# ECOLOGICAL ASSESSMENT OF A TEMPERATE RIVER SYSTEM USING BIOMONITORING TECHNIQUES: A CASE STUDY OF THE BLOUKRANS RIVER SYSTEM, SOUTH AFRICA

A thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (SCIENCE)

of

RHODES UNIVERSITY

by

**TINOTENDA MANGADZE**

November 2019

ORCID ID: 0000-0002-9776-6437

## General Abstract

Escalating pressures from growing human populations and environmental impacts increasingly imperil freshwater ecosystems. The Bloukrans River, which drains an urbanised and agricultural catchment in the Eastern Cape province of South Africa, is no exception. Detailed knowledge of the structure and function of the aquatic ecosystems is required in order to create models and matrices that predict, guide assessment and direct intervention on ecological integrity and water quality management in these systems. The main objectives of this research were to: examine the effects of urbanization on the benthic macroinvertebrate functional feeding guild structure among different stream orders; determine if benthic diatoms can be used as effective and reliable indicators of ionic composition and conductivity in different stream order categories and finally; to evaluate the applicability of the South African Diatom Index (SADI) and other indices in the Eastern Cape region of South Africa. Field studies were carried out to explore temporal patterns in community structure (macroinvertebrates and diatoms) and ecosystem function related to land-use patterns, instream habitat availability, and water quality parameters in the Bloukrans River system across four study periods: February (summer) and July (winter) 2016 and February (summer) and May/July (winter) 2018. The study was conducted along a gradient of impacts from less impacted agricultural headwaters to highly impacted urban sites located immediately downstream of the city of Makhanda.

Macroinvertebrates were separated into functional feeding groups (FFGs) (i.e. collector–gatherer, collector–filterer, scraper, shredder, and predator) which were then used to assess the effects of selected physico-chemical variables and riparian zone condition on FFG organization. Collector–gatherers were the most abundant in the Bloukrans River and represented 71.3 % of the macroinvertebrate assemblages. Stream order 1 less impacted sites

had high abundances of shredders (6.2 %) and predators (14.9 %) compared to the other stream order categories. Results of the Linear mixed effects model (LMM) showed that the most important environmental variables influencing macroinvertebrate FFG distributions were canopy cover, phosphate, total dissolved solids (TDS) and channel width. High canopy cover resulted in an increase in the proportion of shredders ($p < 0.05$), whereas increase in TDS resulted in an increase in the proportion of scrapers, predators and collector gatherers ($p < 0.05$). The FFG ratios indicated that all the eighteen sites were strongly heterotrophic (i.e. streams received additional sources of energy from leaf litter and other organic matter), showed below expected linkage with riparian input and stable substrates were limited. The FFG ratios offered some insights into the overall functioning of Bloukrans River system. Our results highlight the importance of including macroinvertebrate functional diversity as a complementary approach to assess the ecological integrity in management and restoration plans of river systems.

Characteristics of benthic diatom community structure in relation to environmental variables were also analysed using multivariate techniques including the indicator value method and diatom-based indices. Epilithic diatom samples, water chemistry, and physical habitat conditions were characterized for 22 wadeable streams in February (at the end of the rain season when all streams were flowing) and July (dry season) 2016. A gradient of decreasing water quality was observed from stream order 1 sites to stream order 2/3 sites. Diatom community structure and composition closely followed the observed changes in pollution levels across a gradient of increasing organic pollution, eutrophication and ionic composition, with communities from stream order 2/3 sites being different from other communities. As pollution increased, low to moderate pollution-tolerant species such as *Fragilaria tenera*, *Cyclostephanos dubius* and *Gyrosigma acuminatum* were replaced by high pollution-tolerant

species such as *Nitzschia palea*, *Gomphonema parvulum*, *Tryblionella apiculata*, *Diploneis vulgaris* and *Staurosira elliptica.* Multivariate analysis (Canonical correspondence analysis (CCA)) indicated that differences in diatom community assemblages were best explained by calcium, magnesium, pH, phosphate, nitrate, dissolved oxygen, sediment nitrate, conductivity and salinity. These results indicate that diatoms can be used as bioindicators for monitoring highly impacted river systems and to also further examine pollution gradients and impacts of specific/point pollution sources.

In order to further test the application of diatom indices, nine sites with contrasting water quality were sampled along the length river system in February, May and July 2018. Diatom-based indices incorporated in OMNIDIA software were applied to assess the integrity of the water quality as indicated by diatom communities. For comparative purposes, several foreign indices (e.g. the trophic diatom index (TDI), the percentage pollution-tolerant valves (%PTV), biological diatom index (BDI)) and the South African Diatom Index (SADI) were used in the study. From the results, the Percentage Pollution-Tolerant Valves (%PTV) of most urban sites in the Bloukrans River was above the 20% limit indicating the presence of organic pollutants. Although the foreign diatom indices were applicable in the study, the SADI had significant correlations with most water quality variables ($p < 0.05$) compared to other indices such as Watanabe Index (WAT), Biological Index of Water Quality Trophic Index (BIWQ) and Trophic Index (TI)). These results support wider use of the SADI as an indicator of water quality conditions in South African river systems. Finally, the observed variations in diatom community structure and composition as a result of changes in water quality were broadly in agreement with the results of macroinvertebrate FFG structure indicating that the two biological indicators can, and should, be used as complementary techniques in the biomonitoring of rivers and streams in South Africa.

## Acknowledgements

I wish to express my sincere gratitude to all people who have contributed to the accomplishment of this work in different ways. First and foremost, I am greatly indebted to my supervisor Professor William Froneman for his patience, nurturing, always having an open door, guidance and for believing in me throughout the length of the study. His wide knowledge and positive comments from the proposal stage to the end of the thesis has not only shaped my thesis but also my thinking as a scientist. I also sincerely thank him for welcoming me into his department and for facilitating my establishment and comfortable stay in South Africa. I would also like to express my sincere appreciation to my co-supervisor Dr Tatenda Dalu for his continuous support, guidance, insightful ideas and contributions to this research. I am also especially grateful to Dr Ryan Wasserman for his efforts, time and positive criticism in two of the articles in this thesis. A special thanks to Dr Jonathan Taylor for helping me with calibration of diatom indices, insightful comments and support. Finally, I would also like to thank Sandiso Mnguni and Kevin Chikomo for their assistance during the field work.

My bursary was funded by the Organization for Women in Science for the Developing World (OWSD) and Swedish International Development Cooperation Agency (Sida). Many thanks to them for giving me the opportunity to further my studies in South Africa. To all my sisters thank you for your support. Special thanks to Rose Mangadze for the support, encouragement and laughter over the past three years. I am very fortunate to have such a supportive sister. Thanks to my parents for showing interest in what I do and for their continual care throughout my life. Thanks to my friends: Catherine Maware, Rumbidzai Mavunzi, Zviemurwi Chihambakwe, Deogratious Kanongo and Afrah Khairallah for your words of encouragement and motivation throughout the PhD journey. I also extend my inmost gratitude to Chris Mutasa for the encouragement, mentorship and always having my back in every circumstance. To

Louis Botha thank you for your support, coaching and kindness, you made South Africa feel like home.

I am also truly indebted to my two spiritual fathers in the Lord who supported me with prayer, motivational teachings and encouragement for all these years. Above all many thanks to the Almighty God. “A man’s heart plans his way, but the Lord directs his steps” (Proverbs 16:19).

## Preface

Each data chapter in this thesis is a stand-alone and has been published in either local or international peer reviewed journals. As such, there may be some repetition in the introductory sections of the chapters and each chapter includes a reference section. The research reported in this thesis was done in conjunction with other scientists that are listed as co-authors of the mentioned articles. The extent of involvement in each article is as follows:

1) Title: *Use of diatom communities as indicators of conductivity and ionic composition in a small austral temperate river system*

Authors: Tinotenda Mangadze, Ryan J Wasserman and Tatenda Dalu

Published in Water, Air and Soil Pollution, October 2017, No. 228, pages 428–439

Contribution of T Mangadze: Concept, diatom analysis and interpretation and writing of article

2) Title: *Biological monitoring in southern Africa: a review of the current status, challenges and future prospects*

Authors: Tinotenda Mangadze, Tatenda Dalu and William Froneman

Published in Science of the Total Environment, August 2018, No. 648, pages 1492–1499.

Contribution of T Mangadze: Concept, desktop study, data analysis and writing of article

3) Title: *Water quality assessment in a small austral temperate river system (Bloukrans River system, South Africa): application of multivariate analysis and diatom indices*

Authors: Tinotenda Mangadze**,** Jonathan C Taylor, William Froneman and Tatenda Dalu

Published in South African Journal of Botany, August 2019, No. 125, pages 353-359.

Contribution of T Mangadze: Concept, sampling, data analysis and writing of article

4) Title: *Macroinvertebrate functional feeding group alterations in response to habitat degradation of headwater Austral streams*

Authors: Tinotenda Mangadze**,** Ryan J Wasserman, William Froneman and Tatenda Dalu

Published in Science of the Total Environment, August 2019, No. 695, 133910

Contribution of T Mangadze: Concept, data analysis and interpretation and writing of article

# Table of Contents

## List of Tables (Short titles)

## List of Figures (Short titles)

## List of Appendices

## Declaration

I, Tinotenda Mangadze, hereby declare that the work presented in this thesis was carried out in the Department of Zoology and Entomology, Rhodes University under the supervision of Prof. William Froneman and Dr. Tatenda Dalu. The thesis comprises of original work by the author and has not been submitted for examination to any other university.

**TINOTENDA MANGADZE**

**November 2019**

# Chapter 1: General Introduction

## 1.1 Introduction

Global environmental challenges such as climate change, population growth and organic pollution resulting from mining, urbanization, agricultural activities and disposal of untreated sewage and industrial effluent places heavy environmental pressure on most drainage basins worldwide particularly in water scarce southern Africa (Roberts, 2011; Giani et al., 2012). Anthropogenic activities have accelerated the rate of climate change, leading to loss and destruction of habitats with subsequent loss of biodiversity. Moreover, climate change is not just shifting the ultimate structure and function of many freshwater ecosystems but is impacting the distribution and toxicity of chemical pollutants (Noyes et al., 2009). Detailed knowledge of the structure and function of the aquatic ecosystems is therefore, required in order to create models and matrices that predict, guide assessment and direct intervention on ecological integrity and water quality management in these systems (Capmourteres et al., 2018).

The United Nations' Sustainable Development Goals (SDG) of 2015 to 2030 highlights the importance of freshwater ecosystems and aspires for improvements in water quality aimed at 50% reduction in the global disposal of untreated waste by 2030 (United Nations Sustainable Development Goals, 2017). Similarly, at the national level, many countries have revised their policies to achieve sustainability of water resources by implementing national water monitoring programmes (Birk et al., 2012). South Africa has also recognized the importance of freshwater resources for future economic development through the Department of Water and Sanitation (DWS). The DWS performs its duties under the National Water Act (NWA, 6 No. 36 of 1998) and the Water Services Act (WSA, No. 108 of 1997), which was formulated to provide the legal framework for sound, equitable and sustainable water resource and related-services management in South Africa. The NWA states that in order to protect the full range of "goods and services" (e.g. provision of water, disposal of waste, supply of fish, plants and other biota) provided by rivers for humans the entire ecosystem must be conserved. For that reason, the cost-effective national monitoring system, the national River Eco-status Monitoring Programme (REMP), was established to collect information and data on, *inter alia*, the water quality and the health of aquatic ecosystems in South Africa (Roux et al., 1999). The approach of the REMP is to characterise the impacts of multiple stressors on the lotic environments by monitoring the response of biotic communities, with the assumption that the integrity of the biota provides a measure of the ecological integrity of the river system (Roux et al., 1999). Biological communities including diatoms, macroinvertebrates, fish and riparian vegetation are routinely employed to monitor changes in abiotic factors such as habitat, geomorphology, hydrology, and water chemistry to characterize a site and determine its water quality status (DWAF, 2000). These biological indicators are responsive to water chemistry and habitat variability on various scales, making them valuable diagnostic and regulatory tools (Hering, 2006; De La Rey et al., 2008; Yates and Bailey, 2010; Dalu et al., 2016; Mangadze et al., 2016). The most commonly used group employed are, however, the benthic macroinvertebrates because their community structure changes in response to environmental perturbations in predictable ways, which is the basis for development of biological criteria to evaluate human influences (Dallas, 2010). Benthic macroinvertebrates are also suitable because they are mostly available in large numbers, are diverse, have fairly short lifecycles, and vary in their tolerances to pollutants and other aspects of water quality (Day, 2000). The South African Scoring System (SASS), used in biomonitoring, has been demonstrated to be suitable for the assessment of the ecological integrity of river ecosystems throughout the region (Dickens and Graham, 2002; de la Rey, 2008; Odume and Mgada, 2016). Although, macroinvertebrate taxonomically based approach has been effectively used in investigations of impairment, there has been a recent advance in the use of functional analyses as a complementary approach to reflect ecological integrity of aquatic ecosystems (Gessner and Chauvet, 2002). Functional approaches use morphological and behavioural attributes of macroinvertebrates, which are related with feeding and modes of attachment, camouflage, and movement, together with life-history forms (voltinism) and drift propensity for ecological assessments of aquatic systems (Merritt et al., 2002)

That said, Round (1991) lists several reasons why benthic macroinvertebrates may not be suitable for biomonitoring, including amongst others: macroinvertebrate abundance and distribution may vary seasonally; therefore, comparisons of samples taken in different seasons may not be possible; macroinvertebrates have definite habitats and niches, animals may have several diverse life stages and may undergo metamorphosis, animals are mostly motile, and this can result in problems during sampling. Moreover, it is also generally accepted that macroinvertebrate-based indices (such as SASS) do not provide a reliable indication of eutrophication (Bonada et al., 2006). As such, it has been suggested that measurements of the photosynthetic community (e.g. diatom communities) may be more ideally suited for biomonitoring (Kelly, 1998).

Diatoms (class Bacillariophyceae) are more effective biological indicators than macroinvertebrates as they have a shorter generation time and respond rapidly to environmental change. Thus, diatoms are early warning indicators of lotic system deterioration and habitat restoration success (Pan et al., 1996; Hering et al., 2006). Diatoms are species rich assemblages (approximately 100 000 species) (Bennion et al., 2010; Mann and Vanormelingen, 2013). The increases in the number of taxa allows redundancies of information in the data set and increases the assurance of ecological inferences (Ovaskainen and Soininen, 2011). Therefore, numerous diatom-based biotic indices have been developed globally (e.g. Watanabe et al., 1986; Descy and Coste, 1991; Kelly and Whitton, 1995; Gómez and Licuirsi, 2001; Lobo et al., 2004). These indices have universal applicability since they are based on the ecology of widely distributed or cosmopolitan taxa. Differences in climate, geology, longitude and latitude among ecoregions may, however, contribute to variations in the environmental characteristics of lotic systems and may lead to differences in diatom community structure and composition (Potapova and Charles, 2007). Occurrence of endemic species may also necessitate the development of region-specific indices. Currently in South Africa, the South African Diatom Index (SADI), a modified version of the Specific Pollution Sensitivity Index (SPI) which includes indicator and tolerance values for South African endemic species is being employed. However, SADI was only validated and confirmed for a limited number of ecoregions in South Africa. Therefore, there is a need for validation and testing of the SADI in all the country's river systems to ensure that its scores provide an accurate reflection of the exact type of environmental pollution (Holmes and Taylor, 2015). This highlights the need to improve our understanding on the ecological responses of diatoms to different environmental variables in South Africa.

## 1.2 Rationale and significance of the study

The continued deterioration of most freshwater ecosystems, in combination with the failure of physico-chemical variables to offer evidence on the general condition of lotic ecosystems (Bere and Tundisi, 2010) has prompted a change of assessment and management from a regulatory approach to a more integrated and holistic ecosystem approach (Council for Scientific and Industrial Research (CSIR), 2010). While the implementation of biological monitoring programmes in other regions of South Africa are well established (e.g. Dallas 1997; Taylor et al., 2007; Harding and Taylor, 2011; Moyo and Richoux, 2018), there is a paucity of such studies in the Eastern Cape Province of South Africa (Hay et al., 2012).

The Eastern Cape Province has among the highest levels of poverty and unemployment, poor infrastructure development and maintenance (Westaway, 2012). In 2019, the population of Eastern Cape was estimated at 6 712 276 inhabitants by the South African National Statistics Agency. The growth of the cities and towns in the province has led to declining of operations and maintenance of vital water resource infrastructure, such as sewage treatment, garbage collection and urban drainage (Hay et al., 2012). For example, in Makhanda the high average annual range in nutrients, conductivity and low dissolved oxygen (DO) levels reported in most of the rivers are mainly due to the discharge of untreated sewage effluent and urban drainage into the river basins (DWA, 2012; Hill et al., 2015; Dalu et al., 2019). As a consequence, many of the river systems within the province are regarded as ecologically compromised.

The Bloukrans River catchment is characterized by different land-uses which expose the streams to human activities including agriculture, sewage effluent, urbanisation and industrialization (e.g. Odume and Mgada, 2016; Dalu et al., 2017; Nhiwatiwa et al., 2017). The river is economically important as it supplies water for irrigational purposes to the Belmont Valley agricultural farms that cater for the residents of Makhanda, Rhini, Port Alfred and other neighbouring towns. The Makana Municipality Report (2008) states that there is no water quality data available for the Bloukrans River, and data generation and input for this system by the DWA is ongoing. Therefore, there is need for establishing biological monitoring techniques which can be used by water management agencies to characterise the Bloukrans River system considering land-use patterns, discharge of pollutants, and other possible impacts.

## 1.3 Aims and objectives of the study

### *1.3.1 General aim*

The aim of this study is to employ effective biomonitoring methods using diatoms and macroinvertebrate functional groups to characterize the urban and agricultural impacts in the Bloukrans River system in the Eastern Cape Province of South Africa.

### *1.3.2 Specific objectives of the study are as follows:*

1. To summarise current efforts and challenges of monitoring lotic systems using different biomonitoring tools (i.e. macroinvertebrates, diatoms and fish) in southern Africa.
2. To examine the effects of urban land-use activities on the benthic macroinvertebrate functional feeding guild structure among different stream orders in the Bloukrans River system.
3. To determine if benthic diatoms can be used as effective and reliable indicators of ionic composition and conductivity in different stream order categories within the river system.
4. To evaluate the applicability of the South African Diatom Index (SADI) and other foreign indices in the Bloukrans River system, Eastern Cape region of South Africa.

## 1.4 Thesis overview

**Chapter 2:** Provides a summary of the current efforts that have been made to use bioindicators (i.e. macroinvertebrates, diatoms and fish) in monitoring water resources and highlights the challenges in employing these biological monitoring tools in southern Africa. In this chapter, extensive review of literature of these biological monitoring tools over a period of 10 years is used to contribute to our understanding of how organisms respond to different types of environmental variables.

**Chapter 3:** Presents the main findings of a study that employed a functional-based approach to examine the effects of urban land-use activities on macroinvertebrate functional feeding guild structure among different stream orders along the length of the Bloukrans River system.

**Chapter 4:** Presents the main findings of a study that assessed the diatom community structure and composition in relation to ionic composition and conductivity in different stream order categories along the Bloukrans River.

**Chapter 5:** Evaluates the applicability of the South African Diatom Index (SADI) and other foreign indices in monitoring the Bloukrans River system. This chapter highlights the importance of developing region-specific indices for water quality assessments.

**Chapter 6** is a summary of the key findings of this research, with conclusions and suggestions for future directions.

## 1.5 References


Bennion, H., Sayer, C., Tibby, J., Carrick, H., 2010. Diatoms as indicators of environmental change in shallow lakes. In Stoermer EF, Smol JP (eds) The diatoms: applications for the environmental and earth sciences, 2nd edn. Cambridge University Press, Cambridge.

Bere, T., Tundisi, J.G., 2010. Biological monitoring of lotic ecosystems: the role of diatoms. Braz. J. Biol. 70, 493-502.

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., Van De Bund, W., Zampoukas, N., Hering, D., 2012. Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. Ecol. Indic. 18, 31–41.

Bonada, N., Rieradevall, M., Prat, N., Resh, V.H., 2006. Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean climate streams of northern California. J. N. Am. Benthol. Soc. 25, 32–43.

Capmourteres, V., Rooney, N., Anand, M., 2018. Assessing the causal relationships of ecological integrity:a re-evaluation of Karr's iconic Index of Biotic Integrity. Ecosphere 9, e02168. 10.1002/ecs2.2168.

Dalu, T., Bere, T., Froneman, P.W., 2016. Assessment of water quality based on diatom indices in a small temperate river system, Kowie River, South Africa. Water SA 42, 183–193.

Dalu, T., Wasserman, R.J., Tonkin, J.D., Mwedzi, T., Magoro, M., Weyl, O.L.F., 2017. Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macroinvertebrate communities in an austral South African stream. Sci.Total Environ. 607–608, 317–325.

Dalu, T., Magoro, L.M., Wasserman, R., Froneman, W.P., 2019. River nutrient water and sediment measurements inform on nutrient retention, with implications for eutrophication. Sci. Total Environ. 684, 296–302.

Dallas, H. F., Kennedy, M., Taylar, J., Lowe, S., and Murphy, 2010. SAFRASS. South African Rivers Assessment Scheme, WP4. Review Paper.

Dallas, H.F., 1997. A preliminary evaluation of aspects of SASS (South African Scoring System) for the rapid bioassessment of water quality in rivers, with particular reference to the incorporation of SASS in a national biomonitoring programme. Southern Afr. J. of Aquat. Sci. 23, 79-94.

Day J., 2000. Biomonitoring: Appropriate Technology for the 21st Century. 1st WARFSA/WaterNet Symposium: Sustainable Use of Water Resources, Maputo 1-2 November 2000.

De La Rey, P.A., Roux, H., Van Rensburg, L., Vosloo, A., 2008. On the use of diatom-based biological monitoring Part 2: A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation. Water SA 34, 61-69.

Department of Water Affairs, South Africa (DWA)., 2012. Groundwater Reserve Determination in the Mzimvubu to Keiskamma WMA – Western Portion: Intermediate GRDM Assessment. Prepared by Umvoto Africa (Pty) Ltd. On behalf of the Directorate: Reserve Requirements, Department of Water Affairs, Pretoria, South Africa.

Department of Water Affairs and Forestry (DWAF)., 2000. National Biomonitoring Programme for Riverine Ecosystems: Ecological Reference Condition Project: Field-manual. General Information, Catchment Condition, Invertebrates and Water Chemistry. NBP Report Series No. 10. IWQS, Department of Water Affairs and Forestry, Pretoria, South Africa.

Descy, J.P., Coste, M., 1991. A test of methods for assessing water quality based on diatoms. Verhandlungen der Internationalen Vereinigung fürtheoretische und angewandte. Limnologie 24, 2112-2116.

Dickens, C.W.S., Graham, M., 2002. The South African Scoring System (SASS) Version 5 rapid bio-assessment method for rivers. Afr. J. Aquat. Sci. 27, 1-10.

Gessner, M.O., Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. Ecol. Applic. 12, 498-510.

Giani, M., Djakovac, T., Degobbis, D., Cozzi, S., Solidoro, C., Umani, S.F., 2012. Recent changes in the marine ecosystems of the northern Adriatic. Sea. Estuar. Coast Shelf Sci. 115, 1–13.

Gómez, N., Licursi, M., 2001. The Pampean Diatom Index (Idp) for assessment of rivers and streams in Argentina. Aquat. Ecol. 35**,** 173-181.

