

Assessment of phytobenthos in the lower Phongolo River catchment in relation to changing environmental conditions

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

Evelyn E. Gaiser
Think like a Diatom

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Summary

Floodplain ecosystems are important since they provide numerous services and resources, including food, wood and water to humans. The lower Phongolo River floodplain is one such ecosystem and is the most unique and diverse floodplain in South Africa due to its biodiversity and economic value. The floodplain is located in northern KwaZulu-Natal and stretches from the Pongolapoort Dam to the confluence between the Phongolo and Usuthu rivers in the Ndumo Game Reserve (NGR). The Pongolapoort Dam was constructed with the aim to supply nearby towns with water as well as provide irrigation water for sugarcane and cotton plantations. Controlled flood releases were implemented from the dam to ensure that the ecosystem infrastructure is maintained, but there have been no flood releases since December 2014 due to an ongoing drought in the area. The NGR is the only protected section in the floodplain area and is a Ramsar wetland of international importance due to its high biodiversity and unique wetlands.

The lower Phongolo River floodplain is at risk due to increasing human population pressure (extensive fishing, irrigation schemes, water abstraction and agriculture) and spraying of DDT in the floodplain area for mosquito vector control. Only a few studies have focussed on the phytobenthos of South Africa's floodplain ecosystems, with no published work on the diatom community of the lower Phongolo River floodplain. There are various advantages for including diatoms in ecological and ecotoxicology studies as they have a relatively short life span, are sensitive to any changes within their environment, are species rich, can colonise almost all substrata, are found in nearly all aquatic habitats, are primary producers in all aquatic ecosystems and can be preserved for many centuries in sediment due to their siliceous cell wall.

The main aim of this study was to increase our knowledge on the spatial and temporal diatom community structures of the lower Phongolo River floodplain. Ecological and ecotoxicological studies were carried out to assess the influence that flow variation, physico-chemical water quality, changing environmental conditions and insecticides have on the structuring and vitality of the diatom communities.

The results showed that the diatom community had observable differences between river sites during the presence and absence of a flood release event, with flow changes affecting the diatom community the most. During the absence of a controlled flood release, the species had to endure less variation in flow and physico-chemical conditions as there was a more stable environment. Temporal variation was noted across six surveys in the Usuthu River due to more natural flow patterns compared to the Phongolo River. A continuous base flow was maintained in the Phongolo River from the Pongolapoort Dam, which resulted in extremely low and consistent flows for the river. Due to the natural flows, fluctuations in the physico-

chemical water variables were recorded for the Usuthu River with less fluctuations in the physico-chemical water variables for the Phongolo River. Flooding of the Usuthu River into Shokwe Pan resulted in the pan being nutrient enriched. The main drivers structuring the diatom community in Shokwe Pan and the ephemeral pans were nutrients. Nutrients enter these pans as runoff from the surrounding catchment area. Conductivity was the main driver in the structuring of the diatom community of Nyamithi Pan and adjacent Paradise Pan. This was expected as Nyamithi Pan is highly saline (conductivity values between 3500 and 11000 $\mu\text{S}/\text{cm}$) as it is situated on top of marine cretaceous deposits with natural saline groundwater seeping into the pan. Increased conductivity for Paradise Pan is due to the influence of Nyamithi Pan on Paradise Pan. When Nyamithi Pan overflows it fills Paradise Pan, which lies within the catchment area of Nyamithi Pan.

Flooding of the Usuthu River during February 2017 had an influence on the nutrient concentrations of Shokwe Pan, as well as influencing the physico-chemical water variables and diatom community structure of the lower reaches of the Phongolo River and Nyamithi Pan. The influence of an Usuthu River flood on the lower reaches of the Phongolo River and Nyamithi Pan are indicated by similar driving forces (temperature, electrical conductivity and sulphate) shaping the diatom community structure of these sites. As the Phongolo River experienced extremely low flows (flow rate of 4–8.50 m^3s^{-1}) (due to an ongoing drought) during the study period, it had no influence on the floodplain pans. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures remained consistent in the Usuthu River during summer and late summer rainfall seasons, with shifts noted in these stable isotope signatures in the Phongolo River between these seasons. For the Phongolo River, the $\delta^{15}\text{N}$ increased and $\delta^{13}\text{C}$ decreased between the two seasons. An increase in runoff from intensive agricultural activities within the floodplain area could cause an increase in the $\delta^{15}\text{N}$ in the Phongolo River. The $\delta^{13}\text{C}$ values decreased due to lower food availability and increased feeding (from competition) on periphyton during the late summer rainfall period. The $\delta^{13}\text{C}$ values remained consistent for both floodplain pans (Nyamithi and Shokwe Pans) across seasons with a decrease in the $\delta^{15}\text{N}$ values between summer and late summer rainfall seasons. Decreasing rainfall caused a decrease in influx of water into wetland ecosystems, which resulted in a decrease in nitrogen concentrations. Wetland ecosystems are seen as more stable environments than riverine ecosystems when considering changes in the nitrogen concentrations. This is due to the nitrogen spiralling effect where nitrogen is transported downstream within river ecosystems but remains within the same wetland ecosystem.

A sediment core from Nyamithi Pan was dated as 1083 years old and indicated changing environmental conditions. Dominant diatom species identified within the core include *H. coffeaeformis*, *C. meneghiniana*, *D. elliptica*, *Fragilaria* sp., *Navicula* sp., *Nitzschia* sp. and *N. palea*. The relative abundances of these species decreased and fluctuated less in the more

recent (i.e. past 300 years) section of the sediment core. An increase in the relative abundance of *D. elliptica* and decreased relative abundances of *H. coffeaeformis*, *C. meneghiniana*, *Fragilaria* sp., *Navicula* sp., *Nitzschia* sp. and *N. palea* indicates a freshening (decreasing salinity) and decreasing nutrient concentrations of Nyamithi Pan. Less extreme fluctuations in the species' relative abundances are due to annual flooding events in the floodplain that results in fluctuations between desiccation and inundation of the floodplain pan. This is supported by the co-occurrence of salinity tolerant and in-tolerant species and lower relative abundance of the dominant species.

Exposures to DDT, Deltamethrin and a mixture negatively influenced the diatom vitality during laboratory and microcosm exposures irrespective of the exposure concentration. The vitality and functioning of the diatom cells were influenced by these insecticides through changes that occurred to their chloroplast. These insecticides had a phototoxic effect on the diatom community and caused the chloroplasts of these organisms to either distort, dissolve or to have reduced the chlorophyll- α concentrations. For both exposure studies the percentage dead cells were higher in the exposed samples compared to the controls. The insecticides had a negative effect on diatom metrics (life-forms, ecological guilds and size classes), which resulted in a significant decrease in some diatom metrics after exposure to the selected insecticides. Results from both exposure studies indicated that diatoms are effective bio-indicators of pesticide exposure and could be used in ecotoxicology studies.

Overall the results show the importance of including diatom analysis in floodplain ecosystem studies. It is important to study the floodplain as a whole and not only focus on either the river or floodplain pans. Changes in the environmental variables (biological, chemical and physical) will influence the diatom community structure and vitality. Long term analysis of the diatom community within the lower Phongolo River floodplain is essential, especially to study the change and influence on the diatom community when the Phongolo River floods the floodplain area (post-drought).

Keywords: diatoms, changing environment, flood release events, paleo-ecology, stable isotopes, confocal microscopy, pesticides, DDT, Deltamethrin, microcosm

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List of Abbreviations

‰ Parts per thousand

α Alpha

β Beta

δ¹³C Carbon stable isotope

δ¹⁵N Nitrogen stable isotope

γ Gamma

A

AMS Accelerator mass spectrometry

ANOVA Analysis of variance

B

BACON Bayesian accumulation histories

BP Butterfly Pan

C

C Commercial grade

CAC Cold Air Cave

CCA Canonical correspondence analysis

CLSM Confocal laser scanning microscopy

CTCF Corrected total cell fluorescence

CuO Copper oxide

D

DDD 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane

DDE 1,1-dichloro- 2,2-bis(p-chlorophenyl) ethylene

DDT Dichlorodiphenyltrichloroethane

DIC Differential interface contrast

DIN Dissolved inorganic nitrogen

E

EC Electrical conductivity

F

FP Fence Pan

G

GC Gas-chromatograph

GC-μECD Gas-chromatograph micro electron capture detector

H

H High concentration

HCl Hydrochloric acid

K

KMnO₄ Potassium permanganate

L

L Low concentration

LOD Limit of detection

LOQ Limit of quantification

M

MCMC Markov Chain Monte Carlo

N

N Nyamithi

N1 Nyamithi Site 1

N2 Nyamithi Site 2

N3 Nyamithi Site 3

NADPH Nicotinamide adenine dinucleotide phosphate hydrogen

NaOH Sodium hydroxide

NGR Ndumo Game Reserve

NI Nyamithi inflow

nMDS non-metric Multidimensional scaling

NO Nyamithi outflow

Nya Nyamithi Pan

P

P Phongolo River Sites

P1 Phongolo River Site 1

P2 Phongolo River Site 2

P3 Phongolo River Site 3

P4 Phongolo River Site 4

P5 Phongolo River Site 5

P6 Phongolo River Site 6

PAST Paleontological statistics

PCA Principal components analysis

PCNM Principle coordinates of neighbouring matrix

PP Paradise Pan

PR	Phongolo River
PRC	Principle response curve
PSI	Photosystem I
PSII	Photosystem II
S	
S	Shokwe
S1	Shokwe Site 1
S2	Shokwe Site 2
SHCal13	Southern Hemisphere atmospheric curve
Sho	Shokwe Pan
SIMPER	Similarity percentage analysis
T	
T	Technical grade
TP	Total phosphate
U	
U1	Usuthu River Site 1
U2	Usuthu River Site 2
UR	Usuthu River
W	
WHO	World Health Organisation

Chapter 1: General introduction

1.1 Introduction

Freshwater ecosystems are vulnerable to environmental change, with declines in biodiversity far greater than terrestrial ecosystems (Oeding & Taffs, 2015). The major driver of environmental change is the impact that human activities have on aquatic ecosystems, due to increased economic and societal demands that growing human populations have on the world's finite water resources (Brand *et al.*, 2009; Lu *et al.*, 2015; Oeding & Taffs, 2015). These demands highlight the impact that humans have on the environment resulting in biodiversity loss, climate change, land use change, excessive abstraction, transformation and transportation of these resources (Brand *et al.*, 2009; Lu *et al.*, 2015). In South Africa many river ecosystems have deteriorated due to societies considering the resource as inexhaustible, which has caused an increase in catchment degradation, pollution and poor water quality (Brand *et al.*, 2009).

Wetland ecosystems are affected by human activities such as forestry, mining and agricultural drainage (Malan & Day, 2012; Kock *et al.*, 2019). Climate change can also affect wetland ecosystems in various ways including changes in ecosystem functions and structures, browning of the water column due to increased dissolved organic matter and cause more severe fluxes in the nutrient levels (Roberts *et al.*, 2019). Wetlands are susceptible to pollution as they act as “sinks” where sediment and water collect (Dallas & Day, 2004; Malan & Day, 2012). These systems serve as important ecosystems providing humans with necessary ecological services including biochemical cycling, water storage and vital resources such as wood, water and food (Kotze, 2010; Matlala *et al.*, 2011), thus indicating the importance and necessity of wetland ecosystems.

The lower Phongolo River has a floodplain of approximately 10 000 ha (13 000 ha at full inundation) and extends from the Pongolapoort Dam to the confluence of the Phongolo and Usuthu rivers in the Ndumo Game Reserve (NGR), a protected area (Kyle, 1996; Smit *et al.*, 2016). Due to its unique wetlands and high biodiversity the NGR is a Ramsar wetland of international importance (Dube *et al.*, 2015; Smit *et al.*, 2016). The floodplain is one of South Africa's most diverse and largest inland floodplains supporting 50 fish species and migrating bird species (Dube *et al.*, 2015; Smit *et al.*, 2016). The human population living within the catchment area is also dependent on the floodplain for fish, firewood, thatch grass, reeds, water, grazing for domestic livestock and agricultural land (Coetzee *et al.*, 2015; Dube *et al.*, 2015). The Usuthu River is the largest river in Swaziland and forms part of the northern

boundary of the floodplain (Jansen van Rensburg *et al.*, 2016). The river is at risk due to over-utilisation from agriculture activities, domestic activities and transport of waste (Jansen van Rensburg *et al.*, 2016). Shokwe Pan is the main floodplain pan of the Usuthu River and receives water from flooding of the Usuthu River and high localised rainfall. Nyamithi Pan is the second largest pan in the NGR and receives water from groundwater seepage, high localised rainfall and flooding of the Phongolo and Usuthu rivers. The significance of the floodplain in terms of its hydrology, ecological and socio-economic importance are further highlighted in Dube *et al.* (2015).

The Pongolapoort Dam's construction was completed in 1973 with the main purpose to provide irrigation water for sugarcane and cotton plantations and to supply nearby towns with water (Smit *et al.*, 2016; Champion & Downs, 2017; Brown *et al.*, 2018). Since its construction, maximum discharges from the dam halved compared to discharges before the construction of the dam (Brown *et al.*, 2018). This has changed the flood frequency and flow volumes of the downstream Phongolo River. According to van Vuuren (2009) and Brown *et al.* (2018) the release of flood water has varied over time but generally influenced the downstream ecosystems as there were changes in the timing of high and low flows, more constant low flows, a decline in flooding frequency and overall less variability in flow resulting in fewer peaks.

Controlled flood releases from the dam were implemented to mimic the pre-dam's flood regime and to ensure the ecosystem infrastructure is maintained (Smit *et al.*, 2016). Since 1998 flood releases were implemented for an October flood release (with flood releases between < 400 and $> 700 \text{ m}^3\text{s}^{-1}$) in order to inundate the NGR wetlands and to meet the floodplain agricultural needs (Brown *et al.*, 2018). A severe drought (Baudoin *et al.*, 2017; Jozini Local Municipality IDP 2017/18-2021/22, 2017) and flood release protocols that are not properly met (Smit *et al.*, 2016) have resulted in no flood releases from the dam since December 2014. Since then, an average base flow rate of $4\text{--}8.50 \text{ m}^3\text{s}^{-1}$ has been maintained. In order to sustain the floodplain's biodiversity, physical and chemical structure and ecosystem processes the environmental flow requirements may not be met (Smit *et al.*, 2016). Therefore, the lower Phongolo River floodplain's natural functioning was disturbed by the construction of the dam (Champion & Downs, 2017).

The floodplain is thus at risk due to water abstraction, land use, agriculture, irrigation schemes, extensive fishing and flood requirements that are not met from controlled flood releases, upstream of the Pongolapoort Dam (Kyle, 1996; Dube *et al.*, 2015; Smit *et al.*, 2016). The

catchment area is also affected by invasive alien plants (Kyle, 1996) and the spraying of DDT in and around NGR for mosquito vector control (Smit *et al.*, 2016; Volschenk *et al.*, 2019).

Recent studies on the Phongolo River floodplain include work by Jaganyi *et al.* (2009), van Vuuren (2009), Dube *et al.* (2015) and Volschenk *et al.* (2019) just to name a few. However, these studies focussed mainly on fish, amphibians, invertebrate communities and water-associated birds (Netherlands *et al.*, 2015; Smit *et al.*, 2016; Welicky *et al.*, 2017; Wolmarans *et al.*, 2018) with limited to no research done on the phytobenthos community of the floodplain.

Phytobenthos are an important basal energy source that occurs on various substrates in aquatic ecosystems (Dalu *et al.*, 2015). As primary producers phytobenthic organisms are influenced by, amongst other factors, nutrient levels, pH and salinity of the aquatic environment (Dalu *et al.*, 2015; 2016). The phytobenthic community consist of bacteria, diatoms, fungi and protozoa (Dalu *et al.*, 2016) with diatoms often used as representatives of the phytobenthos community (Kelly *et al.*, 2008). Diatoms (Bacillariophyta) consist of roughly 66 000 taxa (freshwater and marine) and are the most diverse microalga class (Rimet & Bouchez, 2011; Bichoff *et al.*, 2017). Diatoms have adapted to several life-forms due to environmental pressure, including flow disturbances, grazing and nutrient resources (Rimet & Bouchez, 2011). These life-forms include: colonial, those living in mucous tubules, mobile, planktonic, pioneer and pedunculated (Rimet & Bouchez, 2011). Diatoms have a wide distribution, are mostly thought to be cosmopolitan, have a high abundance, respond rapidly to anthropogenic activities and changes in the environment, and are found in most aquatic and sub-aerial environments (Bichoff *et al.*, 2017; Kock *et al.*, 2019). They are also present in shallow streams that are only a few millimetres deep and occur in aquatic ecosystems with extreme water quality conditions. In aquatic food-web structures they form a vital link between primary producers and benthic consumers (Kock *et al.*, 2019).

Sub-tropical and tropical regions, such as the lower Phongolo River floodplain, have not received as much research attention as temperate regions across the world (Oeding & Taffs, 2015). There is a general paucity of information on South Africa's wetland ecosystems, including the phytobenthos community. Even though samples have been collected on the diatom community of the lower Phongolo River catchment area, there is limited information and no published work on diatom community structure and the influence that changing environmental conditions, within the floodplain, has on the structuring of their community. As diatoms are primary producers they are valuable indicators of the trophic state and ecological status of the ecosystem (Dalu *et al.*, 2015). As such they are also directly influenced by toxicants within the ecosystem, which can be transferred to higher trophic level organisms

when they consume the phytobenthos. Thus it is important to determine the toxicant flow within the ecosystem and the impact it has on the phytobenthos community, as DDT spraying occurs in and around the NGR. As a result of DDT spraying within this area high levels of DDT have been measured in sampled fish tissue (Volschenk *et al.*, 2019).

1.2 Study hypotheses and aims

The paucity of information on the phytobenthos of the lower Phongolo River floodplain and the effect a changing environment has on the phytobenthos has led to the following hypotheses and aims that will be further addressed in the chapters that follow.

Hypothesis 1: Variations in flow and physico-chemical water quality will have an effect on the structuring of the diatom communities in the Phongolo and Usuthu rivers and their associated floodplain pans.

Aim 1: Determine the influence that controlled flood releases have on the diatom community structure of the lower Phongolo River.

Aim 2: Determine whether the diatom communities of the Phongolo and Usuthu rivers will differ due to system-specific physico-chemical factors.

Aim 3: Investigate if the diatom community structures in the pans of the lower Phongolo system will reflect the degree of lateral connectivity with their associated river systems.

Hypothesis 2: Due to differences in connectivity of the rivers and their associated floodplain pans there will be spatial and temporal differences in the stable isotope signatures and diatom communities between the Phongolo River and associated floodplain pan but not between the Usuthu River and associated floodplain pan.

Aim 4: Investigate the diatom communities and their stable isotope signatures in the Phongolo and Usuthu rivers and associated floodplain pans during the summer rainfall and following the late summer rainfall period.

Hypothesis 3: Diatom community structures will reflect paleo-ecological conditions in Nyamithi Pan.

Aim 5: Determine whether a combination of age-depth sediment profiles and diatom community structures can provide insight into the paleo-ecological condition of Nyamithi Pan.

Hypothesis 4: Since DDT and Deltamethrin are insecticides and do not target diatoms, they will not have an effect on the vitality of the diatom community structures.

Aim 6: Assess the effects of increased concentrations of malaria vector control insecticides (DDT and Deltamethrin) on the diatom community structures using a microcosm approach.

Aim 7: Determine if a mixture (DDT 1:1 Deltamethrin) exposure will have a greater influence on the diatom community when compared to single exposures of these insecticides.

Hypothesis 5: DDT and Deltamethrin will inhibit the photosystems of the diatom cells, negatively affecting their vitality as reflected by chlorophyll- α fluorescence.

Aim 8: Undertake a laboratory bioassay to determine the effects that DDT and Deltamethrin have on the chloroplast of a diatom indicator species, *Nitzschia palea*.

1.3 Thesis layout

To address the hypotheses and aims the study consists of the following chapters:

Chapter 1: A general introduction to the study reporting on the use of phytobenthos and an introduction to the study area as well as the hypotheses and aims of the study.

Chapter 2: A description of the study area and in-depth study of the lower Phongolo River floodplain's diatom community and how a changing environment influences the structuring of the diatom community.

Chapter 3: Analysis of the influence that the absence of a flood release event has on the floodplain by studying the changes and differences in the community structure and stable isotope signatures of phytobenthos between river and floodplain pans during the summer rainfall and following the late summer rainfall period.

Chapter 4: Analysis of how the Nyamithi Pan (within the Ndumo Game Reserve) and the surrounding area has changed over the past 1000 years by studying changes in the diatom

community structure within the paleo-environment, and relating these changes to changing environmental conditions.

Chapter 5: A microcosm experiment to determine the influence that low and high concentration exposures of DDT, Deltamethrin and a mixture has on the vitality of an uncontaminated (in regards to DDT and Deltamethrin) diatom community.

Chapter 6: A four-week laboratory-based exposure to determine the influence that DDT, Deltamethrin and a mixture have on the vitality of *Nitzschia palea* through chlorophyll- α and confocal microscopy methods.

Chapter 7: Concluding remarks on the results obtained from the study and recommendations for future studies on the lower Phongolo River floodplain.

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Chapter 2: Spatial and temporal variation of the diatom community composition and the influence of environmental variables on the community distribution within the lower Phongolo River floodplain.

2.1 Introduction

Floodplain ecosystems are some of the most diverse biological ecosystems worldwide supporting a high diversity and abundance of aquatic organisms (Kingsford, 2000; Dube *et al.*, 2017). One of the major drivers of biota and water chemistry between floodplain wetlands and rivers is flooding, as these ecosystems are linked through flood pulsing (Weilhoefer *et al.*, 2008). However, the construction of dams, climate change and human impacts has caused a decline in the aquatic biodiversity within floodplain ecosystems (Kingsford, 2000; Dube *et al.*, 2017).

The construction of dams influences the flow regime of rivers and the volume of water that reaches floodplain wetlands changes their biological, chemical and physical characteristics that affects their ecology (Kingsford, 2000; Uehlinger *et al.*, 2003; Weilhoefer *et al.*, 2008; Dube *et al.*, 2017). Dams influence the functioning and structuring of floodplain ecosystems as flow, an important factor of floodplain ecosystems, is altered (Uehlinger *et al.*, 2003) with flood control protocols implemented within these systems. Changes in floodplain biota can also be driven by climate change and human activities as the majority of dams are constructed for the purpose of supplying irrigation water for agricultural as well as domestic use (Oeding & Taffs, 2014). Loss of biodiversity, declining water quality, altered biological and chemical cycles are driven by human activities causing degradation and modifications of catchments (Oeding & Taffs, 2015). The biodiversity of floodplain ecosystems are vulnerable to human activities such as recreation, land use, industrial, agricultural and domestic runoff (Mirzahasanlou *et al.*, 2019).

Although much research has been done on the lower Phongolo River floodplain, there is no published work on the diatom community composition (Chapter 1). The Ndumo Game Reserve (NGR) is a RAMSAR site of international importance and is situated in the floodplain area. Communities in the floodplain area are dependent on the floodplains, thus posing a potential threat to the floodplain and aquatic ecosystem due to anthropogenic activities (Dube *et al.*, 2015). The construction of the Pongolapoort Dam has altered the flood regime and natural flow of the ecosystem, thus contributing to the turbidity of the river ecosystem of this is a

bottom release dam. Controlled flood releases from the dam have not taken place since December 2014, as the area is experiencing a drought, causing a disruption to the floodplain's functioning. The lower Phongolo River floodplain is both socio-economically and ecologically important, and is threatened through various activities including agriculture and flow modification (Dube *et al.*, 2015; Smit *et al.*, 2016).

Diatoms belong to the class Bacillariophyceae and are the most diverse, ecologically relevant algal group and in terms of biomass they constitute the majority of phytobenthos in certain seasons (Barragán *et al.*, 2018; Rivera *et al.*, 2018). They are sensitive to changes in their environment and respond rapidly to these changes as they have a short life span (2–3 weeks) (Ramanibai & Jeyanthi, 2010; Stevenson *et al.*, 2010). Species are found along a range of environmental conditions with each individual species having different preferences to environmental requirements (Dixit *et al.*, 1992). Numerous factors (biological, climate, hydrological and physico-chemical) influence their distribution within the aquatic environment (Mirzahasanlou *et al.*, 2019). Environmental variables, such as water quality, current velocity, substrata type, temperature, light availability and environmental stressors including nutrients and other physical and chemical parameters affect the distribution of diatom communities within aquatic ecosystems (Tornés *et al.*, 2015; Barragán *et al.*, 2018; Pumas *et al.*, 2018; Rivera *et al.*, 2018). In ecological research it is important to understanding how the diatom community is influenced by these environmental factors (Mirzahasanlou *et al.*, 2019). The spatial and temporal distribution of the diatom community is limited by environmental and geographical factors (Potapova & Charles, 2002). Factors controlling diatom diversity are still poorly understood (Biggs & Smith, 2002), however, there is evidence from the limited freshwater tropical ecosystem studies that the major drivers of periphytic and planktonic microalgae assemblages are environmental conditions (such as nutrients, flow, temperature, pH and conductivity) (Bartozek *et al.*, 2019). Little is known about diatom distribution in floodplain ecosystems.

The aims of this chapter were to 1) determine the influence that controlled flood releases have on the diatom community structure of the lower Phongolo River; 2) determine whether the diatom communities of the Phongolo and Usuthu rivers will differ due to system-specific physico-chemical factors and 3) investigate if the diatom community structures in the pans of the lower Phongolo system will reflect the degree of lateral connectivity with their associated river systems. The hypothesis that will be tested are that variations in flow and physico-chemical water quality will have an effect on the structuring of the diatom communities in the Phongolo and Usuthu rivers and their associated floodplain pans.

2.2 Materials and methods

2.2.1 Site description

The study was done in the lower Phongolo River floodplain area in the north-western corner of KwaZulu-Natal, South Africa (Figure 2.1). The study sites include the Phongolo River, Usuthu River and their associated floodplains downstream of the Pongolapoort Dam. Sites for the study were selected based on previous studies in the area (Dube *et al.*, 2015; Smit *et al.*, 2016). A total of 19 sites were selected including river sites, floodplain pans and ephemeral pans. The Phongolo and Usuthu rivers form the eastern and northern boundary of the Nudmo Game Reserve respectively. The two rivers join in the north-eastern corner of the park. Two floodplain pans associated with the Phongolo River and one floodplain pan associated with Usuthu River were selected for the study. All ephemeral pans selected for the study receive surface water from runoff during high rainfall and were not filled from overflow from either rivers during the research period.

Surveys in July 2013 represented a period just after localised flooding due to rainfall in the catchments while the September 2013 survey was just before the scheduled controlled flood release. Sites sampled during the 2013 survey include Phongolo River Site 1 (P1), Phongolo River Site 2 (P2) and Phongolo River Site 6 (P6). Sampling at all the sites described below was carried out during February (high flow, summer rain), May (following late summer rain), September (low flow) and November 2017 (beginning of rainy season), and February (high flow, summer rain) and May 2018 (following late summer rain). Prior to and during this period there were no flood releases from the dam, with the last flood release being in December 2014.

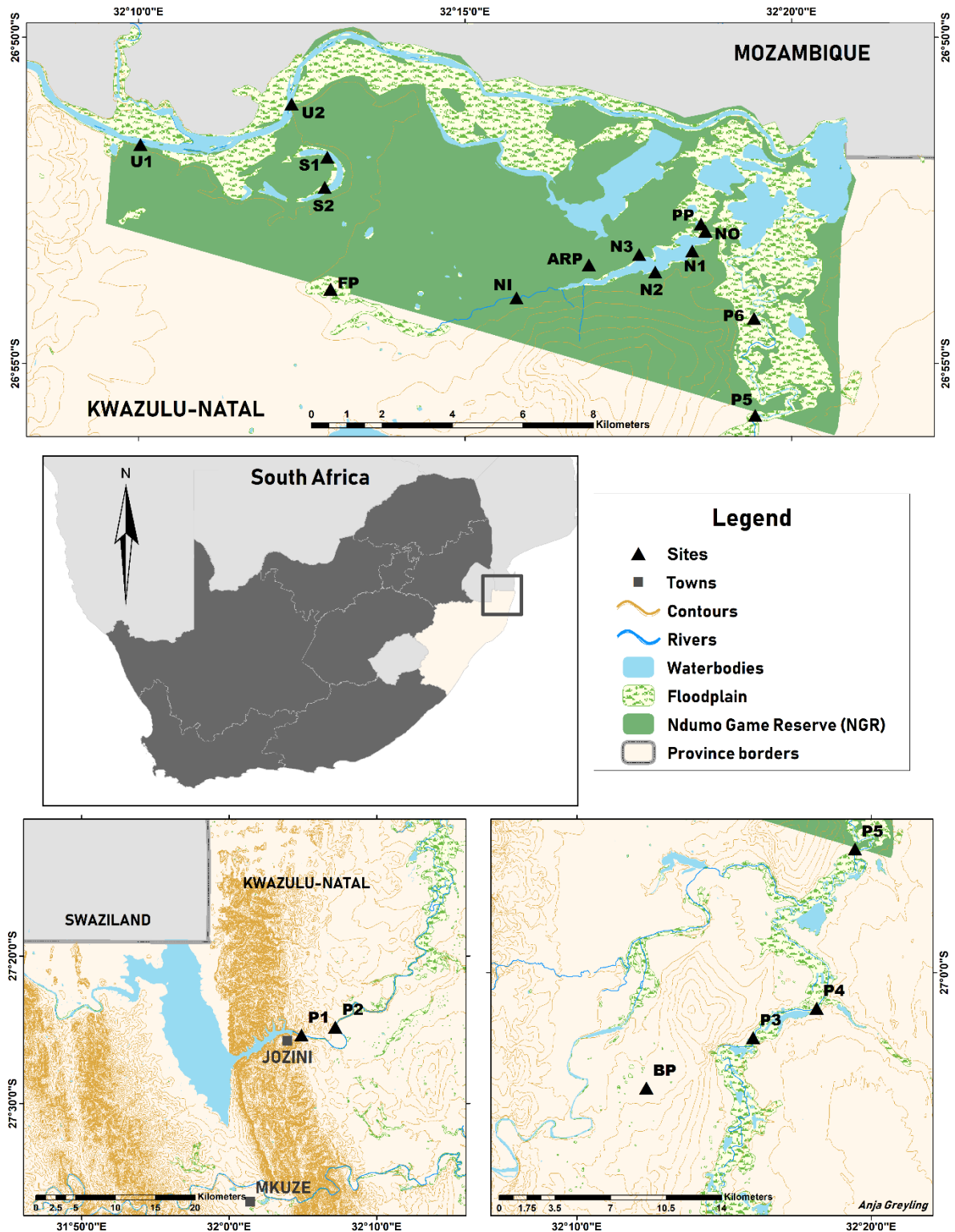


Figure 2.1: Map of lower Phongolo River floodplain area illustrating the sampling sites. P – Phongolo River, U – Usuthu River, N – Nyamithi Pan, S – Shokwe Pan, FP – Fence Pan, ARP – ARP Pan, PP – Paradise Pan, BP – Butterly Pan.

2.2.1.1 River sites

- Phongolo River Site 1 (P1)

The site is the most upstream site of the study and located approximately 1 km downstream of the Pongolapoort Dam situated at S27°25'21.65" E32°04'53.52" (Figure 2.2). A flow of 7 m³/s is maintained at the dam during the low-flow season with a possible 600–800 m³/s flow that can be released during a controlled flood in the high-flow season (Smit *et al.*, 2016). Vegetated islands are present at the site with the substratum consisting mainly of coarse-grained material. The bed is dominated by cobbles and rocks, however, submerged vegetation is also present along the edge of the site as well as in the deeper running waters. Tall reeds are found on the banks of the site with some overhanging trees. A gauging weir (W4H013Q01) (Figure 2.2 A) is situated close to the site with water treatment works adjacent the sampling area. During each survey human impacts were visible with people making use of the area to bathe themselves as well as wash their clothes and cars (personal observation). Diatom samples were retrieved from submerged vegetation (in stream) and sediment. The site was also sampled during July and September 2013.

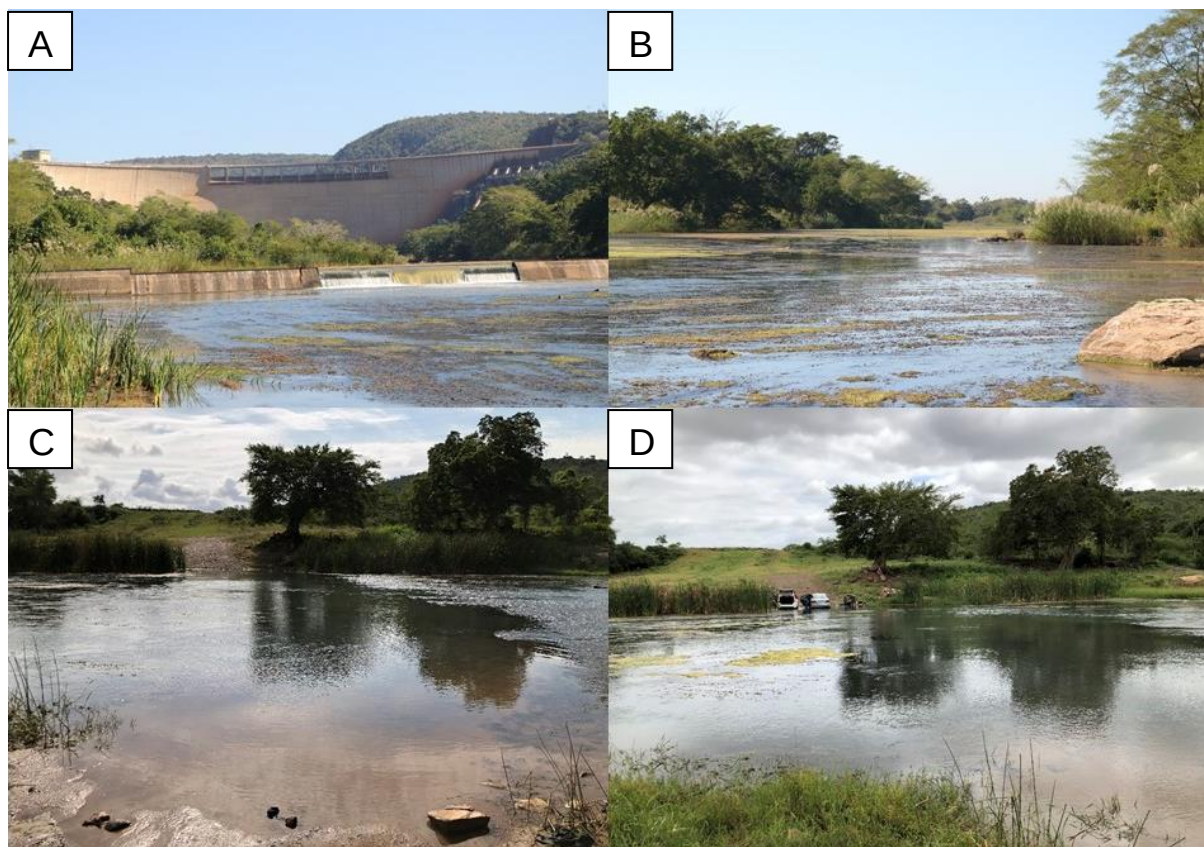


Figure 2.2: Phongolo River Site 1 from upstream (A) to downstream (B). (C) The area where the river is regularly crossed, and (D) an image of the community washing their cars and clothes.

- Phongolo River Site 2 (P2)

The site is situated approximately 12 km downstream of site P1 (Figure 2.3) and located at S27°24'49.50" E32°07'11.42". The area is located at a tented bush camp where various activities, including fishing and canoeing take place. The site is situated in the Kwa Nyamazane conservancy area with this side of the river and riparian vegetation protected, in contrast to the opposite side of the river (Figure 2.3) with no riparian forest present. The areas surrounding the site are impacted by sand mining and agricultural land for sugar cane and cotton farming (Smit *et al.*, 2016). Illegal water abstractions (for subsistence farming) are found just upstream of the site all the way to the NGR. Due to the decrease in the slope, the flow rate is slower than at P1 (Smit *et al.*, 2016). The immediate area at the site consist of reeds with substratum consisting mainly of sand and pebbles. A pool area is situated to the right of the site with riffles to the left. During the survey sediment and reeds were sampled for diatoms. The site was also sampled during July and September 2013.



Figure 2.3: Phongolo River Site 2 from (A) upstream to (B) downstream with agriculture and recreational activities also visible.

- Phongolo River Site 3 (P3)

The Phongolo River Site 3 (S27°02'12.41" E32°15'59.31") (Figure 2.4) is situated adjacent to a high water bridge crossing the Phongolo River. The area is impacted by human activities from the local town (Ephondweni) (roughly 600 m from the site), including fishing, washing of clothes and cars, religious activities and a manmade impoundment for the damming of water. Water is pumped daily from the area for domestic use and road building (personal observation). Substratum present at the site consists mainly of sand and pebbles and rocks are also present. Steep banks are found on both sides of the river with erosion visible. The banks are covered by trees and shrubs with submerged vegetation present at the site. During

low flow there are vegetated islands present at the site. Diatoms were sampled from sediment and the submerged vegetation.

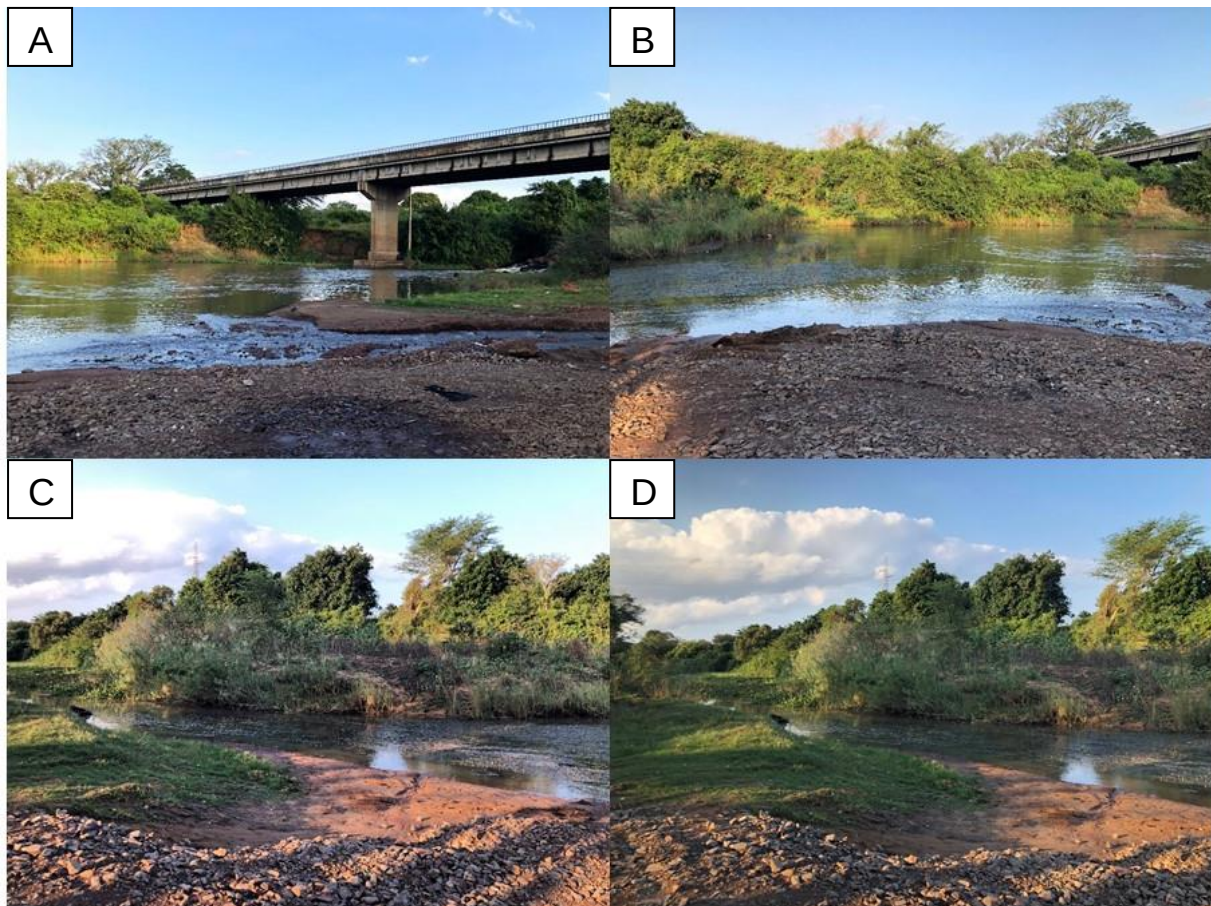


Figure 2.4: Phongolo River Site 3 from (A–D) upstream to downstream, (C–D) with the vegetated island visible.

- Phongolo River Site 4 (P4)

The site (Figure 2.5) is situated approximately 4 km downstream from P3 at S27°01'12.28" E32°18'08.41". A pedestrian walkway to cross the river is present at the site with a gravel road close by. Local community members make use of this area of the river to wash their clothes as it is easily accessible. Cattle were present during almost all sampling surveys as they make use of this area of the river to drink (personal observation; Figure 2.5 E, F). Riffles are present at the site with pools situated upstream and downstream of the site. Riparian vegetation is present surrounding the site with overhanging trees. Samples were retrieved from the eastern bank of the river with submerged vegetation found along the edges of the river and a vegetated island downstream of the pedestrian bridge. The substratum consisted mostly of sand with pebbles, rocks and large boulders were also present. Sediment and submerged vegetation were sampled for diatoms.

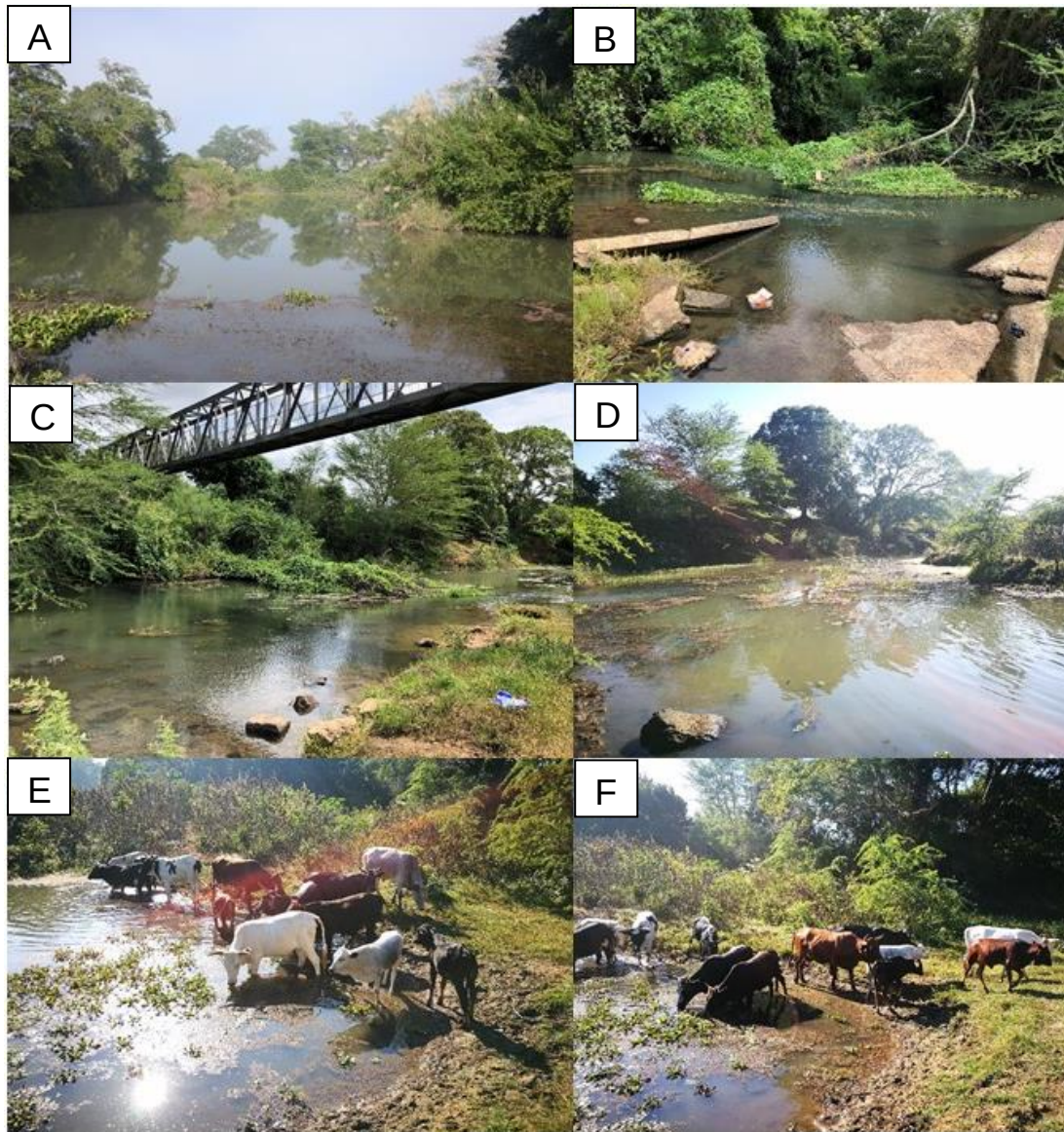


Figure 2.5: Phongolo River Site 4 from (A-B) upstream to (C-D) downstream, with overhanging vegetation, large rocks and boulders and the pedestrian bridge visible. Images shows the presence of (E-F) cattle at the site making use of the river for drinking water.

- Phongolo River Site 5 (P5)

The site (Figure 2.6) is situated at the southern boundary of the NGR as the river enters the reserve at S26°55'47.68" E32°19'26.68". The eastern bank of this site is impacted by agricultural activities from the local community. Water is abstracted from this area for the campsite of the reserve, by farmers for their crops and for parts of Ndumo Town with a new water pumping station installed just upstream of the site. Substratum present consisted largely of clay and sand with sandbanks spread throughout the site. Both banks of the river were impacted by erosion with a large area covered by riparian vegetation and overhanging trees.

Submerged vegetation was present along the edges of the river with shrubs and reeds spread out throughout the area. Diatoms were sampled from submerged vegetation as well as sediment. The site was accessed with a boat during sampling surveys.

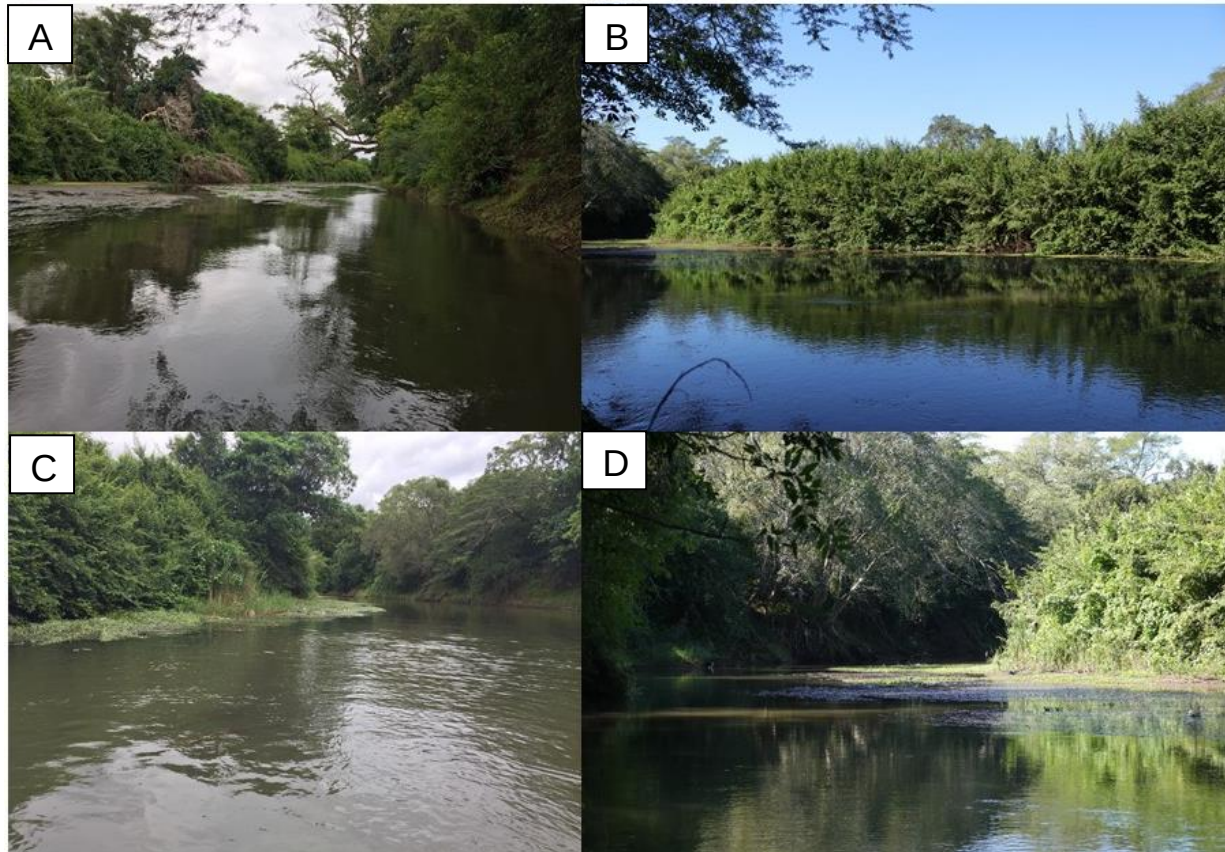


Figure 2.6: Phongolo River Site 5 illustrating the area at the site from (A-B) upstream to (C-D) downstream.

- Phongolo River Site 6 (P6)

Site 6 (Figure 2.7) ($S26^{\circ}53'29.7''$ $E32^{\circ}17'30.9''$) is located within the NGR at the old pump station that provided water to the campsite. The site forms part of the former channel of the Phongolo River prior to formation of the new primary channel splitting away to the east. There was visible erosion on both banks of the river. Riparian vegetation was present with very little submerged vegetation and the substratum consisted mainly of silt and mud. The site receives water during flooding of either the Phongolo- or Usuthu Rivers. The site could only be sampled once in February 2017 when the Usuthu River was in flood and filled the channel. The site was also sampled during July and September 2013.

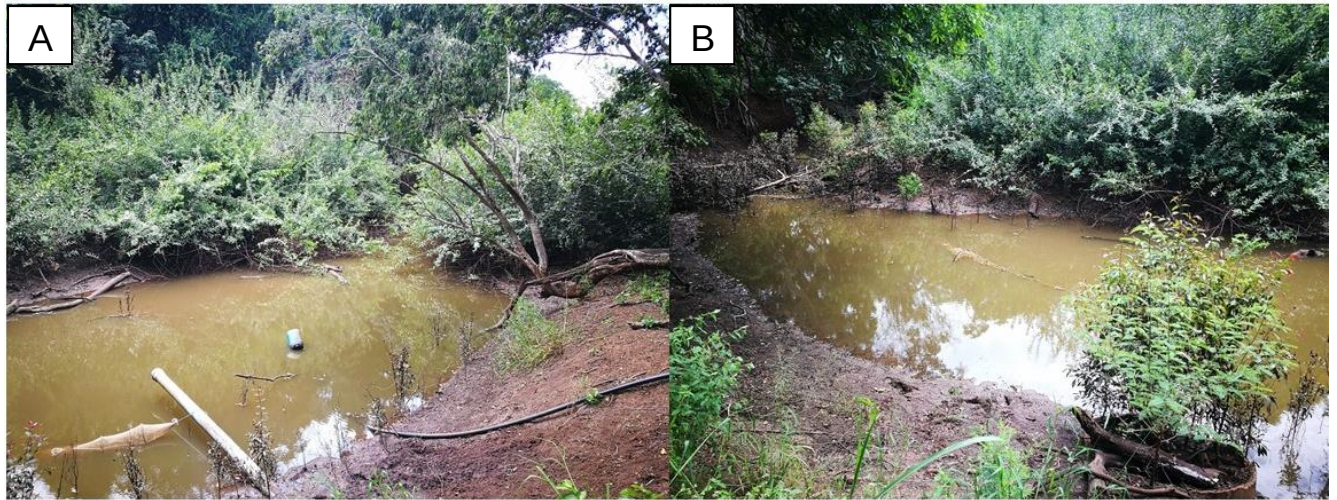


Figure 2.7: Phongolo River Site 6 from (A) upstream to (B) downstream.

- Usuthu River Site 1 (U1)

The site (Figure 2.8 A, B) is situated at S26°51'38.67" E32°10'01.80" and consists of a large channel with sandy substratum. The channel forms the border between South Africa and Mozambique with both banks dominated by reeds and shrubs. Sand bars were present at the site. Little to no submerged vegetation was present and diatoms were sampled from reeds and sand at this site.

- Usuthu River Site 2 (U2)

Usuthu Site 2 (Figure 2.8 C and D) is situated approximately 4 km downstream from U1 at coordinates S26°51'01.41" E32°12'20.69". No vegetated islands were present, however, the riparian vegetation was dominated by tall reeds (Smit *et al.*, 2016). The river forms the border between South Africa and Mozambique. Little submerged vegetation was present and the substratum consisted mainly of sand. Diatoms were sampled from aquatic vegetation and sand during the surveys.

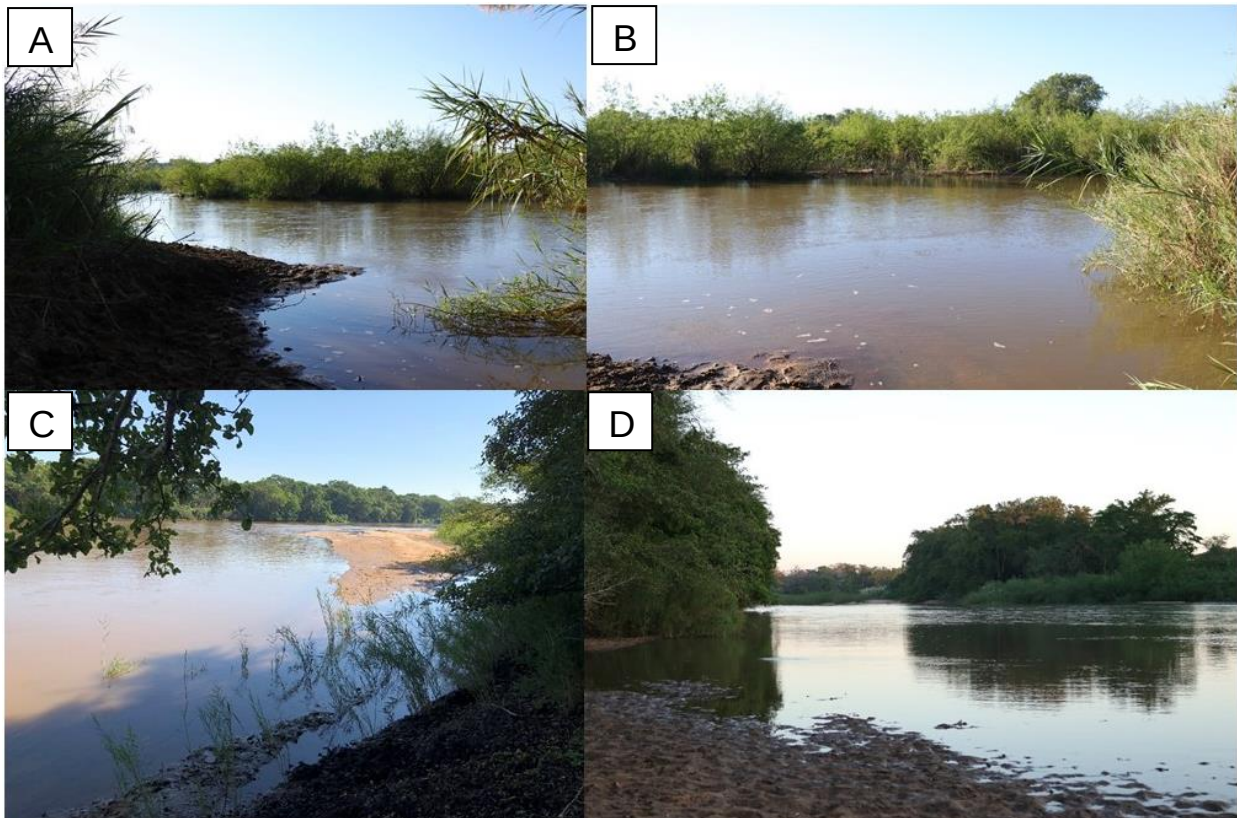


Figure 2.8: Usuthu River Site 1 from (A) upstream to (B) downstream and Usuthu River Site 2 from (C) upstream to (D) downstream. The differences between the two sites are rather pronounced. Mozambique is visible on the opposite side of the river.

