BIOLOGICAL LONGITUDINAL ASPECTS OF THE KABOMPO RIVER, A SIGNIFICANT TRIBUTARY OF THE UPPER ZAMBEZI SUB-CATCHMENT, NORTH-WEST PROVINCE, ZAMBIA

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LOMARIE CATHLEEN JANSE VAN RENSBURG

g20J715

ORCID ID

http://orcid.org/0000-0001-8128-8250

Supervisor: Professor Anthony J. Booth

Co-supervisor: Professor Stephan Woodborne

Co-supervisor: Dr Josephine Pegg

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ABSTRACT

The landscape of the Upper Zambezi Catchment in the remote North-Western Province of Zambia retains many natural features, but current and future anthropogenic activities threaten its integrity. Lack of information relating to the basic ecological functioning of the region hampers conservation efforts. Flooding from the adjacent woody-savanna and open grasslands in the Upper-Zambezi Catchment in the wet season drives crucial processes in the aquatic ecosystems such as the Kabompo River. This thesis describes aquatic food webs and describes the nutrient cycling in this river. A general introduction was based on the seasonality,river morphology, and biodiversity of the Kabompo River.

There is no aquatic food web information available on this river. To address this knowledge gap, sampling of the principal food web components– vegetation, invertebrates, and fish – were collected in the dry season from August to September 2019 across six sites in the Kabompo River. Stable isotope analyses provided proxies for the food web structure at each site, and were the basis of longitudinal comparisons. The analyses suggest that the Kabompo River food webs follow the trophic ordination and nutrient cycling characteristic of the revised-Riverine Productivity Model. Food webs and community assemblages remain structurally similar between sites and provide some evidence of bottom-up productivity-driven trophic dynamics.

To predict the possible landscape-scale processing of nutrient changes present for the aquatic ecosystems of the Kabompo River, a longitudinal comparison between a herbivore, *Labeo cylindricus,* and omnivore, *Synodontis* spp., fish species and their resources (primary producers and invertebrates) was done and showed archetypal fish food web trophic separation (2 ‰ to3 ‰) between species which remains consistent for the headwater sites. The nutrient values change at the convergence between the Kabompo Bridge and the Mwinilunga branches and show a decrease in the trophic separation of the sites below. This change becomes apparent from the last site, Watopa, where the trophic separation re-establishes (2 ‰ to 3 ‰) between

the species. The trend suggests primarily autochthonous production in the headwaterreaches, changing to allochthonous and autochthonous nutrient incorporation after the convergence of the two main branches. Permanent wetlands surrounding the convergence zones may be conducive to more primary producer activity and increased nutrient turnover. Permanent wetlands are a common feature of the Upper Zambezi Catchment tributaries, with the most notable lentic system the Barotse Flood Plains in the south, which shares reach-adjacent characteristics with the Kabompo River. Trends from the 2015 dry season identified for the Barotse Flood Plainssupport the findings of this thesis, where the δ^{13}C values recorded reach depletion of -45 ‰.

Management in the Upper Zambezi Basin and the Kabompo River comprises biannual assessments of water quality and quantity by the Zambezi River Authority, but not of the ecosystem process. This thesis may provide information to address the ecological (food webs and nutrient cycling) dynamics of the Basin rivers. As the first baseline information on the river's biology and ecology, it may present a comparative basis for future assessments under conservation management strategies.

DECLARATION

I, Lomarie Cathleen Janse van Rensburg, hereby declare that this thesis submitted to the Department of Ichthyology and Fisheries Science, Rhodes University, is my original work and has not been previously submitted in any form to another university. I have not included ideas, phrases, passages, or illustrations from another person's work without acknowledging their authorship.

Name: Lomarie Cathleen Janse van Rensburg

Student number: g20j7215

Signed:

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> "All we have to decide is what to do with time that is given us." - Gandalf (*Lord of The Rings, Fellowship of the Ring*, J.R.R. Tolkien)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

CPOM – coarse particulate organic matter

DIC – dissolved inorganic carbon

DIN – dissolved inorganic nitrogen

DOC – dissolved organic carbon

DOM – dissolved organic matter

DON – dissolved organic nitrogen

ENS – effective number of species

FFG – functional feeding groups

FPOM – fine particulate organic matter

OM – organic matter

PIC – particulate inorganic carbon

POC – particulate organic carbon

POM – particulate organic matter

SAIAB – South African Institute for Aquatic Biodiversity

SEAc – Standard Ellipse Area corrected

SIBER – Stable Isotope Bayesian Ellipses in R

TDC – total dissolved carbon

TDN – total dissolved nitrogen

ZISS – Zambian Invertebrate Scoring System

CHAPTER 1: GENERAL INTRODUCTION

Large rivers are critical components of the global environment and provide multiple ecosystem services (Dudgeon 2010), such as water filtration in adjacent wetlands (Hammer and Bastian 2020), terrestrial-aquatic links (Shurin et al. 2006; Talbot et al. 2018), food security (McIntyre et al. 2016; Lima et al. 2020), landscape formation (Rice et al. 2001; Syvitski et al. 2014; ElHalim 2020), biodiversity support (Lima et al. 2020), and nutrient recycling (Dugan et al. 2006; Ward et al. 2013; Talbot et al. 2018). Aquatic ecosystems depend on intricate interactions for these services to continue (Oliver et al. 2015). The alteration of river flow can disrupt these processes and potentially lead to ecosystem service loss (Egoh et al. 2012; Datry et al. 2018; Tonkin et al. 2018; Zeiringer et al. 2018; Tickner et al. 2020). Knowledge of nutrient cycling provides a background for developing promising management approaches to maintain the integrity of riverine ecological interactions (Thorp et al. 1998; Houser 2016).

1.1 Nutrient cycling

Energy flows through organisms in ecosystems as nutrient cycling enables survival, reproduction, and migration, among other activities (Weber and Brown 2013). The interactions that drive energy flow can indicate how ecosystems function because nutrients are selectively bioavailable (Cox 1996; Caraco et al. 1998; Finlay 2001; Weber and Brown 2013, Ward et al. 2013; Cejudo et al. 2018; Chapman 2021a). The primary nutrient cycling processes in aquatic systems that control the breakdown of dissolved organic matter (DOM) are sorption/desorption (particle attachments) and oxidation-reduction (redox) dependent chemistry (Ward et al. 2017). Sorption and desorption can alter the rate of DOM breakdown by preventing or facilitating itsbioavailability (IUPAC 1997; Schaetzel et al. 2017; Ward et al. 2017), and redox reactions area function of energetically efficient chemical reactions in aquatic environments, where either reduction- or oxidation-potential of chemicals is the driving force of the reaction (IUPAC 1997;Ward et al. 2017; Wyness et al. 2021). Both processes drive the cycles of carbon and nitrogenin aquatic habitats.

The carbon cycle

Freshwater ecosystems drive the carbon cycle connecting terrestrial and marine ecosystems (Ward et al. 2017). The products of DOM rely on several factors, such as sorption, desorption, redox state, biological community compositions, photo-oxidation, burning, microbial metabolism, priming and dilution (Zonneveld et al. 2010; Ward et al. 2017).

Microbial nutrient breakdown occurs in the absence (anaerobic) or presence (aerobic) of oxygen with different by-products (Docherty et al. 2006; Sanseverino et al. 2012). Both microbial pathways are present in aquatic habitats, but in general, aerobic breakdown occurs in shallower lotic (rivers and streams) and anaerobic in deeper lentic (dams, lakes, wetlands) systems (Pace and Prairie 2005; Sanseverino et al. 2012). Products from aerobic DOM metabolism are smaller, primarily organic molecules and fewer inorganic molecules, such as carbon dioxide (CO_2) (Kristensen et al. 1995), while anaerobic metabolism creates low molecular weight organic and inorganic products (Kristensen et al. 1995). Most microbial nutrient cycling occurs in association with surfaces in aquatic systems where nutrients are present (Davies et al. 1995; Oliver et al. 2007). Biofilm-inhabiting bacteria actively seek particle adhesion for nutrients (Davies et al. 1995; Oliver 2005, Oliver et al. 2007; Wyness et al. 2018). In some rare cases, sorption and desorption may affect microbial metabolism as particles that bond to surfaces may not always be bioavailable for breakdown (Ward et al. 2017).

The processes of photo-oxidation, dilution, burning and priming weather organic materials (Ward et al. 2017). Photo-oxidation, which is common in lotic environments, is the photochemical breakdown of DOM in the presence of light and oxygen (Spencer et al. 2009; Porcal et al. 2015; Ward et al. 2017; Johannsson et al. 2021). Dilution burning and priming weather organic materials (Ward et al. 2017). Dilution relies on the control of sorption and desorption of DOM breakdown (Tyson 2001; Ward et al. 2017) and burning alters chemical structures (Baldock et al. 2004; Ward et al. 2017). Primingrefers to the six-fold breakdown of vascular plant material to gaseous carbon where ecosystemsintermix and labile carbon inputs into the soil can slow or accelerate the decomposition of soilorganic matter (Kuzyakov et al. 2000; Chen et al. 2014; Ward et al. 2017).

The nitrogen cycle

Nitrogen is most abundant in the atmosphere in the gaseous state of N_2 and becomes bioavailable during biological nitrogen fixation or nitrification (Berman and Bronk 2003). The process of nitrification by bacteria converts the gaseous phase to ions of nitrate (NO_3^-) and ammonia (NH_3) (Equations 1.1, 1.2 and 1.3) (IUPAC 1997; Delwiche 1970). Multicellular organisms use these forms to produce organic molecules that include amino acids and nucleotides (Jaffe 1992). These nitrogen ions flow through food webs before denitrification returns them to the atmosphere as the gaseous state of N_2 (Equation 1.5) (IUPAC 1997; Delwiche 1970).

$$N_2 + 8H^+ + 8e^- + 16ATP = 2NH_3 + H_2 + 16ADP + 16Pi \qquad \text{(Equation 1.1)}$$

The conversion of ammonium to nitrate (IUPAC 1997; Delwiche 1970): where N_2 = Nitrogen gas in the atmosphere; ATP = Adenosine triphosphate; ADP = Adenosine diphosphate; and Pi = inorganic Phosphate.

Oxidation half-reactions:

$$NH_4^+(aq) + 8OH^-(aq) \text{->} NO_2^-(aq) + 6H_2O(l) + 6e^- \qquad \text{(Equation 1.2)}$$

$$NO_2^-(aq) + 2OH^-(aq) \text{->} NO_3^-(aq) + H_2O(l) + 2e^- \qquad \text{(Equation 1.3)}$$

Reduction half-reactions:

$$O_2(aq) + 2H_2O(l) + 4e^- > 4OH^-(aq) \qquad \text{(Equation 1.4)}$$

$$2NO_3^-(aq) + 10e^- + 12H^+ \rightarrow N_2(g) + 6H_2O(l) \qquad \text{(Equation 1.5)}$$

where NH_4^+ is ammonium, NO_2^- is Nitrite, NO_3^- is a nitrate, H^+ represents the ionic form of hydrogen, N_2 is nitrogen, H_2O is water, OH^- is hydroxide, and e^- represents electron transfers.

Denitrification is no longer the only pathway that exists for nitrogen removal, further complicating the tracing and interpretations of food webs (Burgin and Hamilton 2007). In fish food web ecology denitrification is better understood than the other nitrogen removal pathways, described as mainly microbial (Burgin and Hamilton 2007). Therefore, in this thesis, denitrification is considered the main removal pathway for simplicity.

Nutrient cycling in aquatic ecosystems

Aquatic nutrient inputs are either autochthonous (produced in-stream and introduced into the main channel), allochthonous (littoral and terrestrial zone origin), or a combination of both pathways (Kaushik and Hynes 1971; Vander Zanden et al. 2005). Dissolved organic material is a complex mixture of organic molecules comprising carbon, hydrogen, oxygen, nitrogen, phosphorous, and sulphur. It is transported into aquatic ecosystems and includes decomposed soil organic matter, plant residues, and soluble particles (Perdue and Ritchie 2003; Docherty et al. 2006). The organic matter in aquatic habitats occurs as dissolved and as particulate organic matter (POM) which breaks down to fine particulate organic matter (FPOM 1.0 mm - 0.45 μm) accessible to microbes from POM breakdown, and coarse particulate organic matter (CPOM - > 1.0 mm), which is the non-reduced POM that remains (Wetzel 1995; Stelzer et al. 2003; Tant et al. 2013).

Organic matter in rivers is the source of nitrogen and carbon, which are essential nutrients for cellular maintenance (Schoeninger and DeNiro 1984; Gruber and Galloway 2008). The total dissolved carbon (TDC) pool contains small, labile low molecular weight products (carboxylic acids, amino acids, and carbohydrates) which contribute most to carbon cycling, and high molecular weight products (monosaccharides, polysaccharides, and acetyl, among others) (Repeta et al. 2002; Docherty et al. 2006; Berggren et al. 2010; Ward et al. 2017). Totaldissolved carbon (TDC) is categorised into dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) (Gattuso et al. 1998). The total dissolved nitrogen (TDN) pool includesdissolved organic nitrogen (DON) like urea, amino acids, composite organic nitrogen; and dissolved inorganic nitrogen (DIN) like nitrates (NO_3^-) and ammonium ($NH4^+$) (Jaffe 1992; Berman and Bronk 2003; Sipler and Bronk 2015). It is common to express the total pools of TDC and TDN as the sum of all their components such that the TDC is the sum of DOC and DIC; and the TDN is the sum of DON and DIN (Gattuso et al. 1998; Rabalais 2002). These DOM compounds are bioavailable to different organisms.

Heterotrophic bacteria use DOC (0.22-0.70 mm) as an allochthonous energy source, but particulate organic carbon (POC) (> 0.7 mm) accounts for 40% more of the carbon flux in streams as it is generally more labile (Cushing et al. 1993; Stutter et al. 2007; Raymond and Saiers 2010; Drummond et al. 2014; Zhuiykov 2014; Porcal et al. 2015; Brandt et al. 2017). The POC becomes part of the benthos, where bacteria metabolise it further (Drummond et al. 2014). Both DIC and particulate inorganic carbon (PIC) originate from the release of CO_2 and HCO_3 ions through soil weathering and air-water exchanges, but only PIC can be collected (Jones et al. 2002; Finlay 2003; Maberly et al. 2015; Kharbush et al. 2020). All PIC is non-carbonate and combustible (Kharbush et al. 2020). In contrast, all DIC particles play a vital role in the metabolism and community structure of stream autotrophs (Finlay 2003).

Most DON inputs are allochthonous (atmospheric deposition, plant detritus leachate, soils, and terrestrial run-off), while some are autochthonous (phytoplankton, macrophytes, bacteria and microzooplankton) (Jaffe 1992; Rabalais 2002; Berman and Bronk 2003; Sipler and Bronk 2015). These particles subdivide into low molecular weight products (dissolved free amino acids, pteridines, purines, pyrimidines, peptides, and more) and high molecular weight products (nucleic acids, dissolved combined amino acids, proteins, and other) (Antia et al. 1991; Sipler and Bronk 2015). Fixation of DON relies on photo-oxidation, microbial activity, phytoplankton respiration, and abiotic adsorption (Mulholland et al. 2004; Sipler and Bronk

2015; Porcal et al. 2015; Johannsson et al. 2021). The change from DON to DIN is a by-product of microbial activity where specialised microorganisms excrete various forms of nitrogen (Macko and Estep 1984; Wetzel 2001; Rabalais 2002; Camargo and Alonso 2006). Once dissolved, $NH4^+$ is oxidised to NO_3^- in a two-step process primarily by aerobic, chemoautotrophic bacteria from the genera *Nitrosomonas*, *Nitrobacter*, and anammox types (Sharma and Ahlert 1977; Stumm and Morgan 1996; Wetzel 2001; Kartal et al. 2004). Macrophytes, algae and bacteria then use these ions in solution as a resource (Macko and Estep 1984; Howarth 1988; Harper 1992; Paerl 1999; Wetzel 2001; Dodds et al. 2002; Camargo and Alonso 2006; Cejudo et al. 2018).

1.2 Models of energy flow in aquatic systems

Several theoretical models exist, which suggest pathways and characteristics of rivers under various conditions to represent their functioning using food web nutrient cycling (Vannote et al. 1980;Thorp et al. 1998; Thorp and Delong 2006; Tornwall et al. 2015). The theories considered in this thesis are the River Continuum Concept, the Flood Pulse Concept-revised, and the revised-River Productivity Model.

1.2.1 River Continuum Concept

This theory is the oldest conceptualisation of large-scale rivers along a longitudinal gradient (Vannote et al. 1980; Tornwall et al. 2015; Doretto et al. 2020). It is a predictor of upstream-to-downstream changes using three distinct zones and their associated flow velocities, biodiversity, geomorphology,and other spatial and temporal differences (Vannote et al. 1980; Doretto et al. 2020). These zones are headwater (stream order 1-3), middle water (stream order 4-6), and lower water (stream order > 6) reaches (Vannote et al. 1980; Doretto et al. 2020). The characteristics of each reach describe the passage of nutrients from terrestrial to aquatic environments (Vannoteet al. 1980; Doretto et al. 2020). Each reach is a proxy for multiple physical and chemical variables that affect food availability (Doretto et al. 2020). These food particles, POM and primary production, are believed to influence aquatic taxa depending on feeding behaviours. Initially based on macroinvertebrates, this theory made use of functional feeding group classifications over taxonomy (Cummins 1973, 1974; Cummins and Klug 1979; Anderson and Cummins 1979), which enabled improved predictions of energy movement and trophic relationships through aquatic systems (Grubaugh et al. 1996). The main predictions are 1) low-order streams are narrow, shaded, and have more inputs of CPOM (leaf litter) as the primary food source for shredder and collector functional feeding groups, as well as smaller fish that feed on these invertebrates; 2) mid-order streams have more primary production, fewerCPOM inputs due to river width increases leading to FPOM production and scraper, collector- gatherer and other periphyton feeder assemblages with fish that prefer these resources; and 3)high-order stream terrestrial inputs are negligible due to stream size, and in-stream nutrient production is at a peak, attracting collectors and filterers that consume heterotrophic products (Vannote et al. 1980; Doretto et al. 2020). The theory presents methods for testing different hypotheses but is ambiguous in its interpretations (Doretto et al. 2020), and it is limited in its applicability as tests revealed the circumstantial nature of most of its predictions (Barmuta andLake 1982; Statzner and Higler 1985; Doretto et al. 2020).

Alterations to the original theory improved its use, as many tropical studies and its original use of macroinvertebrate communities impeded interpretations (Statzner and Higler 1985; Doretto et al. 2020). One addendum to the theory is the link-discontinuity concept, which suggests a disruption to a large-scale river's longitudinal processes, shifting processes at tributaries (Rice et al. 2001). The additions address the rigorous models that disregard the discontinuous nature of lotic ecosystems (Perry and Schaeffer 1987; Bravard and Gilvear 1996), the importance of regulated and unregulated tributaries (Ward and Stanford 1983, 1995), and recognises the influence of tributaries (Minshall et al. 1983, 1985; Bruns et al. 1984). Two model categories, the river discontinuum and the river-networks-and-meta-community, originated from this simple river longitudinal continuum model (Ruiz-González et al. 2015; Tornwall et al. 2015; Bolpagni et al. 2016; Feio et al. 2017; Curtis et al. 2018; Doretto et al. 2020). The two theories included in this thesis from the river discontinuum category, which considers the influences of local conditions, are the Flood Pulse Concept-revised and revised-Riverine Productivity Model (Junk et al. 1989; Thorp and Delong 1994; Junk 1999; Thorp et al. 2006; Doretto et al. 2020).

1.2.2 Flood Pulse Concept-revised

This theory links river hydraulic flooding dynamics and floodplain nutrient inputs of new terrestrial material into aquatic systems via the littoral zone (edge of inundation) (Ward and Stanford 1983; Sedell et al. 1989; Junk et al. 1989; Spink et al. 1998; Olde Venterink et al. 2006; Keizer et al. 2014). The Flood Pulse Concept-revised emphasises the importance of allochthonous processes in streams from head reaches or floodplains to the rest of a catchment (Junk et al. 1989; Thorp and Delong 1994) but suggests that the main channel is a transportive agent with POM inputs from the head reaches being of lower quality than the nutrient inputs from the adjacent floodplains (Junk 2001).

During flood-pulse-wet-stages, autochthonous nutrient inputs from floodplains are mobilised into the water column (Junk et al. 1989; Kruse et al. 2004; Baldwin and Mitchell 2000; McClain et al. 2003). During flood-pulse-dry-stages, the inundated water drawn down causes high floodplain productivity in the river channel (Junk et al. 1989; Bayley 1995; Junk and Wantzen 2004; Bernal et al. 2013). The expectations for the Flood-Pulse Concept-revised are 1) high primary and secondary production from the conversion of transported DOM by primary producers during flood pulses and 2) the attraction of higher trophic level consumers by DOM cycling across a reach (Junk 2001). Carbon isotope analyses support the predictions from this model in low-gradient river types (Hoeinghaus et al. 2007). Some of the key considerations of this theory are changes in nutrient contributions from each reach and river type due to differences in topography, substrates, vegetation, and other characteristics (Pinay et al. 2000; Steiger and Gurnell 2002; Ward et al. 2002; Hein et al. 2003; Junk and Wantzen, 2004; Loeb et al. 2007, 2008; Chormanski et al. 2011; Southwell and Thoms 2011; Roley et al. 2012; Hudson et al. 2012; Langhans et al. 2013; Noe et al. 2013).

1.2.3 Revised-Riverine Productivity Model

This theory highlights the importance of autochthonous and allochthonous inputs and reduces the importance of upstream nutrient sources from solely allochthonous production pathways (Thorp and Delong 1994). The model for a hypothetical large river includes those with naturally constricted channels and is the most viable of all three models used to describe autotrophic productivity in these channels (Thorp et al. 1998, Humphries et al. 2014). The removal of some restrictions to the original model expands the application and increases the relative importance of autochthonous processes to river consumers – preferring more easily altered autochthonous inputs than refractory allochthonous inputs from the nearby riparian zone (Thorp and Delong 1994; Thorp and Delong 2002; Hoeinghaus et al. 2007). This theory,supported by carbon isotopic ratios, is relevant for high-gradient rivers, reservoirs, and rivers downstream of reservoirs (Hoeinghaus et al. 2007). The theory predicts 1) the domination of local autochthonous and allochthonous (phytoplankton, benthic algae, and aquatic vascular plants and mosses) or direct riparian (i.e., abscised leaves) inputs (as opposed to DOM from decayed matter in flood pulses), 2) that the transformation and storage of DOM are more substantial than transported nutrients from head reaches, 3) DOM assimilated by animals (Thorp and Delong 2002; Hoeinghaus et al. 2007; Humphries et al. 2014), and 4) significant changes between the DOC from reaches and rivers caused by a variety of influences on primary production (channel morphology, floodplain interactions, and riparian vegetation) (Thorp and Delong 2002; Gawne et al. 2007; Humphries et al. 2014).

1.3. Stable isotope analyses in ecology

Stable isotope analysis is used in ecology to trace the pathways of elements in an ecosystem, usually nitrogen and carbon (Jardine et al. 2003; Boecklen et al. 2011). Isotopes are elements with the same atomic number but different numbers of neutrons in their nuclei (IUPAC 1997). Aquatic ecosystems are typically closed, making nutrient tracing possible from source to sink (Jaffe 1992; Rabalais 2002; Berman and Bronk 2003; Durand et al. 2011). As elements pass through organisms in aquatic ecosystems, chemical reactions change the relative proportion of heavier isotopes (^{13}C and ^{15}N) to lighter isotopes (^{12}C and ^{14}N). These can be measured to a high degree of accuracy and sensitivity (per mille (parts per thousand; ‰)) relative to an international standard (Ben-David and Flaherty 2012; Hershey et al. 2017). Stable isotope analysis terminology simplifies isotope expression as relative to the heaviest form (carbon - $\delta^{13}C$; nitrogen - $\delta^{15}N$) (Peterson and Fry 1987; Anderson and Cabana 2007; Michener and Lajtha 2008).

The $\delta^{15}N$ indicates trophic placement as there is a significant stepwise increase (3–4 ‰) per trophic level, and the $\delta^{13}C$ provides information on nutrient carbon sources as their trophic enrichments are not substantial (0 ‰ to 0.5 ‰) (DeNiro and Epstein 1978; Minagawa and Wada 1984; Peterson and Fry 1987; Post 2002; McCutchan et al. 2003; Anderson and Cabana 2007; Pingram et al. 2014). These ratios result from tissue formation, diet-to-tissue discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$), and other chemical processes (Fry 1991; Pinnegar and Pollunin 1999).

1.3.1 Stable isotope analyses of food webs

Stable isotope analyses of aquatic food webs show trophic interactions and energy flow through nitrogen and carbon nutrient tracers (DeNiro and Epstein 1978; Fry 1991; Kendall 1998; Gladyshev 2009). Nitrogen ratios are complex as allochthonous; autochthonous and natural atmospheric nitrogen inputs contribute to the total nitrogen pool and account for significant variations in $\delta^{15}N$ over time (Kendall 1998; Valiela et al. 2000; Vander Zanden et al. 2005). Stable isotope analyses traces the primary producers incorporated nitrogen products into nitrogen constrained aquatic food webs (Fry 1991; Kendall 1998) and show the nitrogen source connected to $\Delta^{15}N$ (Jardine et al. 2003).. Isotope ratios from primary consumers (herbivores) represent the lowest consumer trophic level in an aquatic system as they reflect consumption of the primary productivity (Cabana and Rasmussen 1996; McKinneyet al. 2002; Layman et al. 2007). The ingestion of herbivores by higher trophic level organismspredictably changes the isotope ratio to a higher (enriched) ratio as nutrients pass between trophic groups (Pinnegar and Pollunin 1999; Boecklen et al. 2011; Ben-David and Flaherty 2012). Different primary consumer and producer $\delta^{13}C$ values are associated with distinct types of microbial respiration and carbon (C_3 and C_4) metabolism in a habitat (Sternberg and DeNiro 1983; Pace and Prairie 2005; Sanseverino et al. 2012; Pingram et al. 2014). Carbon originates from terrestrial and *in situ* production by primary producers, with more woody substrates such as trees and their products contributing to lower $\delta^{13}C$ over a longer time period due to slower decomposition (Farquhar et al. 1989; McKee et al. 2002). The carbon in aquatic systems is not constrained (Shurin et al. 2006) and undergo fewer chemical changes between tropic transfers (McCutchan et al. 2003; Ward et al. 2017). Both trends for $\delta^{15}N$ and $\delta^{13}C$ continue across all trophic levels from plants to invertebrates to fish (McKinney et al. 2002; Cole et al. 2004; Moore and Suthers 2005; Schlacher et al. 2005; Northington and Hershley 2006; Bucci et al. 2007). This is apparent in a bi-plot between $\delta^{13}C$ and $\delta^{15}N$ (Figure 1.1) (USGS 2003) which illustrates “who-eats-who” in the food web (Fry 1991). The typical trends in these biplots are primary producersand consumers that occupy the bottom left, and final consumers at the top right of a linear relationship as described by Fry (1991). It is important to note that aquatic ecologies rarely follow thetop-down predator-to-primary producer structure readily seen in terrestrial food webs (Shurin et al. 2006).