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. WRC Report No 1707/1/11 Water Research Commission, Pretoria.

Hay, E.R., Riemann, K., van Zyl, G., Thompson, I., 2012. Ensuring water supply for all towns and villages in the Eastern Cape and Western Cape Provinces of South Africa. Water SA 38, 437-444.

Hering, D., Johnson, R.K., Kramm, S., Schmutz, S., Szoszkiewicz, K., Verdonschot, P.F.M., 2006a. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: a comparative metric-based analysis of organism response to stress. Freshw. Biol. 51, 1757–1785.

Hill, J.M., Motitsoe, S.N., Hill, M.P., 2015. Pollution mapping in Freshwater systems: Using aquatic plants to trace N-Loading. WRC Report no. 2262/1/15. Water Research Commission, Pretoria, pp. 1-35.

Kelly, M. G., B. A. Whitton, 1998. Biological monitoring of eutrophication in rivers. Hydrobiologia 384, 55–67.

Kelly, M. G., Whitton, B. A., 1995. The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. J. Appl. Phycol. 7, 433-444.

Kelly, M.G., 1998. Use of community-based indices to monitor eutrophication in European rivers. Environ. Conserv. 25, 22-29.

Lobo, E.A., Callegaro, V.L., Hermany, G., Bes, D., Wetzel, C.E., Oliveirama., 2004. Use of epilithic diatoms as bioindicator from lotic systems in southern Brazil, with special emphasis on eutrophication. Acta. Limnol. Bras. 16, 25-40.

Makana Municipality., 2008. Integrated Development Plan Draft Review 2008 - 2009.

Mann, D.G., Vanormelingen, P., 2013. An inordinate fondness? The number, distributions, and origins of diatom species. J Eukaryot Microbiol. 60, 414–420.

Mangadze, T., Bere, T., Mwedzi, T., 2016. Choice of biota in stream assessment and monitoring programs in tropical streams: a comparison of diatoms, macroinvertebrates and fish. Ecol. Indic. 63, 128–143.

Merritt, R.W., Cummins, K.W., Berg, M.B., Novak, J.A., Higgins, M.J., Wessell, K.J., Lessard J.L., 2002. Development and application of a macroinvertebrate functional-group approach in the bioassessment of remnant river oxbows in southwest Florida.

Moyo, S., Richoux, N.B., 2017. Macroinvertebrate Functional Organisation Along the Longitudinal Gradient of an Austral Temperate River. J. Afr. Zool. 52,125–136.

Nhiwatiwa, T., Sithole, T., Dalu, T., 2017. Nhiwatiwa, T., Sithole, T., Dalu, T., 2017. Assessment of river quality in a subtropical Austral river system, a combined approach using benthic diatoms and macroinvertebrates. Appl Water Sci. 7, 4785–4792.

Noyes, P. D., McElwee, M. K., Miller, H. D., Clark, B. W., Van Tiem, L. A., Walcott, K. C., Levin, E. D., 2009. The toxicology of climate change: environmental contaminants in a warming world. Environ. Int. 35, 971–986.

Odume, O. N., Mgaba, N., 2016. Statistical analysis of macroinvertebrate community assemblage structure in relation to river health assessment of an urban river, Eastern Cape, South Africa. Aquat. Ecosyst. Health Manag. 4988, 420– 430.

Ovaskainen, O., Soininen, J., 2011. Making more out of sparse data: hierarchical modeling of species communities. Ecology 92, 289–295.

Pan, Y., Stevenson, R.J., Hill, B.H., Herlihy and, A.T., Collins, G.B., 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. J. N. Am. Benthol. Soc. 15, 481-495.

Potapova, M., Charles, D.F., 2007. Diatom metrics for monitoring eutrophication in rivers of the United States. Ecol. Indic. 7, 48-70. Press, New York.

Robert, V.T., 2011. Water quality and effluent discharge in Africa. Afr. J. Aquat. Sci. 89, 42- 54.

Round, F. E., 1991. Diatoms in river water-monitoring studies. J. Appl. Phycol. 3, 129-145.

Round, F. E., 1993. A Review and Methods for the Use of Epilithic Diatoms for Detecting and Monitoring Changes in River Water Quality. Methods for the examination of water and associated materials. HMSO Publications. London.

Roux, D.J., Kleynhans, C.J., Thirion, C., Hill, L., Engelbrecht, J.S., Deacon, A.R., Kemper, N.P., 1999. Adaptive assessment and management of riverine ecosystems: The Crocodile/Elands River case study. Water SA 25, 501–511.

Taylor, J.C., Prygiel, J., Vosloo, A., Pieter, A., Rey, D., Ransburg, L.V., 2007. 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 592, 455-464.

United Nations Millennium Development Goals., 2017. United Nations Millennium Development Goals. Available at http://www.un.org/millenniumgoals/ [Accessed October 10, 2017].

United Nations Sustainable Development., 2016. Sustainable Development Goals: 17 Goals to Transform Our World. Available at: http://www.un.org/sustainabledevelopment/ [Accessed October 10, 2017].

Watanabe, T., Asai, K., Houki, A., 1986. Numerical estimation of organic pollution of flowing waters by using the epilithic diatom assemblage – Diatom Assemblage Index (DIApo). Sci. Total Environ. 55, 209-218.

Westaway, A., 2012. Rural poverty in the Eastern Cape Province: Legacy of apartheid or consequence of contemporary segregationism? Development Southern Africa, 29, 115-125.

Yates, A.G., Bailey, R.C., 2010. Covarying patterns of macroinvertebrate and fish assemblages along natural and human activity gradients: implications for bioassessment. Hydrobiologia 637, 87–100.

## Chapter 2: Biological monitoring in southern Africa: A review of the current status, challenges and future prospects

* This chapter has been published as: **Mangadze, T**., Dalu, T., Froneman, P.W. Biological monitoring in southern Africa: a review of the current status, challenges and future prospects. *Science of the Total Environment* 648, 1492–1499.

**Abstract:** Biological monitoring programmes have gained popularity around the world particularly in southern Africa as they are fast, integrative and cost-effective approaches for assessing the effects of environmental stressors on aquatic ecosystems. This chapter reviews current efforts that have been made to use bioindicators (i.e. macroinvertebrates, diatoms and fish) in monitoring water resources and to summarise the challenges in employing these biological monitoring tools in southern Africa. In South Africa, macroinvertebrate (South African Scoring System (SASS)) and diatom based indices (e.g. South African diatom index (SADI)) have demonstrated their utility in identifying sources of impairment and determining the extent of impacts thus giving natural resource managers a scientifically defensible rationale for developing guidelines for conservation and management. Despite this advancement in South Africa, however, developing regionally appropriate quantitative tools for diagnosing ecosystem health is a pressing need for several other southern African countries. Together with sound scientific research, it is imperative for southern African countries to develop specific legislation and have mandated agencies, with proper training and funding to implement biomonitoring and bioassessments. The advancement and adoption of biological criteria as an integrated approach to assessing the impact of human activities in riverine ecosystems of the southern African region is highly recommended.

**Keywords:** Biomonitoring, bioindicators, southern Africa, macroinvertebrates, diatoms, fish, water quality

## 2.1 Introduction

Characterising the diversity, structure, and function of freshwater ecosystems is becoming increasingly urgent as many ecological systems face escalating pressures from population growth and environmental impacts (Paul and Meyer, 2001; Dudgeon et al., 2006; Taylor et al., 2007a; Bere and Tundisi, 2011a, b; Dalu et al., 2018a, b; Gallardo et al., 2018; Schmeller et al., 2018). There is growing understanding that biologically complex and functionally intact freshwater ecosystems could provide economically valuable ecosystem services to society (Baron et al., 2002; Brown et al., 2018; de Sosa et al., 2018; Vollmer et al., 2018). These services include flood control, transportation, recreation, purification of human and industrial wastes, habitat for plants and animals, and production of fish and other foods and marketable goods (Daily et al., 2000; Baron et al., 2002). The sustainable utilisation of our freshwater ecosystems is, therefore, critical to the development of emerging economies and the well-being of all citizens (Nel et al., 2011). Africa Water Vision 2025 has established common principles to coordinate the efforts of Member States to promote sustainable water use and management of water resources for poverty alleviation, socioeconomic development, regional cooperation, and the environment. However, these principles/targets are aligned with numerous regional development plans that include a doubling of the area of irrigated agriculture and a five-fold increase in overall water use for agriculture, industry and hydropower by 2025 (UN Water, 2003). Given these pressures, the sustainable water use in Africa is of growing importance (Masese et al., 2013), considering that a large proportion of rural populations depend directly on the health of natural resources for their livelihoods.

The management and conservation of freshwater resources in Africa has shifted to an improved understanding of species-environmental relationships, and development of new methodologies and frameworks to assess and monitor the ecological integrity of streams and rivers (Chutter, 1998; Kleynhans, 1999; Dickens and Graham, 2002). According to Cairns et al. (1982), biological monitoring is the regular and systematic use of living organisms as indicators to evaluate changes in the environment. Biological indicators complement the traditional physico-chemical indicators, facilitating a better assessment and management of freshwater ecosystems (Bartram and Balance, 1996; Lobo et al., 2004). The first attempts/studies demonstrated the potential and robustness of macroinvertebrates and fish that could enable their use to monitor river quality. However, attempts to use diatoms as indicators of water quality changes are recent in southern Africa (De la Rey et al., 2004; Taylor et al., 2007b; Phiri, 2007). Since 2005, the use of diatom-based biomonitoring was applied as part of the River Health Programme (RHP) of South Africa (RHP, 2005). After this period, many studies also successfully used diatom communities to evaluate the ecological status and water quality of rivers in other Southern African countries (e.g. Phiri et al., 2007; Bere et al., 2014; Mangadze et al., 2016). For that reason, benthic diatoms, macroinvertebrates and fish have become obligatory bioindicators for use in monitoring river water quality in Southern Africa. To reveal the current status and trends of the research on employing bioindicators (i.e. macroinvertebrates, diatoms and fish) in monitoring water resources and to summarise the challenges in employing these monitoring tools, this chapter analysed the southern African research literature in this field from January 2006 to December 2017 in the Web of Science (WOS) and SCOPUS database, providing important references for researchers in this field to carry out further studies.

## 2.2 Anthropogenic impacts on freshwater ecosystems in southern Africa

The earth's environment since the mid-twentieth century has been considerably affected by rapid human population growth, industrial and agricultural development (Flandroy et al., 2018; Li et al., 2018; Mahmoud and Gan, 2018). Humans have transformed over one-third of the earth's land surface, increased atmospheric carbon dioxide concentrations by nearly 30%, fixed more nitrogen than all-natural terrestrial sources combined and used more than 50% of all accessible surface freshwater (Vitousek et al., 1997). These global effects have both gradual and catastrophic impacts on biodiversity environments that support aquatic life, fish, and fisheries (Scheffer et al., 2001). In southern Africa, aquatic ecosystems are ecologically stressed due to unsustainable land use (Bellinger et al., 2006; Ndiritu et al., 2006; de la Rey et al., 2004, 2008; Janse van Vuuren and Taylor, 2015; Dalu et al., 2015; Mangadze et al., 2016), poor agricultural practices (Walsh, 2000; Dalu et al., 2017a,b,c; Dalu et al., 2015; 2018a,b; Mwedzi et al., 2016), deforestation of the catchments (Tumbare, 2004), pollution from domestic sources and mines (Mangadze et al., 2016; Dalu et al., 2017b,c; Nhiwatiwa et al., 2017a, b), overfishing (Witte et al., 1992), loss of habitat (Dalu et al., 2016) and climate change (Dallas and Rivers-Moore, 2013). Acid rain, nutrient enrichment of aquatic environments, pollution by pesticides, metals, and synthesised toxic substances on local, regional and global scales are some of the results of these anthropogenic activities (Bere, 2007; Mensah et al., 2012). Indeed, freshwater ecosystems are now regarded among the most endangered ecosystems in the world and the decline in freshwater biodiversity is far greater than in most affected terrestrial ecosystems (Sala et al., 2000; Dudgeon et al., 2006). This is not surprising, since the ecological integrity of rivers and other freshwater ecosystems is a direct reflection of all the activities in the catchments they drain (Allanson and Read, 1995; Davies and Day, 1998), and most catchments are subject to an array of ecologically unsustainable land-use and development activities (Minaya et al., 2013).

Existing wastewater treatment facilities in several southern African countries are poorly managed and overloaded and facing serious difficulties in handling the ever-increasing volumes of wastewater generated by increasing urbanisation (Oberholster and Ashton, 2008; Beyene et al., 2009; Simaika and Samways, 2010; Mwedzi et al., 2016). Poor operation and maintenance of wastewater treatment plants in developing countries contribute to the increase in pollutants and nutrients in the aquatic systems (Bere and Mangadze, 2014). In South Africa, management of water resources is governed by the National Water Act (Act 36) of 1998 which states that water extracted from rivers for industrial and municipal use should be returned to rivers as treated effluent based on acceptable standards (Morrison et al., 2001). However, the amounts of phosphorus and nitrogen entering the systems can easily increase above the recovery capabilities of a river (Yount and Niemi, 1990), especially when effluent treatment standards are not met due to lack of adequate infrastructure, poor management and monitoring (Schoeman and Steyn, 2003; Akcil and Koldas, 2006).

Land transformation for agricultural activities also has negative impacts on streams and rivers in southern Africa (Walsh, 2000; Dalu et al., 2015). Sedimentation from agricultural land expansion and deforestation (Choongo et al., 2005; Mambo and Archer, 2007; Dalu et al., 2013), atmospheric deposition and agricultural fertilisers (Walsh, 2000; Hoffman, 2014) all threaten aquatic ecosystems within the subregion. The effects of these land-use changes are manifested in changes in biotic communities, as the patterns of these biota are responsive to the nature of the prevailing physical and chemical conditions (Sponseller et al., 2001). Chemical stressors can result in impaired functioning or loss of a sensitive species and a change in community structure (Beyene et al, 2009; Bere and Tundisi, 2010). As stream ecosystems are changing, it is apparent that there is a need to develop consistent monitoring methods to track these changes and observe the environment within streams (Kearns et al., 2005).

### 2.3 Biological monitoring in southern Africa

Monitoring the negative land-use change consequences, while supporting the production of important resources has become a major priority for researchers and policy makers worldwide, particularly within the developing world (Efiong, 2011; Bere et al., 2016; Dalu et al., 2017a). Biological monitoring programmes have gained momentum in southern African countries as it is a fast, integrative and cost-effective approach for assessing the effects of environmental stressors on aquatic ecosystems (Taylor et al., 2007b; De la Rey et al., 2008; Bere and Tundisi, 2010; Ndebele-Murisa, 2012; Dalu and Froneman, 2016; Mangadze et al., 2017). Biological methods typically integrate responses to combinations of all contaminants and other sources of environmental stress thereby indicating overall effects in aquatic ecosystems (Beyene et al., 2009). Nonetheless, importance must be placed on monitoring methods which have the potential to provide accurate assessments, conservation, and remediation of river ecological integrity and function (Karr and Yoder, 2004). More recently, much attention has been given to the use of three groups of biomonitoring organisms: fish, benthic macroinvertebrates and algae, with the latter group largely limited to diatoms (De Pauw et al., 2006; Resh, 2008; Mangadze et al., 2016).

## 2.4 Methods

### *2.4.1 Bibliographical data*

The databases "SCOPUS" and "Web of Science" were searched for publications on the use of diatoms, macroinvertebrates and fish as biological monitoring tools in the subregion of southern Africa. The search was limited to articles and books published between January 2006 and December 2017. Moreover, the reference lists of identified articles were inspected for further literature. To obtain all literature information for analysis, the searching was conducted with key words such as "biological monitoring", "water quality assessment", "diatoms", "fish", "macroinvertebrates" and "biotic indices". Given that this review focuses on lotic and lentic freshwater systems, publications pertaining to biological monitoring in the marine system, marsh land, coastal waters or groundwater were excluded. Attributes of the selected published journal articles and books that listed characteristics (positive or negative) of using different groups of organisms for biomonitoring were examined and combined. Seventy-four journal articles were found and examined to determine: (1) purported advantages of using each of the three assemblages, and (2) purported disadvantages or limitations in their use. These attributes were then summarized into categories of advantages and disadvantages, and the frequency that they were cited for a particular assemblage was determined (Tables 2.1 and 2.2).

The selection of what or which organism group(s) to use in bioassessments should not be arbitrary, but based on conceptual models and empirical (e.g. dose–response) relationships that characterise the response of the indicator to the stressor of interest as well as quantify the levels of uncertainty associated with the stressor–response relationship (Johnson et al., 2006b). Any assessment and monitoring method developed should not only be scientifically sound but also be cost effective, transferable to different river basins and regional conditions and easy to use (Masese et al., 2013). It should also be within legal and institutional frameworks established to monitor, manage and protect target resources (Johnson et al., 2006a). Therefore, knowledge of how organisms respond to different types of stress can and should be used to design robust and cost-effective monitoring programmes (Hering et al., 2006b).

**Table 2.1:** Percentage number of papers for an assemblage that describes an advantage in biomonitoring in southern Africa. Modified from Resh (2008). Abbreviations, * (0–10%), ** (10–30%), *** (>30%) indicate the percentage of sources for an assemblage group. Inverts – macroinvertebrates.

| **Attribute for use in biomonitoring** | **Algae** | **Inverts** | **Fish** |
|---|---|---|---|
| *Sources* | *25* | *40* | *9* |
| *Widespread*, Group is described as being abundant, common, ubiquitous, etc | *** | *** | * |
| *Diverse*, Group has richness of species and a variety of response to environmental change | *** | ** | * |
| *Important to Ecosystem*, Group has important trophic position or ecological roles | * | ** | *** |
| *Limited Mobility*, Group can be used for inferring local conditions, sedentary or stay within a small area | ** | *** | |
| *Long Generation Time*, Group is useful for tracking changes over time, long term integrators, bioaccumulate toxins | ** | * | *** |
| *Short Generation Time*, Group has rapid responses to change and quick recovery from disturbance | *** | ** | |
| *Recreation value/consumed as human food*, Group has economic value that makes them of interest to the public | | | *** |
| *Easy Taxonomy*, Group is easy to identify and good taxonomic keys are available | * | ** | *** |
| *Easy Sampling*, Group requires low field effort | *** | ** | * |
| *Pre-existing Information*, Group has good background information relating to pollution tolerance, response to disturbance | * | ** | *** |
| *Field examination possible*, Group could be at least partially processed or identified in the field/useful for volunteers | | * | *** |
| *Historical conditions can be indicated*, Group has fossils that can be used to reflect past conditions | | * | |
| *Greater sensitivity to nutrients and herbicides*, Group shows sensitivity to specific plant stresses | *** | ** | |

**Table 2.2:** Percentage number of papers for an assemblage that describes a disadvantage in biomonitoring within southern Africa. Modified from Resh (2008). Abbreviations, * (0–10%), ** (10–30%), *** (>30%) indicate the percentage of sources for an assemblage group, inverts – macroinvertebrates.

| Disadvantageous attribute for biomonitoring | Algae | Inverts | Fish |
|---|---|---|---|
| *Sources* | *25* | *40* | *9* |
| *Sampling Difficulties*, Group requires large effort | ** | *** | ** |
| *Identification*, Group requires expertise for identification, few taxonomic keys available | *** | ** | * |
| *Poor Response Levels*, Group has low sensitivity to disturbance | ** | ** | * |
| *Affected by Non-anthropogenic Conditions*, Group affected by a variety natural factors and changes in physical conditions | ** | ** | * |
| *Mobile*, Group swims, drifts, and not useful as a local indicator, pollution effects may be elsewhere (i.e. spawning grounds) | * | | *** |
| *Problems with Analytical Tools Available*, Group has poor metrics/indices available for analysis | *** | * | ** |
| *Short Generation Time*, Group is a poor integrator, does not show bioaccumulation | | *** | |

## 2.5 Biotic indices

Different sampling protocols and metrics have been developed to evaluate the water quality of rivers and streams in southern Africa. Biotic indices are the most widely used because they are highly robust, sensitive, cost-effective, easy to apply, and easy to interpret (Dallas, 1995, 1997; Chessman et al., 1997; Bonada et al., 2006; Lowe et al., 2013; Bere et al., 2014; Dalu et al., 2016a). Biotic indices are tools for assessing water quality based on the different response of organisms to environmental changes (Borisko et al., 2007).

In South Africa, the South African Scoring System (SASS), developed by Chutter (1998), has been widely employed for biological monitoring. The SASS uses riverine macroinvertebrates to explain the state of water quality. Several southern African countries have subsequently utilised the SASS as a means of assessing the status of river systems (Gratwicke, 1998; Moyo and Phiri, 2002; Muthimkhulu et al., 2005; Ndebele-Murisa, 2012; Bere and Nyamupingidza, 2014; Mwedzi et al., 2016). In Namibia, Botswana and Zambia, SASS has been modified and standardized into the Namibian Scoring System (NASS) (Palmer and Taylor, 2004), Okavango Assessment System (OKAS) (Dallas, 2009) and Zambian Invertebrate Scoring System (ZMSS) (Case study 1, Lowe et al., 2013) to account for additional tropical invertebrate taxa that occur specifically in these regions.

**Case Study 2.1:** SAFRASS biomonitoring scheme, general aspects and benthic macroinvertebrates (ZISS) protocols, a Zambian study

Zambia contains the sources of two of the continent's largest rivers, the Zambezi and Congo Rivers. Infrastructural development initiatives have been identified that will increase freshwater demands in Zambia. In 2011, The United Nations reported that Zambia will have the world's highest predicted population growth rate, coupled with rapidly increased urbanisation, industrialisation and agricultural development. Developing Zambia's water resources without degrading ecosystems is a challenging but prudent goal. A combination of lack of capacity to monitor water resource quality and the potential for increased impacts on these resources make the development of appropriate monitoring tools in Zambia particularly pertinent. No river biomonitoring protocols existed for Zambia prior to the pilot procedure developed by Southern African River Assessment Scheme (SAFRASS) in 2013 (Lowe et al., 2013)

The Zambian Invertebrate Scoring System (ZISS) was developed during the SAFRASS project, with major adaptations to the SASS from South Africa and included family-level taxa identification. The ZISS data sheet with a list of the taxa used for scoring purposes is included in the appendices of the SAFRASS Methods Manual (Lowe et al., 2013). Sensitivity weighting scores were assigned to each family group or taxa and provided the foundation for biotic metrics (Dallas et al., 2010; Lowe et al., 2013). The weighting reflects the sensitivity of taxa to river health impairment. For ZISS, as with SASS5, taxa with high scores represent taxa sensitive to changes in water quality. Conversely, taxa with a low score represent tolerant taxa that are better able to persist under impaired conditions. Sensitivity weightings for ZISS taxa were denoted by expert opinion which included consideration of life history, known ecological and physiological requirements, known examples of response to pollution, and weighting scores from other tested biotic indices. The assignment of macroinvertebrate sensitivity scores to newly developed and incorporated ZISS taxa is detailed in a study by Lowe et al (2013). Further refinement of the sensitivity weighting of some SASS5 taxa for ZISS was based on correlation of taxa occurrence with impacts. ZISS can successfully be applied not only in river channels, but in associated waterbodies such as floodplain lagoons (Moore and Murphy, 2013). However, there is need for refinement of SASS weighting scores for adaptation in Zambia due to different physical characteristics of rivers in Zambia than in South Africa. Moreover, species (and genera) comprising the families used as the indicator taxa are often different in Zambia to those in South Africa and may, therefore, have different sensitivity to pollution (Lowe et al., 2013).

