al., 2015).

The literature review highlights the importance of incorporating economic, social and ecological factors in the implementation of strategies aimed at addressing water scarcity and achieving sustainable water resource management and development. The study focused on assessing the ecological health of selected sites in the Luapula River Basin, which aligned with the reviewed global and regional water challenges. The investigation involved studying benthic macroinvertebrate communities and water quality parameters to gain knowledge on the ecosystem health status of the Luapula River Basin. The literature review underscored the importance of ecological considerations and biomonitoring in assessments of environmental

quality and sustainable water resource management, thereby shaping the approach of this study. The integration of ecological insights into water resource planning and management remains crucial in addressing the complex challenges outlined in the literature review and achieving long-term water sustainability.

CHAPTER THREE: DESCRIPTION OF STUDY AREA

This chapter provides an overview of the study area, aiming to establish a familiarisation with its geographical, environmental, social and economic characteristics relevant to this research.

3.1 Location of the Luapula River Basin

The Luapula River Basin, which is situated predominantly in Zambia and partly in the Democratic Republic of Congo (DRC), is recognised as the fourth largest river basin in the country. It is home to the extensive Luapula River network, responsible for draining the Bangweulu wetlands, where the Chambeshi River ultimately converges. The Luapula River Basin encompasses the entirety of the Luapula Province and certain parts of the Northern Province of Zambia. In its entirety, the Luapula River Basin serves as a transboundary ecosystem, jointly shared by Zambia and DRC, with a respective area coverage of 133,323 square kilometres and 60,063 square kilometres. According to the Water Resources Management Authority (WARMA), the geographical location of the Luapula River Basin is positioned between latitudes 8° 00’ and 13° 30’ South, and longitudes 28° 20’ and 31° 45’ East (WARMA, 2021) (Figure 1).

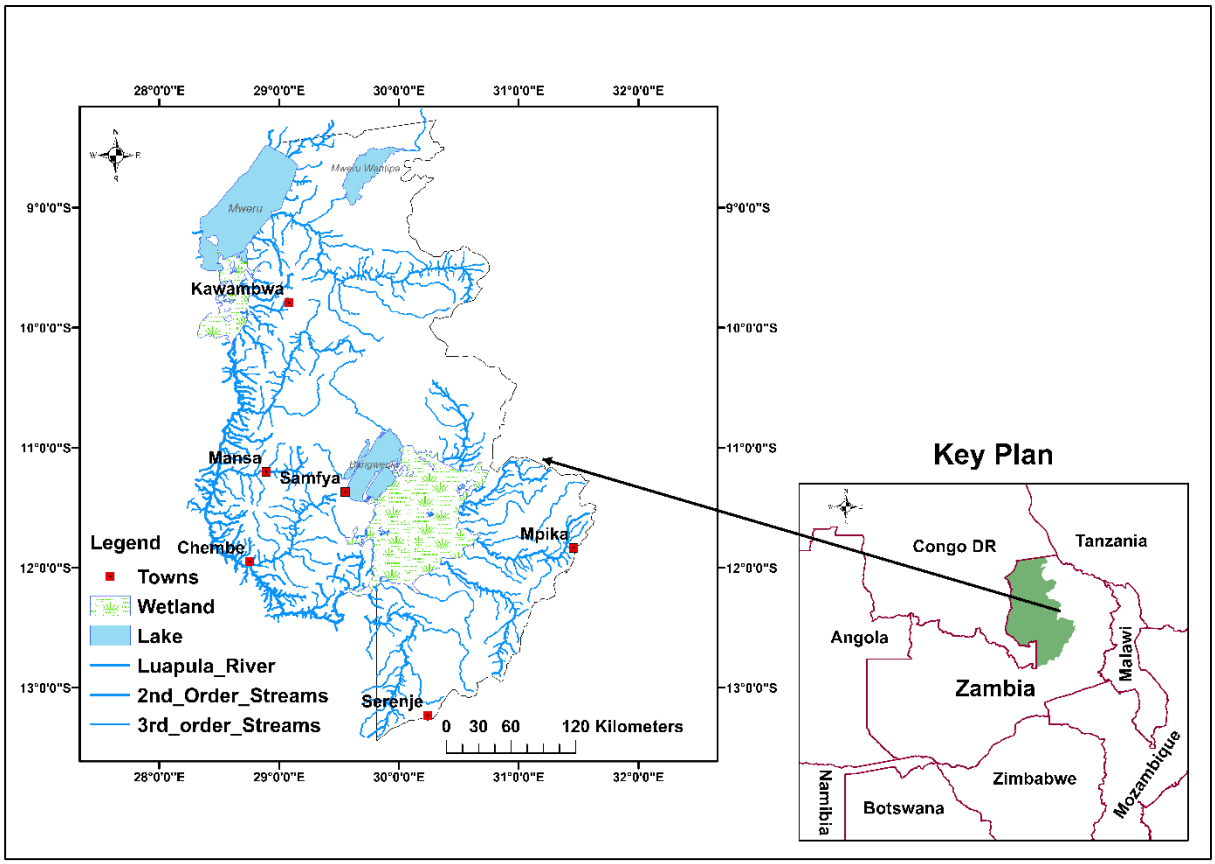


Figure 1: Location of the Luapula River Basin, Zambia.

3.2 Climate

The Water Resources Management Authority (WARMA) describes the country's climate as being mild and sub-tropical as most of the landmass lies on the Central African high plateau at an average altitude of 1,200 m above sea level with mean annual temperatures that range from 19.3 to 24.2 degrees Celsius. According to WARMA (2021), Zambia experiences three distinct seasons: the hot-dry season spanning from August to November; followed by the rainy season from November to April; and finally, the cool-dry season lasting from April to August.

Based on rainfall variations and ecological aspects, Zambia is divided into three agroecological zones, namely 1, 2 and 3. Accordingly, the Luapula River Basin sits on Zambia's 3rd agroecological zone which comprises the Northern, Muchinga, Luapula, Copperbelt and North Western provinces. The 3rd agroecological zone is characterised by precipitation potentials ranging between 1000 mm and 1500 mm. The Luapula River Basin exhibits a tropical climate encompassing three discernible seasons. These include a cool-dry season spanning from April to August, followed by a hot-dry season spanning from August to October and concluding with a warm-wet season that starts in November and ends in April. The average monthly temperature fluctuates between 16 °C and 24 °C during October, with the valley experiencing temperatures surpassing 24 °C during this time (JICA, 1995).

3.3 Hydrology

According to JICA (1995), Zambia has an approximate area coverage of 752,618 square kilometres, and this is divided into six hydrological catchments. The six drainage systems are subunits of transboundary waters which comprise the Zambezi (Kafue, Luangwa, and Zambezi sub-catchments) and the Congo (Chambeshi, Luapula and Tanganyika sub-catchments) systems (WARMA, 2021). Most of these catchments house eco-sensitive areas that constitute headwaters of major rivers that should be protected.

According to the Water Resource Management Authority (WARMA), the Luapula River Basin extends over an area coverage of 113323 km^2 with a mean annual runoff of approximately 30.14 km^3 and a total contribution to surface water potential of 26.5%. The Luapula River Basin is home to Lake Bwangweulu, Mweru and Mweru Wantipa which are three of the major natural lakes in Zambia.

3.4 Terrestrial Ecoregions

The Luapula River Basin belongs to the Zambezian wet-miombo woodlands except for the Bangweulu floodplains which belong to the Zambezian flooded grasslands (Dinerstein et al., 2017).

The Zambezian wet-miombo woodlands, which encompass approximately 70% of Zambia's land area, boast a remarkable abundance of termites. These insects play a vital role in nutrient recycling by constructing nutrient-rich mounds that enhance soil fertility. Dominated by tree species such as *Brachystegia, Julbernardia* and *Isoberlina*, these woodlands feature a diverse vegetative landscape. Key tree species within this ecosystem include *Brachystegia floribunda*, *Brachystegia glaberrima*, *Brachystegia taxifolia*, *Julbernardia globiflora*, *Julbernardia paniculata* and *Isoberlina angolensis* (Chidumayo, 1987; Dinerstein et al., 2017).

The Bangweulu floodplains are part of the Zambezian flooded grasslands, a terrestrial ecoregion known for its diverse mammal population, including large herds of migratory species. These floodplains exist as isolated patches within the wet-miombo woodlands and feature a varied vegetative landscape characterised by a mosaic of grasses and aquatic plants. The wetland is dominated by grasses belonging to the genera *Acrocera*, *Echinochloa*, *Leersia*, *Oryza*, *Phragmites*, *Typha* and *Vossia*, along with *Cyperus papyrus* (Dinerstein et al., 2017).

3.5 Aquatic Ecoregions

The Luapuala River Basin in its entirety belongs to the Bangweulu-Mweru ecoregion. This ecoregion sits on the south-eastern corner and north-eastern stretch of the Democratic Republic of Congo and Zambia, respectively. The ecoregion is a constituent of the southern headwaters of the Congo River and is characterised by an extremely rich and endemic aquatic fauna within the permanent swamps and shallow lakes of the Bangweulu/Mweru systems.

The Bangweulu-Mweru ecoregion is home to 6 major lakes within the broader Bangweulu complex: Lakes Bangweulu; Walilupi; Chifunauli; Kampolombo; Kangwena; and Chale (Bailey and Banister, 1986). The six major lakes all present a depth range of between 3-10 meters with extensive swamp networks that directly aid productivity. The Bangweulu swamps in particular are recipients to inflows from a network of river systems with the Chambeshi River being the largest.

According to Bowmaker et al. (1978), Lake Mweru stands at a depth of 37 m with an area coverage of 4,413 km^2 and has a higher plankton production than Lake Bangweulu. The mouth

of the Luapula River is characterised by a *Vossia* swamp system marked by extensive stands of large ambatch trees. The rest of the shoreline is sandy and rimmed with the sedge *Eleocharis* in shallow waters, with occasional rocky outcrops.

3.6 Landcover and Landuse

The landscape of the Luapula River Basin is characterised by a diverse mosaic of forests, wetlands, water bodies, cultivated lands and expanding urban areas. This description is illustrated in Figure 2, which is based on the 2023 global Esri Sentinel-2 Land Cover image at a 10-meter resolution.

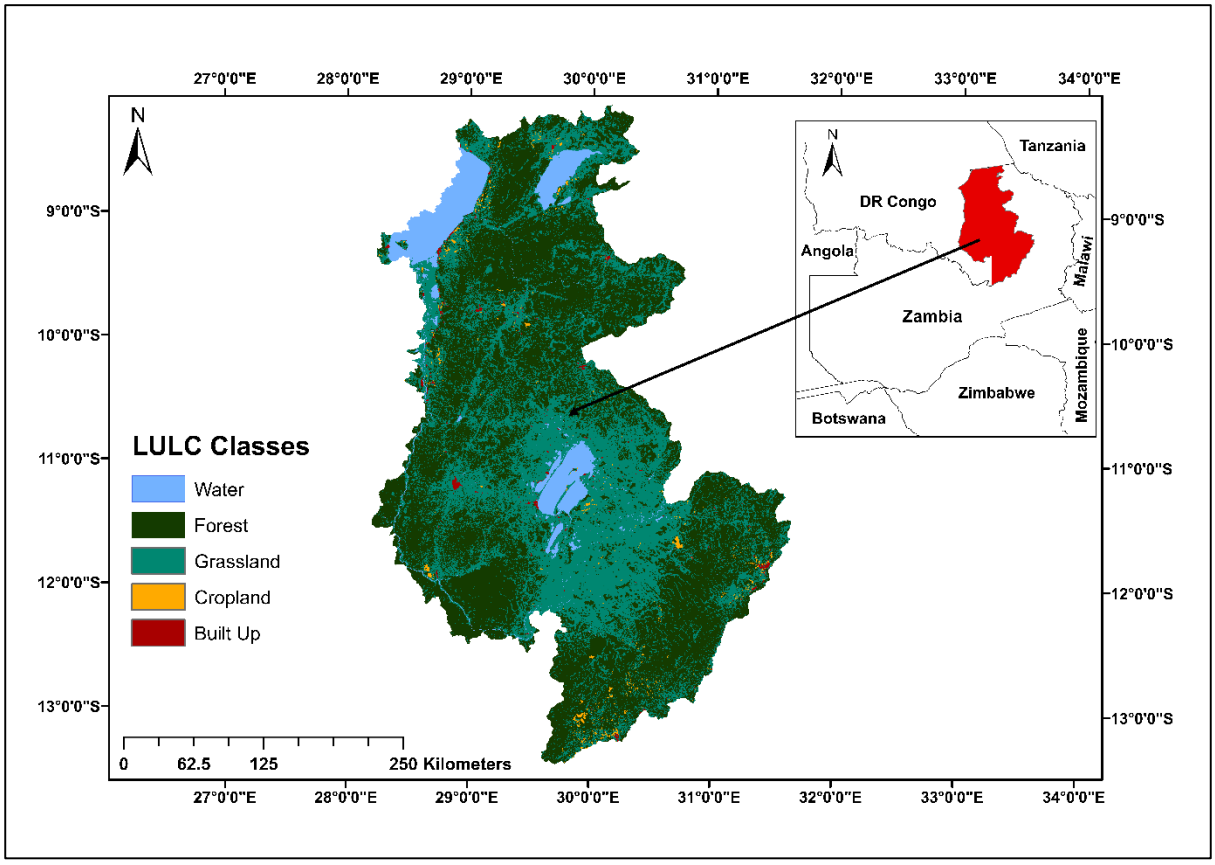


Figure 2: Land Use and Land Cover Classes of the Luapula River Basin, Zambia.

The Luapula River Basin predominantly consists of forests, with grasslands particularly abundant around Lakes Bangweulu and Mweru. The basin encompasses various forest and grassland types as outlined in section 3.4. Additionally, it features significant wetland areas, including the Bangweulu floodplains and the swamps and marshes of Lake Mweru.

The hydrological network of the basin is primarily composed of the Luapula River, its tributaries and associated lakes. These water bodies sustain aquatic ecosystems and provide essential resources for human livelihoods such as fishing, agriculture and transportation.

Agriculture is a one of predominant land use activities in the basin, with a combination of subsistence and commercial farming, particularly prevalent in the southern and northern region of the basin. Smallholder farmers cultivate various crops, including maize, cassava, soya beans, tobacco and vegetables.

Urban centers like Mansa, Samfya, Serenje and Mwense serve as important hubs for administration, commerce and transportation within the basin. Each of these centers exhibits varying degrees of urban development, featuring infrastructure, residential areas and commercial establishments. Additionally, the basin hosts the Musonda Falls hydropower station on the Luongo River in Mwense District. The capacity of the falls was recently upgraded from 5 to 10 Mega Watts, with an annual generation potential exceeding 63 million kilowatt hours of electricity on average. Furthermore, industrial activities such as agro-processing, manufacturing and mining contribute to the local economy but also pose environmental challenges such as pollution and habitat degradation.

3.7 Soils

According to Grimsdell and Bell (1975), the Chambeshi River and much of the area around the Bangweulu swamps including the Lower Luapula River have a granite underlie. However, "the entire region is dominated by recent alluvial deposits, shales, sandstones, quartzites and conglomerates." The Luapula River Basin is also characterized by heavily leached sandy loam soils, with a pH variability range of 3.7 to 4.7 (WARMA, 2021).

3.8 Population and Social-economic Activities

According to the Water Resources Management Authority (WARMA, 2021) and the Central Statistics Office (CSO) records from the 2010 census, the Luapula River Basin is home to approximately 2 million people, representing 15% of Zambia's total population. This region is rich in cultural heritage and historical significance, serving as a hub for various social and economic activities.

The Luapula River Basin hosts several traditional ceremonies that play a vital role in preserving cultural heritage and fostering social cohesion. Among these, the Mutomboko Ceremony, celebrated every July in Mwansabombwe District, is one of the most prominent. This event is deeply rooted in the traditions of the Lunda Tribe and is led by Paramount Chief Mwata Kazembe. Similarly, the Builile Ceremony occurs in September in Chienge District, under the leadership of Senior Chief Puta of the Bwile Tribe. Another culturally significant event is the Ichibwela Mushi Ceremony, celebrated in Serenje District by the Lala and Swaka communities.

Held in October, this ceremony marks the harvest season and the return of families to their villages.

Other notable October ceremonies include the Mabila Ceremony, led by Senior Chief Mununga of the Shila Tribe and the Chishinga Malaila Ceremony, guided by Senior Chief Mushota of the Chishinga Tribe, both held in Chienge District. Additionally, the Kwanga Ceremony in Samfya District is presided over by Senior Chief Mwewa of the N'gumbo Tribe, while the Chabuka Ceremony, under Chief Matanda of the Ushi Tribe, takes place in Mansa District. These ceremonies not only celebrate cultural traditions but also contribute to the region's economic and social vibrancy by attracting visitors, promoting tourism and supporting local businesses.

Economically, the Luapula River Basin is an important contributor to Zambia's development. The basin is a home for activities such as fishing, manganese mining, hydropower generation and the cultivation of crops like sugar, palm oil and bananas. These industries provide livelihoods for many residents and play an important role in the region's economy. The basin is also home to the Musonda Falls Hydro-power Plant, a key source of energy and numerous waterfalls, including Ntumbachushi, Mumbotuta, Lumangwe and Kundabwika, which are popular tourist attractions (WARMA, 2021).

CHAPTER FOUR: METHODOLOGY

The methodology section of this thesis outlines the systematic approach employed to examine the ecological health of selected sites in the Luapula River Basin. The study utilised a combination of benthic macroinvertebrate assemblage and water quality parameters analysis. By explicating the methodological framework, this section provides a comprehensive understanding of the process taken to gather and analyse data. This was intended to ensure that the research findings were suitable for informing evidence-based conservation and management of the Luapula River Basin.

4.1 Data Collection

As guided by the objectives of this study, the data collection process involved the collection of benthic macroinvertebrate taxa and water samples from the twelve selected sampling sites within the three delineated hydro-environmental zones of the Luapula River Basin. Each of the twelve sampling sites was sampled twice during the dry and wet seasons.

4.1.1 Site Selection Within the Hydro-environmental Zones

The selection of sampling sites involved the use of ArcMap (version 10.7.1) to extract stream orders from the RiversATLAS vector file within HydroATLAS-Zambia. In RiversATLAS-Zambia, the core attribute "ZAMBIA_YN", a binary indicator showing values of 1 or 0, where 1 identifies all river reaches that are fully or partially within Zambia, was used to extract river reaches that were required for geospatial analysis at a Zambian extent. Similarly, the core attribute "ZAM_CATCH", a unique identifier that assigns each river reach to one of the six major catchments as defined by the Water Resources Management Authority (WARMA): 1 = Zambezi; 2 = Kafue; 3 = Luangwa; 4 = Luapula; 5 = Chambeshi; and 6 = Lake Tanganyika was used to condense geospatial analysis to the Luapula River Basin.

The extraction of stream orders was aided by the core attribute "ORD CLAS" in RiversATLAS-Zambia. This is an indicator of river order based on the classic stream order system, which is a numeric system used to describe branching levels within a river system: Order 1 represents the main stem river from sink to source; Order 2 represents all tributaries that flow into a 1st Order river; and Order 3 represents all tributaries that flow into a 2nd Order river. This ordering system was used to identify stream networks associated with orders 1, 2 and 3 in order to determine four streams/rivers with perennial flow capacity within each one of the three created hydro-environmental zones (Table 1).

Sampling sites were finally selected based on practical considerations including logistics, perennial flow capacity and accessibility by foot, vehicle or boat.

Table 1: Annual discharge capacity of extracted stream orders in the Luapula River Basin, Zambia.

River/Stream	Minimum Annual Average Discharge (m^3/sec)	Maximum Annual Average Discharge (m^3/sec)
Luapula River	440.842 – 570.153	1273.170 – 1503.240
2nd Order Streams	0.018 – 9.997	122.600 – 189.451
3rd Order Streams	0.014 – 3.344	68.564 – 98.031

4.1.2 Benthic Macroinvertebrate Sampling

The sampling of benthic macroinvertebrates followed the application of the Zambia Invertebrate Scoring System (ZISS) (Dallas et al., 2018; Lowe et al., 2013(a); Lowe et al., 2013(b)), a locally adapted rapid bio-assessment protocol based on the South African Scoring System (SASS) (Dickens and Graham, 2002). Macroinvertebrate sampling was conducted once in the dry season, from October to November 2022 and once in the wet season from March to April 2023. At each of the 12 sampling sites, a kick net with a 2-meter-long metallic handle, a 30cm x 30cm frame and a 1mm mesh size was used to collect macroinvertebrate samples in triplicates within a longitudinal transect of 50 to 100 meters, after which the 3 sampling efforts were combined to form a composite sample. During the sampling process, coordinates of the sampling locations were taken using a handheld Global Positioning System (GPS) and all three ZISS-specified biotopes including vegetation (V), stones (S) and gravel/sand/mud (GSM) were examined at every sampling site where they were available (Appendix 1). The sampling criterion involved hand-picking and visual observation of each biotope for up to 5 minutes. Additionally, a shoreline inspection was carried out to search for mussel shells. Using a handheld lens and a photographic identification guide of common macroinvertebrate taxa in Zambian rivers (Dallas, 2013), all the collected taxa per site were identified to family taxonomic resolution.

For functional analysis, the identified macroinvertebrates were further sorted into functional aggregates in line with the concept of feeding functional groups (FFG) (Cummins et al., 2005; Cummins and Klug, 1979; Merritt et al., 2017; Merrit et al., 1996). The assortment of benthic macroinvertebrates into feeding functional groups was based on the morphological and behavioural mechanisms by which the invertebrates acquire their food resources (Appendices 2 and 3).

4.1.3 Water Sampling

Water sampling was conducted alongside macroinvertebrate sampling. Water samples were collected at each of the 12 macroinvertebrate sampling sites during the dry and wet seasons. Water samples were collected in 500 ml polyethylene bottles, which were thoroughly rinsed with distilled water before sample collection. At the sampling locations, the collection bottles were further rinsed thrice with water from the respective sampling locations. At each sampling location two water samples were collected for nutrient analysis and cation analysis. Nitric acid was added as a preservative to the water bottles meant for cation analysis at a ratio of 1% of the water sample volume. The collected water samples were all stored at 4 °C in coolers before transporting them to the Environmental Engineering Laboratory and Geochemical Laboratory at the University of Zambia for physicochemical and microbial analysis. Water quality parameters such as pH, electrical conductivity (EC, μS/cm), total dissolved solids (TDS, mg/l) and temperature (°C) were measured in situ using a ProDSS YSI xylem brand water quality meter.

4.1.4 Secondary Data

This study used a diverse range of secondary data sources, including books, journals, manuals, articles and reports related to the study. These materials were obtained from various institutions such as district and provincial administration structures, district fisheries stations, the Central Statistics Office, WWF and the University of Zambia library. Additionally, other pertinent data was sourced through the use of the internet. The ambient water specifications and standards for ambient water quality were gathered from the literature provided by the Zambia Bureau of Standards (ZABS). Literature related to this study was also reviewed to gain a comprehensive understanding of the ecological health of the Luapula River Basin. During the review process, the literature was examined to ensure the validity of the research.

4.2 Data Analysis

During data analysis, various biotic and ecological indices were utilised to comprehend the distribution, diversity and abundance of benthic macroinvertebrates across sampling locations in the Luapula River Basin. The analysis also involved the use of taxonomic and functional aggregates of benthic macroinvertebrates as indicators of ecosystem health and function. Additionally, the assessment of water quality was conducted using the weighted arithmetic water quality index based on the measured physicochemical and microbial parameters at each sampling site. Furthermore, the utilisation of multivariate ordinations allowed for the evaluation of the prevalent interactions between water parameters and macroinvertebrate taxa.

4.2.1 Zonation of the Luapula River Basin

For the characterisation of sampling zones, this study utilised four hydro-environmental attributes representing hydrology, physiography, climate and land cover from the BasinsATLAS geospatial data layers within HydroATLAS-Zambia (Lehner, 2020), a regionally adapted version of the global HydroAtlas database. In BasinsATLAS-Zambia, the core attribute "Zambia_YN", a binary indicator showing values of 1 or 0, where 1 identifies all polygons that are fully or partially within Zambia, was used to extract polygons that were required for geospatial analysis at a Zambian extent. Similarly, the core attribute "Zam_Catch", a unique identifier that assigns each polygon to one of the six major catchments as defined by the Water Resources Management Authority (WARMA): 1 = Zambezi; 2 = Kafue; 3 = Luangwa; 4 = Luapula; 5 = Chambeshi; 6 = Lake Tanganyika, was used to condense geospatial analysis to the Luapula River Basin.

The characterisation of the Luapula River Basin into hydro-environmental zones was based on four hydro-environmental attributes including elevation, precipitation, surface water runoff and forest cover extent. Geospatial Information Technology, specifically ArcMap version 10.7.1, was used to transform vector files of the aforementioned hydro-environmental attributes into raster files, which were then used to create a delineated zonal classification of the Luapula River Basin. The hydro-environmental attributes were transformed into a raster format to facilitate geospatial analysis and the natural breaks classification method was employed to optimally group raster hydro-environmental attribute data values into meaningful classes (low, medium and high) based on inherent patterns. Using the reclassify tool in ArcMap version 10.7.1, the natural breaks classified raster outputs were reclassified to rescale values of the earlier generated classes (low, medium and high) to a common scale. Following the reclassification of raster outputs, the class that was earlier assigned low was changed to Zone 1, while the classes representing medium and high were in a similar fashion assigned Zone 2 and Zone 3, respectively. This process was applied to all four raster hydro-environmental input datasets (Table 2).

Table 2: Characterisation of hydro-environmental zones based on hydro-environmental attributes of the Luapula River Basin, Zambia.

Hydro-environmental Zone	Elevation (m)	Average Annual Precipitation (mm)	Average Annual Land Surface Run-off (mm)	Forest Cover (%)
Zone 1	921 - 1092	998 - 1173	36 - 163	0 - 33
Zone 2	1093 - 1296	1174 - 1318	164 - 276	34 - 75
Zone 3	1297 - 1590	1319 - 1510	277 - 394	76 - 100

The reclassified input datasets were further visualised to show the spatial extent of the four input hydro-environmental attributes across the Luapula River Basin (Figure 3).

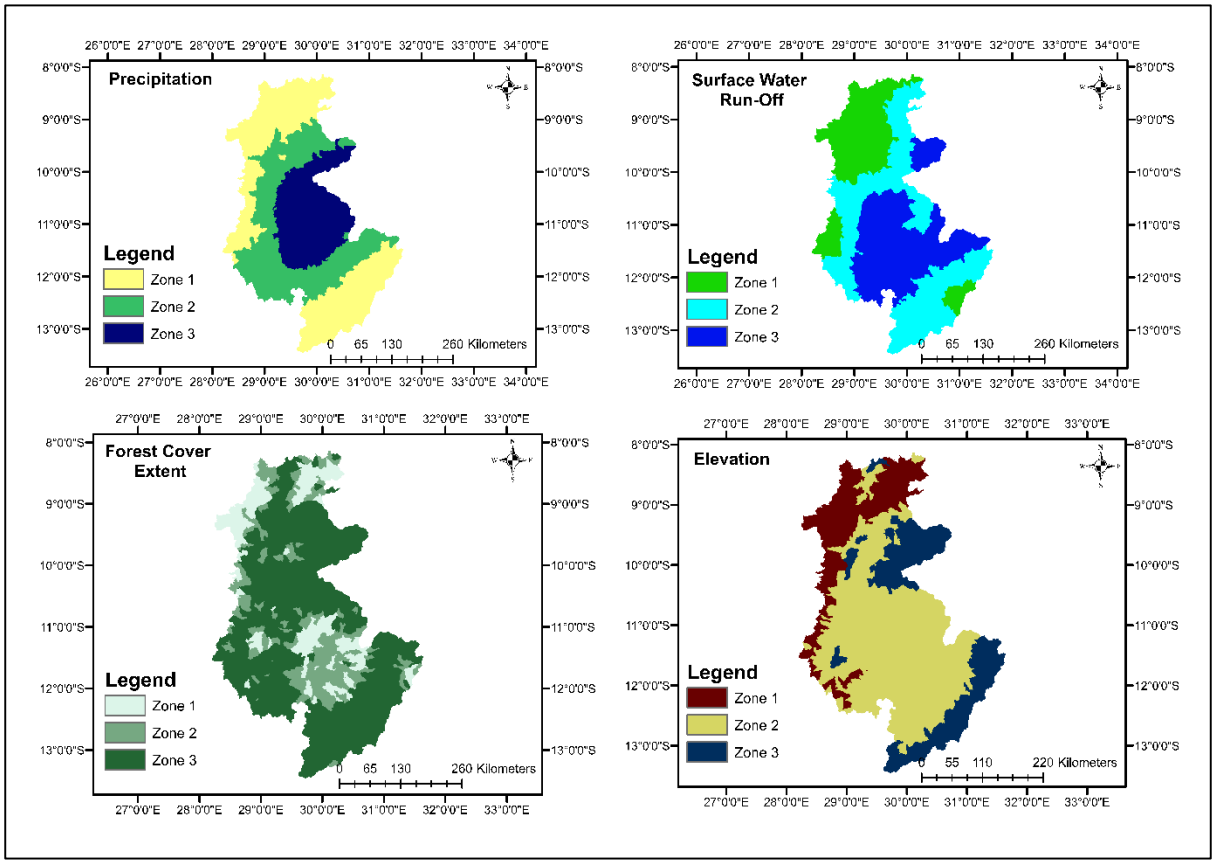


Figure 3: Spatial extent of input hydro-environmental attributes used to delineate the Luapula River Basin into spatial zones.

Using the raster calculator in ArcMap version 10.7.1, the reclassified raster layers were assigned equal weighting and subsequently combined using addition to create an overlay map that synthesized elevation, precipitation, surface water runoff and forest cover extent into one raster output. The generated overlay map was further refined using geoprocessing tools such as merge and dissolve.

4.2.2 Assessment of Taxa Diversity

Biodiversity metrics such as richness, diversity and evenness are important because they provide a quantitative measure of the biodiversity of freshwater ecosystems. These quantitative measures of biodiversity are important for assessing ecosystem health and identifying potential threats to biodiversity. By monitoring these measures over time, valuable insights can be gained regarding the well-being of freshwater ecosystems, as well as the development of tailored strategies for their conservation and management (Loreau, Hector and Isbell, 2022).

Shannon-Weiner Diversity Index was used to generate quantitative expressions of benthic macroinvertebrate taxa diversity. The Shannon-Weiner Diversity Index considers both taxa richness, which refers to the number of different taxa within a community and evenness, a quantitative measure that categorises the relative abundance of different taxa within a biological community. Taxa richness is a quantitative measure of distinct taxa that exist within a particular biological community. This quantitative measure provides a simple measure of biodiversity that does not consider the evenness of each taxon (Stirling et al., 2013).

The Shannon-Weiner Diversity index is related to the concept of uncertainty. It measures the uncertainty associated with predicting the identity of an unknown individual within a highly diverse system. In a diverse and evenly distributed community, an unknown individual could belong to any species, leading to high uncertainty in predicting its identity. The Shannon Diversity Index is represented by the following formula:

$$H = -\sum_{i=1}^{S} p_i * \ln p_i$$ …… Equation 1 (Shannon and Weaver, 1949).

Where:

i. p_i = proportion of individuals of species/taxa i;
ii. ln = is the natural logarithm; and
iii. S = species/taxa richness.

Evenness (J) which represents the balance in terms of the abundance proportion of each taxon within a community was calculated based on Shannon diversity and taxa richness. Evenness is represented by the following equation:

$$J' = \frac{H'}{\log S}$$ …..……..... Equation 2 (Pielou, 1966).

Where:

i. H' = Shannon diversity
ii. Log S = H_{max}

The equation Log S = H_{max} suggests that the natural logarithm of taxa richness (S) is equal to an ecological community's maximum entropy. This is the maximum possible diversity (H_{max}), which occurs when all species are equally abundant (Pielou, 1966).

4.2.3 Assessment of Ecosystem Health and River Condition

This study employed the Zambia Invertebrate Scoring System (ZISS) (Dallas et al., 2018) as a rapid bio-assessment protocol to evaluate the ecosystem health and river condition at selected sampling sites within the Luapula River Basin. The ZISS is a biomonitoring protocol based on the abundance and diversity of aquatic macroinvertebrates. The protocol is based on three metrics, namely:

i. The number of Taxa (families) recorded at a site;
ii. The total ZISS Score (sum of sensitivity weightings for each taxon recorded); and
iii. The Average Score Per Taxon (ASPT) (ZISS Score divided by Number of Taxa).

