# Trophic characteristics of aquatic habitats with different flooding regimes in the Okavango Delta, Botswana

Thesis submitted for the degree of

Doctor of Philosophy

by

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## Declaration

I, Richard Mazebedi, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

## Abstract

In periodically flooded wetland systems, the seasonal flooding regime is the main driver of ecosystem functions. The relationship between a wetlands' hydrology and ecosystem properties is therefore crucial to understand. Food web structure is an important ecosystem property that determines the stability of aquatic populations and hence the resilience of ecosystems to potential threats. While theoretical concepts predicting energy flow and food web dynamics in wetland ecosystems exist, there is still need for empirical research to validate the predictions to better inform local wetland management.

My thesis examined the variability of algal primary productivity and, using stable isotope ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$ respectively), the aquatic food web structure of differently flooded aquatic habitats within the Okavango Delta, in Botswana. The results from *in-situ* algal productivity incubations, showed that primary productivity rate is higher at Lake Ngami compared with Nxaraga lagoon and Phelo floodplain due to greater levels of dissolved nutrients at the lake. The relative importance of basal carbon sources for fish biomass production varied across study sites, but overall, algal sources were the principal basal carbon sources for fish across the study sites. The relative importance of carbon from macrophytes was greatest at Phelo floodplain during high flood. Based on Layman's matrices of food web structure, fish from Lake Ngami had isotopically diverse carbon sources, greater food chain length and greater trophic niche diversity compared to fish from Nxaraga lagoon and Phelo floodplain. There was high degree of trophic niche overlap between different fish feeding guilds at all the study sites indicating opportunistic feeding behaviour among fish in the Delta. Together, the results demonstrate that the diversity of aquatic habitats within the Delta support different pathways of energy flow and different aquatic food web structures, which may be crucial for maintaining diverse ecosystem functions of the Delta.

## Impact statement

The flood pulse that periodically floods the Okavango Delta is the main driver of biological production cycles and ecosystem functions of the Delta. However, natural flooding regime is faced with threats such as large-scale water withdrawals and climate change effects. The ability to predict the effects of the hydrological threats on ecosystem properties or to mitigate the effects of the threats will, however, rely on existing knowledge of the relationship between ecosystem properties and hydrological regimes. My thesis has improved the understanding of ecosystem properties in relation to hydrology within the Okavango Delta by quantifying primary productivity and food web structure in differently flooded habitats in the Delta. The results of Chapter 5, which is based on primary production measurements, are already published as a chapter in a book based on analyses of ecosystem services at Lake Ngami (Kgathi *et al.* 2018). Chapter 6, on the relative importance of basal carbon sources to fish, elucidated important spatial variations of $\delta^{13}C$ and $\delta^{15}N$ rations, which is going to benefit the design of future stable isotope-based studies in the Delta. The results of Chapter 6 showed that the relative importance of carbon sources to fish production vary between fish feeding guilds depending on the aquatic habitat and flood season, these findings are expected to benefit fisheries management in the Delta. Chapter 7 showed that there are high trophic niche overlaps between fish feeding groups, indicating opportunistic feeding strategies, which ensure maximum utilisation of diverse food resources that are availed by seasonal floods. This finding has management implications because it stresses the need for maintaining the natural seasonal flooding regime, hence resources pulses, for which the fishes of the Delta are adapted.

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## GLOSSARY OF KEY TERMS AND ABBREVIATIONS

**Benthic**: the deepest zone of a water body, including the bottom surface and near bottom zone

**Benthic primary productivity**: primary productivity that occurs at the bottom surface and near bottom habitat of a water body

**C:N ratio**: The ratio of carbon mass to nitrogen mass in a matter, it is used to differentiate organic matter from algae and vascular plants. Algal carbon has lower C:N values of 4-10:1 while organic matter has C:N values greater than 10: 1 (Khan *et al*. 2015)

**$C_3$ plants**: Plants whose first photosynthetic molecule is a 3-carbon molecule called 3-phosphoglyceric acid, mostly dicotyledon plants

**$C_4$ plants**: Plants whose first photosynthetic molecule is a 4-carbon intermediate molecule, either malic acid or aspartic acid, mostly grasses

**CAM plants**: plants use both C3 and C4 pathways but separated by the time of the day; at night CAM plants fix carbon dioxide to a 4-carbon intermediate molecule, which is then fixed to a 3-carbon molecule during the day

**DIC:** Dissolved inorganic carbon

**DIN**: Dissolved inorganic carbon

**DO**: Dissolved oxygen

**DOC:** Dissolved organic carbon

**EC**: Electrical conductivity

**Epipelic algae**: algae attached to the bottom surface of a water body

**Epipelic primary productivity**: primary productivity from epipelic algae

***e*GPP**: Epipelic gross primary productivity

***e*NPP**: Epipelic net primary productivity

***e*R**: Epipelic respiration

**Food Chain:** A linear connection of feeding relationships from producers to top consumers

**Food Web:** Feeding relationships made from interconnected food chains

**FWS:** Food web structure, the organisation of feeding relationships within a food web

**GPP**: gross primary productivity, refers to the total $CO_2$ fixed to organic carbon per unit surface area or volume per time during photosynthesis.

**High flood:** High water conditions, when floods are high in the Okavango Delta during winter months

**Low flood**: Low water conditions, when water is restricted to perennial channels and pools

**NPP**: Net primary productivity, refers to $CO_2$ fixed to organic carbon per unit surface area or volume per time during photosynthesis minus carbon which is lost by the photosynthetic organisms through respiration.

of carbon fixed or oxygen produced by plants or algae / bacteria (e.g. g C m-2 yr-1);

**OKACOM**: Okavango River Basin Water Commission

**ORI**: Okavango Research Institute

**Pelagic**: water column

***p*GPP**: pelagic gross primary productivity

***p*NPP**: Pelagic net primary productivity

***p*R**: pelagic respiration

**Primary Productivity**: the conversion of light energy to biochemical energy (starch) per unit of time by photosynthetic organisms.

**R**: Respiration

**SIA:** Stable Isotope Analyses

**SIMM**: Stable Isotope Mixing Models

**TEF:** Trophic Enrichment Factors, change in the value of

**Trophic level**: hierarchical position of an organism in a food chain

# CHAPTER ONE: Wetland ecosystems: Their importance, functioning and threats

Wetland ecosystems are vitally important to humans because they provide various goods and services which communities need to meet their survival needs and for improvement of their wellbeing (Mitsch *et al.* 2015). The sustainability of the benefits that humans derive from wetlands depends on effective management of the ecosystems, which in turn is underpinned by scientific understanding of how the ecosystems function. This chapter discusses the value of wetland ecosystems to humans, their ecological functioning (with a focus on flow of energy and material energy through the ecosystems), and the threats they face. The chapter demonstrates the need for further research on the functioning of wetland ecosystems; a quest that this PhD research contributes towards. This PhD research examines the relationship between aquatic food web structure and flooding regimes, a critical ecosystem feature that determines the dynamins of ecosystem functions such as nutrient cycling, as well as the stability of biological communities.

## 1.1.What are wetlands?

Wetlands can be difficult to precisely define because of their variability in hydrologic conditions, size, location and human influence ( Mitsch and Gossilink 2000, Mitsch *et al.* 2015) Wetlands are also dynamic and their boundaries with adjacent ecosystems can be difficult to define, which adds to the difficulty of defining wetlands ( van der Valk 2006, Golden *et al.* 2017) There is however a detailed and widely accepted definition of wetlands agreed upon by scientists and state federations through an international convention, the Ramsar Convention. The Ramsar definition of wetlands is “areas of marsh, fern, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six meters” (Mitsch and Gosselink 2000, www.ramsar.org). This detailed definition of wetlands, which is indicative of the large variation in forms and function of wetland ecosystems, shall be used in the PhD thesis.

## 1.2. The importance of wetlands to humans

The benefits that humans derive from wetlands, including a range of livelihood, social and economic benefits, that occur as wetlands perform their ecological functions. As part of the landscape, wetlands perform critical functions such as providing habitats for many plant and animal species, and biochemical cycling of important elements such as carbon and nitrogen. The benefits derived by human communities from wetlands include fisheries, clean water and grazing land (Mitsch *et al.* 2015, Costanza *et al.* 2017). It is because of their high value to humans that many wetlands are protected (Mitsch and Gossilink 2000).The fact that wetlands are still the only group of ecosystems with their own international convention, the Ramsar convention (Turner *et al.* 2000, www.ramsar.org), further demonstrates the high importance of wetland ecosystems to humanity. The multiple benefits that human societies derive from wetlands are referred to as ecosystem services (Assessment 2005, Groot *et al.* 2014, Costanza *et al.* 2014) and are discussed in the following sections.

### Ecosystem services from wetlands

Ecosystem services are the benefits that people obtain from ecosystems, and those provided by wetlands are vital for the wellbeing of people and may help to alleviate poverty especially in developing countries (Assessment 2005, Brouwer *et al.* 2014, Mitsch *et al.* 2015). To emphasize the value of ecosystem services and promote sustainable use of natural resources, the value of ecosystem services has even been expressed in monetary terms (Costanza *et al.* 1997), Groot *et al.* 2012, Costanza *et al*. 2014). For example, in 2007 the total value of ecosystem services derived from inland wetlands was estimated at 25,682$/ha/year (Groot *et al.* 2012). The availability of ecosystem services depends on how well a wetland performs its ecosystem functions. An in depth understanding of wetland ecosystem functions and corresponding environmental drivers is therefore crucial to ensure sustainability of ecosystem services derived from wetlands. The ecosystem services that human communities derive from wetland ecosystem functions can be grouped into four categories based on the type of service they provide: provisioning services, supporting services, regulating services, and cultural services (Assessment 2005, Costanza 2014).

### Provisioning services

Provisioning services are goods and services that societies directly derive from wetland ecosystems such as water and food (Assessment 2005, Mitchel *et al.* 2013). Wetland ecosystems avail natural resources that surrounding communities rely on for livelihoods, including the supply of nutritious food, fresh water, fuel-wood, fibre, medicinal and genetic resources among others (Mitsch 2015). The Okavango Delta in Botswana, for example, provides water to almost all households in the Ngamiland region. In addition to using the water for cooking, washing and watering their livestock, the households in the region use the water for flood recession agriculture (Kgathi *et al.* 2005). Other uses of water from wetlands include industrial use, hydropower generation, agricultural and recreational uses (Assessment 2005, Mitchell 2013)

Wetlands are a crucial source of protein for surrounding communities as they provide fish and meat. Globally, inland wetlands alone provide over 10 million tonnes of fish annually (Welcomme 2011). Fish and fish products are an important source of protein especially in developing countries. About 200 million people in the African continent depend on fish as their source of animal protein (Kolding *et al.* 2016). In Malawi about 75% of the total animal protein for both urban and rural communities come from inland fisheries (Assessment 2005, Rebelo *et al*. 2010). Wetlands also provide a sustainable grazing land resource for domesticated animals. In Southern Sudan about 800,000 cattle graze on the Sudd marshes while the coastal wetland of the Niger river supports about 3 million cattle, sheep and goats (Mitchell 2013).

### Supporting services

Supporting services are wetland processes that improve a wetland’s capacity to perform all other ecosystem services (Mitsch *et al*. 2015). Some key supporting services provided by ecosystem processes include primary production, nutrient and water cycling. The ability of an ecosystem to support production of other ecosystem services critically depends on biodiversity including species composition, relative abundance, functional diversity and taxonomic diversity. If these different components of biodiversity are adversely affected, supporting services provided by biodiversity will consequently be reduced and hence the capacity to produce other ecosystem services (Mitchell 2013).

### Regulating services

Regulating services are benefits that societies derived from the effects that wetlands have in controlling or adjusting extreme events and processes in the biosphere. These include regulation of climate, floods, water quality and soil erosion (Mitsch *et al.* 2015). There is a strong feedback relationship between wetlands and climate at local, regional and global scale (IPCC 2007). For example, evapotranspiration from a wetland ecosystem contributes to the net moistening of the atmosphere and hence become a moisture source for the downwind ecosystems (Mitsch and Hernandez 2013). Changes that affect wetland biodiversity and land cover therefore affect climate as well.

Wetlands are sometimes referred to as “landscape kidneys” because of the role they play in maintaining water and air quality (Mitsch and Gossilink 2000, Oliver *et al.* 2016). Macrophytes in wetlands obtain their mineral elements directly from water that flows over and through them. They take up nitrates, phosphates, potassium, calcium and magnesium and small amounts of other elements. As a result, water that passes through a wetland loses dissolved substances, most of which is what people deposit in water as waste. This water cleaning process is important because it prevents or minimizes problems such as eutrophication that would otherwise be caused by nutrient overload in downstream water systems (Mitchell 2013).

### Cultural services

Cultural services include non-material benefits that societies derive from ecosystems. These benefits may be spiritual, recreational, aesthetic, educational, cultural heritage or existence values (Assessment 2005). Many societies have their cultural and religious beliefs linked to ecosystem components such as trees, animals or rivers. These beliefs make the ecosystem components valuable and sacred (Mitsch *et al.* 2015).

### 1.3. Global extent of wetlands

The distribution of wetlands gives an indication of the distribution of ecosystem services derived from them especially at local level. It is therefore important to understand the distribution and extent of wetlands across different parts of the world. The global extent of wetland ecosystems is estimated to be 6 % of the Earth's terrestrial surface ( Junk *et al.* 2013, Russi *et al*. 2013). The major challenge in estimating global extent of wetlands is the variability in coverage estimations due to different definitions of wetlands and incomplete wetland inventories for some regions. For example, global information on peatlands, lakes, dams, major rivers and rice fields is variable and lacking for some inland wetlands and human-made wetlands (Assessment 2005). Table 1.1 presents the two best available estimates of global wetland area: the GRoWI assessment and the WWF/University Global lakes and Wetland Database.

**Table 1.1**: Estimates of Global Wetland Area by Ramsar regions

| *Region* | *1999 Global Review of Wetland Resources* ***(million hectares)*** | *2004 Global lakes and Wetland Database* ***(million hectares)*** |
|---|---|---|
| ***Africa*** | 121-25 | 131 |
| ***Asia*** | 204 | 286 |
| ***Europe*** | 258 | 26 |
| ***Neotropics*** | 415 | 159 |
| ***North America*** | 242 | 287 |
| ***Oceania*** | 36 | 28 |
| Total area | **~1,280** | **917** |

(Assessment 2005).

## 1.4. Extent and distribution of wetlands in Africa

Based on global estimates, African wetlands cover a minimum of 131million hectares (1.31 million km$^2$) (Table 1) within the continent's total land area of 30.3 million km$^2$, with a human population of 944 million people (Junk *et al.* 2013). A large part of African land is arid with low human density. Commonly, African wetlands are formed by large rivers with fringing floodplains and internal deltas (Junk *et al.* 2013). Most African wetlands occur between 15°N and 20°S (Figure 1.1). The unique and biodiverse wetlands in this location include: four major river systems (Nile, Niger, Zaire, and Zambezi); Lake Chad and wetlands of Inner Delta in Mali; the Rift valley lakes (Victoria, Tanganyika, Nyasa, Turkana, Mweru and Albert); the Sudd in Southern Sudan and Ethiopia; and the Okavango Delta in Botswana (Kabii 1996, Mitchell 2013). According to Tiega (2003) the assessment of a full range of African wetlands is made difficult by the inability of some African countries to complete national wetland inventories.

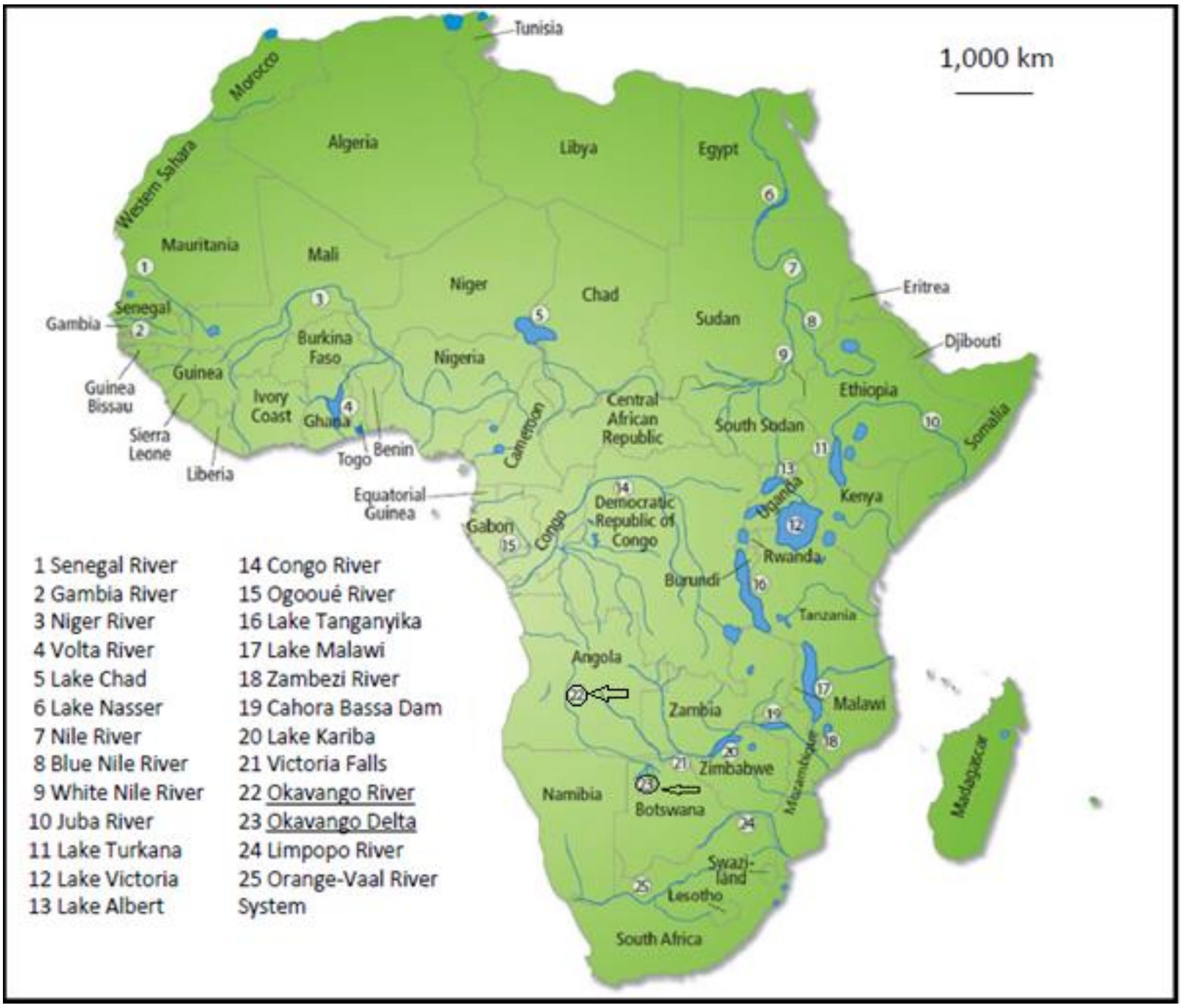


**Figure 1.1:** Distribution of major rivers and lakes in Africa (Van As *et al.* 2012). Okavango River and Delta highlighted.

## 1.5. Wetland classifications

Despite the challenges of incomplete wetland inventories in some African countries, many wetland classifications have been developed and used successfully. According to Scott and Jones (1995) the goals for wetland classification can be summarised as follows "**A**. To provide readily understood terminology for use in scientific research and conservation projects with an international dimension; **B**. To provide a framework for implementing international legal instruments for wetland conservation; **C**. To assist international dissemination of information to as many relevant individuals and organisations as possible".

Wetland classification systems can be divided into two broad approaches: those that are based on the geographical location of wetlands, and those that are not geographically based (US EPA 2002). The geographically based approaches attempt to reduce variability caused by spatial covariance with spatial variables such as climate and geology, topography, climax vegetation, hydrology, and soils. The non-geographically based approaches are based on the unifying wetland characteristics that determine a wetland status and vulnerability at a regional, watershed or ecosystem scale (Finlayson and Van der Valk 1995, US EPA 2002). The choice of a classification criteria often depends on a scientific, management or regulatory interest (Cowardin and Golet 1995)

The Ramsar classification system is a widely used non-geographical classification system that was developed to address the need for a simple global classification (US EPA 2002). The classification was adopted in 1990 following years of negotiation to improve the Ramsar Convention and was further improved in 1999 through Recommendation 4.7 and Resolution VI.5 respectively of the Convention. The system provides the contracting parties to the Ramsar Convention with a simple basis for describing and categorising and hence enabling sustainable management of the ecosystems (US EPA 2002). The Ramsar classification comprises three main wetland habitats: marine and coastal, inland, and human-made wetlands. These main categories are further subdivided into 42 wetland types (Table 1.2).

**Table 1.2**: Ramsar Classification of wetland types (Ramsar 2006)

***Marine/Coastal Wetlands***
A - Permanent shallow marine waters
B - Marine subtidal aquatic beds.
C - Coral reefs.
D - Rocky marine shores.
E - Sand, shingle or pebble shores
F - Estuarine waters
G -- Intertidal mud, sand or salt flats.
H -- Intertidal marshes
I -- Intertidal forested wetlands
J -- Coastal brackish/saline lagoons
K -- Coastal freshwater lagoons
Zk(a) – Karst and other subterranean hydrological systems
***Inland Wetlands***
**L -- Permanent inland deltas**.
M -- Permanent rivers/streams/creeks
N -- Seasonal/intermittent/irregular rivers/streams/creeks.
O -- Permanent freshwater lakes
P -- Seasonal/intermittent freshwater lakes
Q -- Permanent saline/brackish/alkaline lakes.
R -- Seasonal/intermittent saline/brackish/alkaline lakes and flats.
Sp -- Permanent saline/brackish/alkaline marshes/pools.
**Ss --** Seasonal/intermittent saline/brackish/alkaline marshes/pools**.**
Tp -- Permanent freshwater marshes/pools
Ts **--** Seasonal/intermittent freshwater marshes/pools
U -- Non-forested peatlands.
Va -- Alpine wetlands
Vt -- Tundra wetlands
W -- Shrub-dominated wetlands
Xf -- Freshwater, tree-dominated wetlands
Xp -- Forested peatlands
Y **--** Freshwater springs; oases.
Zg -- Geothermal wetlands
Zk(b) – Karst and other subterranean hydrological systems
***Human-made wetlands***
**1 --** Aquaculture **(e.g., fish/shrimp)** ponds
**2 --** Ponds (generally below 8 ha).
**3 --** Irrigated land
**4 --** Seasonally flooded agricultural land
**5 --** Salt exploitation sites
**6 --** Water storage areas
**7 --** Excavations

**8 --** Wastewater treatment areas
**9 --** Canals and drainage channels, ditches**.**
**Zk(c) –** Karst and other subterranean hydrological systems

The Okavango Delta, the study area for this PhD, is a biodiversity-rich seasonally flooded river-floodplain system and it belongs to category L (Permanent inland deltas) of the Ramsar wetland classes. Inland wetlands in category L include lotic systems (e.g. rivers, streams) and lentic systems (e.g. lakes) and their temporal dimension can vary from perennial to ephemeral (Revenga and Kura 2003). Category L wetlands flooded by a seasonal flood wave, are called flood-pulsed wetlands, and are especially ecologically dynamic because of the temporal variability of environmental conditions (Junk *et al.*1989). The following is an overview of biodiversity in inland wetlands ecosystems, providing a context on the level of biodiversity that drives wetland ecosystem functions and hence support ecosystem services that humans derive from the wetlands.

### 1.6. Biodiversity in inland wetlands

The combination of aquatic and terrestrial conditions increases structural diversity in flood-pulsed wetlands, promoting high biodiversity in the ecosystems (Mitsch and Gossenlink, 2000). Inland wetlands have a high level of endemic species diversity, likely due spatial isolation which limits species exchange with other ecosystems. For example, there are over 2000 freshwater fish species indigenous to African wetlands (Skelton 2001). East African Rift Valley lakes (Victoria, Tanganyika and Malawi) further demonstrate the high species endemism in inland wetlands; these lakes harbour hundreds of species of endemic fish, invertebrates and algae, many of which are endemic to a single lake or basin (Cohen *et al.* 2013). More than 700 endemic species of fish have been recorded in these lakes alone. In Lake Tanganyika, for example, 80% of cichlids are endemic. Previous studies have shown that 18 African wetlands rich in biodiversity contained 737 species of amphibians alone (Parish and Looi 1999). Effective management of these biodiversity-rich ecosystems, however, requires in-depth understanding of species interaction dynamics because an upset in the balance of inter-species relations can have huge impacts on the biodiversity levels. For example, the introduction of the Nile perch (*Lates niloticus*) in Lake Victoria in 1954, to boost fisheries, lead to drastic changes in inter-species interactions causing local extinction of over 500 species of cichlid fish (Mkumbo and Marshall 2015), perhaps the largest freshwater extinction event of the 20th century.

Despite the high species richness in wetlands, biodiversity in these systems is poorly known in comparison to terrestrial biodiversity (Parish and Looi 1999). Wetland biodiversity has often been confused with terrestrial life and this may have led to an underestimation of species biodiversity in wetlands (Gopal *et al,* 2000). For example, trees and shrubs that grow in the floodplains of the Amazon river system were for a long time categorized as terrestrial plants, despite the plants spending a larger proportion of time in flooded conditions. (Gopal *et al*, 2000). The importance of wetlands, however, remain evidenced by the considerable portion of landscape biodiversity which they support: freshwater habitats support at least 100,000 species out of the 1.8 million described species of the world (Dudgeon *et al*. 2006); the Prairie Pothole Region (PPR) of North America supports 50-80% of the continent's duck population (Junk 2013); in India, 20% of all biodiversity occurs in inland water systems, while 25% of all Malaysian plant species occur in peat swamps alone (Gopal *et al*. 2000); in African savannas, populations of large ungulates depend on wetlands, such as The Okavango Delta in Southern Africa, as sources of water during the dry season (Junk 2013).

## 1.7. Threats to African wetlands

The biodiversity and natural functioning of wetland ecosystems is faced by many threats, including human and climatic threats (Mitsch and Gossenlink 2000, Mitchell 2013). According to Junk *et al*. (2013) approximately 50% of (mainly inland) wetlands were lost between the year 1900 and 2000. Loss of wetland area means loss of key functions performed by wetlands, and consequently loss of ecosystem goods and services (Zedler and Kercher 2005). African wetlands are especially vulnerable because of general water scarcity and heavy dependence on wetland resources for livelihoods (Kabii 1996, Brouwer *et al*. 2014). Estimates of African wetlands loss ranges from 40% in Cameroon to 70% in Liberia (Chapman *et al.* 2008). There have been efforts to curb wetland loss through formulation of sustainable management strategies for wetlands (Turner *et al.* 2003, Moore *et al.* 2009) However, despite these ongoing efforts to conserve and restore wetland ecosystems there is still global net loss of wetland habitats (Keddy 2010, Davidson 2014). The following is an overview of some of the threats faced by African wetlands.

### Overexploitation

Overexploitation refers to harvesting or use of wetland resources at an unsustainable rate and often leads to ecological problems such as decline in species diversity, reduction of wetland area, and reduced ecological integrity (Lamsal *et al.* 2015). The importance of wetland resources as sources of livelihoods for nearby communities is widely recognised especially in Africa (Opio *et al.* 2011). The common livelihood activities in African wetlands are flood recession cultivation of crops, livestock grazing and fisheries (Mitchell 2013). However, a rapidly increasing human population in Africa, combined with widespread poverty across the continent, puts a lot of pressure on wetland resources (Kabii 1996 Mitchell 2013). According to CIA World Factbook (2011), 32 of the 50 countries with the highest growth rate of human population world-wide belong to the African continent. With such highly expanding human populations, overexploitation of wetland resources can be expected to intensify. Many African lakes have been highlighted as victims of intense hunting and fishing pressure including Lake Victoria, Bangweulu-Mweru, Inner Niger Delta, Niger Delta, Bight Coastal and Volta (Thieme *et al*. 2013). Effective regulation of these livelihood activities requires a sound understanding of wetland ecological dynamics. In the case of over-fishing, for example, a thorough understanding of the influence of potential regulating factors such hydrological factors (Gownaris *et al.* 2017), nutrient levels (Bachmann *et al*.2011), primary production (Hecky *et al*. 2011) and wetland biodiversity levels (Brooks *et al.* 2016), will be vital for sustainable fisheries management.

### Introduced invasive species

Introduction of exotic invasive species into African wetlands is another serious threat to the natural functioning of the wetlands (Thieme *et al.* 2005, Funk *et al*. 2014). The reasons for introduction of exotic species are variable. For example, fish introductions can be undertaken for fish culture (Canonico *et al.* 2005), recreational usage (Courtenay and Welcomme 2006, Hargrove *et al.* 2017) or to improve fisheries yields (Shechonge *et al.* 2019). When these introductions are made, potential negative ecological impacts are often overlooked (Thieme *et al.* 2005). However, changes in species composition caused by introduction of invasive species can result in serious ecosystem impacts such as change in habitat structure (Thieme *et al.* 2005), reduction of native species diversity (Tockner *et al.* 2008, Powell *et al.* 2013), changed nutrient cycling ( Liao *et al.* 2008, Ehrenfeld 2010) and changes to food web structure (Baxter *et al.* 2004, Matveev and Robson 2014).The loss of more than 200 cichilid species from Lake Victoria in 1953 due to introductions of the Nile perch to boast fisheries yield is a classic example of the negative effects of fish introductions (Ogutu-Ohwayo 1990). Introduced aquatic weeds in African wetlands include water hyacinth (*Eichhornia crassipes*) and the Kariba weed (*Salvinia molesta*) (Thieme *et al.* 2005, Hill and Coetzee 2017)). In Botswana, the Department of water affairs is using the weevil *Cyrtobagous slviniae*, which feeds on the weed, to biologically control the weed in the Okavango Delta ( Naidu *et al.* 2000, Kurugundla *et al.* 2016)

### Changes in flooding regimes

The most serious threats faced by flood-pulsed wetlands are those that alter the natural flow regimes (Tockner and Stanford 2002, Wang and Wang 2016). This is because natural flow regimes are the key driver to ecosystem structure and function of the seasonally flooded wetlands (Bunn and Arthington 2002). Flow regimes determine the physical habitat which in turn determines aquatic community composition and life history strategies of aquatic life (Bunn and Arthington 2002). Alteration of flow regimes reduces flood peaks, flooding frequency and duration, and lateral connectivity between seasonal flood plains and perennial channels (McMahon and Finlayson 2003a; Poff and Zimmerman 2010). Climate change and water impoundments are the most serious factors threatening the natural flow regimes of African wetlands (Mitchell 2013), and each are highlighted below.

## Climate change

The world is warming mainly due increasing anthropogenic production of greenhouse gases (IPCC 2007). Most climate models predict a global warming of 1.4 to 5.8°C from 1990 to 2100 (Oyebande, 2013, .IPCC 2013) In most parts of Africa, temperature is expected to increase by about 2°C in the next two decades (Mitchell 2013). In addition to the expected temperature rise, dry spells are expected to be more frequent in Southern Africa because of climate change (IPCC 2014). The likely effects of increased atmospheric temperatures and frequent dry spells is reduced surface water availability, especially because evapotranspiration is expected to increase by 5-10% (IPCC 2014). The expected rise in temperatures and reduced frequency of rainfall events are likely to lead to reduction in flooding extent and duration in wetlands. In river floodplain systems such as the Okavango Delta, reduction in surface water will reduce the extent of floodplain inundation impacting strongly on metacommunity dynamics and the overall ecological functioning of such systems. African lakes can also be expected to be severely affected by future climate change because of their high sensitivity to climate change (Chapman *et al.* 2008). Precise predictions of ecological responses to such climatic effects will depend on knowledge of baseline relationships between hydrology and wetland ecological functioning.

## Water impoundments

Water impoundment means redirecting water from its natural water course into a confined space such as a dam. African wetlands are heavily impacted by impoundments, for example in South Africa more than 1,200 dams with a total capacity of more than 50,000 $m^3$ were built between 1980 and 1990 (Mitchell 2013). Impoundments are the most serious and growing threat to the ecological integrity of river-floodplain ecosystems (Tockner *et al.* 2008). In floodplain systems, biological production depends on the extent of floodplain flooding (Junk 1989, therefore reduced flooding from upstream impoundments can reduce floodplain productivity and biodiversity levels. Impoundments also trap sediments and associated nutrients and therefore disrupting benthic habitats and reducing nutrient levels at the downstream parts of a wetland (Mitchell 2013). In Lake Naivasha in the East Africa rift valley, low water levels resulting from water abstractions for irrigation has led to high silt load, degradation of *Cyprus papyrus* vegetation, and reduced wetland production (Kafumbata *et al.* 2014). While impoundments may increase water reliability, they alter the natural flooding regimes and may reduce the ecosystem services that surrounding communities derive from wetlands.

## 1.8. Conclusions

This chapter has demonstrated that wetlands critically support human existence on earth because of the high level of biodiversity that they support, which in turn support important ecosystem processes that yield valuable goods and services to humans. However, the wetland ecosystems are faced with many threats that necessitate an in-depth understanding of the dynamics ecosystem processes as this will support formulation of effective management policies that safeguard wetland ecosystems against the potential threats. This chapter has also demonstrated that in flood pulsed wetlands, periodic flooding is the main driver of ecosystem functions and hence the relationship between hydrology and wetland process is crucial to understand in the ecosystems. The following chapter, Chapter two, discusses wetland ecosystem properties, with a focus on flow of energy (organic matter) and wetland food web (pathways of energy transfer) dynamics and the use of stable isotope techniques to study wetland food webs.

# CHAPTER TWO: Wetland food webs and the use of stable isotope analyses (SIA) in freshwater food web studies

The objective of this chapter is to provide a theoretical background that is necessary for interpreting subsequent chapters that are based on SIA data generated during this PhD research. The discussions in this chapter will focus on flow of organic matter in wetlands, controls of aquatic wetland food structure in wetlands and the use of the stable isotopes analysis (SIA) approach to study food web dynamics in wetlands. The reasons for choosing the SIA approach as well as natural variability of the selected isotopes are also discussed. The chapter concludes with a critical overview of SIA mixing models, which are increasingly common statistical approaches used to undertake quantitative analyses of freshwater food webs.

## 2.1. Flow of organic matter in wetlands

Within a wetland ecosystem, organic matter is transferred across biodiversity components through feeding relationships among wetland organisms. The complex feeding relationships, that span from plants and other photosynthetic organisms to top predators, are called food webs. These feeding relationships can be tracked by carbon because organic matter contains between 45% and 50% carbon (Kayranli 2010). There are four main forms of carbon that constitute basal carbon pools of wetland food webs: 1. aquatic plants and algae; 2. seston; 3. dissolved organic carbon (DOC), and 4. Inorganic carbon, including dissolved inorganic carbon (DIC) from gaseous end products mainly carbon dioxide and methane (Figure 2.1). The carbon pools can be used to summarise the flow of carbon through wetland ecosystems. Aquatic plants and algae represent active (live) carbon pools that participate in the conversion of DIC to organic carbon through the process of photosynthesis. Seston and dissolved organic matter (DOC) are non-living carbon pools. Seston includes decaying pieces of matter e.g. from plants, algae and microbial cells. Dissolved organic matter (DOC) includes carbon components dissolved in water. Microorganisms mediate processes such as respiration and methanogenesis, that convert organic carbon back to inorganic carbon as well as mineralization of organic carbon (D’Angelo and Reddy 1999, Truu *et al.* 2009) . The basal carbon pools act as energy stocks that support wetland biodiversity because they represent the organic matter that is ultimately consumed by different wetland animals.

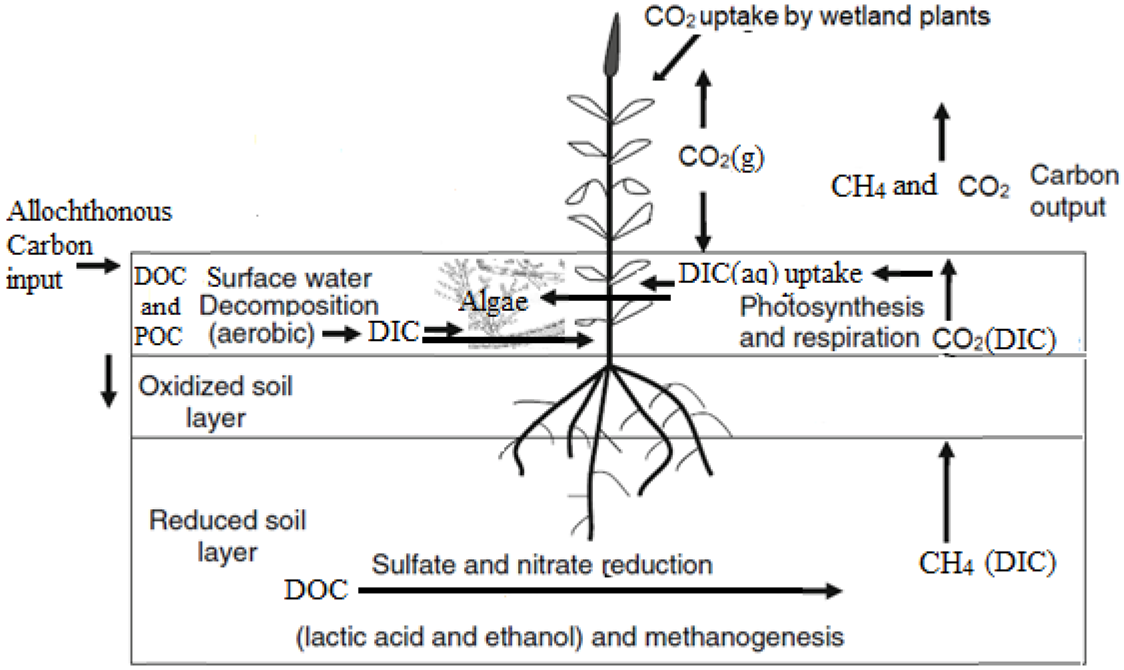


**Figure 2.1:** Different forms of basal carbon sources for wetland ecosystems (Kayranli *et al.* 2010). The arrows indicate the conversion of one form of basal carbon to another through the biological processes indicated.

There are three widely tested theoretical models predicting the relative importance of the basal carbon pools and energy flow dynamics in large river wetlands. These models are the River Continuum Concept (RCC) (Vannote *et al.* 1980), the Flood Pulse Concept (FPC) ( Junk *et al.* 1989) and the Riverine Productivity Model (RPM) (Thorp and Delong 1994). An overview of each is given below.

The RCC (Vannote *et al.* 1980) is the oldest of these models and it predicts that the bulk of basal carbon that supports the aquatic food webs originates from upstream catchments (Fig. 2.2 a). The model predicts that organic matter gradually changes from coarse particulate matter to finer particles in the downstream direction due to biological processing by correspondingly organised functional groups of aquatic organisms. According to this model, the functional groups of aquatic invertebrates along the upstream-downstream axis progressively changes from upstream to downstream such that organisms that break down larger particles (e.g. leaf shredders) occupy the upstream reaches, whereas those that process the finer particles (e.g.

filter feeders) occupy the downstream reaches. The functional organisation postulated by this model allows for efficient processing of organic matter originating from upstream catchments.

Further studies in riverine systems emphasized the importance flood plains as sources of organic matter and the importance of the seasonal floods in mobilising the organic matter to the perennial channels, which in turn led to the development of the Flood Pulse Concept (FPC) (Junk *et al*. 1989) (Fig. 2.2b). The FPC can be described as a cycle of the seasonal flood wave that overflows perennial river channels to flood riparian plains, and a gradual drying of the floodplains until a subsequent flood wave. The riparian flood plains gradually dry mainly because of evaporation and transpiration. The seasonal flooding and subsequent gradual drying in the flood plains results in a diversity of aquatic habitats which include (i) perennial channels (ii) permanently inundated lentic habitats in depressions (iii) seasonally inundated floodplains wetlands and (iv) floodplains at higher elevations that receive only short-term and sporadic inundation during extreme floods (Naiman and Decamps 1997, Lamberti *et al.* 2010). The periodic hydrological linking and disconnecting of floodplains and main channels, causes changes in the physical, chemical and biological features of these aquatic habitats (Junk *et al.* 1989, Bortolini *et al.* 2014) resulting in a remarkable level of spatial and temporal ecological heterogeneity (Junk *et al.*1989, Tockner and Stanford 2002). According to the Flood Pulse Concept, the bulk of the energy that supports river-floodplain food webs originates from floodplains. The annual flood pulse conveys organic matter and nutrients from floodplains to the perennial habitats (Junk *et al*. 1989, Mladenov *et al.* 2005). Seasonal floodplains are a source of nutrients because the alternation between dry and wet phases in soils promotes high nutrient turnover in the habitats (Baldwin and Mitchell 2002, Olde Venterink *et al.* 2002).

Thorp and Delong (1994) developed the Riverine Productivity Model (RPM) based on the observation that the RCC and FPC models best explained energy flow dynamics in small to medium seasonally flooded riverine systems and were less suitable for large, irregularly flooded rivers (Fig.2.2b). They argued that in large rivers with irregular flooding patterns, in-stream production and riparian production are the main source of energy for supporting aquatic food webs. The RPM model postulates that carbon from instream and riparian production is often preferred by aquatic secondary producers as it is more palatable compared to that of particulate organic matter from upstream vegetation and floodplain sources. Based on the RPM model, aquatic primary production is greater in large rivers because there are fewer tree canopies relative to aquatic surface, allowing more sunlight to support aquatic primary producers than in small rivers where tree canopies often overhanging much of the aquatic surface (Cotner *et al*. 2006, Douglas *et. al* 2005, Thorp and Delong 2002).

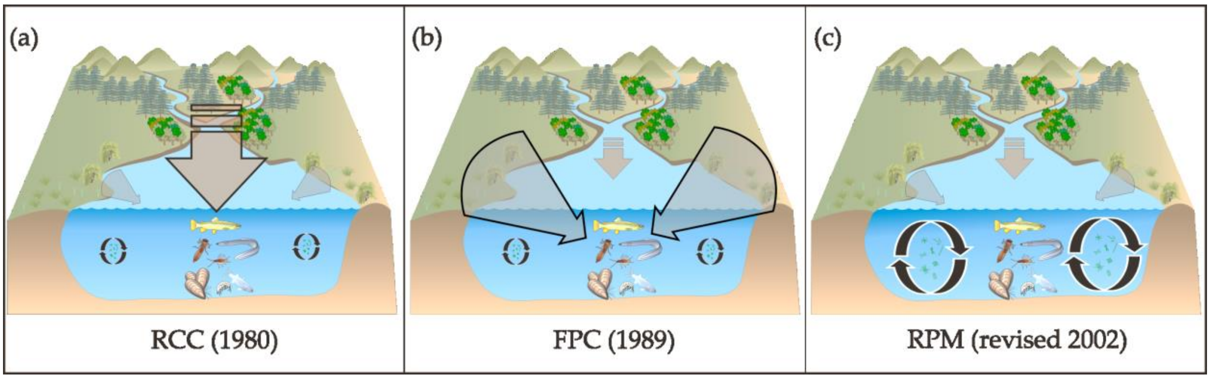


**Figure 2.2**: Three main models explaining energy dynamics in riverine aquatic systems (Pingram *et al.*2014): (a) the River Continuum Concept (RCC); (b) the Flood Pulse Concept (FPC); (c) the Riverine Productivity Model (RPM). The arrows indicate the direction of carbon flow with the size of the arrow indicating the diversity of carbon sources.

Of the three theoretical models above, the energy flow and food web dynamics of the Okavango Delta, can be expected to be best explained by the FPC. This is because the Delta has extensive flood plains and it is flooded on a seasonal basis; the exact phenomenon that FPC predictions are based on. The Delta therefore provides a good opportunity to test aspects of the FPC model.

## 2.2. Wetland food webs

Energy (organic matter) from basal carbon pools is transferred from the carbon pools to other wetland organisms through a network of feeding relationships referred to as food webs (Woodward and Hildrew 2002, Winemiller and Polis 2013). Food web structure (FWS) represents routes followed by energy from primary producers to top predators (Winemiller and Polis 1996, Woodward and Hildrew 2002). The idea that organisms within ecosystems are organised into food webs is well established in ecology (Elton 1927, MacArthur 1955) and has been crucial in advancing ecological knowledge on several subjects such as ecosystem community structure (Kareiva 1985, Moore and Hunt 1988), environmental change and ecosystem function (Winemiller 1990,Schoenly and Cohen 1991). FWS is a critical ecosystem feature because it determines functional attributes such as population stability (Elton 1927, Hansson *et al.* 2013), primary and secondary production (Winemiller *et al.* 2014), material cycling (DeAngelis *et al*. 2002) and contaminant bioaccumulation (Cabana 1994, Tomczyk *et al*. 2018). Because of its strong influence over ecosystem functions, FWS has implications for ecosystem responses to climate change, human perturbations and wetland management practices (Cohen *et al.* 1990, Winemiller 1990, Winemiller *et al.* 2014). A good understanding of the relationship between FWS and environmental factors, can therefore improve prediction of how changes in the environment, such as those that may be caused by climate change and human perturbations, will impact on FWS and ultimately ecosystem functions.

The structure of aquatic food webs in river-floodplain systems is known to be dynamic and varying over different spatial and temporal scales (Woodward and Hildrew 2002). At a regional scale, dispersal ( Pillai *et al.* 2011, Mougi and Kondoh 2016), spatial subsidies ( Holt 2002, Gouhier *et al.* 2010) and landscape heterogeneity (Baiser *et al.* 2013) can influence the structure of food webs. The arrival of a new species into a local food web from a regional species pool can have various effects on the local food webs. For example, the arrival of a new species can mean arrival of new prey resources (expanding the feeding niche) whereas for some species the arrival of the same species can mean the arrival of a new competitor for food resources. Regional subsidies such as the inflow of terrestrial organic matter into a freshwater ecosystem increases the diversity of resources at the base of the local food webs (Holt 2002, Gouhier *et al.* 2010).

A stronger influence on ecosystem FWS is however known to be exerted by local biotic and abiotic factors (Baiser *et al*. 2013). The most important biological interactions shaping food webs are competition and predation (Baiser *et al*. 2013, Winemiller *et al*. 2014). Competition for limited resources can result in local species loss, thereby re-arranging the feeding links. Predation pressure can exert profound top-down trophic cascades that can restructure the entire ecosystem food web (Peterson *et al*. 2008, Kneitel and Miller 2002).