Fish-based indices of biotic integrity have also been developed and used in southern Africa for bioassessments and biomonitoring purposes (Hocutt et al., 1994; Kleynhans, 1999). The index of biotic integrity (IBI) is a holistic multimetric approach that involves the integration of a number of structural and functional attributes or metrics of a fish (Simon and Lyons, 1995) community into a composite index. The Fish Assemblage Integrity Index (FAII) developed by Kleynhans (1999) in the Crocodile River, South Africa, has been used to determine the status of the fish assemblage in relation to human-induced factors. Subsequently, the FAII has been extended to the Fish Response Assessment Index (FRAI) (Kleynhans et al., 2005). The FRAI is an assessment index based on the environmental intolerances and preferences of the reference fish assemblage and the response of the constituent species of the assemblage to particular groups of environmental determinants or drivers. Usually the FRAI is based on a combination of fish sample data and fish habitat data (Kleynhans et al., 2005). The FRAI, has been tested in Elands River (tributary of the Crocodile River, Incomati System) in South Africa. The presence of a fish assemblage and fish habitat features which are similar at all sites, resulted in this reach being selected for FRAI validation (Case study 2, Kleynhans et al., 2007).

**Case Study 2.2:** Validation of FRAI in the Elands River, South Africa

The Elands River is a tributary of the Crocodile River, Incomati System in South Africa. The Elands River is perennial and 5-15 m wide in the reach investigated for this study. The river downstream from the mill was subjected to a severe pollution spill in 1989 (Kleynhans et al., 1992). Sewage spills from the town of Waterval-Boven are known to have also occurred in the past. Land use in the catchment is mostly devoted to eucalypt and pine plantations, vegetable and fruit farming and some tourist accommodation (Kleynhans, 1996). Alien riparian vegetation is abundant along some sections. Impacts on this river reach are generally homogenous and are considered to be minimal to low as far as impacts on the fish assemblages are concerned. Therefore, FRAI was used for validation in 4 sites of Eland River using three terms per each site. The FRAI was calculated according to the approach indicated Kleynhans et al. (2005). Analysis of Catch Per Unit Effort (CPUE) data at all sites indicated that data deviated severely from a normal distribution. There was a significant difference in species among teams at sites 1 and 2 when all species (native and introduced, and regardless of velocity depth preference) were considered. No significant differences were recorded between sites. From the results it was evident that data pooled for all sites or for teams, indicated a FRAI category of B/C when only native species with a high preference for fast flows are considered. However, when all data is pooled the FRAI category increases to "A" which is considered to be the "actual" FRAI category for the reach. It can be concluded that, FRAI's ecological principles, which define assemblage structure and function in relation to present environmental conditions, provide an insight on the status of an assemblage in a given aquatic system, as was shown in the study. It was recommended that more studies be carried out to test the consistency of the FRAI in assessing the frequency of occurrence of native species in a river reach in comparison to the reference (natural) frequency of occurrence in terms of environmental intolerances and preferences in rivers.

Recent studies have also shown the use of diatoms as diagnostic and effective tools for water quality assessments (Harding and Taylor, 2011; Bere and Mangadze, 2014). However, due to lack of information on ecological preferences and tolerances of diatoms in southern Africa, diatom-based biotic indices developed in temperate regions are often applied to assess ecological conditions in tropical context (Bere et al., 2014). Currently, the South African diatom index (SADI) has been developed as a modified form of the Specific pollution sensitivity index (SPI) which includes South African endemic species (Case study 3, Harding and Taylor, 2011). This index however, still requires input from regional studies and validation (Holmes and Taylor, 2015). The use of diatoms as tools to assess ecosystem health in sub-Sahara Africa has also recently been reviewed by Dalu and Froneman (2016b).

**Case Study 2.3:** The South African Diatom Index (SADI) –a preliminary index for indicating water quality in rivers and streams in southern Africa

Biotic indices of diatoms are widely used worldwide to assess the health status of lotic systems (Taylor, 2007a, b, c). These indices are thought to have universal applicability across geographic areas and environments because of the cosmopolitan nature of most diatom species (Harding et al., 2015). There is, however, evidence that diatom indices developed in one geographic area or environment is less successful when applied in other areas (Pipp, 2002). For example, in later catchment-wide studies in South Africa, it was found that some sites, especially those near-natural mountain streams, were dominated by the South African endemic species e.g. *Achnanthidium standeri* (Cholnoky) (Taylor et al., 2007c), for which no tolerance of indicator values were included in the index. This resulted in index scores, based on the composition of these diatoms, being inherently flawed and pointed to the need for a modified index unique to South Africa.

In 2011, a diatom based bioassessment method for South Africa rivers, the South African Diatom Index (SADI) was developed and validated (Harding and Taylor, 2011). The SADI is a modified version of the Specific Pollution Sensitivity Index (SPI) developed in France. The index has been formulated using data from 768 samples collected and analysed. The index has been provided in the form of an upgrade addition to the existing OMNIDIA software, with the intention being that the index will form part of future releases thereof (Harding and Taylor, 2011). Determining tolerance and indicator scores for South African endemics was done using Canonical Correspondence Analysis (CCA) to create a bi-plot of species distribution *vs* water quality variables. Principal Component Analysis (PCA) was used to determine inter-specific relationships within diatom communities. The SADI has proved to be reliable in reflecting general changes in water quality variables in rivers and streams (Harding and Taylor, 2011, 2014). However, SADI is part of the initial steps in the development of diatom based bioassessment tools for rivers and streams of Southern Africa. Ongoing inclusion of results from collections from all over Southern Africa is and will remain necessary for some years in order to increase the robustness of this index.

### 2.6 Biological monitoring challenges

Traditionally, monitoring of water quality deterioration in streams and rivers in Africa has largely been based on physico-chemical analysis (Madrid and Zayas, 2007). This approach is however, costly, time consuming and provides a measure of water at the time of sampling without capturing temporal variations (Rocha, 1992). There is a need for a shift to the use of biological indicators which are cost effective and provide an integrative assessment of ecosystem health status (De La Rey et al., 2004; Taylor et al., 2009). However, inadequate reference information, which is useful as a means of establishing community expectations following restoration initiatives, is a major hindrance to development of river health indices in the region (Bere et al., 2014). For example, diatom-based biotic indices developed in temperate regions are often applied to assess ecological conditions in a tropical context (Taylor et al., 2007a; Bere et al., 2014; Dalu et al., 2016a). Concerns have been raised regarding the transfer of indices developed in one geographic region into another as there may be floristic and faunal differences among regions and potential environmental differences that may modify species responses to environmental change (e.g. Bonada et al., 2006; Ziglio et al., 2006). Field and laboratory testing of borrowed indices is therefore, required to ensure that the scores give a realistic reflection of the specific type of environmental conditions in geographic regions being assessed (Bere et al., 2014).

Modification of biotic indices for use in most African regions is also hindered by incomplete taxonomical resolution and seldom known sensitivity levels of many tropical taxa (Jacobsen et al., 2008). This can also be considered as a setback in the general application of biomonitoring and bioassessment methods (Bere, 2016). Moreover, the absence of biological monitoring experts and lack of necessary resources and equipment (e.g. microscopes, identification guides) in most African countries, represent significant obstacles in ensuring that biomonitoring programmes are included as part of their national policies.

Furthermore, throughout the development of these monitoring tools and methodologies, there is a general lack of regional and international networking among researchers, stakeholders and governing authorities to improve biological monitoring tools (Dalu and Froneman, 2016b). Bere (2016) has argued that diatom-based biological monitoring is not being widely employed because of lack of capacity and training of diatom taxonomists due to limiting funding and job opportunities. Most African research institutions and universities do not have the necessary facilities and expertise to conduct routine diatom analyses (Dalu and Froneman, 2016b). All these challenges have contributed to the difficulty of implementing biological monitoring programmes in most southern African countries.

### 2.7 Conclusion and future directions

It is evident that biological monitoring is now recognised as an appropriate aspect of water resources management and conservation in southern Africa (Phiri et al., 2007; Taylor et al., 2007b; Dallas, 2009; Dalu and Froneman, 2016b; Bere, 2016). However, with increasing human populations and development in catchment areas, degradation of water quality is likely to be exacerbated. These complicated issues associated with protected-areas design and management for freshwaters require energetic and imaginative attention from researchers. The challenge is to mitigate deleterious trends and practices to improve the current condition of water resources (Masese et al., 2013). Maintaining forest buffers or riparian zone restoration areas, which have been found to be useful in improving river health is critical (Kasangaki et al., 2006; Mwedzi et al., 2016), and be a good starting point in most catchments in southern Africa. Riparian buffers protect water resources from non-point source pollution and provide bank stability and aquatic habitats.

Designing more strong and big data sets on autecology and taxonomy of a large number of bioindicators will make biological indices to be more powerful tools for monitoring river health (Dalu and Froneman, 2016b). There is also a need to adapt and strengthen existing biotic indices because they are reliable on identifying sources of impairment and can also be used as a monitoring and evaluation tools to identify streams and rivers where restoration activities are needed and to monitor trends in biotic integrity and biodiversity over time (Lyons et al., 1995). Thus, extensive biomonitoring protocols are still needed in Africa to develop standard methods. To date, the only national programme for assessing ecosystem status of rivers (The River Eco-Status Monitoring Programme (REMP)) has been designed and implemented in South Africa. The REMP replaced the RHP in 2016 and is a component of the National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) of South Africa. Large-scale assessment programmes provide data with sufficient detail, sample size, and scale to fuel an explosion in ecological knowledge (Stevenson, 2014). The data from these surveys should be made available to researchers with the explicit goal of enabling further analysis and interpretation of data and use of data in complementary research projects. Together with sound scientific research, it is imperative for southern African countries to develop specific legislation and have mandated agencies, with proper training and funding to implement biomonitoring and bioassessment.

The true biological monitoring value must be communicated to ensure support and buy-in from all stakeholders and local societies. In South Africa, the Riparian Vegetation Response Assessment Index (VEGRAI) is now being used as part of the suite of models used to assess ecological status (Kleynhans et al., 2007). The relationships between riparian trees, groundwater depth and flood events has also been investigated at some rivers in Namibia and Botswana (Moser, 2006; Obakeng, 2007; Schachtschneider and February, 2010). However, organisms such as amphibians, reptiles, mammals and birds should also possibly be incorporated in biomonitoring programmes in southern African countries. Additionally, challenges still remain with bioindicators such as diatoms as much work still has to be done in terms of diatom tolerances, ecological preferences and ecophysiology studies (Bate et al., 2002; Bere, 2016). They is need to adapt to the South African diatom index (SADI) which includes South African endemic species (Harding and Taylor, 2014).

Environmental and water management efforts should focus on the specific nature of impairment, along with enforcement of existing wastewater discharge standards, restoration of degraded habitats, and mitigation of further degradation, based on accurate assessment and interpretation of component metrics and an understanding of amounts and types of human disturbance. Thus, policies governing environment and water should also consider aquatic ecosystem protection and conservation as a key component in water resources and ecosystem management with the exception of South Africa which has partly implemented these policies through REMP. Scientists need to work more closely with economists and social scientists, as well as biogeochemists, hydrologists, engineers, and policy makers, to better understand how their research can be related to valuation of ecosystem services, developing management strategies, and informing environmental policy.

## 2.8 References


Akcil, A., Koldas, S., 2006. Acid mine drainage (AMD), causes, treatment and case studies. J. Clean. Prod. 14, 1139–1145.

Allanson, B.R., Read, G.H.L., 1995. Further comment on the response of Eastern Cape Province estuaries to variable freshwater flows. Afr. J. Aquat. Sci. 21, 56–70.

Baron, J.S., Poff, N.L., Angermeier, P.L., Clifford, N., Dahm, P.H., Gleick, N.G., Hairston, Jr., Jackson, R.B., Johnston, C.A., Richter, B.D., Steinman, A.D., 2002. Meeting ecological and societal needs for freshwater. Ecol. Appl. 12, 1247–1260.

Bartram J., R., Balance., 1996. Water Quality Monitoring: A practical guide to the design and Implementation of Fresh Water Quality Studies and Monitoring Programmes, UNEP, Taylor & Frances, London

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

Bellinger, B.J., Cocquyt, C., O'Reilly, C.M., 2006. Benthic diatoms as indicators of eutrophication in tropical streams. Hydrobiologia 573, 75–87.

Bere, T., 2007. The assessment of nutrient loading and retention in the upper segment of the Chinyika River, Harare, Implications for eutrophication control. Water SA 33, 279–284.

Bere, T., Mangadze, T., 2014. Diatom communities in streams draining urban areas, community structure in relation to environmental variables. J. Trop. Ecol. 55, 271–281.

Bere, T., Dalu, T., Mwedzi, T., 2016. Detecting the impact of heavy metal contaminated sediment on benthic macroinvertebrate communities in tropical streams. Sci. Total Environ. 572, 147–156.

Bere, T., Mangadze, T., Mwedzi, T., 2014. The application and testing of diatom-based indices of water quality assessment in the Chinhoyi Town, Zimbabwe. Water SA 3, 503–511.

Bere, T., Nyamupingidza, B., 2014. Use of biological monitoring tools beyond their country of origin, a case study of the South African Scoring System Version 5 (SASS5). Hydrobiologia 722, 223–232.

Bere, T., Tundisi, J.G., 2011a. Influence of ionic strength and conductivity on benthic diatom communities in a tropical river (Monjolinho), São Carlos-SP, Brazil. Hydrobiologia 661, 261–276.

Bere, T., Tundisi, J.G., 2011b. 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, 93–102.

Bere, T., Tundisi, J.G., 2010. Biological monitoring of lotic ecosystems, the role of diatoms. Braz. J. Biol. 70, 493–502.

Bere, T., 2016. Challenges of diatom-based biological monitoring and assessment of streams in developing countries. Environ. Sci.Pollut. Res. 6, 5477–5486.

Beyene, A., Addis, T., Kifle, D., Legesse, W., Kloos, H., Triest, L., 2009. Comparative study of diatoms and macroinvertebrates as indicators of severe water pollution, Case study of the Kebena and Akaki rivers in Addis Ababa, Ethiopia. Ecol. Indic. 9, 381–392.

Bonada, N., Rieradevall, M., Prat, N., Resh, V.H., 2006. Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean climate streams of northern California. J. N. Am. Benthol. Soc. 25, 32–43.

Borisko, J.F., Kilgour, B.W., Stanfield, L.W., Jones, F.C., 2007. An evaluation of rapid bioassessment protocols for stream benthic invertebrates in southern Ontario, Canada. Water Qual. Res. J. Can. 42, 184–193.

Brown, C.E., Bhat, M.G., Rehage, J.S., Mirchi, A., Boucek, R., Engel, V., Ault, J.S., Mozumder, P., Watkins, D., Sukop, M., 2018. Ecological-economic assessment of the effects of freshwater flow in the Florida Everglades on recreational fisheries. Sci. Total Environ. 627, 480–493.

Cairns J. Jr., Buikema A. L. Jr., Cherry D. S., Herricks E. E., van der Schalie W. H., Matthews R., Niederlehner B. R., Rodgers J. H. Jr. (1982): Biological Monitoring. (London: Pergamon Press).

Chessman, B.C., Growns, J.E., Kotlash, A.R., 1997. Objective derivation of macroinvertebrate family sensitivity grade numbers for the SIGNAL biotic index, application to the Hunter River system, New South Wales. Aust. J Mar. Freshwat. Res. 48, 159–172.

Choongo, C.K., Syakalima, S.M., Mwase, M., 2005. Coefficient of condition in relation to copper levels in muscle of Serranochromisfish and sediment from the Kafue River, Zambia. Bull. Environ. Contam.Toxicol. 75, 645–651.

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 Water Research Commission, Pretoria.

Daily, G.C., Söderqvist, T., Aniyar, S., Arrow, K., Dasguota, P., Ehrlich, P., Folke, C., Jansson, A., Jansson, B., Kautsky, N., Levin, S., Lunchenco, J., Mäler, K., Simpson, D., Starrett, D., Tilman, D., Walker, B., 2000. The value of nature and the nature of value. Science 289, 395–396.

Dallas, H.F., 1995. An evaluation of SASS (South African Scoring System) as a tool for the rapid bioassessment of water quality. MSc Thesis, University of Cape Town, Cape Town.

Dallas, H.F., 1997. A preliminary evaluation of aspects of SASS (South African Scoring System) for the rapid bioassessment of water quality in rivers, with particular reference to the incorporation of SASS in a national biomonitoring programme. S. Afri. J. Aquat. Sci. 23, 79-94.

Dallas, H.F., Rivers-Moore, N.A., 2013. Ecological consequences of global climate change for freshwater ecosystems in South Africa Climate change and freshwater ecosystems. S. Afr. J. Sci. 110, 1–11.

Dallas, H.F., 2009. Wetland monitoring using aquatic macroinvertebrates. Technical Report 5/2009 Prepared for the Biokavango Project, Harry Oppenheimer Okavango Research Centre, University of Botswana, The Freshwater Consulting Group, University of Cape Town, South Africa.

Dallas, H., Kennedy, M., Taylor, J., S. Lowe, S., Murphy, K., 2010. SAFRASS: Southern African River Assessment Scheme. WP4: Review of existing biomonitoring methodologies and appropriateness for adaptation to river quality assessment protocols for use in southern tropical Africa.

Dalu, T., Magoro, M., Tonkin, J.D., Human, L.R.D., Deyzel, S.H.P., Perissinotto, R., Adams, J.B., Whitfield, A.K., 2018a. Assessing phytoplankton structure within micro-estuaries and micro-outlets, A community analysis approach. Hydrobiologia. 818, 177–191.

Dalu, T., Adams, J.B., Taylor, J.C., Bate, G.C., Nunes, M., Froneman, P.W., Wasserman, R.J., 2018b. An overview of the ecology and status of estuarine microphytobenthos research in South Africa. Afr. J. Mar. Sci. 40, 1–12.

Dalu, T., Bere, T., Froneman, P.W., 2016a. Assessment of water quality based on diatom indices in a small temperate river system, Kowie River, South Africa. Water SA 42, 183–193.

Dalu, T., Bere, T., Richoux, N.B., Froneman, P.W., 2015. Assessment of the spatial and temporal variations in periphyton communities along a small temperate river system, A multimetric and stable isotope analysis approach. S. Afr. J. Bot. 100, 203–212.

Dalu, T., Froneman, P.W., 2016b. Diatom-based water quality monitoring in southern Africa, challenges and future prospects. Water SA 42, 551–559.

Dalu, T., Wasserman, R.J., Magoro, M., Mwedzi, T., Froneman, P.W., Weyl, O.L.F., 2017b. Variation partitioning of benthic diatom community matrices, Effect of multiple variables on benthic diatom communities in an Austral temperate river system. Sci. Total Environ. 601–602, 73–82

Dalu, T., Wasserman, R.J., Tonkin, J.D., Alexander, M.E., Dalu, M.T.B., Motitso, S., Manungo, K.I., Bepe, O., Dube, T., 2017a. Assessing drivers of benthic macroinvertebrate community structure in African highlands, an exploration using multivariate analysis. Sci. Total Environ. 601–602, 1340–1348.

Dalu, T., Wasserman, R.J., Tonkin, J.D., Mwedzi, T., Magoro, M., Weyl, O.L.F., 2017c. Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macroinvertebrate communities in an austral South African stream. Sci. Total Environ. 607–608, 317–325.

Dalu, T., Tambara, E.M., Clegg, B., Chari, L.D., Nhiwatiwa, T., 2013. Modeling sedimentation rates of Malilangwe Reservoir in the south-eastern lowveld of Zimbabwe. Appl. Water Sci. 3, 133–144.

Davies, B., Day, J., 1998. Vanishing Waters. University of Cape Town Press, Cape Town.

De La Rey, P.A., Roux, H., Van Rensburg, L., Vosloo, A., 2008. On the use of diatom-based biological monitoring Part 2, A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation. Water SA 34, 61–69.

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 Pauw, N., Gabriels, W., Goethals, P.L.M., 2006. River monitoring and assessmentmethods based on macroinvertebrates. In: Ziglio, G., Siligardi, M., Flaim, G. (Eds.), Biological Monitoring of Rivers. Application and Perspectives. John Wiley andSons, UK, pp. 113–134.

de Sosa, L.L., Glanville, H.C., Marshall, M.R., Williams, A.P., Jones, D.L., 2018. Quantifying the contribution of riparian soils to the provision of ecosystem services. Sci. Total Environ. 624, 807–819.

Dickens, C.W.S., Graham, P.M., 2002. The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. Afr. J. Aquat. Sci. 27, 1–10.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowle, D.J., Léveˆque, C., Naiman, R.J., Prieur-richard, A.H., Soto, D., Tiassny,andc, M.L.J.S., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163–182.

Efiong, J., 2011. Changing pattern of land use in the Calabar River catchment, south eastern Nigeria. J. Sustain. Dev. 4, 92–102.

Flandroy, L., Poutahidis, T., Berg, G., Clarke, G., Dao, M.C., Decaestecker, E., Furman, E., Haahtela, T., Massart, S., Plovier, H., Sanz, Y., 2018. The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems. Sci. Total Environ. 627, 1018–1038.

Gallardo, B., Bogan, A.E., Harun, S., Jainih, L., Lopes-Lima, M., Pizarro, M., Rahim, K.A., Sousa, R., Virdis, S.G., Zieritz, A., 2018. Current and future effects of global change on a hotspot's freshwater diversity. Sci. Total Environ. 635, 750–760.

Gratwicke, B., 1998. An introduction to aquatic biological monitoring. Zimb. Sci. News 32, 75–78.

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. WRC Report No 1707/1/11 Water Research Commission, Pretoria.

Harding, W.R., Taylor, J.C., 2014. Diatoms as indicators of historical water quality, A comparison of samples taken in the Wemmershoek catchment (Western Cape Province, South Africa) in 1960 and 2008. Water SA 40, 601–606.

Harding, W.R., Archibald, C.G.M., Taylor, J.C., 2015. The relevance of diatoms for water quality assessment in South Africa, a position paper. Water SA 3, 41–46.

Hering, D., Johnson, R.K., Buffagni, A., 2006b. Linking organism groups – major results and conclusions from the STAR project. Hydrobiologia 566, 109–113.

Hocutt, C.H.P.T., Johnson, C., Hay, C.J., van Zyl, B.J., 1994. Biological basis of water quality assessment, the Okavango River, Namibia. Rev. Hydrobiol. Tropics 27, 361–387.

Hoffman, T., 2014. Changing patterns of rural land use and land cover in South Africa and their implications for land reform. J. South. Afr. Stud. 40, 707–725.

Holmes, M., Taylor, J.C., 2015. Diatoms as water quality indicators in the upper reaches of the Great Fish River, Eastern Cape, South Africa. Afr. J. Aquat. Sci. 40, 321–338.

Jacobsen, D., Cressa, C., Mathooko, J.M., Dudgeon, D., 2008. Macroinvertebrates, composition, life histories and production. In, Dudgeon D (ed.) Tropical Stream Ecology. Academic Press, USA. pp. 66–96.

Janse Van Vuuren, S., Taylor, J.C., 2015. Changes in the algal composition and water quality of the Sundays River, Karoo, South Africa, from source to estuary. Afr. J. Aquat. Sci. 40, 339–357.

Johnson, R.K., Hering, D., Furse, M.T., Verdonschot, P.F.M., 2006b. Indicators of ecological change, comparison of the early response of four organism groups to stress gradients. Hydrobiologia 566, 139–152.

