The role of the Usuthu River as refuge for the aquatic biodiversity of the lower Phongolo system

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PREFACE

This study provides the first comprehensive assessment of South Africa's Usuthu River's dynamics and aquatic ecology. It further contributes to the understanding of the Usuthu River and its role and contribution to the environmental quality, community dynamics and pollution of the associated aquatic systems within the Phongolo River Floodplain (PRF).

The PRF is South Africa's largest and most diverse freshwater floodplain system. It is known as a biodiversity hotspot as well as being negatively impacted on by the Pongolapoort Dam and by agricultural activities within the catchment. The dynamics and contribution of the Usuthu River to the PRF are poorly understood and therefore provides an excellent opportunity to assess the rivers water and sediment quality, aquatic community and food web structures together with mercury pollution in the PRF. Furthermore, since the majority of the aquatic systems within the PRF are connected by either the Phongolo River or the Usuthu River, fish movement to associated floodplain systems is possible. Associated floodplain lakes are known as important spawning areas for floodplain riverine fish. Therefore, determining fish migration in a floodplain such as the PRF is an important tool to determine whether fish from the PRF utilises the various systems as refuge areas during periods of drought.

This thesis conforms to the thesis format style of North-West University and consists of an introduction, result chapters and a conclusion. One of the result chapters has already been published in an international accredited peer review journal and co-author consent for use of the publication as part of this thesis and their contributions to the paper is summarised in Table I.

The following chapters are included in this thesis:

- Chapter 1 (unpublished): General introduction.
- Chapter 2 (published): Environmental quality.

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- Chapter 3 (unpublished): Aquatic community structures.
- Chapter 4 (unpublished): Food web structures and fish migration.
- Chapter 5 (unpublished): Mercury bioaccumulation, biomagnification and human health risks.

- Chapter 6 (unpublished): Conclusion and Recommendations.

Table I: Contribution of each author to the article and consent for use as part of this thesis.

Author	Chapter	Contribution	Consent
D. van Rooyen	2	Write-up – original draft, visualization, investigation, data curation.	
R. Gerber	2	Writing – review and editing; visualization; conceptualization; supervision.	
V. Wepener	2	Writing – review and editing; conceptualization; funding acquisition; supervision.	
N.J. Smit	2	Writing – review and editing; conceptualization; funding acquisition; supervision.	

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SUMMARY

Freshwater riverine floodplain systems are one of the most threatened ecosystems. These riverine floodplain systems are also the most productive and biodiverse freshwater systems in the world. Impacts that threaten these systems include altered flow regimes, and various agricultural, domestic, and industrial activities. Furthermore, these impacts may be exacerbated by extreme events such as drought. South Africa's largest and most biodiverse floodplain system, the lower Phongolo River Floodplain (PRF), consists of two large rivers contributing to the downstream associated floodplain lakes. Both the Usuthu and Phongolo rivers, the two largest rivers of the PRF, play important roles in the structure and functioning of the lower PRF aquatic systems and join at the confluence within the only protected area in the PRF, the Ndumo Game Reserve (NGR). More importantly, of these two rivers, the Phongolo River is being heavily regulated by the Pongolapoort Dam while the Usuthu River is not regulated to the same extent. The pressure on the Phongolo River's flow regime was increased by the local drought conditions experienced between 2015 and 2017. Due to these local drought conditions, the most recent flooding of the Phongolo River was in 2022, eight years after the previous flood (2014). The Phongolo River is not only influenced by the dam, but large-scale agricultural activities contribute heavily to degrading the water quality of the river. Over the years, studies have indicated the degradation of water quality of the Phongolo River as a result of these anthropogenic activities. In contrast, the dynamics of the Usuthu River and its natural flow regime remain largely unknown together with its present environmental quality and ecology with the NGR.

Similar anthropogenic activities occur within the Usuthu – Phongolo rivers catchment such as agricultural, industrial and domestic activities. Between the two rivers, the Phongolo River has been the most studied with regard to how the Pongolapoort Dam and agricultural activities have impacted downstream aquatic systems in terms of water quality, ecology, ecotoxicology and human health risks. In contrast, the impacts surrounding the Usuthu River have only been studied in the Eswatini region and therefore less is known of the system within the only protected area in the PRF. Furthermore, the sections of both rivers within the NGR are both different as agricultural activities are adjacent to the Phongolo River within the NGR while no agricultural activities are located near the Usuthu River. Recent studies have shown the impact of the Pongolapoort Dam together with the supra-seasonal drought on the water quality of the Phongolo River and as such indicated that water quality from the Phongolo River has changed considerably and has worsened since the 1970's. The most recent study in the Usuthu River indicated the contribution of agricultural and industrial activities to the upper Usuthu River. Therefore, the main goal of the study was to conduct abiotic and biotic assessments in order to determine the present environmental quality, and aquatic biodiversity of the Usuthu River within the NGR. The influence the natural flow regime has on the downstream floodplain systems was assessed and whether the Usuthu River can act as a refuge for the aquatic biodiversity of the lower Phongolo Floodplain.

The abiotic assessment consisted of water and sediment quality of the various aquatic systems within the PRF. Samples were collected in triplicate from five sites during two surveys (High Flow – HF and Low Flow – LF) within the PRF. Both physical and chemical properties were analysed in the water and sediment samples. Chemical concentrations of various metals from this study were much lower than concentrations in aquatic systems within the Kruger National Park (KNP) that are known to be polluted by extensive mining, industrial and agricultural activities. The highest metal concentrations from the present study were Cu, Fe and Zn as a result of agricultural and industrial practices in the upper catchment. Salt concentrations in Lake Nyamithi was higher compared to the other aquatic systems within the PRF, while the Phongolo River had higher Total Dissolved Solids (TDS) concentrations than the Usuthu River. The Usuthu River was higher in nitrate (NO_3) and nitrite (NO_2) concentrations and phosphates (PO_4) concentrations were higher in the Phongolo River. The primary cause for lower nutrient concentrations in the Phongolo River was attributed to the Pongolapoort Dam that traps nutrient-rich sediments and upstream nutrient supply. The aquatic systems differed in Total Organic Carbon content (TOC) as the floodplain lakes were higher in TOC than their respective rivers with the highest TOC reported in Lake Shokwe. Metal concentrations differed between the systems as higher metal concentrations were measured in Lake Shokwe compared to Lake Nyamithi and its outlet, while the Usuthu River had the highest metal concentrations in sediments during the LF survey.

The analyses of physical and chemical properties lead to a wealth of data that can sometimes be difficult to interpret. Environmental indices were used to summarise the vast amount of data and as such, the Aquatic Toxicity Index (ATI) and Sediment Quality Index (SeQI) were used to determine the water and sediment quality of the aquatic systems within the PRF. The value from the indices indicates the aquatic systems suitability for use and the degree to which the systems are impacted. The Usuthu River during the HF survey had the highest score and Lake Nyamithi outlet the lowest of all the aquatic ecosystems. In general, the variables that contributed the most to lowering the ATI scores were Zn for the metals and Total Dissolved Solids (TDS), ammonium (NH_4) and phosphate (PO_4) for the nutrients. The scores for both floodplain rivers and associated floodplain lakes remained suitable (i.e., a score between 60 and 100) for aquatic biodiversity, with the exception of Lake Nyamithi where its score indicated that water quality is more suitable for hardy fish species such as *Oreochromis mossambicus* and *Clarias gariepinus*. Sediments from the majority of the aquatic ecosystems found to be of acceptable quality (i.e., above 60) and it was only Lake Shokwe and Lake Nyamithi outlet that scored below 60. The variables that contributed to lowering the SeQI scores were Cr, Cu and Ni and was attributed to agricultural activities (Cr and Cu) and coal mines (Ni) in the upper catchment.

The biotic assessments consisted of various aspects such as macroinvertebrate community structures, food web and dietary analyses, fish migration, mercury (Hg) bioaccumulation and human health risks. Aquatic biota are influenced by anthropogenic stressors and therefore studying aquatic biota provides valuable information as to the degree that freshwater systems are influenced by anthropogenic activities. Macroinvertebrate community structures were assessed for both floodplain rivers and associated lakes while also determining the influence of the natural flooding regime on macroinvertebrate community structures by means of different multivariate analyses. Community structures between the regulated Phongolo River and unregulated Usuthu River were not different, however, the taxa in the Usuthu River were more sensitive towards pollution while in the Phongolo River, the taxa were more pollutant tolerant. Furthermore, the Phongolo River inside the NGR had a higher number of taxa than outside the NGR. In the floodplain lakes of the PRF, the Phongolo River associated floodplain lakes were higher in diversity compared to Lake Shokwe, however, these floodplain lakes shared similar taxa such as the backswimmers (*Anisops* sp. A), the water scavenger beetle (*Berosus* sp.) and Oligochaeta. The 2017 and 2018 surveys in Lake Nyamithi differed in structure and diversity as the 2017 survey had higher number of taxa, Pielou's Evenness Index, Margalef's Species Richness, Shannon-Wiener Diversity Index and Simpson's Index than the 2018 survey.

The influence from the Usuthu River was demonstrated through the higher macroinvertebrate diversity of Lake Shokwe during the HF survey. Moreover, due to no flooding of the Phongolo River during the study period, any changes in the macroinvertebrate diversity in Lake Nyamithi indicates the influence from the Usuthu River. Most importantly, variation partitioning analysis showed that in terms of water quality, habitat preference and type of system; habitat preference were the main contributing factor in structuring the macroinvertebrate community structures of aquatic systems within the PRF.

Stable carbon and nitrogen isotopes analyses are a useful tools in determining the consumer diets, tracing anthropogenic impacts, trophic relationships, constructing food web models and indicating how energy flows through an aquatic system. The aim of this biotic assessment was to determine the food web structures and consumer diets from fish of the two floodplain rivers and associated floodplain lakes and to determine the effect of the impaired flow regime on these food web structures and consumer diets. Additionally, the aim was to determine whether there was any biological connectivity (using Strontium (Sr) isotope ratios) between the aquatic systems within the PRF. The key findings were similar to the macroinvertebrate community structures of floodplain rivers; i.e. there was no difference in food web structure between the two rivers, however, different food web components were collected. Furthermore, food web components from the Usuthu River were significantly enriched in nitrogen and correspond with the water quality of the system. Although it is well known that the Phongolo River below the Pongolapoort Dam receives nitrogen inputs through organic fertilisers from the downstream agricultural activities, the impoundment further contributed substantially to the lower nitrogen values. Consumer diets from the Usuthu River comprised of an integrated mixture of food sources with aquatic vegetation (38%) and macroinvertebrates (50%) being consumed the most. Conversely, consumers from the Phongolo River consumed mostly C_3 plants (37%) while C_4 plants were the least consumed (6%).

Dissimilar food web structures were determined in the floodplain lakes within the PRF as food web components (particularly the tigerfish (*Hydrocynus vittatus*), sharptooth catfish (*Clarias gariepinus*) and the dwarf tigerfish (*Brycinus imberi*)) from Lake Shokwe were one trophic level higher than the food web components from Lake Nyamithi. Only one fish species, *H. vittatus* was at the top of the food web in Lake Shokwe whereas in Lake Nyamithi two species (*H. vittatus* and *C. gariepinus*) occupied the top of the food web. Isotopic signatures in food web components from Lake Shokwe were significantly different to Lake Nyamithi's food web components, with the exception of $\delta^{15}N$ signatures in macroinvertebrates. The diet of consumers within Lake Shokwe were similar to its associated river and preferred an integrated mixture of food sources as detritus were consumed the most (46%) and macroinvertebrates were the least consumed (<10%).

Between two different surveys (2017 vs 2018), Lake Nyamithi was also dissimilar in food web structures. No primary producers could be collected during the 2018 survey as a result of the low water levels during the 2018 LF survey, whereas during the 2017 survey, a large variety of primary producers such as detritus, biofilm, leaf litter, and plant material (Trapaceae, Poaceae, Cyperaceae and Nymphaeceae) were collected. Furthermore, *H. vittatus* and *C. gariepinus* occupied the top of the food web during the 2018 survey while in the 2017 survey, *Enteromius toppini* occupied the top of the food web. Consumer diets in Lake Nyamithi between the various surveys preferred different food sources. During the 2017 survey, C_4 plants (32%) and macroinvertebrate (36%) were relatively equally preferred by consumers. Conversely, in the 2018 survey consumers preferred a larger variety of dietary constituents, such as, detritus (30%), aquatic vegetation (29%) and macroinvertebrates (29%) while fish were the least consumed food source (12%).

Strontium isotope ratios in *C. gariepinus* indicated movement between the various aquatic systems within the PRF. Moreover, the $^{87}Sr/^{86}Sr$ ratios between the Usuthu River and Lake Shokwe were very similar indicating the movement between the two systems while the large variation in $^{87}Sr/^{86}Sr$ ratios in the Phongolo River indicated *C. gariepinus* moved between the Phongolo and Usuthu River (due to slight overlap in signature) as well as the Phongolo River tributaries.

Mercury in freshwater systems across South Africa has been thoroughly studied due to the many coal powerplant and gold mines in Southern Africa. The final biotic assessment was to determine Hg bioaccumulation and biomagnification in the biota from the various aquatic systems within the PRF. Furthermore, human health risks associated with the consumption of fish muscle were also determined. Sediments from the systems within the PRF had detectable Hg concentrations apart from Lake Nyamithi. These concentrations were all positively correlated with TOC. Aquatic biota from the various aquatic systems indicated detectable Hg concentrations while predatory fish bioaccumulated the highest Hg concentrations in muscle tissue. Similar to the positive relationship between Hg in sediment and TOC, Hg in fish had positive relationships with fish length and trophic position. With its positive relationship with trophic position, trophic magnification factors (TMF) were determined and indicated biomagnification of Hg through the food webs in all aquatic systems apart from Lake Nyamithi which indicated bio-dilution of Hg. Moreover, as fish from the PRF form an integral part of the diet of the local communities in and around the floodplain, the consumption of fish from these aquatic systems could be alarming. However, human health risks indicated low to no risks associated with the consumption of Hg-contaminated fish.

The present study found that the environmental quality of the Usuthu River and its associated floodplain lake, Lake Shokwe were higher than the Phongolo River and Lake Nyamithi. This coincided with the natural flooding regime of the Usuthu River together with less of an impact from the upper catchment anthropogenic activities. This subsequently influenced the aquatic biota from these downstream aquatic systems while the high diversity of the HF survey indicated the importance of hydrological connectivity in riverine floodplains. The hydrological connectivity in the PRF further indicated the importance of the natural flooding of the Usuthu River and its role in the structure and functioning of the downstream aquatic systems. Therefore, with its strong flows and high hydrological connectivity between the rivers and associated floodplain lakes, enhances the possibility of fish movement within the lower PRF.

More importantly, Hg analyses indicated detectable Hg concentrations in aquatic biota as well as biomagnification, however, the study found no potential human health risk associated with the consumption of fish from the PRF. The $^{87}Sr/^{86}Sr$ ratios in the otoliths of *C. gariepinus* indicated the movement between the various aquatic systems within the PRF. This furthermore indicated the possibility of the Usuthu River serving as a refuge area for aquatic biodiversity of the lower PRF.

Keywords: Water quality, environmental quality, aquatic community structures, aquatic food webs, natural flooding regime, bioaccumulation, biomagnification, mercury, human health risk, regulated rivers, floodplains

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ABBREVIATIONS

$\delta^{13}C$:	Carbon stable isotope
$\delta^{15}N$:	Nitrogen stable isotope
$\delta^{15}N_{component}$:	Mean $\delta^{15}N$ of each separate food web component
$\delta^{15}N_{reference}$:	Mean $\delta^{15}N$ of shrubs of each individual
$^{87}SR/^{86}SR$:	Strontium Isotope
AAS:	Atomic Absorption Spectrometry
ANOVA:	Analysis of Variance
ANZECC:	Australian-New Zealand Environment Conservation Council
ATI:	Aquatic Toxicity Index
ATSDR:	Agency for Toxic Substances and Disease Registry
CCA:	Canonical Correspondence Analysis
CCME:	Canadian Council of Ministers of the Environment
CRM:	Certified Reference Material
DO:	Dissolved Oxygen
DWA:	Department of Water Affairs
EA-IRMS:	Elemental Analyser Isotope Ratio Mass Spectrometry
EDI:	Estimated Daily Intake
FIMS:	Flow Injection Mercury System
FWL:	Food Web Length
GIS:	Geographic Information Systems

HCB:	Hexachlorobenzene
HCl:	Hydrochloric Acid
HF:	High Flow
Hg:	Mercury
HNO_3:	Nitric Acid
HQ:	Hazard Quotient
ICP-MS:	Inductively Coupled Plasma Mass Spectrometry
ISQG:	Integrated Sediment Quality Guidelines
KNP:	Kruger National Park
LF:	Low Flow
LOD:	Limit of Detection
LOQ:	Limit of Quantification
MCMC:	Markov Chain Monte Carlo
MeHg:	Methylmercury
MRL:	Minimum Risk Level
multipatt:	Multi-pattern analysis
$NaBH_4$:	Sodium Borohydride
NGR:	Ndumo Game Reserve
nMDS:	non-metric Multidimensional Scaling
NWU:	North West University
NYA:	Lake Nyamithi

OCP:	Organochlorine Pesticides
PCA:	Principal Component Analysis
PMC:	Phongolo Main Channel
PRF:	Phongolo River Floodplain
PSC:	Phongolo Secondary Channel
RDA:	Redundancy Analysis
RSD:	Relative Standard Deviation
SANBI:	South African National Biodiversity Institute
SE:	Standard Error
SeQI:	Sediment Quality Index
SH:	Lake Shokwe
SIAR:	Stable Isotope Analysis in R
SIMPER:	Similarity Percentage Analysis
SL:	Standard Length
TDI:	Tolerable Daily Intake
TDS:	Total Dissolved Solids
THg:	Total Mercury
TMF:	Trophic Magnification Factor
TOC:	Total Organic carbon Content
TP:	Trophic Position
TWQG:	Target Water Quality Guidelines

UNEP: United Nations Environmental Programme

USEPA: United States Environmental Protection Agency

VLIR-UOS: Vlaamse Interuniversitaire Raad-Universitaire Ontwikkelingssamenwerking

vif: variance inflation factor

WHO: World Health Organization

WRC: Water Research Commission

WRG: Water Research Group

CHAPTER 1
General introduction

1.1. General background

Floodplain ecosystems are regarded as the most threatened freshwater ecosystems (Whittington *et al*., 2013). Floodplains are also the most productive and diverse freshwater ecosystems, providing invaluable ecosystem services (Dube *et al*., 2017; O'Brien *et al*., 2019). These highly productive and diverse ecosystems have come under increasing pressure from human activities causing changes to their structure and functioning, water quality, and aquatic diversity (Beatty *et al*., 2017; Schneider & Petrin, 2017; Malherbe, 2018; Milner *et al*., 2019). The impacts (agriculture, erosion, boreholes, increased nutrients from livestock and agricultural runoff and industrial activities) on wetlands across the world have been recognised as early as the 1970's as the Ramsar Convention was established with the idea of protecting important wetlands (Ramsar Secretariat, 2016).

River floodplains are known as one of the most diverse freshwater ecosystems as a wide range of habitats and gradients of hydrological connectivity are created by their hydrological dynamics (Ward *et al*., 1999; Tockner & Stanford, 2002; Helfield *et al*., 2012). Numerous factors can affect the normal functioning of floodplain ecosystems such as flow alterations, sediment, and rock erosion as well as anthropogenic impacts such as the construction of dams, agricultural, domestic and industrial activities (de Necker *et al*., 2019; Forio & Goethals, 2020).

1.1.1. Importance of flood regime for floodplain rivers

Many of the world's large rivers (approximately >60%) have been regulated through impoundments (Lytle & Poff, 2004; Schneider & Petrin, 2017; Belmar *et al*., 2019; Gillespie *et al*., 2019). River regulation not only causes changes to the structure and functioning of a river but also results in changes in the water quality that in turn affects the aquatic biota (Schneider & Petrin, 2017; Beatty *et al*., 2017; Milner *et al*., 2019). Flow regimes have shaped a wide range of species characteristics such as life histories, behaviours, and morphologies of aquatic and riparian species. More importantly, a river's flow regime is essential for in-stream and floodplain ecosystem processes and further contributes to the river's functioning and to provide ecosystem services (Palmer & Ruhi, 2019).

In contrast, unregulated rivers (free-flowing) are rivers that are not controlled by impoundments and their flows are largely natural and dependent on climatic conditions within the catchment (Jones *et al*., 2007). These aquatic environments are dynamic and highly variable as water levels continuously fluctuate with precipitation and season (Tronstad *et al*., 2005). Furthermore, aquatic species residing within unregulated rivers are able to tolerate these variabilities (Tronstad *et al*., 2005). Importantly, Milner *et al*. (2019) discussed that unregulated tributaries can modify ecological and physical conditions in the main channel through inputs of sediment, water, organic matter, and nutrients.

In riverine floodplain systems, the flooding regime plays an important role in connecting the river to its floodplain (Agostinho *et al*., 2009). As such, river connectivity influences floodplain diversity and productivity with periodic inundation through flooding causing high floodplain productivity and the high-energy flows causing high habitat heterogeneity and therefore high biodiversity (Opperman *et al*., 2010). Natural flooding of a river also increases nutrient inputs through the decomposition of detritus and thus maintains the high productivity of aquatic species (Agostinho *et al*., 2009).

The PRF (13 000ha at full inundation), a biodiversity hotspot, is South Africa's largest and most biodiverse floodplain ecosystem (Dube *et al*., 2015). It extends from below the Pongolapoort Dam up to the confluence of the Phongolo and Usuthu rivers within the NGR and continues into Mozambique (Dube *et al*., 2015; Acosta *et al*., 2021). Approximately 40% of the floodplain is situated in South Africa while the remaining 60% is located within Mozambique (Merron *et al*., 1993; SA Wetlands Conservation Programme, 1996; Dube *et al*., 2015). The PRF is habitat to 50 different fish species, five terrestrial plant communities, and 54 families of aquatic macroinvertebrates indicating its high biodiversity (Dube *et al*., 2015; Acosta *et al*., 2021).

1.1.2. Usuthu – Phongolo rivers catchment

The Usuthu – Phongolo rivers catchment lies predominately within the KwaZulu-Natal Province while the northern part of the catchment lies in the Mpumalanga Province (Figure 1.1). The catchment borders Eswatini and Mozambique and includes only one protected area within the PRF (Dennis & Dennis, 2009). The NGR is the only proclaimed conservation area (102 km^2) situated within the PRF protecting the five distinct wetland types that range from freshwater, over saline to brackish, permanent and intermittent as well as pools, rivers, lakes, and riparian forests (Acosta *et al*., 2021). The lower PRF forms the largest part of this catchment and consists of two large rivers, the Usuthu and Phongolo rivers. These are also the two largest rivers within this catchment and both flow in an easterly direction (Dennis &

Dennis, 2009). The Phongolo River flows predominantly within the KwaZulu Natal Province, South Africa, and originates in the Wakkerstroom area whereas the Usuthu River originates in the Mpumalanga Province, South Africa, moving eastwards through Eswatini before entering the KwaZulu-Natal Province where it forms the international boundary between South Africa and Mozambique before joining the Phongolo River at the confluence in the north-eastern section of the NGR. These rivers become the Maputo River as it enters Mozambique at the confluence before draining into Maputo Bay (Vilane & Tembe, 2016).

In the PRF, the construction of the Pongolapoort Dam and agricultural activities in the Phongolo River catchment contributed significantly to the form and functioning of the floodplain ecosystem (see Figure 1.1) (de Necker *et al*., 2019). The Pongolapoort Dam was constructed in 1973 for the sole purpose of supplying sufficient water to irrigate 40 000 to 50 000 ha of land in the area known as the Makhathini Flats (de Necker *et al*., 2019). It has been well-documented that impoundments alter the flow regime of large rivers (Poff *et al*., 1997; Bunn & Arthington, 2002; Lankford *et al*., 2010; Castello & Macedo, 2015; Smit *et al*., 2016; de Necker *et al*., 2019). In addition to the altered flow regulation, impoundments also cause a change in the downstream water quality of aquatic ecosystems (de Necker *et al*., 2019). Furthermore, the large-scale agricultural activities in and around the PRF are a major source of water quality pressures that could increase metal concentrations (Mthembu *et al*., 2020) and organochlorine pesticides (OCP's) concentrations such as lindane and hexachlorobenzene (HCB) (Wepener *et al*., 2012; Volschenk *et al*., 2019).

Therefore, with the ever-increasing pressures from human-driven activities and localised drought on the PRF, it is essential to assess and monitor the form and functioning of the floodplain ecosystems (Zhang *et al*., 2018; Chetty & Pillay, 2019; Marshall & Negus, 2019). The use of water quality (Dallas, 2000), fish and macroinvertebrates (Dickens and Graham, 2002; Naigaga *et al*., 2011; Gerber *et al*., 2016; Erasmus *et al*., 2021), and food web structures (Layman *et al*., 2012; de Necker *et al*., 2022a) provide useful data in assessing aquatic ecosystem health.

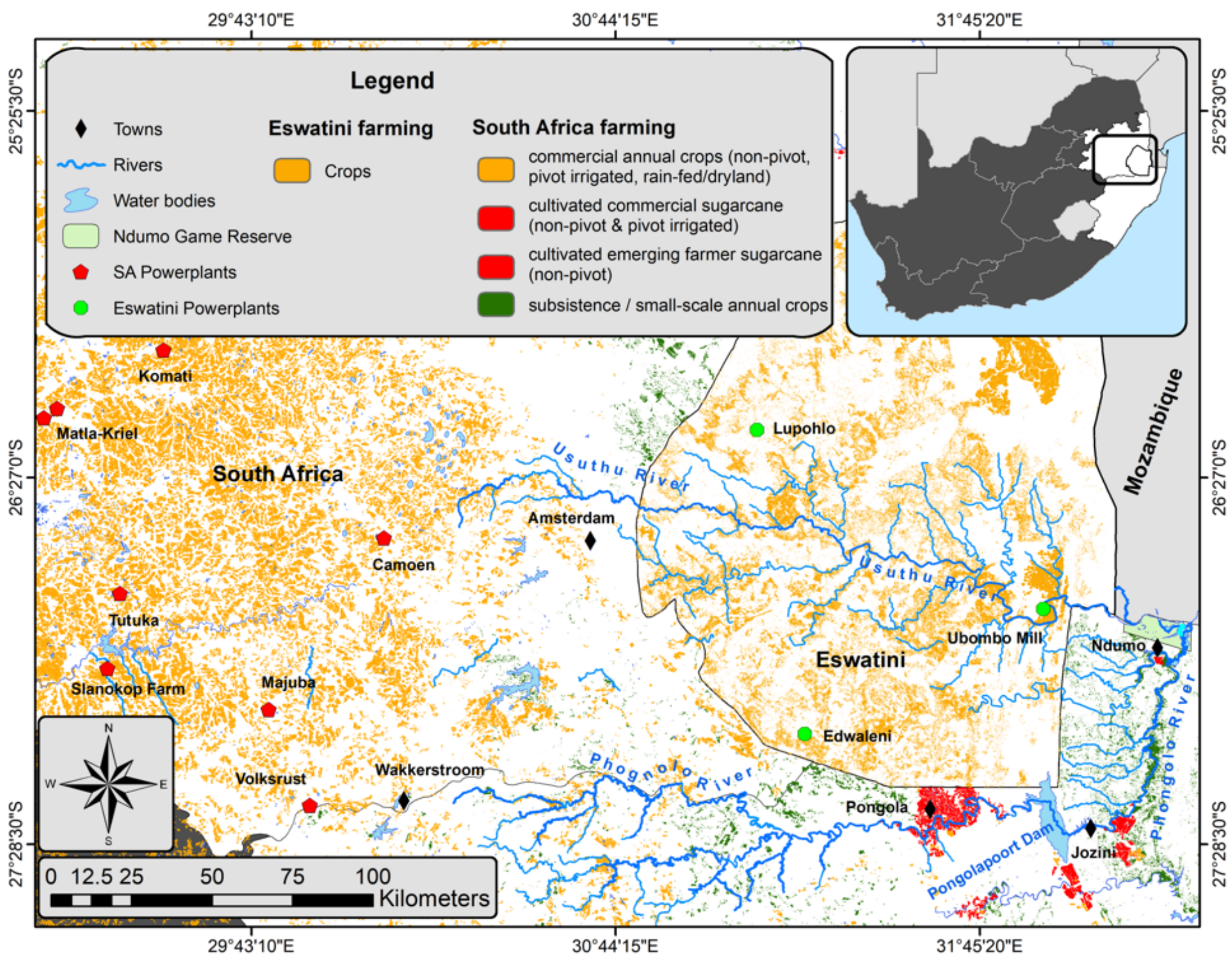


Figure 1.1: Map of the Usuthu – Phongolo rivers catchment area that includes both the Phongolo and Usuthu rivers and their associated impacts in the upper catchments.

1.2. Study area

1.2.1. Phongolo River

The lower Phongolo River and its associated floodplain starts below the Pongolapoort Dam below the town of Jozini. Downstream of the dam, the lower Phongolo River flows approximately 80 km to the confluence of the Usuthu River before entering Mozambique (de Necker *et al*., 2019). Before the construction of the dam, the PRF was subjected to summer flooding which created a mosaic of environments for aquatic biota (de Necker *et al*., 2019). Prior to- and just following the completion of the Pongolapoort Dam, much research was conducted on the nature and timing of flood releases from the dam to mimic its natural floods (Coke, 1970a; Coke, 1970b; Heeg *et al*., 1980; Heeg & Breen; 1982; Van Vuuren, 2009; DWS, 2014; Dube *et al*., 2015). However, due to droughts and the Domoina cyclone in 1984, the success of the intended flooding regime and the management thereof remains uncertain (de Necker *et al*., 2019). The water quality and biodiversity of the PRF is threatened through natural and anthropogenic stressors from industrial, forestry, agriculture, and mining activities, and the influence of the Pongolapoort Dam (de Necker *et al*., 2019). Furthermore, the supra-seasonal drought that South Africa experienced between 2015 and 2017 also resulted in water quality and biological alterations in the Phongolo River (de Necker *et al*., 2019). The typical habitat types along the Phongolo River were mainly dominated by riparian vegetation (Figure 1.2a) and overhanging trees (Figure 1.2c & d). Furthermore, along the edges of the river, submerged vegetation (Figure 1.2b) dominated this section of the river together with reeds and shrubs (Figure 1.6).

Over the years, the PRF has been thoroughly studied and includes studies on social aspects (Jaganyi *et al*., 2009; Lankford *et al*., 2011) and studies in the upper catchment (DWAF, 2008; van der Laan *et al*., 2012; DWS, 2014). Moreover, the Water Research Commission (WRC) funded an extensive research project between 2012 to 2014 to assess the various aspects (ecology, ecotoxicology, and health risks) of the lower Phongolo River Floodplain as a result of the construction of the Pongolapoort Dam (see Smit *et al*., 2016). More importantly, through funding from the WRC and Vlaamse Interuniversitaire Raad-Universitaire Ontwikkelingssamenwerking (VLIR-UOS), it was only until recently that the biodiversity and ecology of the lower PRF together with the effects of the river regulation during a supra-seasonal drought was extensively studied (see de Necker, 2019). The majority of these studies focussed on the Phongolo River and its associated floodplain wetlands. In contrast, very little to no research has focussed on the Usuthu River and its biodiversity and natural flooding regime therefore less is known about the present environmental condition and dynamics of the Usuthu River.

Figure 1.2: Photographs of different habitat types found in the Phongolo River within the Ndumo Game Reserve. Photograph a – riparian vegetation. Photograph b – submerged vegetation. Photograph c & d – overhanging trees.

1.2.2. Usuthu River

The Usuthu River system in the Eswatini region has a catchment area of approximately 2682 km^2 and although weirs are located in the upper catchment, it is a largely unregulated river as it does not have any large impoundments such as the Pongolapoort Dam regulating its flow. The Usuthu River is the largest river that flows through Eswatini and is predominantly used for irrigation, domestic purposes, livestock watering, industrial activities, tourism and fish farming (Kowalkowski *et al*., 2007; Vilane & Tembe, 2016). The Usuthu River adds significant value to the Eswatini economy (Mthimkhulu, 2018). Although the various commercial activities located along the Usuthu River add much value, these human-driven activities also negatively impact the river through water quality changes due to pollutant inputs and impacts on aquatic biota

(Magagula *et al*., 2010). However, it is assumed that the intensity of these human-driven activities is lower compared to the Phongolo River.

Since the Usuthu River is an unregulated river, its flooding regime is typical of free-flowing rivers and is largely natural and controlled by climatic conditions. During the high flows of 2006, the Usuthu River broke its southern banks within the NGR and diverted flows through Lake Banzi before joining its main channel and the Phongolo River at the confluence (Figure 1.6) (Birkhead et al., 2018). The lower Usuthu River is home to 24 different aquatic macroinvertebrate families and 34 fish species of which two fish species are of conservation concern (African Development Bank, 2000; Magagula *et al*., 2010).

Inside the NGR, the Usuthu River feeds a series of floodplain lakes namely, Lake Shokwe and Lake Banzi (Figure 1.6) (Whittington *et al*., 2013). A recent study by de Necker *et al*. (2021) indicated that the Usuthu River also contributes to Lake Nyamithi's water supply. The typical habitat types along the Usuthu River were dominated by overhanging trees (Figure 1.3a) and reed beds (Figure 1.3b) with little to no submerged vegetation (Figure 1.3c). Furthermore, in sections of the river, the river is also dominated by dead plant material and driftwood.

Figure 1.3: Photographs of different habitat types found in the Usuthu within the Ndumo Game Reserve. Photograph a – overhanging trees. Photograph b – the dense reed beds. Photograph c – little to no submerged vegetation. Photograph d – dead plant material and driftwood.

1.2.3. Associated floodplain lakes of the Usuthu and Phongolo rivers

1.2.3.1. Lake Shokwe

Lake Shokwe is an oxbow lake with a closed drainage system and is situated within the NGR and was sampled during the HF (June 2018 – Figures 1.4 a & b) and LF (November 2018 – Figures 1.4 c & d) Lake Shokwe is the first of a series of floodplain lakes that receives water from the Usuthu River during high localised rainfall and through flooding (Figure 1.6). Along the edge of the lake, it is dominated by dense reed beds while grassland woodlands dominate the surroundings of the lake (Whittington *et al*., 2013). On the eastern banks, the lake is surrounded by a fever tree (*Acacia xanthophloea*) forest while on the western bank, a fig tree (*Folia capreifolia* and *Ficus sycomoros*) forest surrounds the lake (Haddad *et al*., 2006; Whittington *et al*., 2013). The dominant aquatic vegetation present in the lake was Nymphaeceae and Onagraceae. Lake Shokwe accounts for a large percentage/number of the Usuthu River's crocodile population (Calverley & Downs, 2014) while the smallest pod of *Hippopotamus amphibius* resides within Lake Shokwe (Fritsch *et al*., 2021).

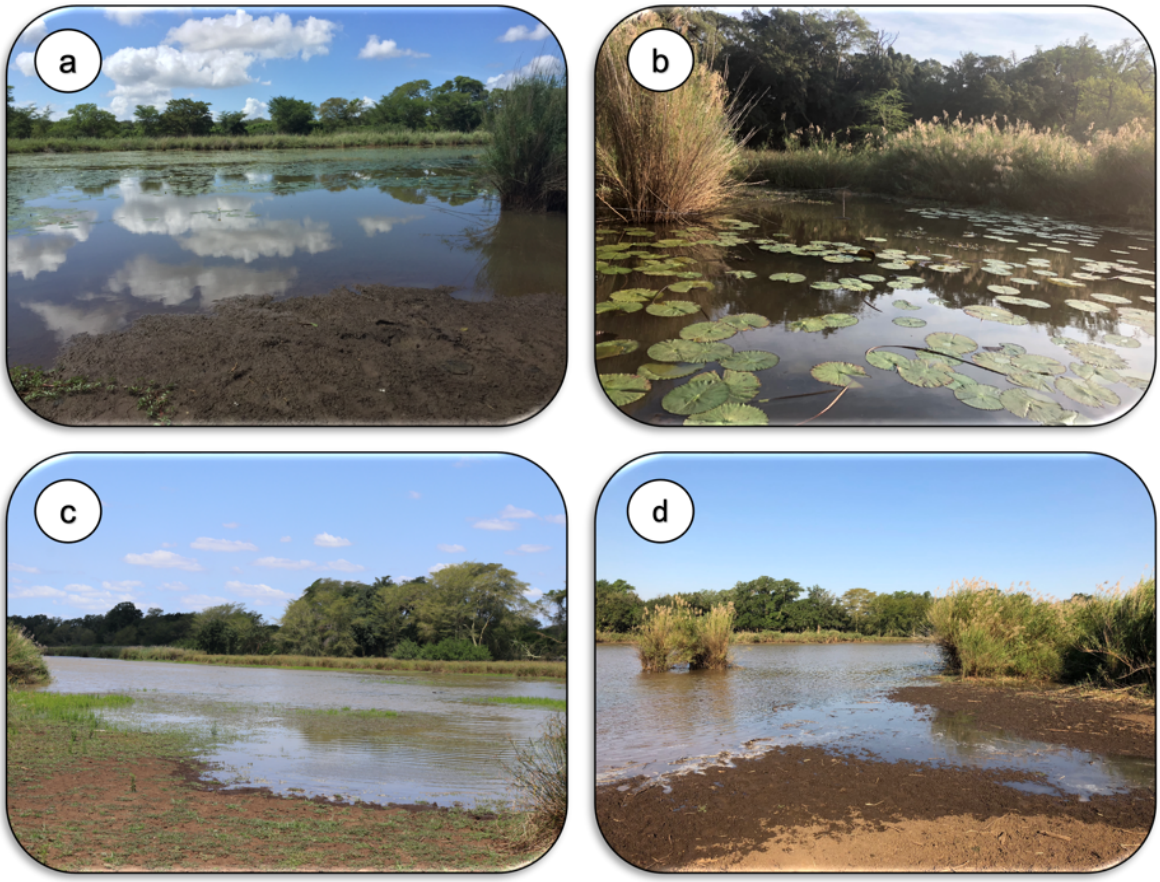


Figure 1.4: Photographs of the Lake Shokwe (Usuthu River associated floodplain lake) during June (a & b – High Flow) and November 2018 (c & d – Low Flow) within the Ndumo Game Reserve.

1.2.3.2. Lake Nyamithi

Lake Nyamithi (Figure 1.5a) is a naturally saline floodplain lake located in the NGR (de Necker *et al*., 2021) and it is the largest (183.4 ha), semi-permanent floodplain lake within the reserve (Heeg and Breen, 1982; Calverley & Downs, 2014). Lake Nyamithi is of high ecological importance as 59 wetland-dependant bird species have been recorded in and around the lake, while it is also home to a large population of hippopotami and the highest density (3rd largest population) of crocodiles (*Crocodylus niloticus*) in South Africa (Calverley & Downs, 2014; de Necker *et al*., 2021). Lake Nyamithi has both a natural inlet and an outlet that was artificially raised to regulate the water level of the lake. This weir has been damaged to the extent that during the study period it was no longer functional. During the high flow periods and flood events, water from a small, localised catchment enter the lake through the inlet, while water from the Usuthu and Phongolo rivers enters the lake through the "outlet". During the low flow periods, water flows out of the lake at the outlet (Figures 1.5b and 1.6).

The lake has a very unique saline characteristic (approximately 5000 µS.cm^{-1}) as it has an ancient marine bed, which contributes to its unique abiotic and biotic composition (Acosta *et al*., 2021; de Necker *et al*., 2021). The lake receives much of its water from the flooding of the Phongolo River following flood releases from the Pongolapoort Dam, while localised rainfall in the catchment and back flooding from the Usuthu River also contributes to Lake Nyamithi's water supply (de Necker *et al*., 2022a).

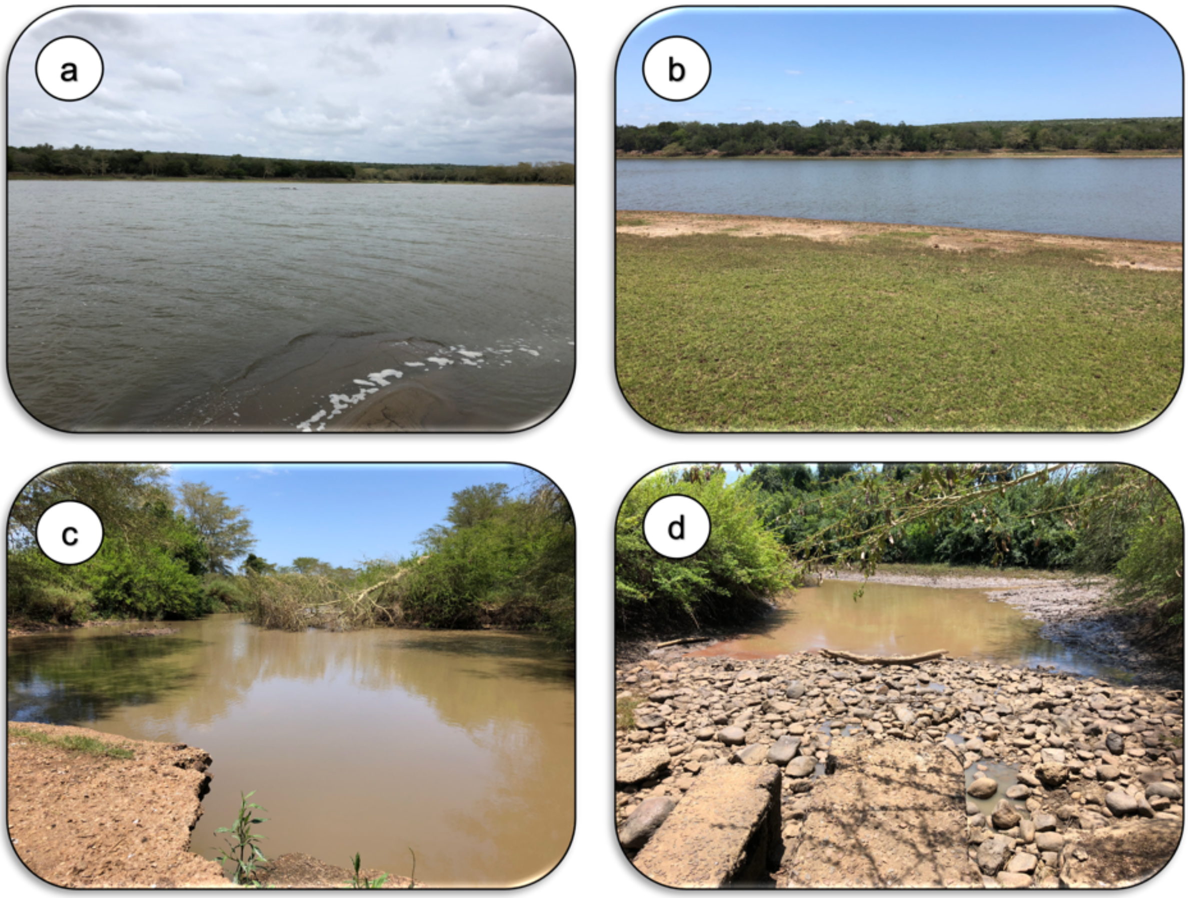


Figure 1.5: Photographs of the Lake Nyamithi site within the Ndumo Game Reserve. Photographs (a & b) – Lake Nyamithi and (c & d) – Lake Nyamithi outlet.

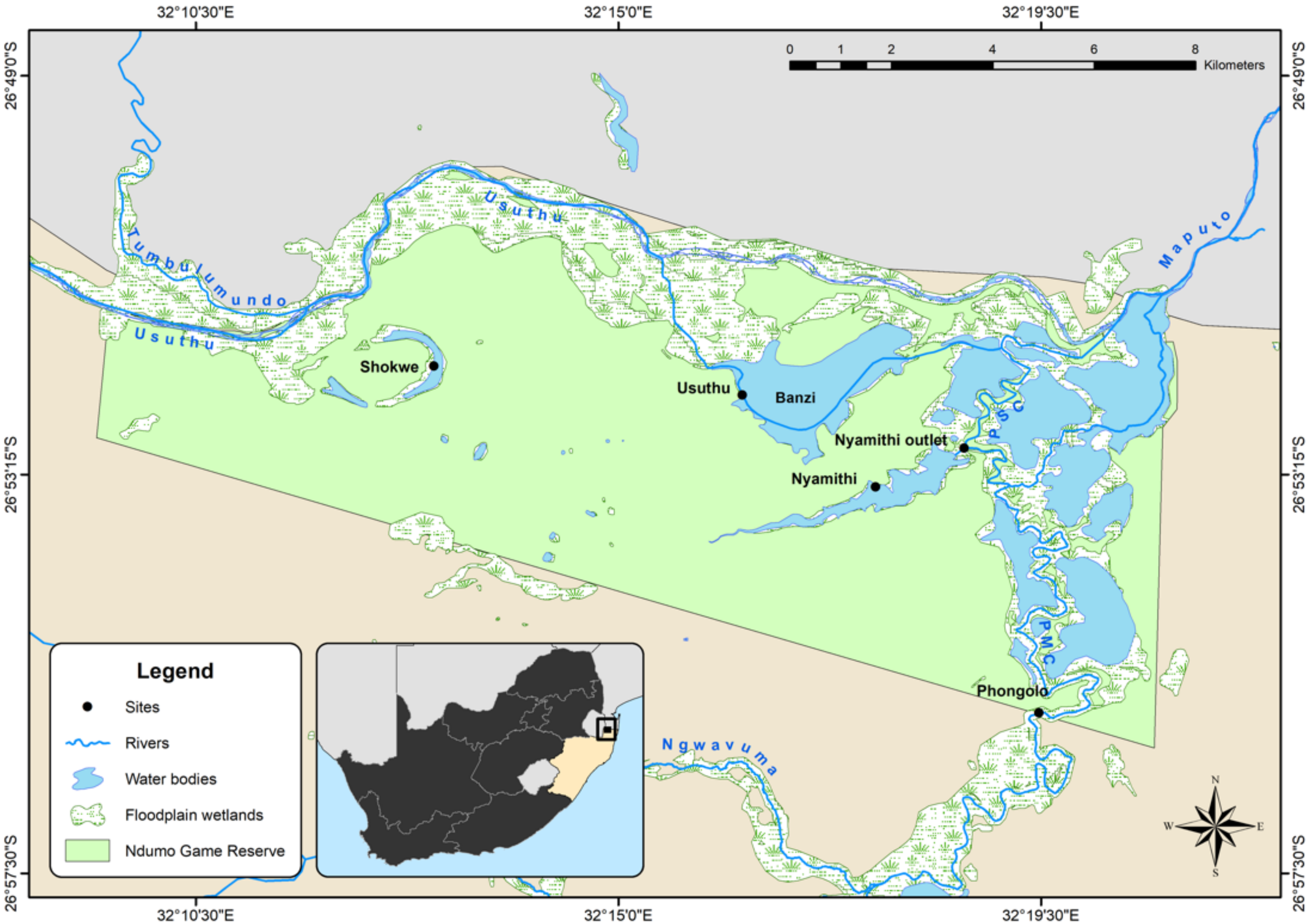


Figure 1.6: Map of the confluence of the Phongolo and Usuthu rivers and their associated floodplain lakes in the Ndumo Game Reserve. PMC – Phongolo main channel & PSC – Phongolo secondary channel.

1.3. Problem statement

As mentioned in section 1.2, to date the majority of the research on the PRF has focused on the Phongolo River, and the impact of the Pongolapoort Dam, while the present environmental quality, ecology and dynamics of the Usuthu River has received much less attention and been severely understudied. Furthermore, the Usuthu River system shares several similar anthropogenic impacts in its upper catchment as the Phongolo River, however, less is known about how these impacts affect the downstream aquatic systems.

In light of the supra-seasonal drought South Africa has experienced in recent years, the contribution and importance of the contributions of the Usuthu River to the associated floodplain lakes and specifically Lake Nyamithi is largely unknown. Moreover, less is known about the aquatic diversity and present environmental quality of the lower Usuthu River. Furthermore, less is known about the dynamics of its associated floodplain lake (Shokwe) within the NGR. Thus, the main aim of this study was to assess the environmental quality and aquatic biota of the Usuthu River, the effect the natural flooding regime has on the aquatic ecosystems within the PRF, and whether the Usuthu River acts as refugia for the lower Phongolo Floodplain aquatic biodiversity when the Phongolo River's flow is reduced. In addition, with the extensive knowledge on the lower Phongolo River, it provides a need to understand the Usuthu River system and to what degree its natural flooding regime impacts downstream aquatic systems.

1.4. Study hypotheses and aims

1.4.1. Study hypotheses

The general hypotheses tested for the present study:

- **Chapter 2:** (1) Water and sediment quality of the Usuthu River and its associated floodplain lake, Lake Shokwe, are in a better ecological condition than the Phongolo River and Lake Nyamithi and is reflected in the water and sediment quality indices; and (2) the hydrological period (i.e., flow) influences the metal concentrations in water and sediments of these systems.
- **Chapter 3:** (1) The macroinvertebrate community structure of the Usuthu River differs from the Phongolo River; (2) the macroinvertebrate community structures of the two largest floodplain lakes within the NGR reflect the influence of the Usuthu River; (3) hydrological connectivity will have a positive effect on the macroinvertebrate community composition

of the Usuthu River associated floodplain lake; (4) the influence of the Usuthu River on Lake Nyamithi will reflect a sustained recovery in macroinvertebrate community structures and lastly, (5) the nature of the aquatic ecosystems (lotic/lentic) drives the structure of macroinvertebrates communities within the NGR.

- **Chapter 4:** The aquatic systems of the PRF are resilient and the food webs will not be affected by the drought.
- **Chapter 5:** (1) Aquatic biota will indicate Hg bioaccumulation and that biomagnification has occurred through the food web; and (2) due to the biomagnification of Hg, there will be human health risks associated with the consumption of fish from the lower PRF.

1.4.2. Study aims

To test the above hypotheses, the study aims were to:

- **Chapter 2:** Provide the first assessment of metals in water and sediments from the two rivers and lakes in the PRF and thereby provide initial (baseline) concentrations. To achieve this, the physical and chemical properties of water and sediment of both rivers and lakes were assessed. Furthermore, two environmental indices were used, namely the Aquatic Toxicity Index (ATI) and Sediment Quality Index (SeQI) for water and sediment quality, respectively, to calculate scores to provide indication of ecological condition.
- **Chapter 3:** Determine the macroinvertebrate community composition of the Usuthu River and as a result of its natural flooding regime, how this natural flow influences the macroinvertebrate community structures of associated freshwater ecosystems.
- **Chapter 4:** 1) Assess the food web structures of the two main rivers and floodplain lakes in the lower PRF and 2) determine whether the impaired flow regime affects the food web structure of the Phongolo River (and associated Lake Nyamithi) when compared to the Usuthu River and its associated floodplain lake, 3) to determine whether there is a difference in piscivorous fish diets between the Usuthu and Phongolo rivers and 4) determine the biological connectivity between the systems using Sr isotope ratios in African sharptooth catfish as an indicator of migration between the systems.
- **Chapter 5:** Provide a novel understanding into the Hg concentrations in sediment and aquatic biota and their potential risk to the local communities of the PRF ecosystem. Furthermore, this chapter provides baseline Hg concentrations in the aquatic systems of the PRF.

1.5. Layout of thesis

This study has been divided into seven chapters:

- **Chapter 1:** General Introduction on the background of the study.
- **Chapter 2:** An assessment of water and sediment quality of aquatic ecosystems within the PRF was undertaken. This involved the sampling and analyses of water and sediment from both rivers and associated floodplain lakes during different hydrological (flow) periods. Using multivariate statistical techniques, ionic compositions, as well as water and sediment quality indices of the environmental condition of the ecosystems were evaluated.
- **Chapter 3:** Ecological importance of the natural flooding regime of the Usuthu River to the macroinvertebrate community structure of aquatic ecosystems within the Ndumo Game Reserve. This involved the sampling of macroinvertebrates from the Usuthu River and associated floodplain lakes (Shokwe and Nyamithi). Macroinvertebrate community structures, species richness, and abundances were determined for both rivers and associated floodplain lakes during the November 2018 survey. Using various multivariate analyses, both rivers and associated floodplain lakes were assessed for similarity between the various aquatic systems and what drives these macroinvertebrate community structures.
- **Chapter 4:** A comparison of aquatic food webs and dietary analyses of the aquatic systems associated with the NGR. This involved the sampling of primary producers, macroinvertebrates and fish from the Usuthu River and associated floodplain lakes to determine and compare the food web structure and dietary analysis of the various aquatic systems.
- **Chapter 5:** Bioaccumulation and trophic transfer of total mercury through the aquatic food webs of an African sub-tropical wetland. Aquatic vegetation, macroinvertebrates and fish species were sampled from both rivers and associated floodplain lakes to determine Hg concentrations in the food webs. Furthermore, biomagnification of Hg was assessed to determine whether there are potential human health risks with the consumption of fish from the lower Phongolo Floodplain.
- **Chapter 6:** Concluding remarks on the study results as well as conclusions drawn and recommendations.
- **Chapter 7:** A list of all the references used in the thesis.

CHAPTER 2
An assessment of water and sediment quality of aquatic ecosystems within South Africa's largest floodplain

2.1. Introduction

Floodplain wetlands are one of the most threatened ecosystems in the world and have declined globally in recent times (Whittington *et al*., 2013; Adame *et al*., 2019). These highly productive and dynamic ecosystems are under increasing threat from the alteration of flooding regime through dams, diversion of flow upstream of a river, and river mismanagement (Kingsford, 2000; Dube *et al*., 2015). The negative impacts on riverine floodplains extensive and can result in changes in structure and functioning of a river, water quality, and subsequently changes in aquatic biota (Schneider & Petrin, 2017; Beatty *et al*., 2017; Milner *et al*., 2019).

There are 23 wetlands in South Africa that fall under the protection of the Ramsar Convention and are all currently preserved as either provincial or national protected areas (Malherbe, 2018). The NGR, which includes the lower PRF is one such area. The PRF is South Africa's largest floodplain (13,000 ha at full inundation), situated in northern KwaZulu-Natal, and extends from the Pongolapoort Dam to the confluence of the Phongolo and Usuthu rivers inside the NGR to the border of Mozambique (Acosta *et al*., 2021).

Aquatic ecosystems offer essential resources for human wellbeing while providing a habitat for aquatic biota (Cañedo-Argüelles *et al*., 2019; O'Brien *et al*., 2021). This is especially true for the PRF where the current population (approximately 180 000 people) is strongly dependent on the floodplain (Smit *et al*., 2016; Birkhead *et al*., 2018). The ecological value of the PRF is high, in particular for providing highly productive soils, vegetation (i.e., grass and reeds) for livestock grazing and construction materials, and fish from the floodplain depressions and lakes (Lankford *et al*., 2010; Coetzee *et al*., 2015; Nkhatha *et al*., 2017). However, these aquatic systems face numerous threats that can change or deteriorate water quality (Kowalkowski *et al*., 2007). These threats include altered flows, sediment, and rock erosion, and human-driven impacts such as the construction of dams, agriculture, domestic and industrial wastewater (de Necker *et al*., 2019). The influence of the construction of the Pongolapoort Dam on the flows and general water quality of the lower PRF are well documented (Lankford *et al*., 2010; Smit *et al*., 2016; de Necker *et al*., 2019; O'Brien *et al*., 2021). The northeastern regions of South Africa have been experiencing drought and below-average rainfall conditions since 2015. The latest flood release from the dam occurred recently in 2022, eight years since the last release in 2014, the present study occurred in between those two releases (de Necker *et al*., 2021). Despite the persistent below-average rainfall conditions, de Necker *et al*. (2022a) demonstrated that the ecology of the system has remained stable, demonstrating the resilience and resistance of the PRF.

A major source of water quality stressors in the PRF is large-scale agriculture activities. A recent study by Mthembu *et al*. (2020) suggests that these agricultural activities contribute to increased metals in the aquifers in the northern Kwa-Zulu Natal region, which includes the PRF. Whilst there are similar human activities in the upper catchments of both rivers (e.g. industrial, forestry, agriculture, and mining), the intensity is lower in the Usuthu River than in the Phongolo River (DWA, 2009; Annual Water Quality Report, 2014; Vilane and Tembe, 2016; de Necker *et al*., 2019). In addition, since the flows of the Usuthu River is not regulated by any large impoundments, the nutrient and sediment transport will not be influenced to the same degree as the highly regulated Phongolo River (Dube *et al*., 2015; Van Cappellen and Maavara, 2016).

Water and sediment quality assessments often lead to a wealth of data and information which can sometimes be difficult to interpret (Wepener *et al*., 1999, Gerber *et al*., 2015a). As such, indices are applied to reduce data to a single index value for easier interpretation. The ATI scoring system was developed by Wepener *et al*. (1992) to evaluate the water quality in terms of an aquatic ecosystem's suitability to maintain aquatic biota, specifically fish. As such, the ATI aids in reflecting the effect of water quality for a specific use such as the aquatic environment while this index is an effective method to monitor trends in water quality (Wepener *et al*., 1999).

The SeQI system was developed by the Canadian Council of Ministers of the Environment (CCME) to assess the degree to which human-driven activities affect sediment quality (CCME, 2007). The SeQI is an effective tool to assess sediment quality to its relative desired state (Gerber *et al*., 2015b).

The assessment of long-term (2009-2017) water quality changes in the system (de Necker *et al*., 2019) did not include the levels of metals in water and sediments of the systems of the PRF. This study was therefore undertaken to provide the first assessment of metals in water and sediments from the two rivers and selected lakes in the PRF and thereby provide initial (baseline) concentrations. To achieve this, the physical and chemical properties of water and sediment of both rivers and lakes were assessed using the two indices, i.e., ATI and SeQI for water and sediment quality respectively. The hypotheses were that water and sediment quality of the Usuthu River and its associated floodplain lake, Lake Shokwe, would be in a better state than the Phongolo River and Lake Nyamithi and this will be reflected in the water and sediment quality indices; and that the hydrological period (i.e., flow) would influence the metal concentrations in water and sediments of these systems.

2.2. Materials and Methods

2.2.1. *Study site and sampling*

The sampling regime was selected according to the environmental flow conditions in the Phongolo floodplain (Dube et al., 2015). To obtain an indication of metals in water and sediments during these distinct flow conditions, sampling was undertaken during the high (June 2018) and low (November 2018) flow periods in the Usuthu, Lake Shokwe, and Lake Nyamithi (see Figure 1.6 in Chapter 1, section 1.2 for study area map and site locations). There was no water in the secondary channel of the Phongolo River during the two surveys and therefore only the main channel of the Phongolo River was sampled during the HF period. Lake Nyamithi could only be sampled during the LF period. Since there was no connection between Lake Nyamithi and the Phongolo River during the sampling period, it is assumed that the results from this period indicate the prevailing water quality of the Lake.

Water samples were collected at a depth of 50 cm from three random sampling points at each of the sites in 500 mL acid-washed polypropylene bottles. Surficial sediment samples at < 50 cm depth were collected from the top 10 cm using a stainless-steel auger. The top 10 cm represents the most recent sediment deposition and therefore the biologically active metals (Wright & Mason, 1998). Sediment samples were transferred into 250 mL acid-washed polypropylene jars. To reduce variability due to the time of day, samples were collected at the same time each day (i.e., between 09:00 and 10:00 am) and frozen and stored at -20 °C until further analysis.

2.2.2. *Water quality analyses*

Dissolved oxygen (DO; mg/L), TDS (mg/L), pH, temperature (°C), and electrical conductivity (µS/cm) were measured *in situ* at each site with the aid of Extech EC500 pH/Conductivity and Extech D0600 Dissolved Oxygen meters. In the laboratory, water samples were allowed to thaw to room temperature and analysed using Merck photometric test kits and a Merck Pharo 100 Spectroquant. Samples were analysed for nitrate (NO_3-N, 109713), nitrite (NO_2-N, 114776), sulphate (SO_4, 114791), turbidity (measured in NTU), chemical oxygen demand (COD, 101796), chloride (Cl, 114897), ammonium (NH_4-N, 114752), orthophosphate (PO_4-P, 114848) and total hardness (TH, 100961).

