MACROINVERTEBRATE FUNCTIONAL AND STRUCTURAL RESPONSES TO HUMAN DISTURBANCE AND FLOW CESSATION IN AFROMONTANE-SAVANNAH RIVERS

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2025

DECLARATION

Declaration by the student

This thesis is my original work and has never been presented for the award of an academic degree in any other university and should not be copied or reproduced in any format without written authority from the author and/or the University of Eldoret.

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DEDICATION

I dedicate this work to my dear late mother, Mrs. Jane Damaris Achieng Omondi, for always believing in me and encouraging me throughout my life. I also dedicate it to her two lovely sisters, Mrs. Joan Owade and Mrs. Emily Omondi, who took over and ensured I received the best education possible.

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ABSTRACT

Freshwater ecosystems in the Afrotropics are increasingly threatened by human activities and disturbances, including agriculture, livestock grazing, water abstraction, and sand harvesting. These activities degrade habitat quality, alter flow regimes, and influence the composition and functioning of aquatic communities. This study assessed the structural and functional responses of macroinvertebrate communities to varying levels of human disturbance, flow permanence, and seasonality in the Wundanyi-Bura catchment, a representative Afromontane-savannah River system in southeastern Kenya. Macroinvertebrates were sampled from 18 study sites categorized by varying disturbance levels (low, moderate, high), flow duration type (permanent vs seasonal), and season (dry vs wet). Physical and chemical water quality parameters, habitat characteristics, and land-use patterns were also quantified. Functional composition was evaluated using Functional Feeding Groups (FFGs) and 14 biological traits comprising 52 ecological trait attributes. Results showed significant degradation in water and habitat quality with increased disturbance, particularly in the lower river reaches. Functional trait analyses revealed that disturbed and seasonal sites were dominated by resilient taxa such as burrowers, predators, and collector-gatherers, while less disturbed, permanent sites had higher proportions of sensitive taxa like shredders and scrapers. Flow variability and seasonality strongly influenced trait distributions and ecosystem attributes, including trophic dynamics, organic matter processing, and top-down control. Multivariate analyses (ANOSIM, NMDS, SIMPER) and trait-based approaches provided robust indicators of ecological integrity and disturbance gradients. This study underscores the value of integrating functional traits and FFG ratios in biomonitoring and river health assessment. It provides crucial baseline data for the Afrotropics, where biomonitoring frameworks are still underdeveloped, and highlights the need to consider both structural and functional metrics in the conservation, restoration, and management of freshwater ecosystems under increasing anthropogenic pressures.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS, ACRONYMS, AND SYMBOLS

ANOVA - Analysis of variance

BFPOM -Benthic fine particulate organic matter

CPOM - Coarse particulate organic matter

CCA - Canonical correspondence analysis

DO - Dissolved oxygen

DOC- Dissolved organic carbon

EC - Electrical conductivity

EMCA- Environmental Management and Coordination Act

EPT - Ephemeroptera, Plecoptera and Trichoptera

FAO - Food and Agriculture Organisation

FFGs - Functional Feeding Groups

FPOM - Fine particulate organic matter

GPS - Global positioning system

NMDS - Non-metric multidimensional scaling

PCA - Principle component analysis

POM - Particulate organic matter

PPT - Parts per thousand

RCC - River continuum concept

RDA - Redundancy analysis

RLQ - R-environmental descriptors of the sampling sites; L-taxon abundances of samples; Q-taxa traits

SD - Standard deviation

SDGs - Sustainable Development Goals

SE - Standard error

SRP - Soluble reactive phosphates

TDS - Total dissolved solids

TFPOM - Transport fine particulate organic matter

TP – Total phosphorus

TSS - Total suspended solids

UoE - University of Eldoret

YSI - Yellow Spring Instruments

CHAPTER ONE

INTRODUCTION

1.1 Background information

The state of a river can range from pristine to a substantially degraded river along a continuous gradient of impairment. Accurately determining the biological health of rivers and streams in Afro-montane savannah rivers is the most crucial and vital task in repairing their condition and restoring them, according to Young et al. (2008). This will eventually make it possible to identify and analyse the causes of the river's environmental degradation as well as the extent to which restoration initiatives are effective and ideal. Resource managers can employ a variety of accurate indicators to assess or track a river's health (Barbour *et al.,* 1999; Boulton & Lake, 2008).

Changes in environmental circumstances and species-specific adaptations or interactions for habitat selection and resource utilization are the main vital causes of the seasonal and geographical variability seen in aquatic macroinvertebrate populations (Masese *et al.,* 2025a; Sitati *et al.,* 2021). However, water quality, channel geomorphology, and stream habitat quality are all impacted by animal-related activities in or around streams and rivers, and this is due to grazing, crossing, and watering (Fierro *et al.,* 2017; Iteba *et al.,* 2021; Masese *et al.,* 2023). Research on how aquatic ecosystems react and interact to pressures in rivers and streams has become more prevalent worldwide, but development in the Afro-montane savannah rivers has been slower. In particular, the making of bio-indices tools for temporary channels is still limited (Dalu and Masese, 2025).

While earlier research, as stated by (Vannote *et al.,* 1980), in the RCC acknowledged the usefulness of utilising (FFGs) as indicators of ecosystem health and functioning, previously there has been a rise in the adoption of species functional metrics and traits matrices as substitutes for assessing community function response to various disturbance gradients (Fierro *et al.,* 2017). Land use changes, riparian vegetation loss, urbanisation are examples of disturbances that contribute to environmental degradation (Sitati *et al.,* 2021). However, earlier research by (Mathers, Hill, *et al.,* 2017; Merritt *et al.,* 2017; Wagenhoff *et al.,* 2012) demonstrated this change. Researchers may further assess the general ecological state of these systems by considering the structural and functional distribution of macroinvertebrates, which in turn makes evaluations of these elements essential (Makaka *et al.,* 2018).

More information is still required to support current biomonitoring indices, and this will make it easier to create new indices for Afro-montane savannah rivers, especially when considering both human and livestock disturbance from rural agricultural activities and human activities on aquatic communities. The Taita Hills region of Kenya, which is like many other tropical river catchments, is highly threatened by human settlements, animal grazing, water abstraction, and sand harvesting that destroy protected forests and other vulnerable ecosystems (MoALF, 2019).

The study was done in the Wundanyi and Bura rivers, which are termed to be Afromontane-savannah rivers. This is because they drain a gradient in land use, height, and rainfall, with the semi-arid savannah lowlands and the more forested higher reaches. The middle and lower reaches are subject to various human activities, including mixed farming, sand harvesting, water abstraction, and agro-pastoral activities, but the higher reaches are more protected with less access to the streams; therefore, they experience less disturbance (Hohenthal *et al.,* 2015). Frequent biomonitoring is thus necessary because these upstream-downstream alterations to natural circumstances brought about by both human and livestock activities, terrestrial vegetation, and rainfall patterns may end up leading to adverse effects on in-stream habitat as the composition and organisation of aquatic communities.

1.2 Statement of the problem and justification of the study

Land use practices, which can be livestock activities and agricultural activities, can have various impacts on freshwater ecosystems. This is because of the introduction of nutrients and organic matter, water flow patterns, and degradation of in-stream habitats. These changes eventually reduce water quality and, in turn, affect the physical environment, disrupt aquatic ecosystems, and therefore lead to the extinction or replacement of sensitive species by more tolerant ones, eventually resulting in a decline in biodiversity (Cornejo *et al.,* 2019. The difficulties largely affect the structure and well-being of the ecosystems, which include the further breakdown of organic materials and relationships between different levels of the food chain.

As a result, it is critical to include macroinvertebrate features and their ecological preferences while undertaking biomonitoring research in freshwater ecosystems, particularly apply to when the primary goals are to find and conserve ecological integrity (Vinagre *et al.,* 2017). Organisms with different traits are critical for the correct functioning of ecosystems. This is because they serve as a reliable indication of pollution's influence on processes that include the nutrient cycle, energy transfer, and carbon and material mobility. Various researchers have used biomonitoring methodologies such as organism traits and feeding habits to investigate the mechanisms that shape their assemblages and the impact of disturbances on these communities (Kuzmanovic *et al.,* 2017; Scotti *et al.,* 2020; Wang et al., 2019).

Therefore, this research is crucial as it will provide vital data on FFGs and trait distribution in Wundanyi-Bura streams, and can be used for assessing land-use impacts and flow cessation on the FFGs communities in Wundanyi and Bura rivers.

1.3 Study Objectives

1.3.1 Overall Objective

To assess the Structural and Functional Composition of Macroinvertebrate Traits in Wundanyi and Bura rivers and their use as Indicators of Livestock Disturbance of Streams and Rivers**.**

1.3.2 Specific Objectives

The specific objectives of the study are to:

i. Determine whether water quality and habitat quality degrade with increasing levels of human disturbance in the Wundanyi and Bura rivers.
ii. Evaluate whether macroinvertebrate communities responded structurally and functionally to human disturbance, seasonality, and flow permanence
iii. Determine whether ecosystem attributes of Afromontane-savannah rivers are influenced by human disturbance, seasonality, and flow permanence
iv. Examine the spatial and seasonal distribution patterns of macroinvertebrates' traits and ecological preference in relation to disturbance, seasonality, and flow permanence in Wundanyi and Bura rivers

1.4 Hypotheses

H_{A1} The ratios of FFGs can be used as surrogates of ecosystem attributes and, at the same time, serve as a useful tool as indicators of the ecological health of streams influenced by different levels of human disturbances, seasonality, and flow permanence

H_{A2} Low-disturbed sites with intact riparian zones would have a higher diversity and abundance of shredders than disturbed sites devoid of riparian zones and inputs of terrestrial organic matter

H_{A3} Flow variability will be a major variable structuring the functional and structural organisation of macroinvertebrates, with seasonal river reaches dominated by predators and collector-gatherers, and permanent river reaches dominated by scrapers and collector-filterers

H_{A4} Sites with high disturbance would differentially influence trait communities between the seasons due to changes in flow velocity, food availability, sedimentation, and hydro-morphological conditions

CHAPTER TWO

LITERATURE REVIEW

2.1 Water quality and habitat quality in streams and rivers

Freshwater ecosystems support an extraordinary diversity of life, harbouring around forty per cent of global different fish species and about a quarter of all vertebrates (Dudgeon, 2006; Amezaga *et al.,* 2002). Despite their ecological value and their biodiversity, these habitats, which a home to some of the world's most threatened species, are currently threatened by extensive activities of both human and livestock activities. This is because changes in land use activities at the riparian zone have had the greatest impact on freshwater ecosystems of any biological environment. Livestock activities have harmed around 80% of rivers and streams, causing flow variations and deteriorated habitat conditions (Dudgeon, 2006; Sitati *et al.,* 2021).

The quality of water is defined as a combination of physical, chemical, and biological qualities that determine its utility and suitability for various uses (Bouwer 2000). Temperature fluctuations, dissolved oxygen concentrations, salinity, turbidity, COD and BOD, pH, nutrient concentrations (phosphates and nitrates), EC, and the presence of metals and OM are just some of the water parameters that affect the water quality (Wilhm & Dorris, 1968). According to the (EMCA) of 1999, acceptable limits for domestic water use in Kenya include nitrates (10 mg/L), ammonia (0.5 mg/L), nitrites (3 mg/L), total dissolved solids (1200 mg/L), total suspended solids (30 mg/L), phosphates (30 mg/L), and aluminum (5 mg/L). In Kenya, people live around these freshwater ecosystems, because, as the population grows, we the people and our livestock rely primarily on these threatened ecosystems for water for daily use.

But this close proximity often leads to agricultural and domestic pollution (Masese *et al.,* 2009; 2014; Minaya *et al.,* 2013). As both organic and inorganic pollutants are introduced into the river channels, changing the water chemistry and altering aquatic life, these similar trends have been observed throughout the Wundanyi and Bura rivers (Gubamwoyo *et al*., 2025; Pellikka *et al*., 2013). Habitat degradation is an urgent issue, especially when there is the removal of riparian vegetation and forests from river catchments. Hydrology, sediment movement, channel alteration, and the variety of aquatic ecosystems and structures have all undergone substantial modifications as a result of these livestock and human activities along the river banks (Allan, 2004).

Issues such as buried littoral zones, altered floodplain dynamics, and smothered streambeds have resulted from increased runoff and sediment input (Dudgeon, 2006). According to Naiman *et al*. (2005), Dobson *et al*. (2002), and Mathooko & Kariuki (2000), riparian buffers are crucial for preserving the health of streams this is because they filter sediments, regulate temperature and nutrient inflow, therefore providing organic matter such as leaves and wood debris, all of which end up to be essential for energy flow and ecological balance.

It is widely established that benthic macroinvertebrates are reliable bioindicators of water quality. This is due to their ability to exist in a range of habitats, as well as their variable vulnerability to various pollutants, which makes them important in assessing ecosystem health (Allan, 2004). According to a recent study, even natural changes in freshwater systems appear to lead to the loss or diminishing of these ecosystems (Hawkins *et al.,* 2015).

The regular pattern of species turnover throughout time provides critical and vital information on ecological integrity (Hepp *et al.,* 2016). Ecological stability, therefore, refers to river systems' ability to sustain the species turnover and integrity in the plight of environmental degradation. (Masese *et al.,* 2014; Sitati *et al.,* 2021). Some rivers, however, have a low species richness while maintaining functional stability, and while having a steady physical attribute. In contrast, more dynamic and diverse biological communities, which contribute to overall stability, are commonly found in systems with significant environmental fluctuation (Vannote et al. 1980).

Degradation of freshwater resources is intensifying across various regions, including the upper catchments of Wundanyi and Bura. Environmental de in areas such as Mwatate, Taveta, Tsavo West, and Bura is largely driven by deforestation, riparian clearing, and agricultural expansion, particularly maize farming, sisal plantations, cattle grazing, horticulture, and sand harvesting. Urban growth further exacerbates these pressures (Gubamwoyo *et al.,* 2025; Pellikka *et al.,* 2018). Runoff from these land uses introduces high loads of nutrients like phosphates, nitrates, and organic carbon into rivers, accelerating eutrophication. This leads to excessive algal growth, algal blooms, and, in turn, leads to hypoxia in aquatic environments (Okungu & Opango, 2005).

This research aims to understand the key physical, chemical, and biological variables altering water quality and habitat quality in the Wundanyi and Bura rivers. It also aims to explore how these environmental conditions shape the composition, diversity, function, and ecological traits of macroinvertebrate assemblages in these freshwater systems.

2.2 The effects of human activities and livestock activities on riverine macroinvertebrates

Human activities are a concern worldwide because of their various negative impacts on aquatic ecosystems, resulting in biodiversity loss, altered ecosystem functions, and disruptions to food webs (Dudgeon, 2006; Mpopetsi *et al.,* 2025b; Reid *et al.,* 2019). Human activities affect aquatic habitats through altered flow regimes, increased loading of OM, introduction of exotic species, and altered physical attributes of aquatic ecosystems (Malapane *et al.,* 2025; Shelton *et al.,* 2025). These factors collectively alter biological communities, leading to a decrease in diversity, changes in composition, and shifts in the distribution of macroinvertebrates (Allan, 2004; Dudgeon, 2006; Reid *et al.,* 2019). The effects of these perturbations, however, depend on the sensitivity or susceptibility of the different macroinvertebrate taxa (Eady *et al.,* 2014; Gasith & Resh, 1999).

Anthropogenic activities such as land use affect sensitive macroinvertebrate taxa such as EPT by reducing their abundance or diversity while increasing the proportion of tolerant taxa such as some Diptera (e.g., Chironomidae), Oligochaeta, and some molluscs (Akamagwuna *et al.,* 2023; Dudgeon, 2006; Lubanga *et al.,* 2021; Raburu *et al.,* 2009; Sitati *et al.,* 2021a, b, ; Yegon *et al.,* 2021). Similarly, various research have found out that some shredders adapted are to cooler and forested streams (Masese *et al.,* 2014; Minaya *et al.,* 2013; Yegon *et al.,* 2021), thus susceptible to human activities that would lead to an increase in water temperature or limit the input of CPOM. Overall, sensitive macroinvertebrate taxa provide a favorable response to anthropogenic activities.

In Africa, land use changes such as deforestation, agriculture, and urban development are major human activities that affect rivers. Clearance of Land for human settlement and agricultural activities is rapidly expanding, driven by increasing human population growth and food demand, which is projected to double by 2050 (FAO, 2013; World Bank Group, 2020). In these systems, land use change has a strong influence on the diversity of macroinvertebrate taxa because it influences water quality, (Masese *et al.*, 2014a,b; Mwaijengo *et al.,* 2020; Sitati *et al.,* 2021). Examples of anthropogenic activities and their impacts on these freshwater ecosystems have been outlined in Table 1 below.

Table 1: Major anthropogenic activities and their impacts on Freshwater ecosystems

Human activity	Impacts on streams	Impacts on macroinvertebrates	References
Urbanization	Deterioration of water quality due to effluent and sewage release, loss of natural habitats, and increased impervious surfaces	The higher runoff and pollution levels impact macroinvertebrate diversity by reducing the sensitive taxa.	(Sterling *et al.,* 2016); Sitati *et al*., (2021a, b)
Agriculture	Reduces habitat heterogeneity, increases pollution through input of agricultural chemicals, increases runoff leading to altered stream flow and sedimentation, and warmer thermal regimes in streams due to the removal of vegetation	Siltation clogs gills, smothers eggs and nests, and affects visibility, thus affecting the breathing, feeding, and reproduction of invertebrates. Increased nutrients and temperature, and loss of habitats, reduce macroinvertebrates richness	(Akamagwuna *et al.,* 2023; Fuge`re *et al.,* 2018; Minaya *et al.,* 2013; Sitati *et al.,* 2021; Yegon *et al.,* 2021)
Deforestation	Removes vegetation cover, alters microhabitats, changes soil composition, and alters temperature regimes	Reduces the quantity and variety of shredders by reducing CPOM availability and negatively affecting detritivores. Changes in temperature affect the life cycles, reproduction, and survival of macroinvertebrates	(Benstead *et al*., 2003; Kasangaki *et al.,* 2008; Lubanga *et al.,* 2021; F. O. Masese *et al.,* 2014; Sitati *et al.,* 2021; Yegon *et al.,* 2021)
Mining and quarrying	Leads to habitat destruction, pollution, and deterioration of water quality	Reduces available habitats and deteriorates water quality, affecting the macroinvertebrate	(Eyankware *et al*., 2020)

		community composition	
Damming	Alters natural flow regimes and results in upstream-downstream shifts in biotic and abiotic patterns and processes.	Affects the habitats and alters energy inputs in streams by inhibiting FPOM transport, thus affecting longitudinal patterns of these organisms	(Bredenhand & Samways, 2009; Munzhelele *et al.,* 2024; Mwedzi *et al.,* 2016; Mwitalemi *et al.,* 2024)
Channelization and drainage	Modify river and stream morphology	Reduces habitat complexity for macroinvertebrates	(Gomes *et al.,* 2023; Osmundson *et al.,* 2002)
Chemical pollution	Increased pesticides, heavy metals, and industrial chemicals	Increased toxicity to macroinvertebrates reduces diversity and alters community composition	(Oltramare *et al.,* 2022; Osano *et al.,* 2003; Twesigye, *et al.,* 2011)
Plastic pollution	Increased microplastics in aquatic environments. Leads to habitat alteration and fine particle distribution.	Can be ingested by macroinvertebrates, affecting their health and biological functions	(Akindele *et al.,* 2023; Oceng *et al.,* 2023);
Introduction of non-native species	Increase of invasive species, resource and habitat partitioning	Invasive species, i.e., invasive mussels, can outcompete native species for resources and alter food webs through predation, leading to declines or extinctions of native populations	(Geray *et al.,* 2015; Rivers-Moore *et al.,* 2018)

2.3 Exploring the morphological and functional composition of macroinvertebrates

Understanding how macroinvertebrates respond at a functional level in streams is crucial for effective water management as global flow intermittency increases. A major key advantage of utilizing this approach, such as identifying and studying macroinvertebrates, is to ideally understand its focus on invertebrates that are consistently susceptible to aquatic degradation. Species that inhabit these ecosystems are an ideal indicator of the environment and water for the future and present. This further allows for the identification of different forms of disturbances that might otherwise be overlooked (Taylor *et al.,* 2005).

Macroinvertebrates have widely been recognized as effective for biomonitoring; this is primarily due to their favorable ecological and biological traits. Their taxonomic and trophic variety, along with their abundance and slow movement, make them the most preferred organisms for biomonitoring. However, their diverse lifespans, susceptibility to various contaminants, and their ability to reflect at both cumulative and interactive pollution impacts increases their use as ecological bioindicators. From a practical point of view, these organisms are quite easy and inexpensive to sample, and identification to the family level is usually simple. Therefore, their critical position in aquatic food webs, as well as the large amount of scientific research supporting their usage, emphasizes their importance in ecological evaluations (Pavluk *et al.,* 2000; Resh, 2008; Wenger *et al.,* 2009).

Benthic macroinvertebrates contribute substantially to nutrient cycling within freshwater systems. Bacteria, diatoms, aquatic plants, invertebrates, and CPOM and FPOM) all rely on them for degradation. These organisms provide food for fish, and other organisms such as water ducks and birds (Pavluk *et al,* 2000; Vannote *et al.,* 1980).

FFGs are commonly used by researchers to understand their ecological roles of macroinvertebrates by categorizing them based on their eating behavior and physical characteristics, especially those that related to food acquisition (Cummins & Klug, 1979). Adaptations such as unique mouthparts and their ecological roles, like resource-use strategies, are also crucial to include in this categorization system (Merritt & Cummins, 2007).

However, Masese *et al.* (2014) pointed out that it can be very challenging to categorize macroinvertebrates into FFGs, particularly when lack of information on their morphological traits or feeding habits are limited. The morphology of feeding habits and the identification of gastrointestinal contents are usually the basis for an assignment. Yet, with very few exceptions, doing fresh analyses, the majority of research in tropical regions is mostly drawn upon from current literature.

Macroinvertebrate assemblages are also often utilized as ideal measures of ecological responses to land-use change. Metrics such as taxonomic richness, biomass, and functional diversity often reflect the degree of disturbance in aquatic systems. Numerous studies have demonstrated a consistent pattern: as human-induced pressures like pollution and habitat alteration increase, sensitive taxa such as (EPT) declines, while more tolerant groups like Diptera and Oligochaeta tend to dominate (Masese *et al.,* 2009; Masese *et al.,* 2014; Raburu *et al.,* 2009).

The present study investigates how macroinvertebrate composition, diversity, and functional group distribution vary across a gradient of land use along a river continuum. Study sites include minimally impacted headwaters, moderately disturbed mid-reaches, and heavily altered downstream segments characterized by intensive agriculture, particularly large-scale maize cultivation, and livestock grazing. This spatial approach provides a comprehensive understanding of how anthropogenic pressures influence aquatic biodiversity and ecosystem function.