In the Zambia Invertebrate Scoring System (ZISS) as with the South African Scoring (SASS) from which the ZISS is adapted, the taxa with high sensitivity weightings represent taxa that sensitive to changes in water quality or river condition. Conversely, taxa with a low sensitivity weighting represent tolerant taxa that can persist under impaired conditions. This is based on a sensitivity scale derived from the tolerance of macroinvertebrate taxa to impairment. The sensitivity scale, herein known as the ZISS sensitivity scale, ranges from 1 to 15, where:

i. 1-5 Highly tolerant to impairment;
ii. 6-10 Moderately tolerant to impairment; and
iii. 11-15 Very low tolerance to impairment.

The ZISS data sheet provided by Lowe et al. (2013) was used as a reference document for taxon sensitivity scores and as a data collection tool. The final interpretation of the status of the ecosystem and river condition was based on a key provided by Chutter (1993) (Table 3).

Table 3: Interpretation of ZISS Score and ASPT for Ecosystem Health and River Condition Assessment (Chutter, 1993).

ZISS Score / ASPT Classes		
ZISS Score	**ASPT**	**Interpretation**
100	>6	Water quality natural; habitat diversity high
<100	>6	Water quality natural; habitat diversity reduced
<100	<6	Borderline good/bad water quality
50 – 100	<6	Some deterioration in water quality
<50	Variable	Major deterioration in water quality

4.2.4 Assessment of Ecosystem Function

The classification system of macroinvertebrate feeding functional groups (FFGs) was based on their feeding strategies and resource utilisation. Understanding these FFGs is important for

assessing ecosystem function of aquatic ecosystems. After the assortment of macroinvertebrate samples into taxonomic orders and families as described in section 4.1.2, the identified families were further aggregated into five feeding functional groups namely, gathering collectors, filtering collectors, shredders, scrappers and predators. This was achieved using functional guides as provided by (Cummins et al., 2005). However, Cummins et al. (2005) classified some families into more than one group. In such cases, this study classified the organism based on the genus that is more abundant in tropical countries (Hart, 2015; Lowe et al., 2013a).

The application of FFGs as proxies of ecosystem attributes was achieved through the use of FFG ratios, which were calculated by comparing the abundance of different FFGs sampled at each site. As prescribed by Cummins et al. 2005 and Merritt et al. (2017) the calculated FFG ratios were used to assess the status of five ecosystem attributes, including autotrophy to heterotrophy index (P/R index), coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) index (CPOM/FPOM index), FPOM in transport (suspended) to FPOM storage in sediments (deposited in benthos) (TFPOM/BFPOM), substrate channel stability (channel stability) and predator-prey index. The classification of macroinvertebrates into feeding functional groups and the subsequent calculation of feeding functional group ratios was done as shown in Appendices 2 and 3.

4.2.5 Water Quality Analysis and Water Quality Index

Laboratory analyses were conducted at the Geochemical and Environmental Engineering Laboratories at the University of Zambia. Water quality parameters, including electrical conductivity (EC), turbidity, total hardness, calcium, magnesium, chloride, sodium, potassium, iron, nitrates, phosphates, sulphates and faecal coliform, were analysed following standards prescribed by the American Public Health Association (APHA) (APHA, 1998). Sodium was not successfully measured during the wet season due to unforeseen technical challenges. By implication sodium measurements were excluded from analysis requiring seasonal comparisons.

The arithmetic weighted Water Quality Index (WQI), developed by Brown et al. (1972) and Horton (1965) was utilised for calculating water quality. WQI computations were performed for all sampled sites during the wet and dry seasons using an automated electronic Excel spreadsheet. Nine variables, including EC, turbidity, total hardness, calcium, magnesium, chloride, potassium, iron and faecal coliform, were used for computing the WQI. Each contributing variable was assigned its unique rating based on the ZABS ambient water quality

specifications for the Luapula River Basin. Each of the 12 sampling sites across three hydro-environmental zones was indexed following the steps below as applied at site 1 during the dry season.

Step 1: Calculate the unit weight factors (*wi*) for each parameter. On summation of all selected parameters, unit weight factors *wi* = 1 (Table 4).

wi = K/Sn

Where:

i. *wi* is the unit weight for the nth parameter;
ii. Sn is the standard desirable value of the n^{th} parameter according to ZABS ambient water quality specifications for the Luapula River Basin; and
iii. K is the reciprocal of the sum of the reciprocals of the standard desirable values for all parameters.

$$K = \frac{1}{\left(\frac{1}{51} + \frac{1}{52} + \frac{1}{53} + \cdots + \frac{1}{5n}\right)}$$ …… Equation 3 (Brown et al., 1972; Horton, 1965)

Table 4: Relative unit weights for selected water parameters at site 1 during the dry season in the Luapula River Basin, Zambia.

Site 1					
Parameters	sn	1/sn	∑1/sn	K=1/(∑1/sn)	*wi* = K/Sn
EC	100	0.010	1.680	0.595	0.006
TDS	45	0.022	1.680	0.595	0.013
Turbidity	10	0.100	1.680	0.595	0.060
Calcium	30	0.033	1.680	0.595	0.020
Magnesium	35	0.029	1.680	0.595	0.017
Iron	0.7	1.429	1.680	0.595	0.850
Cl	30	0.033	1.680	0.595	0.020
Total Hardness	250	0.004	1.680	0.595	0.002
Faecal Coliforms	50	0.020	1.680	0.595	0.012
		1.680			1

Step 2: Calculate the quality rating scale (*qi*) value representing each measured parameter's water quality rating scale. The quality rating scale (*qi*) converts the actual concentration value to a scale of 0 to 100 (Table 5). The formula for *qi* is:

$$qi = \left(\frac{V_a - V_i}{V_s - V_i}\right) \times 100$$ ……. Equation 4 (Brown et al., 1972; Horton, 1965)

Where:

i. *Va* is the actual value of the water quality parameter obtained from the analysis;
ii. *Vi* is the Ideal value (usually 0 for most parameters except pH and DO); and
iii. *Vs* is the same as "sn" which is the standard desirable value of the n^{th} parameter (as per ZABS ambient water quality specifications for the Luapula River Basin).

For specific parameters like pH and Dissolved Oxygen (DO), the ideal values differ as folows:

i. For pH, the ideal value Vi is 7 (neutral); and
ii. For DO, the ideal value Vi is the saturation value at a given temperature and pressure.

By calculating the *qi* values for each parameter, a normalised score is obtained that indicates the quality of each parameter on a scale from 0 to 100. These *qi* values are then used in subsequent steps to compute the overall Water Quality Index (WQI).

Table 5: Water quality rating scale values for water parameters at site 1 during the dry season in the Luapula River Basin, Zambia.

Site 1					
Parameters	sn	Ideal Values (V_i)	Mean conc.Value (V_a)	V_a/Sn	(V_a/Sn)*100 = *qi*
EC	100	0	34.000	0.340	34.000
TDS	45	0	24.000	0.533	53.333
Turbidity	10	0	2.980	0.298	29.800
Ca^{2+}	30	0	8.000	0.267	26.667
Mg^{2+}	35	0	4.800	0.137	13.714
Fe^{2+}	0.7	0	0.580	0.829	82.857
Cl	30	0	13.000	0.433	43.333
Total Hardness	250	0	40.000	0.160	16.000
Faecal Coliforms	50	0	0	0	0

Step 3: Calculate the Sub-Index and Water Quality Index (WQI)

To calculate the sub-index scores for each parameter, the unit weights (*wi*) of each parameter as determined in Step 1 were multiplied by the respective quality rating scale values (*qi*) calculated in Step 2. The overall water quality index (WQI) is a summed up score of sub-indices of each considered parameter. It sums the weighted contributions of each parameter to the overall water quality (Table 6). The water quality index is represented by the formula:

$$\mathrm{WQI} = \sum_{i=1}^{n} w_i q_i$$ ……. Equation 5 (Brown et al., 1972; Horton, 1965).

where:

i. WQI = a number between 0 and 100;

ii. q_i = the quality of the i^{th} parameter, a number between 0 and 100;

iii. w_i = the unit weight of the i^{th} parameter, a number between 0 and 1; and

iv. n = the number of parameters.

Table 6: Sub-index scores of water parameters and the overall water quality index score at site 1 during the dry season in the Luapula River Basin, Zambia.

SITE 1			
Parameters	*wi* = K/Sn	Vn/Sn*100 = *qi*	*wiqi*
EC	0.006	34.000	0.204
TDS	0.013	53.333	0.693
Turbidity	0.060	29.800	1.788
Ca^{2+}	0.020	26.667	0.533
Mg^{2+}	0.017	13.714	0.233
Fe^{2+}	0.850	82.857	70.428
Cl	0.020	43.333	0.867
Total Hardness	0.002	16.000	0.032
Faecal Coliforms	0.012	0	0
	1		**75**

The index was classified on a scale of 0 to 100, with 0 representing excellent water quality and 100 representing very poor water quality (Brown et al., 1972; Boah et al., 2015; Budhlani, 2015) (Table 7). The water quality index was calculated using parameters measured during the dry and wet seasons to capture seasonal variations across the three zones. The calculated WQI scores for dry and wet seasons ranged from 16 to 128 and 2 to 114, respectively.

Table 7: Scale and interpretation of water quality index scores based on Brown et al. (1972), (Horton, 1965), Boah et al. (2015) and Budhlani (2015).

WQI Score	**Status Interpretation**
0 – 25	Excellent
26 – 50	Good
51 – 75	Poor
76 – 100	Very Poor
Above 100	Unsuitable for drinking

4.2.6 Statistical Analysis

A suite of statistical methods, including MANOVA, ANOVA, Pearson Correlation, SIMPER, and CCA was used to explore the relationships between water quality parameters and macroinvertebrate assemblages. These tools, applied via the 'car' package in R-studio, facilitated the analysis of spatial and temporal variations across hydro-environmental zones, elucidating community composition and ecological gradients.

MANOVA and ANOVA compare group means. MANOVA examines differences in multivariate means across groups, assuming multivariate normality, equal covariance matrices and linearity, while ANOVA evaluates differences in univariate means under the assumptions of normal distribution, equal variances and independence. Both tests assess null hypotheses of no differences among groups.

Using the cor() function in R-studio version 4.3.2, Pearson Correlation was applied to measure the linear relationships between macroinvertebrate ecosystem attributes and water quality parameters. The Pearson Correlation quantifies the strength and direction of association between two variables, indicating how much the variable changes as the other variable changes. It is a parametric measure and is most appropriate when the variables are normally distributed and have a linear relationship (Pearson, 1895).

To measure the contribution of different macroinvertebrate taxa to the dissimilarity between samples across the hydro-environmental zones, this study utilised SIMPER (Similarity Percentage) analysis in PAST Statistical software version 4.03. SIMPER analysis as a multivariate analysis protocol provides valuable insights into community composition and structure as it allows for the identification of key taxa that are responsible for the observed differences between hydro-environmental zones. The application of SIMPER ANALYSIS on the macroinvertebrate dataset was based on the Bray-Curtis dissimilarity distance measure. Bray-Curtis dissimilarity takes into account both the presence and abundance of taxa, making it suitable for comparing samples with different taxa richness or abundances. It quantifies the compositional differences between samples by considering the abundances or occurrences of species present in each sample. The output of a SIMPER (Similarity Percentage) analysis includes several components that help interpret ecological variation. The overall average dissimilarity score provides a numerical measure of the average level of dissimilarity between zones, indicating ecological differences. The analysis also presents a list of taxa ranked by their contribution to the observed dissimilarity, thereby highlighting in descending order which taxa are most responsible for the observed differences. Each taxon's percentage contribution shows

the relative importance of each in driving community differences. The cumulative percentage sums these contributions to determine which taxa collectively account for a significant portion of the total dissimilarity, often up to a predefined cut-off point, which in this was placed at 80% in order to capture a wider base of macroinvertebrate interactions.

Canonical Correspondence Analysis (CCA) in PAST Statistical software version 4.03 was used to investigate the relationship between the abundance of macroinvertebrate taxa and water quality variables and altitude. CCA is a multivariate method that allows for visualisation and interpretation of the distribution patterns of taxa along environmental gradients. It identifies the linear combinations of environmental variables (canonical axes) that explain the variation in taxa composition among samples. These canonical axes are then used to plot taxa and samples in a multidimensional space, which helps to understand how species abundances change along environmental gradients. It's important to note that CCA is a constrained ordination technique, which means that it aims to find the linear combinations of environmental variables that have the highest correlation with taxa abundances (ter Braak and Verdonschot 1995). The data on macroinvertebrate composition and water quality, including altitude, were transformed before conducting canonical correspondence analysis (CCA). The Hellinger transformation was used for the macroinvertebrate data to address the issue of double zeroes, which can affect the analysis. This was achieved by using the decostand() function from the vegan package in R-studio version 4.3.2. This transformation involves taking the square root of the relative abundance of each species to stabilise variances and make the data more suitable for analysis. By reducing the dominance of highly abundant species and giving more weight to rare species, the Hellinger transformation allows for a more balanced representation of species composition (Appendices 4 and 5).

The Hellinger transformation is represented by the following formula:

$$D_{\text{Hellinger}}(x_1, x_2) = \sqrt{\sum_{j=1}^{p} \left[\sqrt{\frac{y_{1j}}{y_{1+}}} - \sqrt{\frac{y_{2j}}{y_{2+}}} \right]^2}$$ …… Equation 6 (Legendre and Gallagher, 2001)

where:

i. x_1 and x_2 are the two distributions being compared;
ii. y_{1j} and y_{2j} are the abundances of the j^{th} species in distributions x_1 and x_2, respectively;
iii. y_{1+} and y_{2+} are the total abundances (sum of all species) in distributions x_1 and x_2, respectively; and

iv. p is the number of species.

Similarly, standardisation of water quality parameters and altitude is important to ensure that each parameter contributes equally to the analysis, regardless of its original scale or units. This was achieved by subtracting the mean and dividing it by the standard deviation for each parameter using the scale() function in R-studio version 4.3.2. This process eliminates the influence of differing magnitudes and units (Appendices 6 and 7), allowing CCA to accurately assess the relative importance of each parameter in explaining variations in macroinvertebrate communities.

CHAPTER FIVE: RESULTS AND DISCUSSION

This section provides details of the outputs and interpretations of the methodologies used in the assessment of ecological health, function and water quality of the Luapula River Basin.

5.1 Spatial Heterogeneity

This subsection focuses on the variation of hydroenvironmental attributes and macroinvertebrate composition and abundance across the three spatial extents within the Luapula River Basin.

5.1.1 Geospatial Zonation of the Luapula River Basin

The delineation of the Luapula River Basin into hydro-environmental zones by utilising geospatial layers of elevation, precipitation, surface water runoff and forest cover extent produced an overlay map depicting the three distinct spatial zones (Figure 4) with equal pixel range. Each spatial extent was assigned a specific colour for easy identification: blue for Zone 1; yellow for Zone 2; and green for Zone 3.

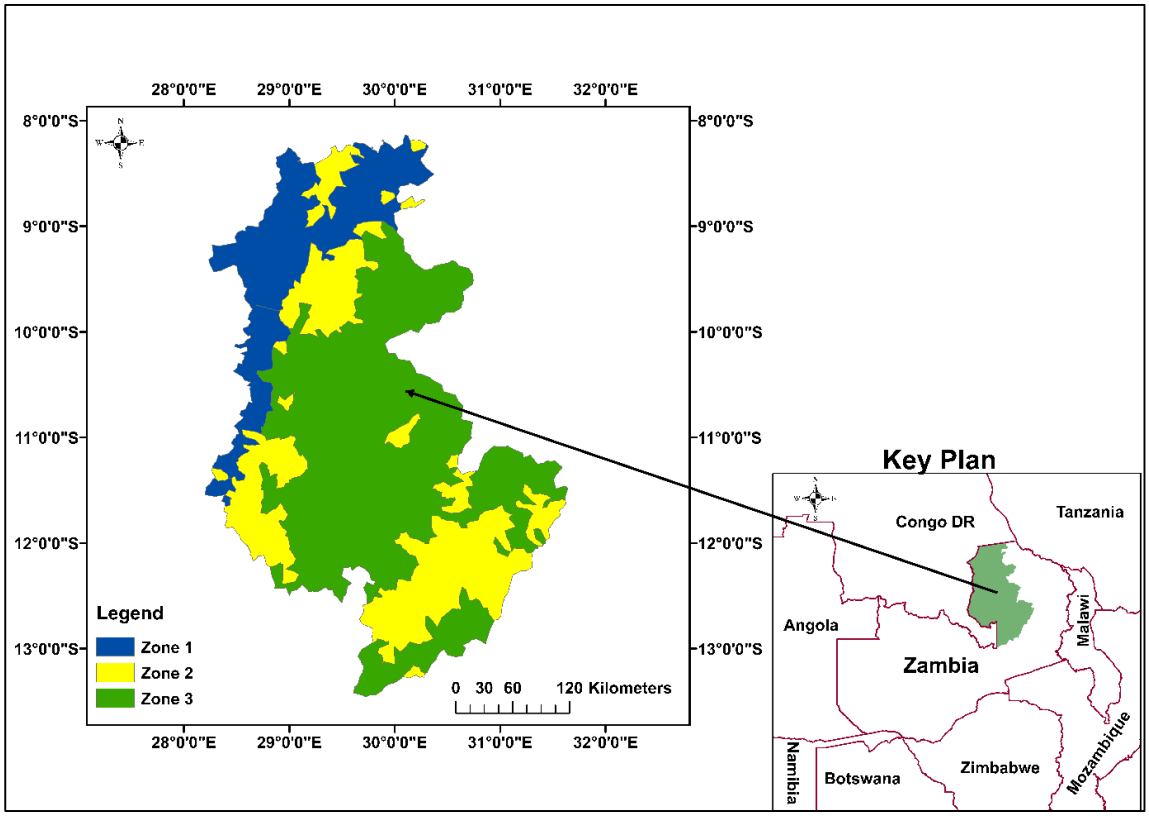


Figure 4: Spatial extent of synthesised hydro-environmental zones of the Luapula River Basin, Zambia. Blue = Zone 1, Yellow = Zone 2, Green is Zone 3.

Based on the classical ordering system in HydrATLAS Zambia, four streams/rivers with perennial flow capacity were selected from each hydro-environmental zone. The final

extracted stream network consisted of streams and rivers associated with order classes 1, 2 and 3 (Figure 5).

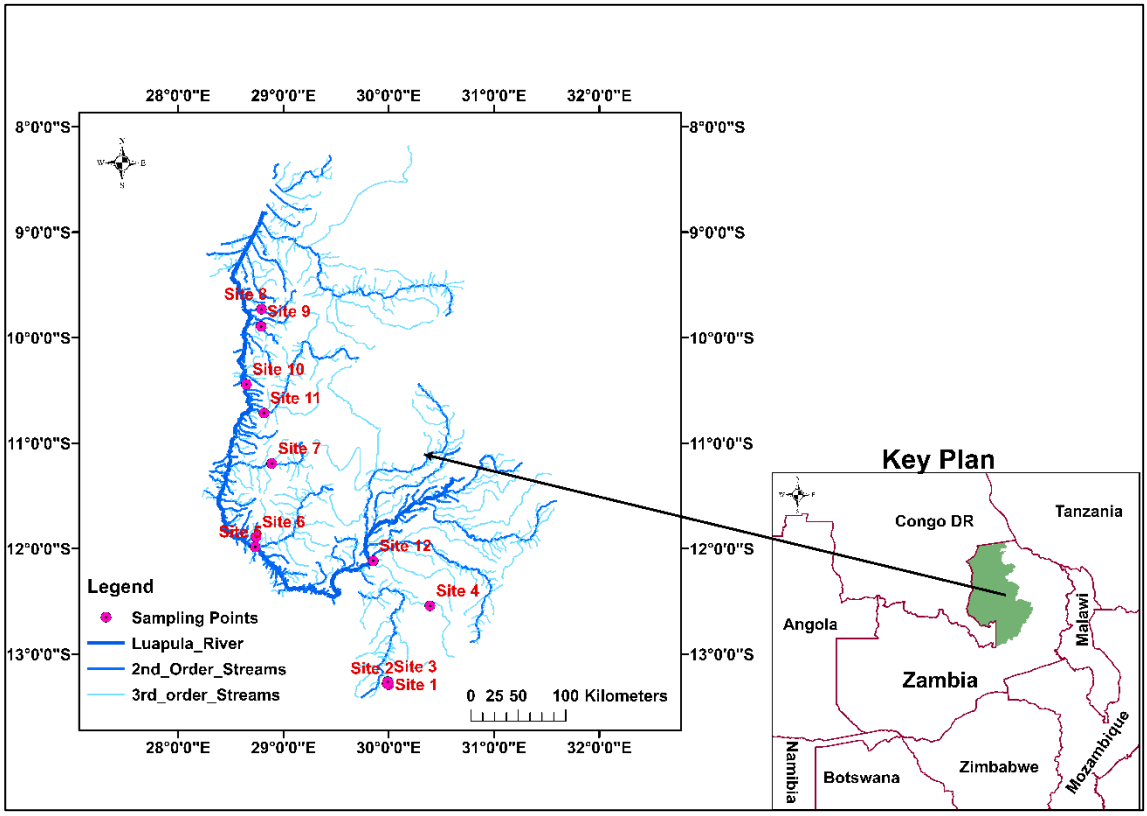


Figure 5: Extracted stream orders and sampling sites in the Luapula River Basin, Zambia.

The culmination of the geospatial zonation process resulted in the creation of a map that overlaid the extracted stream network onto the synthesised spatial extents (Figure 6). The map integrates the delineated hydro-environmental zones with the selected streams and rivers possessing perennial flow capacity. Through this overlay, the spatial distribution of water bodies and their corresponding hydro-environmental contexts became visually apparent, providing a visualisation of the spatial extents of the selected perennial stream/river networks within the Luapula River Basin.

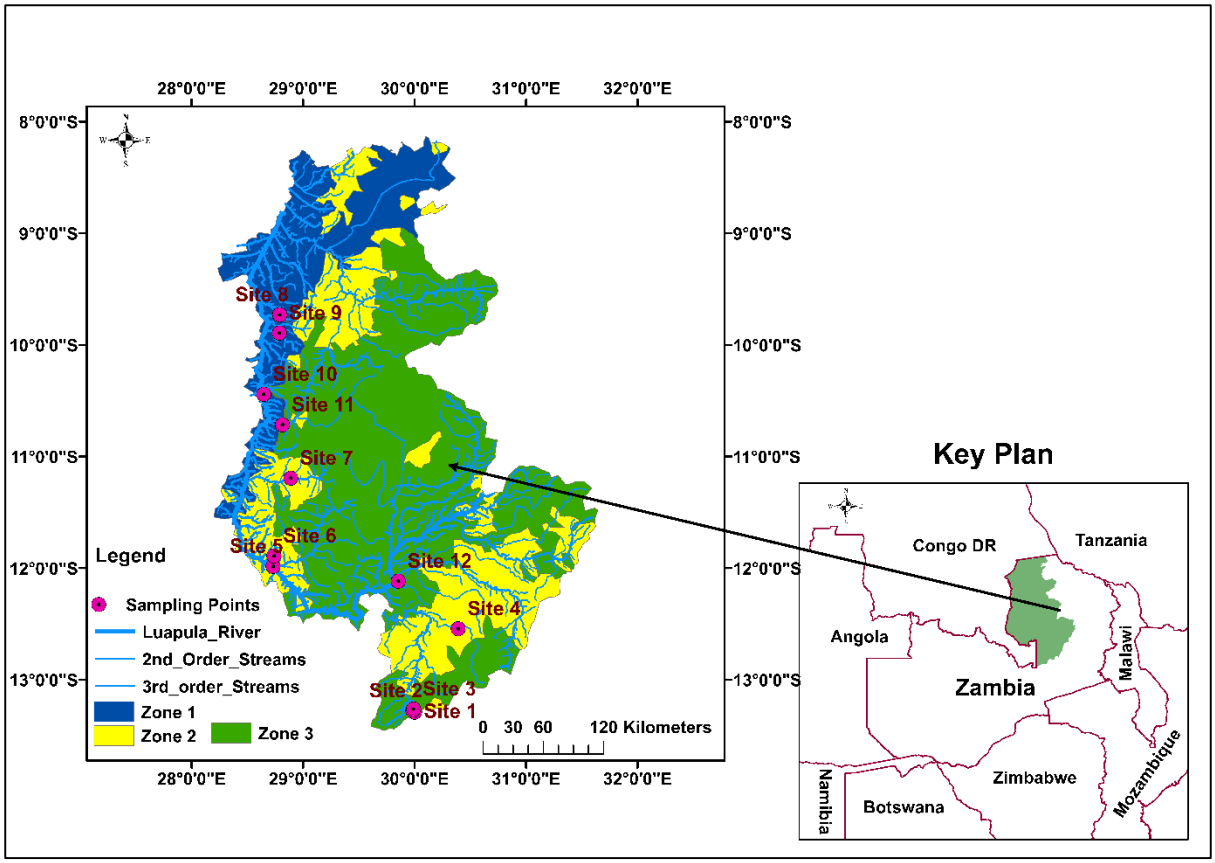


Figure 6: Overlaid map showing hydro-environmental zones, stream/river network and selected sampling sites in the Luapula River Basin, Zambia.

5.1.2 Interpretation and Discussion

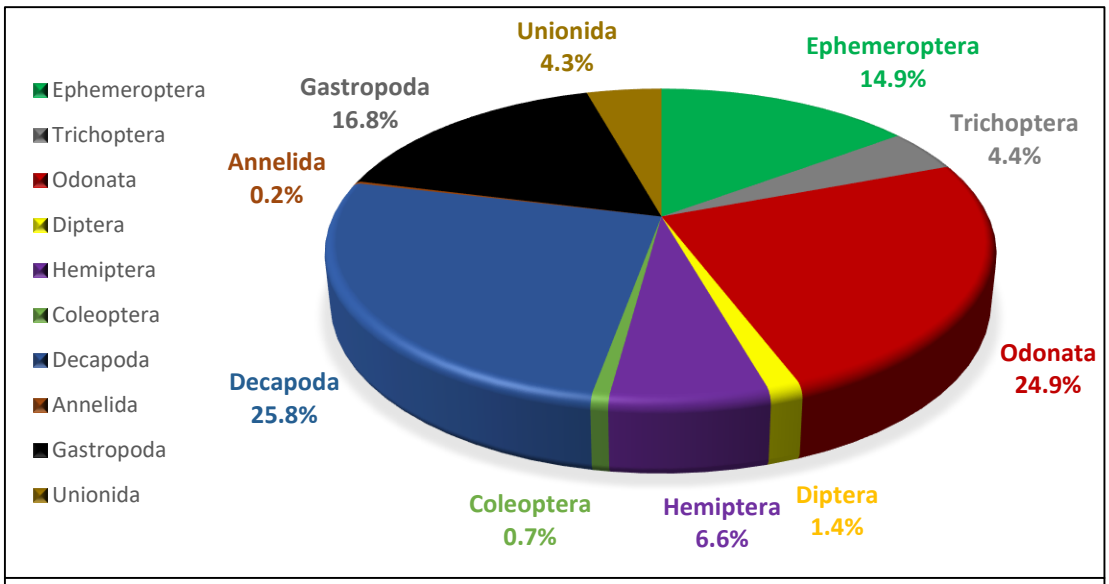
Section 5.1.1 describes the importance of dividing the Luapula River Basin into three distinct hydro-environmental zones based on four key attributes: precipitation; surface water runoff; elevation; and forest cover extent. This spatial division allows for an informed understanding of the environmental heterogeneity within the basin. Zone 1, encompassing regions with a low score range across the four input attributes, represents areas with minimal surface water runoff, lower precipitation, low altitude and sparse vegetation. In contrast, Zone 3, characterised by a high score range of the input attributes, includes regions with increased surface water runoff, high precipitation, high altitude and increased forest cover extent. Zone 2, with mid-range scores, indicates areas that fall between these two extremes. This zonal classification is essential for targeted ecological assessment and water resources management strategies as it helps to identify areas with specific environmental conditions and requirements within the Luapula River Basin. In Qinling-Daba Mountains of China's North-South Transitional Zone, Yin et al. (2022) assert that scientific and objective assessment of the quality of the ecological environment is a complex endeavour, as no single environmental element can comprehensively capture its entirety. Yin et al. (2022) further submitted that eco-environmental quality

encompasses a multifaceted interplay of various ecological components including biotic and abiotic aspects of the natural environment, and that each of these elements contributes uniquely to the overall health and resilience of ecosystems. Truchy et al. (2019) noted that although the significance of space in regulating ecosystem functioning is widely acknowledged in theory, it is seldom explicitly addressed in empirical research. Factors such as community composition, environmental variability and spatial organisation can all impact ecosystem functioning and the delivery of ecosystem services (Truchy et al., 2019).

5.2 Benthic Macroinvertebrates Abundance and Distribution

During the dry and wet seasons, a total of 1385 and 1473 macroinvertebrate individuals were collected across the 12 sampling sites, respectively (Appendices 8 and 9). In the dry season, the 1385 individuals were classified into 10 macroinvertebrate taxonomic orders, while in the wet season, the 1473 macroinvertebrate individuals were classified into 9 major macroinvertebrate taxa groups (Figures 7a and 7b). The dry season composition comprised of the orders Ephemeroptera (mayflies), Trichoptera (caddisflies), Odonata (dragonflies and damselflies), Hemiptera (true bugs), Coleoptera (beetles), Decapoda (crustaceans), Diptera (flies), Annelida (segmented worms), Unionida (bivalves) and Gastropoda (snails), while the wet season composition comprised of the orders Ephemeroptera (mayflies), Trichoptera (caddisflies), Odonata (dragonflies and damselflies), Hemiptera (true bugs), Coleoptera (beetles), Decapoda (crustaceans), Diptera (flies), Unionida (bivalves) and Gastropoda (snails). Furthermore, the 10 taxonomic orders in the dry season were classified into 21 families of benthic macroinvertebrates, while the wet season collection accounted for 25 families of benthic macroinvertebrates (Figures 8a and 8b).

(a)



(b)

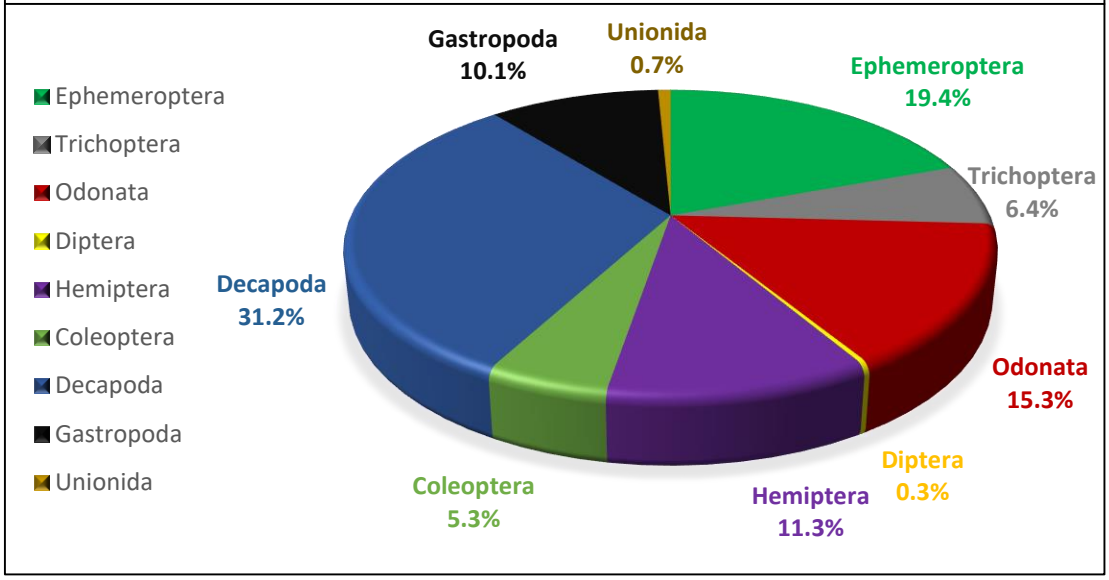


Figure 7: Pie-Chart showing the (a) dry season and (b) wet season benthic macroinvertebrate taxa abundances at order taxonomic resolution collected from all the selected sites of the Luapula River Basin, Zambia.