Habitat characteristics such as habitat size and primary productivity have been shown to influence FWS by determining species composition and diversity (Holt *et al*. 1999, Baiser *et al*. 2013). Primary production is one of the factors whose influence on FWS is most supported by empirical evidence (Post 2002, Vander Zanden and Fetzer 2007, Takimoto *et al*. 2012). Primary production is known to determine the number of trophic levels within a food web due to the inefficiency with which energy produced by primary producers is transferred to higher trophic levels. The efficiency of energy transfer between trophic levels, ranges from 2 to 50% (Christensen and Pauly 1995), hence it limits the number of trophic levels in an ecosystem. Food webs of highly productive ecosystems are therefore expected to have more trophic levels (longer food chains) than less productive systems. Ecosystem size is another important factor believed to strongly influence FWS. Larger ecosystems provide greater ecosystem space, hence more trophic niches and refuge areas which stabilise trophic interactions (Post *et al.* 2000). Larger ecosystems therefore support a greater diversity of feeding pathways compared to smaller ecosystems (Post *et al.* 2000).

Disturbance regime is one of the local factors also known to influence FWS (Takimoto *et al.* 2012). Disturbance refers to a discrete event that results in disruption of the ecosystem processes, community structure and resource availability (White and Pickett 1985, Banks *et al.* 2013, Attard *et al.* 2018). Based on theoretical models (Pimm and Lawton 1977), longer food chains have been shown to be less resilient to disturbance events, therefore ecosystems that experience a high frequency of disturbance events have shorter food chain lengths (Sabo *et al.* 2010, Takimoto *et al*. 2013). Examples of disturbance include unpredictable and destructive events such as drought (Ledger *et al.* 2012). However, regular and predictable events which do not involve high mortalities, such as a seasonal flood wave in floodplain systems, may not be viewed as a disturbance event because floodplain organisms have evolved adaptive life history strategies that synchronise with the timing and duration of the floods (Winemiller 2014).

The concept of FWS has certainly advanced our knowledge of wetland food webs and ecosystem functions, especially in relation to environmental factors. However, the knowledge advancement has been made possible by development of scientific methodologies which enabled field and laboratory testing of ecological predictions based on the concept of FWS. The SIA approach to studying FWS, which is currently the widely used approach and the approach chosen for my PhD study, is discussed in the following section.

## 2.3. The SIA approach to studying FWS

### What are stable isotopes

Stable isotopes are two or more non-radioactive forms of an element with differing atomic mass due to unequal neutron numbers (Oulhote 2011, Perkins *et al.* 2014). Therefore, for elements with isotopes, there are light and heavy isotopes of which the former are the most abundant ones. In the case of carbon isotopes, the relative abundance of the light carbon isotope ($^{12}C$) is 98.89% whereas the heavier isotope ($^{13}C$) has an abundance of only 1.11% (West *et al.* 2006). Despite differences in atomic mass, stable isotopes show similar properties such that they can replace each other in chemical and biochemical reactions. This is because the atomic mass difference is caused by differences in the number of neutrons, which do not actively participate in chemical reactions (Peterson and Fry 1987). Due to their greater weight, heavy isotopes diffuse relatively slowly and hence react relatively slowly, resulting in end products of biochemical reactions having a greater ratio of light isotopes than heavier isotopes; a phenomenon called isotopic fractionation (Tiunov 2007, Perkins *et al.* 2014). Biological and geochemical processes involve fractionation, and consequently there are patterns of isotopic composition between materials within an ecosystem and between ecosystems.

The relative proportions of stable isotopes in natural materials varies within very small ranges. This variation is expressed as per mil (‰) deviation of the ratio of isotopes in a sample from the ratio of the isotope in an international standard (i.e. delta (δ) notation):

$\delta^{n}E = [(R_{sample}-R_{standard}/(R_{standard}))]*1000$ which is equal to: Equation 2.1

$\delta^{n}E = (R_{sample}/R_{standard}-1)*1000$

where E is the element such as carbon (C) or nitrogen (N), *n* is the atomic mass of the heavier isotope and R is the ratio of heavy to light isotopes (Equation 2.1).

### Use of stable isotope in ecological studies

The ratio of stable isotopes have been found to vary in predictable patterns between natural materials and this predictable variation has been used in important scientific investigations such as animal movement and migration patterns (Hobson 1999, Rubenstein and Hobson 2004, Hoffman 2016), resource partitioning (Jackson *et al.* 1995, Young *et al.* 2010, Pool *et al*. 2017) and material fluxes in the biosphere (Peterson and Fry 1987, Roach and Winemiller 2015). The focus of this chapter is on the use of SIA on food web studies (Peterson and Fry 1987, Post *et al*. 2002, Layman *et al.* 2012).

The most commonly used isotopes in food web studies are of carbon (C) and nitrogen (N), even though sulphur (S), oxygen (O) and deuterium (D) may be useful in some cases (Oulhote *et al.* 2011). The ratio of a heavier carbon isotope ($^{13}C$) to a lighter one ($^{12}C$), expressed as $\delta^{13}C$, varies considerably among types of primary producers such as plants with different photosynthetic pathways ($C_3$ and $C_4$ plants) but changes very little across feeding hierarchies of consumers depending on the same primary producer. Trophic ecologists therefore use $\delta^{13}C$ for tracking original sources of dietary carbon (Gladyshev 2009, Perkins *et al.* 2014). The relative proportion of nitrogen isotopes ($^{15}N$ to $^{14}N$), expressed as $\delta^{15}N$, is used to estimate trophic position of organisms because it increases with each trophic transfer, resulting in consumers ranking high in the feeding hierarchy having greater $\delta^{15}N$ values (Gladyshev 2009, Perkins *et al.* 2014). General patterns of $\delta^{13}C$ and $\delta^{15}N$ are now known for different groups of organisms and different ecosystem types, and food web studies benefit from these known patterns in tracing carbon sources and examining the structure of food webs.

The SIA approach has been used in several subdisciplines of food web ecology. The SIA approach is often used to examine resource partitioning (Genner *et al.* 1999, O'Brien *et al.* 2000, Waas *et al.* 2010, Lazada *et al.* 2017, Pool *et al.* 2017), to assign species to trophic positions (Minagawa and Wada 1984, Fry 1991, Post 2002, Hoenghaus and Zeug 2008) and to construct diets (Minson *et al.* 1975, Tieszen et al 1983, Samelius *et al.* 2007). The application of SIA approach in many ecological disciplines indicates high significance in ecological studies.

The SIA approach offers several advantages to food web studies over traditional methods, such as gut content analyses and behavioural observations. The SIA approach can reveal integrated diet over temporal scales ranging from days to years depending on the tissue selected for analyses (Hesslein *et al.* 1993, Phillips and Eldridge 2006, Lazada *et al.* 2017). On the other hand, conventional methods reveal recently ingested diet and no information about its incorporation into consumer tissue. Visual examination of gut contents or faecal matter yields dietary information of recently consumed prey and may not accurately reflect the relative importance of prey items to the consumer (Votier *et al.* 2003). Retention times and digestibility of prey varies, therefore evaluating the importance of dietary items based solely on the rate of their ingestion can lead to inaccurate conclusions about diet of a consumer (Bearhop *et al*. 2004). Identification of partially digested prey items may also be erroneous (Bearborp *et al.* 2004). Other conventional methods such as behavioural observations can also yield erroneous data because some organisms can feed noticeably on one prey but feed unnoticeably on other (Caunt *et al.* 2008). However, while SIA quantifies dietary niches better than traditional methods (Layman *et al*. 2012), the approach may not yield other useful ecological information such as feeding behaviour of an organism, which may be required for habitat restoration for an endangered species (Lindell 2008). Therefore, depending on the ecological information sought, the two approaches can be complementary.

The usage of SIA approach food web research is based on known patterns of variability in values of stable isotopes between different environments (e.g. terrestrial vs aquatic), prey resources (e.g. $C_3$ vs $C_4$ plants) and consumers (e.g. herbivore vs carnivore). The following sections discusses drivers of isotopic variation in different environments and in organic matter.

## Controls of $\delta^{13}C$ values in freshwater

Whereas terrestrial primary producers fix carbon from atmospheric $CO_2$, aquatic primary producers fix carbon from dissolved inorganic carbon (DIC). Consequently, the carbon isotope ratios of organic matter from aquatic photosynthesis is determined to some extent by the isotope ratios of the fixed DIC. The carbon isotopic composition of DIC is a function of relative abundance of dissolved inorganic carbon species: $CO_2$ (aq), $H_2CO_3$, $HCO_3^-$, and $CO_3^{2-}$ (Equation 1).

$$\begin{array}{lllllllll} & & & & \text{Carbonic acid} & & \text{Bicarbonate} & & \\ CO_2\,(g) & + & H_2O\,(aq) & \leftrightarrow & H_2CO_3\,(aq) & \leftrightarrow & HCO_3\,(aq) & + & H^+\,(aq) \\ & & & & & & \updownarrow & & \\ & & & & & & \text{Carbonate} & & \\ & & & & & & CO_3^{2-}\,(aq) & + & 2H^+\,(aq) \end{array} \qquad \textbf{Equation 1}$$

The dominant forms of DIC that occur in the pH range of freshwater are $CO_2$ and $HCO_3^-$, with the former being more abundant in water pH less than 7 and the latter being more abundant in water pH greater than 7 (Fig. 2.3). DIC that is dominated by $CO_2$ has distinctively lower $\delta^{13}C$ values than that is mainly composed of $HCO_3^-$ because formation of $HCO_3^-$ involves greater fractionation of heavier carbon isotopes (Cerling *et al.* 1991). It has been determined experimentally that most of fractionation occurs during the dissociation of $CO_2$ to $HCO_3^-$, hence dissolved $CO_2$ is about 9‰ less enriched than $HCO_3^-$ (Mook *et al.* 1974). Average $\delta^{13}C$ values of freshwater DIC range from 27 ‰ to 0‰, representing DIC derived from oxidation of $C_3$ organic matter and DIC derived from dissolution of limestone rock respectively (Aucour *et al.*1999, Leng and Marshall 2004*)*

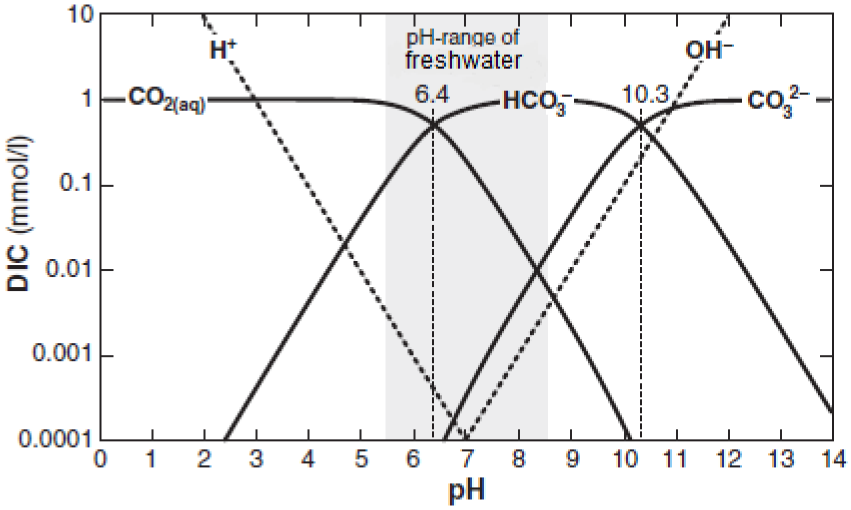


**Figure 2.3:** Speciation of DIC as a function of water pH. The dominant DIC species within the pH range of freshwater are dissolved carbon dioxide ($CO_2$) and carbonates ($HCO_3^-$). (Schulte *et al.* 2011)

### Controls of SIA values in the aquatic environment of lentic ecosystems

In lentic freshwater systems (i.e., lakes and ponds), $\delta^{13}C$ values of DIC ($\delta^{13}C_{DIC}$) are influenced by gas exchange, aquatic metabolism (Mariotti 1991, Leng and Marshall 2004) and dissolution of mineral carbonates (Aucour *et al.* 1999, Cerling *et al.* 2015). The $\delta^{13}C_{DIC}$ values of lentic ecosystems at a given time are determined by the relative influence of these factors.

**Gas exchange**: The flux of atmospheric $CO_2$ into lakes involves fractionation of carbon isotopes during the diffusion of $CO_2$ across the air-water boundary and during dissociation of the $CO_2$ to $HCO_3^-$. Fractionation of carbon isotopes at the air-water interface yields $\delta^{13}C$ ranging from 8 to 6‰ ( Cerling *et al.* 1991, Bade *et al.* 2004) whereas $\delta^{13}C$ of the corresponding $HCO_3^-$ is about 13‰ (Herczeg and Fairbanks 1987). Therefore, air invasions into water contributes to the variability of $\delta^{13}C$ in lentic ecosystems.

**Aquatic metabolism**: Relative rates of aquatic photosynthesis and respiration influence seasonal and inter-lake variation of $\delta^{13}C_{DIC}$ in lakes. $^{12}C$ is preferentially fixed during aquatic photosynthesis, and consequently increases the relative proportion of $^{13}C$ in the remaining DIC.

At high photosynthetic rates, rapid uptake of dissolved $CO_2$ also has the effect of increasing water pH, which in turn increases the relative proportion of $HCO_3$ (see Fig 2.3) resulting in a more $^{13}C$ enriched DIC (Bade and Cole 2006). The effect of aquatic respiration on DIC depends on the origin of the organic matter being respired. Since most aquatic producers use the $C_3$ pathway, respiration of detritus from the autochthonous organic matter will yield $CO_2$ with $\delta^{13}C$ ratios of about –26‰. Dissolution of respired $CO_2$ from terrestrially derived organic matter yields $\delta^{13}C_{DIC}$ values ranging from –26 to –9‰ depending on the relative proportions of $C_3$ and $C_4$ plants in it (Mariotti, 1991). Organic matter derived from terrestrial $C_3$ plants has $\delta^{13}C$ values ranging from –20 to –30‰ whereas organic matter from terrestrial $C_4$ plants ranges from –17 to –9‰ (Leng and Marshall 2004).

Methane production (methanogenesis) and methane oxidation in lakes have different influences on lake DIC and are dependent on dissolved oxygen levels. Methanogenesis occurs in anaerobic sediments (Fig. 2.4c) and anoxic overlaying water (Fig 2.4b) during lake stratification through microbial fermentation of acetate (Ward *et al.* 1978, Oh and Martin 2016) and reduction of $CO_2$ using $H_2$ as source of electrons (Kim *et al.* 2015). Due to differential fractionations of the enzymes involved in the methanogenesis pathways, the $\delta^{13}C$ value of methane produced through acetate fermentation is –36‰ whereas the $\delta^{13}C$ of methane produced through $CO_2$ reduction ranges from –77 to –60‰ (Sugimoto and Wada 1993). When methane is exposed to aerobic conditions in the water column (Fig 2.4 a), such as during enough mixing in lakes, methanotrophic bacteria oxidise methane to $CO_2$ (Jones and Grey 2011). The $\delta^{13}C_{DIC}$ dominated by $CO_2$. derived from methane oxidation has distinctively more negative $\delta^{13}C$ values, resembling the $\delta^{13}C$ values of the substrate $CH_4$ (Whiticar 1999, Nykänen *et al.* 2014).

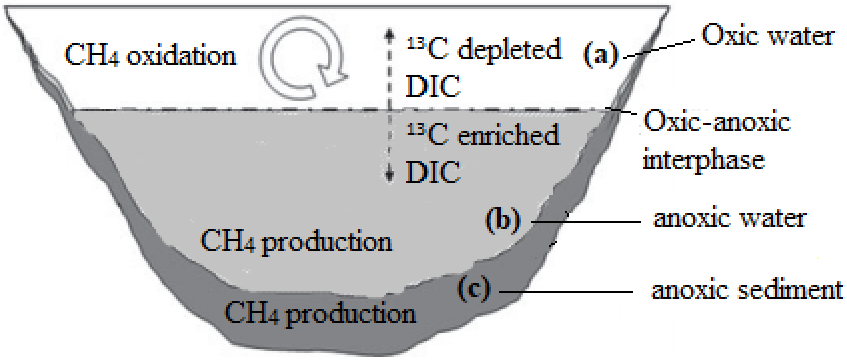


**Figure 2.4:** A schematic diagram of cross section of a stratified lake, showing the anoxic zones of methane production and oxidation and the effect of the processes on DIC values at the respective sections of the lake. (re-drawn from Jones and Grey 2011)

**Dissolution of mineral carbonates**: Dissolution of mineral carbonates from weathering carbonate bedrocks can increase $\delta^{13}C_{DIC}$ values of freshwaters because the carbonate rocks are highly enriched in $^{13}C$ δ13C pattern of dissolved inorganic carbon in a small granitic catchment: the Strengbach case study (Amiotte-Suchet *et al.* 1999). The $\delta^{13}C_{DIC}$ values in freshwater typically varies between +1 and 1‰ (Swart 2015).

### Controls of $\delta^{13}C$ in the aquatic environment of lotic ecosystems

The DIC in lotic (river) systems is derived from two major sources, namely riverine metabolic processes (biogenic DIC) and weathering of carbonate containing minerals (Ancour *et al.* 1999, Cerling *et al.* 2015) (Fig 3.4). Biogenic DIC is derived from autotrophic respiration or respiration of allochthonous organic matter and has a typical $\delta^{13}C$ value of –27‰ in landscapes dominated by $C_3$ plants (O'Leary 1998). Biogenic DIC in soil moisture has higher $\delta^{13}C$ values (up to 4‰ greater than $\delta^{13}C$ from aquatic respiration) due to greater interaction with atmospheric $CO_2$ (Ceiling 1991). DIC derived from weathering of carbonate rocks has higher $\delta^{13}C$ values which typically average 0‰ (Clark and Fritz 1997), but when the carbonate rocks are dissolved by carbonic acid made from soil derived $CO_2$, the isotopic mixture from carbonate dissolution is about 12‰ (Clark and Fritz 1997). The DIC from carbonate dissolution can even have higher $\delta^{13}C$ values when the carbonate rock is dissolved by a non-carbon-based acid such as sulphuric acid during acid rain (Li *et al.* 2010). The relative contribution of these different sources influence variability of $\delta^{13}C$ in lotic systems.

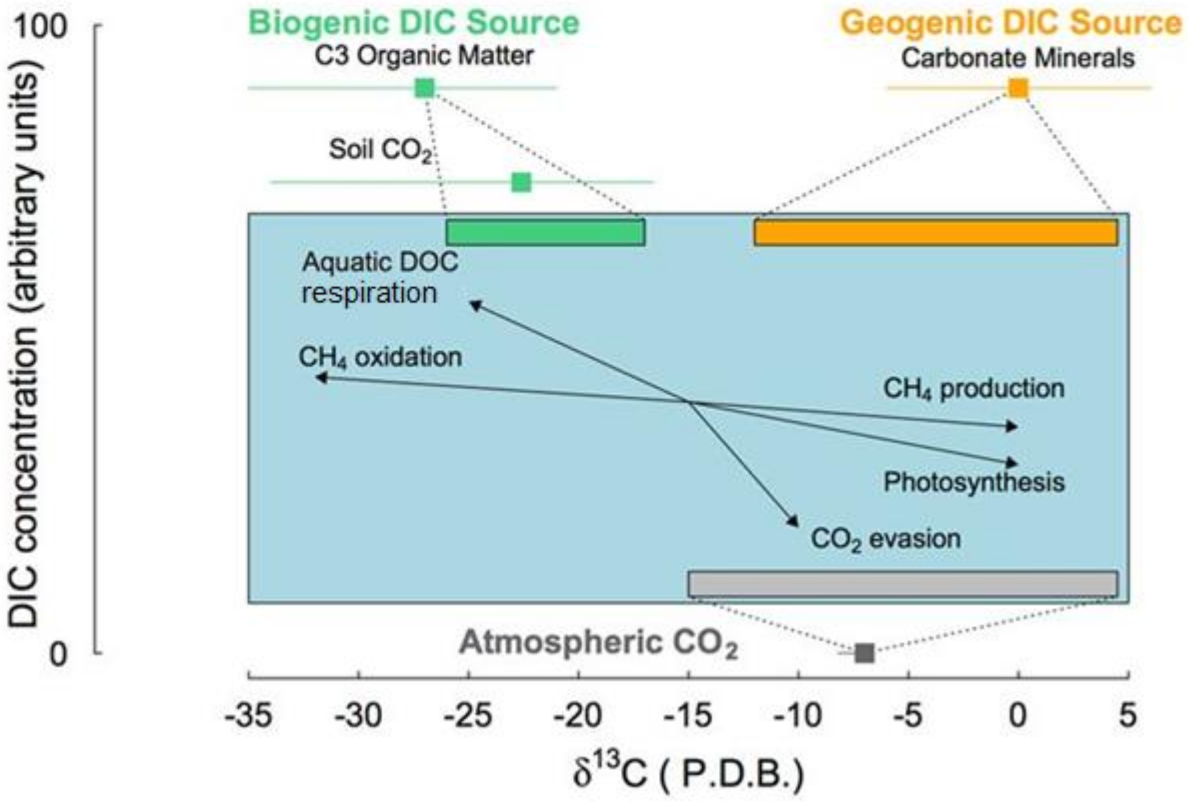


**Figure 2.5:** A conceptual illustration of biogeochemical processes controlling δ13$C_{DIC}$ values in lotic systems (Campeau *et al.* 2017). On the x-axis are the reported range of $\delta^{13}C_{DIC}$ values in lotic systems and on the y-axis is a gradient in DIC concentration with arbitrary units.

The values of $\delta^{13}C_{DIC}$ in lotic systems tend to increase in the downstream direction in response to several factors including $CO_2$ degassing, aquatic photosynthesis, carbonate dissolution and organic matter decomposition (Tamooh *et al.* 2013, Telmer and Veizer 1999, Zou 2016). Carbon dioxide partial pressure ($pCO_2$) in the atmosphere is low compared to $pCO_2$ in many rivers (Aucour *et al*. 1999, Mayorga *et al.* 2005, Hotchkiss *et al*. 2015) therefore exposure of river water to air results in degassing (evasion) of $CO_2$. The loss of $CO_2$ reduces DIC concentration in the river while increasing the proportion of $^{13}C$ in DIC. The loss of $CO_2$ also raises water pH, thereby increasing carbonate dissolution. Riverine photosynthesis uses up $CO_2$ in water and it has the same effect as $CO_2$ degassing. In some cases, spatial variability in $\delta^{13}C_{DIC}$

values can be influenced by anthropogenic inputs of nitrates and phosphates which act through elevating riverine photosynthesis rates (Flintrop *et al.* 1996).

Several studies have demonstrated the influence of riverine geochemistry on the downstream increase of $\delta^{13}C_{DIC}$ in rivers. In a turbulent high-altitude tributary of Tana River in Kenya, the Satima springs, a strong downstream increase of $\delta^{13}C_{DIC}$, which co-varied with partial pressure of $CO_2$ ($pCO_2$), was attributed to rapid degassing of $CO_2$ (Tamooh *et al.* 2013). Still within the Tana River system, a downstream increase of $\delta^{13}C_{DIC}$ and suspended organic matter were observed indicating that respiration of the organic matter influenced the downstream increase in $\delta^{13}C_{DIC}$ values (Tamooh *et al.* 2013). In Lagan River, Northern Ireland, downstream increase in water pH influenced downstream increase in limestone dissolution and consequently increased $\delta^{13}C_{DIC}$ values in the downstream direction (Barth *et al.* 2003). The Ottawa River in Canada showed downstream increase in $\delta^{13}C_{DIC}$ values and the trend was also ascribed to downstream increase of carbonate dissolution (Telmer and Veizer 1999). According to Finlay (2003), in small streams $\delta^{13}C_{DIC}$ is controlled primarily by outgassing (evasion) of excess $CO_2$, and microbial respiration primarily control $\delta^{13}C_{DIC}$ whereas in large rivers algal photosynthesis and $CO_2$ exchange with the atmosphere are more important.

Flood pulsed river-floodplain ecosystems show strong seasonal $\delta^{13}C_{DIC}$ variation because of seasonal changes in the extent of connectivity between river channels and their surrounding flood plains. The flooding stage determines the effects of factors that influence $\delta^{13}C_{DIC}$ values including primary production rates, proportion of ground water relative to discharge volume, and decomposition of organic matter (Roach *et al*. 2016). Seasonal patterns of $\delta^{13}C_{DIC}$ have been observed by several studies. In the downstream part of the Okavango Delta in Botswana, (Akoko *et al.* 2013) observed lower $\delta^{13}C_{DIC}$ (by c. 4 ‰) values due to increased organic matter decomposition during high flood. Seasonal variations of $\delta^{13}C_{DIC}$ values were observed in St Lawrence River in Canada, of 6.8‰ in spring and 1.0‰ in autumn, due to greater supplies of lower $\delta^{13}C_{DIC}$ from watersheds and ground water, lower in situ photosynthesis, and higher rates of organic matter decomposition during spring (Hélie *et al.* 2002). Zhong *et al.* (2017) attributed seasonal $\delta^{13}C_{DIC}$ in Wujiang River, China, to the mixed effects of dissolution of respired $CO_2$ in the soil and increased carbonate weathering as the floods expand during the high flood stage.

## Variability of $\delta^{13}C$ values in organic matter

### Allochthonous organic matter

The terrestrially derived organic carbon in freshwater systems vary in $\delta^{13}C$ signatures depending on the photosynthetic pathway through which it was produced. Different photosynthesis pathways among terrestrial vegetation, namely $C_3$, $C_4$ and Crassulacean acid metabolism (CAM), fractionate carbon isotopes to differential extents ( O'Leary 1988, Bunn and Arthington 2002, Cerling *et al.* 2015). The difference in fractionation of carbon isotopes between the photosynthetic pathways results from differences in the sequence of fractionation steps and differences in photosynthetic enzymes used.

Fractionation of carbon isotopes during $C_3$ photosynthesis occurs at the diffusion, dissolution and carboxylation steps. The diffusion of $CO_2$ through the stomatal pores within the mesophyll involves less fractionation and is reversible since $CO_2$ can diffuse back into the atmosphere. The next step is the dissolution of internal $CO_2$ and its diffusion to the chloroplast, where carboxylation occurs. The dissolution step also involves less fractionation. The total kinetic fractionation that results from diffusion and dissolution of $CO_2$ is about –4.4‰ (Marshall *et al,* 2007). Greater fractionation of up to –29‰ occurs in an irreversible step within the chloroplast when the enzyme RuBP carboxylase converts the dissolved $CO_2$ to carboxylate, an intermediate 3-carbon molecule (3-phosphoglycerate) (O'Leary 1988). These fractionation steps result in a mean $\delta^{13}C$ ratio of about –28‰ in $C_3$ plants (range –33‰ to –22‰).

The $C_4$ pathway, which is utilised mainly by grasses, involves two sequential and irreversible steps of carboxylation. Like the $C_3$ pathway, $CO_2$ initially diffuses into the leaf mesophyll through stomata. The internal $CO_2$ is then taken up by phosphoenolpyruvate (PEP) carboxylase and combined with PEP to form either malate or aspartate within the mesophyll. The fractionation effect of PEP carboxylase is only about –2.2‰ (O'Leary 1988). The product of this carboxylation is then transported to the bundle sheath cells, where it is decarboxylated. The $CO_2$ produced from the decarboxylation is picked by RuBP carboxylase and used in a series of reactions that lead to production of starch. The buddle sheath is almost a closed system such that all the $CO_2$ produced within the cell is fixed into organic matter. Therefore, while RuBP carboxylase is capable of large fractionation, its effect is not expressed in $C_4$ plants. $C_4$ plants are more $^{13}C$ enriched with mean $\delta^{13}C$ ratios of about –13‰ (range –15‰ to –8‰) ( O'Leary 1988, Fantle *et al.* 1999, Khan *et al.* 2015) . The isotopic difference between $C_3$ and $C_4$ plants has been used to distinguish grazers from browsers in African savannas (Ambrose and DeNiro 1986, Cerling *et al.* 2015) and in diet reconstructions of herbivores (Koch *et al.* 1994, Lüdecke *et al.* 2016).

The CAM pathway uses the same carboxylating enzymes as the $C_4$ pathway and in the same order. The difference between the two pathways is that CAM pathway partition the activities of the enzymes between the day when their stomatal pores are closed to reduce water loss, and during the night when their stomatal pores are open. At night CAM plants fix $CO_2$ into $C_4$ acids the same way as $C_4$ plants. During the day when their stomatal pores are closed, the $C_4$ acids of CAM plants are decarboxylated and the $CO_2$ produced is re-fixed by RuBP carboxylase. Since CAM plants fix $CO_2$ using the same order of enzymic action as $C_4$ plants, they have $\delta^{13}C$ value of –11‰. However, some species are facultative CAM plants, using the $C_3$ pathway when conditions are favourable and the CAM pathway during draught. Facultative CAM plants have a $\delta^{13}C$ values intermediate of obligate $C_3$ plants and obligate CAM plants, with the exact $\delta^{13}C$ value depending on the proportion of the respective photosynthesis pathways (Osmond *et al.* 2006). Organic matter from CAM plants and $C_4$ plants are hard to differentiate because of their close $\delta^{13}C$ values.

### Autochthonous organic matter

There is high variability of $\delta^{13}C$ values in freshwater organic matter due to isotopic variability of DIC in freshwater systems, as demonstrated in preceding sections, and due to biochemical and morphological differences among aquatic primary producers, which determine the form of C they take up ( France 1995, Bade *et al.* 2004, Chen *et al.* 2016). To aquatic primary producers, $CO_2$ is available in different isotopic forms which are mainly determined by water pH. It follows that $\delta^{13}C_{DIC}$ is influenced by several environmental factors that influence freshwater pH leading to high $\delta^{13}C_{DIC}$ variability across freshwater systems and seasonally within freshwater bodies. The reason why $\delta^{13}C_{DIC}$ determines $\delta^{13}C$ values of autochthonous organic matter is that $CO_2$ diffuses more slowly in water environment than in air, which limits its availability to photosynthesising cells of aquatic primary producers. Due to limited $CO_2$ availability there is little fractionation of $CO_2$ isotopes and as a result, the fixed carbon will have $\delta^{13}C$ values that are like those of the DIC. Biochemical and morphological features of aquatic primary producers determine their abilities to uptake different forms of the DIC, and differences in these features therefore leads to variability of $\delta^{13}C$ signatures of primary producers since different DIC forms are isotopically distinct. For example, diatoms are more enriched in $^{13}C$ than other phytoplankton because they actively take up $HCO_3^-$ (Tortell *et al.* 1997). Aquatic macrophytes are generally more enriched compared to phytoplankton and benthic algae because macrophytes have relatively thicker walls which present a relatively stronger barrier for molecules to diffuse across. The mean values of $\delta^{13}C$ ratios for macrophytes ranges from –29.2 to –10.1‰, (Piola *et al.* 2008) whereas for phytoplankton the mean value ranges from –32 to –15.8‰, and for benthic algae the mean is about –44‰ (Finlay 2004).

## Controls of $\delta^{15}N$ values in fresh water

The $\delta^{15}N$ varies across freshwater ecosystems and temporally within freshwater ecosystems due to several factors including microbial nitrogen transformations, phytoplankton productivity rates and allochthonous inputs (DIN) (O'leary 1998, Gu *et al.* 2009, Chen *et al.* 2016). For some of these factors, the influence on $\delta^{15}N$ of aquatic primary producers can be synergistic whereas the effects of some factors may oppose each other ( Gu and 1999, Chen *et al*. 2016, Botrel *et al*. 2017). The interpretation of $\delta^{15}N$ values of an ecosystem may therefore not be straight forward, requiring a good understanding of the mechanisms through which these factors influence $\delta^{15}N$ values.

Microbial nitrogen cycling includes ammonia production from organic matter decomposition, nitrification of ammonia and denitrification of nitrates (Ader *et al.* 2016). Decomposition of organic matter (proteins) under anaerobic conditions yields ammonia with minimal isotope fractionation (Prokopenko *et al.* 2007, Möbius 2013). Nitrification occurs when the ammonia is sequentially decomposed to nitrites ($NO_2$) and then nitrates ($NO_3$), which is associated with no isotopic fractionation when all the ammonia is converted to nitrates. However, in rare cases when ammonia is partially nitrified, the residual ammonia gets enriched in $^{15}N$ due to large fractionation involved during the first nitrification step (about −41‰ for ammonia to −13‰ for nitrites (Casciotti *et al.* 2003; Santoro and Casciotti 2011). Denitrification occurs when nitrates are reduced to gaseous nitrogen species ($N_2$ or $N_2O$) under anaerobic conditions involving a nitrogen isotopic fractionation of 15 to 30‰, resulting in the $^{15}N$ enrichment of the residual nitrates (Brandes *et al.*1998, Voss *et al.* 2001). Atmospheric nitrogen can be fixed back into aquatic systems by cyanobacteria using nitrogenase enzyme, which has less fractionation power (Delwiche and Steyn 1970). Due to the less fractionation (1 to 7‰) involved in $N_2$ fixation, the $\delta^{15}N$ of cyanobacteria is close to 0‰. (O'leary 1998, Vuorio *et al.* 2006). The $\delta^{15}N$ values of DIN in lakes can vary temporally following a sequence of annual dominant N processes (Hadas *et al*. 2009). The microbial mediated nitrogen transformations and associated nitrogen fractionations are summarised in figure 2.6.

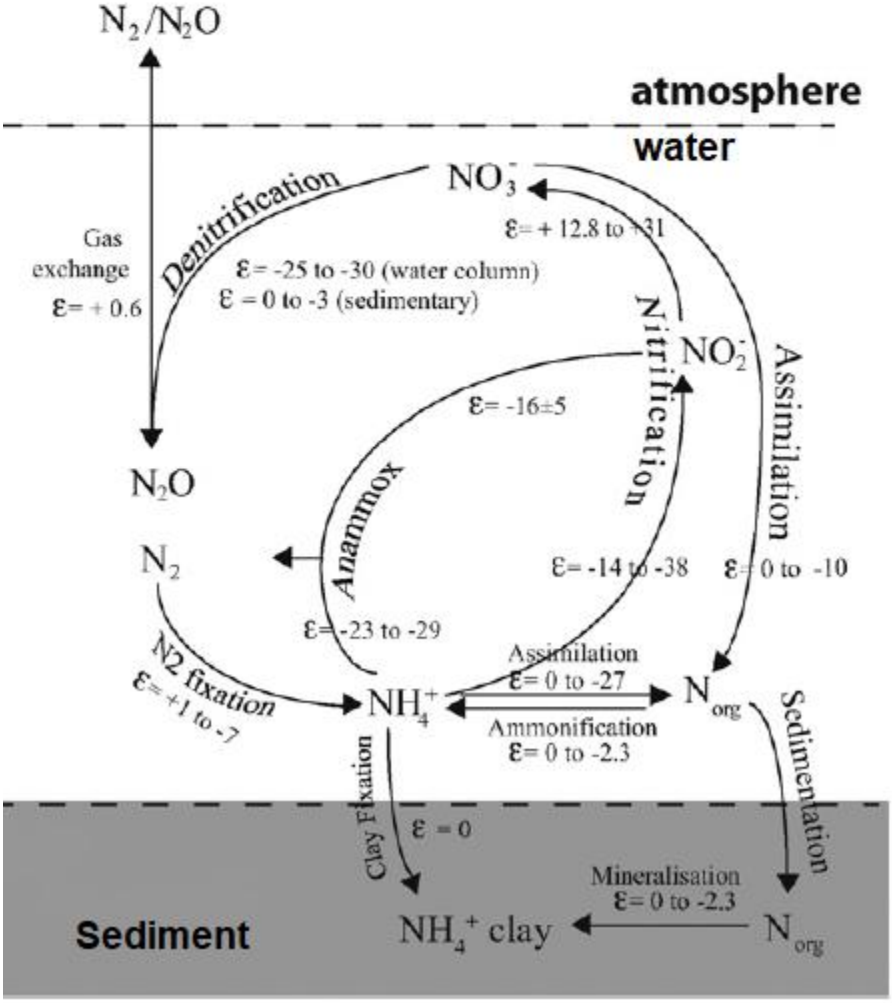


**Figure 2.6**: Microbial nitrogen transformations in an aquatic environment with associated isotopic fractionation values (Ader *et al.* 2016)

In seasonally flooded floodplain rivers, the flood pulse influences temporal variability in N availability and transformations (Koschorreck and Darwich 2003, Shrestha *et al.* 2012). Seasonal inundation of floodplains is associated with decomposition of detritus from aquatic vegetation that dies off during the dry phase and the terrestrial vegetation that grows on the plains when they are dry (Junk *et al.* 1989). The decomposition of the floodplain vegetation, which is facilitated by the seasonal floods, serves as periodic supply of substrate for N oxidation (Koschorreck and Darwich 2003). The seasonal floods also influence changes in soil redox conditions and microbial community structure, hence promoting different N transformation pathways (Pinay *et al*., 2002, Koschorreck and Darwich 2003, Wilson *et al.*

2011, Shretha *et al* 2012). The influence of the flood pulse on N availability and soil microbiology can therefore influences seasonal variability of $\delta^{15}N_{DIN}$ in floodplain rivers.

Rates of phytoplankton photosynthesis also influence $\delta^{15}N$ values of DIN because phytoplankton preferentially uptakes $^{14}N$ leading to progressive concentration of $^{15}N$ in the residual DIN pool (Granger *et al.* 2008, Gu 2009). Factors that affect rates of primary production, such as nutrient levels, temperature and light availability, therefore influence values of $\delta^{15}N_{DIN}$ in freshwater (Gu 2009). Syväranta *et al.* (2006) observed a seasonal $^{15}N$ enrichment in phytoplankton in lake Jyvasjarvi (Finland) which correlated with decrease in nitrate concentration, indicating that the residual DIN pool was progressively getting $^{15}N$ enriched as DIN got limiting. In a review based on data from 42 lakes of different trophic status, Gu (2009) also found a significant relationship between $\delta^{15}N_{DIN}$ values of lakes and rates of phytoplankton productivity, further demonstrating the effect of the effect of phytoplankton photosynthesis on $\delta^{15}N$ values of DIN. In river floodplains $\delta^{15}N_{DIN}$ values are likely to spatially and temporally vary corresponding to the lateral and seasonal variation in primary productivity rates (Junk 1989, Thorp and Delong 1994, Adame *et al.* 2017).

Freshwater $\delta^{15}N_{DIN}$ values are also influenced by inflow of nutrients from external sources and can be used to trace the origin of organic pollutants (Gu 2009, Gebus and Alas 2015). It is possible to trace the origin of organic pollutants because different sources of nitrates have different $\delta^{15}N$ values (Fig. 2.8). The range of $\delta^{15}N$ are known for nitrates derived from septic sewage (+10‰ to +25‰; Kendall 1998) mineral fertilisers (3 to +2 ‰, Lindenbaum 2012) and from atmospheric deposition ($\delta^{15}N$ from 0 to +2‰, Victoria *et al.* 2004). The $\delta^{15}N$ values have been used to identify external nitrate loading from domestic, industrial and agricultural effluents in lakes at proximity with urban settlements (Carpenter *et al.* 1998; Anderson and Cabana 2005).

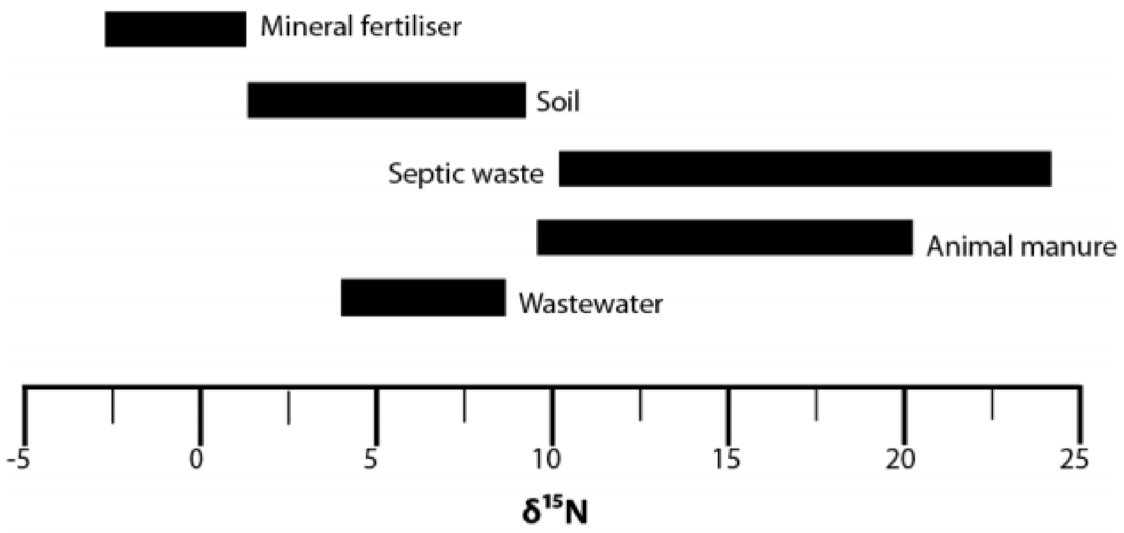


**Figure 2.7**: Ranges of δ15N for different anthropogenic sources of N (Lindenbaum 2012)

### Variability of $\delta^{15}$N values in primary producers

The $\delta^{15}$N values of organic matter in freshwater is influenced by $\delta^{15}$N of dissolved nitrates (DIN) in the water body, environmental settings and phytoplankton community structure and growth rates. As discussed in the previous section, the $\delta^{15}$N value of dissolved nitrates varies due to water body metabolism processes and external factors. Assimilation of the DIN by aquatic primary producers therefore influences $\delta^{15}$N values that corresponds to the $\delta^{15}N_{DIN}$ values (Gu 2009). Anthropogenic nutrient sources (Figure 2.8) also influence $\delta^{15}$N values of freshwater organic matter through altering $\delta^{15}$N values of DIN. The $\delta^{15}$N of organic matter from lakes surrounded by human land use has been shown to be more influenced by nitrates from sources such as agriculture and domestic discharges, than lakes further away from human settlements (Capenter *et al.* 1998, Anderson and Cababa 2005). Therefore, $\delta^{15}$N of freshwater organic matter can vary between systems depending on their locations relative to human influence.

Seasonal variation in phytoplankton productivity and community composition, corresponding to temporal variability in nutrient and light availability, can influence seasonal $\delta^{15}$N in autochthonous organic matter (Syväranta *et al.* 2006). Organic matter synthesised during periods of high rates of aquatic photosynthesis is relatively more enriched in $^{15}$N because fast growing phytoplankton has less discrimination against $^{15}NO_3$ ( Owens 1987, Binhe *et al*. 2011). Additionally, seasonal variation of organic matter $\delta^{15}N$ values occur because as phytoplankton preferentially assimilates $^{14}NO_3$ during photosynthesis, it progressively concentrates $^{15}NO_3$ thereby increasing its rate of assimilation over time (Peterson and Fry 2003). The temporal influence of primary productivity rates and organic matter $\delta^{15}N$ signatures may also be confounded by seasonal changes in phytoplankton community structure such as changes in the abundance of cyanobacteria (Ferber *et al.* 2004, Gardner *et al.* 2017). Cyanobacteria can fix atmospheric nitrogen when dissolved nitrates are limiting, and the fixation involves less discrimination against the heavier nitrogen isotope hence yielding organic matter of $\delta^{15}N$ values close to 0‰. Lakes differing in primary productivity rates (different trophic states) have been shown to have different levels of cyanobacteria abundance, which can also influence spatial variation in $\delta^{15}N$ values of lake autochthonous organic matter.

### Values of $\delta^{13}C$ and C:N ratios distinguish autochthonous from allochthonous organic matter

The $\delta^{13}C$ values and the ratio of carbon to nitrogen isotopes (C:N) can be used to distinguish between autochthonous organic matter and allochthonous in freshwater food webs. Aquatic primary producers fractionate carbon isotopes to a lesser degree than terrestrial plants due to low availability of $CO_2$ that results from slower diffusion rates in the water medium and hence they are more $^{13}C$ enriched compared with terrestrial plants. The $\delta^{13}C$ values of terrestrial plants range from –8 to –30‰ whereas aquatic primary producers range from c. –10‰ (e.g. *Najas marina*, Piola *et al.* 2008) to –44‰ (e.g. epilithic microalgae, Finlay 2004). Aquatic primary producers have lower $\delta^{13}C$ values because they derive their carbon from DIC (Bade *et al.* 2006, Schilder *et al.* 2017) which is has a variable $\delta^{13}C$ ratio but generally lower than of atmospheric air. Autochthonous and allochthonous organic matter can also be distinguished based on C:N ratios. The presence of cellulose in vascular plants increases the ratio of carbon molecules relative to nitrogen molecules (C:N ratios) in their tissues, making the C:N ratios distinctively greater than those of aquatic, non-vascular producers. The C:N ratios of non-vascular plants range between 4 and 10 whereas vascular plants have C:N ratios of over 20 (Figure 2.8) The differences in $\delta^{13}C$ and $\delta^{15}N$ values and the C:N ratios between terrestrial and allochthonous sources of organic matter has been used to examine the relative importance carbon sources to the diets of aquatic organisms ( Hoeinghaus *et al.* 2007, Correa and Winemiller 2018) .