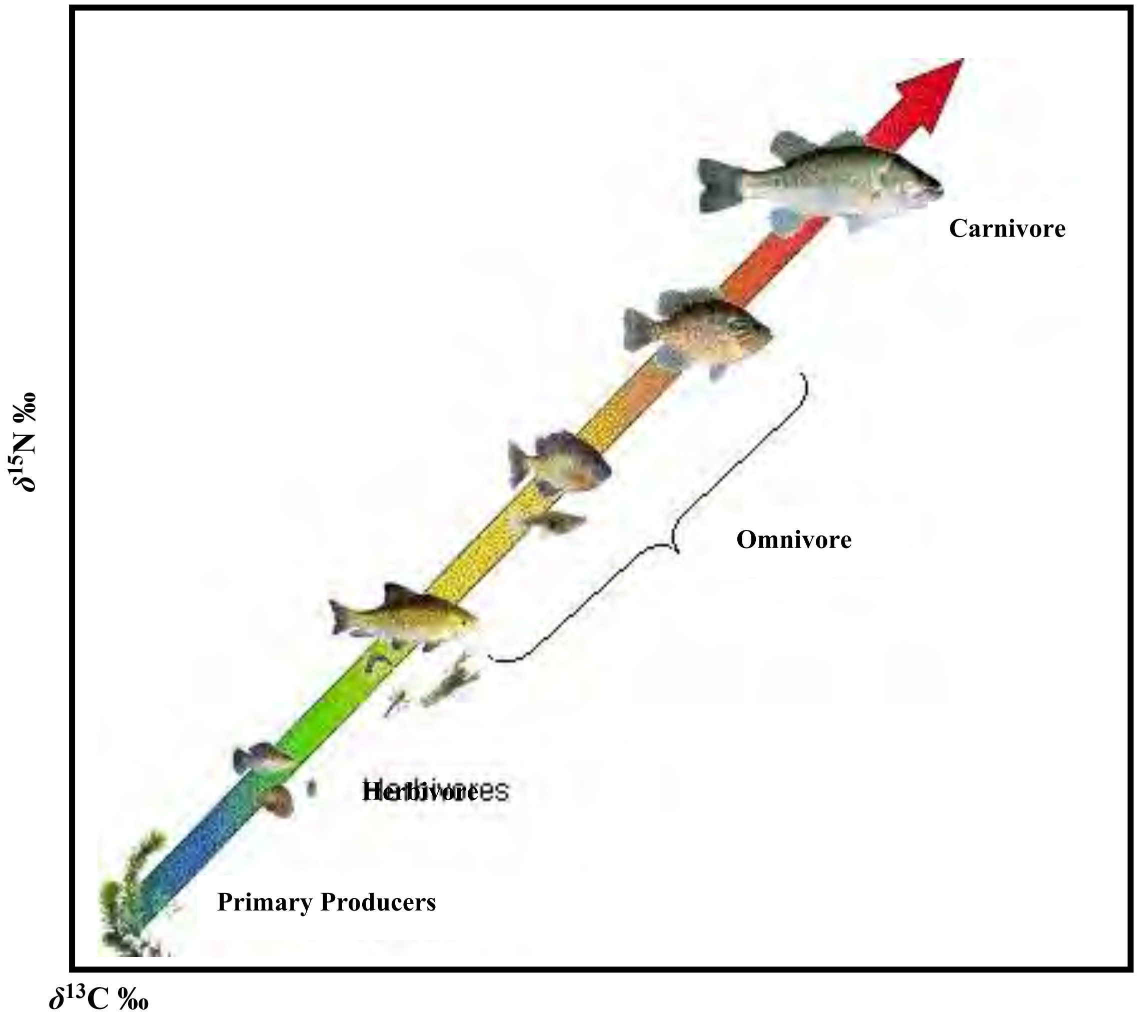


Figure 1.1 The expected increases in stable isotope (δ^{13}C and δ^{15}N, respectively) compositions in the tissues of aquatic biotas (USGS 2003).

Primary producers (plants, algae, diatoms)

In riverine environments, autotrophic and heterotrophic organisms rely on the TDN (Peterson and Fry 1987; Berman and Bronk 2003), and macroalgae are the best indicators for DOM nitrogen sources (Cole et al. 2004). The δ^{13}C reflects the photosynthetic differences of plants and is not dependent on dissolved nutrients (Farquhar et al. 1989; McKee et al. 2002). Algal isotopic ratios in large sub-tropical rivers range between -37.2 ‰ to -23.4 ‰ which depends on the photosynthetic pathway (more C_3 input from nearshore terrestrial or more C_4 input from DOM) (O'Leary 1988; Farquhar et al. 1989; Hamilton and Lewis 1992, Taylor et al. 2017). The ratios for primary productivity associate more positive ratio values with the C_3

metabolism pathway (Sternberg and DeNiro 1983), while relative extremes of negative δ^{13}C values (-24 ‰ to -68 ‰) may be evidence of either anaerobic-powered microbial systems (Sheng et al. 1997; Schubert et al. 2011; Ward et al. 2017). Anaerobic metabolism in these semi-lentic features may be possible as the presence of greenhouse gasses such as methane are recorded for some of the Upper-Zambezi flood plains (Teodoru et al. 2015).

Siliceous microalgae diatoms form a substantial portion of the microphytobenthos and are good indicators of water health (Cox 1996). They gather nutrientsinto their frustules, and their isotopic ratios directly reflect the relative isotope availability in the nutrient pool (Kröger et al. 1999; Kröger et al. 2000; Kröger et al. 2002). Unlike macroalgae, diatoms are regarded as unreliable indicators of δ^{15}N and δ^{13}C from DOM as theypreferentially biomineralise ^{14}N (Δ^{15}N of 3 ‰ to 15 ‰) (Karsh et al. 2003; Needoba et al. 2003; Needoba and Harrison 2004; Leng and Swann 2010).

Invertebrates

Aquatic invertebrates are often placed into functional feeding groups to simplify ecological interpretations (Vannote et al. 1980; Rosi-Marshall and Wallace 2002). These groups, based on food preference and feeding morphology, focus on preferences for DOM, CPOM, FPOM, periphyton (cyanobacteria, detritus and microphytes attached to submerged surfaces), filamentous algae, inorganic silt, leaf fragments, and prey (aquatic invertebrates, amphibians, and fish) (Cummins 1974; Palmer et al. 1993; Tomanova et al. 2006). This categorisation simplifies interpretations but may cause miscomprehension between trophic positions elucidated from feeding groups and actual isotope values (Jepsen andWinemiller 2002).

Fish

Fish are sensitive to turbidity, nutrient changes, microhabitat loss, temperature flux, and parasitism, making them good indicators of aquatic habitat change (Depledge and Galloway 2005; O'Brien et al. 2009; Arthington and Balcombe 2011). Stable isotope analyses of food webs indicate nitrogen incorporation into aquatic habitats above those of basal producers and low-level consumers (McClelland et al. 1997; Jardine et al. 2005), and their larger body sizes and longer life histories relative to invertebrates decrease variability in stable isotope measurements (McClelland et al. 1997; Jardine et al. 2005). Fish body size is important as it relates to tissue turnover rate, which is the change in mass (growth half-life; $G_{0.5}$) or isotopic half-life in days (half-life time in days; $T_{0.5}$) (Busst and Britton 2018) which is the time it takes for a sample (tissue from fish) to reach 50% of itsequilibrium with a new diet (Vander Zanden et al. 2015). Depending on the size of the fish within a species or population the fin tissue turnover rates for different types (rays and membranes) show a daily variation for nitrogen, which requires consideration during interpretations (Hayden et al. 2015). In an example study on an armoured catfish species the daily nitrogen variation ranged between 0.5% to 3.8% (McIntyre and Flecker 2006).

1.4 Interpretations across a longitude

Carbon and nitrogen isotopes both contribute to the overall river landscape dynamics, but carbon indicates local, and nitrogen longitudinal river processes (Finlay 2001; Hadwen et al. 2010). Carbon inputs are highly variable across spatial scales (Hadwen et al. 2010). Nitrogen, constrained in aquatic ecosystems, undergo significant trophic transfer effects, and change slower over space and time, which enables a longer view of whole systems (Finlay 2001). The distinction between each river nutrient model relies on the isotope values along a reach (Finlay 2001; Woodward and Hildrew 2002; Hadwen et al. 2010). As there are differences in nutrient contributions and cycling within a river, certain considerations, such asbaseline corrections, fractionation, discrimination factors, and isotopic predictions for each model become a necessity.

1.4.1 Baseline corrections

Determining the trophic baseline of a food web and correcting for shifts in the baseline allows an accurate comparison between community δ^{15}N and δ^{13}C (Cabana and Rasmussen 1996; Finlay2001; Post 2002; Layman et al. 2012). Variation in individual organismal δ^{15}N and δ^{13}C values provides little information on the absolute trophic positions or ultimate carbon sources (Post 2002). This is due to the considerable variation among ecosystem base δ^{15}N and δ^{13}C from which organisms draw their nutrients (Post 2002). Comparing trophic position values and food chain lengths can infer differences between systems, particularly sites that vary in physical andchemical properties (Layman et al. 2012). Complications arise when any trophic structuralchanges found are not ascribed to any stressor that allows shifts in baseline ratios, which may occur at both the system and organismal levels (Cabana and Rasmussen 1996; Fry and Davis 2015). A variety of stressors in aquatic systems (contaminants; acidity, dissolved oxygen content, acidity, temperature, and invasive species) affect species richness (Ford 1989) and shorten (Odum 1985; Weinstein and Birk 1989) or lengthen food chains (Cabana and Rasmussen 1996). Baseline corrections are necessary to allow accuracy in comparisons between communities at different spatial habitats and time scales (Cabana and Rasmussen 1996; Post 2002; Anderson and Cabana 2007; Woodward et al. 2012). Typical baseline corrections use the average δ^{15}N andδ^{13}C of baseline species, such as primary consumers and long-lived invertebrates in fish ecology (Layman et al. 2012). The averages across all sites provide relative offset values, and subsequently all organisms per site are corrected either by addition or subtraction to isotope ratios relative to the offset from the averages (Layman et al. 2012).

1.4.2 Isotope discrimination factors and fractionation

Discrimination factors describe the change in isotope values as elements pass through tissues when more (enrichment) or less (depletion) of the heavier isotope (δ^{13}C or δ^{15}N) remains and contributes to the final ratio value (Peterson and Fry 1987; Michener and Lajtha 2008). In ecology, enrichment and depletion arise from different intra-tissue chemical reactions, including enzymatic activity after dietary uptake (Peterson and Fry 1987; Michener and Lajtha 2008).

Freshwater fish tissue typically presents a $\Delta^{15}N$ within the range of 3 ‰ to 5 ‰ (Pinnegar and Pollunin 1999). Trophic relationships between fish and ontogeny shifts in feeding, identified from gut content isotopes, show the importance of macroinvertebrates to fish feeding ecology (Davis et al. 2012; Taylor et al. 2017). Vander Zanden et al. (1999) showed that 69% of the variability in food chain length (calculated using $\delta^{15}N$ values of fish) was correlated to fish species richness in boreal lakes. Isotope values present food web structures for all organisms present across isotopic niches (Jackson et al. 2011). The use of $\Delta^{13}C$ and $\Delta^{15}N$ allow more accurate interpretations of consumer dynamics such as predator-prey interactions prevent incorrect interpretation from mixed diets (Minagawa et al. 1984; Vander Zanden and Rasmussen 2001; Stock et al. 2018). Bayesian-isotope mixing models are sensitive to variations in $\Delta^{13}C$ and $\Delta^{15}N$ and are crucial for accurate estimations and use of the derived factors (Bond and Diamond2011; Stock et al. 2018). Modelled food webs may indicate patterns and trends in the specific ecosystem along a longitudinal gradient characteristic of nutrient changes (Thorp et al. 1998; Jackson et al. 2011). Evaluating longitudinal river aspects, at different stream orders, is a common way in which large scale river nutrient dynamics across a landscape are elucidated (Vannote et al. 1989; Thorp et al. 2006; Hadwen et al. 2010; Doretto et al. 2020).

1.4.3 Determining the river nutrient model of best fit

In ecology, evaluating the river nutrients characteristics of each model provides an idea of the best model for the biological data (Thorp et al. 1998). This thesis focuses on the isotope values of selected samples and includes considerationsfor both dissolved and particulate organic matter from terrestrial and in-stream sources.

The River Continuum Concept model suggests the reliance of downstream reaches on contributions from headwater reaches and includes the refractory breakdown of FPOM toward lower reaches (Vannote et al. 1980; Thorp et al. 1998; Tornwall et al. 2015; Doretto et al. 2020). In headwaters, less nutrient breakdown will contribute less negative δ^{13}C and higher δ^{15}N values (Farquhar et al. 1989; Hamilton and Lewis 1992; Wetzel 1984; 1995). As stream order increases, the expected change to the nutrient profile for the River Continuum Concept is nitrogen isotope depletion due to denitrification processes (Wetzel 1984; 1995; Finlay 2001; Chapman 2021a). This gradual, constant trend of δ^{13}C and δ^{15}N depletion would be expected in a large river as fast-flowing upper reaches become slower and wider channels downstream with more nutrient breakdown and with a wider nutrient base (Vannote et al. 1998; Thorp et al. 1998; Berman and Bronk 2003; Tornwall et al. 2015; Ward et al. 2015; Doretto et al. 2020).In contrast, both the Flood Pulse Model-revised and the revised-Riverine Productivity Model are river discontinuum models and suggest external factors influence nutrient contributions andthat local nutrient sources are not reliant on the upper reaches (Thorp et al. 1998; Junk 2001; Tornwall et al. 2015). For these models, the δ^{15}N trend is of greater importance, and enriched values are expected across a reach due to the attraction of higher feeding groups (Flood Pulse concept-revised) or local productivity (revised-Riverine Productivity Model) (Junk 1984; Bayley and Sparks 1989; Junk et al. 1989). The distinguishing difference for the δ^{15}N trend between the models is a relative similarly enriched values at all localities for the Flood Pulse Concept-revised as opposed to highly variable values for the other models (Thorp et al. 1998).. Both models predict different δ^{13}C values at localities along a reach due to the high variability and continuous inputs of carbon resources from DOM (Pingram et al. 2012; Tornwall et al. 2015). Determining the likelier model then relies on external factors such as seasonality (flooding in high flow), floodplains, and channel size considerations (Junk 1984; Bayley 1989; Junk et al. 1989; Thorp et al. 1998; Pingram et al. 2012).

1.5 The Upper Zambezi sub-catchment, Zambia

Within Zambia, the Upper Zambezi sub-catchment falls under a dual legal system governed by a centralised government and traditional customs from adjacent regions (Sambo et al. 2021). Here chiefs and their advisors govern districts, and the governance system contributes to bush-grass (veld) burning, unsustainable agriculture, and overfishing as management roles are not based on management experience (Masaiti 2018; Simabwachi 2019). Few environmental laws exist in the Zambian North-West Province except for fishing in the more accessible Barotseland area (Linell et al. 2019). The Zambian Natural Conservation Act protects all-natural environments, including their wildlife, and aims to prevent pollution (Chomba et al. 2011). The unnecessary and illegal veld-burning practices in the Upper Zambezi sub-catchment by the general population may be a result of previous colonial management but is an understudied cultural phenomenon that goes unregulated although condemned by Law 330 under the Offences, Penalties, and Forfeitures (XXXIV) section of the Amended Act (PCAZ 1994; FAO1998; Eriksen 2007; Milupi et al. 2020; Sambo et al. 2021). Subsistence farming in Zambia sustains the bulk of the rural population but contributes less to land-cover destruction than large artificial reservoirs, such as the lakes Cabora Bassa, Kariba, Itezhi-Tezhi and the Kafue Gorge(Balon and Coche 1974; Timberlake 2000). Increasing populations require increased hydropower and irrigation, which affect fisheries (Tweddle and Peel 2015). These effects maycontribute to the under-utilisation of the Upper Zambezi sub-catchment fisheries as yields are approximately 7 500 tonnes per annum, an estimated half the 14 000 tonnes possible (Tweddleand Peel 2015; AU-IBAR, AUDA-NEPAD, EU 2021). Moreover, the Zambian Natural Conservation Act and its stipulations are under the charge of the Zambezi River Authority (ZRA 2021). This single transboundary institution manages the whole Zambezi River but employs no ecosystem functioning information to assess river health (ZRA 2021).

The damage caused by these unsustainable practices is unquantifiable owing to the lack of information on the ecosystem function of the landscape. Sustainable management is only feasible when the whole ecosystem function is known (Lackey 1998; Udy et al. 2006; Oliver et al. 2015; Truchy et al. 2015). This thesis aims to address the information gap on the ecosystem functioning of the Kabompo River by use of nutrient cycling to function as a proxy for the larger Upper-Zambezi Basin rivers.

1.6. Information gap

No information exists on the food webs and nutrient sources of the Kabompo River. The dominant studies for the Upper Zambezi sub-catchment include taxonomy on tigerfish (*Hydrocynus vitattus*), tilapia, and yellow fishes (*Labeobarbus* spp.) (Winemiller 1991; Winemiller and Kelso-Winemiller 1994, 1996; Økland et al. 2003, 2007). Currently four fish food web studies related to the Upper-Zambezi sub-catchment exists (Winemiller and Kelso-Winemiller 2003; Taylor et al. 2017; Peel et al. 2019a, Peel et al. 2019b). Of these only Taylor et al. (2017) and Peel et al. (2019a) contain isotope analysis. This highlights the need for nutrient baseline data for the Upper Zambezi sub-catchment to inform on system dynamics, which isotope data can provide.

1.7 General aim

This thesis aims to describe the food webs of the Kabompo River, Zambia, and the potential landscape-scale nutrient processing that occurs in the river across a longitudinal gradient in the Zambezi dry season (August to September 2019).

1.8 Thesis outline

The thesis provides a detailed description of the Kabompo River in the broader context of its importance as a large tropical tributary and information on literature gaps in the Upper Zambezi sub-catchment. Chapter 1 is a general introduction that overviews the nutrient cycling process and models under which river function is viewed. Chapter 2 is a case study and site description.

Chapter 3 investigates food webs found in the Kabompo River. The aim of the Chapter is to show and compare food webs for seven longitudinal sites of the river from $\delta^{13}C$ and $\delta^{15}N$ values. Chapter 4 concentrates on nutrient cycling within the Kabompo River. It aims to infer a model that describes the nutrient dynamics by comparing isotope data of two common fish species and their putative resources along a longitudinal transect. The two species used were a herbivore (*Labeo cylindricus*) and an omnivore (*Synodontis* spp.). Chapter 5 is the general discussion section and provides a discussion of the significance of the possible food web structures and landscape nutrient cycling in the Kabompo River during the Zambian dry season for management information and to suggest considerations for future studies from the limitations of this thesis.

CHAPTER 2: CASE SCENARIO: THE KABOMPO RIVER

The Upper Zambezi is a sub-catchment of the Zambezi River and has a human population of almost a million people (ZDP 2015; Sambo et al. 2021; Senzanje and Dirwai 2020). There is a need to conserve this intact landscape as the current anthropogenic development threatens its integrity (WWF 2012; Finlayson et al. 2018). Currently, there is no available Upper Zambezi sub-catchment data apart from hydrological, weather, and water physiochemistry (WWF 2012; ZRA 2021). A significant tributary of this sub-catchment, the Kabompo River, represents the region with the most substantial gap in information for the river continuum of the Zambezi River.

2.1 The Kabompo River

Location

The Kabompo River is the most northerly tributary of the Upper Zambezi sub-catchment (UNEP 2010). It flows entirely within Zambia (14° 02' 00" S and 23° 37' 30" E) and stretches from its origin in the Zambian-Congo watershed area to the Barotseland Flood Plain, where it ends near the town of Lukulu (UNEP 2010) (Figure 2.1). The river is 440 km long and covers approximately 72 000 km^2. It lies at an elevation of 1000 to 1400 m above sea level and has multiple floodplains during the wet season (UNEP 2010; Muzumara 2011; Siwila et al. 2013).

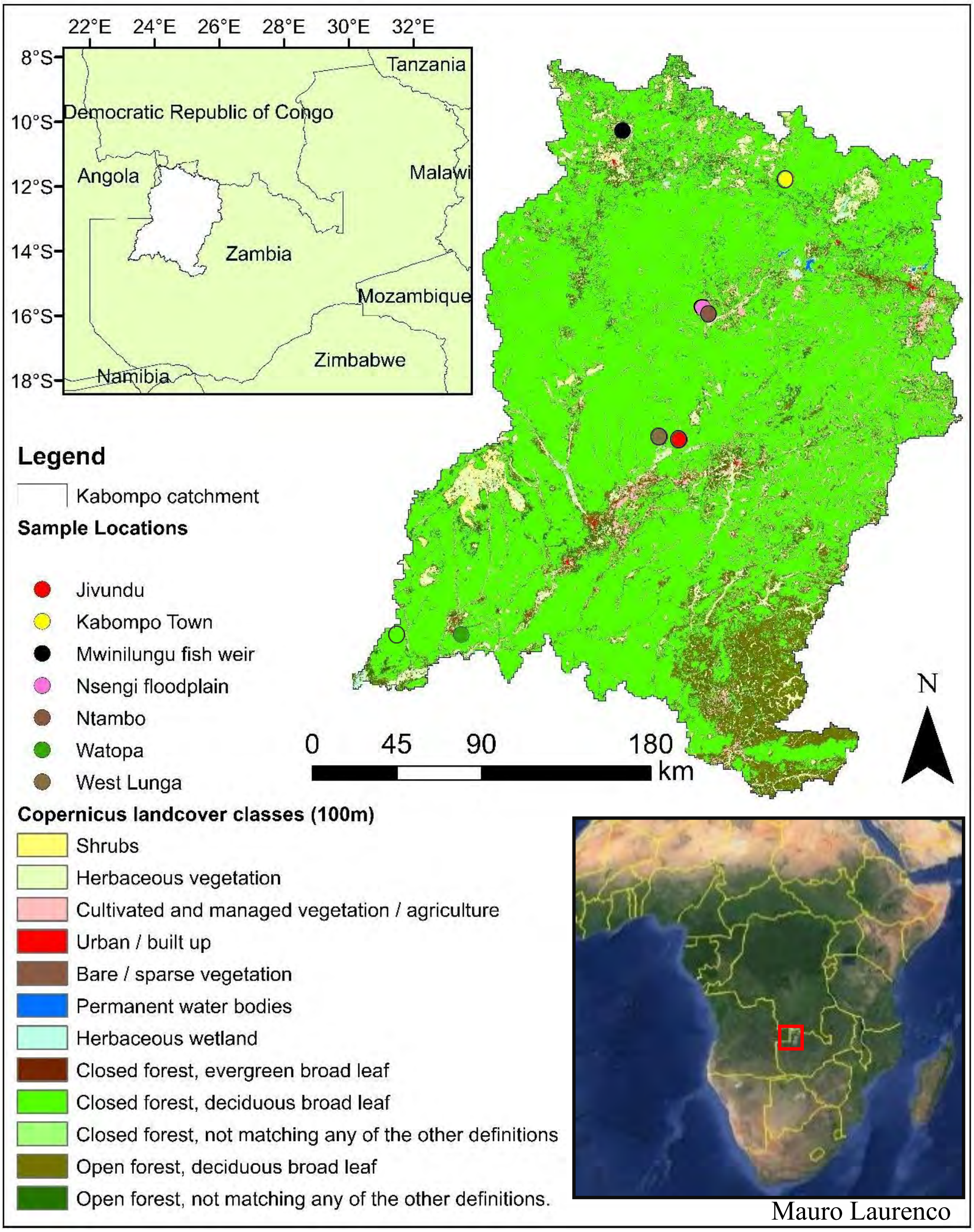


Figure 2.1 The Kabompo River Basin with different landcover considerations in the dry season (August to September 2019) and sampling locations from the headwaters to last site indicated with coloured circles. (Map courtesy of Dr Mauro Laurenco).

Seasonality

The wet season extends from November to April and the dry season from May to October (Tiffen and Mulele 1994; Hachigonta and Reason 2006; Libanda et al. 2019). There is no notable change over time (climate projections for period 2006-2100) in the mean annual precipitation, which remains at 1200 mm(Ndhlovu and Woyessa 2020). Flooding over the river's banks creates grassland floodplains, and drying exposes submerged vegetation, fallen trees and sandbanks (Økland et al. 2005; Beilfuss 2012). The wet season can raise water levels by 5 m after high rainfall, supporting flood-driven and rain-fed agriculture (Beyer et al. 2016).

Landscape description

The geological age of the Kabompo River Basin is 2–5 billion years and is purported to be the oldest part of the Zambezi River Basin (Moore et al. 2007, 2012; Burroughs et al. 2019). The well-drained Kalahari sands group is the most distinguishing geological aspect, but large boulders and deep, narrow sections such as the Kabompo Gorge diversify the sand landscape (Thomas and Shaw 1988; Beilfuss 2012). Seasonal changes in river flow cause the formation of oxbow lakes, swamps, grassland-wetlands, and exposed sandbanks large enough for vegetation growth to create small delta islands (Moore et al. 2007; Zwidofhelangani 2020). The inner edges of the meanders have more extensive deposits of sand and gravel, while the bow edges of these meanders are deeper excavated channels (Zwidofhelangani 2020).