(a)

(b)

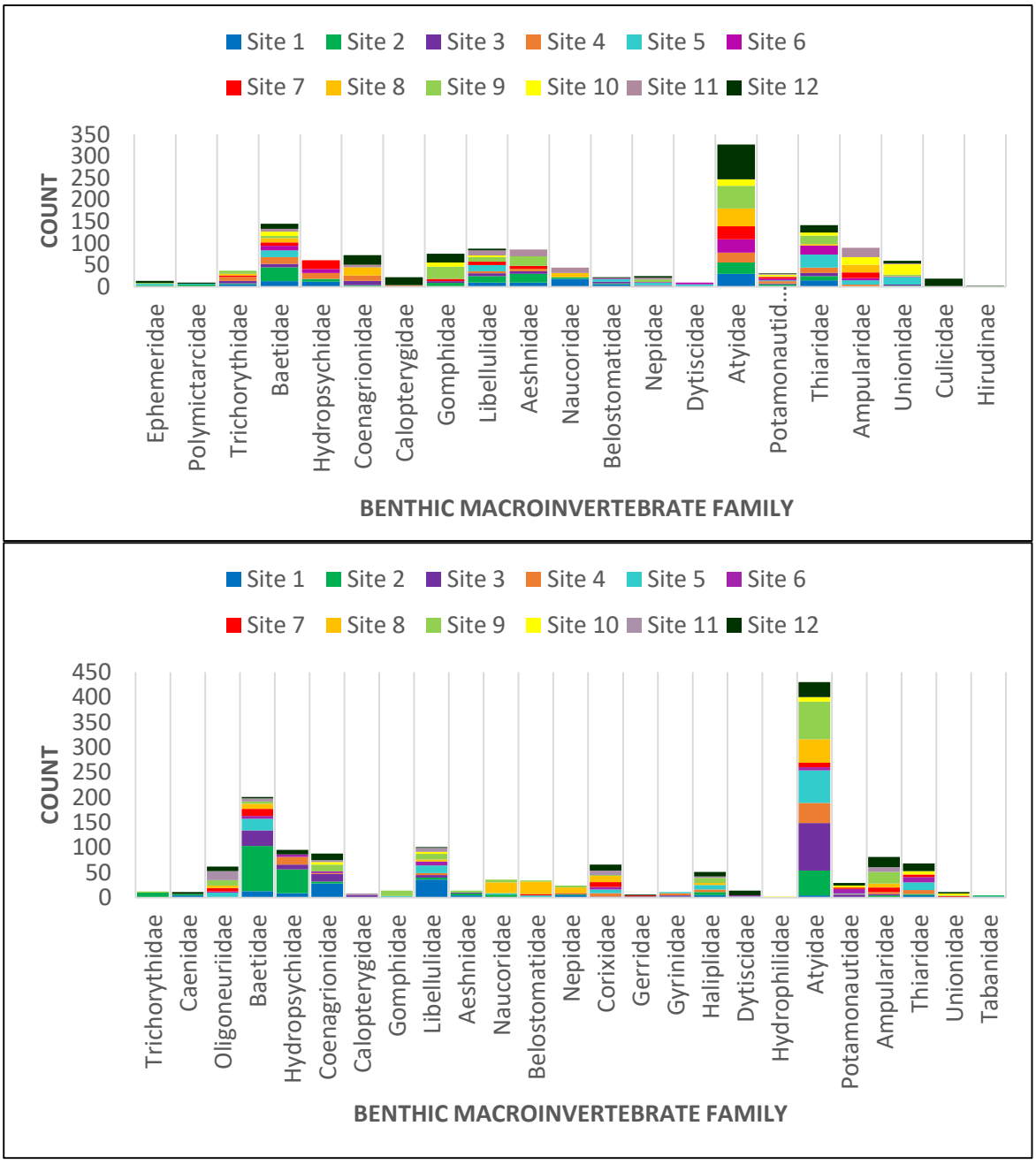


Figure 8: The Bar graph showing (a) dry season and (b) wet season site specific benthic macroinvertebrate family abundances across the selected sites of the Luapula River Basin, Zambia.

The order Decapoda had the highest percentage of benthic macroinvertebrate abundance in both the dry and wet seasons, contributing 25.8% and 31.2% of the overall abundance, respectively. This group consisted of two families, *Atyidae*, which is moderately tolerant to perturbation and *Potamonautidae*, a highly tolerant taxon to perturbation. It is important to note that the *Potamonautidae* family only accounted for 2.2% and 2.0% of the total family abundance in the dry and wet seasons, respectively. However, *Atyidae* had the highest

abundance, representing 23.6% and 29.2% of all collected individuals in dry and wet seasons, respectively. In the dry season, the family *Atyidae* was identified in 9 sampling sites, that is, site 1, site 2, site 4, site 6, site 7, site 8, site 9, site 10 and site 12. Notably, site 12 had the highest count of 80 individuals whereas site 10 had the lowest count of 15 individuals for this family. During the wet season, the *Atyidae* family was found in all sampling sites except for site 11. The family was most abundant in site 3, with 95 individuals recorded, whereas the lowest abundance was recorded in site 1 with only 4 individuals.

The order Odonata encompasses both damselflies and dragonflies, which belong to the suborders Zygoptera and Anisoptera, respectively. In the dry season, their abundance represented 24.9% of the total count, whereas in the wet season, it was 15.3%.

The suborder Anisoptera comprised three families: *Gomphidae*; *Libellulidae*; and *Aeshnidae*. During the dry and wet seasons, the family *Libellulidae*, which ranks as being highly tolerant to perturbation on the ZISS sensitivity scale, was found to be the most abundant family within the Odonatans. A total of 88 individuals were counted during the dry season, whereas 101 individuals were counted during the wet season, representing 6.4% and 6.9% of the total number of macroinvertebrate individuals counted, respectively. The distribution of *Libellulidae* in the dry season was found in all the sampling sites except for site 6 and site 8. The presence of *Libellulidae* was recorded in all the sampling sites during the wet season, except for site 7. The distribution of the family *Gomphidae,* which is classified as being a moderately tolerant taxon to perturbation, was restricted to only two sites, site 5 and site 9 in the wet season, with a collective abundance count of 14 individuals representing 1% of the total individual count. Conversely, the dry season effort presented a much wider distribution of this family, with a registered presence in 6 sampling sites, namely sites 2, 3, 7, 9, 10 and 12. This presence represented a total abundance count of 76 individuals, which accounts for 5.5% of the total dry season collection. The percentage abundance of the family *Aeshnidae*, which is moderately tolerant to perturbation, decreased from 6% in dry season to 1% in wet season. In terms of distribution, the family *Aeshnidae* was found in sites 1, 2, 3, 4, 7, 9 and 11 in the dry season, whereas the wet season had a confirmed presence in sites 1, 2, 3 and 9.

The suborder Zygoptera was represented by two families: *Coenagrionidae*, which is highly tolerant to perturbation; and *Calopterygidae*, which is moderately tolerant. During the dry season, the family *Coenagrionidae* was identified in six sampling sites, namely sites 2, 3, 4, 8, 11 and 12. This distribution accounted for a total of 73 individuals, which represented 5.3% of

the overall dry season collection. In contrast, during the wet season, the family *Coenagrionidae* was present in all the sampling sites except sites 5, 7 and 8, with a total abundance of 88 individuals, accounting for 6% of the overall wet season collection. During the dry and wet seasons, the family *Calopterygidae* was identified in two different sampling sites. These sites were sites 4 and 12 for the dry season and sites 3 and 11 for the wet season. A total of 22 individuals were identified during the dry season, which accounted for 1.6% of the total dry season collection. In contrast, a total of 8 individuals were identified during the wet season, which represented 0.5% of the total wet season collection.

The order Ephemeroptera consists of a group of aquatic macroinvertebrates commonly known as mayflies. Mayflies undergo a nymphal stage before becoming winged adults. They play a vital role in freshwater ecosystems as their nymphs help in nutrient cycling and serve as an essential food source for various aquatic and terrestrial organisms. Adult mayflies are also a crucial part of the food chain and they are consumed by birds, bats and other insect-eating predators. Additionally, this order of macroinvertebrates is important as it comprises taxa that are considered to be highly sensitive to ecological perturbations, making them very important indicators of ecosystem health. During the dry and wet season, the order Ephemeroptera was represented by six families, namely *Ephemeridae, Polymictarcidae, Trichorythidae, Batidae, Caenidae* and *Oligoneuriidae*. This taxa recorded a dry season abundance score of 206 individuals, representing 14.9% of the total dry season count, whereas the wet season sampling campaign was characterised by a total abundance of 286 individuals, representing 19.4% of the total wet season count. The *Batidae* family had a wider distribution across both seasons. During the dry season, this family was found in all sampling sites, making up 10.5% of the total collection. In contrast, during the wet season, the *Batidae* family was found in all sites except for sites 4 and 10. The wet season distribution accounted for a total of 201 individuals, which represented 13.6% of the overall wet season collection. The family *Trichorythidae* was observed to have decreased in abundance from the dry season to the wet season. During the dry season, a total of 37 individuals were recorded, which represented 2.7% of the overall abundance count. However, during the wet season, the abundance of the family *Trichorythidae* declined to only 12 individuals, representing 0.8% of the total wet season sampling effort. The distribution of this family was widespread during the dry season, with confirmed presence in sites 1, 2, 3, 4, 7, 8 and 9. However, during the wet season, its presence was only confirmed in sites 2 and 9. The families *Ephemeridae* and *Polymictarcidae* were found only during the dry season sampling campaign, with *Ephemeridae* having a total of 14 individuals in sites 2, 5, 9

and 12, which made up just 1% of the dry season's total abundance count. Meanwhile, *Polymictarcidae* had a total of 10 individuals in sites 2, 5 and 12, accounting for 0.7% of the overall abundance count. In contrast, the families *Caenidae* and *Oligoneuriidae* were only present in the wet season. *Caenidae* had a total of 11 individuals in sites 1, 2 and 12, which accounted for only 0.7% of the overall abundance count. *Oligoneuriidae*, on the other hand, had a wider distribution and was present in sites 5, 6, 7, 8, 9, 11 and 12. This family had a total abundance of 62 individuals, representing 4.2% of the overall abundance count.

Gastropoda is a large class of invertebrates within the phylum Mollusca, which includes slugs and snails. These organisms play an important role in marine, freshwater and terrestrial ecosystems. They are a source of food for many animals including humans. Additionally, gastropods aid in the recycling of nutrients and contribute to the cleaning of the environment. The seasonal distribution of Gastropoda was represented by two families, *Thiaridae* and *Ampularidae*. In the dry season, Gastropoda recorded a total abundance of 232 individuals, which accounted for 16.8% of the overall abundance count. However, in the wet season, there was a decline to 149 individuals, which accounted for only 10.1% of the overall abundance count. The family *Thiaridae*, which is ranked as highly tolerant on the ZISS sensitivity scale, recorded a total abundance of 142 individuals in the dry season and 68 individuals in the wet season. These abundance scores translated to 10.3% and 4.6% of the respective seasonal abundance total counts. *Thiaridae* was found in all sites during the dry season except for sites 7 and 11. During the wet season, *Thiaridae* was present in sites 1, 2, 4, 5, 6, 7, 10 and 12. The family *Ampularidae*, also ranked as highly tolerant on the ZISS sensitivity scale, recorded a dry season abundance score of 90 individuals across sites 4, 5, 6, 7, 8, 10 and 11. However, the wet season abundance count decreased to 81 individuals, with a confirmed presence in all sites except for sites 1, 5, 6 and 10. The dry season abundance count represented 6.5% of the total dry season count, whereas the wet season was characterised by 5.5% representation of the total wet season abundance count.

Hemiptera, often referred to as "true bugs," are a large and diverse order of insects. They are found in most terrestrial and freshwater habitats, making them one of the most successful and adaptable groups of insects. Hemiptera play a vital role in ecosystems, serving as both herbivores and predators. They are also a valuable food source for other animals, including birds, reptiles and amphibians. However, some hemipteran species can be pests and vectors of diseases. During the dry and wet season sampling campaigns, the order Hemiptera was represented by the families *Naucoridae, Belostomatidae, Nepidae, Corixidae* and *Gerridae*. In

the dry season, the order Hemiptera recorded an abundance score of 91 individuals, representing 6.6% of the total dry season abundance count. The wet season sampling effort yielded an abundance count of 166 individuals, representing 11.3% of the total wet abundance count. The family *Naucoridae* which is ranked as moderately tolerant on the ZISS sensitivity scale had a higher abundance count of 44 individuals during the dry season compared to 36 individuals in the wet season. The dry season abundance was distributed across sites 1, 2, 8 and 11, which accounted for 3.2% of the total dry season count. The wet season abundance accounted for 2.4% of the overall wet season count and was distributed across sites 2, 5, 8 and 9. The family *Belostomatidae* which is ranked as highly tolerant on the ZISS sensitivity scale was distributed across sites 1, 2, 3, 4, 5, 6, 9, 11 and 12 in the dry season and across sites 5, 7, 8 and 9 in the wet season. The dry abundance was recorded at 22 individuals, representing 1.6% of the overall dry season abundance count. The wet season recorded an abundance count of 34 individuals, representing 2.3% of the overall wet season count. During the dry season, the family *Nepidae* recorded an abundance of 25 individuals, accounting for 1.81% of the total count of 1385. *Nepidae* was observed across sites 2, 3, 5, 6, 9, 11 and 12. In contrast, during the wet season, *Nepidae* exhibited an abundance of 24 individuals, constituting 1.63% of the total count of 1473. Their presence was confirmed in sites 1, 2, 7, 8 and 9 during the wet season. Additionally, other Hemiptera families were observed during the wet season, namely *Corixidae* and *Gerridae*. The family *Corixidae* with an abundance of 66 individuals, accounted for 4.49% of the total count of 1473. This family was distributed across multiple sites including sites 3, 4, 5, 6, 7, 8, 9, 11 and 12 suggesting its adaptability to diverse aquatic environments. The Gerridae family, comprising a total of six individuals, accounted for 0.41% of the total count. The distribution of this family was limited with a confirmed presence only in sites 1, 7, and 12.

Coleoptera, a diverse order of macroinvertebrates commonly known as beetles, consists of families that significantly contribute to the ecological dynamics of aquatic ecosystems. Beetles contribute significantly to ecosystem functioning and maintaining ecological balance. Coleoptera was represented by the family *Dytiscidae* in the dry season, accounting for 0.72% of the overall dry season sampling effort and the families *Gyrinidae*, *Halipidae*, *Dytiscidae* and *Hydrophilidae* in the wet season, accounting for 5.3% of the overall wet season effort. In the dry season, the family *Dytiscidae*, ranked as being highly tolerant of the ZISS sensitivity scale, exhibited an abundance of 10 (0.72%) individuals out of a total count of 1385, with a distribution concentrated in sites 5 and 6. In contrast, the wet season revealed a broader spectrum of Coleoptera families, each contributing to the overall macroinvertebrate abundance.

The family *Gyrinidae*, ranked as being highly tolerant on the ZISS sensitivity scale, displayed an abundance of 11(0.75%) individuals out of a total count of 1473. This family was distributed across sites 1, 3, 4 and 5, indicating a wide geographic presence during the wet season. Another significant family identified during the wet season was *Haliplidae*, ranked as being highly tolerant on the ZISS sensitivity scale. *Haliplidae* recorded an abundance of 51 (3.5%) individuals out of the total wet season count of 1473. *Haliplidae* displayed a more extensive distribution, spanning sites 1, 2, 4, 5, 8, 9, 11 and 12. This widespread presence suggests adaptability to various aquatic environments and habitat types, making *Haliplidae* a versatile contributor to the wet season macroinvertebrate community. The family *Dytiscidae*, previously observed during the dry season, continued to be present in the wet season, contributing 14 (1%) individuals to the total count of 1473. Its distribution included sites 5, 6 and 12 during the wet season. *Hydrophilidae*, ranked as being highly tolerant on the ZISS sensitivity scale, exhibited an abundance of 10 (0.13%) individuals during the wet season. This family was predominantly found in site 10, showcasing site-specific preferences within the wet season habitats.

The order Trichoptera, commonly known as caddisflies are sensitive to environmental changes and are useful bioindicators for assessing water quality in aquatic ecosystems. The order Trichoptera was represented by the family *Hydropsychidae* in both the dry and wet season sampling campaigns. During the dry season, the family *Hydropsychidae* exhibited an abundance of 61 individuals, constituting 4.4% of the overall abundance count and were distributed across sites 1, 2, 4, 6, and 7. In the wet season, the abundance increased to 95 individuals representing 6.5% of the overall abundance count, with a broader distribution across sites 1, 2, 3, 4, 6 and 12.

The order Unionida represents a monophyletic order of aquatic bivalve mollusks also known as freshwater mussels. This order was also represented by the family *Unionidae* during the dry and wet season sampling campaign. In the dry season, the abundance of the family *Unionidae* peaked at 60 individuals, representing 4.3% of the total abundance count. This family had a confirmed presence across sites 2, 3, 5, 6, 8, 9, 10, 11 and 12. In the wet season, *Unionidae* exhibited reduced abundance of 11 individuals representing 0.7% of the overall abundance count. The wet season distribution was accounted for across sites 1, 7, 9, 10 and 12.

The order Diptera was represented by the family *Culicidae* known for mosquitoes in the dry season and the family *Tabanidae* known for horseflies in the wet season. This order was characterised by lower abundance percentages across both seasons. The family *Culicidae*, with

19 individuals at site 12 represented 1.4% of the overall macroinvertebrate community during the dry season. The wet season sampling effort was represented by the family *Tabanidae*, which had 4 individuals, representing 0.3% of the total count in sites 1 and 2.

5.2.1 SIMPER (Similarity Percentage) Analysis

During the dry season, SIMPER analysis showed a 56.67% average dissimilarity across the Luapula River Basin's three zones. This midrange dissimilarity highlights the ecological heterogeneity among zones, evidenced by variations in macroinvertebrate taxa composition and abundances. At 80% cut-off point of the cumulative percentage, the following taxa, listed in descending order, contributed to 80% of the observed variations across the three hydro-environmental zones: *Atyidae*; *Ampularidae*; *Thiaridae*; *Aeshnidae*; *Hydropsychidae*; *Gomphidae*; *Coenagrionidae*; *Unionidae*; *Baetidae;* and *Naucoridae* (Table 8).

Table 8: Similarity percentage analysis showing dry season taxon contribution percentage to macroinvertebrate taxa variation across the hydro-environmental zone in the Luapula River Basin, Zambia.

Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean 3	Mean 2	Mean 1
Atyidae	10.65	18.79	18.79	34	20.8	27
Ampularidae	5.134	9.06	27.85	0	8.25	14.3
Thiaridae	4.935	8.708	36.56	12.3	15.8	7.5
Aeshnidae	3.959	6.986	43.54	9	3	9.5
Hydropsychidae	3.742	6.603	50.15	4.5	10.8	0
Gomphidae	3.737	6.595	56.74	8.25	1.25	9.5
Coenagrionidae	3.595	6.343	63.08	9	3	6.25
Unionidae	3.279	5.786	68.87	2.75	4.75	7.5
Baetidae	3.056	5.393	74.26	16.3	12.3	7.75
Naucoridae	2.837	5.006	79.27	5.5	0	5.5
Libellulidae	2.559	4.516	83.78	8.75	6.75	6.5
Trichorythidae	1.652	2.915	86.7	3.5	3	2.75
Belostomatidae	1.284	2.266	88.96	3.5	2	0
Calopterygidae	1.185	2.091	91.06	4.5	1	0
Nepidae	1.119	1.974	93.03	2	1.75	2.5
Culicidae	0.99	1.747	94.78	4.75	0	0
Dytiscidae	0.7759	1.369	96.15	0	2.5	0
Potamonautidae	0.7462	1.317	97.46	2.25	3.75	1.75
Ephemeridae	0.7039	1.242	98.71	2.25	0.75	0.5
Polymictarcidae	0.5775	1.019	99.72	2	0.5	0
Hirudinae	0.1563	0.2758	100	0.75	0	0

During the wet season, the SIMPER analysis revealed an overall average dissimilarity score of 63.33% across the three hydro-environmental zones of the Luapula River Basin. This suggests an increased level of heterogeneity in the ecological conditions of the hydro-environmental zones, as indicated by variations in macroinvertebrate taxa composition and abundances compared to the dry season. At 80% cut-off point of the cumulative percentage, the following taxa were identified in descending order of contribution: *Atyidae*; *Baetidae*; *Hydropsychidae*; *Coenagrionidae*; *Libellulidae*; *Ampularidae*; *Oligoneuriidae*; *Thiaridae*; *Corixidae*; and *Belostomatidae*. Together, these taxa accounted for 80% of the observed variations across the three hydro-environmental zones (Table 9).

Table 9: Similarity percentage analysis showing wet season taxon contribution percentage to macroinvertebrate taxa variation across the hydro-environmental zone in the Luapula River Basin, Zambia.

Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean 3	Mean 2	Mean 1
Atyidae	14.46	22.82	22.82	44.8	30	32.8
Baetidae	8.278	13.07	35.9	34.3	10.8	5.25
Hydropsychidae	4.993	7.885	43.78	18.8	5	0
Coenagrionidae	4.393	6.936	50.72	15	1.25	5.75
Libellulidae	4.015	6.339	57.06	11.8	6.75	6.75
Ampularidae	3.532	5.577	62.63	6.75	3.25	10.3
Oligoneuriidae	3.046	4.81	67.44	2.25	4.75	8.5
Thiaridae	3	4.736	72.18	5.5	9.75	1.75
Corixidae	2.518	3.977	76.15	3.5	7.25	5.75
Belostomatidae	2.01	3.174	79.33	0	1.75	6.75
Naucoridae	1.889	2.982	82.31	1.5	0.75	6.75
Haliplidae	1.815	2.866	85.18	5	3.5	4.25
Potamonautidae	1.501	2.371	87.55	2.75	3.5	1
Nepidae	1.272	2.008	89.56	1.75	0.5	3.75
Dytiscidae	0.9521	1.503	91.06	2.5	1	0
Caenidae	0.7559	1.193	92.25	2.75	0	0
Aeshnidae	0.7509	1.186	93.44	2.5	0	1
Gomphidae	0.7465	1.179	94.62	0	0.75	2.75
Unionidae	0.7	1.105	95.72	1	0.5	1.25
Gyrinidae	0.6755	1.067	96.79	1	1.75	0
Trichorythidae	0.5997	0.9469	97.74	2.5	0	0.5
Calopterygidae	0.5449	0.8603	98.6	1.25	0	0.75
Gerridae	0.4011	0.6333	99.23	1	0.5	0
Tabanidae	0.2619	0.4135	99.64	1	0	0
Hydrophilidae	0.2263	0.3573	100	0	0	0.5

5.2.2 Interpretation and Discussion

Section 5.2 details the seasonal variations in the diversity and distribution of macroinvertebrates in the Luapula River Basin, emphasising how specific site conditions influence taxa biodiversity and distribution. Notably, the Decapoda order, particularly the *Atyidae* family, remained dominant in both seasons, showcasing their resilience to fluctuating water levels across various habitats within the basin. In contrast, Odonata declined in the wet season, likely due to water flow and habitat structure alterations. However, the *Libellulidae* family within this order showed stability across seasons, indicating their adaptability to environmental changes. The increased abundance of Ephemeroptera during the wet season suggests improved water quality and better habitat conditions. On the other hand, the decline in Gastropoda, represented by the families *Thiaridae* and *Ampularidae,* indicates the negative impact of increased water flow on their resource base and substrate stability. The order Hemiptera, especially the families *Corixidae* and *Gerridae,* thrived during the wet season, reflecting their adaptability to higher water levels and associated habitats. Furthermore, the wet season saw an increase in Coleoptera diversity and contrasting responses among Trichoptera and Unionida, illustrating how different taxa respond to seasonal changes. These findings highlight the interplays of environmental factors that affect the diversity and distribution of macroinvertebrates in the Luapula River Basin, with each taxonomic group responding uniquely to seasonal variations. The findings from the Luapula River Basin show that macroinvertebrate assemblage was dominated by taxa that are tolerant and moderately tolerant to environmental disturbances such as Decapoda, Odonata, Gastropoda and Hemiptera. Arimoro et al. (2011) also reported that the dominant benthic invertebrates in Ajijiguan Creek in Nigeria were primarily aquatic insects (Ephemeroptera, Hemiptera, and Odonata) and decapods. In the Mohlapitsi River in South Africa, Raphahlelo et al. (2022) also confirmed the dominance of macroinvertebrates tolerant to disturbance and pollution, with the Thiaridae family belonging to the order Gastropoda being the most abundant, particularly in more disturbed sites. Raphahlelo et al. (2022) also reported the high abundance of the *Baetidae* family throughout the Mohlapitsi River as was observed in the Luapula River Basin. This high abundance was attributed to nutrient enrichment and *Baetidae's* association with moderate pollution level. Odountan et al. (2019) also confirmed the dominance of taxa from the orders Mollusca, Crustacea and Polychaeta in the dry season in Lake Nokoué, a RAMSAR site of Benin. The findings in the Luapula River Basin agree with the observed dominance of the class Mollusca represented by the orders Gastropoda and Unionida in the dry season. However, the order Decopoda (Crustacea) had a higher overall abundance in the wet season compared to the

dry season. Furthermore, the reported prevalence of the orders Coleoptera and Ephemeroptera in Lake Nokoué during the wet season conforms to the observations made in the Luapula River Basin. Masese et al. (2009) also confirms an increased succession of pollution-sensitive taxa such as Ephemeroptera during the rainy season and a dominance of pollution-tolerant taxa Gastropoda during the dry season in the Moiben River in Kenya. Masese et al. (2009) assert that while natural conditions like spates and floods play a role in shaping macroinvertebrate assemblage in the Moiben River, human activities exacerbate these effects. Additionally, during the dry season, declining discharge, lower pH, rising temperatures and reduced dissolved oxygen further stress aquatic ecosystems.

In section 5.2.1, as indicated earlier, the dissimilarity scores of 56.67% in the dry season and 63.33% in the wet season speak to the extent of the existing variations in macroinvertebrate composition across the 3 zones. During the dry season, the dissimilarity score shows that, on average, the macroinvertebrate communities in each zone differ by slightly more than half from one another in terms of the macroinvertebrate composition and their abundance. This suggests that while there are clear differences in the communities among the zones, there are also considarable similarities. The moderate level of variation implies that while each zone has unique factors influencing its macroinvertebrate community, there are commonalities that connect the zones, possibly due to shared hydrological and ecological processes or similar habitat types within the broader extent of the basin. In contrast, the heightened dissimilarity during the wet season suggests that the ecological conditions are more varied than in the dry season. The increased variability can be attributed to several factors characteristic of the wet season, such as higher water flows. The higher dissimilarity score reflects how the wet season's environmental changes amplify the differences among zones, contrasting with the more stable and moderate variation observed during the dry season. This increased heterogeneity underscores the influence of seasonal changes on the distribution and diversity of macroinvertebrate communities. A study that was tracking the effects of habitat attributes on species distribution across space and time on the subtropical coast of São Paulo in Brazil found that habitats were the main drivers of species turnover, with tidal flats harbouring a transitional assemblage between mangroves and sandy beaches (Brustolin et al., 2022). This study confirmed that habitat attributes may induce distinct mechanisms of meta-community assembly at coastal soft-bottom ecosystems. This means that the characteristics of habitats both biotic and abiotic can influence how biological taxa within these ecosystems interact and organise themselves spatially. In the Heihe River of Northwest China, Wang et al. (2021) found a diverse

array of macrobenthic species, with Arthropoda and mollusks dominating the assemblages. In this study Wang et al. (2021) observed a significant increase in standing stock from the upstream to midstream regions, which was attributed to the influence of spatial gradients on community structure. Wang et al. (2021) further highlight the complex interplay between natural conditions and human disturbances along the river as was evidenced by the association between macrobenthic composition and environmental factors such as biochemical oxygen demand (BOD5), water, temperature and nutrient levels. While both studies underscore the importance of environmental factors in shaping freshwater biodiversity, they differ in their focus and methodologies. The Heihe River study emphasises the influence of abiotic conditions and human disturbances on macrobenthic communities, whereas the Luapula River Basin study highlights the role of hydro-environmental zones and seasonal variability in structuring macroinvertebrate assemblages. Together, these findings underscore the need for integrated approaches in the assessment of ecosystem health and function.

5.3 Biodiversity and Ecosystem Health Assessment

This section provides details regarding the outcome and interpretation of the biodiversity and ecosystem health assessment based on taxonomic macroinvertebrate assemblage across the hydro-environmental zones using biodiversity indices and river/stream health indicators (Appendices 10, 11, 12 and 13).

5.3.1 Taxa Richness

The presented taxa richness scores are based on the number of different types of macroinvertebrate families that were collected at each of the sampling sites in the three hydro-environmental zones during the dry and wet seasons (Figure 9).

Zone 3 had the highest average richness scores with 13 and 14 unique macroinvertebrate family taxa during the dry and wet seasons, respectively. Zone 2 recorded an average of 11 unique macroinvertebrate family taxa during the dry season and 10 during the wet season. Zone 1 had the lowest average richness scores with 9 and 10 unique macroinvertebrate family taxa across the dry and wet seasons, respectively. An increase in richness scores as observed in zone 3, indicates the potential of a diverse presence of benthic macroinvertebrate communities within a particular spatial extent. This may also be indicative of a more resilient ecosystem with a wider range of resources and habitats capable of supporting a diverse collection of organisms. Moreover, a significant level of richness may also indicate a higher level of biodiversity, which plays a critical role in maintaining the stability and resilience of ecosystems.

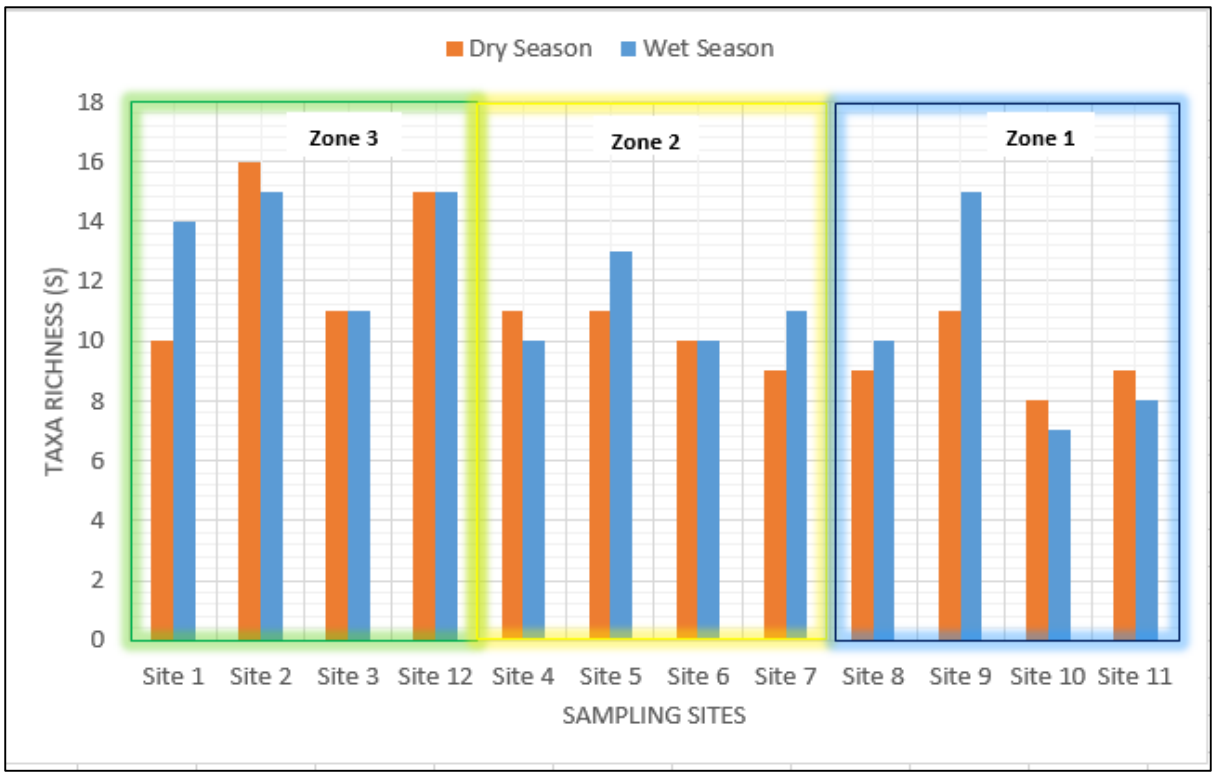


Figure 9: The bar graph shows the richness scores based on family taxa of benthic macroinvertebrate communities across the three hydro-environmental zones of the Luapula River Basin, Zambia.

5.3.2 Evenness Index

Evenness scores were calculated across hydro-environmental zones of the Luapula River Basin and the results were analysed seasonally (Figure 10).