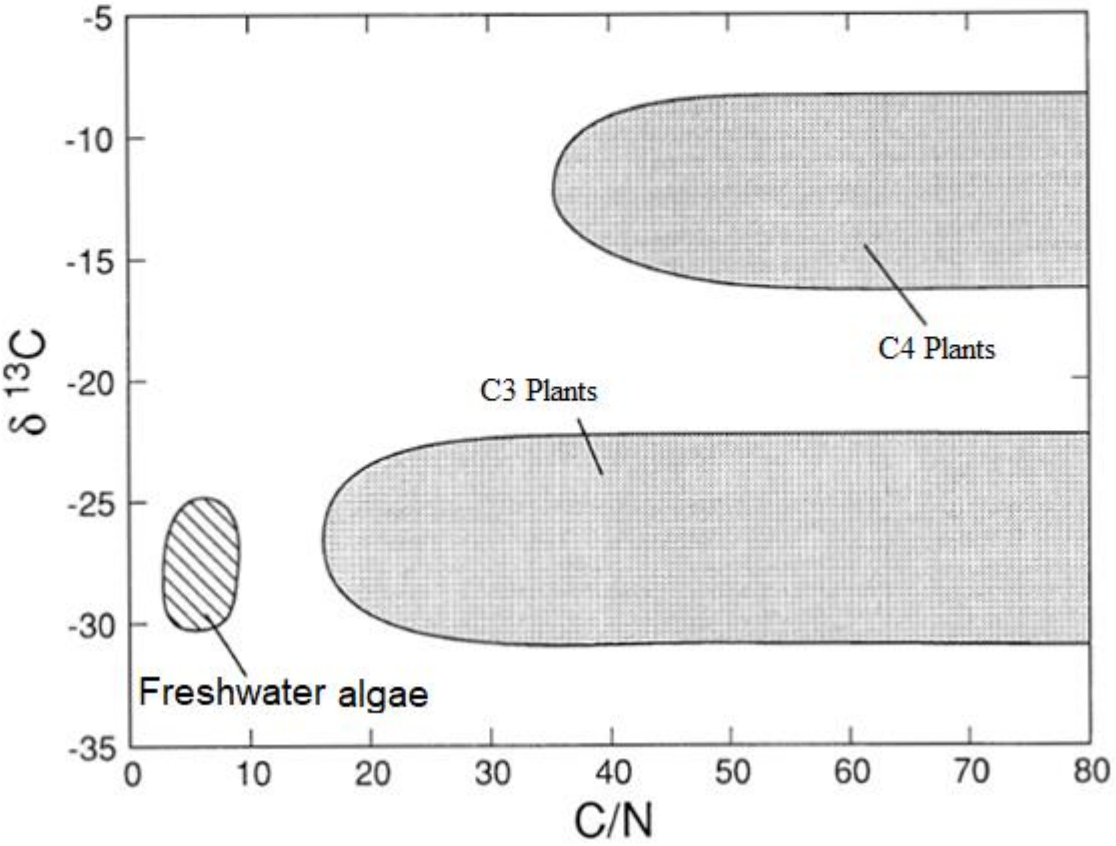


**Figure 2.8**: The range of C:N ratios for aquatic and allochthonous primary producers (from Meyers and Verges 1999).

### Variation of stable isotope values among tissues of a consumer

The values of $\delta^{13}$C and $\delta^{15}$N can also vary between different tissues of the same consumer and this variability has implications for SIA methodologies and for interpretation of SIA results in food web studies. Some SIA methodologies, such as removal of lipids (Tarroux *et al.* 2010) or mathematical corrections for lipid content (Logan *et al.* 2008) when a muscle tissue is used for SIA, are based on the knowledge that there is isotopic difference between lipid and muscle tissues of a consumer. Consumer tissues fractionate C and N isotopes to different degrees; therefore, a consumer's isotopic signature will reflect that of its diet to different degrees depending on the tissue selected for SIA. The isotopic difference between tissues is known to be mainly driven by isotopic routing and differences in biosynthesis pathways and isotopic turnover rates among consumer tissues (Gannes *et al* 1997, Layman 2012, Wolf *et al.* 2015).

Isotopic routing refers to the phenomenon that carbon skeletons of dietary macromolecules (protein, lipids and carbohydrates) are not homogenised before being allocated to different tissues of a consumer but are rather routed differently to specific tissues (Kotch *et al* 1994, Layman *et al*. 2012). A hypothetical example to illustrate routing phenomena is the distribution of carbon skeletons from dietary proteins to consumer tissues (Gannes *et al.* 1998). If carbon skeletons are used to synthesise a consumer tissue without first mixing with carbon skeletons from other macromolecules (carbohydrates and lipids) then the $\delta^{13}$C of consumer protein tissue will reflect that of dietary protein. In contrast, if the carbon skeletons from dietary protein are first mixed with carbon skeletons from other dietary macromolecules, then the $\delta^{13}$C of the tissue protein will reflect the $\delta^{13}$C of bulk diet. One of the factors known to influence the degree of mixing between protein carbon skeletons and carbon skeletons from other macromolecules is the proportion of protein in consumer diet (Tieszen 1993, Podlesak and McWilliams 2006, Boecklen *et al.* 2011). The protein $\delta^{13}$C signature of animals feeding on high-protein diet tend to resemble $\delta^{13}$C signature of dietary protein because they use carbon skeletons from dietary protein to synthesise amino acids. Animals relying on low-protein diets, in contrast, will have protein $\delta^{13}$C resembling that of bulk data because they synthesise amino acids using carbon skeletons from other dietary macromolecules (Ambrose and Norr 1993, Podlesak and McWilliams 2006).

Differences in biosynthesis pathways and isotopic turnover rates influence isotopic differences between tissues of a consumer. Isotopic differences that result from differences in biosynthesis pathways can be illustrated by lipid synthesis. Amino acids synthesised by bacteria from an isotopically homogenous carbon source showed different ratios of carbon isotopes due to differences in biochemical synthetic pathways (Macko *et al.* 1987). In higher animals, this influence is demonstrated by isotopic differences in tissues due to their differences in amino acid composition. For example, collagen is $^{13}$C enriched relative to other body tissues because it contains the highest amount of glycine (33%), a $^{13}$C-enriched (+8‰) amino acid (Hare *et al.* 1991). Some tissues such as hair and feathers are metabolically inert and therefore they store consumer’s isotope ratios at the time of deposition. If the time taken to produce such metabolically inert tissues is known, the tissues provide a resource utilisation timeline for the consumer being studied (Bearhop *et al.* 2004). Consumer tissues vary in terms of metabolic rates and tissue turnover and therefore integrate resource use at different time scales (Boecklen *et al.* 2011, Layman *et al.* 2012). For example, following a diet switch, fish muscle tissue will reflect the isotope ratios of the new diet in about a month, whereas in blood plasma the isotope ratio of the new diet will be reflected within a shorter time scale of days to weeks (MacNeil, Drouillard and Fisk, 2006; Phillips and Eldridge, 2006). The knowledge of how tissues of a consumer vary isotopically, and the temporal scales at which they can track consumer diet, is therefore crucial for tissue selection for SIA in food web studies.

## 2.4 Stable isotope mixing models

### What are mixing models

SIA studies use stable isotope mixing models (SIMMs) to examine the importance of different prey to consumer's tissue production. SIMMs are based on a premise that the isotopic signature of a consumer tissue is similar to that of assimilated food item from which the tissue was synthesised. This premise is famously expressed as "you are what you eat plus a few per mil" (DeNiro and Epstein 1976). The "plus a few per mil" part of the premise refers to the difference in $\delta^{13}C$ and/or $\delta^{15}N$ between the consumer tissue and the dietary items, which results from trophic discrimination (Newsome *et al.* 2010). Trophic discrimination includes fractionation of diet isotopes during assimilation and other processes that favour incorporation of one isotope over the other into a consumer tissue, such as isotope routing (refer to previous section). SIMMs calculate the proportional contributions of prey items to the consumer's tissue using the $\delta^{13}C$ and/or $\delta^{15}N$ data of the prey items and of the consumer's tissue. The consumer tissue is viewed as an isotopic mixture derived from assimilated food items (sources) with different isotopic signatures (Phillips 2012). To illustrate the procedure for most mixing models, I will use an example a simple linear mixing model taken from Phillips (2012), the example involves using one isotope ($\delta^{13}C$) from a consumer that relies on two prey items (A and B). The mixing model for such a scenario can be expressed as follows:

$$\delta^{13}C_{mix} = p_1\, \delta^{13}C_A + p_2\, \delta^{13}C_B \qquad (1)$$

$$p_1 + p_2 = 1$$

The equation above expresses the consumer $\delta^{13}C$ value ($\delta^{13}C_{mix}$) as the sum of $\delta^{13}C$ values of prey items A and B ($\delta^{13}C_A + \delta^{13}C_B$ respectively) weighted by their diet proportions ($p_1$ and $p_2$ respectively). Equation 1 above can be re-arranged as

$$p_1 = (\delta^{13}C_{mix}\ p_2\,\delta^{13}C_B)/\ p_1\ \delta^{13}C_A\ p_2\,\delta^{13}C_B) \qquad (2)$$

$$p_2 = 1\ p_1$$

Consider a herbivore muscle tissue with $\delta^{13}C$ value of –21‰ feeding on food sources A ($C_3$ plants, –25.5‰) and B (C4 plants –15.5‰). After correcting for trophic discrimination of 0.5‰ on the isotope values of prey items we will have $\delta^{13}C$ values as 25‰ and 15‰ for $C_3$ and $C_4$ plants respectively. Substituting the $\delta^{13}C$ values into the model (equation 2) gives the results of relative proportions of $C_3$ and $C_4$ plants to herbivore muscle tissue as 0.6 and 0.4 respectively, which can be expressed in percentage form as 60% and 40%. The basic mixing model can be graphically represented (Fig. 2.10). In a situation where the isotope value of the tissue falls outside the range of isotopic values of the food sources, for example if the $\delta^{13}C$ for the herbivore's tissue in the above illustration was –37‰, the model will still yield values, but there would be negative values and values over 100%, which would not make ecological sense. So, it is a pre-requisite for running SIMMs that the tissue isotopic value of the mixture (tissue) must fall within the range of isotopic values of its sources (Phillips *et al.* 2014). The trophic position of a consumer is calculated the same way as in this example using $\delta^{15}N$ values of a consumer tissue and of food sources.

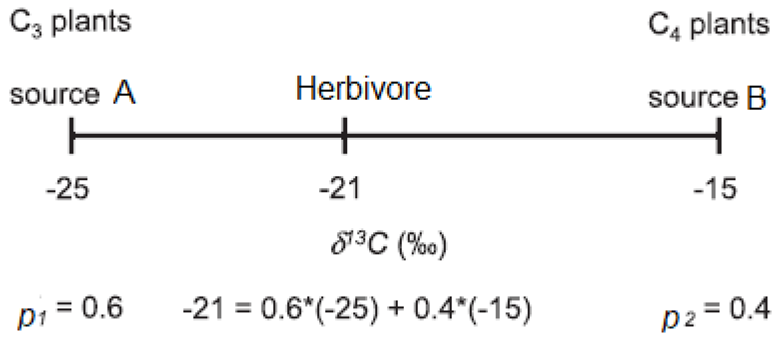


**Figure 2.9**: A diagram for simple mixing model based on one isotope value (δ13C) and two food sources ($C_3$ and $C_4$ plants) in the diet of a herbivore (Phillips 2012). The herbivore tissue is made from 60% C3 and 40% C4 plants as determined by the model.

The number of sources that can be used in SIMMs is constrained by the number of isotopes that are used for the analyses. In the basic mixing model illustration above, one isotope ($\delta^{13}C$) is used to trace the contributions of two sources ($C_3$ and $C_4$ plants) for which there is a mathematical solution for the unknown terms (*p1* and *p2*). In general, if there are *n* isotope tracers (elements), relative contributions of *n*+1 sources can be determined. However, if the number of sources is greater than *n* +1, then the model would have too many unknowns and would yield an infinite number of solutions (Phillips and Gregg 2003). However, since mixing models constrain possible isotopic contributions to 1 (or 100%), they can still be used to find multiple combinations of isotopically feasible source contributions and hence put bounds on possible diet contributions (Phillips 2012).

### Assumptions of mixing models

The two main assumptions made by SIMMs are that there is isotopic homogenisation of assimilated diet and that all sources are included when proportions of sources to the tissue are calculated. The calculations of isotopic contribution of different food sources to the isotopic value of consumer tissue isotope assume that the isotopes of the sources are from an isotopically homogenous assimilated diet (Martınez del Rio *et al.* 2009). However, studies have shown that some elements are channelled to certain tissues in larger proportions than to other tissues, a phenomenon called isotopic routing (refer to Section 2.4.1). The phenomenon of isotopic routing can therefore lead to over- or under-estimation of proportional contribution of some food sources depending on which consumer tissue is chosen for SIMMs (Layman *et al.* 2012). Trophic ecologists are aware of this caveat and they select consumer tissues that are suitable for their investigations (Phillips *et al.* 2014). The second assumption made by SIMMs is that all sources are included in the analysis; exclusion of a source can lead to biased proportion estimates or failure for the model to find solutions based on the included sources (Phillips and Gregg 2003). To overcome this challenge, SIA studies collect all potential prey items and include their isotope values when running SIMMs (Phillips *et al.* 2014). Information about of the feeding ecology of the consumer being investigated as well as the system being studied is therefore critical for running successful SIMMs.

### Overview of mixing model approaches

SIMMs evolved over time, with new models attempting to address challenges that could not be resolved by older models, resulting in three main mixing model approaches, namely, geometric, linear and Bayesian mixing models (Layman *et al.* 2012). Geometric approaches are based on Euclidean distances between consumer and prey isotopes in an isotopic niche space. Several studies have used this approach to quantify the relative contribution of three or more food sources to a consumer's assimilated diet (Kline *et al*. 1993, Peterson and Howarth 1987, Whitledge and Rabeni, 1997). Based on the approach, the relative contribution of the sources to consumer assimilated diet increases with decreasing distance between consumer isotopes and sources isotopes on the plot. While the technique yields interesting and easily interpretable visual representation of relative contribution of sources, the technique has been criticised for underestimating the contribution commonly used food sources and overestimating the contribution of rarely used food sources (Szepanski *et al.* 1999).

Linear mixing models are linear expressions that calculate the relative contribution of sources to a consumer tissue based on stable isotope values of the consumer tissue and of food sources (Phillips 2001), (see the example in Section 2.4.1). The computations of the models are mostly run by computer software and the models have been developed to account for uncertainty (error) associated with estimations of source contributions to consumer diet (Isoerror: Phillips and Gregg 2001). Most SIA food web studies use isotopes of C and N as tracers and the linear models initially assumed equal concentrations of the elements per source, which may be erroneous. Models were further developed therefore, to account for differential concentration of tracer elements in sources (Phillips and Koch 2002). To provide a model that better handled the complexity of natural systems, where consumers typically rely on many sources, Phillips and Gregg (2003) developed a model called IsoSource, which better estimated the relative contributions of many sources. The IsoSource does not yield point estimates of the contributions, instead it produces a range of values for feasible contributions of each source. The IsoSource model computes possible combinations of source proportions adding up to 100% in increments of 1% and then predicts an isotope value (of the consumer tissue) for each combination using mass-balance equations. The predicted isotope values within the tolerance range of 1% around the known consumer isotope value are then recorded. Advantages of this model are that it is freely available, user friendly and can be adjusted to calculate relative contributions of grouped sources. The major problem with IsoSource was not intrinsic to the model itself, but the interpretation of its output. Users of the model often used measures of central tendency such as the mean or median of the output as the point estimate of the contribution of the sources (i.e. definitive solution) even though the structure of the model does not justify that (Phillips and Gregg 2003). Other similar models include SOURCE and STEP (Lubtekin and Simenstad (2004), the Moore-Penrose pseudoinverse model (Hall-Aspland, Hall and Rogers, 2005a; Hall-Aspland, Rogers and Canfield, 2005b) and the linear programming model (Bugalho *et al*. 2008). Unlike IsoSource, which calculates values for possible combinations within tolerance range around the consumer isotope value, The SOURCE and STEP models only determines the outer bounds of the proportions contributed by possible source combinations in an isotopic niche space. The output of these models is smaller compared to that of IsoSource but is somewhat similar (Maier and Simenstad 2009). The Moore-Penrose pseudoinverse model yields point estimations of source contributions but based on one element tracer and algebra matrix. The point estimations from pseudoinverse model are however like point estimates from IsoSource (S.A. Hall-Aspland, in Layman *et al.* 2012). The linear programming model (Bugalho et al., 2008) produces a similar output to that produced by IsoSource, but unlike IsoSource, which uses iterations for the output, the linear programming model uses linear algorithms to calculate the source contributions. The linear programming software is not as frequently used as IsoSource likely because it is not freely available.

Bayesian mixing models (Jackson *et al.* 2009; Moore and Semmens 2008; Parnell *et al.* 2010; Solomon *et al.* 2011) were later developed to estimate error associated with input variables during calculation of source contributions, a limitation that is associated with linear models. Bayesian models are developed within the Bayesian framework, which enables incorporation of priors (known information about input parameters) in the equation before calculation of the likelihood output. Therefore, the Bayesian Models allows incorporation of information such as variability of stable isotope values of sources and variability of trophic discrimination factors, which is very important for making ecological conclusions. Unlike IsoSource, Bayesian models such as MixSIR (Mooreand Semmens 2008; Ward et al. 2010), SIAR (Parnell *et al.* 2010), MixSIAR (Stock and Semmens 2013) and SIBER (Jackson *et al.* 2011) produce probability distributions that allow calculations for measures of central tendency such as the mean or median from the model output. Bayesian mixing models are freely available and are currently the widely used mixing models in food web research. There are a few challenges though associated with using these models which limit their applicability in systems or organisms where a lot of supplementary ecological information is not known. For example, they require information about turnover rate and macro-nutrient composition of the consumer tissue. For the models to effectively calculate proportional contribution of each source, the food sources must have significantly different isotope values. This can be a challenge because in natural systems potential food sources can be numerous and with overlapping isotope signatures. To constrain the output of the models, it is often necessary to group the numerous sources, which can also be challenging because it requires extensive background ecological information about the system being studied. Despite these challenges, Bayesian mixing models are the currently regarded as the best mixing models for food web studies.

Spatial based Bayesian mixing models incorporate continuous variables such as distance gradients to delineate contributions of isotopically overlapping sources (Francis *et al*. 2011, Gray *et al*. 2011, Rasmussen 2010). Some authors categorise spatial based mixing models separately from Bayesian methods because of their explicit application for separating sources based on spatial gradients. However, these models, regardless of the input data they use to delineate sources, are developed based on the Bayesian framework. The spatial based mixing models rely on differential spatial patterns of stable isotope values of food sources, such that contributions sources with distinct spatial gradients can be calculated and used as part of input parameters for the model. For example, in lotic systems autochthonous producers may show isotopic gradients from upstream to downstream whereas terrestrial sources do not have such spatial gradients. Rasmussen (2010) and Gray *et al.* (2011) used the different spatial gradient signatures to delineate contributions of allochthonous and autochthonous basal carbon sources to invertebrate consumers along a river gradient. Francis *et al*. (2011) used the approach to examine the relative importance of different food sources for zooplankton along a vertical distance (depth) in lakes. The major advantage that this approach has is that the models can separate contributions of sources which are isotopically overlapping. However, the models require value inputs of source isotopic spatial gradients which may be difficult to develop or may even not exist in some systems. The models also require the sources to be the same along the spatial gradients, which may not be the case in natural river systems. These constraints limit the application of spatial models in food web research.

Stable isotope data for this study was analysed using the Bayesian the Bayesian mixing models because the approach allows incorporation of variance in stable isotope data parameters and therefore is suitable for natural systems. The Okavango Delta, where stable isotope data for this study was collected, is a highly heterogenous and dynamic system and substantial amounts of variation in stable isotope data can be expected. A comprehensive description of the study area will be provided in the following chapter, **Chapter 3**. While spatial based mixing models would also allow incorporation of variance of the input variables, spatial based mixing models were not used in this study because the aim of my study was to compare food web properties of different sites as opposed to tracing change in food web properties along an environmental gradient; a situation which would necessitate the use of the spatial mixing models.

## CHAPTER THREE: The Okavango Delta: Study area

A common phenomenon among African large rivers, except coastal rivers in West Africa and the Congo-Zaire System, is that they flow through arid landscapes (Welcomme 2003). The Okavango river basin is no exception - the river system is a very large, endorheic system which originates and terminates within Africa's largest desert, the Kalahari Desert in Southern Africa. The study area for this PhD research is the Okavango Delta (hereafter referred often to as the Delta), the downstream part of the Okavango river basin located in Northern Botswana (Figure 3.1). The Delta is located between 19° 20° S and 22° 24° E (Gumbricht *et al.* 2001). The following sections describe the environmental setting of the Delta, including its geology, climate and flooding regimes.

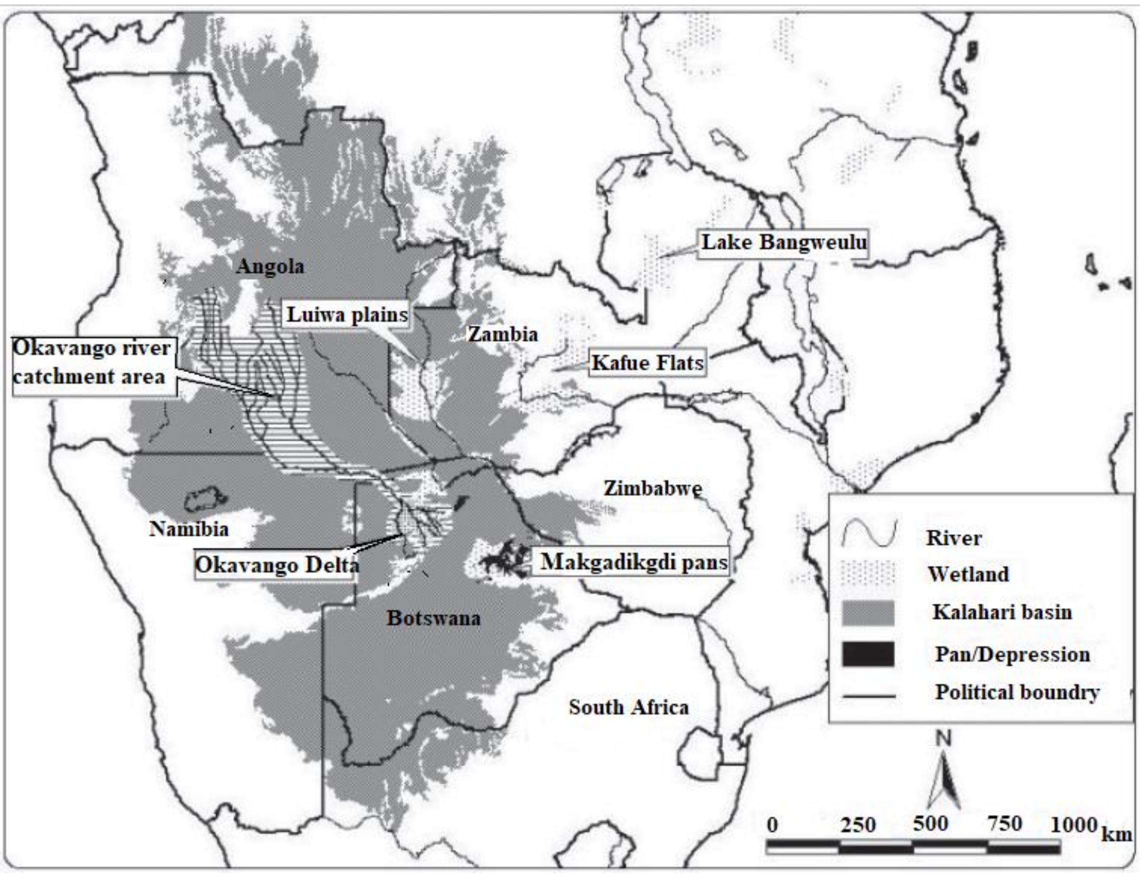


**Figure 3.1**: The position of the Okavango within the Kalahari basin (Ramberg *et al.* 2006)

## 3.1 Geology

The Okavango River basin is formed over fine-grained sands and silts of Cenozoic age which are known as the Kalahari beds ( Shaw and Thomas 1993). The Kalahari bed is 300m thick and comprises semi-consolidated layers of detritus mixed with calcrete and silcrete (Hutchins *et al*. 1976, Shaw and Thomas 1993). The river basin is of Quaternary age, about 2.6 million years old ( Junk *et al.* 2006) and may have been connected to other river systems such as the Orange and Limpopo Rivers in the past, but its course has repeatedly been altered by tectonic activity (McCarthy 1992). The tectonic events involved southward with extension of the great East African Rift Valley, causing rifting of a segment of earth crust along the Kunyere and Thamalakane faults (McCarthy *et al.* 1992). This formed a graben with a northern boundary defined by the Gumare Fault and the southern (downstream) boundary defined by the Kunyere and Thamalakane faults (Figure 3.2). The Okavango Delta wetland is formed as the Okavango River deposits sediments into the graben to form a gentle alluvial fan (McCarthy & Ellery, 1994). Two depressions were formed due to the alluvial fan curving away from the Kunyere Fault line in the south east of the graben: Lake Ngami in the southwest, and Mababe depression in the northwest of the graben (Figure 3.2).

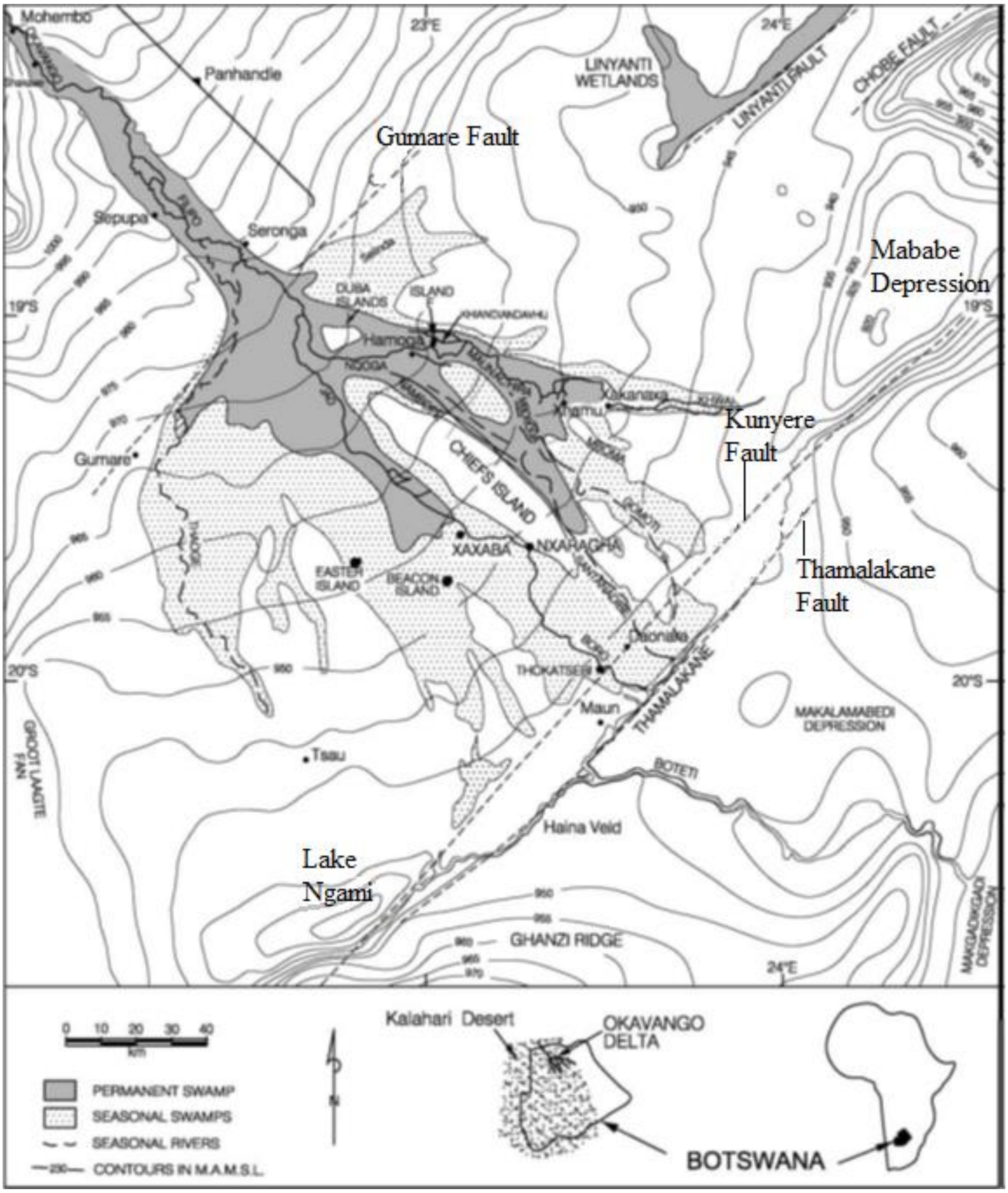


**Figure 3.2**: The Okavango Delta wetlands and the fault lines and contours associated with its geology (Bjorkvald and Boring 2002). The Mababe and Ngami depressions are both highlighted, as are the northern (Gumare) and southern (Kunyere and Thamalakane) fault lines

## 3.2 Climate and hydrology

The climate of the arid Kalahari is characterised by hot summers, and warm winters with cold nights. Monthly maximum temperatures occur between October and January, with the mean temperatures ranging between 32 and 35 °C (Fig 3.3). However, daily maximum temperatures can reach as high as 43 °C during the summer months (Bhalotra 1985, Moses 2017). Winter starts from May and ends at the end of July. During winter, the mean maximum temperature ranges between 25.3 °C and 28.7 °C while mean minimum temperature ranges between 7.0 °C and 10.0 °C (Ellery *et al.* 1991, Moses 2017).

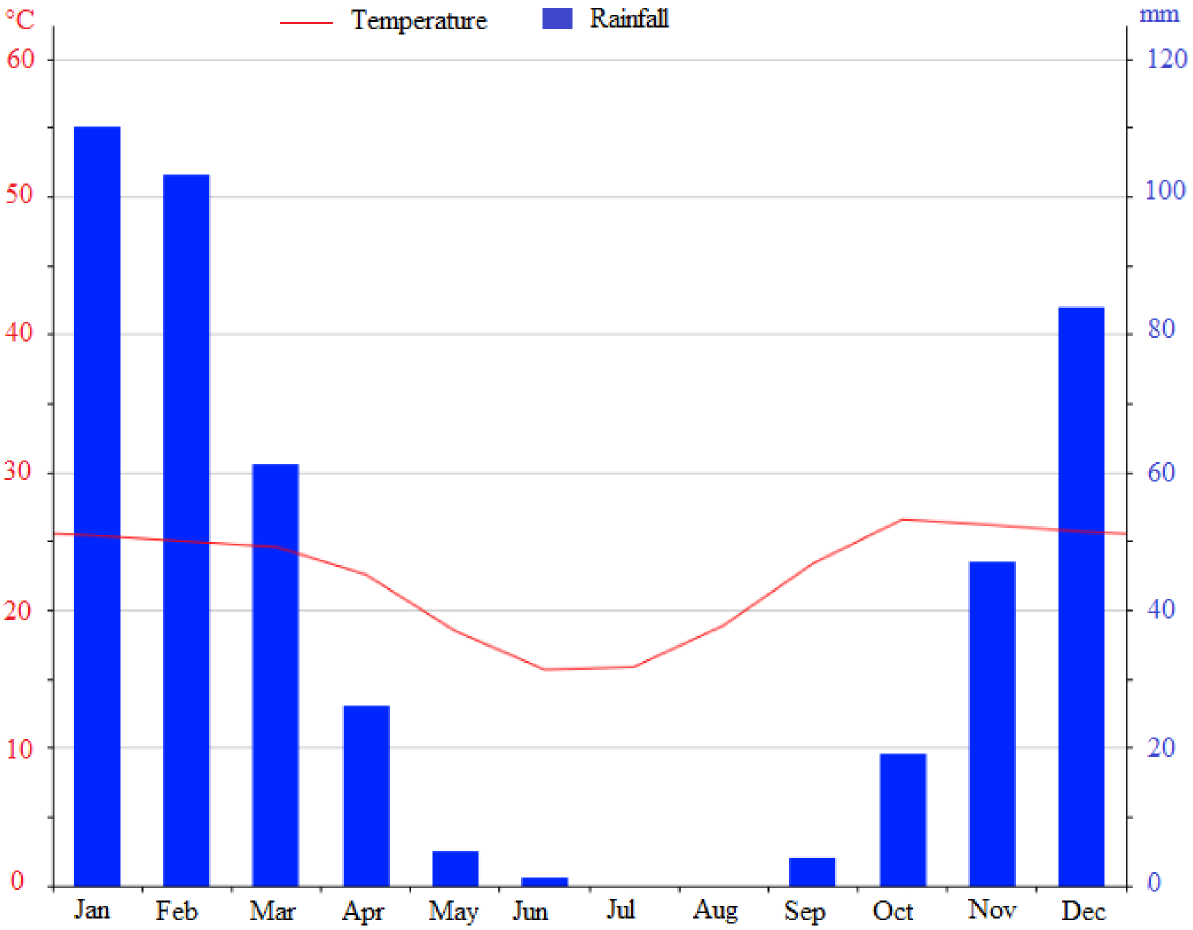


**Figure 3.3**: Monthly average precipitation and temperatures for the Okavango Delta (www.climate.org)

It is perhaps better to describe the climate seasons of the Okavango Delta as wet and dry, as opposed to winter and summer, because seasonal contrasts are more expressed by variations in precipitation than by variations in temperatures (Thomas and Shaw 1991, Van As *et al*. 2012). The rain falls mainly during summer months between November and April, with January being the wettest month (McCarthy *et al* 1998) (Figure 3.3). The summer rainfalls in Botswana mostly occur as thunderstorms in the late afternoons and early evenings, often with volumes of less than 10 mm (Thomas & Shaw 1991). Rainfall amounts increase from south to north and from west to east, with rainfall averaging 150 mm per annum in drier parts and between 500 and 800 mm per annum in the northern parts (Van As *et al.* 2012) and the north-eastern corners about 650 mm (Thomas and Shaw 1991).

### 3.3 Flooding dynamics

The yearly rainfall contributes about $5.2\times10^9$ $m^3$ of water to the Delta, but the bulk of the water that floods the Delta ecosystem, about $9.20\times10^9$ $m^3$, is supplied by the Okavango River (McCarthy *et al*. 1998). The annual flood wave from the Okavango River originates from catchments in the Angolan highlands, associated mainly with the Cubango and Cuito rivers. The water from these river systems flow though Namibia and converge into one river, the Okavango River before entering Botswana on the Northern side in Mohembo village. About 100 km after entering Botswana, the Okavango River spreads out into a swampy system forming the Okavango Delta. The upstream riverine part of the Delta is referred to as the Panhandle whereas the downstream swampy system is referred to as the Fan. Seasonal water level fluctuations in the Panhandle may be as high as 2m due to the lateral confinement of the major channel whereas water fluctuations of only 0.15m occur in the Fan region due to less lateral confinement, water in the Fan region spreads easily to inundate permanent swamps and seasonal floodplains (Ellery and McCarthy 1994). Due to the low topographic gradient (1:5500) of the Delta the flood pulse takes about four months to go through the Delta reaching distal downstream sites near Maun town between June and August (McCarthy 1992, McCarthy *et al*, 1997). The graphs below (Figure 3.4) show the temporal separation of the flooding of the Delta’s upstream and downstream parts.

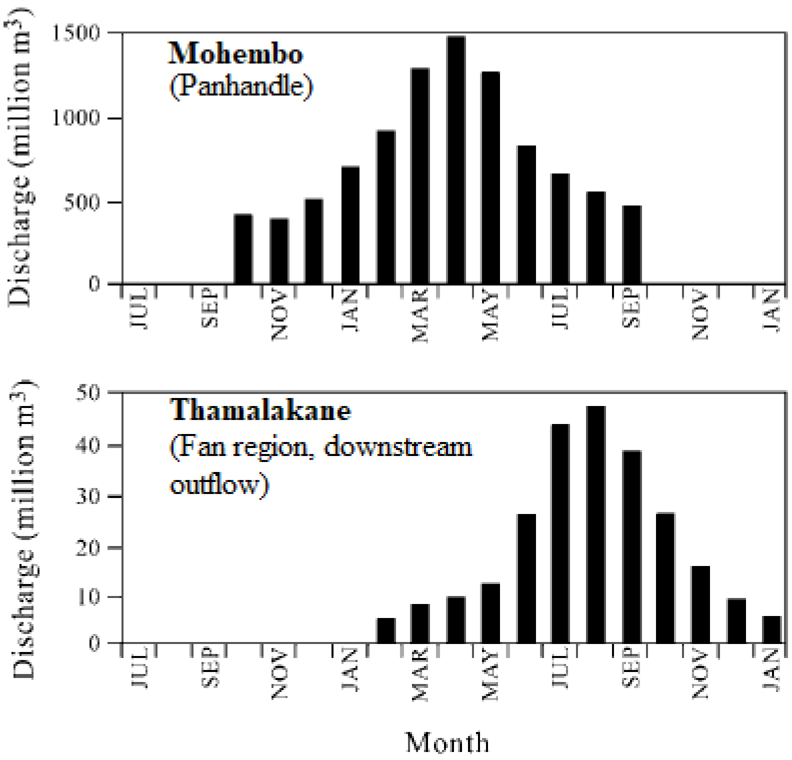


**Figure 3.4**: Monthly discharge volumes at Mohembo and Thamalakane, indicating the temporal separation in flooding between the Panhandle region (upstream) and the outflow channel of the Fan region (Thamalakane river) (Appleton *et al*. 2008).

The wetted area of the Okavango Delta typically varies seasonally in size from 5000 km$^2$ during base flow to 13,000km$^2$ during high water conditions, but the flooding extent can reach up to 28,000 km$^2$ during exceptionally wet years (McCarthy *et al.* 1998, Wolski and Murray-Hudson 2008). The volume of the yearly flood reaching the Delta varies annually due to variations in rainfall in Southern Africa (Mazwimavi and Wolski 2006). Inter-annual variability in the Delta's flood levels is further influenced by the fact that years of high flooding tend to raise the water table of the Delta favouring extensive flooding in the year that follows even when the incoming flood wave is relatively smaller (McCarthy *et al.* 1998). About 98% of the flood wave that floods the Delta is lost through evapotranspiration (1,500mm) (Mazwimavi and Wolski 2006). The remaining water exits the Delta through outflow river channels including Thamalakane, Boteti rivers and Kunyere rivers, with the latter directing water to Lake Ngami (Fig. 3.5). Internal redirection of floods within the Delta due to tectonic movements also adds to the dynamic flooding of the Delta's wetlands. For example, redirection of water in the Delta to the western distributaries that flow into Lake Ngami causes considerable intra- and inter-annual water level fluctuations in the lake (Shaw *et al.* 2003, Wolski *et al.* 2014).

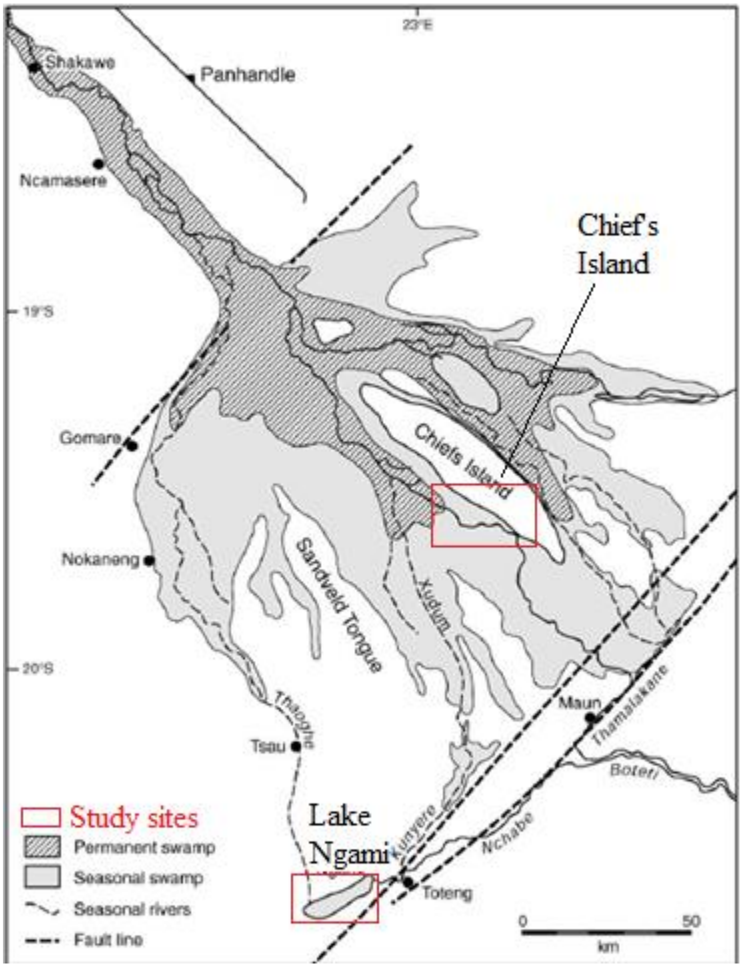


**Figure 3.5**: The main hydrological features of The Delta, showing main channels, floodplains and Lake Ngami (Wolski and Murray-Hudson 2008)

### 3.4. Physical environment and Vegetation

Different regions of the Delta, including The Panhandle, Fan region and Lake Ngami have different physical and vegetation characteristics. The main river channels of the Panhandle region are between 50 to 100m wide, 3-4m deep and fast flowing (0.6 to 0.8 m $s^{-1}$) (Fig. 3.6). The Panhandle is characterised by sandy beds which are flanked by dense stands of *Cyprus papyrus* (papyrus sedge, $C_4$) and *Phragmites australis* (common reed, $C_4$). Swamps that flank the main channels are characterised by species able to grow on semi-consolidated Kalahari sediments.

**Figure 3.6**: Okavango River at Mohembo in the Panhandle region (Photograph: author's own)

In the Fan region, the permanent swamps have main distributary channels of sandy beds, 15 to 50 m width and 2 to 4m depth, which are also flanked by dense vegetation of *C. papyrus* and *P. australis*. The main distributary channels in the Fan region are the Nqoga and Maunachira channels on the north eastern part, and the Boro channel on the central part (Fig. 3.7). The permanent swamp is characterised by large lagoons and backwater habitats. Floating vegetation in the open water of the habitats is dominated by *Nymphea nouchali* (water lily, $C_3$) (Fig. 3.5) and *Trapa natans* (water chestnut, $C_3$) while submerged aquatic vegetation is dominated by *Potamageton spp*. (pond weed, $C_3$) or *Rotala myriophylloides* ($C_3$) (Dallas and Mosepele 2007). Wetland plant species including *Typha spp*., *Vossia cuspidate* (Hippo grass, $C_4$), and

*Ficus verruculosa* (water figs, $C_3$) are common on the margins of the permanent swamp area (Ellery *et al.* 1992, Dallas and Mosepele 2007).

**Figure 3.7**: A lagoon within a permanent swamp in the Fan region, with floating vegetation of N. nouchali (Photograph: author’s own)

Seasonal swamps are at the distal parts of the Fan region and are seasonally flooded as the flood wave overspill the permanent swamp. The seasonal swamp vegetation mainly comprises herbaceous plant species with the plant communities changing with distance from river channels or length of inundation (e.g. see Figure 3.9). The vegetation communities are described in detail by Bonyongo *et al*. (2000). Sedges such as *Cyperus articulates* (Ribbed sedge) and *Schoenoplectus corymbosus* (Bulrush) dominate the deepest and most frequently flooded sites, of depths ranging from 0.50- 1.0 m during high water conditions typically between July- September. Other common plant species in the frequently inundated floodplains include *Miscanthus junceus* (Silver grass, $C_4$) and *Panicum repens* (Torpedo grass, $C_4$).

The shallower (0.1 to 0.25m deep) and less frequently inundated floodplains are mostly vegetated by grass species, mostly by *Setaria sphacelate* (Bristle grass, $C_4$) and *Eragrostis illanwella* (Cane grass). The type of floodplains experiences moderate grazing from lechwe during flooding, but there can be heavy grazing from bulk grazers such as buffalo, zebra and wildebeest and hippopotamus during the dry season. Bordering the frequently inundated floodplains are the relatively elevated grasslands which are rarely flooded. Common grass species on the rarely flooded plains include *S. sphacelate* and *E. illamoena*. The vegetation forms a belt around the secondary floodplains, linking floodplains and island woodlands, and is made of tall grasses of up to 2m, mostly *Veliveria nigritana* (Adrenaline grass, $C_4$) and *S. sphacelala* species. The vegetation gets temporarily flooded by local rains, and by overspills from the flood wave during years of extensive flooding. Other common grass species in the grasslands include the perennial grass *Imperata eylilldrica* (Cogon grass, $C_4$). *S. sphacelata* and *E.s inamoena.* Ellery *et al.* (1992), has demonstrated that in terms of percentage cover $C_3$ plants are more abundant in deep aquatic habitats, dominating deep submerged species, floating leaved, and tall emergent species, whereas $C_4$ grasses are more abundant in shallow floodplains, dominating emergent vegetation rooted in peat.

Islands are common in the Fan region and they range from small islands formed from termite mounds that form between secondary distributary channels, to large islands such as the Chief's Island, which is formed from tectonic activity. Tree vegetation within the Fan region is confined to Islands, where the vegetation is typically zoned with broad leaved evergreen trees occupying the Islands' margins, deciduous trees and palm trees forming the inner ring and grass lands occupying the central part of the islands (e.g. see Figure 3.9).

**Figure 3.8:** A seasonal swamp in the lower part of the Fan region (Photograph: author's own).

**Figure 3.9**: Palm trees and other wood vegetation within an Island in the Fan region (Photograph: author's own).

### 3.5. Biodiversity of the Okavango

The Delta's seasonal flooding regime makes it a highly heterogeneous and dynamic ecosystem that supports a high level of biodiversity. For aquatic habitats, the flooding regime involves accumulation of nutrients during the dry phase and sudden mobilisation of the nutrients during the flooding stage, promoting high level of aquatic biological productivity during high flood (Høberg *et al.* 2002). The seasonal flooding regime influences predictable cycles of vegetation successions in flood plains to which animal communities are adapted to. The Delta is also a crucial source of water for wildlife in the arid area of northern Botswana, especially during the hot, dry season when there are limited water sources in the area (Ramberg *et al.* 2006). The number of identified species in the Delta ecosystem is 1,300 for plants (about 220 species on permanent swamp and 90 on seasonal swamp), 71 for fish, 33 for amphibians, 64 for reptiles, 444 for birds and 122 for mammals (Ramberg *et al.* 2006).

The Delta's biodiversity includes globally threatened and vulnerable species. The Delta has the world's largest population of elephants (*Loxodonta africana*) (Thouless *et al*. 2016) and other large mammal species which are globally threatened such as black and white rhinos (*Diceros bicornis* and *Ceratotherium simum* respectively), wild dogs (*Lycaon pictus*) and cheetahs (*Acinonyx jubatus*) (Richter and Passineasu 1979, Ramberg *et al.* 2006). The Delta is also home for threatened bird species such as slaty egrets and wattled cranes (Ramberg *et al*. 2006). For its global biodiversity significance, the Okavango had been declared a Ramsar site (https://www.ramsar.org) and more recently, in 2014, it was listed as the 1000th UNESCO World Heritage Site (https://whc.unesco.org/en/list/1432 ).

