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Water Quality Report: Diatoms (2018-2021)

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Overview

Droughts and floods are some of nature's most challenging conditions to overcome for many organisms. The conditions created by these events are extremely difficult to survive for some organisms because it forces them to cope with situations beyond their tolerance range. Those that cannot survive the conditions they are in will perish if they cannot relocate or adapt. These naturally occurring conditions are becoming increasingly common due to global warming. Global circulation cycles such as the hydrological cycle, are thrown out of balance and are becoming increasingly unpredictable. It is therefore necessary to evaluate the effects of these conditions on natural ecosystems as well as to mitigate the effects thereof.

Diatoms are excellent bio-indicator organisms and have been employed as such for many years. Diatoms respond well to changes in environmental conditions such as habitat alterations, elevation changes, nutrient and pollutant changes as well as elevated toxicant levels. Diatoms also have very short generation times which allow us to evaluate the effects of the aforementioned beyond one generation. Additionally, diatoms not only respond well to these changes, but they also respond very predictably. It is therefore possible to use diatoms as indicators of changes in environmental constituents, whose concentrations in respective systems such as rivers are altered by droughts and floods. Floods and droughts have many, generally unpredicted, effects on water quality variables that very much depend on geomorphology, geology and other catchments characteristics, when considering rivers.

This study evaluated changes in diatom community structures, both in terms of species prevalence as well as abundance. Diatom indices are the tools that we employ to mathematically calculate water quality based on the characteristics of diatom community compositions. In this study, four diatom indices were calculated. However, one index, the SPI or Specific Pollution sensitivity Index is by far the best index to use for determining water quality and was consequently preferred in this study.

Diatom index scores (SPI scores) were thus calculated for each site along each river across all years. These were compared to one another as well as with *in situ* water quality taken, to determine how diatom community structures correlate with different water quality variables. In order to do this, Correlation analysis was conducted by using STATISTICA to show relationships between diatom index scores and water quality parameters. Multivariate analysis was conducted using CANOCO to show relations between diatom community composition and water quality.

Introduction

The Kruger National Park (KNP) is one of Africa's most prestigious parks. It hosts many species across many taxonomic levels and due to its scale mostly maintains the natural order of ecosystems within it. The park is invaluable in its contribution to the protection of the remaining natural ecosystems on the planet. However, with global climate change, the functioning of these ecosystems is under threat. Not only is it under threat by climate change, but also by anthropogenic influence such land-use, mining, agriculture and industries to name a few.

The KNP implemented the River Health Programme (RHP) in 1994, as set forth by the Department of Water Affairs (DWAF), to ensure higher water quality for the rivers that are found within the park (Mohlala *et al*., 2014). The perennial rivers running through the park are subject to upstream practices that include agriculture, industry, and mining. Additionally, the park is also vulnerable to effects of droughts and floods as they can severely impact the functioning of an ecosystem.

The park has a history of 30 years of applied aquatic ecosystem research, however, each one of its rivers has a unique set of challenges. The KNP is positioned between two trans-boundary river basins. The Letaba, Olifants, and Luvuvhu rivers all contribute to the Limpopo Basin, while the Sabie and Crocodile rivers contribute to the Incomati Basin (Riddell *et al*., 2019). A study conducted by Riddell *et al*. (2019) set out to provide an overview of the challenges facing large protected areas, like the KNP, in terms of effects of upstream pollutants on the viability of aquatic ecosystems. The responsibility to conserve the ecosystems within the KNP remains that of the park, and the use of diatoms and other bioindicators of water quality, revealed the paramount importance of conserving aquatic ecosystems in terms of exposure to pollutants (Riddell *et al*., 2019).

Anthropogenic factors, as mentioned, as well as the natural occurrence of droughts and floods, influence the prevalence and concentration of pollutants in lotic ecosystems. Mining, agriculture and industries all serve as direct or indirect sources of pollution. Industries mainly release effluent into rivers that contain organic pollutants. Mining often creates opportunity for acid mine drainage, which contains toxicants such as heavy metals that can negatively influence biota in lotic systems. Agricultural practices serve as indirect sources of pollution. Nutrients applied during farming wash-off with rainwater and often flow into rivers as nutrient rich effluent that can cause significant loading of excess nitrogen and phosphorous in the water.

Due to global climate change, including anthropogenic influences such as the burning of fossil fuels, the cycles that govern our planet are under threat. The hydrological cycle, that governs the circulation of water around the globe, through all its phases, is greatly impacted by climate change (Sohoulande *et al*., 2015). The components of the hydrological cycle, which include precipitation, transpiration, and evapotranspiration among others, are all affected. However, the magnitude of these effects on the respective components differs distinctly temporally and spatially (Sohoulande *et al*., 2015). These changes within the system cause infrequent and unpredictable climatic events such as droughts and floods. The frequency and magnitude of these events are also changing and thus, calls for the evaluation of the effects of these natural phenomena.

Drought conditions and flood events also impact the water quality of the Park's rivers. Flooding can have beneficial or detrimental effects on the water quality of rivers. Primary beneficial effects include an increase in discharge, which can increase nutrient transport to increase the metabolic activity of algae and other aquatic organisms. Conversely, the increased discharge can strengthen the adsorption and transport of contaminants which can be detrimental to the water quality of rivers and are thus necessary to evaluate (Dalu *et al*., 2014). Additionally, increasing stream velocity can aid in the removal of contaminants through transport, however, these contaminants can be deposited further downstream where they can have an exacerbated effect on the aquatic system. Furthermore, increased stream velocity can interfere with diatom assemblage immigration rates as well as recolonisation rates, as high flow conditions cause sloughing of diatom cells reducing the biofilm available to grazers (Dalu *et al*., 2014). Similar to floods, droughts can also have beneficial and detrimental effects on water quality, although the beneficial effects are very limited.

During drought conditions, high evaporation rates of surface waters occur due to high atmospheric temperatures and low-flow conditions caused by a lack of precipitation (van Vliet & Zwolsman, 2008). These low-flow conditions coupled with high atmospheric temperatures may contribute to the creation of eutrophic environments with warmer surface water temperatures, an increase in nutrient concentration and an increase in suspended solids. This increases the primary productivity of algae, however, the solubility of oxygen produced by algae is hindered by the high surface water temperatures (van Vliet & Zwolsman, 2008). Additionally, cyanobacterial blooms are also common in these conditions that cause water quality to deteriorate further by the release of cyanotoxins into the system. A higher concentration of total suspended solids causes higher adsorption rates for some heavy metals and nitrates that can be beneficial for water quality, however, the increase in suspended material can also increase the adsorption of toxicants and pollutants that can be detrimental to the aquatic system, and since flow conditions are very low, the residence time of these toxins and pollutants is also much longer (van Vliet & Zwolsman, 2008). Low-flow conditions can also decrease the dissolved oxygen concentration due to a lack of turbulent mixing.

The extent of these aforementioned impacts can to a certain degree be determined by using diatoms as bio-indicators. Diatoms are often the dominant primary producers in lotic systems and respond rapidly and directly to growth stimulants such as nutrients, habitat alteration, and stressors like contaminants and pollutants (Dalu & Froneman, 2016; Taylor, 2007). Therefore, diatoms are useful bioindicators organisms for water quality changes and have been used as such for several decades around the globe. Diatoms have since 2005 formed part of the DWS biomonitoring programme, or River Health Program (RHP), due to the cost-effectiveness and ease of use in some aspects (especially sampling which is rapid and relatively easy).

By using diatoms as bioindicators, with their well-established links to water quality changes, some of the effects of these events may be evaluated in the short term within the KNP. Aquatic communities themselves, including the diatoms, can only be conserved if the environment as a whole is conserved and part of this conservation is the maintenance of a certain standard of water quality. While the diatoms have value as indicators of pollution and as a food source they also have intrinsic value. The conservation status of diatoms and other algae in South Africa is almost entirely unknown.

Study area

The Kruger National Park (KNP) serves as the study area. Five perennial rivers were sampled within the KNP at different sites along each river (Figure 1). The rivers sampled were: The Luvuvhu River (4 sites), Letaba River (3 sites), Olifants River (3 sites), Sabie River (6 sites) and the Crocodile River (4 sites). Sampling was conducted in September of 2018 and 2019, when drought conditions were experienced, as part of the KNP internal monitoring program. Samples were also taken during September of 2020 and 2021 to assess the high rainfall events that were experienced in February of both years. It is important to note that fewer samples were taken during 2020 due to Covid-19 lockdown restrictions. Along each River, the same sites were sampled across all years during the same time of the year for better interpretation of results. In each of the rivers, there were multiple sample sites amounting to 20 potential sites for each year with only one sample taken at each site in the respective rivers. During 2020 not all sites were sampled, with samples only being collected from seven sites. This creates a potential problem where results can be misinterpreted due to the small sample size. Water quality data was also not collected during 2020. However, this creates a unique opportunity to discuss the results of 2020 independently of water quality as diatoms have already been proven to correlate well with water quality variables.

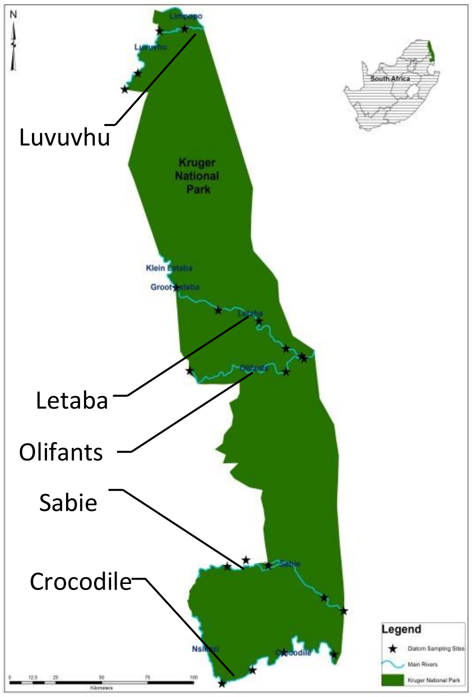

Figure 1: Map for sampling sites in the Kruger National Park. Black dots = Sites. Blue = Rivers. Credit: Hendrik Sithole

Objective

The overall objective of this study is to evaluate the impact of drought (2018, 2019) and flood (2020, 2021) events on the water quality of five perennial rivers within the KNP, in terms of trophic status, salinity and organically bound nutrients, by using diatoms as bioindicators.

Key Questions

- Does water quality, in terms of trophic status, salinity and organic material, change due to drought and flood events?
- Do droughts and floods alter diatom community composition?
- Do diatom index scores accurately reflect water quality changes between sites and rivers?

Information regarding the effectiveness of diatoms as bioindicators of water quality is well supported and has been utilised by the KNP as a part of the internal monitoring programme to assess diatom communities within rivers, as well as their responses to water quality in the Makuleke wetlands. Also, the relationship between diatom taxa and water quality variables is well documented and has been used in many studies pertaining to water quality changes due to droughts and floods. The use of diatoms to assess the ecological status of the park's rivers is also underway, although still in its infancy. Thus, the use of diatoms as bioindicators of should be accurate in evaluating the effect of drought and flood conditions on the trophic status of rivers within the park.

Materials & Methods

Sampling:

During the course of this project, samples were collected from various sites, as determined by the KNP's internal monitoring programme, along five perennial rivers in the park. Diatom samples from solid substrates (epilithic) were taken at each site according to the following procedure.

Diatom sampling entails scraping material from 5-10 rocks or boulders, on the exposed side of the rock, with a clean toothbrush, into a sample tray. The sample, together with some river water, was poured into a labelled (date and location) clean sample vial and preserved with 70% ethanol for later analysis. A single sample taken at each site is considered representative of the environmental conditions of the site. If conditions allowed, sub-samples were collected from different biotopes that were available, such as pools and rapids, for comparison as well as to determine if some biotopes serve as habitats for species not found in flowing waters, these types of subsamples were mostly taken in 2020. In conjunction with diatom samples, chemical water quality data were also collected, *in situ*, at sites for later comparison with diatom community composition. pH, electrical conductivity (EC) and temperature (°C) was measured by using a multi-meter. Dissolved oxygen (DO) was measured by using an oxygen meter. Water samples (1L) were also be taken at the sites for laboratory analysis to determine the amount of inorganic compounds (ammonia, ammonium, chloride, and sodium) present for later comparison with diatom community composition.

Laboratory procedures:

Samples were prepared by using the hot potassium permanganate and hot hydrochloric acid method (Taylor *et al*., 2007). After treatment with chemicals, the samples were rinsed by use of centrifugation until circumneutral. The samples were then poured into a clean sample vial that was labelled accordingly. Thereafter, a small amount of dilute sample (400µl) was placed on a coverslip and allowed to air dry for 24 hours. The dried coverslip was then mounted to a microscope slide by using pleurax (refractive resin).

The microscope slides were analysed by using a Nikon 80i microscope under 100X magnification. On each slide 400 cells were counted to represent the community structure of selected sites. Species were identified to genus as well as to species level if possible. Images of species were collected for archiving and confirmation of identifications.

Water samples taken were analysed by using the appropriate water testing kits for inorganic and organic compounds.

Data analysis:

Correlation analyses and multivariate analysis was performed on the data. Correlation analysis was conducted using STATISTICA to determine the relation between diatom index scores and water quality (Taylor *et al*., 2007). Multivariate analysis was conducted using CANOCO, which uses canonical correspondence analysis as an ordination method to show the relationship between diatom community composition and water quality. Diatom index scores were calculated using Omnidia v 5.3 (Taylor *et al*., 2007). The index scores were be used to determine water quality impacts for sites along selected rivers. The water quality obtained by using diatoms can be compared between 2018, 2019, 2020 and 2021 to determine which rivers had lower water quality and which have higher water quality in terms of trophic status, ionic concentrations and organic material.

Results & Discussion

Diatom Community Composition:

2018/2019

Overall, 146 taxa were found of which 16 were dominant across all sites and had an abundance greater than 5%. The remaining 130 taxa had abundance lower than 5% across all sites and therefore do not contribute as much to the Specific Pollution sensitivity Index calculation (SPI)

Table 1: The most abundant diatom species and genera across all sites for 2018 and 2019.

Abbreviation	Name of taxa	Trophic preference or range
ACHD	*Achnanthidium* Kützing	Oligo- to eutrophic
CKOL	*Cymbella kolbei* Hustedt	Oligotrophic
CPED	*Cocconeis pediculus* Ehrenberg	Meso- to eutrophic
CPLA	*Cocconeis placentula* Ehrenberg	Meso- to eutrophic
CTGL	*Cymbella turgidula* Grunow	Oligo- to mesotrophic
ENLS	*Encyonopsis leei var. sinensis* Metzeltin & Krammer	Oligo- to mesotrophic
ESOR	*Epithemia sorex* Kützing	Meso- to eutrophic
FRAG	*Fragilaria* Lyngbye	Oligo- to eutrophic
GVNU	*Gomphonema venusta* Passy, Kociolek & Lowe	Oligo- to mesotrophic
NAVI	*Navicula* sp.	Oligo- to eutrophic
NIFR	*Nitzschia frustulum* (Kützing)	Eutrophic
NITZ	*Nitszchia* Hassall	Oligo- to eutrophic
NMCY	*Navicula microlyra* Cholnoky	Oligo- to mesotrophic
PRST	*Planothidium rostratum* Lange-Bertalot	Oligo- to mesotrophic
RABB	*Rhoicosphenia abbreviata* Lange-Bertalot	Eutrophic
TFAS	*Tabularia fasciculata* (Agardh) Williams & Round	Meso- to eutrophic

2020

Overall, 115 taxa were counted and identified to genus level, some of which were also identified to species level. 16 of the taxa present represents 4% or more of the diatom valves counted and will therefore have the greatest influence on the calculation of diatom indices.