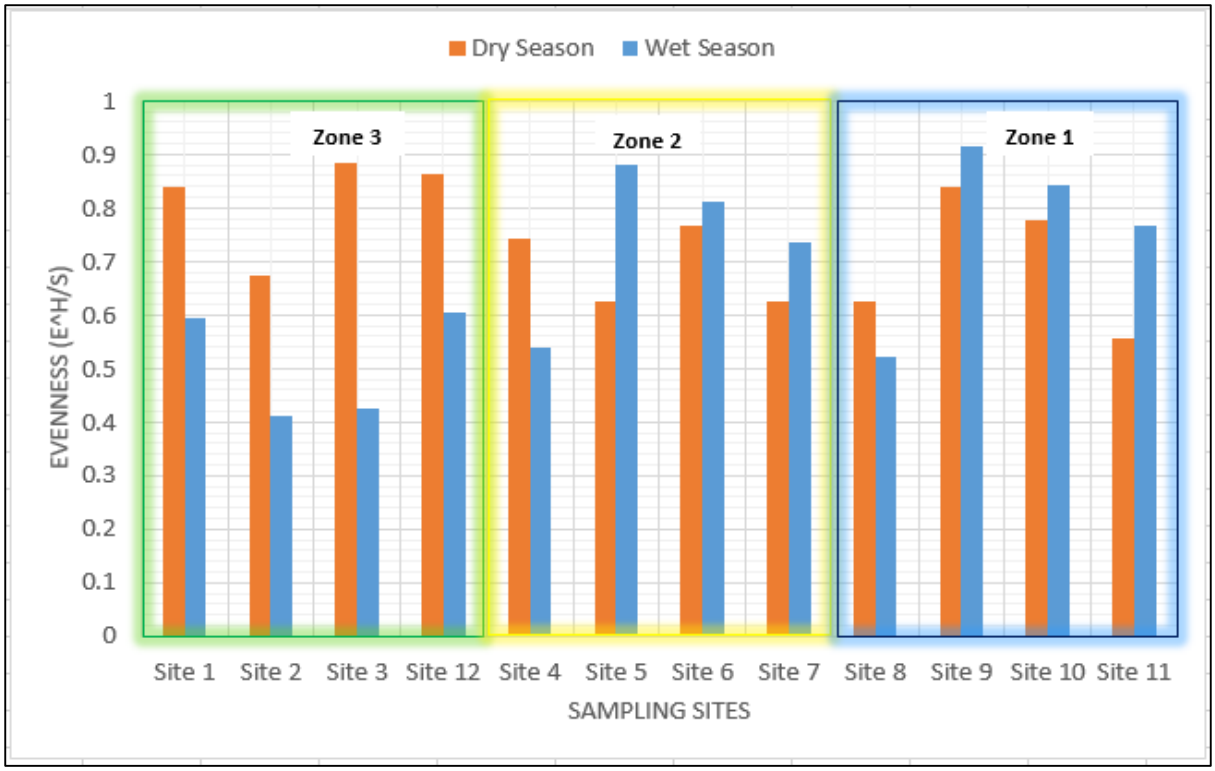


Figure 10: The bar graph shows the evenness scores based on family taxa of benthic macroinvertebrate communities across the three hydro-environmental zones of the Luapula River Basin, Zambia.

The findings showed that Zone 3 had moderate to high evenness scores across wet and dry seasons, with a wet season mean evenness of 0.549 and a dry season mean evenness of 0.739. This suggests a relatively balanced distribution of macroinvertebrate family taxa in respective sampling sites of Zone 3. However, it was noted that evenness scores were relatively lower during the wet season compared to the dry season. This observation was more prominent at sites 2 and 3 where the evenness scores were below 0.5. Furthermore, the low evenness scores at these two sites were reflective of the presence of dominant taxa within these communities as evidenced by high abundances of the families *Baetidae, Hydropsychidae* and *Atyidae* at site 2 and the families *Baetidae* and *Atyidae* at site 3. In Zone 2, the wet season mean evenness was 0.709 whereas the dry season mean evenness was 0.749. This indicates that evenness across the two seasons did not experience much variation in this hydro-environmental zone. However, site-specific seasonal variations were quite noticeable, especially at sites 4 and 5 where the evenness was higher during the dry season compared to the wet season and site 6 where the

evenness was higher during the wet season. On the other hand, site 7 did not display much variation and remained resilient from the dry to wet season. Despite the observed seasonal fluctuations, the distribution of macroinvertebrate taxa in these communities was fairly balanced. Zone 1 recorded high evenness scores, with a wet season mean evenness of 0.753 and a dry season mean evenness of 0.717. Site-specific evenness scores were consistently higher across the two seasons, indicating the presence of well-balanced proportions of macroinvertebrate taxa. This means that benthic macroinvertebrate communities in this zone are evenly distributed across the dry and wet seasons.

5.3.3 Shannon- Weiner Diversity Index

As indicated earlier, the Shannon-Weiner Diversity Index was used to calculate site-specific quantitative scores of macroinvertebrate taxa diversity across the hydro-environmental zones of the Luapula River Basin (Figure 11).

In Zone 1, the mean diversity scores during both the wet and dry seasons were consistently below 2.0, indicating relatively low diversity levels. However, there were notable variations among sites within the zone. For instance, sites 8 and 9 experienced an increase in diversity during the wet season, with diversity scores rising from 1.728 to 1.995 and from 1.929 to 2.054, respectively. On the other hand, site 10 exhibited a slight decrease in diversity from 1.905 to 1.856 during the same period. In contrast, site 11 demonstrated stability in diversity, maintaining a diversity score of 1.945 in the dry season and 1.908 in the wet season.

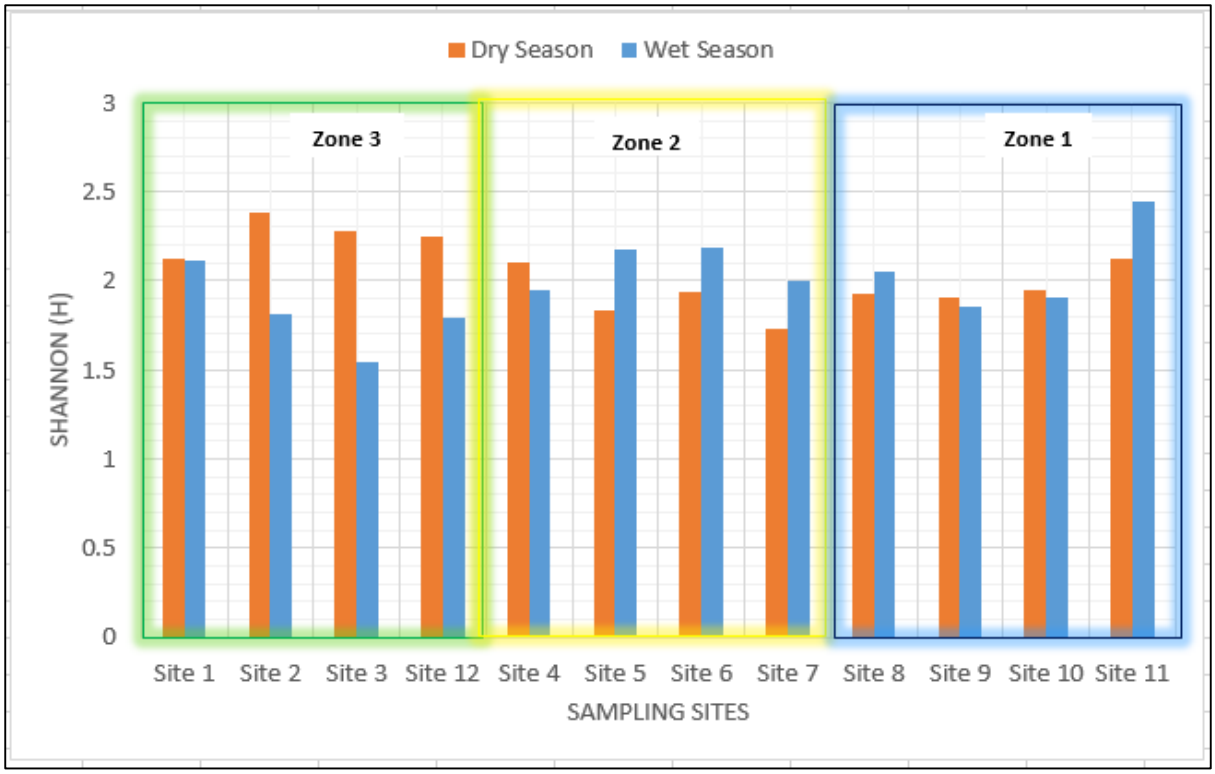


Figure 11: The bar graph shows the Shannon diversity scores based on family taxa of benthic macroinvertebrate communities across the three hydro-environmental zones of the Luapula River Basin, Zambia.

Zone 2 showed increased diversity with mean diversity scores of 2.0 during both the wet and dry seasons. However, there were notable variations among sites within this zone. Sites 4 and 5 experienced a decrease in diversity from the dry season to the wet season. Specifically, site 4 recorded a diversity score of 2.250 in the dry season, which decreased to 1.797 in the wet season. Similarly, site 5 showed a decrease from 2.102 in the dry season to 1.949 in the wet season. In contrast, sites 6 and 7 showed an increase in diversity during the wet season, with diversity scores rising from 1.830 to 2.175 and from 1.933 to 2.191, respectively. In Zone 3, there was a noticeable decrease in mean diversity from 2.22 in the dry season to 1.98 in the wet season. However, individual sites within the zone showed varying diversity patterns. Site 12 stood out with the highest diversity scores in both seasons, recording values of 2.442 in the wet season and 2.124 in the dry season. Conversely, sites 2 and 3 experienced a decrease in diversity from 2.380 in the dry season to 1.818 in the wet season and from 2.275 in the dry season to 1.540 in the wet season, respectively. In contrast, site 1 demonstrated stability in diversity, maintaining a score of 2.125 in the dry season and 2.118 in the wet season.

5.3.4 Assessment of Ecosystem Health

The calculated ZISS Index scores across the 12 sampling sites of the Luapula River Basin were segregated according to their resident hydro-environmental zones (Figure 12). The calculated ZISS score and ASPT indicated that most rivers/streams that were assessed in this study showed signs of moderate deterioration in health condition. This was based on the observation that across all the sampling sites in the hydro-environmental zones, the ZISS score was consistently below 100 during the dry and wet seasons. The ASPT was also observed to be below 7 across all the sampling sites during the dry and wet seasons. However, this observation excludes site 4 in the wet season, sites 6, 8 and 11 in the dry season and site 10 in both the dry and wet seasons. Site 4 in hydro-environmental zone 2 was classified as having major deterioration in water quality in the wet season because the ZISS score was 39 and the ASPT was 4. This observation was a complete departure from the dry season characterisation, where the ZISS score was 62 and the ASPT was 6. Sites 6, 8 and 11 were all classified as having major deterioration in water quality in the dry season. This was due to the ZISS scores and ASPT scores being below 50 and 6, respectively. However, all three sites were observed to experience an improvement in water quality with the change in season. During the wet season, all three sites recorded an increase in the ZISS scores and ASPT scores and were subsequently classified as having moderate deterioration in water quality and health condition. Site 10 in hydro-environmental zone 1 experienced consistently low ZISS index scores during both the dry and wet seasons. The ZISS scores and ASPT scores were consistently below 50 and 6 during the dry and wet seasons, respectively. Therefore, this site was classified as having major deterioration in water quality during the dry and wet seasons.

Overall, it was observed that all sites in hydro-environmental zone 3 were classified as having moderate deterioration in health condition in the dry and wet seasons. In contrast, hydro-environmental zone 1 generally experienced major deterioration in health condition, especially during the dry season and a notable improvement during the wet season. Hydro-environmental zone 2 was observed to experience moderate deterioration in water quality across the dry and wet seasons, with notable upsets in site 4 in the wet season and site 6 in the dry season.

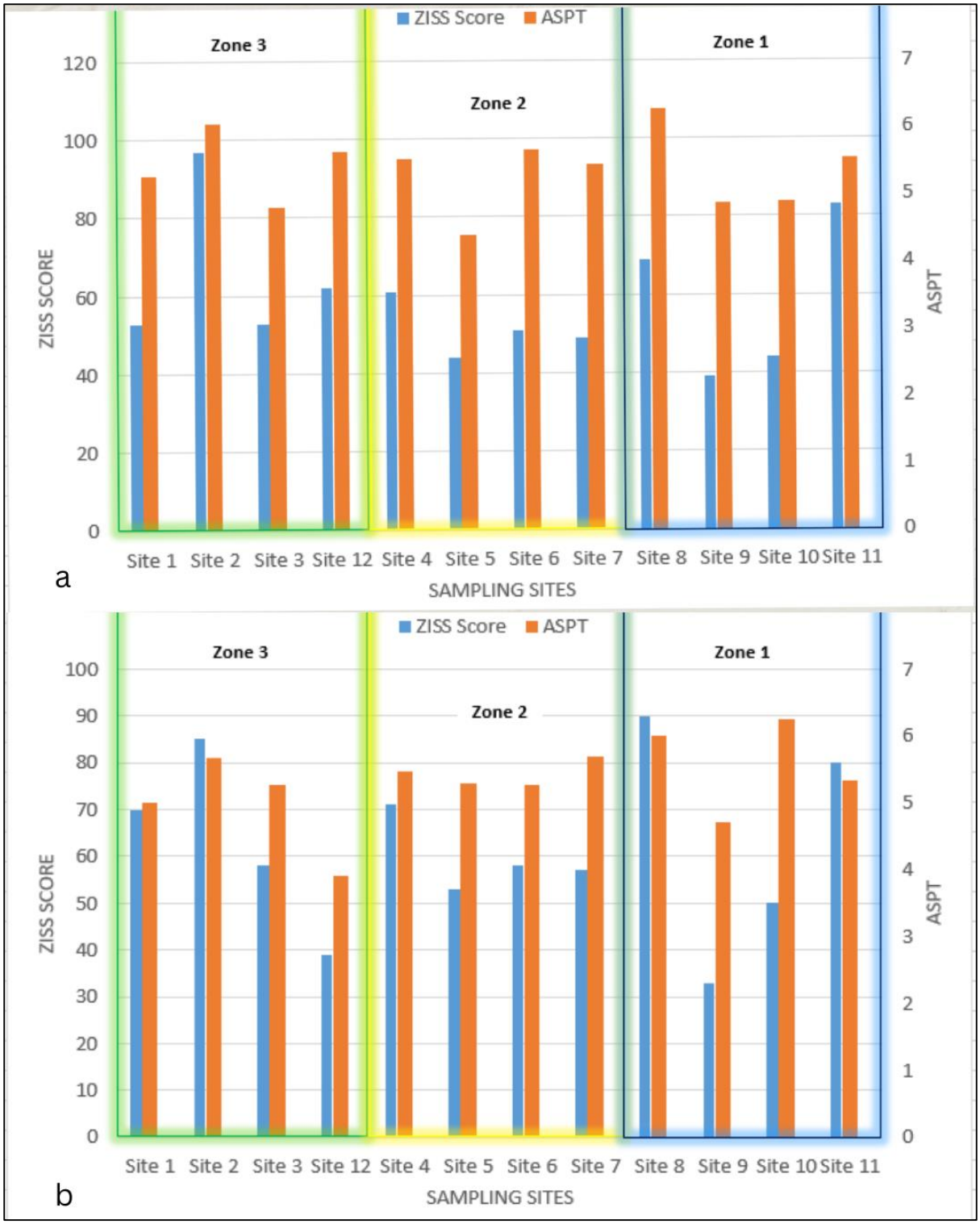


Figure 12: The bar graph shows the (a) dry season and (b) wet season scores of stream health indicators of selected sites across the hydro-environmental zones of the Luapula River Basin, Zambia.

5.3.5 Spatial-Temporal Variation of Biodiversity and Stream Health

To understand the possibility of existing variations in the calculated diversity indices and stream/river health indicators, MANOVA as indicated before, was employed to test the equality of means of the dependent variables across the hydro-environmental zones and seasonal variation (Table 10). The dependent variables were richness, evenness, Shannon Diversity, ZISS score and ASPT whereas the independent variables were hydro-environmental zones and seasonal variation.

Table 10: MANOVA output showing statistical variations in biodiversity metrics and stream health indicators within a spatial-temporal context in the Luapula River Basin, Zambia. Statistical Significance Codes: '***' (p< 0.001), '**' (p< 0.01), '*' (p< 0.05) and '.' (p< 0.1)

	Degree of freedom	Pillai's trace	Approximate F-Statistic	Numerator Degree of freedom	Denominator Degree of freedom	Pr (>F)
Zone	1	0.57037	4.2482	5	16	0.01195 *
Season	1	0.25777	1.1113	5	16	0.39306
Zone:Season	1	0.20192	0.8096	5	16	0.55963
Residuals	20					

The MANOVA output (Table 10 above) shows that the effect of the "Zone" variable was found to be statistically significant (p-value < 0.05), suggesting that there were significant differences in the means of the dependent variables across the three hydro-environmental zones. In contrast, the effect of the "Season" variable was not statistically significant (p-value > 0.05), indicating that there were no significant differences in the means of the dependent variables in the dry and wet seasons. Similarly, the interaction effect between "Zone" and "Season" was not statistically significant (p-value > 0.05). This suggests that the combined effect of "Zone" and "Season" was not significantly different from the expected individual effects of the hydro-environmental zones and change in season.

The MANOVA output (Table 10 above) only provides a general overview of factors responsible for the observed statistical variations among the dependent variables. To extract the dependent variables that were responsible for the observed statistical variations, an ANOVA test was conducted on each dependent variable (Table 11). The ANOVA results show that the zone factor had a significant effect on both the macroinvertebrate family richness (p-value < 0.01) and the ZISS Score (p-value < 0.05). This indicates that there are notable statistical differences in the macroinvertebrate family richness and the ZISS scores among the hydro-environmental zones. It was also observed that the influence of the season factor and the interaction between zone and season had no statistical significance for both richness and ZISS

score. As for Shannon Diversity, the zone factor did not reach conventional significance levels (p-value = 0.07092) but showed a trend towards significance at the 0.1 confidence level. This suggests a potential influence of the hydro-environmental zones on Shannon Diversity. It was also observed that the influence of the factors zone, season and their interaction had no statistical significance on evenness and ASPT.

Table 11: ANOVA outputs showing statistical variations of isolated biodiversity metrics and stream health indicators within a spatial-temporal context in the Luapula River Basin, Zambia. Statistical Significance Codes: '***' (p< 0.001), '**' (p< 0.01), '*' (p< 0.05) and '.' (p< 0.1)

Response Richness:					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	56.25	56.25	12.184	0.002305 **
Season	1	3.375	3.375	0.731	0.402671
Zone:Season	1	0	0	0	1
Residuals	20	92.333	4.617		
Response Evenness:					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	0.03336	0.033361	1.6887	0.2086
Season	1	0.02513	0.025129	1.272	0.2727
Zone:Season	1	0.05103	0.051031	2.5831	0.1237
Residuals	20	0.39511	0.019756		
Response Shannon Diversity:					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	0.141	0.141	3.6386	0.07092 .
Season	1	0.01944	0.019437	0.5016	0.48698
Zone:Season	1	0.10433	0.104329	2.6923	0.11647
Residuals	20	0.77502	0.038751		
Response ZISS Score:					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	1369	1369	5.2123	0.0335 *
Season	1	63.4	63.37	0.2413	0.6286
Zone:Season	1	30.3	30.25	0.1152	0.7379
Residuals	20	5253	262.65		
Response ASPT:					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	0.0625	0.0625	0.1463	0.7061
Season	1	0.1667	0.16667	0.3902	0.5392
Zone:Season	1	0.5625	0.5625	1.3171	0.2647
Residuals	20	8.5417	0.42708		

5.3.6 Interactions Between Biodiversity and Stream Health

As indicated earlier, to understand the interactions between the biodiversity metrics and stream health indicators, the Pearson Correlation Coefficient (PCC) was employed to provide insights into the strengths and direction of the relationships between the ecological indices in both the dry and wet seasons (Table 12).

Table 12: Dry season and wet season Pearson Correlation matrix between biodiversity metrics and indicators of stream health of the Luapula River Basin, Zambia.

Dry Season					
	Richness	Evenness	Shannon Diversity	ZISS Score	ASPT
Richness	1	-0.42575	0.692119	0.955477	0.499267
Evenness	-0.42575	1	0.350952	-0.43079	-0.08121
Shannon Diversity	0.692119	0.350952	1	0.646591	0.462504
ZISS Score	0.955477	-0.43079	0.646591	1	0.677718
ASPT	0.499267	-0.08121	0.462504	0.677718	1
Wet Season					
	Richness	Evenness	Shannon Diversity	ZISS Score	ASPT
Richness	1	-0.63629	0.349537	0.938883	0.171077
Evenness	-0.63629	1	0.483114	-0.5699	-0.08675
Shannon Diversity	0.349537	0.483114	1	0.337192	0.010848
ZISS Score	0.938883	-0.5699	0.337192	1	0.471824
ASPT	0.171077	-0.08675	0.010848	0.471824	1

The analysis revealed positive correlations between richness with the ZISS Score and Shannon Diversity during the dry and wet seasons. However, the correlation between richness and Shannon Diversity was weaker in the wet season (0.35) compared to the dry season (0.69). This suggests that as the number of macroinvertebrate taxa increases, there is a simultaneous increase in both the overall river/stream sensitivity score and the diversity of taxa present. Conversely, evenness exhibited a negative correlation with richness. During the dry season, a moderate negative correlation of -0.43 was observed, indicating a potential trade-off or competitive interaction between the number of macroinvertebrate families and the evenness of their distribution. In the wet season, an increased negative correlation of -0.64 was noted, suggesting an even more pronounced trade-off between richness and evenness during this period. Similarly, the association between evenness and the ZISS Score displayed a moderate negative correlation of -0.43 in the dry season, strengthening to -0.57 in the wet season. Shannon Diversity and evenness showed a positive correlation in both seasons, with a weaker correlation coefficient of 0.35 in the dry season and a relatively pronounced relationship with a correlation coefficient of 0.48 in the wet season. This suggests that as the number of

macroinvertebrate taxa increases, the distribution of individuals among families becomes comparatively more balanced.

Examining the interaction between the ZISS Score and the Average Score per Taxon (ASPT), a strong positive correlation of 0.68 was found in the dry season, indicating that as the stream sensitivity increases, the average score per taxon also tends to rise. In the wet season, this relationship weakened, with a positive correlation of 0.47. In both seasons, the ZISS Score demonstrated a strong positive correlation with richness, Shannon Diversity and ASPT. Conversely, evenness showed a significant negative correlation with both richness and the ZISS Score.

5.3.7 Interpretation and Discussion

In sections 5.3.1 to 5.3.3, The findings highlight the varying biodiversity and distribution patterns of macroinvertebrates across different hydro-environmental zones in the Luapula River Basin. In Zone 1, the ecosystem's low average taxa richness coupled with high average evenness suggests a specialised but vulnerable community. High evenness indicates that the taxa present are well-balanced in terms of abundance, but the low richness implies a limited number of taxa, making the ecosystem susceptible to disturbances. In contrast, Zone 2 demonstrates a stronger and more diverse ecosystem, with increased biodiversity and minimal seasonal fluctuations in average evenness. The presence of site-specific variations within different locations in this zone indicates varying effects of seasonal changes, showcasing the adaptability of different taxa to diverse environmental conditions and ecological preferences. The high average taxa richness in Zone 3 indicates an even stronger and diverse ecosystem, with a variety of resources and microhabitats supporting diverse taxa. However, the wet season brings slight imbalances due to the dominance of certain families, such as *Baetidae*, *Hydropsychidae* and *Atyidae*, suggesting that some taxa may outcompete others during this period. The findings further emphasise the influence of spatial conditions and temporal variations on the macroinvertebrate diversity structure within the basin.

Assessments of spatial-temporal variation of biodiversity metrics as shown in section 5.3.5 indicate that taxa richness was statistically significantly varied across the hydro-environmental zones but not along changes in season. In contrast, Shannon diversity and evenness did not express statistically significant variation with respect to factors of zones or seasons. Shannon diversity was however observed to show a trend towards significance at 0.1 confidence level, suggesting a potential influence from hydro-environmental zones. This finding speaks to the

importance of considering a diversity of scales than a single scale in biodiversity assessments as postulated by Brown et al. (2007). Brown et al. (2007) further assert that species richness as a measure of biodiversity is affected by several factors including geographic, abiotic and biotic factors, and this admission is informed by observed variations in small-scale species richness. In line with this assertion, the observed statistically significant variation of taxa richness across the three hydro-environmental zones can be attributed to their separation on the basis of physiography, hydrology, climate and land cover. This finding is further affirmed by a study conducted in Central and Northern Finland by Astorga et al. (2011) who found out that elevation range was the most important regional correlate of macroinvertebrate taxonomic richness, explaining as much as 31.2% of the variation in riffle sites in near-pristine forest streams. Astorga et al. (2011) also found that landscape variables such as percent of forest cover were related to the taxonomic richness in certain drainage systems.

This study also revealed contrasting biodiversity characterisation by taxa richness and taxa evenness. Zone 3 was characterised by relatively higher taxa richness scores with varying taxa evenness scores across the dry and wet seasons, while on the other hand zone 1 recorded relatively low taxa richness scores with relatively higher taxa evenness scores across the dry and wet seasons. In line with this observed contrast, Morris et al. (2014) postulate that compound indices discriminate between sampling areas better than simple diversity measures. To reaffirm this submission, this study found that the Shannon diversity index (compound index) showed that zone 3 had a comparatively higher diversity score than zone 1. However, what also comes out is the observed stability in the taxa abundance proportions in zone 1 compared to the seasonally varied taxa abundance proportions in zone 3.

Correlation analysis also revealed that Shannon diversity was positively correlated to taxa richness and evenness. Similar trends of interaction were observed by Morris et al. (2014) in the temperate grasslands of German and by Heino et al. (2008) in North-Eastern Finland. Conversely, evenness was negatively correlated to taxa richness across seasons compared to the findings by Heino et al. (2008) where evenness showed a very weak positive correlation with species richness. Morris et al. (2014) on the other hand found that evenness had a directionally varied interaction with other indices of diversity including species richness.

In section 5.3.4, the Zambia Invertebrate Scoring System (ZISS) was used for a rapid bio-assessment of the Luapula River Basin, revealing moderate to major deterioration in river/stream health across the 12 sampling sites. This conclusion was based on ZISS scores

consistently below 100 and ASPT scores consistently below 7, as detailed in section 4.2.3. Specifically, sites 6, 8 and 11 were classified as experiencing major deterioration in river/stream health, with ZISS scores below 50 and ASPT scores below 6 during the dry season, while site 4 exhibited similar major deterioration in the wet season. These low ZISS and ASPT scores indicate that the macroinvertebrate communities at these sites are tolerant to environmental stressors and changes in water quality, resulting in decreased diversity and overall health metrics for the rivers and streams. Seasonal fluctuations, such as rainfall patterns and water flow, likely impact water quality parameters, influencing the health of these aquatic ecosystems. Site 10 showed signs of major deterioration in river/stream health during both the dry and wet seasons, with ZISS scores below 40 and ASPT scores of 5 in both seasons. This site may be heavily influenced by significant pollution sources, such as fuel discharge and increased human presence, as it is located at the border area between Zambia and the DR Congo, characterised by active water transport and trade between the two countries. The observed low scores in ZISS score and ASPT can be attributed to the lower overall abundance of sensitive taxa during both the dry and wet seasons (Chutter, 1993; Dickens and Graham, 2002; Dallas et al., 2018). Both the dry and wet season were dominated by moderately tolerant and highly tolerant macroinvertebrate taxa. This observation agrees though contextually with the observation made by Dallas et al. (2018) in the Bangweulu-Mweru freshwater ecoregion, to which the Luapula River Basin belongs. Dallas et al. (2018) notes that the vegetative samples collected from larger and higher order streams generally had lower ZISS score and taxa richness compared to smaller and lower order streams. However, it is important to note that the underscored observation is only compared on the basis of the generally observed low ZISS scores and taxa richness and not on the basis of the specified biotope or stream orders.

Sections 5.3.5 and 5.3.6 describe how spatial-temporal variation assessments help in understanding how biological assemblages are influenced by environmental gradients as embedded within the hydro-environmental zones across season change and how correlation assessments help to quantify the strength and direction of relationships between indicators of stream health (ZISS score and ASPT) and diversity indicators (richness, evenness and Shannon Diversity) across changing seasons, respectively. The statistically significant effect of the "Zone" variable (p-value < 0.05) indicates that there are notable variations in the stream health indicators and diversity indices across the different hydro-environmental zones. This suggests that the input hydro-environmental attributes of each zone such as precipitation, runoff, elevation and forest cover extent play a significant role in shaping the benthic

macroinvertebrate communities of the Luapula River Basin. In contrast, the non-significant effect of the "Season" variable (p-value > 0.05) implies that there are no significant differences in the means of the dependent variables between the dry and wet seasons. This indicates that the seasonal changes may not have a substantial impact on the overall composition and diversity of macroinvertebrate communities in the Luapula River Basin. The lack of statistical significance in the interaction effect between "Zone" and "Season" (p-value > 0.05) suggests that the combined influence of hydro-environmental zones and seasonal variation does not differ significantly from the expected individual effects. This implies that the effects of zone and season on benthic macroinvertebrate communities may act independently rather than interactively, highlighting the importance of considering these factors separately in macroinvertebrate community assessments of the Luapula River Basin. Spatial-temporal variation assessment indicated that both ZISS score and taxa richness were statistically significantly varied across the hydro-environmental zones but not between seasons. This finding partially aligns with the findings of Kaaya et al. (2015), who observed a statistically significant difference in the TARISS score (Tanzania River Scoring System), ASPT and taxa richness across the reference sites and test sites of the Panjani River Basin, Wami-Ruvu River Basin and Rufiji River Basin of Tanzania. However, this study did not establish a statistically significant output concerning the variation of ASPT across the hydro-environmental zones or seasons in the Luapula River Basin. However, this study did find that ASPT was strongly correlated with ZISS score during both the dry and wet seasons, indicating that as the stream sensitivity score increases, the average score per taxon also tends to rise. This study also showed that ASPT has a weak correlation with taxa richness. These findings are in agreement with the findings of Dallas et al. (2018) and Dickens and Graham (2002) in Zambia and South Africa, respectively. According to Dallas et al. (2018), factors responsible for the observed changes in ASPT and taxa richness seem to operate independently and therefore changes in one or both metrics may be indicative of an impact on river/stream condition. Dickens and Graham (2002) also submit that the consistency that characterises ASPT even in the face of other metrics changing due to prevailing environmental conditions makes it a more repeatable measure of river/stream health. Dallas et al. (2018) and Dickens and Graham (2002) also acknowledge the lack of statistically significant variation in ASPT scores across all biotopes. In support of the underscored argument, Chutter (1993) and Dallas (1997) also submitted that ASPT is less affected by low density because the presence of a few organisms with higher sensitivity scores will influence an appropriate score. However, Chutter (1993) also advises that the consideration of ASPT as a common choice of SASS measure should be approached

cautiously because ASPT may be a more responsive measure when dealing with less impacted sites but ZISS score may be more responsive when dealing with impacted sites. Fourie et al. (2014) and Dallas (2004) collaborate the observed lack of statistically significant seasonal variation in ZISS metrics across the selected sites of the Luapula River Basin. Fourie et al. (2014) found that the SASS metrics including SASS score, ASPT and number of taxa in the relatively unpolluted Skeerpoort River in the North-Western Province of South Africa displayed a lack of statistical seasonal variation and this was attributed to generally similar water levels and the absence of major rainfall/flooding events during the sampling period. Dallas (2004) on the other hand found that the SASS metrics did not present a significant seasonal variation in Mpumalage, a summer rainfall area in South Africa, while significant statistical variation was observed for ASPT and the number of taxa in the Western Cape, a winter rainfall area in South Africa. Fourie et al. (2014) further postulate that macroinvertebrate sampling should not only consider the health of freshwater ecosystems but also the region, because the availability of macroinvertebrates depends on habitat availability, climate and regional differences. Based on this submission, it can also be postulated that the observed statistically significant variation in ZISS score and number of taxa across the zones can be attributed to the varied hydro-environmental attributes including elevation, precipitation, surface water runoff and forest cover extent the basis upon which the hydro-environmental zones were synthesised.

5.4 Feeding Functional Groups

The overall abundance of the five Feeding Functional Groups (FFGs) (Appendix 14) across the sampling sites for the dry and wet seasons is presented in Figure 13. Additionally, site-specific abundances of the FFGs across the two seasons are presented in Figure 14. The outcome of the dry and wet season data analysis shows that gathering collectors were the most dominant feeding functional group, with an overall representation of 37% in the dry season and 48% in the wet season. In both the dry and wet seasons, this FFG had a registered presence across all the sampling sites, with the highest abundances recorded at sites 1, 2, 4, 7, 8, 9 and 12 in the dry season and sites 2, 3, 4, 5, 8 and 9 in the wet season. In contrast, during the dry season, shredders accounted for only 2% of the total abundance of functional feeding groups (FFG), with a recorded abundance score of 31 individuals. However, during the wet season, this score increased to 5% of the total FFG abundance, with a recorded abundance score of 79 individuals. The shredders were found to have the lowest abundance across all 12 sampling sites. During the dry season, the highest relative counts for shredders were recorded at sites 2,

4, 6, 7 and 10. On the other hand, during the wet season, sites 2, 5, 6, 9 and 12 had the highest relative counts for shredders. Shredders and gathering collectors are well adapted to processes of detritus processing within aquatic ecosystems.

