Using the South African Diatom Index (SADI) to determine the present ecological status of the Crocodile River, Kruger National Park

by

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Declaration

I declare that this thesis is my own work. It is being submitted for the Master of Science degree at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree for examination at any other university.

S. Thamae 11 August 2023
(Signature of candidate)

Abstract

The Crocodile River in the Mpumalanga Province of South Africa is a river of great economic significance, while providing support to the surrounding aquatic and riparian ecosystems through ecological processes of chemical, hydrological, and geomorphological nature. This river forms part of the Inkomati River Basin, which serves as a transboundary basin shared between the Republic of South Africa, Mozambique and Eswatini. The importance of the effective management of transboundary water resources, from an African perspective, cannot be stressed enough due to the water-scarce nature of the Southern African region, particularly South Africa. Incorporating Integrated Water Resources Management (IWRM) and Strategic Adaptive Management approaches into the governance of water resources can aid in the protection of both the quality and quantity of the country’s freshwater reserve. Good governance of water resources is essential in the conservation of aquatic and riparian ecosystem biodiversity, as well as meeting the basic human needs reserve, which is essential to meet people’s daily drinking, food preparation and personal hygiene requirements. The Crocodile River is not immune to pollution of anthropogenic origin, such as urbanisation, mining, agriculture, and industrial by-products. The above mentioned constitutes some of the direct and indirect results of large-scale stresses that are exerted on a river system, mainly owing to environmental factors such as landscape, demographic, atmospheric and hydrologic changes. A few practical examples of these factors include changing population dynamics and resultant land-use requirements, accompanied by compromised riparian vegetations arising from the altered land-use. All this necessitates the regular monitoring of the quality of water in this river system. The outcome of regular river monitoring is essential to the protection of this resource through regulation and policy. The use of physico-chemical parameters to determine the health of the Crocodile River has assisted in identifying compromised aquatic and riparian ecosystems and ultimately recommending relevant mitigation strategies necessary in maintaining an acceptable standard of water quality.

Incorporating biomonitoring techniques, wherein aquatic microorganisms are used to infer water quality, as a tool to assess the health of a river ecosystem has proven useful, mainly due to the sensitivity of periphyton assemblages (algae, cyanobacteria, diatoms) to changing river conditions, based on nutrients and physico-chemical parameters. The use of these organisms, in bio-assessments of aquatic ecosystems has been key to overall river health monitoring. This study highlights how diatoms, through their published ecological data, can contribute to the Resource Directed Measures method of determining the Present Ecological Status of a river, using the Crocodile (East) River as a case study.

The current study was developed to assess the ecological category of the Crocodile River, along the southern boundary of the Kruger National Park. Four sampling sites were identified for the study, from which water samples were collected during September 2019, October 2019, and March 2020 sampling sessions. The basis of this was to investigate the changes in diatom communities and dominant microphytobenthos (MPB) groups (based on the tolerance to fluctuating environmental conditions amongst the various species) in response to the spatio-temporal changes in the quality and quantity of water at the four sites throughout the study period. These results were then compared to past studies to determine if there has been a change in river health over the past decade. Physico-chemical variables were measured *in situ* using a YSI Professional Plus (Pro Plus) multi-parameter instrument, which included temperature, pH, electrical conductivity, and dissolved oxygen. The benthic microalgal biomass of cyanobacteria, green algae and diatoms was quantified from the fluorescent signatures of the groups *in situ* using a bbe BenthoTorch The bbe BenthoTorch is a hand-held apparatus that uses *in situ* quantification of chlorophyll-a fluorescence as an index of benthic algal biomass. The diatoms present in the samples were later prepared and isolated for microscopic identification and individual counts.

The Relative Abundance (RA%) of dominant diatom species and the ecological category of each sampling site was determined using OMNIDIA software based South African Diatom Index (SADI). Ecological categories using the SADI range from A (good quality) to E (bad quality). Data analyses include the use of ordination plots (CCA and PCA) to evaluate the response of the dominant diatom species to changing environmental variables and the interspecific relationships between the diatom species in each assemblage, based on their ecological requirements.

The study revealed that the ecological status of the Crocodile River when compared to previous studies had remained the same; C (moderate quality). This finding supports the use of the South African Diatom Index (SADI) in determining the Present Ecological State of the Crocodile River, in the Kruger National Park. There have been similar studies in other river systems within the Kruger National Park, wherein diatoms (specifically diatom-based index scores) were used to infer the water quality, at the time, in comparison to historic / benchmark water quality parameters. These studies were conducted in the Olifants, Letaba and the Sabie rivers of the park. The viability of these studies is motivated by benthic diatoms being particularly sensitive to changes in water quality, making them an ideal indicator of river health that is complementary to the current suite of biomonitoring tools. This method has immense potential in South Africa, provided that more focus is placed on diatoms and investment made

in capacitating researchers and diatom taxonomists with the skills to perpetuate this vast field of study.

Key words: Biomonitoring, diatom-based indices, microphytobenthos, physico-chemical variables, water quality indicator, conservation, biodiversity

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1. Introduction

Water resources management from a South African perspective

The African continent has been widely recognised for its wealth in natural resources (Moti, 2019). Several of these commodities are conserved through varying resource management and/or conservation efforts (Ochola *et al.*, 2010); usually realised by the implementation of various legislation and policies. One of the main resources with which Africa is greatly concerned, is water. South Africa has been identified as a water scarce country (De La Rey *et al.*, 2008; Ololade, 2018) and is still shrouded by human rights matters surrounding this resource. Issues such as the lack of equitable access to clean water and sanitation remains a sore point for some of the country's inhabitants (Ololade, 2018). The 2018 General Household Survey (GHS) revealed that only 89.0% of South African households had access to drinking water (Department of Statistics South Africa, 2018). Integrated Water Resources Management (IWRM), in line with the Sustainable Development Goals (SDG), has highlighted the importance of a global, multidisciplinary approach to the successful management of global water resources, and in so doing, has aimed to ensure the sustainable use of this resource to cater for future generations (Sadoff *et al*., 2020). It is, therefore, crucial to consider the need to govern this resource on a regional scale, thus encouraging transboundary water governance. This collaborative administration can enhance both regional integration and socio-economic development within the Southern African Development Community (SADC) (Jacobs and Nienaber, 2011), and could very well be one of the cornerstones to the effective management and conservation of water resources within the Southern African region.

Another integral part of water resources management is the protection of freshwater sources, from perspectives of both water quality and quantity. The conservation of freshwater should be widely promoted through sustainable use; such as a notable reduction in the unnecessary use of clean water (e.g. for washing cars and watering lawns), encouraging the use of greywater (where applicable), as well as the reuse of wastewater (mainly for non-drinking purposes) that has been treated through various technologies such as Reverse Osmosis (RO) (Adewumi *et al*., 2010). To aid in the regulation of this asset, careful consideration needs to be made in the fair allocation of water to meet the ecological and basic human needs reserves for the country. Dalu and Froneman (2016) emphasised the importance of regular monitoring to ensure the health of river systems and the aquatic ecosystems occurring therein. The importance of regular monitoring in Africa is paramount, as the results can aid in strategic adaptive management, helping to mitigate impacts associated with economic development (Rogers and Luton, 2011; Kingsford and Biggs, 2012; Dalu and Froneman, 2016).

Translation of South Africa's water legislation

South Africa's widely acclaimed National Water Act (Act No.36 of 1998) is a vital tool to the issuance of water for the ecological reserve and the basic human needs reserve (De la Harpe and Ramsden, 1998); the combined water is termed the freshwater Reserve. The difference in these water uses lies in that the former refers to the water that will remain to serve the aquatic ecosystems of the country, when the portion allocated for use in households (for basic human needs) has been met (Bourblanc, 2015). In determining the Reserve, the South African government need to take into account the transboundary obligations of some rivers, such as the Crocodile River which ultimately supplies water to Mozambique, so as to better manage the resource remaining within the catchment; through water-use registrations and licensing (Rogers and Luton, 2011). The Department of Water and Sanitation (DWS), as the custodians of the country's freshwater resource, is mandated to ensure that sustainability remains at the forefront of the country's water resources management strategies. Ensuring that sufficient water is available to meet the needs of current and future users involves a series of steps, one of which is classification. Classification enables government to mandate the necessary level of protection to a water resource, which will ultimately inform the management of that resource. This is then followed by processes which include determination of resource quality objectives (usually setting the required standards around the state of the water) and then finally, the determination of Reserve, which brings about allocation of water for various ecological and human uses (De la Harpe and Ramsden, 1998). The decentralisation of the administration of water to the various Catchment Management Agencies (CMAs) has supported an inclusive approach to the management of this resource, thus prioritising the needs of all affected users, current and prospective alike (De la Harpe and Ramsden, 1998; King and Pienaar, 2011).

The Inkomati-Usuthu Catchment Management Agency (IUCMA) is one of nine of the recently proclaimed Water Management Areas (WMAs) in the country. It is one of only two established Catchment Management Agencies (CMAs), alongside the Breede-Gouritz. In addition to supporting meaningful water-orientated stakeholder relations, these structures have been intrinsic to water resources protection by establishing and overseeing the Resources Directed Measures (RDM), prescribed to rivers of major ecological importance (Meissner *et al.*, 2017). In addition to this, the CMAs, or DWS in instances where no CMA has been developed, have a responsibility to encourage meaningful collaborations around the use and overall conservation of the water resources in their designated Water Management Area (King and Pienaar, 2011). One of the rivers of considerable socio-economic concern in the IUCMA is the Crocodile River, which is located in the Mpumalanga Province of South Africa, and occurs in the Crocodile River Catchment (CRC) (Retief, 2014). The extensive size and vastness of this catchment area – approximately 10 450 km^2 (Soko and Gyedu-Ababio, 2015) - renders it highly susceptible to pollution by anthropogenic activities. Contamination from extensive mining activity, rapid urbanisation and upstream farming ventures contribute greatly to the deterioration in the water quality of this river (Ashton *et al.*, 1995; Palmer *et al.*, 2013; Roux and Selepe, 2013; Riddell *et al.*, 2019), and in turn, has a direct impact on the biodiversity and overall ecological function of this system (Kleynhans, 2007).

Crocodile River Catchment water quality predicament

Due to the high ecological importance of the Crocodile River (Soko and Gyedu-Ababio, 2015) and associated tributaries, the routine monitoring and subsequent data analysis has been essential in the determination of its Resource Quality Objectives (RQO). These targets are necessary to improve the ecological status of the river through studying its Present Ecological State (PES) and identifying management and/or rehabilitation programs for either the upkeep or the improvement of this system (Harding and Taylor, 2011; Malan and Day, 2012; Palmer *et al.*, 2013). While more conventional models and indices such as the Fish Response Assessment Index (FRAI) and the Macroinvertebrate Response Assessment Index (MIRAI), to name a few, have been preferred in making a conclusion on the ecological status of a river (Kleynhans, 2007), there is an alternative method with great potential, available for the effective spatial and temporal assessment of the quality of water in a river. This biomonitoring option is gaining popularity (Dalu and Froneman, 2016) and includes the study of diatom assemblages and how they are affected by the changes in the quality of water in a river (Shikwambana *et al.*, 2021). The technique can be employed alongside the use of typical physico-chemical water quality parameters, and offers a complementary bio-indicator index to the more established macroinvertebrate-based SASS5 index, which is already acclaimed as a definitive indicator of the health of a stream or river system, over time (Riddell *et al.*, 2019). Although affordable and reliable in inferring water quality (Harding and Taylor, 2011), this method remains largely unexplored in South Africa, due to the potential complexities around the accurate taxonomic verification of diatom groups, the lack of understanding of their ecology (Dalu and Froneman, 2016) as well as its time-consuming nature (Harding and Taylor, 2011). The prevalence of these organisms can aid in the understanding of the quality of a body of water at a specific region during a certain time. This deduction is attributed to their sensitivity and resultant response to the changes in quality of the associated system (Harding and Taylor, 2011; Snow, 2016), making the microphytobenthos (MPB) effective indicators of the wellbeing of an ecosystem (Nunes *et al.*, 2019). The essence of this study lies in the development and mainstreaming of effective, reliable, time-efficient water quality monitoring tools to aid in higher level management of water resources in both South Africa, and beyond.

Diatoms

In addition to using water quality assessment techniques that rely on water chemistry studies, biomonitoring, wherein aquatic microorganisms are used to infer water quality, has proven to be a reliable assessment of river ecosystem health (Dalu and Froneman, 2016). Due to the sensitivity of periphyton assemblages (algae, cyanobacteria, diatoms) to changing river conditions, based on nutrients and physico-chemical parameters, these organisms have become an invaluable asset to the bio-assessment of aquatic ecosystems and subsequently, key to river health monitoring (Weilhoefer and Pan, 2006; Dalu and Froneman, 2016; Thacker and Karthick, 2022).

Bacillariophyceae, commonly known as diatoms, are easily distinguished from other algae on the basis of the species-specific biogenic silica making up the cell walls of the organisms, their oil-chrysolaminarin storage faculties and the extraordinary pigments used in their photosynthesis process (Taylor *et al*., 2007b). Diatoms have proven to be more dependable in inferring water quality in relation to other micro-organisms used in biomonitoring. This is because their presence is irrespective of seasons and flow conditions. The published literature detailing their ecological preferences provides an understanding of the general water quality in the areas where varying species are found in abundance (Shikwambana *et al.*, 2021). They contribute greatly to the food chain, by serving as a food source for several aquatic microorganisms (Taylor *et al.*, 2007b). Diatoms can be either benthic in nature, belonging mainly to the pennate species, or occur predominantly in the water column of freshwater systems, as part of the centric diatom species (Taylor *et al.*, 2007b).

The DWS initially acknowledged the desire to use these organisms (in conjunction with historical water quality data) to infer water quality and river health; although not formally adopted as a component of the River Health Programme (RHP) (Harding *et al.*, 2005; Taylor *et al.*, 2007a; Taylor *et al*., 2007c; Harding and Taylor, 2014). The River Health Programme was initiated in 1994 as a part of the South African National Aquatic Ecosystem Biomonitoring Programme (NAEBP) and was rolled out in several phases leading up to its implementation. The programme was oriented around the health of riverine ecosystems, and for that reason, got known as the River Health Programme (RHP). Several other monitoring programmes coexist with the RHP, which prompted the all-inclusive title: “National Aquatic Ecosystem Health Monitoring Programme (NAEHMP)”, rightfully used when referring to all biomonitoring programmes, beyond riverine ecosystems (Strydom *et al.*, 2006). Table 1 below outlines the timeline leading to the implementation of the programme.

Table 1: Stages of the establishment of the River Health Programme (1994-2004). (Source: Strydom *et al*., 2006)

Year	**RHP Phase**
1994	Framework Design Phase
1995	Conceptual Design Phase
1996	Pilot Implementation Phase (Crocodile River, Mpumalanga)
2000	Anchoring phase (Few other provinces)
2004	National Coverage Phase

The use of diatoms is through the South African Diatom Index (SADI), which is a modification of the Specific Pollution-sensitivity Index (SPI). The reliability of the SPI is, in comparison with other indices, supported by its inclusion of many more taxa of varying characteristics, thus yielding a higher resolution. Several South African endemic species have been added to the existing SPI repository, to establish the SADI as we know it (Harding and Taylor, 2011). According to Dalu and Froneman (2016), this method has several shortcomings, pertaining mainly to the application of 'diatom-based monitoring' in ecological studies. The limitations are suspected to have arisen from the lack of training of diatom taxonomists, thus resulting in low capacity, as well as existing taxonomic specialists opting to restrict themselves to the identification and naming of species without further venturing into the ecological classification of these species (Dalu and Froneman, 2016). Several studies have proven the efficacy of diatoms as indicators of water quality based on their response to changing environmental variables. One such study suggests the potential of diatom studies in accurately inferring river health and better understanding the variability of diatom assemblages and their associated ecology (Holmes and Taylor, 2015). The preliminary result of an alternative, ongoing study around the response of diatom communities to anthropogenic activity in shallow water bodies, has qualified these microorganisms to be reliable as a component of existing freshwater biomonitoring tools (Phiri *et al.*, 2007). The evidence of this is however dependent on further, extensive research on the topic (Phiri *et al.*, 2007). The same study conducted by Phiri *et al*. (2007) portrays the stark differences in pollution tolerance and sensitivity to anthropogenic disruption between the various genera of diatoms. They have suggested that samples obtained from outlying areas with reduced human interference yielded larger diatom abundance and proportionally higher diversity and community evenness (Phiri *et al.*, 2007). The reproduction of diatoms occurs over an incredibly short time, making their response to the changes in environmental conditions an adequate indicator of the current state of a system (Harding and Taylor, 2011). The outcome of related diatom research is likely to contribute positively to the management of rivers within National Parks, such as the Kruger National Park. Integrated Water Resource Management (IWRM) principles are central to the decisionmaking processes of the Inkomati-Usuthu Catchment; and includes the adoption of the Strategic Adaptive Management approach to manage freshwater resources that form part of the catchment (King and Pienaar, 2011).

1.1. Project aim

The aim of this study is to determine the ecological category of the Crocodile (East) River, along Kruger National Park's southern boundary, using the South African Diatom Index.

