Diatoms as water quality indicators in the upper reaches of the Great Fish River, Eastern Cape

M. Holmes

22331948

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Supervisor: Dr JC Taylor

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PREFACE

The article format was chosen for this thesis. Research for this report was conducted under the guidance of Dr J.C. Taylor who is listed as co-author in the article. The article has been submitted to the African Journal of Aquatic Science with the title “**Diatoms as water quality indicators in the upper reaches of the Great Fish River, Eastern Cape, South Africa”.** The article is Chapter 3 in this thesis.

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ABSTRACT

The Karoo has been exposed to some form of agricultural activity since the early 1900s. This has resulted in the modification of the rivers in this region. Modifications include weirs, dams and removal of riparian vegetation either for planting crops or livestock grazing. These modifications, together with other agricultural effects such as overgrazing, have impacted the quality of the water in the rivers.

Chemical analysis is expensive and does not integrate all the biotic factors affecting aquatic ecosystems. For farmers to even consider monitoring their effects on the water quality a less expensive and more integrated form of monitoring needs to be considered. For most farmers the absolute values offered by the traditional chemical analysis are meaningless. Diatoms indices, on the other hand, can provide a more meaningful analysis of their water quality.

With this in mind the main aim of this study was to determine if diatoms could be used for biomonitoring in the semi arid conditions in the Karoo. The section of the Great Fish River which is spring fed was chosen for monthly monitoring from 2010 to 2012. Biological samples, and water samples for physical and chemical analysis were collected simultaneously from 5 sites. A total of 51 diatom genera with 269 taxa were identified. Dominant taxa (>5% relative abundance at all sites over all samples) were *Amphora pediculus* (Kützing) Grunow, *Craticula buderi* (Hustedt) Lange-Bertalot, *Fragilaria biceps* (Kützing) Lange-Bertalot, *Nitzschia frustulum* (Kützing) Grunow, *Nitzschia paleacea* (Grunow) Grunow in van Heurck, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot and *Rhopalodia gibba* (Ehrenberg) O.Müller. These species are predominantly pollution tolerant diatoms.

The Generic Diatom Index (GDI), the Specific Pollution sensitivity Index (SPI), the Biological Diatom Index (BDI) and the % Pollution Tolerant Valves (%PTV – part of the UK Trophic Diatom Index TDI) were used to infer that the river water was impacted by agricultural activity. These European diatom indices (GDI, BDI and %PTV) were used. The SPI with the South African Diatom Index (SADI) database was evaluated. Significant correlations were established between the indices and pH, NO_3-N and NH_4-N. Canonical Correspondence Analysis (CCA) was performed to determine the most important environmental variables. These were Electrical Conductivity (EC) and NO_3-N with the same gradient, followed by pH and PO_4 again with an almost identical gradient. It was concluded that decades of agricultural activity has had a negative impact on the water quality and the main drivers for the diatom community composition was EC and NO_3-N. This result is validated by the abundance of *Nitzschia* species. A large number of deformed valves were encountered. Neither the chemical analysis nor the diatom indices were able to explain the high number of deformed valves and the severity of these deformities observed.

KEYWORDS: agricultural impacts, aquatic ecosystem, biomonitoring, deformed diatoms, epilithic diatoms, freshwater, Karoo

UITTREKSEL

Die Karoo is blootgestel aan landbou-aktiwiteite sedert die vroeë 1900's. Dit het dit 'n verandering in riviere veroorsaak. Veranderinge sluit keerwalle, damme en verwydering van oewerplantegroei wat vir of aanplanting van gewasse of veeweiding gebruik word, in. Hierdie veranderinge, tesame met ander landbou-effekte soos oorbeweiding, kan 'n afname in die kwaliteit van die rivierwater veroorsaak.

Chemiese analises is duur en sluit nie al die biotiese faktore wat die akwatiese sisteem affekteer in nie. As boere hul invloed op waterkwaliteit wil oorweeg, sal 'n minder duur en 'n meer geïntegreerde vorm van monitering oorweeg moet word. Vir meeste boere is die absolute waardes, wat deur tradisionele chemiese analises verkry word, nie sinvol nie. Aan die ander kant, kan diatoomindekse 'n meer betekenisvolle analise van hul waterkwaliteit gee.

Hiermee in gedagte, was die hoofdoel van hierdie studie om te bepaal of diatome vir biomonitering in semi-droë toestande van die Karoo gebruik kan word. Die deel van die Groot Visrivier, wat gedurende die lente gevoed word, is maandelikse gemoniteer vanaf 2010 tot 2012. Biologiese monsters, en watermonsters vir chemiese en fisiese analises, was terselfdertyd in die 5 gebiede versamel. 'n Totaal van 51 diatoom genera met 269 taksa is geïdentifiseer. Dominante taksa (>5% relatiewe volopheid in al die monsters by al die gebiede) was *Amphora pediculus* (Kützing) Grunow, *Craticula buderi* (Hustedt) Lange-Bertalot, *Fragilaria biceps* (Kützing) Lange-Bertalot, *Nitzschia frustulum* (Kützing) Grunow, *Nitzschia paleacea* (Grunow) Grunow in van Heurck, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot en *Rhopalodia gibba* (Ehrenberg) O. Müller. Hierdie spesies is hoofsaaklik besoedeling-tolerante diatome.

Die diatoomindekse, naamlik die Generiese-diatoomindeks (GDI), die Spesifieke Besoedelingsensitiwiteitsindeks (SPI), die Biologiese-diatoomindeks (BDI) en die Persentasie Besoedelingtolerante Valvas (%PTV – deel van die VK se Tropiese-diatoomindeks TDI) is gebruik om 'n gevolgtrekking te maak dat die rivier deur landbou aktiwiteite geaffekteer is. Hierdie Europese Diatoomindekse (GDI, BDI en %PTV) is gebruik. Die SPI tesame met die Suid-Afrikaanse Diatoomindeks (SADI) databasis is geëvalueer. Betekenisvolle korrelasies tussen die indekse en pH, NO_3-N en NH_4-N is vasgestel. "Canonical Correspondence Analysis (CCA)" is gebruik om die belangrikste omgewingsvfaktore te bepaal. Elektriese Geleiding (EC) en NO_3-N het dieselfde gradiënt gehad asook pH en PO_4 wat byna dieselfde gradiënt gehad het.

Die gevolgtrekking word dus gemaak dat dekades van landbou-aktiwiteite ‘n negatiewe effek op waterkwaliteit gehad het. Die hoofdrywers vir die diatoomgemeenskapsamestelling was EC en NO_3-N. Hierdie resultaat is bevestig deur die volopheid van *Nitzschia* spesies. ‘n Groot hoeveelhuid van valvas wat misvorm was is gesien. Die hoë aantal misvormde valvas of die graad van die misvormdheid wat waargeneem was, kon nie deur die chemiese analises of die diatoomindekse verklaar word nie.

SLEUTELWOORDE: akwatiese ekosisteem, biomonitering, epilitiese diatome, Karoo, landbou-effekte, misvormde diatome, varswater

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CHAPTER 1 INTRODUCTION

1.1 Background

Most rivers in South Africa are eutrophic or face the threat of eutrophication and have been severely modified (Davies and Day, 1998; Taylor, 2004). These modifications include the construction of many dams and weirs. Cultural eutrophication occurs when a water body is loaded with allochthonous nitrogen and phosphorus with the latter usually having more of an impact. This in most instances occurs due to human impacts (Taylor, 2004). These changes have negative effects on the aquatic ecosystem and cause an imbalance in the trophic relationships with the system (Smol, 2009). With only ~0.01% of the earth's water contained in freshwater rivers and lakes (Smol, 2009) it is imperative that negative impacts be monitored and minimised where possible. In South Africa the average annual rainfall, for the country as a whole, is 464mm which is less than the global average of 860mm (SouthAfrica.info, 2013). Water scarcity in South Africa is nothing new but the increased pressure on this resource requires ongoing monitoring.

Freshwater ecosystems are influenced by the catchment size and extent, drainage, air pollution and groundwater inflows (Smol, 2009). The Great Fish River has a large catchment area of 30 000km^2 (ewater.co.za, 2005) with many smaller tributaries which, together with the altitude variation, often results in flooding. The largest tributary, the Willem Burgers River, is situated in the northern part of the Sneeuberg mountain range and enters the Great Fish River on the farm Glen Owen. This convergence is between the second and third sample sites of this study.

The author of the present study was unable to find previous research on diatoms on the upper section of the Great Fish River. Previous research in the vicinity was done by Archibald during the period 1967 to 1969 on the section of the Great Fish River catchment outside the current study sites (Archibald, 1983). His study was performed prior to the building of the irrigation tunnel to bring water from the Gariep Dam into the Great Fish River. This inter-basin transfer from the Orange River is done through the Teebusspruit and Brak River before entering the Great Fish River. This tunnel is situated near Steynsburg (Eastern Cape Province) which is approximately 100km north of the lowest site in this study, Retreat. This inter-basin transfer changes the characteristics of the water downstream where it becomes a larger fast flowing river. The last sample site in this study, Retreat, is 5km before the confluence of these two rivers.

1.2 Biomonitoring and diatom index development

Historically 'water quality' has mostly been interpreted through chemical analysis. Although providing valuable information chemical analysis on its own does not give a holistic picture of conditions in the river system (Cholnoky, 1968, Kriel, 2008). Chemical analysis only reveals the 'water quality' at the time of sampling whereas diatom analyses, or other bioindicator techniques, reveal the actual effects of pollutants in the river (Taylor 2004). Biomonitoring is not a new concept. In 1901 the saprobian system was suggested by Lauterborn which classified a slowing moving water body into different zones depending on increasing decomposition using, at first, algae, and then later other organisms (Harding and Taylor, 2011). Although this system used an individual species based approach, it resulted in the development of the first numeric biotic indices. From here on indices were improved and increased in use. In 1949 two different researchers, although working independently, developed a similar index which was to gain popularity. This index is today known as the Shannon Wiener Index (Wiener, 1948; Shannon and Weaver, 1948). This index was used to measure diversity in a freshwater stream. There were several disadvantages in using this index. The values were affected by seasonality and taxonomic identification and as a single species was used to calculate the index value, no community composition information was used. Problems also arose when species that were not in the calculation were ignored resulting in lost or skewed data. Biotic indices were steadily improved upon and in 1961 Zelinka and Marvan developed a formula which is the basis of several biotic indices today. Importantly, they assigned an 'indicator weight' to different organisms.

The Generic Diatom Index (GDI Coste and Ayphassorho, 1991) functions at a genus level and contains 174 taxa (Taylor, 2004). This index does not require identification to species level but instead allows for identification to genus level only. The more inclusive Specific Pollution sensitivity Index (SPI) takes factors such as salinity, eutrophication and organic pollution into account. This index is based on improvements by Coste (CEMAGREF, 1982) to Descy's Index. This index takes 2035 taxa into account (Taylor, 2004) and has been adapted to include taxa endemic to and commonly found in South Africa and is known as the South African Diatom Index (SADI) (Harding and Taylor, 2011). The parameters evaluated in the Biological Diatom Index (BDI) are pH, EC, BOD, percentage of oxygen saturation and nutrients (NH_4, NO_3 and PO_4) (Debenest *et al*., 2008). Developed in France, it allowed for standardisation of the methods used in that country for the determination of water quality. It originally included 209 taxa in index calculations which did not produce reliable results for brackish water. This and other shortcomings were addressed and a revised BDI-2006 (Besse-Lototskaya *et al*., 2011) which contained 838 ‘key species’ as well as 146 ‘abnormal forms’. The authors also found that when tested against the SPI it produced comparable results. According to Besse-Lototskaya *et al*. (2011) the parameters taken into account are pH, EC, dissolved oxygen, biological oxygen demand, ammonium, orthophosphates and nitrates. The Percentage Pollution Tolerant Valves (%PTV) is part of the UK Trophic Diatom Index (TDI)(Kelly and Whitton, 1995) and reflects organic pollution and has a maximum value of 100. Values above 20% indicate possible impact from organic pollution.

As mentioned above, water quality was historically determined by the chemical analysis and determining physical variables. This is far from ideal as these variables are influenced by various factors and only provides a ‘snap-shot analysis’. In 1994 the River Health Programme (RHP) was launched by DWAF. This programme sought to use bioindicators (fish, aquatic invertebrates, riparian vegetation) to monitor the health of the different river ecosystems in South Africa. The South African Scoring System (SASS) and the Fish Health Index (FHI) were introduced in an attempt to give a holistic view of the health of aquatic ecosystems. The communities that live in the water are constantly exposed to the conditions in the water and their analysis would provide an integrated analysis (Chutter, 1998). Historically macro and microinvertebrates have been suggested as bioindicators (Dickens and Graham, 2002). Taylor *et al*. (2007c) found that diatom autecological information could be included in a South African Diatom Index (modified SPI) which could be used for biomonitoring under South African conditions (Harding and Taylor, 2011). Endemic species were added and indicator and tolerance values assigned to them for this index.

Diatoms have specific environmental tolerances. A number of different diatom indices have been developed, mainly in Europe. As these indices are based on abundance of each species it is necessary to establish a list of the species that occur in a water body. According to De la Rey (2007) species diversity in a system will be reduced in the face of pollution and that highest diversity would be at mesotrophic levels. Archibald (1972) suggested that using species diversity alone would not give a true reflection of pollution. These tolerance levels among the diatoms allowed for a number of diatom indices to be developed which in turn allow for the inference of water quality (Kalyoncu and Serbetci, 2013) through allocation of numeric values. Diatom indices use the autoecological values assigned to specific taxa to allow for quantification based on the relative abundances of a species and its response to environmental variables. A number of these diatom indices have been tested in Europe, North America and South Africa.

The nature of the river in this study area is not conducive to making use of invertebrate based indices such as SASS5. During dry periods there is little or no water while during extreme rain periods the river floods regularly. These fluctuations make it difficult for invertebrate communities to establish. Riprarian vegetation has been modified and overgrazed. River conditions (mostly shallow, lack of cover, lack of cobble beds and riffles) are not conducive to the survival of fish populations.

1.3 Diatoms

Bacillariophyta, commonly referred to as diatoms, are unicellular organisms that are autotrophic. Their unique morphology (ornamented silica cell walls) makes them ideal for biomonitoring as this characteristic allows identification to species level. As a large part of the epilithic algal community, they are the base of the food chain and are responsible for generating some of the oxygen in the water (Round *et al*. 1990; Taylor, 2004; Busch, 2011). They are sensitive to nutrient concentration changes (Pan *et al*., 1996). Diatoms are abundant in many aquatic or moist habitats and occur over a wide range of trophic levels. The diatom communities usually consist of a fair number of species and their response to an environmental impact is rapid. Epilithic diatoms are unable to move out of their environment and would therefore be excellent indicators of water quality at a particular site. Although broad scale factors will have an impact on the diatom community composition it is the local conditions that will have more of an influence (Bere *et al*., 2013). The correlation between diatoms and the environmental variables is quantifiable and this makes them good indicators (McCormick and Cairns, 1994; Pan *et al*., 1996; Bere and Tundisi, 2011). According to Taylor *et al.* (2005a) diatoms are, in certain circumstances (e.g. concrete canals), the favoured method of biomonitoring.

Diatom flora identification and taxonomy in South Africa has been ongoing since the middle of the 19th century. However, research on South African diatom flora stalled after the work of Cholnoky (1952-1972; Kriel, 2008), Giffen (1960s – 1970s), Schoeman and Archibald in the 1970s and 1980s but in the mid 1990s (Bate *et al*. 2002) began to revisit the subject (Taylor, 2004). Since then several authors have found that diatoms can be used as biomonitoring organisms in freshwater in South Africa. (Bate *et al*., 2004a; 2004b; Dalu *et al*., 2014; De la Rey *et al*., 2004; De la Rey, 2007; Kriel, 2008; Harding and Taylor, 2011; Taylor, 2004; Taylor *et al*., 2005a; 2005b; 2007a; 2007b; 2007c; 2007d; 2009; Walsh, 2008; Walsh and Wepener, 2009).

This study sought to determine if three diatom indices (the GDI, SPI and BDI) together with the Percentage Pollution Tolerant Valves (%PTV) could successfully be used to infer water quality in this river. These indices were chosen based on the research of Taylor (2004) who found them to be suitable for South African conditions. To calculate index scores diatoms were identified using light microscopy and counted. Physio-chemical analysis is costly and needs to be done at regular intervals. Diatoms provide a cost effective, less time consuming, method to monitor the river. The collection of a diatom sample does not require a specialist and laboratory preparation is more cost effective than a range of chemical analyses. Diatoms not only respond to physio-chemical factors but also the interaction of a host of environmental factors and could be used by farmers as an 'early warning system'. This will allow for effective management by the farmer using the interpretation from the diatom indices. Archibald (1972) suggested that the autecological preferences of the dominant diatom species in the diatom community determine the water quality. It is, however, imperative that diatom identification be done by correctly to ensure the validity of the data being presented. Some argue that this technique is too specialised but samples can be taken by someone who has undergone brief training as shown by Taylor *et al*., 2009. The samples can then stored and preserved for a trained person to analyse them. One diatom sample can provide more information than can be gleaned from the chemical analysis.

1.4 Aims and objectives of the study

Expensive chemical analysis present absolute values and are are not easily interpretable. In addition, they do not give a true account of what is happening in the rivers as they do not take the synergistic effects into account. Diatom indices give a time integrated result and can be more cost effective than a regular multi parameter chemical water analysis. Results can also be presented in report format which are more easily interpreted by water managers.

The aims and objectives of this study are:

1. To compile a list of the diatom species occurring in the upper reaches of the Great Fish River.
2. To determine the dominant diatoms species in the study area.
3. To determine if there are any endemic species in the study area.
4. To evaluate the use of diatom indices as bioindicators for this section of the Great Fish River.
5. To determine if the diatom community composition shows seasonality in this section of the Great Fish River.
6. To determine if agriculture has had an impact on the water quality.

1.5 References

Archibald, R. E. M. (1972). Diversity in some South African diatom associations and its relation to water quality. *Water Research*, 6(10), 1229-1238.

Archibald, R. E. M. (1983). *The diatoms of the Sundays and Great Fish rivers in the Eastern Cape Province of South Africa.* (Bibliotheca Diatomologica. Band 1 ed.). Vaduz: J Cramer.

Bate, G. C., Adams, J. B., Van der Molen, J. S. (2002). *Diatoms as indicators of water quality in South African river systems*. Water Research Commission Report No. 814/1/02. Pretoria, South Africa: Water Research Commission.

Bate, G. C., Smailes, P. A., Adams, J.B. (2004a). A water quality index for use with diatoms in the assessment of rivers. *SA Water,* 30(4), 493.

Bate, G. C., Smailes, P. A., Adams, J. B. (2004b). *Benthic diatoms in the rivers and estuaries of South Africa*. Water Research Commission Report TT234/04. Pretoria, South Africa: Water Research Commission.

Bere, T., Tundisi, J. G. (2011). Influence of land-use patterns on benthic diatom communities and water quality in the tropical Monjolinho hydrological basin, São Carlos-SP, Brazil. *Water SA,* 37(1), 93-102.

Bere, T., Phiri, C., Kadye, W. T., Utete, B. (2013). Benthic diatom assemblages in mountain streams: community structure in relation to environmental and human pressures. *African Journal of Ecology*, 51, 625-634.

Besse-Lototskaya, A., Verdonschot, P. F. M., Coste, M., Van de Vijver, B. (2011). Evaluation of European diatom trophic indices. *Ecological Indicators,* 11, 456-467.

Busch, M. (2011). The impacts of herbicides on the diatom communities found within the Burt Lake channel. University of Michigan Biological Station. https://deepblue.lib.umich.edu/bitstream/handle/2027.42/89441/Busch_Michelle_2011.pdf?sequence=1 . Accessed 26 Apr 15.

CEMAGREF, (1982), *Etude des méthods biologiques quantitatives d'appréciation de la qualité deseaux.* Rapport Division Qualite des Eaux Lyon-A.F. Bassin Rhône-Méditerranée-Corse *[A study on the biological methods of qualitative assessment of water quality.* A report of the Water Quality Division Lyon-Outflow Rhône River section catchment], Pierre-Bénite, 218 pp.

Cholnoky, B. J. (1968). *Die ökologie der Diatomeen in Binnengewässern* (p. 699). Lehre: Cramer.

Chutter, F.M. (1998). *Research on the Rapid Biological Assessment of Water Quality Impacts in Streams and Rivers*. Water Research Commission Report No. 422/1/98. Pretoria, South Africa: Water Research Commission.

Coste M., Ayphassorho H. (1991), *Etude de la qualité des eaux du bassin Artois Picardie à l'aide des communautés de diatomées benthiques (Application des indices diatomiques),* Rapport Cemagref Bordeaux – Agence de l'Eau Artois Picardie, 227 pp. [in French]

Davies, B., Day, J. (1998). *Vanishing waters*. Cape Town, South Africa: UCT Press.

Dalu, T., Froneman, P. W., Chari, L. D., Richoux, N. B. (2014). Colonisation and community structure of benthic diatoms on artificial substrates following a major flood event: A case of the Kowie River (Eastern Cape, South Africa). *Water SA*, 40, 471-480.

Debenest, T., Silvestre, J., Coste, M., Delmas, F., Pinelli, E. (2008). Herbicide effects on freshwater benthic diatoms: Induction of nucleus alteration and silica cell wall abnormalities. *Aquatic Toxicology,* 88(1), 88-94.

De la Rey, P. A., Taylor, J. C., Laas, A., Van Rensburg, L., Vosloo, A. (2004). Determining the possible application value of diatoms as indicators of general water quality: A comparison with SASS 5. *Water SA*, 30, 325-332.

De la Rey, P. A. (2007). Evaluation of the applicability of diatom based indices as bioindicators of water quality in South African rivers. Unpublished Phd thesis, North-West University).

Department of Water Affairs and Forestry. (1994). South African River Health Programme, https://www.dwaf.gov.za/iwqs/rhp/goal.html

Dickens, C. W., Graham, P. M. (2002). The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science*, 27(1), 1-10.

ewater.co.za (2005) http://www.ewater.co.za/misc/RiverECGreatFishNEW/defaultgeo.htm. Accessed 19 Mar 2016.

Harding, W.R., Taylor, J.C. (2011) The South African Diatom Index (SADI) – A Preliminary Index for Indicating Water Quality in Rivers and Streams in Southern Africa. Water Research Commission Report No. 1707/1/11. Pretoria, South Africa: Water Research Commission.

Kalyoncu, H., Şerbetci, B. (2013). Applicability of diatom-based water quality assessment indices in Dari stream, Isparta-Turkey. *World Academy of Science, Engineering and Technology,* 7, 188-196.

Kelly, M. G., Whitton, B. A. (1995). The trophic diatom index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, 7, 433-444.

Kriel, G. P. (2008). *Biological indicators of water quality in an urban waterway: Can diatoms reflect short term spatial and temporal changes in water quality?* Unpublished MSc, North West University, Potchefstroom.

McCormick, P.V., Cairns, J. Jr. (1994). Algae as indicators of environmental change. *Journal of Applied Phycology,* 6: 509-526.

Pan, Y., Stevenson, R. J., Hill, B. H., Herlihy, A. T., Collins, G. B. (1996). Using diatoms as indicators of ecological conditions in lotic systems: A regional assessment. *Journal of the North American Benthological Society,* 15(4), 481-495

Round, F. E., Crawford, R.M., D.G. Mann. (1990). The Diatoms Biology and morphology of the genera. Cambridge University Press, Cambridge: 747 pp.

Shannon, C.E., Weaver, R.W. (1948) *A mathematical theory of communication*. The University of Illinois Press, Urbana, Illinois.

Smol, J. P. (2009). *Pollution of lakes and rivers: a paleoenvironmental perspective*. John Wiley & Sons, Oxford.

SouthAfrica.Info. (2013). http://www.southafrica.info/travel/advice/climate.htm#.VTynKZOju8B. Accessed 26 Apr 2015

Taylor, J.C. (2004). *The application of diatom-based pollution indices in the Vaal catchment.* Unpublished MSc dissertation, North-West University, 121 pp.

Taylor, J. C., Harding, W. R., Archibald, C. G. M., Van Rensburg, L. (2005a). Diatoms as indicators of water quality in the Jukskei-Crocodile river system in 1956 and 1957, a re-analysis of diatom count data generated by BJ Cholnoky. *Water SA,* 31(2), 237-246.

Taylor, J. C., De la Rey, P. A., Van Rensburg, L. (2005b). Recommendations for the collection, preparation and enumerations of diatoms from riverine habitats for water quality monitoring in South Africa. *African Journal of Aquatic Science,* 30(1), 65-75.

Taylor, J. C., Janse van Vuuren, M. S., Pieterse, A. J. H. (2007a). The application and testing of diatom-based indices in the Vaal and Wilge rivers, South Africa. *Water SA,* 33(1), 51-60.

Taylor, J. C., Harding, W. R., Archibald, C. G. M. (2007b). A methods manual for the collection, preparation and analysis of diatom samples. *Water Research Commission* Report TT281/07*.* Pretoria, South Africa: Water Research Commission.

Taylor, J. C., Prygiel, J., Vosloo, A., De la Rey, P. A., Van Rensburg, L. (2007c). Can diatom-based pollution indices be used for biomonitoring in South Africa? A case study of the Crocodile west and Marico Water Management area. *Hydrobiologia,* 59, 455-464.

Taylor, J. C., Harding, W. R., Archibald, C. G. M. (2007d). *An illustrated guide to some common diatom species from South Africa.* Water Research Commission Report TT282/07. Pretoria, South Africa: Water Research Commission.

Taylor, J. C., Pope, N., Van Rensburg, L. (2009). Community participation in river monitoring using diatoms: A case study from the Buffelspoort Valley Conservancy. *African Journal of Aquatic Science,* 34(2), 173-182.

Walsh, G. (2008). *Diatom, macroinvertebrate and riparian vegetation community structure responses in agriculturally impacted rivers.* Unpublished MSc, University of Johannesburg, Johannesburg.

Walsh, G., Wepener, V. (2009). The influence of land use on water quality and diatom community structures in urban and agriculturally stressed rivers. *Water SA,* 35(5), 579-593.

Wiener, N. (1948). *Cybernetics*. Wiley, New York.

Zelinka, M., Marvan, P. (1961). Zur Präzisierung der Biologischen Klassifikation der Reinheid Fliessender Gewässer, *Arch. Hydrobiologica*, 57, 389-407.

CHAPTER 2 MATERIALS AND METHODS

The Eastern Karoo, as a sensitive ecosystem, receives an average rainfall of 200 - 400mm per annum (Department of Environmental Affairs and Tourism, 2000). Rainfall within the area varies as higher altitude areas receive more rainfall than the lower elevation plains. The geology of this area is varied and the soil contains high levels of lime. This has led to higher levels of calcium carbonate in the surface, and often borehole, water. It falls within the Karoo Supergroup (Beaufort Group) with dolerite intrusions from the Jurassic age. According to Laker (2005) the quality of soils in South Africa is limited due to the parent material from which they form. Clark *et al*. (2009) describe the soils in the Sneeuberg as shallow and nutrient poor. This renders them prone to degradation and increases the particulate content of the water. Historically this area carried large numbers of sheep and goats due to the vegetation mix. This resulted in overgrazing in large parts of the study area. According to Rowntree and Foster (2009), badland erosion has expanded in semi-arid catchments since 1945.

The Great Fish River catchment is in the Sneeuberg mountain range in the north Eastern Cape Province and enters the Indian Ocean north east of Port Alfred. It is one of the most important rivers in the province and supports many different types of activities. Under normal conditions the upper reaches of the river is narrow with a slow to moderate flow in the catchment and becomes wider downstream. For most of the time, regardless of season, this part of the river is very shallow with a low flow until the water from the Orange – Fish inter basin transfer (via the Great Brak River) enters into the river. This has resulted in intensive cultivation and irrigation of crops on the areas in the valleys with the main crops being lucern, maize and pastures (ewater.co.za, 2005). Most of the area (94%) in the catchment is utilised for livestock farming (ewater.co.za, 2005). There is very little vegetation cover (with the exception of *Populus x canescens* and *Salix babylonica* stands) resulting in increased UV radiation for aquatic organisms. For most of the study area the water depth was usually less than 30cm and deep pools usually only occurred in front of the weirs. These types of modifications affect the microhabitats for various aquatic organisms. In the summer months there are almost continuous green algal blooms along most of the study section which are only washed away during flooding events (pers. observation). The agricultural activity becomes more intense after the Great Brak River joins the Great Fish River which has provided a permanent water supply with which to grow crops. Agricultural activities include herbivorous production (livestock and game), crops (lucerne, corn, wheat and various grass pastures) and dairy. Pit latrines in staff compounds and lack of sewage management are common in the study area (pers. observation). All these activities have a potential impact on the river water quality.

2.1 Site information

Three sites were originally chosen along the Great Fish River. This was later increased to five once permission from the landowners had been secured. These sites cover that part of the river reliant on spring/fountain water.Site selection was limited due to acessability (Figure 2-1). When the spring/fountain dries, so does this part of the river. This section of river is before the confluence of the Great Brak River and the Great Fish River. The Great Brak River brings in water from the Gariep Dam for irrigation purposes (Orange-Fish inter basin transfer). The wool boom, which started in 1952, allowed many farmers to be in the financial position to buy tractors and implements (A. Olivier, personal communication, 2015). This is when many more fields were planted along the river banks.These fields were often ploughed and planted with annual crops. This required the application of fertilisers, herbicides and pesticides. Today the majority of the crops planted along the river in the study area are lucern, maize and pasture grasses.

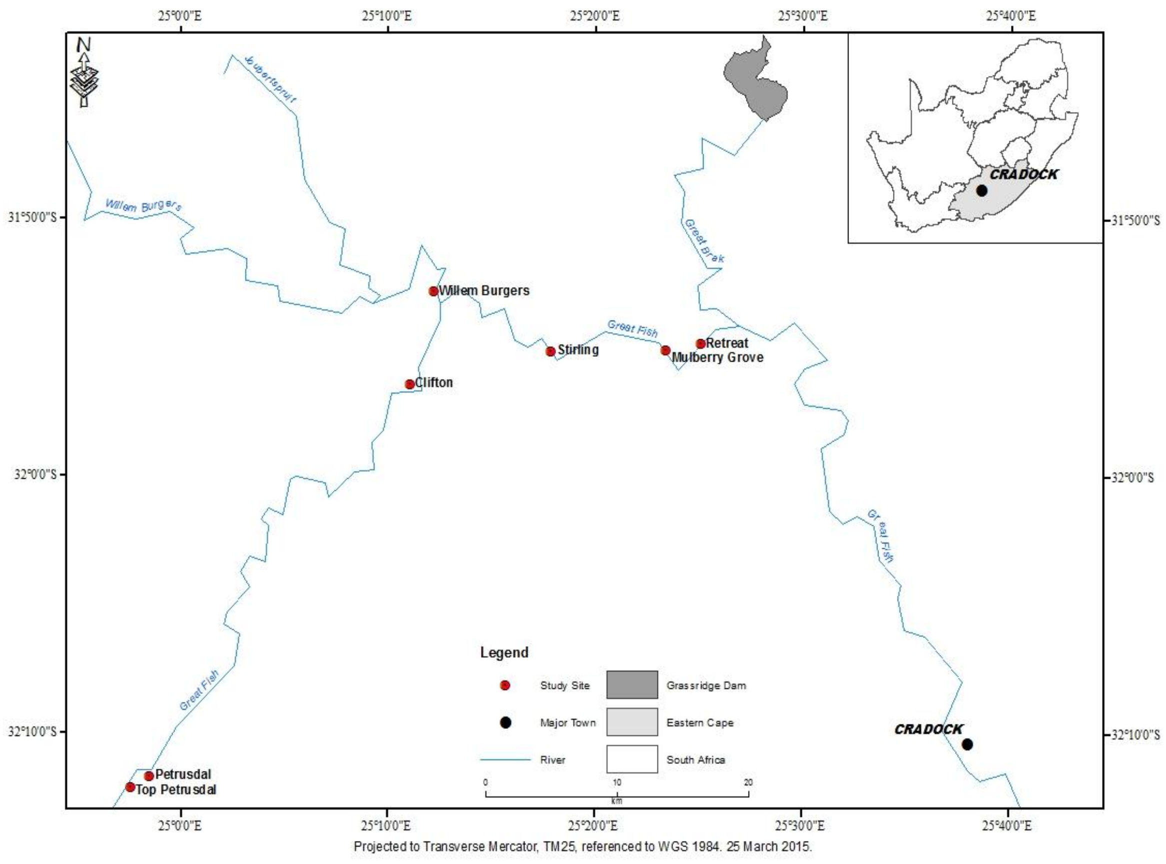


Figure 2-1: Sample sites along the Great Fish River

2.1.1 Petrusdal

The site Petrusdal is the highest site (1581m a.s.l) and is situated on the slopes of Nardousberg in the Eastern Cape. This site was chosen as it is near the peak where the Great

Fish River starts. The farmer allowed unlimited access and the terrain was more accessible than the farm on which the summit is situated. The topography of the area (steep slopes) can result in large amounts of water entering into the river at a substantial velocity during the rainy season. As with four of the five sites the water level is shallow (usually <30cm) and unless there has been heavy rain, there is a low flow. Upstream of the sampling site is a large stand of grey poplars (*Populus x canescens*). This is a common feature of the study area. These trees were originally planted as wood for ox wagon spares and housing in the early 1900's (A.Olivier, personal communication, 2015). The first storage dam on this site was built in the 1920's (A. Olivier, personal communication, 2015). Co ordinates of this site are 32° 11.675'S and 24^{0} 58.539'E. The sampling site receives full sun. The rainfall at this site is higher than those at lower elevation and in addition to the regular winter snowfalls, results the river flowing almost all of the year. The rainfall measured by the farmer for the calendar year 2010 was 515mm and for 2011 was 868mm (A.Oliver, personal communication, 2011 and 2012). This is a livestock and irrigation farm as are all the farms between this site and the next. According to A. Olivier (personal communication, 2015) many of the weirs on the study section of the river were built by the Italian Prisioners of War detained by the South African government during the Second World War. Figure 2.1 and 2.2 are of Petrusdal while Figure 2.3 and 2.4 are of the river moving downstream from this site.

Figure 2-2: Photograph of Petrusdal sample site taken from farm road down towards site

Figure 2-3: Photograph of irrigated fields to the edge of the river bank between Petrusdal and Clifton. Note the debris (between arrows) from a flooding event in February 2011 (pers. observation). (GFR = Great Fish River)

Figure 2-4: One of the large stands of *Populus x canescens* (grey poplar) found along the Great Fish River

2.1.2 Clifton

Clifton is the next site downstream (1129m a.s.l). The sampling site is situated below the weir and receives mostly sun (about 70%). Shading occurs in winter when the sun is lower and the site is then shaded by the weir wall and the willow trees (*Salix babylonica*). The co ordinates for this site are 31° 56.454'S and 25° 11.102'E. The rainfall is less than the first site. In 2010 the measured rainfall was 375mm and for 2011 it was 563.5mm (R.Holmes, personal communication, 2011 and 2012). Fifteen years ago this farm was converted from livestock and crops to game farming. Although the fields are still irrigated they are not replanted and fertilised and have been left to revert to a more natural state. Erosion and overgrazing of the riparian area was remediated by the landowner starting in 1994. Figure 2-5 and 2-6 show the sampling site Clifton. Figure 2-7 shows the condition of the river bank from decades of livestock farming while Figure 2-8 shows the same rehabilitated river bank in 2007 which is several years after conversion to wildlife only.

Figure 2-5: Photograph of Clifton sample site

Figure 2-6: Photograph of a section of the river banks, Clifton. Photograph taken in 1994

Figure 2-7: Photograph of the same section of the river banks as in Figure 2-7, Clifton, 2007

2.1.3 Stirling

Stirling is situated at an altitude of 1039m a.s.l. and contains water from the primary Great Fish River catchment as well as the Willem Burgers River which is the secondary catchment for the Great Fish River. The sampling site with co ordinates 31° 55.167'S and 25° 17.904'E is not shaded. The rainfall recorded at this site for 2010 was 406mm and for 2011 it was 414mm (R.

Holmes, personal communication, 2011 and 2012). The surrounding veld and riparian areas of this farm were badly overgrazed until rehabilitation work began in 1999. In 2005 it became exclusively a game farm. All the farms from Clifton downstream to Stirling are livestock and irrigation farmers. Figures 2-9 to 2-11 illustrate the sample site at Stirling.

Figure 2-8: Photograph of Stirling sample site after flooding event which brought in cobbles (February 2011)

Figure 2-9: April 2011 Stirling sample site after another flooding event (Note: arrows showing debris in trees for height of water). The river channel changed once again. This resulted in the sample site moving a few meters left or right each time.

2.1.4 Mulberry Grove

Mulberry Grove is situated at 993m a.s.l. and received 299mm of rainfall (J.C. Holmes, personal communication, 2011) in 2010. The landowner did not provide rainfall figures for 2011. The co ordinates for this site are 31° 55.116'S and 25° 23.407'E. Land use from Stirling to Mulberry Grove is slowly being converted from livestock and irrigation to game farming. This is resulting in the previously planted and irrigated lands being left unplanted upstream of the site. Mulberry Grove, however, has several hectares of irrigated fields mostly in the riparian zone. Most of the fields in this zone are under drip irrigation in which the farmer adds phosphoric acid to his water before irrigation to reduce the pH (Center for Agriculture, Food and the Environment, 2015). Figures 2-12 and 2-13 illustrate the sample site Mulberry Grove.