Johnson, R.K., Hering, D., Furse, M.T., Clarke, R.T., 2006a. Detection of ecological change using multiple organism groups, metrics and uncertainty. Hydrobiologia 566, 115–137.

Karr, J.R., Yoder, C.O., 2004. Biological assessment and criteria improve total maximum daily load decision making. J. Environ. Eng. 130, 594–604.

Kasangaki, A., Babaasa, D., Efitre, J., McNeilage, A., Bitariho, R., 2006. Links between anthropogenic perturbations and benthic macroinvertebrate assemblages in Afromontane forest streams in Uganda. Hydrobiologia 563, 231–245.

Kearns, F.R., Kelly, N.M., Carter, J.L., Resh, V.H., 2005. A method for the use of landscape metrics in freshwater research and management. Landsc. Ecol. 20, 113–125.

Kleynhans, C.J., Schulz, G.W., Engelbrecht, J.S., Rousseau, F.J., 1992. The impact of a paper mill effluent spill on the fish populations of the Elands and Crocodile Rivers (Incomati System, Transvaal). Water SA 18, 73-80.

Kleynhans, C.J., 1999. The development of a fish index to assess the biological integrity of South African rivers. Water SA 25, 265–278.

Kleynhans, C.J., Thirion, C., Moolman, J., 2005. A Level I River Ecoregion classification System for South Africa, Lesotho and Swaziland. Report No. N/0000/00/REQ0104. Resource Quality Services, Department of Water Affairs and Forestry, Pretoria, South Africa

Kleynhans, C.J., Mackenzie, J., Louw, M.D., 2007. River Ecoclassification: Manual for Ecostatus Determination (Version 2) Module F: Riparian Vegetation Response Assessment Index (VEGRAI). WRC Report No. TT 329/08. Water Research Commission, Pretoria.

Li, H., You, S., Zhang, H., Zheng, W., Zou, L., 2018. Investigating the environmental quality deterioration and human health hazard caused by heating emissions. Sci. Total Environ. 628, 1209–1222.

Lobo, E.A., Callegaro, V.L., Hermany, G., Bes, D., Wetzel, C.E., Oliveirama., 2004. Use of epilithic diatoms as bioindicator from lotic systems in southern Brazil, with special emphasis on eutrophication. Acta Limnol. Bras. 16, 25–40.

Lowe, S., Dallas, H., Kennedy, M.P., Taylor, J.C., Gibbins, C., Lang, P., 2013. The SAFRASS biomonitoring scheme, general aspects, macrophytes (ZMTR) and benthic macroinvertebrates (ZISS) protocols. SAFRASS Deliverable Report to the African, Caribbean and Pacific Group of States (ACP Group), Science and Technology Programme, Contract No AFS/2009/219013. University of Glasgow, Glasgow.

Lyons, J., Navarro-Pèrez, S., Cochran, P., Santana, E., Guzman-Arroyo, M., 1995. Index of biotic integrity based on fish assemblages for the conservation of streams and rivers in West-central Mexico. Conserv. Biol. 9, 569–584.

Madrid, Y., Zayas, Z.P., 2007. Water sampling, Traditional methods and new approaches in water sampling strategy. Trends Anal. Chem. 26, 293–299.

Mahmoud, S.H., Gan, T.Y., 2018. Impact of anthropogenic climate change and human activities on environment and ecosystem services in arid regions. Sci. Total Environ. 633, 1329–1344.

Mambo, J., Archer, E., 2007. An assessment of land degradation in the Save catchment of Zimbabwe. Area 39, 380–391.

Mangadze, T., Bere, T., Mwedzi, T., 2016. Choice of biota in stream assessment and monitoring programmes in tropical streams, A comparison of diatoms, macroinvertebrates and fish. Ecol. Indic. 63, 128–143.

Mangadze, T., Wasserman, R.J., Dalu, T., 2017. Use of diatom communities as indicators of conductivity and ionic composition in a small Austral temperate river system. Water, Air Soil Pollut. 228, 428.

Masese, F.O., Omukoto, J.O., Nyakeya, K., 2013. Biomonitoring as a prerequisite for sustainable water resources, a review of current status, opportunities and challenges to scaling up in east Africa. Ecohydrol. Hydrobiol. 13, 173–191.

Mensah, P.K., Palmer, C.G., Muller, W.J., 2012. Lipid peroxidation in the freshwater shrimp Caridinaniloticaas a biomarker of Roundup herbicide pollution of freshwater systems in South Africa. Water Sci. Technol. 65, 1660–1666.

Minaya, V., McClain, M.E., Moog, O., Omengo, F., Singer, G.A., 2013. Scale-dependent effects of rural activities on benthic macroinvertebrates and physico-chemical characteristics in headwater streams of the Mara River, Kenya. Ecol. Indic. 32, 116–122.

Moser, P.M., 2006. Regeneration and utilization of Faidherbia albida and Acacia erioloba along ephemeral rivers of Namibia. PhD dissertation. Ecol. Dev. Series No 42. Cuvillier Verlag, Göttingen.

Moore, I., Murphy, K.J., 2013. Testing a new invertebrate-based river biomonitoring scheme for tropical rivers in Africa. The 14th Congress of the Brasil Limnological Society, Bonito, Brasil.

Morrison, G., Fatoki, O.S., Persson, L., Ekberg, A., 2001. Assessment of the impact of point source pollution from the Keiskammahoeksewage treatment plant on the Keiskamma River - pH, electrical conductivity, oxygen- demanding substance (COD) and nutrients. Water SA 27, 475–480.

Moyo, N.A.G., Phiri, C., 2002. The degradation of an urban stream in Harare, Zimbabwe. Afr. J. Ecol. 40, 401–404.

Muthimkhulu, S., Dallas, H., Day, J., Hoko, Z., 2005. Biological assessment of the state of the water quality in the Mbuluzi River, Swaziland. In, Mathews K, Nhaphi I (eds) Water and Wastewater Management for Developing Countries. Selected proceedings of the Water and Wastewater Management for Developing Countries Conference, Zimbabwe. IWA Publishing London, UK.

Mwedzi, T., Bere, T., Mangadze, T., 2016. Macroinvertebrate assemblages in agricultural, mining, and urban tropical streams, implications for conservation and management. Environ. Sci. Pollut. Res. 23, 11181–11192.

Ndebele-Murisa, M.R., 2012. Biological monitoring and pollution assessment of the Mukuvisi River, Harare, Zimbabwe. Lakes Reserv. Res. Manag. 17, 73–80.

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. Biodivers. Conserv. 15, 3267–3293.

Nel, J.L., Driver, A., Strydom, W., Maherry, A., Petersen, C., Hill, L., Roux, D.J., Nienabe, S., Van Deventer, H., Swartz, E., Smith-Adao., 2011. Atlas of Freshwater Ecosystem Priority Areas in South

Africa, Maps to Support Sustainable Development of Water Resources. WRC Report NoTT 500/11.Water Resources Commission, Pretoria.

Nhiwatiwa, T., Dalu, T., Brendonck, L., 2017a. Impact of irrigation-based sugarcane cultivation on the Chiredzi and Runde Rivers quality, Zimbabwe. Sci. Total Environ. 587–588, 316–325.

Nhiwatiwa, T., Sithole, T., Dalu, T., 2017b. Assessment of river quality in a subtropical Austral river system, a combined approach using benthic diatoms and macroinvertebrates. Appl. Water Sci. 7, 4785–4792.

Obakeng O.T., 2007. Soil moisture dynamics and evapotranspiration at the fringe of the Botswana Kalahari, with emphasis on deep rooting vegetation, PhD Dissertation No. 141, University of Twente (ITC), The Netherlands.

Oberholster, P.J., Ashton, P.J., 2008. State of the Nation Report, An Overview of the Current Status of Water Quality and Eutrophication in South African Rivers and Reservoirs Parliamentary Grant Deliverable. Council for Scientific and Industrial Research (CSIR), Pretoria.

Palmer, R.W., Taylor, E.D., 2004. The Namibian Scoring System (NASS) Version 2 rapid bio-assessment method for rivers. Afr. J. Aquat. Sci. 29, 229–234.

Paul, M.J., Meyer, J.L., 2001. Streams in the urban landscape. Annu. Rev. Ecol. Systemat. 32, 333–365.

Pipp, E., 2002. A regional diatom-based trophic state indication system for running water sites in upper Austria and its overregional applicability. Ver. Int. Ver. Limnol. 27, 3376–3380.

Phiri, C., Day, J., Chimbari, M., Dhlomo, E., 2007. Epiphytic diatoms associated with a submerged macrophyte, Vallisneria aethiopica, in the shallow marginal areas of Sanyati Basin (Lake Kariba), a preliminary assessment of their use as biomonitoring tools. Aquat. Ecol. 41, 169–181.

River Health Programme. State-of-Rivers Report: Monitoring and Managing the Ecological State of Rivers in the Crocodile (West) Marico Water Management Area. Department of Environmental Affairs and Tourism, Pretoria, 2005.

Resh, VH., 2008. Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. Environ. Monit. Assess. 138, 131–138.

Rocha, A.A., 1992. Algae as indicators of water pollution. In, Cordeiro-Marino M, Azevedo MTP, Santána CL, Tomita NY, Pastino EM (eds) Algae and Environment, A General Approach. Sociedade Brasileira de Ficologia, CETESB, São Paulo.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, R., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke,L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D.,Mooney, H. A., Oesterheld, M., Poff, N. L., Sykes, M. T.,Walker, B. H., Walker, M. & Wall, D. H. (2000). Global Biodiversity Scenarios For The Year 2100. Science 287, 1770–1774.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature 413, 591–596.

Schachtschneider, K., February, E.C., 2010. Water Sources for trees along the ephemeral Kuiseb River in Namibia differ for species and age groups. J. Arid Environ. 74, 1632–1637.

Schmeller, D.S., Loyau, A., Bao, K., Brack, W., Chatzinotas, A., De Vleeschouwer, F., Friesen, J., Gandois, L., Hansson, S.V., Haver, M., Le Roux, G., 2018. People, pollution and pathogens–Global change impacts in mountain freshwater ecosystems. Sci. Total Environ. 622, 756–763.

Schoeman, J.J., Steyn, A., 2003. Nitrate removal with reverse osmosis in a rural area in South Africa. Desalination 155, 15–26.

Simaika, J.P., Samways, M.J., 2010. Large-scale estimators of threatened freshwater catchment species relative to practical conservation management. Biol. Conserv. 143, 311–320.

Simon, T.P., Lyons, J., 1995. Application of the index of biotic integrity to evaluate water resource integrity in freshwater ecosystems In, Davis, WS, Simon, TP (eds), Biological Assessment and Criteria Tools for Water Resource Planning and Decision Making. Lewis Press, Boca Raton, FL.

Sponseller, R.A., Benfield, E.F., Valett, H.M., 2001). Relationships between land-use, spatial scale and stream macroinvertebrate communities. Freshw. Biol. 46, 1409–1424.

Stevenson, J., 2014. Ecological assessments with algae: a review and synthesis. J. Phycol. 50, 437–461

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

Taylor, J.C., Pope, N., Van Rensburg, L., 2009. Community participation in river monitoring using diatoms, a case study from the Buffelspoort Valley. Afr. J. Aquat. Sci. 34, 173–182.

Taylor, J.C., Harding, W.R., Archibald, C.G.M., 2007c. An Illustrated Guide to Some Common Diatom Species from South Africa. WRC Report No TT 282/07 Water Research Commission, Pretoria.

Taylor, J.C., Prygiel, J., Vosloo, A., Pieter, A., Rey, D., Ransburg, L.V., 2007a. 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 592, 455–464.

Tumbare, M.J., 2004. The Zambezi River, Its threats and opportunities the Zambezi River, Its threats and opportunities. The 7th River Symposium Brisbane, Australia.

UN-Water Africa., 2003. The Africa Water Vision for 2025, Equitable and Sustainable Use of Water for Socioeconomic Development. Economic Commission for Africa, Addis Ababa, Ethiopia.

Vitousek, P.M., Aber, J., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, G.D., 1997. Human alteration of the global nitrogen cycle causes and consequences. Ecol. Appl. 7, 737–750.

Vollmer, D., Shaad, K., Souter, N.J., Farrell, T., Dudgeon, D., Sullivan, C.A., Fauconnier, I., MacDonald, G.M., McCartney, M.P., Power, A.G., McNally, A., 2018. Integrating the social, hydrological and ecological dimensions of freshwater health: The Freshwater Health Index. Sci. Total Environ. 627, 304–313.

Walsh, C.J., 2000. Urban impacts on the ecology of receiving waters, a framework for assessment, conservation and restoration. Hydrobiologia 431, 107–114.

Witte, F., Goldschmidt, T., Wanink, J.H., Oijen, M.V., Goudswaard, K., Witte-Maas, E., Bouton, N., 1992. The destruction of an endemic species flock, quantitative data on the decline of the haplochromine cichlids of Lake Victoria. Environ. Biol. Fishes 34, 1–28.

Yount, J.D., Niemi, G.J., 1990. Recovery of lotic communities and ecosystems from disturbance- A narrative review of case studies. Environ. Manag. 14, 547–569.

Ziglio, G., Siligardi, M., Flaim, G., 2006. Biological monitoring of rivers, application and perspectives. In, Ziglio G, Siligardi M, Flaim G (eds) Water Quality Measurement. John Wiley and Sons, Chichester, UK.

# Chapter 3: Macroinvertebrate functional feeding group alterations in response to habitat degradation of headwater Austral streams

* This chapter has been published as: **Mangadze, T**., Wasserman, R.J., Froneman, P.W., Dalu T., 2019. Macroinvertebrate functional feeding group alterations in response to habitat degradation of headwater Austral streams. *Science of the Total Environment,* 695, 133910.

**Abstract:** Protecting the structural and functional integrity of lotic ecosystems is becoming increasingly important as many ecological systems face escalating pressures from human population growth and environmental impacts. Knowledge on the functional composition of macroinvertebrates in austral temperate streams is generally lacking hindering the design and implementation of water management and restoration goals. Therefore, this study examined the effects of urban land–use activities on the benthic macroinvertebrate functional feeding guild structure among different stream orders in an austral river system (Bloukrans River) in the Eastern Cape Province of South Africa. Water quality and macroinvertebrate community data were collected across two seasons from 18 sites in two different stream order categories (i.e. 1, 2+3), following standard methods. Macroinvertebrates were separated into functional feeding groups (FFGs), which were then used to assess the effects of riparian condition on FFG organization. Linear mixed effects model (LMM) results demonstrated that total dissolved solids (TDS), canopy cover, phosphate and channel width were the key variables that described the major sources of variation in macroinvertebrate FFGs. Based on FFG proportions, collector–gatherers were the most abundant in the Bloukrans River and represented 71.3% of the macroinvertebrate assemblages. The FFG ratios indicated that all the eighteen sites were strongly heterotrophic (i.e. streams received additional sources of energy from leaf litter and other organic matter), showed below expected linkage with riparian input and stable substrates were limited. The FFG ratios offered some insights into the overall functioning of Bloukrans River system. Overall, this chapter highlights the importance of including macroinvertebrate

functional diversity as a complementary approach to assess the ecological integrity in management and restoration plans of river systems.

**Keywords:** Land–use, water quality, biological indices, functional feeding groups, Bloukrans River, macroinvertebrates

### 3.1 Introduction

Human activities, such as agriculture, urbanisation, and industrialisation, threaten and/or impair stream ecosystem functioning (including protected areas) worldwide (Feld, 2013; Santos et al., 2015; Li et al., 2018; Mahmoud and Gan, 2018; Fernandes et al., 2019). In developing countries, the increased production of semi or untreated sewage effluent linked to urbanisation and the extensive utilisation of fertilisers in farming within watersheds has led to the overfilling of receiving water bodies with allochthonous nutrients, mainly nitrates and phosphates (Ndiritu et al., 2006; de la Rey et al., 2008; Gutiérrez–Cánovas et al., 2019; Dalu et al., 2019). Land-use conversation (e.g. forest to urban) has further limited the role of riparian environments as biological buffers against chemicals, sediment, and nutrients from adjacent land-use areas (Valle Junior et al., 2015; Pacheco and Fernandes, 2016; Fierro et al., 2017; Mangadze et al., 2019). Newbold et al. (1980) highlighted the implications that degradation of riparian vegetation can have on physical properties of lotic systems. Moreover, alterations in the adjacent riparian vegetation can modify the complex biotic and abiotic processes that direct the function and characteristics of aquatic communities such as macroinvertebrates (Utz et al., 2009).

Benthic macroinvertebrate assemblages have been widely used to quantify ecological impacts of urbanisation by examining changes in functional feeding guilds along with species richness and tolerance to pollution (Gutiérrez-Cánovas et al., 2013; Fierro et al., 2017; Moyo and Richoux, 2017). For example, Miserenduno and Masi (2010), observed significant differences between shredders and collector gatherers biomass in Patagonian streams under different land uses (pasture conversion, native, or exotic vegetation and urbanisation). Similarly, Fu et al. (2015) found that shredder and predator abundances were significantly lower in urban streams of Dongjiang River basin, China. Various ratios of functional groups have also been successfully used as ecosystem attributes in southern Appalachian streams (Stone and Wallace, 1998; Wagner, 2001). Moreover, Berthon et al. (2011) lists several advantages of using functional based approach in water quality assessment. These include among others: the technique does not involve finer species-level taxonomy which can be demanding; functional group metrices have similarly performed well as metrices for species-level identification.

Although the use of FFGs has gained attention in other regions of the world (e.g. Rosi–Marshall and Wallace, 2002; Jiang et al., 2011; Imberger et al., 2014), little is known about how FFG structures are altered by different river water qualities influenced by anthropogenic activities in austral temperate river systems. There is also very little known about FFG structures in temporary rivers that are artificially made permanent through supplemental flow. Furthermore, due to lack of taxonomic information on macroinvertebrates in developing countries, FFG classifications are often based on temperate-zone keys (Farrel et al., 2015). Differences in environmental conditions may result in macroinvertebrates changing their mode of feeding to adapt in the different environments (Tomanova et al., 2006). Therefore, clarification of ecological requirements of endemic macroinvertebrate functional groups is required to develop FFG classification unique to the region.

The present study focused on the effects of urban land-use induced changes in water quality on macroinvertebrate functional feeding guild structure in the Bloukrans River, a highly impacted river in the Eastern Cape Province of South Africa. The river is subject to different land-use practices i.e. agricultural, urban, open canopy, forest sites and private game reserves within its catchment area and has been the focus of previous investigations which showed strong evidence that the ecological integrity of river is being threatened (e.g. Muskett et al., 2016; Odume and Mgada, 2016; Dalu et al., 2017b; Nhiwatiwa et al., 2017). The consequences that these human activities are having on macroinvertebrate functional structure of the endemic biota are not clearly known.

The aim of this study was to assess the effects of urban land–use on the benthic macroinvertebrates functional feeding guild structure in the Bloukrans River system located in the Eastern Cape Province of South Africa. It is hypothesized that the proportion of shredders and predators will decrease with changes in water quality as a result of the surrounding land-use patterns. The outcomes from this study can contribute to our understanding on aspects of organic matter processing, energy flow and trophic relationships, with implications for management activities essential for local biomonitoring programmes.

## 3.2 Materials and methods

### *3.2.1 Study area*

The study was conducted in the Bloukrans River system in the Eastern Cape Province, South Africa (Figure 3.1). Average annual precipitation in this area is generally < 650 mm, with the peak in rainfall occuring in summer (November to April). The air temperatures range between 1.5 and 43 °C. Site selection was done by selecting all probable access points (bridges and road networks) to perennial rivers using topographical maps (1:50000) and then picking stratified random samples ($n$ = 18; Figure 3.1) basing on methods by Barbour et al. (1999). The criterion for picking sampling sites was to evaluate the influence of urban land–use activities on functional feeding groups (FFGs). Catchment delineation was based on stream size whereby sampling sites were picked based on the relative abundance of the diverse stream order categories (Strahler 1957) in the basin. A total of 18 sites were sampled i.e. 13 first order (less impacted, $n$ = 4; and moderately impacted urban streams, $n$ = 9), 2 s order and 3 third–order streams/rivers (highly impacted downstream areas). The less impacted stream order 1 sites were located in the upper forested reaches of the catchment with intact riparian vegetation and no wastewater discharge. The moderately impacted stream order 1 sites were located in the middle reaches of the catchment in moderately polluted urban area with sites in both urban and forested catchments but located in urban areas. Finally, highly polluted stream order 2 and 3 sites were located within the urban area with degraded and eroded riverbanks linked to point and diffuse sources of pollution such as industrial and municipal wastewater discharge. Since the numbers of stream order 2 and 3 were low, these sites were combined and categorised as stream order 2/3. Samples were collected on two occasions, once in February (rainy season, high flow) and July (dry season, low flow) 2016.

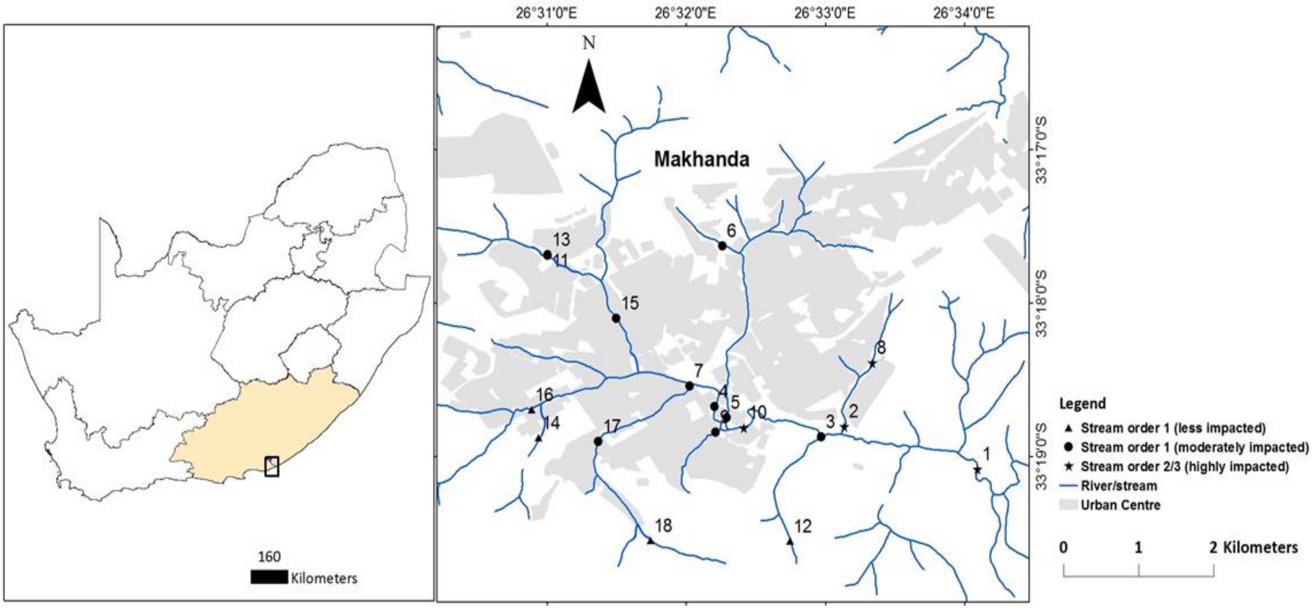


**Figure 3.1:** The location of the study area and sampling sites.

### *3.2.2 Water sampling and analysis*

The physical and chemical variables analysed at each site were pH, total dissolved solids (TDS), dissolved oxygen (DO), conductivity, salinity, nitrate, phosphate, water temperature, channel width, depth, embeddedness and water flow. At each site, water temperature, electrical conductivity, salinity, pH, total dissolved solids (TDS) and turbidity were measured in the field using a multi-parameter water quality probe (PCTestr 35, Eutech/Oakton Instruments, Singapore) and a portable turbimeter (AL250T–IR, Aqualytic, Germany). DO meter (DO 850045, Per Scientific, Taiwan) was used to measure dissolved oxygen (DO) levels. Channel width and river flow velocities were measured with a tape measure and Flo–mate portable flowmeter Model 2000 (Marsh McBirney, Maryland, US), respectively. Depth was determined using a graduated measuring rod. River substrate embeddedness was estimated according to Platts et al. (1983). Integrated water samples were collected at each site from the two coastal zone boundaries and main channel centre to determine nitrate and phosphate levels. Triplicate water samples (500 ml) were collected at each site and placed on ice in the dark awaiting further nutrient (phosphates and nitrates) processing in the laboratory. In the laboratory, nitrate and phosphate concentrations employed as proxies for land–use activities were analysed using an HI 83203 multiparameter bench photometer (Hanna Instruments Inc., Rhode Island) within 12 h of collection.

