UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Assessing water quality using benthic diatoms as bioindicators in the Sabie River (Kruger National Park).

by

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Abstract

The Sabie River's headwaters originates in the Mpumalanga escarpment and flows eastward into the Corumana dam in Mozambique – the river has a shared tripartite agreement between South Africa, Swaziland and Mozambique and South Africa needs to ensure that the water quality of the Sabie River is up to standard and no conditions are violated. The majority of the Sabie River to the west of the Kruger National Park (KNP) is affected by human activities such as informal settlements, urbanisation, industry, small-scale farming, and discharge from the wastewater treatment plant. The 110 km length of the river within the KNP is the main water supply for wildlife, agriculture, and ecotourism in the area.

The study proposed to determine the temporal and spatial changes in water quality along the Sabie-Sand River system, in KNP, using benthic diatom assemblages as bioindicators. In addition, to relate microphytobenthos biomass and composition, using chlorophyll *a* fluorescence as an index, to environmental conditions. Upstream disturbances and environmental changes affect the functioning and processes of microphytobenthos (MPB) communities within river systems. Biomonitoring provides a direct measure of river health and allows for the understanding of environmental effects on water quality. Biomonitoring adds a valuable component to traditional physico-chemical sampling and is essential for Integrated Water Resource Management (IWRM) and a crucial component for assessing water quality and river health.

Physico-chemical variables and benthic diatom collection was done in accordance with the SANParks rangers at five sites along the Sabie River within the KNP. Historic diatom slides (1983 and 1985) were obtained from the South African National Diatom Collection housed at the North-West University. Samples were collected in September and October 2019 (low rainfall season) and March 2020 (high rainfall season). Ten stones were selected for conventional collection of MPB and sampled using the BenthoTorch. Physico-chemical parameters including temperature (^{o}C), dissolved oxygen (% and $mg.l^{-1}$), conductivity ($\mu S.cm^{-1}$) and pH were measured *in situ* using the YSI Professional Plus multiparameter probe. Water samples were collected *in situ* in triplicates and filtered (0.45 µm pore size syringe filter). Chemical analysis as well as benthic diatom slide preparation and analysis was carried out at the University of the Witwatersrand. Overall water quality in relation to benthic diatom species assemblages was analysed using OMNIDIA.

Water quality decreased after the flash flood in February 2020 – nutrients (ammonium and orthophosphate) and turbidity increased and dissolved oxygen decreased, although it was relatively high (> 6 $mg.l^{-1}$) throughout the study period and met the Target Water Quality Range (TWQR). The TWQR was met by ammonium (0.007 $mg.l^{-1}$) during the low flow period (September 2019), however concentrations increased into the high flow period (0.1424 $mg.l^{-1}$). Orthophosphate concentrations (< 0.005 $mg.l^{-1}$) in September 2019 characterised the river system as oligotrophic - limited growth of aquatic plants and blue-green algae, however the system exhibited mesotrophic conditions during the wet season (0.054 $mg.l^{-1}$). Conductivity once corrected for temperature increased from upstream to downstream (103.30 – 155.30 $\mu S.cm^{-1}$) - the Sabie River met the Resource Quality Objective (RQO) for conductivity (< 300 $\mu S.cm^{-1}$). The Sabie River has become more alkaline than what has been recorded in the past. To identify the environmental variables that contributed most to the overall variability in diatom community assemblages, the Canonical Correspondence Analysis (CCA) plot was used. The most important environmental variables in the CCA plot that showed axis ordination and therefore influenced diatom species distribution along the Sabie River were conductivity, dissolved oxygen ($mg.l^{-1}$), ammonium, and orthophosphate concentrations. It should be noted that the environmental variables were only able to explain 47.1% of the variation in diatom distribution along the Sabie River – further studies need to be conducted to assess what other factors may be influencing diatom distribution and composition.

A total of 70 benthic diatom species were identified along the Sabie River within the KNP boundaries (from the 1983, 1985, 2019 and 2020 sampling periods). The five most abundant diatom species sampled during 1983 and 1985 were *Achnanthidium minutissimum*, *Cymbella kolbei*, *Gomphonema venusta*, *Navicula heimansoides*, and *Cocconeis placentula*. In 2019 and 2020; *Cymbella turgidula*, *C. placentula*, *Planothidium rostratum*, *Encyonopsis leei* var. *sinensis,* and *Nitzschia frustulum* were dominant. In 1983 and 1985 the dominant species recorded preferred oligotrophic to mesotrophic waters with low to moderate electrolyte content. Whereas the dominant species of 2019 and 2020 preferred oligotrophic to eutrophic waters with low to high electrolyte content. It is evident that the diatom community composition has changed along the Sabie River over the last 36-years because of changing water quality.

The BenthoTorch allowed us to rapidly study the MPB community composition and biomass and as a result the water quality along the Sabie River. Benthic diatom cell density decreased from the low flow period September 2019 (7280 $cells.mm^{-2}$) and October 2019 (6025 $cells.mm^{-2}$) to the high flow period in March 2020 (2577 $cells.mm^{-2}$), however, cyanobacteria density showed the opposite trend increasing from September 2019 (12256 cells.mm^{-2}) and October 2019 (9425 cells.mm^{-2}) to March 2020 (21390 cells.mm^{-2}). The change in MPB community assemblage as a result of the flash flood could be a cause for concern as an increase in cyanobacteria can decrease the aesthetic value of the river and affect the quality of the water that is consumed by humans and wildlife.

The study showed that benthic diatoms are able to indicate temporal (short and long term) and spatial changes in water quality along the Sabie River and it is apparent that diatom composition is influenced by environmental parameters and this finding emphasizes the importance of incorporating benthic diatoms as bioindicators into future water quality monitoring programmes within the KNP. The change in water quality after the flash flood was due to increased surface runoff from higher up in the catchment, as well as increased water velocity that resulted in mixing of riverbed sediments into the water column and biological activity within the river system due to increased water temperatures. The SPI and BDI scores showed that the water quality of the Sabie River has degraded from good quality in 1983 and 1985 (EcoClassification B) to medium quality in 2019 and 2020 (EcoClassification C). KNP management needs to ensure that the Sabie River's water quality is monitored and managed preferably with the inclusion of benthic diatoms as bioindicators and the BenthoTorch as it was able to provide rapid assessment of the Sabie Rivers water quality – that was supported by physico-chemical and biomonitoring using benthic diatoms. The novel use of the BenthoTorch in the Sabie River means that further studies should be conducted to understand its potential for monitoring water quality in the KNP. All stakeholders within the park and at a catchment level need to be involved in preventing further degradation of the Sabie River – it is apparent that the water quality of the Sabie has degraded since 1983.

KEY WORDS: Microphytobenthos, benthic diatoms, physio-chemical variables, BenthoTorch, Sabie River, water quality, Kruger National Park.

Declaration

I (Sarah Oxley) hereby declare that all the research done for my master's dissertation titled *Assessing water quality using benthic diatoms as bioindicators in the Sabie River (Kruger National Park)* is my own work. All the relevant sources have been cited in a complete reference list.

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Abbreviations

ANOVA - Analysis of variance

BDI - Biological Diatom Index

CCA - Canonical Correspondence Analysis

CMA - Catchment Management Agencies

DWAF - Department of Water Affairs and Forestry

DWS - Department of Water and Sanitation

DOM - Dead Organic Matter

DO - Dissolved oxygen

EC - Electrical conductivity

EPS - Extracellular Polymeric Substance

FHI - Fish Health Index

FEPA - Freshwater Ecosystem Priority Areas

GDP - Gross Domestic Product

IWRM - Integrated Water Resource Management

KNP - Kruger National Park

KNPRRP - Kruger National Park Rivers Research Programme

MAP - Mean Annual Precipitation

MAR - Mean Annual Runoff

MPB – microphytobenthos

MSL – Mean Sea Level

NAEHMP - National Aquatic Ecosystem Health Monitoring Programme

NBPAE - National Biomonitoring Programme for Aquatic Ecosystems

NDP - National Development Plan

NWA - National Water Act

PA - Protected Areas

RQO - Resource Quality Objectives

REMP - River Eco-Status Monitoring Programme

RHP - River Health Programme

SADI - South African Diatom Index

SANDC - South African National Diatom Collection

SANParks - South African National Parks

SASS5 - South African Scoring System version 5

SPI - Specific Pollution Index

SE - Standard error

SAM - Strategic Adaptive Management

SDGs - Sustainable Development Goals

TDS - Total Dissolved Solids

TSS - Total Suspended Solids

TWQR - Target Water Quality Range

RVI - Riparian Vegetation Index

UNEP - United Nations Environment Programme

WMA - Water Management Area

Chapter 1: Introduction

1.1 Water quality issues

Water pollution is one of the main challenges we are facing in the 21st century (Ezbakhe, 2018). With over two billion people worldwide affected by water stress, numbers are expected to rise because of poor infrastructure from government, environmental degradation, overutilization, population growth and climate change (United Nations, 2015). Globally, the threat to freshwater systems receives less attention than marine and terrestrial environments, even though freshwater ecosystems are regarded as extremely vulnerable (Davies and Day, 1998). Continuous threats to freshwater systems reduce their ability to be restored and contributes to further deterioration in water quality (Davies and Day, 1998). Water is one of South Africa’s most limited resources and this limitation causes constraints on social and economic development (Driver *et al*., 2011). Water quality threats are exacerbated because South Africa is a water-scarce, semi-arid country (Ricciardi *et al*., 2009). Annual precipitation in South Africa is 450 mm, considerably lower than the global average of 860mm (DWAF, 2004). Another issue of concern is that the evaporation rate (1110 - 3000mm) of the country exceeds the mean yearly rainfall (Davies and Day, 1998). According to the United Nations Environment Programme (UNEP), 20 - 40% of South Africa’s water is extracted from the river in which it occurs, and this is expected to increase to over 40% due to agricultural and industrial activities (García-Rodríguez *et al*., 2007).

Most rivers in South Africa have been modified for agricultural, industrial, and drinking water purposes (Bate *et al*., 2002). Runoff and discharge from farmlands and sewage plants along with increased erosion are anthropogenic factors causing increases in Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) in many rivers of South Africa (Bate *et al*., 2002). Organic enrichment from urbanization, from increased wastewater and inorganic pollutants entering freshwater systems, leads to fluctuations of various chemical (dissolved oxygen, nutrient levels) and physical TDS and TSS aspects (Dallas and Day, 2004). Elevated concentrations of contaminants such as metals and pesticides may also be of concern (Dalu and Froneman, 2016; Pandey *et al*., 2018). These factors threaten the ecological integrity and functioning of freshwater ecosystems and biotic communities (Bere and Tundisi, 2010), which in turn affects important ecosystem services provided by aquatic systems (Giller, 2005; García-Rodríguez *et al.,* 2007).

Anthropogenic disturbances are well documented in developed countries, however, in developing countries such as South Africa, there is a large knowledge gap in terms of available data, which is important for appropriate management practices (De la Rey *et al*., 2004). Management is required to balance human needs and ecological integrity through sustainable development (Driver *et al*., 2011), as the progressive degradation of aquatic ecosystems poses threats to human health and the environment (both current and future) and hinders economic and social growth (Ezbakhe, 2018).

1.2 Water quality management

1.2.1 Sustainable Development Goals

The 2030 Agenda for Sustainable Development aims to ensure social development, environmental sustainability, and economic prosperity of the country (United Nations, 2015). Sustainable development contains both social and environmental agendas that are intertwined (Ezbakhe, 2018). More specifically, Sustainable Development Goals (SDGs) aim to ensure the conservation of species, habitats, landscapes, and water bodies, along with ecosystem processes responsible for generating and maintaining diversity for present and future generations (Driver *et al*., 2011). Sustainable management of freshwater resources and ecosystems are included in SDG 6 of the agenda, which aims to "ensure availability and sustainable management of water and sanitation for all" (United Nations, 2015). Goal 6.3 aims to improve water quality through the reduction of pollution, hazardous chemicals and wastewater released by constant wastewater treatment monitoring (Stats SA, 2017). However, many countries lack sufficient water quality monitoring, which limits access to information. The SDGs aim to reduce this gap (United Nations, 2015). Goal 6.5 aims to implement Integrated Water Resource Management (IWRM), an international initiative that South Africa is attempting to implement through the National Water Act (NWA, Act 36 of 1998). IWRM is a strategy put in place to achieve the sustainable use of resources at catchment, regional, national, and international levels (DWAF, 2003). Transboundary cooperation is required along with each country's unique input that is based on political, social, environmental, and economic circumstances (United Nations, 2015). Lastly, Goal 6.6 ensures the protection and restoration of water-related ecosystems such as wetlands, rivers and lakes that are affected by exploitation. Subgoal 6.6b is crucial as it focuses on local communities and aims to support and encourage participation in order to improve water and sanitation management (United Nations, 2015). As demands on freshwater ecosystems increase, proper ecosystem monitoring is essential for the sustainable use of these ecosystems (United

Nations, 2015). The challenge for South Africa in terms of the SDGs is linking the goals to South Africa's National Development Plan (NDP) Goals that aim to; ensure all South Africans have access to clean running water in their homes and that there is enough water for agriculture and industry; invest 10% of the Gross Domestic Product (GDP) on transport, energy, and water; ensure environmental sustainability and resilience to future degradation and improve water management at a regional level (NDP, 2011).

1.2.2 The National Water Act

The NWA ensures that the nation's water resources are protected, used, developed, conserved, managed and controlled at catchment and regional levels while taking the following factors into account;

a) basic human needs and provisions for future generations;
b) promoting the efficient, sustainable and beneficial use of water for the public;
c) facilitating social and economic development;
d) protecting aquatic ecosystems and their biological diversity;
e) reducing and preventing pollution and degradation of water resources.

To meet the objectives of the NWA, part one of chapter 14 states that monitoring, assessment, and information on various aspects of water resources are required (DWAF, 1998). Under this Act, any activities that pollute or degrade water resources require a license issued by the Department of Water and Sanitation (DWS; formerly the Department of Water Affairs and Forestry) (De la Rey *et al*., 2004). Continued and coordinated monitoring of water resources through the collection of information and data using established and standardized procedures from management and water users is essential for the objectives of the Act to be met and for decision making (DWAF, 1998).

1.2.3 The Millennium Ecosystem Assessment

Ecosystem services are assets and services that people freely gain from natural environments (MA, 2003). Ecosystems are intricately linked and influenced by human management and activities (Kingsford and Biggs, 2012). Increasing pressures on degraded ecosystems diminishes the prospect of sustainable development. Changes in ecosystems affect humans as well as other species, through the alteration of nitrogen, phosphorus, and carbon cycles. The Millennium Ecosystem Assessment recognizes four categories in terms of ecosystem services provided (MA, 2003).

1) provisioning services (ecosystem goods that people derive directly from aquatic ecosystems such as food products and drinking water);
2) cultural services (services such as the cultural, religious and aesthetic well-being derived directly from aquatic ecosystems);
3) regulating services (indirect services such as water purification, flood attenuation, flow regulation);
4) supporting services (additional ecosystem services such as nutrient and water cycling, soil formation and primary production).

Based on many international (e.g. IWRM and SDGs) and national (e.g. NWA and National Water Resource Strategy) guidelines and principles, the implementation of monitoring protocols in the management of aquatic ecosystems have been recognized as being key to the sustainable use of these ecosystem services. Protected Areas (PA) are an important factor to ensure that the provisions of ecosystem services are maintained, ensuring clean water, flow regulation and aesthetic value of the landscape. PAs are also extremely important for conserving biodiversity and mitigating against climate change (Balfour *et al*., 2016).

1.3 Freshwater ecosystems

Biogeochemical cycles at regional and global scales are dependent on aquatic ecosystems (Stevenson, 2014). Freshwater systems act as both transport and storage sites enabling them to be utilized for drinking water, fishery resources, irrigation supplies and wastewater removal (Bere and Tundisi, 2010). Aquatic ecosystems are characterized by physical, chemical, and biological processes (Straskraba and Tundisi, 1999), and longitudinal differences along aquatic systems result in complex chemical and biological communities (Dalu and Froneman, 2016). The interactions between these parameters create a degree of complexity downstream that is reflected in monitoring practices (Wehr and Descy, 1998; Dalu and Froneman, 2016). Upstream disturbances such as agriculture, urban development or flooding affect the functioning and processes of biotic communities within these systems. This causes changes in microphytobenthos (MPB) species assemblages and, increases the abundance of pollutant-tolerant species (Dalu and Froneman, 2016; Pandey *et al*., 2018). Freshwater biodiversity is a valuable parameter for ecological succession and is driven by the climate and nature of the landscape (Marques, 2001; KNP, 2008).

Anthropogenic and environmental changes have direct impacts on water quality and phytoplankton community structure (Bere, 2007; Dallas, 2008; Dalu and Froneman, 2016). Microphytobenthos species have different physiological requirements and show varying responses to changes in light, temperature, and nutrient levels (Ganai and Parveen, 2014). Ganai and Parveen (2014) showed that the most important factors affecting phytoplankton distribution were temperature, Total Dissolved Solids (TDS), Dissolved Oxygen (DO), and alkalinity. The pollution of water and changes in water chemistry affects species diversity, (De la Rey *et al*., 2008), resulting in unstable phytoplankton community assemblage with low diversity, especially in extremely oligotrophic waters (Van Dam, 1982). In addition, certain diatoms and cyanobacteria can form blooms that clog water filters and produce toxins that ultimately affect the taste, smell, and quality of drinking water, alter the aesthetic value, and make the water harmful for wildlife and humans (Bate *et al*., 2002; Stevenson, 2014).

With shifts in rainfall patterns and part of South Africa becoming drier it is important for rivers to remain flowing to ensure a constant supply of water and to mitigate against the impacts of flash floods (Balfour *et al*., 2016). Disturbances in the forms of droughts and floods play a central role in determining the aquatic biota structure (Lake, 2000). Floods result in the destruction and creation of habitat patches by redistributing bottom materials from sediments to boulders, plants, and biota (Lake, 2000). These are short term events that temporarily disturb the biota of a river and recovery is generally rapid (Lake, 2000). Both predictable (seasonal) or unpredictable**,** the floods of 2000 that affected much of South Africa and the Sabie River were the highest magnitude floods recorded and are estimated to return in 60 years and shows the unpredictability of river catchments (Heritage *et al*., 2001).

Although no universal definition has been defined for droughts, for the purpose of this study a drought is a period where a river has an insufficient flow of water to the point where the ecosystems and aquatic organisms are stressed or killed (Esfahanian *et al*., 2017). A drought develops due to reduced precipitation and results in reduced run-off, soil moisture, river flow and groundwater levels (Lake, 2000). Bond *et al*. (2008) found that river systems in Australia that were impacted by anthropogenic disturbances such as water extraction and the introduction of alien species had reduced resilience to drought. Reduced water flow caused by anthropogenic or natural disturbances can reduce water quality by increasing water temperatures, reducing DO concentrations, and causing pollutants to be more concentrated (Lake, 2000). Healthy river systems ensure that important ecosystem services are regulated (MA, 2003; Nel *et al*., 2011).

Water bodies can be classified as oligotrophic (low nutrients), mesotrophic (moderate nutrients), eutrophic (high nutrients), and hypertrophic (extremely high nutrient levels) (Matthews and Bernard, 2015). Waters that are eutrophic or hypertrophic are affected by cyanobacteria blooms, turbidity, oxygen depletion, taste and odour problems, and overall loss of biodiversity (Matthews and Bernard, 2015). Environmental and anthropogenic disturbances and their effect on water quality have captured the attention of scientists and the public who aim to develop faster and more appropriate biomonitoring techniques, which allow for constant monitoring of aquatic ecosystems (Bere, 2007; Passy, 2007). Through a proactive approach to satisfy the national biodiversity target for South Africa's freshwater ecosystems, at least 20% of each freshwater ecosystems and Freshwater Ecosystem Priority Areas (FEPAs) should be maintained in good condition (Nel *et al*., 2011). FEPAs deal with conservation of ecological processes, conservation of ecosystem types and species associated with freshwater systems (Roux and Nel, 2013).

Chapter 2: Literature Review

2.1 Water quality monitoring

Traditional practices for monitoring water quality involve measurements of chemical and physical variables; however, these methods only provide an instantaneous measurement, therefore restricting knowledge of water conditions prior to sampling (Bere and Tundisi, 2010). The use of chemical measurements has been criticized as it does not provide information on the impacts of environmental stresses on organisms and is only able to provide the degree of contamination (García-Rodríguez *et al.,* 2007; Wolska *et al*., 2007). Chemical components in a river system are easily diluted by rainwater or influenced by mine, sewage, or stormwater runoff, or they may become concentrated during times of drought or low flow (Taylor *et al*., 2007b). These factors result in a fragmented view or snapshot of the river's health being captured (Rocha, 1992; Taylor *et al*., 2007b). Alternatively, the use of aquatic organisms (bioindicators) such as diatoms are crucial for water quality and river health monitoring, as they provide an accurate assessment of anthropogenic and natural changes (De la Rey *et al*., 2004; Dalu and Froneman, 2016; Dalu *et al*., 2016).

2.2 Biomonitoring using diatoms

The National Biomonitoring Programme for Aquatic Ecosystems (NBPAE) was initiated in 1996 to address shortcomings in standard physical and chemical monitoring methods (Bate *et al*., 2002). The objectives were to design a monitoring programme to monitor the health of aquatic ecosystems in South Africa and provide reliable information for the management of these systems using bioindicators (Bate *et al*., 2002). To date, biomonitoring in South Africa largely consists of protocols that have been included in the River Eco-Status Monitoring Programme (REMP), which replaced the River Health Programme (RHP) in 2016 and is now a component of The National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) (De la Rey *et al*., 2004). The NAEHMP is a national programme managed by the Department of Water and Sanitation (DWS) – Resource Quality Information Services with support from the Water Resource Commission and various regional and provincial authorities. It includes the use of macroinvertebrates using the South African Scoring System version 5 (SASS5), Riparian Vegetation Index (RVI) and Fish Health Index (FHI) to assess water quality (De la Rey *et al.*, 2004). Although some research has used diatoms to indicate water quality, the South African Diatom Index (SADI) has not become an integral part of the REMP. This has been attributed to limited baseline information on diatom community composition and ecological requirements (Dalu and Froneman, 2016), coupled with a lack of expertise in the country (Uys *et al*., 1996).

Biomonitoring allows us to observe temporal and spatial changes in river health to assess the impacts of specific anthropogenic stressors on these freshwater ecosystems (Parmar *et al*., 2016). Biomonitoring has become an integral component for assessing water quality and provides an integrated and holistic measure of river health (Chutter, 1998; Bate *et al*., 2002; Ndiritu *et al*., 2006). It not only provides a direct measure of ecological integrity through the integration of various stressors but also allows for long-term environmental effects to be detected, as sampling can reflect both past and present water quality, which allows for more accurate detection of environmental changes (both positive and negative) (De la Rey *et al*., 2004; Bere and Tundisi, 2010; Dalu and Froneman, 2016). Biomonitoring adds a valuable component to traditional physico-chemical sampling (Pandey *et al*., 2018) and is essential for IWRM and a crucial component for assessing water quality and river health (Passy, 2007; Ricciardi *et al*., 2009).

2.3 Bioindicators

Bioindicators are organisms with specific environmental preferences. The advantages of using bioindicators to predict the eco-status of a river are as follows; a) biological impacts can be determined; b) synergetic and antagonistic impacts of various pollutants can be monitored; c) organisms can be easily counted, due to their prevalence; and d) it is an economically viable method when compared to specialist monitoring (Parmar *et al*., 2016). Aquatic biota respond to environmental stresses such as the transmission of light, changes in temperature or suspended solids (Parmar *et al*., 2016). Their resilience and recovery trajectory from these stresses along with their key ecological role at the base of the food web, diverse assemblages, and rapid response to environmental fluctuations, makes algal biofilms and in particular, diatoms (Bacillariophyta), a crucial component for water quality monitoring of South African Rivers (De la Rey *et al*., 2004; Harding *et al*., 2004; Passy, 2007; Pandey *et al*., 2018).

2.3.1 Diatoms

Diatoms comprise the major component of microphytobenthos (MPB), which are a distinctive group of mainly unicellular, autotrophic algae, with chloroplasts that contain photosynthetic pigments chlorophyll *a* and *b* (Janse Van Vuuren *et al*., 2006). Diatoms perform essential photosynthetic and microbial functions as primary producers at the base of the food web

(Harding *et al*., 2004; Dalu and Froneman, 2016), representing an important carbon and energy source for secondary consumers, as well as being a food source for protozoa, invertebrates, small fish, and tadpoles (Hoffman, 2013; Tan *et al*., 2014). Diatoms are also the main chemical modulators in freshwater ecosystems (De la Rey *et al*., 2004). They reproduce rapidly with one of the shortest generation times of any biological indicator, taking only two to three weeks to reflect measurable change (Kelly *et al*., 1998) and respond rapidly to environmental changes in terms of pollution and restoration success, therefore providing the assessment of both short- and long-term water quality and environmental changes (Dalu *et al*., 2014a; Harding and Taylor, 2014; Dalu *et al*., 2016). Specific water quality problems such as eutrophication, organic pollution, and metal contamination can be monitored using diatoms (Tilman *et al*., 1982). Their easily identifiable species-specific silica cell walls further aid in their use as bio-indicators (Janse Van Vuuren *et al*., 2006; Dalu and Froneman, 2016).

Diatoms host a diverse range of species, being the most speciose group of microbial eukaryotes and their geographic distributions range from cosmopolitan to narrow endemic (Vanormelingen *et al*., 2008). Diatoms are sub-cosmopolitan species, with many South African species being found in Europe (Kelly *et al*., 1998). Therefore, specific environmental conditions influence species/ taxa presence and abundance more so than the geographical range (Kelly *et al*., 1998). The use of diatom indices from other parts of the world is possible and made simpler due to the proper development of indices such as the SADI. Taylor *et al*. (2007b) found that 98% of diatoms species sampled in the Vaal and Wilge rivers, South Africa, were cosmopolitan. They occur throughout the year in all freshwater ecosystems, making seasonal studies possible (Bate *et al*., 2002). Diatoms are easily spotted as a brownish film on underwater surfaces and excrete a mucilage, Extracellular Polymeric Substance (EPS), which makes the film feel slimy (Hoffman, 2013). They can be found in sediments, on stones-in-current and on most natural surfaces, as well as on artificial substrates within aquatic systems. These factors make monitoring of both natural and anthropogenic changes in aquatic ecosystems possible (De la Rey *et al*., 2004; Parmar *et al*., 2016).

Using epilithic (benthic) diatoms is a preferred collection technique; as epilithic substrata occurs across the length of a river (Kelly *et al*., 1998); the collection is easy (Karthick *et al*., 2010); and the diatom ecology and major diatom-based indices are well understood (Round, 1993 cited by Harding and Taylor, 2011; Bate *et al.*, 2002; Dalu *et al*., 2016). The distribution and abundance of benthic diatoms are often correlated to environmental factors such as water chemistry, nutrients, light, grazing (Lange *et al*., 2011), substrata and flow velocity (Pillay and Naidoo, 2018).

Diatoms are photoautotrophic and are therefore directly affected by changes in nutrients (Pan *et al*., 1996) and light availability (Tilman *et al*., 1982). Nutrients control benthic diatom community structure (Tilman *et al*., 1982), with nitrogen and phosphate being actively assimilated by benthic diatoms for reproduction and growth (Walmsley, 2000; Hoffman, 2013). Benthic diatoms are sensitive to a wide range of water variables such as pH, conductivity, and nutrients (nitrogen and phosphorus) (Pan *et al*., 1996; Bate *et al*., 2002). Their sensitivity to environmental disturbances (García-Rodríguez *et al*., 2007) results in each taxon displaying a specific optimum and tolerance to specific stressors, making them powerful indicators of water quality in freshwater systems (García-Rodríguez *et al*., 2007; Taylor *et al*., 2007b,c; Bere and Tundisi, 2010; Sawaiker and Rodrigues, 2017). Passy (2007a) used diatom ecological guilds (low, high, and motile) based on their potential to tolerate nutrient limitations and physical disturbance. Guilds showed specific distribution patterns; low profile favoured nutrient-poor, high disturbance habitats; high profile favoured the opposite, and motile guilds increased along nutrient gradients and decreased along disturbance gradients. Epilithon habitats were dominated by high profile guilds. The predictability of these ecological guilds is promising for understanding the ecological change of human-impacted aquatic ecosystems. Pandey *et al*. (2018) used taxonomic (assemblage, structure, richness, and diversity) and non-taxonomic (cell size, cell health status, lipid bodies, and valve deformities) metrics to assess water quality. Additionally, B-Béres *et al.* (2016) successfully used morphological traits such as cell size and biovolume to determine distribution and abundance of benthic diatoms. Diatom assemblage structure along with the size and number of lipid bodies and abnormal diatom frustules were powerful indicators of ecological change (Falasco *et al*., 2009).

2.4 History of diatoms

Diatoms have been used as indicators of water quality in South Africa from the early 1950's by Cholnoky (1953) and soon afterward by Archibald (1972) and Schoeman (1979). Research on diatoms was restarted by Bate *et al.* (2002) who attempted to apply the European diatom index to monitor water quality in South African river systems. He concluded that benthic diatoms could be a useful addition to the NBPAE, as diatoms give a time-integrated indication of specific water quality components. Harding and Taylor (2014) compared diatoms from Cholnoky and Claus (1961) and found that the diatom species assemblage remained relatively unchanged after 48 years. Historically curated diatoms samples are extremely useful for understanding ecosystem change either positive or negative. These studies have resulted in South Africa having one of the most comprehensive collections of diatoms in the world. South Africa's diatom samples are stored at the North-West University in Potchefstroom, in the South African National Diatom Collection (SANDC) and, are well preserved for future analysis (Harding *et al*., 2004). In addition, benthic diatoms have been utilised as bioindicators of river health worldwide, including countries within Europe (Kelly *et al*., 1998) and North America (Stevenson and Pan, 1999), and countries closer to South Africa such as Kenya (Ndiritu *et al*., 2003; Ndiritu *et al*., 2006; Triest *et al*., 2012), Zambia (Lang *et al*., 2012) and Zimbabwe (Bere *et al*., 2014; Bere and Mangadze, 2014) However, large parts of Africa have largely been ignored despite the cost-effective technique being successfully practised (Cocquyt *et al*., 2013). Diatoms can provide information on aquatic biodiversity, which is important for understanding the structure and functioning of the system, as well as ensuring the goals of the NWA are met (DWAF, 1998; García-Rodríguez *et al.,* 2007).

2.5 OMNIDIA database

The OMNIDIA database is a useful tool for determining the ecological status of rivers in South Africa (Taylor *et al*., 2007; Harding and Taylor, 2014; Snow, 2016). Harding and Taylor (2014), who developed the South African Diatom Index (SADI) through the incorporation of relevant ecological characteristics of endemic South African species using existing OMNIDIA software (Lecointe *et al*., 1993), recognised that the robustness of the index depends on the continual improvement through the addition of results from all over the country. The OMNIDIA database, which originated in France, provides a useful software platform for diatom researchers to manage their inventories derived from water samples, as well as to calculate diatom index scores. The OMNIDIA database includes more than 23000 diatom species, over 800 genera and is used to calculate the index scores of 18 different indices (OMNIDIA, 2018). Diatom assemblage structure ranges from sensitive to tolerant species, and respective tolerance levels to changing water chemistry allow for water quality to be determined from diatom species assemblages (Pandey *et al*., 2018).

2.6 BenthoTorch

Chlorophyll *a* can be used as an alternative to measuring the abundance of microalgae, phytoplankton, and microphytobenthos (MPB), present in river systems (Taylor *et al*., 2007). Through the measurement of chlorophyll *a*, we can estimate the number of benthic algae in the water (Echenique-Subiabre *et al*., 2016). This premise makes up the basis of the BenthoTorch. Until recently, water quality monitoring using chlorophyll *a* concentration as an index of total microalgal biomass and community composition has traditionally only been calculated using spectrometry and light microscopy (Pinto *et al*., 2001; Echenique-Subiabre *et al*., 2016; Snow, 2016). The BenthoTorch is now being used to measure *in situ* MPB biomass, with a range of benefits from optimised field sampling to improved ecological status assessment (Kahlert and Mckie, 2014; Echenique-Subiabre *et al*., 2016; Snow, 2016;). This method allows for an alternative to tedious microscope observations (Echenique-Subiabre *et al*., 2016).

This portable device captures concentrations on an LCD screen in under 20 seconds (Echenique-Subiabre *et al*., 2016). The BenthoTorch can quantify major MPB groups, such as cyanobacteria (Cyanophyta), green algae (Chlorophyta) and diatoms (Bacillariophyta), on different substrates including rocks and sediment (Pinto *et al*., 2001; Echenique-Subiabre *et al*., 2016; Snow, 2016). Quantification of fluorescence is emitted from algae due to the presence of pigment signatures and chlorophyll under artificial illumination of different wavelengths (LEDS), which provides MPB biomass and composition (Kahlert and Mckie, 2014). This method is extremely rapid and cost effective for determining environmental disturbances resulting in water quality changes, as pollution and eutrophication result in high chlorophyll *a* levels, resulting in dense MPB communities (Tilman *et al.*, 1982; García-Rodríguez *et al.*, 2007). Pollution inhibits the growth of sensitive organisms, while more tolerant organisms are able to survive (Harding and Taylor, 2011). This creates inter-species competition and ultimately changes in community structure composition. The changes in community structure from diatom-dominant to cyanobacteria-dominant allows researchers to assess the water quality of a particular system (Harding and Taylor, 2011; Snow, 2016). Cyanobacteria blooms are on the rise globally as a result of inefficient wastewater treatment, increased use of fertilizers (Lürling *et al*., 2018), and climate change (Paerl and Huisman, 2009). Elevated water temperature favours the growth of cyanobacteria over other MPB (Paerl and Huisman, 2009).