The Zambian Forest covers an area of 752,600 km² (approximately 45,610 ha) (Sambo et al. 2021). Tree species across 38 genera and 19 families (like the woody-savannah woodlands of Angola) dominate the habitat surrounding the Kabompo River (Figure 2.1) (Gonçalves et al. 2017). Colloquially known as the Miombo forests, the tree genera of *Brachystegia* and *Julbernardia* account for 60% of the total Zambian forested areas (Sambo et al. 2021).

Biodiversity: fish and invertebrates

There is no information available on how ecosystems function in the Kabompo River catchment, and the primary ecological details are on a select few animal species and the annual number of fish catches made (Sambo et al. 2021). Fish protein makes up more than 20% of all animal protein intake by the Zambian population and is essential for the subsistence of the general public (Musumali et al. 2009; Sambo et al. 2021). Surveys suggest that human populations depend on subsistence fishing year-round, which amounts to a seasonal change in fish abundance and size over time (Sambo et al. 2021). Decreases in fish catch and size in the Barotseland are a function of over-catch and landscape alteration in the Upper Zambezi sub-catchment (Kampata et al. 2013; Kabisa 2018).

Studies for the Upper Zambezi sub-catchment provide an estimation of the fish species present, including the Kabompo River (Kelley 1968; Duerre 1969; Winemiller and Kelso-Winemiller 1996; Winemiller and Kelso-Winemiller 2003; Tweddle 2010; Peel et al. 2019a) and Lake Liambezi, south of the Kabompo River (Taylor et al. 2017; Peel et al. 2019a), with 77 species reported(Huntley et al. 2019). These include species from the families Cichlidae, Clariidae, Schilbeidae, Mochokidae, Claroteidae, Hepsetidae, Alestidae, Mormyridae, and Cyprinidae (Huntley et al.2019). All invertebrates recorded for this region are listed in the Zambian Invertebrate ScoringSystem (ZISS) (Dallas et al. 2018).

Human impact

The Kabompo River is non-commercial (Mutamba 2007) and one of the most isolated areas of southern Africa (Negi 2011; Caperon and Brand-Correa 2020). The population relies heavily on what the forest and river provide with limited economic activity in the region (Mutamba 2007; Kampata et al. 2013; Sebukeera et al. 2017). Land use is not intensive, with subsistence farming in the growing season (190 days in the wet season), limited charcoal production (Mutamba 2007; Kampata et al. 2013; Day et al. 2014) and use of shrubs for medicine, food, and other purposes (Sambo et al. 2021). Pressure from external sources isslowly depleting the vegetation cover in response to increasing demand for charcoal, for marketable ‘Makula’ or rosewood (*Dalbergia* spp.), and the increased intensive construction of traditional mokoros (fishing vessels carved from whole trees) using modern equipment (Kampata et al. 2013). Increased mining activity impacts the landscape as population growth catalyses more needs (Beilfuss 2012; Sambo et al. 2021). The Kabompo River has limited infrastructure that impedes flow, but increased mining activities require more energy. Plans tobuild a hydroelectric power plant in the Kabompo Gorge with the potential to generate 40 MWare underway (Mwanza et al. 2017).

Mwinilunga Town is an Upper Zambezi sub-catchment community close to the Democratic Republic of the Congo. It is an example of prominent levels of land-cover removal by anthropogenic activity (Figure 2.1) (Peša 2019). The land-cover change may be due to the intensive farming of dambo-type soils (Peša 2019). The practices of planting monocultures for food (maize fields and fruit tree groves), building (schools, churches, and homes), and the removal of Mukula or rosewood (*Dalbergia* spp.) displace native vegetation and result in habitat fractionation in an otherwise dense tropical forest (Sambo et al. 2021). The trade of ‘Mukula’ is the only known information on the effects of deforestation with commercial relevance (Sambo et al. 2021). Burning of the forests is most destructive during the dry seasons but continues into the wet season (Holden 2001; Eriksen 2007). Despite massive deforestation, the carbon sink of the Miombo forest canopy remains unchanged (Pelletier et al. 2018; Sambo et al. 2021). The reason for this is still unclear as complete ecosystem nutrient cycling information is unavailable.

2.2 The importance of the Upper Zambezi sub-catchment

Southern Africa hosts adjoining global biodiversity hotspots (Tinley 1977; Timberlake 2000; Mittermeier et al. 1998, Küper et al. 2004; Mittermeier et al. 2011; WWF 2012; Rivers-Moore et al. 2021). These are the lakes of Malawi; the Okavango swamps; the Barotse floodplains and woodlands of the Palaeo-Upper Zambezi; the Middle Zambezi and Luangwa valleys; and the Zambezi-Delta ('Gorongosa' or 'Cheringgoma') (Timberlake 2000). The Palaeo-Upper Zambezi sub-catchment basin area is the second most important 'hotspot' of this region that encompasses all the floodplains and swamps of Bangeweulu, Okavango, Kafue, Basunga, and Barotseland (Podgorski et al. 2013; Schleiss et al. 2017). Here the dambos and grasslands are the most extensive and undisturbed habitat left on the continent (Timberlake 2000; Moore et al. 2007). The well-documented Okavango swamps are the largest, richest swamps in the region (Timberlake 2000; Musilová et al. 2013). They are outside the present-day Upper Zambezi sub-catchment basin but connected to it through hydrology and biology (Timberlake2000; Musilová et al. 2013). The high-lying woody-savannah (Miombo) ecoregion covers 70%of the landscape and is rich in reptiles, amphibians, Reduncine antelope, and endemic vegetation (suffrutices and bulb-types) (Timberlake 2000; Moore et al. 2007). The Barotselandregion, where three biomes, the Kalahari, Congolian, and East African Coastal, meet, is vital to this reptile and amphibian life. Most of the national parks in the 520 000 km^2 of the Kavango-Transfrontier Park Area cover the Middle and Lower Zambezi and the Kavango catchments, but not the Upper Zambezi landscape in the North-West Province (Linell et al. 2019). This region is remote, biodiverse, supports a large subset of the rural Zambian population and needs sustainable management to adhere to the United Nations Sustainable Development Goals (UNGA 2015). The persistence of ecosystem services with sustainable river management practices could contribute to meeting these goals.

2.3 Sampling locations

The sampling for this thesis aimed to fill in knowledge gaps inthe Kabompo River across the upper, middle, and lower sections in the dry season (August to September) of 2019. The locations chosen are centred in the middle reaches of the Kabompo River and were between the Ntambu Flood Plains (Ntambu Camp) and the southern edge of the West Lunga National Park at Jivundu Town. The Jivundu localityenabled easy access to the lower section of the West Lunga River. The upper section was classified as above Kabompo Gorge at the T5 road (main Kabompo branch) and near Mwinilunga Town (West Lunga branch); the middle section included the Ntambu Flood Plainand Jivundu Town locality, and the lower section was at a set of rapids upstream from Watopapontoon. The rapids were chosen for sampling due to the large, deep, and slow-flowing habitats downstream that could not be waded. The seven sites represented in this thesis are upstream to downstream: Mwinilunga Fish Weir (MFW), Kabompo Bridge (KB), Ntambu Camp (NC), Nsengi Flood Plain (NFP), West Lunga (WL), Jivundu (J), and Watopa (W) (Figure 2.2; Table2.1).

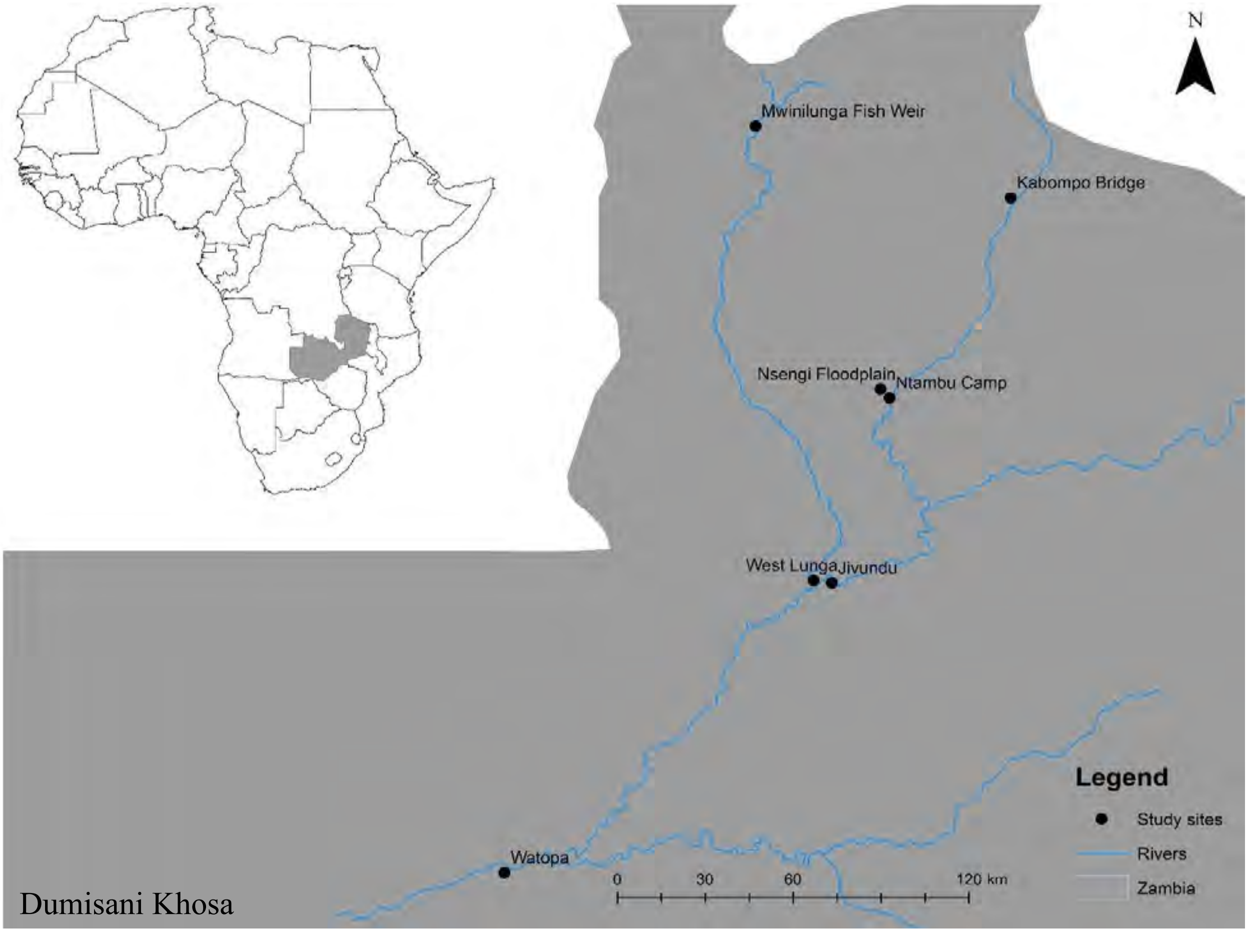


Figure 2.2 Longitudinal sampling sites for the Kabompo River in the Upper Zambezi sub-catchment basin, Zambia. (Map courtesy of Dr Dumisani Khosa)

Table 2.1 Site names, -codes, and -coordinates for all sampling sites along a gradient for the Kabompo River from the headwater in the Zambia-Congo watershed to downstreambefore Lukulu Town at the northern border of the Barotse Flood Plains. A brief habitat description for each site is provided.

Site name	Site code	GPS coordinates	Habitat description
Mwinilunga Fish Weir	MFW	11°39’41.72” S; 24°28’00.94” E	Shallow rocky riffle and gravel bar with marginal vegetation.
Kabompo Bridge	KB	11°53’18.97” S; 25°15’00.64” E	River narrow (+/-10m wide), dominated by rocky bottoms, current fast flowing, limited marginal vegetation, phragmites, and riparian forest.
Nsengi Flood Plain	NFP	12°29’38.43” S; 24°51’03.41” E	Small sections surrounded by massive floodplains, large sections of *Phragmites* swamps, and small pools.
Ntambu camp	NC	12°31’17.49” S; 24°52’43.04” E	River 20–25 m wide, extensive grass-dominated floodplains with a few trees along the banks, *Phragmites* present on riparian edge.
West Lunga	WL	13°05’57.73” S; 24°38’42.41” E	The river is < 10 m wide, has a faster flowing current, many rocks and gravel beds, and crops adjacent to river edges
Jivundu	J	13º05’55.23” S; 24º44’19.68” E	The river is clear, current slow flowing with a deep main channel and abundant algal blooms
Watopa	W	14º01’27.68” S; 23º41’48.48” E	Rocky rapids (+/- 50 m in length), slow and fast flowing sections, bedrock and side channels containing cobble and sand. Abundant marginal vegetation and numerous small islands in the main channel.

2.4 Habitat descriptions

Mwinilunga Fish Weir

A large fish weir upstream from Mwinilunga Town is surrounded by local community activities, including fishing and crop cultivation. The area included rocky habitats and Miombo woodland cover along the riparian edges. The area around the weir was sampled; the habitat sampled was a shallow, rocky riffle and adjacent marginal vegetation (Table 2.1; Figure 2.3A, B, and C).

Kabompo Bridge

This site was a headwater environment near a bridge approximately 10 m in width with a sampled habitat that was rocky bottom dominated by a fast-flowing current. The limited vegetation cover included riparian forests and *Phragmites* (reeds). Sampling was difficult, possibly due to low water conductivity from lower salinity. Owing to the small local human population around this area, some subsistence fishing occurs (Table 2.1; Figure 2.4A, B, and C).

Ntambu Camp

Extensive floodplains surrounded this sampling location. The region was grassland-dominated, with only a few trees present. The sampled habitat included a part of the river 20–25 m in width. Riparian vegetation included *Phragmites* and trees along the banks. A small human population resides here, reliant on subsistence fishing (Table 2.1; Figure 2.5A, B, and C).

Nsengi Flood Plain

The small tributary of the Kabompo River, surrounded by extensive floodplains relative to its size, included large sections of *Phragmites* swamps which were too difficult to sample. The area lies within the large Masele Matebo Game Management Area, and fishing activity is low, but resource use does occur. The habitat sampled was small pools and swampy regions (Figure 2.6A, B, and C). The pools, dominated by aquatic vegetation (lily pads), were shallow, and sampling was never more than waist depth (Table 2.1; Figure 2.6A, B, and C).

West Lunga

Sampling at this site included buying fish directly from the fishers (caught at the site) on the river to supplement samples due to high fishing pressure by local fishers. This area was much narrower than the Kabompo Bridge site (<10 m in width) and had a stronger current. The region, dominated by different subsistence activities, includes strong fishing pressure (predominantly line fishing) and crop cultivation (cleared land sections) along one bank. Cropsincluded cassava and maize. Although this region was inside the West Lunga Nature Reserve Park and was still natural, the obscure veld-burning activities (Eriksen 2007) occurred here. The habitats sampled included riparian vegetation, rocks, and gravel beds (Table 2.1; Figure 2.7A, B, and C).

Jivundu

This site included sampling at a few different sites around the camp area. The river was clear and slow flowing with a deep main channel. A pontoon was near the campsite, but few other disturbances apart from extensive bush burning occurred. Local resource use in subsistence fishing was low in this area as it was on the side of the West Lunga Nature Reserve Park (Table 2.1; Figure 2.8A, B, C, and D).

Watopa

This site had the widest variety of habitats sampled. The rocky rapid comprised bedrock and side channels with cobbles and sand habitats. There was an abundance of marginal vegetation and numerous small islands in the main channel. The small side channels with rapids were rocky with considerable amounts of marginal vegetation. Both slow- and fast-flowing sections in this habitat were sampled. The large rapids in the main channel were sampled to collect more rock-dwelling species. This section of rapids was 50 m in length and about as wide as the river is at the site. There is a relatively higher human population than the other areas, and more clearing for crops and grazing was evident (Table 2.1; Figure 2.9).

Figure 2.3 Mwinilunga Fish Weir, a headwater site on the Kabompo River, Zambia, from A) above, B) upstream, and C) downstream of the weir.

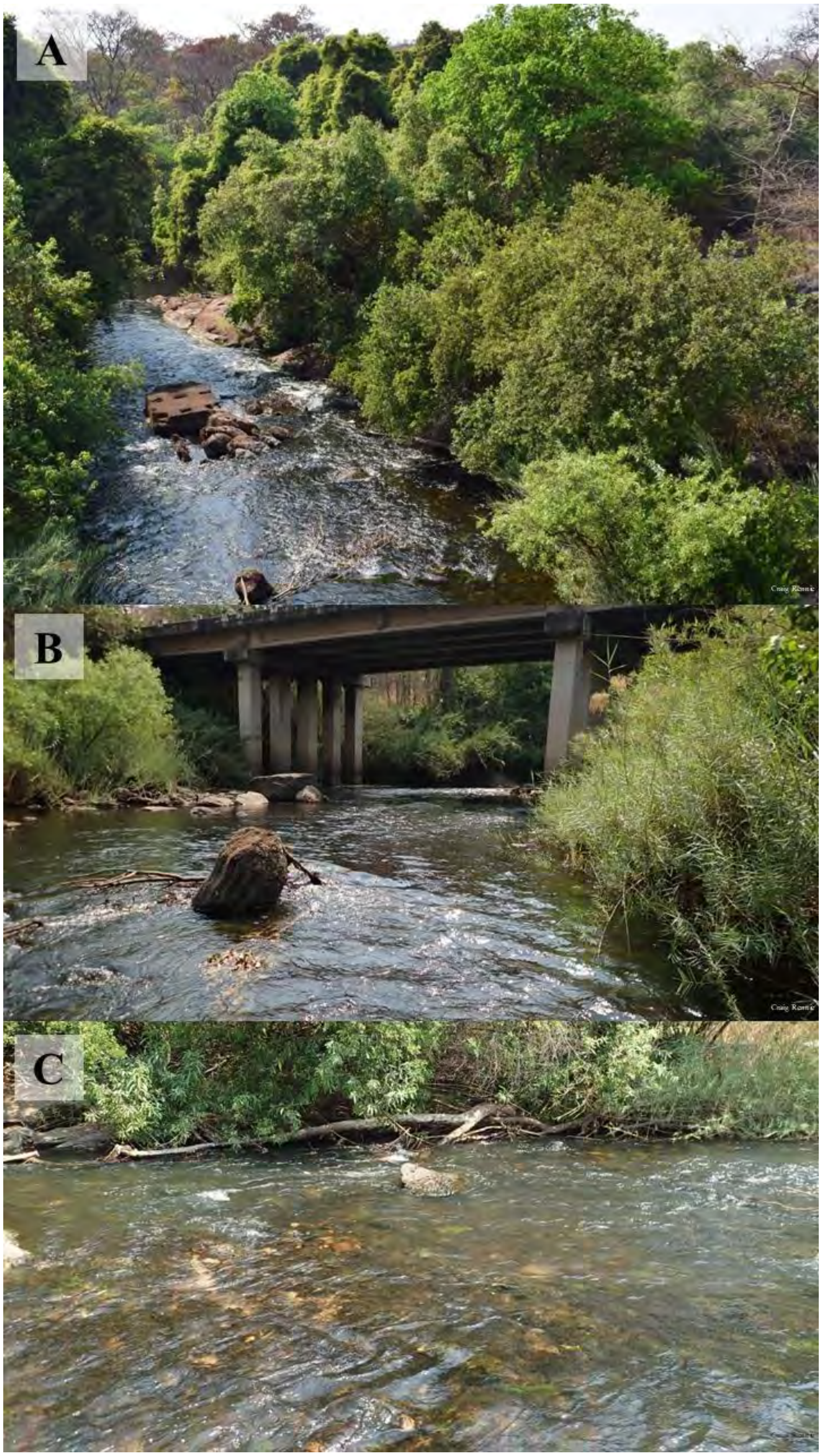


Figure 2.4 Kabompo Bridge site on T5 road, a headwater site on the Kabompo River, Zambia, as seen from: A) above, B) upstream, and C) downstream.

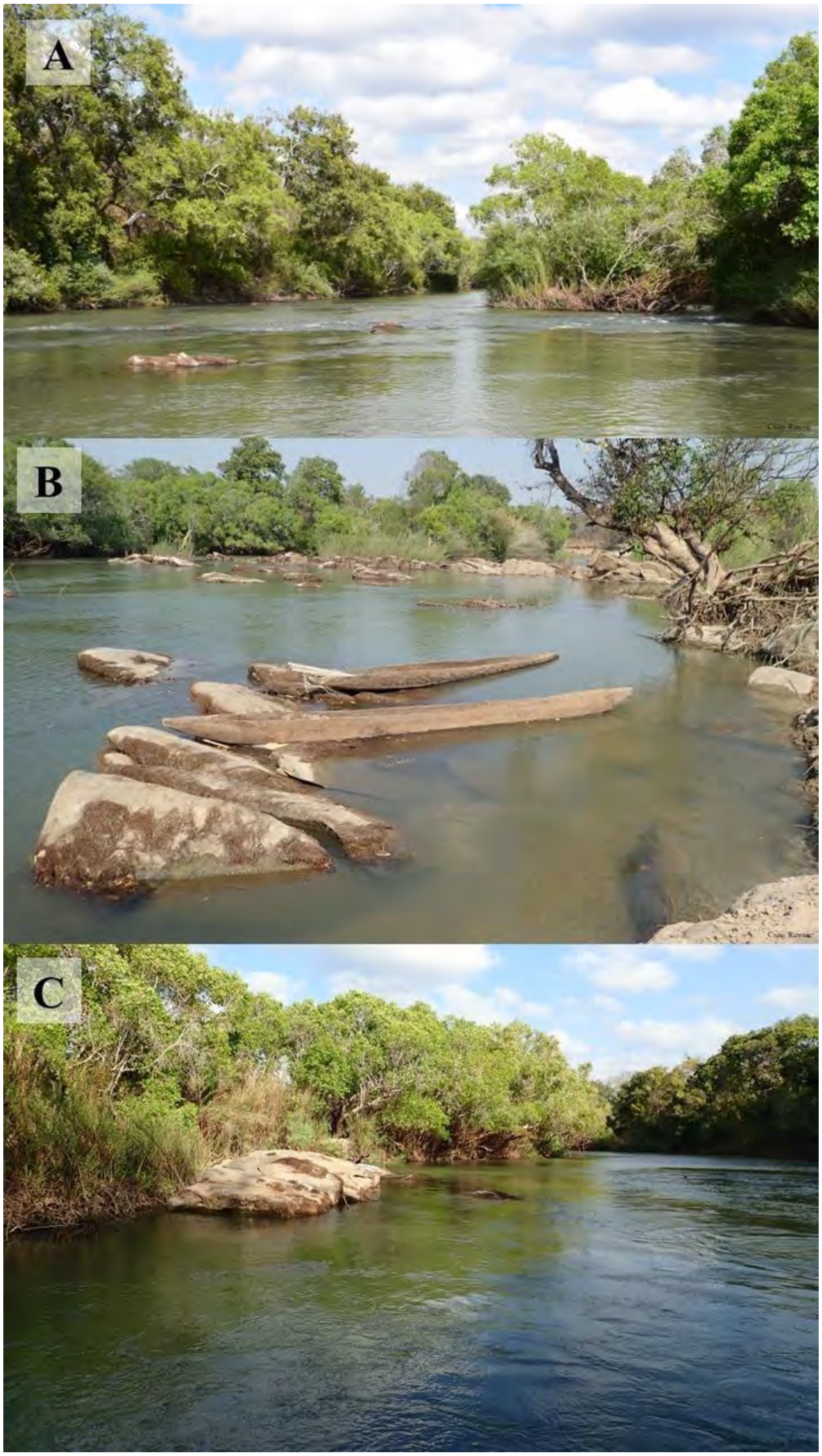


Figure 2.5 Ntambu Camp at Ntambu Flood Plain on the Kabompo River, Zambia, from A) downstream, B) rocky habitats, and C) upstream.

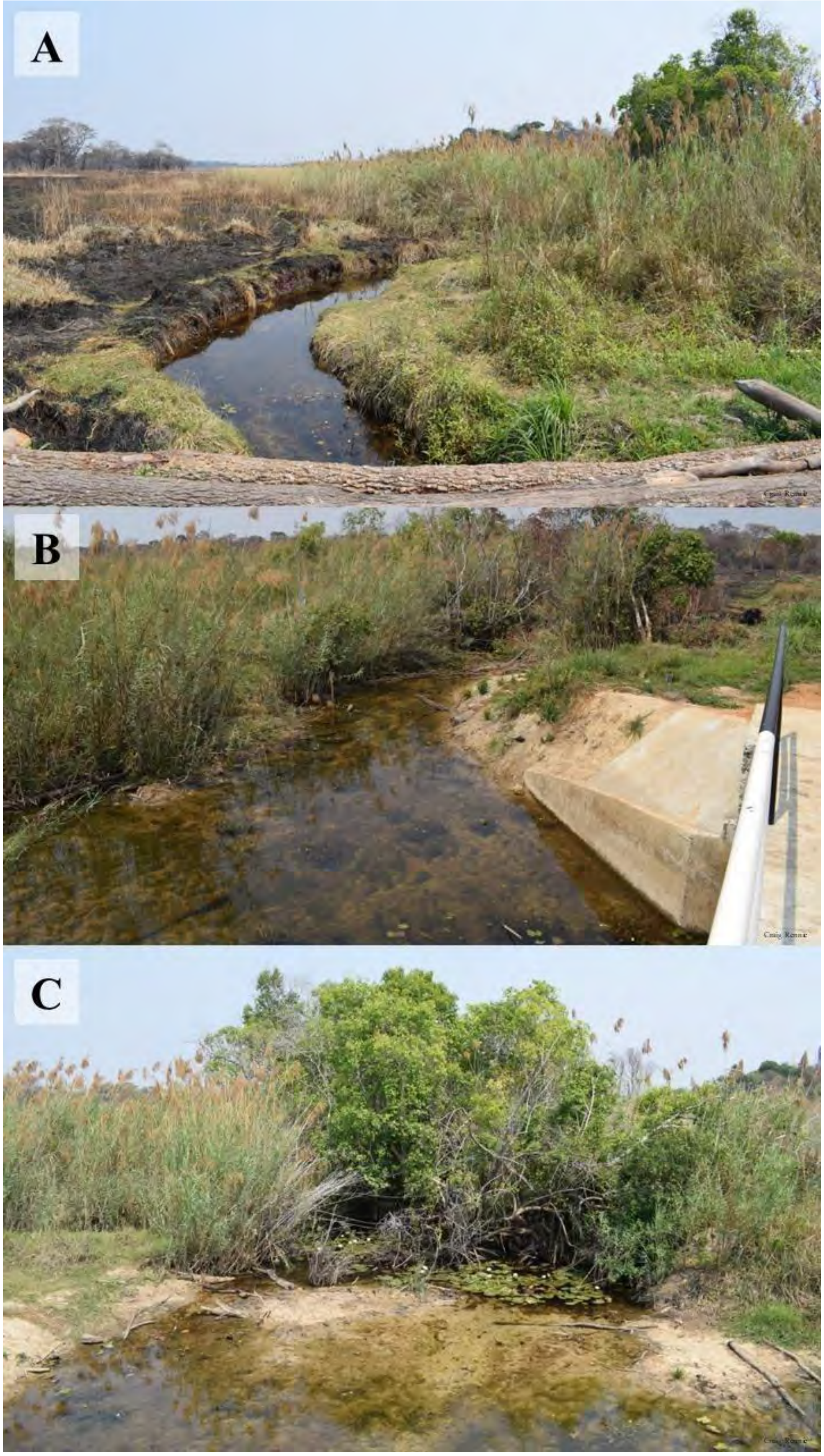


Figure 2.6 Nsengi Flood Plain site on the Kabompo River, Zambia, with a view of the A) pools, B) wetlands next to a small bridge, and C) aquatic and riparian vegetation.