Table 2: The most abundant diatom taxa across all sites, representing 4% or more of the community composition.

Abbreviation	Name of taxa	Trophic preference or range
ACHD	*Achnanthidium* sp.	Oligo- to eutrophic
ADMI	*Achnanthidium minutissimum* Kützing	Oligotrophic
ADUL	*Anorthoneis dulcis* Hein	Oligotrophic
CPED	*Cocconeis pediculus* Ehrenberg	Meso- to eutrophic
CPLA	*Cocconeis placentula* Ehrenberg	Meso- to eutrophic
CTGL	*Cymbella turgidula* Grunow	Oligo- to mesotrophic
ENLS	*Encyonopsis leei* var. *sinensis* Metzeltin & Krammer	Oligo- to mesotrophic
ENMI	*Encyonema minutum* DG Mann	Oligo- to mesotrophic
FUNG	*Fragilaria ungeriana* Grunow	Oligo- to mesotrophic
GNUN	*Gomphonitzschia ungeri* Grunow	Oligo- to mesotrophic
GPAR	*Gomphonema parvulum* Kützing	Eutrophic
MVAR	*Melosira varians* Agardh	Meso- to eutrophic
NAVI	*Navicula* sp.	Oligo- to eutrophic
NITZ	*Nitzschia* sp.	Oligo- to eutrophic
RGIB	*Rhopalodia gibba* Ehrenberg	Meso- to eutrophic
TFAS	*Tabularia fasciculata* (Agardh) Williams & Round	Meso- to eutrophic

2021

Overall, a total of 99 species were identified and counted during 2021. Twenty-three of the species had abundance greater than 2% across all sites and contributed the most to the calculation of the Diatom index scores. For 2018 and 2019, the chosen abundance threshold was set at 5%. the same threshold was chosen at first for the year 2020 and 2021, however, this threshold included too many species for the CCA plot. Therefore, in attempt to declutter the CCA plot, a threshold of 2% was used.

Table 3: The most abundant diatom taxa across all sites, comprising 2% or more of the community composition.

Abbreviation	Name of taxa	Trophic preference or range
ACHD	*Achnanthidium* sp.	Oligo- to eutrophic
ADCR	*Achnanthidium crassum*	Oligo- to mesotrophic
ADMI	*Achnanthidium minutissimum* Kützing	Oligotrophic
ADUL	*Anorthoneis dulcis* Hein	Oligotrophic
CPED	*Cocconeis pediculus* Ehrenberg	Meso- to eutrophic
CPLA	*Cocconeis placentula* Ehrenberg	Meso- to eutrophic
CTGL	*Cymbella turgidula* Grunow	Oligo- to mesotrophic
CTUM	*Cymbella tumida* (Brébisson) Van Heurck	Oligo- to mesotrophic
CYMB	*Cymbella* spp.h	Oligo- to mesotrophic
ENLS	*Encyonopsis leei* var. *sinensis* Metzeltin & Krammer	Oligo- to mesotrophic
FUMP	*Fallacia umpatica* Cholnoky	Meso- to eutrophic
GOMP	*Gomphonema* spp.	Oligo- to eutrophic
GPAR	*Gomphonema parvulum* Kützing	Eutrophic
GPRI	*Gomphonema pumulim* var. *rigidum Reichardt & Lange-Bertalot*	Meso- to eutrophic
GVNU	*Gomphonema venusta* Passy, Kociolek & Lowe	Oligo- to mesotrophic
KPLO	*Kolbesia ploenensis* (Hustedt) Kingston	Oligo- to mesotrophic
NAMP	*Nitzschia amphibia* Grunow	Eutrophic
NAVI	*Navicula* sp.	Oligo- to eutrophic
NDIS	*Nitzschia dissipata* (Kützing) Grunow	Oligo- to mesotrophic
NITZ	*Nitszchia* spp.	Oligo- to eutrophic
NROS	*Navicula rostellata* Kützing	Eutrophic
NVDA	*Navicula vandamii* Schoeman & Archibald	Eutrophic
SSEM	*Sellaphora seminulum* (Grunow) DG Mann	Eutrophic

Trophic preferences for taxa are extrapolated from sources such as (Taylor *et al.,* 2007; Cocquyt & Taylor, 2016)

Site numbers only pertain to specific years. Corresponding site numbers for years should be used in corresponding graphs and figures pertaining to the results of the same year.

Water quality Measured:

Table 4: Water quality measured, *in situ*, during 2018 and 2019.

Year	River	Site name	Nr	Collection nr	Salinity	pH	EC (µs/cm)	Turbidity
2018	Sabie	Sekorongwane	23	20-077	58.1	9.04	126.8	84
		Tinga	21	20-074	60.2	8.86	132.3	79
		Lubye Lubye	36	20-091	65.6	8.39	144.6	15
		Antholysta	17	20-070	61.9	8	133.8	71
		Sabiepoort	24	20-078	79.2	7.71	156	48
	Crocodile	Nsikazi	26	20-081	236	8.57	501	83
		Malelane	27	20-082	192	8.68	413	76
		Marula	33	20-088	206	8.41	440	81
		Nkongoma	32	20-087	275	8.79	593	86
	Olifants	Mamba	28	20-083	269	8.8	577	24
		Balule	20	20-073	292	8.89	613	21
		Confluence	34	20-089	276	8.79	645	76
	Letaba	Lonely Bull	30	20-085	227	8.78	598	62
		Confluence	19	20-072	252	9.93	537	97
	Luvuvhu	Dongadziva	25	20-079	66.6	7.96	146.8	N/A
		Xindzivhani	29	20-084	74.7	8.3	158.3	N/A
		Mutale (Outpost)	35	20-090	71.8	8.61	152.8	N/A
		Bobomane	22	20-076	78.5	8.69	160.9	N/A
2019	Sabie	Sekorongwane	7	20-059	55.5	8.6	121.5	89
		Tinga	8	20-060	60.1	8.45	128.1	75
		Lubye Lubye	6	20-058	72.8	8.3	153.4	18
		Antholysta	31	20-086	68.5	8.41	143.6	62
		Sabiepoort	5	20-057	71.8	7.6	154.8	57
	Crocodile	Nsikazi	1	20-053	241	8.71	518	88
		Malelane	2	20-054	228	8.95	485	86
		Marula	3	20-055	254	8.43	582	80
		Nkongoma	4	20-056	392	9.08	817	97
	Olifants	Mamba	16	20-069	372	9.21	773	65
		Balule	14	20-067	365	8.95	762	58
		Confluence	9	20-061	340	8.89	705	59
	Letaba	Lonely Bull	11	20-063	285	8.2	598	62
		Confluence	18	20-071	255	9.58	537	97
	Luvuvhu	Dongadziva	12	20-065	77.6	8.26	164.4	N/A
		Xindzivhani	15	20-068	78.5	8.33	166.3	N/A
		Mutale (Outpost)	10	20-062	126	8.4	267	N/A
		Bobomane	13	20-066	102	8.84	216	N/A

Table 5: Water quality parameter values measured and calculated during 2021.

				in situ						*Lab*			
				ppm	µs/cm		ppm	mg/l	°C	mg/l	mg/l	mg/l	mg/l
Year	**River**	**Site**	**Nr**	**Na+**	**EC**	**pH**	**Nitrate**	**DO**	**Temp**	**Chloride**	**Nit-Ammonia**	**Sulfate**	**Phosphate**
2021	**Sabie**	Sekerongwane	1	7	148	7.35	16	4.7	21.6	0.2	0.24	69.5	0.08
		Tinga	2	9	165	7.55	13	5.12	24.8	1	0.32	83.6	0.13
		Sand	3	30	234	8.24	14	5.29	27.9	10.8	0.06	69.8	0.03
		Lubye Lubye	4	12	176	8.21	7	6.56	20.8	2.8	0.18	69.6	0.17
		Antholysta	5	16	178	8.37	12	5.98	28.1	6	0.16	92.4	0.07
		Sabiepoort	6	13	209	7.37	8	5.37	23	2.8	0.06	72.9	0.1
	Crocodile	Malelane	7	40	518	7.28	17	6.34	18.7	35.9	0.31	198	0.53
		Marula	8	48	570	8.11	11	6.75	19	11.5	0.04	90	0.07
		Nkongoma	9	69	742	8.23	17	7.13	21	39.4	0.18	94.1	0.05
	Olifants	Mamba	10	72	852	7.72	17	8.39	24	24.9	0.19	166	0.28
		Balule	11	49	738	8.42	21	5.73	27.6	25.2	0.18	129	0.06
		Confluence	12	60	620	8.44	20	7.19	24.2	35.4	0.32	537	0.12
	Letaba	Lonely Bull	13	54	335	6.31	14	6.62	20.3	17.4	0.17	69.1	0.07
		Klipkoppies	14	49	476	8.75	15	7.4	27.6	26.2	0.16	70.9	0.13
		Confluence	15	70	502	7.76	23	5.3	24.4	57	0.38	89.8	0.38
	Luvuvhu	Dongadzhiva	16	11	145	7.73	15	5.21	24.7	1.7	0.15	67.6	0.03
		Xindizivhani	17	11	142	8.04	10	5.77	25.5	1.8	0.15	68.4	0.02
		Mutale/Outpost	18	48	145	6.33	14	6.16	21.9	11.7	0.21	68.4	0.04

Water quality as calculated from diatom indices:

Most diatom indices are calculated based on a weighted average equation by Zelinka & Marvan (1961) and have the following form:

$$index = \frac{\sum_j^n = 1^{a_j s_j v_j}}{\sum_j^n = 1^{a_j v_j}}$$

Where $\boldsymbol{a}_{j}$ represents the abundance of species $\boldsymbol{j}$ in a sample, $\boldsymbol{s}_{j}$ represents the pollution sensitivity of species $\boldsymbol{j}$ in a sample, and $\boldsymbol{v}_{j}$ represents the indicator value for species $\boldsymbol{j}$ in a sample. The performance of these indices depends on the indicator values and pollution sensitivity of species based on literature and other sources (Taylor, 2004). The values for **s** and ***v*** can range from one to the uppermost value of **s**. the number of diatoms used in different indices will also influence these numbers.

The functioning of diatom indices is a follows: The most abundant diatom species in a sample will be the species with the best tolerance for the conditions in which they occur, thus a determinant can be made from the average of the tolerance ranges for taxa present in that sample, weighted against their abundance (Taylor, 2004). Species that are found more frequently have a higher influence on the results than a species that is rare; in addition, an indicator value further strengthens or weakens the influence of certain species. A higher indicator value will result in a higher influence on water quality.

The Specific Pollution sensitivity Index (SPI), which is the preferred index for this study, allocates a score to each of the genera, or species, occurring in a specific biotope subjected to environmental pressures to determine the degree of nutrient, ionic and organic pollution present. The magnitude of the environmental disturbance and autecology of taxa will determine the diatom community composition (Tornés *et al*., 2015). The index is very reliable and has been used in the park previously by Shikwambana *et al* (2021). The index allocates a score to each site based on the abundances and autecology of each of the taxa counted. This score is scaled from 0 – 20 and given a corresponding ecological class (A - E).

Table 6: Ecological classes for SPI scores. Redrawn from (Shikwambana *et al*,. 2021).

Ecological Class	Water Quality	Trophic level	SPI Score
A	High	Oligotrophic	> 17
B	Good	Oligo-mesotrophic	15 – 17
C	Moderate	Mesotrophic	12 – 15
D	Poor	Meso-eutrophic	9 – 12
E	Bad	Eutrophic	< 9

Table 7: Diatom index scores for sites and rivers during 2021.

		GDI				SPI				BDI				%PT			
River	**Site**	**2018**	**2019**	**2020**	**2021**	**2018**	**2019**	**2020**	**2021**	**2018**	**2019**	**2020**	**2021**	**2018**	**2019**	**2020**	**2021**
Sabie	**Sekorongwane**	7.2	7.4	10.1	14.6	5.3	6.8	9.0	14.8	13.3	16.4	14.7	15.5	7.7	0.8	1.6	1.8
	Tinga	14.3	14.1	-	17.2	13.6	14.7	-	17.1	13.8	13.3	-	15.9	1.3	1.8	-	0.5
	Lubye Lubye	13.4	15.3	-	17.1	15.3	16.8	-	16.6	11.5	14.5	-	16.2	15.5	11.6	-	0.8
	Antholysta	16.4	16.2	15.6	15.5	14.6	15.5	16.2	14.5	13.3	13.0	15.7	14.0	2.2	2.5	3.0	0.8
	Sabiepoort	2.3	4.8	-	11.7	2.1	3.7	-	9.4	7.0	10.2	-	9.2	8.7	4.5	-	43.4
Crocodile	**Nsikazi**	11.5	7.6	-	-	12.1	8.1	-	-	15.2	14.1	-	-	0.5	8.8	-	-
	Malelane	10.7	12.2	-	9.1	10.4	13.5	-	8.9	13.5	15.0	-	14.0	14.4	0.0	-	12.0
	Marula	6.7	10.2	12.9	12.3	5.5	10.0	15.2	13.0	12.8	14.0	15.3	15.2	1.2	6.5	0.3	0.3
	Nkongoma	11.7	11.0	-	11.3	12.1	10.8	-	13.9	14.0	13.7	-	14.4	4.5	5.7	-	6.0
Olifants	**Mamba**	3.2	7.3	-	3.1	3.0	10.0	-	3.1	13.5	10.4	-	14.0	0.7	32.5	-	0.0
	Balule	8.4	3.0	-	3.5	8.7	4.8	-	3.5	13.8	5.4	-	12.8	2.5	48.5	-	0.5
	Confluence	12.6	12.0	-	9.6	14.9	14.0	-	10.0	14.9	9.8	-	14.5	1.5	24.4	-	0.8
Letaba	**Lonely Bull**	3.2	10.6	11.0	11.9	2.6	10.9	11.2	12.6	8.8	14.8	12.2	14.7	1.2	0.0	8.2	4.2
	Klipkoppies	12.0	12.4	-	12.5	10.1	14.1	-	14.2	12.4	15.2	-	15.1	2.8	0.2	-	2.0
	Confluence	14.9	1.9	-	10.8	13.3	1.6	-	12.7	11.5	11.7	-	13.8	0.7	3.2	-	12.0
Luvuvhu	**Dongadziva**	16.2	17.2	16.4	17.2	16.5	17.3	16.8	18.4	20.0	20.0	20.0	19.8	0.3	0.0	0.8	0.2
	Xindzivhani	15.5	17.4	15.5	17.0	15.6	16.6	16.8	18.0	13.1	16.3	17.8	19.2	0.2	0.5	2.0	0.0
	Mutale (Outpost)	12.0	13.4	14.1	14.3	11.6	13.8	15.0	16.4	12.5	14.4	15.4	15.7	3.0	4.5	3.3	0.0
	Bobomane	10.8	13.2	-	-	10.7	13.4	-	-	10.3	14.5	-	-	15.5	0.0	-	-

Limpopo Catchment

Luvuvhu River

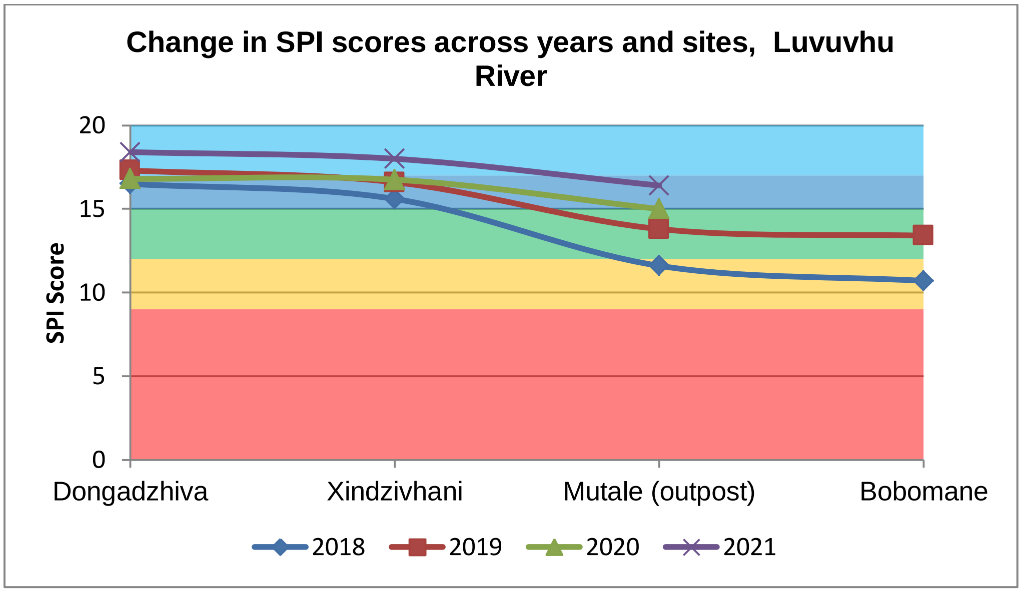

Figure 2: SPI score profile for sites along the Luvuvhu River across all sampling years.