Nutrient pulses caused by storms may further increase cyanobacteria growth (Lürling *et al*., 2018). Lürling *et al*. (2017) found that the addition of nutrients and elevated temperatures in an urban pond resulted in increased cyanobacteria biomass, however the addition of nutrients alone also increased cyanobacteria biomass. In general, low−nutrient water bodies will probably be more resilient to the expected adverse effects of warming than eutrophic waters and are unlikely to cause cyanobacterial blooms under warmer conditions (Brookes and Carey, 2011 cited by Lürling *et al*., 2018).

2.7 Study Site information

2.7.1 Kruger National Park

To assess the ecological integrity of Kruger National Park’s (KNPs) water, an understanding of the complex relationships between diatom community assemblages, environmental as well as anthropogenic factors, need to be established (Gevrey *et al*., 2004). The sustainable use of South Africa’s aquatic ecosystems, especially within nature reserves, requires continuous and accurate monitoring of river ecological status. Rivers provide important ecological functions and ecosystem services, both within the park and beyond its boundaries (KNP, 2008). Protected area networks and environmental management has become an essential part of aquatic ecosystem conservation (Kingsford and Biggs, 2012). Catchment conservation is extremely challenging as the volume and quality of water is affected by processes upstream and outside the reserve and as a result Catchment Management Agencies (CMAs) and Water User Associations ensure that park management actively engages with upstream users (Pollard *et al*., 2011; Kingsford and Biggs, 2012). Effective conservation requires management of the entire system, this includes processes and threats outside their boundaries (Nel *et al*., 2009). This is largely because aquatic organisms depend on flow, temperature, sediment, and nutrient regimes of the entire river system (Kingsford and Biggs, 2012).

The KNP adopted IWRM and Strategic Adaptive Management (SAM) in the 1990’s due to persisting water quality issues within the reserve caused by extensive phosphate and copper mining upstream (Riddell *et al*., 2019). Emphasis is now placed on a proactive rather than reactive approach to ensure long term goals are achieved. SAM monitors trends and then demands discussions for specific goals to be met, which is followed by joint decisions made by researchers and management (Pollard *et al*., 2011). To ensure that rivers are well managed within protected areas such as the KNP, Resource Quality Objectives (RQOs) and monitoring requirements at a finer scale should be incorporated into management plans. KNP maintains ecosystem sustainability by monitoring the ecological status of rivers using different biota as water quality indicators. The Kruger National Park Rivers Research Programme (KNPRRP), a collaborative effort between resource managers, funding agencies and researchers, was put into place to monitor and manage several catchments with the main focus on the Sabie-Sand Catchment. An important focus has been on upstream impacts, as conservation within the reserves relies on the conservation of the catchment as a whole; unfortunately, the KNPRRP came to an end in 1992 (van Wyk *et al*., 2001). The REMP, previously known as the RHP, was put in place by the

Department of Water Affairs in 1994 to assess the ecological status of KNP rivers (DWAF, 1998). Understanding river flow, duration, and frequency along with longitudinal connectivity are essential for reserve and catchment management (KNP, 2008). FEPAs have selected 50% of the KNP river length, making it an important conservation area for achieving freshwater ecosystem conservation goals. Within the reserve 82% of rivers are A/B class (natural or good quality), 15% in class C (moderate quality) and 3% in class D (poor quality) (KNP, 2008). The reach of the Sabie River within the KNP was given an Ecostatus B that indicates good quality water in 2011 (ICMA, 2012).

2.7.2 Water quality and its effect on KNP

The land bordering KNP is occupied by game reserves, farms and settlements that are continuously expanding, resulting in little to no expansion for the water reserve (van Wilgen and Herbst, 2017). The rivers within the park are under threat from upstream influences such as mining, rapidly expanding urbanization, water abstraction, impoundments, pollution, and global change, causing concern for KNP management (Kleynhans *et al*., 2013; Roux *et al*., 2017; van Wilgen and Herbst, 2017). The Sabie River flows through more than 74000 ha of commercial forestry plantations (pine and eucalyptus trees) near Hazyview (Heritage and Moon, 2000). Negative impacts on ecological important rivers such as the Sabie could impact tourism and wildlife. To date there has been negligible research published on benthic diatoms within the lowveld of the Mpumalanga and Limpopo provinces; more specifically KNP. This may be attributed to the fact that diatoms as bioindicators only became part of the REMP in 2019 to ensure temporal sampling of large perennial rivers (Riddell *et al*., 2019). Including diatoms in the SAM and the REMP protocols is valuable to the management of KNP in order to understand aquatic ecosystem change and respond proactively (Rogers and Biggs, 1999).

2.8 Study aims

The aim of this study is to determine the temporal and spatial changes in water quality along the Sabie-Sand River system, in Kruger National Park, using epilithic (benthic) diatom assemblages as bioindicators. In addition, to relate microphytobenthos biomass and composition, using chlorophyll *a* fluorescence as an index, to environmental conditions.

2.9 Hypothesis

The Sabie River is noted as one of the most pristine rivers in the country – however anthropogenic disturbances from higher up in the Sabie-Sand catchment will result in water quality degradation from 1983 to 2020, and therefore a change in benthic diatom composition. Microphytobenthos (MPB) community composition will change (increase in cyanobacteria) during the high flow period as a result of changing water quality – increased nutrients from surface runoff as well as increased water temperature.

2.10 Objectives

1) Identify benthic diatoms using microscope analysis to species level collected from the Sabie-Sand River catchment over broad spatial and temporal ranges.

   a. Compare temporal changes in benthic diatom species assemblages using historic samples from 1983 and 1985, to more recent 2019 and 2020 sampling periods.

   b. Investigate benthic diatom functional traits, nutrient limitations, and disturbance gradients across sampling sites for the 2019 and 2020 sampling periods.

2) Relate changes in diatom community structure to changing environmental conditions using river discharge, temperature, pH, electrical conductivity, dissolved oxygen, water clarity, ammonium, nitrates + nitrites (Total Oxidised Nitrogen: TON), and orthophosphate.

   a. Physico-chemical variables and diatom community structure at five sampling sites within the KNP and across three sampling periods (September 2019, October 2019, and March 2020) will be assessed using a multilinear CCA plot.

3) Develop a detailed diatom species list, including species names and images, with associated ranges of environmental parameters.

   a. Contribute to the SADI and limited knowledge on diatoms within KNP.

4) Utilise a BenthoTorch to measure microphytobenthos (green algae, cyanobacteria, and diatoms) biomass and group composition (diatoms, cyanophytes, and chlorophytes) at respective sampling sites, to monitor overall water quality.

5) Utilise benthic diatoms as bioindicators to determine water quality at specific sampling sites using the SADI for the continuous biomonitoring of rivers within KNP and to assess impacts of upstream users on the rivers within the reserve.

Chapter 3: Study site description and selection

3.1 Study area

The study sites are located along the Sabie-Sand Catchment that is approximately 7096 km^2 and lies within the larger Inkomati Water Management Area (WMA) (Heritage and Moon, 2000). The KNP rivers extend from South Africa into Swaziland and Mozambique and are managed by a tripartite agreement between the three nations. Any country who abstracts too much water or pollutes the water excessively, violates the terms of the agreement (van Wilgen and Herbst, 2017). The perennial Sabie River flows from 2053 m above Mean Sea Level (MSL) in the Mpumalanga escarpment on the eastern slopes to an altitude of approximately 120 m, in the lowveld. The Sabie River forms the upper boundary between KNP and rural areas in Mpumalanga (Heritage and Moon, 2000). The Sabie River is joined by the smaller Sand River tributary and flows out of the KNP at the Mozambique border in the east into the Corumana dam (Roux *et al*., 2017).

The Inyaka Dam located higher up in the Sabie-Sand catchment was completed in 2000 to meet the high-water requirements of KNP and supporting rural communities. The 110 km long Sabie River within the reserve has remained relatively unaltered, being the main water supply for wildlife and conservation and minor flow modifications have been made for potable water supply for tourists (Heritage and Moon, 2000; Roux *et al*., 2017). The Sabie River is considered the most pristine and diverse river in South Africa as well as the most ecologically important (ICMA, 2012; Riddell *et al*., 2019). The Sand River catchment has the highest percentage of afforestation of any catchment in South Africa (Pollard *et al*., 2011). The Sand River is also subjected to over-abstraction and elevated nutrient levels possibly caused by rural settlements (Stolz, 2018). The Bushbuckridge treatment plant abstracts water from the system, resulting in the Inyaka Dam water being in high demand, with the water reserves for the Sabie River already allocated, the system may become water-stressed in the near future (Mallory *et al*., 2013). The Sabie is one of the least modified of the five perennial rivers that flow through the KNP, with over 70% of its natural Mean Annual Runoff (MAR) remaining in the river. Only 30% is utilised by forestry and the freshwater

Reserve (Sinclair and Walker, 2003). Much of the catchment to the west of the KNP is affected by human activities such as forestry, trout fishing, urbanisation, industry, and discharge from wastewater treatment plants (Rivers-Moore *et al*., 2004).

3.1.1 Landscape and climate

Kruger National Park is a longitudinal north-south reserve, the five main perennial (Luvuvhu, Letaba, Olifants, Sabie-Sand and Crocodile) rivers flow in a west to east direction (KNP, 2008). The lowveld's Mean Annual Precipitation (MAP) averages 600 mm, but the perennial source rivers in the Drakensberg mountains receive a higher average of 2000 mm (DWA, 2013). The KNP is located in the lowveld between the Drakensberg escarpment to the west and Mozambique to the east (Sinclair and Walker, 2003). The rainfall gradient for the Sabie-Sand Catchment follows a west-east aridity gradient. The topological differences along the catchment and within the reserve result in climate changes (Stolz, 2018). The west of the reserve is underlain by granitic rock and the east basaltic. These formations are separated by a thin north-south line of sedimentary rock (Sinclair and Walker, 2003). Steep bedrock and gently sloping segments influence the degree of alluviation (Heritage and Moon, 2000). The Sabie River is underlain by a wide variety of bedrock, sedimentary, intrusive, and extrusive igneous and metamorphic rock (Heritage and Moon, 2000). The diversity in soils is due to high variation of bedrock, energy regimes and factors such as climate, geology, water discharge and sediment influx, bed and bank characteristics, and vegetation development (Heritage and Moon, 2000). This results in varied geomorphology along the river creating an environment that can support a great diversity of biota (Heritage and Moon, 2000; Sinclair and Walker, 2003). The Sabie River is the most diverse in terms of fish communities and second to the Luvuvhu River in terms of benthic invertebrate diversity (Sinclair and Walker, 2003).

3.2 Sampling sites

Sampling was conducted along the Sabie River, primarily based on access from scientific services at Skukuza, at five sampling sites that overlapped with Shikwambana (2016) sampling sites. Sites were aligned with South African National Parks (SANParks) and REMP sites. All five sites were located within the KNP boundaries. Flow gauging weirs near the sampling sites include X3H021 (Kruger Gate) and X3H015 (Lower Sabie) along the Sabie River. The Sand River was not included in the study as the river flow was too low during the sampling period. Sampling was conducted in September 2019 and October 2019 (dry seasons) and in March 2020 (wet season).

Sampling was conducted during the dry season to provide the best insight into excessive abstraction and nutrient concentrations and during the wet season to assess the water quality over a wider temporal scale. Sampling conducted in March 2020 followed floods that occurred in February 2020, where river discharge at the Lower Sabie weir (X3H015) peaked at 1246 $m^3.s^{-1}$ on the 11/02/2020 - the base flow at the Lower Sabie weir in February 2020 was 2.53 $m^3.s^{-1}$. The flood in February 2020 may have resulted in benthic diatom communities being displaced (Taylor *et al*., 2007).

Sampling sites were selected based on the safety of the site and were selected by research technicians and SASS5 accredited researchers of SANParks.

Table 3.1: Names and coordinates of sampling sites along the Sabie River during the 2019 – 2020 sampling periods.

Site	**Location**	**GPS coordinates**
1	Paul Kruger Gate	24°59.30'S, 31°17.54'E
2	Tinga	24°58.24'S, 31°30.30'E
3	Antholysta	24°58.01'S, 31°44.10'E
4	Lower Sabie Bridge	25°05.96'S, 31°53.14'E
5	Sabiepoort	25°11.04'S, 32°01.81'E

3.2.1 Sabie River

The Sabie River is a semi-arid bedrock influenced system, consisting of a macro-channel that has been incised into the width of the bedrock and multiple active channels within its confines (Heritage *et al*., 1997; Heritage *et al*., 2001). The Sabie has stretches of unfractured bedrock, bedrock outcrops often dominate areas and determine slope gradients, resulting in sediment accumulation in and along the macro and active channels (Heritage and Moon, 2000; Heritage *et al*., 2001). The macro-channels are controlled by large magnitude, low frequency events such as floods, whereas the active channels are constantly changing in response to flow and sediment deposition (Heritage *et al*., 1997). Sites 1 to 5 are located within the KNP and anthropogenic disturbances are generally from upstream users and not directly within the park (Heritage and

Moon, 2000). The sampling sites within the KNP are characterised as anastomosing, sand dominated with multiple channels, and large shallow pools (ICMA, 2012).

Site 1 - Paul Kruger Gate (24°59.30'S; 31°17.54'E)

The first site is located upstream of the Paul Kruger Gate in KNP, downstream from where the Sabie River enters the park, and this site can be classified as the headwaters of the Sabie River within the park. This section of the river had a relatively narrow channel with a strong flow. The site had a main channel and a slightly sandy pool with cobbles and stones on the right-hand side. The canopy was partially closed with tall trees and lowveld reeds (*Phragmites mauritianus*) being highly abundant and dominant along the river due to the accumulation of sediments and nutrients.

Site 2 - Tinga (24°58.24'S; 31°30.30'E)

Located downstream of Paul Kruger Gate, the river at this site was wide with a slow flow and instream vegetation (*P. mauritianus* and trees). Embedded bedrock was covered with a lot of sand and silt, making this site the most sedimented of the five. An open canopy dominated by *P. mauritianus*, and scattered trees were present along the outer banks. Samples were collected from the right-hand channel from cobbles and stones.

Site 3 - Antholysta (24°58.01'S; 31°44.10'E)

The third site was located downstream of where the Sand and Sabie Rivers meet. The river was wide and had areas with rocks and cobbles. The canopy was open and in stream vegetation was dominated by *P. mauritianus*. The river bank was woody and dense with dry side channels dominated by sand and silt.

Site 4 - Lower Sabie (25°05.96'S; 31°53.14'E)

Site four was located at the confluence of the intermittent Lubye Lubye Stream and Sabie River near the Lower Sabie Rest Camp. The site had a wide and deep channel with large rapids down the left-hand side of the river channel and smaller sandy pools along the right-hand side. Filamentous algae were noted at this site along with very turbid waters. The canopy was open with a small island of in stream vegetation, again dominated by *P. mauritianus*.

Site 5 - Sabiepoort (25°11.04'S; 32°01.81'E)

The last site was located at the lowest part of the Sabie River, close to the Mozambique border running parallel to the Lebombo mountain range. This section of the river was braided with vegetation (*P. mauritianus*) separating the two channels. The sampling site had a large pool above and below it with an accumulation of Dead Organic Matter (DOM) due to the gradual slope and broad channel that allows for the formation of dense reedbeds that are infrequently eroded away by floods. This site had small amounts of filamentous algae and was the only site with water hyacinth (*Eichhornia crassipes*) present.

Historic sampling sites along the Sabie River

The following sites were given unique numbers and were obtained from the North-West University. Diatoms were collected at specific locations along the Sabie River during 1983 and 1985.

Site 6 - upstream of Paul Kruger Gate

Diatoms were collected from stones in current and the site was located below Lisbon Estates along the Sabie River. Samples were collected on the 2/3/1983. The water quality parameters were as follows; pH 8.02, temperature 26.0^{o}C.

Site 7 - located 3km West of the Paul Kruger Gate

Diatoms were collected from stones in slow current and the site was located opposite the Lisbon Estate along the Sabie River. Samples were collected on the 12/9/1985. The water quality parameters were as follows; pH 7.76, temperature 23.0^{o}C.

Site 8 - upstream of Lower Sabie Rest Camp

Diatoms were collected from deep rapids on the 2/3/1983 and the water quality parameters were as follows; pH 8.0, temperature 29.2^{o}C.

Site 9 - upstream of Lubye Lubye confluence (Lower Sabie)

Diatoms were collected from deep rapids on the 10/9/1985 and the water quality parameters were as follows; pH 7.4, temperature 23.0^{o}C.


Site 1 - 2019
Site 1 - 2020
Site 2 - 2019
Site 2 - 2020
Site 3 - 2019
Site 3 - 2020

Figure 3.1: Photographs of the sampling sites in 2019 and 2020 along the Sabie River within KNP.

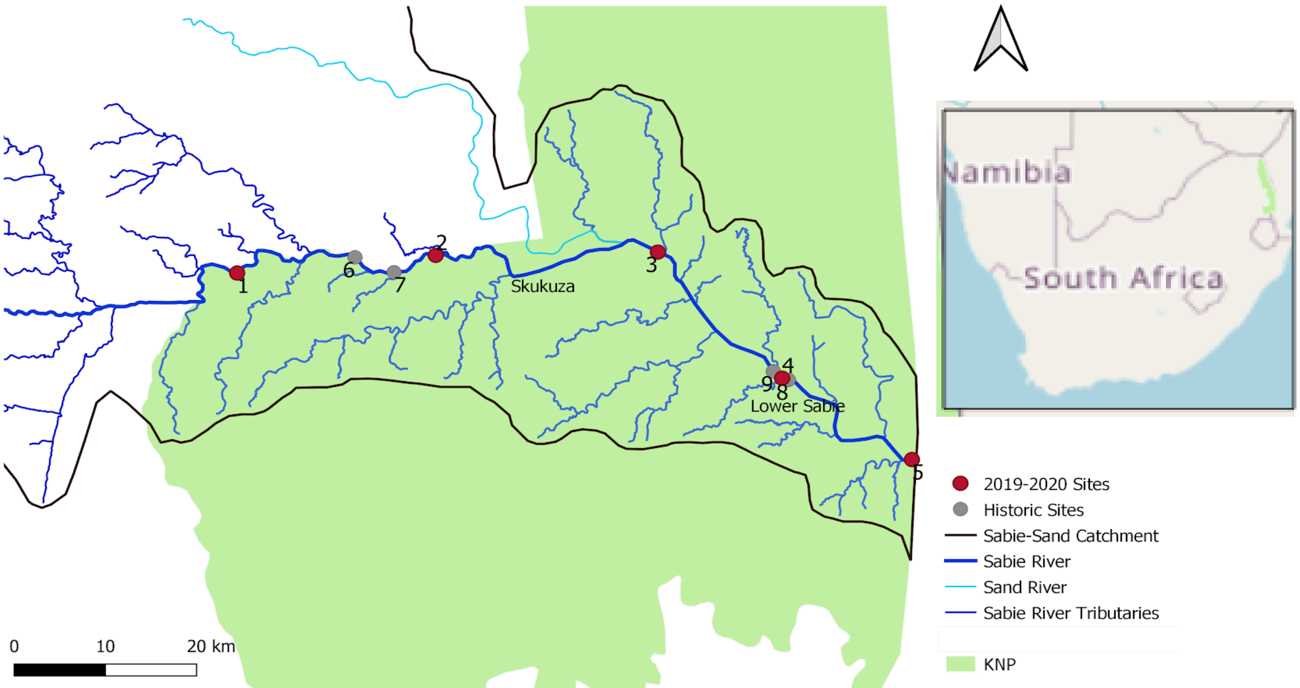


Figure 3.2: Map of the Sabie-Sand Catchment with the historic and present sampling sites.

Chapter 4: Methods and materials

Physico-chemical and diatom collection was done in accordance with the SANParks rangers at the five sites along the Sabie River within the KNP. Historic diatom slides were obtained from the North-West University.

4.1 Physico- chemical variables

4.1.1 Nutrient collection

At each sampling site filtered (0.45 μm pore size syringe filter) water samples were collected from just below the water surface in triplicate from the different sampling sites and then frozen. *In situ* measurements of temperature, dissolved oxygen (DO), electrical conductivity (EC) and pH were measured using a YSI Professional Plus multiparameter probe. The YSI probe was placed instream where it logged measurements every 30s throughout each sampling period. A clarity tube was used to measure the clarity of the water at all sampling sites.

4.1.2 Nutrient analysis

Samples were analysed for orthophosphates and silicate using the methods described by Grasshof *et al*. (1999), for nitrates and nitrites (TON) based on the method described by Bate and Heelas (1975), and ammonium using the method from Parsons *et al*. (1984). All samples were defrosted prior to nutrient analysis.

4.1.2.1. Ammonium

4.1.2.1.1 Standard series

A standard solution was made by adding 0.535 g of ammonium chloride (NH_4Cl) powder to a 1 litre volumetric flask, followed by 1 litre of deionised water. Then 0, 0.1, 0.2, 0.4, 0.6 and 0.8 ml were added to six separate 100 ml volumetric flasks, respectively, creating concentrations of 0, 10, 20, 40, 60 and 80 μm NH_4Cl solutions. The concentrations for all nutrient analyses were initially measured as micromoles (μM) then converted to $mg.l^{-1}$, so ammonium and TON are expressed as $mg\ N.l^{-1}$ and soluble reactive phosphorus/orthophosphates as $mg\ P.l^{-1}$. The standard solutions were treated the same as the field samples from this point onwards.

4.1.2.1.2 Chemical preparation

Phenol solution was prepared under the fume hood by dissolving 20 g of crystalline phenol granules in 200 ml 95% ethanol, the solution was swirled. Sodium nitroprusside solution was prepared by dissolving 1 g in 200 ml deionised water. Lastly, 10 g sodium citrate ($C_6H_5O_7Na_3$) and 0.5 g sodium hydroxide (NaOH) were dissolved in 50 ml of deionised water, 20 ml was then added to 5 ml of fresh sodium hypochlorite solution (10 – 14%) to create the oxidising solution.

4.1.2.1.3 Sample analysis

Two and a half millilitres were drawn from each sample (standard series and field) using a pipette, placed into test tubes in a rack, and each test tube was labelled with the site name and date. Phenol solution (0.1 ml) was added to each sample, followed by 0.1 ml of sodium nitroprusside solution and lastly 0.25ml of oxidising solution. After each addition of a new solution the test tubes were swirled using a vortex mixer. The samples were covered and placed in the dark for an hour; samples are stable for 24 hours. The absorbance of each sample was determined using an UV-1100 spectrophotometer at a wavelength of 640 nm.

4.1.2.2 Orthophosphates

4.1.2.2.1 Standard series

To create the stock solution, 0.0816 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 100 ml of deionised water. To create the standard series, 2.500, 2.375, 2.250, 2.000, 1.750, 1.500 and 1.250 ml of deionised water were added to the respective standard series test tubes in this order, followed by 0, 0.125, 0.250, 0.500, 0.750, 1.000 and 2.500 ml of stock solution.

4.1.2.2.2 Chemical preparation

The following reagents were prepared to create the mixed reagent.

1) Ammonium molybdate solution; 15 g ammonium molybdate ($(NH_4)_6MO_2O_{24}.4H_2O$) was added to 500 ml deionised water.

2) Sulfuric acid solution; 140 ml concentrated sulfuric acid (H_2SO_4) was added to 900 ml of deionised water. [Concentrated acids must always be added to the water, not the other way around.]

3) Ascorbic acid solution; 1.35 g ascorbic acid ($C_6H_8O_6$) was added to 25 ml deionised water.

4) Potassium antimony tartrate solution; 0.34 g potassium antimony tartrate ($C_8H_8KO_{12}Sb$) was added to 250 ml deionised water.

The mixed reagent was created by adding 5 ml of ammonium molybdate solution, 12.5 ml sulfuric acid solution, 5 ml ascorbic acid solution and 2.5 ml potassium antimony tartrate solution, the solution was then swirled.

4.1.2.2.3 Sample analysis

To prepare for the absorbance readings, 2.5 ml of each sample (standard series and field) were added to respective test tubes, followed by 0.25 ml of mixed reagent. The samples were swirled and left to stand for at least five minutes and not longer than 30 minutes. The absorbance readings were read at a wavelength of 885 nm using a spectrophotometer.

4.1.2.3 Total Oxidised Nitrogen (TON)

Preparing copper cadmium powder;

The cadmium powder was washed with 5% HCl (15.2 ml of 33% HCl diluted to 100 ml) and then rinsed with deionised water. The powder was washed with 0.5% copper sulfate pentahydrate ($CuSO_4.5H_2O$) (1.248 g in 1 l distilled water) until the cadmium powder turned from a silvery to dark grey. The dark powder was washed with 0.0007 N HCl (1.4 ml/2 l) containing 0.005 M (1.861 g/l) Na_2EDTA. The excess precipitated copper was decanted until the supernatant was clear and the copper cadmium a silvery grey colour. The copper cadmium was stored under a weak acid-EDTA solution in a sealed air proof flask. To prepare the buffer, 21.4 g/l NH_4Cl adjusted to pH of 9.6 with NH_4OH.

4.1.2.3.1 Standard solution

To prepare the standard stock solution, 0.51 g of potassium nitrate (KNO_3) was added to 1 l of deionised water and then 0, 0.5, 1, 1.5 and 2 ml of the stock solution were added to separate flasks with 50 ml of deionised water; resulting in 0, 50, 100, 150 and 200 µM NO_3 solutions, respectively.

4.1.2.3.2 Chemical preparation

Three millilitres of sample (field and standard series) were placed into respective labelled test tubes. Two millilitres of buffer were added to each test tube, followed by ~ 2 g copper cadmium. The samples were agitated for 10 minutes. New or acid-stripped test tubes were placed on racks and 1 ml of the sample, followed by 1 ml of sulfanilimide solution (1 g sulfanilimide added to 100 ml 1.5 N HCl (15 ml of HCl plus 100 ml deionised water)). One millilitre diamine hydrochloride solution (0.02 g N1-naphthyl-diamine hydrochloride to 100 ml of deionised water) was added to each sample. The samples were swirled after each addition of a reagent. The colour was allowed to develop for at least five minutes and then read at a wavelength of 540 nm using a spectrophotometer.

4.2 River discharge and velocity

The Hydrological river discharge data for the Paul Kruger Gate weir (X3H021) (24°58.11'S, 31°30.92'E) and Lower Sabie weir (X3H015) (25°8.97'S, 31°56.44'E) obtained from the Department of Water Sanitation (DWS) website (http://www.dwa.gov.za/Hydrology/Verified/HyStations.aspx?Region=X&StationType=rbRiver) over the study period. The Paul Kruger Gate weir has operated since 1990 and Lower Sabie weir since 1987 (Heritage *et al*., 1997), therefore, 1983 and 1985 water quality and flow data were not available, and current issues with the probe have resulted in data collection up until 19/03/2020. *In situ* water velocity measurements were made at the precise sampling locations of diatom collection using a Transparent Velocity Head Rod (TVHR); available from Groundtruth, Pietermaritzburg).

4.3 Rainfall data

Monthly rainfall data for Skukuza and Lower Sabie were obtained from the SANParks website (http://dataknp.sanparks.org/sanparks/). Rainfall data for March 2020 was not available.

4.4 Diatoms

4.4.1 Diatom collection

Benthic diatoms (those living on rocks) can be identified by a golden-brown mucilaginous layer (Taylor *et al*., 2007). Microphytobenthos biomass and group composition (cyanobacteria, diatoms, and green algae) were measured using a handheld spectrofluorometer (bbe BenthoTorch) and the conventional collection method used to collect benthic diatoms. Ten rocks or stones (< 20 cm) preferably free of filamentous algae were selected. When no rocks or stones where present bedrock was used. The selected stones were measured using the BenthoTorch and care was taken to avoid damaging the biofilm layer (Kahlert and Mckie, 2014). From the same spot on the stones, benthic diatoms were scraped into a plastic tub using a toothbrush, which created a brown liquid that was poured into a small screw top container. The container was filled with 95% ethanol to make a final concentration of 70% ethanol. The containers were sealed and placed in the refrigerator until future use for microscope slide preparation. This method was chosen as it provides a direct comparison between the two methods.

4.4.2 BenthoTorch measurements

The BenthoTorch emits three different wavelengths (470, 525, and 610 nm) from seven LED’s. An additional LED using 700 nm is emitted to compensate for the effects of background reflection. The results are quantified as $\mu g.cm^{-2}$ by a built-in algorithm that is based on the fluorescence responses to all different excitation wavelengths (Echenique-Subiabre *et al*., 2016). The algorithms have been calculated using characteristic spectral fingerprints (fluorescence spectral signature) of each MPB group (Echenique-Subiabre *et al*., 2016). The pre-calibrated and pre-programmed factory settings were used for all measurements.

4.4.3 Conventional analysis

The benthic diatom samples were prepared using the hot hydrochloric acid (HCl) and potassium permanganate (KMnO4) technique (Taylor *et al*., 2007). The refrigerated samples settled for 24 hours at room temperature; the supernatant liquid was decanted, leaving only the diatom material behind. After shaking the solution, 1 ml of diatom solution was extracted and added into each test tube, followed by the addition of 1 ml of saturated $KMnO_4$. The samples were shaken and left to stand for 24 hours. A fume cabinet was used and 1 ml of concentrated HCL (32%) was added to each test tube. The samples were placed into a large beaker and boiled at 80^{o}C for one hour until the diatom solution became clear. After the oxidation of organic material, a jet of distilled water was added from the wash bottle, to fill each test tube and mix the contents. The test tubes were centrifuged at 3000 rpm for five minutes, the supernatant was decanted in a single motion leaving the pellet behind and the test tubes were filled with distilled water equating to 10 ml per test tube. In total the process was repeated five times. This process was done to oxidise organic material, neutralise the pH and isolate the diatom frustules. The diatom solution for each respective site was pipetted into a 1.5 ml Eppendorf for storage in the dark.

4.4.4 Slide preparation

The coverslips and slides were cleaned with ethanol, then the dry coverslips were placed onto a clean surface where deionised water was slowly pipetted onto the coverslips until a meniscus formed. One drop of diatom solution was pipetted into the meniscus of water on the respective cover slip and mixed to distribute the diatoms evenly. In cases where the diatom solution was saturated with material and cloudy, one drop of solution was added to the cover slip and then three quarters of the water was pipetted off and replaced with fresh distilled water to dilute the sample. This process was done to ensure diatom cells were not overly abundant one each slide, which would complicate identification of frustules under the microscope. The coverslips were left to dry overnight in a dust-free environment allowing the water to evaporate, leaving behind only the diatom frustules. A clean microscope slide was placed onto a hot plate and a drop of mountant (Pleurax; refractive index = 1.73) was added to the slide followed by the diatom-coated cover slip. The hot plate was heated to 180ºC for 2 – 5 minutes to evaporate the alcohol within the Pleurax; the process was complete once no more bubbles were seen coming off the slide. The slides were labelled with respective collection information.

The slides were viewed using a light microscope at 1000x magnification with oil immersion and a touch screen camera attachment. For diatom community analysis in South Africa, counting 400 valves per slide is recommended to produce semi-quantitative data for future analysis (Taylor *et al*., 2007). Analysis was started at the top left corner of the slide and the microscope was moved vertically until at least 400 frustules had been analysed. All diatoms within the visible field of view were identified to species level where possible. Pictures of the diatoms present in the field of view were taken using the Touchscope.

4.4.5 Diatom Ecological guilds

The low-profile guild comprises species of short stature, including prostrate (adhering to the substrate with the entire valve surface), adnate (apically attached but parallel to the substrate), erect (apically attached but perpendicular to the substrate), solitary centric, and slow-moving species (Passy, 2007a). All species of *Cymbella* encountered in this study did not form long stalks and were therefore included in the low-profile guild. The high-profile guild encompassed species of tall stature, including erect, filamentous, branched, chain-forming (spines), tube forming, stalked, and colonial centric, and the motile guild included comparatively fast-moving species (Passy, 2007a; B-Béres *et al.*, 2016). Guilds were obtained from Berthon *et al*. (2011) as not all the species in the study were included in the Passy (2007a) list.

4.4.6 OMNIDIA analysis

The diatom indices were established using Omnidia v.3.1 software (Lecointe *et al*., 1993), which utilises diatom species count data. For the data to be run through OMNIDIA a specific format is required; the first column contains site information, the second a four-letter code for the different species and the third, the counts for each slide totalling 400. The SADI was used to determine the ecological health (Table 4.1). (EcoClassification) of each sampling site where A is pristine (high quality), and E is severely degraded (bad quality). EcoClassification was used to compliment the physico-chemical drivers when analysing water quality (Harding and Taylor, 2011). The Specific Pollution Index (SPI) scores are equivalent to the SADI scores with the addition of endemic South African species (Harding and Taylor, 2014). The SPI makes use of 2035 taxa (Harding and Taylor, 2011).

Table 4.1: SADI scores to interpret ecological health based on SPI scores.