2.4 Macroinvertebrate traits

Apart from FFGs, macroinvertebrates are also classified in terms of their physiological or behavioral adaptations or traits that enable them to thrive in various environments and habitats. As FFGs, classifying macroinvertebrates based on traits offers a mechanistic alternative to traditional taxonomy-based classifications (Haybach *et al.,* 2004). Traits typically fall into two categories: biological and ecological traits. Biological traits include characteristics like life cycle, physiological and behavioral attributes such as maximum body size, lifespan, feeding and reproductive strategies, and type of mobility. On the other hand, ecological traits relate to habitat preferences such as flow velocities, substrate type, tolerance to environmental conditions such as temperature and dissolved oxygen concentration, resistance to organic pollution, and biogeographic distribution (Menezes *et al.,* 2010).

2.5 Macroinvertebrate Trait-Based Approaches

The (TBA) is founded on principles of theoretical ecology, notably the habitat template concept (Townsend & Hildrew, 1994), the habitat filtering concept (Poff, 1997), and various functional ecology frameworks such as functional diversity, redundancy, and uniqueness (Mason et al., 2005; Schmera *et al.,* 2017). Traits facilitate interactions between organisms and their environments, suggesting that trait utilization could transform descriptive community ecology into a predictive discipline (Paillex *et al.,* 2017; Verberk *et al.,* 2013).

Consequently, traits that mediate these interactions are pivotal for forecasting biotic responses to environmental changes and diagnosing environmental stressors through mechanistic links. The TBA promises to significantly enhance the diagnostic precision of freshwater biomonitoring indices, advocating for its integration into existing biomonitoring methodologies or the creation of new tools based on TBA principles, thus advancing biomonitoring science and practice (Akamagwuna *et al.,* 2019; Cornejo *et al.,* 2020). Additionally, traits enable connections between ecosystem well-being (Hevia *et al.,* 2017), shedding light on species resource utilization, niche partitioning, and the diverse effects on biodiversity on ecosystem processes (Cornejo *et al.,* 2020; Price *et al.,* 2019; Ying *et al.,* 2020). Moreover, traits allows for predicting the ecological consequences of non-native species introductions (Carbonell *et al.,* 2017).For example, a consistent decline in the populations of scrapers and filterers has been noted as fine sediment levels increase (Sutherland *et al.,* 2012). Additionally, large-bodied taxa, have external and exposed gills, and are associated with stony substrates have shown intolerance to high levels of FPOM (Bona *et al.,* 2016; Buendia *et al.,* 2013).

Characteristics of an organism determine its interaction with the surrounding environment and can offer a detailed understanding of how different taxa interact with their habitats (Pilière *et al.,* 2016). In sub-Saharan Africa, the utilization of organism traits for monitoring freshwater ecosystems is still relatively underdeveloped. (Statzner & Bêche, 2010) stressed the significance of selecting traits that are directly linked to specific stressors. For instance, in the Tsitsa River and its tributaries, (Akamagwuna *et al.,* 2019), found that sedimentation caused by soil erosion and unregulated livestock grazing is a primary water quality stressor. This situation presents an opportunity to apply (TBA) in environments predominantly impacted by a single stressor (Table 2).

Table 2: Predictions of trait attribute assemblage responses to a gradient of fine sediment stress (−) indicates decreased abundance and (+) increased abundance (Akamagwuna et al., 2019).

Trait category	Trait attributes	Predicted response	Mode of stress	Supported reference
1.Gill type	Filamentous gill (FIL_g) Plate-like (PL-G) Lamellate gill (LA_G) Operculate gill (OP_G)	− − − +	Physical abrasion and clogging of exposed gill surfaces by fine particles Abrasion and clogging of exposed gills Abrasion and clogging of exposed gills Internal respiratory organs are protected from abrasion	Larsen *et al.,* 2011) Townsend & New Zealand, 2008) Larsen *et al.* (2011) (Corbin & Goonan, 2010)
2.Locomotion	Burrower (B1) Crawlers (CRA), Sprawlers Clingers/ climbers (B4) Swimmers (SWI)	+ − − + +	Adapted to burial and the modified substrate Covered substrate and burial by fine particles Covered substrate and burial by fine particles Burial by fine particles and modified substrate Actively swim away from high turbidity due to fine sediment	(Mondy & Usseglio-Polatera, 2013) (Buendia *et al.,* 2013; Mathers, Rice, *et al.,* 2017) (Rabení *et al.,* 2005; Sutherland *et al.,* 2012) Rabení *et al.* (2005) Sutherland *et al.,* (2012) and Buendia *et al.,* (2013)
3. Food preference	Detritus (FPOM)	+ +	Increased availability of fine organic matter	−

Trait category	Trait attributes	Predicted response	Mode of stress	Supported reference
	Detritus (CPOM) Macrophytes / algae Animal materials (AN_M)	+ +	Increased availability of organic matter Reduced primary productivity and food quality Increased turbidity	(Doretto *et al.,* 2017) (Graham, 1990) −
4. Feeding habits	Filter feeder (FI_F) Grazers/scrapers (GRA) Shredders (SHR) Predators (PRE)	− − − −	Clogging of the filter feeding net Decreased quality and quantity of fine organic food particles Decreased quality and availability of leaf materials due to fine particles Reduced visual capacity due to increased turbidity	Rabení *et al.,* (2005) (Oliver *et al.,* 2012), (McKenzie *et al.,* 2024) (Descloux *et al.,* 2014) and Doretto *et al.,* (2017) (Bona *et al.,* 2016)
5. Maximum body size	<5mm (SIZE 1) and >5-10 mm (SIZE 2) >10–20 mm (SIZE 3) and > 20 mm (SIZE 4)	+ −	Decreased interstitial spaces and colmation Increased clogging and reduction of interstitial spaces	Buendia *et al.,* (2013) (Bryce *et al.,* 2010)

2.6 Classification and representation of traits

The classification and representation of traits should accurately reflect their true ecological significance to ensure meaningful ecological analysis and interpretation. Traits in organisms can be broadly classified into two types: categorical and continuous. Categorical traits exhibit distinct and compartmentalized forms. For instance, body shape can be categorized into streamlined, ovate, cylindrical, or combinations of these categories, especially when multiple life phases of the organism exhibit varying shapes (Akamagwuna *et al.,* 2023; Akamagwuna & Odume, 2020). These distinct categories help differentiate between the forms and are essential in studies requiring precise classification. On the other hand, continuous traits are characterized by a gradient and are inherently less compartmentalized. These traits include a wide range of characteristics such as body size, the number of eggs produced, flight distance, lifespan, age at maturity, depth preference, turbidity preference, flow preference, number of offspring per reproductive event, and the number of egg masses, among others (Table 3). Continuous traits often vary along a spectrum and can give accurate data on the organism's biology and ecological interactions.

Classification of macroinvertebrates into traits in African rivers is in its infancy. The macroinvertebrates trait database for Southern Africa was developed by Odume et al., 2023. It is the only one in Africa (Table 3). The database addresses the need for both categorical and continuous data by primarily categorizing most continuous traits to facilitate ease of analysis. This approach enhances the ecological relevance and applicability of the trait data, facilitating deeper insights into organismal biology and ecological interactions.

2.7 The selection and justification for choosing specific traits

Depending on the level of detail in trait data collection, a single taxon may possess a multitude of traits (Table 3). As a result, only characteristics thought to be of relevance in the context of biomonitoring are taken into account. For example, Statzner and Bêche (2010) have continuously emphasized the need to avoid compiling characteristic data needlessly, since this might compromise the effectiveness of TBA in biomonitoring applications. Therefore, easy measurement, observation, and analysis, their mechanistic relationship to anthropogenic stressors, their predictive power, their potential interactions with ecosystem function, and the availability of trait data are some factors that affect the choice of traits. Despite their being useful indicators of ecosystem health and functioning, macroinvertebrates are difficult to use since many tropical streams lack sufficient data on their structural and functional diversity (Boyero *et al.,* 2009b; Masese *et al.,* 2014).

The macroinvertebrate FFGs and characteristics of Kenyan streams are still still underdeveloped, therefore leading to difficulties in utilizing and understanding them as surrogates of ecosystem parameters. The Wundanyi and Bura rivers are low, moderate, and high-disturbed rivers with a variety of land uses along the river gradient. This study therefore investigated macroinvertebrate FFGs and traits of these rivers along temporal (wet and dry season) and spatial (land use and longitudinal) scales, using FFGs ratios as an alternative for ecosystem attributes.

Table 3: Traits and trait attributes captured in the database for southern African freshwater invertebrates. Source: (Odume et al., 2023).

Biological trait		**Behavioral trait**		**Ecological preference**	
Trait	No. of trait attributes	Trait	Number of trait attributes	Trait	Number of trait attributes
Maximum body size	6	Mobility	8	Velocity preference	5
Body shape	6	Functional feeding group	11	Depth preference	5
Respiration	8	Attachment mechanism	4	Habitat preference	8
Potential no. of generations per year (voltinism)	5	Mode of dispersal	4	Water quality preference	4
Reproduction type Age to maturity Adult life span Number of offspring per reproductive event Reproduction type – number of egg masses Aquatic stage Resistance form Body armor	6 6 5 6 3 8 6 5	Dispersal potential (non-adult stages in metres) Dispersal potential (adult stages in metres) Emergence season Emergence duration Exit the water temporarily Oviposition behaviour Diapause Drift	6 6 5 5 3 10 2 4	Hydraulic preference Preferred reproductive periods pH preference Thermal preference Critical thermal maximum (oC) Incipient lethal upper limit (oC) Optimal temperature of emergence (oC) Saprobic preference Stream zonation preference Biological distribution/ endemism Trophic status preference Turbidity preference	5 5 4 3 5 6 4 3 5 4

Table 4: Selected traits, trait attributes, and the predicted responses to disturbance for invertebrate communities; (− and +) indicate potential decreases or increases of the proportion of trait attributes in the face of disturbance, and ± indicates either a potential increase or decrease (Castro et al., 2018; Feio & Dolédec, 2012; Mondy & Usseglio-Polatera, 2013; Scotti et al., 2020).

Trait and trait attributes	Code	Predicted responses	Rationale
Body size (mm) Very small (≤5) Small (5 – 10) Medium (10 – 20) Large (20 – 40) Very large (>40)	 Size 1 Size 2 Size 3 Size 4 Size 5	 + ± - - -	Rapid population growth and resilience capacity increase (Feio Small (5 – 10) SIZE & Dol´edec, 2012) Sensitivity to unstable habitat and colonization by fine sediment from agricultural activities (Bryce *et al.,* 2010; Akamagwuna *et al.,* 2019)
Voltinism Semi-voltinism (<1 generation per year) Univoltinism (1 generation per year)	 SEM UNI	 + -	A shorter life cycle increased population recovery and turnover per year after disturbance (Dol´edec *et al.,* 2006; Townsend et al., 2008) Decreased turnover and recovery in adverse conditions (Townsend *et al.,* 2008)
Life-cycle duration Less than one year (<1year) greater than one year (>1 year)	 LEY GR_Y	 + -	Rapid population growth and increase of resilience capacity after disturbance (Townsend & Hildrew, 1994) Decreased recovery time and population after disturbance (Dol´edec *et al.,* 2006)
Aquatic stages Adult Larvae Nymphs Pupa	 ADU LAR NYM PUP	 + - - -	Life stage is less fragile and vulnerable to disturbance (Mondy & Usseglio-Polatera, 2013) Increasing harsh environmental conditions from agricultural disturbance (Mondy & Usseglio-Polatera,2013)
Respiration Gills Tegument Spiracles (aerial)	 GIL TEG SPI	 – – +	Sensitivity to decreasing oxygen availability due to nutrient input (Feio & Dol´edec, 2012; Mondy & Usseglio-Polatera, 2013)

Lungs Haemoglobin	LUN HAE	+ +	Increased resilience to decreasing oxygen availability from organic pollution (Feio & Dol´edec, 2012; Mondy & Usseglio-Polatera, 2013)
Oviposition behaviour Endophytic oviposition Exophytic oviposition	 EN_O EX_O	 + –	Eggs are protected from harsh environmental conditions and predation (Diaz *et al.,* 2008; Akamagwuna, 2021) Increase egg exposure to adverse conditions from agricultural disturbance and predation (Diaz *et al.,* 2008; Akamagwuna, 2021)
Body shape Streamlined Flattened Spherical Cylindrical	 STR FLA SPH CYL	 + + – –	Resilient to current effects resulting from flow alteration for agriculture and unstable habitat (de Castro *et al.,* 2018) Sensitivity to drift by the current
Body amour Soft and exposed Sclerotised Cased/tubed	 SO_E SCL CAS	 – ± +	Sensitivity of soft and exposed body surfaces to abrasion by fine sediment (Jones *et al.,* 2012) Body surface protected by chitinous scleritin from fine particles during high agricultural disturbance (Akamagwuna *et al.,* 2019)
Feeding groups Shredders Gatherers Filter-feeders Scrapers/grazers Predators	 SHR GAT FI_F SCR PRE	 – – ± – –	Sensitivity to food web change from reduced basal resources availability resulting from riparian habitat alteration (Feio & Dol´edec, 2012; Mondy & Usseglio-Polatera, 2013) Sensitivity to food web changes due to increased organic input (Mondy & Usseglio-Polatera, 2013) Decreased quality and quantity of fine organic food particles (Wilkes *et al.,* 2017) Reduced visual capacity due to increased turbidity and biomagnification (Waters, 1995)
Food types Fine particulate organic matter (FPOM) Coarse particulate organic matter (CPOM) Macrophytes Animal materials	 FPO CPO MAC AN_M	 ± – – ±	Increased input of particulate organic matter Reduced primary productivity and food quality (Graham, 1990) Sensitivity to increased turbidity

Locomotion Crawler Sprawler Swimmer Skater Burrower Climber/clinger	 CRAW SPR SWI SKAT BUR CLI	 – – ± + + +	Sensitivity to burial effect by sediments (Rabení *et al.,* 2005) Increase habitat utilisation as a refuge during increased disturbance from hydrological alteration (de Castro *et al.,* 2018)
Substrate attachment Permanently attached Temporarily attached Free-living **Habitat preference** Stones Sediment (gravel, sand and Mud) Vegetation	 PE_A TE_A FR_L STO SED VEG	 – ± + – – +	Sensitivity to decreased availability of clean and stable substrate due to hydrological modification Resilient to decreased availability of clean and stable substrate and ability to move away from areas of increased perturbation, able to move away from disturbed environments (Wilkes *et al.,* 2017) Sensitivity to unstable and clean substrates due to fine particles Resilient to substrate modification
Velocity preference (m/s) Very fast-flowing (>0.6) Moderately fast flowing (0.3 – 0.6) Slow flowing (1 – 0.3) Very slow-flowing (0.1 – 1)	 VF_F MF_F SF VS_F	 + ± – –	Adaptation to drift by water current changes from hydrological and flow alteration (Shieh *et al.,* 2012) Sensitivity to hydrological and flow alteration (Shieh *et al.,* 2012)

CHAPTER THREE

METHODOLOGY

3.1 Study Area

South-eastern Kenya's Taita Taveta County is home to the rugged Taita Hills region (Fig. 1). The savannah grasslands, which are a part of the Tsavo National Park, surround the region, which has a surface area of about 1000 km2. According to (Hohenthal *et al.,* 2015; Pellikka, 2013), the region has an average elevation of 1500 meters above sea level (m.a.s.l.), with the lowest point in the surrounding low-lying plains (600 m.a.s.l.) and the highest point in Vuria (2208 m.a.s.l.). The intertropical convergence zone influences the region's bimodal rainfall pattern, with the first rainy season (long rains) from March to June and the second rainy season (short rains) from October to December (Hohenthal *et al.,* 2015; Pellikka *et al.,* 2009). The low-lying plains in the region receive an average rainfall of 500 mm per year, whereas the mountainous area receives 1500 mm annually (Pellikka *et al.,* 2013). Even though it is under increasing strain and only has around 1 % of the original forest cover left, the Wundanyi and Bura river catchments form an important natural conservation area with native rainforest and a high diversity of fauna and flora (Hohenthal *et al.,* 2015). Given that it is the source of several important springs and river systems, including the Voi and Mwatate rivers, the Taita Hills region is also a significant source of water in Taita Taveta and other coastal Counties in Kenya (Pellikka *et al.,* 2013)

The population of the County is estimated at 340,671 persons according to the 2019 national census (Kenya National Bureau of Statistics, 2019), with population densities ranging from 14 persons per km2 in the lowlands to >117 persons per km2 in the uplands. The lowland areas of the County outside the national parks are occupied by farms, ranches, estates, and wildlife sanctuaries. The County has approximately 25 ranches. The major land uses in the ranches are cattle grazing and mixed crop farming, intensive cultivation of crops such as maize and bananas is practiced in the highlands. Free-range livestock grazing regime is commonly practiced in the lowlands with animals watering directly from the rivers and artificially constructed water pans (Thornton *et al.,* 2002; Waiswa, 2020). The foothill and lowland zones are dominated by shrubs, sisal plantations, and acacia tree species with dryland agriculture and livestock grazing (Pellikka *et al.,* 2018).

The Wundanyi-Bura rivers are a typical Afromontane River arising in forested uplands where human activity is minimal and livestock do not access the watering points, as zero-grazing is the most practiced form of a livestock production system. However, human activity increases in the middle and lower reaches. Because of the high human population and that of their livestock, the river is influenced by various human activities along its reach, including farming, human settlements and small urban centers, sand mining, water abstraction for irrigation and domestic use, animal grazing and watering, bathing and laundry washing (Waiswa, 2020). Changes in the rainfall amount between the uplands and lowlands, coupled with excessive water withdrawals, have converted once permanent river reaches into seasonal ones that experience cessation of flows during periods of prolonged droughts.

3.2 Study Design

To analyze the effects of human disturbance (disturbance category) caused by mixed crop farming, water abstraction, sand harvesting, livestock grazing, and climate-driven changes in seasonality, and flow variability (permanence) on macroinvertebrate communities, sampling

Study sites were grouped into 3 different levels of disturbance (low, moderate and high disturbance), this was based on catchment land use, and reach scale, human and livestock influences. Further, the sites were grouped according to river type/flow permamanence (permanent flows) and those whose flows ceased during the dry seasons (seasonal). Eventually, all sites were sampled during the dry and wet seasons. In total, 18 sites were selected for sampling (Fig. 1) and Table 5): and grouped as follows: low disturbance (n = 7), moderate disturbance (n = 4), and high disturbance (n = 7) (Table 5). On flow permanence, sites with permanent and seasonal flows were (n = 9). All the 18 sites were sampled for macroinvertebrates and physical habitat conditions during the wet and dry seasons. All the sampled sites traversed a land-use gradient from the forested upper reaches, through the mixed of agricultural activities, livestock activities, sand harvesting, water abstraction, and urbanization. The lower reaches were largely agricultural and wildlife conservancy with some sites frequented by wildlife (Table 5; Plates 1-4).

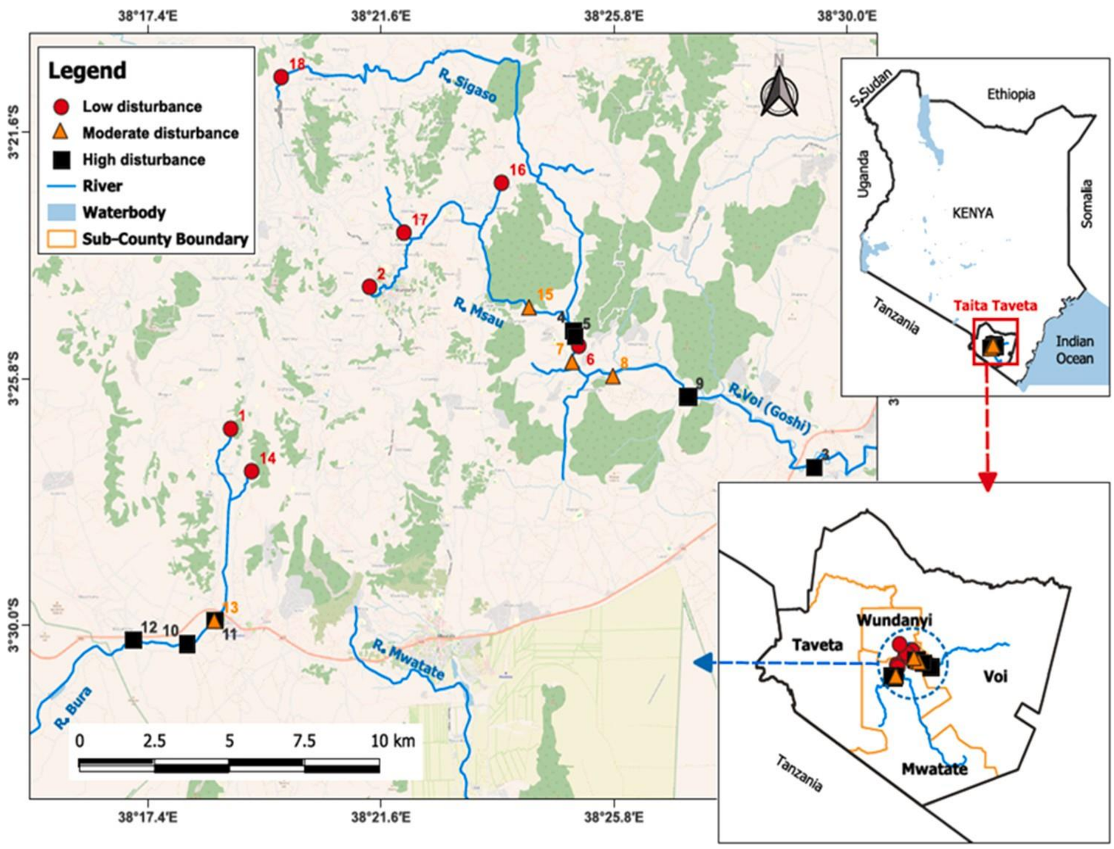


Figure 1: Study sites along the Wundanyi and Bura Catchment, Kenya

Table 5: Description and characteristics of the site categories.