The presence of scrapers and filtering collectors helps control algal populations and the accumulation of suspended organic matter in aquatic ecosystems, respectively. From across the 12 sampling sites, scrapers recorded an abundance score of 235 individuals in the dry season, representing 17% of the collective total. In the wet season, the abundance score of the scrapers was reduced to 215 individuals, representing 14% of the collective total. The filtering collectors on the other hand recorded an abundance score of 140 and 168 individuals in the dry and wet seasons, respectively. This accounted for 11% of the collective seasonal abundances for both sampling campaigns.

Predators were the second dominant group during the dry and wet seasons. In the dry season, the highest predator counts were observed in sites 1, 2, 9 and 11 while in the wet season, sites 1, 8, and 9 showed the highest predator counts. The dry season had a total predator abundance of 449 individuals which represented 33% of the overall total. Conversely, the wet season had an abundance of 351 individuals which represented 22% of the total.

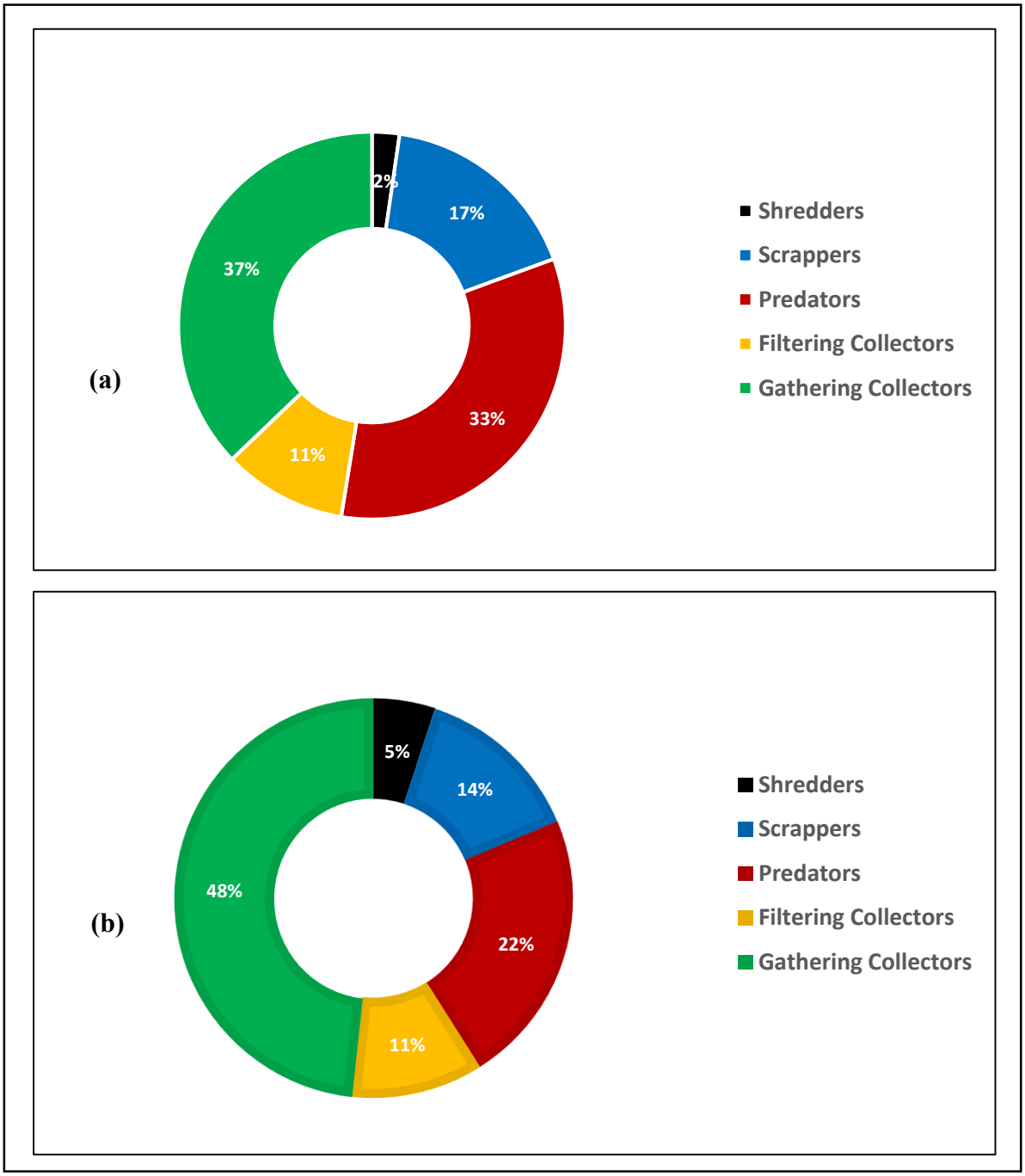


Figure 13: Pie-chart showing the dry season (a) and wet season (b) feeding functional group abundances collected from all the selected sites of the Luapula River Basin, Zambia.

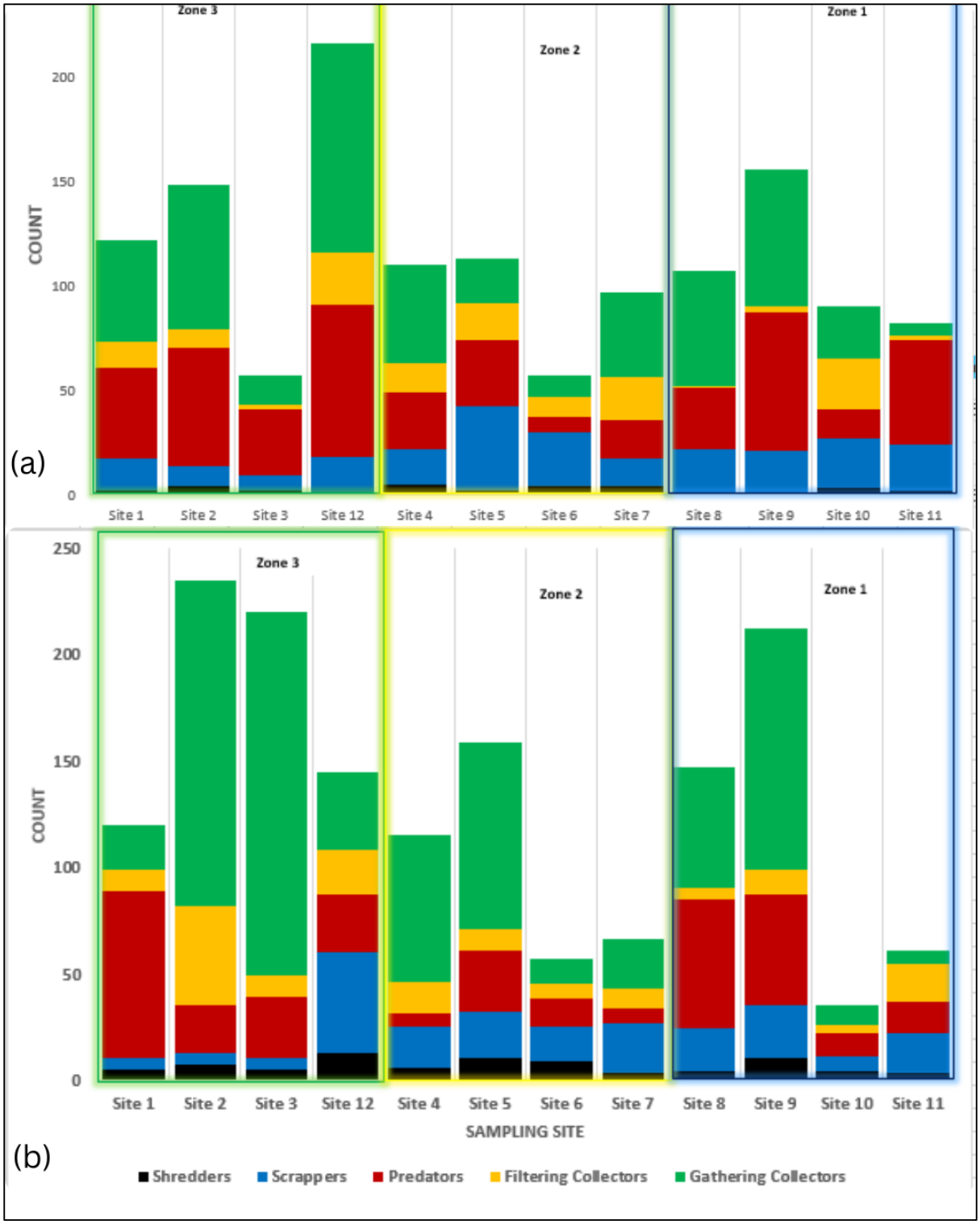


Figure 14: The bar graph showing dry season (a) and wet season (b) site-specific feeding functional group abundances across the selected sites of the Luapula River Basin, Zambia.

5.4.1 Spatial-Temporal Variation of Functional Feeding Groups

The MANOVA analysis (Table 13) indicates that the seasonal factor had a statistically significant impact on the structure of functional feeding groups (FFGs) (p-value < 0.05). This suggests that as the seasons changed, there were noticeable differences in the means of the FFGs, showing that seasonal variation plays an important role in influencing the FFGs. However, the analysis also revealed that neither the zone factor nor the interaction between zone and season had a statistically significant effect on the FFGs (p-value > 0.05). This means that while the season alone affects FFG distribution and abundance, the combined effect of season and zone does not significantly alter the FFGs, nor does the zone factor by itself.

Table 13: MANOVA output showing statistical variations in the structure of functional feeding groups across the hydro-environmental zones and seasons in the Luapula River Basin, Zambia. Statistical Significance Codes: '***' (p< 0.001), '**' (p< 0.01), '*' (p< 0.05) and '.' (p< 0.1).

	Degree of freedom	Pillai's trace	Approximate F-Statistic	Numerator Degree of freedom	Denominator Degree of freedom	Pr (>F)
Zone	1	0.30849	1.4275	5	16	0.26748
Season	1	0.5271	3.5668	5	16	0.02339 *
Zone: Season	1	0.07352	0.2539	5	16	0.93163
Residuals	20					

The ANOVA test further demonstrated that the observed seasonal variations in the structure of functional feeding groups (FFGs) were primarily driven by shredders (p-value < 0.001) (Table 14). This suggests that shredders were the main contributors to the differences in FFG structure with change in season. In contrast, the p-values for the other FFGs, including scrapers, predators, filtering collectors, and gathering collectors, were all above 0.05. This indicates that these groups did not experience significant changes due to seasonal variations, and their distribution and abundance remained relatively unaffected by the change in seasons.

Table 14: ANOVA outputs showing statistical variations of functional feeding groups across change in zone and season in the Luapula River Bain, Zambia. Statistical significance Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Response: Shredders				
	Df Sum Sq	Mean Sq	F value	Pr(>F)
Zone	1 7.562	7.562	1.208	0.2847862
Season	1 96.000	96	15.3344	0.0008563 ***
Zone:Season	1 3.063	3.063	0.4892	0.4923463
Residuals	20 125.208	6.26		
Response: Scrappers				
	Df Sum Sq	Mean Sq	F value	Pr(>F)
Zone	1 132.25	132.25	1.2138	0.2837
Season	1 12.04	12.042	0.1105	0.743
Zone:Season	1 56.25	56.25	0.5163	0.4807
Residuals	20 2179.08	108.954		
Response: Predators				
	Df Sum Sq	Mean Sq	F value	Pr(>F)
Zone	1 256.0	256	0.4985	0.4883
Season	1 400.2	400.17	0.7793	0.3878
Zone: Season	1 49.0	49	0.0954	0.7606
Residuals	20 10270.2	513.51		
Response: Filtering Collectors				
	Df Sum Sq	Mean Sq	F value	Pr(>F)
Zone	1 280.56	280.562	2.8746	0.1055
Season	1 32.67	32.667	0.3347	0.5694
Zone:Season	1 60.06	60.063	0.6154	0.442
Residuals	20 1952.04	97.602		
Response: Gathering Collectors				
	Df Sum Sq	Mean Sq	F value	Pr(>F)
Zone	1 4796	4795.6	2.468	0.1319
Season	1 2731	2730.7	1.4053	0.2497
Zone:Season	1 856	855.6	0.4403	0.5146
Residuals	20 38862	1943.1		

5.4.2 Interpretation and Discussion

The incorporation of the functional aspect of macroinvertebrate community organisation in this study follows the submission by Cummins et al. (2005) and Cummins (2016), which underscores the effectiveness of considering macroinvertebrate morphological and behavioural adaptations in food resource acquisition in determining ecosystem health and function. The increasing dominance of gathering collectors from the dry to the wet season indicates that the ecosystem is rich in fine particulate organic matter (FPOM). This suggests that the ecosystem consistently supplies an abundance of FPOM, which is a vital resource for these organisms.

This observation is significant because gathering collectors play a key role in nutrient cycling by breaking down organic matter and transforming it into forms that can be used by primary producers. This supports primary production and ensures that nutrients are efficiently recycled within the ecosystem. Therefore, the conversion of decomposing organic matter into usable nutrients by gathering collectors is important for the productivity and stability of the ecosystem. During the dry season, there was a low abundance of shredders, which suggests a scarcity of coarse particulate organic matter (CPOM) and poor riparian zone functionality or increased microbial activity. However, their numbers slightly increase during the wet season, indicating a temporary improvement in the availability of CPOM due to seasonal changes such as increased runoff and water flow. This increase in the wet season is likely caused by organic debris being washed into the water from surrounding areas, providing more food resources for the shredders. Overall, while the abundance of shredders is generally low, the wet season conditions briefly improve the habitat suitability for these organisms. In contrast, the decrease in predator abundance from the dry to the wet season suggests that the increased water flows characteristic of the wet season might disturb predator habitats and change the availability of prey. Predators play an important role in controlling prey populations and maintaining ecological balance. Therefore, a decrease in their numbers could have ripple effects within the ecosystem, potentially impacting the overall health and stability of the ecosystem. The consistent presence of scrapers and filtering collectors during the dry and wet seasons indicates a stable level of algal growth and suspended organic matter, suggesting an ecosystem that maintains a balance of these two resources despite seasonal variations. In the study of the Dwars River and Spekboom River in the Olifants River system in South Africa, Addo-Bediako (2021) found that the distribution of macroinvertebrate feeding functional groups showed longitudinal differences, with gathering collectors and filtering collectors being the most abundant, and shredders and scrapers the least abundant. In contrast, the Luapula River Basin exhibited a different pattern of dominance in macroinvertebrate abundance, with the order being gathering collectors > predators > scrapers > filtering feeders > shredders in both the dry and wet seasons. This observation aligns with the findings in a recent study by Makgoale et al. (2022), who found that gathering collectors were the dominant feeding functional group, while shredders were the least abundant in the Steelpoort River in South Africa. Makgoale et al. (2022) attributes the predominance of gathering collectors to their ecological adaptability and proficiency in inhabiting diverse environments, including swift water habitats. In contrast, the low number of shredders was attributed to the possible replacement of shredder activity by effective microbial activities at high temperatures as was postulated by Kaboré et al. (2016)

and Madomguia et al. (2016). The replacement of shredder activity by effective microbial activities at high temperatures in tropical streams compared to temperate streams is reaffirmed by Addo-Bediako (2021) in reference to a study by Deemool and Prommi (2015) in the Mae Klong watershed in Thailand. Less/loss of riparian vegetation resulting from human activities is another possible factor affecting shredder populations.

Makgoale et al. (2022) also observed that despite the observed site-specific variations in the distribution of macroinvertebrate functional feeding groups with change in season, the observed seasonal variations were not statistically significant. Similarly, the abundance of the feeding functional groups was observed to increase in the wet season compared to the dry season in the selected sites of the Luapula River Basin. Despite the observed site-specific variations with change in season, all the FFGs were not statistically significantly affected by the change in season except for shredders (p-value < 0.001). Makgoale et al. (2022) further submitted that the distribution pattern of benthic macroinvertebrate feeding functional groups in the Steelpoort River did not conform with the predictions of the River Continuum Concept (RCC) by Vannotte (1980) because the Steelpoort River, which is a low order river was dominated by predators and gathering collectors, instead of gathering collectors and shredders as predicted by the River Continuum Concept. The River Continuum Concept describes the entire river system as a continuously integrating series of physical gradients and associated biotic adjustments as the river flows from headwater to mouth. The concept further states that, as the physical gradient of a river changes from source to mouth, chemical systems and biological communities shift and change in response. The concept thus allows for the prediction of how relative FFG abundance changes along a river gradient with changing physical and chemical systems. In the same vein, the RCC predicted co-dominance of gathering collectors and shredders was not observed in the Dwars River and Spekboom River of the Olifants river system in South Africa (Addo-Bediako, 2021). Similarly, the observed co-dominance of gathering collectors and predators in the selected sites of the Luapula River Basin in both the dry and wet season did not align with the RCC predicted distribution of macroinvertebrate functional feeding groups. Makgoale et al. (2022) attributed the non-conformity to the River Continuum Concept to the prevailing land use changes within the catchment such as agriculture, urbanisation, industries and mining. Additionally, the study emphasised on the importance of considering land use changes' impact on macroinvertebrate communities when formulating policies. Similarly, Addo-Bediako (2021) attributes the non-conformity of the relative abundance and richness of the feeding functional groups to the river continuum concept

to degradation of the catchment resulting from human activities which also affected the quality of the water.

5.5. Assessment of Ecosystem Function Based on FFG Ratios

The application of FFGs as proxies of ecosystem attributes is achieved through the use of FFG ratios, which were calculated by comparing the abundance of different FFGs sampled at a particular site (Table 15). Table 15 provides insights into the dry and wet season functional dynamics of ecosystem attributes as measured by FFG ratios within the hydro-environmental zones in the dry and wet seasons.

Table 15: Dry and wet season ecosystem attributes measured based on FFG ratios in the selected sites of the Luapula River Basin, Zambia.

Dry Season						
Zone	**Site**	**P/R**	**CPOM/FPOM**	**TFPOM/BFPOM**	**Channel Stability**	**Top-Down Control**
ZONE 3	**1**	0.24	0.03	0.24	0.53	0.56
	2	0.12	0.05	0.13	0.26	0.61
	3	0.39	0.13	0.14	0.56	1.28
	12	0.13	0.01	0.25	0.42	0.51
ZONE 2	**4**	0.26	0.08	0.30	0.60	0.33
	5	0.98	0.05	0.86	2.52	0.40
	6	1.08	0.20	1.00	2.57	0.14
	7	0.20	0.07	0.49	0.73	0.24
ZONE 1	**8**	0.37	0.02	0.02	0.39	0.37
	9	0.29	0.01	0.05	0.34	0.73
	10	0.46	0.06	0.96	1.71	0.18
	11	2.20	0.25	0.33	3.00	1.56
Wet Season						
ZONE 3	**1**	0.14	0.16	0.48	0.58	1.93
	2	0.03	0.04	0.31	0.33	0.10
	3	0.03	0.03	0.06	0.09	0.15
	12	0.66	0.22	0.57	1.36	0.23
ZONE 2	**4**	0.21	0.07	0.22	0.45	0.06
	5	0.20	0.10	0.11	0.33	0.22
	6	0.57	0.47	0.58	1.10	0.30
	7	0.69	0.09	0.39	1.27	0.12
ZONE 1	8	0.30	0.06	0.09	0.41	0.71
	9	0.19	0.08	0.11	0.30	0.33
	10	0.41	0.31	0.44	0.85	0.46
	11	0.70	0.13	3.00	4.11	0.33

The autotrophy to heterotrophy ratio (P/R index) scores revealed that all sampling sites in hydro-environmental Zone 3 were heterotrophic during both dry and wet seasons, as were all sites in Zone 1 and Zone 2 during the wet season. This indicates that the scores were below the autotrophy threshold of 0.75 as defined by Cummins et al. (2005). This means that organic

matter decomposition sourced from leaf litter and detritus serves as the primary energy source for the considered aquatic ecosystems. Consequently, autotrophic production driven by algae and rooted aquatic plants likely plays a lesser role in the energy dynamics of these stream ecosystems. However, during the dry season, sites 5 and 6 in Zone 2 and site 11 in Zone 1 displayed P/R index scores exceeding 0.75, indicating autotrophic conditions where primary production exceeds respiration. This suggests that these stream/river ecosystems produce more organic carbon than they consume.

The coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) ratio which measures the ratio of CPOM to FPOM in streams, indicated that most sampled streams, except for site 11, had index scores below the predicted threshold of 0.25 during the dry season (Cummins et al., 2005). This threshold is associated with normal shredder activity, typically found in functioning riparian zones. Shredders play a crucial role in breaking down CPOM into FPOM, providing an energy source for downstream organisms. The low index scores across all hydro-environmental zones suggest low shredder populations and weak association with riparian zones, indicating minimal contribution to organic matter supply. However, site 11 during the dry season exhibited a normal shredder association, as did sites 6 and 10 during the wet season.

The ratio of transport fine particulate organic matter (TFPOM) to benthic fine particulate organic matter (BFPOM) provides insight into the movement and accessibility of FPOM in supporting filtering taxa within stream/river ecosystems. During the dry season, all sampled sites in hydro-environmental Zone 3, along with sites 4 and 7 in Zone 2 and sites 8, 9 and 11 in Zone 1, exhibited TFPOM to BFPOM index scores below the predicted threshold of 0.5. This suggests reduced loading of suspended FPOM across the three zones, leading to insufficient support for filtering collectors and potential impacts on energy flow through trophic levels. Conversely, sites 5 and 6 in Zone 2 and site 10 in Zone 1 displayed index scores exceeding the threshold, indicating good quality FPOM availability for filtering collectors during the dry season. During the wet season, TFPOM to BFPOM index scores surpassed the predicted threshold of 0.5 at site 12 in Zone 3, site 6 in Zone 2 and site 11 in Zone 1, while it approached the threshold (borderline) at site 1 in Zone 3. This means that the change in temporal orientation from the dry season to the wet season elevates the suspended FPOM load available to the system. This seasonal improvement in FPOM availability is most beneficial to the success of filtering collectors.

The FFG surrogate ratio for channel stability is an indicator of the stability of stream or riverbed environments. It differentiates between the habitat needs of various macroinvertebrates: scrapers and filtering-collectors versus gathering-collectors and shredders. Scrapers and filtering-collectors depend on stable, nonshifting surfaces for feeding and attachment, such as rocks or stable plant material. In contrast, gathering-collectors and shredders inhabit the interstices of sediments and accumulations of organic litter, which are less stable and more variable. The substrate stability ratio indicates the presence of stable substrates such as bedrock, boulders, cobblestones or large wood debris when the value exceeds the predicted threshold of 0.50 (Cummins et al., 2005). In Zone 3, during the dry season, sites 1 and 3 exhibited adequate stable substrates, while site 12 had marginal stability, and site 2 was unstable. However, during the wet season, substrate quality improved at sites 12 and 2 with increased water flow, whereas it declined at site 3. In Zone 2, all sites had index scores above the predicted threshold during the dry season. Sites 5 and 6 had abundant stable substrates, while sites 4 and 7 had adequate stability. In the wet season, however, increased water flow affected the stable substrates at sites 4 and 5, leading to reduced stability rankings. In Zone 1, sites 10 and 11 consistently scored above the predicted threshold during both the dry and wet seasons, maintaining adequate to abundant stable substrates throughout. During the dry season, sites 8 and 9 had predominantly unstable substrates. However, during the wet season, these sites showed a relative improvement in the presence of stable substrates.

The top-down control ratio highlights the relationship between predator population density and prey population dynamics within an ecosystem. In a balanced ecosystem, the population density of predators is closely tied to the availability of prey. Sufficient prey is necessary to sustain a healthy predator population. If predator density is too high, it can lead to over-predation, which can deplete prey populations and eventually cause a decline in predator numbers due to starvation. During the dry season, Zones 3 was characterised by a high top-down ratio across all sites. Similarly, Zones 2 and 1 were characterised by a high top-down ratio except for sites 6 and 10 respectively. This means that most sites recorded a top-down ratio that exceeds the prescribed threshold of 0.1 – 0.2 according to Merritt et al. (2017). A high top-down ratio reflects the abundance of prey species having short life cycles and therefore populations that turn over rapidly to continuously renew the food supply for the longer-lived predators. In contrast, Sites 6 and 10 recoded top-down ratios that are within the prescribed threshold of 0.1 – 0.2, indicating that there is a balance at the stream site between prey taxa with long and short life cycles. In the wet season, Zone 1 was characterised by top-

down ratios above the threshold of 0.1 – 0.2 across all sites. This means that these sites were hosts to prey taxa with short life cycles and rapid turnovers. Zones 2 and 3 were characterised by fluctuations in the top-down ratio. In zone 2, sites 5 and 6 and sites 1 and 12 in zone 3 were characterised by a high top-down ratio, indicating that these sites host polyvoltine prey populations. Sites 2 and 3 in zone 3 and site 7 in zone 2 showed normal predator to prey balance, indicating a balance bwtween at the stream site between prey species with long and short life cycles. In zone 2, site 4 recorded a top-down ratio below the prescribed threshold of 0.1 – 0.2. This suggests a prevalence of prey population with reduced predation pressure at this site.

5.5.1 Spatial-Temporal Variation of Ecosystem Attributes

Multivariate analysis of variance (MANOVA) was employed to investigate the influence of zone, season and their interaction (Zone: Season) on ecosystem function, as measured by FFG ratios, in selected sites of the Luapula River Basin (Table 16).

Table 16: MANOVA output showing statistical variations of ecosystem attributes as measured by FFG ratios within a spatial-temporal context in the Luapula River Basin, Zambia.

	Degree of freedom	Pillai's trace	Approximate F-Statistic	Numerator Degree of freedom	Denominator Degree of freedom	Pr (>F)
Zone	1	0.25088	1.07166	5	16	0.4122
Season	1	0.34026	1.65037	5	16	0.2037
Zone:Season	1	0.11351	0.40974	5	16	0.8351
Residuals	20					

The statistical analysis revealed that neither the zone nor the season factor showed a statistically significant effect on FFG ratios (p-value > 0.05). Thus, the individual effects of zone and season on FFG ratios do not reach conventionally accepted levels of statistical significance. Additionally, the interaction between zone and season as assessed by the zone:season term did not yield any significant effect (p-value >0.05). These results suggest that the combined impact of zone and season on ecosystem function does not significantly deviate from what would be anticipated based on the individual effects of each factor.

5.5.2 Interpretation and Discussion

The key ecosystem attributes evaluated in this study included the autotrophy to heterotrophy index, the ratio of coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) and the ratio of transported to deposited FPOM, the sustrate stability ratio and top-down ratio as described by Cummins et al. (2005) and Merritt et al. (2017). Feeding Functional

Group ratios have been used as surrogates for stream/river ecosystem attributes such as the balance between autotrophy and heterotrophy, linkage with riparian inputs and geomorphic stability (Cummins et al., 2005; Merritt et al., 2017).

The autotrophy to heterotrophy index provides a measure of the balance between primary production (autotrophy) and the consumption of organic matter (heterotrophy) in aquatic ecosystems. In the Luapula River Basin, the index results revealed that most sampled sites were predominantly heterotrophic during both the dry and wet seasons. This predominance of heterotrophy indicates that these ecosystems rely heavily on external organic matter inputs, such as leaf litter, dead organisms and other organic debris, for their sustenance. In contrast, sites 5, 6 and 11 deviated from this trend, showing autotrophic conditions only during the dry season. This observation suggests that these sites have higher rates of primary production during the dry season, likely due to enhanced conditions which facilitate the growth of algae and aquatic plants. These autotrophic conditions indicate that, in the dry season, these ecosystems are more self-sustaining, with primary producers forming the base of the food web and supporting higher trophic levels through photosynthesis. The overall dominance of heterotrophy across most sites, however, highlights the importance of external organic matter inputs in maintaining the ecological balance of the Luapula River Basin. It suggests that the basin's aquatic ecosystems are largely driven by the decomposition and recycling of organic matter, rather than by in situ primary production as confirmed by Cummins et al. (2005) in the neotropical streams/rivers of South Brazil. Cummins et al. (2005) attributed this predominance to allochthonous detritus serving as the primary food source for invertebrate communities. Yule (1996) also noted that in Konaino Creek on Bougainville Island in New Guinea, allochthonous detritus, mainly in the form of fine particulate organic matter resulting from terrestrial degradation was the primary energy source.

Factors contributing to the heterotrophic state of aquatic systems include low primary production, nutrient limitations and shaded conditions caused by canopy cover or turbidity. In a study investigating bacterial nutrient constraints in Florida Bay, in the USA, Cotner et al. (2000) observed that the growth of bacteria and biomass production in the eastern and southern regions of Florida Bay was primarily restricted by inorganic phosphorus. The introduction of nutrients to these systems was also observed to enhance productivity and biomass accumulation, particularly during summertime. In addition, the presence of abundant seagrasses in Florida Bay is thought to foster heterotrophic bacterial production over phytoplankton production by releasing dissolved organic carbon, thus increasing bacterial

competitiveness for limited inorganic phosphate, particularly in the low turbidity and high-light eastern bay areas. Dodds (2006) investigated the trophic state of flowing waters, particularly focusing on rivers and streams. In this study, it was found that the regulation of the autotrophic state is heavily influenced by phosphorus and nitrogen, with benthic algal biomass positively correlated with gross primary production in streams. Chlorophyll levels, indicative of nutrient availability, were also influenced by reference nutrient concentrations, with high levels leading to elevated chlorophyll levels, while average concentrations resulted in lower chlorophyll concentrations. Dodds (2006) also submits that the variability in autotrophic state was attributed to factors such as canopy cover in small pristine streams, whereas heterotrophic state, reliant on allochthonous or autochthonous production, exhibited less variability. Furthermore, Dodds (2006) asserts that both autotrophic and heterotrophic states can be affected by nutrient enrichment, with stoichiometry (carbon, nitrogen, and phosphorus) playing a significant role in trophic state dynamics. The study by Hill and Knight (1988) investigated nutrient and light limitations of algae in two Northern California streams. This research revealed that forested streams with dense forest shade pose a challenge to primary producers such as algae and associated grazing invertebrates. The study demonstrated that periphyton productivity is strongly influenced by light availability. The open sites, with higher light availability, exhibited significantly higher productivity as evidenced by faster snail growth and lipid accumulation. Hill and Knight (1988) also observed that both streams had low nitrogen levels and very low N:P ratios (nitrogen to phosphorous ratios) and nutrient co-limitation, particularly by nitrogen and phosphorus was a common occurrence for both green algae and macrophytes. The collective findings as submitted above underscore the complex interactions between nutrient availability, light conditions and ecosystem dynamics in aquatic systems. This provides insights into the potential factors driving the heterotrophic status of most aquatic systems in the Luapula River Basin.