Ecological Category (EC)	Class	SPI
A	High quality	> 17.3
A/B		16.8-17.2
B	Good quality	13.3-16.7
B/C		12.9-13.2
C	Moderate quality	9.2-12.8
C/D		8.9-9.1
D	Poor quality	5.3-8.8
D/E		4.8-5.2
E	Bad quality	< 4.8

4.4.7 Biological Diatom Index (BDI)

The calculated Biological Diatom Index (BDI) scores ranges from 1 to 20, and water quality is categorised as one of five quality classes (Table 4.2) (Berthon *et al*., 2011). Additionally, the BDI has combined morphologically similar species that are difficult to identify by a non-specialist, and only 209 diatom taxa are used (Harding and Taylor, 2011).

Table 4.2: BDI scores to interpret water quality.

Water Quality Class	BDI
Very good quality	17 - 20
Good quality	13 - 16.9
Medium quality	9 - 12.9
Bad quality	5 - 8.9
Very bad quality	1 - 4.9

4.4.8 Diatom diversity and evenness scores

Table 4.3: Shannon Weaver Index representing water quality.

Shannon index	Water quality
> 3	Low pollution
1 - 3	Moderate pollution
< 1	High pollution

Evenness is constrained between 0 and 1, the less variation in a community the higher the evenness value (Gökçe, 2016).

4.4.9 Diatom distribution (CCA plot)

Only benthic diatom species that were present at a minimum of two sites and had a relative abundance > 1% were included in the CCA plot (Bere and Mangadze, 2014). Additionally, a preliminary DCA was run on the species data for the 2019 and 2020 sampling sessions and the gradient lengths were > 4 standard deviations, which shows that the species exhibit multilinear responses (ter Braak, 1986) and therefore the use of a CCA triplot to understand diatom species distribution in terms of environmental variables was considered appropriate. The CCA plot was reduced in species numbers due to the inclusion rules utilised in CANOCO to a 15% species fit range and 1% species weight range. Only limited environmental data were present for the 1983 and 1985 sampling periods along the Sabie River and therefore a PCA plot was used to determine inter-specific relationships within diatom communities. Environmental variables are not imperative for assessing diatom species tolerance and distribution (ter Braak and Verdonschot, 1995). The distribution of diatoms in the 2019 and 2020 CCA plot was used to determine the tolerance and distribution of species relative to each other – for example *Fragilaria ungeriana* was ordinated next to *Cymbella kolbei* in 2019 and 2020 so we can assume with some confidence that the two species have similar habitat requirements (ter Braak and Verdonschot, 1995).

4.5 Nutrient classification

According to DWAF (1996b) the TWQR for orthophosphates should only be determined after site specific studies. Analysis of orthophosphates should be coupled with its ratio to nitrogen – unimpacted streams have a N:P ratio greater than 25 – 40:1 and impacted or eutrophic rivers a N:P ratio of less than 10:1 (DWAF, 1996a). However, the Resource Quality Objectives (RQO) for the Sabie and Sand River system in terms of orthophosphates requires average levels to be less than 0.015 $mg.l^{-1}$ (DWS, 2016).

Table 4.4: Ammonium, TON, and orthophosphates concentrations for the TWQR and the trophic status of the water.

	Ammonium	**Orthophosphates**	**TON**
TWQR	0.07		0.5
Oligotrophic		< 0.005	< 0.5
Mesotrophic		0.005 - 0.025	0.5 - 2.5
Eutrophic		0.025 - 0.25	2.5 - 10
Hypertrophic		> 0.25	> 10

Table 4.5: Mineral content of water and its respective trophy classification.

Trophy classification	Mineral content ($\mu S.cm^{-1}$)
Oligotrophic	50 - 100
Mesotrophic	100 - 500
Eutrophic	> 500
Hypertrophic	> 1000

Taylor *et al*. (2007a).

The Sabie River RQO for conductivity, states that the river must have conductivity levels less than or equal to 300 $\mu S.cm^{-1}$ 95% of the time (DWS, 2016).

4.6 Statistical analysis

Analysis of variance (ANOVA), using Statistica 64, was used to test whether there were significant differences between physico-chemical parameters between sampling sites and between sampling sessions. Data that were not normal were transformed accordingly; ammonium (square root transformation), velocity (log transformation), and pH (cubed transformation). To be able to identify the environmental variables that contribute most to the overall variability in the data, multivariate techniques were used. These can then be used to establish which environmental variables best explain the variation in diatom community assemblages. The statistical technique for this study focused on the Canonical Correspondence Analysis (CCA) using CANOCO 5.5. CCA analysis does not consider habitat preferences of diatoms to be unimodal functions of habitat variables, a potential limitation of other multivariate techniques (ter Braak and Verdonschot, 1995).

Chapter 5: Results

Monitoring water quality involves chemical and physical measurements that provide a snapshot of a river's health (Rocha, 1992; Taylor *et al*., 2007b; Bere and Tundisi, 2010). Bioindicators in the form of MPB reflect the environmental stress on aquatic organisms and provide an accurate assessment of anthropogenic and natural changes (De la Rey *et al*., 2004; Dalu and Froneman, 2016; Dalu *et al*., 2016). Including bioindicators (diatoms) and other MPB along with other water quality monitoring techniques allows us to assess and monitor the water quality of the Sabie River.

5.1 Rainfall

Kruger National Park receives summer rainfall with an average Mean Annual Precipitation (MAP) of 600 mm that predominantly occurs during the months of November to March (DWA, 2013). Figure 5.1 illustrates the long-term average monthly rainfall and the current rainfall of Skukuza and Lower Sabie during the study period (September 2019 - March 2020). During the study period dry conditions were experienced in September and October of 2019 and wet conditions peaked in February 2020, a month before the March 2020 sampling period. During the sampling periods of September and October 2019, Skukuza received 0% and 62.7% of its average monthly rainfall, and Lower Sabie received 67.3% and 132.9%, respectively. An increase in rainfall during October 2019 can be seen for both Skukuza and Lower Sabie as the wet season began. The MAPs for Skukuza and Lower Sabie for 2019 were 550.4 mm/a and 602.6 mm/a, respectively – with Lower Sabie receiving more rainfall than Skukuza.

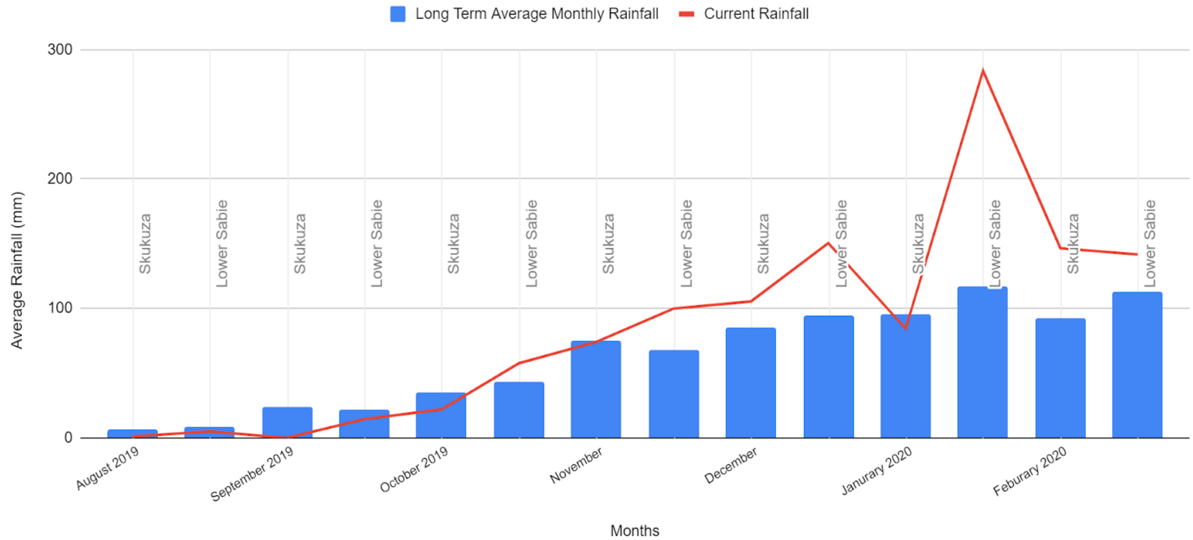


Figure 5.1: Average long-term monthly rainfall (mm) and rainfall recorded during the study period (current) along Sabie River at the Skukuza and Lower Sabie monitoring sites.

5.2 Physico-chemical parameters of the Sabie River within the KNP.

Ammonium and orthophosphate concentrations were higher in March 2020 compared to the low flow sampling periods, in contrast, TON concentrations were the highest during the low flow months. EC showed a weak increase from west to east, peaking at Lower Sabie during low flow months, however, once corrected for temperature the west-east trend was strong. DO was relatively high (> 6 $mg.l^{-1}$) throughout the study and decreased from west to east during the low flow months. pH was slightly basic throughout the study and did not exceed 9, a pH higher than 9 would favour the conversion of ammonium to toxic ammonia. Temperature ranged from 18.7°C to 31.0°C, peaking at Lower Sabie (which would have contributed to elevated EC at this site). Clarity was highly variable between sites during the dry months and consistently lowest at Lower Sabie. Water velocity was lowest, and similar, during the two dry months.

To better represent the changes in ammonium, orthophosphates, and TON levels over the three sampling periods, from the low flow to high flow periods box plots were utilised.

There were no significant differences in ammonium concentrations between the five sites along the Sabie River during each of the three sampling sessions (Figure 5.2). There were temporal differences between sites 1 ($p < 0.04$), 3 ($p < 0.02$), 4 ($p < 0.05$) and 5 ($p < 0.01$) that all showed increased ammonium levels from September 2019 to March 2020. Ammonium levels increased for site 5 ($p < 0.02$) when comparing October 2019 to March 2020, the other four sites showed no statistical changes.

The Target Water Quality Range (TWQR) for ammonium concentration is 0.007 $mg.l^{-1}$ (Table 4.4), the Sabie River showed ammonium levels increasing from the low flow period to the high flow period ($F = 30.57$, $p < 0.01$) (Figure 5.2). There were no significant differences in ammonium concentrations between the two low flow sampling sessions (September and October 2019) ($p = 0.81$)) but they did increase significantly from the low flow to the high flow sampling session, September 2019 (0.0050 ± 0.003 $mg.l^{-1}$) to March 2020 (0.1424 ± 0.018 $mg.l^{-1}$) ($p < 0.01$), and October 2019 (0.0169 ± 0.005 $mg.l^{-1}$) to March 2020 ($p < 0.01$).

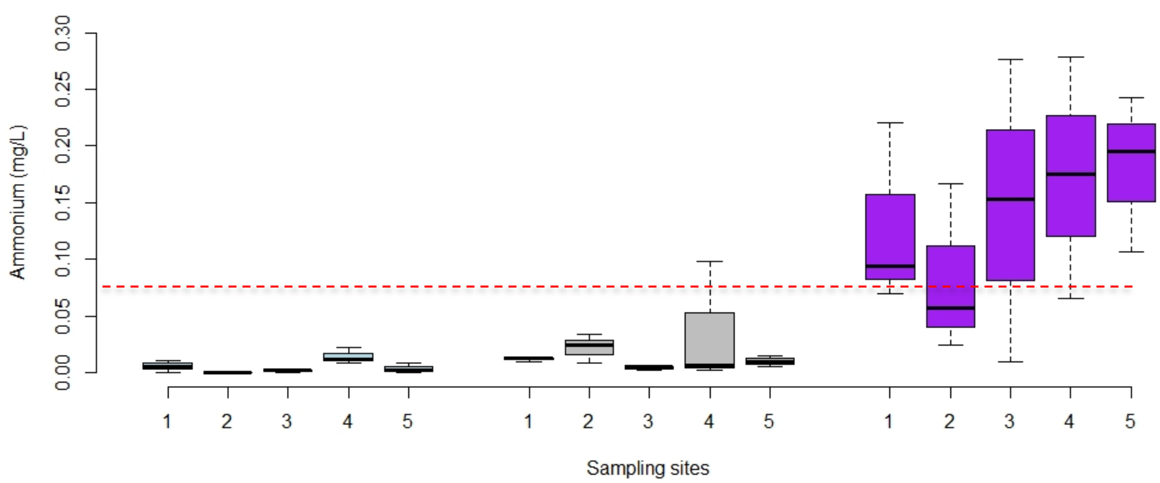


Figure 5.2: Box and whisker plot illustrating the mean ammonium concentration ($mg.l^{-1}$) across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River with the KNP. The TWQR of 0.07 $mg.l^{-1}$ is indicated by the dashed red line.

There were differences in orthophosphate concentrations across the sites and sampling periods ($F = 36.98$, $p < 0.01$) (Figure 5.3). There were no temporal or spatial differences in phosphate levels between the sites in September 2019 and October 2019. However, there were differences between sites in March 2020; site 1 had the lowest concentration compared to site 2 ($p < 0.01$), 4 ($p < 0.01$), and 5 ($p<0.01$). Site 3 had lower levels compared to site 2 ($p < 0.01$), 4 ($p < 0.01$), and 5 ($p < 0.01$). From September 2019 to March 2020, sites 2 ($p < 0.01$), 3 ($p < 0.01$), 4 ($p < 0.01$), and 5 ($p < 0.01$) showed increased phosphate levels. Sites 2 ($p < 0.01$), 3 ($p < 0.01$), 4 ($p < 0.01$) and 5 ($p < 0.01$) also showed increased phosphate levels from October 2019 to March

2020. Site 1 showed no change in orthophosphate concentrations from the low flow periods to the high flow periods.

During the September 2019 sampling period orthophosphate concentrations ranged from 0 to 0.001 $mg.l^{-1}$ across the five sites. These levels (< 0.005 $mg.l^{-1}$) result in the system being classified as oligotrophic – limited growth of aquatic plants and blue-green algae. During the October 2019 sampling period, levels ranged from 0.001 to 0.018 $mg.l^{-1}$, sites 1 and 5 exhibited mesotrophic conditions (0.005 – 0.025 $mg.l^{-1}$) and sites 2, 3, and 4 were oligotrophic. In March 2020, orthophosphate concentrations across all five sites ranged from 0.011 to 0.087 $mg.l^{-1}$ exhibiting mesotrophic – eutrophic conditions (Table 4.4).

The RQO of the Sabie River system for orthophosphates requires average concentrations to be less than 0.015 $mg.l^{-1}$. Average orthophosphate concentrations along the Sabie River increased significantly ($p < 0.01$) from the low flow periods September 2019 (0.0002 ± 0.000 $mg.l^{-1}$), and October 2019 (0.0083 ± 0.004 $mg.l^{-1}$) to the high flow period March 2020 (0.0535 ± 0.013 $mg.l^{-1}$). Concentrations did not change between the two low flow periods ($p > 0.41$).

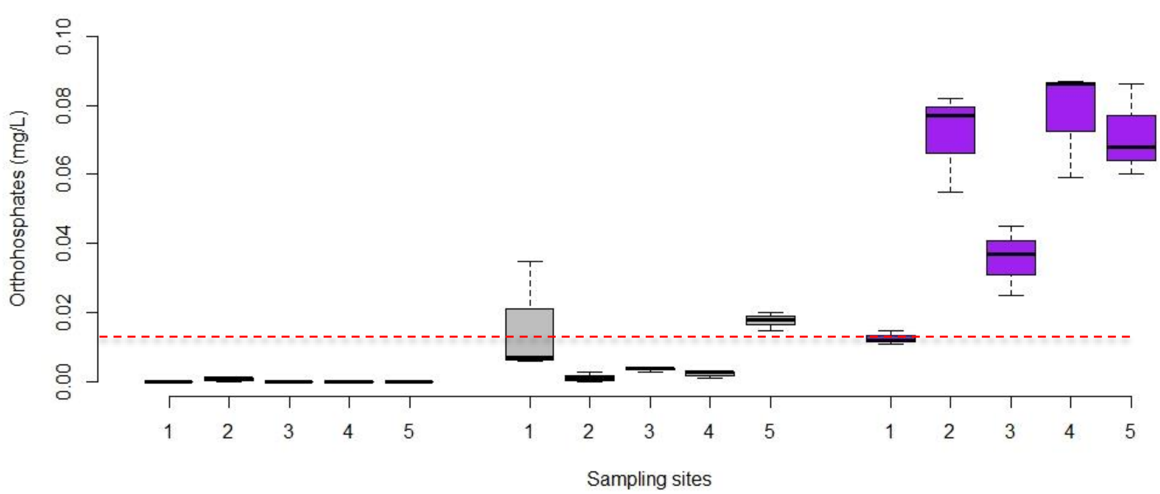


Figure 5.3: Box and whisker plot illustrating the mean orthophosphate concentrations ($mg.l^{-1}$) across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River with the KNP. The RQO of 0.015 $mg.l^{-1}$ is indicated by the dashed red line.

Total Oxidised Nitrogen (TON) concentrations for all sampling seasons and sites showed no temporal and spatial variation ($F = 0.831$, $p > 0.632$) (Figure 5.4). TON concentrations along the Sabie River were in alignment with the TWQR (< 0.5 $mg.l^{-1}$) during the study period. In September 2019 (0.636 ± 0.080 $mg.l^{-1}$), and October 2019 (0.834 ± 0.114 $mg.l^{-1}$), the low flow sampling periods were classified as mesotrophic and the March 2020 (0.379 ± 0.024 $mg.l^{-1}$) high flow sampling period as oligotrophic (Table 4.4).

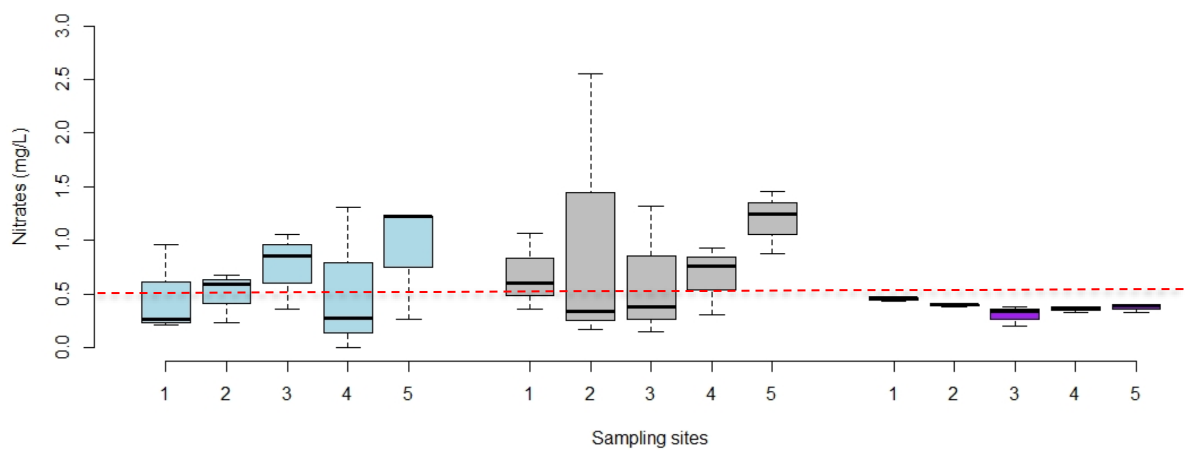


Figure 5.4: Box and whisker plot illustrating mean TON (indicated as nitrates) concentrations ($mg.l^{-1}$) across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP. The TWQR of 0.5 $mg.l^{-1}$ is indicated by the red dotted line.

There were significant differences in velocity between sites and sampling periods ($F = 3.822$, $p < 0.01$) (Figure 5.5). Average river velocity during the low flow periods September 2019 (0.54 ± 0.14 $m^3.s^{-1}$) and October 2019 (0.51 ± 0.08 $m^3.s^{-1}$) remained constant. However, average river velocity increased significantly from the low flow periods to the high flow period – March 2020 (1.87 ± 0.27 $m^3.s^{-1}$). Overall, there was only slight flow variation seen for each sampling site, with site 2 exhibiting increased flow from September 2019 to March 2020 ($p < 0.01$) and from October 2019 to March 2020 ($p < 0.03$).

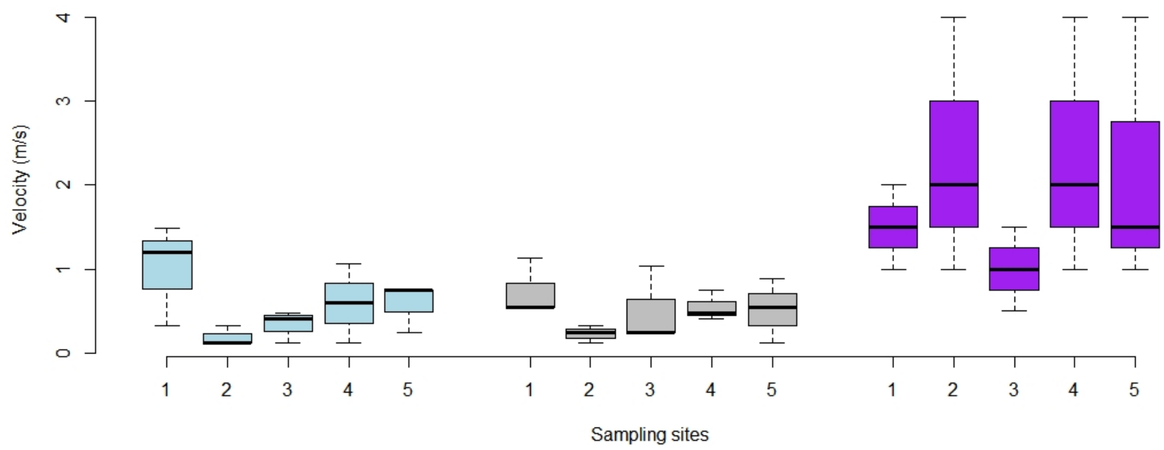


Figure 5.5: Box and whisker plot illustrating of mean river velocity ($m^3.s^{-1}$) across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

In February 2020, the Sabie River experienced a large peak in rainfall (Figure 5.1) and maximum flows at the Lower Sabie and Kruger Gate weirs were recorded at 1246 $m^3.s^{-1}$ and 102 $m^3.s^{-1}$, respectively. The average flow at Lower Sabie in February 2020 was 115.59 $m^3.s^{-1}$ and 18.31 $m^3.s^{-1}$ at Kruger Gate. In March 2020, the average flow at Lower Sabie was 21.70 $m^3.s^{-1}$ and 8.73 $m^3.s^{-1}$ at Kruger Gate. The river velocity during the high flow period was greater than expected compared to the normal base flow of the Sabie River. The baseline flows for the Sabie River during the dry period; were 2.472 and 1.651 $m^3.s^{-1}$ for September 2019 at the Lower Sabie and Kruger Gate weirs, and 1.651 and 1.858 $m^3.s^{-1}$ for October 2019, respectively. During the high flow period of March 2020 river flow was 21.700 $m^3.s^{-1}$ for Lower Sabie and 8.726 $m^3.s^{-1}$ for Kruger Gate weirs (Table 5.1).

Table 5.1: Mean, minimum and maximum monthly river velocity ($m^3.s^{-1}$) recorded at the two weirs along the Sabie River at X3H015 (Lower Sabie) and X3H021 (Paul Kruger Gate) sites.

Season		X3H015	X3H021
August 2019	Mean	2.320	2.112
	Min	1.774	1.964
	Max	2.910	2.290
September 2019	Mean	2.472	2.204
	Min	1.300	1.762
	Max	5.788	3.220
October 2019	Mean	1.651	1.858
	Min	0.016	0.006
	Max	5.788	3.220
November 2019	Mean	8.981	4.240
	Min	0.013	0.002
	Max	37.300	6.925
December 2019	Mean	17.674	4.959
	Min	0.013	0.002
	Max	37.300	7.924
January 2020	Mean	33.720	8.735
	Min	0.013	0.002
	Max	40.190	11.420
February 2020	Mean	115.589	18.308
	Min	2.532	6.067
	Max	1246.000	102.000
March 2020	Mean	21.700	8.726
	Min	21.700	5.049
	Max	21.700	10.860

In situ sampling at site 2 (downstream of Paul Kruger Gate) can be compared to the Paul Kruger Gate weir (X3H021) and *in situ* sampling at site 3 (Lower Sabie) can be compared to the

Lower Sabie weir (X3H015). Unfortunately, the X3H015 and the X3H021 weirs only started capturing flow data from 1986 and 1990 respectively and therefore there was no flow data available for the 1983 and 1985 sampling periods. The flow data captured *in situ* and by the weirs along the Sabie River had similar readings except for the March 2020 flow data where the weirs captured river flow at a higher velocity than the *in situ* sampling (Table 5.2).

Table 5.2: Mean river velocity ($m^3.s^{-1}$) measured *in situ* at site 2 and 3 and river velocity recorded along the Sabie River at the Paul Kruger Gate (X3H021) and Lower Sabie (X3H015) weirs.

Month	**Sites**	**River velocity ($m^3.s^{-1}$)**	
		In situ	**Flow weirs (X3H021 and X3H015)**
September 2019	2	0.19	1.92
	3	0.34	1.98
October 2019	2	0.23	N/A
	3	0.50	1.10
March 2020	2	2.33	8.01
	3	1.00	21.70

Site 1 of the Sabie River in September 2019 was significantly clearer (91.0 cm) when compared to sites 3 (73.0 cm) ($p < 0.01$), 4 (19.0 cm) ($p < 0.01$), and 5 (55.5 cm) ($p < 0.01$) (Figure 5.6). In October 2019, site 1 (99.0 cm) had the best water clarity compared to site 2 (66.5 cm) ($p < 0.01$), 3 (82.0 cm) ($p < 0.01$), 4 (44.0 cm) ($p < 0.01$), and 5 (61.5 cm) ($p < 0.01$). Site 4 was the most turbid site during September and October 2019. There was no variation in clarity between sites 1 (39.5 cm), 2 (43.5 cm), 4 (44.5 cm), and 5 (38.0 cm) during March 2020 and site 3 (30.0 cm) had the most turbid water.

From September 2019 to October 2019 site 2 ($p < 0.01$) showed an increase in turbidity, whereas site 4 showed a decrease ($p < 0.01$). From the low flow period of September 2019 to the high flow period of March 2020, sites 1 ($p < 0.01$), 2 ($p < 0.01$), 3 ($p < 0.01$), and 5 ($p < 0.01$)

exhibited increased turbidity, and site 4 ($p < 0.01$) showed the opposite. Clarity was reduced at sites 1 ($p < 0.01$), 2 ($p < 0.01$), 3 ($p < 0.01$), and 5 ($p < 0.01$) from October 2019 to March 2020.

The average clarity of the Sabie River did not change during the low flow periods, it did however decrease from the low flow periods, September 2019 (64.6 cm) ($p < 0.02$) and October 2019 (70.6 cm) ($p < 0.01$), to the high flow period, March 2020 (39.1 cm) ($F = 7.208$, $p < 0.01$).

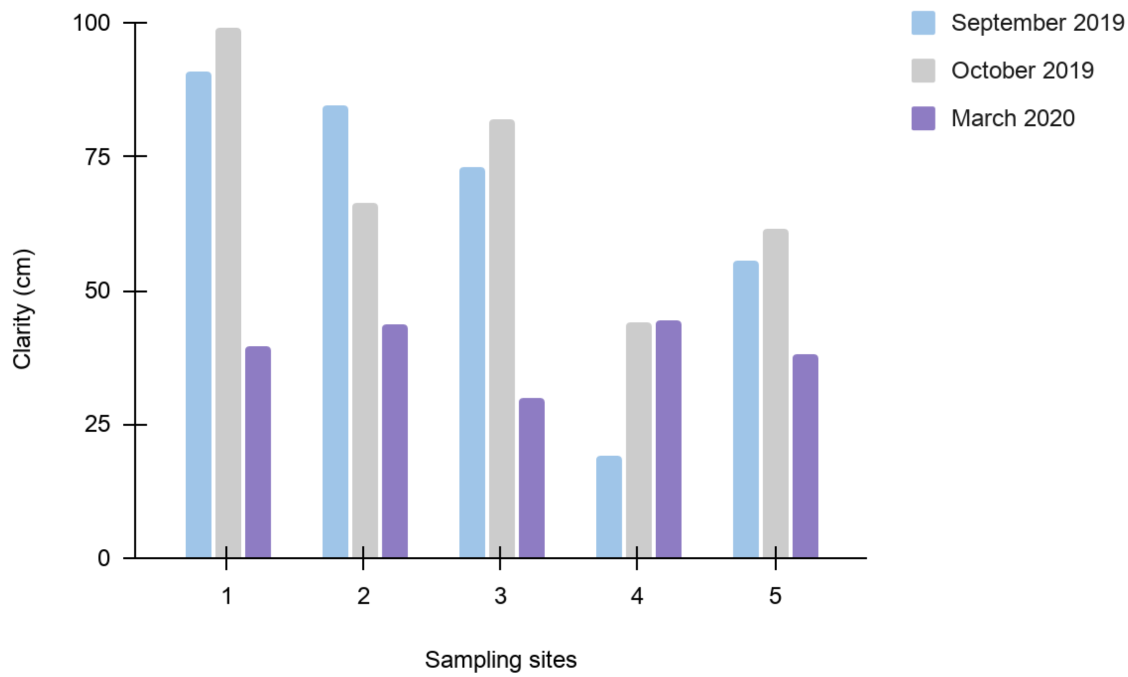


Figure 5.6: Bar graph illustrating mean clarity readings (cm) across the five sites during the three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Specific conductivity ($\mu S.cm^{-1}$) increased from the upstream site 1 to the downstream site 5 for all three sampling periods ($p < 0.01$) (Figure 5.7).

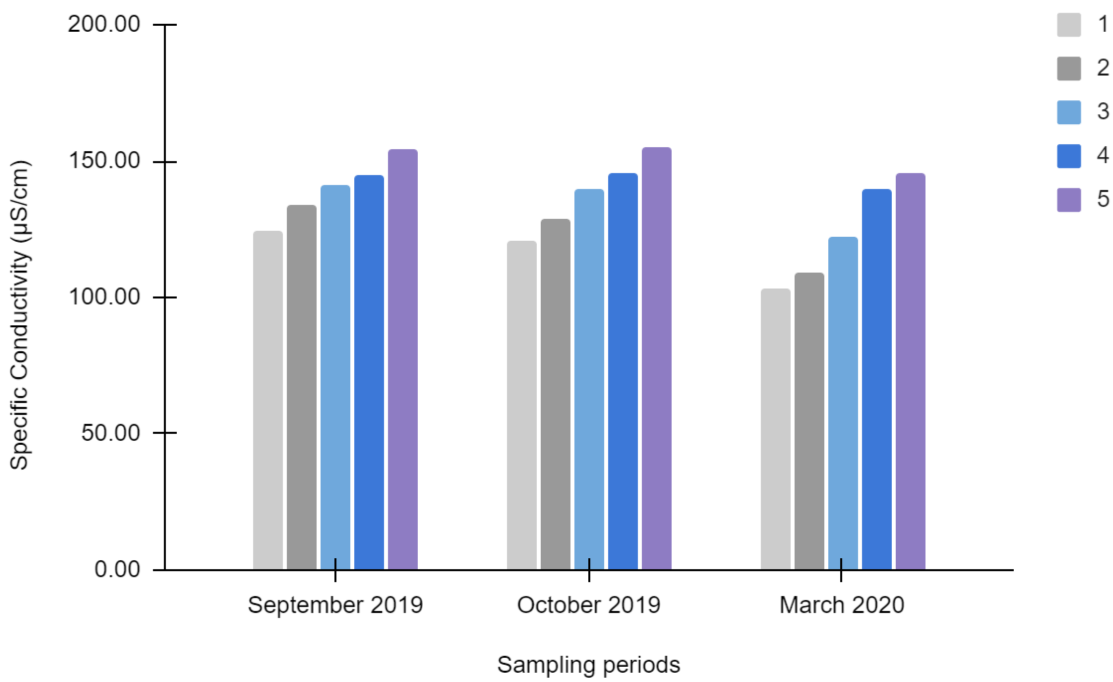


Figure 5.7: Bar graph illustrating the mean Specific Conductivity (µS.cm^{-1}) across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Conductivity showed temporal and spatial variation across the sampling periods and between the sites ($F = 32249$, $p < 0.01$), and concentrations ranged from 101.73 to 158.87 (µS.cm^{-1}), which results in the water being classified as mesotrophic (Table 4.5). The Sabie River met the RQO for conductivity, levels were below 300 µS.cm^{-1} 95% of the time. Average conductivity levels did not change from the low flow periods, September 2019 (133.00 ± 7.17 µS.cm^{-1}) and October 2019 (139.30 ± 7.58 µS.cm^{-1}) to the high flow period, March 2020 (130.76 ± 11.57 µS.cm^{-1}) ($F = 0.848$, $p > 0.44$) (Figure 5.8).

Conductivity increased downstream from sites 1 (101.73 µS.cm^{-1}) to 5 (158.87 µS.cm^{-1}) for the March 2020 sampling period, however the trend was not present in September 2019, and October 2019. Site 4 had the highest conductivity in September (152.13 µS.cm^{-1}), and October 2019 (156.13 µS.cm^{-1}) while site 1 had the lowest conductivity for all three sampling periods ($p < 0.01$). Sites 1 ($p < 0.01$), 2 ($p < 0.01$), and 3 ($p < 0.01$) had conductivity levels that decreased from September 2019 to March 2020, and site 4 ($p < 0.01$), and 5 ($p < 0.01$) showed increased conductivity. From October 2019 to March 2020 temporal variation occurred as sites 1 ($p < 0.01$), 2 ($p < 0.01$), and 3 ($p < 0.01$) had increased conductivity levels and at site 5 ($p < 0.01$) levels decreased. Site 4 showed no variation in conductivity levels.