### *3.2.3 Macroinvertebrate sampling*

Benthic macroinvertebrates sampling followed the South African Scoring System version 5 protocol (SASS5; Dickens and Graham, 2002). Macroinvertebrates were mainly quantitatively sampled at riffle habitats using a hand–held kick net (dimension 30 × 30 cm, mesh size 500 μm, 1.5 m handle, 3 replicates per site). Where available, all other key habitats recognized by Dickens and Graham (2002) were sampled i.e. marginal vegetation and pools. Samples collected were then pooled together, and the macroinvertebrates were identified to family level excluding Oligochaeta and Hirudinea which were identified to class level following studies by Gerber and Gabriel (2002). Macroinvertebrate families that could not be identified in the field were preserved in 70 % ethanol for subsequent identification in the laboratory. Functional feeding groups of macroinvertebrate taxon were placed into an FFG class based on studies by Merrit and Cummins (1996a), Cummins et al. (2005) and Merrit et al. (2008). Functional feeding group (FFG) categories employed in this study were: collector–gatherer, collector–filterer, scraper, shredder, and predator.

### *3.2.4 Statistical analysis*

To assess the differences in environmental variables among the two stream order categories and sampling periods (summer and winter), a linear mixed-effects (LMM) model was employed, with season and stream order category as fixed effects and site as a random effect (Pinheiro et al., 2012). Model selection was conducted using the Akaike Information Criteria (AIC) with maximum likelihood estimation (Pinheiro and Bates, 2000). After testing for normality and homoscedasticity of residuals for each response variable, all the data was log-transformed before analysis to meet the assumptions. The analysis was performed using the '*nlme*' and '*effects*' packages in R statistical package version R 3.2.3 software (R Core Team, 2014).

The percentage contribution of each FFG to the different communities was determined per stream order category i.e. stream order 1 sites (less impacted and moderately impacted) and stream order 2/3 (highly impacted) sites. Since some macroinvertebrate taxa have different trophic roles, taxa with more than one FFG classification were divided equally among the possible functional groups before the proportions were determined (e.g. four baetids were calculated as 2 scrapers and 2 collector–gatherers) (Dudgeon, 1994). As changes in water quality of lotic systems as a result of the surrounding land-use patterns will affect the resulting nutritional resource base of river systems and impact relative abundances of the FFGs (Cummins et al., 2005), important information about ecosystem function relating to changes in basal resources can be captured using FFG ratios as surrogates (Wagner, 2001; Cummins et al., 2005). Therefore, we used FFG ratios to estimate ecosystem parameters in the Bloukrans River (Table 3.1). FFG metrics for gross primary production as a proportion of community respiration i.e. production/respiration ratio (P/R), the degree of top-down control of predators on prey i.e. predator/prey ratio (P/P), the ratio of shredders to total collectors (Σ (shredders) / Σ (collector filters and gatherers)) as a proxy to estimate the coarse particulate organic matter/ fine particulate organic matter (CPOM/FPOM) and streambed stability (channel stability ratio) were calculated for the two stream order categories. The FFG ratios relative to the proposed threshold values are given in Table 3.1.

**Table 3.1:** Functional feeding group ratios as indicators of stream ecosystem attributes (from Cummins et al., 2005).

| **Metric** | **Metric definition** | **Expected response** |
|---|---|---|
| Autotrophy to heterotrophy index (P/R) | Scrapers to shredders + total collectors | Autotrophic > 0.75 |
| Coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) index (CPOM/FPOM) | Shredders to total collectors | Normal shredder association linked to functioning riparian zone > 0.25 |
| Substrate (channel) stability | Scrapers + filtering collectors to shredders + gathering collectors | Stable substrates (e.g., cobbles, boulders, large woody debris, rooted vascular plants) plentiful > 0.50 |
| Predator prey ratio (P/P) | Predators to total of all other functional groups | < 0.15 indicates a normal predator/prey ratio |

Non–metric multidimensional scaling (*n*–MDS) was performed on a Bray–Curtis (BC) dissimilarity matrix, computed from square root transformed macroinvertebrate FFG abundance data (Legendre and Legendre, 1998; Anderson, 2006). The *n*–MDS is a strong non–

parametric ordination technique for datasets with modest sample sizes and a large number of variables with the BC dissimilarity being the most commonly used dissimilarity measure for abundance data in ecology (Legendre and Legendre, 1998; Clarke and Warwick, 2001). The *n*–MDS was analysed using Palaeontological Statistics Software (PAST) Version 2.16 (Hammer et al., 2012).

Principal component analysis (PCA) was used to select environmental variables that describe the major sources of variation in macroinvertebrate functional groups while minimizing redundancy. The PCA was based on the Pearson's correlation matrix (i.e. centred and standardised to ensure that all variables contributed equally). Six variables (DO, temperature, phosphate, TDS, channel width and canopy cover) were selected based on inspection of their loadings with respect to first and second axis, after screening for collinearity through inspection of the correlation matrix. Only the variable with the highest loading was selected for correlated variables ($r > 0.8$). PCA analysis was carried out using SPSS (IBM Corp., 2011). Linear mixed effects models (LMMs, *lme* function within *nlme* package, Pinheiro et al., 2012) were then used to assess the relationship between FFG proportions and significant environmental variables from the PCA. Sampling site was included as a random effect to account for potential non-independence due to repeated measures at each site through time.

## 3.3 Results

### *3.3.1 Water quality*

The mean and standard deviation values of the physico–chemical variables recorded for the stream order categories over the two seasons (summer and winter) are presented in Table 3.2. Nitrate and TDS concentrations were generally high in all the stream order categories over the two seasons (Table 3.2), while phosphate concentrations were elevated in stream order 2/3 sites for both seasons (Table 3.2). Turbidity, salinity and conductivity were similar for the two seasons across the catchment (Table 3.2), with the stream order 2/3 sites being higher than the stream order 1 less impacted sites (Table 3.2). The linear mixed effects model (LMM) results showed significant seasonal differences for temperature, phosphate, DO, SOM and SOC while significant sampling order category variation was observed for phosphate, pH, DO and channel width (Table 3.3).

**Table 3.2:** Mean variation in water column and physical variables (± standard deviation) over two seasons for the two sampling order categories. Abbreviations: TDS – total dissolved solids, DO – dissolved oxygen.

| Variable | Stream order 1 | | | | Stream order 2/3 | |
|---|---|---|---|---|---|---|
| | Less impacted | | Moderately impacted | | Highly impacted | |
| | Winter | Summer | Winter | Summer | Winter | Summer |
| *Water Chemistry* | | | | | | |
| Temperature (°C) | 12.96 ± 2.21 | 18.45 ± 0.56 | 13.52 ± 1.96 | 19.46 ± 0.83 | 14.24 ± 1.4 | 19.46 ± 0.72 |
| Conductivity (µS $cm^{-1}$) | 1994.25 ± 1866.25 | 1274.58 ± 1480.4 | 1012.26 ± 540.36 | 1187.41 ± 584.3 | 2048.47 ± 409.74 | 1761.47 ± 490.53 |
| DO (mg $L^{-1}$) | 9.12 ± 0.45 | 5.8 ± 2.17 | 6.38 ± 1.63 | 4.91 ± 2.59 | 6.35 ± 1.51 | 3.91 ± 2.78 |
| Turbidity (mg $L^{-1}$) | 8.2 ± 3.6 | 16.74 ± 15.1 | 23.46 ± 23.48 | 24.62 ± 20.02 | 14.6 ± 8.29 | 16.18 ± 7.25 |
| pH | 7.63 ± 0.49 | 7.31 ± 0.77 | 7.8 ± 0.35 | 7.91 ± 0.45 | 8.02 ± 0.17 | 8.01 ± 0.28 |
| Salinity (mg $L^{-1}$) | 1035.5 ± 1538.08 | 1005.92 ± 16.51 | 494.59 ± 270.03 | 589.11 ± 300.26 | 999.07 ± 173.98 | 807.73 ± 180.37 |
| TDS (mg $L^{-1}$) | 722.7 ± 382.43 | 869.52 ± 439.43 | 1440.25 ± 1079.46 | 1358.75 ± 1183.72 | 1461.87 ± 286.76 | 1250.87 ± 344.25 |
| Nitrate (mg $L^{-1}$) | 23.28 ± 37.04 | 12.83 ± 13.13 | 61.51 ± 78.5 | 18.12 ± 19.58 | 78.5 ± 62.19 | 53 ± 80.86 |
| Phosphate (mg $L^{-1}$) | 0.8 ± 0.32 | 0.73 ± 0.81 | 2.84 ± 2.35 | 7.2 ± 7.67 | 4.34 ± 3.33 | 8.14 ± 4.34 |
| *Hydromorphology* | | | | | | |
| Channel width (m) | 1.08 ± 0.58 | 0.48 ± 0.29 | 0.6 ± 0.36 | 0.87 ± 0.48 | 1.87 ± 1.09 | 1.95 ± 1.43 |
| Water flow (m $s^{-1}$) | 0.17 ± 0.09 | 0.07 ± 0.02 | 0.19 ± 0.17 | 0.2 ± 0.16 | 0.43 ± 0.65 | 0.44 ± 0.37 |
| Water depth (m) | 0.09 ± 0.07 | 0.08 ± 0.05 | 0.09 ± 0.07 | 0.1 ± 0.06 | 0.08 ± 0.05 | 0.12 ± 0.04 |
| Canopy cover (%) | 0.19 ± 0.22 | 0.54 ± 0.43 | 0.31 ± 0.4 | 0.32 ± 0.4 | 0.28 ± 0.35 | 0.25 ± 0.33 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Embeddedness | 1.75 ± 1.5 | 1.25 ± 0.5 | 1.67 ± 1 | 3 ± 1.94 | 2.4 ± 1.67 | 2.6 ± 2.19 |
| *Organic matter content* | | | | | | |
| Sediment organic matter (%) | 39.19 ± 25.15 | 11.71 ± 11.09 | 15.2 ± 12.58 | 5.38 ± 3.06 | 19.23 ± 7.88 | 2.71 ± 1.41 |
| Sediment organic carbon (%) | 22.73 ± 14.59 | 6.79 ± 6.43 | 8.82 ± 7.3 | 3.12 ± 1.78 | 11.15 ± 4.57 | 1.57 ± 0.82 |

**Table 3.3:** Results of linear mixed-effects model (LMM) for each response variable, with season and stream order categories as fixed factors and site as random effect.

| Variables | Season | | Stream Order | |
|---|---|---|---|---|
| | ***t*-value** | ***p*-value** | ***t*-value** | ***p*-value** |
| *Water Chemistry* | | | | |
| Temperature | 11.87 | 0.01 | 0.92 | 0.36 |
| Conductivity | 0.58 | 0.56 | 0.64 | 0.53 |
| Dissolved oxygen | -4.2 | 0.01 | -5.24 | 0.01 |
| Turbidity | 0.78 | 0.44 | 1.56 | 0.13 |
| pH | -0.12 | 0.9 | 2.51 | 0.12 |
| Salinity | 0.10 | 0.92 | 1.25 | 0.22 |
| Total dissolved solids | -0.03 | 0.98 | 1.25 | 0.22 |
| Nitrate | -1.69 | 0.09 | 1.94 | 0.06 |
| Phosphate | -3.08 | 0.01 | 5.19 | 0.01 |
| *Hydromorphology* | | | | |
| Water flow | 0.09 | 0.93 | 1.52 | 0.13 |
| Channel width | -0.14 | 0.89 | 2.13 | 0.05 |
| Water depth | 0.64 | 0.52 | -0.89 | 0.37 |

### *3.3.2 Macroinvertebrate FFG structure–environmental relationships*

A total of 25 macroinvertebrate families, representing 8 orders were identified across the sampling site categories over the two seasons (Table 3.4). Two classes were also recorded i.e. Oligochaeta and Hirudinea which were not ranked beyond class level. The total number of families among the sampling sites ranged between 2 and 16 during the study period. Macroinvertebrate FFG structure among sampling site categories in the two seasons varied significantly between stream order categories (Figure 3.2). From the PCA, the first two principle components explained 71.92% of the total variance. The first component (PC1) explained 56.82% of total variance and had strong positive loading (> 0.75) of TDS, phosphate, channel width and canopy cover while moderate positive loading (0.5–0.75) of DO (Table 3.5). Second component (PC2) explained 15.1 % of the total variance and had moderate positive loading (0.5–0.75) of temperature and DO (Table 3.5).

**Table 3.4:** The functional feeding groups (FFG)—predators (P), collector–gatherers (CG), collector–filterers (CF), shredders (SH), and scrapers (SC)—assigned to the genera of aquatic macroinvertebrates analysed in the present study. L – Larvae, A – Adult.

| **Order/family** | **Functional Feeding Group** |
|---|---|
| **Annelida** | |
| Hirudinae | PR |
| Oligochaetae | CG |
| **Coleoptera** | |
| Gyrinidae | PR |
| Hydrophilidae | PR(L), generally CG (A) |
| Helodidae | SC |
| Potamonautidae | SH |
| **Diptera** | |
| Chironomidae | CG |
| Dixidae | CG |
| Psychodidae | CG |
| Simuliidae | CF |
| Sphaeriidae | CF |
| Tipuliidae | SH |
| **Ephemeroptera** | |
| Beatidae | CG; SC |
| Leptophlebidae | CG |
| **Tricoptera** | |
| Hydropsychidae | CF; SC |
| **Hemiptera** | |
| Belostomatidae | PR |
| Corixidae | PR; SC |
| Gerridae | PR |
| Notonectidae | PR |
| **Mollusca** | |
| Ancylidae | SC |
| Physidae | SC |
| **Odonata** | |
| Aeshnidae | PR |
| Coenagrionoidae | PR |
| Corduliidae | PR |
| Libellulidae | PR |

**Table 3.5:** Principle component analysis results for physico-chemical variables in the Bloukrans River. Bold and italic values indicate strong (> 0.75) and moderate (0.5–0.750) loadings, respectively.

| **Variables** | **Principle component** | |
|---|---|---|
| | **PC1** | **PC2** |
| Eigen value | 5.72 | 1.73 |
| Variance (%) | 56.82 | 15.1 |
| Cumulative variance | 56.82 | 71.92 |
| | *PCA loading* | |
| *Water chemistry* | | |
| Temperature | -0.04 | **0.72** |
| Conductivity | 0.07 | 0.13 |
| Dissolved oxygen | *0.53* | *0.55* |
| Turbidity | 0.43 | 0.27 |
| pH | 0.06 | 0.02 |
| Salinity | 0.43 | 0.26 |
| Total dissolved solids | **0.93** | 0.41 |
| Nitrate | 0.14 | 0.08 |
| Phosphate | **0.84** | 0.43 |
| *Hydromorphology* | | |
| Width | **0.88** | 0.27 |
| Flow | 0.47 | 0.21 |
| Depth | 0.14 | 0.64 |
| Canopy cover | **0.77** | 0.26 |
| Embeddedness | 0.43 | 0.21 |
| *Organic matter content* | | |
| Sediment organic matter | 0.47 | 0.26 |
| Sediment organic carbon | 0.43 | 0.21 |

Of the six predictor variables included in the LMM model, only four (phosphate, TDS, channel width and canopy cover) had significant influence on FFG proportions (Table 3.6). Increase in TDS resulted in an increase in the proportion of scrapers, predators and collector gatherers ($p < 0.05$ in all cases). High phosphate concentrations increased the proportion of collector gatherers ($p < 0.001$). Increase in channel width led to an increase in scrapers, collector gatherers and filterers ($p < 0.05$ in all cases). High canopy cover resulted in an increase in the proportion of shredders ($p < 0.05$).

**Table 3.6:** Results from linear mixed effects models including macroinvertebrate functional feeding group (FFG) proportion as dependent variable, with the physico-chemical parameters as independent variables and site included as a random effect.

| Variable | Estimate | Standard error | *t*-value | *p*-value |
|---|---|---|---|---|
| **Proportion of Collector gatherers** | | | | |
| Phosphate | 20.40 | 6.46 | 3.16 | 0.01 |
| Channel width | 118.85 | 39.34 | 3.02 | 0.01 |
| **Proportion of Collector filterers** | | | | |
| Channel width | 11.15 | 15.05 | 1.77 | 0.01 |
| **Proportion of Scrapers** | | | | |
| Channel width | 5.63 | 2.22 | 2.53 | 0.02 |
| Total dissolved solids | 6.85 | 4.08 | 1.87 | 0.05 |
| **Proportion of Predators** | | | | |
| Total dissolved solids | -0.01 | 0.02 | -2.74 | 0.05 |
| **Proportion of Shredders** | | | | |
| Canopy cover | 6.65 | 2.793 | 2.37 | 0.02 |

*3.3.3 Proportions and distribution of FFGs*

The most common FFG in the entire river were the collector–gatherers (71.3%; Figure 3.3) followed by collector–filters which were most abundant in the stream order 1 less impacted sites (44.2%). Stream order 1 less impacted sites also had high abundances of shredders (6.2%) and predators (14.9%) compared to the other stream order categories (Figure 3.3). The relative contributions of all FFGs for the stream order categories observed over two seasons is given in Table 3.7.

**Table 3.7:** Relative abundance (RA) of functional feeding groups (FFGs) of macroinvertebrates over two seasons for the sampling order categories (from stream order 1 sites (less impacted and moderately impacted) – stream order 2/3 highly impacted downstream sites). The functional feeding groups (FFG)—Filterers (CF), collector–gatherers (CG), predators (PR), scrapers (SC) and shredders (SH) —assigned to the families of aquatic macroinvertebrates analysed in the present study.

| **FFGs** | **Stream order1** | | | | **Stream order 2/3** | | **Total** |
|---|---|---|---|---|---|---|---|
| | **Less impacted** | | **Moderately impacted** | | **Highly impacted** | | |
| | **Winter** | **Summer** | **Winter** | **Summer** | **Winter** | **Summer** | |
| PR | 16 | 59 | 15 | 72 | 1 | 51 | 214 |
| FT | 96 | 152 | 586 | 165 | 113 | 98 | 1210 |
| SC | 15 | 50 | 110 | 11 | 39 | 61 | 286 |
| SH | 4 | 145 | 5 | 32 | 1 | 13 | 200 |
| CG | 116 | 962 | 1267 | 61 | 987 | 1358 | 4751 |
| Total | 247 | 1368 | 1983 | 341 | 1141 | 1581 | 6661 |
| RA (%) | 3.71 | 20.54 | 29.77 | 5.12 | 17.13 | 23.74 | 100 |

Based on the calculated ratios of the FFGs as surrogates of five ecosystem attributes (see Table 3.1), all stream site categories had P/R < 0.75 indicating that streams were heterotrophic (Table 3.8). All stream site categories also had CPOM/FPOM < 0.25 indicating low shredder abundance and poor linkage to riparian inputs. Whilst, the FFG ratio for channel stability showed that only stream order 1 less impacted sites in winter and moderately impacted sites in summer were above the expected threshold (Table 3.8). Predator–prey ratios (0.01–0.07) were normal for all stream site categories over the two seasons.

**Table 3.8:** Ratios of macroinvertebrate functional feeding group (FFG) analysis of collections from Bloukrans stream/river stream order categories. FFG (see Table 3.1): P/R = Production/Respiration ratio; P/P = Predator/Prey ratio; CPOM/FPOM = Coarse particulate organic matter/ fine particulate organic matter; channel habitat stability = scrapers + filtering collectors/gathering collectors + shredders.

| Ratios (FFGs) | Stream order 1 | | | | Stream order 2/3 | | Entire River |
|---|---|---|---|---|---|---|---|
| | Less impacted | | Moderately impacted | | Highly impacted | | |
| | Winter | Summer | Winter | Summer | Winter | Summer | |
| P/R | 0.07 | 0.04 | 0.06 | 0.04 | 0.04 | 0.04 | 0.03 |
| CPOM/FPOM | 0.02 | 0.01 | 0.01 | 0.14 | 0.01 | 0.01 | 0.02 |
| Channel Stability | 0.92 | 0.18 | 0.54 | 1.89 | 0.15 | 0.12 | 0.3 |
| P/P | 0.07 | 0.05 | 0.01 | 0.03 | 0.01 | 0.03 | 0.03 |

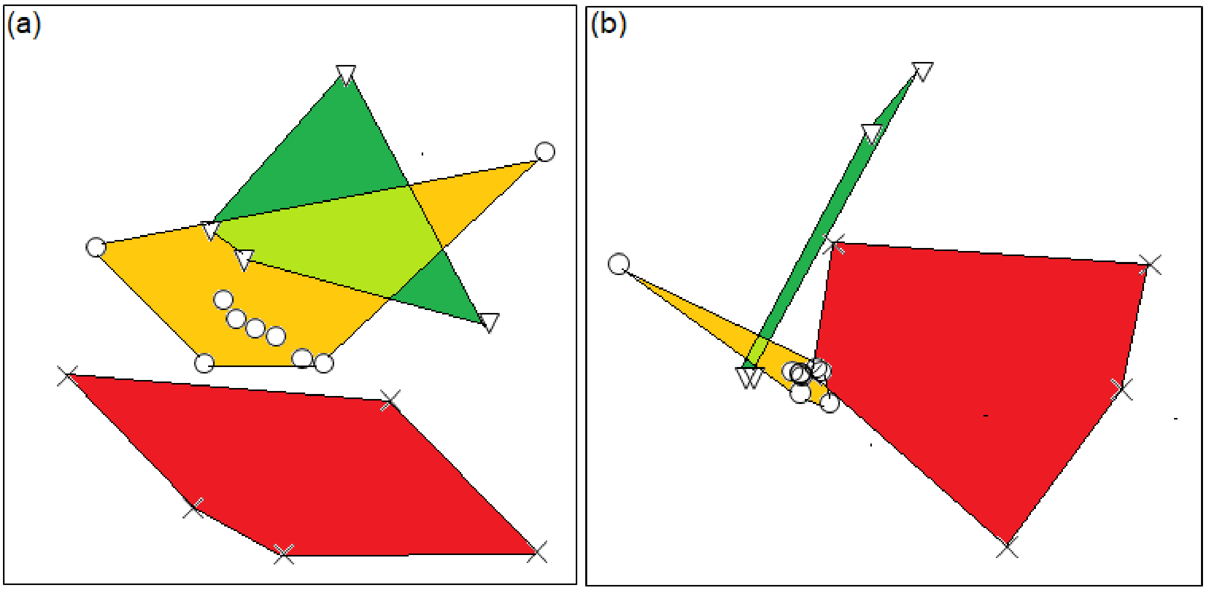


**Figure 3.2:** Two- dimensional *n*-MDS plot of macroinvertebrates functional feeding groups over two seasons **(a)** - winter season and **b) -** summer season in the Bloukrans river system. Symbols represent each stream order category: *crosshatch* stream order 2/3 sampling sites (red coloured eclipses), *down-triangle* stream order 1 less impacted sampling sites (green coloured eclipses) and *circle* stream order 1 moderately impacted sites (orange coloured eclipses).