Site group	Sites	Flow Permanence	Location (Latitude, Longitude)	Characteristics
Low Disturbance	Wundanyi River	Permanent	3° 26' 39"S, 38° 18' 53' E	Forested catchment with > 80% under forest -Riparian zone intact with >70% instream canopy cover – Water is clear with a stony, sand substrate - major human activity through Agriculture
	Ruma River	Permanent	3° 24' 14' S, 38° 21' 23' E	
	Musau Mwasafu	Seasonal	3° 25' 11' S, 38° 25' 10' E	
	Nyolo	Permanent	3° 27' 22' S, 38° 19' 16' E	

Site group	Sites	Flow Permanence	Location (Latitude, Longitude)	Characteristics
	Lembenyi	Permanent	3° 22' 27’ S, 38° 23' 46’ E	
	Lagale 1	Permanent	3° 23' 18’ S, 38° 22' 0’ E	
	Lagale 2	Permanent	3° 13' 53’ S, 38° 12' 56’ E	
Moderate Disturbance	Mwakinyungu	Seasonal	3° 25' 45’ S, 38° 25' 46’ E	-Moderate disturbance catchment with > 70% under moderate disturbance -Riparian zone is less intact with livestock and agricultural activities along the banks -Mucky water with a lot of sedimentation due to sand harvesting and OM loading due to livestock watering -Some other human activities include water abstraction, bathing, washing clothes, animal grazing, and sand harvesting
	Mlalenyi	Seasonal	3° 29' 55’ S, 38° 18' 35’ E	
	Bura Mission	Seasonal	3° 29' 55’ S, 38° 18' 35’ E	
	Kwa Muindi	Permanent	3° 24' 35’ S, 38° 24' 16’ E	
High Disturbance	Musau Maganga	Seasonal	3° 25' 10’ S, 38° 25' 9’ E	Located in agricultural catchment but agriculture and forest both are less than 60% -Agricultural practices at the catchment but some with intact riparian zones.
	Musau Ndembonyi	Seasonal	3° 25' 30’ S, 38° 25' 2’ E	
	Musau Bridge	Permanent	3° 26' 6’ S, 38° 27' 8’ E	
	Kwa Mdoe	Seasonal	3° 30' 19’ S,	

Site group	Sites	Flow Permanence	Location (Latitude, Longitude)	Characteristics
			38° 18' 5’ E	-Some with banks with undercuts.
	Mwashuma	Seasonal	3° 30' 15’ S, 38° 17' 7’ E	-Used for water abstraction for domestic use and animal watering points -Mixed canopy of both indigenous and exotic trees High livestock activities

Plate 1: Sites of low disturbance 1 Sites of low disturbance. Plate A shows the Wundanyi River, while Plate B shows the Nyolo site.

Plate 2: Sampling sites of moderate disturbance. Plate A is Msau Maganga, while Plate B is Msau Ndembonyi (Credit: Godfrey Owuor, 2022).

Plate 3:Sampling sites of high disturbance. Plate A is for Msau Munda, while Plate B is for Msau Bridge. (Credit: Godfrey Owuor, 2022)

Plate 4: Illustration of the effects of seasonality on streams. Dry river beds with occasional pools were observed. Plate A is the Msau Mwakinyungu, while Plate B is the Msau Bridge. (Credit: Godfrey Owuor, 2022).

3.3 Analysis of catchment land-use

Land-use classification and catchment maps were generated using QGIS version 3.14. The semi-automatic classification plugin was used to download Sentinel-2 images for the six catchments of the streams used in this study according to Congedo (2020a, b). The satellite images were pre-processed for four categories of predominant land use in the study area (forest, grassland, cropland, and built-up areas or bare). For all sampling sites, catchments were delineated as the entire landscape contributing surface water to the site. The area of land use category in the catchments of the sampling site was calculated and utilized to determine the percentage of every land use per sampling site.

3.4 Measurement of physicochemical parameters and laboratory Analysis

Field sampling was done during wet and dry seasons (December-January 2021/2022), dry season months were (June-August 2022). Both macroinvertebrates and water quality parameters were sampled within a 100-m representative reach at the sampling sites. Some water quality parameters were measured in situ using a YSI multiprobe water quality meter (556 MPS; Yellow Springs Instruments, Yellow Springs, OHIO, USA), including dissolved oxygen concentration (DO, mg/L), temperature (◦C), electrical conductivity (EC, µS/cm), salinity mg/L, TDS, mg/L, and pH. Nutrient analyses, filtered water samples through pre-weighed and pre-combusted Whatman GF/FF glassfiber filters (0.42 mm thickness, 0.7 µm pore size, and 47 mm diameter) were drawn per site in acid-washed HDPE bottles, and later fixed with sulphuric acid. These filtered water samples were later used to analyse nitrite (mg/L), nitrates (mg/L), ammonium (mg/L), SRP (mg/L), and DOC (mg/L. Volumes of filtered water samples through the GFF filters were recorded, and the filters with attached sediments were retained for the analysis (TSS, mg/L) and (POM, mg/L). Samples of unfiltered water were collected in HDPE bottles (500 ml) and transported to the laboratory for analysis of TP (mg/L).

Each study site, measurements of water depth (m), velocity (m^3/s), and river width (m) were taken. Cross-sectional width measurements were made using a measuring tape, whereas velocity and depth were measured using a meter rule and velocity plank. Stream discharge (m^3/s) was determined by the velocity area method (Wetzel & Likens, 2000). According to (Lakew & Moog, 2015), the substrate in the sampling sites was characterized by identifying the substrate types that constituted >5 % coverage of the study site.

Qualitative and habitat evaluation were used to assess the quality of riparian and instream habitats as outlined by Rankin *et al.,* 1995, which has been improved for the Lake Victoria Basin (Raburu & Masese, 2012). In addition, all animal (livestock) and human activities in the vicinity of the sampling sites were recorded and used in the calculation of habitat quality. These include livestock grazing or watering, sand harvesting, water withdrawals, channelization, laundry washing and washing of vehicles, etc.

APHA, 2005 laboratory protocols were used in the laboratory for the determination of nutrients in water samples. The data were divided into two categories or datasets: physicochemical variables and nutrients. The data on physicochemical measures included pH, DO (mg/L), temperature (◦C), salinity (mg/L), EC (µS/cm), TDS (mg/L), TSS (mg/L), and POM (mg/L). The second dataset was on nutrients and included ammonium NH_4^+ (mg/L), nitrates (NO_3) (mg/L), nitrite (NO_2^-) (mg/L), SRB (mg/L), total phosphorus (mg/L), and DOC (mg/L). These variables have demonstrated their sensitivity to broader catchment-scale influences, such as agricultural or urban land use, as well as local-scale disruptions like wastewater disposal, removal of riparian zones, livestock disturbances, and other stressors, as evidenced by previous studies (Hwang et al., 2016; Iteba et al., 2021; Minaya et al., 2013; Wanderi et al., 2022).

3.5 TSS, POM, and DOC Analysis

Embedded sediments on GF/F filters were dried at 60◦C for seventy-two hours to reach constant weight; they were then re-weighed, and the filters' weight was subtracted for TSS analysis. Filters were ashed in a muffle furnace at 450 ◦C for 4 h and re-weighed for the POM analysis so as to distinguish the difference between TSS and ash-free-dry mass/weight.

TSS (mgL 1) = ((A B) / V) ∗ 106 …Equation 1

POM (mgL 1) = ((C B) / V) ∗ 106 …Equation 2

Where: A = mass of filter + dried residue (g), B = dry mass of filter (g), and V = volume of sample filtered (L), B = dry mass of filter (g), C = Weight of ashed filter (g), and V = volume of sample filtered (L).

DOC analysis, the water samples were initially filtered in situ using GF/F filters stored in new or acid-washed 60 mL HDPE bottles and stored in the cold at freezing temperatures. Measurements for DOC concentrations were determined using the Shimadzu TDN analyzer unit (TNM-1) at the International Livestock Research Institute (ILRI), Nairobi, which is also equipped with a total dissolved organic carbon analyzer (TOC-V CPN). The autosampler for the Shimadzu instrument utilizes chemiluminescence for TDN oxidative combustion for DOC.

3.6 Macroinvertebrate sampling and identification.

The sampling of macroinvertebrates was done using a SASS net (mesh size: 1000 µm), a semi-quantitative sampling protocol as outlined by Dickens & Graham, 2002. The biotopes that were identified and sampled at each sampling site were: (1) GSM: gravel, sand, and mud; (2) STONES: bedrock, boulders, cobbles, and pebbles; (3) VEG: in stream and marginal vegetation (Masese et al., 2021). Sampling process entailed disturbing and kicking the bottom of the net upstream, allowing water currents to wash the dislodged invertebrates. 3 replicates per biotope (9 kick samples per site) were collected. 398 macroinvertebrate kick samples from the biotopes were collected, sorted in the field, and thereafter preserved in 75 % ethyl ethanol. In the laboratory, macroinvertebrate samples were sorted, counted, and eventually identified to the genus level. Identifications were done with the aid of keys from several guides (de Moor *et al.,* 2003a; de Moor *et al.,* 2003b; Merritt *et al.,* 2008).

Allocation of FFGs was based on studies by (Fry, 2022; Dobson *et al.,* 2002; Masese *et al.,* 2014; Merritt *et al.,* 2008), where 5 FFGs were allocated based on numerical abundance (collector–gatherers, and collector–filterers {filterers, predators, scrapers, and shredders). Of the 5 FFGs, their use as surrogates of ecosystem attributes was determined using various ratios of FFGs based on numerical abundance according to Cummins *et al.* (2005) as:

i. (Production/respiration [P/R]) index, which was determined as the scrapers to (shredders + total collectors) ratio;

ii. Coarse particulate organic matter and fine particulate organic matter (CPOM/FPOM) index was calculated as the ratio of shredders to total collectors;

iii. Ratio of predators to prey (total of all other groups) was used to calculate the top-down control index;

iv. Filterers to gatherers ratio was used to determine the transport fine particle organic matter and benthic fine particulate organic matter (TFPOM/BFPOM) index;

v. Scrapers + Filterers: Shredders + Gatherers ratio was used to determine the stable channel index.

3.7 Trait and ecological preference selection and fuzzy coding

14 biological traits were selected and categorized into 52 distinct trait attributes based on ecological preferences (Tables 3 & 4). These traits encompassed aspects of life history (such as duration of life cycle, stages spent in water, and frequency of generation), morphological and physiological features (including body form, size, protective structures, respiratory modes, and attachment mechanisms), behavioral patterns (feeding strategies, egg-laying methods, and movement), as well as ecological preferences (preferred substrates, dietary preferences, and flow velocity affinities).

Trait categories were selected and identified based on their known functional responses with anthropogenic stressors like grazing, agricultural activities, sediment influx, hydrological changes, and changes in environmental parameters such as turbidity, pH, D.O. concentrations, and canopy cover. The availability of data and its relevance to stressor reaction affected attribute selection. (Ding *et al.,* 2017; McKie *et al.,* 2018; Poff *et al.,* 2006; Scotti *et al.,* 2020; Wang *et al.,* 2019). Trait data were provided for 70 taxa at the genus level. Where the genus-level data were lacking, family-level data were used instead. Average characteristic of affinities for taxa that were only recognized at the family level were identified by using data from every known genus in the family, but this only applied to a few taxa. Individuals that were identified to genus level but had no specific genus-level trait data were, in turn, subsequently given family-level information.

Trait assignments were primarily sourced from the South African macroinvertebrate trait database (Odume *et al.,* 2018), supplemented by direct lab observations and a range of published sources (Desrosiers *et al.,* 2019; Mondy & Usseglio-Polatera, 2014; Odume *et al.,* 2023; Palmer & O'Keeffe, 1992; Usseglio-Polatera *et al.,* 2000).

Trait attributes were assigned to each taxon using fuzzy coding (Appendix 3), which enables the representation of a taxon's partial affinities across multiple trait attributes. This method employs affinity scores ranging from 0 to 5, where 0 signifies no association and 5 represents a strong association with a specific trait. Scores of 1 and 3 reflect weak and moderate associations, respectively. Fuzzy coding accommodates intra-family variation and differences across developmental stages (Chevene *et al.,* 1994), while also addressing the uncertainty inherent in literature-derived data and avoiding oversimplified, single-trait assignments (Mondy & Usseglio-Polatera, 2014).

3.8 Statistical analyses

Analyses were performed with R version 4.3.0 (R Development Core Team, 2017), using the packages vegan (Oksanen et al., 2013), sem (Fox, 2006), and deSolve (Soetaert *et al.,* 2010). Figures created in Sigma Plot (Version 12), MS Office Excel (2016), and R version 4.3.0 (R Development Core Team 2017). Means ± standard deviation and plots were used to present spatial and temporal variation in water quality and habitat quality variables at different site categories (disturbance gradients- High, Moderate, and Low), seasonality, and flow permanence. ANOVA was used to test for differences in physicochemical and habitat variables among disturbance gradients (low, moderate, and high), seasons (dry and wet), and flow permanence (permanent vs seasonal), with disturbance gradients and seasons as main factors and disturbance gradients × season interaction term. Where there were no significant seasonal differences, the data were pooled, and one-way ANOVA was used to test for differences among disturbance categories, followed by Tukey's multiple post-hoc comparisons of means. Before analysis, count data were log (x + 1)-transformed, while the rest of the response variables were log-transformed to meet normality assumptions. To reduce the dimensionality of physicochemical and environmental variables data PCA was used. Two PCs were further used to characterize the physicochemical and habitat quality factors. PERMANOVA, which is based on Bray-Curtis dissimilarity matrices, was used to further evaluate PCAs (McArdle & Anderson, 2001). The average rank similarities of macroinvertebrate FFGs were again examined across disturbance site categories, seasons, and flow permanence using ANOSIM, with duplicate disturbance gradients nestled inside seasons. ANOSIM computes the R-statistic, which is a test statistic that ranges between 0 and 1; higher values indicate greater differences across factors.

NMDS was then utilized to show macroinvertebrate functional composition across different disturbance gradients, seasons, and flow permanence (Clarke & Gorley 2006). Coefficients and dissimilarity matrices were calculated using Bray-Curtis (Bray & Curtis, 1957) for two sets of data, which entailed untransformed abundance data and presence-absence data for FFGs. The fit of the ordination was determined by the size of the related stress value (< 0.2, which indicates an excellent ordination) (Kashian *et al.,* 2007). SIMPER analysis was done to verify the key macroinvertebrates that were observed differences between disturbance site gradients, habitat, water quality, and flow permanence (permanent vs seasonal).

The % contribution of FFGs to the overall dissimilarity was quantified between the disturbance site gradient and flow permanence per season. SIMPER, which is a restrictive pairwise analysis between two-factor levels, was used (Clarke and Warwick 2001). In this case, comparisons were done between high and moderate, high and low, and lastly moderate and low disturbance levels. For flow permanence, comparisons were between seasonal and permanent sites per season.

RDA was ideally performed to determine the correlations and interactions between macroinvertebrate assemblages and environmental variables. The results were shown as triplots, with plotted points for species, FFGs, and disturbance site categories linked to physicochemical and habitat factors represented as rays. Before performing RDA, the gradient length in SD units was determined using Detrended Correspondence Analysis to determine the applicability and suitability of CCA (Ter Braak & Smilauer, 1998), but because the gradient length was less than 3 SD, RDA was used instead of CCA to evaluate which elements were important for the groups of FFGs among disturbance site categories (Ter Braak & Smilauer 2004).

RLQ (R-environmental descriptors of the sampling sites; L-taxon abundances of samples; Q-taxa traits) analysis was used to investigate the relationships between traits and ecological preferences and disturbance categories during the dry and wet periods. RLQ analysis is a step-by-step ordination carried out on three ordination analyses that uses three separate data matrices (Dol´edec *et al.,* 1996). CA is the first ordination, and it uses the taxon abundance (L-table). Second ordination uses the physicochemical data (PCA R-table). This ordination procedure is used to link the taxonomic data to the physicochemical data with the sample scores from the CA results. H-S analysis is the third ordination, which links the taxonomic data to the trait data using the taxon scores obtained from the CA as row-weights. Lastly, the RLQ model produces ordinations simultaneously on the three separate ordinations (CA, PCA, and H-S). All the macroinvertebrate taxa, traits, and environmental variables from each site were used in the RLQ model.

To understand and corroborate the identification of characteristics and preferences susceptible to or tolerant to pollution, a multivariate combination of fourth-corner and RLQ was used to clarify a pair-to-pair link between traits and environmental factors. This analysis revealed whether characteristics and physicochemical factors had positive or negative associations or interactions.

Fourteen environmental variables and nine habitat quality variables were included in the analyses respectively: DO (mg/L), pH, Temperature (^{o}C), electrical conductivity (µS/cm), TDS (mg/L), TSS (mg/L), POM (mg/L), TP (mg/L), salinity (mg/L), DOC (mg/L), Nitrate (mg/L), Nitrate (mg/L), SRP (mg/L), and Ammonia (mg/L); Wetted width (m), Mean depth (m), Discharge (m/s), velocity (m^3/s), substrate, Instream canopy cover, channel morphology, riparian zone and bank erosion, pool/glide and riffle/run quality..

Two-way MANOVA tested the significant influence of site category and period on the physicochemical variables. Post hoc comparison tests identified sites pairs that significantly differed. Before MANOVA, Shapiro-Wilk’s and Levene’s evaluated the environmental dataset’s normality and homogeneity of variance.

CHAPTER FOUR

RESULTS

4.1 Physicochemical variables and habitat quality

There was a significant difference ($p < 0.05$) in discharge, water velocity, riparian zone, and bank erosion, and pool and riffle quality recorded along the disturbance gradient (Table 6). Discharge (5.3 ± 2.0m /s), velocity (0.5 ± 0.03–1 m/s), and riparian zone and bank erosion (7.2 ± 0.2), pool and riffle quality (6.7 ± 0.5) were significantly higher at high disturbance sites, whereas substrate type or diversity (13.98±1.0) was significantly higher in low-disturbance sites. Moderate disturbance sites recorded the lowest values of discharge and velocity and low-disturbance sites recorded the highest values on stream substrate and channel morphology (Table 6). There were significant differences in physicochemical variables ($p < 0.05$) for DOC, total phosphorus, pH, and EC between site categories (high-, moderate-, and low-disturbance). The highest values of DOC (27.93±8.14 mg/l) were recorded at moderate disturbance sites, total phosphorus (1.1 ± 0.34 mg/l), and EC (718.81±22.46 μS/cm) recorded the highest values at high disturbed sites whereas pH (8.16±0.1) had the highest values at low disturbance sites. The low disturbance category recorded the lowest values for DOC and EC while total phosphorus and pH recorded the lowest values at moderate disturbance and high disturbance sites, respectively, whereas TSS, POM, nitrite, and nitrate were higher during the dry season (Table 2). There were significant variations in water quality parameters between permanent and seasonal sites during both dry and wet seasons. Among the physicochemical parameters, only temperature (◦C), DOC (mg/L), and ammonia (mg/L) significantly differed between the two site categories during the dry season. Permanent sites had a lower temperature (23.9 ± 0.9 ◦ C) than seasonal/intermittent sites (28.0 ± 1.1 ◦ C).

DOC and ammonia had a similar trend with permanent sites having significantly lower levels (DOC: 8.6 ± 1.8_mg/L; ammonia: 2.1 ± 0.6_mg/L) than seasonal sites (DOC: 24.3± 7.3; ammonia: 4.7 ± 1.1_mg/L). In the wet season, significant differences ($p < 0.001$), were observed in temperature, EC, and total phosphorus, with seasonal streams exhibiting higher temperatures (26.5 ± 0.91), TDS (88.0 ± 11.91), and EC (698.2 ± 2.69). In contrast, permanent sites had lower TP (0.3 + 0.13) than seasonal sites (0.9 + 0.23). Conversely, levels of TDS (mg/L), salinity (mg/L), DO (mg/L), pH, TSS (mg/L), POM (mg/L), and DOC (mg/L), were not significantly different between the two flow permanence categories in the wet season (**Table 7**).

Table 6: Means (± SE) of habitat quality, water quality physico-chemical variables, and stream size variables in the different disturbance categories in the Bura and Wundanyi rivers.

	Level of disturbance			ANOVA test	
Parameter	Low	Moderate	High	F	P value
Habitat quality					
Wetted width (m)	5.2±1.1 a	2.9±0.8a	6.0±1.23 a	3.88	0.14
Mean depth (m)	0.1±0.02 a	0.1±0.01 a	0.1±0.01 a	2.12	0.35
Discharge (m^3/s)	2.0±1.42c	0.3±0.1b	5.3±2.0a	6.77	0.03 *
Velocity (m^3/s)	0.3±0.04b	0.3±0.03b	0.5±0.03a	12.23	0.002 *
Substrate	13.98±1.0 a	12.4±1.2 a	11.4±0.8 a	2.84	0.24
Instream cover	10.3±0.4 a	9.6±0.7 a	9.5±0.5 a	1.14	0.57
Channel morphology	7.9±0.1 a	7.0±1.01 a	6.6±0.8 a	3.7	0.16
Riparian zone and bank erosion	5.9±0.2b	5.8±0.3b	7.2±0.2a	13.46	0.001 *
Pool and riffle quality	5.2±0.4a	4.6±0.2b	6.7±0.5a	7.35	0.03 *
Water quality					
Ammonium (mg/L)	1.8±0.52 a	4.0±2.06 a	3.8±0.38 a	1.89	0.17
SRP (mg/L)	0.1±0.03 a	0.1±0.04 a	0.2±0.02 a	0.52	0.6
Nitrates (mg/L)	0.6±0.11	0.3±0.06 a	0.6±0.06 a	1.6	0.22
Nitrite (mg/L)	0.5±0.09 a	0.3±0.1 a	0.5±0.07 a	1.13	0.34
DOC (mg/L)	7.0±0.71b	27.9±8.14a	23.1±4.87a	6.01	0.01*
TP (mg/L)	0.4±0.15b	0.2±0.05b	1.1±0.34a	3.96	0.03*
POM (mg/L)	20.6±3.66 a	26.9±11.14 a	81.3±45.11 a	1.37	0.27
TSS (mg/L)	70.5±11.34 a	57.4±15.91 a	96.3±30.89 a	0.73	0.49
pH	8.2±0.1b	7.9±0.19a	7.8±0.06a	3.21	0.05
DO (mg/L)	5.7±0.13 a	6.3±0.38 a	6.0±0.28 a	1.28	0.29
Salinity (mg/L)	0.4±0.04 a	0.3±0.05 a	0.3±0.04 a	2.08	0.14
TDS (mg/L)	82.7±11.1 a	111.5±24.26 a	96.7±8.82 a	1.02	0.37
Electrical conductivity (µS/cm)	657.8±11.91 b	687.7±5.24b	718.8±22.46a	3.78	0.03*
Temperature (°C)	23.6±0.78 a	25.6±1.46 a	26.5±0.93 a	2.62	0.09
Landcover/Land use					
Bare ground	0.86±0.83	0.01±0.01	0.02±0.01	2.24	0.15
Built	0.93±0.38	2.84±1.92	0.64±0.05	1.85	0.21
Cropland	68.35±17.42	73.27±6.09	62.16±7.2	0.19	0.83
Grassland	6.93±5.64	7.05±2.84	13.8±3.83	0.78	0.48
Shrubs	18.03±16.57	9.05±3.93	13.61±2.87	0.15	0.87
Tree cover	5.39±1.29	7.76±3.66	9.85±2.27	1.11	0.36

Key: ***SRP= soluble reactive phosphorus, TSS= total suspended solids, POM= particulate organic matter, TDS= total dissolved solids, TP= total phosphorus, TSS= total suspended solids, DOC=dissolved organic carbon, DO=Dissolved Oxygen. *P-values marked with asterisks are significantly different among site categories at p < 0.05.***

Table 7: Means (± SE) of habitat quality, water quality physico-chemical variables, and stream size variables in the two flow permanence categories (seasonal and permanent) in the Bura and Wundanyi rivers (For abbreviations and acronyms, refer to Table 6).

