Diatom diversity and response to water quality within the Makuleke Wetlands and Lake Sibaya

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Diatoms matter! They produce approximately 30 % of the world's oxygen, and they are useful tools in detecting and forecasting the pace of environmental change. If we succeed in relating to this remarkably different organism, we might even hope to relate to one another for the good of all creation.

Evelyn E. Gaiser
Think Like a Diatom

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Abstract

All forms of life are dependent on water for survival. South Africa is a water scare country, due to seasonal rainfall and high temperatures, thus it is important to manage water resources in such a way that it benefits the needs of humans and maintains the integrity of aquatic ecosystems. Agriculture activities, industrial activities and poor sanitation are some of the threats to water resources. Biomonitoring is one of several methods used to evaluate changes within aquatic ecosystems and makes use of the organisms found within the ecosystem to monitor the ecological integrity of that ecosystem — terrestrial or aquatic. As the aquatic organisms are continuously exposed to the environmental conditions within the ecosystem they are constantly exposed to the effects of pollution in the ecosystem which in turn modifies community structure. Biomonitoring could potentially be applied to all aquatic ecosystems including rivers, lakes, oceans, estuaries and wetlands.

Wetlands are important ecosystems as they are areas with a large variety of biota and provide numerous resources and ecological services for humans. However, wetlands are susceptible to nutrient enrichment and pollution as materials are brought into the ecosystem by water, wind and humans in the catchment area. As wetlands acts as 'sinks' sediment and pollutants, including nutrients, accumulate in wetland ecosystems. These pollutants enter the ecosystem through runoff, seepage, direct application or are wind driven. As humans make use wetlands as a source of food and water and wetlands support an abundance of biota it is important to monitor the health of these aquatic ecosystems.

Monitoring of wetland biota can be problematic at times as the variability in depth and inundation time does not allow some systems to support fish and/or macroinvertebrates. For this reason, diatoms are useful as biological indicators to monitor wetlands as they are microorganisms. Furthermore, diatom communities are species rich, respond rapidly to changes in the environment, are easy to collect, abundant and are the most diverse algae group. There is a paucity of aquatic biodiversity information on South Africa's Ramsar wetlands and specifically the diatom communities.

The present study focused on two Ramsar wetlands in South Africa namely Lake Sibaya and the Makuleke Wetlands. The aims of the study were to determine the distribution and occurrence of diatoms in the Makuleke Wetlands and Lake Sibaya in relations to water quality and secondly, to determine if European diatom-based indices for indicating wetland water quality conditions.

Water and diatom samples were collected from the Makuleke Wetlands during a wet (April 2015) and dry season (September 2015). Lake Sibaya was sampled during a winter (July 2015) and two summer seasons (November 2015 and February 2016). The nutrient concentrations present in the water column were measured. Diatom taxa from both wetlands were identified and indicator species were used to determine the trophic level and ecosystem quality of these wetlands.

Measured phosphate and inorganic nitrogen concentrations indicated both Lake Sibaya and the Makuleke Wetlands as nutrient enriched. The diatom community and indices (Specific Pollution sensitivity Index (SPI) and Generic Diatom Index (GDI)) correlated with the measured water quality and indicated both sites as nutrient enriched. The measured water quality variables indicated the wetlands to be either mesotrophic, eutrophic or hypertrophic. Diatom indices indicated that the study sites were in a bad/poor quality state with dominant diatom species occurring in polluted and nutrient enriched ecosystems. Thus, both wetlands are undoubtedly enriched with nutrients, however, it is unsure if these levels can be considered natural for these systems as nutrient accumulation is a key feature of wetlands.

Diatom taxa identified in the Makuleke Wetlands ranged from 12 – 20 species between the pans with a total of 70 species identified in the wetland as a whole. A total of 59 species were identified in Lake Sibaya with a ranging from 20 – 35 species identified at the sampling sites. Dominant diatom species in the Makuleke Wetlands included *Aulacoseira granulata, Gomphonema parvulum, Navicula* sp. and *Nitzschia* sp. Dominant species at Lake Sibaya included *Cocconeis placentula, Epithemia adnata* and *Gomphonema* sp. Dominant species for both wetlands were indicators of nutrient enriched ecosystems and tolerant of generally polluted conditions.

The diatom community (dominant species and diatom indices) and water quality indicated increased nutrients in the studies wetlands, suggesting a declining ecosystem quality. It is concluded that methods for diatom community analysis and water quality analysis were successfully applied and indicated both wetland ecosystems as nutrient enriched, however there are doubts as to whether this can in turn be viewed as indicating poor ecosystem health in general. Thus it is recommended that further in-depth studies be completed on diatom community structure and water quality of wetland ecosystems to determine how to define natural conditions. This will enable better understanding of the nutrient levels within wetlands as well as the use of diatoms as bio-indicators for wetland ecosystems.

Key words: Biomonitoring, wetlands, diatoms, water quality, nutrient enrichment

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List of abbreviations

AEC	Alternative ecological category
ASL	Above sea level
CCA	Canonical correspondence analysis
DEAT	Department of Environmental Affairs and Tourism
DIC	Differential interference contrast
DO	Dissolved oxygen
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
FRAI	Fish Response Assessment Index
GDI	Generic diatom index
HCl	Hydrogen chloride
IBI	Index of Biotic Integrity
$KMnO_4$	Potassium permanganate
KNP	Kruger National Park
LM	Light microscope
MAP	Mean annual precipitation
MAR	Mean annual runoff
MIRAI	Macro-Invertebrate Response Assessment Index
NAEHMP	National Aquatic Ecosystem Health Monitoring Programme
NBPAE	National Biomonitoring Programme for Aquatic Ecosystems
NH_4	Ammonium
nMDS	Non-metric multidimensional scaling

NO_2	Nitrite
NO_3	Nitrates
NWA	National Water Act
OC	Oxygen concentration
%PTV	Percentage Pollution Tolerant Valve
PCA	Principle components analysis
PES	Present ecological status
PO_4	Phosphate
RDA	Redundancy analysis
REMP	River Ecostatus Monitoring Programme
RHP	River Health Programme
SASS 5	South African Scoring System Version 5
SEM	Standard error of the mean
SPI	Specific pollution sensitivity index
TDI	Trophic diatom index
VEGRAI	Riparian Vegetation Response Assessment Index
WQ	Water quality
WQG	Water quality guidelines
WRC	Water Research Commission

Chapter 1 — Introduction

1.1 Water use and management

Water is essential for all forms of life and millions of people die every year due to a lack of clean water (Curry, 2010). It is a limited and scarce resource in South Africa due to seasonal rainfall and high temperatures (Dallas and Day, 2004). South Africa has virtually no natural permanent standing fresh water, with rivers and associated impoundments being exploited as a fresh water source (Dallas and Day, 2004). The country has a mean annual rainfall of 497 mm (with a world mean annual rainfall of 860 mm) and water resources are under pressure as the mean annual evaporation (1100 mm to 3000 mm) exceeds the mean annual rainfall (Dallas, 2000; Mantel *et al.*, 2010). The rivers in South Africa are also under immense pressure (as a source of water and owing to pollution) due to an increase in human population (Dallas, 2000; Dallas and Day, 2004). Increased human population adds pressure to the water sources as an increase in population increases the need for clean water (Curry, 2010) and this increases the agriculture activity as a source for food production. Thus, water needs to be managed in such a way that it benefits human needs as well as maintaining the integrity of the aquatic ecosystem (Mantel *et al.*, 2010). South Africa is currently facing a drought (2016) causing a decrease in water levels across the country (Mawu, 2016; Writer, 2016) which is a great cause for concern and highlights the importance of correct water management in the country.

As South Africa is a water scarce country, its water resources should be properly managed as the human population relies on these sources (rivers) for its water needs (Dallas and Day, 2004). The Department of Water and Sanitation (DWS), previously the Department of Water Affairs and Forestry (DWAF), developed the National Water Act (NWA) in 1998 to ensure that water is managed and used in such a way that the ecosystem and humans benefit from it (DWAF, 1998). The aim of the NWA was to derive a set of criteria for water quality and develop procedures for the protection of South Africa's freshwater ecosystems (DWAF, 1998).

Insufficient freshwater is due to five main causes, namely: industrial pollution, sanitation, increase in human population, disproportional distribution of water and climate change (Dallas, 2000; Curry, 2010). According to Curry (2010), certain races and income groups are worst affected by lack of sanitation and industrial pollution. Disproportional water distribution and climate change contributes to the country's water scarcity and inadequate dissemination, while uneven rainfall patterns account for a portion of the population having insufficient access to water (Curry, 2010).

As 70 % of the world is covered with water it is difficult to understand why there is water scarcity, however, only 2 % of this water is salt-free and only a third of this 2 % is available water for human use (Curry, 2010). This highlights the need for proper management and monitoring of our water sources to ensure clean water for future use.

1.2 Biomonitoring

According to Li *et al.* (2010), biomonitoring is defined as the method to determine the changes or conditions of the environment through the use of living organisms or their responses to environmental variables. Dalu and Froneman (2016) defined biomonitoring as a method that assesses the response of aquatic organisms to change in the environment.

For proper management of water the complete spectrum of information on an aquatic ecosystem is needed, thus bacteriological, chemical and physical measurements form the basis of monitoring of an aquatic ecosystem (Li *et al.*, 2010). However, as running water's hydrology undergoes rapid changes, the impacts of various environmental factors and long-term sustainability are difficult to measure (Li *et al.*, 2010). It has been proven that traditional monitoring techniques (bacteriological, chemical and physical measurements) can be supplemented by biomonitoring techniques as aquatic organisms are constantly exposed to the conditions in their direct environment (Li *et al.*, 2010). Organisms in the ecosystem reflect past and present conditions of the ecosystem as they are continuously exposed to pollutants in the ecosystem (Dalu and Froneman, 2016), making them ideal for monitoring of an aquatic ecosystem. When monitoring an ecosystem, the aquatic organisms in the ecosystem provide an indication of a broad spectrum of the impacts on the ecosystem as they help to detect the long-term effects of changes to the environment on the ecosystem (Dallas, 2000; Dalu and Froneman, 2016).

Biomonitoring programmes are implemented to assess the quality of an ecosystem through use of aquatic organisms. The National Biomonitoring Programme for Aquatic Ecosystems (NBPAE) was initiated in 1996 by DWAF, Department of Environmental Affairs and Tourism (DEAT) and the Water Research Commission (WRC) (Bate *et al.*, 2004). The objective of the NBPAE was to develop a programme to assess the health of aquatic ecosystems and to provide information to manage the water sources throughout the country (Bate *et al.*, 2004). In 2016 the NBPAE was replaced by the National Aquatic Ecosystem Health Monitoring Programme (NAEHMP). A programme designed to monitor aquatic ecosystems in South Africa, known as the River Health Programme (RHP), was developed by the WRC, DEAT and DWAF in 1994 (Dallas, 2005). Various biota (riparian vegetation, macro-invertebrates and

fish) were incorporated into the RHP (Dallas, 2000). The indices included in this programme included the Riparian Vegetation Response Assessment Index (VEGRAI), South African Scoring System Version 5 (SASS 5), Macro-Invertebrate Response Assessment Index (MIRAI) and Fish Response Assessment Index (FRAI) (Dallas, 2005). The RHP was replaced in 2016 by the River Ecostatus Monitoring Programme (REMP).

All the above mentioned indices are limited to the availability of the representative biota and by habitat availability at the study area. Thus, it is necessary to make use of an index that will have biota available in all (or at least most) aquatic ecosystems. A biological indicator (bio-indicator) must have a rapid reaction to unexpected changes in the environment and not only indicate long-term conditions of the environment (Li *et al.*, 2010; Stevenson *et al.*, 2010). As biomonitoring provides an overview of the conditions of the environment it can effectively be used to aid in management of our aquatic ecosystems (Dalu and Froneman, 2016), and diatom-based biomonitoring may be of particular use in wetlands as they have a rapid response and the environments often lack requisite habitats and refuge for higher organisms.

1.3 Wetlands

Owen *et al.* (2004) stated that it is difficult to define a wetland; however, the Ramsar Convention for Wetlands of International Importance constructed a definition for wetlands as "areas of marsh fen, 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 metres" (see Matthews, 2013). Alternatively, the National Water Act (NWA) (1998) defines a wetland as "land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil". A wide variety of aquatic ecosystems are covered by the term wetland namely: floodplains, saline lakes, tree covered swamps and high altitude rainpools (Dallas and Day, 2004).

Wetlands are found across the world and approximately 6 % of the world's surface is made up by wetlands (Matlala *et al.*, 2011). A wetlands' condition is determined by geomorphology, hydrology, seasonal presence of water, level of present water and the presence of basins and depressions (Dallas and Day, 2004). In rivers, differences in chemical and physical conditions are observed longitudinally, but in wetlands these conditions can also be observed vertically through the wetland system (Dallas and Day, 2004). Important ecological services — such as water storage, maintenance of biodiversity, biogeochemical cycling and maintenance of biotic

productivity — are provided by wetlands (Matlala *et al.*, 2011), thus illustrating the importance of wetland systems.

Wetland systems are susceptible to pollution as they act as 'sinks' where water and sediment accumulate (Dallas and Day, 2004; Malan and Day, 2012; Dalu and Froneman, 2016). Materials are brought into wetlands by wind, water, and humans influence in the catchment of the wetland, thus causing a potential build-up of pollutants in wetland's water and sediment (Dallas and Day, 2004). As suspended material accumulates in wetlands their bed becomes composed of sand or fine mud with a result that that they exhibit fewer biotopes than rivers (Dallas and Day, 2004).

According to Kotze (2010), many people in South Africa are dependent on wetlands. It is thus necessary to monitor and correctly manage wetland ecosystems in the country. Unfortunately the water quality (WQ) data for wetlands is not as comprehensive and well documented as for riverine systems (Malan and Day, 2012). As they act as 'sinks', their range of values for WQ are poorly understood and it is unknown under which conditions the system is natural or polluted (Dallas and Day, 2004; Malan and Day, 2012; Dalu and Froneman, 2016). Several research initiatives have recently been undertaken on wetlands, which include research on biotic indicators (e.g. micro- and macro-invertebrates, diatoms and macrophytes) and visible indicators to assess impacts on a wetland to establish the conditions of the wetland (Malan and Day, 2012).

As wetlands provide an area where a large variety of biota occurs, it is necessary to assess the health of these systems to guarantee the conservation of the biota in wetlands (Matlala *et al.*, 2011).

1.4 Diatoms

Aquatic ecosystems are vital for the survival of all living organisms. The microscopic organisms present in aquatic ecosystems are useful bio-indicators as they serve as early indicators on the health of the ecosystem due to them being primary producers (Dalu and Froneman, 2016). These primary producers include the organisms known as diatoms. Taylor *et al.* (2005) describes diatoms as: "a key component of aquatic ecosystems and constitute a fundamental link between primary (autotrophic) and secondary (heterotrophic) production". Diatoms are unicellular micro-organisms (algae) which are photosynthetic and pigmented (sometimes forming colonies), and can be observed worldwide in nearly all aquatic and sub-

aerial environments (Round *et al.*, 1990; Dixit *et al.*, 1992; Hoagland, 1993; Kröger and Sumper, 1998; Taylor *et al.*, 2005; 2007; Smucker and Vis, 2011; John, 2012).

1.4.1 History of diatom research

As the first microscopists examined the world of microorganisms in the 17th century they discovered diatoms and were interested in their movements (John, 2012). The diatoms were initially included under the animal kingdom because of their motile unicellular forms and protoplast (Round *et al.*, 1990; Smol and Stoermer, 2010), which was according to Round *et al.* (1990) "...interpreted as representing the internal organs of, an animal, complete with digestive system". The first diatom, probably *Tabellaria flocculosa* Kützing (1844) according to descriptions and diagrams, was reported by an unknown English country gentleman using a simple microscope in 1703 (Round *et al.*, 1990). Baker's *Employment for the microscope* in 1753 contained the next certain description of a diatom (Round *et al.*, 1990). Baker identifies both *Oscillatoria* (cyanobacteria), which he describes as 'Hair-like insects', as well as the diatom *Craticula cuspidata,* which he described as an 'Oat-animal' (Round *et al.*, 1990). One of O.F. Müller's species, *Bacillaria paxillifer* O Müller (originally as *Vibrio paxillifer* O Müller), served as type of the first diatom genus, *Bacillaria* Gmelin, and he also included two other diatoms in *Vibrio* (Round *et al.*, 1990). One of these two species was *V. tripunctatus* which was thought to be a *Navicula* species, but it could have been a *Nitzschia* species due to difficulty in interpreting Müller's illustrations (Round *et al.*, 1990). Müller called these three *Vibrio* species 'animalcula infusoria' as to him they were animals (Round *et al.*, 1990).

It was only at the end of 1844 when Kützings monograph seemingly ended the uncertainty on whether diatoms were animals or plants (Round *et al.*, 1990; John, 2012). At this stage all diatoms were subsequently treated as plants (whether they were motile or non-motile, colonial or unicellular) and classified as algae. However, diatoms are now classified under the kingdom Protista and in the class Bacillariophyceae (see Julius and Theriot, 2010; Smol and Stoermer, 2010; Dalu and Froneman, 2016). The progression of diatom studies was limited by the technical development of the microscope during the period 1844 – 1900 (Round *et al.*, 1990). However, during this period many diatom genera were described by Grunow and Cleve and it was seen as the golden age of diatom studies (Round *et al.*, 1990; Julius and Theriot, 2010). Development of microscope lenses benefitted from diatom collection as the lenses were tested and developed in terms of resolving power using diatoms as test objects (Round *et al.*, 1990). Interest in diatoms soared amongst amateur microscopists and it became competitive to resolve the finer structures of the diatom valve (Round *et al.*, 1990).

During the latter half of the 19th century sound classifications of diatoms were already established (Round *et al.*, 1990). The development of limnology as a science created a great interest in the role of diatoms in freshwater and according to Round *et al.* (1990), "only now are we beginning to appreciate the full impact of diatom growth on the complete physical, chemical and biological background of a body of freshwater". Thus diatoms remain a valuable bio-indicator as they provide information on pH changes and indicate past environmental conditions (Round *et al.*, 1990; Julius and Theriot, 2010).

1.4.2 Diatom structure and cell division

All diatoms have a similar cellular structure. Freshwater diatoms occur in two main groups, namely, centric and pennate diatoms, each specially adapted to occur in different habitats (Kröger and Sumper, 1998; Taylor *et al.*, 2005; 2007; Julius and Theriot, 2010). Figure 1-1 illustrates the structure of a pennate and centric diatom. More detailed illustrations of these two diatoms are given in Figure 1-2. Due to their specific storage products, siliceous cell wall, and photosynthetic pigments, diatoms are considered unique amongst other algae (Taylor *et al.*, 2005; 2007; Dalu and Froneman, 2016). The cell wall (also called a frustule) of the diatom has an interesting design, it is composed almost entirely of biogenic silica with several other trace elements (Round *et al.*, 1990; Dixit *et al.*, 1992; Kröger *et al.* 1994; Kröger and Sumper, 1998; Julius and Theriot, 2010; Smol and Stoermer, 2010; Dalu and Froneman, 2016). The wall consists of valves and girdle bands, the former are multipartite and the latter are thinner linking structures (Round *et al.*, 1990; Dixit *et al.*, 1992; Smol and Stoermer, 2010). Of the two valve halves there is a younger valve called the hypovalve and an older valve called the epivalve, and these halves are bound together by the girdle bands (Round *et al.*, 1990; Kröger *et al.* 1994, Kröger and Sumper, 1998; Julius and Theriot, 2010). Girdle bands act as belt-like elements that link these valves together (Dixit *et al.*, 1992). Of the two valves the hypovalve will usually be the smaller one, this is due to the fact that the girdle bands are mostly parallel and cylindrical (Round *et al.*, 1990; Julius and Theriot, 2010).

Reproduction in diatoms is primarily asexual mitotic division (Julius and Theriot, 2010). Through a form of exocytosis, new parts of the cell wall, which are formed in the protoplast, are added to the existing cell wall (Round *et al.*, 1990; Kröger *et al.* 1994). According to Round *et al.* (1990), "When the cell divides, the hypocingulum of the parent cell becomes the epicingulum of the one daughter cell, and the parental epicingulum becomes the epicingulum of the other daughter cell".

A diatoms best known biological feature, according to Round *et al.* (1990), is that "the production of new frustule components within the confines of the parental cell wall usually leads to a decline in mean cell size". In sexual reproduction a new frustule is produced through a process called auxosporulation, the process where a special cell expands in a controlled way (Round *et al.*, 1990; John, 2012). This is the only way that a diatoms size can be restored. The process of asexual reproduction or cell division is described visually in Figure 1-3. The process of cell division differs between species and environmental conditions and can take anywhere between 8 to 24 hours to complete (John, 2012).

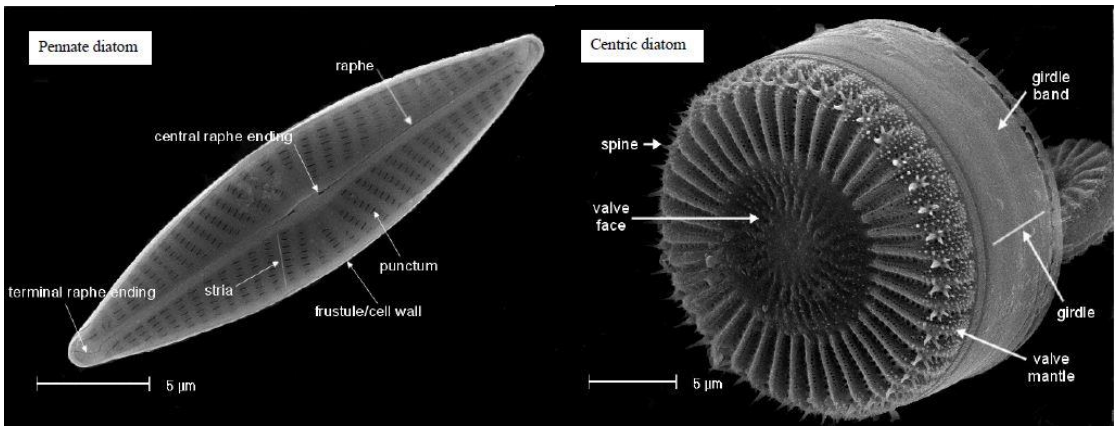

Figure 1-1. Generic structure of a pennate (left) and centric (right) diatom. (Source: Taylor *et al.*, 2005).

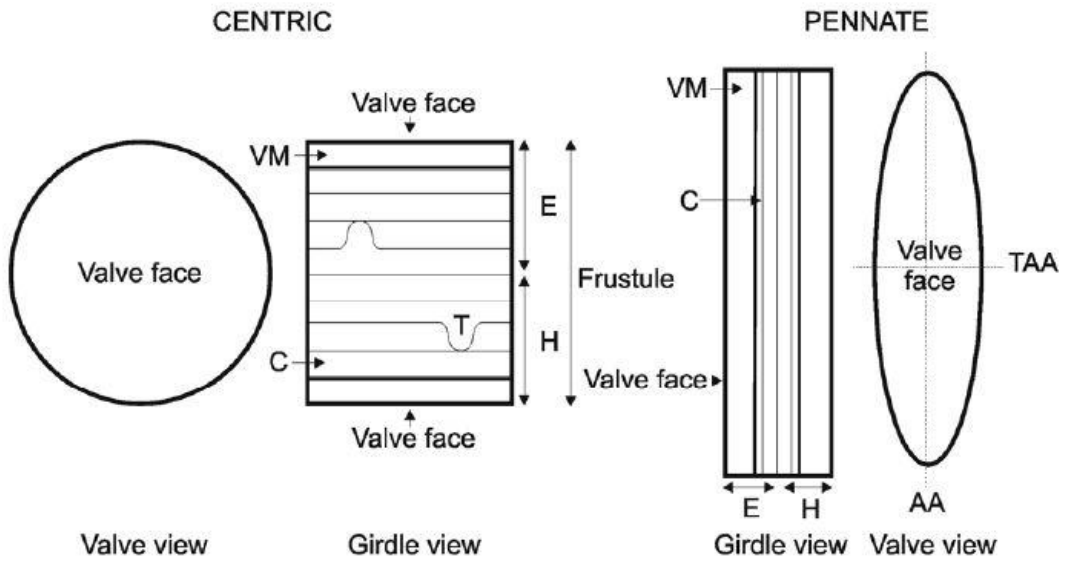

Diagram of diatom cells to show the relationships of the siliceous components.
E = epivalve + epicingulum; **H** = hypovalve + hypocyngulum; **C** = copulae or girdle bands;
VM = valve mantle. Copulae (girdle bands) may have a tongue- like extension (**T**) which inserts into any space between the ends of the adjacent split copula. **AA** = apical axis, **TAA** = transapical axis

Figure 1-2. A detailed diagram of both a centric (left) and pennate (right) diatom. (Source: Taylor *et al.*, 2007).

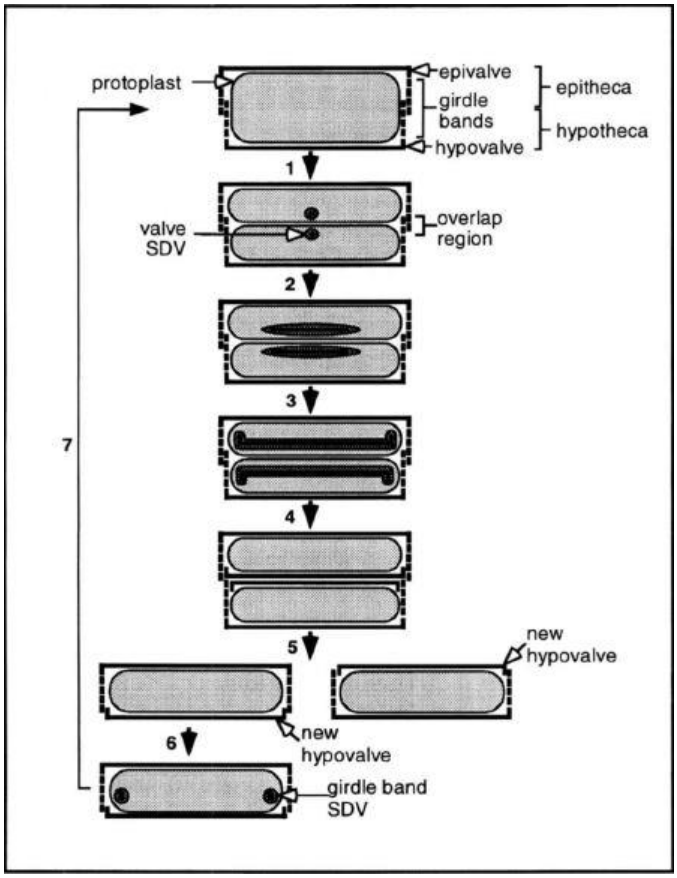

Figure 1-3. A detailed diagram illustrating cell division in diatoms. (Source: Kröger and Sumper, 1998).

1.4.3 Why use diatoms as bio-indicators?

According to Dixit *et al.* (1992), there are certain criteria used to determine if an organism would be ideal to use as a bio-indicator in aquatic ecosystems. When choosing an organism as a bio-indicator the organism should be able to help quantify the rate of deterioration in water quality, the organism should still be applicable over a large geographical region, it should be simple to use, the organism should be sensitive to any changes either in the biotic or abiotic environment, and researchers should be able to base conclusions on the response of the bio-indicator to environmental variables (Dixit *et al.*, 1992; Reid *et al.*, 1995).

Diatoms' use as bio-indicators to monitor past and present conditions of aquatic ecosystems has been widely studied over the past two decades (Morales *et al.*, 2001). These studies have revealed several reasons as to why diatoms should be used as bio-indicators, including:

1) Diatoms have a worldwide distribution (Dixit *et al.*, 1992; Reid *et al.*, 1995).

2) Diatoms are at the base of the food web and are primary producers, thus any change in the community composition of diatoms would be reflected in higher trophic levels (Stevenson *et al.*, 2010; Dalu and Froneman, 2016).

3) They are present in nearly all aquatic ecosystems (freshwater, brackish and marine) as well as terrestrial habitats (Dixit *et al.*, 1992; Reid *et al.*, 1995; Morales *et al.*, 2001; Julius and Theriot, 2010; Smol and Stoermer, 2010; Stevenson *et al.*, 2010).

4) Diatoms can be used to monitor changes in the aquatic environment as they respond to stimulants in their environment, such as habitat alterations, physical factors (elevation and habitat), nutrients and contaminants (Stevenson *et al.*, 2010; Dalu and Froneman, 2016).

5) Diatoms respond rapidly to any changes in their environment due to their short life span. They have one of the shortest life spans (2 weeks) of all aquatic indicators. Thus they serve as early indicators of habitat restoration and pollution (Dixit *et al.*, 1992; Morales *et al.*, 2001; Stevenson *et al.*, 2010; Dalu and Froneman, 2016).

6) Individual species have their own specific water quality requirements and they show a broad range of tolerance along a gradient of aquatic productivity (Dixit *et al.*, 1992).

7) There is comprehensive documentation on diatom taxonomy and identification (Reid *et al.*, 1995).

8) Diatom communities are species rich (Stevenson *et al.*, 2010).

9) Due to their high abundance, only a small but representative sample would be sufficient for analysis (Dixit *et al.*, 1992; Julius and Theriot, 2010).

10) Diatoms are easy to collect in wetlands as they are abundant, dominant, and the most diverse algae group (Alakananda *et al.*, 2011).

11) There is no deterioration of prepared microscope slides thus making them almost permanent records of conditions prevailing at the time of sampling (Reid *et al.*, 1995; Stevenson *et al.*, 2010).

Diatoms are useful bio-indicators of aquatic ecosystems as they satisfy all the criteria listed above needed for an ideal bio-indicator (Dixit *et al.*, 1992). Due to their rapid response to chemical and physical changes (pH, organic nutrients, conductivity, etc.), diatoms can be used to determine short term environmental changes (Stevenson *et al.*, 2010; Alakananda *et al.*, 2011). Thus, diatoms as bio-indicators are time efficient (especially sampling), simple to use, and ensure that regular monitoring can take place over time (Gell *et al.*, 2002).

Any changes in the physio-chemical parameters in the water would cause a change in the community structure of diatoms (Passy, 2007). Environmental factors influence the growth of each individual diatom species (Dixit *et al.*, 1992) and it is the understanding of the relationship between these factors and diatoms that makes diatoms an ideal bio-indicator (Brazner *et al.*, 2007; Smol and Stoermer, 2010; Julius and Theriot, 2010).

1.4.4 Diatoms as indicators for wetlands

Fish and macro-invertebrates are usually hard to use as bio-indicators in wetlands due to the fact that the water body is generally not deep enough to support fish and large organisms and is thus dominated by microscopic organisms (Matlala *et al.*, 2011). A biomonitoring technique must thus be applied to assess these microscopic organisms (Matlala *et al.*, 2011).

According to Matlala *et al.* (2011), “Studies of diatoms which were conducted in wetlands have thus far shown strong correlations between changes in physical and chemical parameters with diatom composition”. It can also be seen in palaeoecological work that past environmental conditions can be determined by comparing modern diatom flora to present environmental conditions (La Hée *et al.*, 2012). Diatoms can be used to determine the long term (years) environmental conditions of wetlands as they can be preserved for a long period of time due to the nature of the silica cell wall (as previously mentioned) (Julius and Theriot, 2010; Smol and Stoermer, 2010; Matlala *et al.*, 2011).

Diatom indices have been developed over the past three decades to provide information on eutrophication, nutrient status, acidification, general water quality and organic pollution in rivers and lakes. Numerous international studies have made use of diatoms as a bio-indicator for ecological assessment of pollution and environmental conditions (Stevenson *et al.*, 2010;

Dalu and Froneman, 2016). Pan and Stevenson (1996) stated that in lakes and streams environmental conditions were successfully determined though use of diatoms as bio-indicators. To monitor water quality changes in the Everglades wetlands system, periphytic diatoms were used as the basis of an effective monitoring tool (La Hée *et al.*, 2012). From the study by La Hée *et al.* (2012) it was shown that diatoms can be effectively used as bio-indicators to determine the water quality of karstic wetlands in the Caribbean. Over the past few years, diatoms have successfully been implemented to evaluate past and present ecological conditions across the world (Dalu and Froneman, 2016). In southern Africa biomonitoring methods have generally made use of macrophytes, macro-invertebrates and fish as bio-indicators for ecosystem monitoring (Dalu and Froneman, 2016). According to Dalu and Froneman (2016), diatoms are not routinely used as bio-indicators in African waters. Béla Jenö Cholnoky (1899 – 1972) (referred to as South Africa's father of diatomology), argued that diatom communities can provide information on a water body's physio-chemical features and should be implemented in monitoring the physico-chemical characteristics of an aquatic ecosystem (Dalu and Froneman, 2016). As diatoms provide comparable data, are accurate and cost effective, the method has been added to South Africa's RHP (Dalu and Froneman, 2016). The use of benthic diatoms for trophic condition assessment and monitoring biotic integrity has been routinely applied in South Africa for monitoring of estuarine and freshwater systems (Dalu and Froneman, 2016). In South Africa diatom use for biomonitoring has been recognised (Dalu and Froneman, 2016) and during the State of the Rivers Report for the Crocodile West/Marico catchment, diatoms were successfully used as one of the biological indicators for the first time in 2005 (River Health Programme, 2005).

Algae (particularly diatoms) are used as bio-indicators in coastal wetlands as they respond to environmental changes over a period of weeks to months, thus giving a short term assessment of the small deviations that occur in the ecosystem (Gaiser *et al.*, 2005). Diatoms can be used to accurately determine the salinity of a system (Gaiser *et al.*, 2005). It should be noted that such a salinity gradient has only been completed for South Florida, USA, and it would be problematic applying this gradient in other countries and poorly explored wetlands (Gaiser *et al.*, 2005). It is, in general, potentially problematic to simply apply diatom-based monitoring systems developed elsewhere in the world to the South African situation. Thus, the present study will examine the use of existing diatom indices for rivers and will critically assess them in wetlands in terms of value as a tool for such ecosystems.

1.5 Study rationale

1.5.1 Problem Statement

The Ramsar convention was established in 1971 and is the oldest modern intergovernmental environmental agreement globally (Matthews, 2013; RCS, 2016). There are currently 169 Contracting Parties that form part of the convention (as of January 2016) (RCS, 2016). Countries that form part of the convention include Australia, Germany, Belgium, New Zealand, South Africa and United States of America (RCS, 2016). South Africa joined the convention on 21 December 1975 (RCS, 2016) and currently has 22 sites. Figure 1-4 shows the location of all 22 sites in South Africa. False Bay Nature Reserve is the latest wetland to be included to the list of South African Ramsar wetlands (Ramsar, 2015).

A WRC workshop in 2013 concluded that there is a paucity of aquatic biodiversity information on South Africa's Ramsar wetlands, including information deficiency on diatom communities. In the Makuleke Wetlands and Lake Sibaya, limited aquatic sampling has been completed in recent years (Deacon, 2007). Also very little information is known on diatoms in South African wetlands, with diatom studies largely being completed on rivers rather than wetlands. Therefore, this project aims to increase information on the available diatom community from selected Ramsar sites (Makuleke Wetlands and Lake Sibaya), by studying water quality and diatom communities present.

Both Lake Sibaya and the Makuleke Wetlands are impacted by anthropogenic activities from rural settlements in their surroundings. Due to Lake Sibaya's endorheic nature (Ward and Kyle, 1990; Humphries and Benitez-Nelson, 2013) and its increased rural development and forestry, it is particularly susceptible to pollution (Ward and Kyle, 1990; Humphries and Benitez-Nelson, 2013; DWS, 2015a). Spraying of herbicides and pesticides also occurs just outside of the Makuleke Wetlands. This is due to spraying within rural towns and the resulting toxins are transported via the Luvuvhu River to the wetlands. The Luvuvhu River flows through over-populated settlements and villages leading to high levels of pollution in the river and causing diseases in animals (Smit *et al.,* 2013).