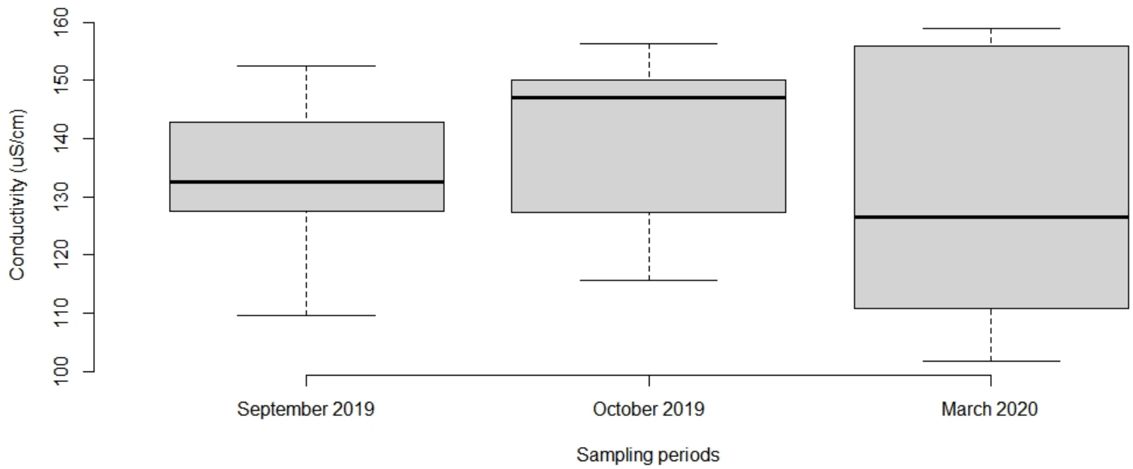


Figure 5.8: Box and whisker plot illustrating mean conductivity ($\mu S.cm^{-1}$) of the five sites for the three different sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Over the three sampling periods, September 2019, October 2019, and March 2020 the five sites were not always sampled at the same time, therefore, time of day may have influenced increases and decreases in water temperature to a small degree. The sites were sampled at the following times: September 2019 – 1 (8:30), 2 (11:45), 3 (7:00), 4 (13:00), and 5 (8:30), October 2019 - 1 (9:00), 2 (10:30), 3 (16:00), 4 (15:00), and 5 (12:30), and March 2020 - 1 (10:30), 2 (12:00), 3 (8:30), 4 (16:00), and 5 (13:30). Only two sites were sampled at different times during the study namely, site 3 in October 2019 was sampled in the afternoon compared to the morning sampling sessions in September 2019 and March 2020, and site 5 in September 2019 was sampled in the morning compared to the afternoon sampling times of October 2019 and March 2020.

The average temperature for the Sabie River increased ($F = 12.89$, $p < 0.01$) from September 2019 (22.36 ± 1.64ºC) to October 2019 (25.43 ± 1.18ºC) ($p < 0.01$), and from September 2019 to March 2020 (27.61 ± 1.25ºC) ($p < 0.01$), and no changes occurred from October 2019 to March 2020 ($p < 0.01$) (Figure 5.9). Site 4 had the highest temperature ($p < 0.01$)

and site 1 had the lowest temperature ($p < 0.01$) for all three sampling periods (September 2019, October 2019, and March 2020).

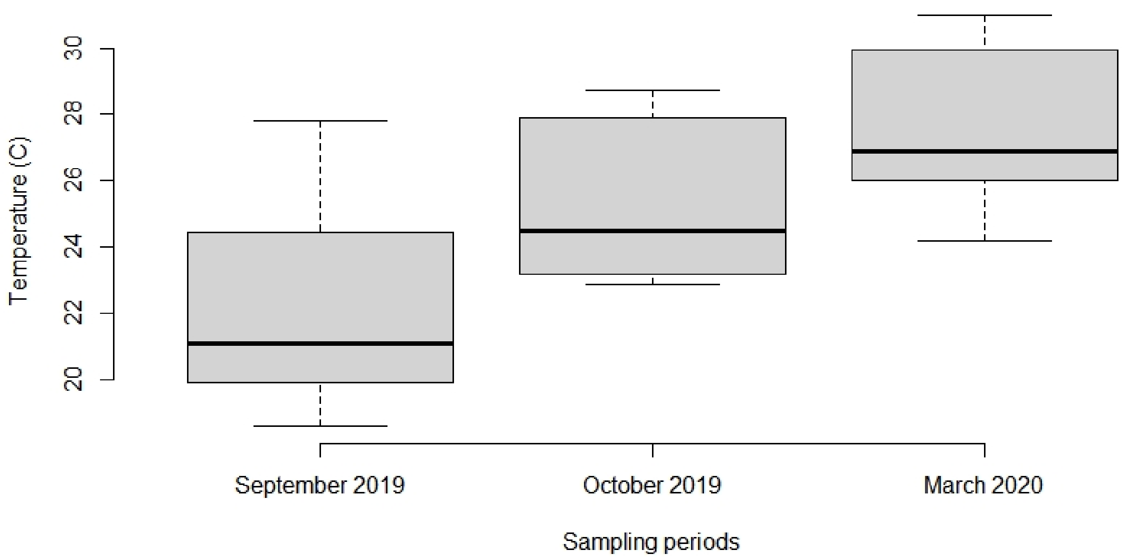


Figure 5.9: Box and whisker plot illustrating the mean temperature (°C) of the five sites for the three different sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

In September 2019, DO concentration decreased from upstream to downstream (Figure 5.10). Site 1 had the highest DO concentrations (12.34 mg.l^{-1}), compared to site 2 (11.52 mg.l^{-1}) ($p < 0.01$), 3 (10.80 mg.l^{-1}) ($p < 0.01$), 4 (10.55 mg.l^{-1}) ($p < 0.01$), and 5 (7.46 mg.l^{-1}) ($p < 0.01$), and in comparison site 5 had the lowest DO concentrations. In October 2019, DO concentrations were higher for site 1 compared to site 4 ($p < 0.01$), and 5 ($p < 0.01$). Site 2 had higher levels compared to site 3 ($p < 0.02$), 4 ($p < 0.01$), and 5 ($p < 0.01$). Site 5 had the lowest DO concentrations (8.14 mg.l^{-1}). There were no discernible spatial trends in DO concentrations between the five sites in March 2020.

From September 2019 to October 2019, DO concentrations decreased for the first four sites except site 5 that exhibited increased levels ($p < 0.01$). When comparing September 2019 to March 2020, sites 1 to 4 had decreased DO concentrations. All five sites showed decreased DO concentrations when comparing October 2019 to March 2020. Average DO concentrations decreased from September 2019 (10.53 ± 0.83 mg.l^{-1}) to October 2019 (8.79 ± 0.24 mg.l^{-1}) ($F = 47.91$, $p < 0.01$), and from September 2019 to March 2020 (6.79 ± 0.08 mg.l^{-1}) ($p < 0.01$), as well as from October 2019 to March 2020 ($p < 0.01$). Overall, average DO concentrations decreased from the low flow periods of September 2019, and October 2019 to the high flow period of March 2020.

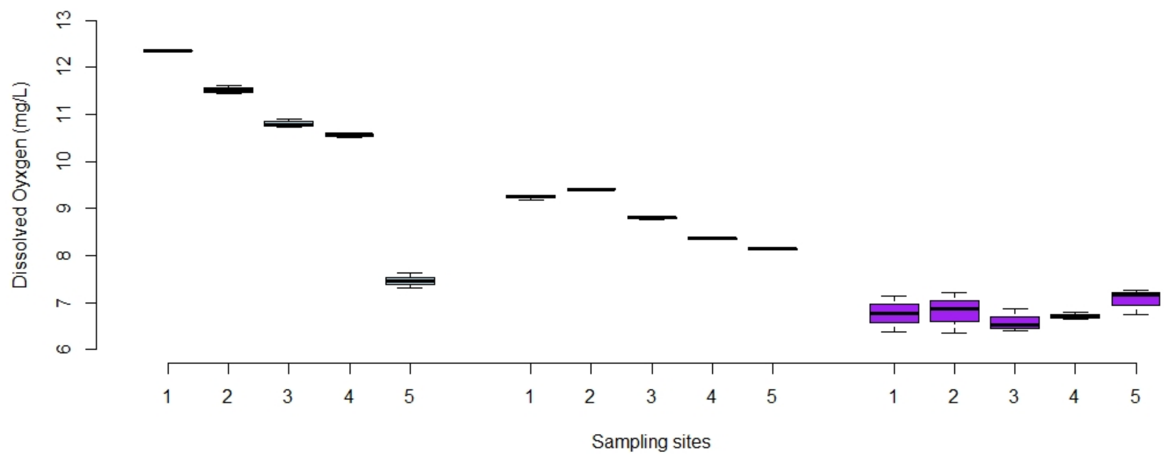


Figure 5.10: Box and whisker plot illustrating the mean dissolved oxygen (mg.l^{-1}) across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

During September 2019, site 5 (8.50) had the highest pH compared to sites 1 (7.97) ($p < 0.01$), 2 (8.07) ($p < 0.01$), 3 (7.79) ($p < 0.01$), and 4 (8.14) ($p < 0.01$) (Figure 5.11). In October 2019, site 3 (8.34) had the highest pH compared to sites 1 (7.97) ($p < 0.01$), 2 (7.73) ($p < 0.01$), 4 (7.69) ($p < 0.01$), and 5 (7.49) ($p < 0.01$). Site 5 had the lowest pH in October 2019. In March 2020, site 3 (8.66) had the highest pH compared to sites 1 (7.78) ($p < 0.01$), 2 (8.03) ($p < 0.01$), 4 (7.69) ($p < 0.01$), and 5 (8.10) ($p < 0.01$).

From September 2019 to October 2019 sites 2 ($p < 0.01$), 4 ($p < 0.01$), and 5 ($p < 0.01$) showed decreased pH levels and site 3 ($p < 0.01$) exhibited an increase in pH. From September 2019 to March 2020, pH increased for site 3 ($p < 0.01$) and decreased for site 5 ($p < 0.01$). From October 2019 to March 2020, sites 2 ($p < 0.01$), 3 ($p < 0.01$), 4 ($p < 0.04$), and 5 ($p < 0.01$) had increased pH. The pH across the sampling periods showed seasonal and temporal variation ($F = 3.81$, $p < 0.03$) and the average pH in September 2019 was (8.10 ± 0.12), October 2019 (7.84 ±

0.14), and March 2020 (8.11 ± 0.15). The only significant difference in average pH was between the months of October 2019 and March 2020 ($p < 0.05$).

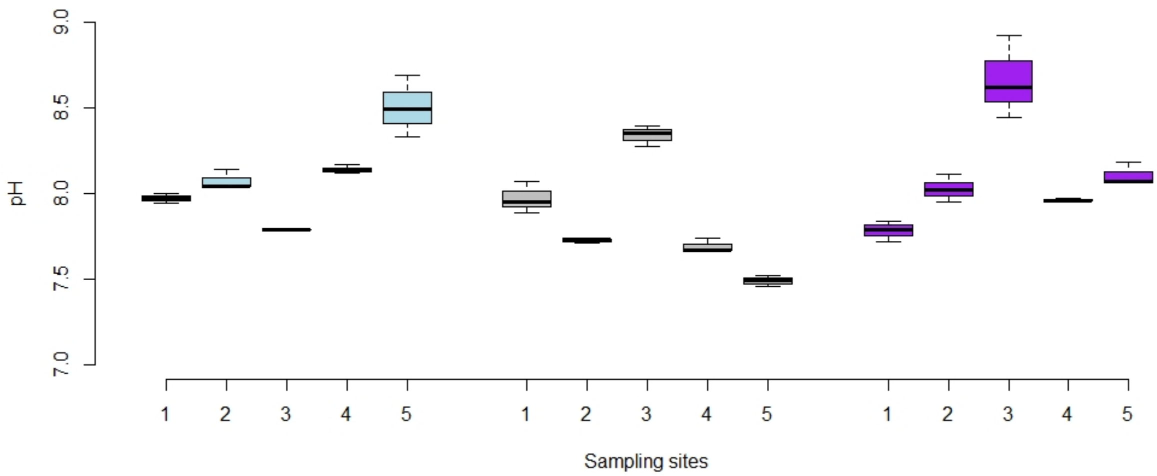


Figure 5.11: Box and whisker plot illustrating the mean pH across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

5.3 Diatom biomass and composition

A total of 44 diatom species, restricted to species with relative abundances > 1% and occurrence at a minimum of two sites, were recorded from the diatom slides collected in 1983 and 1985. Sixty-five species were recorded during the 2019 – 2020 sampling periods. A total of 70 diatom species were identified along the Sabie River located within Kruger National Park's boundaries. Of those an overlap of 39 species were recorded in both the early and latter sampling periods. The five most abundant diatom species sampled during 1983 were *Cymbella kolbei*, *Planothidium rostratum*, *Cocconeis placentula*, *Staurosira elliptica*, and *Staurosirella pinnata*. In 1985; *Achnanthidium minutissimum*, *Gomphonema venusta*, *Navicula heimansoides*, *C. placentula*, and *Cymbella kappii*. In 2019; *Cymbella turgidula*, *C. placentula*, *Encyonopsis leei* var. *sinensis*, *Planothidium rostratum*, and *Navicula microcari*. In 2020; *C. turgidula*, *C. placentula*, *C. kolbei*, *Navicula germainii*, and *Eolimna subminuscula*.

In 1983 the most dominant species (*C. kolbei*) is found in oligotrophic, alkaline waters. The other four dominant species (*P. rostratum*, *C. placentula*, *S. elliptica*, and *S. pinnata*) occur in clean waters with low to high electrolyte content, as well as mesotrophic and eutrophic waters. In 1985 the most dominant species (*A. minutissimum*) occurs in nutrient-poor waters with moderate electrolyte content. The other four dominant species (*G. venusta*, *N. heimansoides*, *C. placentula*, and *C. kappii*) occurs in oligotrophic to mesotrophic (low to moderate electrolyte content), weakly alkaline and acidic waters. In 2019 and 2020 the most dominant diatom species (*C. turgidula*), is found in oligotrophic – mesotrophic waters with low – moderate conductivity and it can tolerate organic pollution. In 2019 the other four dominant species (*C. placentula*, *E. leei var. sinensis*, *P. rostratum*, and *N. microcari*) are found in low to electrolyte rich waters and in 2020 the dominant diatoms species (*C. placentula*, *C. kolbei*, *N. germainii*, and *E. subminuscula*) generally occur in eutrophic, electrolyte rich waters and can tolerate pollution.

Table 5.3: Relative abundances (%) of benthic diatom species recorded at five sites across three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Diatom Species	Sites														
	September 2019					October 2019					March 2020				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Achnanthidium affine	1.75	0.00	1.75	0.25	0.50	3.25	1.00	1.00	0.50	0.00	2.00	0.00	1.25	0.00	0.00
A. crassum	21.75	1.25	0.25	0.50	0.00	9.75	5.50	1.00	4.25	0.00	15.00	1.50	0.25	0.50	0.00
A. exiguum	0.25	0.00	0.50	0.00	0.00	0.00	0.50	0.25	1.25	1.00	0.25	0.25	0.25	0.75	0.75
A. minutissimum	1.75	4.00	10.50	1.00	0.00	2.25	13.25	6.50	6.25	0.25	1.25	0.00	0.00	0.00	7.50
A. straubianum	0.00	0.00	1.00	0.50	0.00	0.00	1.00	0.75	3.25	1.00	0.00	0.00	0.00	0.00	1.50
Anorthoneis dulcis	0.00	0.25	0.75	0.25	0.00	0.00	0.00	0.25	0.75	0.00	0.00	0.00	0.00	0.25	0.00
Caloneis bacillum	0.00	0.00	0.25	0.25	0.00	0.00	0.25	0.50	0.00	0.25	0.00	0.00	0.00	0.00	0.00
Capartogramma crucicula	0.00	0.00	0.25	0.25	0.50	0.00	0.25	0.50	0.25	1.25	0.25	0.25	1.50	0.00	0.25
Cocconeis placentula	4.00	2.00	2.75	2.75	36.00	2.75	6.50	3.50	3.50	16.25	9.50	6.50	6.50	2.25	18.00
Cymbella kappii	0.00	0.00	0.00	0.00	0.00	3.75	2.25	0.00	0.00	0.00	1.00	6.25	3.00	0.50	0.00
C. kolbei	0.75	0.50	0.25	18.75	0.00	4.00	4.00	0.75	1.50	0.00	3.75	20.75	7.75	1.25	0.00
C. tumida	1.25	2.25	2.75	9.25	0.50	2.50	6.75	8.00	2.50	0.75	0.25	0.00	0.00	0.50	0.00
C. turgidula	6.75	2.25	6.00	24.75	0.00	26.75	10.75	16.25	3.00	2.25	13.75	38.50	8.00	2.50	0.50
Diadesmis confervaceoides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	0.25	0.00	0.00	0.00	0.50
Encyonema minutum	1.00	0.00	0.25	0.25	0.50	0.25	0.00	0.00	0.50	1.00	2.25	0.00	0.25	0.75	0.25

Encyonopsis leei var. *sinensis*	3.75	23.50	25.00	18.25	0.25	7.75	4.25	9.75	4.00	0.00	3.25	0.50	0.00	0.00	0.00
Eolimna minima	0.00	1.00	0.00	0.00	0.25	0.50	0.25	0.50	0.00	2.50	0.25	0.50	0.25	0.25	0.00
E. subminuscula	0.50	0.50	0.00	0.50	2.25	0.00	0.00	0.00	1.50	1.50	0.25	0.00	1.00	21.00	6.50
Fallacia insociabilis	0.25	5.50	4.25	0.25	0.00	2.25	3.50	1.50	3.00	6.25	1.25	3.00	1.50	1.50	4.25
F. pygmaea	0.00	2.50	6.75	5.00	0.00	0.25	1.25	3.75	14.00	0.25	0.00	0.25	1.00	4.75	1.25
Fragilaria biceps	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.25	0.00
F. capucina	0.00	0.25	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	2.00	1.00	0.00	0.00	0.00
F. parasitica	0.00	0.00	0.00	0.00	0.00	2.25	0.25	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00
F. ulna var. acus	0.00	0.00	0.00	0.00	0.25	0.75	0.50	0.00	0.00	0.25	0.50	0.00	0.00	0.00	0.00
F. ungeriana	0.00	0.00	0.50	1.00	0.00	3.75	2.00	0.75	0.50	0.00	7.25	0.75	0.00	0.00	0.00
Geissleria decussis	0.75	0.50	0.00	0.00	0.00	0.50	0.50	0.50	0.50	0.50	0.00	0.25	0.00	0.25	0.50
Gomphonema minutum	2.25	0.00	0.25	0.75	3.25	0.25	0.25	0.25	0.00	1.00	0.25	1.50	2.00	0.50	1.00
G. parvulum	1.75	0.75	0.00	0.00	2.75	0.25	1.25	0.75	0.50	1.25	0.25	0.00	0.75	12.50	4.50
G. pumilum var. rigidum	1.00	0.50	0.25	0.00	1.75	1.00	0.00	0.25	0.50	0.50	0.00	0.00	0.00	0.25	0.25
G. venusta	6.00	1.00	0.00	0.00	5.25	1.75	0.25	0.00	0.00	0.75	0.50	0.00	0.25	0.00	0.75
Gyrosigma acuminatum	0.25	0.50	0.25	0.00	0.50	0.25	0.00	1.00	0.00	0.50	0.50	0.00	1.00	0.00	0.50
Hippodonta capitata	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.25
Karayevia ploenensis	0.00	0.00	0.00	0.00	0.25	0.00	0.25	0.00	0.00	1.25	0.00	0.25	0.00	0.00	0.75
Navicula capitatoradiata	0.75	2.50	2.25	0.50	0.00	0.75	0.00	1.25	0.50	0.00	0.75	0.50	0.00	0.00	0.00
N. cryptocephala	0.00	0.25	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.25
N. cryptotonella	1.00	1.00	0.00	0.00	0.75	3.75	1.00	0.00	0.25	0.50	1.25	0.00	0.25	0.50	1.25

N. germainii	1.00	1.00	1.50	0.75	0.50	0.00	0.00	0.00	0.50	0.50	2.00	3.50	20.75	3.75	6.25
N. gregaria	2.00	1.75	0.50	0.00	0.50	1.25	0.25	1.00	0.00	0.75	0.25	2.00	3.50	0.25	2.25
N. heimansioides	8.25	4.25	2.75	0.00	0.00	6.00	1.25	0.00	0.00	0.00	2.50	0.00	0.00	0.00	0.00
N. microcari	4.50	19.50	13.50	0.25	0.25	2.50	7.25	9.75	1.50	0.00	4.50	0.75	0.25	0.00	0.50
N. rostellata	1.25	2.75	1.50	0.50	1.00	0.00	0.50	6.00	1.00	1.50	1.50	1.25	4.25	1.25	1.75
N. schroeteri	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	1.00	0.00	2.00	9.50	2.25	1.50
N. veneta	0.25	2.00	1.00	0.50	0.75	0.75	1.50	3.75	1.00	0.50	0.00	0.00	7.50	4.00	1.50
Nitzschia acidoclinata	0.25	0.00	0.00	0.25	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N. archibaldii	0.00	0.00	0.00	0.00	1.25	0.25	0.00	0.25	0.00	0.50	0.50	0.75	0.75	4.50	0.25
N. aurariae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.25	0.00	0.00	0.00
N. capitellata	0.25	0.00	1.00	0.00	0.75	0.50	0.75	0.25	0.75	0.25	3.00	0.50	1.25	1.25	1.25
N. dissipata var. media	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	1.75	0.00	0.00
N. filiformis	0.25	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.50	0.00	0.25	0.25	0.00	0.00
N. frustulum	3.50	1.00	3.75	7.75	6.00	0.00	2.75	2.00	28.25	3.25	1.25	0.00	0.75	8.00	5.25
N. iremissa	0.00	0.00	0.25	0.75	0.00	0.00	0.00	0.25	0.00	0.00	0.50	0.75	0.00	0.25	0.25
N. liebertruthii	2.50	0.00	0.00	0.00	5.25	0.25	0.00	0.00	0.00	0.75	1.00	0.00	0.25	0.25	0.00
N. linearis	0.50	0.25	0.00	0.00	0.25	0.25	1.25	2.00	0.50	0.50	0.50	0.25	0.25	0.00	0.00
N. linearis var. subtilis	0.25	0.00	0.00	0.50	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N. palea	15.00	0.25	0.00	0.00	20.50	0.25	1.75	2.00	0.25	3.00	6.25	1.25	3.50	14.75	1.75
N. sigma	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.25
Planothidium engelbrechtii	1.25	2.50	3.00	0.25	0.25	0.75	4.00	2.25	4.00	8.75	1.50	0.00	0.25	0.25	2.25

P. frequentissimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50	0.00	0.25	0.00	0.25	7.25
P. rostratum	1.50	10.00	4.25	2.75	4.25	3.50	7.75	8.50	6.75	31.50	4.00	3.75	4.50	5.25	7.25
Pleurosigma salinarum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.50	1.50
Sellaphora pupula	0.00	0.50	0.00	0.25	0.25	0.00	0.75	0.00	0.50	1.00	0.00	0.00	0.25	0.75	0.50
S. seminulum	0.00	0.25	0.00	0.25	0.25	0.50	0.00	0.00	0.25	0.25	0.00	0.00	0.75	0.25	6.50
Staurosira elliptica	0.00	0.00	0.00	0.00	0.00	0.50	0.00	1.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Surirella angusta	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.25	0.00	1.50	0.00	0.00
Tabularia fasciculata	0.00	1.00	0.00	0.25	0.00	0.75	1.00	0.50	0.75	0.25	0.00	0.00	0.00	0.00	0.00

Table 5.4: Relative abundances (%) of the historic benthic diatom species recorded at two sites in March 1983 and two sites in September 1985 along the Sabie River within the KNP.

Diatom Species	Sites			
	March 1983		September 1985	
	6	8	7	9
Achnanthidium crassum	0.00	0.00	0.00	0.25
A. exiguum	0.00	1.25	1.25	0.00
A. minutissimum	0.25	0.00	24.75	54.00
A. straubianum	0.50	1.50	0.00	0.00
Amphora inariensis	0.00	1.50	0.00	0.00
Anorthoneis dulcis	0.00	0.00	1.25	0.75
Capartogramma crucicula	0.00	0.75	0.00	0.00
Cocconeis placentula	1.00	17.25	3.75	4.25
Craticula accomoda	0.25	0.00	0.50	0.00
Cymbella kappii	0.25	0.00	1.75	4.50
C. kolbei	65.75	0.00	4.25	2.00
C. turgidula	4.25	0.25	0.00	0.00
Encyonema minutum	0.25	0.00	0.50	2.75
Eolimna minima	0.25	1.25	0.00	0.00
E. subminuscula	0.00	0.25	0.25	0.50
Fallacia insociabilis	0.00	2.75	1.00	1.50
Fragilaria capucina	0.00	0.00	0.00	1.25
F. ungeriana	1.75	0.00	0.75	0.50
Gomphonema parvulum	3.25	2.50	0.50	2.75
G. venusta	0.25	0.75	19.25	2.75
Gyrosigma acuminatum	0.00	0.00	0.50	0.00
Hippodonta capitata	0.00	0.00	1.00	0.00
Navicula capitatoradiata	0.75	0.00	4.25	1.00
N. cryptocephala	0.00	0.00	0.75	0.00

N. cryptotonella	0.00	0.00	1.50	1.25
N. gregaria	0.00	0.00	0.75	0.50
N. heimansioides	0.00	0.00	12.00	1.00
N. rostellata	3.75	3.75	1.00	2.75
N. schroeteri	2.00	0.25	0.00	5.50
N. veneta	1.25	1.50	3.25	2.00
Nitzschia archibaldii	0.50	0.00	0.00	0.00
N. dissipata var. media	1.25	0.00	1.75	3.25
N. frustulum	1.75	2.00	0.00	0.25
N. iremissa	1.00	0.50	0.25	0.00
N. lancettula	0.50	2.25	0.25	0.00
N. linearis	0.00	0.50	1.25	1.50
N. palea	2.75	1.25	1.75	0.00
N. valdecostata	1.25	0.25	0.75	0.00
Planothidium engelbrechtii	0.00	2.25	2.00	0.00
P. rostratum	0.75	26.25	4.00	1.25
Sellaphora pupula	0.25	2.00	0.00	0.00
S. seminulum	0.75	1.50	0.25	0.00
Staurosira elliptica	0.50	14.00	0.00	0.00
S. pinnata	1.50	7.50	0.50	0.00

Cymbella kolbei was the dominant species (32.88%) during the 1983 sampling period, which is a species typically found in oligotrophic, alkaline waters (Figure 5.12a, Table 5.4). *Planothidium rostratum*, the 2nd most dominant species, prefers waters with low to moderate electrolyte content. *Cocconeis placentula* prefers mesotrophic to eutrophic waters as well as unpolluted waters. *Staurosira elliptica* occurs in electrolyte-rich waters, and lastly *Staurosirella pinnata*, the 5th most dominant species (4.50%) in 1983 is found in clean waters with moderate to high electrolyte content. *Achnanthidium minutissimum* was the most abundant species (39.38%) in 1985 (Figure 5.12b, Table 5.4), which occurs in nutrient-poor waters with moderate electrical conductivity. *Gomphonema venusta* is a species found in circumneutral, oligotrophic to mesotrophic waters with low to moderate electrolyte content. *Navicula heimansoides* occurs in oligotrophic, electrolyte-poor waters, with weakly acidic to circumneutral waters. *C. placentula*, *P. rostratum* and lastly *Cymbella kappii* (3.13%) are found in weakly alkaline oligotrophic to mesotrophic waters with low to moderate electrolyte content.

Cymbella turgidula (9.90%) was the most abundant species during the dry period (September – October) of 2019 (Figure 5.12c, Table 5.3). This species prefers waters with low conductivity and organic pollution. *C. placentula*, and *Encyonopsis leei* var. *sinensis* occurs in oligotrophic to mesotrophic waters with low to moderate electrolyte content. *P. rostratum* is found in waters with low to moderate electrolyte content. *Navicula microcari* (5.92%) occurs in moderate to electrolyte-rich waters. During the 2020 wet season sampling period, *C. turgidula* (12.77%) was the most dominant species (Figure 5.12d, Table 5.3). *C. placentula*, *C. kolbei*, and *Navicula germainii* occurs in eutrophic, electrolyte-rich waters. Lastly, *Eolimna subminuscula* (5.81%) is typically found in electrolyte-rich waters and can tolerate pollution.

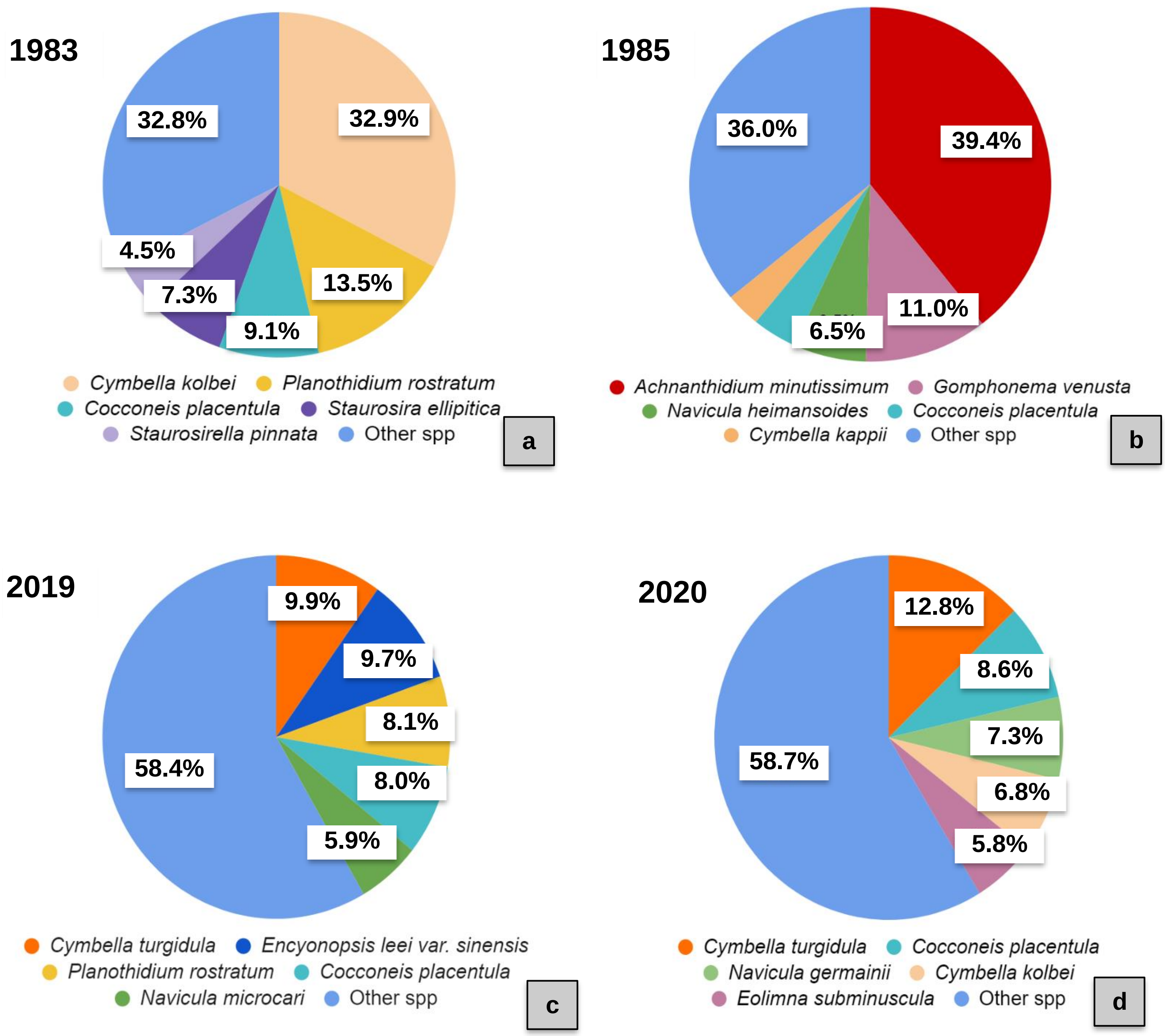


Figure 5.12: Relative abundances of the five most abundant diatom species and their composition relative to other species for the historic samples of **a)** 1983, and **b)** 1985, and recent samples from **c)** 2019 and **d)** 2020.

The five most abundant diatom species sampled during 1983 includes; *Cymbella kolbei* (A), *Planothidium rostratum* (B), *Cocconeis placentula* (C), *Staurosira elliptica* (D), and *Staurosirella pinnata* (E) (Figure 5.13). In 1985; *Achnanthidium minutissimum* (F), *Gomphonema* *venusta* (G), *Navicula heimansoides* (H), *Cocconeis placentula* (C), and *Cymbella kappii* (I) (Figure 5.13).