2.2.1.3 Floodplain pan sites

Pan sites selected for the study include floodplain pans from both the Phongolo and Usuthu rivers as well as ephemeral pans. A total of 11 floodplain pan sites were selected for this study with the majority of these pans inside the NGR. Outside of the NGR there were no inundated floodplain pans due to the abstraction of water by the local community for irrigation for subsistence agriculture.

Phongolo River associated pans

- Nyamithi Pan

Nyamithi Pan is the second largest floodplain pan within the NGR and it is situated approximately 4 km north-east of the reserves main entrance (Smit *et al.*, 2016). Due to the size of the floodplain pan, five sites were selected namely Nyamithi inflow (NI) (S26°53'59.75" E32°15'47.44"), Nyamithi Site 1 (N1) (S26°53'16.66" E32°18'28.87"), Nyamithi Site 2 (N2) (26°53'35.91" E32°17'54.86"), Nyamithi Site 3 (N3) (26°53'19.71" E32°17'40.15") and Nyamithi outflow (NO) (S26°52'58.42" E32°18'41.03"). Nyamithi Pan was the only permanent lentic site during this study.

Nyamithi's inflow water had a red colouration due to the suspension of red clay particles present in the channel (Figure 2.9). It forms part of the channel that feeds Nyamithi Pan as it drains water from the localised catchment area inside the NGR into Nyamithi Pan. Water is artificially dammed up at the site due to a low water bridge on the tourist road that passes close by the site. The vegetation around the pool mainly consisted of bushes and shrubs with tall grass. The edge of the pool was surrounded by riparian vegetation. During the low-flow months, the pan dries out as it mainly receives water from precipitation. Water lilies and submerged vegetation are present with the substratum consisting mainly of silt and clay. Diatoms were sampled from water lilies, submerged vegetation and sediment (consisting mainly of clay) during the surveys.

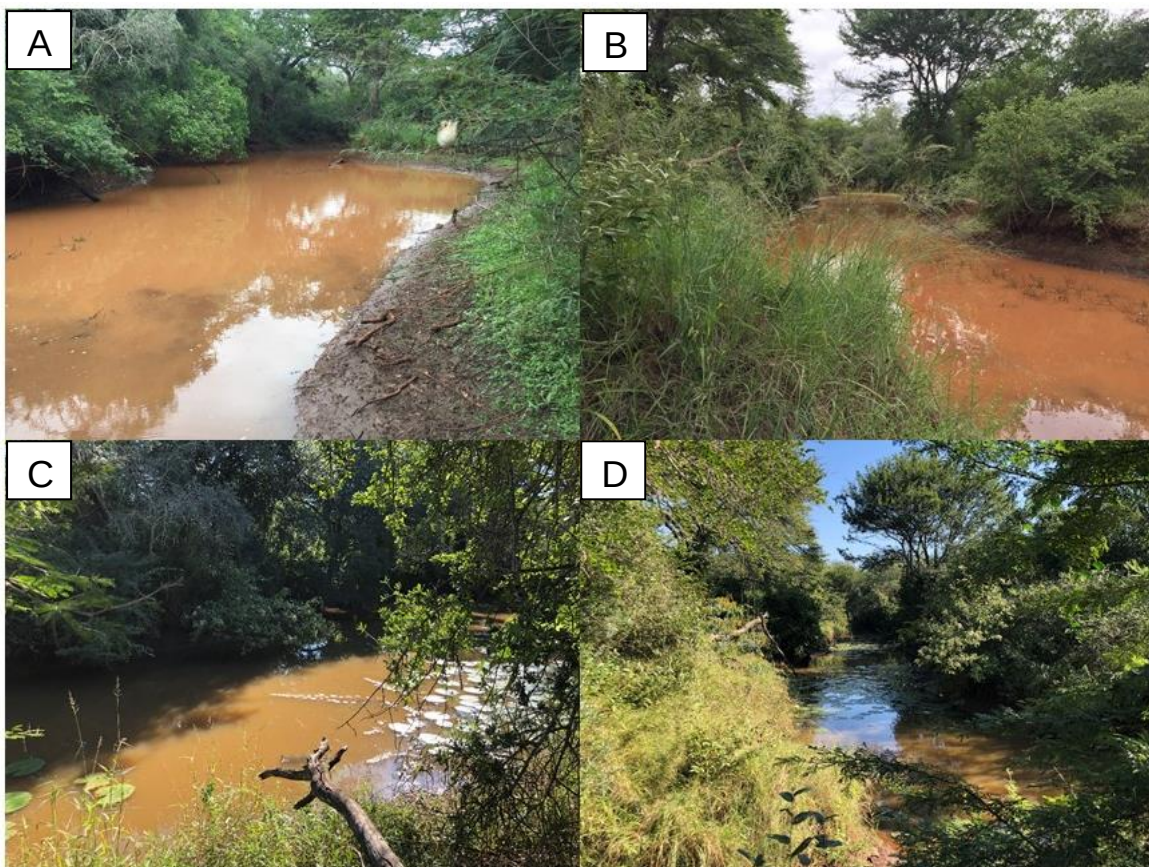


Figure 2.9: Nyamithi inflow site during (A–B) February 2017 and (C–D) May 2018.

The banks on the south-eastern side of Nyamithi Pan (Figure 2.10) are steeper than the opposite bank, with banks vegetated by Lowveld Riverine Forest (Smit *et al.*, 2016). A large number of fever trees (*Vachellia xanthophloea*) were present on the north-western side of the pan, which is periodically flooded (Smit *et al.*, 2016). The pan is occupied by large numbers of crocodiles (*Crocodylus niloticus*) and hippopotami (*Hippopotamus amphibious*). Streams on the south-western side are the main source of water feeding into the pan (Kyle, 1996). The

pan had a high salinity due to the leaching of salt from the substratum as it lies on an ancient/historic marine cretaceous bed (Kyle, 1996). Nyamithi Sites 1 and 2 are both situated on the southern bank of the pan with Nyamithi Site 3 on the northern bank. Nyamithi outflow (Figure 2.10 D, E) is situated at the channel that connects the Phongolo River with the pan (Smit *et al.*, 2016). A weir is present at the site in order to control the outflow of water from the pan (Whittington *et al.*, 2013). According to Smit *et al.* (2016), erosion that occurs on the banks is due to flood recession when water flows out of the pan or floods into the pan. Submerged vegetation and sediment were used to sample diatoms from all sites.

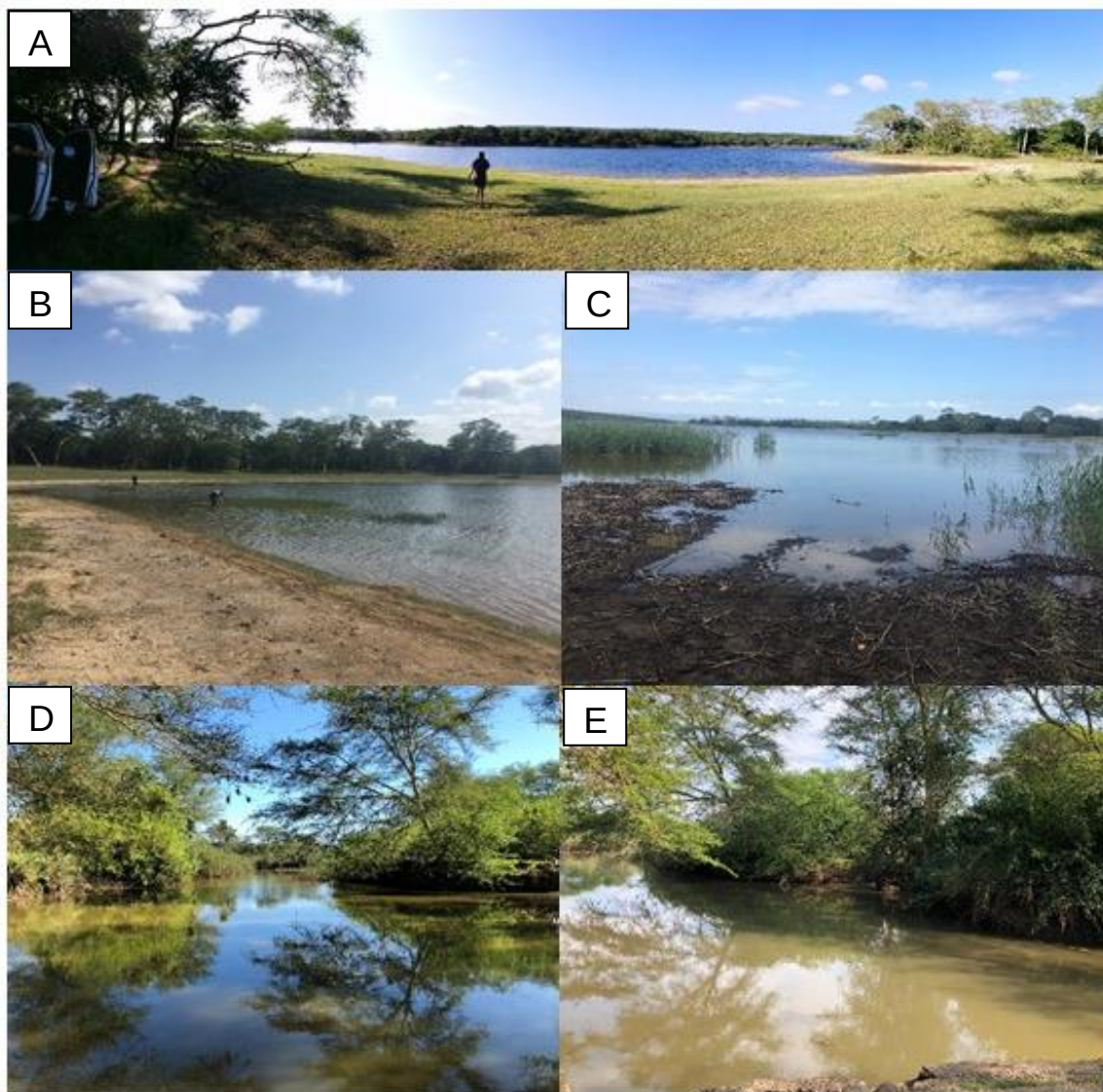


Figure 2.10: (A) A 180° view of Nyamithi Pan. The selected sampling sites of the Nyamithi Pan with (B) Nyamithi 2, (C) Nyamithi 3 and (D–E) Nyamithi outflow.

- Paradise Pan (PP)

Paradise Pan (Figure 2.11) is located just north of NO and is situated in an open grassland. Tall grass and reeds dominated the edge of the pan with grass fields in the surrounding area. The substratum consisted mainly of clay with no vegetated island present at the site. No submerged vegetation was visible and due to the clay, it was difficult to access the site so samples were retrieved from reeds on the edge of the pan. The site could only be sampled once as it only receives water from Nyamithi Pan when it overflows during very high flows.

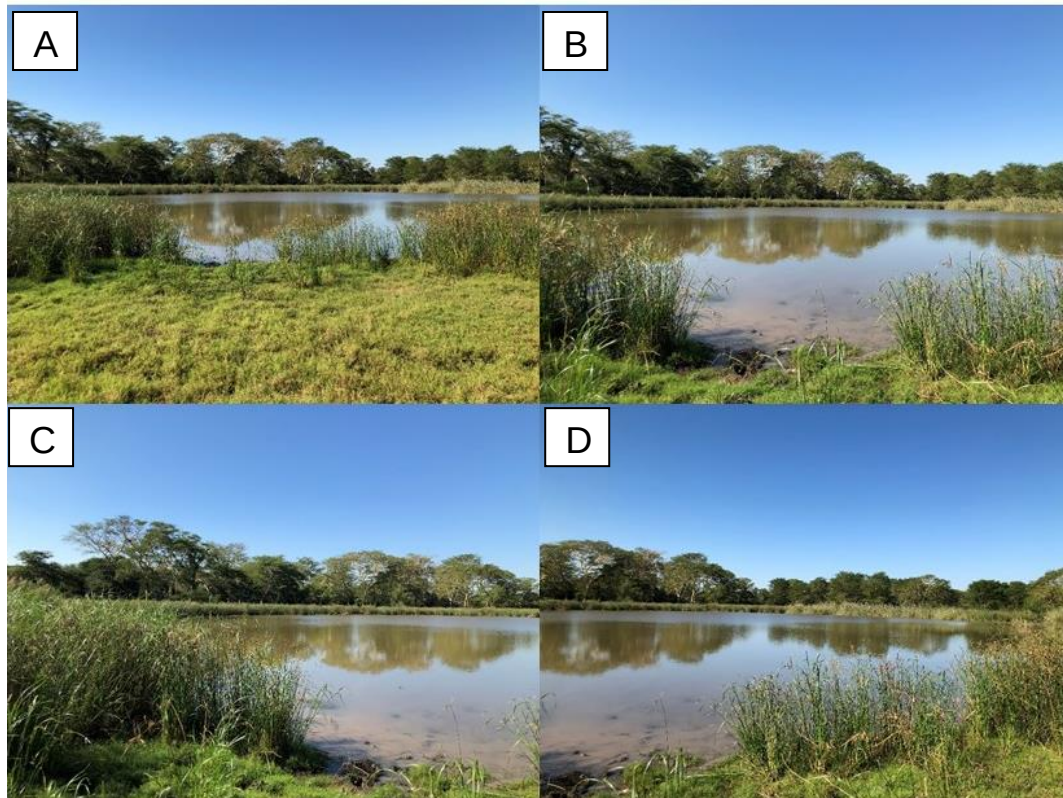


Figure 2.11: (A) Image showing Paradise Pan with the (B) sampling area illustrated in image. Images of the (C) left and (D) right banks with the tall reeds around the edge of the pan clearly visible.

Usuthu River associated pan

- Shokwe Pan

Shokwe Pan is situated within the Usuthu River floodplain and two sites were selected within the pan. These sites include Shokwe Site 1 (S1) (S26°51'52.07" E32°12'52.67") and Shokwe Site 2 (S2) (S26°52'18.17" E32°12'51.15") (Figure 2.12 A, B). Both sites were situated on the western side of the pan and were dominated by dense reed beds. Grassland woodland dominated the area surrounding the floodplain (Whittington *et al.*, 2013), with fig tree forest on the western and fever tree forest on the eastern banks of the pan. Little riparian vegetation was present on the edge of the pan. Substratum at the site consisted mostly of silt with water

lilies being the dominant vegetation within the pan. The pan is a closed drainage system, receiving water during natural flooding of the Usuthu River and local rainfall (Whittington *et al.*, 2013). There were many *H. amphibious* within the pan. Diatoms were sampled from the reeds on the water's edge as well as sediment (coarse mud).



Figure 2.12: Photograph of (A) Shokwe Site 1 and (B) Shokwe Site 2. Little riparian vegetation are present with reeds at the edge of the pan.

Ephemeral pans

- ARP

ARP (Figure 2.13) is situated at coordinates S26°53'28.48" E32°16'55.13" and is 100 m north of Nyamithi Pan. This is a temporary pan that only has water for short periods of time during high local rainfall events. The pan only receives water from rainfall and local runoff and were not filled from Nyamithi Pan. The pan is shallow with a maximum depth of approximately 40 cm and the substratum consists mainly of clay and silt. Due to the shallow nature of the site, the water column is turbid. The site is surrounded by grass and tall trees with submerged vegetation and grasses present within the pan. The site could only be sampled twice as it was dry during the rest of the surveys. During surveys, diatoms were sampled from submerged vegetation.

- Fence pan (FP)

Fence Pan (Figure 2.14) (S26°53'48.76" E32°12'58.38") is situated on the southern border of the NGR about 5 km from the main gate of the reserve. This site only has water during high local rainfall. The pan is shallow with the deepest part roughly 1.5 m. It is surrounded by extensive riparian vegetation with water lilies and submerged vegetation present. Overhanging trees surround the water's edge, with shrubs and bushes present within the area of the pan resulting in increased detritus. The substratum consists mainly of silt and clay. Sediment, water lilies and reeds were used to sample diatoms from.

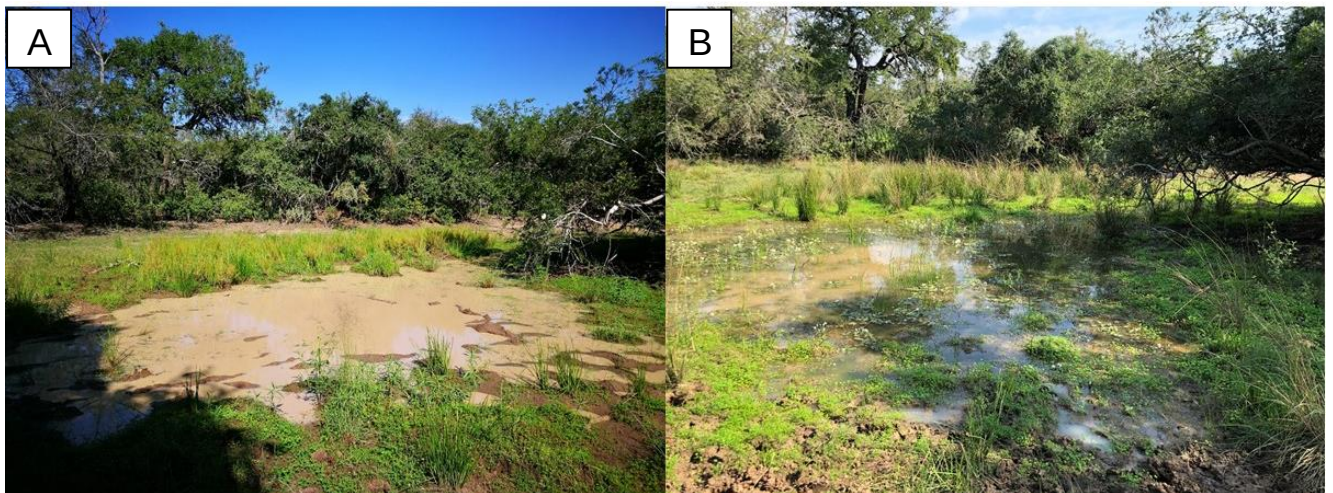


Figure 2.13: ARP site during (A) February 2017 and (B) May 2018.



Figure 2.14: Fence pan with the water lilies and riparian vegetation clearly visible.

- Butterfly Pan (BP)

Butterfly Pan (Figure 2.15) ($S27^{\circ}03'56.60''$ $E32^{\circ}12'22.24''$) is located approximately 26 km outside of the NGR next to a busy road and is in close proximity to housing. This results in the site being impacted by human disturbance with a great deal of litter present in and around the site. Substratum at the pan consists of sand and silt with the surrounding vegetation consisting

mainly of grass. Submerged vegetation was present at the site with grass patches present within the water column. The pan only has water during high local rainfall events. Domestic animals such as chickens and cattle make use of the pan for drinking water (personal observation). Diatoms were sampled from the site from submerged vegetation.

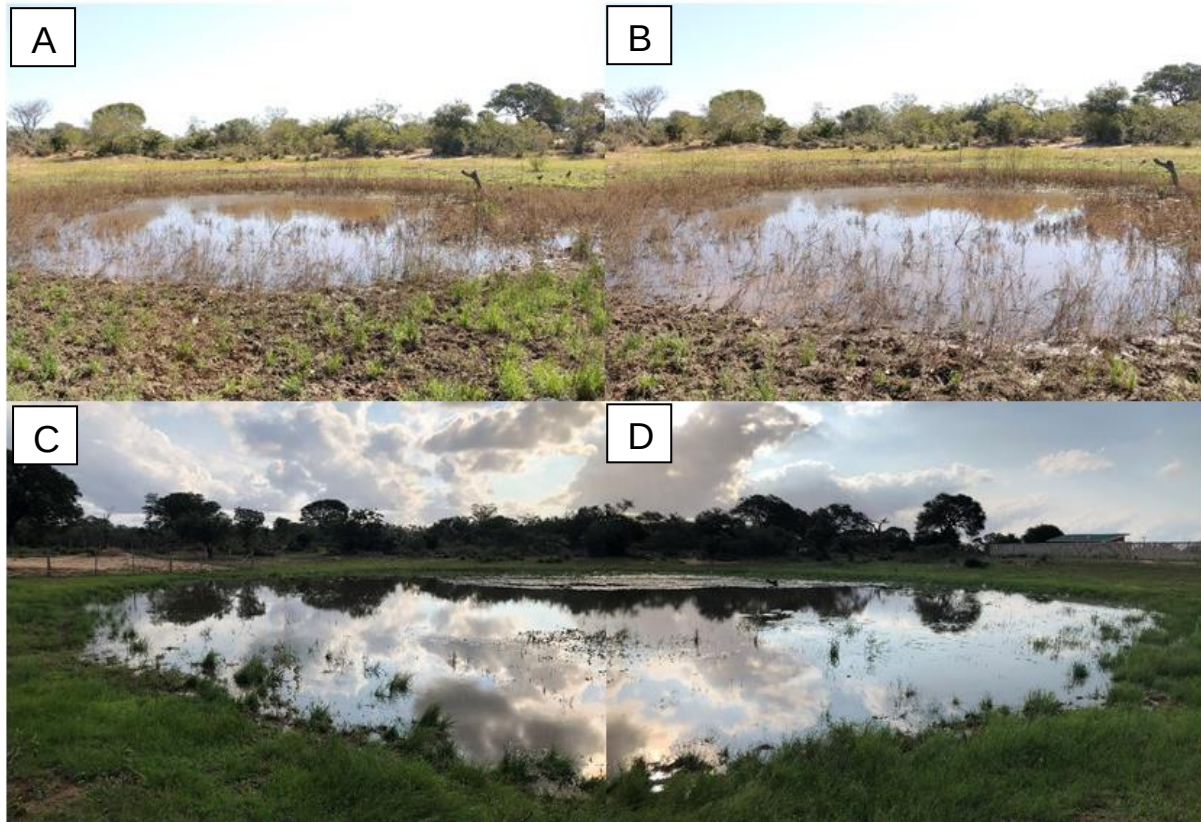


Figure 2.15: Butterfly pan as it was drying out in (A–B) May 2017 and a few weeks after heavy rainfall in (C–D) February 2018.

2.2.2 Physico-chemical water quality

The *in situ* water quality was measured at each site before diatoms were collected. Dissolved oxygen and temperature were measured with an Extech DO610 meter (Extech Instruments, A FLIR Company, USA) and an Extech EC610 meter was used to measure the electrical conductivity, total dissolved solids, salinity and pH. After *in situ* measurements were taken, 500 mL of site water was collected in pre-cleaned sample bottles for nutrient analysis. Water samples were immediately frozen for transportation back to the laboratory. Defrosted water samples were filtered, and nutrient analysis was performed with relevant Merck test kits and a Merck Spectroquant Pharo 300 UV-VIS Spectrophotometer (Merck KGaA, Germany). The following test kits, with corresponding test kit numbers, were used to analyse all water samples: ammonium (1.14752.0001), nitrate (1.09713.0001), nitrite (1.14776.0001), total

phosphate (1.14848.0001) and sulphate (1.14791.0001). Nutrient analysis was done on the key elements (N, P and S) of each molecule.

2.2.3 Diatom sampling, preparation and analysis

For the study two diatom assemblages were sampled namely 1) Epipsammon – diatoms that occur between and on sand particles and 2) Epiphyton – diatoms occurring on macrophytic plants (Taylor *et al.*, 2005). The collection, preparation and analysis of all diatom samples was done according to Taylor *et al.* (2005).

Epipsammic diatoms were sampled using a 5 mm Ø hard plastic tube with a rubber bulb. Air was resiled from the tubing by pressing in the bulb and the end of the tube was rested on the sediment surface and drawn lightly over the surface of the sediment for approximately one metre while slowly and constantly releasing pressure to ensure the top layer of sediment was sucked into the tube. The sample was then transferred to marked sterile honey jars and preserved with 70% ethanol (final concentration not exceeding 20%).

Epiphytic diatoms were sampled from the submerged stems of macrophyte plants. The submerged stems, approximately 10–15 cm, from 10 plants were retrieved and placed in resealable bags with 50 mL of water from the site. After vigorously rubbing the stems, the sample water was transferred to marked honey jars and preserved as described for the epipsammic samples.

The preserved samples were transported back to the laboratory and left for 24 hours to settle after which the supernatant was decanted. Due to high levels of organic matter in South African diatom samples, the preferred method for slide preparation is the hot hydrochloric acid (HCl) and potassium permanganate (KMnO₄) method. Shaken samples were left overnight in marked test tubes with 2–3 mL of KMnO₄. Hydrochloric acid (32%) was added to the samples and then boiled in a hot water bath until the sample cooked clear. To determine if all the organic matter was removed, one drop of hydrogen peroxide was added to each sample. Samples were washed through four washing cycles with distilled water and then pipetted onto cleaned coverslips and left overnight to dry. Coverslips were mounted to microscope slides using Pleurax (Refraction index, 1.73).

A Nikon 80i compound light microscope equipped with differential interface contrast (DIC) and a 100x 1.4N.A. oil immersion objective was used to view the microscope slides. Diatoms were identified to species level where possible and counted until a total number of ~400 diatom

valves or the entire microscope slide was counted. Diatoms were identified with the aid of Taylor *et al.* (2007) as well as the website diatoms.org.

2.2.4 Statistical analysis

A Shapiro-Wilk test was used to test for normality of data. Significant differences between normally distributed data were tested with a parametric one-way Analysis of variance (ANOVA) and a non-parametric ANOVA analysis was used to test for significant differences between non-normally distributed data. Diversity indices (Shannon diversity index, Margalef species richness, Pielou's evenness score and Fisher alpha) were determined using Primer Version 7 and global Whittaker beta diversity with PAST (Paleontological Statistics) Version 3.26. The alpha (α) index represents the diversity of each site and beta (β) index the regional diversity between sites. The gamma (γ) index was used as well and represent the total number of species sampled at each site. Significant differences between diversity indices and physico-chemical water quality were determined with a one-way ANOVA and Tukey's multiple comparison test. The dominant diatom species, as well as the percentage similarity and dissimilarity among samples were determined through a similarity percentage analysis (SIMPER) analysis.

Temporal and spatial differences among waterbody types (i.e. rivers and pans) and sites were determined through non-metric multidimensional scaling (nMDS) plots as well as a Bray Curtis similarity matrix. Spatial and temporal variance among sites and species were determined using Canoco Version 5. An unconstrained principal components analysis (PCA) was used to determine the influence of environmental variables on the seasonal sampled sites.

2.3 Results

A total of eight surveys were successfully completed during 2013 (two surveys) and 2017/18 (six surveys). A total of 19 sites were sampled during these surveys. Physical-chemical water quality variables could not be obtained for some sites during 2017/18. Mean values were used for these sites and sites with mean values are indicated in Appendix A, Table A-1.

2.3.1 Monthly flow of the Phongolo River

During sampling in 2013 there were still regular controlled flood releases from the Pongolapoort Dam (Figure 2.16). During the July 2013 sampling an average flow of 21 m³/s was recorded with an average of 19.7 m³/s flow recorded during the September sampling survey. From December 2014, no controlled flood releases occurred with an average recorded flow of 9.25 m³/s during the 2017/18 surveys.

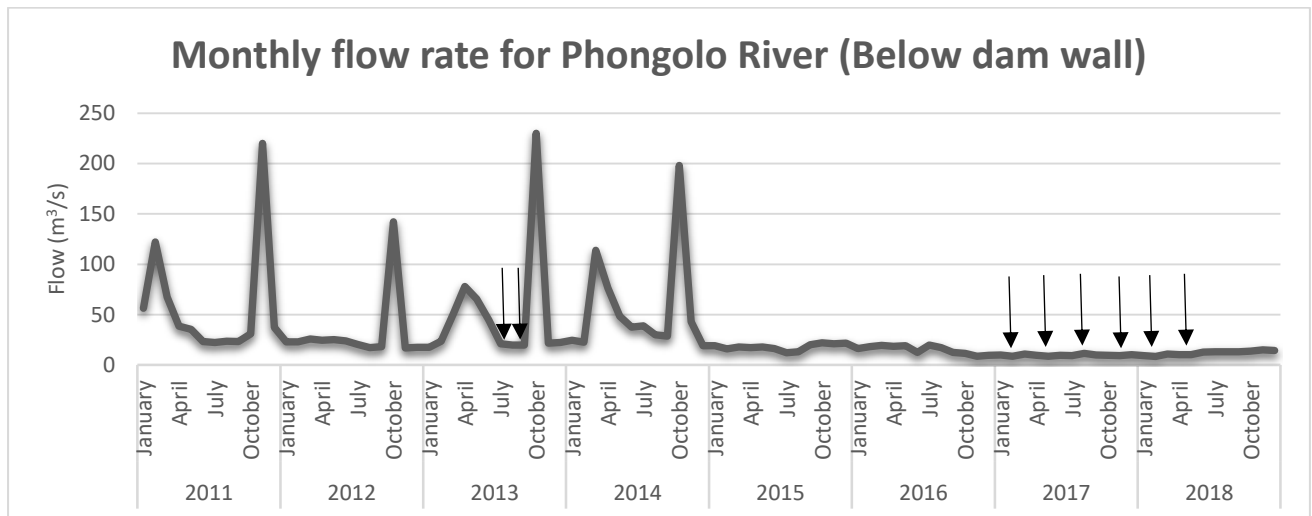


Figure 2.16: Monthly discharges (m³/s) of the Phongolo River. Measurements are from below the dam wall at gauging station W4H013Q01. Arrows indicate periods when sampling took place.

2.3.2 Flood release (2013) versus the absence of a flood release (2017/18)

2.3.2.1 Environmental variables

Mean physico-chemical water variables for sites sampled in 2013 differed from the sites sampled in 2017/18. It is clear that during the 2017/18 surveys nitrate and total phosphate values were much higher compared to 2013 (Figure 2.17 A, B). Measured ammonium and sulphate values were higher during 2017/18 compared to 2013 for all sites (except P6) (Figure 2.17 C, D). The pH remained relatively consistent throughout the study, with significantly ($p < 0.05$) higher pH values for P1 (2017/18) compared to P1 and P2 in 2013 (Figure 2.17 E). All sites in 2013 had significantly ($p < 0.05$) higher flow rates compared to 2017/18 surveys (Figure 2.17 F).

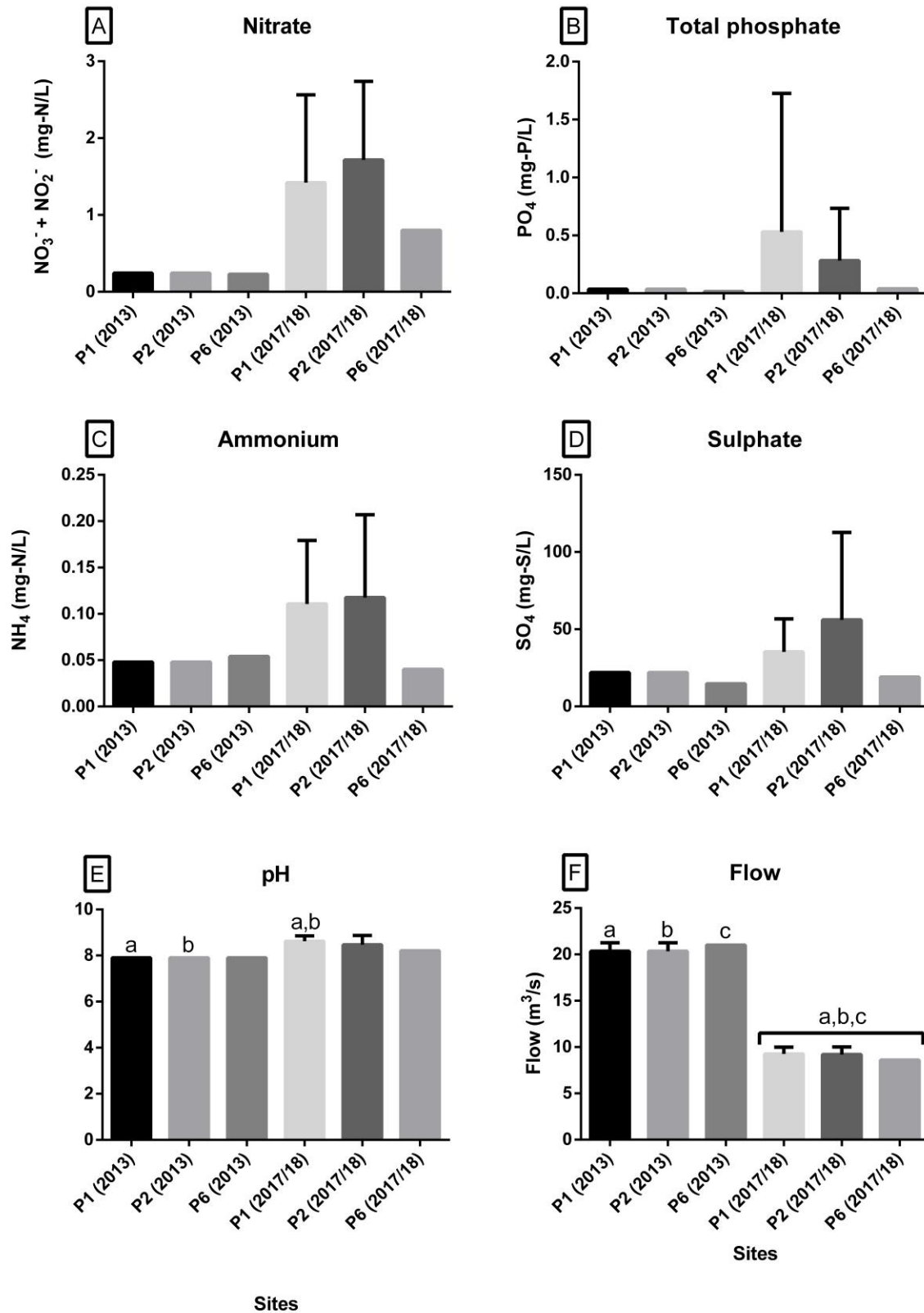


Figure 2.17: Mean $\pm$ SEM of (A) nitrate, (B) total phosphate, (C) sulphate, (D) ammonium, (E) pH and (F) flow data from sites sampled during 2013 and 2017/18. Bars represent the mean of seasonal samples collected from the sites. Bars with common superscript differ significantly ($p < 0.05$).

2.3.2.2 Diatom diversity

During the 2013 surveys a combined total of 66 species were identified. A complete list of species identified during the 2013 surveys is presented in Appendix B, table B-1.

There was no significant differences between the diversity indices for the sites sampled during 2013 and 2017/18 (Figure 2.20). Site P6 had the highest total number of species and Margalef species richness compared to the other sites for both the 2013 and 2017/18 surveys (Figure 2.20 A, B). Both Pielou's evenness and Shannon diversity index remained similar between all sites and surveys (Figure 2.20 C, D).

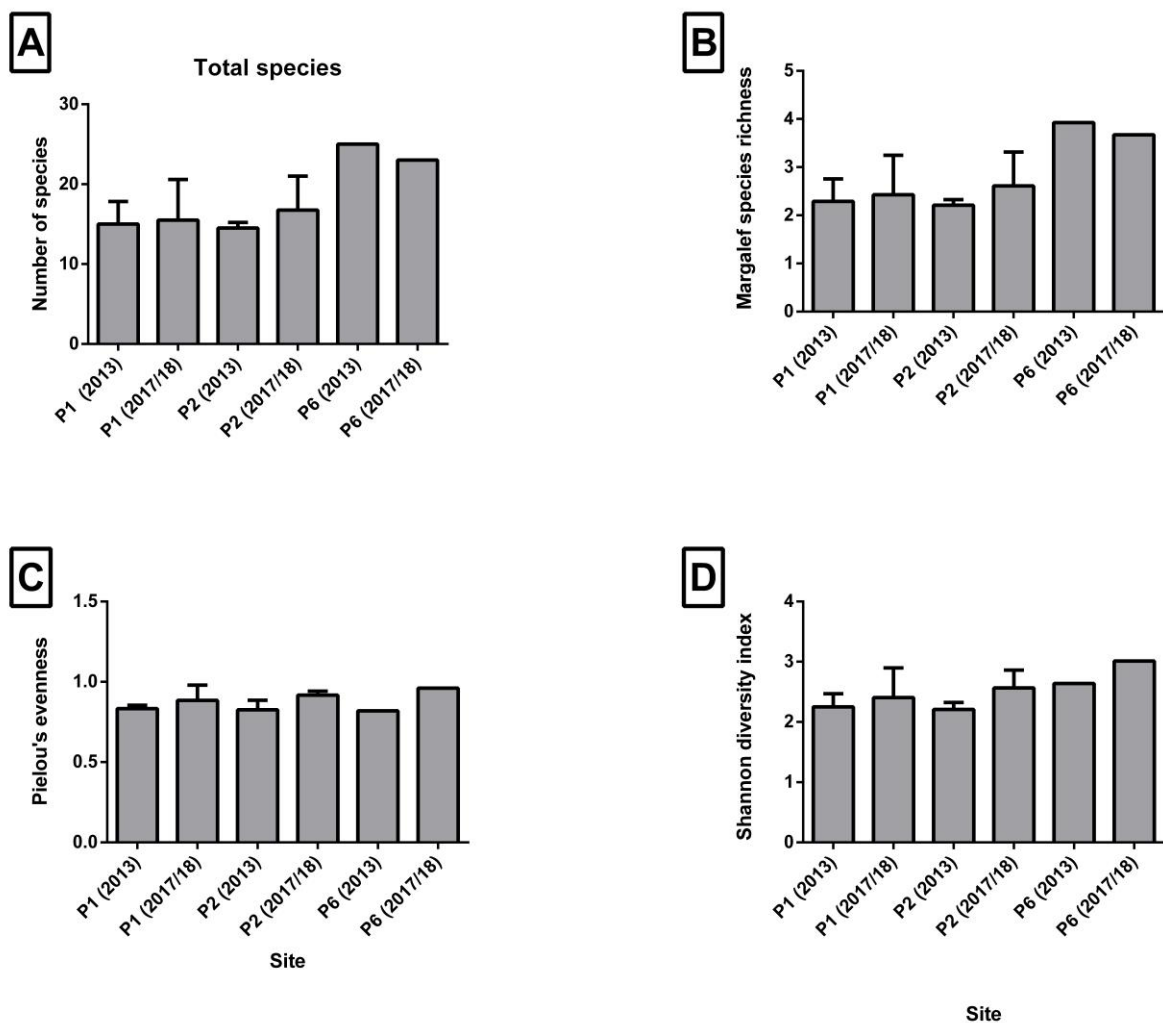


Figure 2.18: Mean $\pm$ SEM for (A) Total species, (B) Margalef species richness, (C) Pielou's evenness and (D) Shannon diversity index. Bars for 2013 represent the means of two seasonal surveys and bars for 2017/18 represents the means for six seasonal surveys.

The regional (gamma) diversity of diatom taxa (for all sites) increased during the absence of a flood release (Figure 2.21). Beta diversity were slightly higher during the absence of a flood release ($\beta = 4.6$) compared to the presence of flood release events ($\beta = 1.8$). The alpha diversity between the presence (3.5 ± 0.6) and absence (3.6 ± 0.3) of a flood release remained relatively the same. A paired t test indicated that there was no significant ($p = 0.36$) differences between the means of the diversity indices.

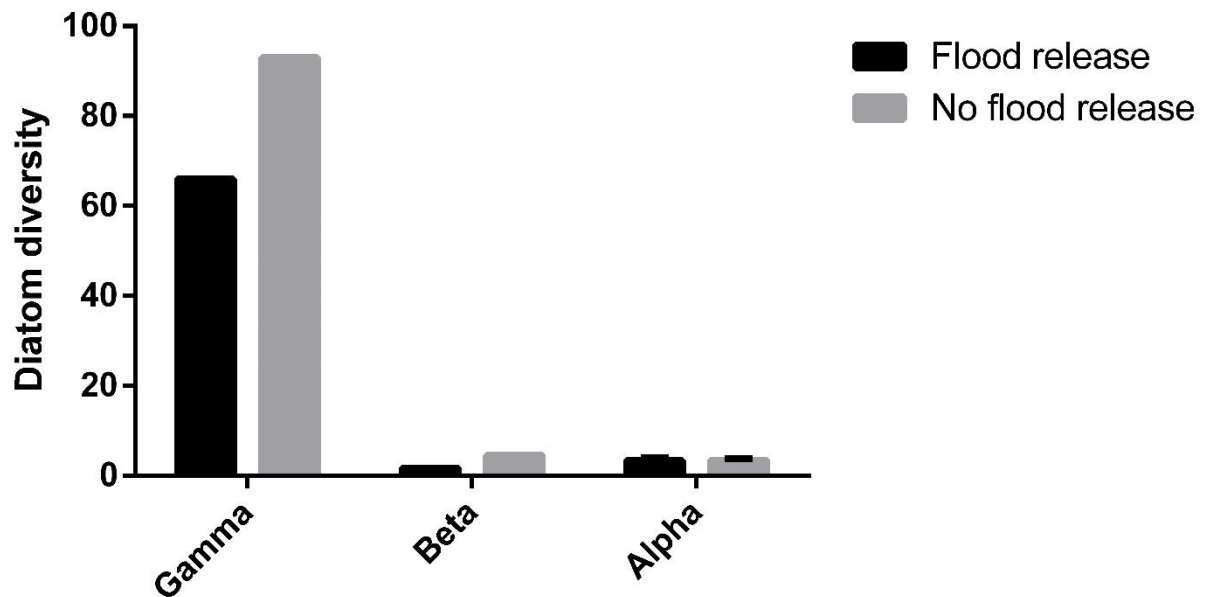


Figure 2.19: The diatom taxa diversity of the sites sampled during the presence and absence of a flood release event.

A non-metric multidimensional (nMDS) biplot based on Bray Curtis similarity coefficients was constructed between sites and surveys to determine differences between sites. There were very clear temporal differences between sites sampled during 2013 and 2017/18 (Figure 2.24). Based on the SIMPER analysis sites sampled during 2013 had a 41.54% similarity with *Cocconeis placentula*, *Cocconeis pediculus* and *Gomphonema munitum* the dominant species. During 2017/18, *Cocconeis placentula*, *Gomphonema insigne* and *Aulacoseira granulata* were the dominant species with a similarity of 27.81%. The two survey periods had an 86.36% dissimilarity.

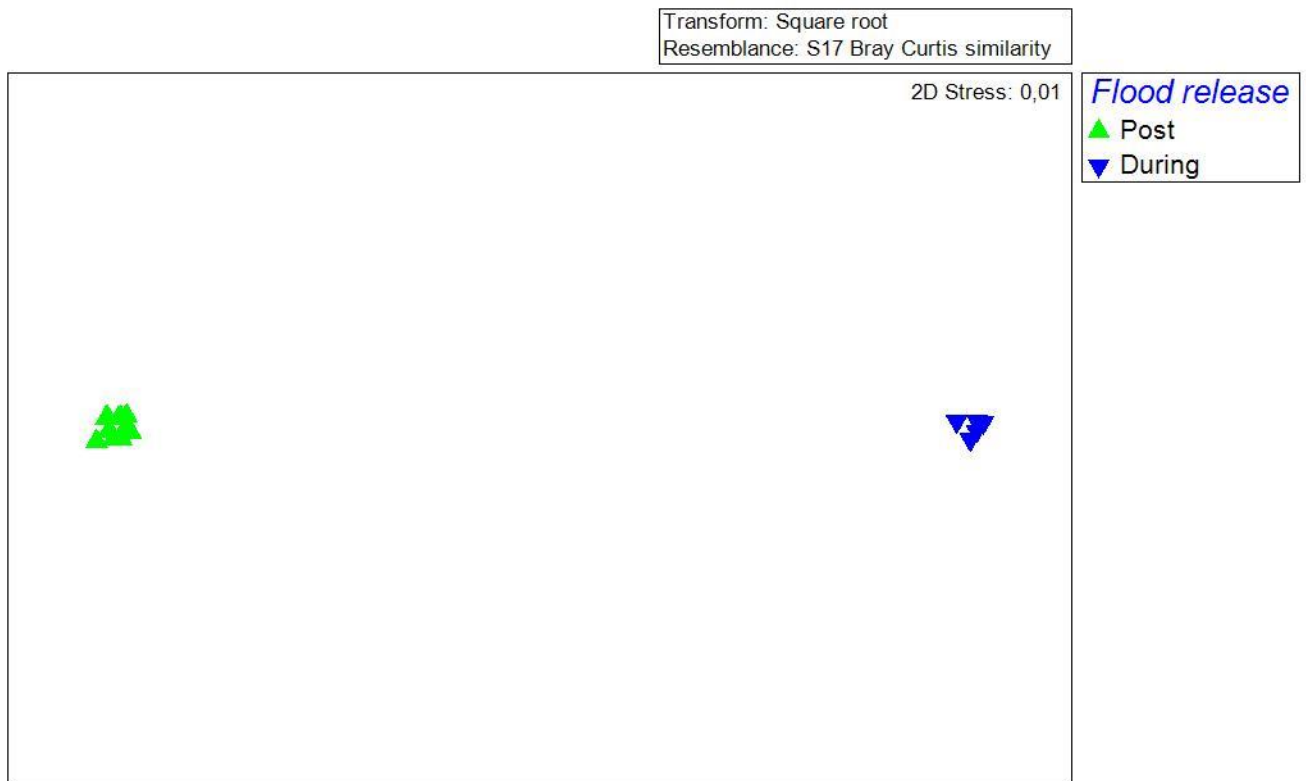


Figure 2.20: An nMDS illustrating temporal difference between sites sampled during a flood release event (2013) and sites sampled after a flood release event (2017/18).

2.3.3 Spatial and temporal connectivity between aquatic ecosystem types for 2017/18

2.3.3.1 Environmental variables

There were no significant ($p < 0.05$) differences for the mean temperature and percentage oxygen saturation for sites sampled during 2017/18, with both variables remaining relatively consistent throughout the study (Figure 2.18 A, B). The three Nyamithi sites (N1, N2, and N3) had significantly ($p < 0.05$) higher conductivity values compared to the other sites, with mean conductivity values $> 6000 \mu\text{S/cm}$ (Figure 2.18 C). The pH values remained relatively consistent between sites with significantly higher ($p < 0.05$) pHs at predominantly ephemeral pan sites (Figure 2.18 D).

Mean nitrate, nitrite and total phosphate values had no significant ($p < 0.05$) differences between sites sampled (Figure 2.19 A, B). Nitrite values remained relatively consistent between sites with the highest nitrite values measured for site P1. Ammonium values were significantly ($p < 0.05$) higher at site NI compared to sites P3, P4, P6, N1 and N2 (Figure 2.19 C). Site S1 had the highest total phosphate values compared to the other sites (Figure 2.19 D).

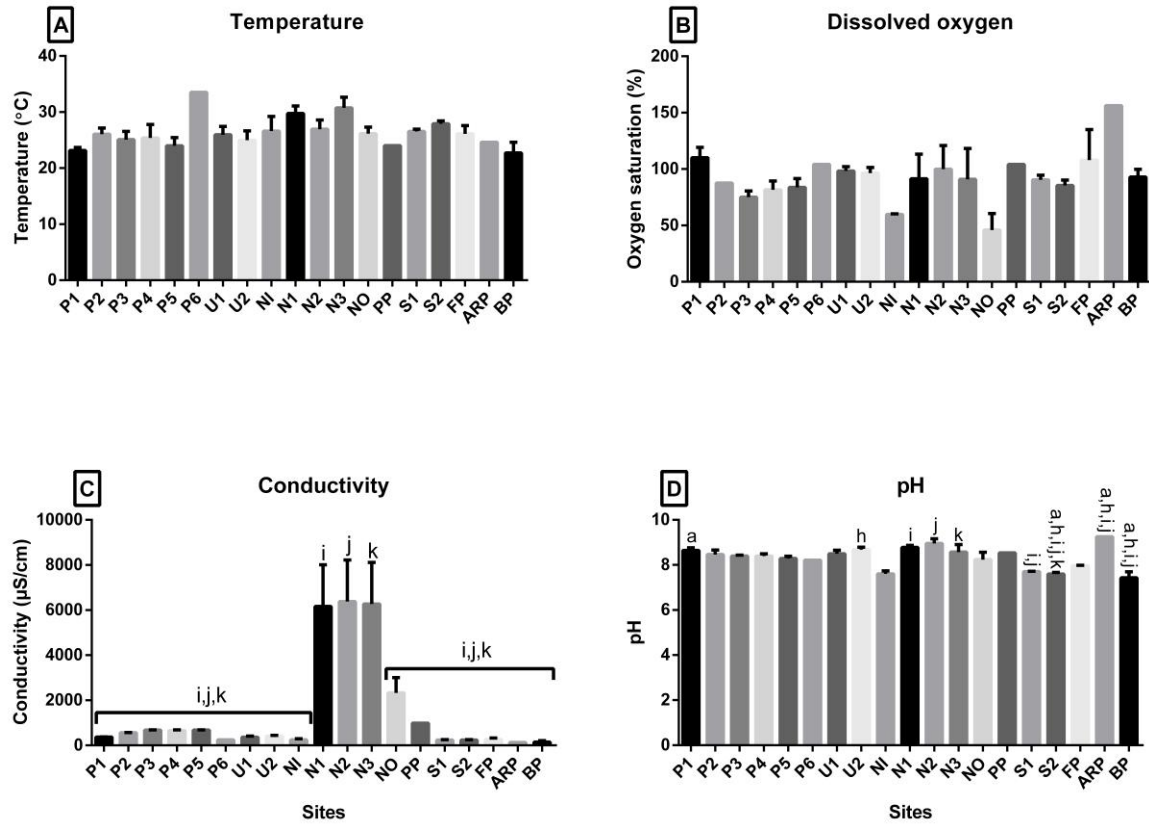


Figure 2.21: Mean $\pm$ SEM for (A) Temperature, (B) Percentage oxygen saturation, (C) Conductivity and (D) pH. Bars represent the means of six seasonal surveys at all sites (except P6) during 2017/18. Bars with common superscript differ significantly ($p < 0.05$).

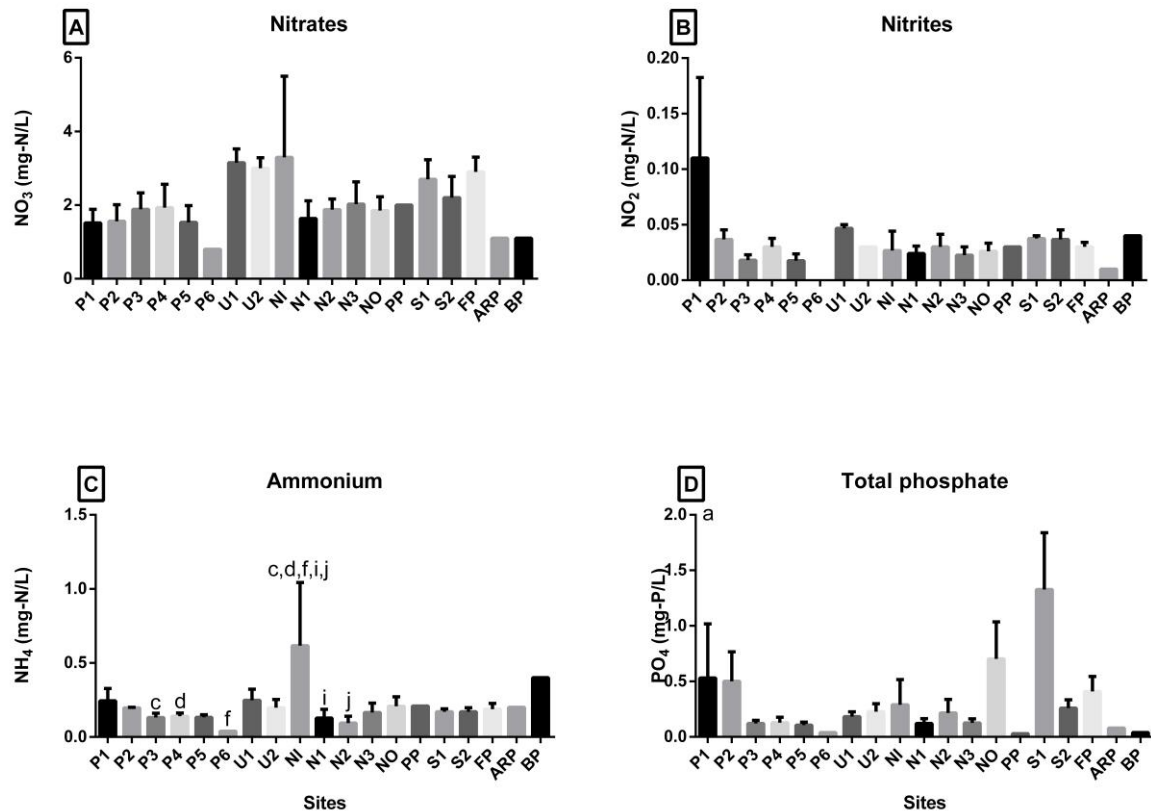


Figure 2.22: Mean $\pm$ SEM for (A) Nitrate, (B) Nitrite, (C) Ammonium and (D) Total phosphate. Bars represent the means of six seasonal surveys at all sites (except P6) during 2017/18. Bars with common superscript differ significantly ($p < 0.05$).

2.3.3.2 Diatom diversity

During the 2017/18 surveys a combined total of 204 species were identified. A complete list of species identified during the 2013 and 2017/18 surveys is presented in Appendix B.

Variance was noted in the total number of species and Margalef species richness with significantly ($p < 0.05$) higher total number of species and richness for S1 compared to FP (Figure 2.22 A, B). Less variance was noted between the Pielou's evenness and Shannon diversity index for the sites sampled, with no clear dominant taxa shown by the scores (Figure 2.22 C, D). No significant ($p < 0.05$) differences between the sites were noted for these indices.