A clear decrease in water quality is immediately observed in terms of flow from upstream to downstream (Figure 2). It is evident that, from West to East, the water quality decreases from high quality to poor or moderate quality for 2018 and 2019. No data is available for this site during 2020 and 2021.

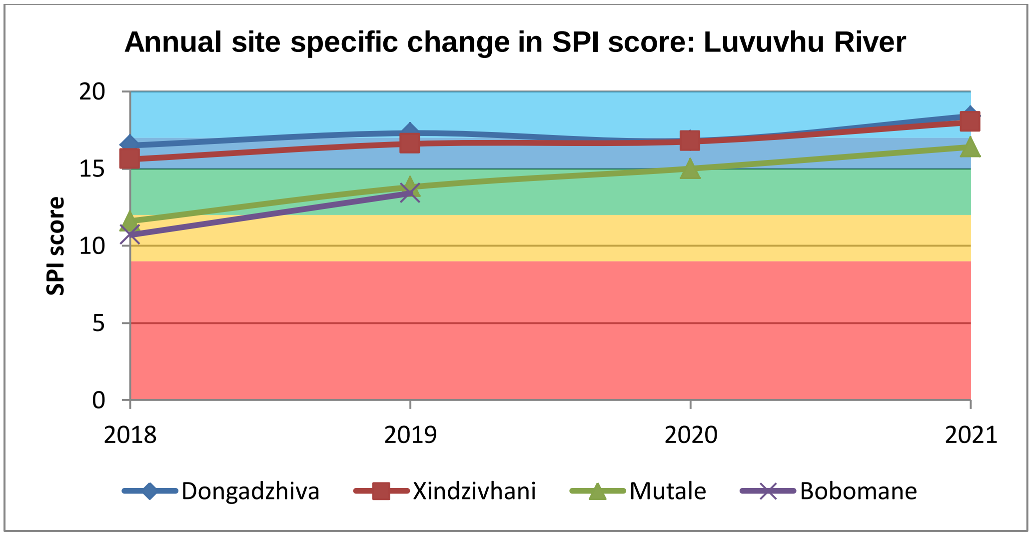

Figure 3: Annual changes in SPI scores for sites specifically in the Luvuvhu River.

Figure 3 illustrates how the SPI score has increased across all sites that have data from 2018 to 2021.

During the drought years of 2018 and 2019, the diatom communities in the Luvuvhu River were comprised of species that are able to tolerate elevated nutrient contents like *Tabularia fasciculata*, *Nitzschia frustulum* and *Achnanthidium saprophilum*, present at the Bobomane site during 2018 (Table 1 & 8). Other species present during 2019 that prefer elevated EC and higher trophic levels are *Cocconies placentula* and *Geissleria decussis*. Electrical conductivity was also higher across all sites during 2018 and 2019 than in 2021 (Tables 4 & 5).

The diatom communities present during 2020 and 2021, however, are comprised of species that prefer meso- to oligotrophic conditions as well as low to moderate electrical conductivities including *Achnanthidium minutissimum* and *Encyonopsis leei* var. *sinensis* (Table 2,3 & 8). The electrical conductivity, even when high in 2018 and 2019, are not as high as the EC for other Rivers such as the Olifants and Crocodile Rivers.

The Luvuvhu River is one of the healthiest Rivers in the KNP. Of the 14 sites sampled in this river across all years, only two sites (Mutale and Bobomane, 2018) have experienced poor water quality. Two of the remaining sites have moderate water quality (Mutale and Bobomane, 2019) and the rest all have high water quality (10 Sites). The autecology of diatoms has therefore successfully been used to determine water quality for the Luvuvhu River in terms of trophic level and levels of organic pollution. The river maintains moderate to low trophic levels and contains little organic substances. Additionally, water quality during droughts years, where water quality constituents are more concentrated and eutrophic conditions are more common, is generally lower. As high rainfall events of 2020 and 2021 were experienced, the water quality has increased. The flooding events have greatly increased the water quality for the Luvuvhu River.

Letaba River

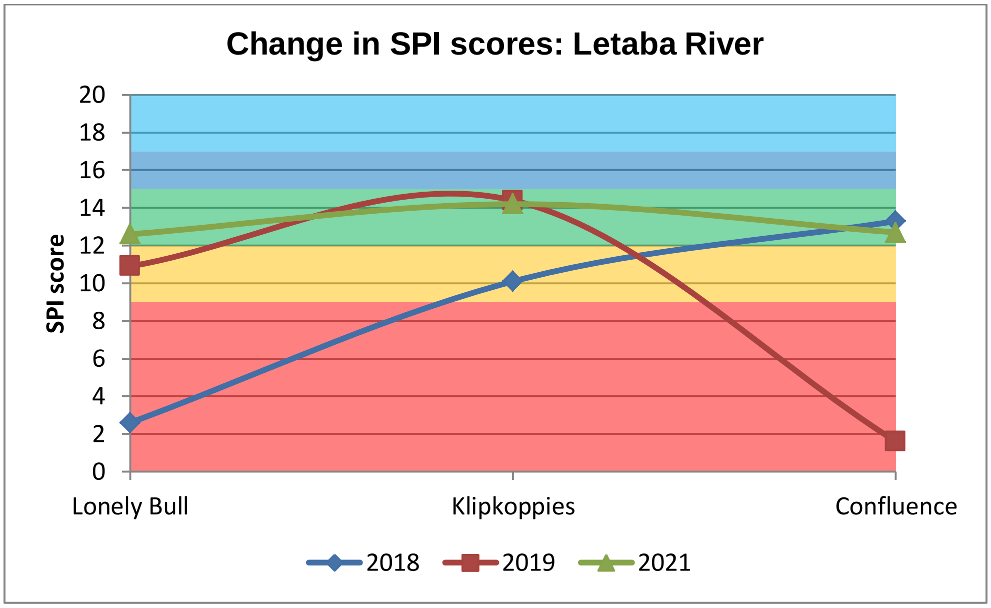

Figure 4: SPI Score change with in terms of flow direction across all years for the Letaba River.

In general higher water quality is observed for 2018 and 2021 across all sites in the Letaba River. However, during 2019 a radical decrease in SPI score is observed for the confluence site. This will be discussed further below.

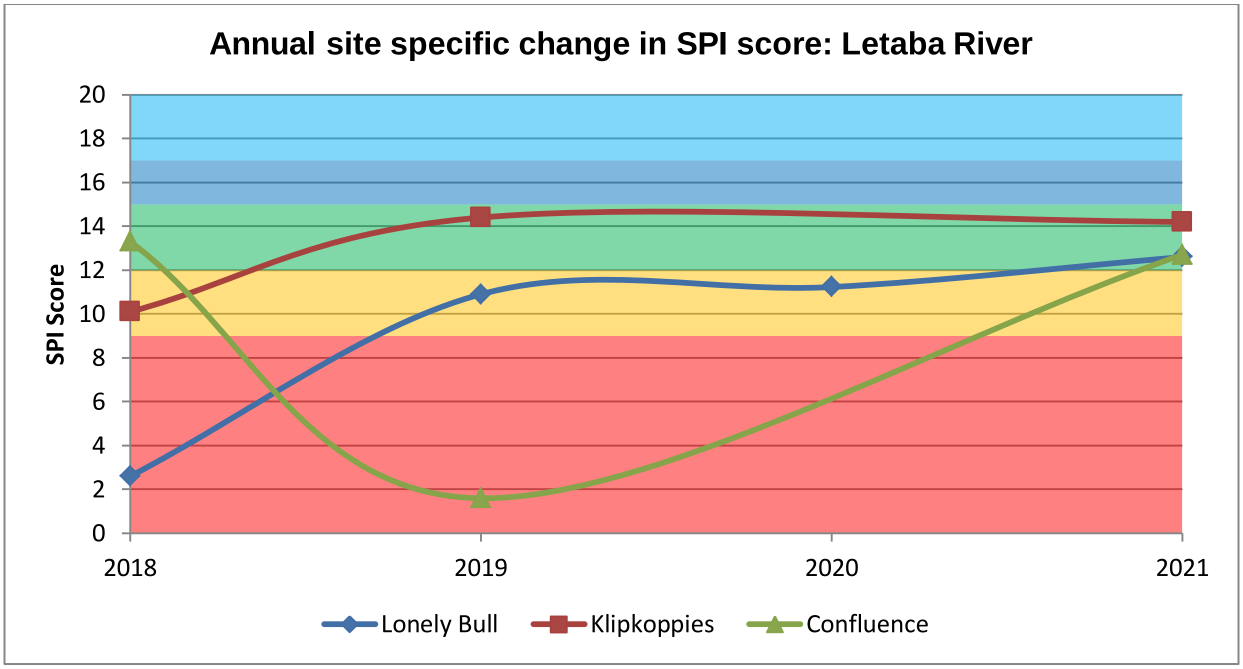

Figure 5: Annual changes in SPI scores for sites in the Letaba River

From this figure an increase in water quality is observed from 2018 to 2019 at two of the sites. The other site, Confluence, has a sharp decrease, as we saw in the previous figure. From 2019, however, a general increase or stabilisation in water quality is observed across all years for all sites in the Letaba River. During 2018 the diatom community in the River is dominated by taxa like *Nitzschia, Cocconeis placentula, Epithemia sorex* and to a lesser degree *Cymbella turgidula* (Table 9). The former three taxa are tolerant of elevated nutrients, elevated EC and higher trophic levels (Table 1). In 2019 the community was similarly dominated by the previously mentioned taxa. However, *Cocconeis placentula* increased in abundance during 2019 (Table 9) and has dominated two of the sites (Lonely Bull and Klipkoppies during 2019). The remaining site, Confluence, has been dominated by a 91% abundance of *Nitzschia.* This explains the sharp drop in the SPI score for 2019.

During 2020, *C. placentula* dominated all biotopes at the Lonely bull site in the Letaba River. However, the abundances are lower than in 2018 and a more evenly spread community composition observed (Table 9). The community composition is similar to that of 2020 for the same site, however, the evenness of taxa is higher. During 2021, similarly *C. placentula* dominates by a large degree. This taxon prefers meso- to eutrophic conditions and moderate EC. The water quality is therefore considered moderate across all sites in 2021.

Overall the water quality for the Letaba River has also increased. None of the sites have poor water quality in 2021 as opposed to 2018 and 2019. It is clear that this river has also experienced an increased water quality due to the high rainfall events of 2020 and 2021.

Olifants River

No samples were taken in the Olifants River during 2020.

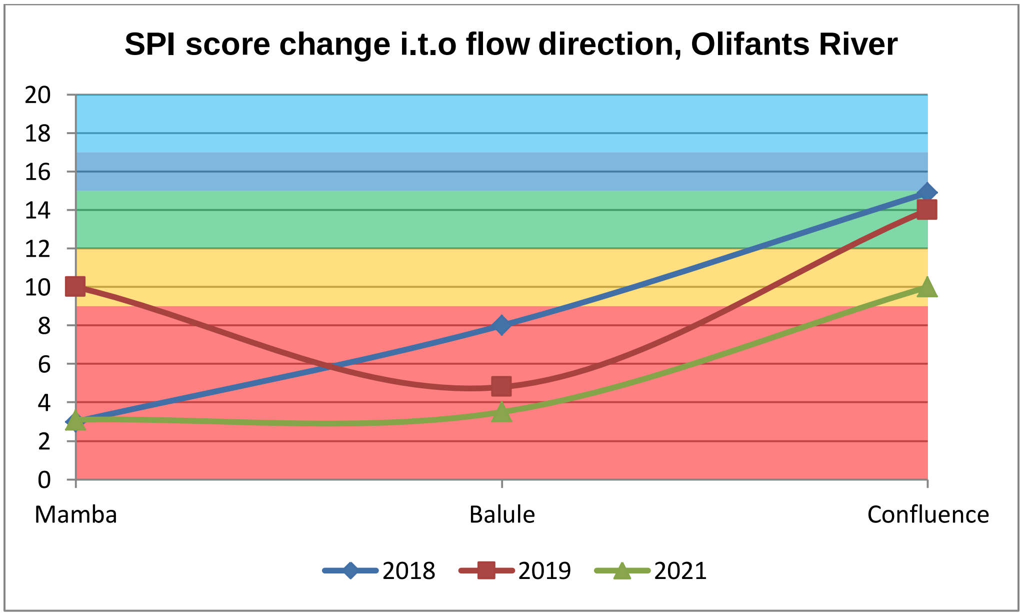

Figure 6: SPI Scores for sites in the Olifants River across sampling years.

From this figure we can see an increase in water quality in the direction of flow from West to East. The water quality has increased from bad/poor to poor/moderate from 2018 to 2021.

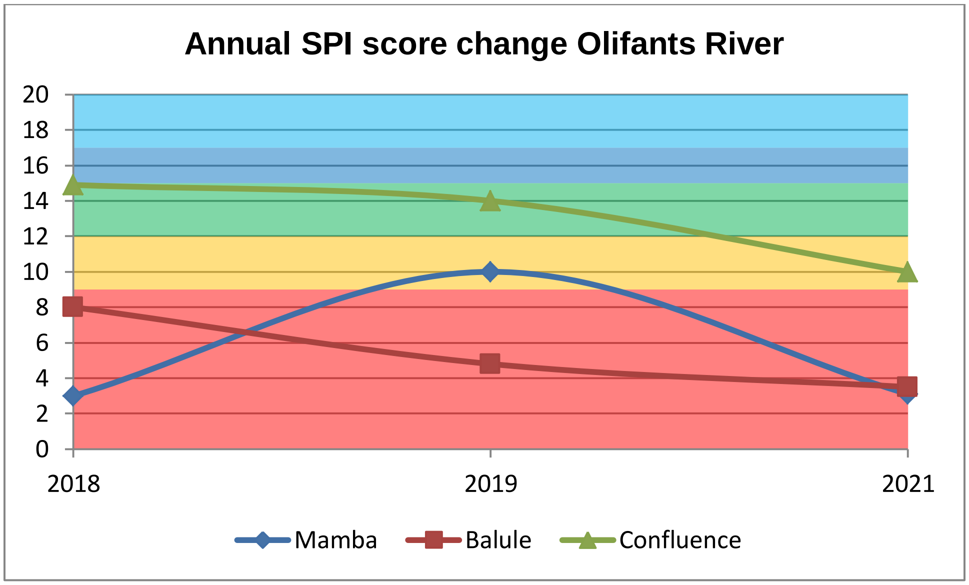

Figure 7: Annual changes in site specific SPI scores.