Parameter	Season	Permanent	Seasonal	t	*P value*
Wetted width (m)	Dry	5.0±1.5	3.8±0.7	0.666	0.511
	Wet	3.7±1.3	8.3±1.64	2.253	0.032*
Mean depth (m)	Dry	0.1±0.01	0.1±0.02	1.458	0.155
	Wet	0.2±0.04	0.1±0.02	0.426	0.673
Discharge (m³/s)	Dry	0.9±0.6	0.8±0.41	0.12	0.905
	Wet	0.5±0.24	10.5±2.87	3.706	0.001*
Velocity (m³/s)	Dry	0.3±0.04	0.4±0.03	1.542	0.134
	Wet	0.3±0.03	0.5±0.05	4.314	0.000*
Substrate	Dry	14.3±1.01	10.9±0.82	2.474	0.019*
	Wet	13.9±1.08	12.0±0.62	1.353	0.186
Instream cover	Dry	10.3±0.39	8.0±0.51	3.647	0.001*
	Wet	10.2±0.52	10.9±0.39	1.026	0.313
Channel morphology	Dry	8.0±0.14	4.9±1.02	3.486	0.002*
	Wet	7.9±0.17	7.9±0.18	0.183	0.856
Riparian zone	Dry	5.8±0.22	6.9±0.29	3.288	0.003*
	Wet	6.1±0.31	6.7±0.23	1.692	0.101
Pool and riffle quality	Dry	5.0±0.38	5.8±0.6	1.209	0.236
	Wet	5.1±0.4	6.3±0.5	1.876	0.07
Temperature (°C)	Dry	23.9±0.89	28.0±1.11	2.82	0.007*
	wet	22.4±0.54	26.5±0.91	4.07	0.001*
Electrical conductivity (µS/cm)	Dry	655.2±19.7	735.7±41.2	1.89	0.068
	wet	669.0±5.11	698.2±2.69	4.86	0.001*
TDS (mg/L)	Dry	93.3±10.86	108.3±22.92	0.63	0.531
	wet	84.1±13.1	88.0±11.91	0.21	0.832
Salinity (mg/L)	Dry	0.3±0.04	0.2±0.04	1.73	0.094
	wet	0.3±0.04	0.4±0.03	0.22	0.83
DO (mg/L)	Dry	5.9±0.24	5.7±0.37	0.52	0.609
	wet	6.0±0.24	6.4±0.27	1.11	0.274
pH	Dry	8.1±0.13	8.0±0.13	0.70	0.491
	wet	8.1±0.13	7.7±0.12	1.82	0.079
TSS (mg/L)	Dry	89.8±15.55	95.1±54.59	0.10	0.919
	wet	72.6±14.75	62.0±15.82	0.41	0.628
POM (mg/L)	Dry	25.3±7.17	95.3±57.41	1.37	0.18
	wet	27.1±6.23	19.7±2.78	1.04	0.305
TP (mg/L)	Dry	0.4±0.2	0.7±0.35	0.71	0.483
	wet	0.3±0.13	0.9±0.23	2.62	0.014*
DOC (mg/L)	Dry	8.6±1.79	24.3±7.34	2.32	0.027*
	wet	9.8±2.2	27.0±7.3	2.38	0.024*
Nitrite (mg/L)	Dry	0.3±0.08	0.3±0.06	0.04	0.972
	wet	0.6±0.14	0.6±0.11	0.3	0.766

Nitrates (mg/L)	Dry	0.6±0.12	0.5±0.06	0.82	0.417
	wet	0.5±0.15	0.5±0.06	0.03	0.975
SRP (mg/L)	Dry	0.1±0.05	0.1±0.05	0.24	0.812
	wet	0.1±0.02	0.2±0.03	2.59	0.015*
Ammonium (mg/L)	Dry	2.1±0.58	4.7±1.1	2.21	0.035*
	wet	1.6±0.66	3.0±0.65	1.54	0.133

The PCA biplot for habitat quality and water quality data collected during the dry and wet seasons identified variables that were associated with the different disturbance categories (Fig. 2). The principal component 1 (PC 1) for habitat quality explained (29.9 %), while that for water quality explained (23.3 %) of the total variation in the study area during the dry and wet seasons (Fig. 2I). The principal component 2 (PC 2) for habitat quality explained (23.1 %), while that for water quality explained 15.7 % of the total variation for the disturbance category (Fig. 2I). Consequently, the PCA biplots for habitat quality and water quality data collected during both the dry and wet seasons identified variables that differed between the seasons. The principal component 1 (PC 1) for habitat quality explained (29.9 %), while that for water quality explained (23.3 %) for the total variation in the study area during sampling seasons. The principal component 2 (PC 2) for habitat quality explained (23.1 %), while that for water quality explained (15.7 %) of the total variation for sampling seasons (Fig. 2 II). Similarly, the PCA biplot for habitat quality and water quality data sampled during both wet and dry seasons identified variables that differed between the flow permanence category (Fig. 2II). The principal component 1 (PC1) for habitat quality explained (25.3 %), while that of water quality explained (25.8 %) for the total variation in the study area. The principal component 2 (PC2) for habitat quality explained (20.8 %), while that for water quality explained (19.3 %) of the total variation in f low permanence (Fig. 2C). 3 For habitat quality and stream size variables, water velocity (0.5 ± 0.05m /s), discharge (10.5 ± 2.87 m 3 /s), and river width (8.3 ± 1.64 m) were higher during the wet seasons

(Table 3), while for water quality, TSS (95.1 ± 54.59 mg/L), POM (95± 57.41 mg/L), and nutrient (0.6 ± 0.12 mg/L) concentrations were higher during the dry season (Table 3). There were significant differences in physicochemical variables between seasonal and permanent sites (PERMANOVA, F = 3.38, d. f. = 1, p = 0.001). Among the disturbance categories (PERMANOVA, F = 1.9, d. f. = 2, p = 0.02), further, there was no noted interaction effect on river permanence and disturbance levels (PERMANOVA, F = 1.03, d. f = 2, p = 0.52). There were significant differences in habitat quality variables among the disturbance categories (PERMANOVA, F = 4.86, df = 2, p = 0. 001), as well as with river permanence (PERMANOVA, F = 2.62, df =1, p = 0.004), with no significant river type × disturbance interaction (PERMANOVA F = 0.56, df = 2, p = 0. 3).

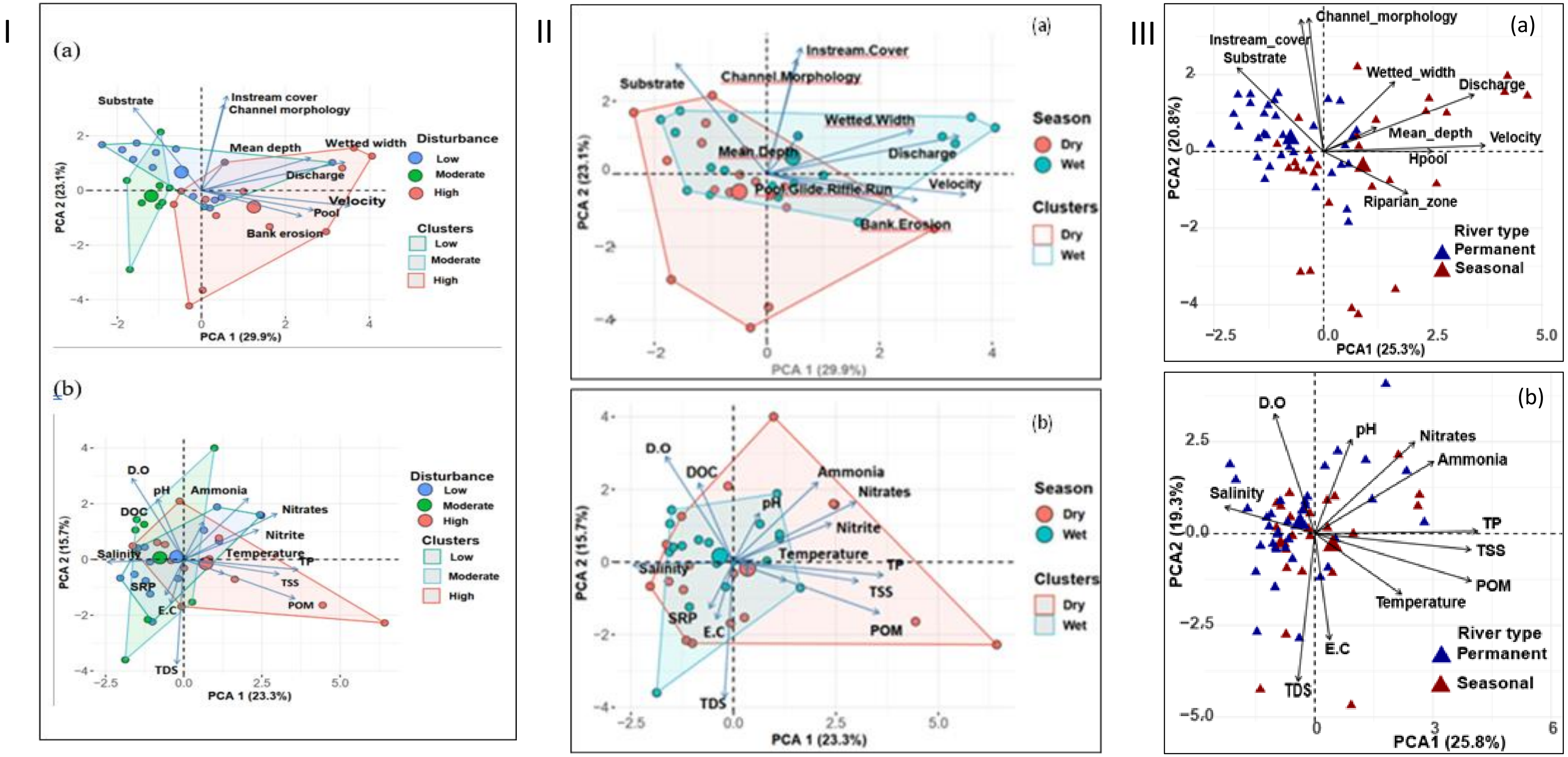


Figure 2: PCA biplot for habitat quality(a), water quality variables between disturbance categories (i), seasons (ii), and flow permanence (iii) in the Wundanyi and Bura rivers.

4.2 Composition of macroinvertebrates FFGs

FFGs allocated in the Wundanyi and Bura rivers were scrapers, collector-gatherers (gatherers), collector-filterers (filterers), predators, and shredders. For the disturbance categories (low, moderate, high), a shift in the abundance of the macroinvertebrate FFGs was observed. Predators had the highest abundance in the low disturbed site categories (1829 (a) (b) I II III 60 individuals), this was followed by shredders and scrapers (870 individuals). Whereas filterers were the least abundant (409 individuals), especially at the moderate disturbed site categories, this pattern of predators was also observed with an abundance of (2865) species, followed by scrapers (2377) and shredders (507).

Filterers were observed to be the least abundant, with 225 individuals. At high disturbance site categories, predators were the most common with (4447 individuals), followed by scrapers (3212 individuals) and gatherers (1221 individuals).

Dry season, predators were abundant with 5224 individuals, followed by scrapers (2762 individuals) and shredders (1021 individuals). Filterers were the least abundant with 104 individuals. A different scenario of patterns was noticed wet season, where predators (4801 individuals) were abundant, followed by scrapers (4117 individuals) and gatherers (1545 individuals), with filterers having the lowest abundance of (636 individuals). In permanent versus seasonal streams, predators were the most abundant with 4231 individuals at permanent sites, followed by scrapers with an abundance of 3040 individuals, and shredders with an abundance of 3040 individuals. Gatherers were the least abundance of 563 individuals.

At seasonal sites, predators also recorded the highest abundance (5794 individuals), followed by scrapers (3839 individuals), and gatherers (1459 individuals), while filterers were least abundant with 171 individuals. Predators were highest in the high disturbance sites categories in the dry season, but this decreased in low- and moderate-disturbance sites (Fig. 3a), a trend that was also observed in the wet season (Figure 3b). Similarly, scrapers were abundant in the high disturbance sites during dry and wet seasons. They decreased in moderate and low disturbance (Figure 3a, b). Shredders' numerical abundance increased in low disturbance sites and decreased in high disturbance sites during the wet and dry seasons (Figure 3a, b). In flow permanence, predators were the most abundant in both the categories (permanent and seasonal) (Figure: 3c). Scraper's abundance was high in seasonal rivers but decreased in permanent rivers a pattern that was observed in both predators and gatherers (Figure: 3c). Shredder abundance was low in seasonal rivers but increased in permanent rivers, this was also a similar pattern in filterers (Figure: 3c).

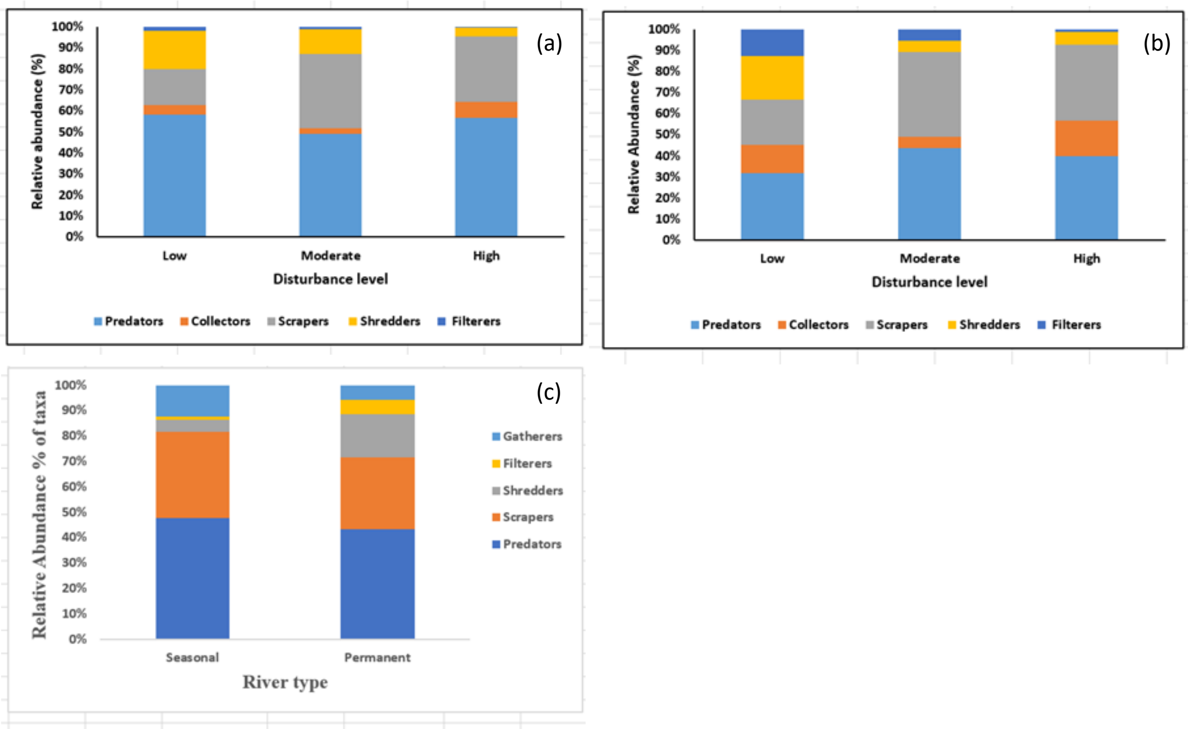


Figure 3: Changes in the relative abundance of Macroinvertebrates FFGs in different sites grouped according to the levels of disturbance, seasonality (dry (a), wet(b)), low, moderate, and high), and flow permanence (Permanent and seasonal).

4.3 Relationships between water quality and FFGs

There were significant differences in the functional composition of macroinvertebrates for untransformed richness data of FFGs among the river disturbance categories as identified by ANOSIM analysis (R-statistic=0.26, $p = 0.001$), seasons (R-statistic=0.15, $p = 0.001$), and between river type categories (R-statistic=0.18, $p = 0.002$). The NMDS ordination of FFGs abundance data identified and grouped the macroinvertebrates, even though some overlaps were observed in wet and dry seasons and between disturbance site categories (Fig. 4). Shredders were associated with low disturbance sites, which are dominated by sensitive taxa that included Decapoda, Diptera, Ephemeroptera, and Lepidoptera. Predators were mostly found in highly disturbed sites, and mainly consisted of Coleoptera, Diptera, Hemiptera, Odonata, and Trichoptera. Moderately disturbed categories had all the FFGs present. In dry season, a high number of predators was observed, primarily consisting of Hemiptera, Odonata, Coleoptera, and some Trichoptera. In wet season, all groups of the FFGs were present. A noticeably high dominance of predators in temporary (seasonal) sites that experienced flow cessation was observed. Predators were dominated by Odonata (e.g., Gomphidae, Lestidae, Libellullidae, and Cordulegastridae families), Hemiptera (e.g., Naucoridae, Corixidae, Pleidae, Notonectidae) and Diptera (e.g., Tabanidae). Permanent sites had all the groups of FFGs which were dominated by Odonata (Gomphidae), Ephemeroptera (Baetidae), Decapoda (Potamonautidae), Diptera (Chironomidae), and Trichoptera (Hyropsychidae).

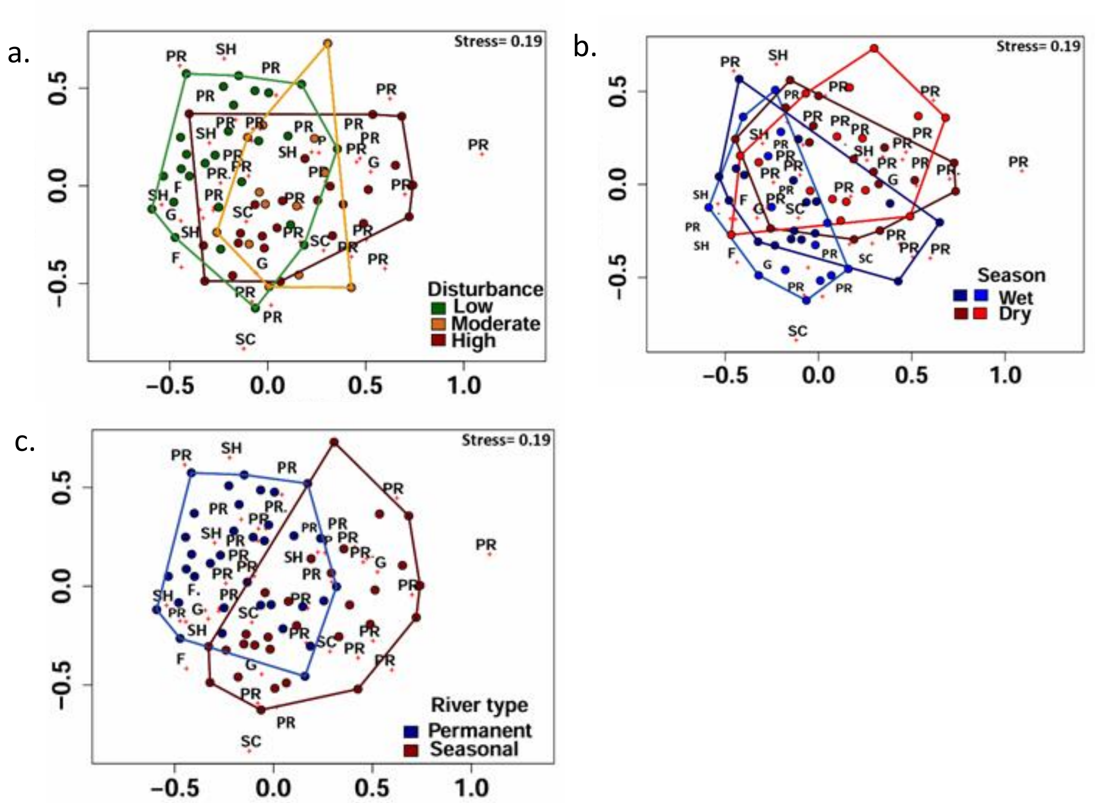


Figure 4: Plots of nMDS based on *numerical* abundance data of FFGs in Wundanyi and Bura rivers according to (a) disturbance gradient (low, moderate, and high), (b) seasons (wet and dry) and (c) river type or flow permanence (permanent and seasonal). Pr = predators, Sh = shredders, Sc =scrapers, F = collector-filterers, G = collector-gatherers.

Pairwise SIMPER comparison of FFGs between low-disturbance site and high-disturbance site categories identified scrapers (34.8 %) and predators (31.2 %) as the greatest dissimilarity between the two site categories during the wet season, with a high abundance of scrapers in high-disturbance sites (Table 8). Predators (29.8 %) and scrapers (28.8 %) had the greatest dissimilarity between moderate- and high-disturbance sites in wet season, with a high abundance of predators in moderate disturbance sites (Table 8). Comparison between low- and moderate disturbance sites identified scrapers (34.9 %) and predators (30.4 %) as having the greatest dissimilarity between the two site categories, with high abundance of scrapers in moderate-disturbance sites in wet season (Table 8). In dry season, scrapers (34.9 %) and predators (33.6 %) the highest dissimilarity between low- and moderate-disturbance sites, where both scrapers and predators had a high abundance in the low-disturbance sites (Table 4). In comparison between moderate- and high-disturbance sites, again, scrapers (41.7 %) and predators (34.8 %) were observed to have the greatest dissimilarity between the two disturbance sites, with both (scrapers and predators) having a high abundance in moderate-disturbance sites (Table 8). Predators (51.6 %) and scrapers (15.4 %) were observed to have a great dissimilarity between permanent and seasonal rivers, with predators having a high abundance in seasonal rivers in dry season (Table 9). In wet season, again, predators (46.1 %) and scrapers (23.4 %) were observed as having a great dissimilarity between permanent and seasonal rivers, with a high abundance of predators in seasonal rivers (Table 9).