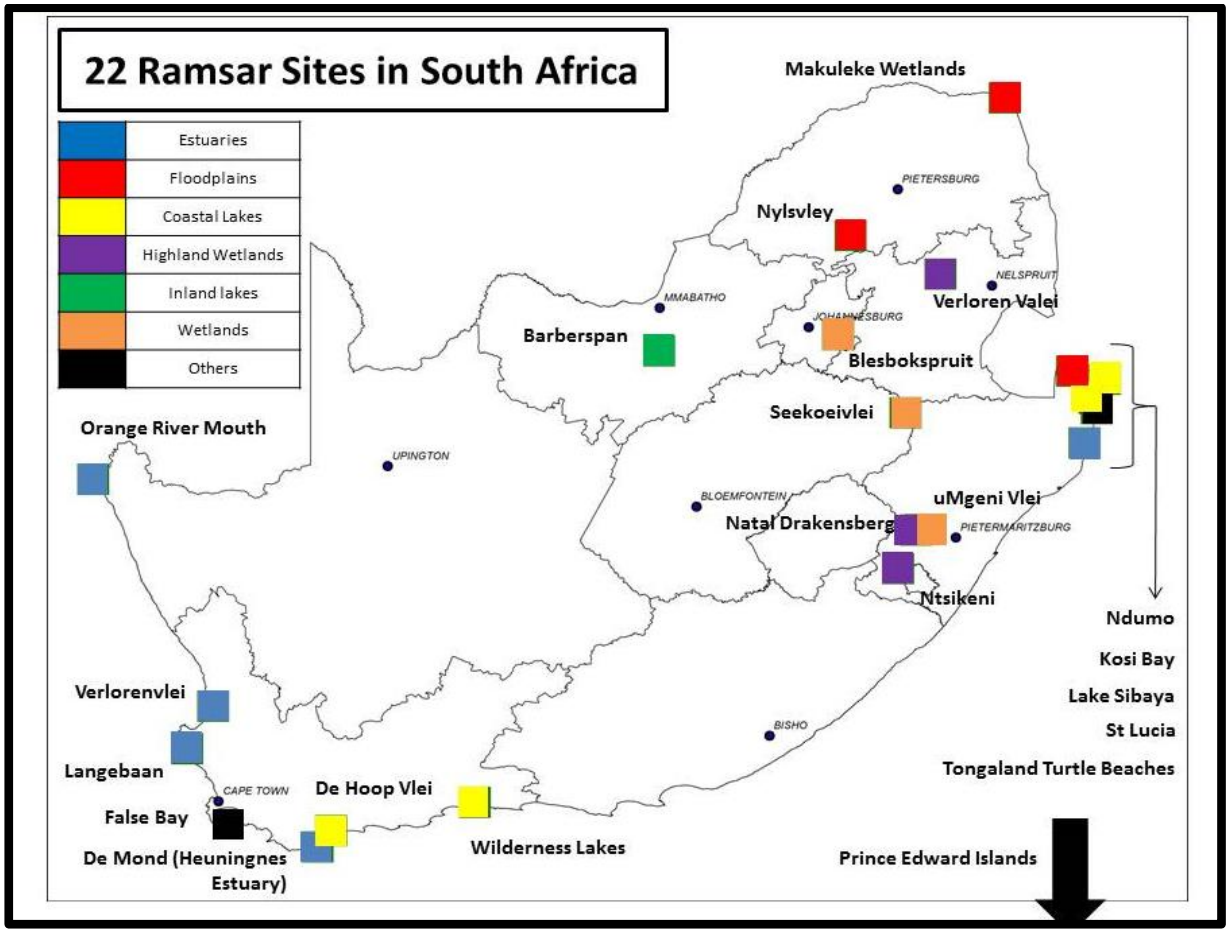

Figure 1-4. Map indicating the location of South Africa's 22 Ramsar wetlands (Source: Dr. Wynand Malherbe).

1.5.2 Hypothesis

For this study the following hypothesis was proposed:

1. Lake Sibaya will be impacted due to the development of rural areas and forestry development.
2. Makuleke Wetlands will be impacted as the wetland receive water from catchment outside of the park (Limpopo and Luvuvhu Rivers).
3. Diatoms will successfully be implemented to assess the quality of the two wetland ecosystems.

1.5.3 Aims and objectives

The aims of the study are to determine the distribution and occurrence of diatoms in the Makuleke Wetlands and Lake Sibaya in relations to water quality; and to determine the use of European diatom-based indices for indicating wetland water quality conditions.

To achieve these aims the follow objectives were set in place:

1. Sample diatoms from both study areas, with several sample sites within each area.
2. To determine the water quality of each site.
3. Identify the diatoms collected as well as the community structure.
4. Statistically compare the water quality and diatom community structure.

Chapter 2 — Methodology

2.1 Site selection

The study was undertaken at two localities, namely, Lake Sibaya and Makuleke Wetlands. At each locality numerous study sites were selected to ensure that results were representative of the entire system. A short description of each study area is presented below while more detailed site descriptions are presented in Chapters 3 and 4 for Lake Sibaya and Makuleke Wetlands respectively.

2.1.1 Lake Sibaya

Lake Sibaya (Figure 2-1) is situated in the KwaZulu-Natal Province on the east coast of South Africa (geographical co-ordinates S27.3485, E32.6842). The lake is situated on the Maputaland coastal plain 430 km north-east of Durban with vegetated dunes cutting it off from the sea (Ward and Kyle, 1990; Combrick *et al.*, 2011; Humphries and Benitez-Nelson, 2013; Stager *et al.*, 2013). It is estimated that the lake only takes up 60 – 70 km^2 of the catchment area (Ward and Kyle, 1990; Humphries and Benitez-Nelson, 2013) and is roughly 20 m above sea level (Bowen, 1978; Combrick *et al.*, 2011; Stager *et al.*, 2013). The mean depth of the lake is 13 m with a maximum depth of 40 m (Bowen, 1978). Lake Sibaya was designated as a Ramsar wetland on the 28th of August 1991 and is South Africa's largest natural freshwater lake (Ward and Kyle, 1990; Combrick *et al.*, 2011; Stager *et al.*, 2013). In KwaZulu-Natal, the second largest *Crocodylus niloticus* (Nile crocodile) and *Hippopotamus amphibius* (Hippopotamus) populations are found at the lake (Ward and Kyle, 1990). The lake supports a large diversity of fauna and flora and has the ability to support hundreds of *C. niloticus*, large mammals, birds and approximately 250 *H. amphibius* (Ward and Kyle, 1990; Humphries and Benitez-Nelson, 2013). Measures were taken to manage and protect the lake as it is state land and serves as a tourist destination (Ward and Kyle, 1990). Further measures were also taken to erect an electric fence around the lake, with a third of the lake already fenced in 1990 (Ward and Kyle, 1990).

At Lake Sibaya four sites were selected for the assessments namely Lake Sibaya 1 (LS1), Lake Sibaya 2 (LS2), Lake Sibaya 3 (LS3) and Lake Sibaya 4 (LS4) (see Figure 2-1).

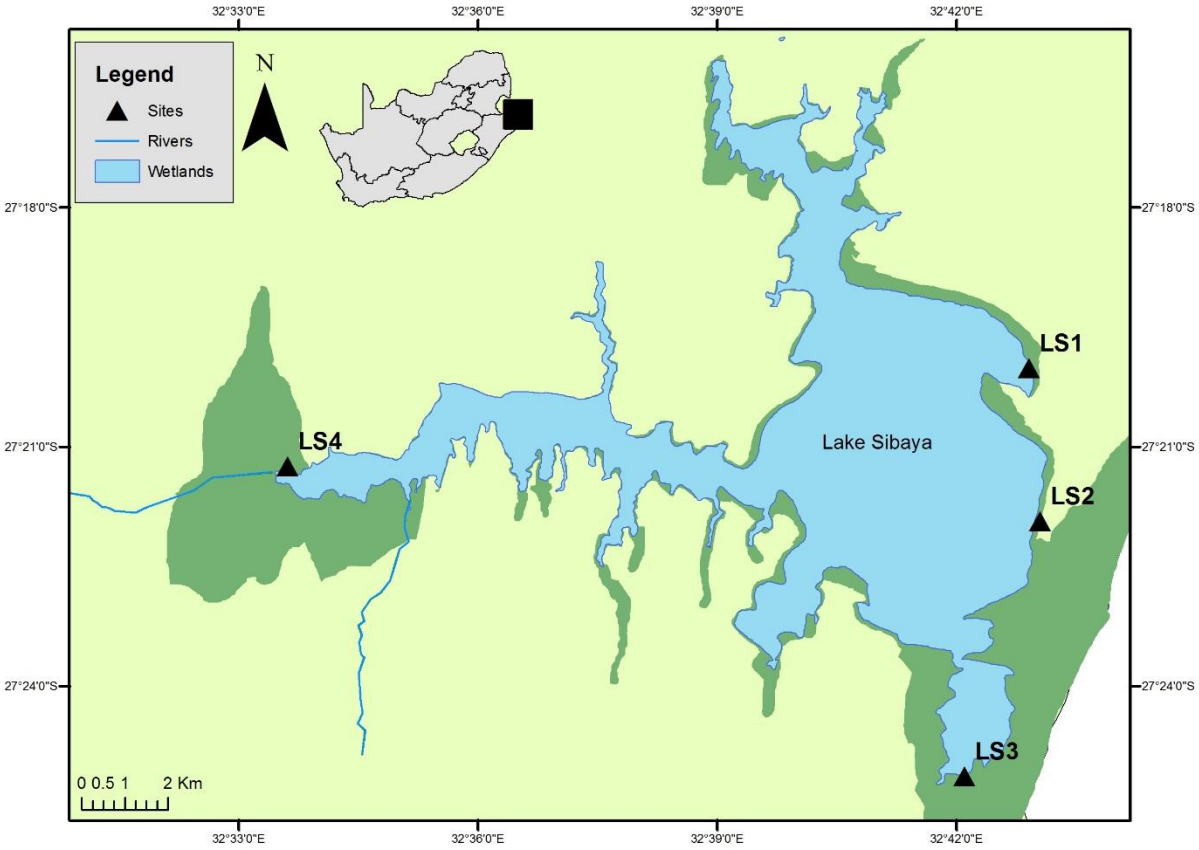

Figure. 2-1. Map of Lake Sibaya indicating where the lake and the sampling sites are situated. (LS = Lakes Sibaya) (Source: Dr. Wynand Malherbe).

2.1.2 Makuleke Wetlands

The Makuleke wetlands (Figure 2-2) are located in the Limpopo Province in the north-eastern corner of South Africa (geographical co-ordinates S22.4000, E31.1969), with Zimbabwe and Mozambique bordering the wetland (Deacon, 2007). They are situated in the northern Kruger National Park and are seen as the jewel of this area of the park (Hilton-Baber and Berger, 2007). The Makuleke Conservancy is classified as a sandveld environment and is distinguished by its diversity of plant and animal species, central African vegetation and alluvial flood plains (Hilton-Baber and Berger, 2007).

The Makuleke wetland system is classified as a floodplain vlei as it comprises of a grassy floodplain and a riverine area (Deacon, 2007). The floodplain is located between the Limpopo River (to the north) and Luvuvhu River (to the south) (Hilton-Baber and Berger, 2007). The floodplain, together with its pans, is important as it maintains the floodplain and riparian vegetation and also recharges the groundwater (Deacon, 2007). The Makuleke Wetlands are 7 757 ha in size and were designated as a Ramsar wetland on 22 May 2007 (Deacon, 2007).

Ten pans were selected in the Makuleke Wetlands for this study namely Banyini, Makwadzi, Hulukulu, Nhlangaluwe, Jachacha, Mapimbi, Gila, Reedbuck Vlei, Nwambi and Hapi (see Figure 2-2).

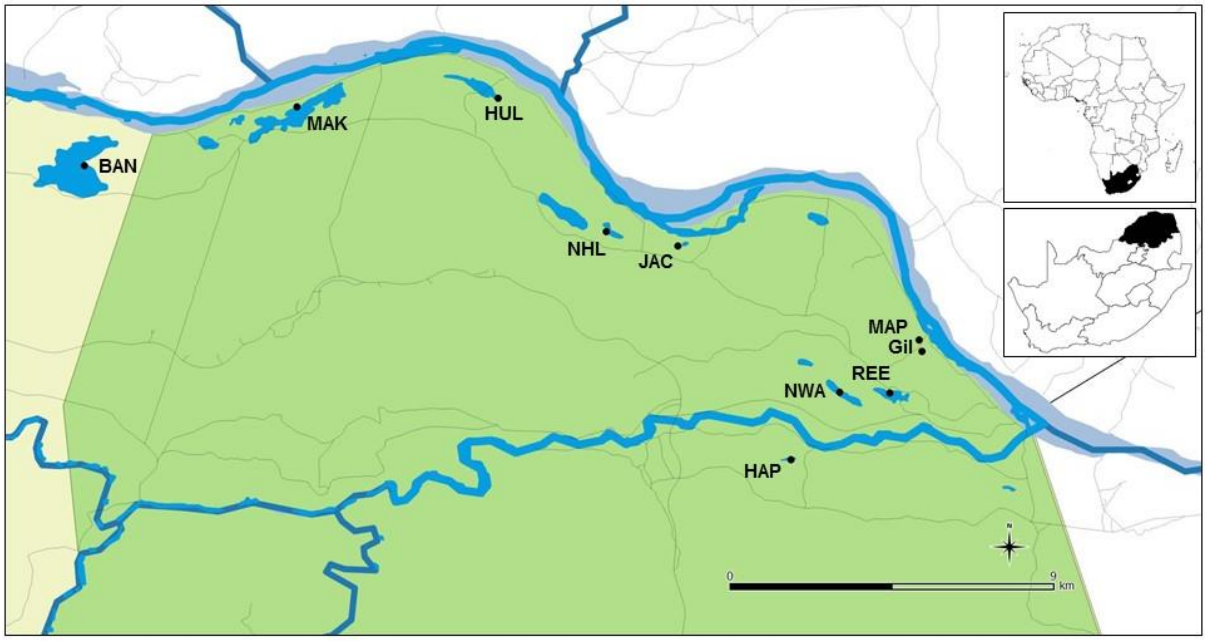

Figure. 2-2. Map of the Makuleke Wetlands within the Kruger National Park. Pans sampled are indicated. (Source: Kelly Shannon Dyamond). (BAN = Banyini Pan, MAK = Makwadzi pan, HUL = Hulukulu Pan, NHL = Nhlangaluwe Pan, JAC = Jachacha Pan, MAP = Mapimbi Pan, GIL = Gila Pan, REE = Reedbuck vlei Pan, NWA = Nwambi pan and HAP = Hapi Pan).

2.2 Water quality

At each study site water quality was measured using Extech DO610 (measures dissolved oxygen and temperature) and Extech EC610 (measures pH, total dissolved solids, salinity, electrical conductivity and temperature) probes. The *in situ* physical and chemical variables were measured before the sampling of diatoms was completed. Each meter was calibrated and cleaned before measuring the electrical conductivity, temperature, total dissolved solids, salinity, pH, oxygen saturation and oxygen concentration. Water samples (500 ml) were also collected, in honey jars, at each site, frozen and returned to the laboratory for nutrient analysis.

2.2.1 Laboratory analysis

Water samples brought back from the field were analysed using a Merck Spectroquant Pharo 300 UV-VIS Spectrophotometer and the relevant test kits. The following nutrients (with their kit method number) were analysed: alkalinity (1.11109.0001), ammonium (1.14752.0001), nitrate (1.09713.0001), nitrite (1.14776.0001), phosphates (1.14848.0001) and sulphates (1.14791.0001), using standard accredited methods according to their relevant test kits.

2.3 Diatoms

Diatom collection, preparation and analysis described in this section were according to the methods described in Taylor *et al.* (2005).

2.3.1 Locating diatoms in the field

Diatoms can be collected from various substrates in an aquatic ecosystem. Before diatoms can be sampled, it is important to identify which substrates are colonised by diatoms, as one of the most common errors when sampling diatoms is the sampling of un-colonised substrates (Taylor *et al.*, 2005; 2007). To detect diatom communities in the environment there are two methods, namely: by sight, where substrata are covered by a thin golden-brown layer; or by touch, when substrata feel slimy or mucilaginous.

Diatom communities occur in microhabitats (Taylor *et al.*, 2005) and they can colonise several different microhabitats from periphytic and planktonic to aerophilic habitats (John, 2012). From these habitats mentioned, most samples are generally taken from periphytic habitats which include rooted stems of vegetation, solid substrata (i.e. rocks, wood, etc.) and epipelon habitat (including damp exposed sediment) (Taylor *et al.*, 2005; John, 2012). These habitats are termed metaphyton (i.e. diatoms that are attached to submerged substrata or larger algae and water plants) (John, 2012). Diatoms can also colonise man-made objects that are found within the water body, such as plastic (Taylor *et al.*, 2005; 2007).

2.3.2 Diatom collection

Diatoms were sampled from Lake Sibaya during a winter (August 2015) and two summer (December 2015 and February 2016) seasons. Sampling from Lake Sibaya was dependent on substrate availability. Diatoms were sampled from Makuleke Wetlands during a dry and a wet season in April and September 2015 respectively. Sampling from pans in the Makuleke Wetlands was water level dependent. Diatoms were sampled preferably from submerged aquatic vegetation, but if no vegetation was available stones were used.

At each site, ten submerged stems (showing diatom growth), 10 – 15 cm in length, were retrieved and inserted into zip lock bags with 50 ml of water. The stems were rubbed vigorously in the bag in order to detach the diatoms from the stems. Stems were then removed and the water containing the diatom sample was transferred to collection jars and 70 % ethanol was added to preserve the sample at a final concentration not exceeding 20 %. Sampling methods were according to Taylor *et al.* (2005).

If vegetation was not available, stones were used with an approximate diameter of 15 – 25 cm. Five stones, showing diatom growth, were retrieved and a toothbrush was used to scrape the stone surface and remove the diatoms. The stones and toothbrush were rinsed with 50 ml sample site water into a tray. The sample was then transferred from the tray to collection jars and 70 % ethanol was added to preserve the sample at a final concentration not exceeding 20 %. Preserved samples were transported in a fridge to ensure the samples stayed cool.

2.3.3 Diatom slide preparation

The diatom microscope slides were prepared using the hot hydrochloric acid (HCl) and potassium permanganate ($KMnO_4$) method (Taylor *et al.*, 2005). Samples from South Africa typically have high levels of organic material, thus it was recommended by Taylor *et al.* (2005) to make use of this method as it produces consistent results.

On return to the laboratory the samples were left to settle for 24 hours after which the clear supernatant liquid was decanted. Test tubes were clearly marked with sample numbers and depending on the material concentration in the sample, 5 – 10 ml of the shaken sample was poured into the test tubes. Potassium permanganate (10 ml) was added to the test tubes, mixed and left to stand for 24 hours. After the samples changed colour from purple to brown, 5 – 10 ml of HCl (32 %) was added to the sample. The sample was boiled on a hot plate for 1 – 2 hours until the sample solution became transparent. One drop of hydrogen peroxide was added to the solution to ensure that no organic material remained in the solution.

The sample was transferred to 10 ml centrifuge tubes and topped up to the 10 ml mark with distilled water if necessary. Samples were centrifuged at 2500 rpm for a period of 10 minutes to rinse the sample. The supernatant was decanted and the pellet was resuspended in 10 ml distilled water for another wash cycle. This washing process was completed four times. After the fourth wash the supernatant was decanted and one drop of 10 % ammonium chloride (NH_4Cl) was added to the sample. The NH_4Cl neutralises the electrostatic charge between the diatoms to ensure there will be no aggregation as the material dries on the slide.

Round cover slips were cleaned with ethanol and ~ 1 ml of the dilute diatom suspension was pipetted onto the cover slip. On the following day (after the covers slips had dried) the cover slips were placed on a warm hotplate for 5 seconds to ensure all the excess NH_4Cl sublimated. While the cover slips and hotplate were allowed to cool down, the microscope slides were prepared by cleaning with 70 % ethanol and labelling each slide. The cover slips were placed on the hotplate, once it had reached ~ 90 ºC, with 1 – 2 drops of Pleurax (mounting media)

(Taylor *et al.*, 2005). Pleurax was used as it has a high refractive index (1.73; Taylor *et al.*, 2005). The microscope slide was then lowered, face down, onto the cover slip to allow the cover slip to attach to the slide. The microscope slide was then inverted and placed on the hotplate and left to allow the Pleurax to boil for a few minutes. The completed slide was subsequently removed and allowed to cool.

After the sample was collected and prepared, the microscope slide was viewed under a light microscope (LM). The slides contained diatoms that were in good condition, as well as diatoms that were fractured, broken and orientated at different angles (Taylor *et al.*, 2005; 2007). Under the microscope, diatoms were viewed in two different views namely the valve view and the girdle view (John, 2012). The face view of the diatom is the valve view and a side on view is the girdle view (John, 2012).

2.3.4 Diatom identification

The prepared microscope slides were viewed using a Nikon 80i compound light microscope equipped with differential interference contrast (DIC) and a 100x 1.4 N. A. oil immersion objective. Diatoms were identified using Taylor *et al.* (2007). After identification the diatom species were counted until a total number of ~ 400 diatom valves or the entire microscope plate were counted (Taylor *et al.*, 2005).

2.3.5 Diatom indices

For this study OMNIDIA version 5.3 (Lecointe *et al.*, 1993) was used to aid in the calculation of diatom indices. The indices used for this study included the Generic Diatom Index (GDI) (Coste and Ayphassorho, 1991), the Specific Pollution sensitivity Index (SPI) (CEMAGREF, 1982), the Trophic Diatom Index (TDI) (Kelly and Whitton, 1995) and the Percentage Pollution Tolerant Valves (%PTV) (Kelly and Whitton, 1995). For the GDI and SPI indices, a score is given between 0 – 20 to indicate the quality of the system. Table 2-1 indicates how each score should be interpreted in regards to the quality of the system. The TDI and %PTV are measured on a scale from 0 – 100. Interpretation of the TDI score is given in Table 2-2 whereas Table 2-3 indicates the interpretation of the %PTV score.

These indices were included for the following reasons (Matlala, 2010):

- GDI — the index base its final score on a diatom taxon's tolerance (at genus level) to pollution.
- SPI — this index includes the most number of species, with more than 1400 species included.

- TDI — this index classifies diatoms into five different sensitivity categories with regard to nutrient status.
- %PTV — the index is an illustration of the degree of organic pollution vs eutrophication as it indicates the diatoms in the community which are tolerant to pollution.

Table 2-1. Table used to interpret the Generic Diatom Index (GDI) and Specific Pollution sensitivity Index (SPI) and thus determine the quality and trophic level of the ecosystem.

Index Score (up to 20)	**Ecosystem quality**	**Trophic level**
> 17	High quality	Oligotrophic
15 – 17	Good quality	Oligo-mesotrophic
12 – 15	Moderate quality	Mesotrophic
9 – 12	Poor quality	Meso-eutrophic
< 9	Bad quality	Eutrophic

Table 2-2. Table used to interpret the Trophic Diatom Index (TDI) score for determination of the trophic level of the ecosystem.

Index Score	**Trophic level**
0 – 20	Oligotrophic
21 – 40	Oligo-mesotrophic
41 – 60	Mesotrophic
61 – 80	Meso-eutrophic
> 80	Eutrophic

Table 2-3. Table used to interpret the percentage Pollution Tolerant Valve (%PTV) for determination of the ecological status of the ecosystem.

Index Score	Ecological status
< 20	Site free from organic pollution
21 – 40	Some evidence of organic pollution
41 – 60	Organic pollution likely to contribute to eutrophication
> 61	Heavily contaminated with organic pollution

2.4 Statistical analysis

2.4.1 Univariate analyses

GraphPad Prism Version 5 was used to determine the average and standard error of the mean (SEM) for the water quality variables and the diatom index scores. A two-tailed Pearson correlation coefficient ($p < 0.05$) between diatom indices and environmental variables, as well as a one-way Anova test and Tukey's multiple comparison test, were performed to determine the response of the diatom indices to the environmental variables.

Omnidia were used to calculate the diatom indices, as well as to determine the number of species used to calculate each index. Primer Version 7 was used to calculate the Shannon diversity index, Margalef species richness, and Pielou's evenness score for each study site across the study. Simper analysis was performed to determine the dominant diatom species across seasons, as well as calculate if there were similarities or variances between dominant species in each season sampled.

2.4.2 Multivariate analyses

Canoco Version 5 was used to determine the temporal and spatial variation between water variables, sites sampled, and species identified. The variation was analysed through the use of an unconstrained principle components analysis (PCA), constrained redundancy analysis (RDA) and a constrained canonical correspondence analysis (CCA). The PCA technique was used to determine the influence of the environmental variables on the sampled sites. The RDA was used to determine the correlation between the environmental variables and the diatom indices. The technique determines the influence that the environmental variables have on the diatom indices. The CCA technique was used to determine the influence the environmental variables have on the diatom species. The temporal difference was determined through a non-metric multidimensional scaling (nMDS) plot and a Bray Curtis similarity matrix. This technique determines similarity between samples collected during different seasons.

Chapter 3 — Lake Sibaya

3.1 Site description

Lake Sibaya is situated 430 km north-east of Durban in Zululand on the Maputaland coastal plain (Allanson, 1979; Bruton, 1979; Ward and Kyle, 1990; Humphries and Benitez-Nelson, 2013; Stager *et al.*, 2013). It commences in Mtunzini (south of Richards Bay) where it broadens out northward into Mozambique and occupies almost half the width of Mozambique (Allanson, 1979). The western side of the lake is very flat making it difficult to define the boundary of the catchment (Ward and Kyle, 1990). High dune forest separates the eastern side of the lake with the ocean (Ward and Kyle, 1990; Combrick *et al.*, 2011; Humphries and Benitez-Nelson, 2013; Stager *et al.*, 2013). Lake Sibaya is classified as a coastal freshwater lake with a surface area of 60 – 70 km^2 and total catchment area of 530 km^2 (Bowen, 1979; Bruton, 1979; Ward and Kyle, 1990; Humphries and Benitez-Nelson, 2013; Stager *et al.*, 2013). The lake has a maximum depth of 43 m, with a maximum altitude of 20 m above sea level (Bowen, 1979; Bruton, 1979; Ward and Kyle, 1990; Combrick *et al.*, 2011; Humphries and Benitez-Nelson, 2013). Offshore marine canyons suggest that a large river once connected the lake to the sea (Ward and Kyle, 1990). This large river is possibly the Phongolo River which is now diverted northwards (Ward and Kyle, 1990).

There are five main regions (Figure 3-1) into which the lake is divided (Allanson, 1979; Bruton, 1979; Combrick *et al.*, 2011; DWS, 2015b), namely the: Main Basin (which contains the deepest water and compromises 56 % – 59 % of the lake's area); the Southwestern Basin and Southern Basin (compromises approximately 9 % of the lake area); and the Northern and Western Arms (extremely dendritic regions and compromising 12 – 20 % of the lake area).

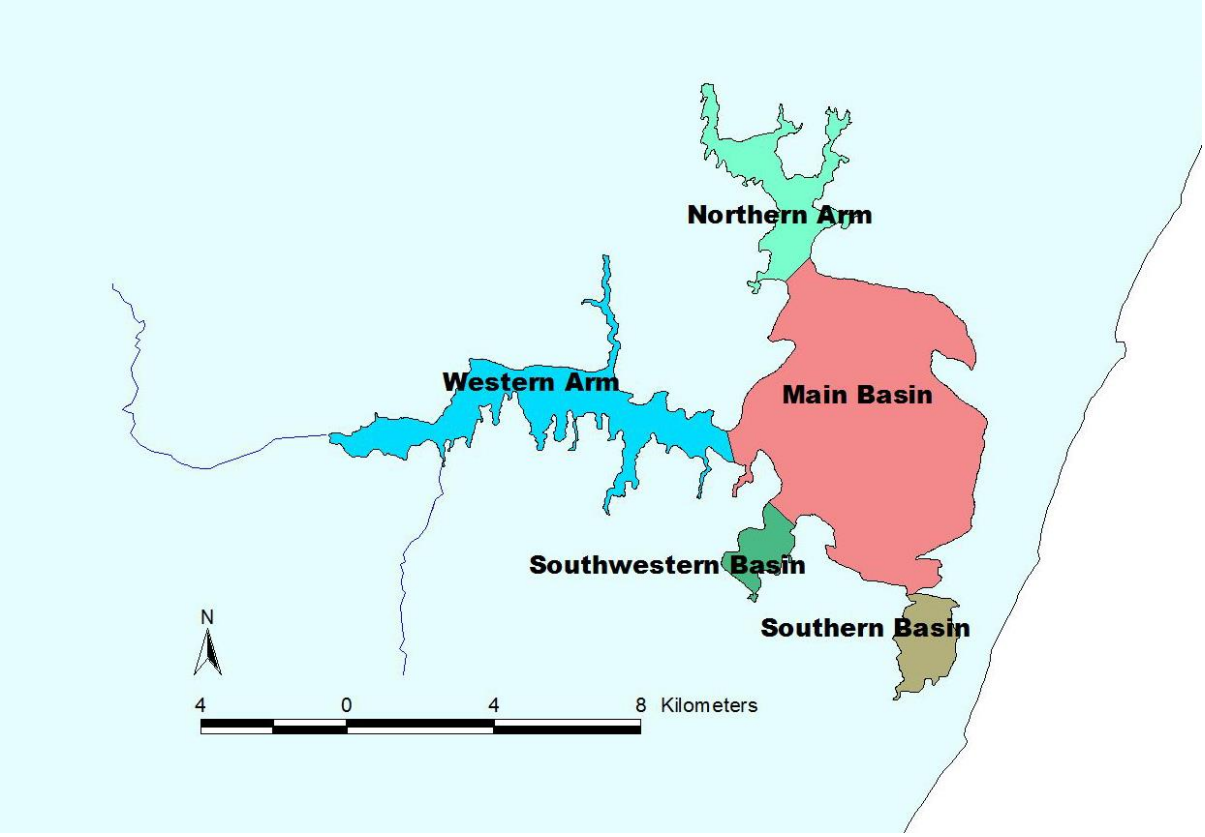

Figure 3-1. Map indicating the five regions of Lake Sibaya. (Source: DWS, 2015b).

3.1.1 Geology

The coastal plain of Lake Sibaya is made up of Tertiary and recent sand, with Cretaceous and Palaeocene sediments forming the main rock underlying the coastal plain (Allanson, 1979; Ward and Kyle, 1990; Humphries and Benitez-Nelson, 2013). The Cretaceous sediments, according to Allanson (1979), are overlaid with "relatively thin, discontinuous Tertiary shallow marine and beach deposits, with erosive unconformity". A variety of depositional environments are represented by Quaternary sediments, which postdates the Cretaceous and Palaeocene sediments (Allanson, 1979). Major physiographic elements are constituted by these environments which include Aeolian dune, lagoonal, shore zone, fluviatile and paludal (Allanson, 1979).

3.1.2 Hydrology

The seasonal mean annual precipitation of Lake Sibaya for July 2014 to June 2015 was 500 – 2000 mm and 100 – 200 mm for July 2015 to November 2015 (Appendix A, Figures A-1 and A-2) (SAWS, 2016) with an mean rainfall of 900 mm/yr (Humphries and Benitez-Nelson, 2013, Stager *et al.*, 2013). Furthermore, the mean annual precipitation decreases over the catchment in a westerly direction with 1200 mm in the south to only 700 mm in the west (Allanson, 1979; Humphries and Benitez-Nelson, 2013). Most of the rain in the area falls

between January and March, but rain occurs year round (Allanson, 1979). Directly on the surface of the lake the annual evaporation of 1420 mm/yr exceeds the annual precipitation (Allanson, 1979; Ward and Kyle, 1990). A small amount of water is lost to the sea through seepage (Allanson, 1979; Ward and Kyle, 1990). The western arm of the lake is fed by the Mseleni River (Stager *et al.*, 2013). The water budget is affected by groundwater due to the fact that recent and Tertiary sand, which are porous, make up the floor of the lake (Allanson, 1979; Humphries and Benitez-Nelson, 2013; Stager *et al.*, 2013). Although it has not yet been calculated, Allanson (1979) reported that there is groundwater inflow into the lake.

3.1.3 Vegetation

A dune forest is situated on the eastern side of the lake with 147 species found in the forest (Allanson, 1979; Ward and Kyle, 1990; Combrick *et al.*, 2011). The forested dunes are the coastal plains most striking feature as they attain a considerable height, even though they rarely exceed 1 km in width (Allanson, 1979). The dune forest reaches heights of 183 m in the St. Lucia vicinity, while Lake Sibaya's highest peak is 134 m (Allanson, 1979; Combrick *et al.*, 2011). The dune crest, in this area, was most probably fashioned by southerly winds when the sea level was lower and there was an expanse of sand on the ocean side (Allanson, 1979). However, the vegetation of the dune forest is mostly influenced by the north-easterly sea breezes (Allanson, 1979). This is due to salt spray, picked up from sea breezes, that is deposited on the vegetation (Allanson, 1979). This wind and sea salt influences the entire KwaZulu-Natal and Zululand vegetation resulting in deformed bushes and low-growing shrubs, all of which has a hedged or uniform canopy (Allanson, 1979). The species occupying the KwaZulu-Natal and Zululand regions are the same as at Lake Sibaya (Allanson, 1979). All these species bind to sand and are salt tolerant (Allanson, 1979).

Canopy, sub-canopy, shrub and herb layer, and coastline vegetation are the strata which comprises the dune forest (Allanson, 1979). The coastline vegetation (dominated by thicket, coastal forest and dry grassland) plays an important role and is found in relatively unstable sand on a narrow belt between the dune scrub and high tide level (Allanson, 1979; Stager *et al.*, 2013). The species that makes up the coastline vegetation are mostly widespread between KwaZulu-Natal and Zululand (Allanson, 1979).

3.2 Site selection

Four study sites were selected at Lake Sibaya namely Lake Sibaya 1 (LS1), Lake Sibaya 2 (LS2), Lake Sibaya 3 (LS3) and Lake Sibaya 4 (LS4). Sites were selected based on accessibility, availability of substrata, and representative of the wetlands. The sites were sampled over three surveys. The first survey in August 2015, the second in December 2015 and the third in February 2016. Figure 3-2 indicates the location of all four sites. Lake Sibaya 1 and 2 are located within the main basin of the lake on the eastern side. Lake Sibaya 3 is situated in the southern basin and Lake Sibaya 4 is situated in the western arm.

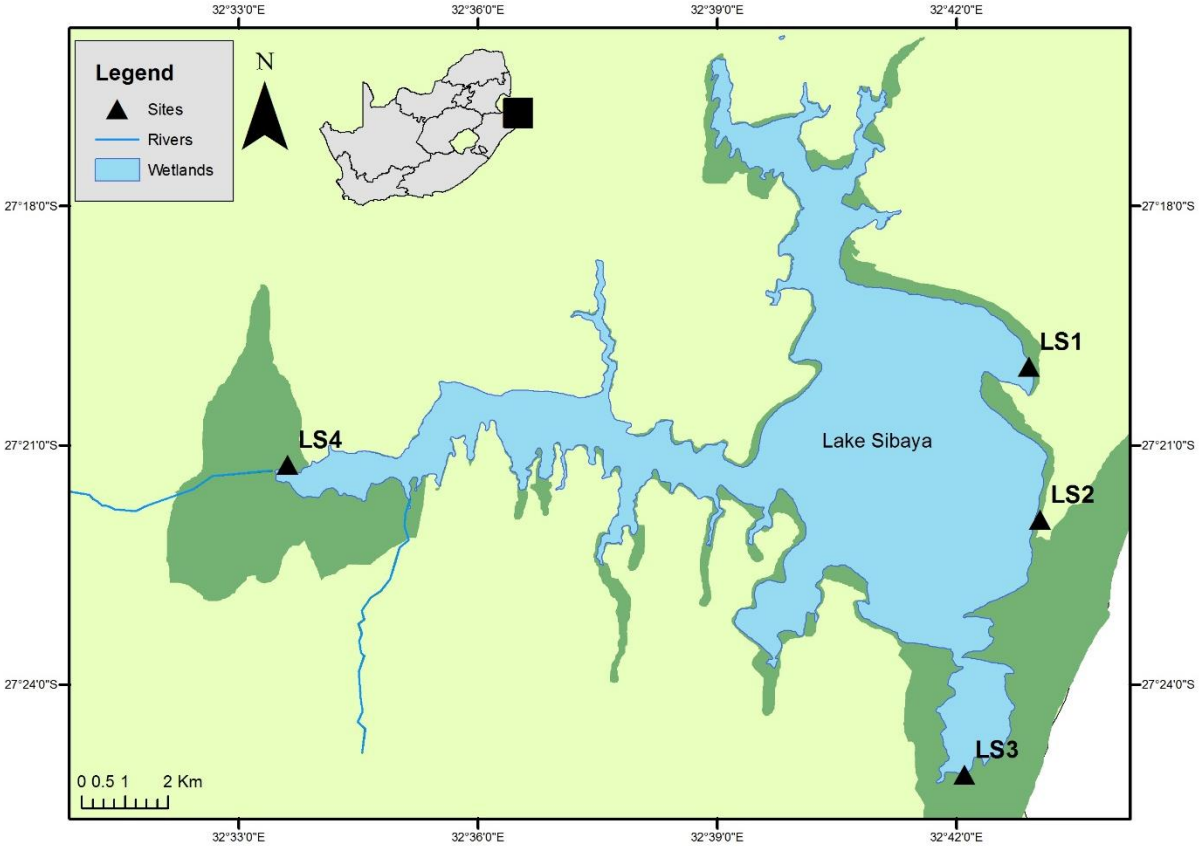

Figure 3-2. Map indicating the location of the four sites (LS 1 – 4) sampled at Lake Sibaya. (LS = Lake Sibaya) (Source: Dr. Wynand Malherbe).

3.2.1 Lake Sibaya 1 (LS 1)

Lake Sibaya 1 (Figure 3-3 A – B) is located at geographic coordinates S27.33361, E32.71512. The site, LS 1, is located in the north-east corner of the main basin. Grasslands surround the site, with tall reeds growing within the sampling site. The site is approximately 3.6 km from the ocean. During all three surveys flamingos and other water birds were present at the site. Sandy sediments are present at the site (Allanson, 1979; Humphries and Benitez-Nelson, 2013).

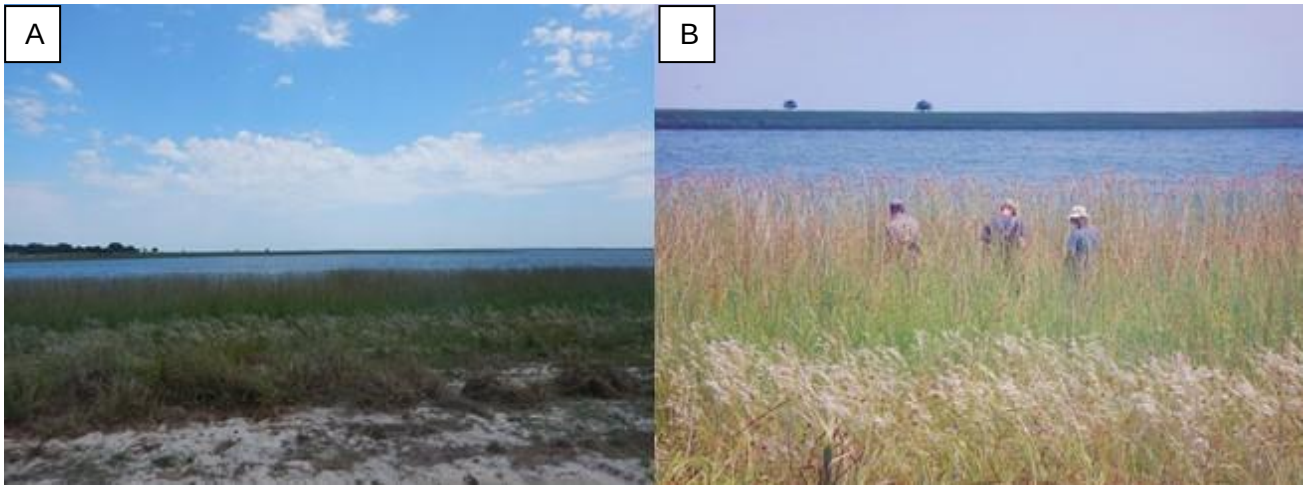

Figure 3-3. Lake Sibaya 1 during the third survey in February 2016 (A – B) (Photo credit: Serita van der Wal).