Regional conservation initiatives include the Okavango River Basin Water Commission (OKACOM), which is an agreement that commits the three riparian countries (Angola, Botswana and Namibia) to ecologically friendly and sustainable management practices within the Okavango River basin. Within Botswana, the Delta is within a protected area of Moremi Game Reserve and managed primarily by Botswana's Department of Wildlife and National Parks (DWNP). The DWNP protects the biodiversity of the Delta through permits that limit utilisation of biodiversity resources (Mosepele & Mosepele, 2005). For example, the DWNP prohibits fishing within Moremi Game reserve but in the Delta's terminal wetlands, like Lake

Ngami, that are outside Moremi game reserve boundaries, the DWNP permits only certain type of fishing gear, hence controls the size and amount of fish that can be harvested per person during the fishing season. The DWNP also has an anti-poaching section that actively surveys the protected area to protect its biodiversity against poachers (DWNP 2000). A month after the current Botswana government took the presidential position, the Botswana government withdrew military weapons and equipment from the DWNP (Kgalemang 2018). The withdrawal of military weapons from DWNP was followed by reports of alarming numbers of elephant killings by poachers, 87 killings within a month (Kgalemang 2018). The reports about the elephant killings made international headlines but the Botswana government refuted the reports due to lack of substantial evidence for the reported high elephant killings (Leithead 2019). The DWNP is currently working jointly with the Botswana Defense Force (BDF), which is in possession of military weapons, to protect the biodiversity of Botswana.

Based on the definition of biodiversity as "…the variety and variability among living organisms, the ecological complexes in which they occur, and the ways in which they interact with each other and their environment" (Ramesh 2003), the biodiversity of the Okavango is only partly understood. The Delta's biodiversity is not well understood because while there has been a comprehensive review of its species diversity (Ramberg *et al.* 2006) and extensive research on its physical environment such as hydrology (McCarthy et al. 1998, Shaw *et al.* 2003, Mazwimavi and Wolski 2006, Wolski *et al.* 2014) and vegetation (Ellery *et al.* 1992, Bonyongo 2000, Ellery and Tacheba 2003, Mackay *et al.* 2011, Tsheboeng *et al.* 2016), information on the interaction dynamics of the diverse species of the Delta is still limited. Despite the incomplete understanding of the Delta's biodiversity, there is consensus that the seasonal flooding regime is the overall driver of biodiversity dynamics in the Delta (Ramberg *et al.* 2006, Lindholm *et al.* 2007, Davidson *et al.* 2011). Considering the hydrological threats as discussed in Chapter 1 (Section 1.7) and the general lack of research on interspecific relationships, the role of flooding regimes on the interdependencies of the species of the Delta is crucial to understand.

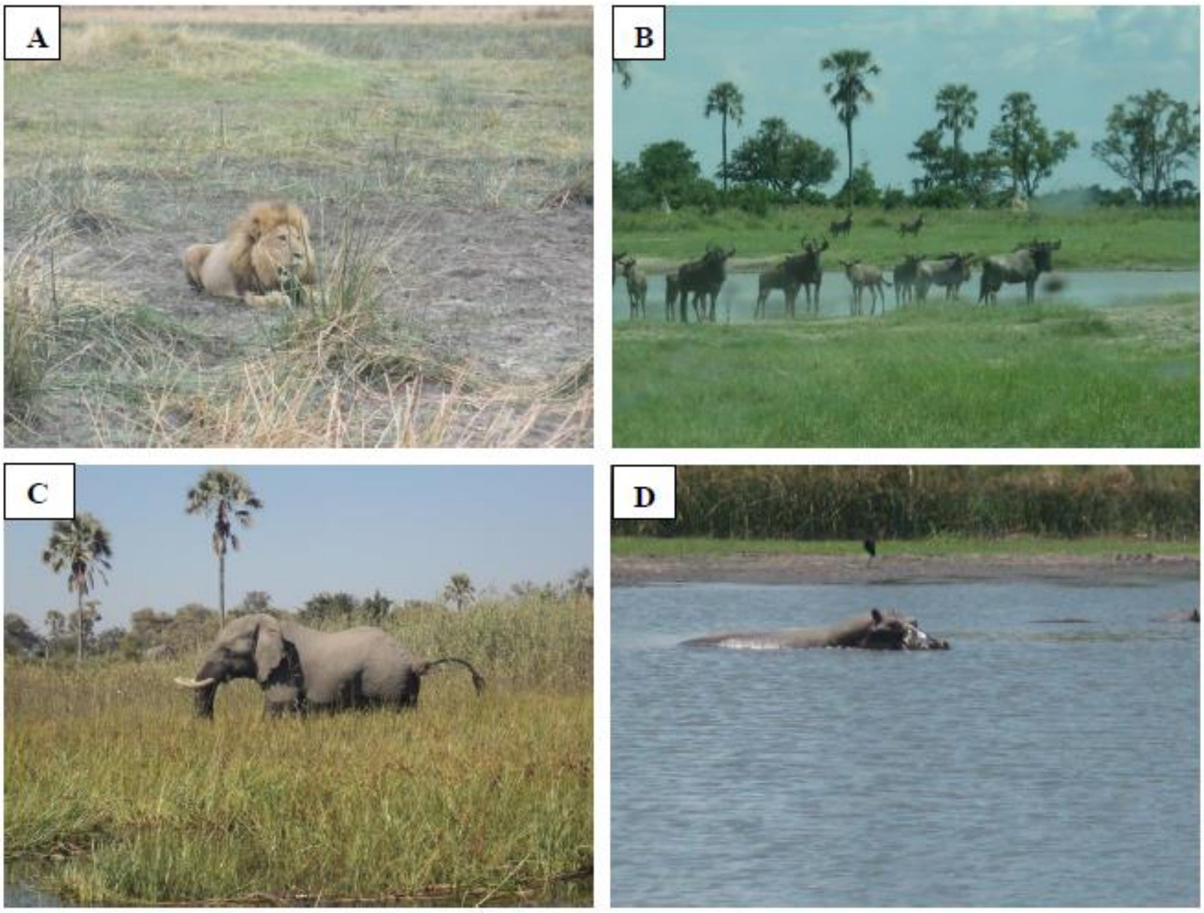


**Figure 3.10**: Some of the biodiversity of Okavango Delta in the Chief's Island region, here showing A: a male lion lying on a dry floodplain, B: Wildebeest near a floodplain pool, C: an elephant walking across the reeds of Boro river and D: a hippopotamus in a floodplain pool. (Photographs: author's own)

### 3.6 The flood pulse and aquatic ecology of the Okavango Delta

Previous studies in the Okavango Delta have demonstrated that the seasonal flooding regime determines ecological dynamics, including species diversity and distributions, in the Delta (Merron 1991, Lindholm *et al.* 2007, Mosepele *et al.* 2009, Davidson *et al*. 2012). The arrival of the flood wave increases the area of aquatic environment and increases habitat complexity, creating new habitat niches for different aquatic taxa. The complexity of aquatic habitats results from vegetation succession that occurs after the arrival of floods, which involves die-back of terrestrial species and rapid growth of wetland reeds and macrophytes. Mladenov (2007) has demonstrated that during high flood conditions, there is greater concentration of dissolved nutrients in seasonal floodplains than in perennial channels. Marazzi (2014) found that greater levels of dissolved solutes promotes greater algal abundances in seasonal floodplains during high water conditions. The level of nutrients in perennial lagoons and channels has, however, been shown to increase with the arrival of the floods leading to improved biological production (Cronberg *et al.* 1995). In flood plains, the arrival of floods facilitates hatching of zooplankton egg banks (Siziba *et al*. 2013) and promotes high zooplankton abundance because of the high primary production rates associated with the floods (Mosepele *et al.* 2009). Fish of the Okavango Delta migrate from permanent channels and perennial pools to spawn in the vegetated floodplains during the high-water conditions because the dense floodplain vegetation and zooplankton provide refuge from piscivores and provides food resources for juvenile fish. The seasonal flood wave is therefore considered a major driver abundance and distribution dynamics of aquatic biota in Delta (Merron *et al.* 1990, Mosepele *et al.* 2009)

Despite the research efforts to understand the role of the seasonal flood pulse on the ecology of the Delta, few studies have investigated its aquatic food webs, although notable exceptions include Gilmore (1979), Lindholm *et al.* (2007) and (Taylor *et al*. 2017). Gilmore *et al.* (1979) examined fish diets at monthly intervals for a year at two lagoons in the lower Boro and concluded that the Delta's food webs are balanced with a greater proportion of herbivorous fish compared to fish of higher feeding levels. The study also observed that fish of the Delta temporally switch diet, a phenomenon that allows them to adapt to the seasonally dynamic nature of the Delta's aquatic environment. Lindholm *et al.* (2007) examined the basic food web properties at Phelo flood plain in the Chief's Island region during two consecutive years, the first with a small flood wave and the following year with a large flood wave. The study demonstrated that flood size is an important factor determining basic properties of food webs in the Delta. Primary production was shown to be higher during a year of less flooding due to greater nutrient concentrations (resulting from low dilution), whereas the year of greater flooding favoured fish production due to improved access to food and habitat resources in the floodplains. The accessibility of floodplains and the generally greater inundation periods of aquatic habitats during a year of a large flood wave ensured fish reproductive success. More recently, Taylor *et al.* (2017) compared the aquatic food web structure of one of the Delta's tributaries, the Kavango river, with that of Kwando and Zambezi rivers to examine the influence of different flooding regimes on the structure of aquatic food webs. The comparative study showed that fish of the Kavango river utilised a broader range of basal carbon sources including riparian $C_4$ grasses and that among the other rivers, Kavango river had relatively high trophic diversity. Although the study was based on data that was collected only once at each of the rivers, the results have demonstrated the strong influence of flooding regimes on aquatic food web structure within the Okavango River basin.

Despite the contribution of the food web studies undertaken within the Okavango system thus far, our understanding of the Delta's aquatic food webs is still limited. The food web studies undertaken within the Okavango Delta, have only examined some of the basic food web properties only helping us to have a general idea of the food webs. Fundamental properties of food web structure such as food chain length, relative importance of different carbon sources, as well as niche sizes of aquatic consumers have not been quantified. Quantification of these food web properties against flooding regimes is fundamental to understanding dynamics of the Delta's ecosystem function. My PhD contributes towards this understanding by quantifying properties of food web structure, during different flooding phases, in aquatic habitats of different flooding regimes within the Delta.

# CHAPTER FOUR: Study objectives, design and the structure of thesis results

This PhD research is based on the hypothesis that aquatic food webs in flood pulsed ecosystems vary across habitats with contrasting flooding regimes. I test the hypothesis through quantifying food web properties of aquatic habitats with different flooding regimes in the Okavango Delta during high flood and low flood seasons. The specific objectives of the thesis are as follows:

## 4.1. Study objectives

1. To examine variability of primary productivity at the selected study sites of different flooding regimes during high flood and low flood
2. To compare the relative contribution of different basal carbon sources to aquatic food webs at the study sites during high flood and low flood
3. To compare aquatic food web structure, using food web matrices, at the study sites during high flood and low flood

## 4.2 Study design

### Rationale for study site selection

To address the above aims, sampling sites were chosen from two regions of the Delta with different flooding regimes: i) the Chief's Island in the mid part of the Delta where there are perennial channels and flood plains of variable water permanence regimes and ii) Lake Ngami, which is a semi-ephemeral lake at the downstream southwest end of the Delta.

One of the reasons for choosing study sites from the Chief's Island region is because the region as a high diversity of aquatic habitats with varying water permanence regimes, suiting my study's objective of comparing food web properties across flooding regimes. Another major reason for choosing sites from the Chief's Island is because it is a hydrological monitoring site for the Okavango Research Institute (ORI) and has been a study site for many ecological studies undertaken by ORI researchers and international researchers, hence providing an opportunity for building onto previous research and where possible an opportunity to use previously collected data. In fact, previous isotope data do exist for the selected sites at the Chief's Island, collected during the latter stages of previous programmes, but I did not use it because the statistical methods that I chose to use (Bayesian mixing models), required the isotope data sets of prey and consumers to have been collected at the same time (see chapter 2).

Lake Ngami was chosen because of its unique flooding regime, it is semi ephemeral on long term basis. The lake has been ephemeral throughout the 20th century with some extended periods of dryness, for example the lake was completely dry from 1989 to 2004 (Shaw *et al.* 2003, Wolski and Murray-Hudson 2006). Annual floods arrive at this lake in June, reaching their maximum in August and lasting anywhere from October to May. Since flooding in 2004 the lake has been permanently flooded and has been supporting commercial fishing, with the collected fish being sold to local and regional markets such as in Angola and Zambia (Kgamanyane 2015). However, in 2015 the government of Botswana banned the commercial fishing due to fears that the fish resource was being over exploited by illegal fishermen, often from foreign countries (Merron 2018). The ban was lifted in December 2016 and only fishermen with valid fishing licences were permitted to fish from the Lake. A scientific study of Lake Ngami food webs is therefore important to support fisheries management decisions at the lake.

### Rationale for temporal selection

One of the aims of this study was to examine the influence of the seasonal flooding regime on aquatic food webs within the Delta, therefore field data was collected at two extreme ends of the Delta's flooding cycle: during the flooding phase when there is greater water volume and extent (hereafter referred to as high flood) and during the dry phase when water is restricted to perennial aquatic habitats (hereafter referred to as low flood). High flood sampling was undertaken in the winter season between June and August whereas low flood sampling was done during warmer summer months of December and January (See Table 5.1 in the following section).

## 4.2. Thesis results structure

Corresponding to the objectives outlined above, I summarise the relevant data chapters as follows. **Chapter 5** compares primary productivity rates and its explanatory environmental variables across the study sites during high flood and low flood. Primary productivity is an ecosystem property which is very influential on the structure of aquatic food webs. For example, the rate of algal primary production and its distribution between the pelagic and benthic parts of aquatic habitats respectively, determine food chain length and whether aquatic food webs are benthic or pelagic based. Quantifying primary production therefore is a crucial foundation for interpreting patterns of food web properties. This chapter also assesses the influence of potential explanatory factors affecting primary productivity, including dissolved nutrients and water transparency at the selected sites. Part of the data from this chapter has recently been published as a chapter (Mazebedi *et al.* 2018) in a book that analysed the ecosystem services at Lake Ngami (Kgathi *et al.* 2018). **Chapter 6** evaluates the relative contribution of basal carbon sources to the Delta aquatic food webs, based on stable isotope analyses (SIA) approach. The relative contribution of different basal sources is evaluated using results from statistical analyses of carbon and nitrogen stable isotope ratios of different carbon sources at different sites and of fish muscle tissue. Fish were used as a model consumer, based on their dietary plasticity, their general ubiquity across the different aquatic habitats, their significance in linking benthic and pelagic food webs, and their significance as a critical ecosystem service resource. **Chapter 7** compares food web structure between the selected sites at high flood and low flood using fish stable isotope data. Food web comparisons were made through computation of food web matrices (Layman's matrices) which quantify several food web properties. The matrices were statistically compared between the study sites during low flood and high flood to evaluate the role of the flood pulse on food web properties. Trophic niche size of different fish feeding guilds, and the overlaps of the niches, were also calculated to further examine the influence of the yearly flood pulse on aquatic food web properties. **Chapter 8** summarises main findings of this research and discusses their implications for management of the Delta and for the existing aquatic food web theory for flood pulsed river floodplains. The chapter makes recommendations for follow-up research on aquatic food webs of the Delta

# CHAPTER FIVE: Primary productivity and environmental variability of the study sites

## 5.1. Introduction

In river floodplain systems, seasonal floods cause over spilling of water from the perennial channels to seasonal plains. The seasonal floods physically link perennial channels with seasonally flooded plains, and hence facilitates mixing of water, nutrients and sediment between permanent channels and flanking plains. The resulting heterogenous and dynamic environment influences biogeochemical cycling and influences the structure of aquatic communities (Winemiller 2004, Davies *et al.* 2008). During the rise of floods and then their gradual recession, aquatic habitats are created of varying water permanence. These range from perennial water channels, plains retaining surface water for a few months, perennial instreams, and isolated floodplain lagoons (Junk *et al.* 1989, Hill *et al.*, 2016). The natural flooding regime of river floodplains is viewed as the main driver of biological production in flood pulsed floodplain rivers (Junk *et al.* 1989, Tockner *et al.* 1999). However, threats of human disturbance such as large-scale water withdrawals for consumption and industrial use (Mitchel 2013, Kafumbara *et al.* 2014), water regulation (Bunn and Arthington 2002) and of climate change effects (Hamilton 2010, Niang *et al*. 2014) threaten flooding regimes and hence biological productivity of river floodplains. Prediction and management of the ecological impacts of these hydrological threats necessitate an in-depth understanding of the relationships between flooding regimes and ecological processes.

The process through which solar or chemical energy is converted into biochemical energy by photosynthetic and chemosynthetic organisms (Davies 2003), is one of the key ecosystem processes in wetlands, and is influenced by flooding regimes. The process is often referred to as primary productivity when it is measured within periods of less than a year, and as primary production when measured over longer periods. Since the focus of this thesis is more on seasonal variations, the term primary productivity will be used throughout this thesis. Primary productivity is a crucial ecosystem process because it forms the base of aquatic food webs, determines the structure of aquatic communities, and the stability of aquatic ecosystems (Shapiro 1980, Carpenter *et al.* 1987, Post *et al.* 2000). Primary productivity also determines the availability of important ecosystem services such fish yields though bottom-up trophic processes (Horne and Goldman, 1994, Gomes *et al*. 2002, Jackson *et al.* 2013). In floodplain rivers, the bulk of primary productivity is known to occur in seasonal swamps whereas relatively less primary productivity occurs in perennial swamps (Junk 1989, Burford *et al.* 2007, Jackson *et al.* 2013). Flooding frequency and the duration of the floods also determine rates of aquatic primary productivity (Robertson *et al*. 2001, Ahearn *et al.* 2006). The relative importance of instream algal primary productivity versus allochthonous organic matter to aquatic food webs is also known to vary with the stage of flooding (Thorp and Delong 1994, Humphries *et al*. 2014). While the link between primary productivity and wetland hydrology is evident, precise predictions of how primary productivity of a system may respond to human and climatic disturbances will need a thorough understanding of local controls of primary productivity.

At a local scale, several abiotic environmental variables such as the level of dissolved nutrients, water temperature and turbidity (Higgins *et al.* 2014, Hessen 2013, Hall *et al.* 2015) have been identified as important determinants of algal primary productivity rates in freshwater systems. Generally, an increase in the level of dissolved nutrients, including nitrates and phosphates, promote growth and biomass of algal cells in the water column (Hansson 1992). Phosphate is particularly important in freshwater systems where it occurs in low levels and can be a limiting factor to rates of primary productivity (Carpenter and Capone 1983, Elser *et al.* 2007, Moss *et al.* 2013). Physical processes such as resuspension of sediments, release nutrients trapped in benthic sediments and therefore can influence the level of dissolved nutrients in the water column (Orihel et al. 2015). Biotic factors such as food web interactions also influence changes in rates of algal primary productivity. An increase in the abundance of large-bodied zooplankton such as *Daphnia* spp. (Mazumber and Havens 1988) and filter feeders such as dressenids (Higgins *et al.* 2014) have been shown to suppress phytoplankton biomass through predation pressure, and ultimately reduce primary productivity in the water column. The high environmental heterogeneity of floodplain habitats can be expected to vary greatly in algal primary productivity.

Algal primary productivity can also be described in terms of its distribution between the water column and the benthic surface of an aquatic habitat, referred to as an autotrophic structure (Althouse *et al*. 2014). Autotrophic structure is a very important ecosystem property because it can determine whether aquatic food webs are pelagic based or benthic based (Brothers *et al.* 2016). This key ecosystem property is generally under-studied in freshwater systems but has progressively gained recognition in recent times (Vadeboncoeur *et al*. 2001, Hilting *et al*. 2013, Althouse *et al.* 2014, Brothers *et al.* 2016). Autotrophic structure can be affected by both abiotic and biotic factors. For example, an increase in dissolved nutrients has been shown to promote high pelagic primary productivity while at the same time supressing benthic primary productivity because of the increase in water turbidity that results from high phytoplankton density limits light availability to lower depths (Vadeboncoeur *et al*. 2001, Althouse *et al.* 2014). Biotic factors, such as increased grazing pressure that may result from establishment of an invasive grazer such as zebra mussel (Higgins and Zanden 2010) or compositional change of zooplankton communities from small bodied to large bodied zooplankton (Higgins *et al.* 2014), can promote benthic productivity by reducing turbidity from high phytoplankton densities, and hence increasing light availability for benthic algae. Given its strong link with aquatic food web dynamics, knowledge of autotrophic structure patterns can very useful for comprehending spatial and temporal variations of aquatic food web properties.

This chapter aims to examine variability of pelagic and epipelic algal primary productivity as well as physical and chemical parameters across sites of varying flooding regimes in the Okavango Delta. Variability of physical and chemical parameters are examined to evaluate the influence of potential explanatory variables on primary productivity and distribution. Since physical and chemical variables are known to influence the composition of aquatic communities (Townsend *et al.*1997), the results on environmental variables will also be useful for environmental characterisation of sites to aid interpretation of food web properties in the chapters that follow. Pelagic primary productivity is expected to increase with the level of dissolved nutrients such that floodplains and can be expected to have higher pelagic primary productivity that the perennial channels. Since the concentration of dissolved solutes increases with the downstream distance (Sawula *et al.* 1991, Masamba and Mazwimavi 2008, Mackay *et al.* 2011), higher pelagic primary productivity can also be expected at downstream sites due to a greater concentration of dissolved nutrients. Epipelic primary productivity is however expected to show an opposite trend to that of pelagic primary productivity, because at sites of greater pelagic primary productivity, hence greater turbidity, there is relatively low amounts of light available for benthic photosynthesis. Because I was able to undertake work on Lake Ngami due to it being permanently flooded only since 2004, there is a special focus on seasonal variability on epipelic and pelagic primary production there.

## 5.2. Objectives

1. To examine the variability of pelagic and epipelic primary productivity across study sites during high and low flood phases
2. To focus in on understanding epipelic and pelagic primary productivity in the recently flooded Lake Ngami

## 5.3. Methods

### Study sites

Field data for this PhD research was collected from two areas of the Delta – the Chief's Island area, and Lake Ngami (Fig. 5.1). At Chief's Island, data were collected from a perennial lagoon in the Boro main channel (Nxaraga lagoon) and from a seasonal floodplain (Phelo floodplain) with a central perennial lagoon (Hippo pool). The study sites are described as follows.

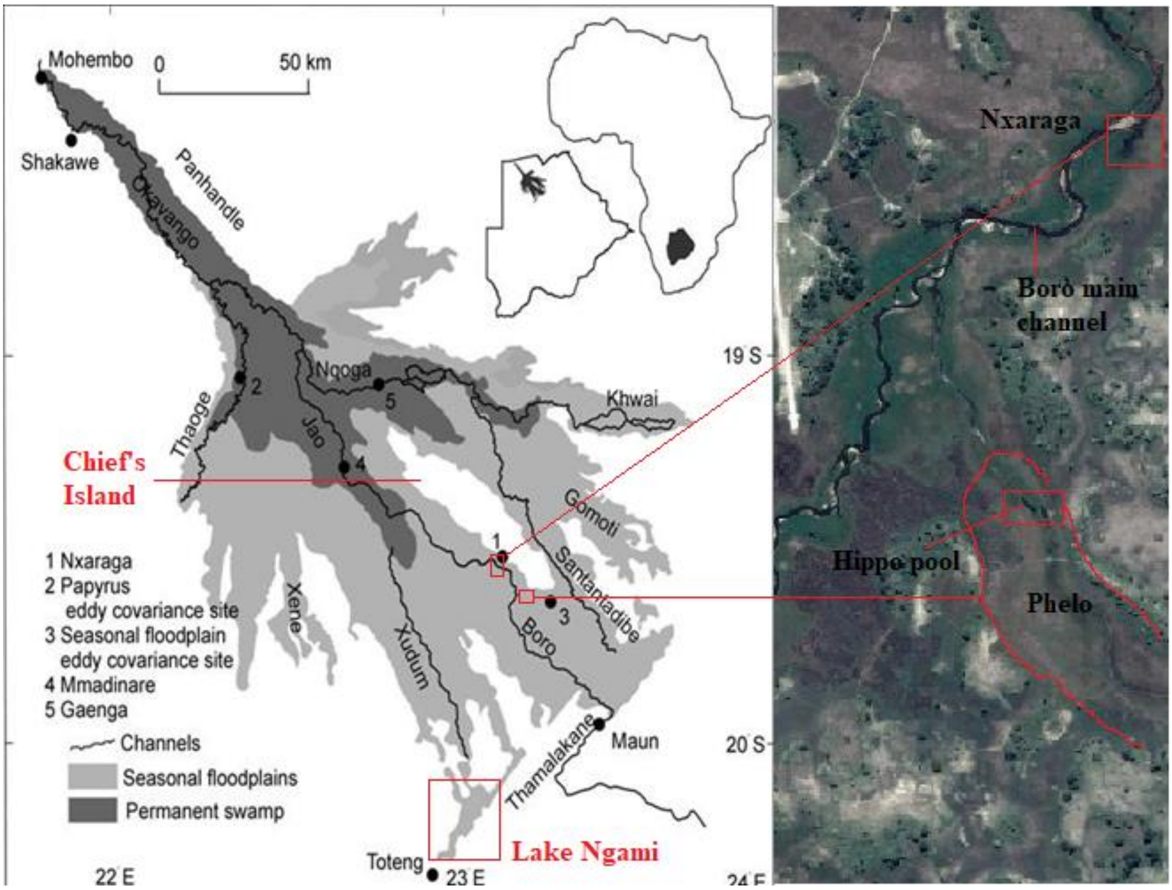


**Figure 5.1**: Map of the Okavango with study sites marked in red rectangles

## Phelo floodplain

Phelo (19°31'S, 23°05'E) is a seasonally inundated floodplain of about 0.8 km$^2$ on the south west of Chief's Island, in the mid region of the Okavango Delta. Phelo floodplain flanks the Boro main channel on the west and connects with the main channel at high flood season through a narrow side channel of about 0.5 km straight line distance. The annual flood pulse inundates the floodplain every year, during the drier winter months from May to August, whereas low water conditions occur during hot summer months between September and February. The average maximum temperature for the hottest month (December) is 35 °C and the average minimum temperature for the coldest month and occurs in July (Mogotsi *et al.* 2011). From late September, the vegetated plains dry off, and surface water becomes restricted only to a small central pool. The central pool of about 50m diameter at high flood and half the size at low flood, is commonly referred to as the Hippo pool (Figure 5.2). It is a home to a group of hippopotami which keep the pool open with few sparsely floating *Nymphea* species and stands of reeds growing around. During dry, hot months, Hippo pool is shallow and muddy measuring only up to 80 cm in depth. During the low water condition, the grassy plains are grazing grounds for herbivores, mostly impala and elephants. Local rains ranging from 250 to 650mm (Bhalotra 1987, Darkoh 1999) occur during the hot months from November to February, and they serve to occasionally saturate floodplain soils and slightly increase water levels of Hippo pool.

**Figure 5.2**: Phelo floodplain and hippo pool lagoon during high flood season. (Photographs: Author's own).

**Nxaraga lagoon**

Nxaraga lagoon (19°32' S 23° 1' W) is a perennial pool within the Boro channel on the south west of the Chief's Island (Figure 5.3). It is about the same size as Hippo pool with water depth varying between c. 2m at high flood and 1.5m at low flood. The high flood and low flood climatic conditions are like those of Phelo floodplain, given their nearby locations. The lagoon is surrounded by relatively dense emergent vegetation of reeds (mostly *Schoenoplectus corymbosus* and *Cyprus articulatus*), *Miscanthus* and *Vossia*. (Bonyongo *et al.* 2001).

**Figure 5.3**: Nxaraga lagoon. (Photograph: Author’s own).

## Lake Ngami

Lake Ngami (20°30' S 22° 44' W) is an endorheic lake located at the distal end of the south west tributary of the Delta, the Xudum tributary. The lake is an open water body about 20km long and 8 km wide. The lake has a maximum depth of about 3m in the central open region, with the shallower, littoral region occupied mainly by drowned *Acacia* species woodland (Figure 5.4). The lake is virtually devoid of aquatic vegetation apart from a few peripheral islands of *Typha* sp. Annual floods fill the lake from around June and it reaches its maximum flooding in August before declining noticeably from October. The extent of lake flooding depends on the size of the flood wave reaching the Panhandle and on the redirection of water in the Delta to the western distributaries that flow into the lake (Shaw et al. 2003; Wolski *et al*. 2014). The lake has been ephemeral throughout the 20th century with some extended periods of dryness, including from 1989 to 2004 (Shaw *et al.* 2003, Wolski and Murray-Hudson 2006). It has only been permanently flooded since 2004.

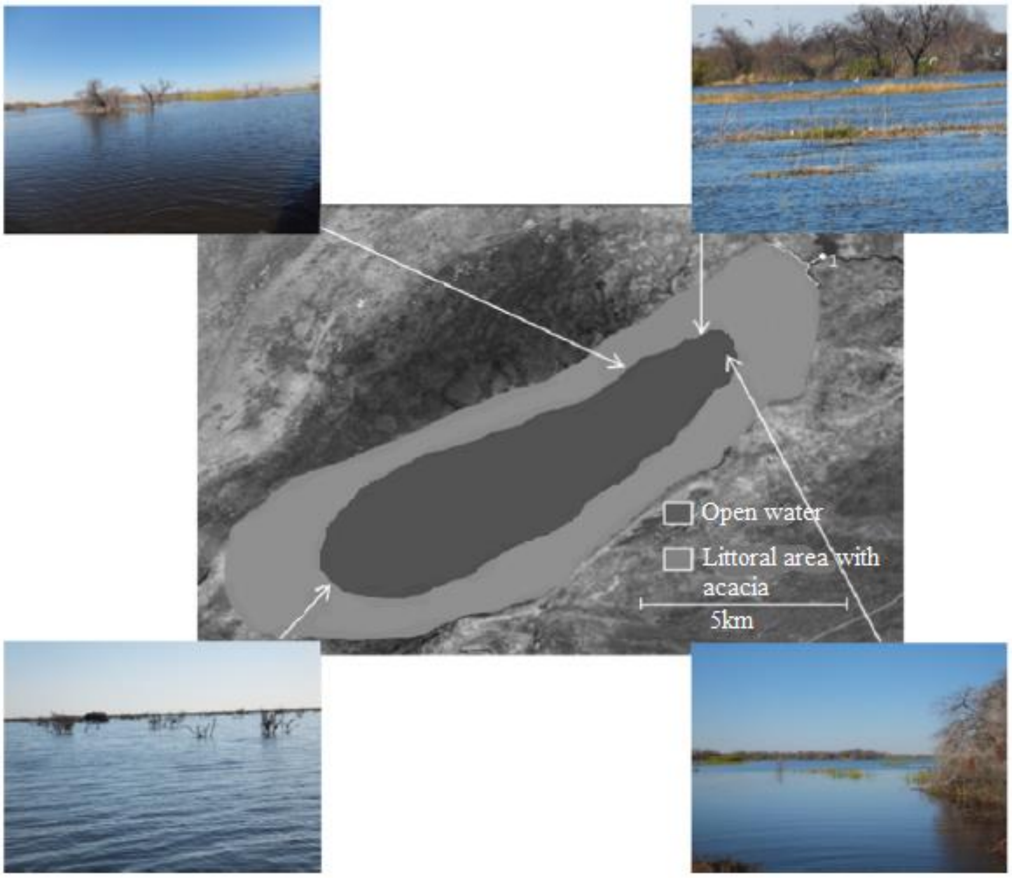


**Figure 5.4**: Lake Ngami picture showing open water and littoral area with dead acacia (Re-drawn from Meier *et al.* 2014). (Photographs: Author's own).

### Methods for measuring primary productivity and selected environmental variables

Algal primary productivity and associated environmental variables, whose quantities either influence (e.g. dissolved nutrients) or are influenced by primary productivity rates (e.g. water turbidity) were measured at selected points from the study sites. Both primary productivity data and data for environmental variables were collected during high flood (August 2015, two fieldwork trips lasting a week each) and low flood (January 2016, two fieldwork trips one lasting two days and the other lasting a week) (Table 5.1). Environmental variables include secchi depth (Secchi, m), water temperature (Temp, °C), water depth (depth, m), chlorophyll-a concentration (Chla, $\mu$g/l), sedimentation rate (g/m$^2$/month), dissolved silica (DSi, mg/l), dissolved nitrates ($NO_3^-$, mg/l), dissolved phosphates ($PO_4^{2-}$, mg/l), conductivity (EC, $\mu$S/m, were water pH (pH), alkalinity ($HCO_3^-$, mg/l) and dissolved oxygen (DO, mg/l). Algal primary productivity and some of the selected environmental variables were determined on site whereas for some environmental variables, such as dissolved nutrients, water samples were collected for laboratory analyses (Laboratory variables) (Table 5.1). Specific methods for the measurements of primary productivity and the enviromental variables are described in the following sections.

**Table 5.1**: Field work dates and the number of measurements done for each selected parameter during each field work campaign. *n.m* indicates that a parameter was not measured during the fieldwork campaign.

| | **Parameter** | **Ngami** | **Nxaraga** | **Phelo** |
|---|---|---|---|---|
| **Campaign1:**<br>**High flood (**29 Jun to 18 Aug 2015) | Pelagic productivity incubations | 15 | 6 | 6 |
| | Epipelic productivity incubations | *n.m* | *n.m* | *n.m* |
| | pH, DO, EC, Temp, Depth | 16 | 8 | 12 |
| | Sedimentation traps | 9 | 6 | 3 |
| | Laboratory variables | 20 | 16 | 13 |
| **Campaign 2:**<br>**Low flood (**11th to 29th Jan 2016) | Pelagic productivity incubations | 15 | 6 | 3 |
| | Epipelic productivity incubations | 3 | 3 | 3 |
| | pH, DO, EC, Temp, Deptrh | 90 | 50 | 50 |
| | Sedimentation rate | *n.m* | *n.m* | *n.m* |
| | Laboratory variables | 13 | 5 | 10 |
| **Campaign 3:**<br>**High flood:** | Pelagic primary productivity | *n.m* | *n.m* | *n.m* |
| | Epipelic primary productivity | 6 | 3 | 5 |
| | pH, DO, EC, Temp, Depth | 6 | 3 | 5 |
| | Sedimentation rate | *n.m* | *n.m* | *n.m* |
| | Laboratory variables | *n.m* | *n.m* | *n.m* |

## Pelagic primary productivity

Pelagic primary productivity was estimated using the light and dark bottles method (Wetzel and Likens 2010). To estimate productivity, six clean 300ml glass bottles, three transparent (light bottles) and three light insulated bottles (dark bottles), were filled with instream water by dipping them to subsurface depth. The dark bottles were covered with aluminum foil then wrapped in black insulation tape. After filling all the bottles with water, they were stoppered while underwater and then incubated for three hours at 0.5m depth. The incubation was terminated by adding five drops of Lugols's iodine solution. Dissolved oxygen (DO) was measured before and after incubation using a DO probe (Probe Type: LDO10101) connected to a Hach HQ40d multi-parameter field meter (±0.1 to 8 ppm; ±0.2 above 8 ppm). Gross primary productivity (GPP), net primary productivity (NPP) and respiration (R) were then calculated based on the change in DO concentration during the incubation time using the equations 1-3:

$$\text{GPP (mgC/l/h)} = \frac{[(LB)-(DB)]\,(0.375)}{(PQ)(t)} \tag{1}$$

$$\text{NPP (mgC/l/h)} = \frac{[(LB)-(IB)]\,(0.375)}{(PQ)(t)} \tag{2}$$

$$\text{R (mgC/l/h)} = \frac{[(IB)-(DB)]\,(0.375)}{(t)} \tag{3}$$

Where:

LB = final DO concentration in light bottle
DB = final DO concentration in dark bottle
IB = initial concentration of DO
t = time in hours
PQ = Photosynthetic coefficient of 0.12
0.375= the ratio of moles of carbon to moles of oxygen (12mg/32mg)

**Epipelic primary productivity**

Incubations for epipelic primary productivity measurements were made on selected sampling locations towards the water margins at all study sites. Preferred depths did not exceed waist level for several reasons: (i) previous research has shown epipelic primary productivity to be greatest at shallow peripherial sites (Althouse *et al.* 2014); (ii) it is logistically challenging to install and monitor epipelic incubations at depths above waist level, a challenge that can lead to errornous estimates; (iii) there is increased risk of animal attack from corocodiles and hippos in deeper central sites where their movements may be more unnoticeable. Epipelic primary productivity and respiration were estimated in a similar method to that used for pelagic measurements, except that for epipelic estimations acrylic hemisphere domes were used (Figure 5.5) (Althouse *et al.* 2014). The domes were of 30 cm diameter and 7l volume, and each had a 220v powered water pump attached on the inside to homogenise water during incubations. The epipelic productivity incubations were made by pressing the base of the hemisphere domes into the benthic substrate by 10cm where there was no emergent vegetation to ensure that the estimations were mainly for epipilic primary production. The epipelic productivity measurements were therefore measuring mainly epipelic primary production. A DO meter probe was then inserted at the top opening of the dome tightly to reduce water exchange between the inside and the outside of the domes. DO measurements were then recorded *in situ* at 15-minute intervals for an hour.

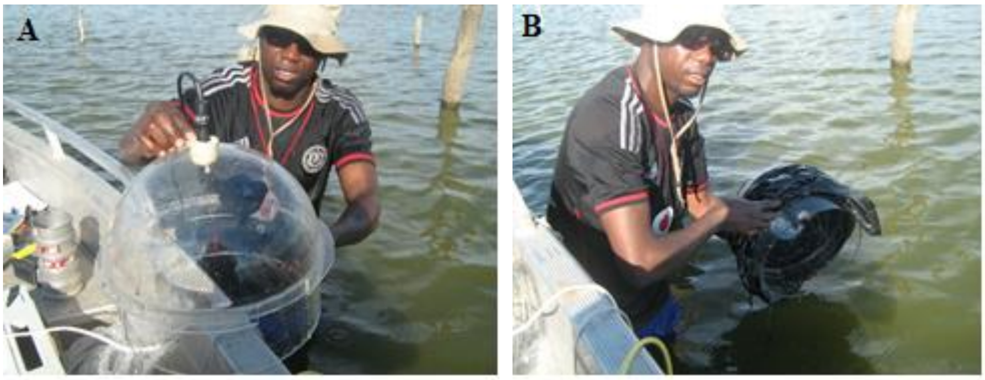


**Figure 5.5**: Acrylic hemisphere domes that were used for epipilic productivity incubations. In A the dome is transparent for light incubations, in B the dome is covered with a black plastic for dark incubations. (Photographs: Author’s own).

## Environmental variables measured in the field

A Hach HQ30d multiparameter field meter was used to measure water pH, DO, EC, and temperature *in-situ* at each study site. To ensure accuracy of the measurements, the meter was re-calibrated for each field trip. Water depth was measured using a calibrated pole approx. 3.5m in length. A calibrated wood pole was prefered for measuring veritical water depth to avoid inaccuracies that may result from reflections from submerged plants when using equipment based on signal transmission and reflection, such as echo sounder. Water clarity was measured using a 20cm diameter Secchi disk attached to a 3m tape measure. The parameters were measured during sunny conditions, between 11 am and 3pm, in August 2015 at high water conditions (Highflood) and at the same times of day in January 2016 at low water conditions (Low flood).

Sediment traps were deployed for a month at each site to estimate the rate of sedimentation. Sediment traps were each made from three PVC tubes of length 30 cm and of 5 cm diameter (Fig. 5.6) making a recommended length-diameter ratio of 6 (Ardiles *et al.* 2013). The tubes were each closed tightly at the bottom with a PVC lid. The trap was then set at 50cm below water surface by tying the tubes onto a 3m wooden thatching pole and hammering the pole about 30 cm into the benthic substrate. The traps were set for a month at each site. The trapped sediments were then collected into a glass beaker, put in a fridge and allowed to settle overnight. The supernatant was then decanted, and the remaining sediment was freeze-dried and weighed. Sedimentation rate was then estimated as sediment dry weight per area of trap per day (g $m^{-2}$ $day^{-1}$). The dried sediments were ground and kept in cold dark storage until it was analysed for stable isotopes of carbon (C), (N) and C:N ratios. The C:N ratio of sediments can be used as a measure of sediment biodegradability ( Meyers and Eadie 1993, Danger *et al*. 2012), with lower C:N values indicating more degradable sediments, and high C:N values indicating less degradable ones. Data on sediment traps is only available for high flood because sediment traps deployed at low flood were found missing, probably destroyed by animals.

**Figure 5.6**: Sediment traps made from PVC pipes tied to a 3m pole. (Photographs: Author's own)

### Environmental variables measured in the labioratory

For measurements of dissolved nutrients; $NO_3^-$, $PO_4^{2-}$ and DSi, three water samples were collected at subsurface depth of 20 cm, into acid washed 300ml bottles at each sampling location of the study sites. At each study site, water was also collected into a 1 liter bottle for laboratory determination of Chla. The bottles containing the water samples were stored in an ice filled cool box for transportation to Okavango Research Institute's (ORI) environmental laboratories. At ORI, parameters were analysed by ORI staff following standard methods as described in (American Public Health Association (APHA), 1999).

### Statistical analyses

Data for both primary productivity and environmental variables were initially tested for normality using Shapiro-Wilk test in SPSS 24. After determining that all the datasets were not normally distributed, non-parametric statistical tests were used. A Kruskal-Wallis test was used for comparisons of primary productivity and respiration measurements across study sites, benthic and pelagic habitats, and between flood seasons. Non-parametric tests such as Kruskal Wallis test are suitable for comparing non-normally distributed data (Sheskin 2010). The statistical comparisons together with summary statistics, were all performed in R software (R core team, 2018). Comparisons were visualised by box plots drawn using ggplot2 package in R software version 3.4.0 ( R Core Team 2018).

## 5.4. Results

### Spatial and seasonal variability of primary productivity

### Pelagic primary productivity

Pelagic productivity was greatest at lake Ngami with an average of *p*NPP of 0.062 ± 0.05 and 0.11 ± 0.01 mgC/l/h during high flood and low flood respectively compared to 0.025± 0.01 and 0.016 ± 0.01 mgC/l/h at Nxaraga lagoon during high flood and low flood respectively and 0.028 ± 0.05 and 0.043 ± 0.01 mgC/l/h during high flood and low flood respectively at Phelo flood plain (Table 5.2). Significant differences between Nxaraga lagoon and Phelo flood plain in terms of pelagic primary productivity parameters were only observed during low flood when *p*NPP was greater in Hippo pool at Phelo floodplain (Mann-Whitney test, $p = 0.02$). Pelagic respiration was greater in Lake Ngami across both flooding seasons whereas rates of pelagic respiration were not significantly different between Nxaraga and Phelo floodplain (Table 5.2). In terms of seasonal differences at each site, pelagic primary productivity was generally greater during low flood season at all study sites although the seasonal difference was significant only for Lake Ngami and Phelo flood plain (Table 5.2).

**Table 5.2**: Comparisons of pelagic primary productivity parameters within each study site and across study sites during high flood and low flood. For each study site, significant change in seasonal parameters are indicated by arrows which point up or down to show direction of change in the level of the parameter relative to its level during the other flood season. Arrows in bold indicate significant seasonal difference based on Mann-Whitney comparisons at p = 0.05. The results of statistical comparison of the parameters across study sites using Mann-Whitney test are shown in separate columns with significant values in bold.

| | Lake Ngami (**L**) | Nxaraga (**N**) | Phelo (**P**) | Mann-Whitney test, *p* = 0.05 | | |
|---|---|---|---|---|---|---|
| | mgC/l/h ±SD | mgC/l/h ±SD | mgC/l/h ± SD | | | |
| | | | | **L** vs **N** | **L** vs **P** | **N** vs **P** |
| **High flood** | n= 15 | n = 9 | n= 9 | | | |
| *p*GPP | 0.11 ± 0.07**↓** | 0.044± 0.02↓ | 0.049± 0.03**↓** | **0.0005** | **0.001** | 0.5 |
| *p*NPP | 0.062 ± 0.05**↓** | 0.025± 0.01↑ | 0.028 ± 0.05↓ | **0.02** | **0.05** | 0.7 |
| *p*R | 0.051 ± 0.05↓ | 0.019 ± 0.01↓ | 0.021 ± 0.05↑ | **0.05** | **0.007** | 0.9 |
| **Low flood** | n= 15 | n= 6 | n= 3 | | | |
| *p*GPP | 0.17 ± 0.03**↑** | 0.049 ± 0.01↑ | 0.075 ± 0.01**↑** | **0.0009** | **0.002** | **0.02** |
| *p*NPP | 0.10 ± 0.01**↑** | 0.016 ± 0.01↓ | 0.043 ± 0.01↑ | **0.0008** | **0.002** | **0.02** |
| *p*R | 0.058 ± 0.02↑ | 0.033 ± 0.01↑ | 0.032 ± 0.01↓ | **0.003** | **0.007** | 0.9 |

## Epipilic primary productivity

While there were variations in epipilic gross primary productivity (*e*GPP) and epipelic respiration (*e*R) between sites and between seasons, there was no significant difference in epipelic net primary productivity (*e*NPP) between study sites during all flood seasons and seasonal differences in *e*NPP were only significant in Lake Ngami (Fig. 5.7). In all the study sites *e*NPP was below zero, with more negative values observed for Phelo floodplain (high flood *e*NPP: -0.077 ± 0.08 mgC/l/h, low flood *e*NPP: -0.065 ± 0.03 mgC/l/h) and Lake Ngami (high flood *e*NPP: -0.068 ± 0.06 mgC/l/h, low flood *e*NPP: -0.12 ± 0.09 mgC/l/h) compared to Nxaraga lagoon (high flood *e*NPP: -0.035 ± 0.02 mgC/l/h, low flood *e*NPP: -0.058 ±0.01 mgC/l/h). *e*GPP and *e*R were greater at lake Ngami compared to the other study sites although the level of R at the Lake was comparable to that measured at Phelo flood plain during both high and low flood (Table 5.3, Fig. 5.7). In terms of seasonal variations, *e*NPP at Lake Ngami was significantly greater during high flood than during low flood whereas at Nxaraga lagoon and Phelo floodplain *e*NPP only slightly increased and decreased respectively during high flood compared to low flood (Table 5.3, Fig. 5.7). *e*GPP was generally greater at high flood across all the sites even though the seasonal differences were statistically significant for Lake Ngami and Phelo flood plain only. At Nxaraga lagoon, *e*R was significantly greater during low flood (Table 5.3, Fig. 5.7).