Figure 2.7 West Lunga site on the Kabompo River, Zambia, where A) is a view from downstream, B) a side view from the bank, and C) fish sold by local fishers.

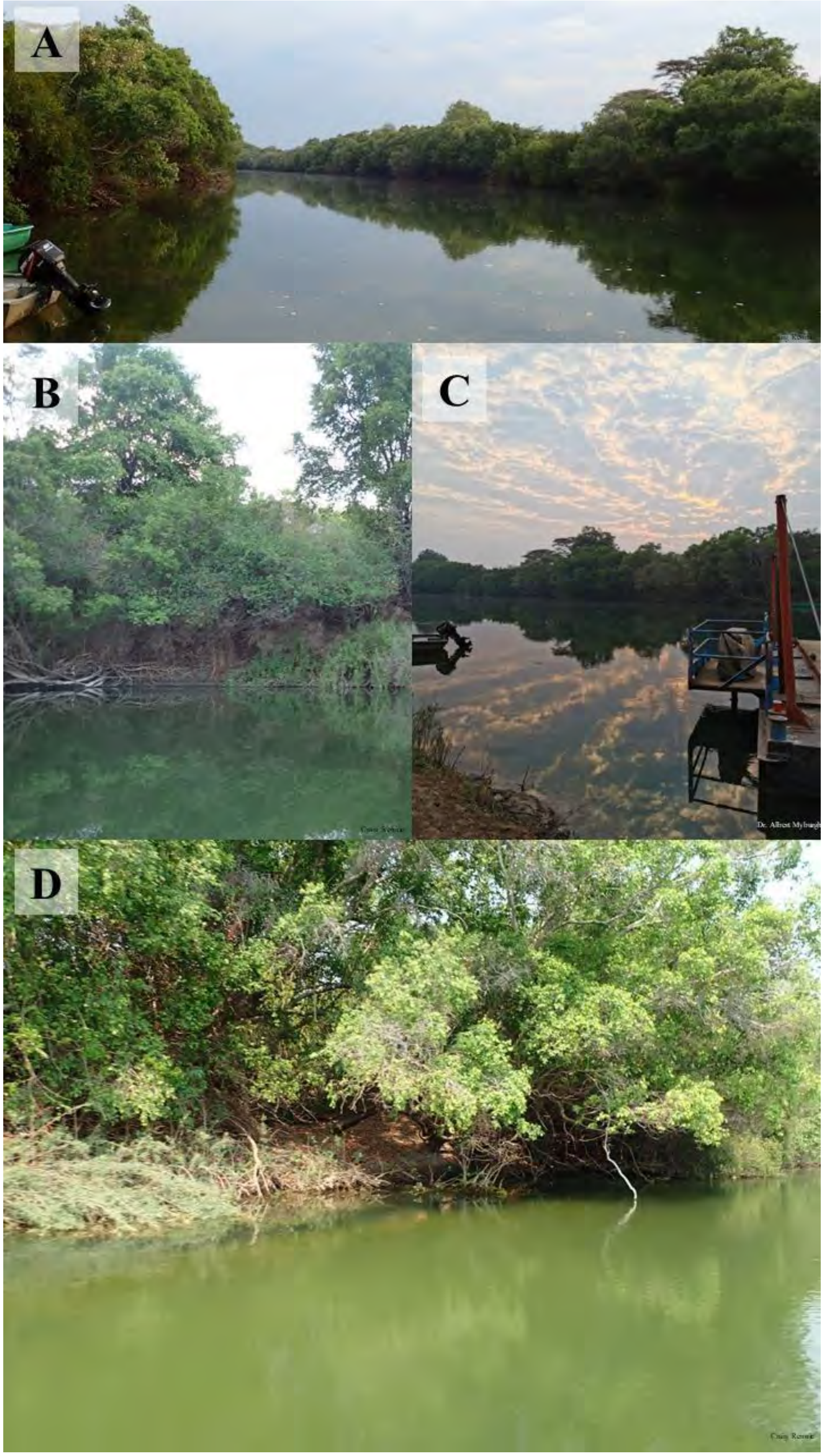


Figure 2.8 Jivundu site near Jivundu Camp on the Kabompo River, Zambia, with A) a view from upstream, B) a view from the campsite bank, C) the pontoon, and D) a view from the bank opposite the campsite.

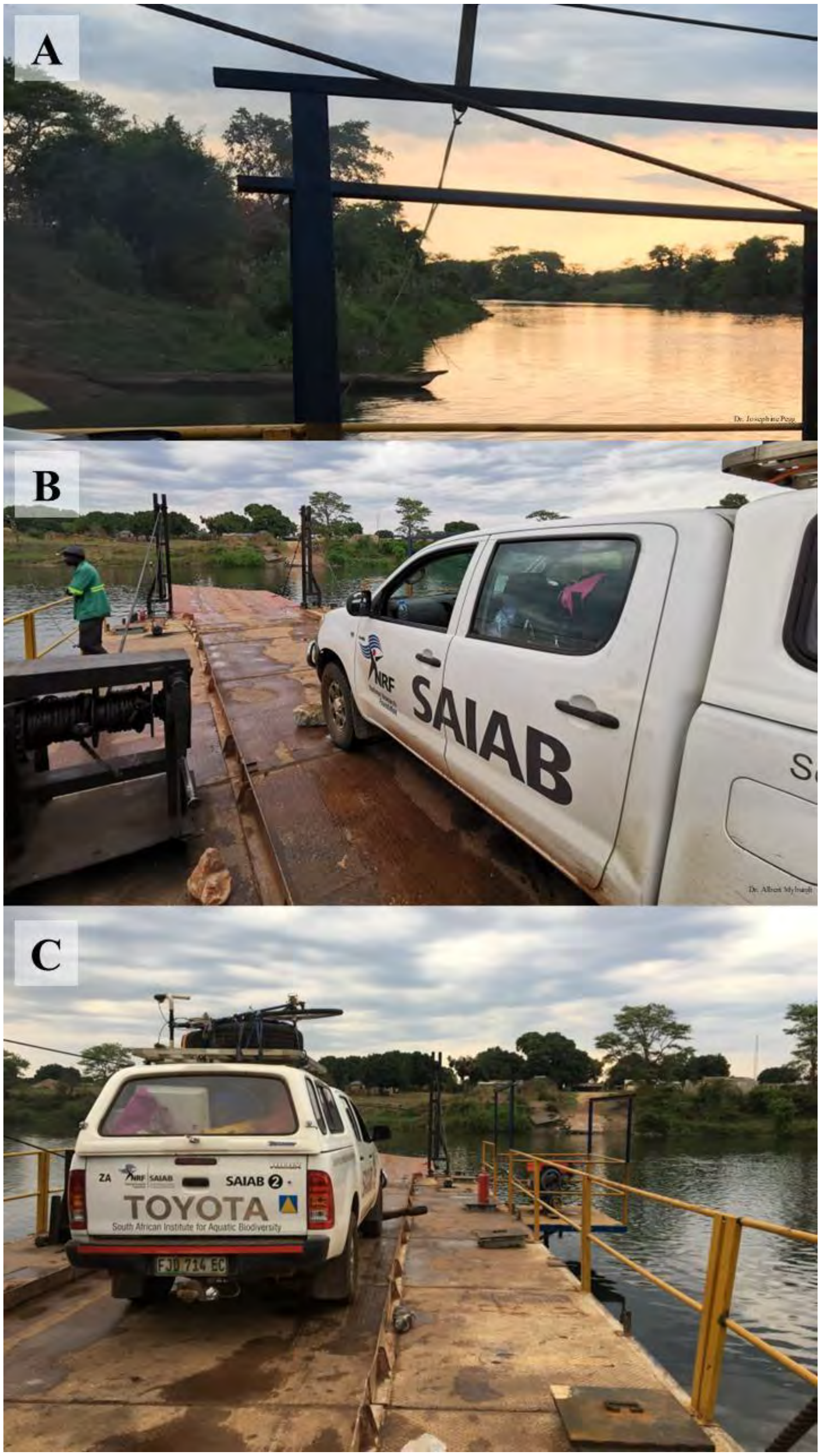


Figure 2.9 Watopa was the last site sampled on the Kabompo River, Zambia, with a view from A) downstream of banks and Mokoros (hollowed out tree canoes) used for fishing by local fishers, and B), and C) view from vehicle pontoon to opposite bank with human settlement.

CHAPTER 3: FOOD WEBS OF THE KABOMPO RIVER, ZAMBIA

Introduction

Freshwater systems in southern Africa support a high diversity of aquatic species and high endemism (Dallas and Rivers-Moore 2014; Darwall et al. 2018). These species provide direct (i.e., fisheries) and indirect (i.e., water purification) ecosystem services to people, and the conservation of these services maintains the livelihood of dependent communities (Dallas and Rivers-Moore 2014; Darwall et al. 2018). Monitoring aquatic ecosystems is one of the tools used in their protection, asit enables environmental degradation to be detected and measured (Levin et al. 2019). Monitoringecosystem health (ecosystem structure and function) can highlight how human activities interfere withnatural processes and may help provide information on which interventions are feasible to rectify this (Udy et al. 2006).

The ecological functionality of a freshwater ecosystem depends on interactions and relationships between organisms that determine the flow of nutrients from a primary producer to the top of a food web (De'Ath 2002; Nilsson and Renöfält 2008; Santos et al. 2010). By using stable isotope analysis, the functioning of food webs can be studied as such analysis reflects the relationships between consumers and their diet (O'Reilly et al. 2002; Jardine et al. 2003; Middelburg 2014). Stable isotope analysis makes the relative measurement of ecosystem health possible as it establishes the structure and clarifies the trophic interactions between organisms, including effects such as trophic cascades (Bunn and Davies 2000; Udy et al. 2006). Isotope ratios from resource materials that include macrophytes, algae, detritus, and biofilm indicate thepotential basal nutrient sources for food webs (Minagawa and Wada 1984).

Functional feeding groups simplify food we analyses by placing species into larger groups based on food acquisition from particle size and diet preferences. These placements, which sometimes do not align with organismal isotope values, remain isotopically unique between trophic levels and are beneficial for viewing energy flow through food webs in a simplified manner (Vannote et al. 1980; Rosi-Marshall and Wallace 2002; Boecklen et al. 2011; Ben-David and Flaherty 2012). Functional feeding groups are used in food webs as they focus on the whole function rather than species- specific aspects and allow a view of whole ecosystems.

The Kabompo River and its adjacent habitats are a southern African biodiversity 'hotspot' and are the main tributaries of the Upper Zambezi sub-catchment in Zambia (Midgley and Thuiller 2011; Beilfuss 2012, Darwall and Freyhof 2016; Rivers-Moore et al. 2021). The Kabompo is a 440 km long, perennial river within the North-West Province that flows entirely within Zambia (Beilfuss 2012; Rivers-Moore et al. 2021). This region needs conservation efforts driven by ecological understanding to ensure the persistence of ecosystem services with sustainable river management (Tweddle 2010; Rivers-Moore et al. 2021). The Kabompo, a seemingly pristine river with limited human impacts, suggests still intact ecological functionality, which is rare, as – globally – most freshwater systems are heavily altered (Dudgeon et al. 2006; Beilfuss 2012; Rivers-Moore et al. 2021). This Chapter aimed to look at the ecological functioning of the Kabompo River through its food web structures since there is no current information available (Tweddle 2010; Taylor et al. 2017; Dallas et al. 2018; Peel et al. 2019). The objectives of this Chapter were to assess fish and invertebrate diversity found in the river, describe the food web structures using stable isotope analyses, and compare these between sites along a longitudinal gradient. Fish food webs for the Kabompo River were expected to follow the typical top-down trophic structures seen in most neotropical and tropical rivers (Minagawa and Wada 1984; Jepsen and Winemiller 2002; Douglas et al. 2005).

Materials and Methods

Sampling locations

Seven sampling locations were chosen for the Kabompo River based on reconnaissance by boat in the dry season of August to September 2019. The locations occur at a gradient from the headwater streams to downstream of the main branches that create the river. They were assigned the identifiers of Mwinilunga Fish Weir (MFW), Kabompo Bridge (KB), Ntambu Camp (NC), Nsengi Flood Plain (NFP), West Lunga (WL), Jivundu (J), and Watopa (W) (see Figure 2.2.; Table2.1; Chapter 2).

Ethics

Ethical approval was granted by the SAIAB Animal Ethics Committee (Reference number 25/4/1/7/5_2019/08), and research permits were obtained from the Zambian Department of Fisheries. The dried and donated samples from the survey were analysed under the Rhodes University ethics reference number 2021-2709-5994.

Sampling

Reconnaissance of the Kabompo River (main and West Lunga Branches) identified areas for possible electrofishing and netting activities, which were approximately < 1 m deep). These areas represented many dry season habitats that included backwaters, shallow main channel margins, stony riffles, and side channels favourable for biodiversity sampling. Wading-depth safety was essential for in-water sampling due to the threat of Nile crocodiles (*Crocodylus niloticus*) and hippopotamuses (*Hippopotamus amphibius*). To avoid sampling bias, complete biodiversity sampling of fish communities, macroinvertebrates, and their putative resources was conducted by the same individual at each habitat.

Fish

Complete biodiversity sampling was the objective, and four main sampling methods were used, based on habitat type for the fish collection. For open areas and rocky substrates electrofishing with a D-framed net (D net) was the most effective method. A SAMUS 725M 12V DC backpack Electrofisher(Frequency: 50-80 Hz; Duration: 30-300 microseconds) and a D-frame (70 cm x 80 cm) net in a two-person team were used where one person carried and operated the backpack, and the other collected stunned fish downstream of the sampling area. Both the area and time electro-fished wereunrecorded. In thick vegetation (i.e., lily pad beds) collections were made by thrusting a D- (70 cmx 80 cm) net into the area three to five times. Larger mainstem sections required angling (with chicken liver or artificial lures) for larger predatory fish, such as, tigerfish (*Hydrocynus vittatus*) and large catfish (*Clarias* spp.). In addition, fish samples were bought at the West Lunga site from localfishers to increase fish sample sizes when species were identified from their catches that were missed by the other sampling methods.

All fish caught were processed on-site immediately to reduce stress. The fish were kept in river water, which was replaced at regular intervals, and kept in the shade in 20-litre buckets. A caudal fin clip was taken per individual for isotope analysis as a non-lethal sampling technique (Sanderson et al. 2009; Jardine et al. 2011; Galván et al. 2015). Fish too small for a fin clip were euthanised (as under the SAIAB sampling ethics – reference number 2019-0270-2016) with clove oil and taken whole. Under the sampling ethics and Central Limit Theorem ecological-rule-of-thumb (Martínez-Abraín 2014), a maximum of thirty samples (fin clip and whole fish) were collected per species. Species and genera identification were based on the best available literature and identification keys (Skelton 1996; Bruwer and van der Bank 2003; Tweddle et al. 2004; Tweddle et al. 2012).

Macroinvertebrates

Invertebrates were sampled using the methodology described by the Zambian Invertebrate Scoring System (ZISS) (Dallas et al. 2018) based on an adaptation from the South African Scoring System version 5 (SASS5) (Dickens and Graham 2002). Where possible, three biotypes within the selected sampling sections were identified (stone, vegetation, and gravel-sand-and-mud). Invertebrates were sampled using the methodology described by the Zambian Invertebrate Scoring System (ZISS) (Dallas et al. 2018) based on an adaptation from the South African Scoring System version 5 (SASS5) (Dickens and Graham 2002). Sampling included any stony substrate > 15 mm in and out of current for 2 min each, vegetation (riparian and aquatic) for 2 min within a 2 m2 quadrant for wadable and > 20 m area for non-wadable rivers, and gravel-sand-and-mud for 1 min (Dallas et al. 2018). Samples were collected with a 300 mm x 300 mm frame with 1 mm x 1 mm mesh size SASS5 net. These samples were identified into family taxa using the ZISS in-field identifying guide (Dallas et al. 2018). These samples were identified into family taxa using the ZISS in-field identifying guide (Dallas et al. 2018).

Resource material

Resource material, including vegetation(macrophytes), algae, detritus, and biofilms were collected at each site through random selection of the predominant types. These samples were only placed into groups related to the abovementioned identifiers and not any lower classification. Algae was the most collected type, with the exception of no algae collected at site Watopa, a minimum of n = 4 samples of algae were collected at each site. Other vegetation such as macrophytes (reeds), biofilm, and detritus were collected at random if present with approximately n = 2 of each type per site. . These samples were only placed into groups related to the abovementioned identifiers and not any lower classification.

Fish tissues, whole invertebrates, and resource samples were stored in a freezer in the field and not preserved in alcohol to prevent compromising them for isotope analysis (Butler et al. 2018; Chua et al. 2020). The samples were later dried at 55°C for 48 hours and packed in Zambia before being transported to the NRF-iThemba LABS in Johannesburg, South Africa.

Laboratory sample preparation

The dried samples (Rhodes University Ethics reference number 2021-2709-5994) were prepared for stable isotope analysis at the Environmental Isotope Laboratory of NRF-iThemba LABS (Johannesburg, Gauteng) and the Stable Isotope Laboratory on the Hatfield campus of the University of Pretoria (Pretoria, Gauteng) facilities.

Lipid extraction

All pre-dried (at 55°C for 48 hours) fish samples received were lipid extracted (Sotiropoulos et al. 2004; Logan et al. 2008). Fin and whole fish tissue samples were homogenised and split into two separate aliquots (one for lipid extraction and the other for analysis without lipid extraction). Lipids were then extracted from one split sample using the modified Folch (1957) and Hussey et al. (2012) methods. Within the sampling tube, 2–3 ml of chloroform: methanol (2: 1) solution was added to the sample. Sample mixtures were shaken vigorously and then allowed to settle before the chloroform: methanol (2: 1) solution (containing the dissolved lipids) was removed using a syringe. This process was repeated three times per sample or until the sample-mixture liquid was clear. The lipid-extracted tissue samples were then re-dried at 70°C in a dry oven for a minimum of 30 hours. No lipid extraction was conducted for invertebrates.

Stable isotope analysis

Dried invertebrates and resource samples were ground to a fine powder using a mortar and pestle for the invertebrates and fish, and a Beadbug microtube homogeniser (Benchmark Scientific, Sayreville NJ, U.S.A.) for resources samples. These instruments were cleaned with a 70% ethanol solution between samples.

Aliquots of 1.0-1.1 mg of resources samples and 0.55-0.60 mg of both invertebrate and fish samples were massed on a Mettler Toledo MX5 scale (Thermo Fisher, Bremen, Germany) and placed in D1006 Tin Capsules. The samples were analysed using a Flash EA 1112 Series elemental analyser coupled to a Delta V plus isotope ratio Mass spectrometer by a Conflo IV interface at the Stable Isotope Facility located on the Hatfield campus of the University of Pretoria. All samples were calibrated against laboratory standards (Merck Gel: $\delta^{13}C$ = -20.57 ‰, $\delta^{15}N$ = 6.8 ‰, C% = 43.83, N% = 14.64 and Urea: $\delta^{13}C$ = -10.57 ‰, $\delta^{15}N$ = -6.15 ‰, C% = 50.35, N% = 11.86%). The reference standards against which results are reported are atmospheric nitrogen for $\delta^{15}N$ and Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$.

Calibrations

Measurements of stable carbon and nitrogen isotope ratios are given as $\delta^{13}C$ or $\delta^{15}N$ = $[(R_{sample}/R_{standard}) - 1] \times 1000$, where R represents $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively (Peterson and Fry 1987; Kendall and McDonnell 1998). Results (for $\delta^{13}C$ and $\delta^{15}N$) were corrected to account for potential machine drift or in-laboratory standard related drift.

Statistical analyses

Data were normalised to the invertebrates but not the resource material, although the resources (biofilms, algae, detritus and macrophytes) form the base of the system. This was done as not all invertebrates feed on the resources, and normalising to the invertebrates eased interpretations. All analyses were conducted in the Microsoft© Excel 2010 and the *R* (version 4.0.5) statistical programme in *Rstudio* (Rstudio Team 2020) using packages sourced from the literature (Jackson et al. 2011). All Ellipses used in overlap comparisons for isotopic niches were created using the package from SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson et al. 2011). Packages for the food web analyses included SIBER (Stable Isotope Bayesian Ellipses in R), rjags (to provide access to the JAGS - Just Another Gibbs Sampler - library for Bayesian data analysis), and ggplot2 (grammar of graphics plot 2 for visualisation).

Fish and invertebrate diversity

Fish and invertebrate diversity across sites was calculated using the Shannon-Wiener Diversity Index (*H'*) (Equation 3.1) and Simpson's Diversity Index (*D*) (Equation 3.2) (Keylock 2005). The Shannon-Wiener Index represents the heterogeneity of an assemblage based on the number of species present and their relative abundance, with high numbers indicating higher heterogeneity (Keylock 2005). Simpson's Diversity Index calculates diversity in terms of distance, where $D = 1$ indicates the highest possible diversity and zero the lowest (Keylock 2005). These indices are frequently used in diversity determination and can estimate richness regardless of taxon level (Hill 1973; Izsák and Papp 2000; Andelman and Willig 2003; Keylock 2005; Wilsey et al. 2005; Magurran 2013).

$$H' = \sum_{i=1}^{S} p_i \ln p_i \qquad \text{(Equation 3.1)}$$

$$D = \sum_{i=1}^{S} \frac{N(N-1)}{n(n-1)} \qquad \text{(Equation 3.2)}$$

where S is the number of species in a community, p_i refers to the proportion of the entire community made up of species i, n is the total number of organisms of a particular species, and N refers to the total number of individuals of all species (Keylock 2005).

Shannon's Diversity Index (*H*') can be supplemented for better interpretation of diversities with the Shannon Equitability Index (E_H) (Equation 3.3) and the effective number of species (*ENS*) (Equation 3.4) (MacArthur 1965; Hill 1973; Peet 1974) and which were calculated. The value of p isthe proportion of the community that comprises a species and is frequently used alongside evenness to discern true diversity (Keylock 2005). The evenness (equitability) index shows similarity in abundances of different species in a community (Magurran 2013), and the effective number of species shows equally common occurring species within a sampled population, which provides value to an index (MacArthur 1965; Hill 1973; Peet 1974). The effective number of species is referred to as the true diversity and indicates how many species in a population are dominant. Fish were identified to genera and species level, but only genera were used for analysis. Invertebrates were only identified to the family level (Keylock 2005). Fish and invertebrate diversity are not directly compared owing to different classification levels (Keylock 2005).

$$E_H = H / \ln S \qquad \text{(Equation 3.3)}$$

$$ENS = e^H \qquad \text{(Equation 3.4)}$$

where H' denotes the Shannon-Wiener Index value and S is the total number of unique species.

Functional feeding groups

Fish and invertebrates were placed into functional feeding groups based on gut content analysis from previous studies (Cummins and Klug 1979; Taylor et al. 2017). The identified fish were first placed into a more diverse range of feeding groups based on Taylor et al. (2017) and later sorted into broader groups (predators, omnivores, invertivores, and herbivores) for analysis (Table 3.1).

Table 3.1 Functional feeding groups (FFG) of sampled organisms from literature for invertebrates (Cummins and Klug 1979) and fish (Taylor et al. 2017) across sites:MFW) Mwinilunga Fish Weir; KB) Kabompo Bridge; NC) Ntambu Camp; NFP) Nsengi Flood Plain; WL) West Lunga; J) Jivundu; and W) Watopa for the Kabompo River on which the Stable Isotope Bayesian Ellipses in R (SIBER) analyses were conducted.