1.1.1. *Significance of research*

This study will make use of physico-chemical water quality parameters, in conjunction with the identification and evaluation of benthic diatom assemblages and chlorophyll *a* biomass, occurring at three sampling sites along the Crocodile River and one site in the Nsikazi River, a tributary of the Crocodile River, within the Kruger National Park (KNP). Riddell *et al.* (2019) indicated that diatom analysis, as described by Taylor *et al*. (2005), is already in use in the KNP. The information gained from the proposed study should, in addition to the work done by Shikwambana *et al.* (2021), therefore further highlight the importance of diatoms as a socio-technical tool in the River Eco-status Monitoring Programme (REMP), and contribute to KNPs strategic adaptive management and sustainable use of the Park's freshwater resources.

1.1.2. Objectives

The objectives for this study include:

1 Measuring *in situ* benthic chlorophyll *a* biomass using a bbe (biological, biophysical, engineering) BenthoTorch on submerged rock surfaces at all sites and using the fluorescent pigment signatures from the bbe BenthoTorch measurements to determine the relative abundances of the three dominant benthic microalgal assemblages (diatoms, chlorophytes (green algae) and cyanobacteria).

2 Determining spatial changes in physico-chemical water quality parameters (temperature, pH, electrical conductivity, dissolved oxygen), in response to wet and dry seasons; compared to historical/ benchmark values, to understand the Present Ecological State of the Crocodile River.

3 Determining the strength of association between spatio-temporal changes of diatom assemblages to physico-chemical water quality parameters.

4 Using benthic diatom-based indices (South African Diatom Index, in particular) in relation to the prevalent diatom species; to determine the ecological category of the Crocodile River.

1.1.3. Predictions

1. It can be anticipated that pH and Electrical Conductivity (EC) have significant influences on the variety of diatom taxa occurring in each site (Schneider *et al.*, 2013).
2. Dissolved Oxygen (DO) concentrations are expected to decrease during months where increased temperature and rainfall are recorded. This can be attributed to the rise in phytoplankton colonies (particularly cyanobacteria), which thrive in conditions with increased temperature and nutrient overload primarily derived from the return flows of irrigated agriculture (Safieh *et al.*, 2020).

1.2. Study area

The Crocodile River catchment consists of several major tributaries to the Crocodile River, which include the Elands, Kaap and Nel rivers. The annual rainfall within the catchment varies on the basis of the landscape, with the mountainous region yielding approximately 1 200 mm, and the lowveld receiving only about 600 mm. The overall average rainfall for the catchment has been determined at 880 mm (Deksissa *et al*., 2003). The Crocodile River, in particular, forms the southern border of the Kruger National Park, where it eventually joins the Inkomati River, close to Komatipoort, and later discharges into the Indian Ocean at Maputo, Mozambique (Strydom *et al.*, 2006). The by-products of human activity and commercial processes such as urbanisation, industry, afforestation, and the generation of power can influence the quality of the water differently at various points of the river (Soko and Gyedu-Ababio, 2015). Climate variability in the form of unpredictable rainfall and changing land use resulting from mining and/or agriculture also contribute to declining water quality (Samuel Che *et al.*, 2022), resulting in alterations to the natural flow conditions of the river (Deksissa *et al*., 2003). This ultimately influences the health of the river. The abstraction of water upstream due to heavy industrial and agricultural demands may further exacerbate declining water quality conditions (Sahula, 2015), which will ultimately have an impact on downstream water uses in the way of low-flowing, contaminated water.

The River Health Programme (RHP), which has become the River Eco-status Monitoring Programme (REMP), has contributed greatly to the management of water through providing data through the regular monitoring at designated sites. The result of an assessment conducted in 1996, around the health of the Crocodile River, revealed that the river ecosystems of this river were in a good to moderate condition. The water quality, as determined by the general health of the fish in the system, was good (Strydom *et al.*, 2006), whereas the health of the riparian vegetation in the ecoregions associated with the current study (Ecoregions 5.06, 5.07 and 6.01) continued to decline over time, with that of the southern banks in a fair to poor condition, by 1999 (WRC, 2001). It is for this reason that it remains imperative to have ways in which water quality can be monitored, to ensure the equitable management of the resource, and careful determination of reserves.

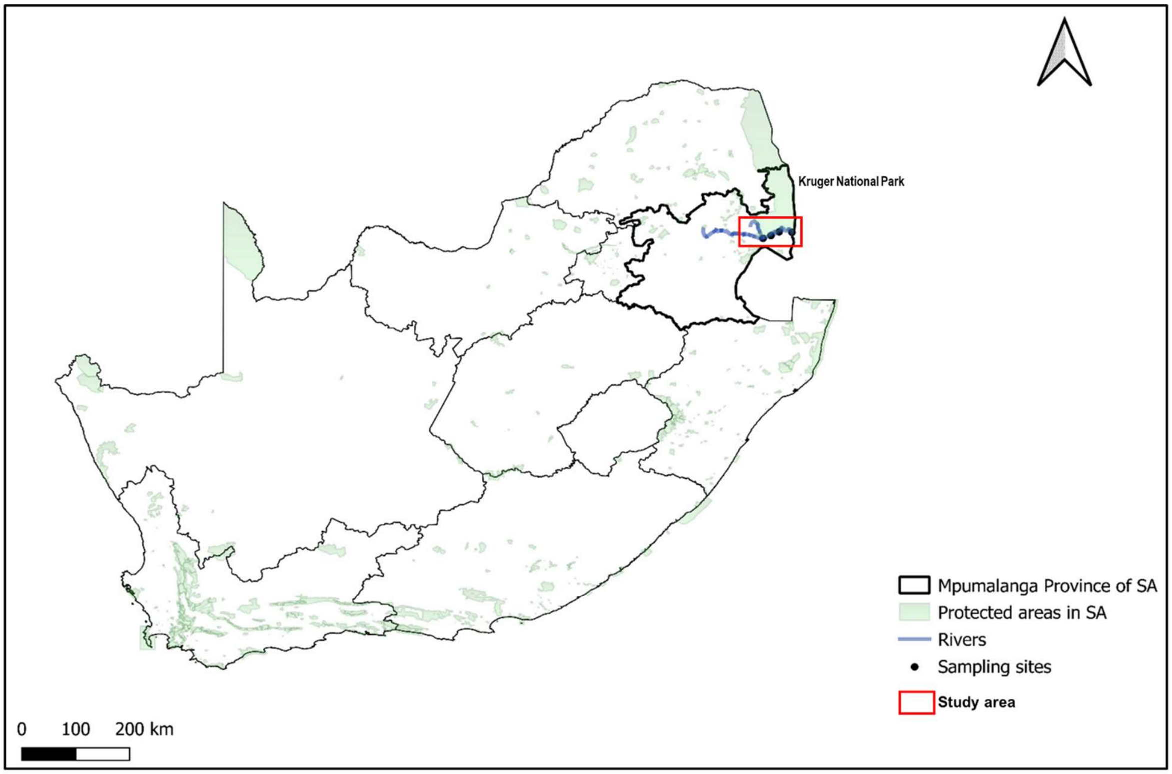
The sampling for the current study was carried out at Malelane high water bridge, Nsikazi, Marula and Nkongama, which are all points along the Crocodile River within the Kruger National Park (KNP) (Table 2).

The landscapes and vegetation associated with the sampling sites varies from mixed woodlands and thorn thickets (46%), to mountain bushveld in some areas (*Krugerpark Ecosystem - Kruger Park Wildlife and Vegetation*, 2022), as well as approximately 8.8% of grasslands (Roux and Selepe, 2013). Months with warmer weather (September to May), are characterised by higher humidity levels, while the remaining months (June – August) are predominantly cold and dry (Venter and Gertenbach, 1986).

Table 2: Study site coordinates (sample Site 1 to Site 4)

No.	Site location	GPS coordinates
1	Nsikazi	25°31'19.9"S 31°22'06.6"E
2	Malelane high water bridge	25°27'38.9"S 31°32'04.9"E
3	Marula	25°22'47.6"S 31°42'20.9"E
4	Nkongama	25°23'28.3"S 31°58'34.7"E

The map below, Figure 1, illustrates the various sampling sites within the Mpumalanga Province.



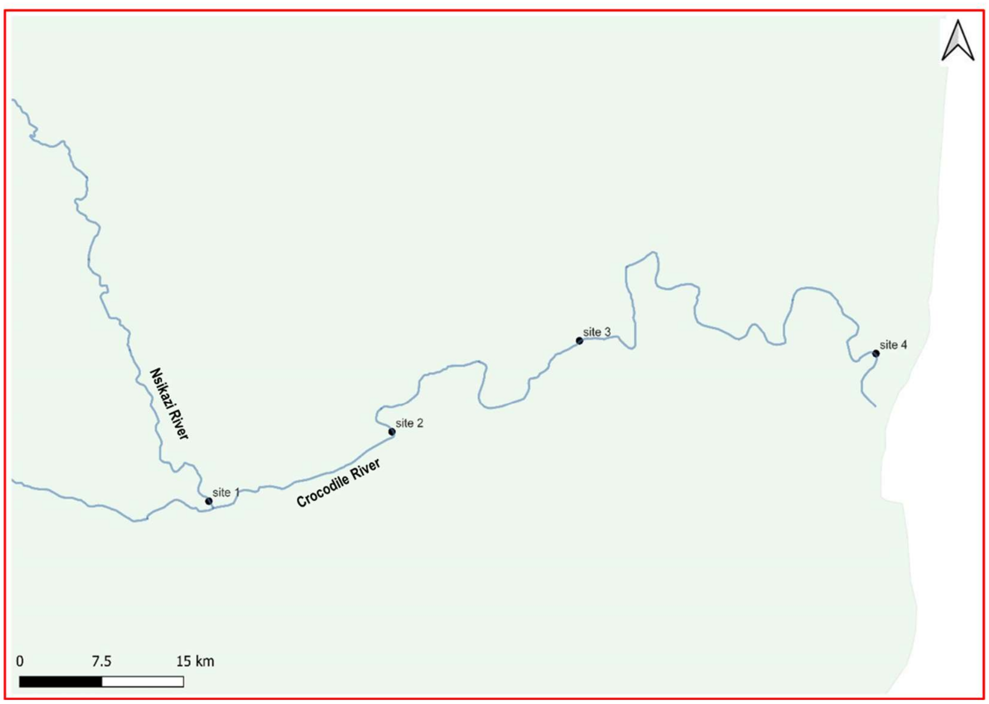


Figure 1: Map of South Africa depicting sample collection sites along the Crocodile River within the Kruger National Park (KNP). Note that Site 1 was located in a small tributary of the Crocodile River. Study area provided as a magnified view depicting sample collection sites in relation to the Crocodile River and Nsikazi River

The main sources of pollution into the Crocodile River at sites 1 and 2 are through tributaries and streams containing wastewater effluent and nutrient-rich return flows from irrigated agricultural activities occurring in the vicinity of Malelane and Matsulu, along the southern banks of the river. The presence of sugarcane farming, citrus orchards, mining and residential settlements constitute a great risk to the quality of the water in this part of the river, with high levels of organic and thermal pollution as a result (Roux and Selepe, 2013). There is slightly better control of the pollution within the Kruger National Park as it is a protected area with a greater focus on conservation (Riddell *et al.*, 2019), although wastewater associated with industrial and household activities extending to the Gauteng Province remains a great threat of pollution to this river (Majdi *et al.*, 2022). The parts of the river that occur within the Kruger National Park thus remains largely natural and relatively unmodified (Louw, 2014). This is despite enduring all anthropogenic, upstream impacts (Riddell *et al.*, 2019).

Sampling was conducted in September 2019, October 2019, and March 2020, to realize the effects of the changing seasons on the water chemistry for the predetermined parameters.

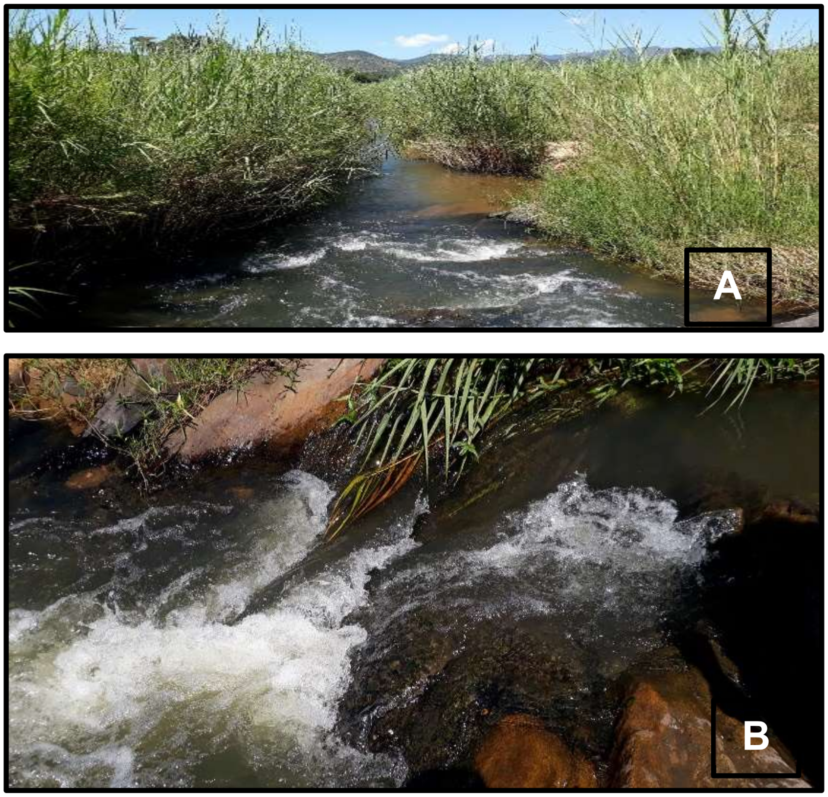


Figure 2: Site 1 – Nsikazi; depiction of sampling location (A = downstream view and B = sampled rock surfaces)

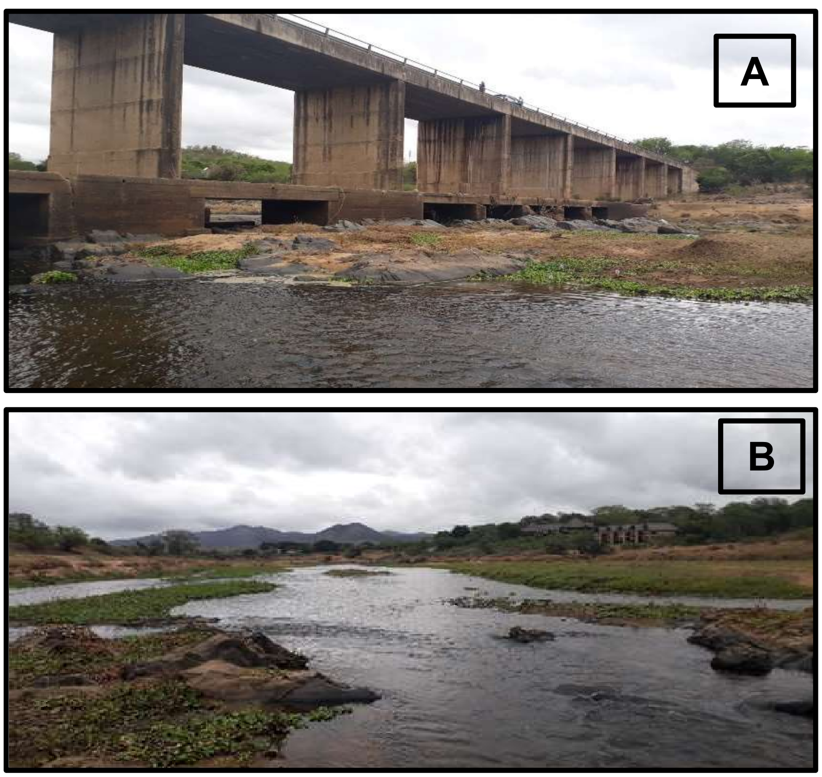


Figure 3: Site 2 - Malelane high water bridge; depiction of sampling location (A = location of site just upstream of the bridge and B = upstream view)

Figure 4: Site 3 – Marula; depiction of sampling location (A = downstream view and B = upstream view)

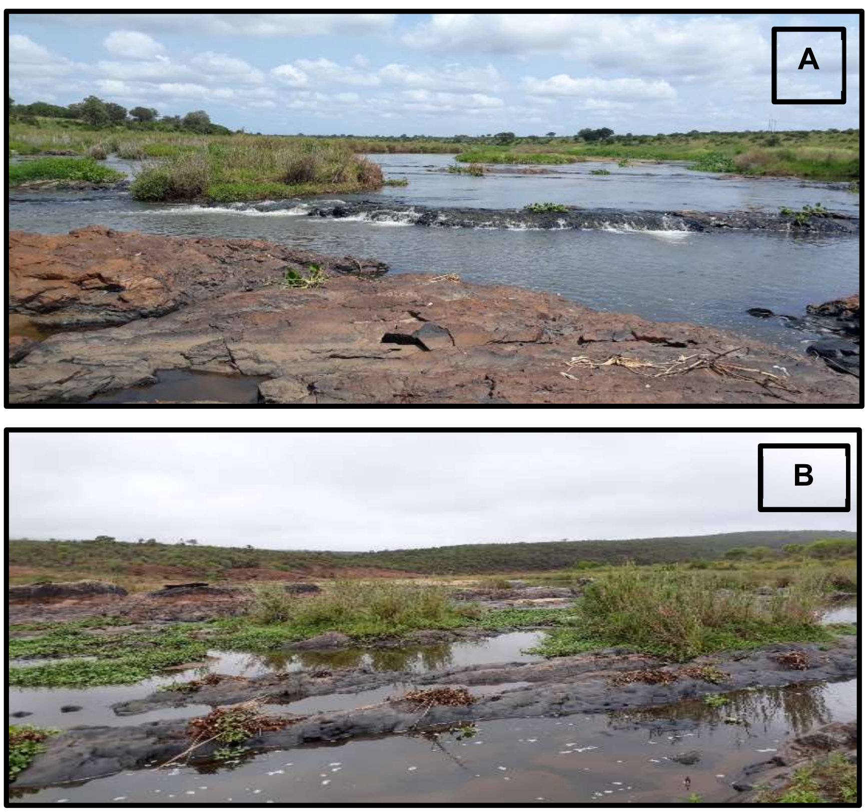


Figure 5: Site 4 – Nkongama; depiction of sampling location (A = upstream view and B = downstream view)

2. Materials and methods

2.1 Field sample and data collection

Water quality parameters were measured *in situ* and samples collected for laboratory analyses at several sites along the Crocodile River during three sampling sessions (September and October 2019, and March 2020). This was to identify changes in the physico-chemical conditions and biological communities along the aquatic ecosystem gradient presenting throughout the river (Weilhoefer and Pan, 2006; Dalu and Froneman, 2016).