Figure 2-10: Photograph of Mulberry Grove sample site

2.1.5 Retreat

The property downstream of the previous site is called Retreat. The co-ordinates of the site are 31° 54.864'S and 25° 25.104'E at an altitude of 988m a.s.l. For water purposes, this farm not only receives fountain water from the Great Fish River but is also draws a water allocation for irrigation from the Great Brak River. The landowner runs a dairy with over 1000 cows in addition to small stock and irrigated fields. Intense ostrich farming was also practised here. The sampling site is situated at a low water bridge. This site has pools of water which were often blue green or green in colour. This site is situated approximately 500m from the village called Fish River (or Visriver). Some of these villagers do not have access to toilets and there is no formal sanitation infrastructure in the area. In times of low flow the site was littered with all types of debris (e.g. tyres, empty maize bags, empty plastic bags, rotting vegetation fallen from tractors, bottles, cans and food containers). It was during these times that an unpleasant smell was noted. No rainfall was measured as farmers from this site downstream do not regularly measure rainfall. Figures 2-14 and 2-15 show the sampling site at Retreat. Figure 2-16 shows the large storage dam which can clearly be seen on the Google earth image (Figure 2-15). Figure 2-17 shows the cattle walkway at Retreat.

Figure 2-11: Photograph from low water bridge upstream at Retreat

Figure 2-12: A dam on Retreat fed by the Great Fish River and used for irrigation and dairy. There is a feedlot behind the dam. The Great Fish River is about 400m behind the feedlot.

Figure 2-13: Cattle walkway on the bank of the Great Fish River at Retreat where a substantial amount of livestock excrement is deposited on the rivers edge on a regular basis

2.1.6 Once off sampling sites

Information on the two once off sites is given below

2.1.6.1 Top Petrusdal

Top Petrusdal is the name of the farm upstream from Petrusdal and is the property which encompasses Nardousberg peak. The peak itself is at 2490m a.s.l. while the sampling site was situated at 1666m a.s.l. The co ordinates are 32° 12.129'S and 24° 57.658'E. This part of the river had been subjected to severe flooding in January and February of 2011 and received 384.5mm of rainfall (A. Olivier, personal communiation, 2011) in those two months. This resulted in the weir being totally destroyed. Figures 2-18 and 2-19 show this site.

Figure 2-14: February 2011. Damaged weir near the source of the Great Fish River at Top Petrusdal.

2.1.6.2 Willem Burgers

The site of Willem Burgers is situated along the river of the same name on the property of Glen Owen at 1089m a.s.l. The co ordinates for this site are 31° 52.861'S and 25° 12.248'E. The farms upstream are all livestock farms and have irrigated fields along the river. The landowner at the time was an absentee owner and the lessees were not able to provide any information on rainfall etc. Figures 2-20 and 2-21 show the site while Figure 2-22 shows the green filamentous algae, which is common all along this river.

Figure 2-15: Photograph of Willem Burgers site

Figure 2-16: Green filamentous algae photograph taken in September 2011. This is a common sight in the Willem Burgers River, a tributary of the Great Fish River.

2.2 Sample collection and processing

Sample collection began in February 2010 for three of the 5 sites (Clifton, Mulberry Grove and Retreat). An additional two sites were added a month later (Petrusdal, Stirling) once permission was received from landowners. Monthly sampling, as planned, could not always be performed due to bad weather and driving conditions. Sampling, for the purposes of this project ended, in April 2012. Comparison samples from Top Petrusdal (closer to peak) and Willem Burgers (tributary) were taken in February 2011 and September 2011 respectively. Physical variable readings (pH, EC, TDS and temperature) were taken simultaneously when biological samples were collected. This was done using a Hanna HI98129 All in One hand held tester. Water samples for chemical analysis were stored in a refridgerator and later processed at North-West University, Potchefstroom, using a Palintest 8000 spectrophotometer. The chemical analysis was conducted on all samples from June 2010. Parameters that were tested were nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonia nitrogen (NH_4-N), orthophosphate (PO_4-P), sulphate (SO_4), and calcium as calcium carbonate ($CaCO_3$). Financial constraints of this self funded project prevented collection of the parameters dissolved oxygen and biological oxygen demand. Turbidity was not measured as the water of the upper reaches of the Great Fish River is, for the most part, clear spring water which is rarely more than 30cm deep in most places. Turbidity is not considered a chemical or physical variable. It has no impact on diatom investigations of this project as sampling took place when there was ample light penetration through to the river bed.

2.2.1 Biological samples

Excess sediment was first removed from the chosen substrate by rinsing it in the river water. A knife and toothbrush were then used to remove diatom samples from the rocks. The samples were stored in plastic containers in a refrigerator until processing was done. The diatom samples from February 2010 to September 2010 were processed at the North-West University, Potchefstroom campus. Samples from October 2010 were processed at the National Health Laboratories, Cradock Hospital and on the farm Clifton using the methods described below. Sample processing was done by the author at these different locations. Wet material was stored on the farm Clifton. As can be seen from section 2.2.1.1 below the fact that diatom samples were processed at different locations did not impact the result. All samples were processed by one person using the same methods at each location.

The method used to process the diatom samples was the Hot HCl and $KMnO_3$ as described by Taylor *et al*. (2007b) and is explained in section 2.2.1.1. Slide preparation was also done according to the methods prescribed by Taylor *et al.* (2007b) using Pleurax as the mounting media (2.2.1.2).

2.2.1.1 Hot HCl and $KMnO_3$ method

After allowing for the sample to settle with gravity the supernatant liquid was discarded. The remaining diatom sample was shaken and emptied into a test tube. Approximately 10ml of saturated solution of $KMnO_3$ was added to the diatom sample and left to stand for 24 hours to remove organic material. HCl was added to the test tube while heating on a hot plate. The HCl reacts with the $KMnO_3$ with the addition of heat to allow for oxidation of the organic material in the cells. The test tube was heated until the liquid became clear. A few drops of H_2O_2 were added to ensure that no biological material remained. After cooling, the supernatant liquid was removed and the remaining sample was transferred to a 10ml centrifuge tube. Samples were centrifuged 4 times at 2500rpm for 10 minutes. After each cycle the supernatant liquid was removed and the sample re-suspended and distilled water was added. This ensured that no acid remained. After the last cycle, diatoms were re-suspended and poured into a glass vial for storage. Once the slides had been successfully processed ethanol was added as a preservative and to keep the sample free of bacteria.

2.2.1.2 Slide preparation

A pipette was used to remove a small amount of re-suspended diatom material and placed in a tube. Distilled water was added until the sample was slightly cloudy. A drop of ammonium chloride (NH_4Cl) was added to prevent diatoms from clumping together on the cover slip. Approximately 1.5ml of the resulting solution was placed on a clean cover-slip and allowed to dry overnight. Once dry, the cover slips were placed on a hot plate (~350ºC) for three minutes to allow for sublimation of NH_4Cl crystals. Once cooled, the cover-slip was inspected under 400x magnification (with a 40x objective and 10x eyepiece) to ensure correct density of diatom valves. The cover-slips were returned to a hot plate with low heat (90 - 120ºC) and 1 - 2 drops of mountant (Pleurax) were added. A clean glass slide was placed onto the cover-slip and turned over to be heated for approximately 5 minutes until it set correctly. Slides were then labelled and the diatoms counted.

2.2.2 Identification and counting

A Nikon E100 phase contrast microscope with a Zeiss 100x 1.25 N.A. phase contrast objective and a Nikon 100x brightfield objective was used to count the diatom slides. An eyepiece graticule was used to measure the frustule size.

Sample counts were kept to 400 valves per slide with the number of deformities added to allow for the calculation of percentage deformities (Taylor *et al*., 2007b). When a broken valve had more than two-thirds (2/3) present it was counted as one unit. If there was less than 2/3's of a broken valve present it was ignored. Identification guides and internet databases were used in identification these included: Archibald (1971, 1983), Giffen (1966), Krammer and Lange Bertalot (1987, 1999a, 1999b), Lange Bertalot (1993, 1996), Krammer (1997, 2000), Prygiel and Coste (2000), Bate *et al*. (2004b) and Taylor *et al*. (2007d). The Academy of Natural Sciences database was the main internet resource.

2.2.3 Statistical analysis

Species abundance data was produced using OMNIDIA 5.3 (Le Cointe *et al*., 1993). Relative abundance was calculated and is presented in Appendix 1. Diatom indices were calculated using OMNIDIA version 5.3 (Lecointe *et al*., 1993). OMNIDIA is an accepted database and has been widely used in Europe (Szczepocka and Szulc, 2009) and South Africa (Harding and Taylor, 2011). This software allows for the calculation of 17 diatom indices in addition to species diversity and species eveness.

Indices used for the purposes of this study were Specific Pollution sensitivity Index (SPI; CEMAGREF 1982); Generic Diatom Index (GDI) (Coste and Ayphassorho, 1991); Biological Diatom Index (BDI) and Percentage Pollution Tolerant Valves (%PTV) which forms part of the UK Trophic Diatom Index (TDI)(Kelly and Whitton, 1995). These indices estimate the trophic status of the water. The SPI, GDI and BDI are all assigned values from 1 – 20 and are based on the weighted average formula of Zelinka and Marvan (1961) (Taylor *et al*., 2007b). The lower the value, the poorer the quality of the water (Table 3-2). The %PTV has values from 1 – 100 and contrary to the abovementioned indices the lower the value of the %PTV the better the water quality.

Table 2-1: Interpretation of the scores for %PTV as found in OMNIDIA (Le Cointe *et al.*, 1993).

Percentage of counted valves that are pollution tolerant taxa	Interpretation
<20	No significant organic pollution
21 – 40	Evidence of organic pollution
41 – 60	Significant evidence of organic pollution contributing to eutrophic state of site
>61	Severe organic contamination of site

Pearson correlations were performed on both chemical and physical variables using Statistica 12. The correlation was done to calculate the usefulness for the diatom indices in indicating water quality. The environmental data was measured on the same dates that the diatom samples were collected. The index scores and environmental data were logged before analysis was performed. Physical variables were collected from February 2010 but chemical analysis was only performed from June 2010 due to logistical and financial constraints.

Canonical Correspondence Analysis (CCA) is a multivariate analysis which can be used to relate a group of species to the environmental variables in which they occur by detecting variations in their occurrence. Multiple regression extracts the gradients that are held to be direct linear functions of the environmental variables. The axes of the CCA biplot are a function of the environment. This suggests the correlation between the diatom gradients and axes will show the strength of the overall gradient. Correlation of diatom species with environmental variables gives an indication of a pattern of composition. The environmental variables will show their shared effect on the diatom species composition and can be seen by the direction and length of the vectors (arrows) on the biplot. The CCA expresses the linear relationship between variables and points on the plot (including the axes). CCA was performed using the environmental variables and diatom species. Site names were not included in the analysis as the aim was to determine the environmental variables affecting diatom species composition. This data was log transformed for the analysis in Canoco 4.5 to normalise the distribution. An attempt was made to perform the CCA for each site but as this technique is not suited to over analysis, it resulted in unacceptably high Variance Inflation Factors (VIFs). VIF's <10 are considered acceptable. Any value above that shows that the environmental variable being tested does not have a unique contribution to the analysis. This type of analysis (CCA) has been successfully applied in diatom studies worldwide (Hill *et al.*, 2001, Potopova and Charles, 2002, Rimet *et al*., 2004, Kovács *et al*., 2006, Ndiritu *et al*., 2006, Bona *et al*., 2007, Solak *et al*., 2012, Hlubikova, 2014).

Concerns have been raised when the cosmopolitan species with environmental data in the northern hemisphere are used for analysis in the southern hemisphere (Bate *et al*., 2002). It is suggested that the same species of diatoms will occur where ever their environmental requirements are met. In 1934, a Dutch professor, Baas Becking summed it up 'Everything is everywhere but the environment selects' (Whitfield, 2005).

2.3 References

Academy of Natural Sciences. (2011). *ANSP alage image database.*, 2010, 2011, 2012 and 2013, from http://diatom.acnatsci.org/AlgaeImage/SearchCriteria.asp

Archibald, R. E. M. (1971). Diatoms from the Vaal Dam catchment area, Transvaal, South Africa. *Botanica Marina, XIV*, 17-70.

Archibald, R. E. M. (1983). *The diatoms of the Sundays and Great Fish rivers in the Eastern Cape Province of South Africa.* (Bibliotheca Diatomologica. Band 1 ed.). Vaduz: J Cramer.

Bate, G. C., Adams, J. B., Van der Molen, J. S. (2002). *Diatoms as indicators of water quality is South African river systems*. Water Research Commission Report No. 814/1/02 Pretoria, South Africa: Water Research Commission.

Bate, G. C., Smailes, P. A., Adams, J. B. (2004b). *Benthic diatoms in the rivers and estuaries of South Africa*. Water Research Commission Report TT234/04. Pretoria, South Africa: Water Research Commission.

Bona, F., Falasco, E., Fassina, S., Griselli, B., Badino, G. (2007). Characterization of diatom assemblages in mid-altitude streams of NW Italy. *Hydrobiologia,* 583(1), 265-274.

CEMAGREF (1982). *Etude des méthods biologiques quantitatives d'appréciation de la qualité deseaux.* Rapport Division Qualite des Eaux Lyon-A.F. Bassin Rhône-Méditerranée-Corse *[A study on the biological methods of qualitative assessment of water quality.* A report of the Water Quality Division Lyon-Outflow Rhône River section catchment*]*, Pierre-Bénite, 218 pp.

Center for Agriculture, Food and the Environment, College of Natural Sciences, University of Massachusetts Amherst, (2015) (https://extension.umass.edu/floriculture/fact-sheets/adjusting-alkalinity-acids Date of access 15 Feb 2015.

Clark, V., Barker, N., Mucina, L. (2009). The Sneeuberg: A new centre of floristic endemism on the great escarpment, South Africa. *South African Journal of Botany,* 75(2), 196-238.

Coste M., Ayphassorho H. (1991). *Etude de la qualité des eaux du bassin Artois Picardie à l'aide des communautés de diatomées benthiques (Application des indices diatomiques),* Rapport Cemagref Bordeaux – Agence de l'Eau Artois Picardie, 227 pp. [in French]

Department of Environmental Affairs and Tourism. (2000). Environmental mean potential atlas for South Africa, Mean annual precipitation map.

Giffen, M.H. 1966. Contributions to the diatom flora of southern Africa. II. Diatoms from Hogsback region of the Amatola mountains, Eastern Cape Province, South Africa. *Nova Hedwigia* 21, 123–160.

Harding, W.R., Taylor, J.C. (2011) The South African Diatom Index (SADI) – A Preliminary Index for Indicating Water Quality in Rivers and Streams in Southern Africa. Water Research Commision Report No. 1707/1/11. Pretoria, South Africa: Water Research Commission.

Hill, B. H., Stevenson, R. J., Pan, Y., Herlihy, A. T., Kaufmann, P. R., Johnson, C. B. (2001). Comparison of correlations between environmental characteristics and stream diatom assemblages characterized at genus and species levels. *Journal of the North American Benthological Society*, 20(2), 299-310.

Hlúbiková, D., Novais, M. H., Dohet, A., Hoffmann, L., Ector, L. (2014). Effect of riparian vegetation on diatom assemblages in headwater streams under different land uses. *Science of the Total Environment*, 475, 234-247.

Kelly, M. G., Whitton, B. A. (1995). The trophic diatom index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, 7, 433-444.

Kovács, C., Kahlert, M., Padisák, J. (2006). Benthic diatom communities along pH and TP gradients in Hungarian and Swedish streams. *Journal of Applied Phycology*, 18(2), 105-117.

Krammer, K., Lange-Bertalot, H. (1987). *Bacillariaceae, Epithemiaceae, Surirellaceae neue und bekannte taxa, neue kombinationen und synonyme sowie bermerkungen und erganzungen zu den Naviculaceae*. Berlin. Stuttgard, Germany: J Cramer.

Krammer, K. (1997). *Die Cymbelloiden diatomeen. Eine monographieder weltweit bekannte taxa. teil 1. allegemeines und Encyonema part* (Bibliotheca Diatomologica Band 36 ed.). Germany: J Cramer.

Krammer, K., Lange-Bertalot, H. (1999a). *Susswasserflora von Mitteleuropa. Bacillariophyceae. 2/2 Bacillariaceae, Epithemiaceae, Surirellaceae* (2nd ed.). Germany: Elsevier Spektrum Akademischer Verlag.

Krammer, K., Lange-Bertalot, H. (1999b). *Susswasserflora von Mitteleuropa. 2/1. Bacillariophyceae. Naviculaceae* (2nd ed.). Germany: Elsevier Spektrum Akademischer Verlag.

Krammer, K. (2000). *Diatoms of Europe. The genus Pinnularia*. Ruggell, Germany: A.R.G. Ganter Verlag Kommanditgeselschaft.

Laker, M. C. (2005). *South Africa's soil resources and sustainable development*. REVIEW: Soil and sustainability October 2005. Department of Environmental Affairs: Pretoria.

Lange-Bertalot, H. (1993). *85 new taxa and much more than 100 taxonomic clarifications supplementary to Susswasserflora von Mitteleuropa vol 2/1-4.* (Bibliotheca Diatomologica Band 27 ed.). Hirschberg, Germany: J Cramer.

Lange-Bertalot, H. (1996). *Inconographia diatomologica*. Konigstein, Germany: Koeltz Scientific Books.

Lecointe, C., Coste, M., Prygiel, J. (1993). `Omnidia': Software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia: The International Journal on Limnology and Marine Sciences,* 269-270(1), 509-513.

Ndiritu, G. G., Gichuki, N. N., Triest, L. (2006). Distribution of epilithic diatoms in response to environmental conditions in an urban tropical stream, Central Kenya. *Biodiversity & Conservation*, *15*(10), 3267-3293.

Potapova, M. G., Charles, D. F. (2002). Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. *Journal of Biogeography*, 29(2), 167-187.

Prygiel, J., Coste, M. (2000). *Guide méthodologique pour la mise en oeuvre de l'indice biologique diatomées*. Eau-Cemagref Bordeaux: Etude Agences de l.

Rimet, F., Ector, L., Cauchie, H. M., Hoffmann, L. (2004). Regional distribution of diatom assemblages in the headwater streams of Luxembourg. *Hydrobiologia*, 520(1-3), 105-117.

Rowntree, K. M., Foster, I. D. L. (2009). Using environmental magnestism and radionuclide analysis to deconstruct landscape structure in a small semi-arid catchment, South Africa. *7th International Conference of the International Association of Geomorphologists (ANZIAG).* Melbourne, Australia. 6-11 July 2009.

Solak, C. N., Barinova, S., Acs, E., Dayioğlu, H. (2012). Diversity and ecology of diatoms from Felent creek (Sakarya river basin), Turkey. *Turkish Journal of Botany*, *36*(2), 191-203.

Szczepocka, E., Szulc, B. (2009). The use of benthic diatoms in estimating water quality of variously polluted rivers. *Oceanological and Hydrobiological Studies,* 38, 17-26.

Taylor, J. C. (2004). *The application of diatom-based pollution indices in the Vaal catchment.* Unpublished MSc dissertation, North-West University , 121pp

Taylor, J. C., Harding, W. R., Archibald, C. G. M. (2007b). *A methods manual for the collection, preparation and analysis of diatom samples, version 1.0 / report to the Water Research Commission by J.C. Taylor, W.R. Harding and C.G.M. Archibald* Gezina, South Africa : Water Research Commission.

Taylor, J. C., Harding, W. R., Archibald, C. G. M. (2007d). *An illustrated guide to some common diatom species from South Africa.* Water Research Commission Report TT282/07. Pretoria, South Africa: Water Research Commission.

Whitfield, J. (2005). Biogeography: Is Everything Everywhere? Science Vol 310. (http://www.botany.wisc.edu/courses/botany_940/06EvidEvol/papers/Whitfield2005.pdf) Accessed 26 April 2015

CHAPTER 3 RESULTS – Article published in the to African Journal of Aquatic Sciences, Volume 40 (pp 321- 337)

3.1 Abstract

This study focused on the spring-fed upper reaches of the economically important Great Fish River with the aim of determining if diatoms could be used for biomonitoring in semi-arid conditions in southern Africa. Five sites were monitored monthly from 2010 to 2012. Of the 269 diatom taxa belonging to 51 genera identified, the dominant taxa were mostly those considered to be pollution-tolerant: *Amphora pediculus, Craticula buderi, Fragilaria biceps, Nitzschia frustulum, Nitzschia paleacea, Planothidium lanceolatum* and *Rhopalodia gibba*. A number of diatom based numerical indices were used to infer water quality, including the generic diatom index, the specific pollutionsensitivity index, the biological diatom index, and percentage pollution-tolerant valves, which forms part of the UK trophic diatom index. All index scores showed the Great Fish River to be impacted, and showed significant correlations of diatom species abundance with pH, NO_3-N, electrical conductivity, NH_4-N and $CaCO_3$. Analysis revealed EC and NO_3-N as the main environmental drivers affecting diatom community composition, followed by pH and PO_4-P. The percentage of diatom deformities at all sites was high, at 3.5%. Diatom indices showed the river to be impacted by decades of agricultural activity, which was confirmed by chemical water analysis. Thus diatom indices can be used for biomonitoring in semi-arid areas.

KEYWORDS: agricultural impacts, aquatic ecosystem, biomonitoring, deformed valves, epilithic diatoms, freshwater, Karoo

3.2 Introduction

Water resources, not only in South Africa, are subject to many human impacts such as degradation, chemical contamination, destruction of aquatic habitats, return flow from irrigated lands, abstraction and introduction of exotic species (Dallas and Day, 2004). All these factors affect diatom communities in aquatic habitats, making them a potentially useful biomonitoring group (Taylor, 2004; Álvarez *et al*., 2012).

Diatoms occur abundantly in all types of aquatic habitats, and their short generation time of about two weeks (Round, 1991; Harding *et al*., 2005; Karthick *et al*., 2010) allows them to respond rapidly to environmental changes. Diatoms, like many other species, prefer certain ecological and physical ranges in which to live. If these conditions change and their tolerance levels are exceeded, the composition of the diatom communities will change accordingly. Physical and chemical monitoring reflects environmental conditions at a particular point in time, but do not give a historical perspective or ‘ecological memory’ (Walsh and Wepener, 2009; Szczepocka *et al*., 2014). Therefore biomonitoring is an important tool with which to evaluate environmental changes over time, and one which is cost-effective. It takes many synergistic associations into account and is therefore a more holistic approach of water quality monitoring. A possible flaw of diatoms as bioindicators is that they may be dead at the time of sampling. A way to reduce this possibility is to check the sample for live cells before processing (Karthick *et al*., 2010).

The Great Fish River is one of the most important rivers in the Eastern Cape province, supporting many different types of activities and thousands of irrigation farmers. The chemical composition of its water is influenced by the geology, agricultural runoff and the degradation and destruction of natural riparian habitats. The instream morphology has been changed by weirs and bridges. For the present study, only the eastern slopes of the Nardousberg subcatchment were monitored. The study area section of the Great Fish River is approximately 80 km long and contains several weirs and dams. Livestock is the main agricultural income source, while lands are irrigated from the river.

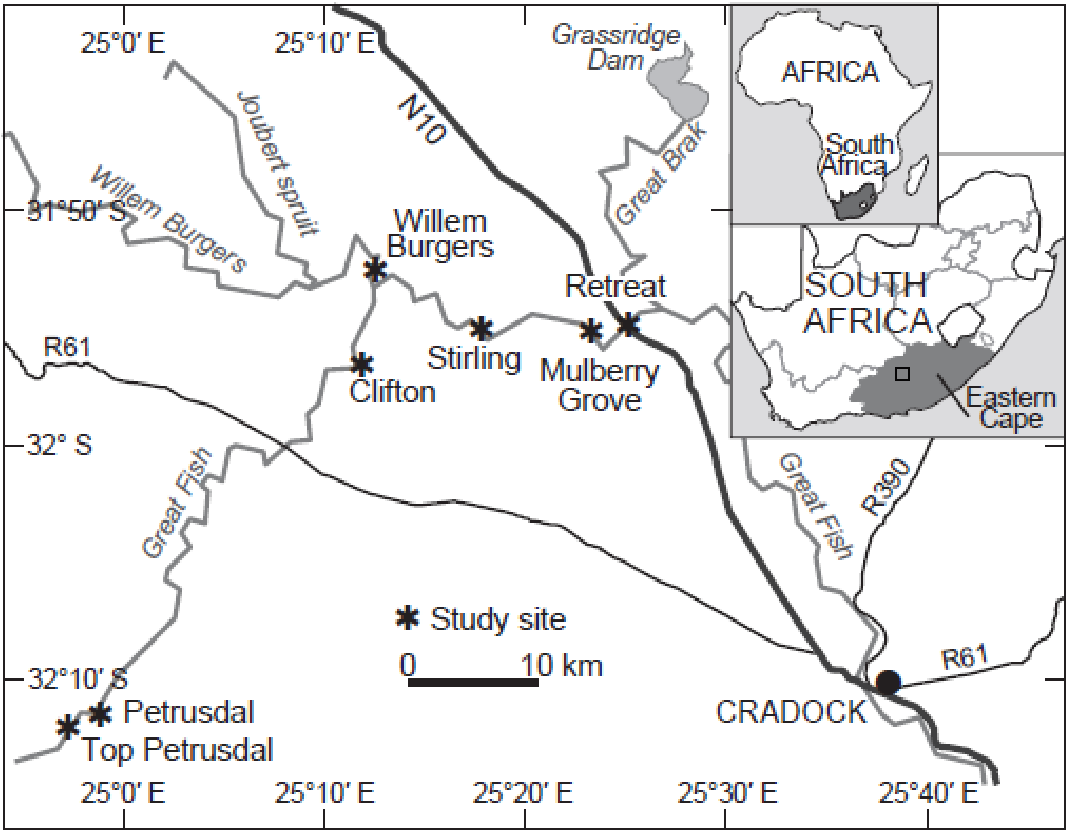
The specific pollution tolerance of many diatom species is known, allowing for quantification of the effects of different variables. These tolerance levels have led to the development of a number of diatom indices which allow for the inference of water quality (Kalyoncu and Şerbetci, 2013) through the allocation of numeric values. European and other diatom indices have been applied successfully in South African rivers and streams by Bate *et al*., (2004), Taylor (2004), Taylor *et al*. (2007a, 2007b), Walsh and Wepener (2009) and Harding and Taylor (2011). However, South Africa does not at present have an entirely unique autecological index which takes the country’s dramatic seasonal fluctuations and local conditions into account. The South African diatom index (SADI) is a modified form of the specific pollution sensitivity index (SPI) which includes South African endemic species (Harding and Taylor, 2011). This index still requires input from regional studies and validation.

The aim of this study was to determine the species composition of the epilithic diatom communities in the upper reaches of the Great Fish River, to determine whether European diatom indices can be used for monitoring water quality in this semi-arid area, and whether they accurately reflected degrees of human impact.

3.3 Materials and methods

The study area is situated within the Nama Karoo biome (Mucina and Rutherford, 2006). Topography in this biome is varied and temperature fluctuations, both daily and seasonally, are not uncommon. Temperatures can range from 45 °C in summer to −15 °C in winter. Rainfall occurs mainly in the warm to hot summer months, with snowfalls occurring on high-lying areas in the cold winter months. This results in dramatic fluctuations in the volume of water in the rivers and in seasonal variations in water temperature. Severe droughts are not uncommon.

3.3.1 Site information



Projected to Transverse Mercator, TM25, referenced to WGS 1984. 25 March 2015.

Figure 3-1: Map showing the upper reaches of the Great Fish River with sample sites

The three original sites of Clifton, Mulberry Grove and Retreat were sampled from February 2010. In March 2010, Petrusdal and Stirling were added once necessary permissions had been received. Sites along the 80 km-long study area were dependent solely on spring water (Table 3-1, Figure 3-1). Petrusdal was chosen as it was more accessible in the mountainous terrain than the actual river source. Clifton, situated 40 km further downstream, was selected to determine if the irrigation of crops in the riparian zone from the Petrusdal site had negative impacts on water quality. These two sites were influenced by water from the Great Fish River only, but the next three sites downstream, namely, Stirling, Mulberry Grove and Retreat, were also influenced by the Willem Burgers River, which is from another subcatchment. Mulberry Grove was situated directly upstream of a dairy with over 1 000 head of cattle, while Retreat was located directly downstream of the dairy. Monitoring these two sites allowed for inference of change of water quality due to the dairy.

Table 3-1: Details of study sites on the Great Fish River

Site name	Coordinates	Altitude (m asl)
Top Petrusdal	32°12.129′ S, 24°57.658′ E	1 666
Petrusdal	32°11.675′ S, 24°58.539′ E	1 581
Clifton	31°56.454′ S, 25°11.102′ E	1 129
Willem Burgers	31°52.861′ S, 25°12.248′ E	1 089
Stirling	31°55.167′ S, 25°17.904′ E	1 039
Mulberry Grove	31°55.116′ S, 25°23.407′ E	993
Retreat	31°54.864′ S, 25°25.104′ E	988

3.3.2 Sample collection and processing

Sampling was performed monthly although this was not always possible for all sites due to weather, flooding events and difficult driving conditions. Monthly samples up to and including January 2012 were used. A follow-up sample in April 2012 was also included. Comparison samples were taken from closer to the source, Top Petrusdal, in February 2011 and the Willem Burgers River in September 2011 (Table 3-1, Figure 3-1). Both were included in the analysis.

All diatom and chemical analysis samples and physical measurements were taken simultaneously.

3.3.3 Physio-chemical samples

Readings for pH, electrical conductivity (EC) and temperature were taken with a Hanna HI98129 meter. Water samples for chemical analysis were taken for later processing at North-West University, Potchefstroom, where they were kept refrigerated. A Palintest 8000 spectrophotometer was used to measure nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonia nitrogen (NH4-N), orthophosphate phosphorus (PO_4-P), sulphate (SO_4), and calcium ($CaCO_3$). Chemical analysis was done on all water samples from June 2010 to April 2012.

3.3.4 Biological samples

Diatoms were sampled near the banks and in the middle of the river at each site. Samples were taken (scrubbed) from cobbles (100–256 mm), except at Stirling which was on bedrock, but where the water was shallow enough with a low flow to allow for scrapings to be collected off the bedrock from five different points. During flood events, cobbles would be washed in at Stirling and these would be sampled and scrubbed in addition to samples taken from the bedrock during low-flow conditions. If the only available substrates were covered with filamentous algae, this was removed before scrubbing. Diatom processing and slide preparation was performed according to Taylor *et al*. (2005, 2007c) using the potassium permanganate/hydrochloric acid method.

3.3.5 Diatom identification and counting

Slides were viewed using a Nokia E100 phase contrast microscope with a Zeiss 100×/1.25 NA phase contrast objective and a Nikon 100× brightfield objective. Frustule measurements were taken using an eyepiece graticule.

Identification was done using guides by Archibald (1971, 1983), Giffen (1966), Krammer and Lange-Bertalot (1987, 1999a, 1999b), Lange-Bertalot (1993, 1996), Krammer (1997, 2000), Prygiel and Coste (2000), Bate *et al*. (2004) and Taylor *et al*. (2007d). The main internet identification source used was the Academy of Natural Sciences (2011) database. Sample counts were of 400 valves per slide which was found to produce a good representation of the diatom community without excessive repetition (Taylor *et al*., 2007d). The total number of deformities was then added to the total of 400 to allow for a percentage to be calculated. Each valve was counted as one unit. If more than two-thirds of a broken valve was present and a positive identification could be made, it was included in the count.

3.3.6 Statistical analysis

Species abundance was calculated using Omnidia v.5.3 (Lecointe *et al*., 1993) and relative abundance was calculated (Appendix 1). Indices used in this study were in accordance with those utilised by Taylor (2004) in his assessment of the applicability of European indices in a South African river. These included the specific pollution sensitivity index (SPI, incorporating the SADI) which has the highest inclusion rate of taxa of all the indices, with toxins, salinity, eutrophication and organic pollution being taken into account; the generic diatom index (GDI) which calculates values at a genus level; the biological diatom index (BDI) which best reflects water quality problems using seven physico-chemical parameters (Debenest *et al*., 2008); and the percentage pollution-tolerant valves (%PTV) which reflects organic pollution and forms part of the UK trophic diatom index (TDI) (Kelly and Whitton, 1995). The first three indices are scored in a range of 0–20 with scores bearing towards 0 indicating an increasing level of pollution or eutrophication (see Table 3- 2). The %PTV index has a maximum value of 100, with any value above 20 indicating an increase in organic pollution (Kelly and Whitton, 1995). Index calculation was done using Omnidia v. 5.3 (species list version 2009; Lecointe *et al*., 1993). The first three indices are calculated using the Zelinka and Marvan (1961) weighted average formula (Taylor *et al*., 2007a), while the %PTV is based on the trophic diatom index, used for monitoring eutrophication in rivers (Kelly and Whitton, 1995). Pearson correlation analysis was done using Statistica 12, and correlation between the indices, as well as the relationship between the indices and the environmental variables, was tested. Canoco for Windows 4.5 (ter Braak and Smilauer, 2003) was used to analyse diatom community composition data and to produce ordination graphs.

Table 3-2: Interpretation of diatom index scores, after Eloranta and Soininen (2002)

Index score	Water quality class
>17	High quality
14–17	Good quality
10–14	Moderate quality
6–14	Poor quality
<6	Bad quality

3.4 Results

3.4.1 Community composition

A total of 269 diatom taxa belonging to 51 genera were identified, excluding the 49 taxa which were not identified even to genus level. Most (99.3%) of the species were cosmopolitan, with only two possible endemic species, viz. *Gyrosigma rautenbachiae* Cholnoky (1.15% relative abundance) and *Nitzschia irremissa* Cholnoky (0.01% relative abundance). Relative abundance data for each sites is given in Appendix 1. Deformed valves scored 1–10% at all sites. When rare species (<1%) were excluded, 64 taxa remained. There were only 26 taxa >5% relative abundance.

Gyrosigma rautenbachiae is found in inland brackish waters that are standing or have a low flow, and can tolerate pollution (Taylor *et al*., 2007d). This species was found in several samples from three sites (Stirling – 7 samples, Mulberry Grove – 11 samples and Retreat – 3 samples) in both low- and faster-flow conditions.

Nitzschia irremissa is thought to be tolerant of pollution and was found only at Retreat in one sample (September 2011) and constituted 0.24% of that particular sample. Environmental variables of that sample were all above the mean, but were not the highest recorded.

Diatom species that were dominant (>5%) when all samples at all sites were taken into account included *Nitzschia frustulum* (Kützing) Grunow (15.8%) and *Rhopalodia gibba* (Ehrenberg) O.Müller (9.6%). Both are cosmopolitan and are tolerant of moderately high EC. While *N. frustulum* is tolerant of changes in osmotic pressure, such as flooding events, *R. gibba* prefers low flow or standing water (Taylor *et al*., 2007d).

Individual sites were then analysed for dominant species. The dominant taxa, with their spectral ranges and mean relative abundances, are listed for the five main sites in Table 3-3. The single sample taken from Top Petrusdal was dominated (>5%) by *Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck (11.8%), *C. placentula* var. *euglypta* (Ehrenberg) Grunow (abnormal form) (11.9%) and *Nitzschia paleacea* (Grunow) Grunow (8.4%). *Cocconeis* spp. are abundant on different types of substrate; they tolerate both flowing and standing meso- to eutrophic waters and can be found at the same sites simultaneously (Krammer and Lange-Bertalot, 1999a). Flooding events at this altitude exert extreme shearing force on the substrates. *Cocconeis* are able to withstand shearing, and this could be the reason why they were dominant at this site, as well as at Petrusdal, at a slightly lower altitude. *Nitzschia* spp. favour water with a constant supply of organic nitrogen. *Nitzschia paleacea*, while being tolerant of heavy levels of pollution, occurs in eutrophic waters with a moderate to high EC.

The dominant species at site Petrusdal in all samples taken were *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow (11.6%), *Nitzschia paleacea* (Grunow) Grunow (6.6%) and *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot (8%). *Planothidium lanceolatum* prefers slightly alkaline conditions (pH >7) with low to moderate EC.

The dominant species at Clifton were *Amphora pediculus* (Kützing) Grunow (11.9%), *Diatoma vulgaris* Bory (9.1%), *Fragilaria biceps* (Kützing) Lange-Bertalot (9%), *Nitzschia frustulum* (Kützing) Grunow (12.5%) and *Nitzschia linearis* (Agardh) W.M.Smith (5.4%). *Amphora pediculus* is alkaphilous, preferring water with moderate EC but tolerating heavy organic pollution. *Diatoma vulgaris* prefers low-flow conditions with a moderate EC and elevated phosphorus/ phosphate levels (Taylor *et al*., 2007b). Van Dam *et al*. (1994) suggested that *F. biceps* prefers eutrophic alkaline waters.