From 2018 to 2021 a decrease in water quality is observed. The Mamba and Balule Sites remain in poor quality, while the Confluence site has decreased from high quality in 2018 to poor quality in 2021. Figure 6 illustrates how the water quality has increased with flow direction, which is also the case for the Letaba and Luvuvhu Rivers. However, although the water quality increased with flow direction, a general decrease in this improvement of water quality is observed from 2018 to 2021.

The Olifants River is dominated by *Nitzschia* spp., *Nitzschia frustulum* and *Cocconeis placentula* (Table 10). These taxa prefer elevated EC and higher trophic levels (Table 1). *N. frustulum* can tolerate great fluctuations in osmotic pressure and *Nitzschia* spp. are in general known to be pollution tolerant. Consequently, the inferred water quality is very poor. The Olifants River has the poorest water quality of all rivers in the park across all sampling years and sites. During 2021, *Nitzschia* similarly dominates the community, it has by far the greatest abundance of taxa in all of the sites sampling during 2021. This is the only river in the park that experienced a decrease in water quality from 2018 to 2021.

Sabie River

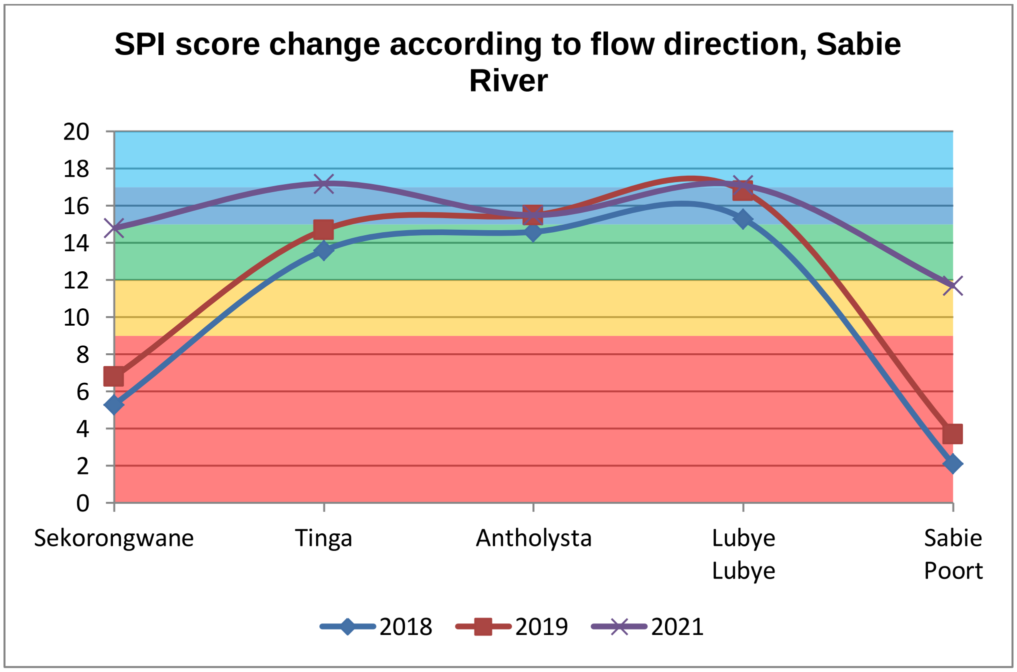

Figure 8: SPI Scores for sites in the Sabie River across sampling years, not including 2020.

Across all sites, a general profile is followed. Water quality is poor at the first site sampled, however, as the river flows downstream, water quality increases and is somewhat stable until it flows from Lubye Lubye to Sabiepoort. A radical decrease in water quality is then observed, across all years.

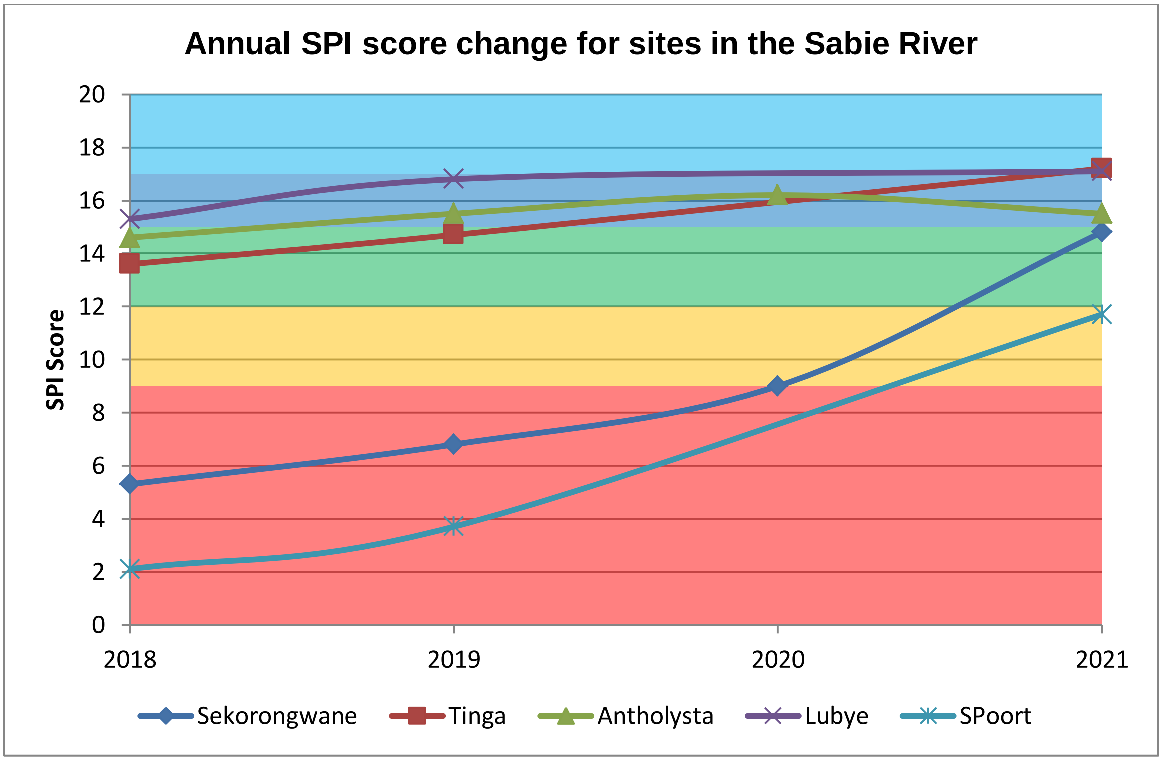

Figure 9: Changes in SPI score for sites in Sabie River, across the whole sampling period.

From this figure a clear increase in water quality is observed for all sites from 2018 to 2021. Although the Sekorongwane and Antholysta sites have the poorest water quality of all sites in the Sabie River, they still have better quality than some sites in the Olifants and Letaba rivers. The profile followed by the SPI score in figure 9 perfectly illustrates how all sites in the Sabie River have experienced an increase in water quality across years sampled as well as with flow direction. During 2018 and 2019, the Sekorongwane and Sabiepoort sites were dominated by *Nitzschia* spp., other taxa including *Gomphonema* and *N. frustulum* were also present. Other sites (Tinga, Anhtolsyta and Lubye Lubye) in the Sabie River during 2018 and 2019 have species such as *Encyonopsis leei* var. *sinensis*, *Cymbella turgidula*, *Achnanthidium* spp., *Cymbella tumida* and some *Navicula* species (Table 1& 11). These taxa prefer oligo- to mesotrophic conditions with low to moderate EC. Consequently, the SPI scores and water quality for these sites in 2018 and 2019 is moderate to high. During 2020, the Sekorongwane sites were similarly dominated by *Nitzschia* spp. as in 2018 and 2019. However, the adundance was lower in 2020.

In 2021 the Sekoronwane site was dominated by *Achnanthidium crassum*, *Navicula* spp. and *Achnanthidium.* Similarly, the Sabiepoort site has also experience in shift in the community structure. *Nitzschia* has been replaced by *Sellaphora seminulum*, *Nitzschia dissipata* and *Cocconeis placentula*. This indicates on an increase water quality for these sites. Additionally, the diversity at the Sabiepoort site for 2021 is the highest enumerated in this study. A total of 50 taxa were counted and identified from a valve count of 400.

Other sites (Tinga, Anhtolsyta and Lubye Lubye) were dominated by *Achnathidium*, *Encyonopsis leei* var. *sinensis*, *A. minutissimum* and *Cymbella turgidula* (Table 11). These taxa are indicative of high water quality as they prefer low- to moderate EC and oligo- to mesotrophic conditions (Table 3). Overall, the water quality in the Sabie River has increased from 2018 to 2021 and diatom community composition supported this conclusion.

Crocodile River

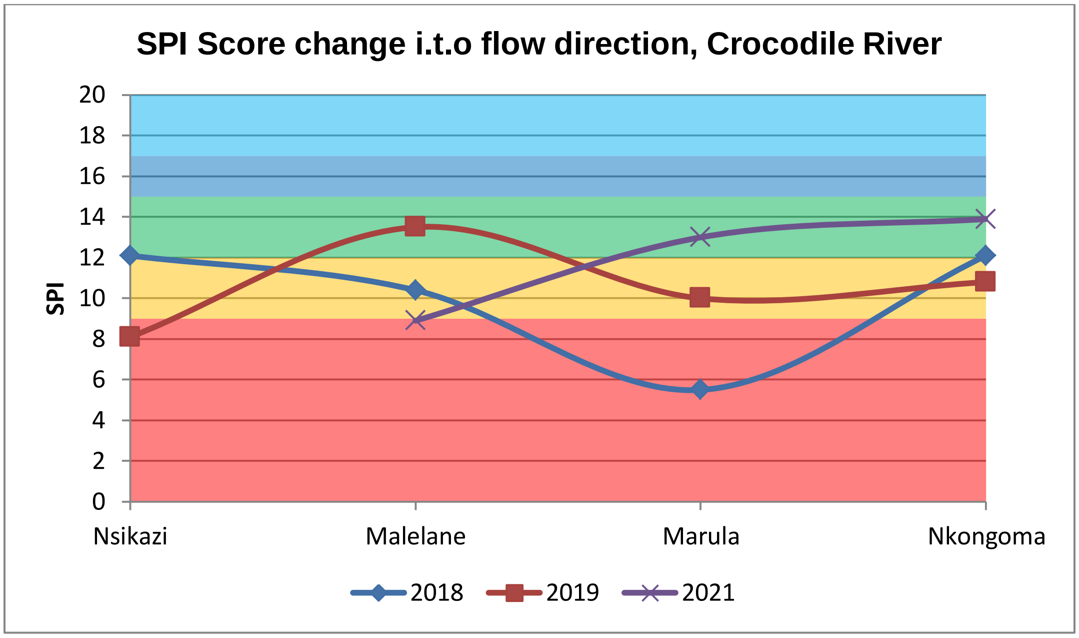

Figure 10: SPI Scores for sites in the Crocodile River across sampling years.

From this figure an increase in water quality is visible from Nsikazi to Malelane in 2019. Thereafter the water quality decreases at the Marula site in both years, after which it slightly increases in quality at the Nkongoma site. Generally, water quality in the Crocodile River has increased from 2018 to 2019. The profile for 2021 shows a clear increase in water quality with flow direction.

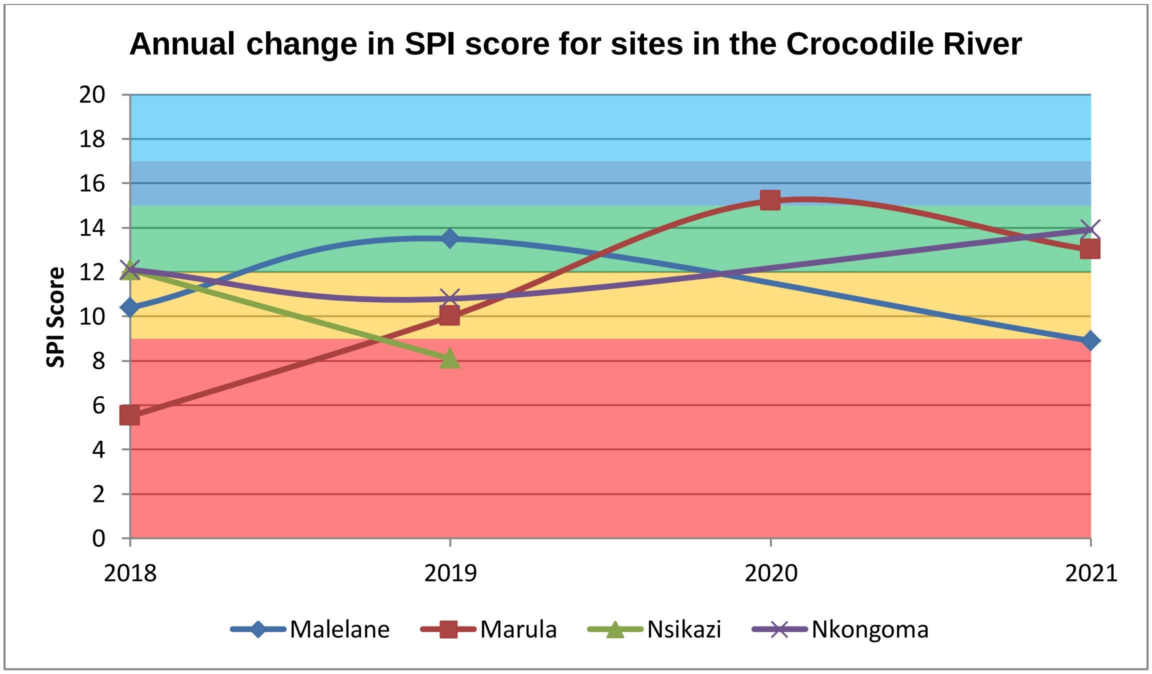

Figure 11: Annual fluctuation SPI score for sites in the Crocodile River.

A general increase in water quality is observed from 2018 to 2021.

Similar to the Olifants and Letaba rivers, the Crocodile River is completely dominated by *Cocconeis placentula* and *Nitzschia* spp (Table 12). These species indicate poor to moderate water quality. Similarly, in 2021, the sites are dominated by the same taxa, with some changes in their abundances. Evidently, water quality has slightly increased from 2018 to 2021 from figure 15. The water quality has increased from bad/poor to poor/moderate.