2.1.1 Water *chemistry*

A YSI Professional Plus (Pro Plus) multi-parameter instrument was used to measure *in situ* water temperature, pH, Dissolved Oxygen (DO), and Electrical Conductivity (EC). These readings were taken at 30 second intervals at each site while other samples were being collected during the three sampling sessions. The minimum number of 30-second replicates was 29.

2.1.2 Chlorophyll a biomass

Benthic microalgal biomass was measured *in situ* using a hand-held bbe BenthoTorch apparatus. Chlorophyll fluorescence readings were taken from the surfaces of ten submerged rocks at each site of varying sizes to establish the average biomass of each distinct group (diatoms, chlorophytes, and cyanobacteria). This method represents a non-destructive technique used to measure chlorophyll-a, the main algal response variable used in aquatic ecosystem health assessments and used as an index of biomass (Maclulich, 1986).

2.1.3 Diatom community structure

Taylor *et al*. (2005) emphasised the importance of the standardisation of the methods involved in the collection, preparation, and the storage of diatoms to ensure results that yield accurate data, which can be used for reference in relevant, future studies. Diatom community structure is greatly influenced by physico-chemical variables such as EC, DO, and pH (Thacker and Karthick, 2022). Shibabaw *et al*. (2021) found that pH had an effect on related water quality parameters, thus posing an indirect influence on the growth of diatom communities, additionally, it can directly result in stress to these organisms, and in so doing impact the succession thereof (Shibabaw *et al.*, 2021).

2.1.4 *Diatom collection*

Representative biomaterial collection included processes wherein a toothbrush was used to sample epithilic diatoms by scraping them off several rock surfaces that had been formerly assessed with the bbe BenthoTorch. This material was collected into a plastic tray. These collection sites were identified as brown and/or green film presenting on submerged surfaces (Macintyre *et al.*, 1996). Due to the inability to work on the samples immediately, they were stored in clear 50 ml plastic conical centrifuge tubes containing ethanol to a final concentration of approximately 70% and then refrigerated, until they could be further prepared for microscopic analysis.

2.2 Laboratory methods

2.2.1 *Diatom preparation*

Diatom preparation was guided by the processes outlined by Taylor *et al*. (2005). The preferred method for the preparation of the diatom samples was the hot hydrochloric acid (HCl) and potassium permanganate ($KMnO_4$) method. From the refrigerator, the samples were allowed to sediment at room temperature for approximately 24 hours, following which, the excess liquid was carefully removed. The diatom material was left behind in the tube. This marks the beginning of the process to oxidise the organic material from the cell in order to successfully identify the distinguishing features of each diatom frustule through microscopy techniques (Taylor *et al.*, 2005). The diatom material was resuspended by agitation, followed by the removal of 1 millilitre (ml) of the diatom mixture into a test tube. 1 ml of saturated $KMnO_4$ was subsequently added to the diatom solution. In a laminar flow cabinet, one millilitre of concentrated (32%) HCl was added to the diatom solution, and this was heated to 80ºC for one hour, until the sample solution was clear. Once cooled, the presumably clean diatom material was re-suspended using distilled water from a wash bottle. The solution was centrifuged at 3000 rpm for five minutes and the supernatant was discarded. The remaining pellet was re-suspended, and the process was repeated five times to ensure that the supernatant has been rid of excess chemicals used in the oxidising process. This will aid in the subsequent long-term preservation of the diatom frustules. The resultant diatom material was stored in a dark environment in 1.5 ml Eppendorf micro-centrifuge tubes for further analysis. Ethanol of a final concentration of 20% was used as a preservative for the diatom material to deter the microbial contamination of the samples.

2.2.2 Slide preparation

Following thorough agitation, an aliquot of the previously prepared diatom solution was removed from the storage tubes and placed on adequately cleaned coverslips, diluted with distilled water and allowed to dry, undisturbed, in a relatively dust-free environment. This was achieved over a 24-hour period. A hot plate was used to heat a microscope slide containing a drop of Pleurax (Refractive Index = 1.7), for approximately 5 minutes, at 180ºC. The coverslips with the diatom frustules were placed on glass microscope slides until the Pleurax began to bubble off. Once adequately cooled, the slides were labelled accordingly, with all the information relevant to the sample. The microscope slides were thereafter viewed under 1000x magnification using a light microscope and the diatom units counted and noted until an approximate value with a minimum of 300 valves had been reached (Taylor *et al*., 2005). The Relative Abundances (RA) of all diatom species were determined from the captured data and diatom index scores determined using OMNIDIA software. Although various literature was used in the identification of the diatom cells, "An illustrated guide to some common diatom species from South Africa" by JC Taylor, WR Harding and CGM Archibald was the most widely used reference throughout this study.

2.3 Data analysis

A major component of the statistical analysis included visualising the general water chemistry variables (EC, pH, DO) using histograms to compare the range of the variables along the river, at the described sampling site during the respective sampling periods. The average values of the physico-chemical properties were determined using open source Jamovi software; The Jamovi project (2022). *jamovi* (Version 2.3) [Computer Software]. (Retrieved from https://www.jamovi.org).

Multivariate data analysis through CANOCO 4.5 software assisted in generating Canonical Correspondence Analysis (CCA) plots for studying the prevalence and distribution of diatom species, as demonstrated through the RA (%), in response to changes in the chemical water quality parameters. The response of various diatom species assemblages to their immediate environmental conditions (water chemistry, pollution, etc.) was thus deduced. The CCA assisted in explaining environmental preferences of each taxa in the form of an ordination plot (ter Braak and Verdonschot, 1995). Ter Braak and Verdonschot (1995), further explains the interpretation of a CCA diagram; that the length of the arrows assocated with the environmental variables can infer the importance of the variable, however, more accurately, it simply indicates the "maximum rate of change of the variable". This then informs us that environmental variables with longer arrows vary more greatly than those with shorter arrows, across the plot (ter Braak and Verdonschot, 1995).

Furthermore, a Principal Component Analysis (PCA) plot based on the environmental input data was used to indicate the correlation between the various species within the diatom assemblages, relative to the differences in the water quality variables at the various sites over the entire sampling period (Della Bella *et al*., 2007).

3. Results and discussion

3.1 Dominant diatom species

A total of 101 diatom species, from 32 genera, were identified from all four sampling sites over the three-month period. The three genera with the most species recorded across all the sites, throughout the sampling period were *Navicula* (17), *Nitzschia* (14) and *Gomphonema* with seven species, although only species with a Relative Abundance (RA) greater than 5% in each community were included in the study, and considered to be dominant (Weilhoefer and Pan, 2006; Taylor *et al*., 2007a). The most dominant species from the three aforementioned genera would thus suggest changes to the quality of the water in the various sampling sites during each sampling period (Shikwambana *et al.*, 2021).

Most of the dominant species encountered across the various sites were cosmopolitan species, meaning that they have a global distribution, however, a few endemic species were found, such as *Gomphonema venusta* Passy. Kociolek & Lowe, which is typically present in oligo-mesotrophic waters, with a distribution spanning the central and northern parts of South Africa (Taylor *et al.*, 2007b) . Taylor *et al*. (2007a) found supporting evidence that most of the dominant diatom species, such as those encountered in this study, which occur in South African rivers had already been recorded in international literature (Taylor *et al.*, 2007a). The results of the current study will indicate that higher relative abundances were calculated for cosmopolitan species with an elevated tolerance for polluted environments (Bere *et al*., 2014).

Table 3: Dominant species encountered during the study period: ecology information adapted from: (Taylor *et al*., 2007b; Levkov, Caput Mihalić and Luc Ector, 2010)

Code	Species	Preferred ecology	RA (%)	Site	Date
CPED	*Cocconeis pediculus* Ehrenberg	Meso-eutrophic	11.8	S2	Sep 2019
CPLA	*Cocconeis placentula* Ehrenberg var. *placentula*	Meso-eutrophic	20.68	S1	Sep 2019
CPLE	*Cocconeis placentula* Ehrenberg var.*euglypta* (Ehr.) Grunow	Meso-eutrophic	59.11	S4	Sep 2019
DKUE	*Denticula kuetzingii* Grunow var.*kuetzingii*	Electrolyte-rich	8.64	S3	Oct 2019
ESBM	*Eolimna subminuscula* (Manguin*)* Moser Lange-Bertalot & Metzeltin	Eutrophic	11.40	S3	Mar 2020
FULN	*Fragilaria ulna* (Nitzsch.) Lange-Bertalot var. *ulna*	Meso-eutrophic	12.46	S1	Mar 2020
GPRI	*Gomphonema pumilum var. rigidum* Reichardt & Lange-Bertalot	Meso-eutrophic	14.58	S2	Mar 2020
GVNU	*Gomphonema venusta* Passy. Kociolek & Lowe	Oligo-mesotrophic	14.97	S2	Oct 2019
MVAR	*Melosira varians* Agardh	Eutrophic	7.48	S1	Oct 2019
NDCI	*Navicula dulcis* Patrick	Eutrophic	11.85	S1	Mar 2020
NETO	*Nitzschia etoshensis* Cholnoky	Electrolyte-rich	6.69	S1	Mar 2020
NPAL	*Nitzschia palea* (Kützing) W.Smith	Eutrophic	5.54	S3	Mar 2020
NPRP	*Nitzschia perspicua* Cholnoky	Saline	7.29	S1	Mar 2020
NSHR	*Navicula schroeteri Meister var. schroeteri*	Eutrophic	6.64	S3	Oct 2019
PLEN	*Planothidium engelbrechtii* (Choln.) Round & Bukhtiyarova	Eutrophic	22.64	S4	Mar 2020
PTRO	*Planothidium rostratum (Oestrup) Round & Bukhtiyarova*	Alkaline	8.81	S4	Mar 2020
RABB	*Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot	Meso-eutrophic	40.51	S1	Sep 2019

3.2 Diatom indices

There are various indices that can be used to deduce ecological water quality classes; including the Specific Pollution Index (SPI) which directly refers to the water quality, and alternatively, the United Kingdom-based Trophic Diatom Index (TDI), which indicates the trophic condition of the freshwater system (Eloranta and Soininen, 2002; Taylor *et al.*, 2007a) as described in Table 4 below. The relevance of the TDI in inferring water quality is through describing how diatoms react to pollution - particularly inorganic pollution (Schneider *et al.*, 2013).

For this study, the SPI , Biological Diatom Index (BDI), TDI and percent Pollution Tolerant Valves (%PTV) values were derived from OMNIDIA software (Lecointe *et al.*, 1993). A maximum value of 20 with the SPI and BDI is indicative of water of superior quality (Taylor *et al.*, 2007a). The %PTV value is a constituent of the TDI and is an indication of the level of organic pollution in a system. A %PTV value of 20 or greater (of 100%) suggests a high level of organic pollution in the associated system (Holmes and Taylor, 2015).

Table 4: Diatom index scores and water quality classification (based on SPI) for the four Crocodile River site over the study period

Sampling session	Site	Sample number	BDI	% PTV	TDI	SPI	Water quality
Sep. 2019	1	S1	13.7	6.8	6.7	12.9	Good
	2	S2	14.0	2.1	9.6	13.5	Good
	3	S3	13.5	2.7	9.5	12.2	Moderate
	4	S4	13.4	9.3	8.9	12.0	Moderate
Oct. 2019	1	S5	12.9	6.9	9.2	12.2	Moderate
	2	S6	11.8	15.0	7.0	10.3	Moderate
	3	S7	12.1	17.6	8.7	10.9	Moderate
	4	S8	12.5	9.0	7.6	11.2	Moderate
Mar. 2020	1	S9	11.6	13.1	8.3	10.3	Moderate
	2	S10	10.7	18.8	7.5	9.9	Moderate
	3	S11	10.1	28.7	6.8	9.2	Moderate
	4	S12	10.9	13.2	5.5	9.5	Moderate
Average			12.27	11.93	7.94	11.18	Moderate

Only one of the 12 samples (representing March 2020) displayed a %PTV over 20% thus suggesting that very few of the species encountered in this study were tolerant to elevated levels of organic pollution, as revealed by the relevant index score. This result is consistent with Holmes and Taylor's (2015) observation; that an increased percentage pollution-tolerant value is mostly accompanied by a low SPI score (Holmes and Taylor, 2015).

Table 5: Specific Pollution-sensitivity Index (IPS) and water quality inference. (Harding and Taylor, 2011)

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

Table 6: Trophic Diatom Index (TDI) translated to trophic state of a river. Adapted from Kelly (1998)

Trophic state	TDI
Oligotrophic	<7
Oligo-mesotrophic	7-10
Mesotrophic	10-13
Meso-eutrophic	13-16
Eutrophic	>16

3.3 CCA – Canonical Correspondence Analysis

The Canonical Correspondence Analysis (CCA) is a multivariate technique that provides visual information about how species interact with their environment, in response to a set of variables. This is particularly useful when trying to understand how a diverse number of species with differing ecological requirements respond to environmental factors (for example, water chemistry parameters) and pollution (ter Braak and Verdonschot, 1995). This technique, alongside information from existing water quality monitoring tools can contribute to developing water resources management strategies.

For this study, a reduced dataset of 17 dominant species was employed in the CCA. This analysis was only applicable to the species with RA ≥ 5%, occurring throughout the study area in the allotted period (September 2019, October 2019, and March 2020), which includes *Achnanthidium exiguum* (Grunow) Czarnecki, *Cocconeis engelbrechtii* Cholnoky, *Gomphonema venusta* Passy. Kociolek & Lowe, *Navicula cryptocephala* Kützing, *Planothidium rostratum* (Oestrup) Round & Bukhtiyarova and *Pseudostaurosira brevistriata* (Grun. in Van Heurck) Williams & Round. The acronyms applicable to all the species displayed in the CCA, can be found in Table 3. Sample 1 is isolated on account of extremely low temperature encountered in Site 1 during September 2019 sampling period. The species occurring in quadrants 3 and 4 of the CCA plot have affinities for low EC and moderate to high water temperatures.

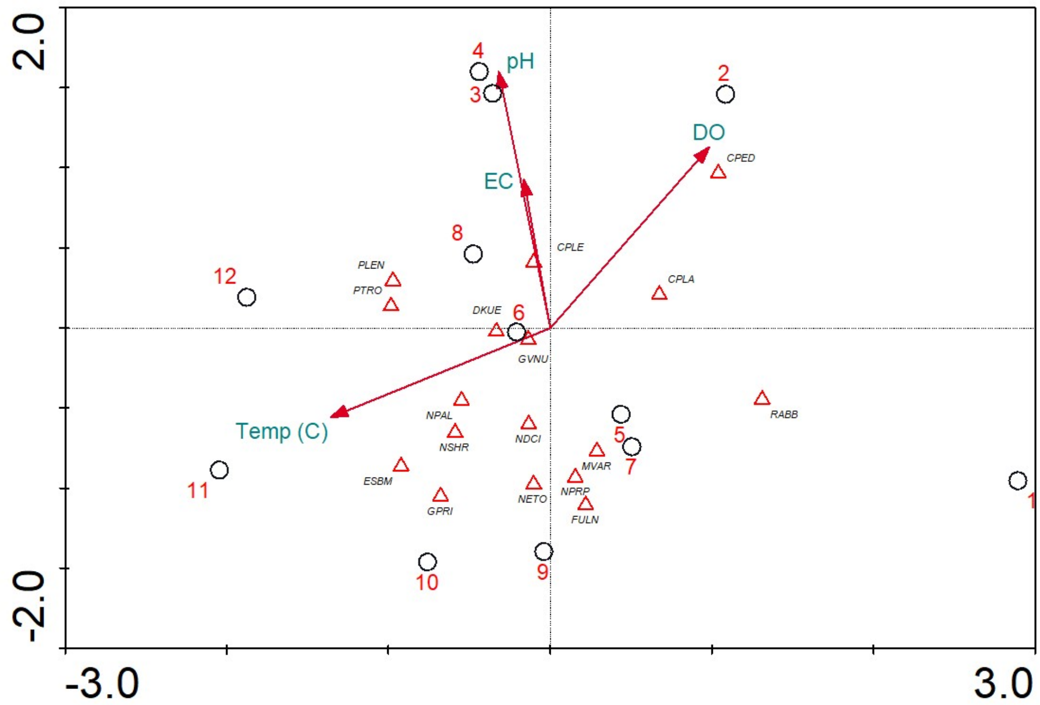


Figure 6: Canonical Correspondence Analysis of physico-chemical parameters on a reduced diatom species dataset (n = 17), indicated by red triangles, for sampling sites 1-4 in the Crocodile River throughout the sampling period, with an indication of species proximity to the various samples (1-12). Samples indicated by clear, numbered circles.