The CPOM to FPOM index, which assesses the ratio of coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) in streams, is an important measure of organic matter composition and its availability in aquatic ecosystems. According to Cummins et al. (2005), a CPOM to FPOM ratio below 0.25 indicates a dominance of fine particulate organic matter, which typically supports gathering/filtering collectors rather than shredders. In the Luapula River Basin, most sampled rivers and streams scored below this anticipated threshold, suggesting that fine particulate organic matter is more prevalent than coarse particulate organic matter. However, during the dry season, site 11 showed a CPOM to FPOM

ratio that supported a normal shredder association, similar to the conditions observed at sites 6 and 10 during the wet season. This observation indicates that at these specific sites and times, there was a sufficient presence of coarse particulate organic matter to support a balanced shredder population. This deviation from the general trend suggests localised variations in organic matter input and availability, potentially due to differences in riparian vegetation, seasonal leaf fall or localised water flow patterns that enhance the deposition and retention of CPOM. Cummins et al. (2005) assert that the expected CPOM to FPOM index threshold typically aligns with normal shredder activity, commonly observed in functioning riparian zones. Shredders play an important role in decomposing CPOM into FPOM, serving as an energy source for downstream organisms. The consistently low index scores across most hydro-environmental zones suggest diminished shredder populations and weak connectivity with riparian function, indicating minimal contribution to organic matter supply. According to Cummins et al. (1989), the connection between riparian vegetation and stream invertebrate shredders is crucial and serves as a cornerstone in evaluations of the relationship between terrestrial and aquatic ecosystems. Shredders, as described by Cummins et al. (1989), are stream invertebrates that consume litter from streamside riparian areas that accumulates in the stream channel. Their feeding behavior is influenced by the structural and biochemical changes in plant litter tissue. Shredders do not specialise in feeding on a specific plant species, but rather on appropriately conditioned litter. Their feeding behavior is dependent on the microbial breakdown of the plant litter tissue. From an ecosystem perspective, the functional unit comprising of shredders, microbes and terrestrial litter plays a key role in converting coarse particulate organic matter (CPOM) of terrestrial origin to fine particulate organic matter (FPOM) that becomes distributed in the aquatic system. The conversion of CPOM to FPOM proceeds year-round in the stream and ensures a continual supply of FPOM to collectors (Cummins et al., 1989). Grubbs and Cummins (1996) investigated the life history and seasonal growth rates of eleven shredder species found in a Pennsylvania mountain stream in the USA. With the exception of two species, each shredder species had a univoltine life cycle, indicating a single annual reproductive cycle. *Tallaperla maria* displayed a semivoltine life cycle, whereas *Pteronarcys proteus* required four years to complete nymphal development. The study also found that shredder species such as *Paracapnia angulata* and *Taeniopteryx maura* showed autumn and/or winter growth patterns, whereas other species such as *Amphinemura nigritta*, showed spring and/or summer growth. The riparian forest surrounding the stream was characterised by a diverse range of woody plant species which impacted the leaf detritus biomass in the stream. This diversity was reflected in the varied seasonal growth patterns

observed in the shredder guild, highlighting the complex relationship between riparian vegetation and shredder population dynamics. In investigating the dynamics of energy sources in four open-canopy streams in Sweden, Leberfinger et al. (2011) examined the dietary preferences of invertebrate shredders using stable isotope analysis in both open sites and those bordered by deciduous trees in the riparian zone. The findings revealed that allochthonous coarse particulate organic matter remained the primary energy source for shredders in both open and wooded sites, suggesting that terrestrial subsidies may play a crucial role in open-canopy stream ecosystems akin to their influence in forested streams. Leberfinger et al. (2011) also observed a higher proportion of biofilm in the diet of shredders at open sites compared to wooded sites, indicating their ability to adapt their diet based on food availability. Wootton et al. (2019) conducted a study on the seasonal dynamics of leaf litter and shredder populations in a tropical stream located in an Australian rainforest ecosystem. The study revealed that shredders are resilient to moderate flooding and they can effectively process green leaves while persisting throughout different seasons. This was observed to contribute to the consistency of detrital-based food webs in the stream. However, the study also found that a one-in-one-year flood event caused a significant reduction in litter packs and altered the composition towards greener leaves. This disturbance led to a decline in shredder abundances. These findings highlight the importance of considering temporal changes in litter composition and decomposition while analysing trophic processes in stream ecosystems.

The ratio of Transport-fine particulate organic matter (TFPOM) to Benthic-fine particulate organic matter (BFPOM) provides insights into the dynamics of FPOM within stream and river ecosystems. This ratio helps to understand the balance between FPOM actively moving within the water column and its accumulation in sediment deposits. High ratios indicate a dominance of suspended FPOM, suggesting an increased availability of organic matter for filter-feeding organisms, whereas low ratios suggest more deposition and storage of FPOM in sediments, which benefits gathering collectors. During the dry and wet seasons, the reduced availability of suspended FPOM across all sites with exception to sites 6, 5 and 10 in the dry season and sites 6, 11 and 12 in the wet season indicates potential limitations for filtering collectors. This reduction in suspended FPOM suggests that the particulate organic matter is settling into the sediments rather than remaining available in the water column. This limitation in suspended FPOM can potentially affect the success of filter-feeding organisms that rely on suspended FPOM for nourishment. On the other hand, the increased deposition of FPOM in sediments can benefit gathering collectors, which feed on this deposited organic matter. The overall

reduction in suspended FPOM and increase in deposited FPOM highlights the observed dominance of gathering collectors across the hydro-environmental zones of the Luapula River Basin. Hutchens et al. (2017) submit that suspended FPOM is readily consumed by filter feeders and serves as a significant pathway for the export of organic matter downstream. A process that facilitates the transfer of energy and nutrients within the aquatic food web. Furthermore, Hutchens et al. (2017) assert that FPOM that settles on the stream bottom exhibits considerable variability across different stream habitats. This variation is attributed to differences in flow dynamics, substrate composition and the input of organic material from riparian vegetation. The variation in FPOM deposition among stream habitats underscores the importance of habitat heterogeneity in supporting diverse assemblages of gathering collectors and by extension maintaining ecosystem functioning within stream ecosystems.

Jin and Ward (2013) evaluated the FPOM retention patterns in Payne Creek, a 2nd order Coastal Plain stream in the USA, under naturally varying hydraulic conditions. The findings revealed that seasonal changes in hydrology and segment-scale variation in channel morphology were the main factors controlling FPOM transport and retention in Payne Creek. This means that shifts in the volume and speed of water flow throughout the year, as well as alterations in the shape and structure of the stream channel, play significant roles in determining how FPOM is transported downstream and how much of it is retained within the stream system. A seasonal influence on suspended FPOM availability was also observed particularly at sites 6, 11 and 12 in the Luapula River Basin. This observation highlights the importance of considering seasonal variations in FPOM dynamics for understanding the functioning of stream and river ecosystems in the Luapula River Basin.

The channel stability ratio, which measures the availability of stable surfaces such as bedrock, boulders, cobbles and large woody debris, shows how conducive the environment is for scrapers and filtering-collectors. A higher ratio indicates more stable surfaces, supporting these groups. This ratio, as referenced in Appendix 3, confirms that the fine particulate organic matter (FPOM) found in the stream originates from natural riparian and in-stream processes. This natural derivation suggests that the FPOM is not significantly influenced by land use impacts, such as agricultural runoff or urban development, which can disrupt natural sediment and organic matter dynamics. Therefore, the channel stability ratio not only highlights the physical stability of the streambed but also provides insights into the ecological health and natural functioning of the riparian ecosystem. In the Luapula River Basin, scores from different locations in the hydro-environmental zones show how important seasonality is in determining

the quality of habitats. In Zones 1 and 3, sites became more stable moving from the dry season into the wet season, showing that habitat conditions improved with higher water flow. However, in Zone 2, the shift from dry to wet season had a different effect, indicating that increased water flow during the wet season could actually lower substrate stability in those areas. Despite the observed lack of statistical significant difference in substrate stability ratios across the zones and seasons, the observed site-specific variations highlight the prevailing relationship between seasonal changes and habitat stability in the basin. Peng et al. (2024) also confirms the influence of seasonality on habitat stability in Yangmei River of the Guangdong Province of Southern China. According to Peng et al. (2024), results for the Yangmei River highlight seasonal and spatial variations in the stability of the river's habitat. Specifically, during the winter and spring seasons, the habitat stability was poor. This seasonal instability was attributed to a variety of factors such as increased runoff, higher water flow rates and sediment movement during these times, which disrupt the riverbed and banks, making it harder for stable habitats to form and persist. Peng et al. (2024) also confirmed variations along the longitudinal gradient of the Yangmei River, with a few sites demonstrating good habitat stability.

The predator-prey relationships in the Luapula River Basin's hydro-environmental zones reflect the influence of seasons and prey reproductive cycles on these interactions. The predominance of prey with short life cycles and rapid turnovers in many areas supports higher predator densities, as evidenced by the high top-down ratios observed during both seasons. However, the presence of balanced sites within each zone underscores the importance of prey reproductive diversity in maintaining sustainable predator-prey interactions within aquatic ecosystems. During the dry season, Zone 3 exhibited a high concentration of predators compared to the number of prey, indicating the presence of many prey taxa with short life cycles. This constant prey turnover supports larger predator populations by providing a steady food source. Similarly, Zones 2 and 1 generally presented the same trend, with the exception of sites 6 and 10, where the ratios suggest a balanced ecosystem characterised by prey reproductive diversity, with some prey having short life cycles and others having long ones. This balance ensures stability in predator-prey interactions. In contrast, the wet season revealed more variability in predator-prey relationships across the different zones. In Zone 1, top-down ratios indicated a dominance of prey taxa with short life cycles and rapid turnovers, supporting higher predator populations with a continuous food supply. Conversely, Zones 2 and 3 exhibited fluctuating top-down ratios, reflecting a mix of ecological dynamics. Sites 1, 5, 6 and

12 recorded top-down ratios indicative of polyvoltine prey populations, which reproduce multiple times a year, ensuring rapid and continuous prey base renewal and sustaining higher predator densities. However, sites 2, 3 and 7 showed more balanced predator-prey dynamics, characterised by a mix of prey taxa with varying life cycles. This balance prevents over-predation and maintains ecosystem stability. Notably, site 4 in Zone 2 recorded a top-down ratio below the threshold of 0.1 – 0.2, suggesting reduced predation pressure. This lower ratio indicates a prevalence of prey taxa with longer life cycles, which do not reproduce as rapidly and are less likely to support high predator densities. Overall, the wet season's variability emphasises the dynamic nature of predator-prey interactions, influenced by the life cycles and reproductive strategies of prey taxa within each zone. In justifying the influence of prey availability on predator distribution, Ceron et al. (2022) looked at how interactions between predators (anurans) and prey (arthropods) vary over space and time. The study focused on how the availability of prey influences these interactions. The findings showed notable differences in how these predator-prey networks are structured across different regions and seasons, mainly because of changes in interactions. The findings further showed that the differences in prey availability played a critical role in shaping the structures of the predator-prey networks, both in different locations and at different times. This emphasises the significance of prey availability in understanding how these predator-prey interactions evolve and the overall network structures. In an effort to validate the hypothesis that water dynamics play a crucial role in predator-prey relationships in aquatic ecosystems, Moussaoui et al. (2018) examined the impact of water level variations on the interactions between prey and predator fish in an artificial lake using a seasonally varying predator-prey model. It was found that increases in water volume hinder the capture of prey by predators, while decreases in water volume facilitate prey capture. The model showed that these changes in water level influenced the rate at which predators hunted, resulting in a system that switched between different states. The study confirmed that the water level variations play a significant role in shaping how fish interact in this ecosystem. Similarly, although the observed variations in top-down ratios across the zones and seasons were not statistically significant in the Luapula River Basin, seasonal influences on predator-prey relationships were quite notable, especially in the dry season where predator abundance was quite high and supported by polyvoltine prey populations. In the Białowieża Primeval Forest in North-East Poland, to see how shifts in species composition influence the survival of predator-prey communities, Saavedra et al. (2016) investigated how seasonal variations in species interactions, especially in predator diets, affect community resilience. As was observed in the Luapula River Basin, Saavedra et al. (2016) found that the

turnover of species has a significant impact on community survival between summer and winter. Importantly, the study showed that changes in species interactions throughout the seasons help maintain a consistent level of community survival despite the turnover of species. It is also important to note that despite the observed influence of prey turnover events on predator distribution in the Luapula River Basin, analysis of spatial-temporal variation did not present statistically significant difference in top-down ratios across zones and seasons. According to Saavedra et al. (2016), the results from Białowieża Primeval Forest suggest that there is a self-regulating mechanism that governs seasonal species interactions and emphasise the importance of monitoring these changes as signs of how species are responding to environmental challenges.

5.6 Assessment of Water Quality

The physicochemical and microbial analysis results for water samples collected from across the 12 sampling sites are tabulated in Appendix 15 and 16. The descriptive statistics included measures of mean, standard deviation, standard error, variance and median to gain insights into the central tendency and variability of the water quality parameters across the dry and wet seasons (Appendices 16 and 17). During the dry and wet seasons nitrates, phosphates and sulphates were all found to be below the detection limit (< 0.01 mg/l). During the wet season total hardness, calcium and magnesium were also found to be below the detection limit (< 0.1 mg/l). Compared to the recommended scores of water quality standards for the Luapula River Basin as specified in the Ambient Water Quality Specifications of the Zambia Bureau of Standards (ZABS). The mean values of the measured parameters were all generally lower than the ZABS recommended catchment scores, except for faecal coliforms and temperature in the wet season (Appendix 18). During the wet season the mean faecal coliform value (57.08 /100ml) was higher than the ZABS recommended ambient catchment value of 50 /100ml and the mean catchment value of 19/100ml. Similarly, during the wet season, the mean temperature score (24.04 °C) exceeded all of the prescribed ZABS specifications, including the catchment mean value (23.2 °C), catchment highest value (23.7 °C) and catchment recommended value (23.5 °C). It is important to note that the ZABS ambient water quality specifications do not include prescribed standards for potassium.

5.6.1 Spatial-Temporal Variation of Water Quality Parameters

A Multivariate Analysis of Variance (MANOVA) was performed to investigate the effects of two independent variables, namely zone and season, as well as their interaction on water quality parameters (Table 17). The results indicate that the impact of zone on the dependent variables

is not statistically significant (p-value > 0.05). In contrast, the variable season had a statistically significant effect on the dependent variables (p-value < 0.001). The interaction between zone and season did not show any significant statistical effect (p-value > 0.05). The analysis shows that season has a statistically significant impact on water quality variables while the effects associated with zone and the interaction between zone and season are not statistically significant.

Table 17: MANOVA output showing statistical variations in water quality parameters within a spatial-temporal context in the Luapula River Basin, Zambia. Statistical Significance Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Degree of freedom	Pillai's trace	Approximate F-Statistic	Numerator Degree of freedom	Denominator Degree of freedom	Pr (>F)
Zone	1	0.57442	1.0123	12	9	0.5044
Season	1	0.97057	24.7311	12	9	0.00002057***
Zone:Season	1	0.62672	1.2592	12	9	0.3713
Residuals	20					

Further analysis was conducted through ANOVA testing to determine which water quality variables drove the observed seasonal variations, as indicated by the MANOVA output. The ANOVA findings, detailed in Appendix 19, highlight the statistical influence of independent variables on various water quality parameters. The results of the ANOVA analysis showed that the season variable had a statistically significant influence on some of the water parameters, including turbidity, total hardness, calcium, potassium, iron, chloride and faecal coliforms. However, parameters like electrical conductivity, pH and total dissolved solids had p-values above 0.05, indicating that the changes in season did not affect them significantly.

5.6.2 Water Quality Index

Numerical expressions of water quality status for all 12 sampling sites were calculated using the weighted arithmetic water quality index method (Table 18). During the dry season, sites 1, 2 and 3 in hydro-environmental zone 3 exhibited poor to unsuitable water quality index scores for drinking water. Among them, sites 2 and 3 recorded the most degraded scores of 128 and 111, respectively. Site 12 was an exception, with a water iron concentration below the recommended ZABS Ambient Water Quality Specifications score of 0.7 mg/l. However, site 12 recorded the highest faecal coliform count of 70 /100 ml, exceeding the recommended score of 50 /100 ml.

Table 18: Seasonal water quality index scores across the sampling sites of the Luapula River Basin, Zambia.

Zone	Site	WQI	
		Dry Season	Wet Season
3	1	75	93
	2	143	6
	3	124	16
	12	25	4
2	4	45	11
	5	18	3
	6	36	128
	7	32	64
1	8	71	102
	9	40	61
	10	21	58
	11	19	21

In the wet season, there was a notable improvement in the overall water quality index scores at all sampling sites in hydro-environmental zone 3, except for site 1. While the other three sites achieved excellent classification, site 1's score increased from 75 in the dry season to 93 in the wet season. This increase was primarily due to a rise in iron concentration from 0.58 mg/l to 0.71 mg/l. In hydro-environmental zone 2, water quality index scores ranged from good to excellent during the dry season and from excellent to unsuitable for drinking during the wet season. Sites 4 and 5 showed an improvement in water quality from the dry to the wet season, while sites 6 and 7 experienced notable deterioration. Site 7's water quality declined from good in the dry season to poor during the wet season, whereas site 6 changed from good in the dry season to unsuitable for drinking in the wet season. The rise in water quality index scores during the wet season was attributed to increased iron concentration and turbidity in both sites, while site 5 maintained an excellent score throughout both seasons. In hydro-environmental zone 1, site 10 scored excellent water quality index ratings during the dry season but declined to poor ratings during the wet season, whereas site 11 was cponsistently in the excellent category across both season. Site 9 changed from good in the dry season to poor water quality in the dry wet season, and site 8 deteriorated from poor in the dry season to unsuitable for drinking during the wet season.

A visualisation of water quality index scores during the dry and wet seasons revealed a pattern where in the dry season, relatively higher scores were observed in sampling sites within hydro-environmental zone 3, with a noticeable transition from site 5 to site 11. Conversely, during the wet season, relatively higher scores are observed in sites 6 and 7 in hydro-environmental

zone 2 and all sites in hydro-environmental zone 1 (Figure 15). This trend suggests that water quality in hydro-environmental zone 3 experiences relative improvement in the wet season compared to the dry season, while water quality in zones 2 and 1 deteriorates relatively during the wet season compared to the dry season. It was further observed that sampling sites in hydro-environmental zone 3 showed higher iron water concentration in the dry season, whereas sampling sites in zones 2 and 1 experience higher iron water concentration in the wet season.

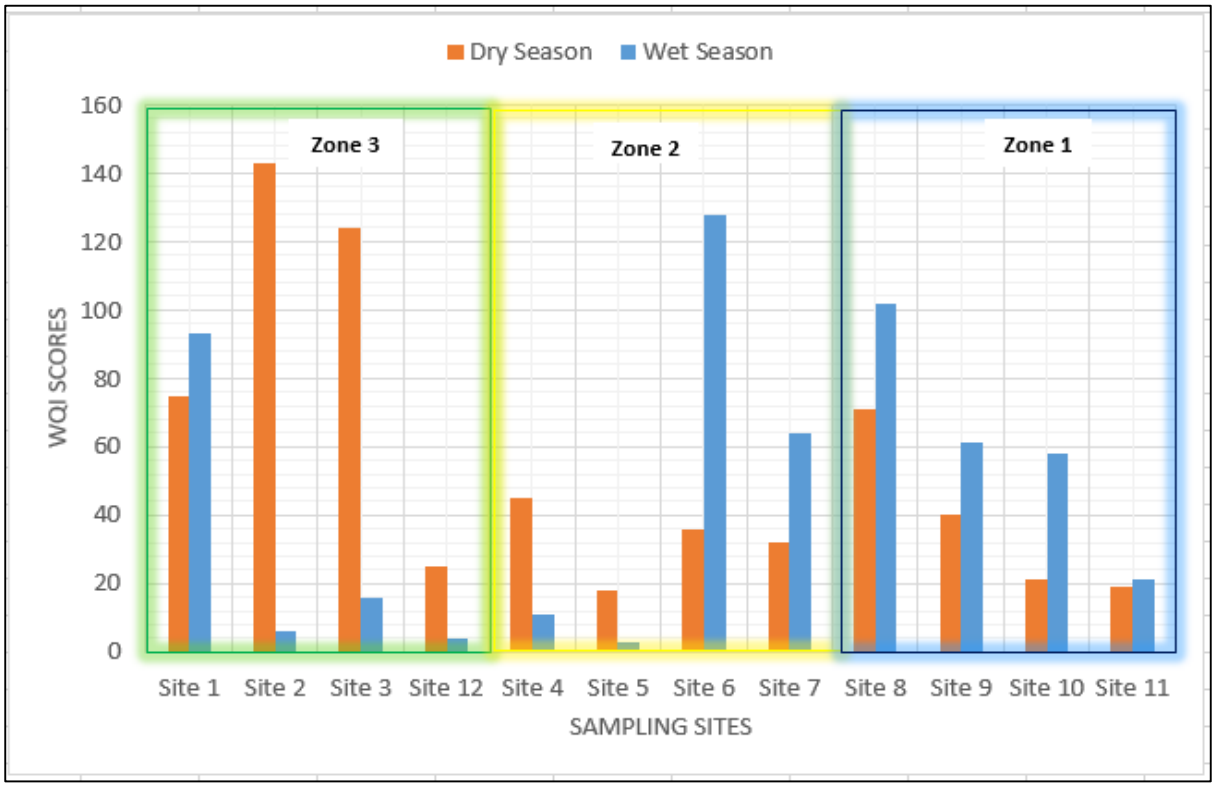


Figure 15: The bar graph shows seasonal water quality index (WQI) scores across the sampling sites of the Luapula River Basin, Zambia.

5.6.3 Water Quality and Macroinvertebrate Interactions

Canonical Correspondence Analysis (CCA) allowed for the visualisation of the relationships between macroinvertebrate taxa distributions and environmental gradients. The generation of triplots enabled the visual interpretation of how different macroinvertebrate families respond to various water quality parameters and altitude in the Luapula River Basin. The CCA analysis also provides an insight in which axes account the for the highest variation of macroinvertbrate structure across the basin (Appendix 21).

During the dry season (Figure 16), the CCA triplot analysis indicated that water quality parameters and altitude along Axis 1 and Axis 2 accounted for 42.75% of the total variation in macroinvertebrate families (Appendix 22). These two axes explained the highest variation

across the sampled sites in the Luapula River Basin. In Quadrant I (positive Axis 1, positive Axis 2), the interaction of altitude and iron with macroinvertebrate families such as *Trichorythidae, Aeshnidae, Naucoridae* and *Hydropsychidae* was positive. This suggests that the distribution and abundance of these families were associated with aquatic habitats at high altitudes with high iron concentrations. In Quadrant II (negative Axis 1, positive Axis 2), the triplot indicated that water quality variables including turbidity, potassium (K^+), chloride (Cl^-), sodium (Na^+) and fecal coliforms influenced the distribution and abundance of macroinvertebrates such as *Gomphidae, Atyidae, Coenagrionidae* and *Calopterygidae*. Turbidity, potassium, sodium and chloride showed a positive influence on the families *Gomphidae, Atyidae* and *Coenagrionidae*, while *Calopterygidae* was more positively influenced by potassium, chloride and sodium than by turbidity. Fecal coliforms showed a negative interaction with these macroinvertebrate families along the Axis 1. This observation suggests that the macroinvertebrates in Quadrant II were associated with aquatic habitats presenting elevated levels of turbidity, sodium, potassium and chloride with reduced levels of fecal coliforms. Additionally, Quadrant II captured the presence of the families *Hirudinae* and *Culicidae,* though their positions on the triplot suggest that their distribution was not solely influenced by the environmental variables associated with this quadrant. In Quadrant III (negative Axis 1, negative Axis 2), the triplot showed negative interactions between the water quality variables TDS (total dissolved solids), EC (electrical conductivity) and calcium (Ca^{2+}) with macroinvertebrate families such as *Unionidae, Belostomatidae, Nepidae, Thiaridae, Ephemeridae* and *Polymitarcyidae*. This output implies that an increase in the concentrations of these water quality variables negatively affects the distribution and abundance of associated macroinvertebrate families. In Quadrant IV (positive Axis 1, negative Axis 2), the distribution and abundance of macroinvertebrate families such as *Ampullariidae, Potamonautidae, Libellulidae* and *Baetidae* were positively influenced by increasing pH and temperature and negatively influenced by increasing magnesium and total hardness levels.

During the wet season (Figure 17), the CCA triplot analysis indicated that water quality parameters and altitude along Axis 1 and Axis 2 accounted for 43.03% of the total variation in macroinvertebrate distribution in the Luapula River Basin (Appendix 22). These two axes explained the highest variation across the sampled sites in the Luapula River Basin during this season. In Quadrant I (positive Axis 1, positive Axis 2), the relationship between faecal coliforms with macroinvertebrate families such as *Nepidae, Ampularidae, Gerridae, Haliplidae* and *Calopterygidae* was positive. This suggests that the distribution and abundance

of these families were associated with aquatic habitats experiencing high faecal contamination. In Quadrant II (negative Axis 1, positive Axis 2), altitude and water quality variables including turbidity, pH, TDS and EC influenced the distribution and abundance of macroinvertebrates such as *Tabanidae, Aeshnidae, Gyrinidae, Hydropsychidae, Coenagrionidae, Libellulidae* and *Baetidae*. Altitude and pH had a positive influence on these families, while EC, TDS and turbidity had a negative influence along the Axis 1. This indicates that the macroinvertebrates in Quadrant II were associated with high-altitude aquatic habitats with enhanced pH levels and lower levels of EC, TDS and turbidity. In Quadrant III (negative Axis 1, negative Axis 2), there were negative relationships between water quality parameters such as potassium (K^+) and iron (Fe^{2+}) with macroinvertebrate families such as *Dytiscidae, Atyidae, Potamonautidae, Thiaridae* and *Unionidae*. This suggests that increasing concentrations of potassium and iron negatively affect the distribution and abundance of these macroinvertebrate families. Although the family Hydrophilidae was associated with Quadrant III, its position indicates that its distribution is not solely limited by potassium and iron concentrations. In Quadrant IV (positive Axis 1, negative Axis 2), the distribution and abundance of macroinvertebrate families such as Belostomatidae, Gomphidae, Naucoridae, Corixidae and Oligoneuriidae were negatively influenced by increasing temperature and chloride (Cl^-) concentration.

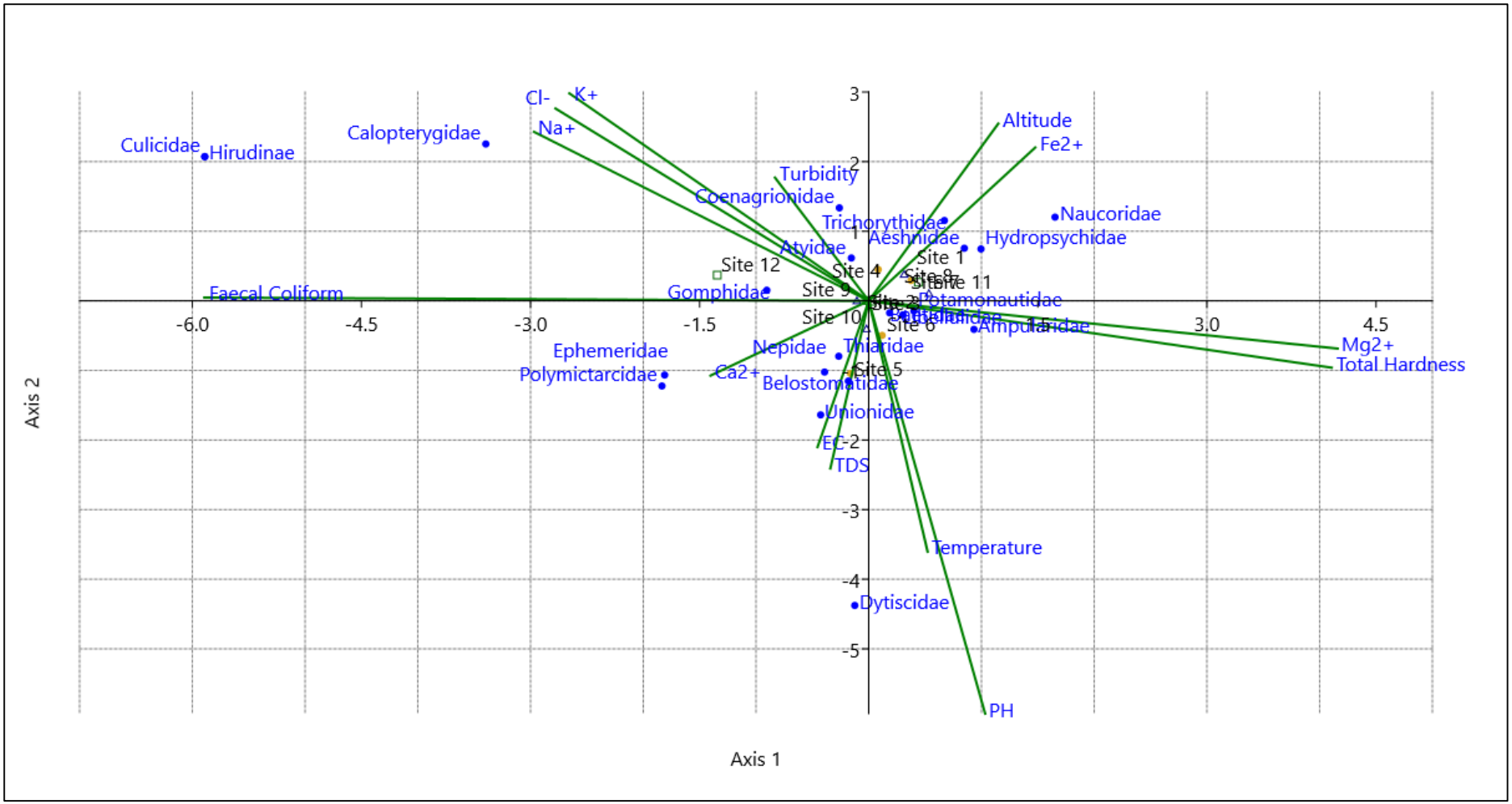


Figure 16: CCA triplot showing the environmental gradients shaping up macroinvertebrate community structure during the dry season in the Luapula River Basin, Zambia.

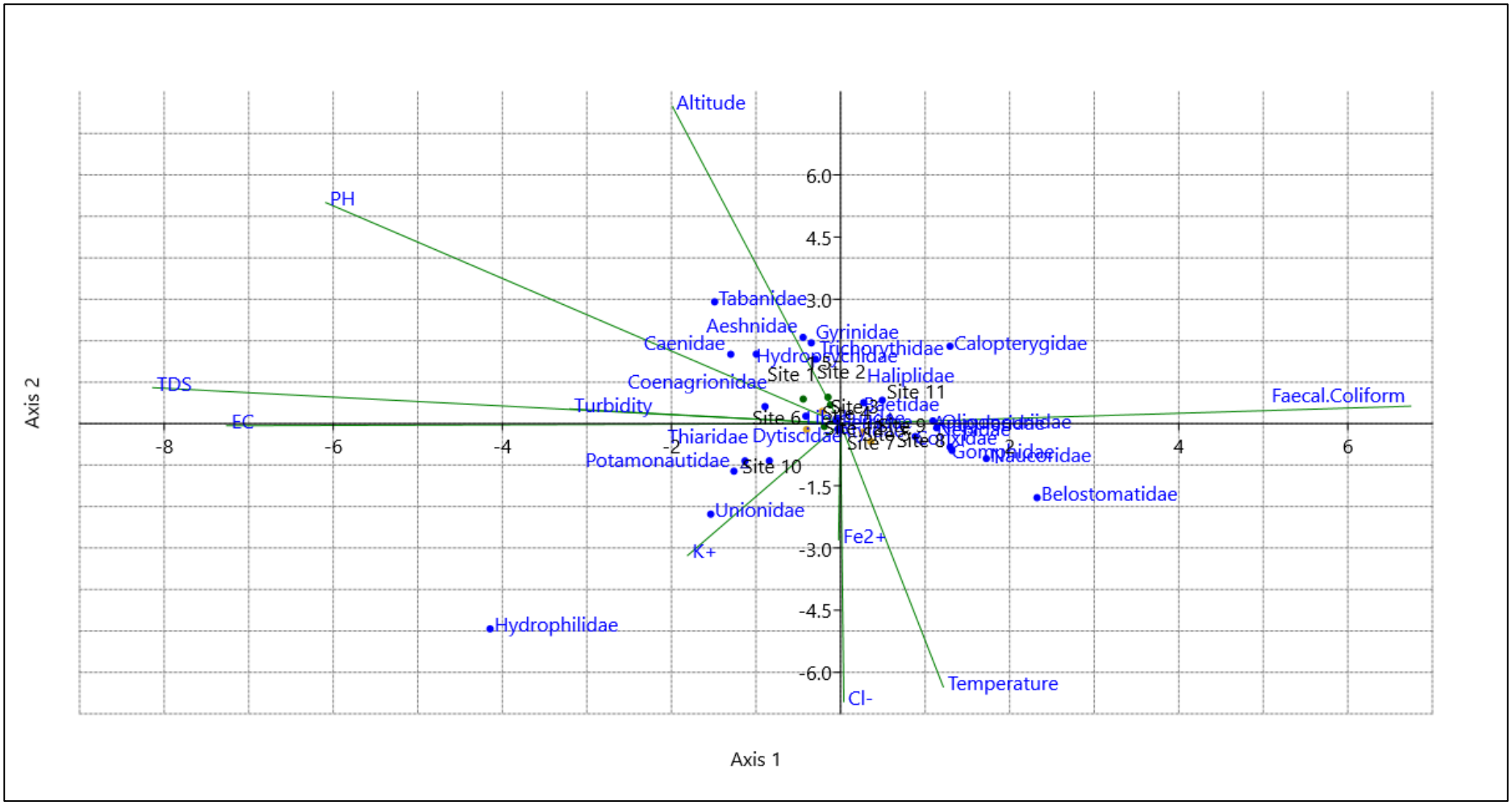


Figure 17: CCA triplot showing the environmental gradients shaping up macroinvertebrate community structure during the wet season in the Luapula River Basin, Zambia.

5.6.4 Interpretation and Discussion

Understanding the intricate relationship between water quality parameters and macroinvertebrate communities is important because the physicochemical and microbial characteristics of water have an influence on the composition and abundance of macroinvertebrate communities in the ecosystems they inhabit. The integration of knowledge on macroinvertebrate assemblage with water quality monitoring allows for a comprehensive understanding of ecosystem health and functionality (Metcalfe, 1989).