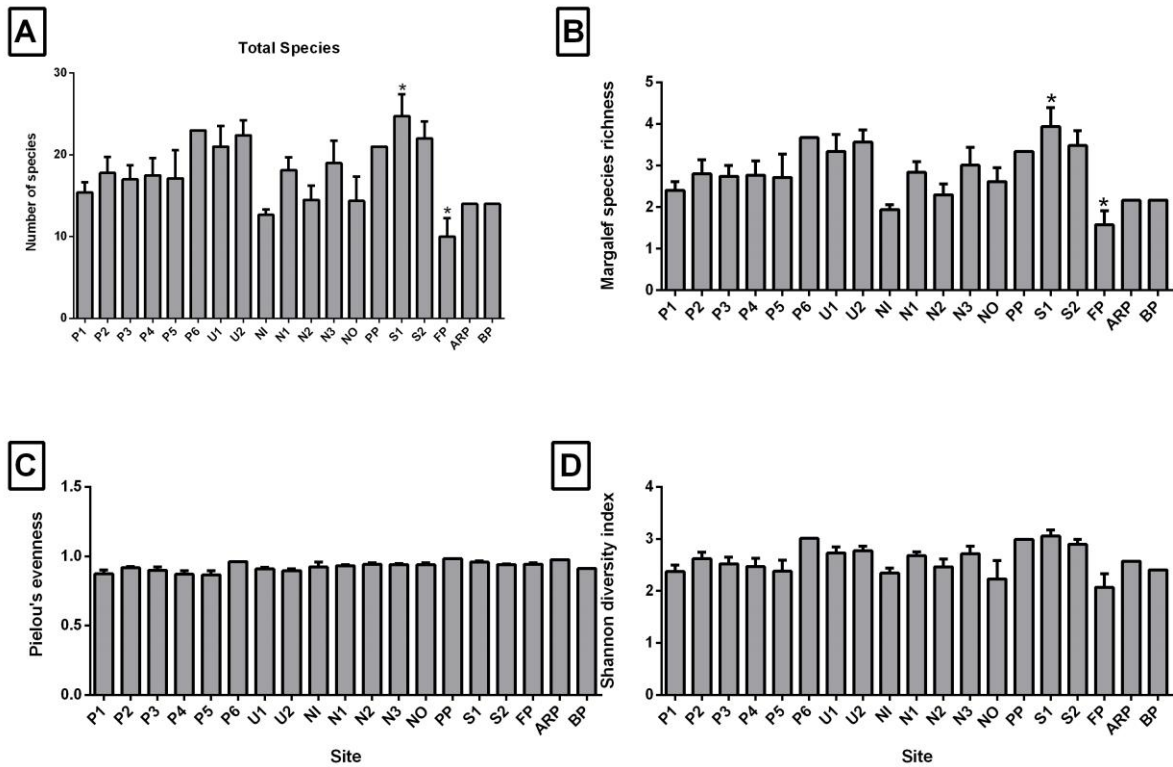


Figure 2.23: Mean $\pm$ SEM for (A) Total species, (B) Margalef species richness, (C) Pielou's evenness and (D) Shannon diversity index. Bars represent the means of six seasonal surveys at all sites (except P6) during 2017/18. Bars with an asterisks differ significantly ($p < 0.05$).

The regional (gamma) diversity of diatom taxa was highest in the Phongolo River and associated pans compared to the Usuthu River and its associated pans and ephemeral pans (Figure 2.23). Phongolo River had the highest gamma diversity and ephemeral pans the lowest. Global beta diversity was highest for the Phongolo River ($\beta = 6.5$) and associated pan sites ($\beta = 6.7$) with the lowest beta diversity for the Usuthu River ($\beta = 1.7$) and associated pans ($\beta = 1.7$) with the ephemeral pans having a beta diversity that was ($\beta = 4.4$) inbetween these sites. Alpha diversity values were highest for the Usuthu River (5 ± 0.4) and associated pans (5.5 ± 0.5) and lowest for the ephemeral pans (2.6 ± 0.4). Alpha diversity values remained relatively consistent between the Phongolo River (3.7 ± 0.2) and associated pans (3.6 ± 0.2). There were no significant ($F = 1.15$, $p = 0.4$) differences between the means of the measured diversity indices.

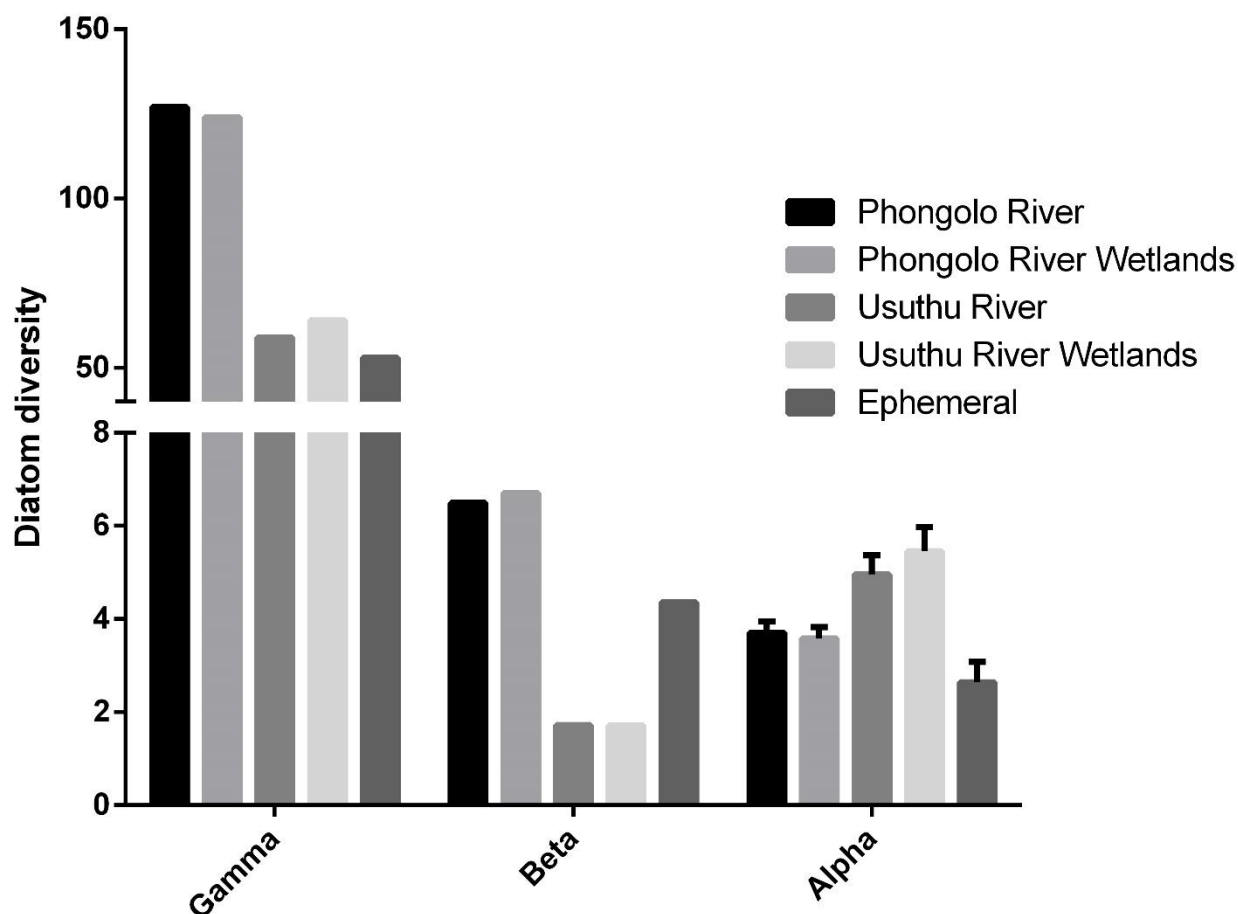


Figure 2.24: The diatom taxa diversity of all the sites sampled during 2017/18.

2.3.3.3 Spatial and temporal variation

To compare whether seasonal variance occurred in the Phongolo River a Principle Components Analysis (PCA) was created to compare sites sampled during the different surveys. Temporal variation was limited with sites sampled during September 2017 and May 2018 separating from the sites sampled in February (except P5 and P6), May and November 2017 and February 2018 on the first axis (explaining 18.06% of data variation) (Figure 2.25). All water quality variables had an equal contribution in the shaping of the diatom community structure. Clear spatial differences were present between sites sampled in February 2017.

Temporal variation was evident for sampled sites in the Usuthu River (Figure 2.26). Sites sampled during February 2017 and 2018 and May 2018 (high flow, during and just following summer rainfall) separated from sites sampled during May 2017, September 2017 and November 2017 (except U2) (low flow) on the first axis (explaining 43.94% of data variation). Temporal variation in the Usuthu River was driven by changes in physico-chemical variables. There was a positive correlation between nitrogen (nitrate and nitrite) and sulphate for the

majority of sites sampled during high flow, and a positive correlation between February 2017 and low flow sites and electrical conductivity, temperature, total phosphate and pH. A high diversity of species was present at both sites for May 2017.

The Shokwe (S) Pan sites sampled during February 2017 separated from the sites sampled during May 2017, February 2018 and May 2018 on the second axis (explaining 9.03% of the variation) (Figure 2.27). Shokwe Pan could not be sampled during September and November 2017 as the pan was dry during these months, however, the pan had a positive correlation with nutrients (nitrite, nitrate, total phosphate and ammonium) before and after drying. Nyamithi (N) Pan sites sampled over all surveys (except NI, N2 and NO February 2017 and NI May 2018) grouped together in the left half of the graph and had a positive correlation with conductivity, pH, sulphate and water temperature. Temporal variation for N pan were noted between February 2017 and all other sampling surveys. Nyamithi Pan sites separated from the ephemeral pans on the first axis (explaining 14.03% of the variation). Spatial and temporal variation were noted between N Pan and S (except May 2018) Pan as they separated on the first axis.

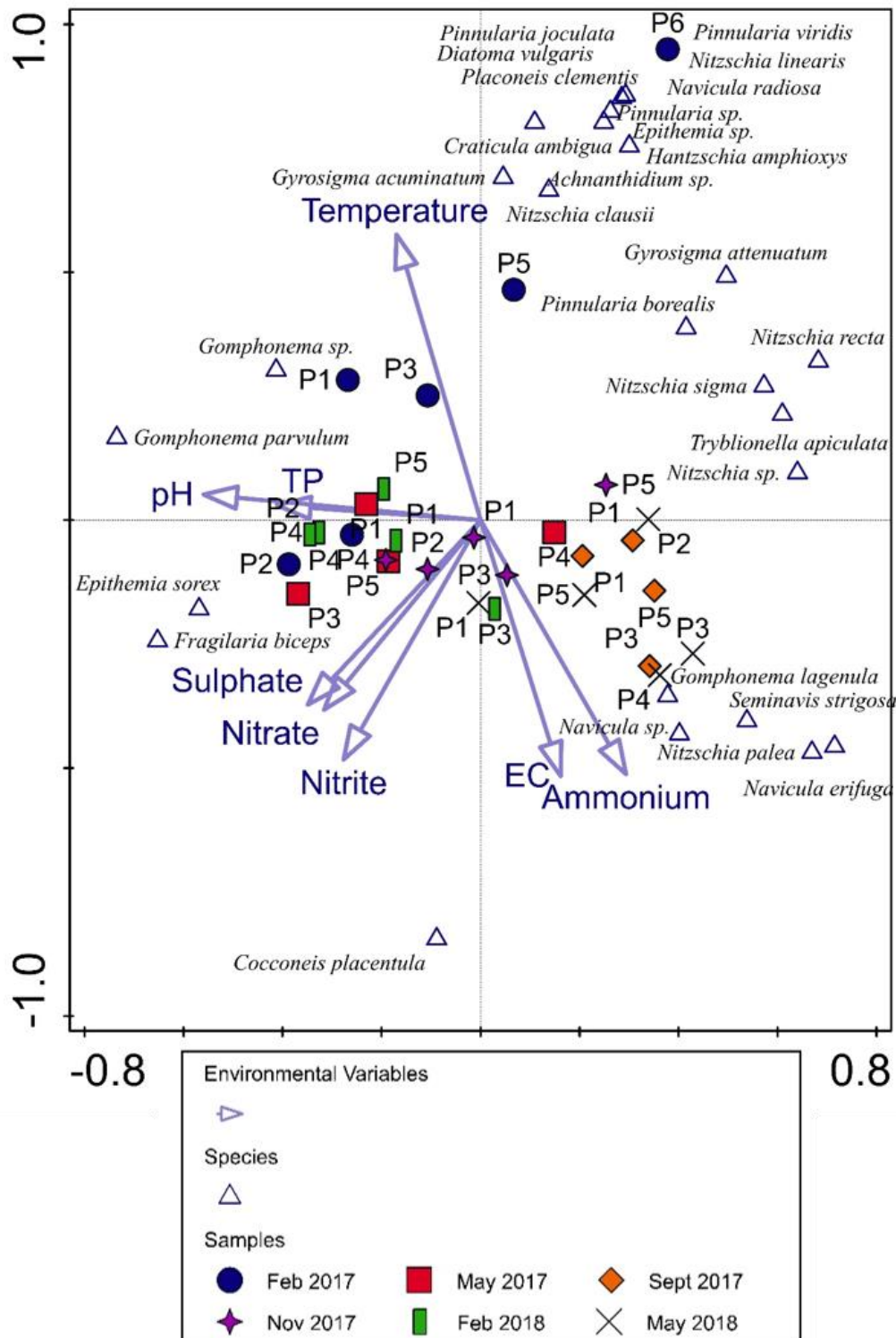


Figure 2.25: Principle Component Analysis (PCA) biplot of the 30 best fitting diatom taxa (triangles) collected from the Phongolo River sites during six surveys from February 2017 to May 2018 with water quality variables (arrows) plotted as supplementary variables. The supplementary variables explain 40.84% of the variation with the first axis explaining 18.06% and the second axis 10.68% of the variation. TP – total phosphate, EC – electrical conductivity. P – Phongolo River Sites.

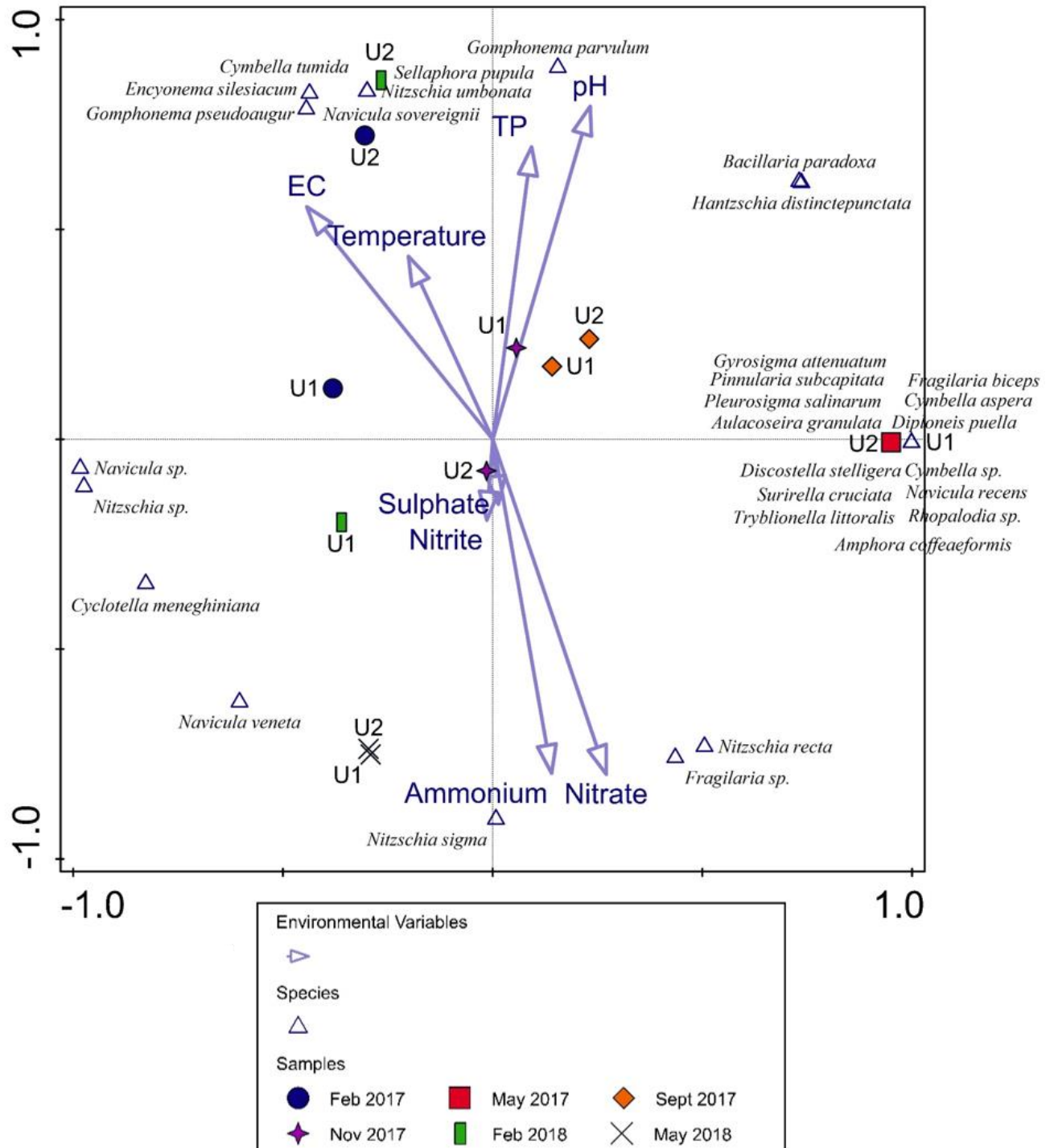


Figure 2.26: Principle Component Analysis (PCA) biplot of the 30 best fitting diatom taxa (triangles) collected from the Usuthu River sites during six surveys from May 2017 to May 2018 with water quality variables (arrows) plotted as supplementary variables. The supplementary variables explain 100% of the variation with the first axis explaining 43.94% and the second axis 30.51% of the variation. TP – total phosphate, EC – electrical conductivity. U – Usuthu River sites.

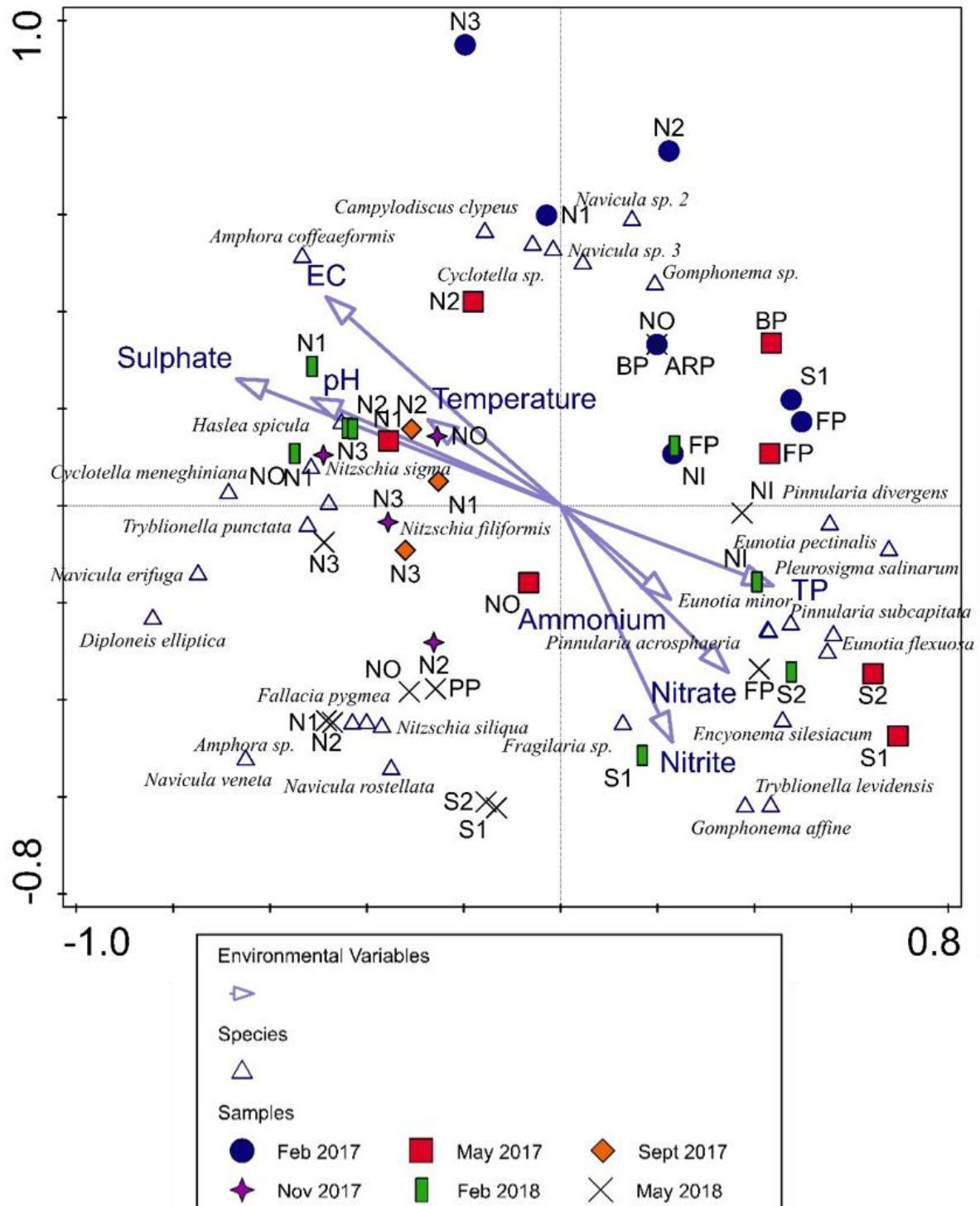


Figure 2.27: Principle Component Analysis (PCA) biplot of the 30 best fitting diatom taxa (triangles) collected from all the floodplain pan sites during six surveys from February 2017 to May 2018 with water quality variables (arrows) plotted as supplementary variables. The biplot explains 31.58% of the variation with the first axis explaining 14.03% and the second axis 9.03% of the variation. TP – total phosphate, EC – electrical conductivity. N – Nyamithi Pan sites, S – Shokwe Pan sites, FP – Fence Pan, BP – Butterfly Pan, PP – Paradise Pan.

2.4 Discussion

2.4.1 Physico-chemical water quality

Due to rapid inter-conversion and co-occurrence, ammonium, nitrites and nitrates will be discussed together as dissolved inorganic nitrogen (DIN) (DWAF, 1996).

2.4.1.1 Presence of a flood release (2013 surveys)

Increased flow during 2013 most likely increased nutrient dilution, spiralling and the flushing of nutrients caused a decrease in nutrient concentrations. Nutrient spiralling can be described as the cycling and transport of nutrients to downstream areas within river ecosystems (Ensign & Doyle, 2006).

The nitrogen values indicated that all sites sampled during 2013 were in an oligotrophic state with values < 0.5 mg/L, with total phosphate values showing that sites P1 and P2 were eutrophic (25–250 $\mu\text{g/L}$) and P6 was mesotrophic (5–25 $\mu\text{g/L}$) (DWAF, 1996). The ratios between nitrogen and phosphate (N:P) are used to determine if an ecosystem is impacted or unimpacted and to determine the limiting nutrient (DWAF, 1996; Kelly, 2001). Both P1 and P2 had ratios of 8:1 with a ratio of 18:1 for P6 during 2013. Ratios of P1 and P2 could be classified as impacted (eutrophic) ecosystems, as their N:P ratio $< 10:1$. The optimum N:P ratio for cell growth in ecosystems are 7:1 (Kelly, 2001). A ratio of 25:1 is an indication of unimpacted ecosystems (DWAF, 1996). P6 ratio was between an impacted and unimpacted ecosystem with a mesotrophic ecological status.

Phosphates may enter the water bodies through runoff and could be attributed to intensive agricultural activities within the floodplain (Kyle, 1996). According to Dallas & Day (2004), phosphate ions bind to the sediment which is transported to the receiving water body by surface flow. The pH and temperature remained within the prescribed limits at all sites during these surveys as they did not vary outside of the expected values (DWAF, 1996).

2.4.1.2 Absence of a flood release (2017/18 surveys)

Nitrogen values for the Usuthu River, Shokwe Pan, FP and NI indicated these sites were in a eutrophic condition with all other sites being mesotrophic (DWAF, 1996). According to the South African Water Quality Guidelines (DWAF, 1996) the measured total phosphate values for P1, P2, NO, Shokwe Pan, FP and NI could be considered as eutrophic (DWAF, 1996). All other sites were characterized as mesotrophic based on the South African water quality guidelines (DWAF, 1996).

The N:P values varied considerably between sites with the majority of the floodplain pan sites ratio's < 10:1. These sites could be considered impacted (eutrophic) when taking these ratios into account. The remaining study area's ratios ranged between 13:1–21:1 indicating sites in a mesotrophic condition. Increased nutrient concentrations within wetland ecosystems are expected as these systems act as “sinks” where sediment and water accumulate (Kock *et al.*, 2019). As described above, intensive agriculture activities within the catchment contribute to increased nutrients within these systems (Kyle, 1996) as they can enter aquatic ecosystems through runoff.

Variations in temperature and pH values between seasons remained within the recommended ranges, for all sites (DWAF, 1996). According to the guidelines the target range for dissolved oxygen to ensure that all aquatic biota's life stages are protected should be 80–120% saturation (DWAF, 1996). All sites were within the target range for percentage dissolved oxygen saturation. Conductivity remained relatively consistent between sites, except for Nyamithi Pan. The significantly higher conductivity measured at Nyamithi is due to the pans saline characteristics. The pan receives some of its water from naturally saline groundwater as the pan is situated on top of marine cretaceous deposits (Kyle, 1996).

2.4.2 Effect of a flood release on diatom diversity (2013 vs 2017/18)

According to Biggs & Smith (2002), diatom community richness as well as spatial and temporal variation is determined by the frequency of flood events. Results from the present study showed that the absence of flood releases since December 2014 resulted in an increase of the diatom diversity and caused a shift in the species present within the Phongolo River, however, the taxa richness (alpha diversity) remained unchanged. Large flood events can also result in the removal of some diatom species due to the stress of currents and bed movement causing diatoms to dislodge from the substratum (Tornés *et al.*, 2015). Species, such as *Achnanthisidium* spp. and *Cocconeis* spp., present during flood release events are those that can withstand flood events as they tightly adhere to the substratum, have a fast growth rate and dominate in disturbed areas (Tornés *et al.*, 2015). According to Tornés *et al.* (2015), species such as *Nitzschia* spp. and *Navicula* spp. are prone to drift away during floods as they are loosely attached to substratum. These species were present in higher numbers during the surveys when there were no flood releases and the water flow was considerably slower. Dube *et al.* (2017) also reported significant changes in macroinvertebrate assemblages within the lower Phongolo River floodplain during flood releases. Differences in the dominant species present were also noted with the presence of *Nitzschia dissipata* during the absence of a flood release. This species normally occurs during post-flood conditions and are normally the dominant species in hard waters (Tornés *et al.*, 2015). These data corresponds with studies

by Uehlinger *et al.* (2003), Tornés *et al.* (2015) and Tudesue *et al.* (2019), which show a decrease in diatom diversity during flood events. Uehlinger *et al.* (2003) also found a persistent shift in the diatom community structure during experimental floods at a dam in the Swiss Alps. Their study suggests that there will be more variability in algal biomass due to flood events. Diatom beta diversity decreased during the presence of a flood release. Bozelli *et al.* (2015) found that the presence of flood releases causes a dilution effect on the environmental variables, such as nutrients and conductivity, within the Amazonian floodplain system and decreased the zooplankton beta diversity. The beta diversity will decrease as taxa dispersal increases in an aquatic ecosystem (Bozelli *et al.*, 2015). One of the main sources of variation, as well as dispersal, within flood-prone rivers is the frequency and timing of floods (Uehlinger *et al.*, 2003). Fewer taxa disperse during the absence of a flood release resulted in an overall increase in the diatom diversity (gamma diversity).

Discharges of water (floods) have an influence on the abiotic factors within the ecosystem which plays a major role in determining the diatom community of the ecosystem. Previous studies have shown that the availability of nutrients has an influence on diatom assemblages (Mirzahasanlou *et al.*, 2019). This is confirmed in the present study when comparing the measured water quality variables for the flood release survey in 2013 to the absence of a flood release in 2017/18 surveys with higher recorded nutrient levels in 2017/18 compared to 2013. Tudesue *et al.* (2019) also stated that variation in diatom community structure can be attributed to variation in environmental conditions. Thus a consistent ecosystem with less fluctuation in environmental variables, such as the abiotic factors, will result in a more stable diatom community with not much variation in the community structure over time.

2.4.3 Spatial and temporal variations

Temporal variations in the Usuthu River were driven by a combination of environmental factors with sites sampled during summer rainfall and high flow (except February 2017) affected by nutrient concentration. The main driver for the Usuthu River during high flow (February and May) was nitrogen, with high concentrations of nitrate measured in the Usuthu River indicating the river as eutrophic. Diatoms sensitivity to nutrient content is well documented with numerous studies reporting on how the community structure is shaped by nutrient concentrations (Ramanibai & Jeyanthi, 2010; Cantonati & Lowe, 2014; Cvetkoska *et al.*, 2018; Rivera *et al.*, 2018). Species such as *F. biceps*, *N. recens*, *A. granulate* and *C. meneghiniana*, associated with the May 2017 survey, are found in eutrophic ecosystems (Taylor *et al.*, 2007). The area experiences summer/autumn rainfall (February and May) and nutrients enter the river as runoff from the catchment area. During high flow (February and May) nutrients are flushed downstream as they enter the river from return flows from sugarcane irrigation

schemes, agriculture practices and discharge of effluents into the river from industries in the upper region (Swaziland) of the Usuthu River (Mhlanga *et al.*, 2006; Fadiran *et al.*, 2008). February 2017 was, however, influenced by increased conductivity measured compared to February 2018 and both surveys in May. During low flow (September and November) a decrease in water levels, flow and runoff resulted in a shift from nitrogen to conductivity, temperature, total phosphate and pH as the drivers of the diatom community structure. An increase in conductivity and pH occur within aquatic ecosystems during low flow, decreased rainfall and periods of drought (Tooth & McCarthy, 2007). This was the case for the study as the pH and conductivity increased (Appendix A) during periods of low flow within the Usuthu River. In their study Pumas *et al.* (2018) found that electrical conductivity was important in determining the structure and distribution of the diatom community in hot springs in northern Thailand. Individual species, as well as diatom assemblages, are directly affected by environmental variables that are highly correlated with conductivity (Pumas *et al.*, 2018). Due to diatoms short life span (2–3 weeks), they respond rapidly to changes within their environment, thus these increases in conductivity and pH will influence the diatom community structure. These results are in accordance with a study done by Bortolini & Bueno (2017) on the São João River, a subtropical river in Brazil, where temporal variation was mainly driven by changes in electrical conductivity, temperature, nutrients and pH.

Limited temporal variation was found in the Phongolo River. During the study period, South Africa experienced the worst drought in the past 23 years (Baudoin *et al.*, 2017). This caused extremely low flows in the floodplain (especially the Phongolo River) (Dube *et al.*, 2017) with only a base flow ($4.54\text{--}8.50\text{ m}^3\text{s}^{-1}$) continuously released from the Pongolapoort Dam. Ecosystems with constant flow and that are not flooded are relatively stable over long periods of time compared to ecosystems that experience variations in flow as well as intermitted flooding (such as the Usuthu River) (Sokal *et al.*, 2010). Ecosystems with consistent flow exhibit higher diversity as flooding of rivers cause dispersal and variation in flood-prone rivers (such as the Usuthu River) (Uehlinger *et al.*, 2003). This was found for the present study as the Phongolo River had a higher diatom diversity (gamma diversity) and beta diversity, due to lower taxa dispersal, than the Usuthu River. As there was little to no temporal variation for the Phongolo River, it suggests that environmental variables among river sites did not vary much between surveys. This is confirmed when studying the water quality data. Measured water quality variables did not differ much between surveys.

The main drivers of diatom taxa in Shokwe Pan were nutrients with dominant diatom taxa (*Nitzschia* sp., *Encyonema* sp., *Fragilaria* sp., *E. silesiacum* and *C. meneghiniana*) including species that occur in nutrient enriched ecosystems (Taylor *et al.*, 2007). As the pan receives

water from natural flooding of the Usuthu River (Whittington *et al.*, 2013) it can result in increased nutrient concentrations as the Usuthu River is nutrient enriched. It is expected that floodplain pans have increased nutrient concentrations as they are normally still standing waters that become eutrophic if not flushed by floodwaters.

Fence Pan (FP) and BP showed no temporal variation with nutrients the main drivers of the ephemeral floodplain pans. Floodplain pans are ecosystems where water and sediment accumulate (Kock *et al.*, 2019) and nutrients enter the floodplain pans from localised runoff from subsistence farming within the floodplain area (Kyle, 1996; Smit *et al.*, 2016; Dube *et al.*, 2017). Measured physico-chemical water quality, for FP and BP, remained consistent over all surveys. Paradise Pan (PP) grouped together with Nyamithi Pan and Shokwe Pan in May 2018. Paradise Pan's diatom community structure was influenced by electrical conductivity. Paradise Pan had increased electrical conductivity compared to all other sites (except Nyamithi), as the pan receives water from Nyamithi Pan when it overflows during very high flow periods. However, as PP and Nyamithi Pan grouped together with Shokwe Pan during May 2018, it suggests that the pan was influenced by the Usuthu River. When the Usuthu River has very high flow, it floods the floodplain area surrounding Nyamithi Pan. Dominant species (*Gyrosigma* spp., *Nitzschia* spp., *Navicula* spp. and *Amphora* spp.) for PP are species that prefer ecosystems with increased salinity and nutrient concentrations (Taylor *et al.*, 2007). This can be an indication that the pan was influenced by both Nyamithi Pan and flooding from the Usuthu River.

The main drivers affecting the distribution of diatom taxa in Nyamithi Pan was sulphate and electrical conductivity. Water is able to conduct an electrical current due to the presence of ions, such as sulphate, that can carry an electrical charge in the water (DWAf, 1996). There is thus a positive correlation between sulphates and electrical conductivity. As mentioned earlier Nyamithi Pan is a natural saline lake within the NGR. Dominant species identified in Nyamithi Pan for all surveys include *C. meneghiniana*, *D. elliptica*, *H. coffeaeformis* and *N. filiformis*. These species occur in ecosystems with increased salinity (Taylor *et al.*, 2007).

Temporal variation between February 2017 surveys and all other surveys for Phongolo River, Nyamithi Pan and Shokwe Pan could not be explained as all variables (physico-chemical variables, monthly rainfall, monthly temperature and flow) remained relatively constant throughout the sampling period. It should be noted that low percentages explain the variations across the PCA's axes, for the Phongolo River, Nyamithi Pan and Shokwe Pan.

2.5 Conclusions

The focus of the chapter was to determine the diatom community structure in the lower Phongolo River floodplain and the influence of environmental variables on the distribution of the community. Results have shown that there was a temporal difference between three river sites sampled during a flood release event and during the absence of a flood release event. These differences in community structure may be attributed to a more stable ecosystem during the absence of flood events with species having to endure less changes in the environment. Temporal variation was noted for the Usuthu River but not the Phongolo River. There was less fluctuation in the physico-chemical water variables in the Phongolo River during the study period, and this can be due to the consistent base flow from the Pongolapoort Dam. The Usuthu River is a more natural ecosystem (in terms of flow) compared to the Phongolo River with more fluctuation in physico-chemical water variables across seasons.

Spatial variation was noted between Nyamithi Pan and the ephemeral pans (except PP) and Shokwe Pan. Different environmental variables were responsible for the structuring of the diatom community in the different floodplain pans. Conductivity, sulphate, pH and temperature were the main drivers in Nyamithi Pan, as with the Phongolo River. Nutrients were the main drivers in the ephemeral pans and Shokwe Pan, as with the Usuthu River. The hypothesis that variations in flow and physico-chemical water quality will have an effect on the structuring of the diatom communities in the Phongolo and Usuthu rivers and their associated floodplain pans is accepted.

2.6 References

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Chapter 3: River and wetland diatom community structures and stable isotope signatures in the lower Phongolo floodplain.

3.1 Introduction

Floodplain wetlands are important ecosystems that provide valuable ecosystem functions (Dube *et al.*, 2017). These ecosystems are highly variable depending on the frequency, magnitude and period of inundation of flood events (Baldwin & Mitchell, 2000). The biological, chemical and physical characteristics of these ecosystems are further influenced by anthropogenic hydrologic modifications to the river, as floodplain wetlands are linked to rivers through flooding events (Weilhoefer *et al.*, 2008). Floodplain wetlands can be described as existing in two phases, namely a connected and an isolated phase (Weilhoefer *et al.*, 2008). The connected phase exists when the river is connected to the wetlands and water is exchanged. During this phase, floodwaters dominate the floodplain and change the wetlands physical structure through sediment deposits, rising water levels and scouring of substrates (Stromberg *et al.*, 1997; Weilhoefer *et al.*, 2008). The isolated phase is characterised by the absence of water exchange and physical separation (at surface water) between the floodplain wetlands and adjacent river (Weilhoefer *et al.*, 2008).

The pans in the lower Phongolo River floodplain are dependent on flooding events for their ecological functioning (Dube *et al.*, 2015). Studies on the influence of flooding events on the structuring and distribution of zooplankton and macroinvertebrates communities in the floodplain area have been done in recent years with some sites of the present study overlapping (Dube *et al.*, 2017, 2019). These studies found that flooding had a relatively low contribution to the structuring of these communities (specifically the zooplankton community) and mainly altered the aquatic environmental conditions of the floodplain. It can therefore be assumed that a change in the environmental conditions of these ecosystems can influence the base of the food web.

Flood events are one of the major drivers of biota in floodplain wetlands, as periodic flooding shapes the communities (especially primary producers such as periphyton) of these ecosystems (Weilhoefer *et al.*, 2008). Floodwaters can either deposit periphyton taxa within the wetlands (Gell *et al.*, 2002; Weilhoefer *et al.*, 2008), flush out the present periphyton community or change the environmental conditions (nutrients, sediment, turbidity) of the wetland ecosystem (Robertson *et al.*, 1999; Weilhoefer *et al.*, 2008) that can lead to different

species dominating the wetland due to unfavourable conditions for the pre-flood assemblages. The changes brought on by flooding cause variations to the floodplain wetlands and their functioning, as well as resulting in shifts of the periphyton community. However, this is largely unknown for the lower Phongolo River floodplain.

Higher trophic level organisms are dependent on periphyton as a food source, making them useful to assess the ecological status and trophic structure of the ecosystem (Dalu *et al.*, 2015). Periphyton communities are regulated by numerous factors including a complex interplay of biological and physico-chemical factors (Passy *et al.*, 1999; Murdock *et al.*, 2004; Dalu *et al.*, 2015). The community consists mainly of algae and are useful in monitoring environmental changes as they are responsive to anthropogenic factors, grazing pressure, temperature, water chemistry, flood disturbances and particularly changes in nutrient concentrations and water flow (Dalu *et al.*, 2015; 2016). These changes result in a modification of the periphyton community that in turn modifies diversity, secondary productivity and trophic interaction throughout the food web (Kratina *et al.*, 2012; Dalu *et al.*, 2016).

Our understanding of aquatic ecosystems can be improved through the use of stable isotope studies (Herman *et al.*, 2000; Zeng *et al.*, 2018). Stable isotope ratios, i.e. $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$, provide information on food webs and trophic interactions (Post, 2002; Nyssen *et al.*, 2005; Koppio *et al.*, 2015). The use of the nitrogen isotope (^{15}N) is useful in determining nitrogen sources in aquatic ecosystems as different nitrogen isotope values are related to different anthropogenic sources (Dalu *et al.*, 2015). Measurement of the nitrogen isotope in periphyton is possible as dissolved organic nitrogen is absorbed and retained in their tissue (Dalu *et al.*, 2015). In periphyton the sorption rate of nitrogen is rapid (Kadlec *et al.*, 2005). Studying the natural variation of the carbon isotope enables us to address important questions such as material cycling, diet composition and trophic structure within ecosystems (Post *et al.*, 2007). Establishing baselines for trophic position and estimation of diet sources can be achieved through carbon variations (Post *et al.*, 2007).

The main aim of this section of the present study was to investigate the diatom communities and their stable isotope signatures in the Phongolo and Usuthu rivers and associated floodplain pans during the summer rainfall and following the late summer rainfall period. The hypothesis that was tested are that due to differences in connectivity of the rivers and their associated floodplain pans there will be spatial and temporal differences in the stable isotope signatures and diatom communities between the Phongolo River and associated floodplain pan but not between the Usuthu River and associated floodplain pan.

3.2 Materials and methods

3.2.1 Study area

The study area is located within the lower Phongolo River catchment in north western Kwazulu-Natal (Figure 3.1). A detailed site description is provided in Section 2.2.1. During the sampling period, there was a prolonged drought resulting in no controlled floods being released from the Pongolapoort Dam. Thus the two sampling events represent summer rainfall (February 2017) during which the Usuthu River was in flood and there was localised filling of the floodplain pans and following late summer rainfall (May 2017).

3.2.2 Physico-chemical water quality

The *in situ* water quality and nutrients were measured at each site before phytobenthos sampling. A detailed description of these measurements is presented in Chapter 2 (section 2.2.2).

3.2.3 Phytobenthos sampling and preparation

Phytobenthos was sampled for diversity as well as stable isotope analysis. Phytobenthos sampling, preparation and stable isotope analysis were done according to Taylor *et al.* (2005) and Dalu *et al.* (2015; 2016).

3.2.3.1 Diversity analysis

For diatom diversity analysis epiphytic diatoms were sampled, prepared and analysed as described in Chapter 2 (section 2.2.3).

3.2.3.2 Stable isotope sampling and preparation

Twenty plant stems (10–15 cm) were randomly collected at each site and gently shaken in the water to remove unwanted loosely attached sediment. Stems were placed in resealable bags together with site water and vigorously rubbed to detach the phytobenthos. The water containing the detached phytobenthos was then transferred to marked falcon tubes and immediately frozen until further processing. In the laboratory, samples were defrosted and freeze-dried for at least 48 hr after all visible unwanted particles were removed. Using a mortar and pestle the freeze-dried sample was ground to a fine, homogenous powder.

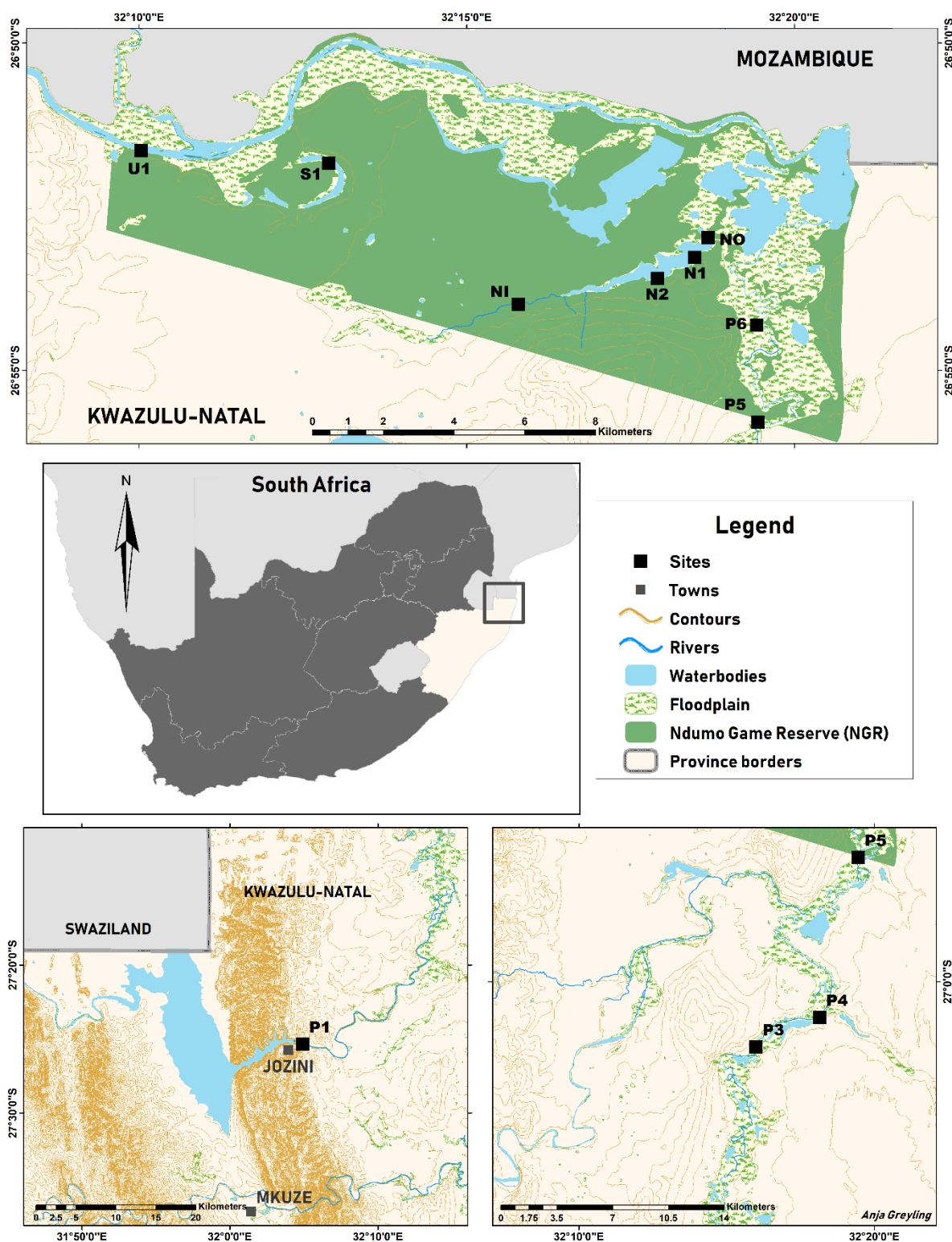


Figure 3.1: Map of the lower Phongolo River floodplain area with sampled sites. P – Phongolo River, U – Usuthu River, N – Nyamithi Pan, S – Shokwe Pan.

3.2.4 Stable isotope analysis

Powdered samples were treated with 2 M hydrochloric acid (HCl) to ensure carbon dioxide release and then dried at 50 °C for 24 hr. Dried samples were weighed (5 mg) and placed in

ultrapure tin capsules. Stable isotope ratios of carbon (^{13}C) and nitrogen (^{15}N) were measured using an Isoprime 100 mass spectrometer coupled with an Elementer elemental analyser. Total N_2 and CO_2 were also measured and values presented as δ -values in parts per thousand (‰) relative to PeeDee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$. Replicate measurements of L-Alanine (laboratory standard) were included for quality control and both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ had a measurement precision of $\pm 0.02 \text{ ‰}$.

3.2.5 Data analysis

Data were tested for normality using the Shapiro-Wilk test. If the data met the requirements they were analysed using a one-way Analysis Of Variance (ANOVA) and Tukey's test for multiple comparisons to test for significant differences ($p < 0.05$) between physico-chemical parameters. For non-parametric data the Kruskal-Wallis test was applied for significance testing. Two-way ANOVA and Sidak's multiple comparisons tests were used to test for significant ($p < 0.05$) differences between the total number of taxa, total number of individuals and diversity indices for the different sites and hydrological conditions. A constrained Canonical Correspondence Analysis (CCA) was carried out to determine seasonal differences, in terms of the diatom community between sampled sites. Physico-chemical water variables were used as explanatory variables for the CCA analysis. To determine the shared and individual influences of physico-chemical water variables and space (distances between sampling sites) a Principle Coordinates of Neighbouring Matrix (PCNM), based on a Euclidean distance matrix, was carried out using Canoco version 5. A two-way ANOVA with Sidak's multiple comparison test was carried out to determine significant differences between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for river and pan sites.

3.3 Results

Water quality and diatom diversity results reported are from the February and May 2017 surveys as these were correlated with the stable isotope data. Also, sites P6 and NI could only be sampled during February as these sites only had water during this time.

3.3.1 Physico-chemical water quality

There were no significant differences in the mean temperature, percentage oxygen saturation and pH between the sampled sites (Figure 3.2 A, B, D). Mean conductivity for N1, N2 and NO were significantly ($p < 0.05$) higher compared to all the sampled sites (Figure 3.2 C). Nitrate, nitrite, total phosphate, ammonium and sulphate had no significant differences in their mean values between sampled sites (Figure 3.3)

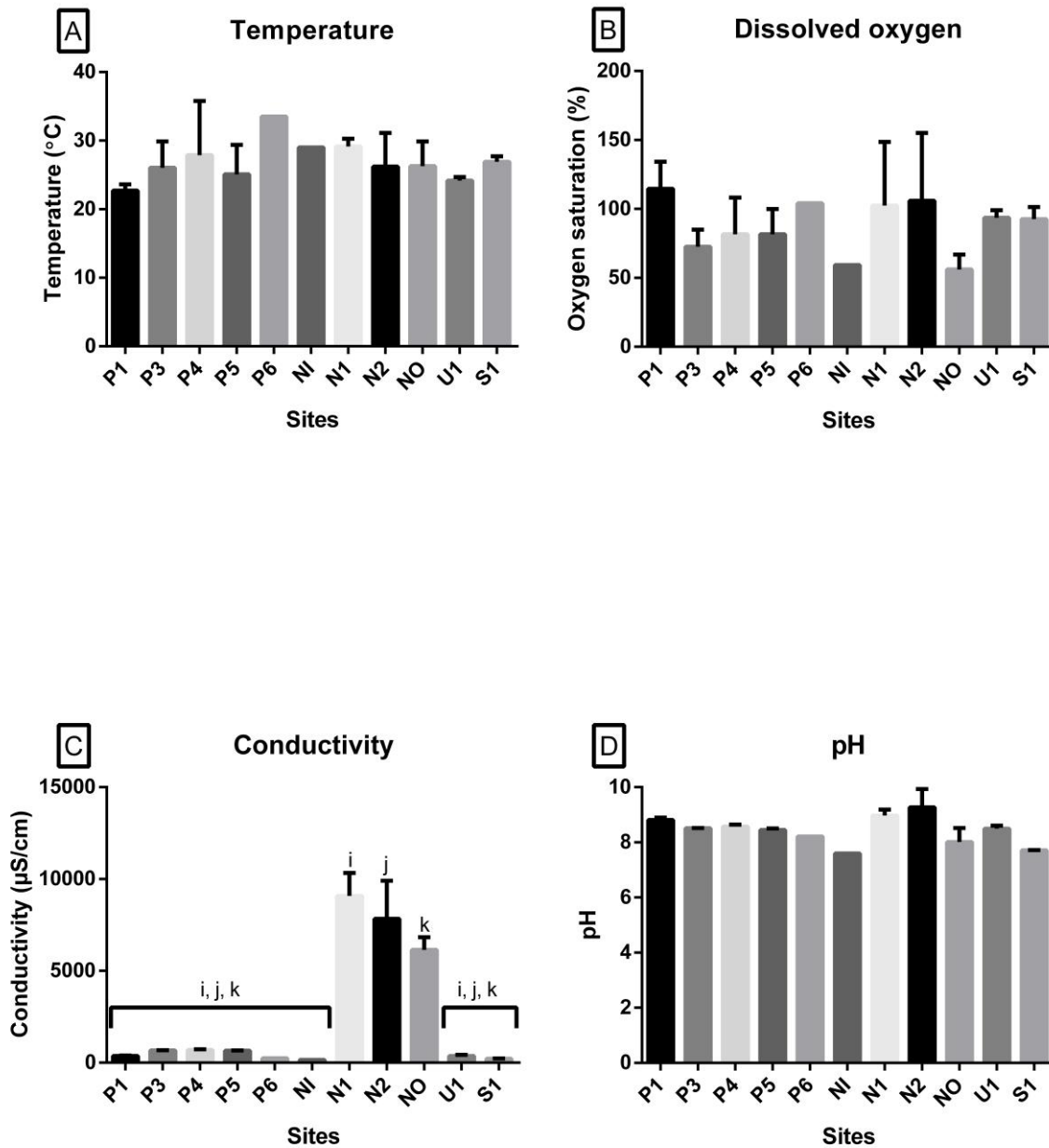


Figure 3.2: Mean $\pm$ SEM for (A) temperature, (B) percentage oxygen saturation, (C) electrical conductivity and (D) pH. Bars represent the means of two seasonal surveys at all sites (except P6) during 2017. Bars with common superscript differ significantly ($p < 0.05$).

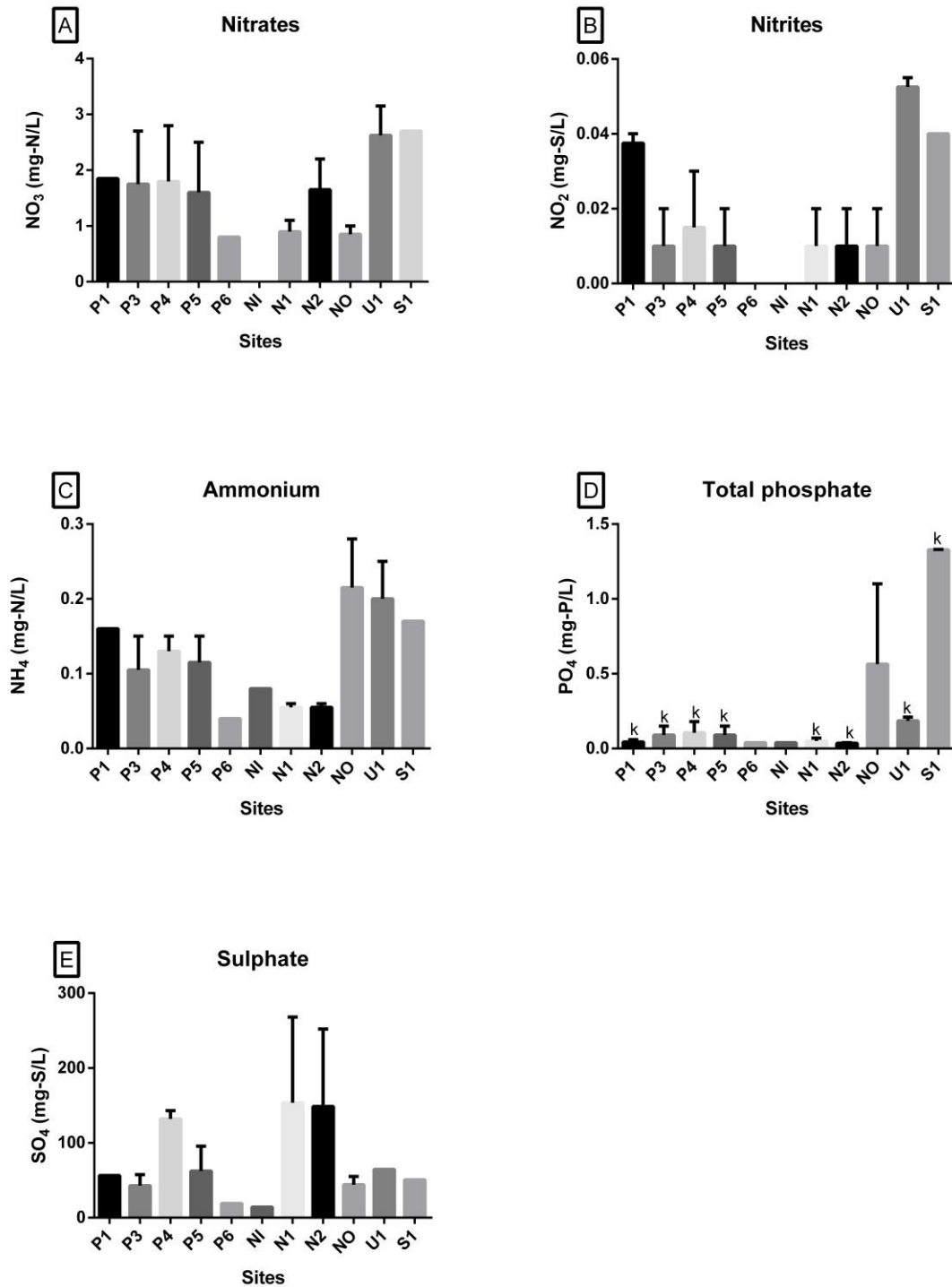


Figure 3.3: Mean $\pm$ SEM for (A) nitrate, (B) nitrite, (C) ammonium, (D) total phosphate and (E) sulphate. Bars represent the means of two seasonal surveys at all sites (except P6) during 2017/18. Bars with common superscript differ significantly ($p < 0.05$).

3.3.2 Diatom community

During the two surveys, a total of 183 diatom taxa were identified. There was a general decreasing trend in the diversity indices for the river sites (except Usuthu River) between February and May, with the opposite observed for the floodplain pan sites with a general increasing trend between the sampling surveys (Figure 3.4). There was no significant total species, total individuals, Shannon diversity and Pielou's evenness differences between sampling surveys for all sites (Figure 3.4).