3.4 PCA – Principal Components Analysis

Because different diatom species in a community respond differently to changes in pollution due to their varying tolerances (Bere and Mangadze, 2014), it is essential to understand the interactions between the various diatom species that occur in an ecosystem. The Principal Components Analysis (PCA) describes these relationships whilst taking into consideration the varying conditions amongst the individual samples. In Figure 7, below, "each arrow (or eigenvector) points in the direction of steepest increase of values for the corresponding species" (ter Braak and Verdonschot, 1995).

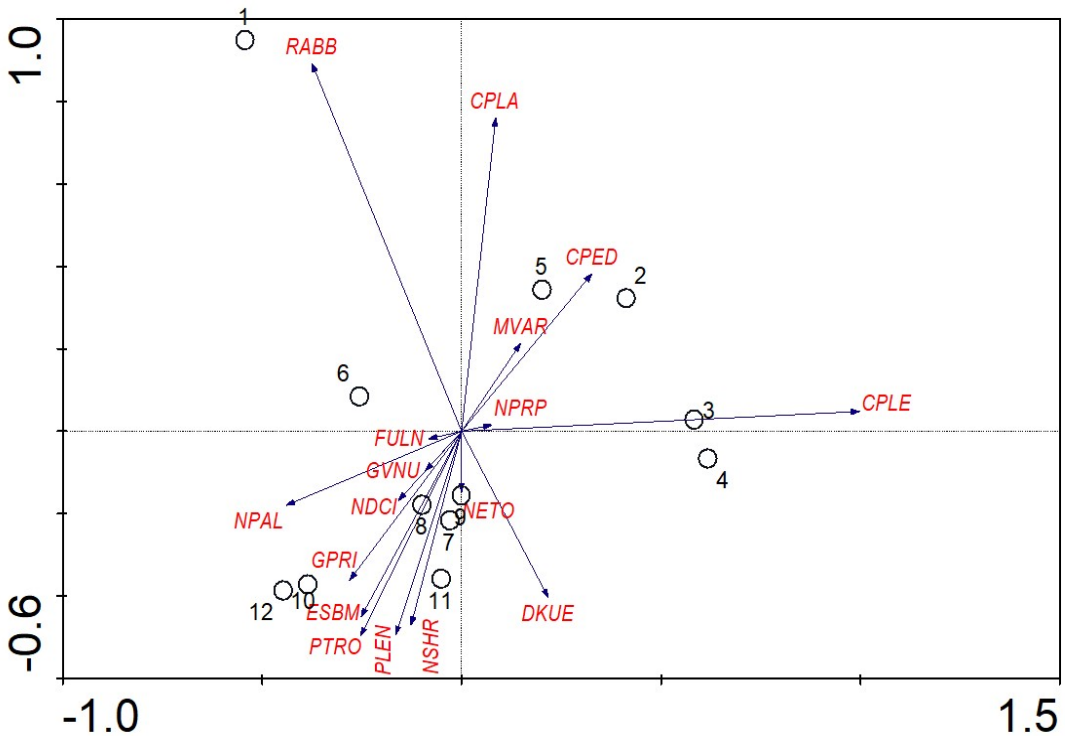


Figure 7: PCA indicating the interspecies relations in response to the physico-chemical variables of the various samples. Samples indicated by clear, numbered circles.

Inferences can be made, such as that sample 1 (S1 – Site 1, September 2019) had the highest RA (%) for *Rhoicosphenia abbreviata* (RABB). This species displayed a negative correlation to *Cocconeis placentula* Ehrenberg *var.euglypta* (Ehr.) Grunow (CPLE), which can be attributed to their vastly differing ecological preferences. Most of the species clustered in the 3rd quadrant of the PCA plot (FULN, GVNU, NETO and NDCI), have an affinity for saline and alkaline waters with a low to moderate electrical conductivity (Taylor *et al.*, 2007b). Species ESBM, NSHR, PTRO and PLEN can be regarded as pollution-tolerant species as the conditions for sample 11 were that of a slightly elevated water temperature (31.72 ºC), thus resulting in a significantly lowered dissolved oxygen concentration (6.47 mg/L).

3.5 Water quality parameters

Throughout the study, various physico-chemical parameters were considered as factors that could elucidate the quality of the water in the system at various points, which include temperature, pH, DO and EC.

Upstream catchment areas and land use pose a threat of pollution to the water in a river system, in addition to effluent discharge and altered flow conditions (Riddell *et al.*, 2019). Irrigation run-off from farms, particularly those located along the southern bank of the Crocodile River in close proximity of the various sampling sites, are likely to have had a direct impact on the quality of the water at these points. The Crocodile River along the southern boundary of the Kruger National Park falls within the Inkomati-Usuthu Water Management Area (WMA) and is vulnerable to pollution from the wastewater effluent at the Komatipoort, Malelane and Mhlatikop WWTW plants, exposed to the effects of industrial, agricultural and urban contamination (Roux and Selepe, 2013).

Table 7: Average values for environmental (physico-chemical) variables measured at sampling sites 1 - 4 throughout the sampling period

Sampling date	Site	Sample number	EC (µS/cm)	Dissolved Oxygen (mg/L)	pH (Units)	Temp (Degree Celsius)
Sep 2019	site 1	S1	465.91	12.47	7.81	18.32
	site 2	S2	478.77	15.74	8.69	23.03
	site 3	S3	497.18	8.74	8.38	20.93
	site 4	S4	860.00	14.58	8.90	25.41
Oct 2019	site 1	S5	556.41	8.88	8.01	22.70
	site 2	S6	450.51	7.98	8.11	23.59
	site 3	S7	447.74	8.52	7.94	23.16
	site 4	S8	704.84	7.40	8.18	21.34
Mar 2020	site 1	S9	510.79	7.53	7.88	25.60
	site 2	S10	384.09	6.48	7.91	28.23
	site 3	S11	428.31	6.47	8.21	31.72
	site 4	S12	516.79	6.92	8.35	28.25

Table 8: Rainfall data for the sampling period derived from the recordings within the areas in close proximity of the sampling sites (*Kruger Climate & Rainfall – Data & Information Resources – Scientific Services – SANParks*, 2022)

Total rainfall (mm/month)			
	September 2019	**October 2019**	**March 2020**
Crocodile bridge	11.0	75.0	12.0
Berg en Dal	0.0	35.0	0.0
Malelane	0.0	35.5	82.7

An overall decrease in the quality of water in the river system can be anticipated during periods with higher rainfall, due to the nutrient-contaminated run-off from the industries and major agricultural holdings found upstream of the catchment (Holmes and Taylor, 2015). Table 7 is a representation of the average values for the environmental variables measured *in-situ* at sampling sites 1 - 4 throughout the sampling period, while Table 8 provides a summary of the average total rainfall recorded at some of the monitoring stations in the Kruger National Park, within proximity of the sampling points. The data recorded in Table 8 pertains only to the months during which the sampling was done (September 2019, October 2019, and March 2020).

3.5.1 Flow velocity

River flow is a significant contributing factor to the establishment and abundance of periphyton community structures in aquatic ecosystems (Plenković-Moraj *et al.*, 2008) and similarly, species diversity (Tan *et al.*, 2013). There is a clear relationship between the flow conditions of a system and the resultant salinity of the water therein (King and Pienaar, 2011). Although limited to a single site, flow data obtained from the Department of Water and Sanitation (DWS) Hydrological Services' website, has been summarised and illustrated in Figure 8 below. This is in the form of a graph depicting the average daily flow conditions for weir X2H016 (Tenbosch Weir), which is located upstream of sampling Site 4; from August 2019 to April 2020. The image serves as a visualisation of how the river flow conditions fluctuated during the sampling sessions for this study, with significantly lower flows during the September 2019 and October 2019 sampling sessions, in relation to the March 2020 sampling session, which yielded higher river flow. These data support the assumption for this study; that an increase in average river flow will elicit a decrease in DO, potentially resulting from turbidity associated with nutrient loading linked to upstream anthropogenic activity, in turn causing a subsequent decrease in EC (Igbinosa and Okoh, 2009).

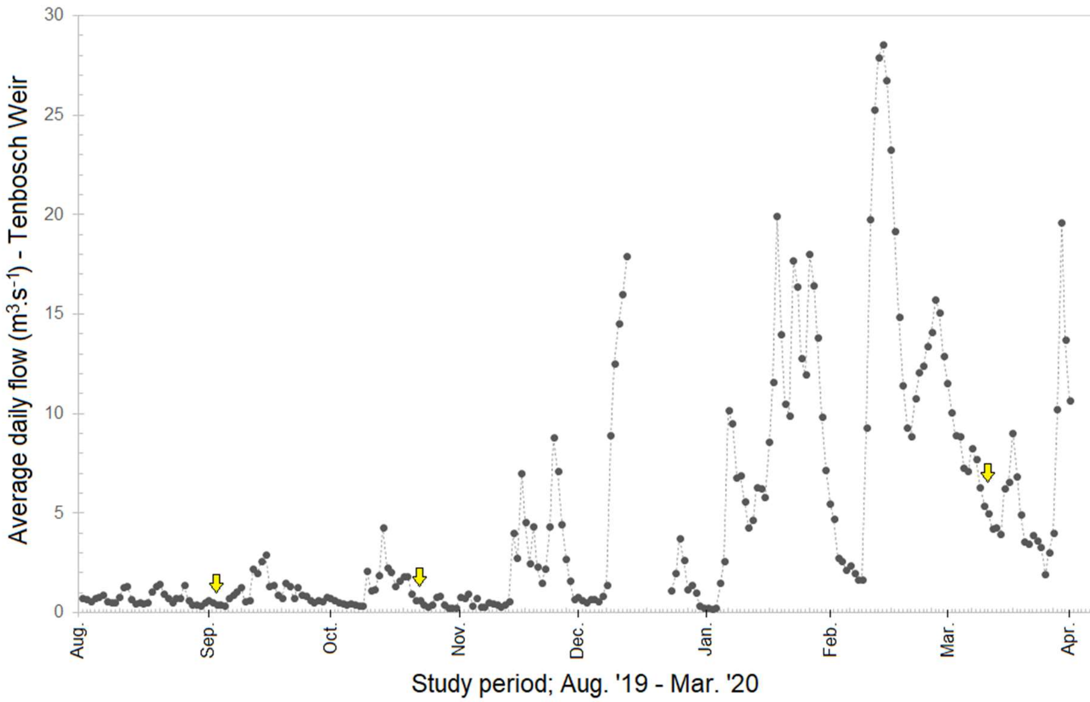


Figure 8: Average daily flow (m^3s^{-1}) at weir X2H016 (Tenbosch Weir) X during period: August 2019 – March 2020. Arrows indicative of the specific sampling dates.

Table 9: Dissolved oxygen and electrical conductivity in response to average daily flow during the three sampling sessions of the study (September 2019, October 2019, and March 2020)

	Average daily flow (m^3s^{-1})	**DO (mg/L)**	**EC µs/cm**
September 2019	0.40	14.58	860.00
October 2019	0.63	7.40	704.84
March 2020	5.00	6.92	516.79

3.5.2 Electrical Conductivity (EC)

The average monthly rainfall data (Table 8), as recorded at Crocodile Bridge, Berg en Dal and Malelane monitoring stations during September 2019, October 2019 and March 2020 have aided in inferring the quality of the system at the sampling sites, in proximity to the stations. The relationship between dissolved ions in water (that influence the electrical conductivity) and rainfall is such that elevated river flows, in response to significant rainfall, result in decreased EC (Malan and Day, 2002). This is supported by the results obtained from the study, wherein the Malelane monitoring station, which was closest to Site 2, with an average monthly rainfall of 82.7 mm (relatively higher than the Crocodile Bridge and the Berg en Dal stations for March 2020) had yielded the lowest EC for that sampling period.

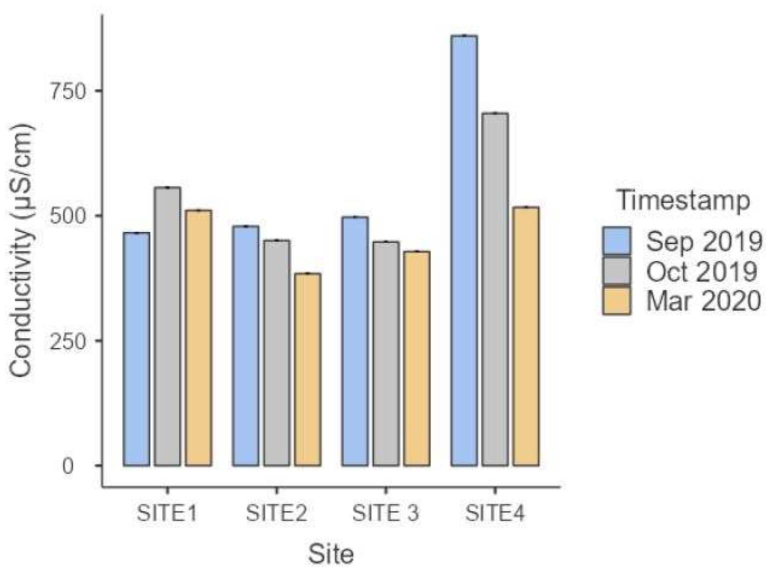


Figure 9: Average electrical conductivity (µS/cm) for each sampling site throughout the sampling period

Sites 2 and 4 of this study overlapped with long-term Ecological Water Requirement (EWR) monitoring sites EWR-C5 and EWR-C6, respectively (DWS, 2016). The Resource Quality Objectives (RQOs) for water quality for sites 2 and 4 state that the 95% percentile of data must not exceed 700 µS/cm (DWS, 2016). This will ensure that the resource quality and ecological categories of the specific reaches of the Crocodile River, is met in the respective resource units (DWS, 2016).

Although the average ECs of sites 1 to 3 remained moderate (range 383-558 µS/cm), Site 4 maintained a consistently high electrolyte content (range 515-861 µS/cm) throughout the sampling period, with average ECs of 860.00 µS/cm, 704.84 µS/cm and 516.79 µS/cm in September 2019, October 2019, and March 2020, respectively. Figure 10 gives an indication of the average EC for each site through the sampling period (September 2019, October 2019, and March 2020), indicating that the RQO for the EWR-C5 had been met, with the stipulated Target Water Quality Range (TWQR) required to keep the system in an Ecological Category "C". However, the conductivity at EWR-C6 frequently exceeded 700 µS/cm (70mS/m) and there is a high chance that the water quality is not meeting the TWQR of Ecological Category "C".

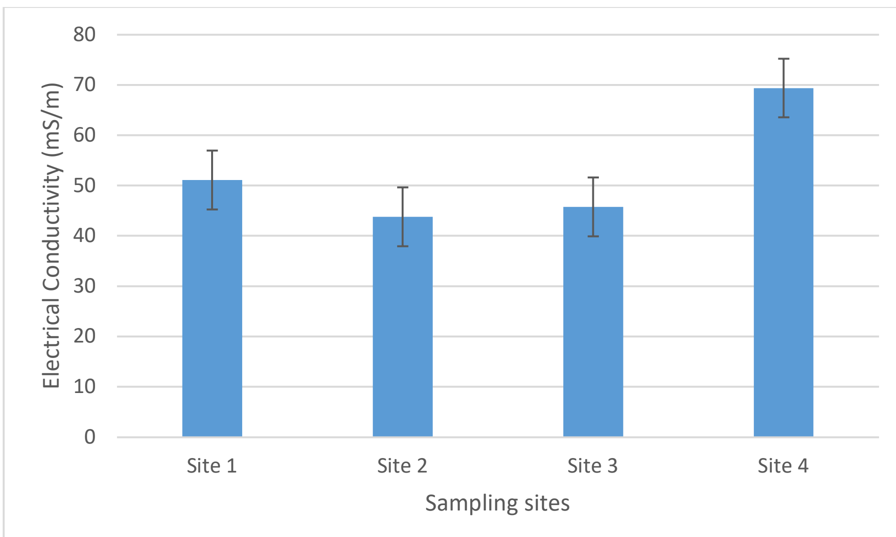


Figure 10: Weighted average electrical conductivity (mS/m) per site throughout the sampling period

3.5.3 Dissolved Oxygen (DO), pH and Temperature

The oxygen requirements for any aquatic species are unique to their specific life stage, metabolic or reproductive needs, making dissolved oxygen a significant contributor to the general health of an aquatic ecosystem (Dallas and Day, 2004). Some diatom taxa have an affinity for water with a higher dissolved oxygen concentration than others. During the study, the DO readings were at their highest in September 2019, with the maximum range measured at Site 2; 15.4 - 16.2 mg/L (Appendix 1), which is considered supersaturated. The CCA (Figure

6) confirms this conclusion, as S2 (Site 2, September 2019), with supersaturated DO concentrations, was dominated by CPED. This is consistent with the ecological characterisation of *Cocconeis pediculus* Ehrenberg, which has been described as an epiphytic cosmopolitan freshwater species, occurring on rock or plant surfaces, mainly in waters with moderate to high electrolyte content (Taylor *et al.*, 2007b; Jahn *et al.*, 2009). The results of a study conducted by Phiri *et al.* (2007) determined a strongly negative correlation of -0.63 between meso-eutraphentic diatoms and dissolved oxygen, likely owing to the dominance of submerged macrophytes (*Vallisneria aethiopica*) present in the associated water system during the sampling period (Phiri *et al.*, 2007). This comes as no surprise, as Holmes and Taylor (2015) were able to comprehensively demonstrate the ecological inconsistency of *Cocconeis* species, globally, due to its ability to tolerate varying levels of pollution (Holmes and Taylor, 2015). This is one of the reasons for which this taxon cannot be solely regarded as a reliable indicator of water quality, especially in this study, due to the lack of nutrient data to support chemical water quality parameters.