Identifier	**Organism**	**FFG**	**SIBER FFG**	**Site**
Algae (globular, filamentous)	Microbes	None	Resources	MFW, NFP, NC, J, W
Detritus (Ash, bark, debris)	Dead vegetation	None	Resources	MFW, WL, NFP
Biofilm	Microbes	None	Resources	MFW, NFP, NC, J, W
Phragmites, leaf litter, and marginal-, submerged- and floodplain vegetation	Vegetation	None	Resources	MFW, KB, WL, NFP, NC, J, W
Pyralidae, Simulidae, Chironomidae, Potomonautidae, Hydropsychidae, Economidae, Bivalves, Lymnaeidae, Physidae, Thiaridae, Ephmeroptera families, oligochaeta	Invertebrate	Collectors (gathering and filtering), Filterers, Shredders, Scrapers, Piercers	Herbivorous Invertebrates	MFW, KB, WL, NFP, NC, J, W
Libellulidae, Gomphidae, belastomatidae, Hydrometridae, Naucoridae, Nepidae, Perlidae, Tabanidae, Dytiscidae, Hydrophilidae, water spiders	Invertebrate	Predator	Predatory Invertebrates	MFW, KB, WL, NFP, NC, J, W
Oreochromis macrochir, O. niloticus, O. andersonii	Fish	Detrivore	Herbivores	WL, J
Labeo cylindricus, Coptodon rendalii	Fish	Herbivore	Herbivores	MFW, KB, WL, NC, J, W
Amphillius johnstonii, A. uranoscopus, Hysopanchax jubbi, Polymyrus spp., P. marianne	Fish	Benthic Invertivore	Invertivores	MFW, KB, NFP, NC, W
Brycinus lateralis, B. imberi, Cyphomyrus spp., C. cubangoensis, C. marianne, Hemichromis elongatus, Hippopotamyrus, H. ansorgii, Kneria polli, Marcusenius altizambesi, Microctenopoma intermedium, Micropanchax johnstoni, Mormyrus lacerda, Nannocharax spp., N. dadgeti, N. micropterus, N. multifasciatus, Opsaridium zambezense, Pharyngochromis acuticeps, P. acutidens, Rhabdalestes maunensis	Fish	Invertivore	Invertivore	MFW, KB, NFP, NC, J, W

Identifier	Organism	FFG	SIBER FFG	Site
Chiloglanis spp., C. fasciatus, Enteromius aeriatus, E. afrovernayi, E. eutaenia, E. eunitaeniatus, E. kerstenii, E. fasciolatus, E. lineomaculatus, E. paludinosis, E. poechii, E. radiatus, Petrocephalus okovangoensis, P. longicapitis	Fish	Insectivore	Invertivore	MFW, KB, WL, NC, J, W
Parauchenoglanis spp., Parauchenoglanis ngamensis, Pseudocrenilabris philander	Fish	Piscivore and Insectivore	Omnivore	MFW, KB, WL, NFP, NC, J, W
Labeobarbus condringtoni, Mastacembalus vanderwaali, Mastacembalus frenatus, Micralestes acutidens, Synodontis spp., S. macrostoma, S. nigromaculatus, S. thamalakanensis, Tilapia sparrmanii	Fish	Omnivore	Omnivore	MFW, KB, WL, NFP, NC, J, W
Clarias spp., Clarias gariepinus, Clarias ngamensis, Clarias theodori, Clarias strapersii, Clariallabes sp., Clariallabes platyprosops, Schilbe intermedius	Fish	Predator-Omnivore	Omnivore	MFW, KB, WL, NC, J, W
Hydrocynus spp., H. vittatus, Serranochromis spp., S. altus, S. angusticeps, S. macrocephalus	Fish	Predator	Predator	MFW, WL, NC, J, W

Isotrophic lines

Isotrophic lines are visual representations of trophic separations between organisms from stable isotope ratios to allow comparisons between different sites (Woodborne et al. 2012). Isotrophic lines are generated from the isotopic values of all organisms part of a food web as described by Woodborne et al. (2012) and provide a method for viewing relative trophic separation within food webs and between sites. . Isotrophic lines were calculated at each site at three trophic levels using the methodology described by Woodborne et al. (2012). All invertebrate samples were used for baseline correction (Chapter 1) and to represent the first isotrophic line (Equation 3.5.1), as no common invertebrate family was found at all sites. It is common practice to use longer lived species (Layman et al. 2012) for baseline correction but as no common species or families were found at all sampling sites the whole invertebrate group was used as a base to the fish food webs. After that, a dietarydiscrimination factor ($\Delta^{15}N$) of 3.4 ‰ was used to calculate enrichments in isotrophic lines (Equations 3.5.2 and 3.5.3) (McCutchan et al. 2003; Busst and Britton 2016; Van der Merwe *et al.(in review)*).

$$\Delta^{15}N= -0.1503\delta^{13}C - 0.0047 \qquad \text{(Equation 3.5.1)}$$

$$\delta^{15}N= -0.1503\delta^{13}C + 3.3953 \qquad \text{(Equation 3.5.2)}$$

$$\delta^{15}N= -0.1503\delta^{13}C + 6.79553 \qquad \text{(Equation 3.5.3)}$$

These lines were calculated once using all the invertebrate data and used at similar positions across all seven compared sites to illustrate the potential changes in food web structure for these sites along a gradient.

Overlap between functional feeding groups

Statistical analysis of stable isotope data was conducted in R (version 4.0.5) using the Rstudio®

interface of the SIBER (Stable Isotope Bayesian Ellipse in R) package. The package generates niche models to show all the isotope observations in the smallest ellipse area possible with a minimum of n = 5 individuals per group is needed to generate a more accurate ellipses at a 40% confidence level (Jackson et al. 2011). For these analyses the SEAc (standard ellipse area corrected) were scaled to a 95% confidence level to include as most of the data. For the overlap statistics the percentages of 95%-overlap between groups were calculated, where ≥50% was considered significant. This package requires a minimum of five samples per group for accuracy.

Results

Assemblage diversity (fish and invertebrates)

Fish richness was highest at the Jivundu site (S = 22) and lowest at the Nsengi Flood Plain site (S = 11) (Table 3.2). Fish diversity was the highest at Jivundu site (*H'* = 2.86) and Mwinilunga Fish Weir site (*D* = 0.97), and the lowest at the Nsengi Flood Plain site (*H'* = 1.98; *D* = 0.82) (Table 3.2). Fish evenness was the highest at sites Jivundu and Watopa (E_H = 0.93) and lowest at Nsengi Flood Plain (E_H = 0.83). The effective number of species for the fish was the highest at the Jivundu site (*ENS* = 17) and lowest at Nsengi Flood Plain (*ENS* = 7) (Table 3.2). The Jivundu site had the highest, and the Nsengi Flood Plain site had the lowest overall richness, diversity, evenness and effective number of species for the fish.

Invertebrate richness was highest at Mwinilunga Fish Weir (S = 26) and lowest at Kabompo Bridge (S = 13) (Table 3.2). Invertebrate diversity was the highest at the Jivundu (*H'* = 3.12; D = 0.98) and Nsengi Flood Plain sites (*D* = 0.98), and the lowest at the Kabompo Bridge (*H'* = 2.38; *D* = 0.92) and Ntambu Camp sites (*D* = 0.92) (Table 3.2). Invertebrate evenness was the highest at Jivundu (E_H = 0.99) and lowest at Watopa (E_H = 0.90). The effective number of species for the fish was the highest at Jivundu (*ENS* = 22) and lowest at Kabompo Bridge (*ENS* = 10) (Table 3.2).

The Jivundu site had the highest overall richness, diversity, evenness, and effective number of species for the invertebrates. All scores indicated a positive correlation which matches with the sampled abundances. Fish diversity for the river showed high diversity for both indices but slightly lower diversity than the invertebrates (Table 3.2). The effective number of species indicated that the invertebrate diversity was closer to the number of invertebrate families collected than the fish diversity for the fish genera collected (Table 3.2).

Table 3.2 Fish and invertebrate diversity across sites at Mwinilunga Fish Weir (MFW), Kabompo Bridge (KB), Nsengi Flood Plain (NFP), Ntambu Camp (NC), West Lunga (WL), Jivundu (J), and Watopa (W) comparing outputs from the Shannon Diversity Index (*H'*) and Simpson's Diversity Index (*D*), including the number of individuals per community (S), evenness (E_H) and effective number of species (*ENS*) values.

Site	**MFW**	**KB**	**NFP**	**NC**	**WL**	**J**	**W**
			Fish				
Genera	17	15	11	20	16	22	20
S	180	106	96	185	79	160	78
H'	2.68	2.28	1.98	2.63	2.49	2.86	2.80
D	0.97	0.88	0.82	0.92	0.90	0.89	0.94
E_H	0.91	0.84	0.83	0.88	0.90	0.93	0.93
ENS	13	9	7	13	12	17	16
			Invertebrates				
Families	26	13	14	18	24	23	22
S	47	27	16	39	40	27	14
H'	3.02	2.38	2.56	2.69	3.00	3.12	2.77
D	0.94	0.92	0.98	0.92	0.93	0.98	0.96
E_H	0.93	0.93	0.97	0.93	0.94	0.99	0.90
ENS	20	10	12	14	20	22	15

Isotrophic lines

From baseline corrected isotope values from upstream (sites Mwinilunga Fish Weir and Kabompo Bridge) to downstream (site Watopa) sites, the isotopic niche space occupied by the food webs remained constant, with δ^{13}C never depleted below -45.0 ‰ and δ^{15}N never enriched above 12.0 ‰ (Figure 3.1). All fish functional feeding groups (Table 3.1) showed a trophic separation of approximately 2.0 ‰ to 4.0 ‰ δ^{15}N (Figure 3.1). Both invertebrate functional feeding groups are at the base of the food webs (Figure 3.1). The whole community structure ranges between -35.0 ‰ to -15.0 ‰ for δ^{13}C and 0.5 ‰ to 12.0 ‰ for δ^{15}N across all sites (Figure 3.1). Site Mwinilunga Fish Weir shows the narrowest δ^{13}C range for all sites, from -35.0 ‰ to -22.0 ‰ (Figure 3.1a). Outliers of extreme negative δ^{13}C (-35.0 ‰ to -45.0 ‰) occur for resources at sites West Lunga (Figure 3.1d), Jivundu (Figure 3.1e), Watopa (Figure 3.1f) and Nsengi Flood Plain (Figure 3.1g). Top predators (*Hydrocynus vittatus* and *Serranochromis* spp.) were not collected at the sites Kabompo Bridge (Figure 3.1b) and Nsengi Flood Plain (Figure 3.1g); invertivore fish were not collected at West Lunga site (Figure 3.1d); nor herbivorous fish at Nsengi Flood Plain site (Figure 3.1g). Omnivorous fish, invertivorous fish, and predatory invertebrate functional feeding groups overlap between isotrophic lines (Figure 3.1).

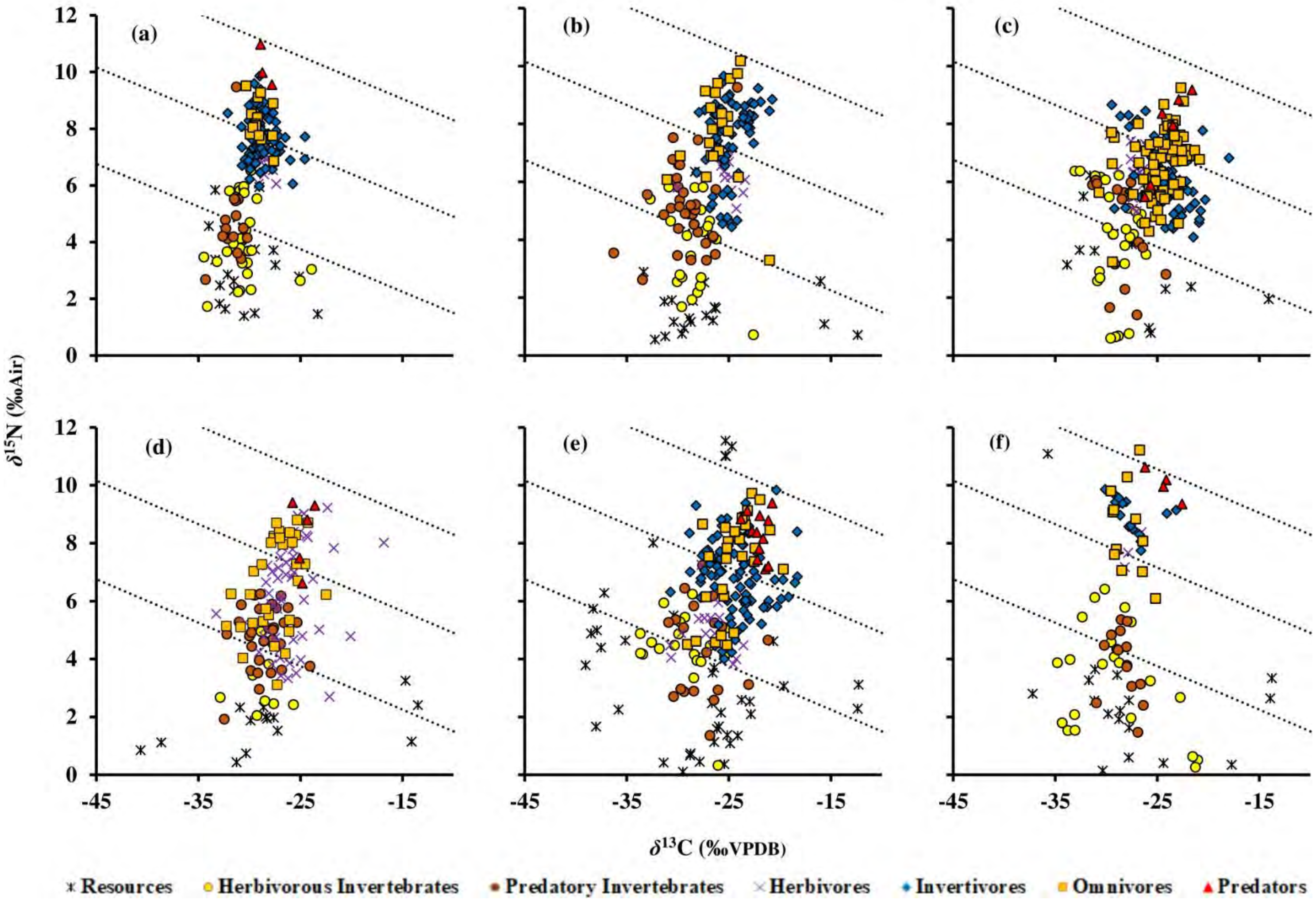


Figure 3.1 Isotrophic lines to indicate classic trophic separation between different functional feeding groups calculated based on total averages of the invertebrate data for sites (a) Mwinilunga Fish Weir, (b) Kabompo Bridge, (c) Ntambu Camp, (d) West Lunga, I Jivundu, (f) Watopa, and (g) Nsengi Flood Plain.

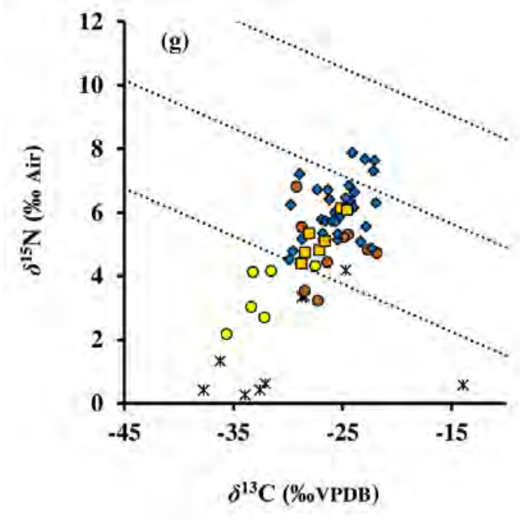


Figure 3.1 Isotrophic lines to indicate trophic separation between classic functional feeding groups calculated based on total averages of the invertebrate data for sites (a) Mwinilunga Fish Weir, (b) Kabompo Bridge, (c) Ntambu Camp, (d) West Lunga, I Jivundu, (f) Watopa, and (g) Nsengi Flood Plain.

Overlap between functional feeding groups

Predator isotopic niche space range higher (> 10 ‰ δ^{15}N) than other functional feeding groups on average and remain relatively high across all sites, with the exception of Ntambu Camp, West Lunga, and Jivundu where overlap with omnivores and invertivores within a similar region (Figure 3.2). The resources had the broadest range of all ellipse areas (Table 3.3.) and range the lowest on δ^{15}N of all groups, on average below the invertebrates (Figure 3.2). The invertebrate groups show significant overlap at Ntambu Camp (ε = 74.8%) and the Jivundu site (ε = 53.2%) (Table 3.3). Invertivores and omnivorous fish feed within the relative same dietary space at all sites (Figure 3.2), but only significantly overlap at Kabompo Bridge (ε = 60.2%), Ntambu Camp (ε = 59.2%) and Jivundu (ε = 56.6%) (Table 3.3). The fish food webs at Watopa show a shift into a higher nitrogen niche space (Figure 3.2). Only West Lunga shows a significant overlap for herbivorous and omnivorous fish (ε = 68.5%) (Table 3.3).

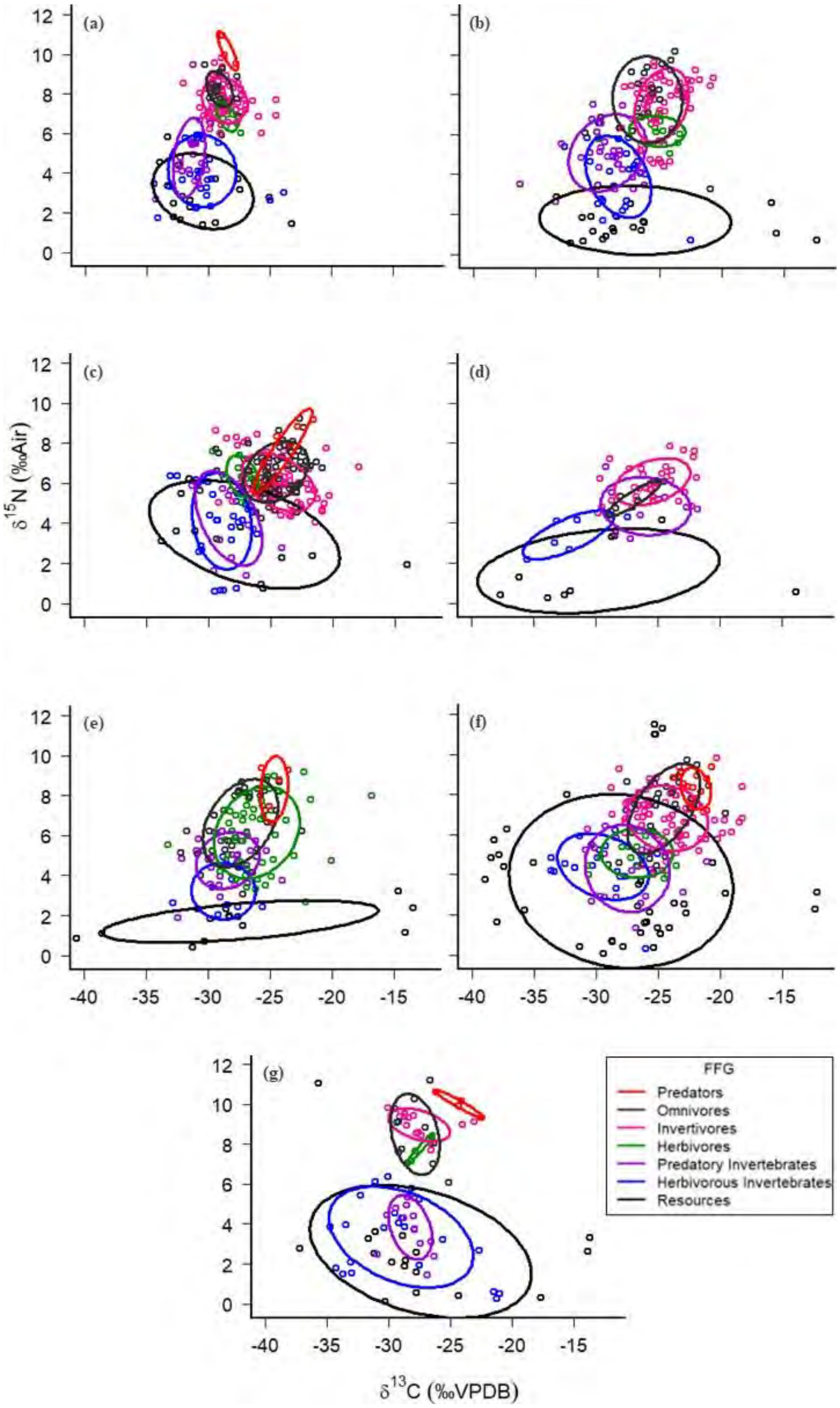


Figure 3.2 Functional feeding group overlap based on baseline corrected δ^{13}C and δ^{15}N values per site of the Kabompo River, Zambia: (a) Mwinilunga Fish Weir, (b) Kabompo Bridge, (c) Ntambu Camp, (d) Nsengi Flood Plain, I West Lunga, (f) Jivundu, and (g) Watopa.

Table 3.3 Stable Isotope Bayesian Ellipses 95%-overlap-probability ε (%) between functional feeding groups Resources I, Herbivorous Invertebrates (HI), Predatory Invertebrates (PI), Herbivores (HV), Invertivores (IV), Omnivores (OV), and Predators (PD) for each site: MFW) Mwinilunga Fish Weir; KB) KabompoBridge; NC) Ntambu Camp; NFP) Nsengi Flood Plain; WL) West Lunga, J) Jivundu; and W) Watopa

Site	**ε**						
	HI -PI	**HV-IV**	**IV-OV**	**OV-PD**	**HV-OV**	**IV-PD**	**HV-PD**
MFW	44.28	27.24	43.17	8.61	43.17	12.10	$9.15e^{-16}$
KB	38.70	39.86	60.24	-	28.88	-	-
NC	74.85	32.65	59.22	37.24	29.30	23.36	19.61
NFP	21.34	-	25.65	-	-	-	-
WL	46.25	-	-	31.52	68.47	-	24.57
J	53.21	31.65	56.61	22.92	36.60	19.91	$6.38e^{-16}$
W	19.96	10.44	38.88	4.77	9.49	$4.038e^{-16}$	$2.58e^{-1}$

Discussion

This chapter showed the isotope food webs structures of the Kabompo River with the potential to contribute information on the ecological functioning of the ecosystem. The objectives were to assess fish and invertebrate diversity found in the river, to describethe food web structures, and to compare the structures between sampled sites across alongitudinal gradient. The food webs in the system do not follow a typical top-down predator-driven structure (Power 1992) but appear more aligned to bottom-up productivity (Pringle and Hamazaki 1998). The results indicate that the food webs separate into clean trophic structures (Minagawa and Wada 1984) across all sites during the dry season and are not dependent on the presence of fish predators as with top-down systems (Minagawa and Wada 1984; Power 1992; Pringle and Hamazaki 1998; Shurin et al. 2006). Nitrogen Isotopes for top predators (*Hydrocynus vittatus* and *Serranochromis* spp.) range across relatively low values of 10 ‰ -12 ‰ that do not suggest nutrient pollution. These same top predators occupy trophic states of 12 ‰ to 20 ‰ δ^{15}N in systems where nutrient pollution was apparent (Woodborne et al 2012; Verhaert et al. 2017) and in these nutrient-loaded systems, primary producers can reach elevated nitrogen values of $\delta^{15}N > 10.00$ ‰ (Motitsoe et al. 2020). This may suggest unimpacted ecological functioning of the Kabompo River food webs as the data suggests limited unnatural stressors to the systems From the diversity indices and calculations, all sites display the same overall diversity for fish and invertebrates, with invertebrates showing a generally higher community diversity (Table 3.2). Unlike Simpson's Index, Shannon-Wiener's Index (*H'*) was more subjective as very high values are sometimes calculated (Keylock 2005). Shannon-Wiener scores ranging from 2.0 to 4.5 are considered high for fish assemblages in tropical rivers (Chowdhury et al. 2019). Scores of 2 to 3 for indigenous fish species in the Surma River, Bangladesh, concludedonly slight stress on the species from pollution, compared to historical diversity estimates thatranged between 3.0 to 4.5 (Chowdhury et al. 2019).

Historical diversity comparisons such as those used for the Surma River were not possible for the Kabompo River, but the diversity of the food webs can be considered high from the values calculated (Table 3.2) (Keylock 2005; Chowdhury et al. 2019). In general, invertebrate diversity is higher than fish diversity but due to the different taxonomic identification (fish to species and invertebrates to family) the differences in diversity was noticeable. The higher invertebrate scores could be indicative of the fine woody detritus (after burning) in the river benthos creating macroinvertebrate suitable microhabitats, but no habitats suitable for fish (stumps, bark, branches, and other woody debris) (Benke and Wallace 2003). Introducing woody debris in restoration projects has been a method to help increase fish diversity and their invertebrate prey (Benke and Wallace 2010). As fish sampling was the principal objective during collection, the lower-than-expected diversity might be attributed to the sampling period and unequal effort, burning events, and fishing practices. The sampling occurred during low flow (in the dry season), and the amount of wood debris available for fish could be less, which would explain the slightly lower diversity (Wohl et al. 2016). Burning the woody-savannah forests in the Upper Zambezi sub-catchment basin in which the Kabompo River lies intensifies during winter (low flow) (Fanshaw 2010; McIntyre et al. 2016) and is a customary practice which may decrease the woody habitats available to fish from burning events (Eriksen 2007).Last, low flow contributes to habitat complexity which would increase fish diversity in the area (Willis et al. 2005), but potential targeting of specific species for subsistence with no new recruitment could decrease diversity (McCann et al. 2016).