Table 8: FFG-ranked SIMPER contributors to percentage dissimilarity (Contrib.%) in the composition between disturbance site categories: Values other than Contrib.% indicate mean abundance. Site categories were defined as (high, moderate, and low disturbance, respectively)

		Dry season				Wet season	
FFGs	**Contrib. %**	**Mean Low**	**Mean High**	**FFGs**	**Contrib. %**	**Mean Low**	**Mean high**
Scrapers	34.9	149	121	Scrapers	34.8	174	239
Predators	33.6	285	285	Predators	31.2	267	253
Shredders	20.5	41	89	Filterers	12.3	17	67
Gatherers	8.8	45	13	Shredders	12	32	77
Filterers	2.3	8	6	Gatherers	9.7	59	69
FFGs	**Contrib. %**	**Mean Moderate**	**Mean High**	**FFGs**	**Contrib. %**	**Mean Moderate**	**Mean High**
Scrapers	41.7	221	121	Predators	29.76	304	253
Predators	34.8	385	285	Scrapers	28.8	324	239
Shredders	18.4	50	89	Gatherers	19.14	169	69
Gatherers	3.8	28	13	Shredders	11.97	106	77
Filterers	1.2	4	6	Filterers	10.33	13	67
FFGs	**Contrib. %**	**Mean Low**	**Mean Moderate**	**FFGs**	**Contrib. %**	**Mean Low**	**Mean Moderate**
Scrapers	44.6	149	221	Scrapers	34.9	174	324
Predators	35	285	385	Predators	30.4	267	304
Shredders	10.4	41	50	Gatherers	18.5	59	169
Gatherers	8.3	45	28	Shredders	13.5	32	106
Filterers	1.7	8	4	Filterers	2.8	17	13

Table 9: FFGs-ranked SIMPER contributors to percentage dissimilarity (Contrib.%) in the composition between flow permanence or river type site categories (permanent and seasonal). Values other than Contrib.% indicate mean abundance.

FFGs	Contrib. %	Mean Permanent	Mean Seasonal
Dry season			
Predators	51.6	17.1	20.8
Scrapers	15.4	1.7	2.8
Shredders	13.3	2.8	2.5
Gatherers	10.0	2.0	2.5
Filterers	9.8	1.4	0.9
Wet Season			
Predators	46.1	17.2	20.0
Scrapers	23.4	2.2	4.8
Collectors	14.8	2.1	3.4
Shredders	10.5	2.7	3.2
Filterers	5.2	1.6	2.0

RDA ordination identified spatial and temporal changes in macroinvertebrate functional abundance in relation to water quality and POM with respect to the disturbance sites (Figure. 5a), seasonality (Figure: 5b) and flow permanence (Figure: 5c). RDA axes 1 and 2 explained 27.2 % on axis 1 and 21.1 % on axis 2 of the associations between water quality and FFGs in the sampling sites. RDA ordination revealed that shredders were linked to dry season and seasonal rivers with a higher POM (Figure: 5b, c), whereas predators, scrapers, and filterers were linked with high levels of pH, nitrite, and ammonia in high disturbance sites (Figure: 5a). Gatherers had a high abundance at high disturbance sites and were linked to seasonal sites and more occurred in dry seasons (Figure: 5a, c).

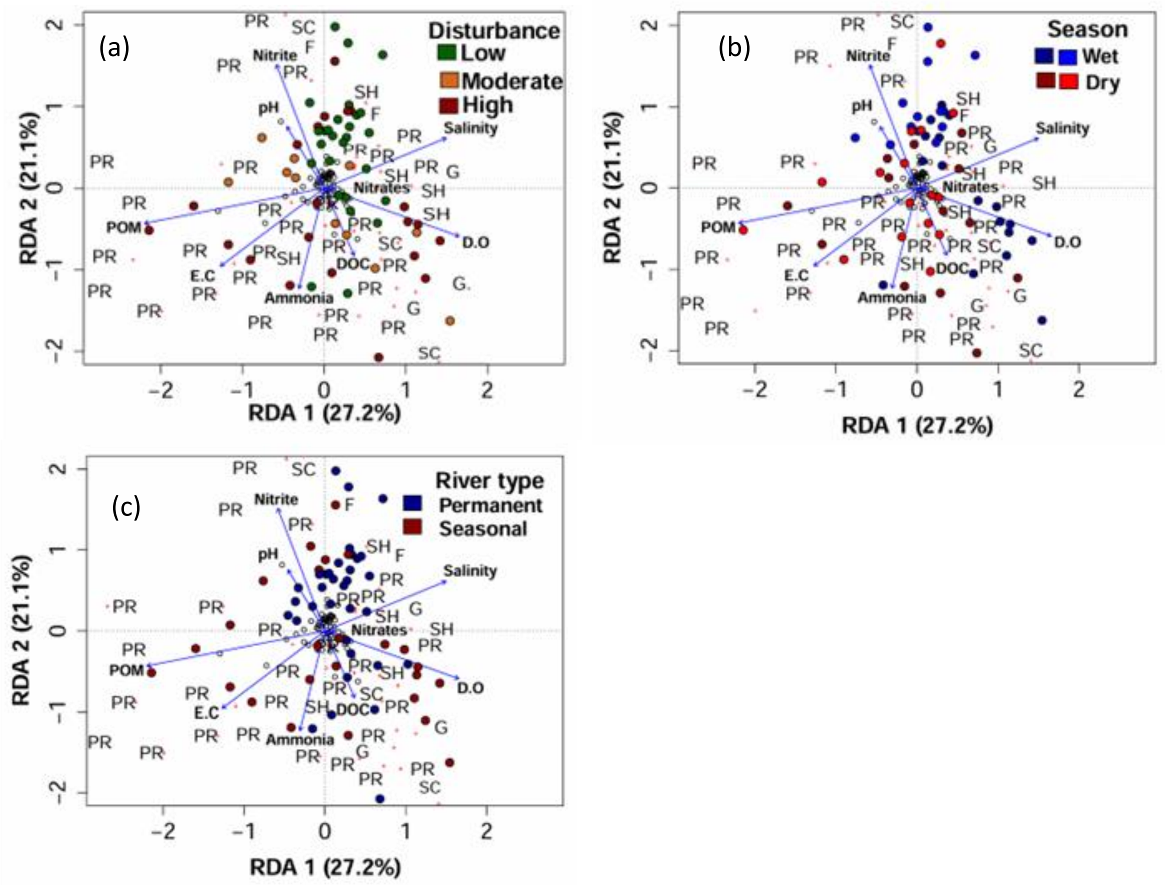


Figure 5: RDA triplot of macroinvertebrate FFGs abundance data to water quality variables in the Wundanyi and Bura rivers. Plots are for a) disturbance categories (low, moderate and high), b) seasons (wet and dry), and c) flow permanence (permanent and seasonal). Pr = predators, Sh = shredders, Sc = scrapers, F = collector-filterers, C = collector-gatherers, E.C. = electrical conductivity, DO = dissolved oxygen concentration, particulate organic matter, DOC = dissolved organic carbon.

4.4 Macroinvertebrates FFGs as used as surrogates of ecosystem attributes

Disturbance and seasonality influenced the functional composition of macroinvertebrates in the sampling sites, and these metrics that were used as surrogates of ecosystem attributes responded similarly (Table 10). Metrics used for disturbance site categories and seasons showed different results for the CPOM/ FPOM index and TFPOM/ BFPOM, with moderate disturbed sites indicating a weak shredder linkage with the riparian zone and with limited FPOM in the water column at high-disturbance sites in dry and wet seasons, but agreed on the channel stability (stable substrate abundance) indices. Seasonality further influenced these metrics of ecosystem functioning. For instance, the metrices indicated that low-disturbance shifted to heterotrophic ($P < R$) in wet season but contradicted on the rest of the sites categories and seasons with data indicating that they were autotrophic ($P > R$).

In both seasons, the top-down control index revealed that all the sites had plenty of predators, indicating that there was a strong top-down control of the system. Irrespective of the disturbance levels, all the study sites had a stable channel and adequate FPOM in (TFPOM) than (TFBOM) in both dry and wet seasons, as revealed by the TFPOM/BFPOM index. However, at the high-and moderate-disturbance sites, the TFPOM/ BFPOM index showed 0.06 and 0.42 in wet and dry sampling occasions, respectively (Table 10). With respect to flow permanence or river type, the P/R metrics were higher than the threshold value ($P/R = 0.75$), meaning that both the permanent ($P/R = 1.08$) and seasonal ($P/R = 1.78$) rivers were auto-trophic. The surrogates for the CPOM/FPOM index for the two river types indicated strong shredder linkage with the riparian zone. In terms of the predominant FPOM, the permanent sites had more FPOM in transport (TFPOM) than in the benthos (BFPOM), while this was not the case for seasonal rivers, as the ratio was below the threshold value (TFPOM/ BFPOM = 0.50). In both river types, there were stable channels and strong top-down controls of the macroinvertebrate community by predators (Table 11).

Table 10: Mean values of stream ecosystem attributes derived from FFG ratios along Bura and Wundanyi rivers in the different disturbance categories during the dry and wet seasons.

Disturbance Level	**Low Disturbance**		**Moderate Disturbance**		**High Disturbance**	
Season	Wet	Dry	Wet	Dry	Wet	Dry
P/R	0.47	**0.88**	**2.52**	**2.21**	**1.49**	**2.58**
CPOM/FPOM	**0.84**	**2.86**	**0.5**	**2.82**	**0.35**	**0.52**
TFPOM/BFPOM	**0.96**	0.39	**1.02**	0.42	0.06	0.06
Channel Stability	**1.02**	**1.03**	**4.29**	**2.48**	**1.59**	**2.72**
T/Down Control	**0.47**	**1.26**	**0.77**	**0.96**	**0.67**	**0.43**

N.B. Ratios are based on numerical abundance. Boldface indicates very strong autotrophic, strong shredder linkage with the riparian zone, heavy suspended loading of FPOM, or good quality of FPOM, stable substrate abundance, and an overabundance of predators with strong top-down control. Boldface identifies those values above the thresholds for that metric. Threshold values for the attributes are: P/R> 0.75, CPOM/FPOM>0.25, TFPOM/BFPOM>0.50, Channel Stability>0.50, Top-Down control>0.20

Table 11: Mean values of stream ecosystem attributes derived from FFG ratios along Bura and Wundanyi rivers between the flow permanence or river type categories (permanent and seasonal).

Flow Permanence	Seasonal	Permanent
P/R	**1.78**	**1.08**
CPOM/FPOM	**0.32**	**1.49**
TFPOM/BFPOM	0.11	**1.01**
Channel Stability	**2.02**	**1.6**
Top/Down Control	**0.97**	**0.72**

N.B. Ratios are based on numerical abundance. Boldface indicates very strong autotrophic, strong shredder linkage with the riparian zone, heavy suspended loading of FPOM, or good quality of FPOM, stable substrate abundance, and an overabundance of predators with strong top-down control. Boldface identifies those values above the thresholds for that metric. Threshold values for the attributes are: P/R> 0.75, CPOM/FPOM>0.25, TFPOM/BFPOM>0.50, Channel Stability>0.50, Top-Down control>0.20

4.5 Spatio-temporal community composition of traits and ecological preferences

The Wundanyi and Bura rivers, trait attributes were analysed for 70 taxa. Twenty-three taxa were assigned trait attributes at the species-level, 48 at the genus-level, and 39 to family-level. The relationships between the traits, macroinvertebrate compositions, and environmental stressors were significant in both sampling periods (wet and dry seasons) (Monte-Carlo test; $p < 0.05$). These relationships were summarised mainly by two RLQ axes, which explained cumulative variances of 93.2% and 83.2% of the model's (Monte-Carlo test) total variability in wet and dry periods, respectively (Table 12). In dry sampling period, one axis explained 96.3% variance of the environmental matrix (R/RLQ), 71.7% of the taxa matrix (RLQ/L) and 77.7% of the attribute matrix (RLQ/Q). In wet sampling period, the axis explained 91.2% variance of the environmental matrix, 58.6% of the taxa data, and 79.3% of the trait attribute data (Table 12). The CAP and MANOVA analyses indicated clear differences in physicochemical parameters across the four site categories (disturbance site categories and flow permanence) during both sampling periods, suggesting that the classification of sites based on land-use cover was generally robust (Table 13). Results from the two-way MANOVA further showed that physicochemical characteristics varied significantly among the three site categories and with flow permanence ($F = 2.37$, $p = 0.04$; $F = 4.18$, $p = 0.04$; Table 13). In addition, a significant interaction effect was detected between site category and sampling period on the measured physicochemical variables.

Table 12*:* RLQ ordination properties, including eigenvalues, variance explained in Axes 1 and 2, covariance, and correlation, projected variances of the environmental data (PCA), species abundance (CA), and trait (Hill–Smith) matrices on two RLQ axes during the dry and wet seasons.

	Wet Season		Dry Season	
RLQ Properties	Axis 1	Axis 2	Axis 1	Axis 2
Variance (RLQ) %	46.07	37.11	70.22	23.07
Cumulative variance (%)	46.07	83.18	70.22	93.29
Eigenvalue	5.701	4.5924	17.55	5.77
Covariance	2.3877	2.143	4.19	2.4
Correlation	0.3752	0.3163	0.51	0.39
Variance R/RLQ (%)	89.35	91.16	94.22	96.31
Variance L/RLQ (%)	50.51	58.65	71.74	67.54
Variance Q/RLQ (%)	46.22	79.27	77.72	72.04

Table 13: Two-way MANOVA results exploring the influences of site category and period (dry and wet) on all ordinated physicochemical measurements.

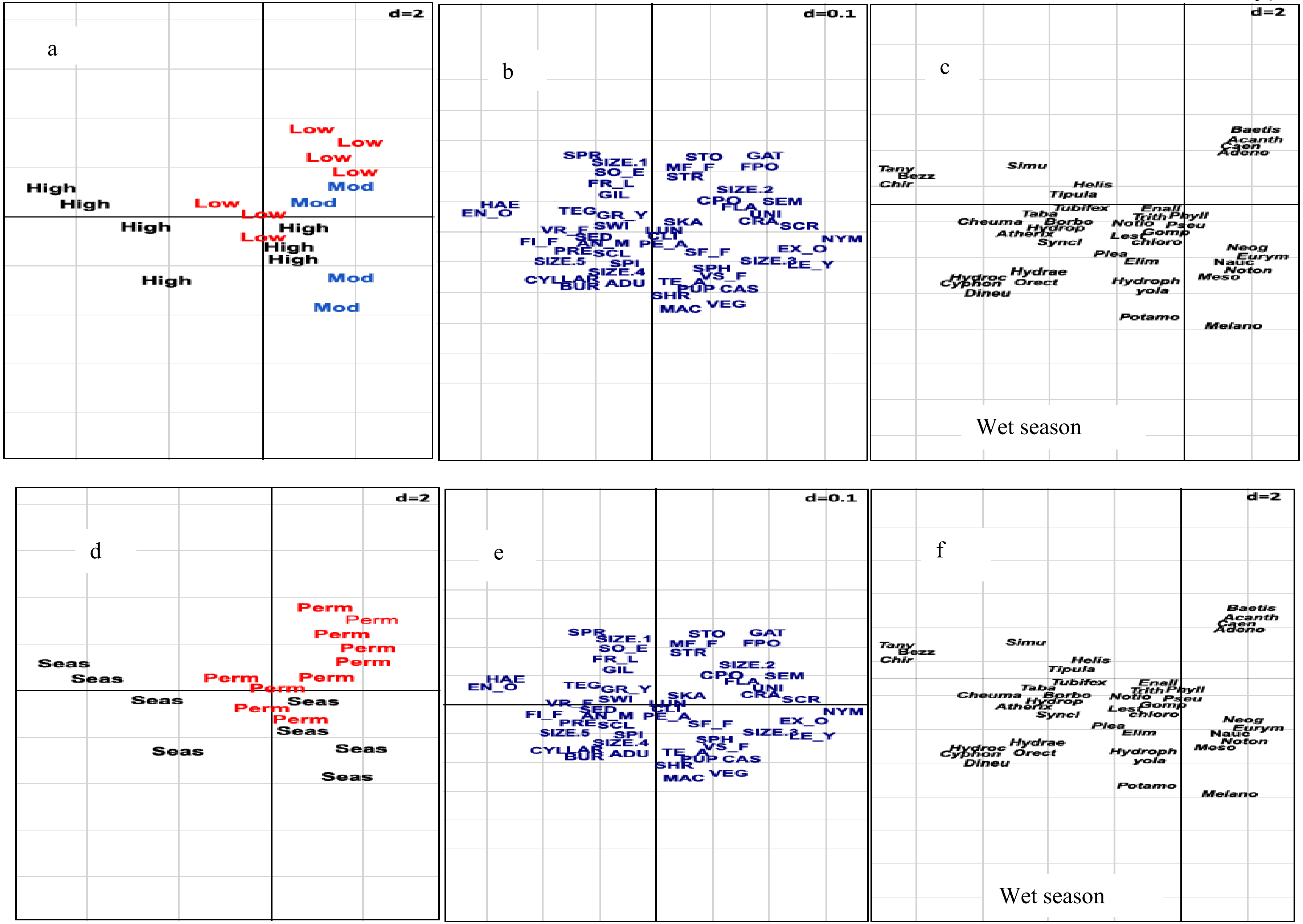
Factor	Wilks' Lambda	F value	Effect (df)	Error (df)	P. values
Disturbance gradient vs season					
Disturbance	0.01	2.16	48	8	0.035
Season	0.21	0.97	24	6	0.571
Disturbance × Season	0.01	2.37	48	12	0.042
River type vs season					
River_type	0.00	2.34	96	22.32	0.012
Season	0.20	0.81	24	5	0.683
River_type:Season	0.05	4.18	24	5	0.042

During the dry period, site categories separated distinctly according to both disturbance and flow permanence (RLQ analysis; **Figure 6g**). Two main clusters were observed: Cluster A, which consisted primarily of high-disturbance sites, and Cluster B, which included sites with high, moderate, and low disturbance. Cluster A was strongly associated with traits such as the presence of haemoglobin, reliance on lungs and spiracles for breathing, an aquatic adult stage, burrowing and swimming behaviours, and preference for soft-bottom sediments (Figure: 6g). These traits were represented mainly by taxa tolerant of disturbances, such as Oligochaeta, *Paragomphus* spp., *Plea* spp., *Tabanus* spp., Vellidae, Tanyanipodinae, Chironomidae, and Larainae, and agreed with predictions of these to increase along the disturbance gradient except for very large body-size (>40 mm) (Table 4). Shredding (SHR), filter-feeding (FI_F), and gathering behaviours (GAT); preference for animal materials (AN_M); soft/exposed body parts (SO_E); and larval aquatic stage (LAR) indicated positive associations with moderate and low disturbed sites (Cluster B; Fig. 4). Similarly, macroinvertebrates with very small (≤5 mm; SIZE1), small (5–10 mm; SIZE2) and medium (10 – 20 mm; SIZE3); body-sizes, a preference for CPOM and sprawling (SPR), were positively associated with the least disturbed sites during wet season. These traits were represented mainly by taxa belonging to the Hydropsychidae, Baetidae, and Leptophlebiidae families and represented indicators of sensitivity to agricultural disturbances. All traits associated with low-disturbed sites, except for very small and small body sizes and a food preference for animal material, supported our predictions (Fig. 4; Table 4).

In the wet period, three different clusters A, B and C were produced, with the high, moderate and low disturbed sites mainly in Cluster A and largely distinct from those in the other categories (RLQ analysis, Fig. 4g). Cluster A sites were positively associated with invertebrates possessing a size 2(5-10), TEA (Temporarily attached), SCR (scrapers), SEM (semi-voltinism), VS_F (very slow flowing), GAT (Gatherers), FPO (Fine particulate organic matter), SPI (Spiracles) (Fig. 4 g,h). Taxa such as Appasus spp. (Appa), Sigara spp. (Sig), Yola spp. (Yol), Plea spp. (Ple), and Oligochaeta (Oli) were linked positively with high disturbance (Fig. 4i). All traits linked to high disturbance, except a preference for very slow-flowing water, were predicted to increase with increasing stress (Table 4).