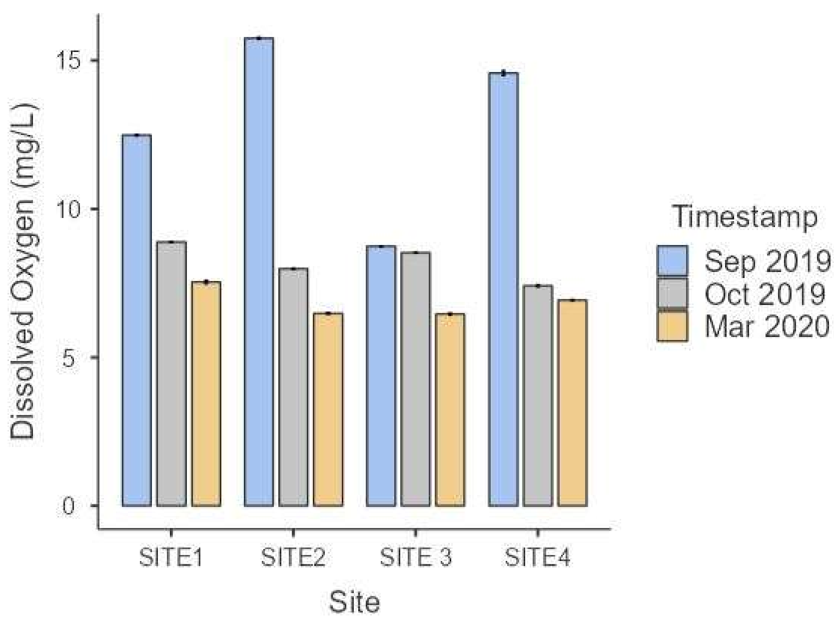


Figure 11: Average dissolved oxygen (mg/L) concentrations per site throughout the sampling period

Diatom assemblages are highly influenced by both temperature and a change in hydrogen ion concentration of an aquatic system, coupled with the associated vegetation cover (Bere and Mangadze, 2014). Temperature has a direct effect on the metabolic activities of diatoms while pH has a more indirect influence on diatom assemblage structures through impacting various other physico-chemical parameters, thus resulting in a response from diatoms in the associated systems (Bere *et al*., 2014). All four sites had slightly elevated pH, i.e., slightly basic, but there was a gradual increase downstream (Figure 12). This is consistent with higher ECs resulting from an increase in dissolved salts, that suggests elevated carbon dioxide demand by primary producers in the river.

All the pH values recorded across all the sampling sites for September 2019, October 2019 and March 2020 were greater than 7.0, however, the results for the correlation matrix (Table 10), indicate that pH has no significant correlation with any of the diatom indices, thus implying a limited direct influence of pH on diatom assemblages.

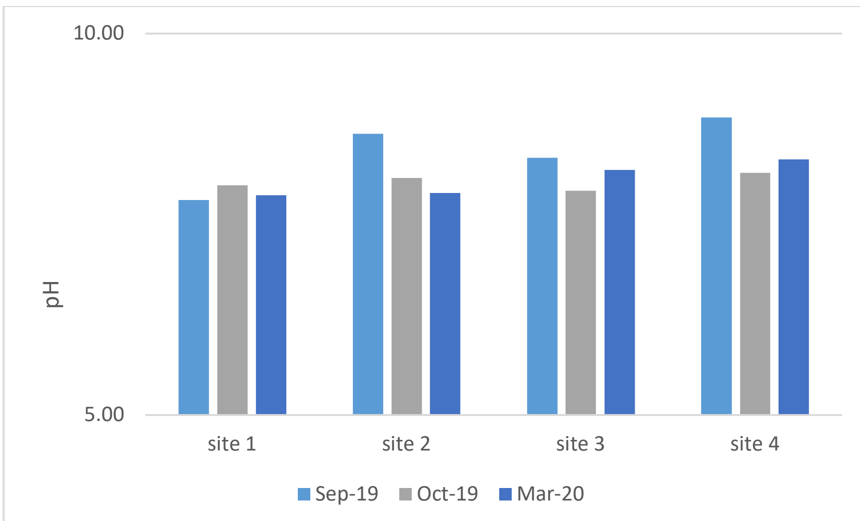


Figure 12: Average pH data per site throughout the sampling period

The temperatures for the sampling periods (spring and autumn months) ranged from 18.2 ºC to 31.8 ºC across all sampling sites throughout the study. The highest recorded temperature reading was at Site 3 during March 2020 (31.8 ºC), referred to as Sample 11 (S11).

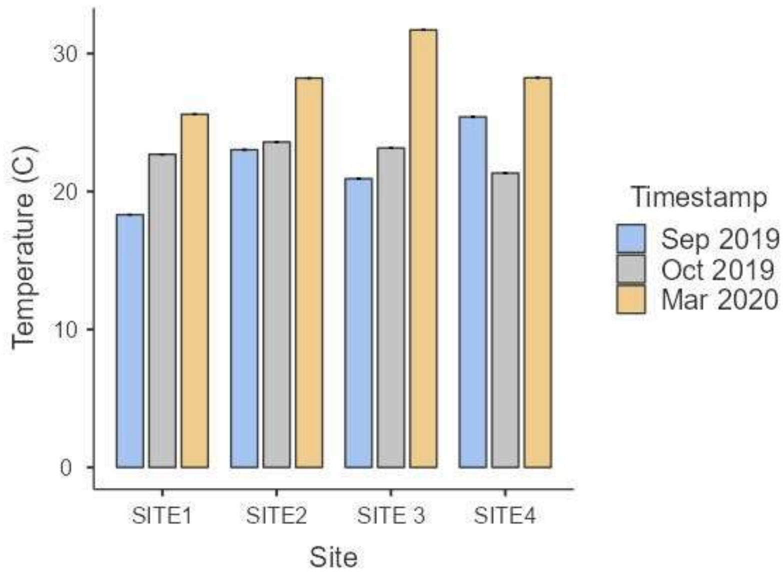


Figure 13: Average temperature (ºC) per site throughout the sampling period

The corresponding %PTV and SPI for the sample (S11) were 28.7 % and 9.2, respectively. This is the highest %PTV of all samples, and well above 20%, indicating that there was significant organic pollution in the system at that site during the specific sampling period. Table 10 demonstrates a strong positive correlation of 0.775 ($p = 0.003$) between temperature and %PTV, which suggests a significantly elevated level of organic matter present in this sample. The diatoms mainly encountered in these conditions included; *Planothidium engelbrechtii* (Cholnoky) Round & Bukhityarova, *Denticula kuetzingii* Grunow var.*kuetzingii* and *Eolimna subminuscula* (Manguin) Moser Lange-Bertalot & Metzeltin, which are all taxa with a tolerance for critical levels of pollution (Taylor *et al.*, 2007b). The main pollutants, as alluded to earlier, may include residential and industrial effluent, as well as the contamination of the river with run-off from irrigation on the agricultural holdings upstream of Site 3, granted its proximity with the town of Malelane.

Water temperature is largely responsible for the types of organisms that are found in a system, and this in turn also influences the dissolved oxygen of an aquatic ecosystem, determined by the number of organisms present in the system. The solubility of dissolved oxygen decreases as water temperature increases (USGS, 2023), which may explain the relationship highlighted in Figure 14 below.

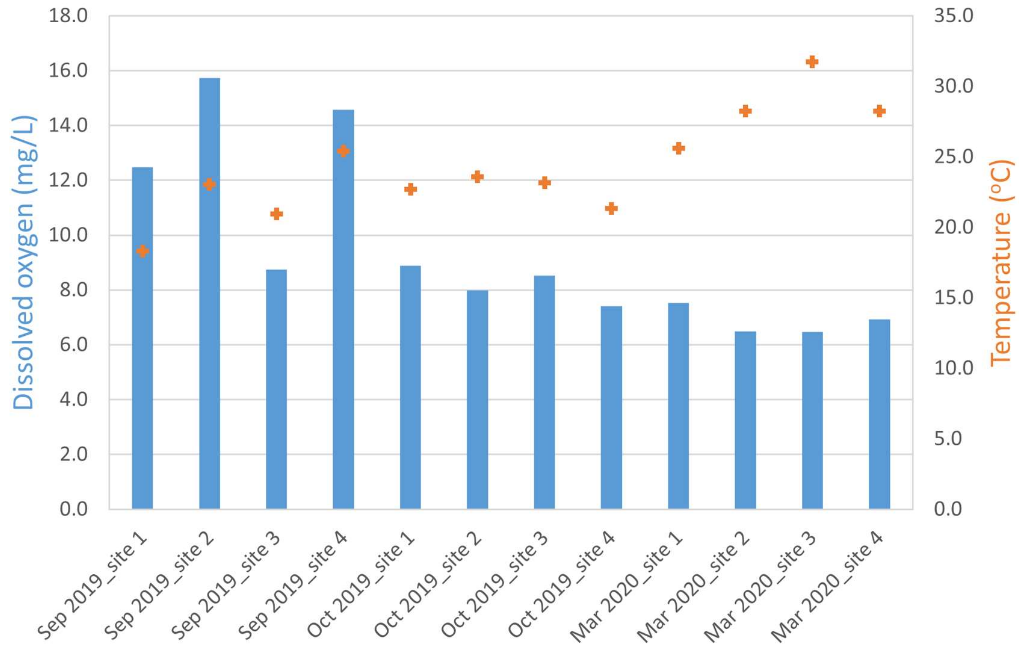


Figure 14: Relationship between dissolved oxygen (mg/L) and temperature (°C) at all four sampling sites throughout the study period; September 2019 to March 2020

Table 10: Pearson correlation results for diatom indices against physico-chemical parameters for the study period. Significant correlations ($p \leq 0.05$) are in bold.

	Diatom index					
	%PTV	**SPI**	**BDI**	**TDI**	**Evenness**	**Diversity**
EC	-0.350 p = 0.265	0.277 p = 0.384	0.397 p = 0.201	0.240 p = 0.453	-0.475 p = 0.119	-0.386 p = 0.215
DO	**-0.614** **p = 0.034**	**0.825** **p <0.001**	**0.807** **p = 0.002**	0.474 p = 0.120	**-0.794** **p = 0.002**	**-0.762** **p = 0.004**
pH	0.323 P = 0.305	0.304 p =0.337	0.359 p = 0.251	0.321 p = 0.309	**-0.598** **p = 0.040**	-0.506 p = 0.093
Temp	**0.775** **p = 0.003**	**-0.780** **p = 0.003**	**0.831** **p <0.001**	-0.369 p = 0.237	0.475 p = 0.119	0.441 p = 0.151

Table 11: Summary of canonical correspondence analyses of physical and chemical parameters, on a reduced dataset (*n* = 17), at sample sites in the Crocodile River over the sampling period

	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**
Eigenvalue	0.25	0.208	0.089	0.021
Species-environment correlations	0.916	0.956	0.866	0.439
Cumulative percentage variance of species data	19.5 %	35.8 %	42.8 %	44.4 %
Cumulative percentage variance of species-environment relation	44.0 %	80.6 %	96.3 %	100.0 %

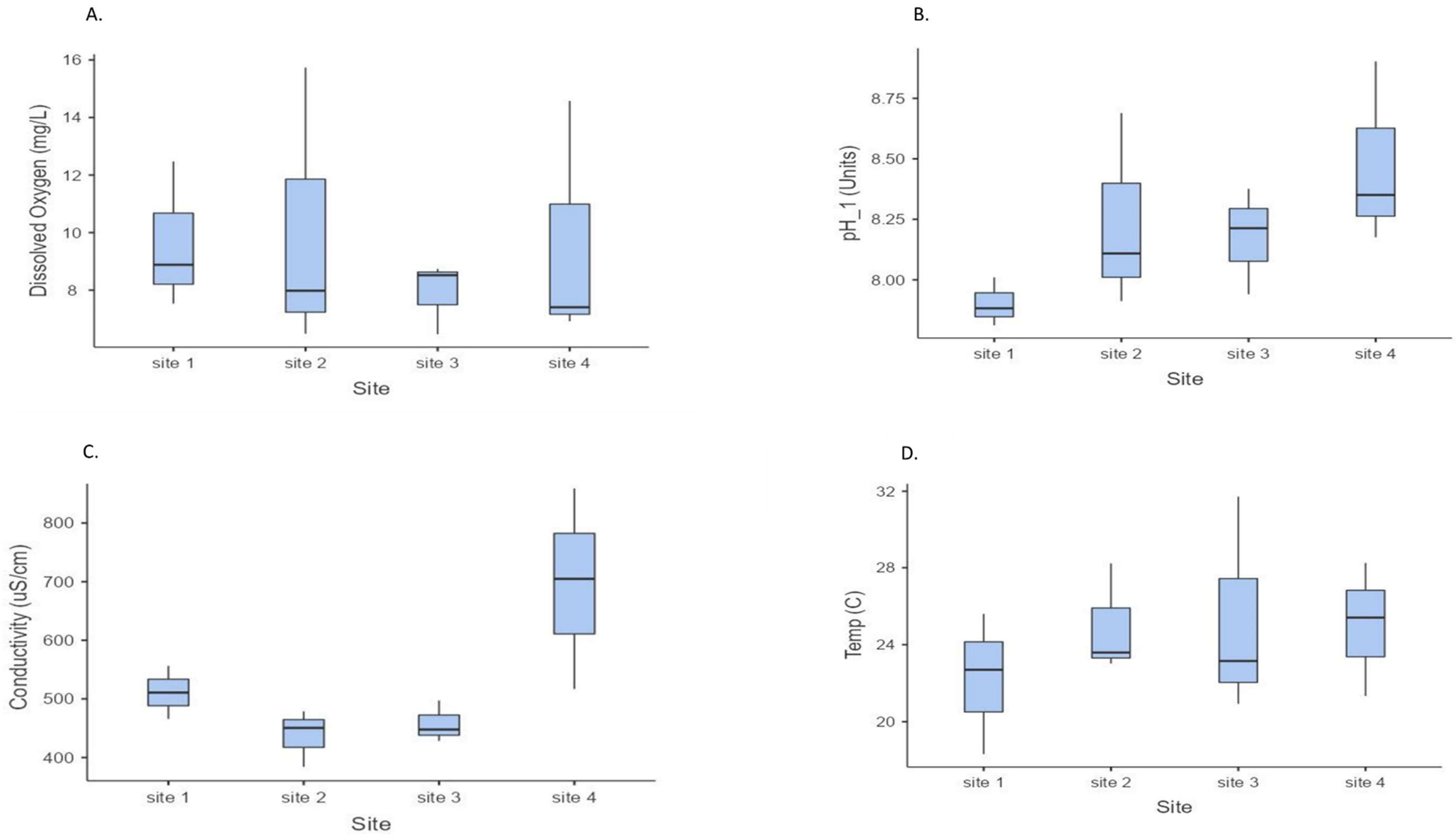


Figure 15: Box and whiskers plots illustrating the average water quality parameters; A. dissolved oxygen, B. pH, C. electrical conductivity, and D. temperature across all sampling sites over the three sampling sessions (September 2019, October 2019, and March 2020)

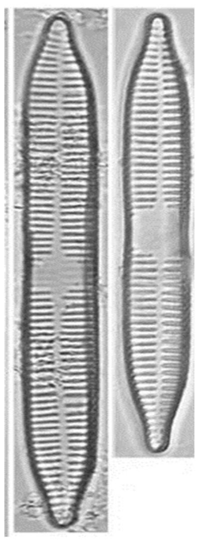
3.6 Species characteristics

Determining the species present in an aquatic ecosystem is crucial to inferring the quality of water in the stream at various points. This task usually involves extensive studies of diatom taxa to correctly identify the organisms, and ultimately verify their specific ecological requirements and/or preferred habitats. Although the microscopic examination and taxonomic identification of species is somewhat demanding, the technique is intended to provide a 'fourth leg' to the River Health Programme suite of monitoring tools (currently invertebrates, vegetation, and fish). This morphological identification is dependent on the shape and structure of the valves and is traditionally based on a light microscopic examination thereof (Taylor *et al.*, 2007b).