The high prevalence of *Nitzschia* and *C. placentula* in the Crocodile, Letaba and Olifants rivers is due to their catchment characteristics. These rivers show an elevation in EC as shown by figure 2.This is possible due to the practices and land-use these rivers are subjected to

Water quality correlations:

The following figures are based on *in situ* water quality data taken during 2021. The reason for this is twofold. Firstly, few *in situ* water quality measurements were taken during 2018/2019 and no data was collected during 2020. Secondly, this data illustrates how electrical conductivity is related to the SPI index as well as which specific water quality constituents contribute most to the electrical conductivity.

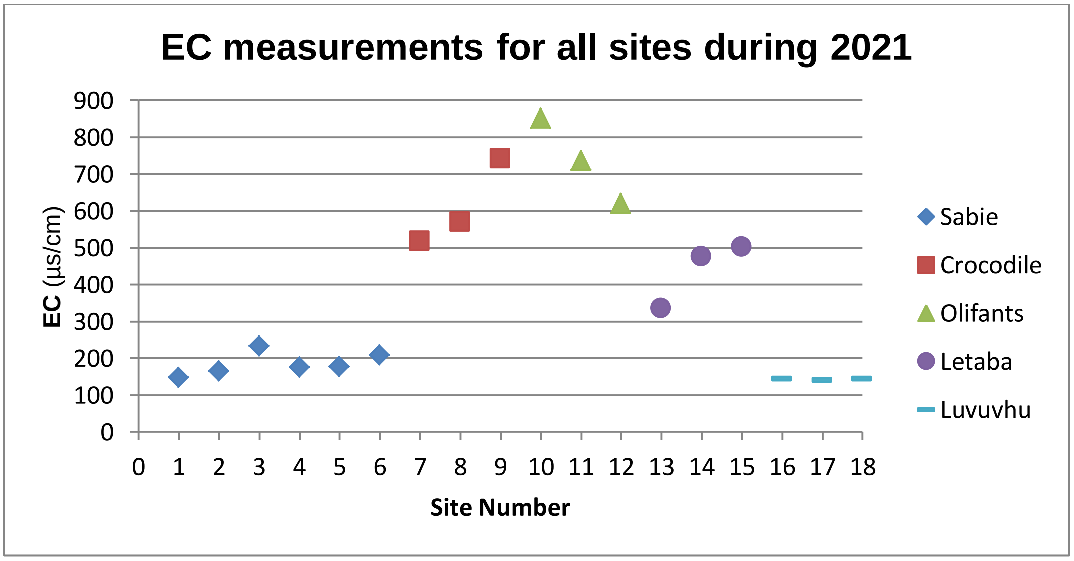

Figure 12: *in situ* electrical conductivity (EC) measurements for all sites in all rivers during sampling in 2021 (Refer to Table 5 for site names).

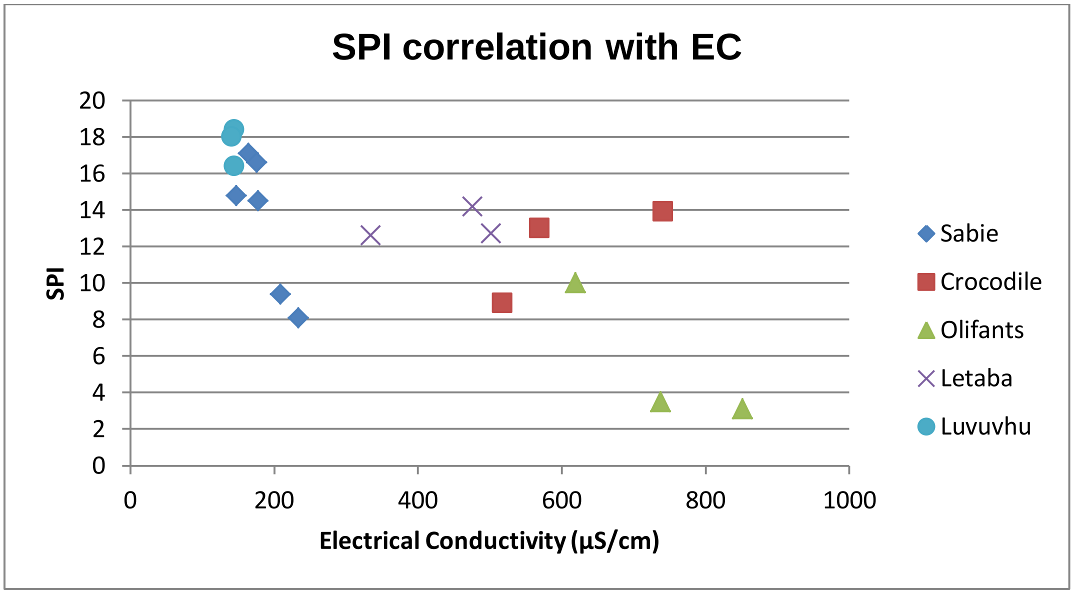

Figure 13: Inverted correlation between calculated SPI scores and *in situ* electrical conductivity measurements.

The water quality, measured *in situ*, differs between sites and rivers. This variation is due to the different physical and chemical properties of the respective river systems including geology, geomorphology, surface water temperature etc. Within a respective system the water quality variables follow a natural variation from one site to the other. Thus, when comparing water quality variables of sites, the comparison is more feasible between sites in the same river than between sites in different rivers.

When analysing the water quality variables collected from the all five rivers, a clear distinction can be made in terms of electrical conductivity (EC). The Crocodile and Olifants Rivers have a high value for EC compared to the Letaba, Sabie and Luvuvhu rivers (Figure 12). However, the EC values for the Letaba River are closer to that of the Crocodile and Olifants Rivers than that of the Sabie and Luvuvhu rivers. **Electrical conductivity** is one of the best parameters to use in correlation with diatom community composition and its responses since this parameter is the **collective measurement of all ions in the water that conduct an electrical charge**. These ions include, but are not limited to, Chloride, Sulphate, Phosphate, Nitrate and Sodium which were measured either with *in situ* measurement or analysed in the laboratory from collected water samples. A Pearson correlation analysis was conducted on the abovementioned ions and electrical conductivity and value for the respective coefficients were obtained (Figure 14). It is evident from this figure that a strong correlation between EC and Chloride, Sodium, Sulphate and Nitrate is present. This suggests that these ions have a great effect in determining the EC for the respective sites within rivers. Of these ions contribute to higher EC, Chloride and Sodium have a very strong correlation with electrical conductivity.

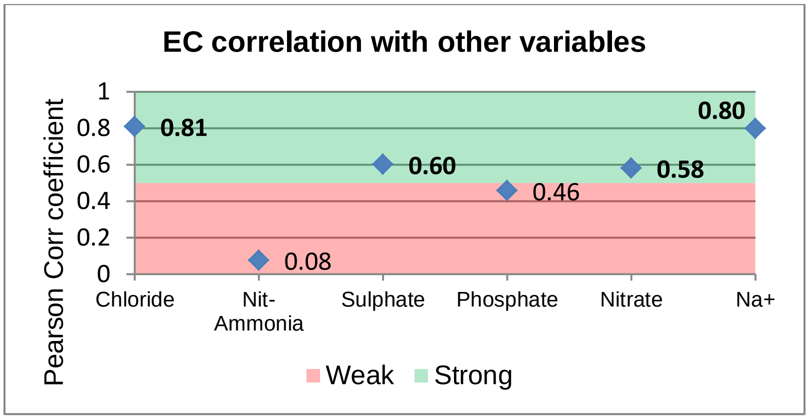

Figure 14: Correlation of laboratory measured water quality variables with *in* situ electrical conductivity values.

R^2 values were also calculated for the above mentioned relationship between electrical conductivity and the respective ions. From (Figure 15) it is evident that a strong correlation is present between EC and Chloride and Sodium. The R^2 value between EC and Sodium is 0.7, which means that 70% of the variation in the data can be explained by the relationship between EC and Na^+. The same is true for EC and Chloride, however, this value is a bit lower at 0.65 but also still represents a strong correlation.

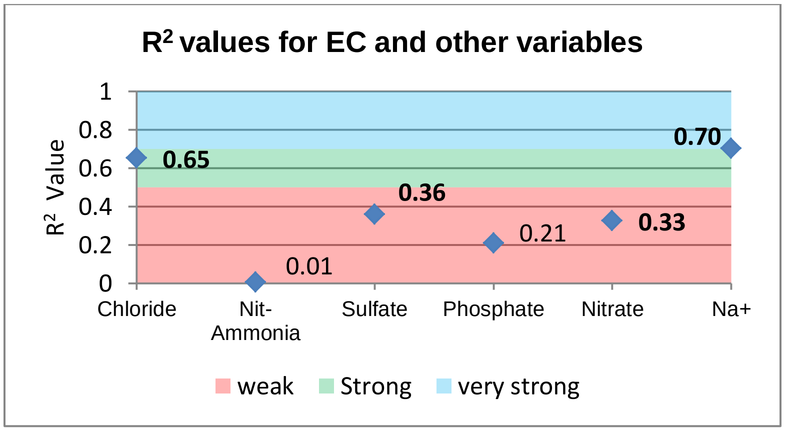

Figure 15: R^2 values for laboratory measured water quality variables and *in* situ electrical conductivity values.

The SPI and the EC measurement taken *in situ* show a very significant relationship (Figure 13). A decreasing trend is immediately visible, as SPI increases, the electrical conductivity decreases. A Pearson correlation coefficient of 0.7 between SPI and EC indicates a high correlation. Additionally, a ***p*** value of 0.004 was calculated for SPI and EC, which is very significant. This means that the calculation of the SPI based on diatom community characteristics and the measurements taken *in situ* have a very significant correlation. This means that the diatom community structure accurately reflects water quality with the index score.

Canonical correspondence analysis (CCA)

2018/2019

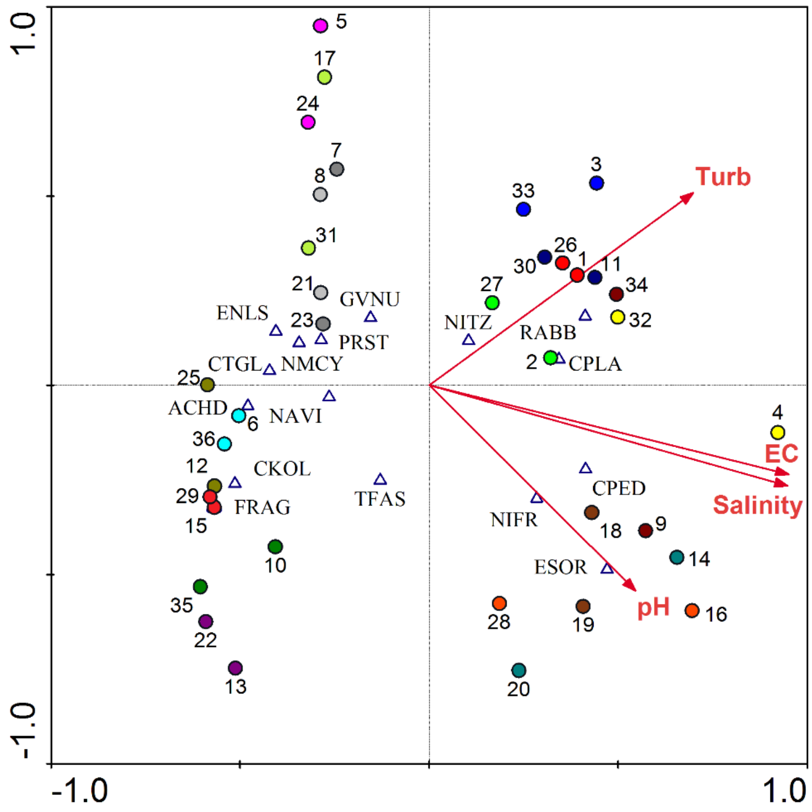

Figure 16: CCA triplot illustrating the relationship between diatom species, water quality variables and sites for 2018 and 2019. (refer to Table 4 for site numbers)

The CCA triplot shows a clear separation of sites along the vertical axes. The sites at the right hand site of the vertical axis show positive correlations with increases in the respective water quality parameters, while the sites on the left hand side show a negative correlation with the water quality parameters. Sites in the Sabie and Luvuvhu rivers, those with the highest SPI score and highest water quality, all appear on the left side of the vertical axis. The rivers with lower SPI scores and consequently lower water quality all appear on the right hand side correlating with increased concentrations of the respective water quality parameters. Sites above the horizontal axis on the left side (Sabie River) all have a strong negative correlation with increase electrical conductivity, pH and salinity. The sites below the axis (Luvuvhu River) seem to have negative correlation with turbidity, this is however not true because no values are available for turbidity in the Luvuvhu River. This can possibly skew the results in the graph, however, there is little alteration to the other vectors as well as to the position of sites and species since there are so many other variables that determine positions for entities on the graph.

2021

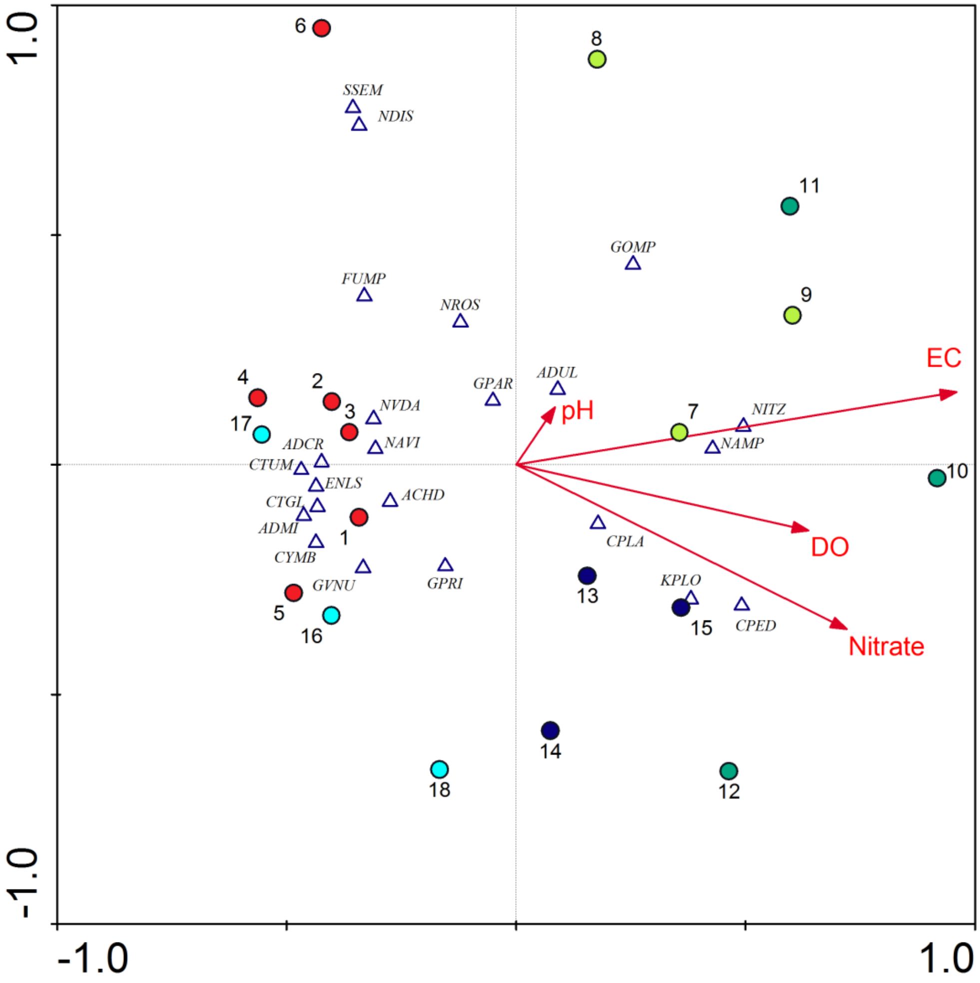

Figure 17: Canonical correspondence analysis between sites, species and water quality variables sampled during 2021. (refer to Table 5 for site names)

From the graph it can be observed that all the sites on the right-hand side of the vertical axis are positively correlated with the water quality variables (pH,EC,DO and Nitrate). The sites on this side of the graph are those sampled in the Crocodile River (light green), the Letaba River (dark blue) and the Olifants River (dark turquoise). The variables having the greatest influence on the position of the sites on the graph are those with the longest vectors and are, in descending order, EC, Nitrate, DO and pH.