The single sample taken from site Willem Burgers was dominated by *Cocconeis pediculus* (20.4%) and *Nitzschia frustulum* (30.8%). *Cocconeis pediculus* tolerates conditions with high nutrient concentrations and moderate organic pollution. Site Stirling was dominated by *Diatoma vulgaris* (7.2%), *Fragilaria biceps* (7.5%), *N. frustulum* (19.9%) and *Rhopalodia gibba* (Ehrenberg) O.Müller (15.3%). *Rhopalodia gibba* is found in a wide range of habitats with a preference for conditions with high phosphates (Marks and Lowe, 2003). The site at Mulberry Grove was dominated by *N. frustulum* (19.4%) and *R. gibba* (27.8%), while site Retreat had *Amphora pediculus* (6.8%), *Craticula buderi* (Hustedt) Lange-Bertalot (18.5%) and *N. frustulum* (19.4%) as its dominant species. *Craticula buderi* occurs in calcareous streams and can tolerate high levels of nutrients as well as high levels of organic pollution.

The average percentages of deformed values were 3.12% at Petrusdal, 3.11% at Clifton, 4.06% at Stirling, 4.05% at Mulberry Grove and 2.92% at Retreat. In this study the average frequency of diatom valve deformities for all sites was 3.5%. According to Morin *et al*. (2012), diatom valve deformity frequencies of over 3.5% could be considered excessive. The severity of valve deformities, however, was of concern (Figure 3-2) and included severe raphe and straie abnormalities. At Petrusdal the highest percentages of deformities occurred in *Nitzschia* (45.9%) followed by *Cocconeis* (22%). Cells that were unidentifiable (code ZZZZ), even to genus level, accounted for 6.6% of the deformities. At Clifton the highest percentages of deformities were identified in *Nitzschia* (53.8%) and *Fragilaria* (17.1%). Unidentified cells contributed 1.4% at this site. *Nitzschia* accounted for 69% of the deformities at Stirling, while *Rhopalodia* and *Fragilaria* deformities at this site were 6.4% and 6.1%, respectively. Unidentified deformed cells at Stirling amounted to 0.9%. At Mulberry Grove *Nitzschia* accounted for 57.8% of deformities, followed by *Rhopalodia* at 25.3%. Deformities of unidentified cells at Mulberry Grove accounted for 0.3% of the cells counted. At Retreat, *Nitzschia* accounted for 74.4% of deformities, with *Craticula* at 12.4% and unidentified cells at 2.9%. Total percentage of deformities per genus over all sites were: *Nitzschia* 61.1%, *Rhopalodia* 7.3% and *Fragilaria* 6.1%.

The total numbers of species recorded at each site for all samples were 184 at Petrusdal, 154 at Clifton, 147 at Stirling, 174 at Mulberry Grove and 175 at Retreat.

No historical comparisons can be made, as this part of the Great Fish River has not been studied before. Archibald (1983) sampled the Theebus/Great Brak, a tributary of the Great Fish, and, of the 20 species he found there, 15 were recorded in the present study. The Theebus/Great Brak River tributary, which lies in a different subcatchment, enters the Great Fish River below the lowest sample site used in this study. Although the geology throughout the

Karoo is similar, the subcatchment sampled by Archibald does not have the altitude and flow variations of the present study area, and therefore his results cannot be applied directly to the Great Fish River.

Table 3-3: Dominant diatom taxa at the five main sample sites in the Great Fish River study area in 2010–2012, with their nutrient spectral ranges, distribution and maximum relative abundances

Code	Taxon	Nutrient spectrum	Distribution at sample sites	Relative abundance (%)
APED	*Amphora pediculus*	Eutrophic	Clifton	11.9
			Retreat	6.8
CPLE	*Cocconeis placentula* var. *euglypta*	Meso- to eutrophic	Petrusdal	11.6
CRBU	*Craticula buderi*	Wide range	Retreat	18.5
DVUL	*Diatoma vulgaris*	Meso- to eutrophic	Clifton	9.1
			Stirling	7.2
FBCP	*Fragilaria biceps*	Eutrophic	Clifton	9.0
			Stirling	7.5
NIFR	*Nitzschia frustulum*	Eutrophic	Stirling	19.9
			Clifton	12.5
			Mulberry Grove	19.4
			Retreat	19.4
NLIN	*Nitzschia linearis*	Meso- to eutrophic	Clifton	5.4
NPAE	*Nitzschia paleacea*	Eutrophic	Petrusdal	6.6
PTLA	*Planothidium lanceolatum*	Eutrophic	Petrusdal	8.0
RGIB	*Rhopalodia gibba*	Eutrophic	Stirling	15.3
			Mulberry Grove	27.8

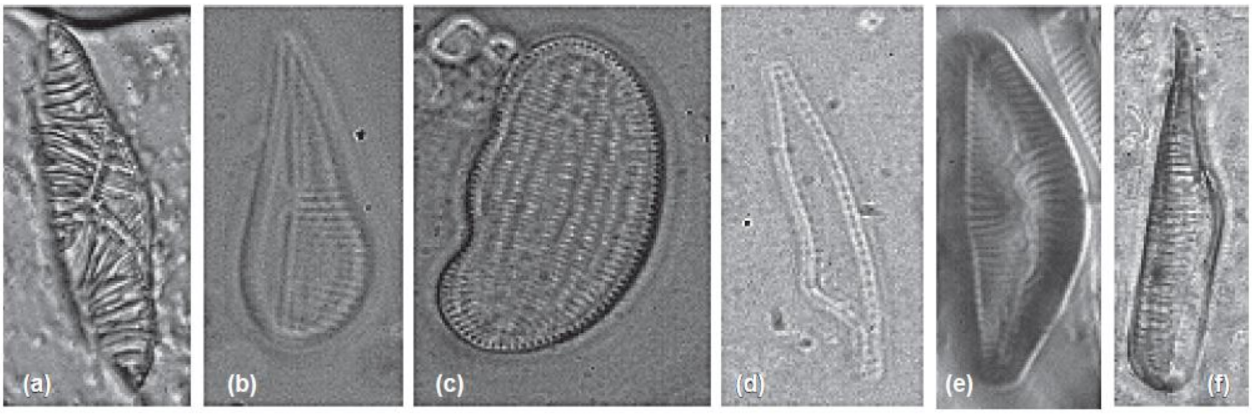


Figure 3-2: Examples of deformed cells: (a) *Rhopalodia* sp., (b) *Amphora veneta*, (c) *Cocconeis placentula*, (d) *Nitzschia* sp., (e) *Cymbella,* (f) *Rhopalodia gibba*

3.4.2. Physio-chemical data

The mean and range of the physical and chemical variables for each site are presented in Table 3-4. According to Davies and Day (1998), the average pH of fresh waters in South Africa is 7.5. The pH was high at all sites, with site Stirling having the highest mean pH of 8.74. However, at all sites a pH of over 9.0 was recorded at least once during the study period. The single reading taken at Willem Burgers (9.4) was surprisingly high, and that at Top Petrusdal was 8.34. The catchments of both these sites lie in the Sneeuberg mountain range. A comparison between pH and rainfall was made to determine if they were correlated, but no correlation was found. Similarly, when pH values were compared to measured rainfall, no correlation was found.

The mean temperature at the headwater site, Petrusdal, was 13.4 °C, which is lower than that further downstream, as expected. The mean EC increased substantially with downstream distance, from 190 to 1 028 µS cm^{-1}, while temperature also showed an increase. The increase of EC and temperature was associated with the increase in biological material and subsequent increase in metabolic rates. The means for NO_2-N and NH_4-N were highest at Retreat (0.082 mg l^{-1} and 0.17 mg l^{-1}, respectively), while the NO_3-N means at Clifton and Stirling, situated upstream from Retreat, were slightly higher at 0.272 mg l^{-1} and 0.261 mg l^{-1}, respectively. The mean for PO_4-P was highest at Retreat (0.648 mg l^{-1}) followed by Clifton (0.567 mg l^{-1}). Clifton was the second highest in altitude, revealing that the mean values for PO_4-P were high not only at the lower sites. The SO_4 mean value was highest at Willem Burgers (69 mg l^{-1}), followed by Mulberry Grove and Retreat (45.7 mg l^{-1} and 38.2 mg l^{-1}, respectively). The highest mean for $CaCO_3$ was measured at Clifton (96.5 mg l^{-1}), followed closely by Retreat and Stirling (94.2 mg l^{-1} and 90.8 mg l^{-1}, respectively).

Table 3-4: Environmental variable values (mean and range) at sample sites on the Great Fish River in 2010–2012

Variable	Sample site						
	Top Petrusdal	Petrusdal	Clifton	Willem Burgers	Stirling	Mulberry Grove	Retreat
Elevation (m asl)	1 666	1 581	1 129	1 089	1 039	993	988
pH	8.34	8.47 (7.73–9.03)	8.48 (7.72–9.12)	9.4	8.74 (8.12–9.25)	8.56 (8.07–9.1)	8.31 (7.5–9.12)
EC (µS cm^{-1})	121	190 (120–243)	705 (367–939)	873	759 (203–912)	939 (197–1 321)	1 028 (650–1 219)
Temperature (°C)	21	13.4 (6.5–20.7)	17.9 (6.6–25.1)	19.7	18.9 (6.6–26)	19.0 (10.1–25.5)	18.2 (10–28.5)
NO_2-N (mg l^{-1})		0.01 (0.001–0.054)	0.038 (0.001–0.237)	0.009	0.028 (0.00–0.109)	0.022 (0.00–0.09)	0.082 (0.001–0.484)
NO_3-N (mg l^{-1})		0.25 (0.021–0.452)	0.272 (0.095–0.409)	0.243	0.261 (0.076–0.499)	0.254 (0.068–0.615)	0.259 (0.084–0.489)
NH_4-N (mg l^{-1})		0.02 (0.00–0.06)	0.075 (0.0–0.56)	0.1	0.105 (0.0–0.76)	0.03 (0.0–0.11)	0.17 (0.0–0.9)
PO_4-P (mg l^{-1})		0.179 (0.020–0.33)	0.567 (0.27–0.99)	0.23	0.451 (0.05–1.08)	0.396 (0.23–0.86)	0.648 (0.34–1.02)
SO_4 (mg l^{-1})		27 (0–147)	29 (0–149)	69	43.3 (3–132)	45.7 (7–117)	38.2 (2–154)
$CaCO_3$ (mg l^{-1})		43.5 (28–81)	96.5 (31–138)	60	90.8 (65–126)	84.1 (47–138)	94.2 (62–214)

3.4.3 Diatom index evaluations

Three diatom indices were evaluated: the generic diatom index (GDI), the specific pollution index (SPI) and the BDI (biological diatom index), as well as the calculation of the percentage pollution-tolerant valves (%PTV) which is part of the trophic diatom index but is not considered an independent index. The SPI was successfully applied in the Mooi River, North-West province (de la Rey *et al*., 2004). For the present study the SADI was incorporated into the SPI. In some cases there was more than a four-point difference between the scores of the SPI and the BDI (Table 3-5), but this could be attributed to the fact that some species are not included in the BDI, which includes approximately 209 species, while the SPI includes all the >2 000 known taxa to which tolerance values have been ascribed (Gomà *et al*., 2005). The general trend of water quality, however, was the same. Taylor *et al*. (2007a) contended that the BDI shows the 'strongest relationship to general water quality.' The GDI scores were close to those of the SPI. Of the 97 samples from the present study, only 30 scored below 20% for %PTV, indicating that this system is dominated by pollution tolerant species and that there are significant sources of organic pollution in the catchment. When %PTV was compared to SPI it showed a correlation in the decrease of SPI scores, where the %PTV was high. The average percentage inclusion of species identified and used in the calculations for the different indices were: GDI – 94.3%, SPI– 95.7%, BDI – 86.3% and %PTV – 65.4%.

The diatom index scores for each site are presented in Table 3-5. All sites were impacted, with Petrusdal (highest upstream site) being impacted to a lesser degree. The decrease in water quality downstream was accompanied by a slight increase in pH, as well as increased levels of NO_3-N. Two sites, Stirling and Mulberry Grove, showed 'good' water quality in the winter and spring of 2010. Ecological indicator classification (Van Dam *et al*., 1994) showed this section of the river to be alkaliphilous (diatoms mainly at pH >7), while its salinity was classed as fresh brackish (salinity <0.9%) with the majority of the diatoms being able to tolerate occasional elevated levels of organic nitrogen occurring in eutrophic water with a moderate oxygen content (>50%). The SPI, considered to be the most inclusive of the diatom indices, has been effectively applied around the world (Blanco and Bécares, 2010; Blanco *et al*., 2012). The studied section of the Great Fish River does not have stratification.

Table 3-5: Average scores per diatom index and water quality classes at the five main sample sites on the Great Fish River in 2010–2012. %PTV = percentage pollution-tolerant valves; GDI = generic diatom index; SPI = specific pollution index, BDI = biological diatom index

Site	%PTV	GDI	SPI	BDI	Water quality class
Petrusdal	27.5	9.3	10.7	10.9	Poor
Clifton	32.4	8.1	9.7	10.2	Poor
Stirling	34.6	8.8	10.4	8.5	Poor/bad
Mulberry Grove	36.6	9.3	11.3	7.5	Poor/bad
Retreat	42.1	5.5	7.2	7.8	Bad

3.4.4 Correlation analysis

Whereas physical variables were sampled monthly from February 2010, chemical analysis could only be performed on the water samples monthly from June 2010; therefore, the data for the correlation analysis with chemical variables were reduced to 48 samples.

Correlation analysis illustrated a significant positive correlation between pH and %PTV (Table 3-6), showing that as the pH increases so pollution-tolerant diatom species increase. A significant negative correlation was shown between pH and BDI, suggesting that as pH increases there will be a resultant decrease in the BDI value and therefore in water quality.

Results of the correlation with chemical variables on the reduced dataset (n = 48) showed significant negative correlations between NO_3-N and BDI, and between NH_4-N and %PTV (Table 3-7). There was also a significant negative correlation between $CaCO_3$ and diversity and evenness, although this is generally considered an unreliable measure of water quality (de la Rey *et al*., 2004; de la Rey, 2007). This shows that as NO_3-N increased the value of the BDI decreased, indicating a decrease in water quality.

Not many of the chemical parameters showed significant correlations with the diatom indices. The relationship between all the indices and NO_2-N, PO_4-P, SO_4 and $CaCO_3$ were insignificant.

Table 3-6: Pearson correlations and probabilities (bracketed) of physical variables and diatom indices for all samples at all sites on the Great Fish River in 2010–2012; n = 98 (casewise deletion of missing data). Correlations in bold type significant at p < 0.05, %PTV = percentage pollution-tolerant valves; GDI = generic diatom index; SPI = specific pollution index, BDI = biological diatom index

Variable	Diatom index					
	%PTV	GDI	SPI	BDI	Diversity	Evenness
pH	**0.3095**	−0.1624	−0.0518	**−0.2357**	−0.0815	0.0129
	(0.0020)	(0.110)	(0.613)	**(0.019)**	(0.425)	(0.899)
EC	−0.0077	−0.0689	−0.0888	−0.1545	**−0.2349**	**−0.2474**
	(0.940)	(0.501)	(0.385)	(0.129)	**(0.020)**	**(0.014)**
Temp.	−0.0065	−0.0492	−0.1108	−0.1504	0.0029	−0.0518
	(0.949)	(0.631)	(0.278)	(0.139)	(0.977)	(0.612)

Table 3-7: Pearson correlations and probabilities (bracketed) of chemical variables and diatom indices for a reduced number of samples from the Great Fish River in 2010–2012; n = 48 (casewise deletion of missing data). Correlations in bold type significant at p < 0.050, %PTV = percentage pollution-tolerant valves; GDI = generic diatom index; SPI = specific pollution index, BDI = biological diatom index

Variable	Diatom index					
	%PTV	GDI	SPI	BDI	Diversity	Evenness
Nitrite (NO_2-N)	0.0367	−0.0755	−0.1559	0.0283	0.165	0.2476
	(0.803)	(0.606)	(0.285)	(0.847)	(0.257)	(0.086)
Nitrate (NO_3-N)	0.2343	−0.2599	−0.247	**−0.4029**	−0.1139	−0.0839
	(0.105)	(0.071)	(0.087)	**(0.004)**	(0.436)	(0.566)
Ammonia (NH_4-N)	**−0.3085**	0.2142	0.1721	0.16	0.0612	0.0688
	(0.031)	(0.139)	(0.237)	(0.272)	(0.676)	(0.639)
Phosphate (PO_4-P)	0.0388	−0.0692	−0.1352	−0.1431	−0.0834	−0.1049
	(0.791)	(0.636)	(0.354)	(0.327)	(0.569)	(0.473)
Sulphate (SO_4)	−0.519	−0.0084	0.0299	0.0686	0.0664	0.0825
	(0.723)	(0.954)	(0.838)	(0.639)	(0.650)	(0.573)
Calcium as $CaCO_3$	−0.1974	0.0537	0.0238	−0.0147	**−0.282**	**−0.2903**
	(0.174)	(0.714)	(0.871)	(0.920)	**(0.050)**	**(0.043)**

3.4.5 Correspondence analysis

Canonical correspondence analysis (CCA) was performed to determine the effect of environmental variables on the diatom species composition (Table 3-8). This is important to determine whether European indices can be used successfully under South African conditions. Acronyms used in the CCA graphs are listed in the relative abundance data (Appendix 1) and are in accordance with those used in Omnidia v. 5.3.

The physical and chemical variables were combined in one ordination on the reduced dataset (n = 48) (Figure 3-3). The main driver of diatom community composition for this study was EC, closely followed by NO_3-N, PO_4-P and pH. Electrical conductivity and PO_4-P had a strong negative correlation with Axis 1 and were more closely aligned to the negative side of the axis (Figure 3-3), indicating a strong relationship to the main pattern. The pH had a strong positive correlation with Axis 1 as well as a strong negative correlation with Axis 2. The NO_3-N had a strong negative correlation with Axis 2. Axis 2 represented a deviation in composition. The strongly correlated environmental variables were closer (smaller angle) to the axis (Figure 3-3). Axis 1 was the most important axis in the species composition, which suggests that EC, PO_4-P and pH were important drivers. A strong correlation existed between temperature and PO_4-P, and a correlation occurred between these two variables and $CaCO_3$ and NO_2-N. The first two axes accounted for 52.7% of the effect of the environmental variables on the species composition ($p < 0.05$). The variance inflation factors (VIFs) were all acceptably low (>10). The weighted correlation matrix is presented in Appendix 4.

Table 3-8: Summary of canonical correspondence analyses of physical and chemical parameters, on a reduced dataset, at sample sites in the Great Fish River in 2010–2012. Species–environment relations given as percentages, i.e. the effect that the environmental variable associated with that axis has on the diatom species composition ($p < 0.05$)

	Axis order			
	1	2	3	4
Eigenvalue	0.421	0.24	0.17	0.125
Species–environment correlation (%)	0.924	0.717	0.666	0.734
Cumulative percentage variance of species data	7.0	11.1	13.9	16.0
Species–environment relation (%)	33.6	52.7	66.3	76.2

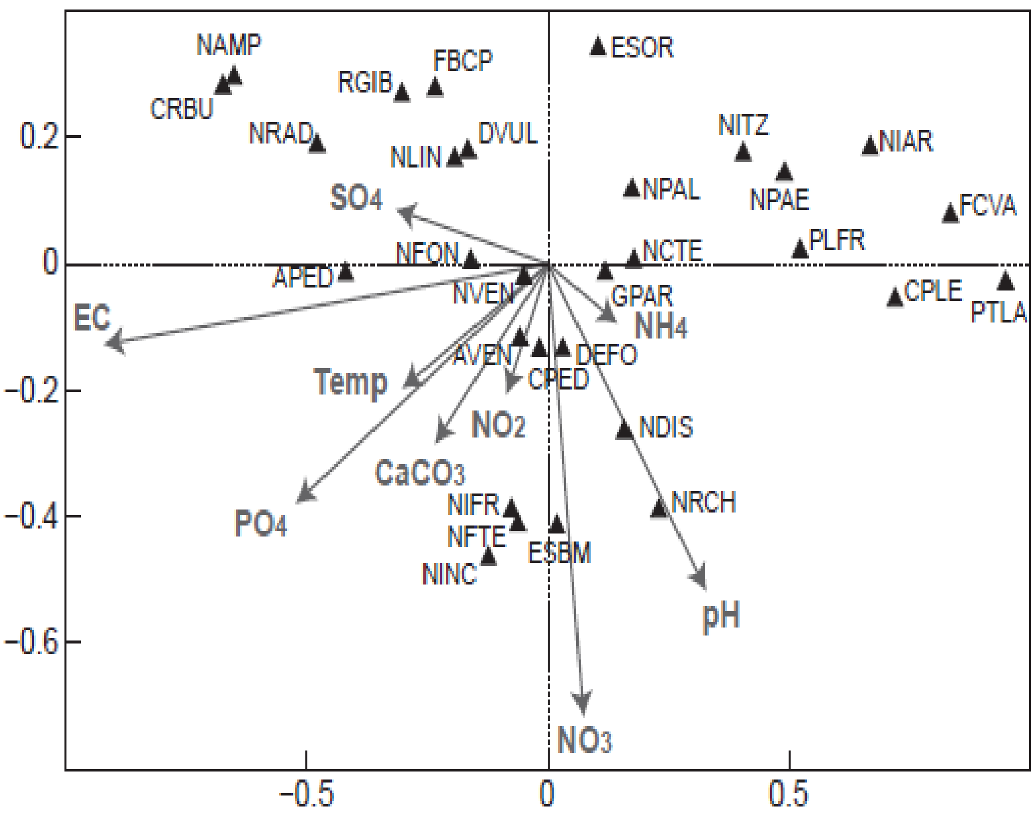


Figure 3-3: Canonical correspondence analysis (CCA) bi-plot of species vs both physical and chemical variables with reduced dataset (n = 48). Refer to Appendix 1 for an explanation of the species codes

Deformities were strongly influenced by a moderate pH and NO_3-N, whereas *Nitzschia dissipata* (Kützing) Grunow and *Navicula reichardtiana* Lange-Bertalot were strongly influenced by higher levels of pH. *Nitzschia frustulum, Nitzschia inconspicua* and their deformed forms, and *Eolima subminuscula* (Manguin) Moser, Lange-Bertalot & Metzeltin, were strongly influenced by high NO_2-N and NO_3-N levels, as expected for those species. *Amphora veneta* was influenced by mid levels of $CaCO_3$ and NO_2-N. *Amphora pediculus* was strongly associated with the higher NO_2-N levels and moderate EC. *Navicula veneta* and *Nitzschia fonticola* were shown to be strongly influenced by moderate levels of EC, and the latter by higher levels of SO_4. *Rhopalodia gibba* was found in the lower range of values recorded for pH, NH_4-N and NO_3-N, as well as high levels of SO_4. It is a freshwater species that contains a nitrogen-fixing bacterium allowing it to live in water with low levels of nitrogen (Kneip *et al*., 2007; Nowack and Melkonian, 2010). *Nitzschia amphibia* Grunow, *Craticula buderi* and *Navicula radiosa* Kützing were all associated with the higher levels of SO_4 and with lower pH levels. Archibald (1971) found *N. amphibia* to prefer a pH of >8, which accounts for it lying in the top left quadrant of the bi-plot (Figure 3-3), as the higher pH values which were >9 are in the bottom right hand quadrant. *Amphora pediculus* had a high affinity to elevated EC, with a moderate association with very high SO_4 levels. Many species preferred the median to lower range of temperature, EC, PO_4-P and $CaCO_3$ measured in this study. Values of the environmental variables measured are given in Table 3-4. Findings shown in this bi-plot of the species in this study (Figure 3-3) agree with known environmental preferences of these taxa from other regions of the world and other parts of Africa, with some exceptions – for example, Bere *et al*. (2013) found that geographical factors, especially altitude, strongly influenced the diatom community.

An additional analysis was done using only species and season (Figure 3-4) to determine if the diatom community composition showed seasonality. This ordination revealed little seasonal influence on the diatom community. Nitzschia frustulum and species related to that group, and Cocconeis placentula var. euglypta only slightly favoured autumn conditions. Under Finnish conditions the latter species was found to prefer summer to autumn conditions (Korhonen *et al*., 2013). Amphora veneta showed a preference for warmer conditions, while most other species were centred in the bi-plot, showing that season was not a major influence on diatom community composition.

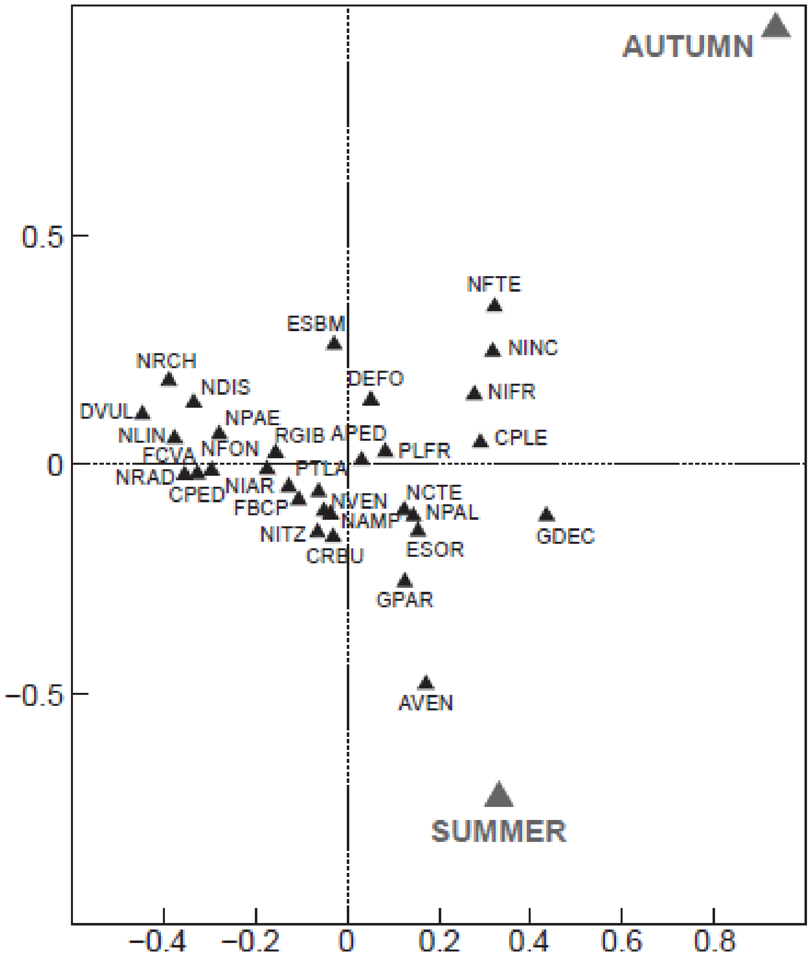


Figure 3-4: Canonical correspondence analysis (CCA) bi-plot of species vs seasonality. Refer to Appendix 1 for an explanation of the species codes

3.5 Discussion

3.5.1 Community composition

Most of the species identified in the present study were pollution-tolerant. These included *Nitzschia palea, Gomphonema parvulum, Mayamaea atomus* (Kützing) Lange-Bertalot, *Navicula crytocephala* Kützing and *Eolima subminiscula. Nitzschia palea* is described by some as a medium pollution-tolerant species (Triest *et al*., 2012; Kalyoncu and Şerbetci, 2013; Bere and Mangadze, 2014), whereas Van Dam *et al*. (1994), Salomoni *et al*. (2006), Potapova and Charles (2007), Lavoie *et al*. (2009) and Bere *et al*. (2014) describe it as an indicator of hypereutrophic conditions. Having an affinity for water with higher EC levels, it was found at all sites and in most of the samples collected in the present study. Besse-Lototskaya *et al*. (2011)

considered this species to be unreliable as an indicator species, due to the possibility of misidentification of the varieties which have different environmental tolerances. Trobajo *et al*. (2009) suggested that this species could be split as, although morphologically similar, its components may not necessarily share the same ecological preferences. Bere *et al*. (2013) found this species and *Nitzschia linearis* at high altitude and high Ca^{2+} levels. The Ca levels (as $CaCO_3$) in this study were much higher than those found in mountain streams in Zimbabwe (Bere *et al*., 2013). In this study the CCA revealed that *N. palea* preferred the lower ranges of measured EC, PO_4-P, $CaCO_3$, NO_2-N and temperature while preferring the median pH range (Figure 3-3).

Gomphonema parvulum is described as being tolerant of extremely polluted conditions in a wide range of waters (Salomoni *et al*., 2006; Taylor *et al*., 2007d; Szczepocka and Szulc, 2009; Urrea-Clos and Sabater, 2012; Bere and Mangadze, 2014) and was found at all sites. It is a good indicator of high organic pollution. This species was found mostly at sites Clifton and Retreat, and the ordination shows it preferred moderate conditions at these sites. In Zimbabwe, Bere *et al*. (2013) found this species at cooler, high-altitude sites that were less impacted than those in this study, in addition to having a higher stream velocity. This study showed that *G. parvulum* had only a slight preference for warmer conditions.

Cocconeis placentula is considered to be an indicator of less-polluted conditions (Salomoni *et al*., 2006) and is known to be sensitive to organic pollution (Szczepocka and Szulc, 2009). The *euglypta* variety was found to be an indicator species in a small, high-mountain stream in the Pyrenees with a low discharge, preferring spring conditions, whereas the nominate variety prefers early to late summer conditions (Gomà *et al*., 2005). *Cocconeis placentula* var. *euglypta* has the same ecology as the nominate variety and was therefore one of the dominant species at site Petrusdal, the highest site and subject to a lower flow except in flooding events. Martinez de Fabricius *et al*. (2003), however, found the nominate variety to prefer high-flow conditions found in summer in South America. In the present study, this species showed only a very slight preference for cooler autumn conditions (Figure 3-4). Gallo *et al*. (2015) considered this species to be a ‘pioneer’, occurring in both low- and high-flow environments. This would explain the abundance of this species at both the high-altitude sites, which are subject to periodic high-velocity flooding events. Birkett and Gardiner (2005) found that *Cocconeis* assemblages dominate upstream locations, while *Nitzschia*, being a pollution-tolerant genus, occurs more commonly in lower locations. While *Cocconeis* was only dominant at the highest location, *Nitzschia* was common at all the sites, including the highest one. Vilbaste and Truu (2003), however, classified *Cocconeis placentula* as being characteristic of eutrophic or hypereutrophicconditions, while Korhonen *et al*. (2013) found them in meso- and eutrophic streams. Wu (1999) found *Cocconeis* to be plentiful in unpolluted waters and Bere *et* *al*., (2014) found it preferred cleaner water with a higher Ca^{2+} (Mangadze *et al*., 2015). Lavoie *et al*. (2004, 2009) found it to be abundant at agriculturally impacted sites. Rimet and Bouchez (2012) found *C. placentula* var. *euglypta* to prefer water in areas with limestone geology. The present study found the species to prefer a lower PO_4-P range with a moderate NO_3-N range, while limestone is common in the geology in this area. The variety *lineata* was found to be sensitive to organic pollution (Szczepocka and Szulc, 2009), while Beltrami (2010) found it to prefer a slightly lower EC and nitrogen levels than the variety *euglypta*, although both were classed as eutrophic indicators in that study. Krammer and Lange-Bertalot (1999a, 1999b) confirmed that both these varieties have been found in conjunction in the same habitats. These *Cocconeis* species have not all shown the same environmental preferences worldwide.

Nitzschia paleacea and *Amphora pediculus* are both tolerant of heavy pollution, while *Nitzschia linearis* and *Diatoma vulgaris* are tolerant of moderate levels of pollution, with a preference for low-flow conditions (Martinez de Fabricius *et al*., 2003). According to Walsh and Wepener (2009), under South African conditions the latter species is found in waters with elevated levels of PO_4-P, and this was confirmed in the present study (Figure 3-3) when measured levels were taken into account. Szczepocka and Szulc (2009) placed *N. paleacea* in a group that is resistant to organic pollution. This study found the species to prefer lower ranges of most variables, but median levels of pH, NH_4-N and NO_3-N. *Amphora pediculus* and *Cymbella* spp. occur in hard water. Kelly *et al*. (1995) suggest that *A. pediculus* is more abundant when nutrients are high but organic pollution levels comparatively lower. This study showed it prefers slightly more than moderate levels of EC and PO_4-P with a higher water temperature (Figure 3-3). *Diatoma vulgaris* is also known to prefer alkaline conditions (Chaïb and Tison-Roseberry, 2012). The present study, however, found it to prefer the lower alkaline range of measured pH and NO_3-N. *Planothidium lanceolatum*, dominant at site Petrusdal, is tolerant of mild pollution while Roubeix *et al*. (2011) found it to be tolerant of herbicide pollution. Weilhoefer and Pan (2008) found this species to be more abundant at lower total phosphate concentrations, although Van Dam *et al*. (1994) found it to be eutrophentic (i.e. tolerating high nutrient concentrations). Our study concurs with the findings of Weilhoefer and Pan (2008). Although only inorganic phosphate was measured in this study, *P. lanceolatum* was found to prefer the lower levels of PO_4-P. Elias *et al*. (2012) found that, while *A. pediculus* preferred summer conditions, *P. lanceolatum* and *N. palea* were not season dependent and *N. inconspicua* was more abundant in autumn. This study found that *A. pediculus*, *P. lanceolatum* and *N. palea* did not exhibit seasonality, but did find that the *N. frustulum/inconspicua* group was more abundant in autumn.

The dominant species in the single sample taken at Top Petrusdal, *Cocconeis placentula* var. *lineata*, *C. placentula* var. *euglypta*, *N. paleacea* and an unidentified *Nitzschia* species, all suggest that this site, although near the peak of Nardousberg, is impacted by human activities. The same impact can be inferred from the one sample, taken for comparison purposes, from Willem Burgers in which *C. pediculus* (20.4%) and *N. frustulum* (30.8%) were dominant. Results of the diatom community composition in this study reveal that there is a high agricultural impact, as *Nitzschia* make up 40.9% of the total diatom community, with only 27.8% belonging to the *Navicula* group (Walsh, 2008). The Epithemiaceae accounted for 11.4% of species identified in all samples at all sites.

When an aquatic ecosystem is impacted by agriculture, *Nitzschia* species dominate (Walsh and Wepener, 2009; Eassa, 2012), while sites with a lower level of impact will be dominated by *Navicula* species. *Nitzschia frustulum*, besides being dominant at three of the sites, was found in most samples. This species is usually found in waters with a high EC and brackish (Archibald, 1971) and is tolerant not only of critical levels of pollution but is also able to survive conditions where there is often a change in osmotic pressure, such as flooding and very low flow (Taylor *et al*., 2007d). It has been suggested that this species is an indicator of medium to high levels of SO_4 and EC (Bate *et al*., 2004). This study showed that *N. frustulum* was influenced by high NO_2-N and NO_3-N levels (Figure 3-3). According to Pan *et al*. (1996), *Amphora pediculus*, *Navicula cryptotenella* and *Navicula gregaria* are indicator species of high pH levels. The present study found *Amphora pediculus* to be an indicator of high EC, while *N. cryptotenella* was slightly influenced by a median pH level and high NH_4-N levels. *Diatoma vulgaris, N. frustulum* and *A. pediculus* were found to be common at agriculturally impacted sites in the Crocodile and Magalies rivers (Walsh and Wepener, 2009), as in the present study.

Sites Clifton and Retreat both had high mean $CaCO_3$ (96.5 mg l^{-1} and 94.2 mg l^{-1}, respectively) but *Craticula buderi* was dominant only at site Retreat. This could be an indication that, although able to live in a wide range of trophic conditions, it can tolerate higher levels of pollution. *Fragilaria* in general is known to need high nutrient concentrations (Mangadze *et al*., 2015). In the present study this taxon is linked to high levels of SO_4 but lower NO_3-N and pH (Figure 3-3). *Rhopalodia gibba* can be an indicator of high phosphate concentrations but, although this species was dominant only at Stirling and Mulberry Grove, three of the sites had a higher P ratio than N. According to Slate and Stevenson (2007), species such as *Nitzschia frustulum, Amphora veneta, Gomphonema affine, Gomphonema gracile, Gomphonema parvulum, Navicula cryptochephala, Navicula cryptotenella and Rhopalodia gibba* are all common in PO_4-P-enriched conditions. They also commonly occur in nutrient-rich waters with high EC (Potapova and Charles, 2003). These species were found in most samples at all sites. This was confirmed by the chemical results and the poor N:P ratio in water. The EC readings at four of the five sites, having a mean value of above 700 µS cm^{-1}, were considered to be on the high side. Species such as *Nitzschia palea, G. parvulum* and *Sellaphora pupula* are indicators of water with high nutrient levels, high organic pollution, poorly oxygenated and low percentage canopy cover (Potapova and Charles, 2003; Bere and Tundisi, 2009; Kalyoncu and Şerbetci, 2013). These three species occurred at all sites but were not dominant when all samples were considered. They did, however, occur in high numbers at times.