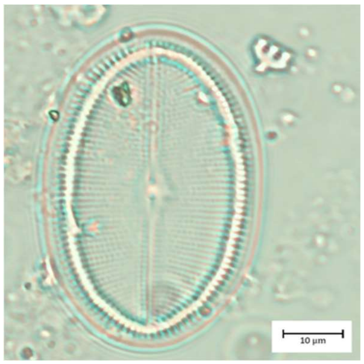
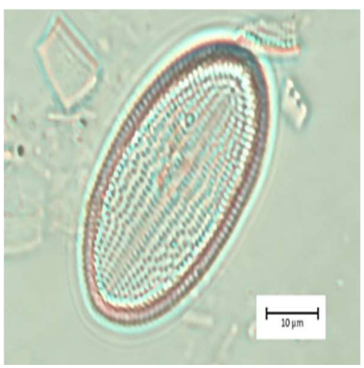
The phenotypic similarities of the inter and intra species, as well as the angles of the cells during identification (valve view vs girdle view) makes it incredibly complex to confidently confirm the various species, however, there is information material in the form of handbooks and literature, available to support identification through elaborately providing details on the valve shape, valve length, valve breadth, striae density, and fibulae density, amongst other information. The ecological data associated with each species can also be used as a guide, in conjunction with other water quality and site-specific background information, to ultimately arrive at a conclusion during cell identification and counting.

Table 12 below provides Light Microscopy (LM) images and a summary of some morphology information of the dominant species encountered in this study. In addition to this, the ecology of the species is included to better understand the environmental requirements of each species or taxa. The primary reference regarding the dimensions of the organisms was Taylor *et al.* (2007b), unless otherwise stated. A 10 μm scale is applicable to all the images displayed in the table.

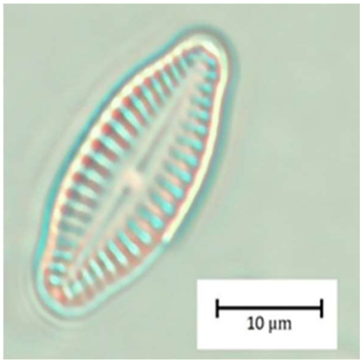
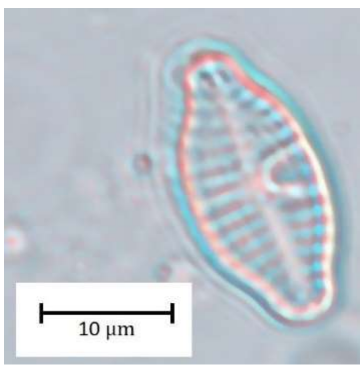
Table 12: Light microscopy images, morphology and ecological information of dominant diatom species identified in this study.

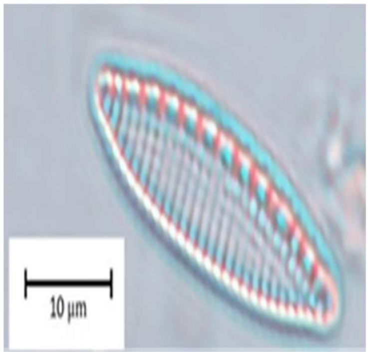
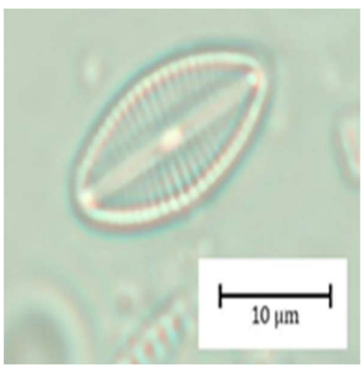
SPECIES	SIZE (VALVE/ STRIAE/ FIBULAE)	MORPHOLOGY/ DESCRIPTION	ECOLOGY
<u>Araphidae - Includes taxa with no true raphe system</u>			
<u>Fragilaria ulna</u> <u>(Nitzsch.) Lange-Bertalot var.</u> *<u>ulna</u>*  **Figure 16** (Taylor *et al*., 2007b)	Length: 50-250 µm Breadth: 2-9 µm Striae density: 7-15 /10 µm	"One of the unique features of this species is its closed girdle bands" (Williams, 2011)	"This is a cosmopolitan taxon found in the benthos of rivers and lakes and is easily suspended in the plankton due to its relatively large surface area. It is often found in mesotrophic to eutrophic, alkaline waters" (Taylor *et al*., 2007b)

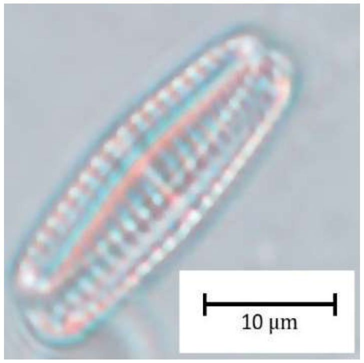
Monoraphidae - Includes taxa with a raphe on only one valve

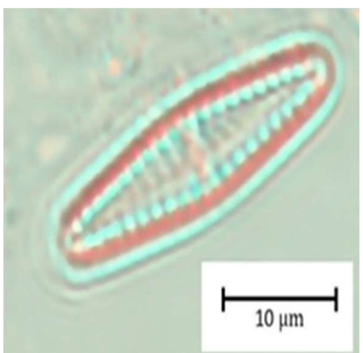
Cocconeis placentula Ehrenberg var. *placentula* 	Length: 7.5-98 µm Breadth: 8-40 µm Striae density: 24-26 /10 µm RV; 20-23 /10 µm RLV	"Valves range from broadly elliptical, elliptical, linear-elliptical to lanceolate-elliptical. Striae composed of funnel-shaped puncta" (Taylor *et al.*, 2007b) "Common feature with *C.pediculus* is the presence of a hyaline ring in the raphid valve" (Jahn *et al.*, 2009)	"This is a cosmopolitan, freshwater taxa" *(Jahn et al.*, 2009). "This species occurs on substrates (plants / wood) in meso-eutrophic standing or moving waters." (Taylor *et al.*, 2007b)
Cocconeis placentula Ehrenberg var.euglypta (Ehr.) Grunow 	Length: 10-46 µm Breadth: 8-40 µm Striae density: 224-226 /10 µm RV; 19-22 /10 µm RLV	"Can be differentiated from other varieties of *C. placentula* due to the presence of only 3-5 puncta per striae"	"*C. placentula* var. *euglypta* is omnipresent, although occurring more frequently in mesotrophic waters. It is tolerant of varying levels of pollution.

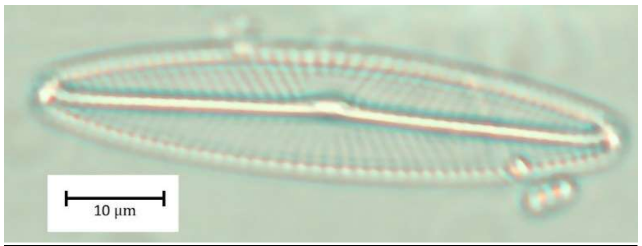
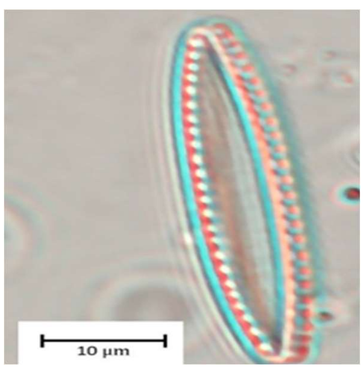
			The species can be found in waters with high conductivity" (Romero and Jahn, 2013)
<u>*Cocconeis pediculus*</u> <u>Ehrenberg</u>  	Length: 5-25 µm Breadth: 2.5-4 µm Striae density: 27-32 /10 µm	"Elliptical valves broader and more strongly curved than those of *C. placentula*. The raphid valves have a well-defined, asymmetrical, rhombic to rounded central area" (Taylor *et al.*, 2007b) "Common feature with *C.placentula* is the presence of a hyaline ring in the raphid valve" (Jahn *et al.*, 2009)	Cosmopolitan, freshwater taxa. Grows naturally epiphytic on filamentous structures (Jahn *et al.*, 2009)

Planothidium engelbrechtii (Choln.) Round & Bukhtiyarova 	Length: 8-18 µm Breadth: 3.5-5.5 µm Striae density: 15-17 /10 µm	"This species was originally described in 1955 from South Africa as *Achnanthes engelbrechtii* Cholnoky. The valves are lanceolate to elliptic–lanceolate and even rhombic–lanceolate with broadly protracted, rounded apices" (Compère and Van De Vijver, 2009)	"The species occurs in waters with increased electrolyte content" (Taylor *et al.*, 2007b)
Planothidium rostratum (Oestrup) Round & Bukhityarova 	Length: 11.0-17.5 µm Breadth: 5-6 µm Striae density: 11-13 / 10 µm (Wetzel *et al.*, 2013)	"Valves are linear-elliptical with rostrate, subrostrate or narrowly subcapitate apices. The RV has a linear axial area that is different to its central area. The raphe is straight with expanded external proximal endings. Multiseriate striae extend throughout both valves"	"Benthic species found in alkaline conditions, in water with a low to moderate electrolyte content. The species is more likely to be found attached to macrophytes" (Taylor *et al.*, 2007b)
Biraphidae - Include taxa with a raphe on both valves			

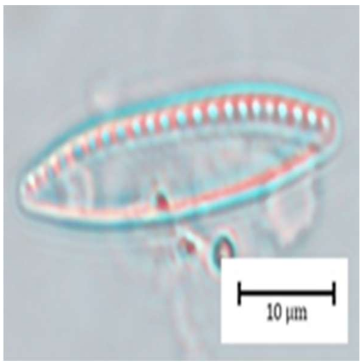
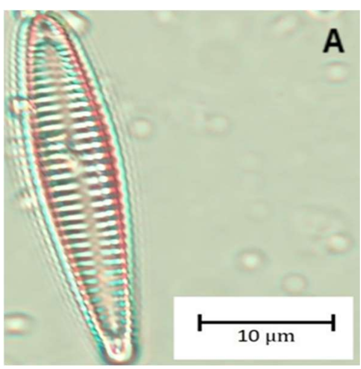
Denticula kuetzingii Grunow var.*kuetzingii* 	Length: 10-120 µm Breadth: 3-8 µm Striae density: 14-18 /10 µm Fibulae density: 5-8 /10 µm	"Linear to lanceolate valve outline, symmetry about the apical and transapical axis, and a canal raphe system subtended by large internal fibulae that partially extend across the valve face from margin to margin, forming partitions within the valve" (Hamsher *et al.*, 2014)	Cosmopolitan species found in water of moderate to high electrolyte content" (Taylor *et al.*, 2007b)
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin 	Length: 7.8-24 µm Breadth: 3.2-5.1 µm Striae density: 15-26 /10 µm	"Valves elliptical to rhombic-elliptical with rounded or acutely rounded, rarely weakly protracted apices. Striae density very variable" (Cook *et al.*, 2014)	"Cosmopolitan species (Sala *et al.*, 2008), more tolerant to heavy organic pollution and eutrophication" (Salomoni *et al.*, 2006)

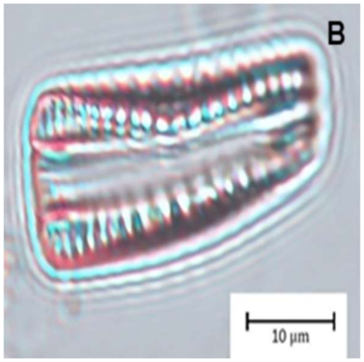
Gomphonema pumilum var. rigidum Reichardt & Lange-Bertalot  ***2 valves**	Length: 12-36 µm Breadth: 3.5-5.3 µm Striae density: 9-12 / 10 µm	Identifiable by a valve outline, which is absent in other *Gomphonema pumilum* variants. "Linear to linear-lanceolate valves with apices that are not protracted. Weakly lateral raphe, with rounded proximal endings"	"Cosmopolitan species found in meso-eutrophic conditions" (Taylor *et al.*, 2007b; Castillejo *et al.*, 2018). Species is not tolerant to elevated levels of pollution.

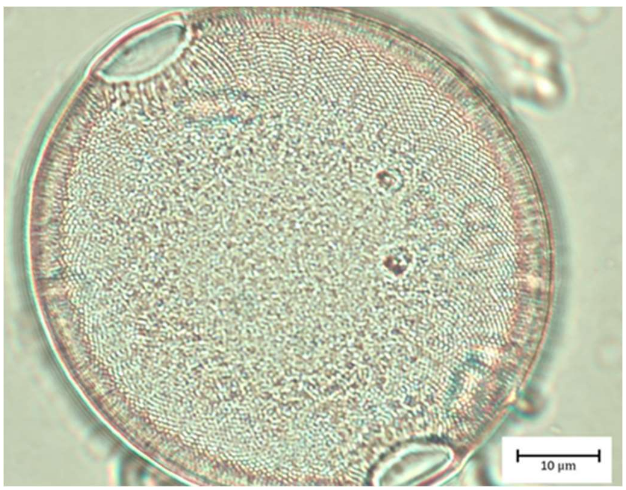
Gomphonema venusta Passy. Kociolek & Lowe (girdle view) 	Length: 30-45 µm Breadth:5-7 µm Striae density: 10-13 /10 µm	"Distinguished from *G. quasicrocodilei* by its broadly lanceolate shape and more acutely rounded head pole" (Passy *et al.*, 1997).	"Endemic species described from northern SA. Oligo to mesotrophic waters – moderate EC" (Taylor *et al.,* 2007b) "Crocodile River, scrapings from stone" (Passy *et al.*, 1997).
Navicula dulcis Patrick 	Length: 7-15.8 µm Breadth: 2.2-3.5 µm *(Potapova, 2013)*	Homonym of *Navicula dulcis* Krasske (1939) and therefore an illegitimate name. Confused with *Navicula perminuta* Grunow in Van Heurck and other small-celled *Navicula* species, which possess terminal raphe fissures characteristic for this genus (Potapova, 2013).	No additional ecology information could be found regarding this species, besides the description provided by the Academy of Natural Sciences of Philadelphia of the species in Sabine

			River, Texas (*Potapova, 2013*)
<u>*Navicula schroeteri* Meister *var. schroeteri*</u> 	Length: 28-42 µm Breadth: 8.0-9.0 µm Central striae density: 9-12 / 10 µm Striae density at ends: 12-14 / 10 µm (Terao *et al.*, 1980)	"Species with linear valves and broadly circular ends. The valves on this species are lanceolate, with a square central region and pointed tip" (Terao *et al.*, 1980).	"Cosmopolitan species occurring mainly in eutrophic conditions with water of a high electrolyte content. The species is tolerant of critical pollution levels" (Taylor *et al.*, 2007b)
<u>*Nitzschia etoshensis* Cholnoky</u> 	Length: 20-60 µm Breadth: 4-5.5 µm Striae density: 40 /10 µm Fibulae density: 15-18 /10 µm	"Lanceolate to linear-lanceolate shape with rounded apices that are never protracted" "The striae are not visible in light microscopy"	"The species occurs in conditions with higher Cl^- and NO_3 concentrations, with low conductivity" (Stenger-Kovács *et al.*, 2014)

Nitzschia palea (Kützing) W.Smith 	Length: 15-70 µm Breadth: 2.5-5 µm Striae density: 28-40 /10 µm Fibulae density: 9-17 /10 µm	"linear lanceolate shape, with rostrate to sub-capitate apices, and all lack central raphe endings, but valve dimensions and pattern densities vary considerably" (Trobajo *et al.*, 2010).	"Widely distributed freshwater species common and often abundant component of benthic diatom communities" (Trobajo *et al.*, 2010) "Described as an indicator species of polysaprobic or hypereutrophic environments, occurring in waters with low concentrations of

			dissolved oxygen” (Salomoni *et al.*, 2006)
Nitzschia perspicua Cholnoky 	Length: 17-25 µm Breadth: 3-4 µm Striae density: 28-40 /10 µm Fibulae density: 15-17 /10 µm	Frustules weakly silicified. Valves narrow-elliptical with bluntly rounded or slightly sub-rostrate apices. Marginal raphe supported by small fibulae. Striae not discernible in LM (Taylor *et al.*, 2007b)	A species commonly occurring in saline waters (Taylor *et al.*, 2007b)
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot 	(**A**: valve view, **B**: girdle view) Length: 10-75 µm Breadth: 3-8 µm Striae density:15-20 /10 µm	“narrow, linear valves with low striae density” (Levkov *et al.*, 2010)	“Mesotrophic to slightly eutrophic rivers and lakes. Freshwater to halophilous brackish water, euryhaline species” (Levkov *et al.*, 2010)



<u>Centrics:</u> single-celled taxa, mostly observed in valve view			
<u>*Melosira varians* (Agardh)</u> 	Diameter: 8-35 µm Depth of mantle: 4-14 µm	filamentous, freshwater, centric diatom (Crawford, 1971)	“Cosmopolitan taxon found in both the benthos as well as the plankton and becomes particularly abundant in eutrophic, occasionally slightly brackish, waters” (Taylor *et al.*, 2007b)

*All species details referenced from Taylor *et al.* (2007b), unless otherwise stated.