All sites on the left hand side of the zero point are those sampled in the Sabie River (red) and the Luvuvhu River (light blue). These sites have a negative correlation with the measured water quality parameters. This is especially true for EC since most sites on the left hand side of the axis have a very small perpendicular distance to the inverse of the vector, only two sites (6 and 18) are outside this cluster. Positive correlations with water quality variables are found with those sites

on the right of the vertical axis. All of these sites are in the Crocodile, Olifants or Letaba rivers. The Crocodile and Olifants rivers have the highest correlation with EC, the Letaba River on the other hand has a neutral to positive correlation with EC. This river has a stronger positive correlation with Nitrates. Although EC and Nitrates do have a correlation coefficient of 0.58 which is biologically still significant (Figure 3).

These CCA graphs illustrates the significant relationship is between diatom species and water quality parameters. The described ecology of the species illustrated in the graph correlates with their relative position to the respective water quality parameters found in the present study. The indices calculated from these ecological preferences such as the SPI are also illustrated in Table 5. The sites on the left of the vertical axis have high SPI scores and consequently, those on the right have lower scores. This illustrates the inverse relationship between SPI scores and water quality parameters, such as EC. Therefore, the ecology of species is well correlated with water quality variables.

Conclusion

Drought and flood conditions have had impacts on the water quality of the KNP Rivers. In general, drought impacted water quality negatively across all sites in all years sampled. High rainfall events on the other hand seem to have increased the water quality for all sites in all rivers except the Olifants River, which is the only river that did not show an improvement in water quality from 2018 to 2021. The rainfall or flooding events seem to have been small and seasonal since they have a beneficial effect on the water quality of the parks rivers. The dilution of nutrients and pollutants in the water, the removal of sediments and consequently captured nutrients within them as well as the increase in flow, are effects small scale floods have on water quality. These types of floods increase water quality and are essential for the wellbeing of lotic ecosystems.

Diatoms are well adapted to deal with small scale flooding events. The community structures of diatoms sampled were accurate in determining the water quality for the respective rivers in terms of trophic level and pollutant tolerance, these communities are also altered because of drought and flooding conditions and consequently reflect the water quality effects created by such events. The SPI has a very high correlation with electrical conductivity, and consequently the ions that can conduct charge in water. It therefore shows that diatom indices, based on diatom abundances and autecology, have high correlations with water quality variables.

The use of diatoms is therefore useful for indicating effects of droughts and floods on the water quality of Rivers in the KNP. Additionally, diatom index scores accurately reflect water quality changes between sites and rivers.

Recommendations:

The SPI has a very high correlation with electrical conductivity, and consequently the ions that can conduct charge in water. It therefore shows that diatom indices, based on diatom abundances and autecology, have high correlations with water quality variables. This means that the diatom community structure accurately reflects water quality by only the calculation of indices. The need to take *in situ* water quality is therefore redundant and can be expensive to conduct, diatoms and other bio-indicators can hence be used in future as an accurate proxy for water quality in such studies.

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Appendix A

Sampling sites during 2021:

Figure 18: Sampling sites. **A**: Sabie – Lubye Lubye. **B**: Letaba – Lonely Bull. **C**: Crocodile – Marula. **D**: Olifants – Balule. **E**: Luvuvhu – Mutale (Outpost).

Appendix B

<u>Micrographs</u>**:**

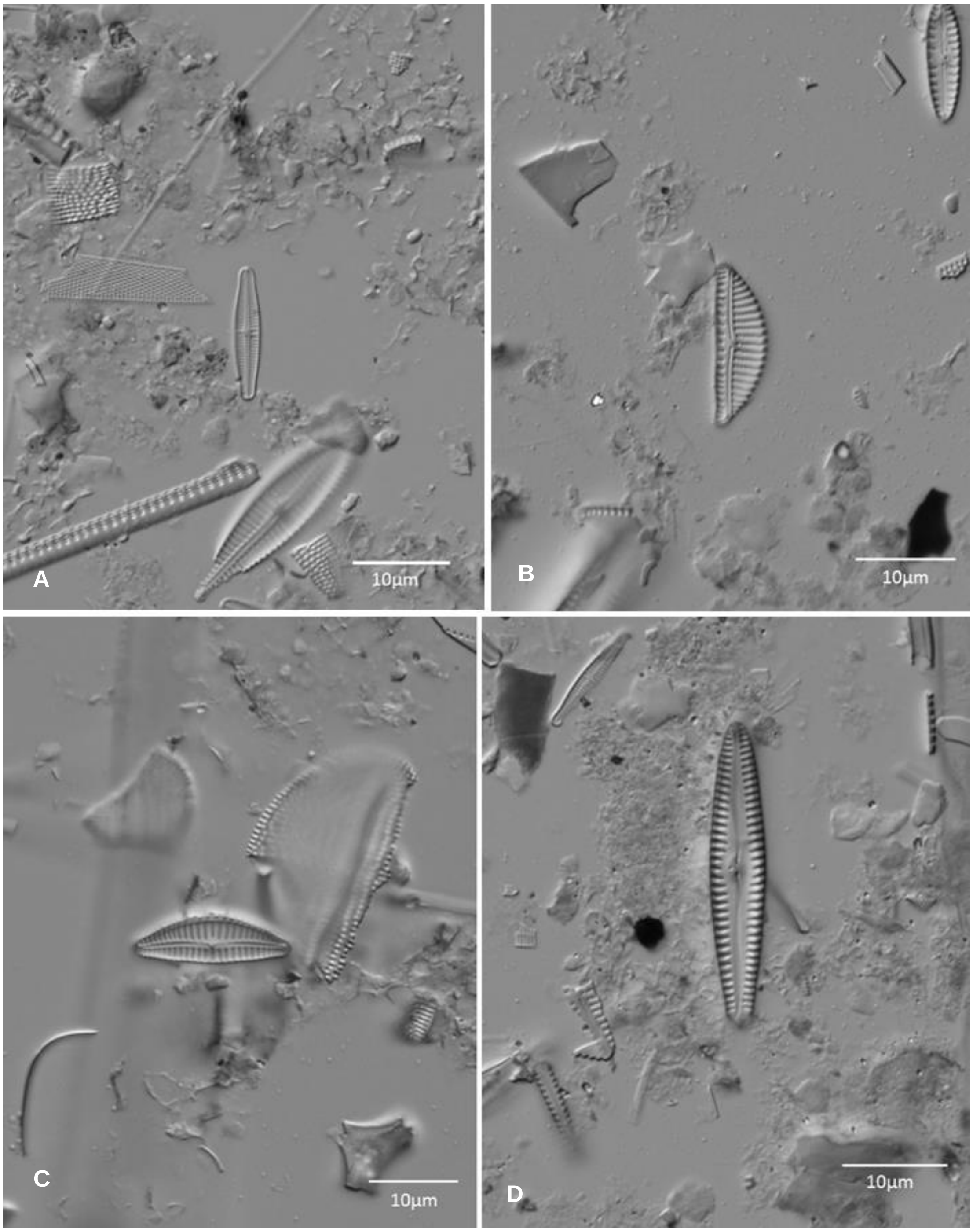

Figure 19: Common oligotrophic species found across all years and sites. A – D. Valve view of cleaned material. **A** - *Achnanthidium minutissimum*. **B –** *Encyonema minutum*. **C –** *Cymbella kolbei*. **D –** *Gomphonema venusta.*

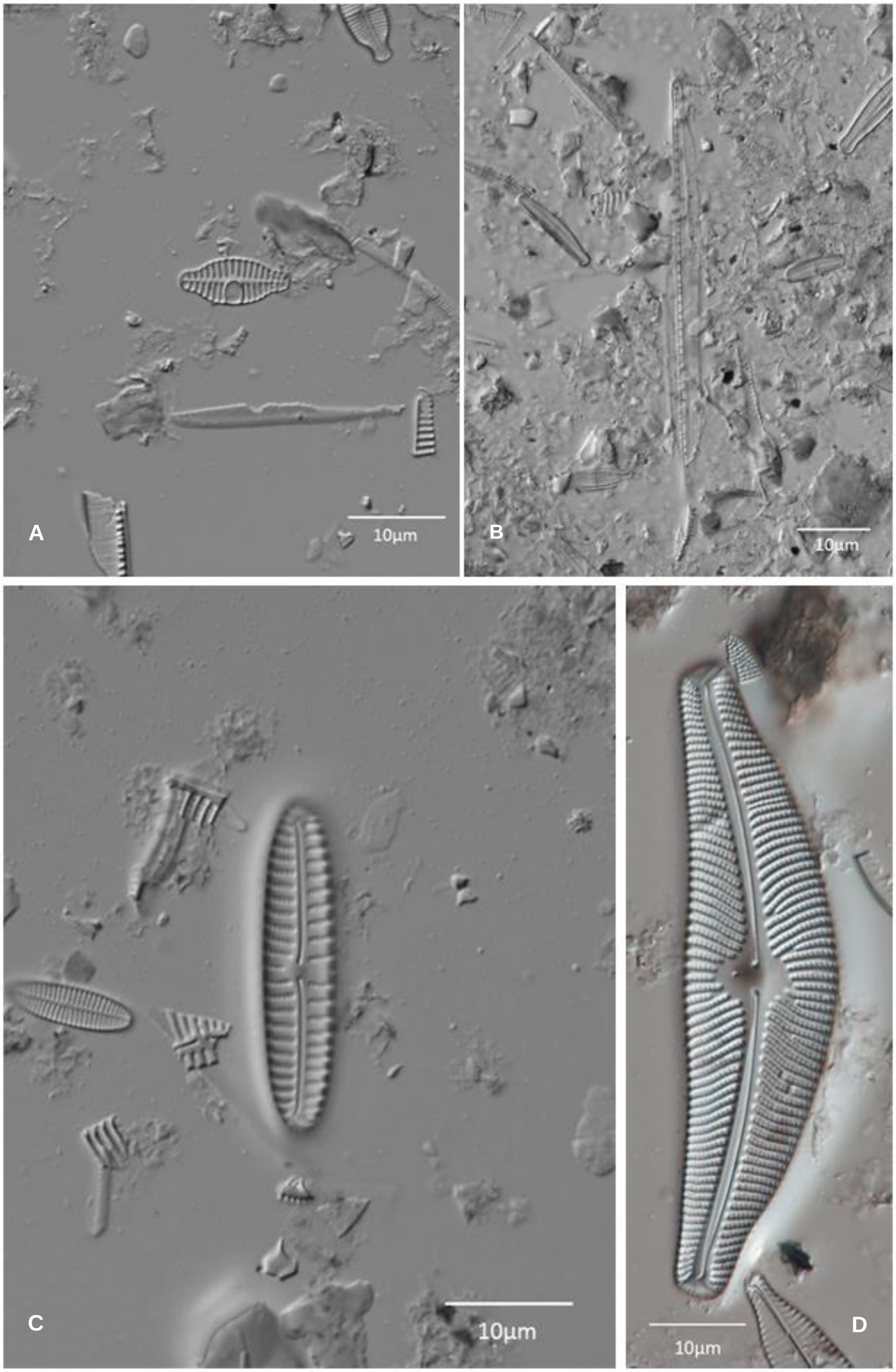

Figure 20: Common Oligo- mesotrophic species found across all years and sites. A – D. Valve view of cleaned material. **A** – *Planothidium rostratum*. **B** – *Nitzschia dissipata*. **C** – *Encyonopsis leei* var. *sinensis.* **D** – *Cymbella tumida.*

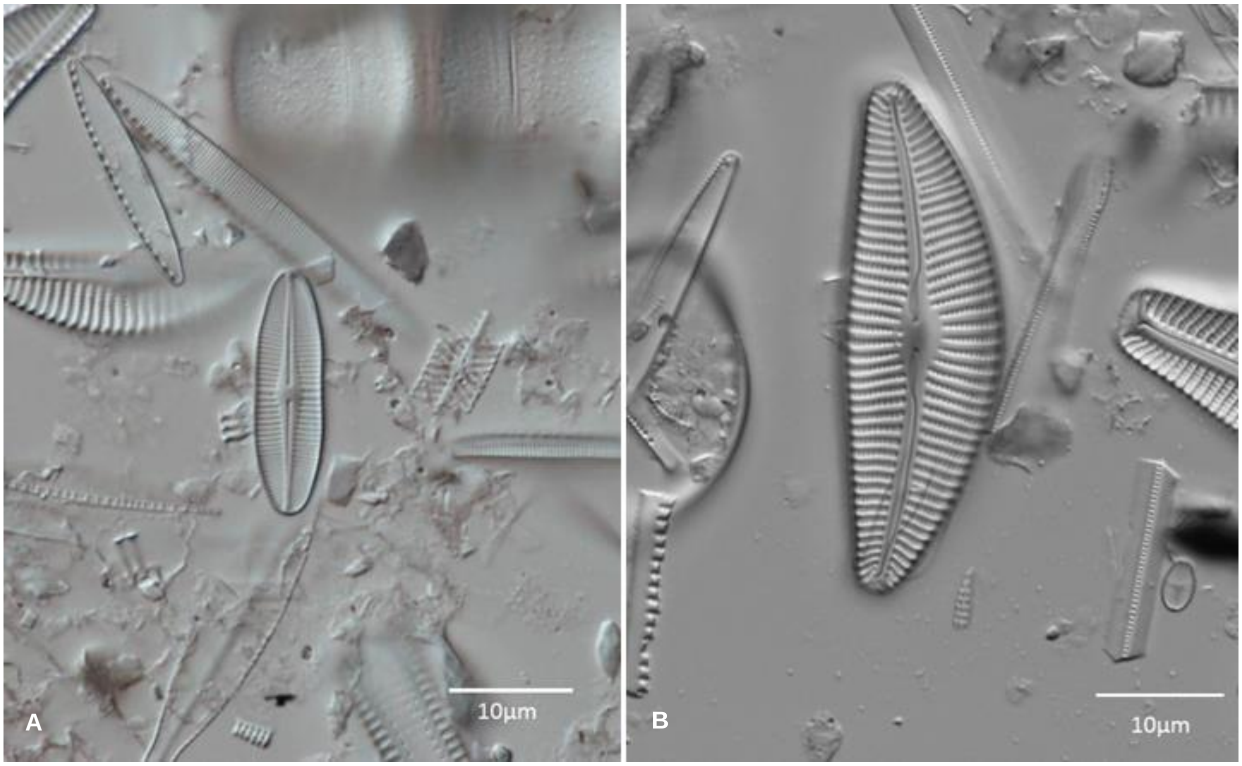

Figure 21: More common oligo- mesotrophic species found across all years and sites. A – B. Valve view of cleaned material. **A** – *Achnanthidium crassum*. **B** – *Cymbella turgidula*.