**Table 5.3:** Comparisons of epipilic primary productivity parameters within each study site and across study sites during high flood and low flood. For each study site, significant change seasonal parameters are indicated by arrows which point up or down to show direction of change in the level of the parameter relative to its level during the other flood season. Arrows in bold indicate significant seasonal difference based on Mann-Whitney comparisons at p = 0.05 using. The results of statistical comparison of the parameters across study sites using Mann-Whitney test are shown in separate columns with significant values in bold

| | Lake Ngami (**L**) | Nxaraga (**N**) | Phelo (**P**) | Mann-Whitney test, *p* = 0.05 | | |
|---|---|---|---|---|---|---|
| | mgC/l/h ±SD | mgC/l/h ±SD | mgC/l/h ± SD | | | |
| | | | | **L** vs **N** | **L** vs **P** | **N** vs **P** |
| **High flood** | n= 9 | n= 5 | n= 12 | | | |
| *e*GPP | 0.10 ± 0.08**↑** | 0.026 ± 0.01↑ | 0.068 ± 0.05**↑** | **0.05** | 0.09 | **0.04** |
| *e*NPP | -0.068 ± 0.06**↑** | -0.035 ± 0.02↑ | -0.077 ± 0.08↓ | 0.42 | 0.64 | 0.56 |
| *e*R | 0.17 ± 0.06↓ | 0.061 ± 0.015**↓** | 0.144 ± 0.08↑ | **0.003** | 0.50 | 0.06 |
| **Low flood** | n= 6 | n= 3 | n= 5 | | | |
| *e*GPP | 0.089 ± 0.2**↓** | 0.021 ±0.01↓ | 0.025 ±0.02**↓** | **0.03** | **0.02** | 0.37 |
| *e*NPP | -0.12 ± 0.09**↓** | -0.058 ±0.01↓ | -0.065 ± 0.03↑ | 0.37 | 0.23 | 1.0 |
| *e*R | 0.21 ± 0.05↑ | 0.079 ± 0.01**↑** | 0.09 ± 0.08↓ | 0.05 | **0.02** | 0.76 |

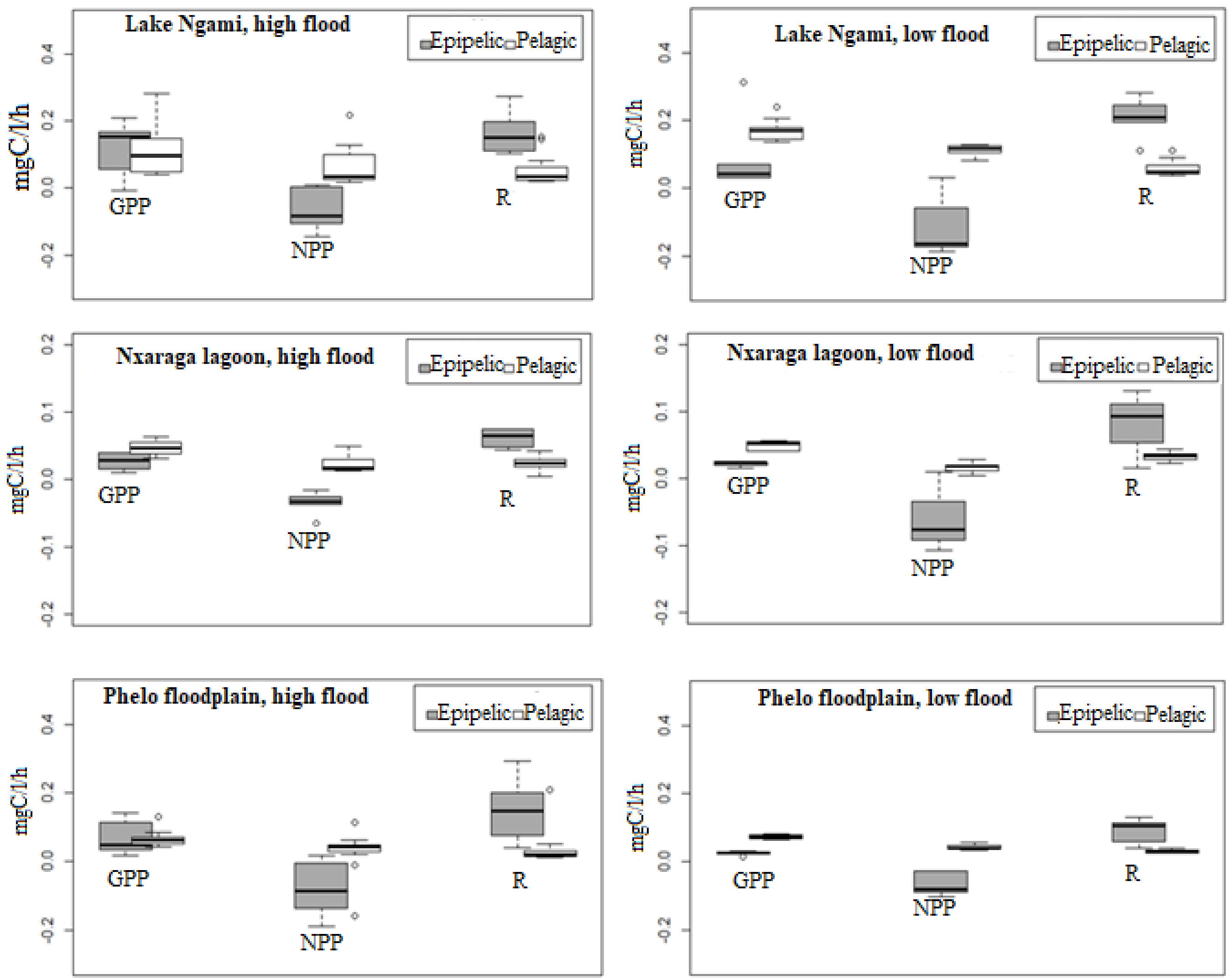


**Figure 5.7**: Box plot comparison of pelagic and epipilic primary productivity parameters within each study site during high flood and low flood. The middle line represents the median, the upper and lower shaded areas represent the upper and lower quartiles respectively, the extending solid line represents the range of the data outside the upper and lower quartile range whereas the dots represent outliers

### Spatial and seasonal variability of environmental variables

Mean values and standard deviations of the environmental variables at each study site during both high flood and low flood are shown in Table 5.4. Dissolved nutrients and solutes in general were greater at lake Ngami compared to Nxaraga lagoon and Phelo floodplain. Statistical comparisons using Mann-Whitney test at $p = 0.05$ showed the concentration of $NO_3^-$, DSi and DOC were significantly greater at Lake Ngami than the other two study sites whereas the level of $PO_4^{2-}$ did not vary significantly across study sites. Alkalinity, turbidity and light attenuation rate were also significantly greater at Lake Ngami compared to at Nxaraga lagoon and Phelo flood plain. Between Nxaraga and Phelo flood plain, there were greater levels of alkalinity, pH, DO, turbidity and EC at Phelo floodplain. The mean depth of sampled sites was greater at Nxaraga compared to Phelo flood plain. Comparisons of these variables across study sites during high flood are visualised by boxplots (Fig. 5.8).

**Table 5.4**: Summary data for environmental variables measured at each study site during high flood and low flood.

| | | N | Ngami<br>mean ± SD | N | Nxaraga<br>mean ± SD | N | Phelo<br>mean ± SD |
|---|---|---|---|---|---|---|---|
| **Highflood** | | | | | | | |
| | Alkalinity | 20 | 127.7±19 | 16 | 51.2±2.2 | 13 | 59.7±6 |
| | Chla | 20 | 29.1±13 | 16 | 15.1±9.7 | 13 | 21.7±11 |
| | Depth | 22 | 2.0±0.5 | 8 | 2.2±0.1 | 18 | 1.5±0.7 |
| | DO | 22 | 7.3±1.4 | 8 | 3.9±0.7 | 18 | 5.2±1.7 |
| | DOC | 20 | 12.5±1.4 | 16 | 4.4±0.5 | 13 | 10.4±1.8 |
| | EC | 22 | 282.9±85 | 8 | 63.1±1.9 | 18 | 103.4±76 |
| | Nitrates | 20 | 0.69±0.1 | 16 | 0.30±0.11 | 13 | 0.36±0.07 |
| | Phosphates | 20 | 0.22±0.07 | 16 | 0.17±0.05 | 13 | 0.20±0.1 |
| | pH | 22 | 7.9±0.4 | 8 | 6.6±0.1 | 18 | 7.0±0.3 |
| | Secchi | 22 | 0.28±0.06 | 8 | 1.5±0.3 | 18 | 1.2±0.1 |
| | Silica | 20 | 20.1±5.1 | 16 | 5.7±3 | 13 | 10.5±9.4 |
| | Temp | 22 | 17.5±2.2 | 8 | 21.0±1.8 | 18 | 20.9±3.9 |
| | Turbidity | 8 | 13.0±7.2 | 9 | 0.758±0.5 | 6 | 4.15±1.9 |
| **Lowflood** | | | | | | | |
| | Alkalinity | 13 | 253.0±44 | 5 | 45.0±4.0 | 10 | 121.8±28 |
| | Chla | 13 | 51.1±14.3 | 5 | 24.2±1.9 | 10 | 30.3±4.1 |
| | Depth | 9 | 1.3±0.5 | 5 | 1.2±0.09 | 10 | 0.4±0.05 |
| | DO | 9 | 10.9±2.1 | 5 | 3.5±2.2 | 10 | 2.6±0.6 |
| | DOC | 13 | 22.5±4.1 | 5 | 8.3±0.5 | 10 | 18.0±1.2 |
| | EC | 9 | 406.5±67 | 5 | 79.0±8.2 | 10 | 301.2±6.8 |
| | Nitrates | 13 | 0.41±0.01 | 5 | 2.2±0.04 | 10 | 3.1±0.17 |
| | Phosphates | 13 | 0.27±0.02 | 5 | 0.55±0.2 | 10 | 1.1±0.03 |
| | pH | 9 | 8.85±0.3 | 5 | 7.1±0.6 | 10 | 7.2±0.1 |
| | Secchi | 10 | 0.13±0.007 | 5 | 0.14±0.008 | 10 | 0.04±0.008 |
| | Silica | 13 | 21.4±1.2 | 5 | 9.6±1.2 | 10 | 11.9±0.6 |
| | Temp | 9 | 31.0±1.6 | 5 | 27.5±1.4 | 10 | 31.3±3.9 |
| | Turbidity | 13 | 62.1±25.0 | 9 | 0.75±0.5 | | |

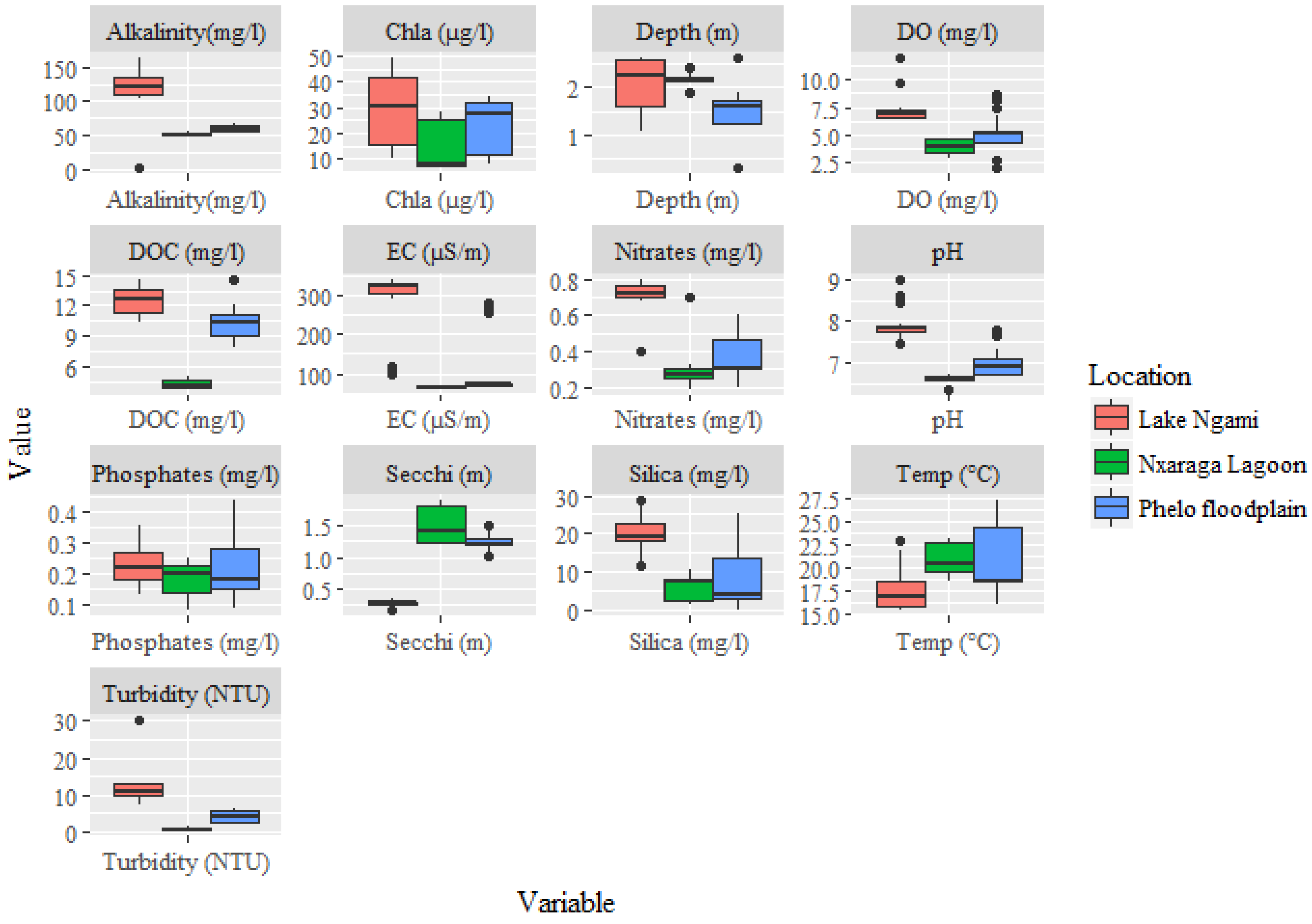


**Figure 5.8**: Box plot comparisons of environmental variables between study sites during high flood. The middle line represents the median, the upper and lower coloured areas represent the upper and lower quartiles respectively, the extending solid line represents the range of the data outside the upper and lower quartile range whereas the black dots represent outliers

Spatial patterns in the level of the measured environmental variables during low flood were like those observed during high flood, except for dissolved nutrients. The concentrations of $NO_3^-$ and $PO_4^{2-}$ were lowest in Lake Ngami and greater in the Chief's Island sites where Hippo pool in Phelo had a significantly greater concentration of the dissolved solutes. With regards to other parameters, there were significantly higher levels of alkalinity, chlorophyll a, DO, DOC, EC and silica (Mann–Whitney U test, $p = <0.05$) compared with samples from Nxaraga lagoon and Phelo floodplain during the flood season. The level of EC was greater in Phelo flood plain compared to those from Nxaraga lagoon). Water temperature did not differ significantly across all study sites (Fig. 5.9).

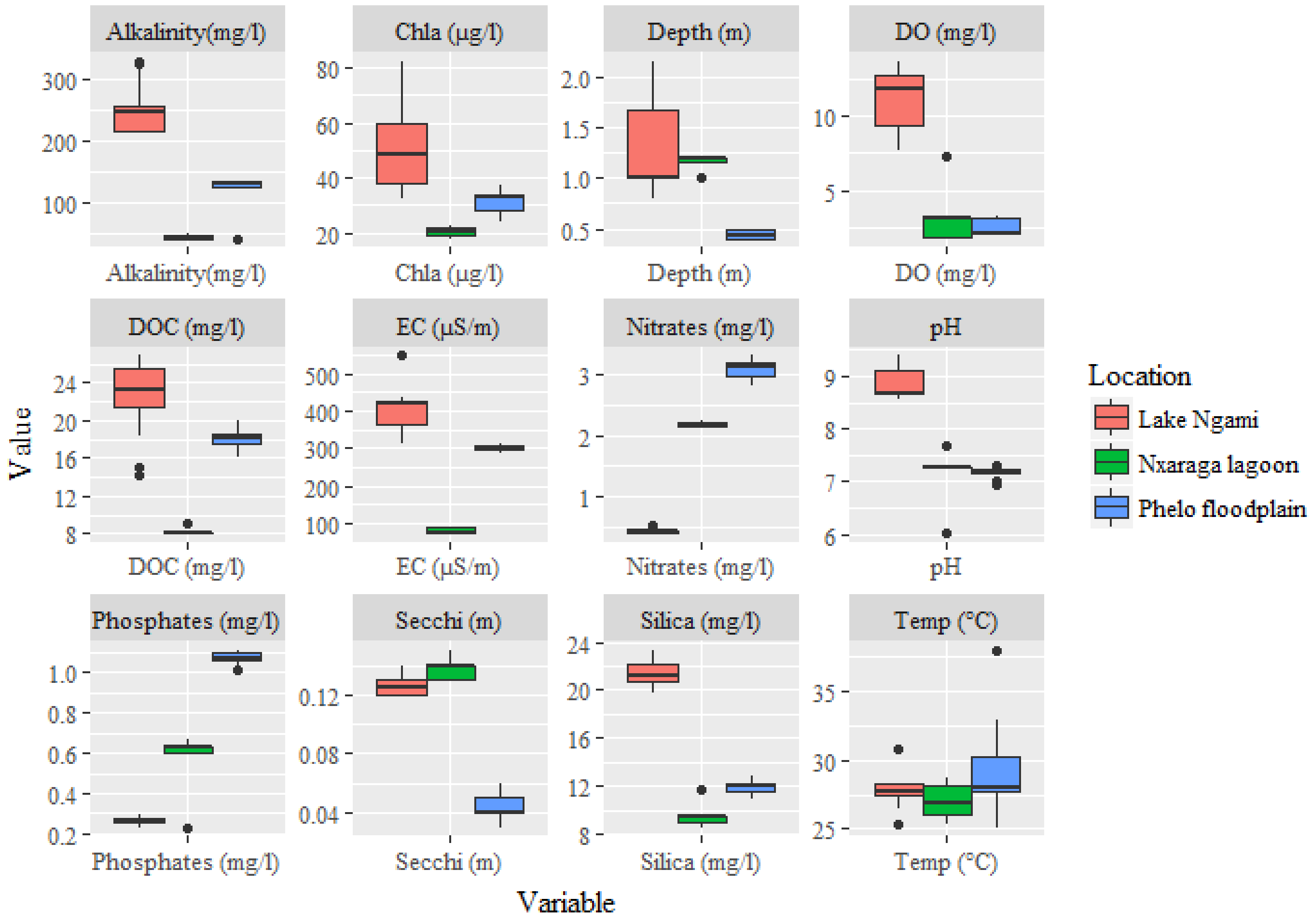


**Figure 5.9:** Box plot comparisons of environmental variables between study sites during low flood. The middle line represents the median, the upper and lower coloured areas represent the upper and lower quartiles respectively, the extending solid line represents the range of the data outside the upper and lower quartile range whereas the black dots represent outliers

### Spatial comparison of sedimentation rate

The results of sediment traps indicate that sedimentation rate was greatest at Lake Ngami, where it was more than double the rate observed at Nxaraga lagoon and Phelo floodplain (Table 5.5). Trapped suspended sediments in Lake Ngami had relatively low amounts of carbon and greater amounts of nitrogen compared to trapped sediments from both Nxaraga lagoon and Hippo pool. Even though sediments from Nxaraga lagoon had relatively greater amounts of nitrogen compared to sediments from Hippo pool, the sediments from Nxaraga lagoon had far greater amounts of carbon, leading to similar C:N ratios for sediments of the two sites.

**Table 5.5:** Sedimentation rate at the three study sites and degradability of suspended organic matter at the respective sites

|  | *n* | Sedimentation rate g $m^{-2}$ $day^{-1}$ | %C | %N | C:N ratio |
|---|---|---|---|---|---|
| Lake Ngami | 12 | 86.0 ± 9 | 6.8 | 4.6 | 1.5 |
| Nxaraga lagoon | 6 | 23.7 ± 8 | 18.9 | 1.6 | 12.0 |
| Phelo floodplain (Hippo pool) | 3 | 24.6 ± 1.0 | 8.9 | 0.6 | 15.0 |

## 5.5 Discussion

### Spatio-temporal variability of primary productivity and respiration

The estimated algal primary productivity in this study is generally comparable to previous estimates of algal primary productivity within the Okavango Delta. Pelagic primary productivity at Phelo flood plain was estimated at 0.028±0.05 mgC/l/h during high flood and 0.043 ± 0.01 mgC/l/h during low flood. Lindholm *et al.* (2007) estimated pelagic primary productivity of 0.033mgC/l/h (reported as 800mgCm$^{-3}$d$^{-1}$) at Phelo flood plain during low flood of a year of less flooding in the Okavango Delta whereas in the subsequent year, which had a larger extent of flooding, they estimated a relatively low pelagic productivity of about 0.005 mgC/l/h (reported as 125mgCm$^{-3}$d$^{-1}$). Like findings of this study, the authors found the concentration of dissolved nutrients at Phelo to be greater during low flood compared to high flood. (Siziba *et al.* 2012) also demonstrated that algal primary productivity is greater at low flood than high flood; the authors recorded chlorophyll a concentration of 41.8 ±8.8 μ/l and 38.6± 7.8 μ/l during low flood and high flood respectively. They also demonstrated that primary productivity in flood plains of the Okavango Delta is higher at low flood compared to high flood phase. Previous studies of algal primary productivity in Lake Ngami could not be found but earlier research at the lake confirm findings of this work that solute levels are greater at Lake Ngami compared to upstream parts of the Delta due to effects of evapotranspiration ( Meier *et al*. 2015, Gondwe and Masamba, 2016).

Respiration rates were greater at Lake Ngami and this can be expected since respiration rates in river-floodplain systems are expected to be higher at downstream locations because allochthonous matter from floodplains gets progressively broken down into smaller sizes that best favour microbial metabolisms at the downstream locations ( Vannote *et al.* 1980). In Macuarie River in Australia, Kobayashi *et al.*( 2011), found oxygen levels to be decreasing in the downstream direction of due to progressive downstream increase in respiration rates.

**Autotrophic structure**

All the study sites had the same autotrophic structure - primary productivity was greatest in the pelagic zone whereas respiration dominates the benthic substrate (Fig.5.7). Net pelagic primary productivity (*p*NPP) in mgC/l/h during high flood was 0.062±0.05, 0.025±0.01 and 0.028±0.05 at Lake Ngami, Nxaraga lagoon and Phelo flood plain respectively whereas epipelic net primary productivity (*e*NPP) was -0.068, -0.035 and -0.077 at the sites respectively. During low flood, the same autotrophic structure was maintained across all the sites with *p*NPP of 0.11 ± 0.01, 0.016 ± 0.01 and 0.043 ± 0.01 mgC/l/h at Lake Ngami, Nxaraga lagoon and Phelo flood plain respectively whereas *e*NPP was -0.12 ± 0.09, -0.035 ± 0.02 and -0.065 ± 0.03 mgC/l/h at respective sites. It was expected that since the Delta is generally an oligotrophic system (Ramberg and Wolski 2008), there would be less pelagic primary productivity which would allow enough light to reach epipelic algae and promote high epipelic primary productivity rates. However, this was not the case; net epipelic primary productivity was negative likely due to the unstable benthic surface. Pelagic primary productivity was variable across the study sites, with Lake Ngami having notably greater pelagic primary productivity than the Chief's Island sites, perhaps indicating that not all aquatic habitats within the highly heterogenous environment of the Delta are oligotrophic.

The negative net epipelic primary productivity across all sites therefore can be explained differently for the study sites. At Lake Ngami, the greater turbidity during high flood (Fig. 5.9) likely limits the light for primary producers at the lake bed. Additionally, the benthic substrate at the lake is a soft muddy sediment which is very unstable and easily moved about by water waves. Substrate stability is a very important determinant of epipelic algae biomass (Peterson 1996, Werner and Köhler 2005). At Nxaraga lagoon, the small and loose sand particles also present a very unstable benthic substrate which may prohibit establishment of epipelic algae on it. Phelo flood plain had a negative epipelic primary productivity (Fig. 5.7) likely due to greater rates of organic matter decomposition. The surface sediments of river floodplain systems like the Okavango Delta, are known for their richness in organic matter, promoting high respiration rates (Baldwin and Mitchel 2000). While the measured epipelic primary productivity at Nxaraga lagoon and Phelo floodplain was negative, total benthic primary production at the sites could be high since there are abundant emergent wetland plants which possibly support a substantial biomass of algal epiphytes.

### Primary productivity at Lake Ngami

Dissolved nutrients are only higher at Ngami during high flood, but phytoplanktonic productivity at the lake was high during both high and low flood (Fig. 5.7). The relatively lower levels of nutrients, especially $PO_4^2$, at the Lake during low flood are likely influenced by greater phytoplankton uptake. The low levels of $PO_4^2$ and $NO_3^-$ at low flood indicate these nutrients may be co-limiting during the warmer months of the low flood season when algal densities are high. Phytoplankton densities measured in Lake Ngami were greater than phytoplankton densities (Chl-a) in the Chief's Island sites, both during high flood and low flood (Fig 5.9), corresponding to the generally greater nutrient levels at the lake (Fig. 5.9). The greater phytoplankton productivity at the lake likely accounts for the bulk of primary productivity that supports the lake's food web whereas aquatic macrophyte production likely dominates aquatic primary productivity at the Chief's Island sites. Compared to Lake Ngami, which virtually lacks aquatic vegetation, the upstream aquatic habitats are characterized by submerged, floating and emergent macrophytes communities ( Ellery *et al.*1992, Bonyongo *et al*. 2000).The long-term flooding variability of Lake Ngami (Shaw *et al*. 2003; Wolski *et al.* 2014) (Shaw *et al.* 2003, Wolski *et al.* 2014) is possibly one of the major factors driving the lake's high phytoplankton productivity. The high variability of the lake filling includes long periods of desiccation during which the lake accumulates nutrients and organic matter as re-colonize the lake bed. Additionally, livestock inhabit the lake bed during desiccation periods (Bendsen and Meyer 2002), and as they graze from nearby grasslands and defecate in the lake bed, they potentially convey nutrients in to the lake bed.

### Resuspension rates

Sedimentation rate at Lake Ngami (86.0 g $m^{-2}$ $day^{-1}$ ± 9) was two-fold greater than sedimentation rate at Nxaraga lagoon (23.7 g $m^{-2}$ $day^{-1}$ ± 8) and Phelo flood plain (24.6 g $m^{-2}$ $day^{-1}$ ± 1.0). The comparatively greater resuspension rates at Lake Ngami possibly result from a combined effect of a strong wave action and very fine muddy sediments of the Lake. Due to its wider open-water space, Lake Ngami experiences strong wind-driven water waves which possibly resuspend benthic sediments at a greater rate than the relatively smaller waves at Nxaraga lagoon and Phelo floodplain's Hippo pool. The sediments at Lake Ngami also had lower C:N ratios, of 1.5 compared to 12.0 and 15.0 for Nxaraga lagoon and Phelo floodplain respectively (Table 5.5), suggesting that not only is sedimentation rate greater at the Lake, but the suspended sediments are more biodegradable. The resuspended sediments at Lake Ngami had a C:N characteristic of algal derived sediments (Meyers and Lallier-Vergès 1999) whereas C:N ratios of sediments from Nxaraga Lagoon and Phelo flood plain are indicative of sediments derived from a mixture of plant and algal organic matter (Meyers and Lallier-Vergès 1999). Lake Ngami sediments can be expected to be algal derived since the lake is visually devoid of aquatic vegetation but with relatively high phytoplankton density. The Chief's Island sites of Nxaraga lagoon and Phelo floodplain are, in contrast, vegetated with a mosaic of wetland plants (Bonyongo *et al*. 2000, Dallas and Mosepele 2007).

## 5.6 Conclusions

The study sites varied in algal primary productivity rates, nutrient levels and environmental characteristics. Lake Ngami had significantly greater levels of algal primary productivity and dissolved solutes than the upstream sites at Chief's Island. However, at low flood, Lake Ngami had lower levels of dissolved solutes than Nxaraga and Phelo floodplain sites perhaps indicating that high phytoplankton nutrient uptake at low flood can override the concentrating effect of evaporation at the lake. The results have also shown that algal primary productivity occurs mainly in the water column whereas the benthic surface is heterotrophic possibly due the unstable substrate of either lose sand particles or soft mud at the study sites. Rates of pelagic primary productivity were also shown to be influenced by the stage of the seasonal floods with greater rates occurring al low flood likely due to progressive nutrient concentrating effect of evaporation as the water levels decline. While it is generally expected that floodplains are sites of greater biological productivity than main channels, these results have demonstrated that downstream lakes can have even greater algal primary productivity because of the progressive concentration of nutrients in the downstream direction. Phytoplankton is likely the major carbon source supporting fish population in Lake Ngami, where fisheries are a crucial livelihood sources, whereas aquatic macrophytes may be relatively more important for food webs of the Chief’s Island sites, where there is relatively low phytoplankton primary productivity and where the aquatic habitats are vegetated by a diversity of wetland plants. The following chapter evaluates the relative importance of different basal carbon sources to aquatic food webs at the study sites.

# CHAPTER SIX: Basal carbon sources supporting aquatic food webs of habitats with different flooding regimes

## 6.1 Introduction

In aquatic food webs, energy from primary producers is transferred to organisms at higher trophic levels through two main pathways: the algal grazer pathway and the microbial loop pathway. In the algal grazer pathway, the transfer of energy trapped by algal photosynthesis to higher trophic levels is initiated by primary consumers who feed on algae. In the microbial loop pathway, energy is released from dead organic matter through microbial decomposition and then channelled into aquatic food webs when phytoplanktonic consumers feed on the microorganisms (Woodward 2009). There is a consensus that much of the energy that supports aquatic food webs is from the algal pathway and there is relatively less energy coming from the microbial loop pathway. In the microbial loop pathway there is a greater number of trophic transfers between the microbes and higher consumers, leading to lower efficiency of energy transfer (Cotner and Biddanda 2002, Woodward 2009, Thompson *et al.* 2012). Basal sources of carbon within the microbial pathway are mostly terrestrially derived organic matter (allochthonous matter) whereas algal the grazer pathway is constituted by different algal primary producers (Woodward 2009). Even though there is a consensus that the algal pathway is the major source of energy for aquatic food webs, the energy from allochthonous carbon sources has been shown to affect several attributes of aquatic food webs including food chain length, the distribution of biomass among trophic levels, and the strength of trophic cascades (Halaj and Wise 2002, Moore *et al.* 2004).

Previous studies, have shown that the relative importance of allochthonous sources to aquatic food webs increases with the abundance of the sources (Solomon *et al.* 2011, Marchese *et al.* 2014, Correa and Winemiller 2018). The most recent review of stable isotope studies on freshwater food webs (Roach 2013), concluded that in oligotrophic flood pulsed wetlands the relative importance of different basal sources to the food webs is a function of environmental variables, principally rates of algal primary productivity and seasonal contribution of terrestrial material to the organic matter pool. Since the main driver of environmental variability in seasonally flooded wetlands is the seasonal flood pulse (Junk *et al.* 1989), quantification of the relative importance of basal carbon sources with respect to the seasonal flooding regime can improve the understanding of aquatic food web dynamics and benefit local wetland management.

Empirical research that was done after Roach's (2013) review demonstrated that the seasonal flood pulse is the main factor influencing the relative importance of basal carbon sources to aquatic food webs in flood pulsed wetlands. Winemiller *et al.* (2014) showed that relative abundance of basal resources varied temporally with respect to the flooding phase due to temporal changes in faunal interactions. Ou and Winemiller (2016) also demonstrated that the contribution of basal carbon sources to assimilated diet production of different fish guilds varied temporally with respect to flooding stage in Lower Mekong Basin (Cambodia). In Lagoa Peixe National Park in Southern Brazil, the seasonal flood pulse, through connecting the estuarine and freshwater habitats, has been shown to increase the importance of allochthonous basal carbon in estuarine habitats during high flood (Garcia *et al.* 2017). In addition to demonstrating the role of the flood pulse in determining the importance of basal resources, results from both Qu and Winemiller (2016) and Garcia *et al.* (2017) have also shown that human modification of wetlands' seasonal flooding regime impacts negatively on aquatic food webs. For example, damming of Sesan River within the Lower Mekong Basin, changed the river's hydrology, channel geomorphology and other factors, leading to fish having to rely on instream basal carbon sources to a greater extent than fish of other rivers within the basin (Qu and Winemiller 2016). While anthropogenic opening of an outlet channel of the Lagoa Peixe National Park terminated the flood pulse and reduced allochthonous carbon from freshwater habitats (Garcia *et al.* 2017). It is evident that the seasonal flood pulse influences the relative importance of basal carbon sources to wetland food webs but through mechanisms that may vary from one wetland system to another. It is therefore necessary to validate theoretical predictions of how the seasonal flooding regime influences energy flow dynamics of local food webs. An in-depth understanding of how the relative importance of basal carbon sources is influenced by flooding regimes can improve forecasting of ecological responses to threats such as hydrological modifications and climate change effects, and hence benefit wetland management.

The diversity of aquatic habitats in the Delta provides a good opportunity to further our understanding on how flooding regimes influence the relative importance of basal resources to aquatic food webs. As we saw in Chapter 3, the Delta is characterised by a diversity of aquatic habitats including seasonal flood plains and pools, permanent swamps and perennially flowing channels. In the Delta, aquatic habitats of different water permanence vary in physical features such as vegetation composition (Bonyongo *et al*. 2000) and water quality (Mackay *et al.* 2011). In terms of water quality variables, seasonal habitats have been shown to have higher levels of solutes including cations, dissolved organic carbon (DOC), nitrates and silica (Mladenov *et al.* 2007; Mackay *et al*. 2011). Results of the preceding chapter, Chapter 5 (Fig. 5d), have also demonstrated the environmental difference between floodplains and perennial channels of the Delta, and the terminal Lake Ngami. The environmental heterogeneity of the Delta is further increased by the progressive increase in the concentration of solutes due to evapo-transpiration (Gondwe and Masamba 2016). Based on literature (Roach 2013), the Delta's environmental variability is expected to influence the relative importance of basal C sources to aquatic food webs.

In the Okavango Delta, the flood pulse is known to be the main driver of environmental heterogeneity, however its influence on aquatic food webs is poorly understood. Because so few studies have been done on aquatic food webs of the Okavango Delta (Høberg *et al*. 2002, Lindholm and Hessen 2007), the structure of its aquatic food web with respect to flooding regimes is not well known. For example, the influence of the flood pulse on the relative importance of different carbon sources is unquantified. Given threats to the Delta's hydrology, such as water abstractions and climate change, it is crucial to understand the relationships between such food web properties and the Delta's flooding regimes. Proposed water development projects including irrigation, hydropower, dams and expansion of community water supplies by Angola, Botswana and Namibia are expected to reduce the flooding extent of the Delta and impact on its ecological functioning (Ashton 2000), although extensive developments have yet to be realised. Reduction in the Delta's flooding extent will likely reduce seasonal aquatic habitats and affect carbon flow dynamics in the Delta. The Delta's seasonal floodplains are known to accumulate terrestrial organic matter during dry conditions and release the carbon to the rest of the wetland aquatic food during high floods (Bonyongo *et al.* 2002; Lindholm *et al.* 2007). An in-depth understanding of the relationship between the Delta's flooding regimes and the relative importance of different basal carbon sources will benefit predictions of how changes in Delta’s hydrology may impact on its aquatic food webs and hence its ecological functioning. Here I hypothesise that (i) the relative importance of algal and non-algal sources to aquatic food webs vary between habitats of different flooding regimes (ii) the relative importance of non-algal sources is greater at high flood than at low flood across the Delta’s habitats.

## 6.2 Objectives

The following objectives were formulated to test the hypotheses above.

1. To compare the relative contribution of different basal carbon sources to fish assimilated diet at differently flooded locations of the Delta

2. To examine the influence of flooding stage on the relative contribution of basal carbon sources to fish assimilated diet at the differently flooded locations.

## 6.3. Methods

### Data collection and processing

The number of samples collected for stable isotope analysis (SIA) including fish, invertebrates and potential carbon sources at each site during high flood and low flood seasons are summarised in Table 6.1 below.

**Table 6.1**: The number of samples of fish, invertebrates and basal carbon sources collected at each study site during high flood and low flood seasons. Overall, n = 187 samples were collected during high flood, and n = 118 samples collected during low flood.

| **Campaign1: High flood**<br>29 June to 18 August 2015 | **Samples** | **Number of samples collected** | | |
|---|---|---|---|---|
| | | Ngami | Nxaraga | Phelo |
| | fish | 27 | 33 | 29 |
| | invertebrates | 14 | 4 | 12 |
| | zooplankton | 2 | 1 | 2 |
| | algae | 7 | 5 | 3 |
| | Macrophytes | 2 | 19 | 10 |
| | sediments | 8 | 5 | 4 |
| **Campaign 2: Low flood**<br>11th to 29th January 2016 | | | | |
| | fish | 34 | 17 | 12 |
| | invertebrates | 4 | 9 | 7 |
| | zooplankton | 2 | 2 | 2 |
| | algae | 4 | 2 | 1 |
| | Macrophytes | 5 | 4 | 4 |
| | sediments | 4 | 3 | 2 |

### Fish

Fish were collected using multi-panel experimental fishing nets consisting of 10m long panels of mesh sizes 22, 28, 35, 45, 57, 73, 93, 118 and 150 mm each (Fig. 6.1). The sampling gear is non-selective and therefore representative of the fish population. The nets were set for 12 hours, from 6 pm in the evening and retrieved at 6am the following morning, a procedure used by previous fish studies in the Delta using the same gear (Mosepele *et al*. 2011, Mosepele *et al.* 2012, Mosepele *et al.* 2016).

**Figure 6.1:** A multi-panel experimental fishing net being set in Lake Ngami at sunset (Photograph: Author's own)

Collecting fish using a procedure that is frequently used in the Delta allows for easy comparisons of my PhD's findings with findings of previous fish research in the Delta. Once the net was retrieved and taken to the base camp, each fish specimen was identified to species level and total length of each fish measured. Fish of each species were counted and the number of individuals for each species was recorded. Thereafter, a scalpel blade and a pair of tweezers were used to cut the muscle tissue from the dorso-lateral region of three fish individuals of adult length per species. Muscle tissue was chosen for measuring isotope ratios because it has an average stable isotope turnover time of 25 days, which allowed for seasonal variations to be detected (Boecklen *et al.* 2011; Vander Zanden *et al.* 2015), also see Chapter 2). The dorso-lateral region contains fewer lipids and inorganic carbonates which may otherwise distort isotopic signatures of fish muscle tissue (Pinnegar and Polunin 1999). A muscle tissue from the dorso-ventral region was therefore considered ideal for examining seasonal isotopic changes in fish. Once cut, the muscle tissue was placed in sterile 5ml vials and kept in a cooler box with ice prior to transportation to ORI laboratories. At ORI laboratories, the samples were stored in a freezer at -20 ˚C until they were shipped to University College London (UCL) where they were further processed and analysed for stable isotopes as described in section 6.3.5

Potential prey resources for fish, including aquatic macroinvertebrates, zooplankton and different basal carbon resources were collected. All potential prey resources for fish were collected because while only data on basal carbon sources is used in this chapter, data on other prey resources is used in the subsequent chapter, Chapter 7, which examines food web structure of the study sites. The methods for collection and processing samples of different prey resources are described below.

### Aquatic macroinvertebrates

Aquatic macroinvertebrates were collected by scooping from aquatic substrate using a scoop net of 1mm mesh size. The net was attached to a 30cm x 30cm square frame with a 2m handle (Figure 6.2). This method is suitable for collecting aquatic macroinvertebrates from diverse aquatic habitats in the Okavango Delta (Dallas and Mosepele, 2007) and was therefore suitable for collecting samples from different vegetation types associated with aquatic habitats of different flooding regimes. Invertebrates were identified to family level, sorted at site, and then placed into sterile 5ml vials containing river water. The macroinvertebrates were left in the vials overnight so that they evacuated their gut contents. On the following day the samples were rinsed with distilled water and then placed into clean vials. The samples were taken to ORI laboratories where they were stored in the freezer at –20 ˚C prior to shipment to the UCL for further processing and laboratory analyses for stable isotopes.

**Figure 6.2**: Richard holding the scoop net that was used for collecting aquatic macroinvertebrates (Photograph: Author's own)

### Zooplankton and phytoplankton

Zooplankton and phytoplankton were collected by sieving water through a stack of sieves with mesh sizes of 300 µm, 150 µm, 90 µm, 75 µm, and 38µm (Fig. 6.3), as described in (Mangaliso *et al.* 2012). Most zooplankton were retained by mesh 90 µm and therefore zooplankton samples were collected from that mesh size and phytoplankton samples were collected from the smallest mesh (38µm). The samples were collected by washing the retained organisms with distilled water into a small plastic vial (5ml). The vials were labelled and put into a cool box with ice and transported to ORI laboratories. At ORI laboratories, the samples were filtered through a pre-combusted (450 ˚C for 8 hours, Hoeinghaus *et al.* (2007)) 0.47µm GF/F glass filter at low pressure, wrapped in aluminium foil and stored in a freezer at –20 ˚C until they were shipped to UCL for stable isotope analyses. A microscopic examination of samples from the 90 µm and 38µm sieves revealed that the samples were mainly composed of zooplankton and phytoplankton respectively with trace amounts of detritus.

**Figure 6.3**: Zooplankton and phytoplankton samples being collected by sieving water through a stack of sieves. (Photograph: Author's own)

### 6.3.4 Basal carbon sources

Potential carbon sources collected at each site were dominant macrophytes, detritus, algae and sediments. Macrophyte samples were collected by cutting green leaf blades or leaf sheaths from different macrophyte species within the flood plain at each site including submerged and emergent plants within the inundated habitats. The collected macrophyte samples included samples from $C_3$ and $C_4$ plants. Detritus that was still attached to plants was cut using a pair of scissors while detached detritus was handpicked. Filamentous algae were collected by hand from the water surface or subsurface depth. Once collected, distilled water was used to wash off detritus and soil from the filamentous algae samples before putting them into labelled zip lock bags. Algal epiphytes were collected by first cutting the stems of macrophytes to which they were attached and then removing the attached algae into 5ml specimen vials using a soft brush and a squeeze bottle (Fig. 6.4). All the collected samples were put into a cool box containing ice prior to transportation to laboratories at ORI. At ORI the samples were stored in a freezer at –20˚C until they were transported to University College London (UCL) for laboratory analyses of stable isotopes.

**Figure 6.4**: Collecting epiphytes from a macrophyte stem into a specimen vial. (Photograph: Author's own)

### 6.3.5 Laboratory processing and SI analyses

Once shipped into the UK, all samples were immediately stored in a freezer at –20°C in University College London's Geography laboratory while awaiting further processing and analyses for stable isotopes to be done at the University's Bloomsbury Environmental Isotope Facility (BEIF). The samples were then further processed and analysed for stable isotopes of carbon and nitrogen as described below.

Once taken from storage, all samples were freeze dried overnight at –60°C in Thermo Modulyo D and Edwards Modulyo freeze-dryers. The freeze-dried samples were immediately ground to a fine powder using a pestle and mortar and then stored in clean glass vials. For macroinvertebrates with shells such as snails and bivalves, a muscle tissue was extracted and processed whilst the shell was discarded as it contains inorganic carbonates which may interfere with organic carbon isotopes values. To examine interference of inorganic carbonates which may be present in exoskeletons of other macroinvertebrates, ground macroinvertebrate samples were initially replicated into pairs. One pair of samples was acidified to remove inorganic carbonates by soaking with a solution of 1% hydrochloric acid overnight, followed by rinsing with distilled water and air drying under a fume hood for 24 hrs. Preliminary stable isotope analyses showed no substantial isotopic difference between the acidified and non-acidified samples. All ground non-acidified samples of macroinvertebrates were therefore subsequently used for stable isotope analyses because acidifying samples has potential to increase values of nitrogen isotopes leading to erroneous interpretations (Bosley & Wainright, 1999). Ground sub-samples were taken to the BEIF laboratory where they were weighed into tin capsules of 6 x 4mm standard pressed weight (OEA laboratories Ltd.).

The samples, together with standard reference materials, were loaded into an elemental analyser (Thermo Finnigan, Flash EA, 1112 series) using an automated sample carousel. Inside the elemental analyser, samples were combusted at 1020 °C and the relative $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of the resulting $CO_2$ and $N_2$ respectively were measured for the samples and standard reference materials. The standards that were used are ANU ($\delta^{13}C$ –10.45‰), USGS 40 ($\delta^{13}C$ –26.39‰, $\delta^{15}N$ = –4.52 ‰ ), USGS 24 ($\delta^{13}C$ -16.05‰, $\delta^{15}N$ = –4.52 ‰), IAEA-600 ($\delta^{13}C$ –27.77‰, $\delta^{15}N$ = +1.0‰), IAEA-N2 ($\delta^{15}N$ = +20.3 ‰), IAEA-N1 ($\delta^{15}N$ = + 0.4 ‰), and Alanine from OEA laboratories ($\delta^{13}C$ –23.33 ‰, $\delta^{15}N$ = –5.56‰),). These standards, which were measured together with each batch of samples analysed, were used for calibration and correction for instrument drift. The results were reported in $\delta^{13}C$ or $\delta^{15}N$ = (Rsample/Rstandard − 1) × 1000 (‰); where R represents the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

## Statistical methods

The $\delta^{13}C$ and $\delta^{15}N$ values of individual basal C sources were each pooled together into aggregate basal sources based on the similarity of stable isotope values of the individual basal C sources and on ecological similarities of the basal sources. For example, individual samples of $C_3$ macrophytes, $C_4$ grasses, or algal sources were pooled together to make aggregate sources when the SI values of the individual samples were not statistically different. Pooling individual sources into aggregate sources improves the ability of mixing models to estimate proportional isotopic contributions of sources to a consumer tissue (Phillips *et al.* 2005). When sources are too many, mixing models perform poorly often producing wide and overlapping estimates of source contributions (Phillips *et al*. 2014). It has been shown that Bayesian mixing models in studies using $\delta^{13}C$ and $\delta^{15}N$ isotopes perform well when the sources do not exceed seven (Stock *et al.* 2018), aggregate sources in this study were therefore limited to five to improve the contribution estimates. The mean and standard deviation values of $\delta^{13}C$ and $\delta^{15}N$ for each aggregate source were then calculated at each study site and used in mixing models.