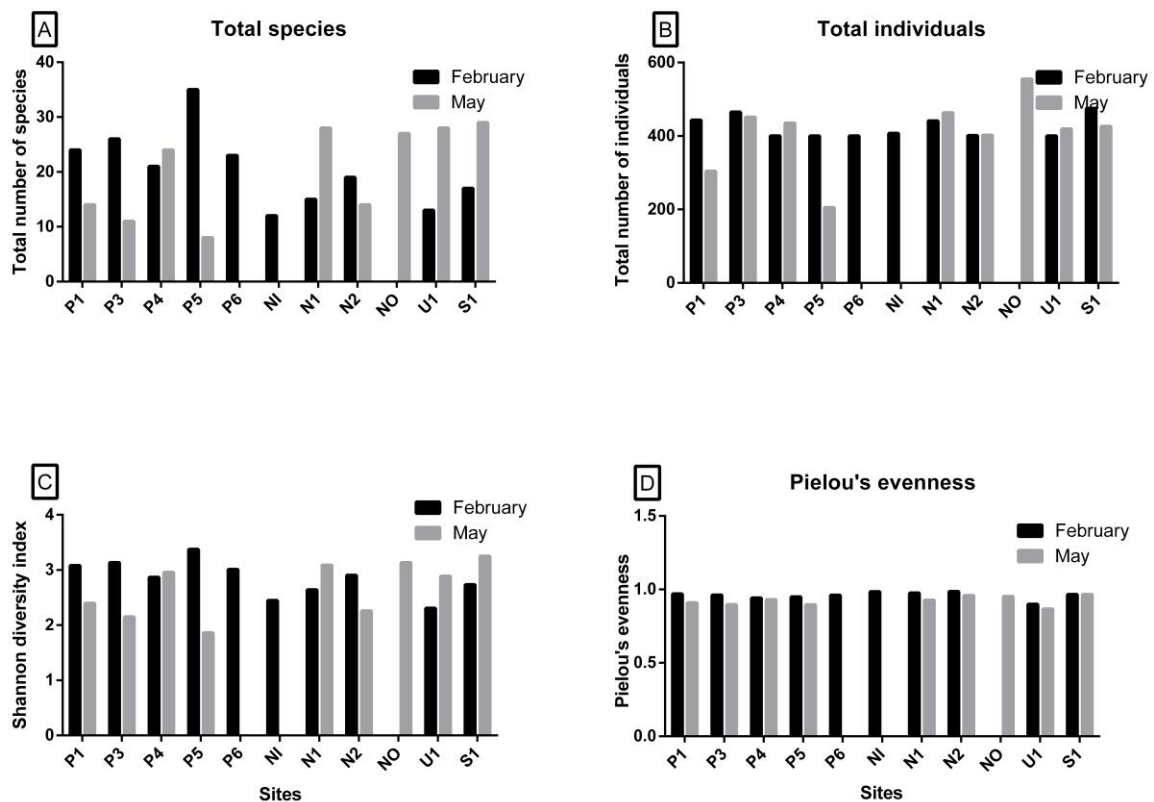


Figure 3.4: (A) Total number of species, (B) total number of individuals, (C) Shannon diversity index and (D) Pielou's evenness for each site after the two sampling periods.

The Canonical Correspondence Analysis (CCA) explained 50.62% of the variation with sampled sites grouping together in three groups (Figure 3.5). In the top half of the graph all the Nyamithi Pan sites from both surveys, P6 (February) and U1 (February) grouped together in group 1. To the right of the graph, Shokwe Pan for both surveys grouped together and the third group in the bottom half of the graph consisted of all the Phongolo River sites for both surveys and U1 (May). Groups 1 and 3 separated across the second axis (explaining 8.41% of the variation) with temperature, electrical conductivity and sulphate the main drivers for group 1. The main drivers for group 3 were nitrogen (ammonium, nitrate and nitrite) and pH with total phosphate the main driver for group 2. Separation across the first axis (explaining

10.03% of the variation) was due to species diversity with the highest species diversity at group 2 and the remaining species evenly distributed between groups 1 and 3 (Figure 3.6).

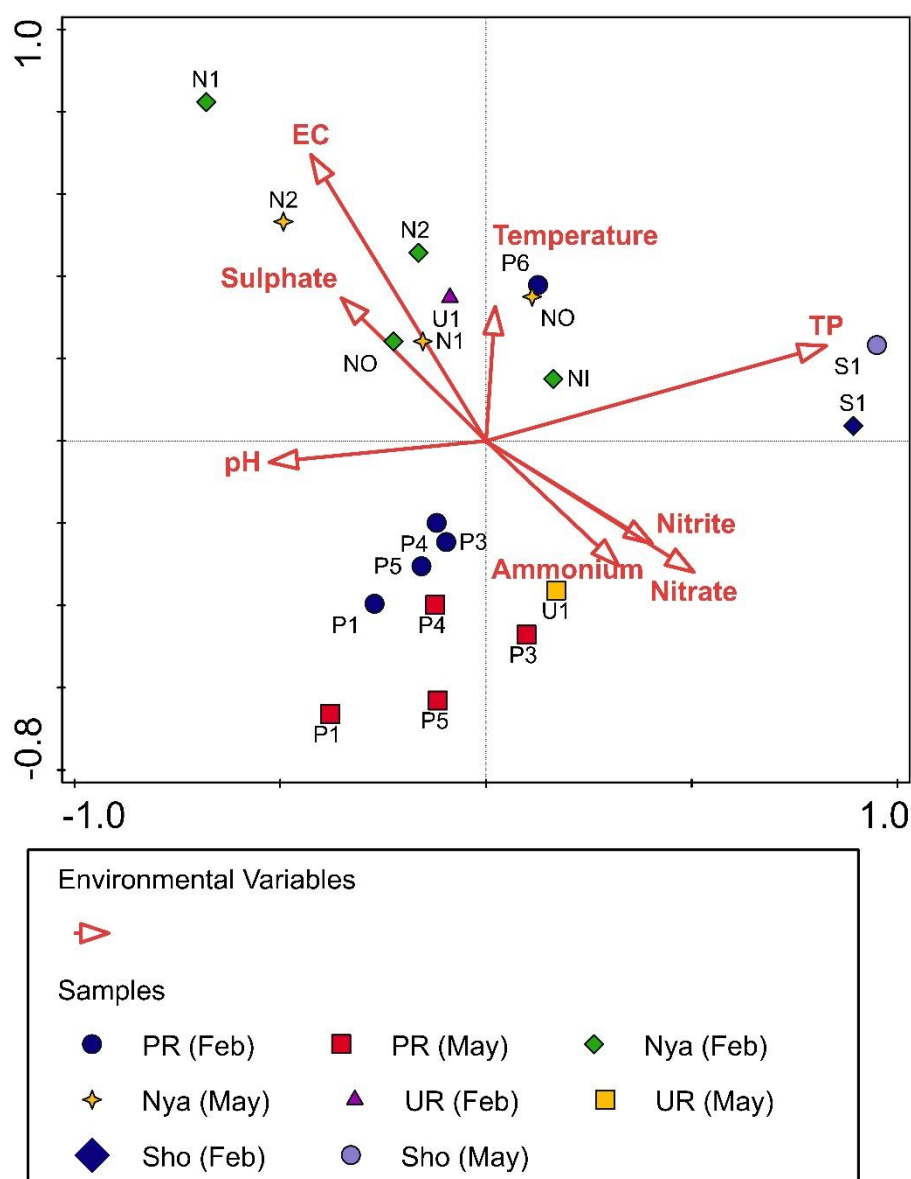


Figure 3.5: Canonical Correspondence Analysis (CCA) biplot comparing physico-chemical water variables to the Phongolo River and associated wetland and Usuthu River and associated wetland during February and May 2017. The biplot explains 50.62% of the variation with the first axis explaining 10.03% and the second axis 8.41% of the variation. TP – total phosphate, EC – electrical conductivity. PR – Phongolo River, UR – Usuthu River, Nya – Nyamithi Pan Sites, Sho – Shokwe Pan Sites.

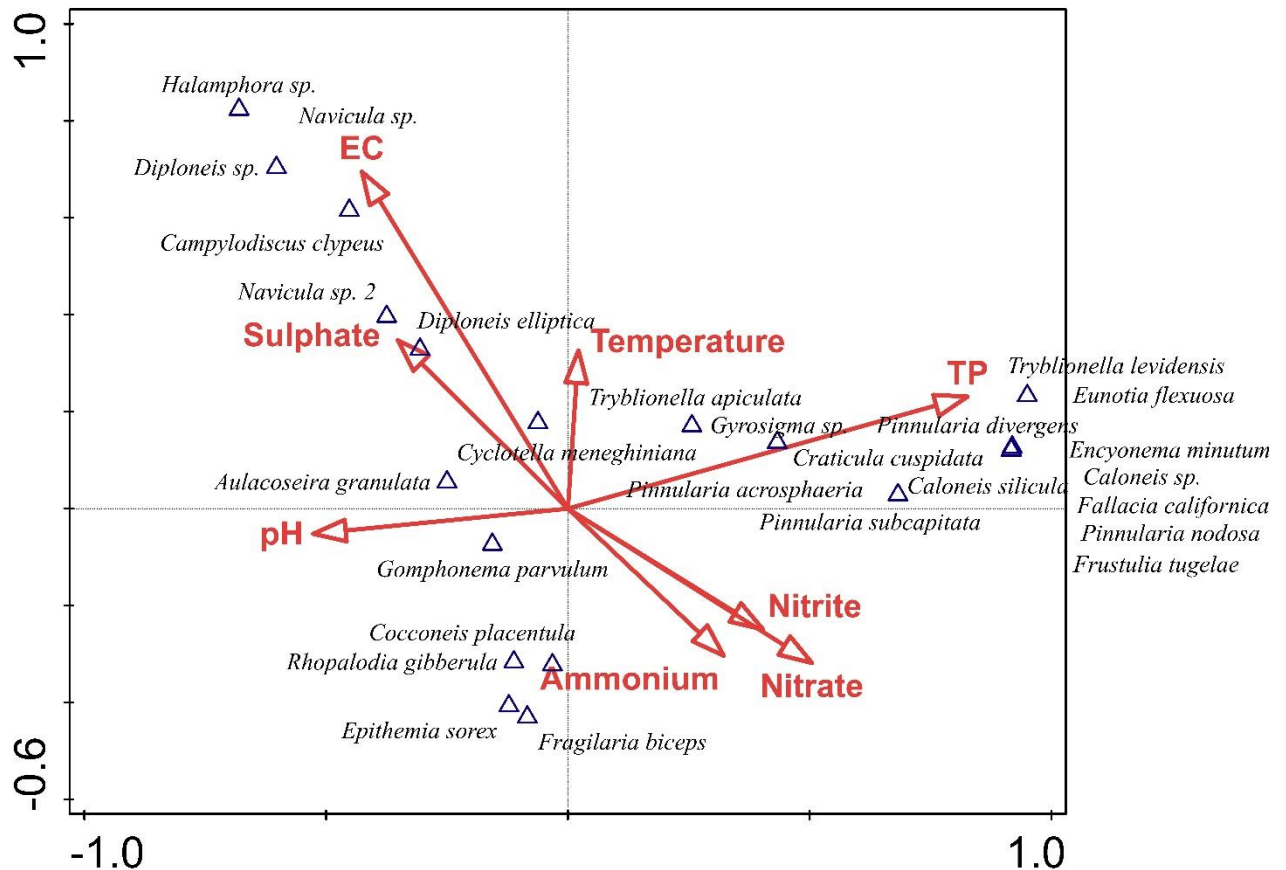


Figure 3.6: Canonical Correspondence Analysis (CCA) biplot comparing physico-chemical water variables to the 30 best fitting diatom species during February and May 2017. The biplot explains 50.62% of the variation with the first axis explaining 10.03% and the second axis 8.41% of the variation. TP – total phosphate, EC – electrical conductivity.

Space (based on each site's geographic coordinates) was included as an extra explanatory variable using a Principle Coordinates of Neighbouring matrices (PCNM) in order to determine the influence of space on the structuring of the diatom community (Figure 3.7). The PCNM explained 100% of the variations with space (based on a Euclidean distance matrix) only explaining 17.1% of the variation and physico-chemical water variables explaining 43.7% of the variation of the diatom community. Combined, physico-chemical water variables and space explained 39.2% of the diatom community variation.

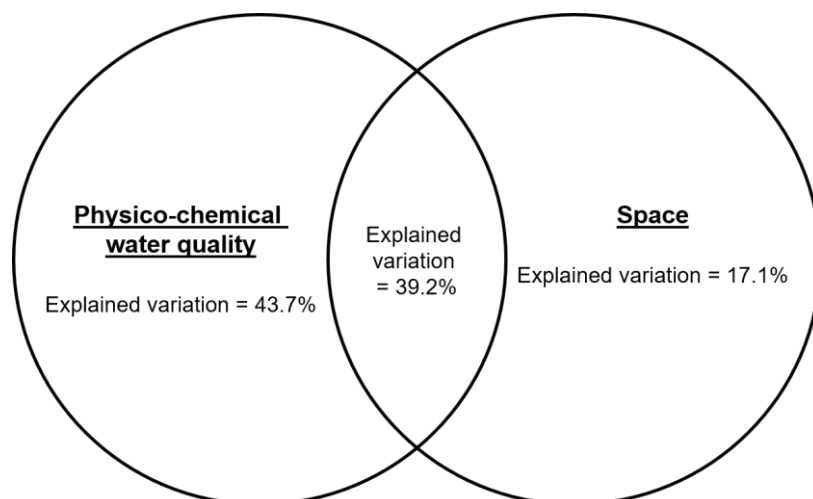


Figure 3.7: Contribution of physico-chemical water variables and space (distance between sites) on the diatom community structuring.

3.3.3 Periphyton stable isotopes

Changes were noted when comparing the stable isotope values between the summer rainfall (February 2017) and late summer rainfall (May 2017) seasons. For the Phongolo River, the $\delta^{13}\text{C}$ values decreased significantly ($p < 0.05$) downstream and $\delta^{15}\text{N}$ values increased significantly ($p < 0.05$) during the summer rainfall period (Figure 3.8 A). Nyamithi Pan's (Phongolo River floodplain pan) $\delta^{13}\text{C}$ values increased significantly and $\delta^{15}\text{N}$ decreased significantly between the inflow and main water body. There were significantly higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the main water body of Nyamithi compared to the Phongolo River during the summer rainfall period. There was a significant decrease in $\delta^{13}\text{C}$ and a significant increase in $\delta^{15}\text{N}$ between the Usuthu River and its associated floodplain pan (Figure 3.8 A). There was no clear grouping of sites during February, however, P6 and U1 were situated close to one another. During May the sites grouped together more compared to February (Figure 3.8 B). The $\delta^{13}\text{C}$ values decreased between site P1 and the other Phongolo River sites. The $\delta^{13}\text{C}$ values increased across the Nyamithi main water body with $\delta^{15}\text{N}$ remaining relatively consistent. Usuthu River and associated floodplain pan grouped closer together compared to February. Significant spatial ($F = 62.24$, $p < 0.0001$) and temporal ($F = 561.6$, $p < 0.0001$) differences were observed between seasonal surveys.

There was an overall decrease in $\delta^{13}\text{C}$ within the Phongolo River between seasons with the $\delta^{13}\text{C}$ remaining relatively consistent within the Usuthu River and the two floodplain pan sites between seasons. Significant ($p < 0.05$) differences for the mean $\delta^{15}\text{N}$ values between the summer rainfall period and the late summer rainfall period were noted for the Phongolo River (increased) and associated floodplain (decreased) pan. The Usuthu River's $\delta^{15}\text{N}$ values remained the same between seasons with a significant decrease in $\delta^{15}\text{N}$ for the Usuthu River's associated floodplain pan.

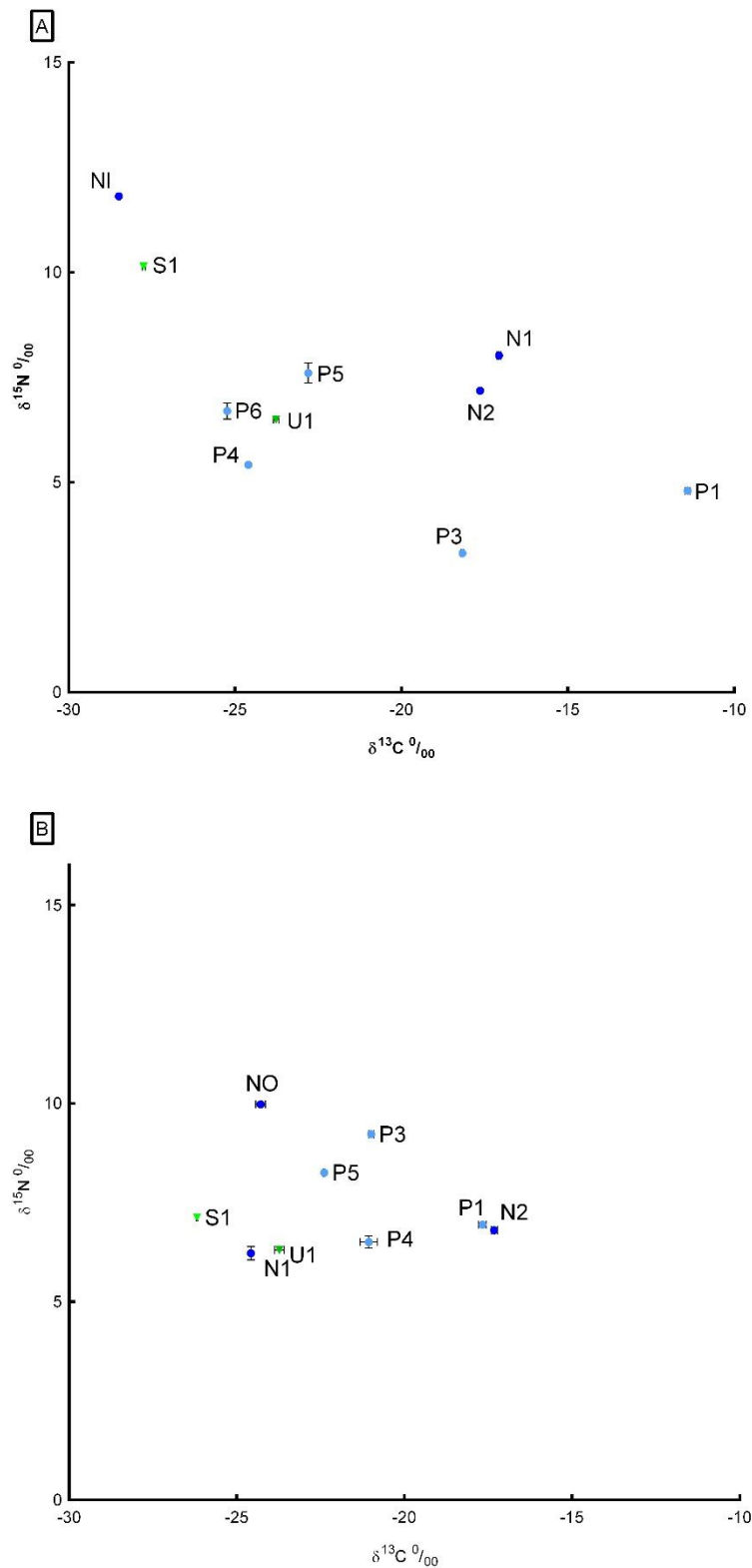


Figure 3.8: Bi-plots indicating the mean and standard error of the mean (SEM) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures for sites sampled during (A) February 2017 and (B) May 2017. Green rectangles – Usuthu River and associated floodplains, Blue circles – Phongolo River and associated floodplains.

3.4 Discussion

3.4.1 Connectivity of rivers and floodplain pans and structuring of diatom communities

The influence of the Usuthu River (February) on the lower Phongolo River (P6 - February) and Nyamithi Pan (both surveys) are visible in the CCA and stable isotope results. During February the Usuthu River was in flood and filled the channel at site P6 and floodwaters pushed into Nyamithi Pan. This was possible as Nyamithi Pan is situated within the floodplain area of site P6, thus flooding of P6 flows into Nyamithi Pan. Temperature, electrical conductivity and sulphate were the main drivers of the diatom community for these sites. *Navicula* sp., *Nitzschia* sp., *C. meneghiniana*, *C. clypeus* and *T. apiculata* were associated with these sites and are species that occur in ecosystems with increased salinity (Taylor *et al.*, 2007). Similar driving forces for these sites indicates the influence of the Usuthu River flood on P6 and Nyamithi as floodplain pan's water quality and diatom assemblages are influenced by the presence and absence of floods (Weilhoefer *et al.*, 2008; Oeding & Taffs, 2015). Weilhoefer *et al.* (2008) and Gell *et al.* (2002) found that mixing of floodwaters and floodplain pan water influenced the communities and physico-chemical water quality of floodplain pans. They also stated that mixing of physico-chemical water variables are dependent on the magnitude of the flood and the concentrations of the physico-chemical variables of both water bodies. Therefore, a low magnitude flood lasting only for a short period of time will not have a prolonged influence on the floodplain pan's physico-chemical water variables (Weilhoefer *et al.*, 2008). For a detailed description of the physico-chemical drivers of the Usuthu River and Nyamithi Pan see Chapter 2 (section 2.4.3).

The Usuthu River's associated pan, Shokwe Pan, grouped separately with total phosphate the main driver of the site for both seasons (as in Chapter 2). Species associated with Shokwe Pan include *E. flexuosa*, *Caloneis* sp. and *C. cuspidate* and are species that are found in either tropical, still standing or eutrophic ecosystems (Taylor *et al.*, 2007). Increased total phosphates in Shokwe Pan are most likely due to the influence of the natural flooding by the Usuthu River (Whittington *et al.*, 2013). The same influence was seen over numerous surveys done during 2017 and 2018 and was discussed in more detail in Chapter 2 (section 2.4.3). The absence of a flood limits the exchange of nutrients and diatom taxa between the river and floodplain pans and results in an isolated wetland ecosystem. During the study, the Phongolo River did not flood into the pans, as only a base flow ($4.54\text{--}8.50\text{ m}^3\text{s}^{-1}$) was released from the Pongolapoort Dam (due to a severe drought) (see Chapter 2, section 2.4.3). Notwithstanding a high diversity and even distribution of species were still obtained during both surveys, including species (*E. sorex*, *R. gibberula* and *C. placentula*) that occur in moderate to high electrolyte, flowing and still standing ecosystems (Taylor *et al.*, 2007).

3.4.2 Stable isotope signatures

The main driving force shaping the diatom community in the Phongolo River during the surveys was nitrogen with an increase in $\delta^{15}\text{N}$ values between the summer rainfall period and the late summer rainfall period. This was coupled to a decrease in $\delta^{13}\text{C}$. In tropical food webs, consumers such as macroinvertebrates and fish receive the majority of their organic carbon from benthic algae (Douglas *et al.*, 2005). Casatti *et al.* (2003) found that small fish fed mainly on diatoms and other autochthonous items in the Paranapanema River in south-eastern Brazil. Clapcott & Bunn (2003) reported that C4 aquatic macrophyte carbon isotope signatures were rarely found in aquatic consumers in Australian tropical rivers. This indicates that primary producers and not aquatic macrophytes, are the major contributors of carbon to aquatic food webs. Therefore the decrease in organic carbon can be attributed to lower food availability and increased competition during the late summer rainfall period, which led to increased feeding on periphyton. Kopprio *et al.* (2015) reported that carbon fractions are higher in diatoms during the warmer months as they use bicarbonate, which is $\sim 8\text{‰}$ more enriched, than CO_2 . Higher $\delta^{13}\text{C}$ values during February was expected as, $\delta^{13}\text{C}$ values are higher during summer months due to isotopic fraction (Fry, 2006).

The increase of $\delta^{15}\text{N}$ in the Phongolo River, especially at the downstream sites, can be attributed to the phenomenon of nutrient spiralling (Doyle, 2005). According to Doyle (2005), nutrient spiralling involves the coupling of nutrient biogeochemical cycling with assimilation, transformation, or physical sorption by biota. This means that when the discharge increases it allows for the release of nutrients back into the water through processes of mineralization or desorption. This effect can cause displacement of nitrogen within river ecosystems as they can easily be transported within the system due to flow (Kadlec *et al.*, 2005). This trend was observed during February where $\delta^{15}\text{N}$ values increased in downstream sites. During the late summer rainfall period (May) the $\delta^{15}\text{N}$ values remained relatively consistent downstream. Seasonality has an influence on the nitrogen cycle by adding nitrogen back into the ecosystem that was stored during the growing season (Kadlec *et al.*, 2005). Agricultural activities (sugarcane, crops and livestock) (Smit *et al.*, 2016; Dube *et al.*, 2017), within the floodplain catchment area, may contribute to the increased nitrogen within the river. In addition animal waste and fertilizers also enter the river through runoff water. Studies by Lake *et al.* (2001) and Jones *et al.* (2018) have related agriculture runoff (animal waste and fertilizer) to increased nitrogen concentrations in aquatic ecosystems. Increase of nitrogen during the late summer rainfall period can also be associated with the drying conditions causing dissolved nitrogen to be more concentrated within the water column resulting in increased $\delta^{15}\text{N}$ values. Increased nitrogen can also be due to runoff from the the surrounding landscape after a rainfall

event. The presence of hippopotami in Nyamithi Pan can have an influence on available nutrients in the pan due to animal waste.

The $\delta^{13}\text{C}$ values remained consistent for the Phongolo River's floodplain pan (Nyamithi Pan) as well as for the Usuthu River and its associated floodplain pan (Shokwe Pan). The diversity of primary producers are influenced by factors such as fluvial dynamics, human activities (agriculture and industrial), degree of seasonal variation, latitude and river size that in turn influences the carbon source contribution to the ecosystem (Zeng *et al.*, 2018). These factors might not have played an important role in the Usuthu River during the sampling period as the $\delta^{13}\text{C}$ values did not change significantly (Zeng *et al.*, 2018). Nitrogen ($\delta^{15}\text{N}$) values remained consistent in the Usuthu River, however, decreased significantly for both the Nyamithi and Shokwe Pans. The decrease of nitrogen was expected for the floodplain pans during the late summer rainfall period. Nitrogen decreases in wetland ecosystems during the transition to dryer conditions and a decrease in rainfall (Baldwin & Mitchell, 2000; Douglas *et al.*, 2005). The decrease can also be caused by sorption of nitrogen by plants or binding to sediment (Kadlec *et al.*, 2005). A rapid decrease in nitrogen, due to particle settlement, was also noted within Australian ponds (Greenway, 2010). Shokwe Pan had higher nitrogen values compared to the Usuthu River during the summer rainfall period. As wetland ecosystems act as "sinks" where nutrients accumulate (Kock *et al.*, 2019), it is expected that the floodplain pan would have higher nitrogen values.

3.5 Conclusion

This chapter examined the connectivity of specific floodplain pans to two river ecosystems in the Ndumo Game Reserve during summer rainfall (February 2017) and following late summer rainfall (May 2017) season. The Usuthu River had an effect on the diatom community structuring of the lower reaches of the Phongolo River and Nyamithi Pan as the flooding during February filled the channel at P6 and floodwaters pushed into Nyamithi Pan. The river also influenced the nutrient concentrations of Shokwe Pan as the pan receives water from the Usuthu River when it is in flood. The Phongolo River had no influence on the floodplain pans as the river had extremely low flows during the study period due to a base flow release that was maintained from the Pongolapoort Dam due to the ongoing drought in the region. There were shifts in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures within the Phongolo River, however, both these signatures remaining consistent in the Usuthu River. Both floodplain pan's $\delta^{15}\text{N}$ values decreased during the late summer rainfall period due to less runoff from the catchment area.

These results highlight how uniqueness the floodplain and how it should be monitored as a whole and not just each river and their associated floodplains individually. Management plans must include the entire floodplain as the Usuthu River can have an influence on not just Shokwe Pan but also the pans associated with the Phongolo River. The hypothesis that due to differences in connectivity of the rivers and their associated floodplain pans there will be spatial and temporal differences in the stable isotope signatures and diatom communities between the Phongolo River and associated floodplain pan but not between the Usuthu River and associated floodplain pan is rejected.

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Chapter 4: Reconstruction of historical diatom community structures in Nyamithi Pan sediment cores.

4.1 Introduction

Environmental changes are increasingly driven by human activities, making it important for us to understand how freshwater ecosystems change over time and space (Lewis & Maslin, 2015; Lami *et al.*, 2018). The understanding of these changes is vital to ensure sustainability of water resources on a global-scale (Lami *et al.*, 2018). Human driven changes and impacts on the environment are region and environment specific, thus it is important to understand the nature of the Anthropocene (Lami *et al.*, 2018). According to Steffen *et al.* (2011) the Anthropocene can be defined as the age in which “...human activity is largely responsible for the exit from the Holocene, that is, that humankind has become a global geological force in its own right”. Various articles have recently referred to the term when discussing issues such as climate change and global environmental issues. (Steffen *et al.*, 2011).

For informed environmental management, a long-term perspective on human interference and climate oscillation is essential (Reinwarth *et al.*, 2013). To accurately determine the response of the environment to human activities, long-term records from the pre-20th century are essential (Lami *et al.*, 2018). However, current studies focus mostly on direct monitoring techniques based on recent (present day) impacted areas of aquatic ecosystems, e.g. the top sediment layers (Lami *et al.*, 2018). Past environmental impacts can be assessed through use of lake sediment (Lami *et al.*, 2018). In order to study the evolution of environmental and climate proxies at the different sediment depths, the age-depth relationship is important. In order to create an age-depth model various methods can be used, however, radiocarbon dating is the preferred method for sediment that contains organic matter and is younger than 50 000 years (Blaauw & Christen, 2011). This approach of age-depth, together with the indicator species such as diatom assemblages has allowed researchers to successfully document the changes (eutrophication, acidification, etc.) of the aquatic environment over time (Saros, 2009).

As mentioned above, past aquatic environments can be reconstructed using biological proxies (e.g. diatoms) that are preserved within the sediment over thousands of years (Ryves *et al.*, 2009). Deep sediment cores represent the diatom community dynamics over time as these sediment records are a proxy of the ecosystems diatom meta-community (Pla-Rabés & Catalan, 2018). Diatoms have been extensively used in order to reconstruct and quantify past

environmental, e.g. water quality (Yun *et al.*, 2017; Bartozek *et al.*, 2019) and climate fluctuations (Blaauw & Christen, 2011; Pla-Rabés & Catalan, 2018). Diatoms are an appropriate microfossil group to reconstruct past environmental conditions through paleo-ecological studies, as species are sensitive to habitat type and water chemistry, communities are diverse, they occupy benthic and planktonic biotopes and they preserve well under certain conditions in sediment due to their silica cell wall (Flower *et al.*, 1997).

Knowledge of the ecological requirements of individual diatom species (McGowan *et al.*, 2018) is used to interpret changes within the natural environment. One such example is determining changes in thermal stratification through increases in *Cyclotella* species (McGowan *et al.*, 2018). Within high arctic ponds changes within the diatom epiphyte communities after the 18th century were attributed to their response to warming (McGowan *et al.*, 2018). Studies have also shown that diatoms are a sensitive bio-indicator of environmental change, as communities change along gradients of lake water pH as well as nutrient availability (McGowan *et al.*, 2018). Pla-Rabés & Catalan (2018) proposed that nutrient availability, light availability, nutrient recycling and contrasting exposure to irradiance will influence the structuring of epilithic diatom communities. The species assemblages present when sediment was deposited gives an indication of the environmental conditions at that time (Saros, 2009). This approach is extremely useful within ecosystems where there are abrupt step changes, however, there are limitations to the approach when changes within the environment are subtle (McGowan *et al.*, 2018).

The Ndumo Game Reserve (NGR) is one such area affected by environmental changes and human activities. The NGR falls within the lower Phongolo River floodplain and is one of the largest and most diverse floodplain ecosystems in South Africa (Dube *et al.*, 2015). However, as the human population that depends on the area over the past 30 years has increased 5 fold (Dube *et al.*, 2015) much pressure has been placed on the floodplain area. Agricultural activities in the surrounding areas have a large impact on the floodplain pans within the NGR as they cause siltation of these pans (Kyle, 1996). Pan ecosystems are not just affected by human activities but climate change may also cause changes to the diatom community (Curtis *et al.*, 2009). According to Roberts *et al.* (2019), the planktonic community change will be further triggered due to 21st century warming, causing a shift in the endemic, heavily silicified diatom community to a lighter, littoral diatom community.

Nyamithi pan is the second largest pan in the NGR (Kyle, 1996) and receives water from numerous sources during flooding, through localised rainfall as well as groundwater seepage. The pan receives water from the south-west when the Phongolo River is in flood and from the

east when the Usuthu River is in flood (Heeg *et al.*, 1978). The NGR groundwater is naturally saline due to underlying marine cretaceous deposits within the NGR area (Heeg *et al.*, 1978). This causes the pan to be highly saline as it receives water from groundwater seepage as well (Heeg *et al.*, 1978). The pan also receives water during localised rainfall within the pan's small catchment area.

The aim of this part of the study was to determine whether a combination of age-depth sediment profiles and diatom community structures can provide insight into the paleo-ecological condition of Nyamithi Pan. The hypothesis is that diatom community structures will reflect paleo-ecological conditions in Nyamithi Pan.

4.2 Materials and methods

4.2.1 Study area

Three sites were selected in Nyamithi Pan, NGR, northern KwaZulu-Natal (Figure 4.1). It is a perennial pan as well as the second largest pan within the reserve (Kyle, 1996). It consists of substrate predominantly made up of sand and mud (Smit *et al.*, 2016). After a flood release from the Pongolapoort Dam, the Phongolo River floods the pan and silt, derived from intensive agricultural activity within the catchment area, is deposited in the floodplain pan.

4.2.2 Core sample collection and age analysis

Core samples were collected during November 2017 from three sites on the banks of Nyamithi Pan using a 50 cm long stainless steel corer with a diameter of 7.5 cm. Core Site 1 was on the northern bank of the pan and a core of 35 cm was retrieved from the site. Site 2 was also located on the northern bank and the sampled core had a total length of 36 cm and Site 3 was on the southern shore of the pan with a total core length of 36 cm sampled. The sites were selected as they showed little disturbance from hippopotami, i.e. no signs of hippopotamus tracks as this could cause sediment mixing. Together with the cores the top layer of the sediment was also sampled for epibenthic biofilm using a syringe with a diameter of 5 mm based on methods described in Taylor *et al.* (2005). The sampled cores were divided into one centimetre slices (Gomes *et al.*, 2017).

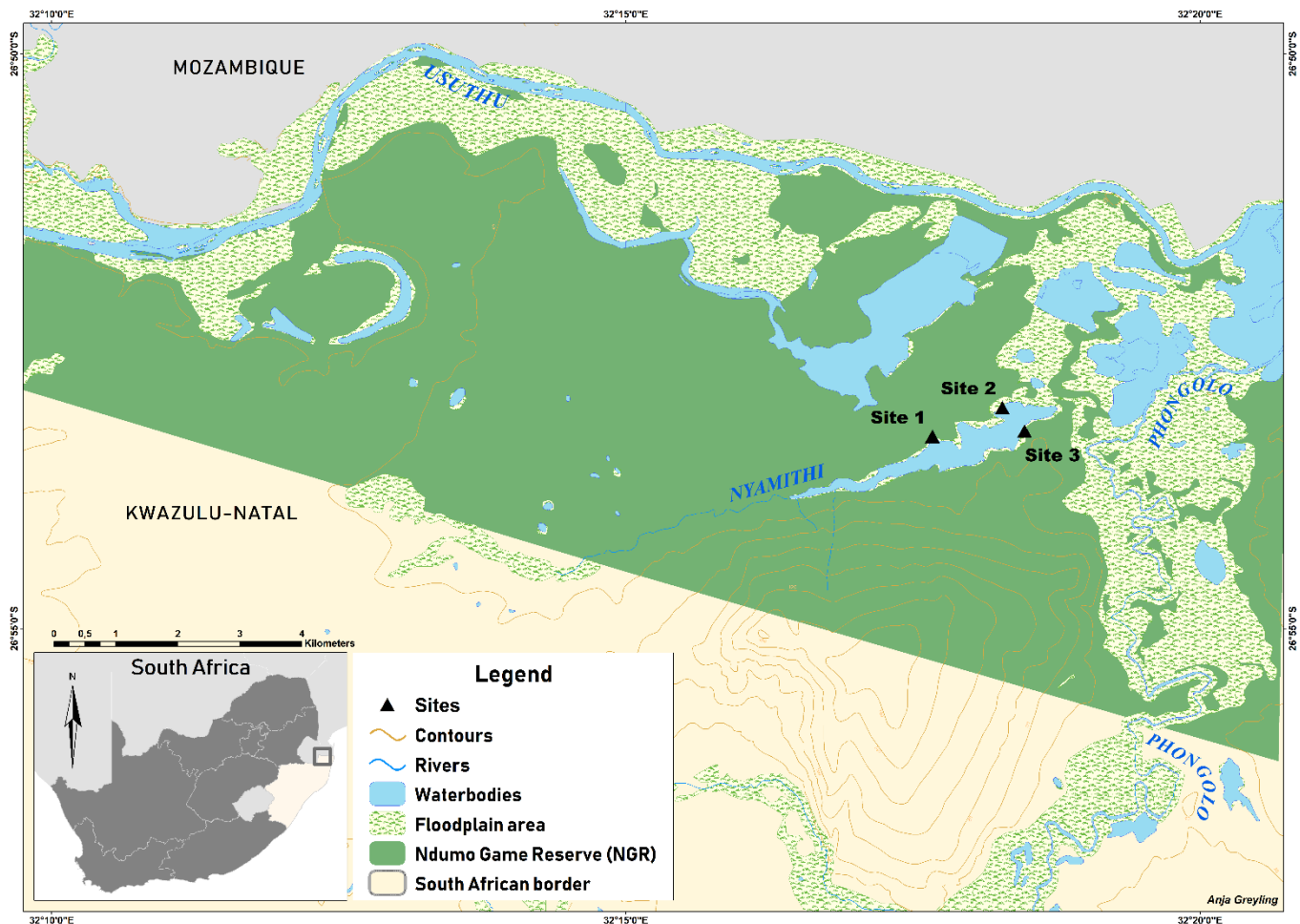


Figure 4.1: Map of the Ndumo Game Reserve with the core sampling sites at Nyamithi Pan.

4.2.3 Carbon dating

Every fifth one cm slice of the core were dated, as a curved plot is used to interpolate an age estimate for each slice's depth (Blaauw & Christen, 2011). According to Blaauw & Christen (2011), this concept is based on the restrictions that sediment can't accumulate backwards in time, and that there is a monotonic increase in the age of sediments with depth.

Carbon dating of selected slices was done according to Woodborne *et al.* (2015) and Woodroffe *et al.* (2015). To remove all carbonate material, sediment slices were put in clearly marked beakers and covered in hydrochloric acid (1% HCl) and heated in a water bath (70 °C) for 45 min while continuously stirring. The supernatant was decanted and samples were washed three times with double distilled water. Samples were covered with weak (< 1%) sodium hydroxide (NaOH) and returned to the water bath (70 °C) for 45 min while continuously stirring where after they were washed three times with double distilled water. Samples were treated one final time with HCl (1%) and heated in a water bath (70 °C) for 30 min, stirred and

washed with double distilled water. After washing, the samples were dried in an oven (70 °C) overnight and dried samples were examined under a Nikon light microscope to ensure all charcoal material has been removed. Approximately 700 mg sample was added to a vacuum pump together with copper oxide (80–90 mg) (CuO) and silver strips (1–2 cm) after which they were combusted at 900 °C. Combusted samples were prepared to graphite and dated through accelerator mass spectrometry (AMS) ^{14}C dating at iThemba laboratory, University of the Witwatersrand (WITS - Johannesburg, South Africa). The original, pre-treated sample's $\delta^{13}\text{C}$ values were measured through stable isotope analysis as described in Chapter 3 (section 3.2.4).

4.2.4 Diatom analysis

Diatom slide preparation was done following the hot hydrochloric acid (HCl) and potassium permanganate (KMnO_4) method described and recommended by Taylor *et al.* (2005). For the preparation of diatom slides, 20 g of each one cm slice was added to a beaker with double distilled water, vigorously mixed to detach diatom cells and left for a few minutes until the heavier sediment had settled. The supernatant was decanted into marked test tubes and microscope slides prepared and analysed as described in Chapter 2 (section 2.2.3).

4.2.5 Statistical analysis

The change in diatom abundances over time were determined and visualised using C2 version 1.7.7 (Juggins, 2007). R version 3.6.1 was used to determine the age of the core samples, with age-depth modelling using BACON (Bayesian accumulation histories) free open source code (Blaauw & Christen, 2013) and calibrated calendar ages calculated using SHCal13 (Southern Hemisphere atmospheric curve). Sedimentation accumulation rates were determined through Markov Chain Monte Carlo (MCMC) iterations estimates through use of BACON. The SIMPER protocol in Primer version 7 was used to determine the dominant diatom species and similarity between core sections based on dominant taxa present.

4.3 Results

4.3.1 Age-depth

The age of the core samples from 1–36 cm ranged from 1863–933 AD (Figure 4.2). Based on the results the deepest core slice at 36 cm is estimated to be 1086 years old. The results indicate that the top centimetre of sediment was dated at 1863, thus 156 years old. Sediment accumulation rates increased in the past 700 years compared to the 300 years preceding that (Figure 4.3 A, B).

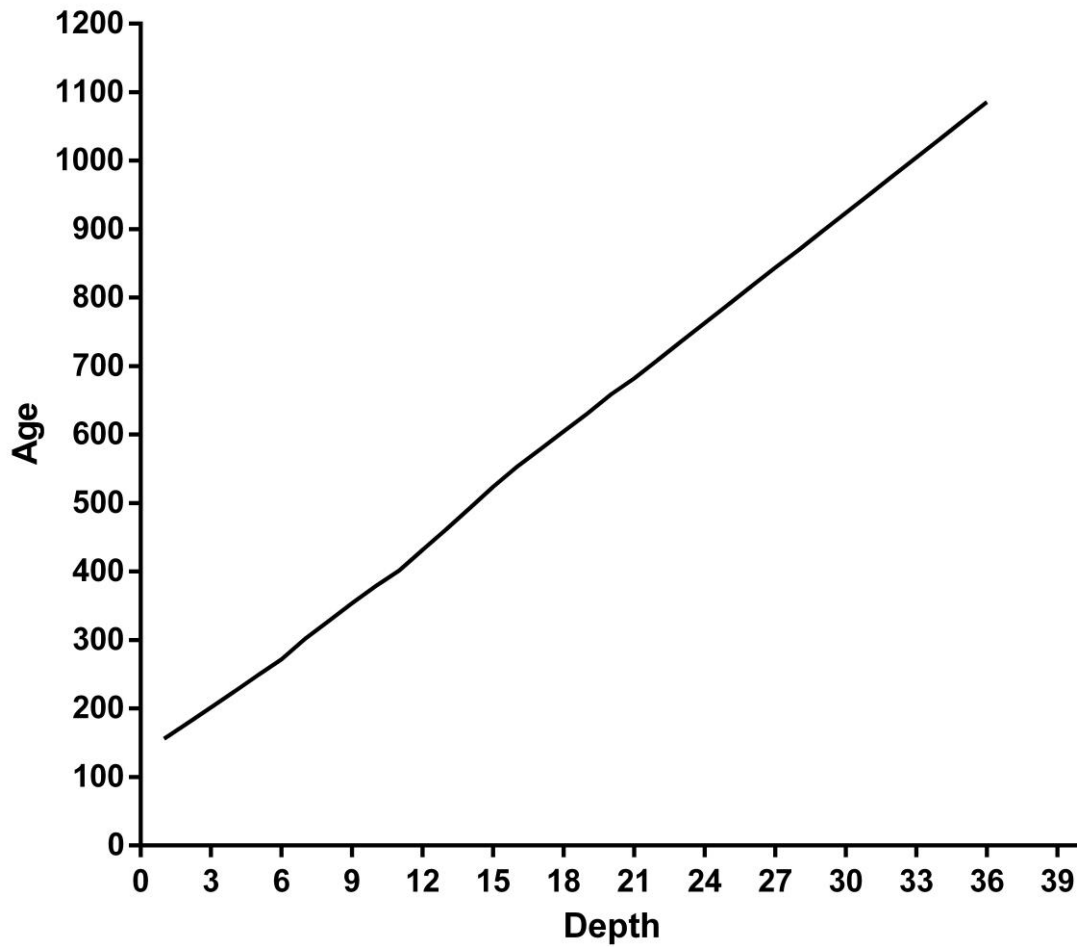


Figure 4.2: Line graph indicating the age (years) of each core slice at different depths(cm).

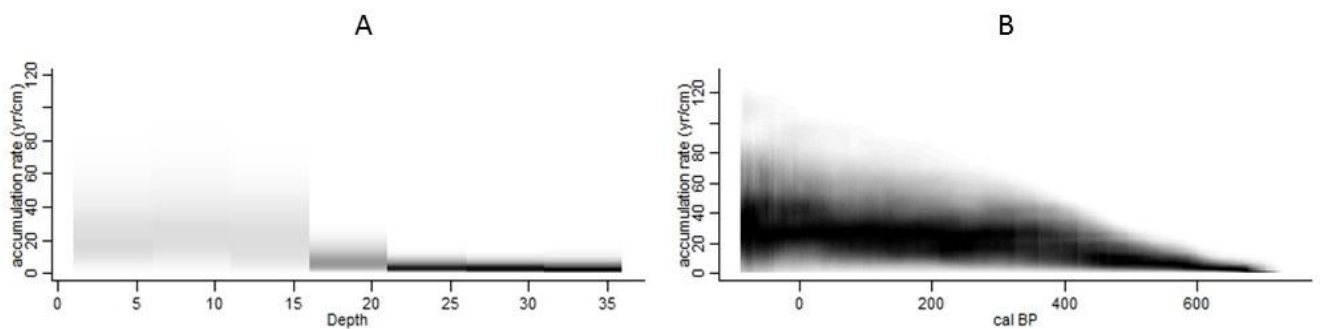


Figure 4.3: Sediment accumulation rate for the core slice at (A) the different depths and (B) cal BP (calendar years before 1950) years. Accumulation rate is indicated as years per centimetre.

4.3.2 Diatom community

For the sediment core; depth 0 cm is the top sediment layer and the deepest slice at 36 cm. No diatoms were present at depths of 21 cm, 22 cm, 24 cm, 25 cm, 27–32 cm and 34 cm (Figure 4.4). As the core gets deeper there is a general decline in total number of individuals counted with exceptions of higher number of individuals at depths of 7 cm, 8 cm, 10 cm, 19 cm and 36 cm.

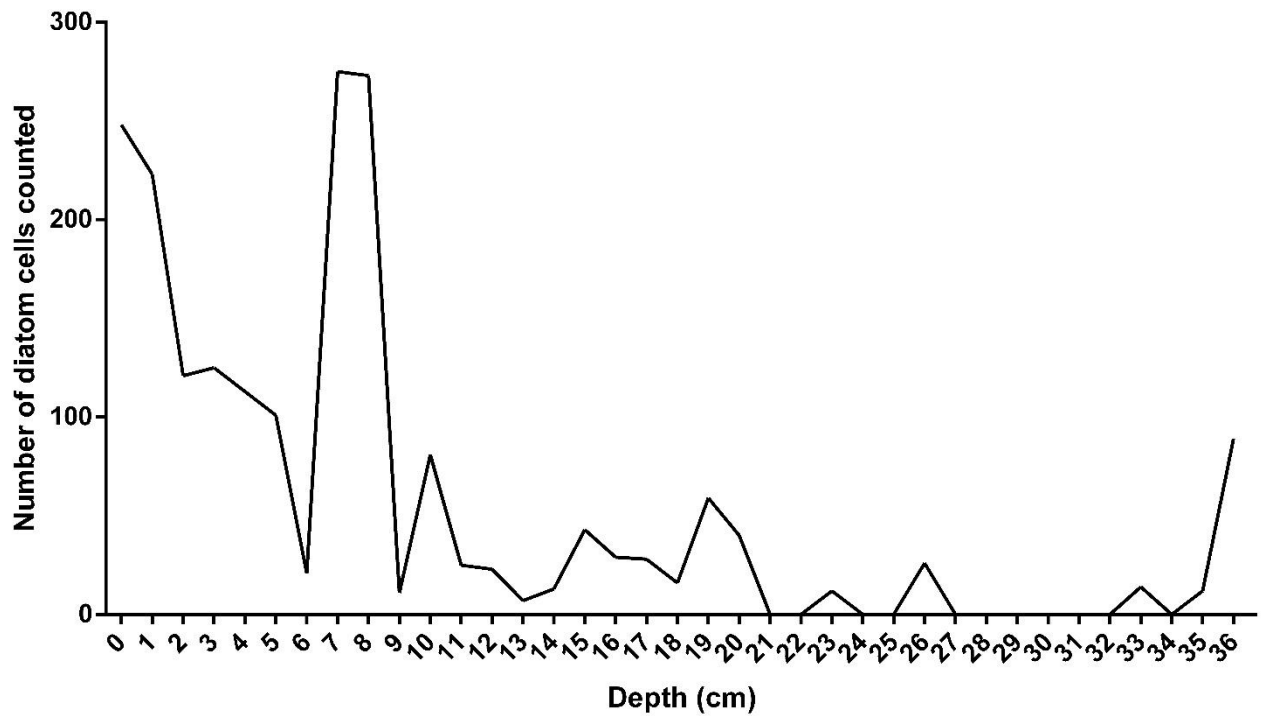


Figure 4.4: Line graph indicating the total number of individuals counted at each depth sampled.

Diatoms could only be identified from one core (Site 2) as the other two cores had no diatom valves or only broken valves that could not be identified. A total of 58 taxa from Site 2 were identified (Table 4.1).

Table 4.1: List of species identified from the core at Site 2.

<i>Achnantheidium</i> sp.	<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot
<i>Halamphora coffeaeformis</i> (Agardh) Kützing	<i>Navicula rostellata</i> (Kützing) Cleve
<i>Amphora fontinalis</i> Hustedt	<i>Navicula</i> sp.
<i>Amphora normannii</i> Rabenhorst	<i>Navicula</i> sp. 2
<i>Amphora</i> sp.	<i>Navicula veneta</i> Kützing
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	<i>Nitzschia cf. archibaldii</i> Lange-Bertalot
<i>Bacillaria paradoxa</i> Gmelin	<i>Nitzschia dissipata</i> (Kützing) Grunow
<i>Campylodiscus clypeus</i> Ehrenberg	<i>Nitzschia filiformis</i> (W. Smith) Van Heurck
<i>Cocconeis placentula</i> Ehrenberg	<i>Nitzschia frustulum</i> (Kützing) Grunow
<i>Craticula cuspidata</i> (Kützing) D.G. Mann	<i>Nitzschia linearis</i> (C. Agardh) W. Smith
<i>Cyclotella meneghiniana</i> Kützing	<i>Nitzschia palea</i> (Kützing) W. Smith
<i>Cymbella aspera</i> (Ehrenberg) H. Peragallo	<i>Nitzschia sigma</i> (Kützing) W. Smith
<i>Diploneis elliptica</i> (Kützing) Cleve	<i>Nitzschia siliqua</i> Archibald
<i>Discostella stelligera</i> (Hustedt) Houk & Klee	<i>Nitzschia</i> sp.
<i>Encynomea</i> sp.	<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot
<i>Epithemia</i> sp.	<i>Parlibellus</i> sp.
<i>Eunotia</i> sp.	<i>Pinnularia</i> sp.
<i>Fallacia pygmaea</i> (Kützing) Sickle & Mann	<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg
<i>Fragilaria</i> sp.	<i>Plagiotropis</i> sp.
<i>Gomphonema insigne</i> Gregory	<i>Planothidium rostratum</i> (Østrup) Lange-Bertalot
<i>Gomphonema parvulum</i> (Kützing) Kützing	<i>Rhopalodia gibba</i> (Ehrenberg) O. Müller
<i>Gomphonema</i> sp. Ehrenberg	<i>Seminavis strigosa</i> (Hustedt) Danieleadis & Economou-Amilli
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	<i>Stauroneis</i> sp.
<i>Haslea spicula</i> (Hickie) Lange-Bertalot	<i>Tabularia fasciculata</i> (C. Agardh) D.M. Williams & Round
<i>Kolbesia kolbei</i> (Hustedt) F.E. Round & L. Bukhtiyarova	<i>Thalassiosira weissflogi</i> (Grunow) Fryxell & Hasle
<i>Navicula cf. germainii</i> (J.H. Wallace) Lange-Bertalot	<i>Tryblionella apiculata</i> (W. Greg.) D.G. Mann
<i>Navicula erifuga</i> Lange-Bertalot	<i>Tryblionella calida</i> (Grunow) D.G. Mann

<i>Navicula libonensis</i> Schoeman	<i>Tryblionella gracilis</i> W. Smith
<i>Navicula radiosa</i> Kützing	

The seven dominant diatom species were determined with a SIMPER analysis (Figure 4.5). These species are *Halamphora coffeaeformis*, *Cyclotella meneghiniana*, *Diploneis elliptica*, *Fragilaria* sp., *Navicula* sp., *Nitzschia* sp. and *Nitzschia palea*. Both *H. coffeaeformis* (1466 AD and 933 AD) and *N. palea* (1557 AD) had peaks in their relative abundances in the lower sections of the core. *Cyclotella meneghiniana*, *D. elliptica* and *Fragilaria* sp. had varying relative abundance down the core with all three species showing peaks further down the core at 1014 AD, 1202 AD and 1283 AD respectively. Both *Navicula* sp. and *Nitzschia* sp. had varying relative abundances down the core with both species having their highest relative abundances at 960 AD. It was clear that for all species their relative abundance decreased upwards into the core (thus from 36–0 cm).

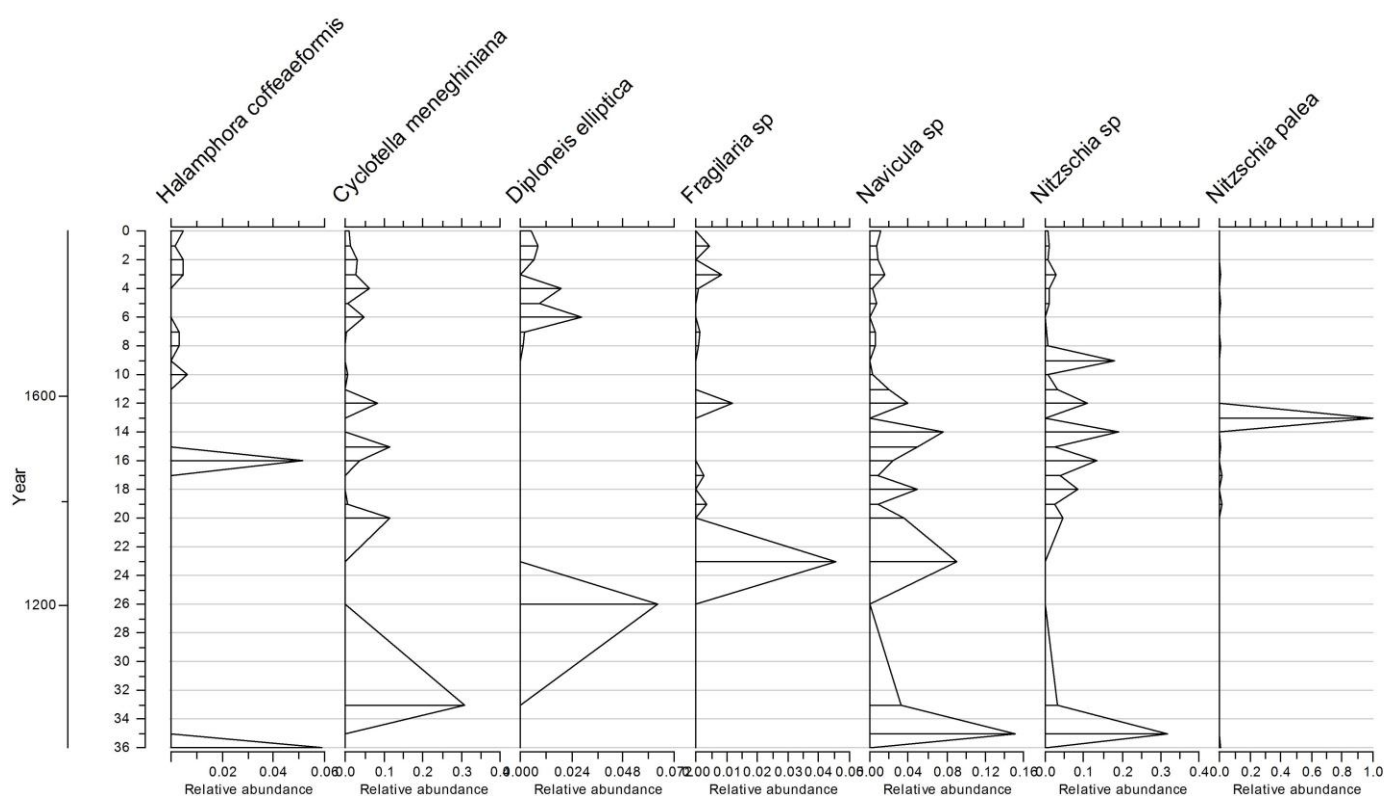


Figure 4.5: Dominant diatom species identified at Site 2. The graph illustrates how the specie's relative abundance changes over depth.