3.2.2 Lake Sibaya 2 (LS 2)

Lake Sibaya 2 (Figure 3-4 A – D) is located at geographic coordinates S27.36564, E32.71747. The site is located in the middle of the main basin on the eastern side. A dune forest is situated between the site and the ocean, with sedges (beach vegetation) between the sampling site and the dune forest. The site is approximately 2 km from the ocean. Sandy sediments are present at the site (Allanson, 1979; Humphries and Benitez-Nelson, 2013). This site could only be sampled once due to a lack of substrata during the last two surveys. Drift wood was sampled during the first survey. The site appeared undisturbed with no clear sign of human or animal interference.

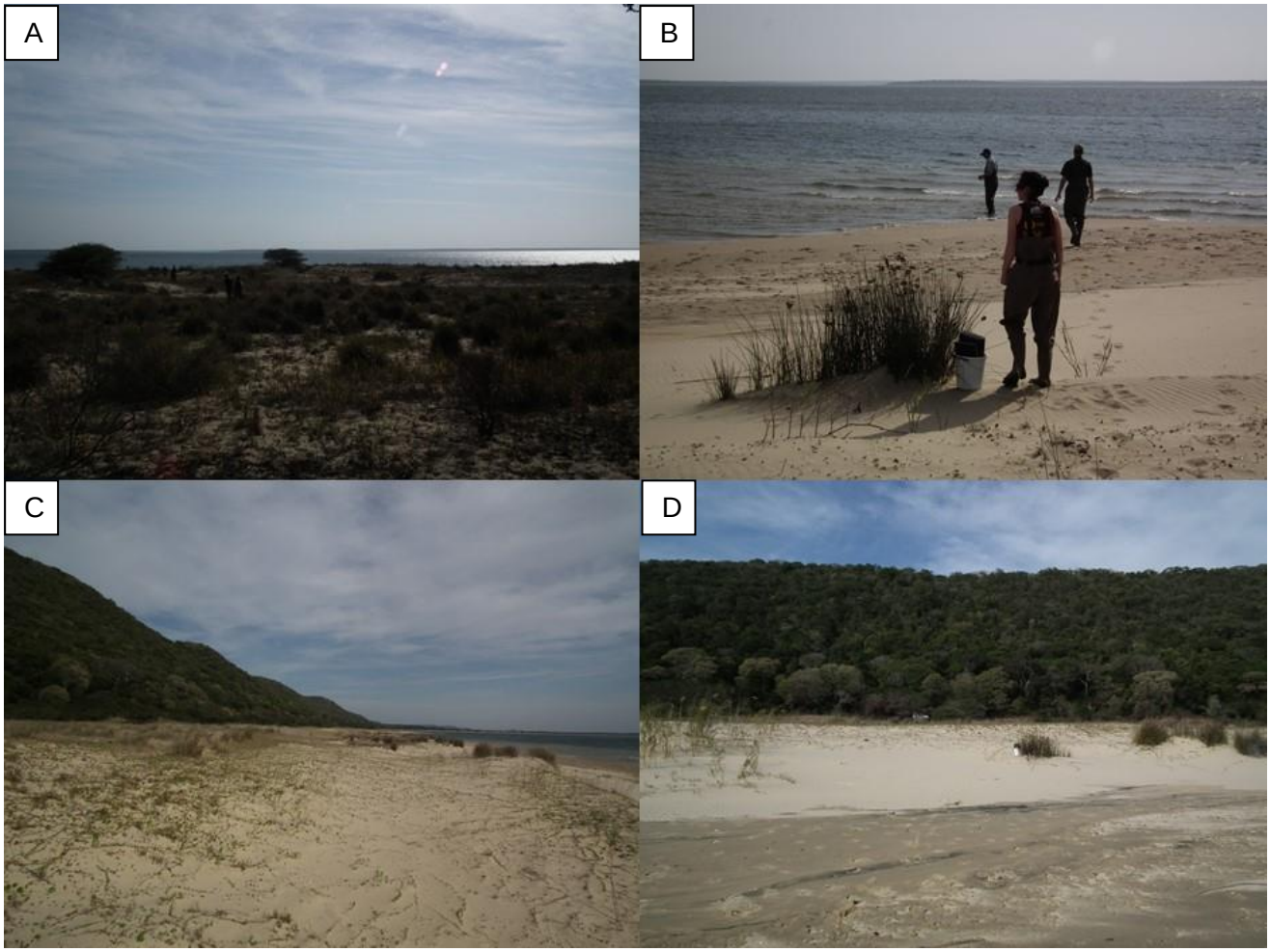

Figure 3-4. Lake Sibaya 2 during the first survey in August 2015 (A – D). (Photo credit: Dr. Wynand Malherbe).

3.2.3 Lake Sibaya 3 (LS 3)

Lake Sibaya 3 (Figure 3-5 A – D) is located at geographic coordinates S27.41886, E32.70162. The site is in the southern basin of the lake and a dune forest is situated between the site and the ocean. The site is approximately 1.7 km from the ocean. Sandy sediments are present at the site (Allanson, 1979; Humphries and Benitez-Nelson, 2013). The beach around the site was open, with patches of sedges. Cattle were observed on the shoreline close to the sampling site, with the shoreline covered in cattle faeces. Submerged water plants were present at the site and sampled during all three surveys. According to DWS (2015b) the southern basin has been influenced by increased rural development and forestry.

Figure 3-5. Lake Sibaya 3 during the first survey in August 2015 (A – D). (Photo credit: Dr. Wynand Malherbe).

3.2.4 Lake Sibaya 4 (LS 4)

Lake Sibaya 4 (Figure 3-6 A – D) is located at geographic coordinates S27.35416, E32.56028. The site is in the western arm of the lake. The site was surrounded by grass fields with tall reeds. The site is approximately 17.3 km from the ocean. Sandy sediments were present at the site (Allanson, 1979; Humphries and Benitez-Nelson, 2013). Water birds were observed at the site during sampling as well as cattle. The site is situated close to a rural community with the community making use of the lake for drinking water, washing clothes, as well as drinking water for cattle and other animals (Humphries and Benitez-Nelson, 2013). A strong sewage smell was present at the site. According to DWS (2015b) the western arm has been influenced by increased rural development and forestry.

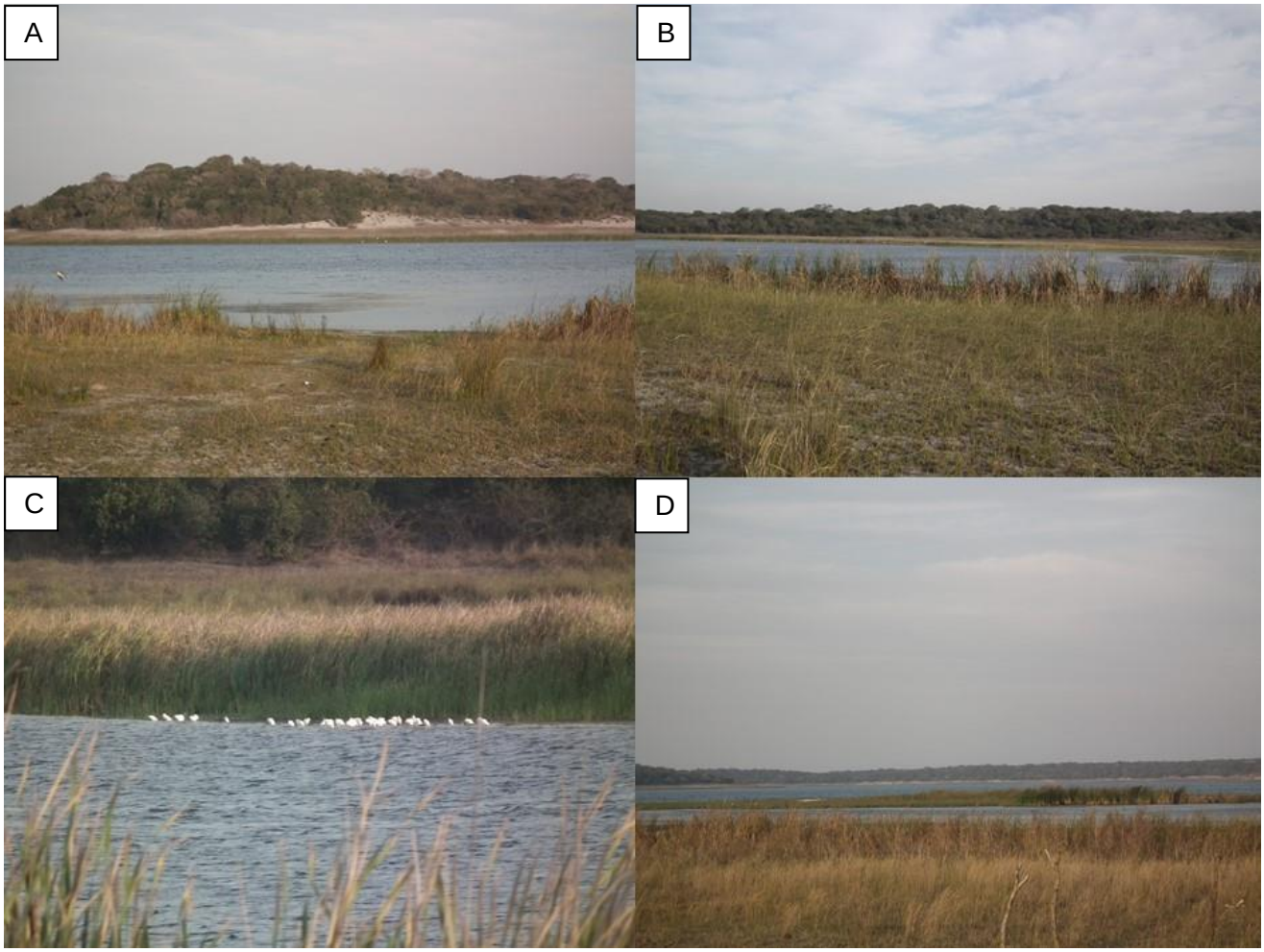

Figure 3-6. Lake Sibaya 4 during the first survey in August 2015 (A – D). (Photo credit: Dr. Wynand Malherbe).

3.3 Results

Water and diatoms samples from all four sites, over three surveys were collected based on the methodology explained in Chapter 2. The following results use an abbreviation of the sites and surveys sampled, i.e. Lake Sibaya 1 survey 2 will be presented as LS 1.2.

3.3.1 Water quality

The water quality results are presented as an average between the surveys for each site for each water quality variable. A full table of water quality results can be found in Appendix B (Table B-1).

3.3.1.1 Nutrients

Nutrients analysed included nitrates (NO_3), nitrites (NO_2), phosphates (PO_4) and ammonium (NH_4). The results obtained for nutrients for the four sites are discussed below. Figure 3-7 (A – D) illustrates the results for NO_3, NO_2, PO_4 and NH_4 in mg/L.

From Figure 3-7 (B) it can be noted that LS 4 had the highest NO_2 concentration in comparison to the other sites, with LS 2 having the highest NO_3 concentration (Figure 3-7 A). There were no significant differences ($p < 0.05$) (one-way ANOVA) between the means of the sites for both NO_2 and NO_3.

Lake Sibaya 1 had the highest PO_4 concentrations and LS 4 the lowest concentration (Figure 3-7 B). There were no significant differences ($p < 0.05$) between the means of this variable at the sites.

Figure 3-7 (D) illustrates the mean NH_4 values for the four sites. It can be observed that LS 4 had the highest NH_4 concentration with LS 2 having the lowest NH_4 concentration. There were significant differences ($p < 0.05$) between the means of LS 2 and LS 4 as well as LS 3 and LS 4.

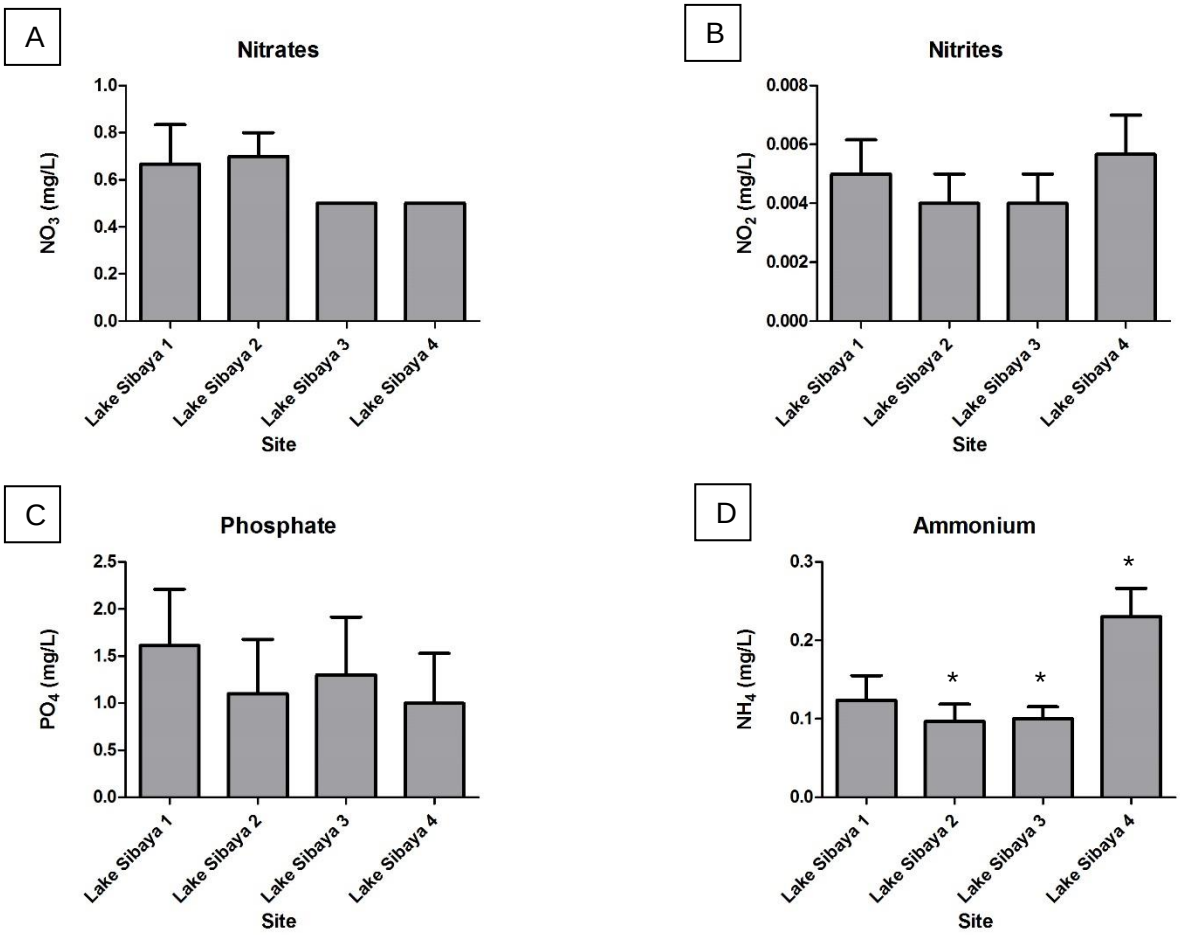

Figure 3-7. A column graph illustrating the mean and standard error of the mean (SEM) for (A) nitrate, (B) nitrite, (C) phosphate and (D) ammonium concentrations (in mg/L) for the four sites over three surveys at Lake Sibaya.

3.3.1.2 Temperature, pH and percentage oxygen concentration

Results for temperature, pH and percentage oxygen concentration (DO) are presented in Figure 3-8 (A – C). The results in Figure 3-8 (A) show that the temperatures were consistent between the four sites. Lake Sibaya 2 had the highest temperature of the four sites. There were no significant differences ($p < 0.05$) between the means of the four sites.

It can be noted from Figure 3-8 (B) that LS 4 had the highest average pH level with LS 2 and 3 having the lowest values. Lake Sibaya 4 had significant differences ($p < 0.05$) with LS 1, LS 2 and LS 3.

From Figure 3-8 (C) it can be noted that LS 1 had the highest percentage of dissolved oxygen saturation with LS 4 having the lowest percentage dissolved oxygen saturation. There were significant differences ($p < 0.05$) between the means of LS 1 and LS 2, LS 1 and LS 3, LS 1

and LS 4, LS 2 and LS 4 and LS 3 and LS 4; however the difference between LS1 and LS4 was approximately 20 %.

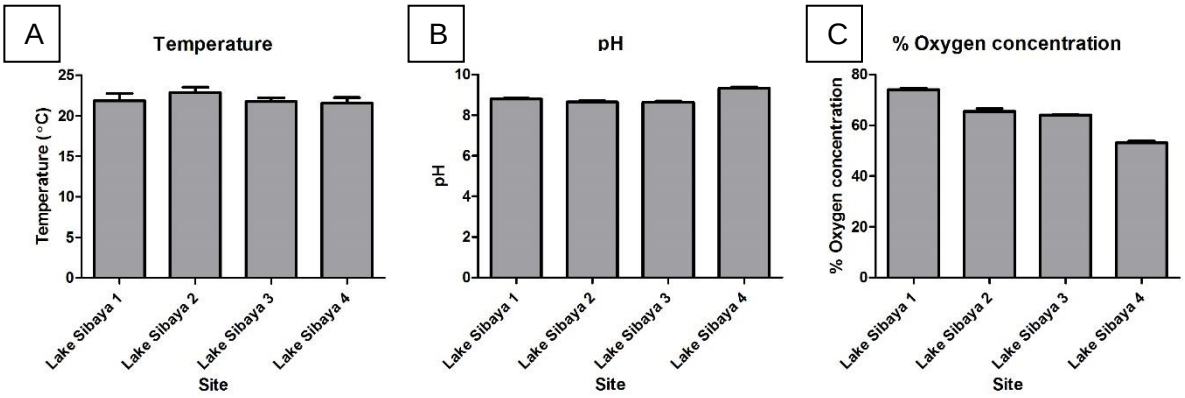

Figure 3-8. A column graph illustrating the mean and standard error of the mean (SEM) for (A) temperature (°C), (B) pH and (C) oxygen concentration (%) for the four sites over three surveys at Lake Sibaya.

3.3.2 Diatoms

The diatom data will be presented in terms of their diversity and abundance and a range of univariate and multivariate statistical analyses will be used in the following sections.

In Table 3-1 a complete species list is provided of all the species sampled in Lake Sibaya over the three surveys undertaken in 2015 and 2016. Species marked in bold are dominant species that contributed the highest (55 % – 100 % weight) to the CCA (Figure 3-15) results. Diatom counts for the selected pans are presented in Appendix C (Table C-1) with figures showing some identified diatom species are presented in Appendix D.

Table 3-1. Diatom species present in the Lake Sibaya system during the three surveys in 2015 and 2016.

Species	**Abbreviation**	**Date described**
Amphora sp. Ehrenberg	AMPH	1844
Amphora veneta Kützing	AVEN	1844
Amphora lacustris R.E.M. Archibald	-	2006
Anomoeoneis sphaerophora (Ehrenberg) Pfitzer	ASPH	1871
Anorthoneis sp. A. Grunow	ANOT	1868
Aulacoseira granulata (Ehrenberg) Simonsen	AUGR	1979
Aulacoseira muzzanensis (Meister) Krammer	AMUZ	1991
Cocconeis pediculus Ehrenberg	CPED	1838
***Cocconeis placentula* (Ehrenberg) Grunow**	CPTG	1884
Cocconeis sp. Ehrenberg	COCO	1837
Craticula sp. Grunow	CRAT	1868

Cymbella cymbiformis Agardh	CCYM	1830
Diploneis sp. Ehrenberg	DIPL	1894
Diploneis ovalis (Hilse) Cleve	DOVA	1891
Diploneis zanzibarica (Grunow) Hustedt	-	1937
Discostella pseudostelligera (Hustedt) Houk and Klee	DPST	2004
Encyonema minutum (Hilse.) D.G. Mann	ENMI	1990
Encyonema sp. Kützing	ENCY	1834
Encyonopsis sp. Krammer	ENCP	1997
Encyonopsis minuta Krammer and Reichardt	ECPM	1997
Encyonopsis subminuta Krammer and Reichardt	ESUM	1997
***Epithemia adnata* (Kützing) Brébisson**	EADN	1838
Epithemia sorex Kützing	ESOR	1844
Fragilaria sp. Lyngbye	FRAG	1819
Fragilaria ulna Lange-Bertalot	FUBI	1980
***Gomphonema* sp. Ehrenberg**	GOMP	1832
***Gomphonema insigne* Gregory**	GINS	1856
Gomphonema parvulum Kützing	GPAR	1849
***Gomphonema pseudoaugur* Lange-Bertalot**	GPSA	1979
Gomphonema sp. 2 Ehrenberg	GOMS	1832
Gomphonema sp. 3 Ehrenberg	GOPS	1832
Gyrosigma acuminatum (Kützing) Rabenhorst	GYAC	1853
Hantzschia distinctepunctata Hustedt	HDIS	1921
Karayevia ploenensis (Hustedt) Bukhtiyarova	-	1999
Mastogloia smithii Thwaites	MSMI	1848
***Mastogloia* sp. 1 Thwaites**	MAST	1848
***Mastogloia* sp. 2 Thwaites**	MASP	1848
***Mastogloia* sp. 3 Thwaites**	MASS	1848
Navicula sp. Bory	NAVI	1822
Navicula cryptotenelloides Lange-Bertalot	NCTO	1993
Navicula interruptestriata Schwabe and Simonsen	NITS	1961
Navicula radiosa Kützing	NRAD	1844
Navicula zanoni Hustedt	NZAN	1949
Navicymbula pusilla Krammer	NCPU	2003
***Nitzschia* sp. Hassall**	NITZ	1845
Pinnularia sp. C.G. Ehrenberg	PINU	1843
Pinnularia subcapitata Gregory	PSEL	1992
Placoneis sp. C. Mereschkowsky	PLAC	1903
Placoneis placentula (Ehrenberg) Heinzerling	PPLC	1908
Rhopalodia sp. O. Müller	RHOP	1895
Rhopalodia gibberula (Ehrenberg) O.Müller	RGBL	1895
Rhopalodia musculus (Kützing) O.Müller	RMUS	1899
Sellaphora sp. Mereschkowsky	SELL	1902
Sellaphora sp. 2 Mereschkowsky	SELS	1902
Sellaphora pupula (Kützing) Mereschkowksy	SPUP	1902

Seminavis sp. D.G. Mann	SMNA	1990
Seminavis strigosa (Hustedt) Danieledis and Economou-Amilli	SMST	2003
Tabularia fasciculata (Agardh) Williams and Round	TFAS	1986
Tryblionella apiculata Gregory	TAPI	1857

The software package Primer Version 7 was used to determine dominant species identified for each survey based on a 70 % contribution. Dominant species identified during the winter (August) survey included *Cocconeis placentula* (23.94 %)*, Epithemia adnata* (18.48 %)*, Gomphonema* sp. (10.91 %), *Gomphonema insigne* (10.39 %) and *Mastogloia* sp. (7.62 %). Dominant species identified during the summer (December) survey included *Gomphonema* sp. (19.88 %), *Navicula interruptestriata* (17.65 %), *Tabularia fasciculata* (11.7 %), *Fragilaria ulna* (9 %)*, Gomphonema pseudoaugur* (7.44 %) and *Anorthoneis* sp. (6 %). Dominant species identified during the summer (February) survey included *Gomphonema pseudoaugur* (21.76 %)*, Cocconeis placentula* (19.54 %)*, Epithemia adnata* (9.95 %), *Seminavis* sp. (7.55 %), *Gomphonema* sp. (7.22 %) and *Epithemia sorex* (6.39 %)*.*

3.3.2.1 Diatom indices

The diatom indices were calculated using the software package Omnidia (Lecointe *et al.*, 1993) for the four sites in order to determine water quality conditions for Lake Sibaya based on the structure of the diatom community. Table 3-2, Table 3-3 and Table 3-4 indicates the interpretation for the index scores with the index scores for each site provided in Table 3-5.

Table 3-2. Table used to interpret the Generic Diatom Index (GDI) and Specific Pollution sensitivity Index (SPI) score in order to determine the ecosystem quality and trophic level of the ecosystem.

Index Score (up to 20)	Ecosystem quality	Trophic level
> 17	High quality	Oligotrophic
15 – 17	Good quality	Oligo-mesotrophic
12 – 15	Moderate quality	Mesotrophic
9 – 12	Poor quality	Meso-eutrophic
< 9	Bad quality	Eutrophic

Table 3-3. Table used to interpret the Trophic Diatom Index (TDI) score to determine the trophic level of the ecosystem.

Index Score	Trophic level
0 – 20	Oligotrophic
21 – 40	Oligo-mesotrophic
41 – 60	Mesotrophic
61 – 80	Meso-eutrophic
> 80	Eutrophic

Table 3-4. Table used to interpret the percentage Pollution Tolerant Valves (%PTV) to determine the ecological status of the ecosystem.

Index Score	Ecological status
< 20	Site free from organic pollution
21 – 40	Some evidence of organic pollution
41 – 60	Organic pollution likely to contribute to eutrophication
> 61	Heavily contaminated with organic pollution

It can be noted from Table 3-5 that LS 4.1 had the highest SPI score and LS 4.2 had the lowest SPI score. Sites LS 1.1 and LS 1.3 scores indicated the site had poor water quality while the score at LS 1.2 indicated a bad quality (Table 3-2). Lake Sibaya 2 (winter) is classified as being of moderate quality. Site LS 3.1 diatom results indicated the site to be of moderate quality with the other two surveys indicating poor quality. All three surveys at LS 4 differed from each other with the first survey having moderate quality, the second survey having bad quality while the third survey had poor quality. The average SPI scores (Figure 3-9 A) were above ten for all the sites except for LS 1 where a score of less than 10 was obtained. Lake Sibaya 2 had the highest SPI score.

The GDI scores from Table 3-5 showed that LS 4.1 had the highest score with LS 2.1 having the lowest score. For LS 1 and LS 3 all three surveys indicated the site had moderate quality while LS 2 was in the poor category (Table 3-2). All three surveys at LS 4 were classified in different categories with the first survey showing the site was of good quality. The second survey indicated LS 4.2 was of poor quality and the third survey indicated the site was in the moderate category. The average GDI scores (Figure 3-9 B) for all sites were higher than 10 with LS 4 having the highest GDI score.

Table 3-5. Specific Pollution sensitivity Index (SPI), Generic Diatom Index (GDI), Trophic Diatom Index (TDI) and percentage Pollution Tolerant Valve (%PTV) scores for each site over the three surveys in 2015 and 2016.

	Indices			
Sites	**Specific Pollution sensitivity Index (SPI)**	**Generic Diatom Index (GDI)**	**UK Trophic Diatom Index (TDI)**	**Percentage Pollution Tolerant Valve (%PTV)**
LS 1.1	9.6	14.9	59	1.7
LS 1.2	8.2	12.8	52.5	0
LS 1.3	10.3	12.5	54.5	0
LS 2.1	12.4	10.9	57.5	0
LS 3.1	12.9	13.6	67.5	0
LS 3.2	11.1	13.4	78	1.6
LS 3.3	11	12.4	63.5	0
LS 4.1	13.5	16.3	70.5	0
LS 4.2	7.4	11.9	52.5	0
LS 4.3	10.2	13.5	62.5	1.9

The TDI values in Table 3-5 shows that all the sites over all three surveys had scores higher than 50. A score of 41 – 60 is an indication of mesotrophic conditions and a score of 61 – 80 is an indication of meso-eutrophic conditions (Table 3-3). Lake Sibaya 1.2 and LS 4.2 had the lowest scores (52.5) and LS 3.2 had the highest score (78). Lake Sibaya 1.1, LS 1.2, LS 1.3, LS 2.1 and LS 4.2 scores indicated mesotrophic conditions and LS 3.1, LS 3.2, LS 3.3, LS 4.1 and LS 4.3 scores indicated meso-eutrophic conditions (Table 3-3). The average TDI score (Figure 3-9 C) for the four different sites was higher than 50 with LS 3 having the highest TDI score. The TDI index correlates with the SPI and GDI indices and indicates the system as nutrient enriched.

The %PTV values in Table 3-5 and Figure 3-9 (D) indicates all sites over all three surveys had percentages lower than 20. A score < 20 indicates that the system did not have organic pollution and nutrients contributed highest to the TDI index (Table 3-4) (Kelly and Whitton, 1995).

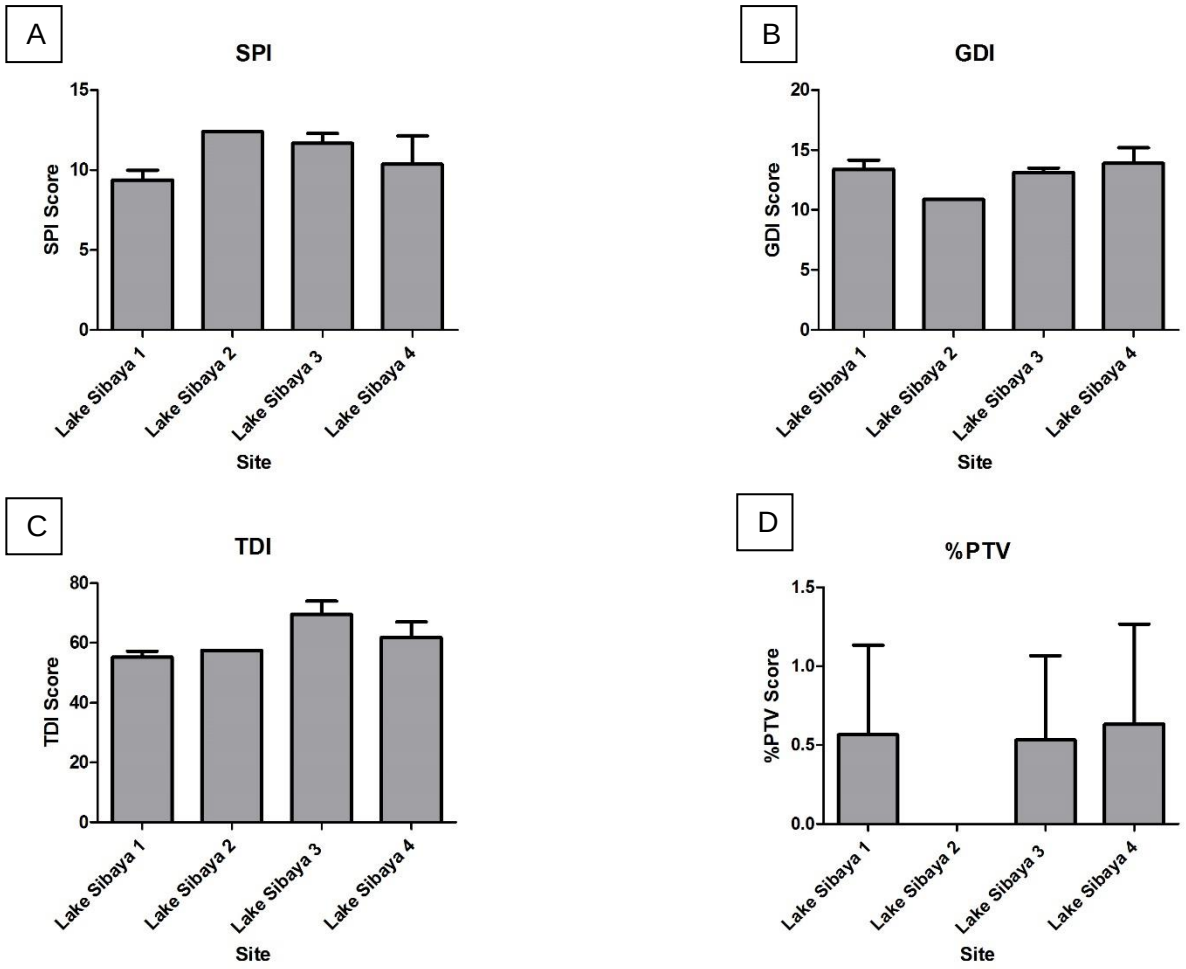

Figure 3-9. Column graph illustrating the average score for (A) Specific Pollution sensitivity Index (SPI), (B) Generic Diatom Index (GDI), (C) Trophic Diatom Index (TDI) and (D) percentage Pollution Tolerant Valve (%PTV) for the four sites in Lake Sibaya for three surveys in 2015 and 2016.

3.3.2.2 Correlation between diatom indices and water quality variables

Table 3-6 provides the correlation results between the diatom indices and the measured water quality variables. Values in bold had a significant ($p < 0.05$) (one-way ANOVA) correlation. It can be noted that the SPI index had a negative correlation with all the variables except temperature. The GDI index had a negative correlation with all the variables except for pH and nitrate. The TDI index had a negative correlation with all the variables except for temperature. However, the opposite was expected as the TDI should have a positive correlation with water quality variables.

Table 3-6. Correlation between diatom indices and water quality variables. Values in bold had a significant ($p < 0.05$) correlation.

	SPI	GDI	TDI
PO_4	**-0.43**	**-0.18**	**-0.05**
NO_3	**-0.24**	**0.38**	**-0.15**
NO_2	**-0.47**	**-0.21**	**-0.47**
Percentage dissolved oxygen	**-0.16**	**-0.18**	**-0.26**
pH	-0.10	0.45	-0.11
Temperature	**0.43**	**-0.11**	**0.02**

3.3.2.3 Diatom diversity indices

Figure 3-10 (A – D) shows the diversity, evenness and richness indices for the four sites over the three surveys at Lake Sibaya. It can be noted that the Pielou's evenness index (Figure 3-10 C) were similar across all sites and surveys, with LS 1.1 having the lowest Pielou's evenness score. However, all of the scores indicate that no dominance by any taxa was present. The Shannon diversity (Figure 3-10 D) between sites was similar across the sites and surveys indicating similar diversity; however, total species (Figure 3-10 A) recorded at each site and survey ranged from 12 to 20 species. The Margalef species richness (Figure 3-10 B) was similar across sites and surveys with LS 4.1 having the lowest richness and LS 4.3 the highest species richness.

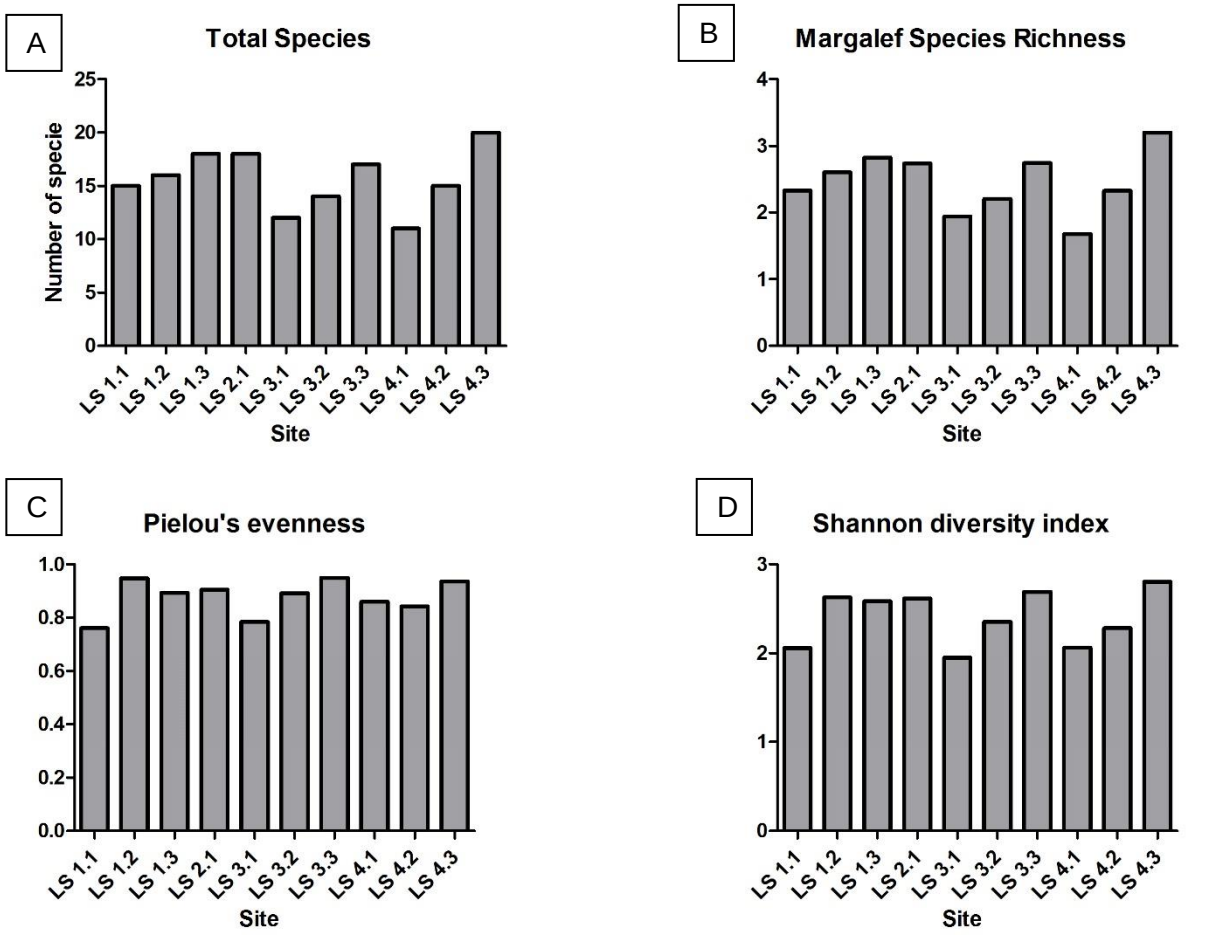

Figure 3-10. Column graphs indicating (A) the total number of species per season, (B) Margalef species richness, (C) Pielou's evenness and (D) Shannon diversity index.