The relative importance of the aggregate basal C sources to fish assimilated diet were estimated using Stable Isotope Analysis in R (SIAR) package (Parnell *et al.* 2010). SIAR is a Bayesian mixing model (see Chapter 2 for introduction to the mixing models) that calculates feasible proportional contributions of sources to tissue production of a consumer. The advantage of using SIAR is that it incorporates the variation associated with the data into the model such as variation in stable isotope values of individual samples (constituting the aggregate source) and variation in trophic enrichment factor (TEF) values (Parnell *et al.* 2010). The SIAR model therefore more accurately represents the variability that exist in the natural systems. The input data for the SIAR package are $\delta^{13}C$ and $\delta^{15}N$ for each potential resource (basal C sources) and their associated standard errors, TEFs and their standard errors, and $\delta^{13}C$ and $\delta^{15}N$ of consumers (fish) (França *et al.* 2011). The SIAR model requires consumers and prey organisms to be on the same trophic level before it calculates the relative contribution, therefore TEFs of all consumers were subtracted from $\delta^{13}C$ and $\delta^{15}N$ values of the fish before loading fish data into the SIAR model. The TEFs of 0.8 and 2.6 were subtracted for $\delta^{13}C$ and $\delta^{15}N$ values respectively and per trophic link. These TEFs are based on average values reported for muscle tissue for a range of aquatic vertebrates and invertebrates (McCutchan *et al.* 2003). The subtraction of TEFs was done as described in (Reid *et al.* 2008), whereby fish were grouped into four trophic levels based on feeding ecologies (Skelton 1997, Mosepele *et al.* 2012, (Froese and Pauly 2017): *detrivores*, *omnivores*, *insectivores* and *piscivores*. The TEFs at each trophic level were calculated as the average of TEF of all possible feeding pathways that lead to the trophic level (Fig. 6.5). The calculated TEFs for each trophic level were then subtracted from the $\delta^{13}C$ and $\delta^{15}N$ values of fish muscle before running the SIAR model

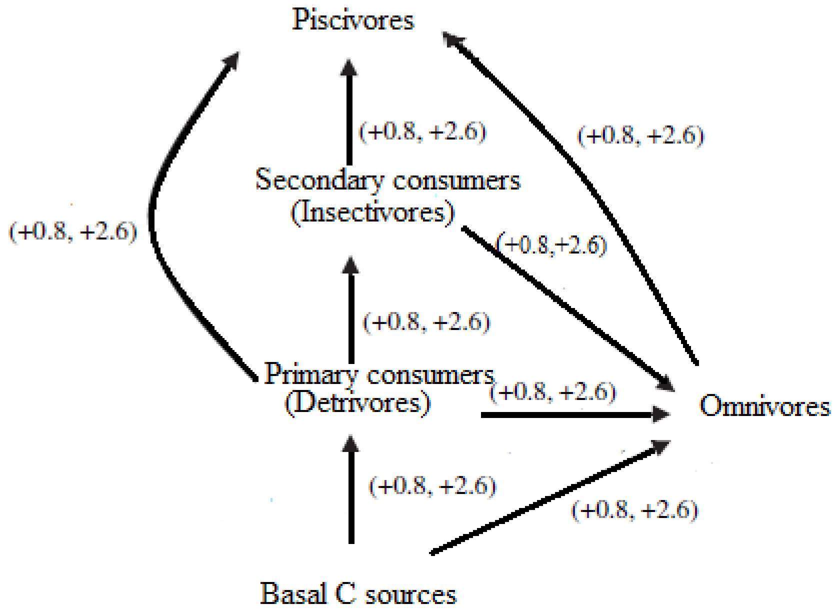


**Figure 6.5**: A simple food web showing TEFs and enrichment possibilities for different fish feeding guilds (Edited from Reid *et al.* 2008). The arrows point from prey to consumer. TEF of each guild is calculated by adding all TEFs of the links (arrows) leading to the guild and dividing it by the number of the routes from which the totals are derived. For example, omnivore TEFs were calculated as [(0.8, 2.6) + (1.6, 5.2) + (2.4, 7.8)]/3 = (+1.6, +5.2). The TEFs were deducted from fish δ13C and δ15N values before loading into SIAR

For each model run, Markov chain Monte Carlo permutations were used to generate plausible simulations of source proportional contributions (Parnell *et al.* 2010). The default iterations in SIAR of 20 000 were used for each model. The range of proportional contributions of each basal C source to assimilated fish diet was described by upper and lower 95% credibility intervals (CIs; Phillips and Gregg 2003, Jackson *et al.* 2009, Parnell *et al.* 2010). The estimated 95% credibility interval are presented as percentages.

## 6.4 Results

### Variability of $\delta^{13}C$ and $\delta^{15}N$ values of basal sources across study sites

A total of 79 samples of basal C source were analysed for $\delta^{13}C$ and $\delta^{15}N$ (Tables 6.1, 6.2). The fewest aggregate sources were from Lake Ngami (6), including only one plant species (*Typha sp*) compared to Nxaraga lagoon and Phelo flood plain where samples from at least 8 plant species were analysed for $\delta^{13}C$ and $\delta^{15}N$ (Table 6.2). Basal sources from Lake Ngami had more negative $\delta^{13}C$ values compared samples from Nxaraga lagoon and Phelo floodplain. The mean $\delta^{13}C$ values of samples from Lake Ngami ranged from –29.45‰ ± 1.6 for *Typha* sp to –5.27‰ ± 2.1 for filamentous algae (Table 6.2). The mean $\delta^{13}C$ values for samples from Nxaraga lagoon ranged from –33.75‰ ±2.6 for filamentous algae to –19.30‰ ± 7.2 for littoral $C_4$ plants. For samples collected from Phelo floodplain, the mean $\delta^{13}C$ values ranged from –27.60‰ ± 2.9 for $C_3$ macrophytes to –17.78‰ ± 5.9 for $C_4$ littoral plants (Table 6.2). Samples from Lake Ngami also had more negative $\delta^{15}N$ values compared to samples collected from the two other study sites. The mean $\delta^{15}N$ values of samples from the lake reached up to +8.55‰ ± 0.9 for algal epiphytes whereas the $\delta^{15}N$ values for basal C sources did not exceed +4.0‰ (Table 6.2).

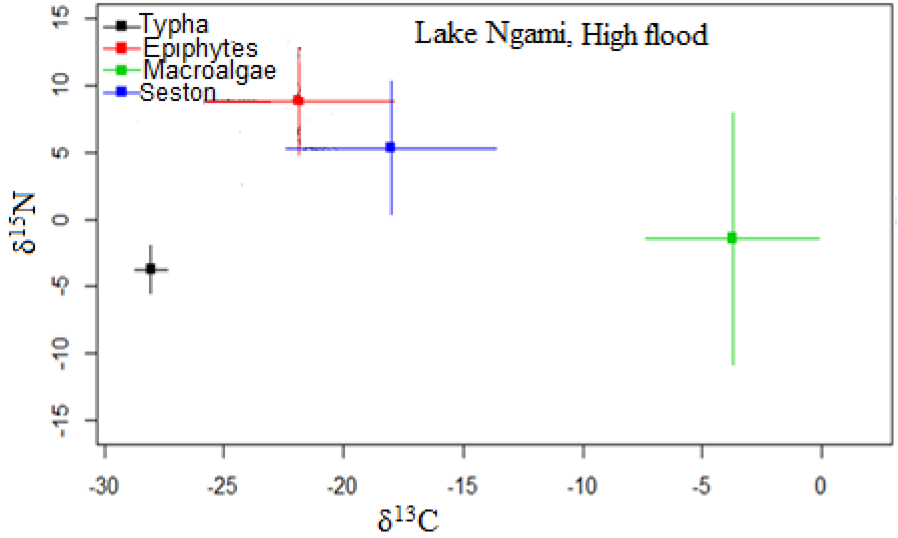
**Table 6.2**: Mean $\delta^{13}C$ and $\delta^{15}N$ values of basal carbon sources from the three study sites

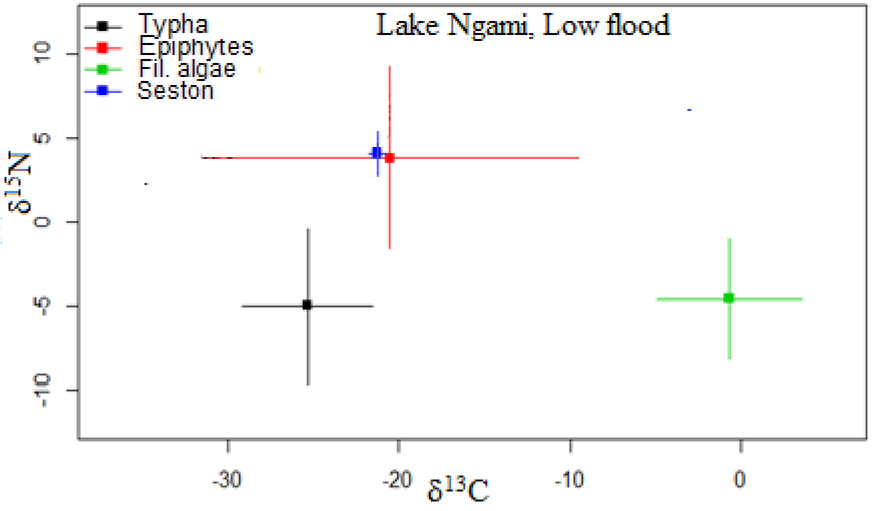
| | Individual C Sources | Habitat/description | Mean $\delta^{13}C$ | Mean $\delta^{15}N$ | C:N | n |
|---|---|---|---|---|---|---|
| **Lake Ngami** | Benthic sediment | Lake bottom | –20.89 ± 3.7 | 3.63 ± 0.6 | 12.8 | 5 |
| | Algal epiphytes | Submerged dead *Acacia* stem | –17.86 ± 0.3 | 4.19 ± 0.2 | 0.12 | 3 |
| | Algal epiphytes | Emergent *Typha* sp | –24.53 ± 0.4 | 8.55± 0.9 | 6.0 | 3 |
| | Phytoplankton | Water column | –22.35 | 4.42 | na | 1 |
| | Filamentous algae | Subsurface filamentous algae | –5.27 ± 2.1 | 2.62 ± 4.1 | 23.30 | 4 |
| | Sediment trap | Water column | –21.47 ± 0.5 | 5.47 ± 2.7 | 9.34 | 6 |
| | *Typha sp* | Emergent $C_4$ reed | –29.45 ± 1.6 | 4.57 ± 1.0 | 41.3 | 3 |
| **Nxaraga Lagoon** | *Ceratophyllum demersum* | Submerged $C_3$ macrophyte | –30.19 ±1.2 | 2.66 ± 2.8 | 17.5 | 2 |
| | Algal epiphytes | Emergent sedges | –24.47 ± 5.0 | 3.17 ± 1.4 | 7.1 | 3 |
| | Filamentous algae | Water column | –33.75 ±2.6 | 2.21 ± 2.0 | 18.9 | 2 |
| | Phytoplankton | Water column | –25.45 | 4.27 | na | 1 |
| | *Miscanthus* sp. | Littoral $C_4$ grass | –14.72 | 0.06 | 29.9 | 1 |
| | *Nymphoea* sp. | Floating $C_3$ macrophyte | –26.17 ± 0.5 | 1.43 ± 1.1 | 16.1 | 2 |
| | *Ottelia ovalifolia* | Submersed $C_3$ macrophyte | –26.58 | 1.87 | 16.5 | 1 |
| | *Schoenoplectus* sp | Emergent $C_4$ reed | –13.06 ± 0.7 | 0.29 ± 0.7 | 74.5 | 3 |
| | *Phragmitis* sp | Littoral $C_4$ reed | –27.15 ± 0.9 | 0.07 ± 1.1 | 33.1 | 3 |
| | *Ludwigia stolonifera* | Floating $C_3$ macrophyte | –27.58 | 2.51 | 28.6 | 1 |
| | *Vossia* sp. | Littoral $C_4$ grass | –23.11 ± 8.5 | 0.32 ± 1.1 | 44.4 | 2 |
| | Sediment_benthic | Lagoon bottom | –23.57 | 1.21 | 15.74 | 1 |
| | Sediment-trap | Water column | –24.39 | 1.18 | 18.8 | 3 |
| **Phelo floodplain** | *Aldrovanda* sp. | Submerged $C_3$ macrophyte | –29.87 | 5.63 | 9.6 | 1 |
| | *Cynodon dactylon* | Emergent $C_4$ grass | –21.25 ± 6.1 | 2.0 ± 3.0 | 40 | 4 |
| | *Lagarosiphon major* | Submersed $C_3$ plant | –30.19 | 4.52 | 13.3 | 1 |
| | *Nymphoea* sp. | Floating $C_3$ macrophyte | –27.13 ± 0.2 | 2.06 ± 0.6 | 16.5 | 2 |
| | *Ottelia ovalifolia* | Submerged $C_3$ macrophyte | –26.78 | 2.56 | 9.8 | 1 |
| | *Penicum ripens* | Emergent $C_4$ grass | –21.25 ± 4.8 | 2.0 ± 4.1 | 39.7 | 4 |
| | *Schoenoplectus* sp | Emergent $C_4$ reed | –17.24 ± 7.3 | 0.93 ± 2.1 | 47.4 | 4 |
| | *Vossia* sp | Littoral $C_4$ grass | –13.73 ± 0.8 | 0.77 ± 0.02 | 66.0 | 2 |
| | *Ludwigia stolonifera* | Floating $C_3$ macrophyte | –28.55 ± 1.0 | 2.58 ± 0.2 | 25.7 | 2 |
| | Algal epiphytes | Emergent reeds | –24.86 ± 1.8 | 3.27 ± 1.8 | 5.2 | 2 |
| | Filamentous algae | Water column | –25.45 ± 1.5 | 3.02 ± 1.6 | 8.5 | 3 |
| | Phytoplankton | Water column | –22.35 | 2.94 | 33.84 | 1 |
| | Sediment_benthic | Bottom of Floodplain | –20.01 ± 1.9 | 1.98 ± 0.2 | 14.2 | 2 |
| | Sediment_trap | Water column | –23.52 ± 0.8 | 1.47 ±1.5 | 13.4 | 3 |

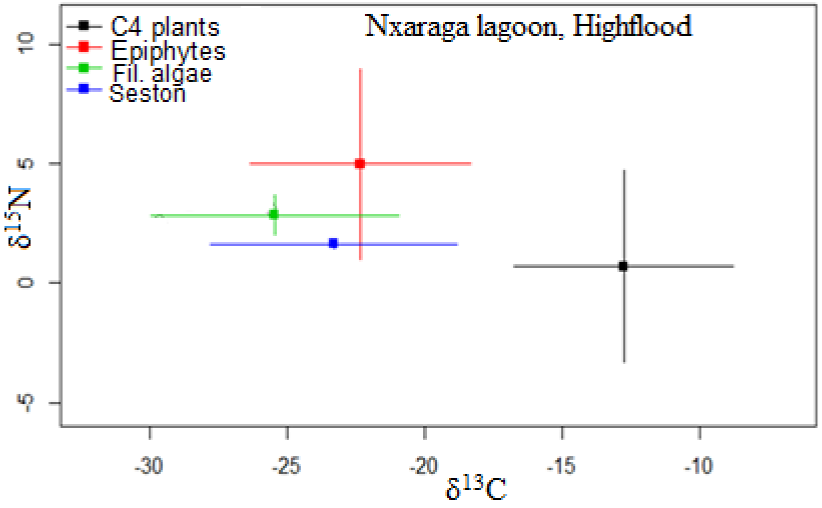
### SI values of aggregate C sources

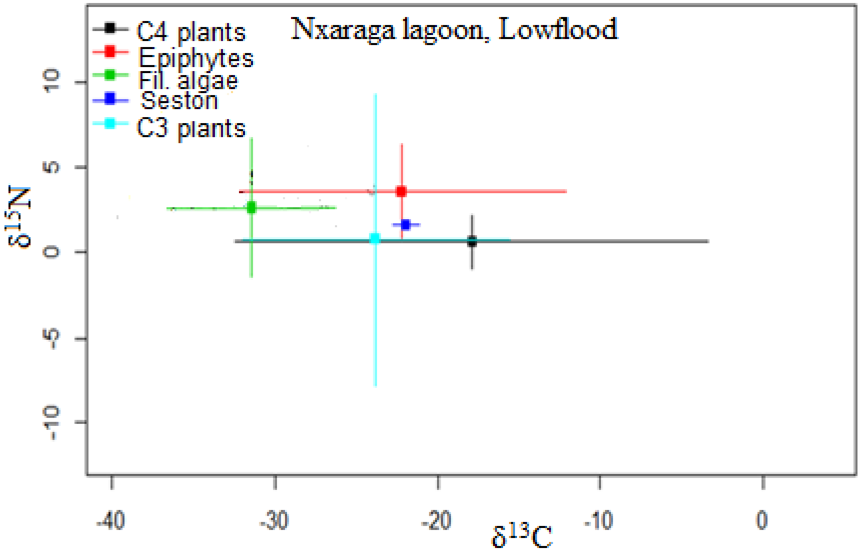
Depending on availability of individual samples, the number of aggregate sources differed between the study sites and in some cases between flood seasons within a study site. The $\delta^{13}C$ values of aggregate sources were least overlapping in Lake Ngami, whereas they showed greater overlap in samples from Nxaraga lagoon and Phelo flood plain compared with those from Lake Ngami (Figure 6.6). While the $\delta^{13}C$ values of aggregate sources were generally less overlapping in Lake Ngami, the $\delta^{13}C$ values of algal epiphytes and particulate organic carbon (seston) were notably overlapping across all seasons with $\delta^{13}C$ values of –19.57‰ ± 3.4 and –20.6‰± 2.2 respectively at high flood and –22.8‰ ± 5.5 and –23.6‰± 0.2 respectively at low flood (Figure 6.6). Filamentous algae at the Lake showed distinctively greater $\delta^{13}C$ values with a mean of –6.0‰ ± 1.8 during high flood and –3.0‰ ± 2.1 during low flood whereas *Typha* sp. had the lowest $\delta^{13}C$ values with mean values of –30.4‰ ± 0.3 and –27.6‰ ± 1.9 during high flood and low flood respectively.

In terms of $\delta^{15}N$ values, the aggregated C sources from Nxaraga and Phelo flood plain had similar range of values and were generally less negative than $\delta^{15}N$ values for basal sources from Lake Ngami sources excluding *Typha sp*. The mean $\delta^{15}N$ values at Nxaraga lagoon ranged from 0.19‰ ± 0.8 for $C_4$ plants collected during high flood to 4.57‰ ± 2.1 for epiphyte samples collected at low flood. In Phelo flood plain, $\delta^{15}N$ values for basal sources ranged from 1.78 ‰ ± 0.3 for sediments to 3.9‰ ± 0.9 for epiphytes collected at high flood (Figure 6.6).


Lake Ngami, High flood
Typha
Epiphytes
Macroalgae
Seston
δ15N
δ13C
15
10
5
0
-5
-10
-15
-30
-25
-20
-15
-10
-5
0


Lake Ngami, Low flood
Typha
Epiphytes
Fil. algae
Seston
δ15N
δ13C
10
5
0
-5
-10
-30
-20
-10
0


Nxaraga lagoon, Highflood
C4 plants
Epiphytes
Fil. algae
Seston
δ15N
δ13C
10
5
0
-5
-30
-25
-20
-15
-10


Nxaraga lagoon, Lowflood
C4 plants
Epiphytes
Fil. algae
Seston
C3 plants
δ15N
δ13C
10
5
0
-5
-10
-40
-30
-20
-10
0

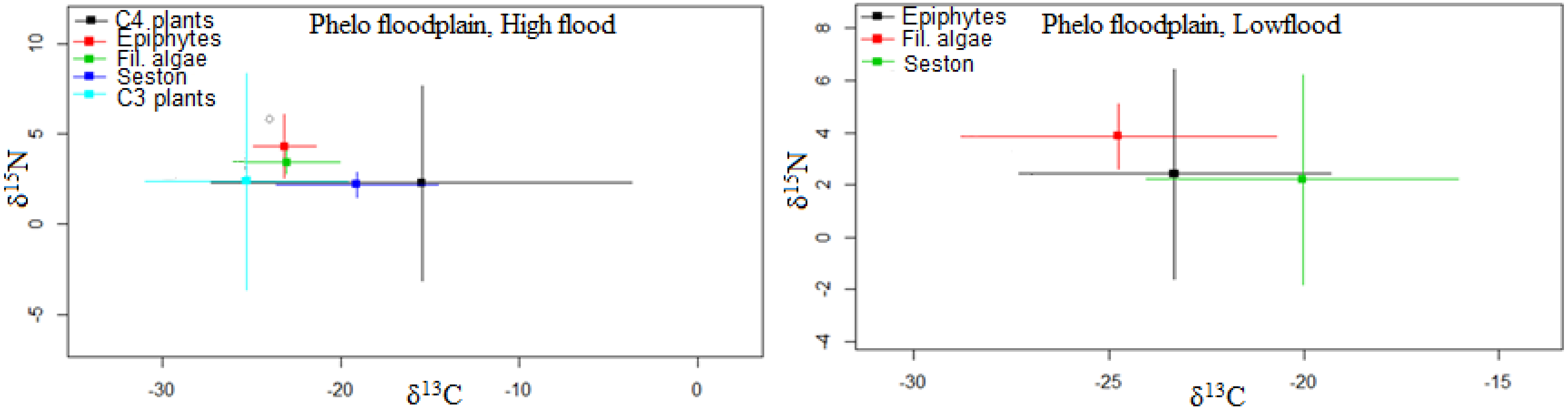


**Figure 6.6**: Cross plots of isotope values of different basal sources of carbon for fish, showing the range of $\delta^{13}C$ and $\delta^{15}N$ for each aggregate source.

## Relative contribution of basal carbon sources to fish assimilated diet

Differences in stable isotope values, mainly $\delta^{13}C$ values, of basal carbon sources facilitated estimations of their relative contribution to fish assimilated diet using the SIAR mixing model (Table 6.3). Based on SIAR estimates, the principal basal carbon sources contributing to fish assimilated diet at Lake Ngami are algal epiphytes and seston. Algal epiphytes were the most assimilated basal carbon source at the lake, contributing an average of 56.4% and 71.2% to fish assimilated diet during high flood and low flood respectively. Among the fish feeding guilds, epiphytes contributed the most to assimilated diet of *omnivores* (63.9%) during high flood and of *insectivores* (85.7%) during low flood. While the contribution of epiphytes to fish assimilated diet at the lake generally increased during low flood, it increased notably for *omnivores* (from 52.9% to 85.7%) and piscivores (from 54.5% to 70.3%) during the flood season.

At Nxaraga lagoon, the most important basal carbon sources to fish were filamentous algae, algal epiphytes and seston. Filamentous algae contributed the most to fish assimilated diet during both high flood and low flood (44.5% and 41.7% respectively). Epiphytes were the second most assimilated basal carbon source by fish at the lagoon, with a greater contribution to fish assimilated diet during low flood (34.5%) than during high flood (20.8%). During high flood filamentous algae contributed the most to assimilated diet of *insectivores* (56.1%) whereas during low flood it contributed the most to assimilated diet of *omnivores* (44.9%). Seston contributed the most to assimilated diet of *piscivores* (20.7%) and *omnivores* (18.7%) during high flood and to assimilated diet of *insectivores* (22%) and *omnivores* (20.9%) during low flood. Overall, the contribution of seston to fish assimilated diet at the lagoon didn't vary much between the flood seasons (16.2% and 18.1% during high flood and low flood respectively).

At Phelo flood plain, basal carbon sources with the highest contribution to fish assimilated diet were epiphytes (29.4%), filamentous algae (26.1%) and $C_3$ macrophytes (21.7%) during high flood, whereas during low flood filamentous algae and epiphytes contributed the most to fish assimilated diet (65.3% and 23% respectively). During high flood, filamentous algae and epiphytes were most assimilated by *piscivores* (32.1% and 35.8% respectively) whereas $C_3$

macrophytes were most assimilated by *omnivores* (30.2%). The contribution of filamentous algae to fish assimilated diet at Phelo floodplain increased by over two-fold at low flood (from 26.1% during high flood to 65.3% during low flood) whereas the contribution of epiphytes to fish assimilated diet was relatively low during low flood (23%) compared to high flood (29.4%).

**Table 6.3**: Results of SIAR model showing the relative contribution (%) of basal C sources to fish trophic groups assimilated diet during high flood and low flood. na = not available

| Site | Consumers | High flood | | | | | Low flood | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fil. algae | $C_3$ plants | $C_4$ plants | Epiphytes | seston | Fil. algae | $C_3$ plants | $C_4$ plants | Epiphytes | seston |
| Lake Ngami | all fish | 3.9 | na | 15.6 | 56.4 | 24.0 | 3.2 | na | 4.9 | 71.2 | 20.6 |
| | Detrivores | 3.4 | na | 18.7 | 54.7 | 23.1 | 7.6 | na | 4.9 | 65.3 | 22.1 |
| | Omnivores | 4.6 | na | 11.0 | 63.9 | 20.5 | 2.9 | na | 9.3 | 63.5 | 24.3 |
| | Insectivores | 4.1 | na | 17.2 | 52.9 | 25.8 | 0.7 | na | 2.3 | 85.7 | 11.1 |
| | Piscivores | 3.5 | na | 15.3 | 54.5 | 26.7 | 1.7 | na | 3.0 | 70.3 | 25.0 |
| Nxaraga | all fish | 44.5 | 12.0 | 6.6 | 20.8 | 16.2 | 41.7 | | 5.5 | 34.5 | 18.1 |
| | Detrivores | 42.1 | 11.2 | 6.7 | 27.7 | 12.3 | 32.1 | na | 5.7 | 45.5 | 16.1 |
| | Omnivores | 39.7 | 16.2 | 8.1 | 18.0 | 18.0 | 44.9 | na | 5.1 | 29.1 | 20.9 |
| | Insectivores | 56.1 | 8.9 | 5.6 | 15.6 | 13.8 | 37.2 | na | 7.9 | 32.8 | 22.0 |
| | Piscivores | 40.0 | 11.8 | 5.9 | 21.7 | 20.7 | 52.7 | na | 3.3 | 30.6 | 13.4 |
| Phelo | all fish | 26.1 | 21.7 | 9.5 | 29.4 | 13.2 | 65.3 | na | na | 23.0 | 11.7 |
| | Detrivores | 23.3 | 24.7 | 10.5 | 28.4 | 13.0 | 68.1 | na | na | 22.1 | 9.8 |
| | Omnivores | 22.9 | 30.2 | 9.1 | 25.4 | 12.4 | 64.9 | na | na | 23.9 | 11.2 |
| | Insectivores | 26.2 | 17.0 | 11.7 | 28.1 | 16.9 | 69.7 | na | na | 19.9 | 10.4 |
| | Piscivores | 32.1 | 14.7 | 6.7 | 35.8 | 10.6 | 58.5 | Na | na | 26.0 | 15.5 |

## 6.5. Discussion

### Isotopic signatures of the analysed basal sources signatures: How the determined $\delta^{13}C$ values compare with literature values

The $\delta^{13}C$ values of the collected basal carbon sources are within the expected the range of $\delta^{13}C$ for freshwater systems (Gladyshev 2009, Chappuis *et al.* 2017) and generally similar to those reported in wetlands. The $\delta^{13}C$ values of $C_3$ macrophytes (–30.19 ‰ to –26.75 ‰) were lower than $\delta^{13}C$ of $C_4$ grasses (–27.25 ‰ to –13.06 ‰), except for *Typha sp* from Lake Ngami which had $\delta^{13}C$ of –29.5 ‰ (Fig. 6.3).A similar range of $\delta^{13}C$ for basal carbon sources have been reported in other African wetlands such Lake Victoria (macrophyte $\delta^{13}C$ mean: –28.5 ± 1.5 ‰,(Masese *et al.* 2015)) and flood pulsed riverine systems in South America namely Parana (range –30.93 ± 8.25‰ to –26.16 ± 0.02‰, Marchese *et al* 2014) and Amazon (macrophyte $\delta^{13}C$ mean: –29.62 ± 1.15‰,(Alves *et al*. 2017)). The $\delta^{13}C$ values for epiphytes (–24.47‰ to –24.86‰) and seston (–21.47‰ to –24.39‰) were greater than the average values reported for other tropical flood pulsed wetlands. For example, lower $\delta^{13}C$ values for epiphytes and seston were reported in Panama wetlands (–30.93‰ and –25.51‰ respectively) (Marchese *et al.* 2014) and in the Amazon floodplains (–25.03 ± 0.78‰ and –30.67 ± 1.72‰ respectively) (Alves *et al*. 2017). A possible explanation for the relatively high $\delta^{13}C$ values for epiphytes and seston is the likely fixation of recycled $CO_2$ by algal epiphytes and by wetland plants at the study sites due to their downstream location within the Delta. Martinelli *et al.* (1991) demonstrated that $\delta^{13}C$ values of local $CO_2$ in floodplain rivers increase in downstream direction due to release of biogenic $^{13}C$-depleted $CO_2$ from plant respiration and decomposition of organic matter. Relatively high $\delta^{13}C$ values for seston could also indicate that a greater proportion of the seston is derived from $C_4$ organic matter, since detritus derived from $C_4$ plants has high $\delta^{13}C$ values than detritus from $C_3$ plants (Finlay and Kendall 2007). Future SIA studies in the Delta, especially those examining the contribution of carbon sources to consumer biomass, should consider the potential isotopic variability of basal carbon sources on the upstream-downstream axis.

## Spatial and temporal patterns of $\delta^{13}C$ values for the collected basal carbon sources

Basal C sources from Lake Ngami had more negative $\delta^{13}C$ and $\delta^{15}N$ values, than basal C sources from the other study sites. As discussed in Chapter 2, differences in $\delta^{13}C$ and $\delta^{15}N$ values between organisms result from the degree to which different organisms discriminate against a heavier isotope. The greater $\delta^{13}C$ and $\delta^{15}N$ values at Lake Ngami is likely due to high phytoplankton productivity rates, which lead to greater concentration of heavier isotopes of C and N and their subsequent uptake by aquatic primary producers. In addition to the likely increase in $\delta^{13}C$ values of primary producers in the downstream direction because of fixation of recycled $CO_2$ as argued above, the $\delta^{13}C$ values of the Lake's basal sources are likely influenced by uptake of the $^{13}C$-enriched DIC pool in the lake. During algal photosynthesis, DIC containing the lighter carbon isotope,$^{12}C$, is preferentially fixed. However, high algal photosynthetic rates rapidly concentrate the $^{13}C$ in the residual DIC pool leading to reduced discrimination against $^{13}C$-containing DIC and hence greater fixation of $^{13}C$ by aquatic primary producers (Finlay *et al.* 1999). The range of $\delta^{13}C$ values for aggregate sources were generally more overlapping in Nxaraga lagoon and Phelo floodplain than in Lake Ngami, although $\delta^{13}C$ values of epiphytes and seston at the lake were notably overlapping. The overlap of algal epiphytes and seston values at the lake could mean that the seston is mostly derived from the algae. The relatively greater overlap of $\delta^{13}C$ values of sources from Nxaraga lagoon and Phelo floodplain could be influenced by a smaller ecosystem size of the two sites compared to Lake Ngami, which likely limits the chance of spatial variability of $\delta^{13}C_{DIC}$ values at the two sites.

Filamentous algae (*Chlorophyta*) collected from Lake Ngami had greater $\delta^{13}C$ (–5.27‰) compared to filamentous algae from Nxaraga lagoon (–33.75‰) and Phelo floodplain (–25.45‰). The isotopic difference could be due the algae fixing dissolved $HCO_3^-$ at the Lake and dissolved $CO_2$ at Nxaraga lagoon and Phelo floodplain. The relatively high pH of Lake Ngami influences greater amounts of $HCO_3^-$ (see chapter 5) in the lake compared to Nxaraga lagoon and Phelo floodplain. Algae is known to have mechanisms of fixing $HCO_3^-$ during photosynthesis (Bade et al., 2004) and this could have influenced high $\delta^{13}C$ values for algae at the lake because $HCO_3^-$ have greater $\delta^{13}C$ than dissolved $CO_2$ (Mook *et al.* 1974, Cerling *et al.* 1991, 2015). According to Raven *et al.* 2002b, there are two groups of *Chlorophyta* identified based on $\delta^{13}C$ values: algae with $\delta^{13}C$ values ranging from −8‰ to −21.3‰ and algae with values ranging from −25.7‰ to −32‰. The first group comprises algal species with ability to fix dissolved $HCO_3^-$ whereas the latter does not the ability to fix $HCO_3^-$. Since the collected Chlorophyta was not identified to species level, there are two explanations to the higher $\delta^{13}C$ values of algae from Lake Ngami: 1) the collected *Chlorophyta* at Lake Ngami consisted of species with $HCO_3^-$– fixing abilities whereas the filamentous algae collected from Nxaraga lagoon and Phelo floodplain did not have $HCO_3^-$– fixing abilities, 2) *Chlorophyta* at all the sites had $HCO_3^-$ fixing abilities but only filamentous algae at Lake Ngami were fixing the $HCO_3^-$ because of its availability at lake Ngami and not at the other sites.

Generally, all basal carbon sources collected from the Lake also showed greater $\delta^{15}N$ values than basal carbon sources collected from other sites. The $\delta^{15}N$ values of the lake's basal carbon sources could be influenced by fixation of N from the anaerobic zone of the lake. During lake stratification, denitrification occurs in the anaerobic part of the lake involving fractionation of +15 to + 30‰, resulting in nitrates of greater $\delta^{15}N$ values (Brandes *et al*. 1998, Voss *et al.* 2001). Mixing of the nitrates from aerobic and anaerobic parts of the lake can lead to greater $\delta^{15}N$ of the basal resources in the lake compared with the $\delta^{15}N$ values of the Chief's Island sites where there may not be stratification or even if there may be stratification, the wind waves, due to small open water space, may not be strong enough to mix the nitrates.

## The relative contribution of collected basal carbon sources to fish assimilated diet

### Spatial patterns

The relative importance of basal carbon sources varied between the study sites, but algal sources were major carbon sources across all the study sites. Based on SIAR estimations, algal sources were the most important basal carbon source for fish across all the study sites (Table 6.3). At Lake Ngami, epiphytes and seston were the most assimilated carbon sources, although the estimated contribution of epiphytes to fish assimilated diet is likely a mixing model artefact as there are too few emergent plants for epiphytes to be an important carbon source for fish in the lake. Filamentous algae were the most important basal carbon source at Nxaraga lagoon and Phelo flood plain (Table 6.3). The relatively greater assimilation of algal carbon sources by fish can be explained by the fact that algal sources lack structural components such as lignin and secondary metabolites such as tannins and are therefore more digestible (Thorp *et al.* 1998,Vadeboncoeur and Power 2017). Previous studies have also found algal sources to be principal carbon sources supporting aquatic food webs in river floodplains (Brito *et al.* 2006; Pettit *et al.* 2011, Thorp and Bowes 2017,Moyo and Richoux 2018). However, there are studies which have found non-algal carbon sources such as macrophytes to be more important than algal sources in river floodplain aquatic food webs depending on the habitat type (Zeug and Winemiller 2008) and the stage of flooding (Roach *et al.* 2015). Zeug and Winemiller (2008) showed that in Brazos River (Texas, USA), algal sources were important in oxbow lakes, whereas macrophytes were more important in flowing channels. Roach and Winemiller (2014) demonstrated that algal sources are more important during the low water conditions whereas aquatic macrophytes are more important during the flooding stage. In this study $C_3$ macrophytes were a major carbon source for fish at Phelo floodplain but were less important to fish at Nxaraga lagoon and Lake Ngami, supporting the observation that the relative importance of non-algal sources to aquatic food webs varies across habitat types. The greater importance of $C_3$ macrophytes to fish at Phelo flood is likely due to greater abundance of $C_3$ organic matter in the floodplain. Floodplains support greater plant biomass than perennial habitats because seasonal replenishment of nutrients (Junk *et al.* 1989, Davies *et al.* 2008).

Filamentous algae was the least important carbon resource for fish at lake Ngami but was the most important carbon source at Nxaraga lagoon and Phelo floodplain. Based on SIAR estimations, the relative contribution of filamentous algae to fish assimilated diet was less than 4% during all flood seasons at Lake Ngami whereas at Nxaraga lagoon it was over 40% during all flood seasons and at Phelo flood plain it was 26% and 65.3% during high flood and low flood respectively. This result was not expected because there is greater algal primary productivity at the lake compared to the other study sites (see Chapter 5) and there is virtual lack of macrophytes, limiting the contribution of non-algal sources at the lake. However, the lower contribution of filamentous algae to fish assimilated diet at the lake could indicate that fish in the lake rely more on carbon derived from phytoplankton (seston) and algal epiphytes, more than on carbon derived from filamentous algae (Table 6.3). This is likely due to smaller fish prey such as zooplankton and macroinvertebrates being able to feed more on phytoplankton than filamentous algae, therefore channelling more planktonic carbon into the aquatic food web as insectivorous fish feed on them. Other studies have also found carbon from phytoplankton to be more important to aquatic food webs than carbon derived from filamentous algae (Truong *et al.* 2017). Lake Ngami has greater phytoplankton primary production than both Nxaraga lagoon and Phelo floodplain (see Chapter 5) and the lack of macrophytes that would provide refuge for the smaller organisms, therefore the plankton pathway may be the major source of energy for the lake's aquatic food web (Segovia *et al.* 2014).

### Temporal patterns

Seasonal variations were observed on the relative contribution of basal sources to fish assimilated diet at all the study sites. At lake Ngami, algal epiphytes and seston were the major carbon sources during all flood seasons, but the relative contribution of algal epiphytes to fish assimilated diet increased during low flood (from 56.4% during high flood to 71.2% during low flood) (Table 6.3). The increase in the relative importance of algal epiphytes is likely due to increased algal production during low flood season. Low water conditions occur during hot summer months (November to February) before the arrival of the flood pulse, leading to higher water temperature and lower water levels and hence influencing high algal densities in the lake (Mazebedi *et al*. 2018). Other studies on freshwater food webs have also shown that the importance algal sources vary seasonally corresponding to the rate of algal primary productivity (Grey and Jones 2001, Reis *et al.* 2017).

Nxaraga lagoon and Phelo floodplain were highly reduced in size during low flood and there was virtually no aquatic vegetation in habitats, hence limiting the contribution of macrophytes to fish assimilated diet (Table 6.3). Low flood sampling was undertaken between December 2015 and January 2016, which was the onset of very strong 2015-16 El Niño-Southern Oscillation (ENSO) event, a climatic period associated with low rainfall amounts and extremely hot atmospheric temperatures (Kogan and Guo 2017, Pomposi *et al.* 2018). The potentially high evaporative rates during the El Niño had reduced both aquatic habitats to small pools of not more than 30m widest diameter, detaching the habitats from littoral reeds. Macrophytes were lacking in the central parts of the pools likely due to hippopotamus movement and activity in the confined small pools. The only macrophyte collected during the sampling period was *Schoenoplactus sp.* ($C_4$) at Nxaraga lagoon which had the least relative contribution to fish assimilated diet at the site amongst the three sources that were evaluated by SIAR. The $C_4$ macrophyte contributed less than 10% to assimilated diet of all fish feeding guilds at Nxaraga lagoon during low flood (Table 6.3). It is noteworthy that despite the lowest relative contribution of $C_4$ carbon to fish assimilated diet at Nxaraga lagoon during low flood, insectivores had assimilated relatively more carbon than other fish guilds, likely due to consumption of terrestrial arthropods (Correa and Winemiller 2018).

The contribution of macrophyte carbon sources to fish assimilated diet was greatest in Phelo flood plain during high flood (Table 6.3). Aquatic macrophytes were the most important basal C sources for omnivores and insectivores at Phelo flood plain during high flood. The greater importance of the plant basal sources in Phelo floodplain is likely influenced by greater abundance of plant derived allochthonous matter in the floodplains. During high flood season, herbivorous invertebrates consume submerged riparian vegetation and the invertebrates are in turn consumed by insectivorous and omnivorous fish. Fish have greater access to terrestrial insects during high flood (Balcombe *et al*. 2005). In Apaporis River in western Amazon, Correa and Winemiller (2018) demonstrated that arthropods were a very important trophic link between basal terrestrial carbon and fish production during high water flood. Decomposing terrestrial vegetation are also an important carbon source in floodplains during high flood (Rayner *et al.* 2010) because detrivorous fish exploit the nutritious microbial biomass associated with the decomposing organic matter (German *et al.* 2010, Lujan *et al.* 2011).

## 6.6. Conclusions

The relative importance of basal carbon sources in supporting aquatic food webs in differently flooded habitats of the Delta was revealed and the influence of the seasonal flood pulse on the relative importance of sources was shown. Algal sources were overall the principal carbon sources to all fish trophic guilds at all the study sites. The relative importance of aquatic macrophytes increased during high flood at Phelo floodplain, confirming predictions of the Flood Pulse Concept (Junk *et al.* 1989) that vascular carbon provides a significant alternative carbon source to floodplain fish during high flood. Overall, the results demonstrate that the relative importance of carbon sources to aquatic food webs in the Okavango Delta vary with respect to habitat hydrology, indicating the need to maintain the different flooding regimes through an undisturbed natural flooding regime.

# CHAPTER SEVEN: Variability of trophic structure across different flooding regimes within the Okavango Delta.

## 7.1. Introduction

The periodic linking of perennial channels with flanking seasonal swamps facilitates a seasonal flux of food and habitat resources to the aquatic food webs in the Okavango Delta. The arrival of floods increases the water volume, habitat complexity and biological productivity (Junk *et al*. 1989, Kruk and Segura 2012), that in turn influence population dynamics and species interactions. The food web dynamics that occur due to the influence of the seasonal flooding are not always obvious and can be complex. For example, while the arrival of the flood pulse increases biological production and prey abundance, the increase in water volume and increased habitat complexity may have a dilution effect and reduce the density of pre-existing prey (Welcomme 1979, Okada *et al.* 2003). The effect of seasonal flooding on the food webs of flood pulsed wetlands can be dynamic and needs to be adequately studied before any theoretical model can be used to describe the food web dynamics. In the Okavango Delta, the relative importance of carbon sources at the base of aquatic food webs (horizontal structure of food webs) have been shown to vary with respect to different flooding regimes (Chapter 6), necessitating an investigation of how the entire food web structure is influenced by the flooding regimes. This chapter builds on Chapter 6 by examining how the feeding relationships of aquatic organisms from basal sources to top predators (the vertical structure of food webs) varies in relation to the different flooding regimes of the Delta.

There are widely used matrices for quantifying food web structure based on stable isotope analyses (SIA), called Layman matrices, which quantitatively reveal important descriptors of food web structure (Layman *et al*. 2007, Jackson *et al.* 2011). The Layman matrices describe food web structure based on SIA data as follows:

(i) The $\delta^{15}$N range (NR) is the difference between $\delta^{15}$N values of the most enriched and the least $^{15}$N enriched consumer, and therefore represents the vertical structure of a food web. A wide $\delta^{15}$N range indicates more trophic links, whereas a narrow range indicates fewer trophic links;

(ii) The $\delta^{13}C$ range (CR) is the difference between the $\delta^{13}C$ values of the most enriched and the least $\delta^{13}C$ enriched consumer. The value represents the breath or variety of C resources supporting a food web. High values of CR are expected in food webs supported by a greater variety of C sources whereas low CR values are expected in food webs relying on a narrow range of C sources.

(iii) The mean distance to centroid (CD) is a measure of trophic diversity within an ecosystem. It is calculated as the average Euclidean distance of each species to the group's ellipse centroid (i.e. group bivariate mean)

(iv) The mean nearest neighbour distance (NND) is the mean of the closest Euclidean distances between species in a $\delta^{13}C$ and $\delta^{15}N$ biplot. It is proxy for trophic similarity between species in a community. Food webs constituted by species with similar trophic ecologies (high trophic redundancy) will have a relatively low NND value, whereas food webs made of species of more divergent trophic ecologies will have a greater NND value.

(v) The standard deviation of the nearest neighbour distance (SDNND) is a measure of the how evenly stable isotope values of consumers are distributed within a $\delta^{13}C$ and $\delta^{15}N$ biplot. It is used as a measure of trophic evenness or trophic segregation. For example, the value of SDNND will be low if there is generally an even level of trophic partitioning within a food web, whereas SDNND will be high if a food web is constituted from several segregations of trophic niches. SDNND is therefore also used to gauge trophic redundancy.

Trophic niches are also important descriptors of food web dynamics because they indicate changes in the use and partitioning of resources between different taxa. A trophic niche can be described as the totality of food resources required by an organism for its survival and reproduction (Newsome *et al.* 2007). Trophic niches result from a species' evolutionary adaptations including morphological, physiological and behavioural adaptations to local environments (Winemiller and Jepsen 1998, Chase and Leibold 2003). Temporal and spatial variations in the physical, chemical and biological environments determine the availability and abundance of resources (including habitat and prey resources) making species' niches variable over time and space. For example, trophic niches have been demonstrated to vary in response to changes in availability of prey (Buren *et al*. 2012), abiotic conditions (Stuart-Smith *et al*. 2004), stage of development (Wener and Hall 1988) and interactions with predators (Siepielski *et al.* 2017). Based on SIA data, trophic niche size is estimated using ellipse areas on a SIA biplot which is usually calculated using the Stable Isotope Bayesian Ellipses package in R (SIBER, Jackson *et al*. 2011).

Fish populations are preferred for studying food web dynamics of river-floodplain ecosystems because they are highly abundant in these ecosystems (Welcomme 1979, Winemiller 2003) and have a high diet plasticity (Balcombe *et al.* 2005, Pool *et al*. 2017). The abundance of fish in river-floodplain systems influences the abundance of their prey and of organisms which prey on them; they therefore exert strong influences on the overall trophic structure (Reynolds *et al.* 2006). Their dietary plasticity also makes them ideal for examining the effects of seasonal environmental changes on aquatic food webs. Quantifying the variability of fish trophic structure can be a significant step towards understanding ecosystem functioning dynamics because there is a strong link between ecosystem functioning and food web structure (Hansson *et al.* 2013)

In the Okavango Delta, there is a high diversity of aquatic habitats that result from both seasonal and long-term flooding patterns (Ramberg *et al.* 2006). These aquatic habitats differ in their water permanence, habitat complexity and structure, and in their aquatic communities. Food web properties of the different aquatic habitats are expected to vary because of their environmental and biological differences. However, there is currently no study that has comprehensively quantified food webs across the hydrological gradients. This study contributes towards the understanding of how the Delta’s aquatic food webs vary with respect to hydrology by quantitatively comparing food web structure across aquatic habitats of different flooding regimes using the SIA approach.