2.2.3. Dissolved metal concentrations

Defrosted water samples (50 mL) were filtered through cellulose nitrate filter paper (0.45 µm pore size). Filtered samples were decanted into 50 mL Falcon tubes and acidified to 1% nitric acid using 50 µL of 65% ultrapure nitric acid (Gerber *et al.*, 2015a). An initial scan was undertaken to identify metals of toxicological importance that were above detection limits. The following metals were selected and measured using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, 7500CE): Al, Mn, As, Cd, Pb, Fe, Cu, Ni, Co, and Zn. Atomic absorption spectrometry (AAS) equipped with Zeeman-effect background correction (PerkinElmer AAnalyst 900) was used to analyse for Cr following the method of Nawrocka and Szkoda (2012). All metal concentrations in water are reported in µg/L.

2.2.4. Sediment quality analysis

Approximately 6 g of dried sediment was weighed and placed in dry, pre-weighed crucibles. Samples were transferred into an incinerator (L 40/11, Nabertherm) at 600 °C for four hours, allowed to cool, and re-weighed. Weights were used to calculate the TOC using the following equation:

% Organic Carbon = [(Mb - Ma) / Mb] x 100

Where: Mb = mass of sediment before incineration (g)

Ma = mass of sediment after incineration (g)

Classes for TOC were classified according to USEPA (2001), as: high (>4%), medium (2–4%), moderately low (1–2%), low (0.05–1%), and very low (<0.05%).

2.2.5. Sediment metal analyses

Sediment samples were freeze-dried (FreeZone®6, Labconco) for approximately 96 hrs at -54 °C. Approximately 0.2 g was weighed and placed in Teflon digestion tubes and 7.5 mL of 32% HCl and 2.5 mL H_2O_2 were added to each sample. Concurrent sample blanks and certified reference material (CRM) were treated in the same manner. The samples were digested at 1,800 W and 200 °C in an Ethos Easy MAXI-44 Microwave Digestion system for 45 min. After digestion, samples were filtered and decanted into 50 mL volumetric flasks and made up to volume with 1% HNO_3 and analysed using an ICP-MS (Agilent Technologies, 7500CE). The same metals were analysed for as in the water samples. The concentrations of metals in sediment samples are reported as mg/kg dry weight.

2.2.6. Quality assurance and quality control

The analytical quality assurance was carried out using CRM (NCS DC 73310 Stream Sediment from the China National Analysis Centre for Iron and Steel) for sediments. The percentage recoveries of the CRM are reported in Table 2.1. The percentage recoveries for Cd, Pb, Cr, Co, and Ni were within 20% of the certified values for the CRM, whilst Mn, As, Cu and Ni recoveries were within 20 and 35% of the certified values. For this study, no correction factor was applied. For each round of digestions, triplicate blank samples were analysed to determine any interferences.

Table 2.1: Total metal concentrations (mg/kg dry weight) in certified reference material (NCS DC 73310) (mg/kg dry weight) and the percentage recovery (%). All values are represented as mean ± standard deviation.

Element	**NCS DC 73310**		
	Measured values	**Certified values**	**Recovery (%)**
Al	1559 ± 738	-	-
Mn	882 ± 29	1400 ± 73	66
As	73 ± 1.9	115 ± 9	69
Cd	3.2 ± 0.1	4 ± 0.4	87
Pb	219 ± 5	285 ± 16	82
Fe	22520 ± 814	-	-
Cr	27 ± 0.8	35 ± 4	78
Co	5.9 ± 0.1	8.8 ± 1.1	77
Ni	8. 4 ± 0.5	13 ± 1.9	77
Cu	842 ± 16	1230 ± 51	71
Zn	318 ± 8.3	498 ± 27	67

2.2.7. Data analysis

2.2.7.1. Major ions (Maucha diagram)

A Maucha diagram was used to display the ionic (milli-equivalents per liter) composition of the water samples, with the star symbol showing the anions and cations in different colours (Figure 2.1) (Maucha, 1932).

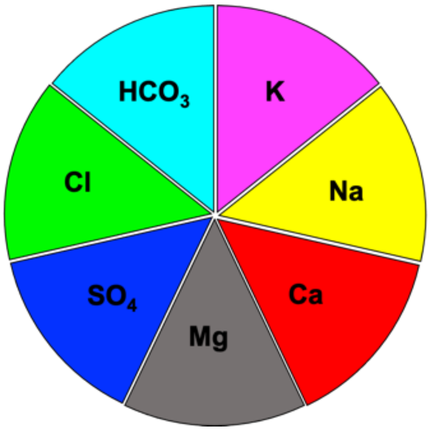


Figure 2.1: A generalised Maucha diagram key adapted from Maucha (1932).

2.2.7.2. Aquatic Toxicity Index (ATI)

The ATI developed by Wepener *et al*. (1992) was applied to the water quality data. Scores were calculated for each of the sampling sites for both surveys. The water quality variables used to calculate the index scores were as follows: pH, TDS, turbidity (log NTU), DO (mg/L), PO_4, NH_4, Zn, Mn, Pb, Ni, Cu, and K. Scores were calculated using a computer-based program, Water 2. Wepener *et al*. (1992) used the Solway Modified Unweighted Additive Aggregation function to calculate the final index scores:

ATI = $1/100\ (1/n \sum_{i=1}^{n} qi)^2$

Where: ATI = final score

n = number of determinants in the indexing system and

qi = the quality of the ith parameter (value between 0 and 100).

Final scores represent a value between 0 and 100 and indicate whether water quality is suitable for aquatic biodiversity. Wepener *et al*. (1992) defined the generic interpretation of the ATI scale as follows: a score of 60 and lower is unsuitable for sustaining aquatic biodiversity, a score between 60 and 100 is suitable conditions for sustaining aquatic biodiversity and a score of 100 indicates pristine conditions.

2.2.7.3. Sediment Quality Index (SeQI)

The sediment quality index provides three components; (1) Scope – referring to the number of variables that did not meet the guidelines, (2) Frequency – the frequency of which the guidelines were not met and (3) Amplitude – the extent of which the guideline was not met. By combining these three components, a single value (between 0 – worst sediment quality and 100 – pristine sediment quality) is produced that describes the sediment quality in relation to the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (ISQG) (CCME, 2007). The following sediment variables were used to derive the index values and quality scores: As, Cd, Cr, Cu, Pb, Ni, Zn, and TOC. For Ni, the sediment guidelines of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC, 2000) were selected since no ISQG guidelines are available from the CCME database. Aluminium, Mn, Co, and Fe were excluded from the calculation as there are no ISQGs for these metals. The international guidelines were used as South Africa does not currently have targeted guidelines for sediment quality. The SeQI scores were calculated as follows:

$$\text{SeQI} = 100 - \left(\frac{\sqrt{F_1^{\,2} + F_2^{\,2} + F_3^{\,2}}}{1.732}\right)$$

Where, F1 – Scope; F2 – Frequency; F3 – Amplitude

The value, 1.732 was used as a divisor as it normalises the values to a range between 0 and 100. For a detailed description of the calculation of F1-3, refer to CCME (2007).

2.2.8. Statistical analyses

The contribution of cations and anions to the water quality were visualised using Maucha diagrams. Differences in spatial and temporal water and sediment data were assessed using analysis of variance (ANOVA). Data were log-transformed to ensure normality and a one-way ANOVA was performed for metal and water quality variables using GraphPad 8 (GraphPad Software, Inc.). The significant differences between groups were determined using Tukey Kramer post hoc analyses. The statistical significance was set at $p < 0.05$. Multicollinearity between metal and nutrient variables was tested utilising a linear regression test of SPSS version 24 (PASW Statistics, IBM, USA) and if present were excluded from the multivariate analysis. Principal component analysis (PCA) was used to interpret site and survey differences in water and sediment quality parameters. A PCA biplot is based on a linear response relating environmental and species variables (van den Brink *et al*., 2003). The results of the ordination are a map of samples on a 2-dimensional basis that reflects the dis/similarities between the sampling sites.

2.3. Results

2.3.1. Water quality

Water quality of the aquatic ecosystems within the NGR are presented in Table A1. Salt concentrations (TDS > Cl > SO_4 > TH) were consistently significantly higher in Lake Nyamithi compared to other sites. Phosphates and SO_4 levels from HF and LF surveys varied in a similar way between the Usuthu River and Lake Shokwe, with lower levels of PO_4 and SO_4 associated with the HF. Conversely, higher NH_4 concentrations were more related to the high flow within each system of the NGR. The water quality at Lake Nyamithi's outlet was similar to Lake Nyamithi during the HF survey with the majority of variables being significantly higher than the rest of the NGR waterbodies.

The PCA biplot representing the general water quality (Fig. 2.2a) shows a separation of Lake Nyamithi from the other sites. Along the first component (explaining 49.4% of the variation) the individual ions and nutrient concentrations differentiate between the sites. The high salinity (measured as TDS) in Lake Nyamithi and its outlet during the HF is responsible for the differentiation between these two sites and the other sites and surveys. The close grouping of the Usuthu River with Lake Shokwe indicates the influence that the river has on the water quality of its associated floodplain lake at the time of sampling (Figure 2.2a). Conversely, Lake Nyamithi does not reflect the influence of either the rivers, on its water quality during either LF or HF conditions (Figure 2.2a).

Dissolved metal concentrations in the four main systems are presented in Table A2. The dissolved metal concentrations revealed limited spatial patterns and no flow-related patterns were observed (Figure 2.2b). The Al, Cu, and Zn concentrations exceeded the target water quality guidelines (TWQG) for aquatic freshwater ecosystems (DWAF, 1996) at all the sites for all the surveys. Together with the aforementioned metals, Cd and Pb exceeded the TWQG in the Usuthu River while Pb exceeded the TWQG in Shokwe. The only metals that were within the TWQGs were As, Cr and Mn.

When the metals were combined with the general water quality parameters, a very similar pattern was observed (Fig. 2.2c). The first component, representing 33.7% of the variation in the data showed a clear separation between the Lake Nyamithi water quality and the other sites. Here the higher metal concentrations were associated with the outlet of Lake Nyamithi. The separation of sites along the second axis (19.8% of the variation) was related to hydrological flow conditions, with very distinct separations between the outlet of Lake Nyamithi. Interestingly, no flow-related separations were observed between the Usuthu River and its associated floodplain lake. It was notable that the Lake Nyamithi water quality appeared to not be influenced by the Phongolo River but rather by the Usuthu River as Lake Nyamithi reflected similar water quality to the Usuthu River. This was because the secondary channel of the Phongolo was not active during the study period and water at the outlet came from the Usuthu River back up into the Phongolo River channel. This was further evidenced by the Nyamithi outlet site displaying similar water quality to the Usuthu River and Lake Shokwe than the Phongolo River.

The ionic chemistry (displayed as Maucha diagrams) indicates similar spatial patterns (Figure 2.3) as was observed in the PCA ordinations. Water bodies were grouped according to water bodies revealing similar concentrations of contributing anions and cations. From the Maucha diagrams, the HF and LF periods contributed different ions to the water chemistry. The Usuthu River and Lake Shokwe, are grouped by the different hydrological periods indicating the influence that the Usuthu River has on its associated floodplain lake (Figure 2.3 – Group 1). The Phongolo River was dominated by Na and Cl, whereas the Usuthu River had high Cl ions, however, the other major ions contributed to the general ionic composition of the Usuthu River. The two floodplain lakes displayed differences in major ionic composition with Na and Cl dominating in Lake Nyamithi (Figure 2.3 – Group 3), while Lake Shokwe (Figure 2.3 – Group 1 and 4) had a variety of contributing ions. The water quality at Lake Nyamithi demonstrated greater variation in ionic composition with distinctly different patterns during high and low flows.

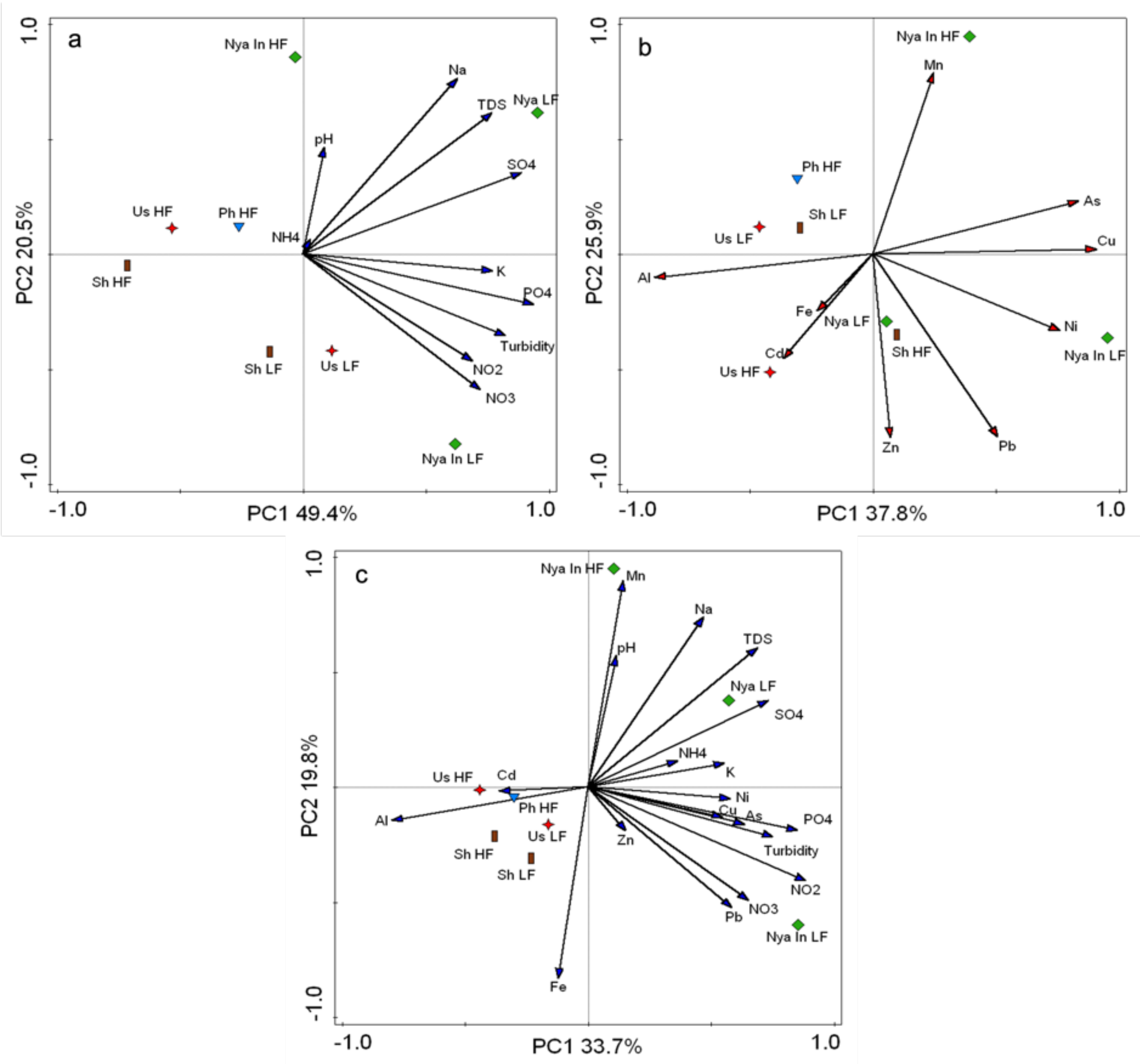


Figure 2.2: Principal Component Analysis biplots of the a) general water quality and b) metal concentrations and c) combined water quality and metal concentrations in the four main aquatic ecosystems (Us=Usuthu River, Sh=Lake Shokwe, Nya=Lake Nyamithi, and Ph=Phongolo River) within the Ndumo Game Reserve. The PCA reflects the spatial as well as flow-related differences in dissolved metal (grey arrows) and general water quality (black arrows) during the high flow (HF) and low flow (LF) surveys. These biplots describe 69.9%, 63.7%, and 53.5% of the total variation in the environmental data, respectively.

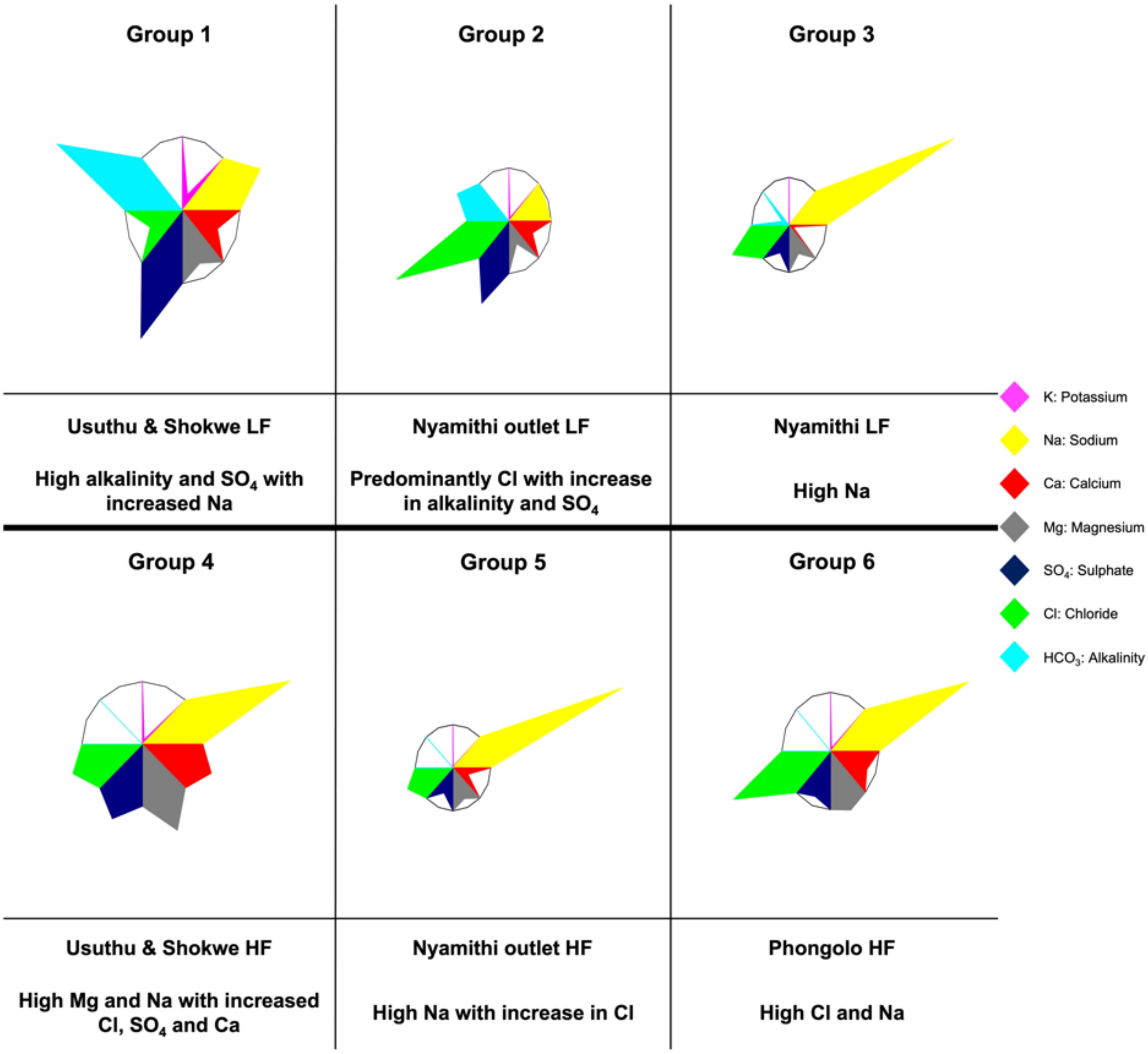


Figure 2.3: Grouping of water bodies in the Ndumo Game Reserve based on ionic composition using Maucha diagrams.

2.3.2. Sediment quality

The percentage organic content and sediment metal concentrations are presented in Table A3. Organic content ranged from 1 to 19.2% between the water bodies, indicating spatial differences. Although not statistically significant, the floodplain lakes had more organic content within sediments, with the highest levels in Lake Shokwe. Limited spatial differences were apparent in sediment metal concentrations. Between the floodplain lakes, most metals were significantly ($p < 0.05$) higher at Lake Shokwe when compared to Lake Nyamithi and its outlet during the LF survey. Although the metals in the Usuthu River sediments were higher during the LF, it was not significantly different from the other sites ($p > 0.05$).

At the time of the surveys, Lake Nyamithi was the only site with Cd concentrations that exceeded the ISQG (0.6 mg/kg) although not significantly different from other aquatic ecosystems of the NGR. Arsenic, Pb, and Zn concentrations were below the ISQG of 5.9 mg/kg, 35 mg/kg, and 123 mg/kg respectively. Chromium concentrations exceeded the ISQG (37 mg/kg) except for the Usuthu River HF survey. Nickel concentrations were compared to ANZECC guidelines (2000) and were below the guidelines in the Usuthu River sediments during both surveys. The concentrations in the Phongolo River HF and Lake Nyamithi LF exceeded the ISQG-Low value of 21 mg/kg while the remainder of the waterbodies surpassed the ISQG-High value of 52 mg/kg during both surveys.

2.3.3. Water and sediment quality indices

The individual ATI scores (a value between 0 and 100) calculated for the water bodies are presented in Table 2.2. The variables contributing to the lowest scores are included. Between both rivers during the high flow survey, the ATI scores indicate spatial differences as the Usuthu HF (79) scored higher than the Phongolo HF (65). The ATI scores for both rivers were above the acceptable levels (60). In Lake Shokwe, both the high (66) and low (67) flow surveys were similar and above the acceptable level. Lake Nyamithi's outlet (HF – 51 and LF – 53) were below the acceptable level of 60. Several variables (e.g., NH_4, PO_4, TDS, turbidity, and Zn) contributed to lowered scores at the different sites, with the lowest rating score of 16 for Zn concentrations across the Usuthu River LF, Phongolo River HF, Lake Shokwe (HF & LF), Lake Nyamithi LF and Lake Nyamithi outlet (HF & LF). The low scores for Lake Nyamithi and the outlet were predominantly due to the TDS concentrations.

The individual SeQI scores (ranging between 0 and 100), the number of variables tested, and those variables that were below the guidelines in the four systems of the NGR are presented in Table 2.3. The index scores ranged from 54 to 100. The Usuthu River system during both surveys had the best sediment quality (HF - 100 and LF 90) while the Phongolo River had a lower SeQI score (68) compared to the Usuthu River. The main factors contributing to lowered scores were Cr, Cu, and Ni concentrations. The SeQI scores from both rivers of the NGR indicate acceptable sediment quality, i.e., scores above 60. The associated floodplain lake (Shokwe) of the Usuthu River had the lowest quality (54) during the HF survey. Lake Nyamithi had a higher score (79) than both Lake Shokwe and the Nyamithi outlet. The lower SeQI for the Phongolo River, Lake Shokwe, and Lake Nyamithi outlet were as a result of Cr, Cu, and Ni failing to meet the guidelines. Chromium and Ni were the driving factors that were responsible for lowering the SeQI score of Lake Nyamithi whereas Cr was the only factor that lowered the index score of the Usuthu River during the LF survey.

Table 2.2: Aquatic Toxicity Index scores of the various Ramsar aquatic ecosystems within the Ndumo Game Reserve and contributing to lower scores variables as well as the corresponding lowest rating score of the aquatic systems of the floodplain.

Sites	ATI Score	Contributing chemical variables	Lowest rating
Usuthu River HF	78.59	Ammonium (34.29)	Ammonium (34.29)
Usuthu River LF	65.46	Orthophosphate (48.68)	Zinc (16)
Phongolo River HF	65.11	Ammonium (62.5)/Orthophosphate (60.41)	Zinc (16)
Lake Shokwe HF	66.49	Ammonium (45.27)	Zinc (16)
Lake Shokwe LF	67.17	Ammonium (75.06)/Orthophosphate (71.46)	Zinc (16)
Lake Nyamithi LF	59.75	Ammonium (58.06)/Orthophosphate (57.58)	Zinc (16)
Lake Nyamithi outlet HF	51.05	TDS (31.62)/Ammonium (34.29)/ Orthophosphate (42.15)	Zinc (16)
Lake Nyamithi outlet LF	53.01	TDS (67.34)/Ammonium (29.37)/Orthophosphate (33.96)	Zinc (16)

Table 2.3: Individual SeQI scores of the various Ramsar aquatic ecosystems within the Ndumo Game Reserve with scope (F1), amplitude (F3), variables as well as variables that failed to meet the CCME (2007) guidelines.

Site	Index score	Scope (F1)	Amplitude (F3)	Variables	Variables failed
Usuthu HF	100	0	0	7(0)	-
Usuthu LF	90	14	0	7(1)	Cr
Phongolo HF	68	43	14	7(3)	Cr, Cu & Ni
Shokwe HF	54	43	49	7(3)	Cr, Cu & Ni
Shokwe LF	56	43	46	7(3)	Cr, Cu & Ni
Nyamithi LF	79	29	9	7(2)	Cr & Ni
Nyamithi outlet HF	59	43	45	7(3)	Cr, Cu & Ni
Nyamithi outlet LF	56	43	45	7(3)	Cr, Cu & Ni

2.4. Discussion

The study evaluates the environmental quality of the regulated Phongolo and unregulated Usuthu rivers and the influence on their two associated floodplain lakes in the NGR during two hydrological periods in 2018. Although the data reflect only the conditions during the study period, this study does provide a departure point for future studies on metals in water and sediment of these systems. The main findings were that the Usuthu River had the better water and sediment quality in relation to environmental quality standards during the two hydrological periods. The Maucha diagrams indicated spatial and flow-related differences between the aquatic ecosystems of the NGR. The multivariate analyses suggested that the water quality of the Usuthu River potentially influences the water quality of the two floodplain lakes within the NGR and were supported by the Maucha diagrams which further suggested that the influence of the Phongolo River on Lake Nyamithi is limited. Environmental quality indices and target quality guidelines indicated spatial and flow-related differences and although most environmental quality variables were within the guideline values, there were certain metals that exceeded these guidelines in both water and sediment.

2.4.1. Water quality

The primary water quality differences were related to the TDS concentrations. The Phongolo River had higher TDS than the Usuthu River and levels were higher than those recorded between 2011 and 2017 (Smit *et al*., 2016; de Necker *et al*., 2019). The higher salt concentrations in the Phongolo River can be attributed to a combination of the prolonged local drought conditions (de Necker *et al*., 2019) and factors affecting increased TDS levels in the groundwater, i.e., silicate weathering, saltwater intrusion, and agricultural activities in the region (Mthembu *et al*., 2020). While in the Usuthu River, the lower TDS and associated salts are related to the relatively unimpacted conditions in the upstream catchment of Eswatini (Rossouw, 2008).

The Phongolo River, a regulated river, had lower nutrient concentrations than the unregulated Usuthu River (Table A1). Despite the advantages (supplies water for industrial, agricultural, and domestic use) that impoundments provide, altering the natural flow regime reduces the nutrient supply of the river (Van Cappellen and Maavara, 2016; Palinkas *et al*., 2019; Zhang *et al*., 2019; de Necker *et al*., 2019). Similarly, de Necker *et al*. (2019) found that the nutrient concentrations decreased significantly in the Phongolo River below the Pongolapoort Dam indicating the dam traps nutrients and nutrient-rich sediments. The higher inorganic phosphate measured in the Phongolo River can be attributed to agricultural practices using phosphate-rich fertilizers (de Necker *et al*., 2019), sewage, and the decomposition of rocks and minerals

(Mthembu *et al*., 2020). The drought has caused the water bodies to dry up and as a result, the hippopotami had to find refuge in the permanent water bodies resulting in increased populations in rivers. Thus, with the absence of agricultural activities close to the lower Usuthu River, the high nitrate concentrations could be attributed to the very high density of hippopotami (average of 49 individuals (Fritsch & Downs, 2020)) in this reach of the river (Stears *et al*., 2018; Mthembu *et al*., 2020).

The nutrient concentrations in both rivers of the NGR were similar to the levels measured in the rivers of the Kruger National Park (KNP) (Gerber *et al*., 2015a). Gerber *et al*. (2015a) explained that the rivers from the KNP are known to be influenced by human-driven impacts outside of the conservation area, a situation that influences the water quality of aquatic ecosystems within the NGR. Thus, the increased nutrient concentrations found in both rivers from the NGR could be attributed to activities upstream and on the border of the NGR. These include domestic animal waste, inorganic fertilisers from small-scale agriculture, and increased human waste due to rapid population growth in the region (de Necker *et al*., 2019; Mthembu *et al*., 2020).

Lake Nyamithi, the largest permanent and natural saline lake of the floodplain, has a unique water chemistry due to its underlying geology, constant water inundation, and the presence of large crocodile (approximately 800 individuals (Calverley and Downs, 2014)) and hippopotami populations. Due to its status, the floodplain lakes of the present study were compared to another Ramsar site (Baberspan) in the North West Province, South Africa (Henri *et al*., 2014). Both lakes of the NGR had higher nutrient concentrations than Barberspan (PO_4: <0.065, NH_4: <0.01, NO_2: <0.070, NO_3: <0.070). Total dissolved solids and Cl from the present study were lower in Lake Shokwe than Barberspan while in Lake Nyamithi, TDS and Cl were higher. The higher concentration of Cl in Barberspan was attributed to the underlying geology and could be exacerbated even further with decreased water levels (Henri *et al*., 2014), while Lake Nyamithi is known for its high salinity and the geology is the main contributing factor (Smit *et al*., 2016). This is because the lake water quality is unique as a result of the salts derived from the underlying ancient marine bed in this region of the PRF (Heeg and Breen, 1982).

Maucha diagrams summarise the major ion ratios present in water samples and are used to compare water chemistry types (van Niekerk *et al*., 2014). Results from the Phongolo River showed that with its higher ratios of Na and Cl, this river is dominated by precipitation (Day and King, 1995). High Na concentrations could be due to irrigation and silicate weathering. This supports the findings by Smit *et al*. (2016) who indicated that increases in salinity in the

Phongolo River were attributed to the return flows from the irrigation schemes, while Mthembu *et al*. (2020) attributed the presence of Na and K to the weathering of silicates.

The unique water chemistry of Lake Nyamithi is highlighted by the fact that Lake Nyamithi does not share the same water chemistry characteristics of either the Phongolo or Usuthu rivers. In this case, the ionic chemistry is driven by the underlying geology (Smit *et al*., 2016). Based on the limited data, the ionic composition at the Lake Nyamithi outlet during the LF survey was distinctly different from the HF ionic composition. This is attributed to the influence of the water quality from the Usuthu River at the outflow during the LF period. However, during the HF the outlet ionic composition was similar to both the Phongolo and Usuthu rivers and this is reflected in the ionic composition of the main water body of the lake. Therefore, there is compelling evidence that, with zero flows between the Phongolo River and the outlet of Nyamithi, the outlet only received water from the Usuthu River, and water quality is influenced during the high and low flow periods.

The metal concentrations recorded in the present study were compared to concentrations in rivers and wetlands of other conservation and Ramsar areas of South Africa (Table 2.4). All these systems are influenced by upstream activities with similar sources of metals to those that may impact on the NGR. The KNP is regarded as a conservation area that receives increased metal concentrations from rivers that flow through it and Gerber *et al*. (2015a) attributed these metal concentrations to extensive mining, urban, industrial, and agricultural practices. Whilst most metals were higher in the KNP rivers, the concentrations of Cu, Fe, and Zn in the NGR water bodies were higher than those measured in the KNP rivers. These higher levels can be attributed to agricultural and industrial practices that can only be found in the upper catchments of both these rivers (DWA, 2009; Annual Water Quality Report, 2014) and the contribution of groundwater concentrations to the surface water (Mthembu *et al*., 2020). The Blesbokspruit wetland (Ramsar site) is in the Gauteng Province and is influenced by extensive mining activities and associated acid mine drainage (Roychoudhury and Starke, 2006; Ambani and Annergan, 2015). This is evident in the higher metal concentrations measured in the Blesbokspruit compared to the current NGR study.

Metal concentrations in both floodplain lakes of the NGR were higher than levels in unpublished data from Seekoeivlei – a Ramsar site in the Free State Province (Table 2.4). Metals in the Seekoeivlei wetland are notably influenced by weathering, erosion, animal excrement, and agricultural fertilisers (unpublished data). Arsenic, Cr, Cu, and Zn were all lower in floodplain lakes of the NGR; Cd, Co, and Pb were similar while Al, Fe, Mn, and Ni concentrations were higher in Barberspan (Henri *et al*., 2014). Barberspan had higher Cu and

Zn concentrations than Lake Shokwe and Lake Nyamithi which can be attributed to coal mining in the catchment (Henri *et al*., 2014). There are no mining activities near the NGR, thus the levels measured cannot be attributed to these activities but are rather a result of geological characteristics, small subsistence farming and to a small degree aerial deposition from coal powerplants (Okedeyi *et al*., 2013; Mthembu *et al*., 2020). Further comparison with similarly less-impacted wetland lakes in Mpumulanga revealed very similar metal concentrations (Table 2.4). The only exception was Zn, which was higher in the Mpumalanga lake and was attributed to erosion due to grazing pressure (Henri *et al*., 2014). In a Ramsar site in Turkey, higher metal concentrations were found than in the present study and were a result of urban and industrial waste and chromium mines (Elmaci et al. 2007; Arslan *et al*., 2010).

2.4.2. Sediment quality

Contaminated sediment can be a potential source of surface water contamination and provides an account of the historic contamination of an aquatic system (Singh *et al*., 2005; Mutia *et al*., 2012; Gerber *et al*., 2015b). Metal concentrations within the NGR were compared to other Ramsar sites with known metal contamination threats (Nyl River floodplain, Blesbokspruit, and Lake Uluabat) and a conservation area (the KNP) which is situated downstream of extensive mining activities (Table 2.5). It was evident that concentrations of most metals in sediments were lower or similar (KNP: Cd, Pb, Fe, Mn, Ni, Zn; Blesbokspruit: Cd, Pb, Fe, Ni) at the NGR sites when compared to the known metal-contaminated systems such as the KNP (Gerber *et al*., 2015b) and Blesbokspruit (Henri *et al*., 2014) (Table 2.5). Lead was the only metal that was higher in sediments from both rivers in this study when compared to the KNP river sediments, while Phongolo River sediments had higher Fe and Mn concentrations than the KNP rivers. When compared to the Nyl River floodplain, the NGR had higher concentrations of Cu and Fe in sediments.

In general, lentic systems have higher sediment metal concentrations than the sediments of the rivers that flow into them. Due to the tendency of metals to adsorb to the organic matter in sediments, they subsequently act as a metal sink (Extence *et al*., 2013; Meena *et al*., 2018; Tendaupenyu and Magadza, 2019). The higher concentration of metals in the sediment of the floodplain lakes can therefore be attributed to the high organic content and while sediments can be transferred to lakes through rivers, there is essentially no export out of lakes therefore lakes act as a sink. The higher salinity in Lake Nyamithi causes salts to form complexes with metals that make adsorption to sediment particles difficult (Wright and Zamuda, 1987; Kumar *et al*., 2015) and therefore is the likely reason for the lower sediment metal concentrations compared to Lake Shokwe.

2.4.3. Water and sediment quality indices

Water quality indices have been applied successfully to summarise the water quality of aquatic systems and provide an indication of the general water quality status (Dede *et al*., 2013; Ismail and Robescu, 2019). The ATI scores calculated for both rivers of the NGR indicated were lower than those calculated for the KNP rivers except for the Usuthu HF survey which was similar to the KNP rivers (Gerber *et al*., 2015a). However, the scores still remained within the water quality limits suitable to sustain healthy aquatic ecosystems. For the rivers of the NGR, nutrients and notably Zn contributed to lowering the scores with Zn contributing to the lowest ATI rating. This is in contrast to the findings by Gerber *et al*. (2015a) who found that metal concentrations had little to no influence on the ATI scores of the known metal contaminated KNP rivers. This poses the question of whether the rating scores for Zn are too stringent since there is no evidence that Zn is responsible for any negative effects related to the water quality that is suitable for fish well-being (Vu *et al*., 2017), with high fish biodiversity present in the system (Acosta *et al*., 2021). Another possibility could be that the ATI was specifically developed for the Olifants River system and that the variables that were included in the index were the problematic variables in the system at that time.

In general, the ATI scores for the NGR floodplain lakes indicated suitable water quality to sustain aquatic biodiversity. The lower scores calculated for Lake Nyamithi (score below 60) are attributed to the higher salinity in this lake. As such, lower scores in Lake Nyamithi means its water quality is suitable for hardy fish species such as Clarias gariepinus and Oreochromis mossambicus (Wepener *et al*., 1992), which were the dominant fish species in the lake during the study period (de Necker *et al*., 2022a). Lake Shokwe had the least number of variables contributing to lowering the ATI score whereas Lake Nyamithi and its outlet had a variety of variables contributing. However, even with these low ATI scores, river-fed floodplain lakes, and in particular the PRF floodplain lakes are important ecosystems for foraging areas, spawning, and breeding of migratory fish (Acosta *et al*., 2021). The ATI scores derived in the aquatic ecosystems within the NGR may be an overestimation of the water quality since it was clear that high nitrate levels were one of the main factors contributing to water quality. In addition, since there is wide-scale use of pesticides in the region, the role of this stressor as a water quality issue remains unknown.

The SeQI scores from both rivers of the NGR indicate acceptable sediment quality, i.e., scores above 60. Scores from the Usuthu were higher than the Olifants and Luvuvhu rivers while similar to the Letaba River whereas the Phongolo River scored lower SeQI values than all three KNP rivers (Gerber *et al*., 2015b). Sediment Quality Index scores of the Lake Nyamithi had a higher index score than the other main floodplain lake. Only Shokwe and the outlet of Nyamithi were below 60, due to having higher concentrations of Cr, Cu, and Ni. All three of these metals in both rivers and floodplain lakes surpassed the ISQG of both the CCME and Anzecc. Chromium and Cu are both associated with agriculture (Agency for Toxic Substances and Disease Registry (ATDSR), 2004; ATSDR, 2012) while Ni is associated with coal mines both land uses are located upstream in the catchments of both rivers (ATSDR, 2005; Rossouw, 2008). Furthermore, the SeQI scores indicated that the sediment quality of aquatic ecosystems within the NGR were suitable to sustain aquatic life. However, from the existing biological data available for the system (Acosta *et al.*, 2021) there is no evidence that these metals pose any risks to decreasing the biodiversity.

Table 2.4: Dissolved metal concentrations (µg/L) of the present study compared to the three rivers (Olifants, Letaba, and Luvuvhu) inside the Kruger National Park, three South African Ramsar sites (Blesbokspruit, Barberspan & Seekoeivlei), international Ramsar sites (Turkey) and the Nyl River floodplain.

Habitat	Source	Site	Dissolved metal concentrations (µg/L)										
			Al	As	Cd	Co	Cr	Cu	Pb	Fe	Mn	Ni	Zn
Floodplain Rivers	Present study	Usuthu HF	21	0.42	1.5	0.13	0.94	6.9	0.3	45	2.4	0.6	21
		Phongolo HF	28	0.77	0.07	0.1	0.66	4.9	0.22	80	2.2	0.6	3.2
		Usuthu LF	117	0.44	0.03	0.15	0.23	4.5	0.2	91	3.8	0.63	6.5
	Dahms-Verster *et al.*, 2018	Nyl River	40-116	-	0-0.76	0-0.71	-	2.1-11	0.5-11	35-916	0.1-17	0.48-10	15-90
Rivers	Gerber *et al.*, 2015a	Olifants River LF	84	0.98	6	3.9	4.4	0.89	2.1	39	4.7	1.6	2.6
		Olifants River HF	55	0.54	8.8	6.6	4.1	0.43	7.4	42	3.4	1.3	2.8
		Letaba River LF	51	0.6	5.8	4.4	4.9	1.2	5.7	42	5.4	1.8	5.4
		Luvuvhu River LF	63	0.25	1.6	0.62	2.6	1	1.4	56	2.4	0.98	3.4
		Luvuvhu River HF	31	0.02	1.2	0.61	0.19	1.1	1.1	12	3	0.72	2
Floodplain lakes	Present study	Shokwe HF	13	0.54	0.1	0.14	1.6	4.1	0.57	30	5.2	1.9	8.2
		Shokwe LF	56	0.59	0.04	0.19	0.37	10	0.5	38	1.1	2.6	6.3
		Nyamithi LF	117	1.4	0.05	0.49	0.28	34	0.86	71	1.5	2.2	13
		Nyamithi outlet HF	9.6	0.83	0.01	0.32	1.1	10	0.38	73	2.7	1.5	5.2
		Nyamithi outlet LF	5.1	0.93	0.02	0.39	0.18	15	0.08	21	109	1.2	8.8
Lake	Henri *et al.*, 2014	Mpumalanga lake (Mp pan H)	19	<5	<1	<1	<5	<5	<1	8	<1	<1	42
Ramsar sites	Roychoudhury & Starke, 2006	Blesbokspruit	-	5.1	4.3	227	4.3	16	1.1	318	653	2122	112
	Henri *et al.*, 2014	Barberspan	<3	<5	<1	<1	<5	27	<1	27	<1	<1	58
	Unpublished data	Seekoeivlei	-	0.001	-	0.0008	0.005	0.003	-	0.072	0.01	0.001	0.002
	Arslan *et al.*, 2010	Lake Uluabat	-	-	3	-	17	118	105	-	-	56	284

Table 2.5: Sediment metal concentrations (mg/kg dry weight) of the present study compared to the three rivers (Olifants, Letaba, and Luvuvhu) inside the Kruger National Park, one South African Ramsar site (Blesbokspruit), one international Ramsar sites (Turkey), and one international floodplains.

Habitat	Source	Sites	Total sediment concentrations (mg/kg dry weight)										
			Al	As	Cd	Co	Cr	Cu	Pb	Fe	Mn	Ni	Zn
River sediment	Present study	Usuthu HF	5855	1	0.03	5.8	24	11	3	7972	186	13	10
		Phongolo HF	19242	2.6	0.08	17	63	39	8.2	30486	601	31	33
		Usuthu LF	7679	1.1	0.06	5.5	38	12	4.6	9656	165	16	14
	Gerber *et al*., 2016	Olifants River LF	13190	4.6	0.11	43	120	33	2.8	26984	241	25	53
		Olifants River HF	27287	2.9	0.08	93	99	22	1.4	18497	127	18	47
		Letaba River LF	14858	1.2	0.08	62	95	19	1.7	11504	157	11	26
		Luvuvhu River LF	12315	1.2	0.16	43	83	35	4.6	19682	279	34	69
		Luvuvhu River HF	11258	0.97	0.07	73	87	25	1.5	19233	128	13	36
Floodplain sediment	Present study	Shokwe HF	48059	4.2	0.13	27	150	57	20	52090	448	86	49
		Shokwe LF	59324	3	0.07	17	155	44	16	38510	281	73	49
		Nyamithi LF	15812	1.8	0.09	7.2	62	16	5.7	14795	253	21	18
		Nyamithi outlet LF	52251	5.5	0.07	28	153	54	19	55074	568	66	50
		Nyamithi outlet HF	35783	5.1	0.08	31	123	58	16	46849	408	57	44
	Dahms-Verster *et al*., 2017	Nyl River Floodplain	-	-	-	-	-	6.1	-	3601	4324	58	149
Ramsar sites	Roychoudhury & Starke, 2006	Blesbokspruit	-	27	0.15	36	191	66	20	3889	828	119	156
	Arslan *et al*., 2010	Lake Uluabat (Turkey)	-	-	0.7	-	58	119	111	-	-	209	171

2.5. Conclusion

This study provides the first evaluation of metal concentrations in water and sediments of different aquatic systems in the PRF over two hydrological periods. It reflects the metals in water and sediments for the study period, however it does provide a baseline for future studies. The study further evaluated the environmental quality of the various ecologically important systems within the NGR and the influence of the regulated Phongolo River and unregulated Usuthu River on their two associated floodplain lakes. Results showed that the Usuthu River had the highest water and sediment quality. Furthermore, the contribution of the Usuthu River to the aquatic ecosystems of the NGR were evident as the multivariate analysis showed that during the HF period, the Usuthu River and the outlet of Lake Nyamithi had similar metal concentrations and similar general water quality. The Maucha diagrams supported these findings while suggesting the influence of the Phongolo River on Lake Nyamithi is limited. With these differences, it is evident that through back flooding from the Usuthu River, water pushes into Lake Nyamithi through the channel between the Usuthu and Phongolo rivers confluence and Lake Nyamithi. Although Lake Nyamithi receives water from the Usuthu River, the water chemistry highlights no change in chemistry, rather keeping its unique chemistry due to the ancient marine bed.

Similarly, environmental quality indices and targeted quality guidelines indicated spatial and flow-related differences between the various aquatic ecosystems within the NGR. The water and sediment quality of the unregulated Usuthu River and its associated floodplain lake, Lake Shokwe, do differ from the regulated Phongolo River and Lake Nyamithi since the ATI scores indicate differences between the Usuthu and Phongolo rivers as a result of higher organic nutrients in the Phongolo River. This was supported by the higher NO_3 levels found in the Usuthu River than in the Phongolo River due to the Pongolapoort dam trapping nutrients and not releasing water. The ATI scores indicate differences between the floodplain lakes, which were due to the natural high salinity of Lake Nyamithi. The SeQI scores further indicated that the Usuthu River sediment quality is higher than that the other four aquatic ecosystems within the NGR. However, based on the indices it is clear that there are no potential ecological risks posed by metals in these systems. Therefore, with the present environmental quality of the various aquatic systems within the PRF been assessed, it is further important to determine the aquatic macroinvertebrate community structures of the various aquatic systems within the PRF as aquatic macroinvertebrates is known to respond to abiotic and biotic factors.

CHAPTER 3
Ecological importance of the natural flooding regime of the Usuthu River to the macroinvertebrate community structure of aquatic ecosystems within Ndumo Game Reserve

3.1. Introduction

Aquatic macroinvertebrates are essential components of riverine ecosystems for a variety of reasons; firstly, they are commonly used in biomonitoring to determine river health, secondly, they are an important food source for higher trophic levels, thirdly, they control and consume periphyton and fourthly, they influence ecosystem processes (Greenwood & Booker, 2015; Dube *et al*., 2017). They are particularly useful in ecological assessments due to their specific traits, they are a diverse group and have different sensitivity and tolerance levels to stressors (Foster *et al*., 2015). Apart from drought, natural flooding (unique to natural aquatic ecosystems) is a major driving force in aquatic community composition (Greenwood & Booker, 2015).

River regulation affects not only the functioning and structure of the river (Schneider & Petrin, 2017) but also causes a change in physical habitats, life histories of aquatic biota are interrupted, restricts migration of fish species and macroinvertebrates, facilitates the invasion and success of introduced species and finally limits or increases lateral and longitudinal connectivity (depending on the type of alteration) (Brooks *et al*., 2011; White *et al*., 2017). Furthermore, river regulation affects water quality (see Chapter 2, section 2.4.1) which in conjunction with the modified flow may cause changes in biota (Schneider & Petrin, 2017; Beatty *et al*., 2017; Milner *et al*., 2019). The environmental quality of the present study (see Chapter 2, section 2.3.1) indicated that the water quality was different between the unregulated and regulated rivers, however, water quality was still suitable for aquatic biota. Poff *et al*. (1997) emphasised that a river's flow regime is essential to sustaining its ecological integrity and biodiversity. High and low flows from natural rivers regulate several ecological processes that in effect restore biological communities which enable species with colonising abilities and fast life cycles to re-establish. Consequently, high flows are unique in such a way that it supplies even more ecological benefits to the ecosystem to sustain productivity and diversity (Poff *et al*., 1997). Aquatic macroinvertebrates are important aquatic biota that have acquired seasonal adaptations to withstand and survive predictable flooding that is not dependent on local conditions (Tronstad *et al*., 2005).

Aquatic community composition is driven by both abiotic and biotic factors which can be susceptible to regime shifts (Peeters *et al*., 2004; Bogan & Lytle, 2011). Drought is seen as a key driver in aquatic community composition, however, although not associated with regime shifts, drought is known to cause changes in local and regional aquatic community dynamics (Bogan & Lytle, 2011). Anthropogenic stressors altering natural patterns of the environment (i.e., natural flow regimes as alluded to above) influence biodiversity composition and therefore, aquatic communities can shift to a lower diversity state. As such, both rivers within the NGR experience anthropogenic impacts from outside the reserve. However, based on the indices, it is evident that there is no potential ecological risk that metals pose in these systems (Chapter 2, section 2.3.4). Although no potential risk arises from the activities in the upper catchment in terms of metals, controlling the flooding regime of large rivers influences aquatic composition not only through water velocity and water levels but water quality as well and therefore it is essential to monitor aquatic community composition. In the face of disturbances, resistance and resilience are the two components influencing the stability of aquatic communities (Rader *et al*., 2008). Van Looy *et al*. (2018) explained that aquatic biota can be resilient either by persisting and resisting the effects or recover rapidly through recolonisation.

As the Usuthu River forms part of the only conserved area on the PRF (for a full description of the PRF and the Usuthu River please see Chapter 1, section 1.2), the disturbances affecting community composition should be minimal. Therefore, the natural river within the conserved area should act as a refuge for aquatic biota. The Usuthu and Phongolo rivers are both important for the two largest floodplain lakes (Lakes Shokwe and Nyamithi) of the NGR (see Chapter 1, section 1.2.3). These floodplain lakes are important habitats for breeding, spawning, and foraging for migratory aquatic species. Acosta *et al*. (2021) explained that the connection between the river and its associated floodplain lakes plays an essential role in the exchange of aquatic vegetation, organic material, and nutrients indicating the flood regime plays a key role in the productivity of wetlands and river health. Hydrological regimes impact the structure and dynamics of communities thus driving biodiversity patterns and ecological function (Dube *et al*., 2019). Floodplain connectivity influences species richness and composition and their functional response (Paillex *et al*., 2013). Dube *et al*. (2019) further explained that floodplain lakes rely on the natural patterns of hydrological connectivity between the river and its associated floodplain lake to maintain biota and ecosystem processes. As such, both rivers within the NGR have an important role to play in maintaining resident biota and the function of these floodplain lakes.

In recent years, de Necker *et al*. (2021) studied the response of aquatic macroinvertebrate communities from Lake Nyamithi to a supra-seasonal drought in the NGR. Historically, Lake

Nyamithi, a natural saline lake, received water predominately from the Phongolo River, and ever since the drought (between 2015 – 2017), the Phongolo River has not flooded as a result of no flood release from the Pongolapoort Dam (de Necker *et al*., 2021). This has led to Lake Nyamithi becoming reliant on the Usuthu River and from localised rainfall (see Chapter 2, section 2.4.1). This large, permanent natural saline lake is a unique floodplain lake in that it does not share the same water quality as its associated river (see Chapter 2, section 2.4.1). Aquatic biota residing in saline lakes are adapted to osmotic stress and disperse when conditions become unfavourable (de Necker *et al*., 2021). The study of de Necker *et al*. (2021) showed that with increased localised rainfall and flooding from the Usuthu River, the macroinvertebrate diversity recovered rapidly with diversity exceeding predrought conditions. As such, this highlights the importance of the Usuthu River in keeping the salinity levels below 8 g/L resulting in the recovery of aquatic macroinvertebrate communities.

The main aim of this chapter was to determine the macroinvertebrate community structure of the Usuthu River and how its natural flooding regime influences the macroinvertebrate community structures of associated freshwater ecosystems. To achieve this aim, the following hypotheses were tested: 1) the macroinvertebrate community structure of the Usuthu River differs from the Phongolo River (the major source of water for Lake Nyamithi during normal rainfall periods); 2) the macroinvertebrate community structures of the two largest floodplain lakes within the NGR reflect the influence of the Usuthu River; 3) hydrological connectivity will have a positive effect on the macroinvertebrate community composition of Usuthu River associated floodplain lake; 4) the influence of the Usuthu River on Lake Nyamithi will reflect a sustained recovery in macroinvertebrate community structures and finally 5) the nature of the aquatic ecosystems (lotic/lentic) drives the structure of macroinvertebrate communities within the NGR.

3.2. Materials and Methods

3.2.1. Study area and sampling

Aquatic macroinvertebrates and water for chemical analyses were collected from the Usuthu River (November – 2018), Lakes Shokwe (June and November – 2018), and Lake Nyamithi (November – 2018). Aquatic macroinvertebrate community structures from the Phongolo River and associated floodplain lakes have already been assessed and therefore will be included in the present study for comparison. Data from the Phongolo River inside (2012, 2013 (Smit *et al.*, 2016) and September – 2016 (de Necker, 2019)) and outside the NGR (September – 2016 (de Necker, 2019)), Phongolo River floodplain lakes (December – 2014 (Dube *et al.*, 2019)) and Lake Nyamithi (November – 2017 (de Necker *et al.*, 2021)) were obtained from previous surveys in the floodplain (Figure 3.1) (see Table 3.1 for survey codes). Refer to Chapter 2, sections 2.2.1 and 2.2.2, for methods regarding water sampling and in situ water quality measurements, respectively. Aquatic macroinvertebrates were collected, using a standard 30 cm x 30 cm dip net, from all available biotopes (vegetation, stones in and out of current; and gravel, sand, and mud) across all sites. Through 40 sweeps, integrated macroinvertebrate samples were collected from each site in triplicate and placed in 500 mL polyethylene jars and fixed with 70% ethanol.

Table 3.1: Different locations sampled during various surveys inside and outside the Ndumo Game Reserve.

Site	Survey Code
Usuthu River 2018	November 2018 (UR 18)
Phongolo River inside NGR (de Necker, 2019)	September 2016 (Ph 16 In)
Phongolo River outside NGR (de Necker, 2019)	September 2016 (Ph 16 Out)
Lake Shokwe 2018	June 2018 (Sh HF) November 2018 (Sh LF)
Lake Nyamithi 2017 (de Necker *et al.*, 2021)	November 2017 (Nya 17)
Lake Nyamithi 2018	November 2018 (Nya 18)
Phongolo Floodplain Lake 1 (Dube *et al.*, 2019)	December 2014 (Ph Fl 1)
Phongolo Floodplain Lake 4 (Dube *et al.*, 2019)	December 2014 (Ph Fl 4)

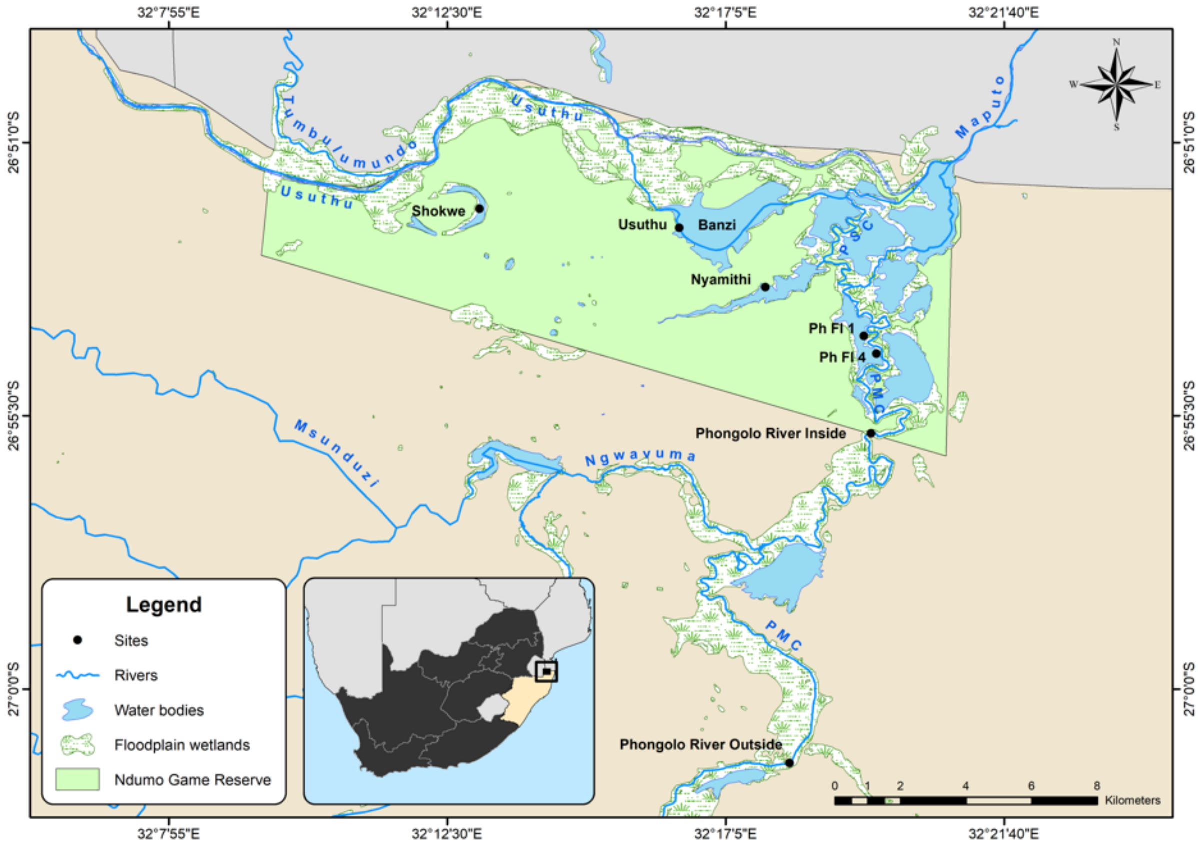


Figure 3.1: Map of the study area within the Ndumo Game Reserve where aquatic macroinvertebrates were collected during the present study (Usuthu River, Lakes Shokwe and Nyamithi) together with sampling sites from previous surveys (Phongolo River, Phongolo floodplain 1 and 4 – Ph Fl and Lake Nyamithi). PMC – Phongolo Main Channel and PSC – Phongolo Secondary Channel.

3.2.2. Laboratory analysis

3.2.2.1. Water quality analysis

The various chemical water analysis methods (nitrate (NO_3), nitrite (NO_2), sulphate (SO_4), turbidity, chemical oxygen demand (COD), chloride (Cl), ammonium (NH_4), orthophosphate (PO_4) and total hardness (TH)) can be found in Chapter 2, section 2.2.2.

3.2.2.2. Macroinvertebrate identification

In the laboratory, macroinvertebrates were washed in a 250 μm mesh size sieve to remove any mud while large pieces of vegetation were picked out by hand and rinsed until clear water drained out the bottom of the sieve (mesh size of 250 μm). All macroinvertebrates were counted and identified to the lowest possible taxonomic level with appropriate identification guides (Day *et al*., 1999; Day *et al*., 2001a; Day *et al*., 2001b; Day and de Moor *et al*., 2002a; Day and de Moor 2002b; Day *et al*., 2003; de Moor *et al*., 2003a; de Moor *et al*., 2003b; Stals and de Moor, 2008) using a Nikon Model C-LEDS dissection microscope.

3.2.2.3. Macroinvertebrate traits

Following macroinvertebrate identification, taxa were classed according to their different traits (functional feeding groups, habitat preference, dispersal, mode of respiration, aquatic life stage, and hydraulic preference) by using the South African Macroinvertebrate Trait Database (Odume *et al*., 2018) (see Appendix B4 – B8 for all taxa and their specific traits).

3.2.3. Statistical analysis

All analyses on aquatic macroinvertebrate diversity were conducted where possible on the lowest taxonomic level. Using untransformed data, various diversity-related indices (Pielou's Evenness index, Margalef species richness, Simpson's index, and Shannon-Wiener diversity) were calculated for each site to determine relationships and abundances within communities. Pielou's evenness index indicates whether there is a dominance of individuals in the community from each site with calculated scores closer to 0 indicating uneven spread while scores closer to 1 indicate complete evenness as taxa are evenly spread with no dominant taxa (Pielou, 1971). Margalef's species richness index determines species richness from a particular site by incorporating taxon richness and abundance (Margalef, 1968). Simpson's index determines, when drawn at random, whether there is a probability that two individuals could belong to the same species. The diversity is low when there is a high probability (Simpson, 1949). The Shannon-Wiener diversity index (Shannon diversity) incorporates both

Pielou's and Margalef's indices to determine overall diversity at each site (Shannon and Weaver, 1964; Clarke and Warwick, 2001). One-way analysis of variance (ANOVA) (GraphPad Prism 8) was used to determine statistical differences ($p < 0.05$) between the diversity indices of the various aquatic ecosystems. Significance was determined through a Tukey Kramer post hoc analysis. Aquatic macroinvertebrate data were normalised by means of square root (√) transformation to reduce the effect of dominant taxa while water quality data were log-transformed ($y = \log (x+1)$) prior to the constrained analysis.