3.3.2.4 Spatial and temporal variation

A non-metric multidimensional scaling (nMDS) plot based on the Bray Curtis similarity matrix (Figure 3-11) and a hierarchical cluster (Figure 3-12) were constructed for the sites sampled over the three surveys. The nMDS plot showed seasonal differences between sites with the sites sampled during the summer season being grouped together. The hierarchical cluster showed a 40 % similarity between the sites sampled in the summer season. During the December and February (summer) surveys there was similarity between the samples. There was no similarity between the winter (August) and summer surveys. It was also noted that the winter samples did not group together showing variation between winter samples.

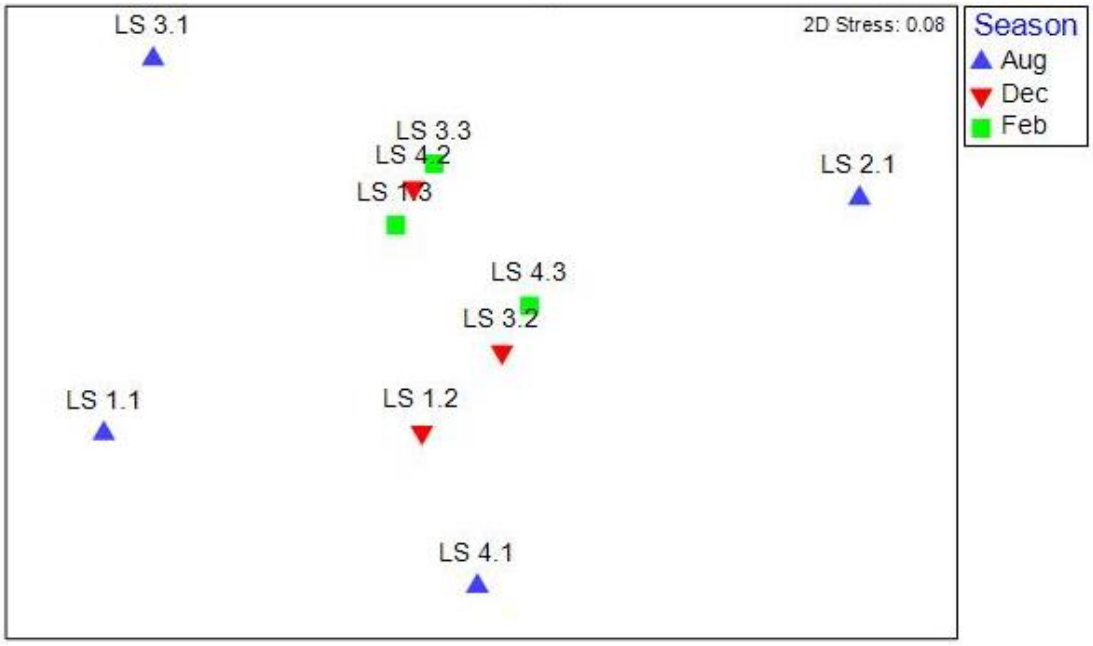

Figure 3-11. Non-metric multidimensional scaling (nMDS) showing the Bray-Curtis similarity between seasonal samples.

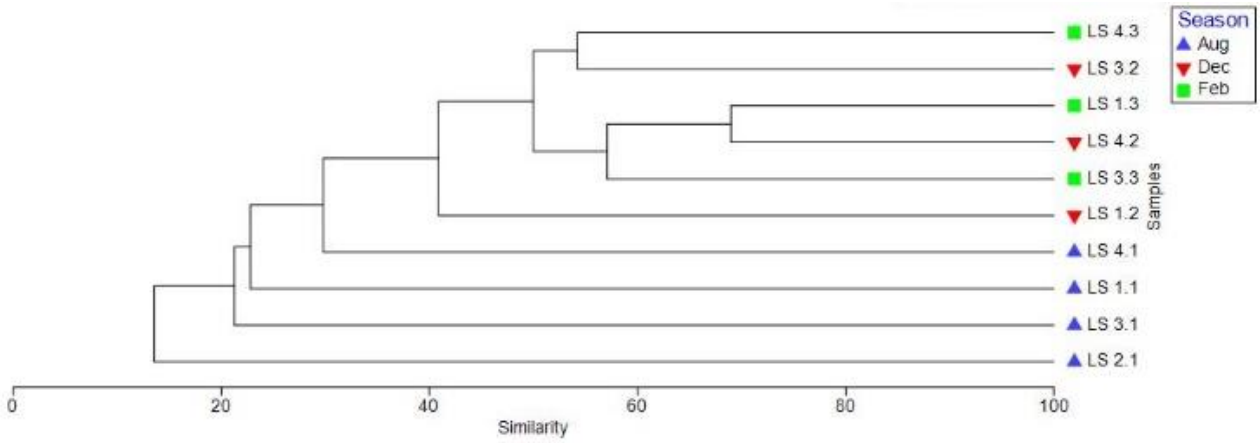

Figure 3-12. Hierarchical cluster showing similarity between seasonal samples.

The influence between water quality and the sampling sites based on the diatom community can be seen in Figure 3-13. It can be noted that LS 1.2, LS 1.3 and LS 3.2 were influenced by nitrates, nitrites and dissolved oxygen. Temperature influenced the sites located in the bottom left quadrant (sites LS 1.1, LS 2.1, LS 3.1, LS 2.3 and LS 3.3). Ammonium concentrations were linked with LS 4.3 and LS 2.2. The first axis explains 82.7 % of the variation and 8.8 % of the variation is explained by axis two. There is a positive correlation between the phosphates, nitrates, nitrites and percentage oxygen. The temperature and ammonium had a negative correlation with one another and the pH had a negative correlation with the phosphates, nitrates, nitrites and percentage oxygen.

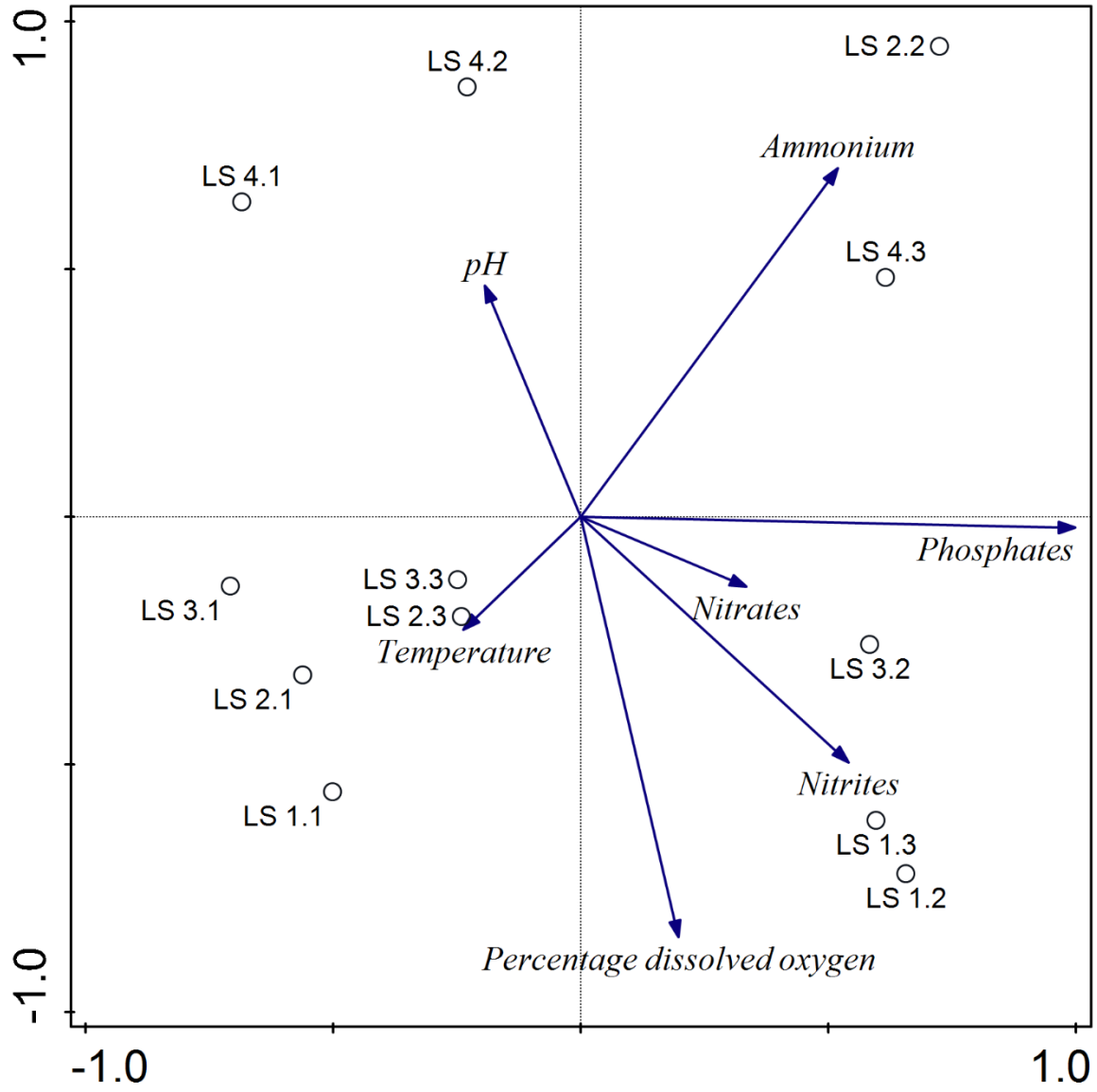

Figure 3-13. Principle components analysis (PCA) biplot illustrating the relationship between water quality and sites sampled.

In Figure 3-14 the correlation between water quality and diatom indices is illustrated using redundancy analysis (RDA). The SPI, GDI and TDI had a negative correlation with the phosphate, ammonium, nitrate, nitrite and dissolved oxygen. However, a Monte Carlo permutation test indicated that there was no significance in the plot ($p = 0.438$). The first axis explained 34.14 % of the variation and the second axis explained 35.18 % of the variation. The total explanatory variables accounted for 35.7 %. This illustrates that the indices were influenced by the water variables, as an increase in the water variables will cause a decrease in the index scores. This negative correlation was expected for the SPI and GDI but not the TDI as there should be a positive correlation between the TDI and nutrients.

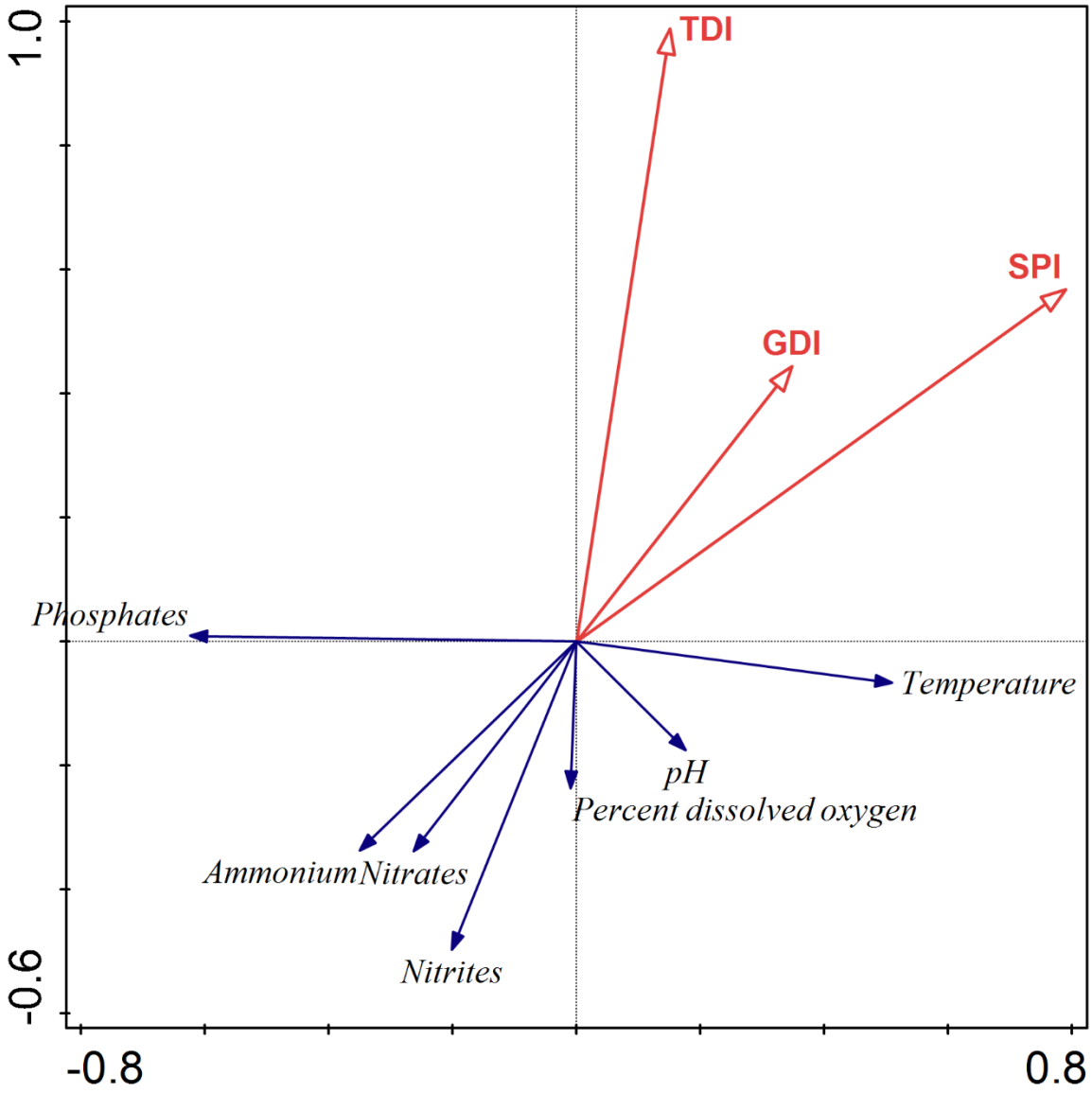

Figure 3-14. Redundancy analysis (RDA) biplot illustrating the correlation between water quality and diatom indices.

From Figure 3-15 it can be seen that the species can be divided into two groups. Group 1 represented diatoms that were related to higher levels of nutrients. This group included the following diatom species: *Gomphonema* sp. (GOMP), *Gomphonema pseudoaugur* (GPSA), *Cocconeis placentula* (CPTG), *Fragilaria ulna* (FUBI), *Navicula interruptestriata* (NITS), *Seminavis* sp. (SMNA), *Encyonopsis minuta* (ECPM) and *Tabularia fasciculate* (TFAS). Group 2 showed diatoms that were related to higher dissolved oxygen. Group 2 diatom species included: *Gomphonema insigne* (GINS), *Cocconeis placentula* (CPTG), *Epithemia adnata* (EADN) and *Fragilaria* sp. (FRAG). The CCA had a P value of 0.718 based on a Monte Carlo permutation. The plot explained 74.3 % of the variation. The first axis explained 20.83 % of the variation with the second axis explaining 39.48 %.

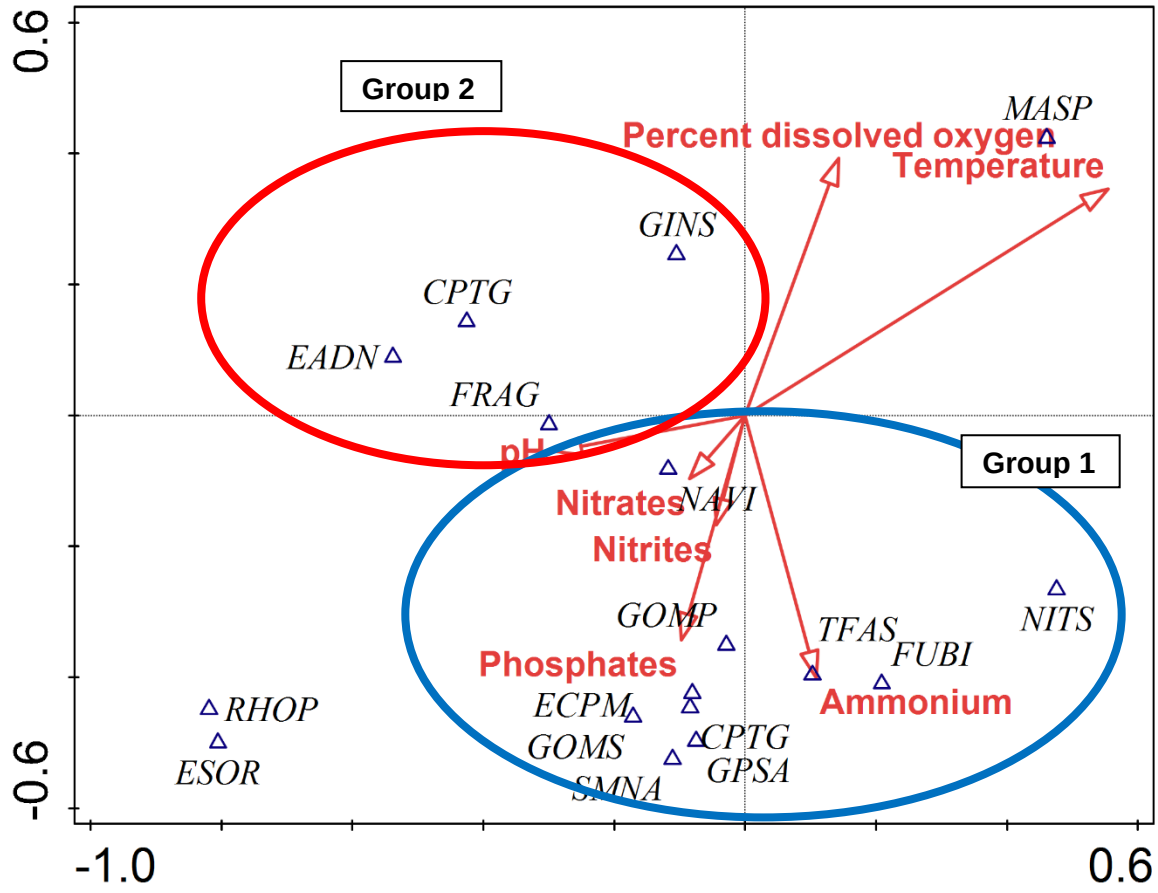

Figure 3-15. Canonical correspondence analysis (CCA) triplot illustrating the correlation between water quality and diatom species. Only species that contributed between 30 % – 100 % of the variation were included for clarity. (Abbreviations in Table 3-1).

3.4 Discussion

3.4.1 Water quality

An aquatic ecosystem's quality can be determined according to different water quality variables. The ratio of these water quality variables can determine a different quality state of the ecosystem. Nutrients are important for aquatic ecosystems as they form the basis for plant growth (Dallas and Day, 2004). With that said, increased nutrients in the system can be problematic and result in eutrophication (Ryther and Dunstan, 1971). The sustainability of aquatic ecosystems are threatened by eutrophication (Humphries and Benitez-Nelson, 2013). As lakes acts as sinks, they are particularly susceptible to nutrient enrichment (Humphries and Benitez-Nelson, 2013).

Nitrites and nitrates will be considered together in the discussion as nitrogen due to co-occurrence and rapid inter-conversion between the two nutrients (DWAF, 1996). The measured nitrogen levels (Figure 3-7 A – B) for all four sites were between 0.5 – 2.5 mg/L. According to South Africa's water quality guidelines (WQG) for aquatic ecosystems (DWAF, 1996), values between 0.5 – 2.5 mg/L are an indication of mesotrophic conditions in the system. When data was compared to a Lake Sibaya report by DWS (2015b) it can be noted that nitrogen data for 1980 to 2015 was in the same range. The DWS (2015b) report on Lake Sibaya was compiled to assist with decision making regarding water resources as the main objective of the report was the determination of the ecological reserve (DWS, 2015b).

Recorded phosphate levels (Figure 3-7 C) in the system were high and indicated hypertrophic conditions (values > 250 µg/L), except for LS 3.1 and LS 4.1 which indicated eutrophic conditions (values 25 – 250 µg/L) according to the South Africa's water quality guidelines (WQG) (DWAF, 1996). The DWS (2015b) reported low phosphate levels over their 1980 – 2015 data collection. Nitrogen and phosphate values were analysed only once per survey and are thus not an accurate representation of these nutrient levels in the system over months and years compared to those presented in the DWS (2015b) report. According to the WQG (DWAF, 1996), single measurements of nutrients are a poor indication of the quality of the system as occasional increases in nutrients are not as important as continuous high concentrations of nutrients. Allanson (1979) studied the dissolved nutrients in the system and found that the concentrations of nitrate were 17 – 32 µg/L and soluble reactive phosphorus were 14 – 25 µg/L. There was no significant evidence of variation in these nutrient concentrations downwards through the water column (Allanson, 1979).

From the DWS (2015b) report it was stated that in the sediment of the western arm (LS 4) there was signs of nutrient enrichment. The development of Mseleni Town, increased human settlement and development of forestry were the cause of this nutrient enrichment (Humphries and Benitez-Nelson, 2013; DWS, 2015a). Humphries and Benitez-Nelson (2013), stated that the main cause of eutrophication in lakes, estuaries and coastal lakes are urban development and land use change. The presence of cattle at both LS 3 and LS 4 may have an influence on nutrient levels as their faeces can enter the site. A study by Humphries and Benitez-Nelson (2013) found over recent years the nutrient concentrations in the lake has increased, especially in the western arm (LS4).

For all four sites the temperature (Figure 3-8 A) did not fluctuate more than 3 °C between winter and summer. The third survey had the highest temperature as it was during the warmer part of summer (February). Temperature is one of the vital environmental variables as it influences chemical reactions and also the metabolic rates of organisms (DWAF, 1996). Allanson (1979) recorded the temperatures of Lake Sibaya from 1968 – 1976 and calculated the average monthly temperatures (DWS, 2015a). The average temperature (from 1968 – 1976) for the months sampled was 19 °C for August, 22 °C for December and 24 °C for February (Allanson, 1979). It should be noted that the recorded temperature values of the present study fell within the limits measured by Allanson (1979). According to the WQG (DWAF, 1996), it would be ideal to measure temperatures over a 24 hour period, during each season, to establish variation. For temperature to have an adverse effect on an aquatic ecosystem the change in temperature should be unnatural (DWAF, 1996).

The pH is determined by the activity of the hydrogen ions in the water (DWAF, 1996; Dallas and Day, 2004). Lake Sibaya 1 – 3 had similar pH levels (Figure 3-8 B) with LS 4 having a higher pH value of more than 9. The higher pH at LS 4 might be due to the anthropogenic activities at this site as mentioned in the site description. Eutrophication is associated with high pH levels due to increased biological activity in the system (DWAF, 1996) and it can be noted from the nutrient data discussed that the system is enriched with nutrients. The toxicity and availability of chemical constituents is also determined by pH (Matlala, 2010).

Oxygen is critical for the survival of all living organisms (DWAF, 1996; Dallas and Day, 2004). Aquatic organisms make use of dissolved oxygen (DO) in the water. Dissolved oxygen and temperature usually have a negative correlation with one another (Dallas and Day, 2004), however, this was not noted during this study. The positive correlation between DO and temperature might be due to photosynthesis and turbulent water (wind action). The higher temperatures and high nutrient values can cause an increase in primary production which will

cause an increase in DO as photosynthesis increases in aquatic vegetation and primary producers (Elwood *et al.*, 1981; Hill *et al.*, 1995). Lake Sibaya 4 had the lowest DO during all three seasons and this might be due to the increased anthropogenic activities at this site, such as waste discharges from the rural development, that could cause a decrease in DO (DWAF, 1996). Low DO concentrations may be linked to nutrient enrichment as well (Humphries and Benitez-Nelson, 2013)

Matlala *et al.* (2011) stated that once-off water quality samples are a snap-shot of the systems quality and at the time of sampling different factors influence the measurements. Therefore, it was suggested to use the diatom community data to determine the health of the system as this data would be more conclusive as each diatom species prefers specific environmental conditions and integrates the effects of pollution in the system over a period of time (de la Rey *et al.*, 2004; Matlala, 2010; Dalu and Froneman, 2016). De la Rey *et al.* (2004) stated that aquatic communities (plant and animal) reflect the ecological integrity of the ecosystem as they reflect the physical and chemical disturbances in the ecosystem over a prolonged period of time.

However, the DWS (2015b) report on Lake Sibaya stated that the water quality for most of the system, except the southern basin and western arm, is in a near pristine condition and not severely impacted based on water quality data alone and not considering the sediment data. However, the southern basin (LS 3) and western arm's (LS 4) water quality have been influenced by increased rural development and forestry (Humphries and Benitez-Nelson, 2013; DWS, 2015a).

Humphries and Benitez-Nelson (2013) stated that as the catchment undergoes development the lake will continue to decline as the pressure on the lake intensifies. The DWS (2015a) report on the lake indicated the present ecological status (PES) and alternative ecological category (AEC). The PES scores were based on the combined water quality parameters (temperature, electrical conductivity, pH, dissolved oxygen, total carbon, total nitrogen and total phosphorus). These statuses can be noted in Table 3-7. The ecological categories are interpreted from A – F with A indicating a natural system and F indicating a critically modified system (Kleynhans and Louw, 2007).

Table 3-7. Present ecological status (PES) and alternative ecological status (AEC) for Lake Sibaya.

Zone	Site	PES	AEC
Main Basin	LS 1 and 2	B/C	C
Southern Basin	LS 3	C	C
Western Arm	LS 4	B/C	C

3.4.2 Diatom communities

Diatoms are effective in indicating the health of a system as they have a short generation time allowing them to respond rapidly to any changes in their environment (Dixit *et al.*, 1992; John, 2012) (see chapter 1). In diatom communities each species has their own specific environmental conditions in which they occur and grow optimally (Dixit *et al.*, 1992; Stevenson *et al.*, 2010; Dalu and Froneman, 2016). The relationship between environmental variables and diatoms is now better understood by researchers, which supports the use of diatom-based approaches (Harding *et al.*, 2005). As the conditions in the system change the more sensitive species (to these changes) will die first with the more resistant species becoming dominant in the system as they do not have to compete for resources (Harding *et al.*, 2005). According to Matlala *et al.* (2011), since living organisms are exposed to their environment they are better indicators of water quality. Thus, the dominant species in the system can be used as a representation of the conditions of the system.

Past studies on diatom communities in Lake Sibaya were completed by Archibald (1966), Allanson (1979) and Stager *et al.* (2013). In Allanson (1979), a preliminary list of algae (benthic and planktonic) is presented. An article by Archibald (1966) lists the species identified during his study including three species recorded in South Africa for the first time and nine new species. The three species recorded in South Africa for the first time included: *Amphora robusta* Gregory, *Navicula cryptolyra* Brockmann and *Stauroneis karstenii* (O. Müller) Hustedt. The nine new species included *Achnanthes breenii* Archibald*, Achnanthes sibayiensis* Archibald*, Amphora lacustris* Archibald*, Cocconeis pusilla* Archibald*, Cyclotella substylorum* Archibald*, Fragilaria exiguissima* Archibald*, Navicula breenii* Archibald*, Navicula sibayiensis* Archibald and *Navicula subpatrickae* Archibald*.* None of the three new South African records were identified in this study but one of the new species was identified in this study, namely, *Amphora lacustris*. Except for the article by Archibald (1966), the only other reference to this species is that of Sánchez Castillo (1993) at the Twelfth International Diatom Symposium in Renesse, Netherlands. A study by Stager *et al.* (2013) found that diatom species are abundant in the lakes shallower water and not in bottom sediment. In May 2004 Stager *et al.* (2013) sampled diatoms in a plankton tow with *Aulacoseira granulata, Nitzschia lacuum* and *Synedra*

delicatissima the dominant species sampled. From the three dominant species sampled *Aulacoseira granulata* was the only species identified in the current study.

Archibald (1966) identified 107 different species, while in the present study only 59 species were identified. Both the present study and Archibald's (1966) made use of aquatic plants as a substrate from which to collect the diatom flora. Some species identified in the present study were not identified by Archibald (1966) or Allanson (1979). This may be due to differences in sampling sites, sampling methods, or due to changes in the aquatic environment between 1966 and 2015/16.

When analysing the present study's diatom community the environmental factors were used to explain variation in the community structure using a CCA ordination technique (ter Braak and Verdonschot, 1995). From Figure 3-15 it can be noted that the diatom species formed two main groups. Group 1 included diatoms that were related to higher levels of ammonium, phosphate and nutrients. Group 2 included diatoms that were related to dissolved oxygen. The *Mastogloia* sp. was associated with higher temperatures and dissolved oxygen. Temperature had an influence on both groups.

The species found in the first group had a high affinity for ammonium, phosphate, nitrogen and pH. The first group species had a negative relationship with dissolved oxygen and temperature. The group included: *Encyonema minutum, Gomphonema* sp., *Gomphonema pseudoaugur, Cocconeis placentula, Fragilaria ulna, Nitzschia* sp. and *Navicula interruptestriata.* These species are found in meso- to eutrophic water and are tolerant of nutrient enrichment (Bellinger *et al.*, 2006; Taylor *et al.*, 2007). The group also included: *Seminavis* sp., *Encyonopsis minuta* and *Tabularia fasciculata* are found in saline and electrolyte rich waters (Taylor *et al.*, 2007).

Figure 3-15 showed that some species (*Cocconeis placentula, Gomphonema* sp. and *Navicula* sp.) are tolerant to pollution thus indicating the water to be in a poor to bad quality. The dominant diatom species (identified over most surveys and having the highest abundance) were *Cocconeis placentula*, *Epithemia adnata*, *Gomphonema* sp., *Gomphonema insigne* and *Gomphonema pseudoaugur*. When studying the ecology of these species it was clear that they occur in waters that are meso- to eutrophic and in electrolyte rich waters (Taylor *et al.*, 2007). The DWS (2015a and b) report indicated that nutrient enrichment is one of the causes of decline in the quality of the system.

Seasonal differences were observed in the study as is shown in Figure 3-11 and Figure 3-12. Survey 2 (December 2015) and survey 3 (February 2016) grouped together for all four sites. Survey 1 (August) was grouped separately. Thus, it can be noted that there was seasonal variation between the surveys.

3.4.3 Diatom indices

Diatom indices were calculated through the aid of the software package Omnidia. Omnidia calculates seventeen different indices based on the species identified and their abundance (Lecointe *et al.*, 1993). Index calculation is grounded on the assumption that diatom communities are influenced by eutrophication, pollution, pH and salinity. As certain sensitive taxa will decline in the presence of pollution, the water quality can be inferred through diatom changes in community composition (Blanco *et al.*, 2012). For this study the indices used were chosen according to the total number of species in the analysis used to calculate the indices final score. As 80 % or more of the species counted were used to calculate the SPI, GDI and TDI indices, they were deemed the most reliable indices and thus included in this study. Omnidia was used to determine the percentage of species used to calculate the final index scores.

Together with the indices, a Pearson correlation coefficient was determined between the diatom indices and the water variables (Table 3-6 and Figure 3-14). The correlation is used to determine the relationship strength between two variables and provides a number between + 1 and – 1 (Rummel, 1976). A positive correlation coefficient will indicate a positive correlation and a negative correlation coefficient a negative correlation. The indices reflect nutrient levels as they all indicate whether a system is eutrophic, meso-eutrophic, mesotrophic, oligo-mesotrophic or oligotrophic. Thus, an increase in nutrients will decrease the index score (negative correlation).

It can be seen (Figure 3-9 A) that LS 2 had the highest SPI score with an average of 12.4 indicating the site is of moderate quality. Lake Sibaya 1 had the lowest average with 9.3 which indicates poor quality. Both LS 3 and 4 are of poor quality. There was a negative correlation between the SPI score and water quality variables. As nutrient enrichment is an indication of a declining ecosystem, the negative correlation was expected (Dalu and Froneman, 2016). The SPI scores and negative correlation relates with the discussed diatom species and water variables as both indicate a nutrient enriched ecosystem.

It can be seen (Figure 3-9 B) that LS 2 had the lowest GDI score of 10.9 indicating a poor quality. Lake Sibaya 4 had the highest score of 13.9 indicating moderate quality. Lake Sibaya

1 and 3 both had scores indicating moderate quality. The GDI index is useful for early detection of pollution in wetlands (Matlala *et al.*, 2011). There was a negative correlation between the GDI score and water quality variables. It was expected that there would be a negative correlation between the GDI and water quality variables as a decrease in the score indicates a decline in water quality (Dalu and Froneman, 2016). The negative correlation and low GDI scores are an indication of a nutrient enriched ecosystem and thus relates to the diatom species identified and measured water variables.

Figure 3-9 (C) and (D) indicated the average TDI and %PTV scores for the different sites and it can be seen that LS 3 had the highest TDI score revealing the trophic level as meso-eutrophic. All the sites had scores higher than 50 indicating the trophic level as either mesotrophic (LS 1.1, LS 1.2, LS 1.3, LS 2.1 and LS 4.2) or meso-eutrophic (LS 3.1, LS 3.2, LS 3.3, LS 4.1 and LS 4.3). The TDI score corresponds with the SPI score, GDI score and dominant diatom species that are signifying meso-eutrophic and mesotrophic conditions. According to Kelly (1995), the TDI has to be carefully interpreted as diatom communities are influenced by medium to long-term changes in its environment which is unrelated to nutrients. TDI scores had a positive correlation with phosphorus in the system (Bellinger *et al.*, 2006) and the high phosphorus levels found in the system (Figure 3-7 C) could be the reason behind the high TDI score. According to the WQG (DWAF, 1996), the phosphate levels recorded for the system are an indication of a hypertrophic system. However, Kelly and Whitton (1995) stated that when the TDI index is used the %PTV index should be taken into account as the %PTV score indicates whether organic pollution or nutrients contributes to the TDI score. As the %PTV were low (< 20 %) it indicated the system is free of organic pollution. As there is no organic pollution, the TDI index then provides a reliable indication on the nutrient levels in the system (Kelly and Whitton, 1995). The high TDI scores thus signified that nutrient enrichment was not caused by organic pollution.

3.5 Conclusion

The chapter focused on the diversity and water quality of the Lake Sibaya system. Studying the diversity of diatom species in the lake, a total number of 59 species were identified in this study. Nearly half the number of species were identified in this study in comparison to Archibald's study (107 species), however, it can be concluded that the lake has a moderate diatom diversity.

The water quality variables revealed that the system was enriched with nutrients, with the nitrogen levels indicating the system as mesotrophic and the phosphate levels indicating a

hypertrophic system. The study by DWS (2015b) found that there was no clear pattern for the nitrogen and phosphate levels over the past 40 years, however, random peaks could be noted for nitrogen and phosphate levels. Studying the diatom community was suggested to determine the quality of the ecosystem, as they are consistently exposed to the environmental conditions in their environment over a period of time.

The diatom community and indices also revealed the ecosystem was enriched with nutrients. Dominant species identified were tolerant of pollution and generally found in nutrient enriched ecosystems. This data correlates with the nitrogen and phosphate data which indicated increased nutrient levels in the ecosystem.

Increased nutrient levels were reported by DWS (2015b) when they studied the sediment of the system. These increased nutrient levels may be due to the development of human settlements, increased forestry or the development of the Mseleni Town surrounding the lake (Humphries and Benitez-Nelson, 2013; DWS, 2015a).

It is thus concluded that the Lake Sibaya system is nutrient enriched. It is, however, difficult to determine if these increased nutrients in the system are an indication of a natural or polluted ecosystem as studies on the nutrient levels of wetlands are limited and poorly understood (Malan and Day, 2012).

Chapter 4 — The Makuleke Wetlands

The Makuleke Wetlands are situated within the Kruger National Park (KNP) and are considered more diverse than the Lake Sibaya system supporting an abundance of living organisms, from the plant to the animal kingdom (Hilton-Baber and Berger, 2007). The following chapter will provide an in-depth discussion on the Makuleke Wetlands.

4.1 Site description

The Makuleke Wetlands are situated in the north of the KNP and the various wetlands are classified as floodplain depressions (Deacon, 2007; Antrobus, 2014). The wetland's boundary falls within the flood level of the Luvuvhu and Limpopo Rivers (Deacon, 2007). The Limpopo River, which is also the border between Zimbabwe and South Africa, is the northern boundary of the Ramsar site (Deacon, 2007; Antrobus, 2014). The border with Mozambique is the eastern boundary of the Ramsar site, with Banyini Pan and the KNP being the western boundary of the wetland (Deacon, 2007). The Hapi drainage line, south of the Luvuvhu River, is the southern boundary of the Ramsar site (Deacon, 2007). The Makuleke wetland comprises a total of 7756.98 ha and the various pans comprise approximately 347 ha of the total area (Deacon, 2007). Banyini Pan has the highest elevation at 235 m above sea level (asl) and the confluence between the Limpopo-Luvuvhu is the lowest at 190 m asl (Deacon, 2007). The wetland falls in the 'Tropical Premontane Arid Thorn Woodland' climate area with winter months being dry and mild to humid and hot during the summer months (Deacon, 2007).