## 7.2. Objectives

Floodplains are characterised by greater biological productivity and complex macrophyte architecture compared to perennial habitats, therefore Phelo floodplain is hypothesised to have greater diversity of carbon sources, more refuges for prey, and greater diversity of feeding pathways compared to both Nxaraga lagoon and Lake Ngami. Aquatic food webs at Lake Ngami were expected to have relatively fewer feeding pathways with many links due to its high algal productivity and lack of macrophyte refuge to provide refuge for fish prey. Nxaraga lagoon was expected to have an intermediate diversity of feeding pathways and carbon sources and of macrophyte refuge. The arrival of the floods is expected to increase carbon sources, habitat space and complexity and hence increase feeding pathways across all the study sites. Using Layman matrices, the above predictions can be stated as follows:

- Aquatic food web of Phelo floodplain will have greater CR, NND and SDNND but smaller NR values compared to aquatic food web of Nxaraga lagoon and Lake Ngami
- Lake Ngami will have the highest NR values and the lowest CR, NND and SDNND values
- Nxaraga lagoon will have intermediate values of all the Layman matrices
- For each study site, Layman matrices are expected to be greater during high flood than during low flood.

To test the above predictions, the following objectives were drawn:

1. To compare Layman's matrices of food web structure across differently flooded habitats during low-water and high-water conditions.
2. To compare the size and overlap of fish trophic niches across differently flooded habitats during low-water and high-water conditions.

## 7.3 Methods

Methods for field collection and laboratory analyses of SI samples are described in Chapter 6, sections 6.3.1 – 6.3.4, and 6.3.5 respectively. Here I focus in on the statistical analyses undertaken to test the predictions detailed above.

### Statistical analyses of SI data

To aid interpretation of SIA results on food web structure, fish diversity and relative abundance of feeding groups (trophic guilds) at each study site was examined using the Simpson diversity index (Simpson-D) and percentage counts of fish trophic guilds, namely detrivores, insectivores, omnivores and piscivores. Fish were assigned into the trophic guilds based on their main prey items using information about their feeding ecology from Skelton (1997), Mosepele *et al*. (2012) and Fishbase (2017). Fish that feed mainly on basal resources including algae, aquatic plants and detritus were put into the group detrivores, fish that rely on basal resources, insects and other fish were put into the group omnivores, fish that feed mainly on insects were put into the group insectivores, whereas fish that feed mainly on other fish were put into the group of piscivores. Diversity calculations were performed using the PAST statistical software (Hammer 2018). Simpson-D was used to compare fish diversity because it measures both species richness and abundance. The values of Simpson-D fall between 0 and 1, where 1 represents complete diversity and 0 represents complete similarity. Unlike other indices such as the Shannon diversity index (H') which may compound species richness and evenness, Simpson D reveals the differences in species abundance (Magguran 2005) making it very useful for this study since the level of prey abundance or consumer abundance can influence trophic interactions (Winemiller *et al.* 2014).

Before any comparisons could be made using SIA data, all $\delta^{13}C$ and $\delta^{15}N$ data sets were tested for normality using Shapiro-Wilk test to determine whether parametric or non-parametric tests were suitable for the comparisons. Most of the SIA data were not normally distributed, often with at least one variable non-normally distributed among the variables being compared. Non-parametric tests were therefore used for the comparisons on all SIA data. Variations of $\delta^{13}C$ and $\delta^{15}N$ values between trophic groups and across study sites were examined using Kruskal -

Wallis test followed by Mann Whitney pairwise comparisons, when the Kruskal-Wallis test showed significant results. The Kruskal -Wallis test is appropriate for evaluating differences among independent variables but will not show which pair(s) of variables are significantly different. Mann Whitney test is suitable for pairwise comparisons of non-normally distributed variables. These tests were computed in R software (R core team, 2018). The statistical comparisons were visualised by bivariate box plots. The $\delta^{13}C$ and $\delta^{15}N$ values of aquatic macroinvertebrates and zooplankton were too few to allow each of the groups to be statistically compared across sites and seasons, therefore the SI data for the groups were pooled. Macroinvertebrates and zooplankton also represent the total invertebrate prey resource for fish, thus it makes ecological sense to combine them when comparing $\delta^{13}C$ and $\delta^{15}N$ values of fish and their prey resources. Aquatic macrophyte, algae and sediment $\delta^{13}C$ and $\delta^{15}N$ values were also pooled together as they represent basal carbon resources for fish. Pooling invertebrate prey and basal resources also allowed comparison of trophic niche size of the prey resources across the study sites using the Stable Isotope Bayesian Ellipses package in R (SIBER, Jackson *et al.* 2011) as some groups did not have enough data points.

Using $\delta^{13}C$ and $\delta^{15}N$ values of fish, five food web structure metrics developed by Layman (2007) and later revised into a Bayesian framework by Jackson *et al.* (2011) were computed using SIBER to compare food web structure across study sites and between flood seasons (Jackson *et al.* 2011). The Layman's matrices are described in the introduction (section 7.1). In addition to Layman's matrices, Total and Standard ellipse areas (TA and SEA, expressed as ‰$^2$), calculated using SIBER (Jackson *et al.* 2011), were used to compare trophic niche size for fish, invertebrates and basal resources within and between sites. The total ellipse area (TA) contains 95% isotopic data points around a bivariate mean of $\delta^{13}C$ and $\delta^{15}N$, and it estimates the total extent of the trophic niche of an organism. The standard ellipse area (SEAc) contains 40% of isotopic data points around the bivariate and is a proxy for core trophic niche space. The subscript "c" indicates that a small sample size correction was applied to SEA calculations so that the estimates are less sensitive to sample size, making comparison of trophic niches of communities with different sample sizes more accurate. The Bayesian estimates of SEA (SEAb) were used to evaluate significance of SEA variation and were derived by bootstrapping of SEA (n = 1,000).

Comparisons of trophic niche overlaps across study sites at high flood and low flood were made using total ellipse area (95% ellipses) for different fish feeding guilds plotted in R using

SIBER. The degree of overlap between trophic niches of the trophic guilds was calculated as the proportion of overlapping area to the total area of the two ellipses being compared. For example, the proportion of trophic overlap between ellipse 1 and 2 would be calculated as follows: Percentage of overlap = [area of ellipse 1 and 2/ (ellipse 1 area + ellipse 2 area)] *100.

## 7.4. Results

### Fish assemblage structure at the study sites

The total number of fish species collected was 20 at Lake Ngami from six net settings, 19 at Nxaraga from six net settings and 24 at Phelo floodplain (Hippo pool) from four net settings (Table 7.1). Based on abundance comparisons of fish collected during both high flood and low flood seasons using Chi squared test, fish abundance was significantly high during low flood at Lake Ngami $\chi^2(2, N=13) =544.8, p= < 0.05$, Nxaraga lagoon $\chi^2(2, N=13) =329.2, p= < 0.05$ and Phelo floodplain $\chi^2(2, N=12) =339.4, p= < 0.05$. Despite the higher fish abundance during low flood, more fish species were collected during high flood compared to low flood, except at lake Ngami where species richness was almost the same (16 and 17 species respectively (Table 7.1)). The abundant species at the Lake Ngami were *Brycinus lateralis, Barbus paludinosus, Barbus unitaeniatus* and *Barbus paludinosus*. At Phelo flood plain and Nxaraga lagoon, 21 and 17 fish species respectively were collected during high flood whereas during low flood 16 fish species and 14 fish species respectively were collected at the study sites (Table 7.1). *Brycinus lateralis* and *Schilbe intermedius* were abundant in both Nxaraga lagoon and Phelo flood plain. *Marcusenius macrolepidotus* common across all study sites but was notably abundant in Nxaraga lagoon during low flood. *Petrocephalus catastoma* and *Barbus paludinosus* were also abundant at Nxaraga lagoon during low flood (Table 7.1).

**Table 7.1:** The number of fishes collected at each site during high flood and low flood, with their species and trophic guild indicated. The number of net settings is indicated by *n*.

| Species | Lake Ngami | | Nxaraga | | Phelo | | Trophic guild |
|---|---|---|---|---|---|---|---|
| | High flood<br>*n* = 4 | Low flood<br>*n* = 2 | High flood<br>*n* = 4 | Low flood<br>*n* = 2 | High flood<br>*n* = 2 | Low flood<br>*n* = 2 | |
| *Oreochromis andersonii* | 3 | 7 | 4 | 0 | 3 | 8 | detritivore |
| *Oreochromis macro* | 2 | 3 | 0 | 0 | 7 | 0 | detritivore |
| *Tilapia rendalli* | 0 | 3 | 1 | 1 | 0 | 4 | detritivore |
| *Tilapia sparrmanii* | 2 | 4 | 1 | 3 | 4 | 16 | detritivore |
| *Micropanchax johnstoni* | 0 | 0 | 0 | 0 | 18 | 2 | insectivore |
| *Brycinus lateralis* | 344 | 382 | 124 | 89 | 112 | 54 | insectivore |
| *Barbus poechi* | 33 | 22 | 23 | 26 | 52 | 0 | insectivore |
| *Hippopotamyrus ansorgii* | 0 | 2 | 1 | 0 | 0 | 0 | insectivore |
| *Marcusenius macrolepidotus* | 10 | 47 | 62 | 144 | 20 | 38 | insectivore |
| *Mormyrus lacerda* | 0 | 0 | 11 | 0 | 10 | 19 | insectivore |
| *Pseudocrenilabrus philander* | 0 | 0 | 0 | 1 | 2 | 0 | insectivore |
| *Petrocephalus catastoma* | 0 | 5 | 15 | 123 | 2 | 0 | insectivore |
| *Pharyngochromis acuticeps* | 2 | 0 | 0 | 0 | 0 | 0 | insectivore |
| *Synodontis spp* | 0 | 0 | 19 | 4 | 10 | 4 | insectivore |
| *Hollandichthys multifasciatus* | 0 | 0 | 0 | 0 | 2 | 0 | insectivore |
| *Barbus multilineatus* | 0 | 0 | 0 | 0 | 7 | 0 | insectivore |
| *Ctenopoma multispine* | 0 | 0 | 0 | 0 | 3 | 0 | insectivore |
| *Barbus paludinosus* | 82 | 575 | 18 | 132 | 0 | 15 | omnivore |
| *Barbus thamalakanensis* | 0 | 0 | 1 | 0 | 11 | 0 | omnivore |
| *Barbus unitaeniatus* | 293 | 465 | 29 | 0 | 34 | 12 | omnivore |
| *Sargochromis condringtonii* | 1 | 1 | 0 | 0 | 0 | 0 | omnivore |
| *Clarius ngamensis* | 0 | 1 | 2 | 2 | 0 | 7 | omnivore |
| *Barbu hassianus* | 0 | 0 | 0 | 0 | 11 | 2 | omnivore |
| *Clarius gariepinus* | 6 | 4 | 5 | 4 | 3 | 11 | piscivore |
| *Hepsetus odoe* | 5 | 3 | 3 | 4 | 6 | 13 | piscivore |
| *Serranochromis thumbergii* | 2 | 4 | 0 | 0 | 0 | 0 | piscivore |
| *Schilbe intermedius* | 20 | 15 | 95 | 64 | 64 | 112 | piscivore |
| *Serranochromis altus* | 3 | 0 | 0 | 0 | 1 | 6 | piscivore |
| *Serranochromis macrocephalus* | 1 | 2 | 0 | 1 | 0 | 0 | piscivore |

Fish diversity was greater in Nxaraga lagoon and Phelo flood plain compared to Lake Ngami during all flood seasons based on Simpson's diversity index (D) (Table 7.2). Species richness (S) was greatest at Lake Ngami although Simpson diversity index (D) was lower compared to the other study sites. At Nxaraga lagoon and Phelo floodplain, the Simpson diversity index did not indicate seasonal changes in fish diversity, however, the species richness was greater during high flood (S = 17 and 21 respectively) than low flood (S = 14 and 16 respectively) (Table 7.2).

The relative abundance of omnivores in Lake Ngami and Nxaraga lagoon was greater during low flood (67.4% and 22.4%) than during high flood (48.4% and 12.1%) whereas at Phelo floodplain the abundance of omnivores was greater during high flood (14.7%) compared to low flood (11.2%) (Table 7.2). At Lake Ngami and Phelo flood plain, insectivores were more dominant during high flood (45.9% and 63.3% respectively) than during low flood (29.6% and 36.2% respectively), whereas at Nxaraga lagoon the relative abundance of insectivores was similar between high flood (61.6%) and low flood seasons (64.7%). Piscivores were better represented in Nxaraga lagoon and Phelo floodplain than in Lake Ngami (Table 7.2); in fact, piscivores were the most dominant guild at Phelo flood plain during low flood (35.0%). Detrivores were the least represented guild and they were better represented at Phelo floodplain where their relative abundance was greater at low flood (8.7%) compared to high flood season (3.7%) (Table 7.2).

**Table 7.2**: Diversity indices and proportion of fish feeding guilds at the study sites during high and low flood seasons

| | Richness S | Simpson's_ D | Dominance | % Detrivore | (%) Insectivore | (%) Omnivore | % Piscivore |
|---|---|---|---|---|---|---|---|
| **Highflood** | | | | | | | |
| L.Ngami | 15 | 0.7 | 0.4 | 0.9 | 45.9 | 48.5 | 4.8 |
| Nxaraga | 17 | 0.8 | 0.2 | 1.5 | 61.6 | 12.1 | 24.9 |
| Phelo | 21 | 0.9 | 0.1 | 3.7 | 63.3 | 14.7 | 19.4 |
| **Lowflood** | | | | | | | |
| L. Ngami | 18 | 0.7 | 0.3 | 1.1 | 29.6 | 67.4 | 1.8 |
| Nxaraga | 14 | 0.8 | 0.2 | 0.7 | 64.7 | 22.4 | 12.2 |
| Phelo | 16 | 0.8 | 0.2 | 8.7 | 36.2 | 11.2 | 44.0 |

### Trophic niche size of fish and prey resources at the study sites

Based on trophic niche indices (expressed in ‰$^2$), which use isotopic niche space as a proxy for trophic niche size, total trophic niche size (TA) and core trophic niche size (SEAc and SEAb) for fish, invertebrates and basal resources were greater in Lake Ngami compared to Nxaraga lagoon and Phelo flood plain (Table 7.3). At each study site, the size of trophic niches reduced with each increase in trophic level, except at Phelo floodplain where there was no difference in the size of trophic niches of invertebrates and basal carbon sources (Figure 7.1).

**Table 7.3:** Stable isotope values and SIBER matrices for different trophic components across study sites. TA = Total ellipse area, SEAc =standard ellipse area with sample size correction, Bayesian standard ellipse area

| | Trophic component | n | TA | SEAc | SEAb |
|---|---|---|---|---|---|
| Lake Ngami | Fish | 61 | 60.2 | 11.4 | 11.4±1.5 |
| | Invertebrates | 22 | 78.1 | 37.2 | 36.6±10.6 |
| | Basal C sources | 30 | 292.6 | 96.9 | 96.9±16.7 |
| Nxaraga | Fish | 50 | 20.5 | 5.7 | 5.8±0.9 |
| | Invertebrates | 16 | 23.8 | 12.6 | 13.6±4.8 |
| | Basal C sources | 38 | 174.1 | 45.2 | 45.3±9.1 |
| Phelo | Fish | 45 | 21.1 | 4.2 | 4.2±0.5 |
| | Invertebrates | 26 | 62.8 | 24.0 | 24.1±5.5 |
| | Basal C sources | 39 | 106.8 | 24.0 | 23.9±3.6 |

Comparisons of core niche size (SEAb) of fish, invertebrates and basal carbon sources is visualised using box plots in Figure 7.1 below.

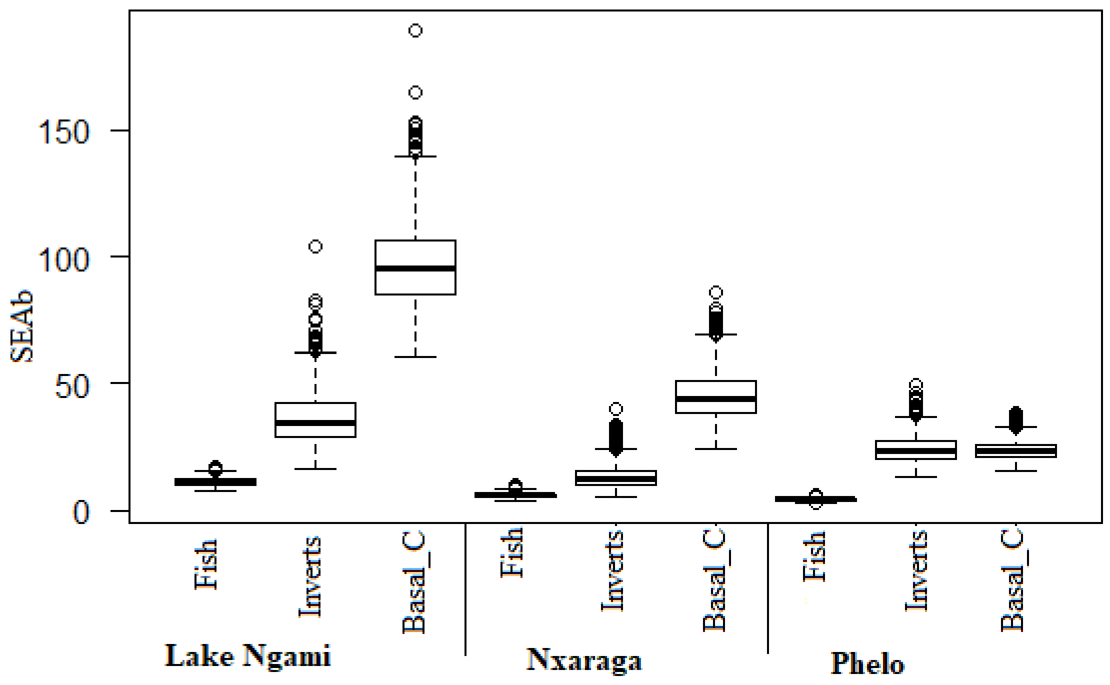


**Figure 7.1:** Within and between-site comparisons of core trophic niches (SEAb) of fish, invertebrates and basal resources across study sites. The black lines represent medians, the vertical size of the boxes are the interquartile ranges, the dotted line represent lowest and highest extremes of the data that do not exceed 1.5 times distance from the middle 50% of the data, and the small circles denote outliers, greater than 1.5 (but less than 3) times distance from the median.

While the change in core trophic niche size of fish guilds with respect to flood season was generally variable across study sites, the core trophic niche size of insectivores and omnivores showed significant increase at high flood across all study sites (Figures 7.2 A-C). The SEAc of insectivores at Lake Ngami, Nxaraga lagoon and Phelo flood plain during high flood was 1.44‰$^2$ (95% CI: 0.1-7.6‰$^2$), 1.18 (95%CI: 0.2-2.6‰$^2$) and 5.86‰$^2$ (95% CI:1.4-10.6‰$^2$) respectively whereas during low flood it was 0.79‰$^2$ (95%CI: 0.4-3.0‰$^2$), 0.32‰$^2$ (95%CI: 0.1-1.0‰$^2$) and 0.15(95%CI: 0.05-0.3‰$^2$) respectively (Fig. 7.2A-C). Given the observed data, there is a 86%, 93% and 100% chance that SEAc of insectivores at Lake Ngami, Nxaraga lagoon and Phelo flood plain respectively will be greater during high flood than during low flood.

For omnivores, the SEAc during high flood was 3.36‰$^2$ (95%CI:0.8-6.3‰$^2$), 2.69‰$^2$ (95%CI:1.2-5.0‰$^2$) and 4.71‰$^2$ (95%CI:2.7-7.1‰$^2$) at Lake Ngami, Nxaraga lagoon and Phelo flood plain respectively whereas during lowflood the SEA for the trophic guild at the respective sites was 1.62 (95%CI: 0.7-2.7‰$^2$), 0.15‰$^2$ (95%CI: 0.3-2.7‰$^2$) and 1.09‰$^2$ (95%CI:0.4-2.1‰$^2$) (Fig. 7.3A-C). Based on the obsevered data, there is a 0.5%, 1.0% and 1.0% probability that SEAc is greater during high flood than at low flood at the study sites respectively.

At Phelo floodplain piscivores and detrivores had a greater SEA during highflood (mean = 3.09‰$^2$, CI =1.5-4.9‰$^2$ and mean=2.40‰$^2$, CI = 1.3- 3.7‰$^2$ respectively) than during lowflood (mean = 1.6‰$^2$, CI = 0.5-3.3‰$^2$ and mean=0.6‰$^2$, CI =0.2-1.3‰$^2$ respectively) whereas at Lake Ngami piscivores and detrivores had a greater SEA during low flood (mean = 13.8‰$^2$, CI = 5.5-23.6‰$^2$ and mean=2.94‰$^2$, CI =1.3-5.4‰$^2$ respectively) compared to high flood (mean = 1.11‰$^2$, CI = 0.5-2.5‰$^2$ and mean=0.85‰$^2$, CI =0.4-1.4‰$^2$ respectively) (Figs. 7.2a and 4c ). The SEA of the trophic guilds did not differ significantly at Nxaraga lagoon between high flood (mean = 2.92‰$^2$, CI = 0.9-5.6‰$^2$ and mean=0.43‰$^2$, CI =1.3-0.8‰$^2$ respectively) and low flood (mean = 3.57‰$^2$, CI = 1.0-7.0‰$^2$ and mean=0.84‰$^2$, CI =0.2-1.6‰$^2$ respectively) (Fig. 7.2B).

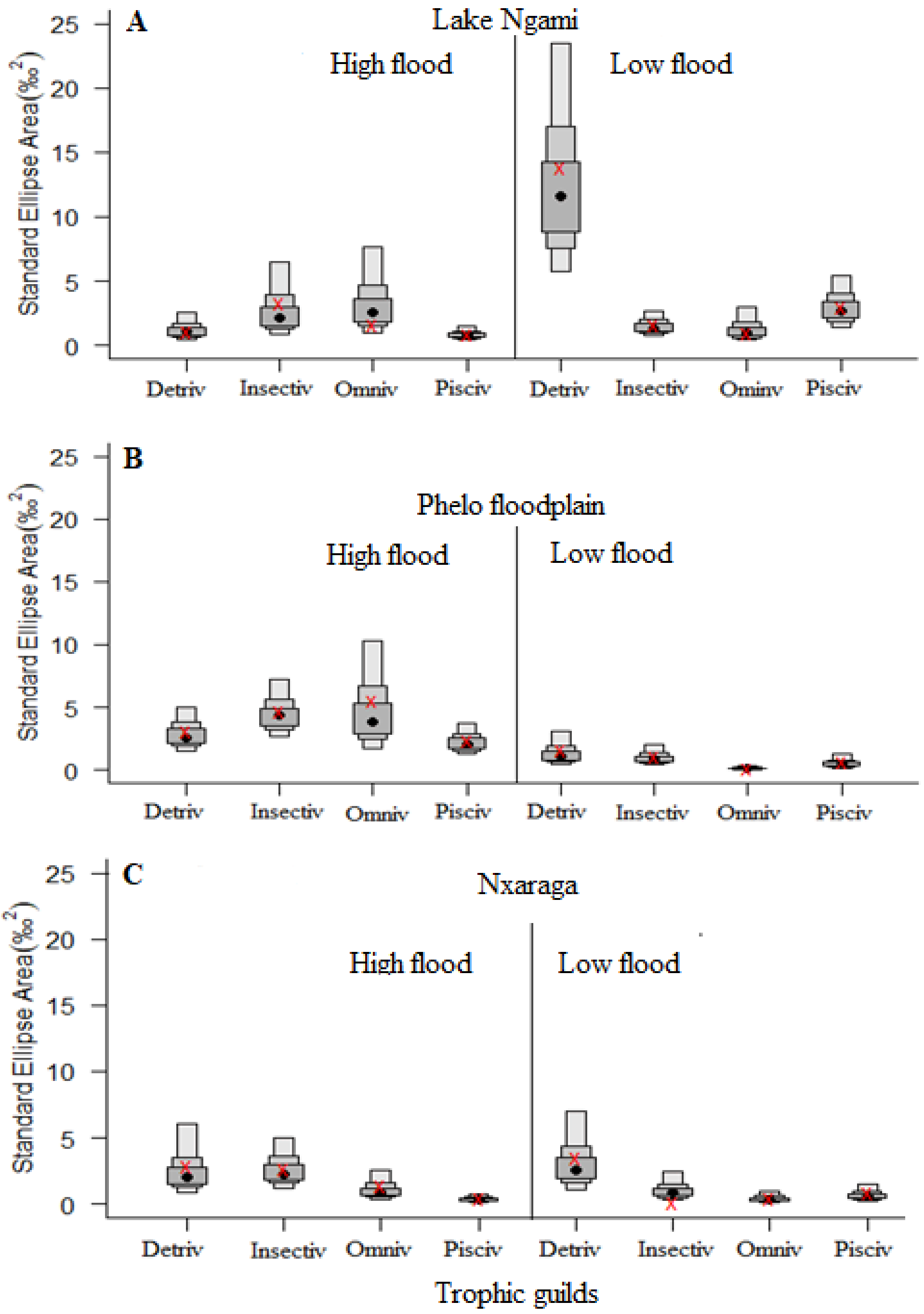


**Figure 7.2A-C**: Comparisons trophic niche size of different fish feeding guilds in Lake Ngami (7.2A), Nxaraga lagoon (7.2B) and Phelo floodplain (7.2C) during high flood and low flood seasons using bayesian standard ellipse areas (SEA) as proxy for the niche sizes. The black points are mean values, the red cross is the mean of size corrected SEA (SEAc) and the boxed areas in decreasing black and white tone represent 95, 75 and 50% confidence intervals around SEA mean.

Comparison of Layman's matrices across study sites and between flood seasons within each study site indicated variability of some food web properties between study and between flood stages at each study site (Fig. 7.3, Table 7.4). During high flood, Lake Ngami had more isotopically distinct carbon sources (C range), greater trophic diversity (TA) and greater trophic segregation (SDNND) than Nxaraga lagoon (Student's t-test, $p<0.005$) (Fig. 7.3, Table 7.4). Compared to Phelo flood plain, the lake had a greater food chain length (N range), greater trophic diversity (CD) and greater trophic segregation (SDNND) (Student's t-test, $p<0.005$) (Fig. 7.3, Table 7.4). During low flood, food chain length (N range) and trophic diversity (TA) were greater at lake Ngami compared to Nxaraga, but Nxaraga had greater trophic redundancy (MNND) than the lake (Student's t-test, $p<0.005$) (Fig. 7.3, Table 7.4). All Layman's matrices greater at lake Ngami than at Phelo floodplain during low flood (Student's t-test, $p<0.005$) (Fig. 7.3). In terms of seasonal variability of food web properties within study sites, at Lake Ngami the number of isotopically distinct carbon sources (C range) and trophic diversity were greater at low flood than high flood (Student's t-test, $p<0.005$) (Fig. 7.3, Table 7.4) . At Nxaraga lagoon, food chain length (N range) and trophic diversity (TA) were greater at low flood while trophic redundancy was lower (greater MNND) than during high flood (Fig. 7.3).The values of Layman's matrices at Phelo floodplain were generally low compared to other study sites and did not show significant seasonal variation (Fig. 7.3, Table 7.4)

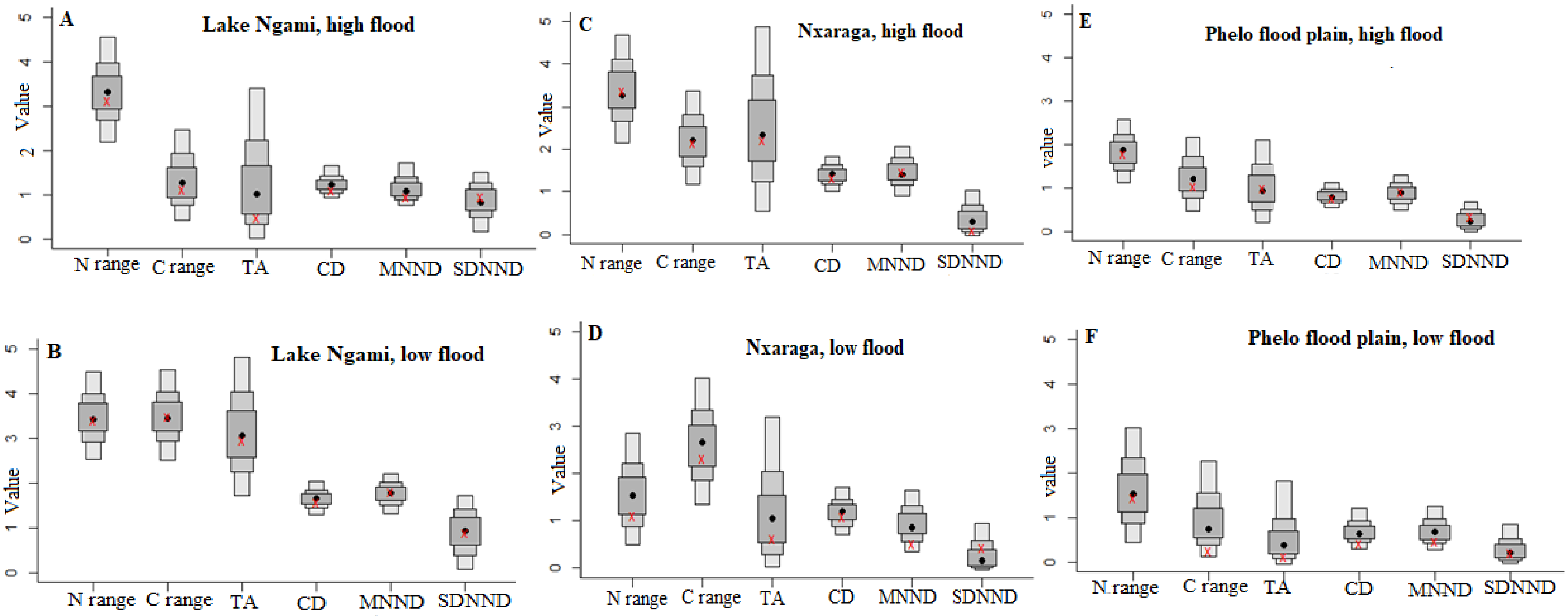


**Figure 7.3** Comparison of Layman's trophic matrices across study sites at different flooding seasons. The red cross is the mean values of the matrics, the black dots are meadian values and the boxed areas in decreasing colour tone respectively represent 95, 75 and 50% confidence intervals around the mean values.

**Table 7.4**: Comparison of Layman community matrices across sites at at Highflood and Lowflood. The arrows pointing up indicate an increase in community matrix value between high and low flood whereas an arrow pointing down indicates a decrease in the matrix

| | **L.Ngami** | | **Nxaraga** | | **Phelo** | |
|---|---|---|---|---|---|---|
| | Highflood | Lowflood | Highflood | Lowflood | Highflood | Lowflood |
| N_range | 3.1 | 3.4↑ | 3.3 | 1.1↓ | 1.8 | 1.5↓ |
| C_range | 1.1 | 3.5↑ | 2.1 | 2.3↑ | 1.1 | 0.3↓ |
| *TA* | 0.5 | 2.9↑ | 2.2 | 0.6↓ | 1.0 | 0.1↓ |
| CD | 1.1 | 1.6↑ | 1.3 | 1.1↓ | 0.8 | 0.4↓ |
| MNND | 1.0 | 1.8↑ | 1.5 | 0.5↓ | 0.9 | 0.5↓ |
| SDNND | 0.9 | 0.9↑ | 0.1 | 0.4↑ | 0.3 | 0.2↓ |

### Niche overlaps of fish trophic groups

The degree of overlap between trophic niches of fish trophic guilds varied between high flood and low flood seasons within and across study sites (Table 7.5; Fig.7.4). At Lake Ngami the largest niche overlap of 33.9% was between omnivores and piscivores during high flood whereas during low flood the largest trophic niche overlap of 30.4% was between insectovores and omnivores. The trophic niche of insectivores at the lake had an overlap of over 20% with omnivore and piscivore trophic niches during all the flooding seasons (Table 7.5). The least niche overlap at Lake Ngami was observed between detrivore*s* and omnivore*s* during both high flood and low flood (4.3 and 6.4% respectively, Table 7.5; Fig 7.4). At Nxaraga lagoon, insectivores and omnivores showed considerably high niche overlaps during both high flood and low flood (21.9% and 39.7% respectively), however the highest overlap during high flood was between detrivores and insectivores (22%) whereas the highest overlap at low flood was between omnivores and piscivores (48.7%). Generally, Phelo flood plain had greater overlaps with all niche overlaps at high flood exceeding 20%. The highest overlaps in Phelo was between detrivores and insectivore*s* during high flood (56.4%) and low flood (44.5%). In Phelo floodplain, the degree of niche overlaps between trophic groups was higher at high flood compared to low flood (Table 7.5; Fig 7.4). Duirng low flood in Phelo flood plain, niche overlaps between omnivores and other trophic groups were the lowest compared to niche overlaps between other trophic groups at low flood (Table 7.5). The comparisons of trophic overlaps between fish guilds are visualised in Figure 7.4.

**Table 7.5**: Percentage niche overlaps of fish trophic groups in study sites at different flooding stages

| Site | Flooding stage | % overlap<br>Detrivore vs insectivore | % overlap<br>Detrivores vs omnivores | % overlap<br>Detrivores vs Piscivores | % overlap<br>Insectivores vs omnivores | % overlap<br>Insectivores vs piscivores | % overlap<br>omnivores vs piscivores |
|---|---|---|---|---|---|---|---|
| L. Ngami | Highflood | 14.2 | 4.3 | 9.4 | 20.9 | 23.9 | 33.9 |
|  | Lowflood | 9.5 | 6.4 | 16.2 | 30.4 | 25.7 | 6.9 |
| Nxaraga | Highflood | 22.0 | 0.24 | 0 | 21.9 | 0 | 6.7 |
|  | Lowflood | 4.1 | 9.5 | 15.1 | 39.7 | 4.0 | 48.6 |
| Phelo | Highflood | 56.4 | 33.7 | 24.3 | 47.2 | 20.6 | 37.5 |
|  | Lowflood | 44.5 | 10.3 | 18.8 | 15.5 | 25.3 | 15.2 |

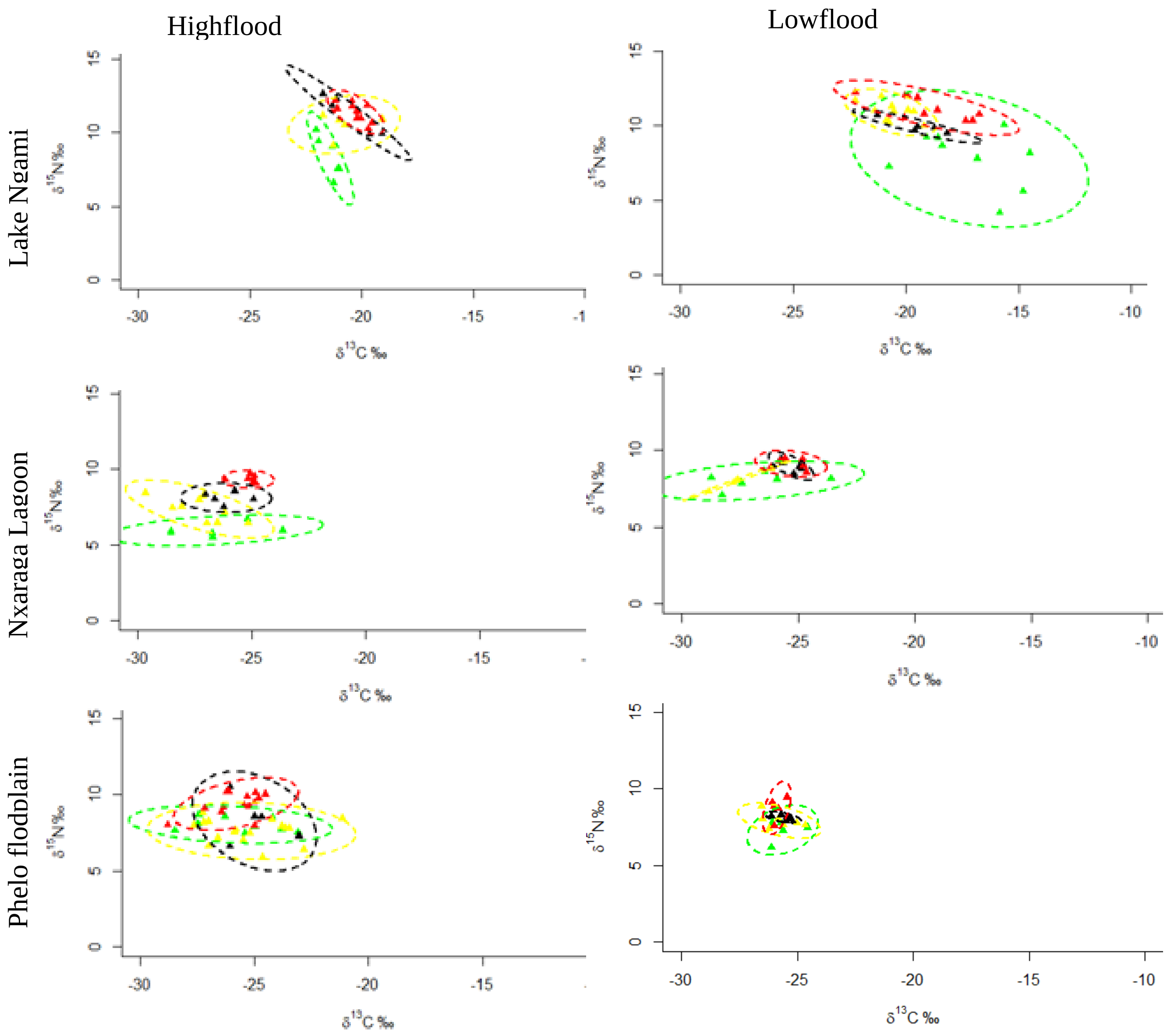


**Figure 7.4** Representation of niche size overlaps between different fish trophic guilds in all study sites using 95% statistical ellipses produced in SIBER. The triangles and dashed ellipsed represent individual species and the niche space of trophic guilds respecitively as follows: red colour =piscivores, black = omnivores, yellow =insectivores and green = detrivores.

## 7.4. Discussion

### Fish assemblage structure at the study sites

A total of 29 fish species, out of the 71 fish species found in the Okavango Delta (Ramberg *et al.* 2008), were collected during this study. Of the collected fish species, 24 species were from Phelo flood plain, 20 species from Lake Ngami and 19 fish species from Nxaraga lagoon. The number of collected fish species is low compared to the number of fish species collected by previous previous fish surveys in the Delta likely due to differences in sampling intensity and study locations. For example, Merron and Bruton (1995) collected 65 species five locations spanning the upstream-downstreams axis of the Delta from the Panhandle at the upstream to Lake Ngami at the distal downstream end. The fish collections by Merron and Bruton (1995) were done over a larger spatial coverage compared to this study in which collections were from Chief's Island and Lake Ngami only. In addition to greater spatial coverage, Merron and Bruton (1995)'s fish collections were done quaterly over a three-year period using multiple gears including gill nets, seine nets and rotenone whereas in this study fish were collected at two flood stages of one using only gill nets. Using the same fishing gear as used in this study, but collecting sampling monthly over a longer period of fours years (2001 to 2005), Mosepele *et al.* (2011) collected 46 fish species in four lagoons in the Delta, including 40 species from Nxaraga lagoon. While the number of fish species collected in the current study is low, the collected species represent the common species in the Delta (Merron and Brutton 1995, Mosepele *et al.* 2017), whose role in aquatic food web dynamics is crucial to understand as they are the ones that mostly support fisheries (Mosepele *et al.* 2011) and because of their ubiquious nature, the common species are critical for energy flow dynamics in the Delta (Høberg *et al.* 2002).

With regard to the relative proprotion of trophic guilds across the study sites, insectivores were the most abundant fish guild at Nxaraga lagoon and Phelo floodplain and were generally abundant across all sites. Hocutt and Johnson (2001) also found insectivores to be generally the most dominant feeding guild in Okavango river upstream of Panhandle. Hocutt and Johnson (2001) observed that insectivores and detrivores occurred in relatively high abundance in the Okavango river and that the guilds temporally replaced one another, with insectivores being more abundant during high flood and detrivores during low flood. In this study, a similar pattern was observed at Phelo floodplain where insectivores were the dominant guild during high flood season but at low flood the dominance switched to piscivores (Table 7.2). During high flood, the aquatic ecosystem expands to vegetated and highly productive flood plains, favouring abundance of insectivores as both insect prey and increased refuge from piscivores increase (Junk *et al.* 1989, Mosepele *et al.* 2011). Omnivores were the most abundant feeding guild in Lake Ngami but were one of the least abundant guilds in the other study sites. The dominance of omnivores in Lake Ngami during both flood seasons is potentially very important for the observed high phytoplankton primary production in the Lake (Gondwe and Masamba 2015, also see Chapter 5 ). In lakes, omnivores do not only favour phytoplanton by supressing macrozooplankton populations, but they also act as nutrient pumps by feeding on benthic nutrients in the form of periphyton and sediment detritus and then exctreting the nutrients in the water column (Drenner *et al.* 1996).

### Seasonal variability of trophic niche size and food web structure: a comparison across study sites

There was an increase in the trophic niche size of insectivores and omnivores at high flood across all sites and whereas for other trophic guilds the change in trophic niche size is variable across sites (Fig. 7.2A-C). An increase in fish isotopic niche size at high flood indicates an isotopically broader prey base, which can be influenced by consumption of more diverse prey or consumption of prey from different and isotopically distinct habitats, or the combination of both factors (Newsome *et al.* 2007). The increase in niche sizes of insectivores and omnivores at high flood season therefore indicates these fish guilds consume more isotopically diverse prey at high flood. The broader prey base of insectivores and omnivores could mean that these fish have high trophic plasticity, allowing them to take advantage of increased prey diversity at high flood. These results could also mean that increased flooding extent allows these fish to forage from different habitats such as shallow flood plain sites and deeper sites in lagoons. As expected, omnivores had greater trophic niche sizes because they are known to exploit a greater range of prey resources across trophic levels (Vadeboncoeur *et al*. 2005, Vandermeera 2006). Fish prey abundance and diversity in the Delta is known to be greater during high flood stage (Høberg *et al.* 2002, Seziba *et al.* 2013). Using the stable isotope approach (Sepúlveda-Lozada *et al.* 2017) have also shown that the trophic niche of most fish trophic guilds increase at highflood phase in a Mexican floodplain system.

The SEA of detrivores in Lake Ngami was exceptionally high during low flood (13.8‰$^2$) compared to high flood season (1.1‰$^2$), indicating a significant increase of trophic niche size of detrivores in the lake during low flood. The increase in fish trophic niche at Lake Ngami detrivores during low flood is likely due to an increased gradient $\delta^{13}C_{DIC}$ variation from lake mouth to the downstream (Meier *et al.* 2015). The high $\delta^{13}C_{DIC}$ gradient at low flood likely increases the variability of algal $\delta^{13}C$ signatures within the lake leading to the increased isotopic niche of the fish. In Nxaraga lagoon and Phelo floodplain, this within-site isotopic variability of algal producers and detrivores is not expected because the water bodies are relatively smaller and not favouring a spatial gradient of algal $\delta^{13}C$ values.

Piscivores' niche sizes showed variable responses to the flooding phase across the study sites. In Lake Ngami, the trophic niche size increased during low flood, in Phelo floodplain the niche size increased at high flood whereas at Nxaraga lagoon the niche size did not change markedly between flooding seasons. The increase in trophic niche size of piscivores in Lake Ngami is likely due to the increased isotopic variation of prey as described above whereas at Phelo the increase is likely due to the increased prey diversity as fish diversity increased during the flood season (Høberg *et al.* 2002, Seziba *et al.* 2013). In Phelo flood plain, fish diversity was greater at high flood (species richness = 21 and Simpson diversity index = 0.85) compared to low flood season (species richness =16 and Simpson diversity index = 0.82). The fish species which were recorded during high flood but not at low flood include *H. multifasciatus, B. multilineatus, P. philander and C. multispine*. Greater fish diversity in Phelo floodplain was expected at high flood based on the predictions of the Flood Pulse Concept (Junk *et al.* 1989) that floodplain inundation leads to high fish diversity as a result of increased biological productivity fueled by nutrient release from allochthonous material that accumulates during the dry period. The higher diversity of fish, which feeds on diverse prey at high flood (Merron and Bruton 1995, Mosepele *et al.* 2011), likely contributed to the greater isotopic niche of piscivores at Phelo floodplain during high flood season.

Comparisons of food web structure based on Layman's food web matrices, showed that the temporal change in food web structure with respect to seasonal flooding regime was similar between Nxaraga lagoon and Phelo flood plain but differed between the two study sites and Lake Ngami (Fig. 7.3, Table 7.4). In Nxaraga lagoon and Phelo flood plain, all Layman's matrices were greater during high flood, indicating greater number of feeding pathways (TA), greater breadth of basal carbon resources (C range), greater number of trophic links (N range) and greater trophic redundancy (NND and SDNND) during the flood season whereas in Lake Ngami the Layman's matrices were greater during low flood season. The increase in feeding pathways and carbon sources at Nxaraga lagoon and Phelo flood plain during high flood was expected because of the high biological productivity that occurs during high flood conditions (Junk *et al.* 1989). At Lake Ngami, greater feeding pathways and carborn sources were not expected a low flood, but the result is likely influenced by high isotopic variability that result from increased phytoplankton productivity (Gondwe and Masamba 2016). In the Amazon flood plain Correa and Winemiller (2014) demonstrated fish shifted their dietary resources seasonally in response to the seasonal flood pulse but corresponding patterns in the vertical food web structure were not obvious.

### Trophic niche overlaps at the study sites

Trophic niche overlaps between different fish trophic guilds were generally variable across sites and flooding seasons, but Phelo floodplain showed generally greater trophic niche overlaps than Nxaraga lagoon and Lake Ngami (Table 7.5 / Fig. 7.4). Trophic niche overlaps occur when fish consume isotopically similar prey resources, therefore they can be indicative of competition for resources or greater trophic plasticity between fish taxa (Jackson *et al.* 2011). The high trophic overlaps in Phelo floodplain may not necessarily indicate competition for resources between trophic groups but rather a greater degree of omnivory among floodplain fish. It is likely that generalist feeding strageties are more common among Phelo floodplain fish, allowing the fish to take advantage of diverse prey resources during high flood. Consistent with findings of this work, Mérona and Rankin-de-Mérona (2008) showed that in the Amazon floodplains, the mean trophic overlap of fish that are generalist feeders was high.