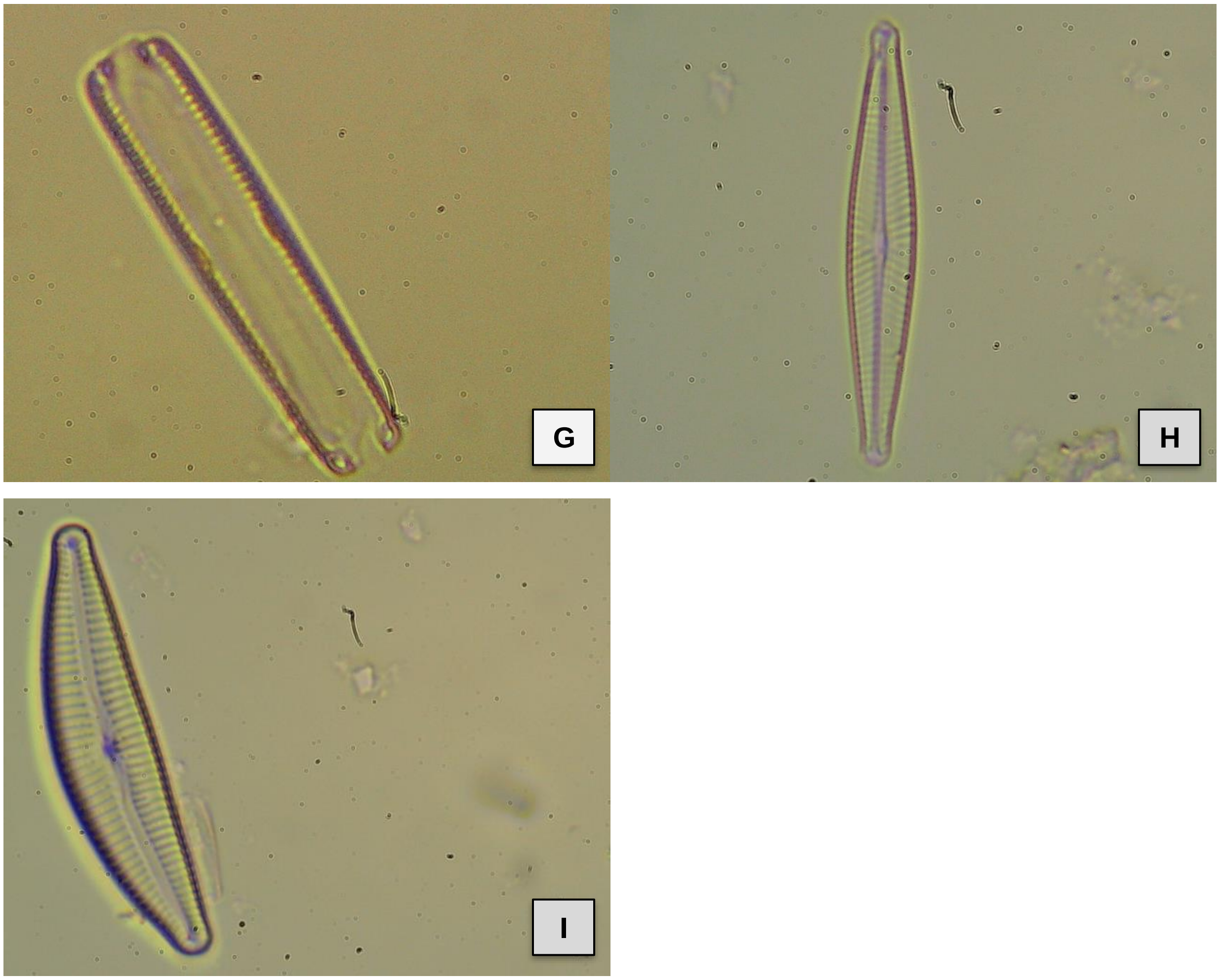
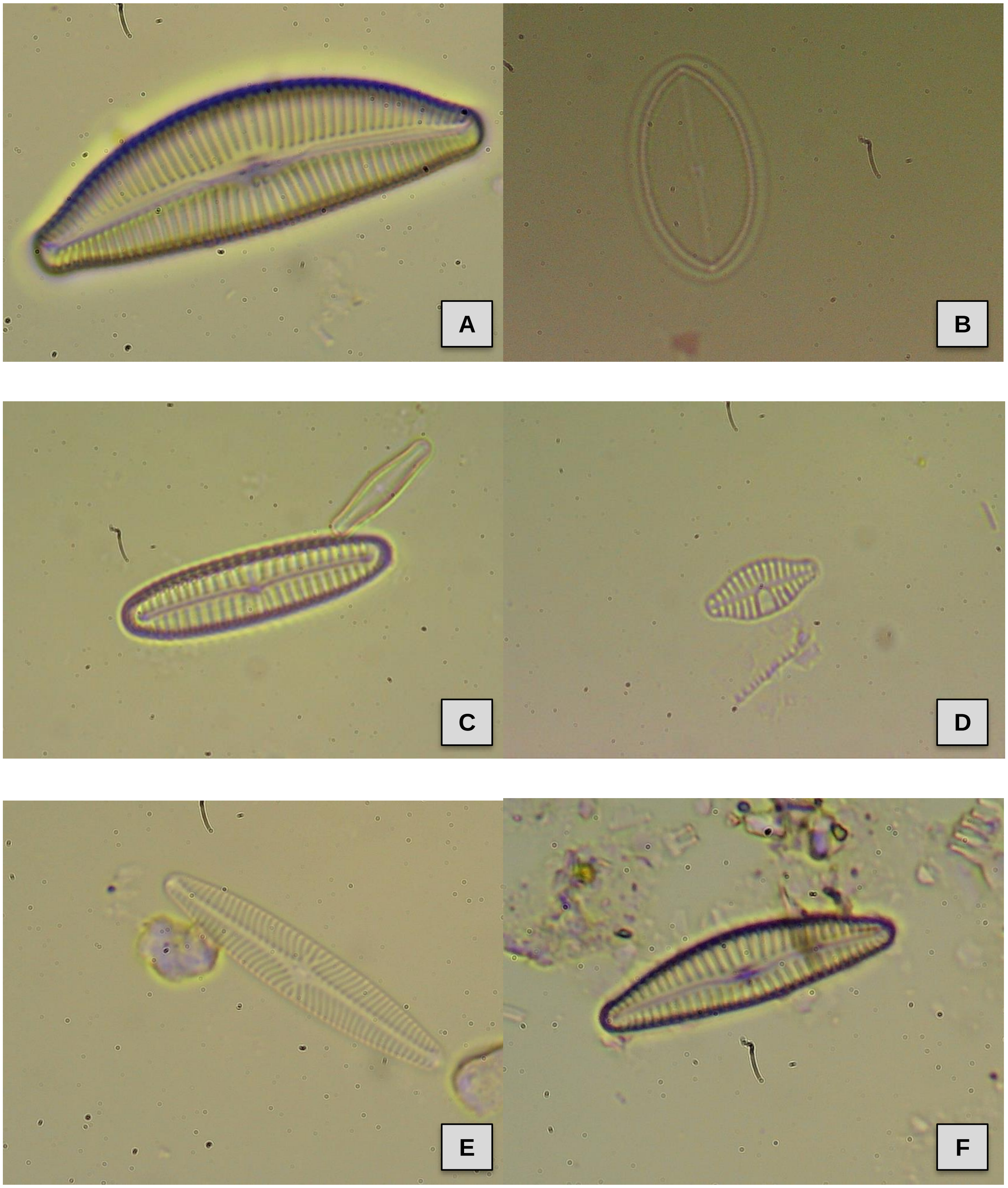


Figure 5.13: Light microscope photos at 1000x magnification with oil immersion of the five most dominant diatom species collected during the 1983 and 1985 sampling periods along the Sabie River within KNP.

The five most abundant diatom species sampled during 2019 include; *Cymbella turgidula* (A), *Cocconeis placentula* (B), *Encyonopsis leei* var. *sinensis* (C), *Planothidium rostratum* (D), and *Navicula microcari* (E) (Figure 5.14). In 2020 the species included; *Cymbella turgidula* (A), *Cocconeis placentula* (B), *Cymbella kolbei* (F), *Navicula germainii* (G), and *Eolimna subminuscula* (H) (Figure 5.14).


A
B
C
D
E
F

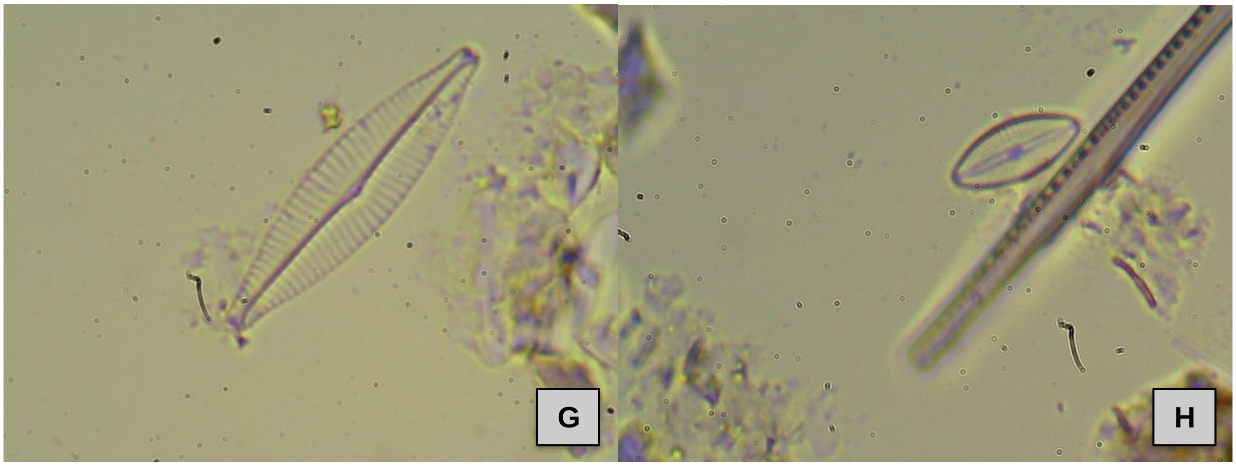


Figure 5.14: Light microscope photos at 1000x magnification with oil immersion of the five most dominant diatom species collected during the 2019 and 2020 sampling periods along the Sabie River within KNP.

5.4 Diversity of diatoms

Diatoms and MPB in general are not only useful as bioindicators in terms of a species ecological tolerance and abundance, but also in terms of the community composition. Knowledge with regards to the species that are present allows us to determine species diversity and biotic index scores that ultimately provide important information with regards to water quality. Shannon diversity showed that the overall water quality of the Sabie River can be classified as moderate. The SPI and BDI scores show that the Sabie Rivers water quality has degraded from 1983 to 2020 (EcoClassification B – C). Diatom species diversity has increased from 1983 to the 2020 sampling period.

The five sampling sites sampled during the study period had a Shannon diversity index ranging from 1.61 to 3.11 indicating waters with low to moderate pollution. These sites were considered to have moderate pollution levels, except for site 2 in September 2019, and sites 1 and 5 in March 2020 that were categorised as having low pollution (refer to Table 4.3). Overall, the Sabie River can be classified as having moderate pollution when interpreting water quality from the diversity index.

Evenness is constrained between 0 and 1, the less variation in a community the higher the evenness value. Evenness along the Sabie River ranged from 0.44 to 0.72 (Table 5.5). Sites with high diversity scores showed higher variation in diatom community structure such as sites 2 and 3 in October 2019, and sites 1 and 5 in March 2020. Site 6 in March 1983 and site 9 in September 1985 had the lowest diversity and low variation in diatom community composition.

The SPI and BDI scores per site showed that site 5 in October 2019 had poor (4.8 – 8.8) and bad (5 – 9) water quality, respectively, and the PTV% indicates organic pollution (> 20%). Seven of the 15 sites during the 2019 and 2020 sampling period had waters classified as good quality according to the SPI and five classified as moderate quality. The BDI classified seven of the sampling sites during the 2019 – 2020 sampling period to have good water quality and six to have medium water quality. The 1983 and 1985 had three sampling periods classified as good quality and one moderate according to the SPI and BDI scores. The following sites had PTV% values > 20%, which indicates organic pollution; site 1 in September 2019, sites 2 and 5 in October 2019, and sites 3 and 5 in March 2020. The historic sampling sites did not have any PTV% scores that exceeded 10.80% (Table 5.5).

Table 5.5: Mean Shannon, evenness, SPI, BDI and PTV (%) scores for all sampling sites and time periods, both historic and latest, along the Sabie River. Refer to Table 4.1 for interpretation of SPI score colour coding.

Season	Sites	Shannon	Evenness	SPI	BDI	%PTV
Mar. 1983	6 (16) PK	1.61	0.44	16.5	15.7	10.8
	7 (20) LS	2.43	0.62	11.8	12.3	10.5
Sep. 1985	8 (27) LS	1.98	0.44	15.2	16.4	5.8
	9 (39) PK	2.58	0.58	14.7	16.5	5.5
Sep. 2019	1	2.84	0.61	11.7	13.5	26.5
	2	2.72	0.57	14.7	13.0	8.8
	3	2.67	0.63	13.5	13.9	8.3
	4	2.30	0.67	11.9	12.5	15.5
	5	2.33	0.52	10.5	10.8	13.5
Oct. 2019	1	2.86	0.55	14.4	13.2	6.0
	2	3.11	0.72	7.1	10.4	41.0
	3	2.97	0.70	12.5	12.5	8.8
	4	2.72	0.54	13.1	13.6	13.8
	5	2.67	0.48	6.6	7.3	63.0
Mar. 2020	1	3.04	0.66	14.0	12.9	5.3
	2	2.22	0.53	15.6	14.8	4.0
	3	2.90	0.65	10.1	9.5	32.5
	4	2.71	0.65	14.9	13.9	5.8
	5	3.02	0.68	6.6	7.3	63.0

The SPI and BDI indices indicated that during the 1983 and 1985 sampling periods the Sabie River had good quality water and had an EcoClassification score of a B. According to the SADI index and this study, the water quality of the Sabie River has degraded and is now classified as moderate quality and is classified as a C. Species diversity was higher during the 2019 and 2020 period compared to 1983 and 1985 (Table 5.6).

Table 5.6: Mean SPI, BDI and Shannon index scores for the historic periods, 1983 and 1985, the most recent periods, 2019, and 2020, along the Sabie River.

Year	SPI	BDI	Shannon index
1983	14.15	14.0 (Good)	2.02
1985	14.95	16.45 (Good)	2.28
2019	11.60	12.07 (Medium)	2.72
2020	12.24	11.68 (Medium)	2.78

5.4.1 Diatom index correlation analysis

Table 5.7: Pearson correlation coefficients between environmental variables and Shannon Weiner diversity, evenness, SPI, and BDI. There were no significant correlations.

Environmental variable	Diversity	Evenness	SPI	BDI
Ammonium ($mg.l^{-1}$)	0.27	0.32	-0.05	-0.22
Orthophosphates ($mg.l^{-1}$)	-0.07	0.02	0.04	-0.11
Nitrates ($mg.l^{-1}$)	-0.04	-0.34	-0.48	-0.35
Conductivity ($\mu S.cm^{-1}$)	-0.13	0.04	-0.36	-0.39
Specific Conductivity ($\mu S.cm^{-1}$)	-0.23	-0.18	-0.45	-0.42
DO ($mg.l^{-1}$)	-0.01	0.04	0.10	0.30
DO (%)	-0.004	0.16	0.11	0.29
pH	-0.16	0.20	0.06	-0.07
Temperature (^{o}C)	0.09	0.34	0.0009	-0.12
Clarity (cm)	0.28	-0.18	0.16	0.23
Velocity ($m^3.s^{-1}$)	-0.08	0.09	0.18	0.07

5.5 Diatom distribution with regards to environmental variables

Environmental variables (orthophosphates, ammonium, temperature, velocity, DO, and EC) were able to explain diatom species distribution along the Sabie River. Sites sampled in September and October 2019 had the highest DO concentrations, and the lowest ammonium, phosphate, and velocity levels, whereas the March 2020 sites showed the opposite trend.

Canonical Correspondence Analysis (CCA) results showed the environmental variables can explain 47.1% of the overall diatom species variation. The Monte-Carlo permutation test for the first canonical axis had eigenvalue = 0.348, F ratio = 1.831 and $p < 0.016$, for all canonical axes, Trace = 1.054, F ratio = 1.726, and $p < 0.01$, confirming that the axes are able to explain the species distribution. The diatom indices utilised in this study, diversity, evenness, SPI and BDI did not show significant correlations to water quality parameters along the Sabie River (Table 5.7). The Marginal effects showed that conductivity, ammonium concentrations, DO ($mg.l^{-1}$), and orthophosphates concentrations had the highest influence on species distribution (Table 5.7). Axis 1 explained 18.6% of the species distribution, and the strongest correlations were with DO ($mg.l^{-1}$) ($r = -0.62$), ammonium ($r = 0.61$), and conductivity ($r = 0.59$). Axis 2 explained 10.6% of the species distribution, orthophosphates ($r = 0.62$) and velocity ($r = 0.60$) had the strongest correlations to axis 2. These environmental variables are important for the ordination of the species along the respective axes. The parameters that have a strong correlation to an axis influence the species distribution along the Sabie River system.

5.5.1 Correlations between environmental variables

The CCA plot illustrated the correlations between the environmental variables. Strong positive correlations (> 0.50) occurred between the following environmental variables; velocity and ammonium ($r = 0.82$), velocity and orthophosphates ($r = 0.91$), as well as orthophosphates and ammonium ($r = 0.82$) (both parameters increased into the wet season (March 2020)) and temperature had correlations with ammonium ($r = 0.62$), orthophosphates ($r = 0.58$) and conductivity ($r = 0.59$). Strong negative correlation occurred between DO ($mg.l^{-1}$) and three parameters, namely; ammonium ($r = -0.72$), orthophosphates ($r = -0.66$) and velocity ($r = -0.61$) (the three parameters increased as DO concentrations decreased), as well as temperature and DO ($mg.l^{-1}$) ($r = 0.54$).

The most significant environmental variables that were utilised in the CCA plot were conductivity, ammonium, DO ($mg.l^{-1}$), orthophosphates ($mg.l^{-1}$), velocity ($m.s^{-1}$) and temperature (ºC). Temperature and velocity were included even though the variables were not significant in terms of the axis ordination, however, their lambda values were high which shows that they were valuable environmental variables. Total Oxidised Nitrogen (TON), clarity, and pH showed no significance in terms of the permutation test and showed low importance in terms of the marginal effect so were excluded from the CCA plot (Table 5.8).

Table 5.8: Marginal effects of the sampled environmental variables along the Sabie River, respective lambda values (indicates the most valuable variables), and p values, which indicate the variables significance in terms of the CCA plot ordination.

Environmental variable	Lambda	P value
Conductivity (µS.cm^{-1})	0.25	0.008
Ammonium (mg.l^{-1})	0.25	0.024
DO (mg.l^{-1})	0.24	0.014
Orthophosphates (mg.l^{-1})	0.24	0.022
Velocity (m.s^{-1})	0.21	0.060
Temperature (ºC)	0.20	0.064
Clarity (cm)	0.19	0.100

Figure 5.15 illustrates that the sites in September 2019 and October 2019 (low flow period) had the highest DO concentrations and were grouped along the DO (mg.l^{-1}) ordination whereas the sites in March 2020 (high flow period) had low DO concentrations and were grouped accordingly. The sites sampled in March 2020 had high ammonium, orthophosphate and velocity levels and were grouped according to those ordinations (top half of the plot) whereas the September 2019, and October 2019 sites had lower ammonium, orthophosphate and velocity levels and were grouped accordingly.

The CCA plot also illustrates diatom species distributions; *Cymbella kolbei*, *Cymbella kappii*, *Cymbella turgidula*, *Fragilaria ungeriana*, *Achnanthidium affine*, and *Achnanthidium crassum*, prefer water with lower conductivity 50 – 130 µS.cm^{-1} and high DO concentrations. Species such as *Navicula heimansoides*, *Navicula capitatoradiata*, *Navicula microcari*, *Tabularia fasciculata*, *Encyonopsis leei* var. *sinensis*, and *Cymbella tumida* preferred slower flowing waters with high DO, and low ammonium and orthophosphate concentrations. *Gomphonema pumilum* *var. rigidum*, *Gomphonema venusta*, *Cocconeis placentula*, *Karayevia ploenensis*, *Sellaphora pupula*, *Achnanthidium exiguum*, *Achnanthidium straubianum*, *Nitzschia frustulum*, and *Anorthoneis dulcis* preferred slow flowing waters with slightly higher conductivity levels (130 – 500 µS.cm^{-1}), and reduced ammonium and orthophosphate concentrations. The following species could tolerate high flowing waters with elevated ammonium, and orthophosphate concentrations; *Gomphonema parvulum*, *Planothidium frequentissimum*, *Nitzschia archibaldii*, *Nitzschia capitellata*, *Sellaphora seminulum*, *Navicula schroeteri*, *Navicula germainii*, *Navicula veneta*, *Navicula gregaria,* and *Eolimna subminuscula*.

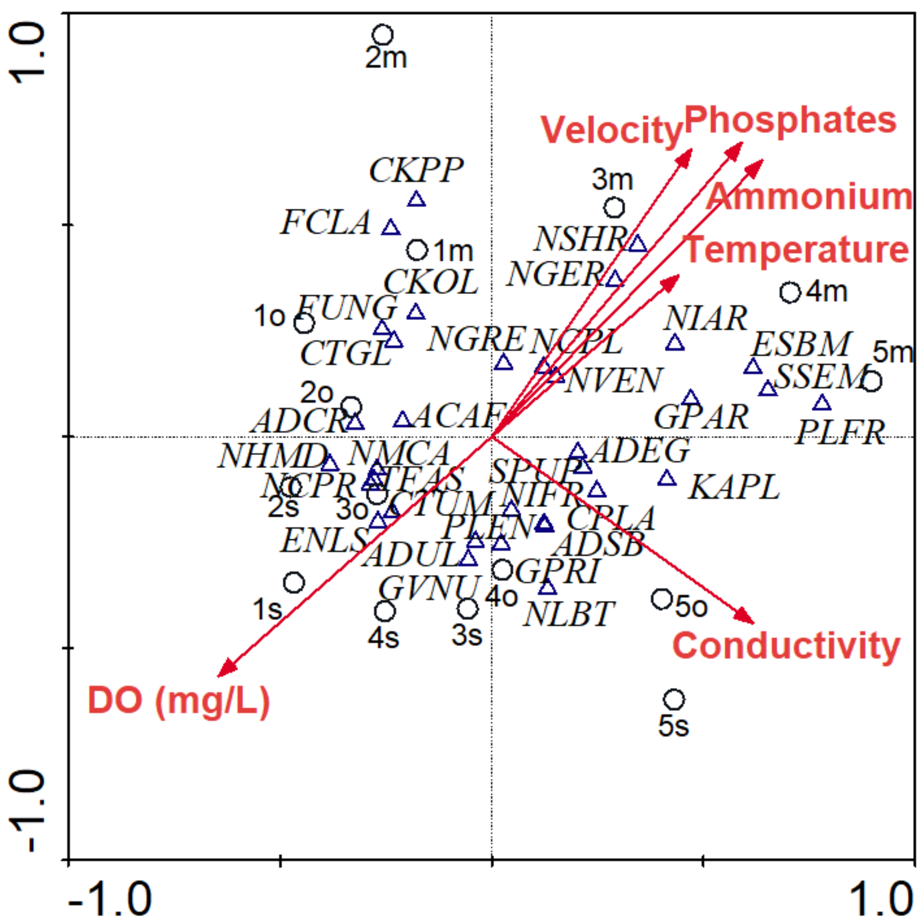


Figure 5.15: CCA triplot (environmental variables, sites, and diatom species) representing the 34 species found along the Sabie River during the three sampling sessions (September 2019, October 2019, and March 2020), and the six most important environmental variables. (1s = Site 1 September 2019, 2s = Site 2 September 2019, 3o = Site 3 October 2019, 4o = Site 4 October 2019, 5m = Site 5 March 2020, and 1m = Site 1 March 2020). The diatom species codes are as follows; *Achnanthidium affine* = ACAF, *A. crassum* = ADCR, *A. exiguum* = ADEG, *A.*

straubianum = ADSB, *Anorthoneis dulcis* = ADUL, *Cocconeis placentula* = CPLA, *Cymbella kappii* = CKPP, *C. kolbei* = CKOL, *C. tumida* = CTUM, *C. turgidula* = CTGL, *Encyonopsis leei* var. *sinensis* = ENLS, *Eolimna subminuscula* = ESBM, *Fragilaria capucina* = FCLA, *F. ungeriana* = FUNG, *Gomphonema parvulum* = GPAR, *G. pumilum var. rigidum* = GPRI, *G. venusta* = GVNU, *Karayevia ploenensis* = KAPL, *Navicula capitatoradiata* = NCPR, *N. germainii* = NGER, *N. gregaria* = NGRE, *N. heimansoides* = NHMD, *N. microcari* = NMCA, *N. schroeteri* = NSHR, *N. veneta* = NVEN, *Nitzschia archibaldii* = NIAR, *N. capitellata* = NCPL, *N. frustulum* = NIFR, *N. liebertruthii* = NLBT, *Planothidium engelbrechtii* = PLEN, *P. frequentissimum* = PLFR, *Sellaphora pupula* = SPUP, *S. seminulum* = SSEM, and *Tabularia fasciculata* = TFAS.

Using the CCA plot with the distribution of diatoms from 2019 – 2020 (Figure 5.15) we can assume with some confidence that the species from 1983 – 1985 had the following environmental requirements as a result of their proximity to the other species in the PCA plot (Figure 5.16). *Cymbella kolbei*, *Cymbella turgidula*, and *Fragilaria ungeriana* prefer water with lower conductivity and higher DO concentrations. *Achnanthidium exiguum*, *Achnanthidium straubianum, Cocconeis placentula, Nitzschia frustulum*, and *Sellaphora pupula*, preferred slightly higher conductivity levels, with reduced ammonium and orthophosphate concentrations as well as slower flowing waters. The environmental requirements for the diatom species in the bottom left corner of the PCA plot showed contrasting environmental requirements when compared to the CCA plot species distribution. For example, *Navicula gregaria*, *Navicula schroeteri*, and *Navicula veneta* prefer high flowing waters with elevated ammonium, and orthophosphate concentrations. *Navicula capitatoradiata*, and *Navicula heimansoides* prefer slower flowing waters with high DO, and low ammonium and orthophosphate concentrations and lastly *Anorthoneis dulcis*, and *Gomphonema venusta* prefer slow flowing waters with slightly higher conductivity levels, and reduced ammonium and orthophosphate concentrations.

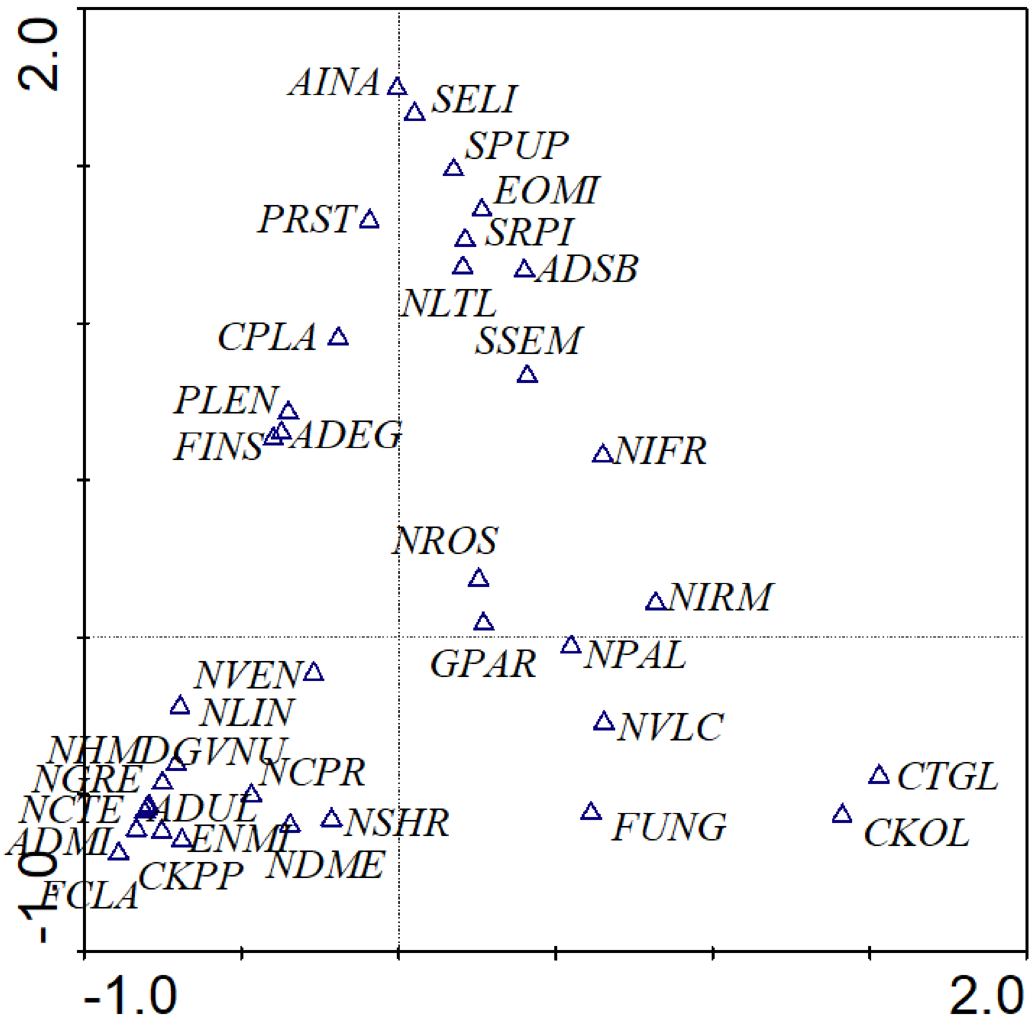


Figure 5.16: PCA plot of the 1983 and 1985 sampling sites along the Sabie River within the KNP, representing 38 diatom species with a 15% fit and 1% species weighting. *Achnanthidium exiguum* = ADEG*, A. minutissimum* = ADMI, *A. straubianum* = ADSB, *Amphora inariensis* = AINA, *Anorthoneis dulcis* = ADUL, *Cocconeis placentula* = CPLA, *Cymbella kappii* = CKPP, *C. kolbei* = CKOL, *C. turgidula* = CTGL, *Encyonema minutum* = ENMI, *Eolimna minima* = EOMI, *Fallacia insociabilis* = FINS, *Fragilaria capucina* = FCLA, *F. ungeriana* = FUNG, *Gomphonema parvulum* = GPAR, *G. venusta* = GVNU, *Navicula capitatoradiata* = NCPR, *N. cryptotonella* = NCTE, *N. gregaria* = NGRE, *N. heimansoides* = NHMD, *N. rostellata* = NROS, *N. schroeteri* = NSHR, *N. veneta* = NVEN, *Nitzschia dissipata var. media* = NDME, *N. frustulum* = NIFR, *N. iremissa* = NIRM, *N. lancettula* = NLTL, *N. linearis* = NLIN, *N. palea* = NPAL, *N. valdecostata* = NVLC, *Planothidium engelbrechtii* = PLEN, *P. rostratum* = PRST, *Sellaphora pupula* = SPUP, *S. seminulum* = SSEM, *Staurosira elliptica* = SELI, and *S. pinnata* = SRPI.

5.5.2 Diatom functional traits

The CCA eigenvalues were 0.046 and 0.054, and environmental parameters can explain 32.4% and 5.5% of the ecological guilds for axis 1 and axis 2, respectively. Ecological guilds showed no significant correlation with the five environmental parameters based on the CCA analysis. Monte-Carlo permutation test for the first canonical axis ($N = 499$, $p = 0.392$), and for all canonical axes ($p = 0.422$). The CCA plot does indicate that high profile guilds prefer high flowing waters, motile profile guilds prefer waters with high conductivity and low-profile guilds are associated with high DO concentrations (Figure 5.17).