4.1.1 Geology and geomorphology

The Makuleke Wetlands are situated in what is known as the Punda Maria-Pafuri-Wambiya area, 70 – 130 km north of the Tropic of Capricorn (Deacon, 2007). The diversity of the landscape features and geographic location contribute to the high biodiversity of this area (Tinley, 1978).

The intrinsic heterogeneity of the area is due to numerous geological features with each characterised by contrasting rock types (Venter, 1990). These rock types include mudstone, basic lavas, quartzite, shale and sandstone (Deacon, 2007; Viljoen, 2015). The Mozambique Plain towards the east is made up of ferricrete, marls, unconsolidated sand, calcrete and boulder beds (Deacon, 2007). Floodplain alluvium occurs at the confluence of the two rivers (Limpopo and Luvuvhu Rivers) to the north (Deacon, 2007). Adjacent floodplains and a well-developed levee characterise the area beside the Limpopo River (Deacon, 2007).

When assessing the rivers that drain the north east of South Africa, it can be seen that the Luvuvhu River differs from the others as it flows over different rock types (Tinley, 1978; Nesbitt, 2014). To the west these rock types include quartzite and sandstone, the eastern rock types are basalt, and the central area rock type consists of sedimentary rocks (Deacon, 2007; Nesbitt, 2014; Viljoen, 2015). The Luvuvhu River is underlain by floodplain alluvium that was formed when most of the Luvuvhu River sediment load was deposited as it exits Lanner Gorge (Deacon, 2007; Smit *et al.*, 2013). Schist calcsilicate rocks, metaquartzite, gneisses and marble are the main types of rock that occur in the Makuleke Wetland (Deacon, 2007).

4.1.2 Hydrology

The Pafuri area is drained by the Limpopo and Luvuvhu Rivers and is characterised by steep gorges and high relief due to the Luvuvhu River's erosive action and the underlying geology's resistance to weathering (Deacon, 2007). The catchment is approximately 5941 km^2 (Smit *et al.*, 2013) and it forms a wide floodplain downstream of Lanner Gorge with numerous ephemeral pans (Tinley, 1978). The Luvuvhu River has recently stopped flowing in the winter months due to forestry, agricultural and mining activities that occur outside the boundaries of the KNP (Deacon, 2007; Smit *et al.*, 2013). It has a mean annual runoff (MAR) of 395 million m^3/a, which is unevenly distributed throughout the catchment (Deacon, 2007), with a mean annual precipitation (MAP) of 608 mm and 1678 mm mean annual evaporation (Smit *et al.*, 2013).

Characteristics of the Limpopo River include a wide, sandy riverbed with a floodplain consisting of many large pans (Deacon, 2007). It should be noted that this is a seasonal river (Deacon, 2007; Nesbitt, 2014). During the summer months (when the river is flowing) the river can have a width of about a kilometre and spill its banks to fill the pans found within in the floodplain (Deacon, 2007). The Limpopo River has a MAR of 2290 million m^3/a (Deacon, 2007).

In the Makuleke area, the prominent feature, namely the extensive riparian forest, is maintained by seepage and ground water from the underlying aquifer and floodplain pans (Deacon, 2007). Most of these pans have catchments large enough to fill them during high floods or heavy local rain fall (Deacon, 2007). When there is high flow in the rivers, the pans closest to them can be filled by ground water seepage (Nesbitt, 2014). High floods and Limpopo River back flooding are the two types of floods that occur on the Luvuvhu River (Deacon, 2007).

4.1.3 Vegetation

River channels, riparian floodplain forests, pans, riverine forests and floodplain grasslands are the various landscapes found within the Ramsar site (Deacon, 2007; Antrobus, 2014). The riverine forest consists of broad, large canopy trees that reach heights of more than 20 m and are mostly confined to the Limpopo and Luvuvhu rivers' banks (Deacon, 2007).

Vachellia xanthophloea (fever trees) and *Faidherbia albida* (ana trees) occur on waterlogged clays in the riparian floodplain woodlands (Deacon, 2007). Floodplain grasslands can be found on the floodplains of both the Limpopo and Luvuvhu Rivers (Venter, 1990).

4.1.4 Ecological significance

The wetlands are of ecological significance as they support a great number of living organisms (Hilton-Baber and Berger, 2007). Makwadzi Pan is home to the last remaining herd of *Hippopotamus amphibius* (Hippopotamus) in the Limpopo River east of Beit Bridge (Nesbitt, 2014). Vulnerable species such as *Python sebae* (African python) and *Crocodylus niloticus* (Nile crocodile) are found within wetlands together with high densities of *Tragelaphus angasii* (Nyala) (Antrobus, 2014; Nesbitt, 2014). Furthermore, bird species that are nationally threatened can be found within the wetland areas (Deacon, 2007). Cycads, critically endangered plant species, are also present within the area (Deacon, 2007). Other species of ecological significance, not dependent on the wetlands, but present in the region include (Antrobus, 2014; Nesbitt, 2014):

1. *Lycaon pictus* (African wild dog) (Endangered)
2. *Leptailurus serval* (Serval) (Near-threatened)
3. *Panthera pardus* (Leopard) (Rare)
4. *Hyaena brunnea* (Brown hyena) (Near-threatened)
5. *Proteles cristata* (Aardwolf) (Rare)

4.1.5 Wetland classification

The wetlands were classified according to the user manual for the classification of wetland systems (Ollis *et al.*, 2013). According to Ollis *et al.* (2013), the manual was developed to provide guidelines, which are user-friendly and can be used in the field, to classify inland aquatic ecosystems. The manual classifies an aquatic ecosystem according to a six-tiered classification system. These tiers are: Level 1 — Systems; Level 2 — Regional Setting; Level 3 — Landscape Units; Level 4 — Hydrogeomorphic Units; Level 5 — Hydrological Regime; and Level 6 — Wetland/Aquatic Ecosystem Characteristics (Ollis *et al.*, 2013). According to the manual, the Makuleke Wetlands are classified as a wetland in the Limpopo plain region

(Ollis *et al.*, 2013). Its landscape is a plain with floodplain depressions as hydrogeomorphic units which are intermittently inundated and seasonally saturated (Ollis *et al.*, 2013). The wetlands are natural and vegetated (Ollis *et al.*, 2013).

The floodplain comprises a total of 31 seasonally flooded pans, all of which are important habitats for animals and birds in terms of feeding and breeding (Antrobus, 2014; Nesbitt, 2014). During the drier winter months the Limpopo River floodplain plays an important role for the system as it holds water well into the dry season (Deacon, 2007). This creates a refuge for wildlife in this region. Migrating birds use this area as a stopover and during the summer and winter it provides water birds with important habitats (Deacon, 2007). Several pans are found within the boundary of the Limpopo River floodplain (Makwadzi being one such example) and are filled during flooding as well as rain (Nesbitt, 2014). These pans are characterised by emergent aquatic macrophytes as well as floodplain vegetation (Deacon, 2007). Other pans, such as Mapimbi, are filled through seepage from the Limpopo River (Nesbitt, 2014). As Mapimbi Pan receives water earlier than other pans (during flooding), it is speculated that fluctuations in the water table influence the hydrology of the pan (Deacon, 2007; Nesbitt, 2014).

Unlike the Limpopo River, there is no prominent evidence that the Luvuvhu Rivers' northern bank has a levee (Deacon, 2007). Thus, these pans are flooded more regularly, have a connection that is more accessible to the river, and these pans are shallower (Deacon, 2007). A drainage line, south of the Luvuvhu River, runs parallel to the river all the way into Mozambique, with Hapi Pan situated in this drainage line (Deacon, 2007).

Mapimbi, Makwadzi, Hapi and Mabvubanye pans have their own drainage lines with substantial catchments. Thus, these systems can completely fill during rainfall events due to runoff from their own catchments (Deacon, 2007).

4.2 Site selection

As mentioned in Chapter 2, ten pans were sampled in the Makuleke Wetlands. The criteria used to select these pans included:

1. Pans should be representative of the entire wetland (east to west).
2. There must be a combination of pans that receive water from both the Limpopo and Luvuvhu River.
3. Pans must be easily accessible.

Ten pans meeting these criteria were selected and included Banyini, Gila, Hapi, Hulukulu, Jachacha, Makwadzi, Mapimbi, Nhlangaluwe, Nwambi and Reedbuck Vlei (Figure 4-1). All the pans were sampled during the wet season; however, only Makwadzi, Mapimbi and Hapi pans were sampled during the dry season as the other pans were too dry to obtain water and diatom samples. A brief description of each pan will be provided in the following sections.

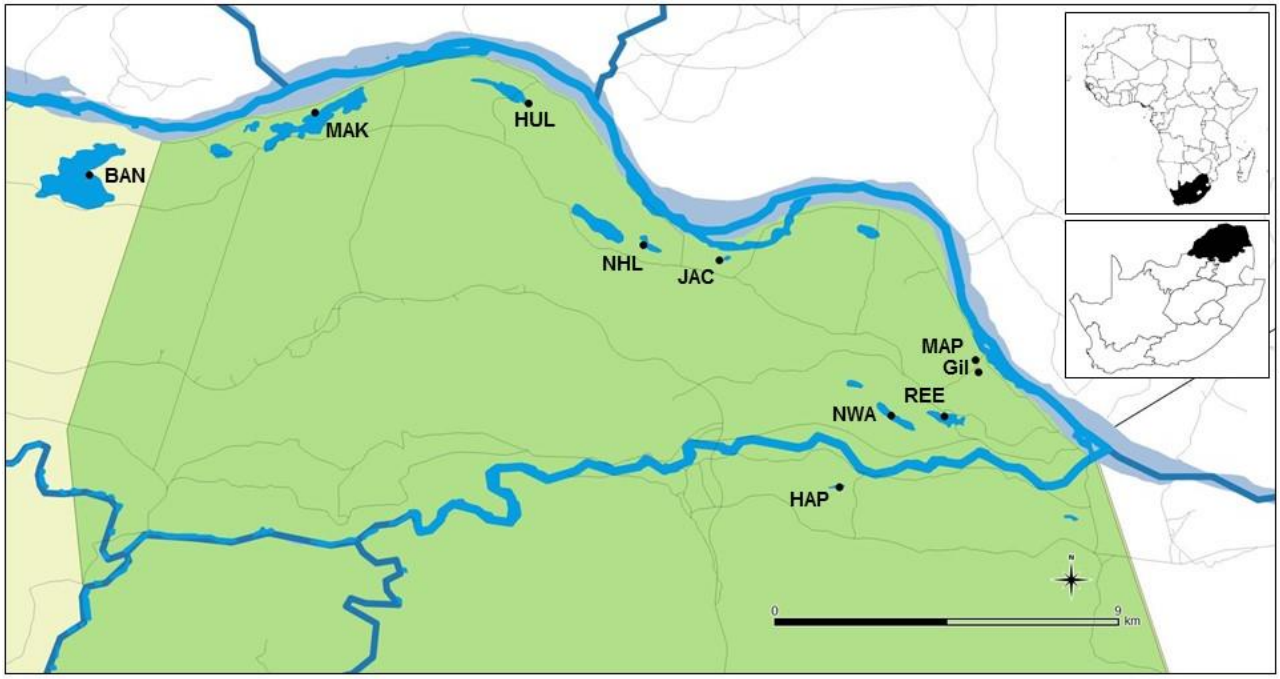

Figure 4-1. Map of the Makuleke Wetlands within the Kruger National Park. Pans sampled are indicated. (Source: Kelly Shannon Dyamond). (BAN = Banyini Pan, MAK = Makwadzi pan, HUL = Hulukulu Pan, NHL = Nhlangaluwe Pan, JAC = Jachacha Pan, MAP = Mapimbi Pan, GIL = Gila Pan, REE = Reedbuck Vlei Pan, NWA = Nwambi pan and HAP = Hapi Pan).

4.2.1 Banyini

Banyini Pan (Figure 4-2 A – D) is located at geographic coordinates S22.3655, E31.07512 and receives its water from the Limpopo River. The pan forms part of the western boundary of the Ramsar site and marks the highest point of the region at 235 m asl (Deacon, 2007). Banyini Pan has a perimeter of 8 km and a size of 162 ha (Deacon, 2007). Due to backflooding of the Limpopo River (approximately every 2 – 3 years), silt is deposited into Banyini Pan (Deacon, 2007).

To the east of the pan lies a large hill with a smaller rocky outcrop to the west. The catchment area around the pan is open grasslands. Reeds were present at the sampling site and were used to sample diatoms. The pan was approximately 1.3 km from the Limpopo River. During both sampling trips herds of *Syncerus caffer* (Cape buffalo) were found within the vicinity of the pan. Evidence that *Loxodonta africana* (African elephant) visit the pan could be seen, while baboons were present in the eastern hills. Due to *L. Africana* and *S. caffer* drinking from the pan, the water was turbid.

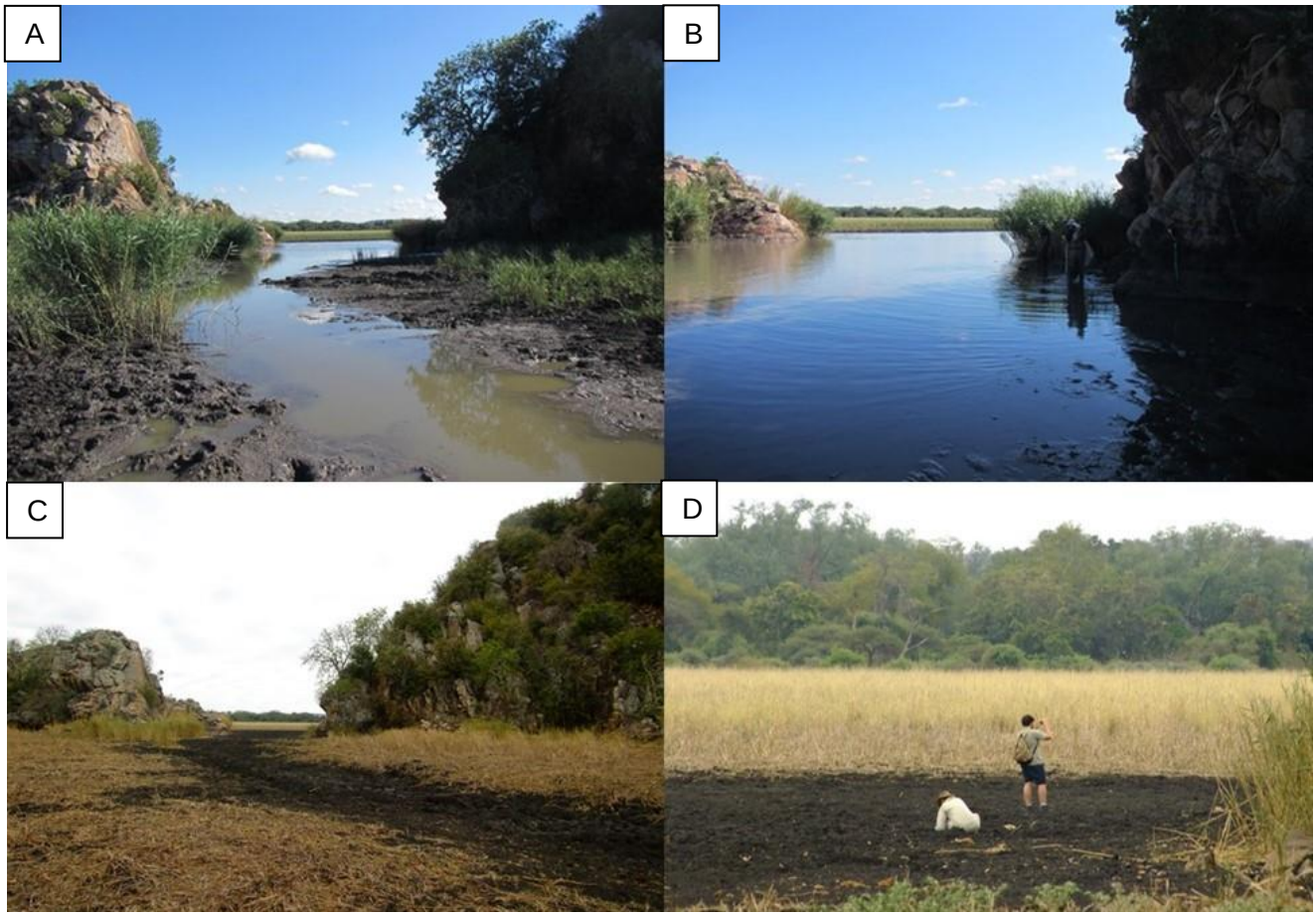

Figure 4-2. Banyini Pan during April 2015 (A – B) (Photo credit: Kelly Shannon Dyamond) and September 2015 (C – D) (Photo credit: Ruan Gerber).

4.2.2 Nhlangaluwe

Nhlangaluwe Pan (Figures 4-3 A – D) is located at geographic coordinates S22.37720, E31.19546 and receives its water from the Limpopo River. The pan is approximately 630 m from the Limpopo River. When the pan is in flood it can become part of an interlinked pan system. This results in Nwankwimbi and Vhembe Bend pans merging with this pan to form one large wetland drainage system (Antrobus, 2014; Nesbitt, 2014). The pan has quite steep banks with moderate tree density on the banks and grasslands surrounding the pan (Antrobus, 2014). The pan is surrounded by trees and grasslands with *S. caffer* present in the area. Diatoms were sampled from vegetation that included grass.

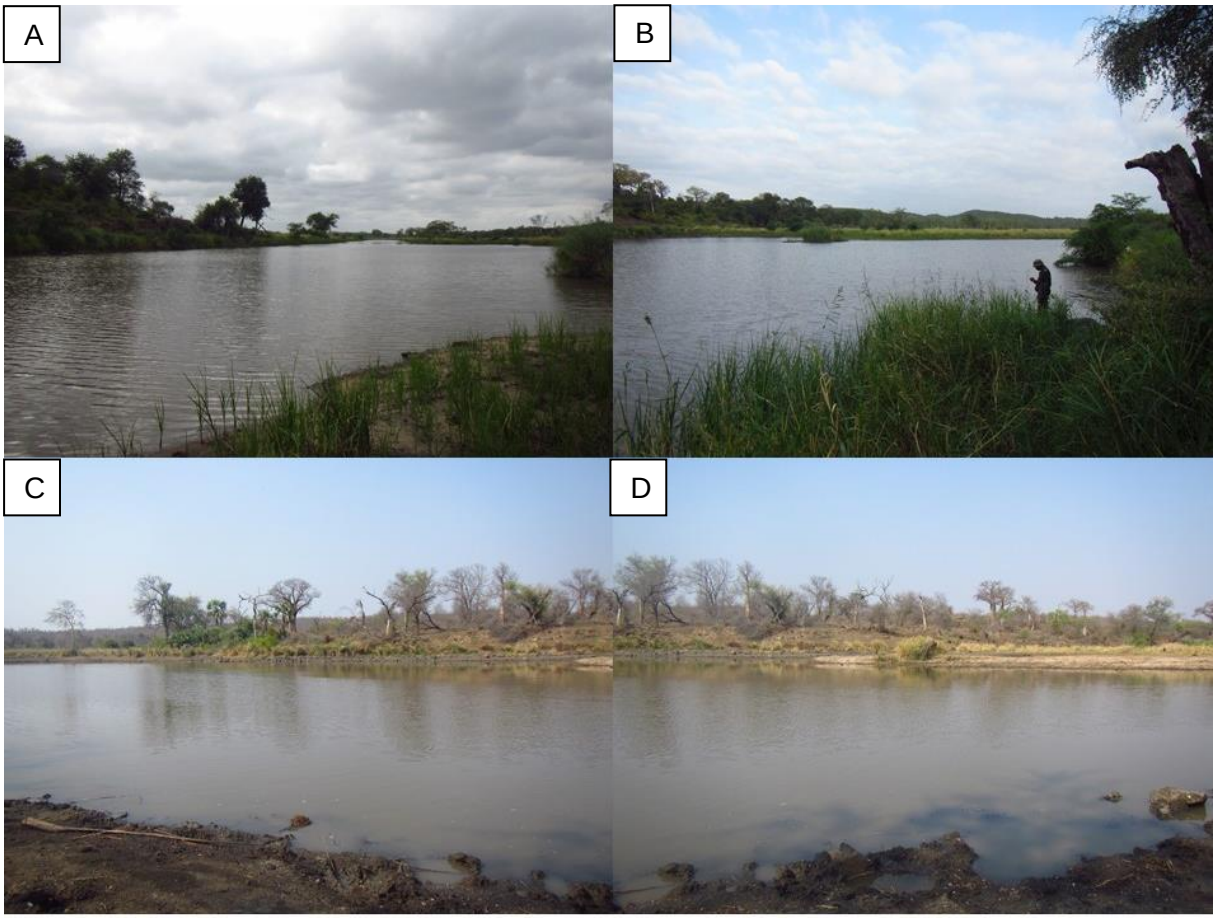

Figure 4-3. Nhlangaluwe Pan during April 2015 (A – B) and September 2015 (C – D). (Photo credit: Kelly Shannon Dyamond).

4.2.3 Makwadzi

Makwadzi Pan (Figure 4-4 A – D) is located at geographic coordinates S22.35014, E31.11875 and receives its water from the Limpopo River. The pan is approximately 150 m from the Limpopo River. Makwadzi Pan, as in the case of Hapi Pan, has its own drainage line from a substantial catchment area (Deacon, 2007). The pan has a depth of approximately 255 cm (Deacon, 2007). During the rainy season the pan is recharged through runoff from its

catchment while it also receives water when the Limpopo River overtops its banks (Nesbitt, 2014). Makwadzi Pan provides refugia for various aquatic birds during breeding seasons (Nesbitt, 2014). The catchment area is open with a high density of trees within the catchment. To the south of the pan there is a large hill, with the Limpopo riparian vegetation to the north. *Crocodylus niloticus* and *H. amphibious* were present in the pan while *S. caffer* (buffalo) were drinking from the pan during both surveys. Diatoms were sampled from vegetation that included grass and sticks from a tree that had fallen into the pan.

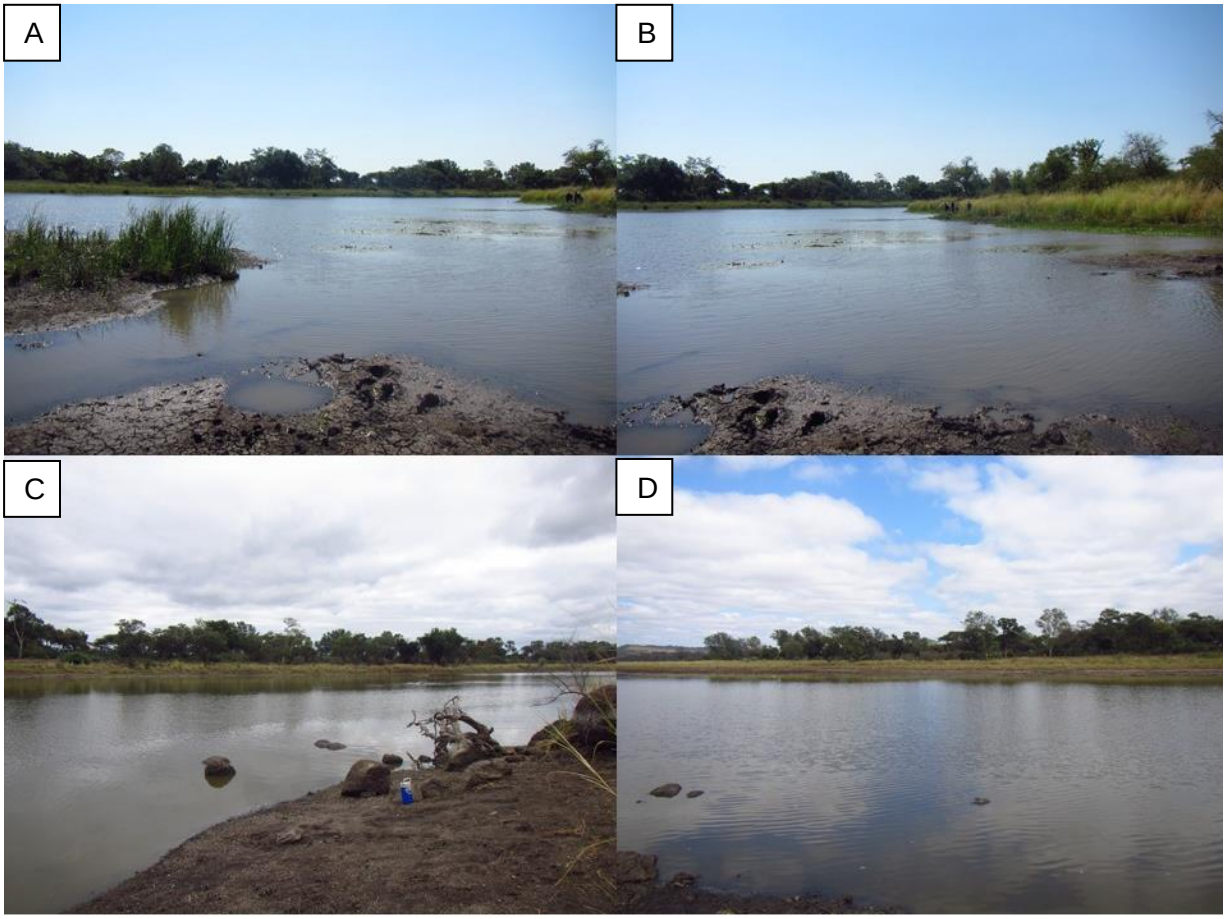

Figure 4-4. Makwadzi Pan during April 2015 (A – B) and September 2015 (C – D). (Photo credit: Kelly Shannon Dyamond).

4.2.4 Hulukulu

Hulukulu Pan (Figure 4-5 A – D) is located at geographic coordinates S22.34245, E31.17545 and receives its water from the Limpopo River. The pan is approximately 650 m from the Limpopo River. Due to backflooding of the Limpopo River (approximately every 2 – 3 years), silt could potentially be deposited into Hulukulu Pan (Deacon, 2007). The banks of the pan have large high canopy trees such as *Vachellia xanthophloea*. The area around the pan is occupied by high canopy trees and shrubs in the riparian forest as it is situated within the Limpopo River riparian zone. *Loxodonta africana*, *Tragelaphus angasii* and *S. caffer* have

been seen in the area during surveys. The area is surrounded by trees (*V. xanthophloea*) and tall grassland and shrubs. Diatoms were sampled from vegetation that included grass.

Figure 4-5. Hulukulu Pan during April 2015 (A – B) (Photo credit: Kelly Shannon Dyamond) and September 2015 (C – D) (Photo credit: Anrich Kock).

4.2.5 Jachacha

Jachacha Pan (Figure 4-6 A – B) is located at geographic coordinates S22.38240, E31.21903 and receives its water from the Limpopo River. The pan is approximately 165 m from the Limpopo River. The southern bank of the pan is steep with a low gradient and a flat northern bank. The pan has grass cover on all its banks. The area surrounding the pan is open grasslands with moderate to low tree densities. *Crocodylus niloticus* were present at the pan. Diatoms were sampled from vegetation that included grass.

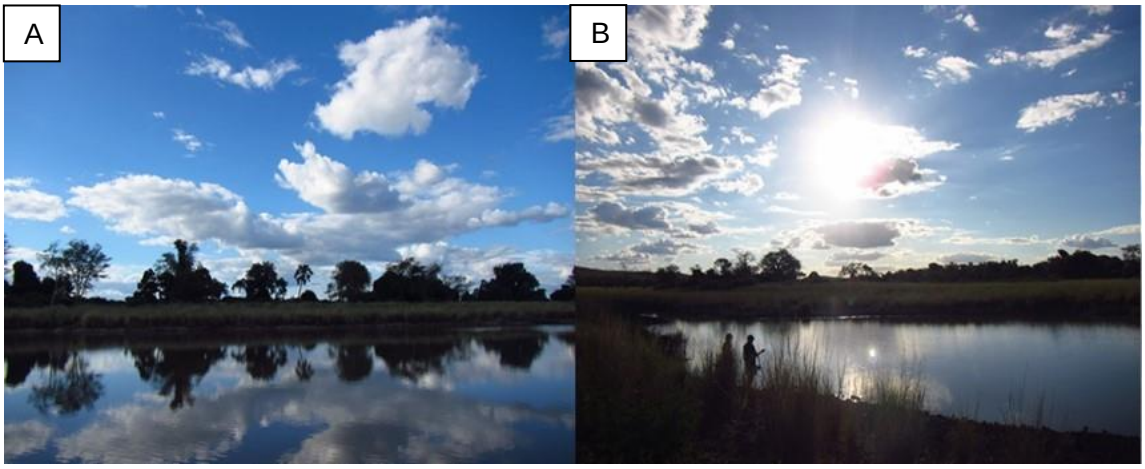

Figure 4-6. Jachacha Pan during April 2015 (A – B) (Photo credit: Kelly Shannon Dyamond).

4.2.6 Mapimbi

Mapimbi Pan (Figures 4-7 A – B) is located at geographic coordinates S22.40286, E31.28006 and receives its water from the Limpopo River. The pan is relatively small (40 m x 150 m) (Gillson and Ekblom, 2008) and has a depth of 310 cm (Deacon 2007). The pan is approximately 150 m from the Limpopo River. Mapimbi Pan is surrounded by tall canopy trees with steep slopes which are highly vegetated (Nesbitt, 2014). The pan is refilled through seepage water received from the Limpopo River (Deacon, 2007; Antrobus, 2014; Nesbitt, 2014) and is situated on the southern bank of the Limpopo within the riparian forest (Antrobus, 2014). The pan can fill completely during normal rains due to runoff (Deacon, 2007). The water has a reddish colour indicating suspended sediment in the water which might have been due to animal disturbance or recent rainfall. Diatoms were sampled from vegetation that included sticks and grass.

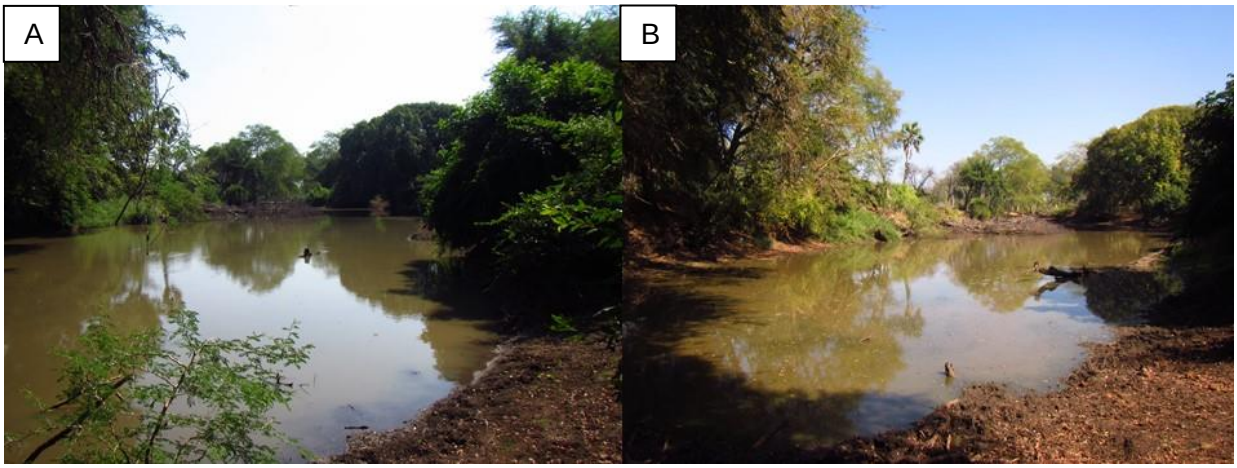

Figure 4-7. Mapimbi Pan during April 2015 (A) and September 2015 (B). (Photo credit: Kelly Shannon Dyamond).

4.2.7 Gila

Gila Pan (Figure 4-8 A – D) is located at geographic coordinates S22.40516, E31.27909 and receives its water from the Limpopo River. Gila Pan has a maximum depth of approximately 170 cm (Deacon, 2007) and is approximately 230 m from the Limpopo River. The banks of the pan are not very steep with a low gradient and are covered by tall grass, shrubs and high canopy trees. The area around the pan is relatively flat grassland with little tree cover. North east of the pan the Limpopo River riparian forest is present. During the survey in April 2015, the pan had a foul smell with algae clearly visible on the water surface. Diatoms samples were collected from stones as no vegetation was available for sampling.

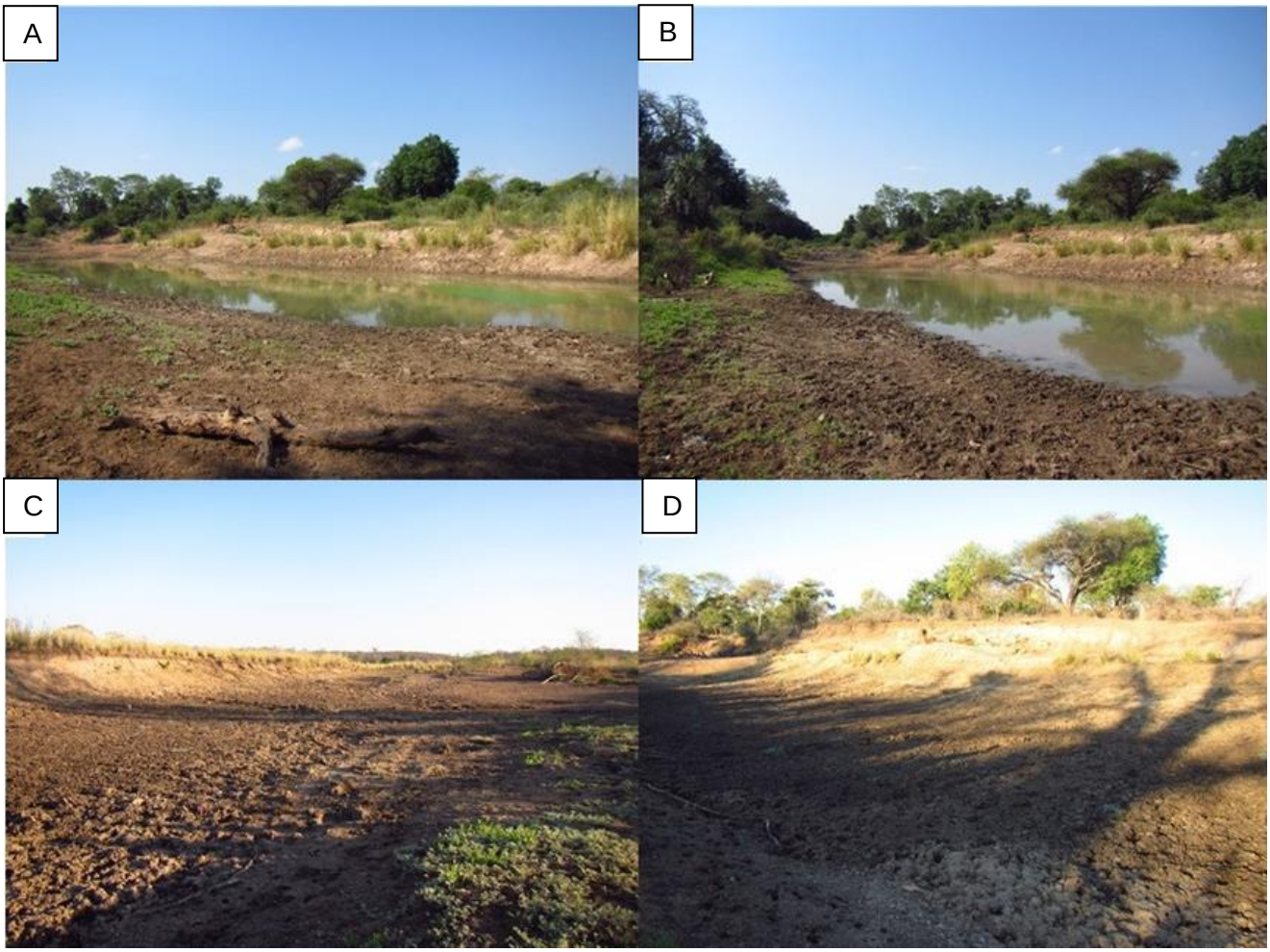

Figure 4-8. Gila Pan during April 2015 (A – B) and September 2015 (C – D). (Photo credit: Kelly Shannon Dyamond).

4.2.8 Hapi

Hapi Pan (Figure 4-9 A – D) is located at geographic coordinates S22.43256, E31.24803 and receives its water from the Luvuvhu River. The pan has a maximum depth of almost 320 cm (Deacon, 2007) and is approximately 660 m from the Luvuvhu River. Hapi Pan is situated in a depression south of the Luvuvhu River and marks the southern boundary of the wetlands (Deacon, 2007). The Luvuvhu River's banks overflow during floods/high flows and fills the drainage line running parallel to the river (Deacon, 2007). The northern bank of the pan is steeper than the southern bank. The banks of the pan are covered by shrubs and high canopy trees which are also present around the area of the pan. Diatoms samples were collected from stones as no vegetation was available for sampling.