From upstream sites (Mwinilunga Fish Weir and Kabompo Bridge) to downstream sites (Watopa), the isotopic positioning of the food webs was similar, with the δ^{15}N values never enrichedabove 12.0 ‰ and δ^{13}C never depleted below -45.0 ‰. The δ^{13}C for all food webs ranged across the same values (Figure 3.1). The δ^{13}C were between -35.0 ‰ and -22.0 ‰ except at West Lunga, Jivundu, and Watopa, which had values more negative than -35.0 ‰ (> -45.0 ‰),which may be an indication of a potential carbon resource alteration from high algal productionin slower currents (Finlay et al. 1999), and floodplain and wetland systems (Neue et al. 1997; Douglas et al. 2005; Tareq et al. 2005). These extreme negative δ^{13}C may indicate the presenceof algal-derived and wetland-derived carbon that regularly include depleted δ^{13}C as these occurin the large sections with slower currents. The same trend was observed at Nsengi Flood Plain,one of the many permanent wetlands in the Upper Zambezi sub-catchment basin (Moore et al.2007). Mwinilunga Fish Weir had the narrowest relative δ^{13}C range, possibly due to high fishing practices indicative of resource input changes possibly from baiting activities (Hardy et al. 2010; Hur et al. 2018). Thissite is at a weir which is likely heavily impacted by the local community, with potential weir pool effects that include resource alteration from bait (Hardy et al. 2010; Hur et al. 2018). The δ^{15}N ranges (< 12.0 ‰) indicate a healthy functioning ecosystem, as expected, with the top predators found on average (even when overlapping with omnivore and invertivore groups) at the top and with subsequent trophic levels below it (Douglas et al. 2005; Sloman et al. 2005; Shurin et al. 2006).”Compared to studies of South African rivers, these nitrogen ranges the range of δ^{15}N ratios in the Kabompo River likely represent a system minimally impacted by pollution. Tiger fish (*Hydrocynus vitattus*) isotope data captured from the Letaba and Olifants Rivers show δ^{15}N that ranges across 13.0 ‰ to 20.0 ‰ (Woodborne et al. 2012), a higher range than the 6.0 ‰ to 11.8 ‰ values for tiger fish (*Hydrocynus vittatus*) in the Kabompo River. The fish δ^{15}N values are not enriched above 13 ‰, characteristic of nutrient pollution (Woodborne et al. 2012; Verhaert et al. 2014), and trophic groups are not separated more than 3 ‰ to 4 ‰, the norm expected in fish food webs (Minagawa and Wada 1984; Douglas et al. 2005). The same effect occurs in the Kabompo, with trophic separation between 2.0 ‰ to 4.0 ‰, indicatingconsumer feeding across two trophic groups (Douglas et al. 2005). At the Kabompo Bridge and Nsengi Flood Plain sites, the top predators were not collected, which could be attributed tothe sampling methods or overfishing, but future investigation is needed to confirm why these predators were absent at these sites. In tropical river systems, the suggestion is that top-down food webs are not affected by trophic cascades when large-bodied predators are no longer present (Pace et al. 1999). In these systems, large predators exert the same pressure on the food web structure as other, smaller-bodied fish and other fish functional feeding groups(Pringle and Hamazaki 1998). This was evident from the food webs where top predators functional feeding group were present that show no clear changes from Kabompo Bridge and Nsengi Flood Plain, where these top predators were absent. . The overlap between the omnivore and insectivorous fish at the Kabompo Bridge site could be seen as an indication of this lack of trophic cascade, with the omnivorous fish isotopic niche shifting up into the expected predatory niche space (Figure 3.2).The results suggest a bottom-up productivity structure for the food webs of the Kabompo Riveras the structures do not apparently rely on top-down predation (Power 1992). The effects of geomorphology should also be considered as these can affect bottom-up nutrient changes and alter results (Nelson et al. 2019).

The invertebrate groups had overlapping niche spaces at Ntambu Camp and Jivundu (Figure 3.2; Table 3.3), which can indicate that both invertebrate functional feeding groups are feeding on the same food resources (Flaherty and Ben-David 2010; Layman et al. 2012), recent high algae growth – elevated levels of algal growth was documented at these sites - that the herbivores feed on (Finlay et al. 1999)or the species classification could have caused an error in the placement of individuals into the wrong functional feeding groups (Tomanova et al. 2006). The ellipse space in ecology assumes that all individuals feed everywhere within the isotopic space identified for a specific species or community, which is not always the case (Blonder et al. 2014; Blonder 2016). Future studies will need to demonstrate if nutrient input and sample sizes (< 30 individuals) influences the niche spaces occupied by communities in the Kabompo River (Nielsen et al. 2018). NsengiFlood Plain illustrated δ^{15}N values within similar average range, but different carbon ranges from the river sites. Different carbon (δ^{13}C) sources at this site formed the base for these foodwebs, potentially due to differential microbial respiration (anaerobic) and terrestrial inputs (Sanseverino et al. 2012).

Conclusion

This chapter shows that the Kabompo River has high biodiversity and suggests a that the food web structure is consistent and relatively stable along the river. The general diversity found in the river seems to be high after analysis, but with no comparable literature on the diversity expectations in the Kabompo River the diversity can only be compared to other sub-tropical systems such as the Surma River. The food web structures follow classic expected structures, with predators found at the top and primary producers and consumers in lower trophic positions. Comparisons between sites indicate food webs that remain structurally unchanged in the dry season along the longitudinal gradient of headwaters downstream to the last site in the Kabompo River. The food webs in this river are unaffected by predator pressures as the presence of this functional feeding group does not affect the general structures across sites. The resulting food webs may be driven by bottom-up productivity may drive structures as the isotope niche ranges remain constant across the gradient, a feature that could contribute to understanding the landscape-level processes part of the river system.

CHAPTER 4: NUTRIENT DYNAMICS ALONG THE KABOMPO RIVER ELUCIDATED USING STABLE ISOTOPE ANALYSIS

Introduction

Nutrient cycling in rivers is complex as they are dynamic systems (Delgado and Follett 2002; Heathwaite 2010) and understanding the nutrient cycling process is important in conserving these threatened freshwater habitats (Maavara et al. 2020), the nutrient levels of which are enriched by flooding or rainfall (Shurin et al. 2006). Large tropical rivers are lotic with lentic features, such as wetlands and pans, which contribute to nutrient profiles through different chemical processes (Bayley 1995; Thomaz et al. 2007). Landscape-level process information, such as longitudinal river flow, is essential to determine nutrient cycling (Bunn and Davies 2000; Teodoru et al. 2015). These landscape-level processes describe autochthonous and allochthonous contributions of nutrients to an aquatic system.

Stable isotope analysis, widely used in ecology, enables nutrient (autochthonous and allochthonous) tracing from source to sink within a landscape using the isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) (Peterson and Fry 1987; Michener and Lajtha 2008). Carbon isotope δ^{13}C values provides a method for tracing carbon sources associated with different microbial and plant carbon metabolism (C_3 and C_4 photosynthesis) (Sternberg and DeNiro 1983; Farquhar et al. 1989; Pace and Prairie 2005; Sanseverino et al. 2012). Plants that utilise the C_3 photosynthetic pathway broadly include woody macrophytes, and plants that utilise the C_4 photosynthetic pathway are mainly crop plants and grasses (Sternberg and DeNiro 1983). Carbon ratios for C_3 range from -21.0 ‰ to -29.0 ‰, C_4 range from -12 ‰ to -16 ‰, microbial anaerobic respiration includes extreme ranges of -24.0 ‰ to -68.0 ‰, and algae ratios range from -23.4 ‰ to -37.2 ‰ depending on more nearshore C_3 or C_4 inputs of dissolved organic matter (DOM) (O'Leary 1988; Farquhar et al. 1989; Hamilton and Lewis 1992; McKee et al. 2002; Taylor et al. 2017). Food web dynamics from nitrogen isotope δ^{15}N values may elucidate energy transfers and clarify trophic positions (Peterson and Fry 1987; Tomanova et al. 2006), with a more enriched nitrogen value indicating a higher trophic position (Peterson and Fry 1987; Michener and Lajtha 2008). The Kabompo River, with many permanent wetlands, supports a high rural population who rely on subsistence activities such as agriculture and fishing (Timberlake 2000; Beilfuss 2012; Zwidofhelangani 2020; Sambo et al. 2021), and understanding the nutrient cycling of the river becomes important for planning conservation management.

This chapter evaluated three theories of nutrient cycling for the Kabompo River: 1) the River Continuum Concept; 2) the Flood Pulse Concept-revised; and 3) the revised-Riverine Productivity Model. The expectations for the River Continuum Concept are headwaters with predominantly terrigenous carbon sources, and mineralisation (nitrification and denitrification) and transferral effects of DOM nutrients from transformation processes downstream, but not in the headwaters (Vannote et al. 1980; Humphries et al. 2014). The Flood Pulse Concept- revised describes the connectivity between a river and its adjacent floodplains as a single dynamic system reliant on both ecology and hydrology (Tockner et al. 2002). It suggests that i) temperature plays a key role in community compositions and habitat structures, ii) expansion-contraction events in reference to inundation and drying of the floodplain play a key role in ecological dynamics (nutrient inputs and removals), and iii) headwater floodplains will differ significantly from lowland floodplains with respect to both different processes controlling habitat heterogeneity and biodiversity assemblages (Tockner et al. 2002). The revised-Riverine Productivity Model reconciles the river heterotrophy paradox, that questions the exclusivity of certain nutrient processing pathways as most riverine habitats are heterogenous (Kempe et al. 1991, Raymond et al. 1997). This model suggests that conversion of transported allochthonous and autochthonous material by microbial activity is the primary driver of the heterotrophic states of a river (Thorp and Delong 2002). The model also posits a weak link between the microbial decomposer pathway and mid- to higher trophic levels via the algal-grazer biomass production pathway (Thorp and Delong 2002). Both carbon and nitrogencontribute to the overall river landscape dynamics, but carbon cycling better elucidates local and nitrogen longitudinal river processes (Finlay 2001; Hadwen et al. 2010). The distinction between each river nutrient model relies on the isotope values along a reach (Finlay 2001; Woodward and Hildrew 2002; Hadwen et al. 2010).

In aquatic habitats, interpretations of isotope values become more complex as contributions and removals become more complex (Thorp et al. 1998). For the purposes of this chapter, nutrient contributions are considered solely in terms of organic matter (OM) and include particulate anddissolved forms of carbon and nitrogen. At local scales there is a perpetual cycling of carbon resources by local biological mechanisms with δ^{13}C values that reflect these fluxes in food webs (Hadwen et al. 2010). The three abovementioned models were evaluated using functionalfeeding group placements of consumers to distil out the most likely processes that occur as indicated by their feeding habits, whereas nitrogen isotope values to assess longitudinal changes in river processes. Carbon values of both fish and invertebrates were used to evaluate site-specific cycling. Invertebrate feeding groups are generally used to determine nutrient cycling processes (Vannote et al. 1980; Kempe et al. 1991, Raymond et al. 1997; Thorp et al. 1998; Thorp and Delong 2002; Humphries et al. 2014). Within these groups, the filterers guild is always dependent on local productivity in the form of fine particulate organic matter (FPOM), and filterers can be the base of a substantial food web (Cummins 1973, 1974; Cummins and Klug 1979; Anderson and Cummins 1979). Grazers are dependent on in situ phytoplankton and biofilm productivity from the bioavailable nitrogen from upstream reaches (Cummins and Klug 1979; Berman and Bronk 2003, Doretto et al. 2020). As the river moves downs stream, there is an assumption that this base will become wider and there will be a niche that expands from filterers towards grazers as in situ productivity is added. The invertebrate guilds are important as they determine whether the FPOM (filterers) or *in situ* productivity (Grazers) support the higher trophic levels in the river. But as fish are the main focus of this chapter, the omnivores, *Synodontis* spp., and algivores as grazers, *Labeo cylindricus*, were used to determine the most likely model.

The assumptions made here where: (1) if the obligate algivore or grazer (*L. cylindricus*) varied linearly, then it was probably the role of mineralisation and denitrification dominating primary productivity, and the river discontinuum models were most appropriate, thus either the Flood Pulse Concept-revised or revised-Riverine Productivity Model; and (2) if the omnivores (*Synodontis* spp.) varied relative to the grazers (*L. Cylindricus*), it would indicate that other nutrient sources played a role and that the food web was not dependent on primary productivity but on local resource assimilation, suggesting the River Continuum Concept was most appropriate.

The aim of this chapter is to evaluate the isotope data across a downstream longitudinal gradient of the KabompoRiver using the functional feeding guilds of inverts and fish, along with the assumptions from the three theories, to determine the model(s) of best fit. This was done by evaluating the fluxes in the depletion and enrichment of the nitrogen isotope values along a gradient with carbon viewed only at local scales. The δ^{15}N enrichment underpins denitrification (change of nitrogenous products that preferentially cycle ^{14}N) and depletion nitrification (suggesting more *in situ* production for biofilm, algae, and phytoplankton) as more nitrogenous products are available to primary producers and consumers. In terms of oxygen content, microbes associated with nitrification are considered aerobes and those with denitrification anaerobes, suggesting possible depths atwhich these processes occur. It was hypothesised that the river system would follow the classic River Continuum Concept as a large continuous river and that some effects from the revised- River Productivity Model would occur in the higher stream orders but not the Flood Pulse Concept-revised, as flooding would be unlikely because the sampling had occurred in the low-flow season. In addition, the analyses of the possible overlaps between the fish and invertebrate groups were used to determine the main contributors of terrestrial nutrients. These analyses helped to visualise feeding preferences of fish and invertebrates and predict if both groups are equally responsible for the same nutrient sources in the river if their isotopic niches show overlap. This may also allow a view of grazing or filter feeding by invertebrates in relation to fish groups.

Materials and Methods

Sample collection, laboratory analysis, and calibrations were completed for fish, invertebrate, and resource materials as described in the Materials and Methods section of Chapter 3.

Statistical analysis

Invertebrate isotope data were used to baseline correct all the other consumer data but not the resource material data (which is the base of the system). The data were baseline corrected to the invertebratesto account for shifts in the bioavailable nutrient pools that may vary across sites (Post 2002; Layman et al. 2012). All analyseswere done in the Microsoft© Excel 2010 and the *R* (version 4.0.5) statistical programme in *Rstudio* (Rstudio Team 2020), and algorithms sourced from the literature (Press et al. 1996; Jackson et al. 2011). The packages for the Kolmogorov-Smirnov tests included hdrcde (Highest Density Regions and Conditional Density Estimation use for both one and to dimensions), and ellipse (for drawing ellipse and ellipse-like confidence regions). The food webs analyses with SIBER (Stable Isotope Bayesian Ellipses in R) included the packages rjags (for the JAGS library - Just Another Gibbs Sampler - library for Bayesian data analysis), and ggplot2 (grammar of graphics plot 2 for visualisation).

Fish and invertebrate communities overlap

Stable Isotope Bayesian Ellipses in R (SIBER) is a Bayesian framework which accounts for uncertainty and incorporates possible data errors from the sampling process (such as smaller sample sizes) (Jackson et al. 2011).It is a derivative of the Convex Hull Area concept common to isotope analysis in ecology but with novel multivariate ellipse-based metrics (Jackson et al. 2011). The compared isotopic niches of the fish and invertebrates were based on both δ^{13}C and

δ^{15}N values in an ellipse biplot (Jackson et al. 2011), to determine the possible terrestrial nutrient contributions of both to the water column (Finlay 2001; Hadwen et al. 2010; Ward et al. 2017)Resource material (vegetation samples) wasadded as a comparative source for both groups. Plots with ellipse areas set at a confidence interval of 75% were created to show overlap between the fish and invertebrate communities at each site and to limit y-axis crossing for the resource material. Statistics from the SIBER plots indicate the percentage overlap between groups using posterior distributions of the fitted ellipses at a confidence interval of 95%.

Longitudinal comparison

Two common fish species (*Labeo cylindricus* and *Synodontis* species) were identified for longitudinal comparisons. Separate graphs were created to show carbon isotope ratios (δ^{13}C) and nitrogen isotope ratios (δ^{15}N). The isotope values of the fish and resource material were normalised to the invertebrates to ease data visualisation as not all invertebrates feed on the resource material. The fish resources (vegetation material andinvertebrates) were separated to indicate different carbon sources for fish-feeding comparisons.Algae, detritus and macrophyte data were combined into the vegetation data series and re- named Primary Producer. To simplify the interpretation of carbon (resources) and nitrogen (trophic positioning) flow at each site, all values for each category (fish, invertebrates and primary producers) were pooled and presented as the average value per group (± 1SE).The values were plotted across six sites (Mwinilunga Fish Weir, Kabompo Bridge, Ntambu Camp, West Lunga, Jivundu and Watopa). The permanent floodplain: Nsengi Flood Plain (NFP) was excluded as it does not form part of the mainstream, and *L. cylindricus* and *Synodontis* spp. Were not presentthere.

Kolmogorov-Smirnov pairwise tests

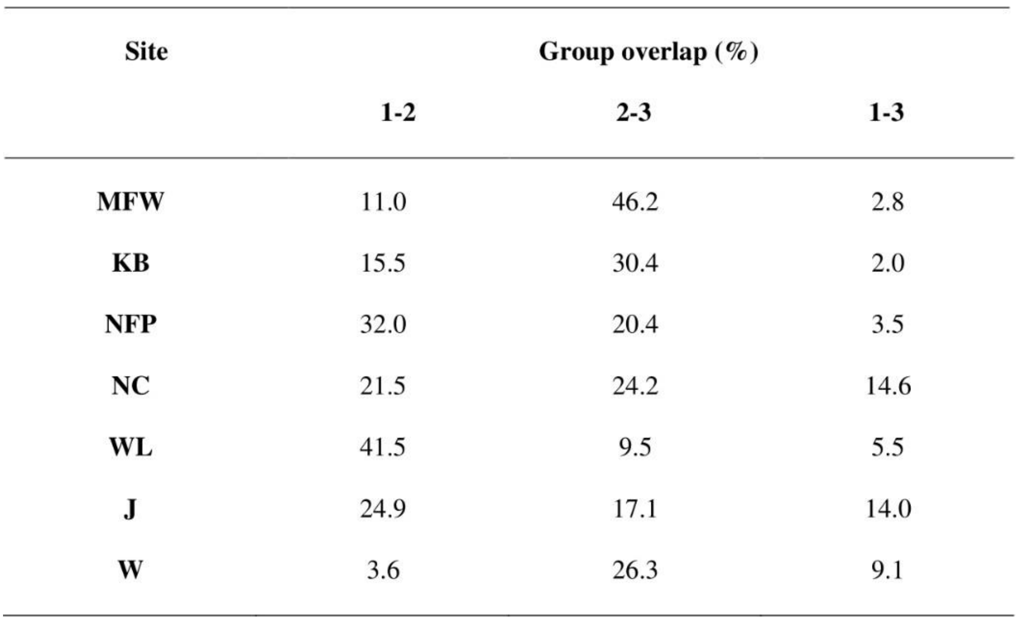
The Kolmogorov-Smirnov test determines the difference between empirical data distribution and a reference distribution. The test creates four different univariate, averaged tests each from the four corners of a square surrounding the data (Press et al. 1996) and calculates the distance between two distributions ($0 \geq D \leq 1$), where $D = 0$ indicates no difference between distributions, and presents the smallest area that encompasses all the observations, but as a convex hull area for visualisation of the data. Two different Kolmogorov-Smirnov tests were used in the non-averaged and baseline corrected data for analysis of both fish species. The first was a one-dimensional Kolmogorov-Smirnov test to evaluate the univariate differences between pairs of sites. This test determined whethersignificant differences exist between the isotope values (δ^{15}N or δ^{13}C) between sites. The second was a two-dimensional Kolmogorov-Smirnov pairwise test to clarify the bivariate (δ^{15}N and δ^{13}C) differences between site distributions. A two-dimensional Kolmogorov-Smirnov convex hull graph was created to visually represent the data output in a two-dimensional space.These are pairwise comparisons between sites where $n(n-1)/2$ comparisons were made for n treatments (sites). For six treatments (Mwinilunga Fish Weir, Kabompo Bridge, Ntambu Camp, West Lunga, Jivundu, and Watopa), there were 15 (6 x 5 / 2 = 15) combinations. The Kolmogorov-Smirnov test code was adapted from the generalised *C* version in Press et al. (1996) and implemented in *R*. The null hypothesis of no significant difference between two site distributions is rejected at the significance level of $p < 0.05$. A two-dimensional Kolmogorov-Smirnov test was not possible for *Synodontis* spp. Between Kabompo Bridge andany other site as only one individual fish was sampled from Kabompo Bridge.

Results

Fish and invertebrate communities overlap

Ellipse plots of overlap (Figure 4.1, Table 4.1) between fish and invertebrate groups at each site show that the proportion of overlap at a 95%-confidence interval was largest at the West Lunga site (41.5%) in the headwater and lowest at the last site, Watopa (3.6%). Nsengi Flood Plain was not part of the main river streams but had the second-largest overlap (32.0%).

Table 4.1 Stable isotope dietary niche overlap percentage (%) at a 95% confidence interval between fish (1) and invertebrate (2) communities at an ellipse confidence interval of 75% across sites. Resource material (3) was added to indicate the nutrient sources of both groups



Site	**Group overlap (%)**		
	1-2	**2-3**	**1-3**
MFW	11.0	46.2	2.8
KB	15.5	30.4	2.0
NFP	32.0	20.4	3.5
NC	21.5	24.2	14.6
WL	41.5	9.5	5.5
J	24.9	17.1	14.0
W	3.6	26.3	9.1

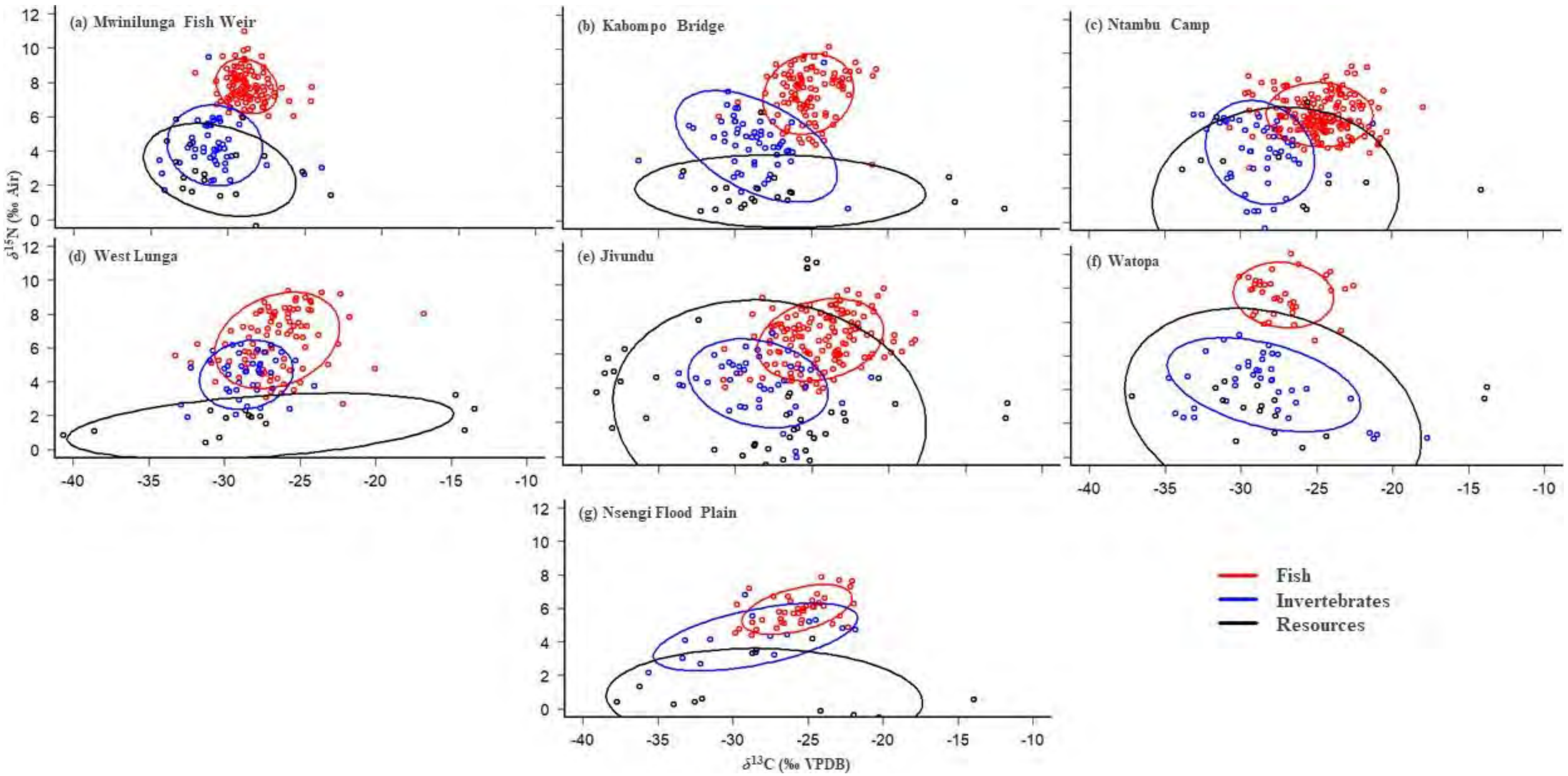


Figure 4.1 Stable isotope niche ellipses based on baseline corrected $\delta^{13}C$ and $\delta^{15}N$ for fish (red), invertebrates (blue) and resource material (black) to show the significance of nutrient sharing along a gradient for the Kabompo River upstream to downstream: (a) Mwinilunga Fish Weir; (b) Kabompo Bridge; (c) Ntambu Camp; (d) West Lunga; I Jivundu; and (f) Watopa. The (g) Nsengi Flood Plain is not part of the main river branches and not part of the gradient.

Longitudinal comparison

The δ^{13}C values pre-averaging range between -31.3 ‰ and -23.4 ‰ for *L. cylindricus* and between -33.4 ‰ and -29.7 ‰ for *Synodontis* spp.; the δ^{15}N pre-averaged values range between 5.0 ‰ and 9.2 ‰ for *L. cylindricus* and between 5.6 ‰ and 10.6 ‰ for *Synodontis* spp. Viewed together as resources for the fish species, both the primary producer and invertebrate pre-averaged δ^{13}C values range from -40.6 ‰ (West Lunga) to -11.7 ‰ (Kabompo Bridge), and 0.1 ‰ to 11.5 ‰ (both at Jivundu) for pre-averaged δ^{15}N.

To ease the longitudinal interpretation, the graphs are based on the values baseline corrected, post-normalisation, to the invertebrate isotope averages (averages for this group are similar between sites). The *Synodontis* spp. at Kabompo Bridge has no error bars as it wasone individual. There is no clear trend for the δ^{13}C averages, but the δ^{15}N averages show a general U-shape trend (Figure 4.2).

The primary producer δ^{13}C averages remain relatively constant across all sites and in line with the invertebrates, with the most enriched averages seen for the Mwinilunga Fish Weir and Kabompo Bridge sites. The average carbon values of the fish show large variation across sites with Mwinilunga Fish Weir showing the largest difference when compared to theother sites. The *L. cylindricus* carbon values have a highly variable trend mimicked by the primary producers from site Kabompo Bridge downstream to the final site Watopa, clustering around the invertebrate carbon values. The *Synodontis* spp. carbon values show no clear trend and display carbon averages at Mwinilunga Fish Weir, West Lunga, and Watopa more depleted than *L. cylindricus*. Onlyat Mwinilunga Fish Weir and Kabompo Bridge were the δ^{13}C values for these two fish similar, at all other sites, *Synodontis* spp. And *L. cylindricus* were separated by a minimum of 1 ‰ to 2 ‰.