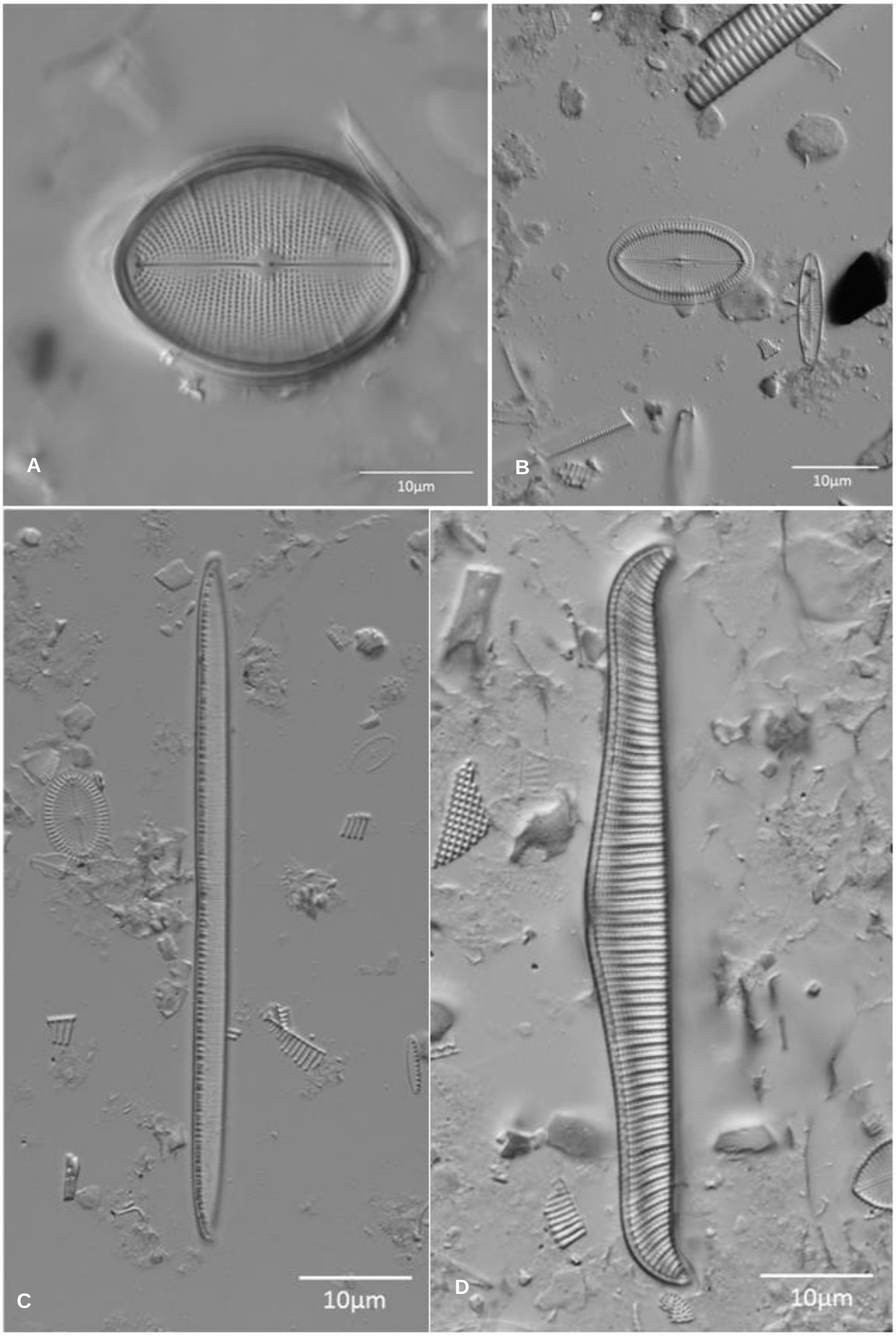

Figure 22: Common meso- to eutrophic species found across all years and sites. A – D. Valve view of cleaned material. **A** – *Cocconeis pediculus*. **B** – *Cocconeis placentula*. **C** – *Nitzschia linearis.* **D** – *Rhopalodia gibba.*

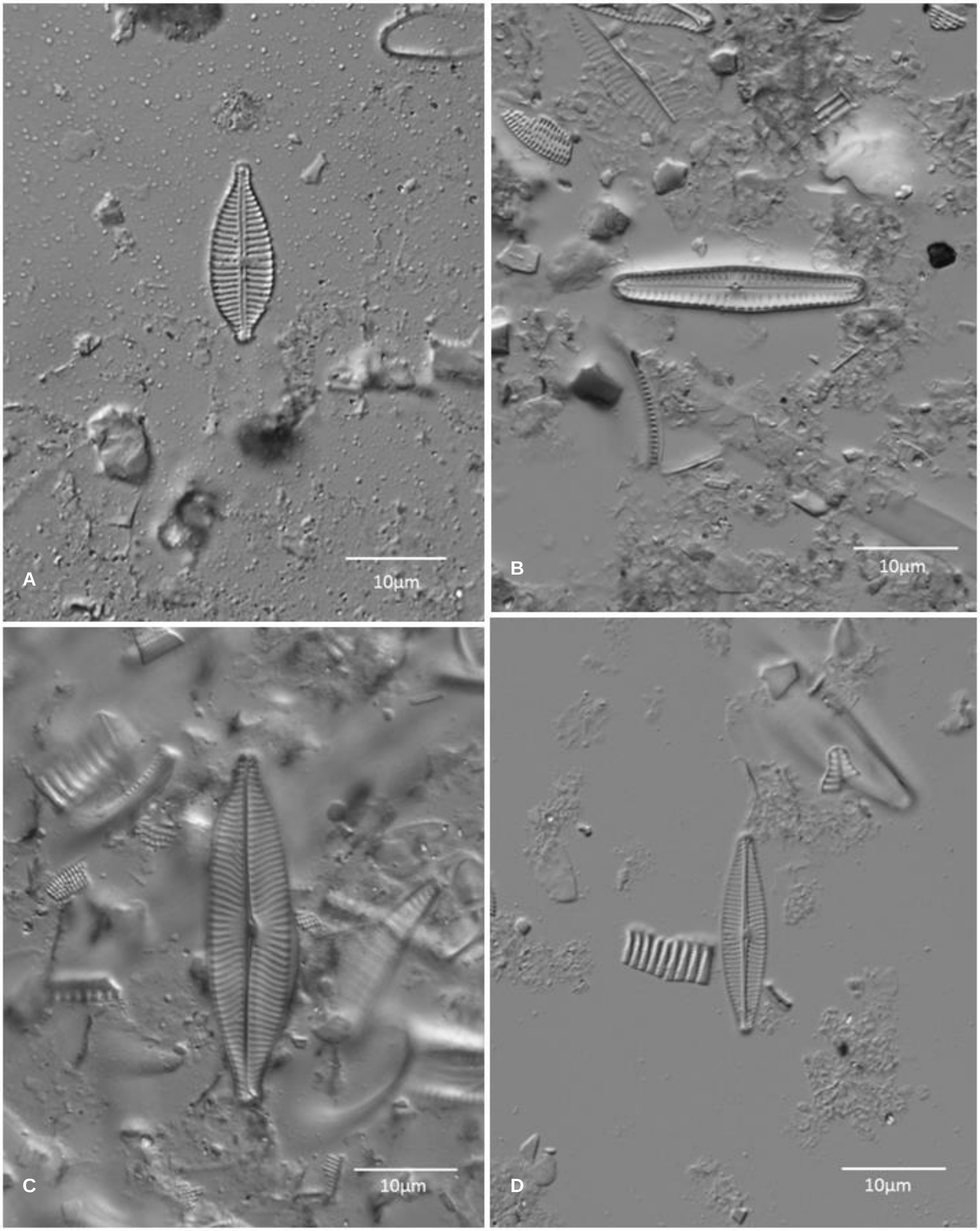

Figure 23: Common eutrophic species found across all years and sites. A – D. Valve view of cleaned material. **A** – *Gomphonema parvulum* var. *lagenula*. **B** – *Gomphonema pumilum* var. *rigidum*. **C** – *Navicula rostellata.* **D** – *Navicula vandamii.*

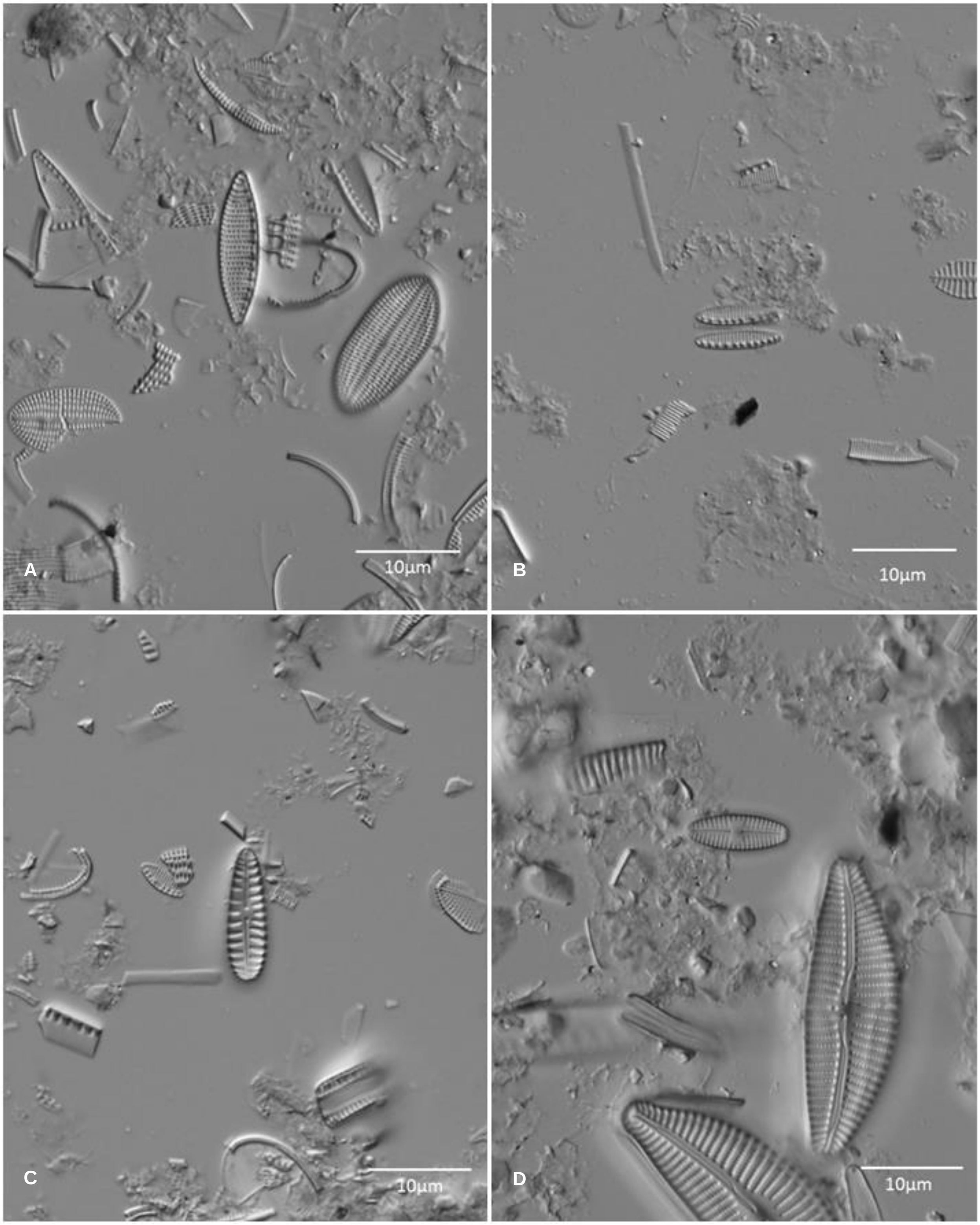

Figure 24: Additional eutrophic species found across all years and sites. A – D. Valve view of cleaned material. **A** – *Nitzschia amphibia*. **B** – *Nitzschia frustulum*. **C** – *Rhoicosphenia abbreviata.* **D** – *Sellaphora seminulum.*

Table 8: Diatom species and corresponding counts for all sites across all years sampled, Luvuvhu River.

Luvuvhu															
2018				**2019**				**2020**				**2021**			
Site Name	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**
Dongadzhiva	*Achnanthidium*	154	39	**Dongadzhiva**	*Achnanthidium*	150	38	**Dongadzhiva**	*Achnanthidim*	196	49	**Dongadzhiva**	***Achnanthidium minutissimum***	**248**	**62**
	Encyonopsis leei var. *sinensis*	72	18.0		*Encyonopsis leei* var. *sinensis*	50	13		*Achnanthidium minutissimum*	59	15		*Achnanthidium*	53	13
	Fragilaria	42	11		*Encyonopsis krammeri*	48	12.0		*Navicula*	35	9		*Encyonopsis leei* var. *sinensis*	19	5
	Encyonopsis krammeri	21	5		*Brachysira vitrea*	46	12		*Brachysira vitrea*	21	5		*Cymbella kappii*	16	4
	Gomphonema venusta	19	5		*Navicula*	38	10		*Encyonopsis leei* var. *sinensis*	7	2		*Gomphonema venusta*	13	3
Xindzivhani	*Achnanthidium*	182	45	**Xindzivhani**	*Achnanthidium*	131	33	**Xindzivhani**	*Cocconeis placentula*	**75**	19	**Xindzivhani**	*Achnanthidium minutissimum*	165	41
	Fragilaria	66	16		*Cymbella turgidula*	107	26.8		*Achnanthidium*	**58**	15		*Achnanthidium*	118	29
	Gomphonema venusta	32	8		*Encyonopsis leei* var. *sinensis*	68	17		*Achnanthidium minutissimum*	**50**	13		*Fragilaria capucina*	22	5
	Encyonopsis leei var. *sinensis*	31	7.7		*Achnanthidium rivulare*	24	6		*Gomphonema venusta*	**35**	9		*Encyonopsis leei* var. *sinensis*	21	5
	Tabularia faciculata	18	4		*Encyonopsis krammeri*	18	5		*Navicula zanonii*	**16**	**4**		*Gomphonema*	16	4
Mutale (Outpost)	*Navicula*	110	28	**Mutale (Outpost)**	*Cocconeis placentula*	121	30	**Mutale (Outpost)**	*Cocconeis pediculus*	68	17	**Mutale (Outpost)**	***Cocconeis placentula***	**215**	**54**
	Fragilaria	77	19		*Achnanthidium*	74	18		*Cocconeis placentula*	66	17		*Achnanthidium*	44	11
	Cymbella turgidula	54	13.6		*Nitzschia*	24	6		*Achnanthidium*	60	15		*Achnanthes*	19	5
	Nitzschia	42	11		*Navicula cryptotenella*	23	5.7		*Cocconeis placentula* var. *lineata*	28	7		*Gomphonema pumilum* var. *rigidum*	19	5
	Cocconeis placentula	21	5		*Fragilaria biceps*	21	5		*Gomphonitzschia ungeri*	24	6		*Gomphonema venusta*	16	4
Bobomane	*Tabularia fasciculata*	64	16.0	**Bobomane**	*Geissleria decussis*	49	12.3	**Xindzivhani (Rapids)**	*Achnanthidium minutissimum*	181	45	**Bobomane**	*N/A*	N/A	N/A
	Cocconeis placentula	37	9.2		*Cocconeis placentula*	48	12.0		*Encyonopsis leei* var. *sinensis*	49	12		*N/A*	N/A	N/A
	Nitzschia frustulum	30	7.5		*Cymbella kolbei*	36	9.0		*Achnanthidium*	33	8		*N/A*	N/A	N/A
	Anorthoneis dulcis	29	7.2		*Nitzschia*	35	8.8		*Fragilaria capucina*	21	5		*N/A*	N/A	N/A
	Achnanthidium saprophilum	27	6.7		*Planothidium rostratum*	25	6.3		*Fragilaria capucina* var. *vaucheriae*	15	4		*N/A*	N/A	N/A

Table 9: Diatom species and corresponding counts for all sites across all years sampled, Letaba River.