The measures of central tendency revealed that the Luapula River Basin undergoes site-specific fluctuations in water quality parameters from the dry to wet season. During the dry season, electrical conductivity, total dissolved solids, chloride, turbidity and potassium concentrations were comparatively higher, potentially due to reduced water levels and the concentration of minerals. In contrast, the wet season was characterised by a decline in the concentration of most water quality parameters, with below detection limit scores of water quality parameters such as total hardness, calcium and magnesium. The pH levels of the water also exhibited a slight shift from slight acidity to slight alkalinity from the dry to wet season, respectively. Additionally, the mean water temperature was observed to be cooler during the dry season compared to the wet season. Notably, the iron levels remained relatively constant throughout the two seasons, indicating that the iron content of the water may not be significantly affected by seasonal changes. The consistently below detection concentrations of nitrates, phosphates and sulphates across all the sampling sites of the Luapula River Basin indicate minimal agricultural runoff, waste discharge and industrial discharge within the Luapula River Basin. The wet season was also characterised by an increase in faecal coliforms especially in zone 1 and zone 3. In Zone 3, this was attributed to the prevalence of fishing camps and wildlife in the Bwangweulu-Luapula systems, whereas Zone 1 was characterised by the prevalence of fishing camps and peripheral human habitations, which are without proper sanitation facilities in the Luapula-Mweru systems. The flooding regime of the Luapula River Basin as experienced in the wet season allows for the transfer of human and animal waste from such peripheral establishments into the main waterways of the Luapula River Basin. Hence the observed increase in faecal coliforms across zone 1 and zone 3 in the wet season. Aliyu et al. (2020) reported similar observations of elevated levels of water quality parameters during the dry season compared to the wet season in River Galma in the city of Zaria, North-western Nigeria. Aliyu et al. (2020) attributed the observed dry season elevations in water quality parameters to evaporation and lack of rainfall. The observed decline in the concentration of water quality

parameters in the Luapula River Basin can be attributed to the findings of Zhao et al. (2018) in the Mun River Basin in Thailand. Zhao et al. (2018) observed that the variations in precipitation patterns between the two seasons had an influence on the spatial distribution of water quality due to the dilution effect of rainfall on pollutant concentrations and water flow. Additionally, Zhao et al. (2018) reported that during the dry season, the quality of water was better compared to the rainy season, especially in the upper regions as compared to the lower areas. In the Bukavu urban rivers of Lake Kivu Basin in the Eastern Democratic Republic of Congo, Bisimwa et al. (2022) found concentrations of phosphates, ammonia, nitrites and nitrates gradually increased from upstream to downstream, indicating heavy pollution of river waters. Nutrient concentrations were highest during the dry season across all rivers, except for specific stations along the Kahuwa and Nyamuhinga Rivers. Bisimwa et al. (2022) further recommended the urgent need for improved liquid and solid waste management strategies, implementation of efficient wastewater treatment plants and development of sewage systems in catchment areas to mitigate cumulative pollution of rivers and lakes. However, the Luapula River Basin's surface water was found to consistently exhibit below-detection concentrations of nutrients in both dry and wet seasons compared to the situation in Lake Kivu Basin. This observation could be attributed to effective natural filtration processes or limited anthropogenic activities impacting water quality in the area. Such findings emphasise the importance of comprehensive biogeophysicochemical water quality assessments in order to clearly understand the dynamics of environmental characteristics of the Luapula River Basin. Aliyev et al., (2023) assert that significant temporal fluctuations in environmental and biological parameters within aquatic systems can be regulated by natural processes, anthropogenic activities or a synergistic interplay of both. In the Serengeti National Park in Tanzania, Strauch (2011) investigated the impact of ecological factors, including seasonal animal activities, rainfall and physiochemical water quality, on the abundance of total coliform (TC) and Escherichia coli faecal bacteria in five rivers. In conformance to the observed influence of seasonality on faecal coliforms in the Luapula River Basin, the findings in the Serengeti National Park revealed significant variation in faecal bacteria levels across different rivers and seasons. In the southern rivers, faecal bacteria levels increased substantially during the wet season compared to the dry season. In contrast, the northern rivers showed few seasonal differences, indicating that large populations of resident wildlife in the north mitigate the seasonal effects of migratory wildlife. These results highlight that wildlife in protected regions can significantly contribute to faecal bacteria contamination in surface waters, posing a considerable threat to human health as the demand for water resources grows.

Spatial-temporal analysis of variance revealed that variations in total hardness, calcium, magnesium, turbidity, chloride, potassium and faecal coliforms measured in the Luapula River Basin were primarily influenced by seasonal changes rather than changes in hydro-environmental zones. In contrast, parameters such as electrical conductivity, pH, temperature, total dissolved solids and iron did not exhibit statistically significant variations concerning the factors of season or zone. These findings are key as they contribute to baseline data on water quality in an understudied catchment and highlight the possible impact of seasonal changes, spatial orientation and human activities on the catchment's water quality. These findings agree with the findings of Aliyev et al., (2023) in the study of spatial and temporal variations in the environmental characteristics of the Turyanchay River in Azerbaijan. This study revealed no significant spatial differences in nutrient (nitrate, ammonium, phosphate) and metal/metalloid (Pb, Cu, Ni, Zn, Mn, Cr, Cd, Fe, Al) contamination levels, as well as chemical (COD and BOD) and biological oxygen demand. However, temporal fluctuations were observed, with higher levels recorded during the warmer months (July and August) and lower levels during the colder months (March and November). In Bua River in Malawi, Balaka and Chagoma (2022) investigated the water quality of the Bua River in Malawi to understand the spatial-temporal variations in water quality across five sites from May 2018 to June 2020, covering three main seasons: warm wet; cool dry; and hot dry. In line with the observed elevation in concentrations of physicochemical water parameters in the Luapula Basin, Balaka and Chagoma (2022) also revealed that while water quality parameters did not show significant spatial variation, temporal differences were significant for some parameters associated with a general elevation in the concentration of considered parameters during the dry season.

To simplify the evaluation process of water quality assessment, the Water Quality Index (WQI) was considered for purposes of calculating an aggregate numerical value that allows for easier interpretation and comparison of water quality across different sites and periods across the hydro-environmental zones in the Luapula River Basin. According to the water quality index, the water quality in Zone 3 ranged from excellent to unsuitable for drinking in the dry and from excellent to very poor in the wet. In Zone 2, the water quality showed a transition concerning seasonal changes. During the dry season, the water quality ranged from excellent to good, whereas in the wet season, there was a deterioration in water quality, with the index scores ranging from excellent to unsuitable for drinking. Zone 1 was characterised by a relative decline in water quality, particularly in the wet season. This was evidenced by the change in the water quality index scores from excellent to poor in the dry season to excellent to very poor

in the wet season. The observed seasonal fluctuations in water quality across the hydro-environmental zones were mainly driven by the presence of elevated iron concentration and turbidity, which were mostly found to be above the recommended ZABS Ambient Water Standards for the Luapula River Basin. Considering the consistent influence of iron on water quality upsets across the three zones, the elevated iron levels were suspected to leach into the streams and rivers from the surrounding geological formations or possibly from industrial discharge, especially from the ongoing manganese mining within the catchment. Accordingly, the elevated levels of turbidity were suspected to be a consequence of the elevated iron presence in the water, increased runoff especially in the wet season and domestic utilisation of streams and rivers by local communities. In the wet, especially in zones 1 and 3, faecal coliforms were also observed to present a notable contribution to upsets in water quality. In Zone 3, this observation was attributed to the establishments of fishing camps and fish trading centers on the peripherals of Lake Bwangweulu and the main Luapula channel. Additionally, Zone 3 is also home to the expanse of the Bangweulu Wetlands, which hosts a diversity of antelopes such as black lechwe, sitatunga, southern reedbuck, tsessebe and oribi. In Zone 1, the proliferation of faecal coliforms is mainly attributed to increased human establishments without proper sanitation facilities along the main river channels within the zone. The observed relative decline in water quality, especially in Zone 1 and sites 6 and 7 in Zone 2 in the wet season is in line with the findings of Opiyo et al. (2022) in the Migori River in Kenya. Opiyo et al. (2022) reveal significant seasonal variability in water quality parameters but no significant spatial differences along the Migori River. The study further revealed that, in the wet season, upstream areas exhibited better water quality compared to downstream locations. The study attributed the observed variations in water quality to both point and non-point pollution sources.

In section 5.6.3, Canonical Correspondence Analysis (CCA) revealed that the distribution of macroinvertebrate families in the Luapula River Basin during the dry season in was influenced by various environmental factors. Factors such as altitude, iron, sodium, chloride, potassium, turbidity, magnesium and total hardness were found to be positively associated with the distribution of different macroinvertebrate families. High altitude habitats with elevated iron concentrations were found to support families such as *Trichorythidae*, *Aeshnidae*, *Naucoridae*, and *Hydropsychidae*. Additionally, environments with high turbidity and elevated levels of sodium, potassium and chloride were favorable for families such as *Gomphidae*, *Atyidae*, and *Coenagrionidae*. During the wet season, the CCA analysis revealed that altitude, faecal coliform levels and pH significantly influenced the structure and distribution of

macroinvertebrate taxa. Macroinvertebrate families such as *Nepidae*, *Ampularidae*, *Gerridae*, *Haliplidae* and *Calopterygidae* were found to be more prevalent in areas with high faecal contamination. On the other hand, high altitude habitats with increased pH levels and lower levels of electrical conductivity (EC), total dissolved solids (TDS) and turbidity were conducive to families like *Tabanidae*, *Aeshnidae*, *Gyrinidae*, *Hydropsychidae*, *Coenagrionidae*, *Libellulidae* and *Baetidae*. The structural composition of macroinvertebrates in the Luapula River Basin is influenced by an intricate interplay of natural processes and human-induced disturbances. As was observed in the Luapula River Basin, Kemp et al. (2014) also confirmed the influence of ecoregions separated on the basis of altitude on the distribution of macroinvertebrate taxa in the Olifants River Catchment in South Africa. Hoang et al. (2010) confirmed that altitude significantly correlated with the taxonomic richness of both insect and non-insect macroinvertebrate communities in the Du River Basin in Northern Vietnam. Hoang et al. (2010) observed that sites above 250 meters were distinct in their community composition, with sensitive taxa found exclusively at these higher altitudes. Kemp et al. (2014) also found that water temperature and ecoregion were the most important factors influencing the distribution of macroinvertebrate families. Warmer temperatures were associated with the Lowveld-Lower region, whereas the Highveld-Upper region were associated with cooler temperatures. In the Luapula River Basin, an increase in temperature was observed to present a limiting effect on the distribution of macroinvertebrate taxa in both the dry and wet seasons. Banda et al. (2023) also found that temperature, pH and electrical conductivity were strongly associated with the diversity of macroinvertebrate taxa in the Barotse Floodplains of Western Zambia, with temperature presenting the strongest influence. In the Luapula River Basin, electrical conductivity was found to present a limiting effect in the distribution of macroinvertebrate taxa in both the dry and wet seasons. pH on the other hand presented a limiting effect in the dry season and a positive association with the the distribution of microinvertebrate taxa in the wet season.

5.7 IWRM and Ecological Considerations

Together, biomonitoring and water quality assessment provide a comprehensive understanding of the state of aquatic ecosystems. They help identify trends, track changes over time and assess the impacts of human activities and natural events on water resources. According to Karr (2006), the twentieth century was largely characterised by a lack of awareness of the collapse of aquatic ecosystems because water was merely viewed as a fluid to be consumed or used as a raw material in agriculture or industry. When monitoring was attempted, it focused on the

presence of chemical contaminants rather than the character of the aquatic biota. Direct biological monitoring and assessment have gained substantial ground with time as an antidote to this lack of awareness. These methods provide a mechanism to directly assess the condition of water bodies, diagnose the causes of degradation, define actions to attain conservation and restoration goals and evaluate the effectiveness of management decisions (Karr, 2006). These insights are essential for making informed decisions in IWRM, which aims to balance the water needs of people, the economy and the environment. Over time, the biotic approach which combines features of saprobic and diversity approaches has emerged as the most favoured method of biological water quality assessment using macroinvertebrates in Europe (Metcalfe, 1989). This approach has evolved from earlier systems like the Trent Biotic Index to more refined methods such as the Biological Monitoring Working Party Score System and the Belgian Biotic Index Method. These methods are now widely used in Europe and are influencing policy decisions related to surface water management. Metcalfe (1989) further adds that macroinvertebrate community assessments are being utilised for various purposes, including water resource management, ambient monitoring and evaluating the effectiveness of pollution control measures. In Africa, macroinvertebrate rapid bioassessment protocols such as the Zambian Invertebrate Scoring System (Dallas et al., 2018), South African Scoring System (Dickens and Graham, 2002), Tanzanian River Scoring System (Kaaya, Day and Dallas, 2015) and Namibian Scoring System (Palmer and Taylor, 2004) which are adapted from earlier systems like the Trent Biotic Index and refined methods such as the Biological Monitoring Working Party Score System and the Belgian Biotic Index Method have become an integral consideration in water management strategies in Southern Africa. These bioassessment protocols utilise macroinvertebrate community assessments to provide rapid, cost-effective and reliable evaluations of water quality and ecosystem health (Barbour et al., 1999). Each system is tailored to the specific ecological and hydrological conditions of its respective region, ensuring accurate and relevant data collection. The continued advancements in sampling methodologies of these bioassessment protocols contribute to improved assessments of water quality, ecosystem health and conservation efforts in freshwater ecosystems (Orozco González, 2023).

This study exploited the taxonomic and functional aspects of macroinvertebrate communities and water quality parameters to understand the ecological health of the Luapula River Basin. These methods have shown that in terms of taxonomic macroinvertebrate community structure, the Luapula River Basin is dominated by taxa that are moderately tolerant and highly tolerant

to environmental disturbances. The dominance of moderately and highly tolerant taxa indicates the resilience of the ecosystem to environmental disturbances but also suggests potential impacts on biodiversity and ecosystem functioning (Burdon et al., 2023). On the other hand, the functional assembly indicates that the Luapula River Basin is dominated by gathering collectors and predators. Gathering collectors feed on fine particulate organic matter, suggesting a high availability of detritus in the ecosystem. Predators, which feed on other organisms, indicate a well-established food web with sufficient prey diversity. This information provides insights into understanding energy flow and nutrient cycling within the ecosystem (Carrasco-Badajoz et al., 2022). Additionally, the application of biotic indices on macroinvertebrate community structure revealed that the Luapula River Basin is characterised by moderate signs of impairment in terms of river condition and water quality, highlighting the areas of concern for conservation and management efforts. From a functional point of view, the biotic indices revealed that the sampled rivers and streams are predominantly heterotrophic systems that depend on external organic matter inputs for energy and nutrients, indicating potential limitations in ecosystem productivity and resilience as disruptions to organic matter inputs could impact biotic communities and ecosystem functions. In the Southern Apalachian Mountains of North Carolina in the USA, Webster et al. (1990) submit that alterations in litter inputs and benthic organic material following forest disturbance highlight the importance of terrestrial organic matter subsidies in stream ecosystems. Webster et al. (1990) also assert that the observed increase in particulate organic matter transport in disturbed streams emphasises the role of external organic matter inputs in driving nutrient cycling and energy flow within aquatic systems. Furthermore, sampled rivers and streams were also found to present a very weak link in the interaction between Shredders and the riparian zones. According to Cummins et al. (1989), Shredders play a critical function in the transformation of large organic plant substrates, such as leaf litter, known as coarse particulate organic matter into smaller particles termed as fine particulate organic matter. This observation justifies the presumption that the Luapula River Basin is characterised by inefficient riparian zones. The assessment of water quality parameters using a water quality index indicates a range of water quality conditions from excellent to unsuitable for drinking in the dry and wet seasons. This underscores the need for improved water quality management and conservation measures to safeguard both ecosystem integrity and human health within the Luapula River Basin.

The principles of Integrated Water Resources Management (IWRM) have a direct bearing on ecological health. By focusing on sustainable management of water resources, IWRM

promotes the health and integrity of ecosystems. Masthura et al. (2023) submitted that Integrated Water Resources Management is a process that integrates water management, land and other related resources in a coordinated way to maximise economic and social welfare equally without sacrificing the sustainability of vital ecosystems. Masthura et al. (2023) further added that while water resources management, particularly through Integrated Water Resources Management (IWRM), is recognised as crucial for sustainable development, its implementation in Indonesia, specifically in the Aceh-Meureudu River Basin, faces significant challenges. Despite the existence of various water management institutions, there are inefficiencies and weaknesses in the implementation of water policies, primarily due to bureaucratic ineffectiveness and conflicts between regulations at different administrative levels. In a study focusing on inter-basin water transfer schemes in southern Africa, Snaddon et al. (1998) highlighted the lack of attention given to the ecological effects of such schemes by administrative, engineering and political circles. Snaddon et al. (1998) further emphasised the need for detailed ecological studies, intelligent and integrated planning and management and strict adherence to Environmental Impact Assessment (EIA) and Integrated Environmental Management (IEM) procedures for both current and future inter-basin water transfer schemes in the region. Snaddon (1999) argues that the ecological and environmental assessments of proposed inter-basin water transfers (IBWTs) have primarily been theoretical and empirical, with limited pre- and post-transfer ecological studies. This has led to a lack of understanding of the potential effects of IBWTs. Snaddon (1999) also notes that as global proposals for IBWTs increase, public opposition is mounting due to growing awareness of the finite nature of water resources within catchments. Snaddon (1999) further submits that initiating a transfer out of a donor catchment could result in a permanent loss of water, impacting future development. Consequently, the costs and benefits of IBWTs are under increasing scrutiny, particularly from communities in donor catchments. In the context of the Luapula River Basin, the findings are consistent with the documented potential ecological impacts of inter-basin water transfers on the donor basins, as previously indicated by Snaddon et al. (1998) and Snaddon (1999). The observed composition of the macroinvertebrate community and water quality variations across the hydro-environmental zones of the Luapula River Basin suggest moderate signs of impairment in ecosystem health and function. This study forms part of a PhD research focusing on the assessment of potential ecological impacts of inter-basin water transfer between the Luapula River Basin and Kafue River Basin. The findings from this study provide a justified basis for a more extensive evaluation of the prevailing ecological profiles and biodiversity of both the Luapula and Kafue River Basins as potential donor and receiver

basins, respectively. Such a comprehensive assessment is essential for determining the ecological viability of transferring water from the Luapula River Basin to the Kafue River Basin, considering the observed moderate impairments in ecosystem health and function as evidenced by the composition of macroinvertebrate assemblages and variations in water quality.

CHAPTER SIX: LIMITATIONS, CONCLUSION AND RECOMMENDATION

6.1 Limitations

i. The reliance on seasonal data may not fully capture long-term ecological trends or extreme events, potentially affecting the reliability of the conclusions.

ii. The observed low abundance of shredders and reliance on external organic matter suggest riparian inefficiency, but the study does not thoroughly explore the underlying causes.

iii. While the study highlights potential risks to ecological health from inter-basin water transfers, it does not quantify these impacts, limiting actionable insights for management.

iv. The positive correlation between taxa richness and ZISS scores implies a link between biodiversity and ecosystem health but does not establish causation, necessitating further investigation.

These limitations highlight the need for expanded temporal and spatial analysis, inclusion of additional parameters and deeper exploration of causal relationships to strengthen conclusions and inform sustainable management practices.

6.2 Conclusion

Based on the findings, the study concluded that:

The Luapula River Basin is characterised by distinct hydro-environmental zones: Zone 1 has low rainfall, elevation, runoff and forest cover, indicating dry lowlands; Zone 3 has high rainfall, elevation, runoff and forest cover, representing elevated, forested regions; and Zone 2 is transitional, with mid-range values. These zones influence macroinvertebrate communities, with dry season dissimilarity at 56.67% and wet season dissimilarity rising to 63.3%, driven.

The Luapula River Basin's macroinvertebrate assemblage is dominated by families from Decapoda, Odonata, Gastropoda, Ephemeroptera, and Hemiptera. The ZISS bioassessment protocol indicates moderate ecological impairment, with a prevalence of tolerant and moderately tolerant families. Hydro-environmental zones significantly influence ZISS scores ($p < 0.05$) and taxa richness ($p < 0.01$), highlighting the importance of elevation, rainfall, runoff, and forest cover in shaping macroinvertebrate communities. A positive correlation between taxa richness and ZISS scores suggests that greater biodiversity corresponds to healthier stream ecosystems.

The macroinvertebrate functional assemblage in the Luapula River Basin is dominated by gathering collectors, predators, and scrapers, with fewer filtering collectors and notably low shredder abundance. This reflects the availability of sediment organic material and prey but indicates limited coarse particulate organic matter, suggesting a weak riparian-shredder link. The ratios of functional feeding groups identify the Luapula River Basin as a heterotrophic system, characterised by an inefficient riparian zone with a generally persistent distribution of polyvoltine prey populations. The low shredder abundance underscores the riparian zone's inadequacy in providing sufficient or suitable organic matter to support a diverse and balanced macroinvertebrate community.

Seasonal means of most water quality parameters in the Luapula River Basin, except temperature and faecal coliforms, met Zambia Bureau of Standards (ZABS) Ambient Water Quality Specifications, indicating overall acceptable water quality despite site-specific variations. Water quality significantly varied by season ($p < 0.001$) but not by hydro-environmental zones ($p > 0.05$). Seasonal changes were driven by turbidity, total hardness, calcium, magnesium, chloride, potassium and faecal coliforms. The water quality index revealed seasonal improvements by zone, with iron, turbidity and faecal coliforms as primary contaminants. Dry season water quality improved in Zone 1 but declined in Zone 3, while the wet season showed the opposite trend. Zone 2 remained transitional between Zones 1 and 3 across seasons.

The proposed inter-basin water transfer could negatively impact the ecological health of the Luapula River Basin, which already shows moderate impairment, relies on external organic matter and has declining riparian function. Significant changes in water flow, quantity, quality and organic matter inputs risk reducing ecosystem stability, biodiversity and ecosystem services. Therefore, water extraction must be carefully managed to avoid exceeding the basin's natural replenishment capacity, ensuring sustainable hydrological and ecological balance.

6.2 Recommendation

This study provided the following recommendations:

i. The Water Management Authority (WARMA) should operationalise its mandate to conserve, preserve and protect water-related environments by adopting biomonitoring techniques in its monitoring frameworks. Current water monitoring frameworks prioritise physicochemical assessments which neglect ecological health considerations. This approach fails to effectively address the conservation, preservation and protection

of water-related environments as it overlooks the ecosystem responses of aquatic organisms to changes in water quality;

ii. The Zambian Government, through the Ministry of Water Development and Sanitation, Ministry of Health, and local water utilities, should launch education campaigns to raise awareness about faecal contamination sources and promote good hygiene practices, including handwashing and safe waste disposal in local communities and fishing camps. This should be coupled with increased promotion of household water treatment methods, such as chlorination and boiling, for safe drinking water;

iii. In light of the observed ineffective riparian support to river/stream ecosystems within the Luapula River Basin, the Zambian Government through the Ministry of Green Economy and Environment working in collaboration with conservation organisations should emphasise restoration efforts to improve sustained organic matter inputs;

iv. Research institutions should replicate this study in the Kafue River Basin to establish a comparative baseline for ecological health using macroinvertebrate assemblages. Future studies should also investigate linkages between riparian vegetation types and functional feeding groups (FFGs). Additionally, comprehensive hydrological and ecological modelling, including biodiversity assessments, is needed for both the Luapula and Kafue River Basins to evaluate water availability and ecological profiles, considering seasonal variations and long-term climate change. Analysing natural flow regimes such as base flows, peak flows and flow variability will provide insights into the potential impacts of water transfers and their effects on ecosystem stability and water supply in these basins; and

v. This study also recommends prioritising and investing in research to adapt and refine existing biomonitoring indices for local environmental conditions and taxonomic needs.

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APPENDICES

Appendix 1: Selected sampling sites with associated location details, land use activities and biotopes in the Luapula River Basin in Zambia. The identified biotopes per site included V = vegetation, S = stone and GSM = gravel, sand and mud. The sites were segregated based on zones where green = Zone 1, yellow = Zone 2 and blue = Zone 1.

						Biotope		
Stream/River	**ID**	**X**	**Y**	**Z**	**Land Use Activities**	**V**	**S**	**GSM**
Musamfwe	**Site 1**	30.00054	-13.2896	1400	Domestic use, charcoal burning	✓	✓	✓
Chisangwa 1	**Site 2**	29.98409	-13.2787	1366	Domestic use, charcoal burning, farming and fishing	✓		✓
Chisangwa 2	**Site 3**	29.98928	-13.2617	1361	Domestic use, animal rearing and farming	✓		✓
Mulembo	**Site 4**	30.39086	-12.5429	1185	Domestic use, road construction and gardening	✓	✓	✓
Chembe	**Site 5**	28.73063	-11.9834	1051	Domestic use, fishing and gardening	✓		✓
Luuo	**Site 6**	28.738	-11.8901	1120	Domestic use, gardening	✓	✓	✓
Mansa	**Site 7**	28.88894	-11.1934	1203	Gardening	✓		✓
Mbereshi	**Site 8**	28.7903	-9.73027	938	Domestic use, gardening	✓		✓
Lufubu	**Site 9**	28.78791	-9.89279	952	Domestic use, animal rearing and gardening	✓		✓
Kashiba	**Site 10**	28.64721	-10.442	927	Domestic use, fishing and transport	✓		✓
Luongo	**Site 11**	28.81413	-10.715	1131	Domestic use, fishing and hydropower generation	✓	✓	
Mukuku	**Site 12**	29.8527	-12.1162	1143	Domestic use, fishing and fish trading	✓		✓

Appendix 2: Feeding functional group (FFG) classes and associated food resources (Cummins et al., 2005; Merritt et al., 2017).

Functional groups	Particle size feeding mechanisms	Dominant food resources	Food size (mm)
Shredders	Chew conditioned litter or live vascular plant or gouge wood	CPOM-decomposing (or living hydrophyte) vascular plants	>1.0
Filtering collectors	Suspension feeders-filter particles from the water column	FPOM-decomposing detrital particles; algae, bacteria, and faeces	0.01-10
Gathering collectors	Deposit feeders-ingest sediment or gather loose particles in depositional areas	FPOM-decomposing detrital particles; algae, bacteria and faeces	0.05-1.0
Scrapers	Graze rock and wood surfaces or stems of rooted aquatic plants	Periphyton-attached non-filamentous algae and associated detritus, microflora and fauna and faeces	0.01-1.0
Predators	Capture and engulf prey or tissue, ingest body fluids	Prey living animal	>0.5

Appendix 3: Feeding functional group ratios as indicators of stream ecosystem attributes (Cummins, Merritt and Andrade, 2005).

Ecosystem attributes	Symbols for ecosystem attributes	Functional feeding group ratios for attributes	General criteria ratio levels
Autotrophy to heterotrophy	P/R	Scrapers+ shredders/Total collectors	Autotrophic, >0.75
Coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM)	CPOM / FPOM	Shredders/Total collectors	Normal shredder association linked to functioning riparian zone, >0.25
Top-down predator control	Top-down predator control	Predator to All other remained FFG	Normal balance between predators and prey range, 0.1-0.2
FPOM in transport to FPOM in storage (deposited in benthos)	TFPOM/BFPOM	Filtering collectors to gathering collectors	FPOM in transport (in suspension) is greater than normal particulate loading in suspension >0.5
Substrate (Channel) stability	Stable channel	Scrapers +Filtering collectors to shredders + gathering collectors	Stable substrates such as boulders and Cobbles and large wood debris vascular plants plentiful >0.5

Appendix 4: Hellinger transformed macroinvertebrate family abundance data as collected during the dry season across the sampling sites in the Luapula River Basin, Zambia.

Dry Season												
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
Ephemeridae	0	0.164399	0	0	0.16294	0	0	0	0.113228	0	0	0.152145
Polymictarcidae	0	0.183804	0	0	0.13304	0	0	0	0	0	0	0.117851
Trichorythidae	0.221766	0.116248	0.324443	0.286039	0	0	0.175863	0.193347	0.21183	0	0	0
Baetidae	0.326431	0.464991	0.374634	0.369274	0.37629	0.339032	0.287183	0.305709	0.179029	0.333333	0.270501	0.235702
Hydropsychidae	0.313625	0.201347	0	0.356753	0	0.321634	0.454077	0	0	0	0	0
Coenagrionidae	0	0.164399	0.418854	0.330289	0	0	0	0.421391	0	0	0.270501	0.319142
Calopterygidae	0	0	0	0.190693	0	0	0	0	0	0	0	0.288675
Gomphidae	0	0.246598	0.264906	0	0	0	0.227038	0	0.423659	0.333333	0	0.30429
Libellulidae	0.286299	0.318357	0.324443	0.213201	0.35199	0	0.287183	0	0.253185	0.210819	0.382546	0.136083
Aeshnidae	0.286299	0.367607	0.324443	0.23355	0	0	0.248708	0	0.375534	0	0.441726	0
Naucoridae	0.384111	0.164399	0	0	0	0	0	0.305709	0	0	0.382546	0
Belostomatidae	0.221766	0.116248	0.264906	0	0.23043	0.15162	0	0	0	0	0	0.096225
Nepidae	0	0.116248	0.187317	0	0.23043	0.107211	0	0	0.196116	0	0.220863	0.136083
Dytiscidae	0	0	0	0	0.23043	0.214423	0	0	0	0	0	0
Atyidae	0.495885	0.419137	0	0.457265	0	0.58722	0.556128	0.619013	0.57735	0.408248	0	0.608581
Potamonautidae	0.128037	0.164399	0.187317	0.213201	0.13304	0.214423	0.203069	0.096674	0.080064	0.182574	0.156174	0.068041
Thiaridae	0.350643	0.259938	0.350438	0.330289	0.51525	0.491304	0	0.167444	0.358057	0.278887	0	0.280542
Ampularidae	0	0	0	0.213201	0.29748	0.239732	0.366088	0.410152	0	0.434613	0.51797	0
Unionidae	0	0.142374	0.187317	0	0.39911	0.107211	0	0.096674	0.138675	0.516398	0.156174	0.166667
Culicidae	0	0	0	0	0	0	0	0	0	0	0	0.296586
Hirudinae	0	0	0	0	0	0	0	0	0	0	0	0.117851

Appendix 5: Hellinger transformed macroinvertebrate family abundance data as collected during the wet season across the sampling sites in the Luapula River Basin, Zambia.

Wet Season	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
Trichorythidae	0	0.206284	0	0	0	0	0	0	0.105118	0	0	0
Caenidae	0.182574	0.112987	0	0	0	0	0	0	0	0	0	0.165521
Oligoneuriidae	0	0	0	0	0.25078	0.187317	0.325669	0.184428	0.246523	0	0.543214	0.248282
Baetidae	0.32914	0.618853	0.420883	0	0.38033	0.324443	0.460566	0.26082	0.166206	0	0.313625	0.143346
Hydropsychidae	0.273861	0.447214	0.239046	0.405999	0	0.296174	0	0	0	0	0	0.248282
Coenagrionidae	0.483046	0.130466	0.29277	0.14825	0	0.229416	0	0	0.278115	0.377964	0.256074	0.298398
Calopterygidae	0	0	0.169031	0	0	0	0	0	0	0	0.221766	0
Gomphidae	0	0	0	0	0.13736	0	0	0	0.246523	0	0	0
Libellulidae	0.547723	0.130466	0.169031	0.209657	0.30715	0.374634	0	0.164957	0.246523	0.338062	0.362143	0.117041
Aeshnidae	0.182574	0.130466	0.106904	0	0	0	0	0	0.148659	0	0	0
Naucoridae	0	0.159787	0	0	0.13736	0	0	0.377964	0.182069	0	0	0
Belostomatidae	0	0	0	0	0.15861	0	0.213201	0.412393	0.105118	0	0	0
Nepidae	0.204124	0.092253	0	0	0	0	0.174078	0.273551	0.148659	0	0	0
Corixidae	0	0	0.106904	0.27735	0.20982	0.296174	0.389249	0.285714	0.105118	0	0.384111	0.286691
Gerridae	0.129099	0	0	0	0	0	0.174078	0	0	0	0	0.117041
Gyrinidae	0.129099	0	0.106904	0.234404	0.11215	0	0	0	0	0	0	0
Haliplidae	0.204124	0.159787	0	0.234404	0.23792	0	0	0.164957	0.23505	0	0.221766	0.248282
Dytiscidae	0	0	0	0	0.11215	0.187317	0	0	0	0	0	0.261712
Hydrophilidae	0	0	0	0	0	0	0	0	0	0.239046	0	0
Atyidae	0.182574	0.461266	0.736788	0.662994	0.63938	0.324443	0.369274	0.565445	0.643712	0.507093	0	0.453298
Potamonautidae	0	0.065233	0.169031	0.104828	0.07931	0.39736	0.213201	0	0	0.338062	0	0.185058
Ampularidae	0	0.130466	0.130931	0.209657	0	0	0.369274	0.233285	0.356471	0	0.404888	0.370117
Thiaridae	0.204124	0.092253	0	0.2965	0.30715	0.439298	0.275241	0	0	0.447214	0	0.32053
Unionidae	0.091287	0	0	0	0	0	0.174078	0	0.074329	0.338062	0	0.143346
Tabanidae	0.129099	0.092253	0	0	0	0	0	0	0	0	0	0

Appendix 6: Standardised water quality parameters and altitude as collected during the dry season in the Luapula River Basin, Zambia.