Macroinvertebrate and water quality data from Dube *et al*., (2019) and an unpublished Ph.D. thesis from de Necker (2019) were used together with the data from this study to compare the macroinvertebrate communities of different aquatic ecosystems of the NGR. During these surveys, the authors studied how the drought, flooding regime, and anthropogenic activities influenced the macroinvertebrate community structure of ephemeral and floodplain lakes together with the Phongolo River. To make the present study comparable, similar sites were selected while similar sampling techniques and analyses were used. A constrained canonical correspondence analysis (CCA) using Canoco v5 was used to determine possible differences between; a) Usuthu River vs Phongolo River, b) Usuthu River associated floodplain lake vs Phongolo River associated floodplain lakes, c) HF & LF surveys of the Usuthu River associated floodplain lake and a redundancy analysis (RDA) was used for d) sampling years (2017 vs 2018) of Lake Nyamithi. Prior to CCA and RDA, water variables were tested for possible collinearity between variables, and in the case of highly collinear variables (>10), they were excluded from the analysis (see Chapter 2, section 2.2.8 for a detailed description of the multicollinearity analysis). As such, the water quality variables that were included for the CCA's and RDA's were the same that were used in Chapter 2, section 2.3.1.

To determine the sensitive and tolerant taxa occurring in the different systems, pie charts were created to compare macroinvertebrate family sensitivity data from both rivers during different surveys (Phongolo River: 2012 – 2013 (Smit *et al*., 2016) and 2016 – 2017 (Unpublished thesis: de Necker, 2019); Usuthu River: present study). As was done by de Necker (2019) for comparison purposes, macroinvertebrates were separated based on sensitivity scores and divided into two large groupings namely, tolerant (0-7) and sensitive (8-15). These sensitivity scores were obtained from Dickens and Graham (2002) where major taxa were allocated sensitivity scores based on their sensitivity to organic pollution.

The percentage (%) in which aquatic macroinvertebrates contributed to dissimilarities between aquatic ecosystems of the NGR was calculated by similarity percentage analysis (SIMPER) using Primer v7. Multiple variation partitioning analysis was applied to determine the unique and shared effects of water quality, habitat preferences, and system type (River or Floodplain Lake) on the macroinvertebrate community composition using the varpart function in the vegan package for R (Oksanen *et al*., 2017; R Core Team, 2017). Prior to the varpart analysis, all water quality variables were analysed for multicollinearity using SPSS version 24 (PASW Statistics, IBM, USA). The highly correlated variables were excluded from the analysis. As such, the water quality variables that were included for the analysis were the same ones that were used in Chapter 2 and for the macroinvertebrate species CCA's and RDA (for more detailed analytical procedures, please see Chapter 2, section 2.2.8 and section 2.3.1). The variation partitioning analysis indicates the portion of variability that the selected explanatory variables (water quality, habitat preference, and system type) explain in the response data (macroinvertebrate communities). For the analysis, variation was partitioned into three components, 1) water quality, to determine the effect of the selected water quality variables, 2) habitat preference, to determine the effect that habitat preference has on community composition and 3) system type, to determine the effect of the type of system on macroinvertebrate community composition. Only the unique effect of each variable can be tested for significance and was tested with partial redundancy analyses.

3.3. Results

3.3.1. Aquatic macroinvertebrate community structure

3.3.1.1. Univariate analyses

3.3.1.1.1. Rivers

A total of 34 different taxa from 30 families were identified from the Usuthu River while the Phongolo River had a total of 38 taxa from 29 families during the 2016 survey within the NGR (Appendix B2). Inside the conservation area, the greatest number of taxa were found in the Phongolo River while outside the NGR, the Phongolo River had the least number of taxa (24) from 23 families. Taxa in the Phongolo River within the reserve consisted largely of midges (Chironomidae), freshwater shrimp (*Caridina nilotica* (Atyidae)), and the invasive pouch snail (*Physa acuta* (Physidae)) while outside the reserve, taxa consisted of *C. nilotica* (Atyidae), damselflies (Coenagrionidae), and the invasive melania snails (*Tarebia granifera* (Thiarida)). The most abundant taxa in the Usuthu River within the NGR were the small minnow mayflies (Baetidae), freshwater shrimp (*C. nilotica* (Atyidae)), whirligig beetles (Gyrinidae), riffle bugs (*Rhagovelia* sp. (Veliidae)), and mayflies (Caenidae).

Similar aquatic macroinvertebrate diversity was found between the Usuthu River and the Phongolo River (Figure 3.2). Outside of the reserve, fewer taxa were found – although not significantly less – compared to sites within the conservation area (Usuthu River: $p > 0.05$ and Phongolo River inside: $p > 0.05$). The Phongolo River inside the NGR during 2016 had the highest number of taxa (38), Pielou’s Evenness Index (0.76), Margalef’s Species Richness (6.39), Shannon-Wiener Diversity Index (2.77), and Simpson’s Index (0.89) (Figure 3.2 a-f). The aquatic macroinvertebrate communities of the Phongolo River inside the NGR during the 2016 survey were statistically significantly higher in Margalef’s Species Richness ($p = 0.0003$) and Shannon Diversity ($p = 0.0255$), however, not in Pielou’s Evenness Index from the Usuthu River inside the reserve.

The abundance of sensitive families decreased in the Phongolo River from 2012 – 2017 (37% – 22%) as was found by de Necker (2019) (Figure 3.3b). The abundance of sensitive families from the Usuthu River (Figure 3.3c) was higher (36%) and similar to the sensitive families reported by Smit et al. (2016) (Figure 3.3a). In the NGR, the two rivers had different tolerant families (Figure 3.3b and Figure 3.3c). A much higher percentage of tolerant families (78%) was found in the Phongolo River while the Usuthu River had 64% tolerant families.

3.3.1.1.2. Floodplain lakes

A total of 24 taxa from 17 families were found in Lake Shokwe during the LF survey, while 52 taxa from 30 families were found during the HF survey of 2018 (Appendix B3). In the associated floodplain lakes of the Phongolo River, a total of 34 different taxa from 18 families were found in Fl 1 and 29 taxa from 18 families in Fl 4 (Appendix B3). The most abundant taxon during the LF survey in Lake Shokwe was the water boatman (*Micronecta* sp. (362 individuals) (Corixidae)) and during the HF survey, Baetidae (151 individuals), and the water boatman (*Agraptocorixa* sp. (214 individuals) and *Micronecta* sp. (482 individuals) (Corixidae)) were the most abundant taxa. The most abundant taxa in Fl 1 were the backswimmers (*Anisops* sp. A (69 individuals)), giant water bugs (*Appasus* sp. (18 individuals)), small minnow mayflies (*Cloeon* & *Procloeon* (18 individuals)), midges (*Orthocladiinae* (68 individuals) and *Tanypodinae* sp. (96 individuals)), aquatic earthworms (Oligochaeta (35 individuals)), and *Agraptocorixa* sp. A (16 individuals) while in Fl 4 the most abundant were *Anisops* sp. A (101 individuals), *Appasus* sp. (29 individuals), bubble snails (*B. forsakaii* (27 individuals)), *Cloeon* & *Procloeon* (39 individuals), and *Tanypodinae* sp. (45 individuals). Similar taxa were shared between the Usuthu River floodplain lake and the Phongolo River floodplain lakes including *Anisops* sp. A, water scavenger beetles (*Berosus* sp.) and Oligochaeta while certain taxa such as *Micronecta* sp. and the diving beetles (*Hydaticus* sp.) were only found in Lake Shokwe.

A total of 30 different taxa from 19 families with a total of 335 individuals were recorded in Lake Nyamithi during 2017 compared to 37 different taxa from 26 families with a total of 2467 individuals in 2018 (Appendix B3). In 2017, the most abundant taxa within the community were *Berosus* sp. A (33 individuals), *C. nilotica* (34 individuals), dragonflies (*B. leucosticta* (27 individuals)), diving beetles (*Hyphydrus* sp. A (34 individuals)) and the seed shrimp (Ostracoda (46 individuals)). The most abundant taxa contributing to the community structure in 2018 were Ostracoda (565 individuals), *Tanypodinae* sp. (259 individuals), *Micronecta* sp. (869 individuals), biting midges (*Bezzia* sp. (164 individuals)) and *Anisops* sp. A (148 individuals). The 2017 survey had a greater number of taxa, Pielou’s Evenness Index, Margalef’s Species Richness, Shannon-Wiener Diversity Index, and Simpson’s Index than the 2018 survey (Figure 3.4 a–f).

There was a difference in taxa of the Usuthu River and its associated floodplain lake during the LF survey, although not significant, and a significant seasonal difference between LF and HF survey in the associated floodplain lake (Figure 3.2a). Shokwe HF had a significantly higher number of taxa (52) (p = 0.0124), Pielou’s Evenness Index (0.63) (p = 0.0091), Margalef’s Species Richness (7.07) (p < 0.0001), Shannon-Wiener Diversity Index (2.51) (p =

0.0005) and Simpson's Index (0.83) (p = 0.0008) to the LF survey while similar to the Usuthu River LF survey. Shokwe LF had the least number of taxa, Pielou's Evenness Index (0.31), Margalef's Species Richness (2.17), Shannon-Wiener Diversity Index (0.77), and Simpson's Index (0.29) (Figure 3.2 a–f). The diversity indices of both Phongolo River floodplain lakes were significantly different from the Usuthu River floodplain lake (Figure 3.2 a–f). Floodplain lakes 1 and 4 were very similar in all indices and were significantly higher than Lake Shokwe indicating a higher diversity of aquatic macroinvertebrate taxa.

3.3.1.2. Multivariate analyses

Multivariate analyses indicated differences in taxa (only the 30 best fitting) between the various sites sampled (Figure 3.5 - 3.7 – total variation of the PCA = 85.01%). The Usuthu River-associated floodplain lake separated from the Phongolo River-associated floodplain lakes along the first axis. Additionally, Lake Nyamithi during the 2018 survey separated from the 2017 survey along the first axis (explaining 47.2% of the data variation) (Figure 3.5). Lake Nyamithi during the 2018 survey separated from the 2017 survey due to the greater diversity of taxa such as the backswimmers (*Anisops* sp. and *Enithares* sp.), *Micronecta* sp., water treaders (*Mesovelia* sp.), Oligochaeta, dragonflies (*Pantala flavescens*), and *Bezzia* sp. Along the 2nd axis (explaining 16% of the total variation in the data), the Usuthu River inside the reserve separated from the Phongolo River and was due to a greater abundance of *Baetidae* sp., caddisflies (*Hydropsyche* sp.), mayflies (*Afrocaenis* sp.), *Caenis* sp., flathead mayflies (*Afronurus* sp.), prong gill mayflies (*Euthralus* sp.), *Rhagovelia* sp., horse flies (*Tabanidae* larvae), blackflies (*Simuliidae* larvae), giant water bugs (*Limnogeton fieberi*), demoiselles (*Phaon iridipennis*), and common fish eating spider (*Nilus margartatus*).

The third axis (Figure 3.6) of the PCA indicated differences between the Usuthu River and the Phongolo River (explaining 12.2% of the data variation). Along this axis, the two largest lakes within the NGR separated from each other during similar surveys and hydrological periods. This separation was due to higher abundances of macroinvertebrate taxa such as the creeping water bug (*Macrocoris* sp.), *Bezzia* sp., Ceratopogonidae, Ostracods, *Baetis* sp., Melania snails (*Melanoides tuberculate*), *Limnogonus* sp., pond skaters (*Naboandelus africanus*), and *Hyphydrus* sp. The biplot representing PC axes 1 and 4 indicated flow-related differences in the Usuthu River-associated floodplain lake (Figure 3.7). This variation is explained by a total of 9.6%. The separation was a result of greater diversity during the high flow survey of Lake Shokwe. The taxa responsible for this separation were Baetidae sp. A and B, *Berosus* sp. A, Oligochaeta., *Mesovelia* sp., *Enithares* sp., *P. flavescens* and *Tanypodinae*. Interestingly, the Phongolo River separated even further along the fourth axis due to higher abundances of taxa such as *P. acuta*, aquatic moths (*Crambidae* sp. A.), common pond snail (*Lymnaea natalensis*), *Lumbriculidae* sp., damselflies (*Pseudagrion* sp. A), worms (*Naididae* sp.) and *Bradinopyga cornuta* while higher abundances of the taxon *Appasus* sp. were associated with the Usuthu River. This indicated different macroinvertebrate community structures between the various aquatic ecosystems.

Percentage similarity analysis using SIMPER indicated that between both rivers inside the reserve, there was an 89.9% dissimilarity in macroinvertebrate community structure (Table 3.2). The taxa responsible for the majority of these dissimilarities were Baetidae, *Rhagovelia* sp., whirligig beetles (*Aulonogyrus* sp.), Chironominae larvae, *Caenis* sp., *C. nilotica*, and *Thermocyclops* sp. The Usuthu River and the Phongolo River outside the reserve also had a high (79.13%) dissimilarity and the taxa contributing to the dissimilarities were Baetidae, *Rhagovelia* sp., *Aulonogyrus* sp., *Caenis* sp., *Pseudagrion* sp. A, *Afrocaenis* sp. and *Afronurus* sp.

The SIMPER analysis showed that the Usuthu River and Lake Shokwe from the same hydrological period were 85.37% dissimilar in macroinvertebrate taxa (Table 3.3). As such, the taxa contributing to the dissimilarity were *Micronecta* sp., Baetidae, *Rhagovelia* sp., *Aulonogyrus* sp., *C. nilotica*, *Caenis* sp. and *Afrocaenis* sp. while, the SIMPER analysis showed that in Lake Shokwe there was a 64.4% dissimilarity in macroinvertebrate diversity and abundances between hydrological periods. The taxa that were responsible for the majority of these dissimilarities were *Agraptocorixa* sp., Baetidae sp. A, *Enithares* sp., *Tanypodinae* sp., *Mesovelia* sp., Baetidae sp. B and water mites (*Neumania* sp.).

Results from SIMPER analysis indicated that the macroinvertebrate composition of Lake Shokwe and the Phongolo River floodplain lakes (Fl 1 (89.25%) & 4 (94.35)) were very different (Table 3.4). The taxa that were responsible for dissimilarities between Shokwe and Phongolo River Fl 1 were *Micronecta* sp., *Tanypodinae* sp., *Anisops* sp. A, *Orthocladiinae* sp., *Appasus* sp., and *Cloeon* & *Procloeon* while taxa such as *Micronecta* sp., *Anisops* sp. A, *Tanypodinae* sp., *Cloeon* & *Procloeon*, burrowing water beetles (*Neohydrocoptus* sp.), *Appasus* sp., *Bulinus forskaii*, and pond skaters (*Neogerris* sp.) were responsible for the dissimilarity between Lake Shokwe and Phongolo River Fl 4.

The SIMPER analysis indicated dissimilarity between the Usuthu River and Lake Nyamithi with an 86.69% dissimilarity (Table 3.5). The taxa responsible for the majority of the dissimilarities were *Micronecta* sp., *Ostracoda* sp., *Baetidae* sp., *Tanypodinae* sp., *Rhagovelia* sp., *Bezzia* sp. and *Aulonogyrus* sp. The analysis also indicated sampling year differences in Lake Nyamithi, as 2017 was 84.04% dissimilar to 2018. Taxa that contributed the most to this dissimilarity were *Micronecta* sp., *Ostracoda* sp., *Tanypodinae* sp., *Anisops* sp., *Bezzia* sp., and Oligochaeta.

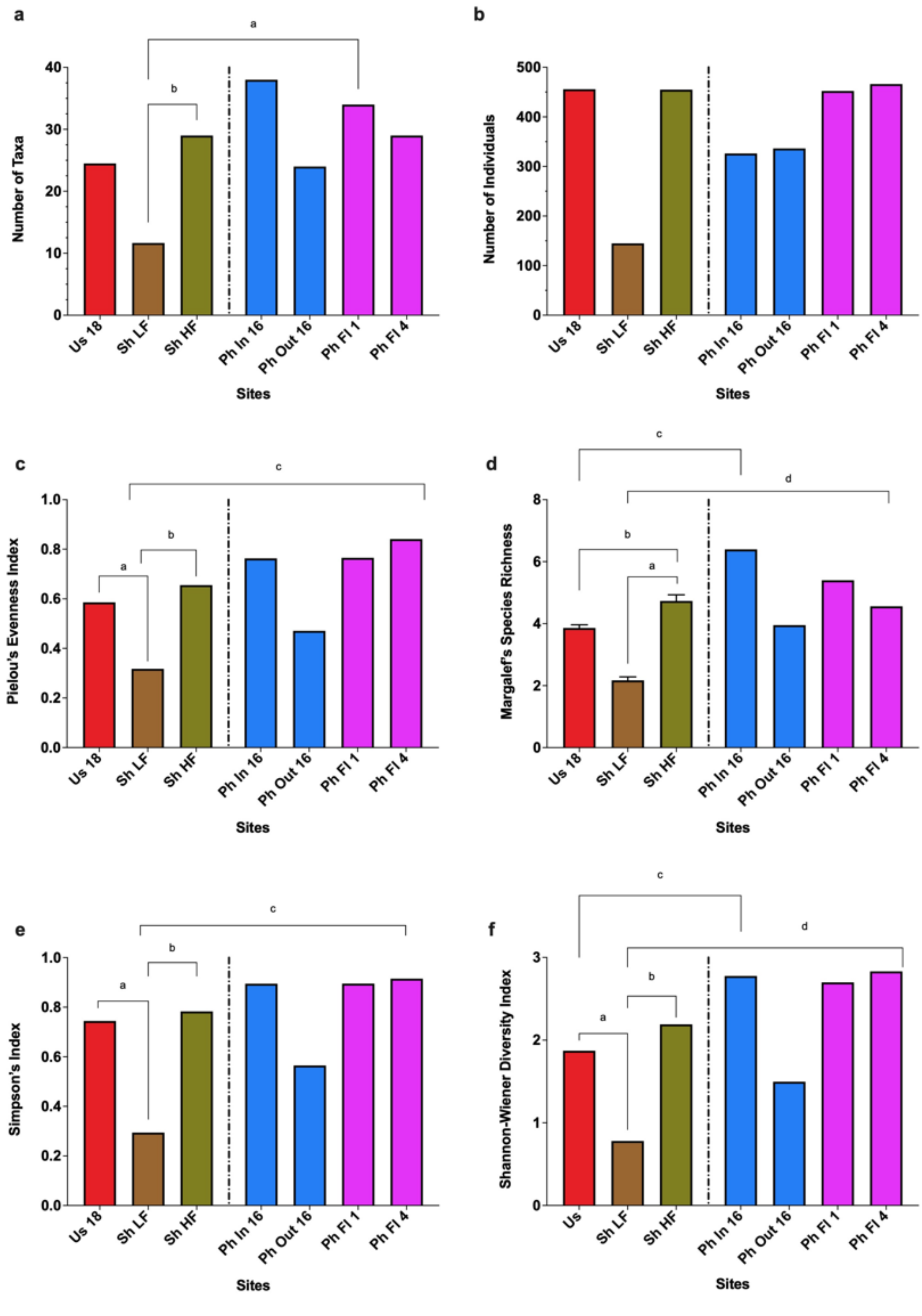


Figure 3.2: Comparison between (a) number of taxa, (b) number of individuals, (c) Pielou's Evenness Index, (d) Margalef's Species Richness, (e) Shannon-Wiener Diversity Index and (f) Simpson's Index of aquatic macroinvertebrates collected in the Usuthu River and its associated floodplain lake within Phongolo River Floodplain. Data of the Phongolo River, in- and outside of Ndumo Game Reserve, during 2016 was used from de Necker (2019) and its associated floodplain lakes during 2014 was used from Dube *et al*., (2019). Superscripts indicate significant differences between sites ($p < 0.05$).

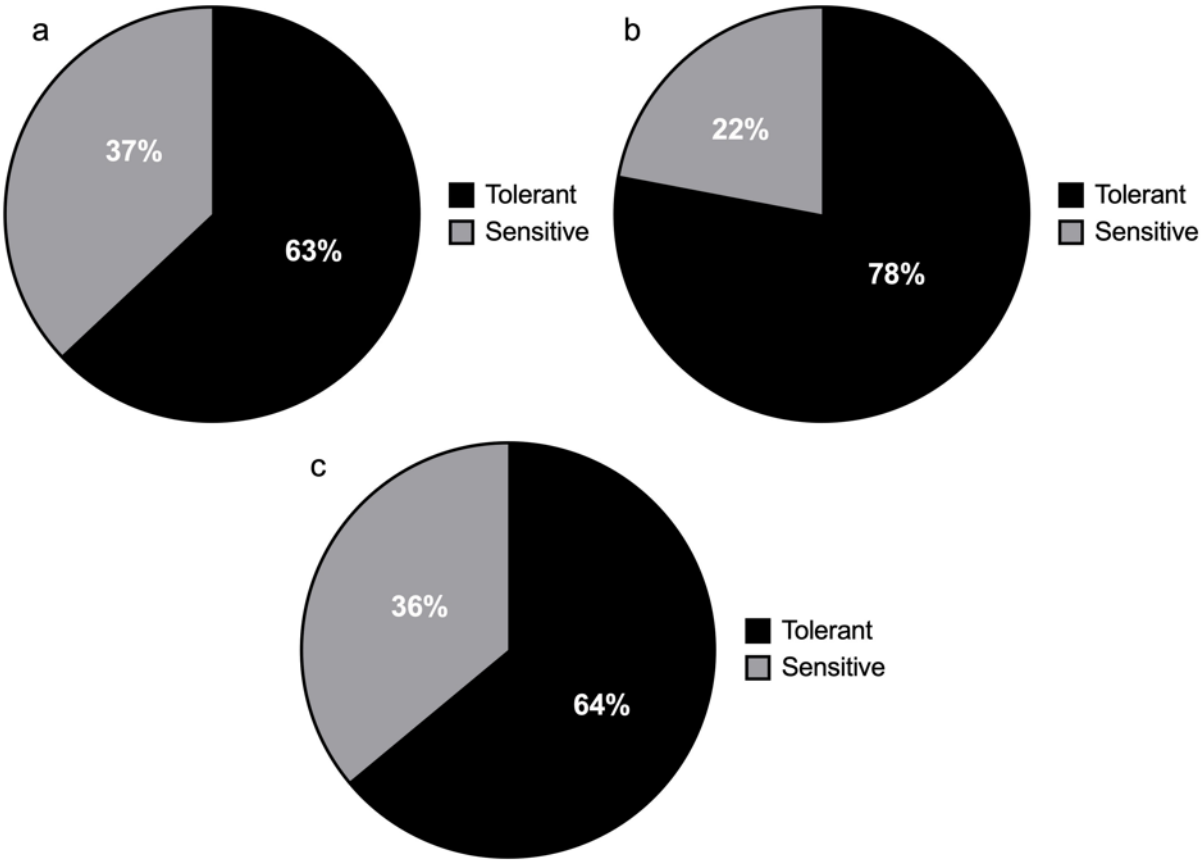


Figure 3.3: Pie charts indicating percentage sensitive and tolerant aquatic macroinvertebrate family scores of different surveys on the Phongolo River during 2012-2013 (a) collected by Smit *et al*., 2016 (b) collected by de Necker, 2019 and the present study on the Usuthu River (c).

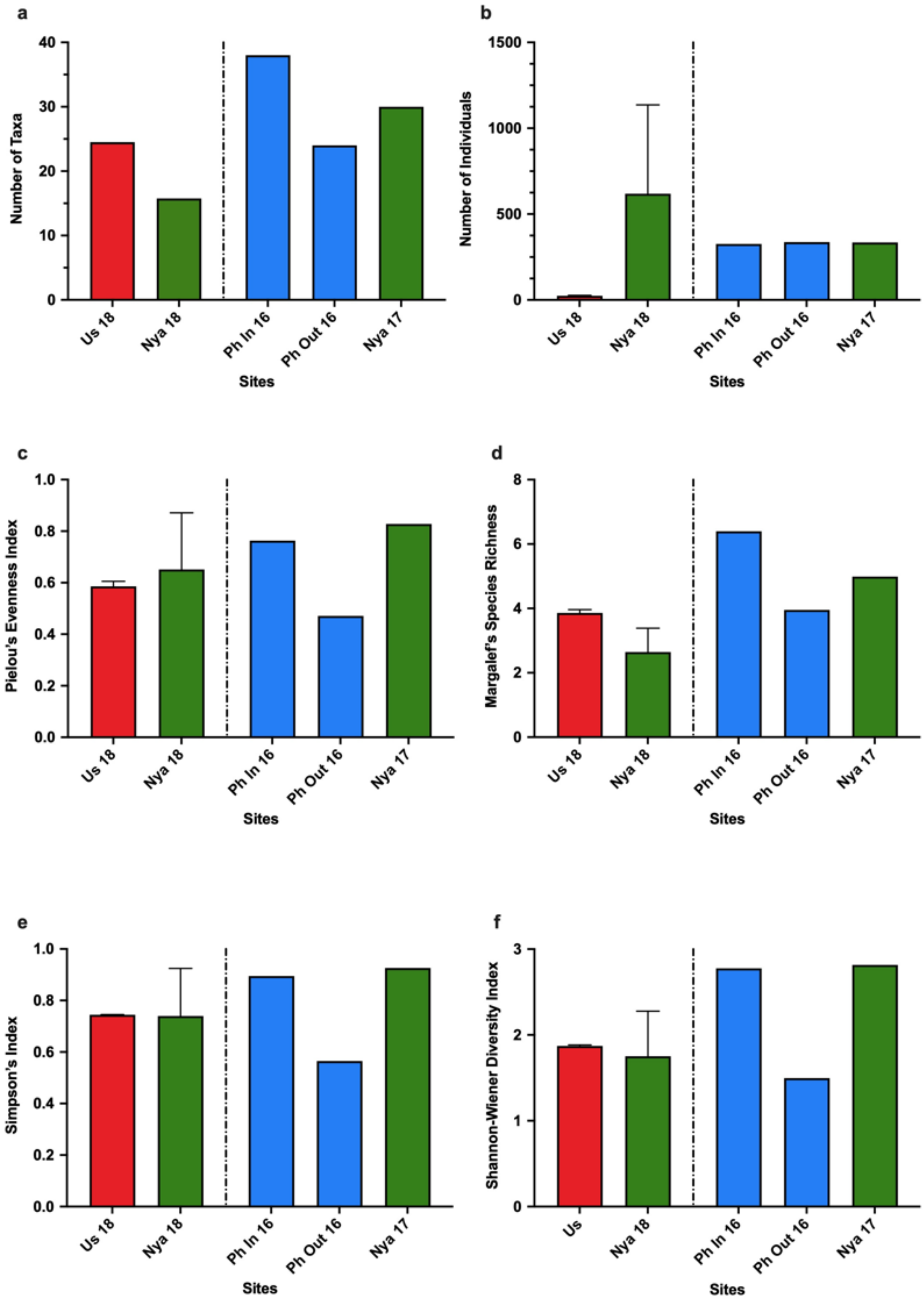


Figure 3.4: Comparison between (a) number of taxa, (b) number of individuals, (c) Pielou's Evenness Index, (d) Margalef's Species Richness, (e) Shannon-Wiener Diversity Index and (f) Simpson's Index of aquatic macroinvertebrates collected in the Usuthu and Phongolo rivers and Lake Nyamithi. Data of the Phongolo River, in- and outside of Ndumo Game Reserve and Lake Nyamithi during 2016 and 2017 was used from de Necker (2019) and de Necker *et al*. (2021).

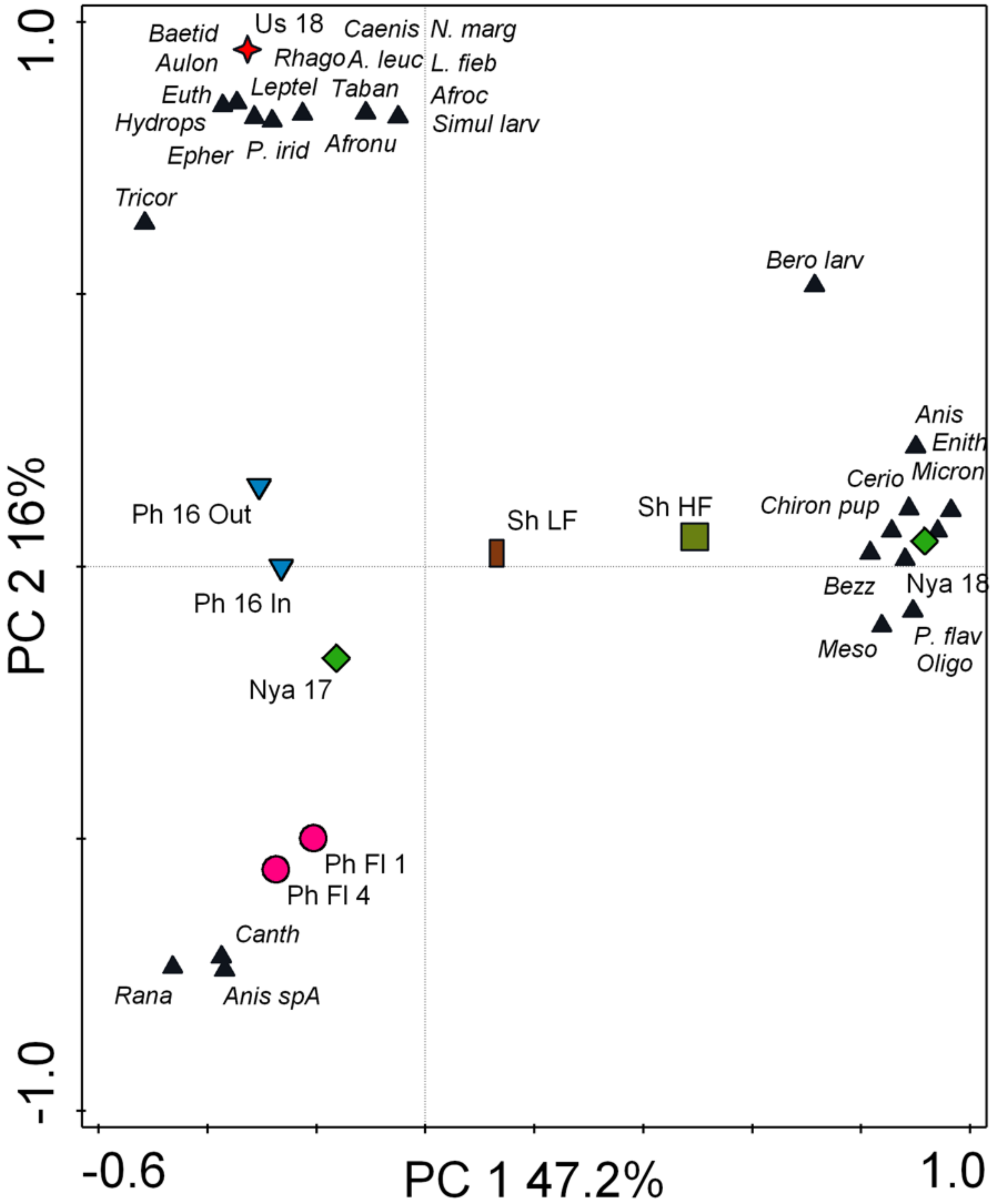


Figure 3.5: Principal Component Analysis of the 30 best fitting aquatic macroinvertebrate taxa (blue arrows) collected from the various sites (Us= red star; Sh LF= brown box; Sh HF= green square; Ph Outside= blue triangle; Ph Inside= blue triangle; Nya= green diamond; Ph floodplain lake= pink circle) inside and outside the Ndumo Game Reserve. The biplot explains 63.2% of the total variation where axis 1 explains 47.2% and axis 2 explains 16% of the variation. Full list of abbreviated macroinvertebrate names is presented in Appendix B2.

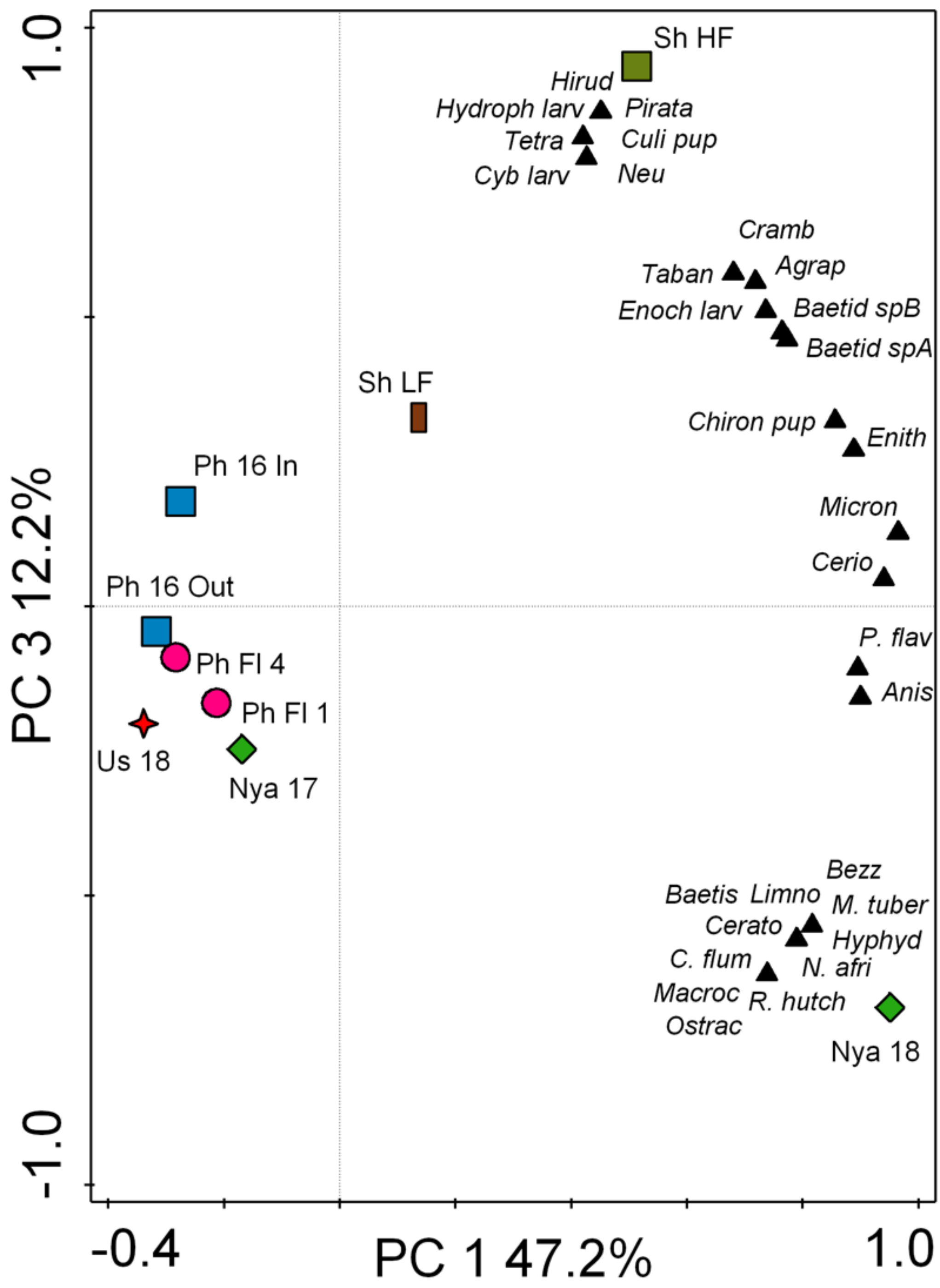


Figure 3.6: Principal Component Analysis of the 30 best fitting aquatic macroinvertebrate taxa (blue arrows) collected from the various sites (Us= red star; Sh LF= brown box; Sh HF= green square; Ph out= orange triangle; Ph Inside= blue triangle; Nya= green diamond; Ph floodplain lake= pink circle) inside and outside the Ndumo Game Reserve. The biplot explains 59.4% of the total variation where axis 1 explains 47.2% and axis 3 explains 12.2% of the variation. Full list of abbreviated macroinvertebrate names is presented in Appendix B2.

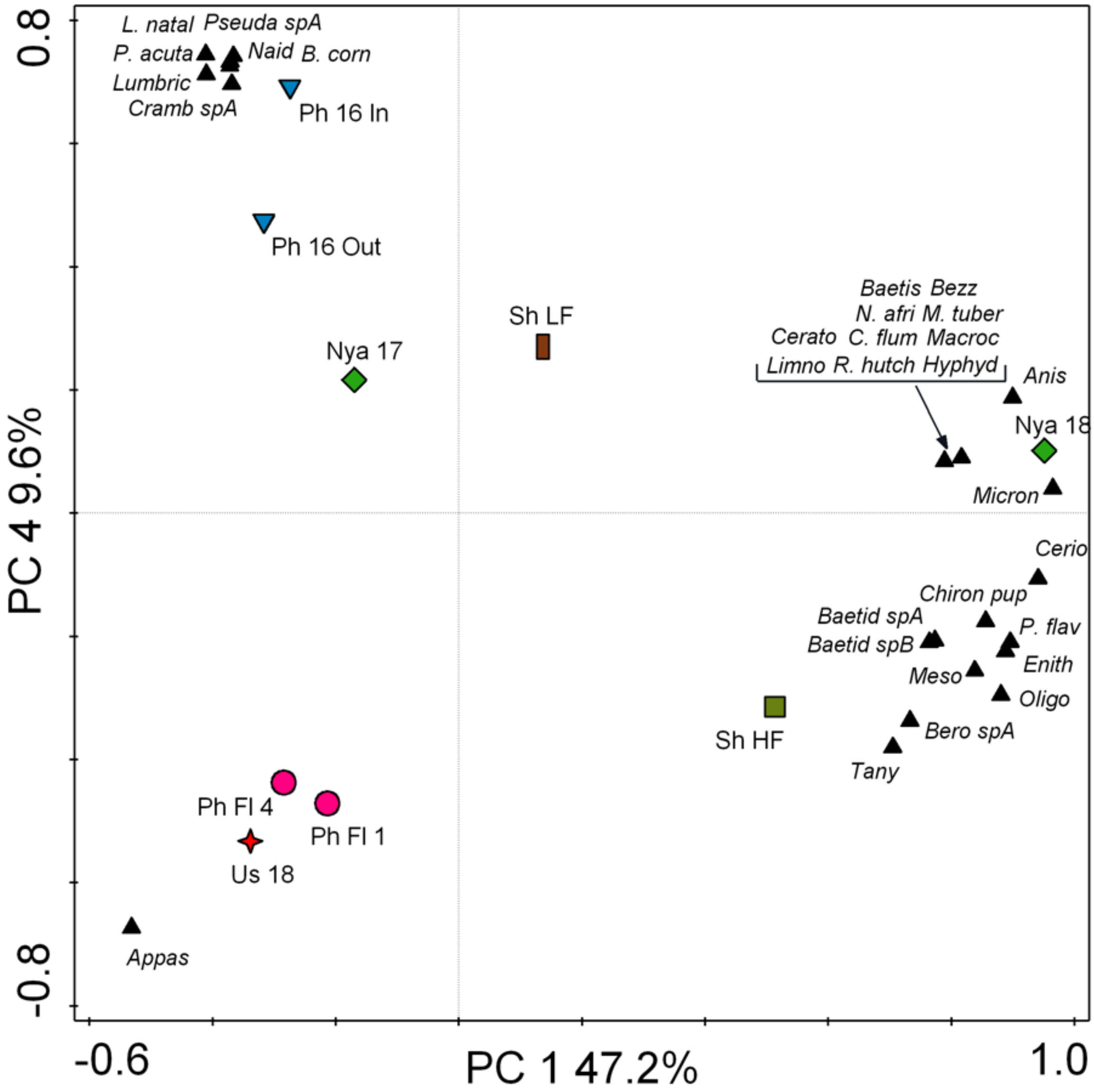


Figure 3.7: Principal Component Analysis of the 30 best fitting aquatic macroinvertebrate taxa (blue arrows) collected from the various sites (Us= red star; Sh LF= brown box; Sh HF= green square; Ph out= orange triangle; Ph Inside= blue triangle; Nya= green diamond; Ph floodplain lake= pink circle) inside and outside the Ndumo Game Reserve. The biplot explains 56.8% of the total variation where axis 1 explains 47.2% and axis 4 explains 9.6% of the variation. Full list of abbreviated macroinvertebrate names is presented in Appendix B2.

Table 3.2: Percentage contribution of the various aquatic macroinvertebrate taxa to between river group dissimilarities calculated by Similarity of percentage analysis (SIMPER). Rivers are in three groups (Usuthu, Phongolo Inside and Outside) and were each determined by nMDS and dendogram analysis.

Site	Overall dissimilarity	Species	Average Dissimilarity	Percentage contribution (%)	Cumulative contribution (%)
Usuthu River vs Phongolo River Inside	**89.89%**	Baetidae	7.76	8.63	8.63
		Rhagovelia sp.	6.31	7.02	15.65
		Aulonogyrus sp.	4.64	5.16	20.81
		Chironominae larvae	4.47	4.98	25.79
		Caenis sp.	4.11	4.57	30.36
		Caridina nilotica	3.54	3.94	34.3
		Thermocyclops sp.	3.37	3.75	38.04
		Afrocaenis sp.	2.73	3.04	41.09
		Chydoridae	2.55	2.84	43.93
		Afronurus sp.	2.41	2.68	46.6
		Appasus sp.	2.2	2.44	40.05
		Pseudagrion sp A	2.03	2.25	51.3
		Tetrathemis polleni	2.03	2.25	53.55
		Culex sp A	1.7	1.89	55.44
		Physa acuta	1.63	1.81	57.26
		Simocephalus serrulatus	1.63	1.81	59.07
		Corbicula sp.	1.55	1.73	60.8
		Nilus margartatus	1.55	1.73	62.52
		Laccocoris sp.	1.39	1.55	64.07
		Allochemis leucosticta	1.3	1.45	65.52
		Cyprididae	1.3	1.45	66.96
		Darwinulidae	1.3	1.45	68.41
		Hydropsyche sp.	1.3	1.45	69.85
		Laccobius	1.3	1.45	71.3
Usuthu River vs Phongolo River Outside	**79.13%**	Baetidae	8.41	10.63	10.63
		Rhagovelia sp.	7.42	9.38	20.02
		Aulonogyrus sp.	6.28	7.93	27.95
		Caenis sp.	4.84	6.11	34.06
		Pseudagrion sp A	3.66	4.62	38.68
		Afrocaenis sp.	3.22	4.07	42.75
		Afronurus sp.	2.83	3.58	46.33
		Appasus sp.	2.58	3.27	49.59
		Caridina nilotica	2.34	2.96	52.55
		Tarebia granifera	2.24	2.83	55.38
		Physa acuta	2.08	2.63	58.02
		Nilus margartatus	1.83	2.31	60.33
		Hydroptila sp.	1.63	2.07	62.39
		Allochemis leucosticta	1.53	1.93	64.33
		Microgomphus mozambicensis	1.42	1.79	66.11
		Oligochaeta sp.	1.29	1.63	67.75
		Anax sp.	1.16	1.46	69.21
		Paragomphus sp.	1.16	1.46	70.67

Table 3.3: Percentage dissimilarities contribution of the various aquatic macroinvertebrate taxa of the Usuthu River and its associated floodplain lake calculated by Similarity of percentage analysis (SIMPER). Groups are based on sites within the Phongolo River Floodplain (Usuthu River, Lake Shokwe LF and Lake Shokwe HF) and were determined by using nMDS and dendogram analysis.

Site	Overall dissimilarity	Species	Average Dissimilarity	Percentage contribution (%)	Cumulative contribution (%)
Usuthu River vs Lake Shokwe LF	85.37%	*Micronecta* sp.	10.22	11.97	11.97
		Baetidae	10.17	11.91	23.88
		Rhagovelia sp.	7.59	8.89	32.77
		Aulonogyrus sp.	6.42	7.52	40.29
		Caridina nilotica	6.31	7.39	47.68
		Caenis sp.	4.94	5.79	53.47
		Afrocaenis sp.	3.29	3.85	57.33
		Afronurus sp.	2.89	3.39	60.72
		Appasus sp.	2.64	3.1	63.81
		Laccocoris sp.	1.67	1.96	65.77
		Pirata sp.	1.67	1.96	67.73
		Allochemis leucosticta	1.56	1.83	69.56
		Hydropsyche sp.	1.56	1.83	71.39
Lake Shokwe LF vs Lake Shokwe HF	64.39	*Agraptocorixa* sp.	6.18	9.6	9.6
		Baetidae sp. A	4.72	7.34	16.94
		Enithares sp.	3.56	5.54	22.47
		Tanypodinae sp.	2.96	4.59	27.07
		Mesovelia sp.	2.35	3.65	30.72
		Baetidae sp. B	2.15	3.35	34.07
		Neumania sp.	2.07	3.22	37.28
		Pseudagrion sp. A	1.94	3.01	40.29
		Tetragnatha sp.	1.74	2.71	43
		Oligochaeta sp.	1.64	2.54	45.54
		Tipula sp.	1.46	2.27	47.81
		Hydrophilus sp. larvae	1.34	2.08	49.89
		Crambidae sp.	1.27	1.97	51.86
		Tabanus sp. B	1.27	1.97	53.83
		Micronecta sp.	1.24	1.92	55.75
		Berosus sp. A	1.23	1.92	57.67
		Anax sp.	1.2	1.86	59.52
		Neogerris sp.	1.2	1.86	61.38
		Clogmia albopunctata	1.12	1.74	63.12
		Anopheles sp.	1.04	1.61	64.72
		Laccobius sp.	1.04	1.61	66.33
		Neomacrocoris sp.	0.94	1.47	67.8
		Anaciaeschna sp.	0.85	1.31	69.11
		Anax imperator	0.85	1.31	70.42

Table 3.4: Percentage dissimilarities contribution of the various aquatic macroinvertebrate taxa between a Usuthu River associated floodplain lake and two Phongolo River associated floodplain lakes calculated by Similarity of percentage analysis. Groups are based on associated floodplain lakes (Lake Shokwe and Ph Fl 1 & 4) and were determined using nMDS and dendogram analysis.

Site	Overall dissimilarity	Species	Average Dissimilarities	Percentage contribution %	Cumulative contribution %
Lake Shokwe vs Ph Fl 1	89.25%	*Micronecta* sp.	12.39	13.88	13.88
		Tanypodinae sp.	6.38	7.15	21.03
		Anisops sp. A	5.41	6.06	27.09
		Orthocladiinae	5.37	6.02	33.1
		Appasus sp.	2.76	3.1	36.2
		Cloeon & *Procloeon*	2.76	3.1	39.29
		Agraptocorixa sp. A	2.6	2.92	42.21
		Anisops sp.	2.44	2.73	44.94
		Lethocerus niloticus	2.44	2.73	47.67
		Caridina nilotica	2.26	2.53	50.2
		Allocotocerus sp.	1.95	2.19	52.39
		Bulinus tropicus	1.84	2.06	54.45
		Hydrophilus sp.	1.84	2.06	56.51
		Pirata sp.	1.84	2.06	58.58
		Anaciaeschna sp.	1.72	1.93	60.51
		Enallagma sp.	1.72	1.93	62.44
		Chironominae sp.	1.59	1.79	64.22
		Hyphydrus sp. A	1.59	1.79	66.01
		Laccophilus sp.	1.59	1.79	67.8
		Oligochaeta sp.	1.5	1.69	69.48
		Hydrachnellae	1.46	1.63	71.11
Lake Shokwe vs Ph Fl 4	94.35%	*Micronecta* sp.	12.28	13.01	13.01
		Anisops sp. A	6.49	6.87	19.89
		Tanypodinae sp.	4.33	4.59	24.47
		Cloeon & *Procloeon*	4.03	4.27	28.75
		Neohydrocoptus	3.59	3.81	32.55
		Appasus sp.	3.48	3.68	36.24
		Bulinus forskaii	3.35	3.55	39.79
		Neogerris sp.	3.03	3.21	43
		Hydrophilus sp.	2.81	2.98	45.98
		Laccophilus sp.	2.81	2.98	48.96
		Bulinus tropicus	2.66	2.82	51.78
		Hyphydrus sp. A	2.58	2.74	54.52
		Anax sp.	2.41	2.56	57.08
		Anisops sp.	2.41	2.56	59.63
		Oligochaeta sp.	2.33	2.47	62.1
		Regimbartia sp.	2.04	2.16	64.26
		Hydrachnellae	1.94	2.05	66.32
		Lethocerus niloticus	1.83	1.93	68.25
		Pirata sp.	1.83	1.93	70.18

Table 3.5: Percentage contribution of the various aquatic macroinvertebrate taxa to within Lake Nyamithi of different survey group dissimilarity calculated by Similarity of percentage analysis. Groups are based on different surveys of Lake Nyamithi (2017 & 2018) and the Usuthu River and were determined using nMDS and dendogram analysis.

Site	Overall dissimilarity	Species	Average Dissimilarities	Percentage contribution %	Cumulative contribution %
Usuthu River vs Lake Nyamithi 18	**86.69%**	*Micronecta* sp.	9.12	10.52	10.52
		Ostracoda sp.	7.82	9.02	19.54
		Baetidae	5.66	6.53	26.07
		Tanypodinae sp.	5.33	6.15	32.22
		Rhagovelia sp.	4.22	4.87	37.09
		Bezzia sp.	4.21	4.86	41.95
		Aulonogyrus sp.	3.57	4.12	46.07
		Anisops sp.	3.34	3.86	49.93
		Caridina nilotica	2.85	3.29	53.22
		Oligochaeta	2.85	3.29	56.51
		Enithares sp.	2.15	2.48	58.99
		Agraptocorixa sp.	2.05	2.37	61.36
		Caenis sp.	1.95	2.24	63.6
		Berosus sp. A	1.86	2.15	65.75
		Afrocaenis sp.	1.83	2.11	67.86
		Baetidae sp. A	1.83	2.11	69.97
		Rhagadotarsus hutchinsonii	1.83	2.11	72.09
Lake Nyamithi 17 vs Lake Nyamithi 18	**84.04%**	*Micronecta* sp.	9.62	11.44	11.44
		Ostracoda sp.	6.21	7.39	18.83
		Tanypodinae sp.	5.93	7.05	25.88
		Anisops sp.	4.45	5.29	31.17
		Bezzia sp.	4.05	4.81	35.98
		Oligochaeta sp.	3.99	4.74	40.73
		Enithares sp.	2.76	3.28	44.01
		Agraptocorixa sp.	2.28	2.72	46.72
		Hyphydrus sp. A	2.13	2.54	49.26
		Baetidae sp. A	2.03	2.42	51.68
		Rhagadotarsus hutchinsonii	2.03	2.42	54.1
		Tarebia granifera	1.93	2.3	56.4
		Brachythemis leucosticta	1.9	2.26	58.66
		Nychia limpida	1.83	2.17	60.83
		Hyphydrus sp. A	1.71	2.04	62.87
		Anisops sp. A	1.67	1.99	64.87
		Chironominae sp.	1.63	1.94	66.81
		Appasus sp.	1.46	1.74	68.55
		Sigara sp.	1.42	1.68	70.23

3.3.2. Macroinvertebrate traits

Trait-based multivariate analyses indicated system (lentic vs lotic) related differences in macroinvertebrate trait composition (Appendix B4) (Figure 3.8 - 3.10 – total variation = 74.5%). Along the first and second axes (Figure 3.8), macroinvertebrate traits resulted in the separation between rivers and floodplain lakes, the separation between both rivers within the NGR and separation between Lake Shokwe and Lake Nyamithi (variation explained by axes 1 & 2 were 25.1% and 19.8%, respectively). Separation was caused by habitat and hydraulic preferences as well as functional feeding groups.

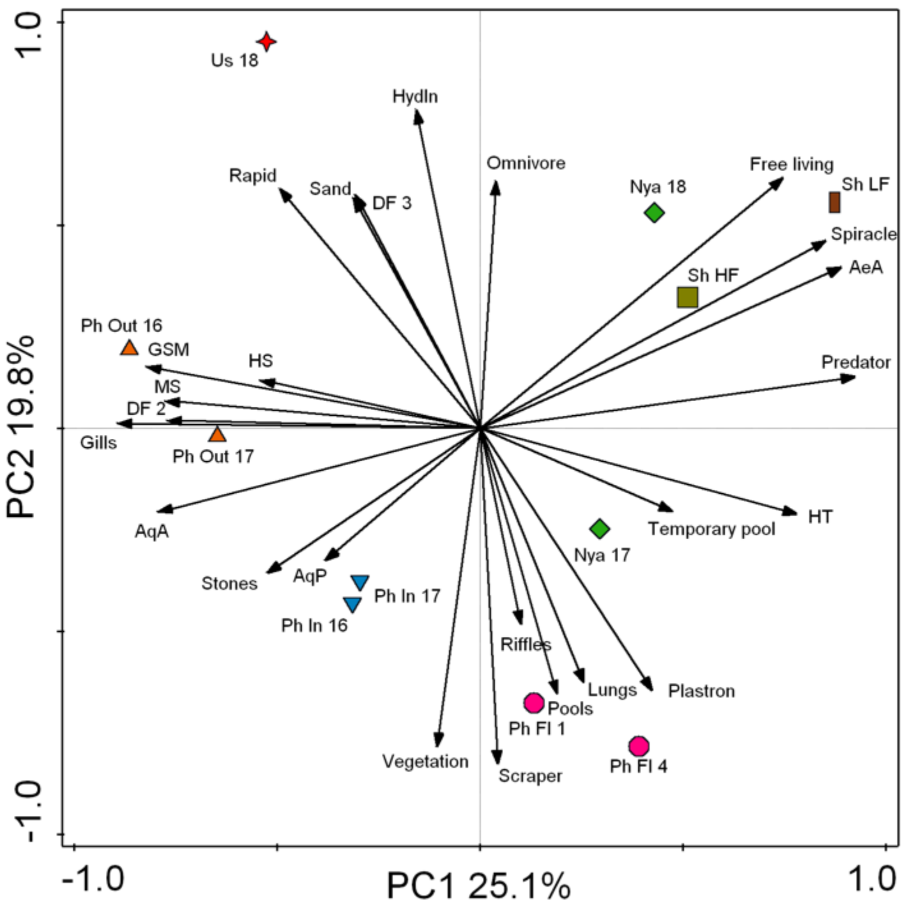


Figure 3.8: A Principal Component Analysis of the 25 best fitting macroinvertebrate traits (black arrows) associated with the various sites (Us= red star; Ph Inside= blue triangle; Ph Outside= orange triangle; Sh LF= brown box; Sh HF= green square; Nya= green diamond and Ph floodplain lakes= pink circle) inside and outside the Ndumo Game Reserve. The biplot explains 44.9% of the total variation in the data with axis 1 explaining 25.1% of the data and axis 2 explaining 19.8% of the variation. Full list of abbreviated macroinvertebrate trait names is presented in B1.

The third axis of the biplot (explained by 16.8% variation of the data) indicated flow-related differences between both rivers within the NGR (Figure 3.9). The Phongolo River September 2016 survey was separated from the February 2017 survey due to different hydrological flow periods. The Usuthu River during the November 2018 survey was similar to the Phongolo River during the same hydrological period. Along the third axis, Lake Shokwe separated from Lake Nyamithi and this separation was due to taxa that preferred different habitat conditions. As such, the Usuthu River floodplain lake was comprised of free-living taxa whereas the taxa in the Phongolo River floodplain lake preferred muddy habitat.

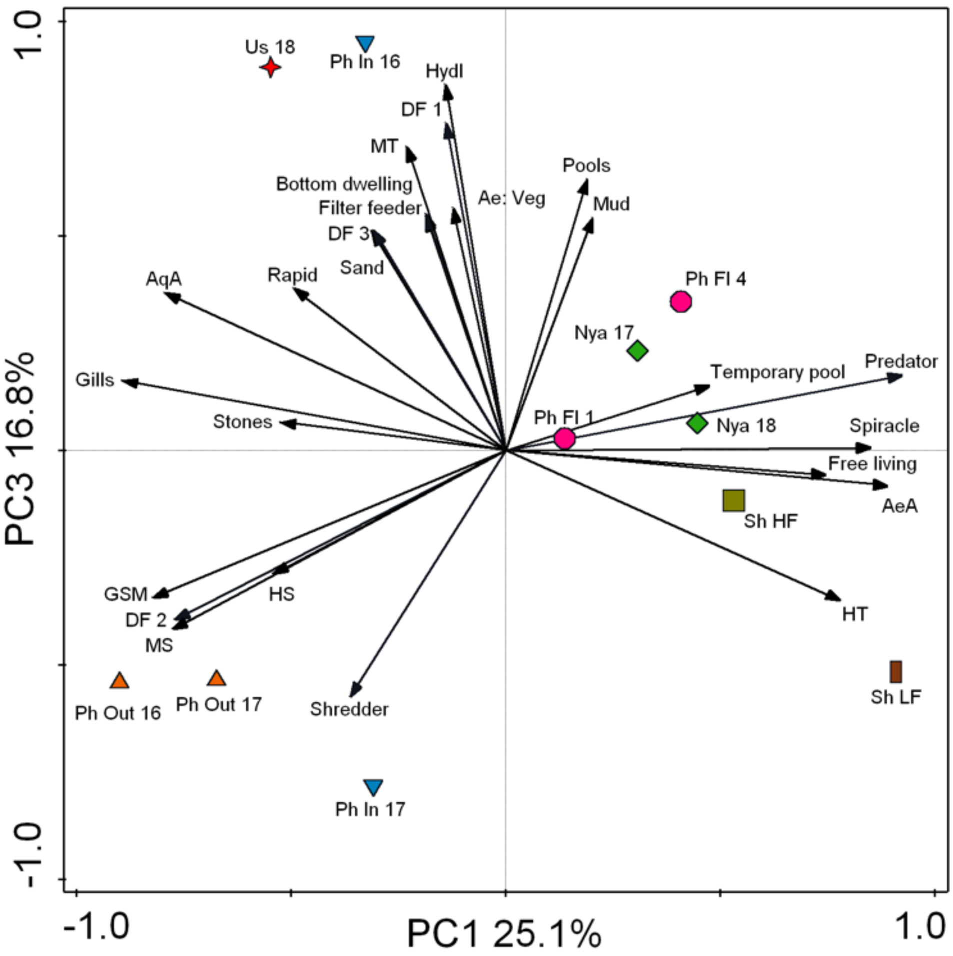


Figure 3.9: A Principal Component Analysis of the 25 best fitting macroinvertebrate traits (black arrows) associated with the various sites (Us= red star; Ph Inside= blue triangle; Ph Outside= orange triangle; Sh LF= brown box; Sh HF= green square; Nya= green diamond and Ph floodplain lakes= pink circle) inside and outside the Ndumo Game Reserve. The biplot explains 41.9% of the total variation in the data with axis 1 explaining 25.1% of the data and axis 3 explaining 16.8% of the variation. Full list of abbreviated macroinvertebrate trait names is presented in B1.

The fourth axis of the biplot (Figure 3.10) indicated spatial differences between both rivers within the NGR (explained 12.8% variation in the data). This separation is caused by habitat and hydraulic preferences, functional feeding groups, and respiration. The biplot indicated differences in the survey as Lake Nyamithi during the 2018 survey separated from the 2017 survey. This separation is due to taxa preferring different habitats and hydraulic conditions.