The influence of the flood pulse on trophic overlaps between fish guilds was variable across study sites, but in general terms, Lake Ngami and Nxaraga lagoon showed greater overlaps during low flood whereas at Phelo floodplain the trophic overlaps were greater at highflood. Trophic overlaps in the Delta is an expected phenomena because flood plain fish species are known to be mostly opportunistic feeders because of the ephemeral nature of the habitats and resources (Lowe-McConnell and Lowe-McConnell 2010, Mortillaro *et al.* 2015). Pool *et al.* (2017) also found high trophic niche overlaps in a flood-pulse river ecosystem of the Tonle Sap Lake(Cambodia), and attributred the trophic overlap to the opportunistic feeding nature of floodplain fish. The results of my PhD research indicate generalist feeding habits among the fish of the Okavango Delta but indicates that the phenomena may be greater in floodplains than perennial sites.

## 7.5. Conclusions

Based on fish SIA data, the structure of aquatic food web has been shown to vary across differently flooded habitats and to be influenced by the seasonal flood pulse. Contrary to expectations that Phelo flood plain will have the greatest trophic niche size and carbon resource range, Lake Ngami had the greatest trophic niche size with the feeding guilds showing more trophic overlap during low flood, whereas Phelo floodplain and Nxaraga lagoon had smaller trophic niche sizes with fish feeding guilds showing greater trophic niche overlap during high flood. Findings from this study will be useful for predicting possible effects on hydrological threats such as climate change effects and large-scale water withdrawals on aquatic food webs structure and energy flow in differently flooded habitats of the Delta.

# Chapter EIGHT: Synthesis

## 8.1. Introduction

The seasonal flood pulse that floods the Okavango Delta every year is known to be the main driver of biological production cycles, ecosystem functions and hence ecosystem services. Like many wetlands in arid environments, the Okavango Delta ecosystem faces several hydrological threats including climate change effects and large-scale water withdrawals to supply human communities. Anticipation and mitigation of the hydrological threats will rely on prior knowledge of the Delta's ecosystem function. The main aim of this research was to examine the relationship between flooding regimes and the structure of aquatic food webs in the Okavango Delta. The dynamics of energy flow and aquatic food web structure are generally not well understood in the Delta. My research contributed to the understanding of energy flow and food web dynamics in Delta's by quantifying aquatic primary production and food web structure across aquatic habitats of different flooding regimes. This synthesis chapter summarises, the key findings in the context of existing theoretical concepts on wetland food webs and management implication.

## 8.2. Discussions

Among chapters based on data generated from this research, **Chapter 5** examined the environmental variability of the selected study sites, focusing on algal primary productivity and nutrient levels. The chapter presents the first quantification of epipelic algal primary productivity and respiration in the Delta, with a special focus on Lake Ngami. Through the Chapter, I demonstrated that the rate of phytoplankton and epipelic primary productivity is greater at Lake Ngami due to greater nutrient levels compared to Chief's Island sites of Nxaraga lagoon and Phelo flood plain. Progressive solute concentration as water transverses the Delta from upstream parts and resuspension of potentially organic-rich benthic sediments are the likely drivers of higher phytoplankton productivity levels at Lake Ngami. Net epipelic algal primary production was negative across all sites possibly due the unstable benthic substrate of the Kalahari sands. My results have also demonstrated that indeed seasonal flooding influences algal primary production in the Delta, but contrary to what I expected based on predictions of the flood pulse concept (Junk *et al.* 1989), primary productivity per volume was higher during low flood than during high flood. The result is likely due to both the greater nutrient concentration and higher seasonal temperatures during high flood. The dilution effect of floods on algal densities and relatively lower temperatures during the high flood season can also explain the lower phytoplankton productivity during high flood season. At Lake Ngami, phytoplankton production is the dominant pathway of carbon fixation into aquatic food web while the contribution of aquatic macrophytes is likely negligible given their virtual absence in the Lake. However, aquatic macrophytes may play a relatively greater role in fixing carbon aquatic food webs at Nxaraga and Phelo floodplain where their abundance is relatively higher.

In **Chapter 6**, I examined the contribution of different basal sources to fish assimilated diet at the study sites during high flood and low flood using the SIA approach. The results showed that the $\delta^{13}C$ and $\delta^{15}N$ values were distinctively more negative at Lake Ngami compared to the values at Nxaraga lagoon and Phelo floodplain. Differences in phytoplankton productivity between Lake Ngami and the two Chief's Island sites is likely to be the main driver of the observed spatial differences in isotope signatures. High rates of phytoplankton productivity increase the uptake rate of heavier isotopes of both C ($^{13}C$) and N ($^{15}N$), making the SI ratios of the elements to be more negative. A Higher rate of phytoplankton productivity at Lake Ngami compared to both Nxaraga lagoon and Phelo floodplain, as shown in Chapter 5, is the likely driver of the isotopic differences between the sites. The relative importance of basal C sources varied between study sites and flood seasons, although in general algal sources were consistently the principal carbon sources for fish. The highlights of the results are i) algal sources were generally the most important sources, ii) filamentous algae at Lake Ngami had distinctively greater $\delta^{13}C$ values and was the least important resource to fish, whereas at Nxaraga and Phelo flood plain filamentous algae was one of the most important basal C sources for fish, iii) the contribution of aquatic macrophytes to fish assimilated diet was relatively higher at Phelo flood plain than other study sites during high flood. Algal sources are expected to be important because they are more digestible and hence better assimilated by aquatic consumers. The lower contribution of filamentous algae to fish assimilated diet at Lake Ngami is likely due to zooplankton feeding preferably on abundant phytoplankton at the lake. The relatively greater importance of macrophyte carbon to fish at floodplain during high flood is consistent with the flood pulse concept (Junk *et al.* 1989), that the seasonal flood pulse facilitates the incorporation of carbon from flood plain vegetation into aquatic food web.

Through **Chapter 7** I have shown that the structure of aquatic food webs, including trophic diversity, trophic redundancy and the degree of trophic niche overlap between fish trophic groups, vary across habitats of different flooding regimes and that at each study site the structure is influenced by the seasonal flood pulse. Based on Layman matrices for food web structure, I have shown that Lake Ngami has a food web structure that has greater trophic diversity and isotopically diverse carbon sources than the other study sites. This was a rather unexpected result, but it is likely influenced by the greater ecosystem size of the Lake and the variability of $\delta^{13}C$ values of DIC (Meier *et al.* 2015) and hence of algal producers. Generally, there was a considerable overlap of trophic niches of fish feeding guilds across all the study sites, but the trophic overlap was relatively more pronounced at Phelo floodplain. The overlap in trophic niches can indicate general feeding strategies (greater feeding plasticity) which is an adaptive phenomenon that suits the environmentally dynamic ecosystem like the Okavango Delta where food resources are seasonally pulsed. The seasonal flood pulse generally increases the trophic niche of fish across study sites, although the extent of the influence varies across trophic groups. The flood pulse is associated with high biological production and creation of new habitats and feeding niches for different aquatic biota in wetlands (Junk 1989, Høberg *et al.* 2002, Balcombe *et al*.2005, Ou and Winemiller 2016), therefore the result is consistent with theoretical expectations.

### 8.3. Conclusions and future research directions

The results of this PhD research have shown that within the Okavango Delta, both primary production and food properties, such as relative important of basal carbon sources and food chain length, vary between differently flooded habitats and are influenced by the stage of flooding. Lake Ngami, an ephemeral lake on decadal scale, had the highest primary production, greater isotopic range of basal carbon sources and longer food chains than Nxaraga lagoon, a lagoon within a perennial channel, and Phelo floodplain, a seasonally inundated floodplain. The greater phytoplankton productivity at Lake Ngami was attributed to progressive concentration of dissolved nutrients as water transverses the Delta to the lake at the downstream end. Potential nutrient input from dung of cattle that inhabit the lake bed during its dry periods may also be a driver for high primary productivity in the lake when the potentially organic-rich sediments are re-suspended. Greater isotopic range of basal carbon sources (C range) compared to other study sites was likely because of high spatial variation of $\delta^{13}C_{DIN}$ values in the lake. The lake's aquatic food web also had longer food chain length (N range) than food webs of the other study sites which can be explained by higher level of phytoplankton productivity at the lake, enabling the lake's food web to support a greater number of trophic links. Across all the study sites, phytoplankton productivity was greater at low flood when dissolved nutrient levels were higher likely because of the concentrating effect of evaporation. The importance of plant-derived carbon to fish was greatest at Phelo floodplain during high flood likely due to decomposition of submersed floodplain vegetation during flooding. Overall, the findings of this PhD research have contributed towards our understanding the Okavango Delta's aquatic food webs dynamics in relation to flooding, the knowledge that can help to better anticipate the response of the Delta's food webs and associated ecosystem services, such as fisheries production, to altered flow regimes.

There are management considerations that can be drawn from this research to ensure sustainability of the ecosystem services that are derived from the Delta. Firstly, the results have demonstrated that Lake Ngami is one the habitats with high phytoplankton productive within the Delta, which in turn supports fish production and associated livelihoods that are derived by local human communities. Large scale water withdrawals in the upstream parts of the Delta should be guarded against as it will lead to reduction of the Lake's aquatic ecosystem space and hence reduce total phytoplankton primary production that supports fish production in the lake. The value of the terminal lakes should therefore be strongly emphasised when assessing potential effects of water withdrawals from the Delta. The results of this research have also shown that the contribution of plant derived carbon to aquatic food webs was greater at Phelo floodplain during high flood, confirming that the lateral expansion of floods is important for facilitating carbon input from seasonal aquatic habitats in the Delta. In general, this research has shown that flooding regimes influence biological production rates and aquatic food web structure, both of which underpin ecosystem functions such as fisheries production. Therefore, management options such as determination of fishing quota for fisheries within the Delta, may benefit from grouping aquatic habitats based on their flooding regimes as proxy trophic characteristics, although the habitat groups will first have to be validated through quantification of the ecosystem properties of interest.

The results of my PhD research are based on data collected from the lower part of the Delta and therefore future examination of aquatic food webs in the Delta may include parts of the Delta, such as the Panhandle region, to further improve our understanding of the Delta's food webs. This is because the Panhandle region is a deep riverine system with flanking floodplains whereas the Fan region in generally swampy region with distributor channels and islands. The different hydrology and associated vegetations and aquatic biota likely translate into different trophic interaction patterns between the respective regions of the Delta. In the current study, the relationship between aquatic food web properties and flooding regimes was examined within one flood cycle. However, based on interannual variations in flooding of the Delta, quantifying aquatic food properties over a multiyear time scale will be very important step towards fully understanding the aquatic food web dynamics of the Delta with respect to flooding regimes.

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# Appendices

## Published Chapter

### *Chapter 7*

# SEASONAL VARIABILITY OF BENTHIC AND PELAGIC PRIMARY PRODUCTION IN LAKE NGAMI, BOTSWANA.

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## ABSTRACT

Primary productivity sustains lake foodwebs and is a key supporting ecosystem service. Baseline knowledge of lake ecosystems' primary production and its relationship with hydrological changes is important for predicting and mitigating effects of hydrological threats. We set out to examine the influence of a seasonal flood pulse on benthic and pelagic primary productivity of Lake Ngami, in northwest Botswana. We made 51 net primary productivity (NPP) measurements, of which 36 were pelagic and 15 were benthic. The lake's average pelagic and benthic NPP were 0.089 ± 0045 mgC/l/h and -0.12 ± 0.10 mgC/l/h respectively. Pelagic net primary productivity (*p*NPP) increased from 0.062 ± 0.05 mgC/l/h at high flood stage to 0.11± 0.07 mgC/l/h at low flood stage. Average benthic respiration (*b*R) was 0.22 ± 0.13 mgC/l/h and was higher than pelagic respiration (*p*R) which was 0.055 ± 0.03 mgC/l/h. The results of this study demonstrated the importance of phytoplankton production as the main pathway through which carbon is fixed into the lake ecosystem. Given that the lake lacks aquatic macrophytes, the phytoplankton forms a very significant resource that supports the lake's fish populations, a critical livelihood resource for surrounding communities. Considering the inter-annual variability of the Okavango Delta's flooding, we recommend monitoring of the lake's primary production to gain insights into its inter-annual primary productivity patterns.

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**Keywords**: Primary productivity, seasonal flooding, Lake Ngami, Okavango Delta

## INTRODUCTION

Lake ecosystems provide multiple and valuable benefits to the surrounding human communities, known as ecosystem services (Millennium Ecosystem Assessment 2005, Costanza et al. 2014). The fisheries in Lake Victoria for example, are an important source of protein, employment and economic networking in East Africa (Downing et al. 2014). The capacity of lakes to sustain these valuable services however depends on their ability to maintain healthy ecosystem functions. An ecosystem function is the availability and interaction of biotic and abiotic components of an ecosystem to produce an important ecosystem outcome (Costanza et al. 1997) such as carbon cycling in lakes. A good baseline understanding of the relationship between lake ecosystem functions and environmental factors is crucial for predicting the effects of environmental change on lake ecosystems and the ecosystem services they provide (Jackson, Woodford, and Weyl 2016).

Lake primary productivity refers to the rate at which energy is fixed into organic matter (by photo- and/chemosynthesis). It is a very important supporting ecosystem service because it forms the base of lacustrine food webs, and determines the structure and stability of lake ecosystems (Post, Pace, and Hairston Jr 2000). The distribution of primary production between pelagic and benthic habitats has progressively gained recognition as a key ecosystem property determining whether lake food webs are pelagic or benthic based (Scheffer 2004, Hilting, Currin, and Kosaki 2013, Althouse, Higgins, and Zanden 2014, Brothers, Vadeboncoeur, and Sibley 2016). The distribution of primary productivity between the lake habitats can be influenced by several lake-specific factors such as depth, water clarity and the composition of the aquatic community (Althouse, Higgins, and Zanden 2014, Higgins et al. 2014). It is therefore a pre-requisite for sustainable management of lake ecosystems to have good understanding of the environmental controls of primary production dynamics.

Through influencing the physical, chemical and biotic environment, water level fluctuations can exert strong influences on primary production (Wantzen, Junk, and Rothhaupt 2008, Liu, Teubner, and Chen 2016, Chastant, Petersen, and Gawlik 2017). Primary productivity in lakes which are seasonally flooded by single source inflows exhibit strong seasonal patterns due to large water level fluctuations. However, empirical research on relationships between lake flooding patterns and primary productivity distribution is generally lacking especially in Southern Africa. Based on the flood pulse concept (Junk, Bayley, and E. 1989), it is likely that seasonal floods increase primary production in Lake Ngami at high flood through nutrient recharge by the arriving flood waters and from nutrients released from the peripheral lake bed during inundation. For Lake Ngami, this prediction is complicated by the fact that the lake fills during winter months when lower temperatures are likely to suppress productivity rates (as opposed to hot summer months which may favour high productivity rates during the lake's low water conditions). In this study, we examine the variability of primary production and its potential explanatory factors with respect to seasonal flooding in Lake Ngami, Botswana. This shallow lake, which is more frequently dry than wet, is very understudied, and yet is a critical source of ecosystem services, a socio-economic focal point in the district (even when it’s dry) and a critical node of the large Ramsar and World Heritage Site, the Okavango Delta.

## STUDY AREA

Lake Ngami is a shallow endorheic lake located at the distal end of the south west tributary of the Okavango Delta (hereafter referred to as the Delta), the Xudum tributary (Fig. 1). The seasonal filling of the lake depends on the seasonal flooding of the Okavango Delta by a flood wave originating from rainfall in the Angolan highlands, and on the redirection of water in the Delta to the western distributaries that flow into the lake (Shaw et al. 2003, Wolski et al. 2014). The volume of the flood pulse that inundates the Delta also varies annually due to variations in rainfall in Southern Africa (Mazvimavi and Wolski 2006). Inter-annual variability in the Delta’s flood levels is further influenced by the fact that years of high flooding tend to raise the water table of the Delta favouring extensive flooding in the year that follows even when the incoming flood wave is relatively smaller (McCarthy, Bloem, and Larkin 1998). These variations in the Delta’s flooding extent consequently lead to highly variable flooding levels of Lake Ngami causing considerable intra- and inter-annual water level fluctuations in the lake. The lake has periods of complete desiccation due to low flood levels in the Delta, for example the years between 1989 to 2004 (Shaw et al. 2003, Wolski and Murray-Hudson 2006) Annual floods arrive between May and June and the lake reaches its peak water level in August and the water level decline becomes noticeable from October. Since 2004 the lake has been permanently flooded and has supported commercial fishing that supplied local and regional fish markets. Fish from the lake has since become a very important source of livelihood for households in Sehitwa and villages surrounding the lake, as well in Maun town. Apart from being a source of fish, the lake is also an important source of water supporting pastoral farming in local communities. Currently (2017) the lake is an open water body about 20km long and 8m wide (Fig. 1). Its maximum depth is about 3m in the middle open parts (Inner Lake) while the shallower peripheral parts are occupied mainly by drowned thorn trees (Outer Lake) (Fig. 1). The lake is generally devoid of aquatic vegetation apart from a few peripheral islands of *Typha sp*. The water is generally turbid compared to upstream parts of the Delta.

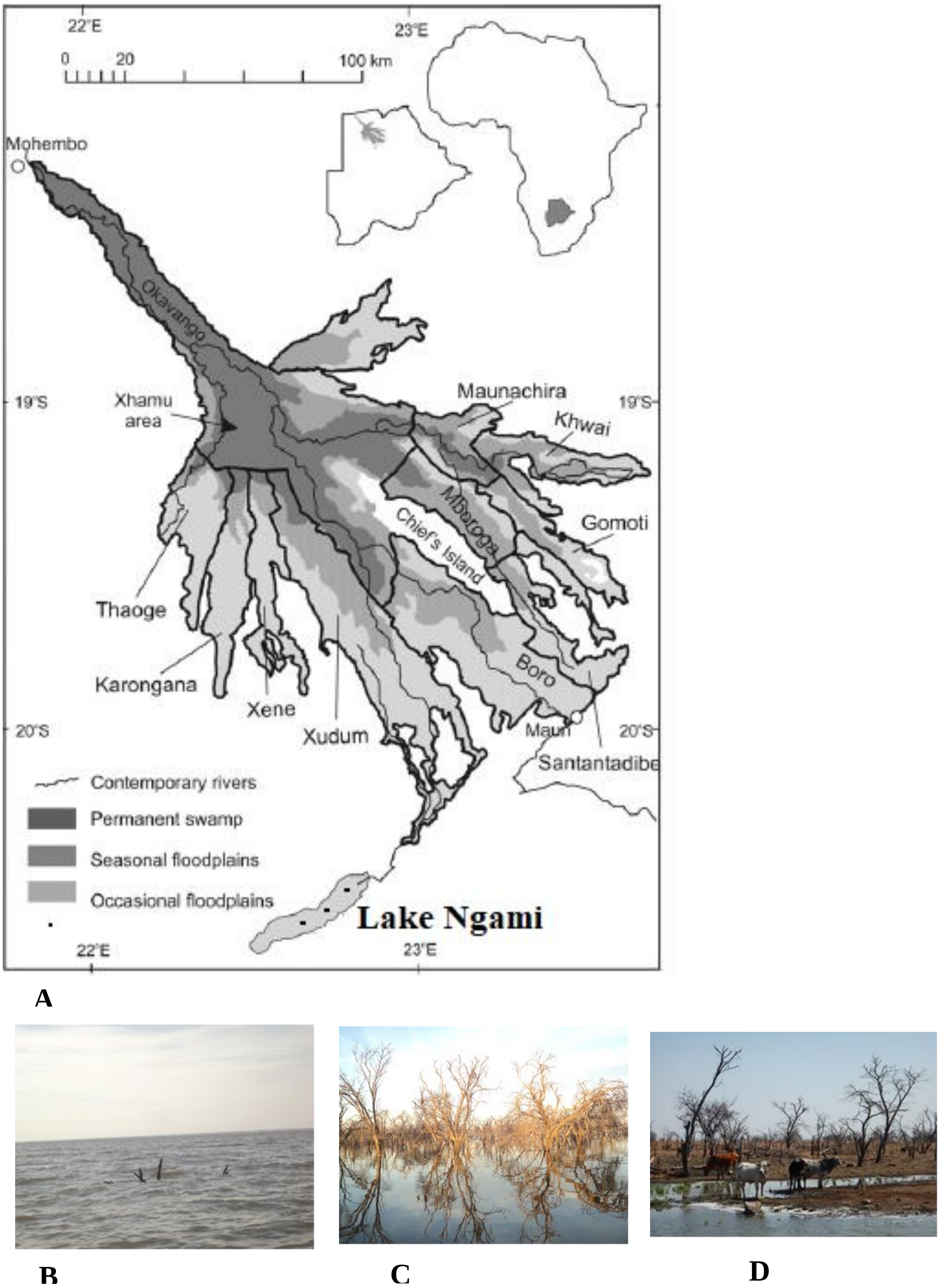

**Figure 7.1. A.** map of the Okavango Delta with the location of Lake Ngami and approximate locations of study sites shown by black points. **B.** The inner open part of the Lake, **C** and **D** outer part of the lake at high flood and low flood conditions respectively.

# METHODS

Sampling points were selected along the upstream-downstream axis of the lake at three regions of the lake: the upstream part, the middle part and downstream part (Fig. 1A). The basis for selecting sites along the upstream-downstream axis was to capture environmental gradients, including solute levels that may result from evaporation as the water moves from upstream to downstream of the lake. At the sampling points pelagic primary productivity was estimated using the light and dark bottles method (Wentzel and Likens 2010). To estimate productivity, we filled six 300ml Wheaton BOD (borosilicate) bottles with lake water at subsurface depth and stoppered them while underwater before incubating them for three hours at 0.5m depth. Three of the six bottles were covered with aluminum foil then wrapped in black insulation tape to shield them from light while the other three bottles were left transparent. The incubation was terminated by adding five drops of Lugols's iodine solution. Dissolved oxygen (DO) was measured before and after incubation using a DO probe (Probe Type: LDO10101) connected to a Hach HQ40d multi-parameter field meter (±0.1 to 8 ppm; ±0.2 above 8 ppm). Gross primary productivity (GPP), net primary productivity (NPP) and respiration (R) were then calculated based on the change in DO concentration during the incubation time using the equations 1-3:

$$\text{GPP (mgC/l/h)} = \frac{[(LB)-(DB)]\,(0.375)}{(PQ)(t)} \tag{1}$$

$$\text{NPP (mgC/l/h)} = \frac{[(LB)-(IB)]\,(0.375)}{(PQ)(t)} \tag{2}$$

$$\text{R (mgC/l/h)} = \frac{[(IB)-(DB)]\,(0.375)}{(t)} \tag{3}$$

Where:

LB = final DO concentration in light bottle

DB = final DO concentration in dark bottle

IB = initial concentration of DO

t = time in hours

PQ = Photosynthetic coefficient of 0.12

0.375= the ratio of moles of carbon to moles of oxygen (12mg/32mg)

Benthic primary productivity and respiration were estimated in a similar method, except that in benthic estimations acrylic hemisphere domes were used (Figure 7.2) (Althouse et al. 2014). The domes were of 30 cm diameter and 7l volume, and each had a 220v powered water pump attached on the inside to homogenise water during incubations. For practical convenience, shallow sites not exceeding 1.5m in depth were selected for the benthic incubations. The benthic incubations were made by pressing the base of the hemisphere domes into the substrate by 10cm. A DO meter probe was then inserted at the top opening of the dome such that there was no water exchange between the inside and the outside of the domes. DO measurements were then recorded *in situ* at 15-minute intervals for an hour.

A. Transparent dome B. Dark (covered) dome

**Figure 7.2.** Acrylic hemisphere domes that were used for benthic DO incubations

To gain further insights into the relationships between primary productivity and physico-chemical lake properties, which vary due to the lake's flooding regime, potential explanatory variables were measured at high flood (August 2015, two fieldwork trips lasting a week each) and low flood (January 2016, two fieldwork trips one lasting two days and the other lasting a week). These include Secchi depth (Secchi, m), water temperature (Temp, °C), chlorophyll-a concentration (Chla, μg/l), dissolved silica (DSi, mg/l), dissolved nitrates ($NO_3^-$, mg/l) and dissolved phosphates ($PO_4^{2-}$, mg/l). The measured primary productivity correlates were water pH (pH), alkalinity ($HCO_3^-$, mg/l), dissolved oxygen (DO, mg/l), conductivity (EC, μS/m) and water depth (depth, m).

The concentration of chlorophyll-a (Chla) is a proxy for algal biomass and indicates a system's photosynthetic capacity. Dissolved nutrients, including $PO_4^2$ and $NO_3^-$ can determine the density of algal communities and hence their influence on primary productivity rates. Dissolved silica (DSi) controls the abundance of diatoms, which are part of a lake's photosynthetic community. Water depth affects other physical properties of a habitat which in turn affect biological processes including primary production, for example a shallow water site will likely warm up from sunlight faster than a deep site. Shallow sites are also likely to experience more mixing from wave action than deep sites. Secchi depth is a measure of water transparency and therefore gives an estimate of sunlight availability to deeper levels in water column. Temperature directly influences primary production by regulating cellular processes involved in photosynthesis. Water pH is positively correlated with photosynthesis and it also determines the form of carbon available for uptake by algae. $HCO_3^-$ is also positively correlated with photosynthesis but it is a measure of the stability of water pH. DO is a function of photosynthesis and respiration while EC is an indication of the level of all dissolved solutes in lake water including dissolved nutrients. Both DO and EC potentially determine the structure of aquatic communities across all trophic levels (Beisner et al. 2006) and hence determine algal densities through top-down trophic interactions (Althouse et al. 2014).

A Hach HQ30d multiparameter field meter was used to measure pH, DO, EC, and temperature at each site. To ensure accuracy of the measurements, the meter was re-calibrated for each data collection field trip, which lasted between two and five days. Water depth was measured using a calibrated pole approx. 3.5m in length, while water clarity was measured using a 20cm diameter Secchi disk attached to a 3m tape measure. The parameters were measured during sunny conditions, between 11 am and 3pm, in August 2015 at high water conditions (Highflood) and at the same times of day in January 2016 at low water conditions (Lowflood) (Table 7.1). For the chemical parameters that required laboratory analyses, namely $NO_3^-$, $PO_4^{2-}$ and DSi, three water samples were collected at subsurface depth of 20 cm, into acid washed 300ml bottles at each sampling point. At each site, water was also collected into 1 liter bottle for laboratory determination of Chla. The bottles containing the water samples were stored in an ice filled cool box for transportation to Okavango Research Institute's (ORI) environmental laboratories. At ORI, the parameters were analysed following standard methods as described in (Eaton et al. 1998).

Data for both primary production and explanatory variables was initially tested for normality using Shapiro-Wilk test for normality in SPSS 24. After determining that all the datasets were not normally distributed, non-parametric statistical tests were used. A Kruskal-Wallis test was used for comparisons of mean values of primary productivity and respiration between benthic and pelagic habitats and between flood seasons. Comparisons of productivity measurements were visualized by box plots drawn using ggplot2 package in R software version 3.4.0 (R Core Team 2017). Comparison of explanatory variables data between flood seasons were made using box plots drawn in ggplot2. Since data for explanatory variables (except for $PO_4^{2-}$and DSi) were non-parametric, Spearman-Rank correlations were run in SPSS 24 to examine correlations between primary productivity and environmental variables during high flood and low flood. Correlations between primary productivity and environmental variables were run for pelagic primary productivity only since most of the measured environmental variables were measured at subsurface depth.

## RESULTS

In total 51 productivity measurements were made, with more measurements done on the pelagic habitat (n = 36) compared to the benthic habitat (n =15). Kruskal-Wallis pairwise comparisons of pelagic and benthic pooled data indicate that, overall, pelagic net primary productivity (*p*NPP) was higher than benthic primary productivity (*b*NPP) ($p = 0.00418$) while benthic respiration (*b*R) was significantly higher ($p = 4.42 \times 10^{-6}$) than pelagic respiration (*p*R) (Table 7.2). However, GPP between the two habitats was very similar and showed no significant difference (p = 0.09).

**Table 7.1.** Comparison of primary productivity and respiration between pelagic and benthic habitats using Kruskal-Wallis test. Significant differences (p < 0.05) are indicated by an asterisk (*)

| Parameters | Pelagic mean (mgC/l/h) +/-SD (n=36) | Benthic median (mgC/l/h) +/-SD, n = 15 |
|---|---|---|
| GPP | 0.14 ± 0.06 | 0.103 ± 0.11 |
| NPP | 0.089 ± 0045* | -0.12 ± 0.10* |
| R | 0.055 ± 0.055* | 0.22 ± 0.13* |

Gross primary productivity (GPP) did not vary between pelagic and benthic habitats both during high flood and during low flood seasons (p = 0.912 and 0.506 respectively). Net primary productivity (NPP) was higher in the pelagic habitat during both high flood and low flood (p = 0.000) whereas respiration was higher in the benthic habitat during all the flood seasons (p = 0.025 and 0.000 for high flood comparison and low flood comparisons respectively) (Table 7.2).

**Table 7.2.** Comparison of primary productivity and respiration between high flood and low flood for both pelagic and benthic habitats using Kruskal-Wallis test. Parameters which are significantly different (p < 0.05) are indicated by an asterisk (*)

| | Pelagic (mgC/l/h) +/-SD | Benthic (mgC/l/h) +/-SD |
|---|---|---|
| **Highflood** | n = 18 | n= 9 |
| GPP | 0.113 ± 0.07 | 0.107 ± 0.08 |
| NPP | 0.062 ± 0.05* | -0.052 ± 0.06* |
| R | 0.51 ± 0.04* | 0.18 ± 0.05* |
| **Lowflood** | n = 18 | n = 6 |
| GPP | 0.15 ± 0.03 | 0.089 ±0.16 |

| | | |
|---|---|---|
| NPP | 0.11± 0.07* | -0.2 ± 0.01* |
| R | 0.058 ± 0.02* | 0.29 ± 0.16* |

Both primary productivity and respiration measurements (GPP, NPP and R) showed higher variability at high flood (Highflood) than at low flood Fig.7.3). Benthic gross primary productivity (GPP) was similar during high flood and low flood whereas pelagic gross primary productivity (GPP) was significantly increased at low flood (Kruskal-Wallis test, p = 0.05). Pelagic respiration was similar between the two flood seasons whereas benthic respiration was higher at low flood (Fig. 7.4).

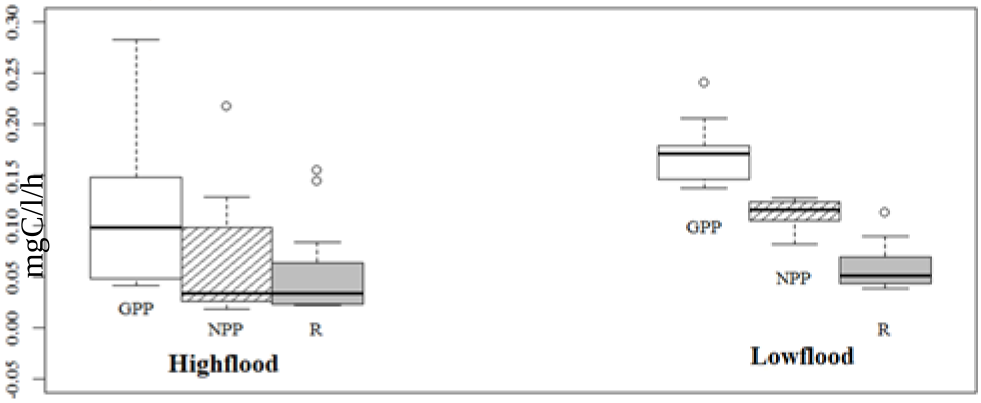

**Figure 7.3**. Comparison of pelagic primary productivity and respiration at Highflood and Lowflood

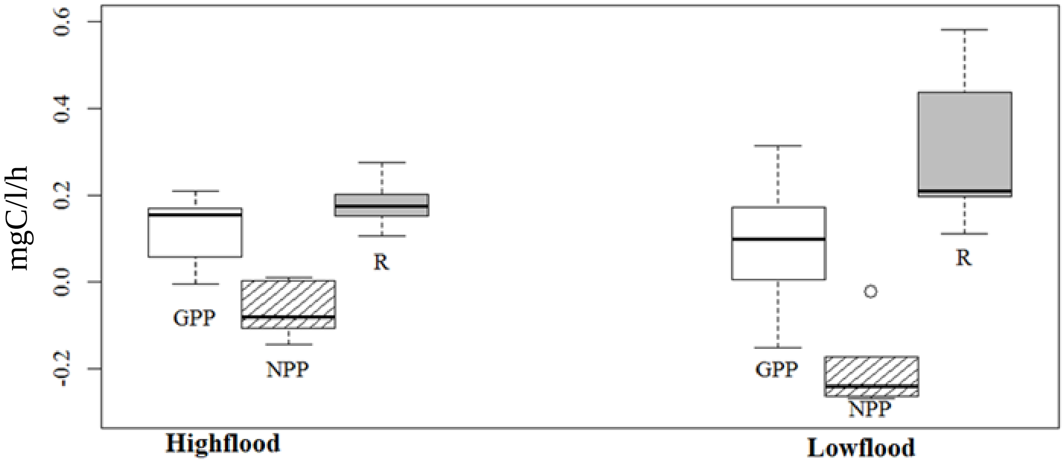

**Figure 7.4**. Comparison of benthic primary productivity and respiration at highflood and lowflood

In general, concentration of solutes in the lake were higher and less variable during low flood than at high flood, as indicated by a significantly higher EC at low flood. Photosynthesis correlates including pH, DO, Temp and Chla were also higher at low flood. Potentially limiting factors, namely nitrates and light availability (Secchi), were higher at high flood than low flood, whereas $PO_4^{2-}$ and DSi did not show significant seasonal changes (Fig. 7.5).

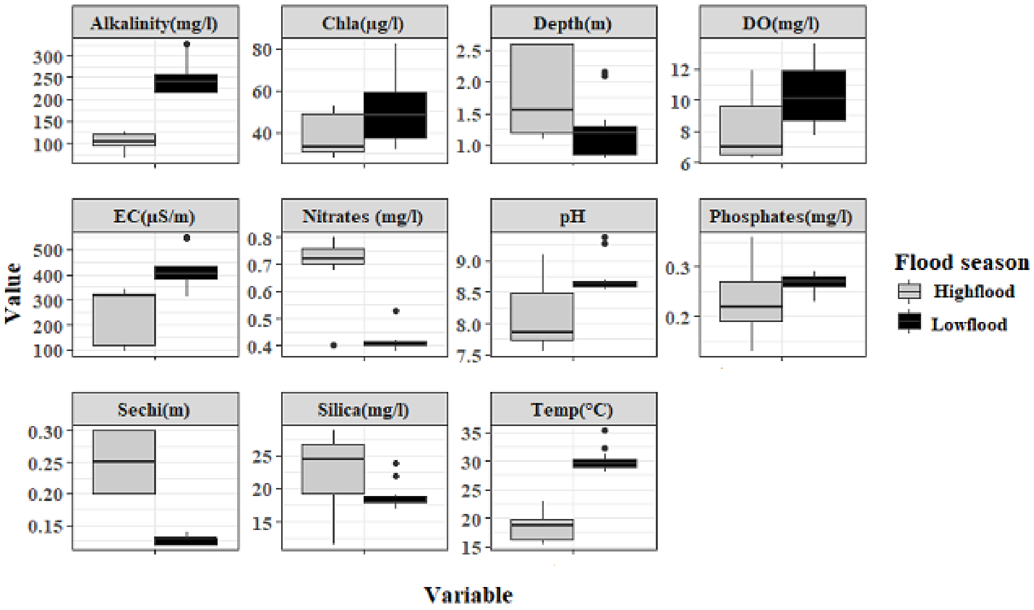

**Figure 7.5**. Comparison of environmental variables at high water level (Lowflood) and low water level (Lowflood) conditions. Note that units for y-axis are given in individual box-plot titles.

Among the measured environmental variables, water temperature showed significant positive correlation with pelagic primary productivity (pNPP) during all the flood seasons. Water depth and Secchi depth showed significant negative correlation with pNPP at high flood whereas pH showed significant positive correlation with pNPP during the season.  At low flood DO and dissolved phosphates were significantly positively correlated with pNPP (Table 7.3).

**Table 7.3.** Spearman-Rank correlations of environmental variables and primary productivity at Highflood and Lowflood.Significant correlations ($p < 0.05$) are printed in bold

| Variable | Highflood (pNPP) | Lowflood (pNPP) |
|---|---|---|
| Alkalinity | -0.397 | -0.0396 |
| Chla | -0.0980 | -0.0726 |
| Depth | **-0.431** | -0.0815 |
| DO | 0.423 | **0.607** |

| | | |
|---|---|---|
| EC | 0.350 | -0.0220 |
| Nitrates | 0.108 | -0.125 |
| pH | **0.568** | 0.0352 |
| Phosphate | 0.324 | **0.489** |
| Secchi | **-0.656** | -0.344 |
| Silica | -0.262 | -0.0840 |
| Temp | **0.614** | **0.633** |

## DISCUSSION

The results of this study indicate that fixation of carbon into Lake Ngami through algal photosynthesis (NPP) occurs mainly in the pelagic habitat (water column) whereas there is a net loss of carbon at the benthic surface. The lake’s average pelagic and benthic NPP were 0.089 ± 0045 mgC/l/h and -0.12 ± 0.10 mgC/l/h respectively. High pelagic primary productivity can be explained by high phytoplankton density as indicated by the Chla concentration of 55.0 ± 14 µg/l. Phytoplankton are the largest component of turbidity in lake systems (Shaw, Mechenich, and Klessig 2004) so while high pelagic phytoplankton densities favour pelagic primary productivity they increase lake turbidity. Consequently, this leads to reduced light penetration (Secchi depth was 0.26m at high flood and 0.13m at low flood) and limits primary production at the benthic surface and deeper levels of the water column too. It is intriguing that GPP was similar between the water column and the benthic surface, which could mean that the two habitats had comparable algal concentrations and that perhaps benthic NPP was suppressed by high respiration rate at the lake bottom.

Benthic respiration (*p*R = 0.22 ± 0.13) was much higher than pelagic respiration (*b*R = 0.055 ± 0.03 mgC/l/h) indicating a significantly higher rate of organic matter mineralisation at the lake bottom compared to the water column. Lake Ngami sediments are probably organic-rich because the remains of terrestrial plant communities that colonise the lake bed during desiccation periods serve as an organic source. Additionally, during desiccation periods livestock that occupy the lake bed may convey organic matter into the lake bed as they graze from surrounding grasslands and defecate on the lake bed ruminate and defecate. With limited resuspension of this organic matter in the lake sediments, respiration may be limited to the organic-rich benthic habitat.

Seasonal variation in pelagic primary production was observed with the Lowflood phase having higher productivity than the Highflood phase. Pelagic net primary productivity (*p*NPP) increased from 0.062 ± 0.05 mgC/l/h at high flood to 0.11± 0.07 mgC/l/h at low flood (Table 7.3). Low water conditions occur during hot summer months (November to February) before the arrival of the flood pulse, leading to higher water temperature. Lower water levels and higher temperature may account for the higher algal density (Chla) (Fig. 4) and higher primary productivity at low flood. The importance of temperature in primary production of the lake is demonstrated by its significant positive correlation with pelagic NPP during both high flood and low flood (Table 7.4). We expected primary productivity to peak at high flood when nutrients are released from terrestrial mineralization to the surface water (Wantzel *et al*. 2008). However, in the Okavango Delta, flood size has been shown to have a significant influence on primary production, smaller floods are associated with higher primary production and high nutrient levels (Lindholm *et al.* 2007). The current study was undertaken during a year of high floods (Fig.5) and has demonstrate that in Lake Ngami, the low flood stage had distinctively eutrophic conditions (high productivity and nutrient levels) compared to the high flood stage.

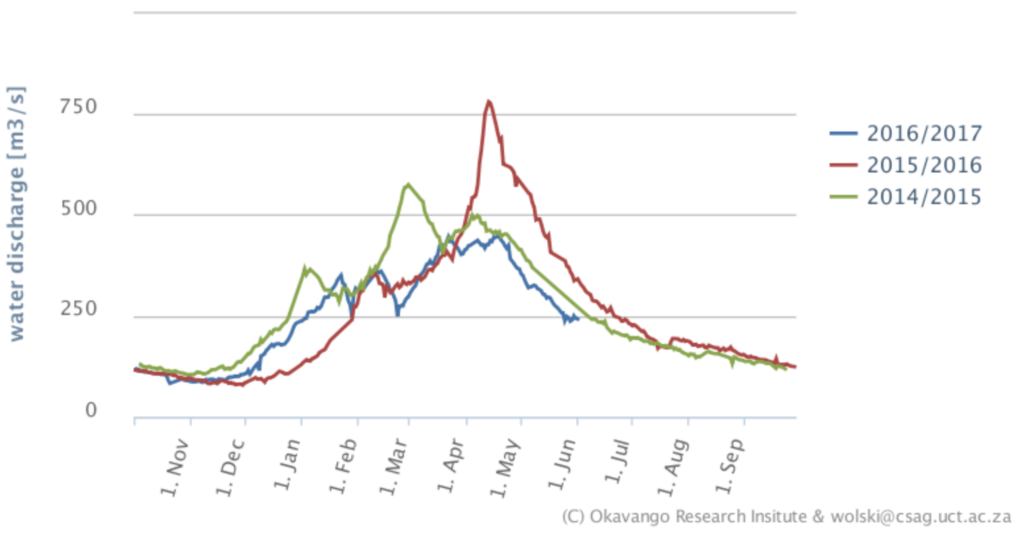

**Figure 7.6**. Okavango Delta flood discharge graph showing discharge levels at Mohembo for the Years 2014 to 2016 (Source: http://www.okavangodata.ub.bw/ori/monitoring/water/index.php)

Despite high levels of productivity at low flood compared to high flood, some of the potentially limiting factors such as water transparency (Secchi) and nitrate levels ($NO_3^-$) were higher at high flood (Fig. 4). The high nitrate levels at high flood are perhaps due to the nutrient-recharge effect of the arriving floods as lake fills and the increased water transparency is caused by the diluting effect of the floods. This diluting effect lowers primary productivity per volume as it dilutes the phytoplankton density at high flood. Our results support this claim because during high water conditions pelagic primary productivity (NPP) rate is negatively associated with Secchi depth (Table 7.5), indicating lower primary productivity at sites with less turbidity and presumably with less phytoplankton concentration. The level of dissolved phosphates ($PO_4^2$) was lower at low flood when phytoplankton densities were high and showed significant positive correlation with primary productivity during low flood The low $PO_4^2$ is possibly due to increased nutrient uptake by the higher algal densities during low flood. This result could mean that $PO_4^2$ was low and limiting at low flood, which is consistent with a widely accepted view that phosphorus is an important limiting factor in freshwater lakes (Elser et al. 2007, Moss et al. 2013). In general, the concentration of solutes in the lake water was higher at low flood. This is shown by higher (EC) at low flood which is almost double that of high flood (Fig. 4). This was expected because of the concentrating effects of evapotranspiration given the openness of the lake and the summer's hot months. Previous studies in the Okavango Delta have also reported higher EC at low flood and have attributed the difference to the effects of evapotranspiration (Mackay et al. 2011, Gondwe, Masamba, and Murray-Hudson 2017).

Our results show that Lake Ngami generally has higher levels of phytoplankton primary production compared to the upstream vegetated parts of the Delta where aquatic macrophyte production likely dominates aquatic primary production. Compared to Lake Ngami, which virtually lacks aquatic vegetation, the upstream aquatic habitats are characterized by submerged, floating and emergent macrophytes communities (Ellery, Ellery, and Verhagen 1992, Bonyongo, Bredenkamp, and Veenendaal 2000). The mean chlorophyll-a concentration recorded both at high flood (45.6μg/l) and low flood (64.2μg/l) in this study far exceeds chlorophyll-a estimates for upstream parts of the Delta. For example, Siziba *et al.* (2012) recorded highest chlorophyll-a concentration of 38.6μg/l from different floodplain types in the Chief's Island region. Lindholm et al. (2007) measured primary productivity of about 0.033mgC/l/h (reported as 800mgC$m^{-3}d^{-1}$) for Phelo floodplain during a highly productive year which reinforces our observation that phytoplankton productivity in Lake Ngami is higher per volume than that of upstream parts of the Delta. This relatively high phytoplankton productivity is key to supporting Lake Ngami fisheries, which are a very important livelihood resource for communities surrounding the lake as well as Maun town. The long-term flooding variability of Lake Ngami is possibly one of the major factors favouring the lake's high phytoplankton production. During desiccation periods the lake accumulate nutrients and organic matter not only through terrestrial trees and grasses that re-colonize the lake bed, but also through cattle and other grazers that occupy the lake bed during the dry period, as they graze from nearby grasslands they act a conveyor belt of nutrients into the lake bed. It is likely that during years when the lake receives flood water the suspension of the accumulated organic matter is the source of nutrients that support high primary productivity in the lake.

## CONCLUSION

The results of this study have shown that phytoplankton production is highly significant in Lake Ngami as a carbon fixation pathway. Given that the lake lacks aquatic macrophytes, the phytoplankton forms a very important resource that supports the lake's food web. Our results have demonstrated that indeed seasonal flooding influences primary production in Lake Ngami, but contrary to what we expected primary productivity per volume was higher at low flood than at high flood. However, this does not necessarily imply that the lake's total primary production is higher at low flood because the larger water volume at high flood provides more production space which could mean more total lake primary production. The results have also demonstrated that respiration occurs mainly at the benthic surface during both high flood and low flood stages. The higher benthic respiration was attributed to high organic matter content of the lake's mud due to the lake's long-term flooding patterns which allow accumulation of organic matter on the lake bed.

Effective natural resource management relies on scientific understanding of ecosystem processes and this study has contributed towards an understanding of primary productivity dynamics in Lake Ngami. However, given the inter-annual variability of the Okavango Delta's flooding, we recommend monitoring of the lake's primary production to gain insights into the lake's inter-annual primary productivity patterns. We also recommend an examination of geochemical processes that take place at the lake's benthic-column interphase which would improve our understanding of the interaction of these two important habitats of the lake.

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