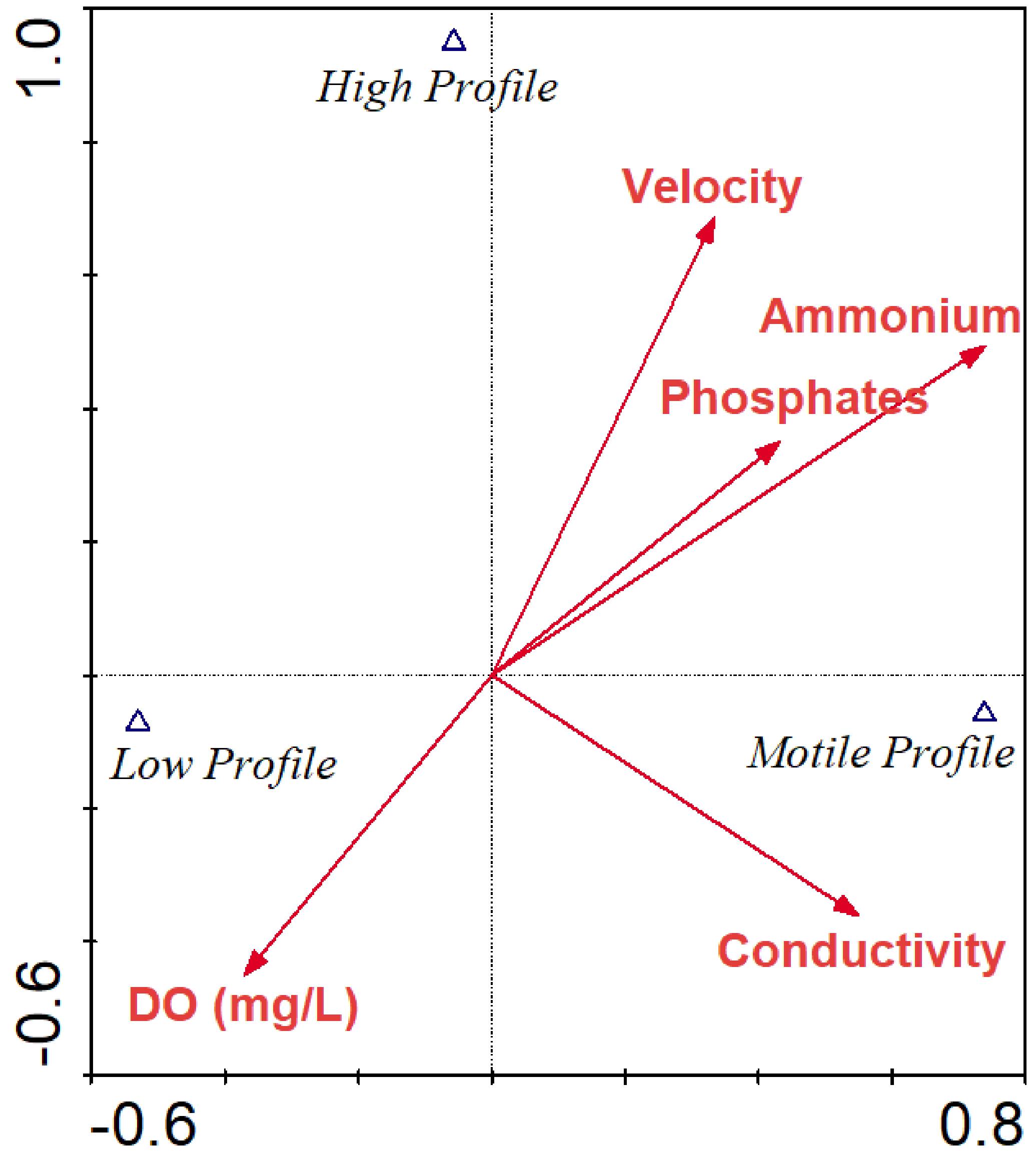


Figure 5.17: CCA plot illustrating the changes in diatom ecological guilds (low, high, and motile profile) and environmental parameters.

The three ecological guilds (low, high, and motile) did not show a linear correlation in response to changing velocity (r_{low} = 0.068, r_{high} = 0.112, and r_{motile} = 0.036) (Figure 5.18).

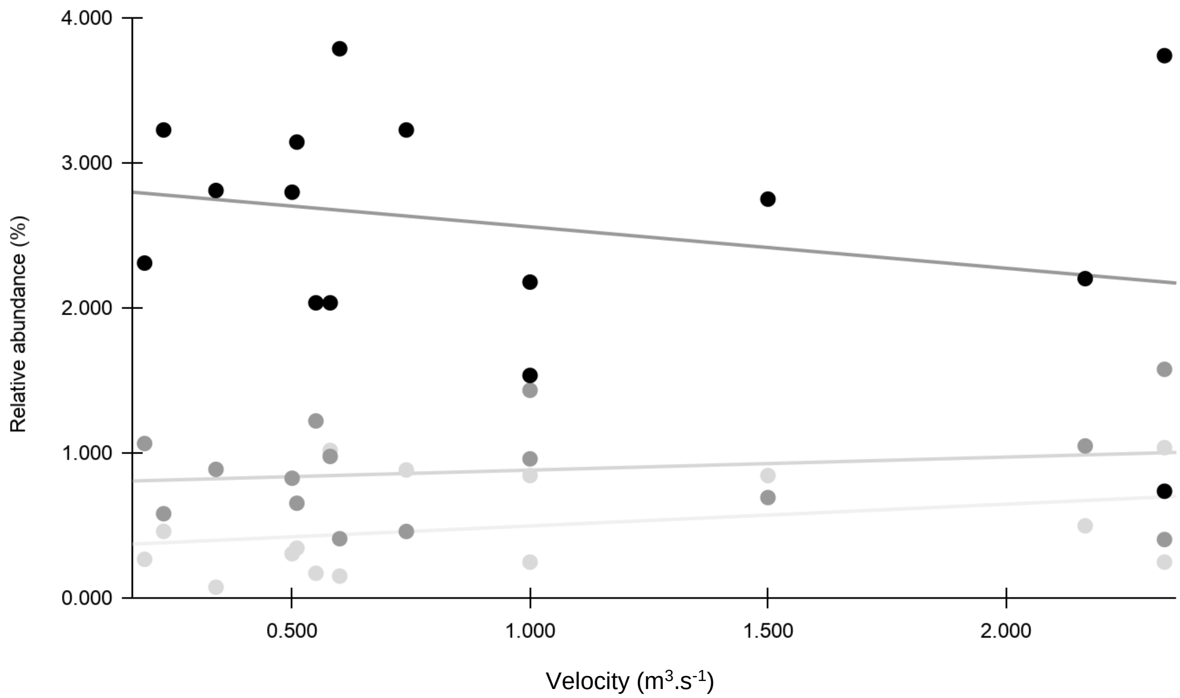


Figure 5.18: Scatter plot illustrating the low, high, and motile profile diatom guilds in relation to river velocity ($m^3.s^{-1}$). Black = low profile (r = 0.068), light grey = high profile (r = 0.112), dark grey = motile profile (r = 0.036).

5.6 BenthoTorch microphytobenthos community composition

The BenthoTorch was used as an alternative to traditional sampling to measure total MPB biomass and composition through the measurement of chlorophyll a based-on pigment fluorescence signatures of diatoms, cyanobacteria, and green algae. Microphytobenthos (MPB) cell density and chlorophyll *a* concentration remained relatively constant from the low flow to the high flow period. Small scale variations occurred between sites. Diatom cell density decreased from the low flow period to the high flow period and cyanobacteria density showed the opposite trend. Diatom abundance was > 50% at site 1, and 3, and cyanobacteria abundance exceeded diatom abundance at sites 2, 4, and 5.

Figure 5.19 illustrates the changes in MPB cell density during the 2019 and 2020 sampling periods (F = 6.907, $p < 0.01$). In September 2019, site 1 (28665.70 ± 2902.87 cells.mm^{-2}) had the highest MPB cell density compared to site 4 (11155.10 ± 1728.42 cells.mm^{-2}), however the other sites showed no variation in MPB cell density. In October 2019, the sites showed no changes in MPB cell density. In March 2020, site 2 (35834.50 ± 2766.40 cells.mm^{-2}) and 4 (42074.80 ±

5168.22 cells.mm^{-2}) had highest cell density of MPB compared to site 3 (14022.40 ± 1981.70 cells.mm^{-2}) ($p < 0.01$ for both), and 5 (12018.70 ± 1088.23 cells.mm^{-2}) ($p < 0.01$ for both). During the low flow sampling sessions (September and October 2019) there were no changes in MPB cell density. Site 2 showed an increase in MPB cell density from October 2019 (17291.80 ± 3116.55 cells.mm^{-2}) to March 2020 and site 4 had the highest cell density in March 2020, compared to September 2019 ($p < 0.01$), and October 2019 (19058.40 ± 3506.28 cells.mm^{-2}) ($p < 0.01$).

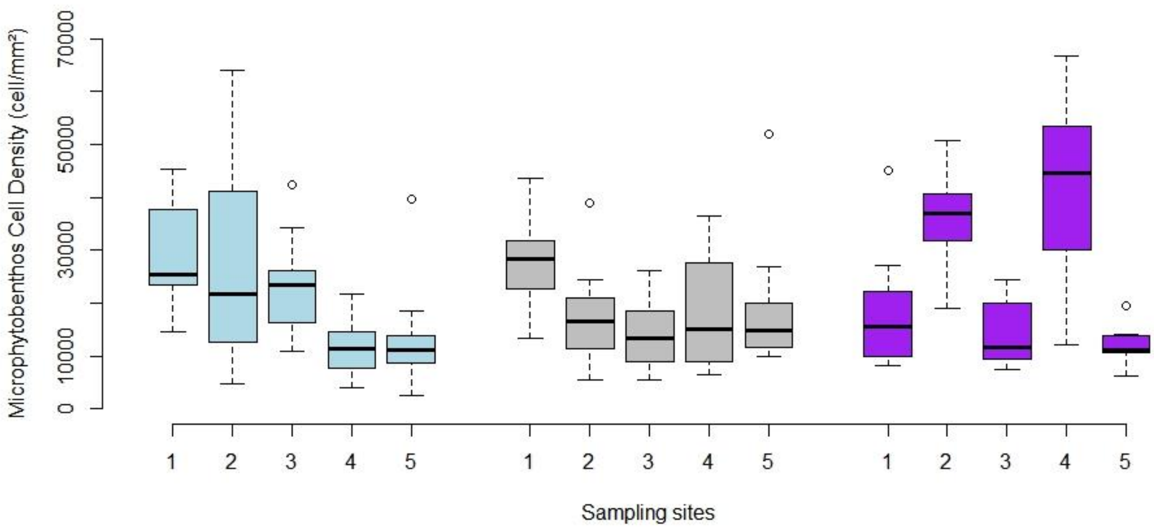
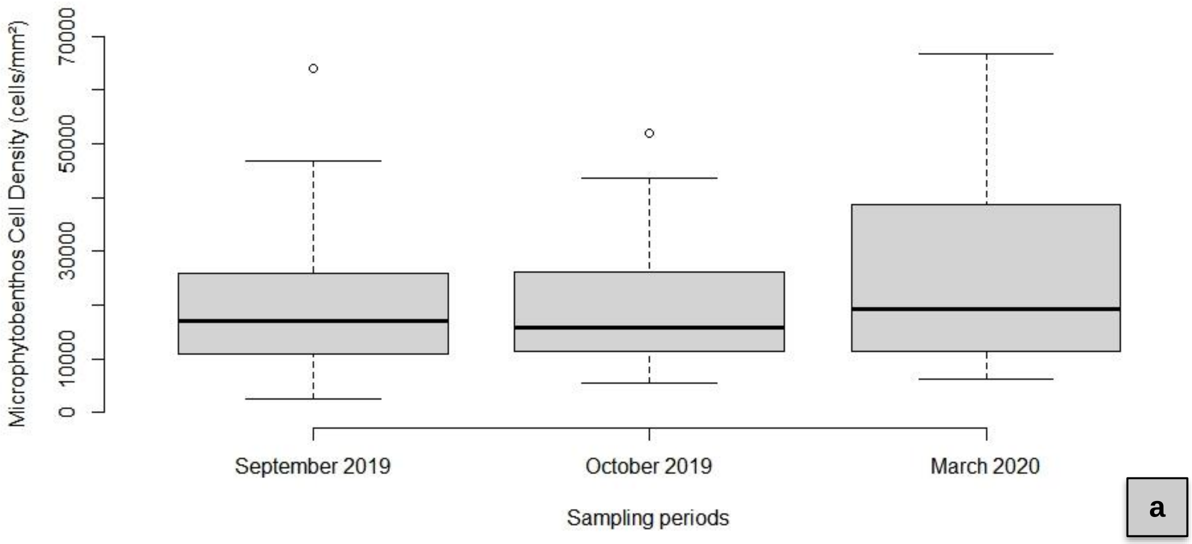


Figure 5.19: Box and whisker plot illustrating the MPB cell density (cells.mm^{-2}) across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Figure 5.20a illustrates the average MPB cell density over the three sampling periods; September 2019 (20695.74 ± 3343.61 cells.mm^{-2}), October 2019 (19351.54 ± 3146.13 cells.mm^{-2}), and March 2020 (24515.78 ± 2896.18 cells.mm^{-2}). There was no significant change in average cell densities ($F = 2.12$, $p > 0.124$) and average chlorophyll *a* ($p > 0.124$) for the period of the study (Figure 5.20b). Average chlorophyll *a* concentration fell within a narrow range between sampling sessions; September 2019 (3.06 ± 0.49 µg.cm^{-2}), October 2019 (3.01 ± 0.60 µg.cm^{-2}), and March 2020 (2.82 ± 0.31 µg.cm^{-2}). The five sites along the Sabie River remained relatively constant in terms of MPB biomass with small scale variation between sites and across sampling periods, from low flow to high flow.



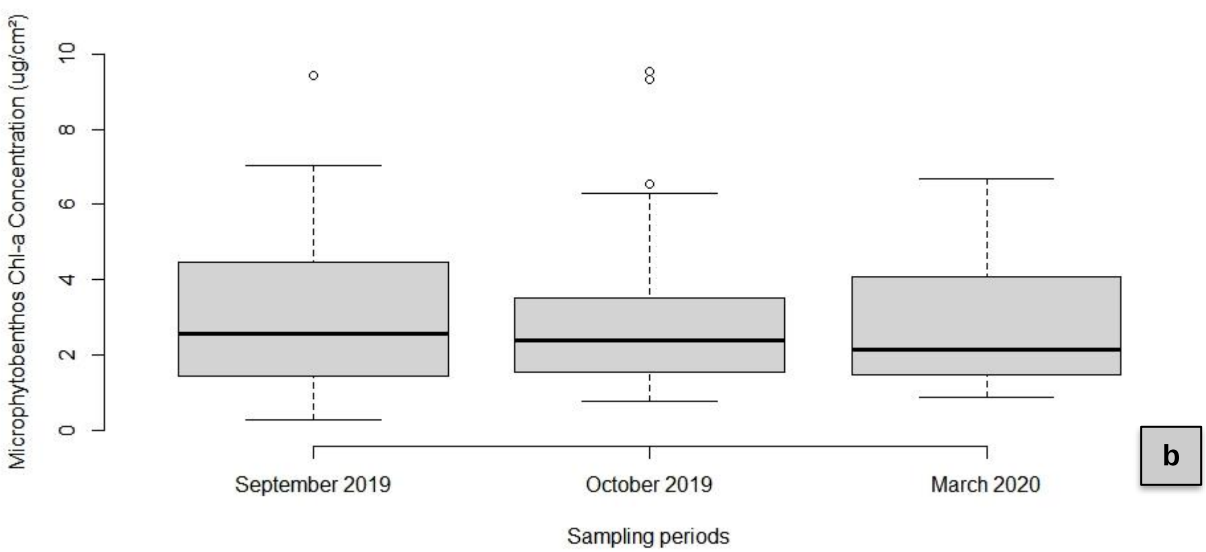


Figure 5.20: a) Box and whisker plot illustrating the average MPB cell density (cells.mm^{-2}) **b)** and average MPB chlorophyll *a* concentration (µg.cm^{-2}) across the five sites and three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Diatom cell density changed significantly from the low flow to the high flow sampling sessions along the Sabie River ($F = 6.011$, $p < 0.01$) (Figure 5.21). From September 2019

(7280.10 ± 1228.19 cells.mm^{-2}) to March 2020 ($p < 0.01$) and from October 2019 (6025.30 ± 1045.27 cells.mm^{-2}) to March 2020 (2576.70 ± 572.68 cells.mm^{-2}) ($p < 0.05$) and) the diatom cell density decreased. There was no change in diatom density between the two low flow sampling sessions: September and October 2019 ($p < 0.636$).

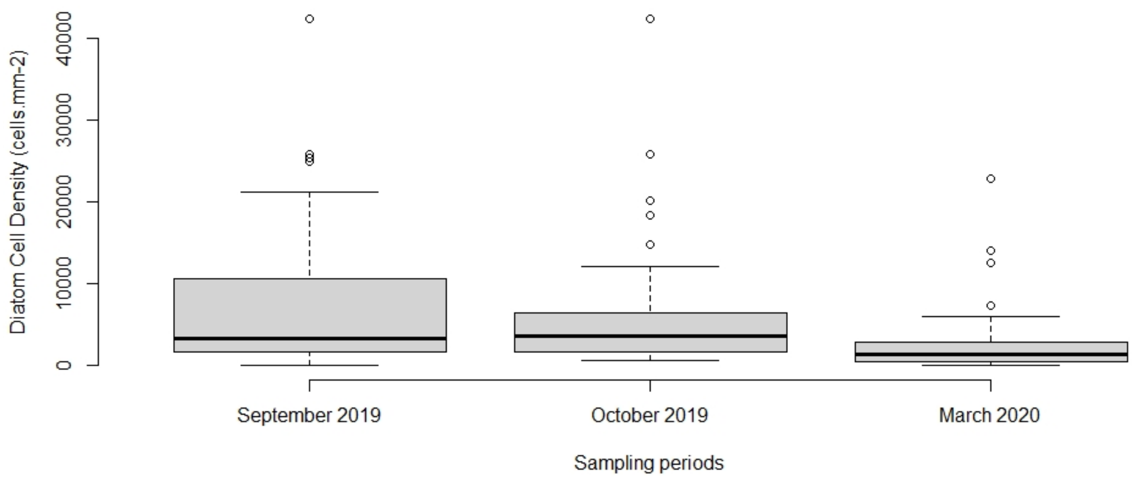


Figure 5.21: Box and whisker plot illustrating the average diatom cell density (cells.mm^{-2}) of the three sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Cyanobacteria cell density changed significantly from the low to the high flow sampling sessions ($F = 13.07$, $p < 0.01$), showing an opposite trend to diatom cell density. Cyanobacteria cell density increased from September 2019 (12256.00 ± 1689.03 cells.mm^{-2}) to March 2020 (21390.00 ± 2251.35 cells.mm^{-2}) ($p < 0.01$) and from October 2019 (9424.49 ± 970.35 cells.mm^{-2}) to March 2020 ($p < 0.01$) (Figure 5.22). There was no change ($p = 0.48$) when comparing the two low flow sampling session cell densities (September and October 2019).

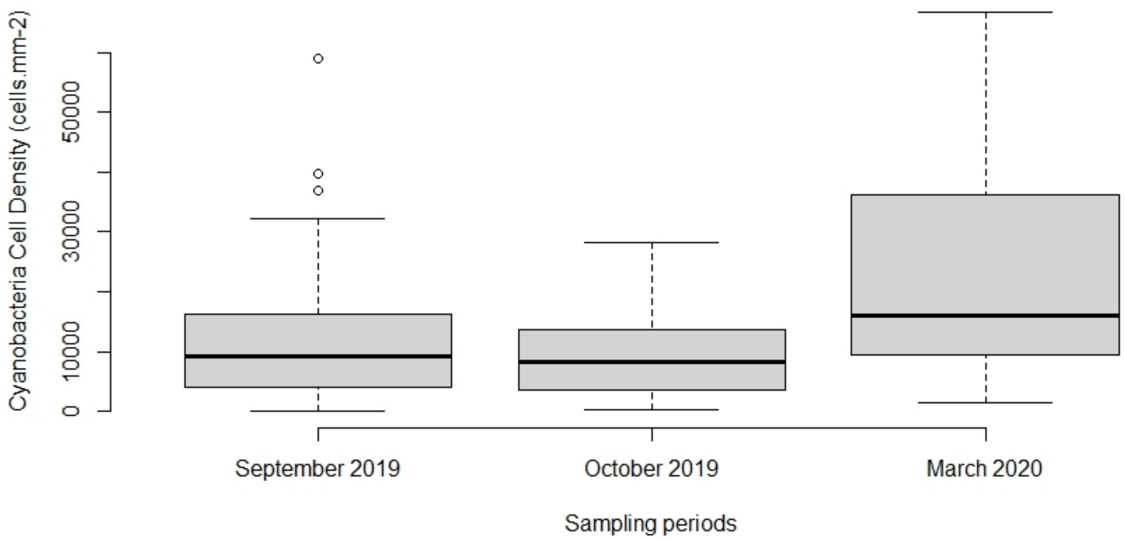


Figure 5.22: Box and whisker plot illustrating the average cyanobacteria cell density (cells.mm^{-2}) of the five sites for the three different sampling periods (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Diatom relative abundance exceeded 50% at sites 1 (53.35%) and 3 (70.93%), while sites 2 (25.21%), 4 (21.49%), and 5 (21.19%) had relatively low diatom composition compared to cyanobacteria. Cyanobacteria was dominant at sites 2 (61.4%), 4 (59.8%), and 5 (63.4%) (Figure 5.23). Even though site 1 had high diatom biomass, the contribution from green algae was low (0.55%) so the cyanobacteria biomass was high too (46.1%). Green algae biomass was highest at sites 4 (18.7%) and 5 (25.4%).

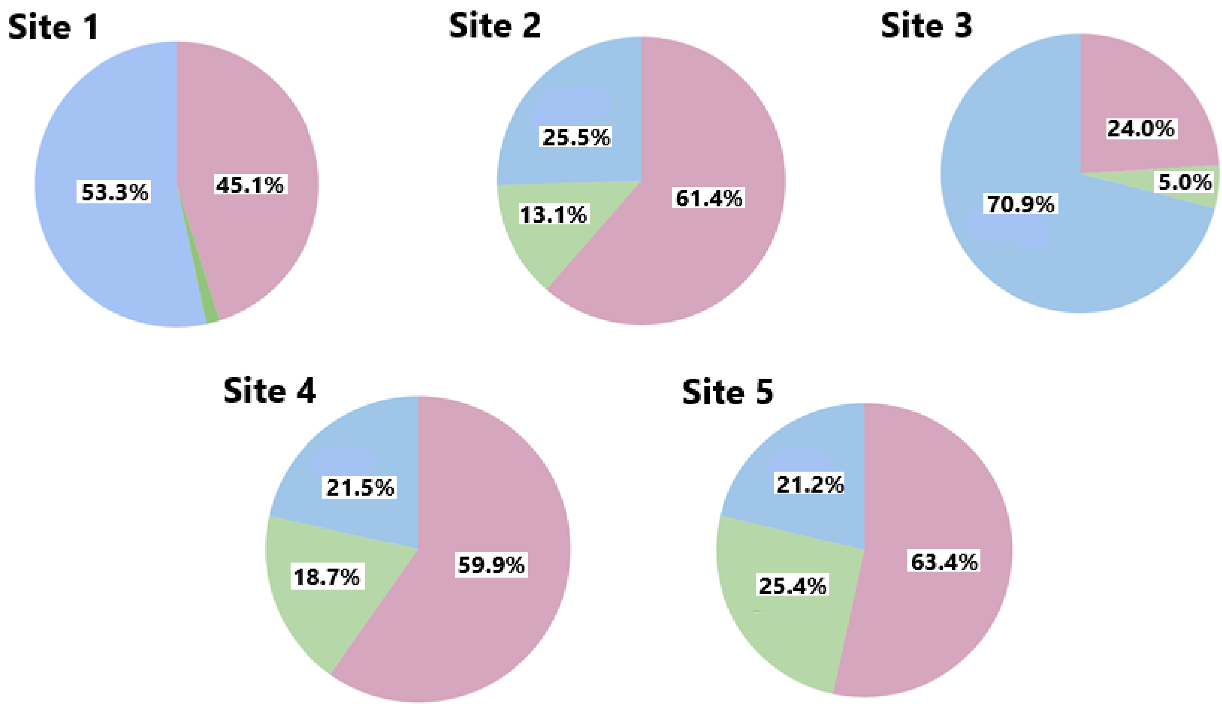


Figure 5.23: Pie charts illustrating MPB (diatoms = blue, cyanobacteria = pink, and green algae = green) percentage composition averaged for the five sites sampled during 2019 and 2020 along the Sabie River within the KNP.

Diatom composition was relatively constant during the low flow periods and decreased from the low flow period to the high flow period; 47.79% in September 2019, 43.24% in October 2019 and 20.39% in March 2020 (Table 5.9). Cyanobacteria composition decreased from 44.99% in September 2019 to 32.07% in October 2019 (where its composition was lower than diatoms), however, the composition showed a large increase in March 2020 (74.03%). Green algae relative abundance varied across the seasons and were the highest during the October 2019 sampling period (24.69%) and the lowest in March 2020 (5.57%).

Diatom composition was relatively constant during the low flow periods and decreased from the low flow period to the high flow period; 47.79% in September 2019, 43.24% in October 2019 and 20.39% in March 2020 (Table 5.9). Cyanobacteria composition decreased from 44.99% in September 2019 to 32.07% in October 2019 (where its composition was lower than diatoms), however, the composition showed a large increase in March 2020 (74.03%). Green algae relative abundance varied across the seasons and were the highest during the October 2019 sampling period (24.69%) and the lowest in March 2020 (5.57%).

Table 5.9: Average MPB community composition (%) for the three sampling sessions (September 2019, October 2019, and March 2020) along the Sabie River within the KNP.

Sampling month	Diatoms	Cyanobacteria	Green algae
Sep. 2019	47.79	44.99	7.22
Oct. 2019	43.24	32.07	24.69
Mar. 2020	20.39	74.03	5.57

Chapter 6: Discussion

6.1 Water quality

Water degradation worldwide is caused by anthropogenic disturbances and only 3% of South Africa's surface waters are oligotrophic, with the majority being classified as mesotrophic (37%), eutrophic (33%), and hypertrophic (28%) (DWA, 2013). Within the KNP, 82% of rivers are A/B class (natural or good quality), 15% in class C (moderate quality) and 3% in class D (poor quality) (KNP, 2008). Water quality parameters such as conductivity, pH, DO, and nutrients are linked and interact in a variety of different ways, in turn changes of these variables affect biotic communities (Dallas and Day, 2004). The flood in February 2020 gave us the unique opportunity to study the effects of the flood on the water quality and MPB community composition and biomass of the Sabie River.

6.1.1 Dissolved oxygen

Aquatic organisms are dependent on water for survival, and more importantly dissolved oxygen is vital for aquatic life (Dallas and Day, 2004). Not all aquatic organisms require the same oxygen concentration and their ability to tolerate low oxygen levels as well as changes in dissolved oxygen depends on the frequency, time of day, and duration (Dallas and Day, 2004). Dissolved oxygen at 5^{o}C should be 12.77 mg.l^{-1} and at 20^{o}C concentrations should be in the range of 9.09 mg.l^{-1} (DWAF, 1996a). DO concentrations below 5 mg.l^{-1} can negatively affect the functioning and survival of biological communities and below 2 mg.l^{-1} may be harmful to aquatic organisms (Chapman, 1996). During the high flow sampling period temperatures ranged from 24.20^{o}C – 31.00^{o}C along the Sabie River, and DO concentrations (6.59 mg.l^{-1} – 7.06 mg.l^{-1}) met the TWQR requirements (Figure 5.10, Appendix A) (DWAF, 1996a). The relationship between temperature and DO was not strongly correlated ($r = 0.29$) during the study period and suggests that decreased DO concentrations in the Sabie River during the high flow period may have been influenced by other environmental factors. Sampling at different times of the day may have influenced the water temperature reading at specific sampling points.

Super saturation (> 100%) can occur in slow moving waters, as a result of oxygen production by plants during photosynthesis. Super saturation is generally an indication of eutrophication in a water body and can cause oxygen bubbles to develop around fish gills causing mortality (DWAF, 1996a). The TWQR requirements for dissolved oxygen states that in order to ensure the protection of all life stages of southern African aquatic organisms that are endemic or adapted to aerobic warm waters ecosystems, concentrations of DO need to fall between 80% and 120% saturation for the lowest instantaneous concentration recorded in a 24-hour period (DWAF, 1996; Dallas and Day, 2004). Non-mobile or less mobile life stages such as eggs and fry are more affected by supersaturated water (Dallas and Day, 2004; Kleynhans *et al*., 2015). During the study period, sites 1, 2, and 4 in September 2019 had DO saturation of 132%, 138%, and 134% respectively. The elevated DO (%) only occurred in September 2019 over the three sites and for the remainder of the sampling period DO (%) fell within the expected TWQR range (80% – 120 %) (Appendix A). Dissolved oxygen concentrations decreased during the high flow period of March 2020 < 7 $mg.l^{-1}$ and the same seasonal trend in DO concentration was recorded along the Cinaruco River in Venezuela (Montoya *et al*., 2006). DO is influenced by three main factors altitude, temperature and photosynthesis by plants and algae (Dallas and Day, 2004). Decreased DO concentrations during the high flow period could be due to the respiration of aquatic organisms and animals, organic waste, aerobic decomposition by microorganisms (microbial activity), the chemical breakdown of pollutants, increased temperature, and the re-suspension of anoxic sediments into the water column (Dallas and Day, 2004). Although the partial pressure of O_2 is lower at higher altitudes, mountainous streams are generally more oxygen rich than lowveld streams due to decreased temperature and organic matter (Nel and Driver, 2015). The lower DO concentrations of the Sabie River may be attributed to the increased photosynthesis, elevated organic matter and temperature associated with lower altitude in the lowveld region of the KNP (Nel and Driver, 2015).

Bere and Tundisi (2011) stated that good water quality typically has DO concentrations between 6.8 $mg.l^{-1}$ and 8.2 $mg.l^{-1}$ and orthophosphate concentrations between 0.002 $mg.l^{-1}$ and 0.0047 $mg.l^{-1}$. Moderate to bad water quality has DO and orthophosphate concentrations < 7 $mg.l^{-1}$ and 0.0126 and 0.0831 $mg.l^{-1}$ respectively. The Sabie River had DO and orthophosphate concentrations within the good quality ranges for the low flow periods (September and October 2019), and within the moderate to bad water quality range during the high flow period (March 2020) (Appendix A).

When the PTV% is above 20% it indicates the presence of organic matter (manure, industrial waste, or sewage) that is generally transported through surface runoff and is degraded by microorganisms (Harding and Taylor, 2011). The most noticeable effect of organic enrichment is reduced DO (Dallas and Day, 2004; Harding and Taylor, 2011). Gradual changes in oxygen concentrations both spatially and temporally can result in certain organisms acclimating, however, high intensity discharge caused by flooding events – may result in plant material (common reeds) being eroded from higher up in the catchment and increasing the organic matter in areas with low river flow or energy, this accumulation of organic matter has an instant effect on biotic communities within the downstream areas of the Sabie River (Dallas and Day, 2004). The flooding event in February 2020 along the Sabie River may have resulted in the low DO concentrations recorded during the March 2020 sampling period as a result of increased organic matter. The following sites had PTV% values > 20%; site 1 in September 2019, sites 2 and 5 in October 2019, and sites 3 and 5 in March 2020 – high flow period. The historic sampling sites did not have any PTV% scores that exceeded 10.80% (Table 5.5). These findings suggest that during the high flow period organic matter washed down from the Sand River may have contributed to the high PTV% at site 3. Site 5 being the most downstream site was a place of accumulation for organic matter during the low and high flow periods. Organic matter has increased in the Sabie River over time and is higher than it was historically – possibly due to increased industrial waste and farming from higher up in the catchment (Riddell *et al*., 2019).

6.1.2 Temperature

Water temperature changes could be a result of anthropogenic disturbances such as stream regulation, removal of riparian vegetation, returning irrigation waters, and global climate change (Mwangi, 2014). Although rivers experience daily and seasonal water temperature fluctuations, aquatic organisms have an optimal range (Dallas and Day, 2004). Increases in water temperature can increase toxicity of certain chemicals potentially leading to sublethal or lethal conditions (Dallas and Day, 2004). Temperature changes can also affect other factors such as oxygen solubility, and microbial activity (Dallas and Day, 2004; Mwangi, 2014). Higher water temperature favours the growth of algae and macrophytes especially when nutrient concentrations are high (Dallas and Day, 2004). De Senerpont Domis *et al*. (2014) found that elevated temperatures coupled with nutrient loading resulted in increased phytobenthos biomass, while elevated temperatures alone resulted in increased growth rates. The Sabie River does not appear to be negatively affected by increased temperatures and nutrients during the wet season as MPB biomass remained relatively constant throughout the study period.

Site 1 was classified as the most upstream site along the Sabie River within the KNP and had the lowest water temperature overall, whereas site 4 located in the low area had the highest water temperatures across all three sampling periods. This could be attributed to the increased canopy cover at site 1 as opposed to site 4. The highest water temperature recorded along the Sabie River were 31^{o}C and 30^{o}C during March 2020 at sites 4 and 5, respectively. Barker (2006) recorded water temperatures in the Sabie River within the KNP during September 2005 (20.5ºC) and March 2005 (25.8ºC), his finding coupled with the current sampling suggest that the Sabie Rivers average water temperatures have increased. To better understand the effects of temperature on the system more continuous sampling will need to be conducted, which will allow for comparison against the TWQR for water temperature determined by the Water Quality guidelines for aquatic ecosystems in South Africa. The TWQR states that the background daily average temperature for a specific site and time may not vary by > 2ºC or > 10% (whichever is more conservative), however establishing specific temperature ranges is challenging due to spatial and temporal variation in temperature with South Africa's inland water sources varying between 5ºC and 30ºC (DWAF, 1996a; Dallas and Day, 2004). The water temperature of the Sabie River remained within the normal range for a river in the lowveld that experiences high ambient temperatures (Dallas, 2008).

6.1.3 Conductivity

The Sabie River sampled within the KNP had conductivity readings between 101.73 – 158.87 µS.cm^{-1} that were half of the RQO and according to Taylor *et al*. (2007a) the river had moderate electrolyte content. Average conductivity levels of each sampling period showed no variation, however, there were changes in conductivity levels between the sites; site 1 had the lowest conductivity levels across all sampling periods, and site 4 had the highest (Appendix A). When corrected for temperature the specific conductivity showed that site 1 had the lowest conductivity and site 5 the highest across all the sampling periods. This may be a result of site 4 and 5 being further downstream, in the "flood plain", and therefore salts have accumulated as opposed to site 1, which is higher up in the river system and can be classified as the upper course. Conductivity is influenced by soil, and geological characteristics of the catchment, the Sabie River is underlain by a wide variety of bedrock, sedimentary, intrusive, and extrusive igneous and metamorphic rock (Heritage and Moon, 2000). Sites 1, 2, and 3 along the Sabie River have Nelspruit Suite Gneiss geology (granitic rock), while sites 4 and 5 are underlain by Karoo Sandstone, and Lebombo Rhyolite respectively that are basalt rocks (Heritage and Moon, 2000; Sinclair and Walker, 2003). Granitic rock has lower EC and less cations compared to basaltic rock and may be a contributing factor to the increased conductivity from the upstream to downstream sites (Figure 5.7) (Chapman, 1996).