3.5.2 Deformed cells

The highest percentages of deformed cells were found at Stirling (4.06%) and Mulberry Grove (4.05%). The site Petrusdal, which is nearest the source, had deformities of 3.12%, followed by Clifton at 3.11%. Retreat deformities accounting for 2.92% of the total number of diatoms counted. A number of deformed and severely deformed cells were encountered during the study (Figure 3-2). Morin *et al*. (2012) suggest the percentage of deformities in a community should not exceed 3.5%. Deformities below 3.5% could be considered natural variations, while values above 3.5% would be considered excessive, indicating contamination. The average percentage of deformities across all sites for this study was 3.5%. These deformities included both changes in shape and outline, as well as disruption of the striae (Figure 3-2). The severity of the deformities found could be linked to the increase in UV radiation (Falasco *et al*., 2009a, 2009b) due to the removal of riparian vegetation or to low silica (Taylor, 2004; Walsh and Wepener, 2009), which was not measured in this study. Esquius *et al*. (2012) found a strong negative correlation between pH and cell deformity in *C. placentula*. High rates of soil erosion can increase the amount of fertiliser, pesticides and herbicides that enter the water (Falasco *et al*., 2009b). Ansara-Ross *et al*. (2008) suggested that pesticides may cause asymmetrical development as they contain endocrine disruptors. Walsh and Wepener (2009) suggested that abnormal cell growth may be a suitable indicator of the health of an ecosystem, while others consider the frustule differences to be natural morphological variation. In this case, however, variations were so severe and persistent that it was unlikely to be a natural morphological phenomenon. Many of the deformities were of *Nitzschia* species, which are pollution-tolerant. The types of deformities found were similar in appearance to those discussed by Falasco *et al*. (2009a, 2009b), but those deformities were found to be caused by Cd. It is highly unlikely that this would be the case here. Similar types of deformities were found by Morin *et al*. (2012), who found the deformities were caused by metal contamination. Although metals were not measured in the present study, they are unlikely to be the cause of these deformities, as there is no industrial activity such as mining or industrial manufacturing in this area. The types of *Nitzschia* deformities noted in this study are similar to those found by Roubeix *et al*. (2011), linking diatom deformities to metolachlor, an organic compound used in certain herbicides.

3.5.3 Diatom-based indices

The SPI and BDI diatom index scores tested in this study show that water quality deteriorates during the rainy periods, possibly due to the influx of nutrients washed in from the surrounding agricultural lands. The average of %PTV, as well as of SPI, show that the Great Fish River water is of poor quality, falling into the meso-eutrophic category. The BDI and SPI showed similar results. Site Retreat repeatedly had the lowest quality, with only one SPI value above 11. The BDI (86.3%) was not as inclusive as the SPI (95.7%). This study confirms the findings of Taylor (2004) that the SPI yielded good results and was the most inclusive diatom index used under South African conditions. It is interesting to note that the %PTV values recorded by Walsh (2008) in the Magalies and Crocodile rivers were lower than those of the Great Fish River, suggesting that this river is more polluted, with more organic nitrogen than the Magalies and Crocodile rivers. The %PTV inclusion rate (65.4%) was not as high as those of the other indices tested. This could result in lower scores than conditions exhibit. An example is *Nitzschia amphibia*, which was found in this study, but was not included in that calculation, as it was not found by Kelly and Whitton (1995) in UK conditions.

Taylor *et al*. (2007a) found the BDI, SPI, GDI and %PTV indices to provide similar results to those obtained in Europe. Bate *et al*. (2002), however, proposed that European indices, and especially the ecological groupings of Van Dam *et al*. (1994), were not applicable under South African conditions. They based their statement on the lack of correlation found in their data and lack of data on South African conditions for the BDI (Bate *et al*., 2004). There has, however, since been an improvement on the SPI for South African conditions by incorporating SA endemic species in the SADI. Almeida (2001) found the SPI to be sensitive in Portugal. Chaïb and Tison-Roseberry (2012) questioned the use of the BDI for non-European conditions, while several authors (Potapova and Charles, 2007; Besse-Lototskaya *et al*., 2011) questioned the variation in environmental prerequisites for individual species on the different continents and eco regions. Kriel (2008) found these indices to be successful in reflecting the water quality in the North-West province. The present study has found these indices to provide valuable insight into the water quality of the Great Fish River. Additional research into ecological preferences of diatom species under South African conditions could enable these indices to become more robust for use in local conditions.

3.5.4 Correlation analysis

Temperature and pH play an important role in the structure of diatom communities (Pan *et al*., 1996; Bere and Mangadze, 2014), while pH also influences many other water chemistry variables. Taylor *et al*. (2007a) found a significant negative correlation between the BDI and pH, as well as EC, in the Vaal and Wilge rivers. The present study revealed a negative relationship between the BDI and pH, but no significant correlation with any of the diatom indices tested was found with EC. Temperature, although a metabolic driver, did not influence the index scores as much as nutrients, which could be due to the difference of the South African climate to that in Europe, upon which these indices are based (Taylor *et al*., 2007b). The CCA analysis, however, showed EC to be one of the main environmental drivers in this system, yet this variable did not have any significant correlation to indices (Table 3-6).

Significant correlations occurred between nutrients NH_4-N and NO_3-N and the %PTV and BDI, respectively, as expected (Table 3-7). Taylor (2004) found a strong correlation between the indices and EC, as well as with nitrogen, when re-analysing diatom samples from the Jukskei–Crocodile river system. Prygiel and Coste (1993) found a significant correlation between two indices (SPI and GDI) and EC and sulphates.

3.5.5 Correspondence analysis

Three rough groupings of diatoms emerge from this analysis (Figure 3-3). In the top right quadrant are those species that prefer the lower spectrum of nutrient values, EC and temperature (less polluted water) measured during this study. The species in the top left quadrant are those that prefer higher SO_4 readings, but at lower pH levels measured (less alkaline). Those species in the bottom quadrants have a common tolerance for moderate to high levels of environmental variables measured during this study. The last group consists of those species that are indicative of eutrophication or elevated levels of pollution. These species include the *Nitzschia frustulum* group, *Navicula reichardtiana*, *Nitzschia dissapata* and *Amphora pediculus*.

Correspondence analysis diagrams with physical and chemical variables differed only slightly when season was added as a covariable. This indicates that season is not a determining factor in the composition of the diatom communities, as was also found in Portugal by Novais *et al*. (2012). The physical variables pH and EC have a larger influence than temperature in diatom community structure. Increased EC values can result in a shift of diatoms species composition to include dominant species such as *Nitzschia palea, Nitzschia dissipata* and *Nitzschia capitellata* (Eloranta and Soininen, 2002; Walsh, 2008; Cohen, 2010). Nitrogen and phosphates played a large role in the composition of diatom assemblages in Italy (Bona *et al*., 2007) and China (Wu *et al*., 2014), as is confirmed by the present study which shows that, of the chemical variables monitored, NO_3-N and PO_4-P have the largest impact. This is unlike the findings by Lavoie *et al*. (2004) who found that NO_3-N and PO_4-P were not important variables in their evaluation of agriculturally impacted streams and rivers in Canada. According to Bate *et al*. (2002), the most important environmental variables affecting rivers studied in the Eastern Cape were pH and EC, which is the same finding as those of Lavoie *et al*. (2004) in Canada, Bere and Tundisi (2009) in Brazil, and Imanpour *et al*. (2013) in Iran. Bere *et al*. (2013) found temperature, NO_3 and Ca to be the main drivers in the relatively unimpacted mountain streams of the Eastern Highlands, Zimbabwe. The present study validates EC and NO_3-N as the main environmental drivers in the Great Fish River, followed by PO_4-P and pH. This shows a similarity with the findings on EC by Bate *et al*. (2004) within the same province, but different to the environmental drivers in Zimbabwe. Conditions in the Eastern Cape province are more semi-arid, whereas Zimbabwe is subtropical. There was also contradiction with other findings for agriculturally impacted rivers (Lavoie *et al*., 2004).

Research conducted in different habitats such as wetlands, estuaries and mangroves has revealed that salinity (EC) and nutrients play a major role in diatom community composition (Underwood *et al*., 1998; Gell *et al*., 2002; Gaiser *et al*., 2005; Della Bella *et al*., 2007), with distinct separation of assemblages between freshwater, marine and mangroves. Zalat and Vildary (2005) found a distinct variation in diatom communities in three different lakes in Egypt based on the EC gradient and nutrient levels, confirming that EC has in fact a major influence on diatom assemblages, regardless of water type.

3.6 Conclusions and recommendations

This study revealed the current physical and chemical signature of this part of the Great Fish River. Extremes in environmental variables were seen, and readings often exceeded the National Water Quality Guidelines target water quality range (DWAF, 1996). This study showed, through correlations and CCA, that diatoms are effective indicators of water quality in the study area. There was no relatively unimpacted reference site with which to compare the analysis, which possibly resulted in poor correlations. The CCA clearly demonstrated the diatom species response to environmental changes. The dominant taxa gave an indication of water quality, which was confirmed by the environmental variables. In addition, the diatom deformities indicated other impacts not detected in the suite of chemical analyses undertaken.

Some potential failings in this study include the reliability of the rock scrapings, as this river system is subject to flooding, albeit at irregular intervals. Not all diatom samples were inspected after collection to confirm that the majority of the cells were in fact alive. Identification of the genus *Achnanthidium* to species level is extremely difficult, and most species were not individually identified but were grouped together as *Achnanthidium* spp. (code ACHD). Fortunately, the number of specimens of this genus (relative abundance = 2.6%) was not large enough to impact the results adversely. Cosmopolitan species accounted for 99.3% of the diatom community. Certain species regarded in this study as indicator species have been found in various environmental conditions by some authors, as compared to those in this study. The increasing amount of data being collected for South African conditions should allow the European diatom indices discussed in the present study to become more robust.

The present study shows that this river has been significantly impacted by decades of farming activities. Irrigated lands are often situated close to the river. As the water evaporates from these fields the remaining salts are washed into the river, thereby increasing its EC and nutrient concentration. Long-term monitoring of this and other rivers in the semi-arid Karoo could improve knowledge of the aboveground water systems. During the course of this study it was found that the local farmers were not aware of the impacts of their activities on the water quality, which in turn affects their ability to farm effectively. As chemical analysis of the water is costly, it is suggested that farmers consider using diatoms for biomonitoring as a more cost-effective alternative for monitoring ecosystem changes. Environmental assessments using diatoms and macroinvertebrates are often the most consistent indicators in aquatic environments, providing complementary information (Blanco and Becares, 2010). Diatoms can be considered a more reliable indicator of river water quality, when assessing eutrophication and organic pollution, than macroinvertebrates (de la Rey *et al*., 2004; de la Rey, 2007; Feio *et al*., 2007) showing a higher sensitivity to nutrient concentrations and biological oxgygen demand (Hering *et al*., 2006). Fish communities in this study area are species poor and only occur in the few larger, deeper pools. In some areas, macrophytes are limited and include exotic species (MH pers. obs.). Fish and macrophytes would, therefore, in the authors' opinion, not be suitable for use as bioindicators in this study area.

The data from this study have contributed towards understanding the Great Fish River system and the challenges it faces. Continued monitoring of this section of the Great Fish River is recommended, as extreme climatic variation, including droughts and excessively rainy periods, in this semi-arid region is common. This study covered a period of two years, of which the beginning was a very dry period but the rest was an extremely wet period for this area. Long-term data will offer a more integrated picture of the river health, as well as more information on the temporal and spatial variation in diatom assemblages in South Africa. An additional recommendation would be to determine which metals occur in the water, as well as organic particulates, in an attempt to determine the cause of the severe diatom deformities observed. The individual diatom environmental preferences show that there is variation between ecoregions. This suggests that calibration or testing of diatom indices on regional scales is necessary and should be considered in future studies.

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3.8 References

Academy of Natural Sciences. (2011). *ANSP alage image database.* http://diatom.acnatsci.org/AlgaeImage/SearchCriteria.asp (accessed 2010, 2011, 2012 and 2013).

Almeida, S. F. P. (2001). Use of diatoms for freshwater quality evaluation in Portugal. *Limnetica*, 20, 205-213.

Álvarez-Blanco, I., Blanco, S., Cejudo-Figueiras C. and Bécares, E. (2012). The Duero Diatom Index (DDI) for river water quality assessment in NW Spain: design and validation. *Environmental Monitoring and Assessment*. 185, 969-981.

Ansara-Ross, T. M., Wepener, V., Van den Brink, P. J., Ross, M. J. (2008). Probabilistic risk assessment of the environmental impacts of pesticides in the Crocodile (west) Marico catchment, North-West Province. *Water SA*, 34, 637-645.

Archibald, R. E. M. (1971). Diatoms from the Vaal Dam catchment area, Transvaal, South Africa. *Botanica Marina, XIV*, 17-70.

Archibald, R. E. M. (1983). *The diatoms of the Sundays and Great Fish rivers in the Eastern Cape Province of South Africa.* Vaduz: J Cramer.

Bate, G. C., Adams, J. B., Van der Molen, J. S. (2002). *Diatoms as indicators of water quality is South African river systems*. Water Research Commission Report No. 814/1/02. Pretoria, South Africa: Water Research Commission.

Bate, G. C., Smailes, P. A., Adams, A. (2004). A water quality index for use with diatoms in the assessment of rivers. *SA Water,* 30, 493-498.

Beltrami, M. E. (2010). Benthic diatoms for ecological water quality assessemtn in Italy, with special reference to Alpine ecoregions. PhD thesis. University of Innsbruck, Austria.

Bere, T., Mangadze, T. (2014). Diatom communities in streams draining urban areas: Community structure in relation to environmental variables. *Tropical Ecology,* 55, 271-281.

Bere, T., Mangadze, T., and Mwedzi, T. (2014). The application and testing of diatom-based indices of stream water quality in Chinhoyi Town, Zimbabwe. *Water SA*, 40, 503-512.

Bere, T., Phiri, C., Kadye, W. T., Utete, B. (2013). Benthic diatom assemblages in mountain streams: community structure in relation to environmental and human pressures. *African Journal of Ecology*, 51, 625-634.

Bere, T., Tundisi, J. G. (2009). Weighted average regression and calibration of conductivity and pH of benthic diatom assemblages in streams influenced by urban pollution - Sao Carlos/SP Brazil. *Acta Limnologica Brasil,* 21, 317-325.

Besse-Lototskaya, A., Verdonschot, P. F. M., Coste, M., Van de Vijver, B. (2011). Evaluation of European diatom trophic indices. *Ecological Indicators,* 11, 456-467.

Birkett, K., Gardiner, S. (2005). The use of epilithic and epiphytic diatoms as indicators of organic pollution in the Cheboygan River, Cheboygan County, Michigan. University of Michigan USA. Available at http://deepblue.lib.umich.edu/handle/2027.42/55043 [Accessed 23 October 2011].

Blanco, S., Bécares, E. (2010). Are biotic indices sensitive to river toxicants? A comparison of metrics based on diatoms and macroinvertebrates. *Chemosphere,* 79, 18-25.

Blanco, S., Cejudo-Figueiras, C., Tudesque, L., Bécares, E., Hoffmann, L., Ector, L. (2012). Are diatom diversity indices reliable monitoring metrics? *Hydrobiologia*, 695, 199-206.

Bona, F., Falasco, E., Fassina, S., Griselli, B., Badino, G. (2007). Characterization of diatom assemblages in mid-altitude streams of NW Italy. *Hydrobiologia,* 583, 265-274.

Chaib, N., Tison-Rosebery, J. (2012). Water quality assessment and application of the biological diatom index in the Kebir-East wadi, Algeria. *African Journal of Aquatic Science,* 37, 59-69.

Cohen, N. (2010). The effect of increased salinity on diversity and abundance of diatoms. The Pennsylvania State University, USA. Available at http://marcellus.psu.edu/resources/PDFs/salinity.pdf [Accessed 26 October 2011].

Dallas, H. F., Day, J. A. (2004). *The effect of water quality variables on aquatic ecosystems: a review.* Water Research Commission Report No. TT224/04. Cape Town, South Africa: Water Research Commission.

Davies, B., Day, J. (1998). *Vanishing waters*. Cape Town, South Africa: UCT Press.

Debenest, T., Silvestre, J., Coste, M., Delmas, F., Pinelli, E. (2008). Herbicide effects on freshwater benthic diatoms: Induction of nucleus alteration and silica cell wall abnormalities. *Aquatic Toxicology,* 88, 88-94.

De la Rey, P. A. (2007). Evaluation of the applicability of diatom based indices as bioindicators of water quality in South African rivers. PhD thesis, North-West University, South Africa.

De la Rey, P. A., Taylor, J. C., Laas, A., Van Rensburg, L., Vosloo, A. (2004). Determining the possible application value of diatoms as indicators of general water quality: A comparison with SASS 5. *Water SA*, 30, 325-332.

Della Bella, V., Puccinelli, C.,Marcheggiani, S.,Mancini, L. (2007). Benthic diatom communities and their relationship to water chemistry in wetlands of central Italy. *Annales de Limnologie-International Journal of Limnology*, 43, 89-99.

Department of Water Affairs and Forestry (DWAF). (1996). *Water quality guidelines. Volume 7. Aquatic Ecosystems.* Pretoria: Department of Water Affairs and Forestry.

Eassa, A. M. (2012). The use of diatom indices for the assessment of Shatt AL-arab river water quality. *Journal of Basrah Researches (Sciences),* 38, 114-124.

Elias, C. L., Vieira, N., Feio, M. J., Almeida, S. F. P. (2012). Can season interfere with diatom ecological quality assessment. *Hydrobiologia,* 695, 223-232.

Eloranta, P., Soininen, J. (2002). Ecological status of some Finish rivers evaluated using benthic diatom communities. *Journal of Applied Phycology,* 14, 1-7.

Esquius, K. S., Altamirano, S. M., Escalante, A. H. (2012). Abnormal forms of *Cocconeis placentula* (Ehrenberg) in a eutrophic shallow lake (BS. AS. province, Argentina). In: Dos Santos Afonso M, Sanchez RMT (eds), *Ciencia y tecnologia ambiental: un enfoque integrador* [Environmental science an technology: an integrative approach; conference proceedings] Argentina y Ambiente May 2012*,* Mar De Plata, Argentina. Buenos Aires: Asociación Argentina para el Progreso de las Ciencias, pp. 250-255.

Falasco, E., Bona, F., Ginepro, M., Hlúbiková, D., Hoffmann, L., Ector, L. (2009a). Morphological abnormalities of diatom silica walls in relation to heavy metal contamination and artificial growth conditions. *Water SA,* 35.

Falasco, E., Bona, F., Badino, G., Hoffmann, L., Ector, L. (2009b). Diatom teratological forms and environmental alterations: A review. *Hydrobiologia,* 623, 1-35.

Feio, M. J., Almeida, S. F., Craveiro, S. C., Calado, A. J. (2007). Diatoms and macroinvertebrates provide consistent and complementary information on environmental quality. *Fundamental and Applied Limnology/Archiv für Hydrobiologie*, 169, 247-258.

Gaiser, E., Wachnicka, A., Ruiz, P., Tobias, F., Ross, M. (2005). Diatom indicators of ecosystem change in subtropical coastal wetlands. *Estuarine Indicators*. CRC Marine Science Series. Boca Raton: CRC Press. pp 127-144.

Gallo, L., Battegazzore, M., Corapi, A., Lucadamo, L. (2015). Changes in epilithic diatom communities and periphytic biomass downstream of a reservoir on a Mediterranean river (Calabria region, S Italy). *Turkish Journal of Botany*. 39, 555-569.

Gell, P.A., Sluiter, I. R., Fluin, J. (2002). Seasonal and interannual variations in diatom assemblages in Murray River connected wetlands in north-west Victoria, Australia. *Marine and Freshwater Research,* 53, 981-992.

Giffen, M. H. (1966). Contributions to the diatom flora of Southern Africa. II. Diatoms from Hogsback region of the Amatola mountains, Eastern Cape Province, South Africa. *Nova Hedwigia* 21, 123-160.

Gomà, J., Rimet, F., Cambra, J., Hoffmann, L., Ector, L. (2005). Diatom communities and water quality assessment in mountain rivers of the upper Segre Basin (la Cerdanya, Oriental Pyrenees). *Hydrobiologia,* 551, 209-225.

Harding, W. R., Archibald, C. G. M., Taylor, J. C. (2005). The relevance of diatoms for water quality assessment in South Africa: A position paper. *Water SA,* 31, 41-46.

Harding, W. R., Taylor, J. C. (2011). *The South African diatom index (SADI) – A preliminary index for indicating water quality in rivers and streams in Southern Africa*. Water Research Commission Report No. 1707/1/11. Pretoria, South Africa: Water Research Commission.

Hering, D., Johnson, R. K., Buffagni, A. (2006). Linking organism groups—major results and conclusions from the STAR project. Furse, M.T., Hering,D., Brabec, K., Buffagni, A., Sandin, L., Verdonschot, P.F.M. (eds). In *The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods. Hydrobiologia,* 566*,* 109-113.

Imanpour, N. J., Sharifinia, M., Ramezanpour, Z. (2013). Biodiversity of diatom population in the Masouleh stream, Guilan, Iran [in Arabic; English Abstract]. *Journal of Taxonomy and Biosistematics,* 5(15), 37-47.

Kalyoncu, H., Şerbetci, B. (2013). Applicability of diatom-based water quality assessment indices in Dari stream, Isparta-Turkey. *World Academy of Science, Engineering and Technology,* 7, 188-196.

Karthick, B., Taylor, J. C., Mahesh, M. K., Ramachandra, T. V. (2010). Protocols for collection, preservation and enumeration of diatoms from aquatic habitats for water quality monitoring in India. *The IUP Journal of Soil and Water Sciences*, 3, 25-60.

Kelly, M. G., Whitton, B. A. (1995). The trophic diatom index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, 7, 433-444.

Kelly, M. G., Penny, C. J., Whitton, B. A. (1995). Comparative performance of benthic diatom indices used to assess river water quality. *Hydrobiologia,* 302, 179-188.

Kneip, C., Lockhart, P., Voß, C., Maier, U. G. (2007). Nitrogen fixation in eukaryotes–new models for symbiosis. *BMC Evolutionary Biology*, 7, 55.

Korhonen, J.J., Köngäs, P., Soininen, J. (2013). Temporal variation of diatom assemblages in oligotrophic and eutrophic streams. *European Journal of Phycology,* 48, 141-151.

Krammer, K. (1997). *Die Cymbelloiden diatomeen. Eine monographieder weltweit bekannte taxa. Teil 1. Allegemeines und Encyonema part* (Bibliotheca Diatomologica Band 36 ed.). Stuttgart: J Cramer.

Krammer, K. (2000). Diatoms of the European inlands waters and comparable habitats. Volumne 1. The genus *Pinnularia*. In: Lange-Bertalot, H. (ed), *Diatoms of Europe*. Ruggell, Germany: A.R.G. Ganter Verlag Kommanditgeselschaft.

Krammer, K., Lange-Bertalot, H. (1987). *Bacillariaceae, Epithemiaceae, Surirellaceae neue und bekannte taxa, neue kombinationen und synonyme sowie bermerkungen und erganzungen zu den Naviculaceae*. Berlin. Stuttgard, Germany: J Cramer.

Krammer, K., Lange-Bertalot, H. (1999a). *Susswasserflora von Mitteleuropa. Bacillariophyceae. 2/2 Bacillariaceae, Epithemiaceae, Surirellaceae* (2nd ed.). Berlin, Heidelberg: Elsevier Spektrum Akademischer Velag.

Krammer, K., Lange-Bertalot, H. (1999b). *Susswasserflora von Mitteleuropa. 2/1. Bacillariophyceae. Naviculaceae* (2nd ed.). Berlin, Heidelberg: Elsevier Spektrum Akademischer Velag.

Kriel, G. P., (2008). *Biological indicators of water quality in an urban waterway: Can diatoms reflect short term spatial and temporal changes in water quality?* Unpublished MSc dissertation, North-West University, Potchefstroom.

Lange-Bertalot, H. (1993). *85 new taxa and much more than 100 taxonomic clarifications supplementary to Susswasserflora von Mitteleuropa vol 2/1-4.* (Bibliotheca Diatomologica Band 27 ed.). Hirschberg, Germany: J Cramer.

Lange-Bertalot, H. (1996). *Inconographia diatomologica*. Konigstein, Germany: Koeltz Scientific Books.

Lavoie, I., Vincent, W. F., Pienitz, R., Painchaud, J. (2004). Benthic algae as bioindicators of agricultural pollution in the streams and rivers of southern Québec (Canada). *Aquatic ecosystem health & Management*, 7, 43-58.

Lavoie, I., Hamilton, P. B., Wang, Y. K., Dillon, P. J., Campeau, S. (2009). A comparison of stream bioassessment in Québec (Canada) using six European and North American diatom-based indices. *Nova Hedwigia*, 135, 37-56.

Lecointe, C., Coste, M., Prygiel, J. (1993). `Omnidia': Software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia: The International Journal on Limnology and Marine Sciences,* 269-270, 509-513.

Mangadze, T., Bere, T., Mwedzi, T. (2015). Epilithic diatom flora in contrasting land-use settings in tropical streams, Manyame Catchment, Zimbabwe. *Hydrobiologia*, 753, 163-173.

Marks, J. C., Lowe, R. L. (1993). Interactive Effects of Nutrient Availability and Light Levels on the Periphyton Composition of a Large Oligotrophic Lake. *Canadian Journal of Fisheries and Aquatic Sciences,* 50, 1270-1278.

Martínez de Fabricius, A., Maidana, N., Gómez, N., Sabater, S. (2003). Distribution patterns of benthic diatoms in a Pampean river exposed to seasonal floods: the Cuarto River (Argentina). *Biodiversity and Conservation,* 12, 2443-2454.

Morin, S., Cordonier, A., Lavoie, I., Arini, A., Blanco, S., Duong, T. T., Sabater, S.(2012). Consistency in diatom response to metal-contaminated environments. In: Barceló, D., Kostianoy, A.G. (eds). *Emerging and priority pollutants in rivers.* Berlin Heidelberg: Springer. (pp. 117-146).

Mucina, L., Rutherford, M. C. (2006). *The vegetation of South Africa, Lesotho and Swaziland.* Pretoria: South African National Biodiversity Institute.

Novais, M. H., Blanco, S., Delgado, C., Morais, M., Hoffmann, L., Ector, L. (2012). Ecological assessment of Portuguese reservoirs based on littoral epilithic diatoms. *Hydrobiologia*, 695, 265-279.

Nowack, E. C., Melkonian, M. (2010). Endosymbiotic associations within protists. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 699-712.

Pan, Y., Stevenson, R. J., Hill, B. H., Herlihy, A. T., Collins, G. B. (1996). Using diatoms as indicators of ecological conditions in lotic systems: A regional assessment. *Journal of the North American Benthological Society,* 15, 481-495.

Potapova, M., Charles, D. F. (2003). Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. *Freshwater Biology,* 48, 1311-1328.

Potapova, M., Charles, D. F. (2007). Diatom metrics for monitoring eutrophication in rivers of the United States. *Ecological Indicators,* 7, 48-70.

Prygiel, J., Coste, M. (1993). The assessment of water quality in the Artois-Picardie water basin (France) by the use of diatom indices. *Hydrobiologia,* 269/270, 343-349.

Prygiel, J., Coste, M. (2000). *Guide méthodologique pour la mise en oeuvre de l'indice biologique diatomées*. Eau-Cemagref Bordeaux: Etude Agences de l'Eau Artois-Picardi.

Rimet, F., Bouchez, A. (2012). Biomonitoring river diatoms: implications of taxonomic resolution. *Ecological indicators*, 15, 92-99.

Roubeix, V., Mazzella, N., Mechin, B., Coste, M., Delmas, F. (2011). Impact of the herbicide metolachlor on river periphytic diatoms: expermimental comparison of descriptors at different biological organization levels. *International Journal of Limnology,* 47, 1-11.

Round, F. E. (1991). Diatoms in river water-monitoring studies. *Journal of Applied Phycology,* 3, 129-145.

Salomoni, S. E., Rocha, V. L., Callegaro, V. L., Lobo, E. A. (2006). Epilithic diatoms as indicators of water quality in the Gravatai River, Rio Grande do Sul, Brazil. *Hydrobiologia,* 559, 223-246.

Slate, J. E., Stevenson, R. J. (2007). The diatom flora of phosphorus-enriched and unenriched sites in an everglades marsh. *Diatom Research,* 22, 355-386.

Szczepocka, E., Szulc, B. (2009). The use of benthic diatoms in estimating water quality of variously polluted rivers. *Oceanological and Hydrobiological Studies,* 38, 17-26.

Szczepocka, E., Szulc, B., Szulc, K., Rakowska, B., Żelazna-Wieczorek, J. (2014). Diatom indices in the biological assessment of the water quality based on the example of a small lowland river. *Oceanological and Hydrobiological Studies,* 43, 265-273.

Taylor, J. C. (2004). *The application of diatom-based pollution indices in the Vaal catchment.* Unpublished MSc dissertation, North-West University, South Africa.

Taylor, J. C., de la Rey, P. A., van Rensburg, L. (2005). Recommendations for the collection, preparation and enumerations of diatoms from riverine habitats for water quality monitoring in South Africa. *African Journal of Aquatic Science,* 30, 65-75.

Taylor, J. C., Janse van Vuuren, M. S., Pieterse, A. J. H. (2007a). The application and testing of diatom-based indices in the Vaal and Wilge rivers, South Africa. *Water SA,* 33, 51-60.

Taylor, J. C., Harding, W. R., Archibald, C. G. M. (2007c). *A methods manual for the collection, preparation and analysis of diatom samples.* Water Research Commission Report TT281/07. Pretoria, South Africa: Water Research Commission.

Taylor, J. C., Prygiel, J., Vosloo, A., De la Rey, P. A., van Rensburg, L. (2007b). Can diatom-based pollution indices be used for biomonitoring in South Africa? A case study of the Crocodile west and Marico Water Management area. *Hydrobiologica,* 59, 455-464.

Taylor, J. C., Harding, W. R., Archibald, C. G. M. (2007d). *An illustrated guide to some common diatom species from South Africa*. Water Research Commission Report TT282/07. Pretoria, South Africa: Water Research Commission.

ter Braak, C. J. F., Smilauer, P. (2003). *CANOCO for Windows, ver 4.51.* Wageningen: Plant Research International.

Triest, L., Lung'ayia, H., Ndiritu, G., Beyene, A. (2012). Epilithic diatoms as indicators in tropical African rivers (Lake Victoria catchment). *Hydrobiologia,* 695, 343-360.

Trobajo, R., Clavero, E., Chepurnov, V. A., Sabbe, K., Mann, D. G., Ishihara, S., Cox, E. J. (2009). Morphological, genetic and mating diversity within the widespread bioindicator Nitzschia palea (Bacillariophyceae). *Phycologia*, 48, 443-459.

Underwood, G., Phillips, J., Saunders, K. (1998). Distribution of estuarine benthic diatom species along salinity and nutrient gradients. *European Journal of Phycology,* 33, 173-183.

Urrea-Clos, G., Sabater, S. (2012). Identifying reference benthic diatom communities in an agricultural watershed (Guadiana River, SW Spain). *Hydrobiologia,* 695, 171-184.

Van Dam, H., Mertens, A., Sinkeldam, J. (1994). A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherland Journal of Aquatic Ecology*, 28, 117-133.

Vilbaste, S., Truu, J. (2003). Distribution of benthic diatoms in relation to environmental variables in lowland streams. *Hydrobiologia,* 493, 81-93.

Walsh, G. (2008). *Diatom, macroinvertebrate and riparian vegetation community structure responses in agriculturally impacted rivers.* Unpublished MSc, University of Johannesburg, Johannesburg.

Walsh, G., Wepener, V. G. (2009). The influence of land use on water quality and diatom community structures in urban and agriculturally stressed rivers. *Water SA,* 35, 579-593.

Weilhoefer, C. L., Pan, Y. (2008). Using change-point and weighted averaging approaches to explore the relationships between common benthic diatoms and in-stream environmental variables in Mid-Atlantic highland streams, USA. *Hydrobiologia,* 614, 259-274.

Wu, J. T. (1999). A generic index of diatom assemblages as bioindicators of pollution in the Keelung River of Taiwan. *Hydrobiologia,* 397, 79-87.

Wu, N., Cai, Q., Fohrer, N. (2014). Contribution of microspatial factors to benthic diatom communities. *Hydrobiologia*, 732, 49-60.

Zalat, A., Vildary, S. S. (2005). Distribution of diatom assemblages and their relationship to environmental variables in the surface sediments of three northern Egyptian lakes. *Journal of Paleolimnology*, 34, 159-174.

Zelinka, M., Marvan, P. (1961) Zur Präzisierung der biologischen Klassifikation der Reinheit fleiessender Gewässer. *Archiv für Hydrogiologie*, 57, 389-407.

CHAPTER 4 DIATOM SPECIES

4 Results and discussion

This chapter will deal with the diatom species identified at the different study sites along the Great Fish River. The Pearson correlations between diatom indices produced from this diatom composition data are presented and discussed in Chapter 3 (Table 3-6 and 3-7) and in Chapter 5 (5.2). The ordination of the environmental variables compared to the diatom community composition data through CCA analysis are presented and discussed in Chapter 3 (Figure 3-3 and 3-4).

4.1 Species composition

In order to calculate the diatom indices the diatom species need to be identified and counted. This project made use of light microscopy with phase contrast optics to identify the different diatom species. A total of 269 taxa were identified. These taxa belonged to 51 genera. When less frequent or rare (<5% relative abundance) species were removed from the ordinations and 26 taxa remained. There were 49 diatom taxa which could not be indentified to genus level but were determined to belong to the class Bacillariophyceae (acronym ZZZZ). This was due to the author not being able ascribe a genus that the species belonged to or due to deformities in which case they were also included in the deformities total (acronym DEFO). The number of species that had a relative abundance of <1% were 173. All species identified were cosmopolitan with the possible exception of two that could possibly be endemic. A full list of species identified is given in Table 4-1.

The species that are named by a South African author are not necessarily endemic. In some cases they were considered endemic when they were identified and named but were later found to occur in other localities around the world. An example is *Cymbella kappii* (Cholnoky) Cholnoky which was described in 1956 from the Natal. In 1995 it was found to occur in Australia (Day *et al*., 1995) and in the USA in 2009 (Bahls). Of the diatom species occurring in the current study site and named by South Africans only two, according to the website Algaebase, that do not have other localities mentioned and could therefore possibly be endemic. These are *Gyrosigma rautenbachiae* Cholnoky (1.15% relative abundance), *Nitzschia irremissa* Cholnoky (0.01% relative abundance).

The diatom community composition from this study cannot be compared to historical data comparison as this part of the river has never been studied before. The sites sampled by Archibald in 1967 – 1969 (1981) were from the Grassridge Dam wall and muddy pools situated at the Fish River Station (A1 in Table 4-2). Grassridge Dam is situated 20km by road north of the study site Retreat and is a storage dam situated on what is today called the Great Brak River. A total of four samples from this site were taken in 1967, 1968 (x 2) and 1969 and the site was recorded as being silted up. According to Archibald (1983) this river know as the Teebusspruit River. The Fish River Station site (A2 in Table 4-2) was also situated on the Teebusspruit River/Great Brak River which is a northern tributary of the Great Fish River. A total of two samples were taken from this site in 1968 and 1969. These samples were taken prior to the Orange-Fish inter basin transfer from the Gariep Dam in 1977 which resulted in a total change of river character. Prior to the inter basin transfer this tributary was a shallow spring fed river which often dried up. Once this irrigation scheme was initiated, the tributary changed to a large brown fast flowing river with permanent water. The inter basin transfer enters into the Teebusspruit and then in to Great Brak River. This water comes from the 83km underground Orange-Fish tunnel at the mountain Teebuss (DWAF, 1975). This tunnel also feeds water into the Sundays River system. Archibald recorded the diatom species that occurred at those sampling stations but unfortunately did not list which ones were dominant in the samples. Table 4-2 lists these species and highlights those species found in the present study.