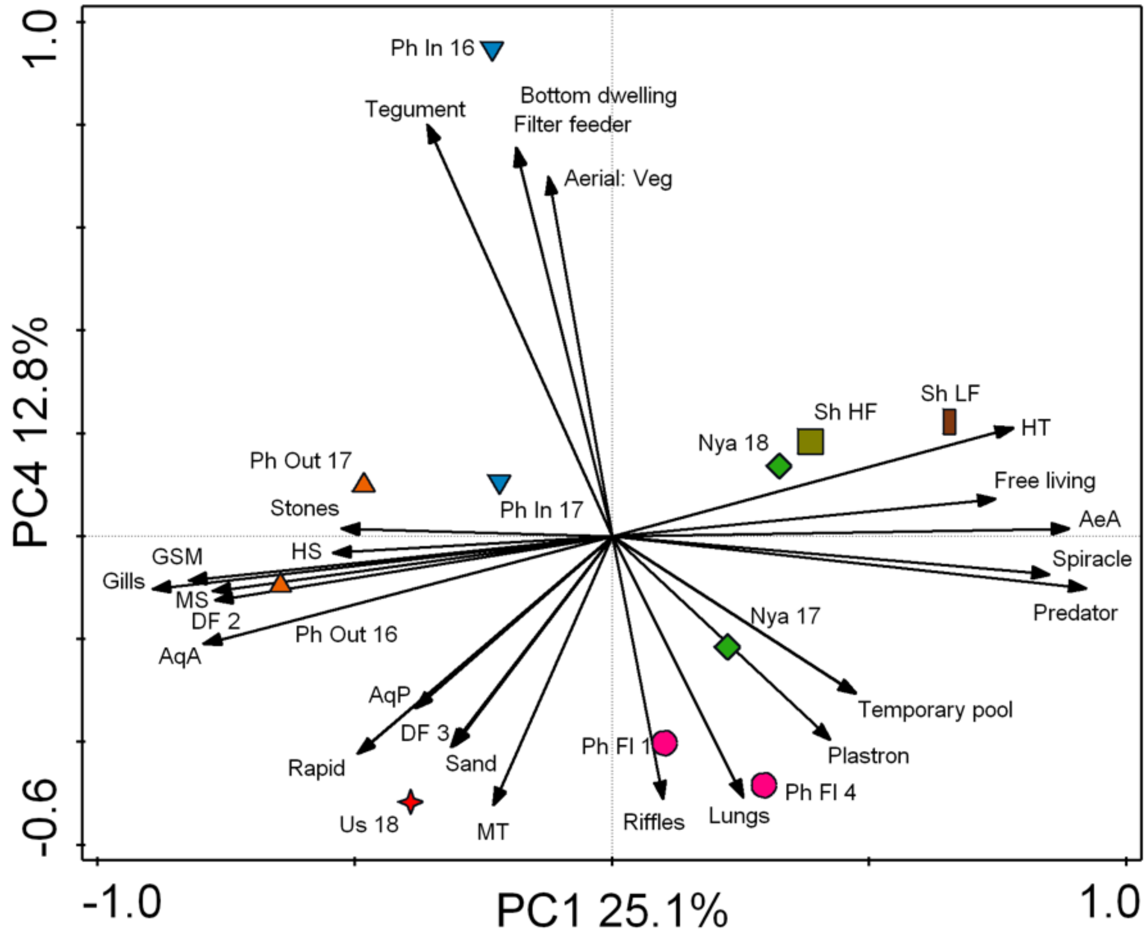


Figure 3.10: A Principal Component Analysis of the 25 best fitting macroinvertebrate traits (black arrows) associated with the various sites (Us= red star; Ph Inside= blue triangle; Ph Outside= orange triangle; Sh LF= brown box; Sh HF= green square; Nya= green diamond and Ph floodplain lakes= pink circle) inside and outside the Ndumo Game Reserve. The biplot explains 37.9% of the total variation in the data with axis 1 explaining 25.1% of the data and axis 4 explaining 12.8% of the variation. Full list of abbreviated macroinvertebrate trait names is presented in B1.

3.3.3. Environmental factors responsible for the structuring of the macroinvertebrate community composition

The CCA triplot indicated differences between the rivers and their associated floodplain lakes as well as differences between the lakes, the triplot explains 44.08% of the total variation (Figure 3.11). On the 1^{st} axis (23.68% of the variation), Lake Shokwe separated from the Phongolo River-associated floodplain lakes. The Phongolo River-associated floodplain lakes correlated with higher diversity while Lake Shokwe correlated with water nutrients. There is also a separation between the Usuthu River and Lake Shokwe along the 2^{nd} axis which is explained by 20.4% of the variation in the data. The HF survey of Lake Shokwe separated away from the LF survey as a result of higher macroinvertebrate diversity.

The CCA triplot indicated differences between the rivers and their associated floodplain lakes as well as differences between the lakes, the triplot explains 44.08% of the total variation (Figure 3.11). On the 1^{st} axis (23.68% of the variation), Lake Shokwe separated from the Phongolo River-associated floodplain lakes. The Phongolo River-associated floodplain lakes correlated with higher diversity while Lake Shokwe correlated with water nutrients. There is also a separation between the Usuthu River and Lake Shokwe along the 2^{nd} axis which is explained by 20.4% of the variation in the data. The HF survey of Lake Shokwe separated away from the LF survey as a result of higher macroinvertebrate diversity.

The variation partitioning analysis indicated that habitat preference had a distinct effect on the macroinvertebrate community composition (Figure 3.13). Although, the effect of habitat was not significant ($p > 0.05$), the amount of unique variation explained by habitat preferences to the community composition was 44%. Contrastingly, both water quality and system type had considerably less contribution with 1% and 2%, respectively. In addition, these two variables had a 12% combined effect, while habitat preference and system type only had a 2% effect. The effect of environmental variables and system type had a combined 8% effect, while 62% were unexplained.

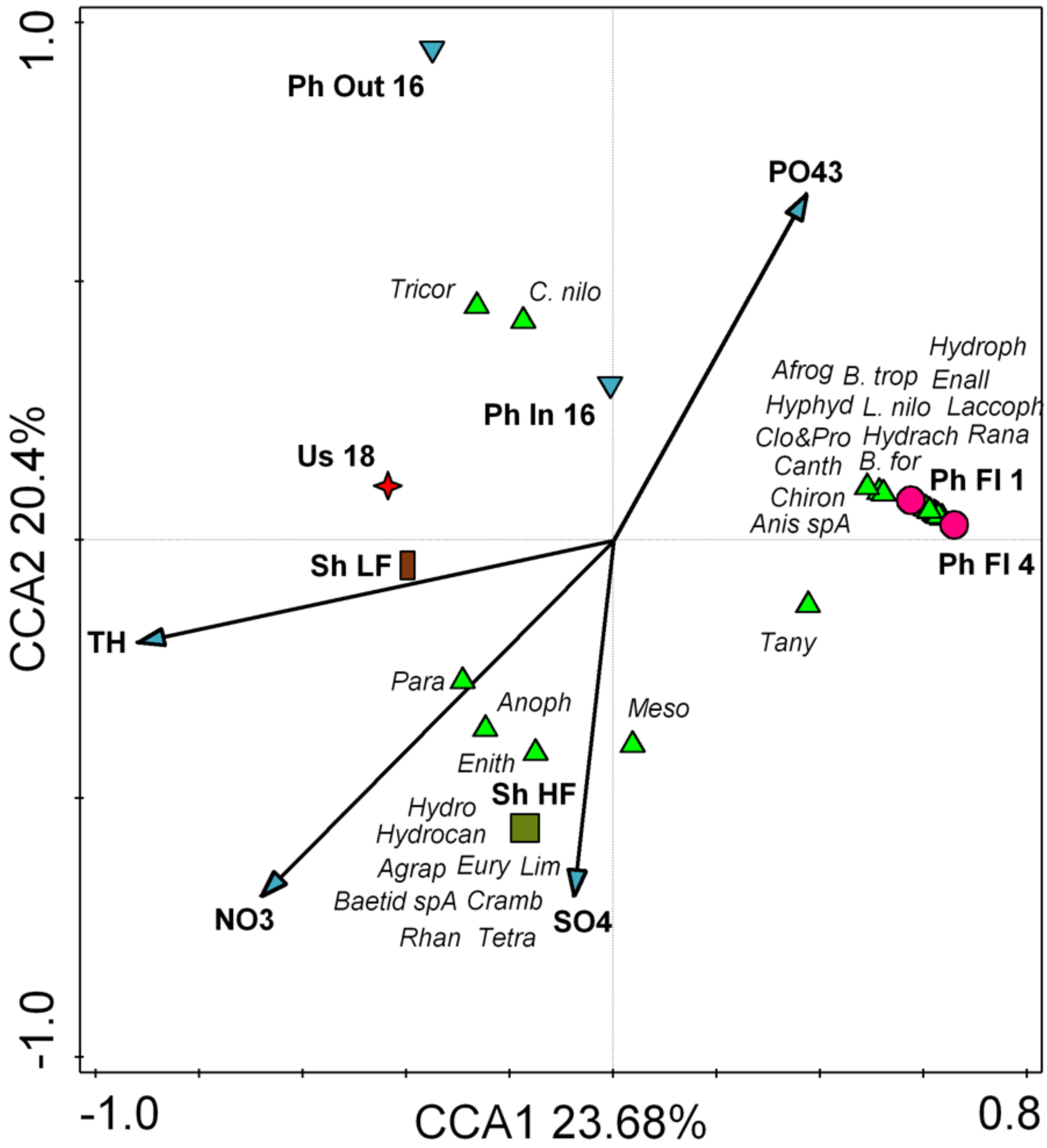


Figure 3.11: Canonical Correspondence Analysis triplot of aquatic macroinvertebrate diversity and selected water nutrients of both rivers and their associated floodplain lakes during various surveys (2014, 2016 & 2018) inside and outside the Ndumo Game Reserve. The triplot explains 44.08% of the total variation in the data with axis 1 explaining 23.68% while the 2nd axis explains 20.4% of the variation in the data. Full list of abbreviated macroinvertebrate names is presented in Appendix B2 & 3.

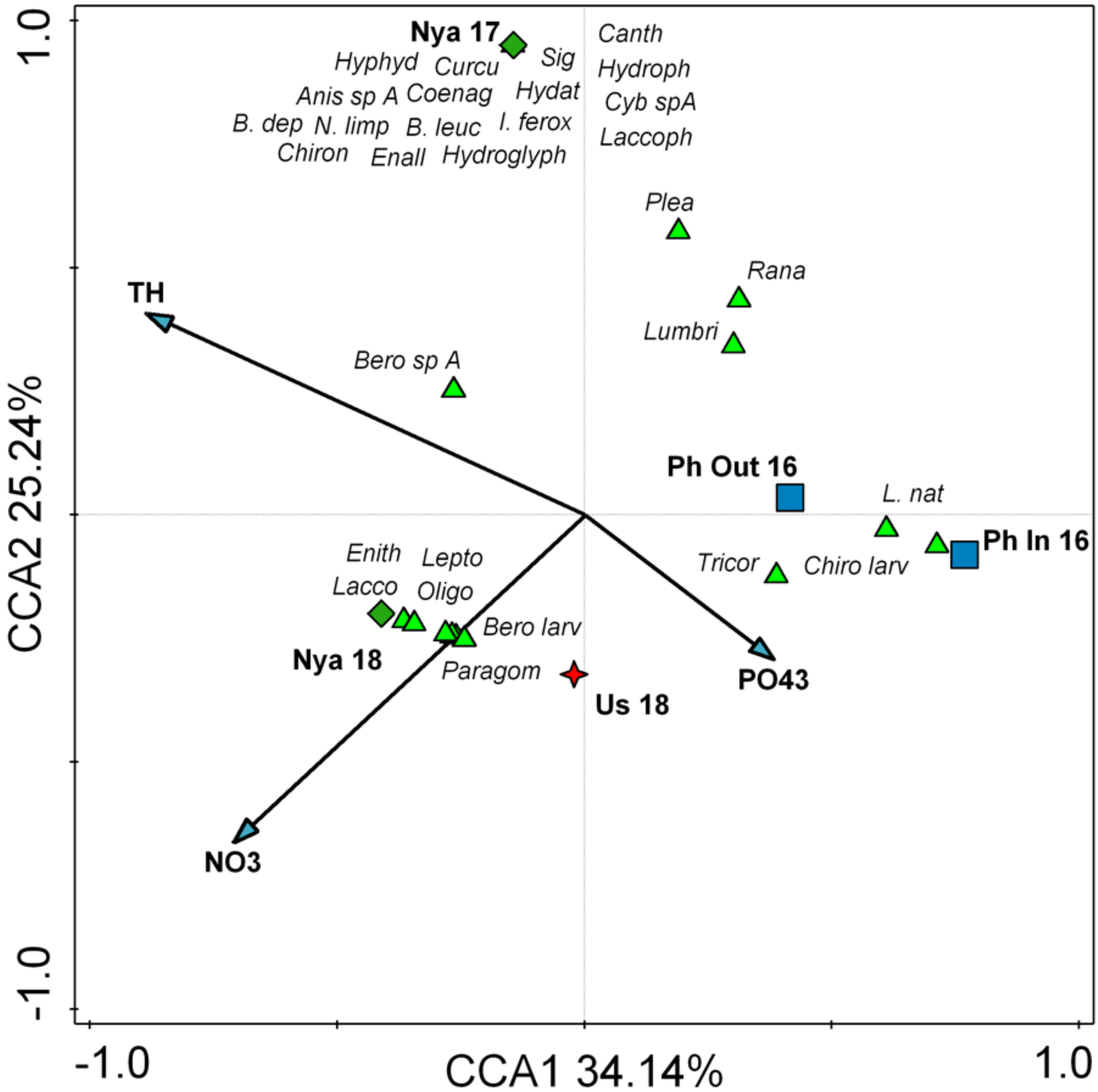


Figure 3.12: Canonical Correspondence Analysis triplot of aquatic macroinvertebrate diversity and selected water nutrients of both rivers and Lake Nyamithi during various surveys (2016, 2017 and 2018). The triplot explains 59.38% of the total variation in the data with axis 1 explaining 34.14% of the variation while 25.24% explains the 2nd axis. Full list of abbreviated macroinvertebrate names is presented in Appendix B2 & 3.

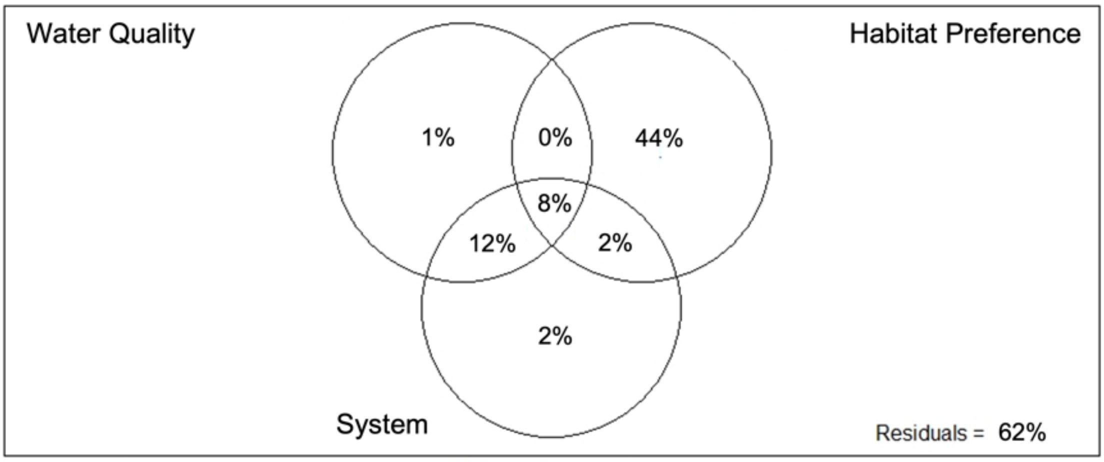


Figure 3.13: Variation partitioning analysis of unique and shared contributions of water quality, habitat preferences and system type (River or Floodplain Lake) on the macroinvertebrate community composition.

3.4. Discussion

This section of the study aimed to determine the inter-relationships between the unregulated Usuthu River and its natural flooding regime on associated floodplain lakes and their resulting macroinvertebrate community structures. Aquatic macroinvertebrate diversity and abundances between the unregulated and regulated rivers were very similar. The influence of the Usuthu River, through hydrological connectivity, on associated floodplain lakes is evident as Lake Showke during the HF survey. The HF survey of Lake Shokwe has shown the positive effect that hydrological connectivity has on macroinvertebrate shown. During the study period, no water from the Phongolo River flooded into Lake Nyamithi. Thus, changes in macroinvertebrate community structures also reflected the influence of the Usuthu River as well as its importance to the lake. Similar macroinvertebrate taxa were found during the 2018 survey and the 2017 survey which shows the sustained recovery of macroinvertebrate communities due to the natural flooding of the Usuthu River.

3.4.1. Macroinvertebrate community structure

The Usuthu River was identified as the main water source of the two largest lakes within the reserve (see Chapter 2) during the study period. Hence understanding the influence the Usuthu plays in the dynamics of these two lakes of the NGR is essential. By identifying the Usuthu River as the main water source for lakes within the reserve, its positive effect could further be seen in the macroinvertebrate diversity of these floodplain lakes. During the HF survey higher taxa richness was found in Lake Shokwe due to continuous incoming water from the Usuthu River, while during the LF, the lake becomes disconnected from the river due to reduced flows. Dube *et al*., (2017) explained that flooding in the Phongolo River increased macroinvertebrate taxa richness in floodplain lakes. Similar findings were noted by Gallardo et al. (2008) who found that macroinvertebrate abundance and richness increased when wetlands within a regulated river floodplain in Spain were connected to the river. Turić *et al*. (2015) explained that flooding periods of rivers shape the structure of aquatic communities and that dry period and short flooding lowers productivity in floodplains. The authors also found that species richness and abundances were higher during prolonged flooding and hydrological connectivity.

Both the Usuthu and Phongolo rivers play important roles in flooding the aquatic wetland ecosystems inside the NGR. However, since the construction of the Pongolapoort Dam, the altered flooding regime has caused much change to the Phongolo River and subsequently caused changes in the NGR floodplain lakes (Smit *et al*., 2016; de Necker *et al*., 2019). Altered flows in rivers are known to cause changes in macroinvertebrate community structures through changes in habitat, vegetation, and water quality (Gillespie *et al*., 2015; Schneider & Petrin, 2017; Gillespie *et al*., 2019). Although the Phongolo River was sampled in September 2016, the present study has shown that, according to the diversity indices, similar macroinvertebrate diversity was found between the regulated Phongolo River and the unregulated Usuthu River. Similarly, Schneider & Petrin (2017) found no differences in macroinvertebrate diversity between a regulated and unregulated river in Norway. Importantly, Schneider & Petrin (2017) found that the changes in water quality due to river regulation were the main cause for changes in macroinvertebrate community assemblages rather than river flow. The authors suggested that changes in physico-chemical parameters as a result of river regulation were equally important to aquatic macroinvertebrate assemblages than changes in river flow. In contrast to previous findings, in the regulated Magpie River, Jones (2013) found greater abundances of benthic macroinvertebrates than in the neighbouring natural rivers which indicated that the Magpie River might not be heavily regulated. The sensitivity score of the Usuthu River was very similar to that of the Phongolo River during the study of Smit *et al*. (2016). The higher abundances of sensitive taxa such as Baetidae, Heptageniidae, and Atyidae are indicative of higher water and habitat quality (Everaert *et al*., 2014; Smit *et al*., 2016; Raphahlelo *et al*., 2022; Rico-Sánchez *et al*., 2022). In the most recent study on the Phongolo River (de Necker, 2019), a higher percentage of pollutant tolerant than sensitive taxa were present in the river. This is probably due to the presence of pollutant-tolerant taxa such as *P. acuta* and Lumbriculidae that flourish in streams that experience organic pollution (Al-Shami *et al*., 2011).

Permanent natural saline lakes (such as Lake Nyamithi) are found all over the world in a myriad of climatic zones, especially regions where evaporation eclipses rainfall (de Necker *et al*., 2021). Differences in diversity indices (Pielou's Evenness Index, Margalef's Species Richness, Shannon-Wiener Diversity Index, and Simpson's Index) were found in the aquatic invertebrate community structure of Lake Nyamithi during the present study and by the study of de Necker *et al*. (2021). Although not significantly higher, Lake Nyamithi during November 2017 was higher in taxa, species diversity, and richness than during the November 2018 survey. Similarly, Velasco *et al*. (2006) and Senner *et al*. (2018) reported lower macroinvertebrate numbers during high salinity in a Mediterranean stream and North America lake, respectively. Families such as Corixidae (*Micronecta* sp.), Dytiscidae, Hydrophilidae (*Berosus* sp.), and Chironomidae are known to be hardy freshwater macroinvertebrates that can tolerate changes to the environment (de Necker *et al*., 2021). Bunn & Davies (1992) similarly found Oligochaeta, Chironomidae, Ostracoda, and Corixidae in a saline river system in south-western Australia and indicated that these taxa are salt tolerant. Ostracods are generally present in the majority of aquatic ecosystems and both non-marine and marine environments and can tolerate extremely harsh conditions that include high salinities (de Necker *et al*., 2021). In a study of saline lakes in northern Tibet, China, Wen *et al*. (2005) found that the most abundant taxon in the saline lakes was Ostracoda (40.9% occurrence) while in Australia, Khan (2003) also found Ostracoda, Mollusca, Corixidae (*Sigara* sp. and *Micronecta* sp.), Hydrophilidae (*Berosus* sp. and *Hydrochus* sp.), Oligochaeta and Ceratopogonidae (*Bezzia* sp.) in the four saline lakes sampled. Khan (2003) further explained that these families are known to dominate aquatic environments with high salinity.

3.4.2. Macroinvertebrate traits

Aquatic macroinvertebrates are excellent indicators of anthropogenic stressors in aquatic systems by responding rapidly to changes in water quality (Carter *et al*., 2017; Calapez *et al*., 2021). As such, between the regulated and unregulated rivers, the flow velocity had effects on the macroinvertebrate traits associated with these rivers. During the present study, the unregulated Usuthu River was comprised of more rheophilous families (Hydropsychidae, Gyrinidae, and Baetidae) than the regulated Phongolo River that was comprised of taxa (Corixidae, Culicidae, Lumbriculidae, and Libellulidae) with a preference for slow-moving water (Schmedtje & Colling, 1996; Fry, 2021). In contrast, the Phongolo River outside the reserve had taxa that preferred fast-flowing water. This was due to the availability of different habitat types that can support macroinvertebrates with a preference for fast-flowing waters (de Necker, 2019). The site outside the reserve had concrete blocks within the channel which created fast-flowing habitats (de Necker, 2019). These data are supported by findings that altered flood regimes in rivers through impoundments change diversity, abundances, and biological traits due to temperature changes, flow velocity, altered substrate, and habitat loss (Belmar *et al*., 2013; Schneider & Petrin, 2017; Belmar *et al*., 2019).

Macroinvertebrates (*Caenis* sp. and *C. nilotica*) within the unregulated Usuthu River had a preference for gravel, sandy, and muddy substrate (Hart *et al*., 2001; Fry, 2021). Habitat preference within the regulated Phongolo River were also gravel, sandy, and muddy substrates, however, taxa (*Anisops* sp., *P. acuta*, *L. natalensis*, and *Pseudagrion* sp.) that had a preference for vegetation were also found (Griffiths *et al*., 2015; Fry, 2021). Shredders were the dominant functional feeding group in the Phongolo River, both inside and outside the reserve. This could be due to the presence of inorganic nutrients and sunlight stimulating periphyton growth and therefore serves as food for shredders (Ferreira *et al*., 2012; van Echelpoel *et al*., 2018). Furthermore, Erasmus *et al*. (2021) explained that specialized feeders such as shredders are possibly more sensitive to anthropogenic changes to natural systems while generalists such as filterers are more tolerant to pollution. The dominant functional feeding groups within the Usuthu River were predators and deposit feeders. The dominance of predators may potentially be linked to the season as higher predator diversity could be found during the dry season (van Echelpoel *et al*., 2018). However, the dominance of predators within the Usuthu River could be a result of the high abundance of these two families (Veliidae & Gyrinidae) rather than the high diversity of predators.

The aquatic macroinvertebrate communities of the floodplain lakes within the reserve species (*Micronecta* sp. (Corixidae), *Enithares* sp. (Notonectidae), Crambidae, Mesoveliidae, and Hydrophilidae) have a preference for slow-moving water such as pools. This agrees with Calapez *et al*. (2021) who found taxa such as *Caenis* sp., Chironomidae, and Oligochaeta to be in high abundance in lentic systems. Also, similar findings were noted by Saha & Gupta (2015) who found that Corixidae and Notonectidae were higher in abundance while Mesoveliidae were also found in three oxbow lakes in India in which most taxa were found during the post-monsoon season (October - December). From these taxa, only Oligochaeta had a high abundance in the lentic systems within the NGR. Macroinvertebrate taxa, diversity, and richness in the two Phongolo River floodplain lakes were significantly higher than in the Usuthu River floodplain lake. The high diversity was because the sampling of those two lakes was after post-controlled flooding of the Phongolo River (Dube *et al*., 2019).

The dominant functional feeding group within the floodplain lakes were predators. Similarly, Ferreira *et al*. (2012) found that the most abundant feeding group within perennial lakes in Mpumalanga were predators. However, macroinvertebrate predators are known to respond positively to reduced flows, thus, higher abundances of predators could be expected in lakes during low connectivity (Gallardo *et al*., 2014; Wilbanks & Mullis, 2022). In the present study, this was specifically true in Lakes Shokwe and Nyamithi during the LF survey. High abundances of predators such as Dytiscidae, Notonectidae, and Corixidae were found in both floodplain lakes. Similarly, Nhiwatiwa *et al*. (2017) found that predators such as Dytiscidae, Notonectidae, and Corixidae were the dominant taxa within the endorheic lakes of Zimbabwe. Nhiwatiwa *et al*. (2017) further explained that the duration of hydroperiod affects the abundance of predators and that longer hydroperiods increase predator abundance.

3.4.3. Environmental factors responsible for structuring macroinvertebrate community composition

It is well known that macroinvertebrates respond rapidly to abiotic and biotic factors (Leigh, 2013; de Necker *et al*., 2016; Acosta *et al*., 2021). Thus, water quality such as nutrients plays an essential role in the structuring of macroinvertebrate taxa (Xu *et al*., 2014; Acosta *et al*., 2021). Xu *et al*. (2014) explained that higher nutrient concentrations greatly affect biodiversity and change the functional feeding group composition of macroinvertebrates. As such, during the present study dissolved nutrients had positive correlations with the Usuthu River, Lakes Shokwe, and Nyamithi during the November survey compared to the Phongolo River, Phongolo River associated lake, and Lake Nyamithi 2017 survey, indicating that nutrients had a higher impact on the macroinvertebrate taxa during the present study compared to the study of de Necker (2019). de Necker *et al*. (2016) found differences in macroinvertebrate structures as a result of seasonal changes and attributed these differences to changes in water quality while Dube *et al*. (2017) found that flooding decreases nutrient and conductivity values.

Similarly, habitat preference also plays an essential role in the macroinvertebrate community composition as macroinvertebrates are sensitive to habitat characteristics (Beauger *et al*., 2006). The variation partition analysis indicated that in the present study habitat preference contributed more than water quality to the structure of macroinvertebrate communities. Carter *et al*., (2017) stated that habitat influences macroinvertebrates equally as much as they are influenced by water quality. Habitat preference such as vegetation affects macroinvertebrate community composition by acting as a food source, providing a substratum for laying eggs, and as a refuge (Gleason & Rooney, 2017).

3.5. Conclusion

The study aimed to determine whether the unregulated Usuthu River had any effect on macroinvertebrate community structures of aquatic systems within the NGR. Results support the hypotheses of the present study in that the naturally flowing Usuthu River had a positive effect on macroinvertebrate community structures. The aquatic macroinvertebrate community structure of the Usuthu River and its associated floodplain lakes from the present study showed differences compared to previous studies of the Phongolo River and associated floodplain lakes (Dube et al., 2019; de Necker, 2019; de Necker et al., 2021). The aquatic macroinvertebrate communities of the Usuthu River are made up of less tolerant taxa and more sensitive taxa compared to a survey in 2016 in the Phongolo River during drought conditions while showing similar tolerant and sensitive taxa to the Phongolo River in pre-drought conditions.

Macroinvertebrate diversity between the two rivers of the NGR was very similar and the hypothesis stating that the macroinvertebrate community structure of the Usuthu River differs from the Phongolo River is thus not supported by the data. The Usuthu River indicated no influence on the macroinvertebrate structure of Lake Shokwe. Instead, the aquatic invertebrate communities of Lake Shokwe during the LF survey were comprised of hardier freshwater macroinvertebrate taxa such as Micronecta sp. (Corixidae), Berosus sp. (Hydrophilidae), Dytiscidae and Oligochaeta that can withstand dry periods. The higher diversity and taxa in the HF survey of Lake Shokwe highlight the importance of the hydrological connectivity to its respective floodplain river. The hypothesis that hydrological connectivity will have a positive effect on the macroinvertebrate community structure of Lake Shokwe is supported by the data.

Due to no water flooding in from the Phongolo River, the macroinvertebrate diversity of Lake Nyamithi was influenced by the Usuthu River. Thus, the hypothesis that the two largest floodplain lakes within the NGR will reflect the influence of the Usuthu River is also supported by the data. The Phongolo River-associated floodplain lakes showed to be different in composition as the lakes had higher taxa, species diversity, and richness compared to the Usuthu River-associated floodplain lakes. Importantly, due to no hydrological connectivity to the Phongolo River, the aquatic community structure of the Phongolo River floodplain lakes is non-existent. Hence, the high diversity was a result of being connected to its associated floodplain river receiving an influx of water. The aquatic macroinvertebrate community structure of Lake Nyamithi consists of unique macroinvertebrate taxa that can resist and be resilient during extreme conditions.

It is clear from the present study that the Usuthu River did have impacts on the aquatic macroinvertebrate community structures of the lentic systems within the NGR. As such, Lake Shokwe highlighted the importance of its respective river while the community structure of Lake Nyamithi showed minimal influences from the Usuthu River. The hypothesis that the influence of the Usuthu River on Lake Nyamithi will reflect sustained recovery is supported by the data as few taxa were shared between the 2017 and 2018 surveys in Lake Nyamithi, demonstrating a partial sustained recovery from the 2017 survey.

The hypothesis that the nature of the aquatic ecosystems (lotic/lentic) drives the structure of macroinvertebrates communities within the NGR is not supported by the data as the variation partitioning analysis indicated that habitat preference was the major driver in structuring macroinvertebrate communities within the NGR.

Therefore, now that the aquatic macroinvertebrate community structures of the two floodplain rivers and associated floodplain lakes been determined, it is important to further investigate the dynamics of these communities through food web structures and dietary analysis.

CHAPTER 4
Comparison of food webs in two linked rivers and lakes of the lower Phongolo floodplain

4.1. Introduction

Food web structures provide an understanding of energy flows through ecosystems as well as insights into the biogeochemical cycle, community structure, and population dynamics of an ecosystem (Mor *et al*., 2018; Ru *et al*., 2020). Importantly, food webs are a summation of species interactions, biodiversity, and ecosystem functions (Rayner *et al*., 2010). Stable isotope analyses of carbon and nitrogen ratios are a useful tool in determining consumer diets, trophic relationships, energy flows, and constructing food web models (Peterson & Fry, 1987; Stewart *et al*., 2017). Furthermore, isotopic signatures reflect the diet of species while also providing an indication of the importance of autochthonous and allochthonous inputs into freshwater ecosystems (Peterson & Fry, 1987; Finlay, 2001). Biota incorporates carbon and nitrogen into their diet through the consumption of resources, carbon isotopes can be used to indicate primary food sources while nitrogen isotopes estimate the relative trophic position of biota on the food web relative to their diet (Doucett *et al*., 1996; Post, 2002; Newsome *et al*., 2007).

The anthropogenic activities in the upper catchments of both rivers (as discussed in Chapter 2, section 2.1) not only influences aquatic macroinvertebrate community structures (Chapter 3, section 3.1) but can influence carbon sources, primary production, nutrient availability, and fish movement (Beatty *et al*., 2017; de Necker *et al*., 2020; Oeding *et al*., 2020). Stable isotope analyses have many advantages such as tracing diets (de Necker *et al*., 2020), determining trophic position (Post, 2002), assess biomagnification of Hg and OCP's (Govaerts *et al*., 2018; Volschenk *et al*., 2019) and flow regulation in aquatic ecosystems (Ruhi *et al*., 2016). When assessing the impact of anthropogenic influences, nitrogen isotopes become an important monitoring tool as each nitrogen source has a typical structure associated with anthropogenic pollution (Griboff *et al*., 2020).

Strontium isotopes are naturally present in the environment and provide useful information on the fish origin, movement patterns, and connectivity between aquatic habitats (Capo *et al*., 1998; Wolff *et al*., 2012; Brennan *et al*., 2015). Strontium ratios vary amongst water bodies as $^{87}Sr/^{86}Sr$ is driven by the underlying geology and weathering of rocks (Wolff *et al*., 2012), and the ratios directly reflect ambient environments (Brennan *et al*., 2015; Jordaan *et al*., 2016). Strontium isotope ratios in the otoliths of fish reflect that of the water and therefore the $^{87}Sr/^{86}Sr$

ratios in water and fish otoliths could be used to indicate the aquatic environments in which they live (Jordaan *et al*., 2016). Thus, by using $^{87}Sr/^{86}Sr$ ratios in fish otoliths, their movement patterns during their life cycle as well as their natal origin can be determined (Brennan *et al*., 2014). Moreover, understanding the natal origin or movement of fish species between aquatic systems within the PRF would provide insights into how river regulation through impoundments influences the movement and origin of important floodplain fish species.

As demonstrated in Chapter 3, natural flood regimes are essential to maintain the biodiversity and ecological integrity of the PRF and influence the dynamics and structure of aquatic species (Dube *et al*., 2019). Flooding of floodplain ecosystems ensures the exchange of organisms between habitats within ecosystems and nutrients (Herwig *et al*., 2006) and according to Taylor *et al*. (2017a), plays an essential role in triggering primary and secondary productivity. Therefore, hydrological connectivity between a river and its floodplain can influence trophic interactions further up in the food web by encouraging consumer movement between the systems. Habitat connectivity is also influenced by the flooding regime, linking the river to its floodplain wetlands, and providing fish the opportunity to move between these systems (Dube *et al*., 2019; Qin *et al*., 2019).

The aquatic ecosystems within Ndumo Game Reserve (NGR), the only conservation area associated with the PRF (Dube *et al*., 2015), should experience less anthropogenic pressures. Water and sediment quality indicated that these systems within the NGR were of acceptable quality to sustain healthy aquatic biodiversity (refer to Chapter 2). However, there were clear demonstrable water quality differences between the unregulated Usuthu River and the flow-regulated Phongolo River (see Chapter 2 also van Rooyen *et al*., 2022). The Phongolo River is a more modified system compared to the Usuthu River, whereby the regulated flow has had an impact on the aquatic biodiversity (Dube *et al*., 2015; Smit *et al*., 2016; de Necker *et al*., 2019).

Therefore, the main aims of this chapter were 1) to assess the food web structures of the two main rivers and floodplain lakes in the lower PRF and 2) to determine whether the impaired flow regime has an effect on the food web structure of the Phongolo River (and associated Lake Nyamithi) when compared to the Usuthu River and its associated floodplain lake (Lake Shokwe), 3) to determine whether there is a difference in piscivorous fish diets between the Usuthu and Phongolo rivers and 4) determine the biological connectivity between the systems using Sr isotope ratios in African sharptooth catfish as an indicator of migration between the systems.

4.2. Materials and Methods

4.2.1. Study area

See Chapter 1, section 1.2 for the study area description. The sites sampled include the Usuthu River and its associated floodplain lake, Lake Shokwe as well as the Phongolo River and Lake Nyamithi (Figure 1.6). Fish, aquatic macroinvertebrates, and aquatic vegetation were collected during two field sampling surveys, namely: June 2018 – high flow (HF) survey and November 2018 – low flow (LF) survey. For the purposes of this study, data were used from previous studies in the region by de Necker (2019), samples from the Phongolo River and Lake Nyamithi were collected during September 2016 (representing LF - de Necker, 2019) and during February 2017 (representing HF - de Necker *et al.*, 2022a), respectively.

4.2.2. Field collections

All field samples were collected using the necessary permits (Permit No's: OP 1582 – 2018 & OP 1585 – 2018) and ethical clearance (Ethics No: NWU-00156-18-A5). From previous surveys, all field samples were collected by de Necker (2019) using the following permits (Permit No's: OP 899/2016 and OP 1075/2017) and ethical clearance (Ethics No: NWU-00264-16-A5).

4.2.2.1. Primary producers

At each of the sites, as many as possible different primary producers that could be found were sampled. These included Poaceae (reeds), Nymphaeceae (water lily's), Onagraceae (African willow herb), detritus, biofilm, and leaf litter, samples were collected by means of hand-picking each primary producer and placed in polyethylene resealable bags. Detritus was more abundant during the dry season corresponding with the LF sampling period, however, it was only present in Lake Shokwe. All primary producer samples collected were frozen in the field and stored at -20 ºC until further analysis.

4.2.2.2. Aquatic macroinvertebrates

Aquatic macroinvertebrates were collected using the method described in Section 3.2.1. For the purposes of this section of the study, macroinvertebrates were only identified at family level using the aquatic macroinvertebrate identification guide of Gerber and Gabriel (2002). The most dominant families (Corixidae, Dytiscidae, Libellulidae, Hydrophilidae, Notonectidae, Belostomatidae, Atyidae, Naucoridae, Nepidae, and Coenagrionidae) were selected, and individuals were hand-picked and collected in 15mL falcon tubes. The most dominant families were selected in order to collect sufficient mass for analysis. A minimum of four replicates were collected from each of the sites for each of the dominant families before being frozen in the field and transported back to the university until further analysis (de Necker *et al*., 2021; de Necker *et al*., 2022a). The family Dytiscidae is a highly diverse family of beetles with an array of different feeding preferences, as such the sampled dytiscids were separated into two groups based on size, i.e., small and large individuals since the different sizes prey on different components of the aquatic food web.

4.2.2.3. Fish

All available fish species were targeted and a minimum of four individuals were collected from each species. Various sampling techniques (rod and line, fyke nets, and cast nets) were applied, and the captured fish were identified to species level using the identification keys in Skelton, (2001). Fish were humanely sacrificed using an approved standard operating protocol for stunning and severing the spinal cord (NWU-00156-18-A5). The standard length (mm) was measured and approximately 2 g of axial muscle tissue was dissected from the dorso-lateral region and placed in polyethylene falcon tubes. Samples were frozen in the field and transported back to the laboratory and kept frozen (-20 ºC) until further analysis.

For the purposes of this study, otoliths were removed from *Clarias gariepinus* (African sharptooth catfish) following the method of Smit *et al*. (2011) as this was a common species sampled across all the sites and is known to migrate Skelton (2001). The tissues surrounding the cranium were removed, exposing the cranium to remove the otoliths from the auditory capsule. Left and right sagittal otoliths from each fish were removed, cleaned with water and soft brush, and stored in 2 mL Eppendorf tubes.

4.2.3. Stable isotope analysis

All samples were placed in a -80ºC ultra-freezer for 24 h prior to being freeze-dried (FreeZone®[6], Labconco) for 72 h. The dried sample was ground into a fine powder using a sterile mortar and pestle. After each sample, all equipment was cleaned using 10% HCl. Finely-ground fish tissue and macroinvertebrate samples were placed in 2 mL Eppendorf tubes prior to treatment for lipid extraction. The samples were placed in the dark at 4 ºC for 12 h after being treated with a 2:1 chloroform:methanol (v:v) solution (Folch *et al*., 1957). Once the lipid extraction was completed, samples were centrifuged (1,500 g) for 2 minutes, the supernatant was discarded, and the pellet was oven dried at 50 ºC for 24 h. Each dried sample was weighed (Sartorius AG, M2P) (primary producers: ± 5 mg, consumers: ± 1 mg, and vegetation: ± 2 mg) before being encapsulated in ultrapure 4 x 6 mm tin caps (IVA Analysetechnik GmbH & Co. KG, Meerbusch, Germany).

Samples were analysed for total carbon and nitrogen content as well as isotopic ratios ($^{13}C/^{12}C$ & $^{15}N/^{14}N$) by dry combusting using an Elemental analyser isotope ratio mass spectrometer (EA-IRMS) (Vario ISOTOPE Select and Isoprime vision). Stable isotope results were derived from the deviation relative to standards (atmospheric N signature and PeeDee Belemnite C signature) and the isotopic values are reported as parts per thousand ($^{0}/_{00}$). Quality control was assured by analysing a laboratory standard (acetanilide) in duplicate after every seventh sample. Normalisation was carried out using international standards USGS40 and USGS41a (both International Atomic Energy Agency, Vienna) (for details see Nachev *et al*., 2017). Replicate measurements of internal laboratory standards resulted in error < 0.06‰ for $\delta^{13}C$ and $\delta^{15}N$.

The available food sources that were collected were pooled into meaningful feeding groups namely: primary producers, small and larger sized macroinvertebrates for the different families, and fish representing algavores, herbivores, insectivores, scavengers, omnivores, and predators. This method is recommended when similarity occurs between isotopic signatures and when sources are logically related, thus providing more reliable results (Phillips *et al*., 2005).

4.2.4. Strontium isotope analysis

The Sr isotopic analyses were conducted using a multi-collector inductively coupled plasma mass spectrometer (Thermo Fisher Scientific Neptune Plus). All chemical procedures were conducted in a Class 100 clean room. The otolith samples were initially washed with Milli-Q water for 10 min in an ultrasonic bath and rinsed three times. The samples were dissolved in 1 mL of 6 N HCl and were dried at 100 °C. The dried samples were then dissolved in 1 mL of 2 N HNO_3. The chemical separation using Sr-spec resin followed the method of Pin *et al.* (1994). Mass fractionation for Sr was internally corrected using $^{86}Sr/^{88}Sr = 0.1194$. Additional corrections were performed by applying a standard bracketing method using NIST987 and normalizing to $^{87}Sr/^{86}Sr = 0.710240$ for NIST 987. The analytical reproducibility (2σ) for the isotopic analyses of natural samples is typically 0.004% for $^{87}Sr/^{86}Sr$.

4.2.5. Data analyses

For each food web component, the trophic position (TP) was determined and compared by calculating the trophic position for each component utilising the mean $\delta^{15}N$ of each of the components. The mean $\delta^{15}N$ signatures were compared to a reference of the base of the food web by using the following equation:

$$TP = \frac{\delta^{15}N_{component} - \delta^{15}N_{reference}}{3.37} + 1$$

Where:

$\delta^{15}N_{component}$ = mean $\delta^{15}N$ of food web component or species

$\delta^{15}N_{reference}$ = mean $\delta^{15}N$ of plant material for each system

3.37 ‰ = the mean trophic enrichment between trophic levels which was estimated by Taylor *et al*. (2017b).

Poaceae served as the base ($\delta^{15}N_{reference}$) of the food web of the Usuthu River while within Lake Shokwe, Onagraceae served as the base, and in Lake Nyamithi, detritus served as the base of the food web. For comparative purposes, the data obtained were compared to unpublished stable isotope data from the Phongolo River and Lake Nyamithi as reported by de Necker (2019).

The food web structures of the aquatic ecosystems of the NGR were graphically constructed using GraphPad Prism 8 (GraphPad Software, Inc) by plotting the mean and standard errors of $\delta^{13}C$ and $\delta^{15}N$ for all sources. Using parametric unpaired student t-tests, significant differences in $\delta^{13}C$ and $\delta^{15}N$ were determined for similar food web components between the floodplain rivers, floodplain lakes of the 2018 survey, and Lake Nyamithi 2017 and 2018. To determine the consumer-food source interactions between the various aquatic ecosystems, a hierarchical Bayesian stable isotope mixing model (MixSIAR; Stock and Semmens, 2016) was applied utilising the $\delta^{13}C$ and $\delta^{15}N$ ratios. Food sources were grouped accordingly into biological groups. The present study compares to the study of de Necker (2019) which collected different types of plants and macroinvertebrates and subsequently grouped them as follows: C_3 and C_4 plants and Group A and B macroinvertebrates. As there were fewer of these plants and macroinvertebrates available during the present study period, the food sources were grouped into overarching groups. These groups consisted of plant material, aquatic macroinvertebrates, and fish. The grouping of multiple food sources is recommended especially when within-group isotopic variation is less than between group variation and/or if sources are related (Phillips *et al*., 2005; Phillips *et al*., 2014; Manfrin *et al*., 2018).

Following the Shapiro-Wilk test for normality, a one-way ANOVA (using Tukey's multiple comparison test) was performed on the $^{87}Sr/^{86}Sr$ ratios of *C. gariepinus* from the various aquatic systems within the NGR using GraphPad Prism 8. Using Tukey's multiple comparisons test, significant differences between sites were determined ($p < 0.05$).

Mixing models were created for each of the sampled aquatic ecosystems and evaluated to determine whether the position of consumers from each aquatic ecosystem was within the convex mixing polygon of potential food sources (Smith *et al*., 2013; Phillips *et al*., 2014; Valladares *et al*., 2017; Lemmens *et al*., 2017). This was done by simulating each mixing polygon region of the aquatic ecosystems using *splancs* and *sp* packages in R (Smith *et al*., 2013; R Core Team, 2017). Consumers were excluded from the final mixing model when individuals were located outside the 95% mixing polygon region.

For each aquatic ecosystem, model runs were performed using the Markov Chains Monte Carlo (MCMC) parameters until three chains of the model converged. Based on the Geweke diagnostic and Gelman-Rubin tests (Stock and Semmens, 2016), convergences were only accepted when <5% of values for the Geweke diagnostic test were outside +/- 1.96 and when the values for the Gelman-Rubin test were <1.05 (Stock and Semmens, 2016). For each of the aquatic ecosystems, the MCMC chain length was 50 000 with a burn-in of 25 000 and a thinning of 25. The MCMC chain length for Lake Nyamithi during the February 2017 survey was 100 000 with a burn-in of 50 000 and thinning of 100 (de Necker, 2019). In the model, process only error terms were used, and consumers were included as fixed effects. Also in the model, the 95% credible intervals are based on the individual level variation for each consumer. Using the stable isotope data of all fish consumers (raw data), and potential food sources (raw data), a Bayesian mixing model was run while using a consistent discrimination factor for $\delta^{13}C$ (1.0 ‰ ±0.25 ‰) (DeNiro and Epstein, 1978; Akamatsu *et al*., 2004) and $\delta^{15}N$ (3.37 ‰ ±1.3 ‰) (Taylor *et al*., 2017b) for all consumers.

4.3. Results

4.3.1. Food web structures

4.3.1.1. Floodplain rivers

A total of 10 fish species (dwarf tigerfish (*B. imberi* (Peters, 1852)), tigerfish (*H. vittatus* (Castelnau, 1861)), southern Churchill (*Petrocephalus wesselsi* (Kramer & van der Bank, 2000)), brown squeaker (*Synodontis zambezensis* (Peters, 1852)), Mozambique tilapia (*Oreochromis mossambicus* (Peters, 1852)), silver catfish (*Schilbe intermedius* (Rüppel, 1832)), sharptooth catfish (*C. gariepinus* (Burchell, 1822)), plump barb (*Enteromius afrohamiltoni* (Crass, 1960)), purple Labeo (*Labeo congoro* (Peters, 1852)) & the bulldog (*Marcusenius macrolepidotus* (Peters, 1852)), representing seven families (Alestidae, Mormyridae, Cyprinidae, Mochokidae, Schilbeidae, Clariidae, and Cichlidae) were collected from the Usuthu River during the November 2018 survey (Appendix C1). During the survey in September 2016, 11 fish species (*P. wesselsi*, *S. zambezensis*, *E. afrohamiltoni*, *H. vittatus*, *S. intermedius*, *B. imberi*, banded tilapia (*Tilapia sparmanii* (Smith, 1840)), and the river goby (*Glossogobius guirius* (Hamilton, 1822)) from seven different families (Alestidae, Mormyridae, Mochokidae, Schilbeidae, Cichlidae and Gobiidae) were collected from the Phongolo River inside NGR (Appendix C2).

Four trophic levels were evident in the food web of the Usuthu River (Figure 4.1a). *Hydrocynus vittatus* was the apex predator and the majority of fish were at a lower trophic level; namely S. *intermedius*, *S. zambezensis*, *C. gariepinus*, *M. macrolepidotus*, *E. afrohamiltoni*, *P. wesselsi* and *B. imberi*. *Labeo congoro* occupied a similar trophic position to the macroinvertebrates while the base of the food web was made up of primary producers (leaf litter, Nymphaeaceae, and Poaceae). The majority of macroinvertebrate families (Potamonautidae, Gomphidae, Atyidae, Gyrinidae, and Belostomatidae) collected occupied the second trophic level while the river prawn (*Macrobrachium* sp.) shared the third trophic level with the majority of fish species (Figure 4.2).

Similar to the Usuthu River, there were four trophic levels in the Phongolo River (Figure 4.1b). In contrast to the Usuthu River, *P. wesselsi* was at the top of the food web while leaf litter, detritus, and biofilm formed the base of the food web in the Phongolo River (Figure 4.1b). In addition, similarly *Macrobrachium* sp. occupied the same trophic level as the majority of the fish. The predatory aquatic macroinvertebrates (Coenagrionidae, Aeshnidae, Naucoridae, and Dytiscidae) were also at least one trophic level above other macroinvertebrates (Noteridae, Hydrophilidae, Physidae, and Atyidae) (Figure 4.3). *Oreochromis mossambicus* was found to be on the same trophic level as many macroinvertebrate families (Coenagrionidae, Tabanidae, Atyidae, Libellulidae, and Lampyridae).

Significant differences in isotopic signatures were found in all but one signature between the rivers of the NGR (Appendix C1 and C2). Only $\delta^{13}C$ signatures in macroinvertebrates were not significantly different between both rivers (p = 0.5208). Food web components from the Usuthu River were significantly enriched in $\delta^{15}N$ (primary producers: $p < 0.008$; macroinvertebrates: $p < 0.0001$ & fish: $p < 0.0001$) while fish from the Phongolo River were significantly $\delta^{13}C$ depleted ($p < 0.0002$) compared to the Usuthu River. The primary producers were significantly $\delta^{13}C$ depleted in the Usuthu River (p = 0.033).

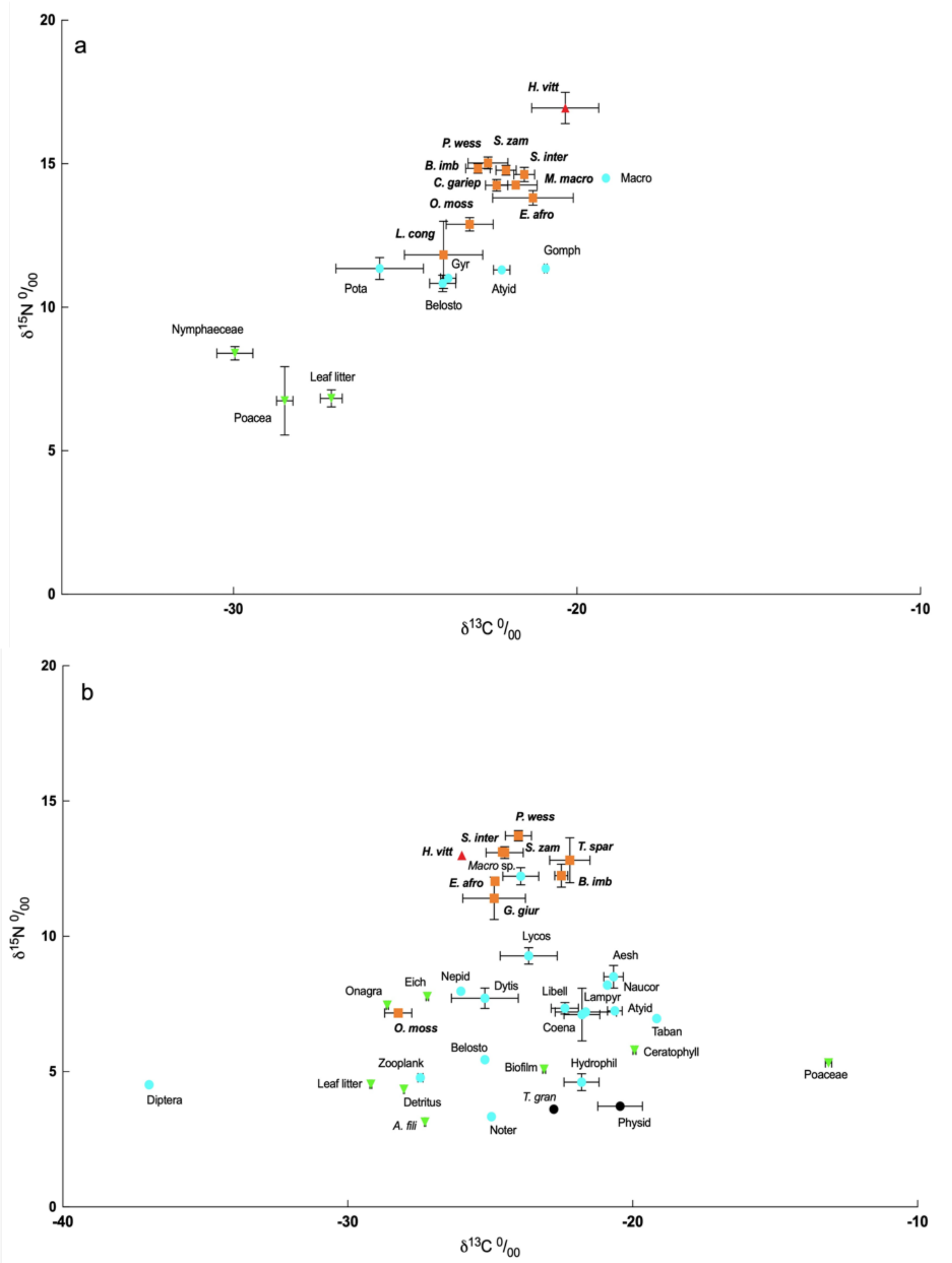


Figure 4.1: Biplots indicating the mean $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures (±1 SE) of the various food web components collected in the Usuthu River (a) during November 2018 and (b) Phongolo River within the Phongolo River Floodplain during September 2016 (de Necker, 2019). Full list of abbreviated macroinvertebrate and fish sample names are presented in Appendix C1 (Usuthu River) and C2 (Phongolo River).

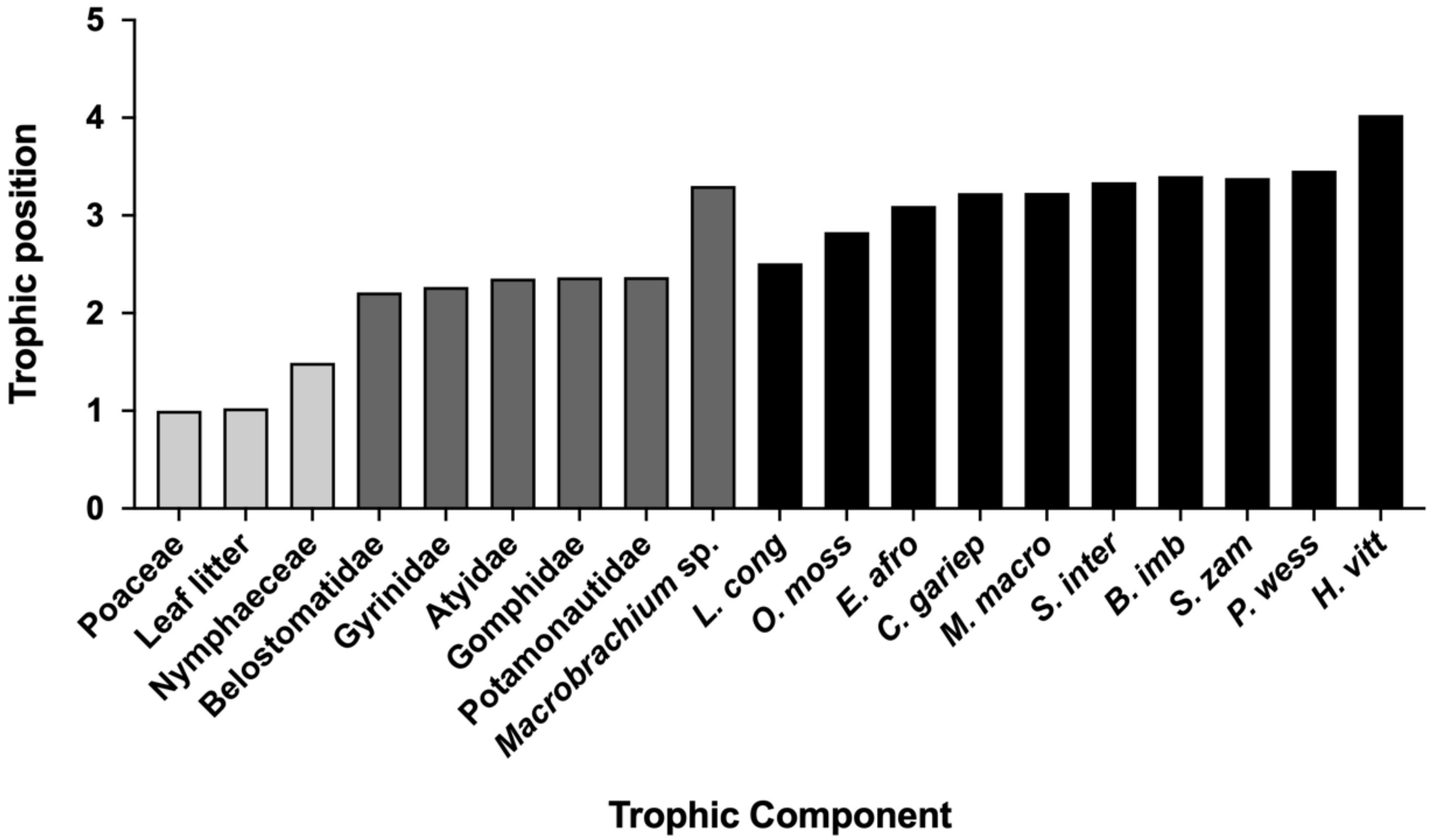


Figure 4.2: Mean trophic level of food web components collected in the Usuthu River within the Phongolo River Floodplain during the November 2018 survey. Colour in the figure indicates the different components collected (Light grey = primary producers, dark grey = macroinvertebrates and black = fish species).

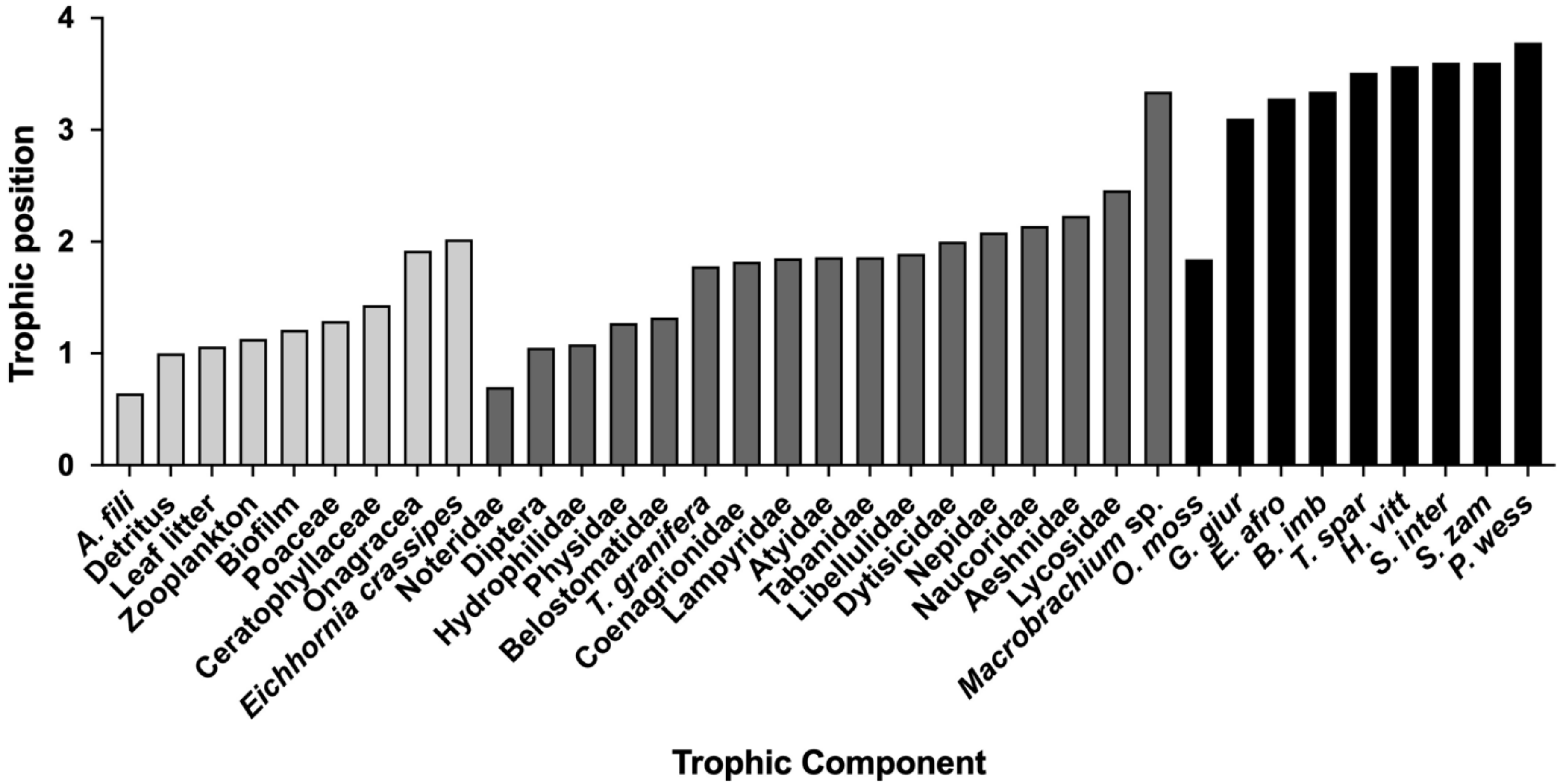


Figure 4.3: Mean trophic level of food web components collected in the Phongolo River within the Phongolo River Floodplain during the September 2016 survey. Colour in the figure indicates the different components collected (Light grey = primary producers, dark grey = macroinvertebrates and black = fish species).