4.4 Discussion

During the present study low abundances of diatom cells (relative to sediment) were found in the deeper layers of the core. This is consistent with the findings of Pla-Rabés & Catalan (2018) who stated that for paleo-ecological samples the number of diatoms counted is generally less when compared to ecological samples. It was only possible to identify diatoms from one of the three cores as two contained either no remnants of diatoms or broken, unidentifiable frustules. This can be due to the bathymetry of lake ecosystems as some diatoms are transported between areas within the lake before they are deposited and this can cause damage to these frustules (McGowan *et al.*, 2018). According to McGowan *et al.* (2018), shifts in diatom abundance are often noted in sedimentary assemblages. Poor records also often exist for sedimentary assemblages as depth and pH can influence diatom distribution throughout a lake (Reavie & Edlund, 2010). Due to this Reavie & Edlund (2010) state that one core within a lake is suitable for paleo-ecological studies.

The absence of diatoms in some of the older core slices may be attributed to increased pH, salinity and temperature which resulted in poor preservation of diatoms (Bennion *et al.*, 2010; Fritz *et al.*, 2010). The preservation of diatoms through the sediment can be problematic as silicon is dissolved when SiO₂ recycling is accelerated. According to Ryves *et al.* (2009), the perfect preservation of diatoms in core samples is often the exception rather than the rule. However, although the record through the column is interrupted, when conditions are favourable for the preservation of valves, the diatom community composition can be used as a relatively accurate proxy for past conditions.

Fluctuations were noted in the relative abundances of all seven dominant species throughout the core sample. An increase in the relative abundance of *D. elliptica* were coupled with a decrease in the relative abundances of *H. coffeaeformis*, *C. meneghiniana*, *Fragilaria* sp., *Navicula* sp., *N. palea* and *Nitzschia* sp., and vice versa. Increased abundances of *D. elliptica* are an indication of decreasing salinity and nutrient concentrations during these periods as the species occurs in moderately saline and lower nutrient concentration ecosystems (Taylor *et al.*, 2007). In contrast, increased abundances of *H. coffeaeformis*, *C. meneghiniana*, *Fragilaria* sp., *Navicula* sp., *N. palea* and *Nitzschia* sp. indicate that the nutrient concentrations and salinity of the ecosystem increases as these species are indicators of eutrophic and high saline ecosystems (Archibald & Schoeman, 1984; Taylor *et al.*, 2007). Fluctuations in physico-chemical water variables and diatom community structure are due to changing environmental conditions of the floodplain area. The study area lies within the temperate-tropical troughs which have experienced a decrease in rainfall from the Medieval Warm Period (1075 AD) to

the Little Ice Age (1805 AD) (Woodborne *et al.*, 2015). The area experienced its driest periods during 1635 AD, 1695 AD and 1805 AD, with an abrupt transition to drier conditions from 1600 AD (Woodborne *et al.*, 2015). During 1280 AD a cultural systems at Mapungubwe collapsed due to drought, however, studies based on the CAC (Cold Air Cave) stable isotope data suggest that the onset of the drought could be at a later stage with an abrupt drought close to 1270-1280 AD (Stager *et al.*, 2013). Over the past ~700 years the KwaZulu-Natal coast has experienced drier conditions based on gray scale records, pollen records and data from the Sibaya conductivity series (Stager *et al.*, 2013). Gomes *et al.* (2017) stated that over the past 2000 years extreme climate fluctuations in climate change were the major driver of changes in the Lake St Lucia system.

The degree of fluctuations decreased in the recent past (from 1691 AD) with a transition to a more stable ecosystem, in terms of less extreme increases in the relative abundance of dominant diatom species. This was an indication that the ecosystem experienced less extreme nutrient and salinity regimes in the recent past with a freshening effect on the pan as the nutrient concentrations and salinity decreased. Fluctuations in the inundation and desiccation of the floodplain pan during the recent past are most likely due to the occurrence of annual flooding events. This is reflected in the lower relative abundances of dominant species and co-occurrence of saline tolerant and in-tolerant species. Increased sedimentation rates closer to recent times were noted for the study and were expected due to human activities, and is consistent with previous observations (Reinwarth *et al.*, 2013). It was reported that agricultural activities increased in the Limpopo Valley during 800-950 AD (Stager *et al.*, 2013). Increased sedimentation rate can also be due to the little Ice Age. In their study, Stager *et al.* (2013) found that due to changes in precipitation during the Little Ice Age, the Verlorenvlei in the Western Cape (South Africa) experienced increased sediment delivery. The Little Ice Age increases sediment delivery to catchment areas due to increased precipitation having a direct influence on erosion rates.

4.5 Conclusion

The change within the Nyamithi system over the past 1086 years is presented in this chapter based on diatom reconstruction. The relative abundances of dominant taxa through the core provide a good indication of the changes over time. Assuming the area was minimally impacted by hunter-gathers, the past eutrophic ecosystem status can be attributed to environmental conditions (Reinwarth *et al.*, 2013). The diatom assemblages reveal a less extreme fluctuation in salinity and nutrient regimes in the pan in the recent past compared to the year 1691 AD and later. Fluctuations in the recent past are caused by annual flooding events in the floodplain area resulting in varying desiccation and inundation of the pan. The hypothesis that the diatom community structures will reflect paleo-ecological conditions in Nyamithi Pan is accepted. The pan transitioned from extreme nutrient and saline fluctuations (933–1960 AD) to less extreme fluctuations in nutrient and salinity regimes (1691–present).

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Chapter 5: A lentic microcosm approach to determine the toxicity of DDT and Deltamethrin on diatom communities.

5.1 Introduction

Diatoms are useful in ecotoxicology studies due to their short life span, the fact that they are the main primary producers in aquatic ecosystems, their rapid response to anthropogenic and environmental disturbances and the cosmopolitan distribution of most species (Reid *et al.*, 1995; Stevenson *et al.*, 2010; Dalu & Froneman, 2016; Pandey *et al.* 2017). As diatom assemblages are species rich, the effects of toxicants can be measured at different levels of ecological organization (individual, community and population level) making the use of diatoms in ecotoxicological studies extremely useful (Stevenson, 2014). To assess the chemical status of aquatic ecosystems using diatoms as bio-indicators may in some circumstances be more advantageous than other organisms (macrophytes, macroinvertebrates and fishes) (Stevenson *et al.*, 2010; Pandey *et al.* 2017). A study by Hering *et al.* (2006) found that fishes, macroinvertebrates and macrophytes were sensitive to hydrological changes in the ecosystem, whereas diatoms were more sensitive to nutrient and organic contamination. Numerous studies from across the world have reported on the sensitivity of diatoms to inorganic contamination (e.g. metals, salts and biologically available nutrients) (Morin *et al.*, 2012) and organic toxicants (atrazine, metolachlor, PAHs, phenols and simazine) (Blanco & Bécares, 2010).

In order to assess saprobic load, trophic load and overall pollution, various bio-assessment tools have been developed based on the taxonomic composition of diatoms (Rimet & Bouchez, 2011). Recent results from ecological studies on the taxonomical composition and physiological activity of algae have shown the potential suitability of diatoms as a bio-assessment tool of pesticide risk (Rimet & Bouchez, 2011). Aside from taxonomic composition, functional groups are useful for pesticide toxicology studies. According to Viktória *et al.* (2017), functional groups are species that have similar ecological and/or morpho-physiological features that are grouped together. The most widely used diatom functional groups (metrics) include life-forms, ecological guilds and size classes (Passy, 2007; Rimet & Bouchez, 2011; Viktória *et al.*, 2017). Diatoms have adapted to several different life-forms (benthic, colonial, mobile, mucous tubules, pedunculate, pioneer and planktonic) as a strategy to resist and survive environmental pressures (Rimet & Bouchez, 2011). Ecological guilds are diatom taxa that have adapted differently to abiotic factors but still live in the same environment (Passy, 2007; Rimet & Bouchez, 2011). There are numerous advantages in using these

metrics as they are easy to use (identification to genus level) and the life-forms and ecological guilds found along numerous gradients can be accurately predicted. These metrics commonly make use of biofilms and they assess pollution levels and not just affinity to concentrations of organic matter and nutrients as with the diatom indices (Rimet & Bouchez, 2011). In the present study functional groups are also used as taxonomic composition can be problematic to use for ecotoxicology studies, as dissimilar species have different physiological responses to different pesticides, also, due to difficulty in identification to species level (Rimet & Bouchez, 2011).

Pesticides are used worldwide for disease and plague control (Margni, 2002; Karataş *et al.*, 2019) and in recent years usage has increased due to their wide spectrum, low cost and aid in increasing food production in the agriculture sector (Pearson *et al.*, 2016; Arslan *et al.*, 2017; Karataş *et al.*, 2019). In industry and agriculture, pesticides are widely used even though they contain highly toxic substances (Karataş *et al.*, 2019). The use of dichlorodiphenyltrichloroethane (DDT) in the past has mainly been for malaria control, however, due to its persistence in the environment, toxicity and accumulation in biota its use has been of concern (Humphries, 2013). Even though the use of DDT to combat agricultural pests has been banned, residues of DDT have been detected globally within water, sediment and aquatic biota (Humphries, 2013). Nevertheless, DDT is still used in some countries, such as South Africa (especially within the lower Phongolo catchment areas) after its complete ban in 1996 and subsequent reintroduction in 2006 for malaria vector control (van Dyk *et al.*, 2010; Humphries, 2013; Smit *et al.*, 2016). During the ban, synthetic pyrethroids replaced DDT. One such pyrethroid is Deltamethrin which was considered a less toxic alternative to DDT (Humphries, 2013). However, aquatic organisms are extremely sensitive to Deltamethrin that has been shown to be more toxic than organophosphates (Karataş *et al.*, 2019). It has been reported that due to Deltamethrin's high absorption rate it is a 1000 times more toxic to fish and other aquatic organisms (Abdelkhalek *et al.*, 2015). However, the agriculture sector still widely uses this highly toxic insecticide and over the past 10–15 years there has been an increase in its use (Abdelkhalek *et al.*, 2015). Even though the insecticide has a short half-life, due to its high evaporation capacity within surface waters (Ensibi *et al.*, 2014; Karataş *et al.*, 2019), it has been observed that Deltamethrin can lead to algal blooms in water as it affects the herbivores within the aquatic environment (Karataş *et al.*, 2019).

The influence of insecticides (in particular DDT) on aquatic ecosystems (Humphries, 2013), fish (Gerber *et al.*, 2016; Smit *et al.*, 2016; Karataş *et al.*, 2019; Volschenk *et al.*, 2019), accumulation in chickens (van Dyk *et al.*, 2010; Thompson *et al.*, 2017) and human health risk (Aneck-Hahn *et al.*, 2007; Eskenazi *et al.*, 2009; van Dyk *et al.*, 2010; Gerber *et al.*, 2016;

Pheiffer *et al.*, 2018) is well documented. However, knowledge of the effect that these insecticides have on non-target species, especially diatoms, is limited. Ullah *et al.* (2018) stated that insecticides affected non-target species causing behavioural disorders, biochemical changes, excitation, oxidative stress, genotoxicity and haematological changes. The aims of this chapter are therefore to 1) assess the effects of increased concentrations of malaria vector control insecticides (DDT and Deltamethrin) on the diatom community structures using a microcosm approach and 2) determine if a mixture (DDT 1:1 Deltamethrin) exposure will have a greater influence on the diatom community when compared to single exposures of these insecticides. The hypothesis tested for this chapter is that since DDT and Deltamethrin are insecticides and do not target diatoms, they will not have an effect on the vitality of diatom community structures.

5.2 Material and methods

5.2.1 Experimental framework

The experiment was carried out using 18 outdoor lentic microcosms each 1.5 m L x 40 cm W x 30 cm H (Figure 5.1). Lentic microcosms were constructed, maintained and sampled according to the guidelines set by the Organisation for Economic Co-operation and Development (OECD) (2006). All microcosms were constructed with approximately 5–7 cm sediment, six stones (10–20 cm in diameter) and macrophytes from a nearby uncontaminated (in terms of DDT and Deltamethrin) slow flowing stream (Gerrit Minnebron River) close to Potchefstroom, South Africa. Each microcosm was filled with 200 litres of borehole water and levels were kept within a 20% range of this volume for the duration of the experiment. All exposures as well as an unexposed control were done in triplicate (Figure 5.1). Macroinvertebrates sampled from stones (out-of-current) and vegetation from Gerrit Minnebron River were randomly distributed between the microcosms. Microscope slides were also added to each microcosm as artificial substrata for diatom colonisation. Microcosms were then left for six weeks to allow for diatom and macroinvertebrate colonisation. The experiment was conducted from August to October 2018. Following the six week colonisation period microcosms were dosed with either Deltamethrin (Dr, Ehrenstorfer GmbH), commercial grade DDT (AVI-DDT 750, Avima (Pty) Ltd) or a mixture (DDT 1:1 Deltamethrin), each at a low (0.1%) and high (1%) concentration. Final concentrations for DDT were 358 µg/L for the high and 35.8 µg/L for the low concentration. For Deltamethrin the final concentration was 1.9 µg/L for the high and 0.19 µg/L for the low concentration exposure. The organisms in the microcosms were exposed for a total of 28 d and sampled pre-exposure, after 96 hr and 28 d.

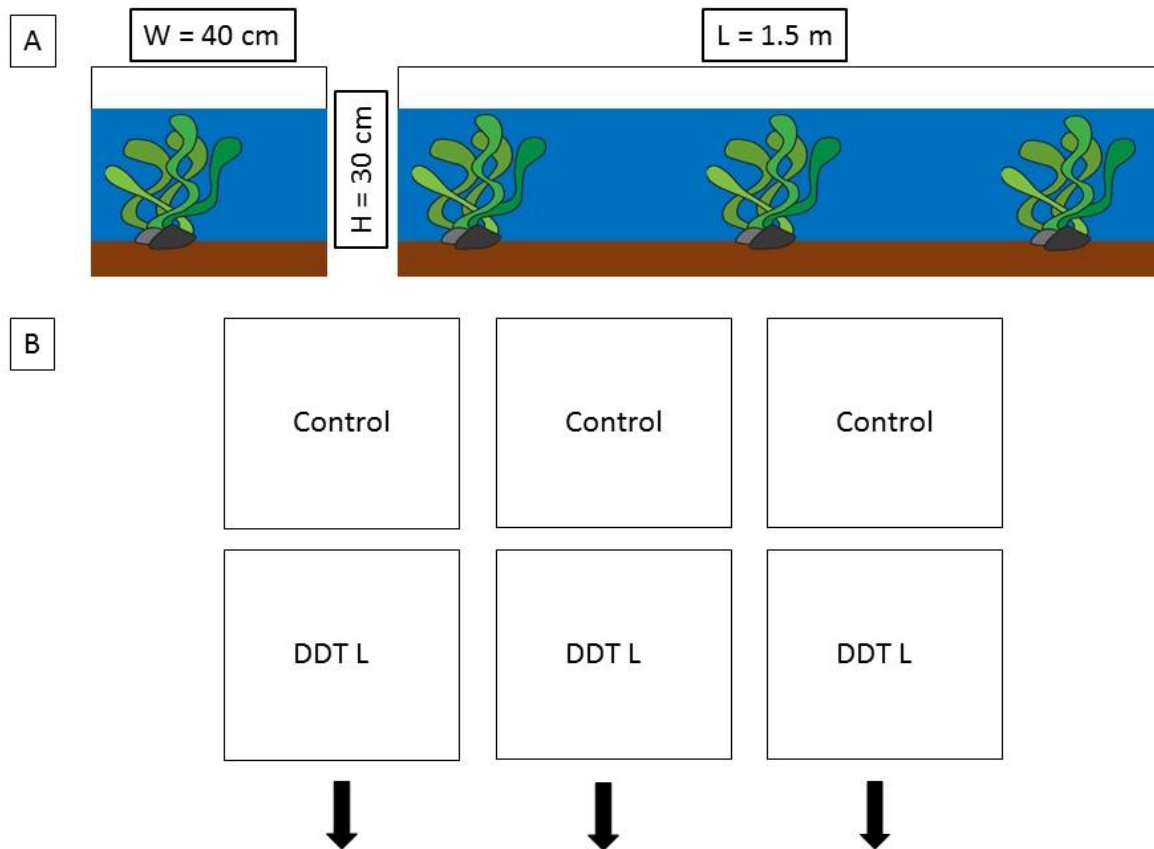


Figure 5.1: Schematic representation of the microcosm (A) dimensions and (B) experimental layout. DDT L – DDT Low concentration.

5.2.2 Physico-chemical variables

Each microcosm's *in situ* water quality variables were measured and 500 mL water samples collected (in pre-cleaned sampling bottles) for nutrient analysis. *In situ* measurements and water collection for the pre-exposure, 96 hr and 28 d analyses were collected in the morning at the same time from each microcosm. *In situ* measurements and nutrient analyses were done as described in Chapter 2 (section 2.2.2).

5.2.3 Diatom sampling and analysis

Diatoms were sampled after the physico-chemical parameters were recorded. Diatom sampling, preparation and analysis were done according to Taylor *et al.* (2005). Diatom samples were collected by scraping the diatoms from the top surface of the glass slides and stones with a brush into a collection tray with 200 mL sample water. Following sampling, the glass slides and stones were discarded in an appropriate manner as organic waste. Samples were subdivided, into sampling jars, in order to determine the percentage dead cells as well as prepare permanent microscope slides for taxonomic examination. The sub sample used

for taxonomic examination was preserved with 70% ethanol (final concentration not exceeding 20%).

Immediately following sampling, the percentage dead cells for each sample was determined. The sample was stirred in order to suspend the diatoms and one drop of the sample was pipetted onto a microscope slide and covered with a cover slide. Slides were viewed using a Nikon Eclipse (E100LED MV R) compound light microscope using 40x objective and diatom cells counted until a total number of 100 valves were counted. Percentage dead cells were calculated as total cells lacking a chloroplast out of a 100 cells. Live cells were defined as cells containing a chloroplast.

Permanent diatom slides were prepared for further taxonomical examination of the cells and to determine if any cell deformities formed due to the presence of the insecticides. Slide preparation and analyses were done as described in Chapter 2 (section 2.2.3).

Identified diatom genera were divided into different distinguishable life-forms, ecological guilds and size classes as set out by Rimet & Bouchez (2011, 2012). Life-forms included benthic, colonial, mobile (species that are able to move as they have a raphe structure), pendunculate and pioneer (species that can colonise bare substrates faster than other species) (Rimet & Bouchez, 2011). Three ecological guilds were selected namely: low-profile (species with short stature), high-profile (species with tall stature) and motile-guild (fast moving species) (Rimet & Bouchez, 2011). Genera were divided into five size classes based on their bio-volumes namely size 1 ($< 99 \mu\text{m}^3$), size 2 ($100\text{--}299 \mu\text{m}^3$), size 3 ($300\text{--}599 \mu\text{m}^3$), size 4 ($600\text{--}1499 \mu\text{m}^3$) and size 5 ($> 1500 \mu\text{m}^3$) (Viktória *et al.*, 2017). Tables 5.1 and 5.2 indicate the different life-forms and ecological guilds and different size classes that each taxa were assigned to, respectively.

5.2.4 Sample extraction and chemical analysis

Chemical analysis was performed on water and sediment samples for DDT, its metabolites (o,p'-DDD, o,p'-DDE, o,p'-DDT, p,p'-DDD, p,p'-DDE, and p,p'-DDT), and Deltamethrin. Sample extraction and chemical analyses were done according to Yohannes *et al.* (2013) and South *et al.* (2019). For the water samples 500 ml water was collected in pre-cleaned sampling bottles and ~500 g surface sediment was collected in pre-cleaned sampling jars.

Table 5.1: Assignment of diatom taxa to different biological metrics (Rimet & Bouchez, 2011, 2012).

	Benthic	Mobile	Colonial	Pendunculate	Pioneer	High Profile	Low Profile	Motile
<i>Achnantheidium</i>	*	*		*	*		*	
<i>Amphipleura</i>	*	*				*	*	
<i>Amphora</i>	*	*			*		*	
<i>Caloneis</i>		*						*
<i>Cocconeis</i>		*					*	
<i>Cymbella</i>	*	*	*	*		*		
<i>Cymbopleura</i>		*	*				*	
<i>Diatoma</i>	*		*	*		*		
<i>Epithemia</i>		*						*
<i>Eunotia</i>		*	*			*		
<i>Fragilaria</i>	*		*	*		*		
<i>Gomphonema</i>	*	*	*	*		*		
<i>Gyrosigma</i>	*	*						*
<i>Mastogloia</i>		*						*
<i>Navicula</i>	*	*						*
<i>Nitzschia</i>	*	*						*
<i>Pinnularia</i>		*						*
<i>Pleurosigma</i>		*						
<i>Rhopalodia</i>		*						*
<i>Stauroneis</i>		*						*
<i>Surirella</i>		*						*
<i>Tryblionella</i>		*						*

5.2.4.1 Water samples

Liquid-liquid extraction was the preferred method used for water samples. Extractions were performed through mixing 50 mL sample water with 50 mL hexane and 100 μ L PCB 143 as internal standard. The water layer was decanted and the hexane supernatant transferred to evaporation flasks. The process was repeated twice and hexane eluate concentrated to dryness in a water bath (36 °C) with a gentle nitrogen flow. The extract was dissolved in 100 μ L n-decane and transferred to GC-vials.

Table 5.2: Assignment of diatom taxa to different size classes (Rimet & Bouchez, 2011, 2012; Viktória *et al.*, 2017).

	Size 1	Size 2	Size 3	Size 4	Size 5
<i>Achnantheidium</i>	*				
<i>Amphipleura</i>					*
<i>Amphora</i>					*
<i>Caloneis</i>			*		
<i>Cocconeis</i>				*	
<i>Cymbella</i>					*
<i>Cymbopleura</i>				*	
<i>Diatoma</i>					*
<i>Epithemia</i>				*	
<i>Eunotia</i>			*		
<i>Fragilaria</i>					*
<i>Gomphonema</i>			*		
<i>Gyrosigma</i>					*
<i>Mastogloia</i>				*	
<i>Navicula</i>					*
<i>Nitzschia</i>			*		
<i>Pinnularia</i>				*	
<i>Pleurosigma</i>					*
<i>Rhopalodia</i>					*
<i>Stauroneis</i>				*	
<i>Surirella</i>				*	
<i>Tryblionella</i>				*	

5.2.4.2 Sediment samples

Five grams anhydrous sodium sulphate and 1 g activated copper (for desulphurization) were added to 5 g freeze dried sediment. Samples were sonicated twice for 30 min with 30 ml hexane:acetone (1:1) and 100 µL PCB 143 as internal standard. The sample was then concentrated to ~2 mL through evaporation with gentle nitrogen flow in a water bath (36 °C). Samples were cleaned-up with a fluorosil column containing anhydrous sodium sulphate and 5 g deactivated silica gel. The column was eluted with 120 mL dicloromethane:hexane (3:7) and eluate concentrated to dryness in a water bath (36 °C) with a gentle nitrogen flow. The extract was transferred to GC-vials after it was dissolved with 100 µL n-decane.

5.2.4.3 Gas chromatography analysis

Analyses of o,p'-DDD, o,p'-DDE, o,p'-DDT, p,p'-DDD, p,p'-DDE, p,p'-DDT and Deltamethrin were carried out using a gas-chromatograph (GC) (HP 6890) equipped with a ⁶³Ni micro electron capture detector (GC-µECD). Separation was achieved using a 0.25 µm film thickness, 250 µm i.d. and 30 m length HT8-MS (SGE) column. Using splitless injection, 1 µL

sample was injected at an inlet temperature of 250 °C. Hydrogen was used as carrier gas and the oven program for the GC was initiated at 100 °C and held for 1 min; ramped up to 200 °C at 20 °C/min; followed by a final 60 °C/min ramp to 260 °C (20 min hold).

5.2.5 Quality control and quality assurance

A seven-point calibration between 1 and 1000 µg/L was done for all compounds and all calibration peaks had a coefficient of variance (r^2) > 0.998. The ratio between the analyte peak area and internal standard (PCB 143) was used for calibration. Both DDT and Deltamethrin were identified by comparing their retention time to the corresponding standard. A blank sample was run after each sample analysis to ensure that there was no interference or cross contamination. An internal standard (PCB 143) was run to monitor the procedural performance and recoveries ranged from 60.33 to 61.29% for water and 68.51 to 73.46% for sediment samples. The limit of detection (LOD) and limit of quantification (LOQ) for the instrument were calculated as 3x and 10x the standard error of the y intercept from the calibration curve (of standard curve) for each compound, respectively and are presented in Table 5.3.

Table 5.3: Limit of detection (LOD) and limit of quantification (LOQ) for gas-chromatograph (GC) (HP 6890), water and sediment for DDT and metabolites as well as Deltamethrin.

	Instrument limits	
	LOD (µg/L)	LOQ (µg/L)
o,p'-DDE	16.69655	55.65518
p,p'-DDE	12.9446	43.14867
o,p'-DDD	15.16905	50.56349
o,p'-DDT	29.55404	98.51346
p,p'-DDD	9.760122	32.53374
p,p'-DDT	35.81305	119.3768
Deltamethrin	42.5926	141.9753

5.2.6 Statistical analysis

Data were tested for normality using the Shapiro-Wilk test. A parametric one-way Analysis Of Variance (ANOVA) was used to test for significant differences between normally distributed data. For non-normally distributed data significant differences were determined using a non-parametric ANOVA. Significant differences ($p < 0.05$) between exposure times for physico-chemical variables and percentage dead cells were done with a two-way ANOVA and Sidak's multiple comparisons test in order to determine the influence of both the concentration and

exposure times. A two-way ANOVA and Dunnett's multiple comparisons test were used to test for significant ($p < 0.05$) differences between total number of taxa, total number of individuals and diversity indices for the different exposure times. Significant differences ($p < 0.05$) between exposure times for abundances and diatom metrics were done with a two-way ANOVA and Tukey's multiple comparisons test. Using Canoco version 5, the diatom data were analysed by Principal Response Curve (PRC). The PRC analysed the data with the insecticide and concentrations as the effects factor and the time after exposure as the temporal factor. Significant difference ($p < 0.05$) of the effects of the insecticides were tested with a Monte Carlo permutation test.

5.3 Results

For all the results the high and low concentrations for the insecticide exposures are represented by the letters "H" and "L" respectively. Physical-chemical water quality values for the microcosm samples during the study are presented in Appendix A (Table A-2 and A-3).

5.3.1 Insecticide concentrations

The DDT and Deltamethrin concentrations were below detection in water samples collected, following 96 hr and 28 d exposure periods (data not shown). Furthermore, no Deltamethrin was recorded in the sediments of any of the control or exposure microcosms (Table 5.4). Of the DDTs, only o,p'- and p,p'-DDD was recorded after both 96 hr and 28 d for the DDT H and Mix H exposures. These two exposures also had detectable o,p'-DDE in the Mix H after 96 hr exposure and p,p'-DDE in the DDT H after 28 d exposure (Table 5.4). Only p,p'-DDE was detected in the DDT L exposure after 96 hr exposure.

5.3.2 Physico-chemical variables

There were no significant temperature, percentage oxygen saturation, TDS and conductivity differences between exposures and exposure time (Figure 5.2). Nitrate, ammonium, sulphate and pH remained relatively constant throughout the exposure period with no significant ($p < 0.05$) differences observed between exposure times (Figure 5.3). Significant ($p < 0.05$) increases were noted for nitrite for both DDT exposures between 96 hr and 28 d (Figure 5.3 B). A decrease in total phosphate (Figure 5.3 B) was observed for all microcosms between 96 hr and 28 d with only DDT L showing a significant ($p < 0.05$) decrease in total phosphate.

Table 5.4: Mean $\pm$ standard error of mean (SEM) of DDT (and metabolites) and Deltamethrin concentrations in sediments ($\mu\text{g/kg}$) after 96 hours and 28 days exposure. ND represents DDT/Deltamethrin not detected; BD represents DDT/Deltamethrin below detection.

	Control		Mix H		DDT L	
	96 hr	28 d	96 hr	28 d	96 hr	28 d
	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM
o,p'-DDE	ND	ND	1.937 $\pm$ 1.455	ND	ND	ND
p,p'-DDE	ND	ND	ND	BD	ND	ND
o,p'-DDD	ND	ND	1.536 $\pm$ 0.331	2.494 $\pm$ 1.123	BD	ND
o,p'-DDT	ND	ND	ND	ND	ND	ND
p,p'-DDD	ND	ND	6.458 $\pm$ 1.875	8.779 $\pm$ 4.016	2.518 $\pm$ 0.931	ND
p,p'-DDT	ND	ND	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND	ND	ND
	DDT H		Deltamethrin L		Deltamethrin H	
	96 hr	28 d	96 hr	28 d	96 hr	28 d
	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM
o,p'-DDE	ND	ND	ND	ND	ND	ND
p,p'-DDE	BD	0.934 $\pm$ 0.246	ND	ND	ND	ND
o,p'-DDD	11.995 $\pm$ 8.349	1.162 $\pm$ 0.949	ND	ND	ND	ND
o,p'-DDT	ND	ND	ND	ND	ND	ND
p,p'-DDD	36.574 $\pm$ 24.119	3.612 $\pm$ 2.949	ND	ND	ND	ND
p,p'-DDT	ND	ND	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND	ND	ND

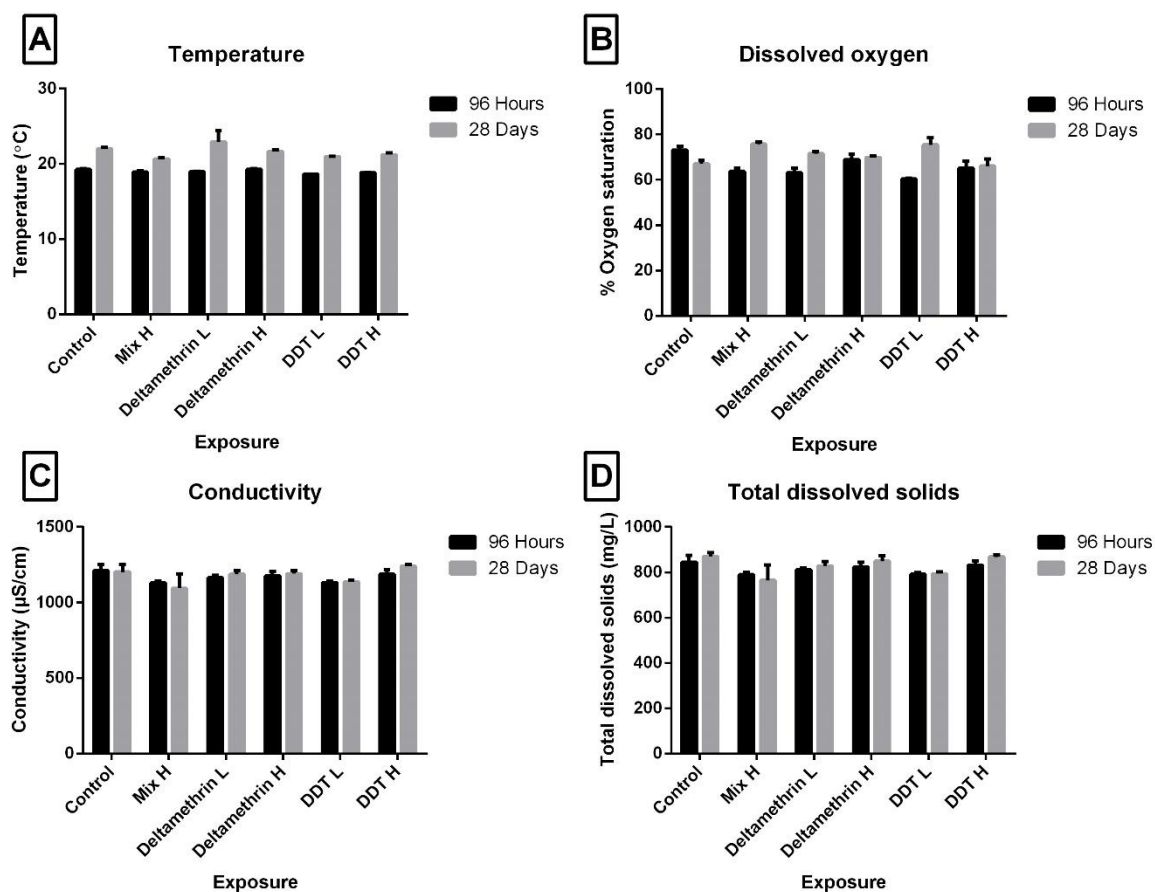


Figure 5.2: Mean and standard error of the mean (SEM) for (A) temperature (early morning, at similar times for each microcosms), (B) percentage oxygen saturation, (C) conductivity and (D) total dissolved solids for the control, Mix (DDT:Deltamethrin), Deltamethrin and DDT exposures after 96 hours and 28 day exposures. L – Low concentration, H – High concentration.

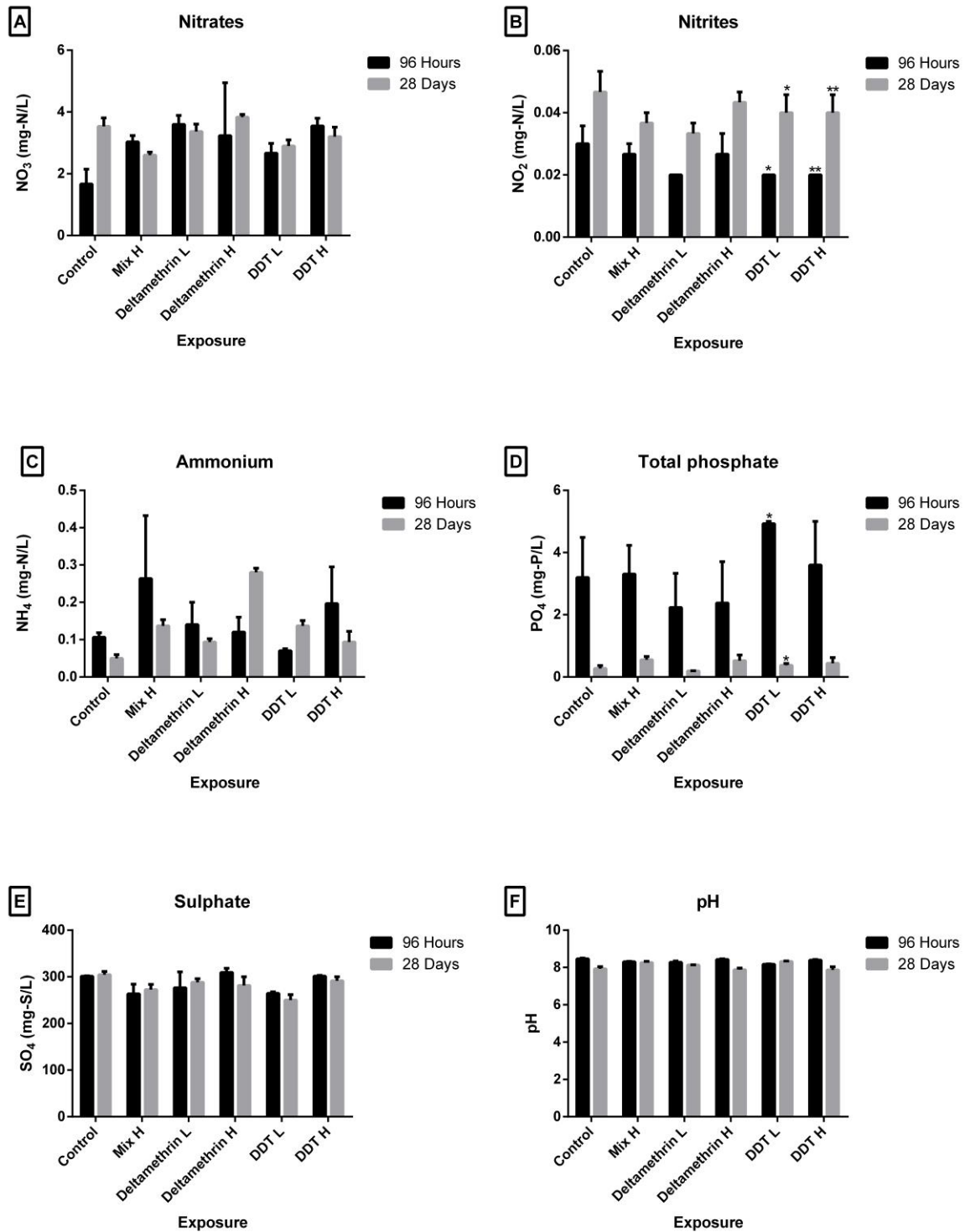


Figure 5.3: Mean and standard error of the mean (SEM) for (A) nitrate, (B) nitrite, (C) ammonium, (D) total phosphate, (E) sulphate and (F) pH for the control, Mix (DDT: Deltamethrin), Deltamethrin and DDT exposures after 96 hours and 28 day exposures. L – Low concentration, H – High concentration. Significant difference ($p < 0.05$) for each exposure is indicated with an asterisks (*).

5.3.3 Diatoms

During the exposures a total 34 diatom taxa were identified (Table 5.5). A complete species list of all identified diatom taxa and counts are presented in Appendix C.

Table 5.5: Complete list of diatom species present in the microcosms during all three (pre, 96 hour exposure and 28 day exposure) surveys.

<i>Achnantheidium</i> sp.	<i>Mastogloia</i> sp.
<i>Amphipleura pellucida</i> (Kützing) Kützing	<i>Navicula</i> sp. 1
<i>Amphora</i> sp.	<i>Navicula</i> sp. 2
<i>Caloneis</i> sp.	<i>Navicula veneta</i> Kützing
<i>Cocconeis pediculus</i> Ehrenberg	<i>Nitzschia linearis</i> (Agardh) W Smith
<i>Cocconeis placentula</i> Ehrenberg	<i>Nitzschia palea</i> (Kützing) W Smith
<i>Cymbella aspera</i> (Ehrenberg) H. Peragallo	<i>Nitzschia recta</i> Hantzsch
<i>Cymboppleura amphicephala</i> (Naegeli) Krammer	<i>Nitzschia sigma</i> (Kützing) W Smith
<i>Diatoma vulgaris</i> Bory	<i>Nitzschia</i> sp.
<i>Epithemia adnata</i> (Kützing) Brébisson	<i>Pinnularia</i> sp.
<i>Eunotia minor</i> (Kützing) Grunow	<i>Pleurosigma salinarum</i> Grunow
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	<i>Rhopalodia gibba</i> (Ehrenberg) O Müller
<i>Fragilaria capucina</i> Desmazières	<i>Rhopalodia musculus</i> (Kützing) O Müller
<i>Gomphonema acuminatum</i> Ehrenberg	<i>Stauroneis smithii</i> Grunow
<i>Gomphonema affine</i> Kützing	<i>Surirela</i> sp.
<i>Gomphonema</i> sp.	<i>Tryblionella apiculata</i> Gregory
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	<i>Tryblionella gracilis</i> W Smith

There was a significant ($p < 0.05$) decrease in total number of species between the Mix H, Deltamethrin L and DDT L compared to the control group after 96 hr exposure (Figure 5.4 A). There was a significant decrease in total number of individuals between the Deltamethrin H and control after 28 d (Figure 5.4 B). There were no significant differences for the Shannon diversity index (Figure 5.4 C) except for Mix H compared to the control after 96 hr. There were no significant ($p < 0.05$) differences for Pielou's evenness between controls and all exposures (Figure 5.4 D).

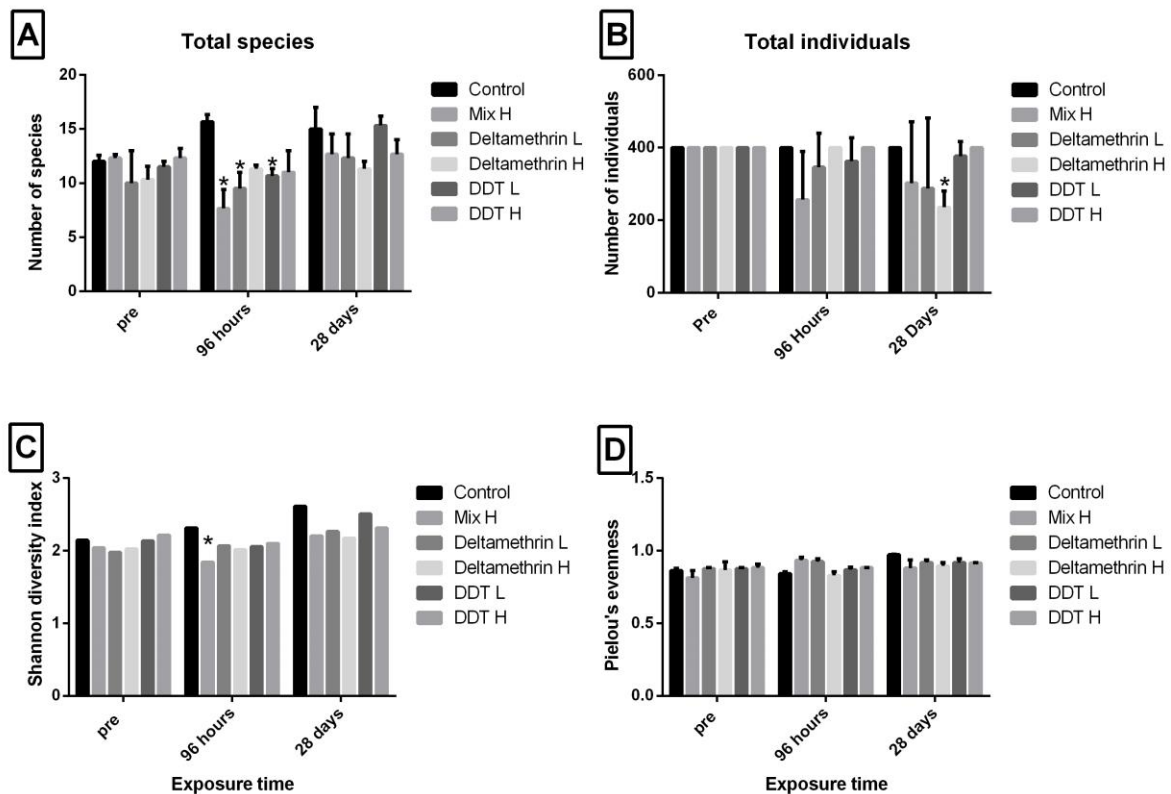


Figure 5.4: Mean and standard error of the mean (SEM) for (A) total number of species, (B) total number of individuals, (C) Shannon diversity index and (D) Pielou's evenness for each exposure after the different exposure times. Significant difference ($p < 0.05$) to the control of each exposure time is indicated with an asterisks (*).

The effects of the insecticides on the diatom taxa over time are presented as a Principle Response Curve (PRC). Species on the right of the PRC indicates each species affinity with the diagram (Figure 5.5). A decrease of species after exposure is associated with a positive weight (number), with an increase of species after exposure with a negative weight. *Achnantheidium* sp. had the highest species weight and thus decreased more in response to insecticides exposure compared to the other species. *Surirela* spp. was the only taxon that had a negative weight and thus increased after exposure. *Eunotia minor* and *Amphora* sp. had the 2nd and 3rd highest species weight and decreased after exposure to the insecticides respectively. Diatom taxa abundance decreased after an acute exposure to all exposures, except Deltamethrin H (Figure 5.5). After the initial (acute) exposure diatom taxa increased up to the 28 d exposure. DDT L had the smallest effect on the diatom taxa with DDT H the highest (Figure 5.5). The PRC results showed that the presence of the insecticides had no significant negative effects ($p = 0.296$) on the diatom community.

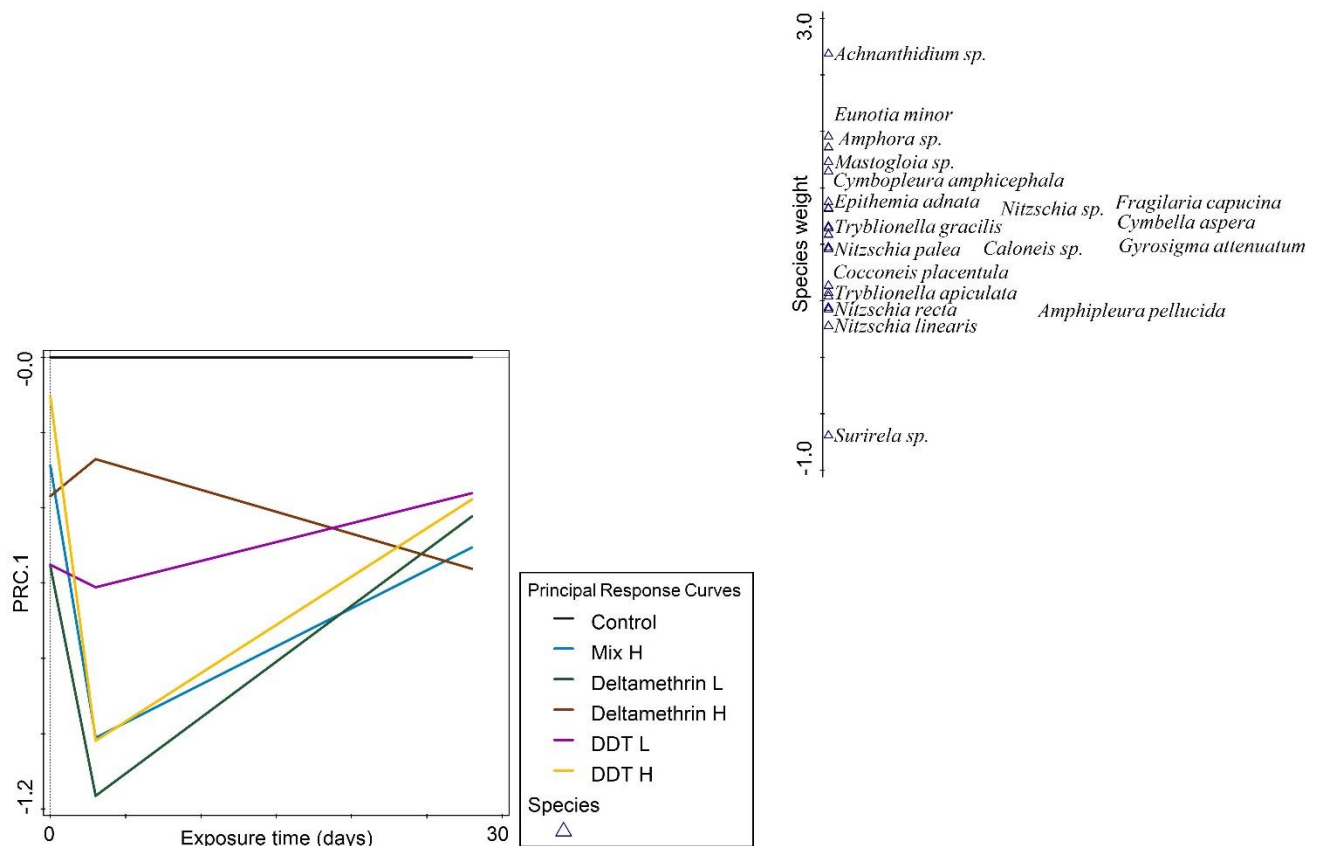


Figure 5.5: Principle response curve (PRC) indicating the effects of the insecticides on the diatom community. The first axis explained 24.1% of the variance.

Two-way ANOVAs based on Sidak's multiple comparison test were carried out to determine significant ($p < 0.05$) differences between insecticide exposures and the control after 96 hr and 28 d. The microcosms for both exposure times had increased percentage dead cells compared to the control (Figure 5.6). After 96 hr, all exposures had significantly ($p < 0.05$) higher percentage dead cells compared to the control. All exposures, except for Mix H and DDT L, had significantly ($p < 0.05$) higher percentage dead cells compared to the control following 28 d of exposure. No significant temporal differences were noted between 96 hr and 28 d exposure. Significant differences ($F = 13.97$, $p < 0.0001$) between insecticide concentrations were noted.

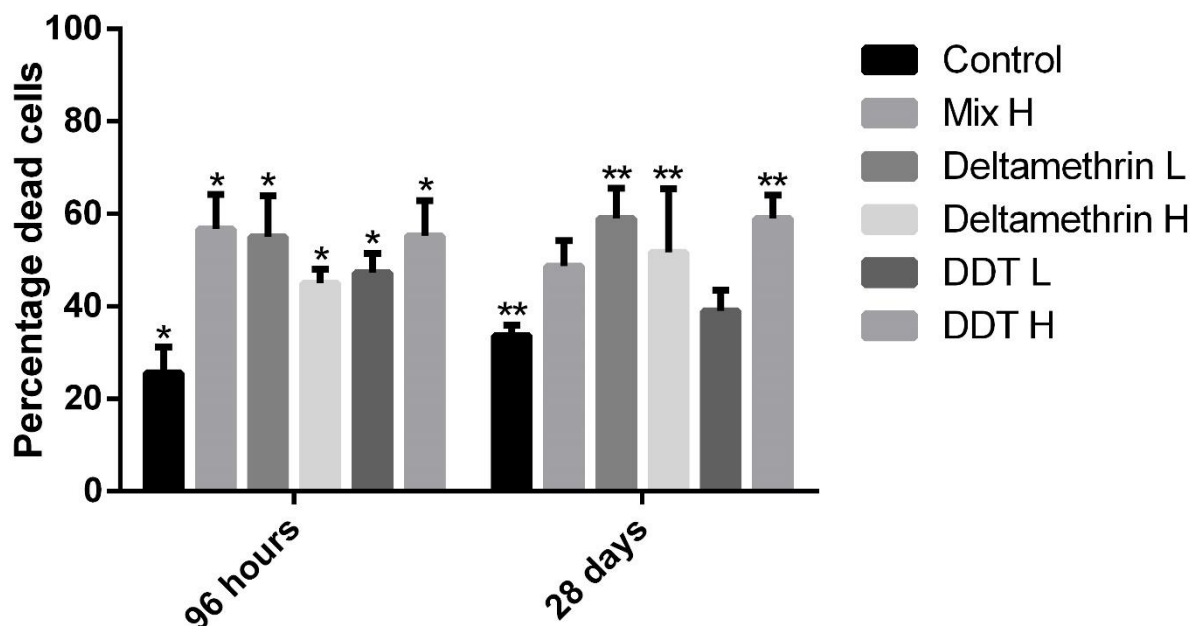


Figure 5.6: Percentage dead cells (mean $\pm$ SEM) for each microcosm after 96 hour, and 28 day exposures to DDT, Deltamethrin, and Mix (DDT:Deltamethrin). L – Low concentration, H – High concentration. Significant difference ($p < 0.05$) from the control is indicated within each respective exposure period with asterisks (*).

Significant ($p < 0.05$) differences for each diatom metric, based on a two-way ANOVA with Tukey's multiple comparisons tests were carried out to determine whether exposure time or type of insecticide exposure had a significant effect ($p < 0.05$) on diatom metrics (Table 5.6). Significant ($p < 0.05$) decreases in the control microcosm were noted for pendunculate and pioneer diatom metrics after 28 d with a significant increase in size class 4. Significant ($p < 0.05$) differences for each exposure were compared to their pre-exposure. After exposure to Mix H the benthic, pendunculate, pioneer (28 d), high profile guild and size 5 had significant ($p < 0.05$) decreases. The pioneer guild and size 5 had a significant ($p < 0.05$) decrease after 28 d exposure to Deltamethrin L. Significant ($p < 0.05$) decreases were noted for pendunculate (28 d), high profile, size 1 (28 d) and size 5 guilds after exposure to Deltamethrin H. Exposure to DDT L caused a significant ($p < 0.05$) increase in the motile guild after 96 hr exposure. Exposure to DDT H caused significant ($p < 0.05$) decreases in the pendunculate (96 hr), high profile and size 5 guild. Size class 4 increased significantly ($p < 0.05$) for all exposures except Deltamethrin H.

No significant differences were noted between insecticide concentrations for all diatom metrics, except for pendunculate ($F = 2.538$, $p = 0.0457$). Temporal differences between exposure periods were noted for benthic ($F = 7.688$, $p = 0.0017$), colonial ($F = 7.162$,

$p = 0.0024$), pendunculate ($F = 20.56$, $p < 0.0001$), pioneer ($F = 29.84$, $p < 0.0001$), high profile ($F = 12.97$, $p < 0.0001$), motile ($F = 3.457$, $p = 0.0423$), size class 1 ($F = 8.164$, $p = 0.0012$), size class 4 ($F = 29.48$, $p < 0.0001$) and size class 5 ($F = 6.849$, $p = 0.0030$) metrics.

Table 5.6: Differences in abundances for each exposure after 96 hours and 28 days. Two-way ANOVA with Tukey's multiple comparisons test were carried out to determine significance ($p < 0.05$) for each exposure time compared to the pre-exposure. L – Low concentration, H – High concentration, hr – hours, d – days.

	Control		Mix H		Deltamethrin L		Deltamethrin H		DDT L		DDT H	
	96 hr	28 d	96 hr	28 d	96 hr	28 d	96 hr	28 d	96 hr	28 d	96 hr	28 d
Benthic	ns	ns	↘	↘	ns	ns	ns	ns	ns	ns	ns	ns
Mobile	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Colonial	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pendunculate	ns	↘	↘	↘	ns	ns	ns	↘	ns	ns	↘	ns
Pioneer	ns	↘	ns	↘	ns	↘	ns	ns	ns	ns	ns	ns
High Profile	ns	ns	↘	ns	ns	ns	↘	↘	ns	ns	↘	ns
Low Profile	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Motile	ns	ns	ns	ns	ns	ns	ns	ns	↗	ns	ns	ns
Size 1	ns	ns	ns	ns	ns	ns	ns	↘	ns	ns	ns	ns
Size 2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Size 3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Size 4	↗	↗	↗	↗	↗	↗	ns	ns	↗	↗	↗	↗
Size 5	ns	ns	ns	↘	ns	↘	↘	↘	ns	ns	↘	↘

↗ = Significant increase; ↘ = significant decrease; ns = no significance

5.4 Discussion

Numerous studies have focused on the effects that herbicides, especially glyphosate and atrazine, have on freshwater diatom species (Pérez *et al.*, 2007; Vera *et al.*, 2012; Wood *et al.* 2014; Lozano *et al.*, 2018), with hardly any recent studies on the effect of insecticides on non-target freshwater diatoms. No studies were found in the literature on the effects of Deltamethrin on freshwater diatoms and to our knowledge the only study focussing on the effects of DDT on these organisms was done by Miyazaki & Thorsteinson (1972). Other studies on the effects of insecticides, mainly DDT, were on algae (Mosser *et al.*, 1972; Ebenezer & Ki, 2014), marine diatoms (Wurster, 1968; Keil & Priester, 1969; MacFarlane *et al.*, 1972; Mosser *et al.*, 1974; Ebenezer & Ki, 2014) and phytoplankton (Wurster, 1968; Wang & Wang, 2005).