3.7 Other microphytobenthos (algae and cyanobacteria)

Microalgal colonies have proven to be a reliable way to determine the stability of an aquatic ecosystem due to their sensitivity to changing environmental variables, including flow conditions. The changes in the quality of an aquatic ecosystem have a direct effect on the microphytobenthos and associated assemblages, thus becoming an important determining factor in the composition of the benthic assemblages in a system (Yang *et al*., 2016)

An emulation of the publication by Schneider *et al*. (2013), meant that the microphytobenthos (MPB) collected at all sampling sites across the study area encompassed diatoms, cyanobacteria and green algae (chlorophyta). The chlorophyll signatures of these three MPB taxa were collected simultaneously and were exposed to similar environmental conditions. Figure 17 illustrates the composition of the various microalgal assemblages, as derived from the bbe BenthoTorch readings from the sampling carried out through the allocated period (September 2019, October 2019, and March 2020). During the study, the cyanobacteria were the most dominant of the three MPB groups at all sites and during all sampling sessions. The cyanobacteria were less dominant during the cooler spring months when river flow was lowest than March 2020, in contrast to the diatoms that were more abundant in September and October 2019 (Figure 17).

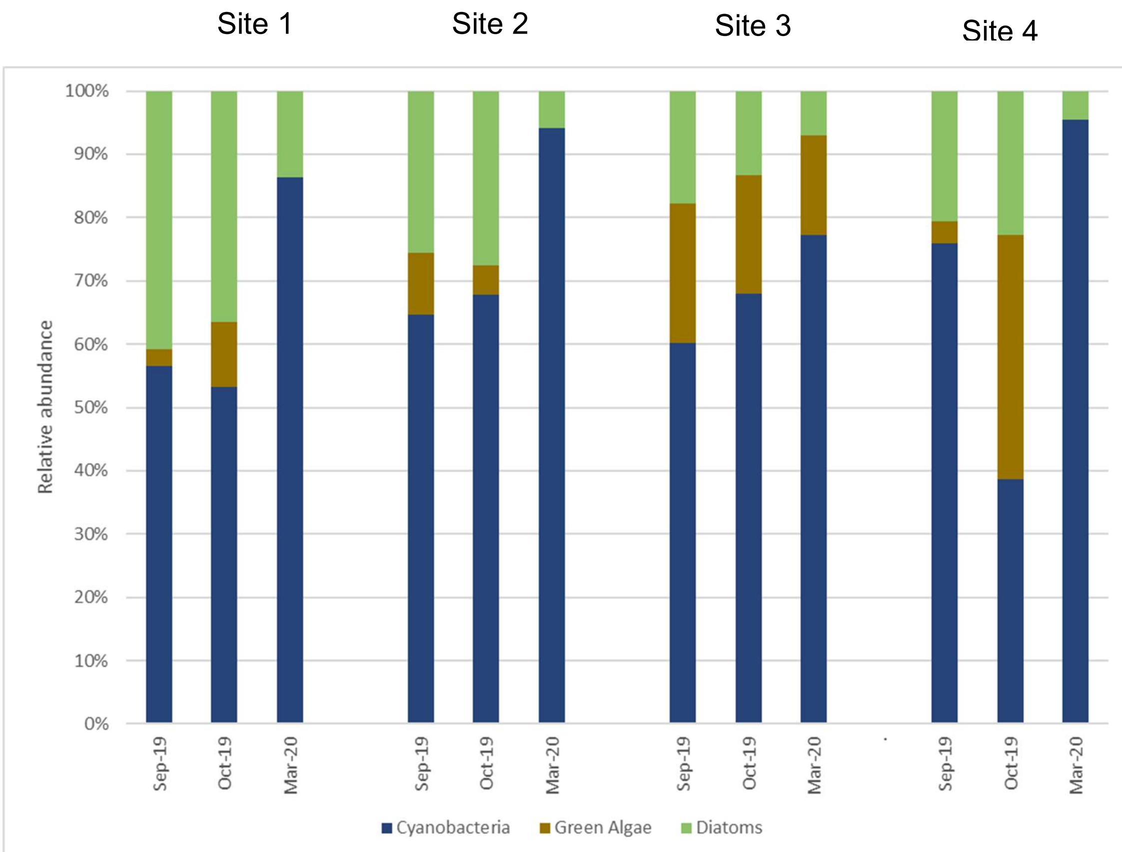


Figure 17: Stacked graph of microphytobenthos relative abundance at the four study sites during each sampling session

A low species richness relating to phytoplankton diversity in an aquatic ecosystem implies water quality that is not compromised, namely, oligotrophic (Ogawa and Ichimura, 1984), whereas elevated cyanobacteria abundance, as observed in March 2020 across all sites, implies that the trophic status of the water body is meso-eutrophic. Nutrient overload and elevated temperature have a direct impact on cyanobacteria being the dominant microorganism within a phytoplankton community (Almanza *et al.*, 2019). Cyanobacteria are frequently associated with hypoxic or anoxic conditions, which is in contrast to most diatom species (Okechukwu and Alex, 2009). This relationship was found in this study, where benthic diatom abundance was elevated in cooler, more oxygenated water than the cyanobacteria (Figure 18).

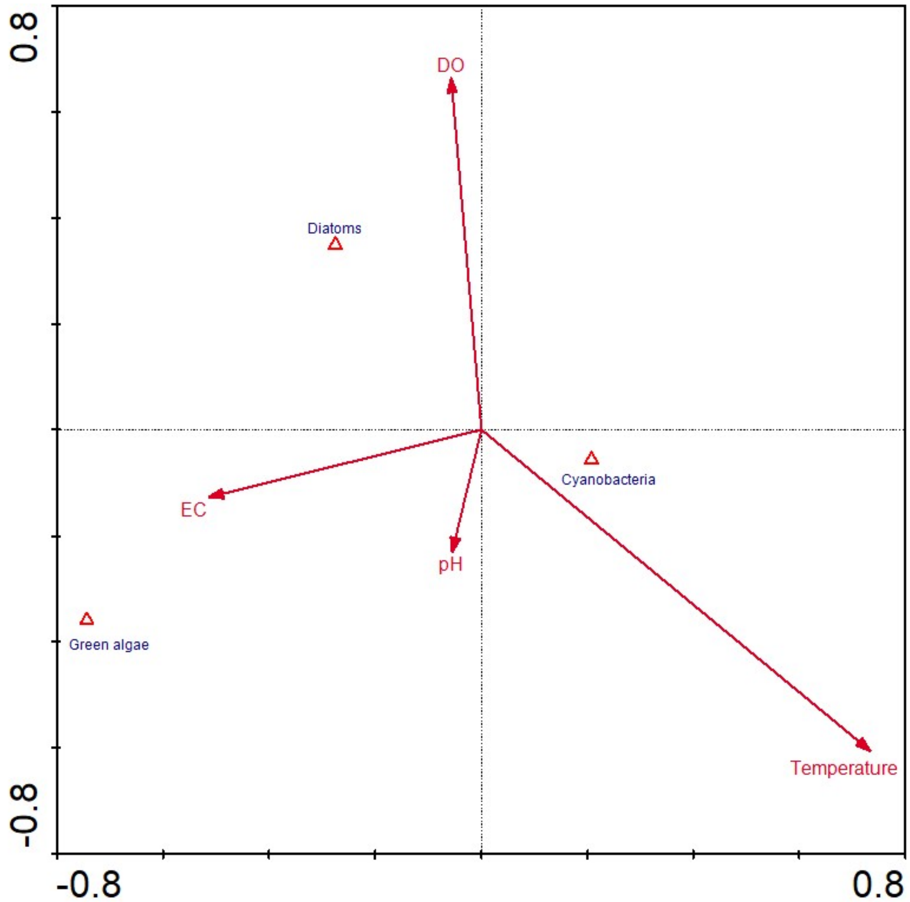


Figure 18: Summary of Canonical Correspondence Analyses of MPB and physico-chemical parameters, for sampling sites 1-4 in the Crocodile River throughout the sampling period

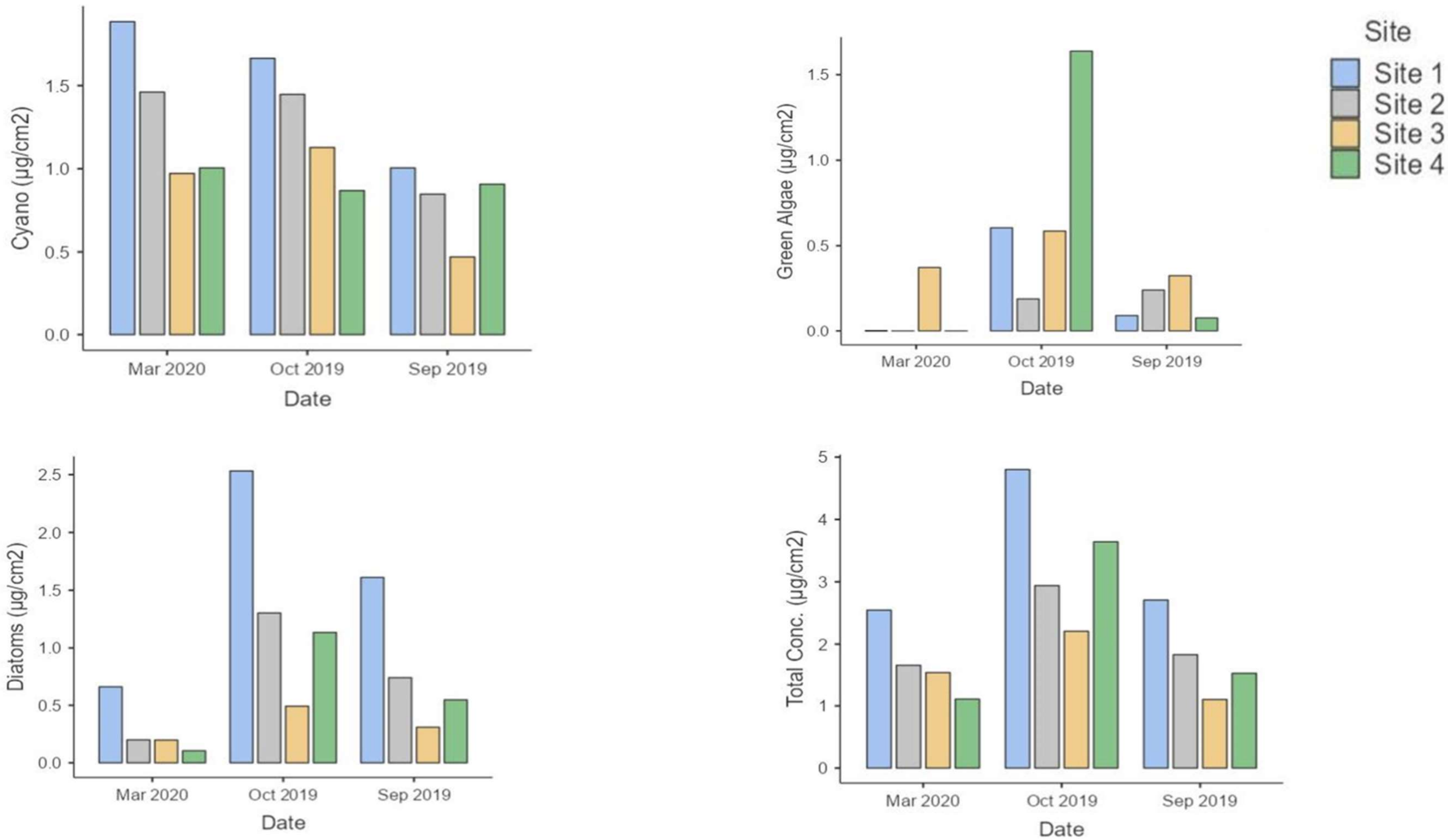


Figure 19: Average relative abundance of cyanobacteria, green algae, diatoms, and total cells across all sampling sites over the study period

4. Conclusion

The aim of this study was to use the South African Diatom Index (SADI) to complement existing water quality indices (Index of Habitat Integrity, Fish Response Assessment Index, South African Scoring System, Macroinvertebrate Response Assessment Index, and Vegetation Response Assessment Index) in determining the PES of the Crocodile River at four predetermined sampling sites in the Kruger National Park. Determining the PES, which serves as evidence regarding the quality of the water in a system at the time, involves the incorporation of the latest (ideally 1-3 years) physico-chemical, environmental and biological data to assess Ecological Category, which range from A (unmodified, natural) to F (critically modified) (King and Pienaar, 2011). In 2017, the Integrated Eco-status' of two long-term sampling sites, X24D-00994 (EWRC5; Malelane) and X24H-00953 (EWRC6; Nkongama) were determined to be category "C", which was neither an improvement nor a decline from the 2012 records at the same points (Table 13). The site between these X24H-0080 (Mbiyamiti), which corresponds with Site 3 of this study, had an Eco-Status of "C" in 2012 (Roux and Selepe, 2013). This implies that the then recommended management actions to maintain the state of the river had been successful at preventing further deterioration. Further evidence of this is provided in the SPI data for Site 2 and Site 4, which ranged from 9.5 (EC score of "B/C") to 13.5 (EC score of "C") during the three sampling sessions (September 2019, October 2019, and March 2020) of the current study.

Literature, in conjunction with the study at hand, have proven that microphytobenthos colonies can be used, with confidence, to aid in concluding the PES of a river system at specific points. This does, however, rely on the rigorous and consistent monitoring of the quality of the water, using the corresponding physico-chemical data gathered over a period. The average SPI score (11.8) from the samples used in this study, places the Crocodile River, at the predetermined sampling sites, at a ecological category "C". This is consistent with the results reported from both 2017 and 2012 periods.

Table 13: Comparison of the integrated Eco-status' of X24D-00994 and X24H-00934 Crocodile River sites from 2012 and 2017 studies (Roux and Selepe, 2013), and corresponding Present Ecological State (PES) for each SQ Reach during the 2017/2018 hydrological year (Thirion and Jafta, 2019).

Study site	EWR site	SQ Reach	Site name	Integrated Eco-status		PES category (2017/2018)
				2012	2017	
2	EWR 5	X24D-00994	X2CROC-MALEL	C	C	D
4	EWR 6	X24H-00934	X2CROC-NKONG	C	C	D

The stable Eco-status of the two sites over the five-year period can be attributed to no further deterioration in the system with ongoing industrial and agricultural activities upstream. These activities cause compromised water quality further downstream, at the sampling points. Despite several reaches of the river being in a protected area, Kruger National Park, the pollution from local WWTWs, agricultural activities, and pollution from neighbouring towns and residential holdings contributes to the resistance of the system to remediation.

In order to motivate for an improvement in the quality of the Crocodile River system, there is a requirement for an integrated (social, ecological, and economic) approach to the management of water resources, wherein CMAs can engage with key stakeholders in promoting learning around Strategic Adaptive Management techniques to effectively support river health (Kingsford and Biggs, 2012; McLoughlin *et al.*, 2021), and promote sustainable water resource management. There may be a benefit to the regulation of quality standards pertaining to the effluent and discharges from commercial, large-scale polluters (Deksissa *et al.*, 2003). Industrial holdings and manufacturing companies are already held to a high standard regarding the emissions associated with their processes. Such companies are required to strictly adhere to the conditions of their water use licenses and ensure little-to-no pollution of natural streams and water systems with contaminated effluent from their manufacturing processes (Sahula, 2015). There is also extensive evidence of the influence of nutrient enrichment on water bodies and the effect of this on the types of microorganisms that exist in abundance in these conditions. In this study, the physico-chemical parameters measured did not include nutrient measurements. The absence of this information inhibited the investigation of the influence of nutrient levels on the quality of the water and subsequently, diatom community structures. Deksissa *et al*. (2003) emphasises the impact of climate on water quality on the basis of rainfall and river flows (Deksissa *et al.*, 2003). Their study has supported the need for flow data, in comparison with temperature and rainfall, in determining the water quality at the various sampling points. This highlights the relevance of comprehensive nutrient and flow data when considering water quality.

The success of using diatom indices as a measure of water quality and ultimately determining the health of a river through PES determination is limited by the shortage of taxonomists and experts who can identify, characterise and classify these microorganisms according to ecological conditions (Dalu and Froneman, 2016). The resolution of this issue would assist in providing further distribution information for studies related to diatoms. There is a need for extensive training on utilising the existing literature to identify and study diatom species and their role in aquatic ecosystems (Harding and Taylor, 2011). In addition to this, there could be a great benefit to utilising molecular methods such as DNA barcoding, to assist in the identification of diatom species and, in-turn, minimize gross misidentifications by novice diatom researchers and students. A recent study by Nunes *et al*. (2019) proved the efficiency of molecular operational taxonomic units (MOTUs) in determining the community diversity in related bioassessments, although more sensitive and specific tests would be required to determine dominant diatom species (Nunes *et al.*, 2019). Reducing errors in identification would require the application of DNA barcoding techniques and the establishment of a comprehensive genetic reference database library for future accurate classification of these species, as necessary. The success of using a diatom DNA barcode database as a tool in routine biomonitoring, according to Rimet (2016), is currently not an option due to the limited ecological data associated with the various diatom species (Rimet *et al.*, 2016).