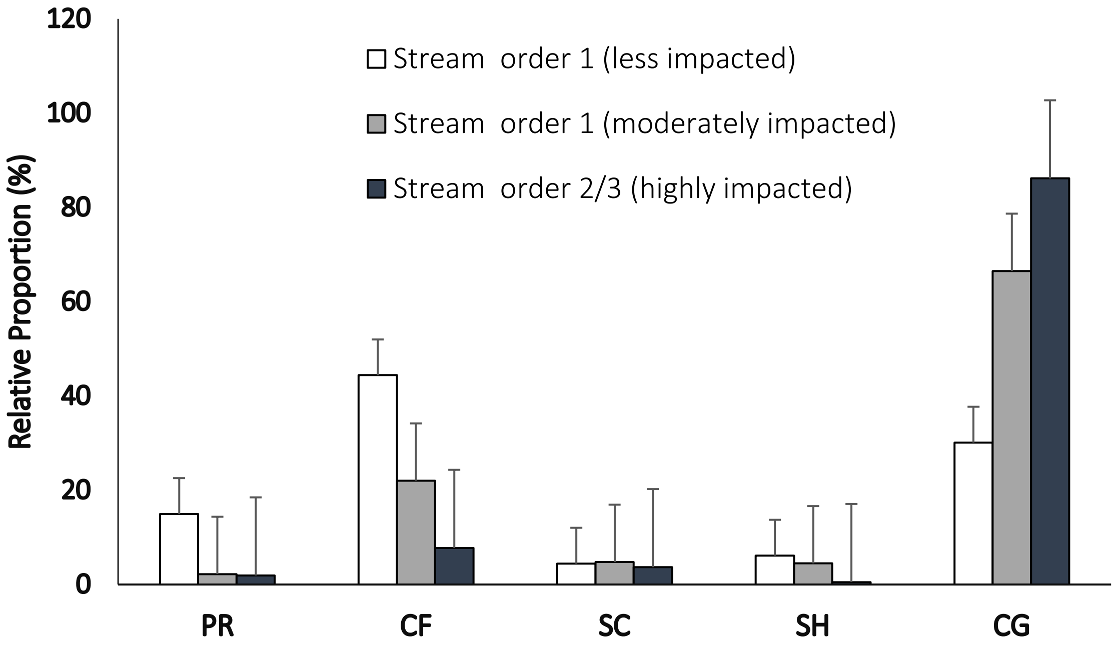


**Figure 3.3:** Proportions (%) (± SD) of different functional feeding groups for the sampling order categories (from stream order 1 sites (less impacted and moderately impacted) – stream order 2/3 downstream sites) in the Bloukrans River. The functional feeding groups (FFG)— Filterers (CF), collector–gatherers (CG), predators (PR), scrapers (SC) and shredders (SH) — assigned to the families of aquatic macroinvertebrates analysed in the present study.

## 3.4 Discussion

*3.4.1 Water quality*

The results of this study show that the spatial differences in physico–chemical variables can be ascribed to the ongoing human influence i.e. urban development as has been observed in other studies (Odume and Mgaba, 2016; Dalu et al., 2017a; Moyo and Richoux, 2017). During the winter season all the sampling sites had higher nitrate concentrations than that of the summer season (Table 3.2). This could be due to organic matter retention resulting from low flows. Moreover, small headwater streams are mainly susceptible to small land-use impacts and minor nutrient inputs in these headwater streams may exceed nutrient demands for benthic communities and lead to saturation of the systems (Weigelhofer, 2017; Dalu et al., 2019). Khan et al. (2017) also noted that there were constant changes in water quality variables, associated with land–use change. Unplanned urbanisation, intense agricultural activities and deforestation are positively linked with carbon, nitrogen and phosphorous related water quality parameters (Dalu et al., 2019). For that reason, recent studies in developed countries are now using spatial data and actual land use maps to plot environmental land use conflicts and classify areas regarding conflict levels based on land use types (e.g. Sanches Fernandes et al., 2018; Fernandes et al., 2019). These collective organic inputs of urban effects also have synergistic effects on water quality that potentially have significant impacts on macroinvertebrate functional feeding interactions and structures in rivers.

### *3.4.2 Functional feeding group (FFG) organisation– environmental relationships*

Macroinvertebrate FFG structure during this investigation closely followed the changes in contamination levels as a result of changing land–use patterns, with stream order 1 less impacted sites being linked with shredders compared to the other stream order categories. These findings are consistent with previous studies that confirmed the negative impacts of land-use change on stream physico-chemical variables and shredder communities (Miserenduno and Masi, 2010; Masese et al., 2014). Likewise, *n*–MDS analysis also reflected the impacts of land–use on macroinvertebrate functional feeding groups in the Bloukrans catchment (Figure 3.2). Other studies in Europe have also successfully used robust multivariate techniques in assessments of water quality and ecological integrity at catchment scale level (e.g. Ferreira et al., 2017; Sanches Fernandes et al., 2018).

Based on the LMM results, there were distinct differences in the environmental variables influencing macroinvertebrates FFGs of the Bloukrans River. The study highlights the significance of phosphate, TDS, channel width and canopy cover in structuring the distribution of macroinvertebrate FFGs. The proportion of scrapers, predators and collector gatherers increased with increases in TDS. Several other authors have also reported that elevated TDS levels have significant and strong correlations with biotic community structure change (Green et al., 2000; Pond et al., 2008). In previous research, conducted in the nearby Kowie River, Moyo and Richoux (2017) observed that the positive association between scrapers and total dissolved solids may be concomitantly related to variations in the algae assemblages that co–occur with changes in dissolved solids.

The proportion of shredders in this study was dependent upon high canopy cover (Table 3.6), indicating that riparian vegetation benefits their subsistence. In this study, the abundance of shredders was very low even in less impacted stream order 1 sites; however, abundances decreased further along the land-use gradient (Table 3.7). Other studies have attributed changes in the distribution and relative abundances of shredders to water temperature, conductivity, and litter input (Palumbo et al., 1987; Ferreira et al., 2012; Masese et al., 2014). Wallace et al. (2015) observed that degradation of riparian cover along urban stream channels can decrease shredder abundance by reducing leaf litter inputs. Shredders rely on allochthonous feeding resources and hence contribute much in the degradation of leaf material dropping into lotic systems from overhanging vegetation (Brasil et al., 2014). Therefore, limited presence of shredders can have a substantial effect on ecosystem function and can change the functioning of aquatic ecosystems through a trophic cascade effect (Chapin III et al., 2000). For example, in a study in North Carolina (USA), Baer et al. (2001) observed that secondary production in streams was reduced (> 70%) after the exclusion of litter. This reduction was associated with changes in the colonization dynamics of macroinvertebrates and detritus processing.

This study highlights the significance of increased phosphate concentrations on collector–gatherer abundance. The abundance of collector–gatherers in Bloukrans River was possibly linked to the increased levels of organic matter, which is enhanced by fine particulate allochthonous inputs of agricultural and urban land–use activities that generally offer significant nutritional value in the downstream areas (Dalu et al., 2017a). Collector–gatherers are generally tolerant to high levels of organic pollution (e.g. Oligocheata and Chironomidae) and have both physiological and physical adaptive structures that allow them to survive in oxygen depleted environments (Statzner and Bêche, 2010). Changes in stream size as shown by the channel width also reflected anthropogenic activities both at the reach and catchment scales. As such, these results showed that increases in the channel width led to an increase in the proportion of scrapers and collectors. Masese et al. (2014) also made a similar observation and noted that in-stream human and livestock activities also widened channels in open-canopy streams. Thus, the LMM results agree with previously reported studies, which demonstrated that environmental variables that are important for taxonomic diversity also impact the functional diversity in river systems.

### *3.4.3 Ecosystem attributes*

The low P/R ratios in the Bloukrans River during both seasons suggested the predominance of heterotrophy over autotrophic production. However, these results were not consistence with field observations as downstream areas (stream order 2/3 sites) had very little or no riparian vegetation and wide-open channels that allowed light penetration enhancing autotrophic production. Most open–canopy streams (i.e. stream order 1 moderately impacted and 2/3 sites) in Bloukrans River receive organic pollution from raw sewage spills from Makhanda and cattle waste (Tinotenda Mangadze, *personal field observation*). These streams were dominated by collector–gatherers (Oligochaetes and Chironomids), which reduced P/R ratios in these potentially autotrophic streams towards heterotrophy. Thus, low FFG ratios for channel stability in stream order 2/3 sites can likely also be attributed to the fact that these streams were extremely heterotrophic which would limit scrapers that are reliant on autotrophy in the form of non–filamentous algae.

There was evidence of weak top–down control (P/P < 0.2 for all stream order categories) along the entire profile of Bloukrans River. Likewise, Hepp and Santos (2009) studied neotropical savanna streams and observed that predators displayed similar distributions with diverse land–uses. The CPOM/FPOM ratio in this study also indicated low riparian integrity, a non–functioning riparian zone as the shredder population was very limited (Table 3.8). Anthropogenic activities within the riparian zones have been demonstrated to be the major contributing factors to a non–functional riparian zone (Masese et al., 2014). Additionally, low abundances of shredders have been attributed to the nutritional quality of leaves (Tomanova et al., 2006; Ferreira et al., 2014) and climate variability as many shredders are adapted to cold water and may be nearer to their thermal maxima in the tropics (Masese et al., 2014). These results agreed with the overall assumption that in headwater streams, shredders are abundant and decrease as a result of land–use practices.

It is worth noting that the FFG ratios results for all stream order categories appeared to be weak during both seasons. This suggests that the classification of FFGs that have been projected for north–temperate regions (from Merritt and Cummins, 1996a, 1996b) will need modification before they can be applied to streams in this region. Different environmental conditions can lead to macroinvertebrates changing their mode of feeding (Rawer–Jost et al., 2000; Kelly et al., 2002). Moreover, taxonomically linked families/species can have diverse diets in different regions (Tomanova et al., 2006). Some authors have noted that some of the functional groupings are not well established (Tomanova et al., 2006; Tierno et al., 2019). For example, the scraper group is composed of taxa inhabiting the trophic roles of herbivores, detritivores and generalists (Rawer-Jost et al., 2000). Thus, the practice of grouping taxa into a single trophic category based on FFG assignment may not be an appropriate use of this concept (Hawkins and MacMahon, 1989). Macroinvertebrate individuals can also use diverse strategies either at one time or during different life stages (Rawer-Jost et al., 2000). This underlines a clear need to improve and standardise FFG assignments as well as to develop FFG classifications unique to the region. Despite these challenges, FFG ratios offered some insights on the overall functioning of Bloukrans river system.

### 3.5 Conclusions

The present study confirmed that alterations of the physico–chemical conditions of a stream disrupt macroinvertebrate feeding group structures and have adverse impacts on the overall stream health. Phosphate, canopy cover, TDS and channel width were significantly associated with changes in macroinvertebrate functional feeding group structures within the Bloukrans River. Results also showed that the dominance of collector–gatherers is associated with the occurrence of point (un–treated sewage effluent) and diffuse (urban land–use activities) pollution sources. The FFG ratios obtained also indicated evidence of human activities in Bloukrans River which has led to a non–functioning riparian zone and the predominance of heterotrophy over autotrophic production. Thus, functional analysis of macroinvertebrate communities may be used to monitor and evaluate land–use change on water ecosystem health in austral South African streams. However, further studies are required to examine the threshold values for heterotrophy vs autotrophy in the austral temperate streams and comparisons among studies will be beneficial for proper classification of FFGs.

## 3.6 References


Anderson, M.J., 2006. Distance–based tests for homogeneity of multivariate dispersions. Biometrics 62, 245–253.

Baer, S.G., Siler, E.R., Eggert, S.L., Wallace, B., 2001. Colonization and production of macroinvertebrates on artificial substrata: upstream–downstream responses to a leaf litter exclusion manipulation. Freshwater Biol. 46, 347–365.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. 2nd edition. US, Environment Protection Agency, Office of Water, Washington DC.

Berthon, V., Bouchez, A., Rimet, F., 2011. Using diatom lifeforms and ecological guilds to assess organic pollution and trophic level in rivers: a case study of rivers in south eastern France. Hydrobiologia 673, 259–271.

Brasil, L.S., Juen, L., Batista, J.D., Pavan, M.G., Cabette, H.S.R., 2014. Longitudinal distribution of the functional feeding groups of aquatic insects in streams of the Brazilian Cerrado Savanna. Neotrop. Entomol. 43, 421–428.

Chapin-III, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C., Diaz, S., 2000. Consequences of changing biodiversity. Nature 405, 234–242.

Clarke, K.R., Warwick, R.M. 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, second ed. PRIMER–E, Plymouth

Cummins, K.W., Merritt, R.W., Andrade, P.C.N., 2005. The use of invertebrate functional groups to characterize the ecosystem attributes in selected streams and rivers in south Brazil. Stud. Neotrop. Fauna Environ. 40, 69–89.

Dalu, T., Magoro, L.M., Wasserman, R., Froneman, W.P., 2019. River nutrient water and sediment measurements inform on nutrient retention, with implications for eutrophication. Sci. Total Environ. 684, 296-302.

Dalu, T., Wasserman, R.J., Tonkin, J.D., Alexander, M.E., Dalu, M.T., Motitsoe, S.N., Manungo, K.I., Bepe, O., Dube, T., 2017b. Assessing drivers of benthic macroinvertebrate community structure in African highland streams: an exploration using multivariate analysis. Sci. Total Environ. 601, 1340–1348.

Dalu, T., Wasserman, R.J., Tonkin, J.D., Mwedzi, T., Magoro, M., Weyl, O.L.F., 2017a. Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macroinvertebrate communities in an austral South African stream. Sci. Total Environ. 607–608, 317–325.

De La Rey, P.A., Roux, H., Van Rensburg, L., Vosloo, A., 2008. On the use of diatom–based biological monitoring Part 2, A comparison of the response of SASS 5 and diatom indices to water quality and habitat variation. Water SA 34, 61–69.

Dickens, C.W.S., Graham, P.M., 2002. The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. Afr. J. Aquat. Sci. 27, 1–10.

Dudgeon, D., 1994. The influence of riparian vegetation on macroinvertebrate community structure and functional organization in six New Guinea streams. Hydrobiologia 294, 65–85.

Farrell, K., van Vuren, J., Ferreira, M., 2015. Do aquatic macroinvertebrate communities respond to land–use effects in the Wilge River, Mpumalanga, South Africa? Afr. J. Aquat. Sci. 40, 165–173.

Feld, C.K., 2013. Response of three lotic assemblages to riparian and catchment-scale land use: implications for designing catchment monitoring programmes. Freshw. Biol. 58, 715–729.

Fernandes, A.C.P., Sanches Fernandes, L.F., Moura, J.P., Cortes, R.M.V., Pacheco, F.A.L., 2019. A structural equation model to predict macroinvertebrate-based ecological status in catchments influenced by anthropogenic pressures. Sci. Total Environ. 681, 242-257.

Ferreira, A.R.L., Sanches Fernandes L.F., Cortes, R.M.V., Pacheco, F.A.L. (2017). Assessing anthropogenic impacts on riverine ecosystems using nested partial least squares regression. Sci. Total Environ. 583, 466-477.

Ferreira, V., Encalada, A.C., Graça, M.A.S., 2012. Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. Freshwater Sci. 31, 945–962.

Ferreira, W.R., Ligeiro, R., Macedo, D.R., Hughes, R.M., Kaufmann, P.R., Oliveira, L.G., Callisto, M., 2014. Importance of environmental factors for the richness and distribution of benthic macroinvertebrates in tropical headwater streams. Freshwater Sci. 33, 860–871.

Fierro, P., Bertrán, C., Tapia, J., Hauenstein, E., Peña-Cortés, F., Vergara, C., Cerna, C., Vargas-Chacoff, L., 2017. Effects of local land-use on riparian vegetation, water quality, and the functional organization of macroinvertebrate assemblages. Sci. Total Environ. 609, 724–734

Fu L, Jiang Y, Ding J, Liu Q, Peng QZ, Kang MY. 2015. Impacts of land use and environmental factors on macroinvertebrate functional feeding groups in the Dongjiang River basin, southeast China. J. of Freshw. Ecol. 31, 21–35.

Gerber, A., Gabriel, M.J.M., 2002. Aquatic Invertebrates of South African Rivers: Field Guide. Resource Quality Services, Department of Water Affairs and Forestry, Pretoria.

Green, J., Passmore, M., Childers, H., 2000. A survey of the condition of streams in the primary region of mountaintop mining/valley fill coal mining. Appendix in Mountain top mining/valley fills in Appalachia. Final programmatic environmental impact statement. Region 3, US EPA. Philadelphia, Pennsylvania.

Gutiérrez-Cánovas, C., Millán, A., Velasco, J., Vaughan, L.P., Omerod, S.J., 2013. Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. Glob. Ecol. Biogeogr. 22, 796–805.

Gutiérrez–Cánovas, C., Arribas, P., Naselli–Flores, L., Bennas, N., Finocchiaro, M., Millán, A. Velasco, J., 2019. Evaluating anthropogenic impacts on naturally stressed ecosystems: Revisiting river classifications and biomonitoring metrics along salinity gradients. Sci. Total Environ. 658, 912–921.

Hammer, O., Harper, D.A.T., Ryan, P. D., 2012. PAST – Palaeontological Statistics, version 2.16.

Hawkins, C.P., MacMahon, J.A., 1989. Guilds: the multiple meanings of a concept. Annu. Rev. Entomol. 34, 423-451.

Hepp, L.U., Santos, S., 2009. Benthic communities of streams related to different land uses in a hydrographic basin in southern Brazil. Environ. Monit. Assess. 157, 305–318.

IBM Corp., 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

Imberger, S. J., Cook, P.L.M., Grace, M.R., Thompson, R.M., 2014. Tracing carbon sources in small urbanising streams: catchment–scale stormwater drainage overwhelms the effects of reach–scale riparian vegetation. Freshwater Biol. 59, 168–186

Jiang, X., Xiong, J., Xie, Z., Chen, Y., 2011. Longitudinal patterns of macroinvertebrate functional feeding groups in a Chinese river system:a test for river continuum concept (RCC). Quat. Int. 244, 289−295

Kelly, D. W., Dick, J.T.A., Montgomery, W.I., 2002. Predation on mayfly nymphs, Baetis rhodani, by native and introduced Gammarus: direct effects and the facilitation of predation by salmonids. Freshw. Biol. 47, 1257–1268.

Khan, A., Jiang, J., Sharma, A., Wang, P., Khan, J., 2017. How Do Terrestrial Determinants Impact the Response of Water Quality to Climate Drivers? —An Elasticity Perspective on the Water Land–Climate Nexus. Sustainability 9, 1–20.

Legendre, P., Legendre, L., 1998. Numerical Ecology, second ed. Elsevier Science B.V., Amsterdam, The Netherlands.

Li, H., You, S., Zhang, H., Zheng, W., Zou, L., 2018. Investigating the environmental quality deterioration and human health hazard caused by heating emissions. Sci. Total Environ. 628, 1209–1222.

Mahmoud, S.H., Gan, T.Y., 2018. Impact of anthropogenic climate change and human activities on environment and ecosystem services in arid regions. Sci. Total Environ. 633, 1329–1344.

Mangadze, T., Dalu, T., Froneman, P.W., 2019. Biological monitoring in southern Africa: A review of the current status, challenges and future prospects. Sci. Total Environ. 648, 1492–1499.

Masese, F.O., Kitaka, N., Kipkemboi, J., Gette, G.M., Irvine, K., McClain, M.E., 2014. Macroinvertebrate functional feeding groups in Kenyan highland streams: evidence for a diverse shredder guild. Nigeria. J. Aquat. Ecol. 5, 67–98.

Merritt, R.W., Cummins, K.W., 1996a. An introduction to the aquatic insects of North America 3rd Edn. Kendall–Hunt, Dubuque, IOWA 862 p.

Merritt, R.W., Cummins, K.W., 1996b. Trophic relations of macroinvertebrates. In: Hauer FR, Lamberti GA, editors. Methods in stream ecology. Academic Press, San Diego.

Merritt, R.W., Cummins, K.W., Berg, M.B., 2008. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Duduque, IA, USA.

Miserendino, M.L., Masi, C.I., 2010. The effects of land use on environmental features andfunctional organization of macroinvertebrate communities in Patagonian low orderstreams. Ecol. Indic. 10, 311–319

Moyo, S., Richoux, N.B., 2017. Macroinvertebrate Functional Organisation Along the Longitudinal Gradient of an Austral Temperate River. J. Afr. Zool. 52, 125–136.

Muskett, P.C., Hill, J.M.,Weyl, P.S.R., 2016. Macroinvertebrate communities associated with duckweed (Lemnaceae) in two Eastern Cape rivers, South Africa. Afr. Aqua. Sci. 41, 481–488.

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. Biodivers. Conserv. 15, 3267–3293.

Newbold, J.D., Erman, D.C., Roby, K.B., 1980. Effects of logging on macroinvertebrates in streams with and without buffer strips. Can. J. Fish. Aquat. Sci. 37, 1076–1085.

Nhiwatiwa, T., Dalu, T., Brendonck, L., 2017. Impact of irrigation–based sugarcane cultivationon the Chiredzi and Runde Rivers quality, Zimbabwe. Sci. Total Environ. 587–588, 316–325.

Odume, O.N., Mgaba, N., 2016. Statistical analysis of macroinvertebrate assemblage structure in relation to river–health assessment of an urban river, Eastern Cape, South Africa. Aquat. Ecosyst. Health Manag. 19, 420–430.

Pacheco, F.A.L., Fernandes, L.F.S., 2016. Environmental land use conflicts in catchments: A major cause of amplified nitrate in river water. Sci. Total Environ. 548, 173–188.

Palumbo, A.V., Mulholland, P.J., Elwood, J.W., 1987. Microbial communities on leaf material protected from macroinvertebrate grazing in acidic and circumneutral streams. Can. J. Fish. Aquat. Sci. 44, 1064–1070.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team., 2012. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-104.

Pinheiro, J.C., Bates, D.M., 2000. Mixed-Effects Models in S and S-PLUS. Statistics and Computing, ed. J. Chambers, et al. 2000, New York: Springer Verlag.

Platts, W.S., Megahan, W.F., Minshall, W.G., 1983. Methods for Evaluating Stream, Riparian, and Biotic Conditions. General Technical Report INT–138, USDA Forest Service. Rocky Mountain Research Station, Ogden, UT.

Pond, G.J., Passmore, M.E., Borsuk, F.A., Reynolds, L., Rose, C.J., 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family– and genus–level macroinvertebrate bioassessment tools. J. N. Am. Benthol. Soc. 27, 717–737.