5.4.1 Physico-chemical variables

All variables (except nitrite and total phosphate) observed in the microcosms remained constant throughout the study with no significant differences between exposure times. A considerable decrease in total phosphate was observed after 96 hr, with a significant decrease measured in the DDT L microcosms. This decrease can be due to the exchange of phosphate between water and sediment (DWAF, 1996; Dallas & Day, 2004) causing a decrease in phosphate suspended within the water column. The activities of the organisms within the microcosm, oxygen-dependent redox interaction, pH and the mineral-water equilibria also has an influence on the total phosphate concentration within the water (DWAF, 1996). Phosphate is accumulated by living organisms (Dallas & Day, 2004) and plays a role in the storage and use of energy within the cells as well as playing a major role in nucleic acids (DWAF, 1996). Phosphate is also used by plants that convert it into cell structures (DWAF, 1996; Dallas & Day, 2004). All the factors could cause the depletion of total phosphate concentration within the water column.

Compared to the South African water quality guidelines (DWAF, 1996) measured dissolved inorganic nitrogen (DIN) (nitrate, nitrite and ammonium) (Figure 5.3) values indicate all microcosms in a eutrophic ecological state after both exposure periods, except for the control which had a mesotrophic ecological state after 96 hr. Measured phosphate (Figure 5.3) concentrations indicated all microcosms in a hypertrophic ecological state after both exposure periods, except Deltamethrin L microcosms which had a eutrophic ecological state (DWAF, 1996; Dallas & Day, 2004).

Nitrogen and phosphate ratios (N:P) can indicate whether a system is impacted or unimpacted (DWAF, 1996; Dallas & Day, 2004). Impacted (eutrophic and hypertrophic) ecosystems typically have N:P ratios less than 10:1, whereas, unimpacted ecosystems have ratios greater than 25–40:1 (DWAF, 1996; Dallas & Day, 2004). Nitrogen and phosphate ratios (N:P) indicated all microcosms as hypertrophic after 96 hr exposure, with ratios ranging from 0.56:1–1.68:1 (DWAF, 1996). After 28 d exposure microcosms were eutrophic (except for Deltamethrin L which was mesotrophic) as indicated by the N:P ratios, with ratios ranging from 5.01:1–18.07:1 (DWAF, 1996).

5.4.2 Chemical analysis

From the results, DDT and its isomers were not detected within the water column but were detected within the sediment. This can be attributed to the low solubility of DDT in water causing it to partition directly from water into the sediment and adsorb to solid and organic particles (Mansouri *et al.*, 2017). DDT can accumulate in sediment-dwelling organisms within the environment (Mansouri *et al.*, 2017), such as the benthic diatoms (see below). The detection of DDD (1,1-dichloro-2,2-bis(p-chlorophenyl) ethane) and DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene) for some exposures can be due to diatoms being able to break DDT down to DDD and DDE (Johnson *et al.*, 1967; Keil & Priester, 1969). Mansouri *et al.* (2017) stated that some microorganisms have the ability to metabolise the metabolites DDE and DDD. Deltamethrin, however, has a short half-life and is broken down rapidly through photolysis as well as microbial degradation (Ensibi *et al.*, 2014; Zhang *et al.*, 2016; Karataş *et al.*, 2019). This together with its high evaporation rate within surface waters (Ensibi *et al.*, 2014; Karataş *et al.*, 2019) explains why Deltamethrin was not detected in the water or sediment of the microcosms.

5.4.3 Diatom vitality

From the data the insecticides had an initial acute negative effect on the diatom community whereafter the community abundance increased. This can be attributed to the diatoms asexual reproduction. As diatoms predominantly undergo asexual reproduction, where two daughter cells are formed through mitosis (Sabater, 2009), the diatom community theoretically doubles after 2–3 weeks due to their short life span. The diatom community has an initial negative response to the insecticides after which the community theoretically doubles in size (due to mitosis), thus causing an increase in the diatom community. However, the percentage dead cells indicate that the insecticides had a negative effect on the vitality of the diatoms with significantly higher percentage dead cells for both 96 hr and 28 d compared to the control.

This was found for both the insecticides' high and low concentrations as well as the mixture exposure.

As the community potentially doubles following asexual reproduction, they are still exposed to the insecticides within the aquatic environment. The insecticides had a negative effect on the diatom taxa that caused cell death. Both DDT (Lee *et al.*, 1976) and Deltamethrin (Bader & Schüller, 1996) inhibit the cell's electron transport reaction. DDT specifically does not inhibit non-cyclic photophosphorylation, whereas Deltamethrin does inhibit photosystem II (PSII) (Bader & Schüller, 1996). However, DDT does inhibit cyclic related phosphorylation at photosystem I (PSI) (Lee *et al.*, 1976). Moreover, DDT's inhibitory effect is associated with the lipid distribution in the cells due to its lipophilic nature that limits it to the lipid rich membranes (Bowes & Gee, 1971), whereas the hydrophilic nature of Deltamethrin does not restrict its distribution in the diatom. Deltamethrin's strong phototoxic inhibition of the photosynthetic electron transport reactions is owed to the two bromides on the halogen side of the molecule, and is considered the stronger inhibitor compared to other pyrethroids (Bader & Schüller, 1996). Photosystem I reactions form nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), which is necessary to fuel the reaction during PSII (Garrett & Grisham, 2008). Photosystem II (a specialized protein complex) is responsible for the production of oxygen through a reaction of electron transfer from water, driven by light energy (Garrett & Grisham, 2008). Inhibition of PSI by DDT therefore causes a stoppage in the production of NADPH to fuel the reaction during PSII, whereas inhibition of PSII by Deltamethrin results in a shutdown of photosynthesis within the cell.

The inhibition of these photosystems were most likely the cause for the significant increase in percentage dead cells compared to the control group. This result was consistent with other studies, e.g. Rimet & Bouchez (2011) who recorded a negative influence of atrazine on the photosynthetic activity of algae. Bérard *et al.* (2003) found that atrazine had the same inhibition action on PSII as Irgarol 1051 (a photosystem II inhibitor).

5.4.4 Diatom metrics

As mentioned previously, functional groups are useful indicators to determine stressors and environmental changes within the aquatic ecosystem (Lukács *et al.*, 2018). The taxonomical composition of biofilms is sometimes difficult to use in ecotoxicological-based studies as each species is physiologically influenced differently by pesticides (Rimet & Bouchez, 2011). Also, taxonomical identification to species level could be problematic. The identification of taxa may not be consistent among studies with taxa from the same genus confused due to small differences e.g. striae densities (Rimet & Bouchez, 2011). This may not be a problem for all

taxa as there are taxa that are morphologically close to one another and grouped together in taxonomical work (Rimet & Bouchez, 2011). However, the use of species compositions is not sufficient to define the tolerance of the community to stressors (Guasch *et al.*, 1998). The use of metrics, such as life-forms, ecological guilds and size classes are ideal as they occur more often than specific taxa (Rimet & Bouchez, 2011). For ecotoxicology studies, trends in metrics can easily be predicted with disruptions to these trends expected with the presence of insecticides (Rimet & Bouchez, 2011).

Rimet & Bouchez's (2011) study found that pesticides had a greater influence on diatom metrics than on species composition. The same results were observed in the present study as the Shannon diversity index and Pielou's evenness index (Figure 5.3 C, D) showed no significant differences over the exposure period. Significant differences were, however, observed between diatom metrics. According to Rimet & Bouchez (2011) the hypothesis for size classes is that smaller genus/species would be more vulnerable to pesticide contamination. Studies have found that metal contamination resulted in the reduction of cell size (Joux-Arab *et al.*, 2000; Cattaneo *et al.*, 2004). We observed a significant increase for size class 4 (including the control) and a significant decrease in size class 5. This trend was also observed by Rimet & Bouchez (2011) who studied the effect of pesticides contamination in rivers. In their study they found that the smaller size classes increased with a decrease in the larger cell sizes. They did, however, state that this trend was weak and that further experiments are necessary.

The pendunculate diatoms (including the control) were negatively affected in the microcosms. This was expected as this life-form has previously been reported as a good ecological indicator of water quality (Rimet & Bouchez, 2011). The abundance of pendunculate diatoms decreases in nutrient rich waters (Rimet *et al.*, 2009) as they can easily absorb dissolved nutrients in the water (Pringle, 1990). This trend was observed for the control microcosms as the pendunculate life-form decreased significantly after 28 d. It is therefore expected that the pendunculate diatoms would be affected by the insecticides in their environment as the diatoms would easily absorb the insecticides. The decrease of pendunculate diatoms in the control microcosms can be attributed to the high nutrient concentrations (section 5.4.1). However, this trend was also observed in the high concentration exposures and not in the low concentration exposures. This significant decrease in pendunculate diatoms for all three high concentration exposures can suggest that insecticides were absorbed and caused mortality.

It was expected that the pioneer diatoms would increase following exposure to insecticides. As more sensitive species are affected by the insecticide contamination they will disappear

and free up space for the pioneer diatoms to colonise the available substrate (Rimet & Bouchez, 2011). However, in this study a significant decrease was observed for pioneer diatoms (including the control) after exposure to Mix H and Deltamethrin L. The decrease of *Achnantheidium* (also pendunculate guild) and *Amphora*, the pioneer diatoms, can be attributed to the eutrophic ecological state (Figure 5.3 and section 5.4.1) of the microcosms during the exposure period, as both species prefer oligotrophic ecosystems (Taylor *et al.*, 2007). The benthic lifeform decreased significantly for the Mix H exposures. Pesticides are known to decrease these diatom life-forms (Lukács *et al.*, 2018). As these life-forms commonly occur on the surface of sediment they are easily affected by insecticides such as DDT and Deltamethrin that both bind to the sediment particles (Mansouri *et al.*, 2017; Placencia *et al.*, 2018).

The abundance of motile diatoms increased significantly during the DDT L exposure. This was expected for this life-form as it consists of pollution-tolerant taxa such as *Navicula* and *Nitzschia*. Also, as the environment changed from a lotic (river) to a lentic (microcosm) system, attached diatom species will give way to the motile species, as attached species are adapted to lotic ecosystems. This result was consistent with other diatom ecotoxicological studies done with atrazine (Guasch *et al.*, 1998; Dorigo *et al.*, 2004), isoproturon (Dorigo *et al.*, 2004) and diuron (Morin *et al.*, 2010). This metric increased for all exposures, however, a significant increase was only measured in the DDT L exposure.

The high profile guild (such as in the pendunculate diatoms) easily absorb dissolved nutrients within the water column (Passy, 2007). According to Rimet & Bouchez (2011) this can likely cause this guild to be more easily affected to insecticides within the ecosystem as they are more readily exposed to them. According to Viktória *et al.* (2017) the high profile guild is sensitive to any disturbances in their environment and will result in a decrease in the guild when exposed to insecticides. This trend was observed for all the high concentration exposures with a significant decrease for the high profile guild.

5.5 Conclusions

This study focussed on the effects of two insecticides (DDT, Deltamethrin and a Mix (DDT: Deltamethrin)) on diatoms using lentic microcosm experiments. Measured physico-chemical water quality variables remained consistent throughout the study with the exception of a considerable decrease in total phosphate for all microcosms. This decrease can be attributed to phosphate exchange between the water and sediment, uptake by algae, as well as oxygen-dependent redox interaction and/or the mineral-water equilibria influence. Measured nitrogen and phosphate values indicated all the microcosms as nutrient enriched and in a eutrophic ecological state following both exposure periods. Measured N:P ratios indicated that all microcosms had a eutrophic ecological state after both exposure periods. DDT and Deltamethrin were not detected within the water column of the microcosms. This can be due to the fact that they accumulated in and are broken down by diatoms or bind to the soil and organic particles. Only the isomers DDE and DDD were detected in the sediment of some microcosms. This can be the result of diatoms breaking down DDT to these isomers.

The diatom community was influenced by the studied insecticides, at both low and high concentrations, as shown in the diatom metrics and percentage dead cells. It was observed that some metrics were significantly influenced by only the high concentrations, and others only by the low concentrations. This was not uncommon as a study by Ebenezer & Ki (2014) found that the marine diatom, *Ditylum brightwellii*, was more sensitive to lower concentrations than high concentrations for the insecticide endosulfan. The percentage dead cells was significantly higher for all the exposures compared to the control. Thus, indicating that the insecticides negatively influenced the vitality of the diatoms. The insecticides break down and dissolve the chloroplast of the cells due to inhibition of the photosystem of the cells. From the results it is clear that diatoms are effective bio-indicators for insecticide ecotoxicology studies with these non-target species negatively influenced by both the low and high concentration exposures. It was originally hypothesized that since DDT and Deltamethrin are insecticides and do not target diatoms, they will not have an effect on the vitality of diatom community structures. The hypothesis is rejected as there was a significant acute and chronic decrease in the diatom vitality after exposure to both insecticides, as well as the mixture. Diatom vitality was equally affected by both the high and low concentration exposures to DDT, Deltamethrin and the mixture.

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Chapter 6: Determining the effects of DDT and Deltamethrin on the vitality of the diatom *Nitzschia palea* (Kützing) W. Smith using an *in situ* chlorophyll fluorescence assay.

6.1 Introduction

Aquatic ecosystems are affected by environmental contamination from agricultural and industrial activities through direct and indirect inputs (DeLorenzo *et al.*, 2001; Nancharaiah *et al.*, 2007). Pesticides are used worldwide, with the agriculture sector particularly dependant on them for plague and disease control (NPIC, 1999; Margni *et al.*, 2002) by killing, mitigating and repelling pests (Margni *et al.*, 2002). Even though pesticides are advantageous to use, they do have negative effects on the environment, human health, and wildlife, especially on non-target organisms (Wilson & Tisdell, 2001; Margni *et al.*, 2002). There is a long list of insecticides used worldwide for pest control (USEPA, 2018), as well as those that have been banned (Ritter *et al.*, 1995). This chapter will focus on two such insecticides, namely dichlorodiphenyltrichloroethane (DDT), a banned organochlorine insecticide; and Deltamethrin, a pyrethroid.

Dichlorodiphenyltrichloroethane and its metabolites persist in the environment for long periods of time (NPIC, 1999). Recent studies showed increased levels of DDT and their metabolites in freshwater fishes (van Dyk *et al.*, 2010; Pheiffer *et al.*, 2018; Volschenk *et al.*, 2019). The unregulated use of DDT and other organochlorine pesticides were banned in South Africa after the country became a signatory to the Stockholm Convention (Bouwman, 2004). Following its banning in 2002 there was an increase in malaria related deaths in South Africa (Ferriman, 2001). This trend was also seen in India (Gunasekaran *et al.*, 2005) and South America (Roberts *et al.*, 1997). Due to the increase in mortality the World Health Organization (WHO) supported the reintroduction of the insecticide in certain parts of South Africa since 2006 (van Dyk *et al.*, 2010; Ansara-Ross *et al.*, 2012).

Deltamethrin is one of the most widely used pyrethroids for agriculture (Kumar *et al.*, 2019). This insecticide is widely used due to its effectiveness at low concentrations, photostability, and low persistence (Abdelkhalek *et al.*, 2015). There are many studies that have evaluated the toxic effects of Deltamethrin to non-mammalian species (Toumi *et al.*, 2013; Abdelkhalek *et al.*, 2015; Kumar *et al.*, 2019). These studies found that Deltamethrin had a toxic effect on the freshwater Nile tilapia (*Oreochromis niloticus*) (Abdelkhalek *et al.*, 2015) and *Daphnia*

magna (Toumi *et al.*, 2013). These study's results indicated that Deltamethrin caused an alteration in serum biochemical parameters, caused lipid peroxidation, suppressed enzyme antioxidant activity, and that this insecticide decreased life history traits, thus acting on the reproduction of organisms (Toumi *et al.*, 2013; Abdelkhalek *et al.*, 2015). Despite its toxicity, it is still widely used for agricultural purposes with a marked increase of its use over the past 10–15 years (Abdelkhalek *et al.*, 2015). In KwaZulu-Natal (South Africa) both insecticides are sprayed as part of an indoor residual spraying program for malaria vector control, with DDT sprayed in traditional and Deltamethrin in western-type houses (Hargreaves *et al.*, 2003) with mixtures of these insecticides found within the environment.

Diatoms are microalgae that fulfil a very important role in aquatic ecosystems through nutrient cycling and utilisation (DeLorenzo *et al.*, 2001). As they are unicellular organisms present at the basis of the food chain, they are easily affected by environmental contamination. Diatoms are especially useful biological indicators of environmental stressors as they respond to many changes within their environment, they have a short lifespan, are present in all aquatic ecosystems, communities are mostly species rich and many taxa have a cosmopolitan distribution (Reid *et al.*, 1995; Stevenson *et al.*, 2010; Dalu & Froneman, 2016). Ecological risk assessments have long been using the effects that environmental contamination has on the growth inhibition of microalgae to determine the impact of these contaminants on the environment (Nancharaiah *et al.*, 2007).

A sensitive and rapid method to assess the toxicity of chemicals on diatoms is the measurement of their fluorescence. Due to the pigments of phototrophic organisms, autofluorescence analysis can be carried out (Neu & Lawrence, 1997). Earlier studies on plants have made use of chlorophyll fluorescent-based assays to test the effects of pollutants on plants (Schreiber *et al.*, 1978). One such method includes the use of confocal laser scanning microscopy (CLSM) (Norton *et al.*, 1998; Nancharaiah *et al.*, 2007; Fricke *et al.*, 2017). This method has the advantage of visually and quantitatively determining the *in situ* fluorescent properties of single celled organisms.

This chapter aims to undertake a laboratory bioassay to determine the effects that DDT and Deltamethrin have on the chloroplast of a diatom indicator species, *Nitzschia palea*. It is hypothesized that DDT and Deltamethrin will inhibit the photosystems of the diatom cells, negatively affecting their vitality as reflected by chlorophyll- α fluorescence.

6.2 Materials and methods

6.2.1 Diatom cultures and exposure

A monoculture of *Nitzschia palea* (Kützinger) W. Smith was obtained from the North-West University's Botany Department. Cultures were grown in a 50% GBG 11 culture medium (Krüger, 1978; Venter *et al.*, 2003). According to Taylor *et al.* (2007), *N. palea* is a cosmopolitan species that is found in heavily polluted and eutrophic water. The cultures were divided into 30 mL clearly labelled subsamples in falcon tubes and stored in a temperature (24 °C) and light (24 hr light on) controlled culture room. A single dose exposure was administered to each sample after two weeks. Samples were exposed to technical grade DDT (Dr, Ehrenstorfer GmbH), commercial grade DDT (AVI-DDT 750, Avima (Pty) Ltd), Deltamethrin (Dr, Ehrenstorfer GmbH), and a mixture (1:1) of commercial DDT and Deltamethrin, each at a high and low concentration. Technical and commercial grade DDT was selected to determine if commercial formulation has the same effect on diatom vitality compared to technical grade due to differences in the active ingredient. Exposures of DDT and Deltamethrin were done at 1% and 0.1%. Concentrations for DDT were 358 µg/L (high) and 35.8 µg/L (low), with Deltamethrin concentrations 1.9 µg/L (high) and 0.19 µg/L (low). An unexposed control and exposed samples were also treated with 3 µg rhodamine-123 dye (0.1 N) to ensure fluorescence of the cell wall (frustule) under a confocal microscope (Kucki & Fuhrmann-Lieker, 2011). The dye ensures that the frustule fluorescence is a contrasting colour to the red of the chloroplast. Rhodamine-123 was used as it does not affect the functionality or vitality of diatoms (Kucki & Fuhrmann-Lieker, 2011). Fifty percent of the culture growth media was replaced weekly after all measurements were done. The samples were exposed to the insecticides for 96 hr, 14 d and 28 d in order for diatoms to complete at least one life cycle.

6.2.2 Chlorophyll- α analysis

To determine the vitality of *N. palea*, the chlorophyll- α concentration was measured and calculated according to the method described in Swanepoel *et al.* (2008). Three mL of sample was filtered using Whatman's glass filters. The filters were added to 10 mL of ethanol (95%) and incubated in a water bath (78 °C) for 5 mins. Samples were allowed to cool to room temperature (in the dark), transferred to cleaned cuvettes and absorbance was determined in triplicate at wavelengths of 665 nm and 750 nm. Three drops of hydrochloric acid (HCl) (0.3 M/L) were added to each sample and read again at the same wavelengths. The total chlorophyll- α was calculated according to the following equation:

$$\text{Chlorophyll-}\alpha \text{ (}\mu\text{g/L)} = \frac{[(A_{665}-A_{750})-(A_{665a}-A_{750a})] \times 28.66 \times V_e}{V_m}$$

Where: A_{665} is the absorbance at 665 nm, A_{750} is the absorbance at 750 nm, A_{665a} is the absorbance at 665 nm after acidification, and A_{750a} is the absorbance at 750 nm after acidification; 28.66 is the ethanol absorption coefficient; V_e is the volume of ethanol (mL), and V_m the volume of sample filtered (mL).

The percentage live cells were calculated relative to the control for each exposure.

$$\text{Percentage live cells (\%)} = \frac{C_e}{C_c} \times 100$$

Where: C_e is the Chlorophyll- α concentration of the sample, and C_c is the Chlorophyll- α concentration of the control.

6.2.3 Confocal laser scanning microscope and image analysis

Samples were centrifuged and washed with distilled water to ensure that there was no dye in the culture medium that could cause background interference while viewing the samples (Kucki & Fuhrmann-Lieker, 2011). Samples were imaged using a Nikon D-Eclipse C1 CLSM with an x60 1.4 NA ApoPlanar oil objective. The microscope was equipped with red Helium/Neon (505 nm/565 nm) and green Krypton (488 nm/515 nm) Spectra-physics lasers. The lasers were used to excite and detect chloroplasts (red) as well as the frustules with the absorbed rhodamine-123 dye (green). A medium pinhole and 3 μs /scan scan speed were used. All CLSM images were acquired using identical settings on Nikon EZ 200 software. Fluorescence intensities of specimens acquired with the CLSM were quantified using ImageJ (Schneider *et al.*, 2012). Mean corrected total cell fluorescence (CTCF) analysis was done according to Gavet and Pines (2010).

6.2.4 Identification of deformities

Permanent slides of the 28 d exposure samples were prepared post-CLSM analysis. This was done in order to determine if any frustule deformities had occurred due to insecticide exposure. Slide preparation was done according to Taylor *et al.* (2005a) using the hot potassium permanganate (KMnO_4) and HCl method as described in Chapter 2 (section 2.2.3).

Prepared slides were viewed under a Nikon 80i compound microscope with an x100 1.4 N.A. oil immersion objective. A total of 100 frustules were counted and the percentage of deformed frustules calculated. Cell deformities were based on morphological characteristics, including cell shape and symmetry.

6.2.5 Sample extraction and chemical analysis

Chemical analysis was performed on the DDT and Deltamethrin stock solutions. Sample extraction and chemical analysis were done according to Yohannes *et al.* (2013) and South *et al.* (2019).

6.2.5.1 Stock solution

Extraction of OCPs and Deltamethrin from stock solutions were done as described in Chapter 5 (section 5.2.4.1).

6.2.5.2 Gas chromatography analysis

Analysis of stock solutions was carried out using gas-chromatograph (GC) as described in Chapter 5 (section 5.2.4.3).

6.2.5.3 Quality control and quality assurance

All stock solutions for the exposures were chemically confirmed. Quality control and assurance were determined as in Chapter 5 (section 5.2.5).

6.2.6 Statistical analysis

To determine significant differences ($p < 0.05$) between groups and exposures, a two-way ANOVA with Dunnett's multiple comparisons *post hoc* test was done. Significant differences ($p < 0.05$) between percentage live cells for each exposure were determined by means of a two-way ANOVA with Tukey's multiple comparisons *post hoc* test. All statistical analyses and graphs were produced using GraphPad Prism version 6.

6.3 Results

In all the results, DDT commercial grade will be reported as DDT C, and the technical grade as DDT T. High and low concentrations for the various exposures are represented by the letters "H" and "L" respectively. DDT stock solutions were chemically confirmed with Deltamethrin stock solution below the detection limit.

6.3.1 Diatom viability (chlorophyll- α)

A common trend between all groups, except Deltamethrin L and Mix L, was observed with significant increases ($p < 0.05$) in the percentage live cells from 96 hr to 14 d, thereafter there was a decrease after 14 d exposure up to the 28 d exposure time (Figure 6.1). Deltamethrin

L and Mix L showed a decrease in percentage live cells after each exposure time, with a significant ($p < 0.05$) decrease between 96 hr and 28 d (Figure 6.1).

Significant differences were observed between insecticide concentrations ($F = 47.22$, $p < 0.0001$) and between exposure periods ($F = 10.41$, $p = 0.0260$). A significant interaction between the insecticide concentration and exposure period was observed ($F = 11.15$, $p < 0.0001$).

6.3.2 Confocal images

A minimum of 15 confocal images ($n = 15$) were taken for each exposure over the experimental time period. Green fluorescence shows the diatom frustule and red fluorescence the chloroplasts. For the control, consistent healthy cells were present with the frustule clearly visible, as well as the two distinct regions containing the chloroplasts (Figure 6.2 A). In the exposure groups changes were noted with either burst chloroplasts (Figure 6.2 B), the absence of intact frustules (Figure 6.2 B), the absence of chloroplasts (Figure 6.2 C) or deformed or shrunken chloroplasts (Figure 6.3 - DDT C H, 14 d). There was however, no clear trend on how *N. palea* reacted to the different insecticides over time. Within the same exposure and time frame there were diatoms showing several of the above mentioned reactions to the insecticide.

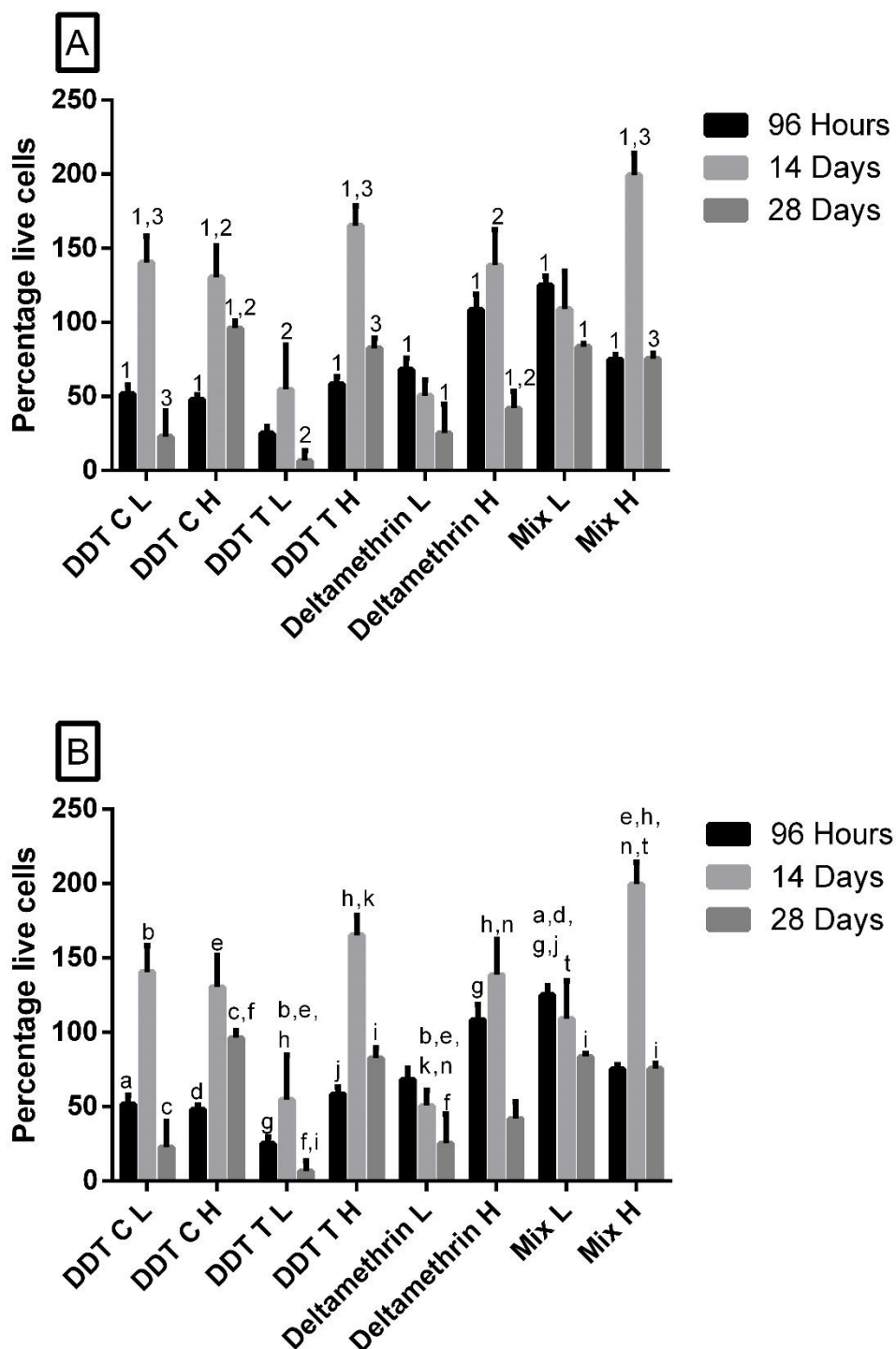


Figure 6.1: Percentage live cells (mean $\pm$ SEM) of *Nitzschia palea* after 96 hr, 14 d, and 28 d exposures to DDT, Deltamethrin, and Mix (DDT: Deltamethrin). (A) Means of columns representing different insecticide exposure groups with common numerals indicating significant differences for exposure period. (B) Means of columns between exposure groups with common letters indicating significant differences between insecticides exposure groups. C – Commercial grade, T – Technical grade, L – Low concentration, H – High concentration.

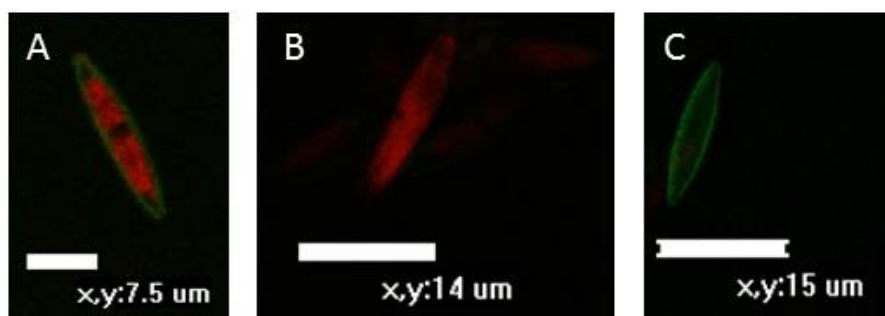


Figure 6.2: Confocal laser scanning microscopy images showing *Nitzschia palea* as a (A) healthy cell, and (B) reactions after exposure to insecticides; frustule dispersion and a burst chloroplast and (C) a cell with no chloroplast present.

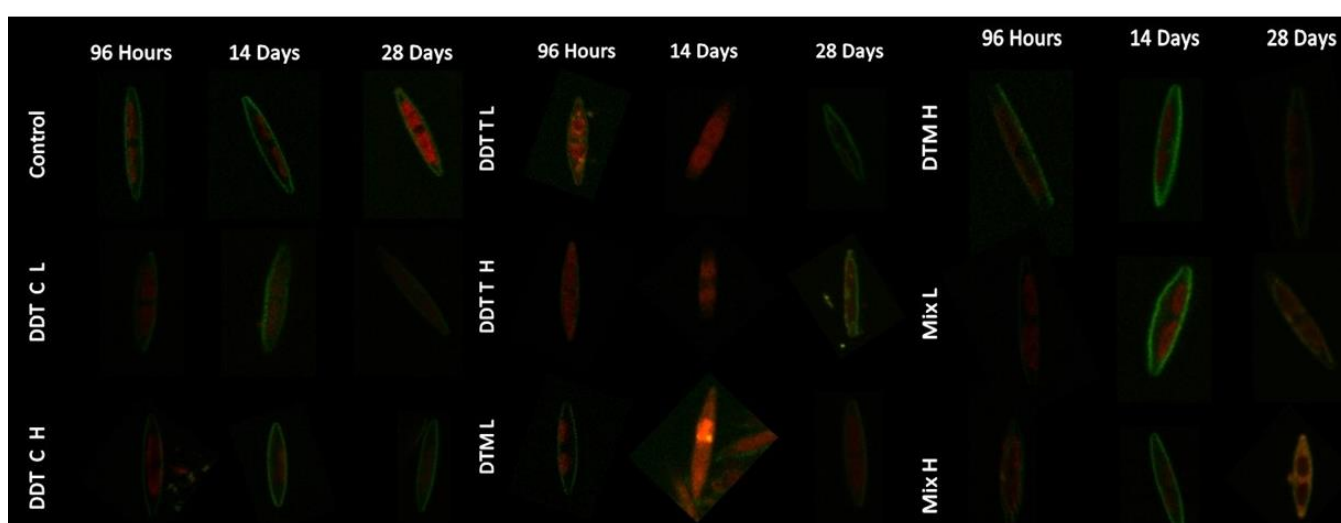


Figure 6.3: Confocal laser scanning microscopy images of the *Nitzschia palea* diatoms exposed to the different insecticides over a time period of 96 hr, 14 d and 28 d. C – Commercial grade, T – Technical grade, L – Low concentration, H – High concentration.

Corrected total cell fluorescence (CTCF) showed that diatoms exposed to the insecticides had a lower fluorescent cell intensity compared to the control over all exposure times (96 hr, 14 d and 28 d) (Figure 6.4). There were significant differences ($p < 0.05$) between all the exposed groups and the control over 96 hr and 14 d (Figure 6.4). Deltamethrin L had the highest intensity compared to the other exposures for both the 96 hr and 14 d exposure, with the exception of DDT T L at 96 hr. The DDT exposures had the lowest intensities from 96 hr to 14 d, except for DDT T L (Figure 6.4). Significant differences were observed between insecticide concentrations ($F = 23.76$, $p < 0.0001$) and between exposure periods ($F = 24.38$, $p < 0.0001$). A significant interaction between the insecticide concentration and exposure period was observed ($F = 6.988$, $p < 0.0001$). It should be noted that there was a significant decrease in the control between 14 d and 28 d.

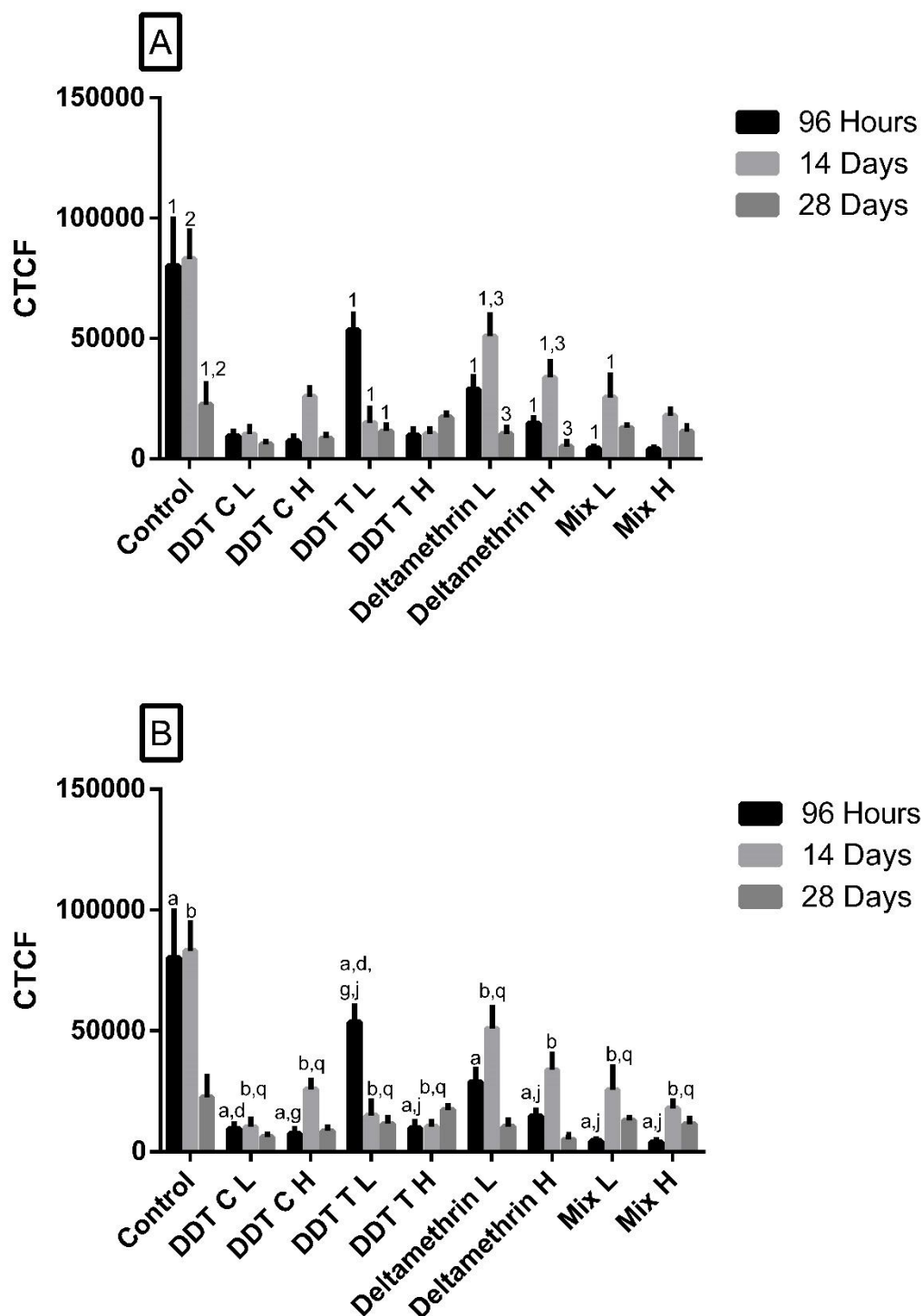


Figure 6.4: Corrected Total Cell Fluorescence (CTCF) of diatom cells for each exposure over the time period of the experiment. (A) Means of columns representing different insecticide exposure groups with common numerals indicating significant differences for exposure period. (B) Means of columns between exposure groups with common letters indicating significant differences between insecticides exposure groups. C – Commercial grade, T – Technical grade, L – Low concentration, H – High concentration.

6.3.4 Diatom deformities

Only the control had no deformed cells (Table 6.1). High concentrations of DDT C and the Mix H had the highest number of cell deformities with a total of 4% deformed cells.

Table 6.1: Percentage deformed diatom frustules from the exposure to the different insecticides following a 28 d exposure.

Group	Control	DDT C L	DDT C H	DDT T L	DDT T H	Deltamethrin L	Deltamethrin H	Mix L	Mix H
Deformed frustules (%)	0	3	4	3	3	1	2	3	4

C – Commercial grade, T – Technical grade, H – High concentration, L – Low concentration.

6.4 Discussion

Insecticide uptake by diatoms takes place through different pathways. Diatom cells are able to absorb and accumulate lipophilic DDT (Wurster, 1968; MacFarlane *et al.*, 1972; Mosser *et al.*, 1972). DDT binds to the cell's lipid-protein membranes by attaching to the photosynthetic mechanisms of the membranes (MacFarlane *et al.*, 1972). Deltamethrin on the other hand is highly water soluble and it seems that the diatoms take up this insecticide through osmotic processes (Fan & Reinfelder, 2003; Wang & Wang, 2005). Baeza-Squiban *et al.* (1987) stated that Deltamethrin can be accumulated in cells and also showed that pyrethroids are toxic to the marine diatom *Skeletonema costatum*. Studies on the effects of insecticides, mainly DDT, have been done on phytoplankton (Wurster, 1968; Wang & Wang, 2005), algae (Mosser *et al.*, 1972; Ebenezer & Ki, 2014) and marine diatoms (Wurster, 1968; Keil & Priester, 1969; MacFarlane *et al.*, 1972; Mosser *et al.*, 1974; Ebenezer & Ki, 2014) and the only study on freshwater diatoms (*Nitzschia* sp. and an unidentified diatom species) by Miyazaki and Thorsteinson (1972). No studies, have been published on the effects of Deltamethrin to diatoms.

The increased cell viability between 96 hr and 14 d exposure can be attributed to the asexual reproduction of diatoms as the *N. palea* population theoretically doubles after 2 to 3 weeks, as described for the diatom community in the previous chapter (Chapter 5, section 5.4.3). With continued exposure to the insecticides after 14 d, the percentage live cells decreases. This decrease represents the negative response of the diatom culture to the insecticides (Lozano *et al.*, 2018). This trend was observed for all the exposures except for Deltamethrin L and Mix L concentrations that showed a steady decrease in cell viability over the exposure period. Pyrethroids are known to be toxic to other aquatic fauna such as fish (Caliskan, 2017; Cengiz

et al., 2017) and invertebrates (Horton *et al.*, 2018; Lidova *et al.*, 2019). Thus the initial toxic effect on the diatoms is of such a nature that the remaining cells were not able to multiply. The population therefore cannot recover and the continuous decrease in viable cells ultimately results in the culture crashing. According to Lozano *et al.* (2018), different periphyton and phytoplankton populations have different reactions to toxicants due to the significant role that biological interactions play within the community as a whole. It was previously shown that different concentrations of the same toxicant can have different effects on the same community. Ebenezer & Ki (2014) found that a marine diatom (*Ditylum brightwellii*) was more sensitive to lower concentrations of the insecticide endosulfan than higher concentrations.

Insecticides affected the chloroplasts of the diatoms and hence their viability and survival. This was due to a decrease in the accumulation of chlorophyll as a result of either degrading or inhibiting chlorophyll biosynthesis (Smedbol *et al.*, 2018). Smedbol *et al.* (2018) stated that a reduction in chlorophyll- α could influence important physiological processes, photosynthesis, as well as cause cell growth reduction. It is evident that these insecticides degrade the chloroplast and the chlorophyll- α is dissolved within the cell and only the cell wall (frustule) remains.

Confocal microscopy was shown to be a feasible method to test the viability of the membrane potential of diatoms (Taylor *et al.*, 2005b). However, very few studies have used this technique to study the influence of insecticides on diatoms. In the present study there was a significant negative effect of exposure concentration and type of insecticide on diatom viability. An interaction between exposure time and insecticide type decreased the cell fluorescence intensities. This decrease in intensity could be due to damage of the chloroplast structure or a reduction in or loss of chloroplasts. The damage to the chloroplast causes a reduction in the photosynthetic output of the cells. The uptake of these insecticides and the effect it has on the diatom community's photosynthetic output are explained in more detail in Chapter 5 (section 5.4.3), however, in short: The uptake of Deltamethrin can be a process of diffusion into the cell (Fan & Reinfelder, 2003; Wang & Wang, 2005) as this insecticide is highly water soluble. DDT on the other hand binds to the cell's lipid-protein membranes and accumulated within the diatom cells (MacFarlane *et al.*, 1972). Exposure to these insecticides damage the chloroplast of the cells due to inhibition of the cells photosynthetic process. The electron transport reaction during photosynthesis is inhibited by both DDT (Lee *et al.*, 1976) and Deltamethrin (Bader & Schöler, 1996). DDT specifically inhibits photosystem I (PSI) and Deltamethrin photosystem II (PSII) of the photosynthesis process (Lee *et al.*, 1976; Bader & Schöler, 1996). Inhibition of PSI will inhibit nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) that fuels the electron transport reaction during PSII (Garrett & Grisham, 2008). While the shutdown of

photosynthesis will result due to inhibition to PSII by Deltamethrin (Garrett & Grisham, 2008). This is seen in the results as there is an immediate decrease in percentage live cells (Figure 6.1) for Deltamethrin L with a delayed effect for DDT exposures.

The effects of DDT and Deltamethrin can thus be ascribed to the reduction in photosynthetic efficiency of the chloroplasts due to the inhibition of PSI and PSII, respectively. This can lead to a reduction in carbon fixation, which results in a reduction in the amount of chlorophyll- α (MacFarlane *et al.*, 1972). MacFarlane *et al.* (1972) also found that DDT reduced and changed the shape of the chloroplast in the diatom cells. Fidalgo *et al.* (1993) also reported that there was a reduction in chloroplast volume after exposure to Deltamethrin. The same was observed in our study for both DDT, Deltamethrin and the insecticide mixture (Figure 6.3). The process of electron transport reaction and carbon fixation was most likely blocked due to the inhibition of the photosystems that results in an interruption of energy production needed for cell growth (UCIPM, 2019). Lipid and protein membrane destruction may be initiated through a chain of reactions caused by highly reactive molecules formed due to inhibition of PSII (UCIPM, 2019). This destruction results in membrane leakage causing rapid disintegration and drying of the cell and cell organelles (UCIPM, 2019). This is visible in the confocal images where either the chloroplast was reduced in size, leaked (burst) inside the frustule and the absence of an intact frustule.

In contrast to the control sample, morphological (shape and symmetry) cell deformities were observed for all the exposures. *Nitzschia palea* diatom cultures used in the present study, is regarded as a “tolerant” species that can withstand extreme conditions, including heavily polluted waters, extreme nutrient loading and temperature variation (Taylor *et al.*, 2007; Sabater, 2009). Thus, the relatively low percentage of deformed cells for *N. palea* (Table 6.1) can be considered as meaningful and demonstrates that the insecticides disturbed cell wall synthesis, thus changing the morphology of cells in the exposed cultures. MacFarlane *et al.* (1972) found similar morphological changes in *Nitzschia delicatissima*. The significance of low percentage deformities was demonstrated by Lavoie *et al.* (2017) where as little as 0.5–2% deformed cells were reported from highly metal polluted sites as well as contaminated (organic contamination, pesticides and increased pH) sites.

6.5 Conclusion

This chapter examined the effects of two insecticides on diatom cultures to determine potential impact of these substances on non-target aquatic organisms. All exposures proved to decrease cell function and vitality via disruption of the photosynthetic process. The insecticides, DDT and Deltamethrin, reduced chlorophyll- α concentrations, distorted and dissolved chloroplasts, as well as resulting in deformed frustules. These changes had an impact on the vitality of the diatoms by most likely restricting asexual reproduction. The results showed that an interaction between the exposure period and insecticide type significantly influenced the diatom vitality at both exposure concentrations. Both insecticides had the same effect on the diatom vitality through different mechanisms as DDT effected PSI and Deltamethrin the PSII photosynthetic processes. From the results obtained it is evident that diatoms are useful for ecotoxicological studies with the effects of both insecticides observed for low and high concentration exposures. The hypothesis that DDT and Deltamethrin will inhibit the photosystems of the diatom cells, negatively affecting their vitality as reflected by chlorophyll- α fluorescence is accepted.

6.6 References

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Chapter 7: Conclusions and recommendations

7.1 Conclusions

The lower Phongolo River floodplain is one of the most diverse and unique floodplains in South Africa and provides numerous socio-economic services to the communities living within the floodplain area (Dube *et al.*, 2015; Smit *et al.*, 2016). Local communities have extensively utilized the Phongolo River floodplain for subsistence farming and used water from the river for irrigation purposes. The main aim for the construction of the Pongolapoort Dam was to provide irrigation water for sugarcane and cotton plantations (Champion & Downs, 2017; Brown *et al.*, 2018). In order to maintain ecosystem services within the floodplain, timed flow releases from the dam were implemented. However, due to a severe drought in the region in recent years (Baudoin *et al.*, 2017; Jozini Local Municipality IDP 2017/18-2021/22, 2017) there have been no flood releases since December 2014. As a consequence the only flow released from the dam was a continuous base flow ($4\text{--}8.50\text{ m}^3\text{s}^{-1}$) resulting in extremely low flows in the Phongolo River. Additional environmental stressors in the region are agricultural activities and indoor residual DDT spraying for malaria vector control (in and around the Ndumo Game Reserve), which resulted in fertilizers and DDT leaking into the floodplain area (Coetzee *et al.*, 2015; Dube *et al.*, 2015; Volschenk *et al.*, 2019). These chemicals, DDT and fertilisers, pose a threat to the humans and aquatic organisms within the floodplain.

Recent studies have focussed on the stress that these factors cause on the environment within the floodplain (Dube *et al.*, 2015; Netherlands *et al.*, 2015; Wolmarans *et al.*, 2018; Volschenk *et al.*, 2019). However, no work has been published on the diatom community from the lower Phongolo River floodplain and the influence that a changing environment has on their community structures. It was, therefore, important to determine the influence that environmental change and insecticides have on the spatial and temporal distribution and vitality of diatom communities in the Phongolo River and associated floodplain pans. This PhD study aimed to answer these questions and concluding remarks will be presented in the following sections.

7.1.1 Hypothesis 1: Variations in flow and physico-chemical water quality will have an effect on the structuring of the diatom communities in the Phongolo and Usuthu rivers and their associated floodplain pans.

The diatom community structure of the Phongolo River differs depending on the presence or absence of flood release events. The physico-chemical water variables change with every

flood release resulting in higher turbidity and changes in nutrient concentrations. Diatom species composition consistently changes in response to the changing environmental conditions. As the physico-chemical water chemistry (*in situ* and nutrient parameters) changes, species that are adapted to these changes are present with species that are not adapted to these new unfavourable conditions disappearing (Ramanibai & Jeyanthi, 2010; Stevenson *et al.*, 2010). Taxa that tightly adhere to the substratum are present during the flood release events as they can endure the increased flow rate. In contrast, the river is a more stable environment (in terms of flow and physico-chemical water chemistry) during the absence of flood release events allowing for more loosely attached and motile species to become established. Species composition remains consistent during the absence of flood release events as they do not have to adapt to ever-changing environments.

Temporal changes in the diatom community structure were found for the Usuthu River, but not for the Phongolo River over the six sampling surveys from February 2017 to May 2018. The Phongolo River experienced extremely low flows due to a severe ongoing drought in the region (Jozini Local Municipality IDP 2017/18-2021/22, 2017) with only a base flow ($4\text{--}8.5\text{ m}^3\text{s}^{-1}$) released from the Pongolapoort Dam. During the present study period the Phongolo River was thus a more stable (constant flow and non-flooding) ecosystem. This was supported by results from the present study that showed that the physico-chemical water variables did not differ significantly between seasons. The Phongolo River had higher gamma diatom diversity and beta diversity as there were fewer taxa dispersal compared to the Usuthu River. Temporal variation in the diatom community for the Usuthu River was most likely caused by various environmental factors over the study period. Main drivers influencing the diatom community structure during the summer high flow (February and May) were nutrients. Conductivity, temperature, total phosphate and pH was the main drivers affecting the diatom community structure during the low flow (September and November) surveys. Intermittent flooding and flow variations cause taxa dispersal and varying environmental variables (Sokal *et al.*, 2010), as was seen in the present study.

Shokwe Pan is situated in the Usuthu River floodplain and receives water during natural flooding of the Usuthu River (Whittington *et al.*, 2013). This natural flooding of the Usuthu River and high nutrient concentrations (during high flow) of the river resulted in increased nutrients in Shokwe Pan. Species identified in the pan, include species that occur in nutrient-enriched ecosystems (*Nitzschia* sp., *Encyonema* sp., *Fragilaria* sp., *E. silesiacum* and *C. meneghiniana*) (Taylor *et al.*, 2007), with nutrients the main driver determining the diatom community of Shokwe Pan. The physico-chemical water variables of the ephemeral pans (Fence and Butterfly pans) remained relatively consistent during the study period with nutrients

the main drivers of their diatom community structures. Nutrients enter these ecosystems through localised runoff from the catchment. Nyamithi and Paradise pans' diatom communities were mainly influenced by electrical conductivity. Nyamithi Pan is a natural saline lake, with saline groundwater seeping into the lake as it is situated on top of marine cretaceous deposits (Kyle, 1996). As Paradise Pan receives water from Nyamithi Pan when it overflows, it can be expected that the pan had increased conductivity similarly to Nyamithi Pan.

The data highlights the importance of implementing management plans for the floodplain area, as well as the importance of flood releases from the Pongolapoort Dam. Proper monitoring plans for the Lower Phongolo River floodplain should be put in place. Regular monitoring of the floodplain must be done in order to ensure ecosystem functions and socio needs are met.

The hypothesis that variations in flow and physico-chemical water quality will have an effect on the structuring of the diatom communities in the Phongolo and Usuthu rivers and their associated floodplain pans, is therefore supported.

7.1.2 Hypothesis 2: Due to differences in connectivity of the rivers and their associated floodplain pans there will be spatial and temporal differences in the stable isotope signatures and diatom communities between the Phongolo River and associated floodplain pan but not between the Usuthu River and associated floodplain pan.

Flooding of the Usuthu River during the summer rainfall season (February 2017) had an effect on the physico-chemical water quality and diatom community structure of the lower reaches of the Phongolo River and Nyamithi Pan. The main drivers affecting the diatom community structure of the three sites were temperature, electrical conductivity and sulphates. Similar driving forces and stable isotope signatures (between Usuthu River and lower reaches of the Phongolo River) for these sites indicates the influence of the Usuthu River on the lower reaches of the Phongolo River and Nyamithi Pan. The mixing of floodwaters with the water in the floodplain pans had an effect on the physico-chemical water quality and community structure of floodplain pans. Flooding of the Usuthu River had an effect on the nutrient concentrations of Shokwe Pan. Nutrients enter the pan during flooding as runoff from the catchment area. As the Phongolo River experienced extremely low flows, it did not have an influence on the floodplain pans.

Assessment of the stable isotope signatures within the lower Phongolo River floodplain revealed decreased $\delta^{13}\text{C}$ and increased $\delta^{15}\text{N}$ between the summer rainfall and late summer

rainfall (May 2017) season. Nitrogen runoff (from agriculture activities within the catchment) into the river was the most likely cause for the increased $\delta^{15}\text{N}$ in the river. A decrease in $\delta^{13}\text{C}$ within the river can be caused by increased feeding pressure of macroinvertebrates and fish on the periphyton. Periphyton is the major source of carbon for higher trophic organisms in aquatic ecosystems (Casatti *et al.*, 2003; Clapcott & Bunn, 2003). During the late summer rainfall season competition for food increases and can result in increased feeding pressure. The $\delta^{13}\text{C}$ values for the Usuthu River, Nyamithi Pan and Shokwe Pan remained relatively consistent between seasons with a decrease in $\delta^{15}\text{N}$ values for both floodplain pans. As the area experienced little to no rainfall during the late summer rainfall season, nitrogen runoff into the wetlands was limited. Desiccation and a decrease of water influx decreased the available nitrogen within floodplain pan ecosystems (Baldwin & Mitchell, 2000; Douglas *et al.*, 2005).

The hypothesis that states that due to differences in connectivity of the rivers and their associated floodplain pans there will be spatial and temporal differences in the stable isotope signatures and diatom communities between the Phongolo River and associated floodplain pan but not between the Usuthu River and associated floodplain pan, is therefore rejected.

7.1.3 Hypothesis 3: Diatom community structures will reflect paleo-ecological conditions in Nyamithi Pan.

Core samples were retrieved from Nyamithi Pan to assess the historical paleo-limnological diatom community structure and to determine how the area had responded to a changing environment. Diatom cells were enumerated from a core sampled from the lake bed of Nyamithi Pan. The core (36 cm) was divided into one cm slices, and the diatom communities identified in each slice as well as radiocarbon dating done on selected slices.