Cluster B consisted mainly of Moderate and Low disturbance sites, and traits such as preference for slow flowing waters, LAR (larvae stage), CLI (climber), ADU (adult), size 3 (10-20), VEG (Vegetation), EX_O (Exophytic oviposition), BUR (burrower), LEY (Less than one year), NYM (Nymphs stage), SED (sediment) substrates. When looking at flow permanence, several traits indicated tolerance to seasonal streams during the wet season, such as haemoglobin possession, endophytic oviposition, adaptation to fast flow (>0.6 m/s), cylindrical shape, burrowing behavior, adult stage prevalence, use of macrophytes or vegetation, pupae, case/tube construction, adaptation to very slow flow (0.1–1 m/s), spherical shape, temporary attachment, short life cycle (<1 year), exophytic oviposition, and lung respiration. In contrast, traits associated with permanent streams in the wet season included spiracles, small body size (≤5 mm), soft and exposed body forms, free-living behavior, gills, tegument, longer life cycles (>1 year), swimming locomotion, univoltinism, crawling, scraping, permanent attachment, flattened body, semi-univoltinism, streamlined shape, moderate flow preference, and reliance on fine particulate organic matter

Cluster C sites were dominated by the occurrence of invertebrates having a permanent attachment (PE_A), preferring macrophytes (MAC) and vegetation (VEG) substrates, exhibiting crawling (CRA), climbing (CLI), scraping (SCR), and shredding (SHR) behaviours, and possessing an aquatic nymph stage (NYM), medium (10 – 20 mm; SIZE 3) and very large (>40 mm; SIZE 5) body sizes (Figure: 4i). These traits were represented by Caenis spp. (Cae), Adenophlebia spp. (Ade), *Crenigomphus* spp. (Cre), *Brachythemis lacustris* (Bra la) and *Pseudocloeon* spp. Traits suggesting tolerance to permanent streams during the dry season included medium and small body sizes (Size 1 and 2), spiracles, semi-voltinism, sprawling locomotion, swimming, endophytic oviposition, gathering feeding habit, fine particulate use, streamlined shape, and long-life cycles.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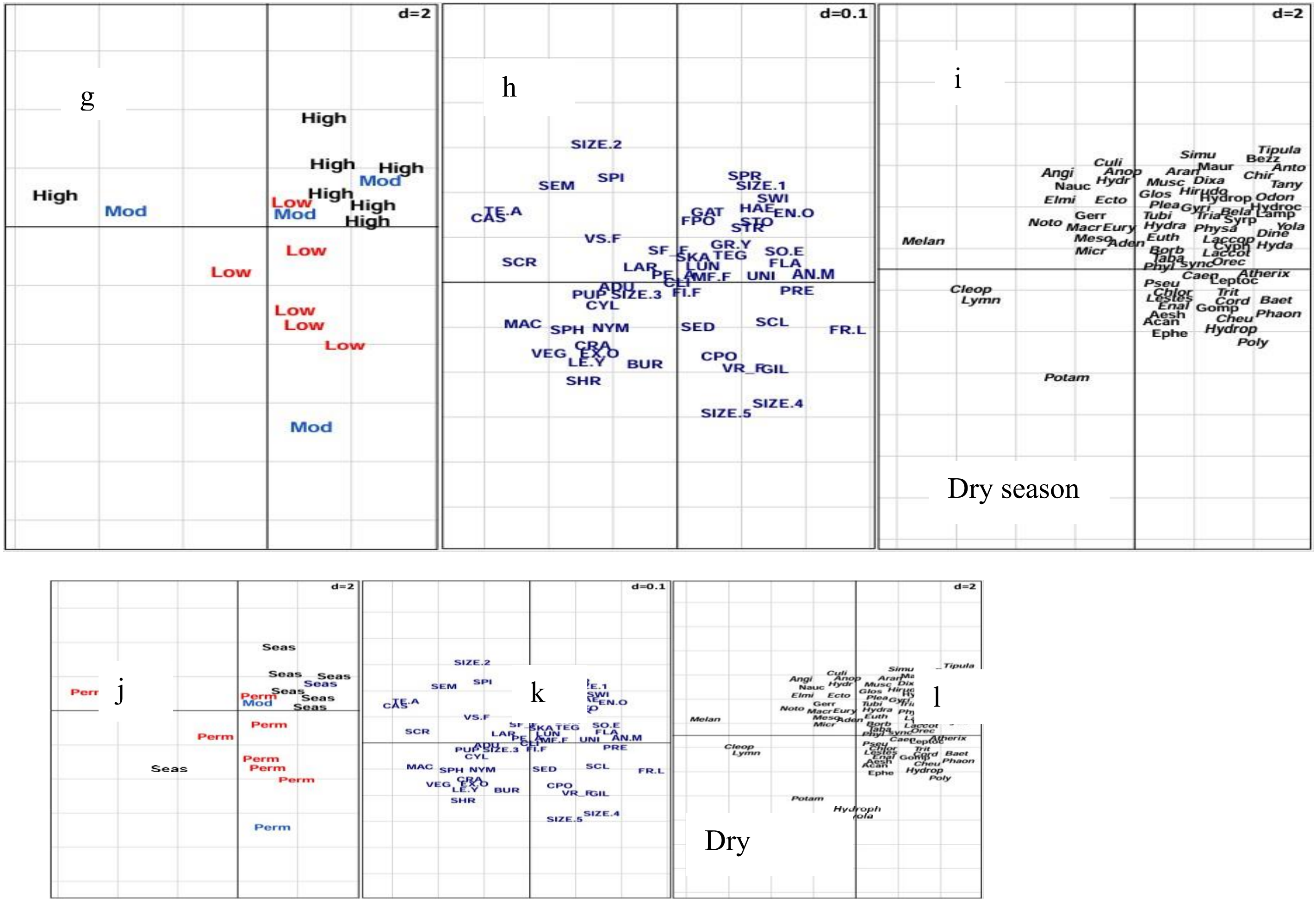

Figure 6: RLQ ordinations showing the associations between site categories (disturbance and river type), traits, and taxa in wet and dry seasons.

In
Figure *6*, the Wet season = (a, b, c, d, e, and f; dry season = g, h, I, k, and l), (disturbance = a, b, c, g, h and I;) river type = (d, e, f, j, k and l). The d value in the upper right corner of the plot is the scale of the graph. Abbreviations for disturbance: Mod = Moderately disturbed category. Abbreviations for flow permanence: Seas = Seasonal, Perm = Permanent rivers: Abbreviations and acronyms for traits: SIZE 1: very small, ≤ 5, SIZE 2: small body (5 – 10), SIZE 3: medium body (10 – 20), SIZE 4: large body (20 – 40), SIZE 5: very large body (>40), LAR: larval aquatic stage, NYM: nymph aquatic stage, PUP: pupa, ADU: Adult aquatic stage, GIL: gills, EN_O: endophytic oviposition, EX_O: exophytic oviposition, TEG: tegument, SPI: spiracle, LUN: lungs, HAE: haemoglobin, SEM: semivoltinism, UNI: univoltinism, LE_Y: < 1 generation year per year, GR_Y: > 1 generation per year, ADU: adult aquatic stage, FPO: fine particulate organic matter, CPO: coarse particulate organic matter, STR: streamlined, FLA: flattened, SPH: spherical, CYL: cylindrical, SO_E: soft and exposed, CAS: cased/tubed, SCL: sclerotised, SHR: shredder, GAT: gatherer, FI_F: filter-feeder, SCR: scraper/grazer, PRE: predator, FPO: fine particulate organic, MAC: macrophytes, AN_M: animal materials, CRA: crawler, CLI: climber/clinger, SPR: sprawler, SWI: swimmer, SKA: skater, BUR: burrower, PE_A: permanently attached, TE_A: temporarily attached, FR_L: free-living, STO: stone, SED: sediment, VEG: vegetation, VF_F: very fast flowing (>0.6), MF_F: moderately flowing (0.3 – 0.6), SL_F: slow flowing (0.1 – 0.3), VS_F: Very slow flowing (0.1 – 1). Aca.th: Acanthiops tsitsa, Ade.au: Adenophlebia auriculata, Ade: Adenophlebia spp., Aes: Aeshna spp., Afr.fe: Afrobrianax ferdyi, Afr.ba: Afronurus barnardi, Afr: Afronurus spp., Afrop: Afroptilum spp., Agr: Agraptocorixa spp., Amp: Amphipoda, Aph: Aphanicerca spp., App: Appasus spp., Athe: Athericidae, Ath: Athripsodes spp., Bae.ha: Baetis harrisoni, Biom: Biomphalaria spp., Bra.la: Brachythemis lacustris, Bra: Brachythemis spp., Bur:

Burnupia spp., Cae: Caenis spp., Cer: Ceratophallus spp., Che.th: Cheumatopsyche thomasetti, Che.af: Cheumatopsyche afra, Che: Cheumatopsyche spp., Chi: Chironomidae, Cor.as: Corbicula astartina, Cre: Crenigomphus spp., Cul.1: Culex spp. 1, Cul.2: Culex spp. 2, Dem: Demoreptus capensis, Din: Dineutus spp., Elm: Elminae, Ena.gl: Enallagma glaucum, Eni: Enithares spp., Eut: Euthraulus spp., Hir: Hirudinea, Hyd: Hydropsyche spp., Lac: Laccocoris spp., Lar: Larainae, Les.pl: Lestes plagiatus, Lim.cr: Limnophila crespusclum, Lym.co: Lymnaea columella, Lym.na: Lymnaea natalensis, Lym: Lymnaea spp., Mac.ca: Macrostemum capense, Mus: Muscidae, Not: Notonecta spp., Oli: Oligochaeta, Ore: Orectogyrus spp., Par.ge: Paragomphus genei, Pot: Potamonautes spp., Pse.sp: Pseudagrion spernatum, Pse pi: Pseudocloeon piscis, Pse: Pseudocloeon spp., Pse.vi: Pseudocloeon vinosum, Sig: Sigara spp., Sim: Simulium spp., Tab: Tabanus spp., Tri.di: Tricorythus discolour, Tri: Tricorythus spp. and Tri: Trithemis spp.

4.6 Identifying invertebrate indicator traits and ecological preferences relevant to different disturbance site categories and flow conditions

Out of the 52 invertebrate traits and ecological preference attributes analyzed, 39 showed significant positive or negative correlations with environmental variables in the combined RLQ and fourth-corner model across disturbance levels and seasons ($p < 0.05$). During the dry season, invertebrate traits such as the possession of endophytic oviposition and free-living behaviors were positively correlated with channel morphology, instream cover, substrate, and salinity, suggesting a stable physical habitat that promotes taxa with these behaviors. Velocity(m^3/s), discharge (m/s), nitrate (mg/L), TSS (mg/L), and run quality were positively associated with traits such as collector-gatherers and multivoltine strategies, suggesting fast-flowing and nutrientrich areas favour opportunistic or fast-reproducing taxa. Temperature (ºC), NH4 (mg/L), TP (mg/L), and Nitrate (mg/L) were associated with VEG (vegetation), SHR (shredders), and MAC (macrophytes), suggesting possibly vegetative cover dwellers, shredders, and macrophyte-associated taxa (Figure 5a).

On the negative correlation, Temperature (ºC), NH4 (mg/L) Mean Depth (m) negatively linked to traits like CAS (cased/tubed), TE_A (temporary attachment), (possibly case builders, piercing mouthparts), suggesting these traits are less favored under warmer, more ammonium-rich, and shallow conditions. SIZE_4 (20-40), SIZE_5 (>40) negatively associated with high flow (Discharge), nutrients (TP), and poor habitat quality, implying smaller organisms are disadvantaged under disturbed conditions (Figure 5a).

Haemoglobin (HAE) was positively associated with salinity in the wet season, whereas burrowers (BUR) showed a positive association with turbidity in the dry season. These traits were deemed indicators of tolerance to different pollutants. Conversely, invertebrate traits having positive associations with high disturbance during both periods, including shredding (SHR) and crawling (CRA) behaviours, medium body size (SIZE 3) and a preference for macrophyte food (MAC; RLQ model; Figure. 4), were either positively associated with DO and canopy cover or negatively correlated with nutrients, TDS, salinity, water temperature, pH and flow velocity during both periods (fourth corner model, $p < 0.05$). For example, crawling behaviour had a significant negative association with turbidity in the dry period, whereas shredding behaviour and a preference for macrophytes showed a significant positive correlation with DO during the dry period. These traits were considered indicators of sensitivity to pollution in the Wundanyi and Bura rivers.

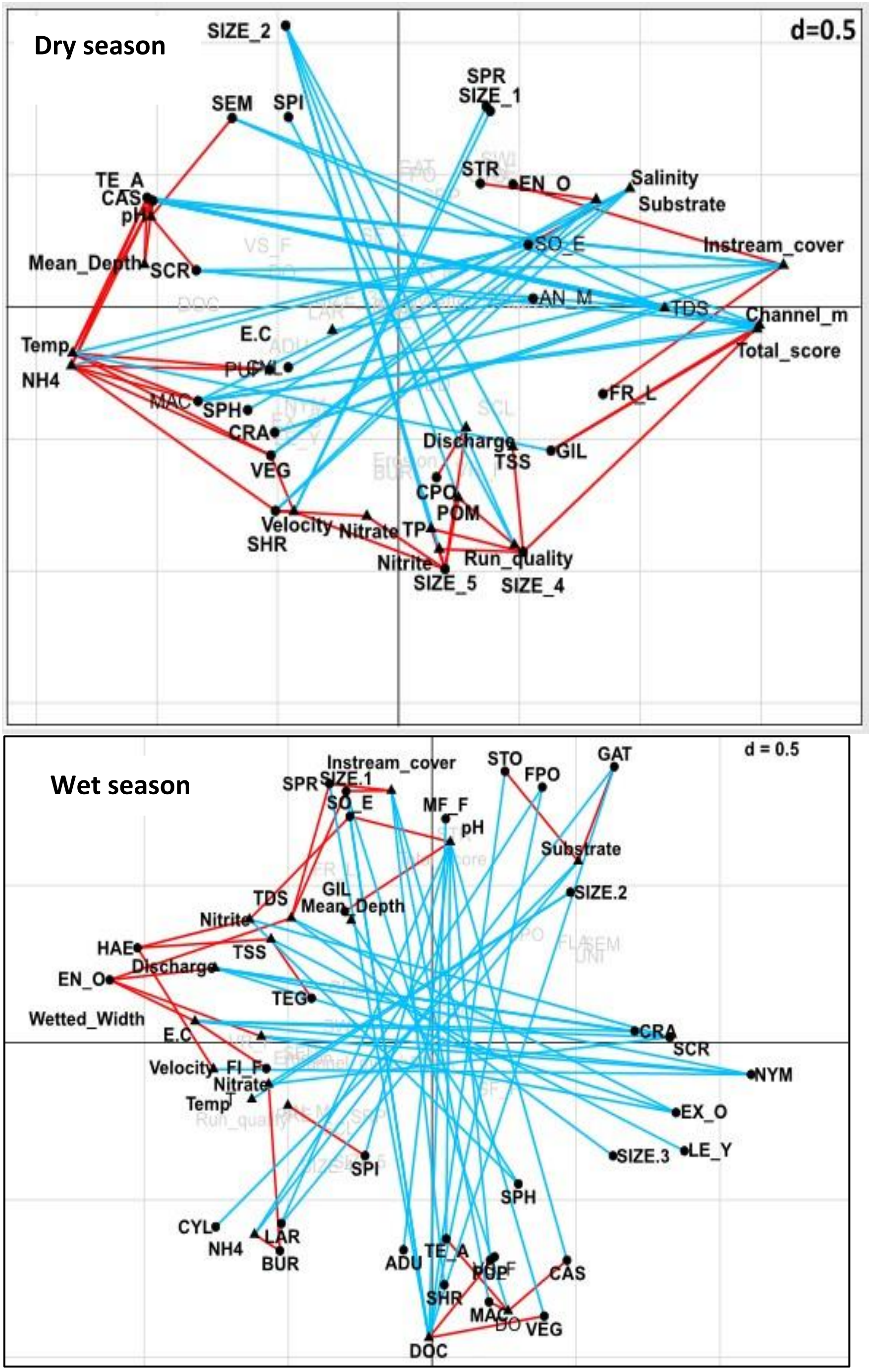

Figure 7: Results of the combination fourth corner and RLQ analysis showing the correlations between macroinvertebrate traits and physicochemical variables in the Kat River. Red/blue boxes represent significant positive/negative correlations ($p < 0.05$), whereas light grey boxes represent no relationships between traits and environmental variables ($p > 0.05$).

CHAPTER FIVE

DISCUSSIONS

The primary aim of this research was to empirically examine the impact of disturbances caused by both human activities and livestock grazing, alongside the influence of stream flow permanence and seasonality on the diversity and composition of macroinvertebrate FFGs and traits in the Wundanyi and Bura rivers. There were significant seasonal and spatial differences in stream size, habitat, and water quality physicochemical variables in the study area caused by human disturbance and flow variability. Macroinvertebrate assemblages responded to these changes with various ratios and metrics of FFGs used as surrogates of ecosystem attributes and ecological conditions. However, the majority of these metrics did not conform to the expected threshold ratios, therefore suggesting that they were not good surrogates of ecosystem attributes. Disturbance, seasonality, and flow permanence all emerged as significant factors shaping the composition of macroinvertebrate FFGs in the study area. During the wet season, there was an increase in the diversity and abundance of macroinvertebrate FFGs compared to the dry season.

5.1 Variation in water and habitat quality

Disturbance, seasonality, and flow permanence exerted significant influences on water and habitat quality variables. Alterations in water quality among different disturbance categories were evidenced by declining DO levels, high temperature, increased EC, and higher levels of POM, TSS, nutrient concentrations, and TDS. The higher temperature observed in the high-disturbance sites and seasonal rivers can be ascribed to the relatively open canopy cover in the riparian zones, whereas the comparatively lower mean water temperature is in low-disturbance sites in forested areas. By reducing the amount of solar radiation that reaches the water's surface, riparian vegetation reduces temperature fluctuations in canopied streams. According to the research, this ecological occurrence is consistent (Aura *et al.,* 2011; López-Carr & Burgdorfer, 2013; Mathooko & Kariuki, 2000). During the dry season, the TSS, POM, and nutrients were higher, because of surface runoff from fields and fertilizer leaching into the rivers, this was anticipated because these factors would be greater during the rainy season. According to Iteba *et al.* (2021) and Yillia *et al.* (2008), these contradictory findings can be attributed to animal disturbance and input of nutrients and organic matter during the dry season, when livestock access to streams and rivers is enhanced as alternative water sources become scarce.

Ideally, seasonal sites (those where flow ceased) had higher EC and TDS, which were attributed to higher water evaporation and water abstraction in these rivers' lower reaches. Seasonality made the impact of perturbations on water quality much worse. Moderate-disturbance sites had the highest levels of DOC concentration, whereas high-disturbance sites had the highest values of TP and EC. Seasonality amplified the disturbance effects on water quality, with high DOC recorded in moderate-disturbance sites, while TP and EC had high values in high-disturbance disturbance, whereas pH recorded higher values in minimally disturbed sites. Therefore, seasonal changes worsened the impact of disturbances on water quality, with different types of disturbances affecting specific water quality parameters (DOC, TP, EC) differently depending on the disturbance level.

5.2 Patterns in structural and functional composition of macroinvertebrates

In comparison to previous studies on the functional composition of macroinvertebrates in Afromontane rivers, interesting patterns were obtained in this study. During the rainy season, the abundance of all FFGs in the Wundanyi-Bura streams increased. This is because the flooding of peripheral vegetation increases the richness and diversity of habitats, giving both rheophilic and pool taxa access to a wider range of flow variations (Dallas, 2007; Masese *et al.,* 2021; Muñoz-Mas *et al.,* 2019). However, there was a plentiful and diverse food resources due to terrestrial runoff during the rainy season (Masese *et al.,* 2009a).

In their research on habitat diversity and macroinvertebrate FFGs in Serra Do Cipo, Brazil, Callisto *et al.* (2001) observed that the wet season significantly increased the abundance of collector filterers, shredders, and collector-gatherers by about three thousand individuals per square meter in comparison to the dry season. The ecological basis for this rise was an increase in OM, which also applied to the collectors. Rainfall provides both allochthonous and autotrophic food supplies for macroinvertebrates by transporting OM from terrestrial areas and depositing it into the river beds (Chakraborty, 2021a, 2021b).

The decline in diversity and abundance of FFGs during the dry season can be attributed to the conditions imposed by the environment's physicochemical features. Moreover, seasonal places with no flows at this period have a higher EC, TDS, and temperature readings. As a result, these features end up having a negative impact on macroinvertebrate diversity and abundance (Masese & Raburu, 2017; Minaya *et al.,* 2013).

Species, such as Hydropsychidae and Simuliidae, rely mostly on water flow to collect food from the water column, and a lack of stable substrate and lower flow velocities might have negative consequences for them (Masese *et al.,* 2021; Thirion, 2016). This is because the hydrologic interaction is weakened during the dry season, therefore resulting in a fall in collector abundance, which may also be attributed to a limited source of food from the terrestrial environment (Benson & Pearson, 2020; Callisto *et al.,* 2001).

Regardless of the kind of disturbance or flow permanence, numerical abundance for predators was consistently seen in all sites. However, the existence of tolerant taxa among the predators, such as Coleoptera, Odonata, and Hemiptera, which are quick colonists and have terrestrial adult forms that can withstand flow cessation, is thought to be responsible for this dominance (Masese *et al.,* 2021). The presence of other tolerant collector taxa, such as Oligochaeta, made it easier for collectors to colonize, especially in areas that were heavily disturbed by both human and livestock activities, which led to an increased level of inputs of organic matter and nutrients (Barbee, 2005; Masese *et al.,* 2021; Mathooko *et al.,* 2005).

5.3 Macroinvertebrate FFGs as surrogates of ecosystem attributes

The functional structure of macroinvertebrate communities was significantly altered by the interplay of disturbance, seasonality, and flow permanence (Tables 8 and 9). Given their temporal and geographical dynamics, in response to disturbance and flow permanence, macroinvertebrate FFGs are important indicators of the ecological health of a river system and the functioning of the same ecosystems. This is because when it came to tracking changes in ecosystem function and integrity brought on by both human and livestock disturbance and seasonal flow fluctuations, the various FFG ratios and indicators used worked well.

The majority of streams were autotrophic (P/R >1), which can be seen to be consistent with previous research in Kenyan streams (Masese *et al.,* 2014; Masese & Raburu, 2017), except sites which were primarily heterotrophic (P/R <1) in less disturbed areas. The P/R ratios in the heterotrophic streams most likely changed towards autotrophy because of the low abundances of collectors (Oligochaeta and Chironomidae). Therefore, autotrophy in most sites throughout both the wet and dry seasons deviates significantly from what would be predicted using other indicators of ecosystem functioning.

This better performance of this metric, which implies a well-preserved riparian zone, can be attributed to the presence of shredders, especially the freshwater crabs (*Potamonautes* sp.) and Tipulidae (Diptera), whose large bodies, even in small numbers, can disproportionately shift the P/R ratio toward greater heterotrophy when biomass is used, and the CPOM/FPOM ratio metric to identify sites as having a well-protected and functioning riparian zone, when in essence they may not (Masese *et al.,* 2014; Sitati *et al.,* 2021).

The findings on the use of the ratios of FFGs as surrogates of ecosystem attributes underscore potential concerns regarding the bias that may be introduced into metrics assessing ecosystem functioning in the presence of large-bodied macroconsumers, such as freshwater crabs. Although macroconsumers, including crabs, crayfish, and shrimps, are frequently categorized as shredders and are recognized for their significant contributions to organic matter decomposition in tropical streams (Crowl *et al.,* 2001; Masese et al., 2014), it is important to acknowledge their omnivorous nature and diverse dietary preferences. This characteristic implies that classifying them as shredders when calculating ecosystem functioning metrics could be potentially misleading. Furthermore, macroconsumers can impose substantial top-down control on other invertebrate populations, as documented by Lancaster *et al.,* 2008. This aspect introduces complexities that could potentially diminish their suitability as reliable indicators of ecosystem condition and functionality. The findings of this study indicate a heightened influence of top-down control by predators in response to increased disturbances and flow variability. Across all site categories and variations in flow permanence, predators were abundant, such as Hemiptera, Coleoptera, and Odonata. Notably, there was an increased abundance of larger-bodied odonates, beetles, and bugs, which are known for their rapid colonization and tolerance to suboptimal water conditions, including in seasonal streams and rivers (Boulton & Lake, 2008). Some coleopteras (beetles) and hemipterans (bugs) can persist in drying pools and possess high mobility, allowing them to fly away and seek refuge in larger, more permanent water bodies (May, 2019; Meena & Kachhwaha, 2024). Certain Odonata species also exhibit resilience to fluctuations in flow and temperature (Hardersen, 2008; Stewart & Samways, 1998), contributing to their prevalence and high diversity across all study sites.

The findings of this study also showed a strong correlation between channel stability and fine particulate organic matter in transport or the water column (TFPOM) versus benthic fine particulate organic matter (BFPOM) metrics as indicators of ecological condition. These metrics consistently indicated a higher presence of FPOM in transport than that deposited on the streambed, highlighting the importance of water movement for transporting organic matter utilized by filter feeders in the study area. These findings suggest that the metric primarily relied on rheophilic taxa such as Hydropsychidae and Simuliidae, both of which are known to be sensitive to flow cessation and rely on water flow to feed. Simuliidae are also considered a food source for predator groups of Hydropsychidae, such as *Cheumatopsyche* spp. (Masese and Raburu, 2017; Rivers-Moore *et al.,* 2007).