Dry Season												
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
EC	-0.26723	2.159504	1.16281	0.469457	-0.05056	-0.18056	-0.65724	-1.39393	-0.91725	0.859468	-0.61391	-0.57057
PH	-1.52332	0.349159	0.052528	0.719948	0.86826	0.868263	0.089607	-1.48624	-0.52219	1.62838	0.126686	-1.17107
TDS	-0.31607	1.73006	0.981476	0.332704	-0.26616	-0.21626	-0.6155	-1.26427	-0.86503	1.779965	-0.66541	-0.6155
Temperature	-0.65922	-0.90139	-1.54716	-0.94175	1.27809	-0.73995	1.035925	0.067268	0.228711	1.600974	0.7534	-0.1749
Turbidity	-1.44813	1.355407	1.106756	0.463372	-1.50408	0.367019	-0.26393	-0.27015	1.541895	-0.24839	-0.69596	-0.4038
Total Hardness	-0.0623	-0.0623	0.768351	-0.39456	-0.39456	-0.0623	-0.39456	-0.89295	-0.89295	0.602221	2.678847	-0.89295
Calcium	0.724824	0.724824	-1.92236	-0.40968	-0.40968	-0.03151	-0.03151	-0.40968	-0.03151	2.237501	-0.78785	0.346655
Magnesium	-0.20845	-0.20845	1.123002	-0.28677	-0.28677	-0.05181	-0.36998	-0.75669	-0.83501	0.104835	2.689412	-0.91333
Chloride	0.177059	1.02694	-1.5227	1.02694	-0.24788	-1.09776	-0.67282	1.02694	-1.09776	1.02694	-0.67282	1.02694
Sodium	0.207471	1.029055	-1.4357	1.029055	-0.20332	-1.02491	-1.02491	1.029055	-1.02491	1.029055	-0.63901	1.029055
Potassium	0.151186	1.018863	-1.56445	1.018863	-0.28265	-1.13061	-0.40097	1.018863	-1.13061	1.018863	-0.73621	1.018863
Iron	0.585645	2.104352	1.657674	-0.24815	-0.81395	-0.4566	-0.51616	0.466531	-0.39705	-0.84373	-0.84373	-0.69483
Faecal Coliform	-0.92879	-0.12696	-0.12696	-0.92879	0.67487	-0.92879	0.674873	-0.92879	1.476702	-0.92879	0.193775	1.877617
Altitude	1.518726	1.313751	1.283607	0.222559	-0.58528	-0.16931	0.331075	-1.26653	-1.18212	-1.33284	-0.10299	-0.03065

Appendix 7: Standardised water quality parameters and altitude as collected during the wet season in the Luapula River Basin, Zambia.

Wet Season												
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
EC	0.238728	0.875335	0.938995	-0.07958	0.68435	-0.71618	-0.58886	-1.54377	-1.22547	1.766584	-0.8435	0.49337
PH	0.842099	1.374989	0.624995	0.585522	0.0921	0.230261	-0.59868	-2.53287	-0.89473	0.072368	0.052631	0.151315
TDS	0.33293	1.032083	1.131962	0.033293	0.13317	-0.06659	-0.66586	-1.66465	-1.26513	1.631357	-0.9655	0.33293
Temperature	-0.94906	-1.03373	-1.28775	-1.16074	1.08312	-0.52569	0.575079	-0.05998	-0.18699	1.083124	1.210136	1.252473
Turbidity	0.493552	0.562636	-0.53739	0.50418	-1.05021	-0.60116	-0.7659	-0.77122	2.260507	1.14985	-0.77122	-0.47362
Chloride	-0.7694	-0.7694	-0.99459	-0.99459	-0.7694	1.482503	0.356551	1.032123	0.806932	1.482503	0.131361	-0.99459
Potassium	0.158595	-1.23704	-0.85641	-0.39966	0.05709	2.814534	0.090928	0.124761	-0.68725	-0.01057	-0.28124	0.226262
Iron	1.022403	-0.95535	-0.70778	-0.84845	-0.97223	1.922661	0.431608	1.275601	0.192477	0.198104	-0.58681	-0.97223
Faecal Coliform	0.636341	-1.30118	0.294427	-0.84529	-0.16146	-1.30118	-0.16146	1.890028	0.704724	-1.07323	1.023845	0.294427
Altitude	1.518726	1.313751	1.283607	0.222559	-0.58528	-0.16931	0.331075	-1.26653	-1.18212	-1.33284	-0.10299	-0.03065

Appendix 8: Dry season macroinvertebrate abundance and distribution in the Luapula River Basin in Zambia.

	Sampling Sites													
Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	Total	%
Ephemeridae	0	4	0	0	3	0	0	0	2	0	0	5	14	1.01083
Polymictarcidae	0	5	0	0	2	0	0	0	0	0	0	3	10	0.722022
Trichorythidae	6	2	6	9	0	0	3	4	7	0	0	0	37	2.67148
Baetidae	13	32	8	15	16	10	8	10	5	10	6	12	145	10.46931
Hydropsychidae	12	6	0	14	0	9	20	0	0	0	0	0	61	4.404332
Coenagrionidae	0	4	10	12	0	0	0	19	0	0	6	22	73	5.270758
Calopterygidae	0	0	0	4	0	0	0	0	0	0	0	18	22	1.588448
Gomphidae	0	9	4	0	0	0	5	0	28	10	0	20	76	5.487365
Libellulidae	10	15	6	5	14	0	8	0	10	4	12	4	88	6.353791
Aeshnidae	10	20	6	6	0	0	6	0	22	0	16	0	86	6.209386
Naucoridae	18	4	0	0	0	0	0	10	0	0	12	0	44	3.176895
Belostomatidae	6	2	4	0	6	2	0	0	0	0	0	2	22	1.588448
Nepidae	0	2	2	0	6	1	0	0	6	0	4	4	25	1.805054
Dytiscidae	0	0	0	0	6	4	0	0	0	0	0	0	10	0.722022
Atyidae	30	26	0	23	0	30	30	41	52	15	0	80	327	23.61011
Potamonautidae	2	4	2	5	2	4	4	1	1	3	2	1	31	2.238267
Thiaridae	15	10	7	12	30	21	0	3	20	7	0	17	142	10.25271
Ampularidae	0	0	0	5	10	5	13	18	0	17	22	0	90	6.498195
Unionidae	0	3	2	0	18	1	0	1	3	24	2	6	60	4.33213
Culicidae	0	0	0	0	0	0	0	0	0	0	0	19	19	1.371841
Hirudinae	0	0	0	0	0	0	0	0	0	0	0	3	3	0.216606
Total	122	148	57	110	113	87	97	107	156	90	82	216	**1385**	

Appendix 9: Wet season macroinvertebrate abundance and distribution in the Luapula River Basin in Zambia.

	Sampling Sites													
Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	**TOTAL**	%
Trichorythidae	0	10	0	0	0	0	0	0	2	0	0	0	12	0.814664
Caenidae	4	3	0	0	0	0	0	0	0	0	0	4	11	0.746775
Oligoneuriidae	0	0	0	0	10	2	7	5	11	0	18	9	62	4.209097
Baetidae	13	90	31	0	23	6	14	10	5	0	6	3	201	13.64562
Hydropsychidae	9	47	10	15	0	5	0	0	0	0	0	9	95	6.449423
Coenagrionidae	28	4	15	2	0	3	0	0	14	5	4	13	88	5.974202
Calopterygidae	0	0	5	0	0	0	0	0	0	0	3	0	8	0.543109
Gomphidae	0	0	0	0	3	0	0	0	11	0	0	0	14	0.950441
Libellulidae	36	4	5	4	15	8	0	4	11	4	8	2	101	6.856755
Aeshnidae	4	4	2	0	0	0	0	0	4	0	0	0	14	0.950441
Naucoridae	0	6	0	0	3	0	0	21	6	0	0	0	36	2.443992
Belostomatidae	0	0	0	0	4	0	3	25	2	0	0	0	34	2.308215
Nepidae	5	2	0	0	0	0	2	11	4	0	0	0	24	1.629328
Corixidae	0	0	2	7	7	5	10	12	2	0	9	12	66	4.480652
Gerridae	2	0	0	0	0	0	2	0	0	0	0	2	6	0.407332
Gyrinidae	2	0	2	5	2	0	0	0	0	0	0	0	11	0.746775
Haliplidae	5	6	0	5	9	0	0	4	10	0	3	9	51	3.462322
Dytiscidae	0	0	0	0	2	2	0	0	0	0	0	10	14	0.950441
Hydrophilidae	0	0	0	0	0	0	0	0	0	2	0	0	2	0.135777
Atyidae	4	50	95	40	65	6	9	47	75	9	0	30	430	29.19212
Potamonautidae	0	1	5	1	1	9	3	0	0	4	0	5	29	1.968771
Ampularidae	0	4	3	4	0	0	9	8	23	0	10	20	81	5.498982
Thiaridae	5	2	0	8	15	11	5	0	0	7	0	15	68	4.616429
Unionidae	1	0	0	0	0	0	2	0	1	4	0	3	11	0.746775

Tabanidae	2	2	0	0	0	0	0	0	0	0	0	0	4	0.271555
Total	120	235	175	91	159	57	66	147	181	35	61	146	**1473**	

Appendix 10: Dry season ZISS scores of macroinvertebrate families in the Luapula River Basin in Zambia.

	Sampling Sites											
Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
Ephemeridae	0	15	0	0	15	0	0	0	15	0	0	15
Polymictarcidae	0	10	0	0	10	0	0	0	0	0	0	10
Trichorythidae	9	9	9	9	0	0	9	9	9	0	0	0
Baetidae	4	4	4	4	4	4	4	4	4	4	4	4
Hydropsychidae	4	4	0	4	0	4	4	0	0	0	0	0
Coenagrionidae	0	4	4	4	0	0	0	4	0	0	4	4
Calopterygidae	0	0	0	10	0	0	0	0	0	0	0	10
Gomphidae	0	6	6	0	0	0	6	0	6	6	0	6
Libellulidae	4	4	4	4	4	0	4	0	4	4	4	4
Aeshnidae	8	8	8	8	0	0	8	0	8	0	8	0
Naucoridae	7	7	0	0	0	0	0	7	0	0	7	0
Belostomatidae	3	3	3	0	3	3	0	0	0	0	0	3
Nepidae	0	3	3	0	3	3	0	0	3	0	3	3
Dytiscidae	0	0	0	0	5	5	0	0	0	0	0	0
Atyidae	8	8	0	8	0	8	8	8	8	8	0	8
Potamonautidae	3	3	3	3	3	3	3	3	3	3	3	3
Thiaridae	3	3	3	3	3	3	0	3	3	3	0	3
Ampularidae	0	0	0	5	5	5	5	5	0	5	5	0
Unionidae	0	6	6	0	6	6	0	6	6	6	6	6
Culicidae	0	0	0	0	0	0	0	0	0	0	0	1
Hirudinae	0	0	0	0	0	0	0	0	0	0	0	3
Total	53	97	53	62	61	44	51	49	69	39	44	83

Appendix 11: Wet season ZISS scores of macroinvertebrate families in the Luapula River Basin in Zambia.

	Sampling Sites											
Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
Trichorythidae	0	9	0	0	0	0	0	0	9	0	0	0
Caenidae	6	6	0	0	0	0	0	0	0	0	0	6
Oligoneuriidae	0	0	0	0	15	15	15	15	15	0	15	15
Baetidae	4	4	4	0	4	4	4	4	4	0	4	4
Hydropsychidae	4	6	4	4	0	4	0	0	0	0	0	4
Coenagrionidae	4	4	4	4	0	4	0	0	4	4	4	4
Calopterygidae	0	0	10	0	0	0	0	0	0	0	10	0
Chlorocyphidae	0	0	0	0	0	0	0	0	0	0	0	0
Gomphidae	0	0	0	0	6	0	0	0	6	0	0	0
Libellulidae	4	4	4	4	4	4	0	4	4	4	4	4
Aeshnidae	8	8	8	0	0	0	0	0	8	0	0	0
Naucoridae	0	7	0	0	7	0	0	7	7	0	0	0
Belostomatidae	0	0	0	0	3	0	3	3	3	0	0	0
Nepidae	3	3	0	0	0	0	3	3	3	0	0	0
Notonectidae	0	0	0	0	0	0	0	0	0	0	0	0
Corixidae	0	0	3	3	3	3	3	3	3	0	3	3
Gerridae	5	5	0	0	0	0	5	0	0	0	0	5
Gyrinidae	5	0	5	0	5	0	0	0	0	0	0	0
Haliplidae	5	5	0	5	5	0	0	5	5	0	5	5
Dytiscidae	0	0	0	0	5	5	0	0	0	0	0	5
Hydrophilidae	0	0	0	0	0	0	0	0	0	5	0	0
Atyidae	8	8	8	8	8	8	8	8	8	8	0	8
Potamonautidae	0	3	3	3	3	3	3	0	0	3	0	3
Ampularidae	0	5	5	5	0	0	5	5	5	0	5	5
Thiaridae	3	3	0	3	3	3	3	0	0	3	0	3

Unionidae	6	0	0	0	0	0	6	0	6	6	0	6
Tabanidae	5	5	0	0	0	0	0	0	0	0	0	0

Appendix 12: Dry season diversity index scores across the sampling sites in the Luapula River Basin in Zambia.

	Sampling Sites											
Indices	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
Taxa_Richness	10	16	11	11	11	10	9	9	11	8	9	15
Individuals	122	148	57	110	113	87	97	107	156	90	82	216
Shannon_Diversity	2.125	2.38	2.275	2.25	2.102	1.83	1.933	1.728	1.929	1.905	1.945	2.124
Evenness_e^H/S	0.838	0.675	0.8843	0.8627	0.7435	0.6236	0.7681	0.6253	0.6259	0.8396	0.7774	0.5576
ZISS Score	53	97	53	62	61	44	51	49	69	39	44	83
ASPT	5	6	5	6	6	4	6	5	6	5	5	6

Appendix 13: Wet season diversity index scores across the sampling sites in the Luapula River Basin in Zambia.

	Sampling Sites											
Indices	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
Taxa_Richness	14	15	11	10	13	10	11	10	15	7	8	15
Individuals	120	235	175	91	159	57	66	147	181	35	61	146
Shannon_Diversity	2.118	1.818	1.54	1.797	1.949	2.175	2.191	1.995	2.054	1.856	1.908	2.442
Evenness_e^H/S	0.5938	0.4108	0.4241	0.6029	0.5403	0.8806	0.8127	0.7351	0.5202	0.9143	0.8428	0.7666
ZISS Score	70	85	58	39	71	53	58	57	90	33	50	80
ASPT	5	6	5	4	5	5	5	6	6	5	6	5

Appendix 14: Abundance and distribution of macroinvertebrate functional feeding groups across the Luapula River Basin in Zambia.

Wet Season					
Site	**Shredders**	**Scrappers**	**Predators**	**Filtering Collectors**	**Gathering Collectors**
Site 1	5	5	79	10	21
Site 2	7	6	22	47	153
Site 3	5	5	29	10	171
Site 12	13	47	27	21	37
Site 4	6	19	6	15	69
Site 5	10	22	29	10	88
Site 6	9	16	13	7	12
Site 7	3	24	7	9	23
Site 8	4	20	61	5	57
Site 9	10	25	52	12	113
Site 10	4	7	11	4	9
Site 11	3	19	15	18	6
Total	**79**	**215**	**351**	**168**	**759**
Dry Season					
Site	**Shredders**	**Scrappers**	**Predators**	**Filtering Collectors**	**Gathering Collectors**
Site 1	2	15	44	12	49
Site 2	4	10	56	9	69
Site 3	2	7	32	2	14
Site 12	1	17	73	25	100
Site 4	5	17	27	14	47
Site 5	2	40	32	18	21
Site 6	4	26	7	10	10
Site 7	4	13	19	20	41
Site 8	1	21	29	1	55
Site 9	1	20	66	3	66
Site 10	3	24	14	24	25
Site 11	2	22	50	2	6
Total	**31**	**232**	**449**	**140**	**503**

Appendix 15: Dry season physicochemical and microbial water quality parameters collected from the sampling sites in the Luapula River Basin in Zambia.

Dry Season													
ID	**EC**	**PH**	**TDS**	**Temperature**	**Turbidity**	**Total Hardness**	**Ca^{2+}**	**Mg^{2+}**	**Cl^{-}**	**Na^{+}**	**K^{+}**	**Fe^{2+}**	**Faecal Coliforms**
Site 1	34	6.17	24	20.8	2.98	40	8	4.8	13	8.58	2.83	0.58	0
Site 2	90	7.18	65	20.2	12	40	8	4.8	15	9.9	3.27	1.09	10
Site 3	67	7.02	50	18.6	11.2	60	2.4	12.96	9	5.94	1.96	0.94	10
Site 4	51	7.38	37	20.1	9.13	32	5.6	4.32	15	9.9	3.27	0.3	0
Site 5	39	7.46	25	25.6	2.8	32	5.6	4.32	12	7.92	2.61	0.11	20
Site 6	36	7.46	26	20.6	8.82	40	6.4	5.76	10	6.6	2.18	0.23	0
Site 7	25	7.04	18	25	6.79	32	6.4	3.81	11	6.6	2.55	0.21	20
Site 8	8	6.19	5	22.6	6.77	20	5.6	1.44	15	9.9	3.27	0.54	0
Site 9	19	6.71	13	23	12.6	20	6.4	0.96	10	6.6	2.18	0.25	30
Site 10	60	7.87	66	26.4	6.84	56	11.2	6.72	15	9.9	3.27	0.1	0
Site 11	26	7.06	17	24.3	5.4	106	4.8	22.56	11	7.22	2.38	0.1	14
Site 12	27	6.36	18	22	6.34	20	7.2	0.48	15	9.9	3.27	0.15	35

Appendix 16: Wet season physicochemical and microbial water quality parameters collected from the sampling sites in the Luapula River Basin in Zambia.

Wet Season													
ID	EC	pH	TDS	Temperature	Turbidity	Total Hardness	Ca^{2+}	Mg^{2+}	Cl^{-}	Na^{+}	K^{+}	Fe^{2+}	Faecal Coliforms
Site 1	36	7.68	25	21.8	6.15	0.1	0.02	0	4	-	0.83	0.71	85
Site 2	46	7.95	32	21.6	6.41	0.1	0.02	0	4	-	0.005	0.007	0
Site 3	47	7.57	33	21	2.27	0.1	0.02	0	3	-	0.23	0.095	70
Site 4	31	7.55	22	21.3	6.19	0.1	0.02	0	3	-	0.5	0.045	20
Site 5	43	7.3	23	26.6	0.34	0.1	0.02	0	4	-	0.77	0.001	50
Site 6	21	7.37	21	22.8	2.03	0.1	0.02	0	14	-	2.4	1.03	0
Site 7	23	6.95	15	25.4	1.41	0.1	0.02	0	9	-	0.79	0.5	50
Site 8	8	5.97	5	23.9	1.39	0.1	0.02	0	12	-	0.81	0.8	140
Site 9	13	6.8	9	23.6	12.8	0.1	0.02	0	11	-	0.33	0.415	88
Site 10	60	7.29	38	26.6	8.62	0.1	0.02	0	14	-	0.73	0.417	10
Site 11	19	7.28	12	26.9	1.39	0.1	0.02	0	8	-	0.57	0.138	102
Site 12	40	7.33	25	27	2.51	0.1	0.02	0	3	-	0.87	0.001	70

Appendix 17: Descriptive statistics of water quality parameters as measured in the dry season across the 12 sampling points of the Luapula River Basin, Zambia.

	EC (µs/cm)	pH	TDS (mg/l)	Temperature (°C)	Turbidity (NTU)	Total Hardness ($mgCaCO_3/l$)	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	Cl^- (mg/l)	Na^+ (mg/l)	K^+ (mg/l)	Fe^{2+} (mg/l)	Faecal Coliform (#/100ml)
N	12	12	12	12	12	12	12	12	12	12	12	12	12
Minimum	8	6.17	5	18.6	2.8	20	2.4	0.48	9	5.94	1.96	0.1	0
Maximum	90	7.87	66	26.4	12.6	106	11.2	22.56	15	9.9	3.27	1.09	35
Sum	482	83.9	364	269.2	91.67	498	77.6	72.93	151	98.96	33.04	4.6	139
Mean	40.17	6.99	30.33	22.43	7.64	41.50	6.47	6.08	12.58	8.25	2.75	0.38	11.58
Standard error	6.66	0.16	5.78	0.72	0.93	6.95	0.61	1.77	0.68	0.46	0.15	0.10	3.60
Variance	532.52	0.29	401.52	6.14	10.35	579.73	4.48	37.56	5.54	2.58	0.26	0.11	155.54
Standard deviation	23.08	0.54	20.04	2.48	3.22	24.08	2.12	6.13	2.35	1.61	0.51	0.34	12.47
Median	35.00	7.05	24.50	22.30	6.82	36.00	6.40	4.56	12.50	8.25	2.72	0.24	10.00
25 percentile	25.25	6.45	17.25	20.30	5.64	23.00	5.60	2.03	10.25	6.60	2.23	0.12	0.00
75 percentile	57.75	7.44	46.75	24.83	10.68	52.00	7.80	6.48	15.00	9.90	3.27	0.57	20.00

Appendix 18: Descriptive statistics of water quality parameters as measured in the wet season across the 12 sampling points of the Luapula River Basin.

	EC (µs/cm)	pH	TDS (mg/l)	Temperature (°C)	Turbidity (NTU)	Cl^- (mg/l)	K^+ (mg/l)	Fe^{2+} (mg/l)	Faecal Coliform (#/100ml)
N	12	12	12	12	12	12	12	12	12
Minimum	8	5.97	5	21	0.34	3	0.005	0.001	0
Maximum	60	7.95	38	27	12.8	14	2.4	1.03	140
Sum	387	87	260	288.5	51.51	89	8.835	4.159	685
Mean	32.25	7.25	21.67	24.04	4.29	7.42	0.74	0.35	57.08
Standard error	4.53	0.15	2.89	0.68	1.09	1.28	0.17	0.10	12.66
Variance	246.75	0.26	100.24	5.58	14.16	19.72	0.35	0.13	1924.63
Standard deviation	15.71	0.51	10.01	2.36	3.76	4.44	0.59	0.36	43.87
Median	33.50	7.32	22.50	23.75	2.39	6.00	0.75	0.28	60.00
25 percentile	19.50	7.03	12.75	21.65	1.40	3.25	0.37	0.02	12.50
75 percentile	45.25	7.57	30.25	26.60	6.36	11.75	0.83	0.66	87.25

Appendix 19: ZABS ambient recommended water quality catchment scores and measured water quality scores of the Luapula River Basin in Zambia.

	ZABS Ambient Water Quality Specifications				Luapula River Basin – Dry Season		Luapula River Basin – Wet Season	
Parameter	**Unit**	**Catchment Mean Value**	**Catchment Highest Value**	**Recommended Value**	**Mean Value**	**Score Range**	**Mean Value**	**Score Range**
EC	**µs/cm**	88	99	100	40.17	8 - 90	32.25	8 - 60
pH		5.4	5.5	5.5 - 8.0	6.99	6.17 - 7.87	7.25	5.97 - 7.95
TDS	**mg/l**	45	50	45	30.33	5 - 66	21.67	5 - 38
Temperature	°C	23.2	23.7	23.5	22.43	18.6 - 26.4	24.04	21 - 27
Turbidity	**NTU**	6	17	10	7.64	2.8 - 12.6	4.29	0.34 - 12.8
Total Hardness	**mg$CaCO_3$/l**	250	300	250	41.5	20 - 106	Nil	Nill
Calcium	**mg/l**	20	35	30	6.47	2.4 - 11.2	Nil	Nil
Magnesium	**mg/l**	23	41	35	6.08	0.48 - 22.56	Nil	Nil
Chloride	**mg/l**	20	40	30	12.58	9 - 15	7.42	3 - 14
Potassium	**mg/l**	-	-	-	2.75	1.96 - 3.27	0.74	0.005 - 2.4
Iron	**mg/l**	0.5	2	0.7	0.38	0.1 - 1.09	0.35	0.001 - 1.03
Nitrates	**mg/l**	0.4	0.8	6	Nil	Nil	Nil	Nil
Phosphates	**mg/l**	-	-	0.04	Nil	Nil	Nil	Nil
Sulphates	**mg/l**	-	-	20	Nil	Nil	Nil	Nil
Faecal Coliform	**#/100ml**	19	100	50	11.58	0 - 35	57.08	0 - 140

Appendix 20: ANOVA outputs showing statistical variations of water quality parameters within a spatial-temporal context in the Luapula River Basin in Zambia. Statistical significance Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Response Total Hardness :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	36	36	0.1142	0.7389
Season	1	10284	10283.8	32.621	1.371e-05 ***
Zone:Season	1	36	36	0.1142	0.7389
Residuals	20	6305	315.3		
Response Calcium :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	0.16	0.16	0.0654	0.8007
Season	1	249.357	249.357	101.9726	2.684e-09 ***
Zone:Season	1	0.16	0.16	0.0654	0.8007
Residuals	20	48.907	2.445		
Response Magnesium :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	1.46	1.458	0.0711	0.792498
Season	1	220.89	220.887	10.7684	0.003731 **
Zone:Season	1	1.46	1.458	0.0711	0.792498
Residuals	20	410.25	20.512		
Response Potassium :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	0	0	1	0
Season	1	24.4118	24.4118	84.0239	1.339e-08 ***
Zone:Season	1	0.6581	0.6581	2.2652	0.1479
Residuals	20	5.8107	0.2905		
Response Iron :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	0.08223	0.08223	0.7825	0.38688
Season	1	0.0081	0.0081	0.0771	0.78409
Zone:Season	1	0.44656	0.44656	4.2499	0.05249 .
Residuals	20	2.1015	0.10508		
Response Chloride :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	9	9	0.7382	0.400416
Season	1	160.17	160.167	13.1374	0.001689 **
Zone:Season	1	25	25	2.0506	0.167592
Residuals	20	243.83	12.192		

Appendix 20 continued.

Response Electrical Conductivity :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	870.2	870.25	2.2646	0.148
Season	1	376	376.04	0.9786	0.3344
Zone:Season	1	16	16	0.0416	0.8404
Residuals	20	7685.7	384.28		
Response pH :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	0.0977	0.09766	0.3952	0.53667
Season	1	0.4108	0.41082	1.6627	0.21195
Zone:Season	1	0.9851	0.98506	3.9869	0.05965 .
Residuals	20	4.9415	0.24708		
Response Total Dissolved Solids :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	451.6	451.56	1.7885	0.1961
Season	1	450.7	450.67	1.7849	0.1966
Zone:Season	1	18.1	18.06	0.0715	0.7918
Residuals	20	5049.7	252.49		
Response Temperature :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	12.96	12.96	2.2663	0.1478
Season	1	15.52	15.5204	2.714	0.1151
Zone:Season	1	1.562	1.5625	0.2732	0.6069
Residuals	20	114.373	5.7187		
Response Turbidity :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	9.533	9.533	0.7339	0.40177
Season	1	67.201	67.201	5.1736	0.03409 *
Zone:Season	1	0.357	0.357	0.0275	0.86999
Residuals	20	259.782	12.989		
Response Faecal Coliform :					
	Degree of Freedom	**Sum of Squares**	**Mean Square**	**F value**	**Pr (>F)**
Zone	1	900	900	0.8382	0.37083
Season	1	12421.5	12421.5	11.568	0.002834 **
Zone:Season	1	506.3	506.3	0.4715	0.500198
Residuals	20	21475.6	1073.8		

Appendix 21: Dry and wet season correspondence canonical analysis eigenvalues showing the influence of each axis on the observed variation in macroinvertebrate structure.

Dry Season	Axis	Eigenvalue	%
	1	0.22788	24.04
	2	0.17784	18.76
	3	0.13842	14.6
	4	0.11658	12.3
	5	0.098903	10.43
	6	0.061831	6.522
	7	0.050642	5.342
	8	0.026808	2.828
	9	0	3
	10	0	2
	11	0.007332	0.7734
Wet Season			
	1	0.25737	24.97
	2	0.18617	18.06
	3	0.15088	14.64
	4	0.11401	11.06
	5	0.099838	9.687
	6	0.074469	7.225
	7	0.057103	5.541
	8	0.04194	4.069
	9	0.029405	2.853
	10	0.019464	1.889

THE UNIVERSITY OF ZAMBIA
DIRECTORATE OF RESEARCH AND GRADUATE STUDIES

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APPROVAL OF STUDY

IORG No. 0005376
NASREC IRB No. 00006465

24th August, 2022

REF NO. NASREC-2022-JUL-023

Nathan Nyambe
The University of Zambia
School of Mines
IWRM Center
P.O. Box 32379
LUSAKA

Dear Mr. Nyambe,

RE: "ECOLOGICAL INTEGRITY OF INTER-BASIN WATER TRANSFER IN THE KAFUE AND LUAPULA RIVER BASINS"

Reference is made to your submission of the protocol captioned above. The NASREC resolved to approve this study and your participation as Principal Investigator for a period of one year.

REVIEW TYPE	**ORDINARY REVIEW**	**APPROVAL NO. NASREC-2022-JUL-023**
Approval and Expiry Date	Approval Date: 24th August, 2022	Expiry Date: 23rd August, 2023
Protocol Version and Date	Version - Nil.	23rd August, 2023
Information Sheet, Consent Forms and Dates	English.	To be provided
Consent form ID and Date	Version - Nil	To be provided
Recruitment Materials	Nil	Nil
Other Study Documents	Questionnaire.	
Number of Participants Approved for Study		

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

CONDITIONS OF APPROVAL

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to NASREC within 5 days.
- All protocol modifications must be approved by NASREC prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to NASREC within 5 working days.
- All recruitment materials must be approved by NASREC prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. HSSREC will only approve a study for a period of 12 months.
- It is the responsibility of the PI to renew his/her ethics approval through a renewal application to NASREC.
- Where the PI desires to extend the study after expiry of the study period, documents for study extension must be received by NASREC at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Documents received within 30 days after expiry will be labelled "late submissions" and will incur a penalty fee of K500.00. No study shall be renewed whose documents are submitted for renewal 30 days after expiry of the certificate.
- Every 6 (six) months a progress report form supplied by The University of Zambia Natural and Applied Sciences Research Ethics Committee as an IRB must be filled in and submitted to us. There is a penalty of K500.00 for failure to submit the report.
- When closing a project, the PI is responsible for notifying, in writing or using the Research Ethics and Management Online (REMO), both NASREC and the National Health Research Authority (NHRA) when ethics certification is no longer required for a project.
- In order to close an approved study, a Closing Report must be submitted in writing or through the REMO system. A Closing Report should be filed when data collection has ended and the study team will no longer be using human participants or animals or secondary data or have any direct or indirect contact with the research participants or animals for the study.
- Filing a closing report (rather than just letting your approval lapse) is important as it assists NASREC in efficiently tracking and reporting on projects. Note that some funding agencies and sponsors require a notice of closure from the IRB which had approved the study and can only be generated after the Closing Report has been filed.

- A reprint of this letter shall be done at a fee.

- All protocol modifications must be approved by NASREC by way of an application for an amendment prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address or methodology and methods. Many modifications entail minimal risk adjustments to a protocol and/or consent form and can be made on an Expedited basis (via the IRB Chair). Some examples are: format changes, correcting spelling errors, adding key personnel, minor changes to questionnaires, recruiting and changes, and so forth. Other, more substantive changes, especially those that may alter the risk-benefit ratio, may require Full Board review. In all cases, except where noted above regarding subject safety, any changes to any protocol document or procedure must first be approved by NASREC before they can be implemented.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of NASREC, we would like to wish you all the success as you carry out your study.

Yours faithfully,

Dr. E. M. Mwanaumo

DR. E. M. MWANAUMO

CHAIRPERSON
THE UNIVERSITY OF ZAMBIA NATURAL AND APPLIED SCIENCES RESEARCH ETHICS COMMITTEE - IRB

CC: Director, Directorate of Research and Graduate Studies
Assistant Director (Research), Directorate of Research and Graduate Studies
Assistant Registrar (Research), Directorate of Research and Graduate Studies