A total of 58 taxa were identified in the core with the deepest slice dated at 933 AD. The abundance of species decreased downward into the core with no species identified at various depths > 20 cm. As increased pH, temperature and salinity negatively affect the preservation of diatoms, it can be an indication that the wetland experienced increased concentrations of these physico-chemical water variables during this time (Bennion *et al.*, 2010; Fritz *et al.*, 2010). Based on the environmental preferences of the species present in the more recent section of the core (i.e. past 300 years) there appears to have been a decrease in salinity and nutrient concentrations in the pan. This is supported by a decrease in the relative abundance of species that prefer increased nutrient concentrations and salinity, with an increase in the relative abundance of *D. elliptica* (an indicator species of low nutrient concentration and

moderate salinity). This suggest less extreme fluctuations in the nutrient and salinity regimes in the recent past. Due to lower relative abundances of the dominant species and the co-occurrence of salinity in-tolerant and tolerant species, these less extreme regimes could be due to annual flooding events in the floodplain. Annual flooding of the pan will result in fluctuations in desiccation and inundation of the floodplain pan. The hypothesis that diatom community structures will reflect paleo-ecological conditions in Nyamithi Pan, is therefore supported.

7.1.4 Hypothesis 4: Since DDT and Deltamethrin are insecticides and do not target diatoms, they will not have an effect on the vitality of the diatom community structures.

DDT is still actively sprayed in and around the Ndumo Game Reserve in the lower Phongolo River floodplain for malaria vector control (Volschenk *et al.*, 2019). However, this insecticide is banned in all other countries with pyrethroids used in its place. The most common of these pyrethroids is Deltamethrin (Humphries, 2013). These insecticides can enter aquatic ecosystems through spray drift as well as surface runoff. Little is known on the effect of these insecticides on non-target species, specifically freshwater diatoms.

Lentic microcosm experiments were done to determine the effects of DDT, Deltamethrin and a mixture (DDT 1:1 Deltamethrin) on the diatom community. Physico-chemical water quality variables remained relatively consistent throughout the exposure period with a considerable decrease in total phosphates between 96 hr and 28 d exposure period. The decrease can be attributed to the oxygen-dependent redox interaction and/or the mineral-water equilibria influence, uptake by algae and exchange of phosphate between water and sediment (DWAf, 1996; Dallas & Day, 2004). Measured nitrogen, phosphate and N:P ratios indicated all microcosm as nutrient enriched and in an eutrophic ecological state during the exposure period.

The insecticides had a negative effect on the diatom metrics and percentage dead cells at both low and high exposure concentrations. The majority of the diatom metrics decreased after exposure to the insecticides and the percentage dead cells for the exposures were significantly higher compared to the control after an acute and chronic exposure period. Both insecticides negatively affected the chloroplast of the diatom cells and inhibited the photosystems, which disrupted the photosynthetic process of the diatom cells. Diatoms were effective bio-indicators to study the effects that these insecticides have on non-target species.

The hypothesis that since DDT and Deltamethrin are insecticides and do not target diatoms, they will not have an effect on the vitality of the diatom community structures, is rejected.

7.1.5 Hypothesis 5: DDT and Deltamethrin will inhibit the photosystems of the diatom cells, negatively affecting their vitality as reflected by chlorophyll- α fluorescence.

Cultures of *Nitzschia palea* were exposed to high (1%) and low (0.1%) concentrations of DDT, Deltamethrin and a mixture of the two (DDT 1:1 Deltamethrin). DDT concentrations were 358 $\mu\text{g/L}$ and 35.8 $\mu\text{g/L}$, with Deltamethrin concentrations 1.9 $\mu\text{g/L}$ and 0.19 $\mu\text{g/L}$, for the high and low exposures respectively. All exposures negatively affected the vitality and cell functioning of the diatoms through disruption of the cell's photosynthetic processes. Inhibition of the cell's photosystems (PSI and PSII) affected the chlorophyll- α of the cells, leading to reduced chlorophyll- α concentrations, distorted and dissolved chloroplast. Changes to the chloroplast decreased cell functioning and influenced the vitality of diatoms. The hypothesis that DDT and Deltamethrin will inhibit the photosystems of the diatom cells, negatively affected their vitality as reflected by chlorophyll- α fluorescence is supported.

7.2 Recommendations

The following recommendations and future potential research are proposed from this study:

- As this study was done during a drought it is recommended that when drought conditions are relieved to do a post-drought study on the changes that occur in the diatom community structure within the lower Phongolo floodplain. It would be important to study the effects of a flooding event on the diatom community structure and to determine if the community would be similar during a flood release compared to the 2013 sampling surveys. More information on the effects that flooding has on the physico-chemical water quality, diatom species structuring and energy flow is necessary in order to better understand the dynamic nature of the floodplain.
- Study the community structures of both the river and floodplain pans during a flood event to determine if the physico-chemical water quality and diatom community between the river and floodplain pans will be similar or how long it will take for equilibrium to be reached during the connected phase.
- Retrieve and analyse core samples from the river and other floodplain pan sites to determine if similar trends in the paleo-limnology of the diatom community is observed throughout the floodplain when compared to Nyamithi Pan.
- Include fatty acid analyses together with stable isotope analysis to assess whether there are differences in energy flow between the river and floodplain pan sites within the floodplain during high and low flow periods.

- Identify the diatom communities/species that are indicators of insecticide contamination, hydrological changes and nutrient enrichment within the floodplain.

7.3 Final thoughts

This study has asserted the importance of including research on the diatom community in the lower Phongolo River floodplain. This is the first time since the 1980s that no controlled flood releases from the Pongolapoort Dam took place for a prolonged period of time. The effect of no controlled flood releases on the diatom community is highlighted in the present study. However, results from the core samples showed that this has most likely happened before. The study shows the importance of including diatom analysis in ecological and ecotoxicological studies of floodplain ecosystems.

The study highlights the importance of further research and conservation of this unique floodplain. Proper implementation of managments plans are essential to ensure ecosystem health. The study highlights the importance of flood releases from the dam and further monitoring is needed to ensure flood releases are implemented to ensure the ecosystem and community benefit from these flood releases.

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Appendix A: Physico-chemical water variables

Table A-1: Physico-chemical (*in situ* and nutrient concentrations) water quality variables measured at each site over the six surveys between February 2017 and May 2018. DNR – Did not read. * – Mean value used

	Date	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (mg/L)	Temperature (°C)	PO ₄ ³⁻ (mg/L)	NO ₂ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	NH ₄ ⁺ (mg/L)	SO ₄ ²⁻ (mg/L)
P1	02/2017	100.8	8.87	395	8.72	262.5	23.6	0.06	0.035*	1.85*	0.16	56.33
P2	02/2017	87.6	7.7	551	8.26	403.5	25.25	0.5	0.035*	1.95*	0.195	92.5
P3	02/2017	63.7	4.72	683	8.52	476	29.9	0.03*	0	0.8	0.06*	28
P4	02/2017	62.7	4.33	735	8.65	511	35.8	0.03	0	0.8	0.11	143
P5	02/2017	68.7	4.34	666	8.39	467	29.4	0.03	0	0.7*	0.08	29
P6	02/2017	104	7.6	246	8.21	172	33.5	0.04	0	0.8	0.04	19
U1	02/2017	97.5	8.48	429.5	8.38*	208.5	24.66*	0.16*	0.055	2.15*	0.15*	64.66*
U2	02/2017	99	8.87	477.5	8.63*	252.5	25.05*	0.265*	0.03*	3*	0.195*	50.33*
N1	02/2017	69.79	5.33	10330	9.19	7220	30.3	0.07	0	0.7	0.06	268
N2	02/2017	71.19	5.78	9910	9.94	6890	31.1	0.04*	0	2.2	0.06	252*
N3	02/2017	63.4	5.05	9900	7.25	7070	29.1	0.04	0	0.8	0.18	220
NO	02/2017	19.5	1.86	3570	7.06	2230	28.3	0.03	0	0.7	0.28	33
S1	02/2017	86.2	7.01	208	7.72*	176	26.15*	1.325*	0.035*	2.7*	0.17*	50.5*
S2	02/2017	80.5	6.44	212	7.585	157	28.3	0.26	0.035	2.2	0.17	54.5
FP	02/2017	107.95	5.37	155.4	7.945	210.8	26.1	0.41	0.03*	2.9	0.185	89.5*
NI	02/2017	59.3	4.92	160.6	7.6	111.3	29	0.04	0	DNR	0.08	14
P1	05/2017	128.5	11.23	346	8.86	245	21.8	0.03*	0.035	1.85*	0.16	56.33*
P3	05/2017	81.3	7.76	656	8.46	459	22.2	0.15	0.02	2.7*	0.15	57.66*
P4	05/2017	100.5	10.44	644	8.46	455	20	0.175*	0.027*	2.8*	0.145*	121*
P5	05/2017	94.5	8.76	642	8.46	450	20.8	0.15*	0.015*	2.5*	0.15	95.66*
U1	05/2017	89.6	8.55	297	8.59	205	23.7	0.21	0.045	3.15*	0.245*	64.66*
U2	05/2017	82.4	7.56	298	8.56	209	23.6	0.27*	0.03*	3	0.195*	50.33

	Date	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Conductivity (μS/cm)	pH	TDS (mg/L)	Temperature (°C)	PO ₄ ³⁻ (mg/L)	NO ₂ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	NH ₄ ⁺ (mg/L)	SO ₄ ²⁻ (mg/L)
N1	05/2017	DNR	DNR	7843	DNR	DNR	28	0.03	0.02	1.1	0.05*	39
N2	05/2017	140.7	6.02	5760	8.6	4000	21.3	0.03	0.02*	1.1*	0.05*	45*
NO	05/2017	48.4	4.09	5450	7.46	3780	22.7	1.1	0.02*	1	0.15*	55*
S1	05/2017	98.8	9.04	234	7.67	154	27.7	1.33	0.035*	2.7*	0.17	50.5*
S2	05/2017	95.1	9.44	276	7.61	190	26.8	0.26*	0.035	2.2	0.17*	54.5*
BP	05/2017	86.1	8.45	211	7.7	146	20.8	0.04*	0.04	1.1	0.4*	30
FP	05/2017	155	12.3	282*	8.02	20	26.2	0.41*	0.03*	2.9*	0.185*	89.5*

P1	09/2017	DNR	DNR	DNR	DNR	DNR	DNR	0.04*	0.40*	1.4*	0.58	45.09*
P3	09/2017	DNR	DNR	676	8.33	DNR	24.4	0.15*	0.02*	2.03	0.15*	67.55
P4	09/2017	DNR	DNR	472	8.08	DNR	20.5	0.175*	0.03*	2.1	0.14	113.66
P5	09/2017	DNR	DNR	688.6	8.1	DNR	23	0.15	0.02*	1.66*	0.15	117.88
U1	09/2017	DNR	DNR	597	8.7	DNR	23.6	DNR	0.08*	2.37*	0.97	51.78*
U2	09/2017	DNR	DNR	583	8.76	DNR	23.9	DNR	0.08*	2.26	0.89*	40.30*
N1	09/2017	DNR	DNR	5356	8.75	DNR	28	0.32	0.03*	2.8*	0.08	300
N2	09/2017	DNR	DNR	9910	8.775	DNR	26.6	0.79*	0.07*	2.2*	0.05*	300
N3	09/2017	DNR	DNR	9900	8.905	DNR	31.1	0.23*	0.03*	3.5	0.08*	300*
NO	09/2017	DNR	DNR	3570	8.725	DNR	27.15	1.81*	0.04*	3*	0.13	210*
P1	11/2017	DNR	DNR	366	8.2	256	23.8	0.05	DNR	0.3	DNR	44
P2	11/2017	DNR	DNR	533	9.06	372	28.4	0.05	DNR	0.4	DNR	-0.063
P3	11/2017	DNR	DNR	DNR	DNR	DNR	DNR	0.09	DNR	0.4	DNR	-0.039
P4	11/2017	DNR	DNR	682	8.7	476	25.7	0.03	DNR	0.3	DNR	-0.065
P5	11/2017	DNR	DNR	671	8.59	474	24.6	0.05	DNR	0.3	DNR	204
U1	11/2017	DNR	DNR	503	9.04	350	30.9	0.07	DNR	DNR	DNR	60
U2	11/2017	DNR	DNR	524	9.03	362	31	0.07	DNR	DNR	DNR	66
N1	11/2017	DNR	DNR	11440	8.51	8000	36.2	0.08	DNR	0.8	0.1	> 300

	Date	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Conductivity (μS/cm)	pH	TDS (mg/L)	Temperature (°C)	PO ₄ ³⁻ (mg/L)	NO ₂ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	NH ₄ ⁺ (mg/L)	SO ₄ ²⁻ (mg/L)
N2	11/2017	DNR	DNR	11030	8.86	7680	29.4	0.07	DNR	DNR	0.05	> 300
N3	11/2017	DNR	DNR	1080	8.87	7510	35	0.08	DNR	DNR	DNR	> 300
NO	11/2017	DNR	DNR	652	8.75	455	24.4	0.06	DNR	1.8	0.03	193

P1	02/2018	DNR	DNR	395	8.76	285	25.3	2.97	0.04	2.9	0.13	71
P2	02/2018	DNR	DNR	551	8.36	387	27.2	0.96	0.05	2.5	0.2	139
P3	02/2018	DNR	DNR	669	8.38	468	26.7	0.23	0.02	3.2	0.08	54
P4	02/2018	DNR	DNR	669	8.28	469	27.9	0.32	0.04	4.5	0.08	173
P5	02/2018	DNR	DNR	682	7.95	469	26.4	DNR	DNR	DNR	DNR	DNR
U1	02/2018	DNR	DNR	262	8.13	184	27.8	0.32	0.05	2.5	0.06	81
U2	02/2018	DNR	DNR	372	8.79	260	24.4	0.45	0.03	2.5	0.06	42
N1	02/2018	DNR	DNR	3830	8.8	2680	28.5	0.13	0.03	DNR	0.06	202
N2	02/2018	DNR	DNR	3860	9.04	2.7	30	0.3	0.03	1.3	0.04	198
N3	02/2018	DNR	DNR	4010	9.15	2750	34.1	0.19	0.03	1.3	0.06	253
NO	02/2018	DNR	DNR	3830	8.92	2670	30.3	0.45	0.03	2.8	0.06	213
S1	02/2018	DNR	DNR	208	7.71	145	26.5	2.58	0.04	4	0.12	55
S2	02/2018	DNR	DNR	212	7.77	147	29.1	0.44	0.02	3.2	0.1	59
FP	02/2018	DNR	DNR	155.4	7.93	109.6	29.7	0.74	0.04	3.6	0.09	115
NI	02/2018	DNR	DNR	218	7.84	154	29.4	0.74	0.06	5.5	1.46	48
P1	05/2018	100.8	8.87	341	8.68	240	21.9	0.03	0.03	0.8	0.19	54
P2	05/2018	87.6	7.7	601	8.16	420	23.3	0.04	0.02	1.4	0.19	46
P3	05/2018	80	6.99	707	8.28	494	22.2	0.07	0.03	2.2	0.22	91
P4	05/2018	83.1	7.31	711	8.21	497	22.3	0.03	0.04	1.1	0.21	47
P5	05/2018	88.1	8.14	718	8.25	494	19.6	0.15	0.03	2.5	0.15	54
U1	05/2018	97.5	8.48	333	8.33	233	22.6	0.1	0.04	3.8	0.43	53
U2	05/2018	99	8.87	331	8.36	245	20.9	0.08	0.03	3.5	0.33	43

	Date	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Conductivity (μS/cm)	pH	TDS (mg/L)	Temperature (°C)	PO ₄ ³⁻ (mg/L)	NO ₂ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	NH ₄ ⁺ (mg/L)	SO ₄ ²⁻ (mg/L)
N1	05/2018	113.2	8.99	1630	8.7	1129	27.5	0.1	0.04	2.8	0.42	152
N2	05/2018	88	7.47	3570	8.51	2490	23.3	0.06	0.03	2.6	0.32	271
N3	05/2018	118.3	9.64	3000	8.66	2050	24.4	0.09	0.03	2.5	0.34	227
NO	05/2018	69.9	5.98	2350	8.53	1630	24	0.09	0.04	1.8	0.42	156
S1	05/2018	86.2	7.01	298	7.73	207	25.8	0.07	0.03	1.4	0.22	46
S2	05/2018	80.5	6.44	241	7.4	167	27.5	0.08	0.05	1.2	0.24	50
BP	05/2018	99.8	8.51	81.9	7.15	56.4	24.6	0.04	0.04	1.1	0.4	30
FP	05/2018	60.9	5.37	452	7.96	312	22.5	0.08	0.02	2.2	0.28	64
NI	05/2018	60.2	5.37	347	7.37	245	21.4	0.09	0.02	1.1	0.31	64
ARP	05/2018	156.2	13.25	130	9.25	91.4	24.6	0.08	0.01	1.1	0.2	85
PP	05/2018	104	9.06	988	8.54	688	24	0.03	0.03	2	0.21	47

Table A-2: Physico-chemical (*in situ* and nutrient concentrations) water quality variables measured at each microcosms after 96 hour exposure period. DNR – Did not read, H – High concentration, L – Low concentration.

Microcosm	Exposure	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Conductivity ($\mu\text{S/cm}$)	pH	TDS (mg/L)	Temperature ($^{\circ}\text{C}$)	PO_4^{3-} (mg/L)	NO_2^- (mg/L)	NO_3^- (mg/L)	NH_4^+ (mg/L)	SO_4^{2-} (mg/L)
1	Mix H	66	6	1149	8.26	805	19.2	3.11	0.03	2.7	0.6	294
2	Mix H	61.2	5.67	1106	8.33	767	18.8	5	0.03	3	0.12	272
3	DDT L	59.7	5.32	1137	8.15	792	18.7	4.79	0.02	3.3	0.08	259
4	DDT L	60.6	5.62	1116	8.14	777	18.7	5	0.02	2.4	0.06	265
5	DDT L	60.8	5.55	1148	8.21	805	18.6	5	0.02	2.3	0.07	270
6	DDT H	70.9	6.61	1154	8.34	810	18.7	5	0.02	3.3	0.07	300
7	Mix H	64	5.96	1136	8.32	797	18.8	1.81	0.02	3.4	0.07	224
8	Deltamethrin L	64.5	5.82	1134	8.35	798	19	0.9	0.02	3.6	0.09	209
9	Deltamethrin L	65.7	6.21	1174	8.35	815	19	1.39	0.02	4.1	0.07	300
10	Deltamethrin L	59.2	5.42	1184	8.14	823	18.9	4.41	0.02	3.1	0.26	320
11	DDT H	60.4	5.52	1252	8.46	874	18.9	0.79	0.02	3.8	0.13	300
12	DDT H	64.2	6	1156	8.39	807	18.9	5	0.02	DNR	0.39	305
13	Deltamethrin H	72.3	6.54	1153	8.47	806	19.2	0.69	0.02	6.6	0.2	310
14	Deltamethrin H	63.8	5.72	1142	8.46	800	19.1	1.44	0.02	2.1	0.07	324
15	Control	76.2	7.01	1132	8.54	788	19.1	0.7	0.02	1.4	0.09	300
16	Control	70.3	6.51	1228	8.44	855	19.2	5	0.03	1	0.13	303
17	Control	72.8	6.62	1270	8.4	890	19.5	3.89	0.04	2.6	0.1	300
18	Deltamethrin H	70.4	6.37	1236	8.36	865	19.5	5	0.04	1	0.09	295

Table A-3: Physico-chemical (*in situ* and nutrient concentrations) water quality variables measured at each microcosms after 28 day exposure period. DNR – Did not read, H – High concentration, L – Low concentration.

Microcosm	Exposure	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Conductivity (μS/cm)	pH	TDS (mg/L)	Temperature (°C)	PO ₄ ³⁻ (mg/L)	NO ₂ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	NH ₄ ⁺ (mg/L)	SO ₄ ²⁻ (mg/L)
1	Mix H	77.2	6.95	906	8.34	630	20.6	0.74	0.04	2.4	0.17	262
2	Mix H	73.7	6.57	1178	8.32	823	20.4	0.39	0.04	2.7	0.12	261
3	DDT L	81.5	7.28	1118	8.36	780	21	0.47	0.05	3.3	0.11	237
4	DDT L	71.3	6.46	1143	8.32	795	20.9	0.26	0.04	2.7	0.14	241
5	DDT L	73.4	6.99	1155	8.29	807	21	0.38	0.03	2.7	0.16	273
6	DDT H	70.3	6.26	1232	8.18	861	20.8	0.3	0.04	2.6	0.1	300
7	Mix H	76.2	7.07	1200	8.13	840	21	0.53	0.03	2.7	0.12	295
8	Deltamethrin L	73	6.48	1130	8.1	788	21.2	0.19	0.03	3.5	0.08	273
9	Deltamethrin L	71.4	6.85	1210	8.15	844	26	0.19	0.04	3.7	0.09	291
10	Deltamethrin L	70.2	5.79	1219	8.14	853	21.6	0.2	0.03	2.9	0.11	300
11	DDT H	68	6.02	1260	7.6	886	21.3	0.22	0.05	3.4	0.04	300
12	DDT H	60.1	5.27	1228	7.84	858	21.6	0.8	0.03	3.6	0.14	275
13	Deltamethrin H	70.1	6.11	1222	7.74	858	21.4	0.85	0.05	4	DNR	300
14	Deltamethrin H	70.9	6.31	1152	7.84	805	21.8	0.51	0.04	3.8	DNR	300
15	Control	65.2	5.87	1209	8	844	22	0.17	0.04	3.9	0.04	300
16	Control	70.1	6.23	1283	8.08	898	22.3	0.46	0.06	3.7	DNR	318
17	Control	65.8	5.73	1118	7.71	870	21.8	0.19	0.04	3	0.06	296
18	Deltamethrin H	68.7	6.07	1201	8.06	886	21.9	0.2	0.04	3.7	0.3	244

Appendix B: Diatom taxa identified and counts for each site for the two surveys in 2013 and six surveys between February 2017 and May 2018.

Table B-1: July and September surveys in 2013

	July 2013	July 2013	July 2013	September 2013	September 2013
	P1	P2	P6	P1	P2
<i>Achnanthes oblongella</i> Oestrup	0	0	0	0	0
<i>Achnanthes subaffinis</i> Cholnoky	0	0	0	0	0
<i>Achnanthes eutrophila</i> Lange-Bertalot	0	0	0	5	0
<i>Achnanthidium exiguum</i> (Grunow) Czarnecki	0	0	0	0	0
<i>Achnanthidium minutissimum</i> (Kützing) Czarnecki	3	9	15	10	0
<i>Amphora ovalis</i> (Kützing) Kützing	0	0	3	0	0
<i>Amphora veneta</i> Kützing	0	0	5	0	0
<i>Cocconeis engelbrechtii</i> Cholnoky	0	25	0	0	23
<i>Cocconeis pediculus</i> Ehrenberg	95	136	0	95	62
<i>Cocconeis placentula</i> Ehrenberg	62	28	45	48	47
<i>Cocconeis placentula</i> var. <i>euglypta</i>	0	0	0	40	0
<i>Craticula buderii</i> (Hustedt) Lange-Bertalot	0	0	0	14	0
<i>Craticula</i> sp.	0	0	0	0	10
<i>Cyclotella meneghiniana</i> Kützing	20	23	10	38	0
<i>Cyclotella ocellata</i> Pantocsek	0	0	0	10	0
<i>Cymbella aspera</i> (Ehrenberg) H.Peragallo	0	14	0	0	0
<i>Cymbella kappii</i> (Cholnoky) Cholnoky	8	0	0	3	16
<i>Cymbella tumida</i> (Brebisson) Van Heurck	0	2	0	0	0
<i>Cymbella turgidula</i> Grunow 1875 in A.Schmidt & al. var. <i>turgidula</i>	0	14	0	0	94
<i>Diadesmis confervacea</i> Kützing var. <i>confervacea</i>	0	3	3	0	0
<i>Diploneis elliptica</i> (Kützing) Cleve	0	0	3	0	0

<i>Discostella stelligera</i> (Cleve et Grun.) Houk & Klee	0	0	0	3	6
<i>Encyonema minutum</i> (Hilse in Rabh.) D.G. Mann	0	0	0	0	0
<i>Encyonema</i> sp.	0	0	3	0	0
<i>Encyonopsis leei</i> Krammer var. <i>sinensis</i> Metzeltin & Krammer	88	0	0	10	31
<i>Eolimna subminuscula</i> (Manguin) Moser Lange-Bertalot & Metzeltin	0	0	0	0	0
<i>Epithemia adnata</i> (Kützing) Brebisson	25	0	0	0	0
<i>Eunotia bilunaris</i> (Ehr.) Mills	0	0	6	0	0
<i>Fallacia pygmaea</i> (Kützing) Stickle & Mann	0	0	6	0	0
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	3	0	0	0	0
<i>Fragilaria capucina</i> Desmazières	0	0	0	0	0
<i>Fragilaria ungeriana</i> Grunow	0	0	0	0	0
<i>Geissleria decussis</i> (Ostrup) Lange-Bertalot & Metzeltin	0	0	3	0	0
<i>Gomphonema gracile</i> Ehrenberg	35	69	42	20	0
<i>Gomphonema lagenula</i> Kützing	0	0	0	0	0
<i>Gomphonema minutum</i> (Ag.) Agardh f. <i>minutum</i>	10	64	97	14	40
<i>Gomphonema parvulum</i> (Kützing)	0	0	35	0	0
<i>Gomphonema pumilum</i> (Grunow) Reichardt & Lange-Bertalot	0	0	0	0	33
<i>Gomphonema</i> sp.	10	0	25	40	6
<i>Gomphonema venusta</i> Passy. Kociolek & Lowe	0	0	0	0	6
<i>Halamphora coffeaeformis</i> (Agardh) Kützing	0	0	30	0	0
<i>Lemnicola hungarica</i> (Grunow) Round & Basson	0	0	4	0	0
<i>Melosira varians</i> Agardh	0	0	0	0	0
<i>Navicula antonii</i> Lange-Bertalot	0	4	14	0	0
<i>Navicula cryptocephala</i> Kützing	0	0	0	0	0
<i>Navicula erifuga</i> Lange-Bertalot	0	0	0	0	0
<i>Navicula gregaria</i> Donkin	0	0	0	0	0
<i>Navicula rostellata</i> Kützing	0	0	0	0	0
<i>Navicula symmetrica</i> Patrick	0	0	62	0	0
<i>Navicula veneta</i> Kützing	0	0	8	0	0

<i>Navicula zanonii</i> Hustedt	0	0	0	6	0
<i>Nitzschia amphibia</i> Grunow f.amphibia	0	0	0	0	0
<i>Nitzschia palea</i> (Kützing) W. Smith	0	0	6	14	0
<i>Nitzschia sigma</i> (Kützing) W. Smith	0	0	0	0	0
<i>Nitzschia</i> sp.	0	0	3	0	0
<i>Placoneis dicephala</i> (W.Smith) Mereschkowsky	0	0	0	0	0
<i>Planothidium frequentissimum</i> (Lange-Bertalot) Lange-Bertalot	0	0	0	0	0
<i>Planothidium rostratum</i> (Oestrup) Lange-Bertalot	0	0	8	0	0
<i>Pleurosigma salinarum</i> (Grunow) Cleve & Grunow	0	2	0	0	0
<i>Reimeria sinuata</i> (Gregory) Kociolek & Stoermer	0	53	0	80	72
<i>Reimeria uniseriata</i> Sala Guerrero & Ferrario	85	0	0	0	0
<i>Rhoicosphenia abbreviata</i> (C.Agardh) Lange-Bertalot	6	0	0	0	4
<i>Seminavis strigosa</i> (Hustedt) Danieleadis & Economou-Amilli	0	0	0	0	0
<i>Surirella ovalis</i> Brebisson	0	0	0	0	0
<i>Tabularia fasciculata</i> (Agardh) Williams et Round	0	4	8	0	0
<i>Tryblionella hungarica</i> (Grunow) D.G. Mann	0	0	6	0	0

Table B-2: February 2017

	P1	P3	P4	P5	P6	U1	U2	NI	N1	N2	N3	NO	S1	FP
<i>Achnanthes inflata</i> (Kützing) Grunow	0	0	0	0	5	0	0	0	0	0	0	0	0	0
<i>Achnanthes</i> sp.	0	0	4	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes standeri</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthidium</i> sp.	0	0	0	0	17	0	0	0	0	0	0	0	0	0
<i>Afrocymbella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora montana</i> Krasske	0	0	14	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora ovalis</i> (Kützing) Kützing	0	0	0	19	0	0	0	0	0	0	0	0	0	0
<i>Amphora</i> sp.	0	0	0	0	0	13	9	0	0	0	0	0	0	0

<i>Amphora</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora strigosa</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora veneta</i> Kützing	0	0	0	8	0	0	0	0	0	0	0	0	0	0
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	22	0	0	14	27	0	0	0	33	18	28	0	0	0
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O Müller) Simonsen	14	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillaria paradoxa</i> Gmelin	0	0	0	0	0	0	6	0	0	0	0	2	0	0
<i>Caloneis bacillum</i> (Grunow) Cleve	0	8	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis silicula</i> (Ehrenberg) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	17	0
<i>Caloneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Campylodiscus clypeus</i> Ehrenberg	0	0	0	0	0	0	0	0	25	18	11	0	0	0
<i>Capartogramma crucicula</i> (Grunow ex Cleve) Ross	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis engelbrechtii</i> Cholnoky	22	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis placentula</i> Ehrenberg	25	20	21	17	0	9	8	0	0	0	12	0	0	0
<i>Craticula accomoda</i> (Hustedt) DG Mann	0	14	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula ambigua</i> (Ehrenberg) DG Mann	0	0	0	0	9	0	0	0	0	0	0	0	0	0
<i>Craticula buderii</i> (Hustedt)	17	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula cuspidata</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	24	0
<i>Craticula</i> sp.	0	0	0	0	0	5	9	0	14	0	0	0	0	38
<i>Craticula subminuscula</i> (Manguin) Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Cyclotella meneghiniana</i> Kützing	0	18	28	22	27	18	10	40	34	23	22	16	49	0
<i>Cyclotella ocellata</i> Pantocsek	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella</i> sp.	0	0	0	0	0	0	0	0	0	25	5	0	0	0
<i>Cymatopleura solea</i> (Brébisson) W. SMith	0	5	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella cymbiformis</i> Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella neocistula</i> Krammer	11	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella simonsenii</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella</i> sp.	0	0	0	8	0	0	0	0	0	0	0	0	0	0
<i>Cymbella subleptoceros</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella tumida</i> (Brébisson) Van Heurck	0	8	0	0	0	0	11	0	0	0	0	0	0	0
<i>Cymbella turgidula</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbopleura</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula</i> sp.	0	0	0	0	0	0	0	0	0	0	3	0	0	0
<i>Denticula subtilis</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diadesmis confervacea</i> (Kützing) DG Mann	0	0	0	4	0	0	0	0	0	0	0	0	0	0
<i>Diatoma vulgaris</i> Bory	0	17	6	9	36	0	0	0	0	23	0	0	28	27
<i>Diploneis elliptica</i> (Kützing) Cleve	24	0	0	0	0	2	9	0	34	0	0	28	0	0
<i>Diploneis puella</i> (Schumann) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis</i> sp.	0	0	0	0	0	0	0	0	22	0	0	0	0	0

<i>Diploneis subovalis</i> Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis zanzibarica</i> (Grunow) Hustedt	0	0	0	5	0	0	0	0	0	0	0	0	0	0
<i>Discostella stelligera</i> (Hustedt) Houk & Klee	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema mesianum</i> (Cholnoky) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema minutum</i> (Hilse) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema neogracile</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema silesiacum</i> (Bleisch) DG Mann	0	0	0	0	0	18	11	0	0	0	0	0	0	16
<i>Encyonema</i> sp.	0	0	0	0	0	0	0	0	0	0	14	14	22	0
<i>Encyonopsis microcephala</i> (Grunow) Krammer	0	0	0	0	0	0	0	28	0	0	0	0	0	0
<i>Eolimna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia adnata</i> (Kützing) Brébisson	26	28	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia sorex</i> Kützing	18	14	16	6	0	0	0	0	0	0	0	0	0	0
<i>Epithemia</i> sp.	0	0	0	0	15	0	0	0	0	0	0	0	0	0
<i>Eunotia flexuosa</i> (Brébisson) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia formica</i> Ehrenberg	14	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia minor</i> (Kützing) Grunow	0	0	32	17	0	0	0	36	0	22	0	0	38	27
<i>Eunotia pectinalis</i> var. undulata (Ralfs) Rabenhorst	0	11	0	0	0	0	0	0	0	0	0	0	24	22
<i>Eunotia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia californica</i> Stancheva and Manoylov	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Fallacia pygmaea</i> (Kützing) Sickle & Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	0	10	23	11	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria capucina</i> var. <i>rumpens</i> (Kützing) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria</i> sp.	0	0	0	2	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot	27	13	22	21	0	0	0	27	0	0	0	0	0	0
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ungeriana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Frustulia tugelae</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema affine</i> Kützing	0	25	39	16	0	0	0	0	0	0	0	0	0	39
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	0	8	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema clavatum</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema exilissimum</i> Lange-Bertalot & Reichardt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema gracile</i> Ehrenberg sensu stricto	0	0	0	0	0	0	0	0	0	0	0	0	48	0
<i>Gomphonema insigne</i> Gregory	37	0	0	0	0	0	0	40	0	0	0	0	36	0
<i>Gomphonema lagenula</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema laticollum</i> Reichardt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema mexicanum</i> Grunow in Van Heurck	0	0	6	0	0	0	0	0	0	0	0	0	0	0

<i>Gomphonema minutum</i> (C.Agardh) C.Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	23	27	25	28	0	20	14	55	48	0	0	16	0	0
<i>Gomphonema pseudoaugur</i> Krammer	14	22	34	0	0	21	22	30	0	0	0	0	0	0
<i>Gomphonema pumilum</i> var. <i>rigidum</i> Reichardt & Lange-Bertalot	9	0	27	0	0	0	0	0	0	39	0	0	0	0
<i>Gomphonema</i> sp.	20	0	10	0	14	0	0	0	0	22	12	0	7	0
<i>Gomphonema venusta</i> Passy, Kociolek & Lowe	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	26	0	0	0	19	0	0	0	0	0	0	0	0	0
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	0	0	0	13	27	0	0	0	0	0	0	0	0	0
<i>Gyrosigma macrum</i> (W. Smith) J.W. Griffith Henfrey	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma scalpoides</i> (Rabenhorst) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma</i> sp.	0	0	0	4	0	0	6	0	0	0	0	0	0	0
<i>Halamphora coffeaeformis</i> (Agardh) Kützing	0	24	0	0	0	0	0	0	0	22	28	10	0	0
<i>Halamphora</i> sp.	0	0	0	0	0	0	0	0	13	0	0	0	0	0
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	0	0	0	0	5	0	0	29	0	0	0	0	0	0
<i>Hantzschia distinctepunctata</i> Hustedt	0	0	15	0	0	25	33	0	0	0	0	0	0	0

<i>Haslea spicula</i> (Hickie) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	21	0	0
<i>Hippodonta</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kolbesia kolbei</i> (Hustedt) F.E. Round & L. Bukhtiyarova	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola mutica</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola</i> sp.	0	5	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastoglia</i> sp.	4	0	0	7	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia dansei</i> (Thwaites) Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia elliptica</i> (Agardh) Cleve	7	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia smithii</i> Thwaites	9	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula erifuga</i> (OF Müller) Bory	0	0	0	0	0	30	10	0	0	0	14	15	0	0
<i>Navicula germainii</i> Wallace	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula gregaria</i> Donkin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula radiosa</i> Kützing	0	0	0	0	31	0	0	0	0	0	17	0	0	0
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	32	0	0	27
<i>Navicula rostellata</i> (Kützing) Cleve	0	0	0	0	0	0	30	0	0	0	0	0	0	0
<i>Navicula sovereignii</i> L.L. Bahls	0	0	0	0	0	0	57	0	0	0	0	0	0	0
<i>Navicula</i> sp.	0	0	16	5	0	42	16	0	32	22	8	0	0	0
<i>Navicula</i> sp. 2	0	0	0	11	0	0	0	0	39	15	19	0	0	0
<i>Navicula</i> sp. 3	0	0	0	7	0	0	0	0	14	0	14	0	0	0
<i>Navicula</i> sp. 4	0	0	0	0	0	0	0	0	25	0	0	0	0	0
<i>Navicula</i> sp. 5	0	0	0	0	0	0	0	0	28	0	0	0	0	0
<i>Navicula</i> sp. 6	0	0	0	0	0	0	0	0	33	0	0	0	0	0

<i>Navicula subrhynchocephala</i> Hustedt	0	0	0	0	0	0	0	0	0	0	7	0	0	0
<i>Navicula symmetrica</i> Patrick	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	0	0	0	0	0	33	0	0	0	0	0	33	0	0
<i>Navicula zanonii</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neidium productum</i> (W. SMith) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia aerophila</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia agnita</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia capitellata</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>archibaldii</i> Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>pellucida</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia clausii</i> Hantzsch	0	38	0	0	24	0	0	0	0	0	0	0	0	0
<i>Nitzschia desertorum</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia dissipata</i> (Kützing) Grunow	31	0	0	15	0	13	15	0	0	32	0	0	0	0
<i>Nitzschia etoshensis</i> Cholnoky	0	0	0	0	0	0	0	0	0	16	26	10	0	0
<i>Nitzschia filiformis</i> (W. SMith) Van Heurk	0	9	0	0	0	0	0	0	0	0	14	28	0	0
<i>Nitzschia fontifuga</i> (n. spec.) Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia frustulum</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	19	0	0	0
<i>Nitzschia hantzschia</i> Krammer & Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	19	0	0	0
<i>Nitzschia intermedia</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia lancetulla</i> Müller	0	0	0	0	0	0	0	0	0	14	22	0	0	0

<i>Nitzschia liebetruthii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia linearis</i> (C. Agardh) W. Smith	0	0	0	14	17	0	0	42	47	0	0	0	0	0
<i>Nitzschia littorea</i> Grunow	0	0	0	0	0	0	0	0	0	0	19	0	0	0
<i>Nitzschia minuta</i> Bleisch in Rabenh	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia nana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	25	0	0	0
<i>Nitzschia palea</i> (Kützinger) W. Smith	0	0	0	0	0	22	14	0	0	0	16	14	0	0
<i>Nitzschia paleacea</i> (Grunow) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia perminuta</i> (Grunow in Van Heurck) H. Perag.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia pura</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia recta</i> Hantzsch	0	24	0	25	22	0	0	0	0	18	0	0	0	0
<i>Nitzschia reversa</i> W. Smith	0	0	0	12	0	0	0	0	0	0	9	4	0	0
<i>Nitzschia sigma</i> (Kützinger) W. Smith	0	0	0	4	13	55	0	0	0	0	27	35	0	0
<i>Nitzschia siliqua</i> Archibald	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp.	17	36	0	2	22	50	15	25	0	14	0	39	41	0
<i>Nitzschia</i> sp. 2	0	21	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	0	0	35	3	0	0	14	0	0	0	8	0	0	0
<i>Pinnularia acrosphaeria</i> W. Smith	0	0	0	0	0	0	0	0	0	0	0	0	21	0
<i>Pinnularia borealis</i> Ehrenberg sensu lato	0	0	0	0	7	0	0	0	0	0	6	0	0	0

<i>Pinnularia divergens</i> W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	36	0
<i>Pinnularia joculata</i> (Manguin) Krammer	0	0	0	5	13	0	0	0	0	0	0	0	0	0
<i>Pinnularia nodosa</i> (Ehrenb.) W.Sm.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia</i> sp.	0	0	0	0	5	0	0	0	0	0	0	9	0	0
<i>Pinnularia subbrevistriata</i> Krammer	0	0	0	0	0	0	0	37	0	0	0	0	27	25
<i>Pinnularia subcapitata</i> Gregory	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia viridiformis</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	31	0
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	0	0	0	4	15	0	0	0	0	0	0	0	0	0
<i>Placoneis clementis</i> (Grunow) EJ Cox	0	0	0	14	11	0	0	0	0	0	0	0	0	0
<i>Placoneis dicephala</i> (W. SMith) Mereschkowsky	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Planothidium rostratum</i> (Oestrup) Round & Bukhityarova	0	0	0	0	0	6	0	0	0	0	0	0	0	0
<i>Pleurosigma salinarum</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	15	27	19	14	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibberula</i> (Ehrenberg) O Müller	0	14	0	19	0	0	0	0	0	0	0	0	0	0

<i>Rhopalodia musculus</i> (Kützing) O Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia operculata</i> (Agardh) Håkansson	0	0	0	15	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sellaphora pupula</i> (Kützing) Mereschkowsky sensu lato	0	0	0	0	0	0	6	0	0	0	0	0	0	0
<i>Sellaphora seminulum</i> (Grunow) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Seminavis strigosa</i> (Hustedt) Danieledis & Economou-Amilli	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis anceps</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	14	0	0	0	0
<i>Stauroneis smithii</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis</i> sp.	0	0	6	0	0	0	0	18	0	0	0	0	0	0
<i>Stenopterobia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirela</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella cruciata</i> A. Schmidt	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella ovalis</i> Brébisson	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synedra</i> sp.	0	0	2	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia fasciculata</i> (C. Agardh) D.M. Williams & Round	0	0	0	0	0	0	0	0	0	0	0	0	8	0
<i>Tabularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella apiculata</i> (W. Greg.) D.G. Mann	0	0	0	0	19	0	5	0	0	26	0	22	18	0
<i>Tryblionella calida</i> (Grunow) D.G. Mann	0	19	0	0	0	0	0	0	0	0	0	0	0	0

<i>Tryblionella</i> <i>coarctata</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella gracilis</i> W. SMith	0	0	0	0	0	0	0	0	0	0	9	0	0	0
<i>Tryblionella</i> <i>hungarica</i> (Grunow) D.G. Mann	11	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella</i> <i>levidensis</i> (W. Smith) Grunow	0	0	0	0	0	0	8	0	0	0	0	0	0	0
<i>Tryblionella</i> <i>littoralis</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella</i> <i>punctata</i> W. Smith	0	0	0	0	0	0	0	0	0	0	0	12	0	0
<i>Tryblionella</i> sp.	0	0	0	0	0	0	0	0	0	18	7	0	0	13

Table B-3: May 2017

	P1	P3	P4	P5	U1	U2	N1	N2	NO	S1	S2	FP	BP
<i>Achnanthes inflata</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes standeri</i> Cholnoky	22	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthidium</i> sp.	0	0	0	0	0	0	0	0	0	0	0	15	0
<i>Afrocymbella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	19	0
<i>Amphora montana</i> Krasske	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora ovalis</i> (Kützing) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora</i> sp.	0	0	0	0	0	0	5	28	10	11	20	0	0
<i>Amphora</i> sp. 2	0	0	0	0	0	0	0	0	18	0	0	0	0
<i>Amphora strigosa</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora veneta</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	30	0	8	0	7	7	0	38	0	0	0	0	0
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O Müller) Simonsen	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillaria paradoxa</i> Gmelin	0	0	15	0	11	11	0	0	0	0	0	0	0
<i>Caloneis bacillum</i> (Grunow) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis silicula</i> (Ehrenberg) Cleve	0	0	0	0	0	0	0	0	0	8	0	0	0
<i>Caloneis</i> sp.	0	0	0	0	0	0	0	0	0	10	0	0	0
<i>Campylodiscus clypeus</i> Ehrenberg	0	0	0	0	0	0	0	12	0	0	0	0	0
<i>Capartogramma crucicula</i> (Grunow ex Cleve) Ross	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis engelbrechtii</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis placentula</i> Ehrenberg	78	121	70	16	13	13	0	0	0	15	26	0	0
<i>Craticula accomoda</i> (Hustedt) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula ambigua</i> (Ehrenberg) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Craticula buderi</i> (Hustedt)	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula cuspidata</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	10	0	23	0
<i>Craticula</i> sp.	0	0	0	0	3	3	14	0	0	0	0	0	0
<i>Craticula subminuscula</i> (Manguin) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella meneghiniana</i> Kützing	0	0	5	0	4	4	32	39	34	0	0	0	0
<i>Cyclotella ocellata</i> Pantocsek	0	0	0	0	0	0	2	0	0	0	0	0	0
<i>Cyclotella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymatopleura solea</i> (Brébisson) W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	0	0	0	0	1	1	0	0	0	0	0	0	0
<i>Cymbella cymbiformis</i> Agardh	0	21	0	0	0	0	0	0	32	0	0	0	0
<i>Cymbella neocistula</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella simonsenii</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella</i> sp.	9	27	0	0	37	37	0	0	6	0	12	0	0
<i>Cymbella subleptoceros</i> Krammer	17	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella tumida</i> (Brébisson) Van Heurck	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella turgidula</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbopleura</i> sp.	0	0	13	12	0	0	0	0	0	0	0	0	13
<i>Denticula</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula subtilis</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diadsmis confervacea</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diatoma vulgaris</i> Bory	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis elliptica</i> (Kützing) Cleve	0	0	0	0	0	0	0	42	28	0	0	0	0
<i>Diploneis puella</i> (Schumann) Cleve	0	0	0	0	24	24	0	0	0	0	0	0	0
<i>Diploneis</i> sp.	0	0	0	0	0	0	0	8	0	0	0	0	0
<i>Diploneis subovalis</i> Cleve	0	0	0	0	0	0	8	0	0	0	0	0	0

<i>Diploneis zanzibarica</i> (Grunow) Hustedt	0	0	0	0	2	2	0	0	0	0	0	0	0
<i>Discostella stelligera</i> (Hustedt) Houk & Klee	0	0	0	0	4	4	0	0	0	0	0	0	0
<i>Encyonema mesianum</i> (Cholnoky) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema minutum</i> (Hilse) DG Mann	0	0	0	0	0	0	0	0	0	13	0	0	0
<i>Encyonema neogracile</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema silesiacum</i> (Bleisch) DG Mann	0	0	0	0	0	0	0	0	0	16	0	0	0
<i>Encyonema</i> sp.	0	0	6	0	0	0	7	0	0	0	14	0	0
<i>Encyonopsis microcephala</i> (Grunow) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eolimna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia adnata</i> (Kützing) Brébisson	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia sorex</i> Kützing	4	43	45	16	0	0	0	0	0	0	0	0	0
<i>Epithemia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia flexuosa</i> (Brébisson) Kützing	0	0	0	0	0	0	0	0	0	19	29	0	0
<i>Eunotia formica</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia minor</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	9	0	0	0
<i>Eunotia pectinalis</i> var. undulata (Ralfs) Rabenhorst	0	0	0	0	0	0	0	0	0	1	10	37	7
<i>Eunotia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia californica</i> Stancheva and Manoylov	0	0	0	0	0	0	0	0	0	30	0	0	0
<i>Fallacia pygmaea</i> (Kützing) Sickie & Mann	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	21	54	0	45	11	11	0	0	0	0	34	0	0
<i>Fragilaria capucina</i> var. rumpens (Kützing) Lange-Bertalot	9	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria</i> sp.	0	0	15	0	6	6	0	0	0	15	0	0	0
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ungeriana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Frustulia tugelae</i> Cholnoky	0	0	0	0	0	0	0	0	0	25	0	0	0
<i>Gomphonema affine</i> Kützing	0	57	6	46	0	0	0	0	48	14	21	26	0
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	27	0	0	50	0	0	0	0	0	0	0	0	0
<i>Gomphonema clavatum</i> Ehrenberg	0	56	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema exilissimum</i> Lange-Bertalot & Reichardt	0	0	0	0	0	0	0	0	24	0	0	0	0
<i>Gomphonema gracile</i> Ehrenberg sensu stricto	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema insigne</i> Gregory	0	0	0	0	0	0	0	0	46	8	0	0	0
<i>Gomphonema lagenula</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema laticollum</i> Reichardt	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema mexicanum</i> Grunow in Van Heurck	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema minutum</i> (C.Agardh) C.Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	11	37	13	0	7	7	59	0	32	0	0	26	12
<i>Gomphonema pseudoaugur</i> Krammer	0	10	0	14	0	0	0	0	16	0	0	0	0
<i>Gomphonema pumilum</i> var. <i>rigidum</i> Reichardt & Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> sp.	20	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema venusta</i> Passy, Kociolek & Lowe	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	0	0	0	0	53	53	0	0	0	0	0	0	0
<i>Gyrosigma macrum</i> (W. Smith) J.W. Griffith Henfrey	0	0	0	0	0	0	26	0	0	0	0	0	0
<i>Gyrosigma scalproides</i> (Rabenhorst) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma</i> sp.	0	0	0	0	0	0	0	0	6	20	16	0	0
<i>Halamphora coffeaeformis</i> (Agardh) Kützing	0	0	23	0	13	13	24	24	0	0	0	0	0
<i>Halamphora</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Hantzschia distinctepunctata</i> Hustedt	0	0	0	0	66	66	0	0	0	0	7	0	0
<i>Haslea spicula</i> (Hickie) Lange-Bertalot	0	0	0	0	0	0	18	0	0	0	0	0	0
<i>Hippodonta</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kolbesia kolbei</i> (Hustedt) F.E. Round & L. Bukhtiyarova	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola mutica</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastoglia</i> sp.	0	0	0	0	0	0	0	25	0	0	0	0	0
<i>Mastogloia dansei</i> (Thwaites) Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia elliptica</i> (Agardh) Cleve	11	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia smithii</i> Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula erifuga</i> (OF Müller) Bory	0	0	0	0	8	8	35	0	0	0	0	0	0
<i>Navicula germainii</i> Wallace	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula gregaria</i> Donkin	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula radiosa</i> Kützing	0	0	0	0	0	0	0	0	27	0	0	0	0
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	0	0	21	0	46	46	0	57	0	10	0	0	0
<i>Navicula rostellata</i> (Kützing) Cleve	0	0	16	0	12	12	7	0	12	0	0	0	0
<i>Navicula sovereignii</i> L.L. Bahls	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp.	0	0	8	0	0	0	10	0	0	0	0	18	24
<i>Navicula</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	17	6
<i>Navicula</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	2
<i>Navicula</i> sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 5	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 6	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula subrhynchocephala</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula symmetrica</i> Patrick	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	23	0	22	0	0	0	6	0	0	0	0	0	0

<i>Navicula zanonii</i> Hustedt	0	0	0	0	0	0	11	0	0	0	0	0	0
<i>Neidium productum</i> (W. SMith) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia aerophila</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia agnita</i> Hustedt	0	0	0	0	0	0	21	0	0	0	0	0	0
<i>Nitzschia capitellata</i> Hustedt	0	0	0	0	0	0	0	0	0	0	3	0	0
<i>Nitzschia</i> cf. <i>archibaldii</i> Lange-Bertalot	0	0	28	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>pellucida</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia clausii</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia desertorum</i> Hustedt	0	0	18	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia dissipata</i> (Kützinger) Grunow	0	0	0	0	0	0	0	0	29	11	25	16	0
<i>Nitzschia etoshensis</i> Cholnoky	22	0	0	0	0	0	0	0	24	0	0	0	29
<i>Nitzschia filiformis</i> (W. SMith) Van Heurk	0	0	0	0	0	0	12	0	15	0	0	0	0
<i>Nitzschia fontifuga</i> (n. spec.) Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia frustulum</i> (Kützinger) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia hantzschia</i> Krammer & Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia intermedia</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia lancetulla</i> Müller	0	0	0	0	0	0	24	46	0	0	0	0	0
<i>Nitzschia liebetruthii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia linearis</i> (C. Agardh) W. Smith	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia littorea</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia minuta</i> Bleisch in Rabenh	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia nana</i> Grunow	0	0	0	0	0	0	28	0	0	0	0	0	0
<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	0	0	0	0	0	0	0	0	9	0	0	0	0
<i>Nitzschia palea</i> (Kützinger) W. SMith	0	0	0	0	6	6	16	0	0	0	0	0	0
<i>Nitzschia paleacea</i> (Grunow) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Nitzschia perminuta</i> (Grunow in Van Heurcj) H. Perag.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia pura</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia recta</i> Hantzsch	0	0	23	0	13	13	0	0	25	14	0	0	0
<i>Nitzschia reversa</i> W. SMith	0	0	15	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia sigma</i> (Kützing) W. Smith	0	0	21	6	18	18	22	0	29	0	0	0	0
<i>Nitzschia siliqua</i> Archibald	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp.	0	0	0	0	0	0	20	15	11	14	25	86	55
<i>Nitzschia</i> sp. 2	0	0	0	0	0	0	6	23	19	0	0	0	35
<i>Nitzschia</i> sp. 3	0	0	0	0	0	0	0	0	16	0	0	0	35
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	0	0	0	0	0	0	0	0	17	0	0	0	0
<i>Pinnularia acrosphaeria</i> W. SMith	0	0	0	0	0	0	0	0	0	13	7	0	0
<i>Pinnularia borealis</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	0	0	19	27
<i>Pinnularia divergens</i> W. SMith	0	0	0	0	0	0	0	0	0	20	38	0	52
<i>Pinnularia jocolata</i> (Manguin) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia nodosa</i> (Ehrenb.) W.Sm.	0	0	0	0	0	0	0	0	0	20	0	0	0
<i>Pinnularia</i> sp.	0	0	0	0	0	0	0	26	12	0	30	44	0
<i>Pinnularia subbrevistriata</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia subcapitata</i> Gregory	0	0	0	0	5	5	0	0	0	30	30	0	61
<i>Pinnularia viridiformis</i> Krammer	0	0	0	0	0	0	0	0	0	0	9	0	0
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis clementis</i> (Grunow) EJ Cox	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis dicephala</i> (W. SMith) Mereschkowsky	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Planothidium rostratum</i> (Oestrup) Round & Bukhityarova	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Pleurosigma salinarum</i> Grunow	0	0	11	0	9	9	24	0	0	11	20	0	0
<i>Pleurosigma</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	0	12	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibberula</i> (Ehrenberg) O Müller	0	0	8	0	0	0	0	0	0	5	0	0	0
<i>Rhopalodia musculus</i> (Kützing) O Müller	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia operculata</i> (Agardh) Håkansson	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia</i> sp.	0	0	0	0	1	1	0	0	0	0	0	0	0
<i>Sellaphora pupula</i> (Kützing) Mereschkowsky sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sellaphora seminulum</i> (Grunow) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Seminavis strigosa</i> (Hustedt) Danieledis & Economou- Amilli	0	0	0	0	0	0	9	19	0	0	0	0	0
<i>Stauroneis anceps</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	16	20	0	0
<i>Stauroneis smithii</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis</i> sp.	0	0	0	0	0	0	0	0	6	0	0	0	0
<i>Stenopterobia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirela</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella cruciata</i> A. Schmidt	0	0	0	0	6	6	0	0	0	0	0	0	0
<i>Surirella ovalis</i> Brébisson	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synedra</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia fasciculata</i> (C. Agardh) D.M. Williams & Round	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella apiculata</i> (W. Greg.) D.G. Mann	0	0	0	0	17	17	7	0	0	10	0	54	42
<i>Tryblionella calida</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella coarctata</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Tryblionella gracilis</i> W. Smith	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella hungarica</i> (Grunow) D.G. Mann	0	0	0	0	0	0	6	0	0	0	0	0	0
<i>Tryblionella levidensis</i> (W. Smith) Grunow	0	0	0	0	0	0	0	0	0	28	59	0	0
<i>Tryblionella littoralis</i> (Grunow) D.G. Mann	0	0	0	0	16	16	4	0	0	0	0	0	0
<i>Tryblionella punctata</i> W. Smith	0	0	10	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella</i> sp.	0	13	15	0	0	0	0	0	4	0	0	0	0