4.3.1.2. Floodplain lakes

In Lake Shokwe, a total of seven fish species (*E. afrohamiltoni*, *C. gariepinus*, *H. vittatus*, *O. mossambicus*, red breasted tilapia (*Coptodon rendalli* ((Boulenger, 1896)), *S. intermedius,* and *B. imberi*) from five different families (Alestidae, Clariidae, Cichlidae, *Schilbeidae* and Cyprinidae) were collected during the November 2018 survey (Appendix C3). The macroinvertebrates collected were Corixidae, Belostomatidae, small and large individuals of Dytiscidae, Notonectidae, and Pisauridae. In Lake Nyamithi, only five fish species (*H. vittatus*, *C. gariepinus*, *B. imberi*, *C. rendalli,* and *O. mossambicus*) from three different families (Alestidae, Clariidae, and Cichlidae) were collected during the November 2018 survey (Appendix C4). In Lake Nyamithi, macroinvertebrate families such as Gerridae, Nepidae, Atyidae, Gomphidae, Aspidytidae, Libellulidae, Belostomatidae, and Notonectidae were collected.

Trophic levels between the floodplain lakes within the NGR were dissimilar as food web components within Lake Shokwe were at least one trophic level higher than the components from Lake Nyamithi. This is specifically true for *H. vittatus*, *C. gariepinus,* and *B. imberi* while *O. mossambicus* and *C. rendalli* occupied similar trophic levels in both floodplain lakes (Figure 4.4a). Macroinvertebrates from Lake Shokwe occupied either the first or second trophic level with the predatory macroinvertebrates (Pisauridae, larger Dytiscidae individuals, and Notonectidae) being at least one trophic level higher (second trophic level) than other macroinvertebrate families (first trophic level). The primary producers had a similar trophic position to Corixidae, smaller Dytiscidae individuals, and Belostomatidae (Figure 4.5). In Lake Nyamithi, *H. vittatus* and *C. gariepinus* were the top predators (Figure 4.4b). No primary producers could be sampled in Lake Nyamithi during the November 2018 survey, thus the macroinvertebrate family, Belostomatidae, formed the base of the food web (Figure 4.7).

Isotopic signatures of aquatic biota within Lake Shokwe were significantly different from Lake Nyamithi except for $\delta^{15}N$ signatures in macroinvertebrates (p = 0.4227) (Appendix C3). Macroinvertebrates and fish from Lake Shokwe were significantly $\delta^{13}C$ depleted (Macroinvertebrates: p < 0.0001; fish: p < 0.0001) compared to macroinvertebrates and fish from Lake Nyamithi, while $\delta^{15}N$ signatures in fish from Lake Shokwe were significantly enriched (p < 0.0001) compared to Lake Nyamithi.

During the 2017 survey of Lake Nyamithi, 10 fish species (*O. mossambicus, H. vittatus*, *C. gariepinus*, threespot barb (*Enteromius trimaculatus* (Peters, 1852)), *E. afrohamiltoni*, *S. intermedius*, *S. zambezensis*, east coast barb (*Enteromius toppini* (Boulenger, 1916)), rednose Labeo (*Labeo rosae* (Steindachner, 1894)) and *B. imberi*) from six different families

(Alestidae, Clariidae, Cichlidae, Cyprinidae, Schilbeidae, Mochokidae) were collected, respectively (Appendix C5). In the February 2017 survey, a large variety of primary producers such as biofilm, detritus, leaf litter, Trapaceae, Poaceae, Hydrocharitaceae sp., and Cyperaceae were collected while macroinvertebrates families such as Hydrophillidae, Dytiscidae, Naucoridae, Corixidae, Coenagrionidae, Tetragnathidae, Belostomatidae, Nepidae and Macrobrachium sp. were collected (Figure 4.6a). During the November 2018 survey, the only macroinvertebrates collected were Gerridae, Nepidae, Atyidae, Gomphidae, Aspidytidae, Libellulidae, Belostomatidae, and Notonectidae. *Hydrocynus vittatus* and *C. gariepinus* were the top predators within Lake Nyamithi during the 2018 survey (Figure 4.6b).

During the 2018 survey, the majority of macroinvertebrates (Belostomatidae, Gomphidae, Nepidae, and Aspidytidae) from Lake Nyamithi occupied the first trophic level while the families Atyidae, Libellulidae, and Gerridae occupied the second trophic level along with two fish species (*C. rendalli* and *O. mossambicus*). Two fish species (*C. gariepinus* and *H. vittatus*) occupied the top of the food web (third trophic level) (Figure 4.7). The majority of fish species were near the top of the food web while *E. toppini* (Cyprinidae) occupied the top of the food web (Figure 4.8). Furthermore, *O. mossambicus* and *L. rosae* were at a similar trophic level to the majority of the primary producers and macroinvertebrates (1^{st} and 2^{nd} trophic level) while biofilm and detritus formed the base of the food web (Figure 4.8).

When comparing surveys in Lake Nyamithi (Appendix C4 and C5), macroinvertebrates were not significantly different in signatures ($\delta^{13}C$: p = 0.6997 & $\delta^{15}N$: p = 0.1326) while the $\delta^{13}C$ signatures in fish were significantly depleted during the 2018 survey compared to the 2017 survey (p = 0.0316).

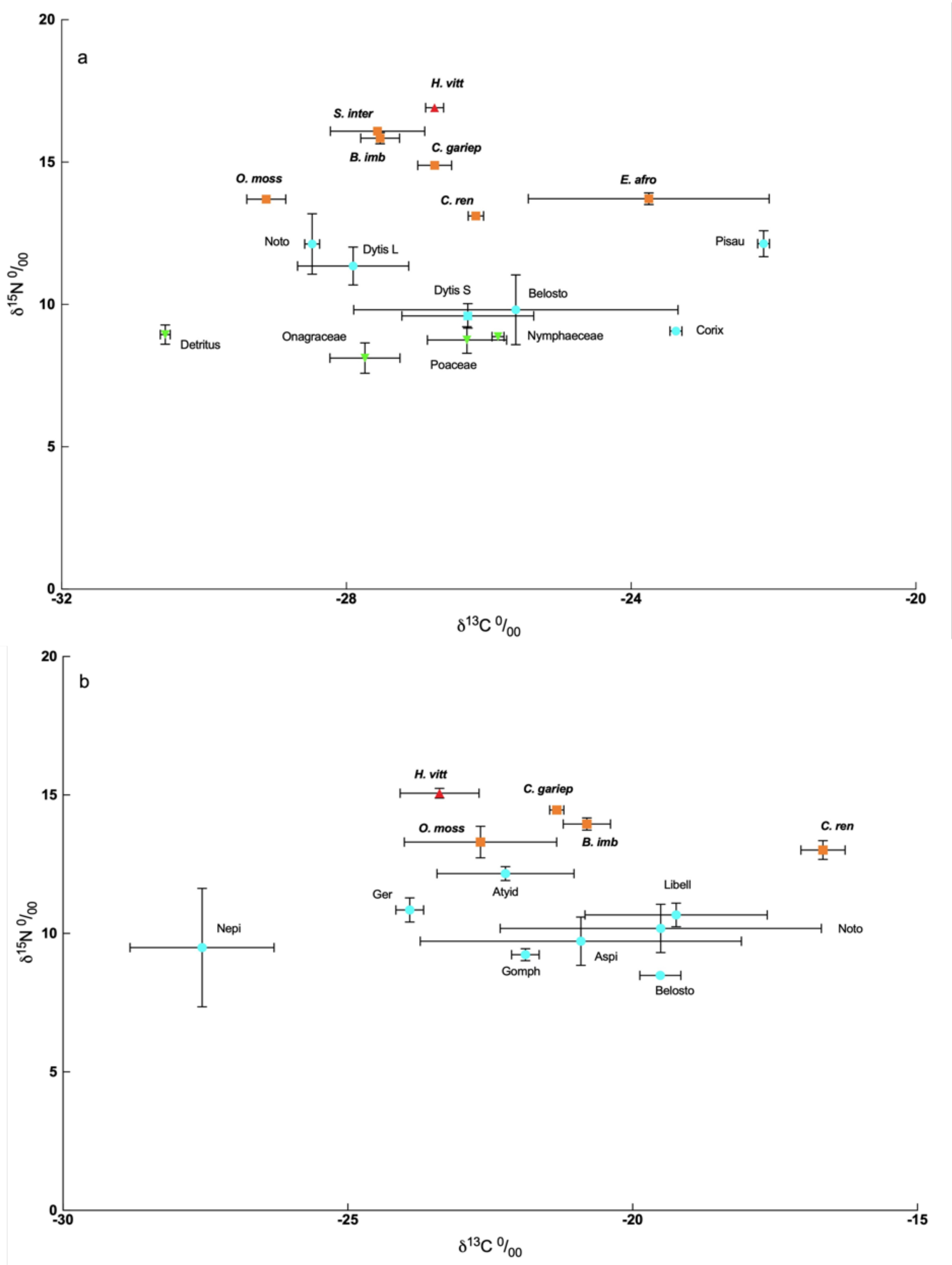


Figure 4.4: Biplots indicating the mean $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures (±1 SE) of the various food web components collected in Lake Shokwe (a) and Lake Nyamithi (b) within the Phongolo River Floodplain during the November 2018 survey. Full list of abbreviated macroinvertebrate and fish sample names are presented in Appendix C3 (Lake Shokwe) and C4 (Lake Nyamithi).

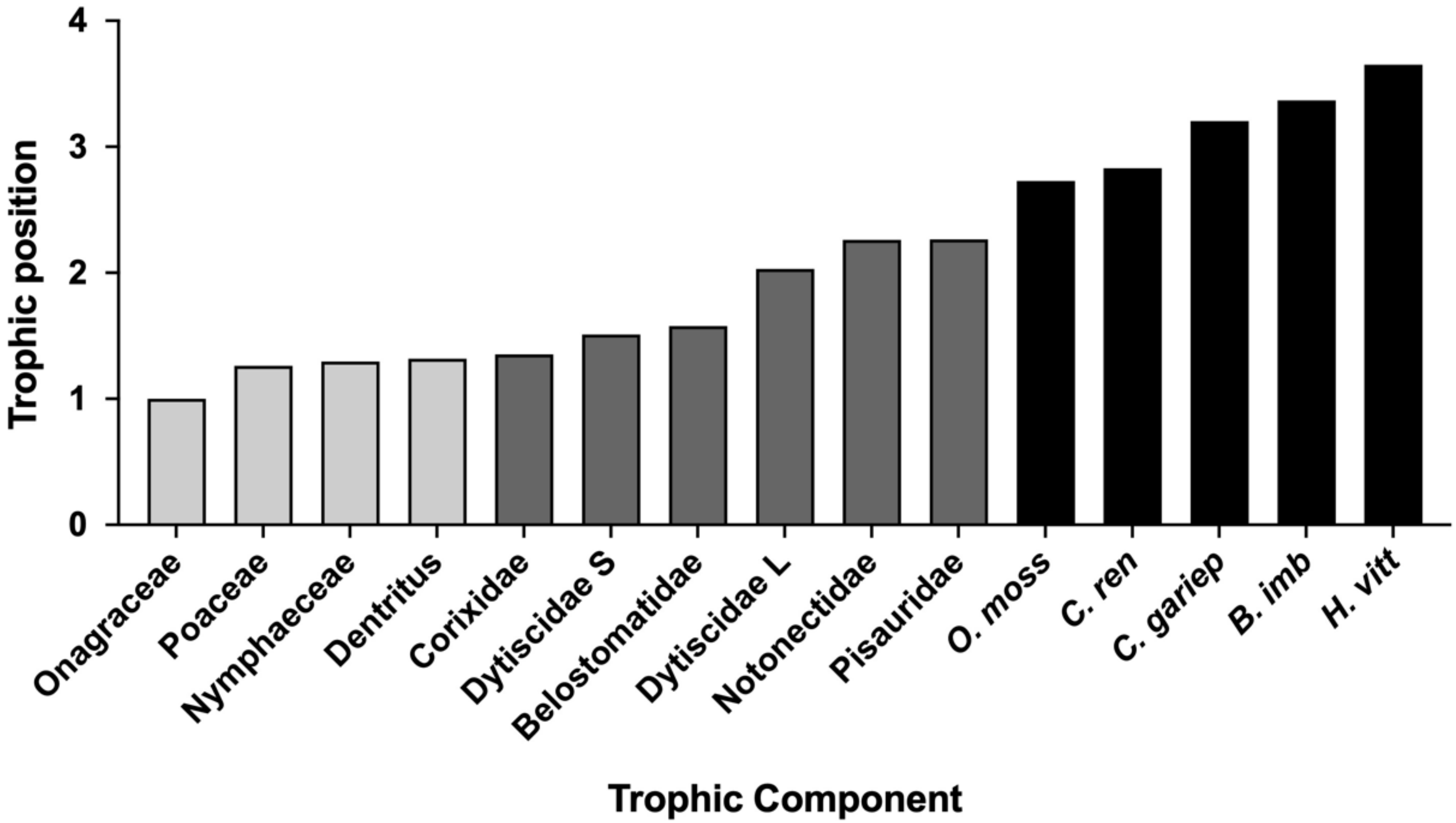


Figure 4.5: Mean trophic level of food web components collected in Lake Shokwe within the Phongolo River Floodplain during the November 2018 survey. Colour in the figure indicates the different components collected (Light grey = primary producers, dark grey = macroinvertebrates and black = fish species).

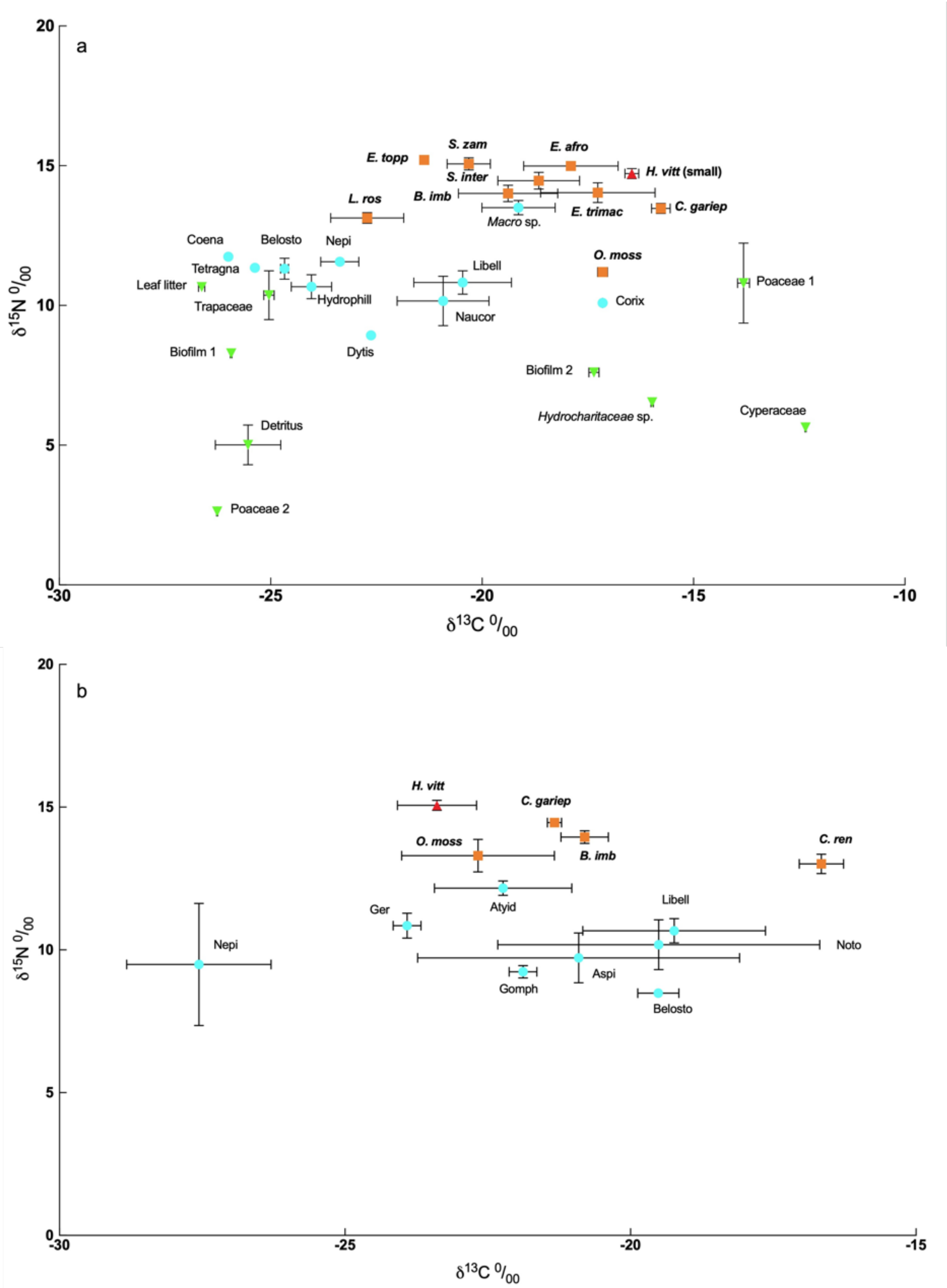


Figure 4.6: Biplots indicating the mean $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures (±1 SE) of the various food web components collected in Lake Nyamithi during February 2017 survey (a) and during November 2018 survey (b) within the Phongolo River Floodplain. Full list of abbreviated macroinvertebrate and fish sample names are presented in Appendix C5 (Lake Nyamithi).

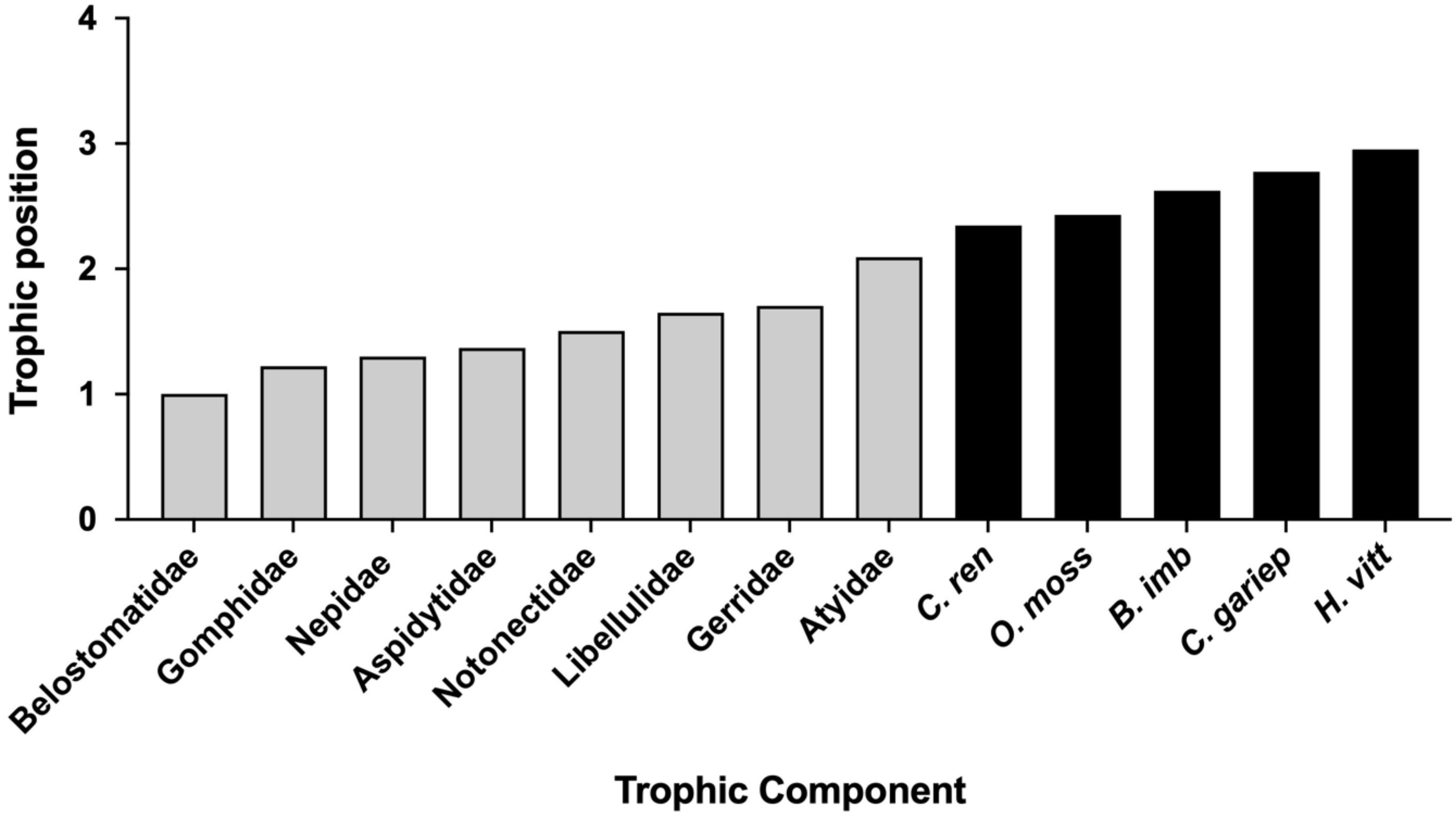


Figure 4.7: Mean trophic level of food web components collected in Lake Nyamithi within the Phongolo River Floodplain during the November 2018 survey. Colour in the figure indicates the different components collected (Light grey = primary producers, dark grey = macroinvertebrates and black = fish species).

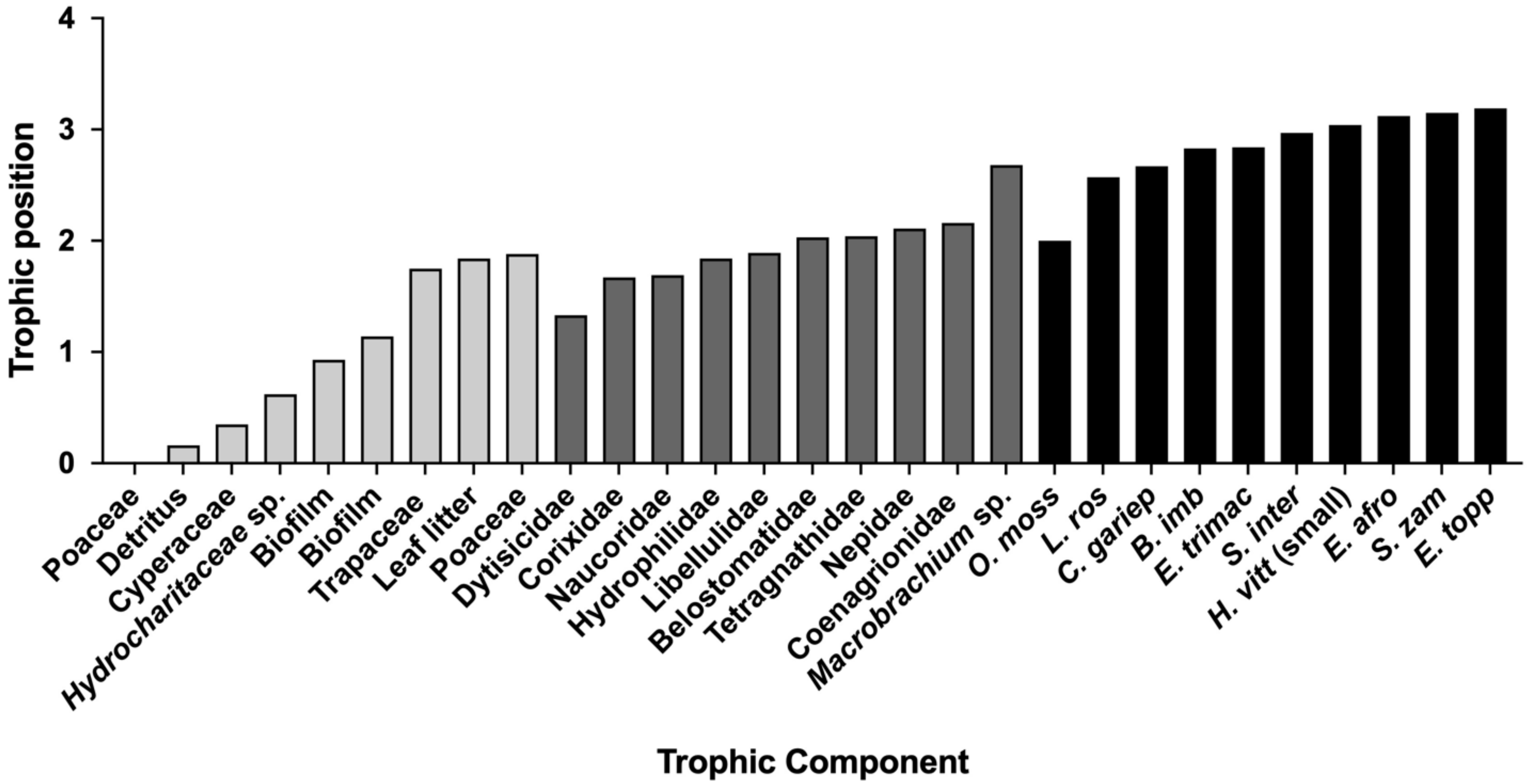


Figure 4.8: Mean trophic level of food web components collected in Lake Nyamithi within the Phongolo River Floodplain during the February 2017 survey. Colour in the figure indicates the different components collected (Light grey = primary producers, dark grey = macroinvertebrates and black = fish species).

4.3.2. *Dietary analysis of fish species*

4.3.2.1. Floodplain rivers

In the Usuthu River, the isoplot and credible intervals indicated that fish consumed an integrated mixture of all sources, with plant material (38%) and aquatic macroinvertebrates (50%) being consumed the most (Figure 4.9a; Table 4.1). Two fish species from the Usuthu River, namely *H. vittatus* and the southern Churchill *P. wesselsi* preyed mainly on fish (55% and 21%, respectively). In contrast with the Usuthu River, the food source that was mainly consumed in the Phongolo River, was C_3 plants as they formed the largest portion of the consumer's diet (37%) and C_4 plants the smallest portion (6%) (Figure 4.9b; Table 4.2). Two species of fish that preyed the most on fish were *P. wesselsi* and *Tilapia sparmanii* (Smith, 1840) (36% and 29%, respectively).

4.3.2.2. Floodplain lakes

During the 2017 survey, fish consumers from Lake Nyamithi equally preferred C_4 plants (32%) and macroinvertebrates (36%) as food sources (Figure 4.10a; Table 4.3). Consumers had less of a preference for C_3 plants (17%) and fish (15%). In addition, C_4 plants formed the largest portion of *O. mossambicus* diet (65%) while small *H. vittatus* preyed the most on lower trophic level fish species (26%). In the 2018 survey, detritus (30%), plant material (29%), and macroinvertebrates (29%) were consumed equally by consumers from Lake Nyamithi. Fish contributed the least (12%) to the diet (Figure 4.10b; Table 4.4). *Hydrocynus vittatus* (21%) together with *C. gariepinus* (18%) preyed on fish from Lake Nyamithi.

Consumers from Lake Shokwe preferred an integrated mixture of food sources with detritus (46%) forming the largest portion of the diet (Figure 4.11; Table 4.5). Plant material (33%) and fish (12%) formed a large portion of the overall diet of consumers while aquatic macroinvertebrates contributed the least (<10%) to their diet. The apex predator *H. vittatus* (30%) and the silver catfish *S. intermedius* (16%) largely preyed on fish.

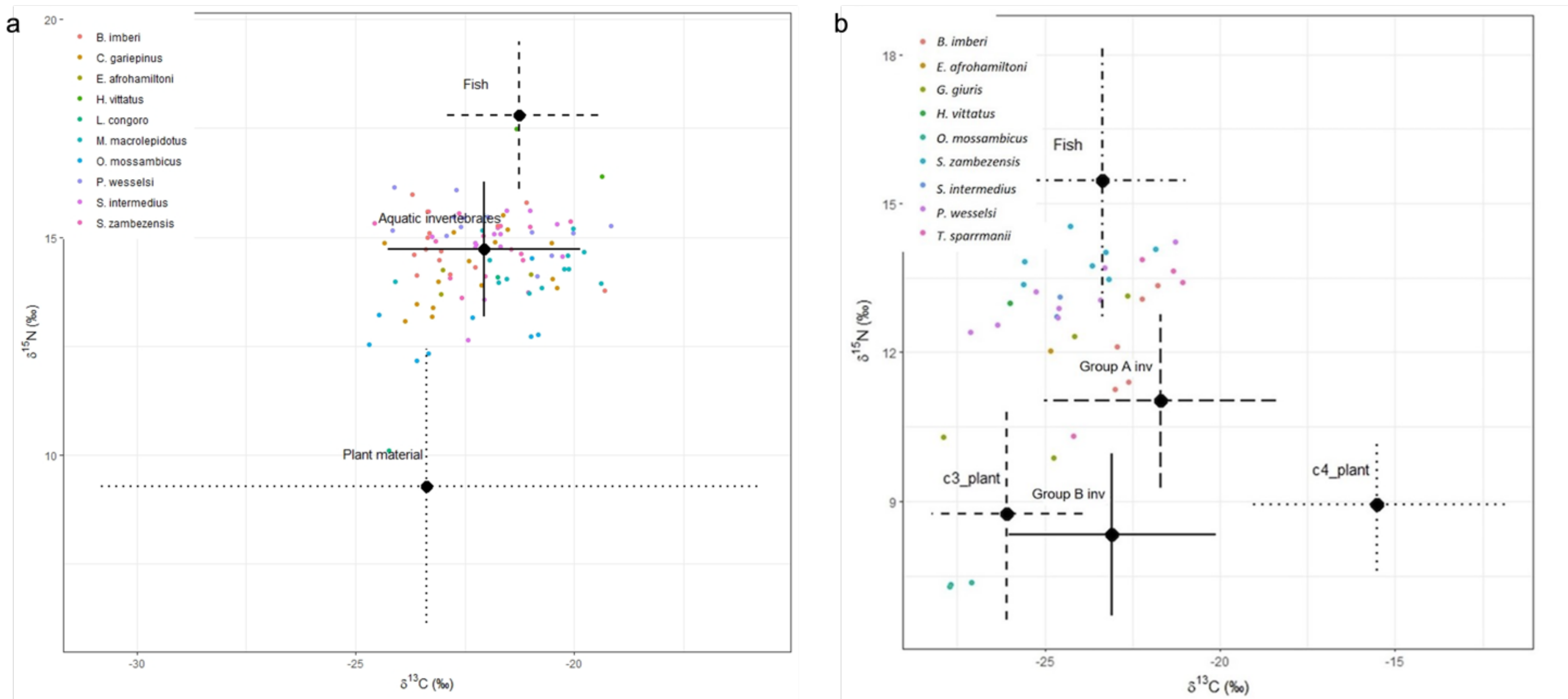


Figure 4.9: Biplot of the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of consumers (dots) and the potential food sources (error bars) collected in the Usuthu River (a) and Phongolo River (b) within the Phongolo River Floodplain during the November 2018 and September 2016 surveys (de Necker, 2019). Fish source and consumer data are abbreviated: Plant material – Aquatic vegetation; C_3 and C_4 plant – Aquatic vegetation; Aquatic invertebrates – Aquatic Macroinvertebrate families and Fish – Fish food sources. Error bars represent 95% confidence intervals. All taxa included in each source are presented in Appendix C3.

Table 4.1: MixSIAR dietary results indicating the contribution of potential food sources (%) that forms part of the diet of fish species collected in the Usuthu River within the Phongolo River Floodplain in November 2018. Data are presented as mean ± standard deviation (95% credible interval).

Sources	*Brycinus imberi*	*Clarias gariepinus*	*Enteromius afrohamiltoni*	*Hydrocynus vittatus*	*Labeo congoro*	*Marcusenius macrolepidotus*	*Oreochromis mossambicus*	*Petrocephalus wesselsi*	*Schilbe intermedius*	*Synodontis zambezensis*
Plant material	36±8.8 (23 – 51)	21±14 (3.3 – 48)	31±19 (4.7 – 66)	25±13 (4.9 – 48)	85±8.8 (75 – 94)	32±14 (5.7 – 55)	75±8.5 (61 – 86)	37±9.9 (21 – 54)	12±7.7 (2.6 – 27)	23±11 (5.6 – 43)
Macroinvertebrates	53±10 (36 – 69)	76±15 (48 – 95)	64±21 (26 – 93)	20±14 (4.6 – 43)	11±8.9 (3 – 23)	62±17 (34 – 92)	22±9.6 (7.9 – 37)	42±12 (23 – 61)	81±9.7 (62 – 94)	68±14 (44 – 89)
Fish	11±3.5 (4.9 – 16)	2.9±2.3 (0.5 – 7.3)	5.1±3.8 (0.8 – 13)	55±13 (36 – 76)	3.3±2.2 (0.6 – 7.5)	6.1±4.3 (0.8 – 14)	3.3±2.2 (0.6 – 7.7)	21±4.3 (14 – 28)	7.2±4.5 (1 – 15)	9.1±4.5 (1.1 – 17)

Table 4.2: MixSIAR dietary results indicating the contribution of potential food sources (%) that forms part of the diet of fish species collected in the Phongolo River within the Phongolo River Floodplain in September 2016 (Adapted from de Necker, 2019). Data are presented as mean ± standard deviation (95% credible interval).

Sources	*Brycinus imberi*	*Enteromius afrohamiltoni*	*Glossogobius giuris*	*Hydrocynus vittatus*	*Oreochromis mossambicus*	*Petrocephalus wesselsi*	*Schilbe intermedius*	*Synodontis zambezensis*	*Tilapia sparmanii*
C_3 plants	33±12 (9.4 – 57)	34±22 (2.8 – 80)	45±24 (2.9 – 84)	37±24 (2.8 – 83)	62±34 (1.4 – 96)	33±13 (4.9 – 57)	33±21 (2.9 – 78)	37±17 (4.4 – 67)	21±14 (2.4 – 54)
C_4 plants	7.5±6.2 (0.3 – 23)	6.3±7.5 (0.1 – 28)	6.8±9.2 (0.1 – 34)	4.4±5 (0.1 – 18)	5±6.2 (0.1 – 22)	2.3±2.6 (0.0 – 9.7)	5.4±5 (0.1 – 28)	4.4±4.7 (0.1 – 16)	11±10 (0.1 – 36)
Group A macroinvertebrates	16±10 (1.1 – 39)	16±16 (0.4 – 60)	14±15 (0.4 – 57)	14±15 (0.4 – 59)	4±3.9 (0.2 – 15)	12±8.8 (0.5 – 33)	15±15 (0.5 – 59)	11±9.8 (0.4 – 37)	21±17 (0.5 – 59)
Group B macroinvertebrates	21±12 (1.5 – 46)	21±18 (0.6 – 67)	23±20 (0.4 – 68)	19±18 (0.6 – 62)	29±33 (0.2 – 91)	16±11 (0.8 – 39)	18±16 (0.5 – 59)	18±13 (0.7 – 48)	18±14 (0.6 – 52)
Fish	23±6.4 (12 – 36)	22±16 (2.2 – 64)	13±8.3 (2.4 – 33)	25±17 (2.8 – 67)	2.9±2.3 (0.4 – 8.4)	36±9.1 (20 – 56)	27±17 (3.2 – 66)	30±9.7 (13 – 52)	29±12 (9.1 – 58)

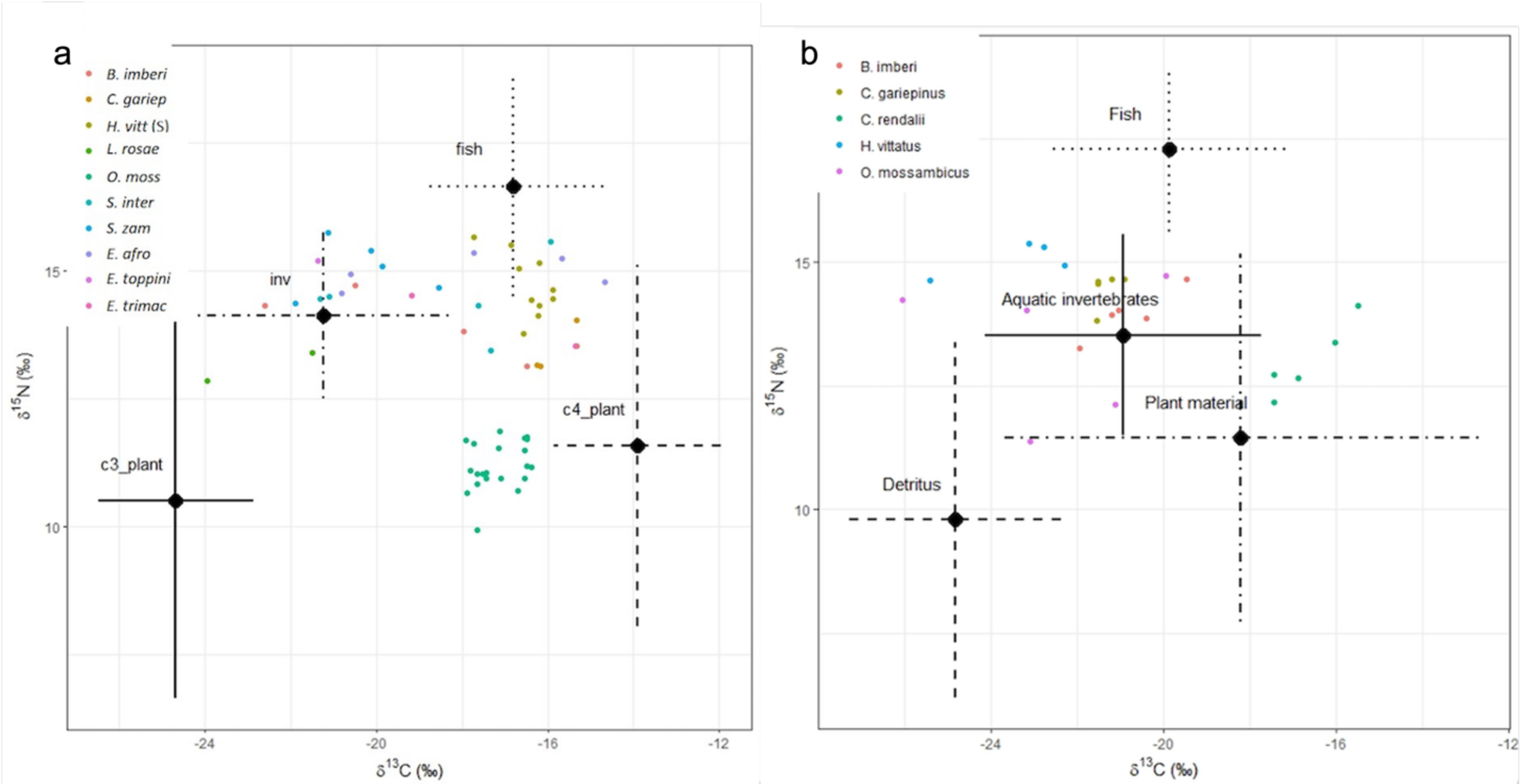


Figure 4.10: Biplot of the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of consumers (dots) and the potential food sources (error bars) collected in Lake Nyamithi 2017 (a) and Lake Nyamithi 2018 (b) within the Phongolo River Floodplain. Fish source and consumer data are abbreviated: Detritus – Detritus; C_3 and C_4 plant – Aquatic vegetation; Plant material – Aquatic vegetation; Aquatic invertebrates – Aquatic Macroinvertebrate families and Fish – Fish food sources. Error bars represent 95% confidence intervals. All taxa included in each source are presented in Appendixes C4 and C5.

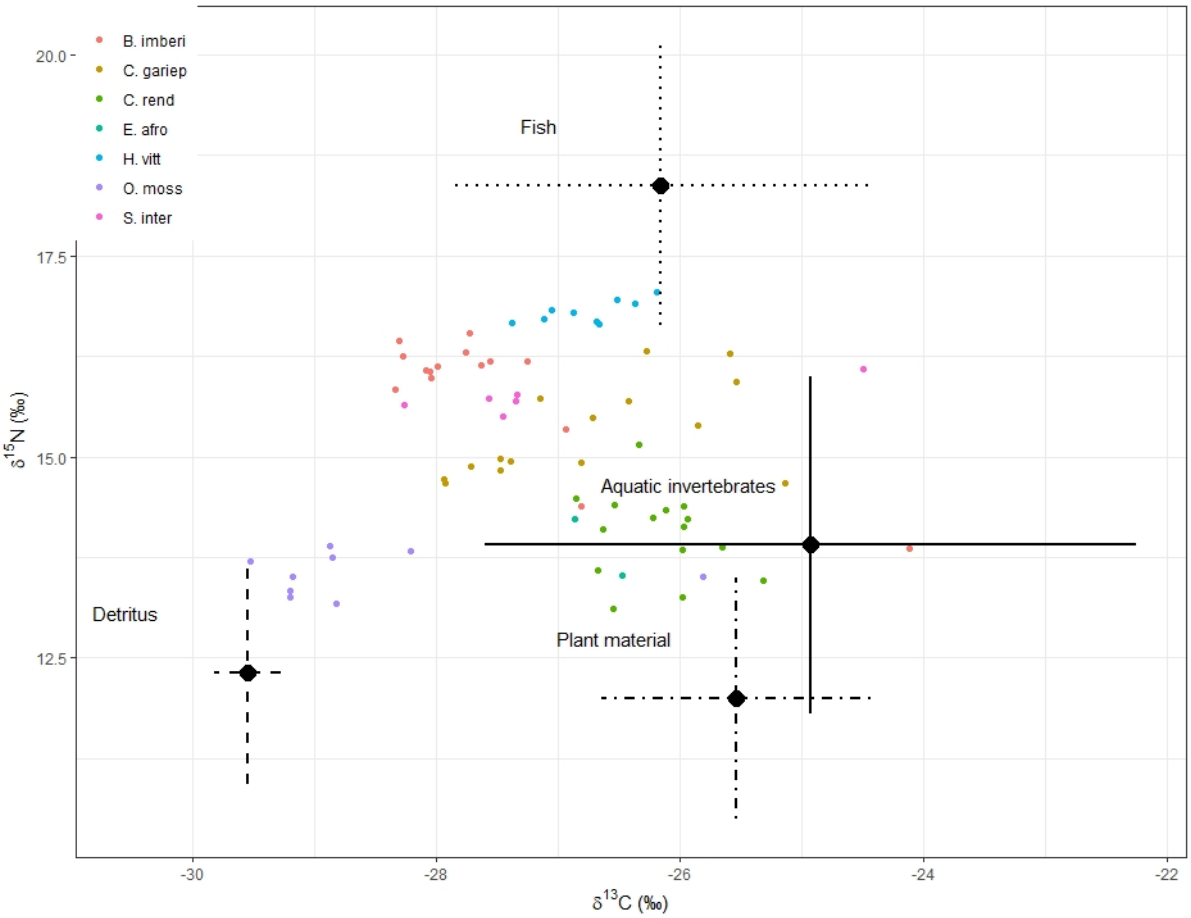


Figure 4.11: Biplot of the δ^{13}C and δ^{15}N isotopic signatures of consumers (dots) and the potential food sources (error bars) collected in Lake Shokwe within the Phongolo River Floodplain during the November 2018 survey. Fish source and consumer data are abbreviated: Detritus – Detritus; Plant material – Aquatic vegetation; Aquatic invertebrates – Aquatic Macroinvertebrate families and Fish – Fish food sources. Error bars represent 95% confidence intervals. All taxa included in each source are presented in Appendixes C3.

Table 4.3: MixSIAR dietary results indicating the contribution of potential food sources (%) that forms part of the diet of fish species collected in Lake Nyamithi within the Phongolo River Floodplain in February 2017 (Adapted from de Necker *et al*. (2022a). Data are presented as mean ± standard deviation (95% credible interval).

Sources	*Brycinus imberi*	*Clarias gariepinus*	*Hydrocynus vittatus* (small)	*Labeo rosae*	*Oreochromis mossambicus*	*Schilbe intermedius*	*Synodontis zambezensis*	*Enteromius afrohamiltoni*	*Enteromius toppini*	*Enteromius trimaculatus*
C_3 plants	17±7.9 (4.8 – 35)	8.1±5.9 (0.9 – 22)	8±4.7 (1.1 – 19)	32±23 (1.1 – 74)	24±5.5 (12 – 34)	12±8.6 (1.2 – 33)	23±13 (1.9 – 50)	8.8±6.5 (1 – 25)	22±18 (1.1 – 64)	11±8.4 (1 – 32)
C_4 plants	29±8.3 (14 – 46)	58±15 (19 – 82)	45±11 (23 – 65)	13±8 (1.8 – 31)	65±4.5 (55 – 73)	24±13 (3.5 – 50)	16±8.8 (2.9 – 35)	25±15 (3.3 – 59)	18±13 (2.1 – 49)	35±20 (20 – 73)
Macroinvertebrates	40±12 (15 – 62)	22±14 (3.4 – 59)	21±9.8 (4.9 – 43)	47±25 (4.4 – 90)	9.2±5.8 (1.6 – 24)	48±17 (12 – 81)	41±18 (7.7 – 77)	44±19 (7.3 – 80)	45±22 (5.7 – 87)	38±22 (4.5 – 83)
Fish	14±6.4 (3.9 – 29)	12±10 (0.9 – 38)	26±11 (6.2 – 49)	7.7±6.4 (0.6 – 24)	2.1±1.4 (0.4 – 5.5	16±12 (1.2 – 48)	19±11 (1.6 – 43)	23±16 (1.4 – 62)	15±13 (0.8 – 47)	16±15 (0.8 – 56)

Table 4.4: MixSIAR dietary results indicating the contribution of potential food sources (%) that forms part of the diet of fish species collected in Lake Nyamithi within the Phongolo River Floodplain in November 2018. Data are presented as mean ± standard deviation (95% credible interval).

Sources	*Brycinus imberi*	*Clarias gariepinus*	*Coptodon rendalli*	*Hydrocyus vittatus*	*Oreochromis mossambicus*
Plant material	27±13 (5.5 – 48)	17±11 (2.7 – 36)	60±25 (2.4 – 88)	13±11 (1.6 – 34)	26±22 (1.4 – 71)
Detritus	28±11 (10 – 45)	26±13 (5.8 – 48)	9.5±7.4 (1.3 – 24)	43±19 (6.4 – 69)	45±24 (3.2 – 78)
Macroinvertebrates	34±16 (9.3 – 60)	39±16 (12 – 66)	26±25 (2.2 – 88)	24±19 (2.9 – 63)	23±23 (2.3 – 82)
Fish	12±4.3 (5.6 – 20)	18±6 (8.3 – 28)	4.1±2.8 (0.7 – 9.4)	21±7 (9.2 – 32)	5.7±3.2 (1.2 – 11)

Table 4.5: MixSIAR dietary results indicating the contribution of potential food sources (%) that forms part of the diet of fish species collected in Lake Shokwe within the Phongolo River Floodplain in November 2018. Data are presented as mean ± standard deviation (95% credible interval).

Sources	*Brycinus imberi*	*Clarias gariepinus*	*Coptodon rendalli*	*Enteromius afrohamiltoni*	*Hydrocyus vittatus*	*Oreochromis mossambicus*	*Schilbe intermedius*
Plant material	23±13 (7.6 – 49)	34±21 (3.3 – 67)	62±15 (34 – 84)	41±24 (3.9 – 80)	31±13 (8.9 – 52)	17±16 (1.1 – 52)	23±16 (3 – 52)
Detritus	56±15 (24 – 75)	42±16 (14 – 66)	22±11 (5.7 – 41)	43±22 (9.2 – 82)	33±12 (12 – 52)	76±18 (37 – 96)	52±17 (21 – 77)
Macroinvertebrates	7.1±6.3 (0.8 – 19)	12±13 (0.3 – 39)	10±11 (0.3 – 31)	12±14 (0.3 – 43)	6.8±5.4 (0.5 – 17)	4.2±5.4 (0.2 – 15)	10±10 (0.4 – 30)
Fish	15±3 (11 – 20)	13±3.4 (8.6 – 19)	5±1.3 (3 – 7.1)	4.4±2.2 (1.7 – 8)	30±5.6 (22 – 40)	2.2±0.9 (0.9 – 3.7)	16±4.2 (10 – 24)

4.3.3. Strontium isotope analysis

The $^{87}Sr/^{86}Sr$ ratios between the Usuthu River and Lake Shokwe were more similar than the Phongolo River and Lake Nyamithi (Figure 4.12). *Clarias gariepinus* from Lake Nyamithi had a very distinct $^{87}Sr/^{86}Sr$ ratio compared to conspecifics from all the other aquatic ecosystems while the *C. gariepinus* Phongolo River has a highly variable signal (Appendix C6). The *C. gariepinus* from the Usuthu River and its associated lake were not significantly different (p = 0.991) in $^{87}Sr/^{86}Sr$ ratios while both rivers were significantly different with the Usuthu River being significantly higher ($p < 0.038$). Between the floodplain lakes of the NGR, *C. gariepinus* from Lake Shokwe had significantly higher ($p < 0.001$) $^{87}Sr/^{86}Sr$ ratios than Lake Nyamithi.

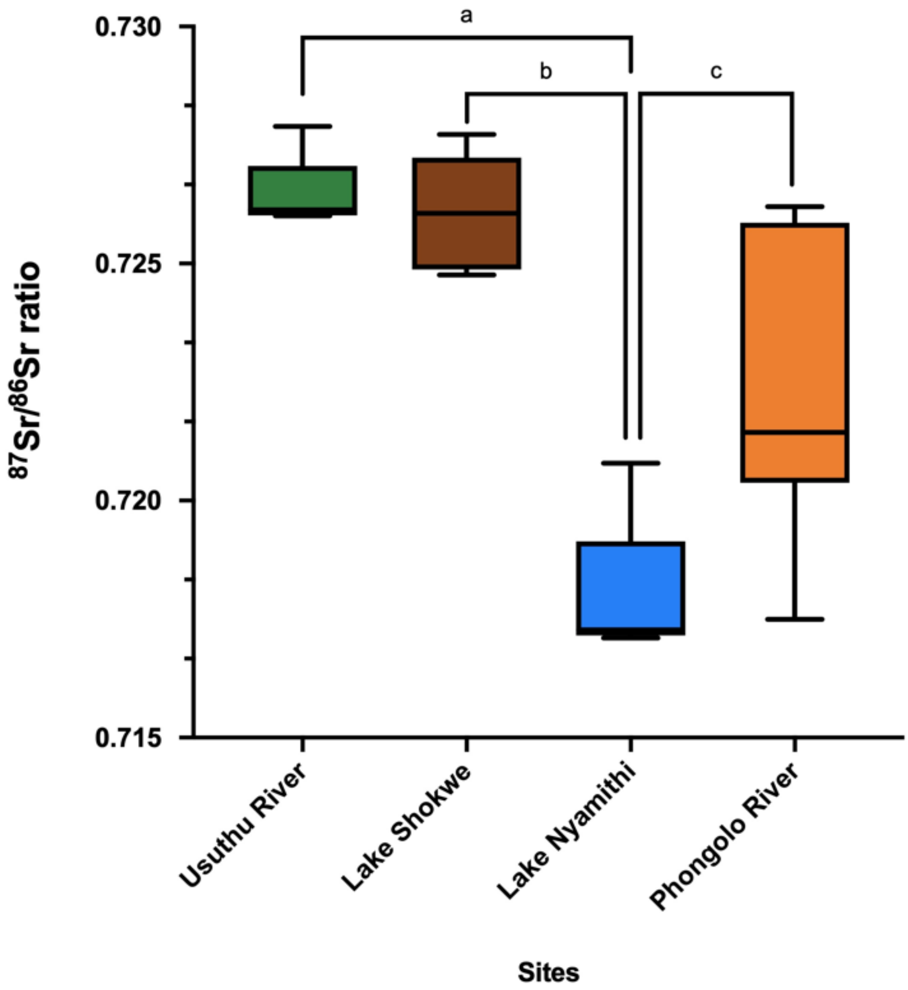


Figure 4.12: Strontium isotope analysis indicating the $^{87}Sr/^{86}Sr$ ratios in otoliths of *Clarias gariepinus* from the various aquatic systems within the Phongolo River Floodplain. Bars with common alphabetical superscript are indicate no significant differences ($p < 0.05$).

4.4. Discussion

Floodplain wetlands across the world are the most productive and biodiverse ecosystems but are also the most threatened (Whittington *et al*., 2013). These floodplain ecosystems face an array of challenges including anthropogenic activities that potentially can influence carbon sources, primary production, nutrient availability, and fish movement (Whittington *et al*., 2013; Beatty *et al*., 2017; Malherbe, 2018; de Necker *et al*., 2020; Oeding *et al*., 2020). These influences were observed in the aquatic systems within the NGR during the present study, including changes in $\delta^{13}C$ and $\delta^{15}N$ isotope signatures, alterations of food sources, and fish movement between the aquatic systems of the PRF. Both the significant enrichment and depletion of $\delta^{15}N$ and $\delta^{13}C$ values, respectively, indicated the difference in anthropogenic impacts associated with both rivers and associated floodplain lakes. Strontium isotopic values of *C. gariepinus* indicated the movement between the various aquatic systems while illustrating the use of the various aquatic systems.

4.4.1. Floodplain rivers

The various food web components of the Usuthu River were significantly enriched in the heavier nitrogen isotope compared to the Phongolo River. This corresponds with findings in Chapter 2 where the unregulated Usuthu River had higher inorganic nutrient levels (NO_3 and PO_4). Increased nitrogen inputs into aquatic systems arise from anthropogenic activities such as agriculture (fertilisers), runoff from rainfall, and human wastewater (Bunn & Boon, 1993; Cabana & Rasmussen, 1996; Jones *et al*., 2018). Moreover, it is often found that agricultural practices near waterbodies lead to nitrogen enrichment as a result of organic fertilisers and pesticides (Dudgeon, 2008; Al-Shami *et al*., 2011). Agricultural activities are also found in the upper catchments of the Usuthu River (Kowalkowski *et al*., 2007; Shiba *et al*., 2020). A previous study on the Phongolo River (de Necker *et al*., 2019) indicated that the river receives numerous nitrogen inputs through agricultural activities. However, de Necker *et al*. (2019) also indicated that the Pongolapoort Dam retains nutrients from the upper catchment and therefore results in lower nutrient concentrations downstream of the dam. Importantly, research has shown that altering the flow of a river reduces the nutrient supply (Van Cappellen & Maavara, 2016; Palinkas *et al*., 2019; Zhang *et al*., 2019; de Necker *et al*., 2019).

Furthermore, animal waste was also found to be increasing nutrients in river systems (Mthembu *et al*., 2020). Although increased nutrient inputs into both systems have been recorded (de Necker *et al*., 2019; Mthembu *et al*., 2020), the natural flooding regime of the Usuthu River and the presence of many hippopotami (*Hippotamus amphibius*) pods in this reach of the river (personal observations) appear to have a much greater influence on the nitrogen isotopic signatures of aquatic biota than the agricultural activities in the Phongolo River. Similarly, Masese *et al*. (2018) found that sites in the Mara River catchment in Kenya where animal numbers were higher, contained higher $\delta^{15}N$ isotopes.

The significantly lower $\delta^{13}C$ in fish from the Phongolo River can be attributed to the reduced discharge and water velocity as a result of the prolonged drought since lower discharge is known to cause fish to rely on riparian plants with lower $\delta^{13}C$ and therefore could result in lower $\delta^{13}C$ signatures in fish (Freedman *et al*., 2013). This was particularly evident as riparian plants in the Phongolo River were lower in $\delta^{13}C$ signatures. Zheng *et al*. (2018) found similar results in a large cross-border river in China, fish species further away from the dam (middle and lower reaches of the river) were depleted in $\delta^{13}C$ values since they relied greatly on riparian plants with lower $\delta^{13}C$. Growns *et al*. (2014) found similar $\delta^{13}C$ depleted values in the shrimp *Paratya australiensis* as the distance from the dam increased. This corresponds with the depleted $\delta^{13}C$ values found in the aquatic biota of the Phongolo River within the NGR which is situated approximately 80 km downstream of the Pongolapoort Dam. Also, Freedman *et al*. (2013) found that fish species below impounded sites on the Allegheny River were depleted in $\delta^{13}C$ isotopes.

Interestingly, as decreased water velocity could cause $\delta^{13}C$ depletion in consumers (as mentioned previously), animals such as hippopotami could increase turbidity in aquatic systems that can limit primary production in rivers (Dutton *et al*., 2018). This could be particularly true for the Usuthu River as a great number of hippopotami pods exist in this reach of the river (Fritsch *et al*., 2020). Furthermore, light availability (directly affected by turbidity) is an important factor for photosynthesis and affects carbon cycling in aquatic plants (Lacoul & Freedman, 2006). Therefore, the lower $\delta^{13}C$ values in the aquatic vegetation of the Usuthu River could be attributed to high turbidity caused by hippopotami in the Usuthu River where primary production could be limited due to light availability. Similarly, Buchmann *et al*. (1996) found that low light conditions lowered $\delta^{13}C$ ratio in C_4 grasses.

Hydrocynus vittatus are commonly the apex predators in rivers and are specialist feeders (Skelton, 2001). Interestingly, the analysis also indicated that *H. vittatus* consumed plant material which is similar to the results obtained by de Necker (2019) where the diet of *H. vittatus* from the Phongolo River consisted of 37% C_3 plant material. Oliveira *et al*. (2006) cautioned that it is possible for consumers such as *H. vittatus* to indirectly incorporate isotopic signatures of plant material when they consume food sources such as macroinvertebrates and insects with similar isotopic signatures as plant material. Therefore, it is very likely that the fish and aquatic macroinvertebrates preyed on by *H. vittatus*, reflect the isotopic signature of plant material within the tigerfish.

4.4.2. Floodplain lakes

In the floodplain lakes of the NGR, more food web components were collected which could be attributed to suitable conditions within the lakes, as the standing water can provide more time for floating and rooted plants to establish. Bornette & Puijalon (2011) explained that the movement of water could cause aquatic plants to be uprooted or break as currents and waves influences plant growth and development. Furthermore, aquatic plant growth in lakes is favoured through exposure to water movement (incoming water from the river) together with light attenuation (Bornette & Puijalon, 2011) as the movement of water reduces particle and nutrient resuspension that potentially limits light penetration (Madsen *et al*., 2001). During the present study, the lack of water movement during the LF survey could enhance favourable conditions (i.e., increased light penetration) for plant growth. Food web components within Lake Shokwe were significantly depleted in $\delta^{13}C$ values and significantly enriched in $\delta^{15}N$ values. Consumer carbon signatures were likely lower because consumers were feeding on other components that were lower in carbon (i.e., riparian vegetation). Carbon depletion can occur when food sources are consumed that are more depleted in carbon (Bunn & Boon, 1993). Significantly enriched $\delta^{15}N$ values in food components of Lake Shokwe corresponds with the findings in the Usuthu River. This is indicative of the influence of the hydrological connectivity of the Usuthu River and its associated floodplain lake.