The nitrogen trend of relative depletion in δ^{15}N between Mwinilunga Fish Weir, Kabompo Bridge, and Ntambu Camp occurs in both the fish species and the primary producers. There is a relative plateau in values from Ntambu Camp to West Lunga for both the *Synodontis* spp. and primary producers before slight enrichment at Jivundu site. From Ntambu Camp to Jivundu, *L. cylindricus* values remain within the plateau until Watopa, where enrichment places both the averages of *L. cylindricus* and *Synodontis* spp. At a higher value than at any other site, and primary producers at the same average value as at Mwinilunga Fish Weir.

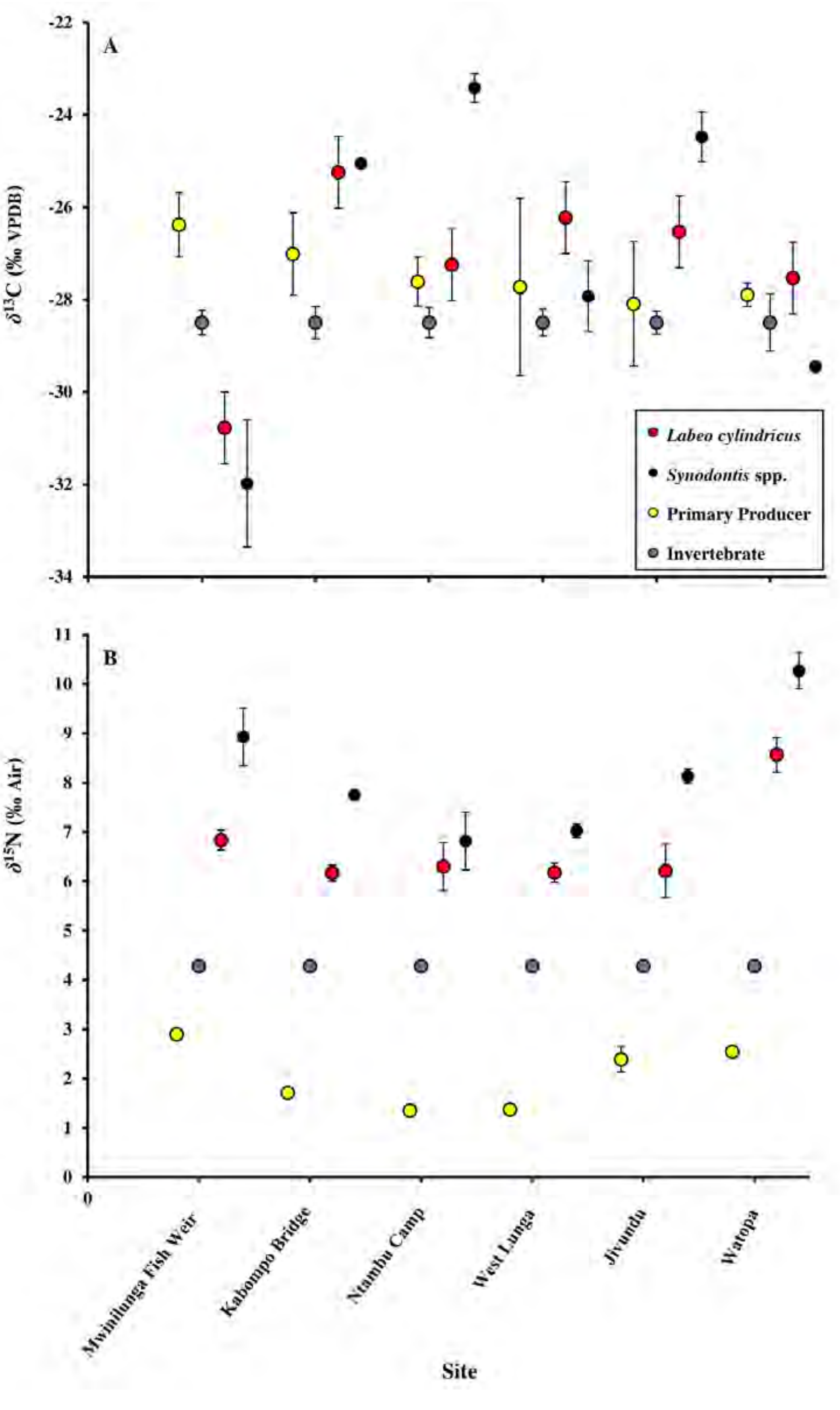
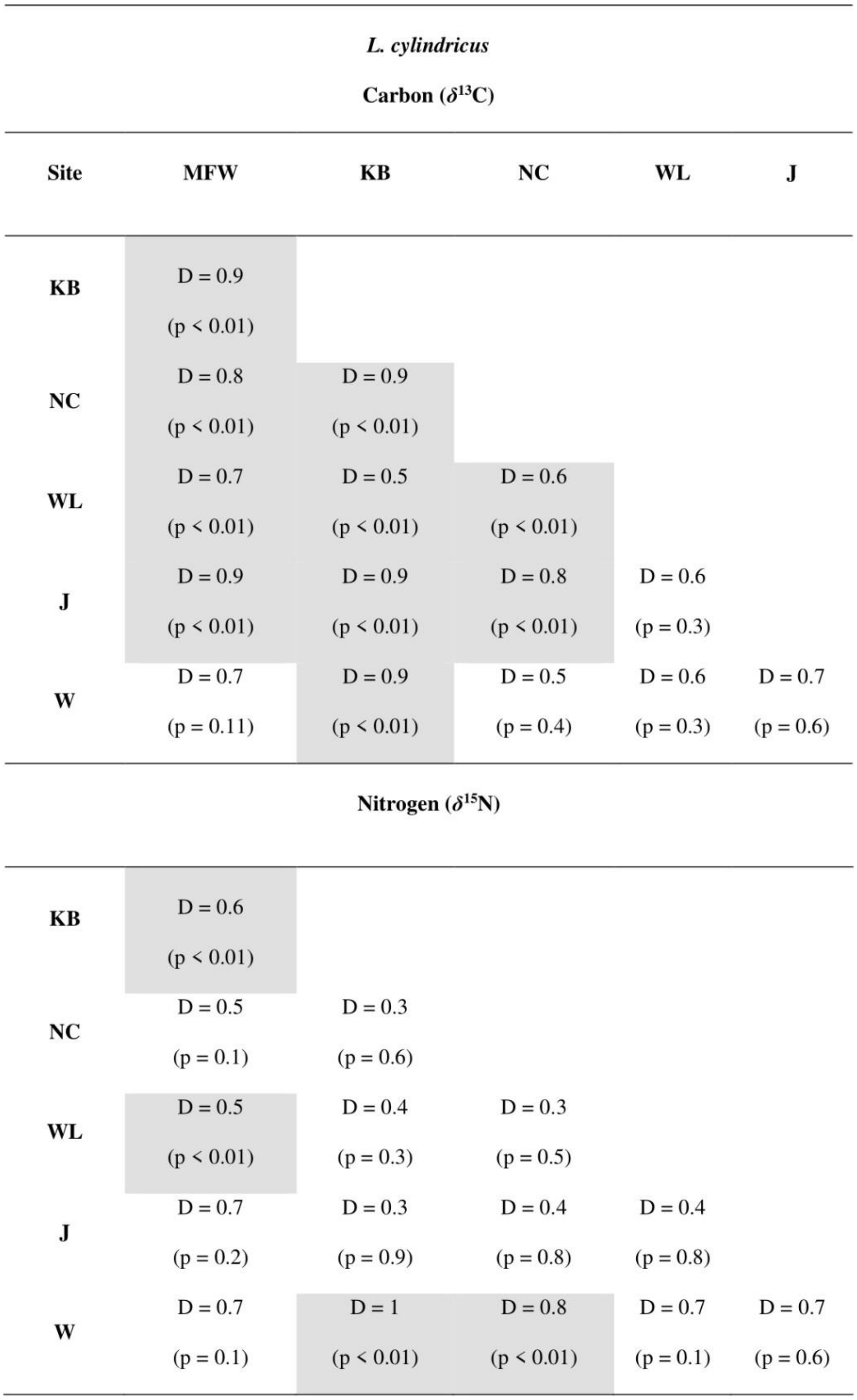


Figure 4.2. Longitudinal comparison of site-specific isotope A) δ^{13}C and B) δ^{15}N averages (with one standard error), normalised to the invertebrates to indicate changes in nutrient dynamics in the Kabompo River from a herbivore *Labeo cylindricus* and omnivore *Synodontis* spp. Alongside their putative resources, primary producers (vegetation, algae, and microorganisms), and invertebrates. Sites are 1) Mwinilunga Fish Weir; 2) Kabompo Bridge; 3) Ntambu Camp; 4) West Lunga; 5) Jivundu; and 6) Watopa.

Kolmogorov-Smirnov-tests

The one-dimensional Kolmogorov-Smirnov test for *L. cylindricus* baseline corrected non normalised isotope values (Table 4.2.1) shows the pairs with significant differences ($p < 0.01$) as MFW-KB ($D = 0.9$), MFW-NC ($D = 0.8$), MFW-WL ($D = 0.7$), MFW-J ($D = 0.9$), KB-NC ($D = 0.9$), KB-J ($D = 0.9$), KB-W ($D = 0.9$), NC-WL ($D = 0.6$) and NC-J ($D = 0.8$) for $\delta^{13}C$; and between MFW-KB ($D = 0.6$), MFW-WL ($D = 0.5$), KB-W ($D = 1.0$) and NC-W ($D = 0.8$) for $\delta^{15}N$.

Table 4.2.1 Pairwise one-dimensional Kolmogorov-Smirnov tests on the baseline corrected, non-normalised data for the effect of each isotope (δ^{13}C and δ^{15}N) separately for *L. cylindricus* (*Labeo cylindricus*) species between sites MFW = Mwinilunga; KB = Kabompo Bridge; NC = Ntambu Camp; WL = West Lunga; J = Jivundu; and W = Watopa where shaded values indicate significant differences.



L. cylindricus

Carbon (δ^{13}C)

Site	**MFW**	**KB**	**NC**	**WL**	**J**
KB	D = 0.9 (p < 0.01)				
NC	D = 0.8 (p < 0.01)	D = 0.9 (p < 0.01)			
WL	D = 0.7 (p < 0.01)	D = 0.5 (p < 0.01)	D = 0.6 (p < 0.01)		
J	D = 0.9 (p < 0.01)	D = 0.9 (p < 0.01)	D = 0.8 (p < 0.01)	D = 0.6 (p = 0.3)	
W	D = 0.7 (p = 0.11)	D = 0.9 (p < 0.01)	D = 0.5 (p = 0.4)	D = 0.6 (p = 0.3)	D = 0.7 (p = 0.6)

Nitrogen (δ^{15}N)

Site	**MFW**	**KB**	**NC**	**WL**	**J**
KB	D = 0.6 (p < 0.01)				
NC	D = 0.5 (p = 0.1)	D = 0.3 (p = 0.6)			
WL	D = 0.5 (p < 0.01)	D = 0.4 (p = 0.3)	D = 0.3 (p = 0.5)		
J	D = 0.7 (p = 0.2)	D = 0.3 (p = 0.9)	D = 0.4 (p = 0.8)	D = 0.4 (p = 0.8)	
W	D = 0.7 (p = 0.1)	D = 1 (p < 0.01)	D = 0.8 (p < 0.01)	D = 0.7 (p = 0.1)	D = 0.7 (p = 0.6)

The one-dimensional Kolmogorov-Smirnov test for *Synodontis* spp. Baseline corrected non normalised isotopes values (Table 4.2.2) shows significant differences ($p < 0.05$) for the pairs MFW-NC ($D = 1.0$), NC-WL ($D = 0.9$) NC-W ($D = 1.0$), and WL-J ($D = 0.8$) for $\delta^{13}C$; and between , NC-WL ($D = 0.6$), NC-J ($D = 1.0$), NC-W ($D = 1.0$), and WL-W ($D = 1.0$) for $\delta^{15}N$.

Table 4.2.2 Pairwise one-dimensional Kolmogorov-Smirnov tests on the baseline corrected, non-normalised data for the effect of each isotope (δ^{13}C and δ^{15}N) separately for *Synodontis* spp. Between sites MFW = Mwinilunga Fish Weir; KB = Kabompo Bridge; NC = Ntambu Camp; WL = West Lunga; J = Jivundu; and W = Watopa where shaded values indicate significant differences.

Synodontis spp.

Carbon (δ^{13}C)

Site	**MFW**	**KB**	**NC**	**WL**	**J**
KB	D = 1.0 (p = 0.7)				
NC	D = 1.0 (p = 0.01)	D = 0.9 (p = 0.2)			
WL	D = 0.8 (p = 0.2)	D = 0.9 (p = 0.3)	D = 0.9 (p < 0.01)		
J	D = 1.0 (p = 0.1)	D = 0.6 (p = 1.0)	D = 0.6 (p = 0.1)	D = 0.8 (p < 0.01)	
W	D = 0.5 (p = 1.0)	D = 1.0 (p = 0.7)	D = 1.0 (p = 0.01)	D = 0.8 (p = 0.2)	D = 1.0 (p = 0.1)
			Nitrogen (δ^{15}N)		
KB	D = 1.0 (p = 0.7)				
NC	D = 1.0 (p = 0.01)	D = 0.9 (p = 0.2)			
WL	D = 0.8 (p = 0.2	D = 0.5 (p = 1.0)	D = 0.5 (p = 0.01)		
J	D = 0.6 (p = 0.6)	D = 1.0 (p = 0.3)	D = 1.0 (p < 0.01)	D = 0.5 (p = 0.3)	
W	D = 0.5 (p = 1.0)	D = 1.0 (p = 0.7)	D = 1.0 (p = 0.01)	D = 1.0 (p = 0.01)	D = 1.0 (p = 0.1)

The two-dimensional Kolmogorov-Smirnov test for both species' baseline corrected, non normalised isotope values (Table 4.2.3) indicates a significant difference ($p < 0.05$) between all site pairs except MFW-J, NC-J, WL-J, J-W and WL-W for *L. cylindricus;* and MFW-WL, MFW-J, and MFW-W for *Synodontis* spp. Comparisons for site Kabompo Bridge (KB) were not possible as only one individual was analysed. The visual representation of the two-dimensional Kolmogorov-Smirnov test shows that the West Lunga (WL) groups of both species dominate the convex hull areas (Figure 4.3). The fish assemblages cover a range from -34.0 ‰ to -17.0 ‰ δ^{13}C and 0 ‰ to 10.0 ‰ δ^{15}N. *Labeo cylindricus* covers the widest (carbon) resource range, and *Synodontis* spp. The highest trophic(nitrogen) position. The apparent shift in the *Synodontis* spp. Convex hull areas from Ntambu Camp to West Lunga to Jivundu show that the omnivores feed from different nutrient sources,while *L. cylindricus* remains in the same general area.

Box plots of the fish species baseline corrected δ^{13}C and δ^{15}N averages, maximums, and minimums non normalised are shown in Figure 4.4.

Table 4.2.3 Pairwise two-dimensional Kolmogorov-Smirnov tests on baseline corrected, non-normalised data for the effect of each isotope (δ^{13}C and δ^{15}N) separately for *L. cylindricus* (*Labeo cylindricus*) and *Synodontis* spp. (*Synodontis* species) between sites MFW = Mwinilunga Fish Weir, KB = Kabompo Bridge; NC = Ntambu Camp; WL = West Lunga; J = Jivundu; and W = Watopa where shaded values indicate significant differences.

L. cylindricus

Carbon (δ^{13}C) and Nitrogen (δ^{15}N)

	MFW	**KB**	**NC**	**WL**	**J**
KB	$D = 0.8$ ($p < 0.01$)				
NC	$D = 0.6$ ($p < 0.01$)	$D = 0.8$ ($p < 0.01$)			
WL	$D = 0.8$ ($p < 0.01$)	$D = 0.5$ ($p = 0.03$)	$D = 0.5$ ($p = 0.05$)		
J	$D = 0.7$ ($p = 0.11$)	$D = 0.8$ ($p = 0.04$)	$D = 0.5$ ($p = 0.3$)	$D = 0.4$ ($p = 0.5$)	
W	$D = 0.8$ ($p = 0.02$)	$D = 1.0$ ($p < 0.01$)	$D = 0.8$ ($p = 0.02$)	$D = 0.6$ ($p = 0.1$)	$D = 0.8$ ($p = 0.2$)

Synodontis spp.

Carbon (δ^{13}C) and Nitrogen (δ^{15}N)

	MFW	**KB**	**NC**	**WL**	**J**
KB	-				
NC	$D = 1.0$ ($p < 0.01$)	-			
WL	$D = 0.8$ ($p = 0.06$)	-	$D = 0.8$ ($p < 0.01$)		
J	$D = 0.7$ ($p = 0.2$)	-	$D = 0.8$ ($p < 0.01$)	$D = 0.8$ ($p < 0.01$)	
W	$D = 0.3$ ($p = 1.0$)	-	$D = 1.0$ ($p < 0.01$)	$D = 0.9$ ($p = 0.03$)	$D = 1.0$ ($p = 0.01$)

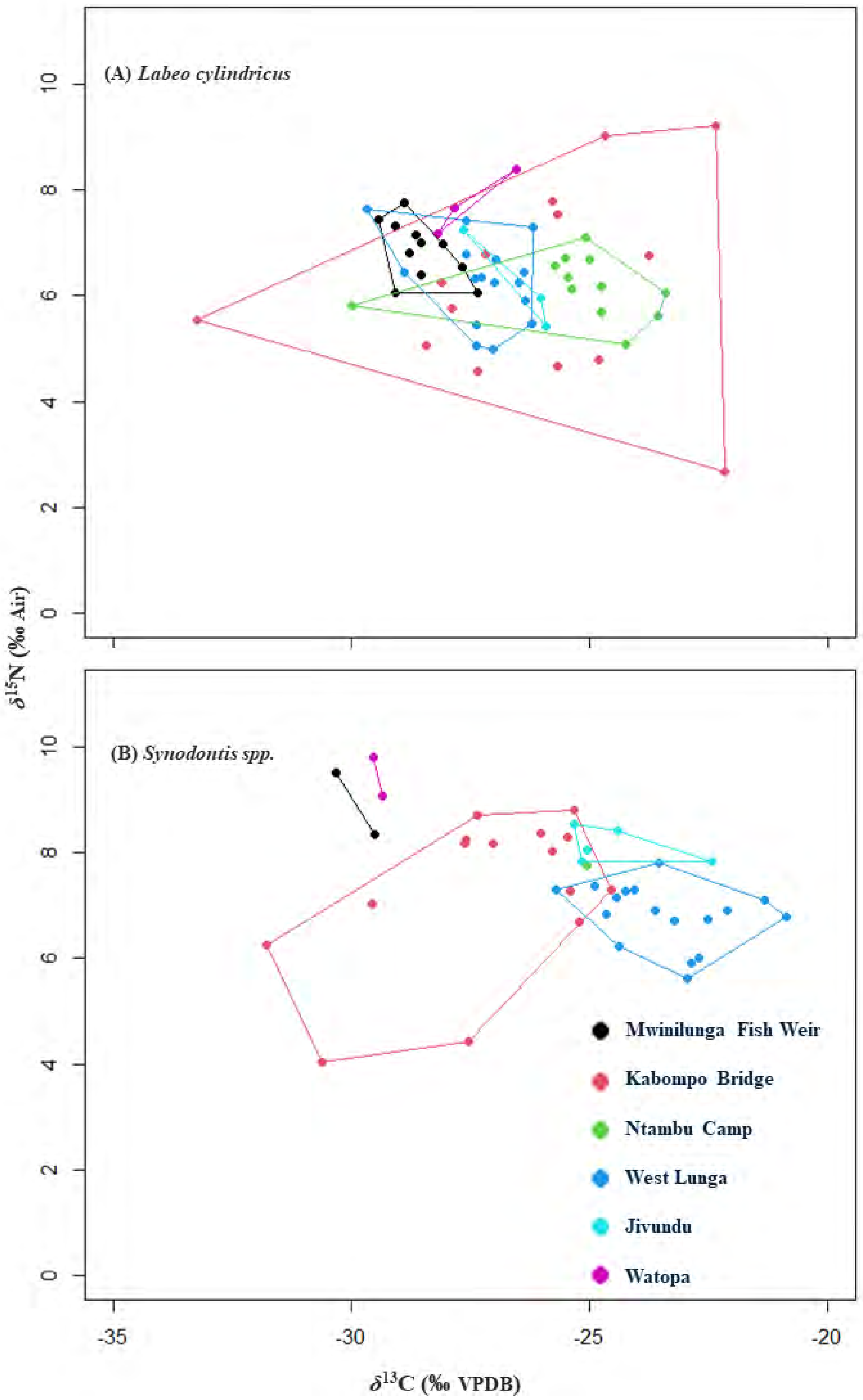


Figure 4.3 Visual representation of two-dimensional Kolmogorov-Smirnov tests to show bivariate (δ^{13}C and δ^{15}N) distribution areas for A) a herbivore *L. cylindricus* and B) an omnivore *Synodontis* spp., respectively across a longitudinal gradient: Mwinilunga Fish Weir; Kabompo Bridge; Ntambu Camp; West Lunga; Jivundu; and Watopa. Values indicate the largest of the niche spaces occupied by the communities from West Lunga.

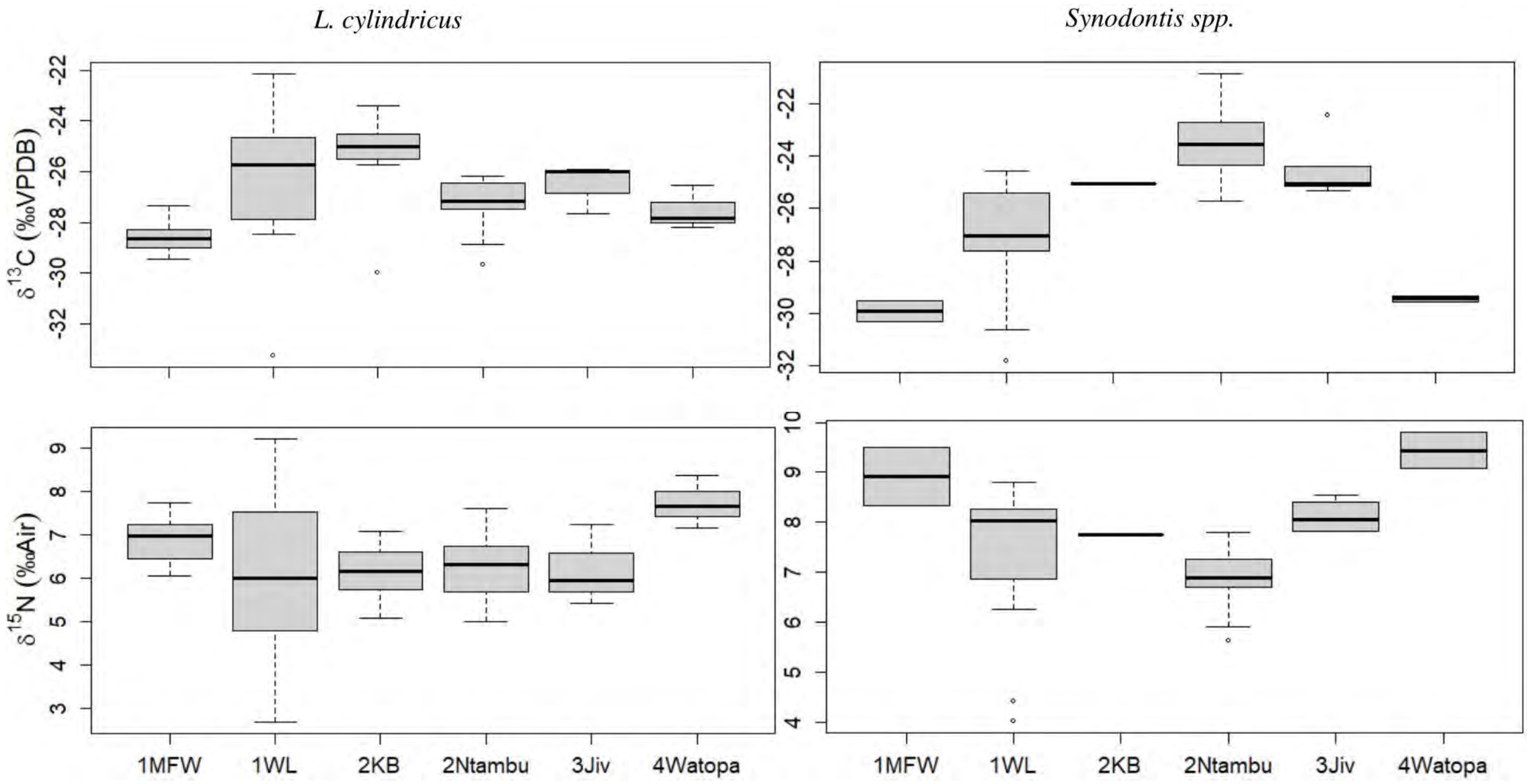


Figure 4.4 The baseline corrected, non-normalised isotope values (δ^{15}N and δ^{13}C) as used for the Kolmogorov-Smirnov two-dimensional pairwise test as averages, maximum, and minimum for A) herbivore *L. cylindricus* (*Labeo cylindricus*) and B) an omnivore *Synodontis* spp. (*Synodontis species*) across a gradient where 1MFW = Mwinilunga Fish Weir; 1WL = West Lunga; 2KB = Kabompo Bridge; 2Ntambu = Ntambu Camp; 3Jiv = Jivundu; and 4Watopa = Watopa..

Discussion

This chapter aimed to better understand the possible nutrient cycling processes that drive the ecosystems of the Kabompo River. A longitudinal river comparison between two common southern African fishes, a herbivore *L. cylindricus* (Weyl and Booth 1999) and omnivore *Synodontis* spp. Complex (Shinkafi et al. 2010) indicate a relative depletion in nitrogen isotope ratios averages at Ntambu Camp and West Lunga sites compared to the other sites. Both species visually occupy the most significant dietary niche space at West Lunga site compared at the other measured sites (Figure 4.3), and there was a significant difference between the combined δ^{13}C and δ^{15}N feeding niches at this site and most other sites (Table 4.2.3).