Table B-4: September 2017. B – Benthic; P – Plant

	P1 B	P1 P	P3 B	P3 P	P4 B	P4 P	P5 P	N1 B	N1 P	N2 B	N2 P	N3 B	N3 P	NO B	NO P
<i>Achnanthes inflata</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes standeri</i> Chohnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	12	12
<i>Achnanthidium</i> sp.	0	0	0	0	0	5	0	0	0	0	0	13	0	0	0
<i>Afrocymbella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora montana</i> Krasske	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora ovalis</i> (Kützing) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora</i> sp.	0	0	0	0	0	0	14	0	0	0	29	0	15	0	0
<i>Amphora</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora strigosa</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora veneta</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	85	81	0	0	88	89	0	0	0	0	0	0	7	0	0
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O Müller) Simonsen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillaria paradoxa</i> Gmelin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis bacillum</i> (Grunow) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis silicula</i> (Ehrenberg) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Caloneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Campylodiscus clypeus</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	8	1	0	0
<i>Capartogramma crucicula</i> (Grunow ex Cleve) Ross	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis engelbrechtii</i> Chohnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis placentula</i> Ehrenberg	60	76	53	62	89	54	59	0	0	0	0	0	0	0	0
<i>Craticula accomoda</i> (Hustedt) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula ambigua</i> (Ehrenberg) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula buderii</i> (Hustedt)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula cuspidata</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula</i> sp.	0	0	0	0	0	0	0	10	10	0	16	0	13	0	0
<i>Craticula subminuscula</i> (Manguin) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella meneghiniana</i> Kützing	0	9	19	14	20	1	0	36	60	12	21	48	28	64	52
<i>Cyclotella ocellata</i> Pantocsek	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymatopleura solea</i> (Brébisson) W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	0	0	0	6	0	0	17	0	0	0	0	3	0	0	0
<i>Cymbella cymbiformis</i> Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella neocistula</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella simonsenii</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella subleptoceros</i> Krammer	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0

<i>Cymbella tumida</i> (Brébisson) Van Heurck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella turgidula</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymboplectra</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula subtilis</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diadesmis</i> <i>confervacea</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diatoma vulgaris</i> Bory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis elliptica</i> (Kützing) Cleve	0	0	0	0	0	0	0	35	40	27	26	37	27	5	11
<i>Diploneis puella</i> (Schumann) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis subovalis</i> Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis zanzibarica</i> (Grunow) Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
<i>Discostella stelligera</i> (Hustedt) Houk & Klee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema</i> <i>mesianum</i> (Cholnoky) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema minutum</i> (Hilse) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema</i> <i>neogracile</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema</i> <i>silesiacum</i> (Bleisch) DG Mann	15	17	0	0	0	16	0	0	0	0	0	0	0	0	0
<i>Encyonema</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonopsis</i> <i>microcephala</i> (Grunow) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eolimna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia adnata</i> (Kützing) Brébisson	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0
<i>Epithemia sorex</i> Kützing	0	0	0	23	0	0	28	0	0	0	0	0	0	0	0

<i>Epithemia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia flexuosa</i> (Brébisson) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia formica</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia minor</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia pectinalis</i> var. undulata (Ralfs) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia californica</i> Stancheva and Manoylov	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia pygmaea</i> (Kützing) Sickie & Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria biceps</i> (Kützing) Lange- Bertalot	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0
<i>Fragilaria capucina</i> var. rumpens (Kützing) Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ulna</i> (Nitzsch) Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	10	12	0	17	33	57	25	0	0	0	0	0	0	6	0
<i>Fragilaria ungeriana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Frustulia tugelae</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema affine</i> Kützing	39	0	0	20	0	0	12	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>angustatum</i> (Kützing) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>clavatum</i> Ehrenberg	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>exilissimum</i> Lange- Bertalot & Reichardt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Gomphonema gracile</i> Ehrenberg sensu stricto	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>insigne</i> Gregory	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>lagenula</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>laticollum</i> Reichart	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>mexicanum</i> Grunow in Van Heurck	22	23	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>minutum</i> (C.Agardh) C.Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>parvulum</i> (Kützing) Kützing sensu stricto	0	0	0	0	12	9	0	0	0	0	0	12	4	8	26
<i>Gomphonema</i> <i>pseudoaugur</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>pumilum</i> var. <i>rigidum</i> Reichardt & Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>venusta</i> Passy, Kociolek & Lowe	0	0	23	0	22	0	0	0	0	7	0	0	0	0	0
<i>Gyrosigma</i> <i>acuminatum</i> (Kützing) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma</i> <i>attenuatum</i> (Kützing) Cleve	17	16	17	14	0	0	12	0	0	0	0	0	0	0	0
<i>Gyrosigma macrum</i> (W. Smith) J.W. Griffith Henfrey	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma</i> <i>scalproides</i> (Rabenhorst) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma</i> sp.	0	0	0	0	12	0	0	0	8	0	7	10	11	22	15
<i>Halamphora</i> <i>coffaeaeformis</i> (Agardh) Kützing	0	15	0	0	21	10	0	9	9	22	21	35	10	0	22
<i>Halamphora</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hantzschia distinctepunctata</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
<i>Haslea spicula</i> (Hickie) Lange-Bertalot	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hippodonta</i> sp.	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0
<i>Kolbesia kolbei</i> (Hustedt) F.E. Round & L. Bukhtiyarova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola mutica</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola</i> sp.	0	0	0	0	0	0	0	0	0	0	57	0	32	0	0
<i>Mastoglia</i> sp.	0	0	19	11	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia dansei</i> (Thwaites) Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia elliptica</i> (Agardh) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia smithii</i> Thwaites	8	0	0	0	0	0	0	0	0	0	53	19	36	0	0
<i>Navicula erifuga</i> (OF Müller) Bory	59	25	19	40	0	22	25	20	0	0	22	0	0	42	32
<i>Navicula germainii</i> Wallace	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula gregaria</i> Donkin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula radiosa</i> Kützing	0	10	0	0	22	14	0	0	0	0	0	0	0	20	19
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula rostellata</i> (Kützing) Cleve	0	0	0	0	0	0	0	8	15	0	0	0	11	0	0
<i>Navicula sovereignii</i> L.L. Bahls	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp.	0	0	33	41	26	14	18	16	0	10	0	36	24	0	14
<i>Navicula</i> sp. 2	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0
<i>Navicula</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Navicula</i> sp. 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula subrhynchocephala</i> Hustedt	0	0	0	0	0	0	0	0	0	0	31	0	0	0	0
<i>Navicula symmetrica</i> Patrick	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	15	18	17	15	0	12	14	13	0	0	0	25	15	0	0
<i>Navicula zanonii</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neidium productum</i> (W. SMith) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia aerophila</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia agnita</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0
<i>Nitzschia capitellata</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>archibaldii</i> Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>pellucida</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia clausii</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia desertorum</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia dissipata</i> (Kützing) Grunow	9	12	26	0	0	0	12	15	21	0	0	0	0	21	57
<i>Nitzschia etoshensis</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia filiformis</i> (W. SMith) Van Heurk	0	15	22	0	0	0	21	12	57	0	0	0	14	0	24
<i>Nitzschia fontifuga</i> (n. spec.) Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia frustulum</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia hantzschia</i> Krammer & Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia intermedia</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia lanceatula</i> Müller	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0
<i>Nitzschia liebetruthii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia linearis</i> (C. Agardh) W. Smith	0	0	0	0	0	0	0	0	0	0	8	0	12	0	0

<i>Nitzschia littorea</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia minuta</i> Bleich in Rabenh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia nana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35
<i>Nitzschia palea</i> (Kützinger) W. SMith	13	20	18	45	18	15	18	0	21	0	37	27	11	15	35
<i>Nitzschia paleacea</i> (Grunow) Grunow	0	0	0	0	0	0	0	11	0	0	21	11	0	0	0
<i>Nitzschia perminuta</i> (Grunow in Van Heurck) H. Perag.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia pura</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia recta</i> Hantzsch	0	0	20	0	0	23	13	0	0	0	16	0	0	0	0
<i>Nitzschia reversa</i> W. SMith	0	0	0	0	0	0	0	11	0	0	0	0	0	72	0
<i>Nitzschia sigma</i> (Kützinger) W. SMith	0	0	19	0	0	0	24	25	27	0	0	14	5	17	0
<i>Nitzschia siliqua</i> Archibald	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0
<i>Nitzschia</i> sp.	15	0	25	31	30	10	18	20	0	15	0	51	25	16	25
<i>Nitzschia</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia umbonata</i> (Ehrenberg) Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia</i> <i>acrosphaeria</i> W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia borealis</i> Ehrenberg sensu lato	0	0	0	0	0	0	13	0	0	0	0	0	0	0	5
<i>Pinnularia divergens</i> W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia joculata</i> (Manguin) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia nodosa</i> (Ehrenb.) W.Sm.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia</i> <i>subbrevistriata</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Pinnularia subcapitata</i> Gregory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia viridiformis</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	0	0	0	0	0	0	0	3	13	0	5	0	8	0	0
<i>Placoneis clementis</i> (Grunow) EJ Cox	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis dicephala</i> (W. SMith) Mereschkowsky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Planothidium rostratum</i> (Oestrup) Round & Bukhtyarova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma salinarum</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	0	0	0	17	0	0	19	0	0	0	0	0	0	0	0
<i>Rhopalodia gibberula</i> (Ehrenberg) O Müller	20	0	0	0	0	0	20	0	0	0	0	0	22	0	0
<i>Rhopalodia musculus</i> (Kützing) O Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia operculata</i> (Agardh) Håkansson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sellaphora pupula</i> (Kützing) Mereschkowsky sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sellaphora seminulum</i> (Grunow) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0
<i>Seminavis strigosa</i> (Hustedt) Danieleadis & Economou-Amilli	13	16	0	15	0	0	0	15	9	0	0	0	0	0	0
<i>Stauroneis anceps</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis smithii</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Stauroneis</i> sp.	0	0	0	0	0	0	7	0	0	10	8	15	0	0	0
<i>Stenopterobia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0
<i>Surirela</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella cruciata</i> A. Schmidt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella ovalis</i> Brébisson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synedra</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia fasciculata</i> (C. Agardh) D.M. Williams & Round	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella apiculata</i> (W. Greg.) D.G. Mann	0	13	19	14	0	0	0	37	45	0	0	13	10	58	16
<i>Tryblionella calida</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	40	0	0	0	0	0	0	0
<i>Tryblionella coarctata</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella gracilis</i> W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella hungarica</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella levidensis</i> (W. Smith) Grunow	0	0	24	15	0	0	0	28	33	0	0	0	14	0	0
<i>Tryblionella littoralis</i> (Grunow) D.G. Mann	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella punctata</i> W. Smith	0	0	0	0	0	0	0	21	32	0	8	0	10	0	0
<i>Tryblionella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table B-5: November 2017. B – Benthic; P – Plant

	P1 B	P1 P	P2 B	P2 P	P3 B	P3 P	P4 B	P4 P	P5 B	P5 P	U1 B	U1 P	U2 B	U2 P	N1 B	N1 P	N2 B	N2 P	N3 B
<i>Achnanthes inflata</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes standeri</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Achnanthyidium</i> sp.	0	0	0	0	11	38	0	0	0	0	0	10	0	8	0	0	0	0	0
<i>Afrocybella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora montana</i> Krasske	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora ovalis</i> (Kützing) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0
<i>Amphora</i> sp.	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	12	23	12	21
<i>Amphora</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora strigosa</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora veneta</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aulacoseira</i> <i>granulata</i> (Ehrenberg) Simonsen	173	75	7	0	10	12	0	3	0	0	19	40	49	38	0	11	0	0	0
<i>Aulacoseira</i> <i>granulata</i> var. <i>angustissima</i> (O Müller) Simonsen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillaria paradoxa</i> Gmelin	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0
<i>Caloneis bacillum</i> (Grunow) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis silicula</i> (Ehrenberg) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Campylodiscus</i> <i>clypeus</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	13
<i>Capartogramma</i> <i>crucicula</i> (Grunow ex Cleve) Ross	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis</i> <i>engelbrechtii</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis</i> <i>placentula</i> Ehrenberg	42	178	0	11	10	125	60	64	56	78	31	19	47	16	11	0	0	0	0
<i>Craticula</i> <i>accomoda</i> (Hustedt) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0
<i>Craticula ambigua</i> (Ehrenberg) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0

<i>Craticula buderi</i> (Hustedt)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula cuspidata</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula</i> sp.	0	0	0	0	0	2	0	0	0	0	8	0	3	0	0	0	0	0	0
<i>Craticula subminuscula</i> (Manguin) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella meneghiniana</i> Kützing	24	5	0	0	0	7	7	7	12	9	17	0	0	0	14	33	26	38	24
<i>Cyclotella ocellata</i> Pantocsek	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymatopleura solea</i> (Brébisson) W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	0	42	24	40	2	0	0	4	5	7	0	0	2	0	0	0	0	0	0
<i>Cymbella cymbiformis</i> Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella neocistula</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella simonsenii</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella</i> sp.	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella subleptoceros</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella tumida</i> (Brébisson) Van Heurck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella turgidula</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbopleura</i> sp.	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	0	0	0	0
<i>Denticula subtilis</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Diadmesmis confervacea</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diatoma vulgaris</i> Bory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis elliptica</i> (Kützing) Cleve	13	0	6	0	0	0	0	0	0	0	0	0	0	0	0	41	0	54	18
<i>Diploneis puella</i> (Schumann) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis subovalis</i> Cleve	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0
<i>Diploneis zanzibarica</i> (Grunow) Hustedt	0	0	0	0	0	0	0	3	1	0	0	0	30	3	0	0	0	0	0
<i>Discostella stelligera</i> (Hustedt) Houk & Klee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema mesianum</i> (Cholnoky) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema minutum</i> (Hilse) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema neogracile</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema silesiacum</i> (Bleisch) DG Mann	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0
<i>Encyonema</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonopsis microcephala</i> (Grunow) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eolimna</i> sp.	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia adnata</i> (Kützing) Brébisson	0	6	10	12	0	0	0	37	16	0	0	0	0	0	0	0	0	0	0
<i>Epithemia sorex</i> Kützing	0	5	12	56	0	23	5	64	115	0	0	0	0	0	0	0	0	0	0
<i>Epithemia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia flexuosa</i> (Brébisson) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Eunotia formica</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia minor</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia pectinalis</i> var. undulata (Ralfs) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia californica</i> Stancheva and Manoylov	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia pygmaea</i> (Kützing) Sickel & Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria biceps</i> (Kützing) Lange- Bertalot	31	2	31	30	0	13	5	42	55	0	0	4	0	14	0	0	0	0	0
<i>Fragilaria capucina</i> var. rumpens (Kützing) Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	14	0	0	0	64	64	74	34	0
<i>Fragilaria</i> sp.	0	0	0	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ulna</i> (Nitzsch) Lange- Bertalot	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria</i> <i>ungeriana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Frustulia tugelae</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>affine</i> Kützing	6	53	0	21	0	24	0	46	26	11	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>angustatum</i> (Kützing) Rabenhorst	0	0	6	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>clavatum</i> Ehrenberg	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>exilissimum</i> Lange- Bertalot & Reichardt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Gomphonema gracile</i> Ehrenberg sensu stricto	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema insigne</i> Gregory	0	27	0	20	0	0	0	47	0	0	0	0	0	0	0	0	0	11	0
<i>Gomphonema lagenula</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema laticollum</i> Reichart	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema mexicanum</i> Grunow in Van Heurck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema minutum</i> (C.Agardh) C.Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	0	0	0	0	0	0	0	0	0	0	12	14	9	0	0	0	0	0	0
<i>Gomphonema pseudoaugur</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	1	6	0	0	0	0	0
<i>Gomphonema pumilum</i> var. <i>rigidum</i> Reichardt & Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema venusta</i> Passy, Kociolek & Lowe	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	0	0	0	4	0	0	0	0	0	11	3	0	0	0	0	0	0	0	6
<i>Gyrosigma macrum</i> (W. Smith) J.W. Griffith Henfrey	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma scalpoides</i> (Rabenhorst) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma</i> sp.	10	0	49	5	3	0	0	0	0	0	0	14	16	4	0	0	0	0	0

<i>Halamphora coffeaeformis</i> (Agardh) Kützing	0	0	9	0	0	0	39	0	0	0	30	14	44	30	0	15	0	0	18
<i>Halamphora</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hantzschia distinctepunctata</i> Hustedt	0	0	0	0	3	0	0	9	0	0	69	57	74	60	0	0	0	0	0
<i>Haslea spicula</i> (Hickie) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hippodonta</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kolbesia kolbei</i> (Hustedt) F.E. Round & L. Bukhtiyarova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola mutica</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola</i> sp.	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0
<i>Mastoglia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia dansei</i> (Thwaites) Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia elliptica</i> (Agardh) Cleve	0	0	0	57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia smithii</i> Thwaites	4	0	12	49	0	0	0	0	0	0	0	0	0	0	0	25	0	39	0
<i>Navicula erifuga</i> (OF Müller) Bory	0	0	26	0	0	0	20	0	0	0	0	16	33	24	0	12	0	0	0
<i>Navicula germainii</i> Wallace	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula gregaria</i> Donkin	0	0	0	0	0	0	0	0	0	0	16	0	0	9	0	0	0	15	0
<i>Navicula radiosa</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	20	0	0	0	10	0	0	0	0	56	0	0	0	0	0	0	0	0	0
<i>Navicula rostellata</i> (Kützing) Cleve	0	0	0	0	0	0	0	0	0	0	25	19	37	23	17	0	0	24	0
<i>Navicula sovereignii</i> L.L. Bahls	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Navicula</i> sp.	0	0	35	14	17	67	25	0	0	22	0	20	9	21	60	0	0	0	27
<i>Navicula</i> sp. 2	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	46
<i>Navicula</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula subrhynchocephala</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula symmetrica</i> Patrick	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	0	0	0	0	0	0	26	0	0	0	0	0	0	0	17	14	54	28	0
<i>Navicula zanonii</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neidium productum</i> (W. SMith) Cleve	0	0	0	0	5	0	0	0	0	0	0	15	0	0	0	0	0	0	0
<i>Nitzschia aerophila</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia agnita</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia capitellata</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>archibaldii</i> Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	32	31	0	41	0	0	0	0	0
<i>Nitzschia</i> cf. <i>pellucida</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0
<i>Nitzschia clausii</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia desertorum</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia dissipata</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	6	30	0	0	0	10	0	0	0	0	0
<i>Nitzschia etoshensis</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia filiformis</i> (W. SMith) Van Heurk	0	0	0	0	0	0	0	0	0	0	44	0	0	19	0	0	0	0	0
<i>Nitzschia fontifuga</i> (n. spec.) Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Nitzschia frustulum</i> (Kützing) Grunow	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia hantzschia</i> Krammer & Lange-Bertalot	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia intermedia</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	20	0
<i>Nitzschia lanceatula</i> Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0
<i>Nitzschia liebetruthii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia linearis</i> (C. Agardh) W. Smith	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia littorea</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia minuta</i> Bleisch in Rabenh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia nana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0
<i>Nitzschia palea</i> (Kützing) W. SMith	0	0	13	0	0	7	21	0	0	0	19	41	0	20	0	21	0	0	0
<i>Nitzschia paleacea</i> (Grunow) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia perminuta</i> (Grunow in Van Heurcj) H. Perag.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia pura</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia recta</i> Hantzsch	0	0	0	0	0	0	0	7	0	0	0	19	0	0	10	40	0	26	0
<i>Nitzschia reversa</i> W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia sigma</i> (Kützing) W. Smith	0	0	17	0	0	0	0	0	0	21	14	0	0	16	0	9	0	0	25
<i>Nitzschia siliqua</i> Archibald	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp.	0	7	33	15	0	0	23	0	0	9	0	26	13	20	53	0	0	22	23
<i>Nitzschia</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Nitzschia</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0
<i>Pinnularia acrosphaeria</i> W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia borealis</i> Ehrenberg sensu lato	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia divergens</i> W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia joculata</i> (Manguin) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia nodosa</i> (Ehrenb.) W.Sm.	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia subbrevistriata</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia subcapitata</i> Gregory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia viridiformis</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	0	0	6	0	0	23	0	0	0	1	0	0	0	0	0	26	87	24	15
<i>Placoneis clementis</i> (Grunow) EJ Cox	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis dicephala</i> (W. SMith) Mereschowsky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Planothidium rostratum</i> (Oestrup) Round & Bukhityarova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma salinarum</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma</i> sp.	5	0	0	0	0	0	0	0	0	0	3	10	15	0	0	0	0	0	0

<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	0	0	13	26	0	0	0	44	62	3	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibberula</i> (Ehrenberg) O Müller	8	0	0	0	0	0	0	0	18	60	0	0	0	0	0	0	0	0	0
<i>Rhopalodia musculus</i> (Kützing) O Müller	0	0	7	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
<i>Rhopalodia operculata</i> (Agardh) Håkansson	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sellaphora pupula</i> (Kützing) Mereschowsky sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42	0	0	0	0
<i>Sellaphora seminulum</i> (Grunow) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Seminavis strigosa</i> (Hustedt) Danieledis & Economou-Amilli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis anceps</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis smithii</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenopterobia</i> sp.	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirela</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella cruciata</i> A. Schmidt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella ovalis</i> Brébisson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synedra</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Tabularia fasciculata</i> (C. Agardh) D.M. Williams & Round	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella apiculata</i> (W. Greg.) D.G. Mann	0	0	2	0	0	0	2	0	0	43	13	13	10	6	26	29	0	0	0
<i>Tryblionella calida</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	8	15	0	0	0	0
<i>Tryblionella coarctata</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella gracilis</i> W. Smith	0	0	0	0	0	0	0	0	0	0	0	4	8	4	13	21	15	0	12
<i>Tryblionella hungarica</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella levidensis</i> (W. Smith) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0
<i>Tryblionella littoralis</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella punctata</i> W. Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table B-6: February 2018.

	P1	P2	P3	P4	P5	U1	U2	N1	N2	N3	NO	S1	S2	NI	FP
<i>Achnanthes inflata</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes standeri</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthidium</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Afrocymbella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora montana</i> Krasske	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora ovalis</i> (Kützing) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Amphora</i> sp.	0	0	18	0	1	10	0	11	0	0	0	0	0	0	0
<i>Amphora</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora strigosa</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora veneta</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	43	0	5	0	7	0	0	0	0	0	0	15	17	0	0
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O Müller) Simonsen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillaria paradoxa</i> Gmelin	0	0	0	0	0	0	6	0	0	0	11	0	0	0	0
<i>Caloneis bacillum</i> (Grunow) Cleve	0	0	0	0	0	0	0	0	0	0	0	9	19	0	0
<i>Caloneis silicula</i> (Ehrenberg) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
<i>Caloneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Campylodiscus clypeus</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Capartogramma crucicula</i> (Grunow ex Cleve) Ross	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis engelbrechtii</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis placentula</i> Ehrenberg	99	51	98	163	137	9	8	0	15	0	0	0	0	40	0
<i>Craticula accomoda</i> (Hustedt) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula ambigua</i> (Ehrenberg) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula buderi</i> (Hustedt)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula cuspidata</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula subminuscula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(Manguin) Lange-Bertalot															
<i>Cyclotella meneghiniana</i> Kützing	0	17	29	9	55	14	6	53	46	48	20	11	18	0	0
<i>Cyclotella ocellata</i> Pantocsek	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymatopleura solea</i> (Brébisson) W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0
<i>Cymbella cymbiformis</i> Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella neocistula</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella simonsenii</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella subleptoceros</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella tumida</i> (Brébisson) Van Heurck	0	0	4	8	3	0	11	0	0	0	0	0	0	0	0
<i>Cymbella turgidula</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbopleura</i> sp.	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
<i>Denticula</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula subtilis</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diadesmis confervacea</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diatoma vulgaris</i> Bory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis elliptica</i> (Kützing) Cleve	0	6	24	0	0	11	0	44	34	34	28	7	0	0	0
<i>Diploneis puella</i> (Schumann) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Diploneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis subovalis</i> Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis zanzibarica</i> (Grunow) Hustedt	0	0	8	0	0	0	1	0	4	4	0	0	0	0	0
<i>Discostella stelligera</i> (Hustedt) Houk & Klee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema mesianum</i> (Cholnoky) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema minutum</i> (Hilse) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema neogracile</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema silesiacum</i> (Bleisch) DG Mann	18	55	0	0	0	0	20	0	0	0	0	26	39	0	0
<i>Encyonema</i> sp.	0	0	0	0	0	0	0	8	21	69	14	26	18	0	0
<i>Encyonopsis microcephala</i> (Grunow) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eolimna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia adnata</i> (Kützing) Brébisson	0	0	0	5	6	0	0	0	0	0	0	0	0	0	0
<i>Epithemia sorex</i> Kützing	10	19	31	28	3	0	0	0	0	0	0	0	0	0	0
<i>Epithemia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia flexuosa</i> (Brébisson) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia formica</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia minor</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	4	12	37	0
<i>Eunotia pectinalis</i> var. <i>undulata</i> (Ralfs) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	25	7	0
<i>Eunotia</i> sp.	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Fallacia californica</i> Stancheva and Manoylov	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia pygmaea</i> (Kützing) Sickle & Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria biceps</i> (Kützing) Lange- Bertalot	10	25	37	58	76	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria capucina</i> var. <i>rumpens</i> (Kützing) Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ulna</i> (Nitzsch) Lange- Bertalot	0	0	0	0	48	0	15	0	0	0	0	12	62	0	0
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria</i> <i>ungeriana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Frustulia tugelae</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>affine</i> Kützing	0	91	0	0	0	0	0	0	0	0	0	15	12	34	17
<i>Gomphonema</i> <i>angustatum</i> (Kützing) Rabenhorst	31	0	0	33	3	0	0	0	0	0	0	8	0	0	0
<i>Gomphonema</i> <i>clavatum</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>exilissimum</i> Lange- Bertalot & Reichardt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>gracile</i> Ehrenberg sensu stricto	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>insigne</i> Gregory	30	0	0	0	0	0	0	0	0	0	0	15	0	0	0
<i>Gomphonema</i> <i>lagenula</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>laticollum</i> Reichart	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> <i>mexicanum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Grunow in Van Heurck															
<i>Gomphonema minutum</i> (C.Agardh) C.Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	0	40	16	28	5	0	42	0	0	0	14	23	0	28	0
<i>Gomphonema pseudoaugur</i> Krammer	0	6	17	0	10	0	49	0	0	0	0	12	10	21	0
<i>Gomphonema pumilum</i> var. <i>rigidum</i> Reichardt & Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> sp.	44	63	0	38	16	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema venusta</i> Passy, Kociolek & Lowe	74	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma macrum</i> (W. Smith) J.W. Griffith Henfrey	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma scalpoides</i> (Rabenhorst) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma</i> sp.	0	0	22	0	4	0	6	0	0	0	5	11	0	0	0
<i>Halamphora coffaeiformis</i> (Agardh) Kützing	0	0	10	0	0	0	0	15	20	34	17	0	0	0	0
<i>Halamphora</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0
<i>Hantzschia distinctepunctata</i> Hustedt	0	0	0	0	0	7	26	0	16	0	0	0	0	0	0

<i>Haslea spicula</i> (Hickie) Lange-Bertalot	0	0	0	0	0	0	0	62	26	61	44	0	0	0	0
<i>Hippodonta</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kolbesia kolbei</i> (Hustedt) F.E. Round & L. Bukhtiyarova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola mutica</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastoglia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia dansei</i> (Thwaites) Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia elliptica</i> (Agardh) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia smithii</i> Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula erifuga</i> (OF Müller) Bory	0	0	10	0	0	39	0	47	46	27	15	8	0	0	0
<i>Navicula germainii</i> Wallace	0	0	0	0	0	0	0	64	0	0	0	0	0	0	0
<i>Navicula gregaria</i> Donkin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula radiosa</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula rostellata</i> (Kützing) Cleve	0	0	0	0	0	0	30	0	22	0	0	0	0	0	0
<i>Navicula sovereignii</i> L.L. Bahls	0	0	0	0	0	0	57	0	0	0	0	0	0	0	0
<i>Navicula</i> sp.	26	0	18	0	0	42	16	0	0	0	0	23	32	33	10
<i>Navicula</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Navicula subrhynchocephala</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula symmetrica</i> Patrick	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	0	0	12	0	0	33	0	0	0	37	33	28	7	0	0
<i>Navicula zanonii</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neidium productum</i> (W. SMith) Cleve	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
<i>Nitzschia aerophila</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia agnita</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia capitellata</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>archibaldii</i> Lange-Bertalot	0	0	0	0	0	0	34	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>pellucida</i> Grunow	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0
<i>Nitzschia clausii</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia desertorum</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia dissipata</i> (Kützing) Grunow	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0
<i>Nitzschia etoshensis</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0
<i>Nitzschia filiformis</i> (W. SMith) Van Heurk	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0
<i>Nitzschia fontifuga</i> (n. spec.) Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia frustulum</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia hantzschia</i> Krammer & Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia intermedia</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia lancetulla</i> Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Nitzschia liebetruthii</i> Rabenhorst	0	0	0	0	0	0	0	0	27	0	0	0	0	0	0
<i>Nitzschia linearis</i> (C. Agardh) W. Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia littorea</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia minuta</i> Bleisch in Rabenh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia nana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia palea</i> (Kützinger) W. Smith	0	0	0	0	0	55	25	21	30	28	19	21	9	117	34
<i>Nitzschia paleacea</i> (Grunow) Grunow	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0
<i>Nitzschia perminuta</i> (Grunow in Van Heurck) H. Perag.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia pura</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia recta</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia reversa</i> W. Smith	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0
<i>Nitzschia sigma</i> (Kützinger) W. Smith	0	0	0	0	0	55	0	26	23	2	29	0	0	0	0
<i>Nitzschia siliqua</i> Archibald	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0
<i>Nitzschia</i> sp.	0	0	19	0	6	50	15	0	47	56	26	20	26	61	24
<i>Nitzschia</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	0	0	0	0	2	0	14	0	0	0	0	22	9	0	0
<i>Pinnularia acrosphaeria</i> W. Smith	0	0	0	0	0	0	0	0	0	0	0	15	12	4	0
<i>Pinnularia borealis</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Pinnularia divergens</i> W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia joculata</i> (Manguin) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia nodosa</i> (Ehrenb.) W.Sm.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia</i> sp.	0	0	0	0	0	0	0	0	0	0	14	8	0	0	0
<i>Pinnularia subbrevistriata</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia subcapitata</i> Gregory	0	0	0	0	0	0	0	0	0	0	0	18	36	11	0
<i>Pinnularia viridiformis</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis clementis</i> (Grunow) EJ Cox	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis dicephala</i> (W. SMith) Mereschkowsky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Planothidium rostratum</i> (Oestrup) Round & Bukhityarova	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0
<i>Pleurosigma salinarum</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	0	22	3	10	18	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibberula</i> (Ehrenberg) O Müller	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0

<i>Rhopalodia musculus</i> (Kützing) O Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia operculata</i> (Agardh) Håkansson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sellaphora pupula</i> (Kützing) Mereschkowsky sensu lato	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
<i>Sellaphora seminulum</i> (Grunow) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Seminavis strigosa</i> (Hustedt) Danielelis & Economou-Amilli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis anceps</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis smithii</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenopterobia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirela</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella cruciata</i> A. Schmidt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella ovalis</i> Brébisson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synedra</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia fasciculata</i> (C. Agardh) D.M. Williams & Round	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella apiculata</i> (W. Greg.) D.G. Mann	0	0	0	0	0	0	5	10	0	0	17	0	0	0	0
<i>Tryblionella calida</i> (Grunow) D.G. Mann	0	0	8	0	0	0	0	0	0	0	0	18	0	0	0

<i>Tryblionella coarctata</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
<i>Tryblionella gracilis</i> W. SMith	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0
<i>Tryblionella hungarica</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella levidensis</i> (W. Smith) Grunow	0	0	3	0	0	0	8	0	0	0	0	12	33	0	0
<i>Tryblionella littoralis</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella punctata</i> W. Smith	0	0	0	0	0	0	0	8	10	0	12	0	0	0	0
<i>Tryblionella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table B-7: May 2018.

	P1	P2	P3	P4	P5	U1	U2	N1	N2	N3	NO	S1	S2	NI	PP	ARP	FP	BP
<i>Achnanthes inflata</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthes standeri</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achnanthidium</i> sp.	0	0	0	0	0	0	0	0	0	0	0	9	9	0	0	0	0	0
<i>Afrocymbella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora montana</i> Krasske	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora ovalis</i> (Kützing) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora</i> sp.	0	0	0	0	8	0	0	8	10	9	15	8	7	0	16	0	0	0
<i>Amphora</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0	0	0
<i>Amphora strigosa</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora veneta</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	12	0	8	0	0	0	0	0	0	0	0	6	10	0	0	0	0	0

<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O Müller) Simonsen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillaria paradoxa</i> Gmelin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis bacillum</i> (Grunow) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis silicula</i> (Ehrenberg) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caloneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Campylodiscus clypeus</i> Ehrenberg	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
<i>Capartogramma crucicula</i> (Grunow ex Cleve) Ross	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis engelbrechtii</i> Chohnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cocconeis placentula</i> Ehrenberg	45	33	143	145	86	12	8	34	30	0	0	0	0	0	0	0	0	0
<i>Craticula accomoda</i> (Hustedt) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula ambigua</i> (Ehrenberg) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula buderi</i> (Hustedt)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula cuspidata</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Craticula</i> sp.	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0
<i>Craticula subminuscula</i> (Manguin) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella meneghiniana</i> Kützing	57	10	8	14	9	10	18	33	35	23	21	10	12	80	16	0	0	0
<i>Cyclotella ocellata</i> Pantocsek	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclotella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymatopleura solea</i> (Brébisson) W. SMith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella cymbiformis</i> Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella neocistula</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella simonsenii</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella subleptoceros</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella tumida</i> (Brébisson) Van Heurck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbella turgidula</i> Grunow	0	4	0	0	0	13	11	0	0	0	0	0	0	0	0	0	0	0
<i>Cymbopleura</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Denticula subtilis</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diademesmis confervacea</i> (Kützinger) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diatoma vulgaris</i> Bory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis elliptica</i> (Kützinger) Cleve	0	9	0	0	0	0	0	56	50	57	109	8	8	0	17	0	0	0
<i>Diploneis puella</i> (Schumann) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis subovalis</i> Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diploneis zanzibarica</i> (Grunow) Hustedt	0	0	6	4	0	7	9	0	0	0	0	0	0	0	0	0	0	0
<i>Discostella stelligera</i> (Hustedt) Houk & Klee	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema mesianum</i>	19	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0

(Cholnoky) DG Mann																		
<i>Encyonema minutum</i> (Hilse) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Encyonema neogracile</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	0
<i>Encyonema silesiacum</i> (Bleisch) DG Mann	0	0	0	0	0	0	0	0	0	0	0	9	5	25	0	0	49	0
<i>Encyonema</i> sp.	0	0	0	0	0	0	0	0	0	0	0	8	6	0	0	0	0	0
<i>Encyonopsis microcephala</i> (Grunow) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eolimna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia adnata</i> (Kützing) Brébisson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia sorex</i> Kützing	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia flexuosa</i> (Brébisson) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia formica</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eunotia minor</i> (Kützing) Grunow	10	0	0	9	0	7	3	0	0	0	14	7	8	0	0	0	16	0
<i>Eunotia pectinalis</i> var. undulata (Ralfs) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	48	0
<i>Eunotia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia californica</i> Stancheva and Manoylov	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fallacia pygmaea</i> (Kützing) Sickel & Mann	10	0	0	0	0	0	0	60	66	53	71	7	7	0	0	0	0	0
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	20	0	8	10	0	0	0	0	0	0	0	0	0	52	0	0	28	0
<i>Fragilaria capucina</i> var. rumpens (Kützing) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Fragilaria</i> sp.	0	0	0	0	0	10	14	0	0	0	0	9	12	0	0	0	0	0
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot	0	0	0	0	15	7	9	0	0	6	10	0	0	55	0	0	33	0
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria ungeriana</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Frustulia tugelae</i> Chohnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema affine</i> Kützing	26	23	0	17	0	0	0	16	10	0	19	20	22	20	0	0	71	0
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema clavatum</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema exilissimum</i> Lange-Bertalot & Reichardt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema gracile</i> Ehrenberg sensu stricto	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema insigne</i> Gregory	25	9	0	0	65	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema lagenula</i> Kützing	21	0	5	31	74	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema laticollum</i> Reichardt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema mexicanum</i> Grunow in Van Heurck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema minutum</i> (C.Agardh) C.Agardh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	0	0	0	0	0	0	0	15	18	0	46	0	0	0	16	0	0	0
<i>Gomphonema pseudoaugur</i> Krammer	28	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0

<i>Gomphonema pumilum</i> var. <i>rigidum</i> Reichardt & Lange-Bertalot	15	0	17	22	95	7	6	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> sp.	0	37	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0
<i>Gomphonema venusta</i> Passy, Kociolek & Lowe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma macrum</i> (W. Smith) J.W. Griffith Henfrey	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma scalpoides</i> (Rabenhorst) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gyrosigma</i> sp.	0	23	0	7	8	16	18	3	5	10	0	0	0	0	36	0	0	0
<i>Halamphora coffeaeformis</i> (Agardh) Kützing	0	0	29	5	0	0	0	0	0	15	0	0	0	0	0	0	0	0
<i>Halamphora</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hantzschia distinctepunctata</i> Hustedt	0	0	4	0	0	5	7	0	0	0	0	0	0	10	0	0	0	0
<i>Haslea spicula</i> (Hickie) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hippodonta</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kolbesia kolbei</i> (Hustedt) F.E. Round & L. Bukhtiyarova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola mutica</i> (Kützing) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Luticola</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Mastoglia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia dansei</i> (Thwaites)																		
Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia elliptica</i> (Agardh) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mastogloia smithii</i> Thwaites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0
<i>Navicula erifuga</i> (OF Müller) Bory	0	16	44	17	0	23	19	7	8	23	0	30	36	0	16	0	0	0
<i>Navicula germainii</i> Wallace	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula gregaria</i> Donkin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0
<i>Navicula radiosa</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	0	0	0	0	0	0	0	0	0	0	0	18	15	0	0	0	0	0
<i>Navicula rostellata</i> (Kützing) Cleve	0	16	0	27	0	28	30	24	20	0	22	27	30	0	0	0	11	0
<i>Navicula</i> <i>sovereignii</i> L.L. Bahls	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp.	26	33	19	9	0	19	24	0	0	13	0	45	32	19	29	0	0	0
<i>Navicula</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> <i>subrhynchocephala</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> <i>symmetrica</i> Patrick	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	0	0	5	0	0	16	14	46	40	50	18	20	20	0	17	0	0	0
<i>Navicula zanonii</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neidium productum</i> (W. Smith) Cleve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia aerophila</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Nitzschia agnita</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia capitellata</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>archibaldii</i> Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> cf. <i>pellucida</i> Grunow	0	0	0	0	0	0	0	11	15	0	0	0	0	0	0	0	0	0
<i>Nitzschia clausii</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia desertorum</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia dissipata</i> (Kützing) Grunow	0	0	0	6	0	14	13	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia etoshensis</i> Cholnoky	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia filiformis</i> (W. SMith) Van Heurk	0	0	0	0	0	19	25	18	18	37	0	0	0	0	0	0	0	0
<i>Nitzschia fontifuga</i> (n. spec.) Cholnoky	0	0	0	0	0	0	0	0	0	0	0	8	8	0	0	0	0	0
<i>Nitzschia frustulum</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia hantzschia</i> Krammer & Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia intermedia</i> Hantzsch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia lancetulla</i> Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia liebetruthii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia linearis</i> (C. Agardh) W. Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia littorea</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia minuta</i> Bleisch in Rabenh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia nana</i> Grunow	0	0	0	0	0	0	0	13	13	0	0	0	0	0	0	0	0	0

<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia palea</i> (Kützinger) W. Smith	0	0	27	21	24	18	11	0	0	0	20	25	25	0	18	0	17	0
<i>Nitzschia paleacea</i> (Grunow) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia perminuta</i> (Grunow in Van Heurck) H. Perag.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia pura</i> Hustedt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia recta</i> Hantzsch	0	3	20	15	16	20	22	0	0	0	0	24	28	0	11	0	14	0
<i>Nitzschia reversa</i> W. Smith	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia sigma</i> (Kützinger) W. Smith	0	46	5	0	0	82	71	0	0	27	20	0	0	0	17	0	14	0
<i>Nitzschia siliqua</i> Archibald	0	0	0	0	0	0	0	17	25	0	0	19	19	0	0	0	0	0
<i>Nitzschia</i> sp.	33	31	27	15	0	23	26	0	0	13	0	41	36	0	34	0	0	0
<i>Nitzschia</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	0	9	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	0
<i>Pinnularia acrosphaeria</i> W. Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia borealis</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia divergens</i> W. Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0
<i>Pinnularia joculata</i> (Manguin) Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia nodosa</i> (Ehrenb.) W.Sm.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia</i> sp.	0	0	0	0	0	0	0	0	0	9	0	5	9	0	0	0	0	0
<i>Pinnularia subbrevistriata</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Pinnularia subcapitata</i> Gregory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia viridiformis</i> Krammer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis clementis</i> (Grunow) EJ Cox	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Placoneis dicephala</i> (W. SMith) Mereschkowsky	0	0	0	0	0	0	0	0	0	0	0	8	8	0	0	0	0	0
<i>Placoneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Planothidium rostratum</i> (Oestrup) Round & Bukhityarova	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma salinarum</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange- Bertalot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	0	0	0	8	0	0	0	0	0	0	0	8	11	0	0	0	0	0
<i>Rhopalodia gibberula</i> (Ehrenberg) O Müller	0	20	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0
<i>Rhopalodia musculus</i> (Kützing) O Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia operculata</i> (Agardh) Håkansson	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sellaphora pupula</i> (Kützing) Mereschkowsky sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Sellaphora seminulum</i> (Grunow) DG Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Seminavis strigosa</i> (Hustedt) Danieledis & Economou-Amilli	0	0	25	5	0	0	0	21	21	0	15	0	0	0	0	0	58	0
<i>Stauroneis anceps</i> Ehrenberg sensu lato	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis smithii</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0
<i>Stenopterobia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirela</i> sp.	0	5	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella cruciata</i> A. Schmidt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Surirella ovalis</i> Brébisson	0	0	0	0	0	12	11	0	0	11	0	0	0	0	0	0	0	0
<i>Synedra</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia fasciculata</i> (C. Agardh) D.M. Williams & Round	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabularia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella apiculata</i> (W. Greg.) D.G. Mann	0	14	0	9	0	13	9	12	10	14	0	9	9	15	13	0	0	0
<i>Tryblionella calida</i> (Grunow) D.G. Mann	0	0	0	0	0	11	10	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella coarctata</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella gracilis</i> W. SMith	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella hungarica</i> (Grunow) D.G. Mann	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0
<i>Tryblionella levidensis</i> (W. Smith) Grunow	0	0	0	0	0	8	12	0	0	0	0	12	8	15	23	0	13	0

<i>Tryblionella littoralis</i> (Grunow) D.G. Mann	0	12	0	0	0	0	0	0	0	13	0	0	0	0	20	0	18	0
<i>Tryblionella punctata</i> W. Smith	0	0	0	0	0	0	0	6	6	0	0	0	0	0	0	0	0	0
<i>Tryblionella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix C: Diatom taxa identified and counts for each Microcosm for the exposure period.

Table C-1: Pre-exposure.

Microcosms	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Achnanthyidium</i> sp.	155	0	22	0	75	21	30	0	83	27	17	80	194	0	118	75	38	0
<i>Amphipleura pellucida</i> (Kützing) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora</i> sp.	92	116	98	0	86	85	96	0	132	94	108	75	16	67	96	78	105	73
<i>Caloneis</i> sp.	57	0	22	0	27	17	13	0	0	19	0	0	55	37	25	20	33	50
<i>Cocconeis pediculus</i> Ehrenberg	14	0	0	0	0	0	0	0	0	7	22	0	12	0	0	0	0	0
<i>Cocconeis placentula</i> Ehrenberg	6	11	13	0	0	0	18	0	29	14	8	20	18	20	20	0	20	23
<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	3	0	0	0	0	0	0	0	0	0	0	0	0	24	0	9	0	0
<i>Cymboplectura amphicephala</i> (Naegeli) Krammer	13	0	26	0	19	12	20	0	0	19	16	0	0	16	13	41	15	18
<i>Diatoma vulgare</i> Bory	5	15	11	0	0	13	5	0	0	11	0	0	0	0	4	0	18	0
<i>Epithemia adnata</i> (Kützing) Brébisson	4	58	73	0	14	48	47	0	26	69	51	34	17	36	35	31	22	18
<i>Eunotia minor</i> (Kützing) Grunow	3	14	10	0	18	0	21	0	0	0	10	20	0	0	0	22	13	31
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	36	20	0	0	48	26	7	0	0	0	0	0	0	57	0	0	94	97
<i>Fragilaria capucina</i> Desmazières	12	16	60	0	84	87	90	0	61	68	86	60	70	52	28	69	27	38
<i>Gomphonema acuminatum</i> Ehrenberg	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Gomphonema affine</i> Kützing	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> sp.	0	59	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	0	34	0	0	0	0	18	0	0	0	11	0	0	0	11	0	15	0
<i>Mastoglia</i> sp.	0	9	0	0	9	0	0	0	10	11	15	15	0	20	0	11	0	10
<i>Navicula</i> sp.	0	0	33	0	0	25	31	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula</i> sp. 2	0	0	21	0	0	10	0	0	0	0	0	16	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia linearis</i> (Agardh) W Smith	0	0	0	0	14	0	4	0	0	17	13	0	18	0	0	9	0	0
<i>Nitzschia palea</i> (Kützing) W Smith	0	0	0	0	6	0	0	0	0	8	0	0	0	19	0	9	0	0
<i>Nitzschia recta</i> Hantzsch	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia sigma</i> (Kützing) W Smith	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp.	0	0	0	0	0	20	0	0	59	36	43	20	0	45	20	15	0	0
<i>Pinnularia</i> sp.	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pleurosigma salinarum</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	29	0	0	0	11	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	7	16	0	0	31
<i>Rhopalodia musculus</i> (Kützing) O Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0
<i>Stauroneis smithii</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
<i>Surirela</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tryblionella apiculata</i> Gregory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Tryblionella gracilis</i> W Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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Table C-2: 96 hour exposure.

Microcoms	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Achnanthyidium</i> sp.	0	0	16	33	30	14	14	0	10	0	0	42	43	91	52	55	91	91
<i>Amphipleura pellucida</i> (Kützing) Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphora</i> sp.	36	19	56	99	87	84	52	103	22	0	0	102	108	102	64	101	87	101
<i>Caloneis</i> sp.	26	0	19	0	10	0	0	20	0	0	0	19	0	11	31	13	3	28
<i>Cocconeis pediculus</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0	0
<i>Cocconeis placentula</i> Ehrenberg	16	0	0	10	0	12	6	17	10	0	0	15	31	15	10	9	9	10
<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	0	0	0	0	0	0	0	0	0	0	0	0	0	9	12	24	0	0
<i>Cymbopleura amphicephala</i> (Naegeli) Krammer	0	26	14	10	0	22	45	28	0	0	0	13	17	10	40	13	26	30
<i>Diatoma vulgare</i> Bory	0	0	0	13	10	0	0	6	0	0	0	10	7	0	5	0	3	0
<i>Epithemia adnata</i> (Kützing) Brébisson	58	43	129	50	41	97	79	73	46	0	0	80	85	56	79	50	64	82
<i>Eunotia minor</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	10	11	7
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	55	0	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0	0
<i>Fragilaria capucina</i> Desmazières	0	0	15	15	0	29	26	35	0	0	0	11	19	0	16	44	23	15

<i>Gomphonema acuminatum</i> Ehrenberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema affine</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> sp.	0	0	0	0	0	0	34	28	0	0	0	0	0	27	0	0	0	0
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	0	0	0	0	14	0	0	0	0	0	0	0	16	0	0	0	0	0
<i>Mastoglia</i> sp.	0	0	6	0	0	0	0	0	0	0	0	0	12	8	9	4	8	8
<i>Navicula</i> sp.	0	32	28	45	0	44	37	30	38	0	0	17	26	0	15	12	18	22
<i>Navicula</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia linearis</i> (Agardh) W Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0
<i>Nitzschia palea</i> (Kützing) W Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0
<i>Nitzschia recta</i> Hantzsch	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	7	0	0
<i>Nitzschia sigma</i> (Kützing) W Smith	0	0	0	0	7	0	14	0	0	0	0	17	0	0	9	0	0	0
<i>Nitzschia</i> sp.	28	0	83	41	53	0	46	34	37	0	0	28	36	29	39	39	25	0
<i>Pinnularia</i> sp.	0	0	0	42	0	0	0	0	43	0	0	0	0	0	0	0	22	0
<i>Pleurosigma salinarum</i> Grunow	0	0	0	0	11	14	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	0	19	34	33	25	45	3	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia musculus</i> (Kützing) O Müller	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis smithii</i> Grunow	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
<i>Surirela</i> sp.	0	0	0	0	0	0	0	0	32	0	0	28	0	0	0	0	0	0

<i>Tryblionella apiculata</i> Gregory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
<i>Tryblionella gracilis</i> W Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	2	6	6	8	6

Table C-3: 28 day exposure.

Microcoms	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Achnanthyidium</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphipleura pellucida</i> (Kützing) Kützing	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	15	13	0
<i>Amphora</i> sp.	9	0	0	10	0	0	14	10	8	10	14	0	10	5	31	18	22	18
<i>Caloneis</i> sp.	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
<i>Cocconeis pediculus</i> Ehrenberg	39	41	31	67	52	56	7	63	56	0	46	86	52	42	47	30	35	36
<i>Cocconeis placentula</i> Ehrenberg	131	93	21	63	55	59	5	66	68	15	61	73	61	50	45	45	43	42
<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	0	21	22	18	5	15	0	23	18	6	44	16	16	10	0	20	17	14
<i>Cymbopleura amphicephala</i> (Naegeli) Krammer	54	58	58	70	60	68	20	74	68	10	81	74	0	0	50	48	44	38
<i>Diatoma vulgare</i> Bory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epithemia adnata</i> (Kützing) Brébisson	0	11	18	24	8	12	8	15	17	0	16	0	0	0	0	13	15	0
<i>Eunotia minor</i> (Kützing) Grunow	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	21	22	0
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	84	30	25	11	13	16	0	0	25	0	15	21	18	8	0	15	24	11

<i>Fragilaria capucina</i> Desmazières	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphonema acuminatum</i> Ehrenberg	0	0	17	10	0	0	0	12	8	0	0	0	0	0	0	0	0	0
<i>Gomphonema affine</i> Kützing	16	11	0	0	8	0	0	12	12	0	0	0	0	0	0	0	0	0
<i>Gomphonema</i> sp.	0	0	25	14	5	15	0	13	17	0	12	0	15	7	28	18	12	12
<i>Gyrosigma attenuatum</i> (Kützing) Cleve	11	20	15	23	20	16	12	21	17	0	26	16	17	9	16	38	42	21
<i>Mastoglia</i> sp.	6	0	12	0	0	0	0	0	0	0	0	20	0	0	28	0	0	0
<i>Navicula</i> sp.	10	38	34	33	33	40	14	34	32	8	27	45	33	22	28	22	19	15
<i>Navicula</i> sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Navicula veneta</i> Kützing	8	0	27	18	16	23	13	16	14	5	0	0	0	0	43	18	17	12
<i>Nitzschia linearis</i> (Agardh) W Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia palea</i> (Kützing) W Smith	11	0	0	0	0	0	0	0	0	0	0	0	0	0	48	18	25	4
<i>Nitzschia recta</i> Hantzsch	0	0	16	0	0	18	0	0	0	0	18	0	0	0	0	0	0	0
<i>Nitzschia sigma</i> (Kützing) W Smith	5	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia</i> sp.	8	13	31	26	32	37	13	34	36	5	22	32	36	34	36	29	21	13
<i>Pinnularia</i> sp.	0	0	0	0	6	0	0	0	0	0	0	17	0	0	0	0	0	0
<i>Pleurosigma salinarum</i> Grunow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	0	11	14	13	0	10	0	7	4	0	0	0	0	0	0	0	0	0
<i>Rhopalodia musculus</i> (Kützing) O Müller	0	12	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stauroneis smithii</i> Grunow	8	15	16	0	9	15	0	0	0	0	10	0	8	4	0	18	12	0

<i>Surirela</i> sp.	0	0	0	0	0	0	0	0	0	0	8	0	6	0	0	0	0	0
<i>Tryblionella</i> <i>apiculata</i> Gregory	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	14	17	0
<i>Tryblionella</i> <i>gracilis</i> W Smith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix D: Stable isotope data from the sampled sites during February 2017 and May 2017.

Table D-1: February 2017. Numbers in brackets represent replicate number.

Site	Sample Weight	C ¹³	N ¹⁵	Site	Sample Weight	C ¹³	N ¹⁵
P1 (1)	5.11	-11.56	4.80	NI (1)	5.06	-28.38	11.76
P1 (2)	5.20	-11.24	4.87	NI (2)	5.17	-28.54	11.81
P1 (3)	5.19	-11.39	4.73	NI (3)	5.11	-28.55	11.86
P3 (1)	5.04	-18.14	3.30	N1 (1)	5.11	-17.12	8.03
P3 (2)	5.01	-18.25	3.35	N1 (2)	5.11	-16.94	8.03
P3 (3)	5.14	-18.14	3.28	N1 (3)	5.08	-17.16	8.00
P4 (1)	5.19	-24.63	5.51	N2 (1)	5.16	-17.67	7.28
P4 (2)	5.16	-24.57	5.39	N2 (2)	5.07	-17.63	7.21
P4 (3)	5.09	-24.60	5.37	N2 (3)	5.20	-17.61	7.05
P5 (1)	5.09	-22.83	7.17	U1 (1)	5.09	-23.94	6.53
P5 (2)	5.02	-22.78	8.15	U1 (2)	5.00	-23.60	6.52
P5 (3)	5.08	-22.79	7.48	U1 (3)	5.14	-23.78	6.42
P6 (1)	5.06	-25.33	6.76	S1 (1)	5.15	-27.63	9.97
P6 (2)	5.13	-25.22	7.07	S1 (2)	5.13	-27.80	10.19
P6 (3)	5.06	-25.18	6.26	S1 (3)	5.04	-27.81	10.25

Table D-2: May 2017. Numbers in brackets represent replicate number.

Site	Sample Weight	C ¹³	N ¹⁵	Site	Sample Weight	C ¹³	N ¹⁵
P1 (1)	5.05	-17.95	7.14	NO (1)	5.17	-23.94	9.93
P1 (2)	5.11	-17.55	6.82	NO (2)	5.14	-24.39	10.18
P1 (3)	5.14	-17.50	6.86	NO (3)	5.11	-24.54	9.79
P3 (1)	5.18	-21.06	9.19	N1 (1)	5.02	-24.63	5.81
P3 (2)	5.14	-21.11	9.34	N1 (2)	5.15	-24.64	6.44
P3 (3)	5.17	-20.82	9.13	N1 (3)	5.11	-24.48	6.39
P4 (1)	5.07	-21.60	6.47	N2 (1)	5.01	-17.25	6.88
P4 (2)	5.13	-20.52	6.83	N2 (2)	5.08	-17.54	6.59
P4 (3)	5.12	-21.05	6.19	N2 (3)	5.12	-17.17	6.93
P5 (1)	5.06	-22.45	8.21	S1 (1)	5.07	-26.23	7.04
P5 (2)	5.15	-22.35	8.37	S1 (2)	5.04	-26.14	7.22
P5 (3)	5.02	-22.38	8.16	S1 (3)	5.02	-26.20	7.09
U1 (1)	5.12	-23.57	6.27				
U1 (2)	5.08	-24.09	6.36				
U1 (3)	5.03	-23.53	6.28				