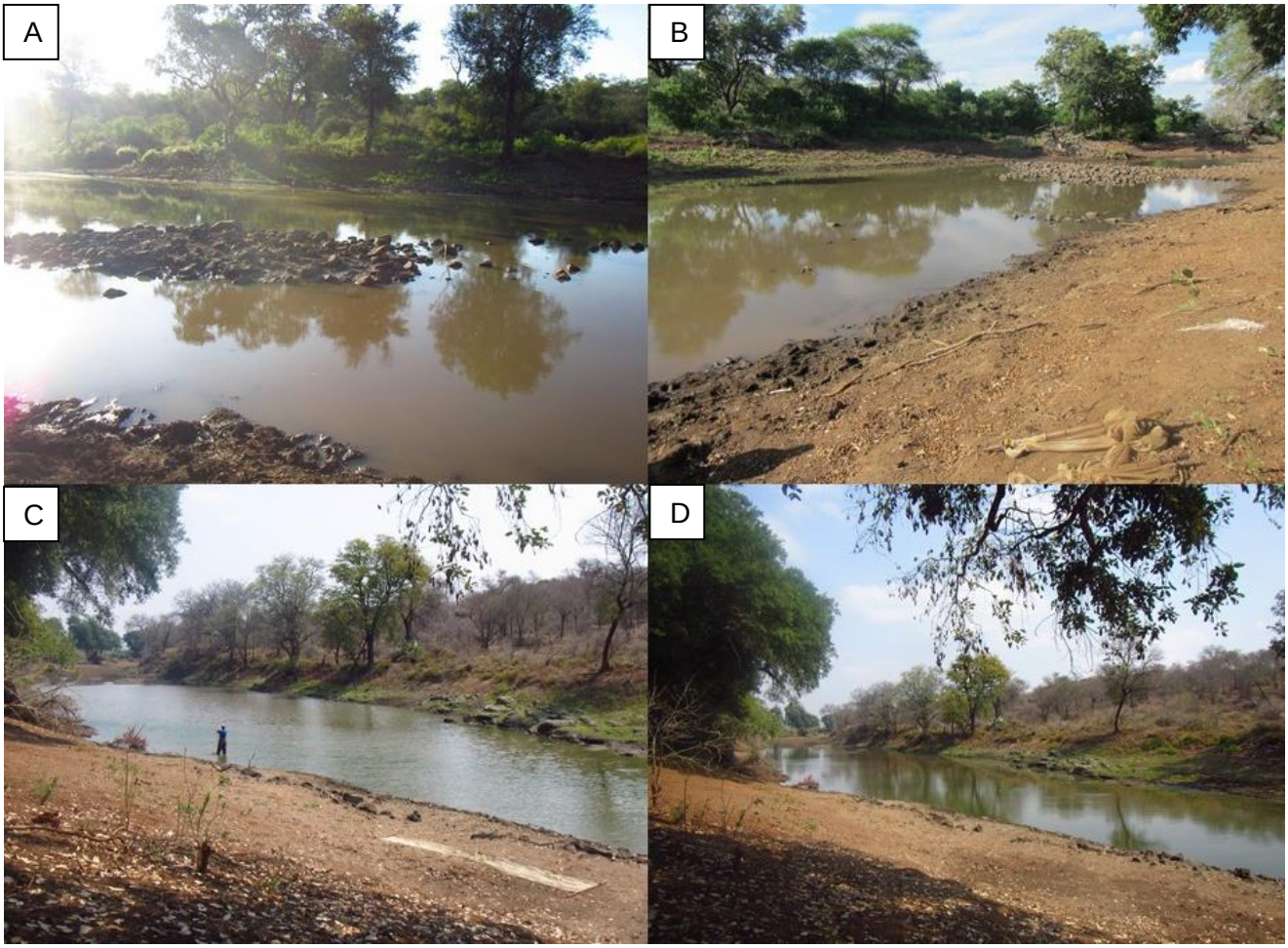

Figure 4-9. Hapi Pan during April 2015 (A – B) and September 2015 (C – D). (Photo credit: Kelly Shannon Dyamond).

4.2.9 Nwambi

Nwambi Pan (Figures 4-10 A – D) is located at geographic coordinates S22.41695, E31.26199 and receives its water from the Luvuvhu River. The pan is approximately 640 m from the Luvuvhu River. Shrubs and high canopy trees are present on the banks of the pan with the surrounding area consisting of large trees and shrubs. Silt is deposited into Nwambi Pan due to backflooding of the Limpopo River (approximately every 2 – 3 years) (Deacon, 2007). The pan is long and narrow stretching from east to west. The water had a reddish colour indicating suspended sediment in the water which might have been due to animal disturbance or recent rainfall during both surveys. *Loxodonta africana* and *C. niloticus* were present at the pan during the second survey. During the second survey, algae were clearly visible on the surface of the water. Diatoms were sampled from vegetation that included submerged vegetation.

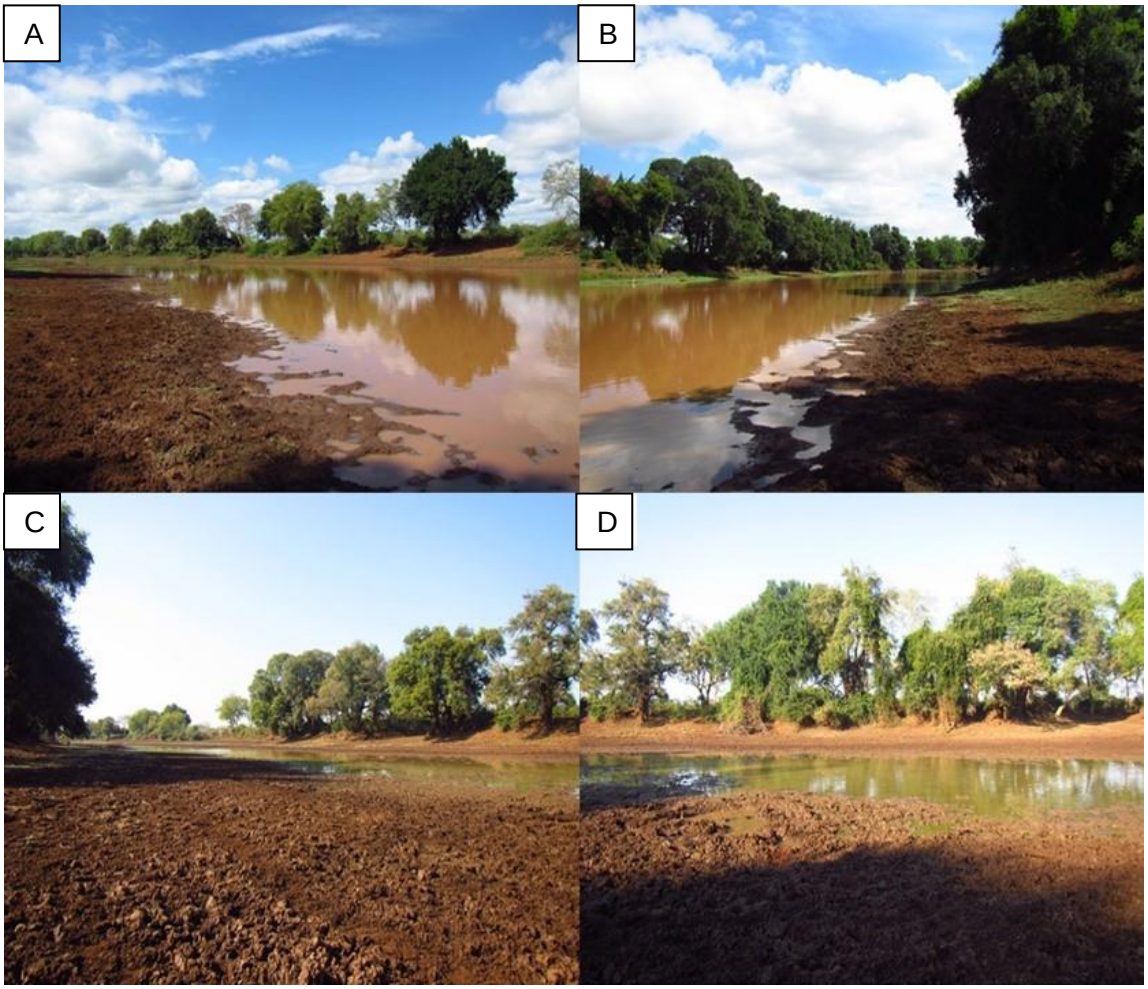

Figure 4-10. Nwambi Pan during April 2015 (A – B) and September 2015 (C – D). (Photo credit: Kelly Shannon Dyamond).

4.2.10 Reedbuck Vlei

Reedbuck Vlei (Figures 4-11 A – D) is located at geographic coordinates S22.41549, E31.27510 and receives its water from the Luvuvhu River. The pan is approximately 1200 m from the Luvuvhu River. Nymphaeaceae (Water lilies) were present in the pan. The area surrounding the pan to the south is open grassland with a *V. xanthophloea* forest situated to the south as well. To the north the area has tree cover and a low gradient higher relief. Diatoms were sampled from vegetation that included water lilies.

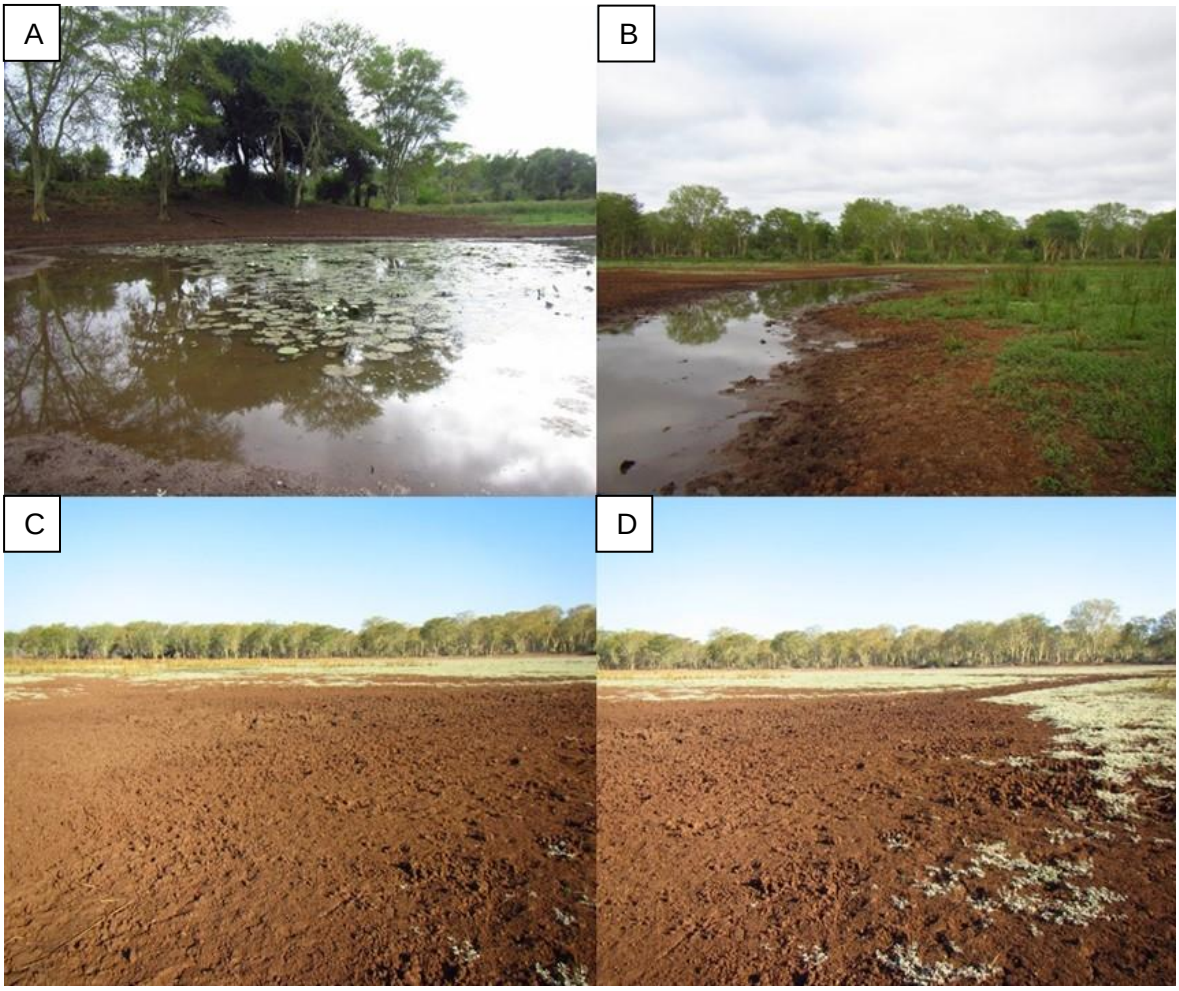

Figure 4-11. Reedbuck Vlei during April 2015 (A – B) and September 2015 (C – D). (Photo credit: Kelly Shannon Dyamond).

4.3 Results

4.3.1 Water quality

Water quality results for the three pans that were sampled twice will be presented as averages. The three pans that were sampled during the dry season are indicated by the number 2 after the pan name i.e. Mapimbi 2. A full table of results can be found in Appendix B (Table B-2).

4.3.1.1 Nutrients

Nutrients analysed included nitrates (NO_3), nitrites (NO_2), phosphates (PO_4) and ammonium (NH_4). Nitrites were not presented graphically as all measurements were below the detection limit of 0.002 mg/L.

The NO_3 concentrations presented in Figure 4-12 (A) indicated that Nwambi Pan had the highest nitrate concentration while Mapimbi Pan had the lowest nitrate concentration. All the pans (except for Mapimbi Pan) had nitrate concentrations higher than 1 mg/L. Pans sampled during the dry season had higher nitrate concentrations than in the wet season. The PO_4 concentrations are presented in Figure 4-12 (B) and it can be noted that the pans sampled during the dry season had the highest phosphate concentrations. Reedbuck Vlei had the lowest phosphate concentration and Nhlangaluwe Pan had the highest phosphate concentration. Figure 4-12 (C) illustrates the ammonium concentrations for the sampled sites. Mapimbi Pan had the highest ammonium concentration with Jachacha Pan having the lowest ammonium concentration. Pans sampled in the dry season had lower ammonium concentrations than in the wet season. All the pans had ammonium concentrations < 1 mg/L, except for Hulukulu, Nwambi and Mapimbi pans.

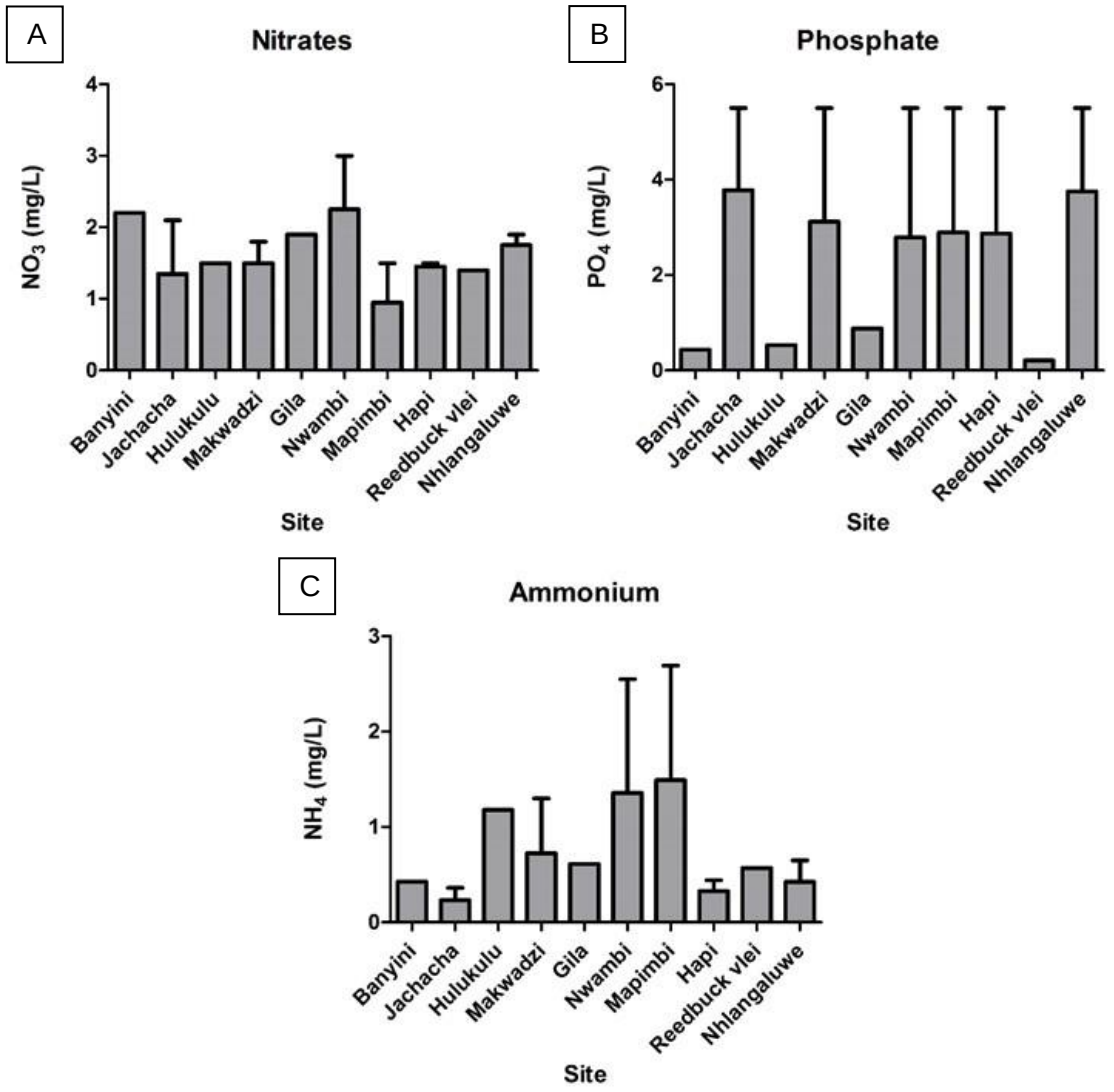

Figure 4-12. Column graphs illustrating the mean and standard error of the mean (SEM) for (A) nitrate, (B) phosphate, and (C) ammonium concentrations (in mg/L) for the ten sites. The sites with SEM values are sites that had water during the dry season.

4.3.1.2 Temperature, pH and percentage oxygen concentration

The temperature values (in °C) measured during the study are presented in Figure 4-13 (A). It can be noted that the sites had similar temperatures. Gila Pan had the highest temperature while Reedbuck Vlei had the lowest temperature. Temperatures were similar for pans sampled during the dry season. From Figure 4-13 (B) it can be noted that Gila Pan had the highest pH while Reedbuck Vlei had the lowest pH level. The pH levels were similar between all of the sites. In Figure 4-13 (C), the percentage dissolved oxygen indicated that the percentage oxygen saturation differed between pans; Gila Pan had the highest percentage dissolved oxygen with Hulukulu Pan having the lowest percentage dissolved oxygen.

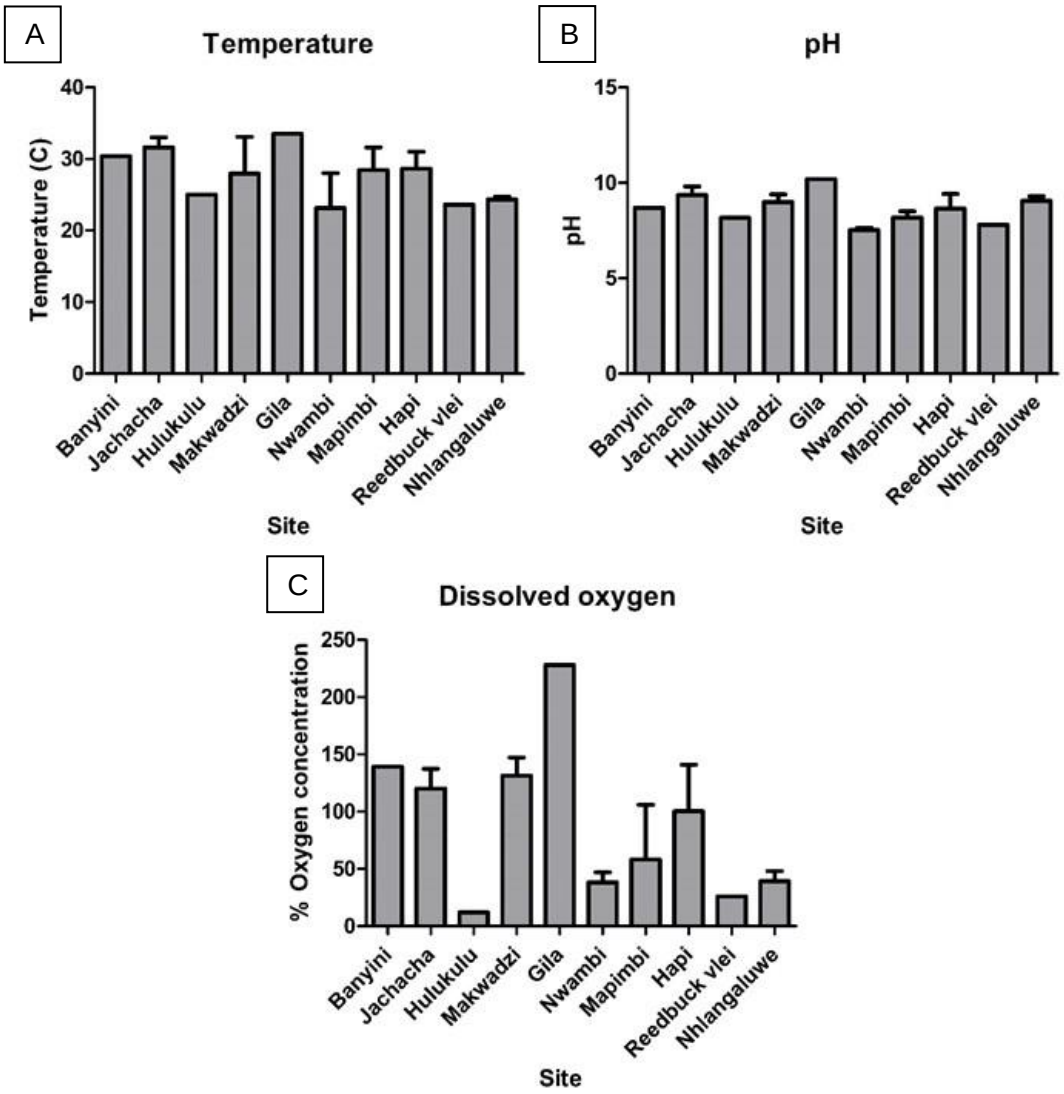

Figure 4-13. Column graphs illustrating the mean and standard error of the mean (SEM) for (A) temperature (°C), (B) pH, and (C) oxygen concentration (%) for the ten sites. The sites with SEM values are sites that had water during the dry season.

4.3.2 Diatom community

In this section all the diatom results will be presented in the form of a species list, univariate diversity indices (Pielou's Evenness and Shannon Index) and multivariate analyses.

4.3.2.1 Diatoms

In Table 4-1, a species list is provided with the taxa identified in the ten pans during the two surveys. Species in bold were dominant species in the wet and dry seasons as well as species that contributed the highest (50 % – 100 % weight) to the CCA (Figure 4-18) results. Diatom counts for the selected pans are presented in Appendix C (Table C-2) with figures showing some identified diatom species are presented in Appendix D.

Table 4-1. Diatom species present in the Makuleke Wetlands from two surveys in April 2015 and September 2015.

Species	**Abbreviation**	**Date described**
Amphora pediculus (Kützing) Grunow	APED	1880
***Aulacoseira granulata* (Ehrenebrg) Simonsen**	AUGR	1979
Caloneis aequatorialis Hustedt	CAET	1962
Cocconeis placentula Ehrenberg	CPTG	1838
Craticula sp. Grunow	CRAT	1868
Craticula accomoda (Hustedt) D.G. Mann	CRAC	1990
Craticula accomodiformis Lange-Bertalot	CACM	1993
Craticula cuspidata (Kützing) D.G. Mann	CRCU	1990
***Cyclotella meneghiniana* Kützing**	CMEN	1844
Cyclotella ocellata Pantocsék	COCE	1902
Cymbella cymbiformis Agardh	CCYM	1830
Diploneis elliptica (Kützing) Cleve	DELL	1891
Discostella pseudostelligera (Hustedt) Houk and Klee	DPST	2004
Eolimna subminuscula (Manguin) Moser, Lange-Bert.and Metzeltin	ESBT	1998
Eunotia formica Ehrenberg	EFOR	1843
***Gomphonema* sp. Ehrenberg**	GOMP	1832
Gomphonema exilissimum (Grunow) Lange-Bertalot and Reichardt	GEXL	1996
Gomphonema insigne Gregory	GINS	1856
Gomphonema lagenula Kützing	GLGN	1844
***Gomphonema parvulum* (Kützing) Kützing**	GPAR	1849
Gomphonema pseudoaugur Lange-Bertalot	GPSA	1979
Gomphonema sp. 2	GOMS	1832
Gyrosigma sp. Hassall	GYRO	1845
Hantzschia amphioxys (Ehrenberg) Grunow	HAFC	1909
***Navicula* sp. Bory**	NAVI	1822
Navicula antonii Lange-Bertalot	NANT	2000
***Navicula erifuga* Lange-Bertalot**	NERI	1985
Navicula germainii Wallace	NGER	1960
Navicula pusilla W.Smith	NPUS	1853
Navicula radiosa Kützing	NRAD	1844
Navicula ranomafanensis (Manguin) Metzeltin and Lange-Bertalot	NRAN	2002
Navicula rhynchocephala Kützing	NRHY	1844
Navicula rosenbergii Oestrup	NRSB	2006
Navicula sp. 1	NASP	1822
Navicula veneta Kützing	NVEN	1844
***Nitzschia* sp. Hassall**	NITZ	1845
Nitzschia acidoclinata Lange-Bertalot	NACD	1976
Nitzschia amphibia Grunow	NAMP	1862
Nitzschia archibaldii Lange-Bertalot	NIAR	1980

Nitzschia capitellata Hustedt	NCTN	1995
Nitzschia dissipata (Kützing) Grunow	NDTG	1862
Nitzschia etoshensis Cholnoky	NETO	1966
Nitzschia filiformis (W.M.Smith) Van Heurck	NFIT	1896
Nitzschia gracilis Hantzsch	NIGR	1860
***Nitzschia hantzschiana* Rabenhorst**	NHAN	1860
Nitzschia intermedia Hantzsch	NINT	1880
Nitzschia irremissa Cholnoky	NIRM	2008
Nitzschia liebetruthii Rabenhorst	NLBT	1864
Nitzschia linearis (Agardh) W.M.Smith	NLIP	1853
Nitzschia microcephala Grunow	NMIC	1878
Nitzschia palea (Kützing) W.Smith	NPAL	1856
Nitzschia paleacea (Grunow) Grunow	NPTG	1881
Nitzschia pusilla (Kützing)Grunow	NIPU	1862
Nitzschia reversa W.Smith	NREV	1853
Nitzschia sigma var. diminuta Grunow	NSDI	1881
Nitzschia sp. 2 Hassall	NIS1	1845
Nitzschia sp. 3 Hassall	NZSS	1845
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	NUMB	1978
Pinnularia subbrevistriata Krammer	PSBV	2000
Pinnularia viridis (Nitzsch) Ehrenberg	PVMI	1891
Placoneis placentula (Ehrenberg) Heinzerling	PLPL	1908
Rhopalodia gibba (Ehrenberg) O.Müller	RGIB	1895
Sellaphora pupula (Kützing) Mereschkowksy	SPUP	1902
Sellaphora stroemii (Hustedt) D.G. Mann	SSTM	2002
Stauroneis anceps Ehrenberg	STAN	1843
Surirella abies Cleve-Euler	SABI	2010
Tryblionella sp. W. Smith	TRYB	1853
Tryblionella calida (Grunow) D.G. Mann	TCAL	1853
***Tryblionella hungarica* (Grunow) D.G. Mann**	THUN	1853
Tryblionella levidensis W.M. Smith	TLEV	1853

4.3.2.2 Diatom indices

The diatom indices indicated in Chapter 2 were determined for the ten pans sampled from the various Makuleke sites. The indices are based on the diatom community composition observed and can be used to determine the quality of these ecosystems. Table 4-2, Table 4-3 and Table 4-4 indicate the interpretation of the diatom index scores which have been provided for the pans and the surveys in Table 4-5.

Table 4-2. Table used to interpret the Generic Diatom Index (GDI) and Specific Pollution sensitivity Index (SPI) score in order to determine the ecosystem quality and trophic level of the ecosystem.

Index Score (up to 20)	Ecosystem quality	Trophic level
> 17	High quality	Oligotrophic
15 – 17	Good quality	Oligo-mesotrophic
12 – 15	Moderate quality	Mesotrophic
9 – 12	Poor quality	Meso-eutrophic
< 9	Bad quality	Eutrophic

Table 4-3. Table used to interpret the Trophic Diatom Index (TDI) score to determine the trophic level of the ecosystem.

Index Score	Trophic level
0 – 20	Oligotrophic
21 – 40	Oligo-mesotrophic
41 – 60	Mesotrophic
61 – 80	Meso-eutrophic
> 80	Eutrophic

Table 4-4. Table used to interpret the percentage Pollution Tolerant Valve (%PTV) to determine the ecological status of the ecosystem.

Index Score	Ecological status
< 20	Site free from organic pollution
21 – 40	Some evidence of organic pollution
41 – 60	Organic pollution likely to contribute to eutrophication
> 61	Heavily contaminated with organic pollution

When studying the SPI scores (Table 4-5), it is clear that Makwadzi Pan had the relatively highest score in comparison to the other sites and surveys and this indicated the pan had poor water quality (Table 4-2). Nwambi Pan had the lowest score indicating a bad quality. From Table 4-5 it can be seen that Jachacha, Hulukulu, Gila, Nwambi, Mapimbi, Hapi, Reedbuck Vlei, Nhlangaluwe, Makwadzi 2 and Hapi 2 pans had SPI scores < 9 signifying the pans to be of bad quality. Banyini, Makwadzi and Mapimbi 2 pans had scores between 9 and 12 indicating poor quality.

For the GDI score (Table 4-5), Makwadzi 2 Pan (dry season) had the highest score while Hapi 2 Pan (dry season) had the lowest GDI score. From the table it can be noted that Banyini, Jachacha, Hulukulu, Gila, Nwambi, Mapimbi, Reedbuck Vlei, Mapimbi 2 and Hapi 2 pans had

GDI scores < 9 indicating a bad quality for these pans (Table 4-2). Makwadzi, Hapi and Nhlangaluwe Pans had scores between 9 and 12 signifying a poor quality. Makwadzi 2 Pan had a score between 12 and 15 indicating a moderate quality.

The TDI values in Table 4-5 showed variance between the sites. Hulukulu Pan had the highest TDI value (52) and Hapi Pan had the lowest value (11). Nwambi, Mapimbi, Mapimbi 2 and Hapi pans had values between 0 – 20 that showed oligotrophic conditions (Table 4-3). Banyini, Jachacha, Makwadzi, Makwadzi 2, Gila, Hapi 2, Reedbuck Vlei and Nhlangaluwe pans' results indicated oligo-mesotrophic conditions and Hulukulu Pan signified mesotrophic conditions.

Table 4-5. Specific Pollution sensitivity Index (SPI), Generic Diatom Index (GDI), Trophic Diatom Index (TDI) and percentage Pollution Tolerant Valve (%PTV) scores for each site over the two surveys in 2015 and 2016. Sites sampled during the dry season are indicated with the number 2.

	Indices			
Sites	**Specific Pollution sensitivity Index (SPI)**	**Generic Diatom Index (GDI)**	**UK Trophic Diatom Index (TDI)**	**Percentage Pollution Tolerant Valve (%PTV)**
Banyini	10.1	8.6	33.5	11.9
Jachacha	7.5	5.5	29.5	23.8
Hulukulu	7.1	9.0	52.0	17.9
Makwadzi	12.0	11.6	30.0	13.9
Makwadzi 2	6.5	12.5	30.0	6.7
Gila	4.5	5.6	24.5	7.2
Nwambi	3.0	7.0	18.0	62.9
Mapimbi	6.4	8.5	16.0	33.9
Mapimbi 2	10.0	4.8	20.0	61.6
Hapi	6.0	9.7	11.0	25.5
Hapi 2	4.5	2.0	26.0	43.3
Reedbuck Vlei	3.5	2.6	34.0	50.3
Nhlangaluwe	4.1	9.7	34.0	2.1

The %PTV values are showed in Table 4-5. Variability was noted in the %PTV between the pans sampled as Banyini, Hulukulu, Makwadzi, Makwadzi 2, Gila and Nhlangaluwe pans had values < 20, that indicated these pans were free from organic pollution (Table 4-4). Some evidence of organic pollution was demonstrated for Jachacha, Mapimbi and Hapi pans. Hapi 2 and Reedbuck Vlei pans had values between 41 and 60 indicating that the eutrophication

was most likely due to organically bound nutrients. Nwambi and Mapimbi 2 pans were contaminated with organic pollution according to the %PTV results.

4.3.2.3 Correlation between diatom indices and water quality variables

In Table 4-6, the correlation between the diatom indices and water variables are presented. From the table it can be seen that SPI had a negative correlation with NO_3, and GDI a negative correlation with temperature. The TDI had a negative correlation with all the variables except NH_4. Values in bold had a significant ($p < 0.05$) (one-way ANOVA) correlation.

Table 4-6. Correlation between diatom indices and water quality variables. Values in bold had a significant ($p < 0.05$) correlation.

WQ variable	SPI	GDI	TDI
PO_4	0.371	0.303	-0.421
NO_3	-0.333	0.184	-0.097
NH_4	0.359	0.162	0.262
Percentage dissolved oxygen	**0.241**	**0.067**	**-0.289**
pH	0.166	0.172	-0.051
Temperature	0.282	-0.058	-0.437

4.3.2.4 Diatom diversity indices

Figure 4-14 (A – D) shows the evenness and diversity indices for the ten pans sampled during 2015 in the Makuleke Wetlands. The species were evenly distributed over the various wetland sites when taking into account the Pielou's evenness score; Makwadzi Pan had the lowest Pielou's evenness score. However, all the scores indicated that no dominance by certain taxa was present. The Shannon diversity between sites was similar across the sites (except for Makwadzi Pan sampled in the dry season) and this indicated similar diversity. However, total species recorded at each site and in each survey ranged from 7 to 18 species. The Margalef species richness differed across sites as Makwadzi 2 Pan had the lowest richness, and Hulukulu Pan the highest species richness.

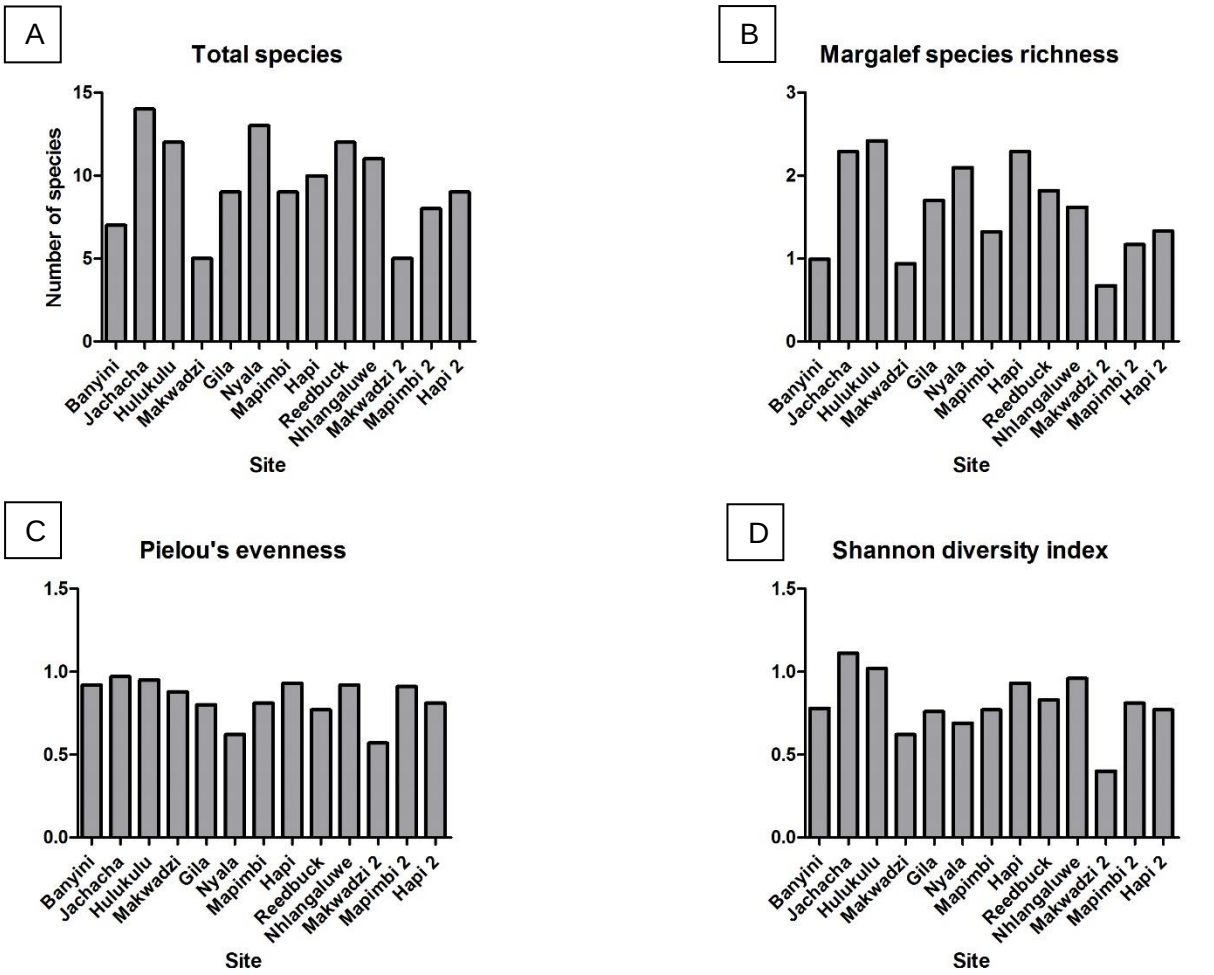

Figure 4-14. Column graphs indicating the (A) total number of species, (B) Margalef species richness, (C) Pielou's evenness, and (D) Shannon diversity index.