Table 4-1: Diatom species list from study sites along the Great Fish River and the acronyms used

Taxa	Code
*Abnormal diatom valve (unidentified) or sum of deformities abundances	DEFO
Achnanthes brevipes var.*angustata* (Greville) Cleve	ABAN
Achnanthidium biasolettianum (Grunow) Lange-Bertalot	ADBI
Achnanthidium crassum (Hustedt) Potapova & Ponader	ADCR
Achnanthidium eutrophilum (Lange-Bertalot) Lange-Bertalot	ADEU
Achnanthidium exiguum (Grunow) Czarnecki	ADEG
Achnanthidium F.T. Kützing	ACHD
Achnanthidium macrocephalum (Hustedt) Round & Bukhtiyarova	ADMA
Achnanthidium minutissimum (Kützing) Czarnecki	ADMI
Achnanthidium saprophilum (Kobayasi et Mayama) Round & Bukhtiyarova	ADSA
Adlafia bryophila (Petersen) Moser Lange-Bertalot & Metzeltin	ABRY
Amphipleura pellucida Kützing	APEL
Amphora C.G. Ehrenberg ex F.T. Kützing	AMPH
Amphora coffeaeformis (Agardh) Kützing	ACOF
Amphora copulata (Kützing) Schoeman & Archibald	ACOP

Taxa	Code
Amphora montana Krasske	AMMO
**Amphora pediculus* (Kützing) Grunow abnormal form	APAB
Amphora species	AMPS
Amphora veneta Kützing	AVEN
Amphora veneta Kützing abnormal form	AVET
Anomoeoneis sphaerophora (Ehrenberg) Pfitzer	ASPH
Aulacoseira granulata (Ehrenberg) Simonsen	AUGR
Aulacoseira granulata (Ehrenberg) Simonsen var. *angustissima* (O.Müller.) Simonsen	AUGA
Bacillaria paradoxa Gmelin	BPAR
Caloneis bacillum (Grunow) Cleve	CBAC
Caloneis Cleve	CALO
Caloneis molaris (Grunow) Krammer	CMOL
Cocconeis C.G. Ehrenberg	COCO
**Cocconeis pediculus* Ehrenberg	CPED
Cocconeis pediculus Ehrenberg abnormal form	CPAB
Cocconeis placentula Ehrenberg abnormal form	CPTG
Cocconeis placentula Ehrenberg	CPLA
**Cocconeis placentula* Ehrenberg var. *euglypta* (Ehrenberg) Grunow	CPLE
Cocconeis placentula Ehrenberg var. *lineata* (Ehrenberg) Van Heurck	CPLI
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow abnormal form	CPEA
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck abnormal form	CPLM
Craticula A. Grunow	CRAT
Craticula accomoda (Hustedt) Mann	CRAC
Craticula accomodiformis Lange-Bertalot	CACM
Craticula ambigua (Ehrenberg) Mann	CAMB
**Craticula buderi* (Hustedt) Lange-Bertalot	CRBU
Craticula molestiformis (Krasske) Lange-Bertalot & Willmann	CMLF
Craticula vixnegligenda Lange-Bertalot	CVIX
Cyclostephanos dubius (Fricke) Round	CDUB
Cyclostephanos invisitatus (Hohn & Hellerman) Theriot Stoermer & Hakansson	CINV
Cyclostephanos species	CYCS
Cyclotella atomus Hustedt	CATO
Cyclotella meneghiniana Kützing	CMEN
Cymatopleura solea (Brebisson) W.Smith	CSOL
Cymatopleura solea (Brebisson) W.Smith var. *apiculata* (W.Smith) Ralfs	CSAP
Cymbella cymbiformis Agardh	CCYM
Cymbella kappii (Cholnoky) Cholnoky	CKPP
Cymbella kolbei Hustedt	CKOL
Cymbella neocistula Krammer	CNCI
Cymbella simonsenii Krammer	CSMO
Cymbella turgida Gregory	CTUR
Cymbella zambesiana Krammer	CZAM
Denticula kuetzingii Grunow	DKUE
**Diatoma vulgaris* Bory	DVUL
Diatoma vulgaris Bory abnormal form	DVUT
Diploneis elliptica (Kützing) Cleve	DELL

Taxa	Code
Diploneis smithii (Brebisson) Cleve	DSMI
Diploneis subovalis Cleve	DSBO
Encyonema caespitosum Kützing	ECAE
Encyonema F.T. Kützing	ENCY
Encyonema minutum (Hilse) D.G. Mann	ENMI
Encyonema silesiacum (Bleisch) D.G. Mann	ESLE
Encyonema species	ENSP
Encyonema volkii (Rumrich. Krammer & Lange-Bertalot) Krammer	EVOL
Encyonopsis buedelii Krammer	ECBU
Encyonopsis cesatii (Rabenhorst) Krammer	ECES
Encyonopsis Krammer	ENCP
Encyonopsis subminuta Krammer & Reichardt	ESUM
Eolimna minima (Grunow) Lange-Bertalot	EOMI
Eolimna minima (Grunow) Lange-Bertalot abnormal form	EOMT
Eolimna subminuscula (Manguin) Moser Lange-Bert.&Metzeltin abnormal form	ESBT
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	ESBM
Epithemia F.T. Kützing	EPIT
*Epithemia adnata (*Kützing) Brebisson	EADN
**Epithemia sorex* Kützing	ESOR
Epithemia sorex Kützing abnormal form	ESXT
Fallacia pygmaea (Kützing) Stickle & Mann	FPYG
Fallacia tenera (Hustedt) Mann in Round	FTNR
Fragilaria H.C. Lyngbye	FRAG
**Fragilaria biceps* (Kützing) Lange-Bertalot	FBCP
Fragilaria biceps (Kützing) Lange-Bertalot abnormal form	FBCT
Fragilaria capucina Desmazieres var. *rumpens* (Kützing) Lange-Bertalot	FCRU
Fragilaria capucina Desmazieres	FCAP
Fragilaria capucina Desmazieres var. *vaucheriae (*Kützing) Lange-Bertalot	FCVA
Fragilaria capucina var. *vaucheriae* (Kützing) Lange-Bertalot abnormal form	FCVT
Fragilaria crotonensis Kitton	FCRO
Fragilaria tenera (W.Smith) Lange-Bertalot	FTEN
Fragilaria ulna (Nitzsch) Lange-Bertalot teratogen form	FULT
Fragilaria ulna (Nitzsch)Lange-Bertalot var. *acus* (Kützing) Lange-Bertalot	FUAC
Fragilaria ulna var. *acus* (Kützing)Lange-Bertalot abnormal form	FUAT
Frustulia L. Rabenhorst	FRUS
Frustulia vulgaris (Thwaites) De Toni	FVUL
**Geissleria decussis* (Ostrup) Lange-Bertalot & Metzeltin	GDEC
GENERA NOT IDENTIFIED	ZZZZ
Gomphonema C.G. Ehrenberg	GOMP
Gomphonema acuminatum Ehrenberg	GACU
Gomphonema affine Kützing	GAFF
Gomphonema angustatum (Kützing) Rabenhorst	GANG
Gomphonema clavatum Ehrenberg	GCLA
Gomphonema gracile Ehrenberg	GGRA
Gomphonema gracile Ehrenberg abnormal form	GGRT
Gomphonema insigne Gregory	GINS

Taxa	Code
Gomphonema italicum Kützing	GITA
Gomphonema lagenula Kützing	GLGN
Gomphonema laticollum Reichardt	GLTC
Gomphonema minutum (Agardh) Agardh	GMIN
**Gomphonema parvulum* (Kützing) Kützing	GPAR
Gomphonema parvulum Kützing abnormal form	GPAT
Gomphonema parvulum var. *exilissimum* Grunow	GPXS
Gomphonema pseudoaugur Lange-Bertalot	GPSA
Gomphonema pseudoaugur Lange-Bertalot abnormal form	GPAA
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	GPUM
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot abnormal form	GPUT
Gomphonema pumilum var. *rigidum* Reichardt & Lange-Bertalot	GPRI
Gomphonema venusta Passy, Kociolek & Lowe	GVNU
Gyrosigma acuminatum (Kützing)Rabenhorst	GYAC
Gyrosigma attenuatum (Kützing) Rabenhorst	GYAT
Gyrosigma rautenbachiae Cholnoky	GRAU
Gyrosigma scalproides (Rabenhorst) Cleve	GSCA
Hantzschia amphioxys (Ehrenberg) Grunow	HAMP
Luticola mutica (Kützing) D.G. Mann	LMUT
*Mayamaea atomu*s (Kützing) Lange-Bertalot	MAAT
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	MAPE
Melosira varians Agardh	MVAR
Navicula J.B.M. Bory de St. Vincent	NAVI
Navicula amphiceropsis Lange-Bertalot & Rumrich	NAAM
Navicula antonii Lange-Bertalot	NANT
Navicula antonii Lange-Bertalot abnormal form	NAOT
Navicula arvensis Hustedt var. *maior* Lange-Bertalot	NAMA
Navicula capitatoradiata Germain	NCPR
Navicula capitatoradiata Germain abnormal form	NCPG
Navicula cincta (Ehrenberg) Ralfs	NCIN
Navicula cryptocephala Kützing	NCRY
Navicula cryptotenella Lange-Bertalot	NCTE
Navicula cryptotenella Lange-Bertalot abnormal form	NCTG
Navicula cryptotenelloides Lange-Bertalot	NCTO
Navicula erifuga Lange-Bertalot	NERI
Navicula exilis Kützing	NEXI
Navicula germainii Wallace	NGER
Navicula gregaria Donkin	NGRE
Navicula gregaria Donkin abnormal form	NGTG
Navicula libonensis Schoeman	NLIB
Navicula microcari Lange-Bertalot	NMCA
Navicula notha Wallace	NNOT
**Navicula radiosa* Kützing	NRAD
Navicula recens (Lange-Bertalot) Lange-Bertalot	NRCS
Navicula reichardtiana Lange-Bertalot abnormal form	NRCF
Navicula reichardtiana Lange-Bertalot	NRCH

Taxa	Code
Navicula reinhardtii (Grunow) Grunow	NREI
Navicula rostellata Kützing	NROS
Navicula small species	NSMZ
Navicula sp.	NASP
Navicula subrhynchocephala Hustedt	NSRH
Navicula symmetrica Patrick	NSYM
Navicula tenelloides Hustedt	NTEN
Navicula tripunctata (O.F.M.) Bory forme teratogene	NTTT
Navicula tripunctata (O.F.Müller) Bory	NTPT
Navicula trivialis Lange-Bertalot	NTRV
Navicula vandamii Schoeman & Archibald	NVDA
Navicula vandamii var. *mertensiae* Lange-Bertalot	NVDM
**Navicula veneta* Kützing	NVEN
Navicula veneta Kützing abnormal form	NVTG
Navicula viridula (Kützing) Ehrenberg	NVIR
Navicula zanoni Hustedt	NZAN
Navicymbula pusilla Krammer	NCPU
Neidium E. Pfitzer	NEID
Neidium productum (W.M.Smith) Cleve	NEPR
**Nitzschia* A.H. Hassall	NITZ
Nitzschia acicularis (Kützing) W.M.Smith	NACI
Nitzschia agnewii Cholnoky	NAGW
Nitzschia agnita Hustedt	NAGN
Nitzschia amphibia Grunow abnormal form	NATG
**Nitzschia amphibia* Grunow	NAMP
**Nitzschia archibaldii* Lange-Bertalot	NIAR
Nitzschia aurariae Cholnoky	NAUR
Nitzschia bacillum Hustedt	NBCL
Nitzschia capitellata Hustedt	NCPL
*Nitzschia clausi*i Hantzsch	NCLA
Nitzschia communis Rabenhorst	NCOM
Nitzschia desertorum Hustedt	NDES
Nitzschia dissipata (Kützing) Grunow abnormal form	NDTG
Nitzschia dissipata (Kützing) Grunow	NDIS
Nitzschia dissipata (Kützing) Grunow var. *media* (Hantzsch) Grunow	NDME
Nitzschia draveillensis Coste & Ricard	NDRA
Nitzschia elegantula Grunow	NELE
Nitzschia filiformis (W.M.Smith) Van Heurck	NFIL
**Nitzschia fonticol*a Grunow	NFON
Nitzschia fonticola Grunow abnormal form	NFOT
Nitzschia frequens Hustedt	NIFQ
**Nitzschia frustulum* (Kützing) Grunow abnormal form	NFTE
**Nitzschia frustulum* (Kützing) Grunow	NIFR
Nitzschia gracilis Hantzsch	NIGR
**Nitzschia inconspicua* Grunow	NINC
Nitzschia inconspicua Grunow abnormal form	NZIT

Taxa	Code
Nitzschia intermedia Hantzsch ex Cleve & Grunow	NINT
Nitzschia irremissa Cholnoky	NIRM
Nitzschia lancettula O.Muller	NLTL
Nitzschia liebetruthii Rabenhorst	NLBT
**Nitzschia linearis* (Agardh) W.M.Smith	NLIN
Nitzschia linearis (Agardh) W.M.Smith abnormal form	NLIA
Nitzschia linearis (Agardh) W.M.Smith var*.* *subtilis* (Grunow) Hustedt	NLSU
Nitzschia microcephala Grunow	NMIC
Nitzschia nana Grunow in Van Heurck	NNAN
**Nitzschia palea* (Kützing) W.Smith	NPAL
Nitzschia palea (Kützing) W.Smith abnormal form	NPTR
Nitzschia paleacea (Grunow) Grunow abnormal form	NPTG
**Nitzschia paleacea* (Grunow) Grunow	NPAE
Nitzschia paleaeformis Hustedt	NIPF
Nitzschia pumila Hustedt	NPML
Nitzschia pura Hustedt	NIPR
Nitzschia pusilla (Kützing) Grunow	NIPU
Nitzschia radicula Hustedt	NZRA
Nitzschia rautenbachiae Cholnoky	NRTB
Nitzschia recta Hantzsch	NREC
Nitzschia rosenstockii Lange-Bertalot	NRST
Nitzschia sigma (Kützing) W.M.Smith	NSIG
Nitzschia siliqua Archibald	NSLQ
Nitzschia solita Hustedt	NISO
Nitzschia sp. 1	NIS1
Nitzschia sp. 2	NIS2
Nitzschia sp. 3	NIS3
Nitzschia sp. 4	NIS4
Nitzschia species abnormal form	NIZT
Nitzschia supralitorea Lange-Bertalot	NZSU
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	NUMB
Nitzschia valdecostata Lange-Bertalot et Simonsen	NVLC
Pinnularia C.G. Ehrenberg	PINU
Pinnularia borealis Ehrenberg	PBOR
Pinnularia divergens W.M.Smith	PDIV
Pinnularia microstauron (Ehrenberg) Cleve	PMIC
Pl*aconeis* C. Mereschkowsky	PLAC
Placoneis dicephala (W.Smith) Mereschkowsky	PDIC
Planothidium engelbrechtii (Cholnoky) Round & Bukhtiyarova	PLEN
**Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot	PLFR
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot abnormal form	PLFT
Planothidium lanceolatum (Brebisson ex Kützing) Lange-Bertalot abnormal form	PTLT
**Planothidium lanceolatum* (Brebisson ex Kützing) Lange-Bertalot	PTLA
Planothidium rostratum (Oestrup) Round & Bukhtiyarova	PTRO
Planothidium Round & Bukhtiyarova	PLTD
Pleurosigma salinarum (Grunow) Cleve & Grunow	PSAL

Taxa	Code
Pseudostaurosira brevistriata (Grunow) Williams & Round	PSBR
Reimeria sinuata (Gregory) Kociolek & Stoermer	RSIN
Reimeria uniseriata Sala Guerrero & Ferrario abnormal form	RUNT
**Rhopalodia gibba* (Ehrenberg) O.Muller	RGIB
Rhopalodia operculata (Agardh) Hakansson	ROPE
Rhopalodia species	RHOS
Sellaphora pupula (Kützing) Mereschkowksy	SPUP
Simonsenia delognei Lange-Bertalot	SIDE
Staurosira elliptica (Schumann) Williams & Round	SELI
Staurosirella pinnata (Ehrenberg) Williams & Round	SPIN
Surirella angusta Kützing abnormal form	SANT
Surirella angusta Kützing	SANG
Surirella brebissonii Krammer & Lange-Bertalot	SBRE
Surirella ovalis Brebisson	SOVI
Tabularia fasciculata (Agardh) Williams	TFAS
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	TWEI
Tryblionella W. Smith	TRYB
Tryblionella apiculata Gregory	TAPI
Tryblionella calida (Grunow) D.G. Mann	TCAL
Tryblionella debilis Arnott ex O'Meara	TDEB
Tryblionella gracilis w. Smith	TGRL
Tryblionella hungarica (Grunow) D.G. Mann	THUN
Tryblionella levidensis Wm. Smith	TLEV
Tryblionella littoralis (Grunow) D.G. Mann	TLIT

Table 4-2: Diatom species collected by Archibald in 1967 – 1969 which he described as being from the Great Fish River

GFR - current study, A1 – Grassridge Dam, A2 – Fish River Station

CODE	Taxa	GFR	A1	A2
AVEN	*Amphora veneta* Kützing	X	X	X
CMEN	*Cyclotella meneghiniana* Kützing	X	X	X
GPAR	*Gomphonema parvulum* (Kützing) Kützing	X	X	X
HAMP	*Hantzschia amphioxys* (Ehrenberg) Grunow			X
MVAR	*Melosira varian*s Agardh			X
NACO	*Navicula accomoda* Hustedt	X	X	X
NAGR	*Navicula agrestis* Hustedt		X	
NCTE	*Navicula cryptotenella* Lange-Bertalot	X	X	X
NFRU	*Navicula frugalis* Hustedt		X	X
NMLL	*Navicula mollis* (WM Smith) Cleve			X
NMUR	*Navicula muralis* Grunow		X	X
NTNE	*Navicula tenella* Brebisson		X	
NROS	*Navicula rostellata* Kützing			X

CODE	Taxa	GFR	A1	A2
NTWY	*Navicula twymaniana* Archibald syn. *Craticula molestiformis* (Hustedt) Lange-Bertalot	X	X	
NFON	*Nitzschia fonticola* Grunow	X	X	X
NINC	*Nitzschia inconspicua* Grunow			X
NINT	*Nitzschia intermedia* Hantzsch			X
NPAL	*Nitzschia palea* (Kützing) W.Smith	X	X	
NPRP	*Nitzschia perspicua* Cholnoky			X
NIPU	*Nitzschia pusilla* (Kützing) Grunow			X
FULT	*Fragilaria ulna* (Nitzsch) Lange-Bertalot			X

Dominant taxa for each site were identified as those that occurred in more than 5% of all the samples for each sample site over the study period. A total of 10 dominant taxa were identified and are listed in Chapter 3 in Table 3-3. Relative abundance data was calculated and used in the calculation of the diatom indices with the OMNIDIA 5.3 database. A summarised table of relative abundance data is in Appendix 1.

4.2 Deformed diatom valves

Deformed valves accounted for 3.5% of the total valves counted. The percentage deformities found during this study is considered to be excessive (Morin *et al*., 2012). The severity of the deformities is unusual. Figures of some of the deformed valves can be seen in Figure 3-2 and Appendix 7. Information on the deformities of each site is listed in Table 4-3. The taxa with the three highest deformed valves over all sites are *Nitzschia* (61.1%), *Rhopalodia* (7.3%) and *Fragilaria* (6.1%).

Table 4-3: Deformed diatom valve information

Site	Genus of highest number of deformed valves	Genus of second high number of deformed valves	Total percentage deformities for site	Unidentified percentage (code ZZZZ)
Petrusdal	*Nitzschia* – 45.9%	*Cocconeis* – 22%	3.12	6.6
Clifton	*Nitzschia* – 53.8%	*Fragiliaria* – 17.1%	3.11	1.4
Stirling	*Nitzschia* – 69%	*Rhopalodia* – 6.4%	4.06	0.9

Site	Genus of highest number of deformed valves	Genus of second high number of deformed valves	Total percentage deformities for site	Unidentified percentage (code ZZZZ)
Mulberry Grove	*Nitzschia* – 57.8%	*Rhopalodia* – 25.3%	4.05	0.3
Retreat	*Nitzschia* – 74.4%	*Craticula* – 12.4%	2.92	2.9

Factors that have been known to contribute to diatom deformity are:

1. UV radiation (Falasco *et al*., 2009a, 2009b)
2. Removal of streamside vegetation (Walsh 2008)
3. Low Silica (Si) levels (Taylor, 2004; Walsh and Wepener, 2009)
4. Heavy metal contamination (Dickman,1998, Falaso *et al*.,2009a, 2009b, Morin *et al*., 2012, Pandey *et al*., 2014).
5. Pesticides/herbicides (Ansara-Ross *et al*., 2008, Debenst *et al*., 2008, Falasco *et al*., 2009a, Roubeix *et al*., 2011).
6. pH (Esquius *et al*., 2012)
7. Acid mine drainage (Muir, 2014)

As there were no tests done for heavy metals, pesticides/herbicides or Si levels it cannot be suggested with certainty what the cause of the deformities were. When taking the characteristics of the study area into account the following assumptions were made regarding each of the above mentioned causes.

1. It is possible that UV radiation could be a contributing cause of the deformities. Part of the 80km stretch of river in this study does receive shade in areas from the large stands of *Populus x canescens* (Figure 2-4). In the summer months, however, there is often filamentous green algae which could be reducing the UV radiation. The water is mainly shallow and mostly clear (except when flooding events occur) resulting in intense UV radiation. It is therefore possible that this could be a contributing factor to the diatom deformities found.
2. The removal of streamside vegetation would indirectly result in increased UV radiation. This, even in conjunction with UV radiation, is unlikely to be the only cause for deformities.

3. Si levels were not measured during this study. Triplett *et al*. (2012) and Ahearn *et al*., (2005) found that river impoundments were responsible for the decrease in Si downstream by trapping nutrients. There are several impoundments along the 80km stretch of river and this could, therefore, also be a contributing factor to the diatom deformities. This, however, does not explain the high percentage deformities found at the highest (most upstream) site (Petrusdal – 3.2%) as there are only two weirs above the sample site.
4. Heavy metal contamination is responsible for a large percentage of diatom deformities around the world. No measurements for heavy metals were taken during this study. At the initiation of sampling it was not foreseen that the diatoms would show these types and severity of deformities. Diatom microscope slides were viewed over an extended period which was after the water, used for chemical analysis, had been disposed of. This area is solely an agricultural area with no industrial activity. The geology of the area does not indicate heavy metals would be present with the exception of iron. It is, however, possible that there could be heavy metal contamination from fertilisers (e.g. Cd). Iron readings taken were deemed insignificant and discontinued after 4 months. The mean values (in mg/L) found for the 4 month period for the sites were: Petrusdal – 0.045, Clifton – 0.01, Stirling – 0, Mulberry Grove – 0.04 and Retreat – 0.045. Although heavy metals cannot totally be ruled out as the cause, given the history and current characteristics of the study area it is highly unlikely that this is the cause.
5. In an agricultural district many landowners use pesticides and fertilisers. As was mentioned in Chapter 2, agricultural activity began in the 1950s when farmers could afford implements used for land transformation. Agrochemicals, however, made their appearance from the early 1900s (Dallas and Day, 2004). Up to the 1990's the South African government had a policy of mandatory pesticide spraying on brown locust hoppers as soon as they were sighted. Cyanide was a common ingredient of sheep dose. Dichlorodiphenyltrichloroethane, also known as DDT, was commonly used by the farming community. Banned for use in 1974 (Ansara-Ross *et al*., 2008) it was and sometimes still is used in this area. Farmers in the study area have been hesitant to share information regarding the agrochemicals they have used in the past and are currently using. Brand names that have been identified include Dimet (active ingredient Chlorsulfuron), Triflurex (active ingredient Trifluralin), Round Up ready Plus (active ingredient Glyphosate), Dinethoate organophosphate (active ingredient Dinethoate), Bulldock (active ingredient Beta-cyfluthrin), Dazzel (active ingredient Diazinon) and Ivomec (active ingredient Ivermectin). Research into the impacts of pesticides and herbicides on the aquatic system is improving in South Africa

(Ansara-Ross *et al*., 2012). These authors indicate that there is extensive persistence of organochlorine pesticides in the aquatic system in South Africa even though they have been banned for a substantial period of time. The organochlorine pesticide group has, up to now, been the main pesticide group to be researched. Awofolu *et al*., (2004) sampled 4 rivers and 1 dam in the Eastern Cape which revealed high levels of certain pesticides downstream of agricultural areas. It is, therefore, highly likely that this could be the cause of the diatom deformities.

6. The study conducted by Esquius *et al*. (2012) focused on the deformities of *Cocconeis placentula*. For this species they found that a decrease in the pH could be positively correlated to the cell deformities. The mean pH readings for their two sites were 7.99 (SD 0.39) and 8.65 (SD 0.43). The mean pH for the current study sites were: Petrusdal - 8.47, Clifton – 8.48, Stirling – 8.74, Mulberry Grove – 8.56 and Retreat – 8.31. Standard deviation for all sites 1.96. The pH measurements for the current study sites are higher than those in Esquius *et al*. (2012). It is therefore unlikely that pH is the cause for the deformities. There is, however, concern that the higher pH readings combined with the NH_4-N could be causing toxicity.
7. Acid mine drainage is not applicable in this area. There is no mining or industry of any kind. This would not be a factor in the deformities of the diatoms in the Great Fish River.

4.3 Diatom indices

Species abundance data was entered into OMNIDIA for the rapid calculation of the diatom indices. The numerical values assigned by the software for the environmental preferences of each species allows for the calculation of the indices. A full list of index scores is presented in Appendix 3.

The diatom indices that were evaluated for the purposes of this study are the Generic Diatom Index (GDI; Coste and Ayphassorho, 1991), the Specific Pollution sensitivity Index (SPI; CEMAGREF 1982) with the SADI database (Harding and Taylor, 2011), the Biological Diatom Index (BDI) and the % Pollution Tolerant Valves (%PTV) which forms part of the Trophic Diatom Index (TDI; Kelly and Whitton, 1995).

The GDI, SPI and BDI all have values that vary from 1 to 20 with the higher score indicating cleaner water. The table with the index score interpretation is found in Chapter 3, Table 3-2. The %PTV is scored from 1 – 100% with the lower value indicating cleaner water. Table 2-1 shows the different scoring categories for the %PTV.

4.3.1 Generic Diatom Index

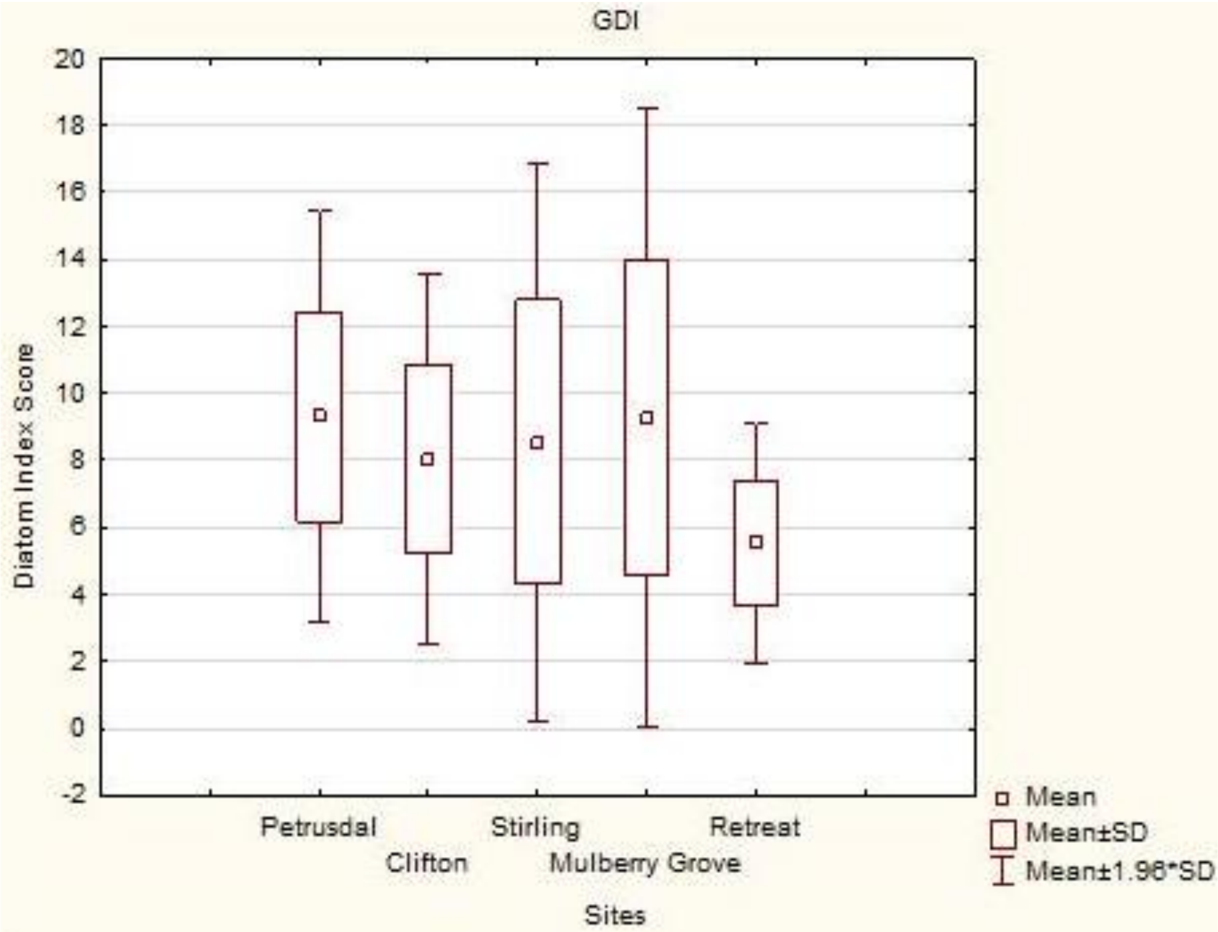


Figure 4-1: Generic Diatom Index (GDI) for all sites along the Great Fish River for the period Feb 10 to Apr 12

The Generic Diatom Index, as the name implies, calculates the scores based on genus level identification and makes use of 174 taxa (Taylor, 2004). As can be seen from Figure 4-1 the mean values for four of the sites (Petrusdal, Clifton, Stirling and Mulberry Grove) are within a 2 point range while the mean value for Retreat is lower and is below 6. This is the threshold for ‘bad’ water quality. The other four sites are within the ‘poor’ water quality range. The minimum and maximum values show a larger range for the first four sites while Retreat shows a lower, narrower range. From this analysis the GDI indicates that Retreat has lower quality water than the other sites. The inclusion rate of genera for this index is 94.9%.

4.3.2 Specific Pollution sensitivity Index

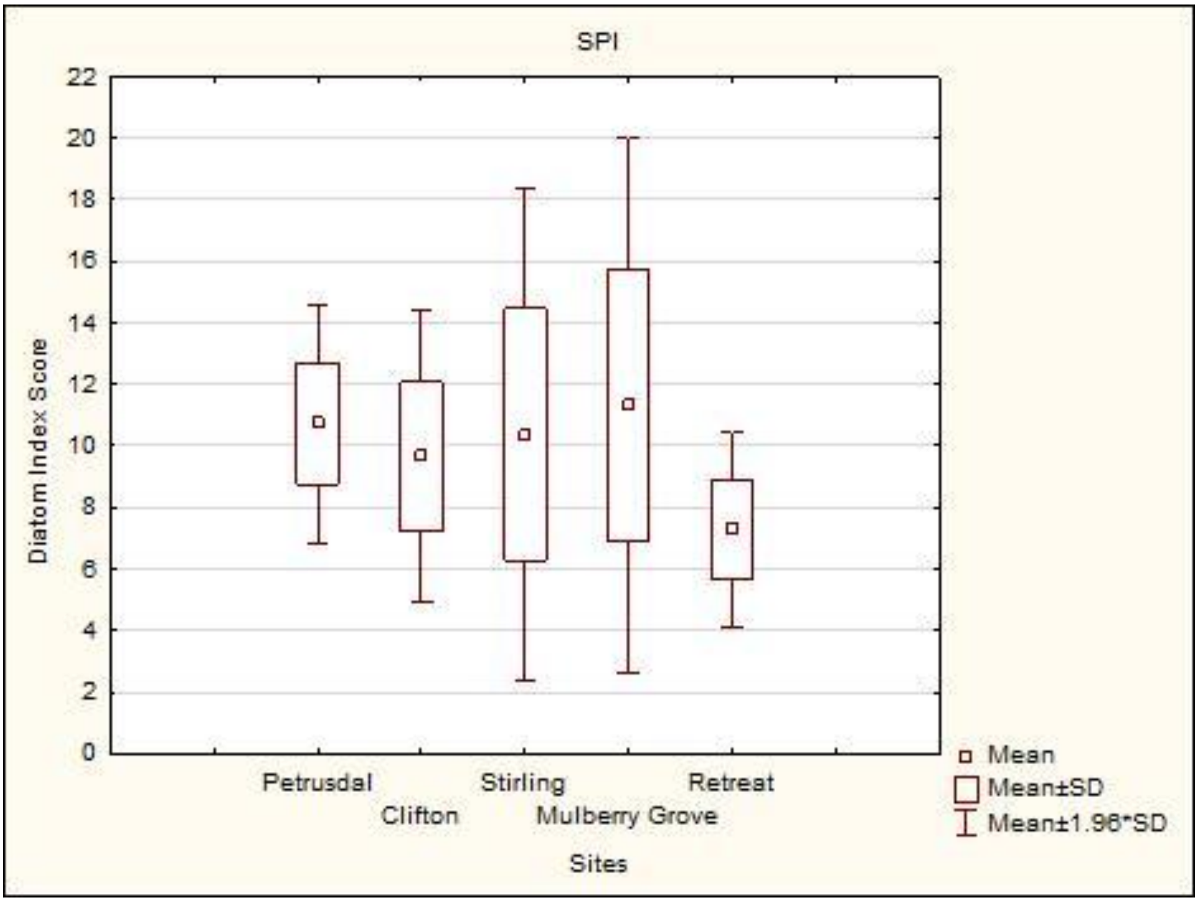


Figure 4-2: Specific Pollution sensitivity Index (SPI) incorporating the SADI for all sites along the Great Fish River for the period Feb 10 to Apr 12

The Specific Pollution sensitivity Index (SPI) takes 2035 taxa into account (Taylor, 2004). For the purposes of this study the South African Diatom Index (SADI) (Harding and Taylor, 2011) was incorporated into the OMNIDIA software as additional data to the SPI for the calculation of this index. By including the SADI autecological information from South African research has been included. From Figure 4-2 it can be seen that the same four sites are again within a 2 point median and the Retreat median value once again scores at least two points lower. Using this index, Retreat would score in the 'poor' category as compared to the 'bad' using the GDI. The SPI shows, as does the GDI, that both Stirling and Mulberry Grove exhibit a wide range when minimum and maximum values. Petrusdal and Clifton show a narrower range of values with the SPI as well as a slightly better score. This index also classifies Petrusdal, Clifton, Stirling and Mulberry Grove as having 'poor' water quality. This index was calculated using 96.7% of the species in all the samples. This index has shown to be the most inclusive for this study and can be considered robust for these conditions.

4.3.3 Biological Diatom Index

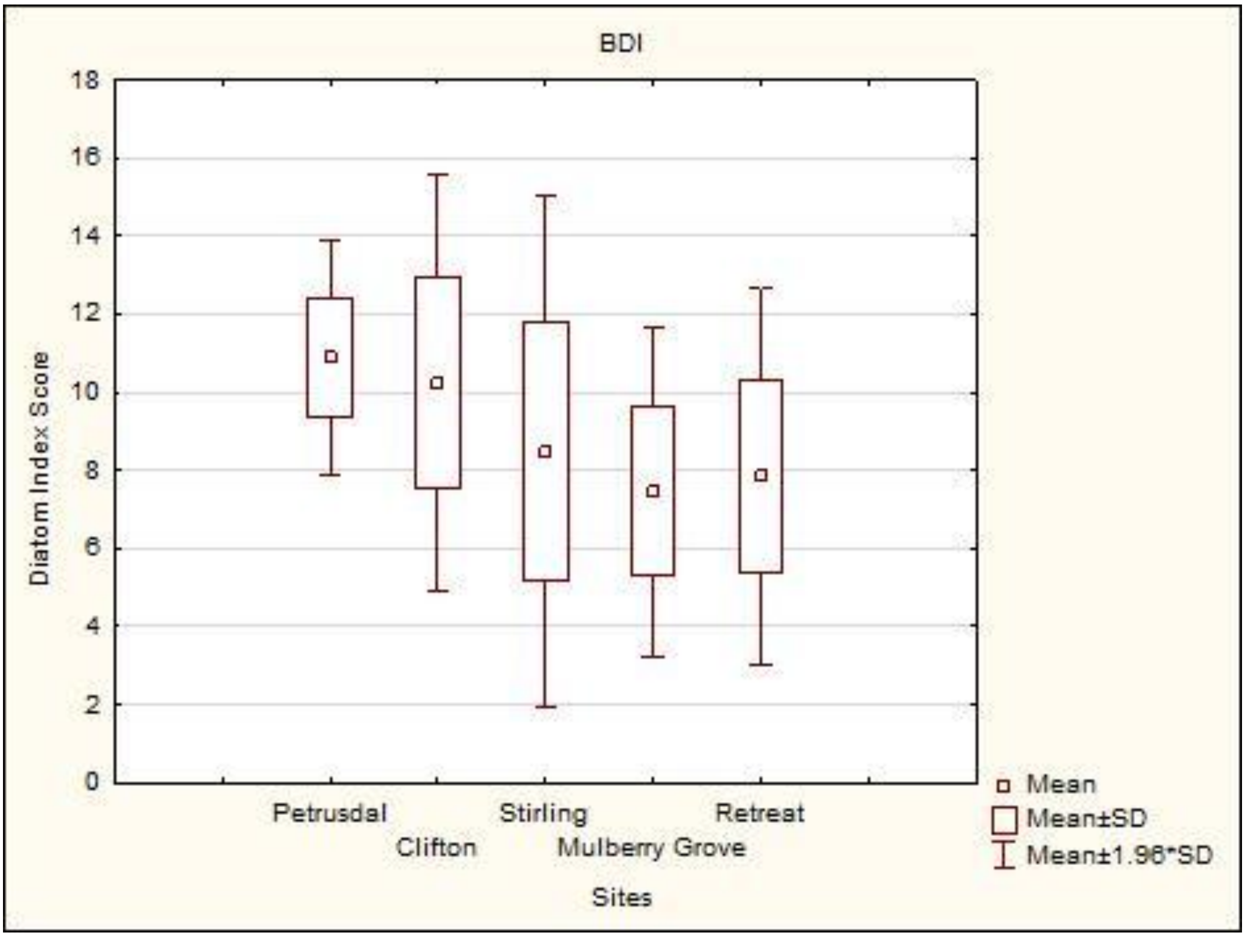


Figure 4-3: Biological Diatom Index (BDI) for all sites along the Great Fish River for the period Feb 10 to Apr 12

This index uses 838 key species with 146 abnormal forms with which to calculate the index values. The mean values for the sites showed more variation within the BDI than the other two indices (Figure 4-3). With this index the lowest mean value was for Mulberry Grove and not Retreat as was found by the GDI and SPI. Although the mean value is not the lowest this index still classifies Retreat as 'poor' quality water. The BDI shows the most variation in values that occurs at Clifton and Stirling and not at Stirling and Mulberry Grove as the GDI and SPI did. This index classifies all the sites in the 'poor' water quality category. The BDI calculation included 86.3% of the species counted. Although not as high a percentage as the SPI, it is an acceptable number.