R Core Team., 2014. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rawer–Jost, C., Böhmer, J., Blank, J., Rahmann, H., 2000. Macroinvertebrate functional feeding group methods in ecological assessment. Hydrobiologia 423, 225–232

Rosi–Marshall, E. J., Wallace, J.B., 2002. Invertebrate food webs along a stream resource gradient. Freshw. Biol. 47 129–141.

Sanches Fernandes, L.F., Fernandes, A.C.P., Ferreira, A.R.L., Cortes, R.M.V., Pacheco, F.A.L., 2018. A partial-least squares - path modeling analysis for the understanding of biodiversity loss in rural and urban watersheds in Portugal. Sci. Total Environ. 626, 1069-1085.

Santos, R.M.B., Sanches Fernandes, L.F., Varandas, S.G.P., Pereira, M.G., Sousa, R., Teixeira, A., Lopes-Lima, M., Cortes R.M.V., Pacheco F.A.L., 2015. Impacts of climate change and land-use scenarios on *Margaritifera margaritifera*, an environmental indicator and endangered species. Sci. of the Total Environ. 511, 477- 488.

Statzner, B., Bêche, L. A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biol.55, 80–119.

Stone, M.K., Wallace, J.B., 1998. Long-term recovery of a mountain stream from clear-cut logging: the effects of forest succession on benthic invertebrate community structure. Freshwater Biol. 39, 151–169.

Strahler, A.N., 1957. Quantitative analysis of watershed geomorphology. Trans. Am. Geophys. Union. 38, 913–920.

Tierno de Figueroa, J. M., López-Rodríguez, M. J., Villar-Argaiz, M., 2019. Spatial and seasonal variability in the trophic role of aquatic insects: An assessment of functional feeding group applicability. Freshwater Biol. 64, 954-966.

Tomanova, S., Goitia, E., Helešic, J., 2006. Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. Hydrobiologia 556, 251–264.

Utz, R.M., Hilderbrand, H., Boward, D.M., 2009. Identifying regional differences in threshold responses of aquatic invertebrates to land cover gradients. Ecol. Indic. 9, 556–567.

Valle Junior, R.F., Varandas, S.G.P., Pacheco, F.A.L., Pereira, V.R., Santos, C.F., Cortes, R.M.V., Fernandes, L.F.S., 2015. Impacts of land use conflicts on riverine ecosystems. Land Use Policy 43, 48-62.

Wagner, P. F., 2001. Legacies of early 20th century logging in southern Appalachian streams. PhD Dissertation, Virginia Polytechnic Institute and State University, Blacksburg,

Wallace, J. B., Eggert, S. L., Meyer, J. L., Webster, J. R., 2015. Stream invertebrate productivity linked to forest subsidies: 37 stream-years of reference and experimental data. Ecology 96, 1213– 1228.

Weigelhofer, G., 2017. The potential of agricultural headwater streams to retain soluble reactive phosphorus. Hydrobiologia 793, 149–160.

# Chapter 4: Use of diatom communities as indicators of conductivity and ionic composition in a small Austral temperate river system

* This chapter has been published as: **Mangadze, T.**, Wasserman, R.J., Dalu, T., 2017. Use of diatom communities as indicators of conductivity and ionic composition in a small Austral temperate river system. *Water, Air and Soil Pollution* 228(11), 428-439.

**Abstract**: The aim of this study was to determine if benthic diatoms can be used as effective and reliable indicators of ionic composition and conductivity in different stream order categories. Samples were collected twice at 22 sampling sites within the Bloukrans River system, Eastern Cape Province, South Africa. The data collected were subjected to multivariate statistical technique i.e. CCA to determine environmental gradients along which the diatom species were distributed as well as to elucidate hypothesized differences in community structure per stream order. Significant differences between the two sampling periods were observed in dissolved oxygen, temperature, Na, B, Ca, Zn, Cu, Cr, K, Fe, phosphate, conductivity, salinity and nitrate, while significant stream order variation was observed for conductivity, salinity, Mg, Ca and sediment nitrates. Study sites were grouped into roughly two broad categories (stream order 1 and 2/3 sites) based on CCA. As pollution increased, low to moderate pollution tolerant species such as *Fragilaria tenera*, *Cyclostephanos dubius* and *Gyrosigma acuminatum* were replaced by high pollution tolerant species such as *Nitzschia palea*, *Gomphonema parvulum*, *Tryblionella apiculata*, *Diploneis vulgaris* and *Staurosira elliptica*. This shows that diatom assemblages are appropriate indicators of ionic composition/conductivity and hydromorphological characteristics (e.g., stream size) of running waters. The results of the study highlight the importance of creating regional calibration datasets which will make it possible to develop finely tuned models to quantitatively infer conductivity and ion concentrations.

**Keywords:** Conductivity, ionic composition, diatoms, stream order, water quality

## 4.1 Introduction

Aquatic environments are essential components of the regional and global biogeochemical cycles (reduction/oxidation transformations) and they serve as sources of water for drinking, fisheries resources and irrigation supplies (Bere and Tundisi, 2010; Bere et al., 2016). However, human civilisation has caused significant land-use changes resulting in inevitable discharge of liquid and solid wastes into water bodies ensuing in adverse effects upon the receiving water bodies (Dudgeon et al., 2006; Dalu and Froneman, 2016, Nel et al., 2018). Changes in the land cover and land management practices have also been regarded as the key influencing factors behind the alteration of the hydrological system, which have led to changes in runoff and water quality (Pan et al., 2004; Dalu et al., 2017a). For example, agricultural land-use often increases landscape vulnerability to surface runoff leading to loss of riparian complexity and in-stream habitats, changes in hydrology and increased inputs of nutrients, herbicides/pesticides and fine sediments into the river (Zhang et al., 2012; Nhiwatiwa et al., 2017).

Ion leaching from irrigated soils can further elevate the naturally high salinity of many rivers in arid and semi-arid zones (Potapova and Charles, 2003; Nhiwatiwa et al., 2017). An increasing number of studies have also shown substantial land-use effects on natural water quality due to activities such as agriculture, urban development, domestic and industrial wastewater discharge (Bere and Tundisi, 2011a; Tan et al., 2014a, b). As human activities degrade lotic systems, the aquatic communities they support are modified to varying degrees (Waite et al., 2000; Mwedzi et al., 2016). Every aquatic organism has particular requirements with respect to the physical, chemical and biological conditions of its habitat. Degradation of these conditions can negatively affect species diversity through a change in species dominance or total loss of sensitive species by death or migration (Beyene et al., 2009). Additionally, water body size has also been shown to be an important factor in determining the structure and function of aquatic ecosystems (Higgins et al., 2005). The Strahler stream order (Strahler, 1957) has been a useful indicator of stream size in stream biology (Miyamoto et al., 2011) regionally, nationally, and globally.

For routine water quality monitoring, diatoms have gained momentum as bioindicators in lotic systems and information about their responses to changes in environmental quality continues to grow (de la Rey et al., 2004; Taylor, 2007; Beyene et al., 2009; Dalu et al., 2016, 2017b, c; Mangadze et al., 2016). Diatoms are commonly used because they are sensitive to specific environmental disturbances such as pH, conductivity and nutrients and therefore diatom species composition and their respective abundance in the community can be used to track changes in the properties of the river system (Potapova and Charles, 2002). Although it is well known that conductivity and ionic composition have a strong influence on distributions of individual diatom taxa (Cholnoky, 1968; Potapova and Charles, 2003; Bere and Tundisi, 2011b), the relative importance of these factors has rarely been studied in Austral temperate river systems. Pollution dynamics in arid Austral river systems are not well understood as flow is highly variable and differences between tributary stream and mainstem river dynamics are often more pronounced than in mesic environments (Dalu et al., 2017b). Thus, existence of physiological conductivity thresholds in diatoms is not only an interesting theoretical question, but also has practical implications and is important to establish water quality guidelines in South African rivers.

A spatio-temporal investigation was conducted on the benthic diatoms present within the Bloukrans River system in relation to ion concentrations. The main aim of this study was to determine if benthic diatoms can be used as effective and reliable indicators of ionic composition and conductivity in different stream order categories. It was hypothesised that, changes in ionic composition and conductivity resulting from different land-use patterns and stream order variation would be reflected in diatom community structure. This information improves our understanding of diatom autecology within Austral temperate rivers with respect to conductivity and major ions.

## 4.2 Materials and methods

### *4.2.1 Study area*

The study was carried out on the Bloukrans River system in the Eastern Cape Province, South Africa (Figure 4.1). The Bloukrans River catchment area is approximately ~ 230 $km^2$, with a total river length of ~ 40 km. The Bloukrans River is located on the eastern periphery of the Mediterranean rainfall area i.e. the warm temperate climatic zone, which covers the southern Cape. Mean annual precipitation in the study area is around 650 mm, with minimum and maximum air temperatures of 1.5 °C and 43 °C, respectively. The river receives pollutants from various point and diffuse sources, such as overflowing sewage drains and litter from the largest town i.e. Makhanda in the Makana Local Municipality, Eastern Cape province of South Africa (Nel et al., 2018). Furthermore, as the river leaves the town, effluent from the Makhanda Wastewater Treatment Works is released into the river, before the river runs through sections of irrigated dairy farming pastures and agriculture (Dalu et al., 2017b). Sampling sites were selected by picking all possible access points (bridges and roads) to perennial rivers using topographic maps (1: 50 000) and then selecting a stratified random sample ($n$ = 22; Figure 4.1) according to Barbour et al. (1999). Stratification was based on stream size whereby sampling sites were selected based on the relative abundance of the different stream orders (Strahler, 1957) in the basin. Thirteen first-order, two second order and seven third-order streams/rivers were sampled. Sampling was done twice, once in February (at the end of the rain season when all the streams were flowing) and July (during the dry season) 2016 to capture the two flow extremes typical of the study area.

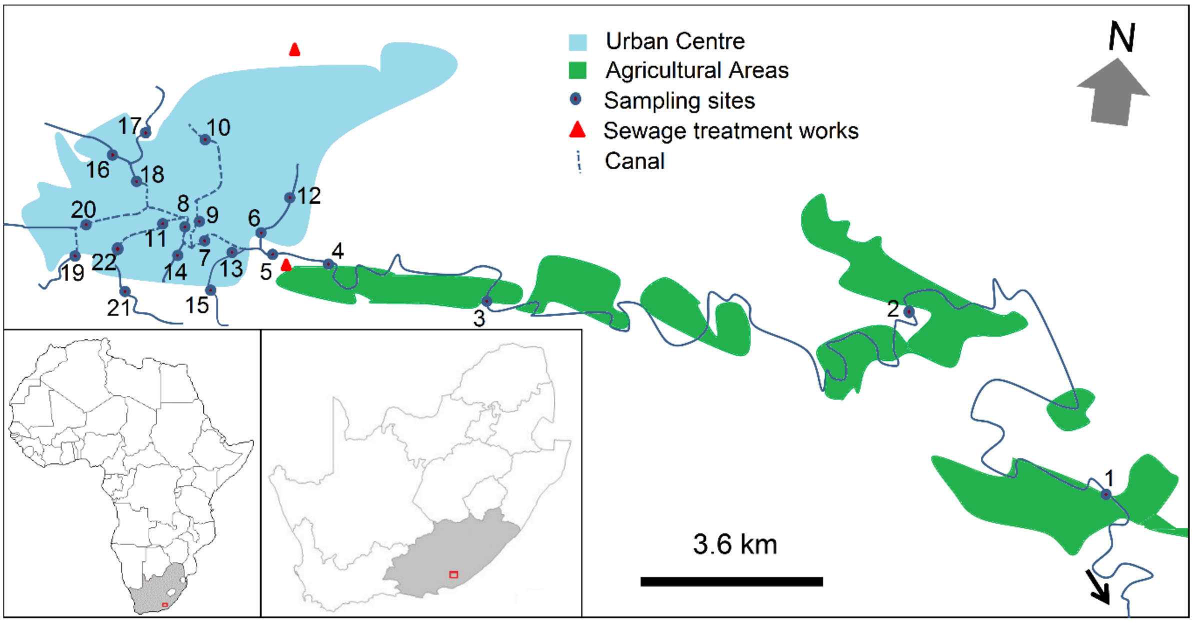


**Figure 4.1:** The location of the study area and sampling sites.

*4.2.2 Physico-chemical variables*

At each site, a portable multi-parameter probe (PCTestr 35, Eutech/Oakton Instruments, Singapore) was used to measure conductivity, pH, salinity, total dissolved solids (TDS) and temperature, while dissolved oxygen (DO) and turbidity were measured using a DO meter (DO 850045, Per Scientific, Taiwan) and turbidity meter (AL250T-IR, Aqualytic, Germany), respectively. Samples of water (500 mL) from each site were collected in triplicate and placed on ice in the dark awaiting further nutrient (ammonium, phosphates and nitrates) processing in the laboratory. Nutrient concentrations, employed as proxies for land-use activities, were analysed using a multiparameter bench photometer (HI 83203, Hanna Instruments Inc., Rhode Island) within 12 h of collection. Channel width and water depth were estimated using a graduated measuring rod. The river substrate embeddedness was determined according to Platts et al. (1983).

Integrated sediment samples (*n* = 2, 1.5kg, depth ~5–10cm) were collected at each site per season using a plastic hand shovel. On site, sediment was placed into polyethylene ziplock bags after removal of overlaying debris. Once on the laboratory, sediment samples were dried in an oven at 60°C, before being disaggregated using a porcelain pestle and mortar and sieved through a <0.075 mm sieve to remove plant roots and other debris. Boron (B), calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) were determined using acid digestion with a 1:1 mixture of 1 N nitric acid ($HNO_3$) and hydrochloric acid at 80 °C for 30 minutes. Chromium (Cr), copper (Cu), iron (Fe), lead (Pb) and zinc (Zn) analyses conducted using 20 mL $HNO_3$ (55%) and 5 mL hydrogen peroxide (30 %) and placed on a heated sand bed (180$^o$C) for eight hours, before being filtered onto a Whatman filter paper. Sediment nitrate concentrations were determined calorimetrically through reduction of nitrate ($SedNO_3^-$) to nitrite ($NO_2^-$) using a copper-cadmium reduction column (AgriLASA, 2004). Whereas, sediment total phosphorus (SedTP) and phosphates ($SedPO_4^{3-}$) concentrations were analysed using a Bray-2 extract method (Bray and Kurtz, 1945). All metal analysis was conducted at a South African National Accreditation System (SANAS) certified laboratory (Bemlab, Cape Town) using an ICP-OES optical emission spectrometer (Varian, Mulgrave, Australia) (see Clesceri et al. (1998) for detailed methodology) for metals and SEAL Auto-Analyser 3 for sediment nitrate concentrations. Sediment organic matter (SOM) and sediment organic carbon (SOC) were assessed based on the modified Walkley–Black method (Chan et al., 2001).

*4.2.3 Diatom sampling*

Epilithic diatoms were sampled on riffles at each site by brushing stones with a toothbrush. Prior to sampling of epilithic surfaces, all substrata were gently shaken in stream water to remove any loosely attached sediments and non-epilithic diatoms. At least 10 pebble (>2–64 mm) to cobble-sized (> 64–256 mm) rocks/stones were randomly collected at each sampling site and brushed, and the resulting diatom suspensions were pooled to form a single sample. The periphyton contents were emptied into a labelled container and preserved in Lugol's iodine for microscopic examination in the laboratory. Two days after collection, the benthic diatom samples were digested using the potassium permanganate and hydrochloric acid method following Taylor et al. (2005) and mounted in Pleurax (JC Taylor, North-West University, Potchefstroom) (r.i. 1.73). A total of 300–650 valves per sample (based on counting efficiency determination method by Pappas and Stoermer (1996)) were identified and counted using an Olympus CX light microscope at ×1000 magnification. The diatoms were identified to species level based on guides by Taylor et al. (2007).

*4.2.4 Data analysis*

A two-way ANOVA was used to test the differences in physico-chemical variables among the two stream order categories and sampling periods (Ho: no difference among stream order categories and sampling periods). Diatom counts from each site were expressed as relative abundances to eliminate the effects of rare species. Input for community analysis included only the diatom taxa that were present in a minimum of two samples and had a relative abundance of ≥1 % in at least one sample. Of the 119 diatom taxa recorded in the 22 sampling stations during the two sampling periods, 101 taxa met this criterion.

Distance-based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001) was conducted using PRIMER v6 add-on package PERMANOVA+ (Anderson et al., 2008). To determine diatom species variation among the stream order categories and period, we used stream orders and sampling period as the main factors in a PERMANOVA with Euclidean dissimilarities as distance measures. Each term in the analysis was tested using 9999 permutations of the correct relevant permutable units (Anderson and ter Braak, 2003), with significant terms investigated using PERMANOVA *t* statistic pair-wise comparisons (Anderson et al., 2008).

Multivariate analyses were performed on the diatom community dataset to specify the main gradients of floristic variation and to detect and visualize similarities in diatom samples in relation to stream order patterns within the catchment. Preliminary de-trended correspondence analysis (DCA) was applied to the diatom community dataset to examine the gradient lengths. The DCA analysis revealed that the gradient lengths were greater than 3 standard deviation units, justifying the use of unimodal ordination techniques (ter Braak and Verdonschot, 1995). Thus, canonical correspondence analysis (CCA) was used to explore the relationships between diatom communities and predictor variables from different study sites. Preliminary CCA analysis identified collinear variables and selected a subset on inspection of variance inflation factors (VIF < 20; ter Braak and Verdonschot, 1995). Monte Carlo permutation tests (9999 unrestricted permutations, $p \leq 0.05$) were used to test the significance of the axis and hence determine if the selected physico-chemical variables could explain much of the diatom data variation, including all the measured physico-chemical variables combined.

The strengths of relationships between diatom assemblages and environmental variables were assessed using the ratios of the first and second Eigen values ($\lambda_1$/ $\lambda_2$). This ratio measures the strength of the constraining variable with respect to the first unconstrained gradient in the assemblage composition data. Large numbers indicate strong responses of biological assemblages to environmental variables, with the strength of relationship being considered very high if $\lambda_1/\lambda_2 > 1$, moderately high if $0.5 < \lambda_1/\lambda_2 < 1$ and weak if $\lambda_1/\lambda_2 < 0.5$ (ter Braak and Prentice, 1988). Once the significant variables with a moderate to high relationship with diatom communities were identified, we quantified their relative influence on diatom communities with the variance partitioning (partial CCA) method. Partial CCA (Borcard et al., 1992) was then used to separate and examine the relative importance of two sets of exploratory variables (ionic composition and conductivity) on the diatom community. Various programs were employed for statistical analyses. Univariate analyses were conducted using SPSS version 16 (SPSS Inc., 2007), while CCA was performed using CANOCO version 5 (ter Braak and Šmilauer, 2002).

## 4.3 Results

### *4.3.1 Physico-chemical variables*

The pH increased slightly down the pristine (upstream) to urban (downstream) gradient, being slightly acidic at the upstream sites and slightly alkaline at downstream sites (Table 4.1). The difference in pH between the two sampling periods and amongst sampling categories was not statistically significant ($p > 0.05$). Significant sampling period differences were observed for DO, temperature, Na, B, Ca, Zn, Cu, Cr, K, Fe, phosphate, conductivity, salinity and nitrate (ANOVA, $p \leq 0.0$), while significant sampling stream order variation was observed for conductivity, salinity, Mg, Ca and SedNO$_{3-}$ (ANOVA, $p \leq 0.05$). The concentrations of Cu, Cr, Zn and SedPO$_4^{3-}$ increased at downstream sites along stream order 2/3 but were not statistically significant ($p > 0.05$, Table 4.1). No significant differences were also observed in physico-chemical factors between the two sampling periods for TDS, Pb, SedTP and $SedPO_4^{3-}$ ($p$ > 0.05).

### *4.3.2 Community composition*

A total of 119 diatom species belonging to 36 genera were recorded. Of the 119 species observed, 101 species were retained for subsequent analysis (present in a minimum of 4 samples and had a relative abundance of ≥1 % in at least 1 sample; Appendix A.1, Table S1). No significant effect of sampling period was found on diatom communities (PERMANOVA: Pseudo-F = 1.118, $p$ = 0.215) nor between sampling period and stream order on diatom communities (pseudo-F = 0.906, $p$ = 0.720). However, PERMANOVA identified a significant effect on stream order on diatom communities (Pseudo-F = 1.368, $p$ = 0.010).

### *4.3.3 Diatom community composition and physico-chemical relationships*

Based on CCAs carried out using individual variables (Table 4.2), the following ions were significantly (Monte Carlo permutation test, $p \leq 0.05$) associated with changes in diatom communities (in order of their decreasing importance, based on the ratio of the first to second axes eigenvalues): $SedNO_3^-$, $SedPO_4^{3-}$, B, K, Fe, Pb, Mg, Na, Ca and phosphate. Thus, ion species associated with eutrophication ($SedNO_3^-$ and $SedPO_4^{3-}$) were amongst the ions associated with changes in diatom communities. Eigenvalues of the first axis in all the analyses ranged from 0.20 to 0.51 with a low to moderate ratio of the first to the second eigenvalue (Table 4.2).