4.3.2.5 Spatial and temporal variation

A non-metric multidimensional scaling (nMDS) plot based on the Bray Curtis similarity matrix (Figure 4-15) was drawn to determine diatom community variation between the wet and dry seasons. It can be seen that there was spatial variation between the different sites while there was also temporal variation between the wet season and the dry season. Pans indicated in red in Figure 4-15 were sampled during the dry season and grouped together on the right hand side, while pans indicated in blue were sampled during the wet season and grouped together on the left of the figure. Variation was noted for pans sampled during each season as seasonal samples did not group together.

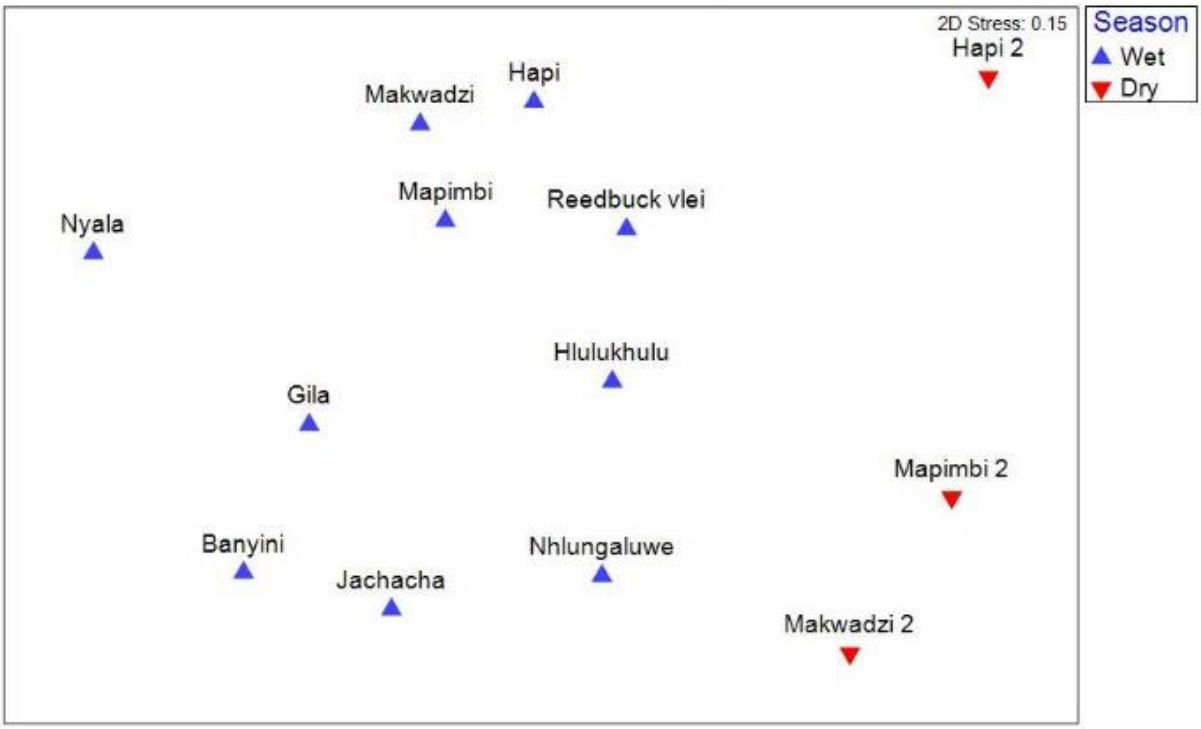

Figure 4-15. Non-metric multidimensional scaling (nMDS) plot based on the Bray-Curtis similarity between the different pans from the different seasons in 2015 from the Makuleke Wetlands.

In Figure 4-16 the relationship between water quality and the pans sampled can be observed. It can be noted that majority of the pans (Group 1) were influenced by temperature and nitrates. Other pans including Makwadzi 2, Hapi 2, Mapimbi 2 (all three sites sampled during the dry season), Jachacha and Nhlangaluwe were grouped together in Group 2 and were influenced by phosphates, pH and ammonium. Both groups were influenced by dissolved oxygen (DO). The first axis explained 60.79 % of the variation and the second axis explained 30.51 % of the variation.

Seasonal variation was noticed in the figure as Makwadzi 2, Hapi 2 and Mapimbi 2 (sites sampled during the dry season) were grouped together in Group 2. This grouping was driven by the higher concentration of phosphates found during the dry season. The majority of the pans sampled during the wet season were grouped together in Group 1 due to the temperature differences.

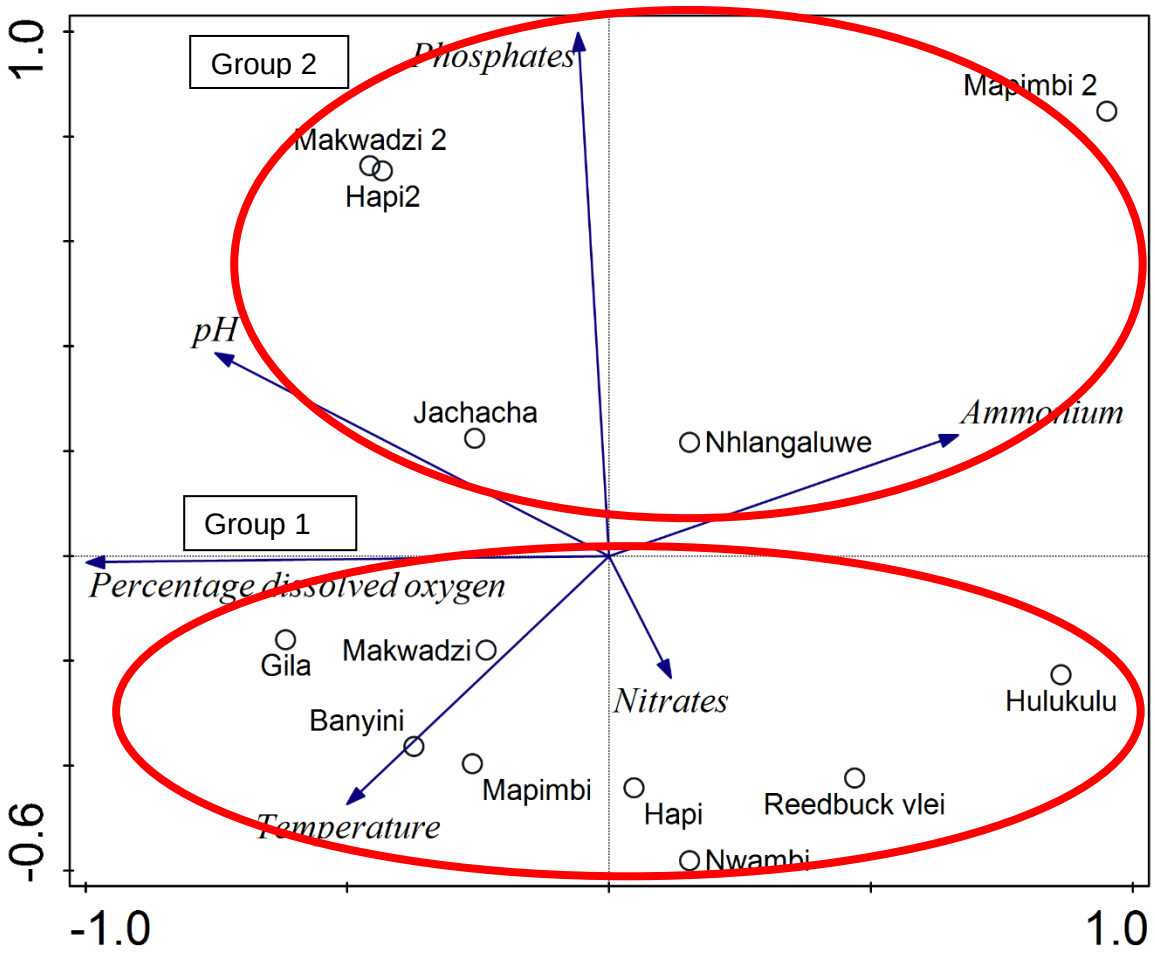

Figure 4-16. Principle component analysis (PCA) biplot illustrating the relationship between water quality and sites sampled for the diatom community from the Makuleke Wetlands for the 2015 sampling surveys.

Figure 4-17 shows the relationship between water quality and diatom indices. The SPI had a negative correlation with phosphates and the GDI had a negative correlation with ammonium and phosphates. The SPI had a positive correlation with ammonium, nitrates and temperature. The GDI had a positive correlation with nitrates and temperature, pH and DO. The TDI had a positive correlation with ammonium and a negative correlation with temperature, pH and DO. The RDA explains 21.4 % of the variation while the first axis explained 16.76 % of the variation and the second axis explained 4.43 % of the variation. A Monte Carlo permutation test indicated that there was no significance in the plot ($p = 0.548$).

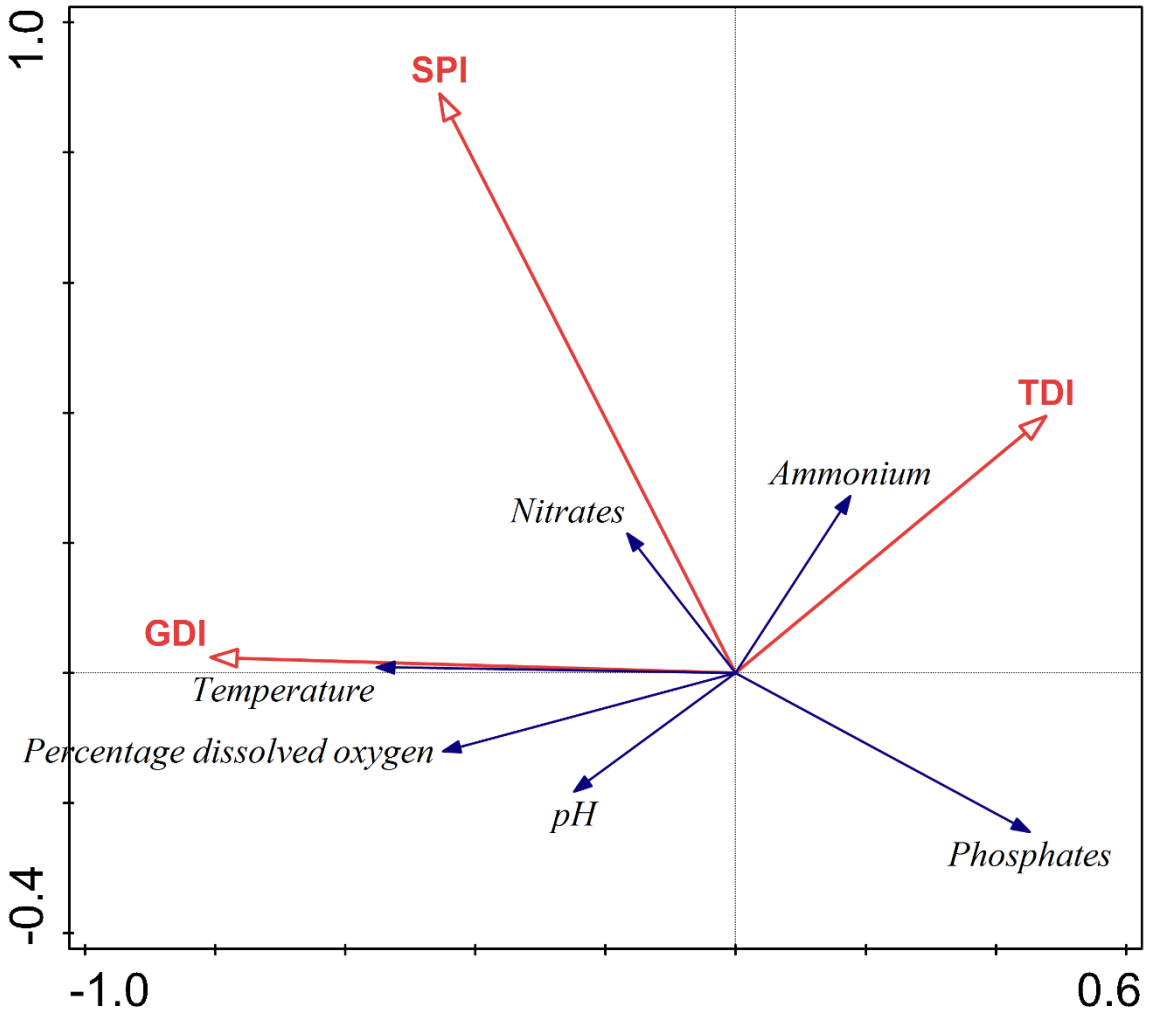

Figure 4-17. Redundancy analysis (RDA) biplot illustrating the correlation between water quality and diatom indices.

In Figure 4-18 a CCA was constructed to determine the relationship between the dominant species (20 % – 100 %) and the water quality variables, specifically the ecosystem and nutrient variables. In the figure, the species were divided into two groups. Group 1 were represented by *Nitzschia* sp. (NITZ), *Gomphonema lagenula* (GLGN), *Pinnularia subbrevistriata* (PSBV), *Navicula* sp. (NAVI), *Cyclotella meneghiniana* (CMEN), *Nitzschia reversa* (NREV), *Nitzschia palea* (NPAL) and *Nitzschia filiformis* (NFIT). Group 1 species are those that are related to higher concentrations of temperature, DO and pH. Group 2 species are represented by *Nitzschia liebetruthii* (NLBT), *Tryblionella hungarica* (THUN), *Nitzschia paleacea* (NPTG), *Navicula erifuga* (NERI), *Gomphonema parvulum* (GPAR) and *Gomphonema* sp. (GOMP). The species in Group 2 included those influenced by higher concentrations of ammonium and phosphate. The CCA had a P value of 0.088 (using a Monte Carlo permutation test) for both axes and explained 52.9 % of the variation; thus, the p value

indicated that the CCA was not significant. The first axis explained 12.36 % of the variation of the CCA and the second axis explained 10.57 % of the CCA's variation.

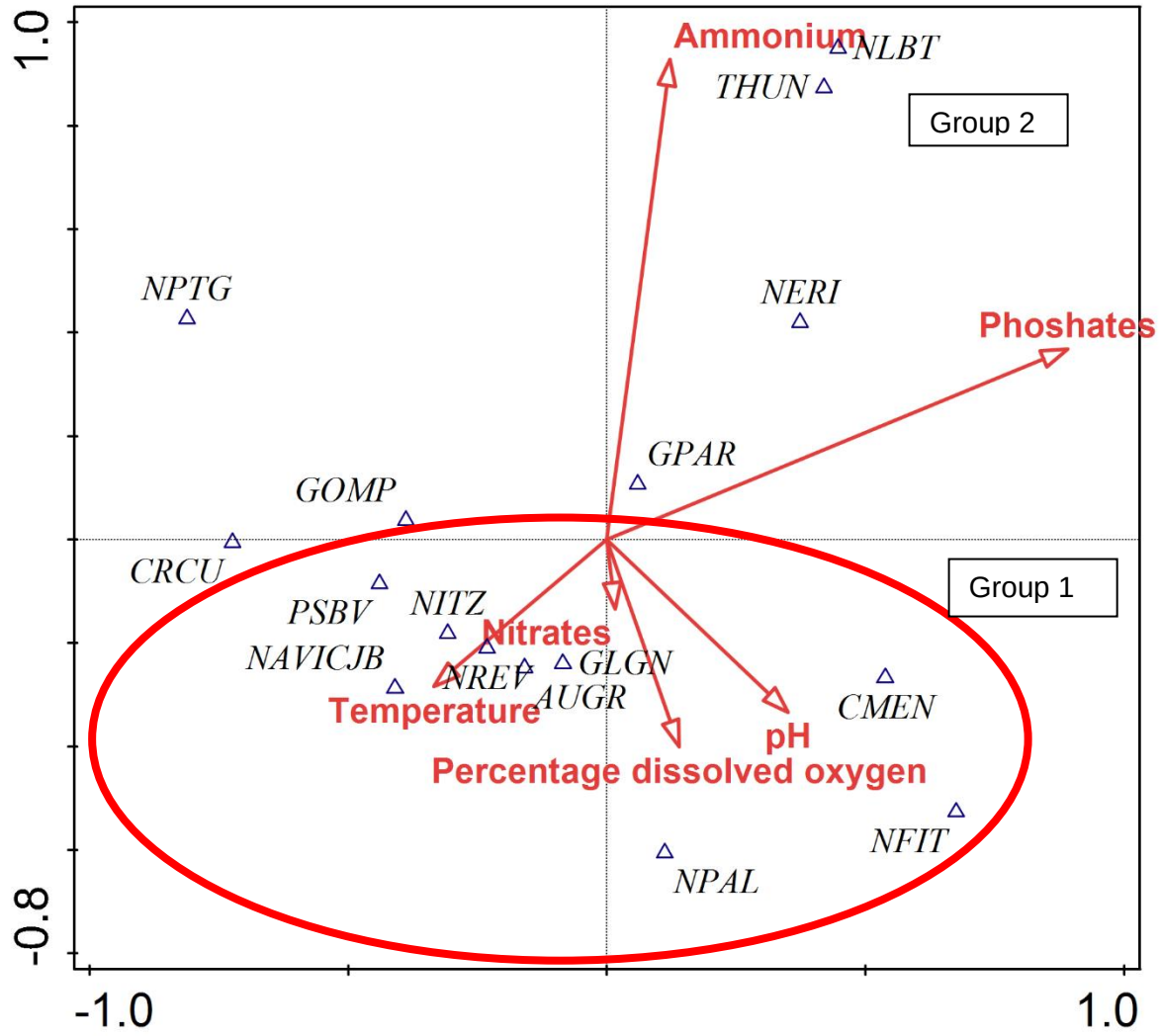

Figure 4-18. Canonical correspondence analysis (CCA) triplot illustrating the correlation between water quality and diatom species identified that had a 20 % – 100 % contribution to the results. (Abbreviations in Table 4-1).

4.4 Discussion

4.4.1 Water quality

All aquatic ecosystems are influenced by water quality variables and numerous environmental variables influence the water quality of the ecosystem. Nutrient concentrations in the ecosystem can cause nutrient enrichment and that can influence the plant growth (Dallas and Day, 2004). Inflow from nutrient enriched ecosystems into the pans influences the final nutrient concentrations of the pans. According to Nesbitt (2014), the regularity and types of inflow that a pan receives impacts the quality of the pan's water. Environmental variables influences the diatom species in the ecosystem as each species has different tolerances to each environmental variable (Dallas and Day, 2004; Dalu and Froneman, 2016). Thus, it can be seen that water variables plays an important role in an aquatic ecosystem.

Nitrogen is one of the more abundant elements found in nature (Dallas and Day, 2004). Nitrogen can be inorganic or bound in organic compounds and most biochemical and biological processes are dependent on it (Dallas and Day, 2004). Nitrite will not be discussed as it was below the detection limit. The nitrate and ammonium will be discussed together as total nitrogen. The total nitrogen was presented in Figures 4-12 (A) and (C) and showed that Jachacha, Mapimbi, Hapi, Reedbuck Vlei, Nhlangaluwe, Makwadzi 2 and Hapi 2 pans had total nitrogen concentrations between 0.5 – 2.5 mg/L, indicating mesotrophic conditions according to South Africa's water quality guidelines for aquatic ecosystems (WQG) (DWAF, 1996). According to the WQG, Banyini, Hulukulu Makwadzi, Gila, Nwambi and Mapimbi 2 pans were in a eutrophic condition as the total nitrogen concentrations were between 2.5 – 10 mg/L. It should be noted that the WQG (DWAF, 1996) were designed for riverine systems and are not wetland specific. Recorded phosphate concentrations (Figure 4-12 B) for Nwambi, Hapi and Reedbuck Vlei pans were between 25 – 250 µg/L which were an indication of eutrophic conditions according to the WQG. Banyini, Jachacha, Hulukulu, Makwadzi, Gila, Mapimbi, Nhlangaluwe and pans sampled during the dry season (Makwadzi 2, Mapimbi 2 and Hapi 2) had phosphate concentrations > 250 µg/L indicating hypertrophic conditions. The three pans sampled during the dry season had the highest phosphate concentrations (as seen previously).

In order to determine the limiting nutrient in the ecosystem the ratio between the nitrogen and phosphorous (N:P) is determined (Kelly, 2001). According to Kelly (2001), the N:P (Redfield ratio) for optimum cell growth is 7:1. The N:P ratios for the current study ranged from 0.2 – 6.5:1. As the ratios are < 5 (except for Banyini, Hapi and Reedbuck Vlei pans) the limiting nutrient in these systems was nitrogen (Kelly, 2001). From the data it can be seen that the

pans have increased nutrients, especially phosphate, which can be expected for pans as nutrient retention is one of the key processes of a wetland (Kotze, 2010). Dallas and Day (2004), Malan and Day (2012) and Dalu and Froneman (2016) explained that, unlike rivers which have flowing water, a wetland environment accumulates materials brought in by humans, water and wind thus acts as a "sink". One of the reasons for the increase in nutrients is due to the fact that there is little water flow out of the basin and therefore wetlands, for the most part, can be considered to have stagnant water (Matlala, 2010). Another reason might be due to decaying plants as well as animal faeces (Davis *et al.*, 2003; Nesbitt, 2014). The higher concentration recorded during the dry season survey could be due to lower water levels, thus the nutrients are more concentrated (Malan and Day, 2012; Nesbitt, 2014). Malan and Day (2012) stated that there is a correlation between ecological categories (the water source's ecological conditions) and the concentration of nutrients.

A study conducted by Smit *et al.* (2013) and Gerber *et al.* (2015) focussed on the Olifants and Luvuvhu Rivers in the KNP. Their study on the Luvuvhu River was to determine if the water quality of the river and the pans studied were comparable. They found that the measured water quality variables (for aquatic ecosystems) were within the target water quality range. There were no changes between flow periods in the concentration of nitrate, nitrite, ammonium and orthophosphate (orthophosphate is a form of phosphorus that aquatic biota utilise directly) (Smit *et al.*, 2013). Gerber *et al.* (2015) found that the water quality did not improve as it flowed through the KNP from the theoretical polluted western boundary to the eastern boundary. During their study, Smit *et al.* (2013) found that between 2009 and 2010 the nitrate concentrations increased from an oligotrophic to a hypertrophic state. Recorded phosphate concentrations for 2009 – 2011 indicated hypertrophic conditions (Smit *et al.*, 2013).

Temperature plays an important role in an aquatic ecosystem as the chemical reactions and metabolic rate of organisms are influenced by it (DWAF, 1996). Temperature is influenced naturally by many factors such as ground water contributions, source of the water flow rate, season and altitude (Dallas and Day, 2004). Optimum growth and reproduction of each species are at different temperatures (Dallas and Day, 2004). It can be noted that fluctuations in temperature between the three pans sampled in the both seasons were within the natural range i.e. temperature did not fluctuate more than 10 °C (DWAF, 1996). As temperatures (Figure 4-13 A) were only recorded once for most pans it is difficult to determine if the temperatures (recorded during each season) were within natural limits and to determine the fluctuation between seasons. The WQG states that it would be ideal to measure the temperature over the course of 24 hours to establish variations (DWAF, 1996).

Hydrogen ion concentration in water determines the pH of a system (DWAF, 1996; Dallas and Day, 2004). Some of the most important ions that impact the water quality of an ecosystem include hydrogen and hydroxyl ions (Dallas and Day, 2004). According to Dallas and Day (2004), the bioavalability of compounds, such as nutrients, is affected by changes in the pH. Thus, the higher pH values for Gila, Makwadzi and Hapi Pan — which had pH levels > 9 — may be due to the nutrient enrichment. Algae were clearly visible on the surface of Gila Pan. Increased biological activity (in a eutrophic ecosystem) can cause elevated pH values (DWAF, 1996) which will increase the dissolved oxygen and decrease the carbon dioxide in the ecosystem. Malan and Day (2012) stated that the relationship between pH and environmental conditions is complex, thus the environmental conditions of a wetland should not be assessed through pH alone.

Aquatic organisms make use of dissolved oxygen (DO) in the water as a supply of oxygen (DWAF, 1996; Dallas and Day, 2004). Jachacha, Makwadzi and Mapimbi Pan had DO levels between 80 – 120 % indicating normal conditions for life according to the WQG. As stated in the WQG, DO levels between 40 % and 60 % are indications of lethal conditions and pans with these concentrations included Nwambi, Hapi and Nhlangaluwe Pans. Hulukulu, Reedbuck Vlei and Mapimbi 2 pans had DO levels < 40 % which are lethal concentrations according to the WQG. Banyini, Gila, Makwadzi 2 and Hapi 2 pans had DO levels > 120 %. According to DWAF (1996), DO levels < 100 % indicates that the system is depleted of DO and levels > 100 % indicates eutrophication. As the percentage dissolved oxygen fluctuated between pans it is difficult to draw a conclusion based on the previous statement. Dissolved oxygen fluctuates due to the time of day the measurement was taken. Photosynthesis increases as the water temperature increases, thus influencing the DO levels in the pans. Malan and Day (2012) also stated that a wetlands environmental condition does not correlate with DO. Thus, the quality assessment of these wetland pans will not be based on the measured DO.

According to DWAF (1996), Dallas and Day (2004) and Matlala *et al.* (2011) the water quality variables should be measured over a period of time and not as a once off measurement as this only provides a snap shot interpretation of the ecosystems quality at the time of measurement. It is thus not recommended to base the health assessment of the ecosystem on these water quality variables alone. Therefore, it is suggested to base the health of the aquatic ecosystem on the diatom community in the ecosystem. The diatoms are continuously exposed to the environmental conditions and as each species prefers specific environmental conditions, the effects of pollution can be determined over a period of time (de la Rey *et al.*, 2004; Matlala, 2010; Dalu and Froneman, 2016).

4.4.2 Diatom communities

The integrity of an aquatic ecosystem can be determined by the organisms present in the ecosystem (Dallas and Day, 2004). One of the groups of organisms that can be used as bio-indicators are diatoms as they are single-celled, have a short life span, and respond rapidly to environmental changes (See Chapter 1) (Taylor *et al.*, 2005; Dalu and Froneman, 2016). It is clear that each species has specific tolerances to environmental factors and the relationship between diatoms and environmental factors is understood (Potapova, 2003; Harding *et al.*, 2005). From this, the quality of the system can be determined based on the species found within the environment. As the water quality conditions changes, species that are more resistant to these changes will thrive and become dominant, while species that cannot tolerate these changes will disappear (Potapova, 2003; Harding *et al.*, 2005; Matlala *et al.*, 2011). The dominant species present in the system can thus be used to determine the quality of the system (Dalu and Froneman, 2016). Malan and Day (2012) stated that using diatom assemblages when monitoring wetlands provides a more comprehensive indication of the nutrient concentrations in a wetland system.

Limited information is available on the diatom community in the area, with no known information on the diatom community in the Luvuvhu River. However, a Limpopo Watercourse Commission (LIMCOM) survey done in 2013 studied the Limpopo River and included diatom studies on the river. Diatom data were retrieved from Mark Graham (Ground Truth) and analysed by Dr. Jonathan Taylor (North-West University). Their study identified 77 diatom species over eight sites. The currently study identified 36 diatom species not identified in their study on the Limpopo River. Thus half of the species may be introduced into the wetlands from the Limpopo River. From the 70 diatom species in this study, no scarce species were identified.

From Figure 4-18, it can be noted that the diatom species were divided into two groups. In Group 1 were species associated with higher concentrations of nitrates and temperature. The species found in Group 1 (*Nitzschia* sp., *Navicula* sp., *Aulacoseira granulata* and *Cyclotella meneghinana*) are species found in nutrient enriched ecosystems and they are tolerant of polluted conditions (Taylor *et al.*, 2007). *Gomphonema parvulum, Navicula erifuga* and *Tryblionella hungarica* are in Group 2 and are all species tolerant of extremely polluted conditions (Taylor *et al.*, 2007). Other species included *Craticula cuspidata* and *Nitzschia palea,* both species that are tolerant to polluted water (Taylor *et al.*, 2007).

The above mentioned species are species tolerant of nutrient enrichment and polluted conditions. In Table 4-1 the dominant species are indicated in bold and include *Aulacoseira granulata, Cyclotella meneghiniana, Gomphonema parvulum, Gomphonema* sp., *Navicula* sp., *Navicula erifuga, Nitzschia* sp., *Nitzschia hantzschiana* and *Tryblionella hungarica*. These species are tolerant of polluted water and found in nutrient enriched water (Taylor *et al.*, 2007).

From the CCA (Figure 4-18), and the preceding paragraph, it can be seen that the species found in the wetland are tolerant of polluted water and thus indicated that the system had poor water quality. This observation agrees with the nutrient concentrations (discussed in previous section) which also indicated a nutrient enriched ecosystem.

In Figure 4-15 and Figure 4-16 it can be noted that there were seasonal differences between the sites. From Figure 4-16 it can be seen that majority of the pans sampled in the wet season (April 2015) were grouped together and the three pans sampled in the dry season (September 2015) were grouped together. During the warmer months the water temperature increases and the dissolved oxygen decreases which has an effect on the aquatic communities (Dallas and Day, 2004). It can be seen from Figure 4-16, that the sites sampled in the dry season were weakly influenced by temperature and temperature had a strong influence on the sites sampled in the wet season. The dominant species for each season were determined with the aid of the software package Primer Version 7. Dominant species identified during the wet season (April 2015) included *Aulacoseira granulata, Gomphonema parvulum, Navicula* sp. and *Nitzschia* sp. Dominant species identified during the dry season (September 2015) included *Navicula erifuga, Nitzschia hantzschiana* and *Tryblionella hungarica.*

4.4.3 Diatom indices

The software package, Omnidia, was used to calculate the diatom indices. The indices' scores are based on diatoms sensitivity to environmental conditions (Blanco *et al.,* 2012). Thus, the water quality can be determined based on the dominant diatom community present, as a decline in water quality will cause a decline in the sensitive taxa's diversity (Blanco *et al.*, 2012). The diatom indices were used to determine the water quality based on the diatom community identified in the ecosystem. For the Makuleke Wetlands, the GDI, SPI and TDI indices were used as the calculation of the index scores included more than 80 % of the species encountered in the analysis.

Table 4-5 shows the index scores for all the studied pans in the Makuleke system. It can be noted that scores of both the GDI and SPI values are low, indicating that all the pans have

either poor or bad water quality. The pans are classified as either meso-eutrophic or eutrophic. Thus, these results correspond to the water quality results for the measured variables that indicated the water quality was poor; in addition the dominant species present in the ecosystem are tolerant of polluted waters and occur in eutrophic waters strengthening this conclusion. Both these indices are based on diatoms sensitivity to nutrient concentrations in the system as they are indications of trophic levels.

The TDI and %PTV values for the pans are presented in Table 4-5. TDI values varied between pans with pans classified as either oligotrophic, oligo-mesotrophic or mesotrophic. The TDI values do not correspond with the SPI and GDI indices or with the dominant species (which indicates nutrient enriched ecosystems). The TDI index has to be carefully interpreted as medium to long-term environmental changes that are unrelated to nutrients, influence the diatom community (Kelly, 1995). The TDI index has to be used together with the %PTV index, as the %PTV indicates whether nutrient enrichment or organic pollution are responsible for the TDI score (Kelly and Whitton, 1995). According to the %PTV values, Nwambi, Mapimbi 2, Hapi 2 and Reedbuck Vlei had organic pollution, with Jachacha, Mapimbi and Hapi pans showing some evidence of organic pollution. Banyini, Hulukulu, Makwadzi, Makwadzi 2, Gila and Nhlangaluwe pans were free from organic pollution. The pans that indicated evidence of organic pollution were pans with high densities of vegetation. Decaying plant matter may be the cause for the organic pollution in these pans. As mentioned in the previous sections, the two rivers (Limpopo and Luvuvhu Rivers) introduce nutrients into the wetlands and may introduce organic pollution as well. Thus the organic pollution in the pans may be caused by inflow from the rivers.

Seeing that the indices are based on diatoms sensitivity to nutrients and nutrient changes in the environment, it is expected that there would be a negative correlation between the variables for the SPI and GDI indices. A positive correlation is expected between the TDI and environmental variables as a decrease in quality should cause an increase in the TDI. This negative correlation was only noted for SPI and NO_3. From the water quality results it was noted that all the water variables were within normal ranges except for total nitrogen and phosphate. The total nitrogen and phosphate concentrations were elevated and indicated eutrophic and/or hypertrophic conditions. In Figure 4-17 the opposite results were noted as SPI had a negative correlation with PO_4. As seen in Figure 4-17, GDI had a negative correlation with PO_4 and NH_4 suggesting that phosphate is one of the primary drivers in the Makuleke wetlands in terms of determining water quality. It should be noted that organic nitrogen was not measured in this study and dominant diatoms in the wetlands are indicators

of the presence organic nitrogen. The TDI had a positive correlation with nitrogen and phosphate. This was expected as higher nutrient values will increase the TDI index.

4.5 Conclusion

The focus in Chapter 4 was on the water quality and diatom community of the Makuleke Wetlands. As the Makuleke Wetlands are situated in a conservation area (Kruger National Park) it was expected that the water conditions would be pristine and a high diversity of diatoms would be present in the system. However, when studying the water quality data the nitrates indicated the system to be in a mesotrophic condition. Phosphate levels indicated either eutrophic or hypertrophic conditions, and according to the Redfield ratio, it was suggested that nitrates were the limiting nutrient in the wetland. The high levels of nutrients may be due to animal faeces, decaying plant and animal matter, or from inflow from the Luvuvhu River as the Luvuvhu River has increased nutrient levels.

Studying the diatom community present in the wetland it was seen that there was a moderate diversity of diatoms in the system, with a total of 70 diatom species identified. The dominant diatom species identified were those tolerant to pollution and occurring in nutrient enriched water. This agrees with the nitrate data which indicated that the system was enriched with nutrients. The diatom indices also classified the system as meso-eutrophic or eutrophic. From this data, it is seen that the system is enriched with nutrients and is in a bad quality state.

It can be concluded that the Makuleke Wetlands are enriched with nutrients as demonstrated by the measured water quality variables and diatom community. However, Malan and Day (2012) stated that little research has been done on the nutrient levels of wetlands. It is thus difficult to determine whether the wetland is polluted or if these increased nutrient levels are normal for the ecosystem as the increase in nutrients is expected for wetlands due to their key process of nutrient retention (Kotze, 2010).

Chapter 5 — Conclusion and Recommendations

5.1 Conclusion

Both the Makuleke Wetlands and Lake Sibaya are important ecosystems as they provide ecological services in their direct environment. The Makuleke Wetlands and Lake Sibaya are two Ramsar wetlands of international importance in South Africa (SA). The Makuleke Wetlands are situated in the Kruger National Park (KNP), a conservation area, and support an abundance of biodiversity including rare and endangered species. Lake Sibaya, situated in the north-east corner of KwaZululu-Natal, is SA's largest natural freshwater lake. Lake Sibaya provides a source of water and food for local residents and also supports the development of forestry and the town of Mseleni. However, little biodiversity information is known on SA's Ramsar wetlands with limited sampling completed on the Makuleke Wetlands and Lake Sibaya, especially for diatoms.

Wetland ecosystems are found across the world in all climatic regions and are important water bodies as they provide crucial ecological services. Wetlands support an abundance of biota and in South Africa many people are dependent on wetland ecosystems. People rely on these systems for their immediate resource needs, such as water and food. Thus, the importance of wetlands cannot be overlooked.

Wetlands are ecosystems that are susceptible to pollution and nutrient enrichment as they are areas where water and sediment accumulate (Dalu and Froneman, 2016); also one of the key features of wetlands is nutrient retention (Kotze, 2010). The Makuleke Wetlands mainly receive its water from catchments outside of the KNP (Limpopo and Luvuvhu Rivers) and are thus threatened by anthropogenic activities such as agriculture, mining and the spraying of pesticides. Due to Lake Sibaya's endorheic nature, and the increase in forestry and rural development, it is susceptible to pollution. The importance of wetland monitoring can thus not be ignored. As these wetlands are threatened by anthropogenic activities, both inside and outside their catchment areas, it is necessary to routinely monitor these wetlands.

Information on the complete spectrum of water quality impacts is necessary for monitoring of an aquatic ecosystem. As organisms in an aquatic ecosystem are continuously exposed over a long period of time to the pollutants and environmental variables in the aquatic ecosystem, they have proved to be a valuable tool for monitoring of the ecosystem (Dallas, 2000). Diatoms are valuable bio-indicators as they provide information on the medium-term conditions of the ecosystem and they respond rapidly to unexpected changes (Li *et al.*, 2010; Stevenson *et al.*,

2010). The diatom community structure and diatom indices provide information on the nutrient and trophic levels of the system and can thus be implemented to monitor the water quality of the ecosystem.

Diatoms, together with water, were sampled from both wetlands during different seasons. Lake Sibaya was sampled during a winter (August 2015) and two summer (December 2015 and February 2016) seasons. The Makuleke Wetlands were sampled during a wet (April 2015) and a dry (September 2015) season. In the preceding chapters, each wetland's water quality and diatom community was analysed and the interaction between the water quality and diatom communities were determined.

Nutrient analysis of Lake Sibaya (Chapter 3) indicated that all four of the study sites were enriched with nutrients, with the total nitrogen concentrations indicating mesotrophic conditions and the phosphate concentrations indicating hypertrophic conditions. The ecosystem was phosphate driven according to the N:P ratio for the ecosystem (Kelly, 2001). The Specific Pollution sensitivity Index (SPI) and Generic Diatom Index (GDI) indicated a declining ecosystem and both indices showed a negative correlation with the measured water quality variables. Both the SPI and GDI indicated the wetland was either in a mesotrophic or meso-eutrophic condition. The Trophic Diatom Index (TDI) indicated the ecosystem was either mesotrophic or meso-eutrophic and the associated percentage Pollution Tolerant Valves (%PTV) indicated that all sites were free of organic pollution. A report by DWS (2015a and b) highlighted nutrient enrichment in the ecosystem's sediment, with the southern basin (LS3) and western arm (LS4) the highest due to the development of rural areas and forestry. The poor water quality and nutrient enriched ecosystem was accompanied by the presence of diatom species (*Cocconeis placentula, Gomphonema* sp. and *Navicula* sp.) which are tolerant to pollution and present in nutrient enriched ecosystems (Taylor *et al.,* 2007).