4.3.4 Percentage Pollution Tolerant Valves

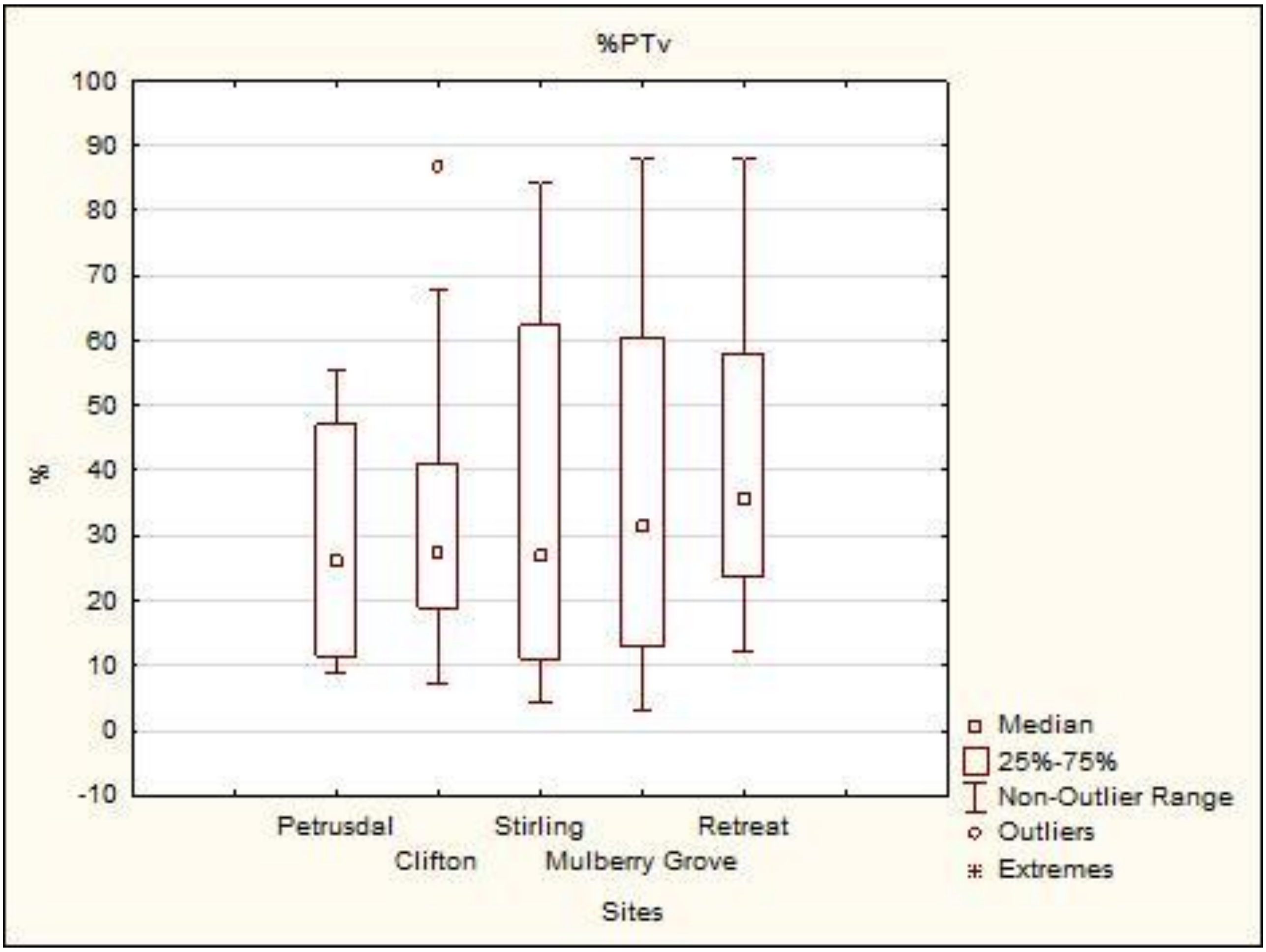


Figure 4-4: Percentage Pollution Tolerant Valves (%PTV) for all sites along the Great Fish River for the period Feb 10 to Apr 12

The %PTV indicates that all sites are influenced by organic nitrogen. Values >20% and above indicate the influence of organic nitrogen. The mean values for all the sites falls within this range. Mean values for the highest site are also >20%. What is of concern are the spikes in values as can be seen from the maximum values which are close to 90% for three sites (Stirling, Mulberry Grove and Retreat) as seen in Figure 4-4. It was not possible to correlate this with rainfall due to the lack of rainfall data from the landowners at Mulberry Grove and Retreat. When the rainfall data for the remaining sites was compared to the %PTV it showed that the increase in the value of %PTV occurred after an increase in rainfall. This confirms that nutrients are washed in to the river from surrounding areas during rains. This index had an inclusion rate of 65.4% and is the lowest out of the indices calculated. This could be due to the fact that certain *Nitzschia* species are not included in the index. An example would be *Nitzschia amphibia* which had a total relative abundance of 6.2% over all sites but is not included in this index. The reason is that this species was not found in the UK rivers on which Kelly and Whitton (1995) based their index. Had this species been included in the index calculation it is likely that the score would have been higher.

4.4 References

Ahearn, D. S., Sheibley, R. W., Dahlgren, R. A. (2005). Effects of river regulation on water quality in the lower Mokelumne River, California. *River Research and Applications*, 21(6), 651-670.

Ansara-Ross, T. M., Wepener, V., Van den Brink, P. J., Ross, M. J. (2008). Probabilistic risk assessment of the environmental impacts of pesticides in the Crocodile (west) Marico catchment, North-West Province. *Water SA*, 34(5), 637-645.

Ansara-Ross, T. M., Wepener, V., Van den Brink, P. J., Ross, M. J. (2012). Pesticides in South African fresh waters. *African Journal of Aquatic Science*, 37(1), 1-16.

Archibald, R.E.M. (1981). An investigation into the taxonomy of the diatoms (Bacillariophyta) of the Sundays and Fish Rivers with ecological observations on the Sundays River. Volumes 1, 2 and 3. PhD, Rhodes University.

Awofolu, R. O., Fatoki, O. S. (2004). Persistent organochlorine pesticide residues in freshwater systems and sediments from the Eastern Cape, South Africa. *Water SA*, 29(3), 323-330.

Bahls, L.L. (2009). A checklist of diatoms from inland waters of the Northwestern United States. *Proceedings of the Academy of Natural Sciences of Philadelphia* 158(1): 1-35.

CEMAGREF, (1982), *Etude des méthods biologiques quantitatives d'appréciation de la qualité deseaux. Rapport Division Qualite des Eaux Lyon-A.F. Bassin Rhône-Méditerranée-Corse [A study on the biological methods of qualitative assessment of water quality. A report of the Water Quality Division Lyon-Outflow Rhône River section catchment]*, Pierre-Bénite, 218 pp.

Cholnoky, B.J. (1956). Neue und seltene Diatomeen aus Afrika. II. Diatomeen aus dem Tugela-Gebiete in Natal. *Österreichische Botanische Zeitschrift* 103: 53-97.

Coste M., Ayphassorho H., (1991). *Etude de la qualité des eaux du bassin Artois Picardie à l'aide des communautés de diatomées benthiques (Application des indices diatomiques),* Rapport Cemagref Bordeaux – Agence de l'Eau Artois Picardie, 227 pp. [in French]

Dallas, H. F., Day, J. A. (2004). *The effect of water quality variables on aquatic ecosystems: a review*. Water Research Commission Report TT224/04. Cape Town, South Africa: Water Research Commission.

Day, S.A., Wickham, R.P., Entwisle, T.J. Tyler, P.A. (1995). Bibliographic check-list of non-marine algae in Australia. *Flora of Australia Supplementary Series* 4: i-vii, 1-276.

Debenest, T., Silvestre, J., Coste, M., Delmas, F., Pinelli, E. (2008). Herbicide effects on freshwater benthic diatoms: Induction of nucleus alteration and silica cell wall abnormalities. *Aquatic Toxicology,* 88, 88-94.

Department of Water Affairs and Forestry. Orange-Fish transfer. (1975). Water transfer schemes in the middle Orange. https://www.dwaf.gov.za/orange/Mid_Orange/orange-f.htm (Accessed 17 March 2015).

Dickman, M. D. (1998). Benthic marine diatom deformities associated with contaminated sediments in Hong Kong. *Environment international*, 24(7), 749-759.

Esquius, K. S., Altamirano, S. M., Escalante, A. H. (2012). Abnormal forms of *Cocconeis placentula* (Ehrenberg) in a eutrophic shallow lake (BS. AS. province, Argentina). In: Dos Santos Afonso M, Sanchez RMT (eds), *Ciencia y tecnologia ambiental: un enfoque integrador* [Environmental science an technology: an integrative approach; conference proceedings] Argentina y Ambiente May 2012*,* Mar De Plata, Argentina*.* Buenos Aires: Asociación Argentina para el Progreso de las Ciencias, pp. 250-255.

Falasco, E., Bona, F., Ginepro, M., Hlúbiková, D., Hoffmann, L., Ector, L. (2009a). Morphological abnormalities of diatom silica walls in relation to heavy metal contamination and artificial growth conditions. *Water SA,* 35, 595-606.

Falasco, E., Bona, F., Badino, G., Hoffmann, L., Ector, L. (2009b). Diatom teratological forms and environmental alterations: A review. *Hydrobiologia,* 623, 1-35.

Guiry, M.D. in Guiry, M.D., Guiry, G.M. (2015) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 17 March 2015.

Harding, W.R., Taylor, J.C. (2011). The South African Diatom Index (SADI) – *A Preliminary Index for Indicating Water Quality in Rivers and Streams in Southern Africa.* Water Research Commision Report No. 1707/1/11. Pretoria, South Africa: Water Research Commission.

Kelly, M. G., Whitton, B. A. (1995). The trophic diatom index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, 7, 433-444.

Morin, S., Cordonier, A., Lavoie, I., Arini, A., Blanco, S., Duong, T. T. and Sabater, S. (2012). Consistency in diatom response to metal-contaminated environments. In *Emerging and priority pollutants in rivers* (pp. 117-146). Springer Berlin Heidelberg.

Muir, E. (2014). Teratological Diatom Deformities in the Periphyton of Colorado Alpine Streams as Indicators of Acid Mine Drainage Contamination. Honors thesis, University of Colorado, USA. Available at http://scholar.colorado.edu/cgi/viewcontent.cgi?article=1169&context=honr_theses [Accessed 28 February 2015].

Pandey, L. K., Kumar, D., Yadav, A., Rai, J., Gaur, J. P. (2014). Morphological abnormalities in periphytic diatoms as a tool for biomonitoring of heavy metal pollution in a river. *Ecological Indicators*, 36, 272-279.

Roubeix, V., Mazzella, N., Mechin, B., Coste, M., Delmas, F. (2011). Impact of the herbicide metolachlor on river periphytic diatoms: expermimental comparison of descriptors at different biological organization levels. *International Journal of Limnology,* 47, 1-11.

Taylor, J. C. (2004). *The application of diatom-based pollution indices in the Vaal catchment.* MSc, North West University, 121pp

Triplett, L. D., Engstrom, D. R., Conley, D. J. (2012). Changes in amorphous silica sequestration with eutrophication of riverine impoundments. *Biogeochemistry*, 108(1-3), 413-427.

Van Dam, H., Mertens, A., Sinkeldam, J. (1994). A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherland Journal of Aquatic Ecology*, 28, 117-133.

Walsh, G. (2008). *Diatom, macroinvertebrate and riparian vegetation community structure responses in agriculturally impacted rivers.* Unpublished MSc, University of Johannesburg, Johannesburg.

Walsh, G., Wepener, V. (2009). The influence of land use on water quality and diatom community structures in urban and agriculturally stressed rivers. *Water SA,* 35(5), 579-593.

CHAPTER 5 ENVIRONMENTAL VARIABLE AND CORRELATIONS

5 Results and discussion

Pearson correlations, as mentioned in Chapter 2, were used to determine if there was a relationship between the measured environmental variables and the diatom indices produced. The table with the complete diatom index scores can be found in Appendix 3. Environmental data and diatom index scores were log transformed before the correlation analysis was performed. Environmental data needs to be normalised and this is done by log transforming the data.

5.1 Environmental variables

5.1.1 Physical variables

5.1.1.1 Water temperature

Water temperature is an important regulator in aquatic ecosystems (Bere *et al*., 2013). According to Pan *et al*. (1996) it is in an important factor that influences diatom community composition. Water temperature is influenced by altitude, water depth, impoundments, canopy cover, stream velocity and dissolved organic matter. The solubility of oxygen in the water, on which aquatic organisms rely, is temperature dependant. The median temperature for the 5 sites is presented in Figure 5.1.

As can be seen from Figure 5-1 there is large fluctuation in water temperature. These fluctuations coincide with the variations in seasonal temperature. The coldest water temperature was measured at Petrusdal in winter (Jun 2010 at 6.5 ºC). Winters can be severe in the study area with ambient temperatures reaching -15ºC just before dawn (pers. observation). The maximum water temperature recorded for the study period of 28.5ºC and was measured at Retreat in December 2011. The mean water temperature for Petrusdal is lower than the other sites, which is expected from a higher altitude site.

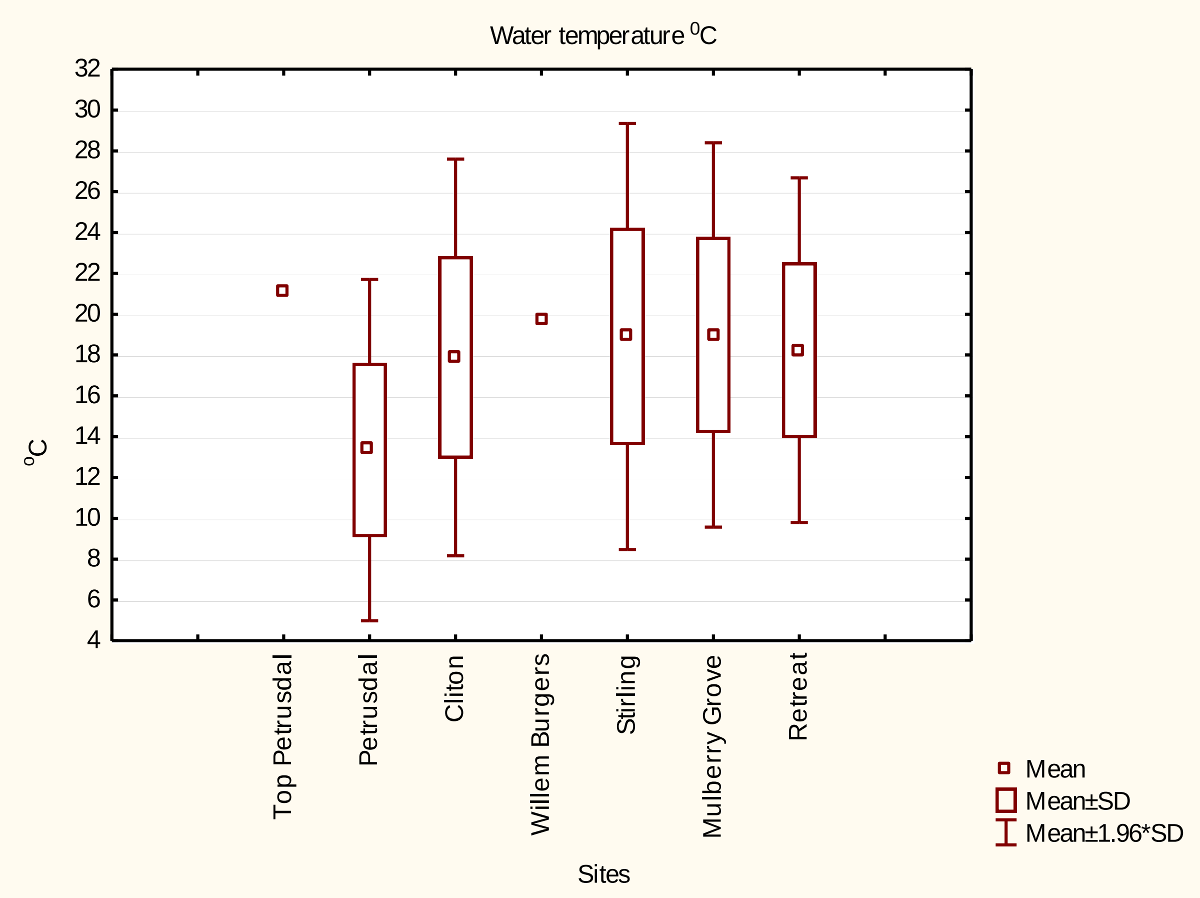


Figure 5-1: Median water temperature at all sites in the Great Fish River from Feb 2010 to Apr 2012

5.1.1.2 pH

The pH of water is a measure of the concentration of H^+ ions in the water and affects multiple metabolic pathways (Dallas and Day, 2004). Geology, soil composition, atmospheric conditions and decaying plant material all affect the pH. The pH of the water plays a major role in determining which species will occur in that water and is affected by the water temperature, inorganic and organic ions. The buffering capacity of the water is determined by the reaction:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2} + 2H^+$$

When the pH is below 6.4, H_2CO_3 dominates while at a pH of 6.4 to 8.6, HCO_3 is dominant. When the pH levels rise above 8.6 up to 10.3, CO_3^{2} will dominate. It is not only pH that has an impact on organisms. The calcium content of the water will play an important role in the buffering of CO_2 and HCO_3^- in that water. Although Ca and Mg are interchangeable in water that contains limestone, the latter does not perform a function in the buffering system of the water (Archibald, 1981).

According to DWAF (1996), the pH of surface water under South African conditions ranges from 6 to 8. The synergistic effects of pH also play an important role in determining diatom community composition. At a pH<8 the ammonium ion (NH_4^+) is not toxic but at a pH of >8 it is converted to a toxic form of ammonia (NH_3^+, DWAF, 1996; Dallas and Day, 2004, Taylor, 2004). The median values for pH can be seen in Figure 5-2.

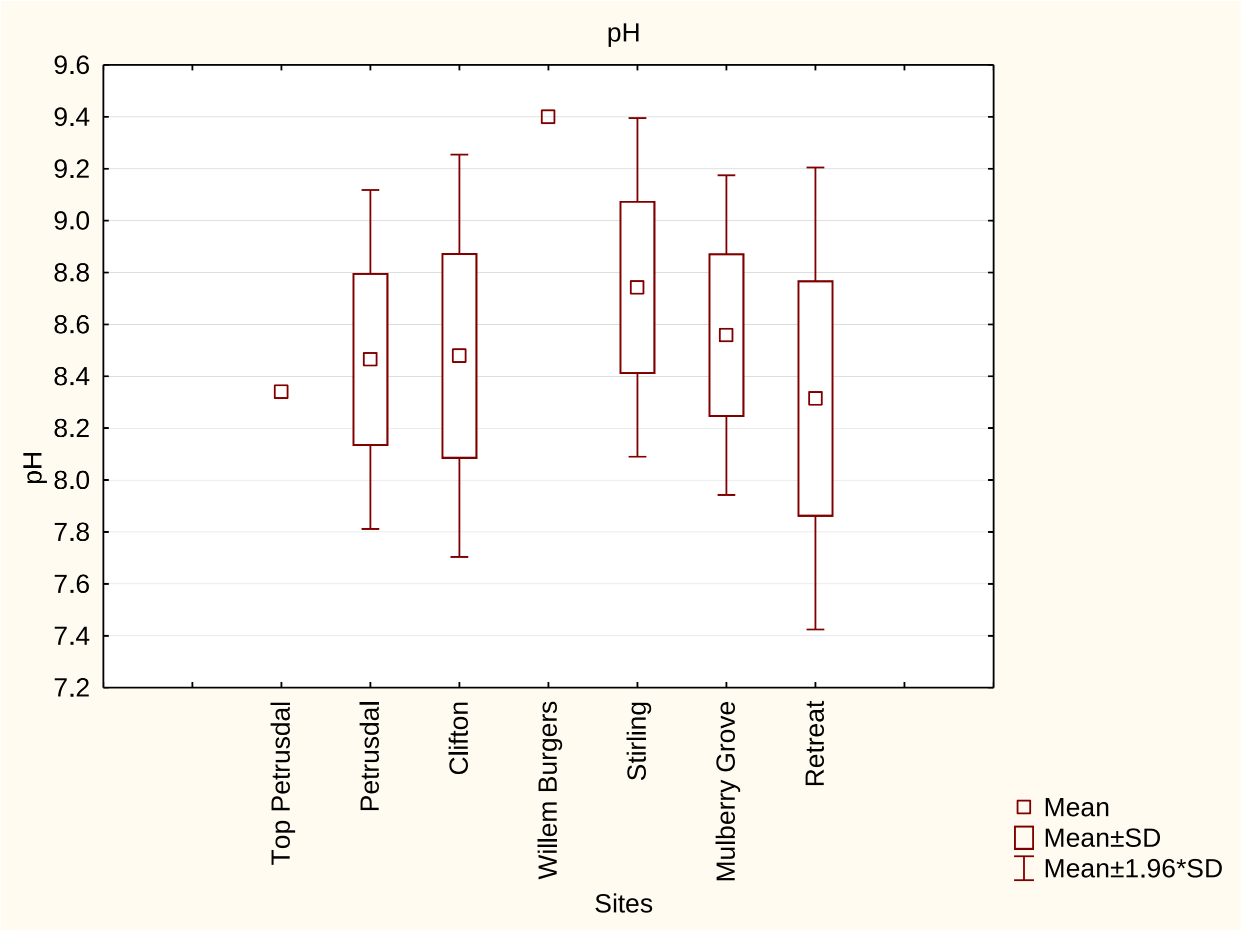


Figure 5-2: Median pH at all sites in the Great Fish River from Feb 2010 to Apr 2012

The median values for pH are, with the exception of the one sample from Willem Burgers, within the range of 8.2 to 8.8. The lowest pH of 7.5 was measured at Retreat in March 2010 during a dry summer. The highest pH reading was the single measurement taken from Willem Burgers. All other sites measured a pH >9 more than once during this study. This variable was determined to be an important driver in the diatom community composition (Figure 3-3). Elevated pH levels may be as a result of eutrophication in which there are increased levels of biological activity, carbon dioxide is taken up and the buffering capactity of the river water is affected.

There are no set values for the Target Water Quality Range (TWQR) for pH. The DWAF guidelines (1996) states that the pH values should not vary more than 5% from the background value which in this case would be Petrusdal as there is no clean reference site. When mean values for the study period are used the pH falls within these values but if monthly values are taken the values do not fall within this range.

Strong correlations between pH and two indices were found (Table 3-6). There was a strong negative correlation with BDI and a strong positive correlation was found with %PTV. The CCA analysis that was performed with physical variables only (Figure 5-6) determined that pH together with EC were the main drivers. When physical and chemical variables were combined (Figure 3-3) it still revealed pH to be an important variable in the diatom community composition.

5.1.1.3 Electrical conductivity

Electrical conductivity (EC) is the ability of the water to conduct an electrical charge through the ions in the water. EC is, therefore, a measure of the total dissolved material within a water body that has an electrical charge (Taylor, 2004). EC is related to water temperature and is regarded as an important indicator of water quality (Dallas and Day, 2004). The major ions found in water that would influence the EC readings are Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, HCO_3^{2-}, Cl^{-} and SO_4^{2-}. It is not only the total amount of the ions that occur in the water that will affect the species composition. The chemical properties of these ions play a large role (Archibald, 1981).The increase of EC and temperature is associated with the increase in biological material and subsequent increase in metabolic rates. Salinity, which is associated with an increase in EC, will affect the osmotic pressure of the water in which the species grow which in turn will have an effect on the composition of the species occurring in that water. The Great Fish River is ephemeral in dry years and this affects the osmotic pressure through increased EC in the water due to evaporation. This osmotic pressure (and EC) then drops dramatically during rainy periods especially when flooding occurs, which is common in semi-arid areas.

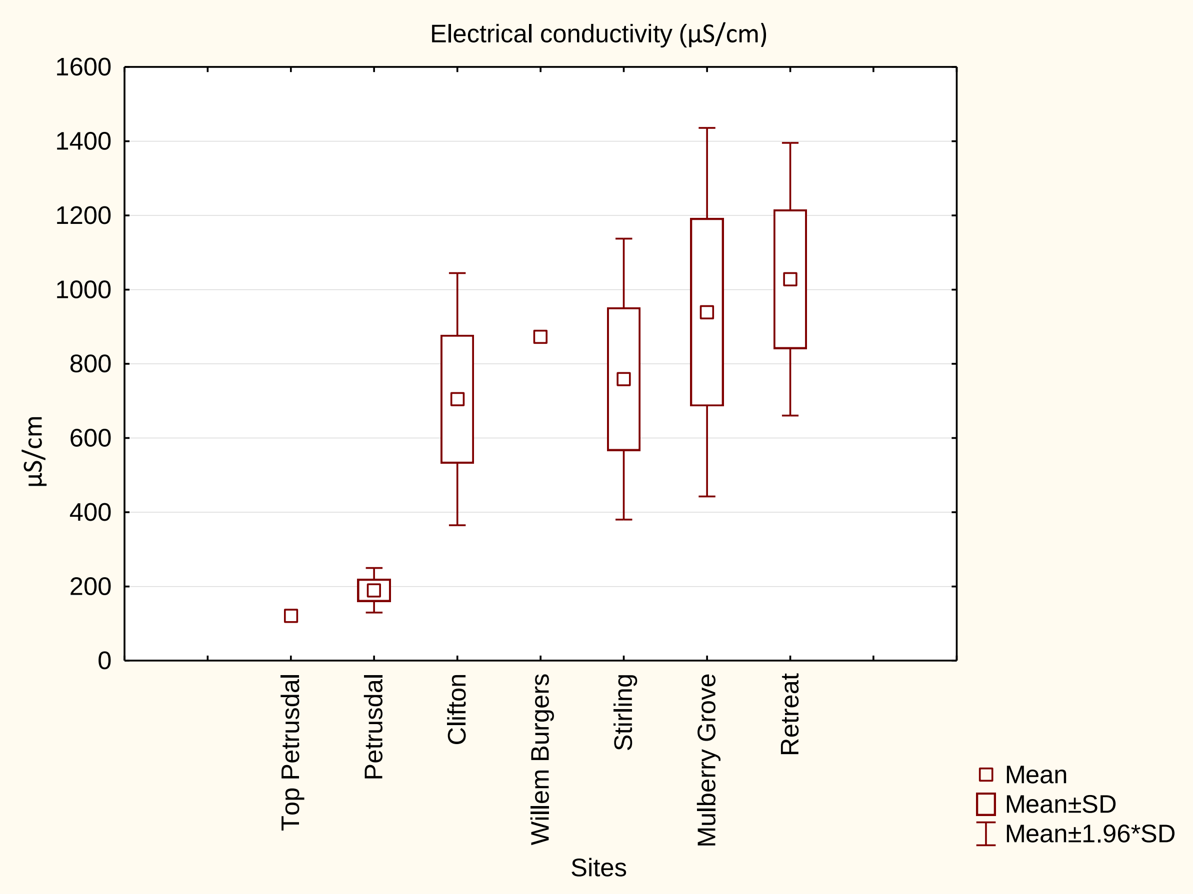


Figure 5-3: Median EC at all sites in the Great Fish River from Feb 2010 to Apr 2012

As the normal cycle values for EC for this section of the river are not known, the DWAF (1996) guideline was used. This source states that concentrations should not change at a particular site by more than 15% from a natural cycle. Given that the EC readings taken over the study period at each site vary more than 15%, it could be inferred that these sites are negatively impacted. There is usually an increase in all nutrients from the headwaters as the river moves downstream but physical parameters will undergo seasonal changes (altitude, temperature). According to Gomá *et al.* (2005), EC is dependent on stream hydrology, decreases with dilution and increases with stream order. The EC values for this study varied from 120 – 1321 µS/cm (Figure 5-3). The higher altitude site had lower EC values, as was expected, than those downstream. The higher end values (lower altitude sites) were much higher than the conductivity values of 612 - 739µS/cm found in the Crocodile and Magalies Rivers (Walsh and Wepener, 2009) which was impacted by agricultural activity. In the present study the increase in EC levels could be attributed to irrigation, stripping of natural vegetation (riparian buffer zone) right to the rivers edge to plant crops and overgrazing of riparian areas.

5.1.2 Nutrients

Nitrogen and phosphates are considered to be growth limiting nutrients for plants. Nitrogen plays an important role in protein synthesis in the plant while phosphates are important for plant metabolism. Phosphates are also responsible for energy transformation in the cell in addition to the synthesis of many organic compounds.

5.1.2.1 Inorganic nitrogen

Abundant in nature, nitrogen plays an important part of many biological processes. The forms of inorganic nitrogen that are commonly tested in chemical analysis and were tested in this study are nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+). Nitrate, rarely in abundance under natural conditions, is taken up by the cell and reduced to nitrite which in turn is reduced to ammonium. Under higher pH conditions (>8) this ammonium is converted to toxic ammonium hydroxide (NH_4OH; DWAF, 1996; Taylor, 2004). For the purposes of this study Total Dissolved Inorganic Nitrogen was calculated as follows: NO_2-N + NO_3-N + NH_4-N. Individual box and whisker plots for NO_2-N, NO_3-N and NH_4-N are presented in Appendix 2.

Nitrogen levels are influenced by agricultural fertilisers, surface runoff and excrement (animal and human). Aquatic organisms rapidly utilise the inorganic nitrogen.

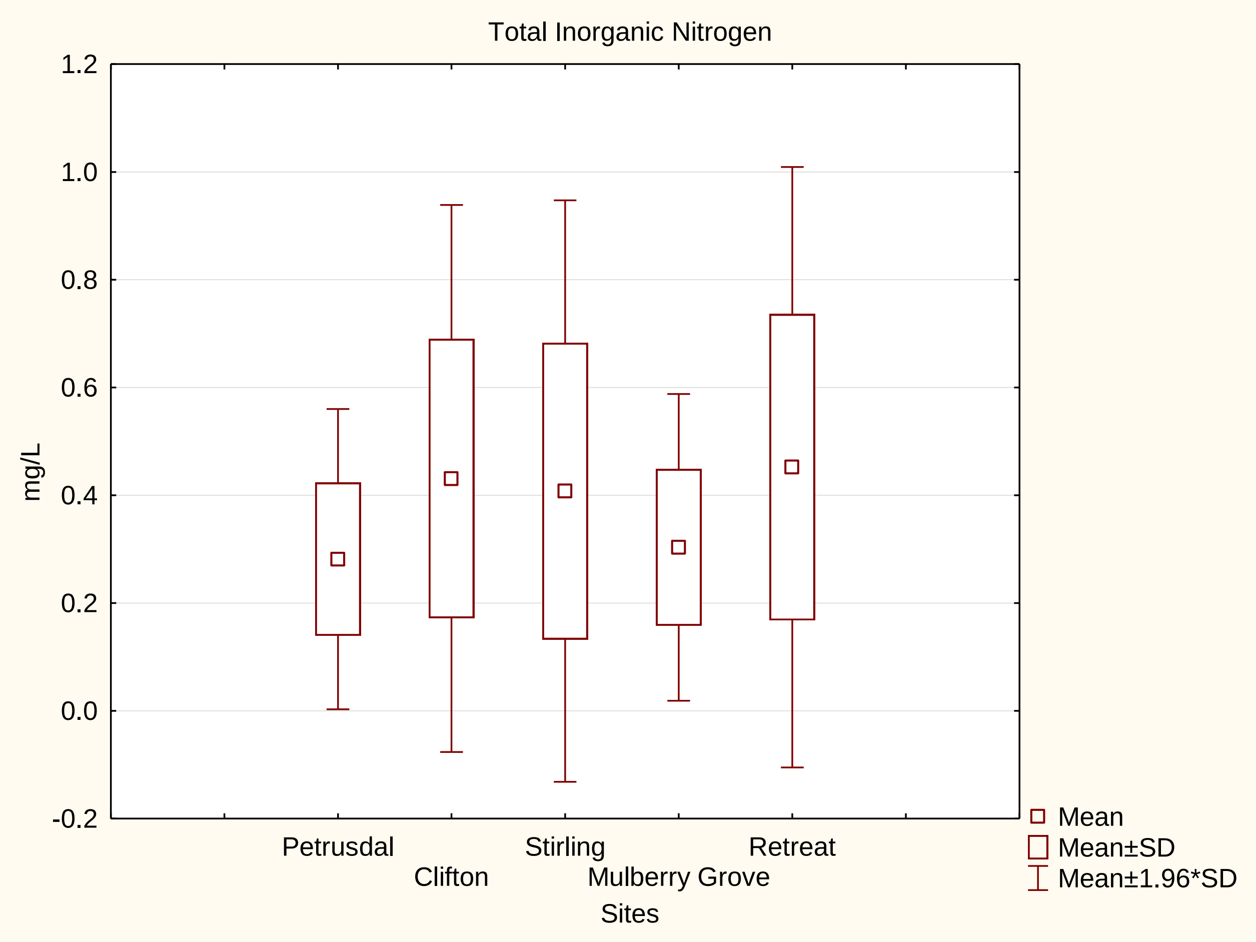


Figure 5-4: Median total inorganic nitrogen in the Great Fish River from Jul 2010 to Apr 2012

Results show that sites Willem Burgers, Stirling and Retreat have a NH_4-N value that is more than 20% higher (or 0,1mg/L) than the standard (DWAF, 1996) while site Clifton is on par with it suggesting that this river is impacted (Figure 5-4). DWAF (1996) considers readings of >10mg/L to be hypereutrophic while Taylor (2004) quotes the amount of <0.5mg/L as being ‘unimpacted’. Of the total dissolved inorganic nitrogen readings taken over the 2 year period only 4 were above 1mg/L. The most abundant diatom genus was *Nitzschia* but the total dissolved inorganic nitrogen readings were considered ‘low’. *Nitzschia* require organic nitrogen and this form of nitrogen is not detected in the chemical analyses that were performed. This suggests that there could be a high organic nitrogen component in this river.

Dallas and Day (2004) suggest that NO_3-N levels in natural surfaces are rarely >0.1mg/L as it (NO_3-N) is being transformed to organic nitrogen by photosynthetic processes. For this study most of the samples were equal to or above the value of 0.1mg/L.

For ammonia and its compounds values of >0.1mg/L are found in natural surface water (Dallas and Day, 2004) in South Africa. Ammonia in 14 samples in this study were equal to or above the level of 0.1mg/L. Toxicity of NH_4, however, is of concern when the values of pH >8 (DWAF, 1996: Dallas and Day, 2004; Taylor, 2004). The pH readings during this study showed that only 9 were below a pH of 8 while 16 of the samples had pH >9. It is not known if this toxicity occurred and if so, what the effects on the diatom communities were.

5.1.2.2 Inorganic phosphate

There is a constant flux of phosphates in a river system as it changes from organic forms to oxidised inorganic forms. Various physical, chemical and biological factors affect this process. Phosphate is an essential plant nutrient and in unimpacted systems will be at low levels. Agricultural runoff is a common source of phosphate into the aquatic system (DWAF, 1996).

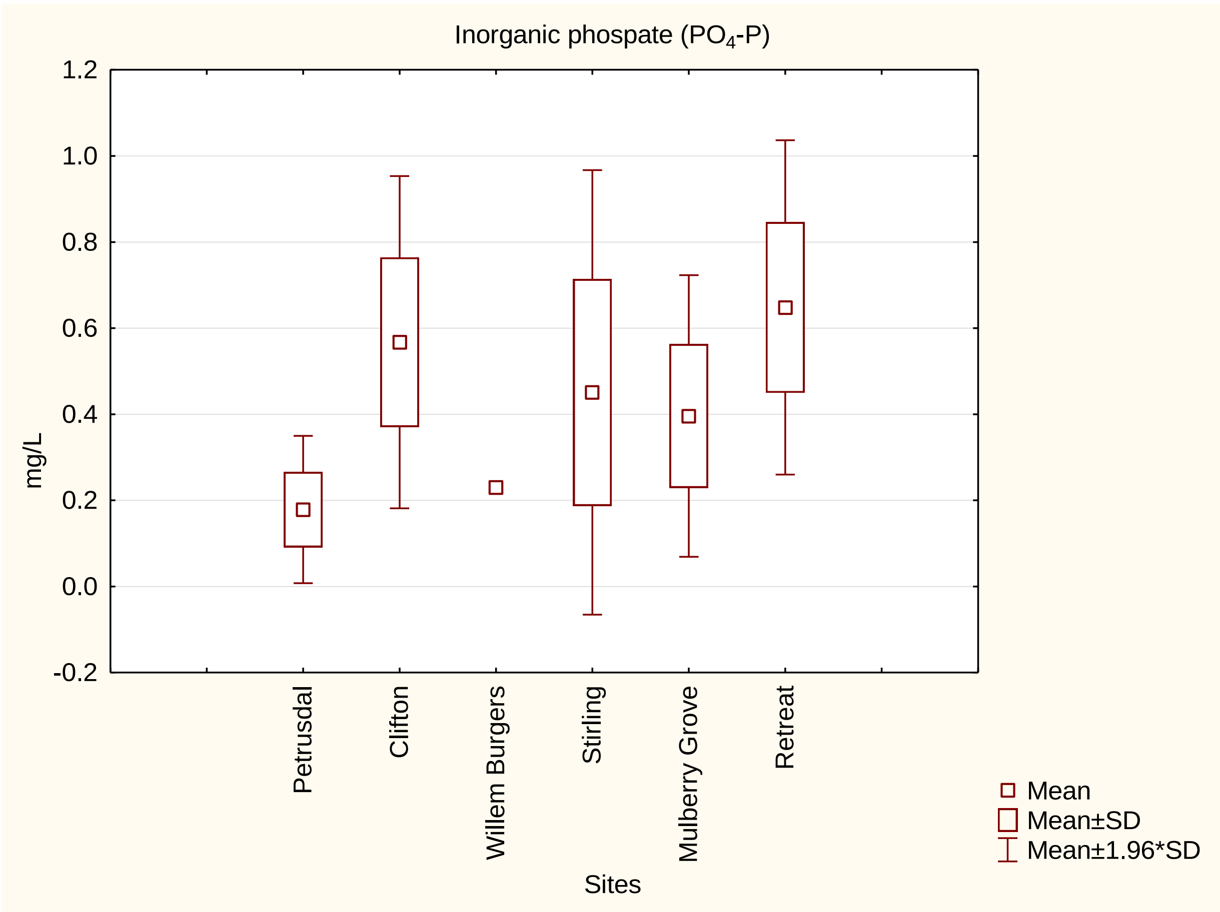


Figure 5-5: **Median inorganic phosphate in the Great Fish River from Jul 2010 to Apr 2012**

According to the TWQR (DWAF, 1996) total nitrogen (TN) and total phosphate (TP) should not change by more than 15% for relevant time of year (i.e. season). Regardless of the fact that this study only measured the dissolved inorganic nutrients, these changed more than 15% within a season (Figure 5-5). From the percentage variation of measured nutrients it could be concluded that this system is impacted.