Lake Nyamithi showed very different results between surveys, with the survey in 2017 (de Necker *et al*., 2022a) having a longer food web (three trophic levels) than the survey in 2018 (two trophic levels). The main difference in food webs was the different primary producers collected between the two surveys. During the 2018 survey, no plant source could be collected as none were present due to the lowered lake levels compared to 2017. Lake Nyamithi is less suitable for plant growth due to its saline sediments (see Chapter 2, section 2.4.1). According to Nielsen *et al*. (2003a and 2003b), aquatic vegetation can be susceptible to increasing salinity. Nielsen *et al*. (2003a) found that germination and establishment of aquatic plants decreased when salinity increased in two wetlands in Australia. Moreover, Nielsen *et al*. (2003a) found that with the increase in salinity, there was a reduction in abundance and ultimately a loss of aquatic plant taxa. In a mesocosm study by Tootoonchi & Gettys (2019), *Hydrilla verticillata* responded negatively to increased salinity. The biomass of *H. verticillata* decreased as the salinity increased. Furthermore, through flooding of rivers, the influx of water could reduce the salinity and therefore facilitate seed replenishment and macrophytes can re-establish. This was particularly evident in the study by de Necker *et al*. (2022a) which found an increase in aquatic macrophytes and macroinvertebrates after Lake Nyamithi received water through back flooding from the Usuthu River in a 2017 survey. Bornette & Puijalon (2011) explained that the movement of water plays a large role in the dispersal of seeds or vegetative fragments of aquatic vegetation. More importantly, connectivity to the river through flooding provides the exchange of nutrient-rich sediment, water, high diversity of macrophytes, macroinvertebrates, and fish (Aarts *et al*., 2004; Paillex *et al*., 2007; Rocha *et al*., 2012).

Fish in Lake Shokwe largely relied on aquatic vegetation and detritus and the apex predator, *H. vittatus*, preferred fish, while also reflecting the isotopic signature of aquatic vegetation and detritus. These findings are similar to what was found in the rivers. Therefore, it is also likely that the aquatic vegetation isotopic signature in *H. vittatus* is a result of feeding on prey that has isotopic signatures similar to those basal sources. Peel *et al*. (2019) explained that one of the pathways for carbon to be assimilated is through aquatic macroinvertebrates feeding on detritus. Detritus was largely the preferred food source for consumers within Lake Shokwe. Detrital material forms part of the primary foundation in many aquatic food webs and aquatic macroinvertebrates are important processors of detritus (Wissinger *et al*., 2021). Therefore, fish from Lake Shokwe could reflect the isotopic signature of detritus through the consumption of macroinvertebrates and fish that had a similar isotopic signature to detritus and aquatic vegetation. Similarly, Adis and Victoria (2001) found that insects reflected the isotopic signatures of both C_3 and C_4 plants they consumed.

Coptodon rendalli, is regarded as an insectivore and herbivore (Skelton, 2001). Aquatic vegetation (62%) made the largest contribution to the diet of *C. rendalli*, followed by detritus (22%) and aquatic macroinvertebrates (10%). This is in line with what Peel *et al*. (2019) found for *C. rendalli* from Lake Liambezi (an ephemeral floodplain lake). They relied primarily on aquatic vegetation, filamentous algae, detritus, and particulate organic matter. Peel *et al*. (2019) further explained that the presence of detritus in the diet of *C. rendalli* was due to the sampled detritus possibly being of submerged aquatic vegetation origin. This could also be true for the large detrital signal found for *O mossambicus* (76%). Similarly, Teferra *et al*. (2003) and Zengeya *et al*. (2011) found that *O. mossambicus* from the Gaborone Dam and the Limpopo River predominantly consumed detritus.

Mixing models indicated that consumers within Lake Nyamithi relied primarily on C_4 basal sources during the drought recovery period in 2017 (de Necker *et al*., 2022a). A study on estuaries (known to have higher salinity than freshwater systems) in East Africa, indicated the same dominance of C_4 plants in consumers' diets (Abrantes *et al*., 2013). Importantly, de

Necker *et al*. (2022a) further indicated that due to consumer's reliance on C_4 basal sources, the lake was more productive after the prolonged drought (2015 – 2017) while during the recovery period (2017) there was a shift from phytoplankton to macrophyte production. During the present study (2018), detritus (30%), aquatic vegetation (29%), and macroinvertebrates (29%) made the largest contribution to the diet of consumers from Lake Nyamithi. Fish as a food source was the least consumed (12%). Similarly, in 2017, de Necker *et al*. (2022a) also found consumers to be non-specific in their feeding behaviour and that they feed across a variety of sources. Moreover, this non-specific omnivorous feeding phenomenon is an indication of stabilisation of food webs, which in turn causes greater adaptability and maintains adaptable and high diversity of biological communities (McMeans *et al*., 2015; Ward and McCann, 2017). In addition, having a flexible feeding behaviour not only ensures survival but also ensures greater biotic interactions (de Necker *et al*., 2022a).

Moreover, data indicated a large variation in $\delta^{13}C$ isotopes across the trophic levels of the floodplain lakes. This indicates that consumers in floodplain lakes feed on a variety of food sources and therefore have a large variation in carbon values. Multiple fish species were on a similar trophic level, indicating that there was likely some overlap in their diet and isotopic niche use. This is in line with the study of de Necker *et al*. (2022a), which also found that *L. rosae*, *C. gariepinus,* and *O. mossambicus* preferred similar resources within Lake Nyamithi. Similarly, Peel *et al*. (2019) found that two species (S. *intermedius* and *Clarias ngamensis* (Castelnau, 1861)) shared a similar isotopic niche space due to similar feeding behaviour (i.e., feeding on a wide variety of decapods, molluscs, insects, and fish).

4.4.3. Strontium isotopes

Strontium isotopes have been successfully used to track the movement of freshwater fish species as well as their natal origin (Capo *et al*., 1998; Hobbs *et al*., 2010; Wolff *et al*., 2012; Brennan *et al*., 2015; Jordaan *et al*., 2016; Feyrer *et al*., 2019; Saygun *et al*., 2022). The chemical composition of otoliths e.g., the $^{87}Sr/^{86}Sr$ ratios are driven by the underlying geology and this results in different $^{87}Sr/^{86}Sr$ ratios from water bodies (Brennan *et al*., 2014; Brennan *et al*., 2015; Jordaan *et al*., 2016). Since floodplain habitats such as Lakes Shokwe and Nyamithi are known to be important spawning habitats for floodplain riverine fish species, (Naus & Adams, 2016), it stands to reason that these systems may act as refugia for fish. This ensures the growth and recruitment of offspring due to favourable environmental conditions in floodplain lakes during drought events (Naus & Adams, 2016).

Clarias gariepinus is known as one of the few African freshwater species to migrate (Mbalassa *et al*., 2015) and have been recorded to migrating between lakes and nearby rivers, e.g., between Lake Edward (Mbalassa *et al*., 2015), Lake Kariba (Bowmaker, 1974) and Lake Victoria (Lung'Ayia, 1994) and associated river systems. Therefore, the $^{87}Sr/^{86}Sr$ ratios in otoliths of *C. gariepinus* were used to determine to what degree fish moved between rivers and floodplain lakes in the NGR. $^{87}Sr/^{86}Sr$ ratios in otoliths of *C. gariepinus* from the Usuthu River and Lake Shokwe (its associated floodplain lake) were very similar indicating that they were restricted to the Usuthu system. The large variation in $^{87}Sr/^{86}Sr$ ratios in otoliths of *C. gariepinus* from the Phongolo River indicates the possibility that the fish migrate between the Phongolo and other rivers such as the Usuthu (due to slight overlap in signal) and other tributaries further upstream. On the other hand, $^{87}Sr/^{86}Sr$ ratios in otoliths of *C. gariepinus* from Lake Nyamithi were notably different compared to all the other systems. This reflects the very specific water chemistry caused by the underlying geology of Lake Nyamithi.

4.5. Conclusion

In this chapter, food web structures of the Usuthu and Phongolo rivers and associated floodplain lakes (Shokwe and Nyamithi) were assessed and compared. Both the rivers and associated floodplain lakes were different in food web structure. Fish from the Usuthu River were nitrogen enriched. This is indicative of the difference in flow regimes and water quality of the two rivers. Food web structure of Lake Shokwe was different from Lake Nyamithi due to different carbon energy sources collected within the lake. This is a result of differences in water quality between the freshwater and saline lake as the high salinity is unsuitable for aquatic plant growth. Furthermore, components within Lake Shokwe were significantly lower in $\delta^{13}C$ while also significantly enriched in $\delta^{15}N$ than components from Lake Nyamithi. The lower carbon values were linked to the consumption of riparian plants with a lower $\delta^{13}C$ ratio while the enrichment in nitrogen could have been influenced by the water quality from the Usuthu River.

The differences in $^{87}Sr/^{86}Sr$ ratios in otoliths from *C. gariepinus* between the Usuthu River and its associated floodplain lake were very similar, and this is indicative of their restricted movement between the Usuthu system. The large $^{87}Sr/^{86}Sr$ signature variation in the Phongolo River indicated that *C. gariepinus* are rather migrating between the Phongolo and other rivers. Furthermore, the slight overlap with the Usuthu River indicates the movement of *C. gariepinus* between the Phongolo and Usuthu rivers. Interestingly, the water chemistry (due to the underlying geology) of Lake Nyamithi was rather the cause for the significantly different $^{87}Sr/^{86}Sr$ ratios in *C. gariepinus*. The data from the $^{87}Sr/^{86}Sr$ isotopes indicated the movement of *C. gariepinus* between the various systems and thus could act as a refuge for one another during periods of drought. However, due to no water sample to compare the $^{87}Sr/^{86}Sr$ ratio in fish otoliths to, there are certain limitations of this part of the study as it is difficult to draw concrete conclusions from the data.

Lastly, since food web structures have been established, Hg concentrations in food web components will be investigated to determine the degree of contamination as well as the transfer of Hg through the food web and subsequently, the human health risks associated with the consumption of Hg-contaminated fish.

CHAPTER 5
Bioaccumulation and trophic transfer of total mercury through the aquatic food webs of an African sub-tropical Ramsar wetland

5.1. Introduction

Mercury is a naturally occurring element (Streets *et al*., 2019). However, due to human activities such as coal and gold mining, as well as coal and fuel combustion, concentrations have increased in the environment (see Chapter 1, section 1.3 for a description of anthropogenic activities in the upper catchments of the Usuthu – Phongolo rivers catchment) (Dabrowski *et al*., 2008, Williams *et al*., 2010, Martinez *et al*., 2018 & Verhaert *et al*., 2019). South Africa is one of the major producers of coal and gold in the world (Oosthuizen *et al*., 2010) and these activities are regarded as the main sources of Hg pollution in Southern Africa (Walters *et al*., 2011; Verhaert *et al*., 2019). While point sources of Hg have been studied extensively in freshwater systems in South Africa (Oosthuizen & Ehrlich, 2001; Papu-Zamxaka *et al*., 2010; Oosthuizen *et al*., 2010; Walters *et al*., 2011; Malehase *et al*., 2016; Govaerts *et al*., 2018; Plessl *et al*., 2019; Verhaert *et al*., 2019), limited research has been completed on waterbodies that can potentially be impacted on by Hg aerial deposition. One such area is the Phongolo River Floodplain (PRF) which falls within South Africa's dominant atmospheric transport pattern that flows from South Africa's Highveld, home to the majority of the country's 18 coal fire power stations, to the east coast of southern Africa (Freiman and Piketh, 2003).

The chemical forms of Hg in the environment affect its biogeochemical fate and transport, bioaccumulation, and toxicity in the environment (Li *et al*., 2012). The predominant Hg species in the aquatic environment are inorganic and elemental Hg (Verhaert *et al*., 2019). Furthermore, a small portion of Hg is methylated by bacteria to form methylated mercury (MeHg) (Shao *et al*., 2012). Methyl mercury is the most toxic form, readily available for uptake, and biomagnifies through the food webs (Verhaert *et al*., 2019). Most importantly, between 75 and 100% of total Hg (THg) found in fish species is present as MeHg (Shao *et al*., 2012; Łuczyńska *et al*., 2017; Arcagni *et al*., 2018; Verhaert *et al*., 2019).

In aquatic systems, Hg adsorbs to suspended particles, which then play an important role in the transport of Hg through aquatic ecosystems and cause the precipitation of particle-bound Hg onto sediments (Ullrich *et al*., 2001). In aquatic systems, the total organic carbon content (TOC) of sediment affects Hg bioavailability as various studies have shown that Hg sediment concentration increases as the TOC percentage increases in sediment (Williams *et al*., 2011; Govaerts *et al*., 2018; Verhaert *et al*., 2019; Gopikrishna *et al*., 2020; Lintern *et al*., 2020). Furthermore, in aquatic systems, the direct route for Hg uptake is predominantly through the diet while trophic pathways also play a significant part in Hg bioaccumulation in biota (Ullrich *et al*., 2001; Kidd *et al*., 2012; Gentès *et al*., 2021). The Trophic Magnification Factor (TMF) is used to determine biomagnification in ecosystems. In addition to size, age, lipid content, and metabolic rates of fish species, biomagnification rates, as well as trophic position (TP) all contribute to the bioaccumulation of Hg in fish species (Kidd *et al*., 2003; Hanna *et al*., 2015; Verhaert *et al*., 2019).

Elevated concentrations of Hg affect the health of not only wildlife but also humans relying on fish as a protein source (Black et al., 2011; Govaerts et al., 2018; Verhaert et al., 2019). The PRF and its natural resources such as fish are of great socio-ecological value (Lankford *et al*., 2010; Acosta *et al*., 2021). Fish of the PRF form an important part of the diet of the local community (Coetzee *et al*., 2015). and are also of conservation importance since many species are at their southernmost distribution range (Acosta *et al*., 2021). Thus, studying Hg in waterbodies associated with the PRF adds to the understanding of the dynamics of diffuse sources of Hg in these aquatic ecosystems as it provides insight into the deposition of Hg that moves from the highveld to the east coast. Furthermore, the environmental indices (as discussed in Chapter 2, section 2.4.3) indicated that water quality remained within limits suitable for healthy aquatic ecosystems. Aquatic macroinvertebrate community structures (see Chapter 3) showed the high biodiversity status of the PRF. Therefore, it is essential to monitor Hg levels in the PRF and the transfer through the food webs determined in Chapter 4, section 4.3.1.

This chapter aims to determine Hg contamination, accumulation, and biomagnification in two rivers and their associated freshwater floodplain lakes in the sub-tropical wetlands of the PRF as well as the human health risks associated with consuming fish from this region. This was achieved by 1) determining the Hg concentrations in sediment and biota; 2) determining the abiotic relationship between Hg sediment concentrations and TOC, 3) determining the biotic relationships between Hg in fish and intrinsic factors such as fish length and extrinsic factors such as diet, trophic position and transfer of Hg, and 4) determining the human health risks associated with consumption of Hg-contaminated fish.

5.2. Materials and Methods

5.2.1. Study area and sampling

The study area, mentioned in previous chapters, were the two floodplain rivers and associated floodplain lakes within the lower PRF (see Chapter 1, sections 1.2 & 1.3 for the study area description and also Figure 1.6). Sediments, aquatic vegetation, macroinvertebrates, and fish were collected from four sites within the NGR during two field surveys in June 2018 during the HF and in November 2018 during the low flow LF.

Sediments were collected from each site in triplicate in 1 L acid-washed polypropylene bottles using a stainless-steel auger and stored at -20 ºC until further analysis (see Chapter 2, section 2.2.1 for methods on how sediment was collected). From each site, all present aquatic plants and organic matter (leaf and grass litter, detritus, grasses, and reeds) were hand-picked and placed in polyethylene resealable bags. All plant samples collected were frozen in a field freezer and stored at -20 ºC until further analysis.

Aquatic macroinvertebrates were collected and identified, using the method described in Chapter 3, section 3.2.2.2. Multiple individuals from dominant macroinvertebrate families of each site were collected in four replicates de Necker *et al*. (2020) and placed in 15 mL Falcon tubes and frozen in a field freezer until further analysis. Representatives of different fish species occurring at each site were targeted using rod and line and cast netting techniques. Captured fish were identified using keys in Skelton (2001), and euthanised by means of cervical transection followed by pithing (Ethics number: NWU-00156-18-A5) and standard length (SL) measured. Axial muscle tissue was removed from the left side of each fish, placed in 15 mL Falcon tubes, and frozen in a field freezer (-20 ℃) before being transported to NWU for further analysis.

Detailed synopses of all macroinvertebrate families and fish species, number of individuals, and size of individuals collected in the waterbodies within the PRF during the present study are provided in Appendixes (D1 – D4).

5.2.2. Total organic carbon content (TOC)

For detailed description on sample preparation, analysis, and TOC calculations, please see Chapter 2, section 2.2.4.

5.2.3. Total mercury (THg)

5.2.3.1. Sample preparation

Sediments, plants, aquatic macroinvertebrates, and fish tissue were freeze-dried (FreeZone®[6], Labconco) for approximately 96 hr at -54 ºC. Dried muscle and sediment samples were digested using approximately 0.2 g tissue and placed in Teflon tubes before adding 7.5 mL 65% HNO_3 (supra pure quality, Merck) and 2.5 mL 37% HCl (supra pure quality, Merck) to each muscle sample, while 7.5 mL 32% HCl and 2.5 mL HNO_3 were added to each sediment sample (Erasmus *et al*., 2020). Samples were digested at 1,800 W and 200 °C in an Ethos Easy MAXI-44 Microwave Digestion system for 45 min. Following digestion, samples were decanted into 50 mL volumetric flasks and diluted with 1% HNO_3 up to 50 mL. Pooled macroinvertebrate families and aquatic vegetation were weighed and approximately 0.08 g of dried samples were placed in a Teflon multiple-cell container before adding 1 mL 65% HNO_3 per cell. Samples were digested under pressure at 60 ºC for 24 h and then decanted into a 15 mL Falcon tube before being diluted to 15 mL with 1% HNO_3 following the method of Wolmarans and van Aardt (1985). Digested samples were stored in 50 mL Falcon tubes at room temperature before analysis.

5.2.3.2. Total mercury analysis

Samples were analysed for THg using cold vapour atomic absorption spectrometry using a Flow Injection Mercury System (FIMS) - FIAS 400 (PerkinElmer, Germany), using $NaBH_4$ as a reducing agent and 3% HCl as carrier. Calibration of the FIMS was performed using a series of seven dilutions of Hg standard (PerkinElmer Pure mercury 1000 µg/mL standard in 10% HNO_3). With this calibration, the concentrations of Hg were calculated in the samples using the regression line with a correlation factor of ≥0.999. The limits of detection (LOD) and quantification (LOQ) were 0.71 ng/g and 2.13 ng/g and were calculated as three and nine times the standard deviation of the blank measurements, respectively. Sample replicates that were below the limit of detection were calculated through the frequency of occurrence method. This method uses the number of replicates below LOD divided by the total number of replicates and then multiplied by the lowest detected value. Importantly, as MeHg constitutes a large percentage of THg, only THg was analysed for the purposes of this study, and this includes both MeHg and inorganic Hg. Furthermore, all Hg reported for this chapter refers to THg.

5.2.3.3. *Quality control and quality assurance*

Quality control and assurance were carried out using certified reference material (CRM) for sediments (NCS DC 73310 Stream Sediment from the China National Analysis Centre for Iron & Steel) and fish (DORM-4 Fish protein certified reference material for trace metals from National Research Council Canada). Percentage recoveries of the aforementioned certified materials were all within a 15% deviation range (Table 5.1). For each round of digestions, relevant procedural blanks (n = 4) and CRMs (n = 3) were prepared in an exact manner as the samples and were run in triplicate for further analysis. The mean concentration of blanks that were analysed for the fish was 0.005 µg/L and for the sediment was 0.006 µg/L. Each sample (procedural blanks, CRMs, as well as sediment, macroinvertebrate, and fish samples) were analysed in triplicate and the mean concentration was used, while the percentage relative standard deviation (%RSD) was less than 10%.

Table 5.1: Recovery rates (%) and detection limits (ng/g) for both sediment and fish Certified Reference Materials.

Metal	Recovery Rates (%)		Detection limits (ng/g)	
	NCS DC 73310	DORM-4	Sediment	Fish
Mercury	111.3	98.7	0.33	0.41

5.2.4. *Stable isotope analysis (^{13}C, ^{15}N)*

Methods regarding stable isotope sample preparation and analysis can be found in Chapter 4, sections 4.2.3 and 4.2.5.

5.2.5. *Statistical analysis*

GraphPad Prism 8 (GraphPad Software, Inc.) was used for the relevant statistical analyses with statistical significance set at $p < 0.05$. All data were tested for normality (Shapiro-Wilk) before log transformation (y = log (x+1)). Differences between Hg concentrations of similar fish species from different sampling sites were evaluated by performing a two-way analysis of variance (ANOVA) and using a Sidak multiple comparisons test. Statistical differences between Hg concentrations of the different species were evaluated by performing a one-way ANOVA using Tukey Kramer post hoc tests. Multiple regression analyses were performed to establish which biological/environmental variable (fish length, trophic position, sediment concentrations, or TOC content) best predicts Hg concentration in fish from the various sampled aquatic systems. For the multiple regression analysis, due to collinearity, Hg sediment concentrations and sediment TOC were removed from the analysis. Using log-transformed data, a Pearson correlation coefficient was calculated for each of the following variables 1) TOC content and sediment Hg concentrations; 2) fish length and fish Hg concentrations; 3) Hg concentrations of sediment and fish and finally 4) trophic position of species and Hg concentrations. From these correlations, only the significant correlations are illustrated in the figures.

5.2.6. Human health risk

Various international organisations (WHO, 2007; UNEP, 2008; ATSDR, 2018) have evaluated the potential risk to human health when consuming contaminated fish tissue. Therefore, the human health risk assessment follows that of Mataba *et al.* (2016) and was performed using the 50th and 95th percentile concentrations. To assess the human health risk associated with the consumption of contaminated fish, the hazard quotient (HQ) was calculated. The HQ value is the ratio between the tolerable daily intake (TDI) of a pollutant (µg/kg body weight per day) and the estimated daily intake (EDI) of a pollutant (µg/kg body weight per day). An HQ value of >1 indicates a high probability of health effects while an HQ value of <1 suggests unlikely health effects. The TDI is based on international guidelines for the oral consumption of pollutants by ATSDR (2018) and WHO (2007). Estimated daily intake is based on the 50th and 95th percentile THg concentrations in fish muscle (µg/g ww), the average adult person's body weight (60 kg) and for children (<6 years) (14.5 kg) (WHO, 2002 – adapted to a more local approach (Heath *et al*., 2004)), and the average consumption of fish per adult person (150 g per day) and per child (50 g per day) (Heath *et al*., 2004). In fish tissue, it's assumed that 75 – 100% of Hg is methylmercury (MeHg) and thus a conversion factor of 1.0 is suggested based on the MeHg/THg proportion (Power *et al.,* 2002; Verhaert *et al*., 2019). In addition to the calculated HQs, the following formula has been used to calculate the maximum amount of pollutant a person can consume per day without posing a risk:

Y = W x M * 1000; Q = Y/C; thus Q = (W x M)/C

Where:

W = weight of the average person (kg)

M = Minimum Risk Level (MRL) for oral intake (mg/kg body weight/day)

Y = maximum amount of pollutant a 60 kg person can consume per day without posing a risk

C = 50th and 95th percentiles of Hg concentrations in fish muscle (µg/g ww)

Q = maximum amount (g) of contaminated fish muscle a 60 kg person can consume per day without posing risk.

5.3. Results

5.3.1. Mercury accumulation in sediment

Mercury concentrations in sediment ranged from below the detection limit (0.33 ng/g dw) to 68 ng/g dw (Table 5.2). The Usuthu River had the highest Hg sediment concentrations with 68 ng/g followed by its associated floodplain lake (Shokwe) during the LF period with 42 ng/g, while Lake Nyamithi had the lowest Hg concentration that was below the detection limits (0.33 ng/g). Concentrations of Hg were significantly higher in the Usuthu River than in Lake Shokwe during the HF period (4.1 ng/g; p = 0.047) and at the inlet of Lake Nyamithi during the LF period (p = 0.047) (Table 5.2).

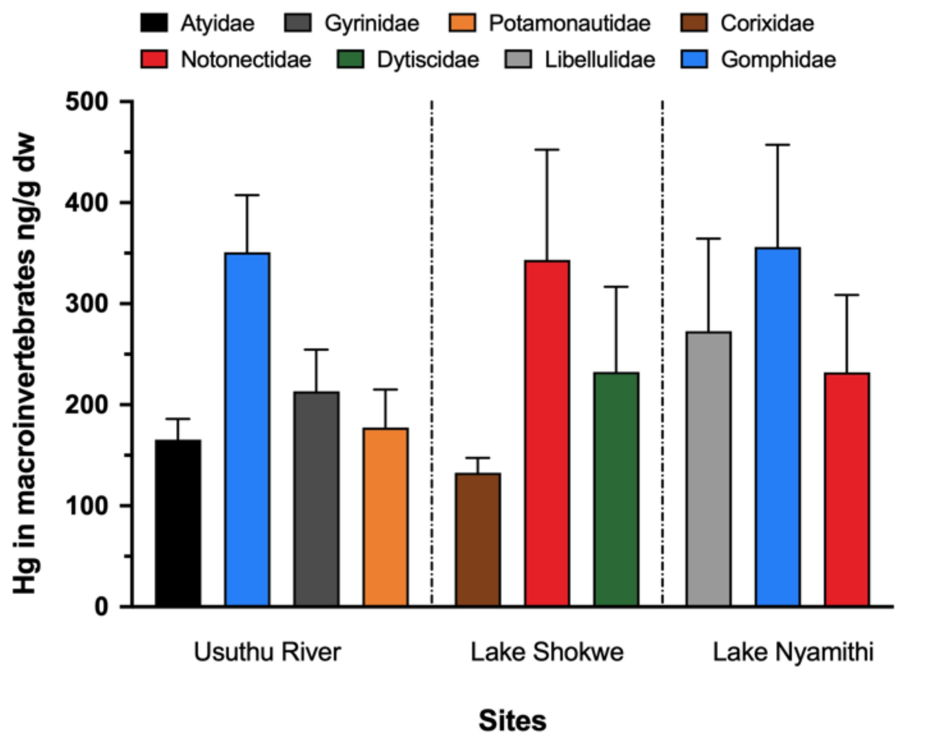
Table 5.2: The total number of samples analysed (N), levels of total organic carbon content (%) and range and median levels of mercury (ng/g dw) in sediment of floodplain rivers and lakes within the Phongolo River Floodplain. Significant differences are indicated by means of different superscript letters (p < 0.05). Limit of detection: LOD.

Site	N	TOC (%)	Hg (ng/g dw)
Usuthu River LF	3	4.02	68 ± 47^{a} (35 – 102)
Phongolo River HF	3	8.86	7.01
Lake Shokwe HF	3	13.1	4.06 ± 6.84^{b} (0.109 – 12)
Lake Shokwe LF	3	19.24	42 ± 11 (30 – 52)
Lake Nyamithi LF	3	6.44	**LOD**
Lake Nyamithi inlet LF	3	8.02	4.3 ± 6.42^{c} (0.2 – 12)

5.3.2. Bioaccumulation of mercury in aquatic biota

5.3.2.1. Aquatic macroinvertebrates

The Hg concentrations in aquatic macroinvertebrates from the aquatic ecosystems of the PRF ranged from 133 to 1300 ng/g dw (Figure 5.1). Within sites, Hg concentrations in Gomphidae from the Usuthu River were significantly higher than in Gyrinidae (p = 0.004), Potamonautidae (p = 0.0006), and Atyidae (p = 0.0003) from the Usuthu River (Figure 5.1). In Lake Shokwe, Hg concentrations in Notonectidae were significantly higher than Hg concentrations in Corixidae (p < 0.05) (Figure 5.1).



Usuthu River	Gyrinidae	Potamonautidae	Atyidae
Gomphidae	*	*	*

Shokwe	Dytiscidae	Notonectidae
Corixidae		*

Figure 5.1: Mean ± SD mercury concentration (ng/g dry weight) in aquatic macroinvertebrates of the Usuthu River and its associated floodplain lakes. Significant differences between macroinvertebrates from a site are indicated in table format by means of an asterisk (*) ($p < 0.05$).

5.3.2.2. Fish

Bioaccumulation of Hg in fish muscle from different fish species from the Usuthu River ranged from 45 to 700 ng/g dw (27 – 362 ng/g ww) (Appendix D1). In the Usuthu River, fish species accumulated Hg in the order of: *H. vittatus* (Castelnau, 1861) > *S. intermedius* (Rüppel, 1832) > *S. zambezensis* (Peters, 1852) > *M. macrolepidotus* (Peters, 1852) > *C. gariepinus* (Burchell, 1822) > *B. imberi* (Peters, 1852) > *O. mossambicus* (Peters, 1852) (Figure 5.2). Only the tigerfish muscle, *H. vittatus*, (700 ng/g dw) had significantly higher concentrations ($p < 0.0001$) than the other species from the Usuthu River. *Schilbe intermedius* muscle had significantly higher Hg concentration than the majority of Usuthu River fish species except for *S. zambezensis* ($p = 0.977$) and *M. macrolepidotus* ($p = 0.073$).

Bioaccumulation of Hg in fish muscle from different fish species from the Phongolo River ranged from 22 – 639 ng/g dw (16 – 342 ng/g ww) (Appendix D2). Fish species in the Phongolo River accumulated Hg in the order of: *H. vittatus* > *S. zambezensis* > *O. mossambicus* > *Coptodon rendalli* (Boulenger, 1896) (Figure 5.2). Similar to the Usuthu River, *H. vittatus* muscle, (639 ng/g dw) had significantly higher concentrations ($p < 0.0001$) than the other species from the Phongolo River. In addition, the muscle of the brown squeaker, *S. zambezensis*, in the Phongolo River was significantly higher in Hg concentrations than the muscle of both *C. rendalli* ($p < 0.0001$) and *O. mossambicus* ($p < 0.0001$).

Bioaccumulation of Hg in fish muscle from different fish species from Lake Shokwe ranged from 96 to 274 ng/g dw (41 to 342 ng/g ww) (Appendix D3). In Lake Shokwe fish species accumulated Hg in the order of: *B. imberi* > *H. vittatus* > *C. gariepinus* > *C. rendalli* > *O. mossambicus* (Figure 5.3). *Brycinus imberi* (274 ng/g dw) had significantly higher concentrations in the muscle than *C. gariepinus* ($p = 0.006$), *C. rendalli* ($p < 0.0001$), and *O. mossambicus* ($p < 0.0001$). The muscle of tigerfish, *H. vittatus*, had significantly higher Hg concentrations than the muscle of both *C. rendalli* ($p = 0.016$) and *O. mossambicus* ($p < 0.001$). Bioaccumulation of Hg in fish muscle from different fish species from Lake Nyamithi ranged from 29 – 377 ng/g dw (18 – 309 ng/g ww) (Appendix D4). In Lake Nyamithi fish species accumulated Hg in the order of: *H. vittatus* > *C. gariepinus* > *B. imberi* > *O. mossambicus* > *C. rendalli*. The muscle of both *H. vittatus* ($p < 0.0001$) and *C. gariepinus* ($p < 0.0001$) had significantly higher Hg concentrations than all the other fish species within Lake Nyamithi (Figure 5.3).

Concentrations of Hg in fish muscle between similar fish species of the different sites indicated significant differences for both *H. vittatus* and *O. mossambicus* (Figure 5.4). The Hg concentrations in the muscle of, *H. vittatus* were significantly higher in the floodplain rivers (659 ng/g dw) than in the floodplain lakes (271 ng/g dw) ($p < 0.0001$) and between the floodplain lakes, *H. vittatus* muscle were significantly higher in Lake Nyamithi (377 ng/g dw) than in Lake Shokwe (224 ng/g dw) ($p < 0.0001$). *Oreochromis mossambicus* muscle were similar in Hg concentrations across the majority of the sites. However, in Lake Shokwe, *O. mossambicus* muscle (96 ng/g dw) had a significantly higher Hg concentration than the *O. mossambicus* muscle from the Phongolo River (33 ng/g dw) ($p = 0.012$).

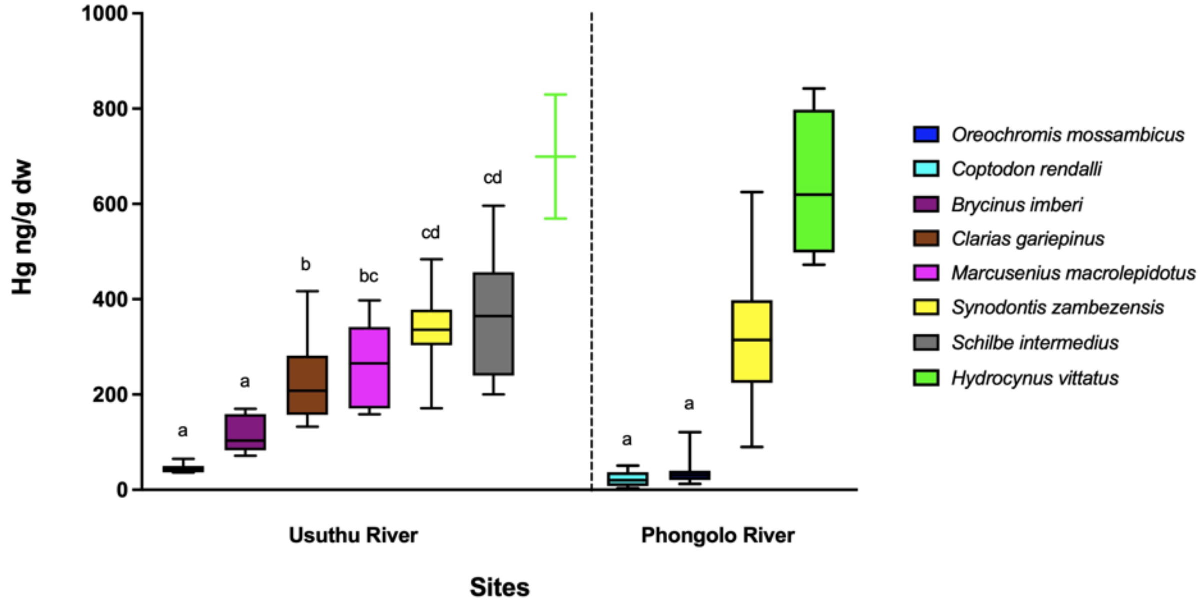


Figure 5.2: Bioaccumulation of mercury concentrations in fish species from the Usuthu and Phongolo Rivers within the Phongolo River Floodplain. Bars with common alphabetical superscripts indicate no significant differences between fish species of a site ($p > 0.05$).

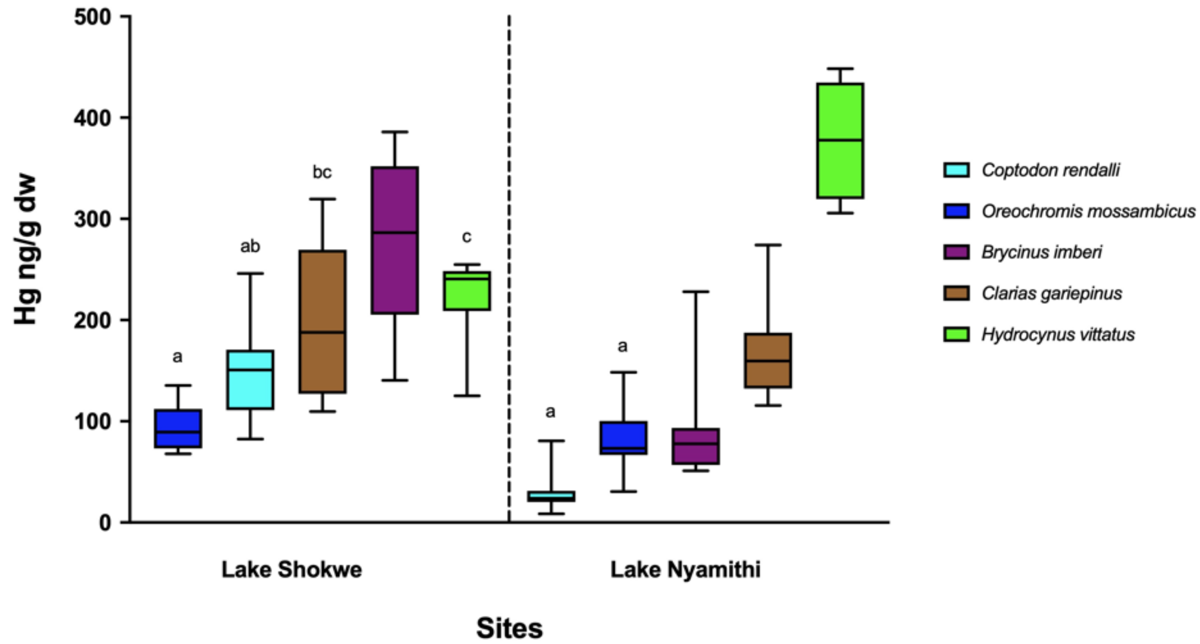


Figure 5.3: Bioaccumulation of mercury concentrations in fish species from the freshwater floodplain lakes within the Phongolo River Floodplain. Bars with common alphabetical superscripts indicate no significant differences between fish species of a site ($p > 0.05$).

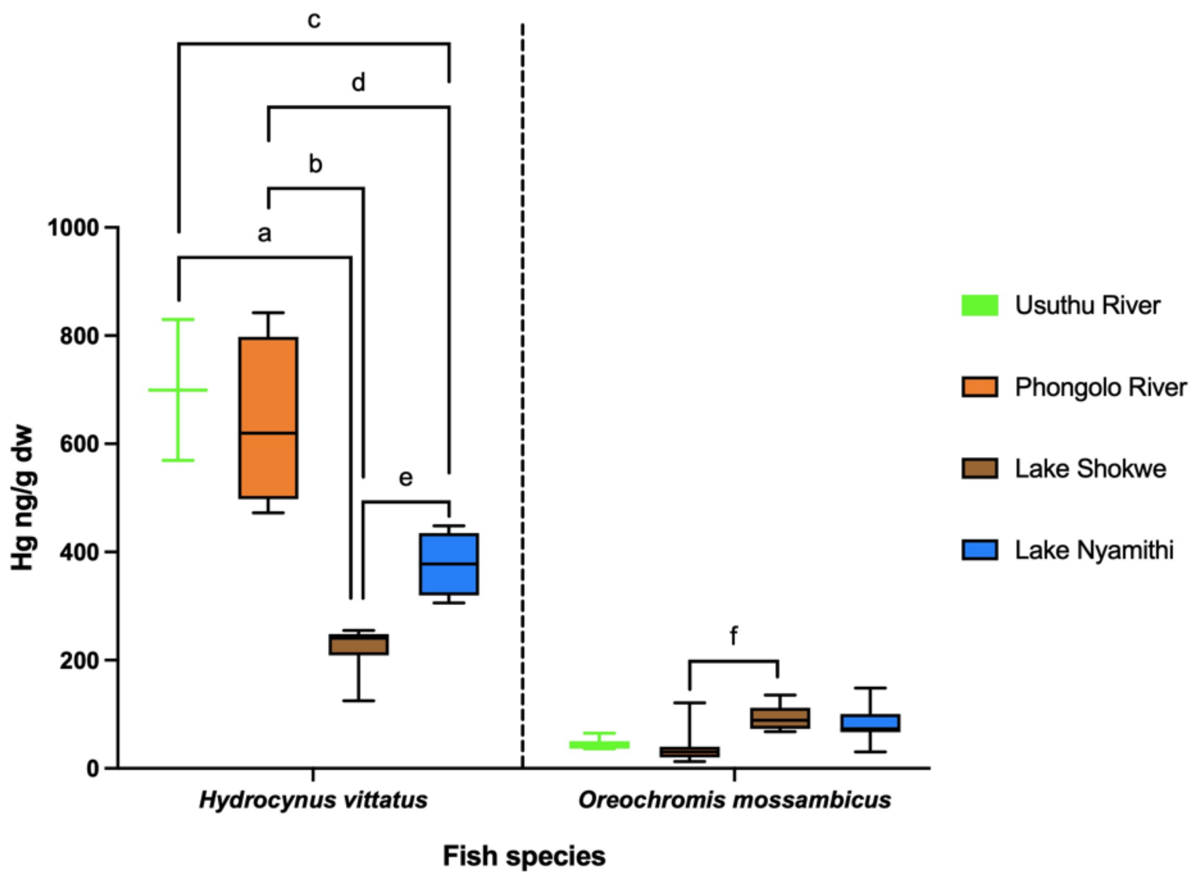


Figure 5.4: Mercury concentrations in muscle tissue (ng/g dw) of similar species between all sites within the Phongolo River Floodplain. Bars with common alphabetical letters indicate no significant differences ($p > 0.05$).

5.3.3. Determining factors of mercury concentrations in fish

5.3.3.1. Mercury in sediment relationship with total organic carbon (%)

The TOC (%) ranged from 2.9 – 9.3% in the different sites of the PRF. Mercury concentrations found in sediment (ng/g dw) from the various sites within the NGR were negatively correlated with TOC (%), however, this correlation was not significant (r = -0.31, p = 0.62).

5.3.3.2. Mercury relationship with fish length

In the Usuthu River, a significant positive correlation was found only between Hg (ng/g ww) in fish muscles and lengths (mm) for *C. gariepinus* (r = 0.7, p = 0.0035) and *S. zambezensis* (r = 0.56, p =0.0296) (Figure 5.6). The only species in the Phongolo River that had a significant positive correlation between Hg muscle concentration and length was *O. mossambicus* (r = 0.53, p = 0.0427) (Figure 5.6).

Significant positive correlations were found in Lake Shokwe in the muscle of majority of the species (*H. vittatus*, *C. gariepinus,* and *O. mossambicus*) (Figure 5.7). The muscle concentrations of Hg in *H. vittatus* (r = 0.86, p = 0.003), *C. gariepinus* (r = 0.8, p = 0.0003) and *O. mossambicus* (r = 0.62, p = 0.0127) had a significant relationship between Hg and length. In Lake Nyamithi, the correlations indicated that the majority of fish species had a positive relationship between Hg muscle concentration and length, while only the muscle of *C. rendalli* had a negative but non-significant relationship.

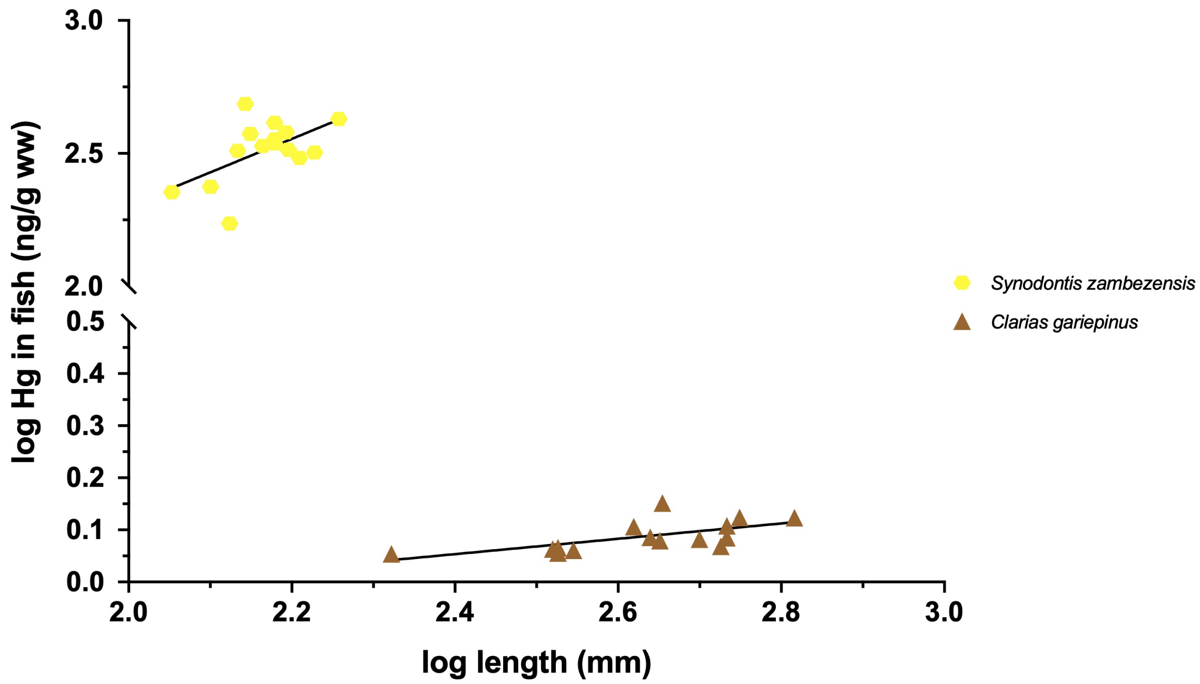


Figure 5.5: Relationship between logarithm mercury (ng/g wet weight) and logarithm length (mm) of different fish species from the Usuthu River within the Phongolo River Floodplain.

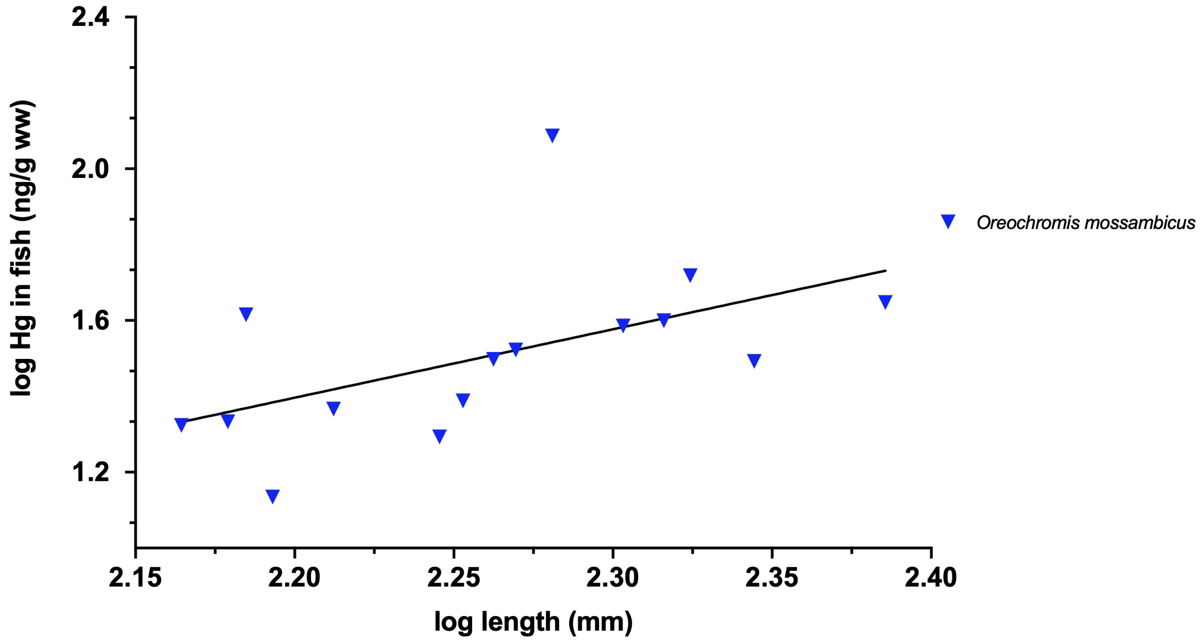


Figure 5.6: Relationship between logarithm mercury (ng/g wet weight) and logarithm length (mm) of different fish species from the Phongolo River within the Phongolo River Floodplain.

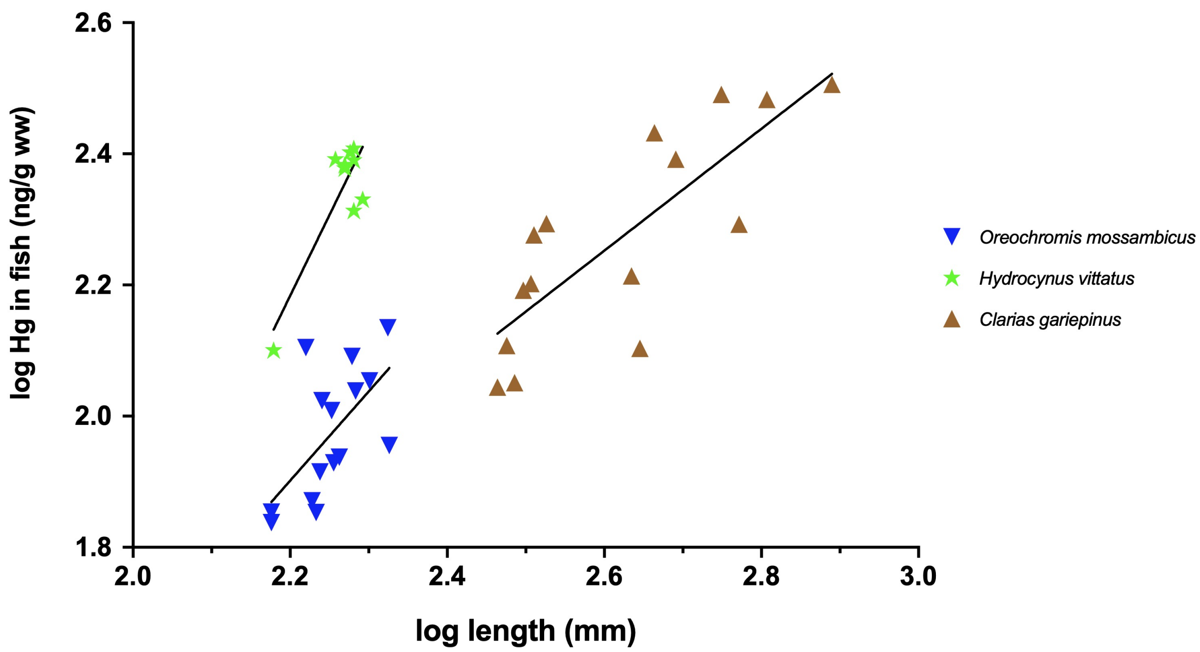


Figure 5.7: Relationship between the logarithm mercury (ng/g wet weight) and logarithm length (mm) of different fish species from Lake Shokwe within the Phongolo River Floodplain.

5.3.3.3. Multiple regression analysis

A multiple regression analysis was performed to investigate, which of the following biological variables; fish length (mm), trophic position (see Chapter 4, section 4.3.1 for the analyses of trophic position), and aquatic macroinvertebrates (ng/g dw) could successfully predict Hg concentrations in fish from the various sites. None of the biological variables could significantly determine Hg concentrations in fish muscle. The final predictive model for the various sites of the NGR was:

Usuthu River: Hg_{fish} = 2.31 – (0.48 * fish length) + (0.26 * trophic position) – (2.31 * macroinvertebrates)

Phongolo River: Hg_{fish} = 5.73 – (5.97 * fish length) – (0.43 * trophic position)

Lake Shokwe: Hg_{fish} = 1.29 – (0.52 * fish length) + (0.62 * trophic position) + (0.06 * macroinvertebrates)

Lake Nyamithi: Hg_{fish} = 1.28 + (0.02 * fish length) + (0.40 * trophic position) + (0.61 * macroinvertebrates)

5.3.4. Trophic positions of organisms and mercury biomagnification through the aquatic food webs of the lower Phongolo floodplain

In the Usuthu River, a significant positive correlation (r = 0.75, p = 0.005) between the trophic position and log Hg (ng/g dw) was observed (Figure 5.8). Based on the slope of this relationship, the calculated TMF was 2.7 indicating biomagnification. In the Phongolo River, a nonsignificant positive correlation (r = 0.82, p = 0.183) between trophic position and log Hg (ng/g dw) was observed. It is important to note that, the TMF calculated for the Phongolo River was only based on using the fish species collected during the present study and not all food web components. As such since only using fish species, the TMF (1.53) indicated biomagnification in the Phongolo River.

In the lakes of the NGR, significant positive and nonsignificant negative relationships between trophic position and log concentrations of Hg (ng/g dw) were found in Lake Shokwe (r = 0.71, p = 0.015) and Lake Nyamithi (r = -0.09, p = 0.841), respectively (Figure 5.10). Trophic magnification factors indicated biomagnification in Lake Shokwe (2.3) and bio-dilution in Lake Nyamithi (0.89).

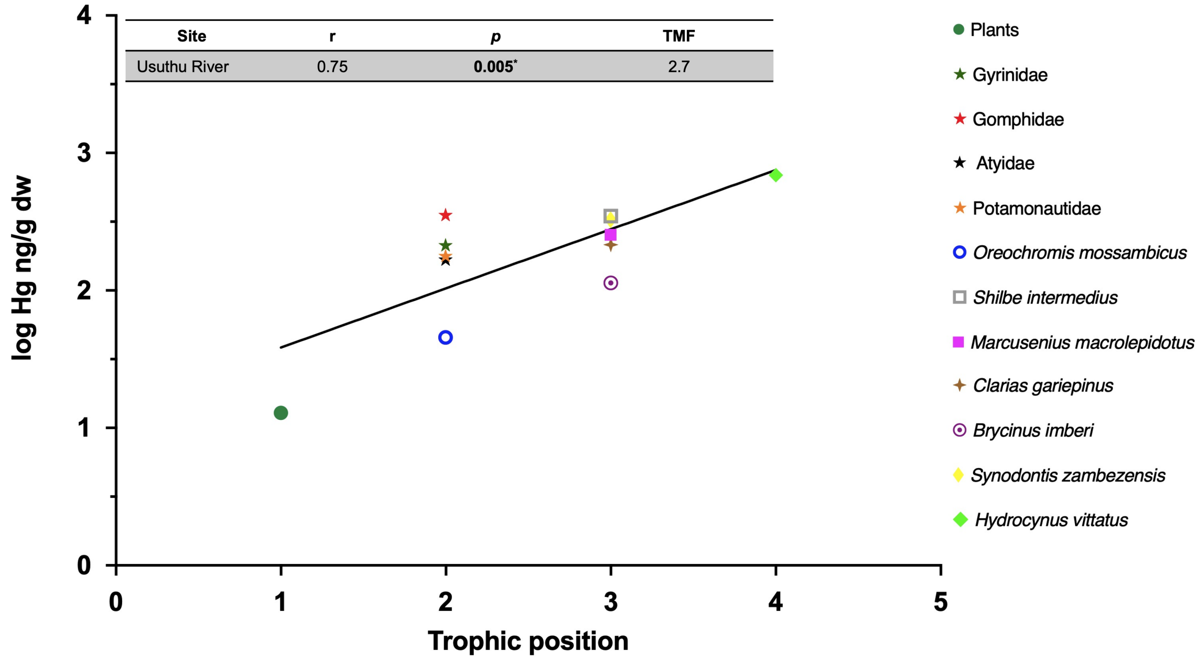


Figure 5.8: Relationship between the mean trophic position and mean logarithm concentrations of mercury in the food webs from the Usuthu River (—) within the Phongolo River Floodplain.

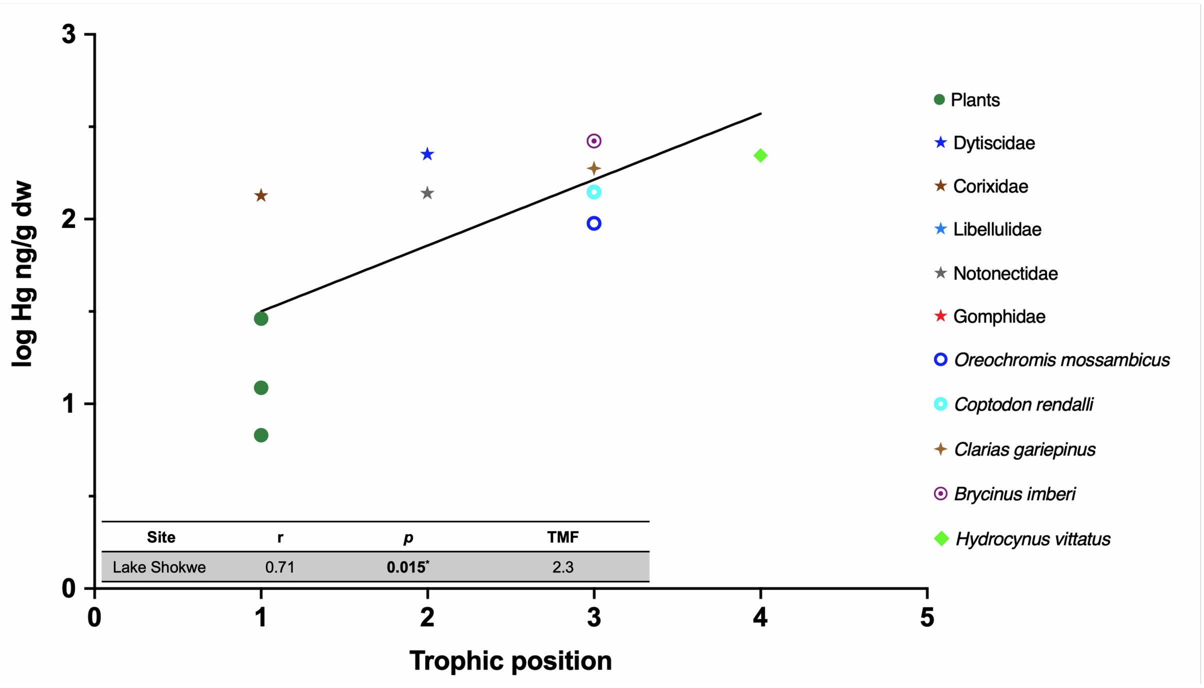


Figure 5.9: Relationship between the mean trophic position and mean logarithm concentrations of mercury in the food webs from Lakes Shokwe (—) within the Phongolo River Floodplain.

5.3.5. Human health risk

The 50th and 95th percentile values, maximum weight of fish muscle (g) that can be consumed by a 60 kg person without posing a health risk together with Hg hazard quotient is shown in Table 5.3. In the Phongolo River, *H. vittatus* (36 g) and *C. rendalli* (450 g) accounted for the lowest and highest edible amount of fish muscle, respectively. People in and around the PRF conservatively consumes a 150 g per day per person of fish muscle (Heath *et al*., 2004). As a result, the maximum edible amount of fish muscle calculated for majority of species at most aquatic systems far exceeds the daily consumption rate of 150 g. The maximum edible amount of fish muscle calculated for *H. vittatus* and *S. zambezensis* is well below the daily consumption rate. This has subsequently resulted in high HQ values (>1). Additionally, even though maximum edible amount of fish muscle was well above the daily consumption rate, the HQ values were still above 1. Furthermore, calculated HQs of all fish in the PRF were the highest in *H. vittatus* (83) and lowest in the *C. rendalli* (6.7) from the Phongolo River. In the Usuthu River, *S. zambezensis* had the highest HQ (70) value while *O. mossambicus* had the lowest HQ value (6.7). Between the floodplain lakes, Lake Nyamithi had the highest and lowest HQ values in *H. vittatus* (63) and *C. rendalli* (17), respectively.

The 50th and 95th percentile values, maximum weight of fish muscle (g) that can be consumed by a 14.5 kg child (<6 years) without posing a health risk together with Hg hazard quotient is shown in Table 5.4. In the Phongolo River, *H. vittatus* (9 g) and *C. rendalli* (109 g) accounted for the lowest and highest edible amount of fish muscle, respectively. Children (<6 years old in and around the PRF conservatively consumes a 50 g per day per person of fish muscle (Heath *et al*., 2004). As a result, the maximum edible amount of fish muscle calculated far exceeds the daily consumption rate of 50 g for majority of the species from all aquatic systems. The maximum edible amount of fish muscle for *H. vittatus*, *O. mossambicus* (Lakes Nyamithi and Shokwe), *C. rendalli* (Lake Shokwe) and *S. zambezensis* are below the daily consumption rate and subsequently resulted in higher HQ values (>1) than the adults. Additionally, the species with the maximum edible amount of fish muscle higher than the daily consumption rate also resulted in above 1 HQ values. Moreover, calculated HQs of all fish in the PRF were the highest in *H. vittatus* (115) and lowest *C. rendalli* (9.2) from the Phongolo River. In the Usuthu River, *S. zambezensis* had the highest HQ value (97) while *O. mossambicus* had the lowest HQ value (9.2). Between the floodplain lakes, Lake Nyamithi had the highest and lowest HQ values in *H. vittatus* (87) and *C. rendalli* (11), respectively.