There is no trend for the averaged values of the fish δ^{13}C along a gradient that indicates a relative fluctuation in the carbon amounts of C_3 to relative extreme negative C_3 (specific microbial and wetland effects) and a possible increased input of terrestrial resources. These fluctuations are an indication of localised mechanisms of carbon cycling (Finlay 2001; Hadwen et al. 2010; Wardet al. 2017). The highly depleted ratios provide evidence for floodplain dynamics as wetlands produce these extreme values from anaerobic metabolism and photodegradation of DOM (Porcal et al. 2015; Johannsson et al. 2021; France et al. 2022). The relative less negative δ^{13}Cof the primary producers suggests input from the woody-savannah terrestrial biomass, remaining within the same relative average values across the gradient. This may suggest the unchanging contributions from the forest canopy across the river longitude even in the dry season. The Upper Zambezi sub-catchment carbon load remains relatively unchanged despite the increase in fire and deforestation in the region (Pelletier et al. 2018). The most significant Δ^{13}C enrichment occur for the fishes from Mwinilunga Fish Weir to Kabompo Bridge. Both these sites are separate head streams that converge at Ntambu Camp. The *Synodontis* spp. Complex retained the highest average value at Ntambu Camp unlike *L.*

cylindricus which converges closer to the primary producers at Ntambu Camp. This may be the first indication of the polyphagous nature of the *Synodontis* spp. And algivorous feeding by *L. cylindricus*.From Ntambu Camp to Watopa, *L. cylindricus* carbon values do not shift dramatically, suggesting a consistent diet among sites, but *Synodontis* spp. shifts between depletion and enrichment. The enrichment of δ^{13}C indicates possible incorporated and transformed terrestrial inputs like the flood 'pulses' in the seasonal swamp of the Okavango Delta, Botswana (Mladenov et al. 2007). The DOM processing in the Okavango Delta, water fed by the Middle-Zambezi River reach, is characterised by higher amounts of plant litter that contribute higher nitrogen (δ^{15}N) and carbon (δ^{13}N) (Mladenov et al. 2007). The final finding suggests that in this large flood plain wetlands there is progressive enrichment of DOM from plant derived material as it progresses downstream and then undergoes some environmental processing (photodegradation and bacterial breakdown) (Mladenov et al. 2007). Flooding in the dryseason, however, is uncommon for the Upper Zambezi sub-catchment basin (Beyer et al. 2016),suggesting *in situ* change to nutrients according to the revised-Riverine Productivity Modelcomparable to the photodegradation of woody matter for the Congo River (Spencer et al. 2009).

The nitrogen isotopes indicated a strong, shared trend and constant trophic separation (1.0 ‰ to 2.0 ‰) between the fish. The primary producers also follow this U-shaped trend for the relative ratios and suggest initial denitrification (enrichment) at Mwinilunga Fish Weir to nitrification (depletion) at Kabompo Bridge to Ntambu Camp and West Lunga, and denitrification (enrichment) at Jivundu and Watopa (Sharma and Ahlert 1977; Lund et al. 1999; Berman and Bronk 2003; Olde Venterink et al. 2006; Burgin and Hamilton 2007). *Labeo cylindricus* showed no change in their average carbon values and remained relatively similar across all sites (except at Watopa),but this is not the case for *Synodontis* spp. (Figure 4.2). Algae with considerable amounts of cyanobacteria have a lower δ^{15}N than other algae, which may explain the depletion seen in *L. cylindricus* from Mwinilunga Fish Weir to Kabompo Bridge, Ntambu Camp, and West Lunga (Hamilton and Lewis 1992). Isotope evidence suggests that the trophic importance of algae in wetlands may be more important than previously imagined, as detritus from vascular plants was thought to play a more significant role in these lentic features (Hamilton and Lewis 1992). The apparent niche separation from the δ^{15}N betweenthe fish diminished at Ntambu Camp and west Lunga before separation re-established along with enrichment from Jivundu to Watopa. This may suggest that the *Synodontis* spp. Groups changed their feeding behaviour due to an abundance of *in situ* production of algae at Ntambu Camp and West Lunga. Subsequent enrichment in nitrogen at the last two sites may indicate a diminished reliance on *in situ* algae production as the *Synodontis* spp. nitrogen values increases. The primary producers and *L. cylindricus* mirror this nitrogen trend which may indicate that primary production relies on new products from denitrification (enriched δ^{15}N) and *L. cylindricus* may be feeding from thesemore enriched products.

Isotope ratios from the Okavango Delta in Botswana indicated similar trends of δ^{15}N enrichment and δ^{13}C depletion (Mladenov et al. 2007). The δ^{15}N trend and the changes in the δ^{13}C show the versatile feeding habits of *Synodontis* spp. And support their polyphagous nature (Shinkafi et al. 2010). The *Synodontis* spp. at the Ntambu Camp and West Lunga sites had similar nitrogen but very different carbon averages, suggesting that the fish species are using totally different resources that use the same nitrogen cycling processes. At the last two sites (Jivundu and Watopa), there was an increase in nitrogenisotope values, which is evidence of denitrification (Lund et al. 1999; Olde Venterink et al. 2006; Burgin and Hamilton 2007). The significant differences in the one- andtwo-dimensional Kolmogorov-Smirnov tests for both species strengthen the longitudinalcomparison observation of similar trophic positioning and account for the relative convergence of the δ^{15}Nvalues, which become more similar across the gradient. From the pairwise Kolmogorov-Smirnov tests, fish communities at upstream Ntambu Camp (except for the one-dimensional Kolmogorov-Smirnov for δ^{15}N) and West Lunga are consistently, significantly different from most other sites. At West Lunga, assemblages of both species dominate the isotopic space in the two-dimensional Kolmogorov-Smirnov comparison, suggesting a change to broader resource assimilation by both species. The one-dimensional Kolmogorov-Smirnov δ^{15}N at Ntambu Camp and West Lunga is more significantly similar (D = 0.5; p = 0.01) for Synodontis spp. which suggests an element of similarity between the communities (Table 4.2.2)." The data suggest that *L. cylindricus* may be consumingalgae and invertebrates on them, and *Synodontis* spp. may be exploiting anything available. The significance of all comparison differences for Watopa, except for δ^{13}C between Ntambu Camp and Watopa (NC-W Table 4.2.1), suggests that this site is consistently different from theother sites in terms of its nutrient contributions and energy transfers.

In headwaters, leaf litter and other terrigenous material typically enter the rivers from allochthonous inputs. Further down the river from the headwaters, channels become wider and incorporate floodplains, wetlands, some *Phragmites* reeds, and more open savannah adjacent habitat products (sages and grasses) (Timberlake 2000; Moore et al. 2012; Zwidofhelangani 2020). The River Continuum Concept model suggests both a nutrient mineralisation and transferral effects from different processes occurred downstream but not within the headwaters (Vannote et al. 1980; Humphries et al. 2014; Tornwall et al. 2015; Doretto et al. 2020). The general trends for the fish δ^{13}C and δ^{15}N do not support this (especially Mwinilunga Fish Weir, which presents a caveat). For the primary producer δ^{13}C, more allochthonous input of nutrients from the upstream was evident as less negative δ^{13}C values indicate direct contributions from the surrounding Miombo-woodlands as coarse particulate organic matter (CPOM) (Timberlake

2000; Bowling et al. 2008). This is normal for C_3 plant litter which has ratios in the -25.0 ‰ to -27.0 ‰ $\delta^{13}C$ range. The lack of overlap between the fish and invertebrate communities could indicate different nutrient incorporation. At West Lunga, the 41.5 % overlap may suggest an abundance of new nutrients to the system from two separate sources. The depletion in nitrogen and depletion in carbon indicates possible incorporation of allochthonous production into the already present autochthonous nutrient provision from the upper reaches of the river at the wider, deeper, and generally slower flowing downstream reaches. The incorporation of CPOM at theheadwater sites and then the breakdown of CPOM to fine particulate organic matter (FPOM) and other inorganic matter along a spatial flow supports the river discontinuum concepts due to the new food webs bases incorporated. The decreased overlap and re-enrichment of carbon at Watopa site support possible autochthonous nutrient incorporation from flooding along with the already present allochthonous products.

Mwinilunga Fish Weir and Watopa are the first and last sites, respectively, in the longitudinal gradient. These sites present the same relative average values for *Synodontis* spp. Across both isotopes, but Mwinilunga Fish Weir is considered a head water reach, and Watopa a higher order downstream reach. A potential explanation is that stream size influences allochthonous inputs into a stream (Wollheim et al. 2006). This effect suggests that the narrower the stream, the greater the effect of outside input, but input significance decreases as a channel becomes larger (Wollheim et al. 2006). All the sites in the Kabompo River are large and diminish support for the stream size suggestion. Alternatively, the *L. cylindricus* $\delta^{13}C$ shows depletion at both sites below the averages of the other sites that would represent wetland conditions and is supported by the enriched $\delta^{15}N$ values (Lund et al. 1999). The third possible explanation is potential flooding pulses of these larger sites that may introduce higher amounts of terrestrial carbon to the water (Mladenov et al. 2007). The lack of flooding in the Upper

Zambezi sub- catchment headwater rivers (Beyer et al. 2016) suggests the conditions of the revised-RiverineProductivity Model*, in situ* nutrient production, as the most probable model of nutrient cycling(Humphries et al. 2014).

Mwinilunga Fish Weir site is a fish weir near the town of Mwinilunga in North-West Zambia, a community that relies heavily on the river and forest (Peša 2019). At fish weir infrastructures, new habitats or 'weir pools' occur, in which organisms obtain nutrients from non-conventional sources, and trophic overlap occurs (Hardy et al. 2010; Hur et al. 2018). Fish are a large part of the daily protein resources for subsistence anglers (Sambo et al. 2021) and fishing with baited traps by communities around the weir would contribute differential resources that would influence the isotope ratios of the whole 'weir pool' ecosystem (Bašić et al. 2015; Mehner et al. 2019). There is no niche sharing between the fish and invertebrates at Mwinilunga Fish Weir. The invertebrates may be feeding on other sources still in the main channel, but the fish are concentrated at this site more than at any other site, suggesting that the depletion in δ^{13}C and enrichment in δ^{15}N is a combined effect of changes in dietary niche space by individuals (Hur et al. 2018) and possible impacts from fishing (Bašić et al. 2015; Mehner et al. 2019). The Watopa site data may indicate effects from both fishing and habitat heterogeneity. During collection from this site, a large community (more individual fish) was present than at the other sites. Observations of this site showed it included the widest range of in-stream habitats sampled, which may contribute to the isotope values collected along with the larger sample size.

Conclusion

The Kabompo River is a relatively pristine river with normal nitrogen isotope values that indicate limited nutrient pollution and with one structure (fish weir) that could impede fish movement. These data support the characteristics expected of the revised-River Productivity

Model for the Kabompo River, where this landscape nutrient-processing model *in situ* productivity is the primary driver of food webs. Here the trends suggest allochthonous, and autochthonous pathways appear to be equally prevalent in the river at each locality and include mineralisation, microbial and terrigenous inputs that may explain the change in the carbon and nitrogen isotopes. The trends suggest high variability in carbon nutrient cycling at local scales and gradual changes to the nitrogen inputs during trophic transfers that are not controlled by top-down predation and rather bottom-up productivity.

CHAPTER 5: GENERAL DISCUSSION

This thesis provided information on the Kabompo River, a main tributary of the remote and understudied Upper Zambezi sub-catchment. The river is large, with stream ordination that increases from the headwaters to downstream sites. It sustains a large subset of the Zambian rural human population and is less well understood than the Barotse Flood Plain and Chobe National Park regions. As a significant tributary, the river is a proxy for biological information on the most northern parts of the sub-catchment. A shortage of biological information here limits the formulation of management approaches. The data collected from the 2019 dry season (August to September) provided the first baseline food web dynamics for the river along a gradient. Food webs from stable isotope analyses show that organisms (fish, aquatic invertebrates, and their resources) organise into typical food web structures that suggest bottom-up primary production-driven functioning. Nutrient cycling from longitudinal comparisons between the fish species *L. cylindricus* (herbivores) and a *Synodontis* spp. Complex (omnivores) indicated the flow of energy was representative of the revised-Riverine Productivity Model and potentially explains landscape-scale processing for the river.

Developing countries in southern Africa, including Zambia, experience management challenges for ecosystem services due to information gaps (Gilman et al. 2004; Dunford et al. 2018). In aquatic ecology, this translates into a lack of knowledge on system functioning (Blocksom and Johnson 2009; Saarikoski et al. 2018). River management of the whole Zambezi catchment, under the legislation of the Zambezi River Authority, bases bi-annual assessments on water quality and quantity parameters (Tumbare and Mukosa 2021). The assessment allows a view of river function only from a physical and chemical perspective and is historically an effective rapid practice common to most countries (Karr 1993; Chapman 2021a, b). However, management that considers all aspects of an ecosystem proves to be more effective at assessing rapidly changing ecosystems found in rivers (Karr 1993; Chapman 2021a, b). The available information for the Kabompo River includes geomorphology, fish taxonomic research, climate change, and hydrology (Shela 2000; Kampata et al. 2013; Beilfuss2012; Ndlovu and Woyessa 2020; Banda et al. 2021).

Fish are reliable indicators of freshwater habitat changes as they respond to direct and indirect environmental changes, including microhabitat loss, nutrient changes, turbidity, parasitism, and temperature flux (Depledge and Galloway 2005; Dudgeon et al. 2006; Wrona et al. 2006; Poff et al. 2007; O'Brien et al. 2009; Arthington and Balcombe 2011; Gruner et al. 2017; Knouft and Ficklin 2017). Knowledge of fish feeding dynamics is beneficial for catchment management plans as the feeding dynamics indicate the flow of nutrients (Gilman et al. 2004), but available information is limited for riverine biota, except for tigerfish (*Hydrocynus vittatus*), tilapia, and yellow fishes (*Labeobarbus*) (Winemiller 1991; Winemiller and Kelso-Winemiller 1994, 1996; Økland et al. 2005, 2007).

A lack of knowledge on system functioning impedes management, as changes to the system's ecological integrity are unquantifiable (Callicott et al. 1999; Blocksom and Johnson 2009; Abbott and Campbell 2009, Abbott et al. 2015; Pahl-Wostl et al. 2021a, b). The underlying cycling process of nutrients for the Kabompo River in the dry season could partly assist with future management planning. This data forms a small subset of the large Upper Zambezi projects aimed at increasing knowledge of the natural and socio-economic nature of the Upper Zambezi sub-catchment basin. The data in this thesis add to the biological information pool for the Upper Zambezi sub-catchment basin, and its findings may assist policymakers in developing better management efforts for the basin.

One of the tools used in ecological environmental assessment surveys, and suggested for the Kabompo River, is the collection of fishes and invertebrates, and an assessment of species or family changes every season. The use of the ZISS is suggested as a rapid test, but a biotic index for the fish species still needs to be developed. Information from the food webs and basic nutrient cycling of the river may provide a starting point for the creation of such an index. The bottom-up driven nature of the Kabompo River food webs may assist in the use of diatoms to assess specific carbon fluxes in the river.

The Kabompo River ecosystem is relatively within expected natural ranges indicated by δ^{13}C (- 10.0 ‰ > x > 40.0 ‰) and δ^{15}N (< 12.0 ‰) values (Chapter 3). The values are not characteristic of nutrient pollution, as in many other southern African countries, such as South Africa (Motitsoe et al. 2020). Nutrient pollution indicated by δ^{15}N values occurs as extremely high (enriched) values in freshwater systems (8.0 ‰ to 38.0 ‰) comparable to the values obtained from food webs that contain large marine species (Montoya 2008; Dailer et al.2010; Orlandi et al. 2014). The nitrogen values from the Kabompo never exceeded 11.5 ‰ δ^{15}N for thelargest predatory fish (*Hydrocynus vittatus*).

The river provided a high species diversity at all sampling sites (Chapter 3). The species captured represent different feeding guilds characteristic of large floodplain river systems (Tweddle 2010; Peel. Et al. 2019; Taylor et al. 2017). These species sort into the guilds of detritivores, herbivores, benthic invertivores, invertivores, insectivores, omnivores, predatory-omnivore, and pure predators (Taylor et al. 2017). The largest predators are from the genera *Hydrocynus* and *Serranochromis*. Overlaps between these functional feeding groups are minimal (Chapter 3). This lack of overlap indicates coexistence between different groups and suggests potentially no resource sharing (Sala and Ballesteros 1997, Colloca et al. 2010). No exotic specieswere caught, such as the Nile Tilapia (*Oreochromis niloticus*) or red-claw crayfish (*Cherax quadraticus*), which are potential alien invaders to the Upper Zambezi sub-catchment (Douthwaite et al. 2018; Madzivanzira et al. 2021).

Longitudinal comparison between a herbivore (*L. cylindricus*) and an omnivore complex (*Synodontis* spp.) reflect the diet of each and the surrounding river nutrient fluctuations (Chapter 4). The data suggest that the *L. cylindricus* groups remain in their feeding trophic position and niches across the Kabompo River (Chapter 4), but that the *Synodontis* spp. groups change their dietary niche and overlap with the feeding niche of the *L. cylindricus* groups as evidenced from the similar nitrogen signatures of both species (Chapter 4). This overlap occurs at only two sites before trophic partitioning re-establishes at the last site, Watopa (Chapter 4). Theresults represent the polyphagous nature of the *Synodontis* spp. Group (Shinkafi et al. 2010). The changes in the fish isotopic values allowed the speculation of the potential presence of an abundance of algae, which in turn is suggested to prompt these omnivores to exploit the algae as a primary resource, along with *L. cylindricus*, an obligate algivore (Taylor et al. 2017)." Niche overlap between guilds is common in fish biology (Sloman et al.2005). Fish can share the same food resource, but several species may exploit exclusive resources throughout a year (Goulding 1980; Prejs and Prejs 1987; Olurin and Awolesi 1991; Mérona and Rankin-de-Mérona 2004; Pouilly et al. 2006; Hahn et al. 2008). Overlap betweenspecies only becomes a problem when a lack of changes to the system, such as mild seasons or poor flooding, force constant overlap, and one species out-competes the other (Cucherousset andOlden 2011; Zengeya et al. 2015; Pennock et al. 2021). This occurs typically due competition, but it is not present in overlap statistics for the Kabompo River (Chapter 3;Chapter 4).

The results suggest that the invertebrates and the fish species may not exploit exactly the same resources in the river, but some small overlap was seen at most sites with the highest overlap at West Lunga (41.5%) (Chapter 4). Except at the Nsengi Flood Plain, stable isotope analysis of the limited overlap between these groups shows niche partitioning (Peterson and Fry 1987; Sepulveda et al. 2012), suggesting that fish feed from a different nutrient source with a higher δ^{15}N than the invertebrates. The limited, not significant overlap between the fish and invertebrate communities and the different δ^{13}C could indicate different nutrient incorporation. At West Lunga, the 41.5 % overlap between the fish and invertebrates may suggest an abundance of new nutrients to the system from two separate sources. The types of individuals collected were mostly the nymphal stages of aquaticinvertebrate families (Lenat and Resh 2001). These nymphs remain submerged, feeding at thebenthopelagic level until maturity (Cummins and Klug 1979; Lenat and Resh 2001). All resources available to these nymphs originate from dissolved resources or other aquatic prey items also reliant on the nutrients in the water column (Cummins and Klug 1979; Lenat and Resh 2001). The Kabompo River is large, and wading is impossible, limiting the types of invertebrate families collected, unlike fish captured by rod and reel.

The fish isotope values reflect the changes in the river-adjacent landscape from Miombo forests (woody-savannah) to wetland-dominated regions. Woody plants reflect the C_3 photosynthesis pathway, and grasses the C_4 pathway, with isotope values more representative of both pathways (Chapter 1; Chapter 4; O'Leary 1988). The C_3 plants retain less of the heavier ^{13}C isotope than the lighter ^{12}C isotope, skewing the ratio towards less negative values (Peterson and Fry 1987; Michener and Lajtha 2008). For wetlands, the additional action of microbial respirationdepletes DOM material to produce a carbon product of extremely negative δ^{13}C value (Sanseverino et al. 2012). These contributions are not always apparent in ecosystems as different contributions to large rivers influence isotopic compositions, but overall, trends infer the dominant contributions (Layman et al. 2012).

Data deviations at the headwater site Mwinilunga Fish Weir could suggest fishing impacts. Isotope values from the headwater stream site Mwinilunga Fish Weir are characteristic for both a wetland (extreme negative δ^{13}C) and river influence (high δ^{15}N). The deviations and overlap between some functional feeding groups suggest potential influences of fishing and ‘weir pool’ effects. Watopa, the final site, presents higher overall δ^{15}Nvalues in the longitudinal comparison (Chapter 4), the lowest overlap, and the second highest fish and third highest invertebrate diversity (, suggesting total niche-partitioning by the lack of overlap between all groups (Chapter 3; Chapter 4). Fishing and/or the difficulty of sampling at this high order stream could explain the low species (fish) and family (invertebrate) individuals collected in the community at Watopa.

The change in isotope ratios from the headwater sites to the downstream sites indicates a change in nutrient contributions. Headwaters characterised by fluctuations in negative δ13C (except Mwinilunga Fish Weir) change to more negative δ^{13}C downstream relative to the headwaters, and the depletion and re-enrichment in δ^{15}N in from upstream to downstream sites reflect a change to the nutrient pool from in situ productivity potential as a result of wetlands and terrestrial contributions. The trend underpins the revised-Riverine Productivity Model characteristics, despite deviations at Mwinilunga Fish Weir for δ^{13}C. This finding suggests that the natural flow of this river incorporates more autochthonous products at all sections along a reach (Doretto et al. 2020) which changes over time due to different metabolic activities or chemicalreactions (Vannote et al. 1980; Humphries et al. 2014; Tornwall et al. 2015; Doretto et al. 2020). These changed products contribute new allochthonous sources of nutrients to the river andchanges to the biotic assemblages are driven by nutrient availability (Vannote et al. 1980;Humphries et al. 2014; Tornwall et al. 2015; Doretto et al. 2020). The revised-Riverine Productivity Model is the suggested form of nutrient cycling in the Kabompo River as the Flood Pulse Concept-revised postulates allochthonous processes to be present in the river fromthe headwaters to the lower reaches throughout. The sampling during the dry season of this river further supports the evidence for the revised-Riverine Productivity Model due to the rarityof flood pulses during this season (Kampata et al. 2013).

Limitations

Limitations in this thesis stemmed mainly from sampling and laboratory work. During the sampling and laboratory preparation period from 2019 to 2021 some samples were damaged, compromised, and data were lost.

Sampling limitations were seasonality and fish abundance. Only one season was sampled for this data set because of the logistical and financial constraints of accessing the region during COVID lockdown. The samples from the dry season all show non-fat fish and resource material, including detritus and ash from burning. This perspective from a single season is considered acceptable for fish assemblages and total food webs as carbon cycling stays the same across seasons in the Upper Zambezi sub-catchment (Zuijdgeest et al. 2015; Zuijdgeest and Wehrli 2017; Lowman et al. 2018), and no fish migration occurs in the dry season to skew results. No water quality data were collected during the sampling period and could have provided an additional benefit to the elucidation of nutrient processes.

Limitations from laboratory work and data include i) data lost from laboratory software, ii) a subset of non-lipid extracted fins, and iii) compromised invertebrate samples. Data loss included the problem of only one *Synodontis* spp. For the site Kabompo Bridge. This single individual could skew interpretations drawn from the longitudinal comparison, but the trends that the *L. cylindricus* groups project suggests that this does not affect the trends from *Synodontis* spp. Group assemblages at other sites. The non-lipid extracted fish fins were not used and only lipid-extracted fins were used for final analysis. Comparisons between a sub-set of lipid extracted and non-lipid extracted isotope values showed no significant differences andsuggested the use of lipid extracted fins for analysis as acceptable to permit correct interpretations. The comparison further suggested a river system with relatively lean fish, a potential effect of dry season sampling. The findings and C: N values (< 4) suggest lipid extraction was unnecessary (Post et al. 2007). Some invertebrate samples had fungus on the dried sample that was cleaned with distilled water, not alcohol, to keep the sample isotope ratios intact (Durante et al. 2020). Distilled water does not affect isotope analysis, but fungal growth may cause change (Peiman et al. 2022). Decomposition of fish tissues shows that only δ^{13}C and not δ^{15}N values are significantly influenced by saprophytic activity (Peiman et al. 2022).

If considerations of human impacts on the environment on the river could be quantified, they would be beneficial in assessing the effect of this stressor. Future studies should also focus on including water quality parameters to accompany the results from isotopic analysis and diversity estimates.

Future research

This Upper Zambezi sub-catchment basin is a prime area for novel ecological and taxonomic research. As the region is limited in biological information, community assemblages and species-specific information is required.

Incorporating a wet season sampling with dry season sampling has the potential to elucidate any change between the seasons and strengthen the current findings. Future endeavours would benefit from the collection of no less than five individuals per species or family as this is the suggested number for accuracy in stable isotope Bayesian ellipse analysis of food webs (Jackson et al. 2011). Further freezing of collected samples at the site of collection may decrease the risk of fungal colonisation of samples before laboratory drying. Finally, the collection of water quality and quantity information during both sampling seasons to supplement findings could enable more precise interpretations. In studies that focus ondiversity for community assemblages, rarefying the fish data for analysis is suggested where abundances are skewed to ensure accuracy in interpretations (Love and Taylor 2004). In these studies, the identification of invertebrates and plants to lower taxonomic level as possible should be done, along with fish species, to increase the validity of interpretations and contribute to the biological information pool. Incorporating infrastructure and geomorphology considerations during sampling (such as weirs, dams, fish ‘kraals’ or any other flow-impeding structures) along with food web data could determine the main drivers of food web structures but must be considered for a more extended sampling period (Nelson et al. 2019).

The collection of algae and diatom samples would provide information on the microbiome communities of the Kabompo River and Upper-Zambezi sub-catchment. These communities often form the large, unknown base of many freshwater systems where many important biogeochemical processes take place. Studies on these organisms have the potential to provide information on water quality changes over time and the microbiomes that support the ecologies of large rivers (Berthon et al. 2011; Fetscher et al. 2014).

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