The Orange River in the Eastern Cape Province experiences large amounts of evaporation that result in increased conductivity levels, increasing by one magnitude seasonally

(Dallas and Day, 2004). The Sabie River did not show large changes in conductivity and this may be because it does not experience large amounts of evaporation that cause large fluctuations in conductivity levels. Barker (2006) found that the Shingwedzi, and Letaba Rivers within the KNP had higher conductivity during the dry season and behaved differently to the Sabie where conductivity levels increased during the wet season due to increased runoff. The Sabie River may have experienced a dilution effect during the wet season especially if incoming waters were low in conductivity and relatively unimpacted by upstream nutrient input or surface runoff (Dallas and Day, 2004; Montoya *et al*., 2006).

6.1.4 Nutrients

Nutrient enrichment of aquatic ecosystems is generally in the form of nitrogen and phosphorus, nutrients that are mineralised by decomposers and result in increased concentrations of dissolved and particulate nutrients in river systems (Dallas and Day, 2004). Phosphorus is considered the main nutrient controlling eutrophication in freshwater systems and naturally enters rivers from the weathering and leaching of rocks and the decomposition of organic matter (DWAF, 1996a; Griffin 2017).

Nitrates are the product of aerobic stabilisation of organic nitrogen and enters water from fertilizers and runoff (Dallas and Day, 2004). Dissolved inorganic nitrogen is seldom present in high concentrations in pristine freshwater systems < 0.1 $mg.l^{-1}$, as it is rapidly taken up by aquatic plants and assimilated into proteins and organic nitrogen in plant cells (DWAF, 1996a; Dallas and Day, 2004). Dissolved inorganic nitrogen is monitored as increases cause aquatic plant and algal growth (DWAF, 1996a). The requirement for nitrate concentrations in aquatic systems of South Africa is 0.5 $mg.l^{-1}$ and concentrations exceeding 4 $mg.l^{-1}$ tend to be associated with eutrophication (DWAF, 1996a). Total Oxidised Nitrogen (TON) concentrations along the Sabie River were in alignment with the TWQR. September and October 2019 (low flow periods) were classified as mesotrophic and the March 2020 (high flow) period as oligotrophic (Figure 5.4) (DWAF, 1996a). The changes in classification from the dry season to the wet season may be due to the dilution effect - increased water flow during the wet season with waters low in nitrate concentrations caused the dilution of the nitrate ions already present in the Sabie River (Girardi *et al*., 2016).

Average orthophosphate concentrations along the Sabie River increased from the low flow period to the high flow period (Figure 5.3). Orthophosphates are seldom found in quantity > 0.005 $mg.l^{-1}$ in pristine waters (DWAF, 1996a; Dallas and Day, 2004). According to the TWQR for aquatic systems, orthophosphate concentrations during the September 2019 period resulted in the Sabie River showing oligotrophic conditions (0.0002 ± 0.000 mg.l^{-1}), while October 2019 (0.0083 ± 0.004 mg.l^{-1}) and March 2020 exhibited mesotrophic conditions (0.0535 ± 0.013 mg.l^{-1}) (DWAF, 1996a). The change in water condition from oligotrophic to mesotrophic may be a result of increased bacterial activity and biological metabolism, coupled with higher water temperatures (Chapman, 1996). Occasional increases in inorganic nitrogen and orthophosphates are less important than continuous high concentrations (DWAF, 1996a). The flow regime of rivers affects the mobility, availability, and distribution of orthophosphates within a river, and during the low flow periods orthophosphate concentrations within the water column were lower due to biotic uptake and settlement from the water column, with stream beds acting as a sink for phosphorus (DWAF, 1996a). High river flow associated with large rainfall events results in orthophosphates being released into the water column, as seen along the Sabie River – where river velocity was positively correlated with ammonium and orthophosphate concentrations (Figure 5.3). The increase in orthophosphates and ammonium concentrations within the Sabie River may also be due to anthropogenic pollution such as surface runoff from the surrounding Sabie-Sand catchment that is affected by human activities such as forestry (pine and eucalyptus trees), urbanisation, industry, and discharge from the wastewater treatment plant (Chapman, 1996; DWAF, 1996a; Heritage and Moon, 2000; Rivers-Moore *et al*., 2004; Stolz 2018; Roux *et al*., 2017). Large rainfall events, as experienced within the Sabie River catchment in February 2020, result in increased surface runoff from upstream activities and are a major source of inorganic nitrogen. These conditions are generally coupled with decreased DO concentrations as seen along the Sabie River during March 2020 (Figure 5.10).

Ammonium is generally not toxic to aquatic invertebrates; however, ammonia is toxic to aquatic life and increases in rivers due to sewage and industrial effluent as well as from runoff from agricultural lands that make use of fertilizers (DWAF, 1996a; Dallas and Day, 2004). Ammonium occurs due to the decomposition of nitrogenous organic matter, and is generally present at concentrations < 0.1 mg.l^{-1} (Dallas and Day, 2004). Along the Sabie River ammonium concentrations increased from the low flow periods of September and October 2019 (0.005 mg.l^{-1} and 0.017 mg.l^{-1}) respectively to the high flow period (March 2020) (0.142 mg.l^{-1}) (Figure 5.2). The ammonium concentrations of the Sabie River are of no concern as the ion is generally nontoxic and was only found in slight excess during the high flow period as a result of agricultural runoff, and industrial and sewage effluent from higher up in the Sabie-Sand catchment (Chapman, 1996).

Despite the increase in nutrients such as ammonium, and orthophosphates during the high flow period, nitrate levels showed no variation. This may be due to the fact that orthophosphates are highly charged and reactive and are able to bind to a number of metal cations and taken up by sediments whereas nitrates are broken down more easily and removed from rivers through nitrification (Griffin, 2017). Overall nutrients such as ammonium, and orthophosphates were present in the Sabie River at low to moderate levels coupled with conductivity levels that were mesotrophic. These water quality parameters result in a river system that is productive with the chance of occasional water quality problems. Nuisance algae and plant growth can occur occasionally in these systems; however, cyanobacterial blooms are seldom toxic (DWAF, 1996a).

6.1.5 pH

The pH of streams is influenced by geomorphological characteristics, making it site specific and biota are generally adapted to these systems whether they are acidic or alkaline (Dallas and Day, 2004). Most freshwater systems in South Africa are relatively well buffered and have a circumneutral pH ranging between 6 – 8 (Dallas and Day, 2004; Barker, 2006). However, large changes in pH can cause potential toxicity of many elements in water, both H^+ and OH^- ions affect the ionic and osmotic balance of aquatic organisms, therefore ensuring pH remains relatively constant is an important contributing factor to good river health (Dallas and Day, 2004). The TWQR states that pH should not vary from the background pH by > 0.5 of a H unit, and the diel and seasonal variations should be considered (DWAF, 1996a). The average pH along the Sabie River as well as for each sampling period did not vary by more than the TWQR specificities (Figure 5.11).

Changes in pH can affect non-metallic ions such as ammonium (the main form of nitrogen assimilated by aquatic plants). When pH levels rise above 8 ammonium ions are converted to toxic ammonia ions (DWAF, 1996a). The pH values during the study ranged from a minimum of 7.49 to a maximum of 8.66, and during the September 2019 (8.10) and March 2020 (8.11) sampling periods the river had an average pH > 8 (Figure 5.11). Overall, the Sabie River appears to be a well buffered river with pH levels showing variation within the neutral range and the TWQR. However, it should be noted that the river is considered alkaline and even though alkaline pollution is less common in river systems compared to acidification it may become a cause for concern in the long term. The Sabie River has become more alkaline compared to previous years; Barker (2006) recorded pH levels along the Sabie River in March 2005 that ranged between 7 and 7.7, Shikwambana *et al.* (2021) recorded average pH levels of 7.69 in 2015 and Erasmus (2018) recorded pH levels in 2016 that ranged from 6.96 at the Kruger Gate, 6.81 at Skukuza, 7.11 at Lower Sabie, and 6.74 at the Mozambique border. Alkaline pollution is caused by industrial effluents (e.g., food canning, and textile production) and anthropogenic eutrophication (high primary production that leads to the depletion of CO_2 during the day) (Dallas and Day, 2004). Increased pH levels can also create favourable conditions for algal blooms and weed growth (Dallas and Day, 2004), during the sampling period weeds such as Water Hyacinth were present at site five near the Mozambique border.

6.1.6 River discharge

High flow periods are common in river systems and the Sabie River is no exception - it experienced changes in flow from the dry season to the wet season and over the years (Figure 5.5). A flood in 1996 had a peak discharge of 2200 $m^3.s^{-1}$ that resulted in the removal of sediments especially in areas where the river was dominated by bedrock (Rountree *et al*., 2000). A flood in 2000 peaked at 3000 $m^3.s^{-1}$ at the Paul Kruger Gate and increased to 7000 $m^3.s^{-1}$ at Lower Sabie (Heritage *et al*., 2001). During the study period high rainfall in February 2020 caused peak river discharge at the Kruger Gate weir 102 $m^3.s^{-1}$ and at the Lower Sabie weir 1246 $m^3.s^{-1}$ (Table 5.1). The increase in peak discharge downstream (Lower Sabie) during March 2020 was the result of increased water input from the Maritsane and Sand River (Heritage *et al*., 2001). RQO in terms of flow for the Sabie River for the respective months were as follows; September (2.762 $m^3.s^{-1}$), October 2019 (2.57 $m^3.s^{-1}$), and March 2020 (6.73 $m^3.s^{-1}$) (DWS, 2016). The flow rate for the Sabie River met the RQO for September 2019 (2.34 $m^3.s^{-1}$), and March 2020 it was more than double (15.21 $m^3.s^{-1}$), however in October 2019 the flow rate was lower than expected (1.75 $m^3.s^{-1}$).

6.1.7 Turbidity

The geomorphology of the landscape influences river flow that not only affects the amount of oxygen in the water because of turbulence but also the rate of erosion which influences turbidity (Dallas and Day, 2004). Reduced clarity in many rivers of South Africa is due to silt, organic matter, inorganic matter, and plankton entering or that are produced in the river system, however, anthropogenic processes such as overgrazing, discharge from farmlands, deforestation, sewage effluent, industrial discharge, surface runoff and erosion increase the quantity of suspended solids and therefore reduces clarity in river systems even further (Chapman, 1996; Heritage and Moon, 2000; Bate *et al*., 2002; Dallas and Day, 2004; Stolz, 2018; Riddell *et al*., 2019).

In general turbidity is not a major concern for South African rivers as turbid rivers are relatively common, however there are cases where increased suspended solids can cause drastic changes in invertebrate community, for example in the Lourens River in the Western Cape during winter (Ractliffe, 1991 cited by Dallas and Day, 2004). Increased turbidity causes reduced light penetration, primary production, and food availability to organisms higher up in the food chain (Henley *et al.*, 2000; Dallas and Day, 2004)**.** More studies need to be conducted to assess how turbidity and suspended solids affect diatom species composition and distribution along the Sabie River.

The increased turbidity in the Sabie River from the low flow periods to the high flow period in March 2020 can be attributed to the February 2020 flood that increased river flow from its base level of 6.07 $m^3.s^{-1}$ to 18.31 $m^3.s^{-1}$ at Paul Kruger Gate and 2.53 $m^3.s^{-1}$ to 115.59 $m^3.s^{-1}$ at Lower Sabie (Table 5.1). Henley *et al*. (2000) stated that increased river flow resulted in mixing of sediments into the water column which in turn increased turbidity during the wet season. Barker (2006) showed that the rivers within the KNP including the Sabie became more turbid during the wet season. Along the Sabie River, site 1 (upper course) had the least turbid waters and site 4 had the most turbid waters during the low flow periods, this may be due to site 4 being located in the “flood plain” of the river system and the accumulation of sediments was greater than at site 1 that was in the upper reach of the river. During the high flow period the clarity of the river was low for all the sites and site 3 was the most turbid – this may be due to the sediment input from the Sand river that joins the Sabie River slightly upstream from site 3 (Figure 5.6). Riddell *et al*. (2019), showed that the Sand River contributed major salt loads to the Sabie River during high peak flows, as the river experiences low flow conditions for most of the year.

Reduced water clarity in other rivers has been attributed to increases in phytoplankton in the water column (DWAF, 1996), however, the Sabie River did not show increases in MPB, and the phytoplankton in the water column was not collected and will need to be sampled in the future to assess the validity of the comment. Turbidity levels in the Sabie River are not a cause for concern as the frequency of suspended solid input into river systems seems to be infrequent and not continuous. The frequency of turbidity will influence how the biota will respond and therefore the community composition, and infrequent inputs often result in communities becoming tolerant while continuous inputs can cause serious consequences (Dallas and Day, 2004). Continued monitoring of the Sabie River is needed to ensure that elevated turbidity levels are not constant, and it is advised as long-term increased turbidity can cause benthic drift (the rate at which benthic organisms move by floating downstream), which could lead to a decrease in benthic organisms and diversity (Quinn *et al*., 1991 cited by Dallas and Day, 2004). The MPB along the Sabie River did not appear to show genetic drift as the cell density from upstream to downstream remained relatively constant throughout the sampling periods (Figure 5.19), however diatom species diversity in March 2020 was higher downstream (site 5) compared to upstream (site 2) (Table 5.5). Reduced light penetration caused by turbid waters, often results in elevated growth of free floating macrophytes (e.g. Water Hyacinth) (DWAF, 1996). Water Hyacinth were not sampled during the study, period; however, the invasive species may become a cause for concern for downstream sites and the Corumana Dam in Mozambique that receives water from the Sabie River.

Girardi *et al*. (2016) concluded that water quality parameters remained relatively constant during low and high rainfall periods in preserved areas. Water quality parameters along the Sabie River exhibited changes between the dry and wet season. Parameters such as ammonium, orthophosphates, temperature, and DO concentration showed changes while TON, conductivity and pH remained relatively constant, however pH has increased since 2015. The water quality of the Sabie River was influenced by stream flow due to the heavy rainfall event. Future studies after a heavy rainfall event need to be conducted to understand the ability of the Sabie River to recover.

6.2 Microphytobenthos

Within a river, diatom community composition is influenced by water quality, available biotopes (habitat diversity), river velocity, seasonal flow of a river, and the historical distribution of species (Dallas and Day, 2004). Water quality parameters such as turbidity, temperature, and nutrients affect individual MPB, and the effect is determined by their specific tolerance limits (Dallas and Day, 2004). The use of the BenthoTorch allowed us to rapidly study the MPB community composition and biomass within the Sabie River to assess the overall water quality.

6.2.1 Microphytobenthos biomass

The World Health Organisation (WHO) has stated that in order to maintain waters with low adverse health risks, cyanobacteria cell counts must be < 20000 cells.ml^{-1} or 10 µg.l^{-1} and levels above 100000 cells.ml^{-1} or 50 µg.l^{-1} could result in adverse health effects (WHO, 2003). The average cyanobacteria cell density for the five sites over the three sampling periods along the

Sabie River were as follows: September 2019 (12256 ± 1689.03 cells.mm^{-2}), October 2019 (9507 ± 988.68 cells.mm^{-2}), and March 2020 (21390 ± 2251.35 cells.mm^{-2}) (Figure 5.22). Overall, the Sabie River has cyanobacteria densities that are less than the WHO levels or slightly higher as in the case with the high flow period of March 2020. Chlorophyll *a* concentrations should average less than or equal to 8.4 µg.cm^{-2} within South African rivers and the Sabie River has chlorophyll *a* concentrations ranging from 2.82 µg.cm^{-2} - 3.06 µg.cm^{-2} that is lower than that stated (DWS, 2016).

Makovinská *et al*. (2014) showed that increased turbidity resulted in reduced chlorophyll *a* concentrations as a result of reduced phytobenthos development. The Sabie River did not show reduced MPB biomass as a result of decreased turbidity during the high flow period of March 2020. Mean MPB chlorophyll *a* concentration and cell density in the Sabie River during September 2019 were 3.06 ± 0.49 ug.cm^{-2} and 20696 ± 3343.61 cells.mm^{-2}, and in October 2019 (low flow periods) 3.02 ± 0.60 ug.cm^{-2} and 19352 ± 3146.13 cells.mm^{-2}, and during the high flow period 2.82 ± 0.31ug.cm^{-2} and 24516 ± 2896.18 cells.mm^{-2} (Figure 5.20a,b). Benthic algal biomass using chlorophyll *a* as an index was measured using the BenthoTorch in Northern Sweden and streams with levels < 0.52 ug.cm^{-2} were classified as oligotrophic (Kahlert and McKie, 2014). The Sabie River has chlorophyll *a* concentrations that are higher than the oligotrophic stream sampled in Northern Sweden, however, the concentrations are still low compared to other studies in France and New Zealand where microphytobenthic chlorophyll *a* levels exceeded 48.6 ug.cm^{-2} (Echenique-Subiabre, 2016). This may be because turbid waters along the Sabie River are seasonal as a result of high rainfall and elevated surface runoff and therefore the lack of continuous turbid waters is a result of the stream's ability to flush out sediments (Hellawell, 1986 cited by Dallas and Day, 2004)**.** The reestablishment of diatom communities in comparison to other algae after a disturbance such as a flood may influence the recovery rate of consumers (invertebrates) as diatoms represent a high-quality food resource (Morin *et al*., 2010; Pandey *et al*., 2018). MPB biomass remained relatively constant in the Sabie system despite changes in environmental parameters and shows that the MPB community appears to recover well after a flood, however, community composition showed larger changes.

Low diatom cell densities (20000 cells.cm^{-2}) have been reported in metal contaminated waters (Gold *et al*., 2002 cited by Pandey *et al*., 2018). Pandey *et al*. (2018) found that low diatom cell densities were not always correlated with impacted sites, and high diatom densities may be the result of nutrient enrichment and may not represent good water quality. Pandey *et al*. (2018) suggests that cell density should be combined with other factors when assessing water quality as understanding the changes in benthic algae composition may provide information on the ecosystem function and structure. The low diatom density in March 2020 may be due to displacement by the February 2020 flood. Further studies should be conducted to assess the rate of colonisation by MPB – especially diatoms and cyanobacteria which will allow for a more in depth understanding of how the two algae groups interact during colonisation.

6.2.2 Microphytobenthos community composition

Microphytobenthos community composition has been shown to change over time due to agriculture, urban development, and flooding. Yang *et al*. (2016) showed that water level fluctuations affect phytobenthos community structure as low flow and drought result in reduced dilution and increased nutrient concentrations. Green algae tend to be more common in summer while diatoms are often more dominant in winter (DWAF, 1996). Changes in MPB community composition in the Sabie River occurred over time, with diatom biomass decreasing and cyanobacteria biomass increasing into the high flow period or summer period. Green algae showed variation in biomass and had the lowest relative abundance (%) of the three algae types. Green algae had the lowest concentration in March 2020 (summer period), and October 2019 (winter period) had the highest concentration (Table 5.9).

Streams in Poland with increased phosphorus concentrations exhibited increased diatom biomass (Kownacki *et al.*, 1985). The Sabie River did not have this correlation despite the increased orthophosphate concentrations during the high flow period. Alternatively, the presence of elevated orthophosphate concentrations (nutrients) in the system during the high flow period coupled with changes in other environmental parameters (conductivity, DO, velocity, temperature, and ammonium) may have caused the MPB community shift, therefore resulting in cyanobacteria becoming more dominant during the wet season (Figure 5.22).

Temperature influences primary production directly (Dallas, 2008). Lürling *et al*. (2017) found that cyanobacteria biomass had an increase of 30% when water temperature rose from 20^{o}C to 25^{o}C, the addition of nutrients further multiplied cyanobacterial biomass. The increase in water temperatures of the Sabie River from 22.36^{o}C in spring, to 27.61^{o}C in the summer coupled with the elevated orthophosphate concentrations may have been the reason for the increase in cyanobacteria biomass during the summer period (Figure 5.22, Table 5.9). De Nicola (1996) cited by Dallas (2008) found that algae composition shifted due to temperature, diatoms were dominate at temperatures < 20^{o}C, green algae between 15^{o}C and 30^{o}C, and cyanobacteria > 30^{o}C. This temperature pattern was not exhibited by the MPB community in the Sabie River and other factors appear to be influencing the composition. Increased nutrients (primarily orthophosphates) in the Sabie River as well as elevated temperatures, coupled with flash floods caused by climate change (Bere, 2007; Dallas, 2008; Dalu and Froneman, 2016) may be a cause for concern as cyanobacteria densities may increase further, resulting in, algal scum and the further depletion of oxygen, especially during the summer months when high rainfall and high river flow conditions are present (Janse van Vuuren *et al*., 2006).

6.3 Diatoms along the Sabie River

6.3.1 Diatom diversity

The Shannon Wiener Diversity Index (H) and species richness are often used to assess the biological integrity of river ecosystems (Pandey *et al*., 2018). Verb and Vis (2000) and Pandey *et al*. (2018) found that the Shannon's H scores (1.6 - 2.0) and species richness were lowest at polluted sites, including sites affected by acid mine drainage. Diversity indices are used to explain community responses to environmental variables and are best utilised alongside other monitoring metrics such as biomonitoring and physico-chemical monitoring because various factors can influence species diversity (Li *et al*., 2010).

The use of diversity indices has been controversial in the past as high dominance species occur in both polluted and pristine waters. However, Dallas and Day (2004) and De la Rey (2008) later stated that species diversity is generally higher in moderately polluted waters and that the relationship between the two is not linear. Nutrient enriched water is generally represented by fewer species with many individuals and pristine waters have more species with fewer individuals (Dallas and Day, 2004). The diatom diversity along the Sabie River increased from 1983 to 2020 (Table 5.6), which may be a result of increased nutrients such as ammonium and orthophosphates entering the Sabie River, especially during the high flow period of March 2020 as a result of surface runoff. Pillay and Naidoo (2018) showed that diatom diversity ranged from 1.2 to 1.5 and decreased at sites with slow water and substrates such as pebbles, gravel, and silt, stating that species diversity changes can be attributed to water chemistry and habitat structure. The diatom slides obtained from 1983 and 1985 were sampled from one biotope whereas the sampling done in 2019 and 2020 ensured that diatom samples were scraped from 10 different pebbles or boulders from different biotopes within a river reach. Therefore, the increased diatom diversity noted for the 2019 and 2020 sampling periods may have been influenced by the variation in sampling techniques and biotopes.

In March 1983 *Cymbella kolbei* had a high relative abundance of 32.88%, and the second most-abundant species with a relative abundance of 13.50% was *Planothidium rostratum*. September 1985 had the lowest diversity of all the sampling periods, *Achnanthidium minutissimum* had the highest relative abundance (39.38%), *Gomphonema venusta* was the second most dominant species making up 11.00% of the community composition (Table 5.4). In September 2019 *Cymbella turgidula* had the highest relative abundance of 9.90%, the second most-abundant species was *Encyonopsis leei* var. *sinensis*, making up 9.68% of the community composition. In March 2020, *C. turgidula* was the most dominant species (12.77%), followed by *Cocconeis placentula* with 8.63% of the community composition (Table 5.3). In 1983 and 1985 the most dominant diatom species *C. kolbei*, *P. rostratum*, *A. minutissimum* and *G. venusta* made up over a quarter of the community composition, however, this composition decreased for 2019 and 2020 with the relative abundance of the most dominant species comprising an eighth of the community composition. The high relative abundance of a single species, as seen for the 1983 and 1985 sampling periods along the Sabie River can affect species diversity and influence community stability during periods of environmental stress such as increased nutrients recorded in the March 2020 sampling period (Lim and Lee, 2017).

6.3.2 Diatom species composition change

Upstream disturbances affect MPB species composition due to changes in water chemistry, which often results in unstable MPB community structure and low diversity and therefore pollution tolerant species becoming more abundant (De la Rey *et al*., 2008; Dalu and Froneman, 2016; Pandey *et al*., 2018). Along the Sabie River the following pollution tolerant species increased in abundance from the 1983 and 1985 sampling periods to the 2019 and 2020 sampling periods; *Nitzschia palea*, *Nitzschia capitellata*, *Navicula gregaria*, *Navicula germainii*, *Fallacia pygmaea*, *Planothidium engelbrechtii*, *Nitzschia frustulum*, *Gomphonema minutum* (wasn't present in 1983 and 1985), and *Eolimna subminuscula*. However, some pollution-tolerant species such as *Gomphonema parvulum*, *Navicula capitatoradiata*, *Eolimna minima*, *Nitzschia linearis*, *Navicula cryptotonella*, *Navicula cryptocephala*, *Hippodonta capitata*, *Navicula veneta*, *Navicula schroeteri*, *Navicula rostellata*, *Nitzschia iremissa*, *Sellaphora pupula*, and *Sellaphora seminulum* did not increase in abundance and their biomass and composition within the Sabie River remained relatively constant or decreased over the 37-year period (Table 5.3, Table 5.4).

The Sabie River contains diatom species that are found in a broad range of water conditions from pristine waters to nitrogen-rich heterotrophic waters (Taylor *et al*., 2007a). In 1983 and 1985 the diatom index scores indicates that the water quality of the Sabie River was good, and the two most dominant species typically occur in pristine waters and waters with low conductivity, such as *Cymbella kolbei* and *Planothidium rostratum*, that were dominant in 1983. *Achnanthidium minutissimum* and *Gomphonema venusta*, present in the samples collected in 1985, are typically found in nutrient-poor, broad range of waters (oligotrophic to eutrophic waters), as well as unpolluted waters (Taylor *et al*., 2007a). In 2019 and 2020 the Sabie River was categorised as having moderate water quality and two species, *Cymbella turgidula* and *Encyonopsis leei* var. *sinensis* that typically occur in oligotrophic to mesotrophic waters with low to moderate electrolyte content were dominant in 2019. *C. turgidula* and *Cocconeis placentula* were the two dominant species in 2020, these two species typically occur in waters with a broad range of physico-chemical conditions, water with low conductivity, as well as mesotrophic to eutrophic waters (Taylor *et al*., 2007a). It is apparent that the diatom species composition of the Sabie River has changed over time which suggests a change in water quality of the river. Shikwambana *et al*. (2021) also showed that diatom composition changed over time along the Sabie River with *C. kolbei* being the dominant species in 1983 and *E. leei* the most dominant species in 2015. It is evident that diatom species composition changes over seasons (low flow to high flow periods) and years (1983 – 2020), and composition change can happen over an even shorter period - Dalu *et al*. (2014) found that benthic diatom colonisation and composition following a flooding event changed over a short period of 28 days along the Kowie River, Eastern Cape. Early colonisers included *A. minutissimum*, *Fragilaria biceps*, and *Fragilaria ulna var. acus* that were dominant after seven days, followed by *A. minutissimum*, *Fragilaria capucina*, and *G. venusta*, categorised as late colonisers who were dominant after 28 days. The variation of benthic diatom species composition over time indicates the complexity of freshwater systems.

The presence of diatoms at a relative abundance of 50% indicates good water quality (Snow, 2016). Of the three MPB groups considered using the BenthoTorch – diatoms, green algae, and cyanobacteria – diatom composition was above 50% for sites 1 (53.35%), and 3 (70.93%), and cyanobacteria were dominant at sites 2 (61.4%), 4 (59.8%), and 5 (63.4%) along the Sabie River over the 2019, and 2020 sampling periods (Figure 5.23). Griffin (2017) showed that 333 monitoring points across South Africa had phosphate concentrations that were tolerable in 1985, bad in 2007, and acceptable in 2013. Phosphate concentrations have shown a considerable decrease regionally in South Africa from 2007 that cannot be pinpointed to a single phosphate source (Griffin, 2017). Stolz (2018) concluded that urban areas, and informal settlements had the largest effect on the Sabie Rivers water quality in comparison to forestry, commercial plantations, and agricultural lands. The Sabie River to date is classified as a system with relatively high species diversity as indicated by the species diversity index, however care needs to be taken by management to ensure that anthropogenic disturbances such as the expansion of urban areas and informal settlements do not result in further degradation of the Sabie River's water quality.

Harding and Taylor (2014) found that relatively undisturbed sites in the Wemmershoek catchment, South Africa showed no changes in diatom community as well as SPI and BDI scores and therefore water quality during their 1960 – 2008 sampling period. The Sabie River was different to the Wemmershoek catchment and showed changes in diatom community composition, and diatom index scores, which provided information on the changing water quality status from 1983 and 1985 to 2019 and 2020. The SPI and BDI scores were able to provide information on the water quality of the Sabie River in 1983 and 1985, where the river was classified as good quality (EcoClassification B), and moderate quality (EcoClassification C) in 2019 and 2020. The Ecological categories for the Sabie River, downstream of the Sand River in 2016 were all A/B (DWS, 2016). A study done by Shikwambana *et al*. (2021) classified the Sabie River in 1983 and 2015 as an EcoClassification A (high water quality) which does not coincide with my findings.

Diatom index scores generally increase in rivers with high flow due to rapid shifts in diatom community structure (Harding and Taylor, 2011). The diatom index scores for the sites sampled in March 2020 along the Sabie River had a higher SPI score than the low flow sampling periods (Table 5.5). The diatom indices utilised in this study, SPI and BDI did not show significant correlations to water quality parameters along the Sabie River (Table 5.7). De la Rey *et al*. (2004) found that there were no correlations between SPI and the following environmental parameters; DO, turbidity, total phosphorus, and total nitrogen. Significant correlations indicate the ability of the indices to show changes in water quality (Taylor *et al*., 2007c). Benthic diatom community composition is constantly changing within the Sabie River, either on a short- or long-term temporal scale and future sampling is recommended to ensure that there is a stronger correlation between environmental parameters and the SPI scores.

The SPI obtained from OMNIDIA classified the low and high flow periods as having moderate water quality (EcoClassification C) during 2019 and 2020. During 1983 and 1985 the

SPI classified the Sabie River as having good quality water (EcoClassification B). The Sabie Rivers water quality has degraded from good quality waters in 1983 and 1985 to moderate water quality in 2019 and 2020. Following the concept of species diversity, it is evident that diatom diversity increased from 1983 to 2020 as a result of changing environmental parameters. According to the physico-chemical variables of the Sabie River during 2019 and 2020, water quality was generally mesotrophic during the low flow and high flow periods. The dominant diatoms along the Sabie River preferred a broad range of waters from low to moderate electrolyte content as well as oligotrophic to mesotrophic waters.

6.3.3 Diatom species distribution with regards to environmental parameters

Canonical Correspondence Analysis (CCA) allows for an integrated understanding of diatom species and their interaction with environmental variables (ter Braak, 1986). Pandey *et al*. (2018) stated that if a sampling sites position on a CCA plot is different to what the environmental parameters are indicating it may be a result of the diatom species indicating more impacted or pristine waters or temporal fluctuations in contamination. The sites sampled along the Sabie River were aligned on the CCA plot with respect to the environmental parameters and the interaction with diatoms species (Figure 5.15).

Riddell *et al*. (2019), lumped two years of diatom data within the KNP to perform the CCA plot at a park scale and found that nitrogen oxides, ammonia and orthophosphates were most strongly associated with diatom presence within the KNP. The data collected along the Sabie River during 2019, and 2020 was grouped per site and the CCA plot was able to explain 47.1% of the overall diatom species distribution. The low variation (18.8%) of Axis 1 and 10.6% for Axis 2 does reduce the certainty in terms of explaining diatom species distribution with regards to the environmental parameters, however it is apparent that the spatio-temporal parameters such as conductivity, ammonium, orthophosphates, velocity and DO concentrations were associated with diatom species distribution along the Sabie River within the KNP (Figure 5.15). Bate *et al*. (2004) showed that only conductivity was correlated to diatom community structure in the Swartkops River in South Africa.

Nitzschia frustulum, and *Eolimna subminuscula* were found to prefer moderate conductivity levels, whereas only the latter could tolerate elevated ammonium, and orthophosphate concentrations. These two species are associated with poor water quality and elevated conductivity (Taylor *et al*., 2007c). *Nitzschia palea*, *Gomphonema parvulum,* and

Sellaphora pupula were found in waters with elevated ammonium, and orthophosphate concentrations as well as moderate conductivity (101.70 – 159.00 µS.cm^{-1}) along the Sabie River (Figure 5.15). *N. palea*, *G. parvulum*, and *S. pupula* were present at sites characterised as having bad water quality along the Monjolinho River in Brazil – these sites were located downstream from an urban area (DO = 0.4 to 1.9 mg.l^{-1}; P > 0.1425 – 0.249 mg.l^{-1}) (Bere and Tundisi, 2011). Pandey *et al*. (2018) showed that *N. palea*, *G. parvulum*, and *N. frustulum*, were also found at high electrolyte sites ranging from 321 – 4007 µS.cm^{-1}. Taylor *et al.* (2007a) classified *G. parvulum* as a pollution-tolerant species found in electrolyte-rich waters. The Sabie River did not exhibit such bad water quality parameters as described in the studies above; however, the species were present in the Sabie River indicating that they have a broad tolerance to pollution.