Table 5.3: The 50th and 95th percentile mercury concentrations (μg/g ww) in different fish muscle, the maximum edible amount of different fish species muscle (g) that can be consumed per day without posing a health risk to a 60 kg adult and the hazard quotient (HQ) for total mercury in fish muscle.

Fish species	Sites	Hg concentrations		Maximum edible amount of fish muscle		Hazard quotient	
		50th percentile (μg/g ww)	95th percentile (μg/g ww)	50th percentile (g)	95th percentile (g)	50th percentile	95th percentile
Clarias gariepinus	Usuthu river	0.05	0.08	360	225	8.3	13
	Shokwe	0.04	0.07	450	257	6.7	12
	Nyamithi	0.03	0.06	600	300	5	10
Hydrocynus vittatus	Phongolo river	0.33	0.5	55	36	55	83
	Shokwe	0.16	0.18	113	100	27	30
	Nyamithi	0.3	0.38	60	47	50	63
Oreochromis mossambicus	Usuthu River	0.03	0.04	600	450	5	6.7
	Phongolo River	0.02	0.08	900	225	3.3	13
	Shokwe	0.06	0.1	300	257	10	17
	Nyamithi	0.05	0.1	360	180	8.3	17
Coptodon rendalli	Phongolo River	0.01	0.04	1800	450	1.7	6.7
	Shokwe	0.11	0.18	164	100	18	30
	Nyamithi	0.02	0.05	900	360	3.3	17
Synodontis zambezensis	Usuthu River	0.28	0.42	64	43	47	70
	Phongolo River	0.2	0.38	90	47	33	63

Table 5.4: The 50th and 95th percentile mercury concentrations (µg/g ww) in different fish muscle, the maximum edible amount of different fish species muscle (g) that can be consumed per day without posing a health risk to a 14.5 kg child (<6 years) and the hazard quotient (HQ) for total mercury in fish muscle.

Fish species	Sites	Hg concentrations		Maximum edible amount of fish muscle		Hazard quotient	
		50th percentile (µg/g ww)	95th percentile (µg/g ww)	50th percentile (g)	95th percentile (g)	50th percentile	95th percentile
Clarias gariepinus	Usuthu river	0.05	0.08	87	54	11	18
	Shokwe	0.04	0.07	109	62	9.2	16
	Nyamithi	0.03	0.06	145	73	6.9	14
Hydrocynus vittatus	Phongolo river	0.33	0.5	13	9	76	115
	Shokwe	0.16	0.18	27	24	37	41
	Nyamithi	0.3	0.38	15	11	69	87
Oreochromis mossambicus	Usuthu River	0.03	0.04	145	109	6.9	9.2
	Phongolo River	0.02	0.08	218	54	4.6	18
	Shokwe	0.06	0.1	73	44	14	23
	Nyamithi	0.05	0.1	87	44	11	23
Coptodon rendalli	Phongolo River	0.01	0.04	435	109	2.3	9.2
	Shokwe	0.11	0.18	40	24	25	41
	Nyamithi	0.02	0.05	218	87	4.6	11
Synodontis zambezensis	Usuthu River	0.28	0.42	16	10	64	97
	Phongolo River	0.2	0.38	22	11	46	87

5.4. Discussion

In the present study, Hg accumulation and biomagnification were determined in the two rivers of the lower Phongolo floodplain and two of their associated floodplain lakes. Sediment from all sites apart from Lake Nyamithi had detectable Hg concentrations and were negatively correlated with TOC in sediment. All biotic compartments had detectable Hg concentrations with the predatory fish species having the highest Hg concentrations. Positive correlations were observed between Hg and fish length as well as trophic position. Trophic magnification factors indicated both biomagnification and bio-dilution in the different sites within the PRF. Biological parameters such as fish length, trophic position, and macroinvertebrates could not successfully predict Hg concentrations in fish muscle from the NGR.

5.4.1. Mercury accumulation

In the sediment, Hg concentrations ranged from below the detection limit to 68 ng/g dw. This is similar to what was found in the Olifants River catchment, which is known to be affected by several anthropogenic effluents in South Africa (Verhaert *et al*., 2019). Prior to the former study, Walters *et al.* (2011) reported that the sediment Hg concentrations (50 ng/g dw) in the Upper Olifants River were the highest in the Olifants River system. However, even the higher Hg concentrations (78 ng/g) that Verhaert *et al*. (2019) attributed to the industrial activities in the Olifants River catchment, were still lower than >200 ng/g reported in sediments of the Incomati River catchment, which is in close proximity to all the major power stations (Somerset *et al*., 2010) and the 40 – 8,000 ng/g Hg measured in sediments of the Taaibosspruit wetland adjacent to a large petrochemical complex (de Klerk *et al*., 2013). The concentrations measured during the current study were also lower than levels measured in sediments from other African rivers (Thigithe River, Gambia River, and Malagarasi River). These rivers were all situated close to gold mines (Taylor *et al*., 2005; Niane *et al*., 2014; Mataba *et al*., 2016). These African studies reported Hg concentrations in freshwater ecosystems impacted by artisanal and small-scale mining to be between 40.3 – 660 ng/g (Taylor *et al*., 2005; Mataba *et al*., 2016; Mason *et al*., 2019). Since there are no gold mining or industrial activities in the catchments of the PRF, the source of Hg in sediments measured during the present study is very likely from atmospheric deposition as the PRF falls within the atmospheric deposition pathway from the Mpumalanga highveld (Freiman & Piketh, 2003; Black *et al*., 2011; Belelie *et al*., 2019). Sediment Hg concentrations of all aquatic ecosystems within the Ndumo Game Reserve are well below the CCME ISQG guidelines of 170 ng/g (CCME, 2007).

Chapter 5

Bioaccumulation and human health risk

Previous research reported that lentic ecosystems have higher Hg concentrations than lotic systems (Gerson *et al*., 2020). Surprisingly, in the present study, an opposite trend was found as fish muscle from the Usuthu River had higher Hg concentrations during the LF survey than in the floodplain lakes. This phenomenon could be due to more recent Hg atmospheric deposition into the Usuthu River as Black *et al*. (2011) explained that newly deposited atmospheric Hg is more bioavailable and labile to sediment and microbes. Furthermore, Lake Shokwe had higher TOC content than the floodplain lakes, however, the lower concentrations were surprising given the positive relationship between TOC and Hg that is well established (de Klerk *et al*., 2013; Chakraborty *et al*., 2015; Verhaert *et al*., 2019). Conversely, de Klerk *et al*. (2013) found a positive relationship between sediment Hg and sediment TOC. Within aquatic systems, the bioavailability and methylation of Hg depend on the microbial activity and organic carbon in the sediment (de Klerk *et al*., 2013). Importantly, Lambertsson and Nilsson (2006) concluded that organic content is an important predictor in explaining Hg methylation. This is due to decaying organic matter increasing microbial activity and in turn creating favourable conditions to stimulate methylation (Olson & Cooper, 1976; Gilmour & Henry, 1991).

The Hg concentrations in macroinvertebrates from the present study were comparable with the Hg concentrations in macroinvertebrates of the Olifants River (Verhaert *et al*., 2019). In the Olifants River Basin, the mean Hg concentrations in Gomphidae ranged from 88 – 690 ng/g dw while in the present study the concentrations ranged from 241 – 432 ng/g dw (Verhaert *et al*., 2019). Although the Olifants River catchment has many anthropogenic pressures that contribute to the Hg concentrations (Verhaert *et al*., 2019), the present study showed that even though there are fewer anthropogenic pressures in and around the PRF, atmospheric deposition could still result in increased Hg concentrations in freshwater systems. Similar Hg concentrations (range: 132.6 – 356.1 ng/g) in macroinvertebrates were found to that of Marziali *et al*. (2021). However, Marziali *et al*. (2021) reported that Hg concentrations in aquatic macroinvertebrates correspond to the intensity of industrial activities, as they increased further downstream closer to the industrial area. Since no industrial activities are found in close proximity to the PRF, the Hg concentrations found in macroinvertebrates of the present study could be attributed to a combination of atmospheric deposition from coal-fired powerplants as well as anthropogenic inputs from coal-fired powerplants further upstream (DWA, 2009; Belelie *et al*., 2019).

Fish from the various aquatic systems of the PRF bioaccumulated higher concentrations of Hg than the sediment. Black *et al*. (2011) reported from the Okavango Delta mean Hg concentrations in piscivorous and non-piscivorous fish of 59 ng/g ww and 19 ng/g ww, respectively. This was considerably lower than what was found in the PRF as concentrations from the present study in piscivorous and non-piscivorous fish were 128 ng/g ww and 108 ng/g ww, respectively. Moreover, the concentrations in the PRF are considerably higher than the European biota environmental quality standards of 20 ng/g ww (European Union, 2014). According to Black *et al*. (2011), the Hg concentrations found in the fish from the Okavango Delta result from atmospheric deposition rather than from point sources. The higher concentrations of the present study could indicate more recent atmospheric Hg deposition since according to Black *et al*. (2011) newly deposited atmospheric Hg result in higher Hg concentrations in fish as Hg is easier methylated and bioaccumulated.

When compared to fish from the Olifants River catchment, the Hg concentrations in fish from the lower PRF were considerably lower. Concentrations in fish from the Olifants River ranged from 100 to 6100 ng/g dw (Verhaert *et al*., 2019), whereas those from the present study varied between 7 and 700 ng/g dw. The study by Verhaert *et al*. (2019) concluded that the concentrations in fish were still lower or similar to studies from impacted regions of Europe and the sub-Arctic (Power *et al*., 2002; Carrasco *et al*., 2011; Nguetseng *et al*., 2015). Furthermore, Verhaert *et al*. (2019) concluded that Hg concentrations found in the Olifants River catchment were from point sources such as agriculture and industrial activities. The lower Hg found in fish of the present study supports the observations made for sediments that the Hg source was most likely atmospheric deposition from coal-fired powerplants. This is supported by Park & Curtis (1997) who indicated that point source Hg inputs result in higher Hg concentrations in fish than atmospheric deposition.

5.4.2. Relationship between mercury and biological parameters

In the present study, a negative relationship was found between sediment Hg concentrations and sediment TOC (%) and contradicts previous research (Williams *et al*., 2011; Govaerts *et al*., 2018; Verhaert *et al*., 2019; Gopikrishna *et al*., 2020; Lintern *et al*., 2020). Total organic carbon content is important in the fate and retention of Hg in sediment as higher Hg concentrations are expected in sediments with higher TOC (Verhaert *et al*., 2019). As such, the organic carbon content in sediment causes Hg to bind to sediments due to Hg's high affinity to organic matter making Hg less bioavailable to aquatic biota (Shao *et al*., 2012; Taylor *et al*., 2012). Within Lake Nyamithi, a natural saline lake, the Hg concentration found could be due to high salinity that makes adsorption difficult and therefore results in lower sediment concentrations (Wright & Zamuda, 1987 & Kumar *et al*., 2015).

The present study showed that Hg concentrations in the aquatic ecosystems from the lower PRF were in some cases positively and in others negatively correlated with fish length. The only two species where Hg concentrations had a significant positive correlation with fish length were *C. gariepinus* and *H. vittatus*. Similarly, Verhaert *et al*. (2019) found that fish occupying a higher trophic position such as *C. gariepinus* had a positive relationship between Hg and fish length. Between conspecific species such as *H. vittatus*, the Hg concentrations were significantly higher in rivers than in lakes and were very similar in both rivers. This can be attributed to the length of the tigerfish as the species in the rivers were much larger compared to the floodplain lakes. This is in agreement with other studies in Lake Victoria and Nantucket where Hg concentrations increased with an increase in size (Taylor *et al*., 2005; Backstrom *et al*., 2020).

5.4.3. Mercury relationship with trophic position

Trophic magnification factors calculated for the present study showed both biomagnification and surprisingly bio-dilution through the food webs. The biomagnification in the present study is consistent with previous reports in subtropical aquatic ecosystems in Africa (Kidd *et al*., 2003; Campbell *et al*., 2004; Poste *et al*., 2008; Govaerts *et al*., 2018; Verhaert *et al*., 2019). Mercury is well known to accumulate through food webs of aquatic ecosystems, as piscivorous fish accumulate more Hg than species at the base of the food web (Zhou & Wong, 2000; Ullrich *et al*., 2001; Oosthuizen & Somerset, 2010; Kidd *et al*., 2012; Verhaert *et al*., 2019).

The TMFs of Hg determined by Verhaert *et al*. (2019) from the Olifants River Basin were higher (1.9 – 4.2) than the present study (0.9 – 2.7). Surprisingly, Hg did not biomagnify through the food web in Lake Nyamithi and consequently showed bio-dilution of Hg. In a study by Volschenk *et al*. (2019), the authors found that certain organochlorine pesticides did not biomagnify either within Lake Nyamithi ecosystem. However, when Gomphidae was removed from the TMF calculation, Hg biomagnifies through the food web in Lake Nyamithi (TMF = 1.63). It remains unclear whether food web processes (species diversity, growth rate, and food web length) or do physico-chemical variables also have an influence on biomagnification (Lavoie *et al*., 2013).

5.4.4. Human health risk

The present study has demonstrated bioaccumulation and biomagnification of Hg in the various fish species from the PRF resulting in potential risk for humans. This is in line with numerous studies confirming the biomagnification nature of Hg as well as the potential human health risk (du Preez *et al*., 2003; Campbell *et al*., 2004; Campbell *et al*., 2010; Verhaert *et al*., 2019). Mercury may affect not only the immune and digestive system but also the nervous system, eyes, skin, kidneys, and lungs (WHO, 2007). Given the Hg concentrations found in the muscle of various fish species and the estimated daily consumption of 150 g per person (Heath *et al*., 2004), a high risk is posed with the consumption of fish from the PRF by Hg. Conversely, a study in the Ga-Selati River indicated no risks when consuming the muscle of either *C. gariepinus* or *S. zambezensis* (Govaerts *et al*., 2018). Verhaert *et al*. (2019) also found that in the Olifants River people will be at risk when consuming fish muscle contaminated with Hg. This is similar to the present study which found high HQ values in all fish species. In the Olifants River, the maximum amount of *C. gariepinus* (19 g), *S. zambezensis* (22 g) and *H. vittatus* (21 g) muscle that could be consumed were below the estimated daily consumption (Verhaert *et al*., 2019). In the present study, only *H. vittatus* (36 g – Phongolo River and 47 g – Lake Nyamithi) and *S. zambezensis* (43 g – Usuthu River and 47 – Phongolo River) was well below to the estimated daily consumption. As mentioned earlier, fish muscle concentrations in the lower Phongolo Floodplain had considerably lower Hg concentrations than reported by Verhaert *et al*. (2019) in the Olifants River Basin. This explains the higher maximum edible amount of fish muscle in majority of the fish species from the present study. However, the HQ values were still above 1. In addition, it is expected that larger fish species are selected for consumption, and as Hg increases with the increase in size causing higher uptake rates (Verhaert *et al*., 2019).

Children are more sensitive and susceptible, than adults, to chronic exposures to elements such as Hg (WHO 2010; Erasmus *et al*., 2020). Children are at risk of neurologic impairment, especially during the early stages of brain development, when exposed to Hg (Barone *et al*., 2021). Given the sensitivities, the human health risk Hg could pose to children, was calculated for children under the age of 6 (WHO, 2002; Heath *et al*., 2004). As was shown with the adults, the Hg concentrations of the present study were higher and lower compared to what Govaerts *et al*. (2018) and Verhaert *et al*. (2019), respectively. However, with children being more sensitive towards trace elements, maximum edible amount of fish muscle that can be consumed as well as the estimated daily intake will be lower compared to the adults. This was highlighted by the human health risk calculated as the HQ values in children were much higher. Similarly, Barone *et al*. (2021) found in marine fish from the Mediterranean area, that both classes (adults and children) are vulnerable to Hg health effects. Additionally, in the Mississippi Lakes, Huggett *et al*. (2000) found that the calculated human hazard indexes for both adults and children were above 1 when consuming fish while also highlighting that adults could consume more fish muscle than children.

5.5. Conclusion

This study provides a novel understanding into the Hg concentrations in sediment and aquatic biota and their potential risk to the local communities of the PRF ecosystem. The present study indicated that, although there are limited anthropogenic influences of Hg, it can accumulate in the freshwater systems through atmospheric deposition. Furthermore, although the present study reported concentrations typical of unpolluted sites, the coal-fired powerplants located in the upper catchments (Mpumalanga Province) of both the Usuthu and Phongolo rivers could still potentially cause Hg concentrations to increase in the freshwater systems within the NGR. The ability of Hg to biomagnify was evident as predatory macroinvertebrates and fish species accumulated high Hg concentrations. Most importantly, even with biomagnification, the potential human health risks associated with consuming Hg contaminated fish from the floodplain was low to no risk. This study provides important baseline data on how atmospheric deposition can influence Hg concentrations in freshwater systems far from its source.

Therefore, the hypothesis stating that the aquatic biota will indicate Hg bioaccumulation and that biomagnification has occurred through the food web is supported by the data. Aquatic biota from the aquatic systems within the PRF had detectable levels of Hg and further biomagnified through the food webs. Furthermore, the hypothesis stating that due to the biomagnification of Hg, there will be human health risks associated with the consumption of fish from the lower PRF is not supported by the data. The present study concluded that there is low to no human health risk associated with the consumption of fish muscle from the PRF.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1. General Remarks

The Usuthu – Phongolo river catchments have similar anthropogenic activities in their upper catchment (see Chapter 1, specifically Figure 1.1). However, the Phongolo River has been for many years, since the construction of the Pongolapoort Dam, extensively studied to determine the degree of influence as a result of the impoundment, the effect of the large-scale agricultural activities and the supra-seasonal drought South Africa has experienced between 2015 – 2017. The Usuthu River, although weirs are located in its upper catchment, is not regulated to the same extent as the Phongolo River and therefore is an unregulated river. There are similar anthropogenic activities in the upper catchment of both rivers (see Figure 1.1). It is assumed that the impacts of these activities on the downstream water and sediment quality and subsequently its aquatic biota of the Usuthu River, to be to a lower degree than the Phongolo River (see Chapter 2). The contribution and importance of the contributions of the Usuthu River to the associated floodplain lakes and specifically Lake Nyamithi is largely unknown. Therefore, due to the extensive knowledge on the lower Phongolo River, it provides the need for a better understanding of the Usuthu River system and to what degree its natural flooding regime impacts downstream ecosystems. This study was aimed at determining the present environmental quality and aquatic biota community composition of the Usuthu River, the effect the natural flooding regime has on the aquatic ecosystems within the PRF and whether the Usuthu River acts as refugia for the lower Phongolo Floodplain aquatic biodiversity. The following sections provides concluding remarks on each hypothesis of the study.

6.2. Specific conclusions

6.1.1. Chapter 2 – Environmental Quality

(1) Water and sediment quality of the Usuthu River and its associated floodplain lake, Lake Shokwe, would be in a better state than the Phongolo River and Lake Nyamithi, and this will be reflected in the water and sediment quality indices; and (2) the hydrological period (i.e., flow) would influence the metal concentrations in water and sediments of these systems:

Altering the flow of a river through impoundments has many negative effects such as trapping nutrient-rich sediment, nutrient retention within the impoundment, degrading water quality, loss of aquatic biodiversity and biota and disconnecting the associated floodplain lakes from its rivers. These effects have been recorded in previous studies in the PRF (see Dube *et al*., 2017; de Necker *et al*., 2019). It has been previously stated that, historically, the Phongolo River's water quality has been affected by the various anthropogenic activities in the upper catchment. Furthermore, the water quality of the Phongolo River has degraded even further due to the increase in anthropogenic activities and the construction of the Pongolapoort Dam (see de Necker *et al*., 2019). The present environmental quality within the NGR and the effect of the natural flooding regime of the Usuthu River is largely unknown. The absence of an impoundment in the Usuthu River catchment is assumed to lessen the impact on the downstream water and sediment quality of the river. This was indicative of the better water and sediment quality in the Usuthu River within the NGR. The cause of the higher water and sediment quality in the Usuthu River is mainly due to fewer agricultural activities in close proximity of the NGR and the lack of flow regulation. The main differences were observed in TDS as well as nutrient concentrations. It has been concluded that silicate weathering, saltwater intrusion and close proximity of agricultural activities have exacerbated the TDS levels in the Phongolo River (see Chapter 2 and van Rooyen *et al*., 2022). In addition, the lack of strong flow and drought conditions have added more stress on the Phongolo River. Furthermore, the Pongolapoort Dam, despite its numerous advantages, has contributed to reduced nutrients in the river through trapping nutrient-rich sediments and reducing nutrient supply. The water quality of the Usuthu River was similar to unimpacted conditions in the upstream catchment of Eswatini. More importantly, due to the lack of agricultural activities located close to the NGR. The increased nutrient concentrations in this particular stretch of the river were instead attributed to hippopotami pods.

The contribution and importance of the Usuthu River is highlighted through the multivariate analyses as similar general water quality as well as metal concentrations were observed between the Usuthu River HF survey and the outlet of Lake Nyamithi as well as Lake Shokwe. The lack of high flow and flooding from the Phongolo River is indicative of the differences in water quality between the Phongolo River and Lake Nyamithi and therefore the influences were limited. This highlights the importance of the natural flooding regime of the Usuthu River to Lake Nyamithi where water pushes into Lake Nyamithi during flooding from the Usuthu River.

Metal concentrations in aquatic ecosystems are known to have a negative impact on the aquatic biota making it essential to monitor in aquatic environments. This is the first study to determine metal concentrations of the aquatic systems within the PRF and provides a baseline for future studies. The low metal concentrations are indicative of fewer anthropogenic inputs into the Usuthu – Phongolo river catchments. Furthermore, the aquatic systems within the

PRF were higher in Cu, Fe and Zn concentrations than the KNP rivers which could be as a result of agricultural and industrial activities within the upper catchment while groundwater concentrations also contributed to the concentrations in water. Sediments acts as a sink for contaminants such as metals and adsorbs to sediment organic matter. Furthermore, contaminated sediment can be a potential source of water contamination. Therefore, analysing metal concentrations in sediment provides a degree of contamination in the aquatic systems as well as historical contamination. As such, metal concentrations in floodplain lakes were low and similar to less-impacted areas and the concentrations indicate a small influence from the geological characteristics and small-scale farming. The floodplain lakes act as a sink for contaminants as rivers transfer sediments into the lakes, and there is no export out of the lakes. This, together with higher TOC, explains the higher metal concentrations in lake sediment than in river sediment.

Therefore, the first hypothesis stating that the water and sediment quality of the Usuthu River and its associated floodplain lake, Lake Shokwe, would be in a better state than the Phongolo River and Lake Nyamithi and this will be reflected in the water and sediment quality indices, is supported by the data. The environmental quality indices indicated differences between the two rivers and associated floodplain lakes. The second hypothesis stating that the hydrological period would influence the metal concentrations in water and sediments of these systems, is not supported by the findings of this study. Multivariate analyses indicated limited spatial but no flow-related differences in dissolved metal concentrations.

6.1.2. Chapter 3 – Aquatic community structures

(1) The macroinvertebrate community structure of the Usuthu River differs from the Phongolo River; (2) the macroinvertebrate community structures of the two largest floodplain lakes within the NGR reflect the influence of the Usuthu River; (3) hydrological connectivity will have a positive effect on the macroinvertebrate community composition of the Usuthu River associated floodplain lake; (4) the influence of the Usuthu River on Lake Nyamithi will reflect sustained recovery in macroinvertebrate community structures and finally (5) the nature of the aquatic ecosystems (lotic/lentic) drives the structure of macroinvertebrate communities within the NGR:

Aquatic macroinvertebrates are essential components of riverine systems and altering the flow regime could significantly impact community composition and structure (Greenwood & Booker, 2015; Dube *et al*., 2017). Apart from drought, natural flooding is a major driver in impacting aquatic macroinvertebrate community composition. Although numerous studies have shown that altering the flow of a river has an impact on the aquatic macroinvertebrate community structure (Gillespie *et al*., 2015; Schneider & Petrin, 2017; Gillespie *et al*., 2019), this impact was not seen in the present study. In a similar study, Schneider & Petrin (2017) found no difference in community structure between a regulated and unregulated river in Norway. There were no differences in macroinvertebrate community structures between the regulated Phongolo River and unregulated Usuthu River, suggesting that the baseflow of the Phongolo River is sufficient in sustaining aquatic community composition. Furthermore, although the Phongolo River has been influenced by anthropogenic activities, the community composition does not seem to be influenced due to the replacement of more tolerant taxa and frequent taxonomic turnover. In addition, the Usuthu River was different in pollutant-tolerant and sensitive taxa as the river was comprised of a higher percentage of sensitive taxa. Higher abundances of sensitive taxa are found in aquatic systems with better water, sediment and habitat quality (Everaert *et al*., 2014; Smit *et al*., 2016; Raphahlelo *et al*., 2022) and corresponds with the findings reported in Chapter 2. This emphasises the importance of the Usuthu River to the aquatic systems within the PRF and with its higher water and sediment quality, the river serves as a refuge area for aquatic biodiversity.

The Usuthu River was identified as the main source of water for the two largest floodplain lakes within the PRF (see Chapter 2), therefore understanding the influence the Usuthu River plays in the dynamics of these associated floodplain lakes is important. The high diversity recorded in the HF survey of Lake Shokwe further showed the important contribution that the Usuthu River has on associated floodplain lakes. The unaltered flow regime of the Usuthu River contributes to the hydrological connectivity of its associated floodplains and further positively impacts the aquatic macroinvertebrate diversity. Wetlands are known for the high macroinvertebrate diversity and abundances during periods of flooding and hydrological connectivity. Hydrological connectivity prompts the dispersal of aquatic macroinvertebrates and fish from the river to floodplain lakes. Therefore, flooding of rivers impacts the diversity and community structure of river floodplain systems (see Amoros & Bornette, 2002).

Saline lakes are known for their distinct aquatic and semiaquatic community composition due to their unique environment. However, during extreme events such as the drought South Africa experienced, fluctuations in salinity affects macroinvertebrate community structures. Macroinvertebrate community structures of Lake Nyamithi were different between surveys (2017 vs 2018) and species residing in saline lakes have special attributes in that they are highly adapted and able to disperse when conditions become unfavourable (de Necker *et al*., 2021). The rainfall and small floods from the Usuthu River caused a decrease in salinity, lowering salinity to more suitable concentrations for macroinvertebrates and subsequently resulted in higher taxa richness, abundances, and diversity (see Chapter 3). Moreover, due to the construction of the Pongolapoort Dam, the flow of the Phongolo River has been altered which impacts the aquatic community structure of the river and floodplain lakes further downstream. In addition, the altered flow regime influences hydrological connectivity and impacts the floodplains aquatic communities and diversity (Tockner *et al*., 1998; Tockner *et al*., 1999). This, together with the drought experienced in South Africa has resulted in no simulated flood release from the dam and has subsequently led to a disconnection from Lake Nyamithi. Therefore, due to freshwater inputs from the Usuthu River into Lake Nyamithi during the study period, any changes in macroinvertebrate community structure in Lake Nyamithi were likely due to the Usuthu River. Although the 2017 vs 2018 surveys were different in aquatic macroinvertebrate community structures, the influences from the Usuthu River were minimal with only a few shared taxa (Oligochaeta, Chironomidae, Ostracoda and Corixidae) between the two surveys. This reflects the partially sustained recovery from the 2017 survey in Lake Nyamithi.

It is well known that macroinvertebrates respond to changes in water quality and as such nutrient concentrations had positive correlations with the macroinvertebrates of the Usuthu River and Lakes Shokwe and Nyamithi. Seasonal changes in water quality greatly affected macroinvertebrate community structures while flooding of rivers decreases nutrient and conductivity levels. Therefore, changes in water quality caused a change in macroinvertebrate community structures. However, habitat preference played a greater role in the structuring of macroinvertebrate communities compared to water quality. Importantly, habitat is a function of water volume and the degree of inundation of the structures that lead to habitat diversity or heterogeneity.

The first part of the hypothesis stating that the natural flooding regime of the Usuthu River will structure and influence the macroinvertebrate community structures of the floodplain lakes within the NGR and community structures of the unregulated river will be different to the regulated Phongolo River is supported by the data. The natural flooding of the regime did influence the macroinvertebrate community structures of floodplain lakes within the NGR. However, the part of the hypothesis that states that the macroinvertebrate community structures will be different between the regulated and unregulated rivers is not supported by the data as diversity indices indicated no differences in community structures. The second hypothesis stating that the macroinvertebrate community structure of the two largest floodplain lakes within the NGR reflect the influence of the Usuthu River, is supported by the data. Aquatic macroinvertebrates from Lake Shokwe during the HF survey showed the influence from the Usuthu River while no flooding of the Phongolo River has caused changes in macroinvertebrate community structure in Lake Nyamithi to be as a result of flooding from the

Usuthu River. The third hypothesis that the influence from the Usuthu River on Lake Nyamithi will reflect sustained recovery is supported by the data as few taxa were shared between the 2017 and 2018 surveys, showing partial sustained recovery. Lastly, the fourth hypothesis stating that the nature of the aquatic ecosystems (lotic/lentic) drives the structure of macroinvertebrate communities within the NGR is not supported by the data as variation partitioning analysis indicated habitat preference to be the major driver in structuring macroinvertebrate communities within the PRF.

6.1.3. Chapter 4 – Food web structures and fish migration

The aquatic systems of the PRF are resilient and the food webs will not be affected by the drought:

Previous research indicated that flow regimes, agricultural, industrial and domestic activities as well as drought have an impact on aquatic food web structures. These impacts range from shifting predator foraging, nitrogen enrichment, carbon depletion, to loss of aquatic species and vegetation. However, the aquatic food webs of the PRF were not significantly different in structure but differed in terms of available food web components. The major differences in the aquatic food webs were in nitrogen and carbon signatures and consumer diets. Aquatic systems in close proximity to agricultural practises will often be subjected to increased nitrogen inputs through the use of organic fertilisers. However, food web components in the Usuthu River were significantly enriched in nitrogen compared to the Phongolo River and corresponds to the findings of Chapter 2 as the Usuthu River had higher inorganic NO_3 and NO_2 concentrations. Furthermore, the Pongolapoort Dam also contributes to the lower nitrogen values in the Phongolo River as the dam traps nutrient-rich sediment and reduces nutrient supply to the river. Although the study of de Necker *et al*. (2019) indicated that the lower Phongolo River still receives nutrient input from the agricultural activities, the Pongolapoort Dam is responsible for trapping nutrient-rich sediments and retains upstream nutrient supply, thereby resulting in overall lower nitrogen values. Due to fewer impoundments in the Usuthu River compared to the Phongolo River, the nitrogen levels are higher since the natural flooding regime allows for sediment and nutrient transport. In addition, there is increased nutrient input from the large number of hippopotami pods concentrated in this reach of the river. This supports the findings by Masese *et al*. (2018) and Mthembu *et al*. (2020) who found enriched nitrogen levels where animal numbers were higher and corresponds with the findings of the higher inorganic nutrients in the Usuthu River (see Chapter 2).

The activities surrounding the PRF may not have severely impacted the aquatic food webs, but did influence consumer diets of the various aquatic ecosystems within the PRF. The consumer diets of the various aquatic systems within the PRF consisted largely of omnivorous feeding which is indicative of a stable food web. This points towards an adaptable food web as a result of the maintenance of a high diversity of biological communities. Furthermore, this non-specific feeding behaviour not only ensures survival but also facilitates biotic interactions. When consumers switch their feeding behaviour to more generalist feeding it prevents certain resources from becoming dominant and stabilises the food web.

Strontium isotopes have been successfully used in the past to track fish movement as well as their natal origin. Therefore, this tool was used in this study to determine whether it was possible to identify refuge and / or recruitment areas for fish. Since the large floodplain lakes, Shokwe and Nyamithi, are known to be important spawning habitats for floodplain riverine fish species, it was reasoned that these systems may act as refugia for fish. Due to favourable environmental conditions during drought events, the floodplain lakes could ensure growth and recruitment of offspring. *Clarias gariepinus* is known as one of the few freshwater species in Africa to migrate between lakes and rivers and have been recorded to migrate from Lake Kariba, Lake Edward and Lake Victoria to associated river systems. The results of the $^{87}Sr/^{86}Sr$ signatures in the otoliths of *C. gariepinus* indicated that they had restrictive movement in the Usuthu system, whereas *C. gariepinus* from the Phongolo River moved freely between various systems such as the Usuthu River as well as tributaries of the Phongolo River. Therefore, the aquatic systems within the PRF act as a refuge for one another during periods of drought.

The data support the hypothesis that the aquatic systems of the PRF are resilient, and the food webs will not be affected by the drought conditions. Aquatic food webs of the PRF were not significantly altered by factors associated with the prolonged local drought conditions. On the contrary, the aquatic ecosystems seemed to be stable and aquatic biota from the PRF were resilient to changes in the aquatic environment. It could therefore be concluded that although not optimal, the baseflow of the Phongolo River was able to sustain a stable food web structure.

6.1.4. Chapter 5 – Mecury bioaccumulation, biomagnification and human health risks

(1) Aquatic biota will indicate Hg bioaccumulation and that biomagnification has occurred through the food web; and (2) due to the biomagnification of Hg, there will be human health risks associated with the consumption of fish from the lower PRF:

Fish from the PRF form an important part of local community diet in and around the PRF. Therefore, determining contaminants such as Hg in the muscle of fish from the PRF provides insights into risks posed through consuming fish from the PRF. Mercury is one of the very few metals that has the ability to biomagnify through the food web and thus may pose a health risk to humans. South Africa is one of the top producers of coal and gold, two activities that are the main contributors to Hg pollution in Southern Africa. Furthermore, these activities not only influence aquatic ecosystems through direct (point source) inputs into rivers but can also enter rivers through diffuse sources by means of aerial deposition. There is limited information on how aerial deposition can potentially impact aquatic ecosystems. The PRF is one such area that has the potential to be influenced by aerial deposition as it falls within the dominant atmospheric transport pattern that flows from South Africa's Highveld to the east coast of South Africa. Mercury accumulated and biomagnified through the food webs of the two rivers and their associated floodplain lakes. Mercury showed a positive relationship with sediment TOC, fish length and TP with the latter indicating the potential human health risks associated with consuming fish from PRF. Although the concentrations measured are reflective of unimpacted systems, the positive relationship with TP and TMF indicated that biomagnification has occurred through the food webs of the PRF. However, even though there was biomagnification, the consumption of Hg-contaminated fish from the PRF does not pose any human health risks at the current levels. This is in contrast to other South African studies that demonstrated human health risks associated with consumption of Hg contaminated fish (see Verhaert *et al*., 2019).

This study provided an understanding into the Hg concentrations in sediment and aquatic biota and their potential risk to local communities in and around the PRF ecosystem. The data supported the first part of the hypothesis stating that components of aquatic systems within the NGR will indicate mercury contamination, bioaccumulation, and biomagnification through the food webs. However, even though Hg accumulated and biomagnified in the aquatic ecosystems of the PRF the human health risk assessment results do not support the hypothesis that there will be a risk when fish from the PRF is consumed.

6.3. Concluding remarks

The regulated lower PRF is impacted by several anthropogenic stressors such as the impoundment of the Phongolo River and agricultural as well as domestic activities. Moreover, over the course of the study, no flood releases from the Pongolapoort Dam took place. Although the baseflow releases from the Pongolapoort Dam seemed to sustain aquatic biodiversity in the lower Phongolo River, the flow was never sufficient to inundate the floodplain wetlands. Therefore, during the drought period the water quality of the system showed a deterioration. The Usuthu River, a largely natural river, has similar stressors to the Phongolo River located in the upper catchment; however, due to unregulated flow, the impacts on downstream aquatic systems were not as severe. Even during the low flow periods, the Usuthu River remains perennial. Because of this continuous flow it allows for the continuous movement and supply of nutrient-rich sediment, inundating downstream systems, improving downstream water quality, and facilitating connectivity to floodplain lakes. This resulted in better water and sediment quality of the Usuthu River, and associated floodplain lake compared to the Phongolo River and its associated floodplain lake (see Chapter 2). It was however surprising that the natural flooding regime of the Usuthu River did not lead to a macroinvertebrate community structure consisting of a higher diversity in comparison with the regulated Phongolo River since both rivers had similar macroinvertebrate community structures (see Chapter 3). However, there was a greater percentage of sensitive taxa in the Usuthu River (e.g., Baetidae, Heptageniidae and Atyidae) indicating better environmental quality. The Phongolo River contained more tolerant taxa (*P. acuta* and Lumbriculidae) and was the result of extreme flow regulation (through the Pongolapoort Dam) and the agricultural activity inputs.

The importance of the Usuthu River to the downstream aquatic systems of the PRF was evident in the influence that it has on the water quality and macroinvertebrate community structures of the floodplain lakes (Shokwe and Nyamithi). This was particularly noticeable with the freshwater inputs on the macroinvertebrate community structures in Lake Nyamithi. The aquatic food webs (as reflected by the stable isotope ratio's) were distinct for each of the systems of the PRF and reflected the effects of the upstream anthropogenic stressors. Furthermore, the $^{87}Sr/^{86}Sr$ ratios reflected the movement of *C. gariepinus* between the aquatic systems of the PRF and demonstrated the role of the floodplain lakes as refugia for fish during periods of drought (see Chapter 4). The NGR is a biodiversity hotspot and a protected area, therefore making it essential to monitor the environmental quality and aquatic biota of the associated systems. Pollutants such as Hg, are detrimental to aquatic environments are known to occur in high concentrations in aquatic biota in regions where anthropogenic pressures are high. The PRF has been identified to fall within the atmospheric pathway of Hg released from the coal-fired powerplants located in the Mpumalanga Province. Therefore, with its tendency to biomagnify it is essential to monitor the levels of Hg in the aquatic systems of the PRF since local communities utilise fish as a protein source. The aquatic ecosystems of the PRF displayed Hg accumulation, bioaccumulation and biomagnification through the food webs. However, no human health risks were associated with the consumption of fish from the PRF, and this is indicative of the overall quality of the aquatic systems within the PRF.

The main aim of the study was to assess the environmental quality and aquatic community composition of the Usuthu River, the effect the natural flooding regime has on the aquatic ecosystems within the PRF and whether the Usuthu River acts as refugia for the lower Phongolo Floodplain aquatic biodiversity when the Phongolo River's flow is reduced. The study indicated the higher environmental quality of the Usuthu River that subsequently positively influenced aquatic biota in the various aquatic systems within the PRF. Furthermore, the high diversity in floodplain lakes during HF emphasized the importance of hydrological connectivity in riverine floodplains. This in effect further demonstrated the large role the natural flooding regime of the Usuthu River plays in the structure and functioning of associated floodplain lakes within the PRF.

6.4. Recommendations

Recommendations for potential future research were determined during the present study.

- Due to the high flows of the Usuthu River during the rainy months, further research should be undertaken to understand the dynamics and influence of the Usuthu River on the downstream aquatic systems during periods of high flow.
- Since this was the first study to report on metal concentrations within the PRF, follow up assessments should take place to include locations outside the NGR to determine the levels outside of the conservation area.
- The study only provided metal concentrations in water and sediment from the aquatic systems of the PRF. Further studies on determining the metal concentrations within fish species of these aquatic systems will provide valuable information on the overall quality of these aquatic systems as fish are excellent biological indicators as they can integrate the effects of harmful environmental changes.
- As a result of its sustained high flow conditions, macroinvertebrate community and food web structures of the Usuthu River were determined during the low flow months, therefore, further investigations should take place in order to determine the structure and differences

between hydrological periods in the Usuthu River and its effect on Lakes Shokwe and Nyamithi.

- Although the study was successful in illustrating the movement of *C. gariepinus* between the aquatic systems during the LF periods as well the utilization of aquatic systems as a refuge, further investigation needs to be done to understand how fish species utilise aquatic systems within the floodplain during periods of high hydrological connectivity. This will provide valuable information on how fish species from the PRF utilises the floodplain during high connectivity.
- Pesticide analyses in the PRF (Bouwman *et al*., 1990; Smit *et al*., 2016; Volschenk *et al*., 2019) indicated the influence of the agricultural activities located along the Phongolo River, however, less is known of the Usuthu River. Further studies should be undertaken to determine the pesticide concentrations within the Usuthu River and fish from the river as well as how agricultural activities in the Eswatini region contributes to downstream concentrations. Furthermore, the analysis of pesticides will provide important baseline data for future studies in the Usuthu River.
- It is important to undertake further research into the ecology of the Usuthu River and its dynamics to fully understand the effect of the natural flooding regime on the aquatic community composition during different hydrological periods.

Importantly, the role of the Usuthu River and its contribution to the PRF associated ecosystems has largely been poorly understood. Therefore, this study provided the first indication of its pivotal role and contributions to the aquatic systems within the PRF. The lack of any impoundments and less agricultural inputs, caused for higher environmental quality and aquatic diversity of the Usuthu River and its associated floodplain lakes and thus forms an essential component to the PRF's diversity. The strong flows positively influence hydrological connectivity and further facilitated the movement of fish, the study highlighted how fish may use the various aquatic ecosystems within the PRF. Therefore, results from this study do indeed show that the Usuthu River has a huge potential to act as a refuge for the aquatic biodiversity of the lower Phongolo Floodplain system when the Phongolo River is under stress.

CHAPTER 7
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Table A1: Water quality parameters (mean ± standard deviation) of the different sites sampled in the Ndumo Game Reserve during the high flow (June 2018) and low flow (November 2018) seasons. Within columns means with common superscripts indicate no significant differences ($p < 0.05$).

Site	pH	Turbidity (NTU)	Sulphates (mg/L)	Phosphates (mg/L)	Nitrites (mg/L)	Nitrates (mg/L)	Chlorides (mg/L)	Ammonium (mg/L)	Chemical oxygen demand (COD) (mg/L)	Total dissolved solids (mg/L)	Total Hardness (mg/L)
Us HF	8.4±0.02	$25±1^{def}$	$43±10^{f}$	$0.07±0.03^{a}$	$0.13±0.15^{a}$	$3.1±0.91^{b}$	$30±0.58^{d}$	$0.43±0.10^{ab}$	$6.3±2.1^{c}$	$239±8.5^{cd}$	$14±4.4^{f}$
Us LF	7.9±0.64	$53±3^{bcd}$	$101±3.8^{de}$	$0.30±0.18^{a}$	$0.04±0.01^{a}$	$4.2±1.5^{ab}$	$23±0.21^{d}$	$0.09±0.01^{e}$	$10±3.3^{c}$	$212±28^{d}$	$123±7.6^{cd}$
Sh HF	7.6±0.23	$21±7.57^{f}$	$49±6.7^{f}$	$0.07±0.01^{a}$	$0.04±0.01^{a}$	$1.8±0.81^{b}$	$24±3.1^{d}$	$0.29±0.1^{bcd}$	$14±5.4^{c}$	$187±28.3^{d}$	$39±7^{f}$
Sh LF	7.7±0.14	$52±10.97^{bcde}$	$105±5.7^{d}$	$0.14±0.04^{a}$	$0.05±0.01^{a}$	$3.7±0.85^{ab}$	$26±1.7^{d}$	0.08^{e}	$15±8.9^{c}$	$220±7^{d}$	$109±20^{cde}$
Ph HF	8.3	83^{ab}	56^{def}	0.21^{a}	0.03^{a}	1.4^{b}	98^{cd}	0.15^{de}	4.2^{c}	494^{cd}	66^{def}
Nya LF	8±0.07	$61±20.0^{abc}$	$1197±27.5^{a}$	$0.23±0.08^{a}$	$0.11±0.01^{a}$	$4±0.95^{ab}$	$2277±81.5^{a}$	$0.18±0.04^{de}$	$41±1.3^{a}$	$7397±402^{a}$	$389±28.7^{a}$
Nya outlet HF	8.5	$39±1.0^{cdef}$	$156±2.5^{b}$	$0.36±0.47^{a}$	0.04^{a}	$1.8±0.6^{b}$	$237±5.6^{b}$	$0.43±0.01^{abc}$	$37±4.20^{ab}$	1630^{b}	$333±17.6^{b}$
Nya outlet LF	8.1	$85±13.65^{a}$	$146±13.8^{bc}$	$0.45±0.25^{a}$	0.24^{a}	$6.6±1.3^{a}$	$184±1.5^{bc}$	$0.54±0.02^{a}$	$11±5.2^{c}$	667^{bc}	$133±3.5^{c}$

Table A2: Dissolved water metal concentrations (µg/L) of aquatic ecosystems within the Ndumo Game Reserve from sampling surveys of June and November 2018. Within columns means±standard deviation with common superscripts indicate no significant differences ($p < 0.05$). Lower than Detection Limit: LD.

Sites	Al	As	Cd	Co	Cr	Cu	Pb	Fe	Mn	Ni	Zn	Mg	K	Na	Ca
Us HF	21±6.4^{a}	0.42±0.02^{e}	1.5^{a}	0.13±0.03^{c}	0.94±0.34^{bc}	6.9±5.1^{b}	0.3±0.14^{a}	45±2.9^{a}	2.4±0.46^{b}	0.6±0.18^{d}	21±20^{a}	13±0.02^{c}	2±0.22^{f}	42±0.46^{c}	17±0.34^{cde}
Us LF	117±14^{a}	0.44±0.05^{e}	0.03±0.01^{bc}	0.15±0.12^{bc}	0.23±0.07^{de}	4.5±1^{b}	0.2±0.1^{a}	91±98^{a}	3.8±4.9^{b}	0.63±0.32^{d}	6.5±3^{a}	11±0.2^{c}	9.9±0.37^{a}	37±0.76^{c}	15±0.32^{ef}
Sh HF	13±0.91^{a}	0.54±0.07^{cde}	0.1±0.05^{b}	0.14±0.04^{bc}	1.6±0.25^{a}	4.1±1^{b}	0.57±0.11^{a}	30±3.3^{a}	5.2±3.6^{b}	1.9±0.63^{abc}	8.2±1.1^{a}	7.8±0.32^{c}	2.9±0.53^{f}	27±1.5^{c}	13±0.84^{ef}
Sh LF	56±14^{a}	0.59±0.23^{cde}	0.04±0.03^{bc}	0.19±0.02^{bc}	0.37±0.09^{de}	11±5.6^{b}	0.5±0.13^{a}	38±11.5^{a}	1.1±0.66^{b}	2.6±0.58^{a}	6.3±2.1^{a}	7.2±1.9^{c}	4.6±1.1^{cde}	44±2.6^{c}	9.8±2.8^{f}
Ph HF	28±19^{a}	0.77±0.09^{bcd}	0.07^{bc}	0.1±0.01^{c}	0.66±0.02^{cd}	4.9±0.08^{b}	0.22±0.13^{a}	80±11^{a}	2.2±0.32^{b}	0.6±0.05^{d}	3.2±0.9^{a}	18±1^{c}	3.1±0.17^{f}	94±5.4^{bc}	22±1.4^{cd}
Nya LF	284^{a}	1.4±0.14^{a}	0.05±0.003^{bc}	0.49±0.12^{a}	0.28±0.05^{de}	34±17^{a}	0.86±0.96^{a}	7±5.8^{a}	1.5±0.31^{b}	2.2±0.29^{ab}	13±11^{a}	313±21^{a}	8.5±0.23^{b}	4279±494^{a}	90±4.9^{a}
Nya outlet HF	9.6±1.7^{a}	0.83±0.04^{bc}	0.01±0.01^{c}	0.32±0.01^{abc}	1.1±0.03^{b}	10±3^{b}	0.38±0.30^{a}	73±0.96^{a}	2.7±0.17^{b}	1.5±0.07^{bcd}	5.2±3.1^{a}	49±1.3^{b}	5.2±0.09^{c}	575±13^{b}	44±0.89^{b}
Nya outlet LF	5.1±3.1^{a}	0.93±0.02^{b}	0.02±0.004^{c}	0.39±0.18^{ab}	0.18±0.02^{e}	15±4.5^{b}	0.08±0.03^{a}	21±1.9^{a}	109±40^{a}	1.2±0.04^{cd}	8.8±0.53^{a}	10±0.19^{c}	5.1±0.03^{cd}	39±0.93^{c}	22±0.45^{c}

Table A3: Sediment metal concentrations (mg/kg dry weight) from the four main aquatic ecosystems of the Ndumo Game Reserve from sampling surveys of June and November 2018. Within columns means±standard deviation with common superscripts indicate no significant differences ($p < 0.05$).

Sites	TOC (%)	Metals										
		Al	As	Cd	Co	Cr	Cu	Pb	Fe	Mn	Ni	Zn
Usuthu HF	1±0.22	5855^{d}	0.96^{d}	0.03^{a}	5.8^{e}	24^{e}	11^{e}	3^{e}	7972^{e}	186^{cde}	13^{d}	9.9^{e}
Usuthu LF	2.9±1.2	7679±6645^{d}	1.1±0.44^{d}	0.1±0.02^{a}	5.5±3.5^{e}	38±22^{e}	12±7.8^{e}	4.6±2.4^{e}	9656±6486^{e}	164±90^{e}	16±11^{d}	14±8.7^{e}
Shokwe HF	13±0.62	48059±7036^{abc}	4.2±0.36^{abc}	0.13±0.1^{a}	27±1.6^{abc}	150±9.2^{abc}	57±1.9^{ab}	20±0.89^{a}	52090±1284^{ab}	447±60^{abc}	86±13^{a}	49±2.5^{ab}
Shokwe LF	19±6.8	59324±13850^{a}	3±0.35^{bcd}	0.07±0.007^{a}	17±2.8^{d}	154±25^{a}	44±7.8^{abcd}	16±2.6^{abc}	38510±6291^{abcd}	281±26^{acde}	73±18^{ab}	49±6.8^{abc}
Phongolo HF	9.3±0.4	19242^{abcd}	2.6^{bcd}	0.08^{a}	17de	63^{abcde}	40^{abcde}	8.2^{abcde}	30486^{abcde}	600^{a}	31^{abcd}	33^{abcde}
Nyamithi LF	6.4±4.9	15812±16998^{cd}	1.8±0.82^{d}	0.1±0.07^{a}	7.2±4.2^{e}	62±48de	16±12^{e}	5.7±4.6^{e}	14795±12537de	252±74^{cde}	21±20^{cd}	19±13de
Nyamithi inlet HF	9.8±2.2	35783±14146^{abcd}	5.1±1.4^{ab}	0.08±0.02^{a}	31±3.2^{a}	123±30^{abcd}	58±13^{a}	16±4.9^{abcd}	46849±12544^{abc}	407±102^{abcd}	57±18^{abcd}	44±17^{abcd}
Nyamithi inlet LF	8±0.29	52251±6009^{ab}	5.4±0.16^{a}	0.07±0.02^{a}	28±3.7^{ab}	152±17^{ab}	54±9^{abc}	19±3^{ab}	55074±4773^{a}	567±105^{ab}	66±7.1^{abc}	50±5.6^{a}

Appendix B1: Full list of macroinvertebrate traits and its abbreviation used in multivariate statistical analysis.

Macroinvertebrate Traits	Abbreviation
Gills	Gills
Tegument/Cutaneaous	Tegu
Aerial: Spiracle	Spiracle
Aerial/vegetation	Ae: Veg
Plastron	Plastron
Aerial: Lungs	Lungs
Gravel, sand and mud	GSM
Mud	Mud
Stones	Stones
Habitat: indifferent	HydI
Vegetation	Veg
Free Living	Free living
Sand	Sand
Pools	Pools
Hydraulic: indifferent	HydrI
Bottom dwelling	Bottom dwelling
Riffles	Riffles
Runs	Runs
Rapid	Rapid
Temporary pools	Temporary pools
Aquatic Active	AqA
Aquatic Passive	AqP
Aerial Active	AeA
Predator	Predator
Scrapers	Scraper
Grazer	Grazer
Filter feeder	Filter feeder
Deposit feeder 1	DF 1
Deposit feeder 2	DF 2
Deposit feeder 4	DF 3
Omnivore	Omnivore
Shredder	Shredder
Highly tolerant	HT
Moderately tolerant	MT
Moderately sensitive	MS
Highly sensitive	HS

Appendix B2: Detailed list of aquatic macroinvertebrates with abbreviations (in parentheses) and families collected during the present study in the Usuthu River together with the Phongolo in- and outside river sites collected by de Necker 2019 within the Ndumo Game Reserve.

Species	**Family**	**Present study**	**de Necker 2019**	
		Usuthu River	Phongolo River In 2016	Phongolo River Out 2016
***Anax* sp.**	Aeshnidae	0	4	4
***Burnupia* sp. (Burnu)**	Ancylidae	0	0	0
Caridina nilotica (C. nilo)	Atyidae	114	12	217
***Acanthiops* sp. (Acanthiop)**	Baetidae	0	0	0
***Baetidae* sp. (Baetid)**	Baetidae	296	2	7
***Cloeon* and *Procloeon* (Clo&Pro)**	Baetidae	0	5	0
***Appasus* sp. (Appas)**	Belostomatidae	20	0	0
***Limnogeton fieberi* (L. fieb)**	Belostomatidae	1	0	0
***Afrocaenis* sp. (Afroc)**	Caenidae	31	0	0
***Caenis* sp.**	Caenidae	70	0	0
Calanoida (Calano)	Calanoida	0	0	1
***Phaon iridipennis* (P. irid)**	Calopterygidae	3	0	0
Chironominae (Chiron)	Chironomidae	0	0	4
Chydoridae (Chydo)	Chydoridae	0	0	0
***Ceriagrion* sp. (Ceriag)**	Coenagrionidae	0	0	0
***Enallagma* sp. (Enall)**	Coenagrionidae	0	0	0
***Pseudagrion* sp. A (Pseuda spA)**	Coenagrionidae	0	17	40
***Pseudagrion* sp. B (Pseuda spB)**	Coenagrionidae	0	0	0
***Corbicula* sp. (Corbic)**	Corbiculidae	0	10	0
***Micronecta* sp. (Micron)**	Corixidae	3	1	0
Crambidae sp. A (Cram spA)	Crambidae	0	1	1
***Anopheles* sp. (Anoph)**	Culicidae	1	0	0
***Culex* sp. A (Cul spA)**	Culicidae	0	12	0
***Mansonia* sp. (Mans)**	Culicidae	0	1	0
***Curculionidae* larva (Curcu larv)**	Curculionidae	0	0	0

Appendix B2: Continued

Species	Family	Present study	de Necker 2019	
		Usuthu River	Phongolo River In 2016	Phongolo River Out 2016
Cyrtobagous salviniae **(C. salv)**	Curculionidae	0	0	1
Neochetina **sp. (Neochet)**	Curculionidae	0	0	0
Neohydromus affinis **(N. affi)**	Curculionidae	0	0	0
Stenopelmus rufinasus **(S. rufi)**	Curculionidae	0	0	0
Thermocyclops **sp. (Thermo)**	Cyclopidae	0	47	0
Cyprididae (Cyprid)	Cyprididae	0	7	0
Cytheroidea (Cyther)	Cytheroidea	0	0	0
Simocephalus serrulatus **(S. serru)**	Daphniidae	0	11	0
Darwinulidae (Darwin)	Darwinulidae	0	7	0
Cybister **sp. (Cyb sp.)**	Dytiscidae	0	4	0
Dytiscidae larva (Dytis larv)	Dytiscidae	0	1	0
Laccophilus **sp. (Laccoph)**	Dytiscidae	0	0	0
Elminae larva (Elmin larv)	Elmidae	0	1	0
Larainae **sp. larva (Larain larv)**	Elimidae	1	0	0
Leptelmis **sp. (Leptel)**	Elmidae	1	0	0
Brachydeutera **sp. pupa (Brachyd pup)**	Ephydridae	0	0	0
Ephydridae larvae (Ephyd larv)	Ephydridae	0	0	0
Microgomphus mozambicensis **(M. mozam)**	Gomphidae	0	1	6
Aulonogyrus **sp. (Aulon)**	Gyrinidae	118	2	0
Peltodytes **sp. (Pelt)**	Haliplidae	0	0	0
Afronurus **sp. (Afronu)**	Heptageniidae	24	0	0
Hirudinae (Hirud)	Hirudinea	0	0	0
Hydrachnellae (Hydrach)	Hydrachnidae	0	1	0
Hydrometra **sp. (Hydrom)**	Hydrometridae	3	0	0
Amphiops **sp. (Amphi)**	Hydrophilidae	0	0	0
Berosus **sp. (Bero)**	Hydrophilidae	2	0	0
Berosus **sp. larva (Bero larv)**	Hydrophilidae	2	0	0

Appendix B2: Continued

Species	Family	Present study	de Necker 2019	
		Usuthu River	Phongolo River In 2016	Phongolo River Out 2016
***Enochrus* sp. larva (Enoch larv)**	Hydrophilidae	0	4	0
***Laccobius* sp. (Laccob)**	Hydrophilidae	8	0	3
***Hydropsyche* sp. (Hydrops)**	Hydropsychidae	7	0	1
***Hydroptila* sp. (Hydro)**	Hydroptilidae	0	0	8
Lampyridae sp. A larva (Lampy spA)	Lampyridae	0	0	0
Lampyridae sp. B larva (Lampy spB)	Lampyridae	0	0	1
Leptoceridae sp. (Lepto)	Leptoceridae	1	0	0
***Euthraulus* sp. (Euth)**	Leptophlebiidae	1	0	0
***Bradinopyga cornuta* (B. corn)**	Libellulidae	0	1	2
***Diplacodes* sp. (Diplac)**	Libellulidae	0	0	0
***Olpogastra* sp. (Olpo)**	Libellulidae	0	1	0
***Orthetrum* sp. (Orthet)**	Libellulidae	0	3	0
***Tetrathemis polleni* (T. poll)**	Libellulidae	0	17	0
***Trithemis* sp. (Trith)**	Libellulidae	0	0	0
***Gomphocythere* sp. (Gompho)**	Limnocytheridae	0	0	0
Lumbriculidae (Lumbric)	Lumbriculidae	0	1	2
***Lymnaea natalensis* (L. natal)**	Lymnaeidae	0	3	2
***Moina micrura* (M. micr)**	Moinidae	1	0	0
Naididae (Naid)	Naididae	0	1	1
***Laccocoris* sp. (Laccoc)**	Naucoridae	8	0	3
***Naucoris* sp. (Nauc)**	Naucoridae	0	0	0
***Ranatra* sp. (Rana)**	Nepidae	0	1	0
***Canthydrus* sp. (Canth)**	Noteridae	0	0	0
***Enithares* sp. (Enith)**	Notonectidae	1	0	0
***Anisops* sp. (Anis)**	Notonectidae	4	14	1
Oligochaeta sp. (Oligo)	Oligochaeta	5	0	0

Appendix B2: Continued

Species	Family	Present study	de Necker 2019	
		Usuthu River	Phongolo River In 2016	Phongolo River Out 2016
Ostracoda (Ostrac)	Ostracoda	0	0	1
***Physa acuta* (P. acuta)**	Physidae	0	11	13
***Nilus margartatus* (N. marg)**	Pisauridae	10	0	0
***Biomphalaria pfeifferi* (B. pfei)**	Planorbidae	0	0	0
***Gyraulus costulatus* (G. cost)**	Planorbidae	0	2	0
***Allocnemis leucisticta* (A. leuc)**	Platycnemididae	7	0	0
***Plea* sp.**	Pleidae	0	1	0
Sciomyzidae larv (Sciom larv)	Sciomyzidae	0	0	4
Scirtidae (Scirt)	Scirtidae	0	0	1
***Simulium* sp. larv (Simul larv)**	Simuliidae	2	0	0
***Sphaerium incomitatum* (S. incom)**	Sphaeridae	0	0	0
Syrphidae larva (Syrph)	Syrphidae	0	1	0
Tabanidae larva (Taban)	Tabanidae	1	0	0
***Melanoides tuberculate* (M. tuber)**	Thiaridae	0	0	0
***Tarebia granifera* (T. gran)**	Thiaridae	0	0	15
***Tricorythus* sp. (Tricor)**	Tricorythidae	1	1	1
***Rhagovelia* sp. (Rhago)**	Veliidae	165	0	0

Appendix B3: Detailed list of aquatic macroinvertebrates with abbreviations (in parentheses) and families collected during the present study and study by de Necker *et al*. (2021) & Dube *et al*. (2019) in the floodplain lakes within Ndumo Game Reserve.