Table 5-1: Values of PO_4-P from studies conducted worldwide

Country	Considered low PO_4-P value (mg/L)	Considered high PO_4-P value (mg/L)	Author
Estonia	0.01	0.05	Vilbaste, 2001
UK	0.02	0.09	Kelly and Whitton, 1995
USA	0.01	0.1	Potapova and Charles, 2007
South Africa	0.005	0.250	DWAF, 1996

The median values for the sites in this study are (in mg/L); Petrusdal – 0.179, Clifton – 0.567, Stirling - 0.451, Mulberry Grove – 0.396 and Retreat – 0.648. Petrusdal falls into the eutrophic category while the remaining four sites fall into the hypereutrophic catergory (DWAF, 1996).

The remaining chemical variable figures for the individual inorganic nitrogen components (NO_2-N, NO_3-N and NH_4-N) as well as SO_4 and $CaCO_3$ are presented in Appendix 2

5.2 Correlations

A correlation analysis allows for determination of the effectiveness of the application of the selected diatom indices compared to the measured environmental variables. The environmental variables were discussed earlier in this chapter (5.1) and the diatom indices were discussed in Chapter 4 (4.4).

5.2.1 Physical variables correlation and correspondence analysis

Pearson correlation between the physical variables (pH, EC and water temperature) and diatom indices (GDI, SPI, BDI and %PTV) were performed on all samples from Feb 10 to Apr 12. The table with the correlations for this analysis are presented in Chapter 3 (Table 3-6). This analysis shows that there is only a positive significant correlation (p=0.05) between pH and %PTV. A strong negative correlation exists between pH and the BDI. The correspondence analysis of both physical and chemical variables to diatom species (Figure 3-3) shows two physical variables to be of almost the same importance for the diatom community composition.

A separate CCA analysis using physical variables and diatom species only showed pH and EC both to be important drivers in the diatom composition. This biplot is presented in Figure 5-6. The CCA reveals a strong negative correlation with both EC and temperature to Axis 1 whereas pH exhibits a strong positive correlation with Axis 1 and a strong negative correlation with Axis 2. The species environmental relation figures indicate that Axis 1 and 2 account for 82.1% of the effect of the environmental variables tested on the species composition. It is well known that pH is an important influencing factor in the diatom community composition as well as in the water chemistry (Pan *et al.*, 1996; Bere and Mangadze, 2014).

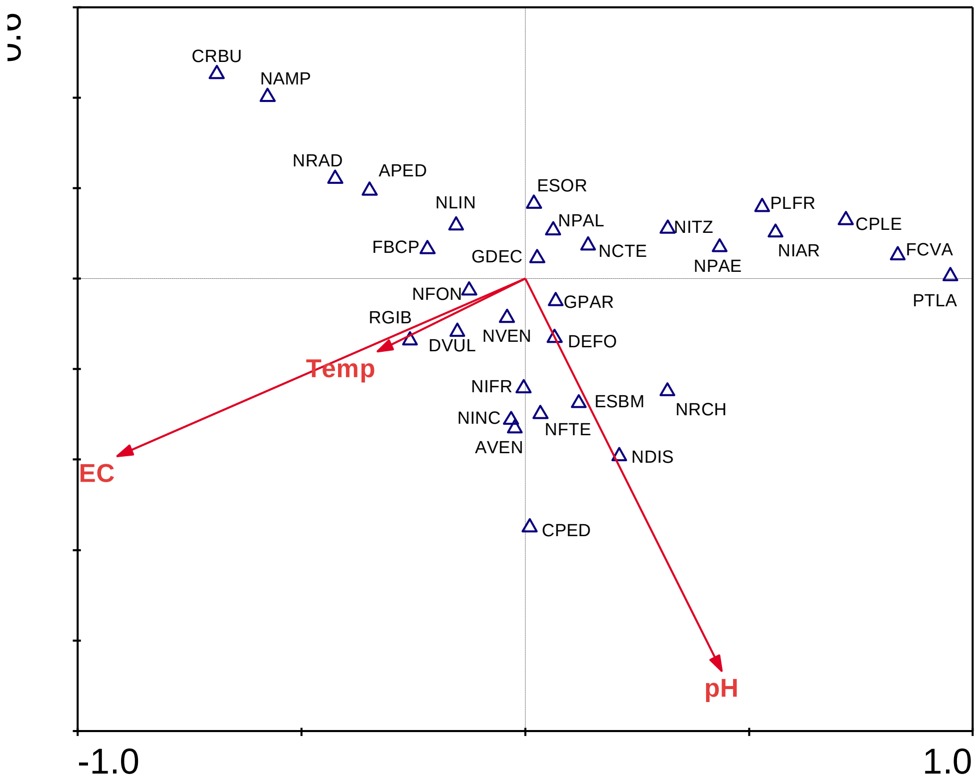


Figure 5-6: **Canonical Correspondence Analysis of species vs physical parameters of all sites and all samples on the Great Fish River from Feb10 to Apr12**

5.2.2 Chemical variables correlation and correspondence analysis

Due to logistical constraints chemical analysis could only be performed from Jun 10. This resulted in a reduced data set for this correlation. A correlation was performed using 48 samples. The chemical variables that were log transformed were NO_2-N, NO_3-N, NH_4-N, PO_4-P, SO_4 and $CaCO_3$. The diatom indices that were evaluated were GDI, SPI, BDI and %PTV. Table 3-7 refers to this section. Only two significant correlations (p=0.05) were found. Both were negative and were between the BDI and NO_3-N and between %PTV and NH_4-N.

A separate CCA was performed on the reduced chemical data set and corresponding diatom samples and is presented in Figure 5-7. This analysis found that NO_3-N and PO_4-P were the most important drivers followed by $CaCO_3$ and SO_4.These last two variables have a strong negative correlation with Axis 1. PO_4-P shows a strong negative correlation with Axis 2 which is the most important gradient in this analysis of community composition. This analysis does not show any strong association with NH_4-N as is found in the correlation. The species environmental relation figures indicate that Axis 1 and 2 account for 63.4% of the effect of the environmental variables tested on the species composition in this analysis. The combined CCA (physical and chemical variables) produced the same results as the individual physical and chemical analyses.

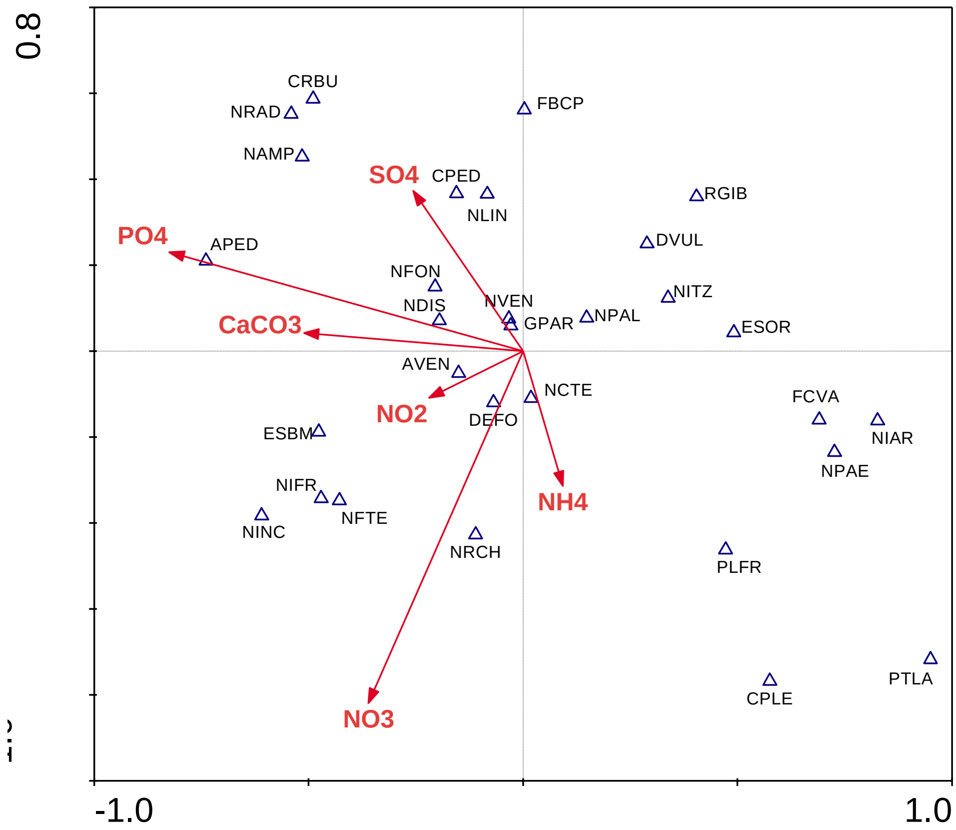


Figure 5-7: Canonical Correspondence Analysis of species vs chemical parameters on a reduced data set (n = 48) on the Great Fish River

5.3 Discussion

The difference that has been shown from the diatom community composition to that of the environmental data and subsequent correlations show that there is another influence in the river which has not been measured by this study. These factors could include the fact that organic nitrogen and organic phosphates were not measured or that there is a toxicant present in the water. The latter would be important in attempting to explain the severe diatom deformities found.

The occurrence of the *Nitzschia* species indicates that there is a constant supply of organic nitrogen in the water. However, there was no significant correlation from the Pearson correlation between any indices and (inorganic nitrogen) NO_3-N or NO_2-N. There was only a significant correlation with NH_4-N. This seems to indicate that there is either another factor that is not being taken into account with traditional analysis or that in the large number of unidentified (to species level) *Nitzschia* valves there are some that prefer cleaner water. As these species are coded to NITZ they are automatically assigned the lower water quality value. It is unlikely that the second point is the case. When the CCA biplots are examined they follow expected patterns.

It is not possible to make an accurate comparison or inference of environmental variables measured in this study to other studies undertaken in South Africa. Each river in South Africa has a different underlying geology, different rates of input and differing land use. It would be of no value to compare these values with those found in, for examples, the Swartkops River in the Eastern Cape (Bate *et al*., 2002). The underlying geology is different as is the rainfall, vegetation biome and land use. This river water quality is affected by industry and wastewater. The sample sites are also situated nearer the estuary of this river which will automatically have different chemical values. The same reasoning excludes comparison of the Buffalo River (Bate *et al*., 2002), Kowie River (Dalu *et al.*, 2014), Kat River (Lerotholi *et al*., 2004) and Sundays River (Bate *et al*., 2002). A study by Von der Meden *et al*. (2005) conducted further downstream on the Great Fish River can also not be compared to this study as this part of the river is largely influenced by the Orange-fish inter basin transfer water.

5.4 References

Archibald, R.E.M. (1981). An investigation into the taxonomy of the diatoms (Bacillariophyta) of the Sundays and Fish Rivers with ecological observations on the Sundays River. Volumes 1, 2 and 3. PhD, Rhodes University.

Bate, G. C., Adams, J. B., Van der Molen, J. S. (2002). *Diatoms as indicators of water quality in South African river systems*. Water Research Commission Report No. 814/1/02. Pretoria, South Africa: Water Research Commission.

Bere, T., Phiri, C., Kadye, W. T., Utete, B. (2013). Benthic diatom assemblages in mountain streams: community structure in relation to environmental and human pressures. *African Journal of Ecology*, 51, 625-634.

Bere, T., Mangadze, T. (2014). Diatom communities in streams draining urban areas: Community structure in relation to environmental variables. *Tropical Ecology,* 55, 271-281.

Dallas, H. F., Day, J. A. (2004). *The effect of water quality variables on aquatic ecosystems: a review*. Water Research Commission Report TT224/04. Cape Town, South Africa: Water Research Commission.

Dalu, T., Froneman, P. W., Chari, L. D., Richoux, N. B. (2014). Colonisation and community structure of benthic diatoms on artificial substrates following a major flood event: A case of the Kowie River (Eastern Cape, South Africa). *Water SA*, 40, 471-480.

Department of Water Affairs and Forestry (DWAF). (1996). *Water quality guidelines. Volume 7. Aquatic Ecosystems.* Pretoria: Department of Water Affairs and Forestry.

Gomà, J., Rimet, F., Cambra, J., Hoffmann, L., Ector, L. (2005). Diatom communities and water quality assessment in mountain rivers of the upper Segre Basin (la Cerdanya, Oriental Pyrenees). *Hydrobiologia,* 551, 209-225.

Kelly, M. G., Penny, C. J., Whitton, B. A. (1995). Comparative performance of benthic diatom indices used to assess river water quality. *Hydrobiologia,* 302, 179-188.

Lerotholi, S., Palmer, C. G., Rowntree, K. (2004). Bioassessment of a River in a Semiarid, Agricultural Catchment, Eastern Cape. In *Proceedings of the 2004 Water Institute of Southern Africa (WISA) Biennial Conference, Cape Town, South Africa* (pp. 338-344).

Pan, Y., Stevenson, R. J., Hill, B. H., Herlihy, A. T., Collins, G. B. (1996). Using diatoms as indicators of ecological conditions in lotic systems: A regional assessment. *Journal of the North American Benthological Society,* 15, 481-495.

Potapova, M., Charles, D. F. (2007). Diatom metrics for monitoring eutrophication in rivers of the United States. *Ecological Indicators,* 7, 48-70.

Taylor, J. C. (2004). The application of diatom-based pollution indices in the Vaal catchment. Unpublished MSc dissertation, North-West University, 121pp.

Vilbaste, S. (2001), Benthic diatom communities in Estonian rivers. *Boreal Environmental Research*, 6, 191-203.

Von der Meden, C.E.O., Palmer, C.G., Davies-Coleman, H.D., Gola, N.P. (2005). Environmental water quality in Makana Municipality, Eastern Cape, South Africa: A case study of local government responsibility for water resource management. Rhodes University, South Africa. Available at https://www.ru.ac.za/media/rhodesuniversity/content/environment/documents/k%20Environmental%20Water%20Quality%20in%20Makana%20Municipality.pdf [Accessed 01 March 2015].

Walsh, G., Wepener, V. (2009). The influence of land use on water quality and diatom community structures in urban and agriculturally stressed rivers. *Water SA,* 35(5), 579-593.

CHAPTER 6 - CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The aims of this study have been addressed and answered:

1. The full list of the species found in the upper reaches of the Great Fish River has been compiled and can be found in Chapter 4, Table 4-1.
2. The dominant species for the different sites were identified in Chapter 3, Table 3-3.
3. Possible endemic species were identified as *Gyrosigma rautenbachiae* Cholnoky and *Nitzschia irremissa* Cholnoky.
4. The use of diatoms indices were proven to be reliable indicators of the water quality. The inclusion rate was GDI – 94.9%, SPI – 95.7% - BDI – 86.3% and %PTV – 65.4%. The water quality can therefore be inferred with a high degree of certainty.
5. Seasonality was shown to have a limited effect on community composition (Figure 3-4)
6. The diatom community composition has shown that agriculture has had a negative impact on the water quality of the upper reaches of the Great Fish River.

The benthic diatom community provides an integrated reflection of the water quality in an area and gives a holistic view of the river. Physio-chemical analyses of the water do not always give an accurate reflection of the water quality (Szczepocka and Szulc, 2009, Harding and Taylor, 2011) especially if only single samples are collected (snapshots). This type of analysis does not take the synergistic effects of the different variables into account. This is supported by the findings of this study in that the measured environmental variables alone do not account for the diatom community composition or the deformities observed.

Photographs of some deformed diatoms are presented in Appendix 7. Although *Nitzschia* sp. were dominant overall (40.9%), this was not the case for each site. At Petrusdal the dominant group was the monoraphids to which one of the dominant species, *Cocconeis placentula* var. *euglypta* belongs. At Clifton *Navicula* spp. accounted for 39.5% of the species while *Nitzschia* spp. accounted for 37.5%. Stirling, Mulberry Grove and Retreat were all dominated by *Nitzschia* spp. This result confirms that the water downstream of the source has increased levels of eutrophication. The fact that there were so many *Nitzschia* species that were not identified to species level could have affected the diatom index scores. All the unknown *Nitzschia* species were classed as NITZ and this code is automatically assigned an ecological value even if some of those *Nitzschia* species may occur in water of a slightly better quality. Taxonomy in this genus is problematic as there are many species that have not been named.

This section of the Great Fish River has periodic inflow and water levels constantly fluctuate. This climate in this area, as well as the geology, are natural factors that threaten to increase salinity in the river along with agricultural activities which increase this risk. This study showed that pH plays an important role in structuring the composition of the diatom community. pH showed strong correlations with two of the indices tested namely the BDI and %PTV. The electrical conductivity (EC) mean increased substantially downstream (from 190 to 1028) while temperature also showed an increase. Irrigation of crops is known to cause an increase in EC (Dallas and Day, 1993). The water from irrigation of crops is utilised by the plants while the ions from the water remain in the soil. NaCl is formed by ionic processes within the soil and washed into the water during the rainy season (Taylor, 2004).

The ecological status of the upper reaches of the Great Fish River was unknown until now. Nel and Driver (2011) listed this river system in the class of 'least threatened'. Plants require certain nutrients for growth with limiting nutrients being nitrogen and phosphate. Eutrophication is caused by an excess of certain nutrients, mainly nitrogen (as nitrate, nitrite and ammonium) and phosphate. The level of these nutrients is constantly changing and once off chemical analysis does not reveal the true ecological status of the river. These nutrients are not toxic but in high levels may affect the structure of the aquatic community. These nutrients play a large role in the diatom community composition. Diatom indices can be successfully used to determine the ecological status of the river as can be seen by this study.

Diatom communities were shown, in this study, to respond to subtle changes in water quality variables concomitant with their known environmental tolerances. Agricultural and related activities together with modifications (direct and indirect) to the studied river and surrounding areas have impacted the quality of the water. The pH in agriculturally impacted areas tends to be higher (7.5 to 9.4 in this study) either as a result of the general soil type in which farming takes place or due to poor farming practices (Lavoie *et al*. 2004). The high levels of EC can be considered a sign of human activities (Taylor, 2004; Bere *et al*. 2013). Although the diatom indices did not show strong correlation with many environmental variables they did show good species environmental relationships in CCA analysis (first two axes explained 52.8%). Overall the diatom indices classified this river as 'poor'. This is in line with the finding that *Nitzschia* spp. dominate the diatom community composition.

Diatoms are more sensitive to subtle changes and fluctuations in nutrients in the water than can be revealed by water chemistry tests and therefore offer a more holistic approach to water quality monitoring. This study has given insight into the impacts of decades of agricultural activity in the studied catchment. In order to prevent further negative impacts, ongoing monitoring of these sites is recommended. The results presented in this study have proven diatoms to be reliable indicators of the water quality assessment in the Great Fish River.

6.2 Recommendations

1. A study to determine if the high pH combined with NH_4-N is causing toxicity which in turn is resulting in diatom deformities and may be affecting other aquatic organisms.
2. A study of the pesticides in the river could yield valuable information regarding the diatom deformities and the possible impacts on other aquatic organisms.
3. A study to determine the sources of the pollution that has been shown to occur in the headwaters.
4. A study to determine why there are such high levels of inorganic nitrogen and inorganic phosphate in a headwater stream.
5. A study to measure the levels of organic nitrogen and organic phosphates or a toxicant that would explain the diatom deformities. This study should also include Si levels to determine if this could be a cause of deformed cells.
6. Long term monitoring will allow for an integrated investigation in to the condition of this river. It may reveal the natural variability of the river.

6.3 References

Bere, T., Phiri, C., Kadye, W. T., Utete, B. (2013). Benthic diatom assemblages in mountain streams: community structure in relation to environmental and human pressures. *African Journal of Ecology*, 51, 625-634.

Dallas, H. F., Day, J. A. (1993). *The effect of water quality variables on riverine ecosystems: a review*. Water Research Commission Report TT61/93. Cape Town, South Africa: Water Research Commision.

Harding, W.R., Taylor, J.C. (2011). The South African Diatom Index (SADI) – A Preliminary Index for Indicating Water Quality in Rivers and Streams in Southern Africa. Water Research Commission Report No. 1707/1/11. Pretoria, South Africa: Water Research Commission.

Lavoie, I., Vincent, W. F., Pienitz, R., Painchaud, J. (2004). Benthic algae as bioindicators of agricultural pollution in the streams and rivers of southern Québec (Canada). *Aquatic ecosystem health & Management*, *7*(1), 43-58.

Nel, J. L., Driver, A. (2011). *South African National Biodiversity Assessment 2011: Technical Report. Volume 2: Freshwater component*. CSIR Report Number CSIR/NRE/ECO/IR/2012/0022/A, Council for Scientific and Industrial Research, Stellenbosch.

Szczepocka, E., Szulc, B. (2009). The use of benthic diatoms in estimating water quality of variously polluted rivers. *Oceanological and Hydrobiological Studies,* 38, 17-26.

Taylor, J. C. (2004). The application of diatom-based pollution indices in the Vaal catchment. Unpublished MSc dissertation, North-West Univeristy , 121pp.

ANNEXURES - APPENDIX 1- RELATIVE ABUNDANCE DATA FOR FEBRUARY 2010 TO APRIL 2012

For the purposes of this appendix only the follow site abbreviations apply: TP = Top Petrusdal; P = Petrusdal; C = Clifton; S = Stirling; WB = Willem Burgers; M = Mulberry Grove and R = Retreat. Possible South African endemic species in bold type.1 Totals for all samples from each site summed and divided by number of samples. + = 0.5–1.0%; ++ = 1.01–10%; +++ = 10.01–20%; ++++ = 20.01–40%; +++++ = 40.01+% of the total cells in the community for that site (<0.5% not included)

Taxon	Code	TP	P	C	WB	S	M	R
Abnormal diatom valve (unidentified)	DEFO	++	++	++	++	++	++	++
Achnanthidium biasolettianum (Grunow) Lange-Bertalot	ADBI		+					
Achnanthidium sp.	ACHD	+	++					+
Amphora pediculus (Kützing) Grunow	APED	+		+++	++	++	+	++
Amphora veneta Kützing	AVEN			+	++	++		+
Amphora veneta Kützing abnormal form	AVET				+			
Aulacoseira granulata (Ehrenberg) Simonsen	AUGR						+	
Cocconeis pediculus Ehrenberg	CPED			+	++++	++	++	+
Cocconeis placentula Ehrenberg	CPLA		++					
Cocconeis placentula var.euglypta (Ehrenberg) Grunow	CPLE	+++	+++	++	++	++	++	
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	CPLI	+++	++					
Craticula buderi (Hustedt) Lange-Bertalot	CRBU		+	+				+++
Cyclostephanos invisitatus (Hohn & Hellerman) Theriot, Stoermer & Hkansson	CINV					+	+	
Cyclotella atomus Hustedt	CATO						+	
Cyclotella meneghiniana Kützing	CMEN							+
Cymbella kappii (Cholnoky) Cholnoky	CKPP			++				
Cymbella neocistula Krammer	CNCI			+				
Cymbella simonsenii Krammer	CSMO			+				
Diatoma vulgaris Bory	DVUL			++		++		
Encyonema sp.	ENSP							++
Eolimna minima (Grunow) Lange-Bertalot	EOMI		+					
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin	ESBM			++	++	++	++	++
Epithemia adnata (Kützing) Brebisson	EADN		++			+	+	
Epithemia sorex Kützing	ESOR	+	++			+	++	
Fallacia tenera (Hustedt) D.G.Mann	FTNR				+			
Fragilaria biceps (Kützing) Lange-Bertalot	FBCP	+	++	++		++		+
Fragilaria capucina var.vaucheriae (Kützing) Lange-Bertalot	FCVA	++	++		+			
Geissleria decussis (Oestrup) Lange-Bertalot & Metzeltin	GDEC		+	++		++	+	
Gomphonema parvulum (Kützing) Kützing	GPAR	++	+	++	++		+	++
Gomphonema parvulum var. *exilissimum* Grunow	GPXS	++						
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	GPUM	+	+		++			
Gomphonema pumilum var. *rigidum* Reichardt & Lange-Bertalot	GPRI		+					
Gyrosigma rautenbachiae **Cholnoky**	**GRAU**						+	
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	MAPE					+		
Navicula antonii Lange-Bertalot	NANT		++	+				+
Navicula capitatoradiata Germain	NCPR		++					
Navicula cryptotenella Lange-Bertalot	NCTE	++	++	+		+	+	+
Navicula erifuga Lange-Bertalot	NERI	+		+			+	+
Navicula exilis Kützing	NEXI	+						
Navicula gregaria Donkin	NGRE		+					
Navicula libonensis Schoeman	NLIB	+	+					
Navicula radiosa Kützing	NRAD			++				++
Navicula reichardtiana Lange-Bertalot	NRCH		+	++				+
Navicula sp.	NASP			+				
Navicula subrhynchocephala Hustedt	NSRH					++		
Navicula vandamii Schoeman & Archibald	NVDA	++						
Navicula veneta Kützing	NVEN		++	++		++	++	++
Nitzschia sp. 1	NITZ	++	++	+	+	+	++	
Nitzschia agnewii Cholnoky	NAGW		++					
Nitzschia amphibia Grunow	NAMP			++				++
Nitzschia archibaldii Lange-Bertalot	NIAR	++	++	+	++	+	+	
Nitzschia capitellata Hustedt	NCPL							+
Nitzschia desertorum Hustedt	NDES						+	+
Nitzschia dissipata (Kützing) Grunow	NDIS		+	+		++	++	+
Nitzschia fonticola Grunow	NFON		+	++	+	++	+	+
Nitzschia frustulum (Kützing) Grunow abnormal form	NFTE			+	++	++	++	++
Nitzschia frustulum (Kützing) Grunow	NIFR	++	++	+++	++++	+++	+++	+++
Nitzschia gracilis Hantzsch	NIGR	++	++		+			
Nitzschia inconspicua Grunow	NINC			++	++	++	++	++
Nitzschia linearis (Agardh) W.M.Smith	NLIN	++	++	++	++	++	++	++
Nitzschia linearis var. *subtilis* (Grunow) Hustedt	NLSU			++	+	+		+

ANNEXURES - APPENDIX 1CONTINUED- RELATIVE ABUNDANCE DATA FOR FEBRUARY 2010 TO APRIL 2012

For the purposes of this appendix only the follow site abbreviations apply: TP = Top Petrusdal; P = Petrusdal; C = Clifton; S = Stirling; WB = Willem Burgers; M = Mulberry Grove and R = Retreat. Possible South African endemic species in bold type.1 Totals for all samples from each site summed and divided by number of samples. + = 0.5–1.0%; ++ = 1.01–10%; +++ = 10.01–20%; ++++ = 20.01–40%; +++++ = 40.01+% of the total cells in the community for that site (<0.5% not included)

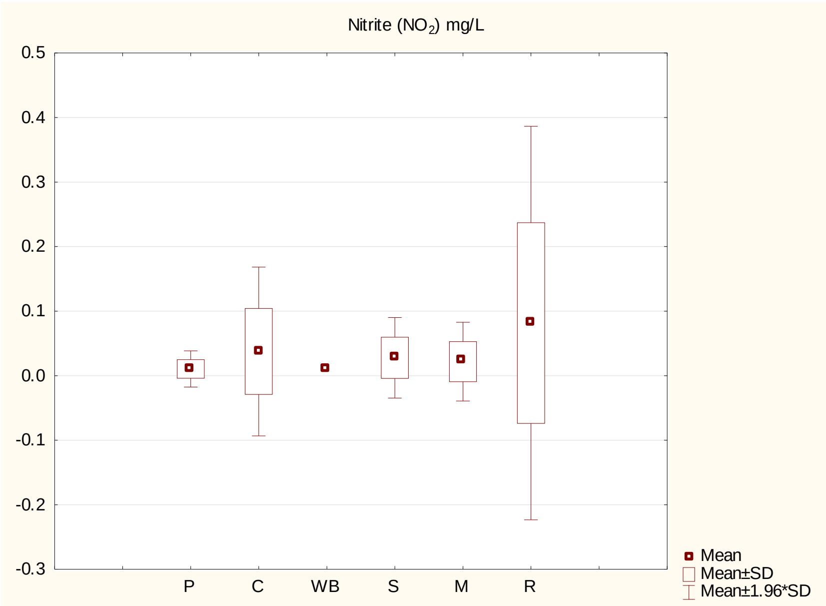
Nitzschia microcephala Grunow	NMIC					+	++	
Nitzschia palea (Kützing) W.Smith	NPAL	++	++	++		++	++	++
Nitzschia paleacea (Grunow) Grunow	NPAE	++	++	++	+	++	++	++
Nitzschia recta Hantzsch	NREC	+	++					
Nitzschia sp. 2	NIS2	++	++	+		++		
Nitzschia sp. abnormal form	NIZT		+	+	++	+		
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	PLFR	++	++	+	+	+	+	+
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	PTLA	+	++	+	++			
Pleurosigma salinarum (Grunow) Cleve & Grunow	PSAL						+	
Reimeria sinuata (Gregory) Kociolek & Stoermer	RSIN					+		
Rhopalodia gibba (Ehrenberg) O.Müller	RGIB	++	++	+		+++	++++	++
Sellaphora pupula (Kützing) Mereschkowksy	SPUP							++
Staurosira elliptica (Schumann) Williams & Round	SELI						+	
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	TWEI			+				
Tryblionella apiculata Gregory	TAPI						+	++

1 The second possible endemic species, Nitzschia irremissa, is not included in the appendix as its relative abundance was <0.05 (0.01188)

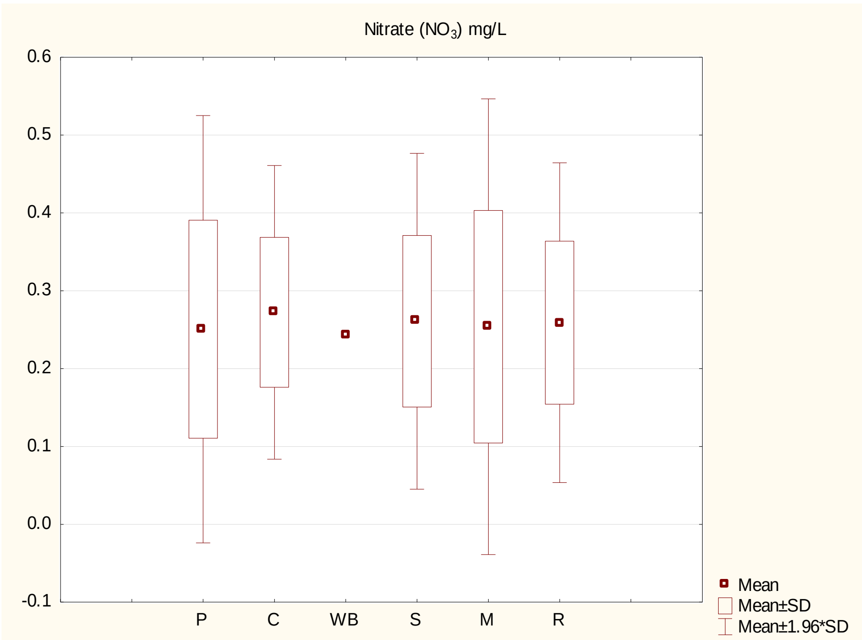
ANNEXURES - APPENDIX 2 - CHEMICAL DATA

For the purposes of this appendix only the follow site abbreviations apply to the box and whisker plots: P = Petrusdal; C = Clifton; S = Stirling; M = Mulberry Grove and R = Retreat.

Box and whisker graphs of NO_2-N, NO_3-N, NH_4-N, SO_4 and $CaCO_3$.



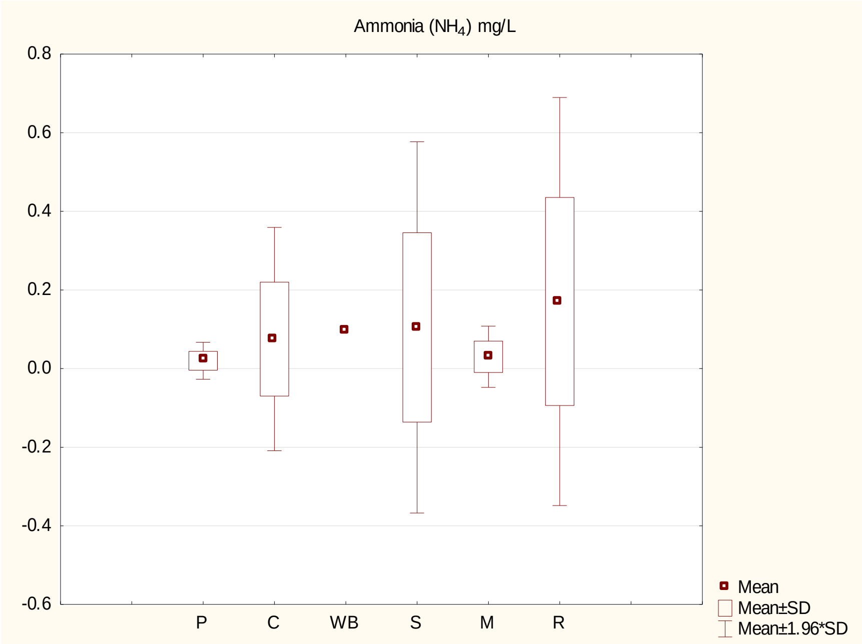
Median total nitrite (NO_2-N) in the Great Fish River from Jul 2010 to Apr 2012.



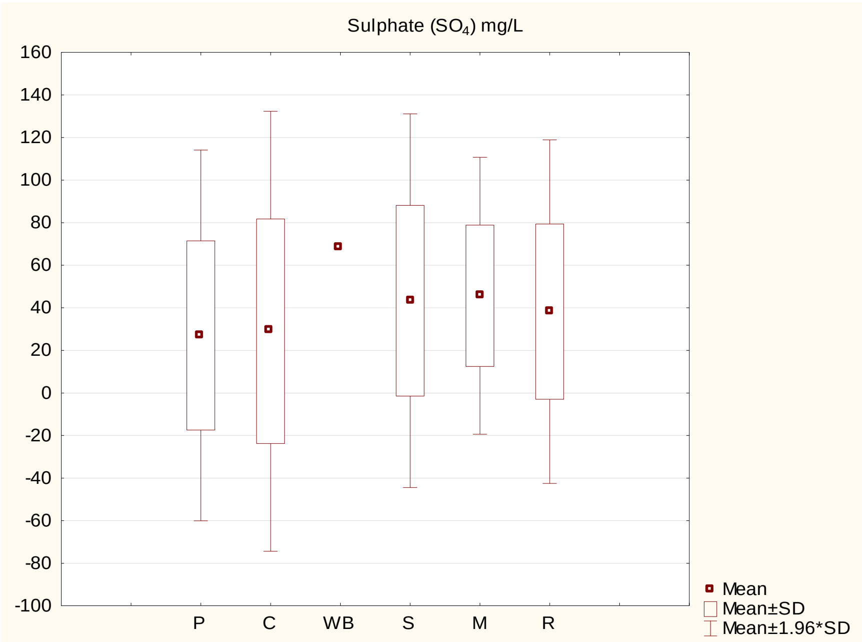
Median total nitrate (NO_3-N) in the Great Fish River from Jul 2010 to Apr 2012

Appendix 2 – Chemical data continued

For the purposes of this appendix only the follow site abbreviations apply to the box and whisker plots: P = Petrusdal; C = Clifton; S = Stirling; M = Mulberry Grove and R = Retreat.