Navicula veneta, *Nitzschia palea*, *Eolimna subminuscula*, and *Nitzschia capitellata* were present in nutrient-rich waters in an urban stream in Cape Town, South Africa (Harding and Taylor, 2011). A similar study by Harding *et al*. (2004) showed that *N. capitellata* was present in poor quality waters. However, along the Sabie River the diatom species was present in moderate electrolyte waters and *N. veneta*, *N. capitellata*, and *E. subminuscula* appeared to prefer waters that have high ammonium (0.143 mg.l^{-1}) and orthophosphate concentrations (0.054 mg.l^{-1}) (Figure 5.15, Appendix A). The *Nitzschia* genus prefers nitrogen-rich, nutrient enriched waters and is generally an indicator of waters with elevated nutrient concentrations, however, they have a broad tolerance and are present in low abundance in oligotrophic waters (Janse van Vuuren *et al*., 2006; Harding and Taylor, 2011). *Navicula* species are found in a range of waters from oligotrophic to eutrophic and extremely polluted waters (Janse van Vuuren *et al*., 2006; Taylor *et al.,* 2007a). Harding and Taylor (2011) showed that high intensity farming resulted in more *Nitzschia* species and low intensity farming more *Navicula* species. *Nitzschia* species were present along the Sabie River in 2019 and 2020 but were not dominant during any of the sampling periods from 1983 – 2020. *Navicula microcari* was a dominant species in 2019 and *Navicula germainii* in 2020. *Navicula* species were present at all the sampling sites and periods along the Sabie River. Small-scale farming practices (summer and winter crops) upstream of the Paul Kruger Gate can be classified as a low intensity disturbance and this may be an indication of the high abundance of *Navicula* species and slightly lower abundance of *Nitzschia* species during the historic periods (1983 and 1985) and the more present sampling periods (2019 and 2020) (Table 5.3, Table 5.4) (Riddell *et al*., 2019).

Gomphonema parvulum, *Navicula gregaria*, and *Navicula schroeteri* were ordinated with high orthophosphate and ammonium concentrations as well as relatively low DO along the Sabie

River (Figure 5.15). Bere and Mangadze (2014) found *G. parvulum*, *N. gregaria*, and *N. schroeteri* present in waters with the same characteristics as the Sabie River and were correlated with high ammonium concentrations (Bate *et al*., 2002). Pandey *et al*. (2018) showed that less impacted sites and sites with large variation in conductivity (electrical conductivity range: 170 – 659 $\mu S.cm^{-1}$) contained *N. gregaria*, and *Navicula capitatoradiata*. Bate *et al*. (2004) also stated that *N. gregaria* was dominant at sites with large variations in conductivity levels in the Swartkops River. Harding *et al*. (2004) showed that *N. capitatoradiata* was present in moderate quality water. This trend was noted for the two species (*N. gregaria* and *N. capitatoradiata*) along the Sabie River that typically occur in waters with moderate electrolyte content, however the conductivity levels during the study period only fluctuated slightly.

The water quality parameters along the Sabie River influenced the following diatom species distribution, these included *Navicula heimansoides*, *Encyonopsis leei* var. *sinensis*, *Fragilaria ungeriana*, *Achnanthidium affine*, *Achnanthidium crassum*, and *Cymbella tumida* as well as *Navicula capitatoradiata*, *Navicula microcari*, and *Tabularia fasciculata* that occurred at sites with moderate electrolyte content and slow flowing waters (Figure 5.15). Taylor *et al*. (2007a,c) described these species occurrence in oligotrophic to mesotrophic waters with low to moderate electrolyte content, these are similar to the parameters used to describe the Sabie River. *N. microcari*, and *T. fasciculata* are species that occur in waters with moderate to high electrolyte content and can tolerate critical levels of pollution (Taylor *et al*., 2007a). *A. crassum* has been described as a species present in slow-flowing waters (Taylor *et al*., 2007a), which was confirmed by the CCA plot of the environmental parameters and diatom distribution along the Sabie River.

Cymbella is a genus known to occur in pristine and unpolluted river systems (Janse van Vuuren *et al*., 2006; Taylor *et al*., 2007b**;** Bere and Mangadze, 2014; Sen *et al.,* 2014; Pillay and Naidoo, 2018). The *Cymbella* species (*Cymbella kolbei, Cymbella kappii, Cymbella turgidula,* and *Cymbella tumida*) as well as *Fragilaria ungeriana* that occurred in the Sabie River showed preference to the sites with lower conductivity and slow river discharge (Figure 5.15). *C. turgidula* has been described as a species that is insensitive to high ammonium concentrations (Bate *et al*., 2004), and although ammonium concentrations increased from the dry season to the wet season (2019 to 2020) along the Sabie River, *C. turgidula* was present at almost all the sampling sites except at site 5 in September 2019, and sites 8 and 9 in September 1985 (Table 5.3, Table 5.4). This shows that ammonium concentrations were not at a level that prevented the presence of *Cymbella*.

Pioneer species and those that prefer well-oxygenated waters, such as *Achnanthidium minutissimum* and *Cocconeis sp*., generally dominate sites after a flood (Harding and Taylor, 2011; Dalu *et al*., 2014). However, along the Sabie River *A. minutissimum* showed extremely low abundance after the flood peak in February 2020, being absent from three of the five sampling sites in March 2020. *C. placentula* showed a relatively constant abundance both before and after the flood (Table 5.3).

Bere and Mangadze (2014) showed that as water quality decreased (decrease in pH and DO with an increase in nutrients and conductivity), species such as *Cymbella tumida*, and *Cocconeis placentula*, were replaced by pollution tolerant species such as *Gomphonema parvulum* and *Nitzschia palea.* Species such as *C. placentula* being replaced by *G. parvulum*, and *N. palea* does not seem to have occurred within the Sabie River as *C. placentula* was considered a dominant species in the 2019 and 2020 sampling periods, whereas *G. parvulum* and *N. palea* were present at lower abundances (Table 5.3, Table 5.4). The lack of a species shift, in terms of *C. placentula* to pollution-tolerant species such as *G. parvulum* and *N. palea*, may be because conductivity and nutrient concentrations remained relatively constant along the Sabie River despite the high flow conditions causing changes. Bere and Mangadze (2014) and Bere *et al*. (2014) showed that species such as *G. parvulum*, and *Navicula gregaria* could tolerate high ammonium and low DO and pH levels, whereas *C. tumida*, *C. placentula*, *Gomphonema pumilum var. rigidum*, *Tabularia fasciculata*, and *Gomphonema venusta* were more sensitive to ammonium, DO and pH, being found in waters with lower pH and ammonium and higher DO. These diatom species in the Sabie River showed similar tolerances and therefore distribution, with regards to ammonium, and DO concentrations (Figure 5.15).

6.3.4 Diatom functional traits

Lange *et al*. (2011) showed that low profile taxa comprised 40% of all diatoms counted, and the competitive advantages of filamentous, erect and stalked forms were less distinct than expected. However, motile diatoms were more abundant in nutrient-rich environments as they can select the most suitable habitat that is limited by physical disturbance (Passy, 2007a; Lange *et al*., 2011). Low profile diatoms are resistant to disturbance but can't tolerate high nutrient levels, whereas high and motile guilds are unable to tolerate disturbance in the form of increased flow but can tolerate nutrient enrichment (Passy, 2007a). Makovinská *et al*. (2014) also showed that motile and low-profile guilds comprised the majority of the diatoms, whereas high profile guilds had a lower proportion. In the Sabie River, motile diatoms comprised 56.96% of the diatoms present, with low profile (26.58%) and high profile (16.46%) diatoms were less prominent. The motile diatoms were ordinated with nutrient-rich sites, the low motile species were ordinated with high DO concentrations and the high-profile guilds ordinated with high water velocity sites along the Sabie River. The high-profile and low-profile guilds were different to what was expected as the high-profile guild should in theory not tolerate high disturbance, such as velocity, and the low-profile guild should be able to tolerate high velocity, but they favoured low-velocity sites (Figure 5.17). The three ecological guilds showed no correlation in response to changing velocity (Figure 5.18). To further assess the functionality of diatom functional traits, samples along the entire Sabie-Sand catchment should be taken as this may provide a better comparison in terms of changing water quality along the river, as well as being an efficient way to assess water quality and environmental changes using diatoms (Passy, 2007a; Lange *et al*., 2011).

6.4 BenthoTorch

The BenthoTorch performance was shown to be correlated to spectrophotometry analysis of chlorophyll *a* when the biofilm layer was < 2 mm or 4 $\mu g.cm^{-2}$, whereas thick biofilms with high chlorophyll *a* biomass (48.6 $\mu g.cm^{-2}$) underestimated biomass possibly due to the limitation of the light emitted by the sensor to penetrate the biofilm (Echenique-Subiabre, 2016). The chlorophyll *a* biomass was relatively low for the Sabie River (1.35 – 4.58 $\mu g.cm^{-2}$) and further comparison of chlorophyll *a* readings obtained using the BenthoTorch and laboratory analyses, will need to be conducted to ensure no underestimations of MPB biomass occurred.

The BenthoTorch resulted in diatoms being overestimated and green algae underestimated in a river in Northern Sweden (Kahlert and McKie, 2014). They concluded that the BenthoTorch computed biomass readings equivalent to conventional analysis. Laboratory errors are eliminated and the rapid analysis using the BenthoTorch allows for increased samples to be obtained (Kahlert and McKie, 2014). The ability of the BenthoTorch to capture chlorophyll *a* readings is limited to the upper layer of MPB, however algae biofilms are complex and range in thickness and further developments of the BenthoTorch need to be done (Echenique-Subiabre, 2016). The BenthoTorch was able to provide a rapid assessment of the overall river health of the Sabie River using MPB biomass and community composition – that was supported by the water quality provided by the physico-chemical parameters and biomonitoring using the benthic diatoms.

Chapter 7: Concluding remarks

Freshwater biodiversity and MPB composition and biomass are influenced by environmental parameters such as water flow, temperature, nutrient regimes, and dissolved oxygen concentrations as well as anthropogenic disturbances and climate change. The February 2020 flood within the Sabie-Sand catchment resulted in an apparent change in water quality. Orthophosphates increased from the low flow periods September 2019 and October 2019 into the high flow period of March 2020 where the orthophosphate concentration was higher than the RQO for the Sabie River. The TWQR was met by ammonium (0.007 $mg.l^{-1}$) during the low flow period (September 2019), however levels increased into the high flow period of March 2020. Average DO concentrations decreased from the low flow periods, into the wet season where concentrations were the lowest – all DO concentrations were within the TWQR for the Sabie River. When corrected for temperature the specific conductivity of the Sabie River increased from upstream to downstream and met the RQO for conductivity (< 300 $\mu S.cm^{-1}$).

A total of 70 benthic diatom species were identified along the Sabie River within the KNP boundaries (from the 1983, 1985, 2019 and 2020 sampling periods). The five most abundant diatom species sampled during 1983 and 1985 were *Achnanthidium minutissimum*, *Cymbella kolbei*, *Gomphonema venusta*, *Navicula heimansoides*, and *Cocconeis placentula*. In 2019 and 2020; *Cymbella turgidula*, *C. placentula*, *Planothidium rostratum*, *Encyonopsis leei* var. *sinensis,* and *Nitzschia frustulum* were dominant. In 1983 and 1985 the dominant species recorded preferred oligotrophic to mesotrophic waters with low to moderate electrolyte content. Where as the dominant species of 2019 and 2020 preferred oligotrophic to eutrophic waters with low to high electrolyte content. It is evident that the diatom community composition has changed along the Sabie River over the last 36-years. It is apparent that the change in diatom community structure is influenced by the change in water quality as indicated by the SPI and BDI scores which states that the water quality has changed from good (1983 and 1985) to moderate quality (2019 and 2020). This study has increased the knowledge around benthic diatoms and water quality and provided a photo reference for future studies along the Sabie River.

It is apparent that diatom distribution and composition is influenced by environmental parameters as shown by the CCA plot and emphasizes the importance of using benthic diatoms as bioindicators when assessing water quality. It should be noted that the environmental parameters were only able to explain 47.1% of the diatom distribution along the Sabie River and future sampling is required to get a better understanding of how the environmental parameters influence diatom species distribution and composition.

The flash flood of February 2020 along the Sabie-Sand Catchment resulted in elevated surface runoff from small scale farming activities and urban settlements that caused increased turbidity and nutrients (orthophosphates and ammonium) coupled with decreased DO concentrations (Heritage and Moon, 2000; Stolz, 2018; Riddell *et al*., 2019). The BenthoTorch was able to show the change in MPB community composition, that shifted from diatom dominant (46%) in the low flow period to cyanobacteria dominant (73%) in the wet season, however MPB biomass (chlorophyll *a*) remained relatively constant over the sampling period which is a positive sign as dense MPB result in waters being at risk of becoming eutrophic. Stolz (2018) concluded that urban areas, and informal settlements had the largest effect on the Sabie Rivers water quality. The pH levels showed a river that is well buffered, however it should be noted that the Sabie River has become more alkaline than what has been recorded in the past. Alkaline waters can create favourable conditions for algal blooms and weed growth (Water Hyacinth) that is already present at the most downstream site of the Sabie River that leads directly into Mozambique. According to this study the Sabie River can be described as a productive system with the chance of occasional water quality problems including nuisance algae and plant growth (Water Hyacinth).

The water quality of a perennial river such as the Sabie that is not only used by South Africa but is an important resource downstream of the KNP where it flows into Mozambique needs to be monitored and the management and sustainable use of the Sabie River’s water needs to happen on a larger catchment scale, with all users and stakeholders coming together to ensure the water quality of the river does not degrade further due to upstream activities. Most of Sabie Rivers water monitoring is focused on macroinvertebrates, riparian vegetation, and fish. There is very little use of diatoms in the KNP monitoring programs and it is evident from this study that benthic diatoms are a useful indicator of river health and water quality change in the Sabie River both temporally (short and long term) and spatially. Future water quality monitoring of the Sabie River should include the BenthoTorch as it provides rapid insight in terms of river health – however, due to this being a novel monitoring practice in the KNP further studies need to be conducted to investigate its potential for monitoring water quality.

Chapter 8: References

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Chapter 9: Appendices

Appendix 1: The mean, minimum, and maximum values of the environmental parameters for the three sampling periods (September 2019, October 2019, and March 2020) over the five sampling sites along the Sabie River within the KNP.

Parameters	Seasons	Site 1			Site 2			Site 3			Site 4			Site 5		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Ammonium ($mg.l^{-1}$)	Sep. 2019	0.006	0.000	0.011	0.000	0.000	0.000	0.002	0.000	0.002	0.014	0.009	0.022	0.004	0.000	0.009
	Oct. 2019	0.012	0.010	0.013	0.022	0.009	0.034	0.005	0.002	0.007	0.036	0.002	0.098	0.010	0.006	0.015
	Mar. 2020	0.128	0.070	0.221	0.083	0.024	0.167	0.146	0.010	0.276	0.173	0.066	0.279	0.182	0.107	0.243
Orthophosphates ($mg.l^{-1}$)	Sep. 2019	0.000	0.000	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Oct. 2019	0.016	0.006	0.035	0.001	0.000	0.003	0.004	0.003	0.004	0.002	0.001	0.003	0.018	0.015	0.020
	Mar. 2020	0.012	0.011	0.015	0.071	0.055	0.082	0.035	0.025	0.045	0.077	0.059	0.087	0.072	0.060	0.086
Nitrates ($mg.l^{-1}$)	Sep. 2019	0.480	0.211	0.965	0.502	0.238	0.674	0.758	0.357	1.057	0.530	0.005	1.308	0.907	0.264	1.229
	Oct. 2019	0.678	0.357	1.070	1.022	0.172	2.550	0.617	0.145	1.321	0.665	0.304	0.925	1.189	0.872	1.454
	Mar. 2020	0.453	0.432	0.467	0.397	0.378	0.412	0.306	0.200	0.379	0.362	0.328	0.386	0.375	0.324	0.405
Conductivity ($\mu S.cm^{-1}$)	Sep. 2019	109.83	109.60	110.10	132.66	132.60	132.80	127.57	127.50	127.60	152.13	151.80	152.50	142.80	142.80	142.80
	Oct. 2019	115.77	115.70	115.90	127.53	127.40	127.80	147.10	146.80	147.40	156.13	156.00	156.30	149.97	149.90	150.00

	Mar. 2020	101.73	101.70	101.80	110.80	110.70	110.90	126.60	126.40	126.90	155.80	155.70	155.90	158.87	158.70	159.00
Specific Conductance ($\mu S.cm^{-1}$)	Sep. 2019	124.70	124.70	124.70	134.10	134.00	134.30	141.43	141.30	141.50	144.63	144.60	144.70	154.33	154.30	154.40
	Oct. 2019	120.53	120.50	120.60	128.63	128.60	128.70	139.63	139.50	139.80	145.90	145.90	145.90	155.30	155.30	155.30
	Mar. 2020	103.33	103.30	103.40	108.77	108.70	108.90	122.13	122.00	122.30	139.80	139.70	139.90	145.40	145.30	145.50
Dissolved Oxygen ($mg.l^{-1}$)	Sep. 2019	12.34	12.34	12.34	11.52	11.44	11.61	10.80	10.73	10.90	10.55	10.51	10.57	7.46	7.30	7.63
	Oct. 2019	9.24	9.18	9.27	9.41	9.39	9.42	8.80	8.75	8.82	8.35	8.33	8.36	8.14	8.12	8.14
	Mar. 2020	6.76	6.38	7.14	6.81	6.34	7.22	6.59	6.39	6.86	6.71	6.64	6.79	7.06	6.75	7.27
Dissolved Oxygen (%)	Sep. 2019	132.40	132.10	132.70	138.03	137.10	138.80	118.50	117.70	119.60	134.10	133.90	134.30	83.83	82.00	85.70
	Oct. 2019	107.53	106.70	108.00	112.93	112.50	113.20	112.03	111.00	112.70	107.97	107.70	108.10	95.20	95.00	95.40
	Mar. 2020	80.70	76.10	85.20	83.87	78.10	89.00	82.53	80.20	85.90	90.37	89.30	91.40	93.17	89.00	96.20
pH	Sep. 2019	7.97	7.94	8.00	8.07	8.04	8.14	7.79	7.79	7.79	8.14	8.12	8.17	8.50	8.33	8.69
	Oct. 2019	7.97	7.89	8.07	7.73	7.71	7.74	8.34	8.27	8.39	7.69	7.67	7.74	7.49	7.46	7.52
	Mar. 2020	7.78	7.72	7.84	8.03	7.95	8.11	8.66	8.44	8.92	7.96	7.95	7.97	8.10	8.06	8.18
Temperature (℃)	Sep. 2019	18.70	18.60	18.80	24.40	24.30	24.50	19.90	19.90	19.90	27.70	27.60	27.80	21.10	21.10	21.10
	Oct. 2019	22.93	22.90	23.00	24.57	24.50	24.70	27.80	27.60	28.00	28.67	28.60	28.70	23.20	23.20	23.20
	Mar. 2020	24.20	24.20	24.20	26.00	26.00	26.00	26.93	26.90	27.00	31.00	31.00	31.00	29.90	29.80	30.00
Clarity (cm)	Sep. 2019	91.00	89.00	93.00	84.50	84.00	85.00	73.00	71.00	75.00	19.00	18.00	20.00	55.50	54.00	57.00

	Oct. 2019	99.00	98.00	100.00	66.50	66.00	67.00	82.00	81.00	83.00	44.00	42.00	46.00	61.50	60.00	63.00
	Mar. 2020	39.50	36.00	40.00	43.50	42.00	47.00	30.00	29.00	31.00	44.50	43.00	44.00	38.00	37.00	42.00
Velocity ($m^3.s^{-1}$)	Sep. 2019	1.00	0.33	1.48	0.19	0.12	0.33	0.34	0.12	0.48	0.60	0.12	1.07	0.58	0.24	0.75
	Oct. 2019	0.74	0.54	1.13	0.23	0.12	0.33	0.50	0.24	1.03	0.55	0.41	0.75	0.51	0.12	0.88
	Mar. 2020	1.50	1.00	2.00	2.33	1.00	4.00	1.00	0.50	1.50	2.33	1.00	4.00	2.17	1.00	4.00

Appendix 2-A: Count data for the diatom species sampled at the five sites over the September 2019, October 2019, and March 2020 sampling periods along the Sabie River within KNP.

Diatom species	September 2019					October 2019					March 2020				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Achnanthidium affine (Grunow) Czarnecki	7	1	4	0	5	0	2	4	8	0	7	13	2	0	0
A. crassum (Hustedt) Potapova & Ponader	87	2	22	0	1	5	0	4	60	0	1	39	17	6	0
A. exiguum (Grunow) Czarnecki	1	0	2	4	1	0	0	1	1	4	2	0	5	1	3
A. minutissimum (Kützing) Czarnecki	7	4	53	1	0	16	0	26	5	1	42	9	25	0	30
A. straubianum (Lange-Bertalot) Lange-Bertalot	0	2	4	4	0	0	0	3	0	4	4	0	13	0	6
Amphipleura pellucida Kützing	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Amphora inariensis Krammer	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Anorthoneis dulcis Hein	0	1	0	0	0	1	0	1	0	0	3	0	3	0	0
Caloneis bacillum (Grunow) Cleve	0	1	1	1	0	0	0	2	0	1	1	0	0	0	0
Capartogramma crucicula (Grunow) Ross	0	1	1	5	6	0	2	2	1	5	1	0	1	1	1
Cocconeis placentula Ehrenberg var. *placentula*	16	11	26	65	26	8	144	14	38	65	11	11	14	26	72
Cymbella aspera (Ehrenberg) H.Peragallo	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
C. kappii (Cholnoky)	0	0	9	0	12	0	0	0	4	0	0	15	0	25	0
C. kolbei Hustedt	3	75	16	0	31	2	0	3	15	0	1	16	6	83	0

C. tumida (Brebisson) Van Heurck	5	37	27	3	0	9	2	32	1	3	11	10	10	0	0
C. turgidula Grunow 1875 in A.Schmidt *et al*. var. *turgidula*	27	99	43	9	32	9	0	65	55	9	24	107	12	154	2
Diadesmis confervaceoides Lange-Bertalot & Rumrich	0	0	0	5	0	0	0	0	1	5	0	0	0	0	2
Encyonema minutum (Hilse in Rabh.) D.G. Mann	4	1	0	4	1	0	2	0	9	4	1	1	2	0	1
Encyonopsis leei Krammer var. *sinensis* Metzeltin & Krammer	15	73	17	0	0	94	1	39	13	0	100	31	16	2	0
Eolimna minima (Grunow) Lange-Bertalot	0	0	1	10	1	4	1	2	1	10	0	2	0	2	0
E. subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	2	2	0	6	4	2	9	0	1	6	0	0	6	0	26
Fallacia insociabilis (Krasske) D.G. Mann	1	1	14	25	6	22	0	6	5	25	17	9	12	12	17
F. pygmaea (Kützing) Stickle & Mann ssp. *pygmaea* Lange-Bertalot	0	20	5	1	4	10	0	15	0	1	27	1	56	1	5
Fragilaria biceps (Kützing) Lange-Bertalot	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0
F. capucina Desmazieres var. *lanceolata* Grunow	0	0	2	0	0	1	0	0	8	0	0	0	0	4	0
F. parasitica (W.Smith) var. *subconstricta* Grunow	0	0	1	0	0	0	0	0	1	0	0	9	0	0	0
F. ulna (Nitzsch) Lange-Bertalot var. *acus* (Kützing)	0	0	2	1	0	0	1	0	2	1	0	3	0	0	0
F. ungeriana Grunow	0	4	8	0	0	0	0	3	29	0	2	15	2	3	0
Geissleria decussis (Ostrup) Lange-Bertalot & Metzeltin	3	0	2	2	0	2	0	2	0	2	0	2	2	1	2
Gomphonema micropus Kützing fo teratogene	9	3	1	4	8	0	13	1	1	4	1	1	0	6	4
G. parvulum (Kützing) Kützing var. *parvulum f. parvulum*	7	0	5	5	3	3	11	3	1	5	0	1	2	0	18
G. pumilum var. rigidum Reichardt & Lange-Bertalot	4	0	0	2	0	2	7	1	0	2	1	4	2	0	1

G. venusta Passy, Kociolek & Lowe	24	0	1	3	1	4	21	0	2	3	0	7	0	0	3
Gyrosigma acuminatum (Kützing) Rabenhorst	1	0	0	2	4	2	2	4	2	2	1	1	0	0	2
Hantzschia amphioxys (Ehrenberg) Grunow in Cleve & Grunow 1880	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Hippodonta capitata (Ehrenberg) Lange-Bert.Metzeltin & Witkowski	0	0	1	0	0	0	0	0	2	0	0	0	0	0	1
Karayevia ploenensis (Hustedt) Bukhtiyarova	0	0	1	5	0	0	1	0	0	5	0	0	0	1	3
Lemnicola hungarica (Grunow) Round & Basson	0	0	0	2	0	0	1	0	0	2	0	0	0	0	0
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Navicula capitatoradiata Germain	3	2	0	0	0	10	0	5	3	0	9	3	2	2	0
N. cryptocephala Kützing	0	0	0	0	0	1	3	0	1	0	0	0	0	0	1
N. cryptotonella Lange-Bertalot	4	0	4	2	1	4	3	0	5	2	0	15	1	0	5
N. germainii Wallace	4	3	0	2	83	4	2	0	8	2	6	0	2	14	25
N. gregaria Donkin	8	0	1	3	14	7	2	4	1	3	2	5	0	8	9
N. heimansioides Lange-Bertalot	33	0	5	0	0	17	0	0	10	0	11	24	0	0	0
N. microcari Lange-Bertalot	18	1	29	0	1	78	1	39	18	0	54	10	6	3	2
N. rostellata Kützing	5	2	2	6	17	11	4	24	6	6	6	0	4	5	7
N. schroeteri Meister var. *schroeteri*	0	0	0	4	38	0	1	0	0	4	0	0	0	8	6
N. veneta Kützing	1	2	6	2	30	8	3	15	0	2	4	3	4	0	6
Nitzschia acicularis (Kützing) W.M.Smith	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0

N. acidoclinata Lange-Bertalot	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0
N. archibaldii Lange-Bertalot	0	0	0	2	3	0	5	1	2	2	0	1	0	3	1
N. aurariae Cholnoky	0	0	0	2	0	0	0	0	0	2	0	0	2	1	0
N. capitellata Hustedt in A.Schmidt *et al*.	1	0	3	1	5	0	3	1	12	1	4	2	3	2	5
N. dissipata (Kützing) Grunow var.*media* (Hantzsch)	0	0	0	0	7	0	0	2	0	0	0	0	0	0	0
N. filiformis (W.M.Smith) Van Heurck var. *filiformis*	1	0	0	2	1	0	2	0	0	2	0	0	0	1	0
N. frustulum (Kützing) Grunow var. *frustulum*	14	31	11	13	3	4	24	8	5	13	15	0	113	0	21
N. iremissa Cholnoky	0	3	0	0	0	0	0	1	2	0	1	0	0	3	1
N. lancettula O.Muller	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0
N. liebertruthii Rabenhorst var. *liebertruthii*	10	0	0	3	1	0	21	0	4	3	0	1	0	0	0
N. linearis (Agardh) W.M.Smith var. *linearis*	2	0	5	2	1	1	1	8	2	2	0	1	2	1	0
N. linearis (Agardh) W.M.Smith var*. subtilis* (Grunow) Hustedt	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0
N. palea (Kützing) W.Smith	60	0	7	12	14	1	82	8	25	12	0	1	1	5	7
N. sigma (Kützing) W.M.Smith	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1
Planothidium engelbrechtii (Cholnoky) Round & Bukhtiyarova	5	1	16	35	1	10	1	9	6	35	12	3	16	0	9
P. frequentissimum (Lange-Bertalot)	0	0	0	6	0	0	0	0	0	6	0	0	0	1	29
P. rostratum (Oestrup) Lange-Bertalot	6	11	31	126	18	40	17	34	16	126	17	14	27	15	29
Pleurosigma salinarum (Grunow) Cleve & Grunow	0	0	0	0	1	0	0	0	0	0	0	0	0	0	6

Pseudostaurosira brevistriata (Grunow in Van Heurck) Williams & Round	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Reimeria uniseriata Sala Guerrero & Ferrario	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1
Sellaphora pupula (Kützing) Mereschkowksy	0	1	3	4	1	2	1	0	0	4	0	0	2	0	2
S. seminulum (Grunow) D.G. Mann	0	1	0	1	3	1	1	0	0	1	0	2	1	0	26
Stauroneis anceps Ehrenberg	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Staurosira elliptica (Schumann) Williams & Round	0	0	0	0	0	0	0	6	0	0	0	2	0	0	0
Surirella angusta Kützing	0	0	0	0	6	0	1	0	1	0	0	0	0	0	0
Tabularia fasciculata (Agardh) Williams & Round	0	1	4	1	0	4	0	2	0	1	0	3	3	0	0
Tryblionella calida (Grunow in Cleve & Grunow) D.G. Mann	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1

Appendix 2-B: Count data for the diatom species sampled at the four historic sites during March 1983, and September 1985 along the Sabie River within KNP.

Diatom Species	March 1983		September 1985	
	Site6	Site8	Site7	Site9
Achnanthidium affine (Grunow) Czarnecki	2	0	0	2
A. crassum (Hustedt) Potapova & Ponader	0	0	0	1
A. exiguum (Grunow) Czarnecki	0	5	5	0
A. minutissimum (Kützing) Czarnecki	1	0	99	216
A. straubianum (Lange-Bertalot)	2	6	0	0
Amphora copulata (Kützing) Schoeman & Archibald	2	0	0	0
A. inariensis Krammer	0	6	0	0
Anorthoneis dulcis Hein	0	0	5	3
Caloneis bacillum (Grunow) Cleve	0	0	3	0
Capartogramma crucicula (Grunow) Ross	0	3	0	0
Cocconeis placentula Ehrenberg var. *placentula*	4	69	15	17
Craticula accomoda (Hustedt) Mann	1	0	2	0
Cymbella aspera (Ehrenberg) H.Peragallo	0	0	2	0
C. kappii (Cholnoky) Cholnoky	1	0	7	18
C. kolbei Hustedt	263	0	17	8
C. turgidula Grunow 1875 in A.Schmidt *et al*. var. *turgidula*	17	1	0	0
Encyonema minutum (Hilse) D.G. Mann	1	0	2	11

Eolimna minima (Grunow) Lange-Bertalot	1	5	0	0
E. subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	0	1	1	2
Fallacia insociabilis (Krasske) D.G. Mann	0	11	4	6
F. pygmaea (Kützing) Stickle & Mann ssp. *pygmaea* Lange-Bertalot	0	2	0	0
Fragilaria biceps (Kützing) Lange-Bertalot	0	0	3	0
F. capucina Desmazieres var. *lanceolata* Grunow	0	0	0	5
F. ulna (Nitzsch) Lange-Bertalot var. *acus* (Kützing) Lange-Bertalot	1	0	0	1
F. ungeriana Grunow	7	0	3	2
Gomphonema parvulum (Kützing) var. *parvulum f. parvulum*	13	10	2	11
G. venusta Passy. Kociolek & Lowe	1	3	77	11
Gyrosigma acuminatum (Kützing) Rabenhorst	0	0	2	0
Hippodonta capitata (Ehrenberg) Lange-Bert.Metzeltin & Witkowski	0	0	4	0
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann	0	3	0	0
Navicula capitatoradiata Germain	3	0	17	4
N. cryptocephala Kützing	0	0	3	0
N. cryptotonella Lange-Bertalot	0	0	6	5
N. germainii Wallace	0	0	2	0
N. gregaria Donkin	0	0	3	2
N. heimansioides Lange-Bertalot	0	0	48	4
N. rostellata Kützing	15	15	4	11
N. schroeteri Meister var. *schroeteri*	8	1	0	22

N. veneta Kützing	5	6	13	8
Nitzschia archibaldii Lange-Bertalot	2	0	0	0
N. capitellata Hustedt in A.Schmidt *et al*.	1	0	0	1
N. dissipata (Kützing) Grunow var. *media* (Hantzsch)	5	0	7	13
N. frustulum (Kützing) Grunow var. *frustulum*	7	8	0	1
N. iremissa Cholnoky	4	2	1	0
N. lancettula O.Muller	2	9	1	0
N. liebertruthii Rabenhorst var. *liebertruthii*	0	4	0	0
N. linearis (Agardh) W.M.Smith var. *linearis*	0	2	5	6
N. palea (Kützing) W.Smith	11	5	7	0
N. valdecostata Lange-Bertalot et Simonsen	5	1	3	0
Planothidium engelbrechtii (Cholnoky) Round & Bukhtiyarova	0	9	8	0
P. frequentissimum (Lange-Bertalot)	0	2	0	0
P. rostratum (Oestrup) Lange-Bertalot	3	105	16	5
Pseudostaurosira brevistriata (Grunow in Van Heurck) Williams & Round	0	5	0	0
Sellaphora pupula (Kützing) Mereschkowksy	1	8	0	0
S. seminulum (Grunow) D.G. Mann	3	6	1	0
Staurosira elliptica (Schumann) Williams & Round	2	56	0	0
S. pinnata Ehrenberg	6	30	2	0
Tabularia fasciculata (Agardh) Williams & Round	0	1	0	2
Tryblionella levidensis W.M. Smith	0	0	0	2