Species	Family	Present study			de Necker *et al*. (2021)	Dube *et al*. (2019)	
		Shokwe LF	Shokwe HF	Nyamithi 2018	Nyamithi 2017	Phongolo FI 1	Phongolo FI 4
***Anax* sp.**	Aeshnidae	0	8	0	0	0	14
***Anax imperator* (A. imp)**	Aeshnidae	0	4	0	0	0	0
***Anaciaeschna* sp. (Anac)**	Aeshnidae	0	4	0	0	7	1
***Cardina nilotica* (C. nilo)**	Atyidae	0	3	4	34	12	7
***Baetidae* sp. A (Baetid spA)**	Baetidae	0	125	31	0	0	0
***Baetidae* sp. B (Baetid spB)**	Baetidae	0	26	6	0	0	0
***Baetis* sp.**	Baetidae	0	0	1	0	0	0
***Cloeon* and *Procloeon* (Clo&Pro)**	Baetidae	0	0	0	0	18	39
***Appasus* sp. (Appas)**	Belostomatidae	0	0	0	16	18	29
***Lethocerus niloticus* (L. nilo)**	Belostomatidae	0	0	0	0	14	8
***Afrocaenis* sp. (Afroc)**	Caenidae	0	0	0	0	0	0
***Caenis* sp.**	Caenidae	0	0	6	0	0	0
***Culicoides* pupae (Culi pup)**	Ceratopogonidae	0	2	0	0	0	0
***Bezzia* sp. (Bezz)**	Ceratopogonidae	0	1	164	3	0	0
***Chironomidae* sp. pupae (Chiron pup)**	Chironomidae	0	2	1	0	0	0
Chironominae (Chiron)	Chironomidae	0	0	0	20	6	4
***Tanypodinae* sp. (Tany)**	Chironomidae	0	49	263	0	96	45
***Orthocladiinae* sp (Ortho)**	Chironomidae	0	0	0	0	68	0
Coenagrionidae sp. (Coenag)	Coenagrionidae	0	0	0	1	0	0
***Ceriogrion* sp. (Cerio)**	Coenagrionidae	0	2	3	0	0	0
***Enallagma* sp. (Enall)**	Coenagrionidae	0	0	0	3	0	0
***Pseudagrion* sp. A (Pseuda spA)**	Coenagrionidae	0	21	0	4	0	0

Appendix B3: Continued

Species	Family	Present study			de Necker *et al.* (2021)	Dube *et al.* (2019)	
		Shokwe LF	Shokwe HF	Nyamithi 2018	Nyamithi 2017	Phongolo FI 1	Phongolo FI 4
***Corbicula fluminalis* (C. flum)**	Corbiculidae	0	0	2	0	0	0
***Agraptocorixa* sp. (Agrap)**	Corixidae	0	214	39	0	0	0
***Micronecta* sp. (Micron)**	Corixidae	362	482	869	10	0	0
***Sigara* sp. (Sig)**	Corixidae	0	0	0	15	0	0
Crambidae sp. (Cramb)	Crambidae	0	9	1	0	0	0
***Anopheles* sp. (Anoph)**	Culicidae	0	6	1	0	0	0
***Culex* sp. (Cul)**	Culicidae	0	2	1	0	0	0
Curculionidae sp. (Curcu)	Curculionidae	0	0	0	1	0	0
Dolichopodidae sp. B (Doli spB)	Dolichopodidae	0	0	0	0	0	2
***Ahaggaria australis* (A. aus)**	Dryopidae	0	0	0	0	0	4
***Cybister* sp. A (Cyb spA)**	Dytiscidae	1	0	0	4	0	0
***Cybister* sp. larvae (Cyb larv)**	Dytiscidae	0	3	0	0	0	0
Dytiscidae larva sp. A (Dyt larv spA)	Dytiscidae	0	0	0	0	1	0
Dytiscidae larva sp. (Dyt larv)	Dytiscidae	0	0	0	0	0	5
***Hydaticus* sp. (Hydat)**	Dytiscidae	3	3	0	3	0	0
***Hydroglyphus* sp. (Hydroglyph)**	Dytiscidae	1	0	0	1	8	19
***Hydrovatus* sp. (Hydro)**	Dytiscidae	0	1	0	0	0	0
***Hyphydrus* sp. A (Hyphyd)**	Dytiscidae	0	0	22	34	6	16
***Laccophilus* sp. (Lacco)**	Dytiscidae	0	0	0	3	6	19
***Rhantus* larvae sp. (Rhan)**	Dytiscidae	0	1	0	0	0	0
Empididae sp. (Empi)	Dytiscidae	1	0	0	0	0	0

Appendix B3: Continued

Species	Family	Present study			de Necker *et al*. (2021)	Dube *et al*. (2019)	
		Shokwe LF	Shokwe HF	Nyamithi 2018	Nyamithi 2017	Phongolo FI 1	Phongolo FI 4
***Larainae* sp. larvae (Larain)**	Elmidae	1	1	0	0	0	0
***Gerris* sp. (Ger)**	Gerridae	0	3	0	0	0	0
***Eurymetra* sp. (Eury)**	Gerridae	0	4	0	0	0	0
***Limnogonus* sp. (Limno)**	Gerridae	0	0	2	0	0	0
***Naboandelus africanus* (N. afri)**	Gerridae	0	0	1	0	0	0
***Neogerris* sp. (Neo)**	Gerridae	0	8	0	0	0	22
***Rhagadotarsus hutchinsonii* (R. hutch)**	Gerridae	0	0	31	0	0	0
***Ceratogomphus pictus* (C. pic)**	Gomphidae	0	0	2	0	0	0
***Ictinogomphus ferox* (I. ferox)**	Gomphidae	0	0	0	2	0	0
***Paragomphus genei* (P. genei)**	Gomphidae	0	0	7	0	0	0
***Dineutus* sp.**	Gyrinidae	2	0	0	0	0	0
***Orectogyrus* sp. (Orecto)**	Gyrinidae	0	2	0	0	0	0
Hirudinea sp. (Hirud)	Hirudinea	1	3	0	0	0	0
Hydrachnella sp. (Hydrach)	Hydrachnidae	0	0	0	0	5	9
***Parasthetops* sp. (Para)**	Hydraenidae	2	6	0	0	0	0
***Hydrometra* sp. (Hydro)**	Hydrometridae	1	0	0	0	0	0
***Allocotocerus* sp. (Alloc)**	Hydrophilidae	0	0	0	0	9	0
***Berosus* sp. (Bero)**	Hydrophilidae	7	31	50	33	16	10
***Berosus* sp larvae (Bero larv)**	Hydrophilidae	0	1	5	0	0	0
***Enochrus* sp. (Enoch)**	Hydrophilidae	3	12	0	0	0	9
***Enochrus* sp. larvae (Enoch larv)**	Hydrophilidae	2	11	4	0	0	0
***Hydrophilus* sp. (Hydroph)**	Hydrophilidae	0	0	0	1	0	0
***Hydrophilus* sp. larvae (Hydroph larv)**	Hydrophilidae	0	10	0	0	0	0
***Helochares* sp. (Helo)**	Hydrophilidae	0	0	0	0	1	0

Appendix B3: Continued

Species	Family	Present study			de Necker *et al.* (2021)	Dube *et al.* (2019)	
		Shokwe LF	Shokwe HF	Nyamithi 2018	Nyamithi 2017	Phongolo Fl 1	Phongolo Fl 4
***Regimbartia* sp. (Regim)**	Hydrophilidae	0	0	0	0	0	10
***Laccobius* sp. (Laccob)**	Hydrophilidae	0	6	0	2	0	0
Leptoceridae sp. (Lepto)	Leptoceridae	0	0	4	0	0	0
***Pirata* sp.**	Lycosidae	8	10	0	0	0	0
***Brachythemis leucosticte* (B. leuc)**	Libellulidae	0	0	0	27	0	0
***Pantala flavescens* (P. flav)**	Libellulidae	0	3	7	0	1	0
Lumbriculidae sp. (Lumbri)	Lumbriculidae	0	0	0	2	0	0
***Mesovelia* sp. (Meso)**	Mesoveliidae	0	31	19	2	1	1
***Moina micrura* (M. mic)**	Moiniidae	0	1	0	0	0	0
***Laccocoris* sp. (Laccoc)**	Naucoridae	0	0	1	13	0	0
***Macrocoris* sp. (Macroc)**	Naucoridae	0	0	6	0	0	0
***Naucoris* sp. (Nauc)**	Naucoridae	1	0	0	0	0	0
***Neomacrocoris* sp. (Neomac)**	Naucoridae	0	5	0	0	0	0
***Laccotrephes* sp. (Laccot)**	Nepidae	0	0	3	0	0	0
***Ranatra* sp. (Rana)**	Nepidae	0	0	0	1	2	5
***Hydrocanthus* sp. (Hydroc)**	Noteridae	0	1	0	0	0	0
***Canthydrus* sp. (Canth)**	Noteridae	0	0	0	4	4	7
***Neohydrocoptus* sp. (Neohyd)**	Noteridae	0	0	0	0	2	31
***Anisops* sp. A (Anis spA)**	Notonectidae	14	26	148	21	69	101
***Anisops* sp. B (Anis spB)**	Notonectidae	0	0	0	0	1	0
***Anisops* sp. C (Anis spC)**	Notonectidae	0	0	0	0	3	0
***Enithares* sp. (Enith)**	Notonectidae	1	89	57	0	0	0
***Nychia limpida* (N. limp)**	Notonectidae	1	0	0	25	0	0
Oligochaeta sp. (Oligo)	Oligochaeta	13	56	119	0	35	0
Ostracoda sp. (Ostra)	Ostracoda	0	0	564	46	0	0
***Afrogyrus* sp. (Afrog)**	Planorbidae	0	0	0	0	1	2

Appendix B3: Continued

Species	Family	Present study			de Necker *et al.* (2021)	Dube *et al.* (2019)	
		Shokwe LF	Shokwe HF	Nyamithi 2018	Nyamithi 2017	Phongolo Fl 1	Phongolo Fl 4
***Bulinus depressus* (B. dep)**	Planorbidae	1	0	0	1	1	0
***Bulinus forskaii* (B. for)**	Planorbidae	0	0	0	0	4	27
***Bulinus natalensis* (B. natal)**	Planorbidae	0	0	0	0	3	0
***Bulinus tropicus* (B. trop)**	Planorbidae	0	0	0	0	8	17
***Nilus margartatus* (N. marg)**	Pisauridae	1	1	0	0	0	0
***Clogmia albopunctata* (C. albo)**	Psychodinae	0	7	0	0	0	0
Plea sp.	Pleidae	0	0	0	3	0	0
***Rhyssemus* sp. (Rhyss)**	Scarabaeidae	2	0	0	0	0	0
***Spercheus* sp. (Sper)**	Spercheidae	0	0	0	0	2	0
***Tabanus* sp. (Tab)**	Tabanidae	0	9	1	0	0	0
***Tetragnatha* sp. (Tetra)**	Tetragnathidae	0	17	0	0	0	0
***Melanoides tuberculate* (M. tuber)**	Thiaridae	0	0	1	0	0	0
***Tarebia granifera* (T. gran)**	Thiaridae	0	0	28	0	0	0
***Limonia* sp. (Lim)**	Tipulidae	0	1	0	0	0	0
***Tipula* sp.**	Tipulidae	0	12	0	0	0	0
Unionicolidae sp. (Unio)	Unionicolidae	2	0	0	0	0	0
***Neumania* sp. (Neu)**	Unionicolidae	0	24	0	0	0	0
***Angila* sp.**	Veliidae	0	1	0	0	0	0
***Rhagovelia* sp. (Rhago)**	Veliidae	0	3	0	0	0	0

Appendix B4: Macroinvertebrate traits associated with taxa found in the Usuthu River within the Ndumo Game Reserve during the November 2018 survey.

Species	Usuthu River					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Afrocaenis* sp.**	Gills	Stones	-	-	DF 3	MT
***Afronurus* sp.**	Gills	Stones	Rifles	AeA	Grazer	HS
Allochemis leucosticta	Gills	Veg	Pools	-	Predator	MT
***Anisops* sp.**	Spiracle	Veg	Pools	AeA	Predator	HT
***Anopheles* sp.**	Ae: /veg	Veg	Indifferent	-	Filter feeder	HT
***Appasus* sp.**	Spiracle	Veg	Pools	-	Predator	HT
***Aulonogyrus* sp.**	Spiracle	Free living	Indifferent	AqA	Predator	MT
***Baetidae* sp.**	Gills	Indifferent	Indifferent	AqA	DF 1	MT
***Berosus* larvae**	Plastron	Veg	Temporary pools	AeA	Scraper	MT
***Berosus* sp. A**	Plastron	Veg	Temporary pools	AeA	Scraper	MT
***Caenis* sp.**	Gills	GSM	-	-	DF 3	MT
Caridina nilotica	Gills	GSM	Indifferent	AqA	DF 2	MS
***Enithares* sp.**	Spiracle	Free living	Pools	AeA	Predator	HT
***Epheron* sp.**	Gills	Sand	-	-	-	-
***Euthraulus* sp.**	Gills	Stones	Pools	-	DF 1	MS
***Hydrometra* sp.**	Spiracle	Veg	Pools	AeA	Predator	MT
Hydropsyche sp.	Gills	Stones	Rapid	-	Predator	MT
***Laccocoris* sp.**	Spiracle	Veg	Pools	AeA	Predator	MS
***Laccotrephes* sp.**	Ae: veg	Veg	Pools	AeA	Predators	HT
***Larainae* sp. larva**	-	-	-	-	-	-
***Leptelmis* sp.**	Plastron	Stones	Riffles	AqA	Scraper	MS
***Leptoceridae* sp.**	-	Indifferent	-	-	Omnivore	MT
Limnogeton fieberi	Spiracle	Veg	Pools	AeA	Predator	HT
***Micronecta* sp.**	Spiracle	Free living	Indifferent	AeA	Predator	HT
Moina micrura	-	-	-	-	-	-

Appendix B4: Continued

Species	**Usuthu River**					
	Respiration	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**
Nilus margartatus	-	-	-	-	-	-
***Oligochaeta* sp.**	-	-	-	-	-	HT
Paragomphus sp.	Gills	Mud	Pools	AqP	Predator	MT
Parasthetops sp.	Spiracle	Stones	Indifferent	-	Scraper	MS
Phaon iridipennis	Gills	Veg	Riffles	AqP	Predator	MS
***Rhagovelia* sp.**	Spiracle	Free living	Pools	AeA	Predator	MT
***Simulium* sp. larva**	Gills	Stones	Indifferent	-	Filter feeder	MT
Tabanidae larvae	Spiracle	Stones	Indifferent	-	Predator	MT
***Tricorythus* sp.**	Gills	Stones	-	-	DF 2	-

Appendix B5: Macroinvertebrate traits associated with taxa found in the Phongolo River inside and outside the Ndumo Game Reserve during previous surveys (de Necker, 2019). Bold dashed lines indicate trait not determined for taxa according to Odume *et al*. (2018) while open space indicate taxa not sampled at the specific site. Please see Appendix B1 for abbreviation of traits.

Species	Phongolo River Inside						Phongolo River Outside					
	Respiration	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**	**Respiration**	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**
***Afronurus* sp.**							Gills	Stones	Riffles	AeA	Grazer	HT
***Amphiops* sp.**	Plastron	Veg	–	AeA	Scraper	MT						
***Anax* sp.**	Gills	Veg	Pools	AqA	Predator	MS	Gills	Veg	Pools	AqA	Predator	MS
***Anisops* sp.**	Spiracle	Veg	Pools	AeA	Predator	HT	Spiracle	Veg	Pools	AeA	Predator	HT
***Appasus* sp.**	Spiracle	Veg	Pools	-	Predator	HT	Spiracle	Veg	Pools	-	Predator	HT
***Aulonogyrus* sp.**	Spiracle	Free living	Indifferent	AqA	Predator	MT						
***Baetidae* sp.**	Gills	Indifferent	Indifferent	AqA	DF 1	MT	Gills	Indifferent	Indifferent	AqA	DF 1	MT
Biomphalaria pfeiferi	–	–	Indifferent	–	Scraper	HT						
***Brachydeutera* sp. pupa**	–	–	–	–	–	–						
Bradinopyga cornuta	Gills	Stones	Pools	AqA	Predator	MT	Gills	Stones	Pools	AqA	Predator	MT
***Burnupia* sp.**	–	Stones	Indifferent	-	Scraper	HT						
***Calanoida* sp.**							–	–	–	–	–	–
***Canthydrus* sp.**	Plastron	Mud	Pools	AqA	Predator	MT						
Caridina nilotica	Gills	GSM	Indifferent	AqA	DF 2	MS	Gills	GSM	Indifferent	AqA	DF 2	MS
***Ceriogrion* sp.**							Gills	Veg	Pools	–	Predator	MT
***Chironominae* larvae**	Gills	Indifferent	Pools	AqA	DF 1	HT	Gills	Indifferent	Pools	AqA	DF 1	HT
***Chydoridae* sp.**	Gills	Mud	Bottom dwelling	–	Filter feeder	–						

Appendix B5: Continued

Species	Phongolo River Inside						Phongolo River Outside					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
Cloeon & Procloeon	Gills	Veg	Riffles	AqA	DF 2	MT						
***Coenagrionidae* sp.**							Gills	GSM	Pools	–	Predator	MT
***Corbicula* sp.**	–	GSM	Indifferent	–	Filter feeder	MT						
***Crambidae* sp. A larvae**	Tegu	Veg	Pools	AeA	Shredder	HS	Tegu	Veg	Pools	AeA	Shredder	HS
***Culex* sp. A**	Ae: veg	Stones	Indifferent	–	Filter feeder	HT						
***Curculionidae* larvae**							Plastron	Veg	Indifferent	AqA	Grazer	–
***Cybister* sp.**	Plastron	Free living	Pools	AqA	Predator	MT						
***Cyprididae* sp.**	–	–	–	–	–	–						
Cyrtobagous salviniae	Plastron	Veg	Indifferent	–	Grazer	–	Plastron	Veg	Indifferent	–	Grazer	–
***Cytheroidea* sp.**	Tegu	Veg	Pools	–	–	–						
***Darwinulidae* sp.**	Tegu	Veg	Pools	–	–	–	Tegu	Veg	Pools	–	–	–
***Diplacodes* sp.**	Gills	Stones	Pools	AqA	Predator	MT						
***Dytiscidae* larvae**	Plastron	Free living	Pools	–	Predator	MT						
***Elminae* larvae**	Plastron	–	–	–	–	–						
***Enallagma* sp.**	Gills	Stones	-	AqP	Predator	MT						
***Enochrus* larvae**	Plastron	Veg	Pools	AeA	Scraper	MT						
***Ephydrid* larvae**	Ae: veg	–	–	–	Scraper	HT	Ae: veg	–	–	–	Scraper	HT
***Gomphocythere* sp.**	Tegu	Veg	Pools	–	–	–						

Appendix B5: Continued

Species	Phongolo River Inside						Phongolo River Outside					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
Gyraulus costulatus	–	Stones	Indifferent	–	Scraper	HT						
***Hirudinea* sp.**	Tegu	–	Pools	–	Predator	HT						
***Hydrachnellae* sp.**	–	–	–	–	Predator	-						
***Hydropsyche* sp.**							Gills	Stones	Rapid	–	Predator	MT
***Hydroptila* sp.**							Gills	Stones	–	–	DF 2	MT
***Laccobius* sp.**	Plastron	Veg	Pools	AeA	Scraper	MT						
***Laccocoris* sp.**							Spiracle	Veg	Pools	AeA	Predator	MS
***Laccophilus* sp.**	Plastron	Free living	Pools	AqA	Predator	MT						
***Lampyridae* sp. A larvae**	–	–	–	–	–	–						
***Lampyridae* sp. B larvae**							–	–	–	–	–	–
***Lumbriculidae* sp.**	Tegu	–	–	–	DF 1	HT	Tegu	–	–	–	DF 1	HT
Lymnae natalensis	–	Veg	Indifferent	–	Scraper	HT	–	Veg	Indifferent	–	Scraper	HT
***Mansonia* sp.**	Ae: veg	Indifferent	Indifferent	–	Filter feeder	HT						
Melanoides tuberculate							–	Stones	Indifferent	–	Scraper	HT
Microgomphus mozambicensis	Gills	Mud	Pools	AqP	Predator	MT	Gills	Mud	Pools	AqP	Predator	MT
***Micronecta* sp.**	Spiracle	Free living	Indiferrent	AeA	Predator	HT						
***Naididae* sp.**	Tegu	–	–	–	Grazer	HT	Tegu	–	–	–	Grazer	HT
***Naucoris* sp.**	Spiracle	Veg	Pools	AeA	Predator	MS						

Appendix B5: Continued

Species	Phongolo River Inside						Phongolo River Outside					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Neochetina* sp.**	Plastron	Veg	Indifferent	AqA	Grazer	–						
Neohydromus affinis	Plastron	Veg	Indifferent	AqA	Grazer	–						
***Olpogastra* sp.**	Gills	Veg	Pools	–	Predator	MT						
***Orthetrum* sp.**	Gills	Mud	Pools	AqA	Predator	MT						
***Ostracoda* sp.**							–	–	–		DF 1	–
***Peltodytes* sp.**	Spiracle	Veg	Pools	AeA	Grazer	MT						
Physa acuta	–	Veg	–	–	–	HT	–	Veg	–	–	–	HT
***Plea* sp.**	Spiracle	Veg	Pools	AeA	Predator	MT						
***Pseudagrion* sp. A**	Gills	Veg	Indifferent	–	Predator	MT	Gills	Veg	Indifferent	–	Predator	MT
***Pseudagrion* sp. B**							Gills	Veg	Indifferent	–	Predator	MT
***Ranatra* sp.**	Ae: veg	Veg	Pools	–	Predator	HT						
***Sciomyzidae* sp. larvae**							–	–	–	–	Predator	–
***Scirtidae* sp.**							–	–	–	–	Filter feeder	–
Simocephalus serrulatus	–	–	–	–	Filter feeder	–						
Sphaerium incomitatum	–	–	–	–	–	HT						
Stenopelmus rufinasus	Plastron	Veg	Indifferent	–	Grazer	–						

Appendix B5: Continued

Species	Phongolo River Inside						Phongolo River Outside					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Syrphidae* larvae**	Ae: veg	GSM	Indifferent	–	Filter feeder	HT						
***Tabanidae* larvae**						MT	Spiracle	Stones	Indifferent	–	Predator	MT
Tarebia granifera							–	–	–	–	Scraper	HT
Tetrathemis polleni	Gills	Stones	Pools	AqA	Predator	MT						
***Thermocyclops* sp.**	Tegu	–	Indifferent	AqA	Predator	–	Tegu	–	Indifferent	AqA	Predator	–
***Tricorythus* sp.**	Gills	Stones	–	–	DF 2	–	Gills	Stones	–	–	DF 2	–
***Trithemis* sp.**							Gills	Mud	Pools	AqA	Predator	MT

Appendix B6: Macroinvertebrate traits associated with taxa found during different hydrological periods in Lake Shokwe within the Ndumo Game Reserve during the present study. Bold dashed lines indicate trait not determined for taxa according to Odume *et al*. (2018) while open space indicate taxa not sampled at the specific site. Please see Appendix C1 for abbreviation of traits.

Species	Lake Shokwe LF						Lake Shokwe HF					
	Respiration	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**	**Respiration**	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**
***Agraptocorixa* sp.**							Spiracle	Free living	Indifferent	–	Predator	HT
***Anaciaeschna* sp.**							Gills	–	Pools	AqA	Predator	MS
Anax imperator							Gills	Veg	Pools	AqA	Predator	MS
***Anax* sp.**							Gills	Veg	Pools	AqA	Predator	MS
***Angila* sp.**							Spiracle	Free living	Pools	AeA	Predator	MT
***Anisops* sp.**	Spiracle	Veg	Pools	AeA	Predator	HT	Spiracle	Veg	Pools	AeA	Predator	HT
***Baetidae* sp. A**							Gills	Indifferent	Indifferent	AqA	DF 1	MT
***Baetidae* sp. B**							Gills	Indifferent	Indifferent	AqA	DF 1	MT
***Berosus* larvae**							Plastron	Veg	Temporary pools	AeA	Scraper	MT
***Berosus* sp. A**	Plastron	Veg	Temporary pools	AeA	Scraper	MT	Plastron	Veg	Temporary pools	AeA	Scraper	MT
***Bezzia* sp.**							Gills	Mud	Indifferent	–	Grazer	MT
Bulinus depressus	–	–	–	–	Scraper	HT						
Caridina nilotica							Gills	GSM	Indifferent	AqA	DF 2	MS
***Ceriogrion* sp.**							Gills	Veg	Pools	–	Predator	MT

Appendix B6: Continued

Species	Lake Shokwe LF						Lake Shokwe HF					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Chironomidae* sp. pupae**							Gills	Indifferent	Pools	AqA	DF 1	HT
Clogmia albopunctata							Ae: veg	Indifferent	–	–	DF 2	HT
***Crambidae* sp.**							Tegu	Veg	Pools	AeA	Shredder	HS
***Culex* sp. A**							Ae: veg	Stones	Indifferent	–	Filter feeder	HT
Culicoides pupae							Gills	Mud	Indifferent	–	Filter feeder	HT
***Cybister* larvae sp.**							Plastron	Free living	Pools	AqA	Predator	MT
***Cybister* sp.**	Plastron	Free living	Pools	AqA	Predator	MT						
***Dineutus* sp.**	Spiracle	Free living	Indifferent	AqA	Predator	MT						
***Empididae* sp.**	–	Stones	Indifferent	–	Predator	MT						
***Enithares* sp.**	Spiracle	Free living	Pools	AeA	Predator	HT	Spiracle	Free living	Pools	AeA	Predator	HT
***Enochrus* sp.**	Plastron	Veg	Pools	AeA	Scraper	MT	Plastron	Veg	Pools	AeA	Scraper	MT
***Enochrus* larvae**	Plastron	Veg	Pools	AeA	Scraper	MT	Plastron	Veg	Pools	AeA	Scraper	MT
***Eurymetra* sp.**							Spiracle	Veg	Pools	AeA	Predator	MT
***Gerris* sp.**							Spiracle	Veg	Pools	AeA	Predator	MT
***Glossosomatidae* sp.**	–	Stones	–	–	Scraper	MS						
***Hirudinea* sp.**	Tegu	–	Pools	–	Predator	HT	Tegu	–	Pools	–	Predator	HT
***Hydaticus* sp.**	Plastron	Free living	Pools	AqA	Predator	MT	Plastron	Free living	Pools	AqA	Predator	MT

Appendix B6: Continued

Species	Lake Shokwe LF						Lake Shokwe HF					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Hydrocanthus* sp.**							Plastron	Veg	Pools	AqA	Predator	MT
***Hydroglyphus* sp.**	Plastron	Free living	Pools	AqA	Predator	MT						
***Hydrometra* sp.**	Spiracle	Veg	Pools	AeA	Predator	MT						
***Hydrophilus* sp. larvae**							Plastron	Veg	Pools	–	Scraper	MT
***Hydrovatus* sp. larvae**							Plastron	Veg	Pools	AqA	Predator	MT
***Laccobius* sp.**							Plastron	Veg	Pools	AeA	Scraper	MT
***Larainae* sp. larvae**	–	–	–				–	–	–	–	–	–
***Limonia* sp.**							Gills	Veg	Indifferent	-	Predator	MT
***Mesovelia* sp.**							Spiracle	Veg	Pools	AeA	Predator	MT
***Micronecta* sp.**	Spiracle	Free living	Indifferent	AeA	Predator	HT	Spiracle	Free living	Indifferent	AeA	Predator	HT
Moina micrura							–	–	–	–	–	–
***Naucoris* sp.**	Spiracle	Veg	Pools	AeA	Predator	MS						
***Neogerris* sp.**							Spiracle	Veg	Pools	–	Predator	MT
***Neomacrocoris* sp.**							Spiracle	Veg	Pools	AeA	Predator	MS
***Neumania* sp.**							–	–	–	–	–	–
Nilus margartatus	–	–	–	–	–	–	–	–	–	–	–	–

Appendix B6: Continued

Species	Lake Shokwe LF						Lake Shokwe HF					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
Nychia limpida	Spiracle	Free living	Pools	AeA	Predator	HT						
***Oligochaeta* sp.**	–	–	–	–	–	HT	–	–	–	–	–	HT
***Orectogyrus* sp.**							Spiracle	Free living	Indifferent	AqA	Predator	MT
Pantala flavescences							Gills	Veg	Pools	AqA	Predator	MT
***Parasthetops* sp.**	Spiracle	Stones	Indifferent	–	Scraper	MS	Spiracle	Stones	Indifferent	–	Scraper	MS
***Pirata* sp.**	–	–	–	–	–	–	–	–	–	–	–	–
Pseudagrion spermatum							Gills	Veg	Indifferent	–	Predator	MT
***Rhagovelia* sp.**							Spiracle	Free living	Pools	AeA	Predator	MT
***Rhantus* sp. larvae**							Plastron	Veg	Pools	–	Predator	MT
***Rhyssemus* sp.**	–	–	–	–	–	–						

Appendix B6: Continued

Species	Lake Shokwe LF						Lake Shokwe HF					
	Respiration	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**	**Respiration**	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**
***Tabanus* sp.**							Spiracle	Mud	Indifferent	–	Predator	MT
***Tapypodinae* sp.**							Gills	Indifferent	–	AqA	Predator	HT
***Tetragnatha* sp.**							–	–	–	–	–	–
***Tipula* sp.**							–	Mud	Indifferent	–	Predator	MT
***Unionicolidae* sp.**	Gills	GSM	Indifferent	–	–	MT						

Appendix B7: Macroinvertebrate traits associated with taxa found in Lake Nyamithi from a previous survey (2017) (de Necker *et al*., 2021) and during the present study (2018). Bold dashed lines indicate trait not determined for taxa according to Odume *et al*. (2018) while open space indicate taxa not sampled at the specific site. Please see Appendix C1 for abbreviation of traits.

Species	**Lake Nyamithi 2017**						**Lake Nyamithi 2018**					
	Respiration	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**	**Respiration**	**Habitat**	**Hydraulic**	**Dispersal**	**Functional feeding groups**	**Sensitivity**
***Agraptocorixa* sp.**							Spiracle	Free living	Indifferent	**–**	Predator	HT
***Anisops* sp. A**	Spiracle	Veg	Pools	AeA	Predator	HT						
***Anisops* sp.**							Spiracle	Veg	Pools	AeA	Predator	HT
***Anopheles* sp.**							Ae: veg	Veg	Indifferent	**–**	Filter feeder	HT
***Appasus* sp.**	Spiracle	Veg	Pools	**–**	Predator	HT						
***Baetidae* sp. A**							Gills	Indifferent	Indifferent	AqA	DF 1	MT
***Baetidae* sp. B**							Gills	Indifferent	Indifferent	AqA	DF 1	MT
***Baetis* sp.**							Gills	Stones	Riffles	AqA	DF 1	MS
***Berosus* larvae**							Plastron	Veg	Temporary pools	AeA	Scraper	MT
***Berosus* sp. A**	Plastron	Veg	Temporary pools	AeA	Scraper	MT	Plastron	Veg	Temporary pools	AeA	Scraper	MT
***Bezzia* sp.**	Gills	Mud	Indifferent	**–**	Grazer	MT	Gills	Mud	Indifferent	**–**	Grazer	MT
Brachythemis leucosticta	Gills	Mud	Pools	AqA	Predator	MT						
Bulinus depressus	**–**	**–**	**–**	**–**	Scraper	HT						
***Caenis* sp.**							Gills	GSM	**–**	**–**	DF 3	MT
***Canthydrus* sp.**	Plastron	Mud	Pools	AqA	Predator	MT						
Caridina nilotica	Gills	GSM	Indifferent	AqA	DF 2	MS	Gills	GSM	Indifferent	AqA	DF 2	MS

Appendix B7: Continued

Species	Lake Nyamithi 2017						Lake Nyamithi 2018					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Ceratogomphus* sp.**							Gills	GSM	Pools	AqP	Predator	MT
***Ceriogrion* sp.**							Gills	Veg	Pools	–	Predator	MT
***Chironimae* sp.**	Gills	Indifferent	Pools	AqA	DF 1	HT						
***Chironomidae pupae* sp.**							Gills	Indifferent	Pools	AqA	DF 1	HT
***Coenagrionidae* sp.**	Gills	GSM	Pools	–	Predator	MT						
Corbicula fluminalis							–	GSM	Indifferent	–	Filter feeder	MT
***Crambidae* sp.**							Tegu	Veg	Pools	AeA	Shredder	HS
***Culex* sp. A**							Ae: veg	Stones	Indifferent	–	Filter feeder	HT
***Curculionidae* sp.**	Plastron	Veg	Indifferent	AqA	Grazer	HT						
***Cybister* sp. A**	Plastron	Free living	Pools	AqA	Predator	MT						
***Enallagma* sp.**	Gills	Stones	–	AqP	Predator	MT						
***Enithares* sp.**							Spiracle	Free living	Pools	AeA	Predator	HT
***Enochrus* larvae**							Plastron	Veg	Pools	AeA	Scraper	MT
***Hydaticus* sp.**	Plastron	Free living	Pools	AqA	Predator	MT						
***Hydroglyphus* sp.**	Plastron	Free living	Pools	AqA	Predator	MT						

Appendix B7: Continued

Species	Lake Nyamithi 2017						Lake Nyamithi 2018					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Hydrophilus* sp.**	Plastron	Veg	Pools	–	Scraper	MT						
***Hyphydrus* sp.**							Plastron	Free living	Pools	AqA	Predator	MT
***Hyphydrus* sp. A**	Plastron	Free living	Pools	AqA	Predator	MT						
Ictinogomphus ferox	Gills	Mud	Pools	AqP	Predator	MT						
***Laccobius* sp.**	Plastron	Veg	Pools	AeA	Scraper	MT						
***Laccocoris* sp.**	Spiracle	Veg	Pools	AeA	Predator	MS	Spiracle	Veg	Pools	AeA	Predator	MS
***Laccophilus* sp.**	Plastron	Free living	Pools	AqA	Predator	MT						
***Laccotrephes* sp.**							Ae: veg	Veg	Pools	AeA	Predator	HT
***Leptoceridae* sp.**							–	Indifferent	–	–	Omnivore	MT
***Limnogonus* sp.**							Spiracle	Veg	Pools	AeA	Predator	MT
***Lumbriculidae* sp.**	Tegu	–	–	–	DF 1	HT						
***Macrocoris* sp.**							Spiracle	Veg	Pools	AeA	Predator	MS
Melanoides tuberculate							–	Stones	Indifferent	–	Scraper	HT

Appendix B7: Continued

Species	Lake Nyamithi 2017						Lake Nyamithi 2018					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Mesovelia* sp.**	Spiracle	Veg	Pools	AeA	Predator	MT	Spiracle	Veg	Pools	AeA	Predator	MT
***Micronecta* sp.**	Spiracle	Free living	Indifferent	AeA	Predator	HT	Spiracle	Free living	Indifferent	AeA	Predator	HT
Naboandelus africanus							Spiracle	Veg	Pools	AeA	Predator	MT
Nychia limpida	Spiracle	Free living	Pools	AeA	Predator	HT						
***Oligochaeta* sp.**							–	–	–	–	–	HT
***Ostracoda* sp.**	–	–	–	–	DF 1	–	–	–	–	–	DF 1	–
Pantala flavescencs							Gills	Veg	Pools	AqA	Predator	MT
***Paragomphus* sp.**							Gills	Mud	Pools	AqP	Predator	MT
***Plea* sp.**	Spiracle	Veg	Pools	AeA	Predator	MT						
***Pseudagrion* sp. A**	Gills	Veg	Indifferent	–	Predator	MT						
***Ranatra* sp.**	Ae: veg	Veg	Pools	–	Predator	HT						
Rhagadotarsus hutchinsonii							Spiracle	Veg	Pools	AeA	Predator	MT
***Sigara* sp.**	Spiracle	Free living	Indifferent	AeA	Predator	HT						
***Tabanus* sp.**							Spiracle	Mud	Indifferent	–	Predator	MT
***Tapypodinae* sp.**							Gills	Indifferent	–	AqA	Predator	HT
Tarebia granifera							–	–	–	–	Scraper	HT

Appendix B8: Macroinvertebrate traits associated with taxa found in two Phongolo River floodplain lakes during previous surveys (Dube *et al*., 2019).

Species	Ph Floodplain 1						Ph Floodplain 4					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
Afrogyrus coretus	Lungs	Veg	Indifferent	–	–	HT	Lungs	Veg	Indifferent	–	–	HT
***Agraptocorixa* sp. A**	Spiracle	Free living	Indifferent	–	Predator	HT	Spiracle	Free living	Indifferent	–	Predator	HT
Ahaggaria australis							Plastron	Stones	Riffles	AqA	–	MS
***Allocotocerus* sp.**	Plastron	Veg	–	AeA	Scraper	MT						
***Anaciaeschna* sp.**	Gills	–	Pools	AqA	Predator	MS	Gills	–	Pools	AqA	Predator	MS
***Anax* sp.**							Gills	Veg	Pools	AqA	Predator	MS
***Anisops* sp. A**	Spiracle	Veg	Pools	AeA	Predator	HT	Spiracle	Veg	Pools	AeA	Predator	HT
***Anisops* sp. B**	Spiracle	Veg	Pools	AeA	Predator	HT						
***Anisops* sp. C**	Spiracle	Veg	Pools	AeA	Predator	HT						
***Appasus* sp.**	Spiracle	Veg	Pools	–	Predator	HT	Spiracle	Veg	Pools	-	Predator	HT
***Berosus* sp. A**	Plastron	Veg	Temporary pools	AeA	Scraper	MT	Plastron	Veg	Temporary pools	AeA	Scraper	MT
Bulinus depressus	–	–	–	–	Scraper	HT						
Bulinus forskaii	–	–	–	–	Scraper	HT	–	–	–	–	Scraper	HT
Bulinus natalensis	–	–	–	–	Scraper	HT						
Bulinus tropicus	–	–	–	–	Scraper	HT	–	–	–	–	Scraper	HT
***Canthydrus* sp.**	Plastron	Mud	Pools	AqA	Predator	MT	Plastron	Mud	Pools	AqA	Predator	MT

Appendix B8: Continued

Species	Ph Floodplain 1						Ph Floodplain 4					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
Caridina nilotica	Gills	GSM	Indifferent	AqA	DF 2	MS	Gills	GSM	Indifferent	AqA	DF 2	MS
***Chironimae* sp.**	Gills	Indifferent	Pools	AqA	DF 1	HT	Gills	Indifferent	Pools	AqA	DF 1	HT
Cloeon & Procloeon	Gills	Veg	Riffles	AqA	DF 2	MT	Gills	Veg	Riffles	AqA	DF 2	MT
***Dolichopodidae* sp. B**							Spiracle	–	–	–	Predator	–
***Dytiscidae* larvae**							Plastron	Free living	Pools	–	Predator	MT
***Dytiscidae* larvae sp. A**	Plastron	Fee living	Pools	–	Predator	MT						
***Enallagma* sp.**	Gills	Stones	–	AqP	Predator	MT	Gills	Stones	–	AqP	Predator	MT
***Enithares* sp.**	Spiracle	Free living	Pools	AeA	Predator	HT						
***Enochrus* sp.**							Plastron	Veg	Pools	AeA	Scraper	MT
***Helochares* sp.**	Plastron	Veg	Pools	AeA	Scraper	MT						
***Hydrachnellae* sp.**	–	–	–	–	Predator	–	–	–	–	–	Predator	–
***Hydrophilus* sp.**	Plastron	Veg	Pools	–	Scraper	MT	Plastron	Veg	Pools	–	Scraper	MT
***Hyphydrus* sp. A**	Plastron	Free living	Pools	AqA	Predator	MT	Plastron	Free living	Pools	AqA	Predator	MT
***Laccophilus* sp.**	Plastron	Free living	Pools	AqA	Predator	MT	Plastron	Free living	Pools	AqA	Predator	MT
Lethocerus niloticus	Spiracle	Veg	Pools	AeA	Predator	HT	Spiracle	Veg	Pools	AeA	Predator	HT
***Mesovelia* sp.**	Spiracle	Veg	Pools	AeA	Predator	MT	Spiracle	Veg	Pools	AeA	Predator	MT

Appendix B8: Continued

Species	Ph Floodplain 1						Ph Floodplain 4					
	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity	Respiration	Habitat	Hydraulic	Dispersal	Functional feeding groups	Sensitivity
***Neogerris* sp.**						MT	Spiracle	Veg	Pools	–	Predator	MT
***Neohydrocoptus* sp.**	Plastron	Mud	Pools	AqA		MT	Plastron	Mud	Pools	AqA	Predator	MT
***Oligochaeta* sp.**	–	–	–	–	–	HT						
***Orthocladiinae* sp.**	Gills	Stones	Runs	AqA	Scraper	HT						
Pantala flavescens	Gills	Veg	Pools	AqA	Predator	MT						
***Ranatra* sp.**	Ae: veg	Veg	Pools	–	Predator	HT	Ae: veg	Veg	Pools	–	Predator	HT
***Regimbartia* sp.**							Plastron	Veg	–	AeA	Scraper	MT
***Spercheus* sp.**	Plastron	Free living	Pools	–	Filter feeder	–						
***Tapypodinae* sp.**	Gills	Indifferent	–	AqA	Predator	HT	Gills	Indifferent	–	AqA	Predator	HT

Appendix C1: Names, abbreviations (in parentheses), number of replicates, mean trophic position, mean δ^{13}C and δ^{15}N isotopic signatures of (±SE) of the various food web components collected in the Usuthu River during November 2018 within the Ndumo Game Reserve.

Components	Species	N	Trophic position	Mean δ^{13}C (±SE)	Mean δ^{15}N (±SE)
Primary Producers	Onagraceae	4	1	-30 (±0.52)	8.4 (±0.24)
	Leaf litter	4	1	-27 (±0.32)	6.8 (±0.3)
	Poaceae	4	1	-29 (±0.24)	6.7 (±1.19)
Macroinvertebrates	Atyidae	4	2	-22 (±0.24)	11 (±0.14)
	Gomphidae (Gomph)	4	2	-21 (±0.04)	11 (±0.03)
	Gyrinidae (Gyr)	4	2	-24 (±0.23)	11 (±0.08)
	Belostomatidae (Belo)	4	2	-24 (±0.38)	11 (±0.29)
	Potamonautidae (Pota	4	2	-26 (±1.28)	11 (±0.38)
	Macrobrachium sp. (Macro)	1	3	-19 (±0)	11 (±0)
Consumers	*O. mossambicus* (*O. moss*)	9	2	-23 (±0.69)	13 (±0.23)
	C. gariepinus (*C. gariep*)	15	3	-22 (±0.33)	14 (±0.20)
	S. zambezensis (*S. zam*)	15	3	-22 (±0.3)	15 (±0.17)
	M. macrolepidotus (*M. macro*)	11	3	-22 (±0.62)	14 (±0.12)
	P. wesselsi (*P. wess*)	15	3	-23 (±0.58)	15 (±0.21)
	S. intermedius (*S. inter*)	15	3	-22 (±0.3)	15 (±0/25)
	L. congoro (*L. cong*)	3	2	-24 (±1.14)	12 (±1.17)
	E. afrohamiltoni (*E. afro*)	4	3	-21 (±1.17)	14 (±0.25)
	B. imberi (*B. imb*)	15	3	-23 (±0.36)	15 (±0.17)
	H. vittatus	2	4	-20 (±0.98)	17 (±0.55)

Appendix C2: Names, abbreviations (in parentheses), number of replicates, mean trophic position, mean $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of (±SE) of the various food web components collected in the Phongolo River during September 2016 within the Ndumo Game Reserve. Adapted from de Necker (2019).

Components	Species	N	Trophic position	Mean $\delta^{13}C$ (±SE)	Mean $\delta^{15}N$ (±SE)
Primary Producers	*Azolla filiculoides* (*A. fili*)	4	1	-27 (±0.02)	3.14 (±0.11)
	Biofilm	5	1	-23 (±0.05)	5.08 (±0.13)
	Ceratophyllaceae (Ceratophyll)	4	1	-20 (±0.05)	5.79 (±0.05)
	Detritus	4	1	-28 (±0.02)	4.35 (±0.03)
	Eichhornia crassipes (*Eich*)	4	2	-27 (0.04)	7.78 (±0.02)
	Leaf litter	4	1	-29 (±0.03)	4.53 (±0.03)
	Onogracea (Onogra)	4	2	-29 (±0.04)	7.45 (±0.01)
	Poaceae	4	1	-13 (±0.11)	5.32 (±0.09)
	Zooplankton (Zooplank)	4	1	-27 (±0.09)	4.78 (±0.13)
Macroinvertebrates	Aeshnidae (Aesh)	4	2	-21 (±0.34)	8.51 (±0.42)
	Atyidae (Atyid)	6	2	-21 (±0.26)	7.24 (±0.15)
	Belostomatidae (Belosto)	1	1	-25 (±0)	5.44 (±0)
	Coenagrionidae (Coena)	4	2	-28 (±0.63)	7.11 (±0.97)
	Diptera	1	1	-37 (±0)	4.52 (±0)
	Dytiscidae	5	2	-25 (±1.17)	7.71 (±0.38)
	Hydrophilidae (Hydrophill)	3	1	-22 (±0.61)	4.61 (±0.32)
	Lampyridae (Lampyr)	2	2	-22 (±1.08)	7.21 (±0.08)

Appendix C2: Continued

Components	Species	N	Trophic position	Mean $\delta^{13}C$ (±SE)	Mean $\delta^{15}N$ (±SE)
Macroinvertebrates	Libellulidae (Libell)	6	2	-22 (±0.48)	7.34 (±0.08)
	Lycosidae (Lycos)	4	2	-24 (±1)	9.28 (±0.3)
	Macrobrachium sp. (*Macro* sp.)	10	3	-24 (±0.63)	12 (±0.32)
	Naucoridae (Naucor)	1	2	-21 (±0)	8.2 (±0)
	Nepidae (Nepid)	1	2	-26 (±0)	7.97 (±0)
	Noteridae (Noter)	1	1	-25 (±0)	3.34 (±0)
	Physidae (Physid)	2	1	-20 (±0.78)	3.72 (±0.02)
	Tabanidae (Taban)	1	2	-19 (±0)	6.96 (±0)
	Tarebia granifera (*T. gran*)	1	2	-23 (±0)	3.61 (±0)
Consumers	*B. imberi* (*B. imb*)	5	3	-23 (±0.23)	12 (±0.43)
	E. afrohamiltoni (*E. afro*)	1	3	-25 (±0)	12 (±0)
	G. giuris (*G. giur*)	4	3	-25 (±1.1)	11 (±0.78)
	H. vittatus (*H. vitt*)	1	4	-26 (±0)	13 (±0)
	O. mossambicus (*O. moss*)	5	2	-28 (±0.48)	7.17 (±0.11)
	P. wesselsi (*P. wess*)	8	4	-24 (±0.46)	14 (±0.19)
	S. intermedius (*S. inter*)	1	4	-25 (±0)	13 (±0)
	S. zambezensis (*S. zam*)	8	4	-24 (±0.65)	13 (±0.22)
	T. sparmanii (*T. spar*)	4	4	-22 (±0.71)	13 (±0.83)

Appendix C3: Names, abbreviations (in parentheses), number of replicates, mean trophic position, mean δ^{13}C and δ^{15}N isotopic signatures of (±SD) of the various food web components collected in Lake Shokwe during November 2018 within the Ndumo Game Reserve.

Components	Species	N	Trophic position	Mean δ^{13}C (±SE)	Mean δ^{15}N (±SE)
Primary Producer	*Hydrocharitaceae*	4	1	-26 (±0.08)	8.87 (±0.12)
	Phragmites sp.	4	1	-26 (±0.56)	8.75 (±0.47)
	Shrubs	4	1	-28 (±0.49)	8.12 (±0.53)
	Detritus	4	1	-31 (±0.07)	8.94 (±0.34)
Macroinvertebrates	Corixidae (Corix)	3	1	-23 (±0.08)	9.06 (±0.08)
	Dytiscidae Small (Dytis S)	4	1	-26 (±0.93)	9.59 (±0.43)
	Dytiscidae Large (Dytis L)	5	2	-28 (±0.78)	11 (±0.67)
	Belostomatidae (Belo)	3	2	-26 (±2.28)	9.81 (±1.23)
	Pisauridae (Pisau)	2	2	-22 (±0.08)	12 (0.46)
	Notonectidae (Noto)	3	2	-28 (±0.10)	12 (±1.06)
Consumers	*O. mossambicus* (*O. moss*)	15	3	-29 (±0.27)	14 (±0.08)
	C. rendalli (*C. ren*)	15	3	-26 (±0.11)	14 (±0.14)
	S. intermedius (*S. inter*)	7	3	-28 (±0.66)	16 (±0.07)
	E. afrohamiltoni (*E. afro*)	4	3	-24 (±1.69)	14 (±0.20)
	B. imberi (*B. imb*)	15	3	-28 (±0.27)	16 (±0.20)
	C. gariepinus (*C. gariep*)	15	3	-27 (±0.24)	15 (±0.15)
	H. vittatus (*H. vitt*)	9	4	-27 (±0.13)	17 (±0.05)

Appendix C4: Names, abbreviations (in parentheses), number of replicates, mean trophic position, mean δ^{13}C and δ^{15}N isotopic signatures of (±SE) of the various food web components collected in Lake Nyamithi during November 2018 within the Ndumo Game Reserve.

Site	Components	N	Trophic position	Mean δ^{13}C (±SE)	Mean δ^{15}N (±SE)
Macroinvertebrates	Gomphidae (Gomph)	4	1	-22 (±0.24)	9.23 (±0.22)
	Gerridae (Ger)	4	1	-24 (±0.24)	11 (±0.44)
	Libellulidae (Libel)	3	2	-19 (±1.60)	11 (±0.42)
	Belostomatidae (Belo)	2	2	-20 (±0.36)	8.48 (±0.10)
	Nepidae (Nepi)	3	2	-28 (±1.26)	9.49 (±2.14)
	Aspidytidae (Aspi)	3	2	-21 (±2.82)	9.72 (±0.87)
	Notonectidae (Noto)	4	3	-20 (±2.82)	10 (±0.87)
	Atyidae	3	3	-22 (±1.20)	12 (±0.25)
Consumers	*C. gariepinus* (*C. gariep*)	5	3	-21 (±0.13)	14 (±0.16)
	B. imberi (*B. imb*)	5	3	-21 (±0.41)	14 (±0.22)
	O. mossambicus (*O. moss*)	5	2	-23 (±1.34)	13 (±0.57)
	C. rendalli (*C. ren*)	5	2	-17 (±0.39)	13 (±0.34)
	H. vittatus (*H. vitt*)	4	3	-23 (±0.69)	15 (±0.17)

Appendix C5: Names, abbreviations (in parentheses), number of replicates, mean trophic position, mean δ^{13}C and δ^{15}N isotopic signatures of (±SE) of the various food web components collected in Lake Nyamithi during February 2017 within the Ndumo Game Reserve.

Site	Components	N	Trophic position	Mean δ^{13}C (±SE)	Mean δ^{15}N (±SE)
Primary Producers	Biofilm 1	3	1	-26 (±0.02)	8.29 (±0.0.01)
	Biofilm 2	6	1	-17 (0.12	7.6 (±0.17)
	Cyperaceaea	4	0.35	-12 (±0.01)	5.63 (±0.02)
	Detritus	12	0.16	-26 (±0.77)	5.01 (±0.71)
	Hydrocharitaceae	4	1	-16 (±0.03)	6.53 (±0.03)
	Leaf litter	4	2	-27 (±0.07)	11 (±0.0.02)
	Poaceae 1	8	2	-14 (±0.14)	11 (±1.43)
	Poaceae 2	4	0	-26 (±0.01)	2.62 (±0.02)
	Trapaceae	8	2	-25 (±0.12)	10 (±0.87)
Macroinvertebrates	Belostomatidae	2	2	-25 (±4.88)	11 (±0.38)
	Coenagrionidae	1	2	-26 (±0.33)	12 (±0)
	Corixidae	2	2	-17 (±2.08)	10 (±0.02)
	Dytiscidae	1	1	-23 (±0.28)	8.93 (±0)
	Hydrophilidae	2	2	-24 (±0.93)	11 (±0.43)
	Libellulidae	3	2	-20 (±2.33)	11 (±0.42)
	Macrobrachium sp.	2	3	-19 (±0.87)	14 (±0.25)
	Naucoridae	2	2	-21 (±1.39)	10 (±0.88)

Appendix C5: Continued

Components	Species	N	Trophic position	Mean $\delta^{13}C$ (±SE)	Mean $\delta^{15}N$ (±SE)
Macroinvertebrates	Nepidae	1	2	-23 (±0.47)	12 (±0.05)
	Tetragnathidae	1	2	-25 (±0.45)	11 (±0)
Consumers	*B. imberi* (*B. imberi*)	4	3	-19 (±1.17)	14 (±0.29)
	C. gariepinus (*C. gariep*)	4	3	-16 (±0.22)	13 (±0.18)
	E. afrohamiltoni (*E. afrohamiltoni*)	5	3	-18 (±1.12)	15 (±0.13)
	E. toppini (*E. toppini*)	1	3	-21 (±0)	15 (±0)
	E. trimaculatus (*E. trimaculatus*)	2	3	-17 (±1.35)	14 (±0.35)
	H. vittatus (small) (*H. vittatus*)	10	3	-16 (±0.16)	15 (±0.18)
	L. rosae (*L. rosae*)	2	3	-23 (±0.86)	13 (±0.19)
	O. mossambicus (*O. mossambicus*)	21	2	-17 (±0.12)	11 (±0.1)
	S. intermedius (*S. intermedius*)	5	3	-19 (±0.97)	14 (±0.3)
	S. zambezensis (*S. zambezensis*)	5	3	-20 (±0.51)	15 (±0.22)

Appendix C6: Species, number of replicates and Mean $^{87}Sr/^{86}Sr$ ratio (±SE) in ototliths of C. gariepinus collected in the various aquatic waterbodies within the Ndumo Game Reserve.

Site	Species	N	Mean $^{87}Sr/^{86}Sr$ ratio (±SE)
Usuthu River	*C. gariepinus*	5	0.7265 (±0.0004)
Phongolo River	*C. gariepinus*	5	0.7227 (±0.001)
Lake Shokwe	*C. gariepinus*	5	0.7261 (±0.0006)
Lake Nyamithi	*C. gariepinus*	5	0.7180 (±0.0007)

Appendix D1: Total number of samples analysed (N), mean ± SD of ranges of length (mm), Hg in dry (dw) and wet (ww) weight (ng/g) of all samples collected from the Usuthu River within Phongolo River Floodplain, South Africa, Range in parentheses. Common superscripts indicate no significant differences (p > 0.05).

Site	Sample	N	Trophic position	Length (mm)	Hg ng/g dw	Hg ng/g ww
Usuthu River	Shrubs	4	1	-	14±7.9 (5.6 – 24)	
	Atyidae	4	2	-	165±21 (139 – 188)	-
	Gomphidae	3	2	-	351±57^{a} (303 – 413)	-
	Gyrinidae	4	2	-	213±41^{a} (156 – 250)	-
	Potamonautidae	4	2	-	177±38^{a} (139 – 223)	-
	O. mossambicus	15	2	19±29 (152 – 235)	45±9.7^{a} (36 – 65)	27±5.9 (20 – 38)
	C. gariepinus	15	3	442±116 (209 – 654)	226±84^{b} (133 – 417)	45±16 (24 – 79)
	S. zambezensis	15	3	147±17 (112 – 180)	334±80^{cd} (171 – 484)	283±65 (149 – 419)
	M. macrolepidotus	11	3	198±37 (151 – 270)	263±85^{bc} (158 – 398)	169±72 (85 – 267)
	S. intermedius	15	3	213±39 (167 – 255)	364±129^{cd} (200 – 596)	239±81 (102 – 410)
	B. imberi	15	3	152±16 (111 – 171)	116±35^{a} (72 – 170)	84±28 (51 – 143)
	H. vittatus	2	4	250±20 (236 – 264)	700±184 (569 – 830)	362±73 (310 – 413)

Appendix D2: Total number of samples analysed (N), mean ± SD of ranges of length (mm), Hg in dry (dw) and wet (ww) weight (ng/g) of all samples collected from the Phongolo River within Phongolo River Floodplain, South Africa, Range in parentheses. Common superscripts indicate no significant differences (p > 0.05).

Site	Sample	N	Trophic position	Length (mm)	Hg ng/g dw	Hg ng/g ww
Phongolo River	*O. mossambicus*	15	-	184±28 (145 – 242)	33±26[a] (13 – 121)	21±16 (7.6 – 75)
	C. rendalli	5	-	137±48 (98 – 202)	22±18[a] (3.3 – 51)	16±16 (2.1 – 44)
	S. zambezensis	15	-	170±24 (120 – 200)	322±134 (90 – 625)	210±92 (54 – 384)
	H. vittatus	4	-	242±63 (150 – 290)	639±157 (473 – 842)	342±137 (200 – 505)

Appendix D3: Total number of samples analysed (N), mean ± SD of ranges of length (mm), Hg in dry and wet weight (ng/g) of all samples collected from Lake Shokwe within Phongolo River Floodplain, South Africa, Range in parentheses. Common superscripts indicate no significant differences (p > 0.05).

Site	Sample	N	Trophic position	Length (mm)	Hg ng/g dw	Hg ng/g ww
Lake Shokwe	*Hydrocharitaceae*	4	1	-	12±5.3 (6.7 – 17)	-
	Phragmites sp.	4	1	-	7.6±6.7 (1.6 – 15)	-
	Shrubs	4	1	-	41±25 (3.3 – 57)	-
	Corixidae	3	1	-	133±15^{a} (116 – 142)	-
	Dytiscidae	4	2	-	232±84^{ab} (155 – 352)	-
	Notonectidae	2	2	-	343±109^{b} (266 – 421)	-
	O. mossambicus	15	3	179±19 (165– 211)	96±22^{a} (68 – 135)	71±16 (53 – 104)
	C. rendalli	15	3	146±21 (120 – 188)	144±44^{ab} (83 – 246)	112±35 (48 – 182)
	B. imberi	15	3	120±9.3 (109 – 147)	274±79 (140 – 386)	167±30 (124 – 214)
	C. gariepinus	15	3	184±13 (290 – 775)	198±74^{bc} (110 – 320)	41±16 (22 – 70)
	H. vittatus	9	4	438±148 (150 – 195)	224±41^{c} (125 – 255)	156±26 (99 – 184)

Appendix D4: Total number of samples analysed (N), mean ± SD of ranges of length (mm), Hg in dry and wet weight (ng/g) of all samples collected from Lake Nyamithi within Phongolo River Floodplain, South Africa, Range in parentheses. Common superscripts indicate no significant differences (p > 0.05).

Site	Sample	N	Trophic position	Length (mm)	Hg ng/g dw	Hg ng/g ww
Lake Nyamithi	Gomphidae	3	1	-	356±101^{a} (241 – 432)	-
	Libellulidae	3	2	-	273±92^{ab} (189 – 371)	-
	Notonectidae	3	2	-	232±77^{ab} (183 – 320)	-
	O. mossambicus	15	2	185±21 (160 – 225)	82±30^{a} (31 – 148)	56±22 (23 – 100)
	C. rendalli	15	2	189±26 (130 – 240)	29±19^{a} (8.5 – 81)	18±13 (4.4 – 53)
	B. imberi	10	3	117±12 (101 – 139)	82±52 (51 – 228)	82±49 (47 – 212)
	C. gariepinus	15	3	596±109 (420 – 790)	168±45 (116 – 274)	35±11 (23 – 64)
	H. vittatus	4	3	172±7.3 (165 – 179)	377±60 (306 – 448)	309±61 (244 – 384)