5.4 Macroinvertebrate traits

This study also examined how macroinvertebrate traits vary in response to human disturbance and flow cessation in streams and rivers. The aim was to enhance existing macroinvertebrate-based biomonitoring programs in the region by incorporating trait-based approaches. Consistent with earlier studies in the Afrotropics (e.g., Edegbene *et al.,* 2020; Sitati *et al.,* 2021) and beyond (Gerth *et al.,* 2017; Wang *et al.,* 2019; Zhang *et al.,* 2021), which have documented the impact of agriculture, human activity, and livestock on river ecosystems, the results show that macroinvertebrate traits respond to the different levels of disturbance and flow conditions, and thus can be used as indicators of ecological conditions in Afromontane-savannah rivers.

Indicators for all disturbance levels and flow permanence categories were found to be specific characteristics and trait features. This is because features that were linked to high disturbance during the wet season, including having hemoglobin, big size (Size 4 and 5), preferring sediment, and being able to swim, were characteristics most likely indicating resistance to unstable or degraded habitats. Characteristics that were linked to moderate disturbance included semi-voltinism and univoltinism (moderate life cycle durations), affiliations with vegetation or macrophytes, medium body size (Size 2), and dependence on coarse particle organic matter (shredders). Moreover, included in this group were characteristics such as case/tube builders, shredders, pupae, nymphal stages, and exophytic oviposition.

Features like spiracles, a small size (Size 1), soft and exposed body forms, free-living habits, gills, long life cycles (>1 year), predatory behavior, and tough outer body coverings (tegument) were common and observed in low disturbance sites, indicating adaptation to stable, high-quality environments. However, in the dry season, characteristics such as medium body size (Size 2), transitory attachment mechanisms, scraping feeding behaviors, semi-voltinism, affinity for very slow-flowing or stagnant water, and dependence on FPOM was noticed in taxa that could withstand considerable disturbance.

Traits linked to moderate disturbance included case/tube building, a range of body sizes (Size 1, 4, and 5). For low disturbance conditions, traits like slow-flowing habitat preference, larval stage dominance, climbing ability, adult presence, medium body size (Size 3), association with vegetation, exophytic oviposition, burrowing, short life spans (<1 year), and sediment preference were frequently observed (Figure: 6 RLQ).

In terms of flow permanence, several traits indicated tolerance to seasonal streams during the wet season, such as haemoglobin possession, endophytic oviposition, adaptation to fast flow (>0.6 m/s), cylindrical shape, burrowing behavior, adult stage prevalence, use of macrophytes or vegetation, pupae, case/tube construction, adaptation to very slow flow (0.1–1 m/s), spherical shape, temporary attachment, short life cycle (<1 year), exophytic oviposition, and lung respiration. In contrast, traits associated with permanent streams in the wet season included spiracles, small body size (≤5 mm), soft and exposed body forms, free-living behavior, gills, tegument, longer life cycles (>1 year), swimming locomotion, univoltinism, crawling, scraping, permanent attachment, flattened body, semi-univoltinism, streamlined shape, moderate flow preference, and reliance on fine particulate organic matter.

In the dry season, traits linked to seasonal streams included temporary attachment, case/tube building, scraping, adult and pupal stages, medium body size (Size 3), fine particulate organic matter use, larval presence, lung respiration, sediment and coarse particulate use, large body sizes (Size 4 and 5), shredding feeding behavior, short life cycles (<1 year), exophytic oviposition, and burrowing. Meanwhile, traits suggesting tolerance to permanent streams during the dry season included medium and small body sizes (Size 1 and 2), spiracles, semi-voltinism, sprawling locomotion, swimming, endophytic oviposition, gathering feeding habit, fine particulate use, streamlined shape, and long-life cycles (>1 year) (Figure: 6 RLQ).

These traits reflect the adaptability of macroinvertebrates to varying levels of disturbance and flow permanence. Traits like haemoglobin, burrowing, and temporary attachment enable survival in disturbed or fluctuating environments, while traits such as gill respiration, crawling, and vegetation reliance indicate stability and higher ecological quality. This study's findings align with previous research (Dolédec *et al.,* 2006; Schäfer et al., 2007; Akamagwuna *et al.,* 2021) and offer valuable insights for biomonitoring and conservation strategies in diverse aquatic systems.

It was expected that medium and large-bodied invertebrates (especially those over 40 mm) would become less common, and that smaller-bodied species would dominate as disturbance increased, an assumption supported by earlier studies (Feio & Dolédec, 2012; Kuzmanovic et al., 2017). Surprisingly, the findings from the Wundanyi and Bura rivers showed that larger-bodied organisms, i.e, crabs, were tolerant of high disturbance. Typically, larger body size is linked to traits like longer life cycles and lower reproductive rates, which reduce the ability of these organisms to quickly recolonize disturbed environments (Larsen & Ormerod, 2010; Feio *et al.,* 2015). However, the way organisms respond to pollution is often shaped by interactions between multiple traits. In the case of large-bodied invertebrates, other traits may have had a stronger influence on their tolerance, potentially overriding the expected disadvantages of their size (Statzner & Bêche, 2010; Odume *et al.,* 2014; Odume, 2020; Akamagwuna,2021). Physiologically, large-bodied animals have a lower surface-area-to-volume ratio compared to smaller species, which reduces their exposure to and absorption of dissolved salts and metals, an important aspect that explains their resilience (Statzner & Bêche, 2010) (Figure: 6 RLQ). Some of these large bodied taxa such as crabs, odonates, coleopterans, and Hemipterans, have air-breathing and are semi-aquatic taxa that can tolerate low DO conditions and can exist in poor water quality. Some of these taxa are also early colonizers and can fly or move away from degraded streams and pools and fully colonize other streams with water flow. This can be seen in crabs, which are semi-aquatic and can obtain a large fraction of their energy needs from the terrestrial environment (Lancaster *et al.,* 2008; Masese and Raburu, 2017). These physiological phenomena could partly explain why medium and large-sized invertebrates were more abundant along the polluted sites in the Wundanyi and Bura rivers.

This scenario has also been observed in neotropical headwater streams, where swimming species are most abundant, especially in areas of agricultural activities and livestock disturbance (Johnson *et al.,* 1997; de Castro *et al.,* 2018). Burrowers are usually known to live in fine sediment, where they eat organic substances in the substrate. These sediments not only supply food, but they also protect them from water from pollution or polluted environment (Leslie & Lamp, 2017).

In this study, traits like haemoglobin and spiracles were found in high numbers at degraded sites along the Wundanyi and Bura rivers, while gill-breathing taxa were noticeably fewer (Figure: 5). Invertebrates with haemoglobin or spiracles thrive in low-oxygenated conditions by supplementing their oxygen intake (Díaz *et al.,* 2008; Statzner & Bêche, 2010). However, food intake and OM enrichment usually reduce DO levels, therefore making it more stressful for organisms that rely on gills for breathing to survive in such conditions. However, gill-breathing species' respiratory surfaces can clog and impede gas exchange, therefore rendering them more vulnerable to fine silt and turbidity as observed in agricultural surface runoff.

The interaction of various features affects how predators adapt to different levels of environmental stressors. For instance, characteristics that aid in survival in low DO may be more valuable than the disadvantages of reduced eyesight in muddy water. According to research, communities' responses to different forms of stress are usually driven by combinations of different attributes rather than individual aspects (Poff *et al.,* 2006; Menezes *et al.,* 2010; Verberk *et al.,* 2013; Odume, 2020).

This study supports the concept that predatory behavior might be an indication of environmental degradation in some various conditions. This is supported by the observations from Akamwugana et al. (2021) in the Kat River, South Africa, where they stated that shredders are sensitive to agricultural disturbance. They further noticed that they were shown to be particularly susceptible to low and moderate disturbances. This they attributed to grasslands, rather than forests, predominating in the highly disturbed areas, and the input of leaf litter and OM, which is an important source of food for shredders.

Human and livestock disturbances had the greatest influence on crawling invertebrates. This is because crawlers move slowly along the streambeds, therefore making them have little to no ability to avoid predators or a deteriorated environment. However, because of their fragility, they were most likely more prevalent in parts of the Wundanyi and Bura rivers with less disturbance and a more stable channel. Crawler populations in temperate streams are diminishing due to land-use pressures, from both human and livestock activities, which is consistent with our findings (Mondy & Usseglio-Polatera, 2013; Yadamsuren *et al*., 2020).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study contributes to the expanding pool of information research regarding the functional arrangement of macroinvertebrates in Afro-tropical streams and their efficacy as bioindicators for assessing ecosystem functionality across various disturbance levels, temporal scales, and flow permanence. Given the challenges in drawing general inferences and conclusions from different categorical responses at different altitudes and land use categories which is also inferred as disturbance, this study proposes that indicator taxa and site-specific environmental factors be included in biomonitoring programs in Afro-montane Savannah rivers.

This work contributes to the increasing amount of research that supports trait-based approaches in freshwater biomonitoring by identifying certain characteristics associated with livestock disturbance in Afrotropical rivers. It is therefore crucial to use caution when interpreting trait-based results. Within a species, the influence of several overlapping qualities can obfuscate distinct patterns, therefore making it difficult to differentiate the effects of particular features. Additionally, the low availability of specific trait information, particularly about life cycle traits, remains a barrier for many regional taxa. It is therefore advised that these findings be expanded and strengthened by larger research conducted in other river systems and geographical regions.

6.2 Recommendations

These are the recommendations from this study:

1. Integrate Trait-Based Biomonitoring into Routine River Health Assessments

Functional trait analysis should be used in integration with conventional taxonomic methods to improve ecological biomonitoring aspects that have currently been used. Characteristics that include life cycle duration, feeding group, mobility, and respiration mode offer more detailed and crucial information about how macroinvertebrate groups react to certain stresses like pollution and hydrological change. More sensitive environmental change detection is made possible by this dual method, especially in systems where species-level identification is insufficient.

2. Prioritize the Conservation and Restoration of Flow Regimes in Seasonal Rivers

Maintaining environmental flows should be the major goal of conservation efforts since flow cessation has a significant impact on macroinvertebrate structure and function, particularly in savannah rivers with distinct dry seasons. This entails controlling water abstraction, safeguarding upstream sources, and reestablishing natural hydrological variability. Ideally, controlling flow regimes preserves biological integrity and ecosystem processes, including organic matter digestion and nutrient cycling in addition to supporting habitat stability.

3. Expand Functional Trait Databases for Afrotropical Aquatic Invertebrates

A significant obstacle to implementing trait-based methods in Kenya or East Africa is the limited availability of robust trait information for native species. This will make it possible to describe the life cycle, physical characteristics, and ecological aspects of Afrotropical macroinvertebrates, particularly endemic and understudied taxa. Therefore, it will enhance the precision of trait-environment models and facilitate the creation of biomonitoring instruments that are pertinent to the region and capture the distinctive features of savannah and Afromontane ecosystems.

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APPENDICES

Appendix I: Macroinvertebrates FFG assignment (Merritt & Cummins, 1996; Dobson et al., 2002, Molina, 2004; Masese et al., 2014a) collected in the Sosiani Kipkaren River during the wet and dry seasons. PRD = Predators, SCR = Scrapers, CG = Collector-gatherers, CF = Collector-filterers, SHR = Shredders.

ORDER	FAMILY	GENUS	FFG
Arachnida	Araneae	Araneae	PRD
Arhynchobdellida	Hirudinae	*Hirudo sp.*	PRD
Coleoptera	Amphizoidae	Amphizoidae	PRD
	Dytiscidae	*Yola sp.*	PRD
	Elmidae	Elminae	SCR
		Larainae	SHR
	Gyrinidae	*Dineutus sp.*	PRD
		Gyrinus sp.	PRD
	Hydraenidae	*Hydraena sp.*	PRD
	Hydrophilidae	Hydrohilinae	PRD
		Hydrophilus sp.	PRD
	Noteridae	Noteridae	PRD
	Scirtidae	*Elodes sp.*	SCR
Collembola	Collembola	Collembola	CG
Decapoda	Atyidae	Atyidae	CG
	Cambaridae	*Cambarus sp.*	CG
	Potamonautidae	*Potamonaute sp.*	SHR
Diptera	Athericidae	Athericidae	PRD
	Ceratopogonidae	*Bezzia sp.*	PRD
		Culicoides	PRD
	Chironomidae	*Chironomus sp.*	CG
		Orthocladiinae	CG
		Tanypodinae	PRD
	Muscidae	*Musca sp.*	CG
	Nepidae	Nepidae	PRD
	Psychodidae	Psychodidae	CG
	Simuliidae	*Simulium sp.*	CF
	Tabanidae	*Tabanus sp.*	PRD
	Tanyderidae	Tanyderidae	CG
	Tipulidae	*Antocha sp.*	SHR
		Hexatoma sp.	PRD
		Limonia sp.	SHR
		Tipula sp.	SHR
Ephemeroptera	Baetidae	*Acanthiops sp.*	SHR
		Baetis sp	SCR

		Centroptiloides	CG
		Tsitsa sp.	CG
	Caenidae	*Afrocaenis sp*	CG
		Caenis sp	CG
	Ephemerythidae	*Ephemerythus sp.*	CG
	Heptageniidae	*Afronurus sp.*	SCR
	Leptophlebiidae	*Adenophlebia sp*	CG
		Euthraulus sp.	SCR
		Leptophlebia sp.	CG
	Oligoneuriidae	*Oligoneuriopsis sp.*	CF
	Polymitarcyidae	*Povila sp.*	CG
	Tricorythidae	*Dicercomyzon sp.*	CG
		Tricorythus sp.	CG
Hemiptera	Belostomatidae	*Belostoma sp.*	PRD
	Corixidae	*Corixa sp.*	PRD
		Micronecta sp.	PRD
	Gerridae	*Eurymetra sp.*	PRD
		Gerris sp.	PRD
		Metrobates sp.	PRD
	Hydrometridae	*Hydrometra sp.*	PRD
	Mesoveliidae	*Mesovelia sp.*	PRD
	Naucoridae	Naucoridae	PRD
	Nepidae	Nepidae	PRD
	Notonectidae	*Notonecta sp.*	PRD
		Plea sp.	PRD
Lepidoptera	Crambidae	Crambidae	SHR
		Parapoynx sp.	SHR
		Synclita sp.	SHR
Mollusca	Lymnaeidae	Lymnaeidae	SCR
	Planorbidae	*Planorbis sp.*	SCR
	Sphaeriidae	*Pisidium sp.*	CF
	Thiaridae	Thiaridae	SCR
Odonata	Aeshinidae	*Aeshna sp.*	PRD
	Chlorolestidae	*Chlorolestidae*	PRD
	Corduliidae	*Cordulia sp.*	PRD
		Corduliidae	PRD
		Phyllomacromia sp.	PRD
	Gomphidae	*Gomphus sp.*	PRD
	Lestidae	*Lestes sp.*	PRD
	Libelluliidae	Libelluliidae	PRD
	Protoneuridae	Protoneuridae	PRD
Oligochaeta	Lumbriculidae	*Lumbricus sp.*	CG
	Tubificidae	*Tubifex sp.*	CG
Plecoptera	Perlidae	*Neoperla sp.*	PRD
Trichoptera	Calamoceratidae	*Anisocentropus sp.*	SHR

	Ecnomidae	*Ecnomus sp.*	CF
	Hydropsychidae	*Cheumatopsyche sp.*	PRD
		Hydropsyche sp.	CF
	Hydroptilidae	Hydroptilidae	SCR
	Lepidostomatidae	*Lepidostoma sp.*	SHR
	Leptoceridae	Atheripceides	SHR
		Leptocerus sp	SHR
		Oecetis sp	PRD
	Philopotamidae	*Chimarra sp.*	CF
	Pisuliidae	*Pisulia sp.*	SHR
	Polycentropodidae	*Polycentropus sp.*	PRD
Tricladida	Planariidae	*Planaria sp.*	PRD

Appendix II: The QHEI form used for qualitative evaluation of sites along the Wundanyi and Bura rivers (Adopted from Rankin, 1995)

QUALITATIVE HABITAT EVALUATION INDEX FIELD SHEET **TOTAL SCORE** ☐

Stream ________ RM________ Date ________ River Code________

Location ________ Scorer's Name ________

1] SUBSTRATE (Check ONLY Two Substrate TYPE BOXES: Estimate % or note every type present):

TYPE	POOL RIFFLE		POOL RIFFLE	NUMBER OF SUBSTRATES
BLDER/SLAB(10)	___ ___	GRAVEL(7)	___ ___	___ 4 or More(3)
BOULDER(9)	___ ___	SAND(6)	___ ___	___ <4 (0)
COBBLE(8)	___ ___	BEROCK(5)	___ ___	
HARDPAN (4)	___ ___	DETRITUS(3)	___ ___	
MUCK (2)	___ ___	ARTIFICIAL(0)	___ ___	
SILT (2)	___ ___			

NOTE: (Ignore sludge that originates from point-sources: Score on Natural substrates)

2] INSTREAM COVER

TYPE (Check all that apply, ALL 1 except deep pools) **AMOUNT** (Check on 1 or 2 and average)

UNDERCUT BANKS	___	DEEP POOLS	___	EXTENSIVE >75% (10)	___
OVERHANGING VEGETATION	___	ROOTWADS	___	MODERATE 25-75%(6)	___
SHALLOWS	___	BOULDERS	___	SPARSE 5-25%(3)	___
ROOTMATS	___	OXBOWS	___	NEARLY ABSENT <5%	___
AQUATIC MACROPHYTES	___	LOGS/WOODY	___		

OMMENTS ________

3] CHANNEL MORPHOLOGY (CHECK ONLY 1 PER CATEGORY OR CHECK 2 AND AVERAGE)

SINUOSITY		STABILITY		MODIFICATIONS/OTHER			
HIGH(4)	___	HIGH (3)	___	SNAGGING	___	MPOUND	___
MODERATE 3)	___	MODERATE (3)	___	ISLANDS	___	LEVEED	___
LOW (2)	___	LOW (3)	___	CANOPY REMOVAL	___	RELOCATION	___
NONE (1)	___			DREDGING	___	BANK SHAPING	___

4] RIPARIAN ZONE AND BANK EROSION (Check ONE per bank or check 2 and AVERAGE per bank)

RIPARIAN WIDTH FLOOD PLAIN QUALITY BANK EROSION

(L R per bank)	L R (Most prominent per bank)		
		CONSERVATION TILLAGE (1)	NONE/LITTLE (3)
WIDE >50m (4)	FOREST, SWAMP (3)	URBAN (0)	MODERATE (2)
MODERATE 10-50m(3)	SHRUB OR OLD FIELD (2)	OPEN PASTURE (O)	HEAVY (1)
NARROW 5-10m(2)	RESIDENTIAL PARK (1)	ROW CROP (0)	
VERY NARROW (1)	FENCED PASTURE (1)		NONE (0)

5] POOL/GLIDE AND RIFFLE/RUN QUALITY

MAX DEPTH (Check 1 only)	MORPHOLOGY (Check 1or 2 and average)	CURRENT VELOCITY (POOL $ RIFFLES) (Check all that apply)	
>1m (6)			
0.7-1m (2)	POOL WIDTH> RIFFLE WIDTH(2)	EDDIES(1)	TORRENTIAL(-1)
0.4-0.7m(2)	POOL WIDTH=RIFFLE WIDTH(1)	FAST(1)	INTERSTITIAL(-1)
0.2-0.4m (1)	POOL WIDTH< RIFFLE WIDTH(0)	MODERATE(1)	INTERMITENT(-2)
<0.2m (pool=0)		LOW(1)	

RIFFLE/RUN DEPTH (CHECK ONE OR CHECK 2 AND AVERAGE) **RIFFLE/RUN SUBSTRATE**

GENERALLY >50cm(4)	___	STABLE (e.g cobble, boulder) (2)	___
GENERALLY 10-50cm(3)	___	MOD, STABLE (e.g. Large Gravel) (1)	___
GENERALLY 5-10cm(1)	___	UNSTABLE (Fine Gravel, Sand) (0)	___
GENERALLY <5cm(0)	___		

Adopted from Rankin (1995)

Appendix III: Fuzzy coding of macroinvertebrate traits in the Wundanyi and Bura rivers.

Code: SIZE 1: very small, ≤ 5, SIZE 2: Small body (> 5 – 10), SIZE 3: Medium body (> 10 – 20), SIZE 4: Large body (> 20 – 40), SIZE 5: Very-large body (>40), LAR: Larvae aquatic stage, NYM: Nymph aquatic stage, PUP: Pupa, GIL: Gills, EN_O: Endophytic oviposition, EX_O: Exophytic oviposition, TEG: Tegument, SPI: Spiracle, LUN: Lung, HAE: Haemoglobin, SEM: Semivoltinism, UNI: Univoltinism, LE_Y: < 1 generation year per year), GR_Y: > 1 generation per year, ADU: Adult aquatic stage, FPO: Fine particulate organic matter, CPO: Coarse particulate organic matter, STR: Streamlined, FLA: Flattened, SPH: Spherical, CYL: Cylindrical, SO_E: Soft and exposed, CAS: Cased/tubed, SCL: Sclerotised, SHR: Shredders, GAT: Gatherers, FI_F: Filter-feeders, SCR: Scrapers/grazers, PRE: Predators, FPO: Fine particulate organic, MAC: Macrophytes, AN_M: Animal materials, CRA: Crawlers, CLI: Climbers/clingers, SPR: Sprawlers, SWI: Swimmers, SKA: Skaters, BUR: Burrowers, PE_A: Permanently attached, TE_A: Temporarily attached, FR_L: Free-living, STO: Stone, SED: Sediment), VEG: vegetation, VF_F: Very fast-flowing (> 0.6), MF_F: Moderately fast-flowing (> 0.3 – 0.6), SL_F: Slow flowing (0.1 – 0.3), VS_F: Very slow-flowing (< 0.1 – 1).