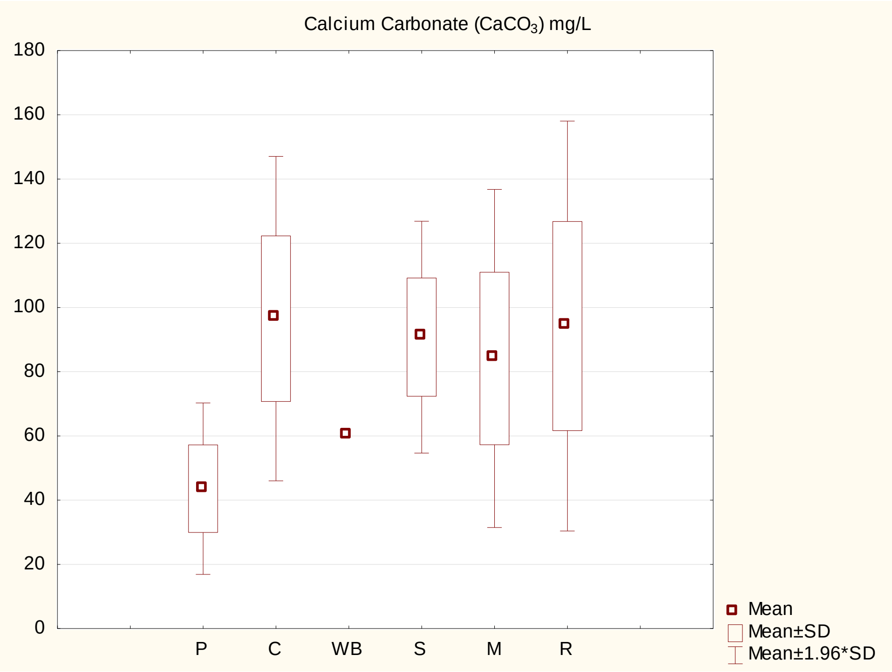
Median total ammonium (NH_4-N) in the Great Fish River from Jul 2010 to Apr 2012.



Median total sulphate (SO_4) in the Great Fish River from Jul 2010 to Apr 2012.

Appendix 2 – Chemical data continued

For the purposes of this appendix only the follow site abbreviations apply to the box and whisker plots: P = Petrusdal; C = Clifton; S = Stirling; M = Mulberry Grove and R = Retreat.



Median total calcium carbonate ($CaCO_3$) in the Great Fish River from Jul 2010 to Apr 2012.

ANNEXURES - APPENDIX 3 – INDEX SCORES

For the purposes of this appendix only the follow site abbreviations apply to the index scores:TP = Top Petrusdal; P = Petrusdal; C = Clifton; WB = Willem Burgers; S = Stirling; M = Mulberry Grove and R = Retreat

	N°	Population	NB spec.	SLA	DESCY	IDSE/5	SHE	WAT	TDI	**%PT**	**GENR**	CEE	**IPS**	**IBD**	IDAP	EPI-D	DI_CH	IDP	LOBO	SID	TID	Diversity	Evenness
R	31	406	45	6.2	7.9	2.52	7.6	9.7	2.1	18	5.9	3.9	5.3	5.8	5.8	6.4	5.2	6.9	16.8	7.2	4.1	3.31	0.6
M	32	412	53	10.6	13.3	3.1	8.9	10.7	11.1	31.6	9.9	6.5	11.1	7	5.7	12.4	7.4	11.4	4.7	12.9	5	4.21	0.73
C	33	402	44	10.6	10.6	3.05	10.6	9.3	4.2	45	7	4.2	8.2	8.8	6.1	9.8	7.6	8.2	9.7	11.2	5.5	4.42	0.81
R	34	405	49	6.5	9.5	2.9	11.1	10	1.9	14.1	6	5.6	5.8	7.2	6.8	8	6.3	7.7	16.3	7.5	3.4	3.04	0.54
M	35	414	55	10.5	13.3	3.11	11.5	10.5	10.8	26.8	10.2	7.7	11.2	7.6	7	12	8.2	11.6	5.3	13.7	5.5	4.54	0.79
S	36	417	47	11.8	13.4	3.43	10.1	9.6	7.7	48	6.9	4.6	8.4	6.5	6.3	9.9	9	10.6	11.8	12.1	4.5	4.14	0.75
C	37	430	27	12.2	13	3.39	14.5	8.4	6.6	14.9	13.3	7.7	11.7	10.6	6.6	12.5	12.1	10.7	4.8	13.7	8.2	2.96	0.62
R	38	405	46	7.7	11.8	3.07	10.3	10.3	3	35.6	4.8	6.7	6	8.2	8.1	7.8	6.2	8.5	16.6	8.7	3.8	3.87	0.7
M	39	406	36	11.7	13.6	3.2	12.2	10.4	16.9	9.9	14.3	8.6	15.9	9.6	8.6	13.9	9.1	12.7	2.2	15.6	6.7	3.05	0.59
S	40	413	42	11.4	13	3.19	10.2	10.3	10.5	25.4	10.8	6.1	12	7.8	6.5	13	7.8	11.5	8	13.7	5.2	4.09	0.76
C	41	418	44	10.9	13.9	3.29	12.2	8.8	5	22.7	9.3	10.5	9.2	10.8	7.8	10.1	8.2	10.2	12.5	11.9	4.2	3.96	0.73
P	42	406	60	11.7	14.9	3.36	13.2	13.4	8.8	18.7	10.1	10.5	11.6	12.7	12.5	12.6	10	10.6	10.2	13.5	7	4.28	0.72
R	43	412	53	8.4	13.8	3.3	11.5	10.5	3.4	34.2	5.6	8.8	6.9	8.1	8.7	8.6	7.3	9.3	14.6	9.5	4.3	4.32	0.75
M	44	417	19	12.6	13.3	3.23	7.3	10.5	18.9	2.9	16.1	9	18.2	9.7	3.7	14.9	5.4	13.9	1.2	16.3	7	1.09	0.26
C	45	409	36	11	16.5	3.35	13.3	7.9	6.2	23.7	9.4	12.6	10.1	12.9	10.3	9.9	10.4	10.5	15.9	12.8	4.1	3	0.58
R	46	411	51	10.2	13.8	3.41	13.3	10.5	4.1	43.3	5.1	10.1	8.9	10.4	10.5	9.4	8.5	9	17.6	12	4.6	3.98	0.7
M	47	422	41	11.7	10.8	3.17	7.5	10.4	15.6	4.5	14.7	8.2	16.3	9.2	5.5	14.6	6.5	13.5	2.3	15.7	6.6	2.36	0.44
S	48	410	19	10.8	19.1	3.16	13.3	12.7	5.4	9.3	13	15.8	14.7	12.9	9.8	13.3	10.5	12.8	8.2	13.9	5.2	2.55	0.6
S	49	406	24	11.5	15.9	3.36	11.8	5.9	13.3	4.4	14.2	13.7	14.9	12.4	9.1	13.9	14.4	13.3	4.3	15.9	6.9	2.18	0.48
C	50	416	34	10.5	17.5	3.24	12.7	12.6	4	25.2	9.7	12.6	9.6	12	9.4	10.5	9.3	11.8	13.3	12.2	4.2	3.41	0.67
R	51	408	45	8.2	14.2	3.46	12.7	10.7	3.6	34.8	5.5	9.2	8.1	10.4	11.6	10	8.5	9.5	15.7	10.4	5.7	3.6	0.66
M	52	409	49	11.3	15	3.14	10.1	10.5	15.8	10.3	14.5	9.7	15.8	9.4	5.4	13.9	10.8	12.8	2.2	15.2	6.3	3.03	0.54
S	53	408	19	10.4	19.5	3.11	13.4	14.3	4.8	4.9	14	16.4	15.2	13.4	9	13.6	11	13.1	5	13.8	6	2.38	0.56
C	54	416	43	11.9	15.6	3.55	13.3	12.5	3.8	26	7.7	13.4	10.9	11.4	12.1	11.5	9	11.5	13	13.4	6.8	4.24	0.78
P	55	400	48	11.2	13.8	3.1	12.2	8.8	7.3	47.5	4.5	8.6	7.7	11.2	8.3	10.6	10.5	9.6	5.6	13.3	7.1	4.27	0.76
R	56	404	47	8.6	14.6	3.5	13.4	11.1	4	26.7	6.4	10.7	9.6	11.4	12.9	11.4	8.7	10.8	12.3	11.6	7.4	3.81	0.69
M	57	411	53	11.6	13.5	3.2	10.9	10.2	14.1	16.1	13.3	8.6	15.8	9.4	6.6	14.1	8.9	13	3.4	15	6.3	3.08	0.54
S	58	413	30	11.8	18.1	3.29	13.3	12	9.1	11.1	13.7	16.6	16.2	11.5	10.5	14.2	10.8	13.1	6.6	15	6.2	2.79	0.57
C	59	406	24	11.9	15	3.68	14.9	9.7	5.8	9.6	13.8	11.8	15.6	13	18.3	14.8	10.2	12.9	5.8	17.3	15.6	3.13	0.68
P	60	407	70	10.3	11	2.96	9.5	9	6.8	55.3	7	6.9	8.8	9.6	9.4	8.8	7.3	8.1	7.7	12	6.2	5.31	0.87
R	61	407	42	10.3	17.1	3.69	13.2	14.8	3.6	12.3	8.9	13.2	11.8	13.4	12.9	12.3	8.8	11.2	14.9	11.5	6.2	3.27	0.61
M	62	422	36	12.7	16	3.28	12.1	10.5	15.8	8.8	14.6	11.6	16.6	8.8	6.3	14.5	12.1	13.6	2.3	16	6.9	2.57	0.5
S	63	427	34	12.5	16.1	3.29	12.2	10.9	13.8	11.2	14.3	14.3	16.7	10	9.2	14.5	9.8	13.4	3.8	15.6	6.4	2.5	0.49
C	64	403	37	12	17.8	3.58	13.2	15.7	2.7	11.2	9.1	14.9	12.9	13.7	14.1	12.8	8.6	11.7	8.6	13.3	7.9	2.91	0.56
P	65	436	48	11.4	13.1	3.18	10.7	11.2	9.5	47	5.5	7.5	7.2	7	8.9	9.6	8.6	9.5	11.1	11.9	6	4.63	0.83
R	66	415	42	8.7	10.9	3.24	12.7	11.9	3.6	19.5	6.2	9.6	7.9	8.5	8.4	9.9	6.6	10.2	15.9	10.3	3.7	3.81	0.71
M	67	411	56	11.8	12.9	3.18	9.6	10.3	13.7	21.4	12.5	9.6	14.4	8.7	6.5	13.7	9.8	12.4	4	15	6.1	3.74	0.64
S	68	405	37	10.9	18.1	3.27	13.1	11.1	6.3	8.4	12.7	14.7	14.2	12.9	11.7	13.3	10.1	12.8	5.9	14.2	7.2	3.47	0.67
C	69	405	41	11.1	15.6	3.41	13.4	9.9	5	7.4	11.3	13.2	12.5	12.9	14	12.5	9	11.7	8.3	14.4	9.4	3.82	0.71
P	70	406	53	10.9	15.8	3.18	10.9	11.5	10.2	32.3	9.8	8.2	11.6	11	9.7	11	9.2	11.2	9.2	13.3	6	4.76	0.83

Appendix 3 – Index scores continued

	N°	Population	NB spec.	SLA	DESCY	IDSE/5	SHE	WAT	TDI	**%PT**	**GENR**	CEE	**IPS**	**IBD**	IDAP	EPI-D	DI_CH	IDP	LOBO	SID	TID	Diversity	Evenness
R	71	409	45	8	13.7	3.27	11.9	12	4.4	22.5	7.5	9.4	8.1	9.4	8.8	9.8	7.2	10.3	14.8	9.5	4.1	4.07	0.74
M	72	405	47	11.4	12.4	3.14	10.3	10.1	13.8	15.3	13.4	10.5	14.9	8.7	6.2	13.9	10.8	12.5	2.8	15.3	5.9	3.47	0.62
S	73	411	49	10.8	13.1	3.11	10.3	10	9.5	22.4	9.4	8.6	11.2	9	6.9	12.6	7.5	11.7	7.3	13.4	5.5	4.23	0.75
C	74	403	39	12.1	18	3.59	12.9	15.8	2.1	9.4	8.3	13.5	12	13.6	13	12	8.1	11.3	11.2	12.4	6.5	2.78	0.53
P	75	409	55	13	16.4	3.44	13.1	11.7	14.8	8.8	14.7	12	15.1	11.4	11.3	14	13.9	12.4	8.2	16	6.9	3.93	0.68
R	76	413	47	8	12.9	3.27	11.5	11.1	3.4	24.9	6.3	9	6.6	7.6	8.1	9.8	6.8	9.9	15.5	9.3	3.9	4.05	0.73
M	77	413	72	10.7	13.7	3.02	9.6	10.6	8.5	31.7	9.4	9.4	10.8	7.8	6.4	12	6.7	10.4	7.1	12.5	5.2	5.03	0.82
S	78	411	46	10	13.5	3	10.1	10.2	8.4	16.8	10.1	9.9	11.2	9.2	6.8	12.1	7.2	11.9	7	13.1	5.4	4.53	0.82
C	79	412	59	10.7	14.1	3.2	12	11.5	3.4	18.9	6.8	11.1	8.2	10.6	10.5	9.8	7	10.1	14.2	12.1	5.4	4.69	0.8
P	80	411	77	10.7	15	3.27	11.4	12.3	8.5	26	10.3	8.8	10.9	10.9	9	11	9.4	10.1	10.6	12.9	6.2	5.36	0.86
TP	81	405	57	11.9	11.2	3.36	10.4	12.8	7.6	30.1	8.1	9.7	9.7	12.5	8.1	12.3	8.6	9.1	8.9	12.2	8.2	4.7	0.81
C	82	412	22	12.8	14.5	3.69	9.6	10	5.2	86.9	1.8	4.4	5.5	4.2	5.8	6.4	9.3	10.6	10.9	11.4	4.5	2.25	0.5
C	83	413	39	12.7	14.2	3.61	10.8	10.1	5.2	68	2.9	4.2	7.6	7.2	7.4	7.7	9.6	9.6	17.2	11.6	4	3.44	0.65
S	84	427	28	12.3	13.5	3.54	9.4	9.5	4.7	80.1	1.9	2.9	4.2	3.5	5.8	6.9	8.4	9.9	14.8	11.1	4.3	2.7	0.56
M	85	418	16	13.2	14.8	3.73	9.9	10.1	4.9	88	1.5	3.1	4.9	3.7	5.8	6.7	9.5	11	12.5	11.5	4.2	1.81	0.45
R	86	419	14	13.2	15.2	3.78	10.2	10.3	4.6	88.1	1.7	5.9	5.5	4.3	5.8	7.2	9.5	11.1	3.7	11.7	4.3	1.75	0.46
P	87	410	45	12	11.2	3.39	7.9	14.6	8.4	29	9.2	10.5	10.4	11.4	7.8	12.4	6.7	8.6	9.1	10.6	8.1	3.71	0.68
R	88	419	26	13.3	15.2	3.83	10.4	10.3	5	82.6	2.2	4	5.8	4.1	5.9	7	10.1	11.2	12.4	11.7	4.2	2.06	0.44
M	89	464	22	12.7	15.2	3.75	10.4	10	5	67.2	2.5	5	4.4	2.2	5.9	7.4	9.4	10.7	3.2	11.7	4.3	2.4	0.54
S	90	450	22	13.1	15.2	3.79	10.8	10.2	5.1	67.6	3.2	4.4	5.4	3	5.7	8.1	10.3	11	2.5	11.9	4.5	2.3	0.52
C	91	428	50	11.8	14.7	3.45	10.1	10.1	6.3	62.9	5.1	5	7.7	4.7	6	9	8.4	10.6	8.5	11.7	4.4	3.79	0.67
P	92	419	52	12.2	13.7	3.51	9.5	14.3	6.9	11	11.7	12.4	10.8	11.1	11.2	13.5	7.8	10	9.5	11.7	6.9	4.13	0.72
C	93	430	33	10.9	16.8	3.55	10.9	12.8	3.2	35.8	6.4	10.7	7.6	7.3	8.2	8.9	8.4	10.8	10.1	11.6	4.8	3.6	0.71
S	94	419	42	9.1	15.4	3.33	9.1	10	4.3	62.3	5.1	6.5	7.1	5.9	7.1	7.3	7.4	9.5	9.9	10.4	4.4	4.05	0.75
R	95	412	42	7.4	14.8	2.59	8.1	10.6	3.9	57.3	7.7	6.7	7.5	8.2	7	7.1	5.5	6	9.4	9.1	3.3	4.34	0.8
M	96	420	49	9.6	14.8	3.09	9.6	10	4.8	66.4	4.7	6.7	7.5	6.8	7.9	6.9	7.1	8.3	13.2	10.4	3.8	4.34	0.77
S	97	425	36	12.2	15.4	3.72	10.2	10.9	4.5	72.7	3.7	8.4	6.9	4.5	7.3	7.3	9.5	10.9	7.6	11.5	4.4	2.78	0.54
C	98	403	28	9.4	14.3	2.93	9.8	13.2	4	49.1	7.5	6.9	9	10.3	7.2	9.1	6.9	8.9	6.4	10.8	5.3	3.73	0.78
P	99	427	27	12.2	10.9	3.13	11.1	11.1	4.6	52.2	3.8	9.9	9.4	8.4	8.6	14.2	6.6	8.7	4	10	8.1	3.32	0.7
R	100	415	18	13.9	15.4	3.83	10.8	11.8	5.4	82.9	2.6	9.2	7.6	5.2	7.1	8	10.6	11.7	3	11.8	4.6	2.11	0.51
M	101	417	45	11.2	13.9	3.27	10.2	13.1	6	46.3	6.9	9.4	9.6	8.8	9.4	9.7	7	9	9	10.6	6.2	4.6	0.84
S	102	417	52	10.8	15.7	3.41	11	13.6	5.4	34.8	8.2	10.3	10	9	9.7	10	8.6	10.6	8.3	11	5.4	4.46	0.78
C	103	418	39	8.9	16.9	2.99	10.8	13.2	2.5	28.9	10	10.3	9.5	10.1	8.6	9.4	7.5	10.2	8.9	11	4.9	4.15	0.79
P	104	416	29	11	13.3	3.33	12.7	12	5.9	33.2	6.9	10.1	10.9	10.3	10.9	12.9	9.7	11.4	5.8	11.1	7.2	3.82	0.79
R	105	421	44	10.2	16	3.38	11.1	13.3	3.3	35.6	7.1	9.7	8.5	8.8	9.8	9.3	7.4	10.1	10.1	10.7	5	4.39	0.8
M	106	422	33	12.1	15.2	3.7	11.3	11.9	4.9	63.7	4.2	6.7	7.9	6.2	8.1	8	9.3	11.1	10.5	11.9	4.8	2.97	0.59
S	107	428	51	10.2	13.9	3.21	10.5	13.6	6.1	32.5	7.9	8.8	7.7	8.1	8	9.5	6.9	8.8	10.4	10.2	5.6	4.69	0.83
C	108	411	47	10.9	16.6	3.4	12.5	13.5	3.5	37.2	8.2	11.6	11.1	11.5	11.4	10.3	9	10.7	12	11.9	5	4.58	0.82
P	109	418	48	10.8	13.8	3.56	12.6	12.6	4.4	18.4	10.2	13	10.8	11.7	12.6	13.5	9.5	11.1	10.2	10.4	6	4.27	0.76
WB	110	432	41	12	14.8	3.56	11.7	12.9	5.1	46.8	5.9	9	8.1	6.8	9.2	9.4	9	10.2	8	11.7	5.1	3.75	0.7

Appendix 3 – Index scores continued

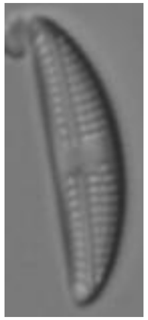
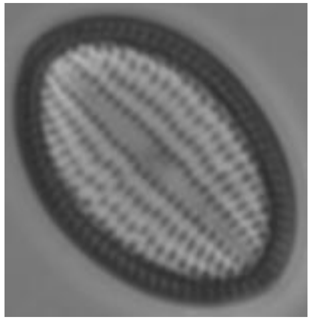
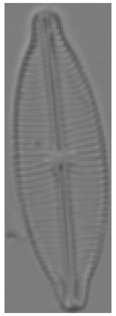
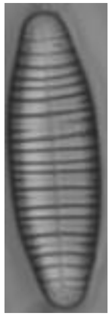
	N°	Population	NB spec.	SLA	DESCY	IDSE/5	SHE	WAT	TDI	**%PT**	**GENR**	CEE	**IPS**	**IBD**	IDAP	EPI-D	DI_CH	IDP	LOBO	SID	TID	Diversity	Evenness
R	111	416	60	8.6	14.8	3.16	10	11.1	4.1	37.7	6.4	6.1	7	7.7	8	7.7	7.9	8.7	10.4	9.3	4.4	4.82	0.82
M	112	408	55	11.4	15.2	3.51	11.5	13.3	5.8	41.7	5.8	8.6	7.6	8.6	9.1	9.2	8.4	9.4	10.9	11.6	5.3	4.38	0.76
S	113	407	46	11.3	15.4	3.53	10.7	14.8	7	27	9.2	9.4	10.1	10.7	8.8	10.5	7.9	9.4	9.7	11	6.2	3.93	0.71
C	114	411	53	10.7	15	3.45	11.4	13.1	5.9	29.4	8.3	9.7	9.7	10.6	9.2	11	8.4	9.7	10.6	11.2	6	4.51	0.79
P	115	406	45	10.4	13.2	3.49	11.8	12.5	4.5	13.1	12.1	13	13.1	12.4	12.5	14.9	8.2	11.3	9.5	8.5	5.6	3.73	0.68
R	116	404	56	9.1	14.4	3.31	10.2	9.3	4.1	44.1	6.1	6.1	6.9	8	6.7	7.1	7.8	9.7	13	10.2	4.2	4.28	0.74
M	117	425	43	13.3	15.8	3.7	11.7	12.1	6.3	44.7	7.8	10.9	11.4	7.4	9	11.7	11.1	12.2	6.8	12.7	5.4	3.6	0.66
S	118	426	50	10.1	12.7	2.79	8.1	11.7	4.8	33.6	6.4	5.6	5	6.5	4.6	7.2	7.1	6.7	7.3	7.6	4.9	4.39	0.78
C	119	425	49	10.6	12.3	3.04	8.5	12	4.9	41.2	6.9	7.5	6.6	7.6	6.9	9.6	6.2	7.7	9.4	8.8	5.2	4.68	0.83
P	120	408	61	10.7	15.1	3.52	10.3	14.2	6.1	11.5	12	12.2	11.8	12.4	10.6	13	8.4	10.3	10.6	11.4	6.9	4.18	0.7
R	121	416	55	11.1	14	3.4	9.8	10.1	4	58.7	4.5	6.9	5.9	5.8	6.6	7.7	7.8	9.5	11.9	10.8	4.1	3.99	0.69
M	122	413	41	12.4	14.9	3.43	9.7	8.7	5.1	57.4	5.3	5.2	6.3	5.9	6.1	7.5	9.4	9.2	6.6	11.6	4.3	3.41	0.64
S	123	407	28	13.4	15.1	3.81	10.2	10.3	5.3	84.3	2.1	4.4	5.9	4.3	5.8	7	10.3	11.1	7	11.7	4.2	1.97	0.41
C	124	408	50	10	11.6	3.04	8.9	10	3.4	39.7	6.4	6.9	6.5	8.8	7.7	8.6	5.8	7.6	13.2	9.5	4.7	4.65	0.82
R	125	421	58	11.1	15	3.51	9.6	9.7	4.7	70.3	4.1	5.6	6.1	4.5	6	7.3	7.9	9.8	6.3	11.1	4.2	3.43	0.59
P	126	416	52	12.1	14.6	3.65	10.8	15.2	7.5	8.9	11.8	13.7	10.9	11.8	11.3	14.3	8.7	10.3	10.1	10.8	7.1	4.09	0.72
C	127	407	47	12.2	18.3	3.71	12.7	15.6	2.6	20.1	8	13.4	10.9	12.6	11.9	11.5	8.9	10.9	14.7	12.1	5.8	3.37	0.61
M	128	423	34	10.4	15	3.43	9.1	8.9	4.6	77.5	4	4.8	5.7	3.8	5.8	6.7	7.5	9.3	3.8	10.9	4.2	2.83	0.56

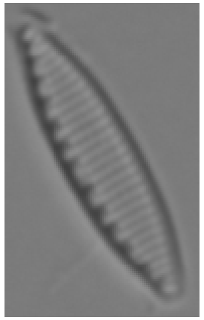
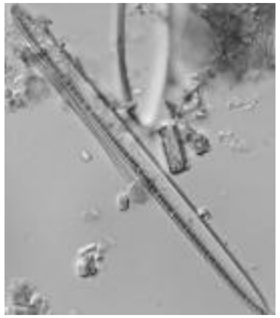
ANNEXURES - APPENDIX 4 – WEIGHTED CORRELATION MATRIX

Weighted correlation matrix for canonical correspondence analyses of physical and chemical parameters, on a reduced dataset, at the five main sample sites on the Great Fish River in 2010–2012. Bold values in columns 1 and 2 (which correspond to axes 1 and 2) indicate a strong correlation

SPEC AX1	1.0000							
SPEC AX2	−0.0992	1.0000						
SPEC AX3	0.0160	0.0980	1.0000					
SPEC AX4	−0.0115	0.0036	−0.1058	1.0000				
ENVI AX1	0.9242	0.0000	0.0000	0.0000	1.0000			
ENVI AX2	0.0000	0.7166	0.0000	0.0000	0.0000	1.0000		
ENVI AX3	0.0000	0.0000	0.6662	0.0000	0.0000	0.0000	1.0000	
ENVI AX4	0.0000	0.0000	0.0000	0.7344	0.0000	0.0000	0.0000	1.0000
NO_2	−0.0794	−0.1483	−0.0113	−0.1969	−0.0859	−0.2069	−0.0169	−0.2681
NO_3	0.0678	**−0.5144**	−0.0623	−0.3369	0.0733	−0.7178	−0.0935	−0.4588
NH_4	0.1339	−0.0670	0.0124	−0.2740	0.1449	−0.0934	0.0186	−0.3731
PO_4	**−0.4838**	−0.2715	−0.2327	0.0476	−0.5235	−0.3789	−0.3493	0.0648
SO_4	−0.2919	0.0623	0.0073	−0.1902	−0.3159	0.0869	0.0109	−0.2590
$CaCO_3$	−0.2178	−0.2042	−0.2704	0.1322	−0.2357	−0.2849	−0.4059	0.1800
EC	**−0.8525**	−0.0912	0.1501	−0.0223	−0.9224	−0.1273	0.2253	−0.0303
Temp.	−0.2798	−0.1410	−0.0113	−0.5373	−0.3027	−0.1967	−0.0169	−0.7316
pH	**0.3036**	**−0.3706**	0.4255	0.2671	0.3285	−0.5172	0.6387	0.3637

ANNEXURES - APPENDIX 5 – DOMINANT DIATOM SPECIES

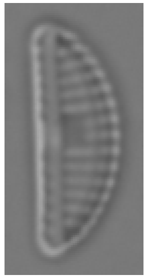




1. **2.** **3.** **4.** **5.** **6.** **7.** **8.** **9.** **10.**

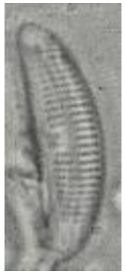
Dominant species (>5% in all samples per site)

NO	SPECIES NAME
1	*Amphora pediculus* (Kützing) Grunow
2	*Cocconeis placentula* var *euglypta* (Ehrenberg.) Grunow
3	*Craticula buderi* (Hustedt) Lange-Bertalot
4	*Diatoma vulgaris* Bory
5	*Fragilaria biceps* (Kützing) Lange-Bertalot
6	*Nitzschia frustulum* (Kützing) Grunow
7	*Nitzschia linearis* (Agardh) W.M.Smith
8	*Nitzschia paleaceae* Grunow
9	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot
10	*Rhopalodia gibba* (Ehrenberg.) O.Müller

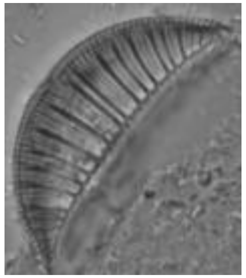
ANNEXURES - APPENDIX 6– PHOTOGRAPHS OF TERATOLOGICAL FORMS OF DIATOM CELLS



AMPS

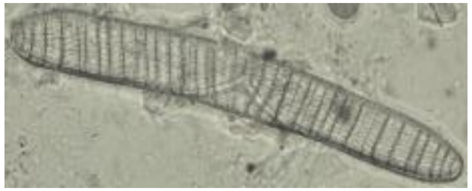


AMPH

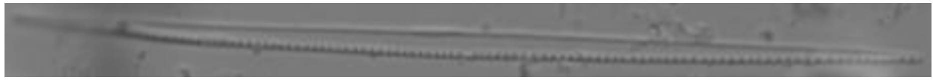


RHOS

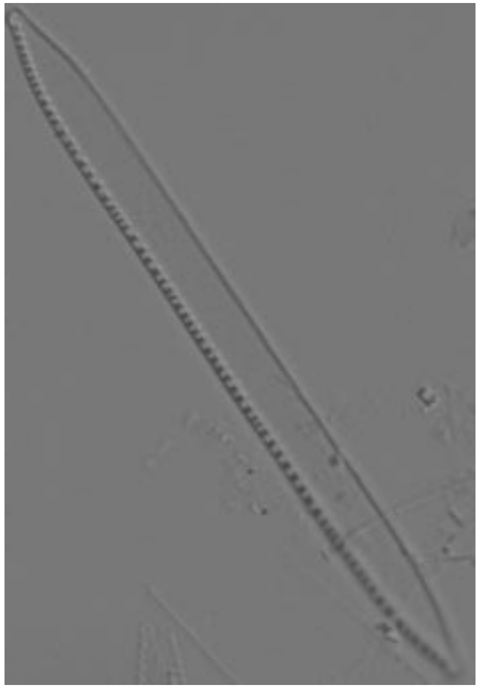
ZZZZ



EPIT L113 B4



NIS2



NIS3

Explanation of codes in Appendix 6	
AMPH	*Amphora* species
AMPS	*Amphora* species similar to *Amphora pediculus* (Kützing) Grunow
EPIT	*Epithemia* species (length 113µm breadth 4µm)
NASP	*Navicula* species
NIS1	*Nitzschia* species 1 (no photo available but as for NIS2 with no central gap in fibulae)
NIS2	*Nitzschia* species 2 (long thin valve with central gap in fibulae)
NIS3	*Nitzschia* species 3 (similar but longer than *Nitzschia umbonata* (Ehrenberg) Lange-Bertalot with no central gap in fibulae)
NIS4	*Nitzschia* species 4 (no photo available but as for NIS3 with central gap in fibulae)
RHOS	*Rhopalodia* species
ZZZZ	Unknown / Genus not identified

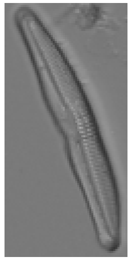
ANNEXURES - APPENDIX 7– PHOTOGRAPHS OF SOME OF THE DEFORMED DIATOMS


ZZZZ 1


AVET

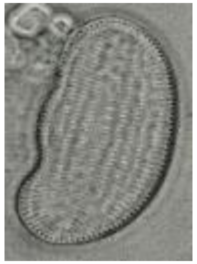

AVET

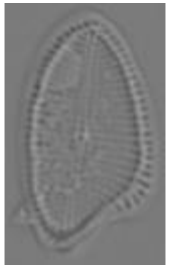

AVET

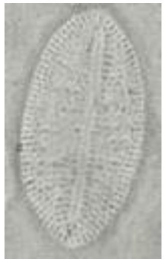

AVET


CRBU

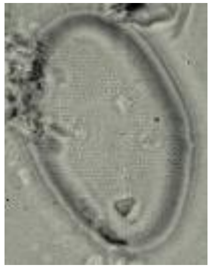

COCO


COCO


COCO

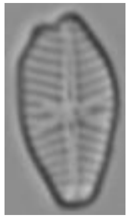

COCO


COCO


COCO


EPIT

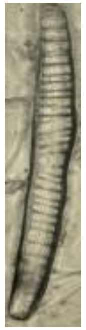

ESXT


GDEC


GDEC


CYMB


EPIT

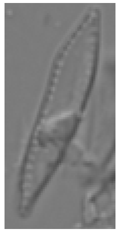

EPIT

Explanation of codes in Appendix 7	
AVET	*Amphora veneta* Kützing abnormal form
COCO	*Cocconeis* spp.
CRBU	*Craticula buderi* (Hustedt) Lange-Bertalot
CYMB	*Cymbella* spp.
EPIT	*Epithemia* spp.
ESXT	*Epithemia sorex* Kützing abnormal form
FRAG	*Fragilaria* spp.
GDEC	*Geissleria decussis* (Ostrup) Lange-Bertalot & Metzeltin
NATG	*Nitzschia amphibia* abnormal form
NFTE	*Nitzschia frustulum* (Kützing) Grunow abnormal form
NIZT	*Nitzschia* sp abnormal form
NLIA	*Nitzschia linearis* (Agardh) W.M. Smith abnormal form
RGIB	*Rhopalodia gibba* (Ehrenberg) O.Müller
ZZZZ	Unknown/Genus not identified

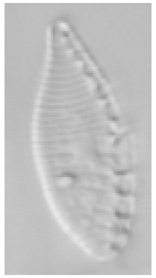
APPENDIX 7 – Photographs of some of the deformed diatoms continued


NFTE


NIZT


NIZT


NIZT

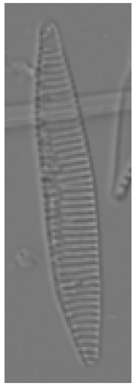

NIZT


NIZT

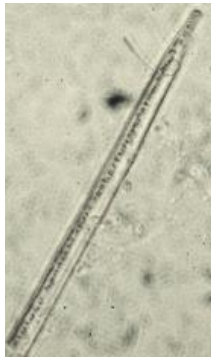

NIZT


NIZT


NIZT

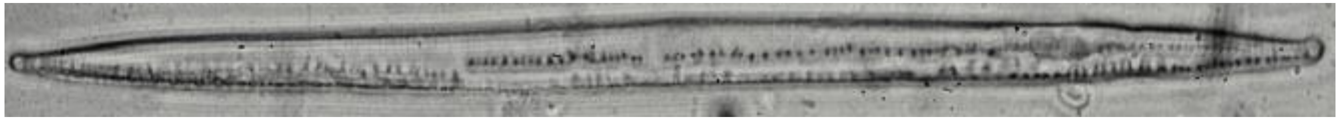

NATG


NIZT

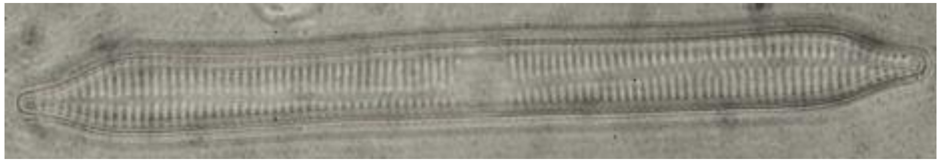

NLIA

Explanation of codes in Appendix 7	
AVET	*Amphora veneta* Kützing abnormal form
COCO	*Cocconeis* spp.
CRBU	*Craticula buderi* (Hustedt) Lange-Bertalot
CYMB	*Cymbella* spp.
EPIT	*Epithemia* spp.
ESXT	*Epithemia sorex* Kützing abnormal form
FRAG	*Fragilaria* spp.
GDEC	*Geissleria decussis* (Ostrup) Lange-Bertalot & Metzeltin
NATG	*Nitzschia amphibia* abnormal form
NFTE	*Nitzschia frustulum* (Kützing) Grunow abnormal form
NIZT	*Nitzschia* sp abnormal form
NLIA	*Nitzschia linearis* (Agardh) W.M. Smith abnormal form
RGIB	*Rhopalodia gibba* (Ehrenberg) O.Müller
ZZZZ	Unknown/Genus not identified

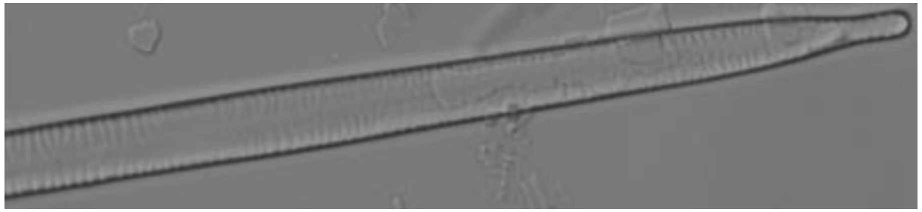
APPENDIX 6 – Photographs of some of the deformed diatoms continued



NLIA



FRAG



FRAG

RGIB

RGIB

Explanation of codes in Appendix 7	
AVET	*Amphora veneta* Kützing abnormal form
COCO	*Cocconeis* spp.
CRBU	*Craticula buderi* (Hustedt) Lange-Bertalot
CYMB	*Cymbella* spp.
EPIT	*Epithemia* spp.
ESXT	*Epithemia sorex* Kützing abnormal form
FRAG	*Fragilaria* spp.
GDEC	*Geissleria decussis* (Ostrup) Lange-Bertalot & Metzeltin
NATG	*Nitzschia amphibia* abnormal form
NFTE	*Nitzschia frustulum* (Kützing) Grunow abnormal form
NIZT	*Nitzschia* sp abnormal form
NLIA	*Nitzschia linearis* (Agardh) W.M. Smith abnormal form
RGIB	*Rhopalodia gibba* (Ehrenberg) O.Müller
ZZZZ	Unknown/Genus not identified