Seasonal variation in the diatom community was noted for Lake Sibaya as summer (December and February) samples grouped together while the winter (August) samples were grouped separately. This temporal variation suggested seasonal differences in community structure. A relatively low number of taxa (59 species) were identified for Lake Sibaya during these surveys compared to Archibald (1966) who identified 107 species. Archibald (1966) described nine new species one of which (*Amphora lacustris* Archibald) was observed in the present study.

Water quality analysis of the Makuleke Wetlands (Chapter 4) water samples indicated a nutrient enriched ecosystem. The measured total nitrogen concentrations showed the pans as either in a mesotrophic or eutrophic condition. Measured phosphate concentration

indicated either eutrophic or hypertrophic conditions were found in the various pans. The N:P ratio for the wetland showed that total nitrogen was the limiting nutrient in the ecosystem (Kelly, 2001). Both the SPI and GDI indicated a poor/bad water quality for the wetland and classified the wetland's trophic level as either a meso-eutrophic or eutrophic condition. The SPI and GDI had a negative correlation with water quality variables. Smit *et al.* (2013) reported nutrient enrichment in the Luvuvhu River, which potentially contributed to the elevated nutrient concentrations in these pans. The TDI values varied between pans and contradicted the SPI and GDI. Measured TDI classified the pans as oligotrophic, oligo-mesotrophic or mesotrophic, however, the TDI for some pans corresponded with the measured total nitrogen indicating a mesotrophic trophic level. Variability was noted between the %PTV for the pans which indicated pans as either polluted with organic matter or free from organic pollution. Pans impacted by organic pollution included those with high densities of vegetation, thus plant decay could have contributed to this result. Animal decay and the addition of faeces could have resulted in an increase in the organic pollution as well.

The observation of the decline of the quality of the ecosystem in the Makuleke wetlands — especially in terms of nutrient enrichment — was supported by the identified diatom community. Recorded diatom species (*Aulacoseira granulata, Gomphonema parvulum, Gomphonema* sp., *Navicula* sp.*, Nitzschia* sp.) are species associated with nutrient enriched ecosystems and are tolerant of polluted conditions (Taylor *et al.,* 2007). Temporal variation was observed between samples collected during the dry and wet seasons suggesting differences in community structure between seasons. Moderate diatom diversity was recorded for the Makuleke Wetlands with 70 species identified. Diatom communities identified differed between pans suggesting different ecological functioning in each pan.

As little information is known on diatom communities in wetlands and as wetlands act as 'sinks' where nutrients accumulate (Malan and Day, 2012), it is difficult to determine if a nutrient enriched wetland is natural or polluted. Also, diatom indices were developed for riverine ecosystems further complicating the interpretation of diatom index scores as a tool to indicate the health of a wetland ecosystem. Further studies are thus required into diatoms as bio-indicators for monitoring wetlands (Malan and Day, 2012) and in terms of determining base-line condition or desired states for pans.

The first hypothesis that Lake Sibaya will be impacted due to the development of rural areas and forestry development was accepted. The diatom community together with water quality variables indicated the lake as nutrient enriched (Chapter 3).

The second hypothesis states, that Makuleke Wetlands will be impacted as the wetlands receive water from catchments outside of the KNP (Limpopo and Luvuvhu Rivers). The hypothesis was accepted as the wetland had increased nutrients as indicated by the water quality analysis and diatom community (Chapter 4).

The diatom community for both wetlands was studied in order to determine if the wetlands were impacted as hypothesised. Thus, the first aim was achieved as the diatom distribution and community structure are documented for both wetlands. A correlation between water quality and the distribution and occurrence of diatoms was successfully determined.

The third hypothesis states, diatoms will successfully be implemented to assess the quality of the two wetland ecosystems. The hypothesis was accepted as the diatom community and indices at both wetlands corresponded with the water quality indicating the ecosystems as nutrient enriched.

As the water quality for both wetlands was effectively determined through use of diatoms as bio-indicators — as addressed in the third hypothesis — the second aim was achieved with limitations. The trophic state (oligotrophic, mesotrophic and eutrophic) of the wetlands was successfully determined with the European diatom-based indices, however, the ecosystem quality was not accurately determined by the diatom indices. The indices corresponded with measured water quality variables and indicated the trophic state of these ecosystems; however, it did not necessarily indicate the quality of the ecosystem. Research by Matlala (2010) and Matlala *et al.* (2011) had similar findings in their study on diatom based biomonitoring of wetland ecosystems.

5.2 Recommendations

The following recommendations are proposed from this study:

- Further studies are required when implementing diatoms as bio-indicator organisms for monitoring wetland ecosystems. A wetland must be classified according to their ecosystem quality rather than trophic level, as nutrient enriched wetlands are not necessarily as a result of anthropogenic impacts.

- Little is known about the diatom community of wetland ecosystems, thus the diatom diversity of the Makuleke Wetlands and Lake Sibaya needs to be investigated further. More study sites should be sampled together with samples over different seasons. It would be recommended to not sample during a dry season.

- Long-term monitoring of the physical-chemical variables of these wetlands is required in order to establish the baseline conditions for these wetlands. Coupled to the water quality monitoring it is suggested that more routine diatom monitoring is implemented to further investigate the occurrence and distribution of diatoms in the wetlands.

- Lastly, diatom community response and water quality for wetlands needs to be established in order to determine the ecosystem health of wetlands. Due to the nature of wetlands, it is difficult to determine whether the ecosystem is natural or polluted.

Chapter 6 — References

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Appendix A

Below weather maps of Lake Sibaya during July 2014 to November 2015.

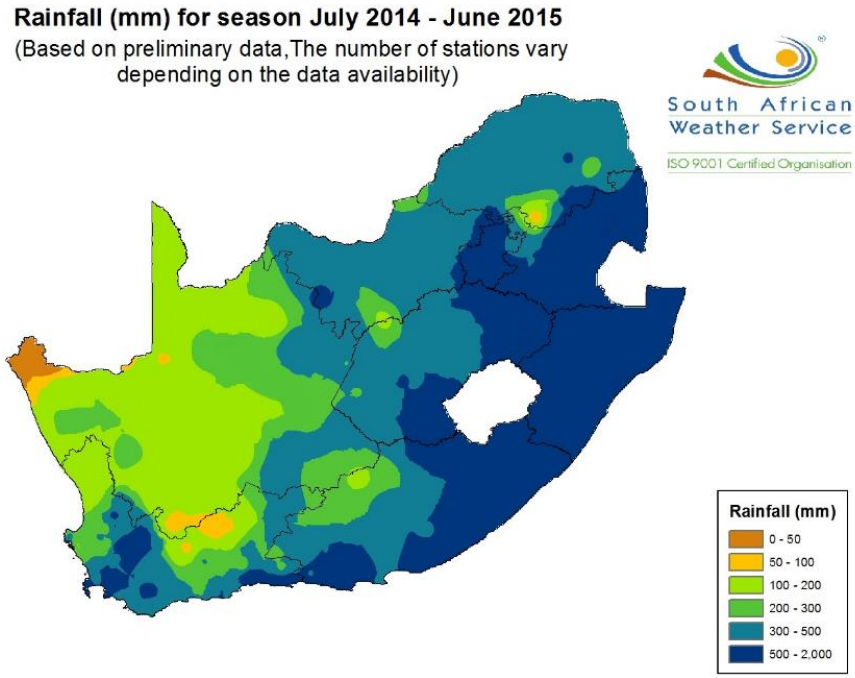

Figure A-1. Map indicating the rainfall (in mm) for South Africa during July 2014 – June 2015. (Source: SAWS, 2016).

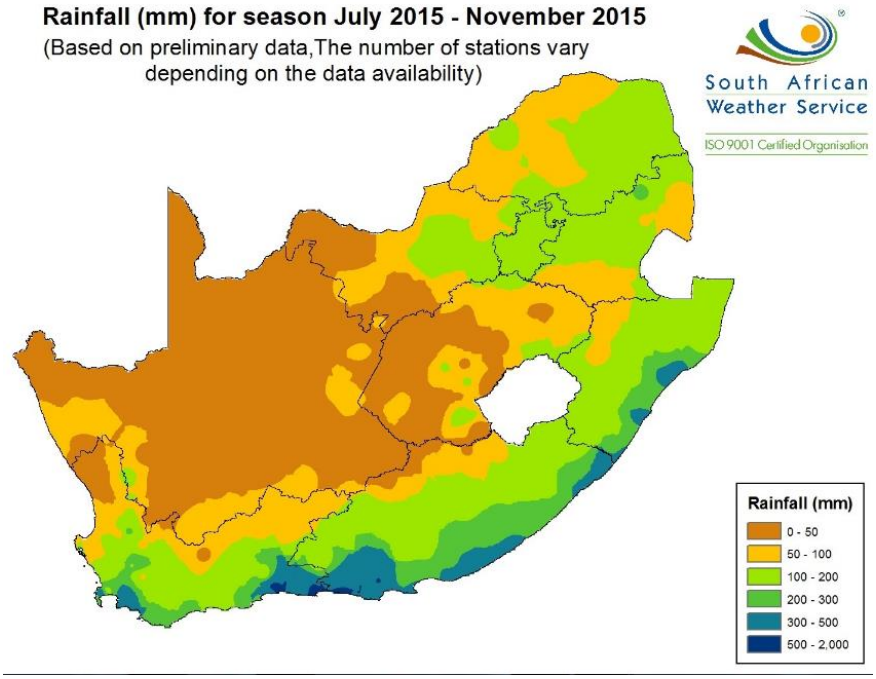

Figure A-2. Map indicating the rainfall (in mm) for South Africa during July 2015 – November 2015. (Source: SAWS, 2016).

Appendix B

Below are included the raw water quality data for Lake Sibaya (Table B1) and Makuleke Wetlands (Table B2).

Table B-1. Water quality data for Lake Sibaya during three surveys in 2015 and 2016. The number after Lake Sibaya indicates the site and the number in brackets indicates the survey number i.e. Lake Sibaya 1 (1) indicates site 1 survey 1.

	Site		
	Lake Sibaya 1 (1)	**Lake Sibaya 1 (2)**	**Lake Sibaya 1 (3)**
Dissolved Oxygen (%)	73.1	75.2	73.8
pH	8.85	8.8	8.72
Temperature (°C)	22.6	20.1	22.9
Phosphates (mg/L)	0.42	2.40	2.33
Nitrates (mg/L)	< 1	1	< 1
Nitrites (mg/L)	0.003	0.070	0.050
Ammonium (mg/L)	0.06	0.16	0.15
	Lake Sibaya 2 (1)	**Lake Sibaya 2 (2)**	**Lake Sibaya 2 (3)**
Dissolved Oxygen (%)	66	63.6	67.2
pH	8.66	8.53	8.75
Temperature (°C)	23.2	21.6	23.8
Phosphates (mg/L)	0.34	2.40	0.73
Nitrates (mg/L)	0.8	0.8	< 1
Nitrites (mg/L)	0.002	< 0.010	< 0.010
Ammonium (mg/L)	0.07	0.8	0.14
	Lake Sibaya 3 (1)	**Lake Sibaya 3 (2)**	**Lake Sibaya 3 (3)**
Dissolved Oxygen (%)	64.5	63.9	64
pH	8.67	8.55	8.69
Temperature (°C)	21.7	21	22.6
Phosphates (mg/L)	0.21	2.33	0.73
Nitrates (mg/L)	< 1.0	< 1	< 1
Nitrites (mg/L)	0.002	< 0.010	< 0.010
Ammonium (mg/L)	0.07	0.12	0.11
	Lake Sibaya 4 (1)	**Lake Sibaya 4 (2)**	**Lake Sibaya 4 (3)**
Dissolved Oxygen (%)	53.1	52.1	54.3
pH	9.4	9.2	9.35
Temperature (°C)	21.5	20.4	22.8
Phosphates (mg/L)	0.23	0.73	2.40
Nitrates (mg/L)	< 1.0	< 1	< 1
Nitrites (mg/L)	0.003	0.007	0.007
Ammonium (mg/L)	0.16	0.28	0.25

Table B-2. Water quality data for Makuleke Wetlands during two surveys in 2015. Pans that were sample during the dry season are indicated with a '2' after the pan name. (UDL = Under Detection Limit).

	Banyini	**Jachacha**	**Hulukulu**	**Makwadzi**	**Gila**
Dissolved Oxygen (%)	139.2	102.8	12	115.7	228
pH	8.7	8.9	8.17	8.59	10.19
Temperature (°C)	30.4	30.2	25	33.1	33.5
Phosphates (mg/L)	0.43	2.05	0.53	0.74	0.88
Nitrate (mg/L)s	2.2	0.6	1.5	1.8	1.9
Nitrites (mg/L)	UDL	UDL	UDL	UDL	UDL
Ammonium (mg/L)	0.425	0.362	1.18	1.3	0.61
	Nwambi	**Mapimbi**	**Hapi**	**Reedbuck vlei**	**Nhlangaluwe**
Dissolved Oxygen (%)	47	105.9	59.6	26	48.1
pH	7.64	8.51	7.9	7.8	8.83
Temperature (°C)	28	31.6	31	23.6	24.7
Phosphates (mg/L)	0.08	0.28	0.24	0.21	2
Nitrate (mg/L)s	3	0.4	1.5	1.4	1.6
Nitrites (mg/L)	UDL	UDL	UDL	UDL	UDL
Ammonium (mg/L)	0.161	0.293	0.442	0.57	0.65
	Makwadzi 2	**Mapimbi 2**	**Hapi 2**		
Dissolved Oxygen (%)	147.2	10.6	141		
pH	9.39	7.85	9.42		
Temperature (°C)	22.8	25.3	26.3		
Phosphates (mg/L)	> 5	> 5	> 5		
Nitrates (mg/L)	1.2	1.5	1.4		
Nitrites (mg/L)	0.04	0.04	0.02		
Ammonium (mg/L)	0.15	2.69	0.21		

Appendix C

Below are included the diatom counts for Lake Sibaya (Table C1) and Makuleke Wetlands (Table C2).

Table C-1. Diatom counts for Lake Sibaya during three surveys in 2015 and 2016. The number after Lake Sibaya indicates the site and the second number indicates the survey number i.e. Lake Sibaya 1.1 indicates site 1 survey 1.

	LS 1.1	**LS 1.2**	**LS 1.3**	**LS 2.1**	**LS 3.1**	**LS 3.2**	**LS 3.3**	**LS 4.1**	**LS 4.2**	**LS 4.3**
Amphora sp. Ehrenberg	0	5	0	42	0	0	0	0	0	15
Amphora veneta Kützing	0	0	0	0	2	0	0	0	0	0
Amphora lacustris R.E.M. Archibald	0	13	0	0	0	0	0	0	0	0
Anomoeoneis sphaerophora (Ehrenberg) Pfitzer	0	16	0	0	0	7	0	11	0	0
Anorthoneis sp. A. Grunow	0	0	0	1	0	0	22	0	4	0
Aulacoseira granulata (Ehrenberg) Simonsen	0	0	0	12	0	0	0	0	0	0
Aulacoseira muzzanensis (Meister) Krammer	0	0	0	0	0	0	11	0	0	0
Cocconeis pediculus Ehrenberg	0	0	0	12	0	0	0	0	0	0
Cocconeis placentula (Ehrenberg) Grunow	0	0	65	0	17	32	9	0	65	11
Cocconeis sp. Ehrenberg	147	12	55	0	0	17	16	65	86	17
Craticula sp. Grunow	0	0	0	0	75	0	0	0	0	0
Cymbella cymbiformis Agardh	0	0	0	0	0	0	17	0	0	0
Diploneis sp. Ehrenberg	0	0	0	0	0	0	0	29	0	0
Diploneis ovalis (Hilse) Cleve	0	0	0	42	0	0	0	0	0	0
Diploneis zanzibarica (Grunow) Hustedt	0	0	0	33	0	0	0	0	0	19
Discostella pseudostelligera (Hustedt) Houk and Klee	0	0	0	0	1	0	12	0	0	0
Encyonema minutum (Hilse.) D.G. Mann	5	0	0	0	0	0	0	0	0	16
Encyonema sp. Kützing	0	0	0	0	0	0	0	54	0	0
Encyonopsis sp. Krammer	0	0	0	0	0	0	0	76	0	0
Encyonopsis minuta Krammer and Reichardt	0	0	22	0	87	0	5	0	14	0

Encyonopsis subminuta Krammer and Reichardt	21	0	0	0	0	0	0	0	0	0
Epithemia adnata (Kützing) Brébisson	11	51	15	0	19	1	0	13	0	14
Epithemia sorex Kützing	0	0	0	0	0	45	0	22	0	18
Fragilaria sp. Lyngbye	4	0	13	0	0	42	0	0	42	0
Fragilaria ulna Lange-Bertalot	0	0	28	0	0	0	41	0	71	39
Gomphonema sp. Ehrenberg	0	28	19	12	0	34	43	26	33	28
Gomphonema insigne Gregory	27	19	69	0	0	0	5	0	16	25
Gomphonema parvulum Kützing	0	0	0	0	0	8	0	0	0	8
Gomphonema pseudoaugur Lange-Bertalot	0	0	0	0	11	83	27	0	21	57
Gomphonema sp. 2 Ehrenberg	0	14	16	0	0	0	0	0	0	21
Gomphonema sp. 3 Ehrenberg	0	14	24	0	0	32	31	3	0	15
Gyrosigma acuminatum (Kützing) Rabenhorst	0	0	1	0	0	0	0	0	6	0
Hantzschia distinctepunctata Hustedt	0	0	0	26	0	0	0	0	0	0
Karayevia ploenensis (Hustedt) Bukhtiyarova	0	12	0	0	0	0	0	0	0	0
Mastogloia smithii Thwaites	5	0	0	0	0	0	0	0	0	0
Mastogloia sp. 1 Thwaites	0	0	14	57	0	0	0	0	0	0
Mastogloia sp. 2 Thwaites	7	14	22	68	0	0	0	0	2	3
Mastogloia sp. 3 Thwaites	0	0	0	0	12	0	0	0	0	0
Navicula sp. Bory	4	18	11	0	36	0	25	0	4	0
Navicula cryptotenelloides Lange-Bertalot	0	0	0	0	17	0	0	0	0	0
Navicula interruptestriata Schwabe and Simonsen	0	0	9	23	0	12	24	0	9	12
Navicula radiosa Kützing	38	0	0	0	0	0	0	4	0	0
Navicula zanoni Hustedt	0	24	0	0	0	0	0	0	0	0
Navicymbula pusilla Krammer	29	0	0	0	0	0	0	0	0	0
Nitzschia sp. Hassall	0	0	8	0	0	0	0	0	9	2
Pinnularia sp. C.G. Ehrenberg	0	0	0	11	0	0	0	0	0	0
Pinnularia subcapitata Gregory	7	0	0	0	0	0	0	0	0	0

Placoneis sp. C. Mereschkowsky	0	0	0	0	0	0	22	0	0	29
Placoneis placentula (Ehrenberg) Heinzerling	0	0	0	32	0	0	0	0	0	0
Rhopalodia sp. O. Müller	0	24	0	0	0	22	0	86	0	11
Rhopalodia gibberula (Ehrenberg) O.Müller	0	12	0	6	0	0	0	0	0	18
Rhopalodia musculus (Kützing) O.Müller	78	0	0	0	1	0	0	0	0	0
Sellaphora sp. Mereschkowsky	0	0	0	48	0	0	0	0	0	0
Sellaphora sp. 2 Mereschkowsky	0	0	0	19	0	0	0	0	0	0
Sellaphora pupula (Kützing) Mereschkowksy	12	0	0	0	0	0	0	0	0	0
Seminavis sp. D.G. Mann	0	0	13	0	14	13	17	0	0	0
Seminavis strigosa (Hustedt) Danieledis and Economou-Amilli	0	0	0	54	0	0	0	0	0	0
Tabularia fasciculata (Agardh) Williams and Round	0	41	6	2	0	17	16	0	26	0
Tryblionella apiculata Gregory	9	0	0	0	0	0	0	0	0	0

Table C-2. Diatom counts for Makuleke Wetlands during two surveys in 2015. Pans that were sample during the dry season are indicated with a '2' after the pan name.

	Banyini	**Jachacha**	**Hulukulu**	**Makwadzi**	**Makwadzi 2**	**Gila**	**Nwambi**
Amphora pediculus (Kützing) Grunow	0	0	0	0	0	0	15
Aulacoseira granulata (Ehrenebrg) Simonsen	0	9	15	1	0	0	21
Caloneis aequatorialis Hustedt	0	0	6	0	0	0	0
Cocconeis placentula Ehrenberg	0	0	0	0	0	0	0
Craticula sp. Grunow	0	0	0	0	0	0	0
Craticula accomoda (Hustedt) D.G. Mann	0	0	0	0	0	26	0
Craticula accomodiformis Lange-Bertalot	0	0	0	0	0	11	0
Craticula cuspidata (Kützing) D.G. Mann	0	0	0	0	0	4	0

Cyclotella meneghiniana Kützing	0	0	0	0	261	0	9
Cyclotella ocellata Pantocsék	0	0	0	0	0	0	0
Cymbella cymbiformis Agardh	0	0	0	0	0	4	0
Diploneis elliptica (Kützing) Cleve	0	0	0	0	0	0	0
Discostella pseudostelligera (Hustedt) Houk and Klee	0	0	5	0	0	0	0
Eolimna subminuscula (Manguin) Moser, Lange-Bert.and Metzeltin	0	0	0	0	0	0	0
Eunotia formica Ehrenberg	0	0	0	0	0	0	0
Gomphonema sp. Ehrenberg	0	29	0	0	0	0	0
Gomphonema exilissimum (Grunow) Lange-Bertalot and Reichardt	0	0	0	0	6	0	0
Gomphonema insigne Gregory	68	0	0	0	0	0	0
Gomphonema lagenula Kützing	0	0	0	1	0	0	14
Gomphonema parvulum (Kützing) Kützing	0	0	0	0	0	0	0
Gomphonema pseudoaugur Lange-Bertalot	22	25	0	34	0	14	0
Gomphonema sp. 2	36	0	0	0	0	0	0
Gyrosigma sp. Hassall	0	0	0	0	0	0	0
Hantzschia amphioxys (Ehrenberg) Grunow	0	0	0	0	0	0	0
Navicula sp. Bory	0	0	0	1	0	0	11
Navicula antonii Lange-Bertalot	0	0	14	0	0	0	0
Navicula erifuga Lange-Bertalot	0	0	5	0	18	2	0
Navicula germainii Wallace	0	2	0	0	0	0	0
Navicula pusilla W.Smith	0	0	0	0	0	0	0
Navicula radiosa Kützing	137	0	0	0	0	0	0

Navicula ranomafanensis (Manguin) Metzeltin and Lange-Bertalot	0	1	0	0	0	0	0
Navicula rhynchocephala Kützing	0	0	0	0	0	0	7
Navicula rosenbergii Oestrup	0	0	0	0	0	0	0
Navicula sp. 1	0	0	0	0	0	0	0
Navicula veneta Kützing	0	26	0	0	0	0	0
Nitzschia sp. Hassall	75	39	12	0	0	42	17
Nitzschia acidoclinata Lange-Bertalot	0	0	0	0	0	0	0
Nitzschia amphibia Grunow	0	14	0	0	0	0	0
Nitzschia archibaldii Lange-Bertalot	0	19	3	0	0	0	0
Nitzschia capitellata Hustedt	0	0	0	0	0	4	0
Nitzschia dissipata (Kützing) Grunow	0	0	0	0	0	0	0
Nitzschia etoshensis Cholnoky	0	0	0	0	0	0	0
Nitzschia filiformis (W.M.Smith) Van Heurck	0	0	0	0	0	0	14
Nitzschia gracilis Hantzsch	0	0	0	0	0	0	0
Nitzschia hantzschiana Rabenhorst	0	0	0	0	11	0	0
Nitzschia intermedia Hantzsch	0	12	0	0	0	0	0
Nitzschia irremissa Cholnoky	0	0	0	0	0	0	0
Nitzschia liebetruthii Rabenhorst	0	14	0	0	0	0	0
Nitzschia linearis (Agardh) W.M.Smith	0	0	0	0	0	0	0
Nitzschia microcephala Grunow	0	0	0	0	0	0	0
Nitzschia palea (Kützing) W.Smith	0	0	0	0	0	0	161
Nitzschia paleacea (Grunow) Grunow	0	0	0	8	0	0	0
Nitzschia pusilla (Kützing)Grunow	0	0	1	0	0	0	0
Nitzschia reversa W.Smith	51	25	0	0	0	0	0

Nitzschia sigma var. diminuta Grunow	0	36	0	0	0	0	0
Nitzschia sp. 2 Hassall	0	0	0	0	0	0	0
Nitzschia sp. 3 Hassall	0	0	0	0	0	0	0
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	0	0	0	0	0	0	0
Pinnularia subbrevistriata Krammer	0	0	7	0	0	0	19
Pinnularia viridis (Nitzsch) Ehrenberg	0	0	4	0	0	0	0
Placoneis placentula (Ehrenberg) Heinzerling	0	0	0	0	0	0	0
Rhopalodia gibba (Ehrenberg) O.Müller	0	0	0	0	0	0	0
Sellaphora pupula (Kützing) Mereschkowksy	41	0	0	0	0	0	0
Sellaphora stroemii (Hustedt) D.G. Mann	0	0	0	0	0	0	0
Stauroneis anceps Ehrenberg	0	0	0	0	0	0	0
Surirella abies Cleve-Euler	0	0	0	0	0	0	31
Tryblionella sp. W. Smith	0	0	0	0	0	0	0
Tryblionella calida (Grunow) D.G. Mann	0	0	11	0	0	0	0
Tryblionella hungarica (Grunow) D.G. Mann	0	0	3	0	16	4	0
Tryblionella levidensis W.M. Smith	0	16	0	0	0	0	12
	Mapimbi	**Mapimbi 2**	**Hapi**	**Hapi 2**	**Reedbuck Vlei**	**Nhlangaluwe**	
Amphora pediculus (Kützing) Grunow	0	0	0	0	0	0	
Aulacoseira granulata (Ehrenebrg) Simonsen	5	0	3	0	0	37	
Caloneis aequatorialis Hustedt	0	0	0	0	0	0	

Cocconeis placentula Ehrenberg	2	0	0	0	0	0
Craticula sp. Grunow	0	0	0	0	0	47
Craticula accomoda (Hustedt) D.G. Mann	0	0	0	0	0	0
Craticula accomodiformis Lange-Bertalot	41	0	0	0	5	0
Craticula cuspidata (Kützing) D.G. Mann	0	0	2	0	5	0
Cyclotella meneghiniana Kützing	0	0	0	0	0	65
Cyclotella ocellata Pantocsék	0	51	0	0	0	0
Cymbella cymbiformis Agardh	0	0	0	0	0	0
Diploneis elliptica (Kützing) Cleve	0	0	0	0	0	16
Discostella pseudostelligera (Hustedt) Houk and Klee	0	0	0	0	0	0
Eolimna subminuscula (Manguin) Moser, Lange-Bert.and Metzeltin	0	0	0	13	0	0
Eunotia formica Ehrenberg	0	0	0	0	4	0
Gomphonema sp. Ehrenberg	0	0	0	0	0	0
Gomphonema exilissimum (Grunow) Lange-Bertalot and Reichardt	0	0	0	0	0	0
Gomphonema insigne Gregory	0	0	0	0	0	88
Gomphonema lagenula Kützing	128	17	7	14	0	0
Gomphonema parvulum (Kützing) Kützing	0	0	0	0	3	0
Gomphonema pseudoaugur Lange-Bertalot	0	0	0	0	4	0
Gomphonema sp. 2	0	0	0	0	0	0
Gyrosigma sp. Hassall	0	0	6	0	0	0
Hantzschia amphioxys (Ehrenberg) Grunow	0	0	0	0	24	0
Navicula sp. Bory	12	0	3	0	0	0

Navicula antonii Lange-Bertalot	0	0	0	0	0	0
Navicula erifuga Lange-Bertalot	0	31	0	0	0	64
Navicula germainii Wallace	0	0	0	0	0	0
Navicula pusilla W.Smith	0	0	0	11	0	0
Navicula radiosa Kützing	0	0	0	0	0	0
Navicula ranomafanensis (Manguin) Metzeltin and Lange-Bertalot	0	0	0	0	0	0
Navicula rhynchocephala Kützing	0	0	0	0	0	0
Navicula rosenbergii Oestrup	11	0	0	0	0	0
Navicula sp. 1	97	0	0	0	0	0
Navicula veneta Kützing	0	0	0	0	0	7
Nitzschia sp. Hassall	11	0	4	0	5	22
Nitzschia acidoclinata Lange-Bertalot	0	123	0	0	0	0
Nitzschia amphibia Grunow	0	0	0	0	0	0
Nitzschia archibaldii Lange-Bertalot	0	0	0	0	0	0
Nitzschia capitellata Hustedt	0	0	0	0	0	0
Nitzschia dissipata (Kützing) Grunow	0	54	0	0	0	0
Nitzschia etoshensis Cholnoky	0	0	0	76	0	0
Nitzschia filiformis (W.M.Smith) Van Heurck	0	0	0	54	0	45
Nitzschia gracilis Hantzsch	19	0	0	0	0	0
Nitzschia hantzschiana Rabenhorst	0	0	0	14	0	0
Nitzschia intermedia Hantzsch	0	0	0	0	0	0
Nitzschia irremissa Cholnoky	0	0	0	0	0	11
Nitzschia liebetruthii Rabenhorst	0	34	0	0	0	0
Nitzschia linearis (Agardh) W.M.Smith	0	0	0	68	0	0

Nitzschia microcephala Grunow	0	0	0	151	0	0
Nitzschia palea (Kützing) W.Smith	0	0	0	0	0	11
Nitzschia paleacea (Grunow) Grunow	0	0	0	0	73	0
Nitzschia pusilla (Kützing)Grunow	0	0	0	0	0	0
Nitzschia reversa W.Smith	0	0	0	0	0	0
Nitzschia sigma var. diminuta Grunow	0	0	0	0	0	0
Nitzschia sp. 2 Hassall	0	0	13	0	0	0
Nitzschia sp. 3 Hassall	0	0	3	0	0	0
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	0	0	0	0	154	0
Pinnularia subbrevistriata Krammer	0	0	0	0	6	0
Pinnularia viridis (Nitzsch) Ehrenberg	0	0	0	0	0	0
Placoneis placentula (Ehrenberg) Heinzerling	0	18	0	0	0	0
Rhopalodia gibba (Ehrenberg) O.Müller	0	0	0	0	4	0
Sellaphora pupula (Kützing) Mereschkowksy	0	0	4	0	0	0
Sellaphora stroemii (Hustedt) D.G. Mann	0	0	0	12	0	0
Stauroneis anceps Ehrenberg	0	0	0	0	3	0
Surirella abies Cleve-Euler	0	0	0	0	0	0
Tryblionella sp. W. Smith	0	0	0	0	16	0
Tryblionella calida (Grunow) D.G. Mann	0	0	6	0	0	0
Tryblionella hungarica (Grunow) D.G. Mann	0	73	0	0	0	0
Tryblionella levidensis W.M. Smith	0	0	0	0	0	0

Appendix D

In the figures below are included some of the diatom species identified in the Lake Sibaya and Makuleke Wetlands. Pictures presented below do not compare in size.

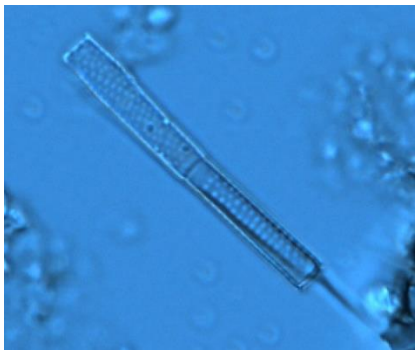

Figure D-1. *Aulacoseira granulata.*

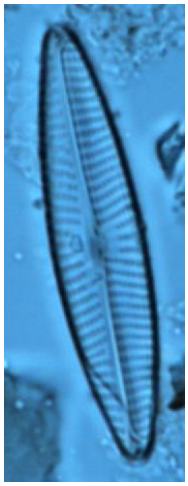

Figure D-2. *Navicula* sp.

Figure D-3. *Nitzschia* sp.

Figure D-4. *Gomphonema parvulum.*

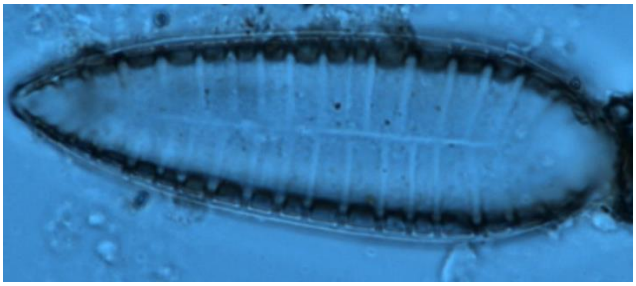

Figure D-5. *Surirella* sp.

Figure D-6. *Pinnularia subbrevistriata*

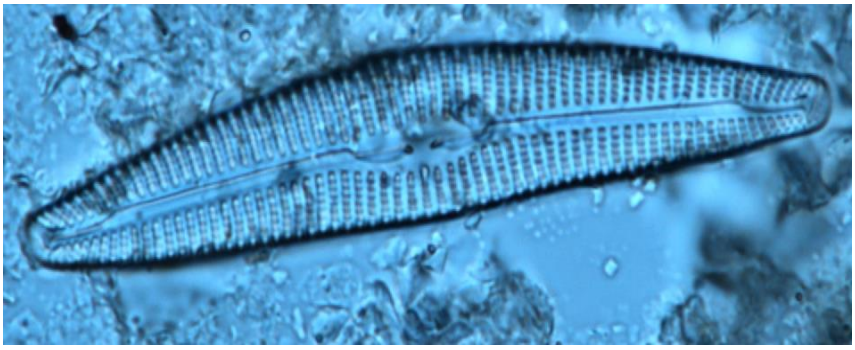

Figure D-7. *Cymbella cymbiformis.*

Figure D-8. *Encyonopsis subminuta.*

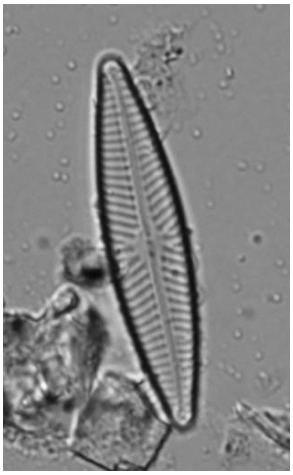

Figure D-9. *Navicula* sp.

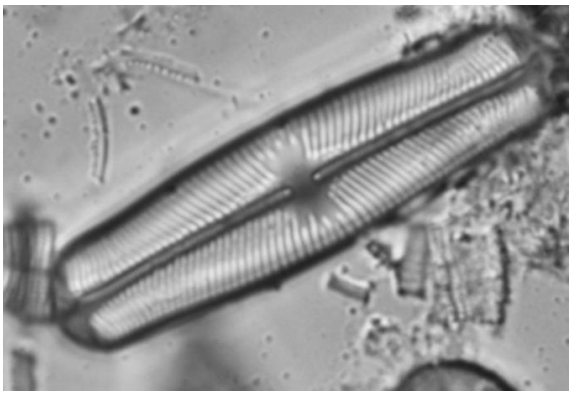

Figure D-10. *Pinnularia subcapitata.*

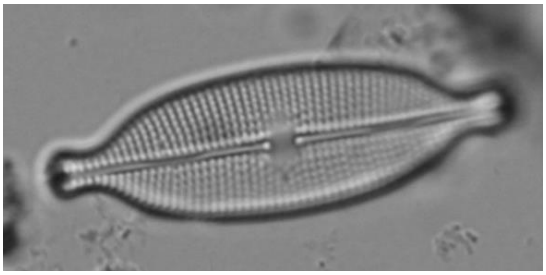

Figure D-11. *Mastogloia* sp.

Figure D-12. *Diploneis zanzibarica.*

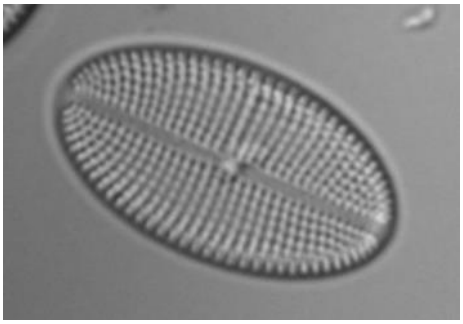

Figure D-13. *Cocconeis subdirupta*

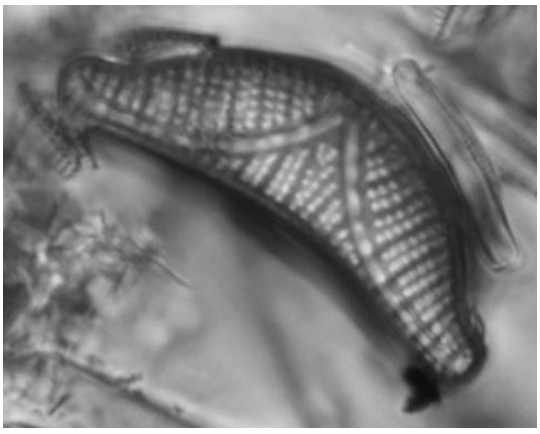

Figure D-14. *Epithemia sorex.*