The presence of varying diatom species in a community is sufficiently definitive of the quality of water at a site, based on each individual species' documented ecological preferences, however, the reliability of the results from OMNIDIA, pertaining to study sites 2 and 4 during the March 2020 sampling period, and study site 1 during the September 2019 sampling period remain questionable, as less than 300 diatoms were counted for these samples; with only 288, 159 and 237 cells counted, respectively. Most of the frustules from these samples were either extensively fragmented, rendering them difficult to correctly identify or they were too ambiguous to make a conclusive identification thereof. To further improve the reliability of this study, more diatoms will need to be collected and counted to get a slightly improved estimation of the species abundance and diversity. This will enable the OMNIDA software to yield more accurate data pertaining to the indices calculated, thus resulting in improvements to the correlation of these diatom data with the physico-chemical information, per site.

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Appendix 1: Values for environmental (physico-chemical) variables measured at sampling sites 1 - 4

Physico-chemical variable	Sampling interval	Site 1					Site 2					Site 3					Site 4				
		mean	median	standard deviation	min	max	mean	median	standard deviation	min	max	mean	median	standard deviation	min	max	mean	median	standard deviation	min	max
EC (uS/cm)	Sep-19	466.000	466.000	0.634	465.000	467.000	479.000	478.000	1.540	478.000	482.000	497.000	497.000	1.660	495.000	500.000	860.000	860.000	0.451	859.000	861.000
	Oct-19	556.000	556.000	0.541	556.000	558.000	451.000	451.000	0.218	450.000	451.000	448.000	448.000	0.114	448.000	448.000	705.000	705.000	0.764	704.000	706.000
	Mar-20	511.000	511.000	0.750	510.000	512.000	384.000	384.000	0.734	383.000	385.000	428.000	428.000	0.643	427.000	429.000	517.000	517.000	1.340	515.000	519.000
DO (mg/L)	Sep-19	12.500	12.500	0.138	12.200	12.700	15.700	15.700	0.221	15.400	16.200	8.740	8.740	0.054	8.610	8.900	16.600	14.500	0.593	13.400	15.900
	Oct-19	8.880	8.910	0.085	8.720	8.990	7.980	7.970	0.095	7.740	8.270	8.520	8.520	0.035	8.450	8.650	7.400	7.360	0.188	7.230	8.400
	Mar-20	7.530	7.390	0.351	7.230	8.490	6.480	6.490	0.135	6.310	6.760	6.470	6.410	0.184	6.240	6.780	6.920	6.900	0.096	6.770	7.240
DO (%)	Sep-19	133.000	133.000	1.650	130.000	136.000	184.000	183.000	2.320	180.000	189.000	98.000	98.000	0.792	96.200	100.000	178.000	177.000	7.090	164.000	195.000
	Oct-19	103.000	103.000	0.958	101.000	104.000	94.200	94.100	1.150	91.300	97.700	99.700	99.700	0.422	98.900	101.000	83.800	83.400	2.140	81.800	95.200
	Mar-20	92.300	90.500	4.230	88.700	104.000	83.300	83.300	1.820	81.000	87.000	88.200	87.400	2.580	85.000	92.600	88.900	88.700	1.240	86.900	92.700
pH	Sep-19	7.820	7.850	0.155	7.560	8.040	8.690	8.740	0.184	8.270	8.910	8.380	8.300	0.219	8.150	9.070	8.900	8.920	0.111	8.620	9.040
	Oct-19	8.010	8.000	0.022	7.990	8.080	8.110	8.100	0.052	8.040	8.210	7.940	7.880	0.110	7.820	8.200	8.180	8.140	0.109	8.050	8.430
	Mar-20	7.880	7.920	0.150	7.560	8.070	7.910	7.900	0.025	7.890	7.960	8.210	8.210	0.032	8.170	8.300	8.350	8.320	0.086	8.310	8.710
Temp (°C)	Sep-19	18.300	18.300	0.072	18.200	18.400	23.000	23.000	0.179	22.700	23.300	20.900	20.900	0.160	20.700	21.200	25.400	25.400	0.230	25.000	25.800
	Oct-19	22.700	22.700	0.000	22.700	22.700	23.600	23.600	0.026	23.500	23.600	23.200	23.200	0.050	23.100	23.200	21.300	21.300	0.050	21.300	21.400
	Mar-20	25.600	25.600	0.080	25.500	25.700	28.200	28.200	0.072	28.100	28.300	31.700	31.700	0.070	31.600	31.800	28.200	28.200	0.115	28.100	28.400

Appendix 2: Diatom counts during the three sampling sessions (September 2019, October 2019, and March 2020) and the corresponding sample ID

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Diatom species name	**Sep-19**				**Oct-19**				**Mar-20**			
	site 1	**site 2**	**site 3**	**site 4**	**site 1**	**site 2**	**site 3**	**site 4**	**site 1**	**site 2**	**site 3**	**site 4**
Aulacoseira ambigua (Grunow) Simonsen	1	0	0	0	10	0	0	0	3	2	0	0
Achnanthidium affine (Grun) Czarnecki	0	0	0	0	0	0	0	1	0	0	0	0
Amphora coffeaeformis (Agardh) Kützing var. *coffeaeformis*	0	2	2	1	0	0	2	0	0	0	0	1
Amphora copulata (Kütz) Schoeman & Archibald	0	0	0	0	0	4	0	0	0	0	0	0
Achnanthidium crassum (Hustedt) Potapova & Ponader	1	3	0	0	3	0	0	7	0	2	3	5
Achnanthidium exiguum (Grunow) Czarnecki	0	0	1	2	0	0	2	3	0	4	1	4
Achnanthidium minutissimum (Kützing)	0	3	0	0	0	0	0	0	0	0	0	0
Achnanthes eutrophila Lange-Bertalot	2	0	0	0	0	0	0	0	0	0	0	0
Amphora inariensis Krammer	0	0	0	0	0	5	0	0	0	2	0	0
Amphora montana Krasske	0	0	0	0	0	0	0	2	0	0	0	0
Amphora species	0	0	0	0	0	3	1	0	0	0	0	0
Amphora pediculus (Kützing) Grunow	0	0	0	0	0	3	0	0	0	0	0	0
Amphora veneta Kützing	0	0	0	1	0	0	2	0	0	0	0	0
Bacillaria paradoxa Gmelin	0	0	0	0	0	0	1	0	0	1	1	0
Capartogramma crucicula (Grun.ex Cl.)Ross	0	0	0	0	0	3	0	0	0	0	0	0
Cocconeis engelbrechtii Cholnoky	5	1	0	3	1	2	2	6	0	1	0	8
Cymbella kolbei Hustedt var. *kolbei*	9	0	0	0	0	0	0	0	4	3	0	0
Cymbella kappii (Cholnoky) Cholnoky	1	0	1	0	0	0	0	0	2	0	0	0
Cyclotella meneghiniana Kützing	0	0	0	0	0	0	0	0	0	0	0	1
Cocconeis species	2	9	0	0	15	1	2	0	0	3	0	2
Cocconeis pediculus Ehrenberg	3	40	0	0	8	2	0	0	0	0	0	0
Cocconeis placentula Ehrenberg var. *placentula*	49	54	33	3	14	16	13	20	9	9	5	7
Cocconeis placentula Ehrenberg var.*euglypta* (Ehr.) Grunow	14	149	178	185	123	51	82	72	87	28	80	15
Cocconeis placentula Ehrenberg var.*lineata* (Ehr.) Van Heurck	0	11	16	5	1	3	5	3	2	6	1	0
Cymbella turgidula Grunow 1875 in A.Schmidt & al. var. *turgidula*	0	1	0	0	1	0	0	0	9	1	0	0
Diadesmis confervaceoides Lange-Bertalot & Rumrich	0	0	4	2	0	10	6	7	0	3	4	6
Denticula kuetzingii Grunow var.*kuetzingii*	0	0	8	12	0	4	26	18	8	0	12	0
Denticula subtilis Grunow	0	0	8	4	0	10	12	6	2	0	6	0

<u>KEY:</u>

S1 – sample 1	S7 – sample 7
S2 – sample 2	S8 – sample 8
S3 – sample 3	S9 – sample 9
S4 – sample 4	S10 – sample 10
S5 – sample 5	S11 – sample 11
S6 – sample 6	S12 – sample 12

*Samples applicable to all data

Diatom species name	Sep-19				Oct-19				Mar-20			
	site 1	site 2	site 3	site 4	site 1	site 2	site 3	site 4	site 1	site 2	site 3	site 4
Denticula sundayensis Archibald	0	2	0	0	0	10	2	0	7	8	2	2
Epithemia adnata (Kützing) Brebisson	0	0	0	0	0	0	2	0	0	0	0	0
Encyonopsis krammeri Reichardt	1	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis leei Krammer var. *sinensis* Metzeltin & Krammer	2	3	1	0	1	0	0	1	0	2	0	0
Eolimna minima (Grunow) Lange-Bertalot	0	0	0	0	0	2	5	0	0	2	1	0
Eunotia pectinalis (Kütz.) Rabenhorst var. *undulata* (Ralfs) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	2
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	1	0	0	0	0	10	17	0	3	15	35	1
Epithemia sorex Kützing	1	0	0	0	9	0	0	0	0	0	0	0
Fragilaria biceps (Kützing) Lange-Bertalot	0	0	1	2	1	0	4	1	0	0	0	2
Fragilaria capucina var. *vaucheriae* (Kütz.) Lange-Bertalot abnormal form	0	0	0	0	4	0	0	0	0	0	0	0
Fallacia insociabilis (Krasske) D.G. Mann	0	1	0	0	0	0	0	0	0	0	0	2
Fallacia pygmaea (Kützing) Stickle & Mann ssp.pygmaea Lange-Bertalot	0	0	0	0	0	2	0	1	0	2	0	5
Fragilaria ulna (Nitzsch.) Lange-Bertalot var. *acus* (Kütz.) Lange-Bertalot	0	0	2	0	2	12	2	2	0	6	19	0
Fragilaria ulna (Nitzsch.) Lange-Bertalot var. *ulna*	6	2	0	0	2	4	2	0	41	2	2	0
Gomphonema affine Kützing	0	0	0	1	0	0	0	2	0	3	10	0
Geissleria decussis (Ostrup) Lange-Bertalot & Metzeltin	0	0	0	1	0	0	0	0	0	0	0	0
Gomphonema laticollum Reichardt	0	0	0	0	0	0	1	0	0	0	0	0
Gomphonema minutum (Ag.) Agardh *f. minutum*	0	0	3	1	0	5	1	2	0	10	2	0
Gomphonema parvulum (Kützing) Kützing var. *parvulum f. parvulum*	0	0	3	12	2	2	4	10	13	5	13	1
Gomphonema parvulum var.parvulum f.saprophilum Lange-Bert. & Reichardt	0	0	0	0	1	0	0	3	0	2	2	0
Gomphonema pumilum var. rigidum Reichardt & Lange-Bertalot	0	0	0	5	0	1	3	1	3	42	2	1
Gomphonema venusta Passy. Kociolek & Lowe	6	15	17	12	11	47	12	24	16	29	6	2

Diatom species name	Sep-19				Oct-19				Mar-20			
	site 1	site 2	site 3	site 4	site 1	site 2	site 3	site 4	site 1	site 2	site 3	site 4
Hantzschia amphioxys (Ehr.) Grunow in Cleve et Grunow 1880	0	0	0	0	0	0	0	1	0	0	0	0
Hippodonta capitata (Ehr.) Lange-Bert.Metzeltin & Witkowski	0	0	0	0	0	0	0	0	0	1	0	0
Hantzschia distinctepunctata Hustedt in Schmidt & al.	0	0	0	0	0	2	0	1	0	0	0	0
Luticola kotschyi (Grunow) in TDI3 Kelly	0	0	1	0	0	0	0	0	0	0	0	0
Melosira species	0	0	0	0	2	0	0	0	0	0	0	0
Melosira varians Agardh	0	0	0	0	24	1	1	0	10	0	0	0
Nitzschia acidoclinata Lange-Bertalot	0	0	0	1	0	0	0	0	0	0	0	0
Navicula agnewii Cholnoky	0	0	0	0	0	0	0	0	5	0	0	0
Navicula sp.	0	3	0	0	0	0	1	0	0	2	0	1
Navicula atomus (Kütz.) Grunow var. *atomus*	1	0	0	0	0	0	2	2	0	4	1	3
Nitzschia capitellata Hustedt in A.Schmidt & al.	0	1	0	2	0	0	1	2	0	2	12	0
Navicula capitatoradiata Germain	0	1	0	1	0	2	0	3	0	1	0	0
Navicymbula pusilla Krammer var *pusilla*	0	0	0	0	0	0	0	0	1	0	0	0
Navicula cryptocephala Kützing	0	0	1	2	0	2	0	2	0	1	0	0
Navicula cryptotenella Lange-Bertalot	0	0	4	7	0	0	3	3	4	0	3	1
Navicula dulcis Patrick	9	7	10	2	14	11	10	6	39	20	14	5
Nitzschia dissipata (Kützing) Grunow var.*media* (Hantzsch.) Grunow	0	0	0	0	0	0	0	0	0	1	0	0
Navicula erifuga Lange-Bertalot	0	0	2	0	0	0	0	0	0	0	1	1
Nitzschia etoshensis Cholnoky	1	0	3	0	5	0	2	1	22	5	2	0
Nitzschia filiformis (W.M.Smith) Van Heurck var. *filiformis*	0	0	0	1	0	2	0	0	0	2	0	0
Navicula germainii Wallace	1	0	1	1	0	0	2	3	1	2	1	0
Navicula heimansioides Lange-Bertalot	3	0	1	1	2	1	4	3	0	1	0	1
Nitzschia frustulum (Kützing) Grunow var.*frustulum*	1	1	1	5	1	7	6	2	3	3	3	0
Nitzschia liebetruthii Rabenhorst var.*liebetruthii*	0	0	1	2	0	5	8	6	0	3	1	0
Navicula libonensis Schoeman	0	0	0	0	0	0	0	0	0	0	0	1
Nitzschia linearis (Agardh) W.M.Smith var.*linearis*	0	2	1	4	0	1	1	2	0	0	4	1

Diatom species name	Sep-19				Oct-19				Mar-20			
	site 1	site 2	site 3	site 4	site 1	site 2	site 3	site 4	site 1	site 2	site 3	site 4
Nitzschia linearis (Agardh) W.M.Smith var.*subtilis* (Grunow) Hustedt	0	0	0	0	0	1	0	0	0	0	0	0
Navicula microcari Lange-Bertalot	1	0	1	0	0	1	0	0	0	0	0	0
Nitzschia obtusa W.M.Smith var. *kurzii* (Rabenhorst) Grunow	4	2	0	0	0	0	0	0	0	0	0	2
Nitzschia palea (Kützing) W.Smith	7	0	3	2	0	12	2	4	0	9	17	3
Nitzschia perspicua Cholnoky	0	2	0	0	16	1	4	0	24	3	0	0
Navicula radiosa Kützing	0	0	1	0	0	3	0	0	0	3	0	0
Navicula rostellata Kützing	0	6	4	1	1	0	3	2	1	0	0	1
Navicula schroeteri Meister var. *schroeteri*	0	1	3	0	0	1	20	4	0	10	16	0
Nitzschia sigma (Kützing) W.M.Smith	0	0	0	0	0	1	0	0	0	0	0	0
Navicula symmetrica Patrick	0	0	0	0	0	0	0	0	0	3	0	0
Navicula veneta Kützing	0	0	0	1	0	2	0	2	0	0	0	0
Navicula viridula (Kützing) Ehrenberg	0	0	2	0	0	1	1	0	0	1	0	0
Nitzschia vanoyei Cholnoky	0	0	0	0	0	0	0	0	0	0	0	2
Nitzschia species	5	1	0	0	3	0	2	0	0	2	0	1
Placoneis dicephala (W.Smith) Mereschkowsky	0	0	0	1	0	0	0	0	0	0	0	0
Planothidium engelbrechtii (Choln.) Round & Bukhtiyarova	0	1	12	11	0	5	6	40	0	4	15	36
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0	0	0	0	0	0	0	1	0	0	0	0
Pleurosigma salinarum (Grunow) Cleve & Grunow	0	0	0	1	0	0	0	0	0	2	0	3
Pseudostaurosira brevistriata (Grun.in Van Heurck) Williams & Round	0	0	0	2	1	0	0	9	0	0	0	3
Planothidium rostratum (Oestrup) Round & Bukhtiyarova	0	4	0	3	0	1	3	5	1	1	6	14
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	96	11	2	8	41	32	7	11	7	3	2	0
Sellaphora pupula (Kützing) Mereschkowksy	0	0	0	1	0	3	0	0	2	0	0	0
Sellaphora seminulum (Grunow) D.G. Mann	0	0	0	0	0	4	0	0	0	0	1	11
Tabularia fasciculata (Agardh)Williams et Round	3	0	0	1	2	0	0	5	0	2	1	2
Tryblionella hungarica (Grunow) D.G. Mann	1	0	0	0	0	0	1	0	0	4	0	0