Taxa	**SIZE 1**	**SIZE 2**	**SIZE 3**	**SIZE 4**	**SIZE 5**	**SEM**	**UNI**	**LE_Y**	**GR_Y**	**ADU**	**LAR**	**NYM**	**PUP**	**GIL**	**TEG**	**BR_S**	**SPI**	**LUN**
Acanthiops tsitsa (Lugo-Ortiz & McCafferty, 1997)	1	5	3	0	0	5	0	5	0	0	0	5	0	5	0	0	0	0
Adenophlebia (auriculata Eaton, 1871)	0	3	5	4	0	5	0	5	0	0	0	5	0	5	0	0	0	0
Adenophlebia spp.	0	3	5	4	0	5	0	5	0	0	0	5	0	5	0	0	0	0
Aeshna spp.	0	1	4	5	3	0	0	5	0	0	0	5	0	5	0	0	0	0
Afrobrianax ferdyi Lee, Philip & Yang, 2003	0	3	5	0	0	0	5	5	0	5	5	5	5	5	0	0	5	0
Afronurus Barnardi (Schoonbee, 1968)	1	5	4	0	0	5	4	5	3	0	0	5	0	5	0	0	0	0
Afronurus spp	1	5	4	0	0	5	4	5	3	0	0	5	0	5	0	0	0	0
Afroptilum spp.	2	5	3	0	0	5	3	5	0	0	0	5	0	5	0	0	0	0

Agraptocorixa sp	1	2	5	4	0	5	0	5	0	5	5	5	5	0	0	0	5	0
Allocnemis spp.	0	0	5	0	0	5	0	0	5	0	0	5	0	5	0	0	0	0
Amphipoda	0	1	5	0	0	0	0	0	0	5	5	5	5	0	5	0	0	0
Ancylidae	0	5	4	0	0	5	0	5	0	5	5	5	5	0	0	0	5	0
Aphericerca spp.	0	2	5	4	0	0	5	5	3	0	0	5	0	5	0	0	0	0
Appasus spp.	0	2	2	4	5	0	0	5	0	5	5	5	5	0	0	0	5	0
Athericidae	0	1	5	1	0	0	0	5	0	2	5	0	0	0	0	0	5	0
Athripsodes spp.	0	0	5	0	0	0	5	0	5	0	5	0	5	5	0	0	0	0
Baetis harrisoni (Barnard, 1932)	2	5	3	0	0	5	3	5	0	0	0	5	0	5	0	0	0	0
Baetis spp.	2	5	3	0	0	5	3	5	0	0	0	5	0	5	0	0	0	0
Biomphalaria spp.	0	0	5	5	3	5	0	5	0	5	5	5	5	0	0	0	5	5
Brachythemis lacustris (Kirby, 1889)	0	0	5	3	0	0	5	5	0	0	0	5	0	5	0	0	0	0
Brachythemis spp	0	0	5	3	0	0	5	5	0	0	0	5	0	5	0	0	0	0
Burnupia spp.	0	5	4	0	0	5	0	5	0	5	5	5	5	0	0	0	5	0
Caenis spp.	3	5	2	0	0	0	5	5	0	0	0	5	0	5	0	0	0	0
Ceratophallus spp.	0	0	5	5	3	5	0	5	0	5	5	5	5	0	0	0	5	5
Cheumatopsyche spp.	0	2	5	4	0	0	5	5	3	0	5	0	5	5	0	0	0	0
Cheumatopsyche thomasetti (Ulmer) 1931	0	2	5	4	0	0	5	5	3	0	5	0	5	5	0	0	0	0
Cheumatopsyche afra (Mosely 1935)	0	2	5	4	0	0	5	5	3	0	5	0	5	5	0	0	0	0
Chironomidae	3	5	1	1	0	0	0	0	0	2	5	0	0	5	0	0	4	0
Corbicula astartina (Martens, 1860)	0	5	4	0	0	5	0	5	0	5	5	5	5	0	0	0	5	0
Crenigomphus spp.	0	3	4	5	1	5	0	0	5	0	0	5	0	5	0	0	0	0
Crocothemis erythraea (Brulle, 1832)	0	0	5	3	0	0	5	5	0	0	0	5	0	5	0	0	0	0
Culex spp 1	0	2	5	0	0	5	0	5	0	2	5	0	0	0	0	5	4	0
Culex spp 2	0	2	5	0	0	5	0	5	0	2	5	0	0	0	0	5	4	0
Demoreptus capensis (Barnard, 1932)	2	4	5	3	0	5	0	5	3	0	0	5	0	5	0	0	0	0
Dineutus spp.	0	0	2	5	4	0	5	0	5	5	5	5	5	0	0	0	5	0
Elassoneuria spp.	0	2	4	5	0	0	5	5	3	0	0	5	0	5	0	0	0	0
Elminae sub-family	2	5	3	0	0	0	5	0	5	5	5	5	5	0	0	0	5	0

Enallagma glaucum (Burmeister, 1839)	0	1	5	5	1	0	5	5	0	0	0	5	0	5	0	0	0	0
Enithares spp. (Notonectidae)	0	2	5	0	0	5	0	5	0	5	5	5	5	0	0	0	5	0
Euthraulus spp	1	3	5	0	0	0	5	5	0	0	0	5	0	5	0	0	0	0
Hydropsyche spp.	0	3	5	4	0	0	5	5	3	0	5	0	5	5	0	0	0	0
Laccocoris spp.	0	5	5	0	0	0	5	5	0	5	5	5	5	0	0	0	5	0
Larainae sub-family	2	5	3	0	0	0	5	0	5	5	5	5	5	5	0	0	0	0
Leeches	0	3	1	0	0	0	0	0	0	5	5	5	5	0	5	0	0	0
Lestes plagiatus (Burmeister, 1839)	0	1	5	5	0	0	5	5	0	0	0	5	0	5	0	0	0	0
Limnophila crespusclum (Tipulidae)	0	5	5	2	0	0	5	0	0	2	5	0	0	5	0	0	4	0
Lymnaea columella (Say, 1817)	0	0	3	5	4	0	0	5	0	5	5	5	5	0	0	0	5	5
Lymnaea natalensis (Krauss, 1 848)	0	0	3	5	4	0	0	5	0	5	5	5	5	0	0	0	5	5
Lymnaea spp.	0	0	3	5	4	0	0	5	0	5	5	5	5	0	0	0	5	5
Macrostemum capense (Walker, 1952)	0	2	5	4	0	0	5	5	3	0	5	0	5	5	0	0	0	0
Muscidae	0	5	0	0	0	0	0	5	0	2	5	0	0	0	0	0	5	0
Notonecta spp.	0	2	5	0	0	5	0	5	0	5	5	5	5	0	0	0	5	0
Oligochaeta	0	0	5	0	0	5	0	0	5	5	5	5	5	0	5	0	0	0
Orectogyrus spp.	0	0	2	5	4	0	5	0	5	5	5	5	5	0	0	0	5	0
Pantala spp.	0	0	5	3	0	0	5	5	0	0	0	5	0	5	0	0	0	0
Paragomphus genei (Selys, 1841)	0	3	4	5	1	5	0	0	5	0	0	5	0	5	0	0	0	0
Plea spp.	5	0	0	0	0	0	0	5	0	5	5	5	5	0	0	5	3	0
Potamonautes spp.	0	0	2	4	5	0	0	5	0	5	5	5	5	5	0	0	0	0
Pseudagrion spernatum (Selys, 1881)	0	1	5	5	1	5	0	5	0	0	0	5	0	5	0	0	0	0
Pseudocloeon glaucum (Agnew, 1961)	2	5	2	0	0	5	3	5	0	0	0	5	0	5	0	0	0	0
Pseudocloeon pisc (Lugo-Ortiz & McCafferty, 1997	2	5	3	0	0	5	3	5	0	0	0	5	0	5	0	0	0	0
Pseudocloeon spp.	2	5	3	0	0	5	3	5	0	0	0	5	0	5	0	0	0	0
Pseudocloeon vinosum (Barnard 1932)	1	5	3	0	0	5	3	5	0	0	0	5	0	5	0	0	0	0
Sigara spp.	1	2	5	4	0	5	0	5	0	5	5	5	5	0	0	0	5	0

Simulium spp.	3	5	0	0	0	0	0	5	0	2	5	0	0	5	0	0	4	0
Tabanus spp.	0	1	2	5	2	0	0	5	0	2	5	0	0	0	0	0	5	0
Tricorythus discolor (Burmeister, 1839)	1	3	5	2	0	5	0	5	3	0	0	5	0	5	0	0	0	0
Tricorythus spp.	1	3	5	2	0	5	0	5	3	0	0	5	0	5	0	0	0	0
Trithemis spp	0	0	5	3	0	0	5	5	0	0	0	5	0	5	0	0	0	0
Veliidae	4	5	3	0	0	5	0	5	0	5	5	5	5	0	0	0	5	0
Yola spp	2	3	5	2	0	0	5	5	0	5	5	5	5	0	0	0	5	0

Taxa	SO_E	CAS	SCL	SHR	GAT	FI_F	SCR	PRE	FPO	CPO	MAC	AN_M	CLI	CRA	SPR	SWI	SKA	BUR
Acanthiops tsitsa (Lugo-Ortiz & McCafferty, 1997)	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Adenophlebia (auriculata Eaton, 1871)	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Adenophlebia spp.	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Aeshna spp.	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Afrobrianax ferdyi Lee, Philip & Yang, 2003	0	0	5	0	0	0	5	0	0	3	5	0	0	5	0	0	0	0
Afronurus Barnardi (Schoonbee, 1968)	5	0	0	0	3	0	5	0	5	3	0	0	0	5	3	0	0	0
Afronurus spp	5	0	0	0	3	0	5	0	5	3	0	0	0	5	3	0	0	0
Afroptilum spp.	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Agraptocorixa sp	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0
Allocnemis spp.	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Amphipoda	5	0	3	0	0	0	0	5	0	0	0	5	0	0	5	0	0	3
Ancylidae	0	5	0	3	0	0	5	0	0	0	5	0	0	5	0	0	0	3
Aphericerca spp.	5	0	0	0	5	0	0	5	0	0	0	3	0	5	3	0	0	0
Appasus spp.	0	0	5	0	0	0	0	5	0	0	0	5	0	5	0	0	0	0
Athericidae	5	0	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	3
Athripsodes spp.	0	5	0	5	3	0	3	0	0	5	4	0	3	5	0	0	0	0
Baetis harrisoni (Barnard, 1932)	5	0	0	0	5	0	3	0	5	3	0	0	0	0	5	0	0	0
Baetis spp.	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Biomphalaria spp.	0	5	0	3	0	0	5	0	0	0	5	0	0	5	0	0	0	3

Brachythemis lacustris (Kirby, 1889)	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Brachythemis spp	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Burnupia spp.	0	5	0	3	0	0	5	0	0	0	5	0	0	5	0	0	0	3
Caenis spp.	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Ceratophallus spp.	0	5	0	3	0	0	5	0	0	0	5	0	0	5	0	0	0	3
Cheumatopsyche spp.	3	0	5	0	0	5	0	3	5	0	0	3	3	0	0	5	0	0
Cheumatopsyche thomasetti (Ulmer) 1931	3	0	5	0	0	5	0	3	5	0	0	3	3	0	0	5	0	0
Cheumatopsyche afra (Mosely 1935)	3	0	5	0	0	5	0	3	5	0	0	3	3	0	0	5	0	0
Chironomidae	5	0	4	0	0	0	0	5	0	0	0	5	0	0	5	5	0	3
Corbicula astartina (Martens, 1860)	0	5	0	3	0	5	5	0	0	0	5	0	0	5	0	0	0	3
Crenigomphus spp.	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Crocothemis erythraea (Brulle, 1832)	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Culex spp 1	5	0	0	0	0	5	0	0	5	3	0	0	0	0	5	0	0	0
Culex spp 2	5	0	0	0	0	5	0	0	5	3	0	0	0	0	5	0	0	0
Demoreptus capensis (Barnard, 1932)	5	0	0	0	3	0	5	0	5	3	0	0	0	5	3	0	0	0
Dineutus spp.	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0
Elassoneuria spp.	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Elminae sub-family	0	0	5	0	0	0	5	3	0	0	5	3	0	5	0	0	0	0
Enallagma glaucum (Burmeister, 1839)	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Enithares spp. (Notonectidae)	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0
Euthraulus spp	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Hydropsyche spp.	3	0	5	0	0	5	0	3	5	0	0	3	3	0	0	5	0	0
Laccocoris spp.	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0
Larainae sub-family	0	0	5	0	0	0	5	3	0	0	5	3	0	5	0	0	0	0
Leeches	5	0	0	0	0	0	0	5	0	0	0	5	0	0	5	0	0	3
Lestes plagiatus (Burmeister, 1839)	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Limnophila crespusclum (Tipulidae)	0	5	0	0	0	0	0	5	0	0	0	5	0	0	5	0	0	0
Lymnaea columella (Say, 1817)	0	5	0	3	0	0	5	0	0	0	5	0	0	5	0	0	0	3
Lymnaea natalensis (Krauss, 1 848)	0	5	0	3	0	0	5	0	0	5	4	0	0	5	0	0	0	3
Lymnaea spp.	0	5	0	3	0	0	5	0	0	0	5	0	0	5	0	0	0	3

Macrostemum capense (Walker, 1952)	3	0	5	0	0	5	0	3	5	0	0	3	3	0	0	5	0	0
Muscidae	5	0	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	3
Notonecta spp.	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0
Oligochaeta	5	0	0	0	5	0	0	0	3	5	0	0	0	3	0	0	0	5
Orectogyrus spp.	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0
Pantala spp.	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Paragomphus genei (Selys, 1841)	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Plea spp.	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0
Potamonautes spp.	0	0	5	5	0	0	3	0	0	5	4	0	0	5	0	0	0	3
Pseudagrion spernatum (Selys, 1881)	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Pseudocloeon glaucum (Agnew, 1961)	5	0	0	0	5	0	3	0	5	3	0	0	0	0	5	0	0	0
Pseudocloeon pisc (Lugo-Ortiz & McCafferty, 1997	5	0	0	0	5	0	3	0	5	3	0	0	0	0	5	0	0	0
Pseudocloeon spp.	5	0	0	0	5	0	3	0	5	3	0	0	0	0	5	0	0	0
Pseudocloeon vinosum (Barnard 1932)	5	0	0	0	5	0	3	0	5	3	0	0	0	0	5	0	0	0
Sigara spp.	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0
Simulium spp.	5	0	0	0	0	5	0	0	5	0	0	0	0	0	5	0	0	0
Tabanus spp.	5	0	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	3
Tricorythus discolor (Burmeister, 1839)	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Tricorythus spp.	5	0	0	0	5	0	3	0	5	3	0	0	0	5	3	0	0	0
Trithemis spp	5	0	0	0	0	0	0	5	0	0	0	5	0	4	0	0	0	5
Veliidae	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	0	5	3
Yola spp	0	0	5	0	0	0	0	5	0	0	0	5	0	0	0	5	0	0

….Continued

Taxa	PE_A	TE_A	FR_L	STO	SED	VEG	VR_F	MF_F	SF_F	VS_F	HAE	EN_O	EX_O	STR	FLA	SPH	CYL
Acanthiops tsitsa (Lugo-Ortiz & McCafferty, 1997)	0	0	5	5	3	1	0	5	3	0	0	0	5	5	3	0	0
Adenophlebia (auriculata Eaton, 1871)	0	0	5	5	3	1	0	5	3	0	0	0	5	5	2	0	0
Adenophlebia spp.	0	0	5	5	3	1	0	5	3	0	0	0	5	5	2	0	0
Aeshna spp.	0	0	5	2	5	3	0	0	5	3	0	0	5	0	3	0	5
Afrobrianax ferdyi Lee, Philip & Yang, 2003	0	5	3	0	0	5	0	0	5	3	0	5	0	0	5	3	0
Afronurus Barnardi (Schoonbee, 1968)	0	3	5	5	3	1	0	5	3	0	0	0	5	2	5	0	0
Afronurus spp	0	3	5	5	3	1	0	5	3	0	0	0	5	2	5	0	0
Afroptilum spp.	0	0	5	5	3	1	0	5	3	0	0	0	5	5	3	0	0
Agraptocorixa sp	0	0	5	2	0	5	0	0	3	5	0	0	5	0	5	0	0
Allocnemis spp.	0	0	5	2	5	3	0	0	5	3	0	0	5	0	5	0	3
Amphipoda	0	0	5	3	5	0	5	3	0	0	0	5	2	3	2	0	5
Ancylidae	0	5	3	0	3	5	0	0	3	5	0	0	5	0	0	3	5
Aphericerca spp.	0	0	5	5	3	1	5	3	0	0	0	0	5	5	3	0	0
Appasus spp.	0	0	5	2	0	5	0	0	3	5	0	0	5	0	5	0	0
Athericidae	0	0	5	5	2	0	0	0	3	5	0	5	3	3	0	0	5
Athripsodes spp.	5	3	5	5	3	1	5	3	0	0	0	5	0	3	0	0	5
Baetis harrisoni (Barnard, 1932)	0	0	5	5	3	1	0	3	5	0	0	0	5	5	3	0	0
Baetis spp.	0	0	5	5	3	1	0	5	3	0	0	0	5	5	3	0	0
Biomphalaria spp.	0	5	3	0	3	5	0	0	3	5	0	0	5	0	0	3	5
Brachythemis lacustris (Kirby, 1889)	0	0	5	2	5	3	0	0	5	3	0	0	5	2	5	0	0
Brachythemis spp	0	0	5	2	5	3	0	0	5	3	0	0	5	2	5	0	0
Burnupia spp.	0	5	3	0	3	5	0	0	3	5	0	0	5	0	0	3	5
Caenis spp.	0	0	5	5	3	1	5	3	0	0	0	0	5	0	5	3	0
Ceratophallus spp.	0	5	3	0	3	5	0	0	3	5	0	0	5	0	0	3	5
Cheumatopsyche spp.	5	3	0	5	3	1	5	3	0	0	0	5	0	5	0	0	2
Cheumatopsyche thomasetti (Ulmer) 1931	5	3	0	5	3	1	5	3	0	0	0	5	0	5	0	0	2

Cheumatopsyche afra (Mosely 1935)	5	3	0	5	3	1	5	3	0	0	0	5	0	5	0	0	2
Chironomidae	0	0	5	2	5	0	5	3	0	0	4	5	2	2	0	0	5
Corbicula astartina (Martens, 1860)	0	5	3	0	3	5	0	0	3	5	0	0	5	0	0	3	5
Crenigomphus spp.	0	0	5	2	5	3	0	0	5	3	0	0	5	0	5	0	3
Crocothemis erythraea (Brulle, 1832)	0	0	5	2	5	3	0	0	5	3	0	0	5	2	5	0	0
Culex spp 1	0	5	3	5	2	0	0	0	3	5	0	5	0	3	0	0	5
Culex spp 2	0	5	3	5	2	0	0	0	3	5	0	5	0	3	0	0	5
Demoreptus capensis (Barnard, 1932)	0	3	5	5	3	1	0	5	3	0	0	0	5	2	5	0	0
Dineutus spp.	0	0	5	0	0	5	0	0	5	3	0	5	0	0	5	0	0
Elassoneuria spp.	0	0	5	5	3	1	5	3	0	0	0	0	5	5	2	0	0
Elminae sub-family	0	0	5	0	0	5	0	5	3	0	0	5	0	0	5	3	0
Enallagma glaucum (Burmeister, 1839)	0	0	5	2	5	3	0	0	5	3	0	0	5	2	5	0	0
Enithares spp. (Notonectidae)	0	0	5	2	0	5	0	0	3	5	0	0	5	0	5	0	0
Euthraulus spp	0	0	5	5	3	1	0	5	3	0	0	0	5	5	2	0	0
Hydropsyche spp.	5	3	0	5	3	1	5	3	0	0	0	5	0	5	0	0	2
Laccocoris spp.	0	0	5	2	0	5	0	0	3	5	0	0	5	0	5	0	0
Larainae sub-family	0	0	5	0	0	5	0	5	3	0	0	5	0	0	5	3	0
Leeches	0	5	3	3	5	0	0	0	3	5	0	5	2	3	2	0	5
Lestes plagiatus (Burmeister, 1839)	0	0	5	2	5	3	0	0	3	5	0	0	5	2	5	0	0
Limnophila crespusclum (Tipulidae)	0	0	5	5	2	0	5	3	0	0	0	5	0	0	5	0	0
Lymnaea columella (Say, 1817)	0	5	3	0	3	5	0	0	3	5	0	0	5	0	0	3	5
Lymnaea natalensis (Krauss, 1 848)	0	5	3	0	3	5	0	0	3	5	0	0	5	0	0	3	5
Lymnaea spp.	0	5	3	0	3	5	0	0	3	5	0	0	5	0	0	3	5
Macrostemum capense (Walker, 1952)	5	3	0	5	3	1	5	3	0	0	0	5	0	5	0	0	2
Muscidae	0	0	5	5	2	0	0	3	5	0	0	5	3	3	0	0	5
Notonecta spp.	0	0	5	2	0	5	0	0	3	5	0	0	5	0	5	0	0
Oligochaeta	0	3	5	3	5	0	0	0	0	5	0	5	2	3	2	0	5
Orectogyrus spp.	0	0	5	0	0	5	0	0	5	3	0	5	0	0	5	0	0
Pantala spp.	0	0	5	2	5	3	0	0	5	3	0	0	5	2	5	0	0
Paragomphus genei (Selys, 1841)	0	0	5	2	5	3	0	0	5	3	0	0	5	0	5	0	3
Plea spp.	0	0	5	2	0	5	0	0	3	5	0	0	5	0	5	0	0

Potamonautes spp.	0	0	5	0	3	5	5	3	0	0	0	0	5	0	0	3	5
Pseudagrion spernatum (Selys, 1881)	0	0	5	2	5	3	0	0	5	3	0	0	5	2	5	0	0
Pseudocloeon glaucum (Agnew, 1961)	0	0	5	5	3	1	0	5	3	0	0	0	5	5	3	0	0
Pseudocloeon pisc (Lugo-Ortiz & McCafferty, 1997	0	0	5	5	3	1	0	5	3	0	0	0	5	5	3	0	0
Pseudocloeon spp.	0	0	5	5	3	1	0	5	3	0	0	0	5	5	3	0	0
Pseudocloeon vinosum (Barnard 1932)	0	0	5	5	3	1	0	5	3	0	0	0	5	5	3	0	0
Sigara spp.	0	0	5	2	0	5	0	0	3	5	0	0	5	0	5	0	0
Simulium spp.	0	3	5	5	2	0	4	5	3	0	0	5	3	3	0	0	5
Tabanus spp.	0	0	5	5	2	0	0	3	5	3	0	5	3	3	0	0	5
Tricorythus discolor (Burmeister, 1839)	0	3	5	5	3	1	5	3	2	0	0	0	5	2	5	0	0
Tricorythus spp.	0	3	5	5	3	1	5	3	2	0	0	0	5	2	5	0	0
Trithemis spp	0	0	5	2	5	3	0	0	5	3	0	0	5	2	5	0	0
Veliidae	0	0	5	2	0	5	0	5	3	0	0	0	5	0	5	0	0
Yola spp	0	0	5	0	0	5	0	0	3	5	0	5	0	0	5	3	0

Appendix IV: Similarity Report

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