LETABA															
2018				**2019**				**2020**				**2021**			
Site Name	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**
Lonely Bull	*Nitzschia*	293	73	**Lonely Bull**	***Cocconeis placentula***	**293**	**73**	**Lonely Bull (Pool)**	***Cocconeis placentula***	**202**	**51**	**Lonely Bull**	***Cocconeis placentula***	**324**	**81**
	Cocconeis placentula	41	10.2		*Nitzschia*	64	16		*Tabularia fasciculata*	83	21		*Nitzschia*	23	6
	Tabularia fasciculata	40	10		*Encyonopsis leei* var. *sinensis*	10	2.5		*Nitzschia*	30	8		*Gomphonema parvulum*	14	3
	Anorthoneis dulcis	8	2		*Kolbesia ploenensis*	9	2		*Gomphonema parvulum*	16	4		*Navicula rostellata*	7	2
	Gomphonema parvulum	4	1		*Tabularia fasciculata*	8	2		*Kolbesia ploenensis*	16	4.0		*Anorthoneis dulcis*	4	1
Klipkoppies	*Cymbella turgidula*	91	22.8	**Klipkoppies**	*Cocconeis placentula*	358	88.0	**Lonely Bull (Rapids)**	***Cocconeis placentula***	**216**	**54**	**Klipkoppies**	***Cocconeis placentula***	**368**	**92**
	Nitzschia	83	20.8		*Nitzschia*	14	3.4		*Gomphonema parvulum*	52	13		*Nitzschia*	11	3
	Navicula cryptotenella	37	9.3		*Kolbesia ploenensis*	8	2.0		*Tabularia fasciculata*	44	11		*Gomphonema parvulum*	7	2
	Rhopalodia gibba	30	7.5		*Hippodonta*	5	1.2		*Rhopalodia gibba*	27	6.8		*Cocconeis pediculus*	3	1
	Navicula veneta	21	5.3		*Rhopalodia gibba*	3	0.7		*Fragilaria ulna*	15	4		*Gomphonema venusta*	3	1
Confluence	*Epithemia sorex*	235	58.5	**Confluence**	*Nitzschia*	367	91.3	**Lonely Bull (Run)**	*Cocconeis placentula*	128	32	**Confluence**	***Cocconeis placentula***	**211**	**53**
	Cocconeis placentula	48	11.9		*Gomphonema parvulum*	13	3.2		*Nitzschia*	63	16		*Kolbesia ploenensis*	64	16
	Nitzschia	42	10.4		*Cocconeis pediculus*	9	2.2		*Rhopalodia gibba*	56	14		*Nitzschia*	34	9
	Achnanthidium saprophilum	30	7.5		*Cocconeis placentula*	8	2.0		*Epithemia sorex*	45	11		*Nitzschia amphibia*	22	6
	Rhopalodia gibba	18	4.5		*Hippodonta*	3	0.7		*Gomphonema parvulum*	28	7.0		*Sellaphora seminulum*	12	3

Table 10: Diatom species and corresponding counts for all sites across all years sampled, Olifants River.

OLIFANTS															
2018				2019				2020				2021			
Site Name	Species	Count	%	Site Name	Species	Count	%	Site Name	Species	Count	%	Site Name	Species	Count	%
Mamba	*Nitzschia*	307	76.2	Mamba	*Cocconeis placentula*	158	39.5	Mamba	*N/A*	N/A	N/A	Mamba	***Nitzschia***	**321**	**80**
	Navicula	48	11.9		*Nitzschia frustulum*	113	28.3		*N/A*	N/A	N/A		*Gomphonema*	23	6
	Navicula cryptotenelloides	19	4.7		*Nitzschia*	34	8.5		*N/A*	N/A	N/A		*Achnanthidium*	15	4
	Rhoicosphenia abbreviata	11	2.7		*Cocconeis pediculus*	21	5.3		*N/A*	N/A	N/A		*Cocconeis placentula*	10	3
	Cocconeis placentula	4	1.0		*Nitzschia dicompressa*	17	4.3		*N/A*	N/A	N/A		*Navicula rostellata*	9	2
Balule	*Nitzschia*	121	30.0	Balule	*Nitzschia frustulum*	191	47.0	Balule	*N/A*	N/A	N/A	Balule	***Nitzschia***	**305**	**76**
	Cocconeis placentula	120	29.8		*Nitzschia*	131	32.3		*N/A*	N/A	N/A		*Gomphonema*	28	7
	Hippodonta	57	14.1		*Cocconeis placentula*	19	4.7		*N/A*	N/A	N/A		*Cocconies placentula*	12	3
	Navicula	21	5.2		*Tabularia fasciculata*	15	3.7		*N/A*	N/A	N/A		*Hippodonta*	10	2
	Kolbesia ploenensis	17	4.2		*Cocconeis pediculus*	10	2.5		*N/A*	N/A	N/A		*Achnanthidium*	5	1
Confluence	*Cocconeis placentula*	281	69.7	Confluence	*Epithemia sorex*	139	34.7	Confluence	*N/A*	N/A	N/A	Confluence	*Nitzschia*	119	30
	Cocconeis pediculus	42	10.4		*Nitzschia frustulum*	92	22.9		*N/A*	N/A	N/A		*Cocconies placentula*	103	26
	Rhoicosphenia abbreviata	40	9.9		*Cocconeis placentula*	42	10.5		*N/A*	N/A	N/A		*Achnanthidium*	59	15
	Nitzschia	5	1.2		*Cocconeis pediculus*	30	7.5		*N/A*	N/A	N/A		*Cocconeis pediculus*	56	14
	Nitzschia frustulum	5	1.2		*Rhopalodia operculata*	26	6.5		*N/A*	N/A	N/A		*Gomphonema*	29	7

Table 11: Diatom species and corresponding counts for all sites across all years sampled, Sabie River.

SABIE															
2018				**2019**				**2020**				**2021**			
Site Name	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**
Sekorongwane	*Nitzschia*	179	45	**Sekorongwane**	***Nitzschia***	**209**	**52**	**Sekorongwane (Rapids)**	*Nitzschia*	126	32	**Sekorongwane**	*Achnanthidium crassum*	133	33
	Gomphonema	61	15.2		*Encyonopsis leei* var. *sinensis*	44	11		*Melosira varians*	81	20		*Navicula*	48	12
	Nitzschia frustulum	30	7		*Achnanthidium rivulare*	35	8.8		*Encyonema minutum*	46	12		*Achnanthidium*	31	8
	Achnanthidium	24	6		*Cymbella turgidula*	19	5		*Achnanthidium*	41	10		*Navicula vandamii*	27	7
	Ulnaria nyanse	18	4		*Navicula*	16	4		*Cymbella turgidula*	24	6		*Nitzschia*	23	6
Tinga	*Navicula*	58	14.5	**Tinga**	*Encyonopsis leei* var. *sinensis*	91	22.75	**Sekorongwane (Run)**	*Nitzschia*	156	39	**Tinga**	*Achnanthidium*	134	33
	Cymbella turgidula	57	14.25		*Navicula*	77	19.25		*Navicula*	36	9		*Encyonopsis leei* var. *sinensis*	77	19
	Achnanthidium	54	13.5		*Achnanthidium*	57	14.25		*Encyonema minutum*	33	8		*Achnanthidium minutissimum*	59	15
	Planothidium rostratum	42	10.5		*Planothidium rostratum*	50	12.5		*Cymbella turgidula*	26	7		*Cymbella turgidula*	30	7
	Encyonopsis leei var. sinensis	36	9		*Cocconeis placentula*	21	5.25		*Achnanthidium*	24	6		*Achnanthidium crassum*	15	4
Sand	*Anorthoneis dulcis*	219	54.1	**Sand**	N/A	N/A	N/A	**Tinga**	N/A	N/A	N/A	**Sand**	*Nitzschia*	93	23
	Cocconeis placentula	56	13.8		N/A	N/A	N/A		N/A	N/A	N/A		*Anorthoneis dulcis*	84	21
	Nitzschia frustulum	24	5.9		N/A	N/A	N/A		N/A	N/A	N/A		*Cocconeis placentula*	33	8
	Gomphonema venusta	22	5.4		N/A	N/A	N/A		N/A	N/A	N/A		*Planothidium*	20	5
	Planothidium rostratum	18	4.4		N/A	N/A	N/A		N/A	N/A	N/A		*Cymbella turgidula*	17	4
Lubye Lubye	*Encyonopsis leei* var. *sinensis*	113	28.2	**Lubye Lubye**	*Cymbella kolbei*	126	31.0	**Lubye Lubye**	N/A	N/A	N/A	**Lubye Lubye**	*Achanthidium crassum*	98	25
	Navicula microlyra	54	13.5		*Encyonopsis leei* var. *sinensis*	89	21.9		N/A	N/A	N/A		*Achnanthidium minutissimum*	77	19
	Nitzschia frustulum	49	12.2		*Nitzschia frustulum*	41	10.1		N/A	N/A	N/A		*Achanthidium*	74	19
	Cymbella turgidula	43	10.7		*Cymbella turgidula*	35	8.6		N/A	N/A	N/A		*Encyonopsis leei* var. *sinensis*	40	10
	Fallacia umpatica	23	5.7		*Navicula microlyra*	31	7.6		N/A	N/A	N/A		*Cymbella turgidula*	27	7
Antholysta	*Encyonopsis leei* var. *sinensis*	86	21	**Antholysta**	*Encyonopsis leei* var. *sinensis*	112	28.1	**Antholysta**	*Achnanthidium minutissimum*	84	21	**Antholysta**	*Cymbella turgidula*	82	21
	Achnanthidium	64	16		*Achnanthidium*	81	20		*Ulnaria nyanse*	51	13		*Encyonopsis leei* var. *sinensis*	57	14
	Cymbella tumida	57	14		*Navicula microlyra*	51	13		*Cymbella turgidula*	44	11		*Cocconeis placentula*	38	10
	Cymbella turgidula	57	14		*Cymbella turgidula*	44	11		*Achnanthidium crassum*	35	9		*Cymbella*	36	9
	Navicula	29	7.1		*Cymbella tumida*	30	7		*Achnanthidim*	33	8		*Cymbella tumida*	29	7
Sabiepoort	*Nitzschia*	323	80.3	**Sabiepoort**	*Nitzschia*	264	65.8	**Sabiepoort**	N/A	N/A	N/A	**Sabiepoort**	*Sellaphora seminulum*	149	37
	Nitzschia frustulum	31	7.7		*Gomphonema lagenula*	40	10.0		N/A	N/A	N/A		*Nitzschia dissipata*	38	10
	Gomphonema lagenula	23	5.7		*Cocconeis placentula*	26	6.5		N/A	N/A	N/A		*Cocconeis placentula*	35	9
	Gomphonema parvulum	4	1.0		*Nitzschia frustulum*	16	4.0		N/A	N/A	N/A		*Navicula rostellata*	28	7
	Navicula heimansioides	3	0.7		*Encyonema minutum*	14	3.5		N/A	N/A	N/A		*Planothidium rostratum*	18	4

Table 12: Diatom species and corresponding counts for all sites across all years sampled, Crocodile River.

CROCODILE															
2018				**2019**				**2020**				**2021**			
Site Name	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**	**Site Name**	**Species**	**Count**	**%**
Nsikazi	*Cocconeis placentula*	268	66.0	**Nsikazi**	*Nitzschia*	119	29.7	**Nsikazi**	*N/A*	N/A	N/A	**Nsikazi**	*N/A*	N/A	N/A
	Nitzschia	44	10.8		*Cocconeis placentula*	99	24.7		*N/A*	N/A	N/A		*N/A*	N/A	N/A
	Gomphonema minutum	41	10.1		*Rhoicosphenia abbreviata*	86	21.4		*N/A*	N/A	N/A		*N/A*	N/A	N/A
	Geissleria decussis	12	3.0		*Nitzschia linearis*	35	8.7		*N/A*	N/A	N/A		*N/A*	N/A	N/A
	Navicula cryptotenella	9	2.2		*Navicula cryptotenella*	20	5.0		*N/A*	N/A	N/A		*N/A*	N/A	N/A
Malelane	*Cocconeis placentula*	206	51.2	**Malelane**	*Cocconeis placentula*	342	82.8	**Malelane**	*N/A*	N/A	N/A	**Malelane**	*Cocconeis placentula*	249	62
	Nitzschia	45	11.2		*Nitzschia*	19	4.6		*N/A*	N/A	N/A		*Nitzschia*	85	21
	Craticula subminuscula	38	9.5		*Encyonopsis leei* var. *sinensis*	11	2.7		*N/A*	N/A	N/A		*Eolimna subminuscula*	26	7
	Gomphonema venusta	30	7.5		*Navicula veneta*	9	2.2		*N/A*	N/A	N/A		*Nitzschia amphibia*	18	5
	Eolimna minima	18	4.5		*Navicula microlyra*	7	1.7		*N/A*	N/A	N/A		*Gomphonema*	8	2
Marula	*Nitzschia*	175	44	**Marula**	*Cocconeis placentula*	203	51	**Marula**	*Cocconeis placentula*	364	91	**Marula**	*Cocconeis placentula*	217	54
	Cocconeis placentula	111	28		*Nitzschia*	52	13		*Gomphonema minutum*	8	2		*Gomphonema*	149	37
	Gomphonema pumilum var. *rigidum*	25	6.4		*Gomphonema venusta*	39	10		*Achnanthidium minutissimum*	5	1.3		*Nitzschia*	22	6
	Navicula viridula	13	3		*Navicula cryptotenelloides*	21	5.2		*Gomphonema pumulum* var. *rigidum*	4	1		*Achnanthidium*	3	1
	Navicula schroeteri	10	2		*Nitzschia amphibia*	15	4		*Kolbesia ploenensis*	4	1		*Navicula schroeteri*	3	1
Nkongoma	*Cocconeis placentula*	245	60.9	**Nkongoma**	*Cocconeis plecentula*	222	55.4	**Nkongoma**	*N/A*	N/A	N/A	**Nkongoma**	*Cocconeis placentula*	197	49
	Anorthoneis dulcis	19	4.7		*Nitzschia*	33	8.2		*N/A*	N/A	N/A		*Anorthoneis dulcis*	86	21
	Gomphonema pumilum var. *rigidum*	17	4.2		*Gomphonema minutum*	32	8.0		*N/A*	N/A	N/A		*Coccoceis pediculus*	26	6
	Nitzschia	17	4.2		*Gomphonema parvulum*	16	4.0		*N/A*	N/A	N/A		*Gomphonema*	21	5
	Navicula vandamii	15	3.7		*Fragilaria biceps*	15	3.7		*N/A*	N/A	N/A		*Nitzschia amphibia*	21	5