**Table 4.1:** Mean (±SD) of physical and chemical variables recorded in all site categories (from stream order 1 and 2/3) during the study period. Significant differences at $p$ < 0.05 (two-way ANOVA) are indicated in bold. Abbreviations: DO – dissolved oxygen, TDS – total dissolved solids, $SedNO_{3-}$ – sediment nitrates, SedTP – sediment total phosphorus, $SedPO_4^{3-}$ – sediment phosphate.

| **Variable** | **Stream order 1** | | **Stream order 2/3** | | **Sampling period** | | **Stream order** | |
|---|---|---|---|---|---|---|---|---|
| | **Summer** | **Winter** | **Summer** | **Winter** | **F** | ***p*** | **F** | ***p*** |
| **Water Chemistry** | | | | | | | | |
| Temperature (°C) | 19.28± 0.90 | 13.40± 2.02 | 20.34± 1.58 | 12.96± 1.94 | **171.2** | **0.001** | 0.350 | 0.500 |
| Conductivity (µS $cm^{-1}$) | 1415.82± 1570.53 | 1975.28± 2010.98 | 1644.89± 551.19 | 1351.13± 438.92 | **4.090** | **0.030** | **5.200** | **0.026** |
| DO (mg $L^{-1}$) | 7.02± 1.75 | 5.38± 2.59 | 6.26± 1.79 | 4.13± 1.98 | **9.904** | **0.003** | 1.861 | 0.180 |
| pH | 7.75± 0.38 | 7.88± 0.48 | 8.67± 0.21 | 7.73± 0.52 | 0.351 | 0.557 | 0.229 | 0.635 |
| Salinity (ppt) | 712.69± 839.80 | 791.02± 670.87 | 806.37± 258.42 | 630.88± 203.96 | **6.055** | **0.016** | **5.250** | **0.029** |
| TDS (mg $L^{-1}$) | 1015.35± 1137.65 | 1123.76± 1153.17 | 1175.96± 390.35 | 966.40± 381.53 | 0.032 | 0.859 | 0.000 | 0.79 |
| Nitrate (mg $L^{-1}$) | 61.42± 74.37 | 28.46± 53.57 | 49.78± 33.51 | 12.73± 8.48 | **4.719** | **0.030** | 0.725 | 0.399 |
| Phosphate (mg $L^{-1}$) | 2.34± 2.10 | 5.63± 6.83 | 5.22± 3.95 | 8.28± 4.61 | **3.875** | **0.056** | **4.404** | **0.042** |
| Ca (mg $L^{-1}$) | 7191.97± 7632.76 | 6.29± 4.74 | 6337.33± 8084.09 | 2.11± 1.07 | **15.914** | **0.001** | **4.064** | **0.039** |
| B (mg $L^{-1}$) | 6.97± 2.71 | 0.91± 0.89 | 5.89± 2.11 | 0.34± 0.18 | **103.34** | **0.001** | 2.793 | 0.100 |
| Fe (mg $L^{-1}$) | 36126.93± 11444.45 | 607.61± 522.44 | 31728.56± 14380.68 | 425.27± 162.31 | **147.057** | **0.002** | 0.691 | 0.911 |
| Mg (mg $L^{-1}$) | 880.92± 615.64 | 2.23± 1.55 | 679.89± 347.87 | 0.90± 0.39 | **46.751** | **0.001** | **7.794** | **0.008** |
| Pb (mg $L^{-1}$) | 20.80± 10.15 | 12.71± 9.85 | 33.78± 50.28 | 14.96± 14.58 | 3.005 | 0.910 | 0.922 | 0.343 |
| K (mg $L^{-1}$) | 227.63± 134.12 | 0.29± 0.29 | 199.44± 84.65 | 0.08± 0.04 | **70.882** | **0.001** | 0.314 | 0.570 |
| Zn (mg $L^{-1}$) | 98.88± 52.14 | 25.52± 24.00 | 117.22± 105.64 | 19.28± 16.30 | **23.857** | **0.001** | 0.120 | 0.731 |
| Na (mg $L^{-1}$) | 133.92± 212.07 | 0.72± 0.64 | 70.89± 51.76 | 0.27± 0.10 | **7.881** | **0.008** | 0.762 | 0.388 |
| Cu (mg $L^{-1}$) | 20.11± 31.24 | 2.89± 2.29 | 21.22± 35.93 | 2.31± 2.25 | **6.460** | **0.015** | 0.007 | 0.935 |
| Cr (mg $L^{-1}$) | 14.99± 6.14 | 8.69± 3.85 | 15.44± 5.74 | 9.21± 4.05 | **16.539** | **0.001** | 0.119 | 0.732 |
| **Sediment Chemistry** | | | | | | | | |
| $SedNO_{3-}$ (mg $kg^{-1}$) | 0.85± 0.88 | 0.21± 0.23 | 0.56± 0.17 | 0.30± 0.32 | **5.286** | **0.027** | **4.253** | **0.052** |
| SedTP (mg $kg^{-1}$) | 500.14± 304.47 | 575.39± 425.52 | 652.44± 565.89 | 451.08± 215.36 | 0.273 | 0.604 | 0.014 | 0.902 |
| $SedPO_4^{3-}$ (mg $kg^{-1}$) | 1530.43± 931.68 | 1760.69± 1302.11 | 1997.11± 1731.62 | 1380.32± 659.01 | 0.273 | 0.604 | 0.14 | 0.902 |

**Table 4.2:** Results of CCAs showing the significance (Monte Carlo 9999 unrestricted permutations $p \leq 0.05$) of the effects and strength of each variable on benthic diatom community composition. Significant variables are highlighted in bold.

| Variable | $\lambda_1$ | $\lambda_2$ | $\lambda_1/\lambda_2$ | $p$ |
|---|---|---|---|---|
| **Sediment Chemistry** | | | | |
| Sediment $NO_3^-$ | **0.30** | **0.50** | **0.6** | **0.04** |
| Sediment $PO_4^{3-}$ | **0.30** | **0.50** | **0.6** | **0.05** |
| **Water Chemistry** | | | | |
| B | **0.30** | **0.51** | **0.59** | **0.01** |
| K | **0.30** | **0.51** | **0.59** | **0.01** |
| Fe | **0.29** | **0.51** | **0.57** | **0.01** |
| Pb | **0.29** | **0.51** | **0.57** | **0.01** |
| Mg | **0.29** | **0.51** | **0.56** | **0.02** |
| Na | **0.29** | **0.51** | **0.56** | **0.01** |
| Temperature | **0.27** | **0.51** | **0.53** | **0.04** |
| Conductivity | **0.27** | **0.51** | **0.53** | **0.01** |
| Salinity | **0.27** | **0.51** | **0.52** | **0.01** |
| Ca | **0.25** | **0.51** | **0.50** | **0.05** |
| Phosphate | **0.26** | **0.51** | **0.50** | **0.05** |
| Total dissolved solids | 0.26 | 0.51 | 0.50 | 0.16 |
| pH | 0.26 | 0.51 | 0.50 | 0.08 |
| Cr | 0.25 | 0.51 | 0.49 | 0.13 |
| Zn | 0.24 | 0.51 | 0.47 | 0.34 |
| Nitrate | 0.23 | 0.50 | 0.46 | 0.30 |
| Dissolved oxygen | 0.22 | 0.51 | 0.43 | 0.30 |
| Cu | 0.21 | 0.51 | 0.41 | 0.50 |

From the partial CCA results, ionic composition alone accounted for 39.6 % of explained variation. Other variables represented 18.0 % of the explained variation. The results showed that 5.8 % of the diatom data variation was shared by ionic composition and conductivity and other variables. Finally, the unexplained variation accounted for 50.3 % (Figure 4.2). The first four axes of the species-environmental plot accounted for 50.7 % of the total variance in the community due to measured environmental variables. Axis 1 and 2 explained 13.8 % and 13.6 % of the diatom species variance, respectively. Monte Carlo unrestricted permutation test indicated that axis 1 (9999 permutations) and axis 2 (9999 permutations of axis 2 with axis 1 as a covariable) were statistically significant ($p \leq 0.05$). Ca, Mg, DO, Sed$NO_3^-$, conductivity and salinity were positively associated with the first axis, while pH, phosphate and nitrate were negatively associated with the first axis. Nitrate, Mg, Ca and DO were positively associated with the second axis, while phosphate, pH, SedNO$_3^-$, conductivity and salinity were negatively associated with the second axis. CCA axis 1 and 2 separated the sampling stations into 2 groups (i.e. sites in stream order 1 and sites in stream order 2/3). The first group generally consisted of less impacted sites in stream order 1. These sites were associated with high flow, low concentrations of phosphates and nitrates, and lower conductivity (Figure 4.2). Sites in stream order 1 were characterised by species such as *Navicula subrhynchocephala* Hustedt, *Rhopalodia gibba* (Ehrenberg) Otto Müller, *Amphora pediculus* (Kützing) Grunow ex A. Schmidt, *Fragilaria crotonensis* Kitton, *Gyrosigma acuminatum* (Kützing) Rabenhorst, *Diadesmis confervacea* Kützing and *Epithemia sorex* Kützing. The second group consisted of highly impacted sites in stream order 2/3. These sites were characterised by diatom species such as *Achnanthidium exiguum* (Grunow) Czarnecki, *Cyclotella ocellata* Pantocsek, *Diatoma vulgaris* Bory, *Eunotia formica* Ehrenberg, *Epithemia sorex* Kützing, *Gomphonema acuminatum* Ehrenberg, *Gomphonema parvulum* (Kützing) Kützing, *Nitzschia palea* (Kützing) W. Smith, *Navicula radiosa* Kützing, *Pinnularia divergens* W. Smith and *Staurosira elliptica* (Schumann) D.M. Williams and Round (Figure 4.2).

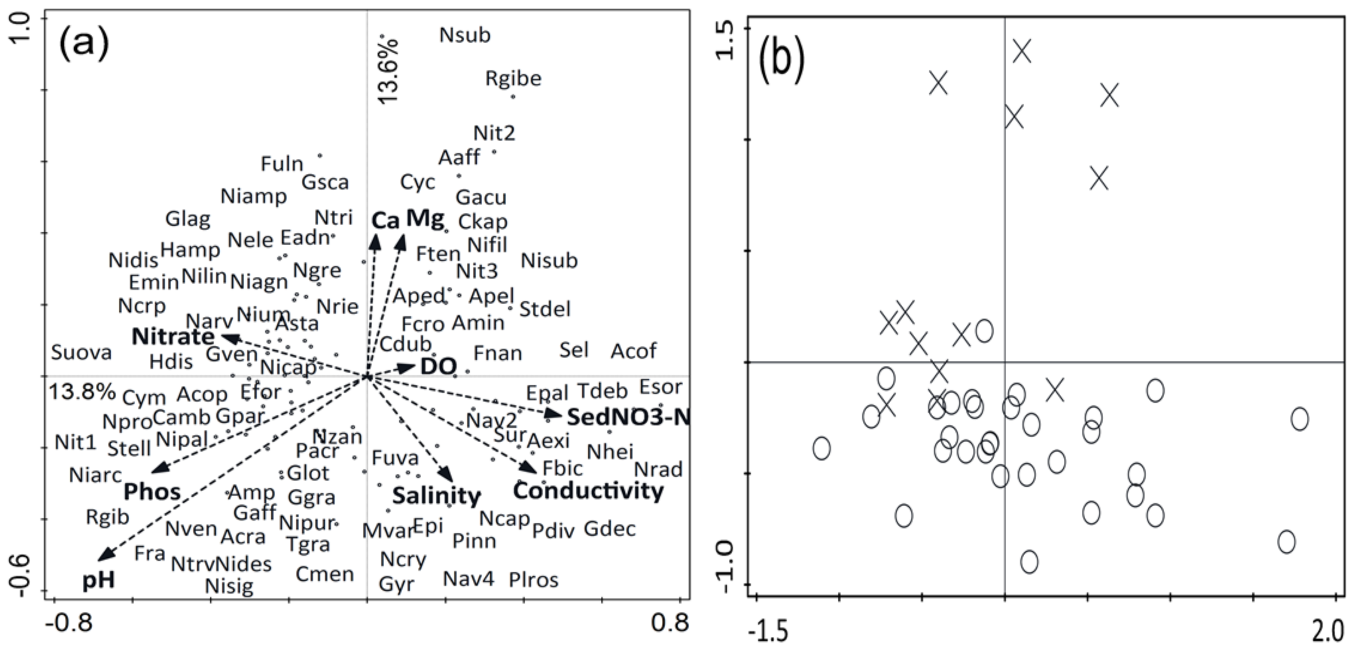

**Figure 4.2:** Ordination diagram based on canonical correspondence analysis (CCA) of 22 sampling stations: (***a***) diatom species composition with respect to nine environmental variables (Ca, pH, phosphate, nitrate, Mg, DO, SedNO$_3^-$, conductivity and salinity), and (***b***) the corresponding stream order patterns: cross – stream order 1 and circles – stream order 2/3 sites. Taxa codes are shown in Appendix A.1, Table S1.

## 4.4 Discussion

### *4.4.1 Diatoms as indicators of ionic strength and conductivity*

The study identified the major effects of anthropogenic activities on physico-chemical variables in streams. Diatom community structure and composition in this study closely followed the observed changes in pollution levels as a result of changes in the stream orders, with less polluted sampling sites being associated with diatom communities that were different from highly polluted sampling sites. PERMANOVA identified significant effect of stream order on diatom communities. The algal communities in this study were primarily affected by metal, organic and nutrient concentrations in the streams resulting from agricultural runoff, mineral runoff, and urban runoff as confirmed by the findings of Dalu et al. (2015, 2017b). Stream order is an important component of understanding relationship between diatom assemblages and eutrophication/ organic pollution as was considered by Pan et al. (1996) in a water basin which consisted of 49 streams from 1$^{st}$ to 3$^{rd}$ order. Thus, in a biomonitoring system, stream order is a relevant typological parameter which influences diatom species numbers and diversity.

Based on the CCAs carried using individual variables, SedNO$_3^-$, SedPO$_4^{3-}$, B, K, Fe, Pb, Mg, Na were the most important ions associated with the distribution of diatom communities in Bloukrans River system (Table 4.2). Temperature, conductivity and salinity were also associated with changes in diatom composition in the study. Other authors have also reported the effects of ionic composition and conductivity in lotic and lentic systems (e.g. Potapova and Charles, 2003; Bere and Tundisi, 2009, 2011a, b). Sed$NO_3^-$, Sed$PO_4^{3-}$ were amongst the ions associated with changes in diatom communities. $PO_4^{3-}$ has a relationship with eutrophication and is considered a useful indicator of this phenomenon in the Pampean streams, Argentina (Gomez and Licursi, 2001). Temperature is also an important driver of metabolic activities in benthic diatoms in lotic systems and has also been identified as an important variable for determining river diatom assemblages in urbanised boreal and tropical regions (Bere and Mangadze, 2014; Ingebrigtsen et al., 2016).

The CCA conducted to explore simultaneous effects of variables showed that diatom species distribution was highly correlated with our measured environmental variables (Figure 4.2). The CCA analysis for the Bloukrans River demonstrated that Ca, Mg, DO, Sed$NO_3^-$, conductivity, pH, phosphate and nitrate best explained the variation in the diatom community structure (Figure 4.2). Based on the CCA, the less polluted sites in stream order 1 were characterised by species such as *Fragilaria tenera* (W Smith) Lange-Bertalot, *Cyclostephanos dubius* (Fricke) Round and *Gyrosigma acuminatum* (Kützing) Rabenhorst. These species are known to prefer waters of lower ionic strength (Lowe, 1974; Taylor et al., 2007; Dalu et al., 2015). However, these sampling sites had high calcium levels which are important in the carbonate-bicarbonate buffering system, and because it brings about precipitation of many heavy metals and excessive phosphates, it is often beneficial to diatom growth (Pearsall, 1932). Sites in stream order 2/3 with high levels of phosphate, pH, Sed$NO_3$-N, conductivity and salinity were characterized by species such as *Nitzschia palea* (Kützing) Smith, *G. parvulum* (Kützing) Kützing, *Tryblionella apiculata* Gregory, *Diatoma vulgaris* Bory and *Staurosira elliptica* (Schumann) Williams and Round. These species have also been frequently recorded in waters that are nutrient rich and poorly oxygenated with high electrical conductivity (Lange-Bertalot, 1979; Kilham et al., 1986; Kobayasi and Mayama, 1989; Round, 1991; Van Dam et al., 1994; Lobo et al., 2004; Bere and Tundisi, 2009). The pH is very influential to diatom community composition, making diatoms highly effective indicators for assessing acidification impacts and changes in streams and lakes (Charles and Smol, 1988; ter Braak and van Dam, 1989; Verb and Vis, 2000). Recent studies of environmental monitoring, using diatom communities in hydrological systems of South Africa, have also proved the importance of eutrophication in structuring benthic diatom communities (Taylor, 2007; Walsh and Wepener, 2009; Dalu et al., 2016). Nutrient enrichment may expand the high end of a taxon's salinity tolerance range (Saros and Fritz, 2000). Thus, shifts in diatom species composition along gradients of ionic concentration and composition may be driven in part by nutrients, and hence the process of eutrophication (Bere and Tundisi, 2011a). Knowledge of how diatom communities respond to different types of stress can and should be used to design more robust and cost-effective monitoring programs (Hering et al., 2006). The information gained through this study provide us with baseline information on urban and agricultural pollution in a developing world context in an Austral temperate environment.

### 4.5 Conclusion and recommendations

The results of this study demonstrate that diatom assemblages are distributed continuously along gradients of conductivity and major ions. Thus, diatoms can be recommended as the preferred bioindicators for monitoring highly impacted river systems and to also further examine pollution gradients and impacts of specific/point pollution sources. We therefore recommend future studies to include other potential factors such as hydromorphological alterations (epifaunal substrate, embeddedness, velocity/depth combinations, sediment deposition, channel flow status, channel alteration and frequency of riffles) that have been found to influence diatom assemblage structure in riverine systems, but were not investigated in the current study. Additionally, future studies should focus on the creation of regional calibration datasets which will make it possible to develop finely tuned models to quantitatively infer conductivity and ion concentrations. Reducing anthropogenic contributions of nutrients to streams and rivers, conscientious land use practices, and conserving habitats is therefore imperative to sustaining stream ecosystems.

## 4.6 References


Agriculture Laboratory Association of Southern Africa (AgriLASA), 2004. Soil Handbook. Agriculture Laboratory Association of Southern Africa, Pretoria.

Anderson, M., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Anderson, M.J., ter Braak, C.J.F., 2003. Permutation tests for multifactorial analysis of variance. J. Stat. Comput. Sim. 73, 85–113.

Anderson, N.J., Brodersen, K.P., Ryves, D.B., McGowan, S., Johanssen, L.S., Jeppesen, E., Leng, M.J., 2008. Climate versus in-lake processes as controls on the development of community structure in a Low-Arctic lake (South-West Greenland). Ecosystems 11, 307–324.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Algal, Benthic Macroinvertebrates and Fish. 2nd edition. EPA 841-B-99-002, US Environmental Protection Agency, Office of Water, Washington DC.

Bere, T., Dalu, T., Mwedzi T., 2016. Detecting the impact of heavy metal contaminated sediment on benthic macroinvertebrate communities in tropical streams. Sci. Total Environ. 572, 147–156.

Bere, T., Mangadze, T., 2014. Diatom communities in streams draining urban areas: community structure in relation to environmental variables. J. Trop. Ecol. 55, 271–281.

Bere, T., Tundisi, J.G., 2009. Weighted average regression and calibration of conductivity and pH of benthic diatoms in streams influenced by urban pollution – Sao Carlos/SP Brazil. Acta Limnol. Bras. 21, 317–325.

Bere, T., Tundisi, J.G., 2010. Biological monitoring of lotic ecosystems: the role of diatoms. Braz. J. Biol.70, 493–502.

Bere, T., Tundisi, J.G., 2011a. Influence of ionic strength and conductivity on benthic diatom communities in a tropical river (Monjolinho), São Carlos-SP, Brazil. Hydrobiologia 661, 261–276.

Bere, T., Tundisi, J.G., 2011b. 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, 93–102.

Beyene, A., Addis, T., Kifle, D., Legesse, W., Kloos, H., Triest, L., 2009. Comparative study of diatoms and macroinvertebrates as indicators of severe water pollution: Case study of the Kebena and Akaki rivers in Addis Ababa, Ethiopia. Ecol. Indic. 9, 381–392.

Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological variation. Ecology 73, 1045–1055.

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59, 39–45.

Chan, K.Y., Bowman, A., Oates, A., 2001. Oxidizible organic carbon fractions and soil quality changes in an Oxic Paleustalfunder different pasture leys. Soil Sci. Soc. Am. J. 166, 61–67.

Charles, D.F., Smol, J.P., 1988. New methods for using diatoms and chrysophytes to infer past pH of low-alkalinity lakes. Limnol. Oceanogr. 33, 1451–1462.

Cholnoky, B.J., 1968. Die Ökologie der Diatomeen in Binnengewässern. Lehre, Cramer.

Clesceri, L.S., Greenberg, A.E., Eaton, A.D., 1998. Standard Methods for the Examination of Water and Wastewater. 20th edition. American Public Health Association, American Water Works Association and Water Environment Federation, Washington DC.

Dalu, T., Bere, T., Froneman, P.W., 2016. Assessment of water quality based on diatom indices in a small temperate river system, Kowie River, South Africa. Water SA 42, 183–193.

Dalu, T., Bere, T., Richoux, N.B., Froneman, P.W., 2015. Assessment of the spatial and temporal variations in periphyton communities along a small temperate river system: A multimetric and stable isotope analysis approach. S. Afr. J. Bot. 100, 203–212.

Dalu, T., Froneman, P.W., 2016. Diatom-based water quality monitoring in southern Africa: challenges and future prospects. Water SA 42, 551–559.

Dalu, T., Wasserman, R.J., Magoro, M., Mwedzi, T., Froneman, P.W., Weyl, O.L.F., 2017a. Variation partitioning of benthic diatom community matrices: Effect of multiple variables on benthic diatom communities in an Austral temperate river system. Sci. Total Environ. 601, 73–82.

Dalu, T., Wasserman, R.J., Tonkin, J.D., Alexander, M.E., Dalu, M.T.B., Motitso, S., Manungo, K.I., Bepe, O., Dube, T.,2017c. Assessing drivers of benthic macroinvertebrate community structure in African highlands: an exploration using multivariate analysis. Sci. Total Environ. 601–602, 1340–1348.

Dalu, T., Wasserman, R.J., Tonkin, J.D., Mwedzi, T., Magoro, M., Weyl, O.L.F., 2017b. Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macroinvertebrate communities in an austral South African stream. Sci. Total Environ. 607–608, 317–325.

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.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163–182.

Gŏmez, N., Licursi, M., 2001. The Pampean Diatom Index (IDP) for assessment of rivers and streams in Argentina. Aquat. Ecol. 35, 173–181.

Hering, D., Johnson, R.K., Buffagni, A., 2006. Linking organism groups – major results and conclusions from the STAR project. Hydrobiologia 566, 109–113.

Higgins, J.V., Bryer, M.T., Khoury, M.L., Fitzhugh, T.W., 2005. A freshwater classification approach for biodiversity conservation planning. Conserv. Biol. 19, 432–445. R.A.

Ingebrigtsen, R.A., Hansen, E., Andersen, J.H., Eilertsen, H.C., 2016. Light and temperature effects on bioactivity in diatoms. J. Appl. Phycol. 28, 939-950

Kilham, P., Kilham, S.S., Hecky, R.E., 1986. Hypothesized resources relationships among African plankton diatoms. Limnol. Oceanogr. 31, 1169–1181.

Kobayasi, H., Mayama, S., 1989. Most pollution-tolerant diatoms of severely polluted rivers in the vicinity of Tokyo. Jpn. J. Phycol. 30, 188–196

Lange-Bertalot, H., 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia 64, 285–304.

Lobo, E.A., Callegaro, V.L.M., Hermany, G., Bes, D., Wetzel, C.E., Oliveira, M.A., 2004. Use of epilithic diatoms as bioindicator from lotic systems in southern Brazil, with special emphasis on eutrophication. Acta Limnol. Bras.16, 25–40.

Lowe, R.L., 1974. Environmental Requirements and Pollution Tolerance of Freshwater Diatoms. United States Environmental Protection Agency, EPA-670/4– 74–005. Cincinnati, Ohio.

Mangadze, T., Bere, T., Mwedzi, T., 2016. Choice of biota in stream assessment and monitoring programs in tropical streams: a comparison of diatoms, macroinvertebrates and fish. Ecol. Indic. 63, 128–143.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82, 290–297.

Miyamoto, H., Hashimoto, T., Michioku, K., 2011. Basin wide distribution of land use and human population: stream order modeling and river basin classification in Japan. Environ. Manage. 47, 885–898.

Mwedzi, T., Bere, T., Mangadze, T., 2016. Macroinvertebrate assemblages in agricultural, mining, and urban tropical streams: implications for conservation and management. Environ. Sci. Pollut. R. 23, 11181–11192.

Nel, H.A., Dalu, T., Wasserman, R.J., 2018. Sinks and sources: assessing microplastic abundance in river sediment and deposit feeders in an Austral urban river system. Sci. Total Environ. 612, 950–956.

Nhiwatiwa, T., Dalu, T., Brendonck, L., 2017. Impact of irrigation-based sugarcane cultivation on the Chiredzi and Runde Rivers quality, Zimbabwe. Sci. Total Environ. 587–588, 316–325.

Pan, Y., Herlihy, A., Kaufmann, P., Wigington, J., Van Sickle, J., Moser, T., 2004. Linkages among land-use, water quality, physical habitat conditions and lotic diatom assemblages: A multi-spatial scale assessment. Hydrobiologia 515, 59–73.

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. J. N. Am. Benthol. Soc. 15, 481–495.

Pappas, J.L., Stoermer, E.F., 1996. Formulation of a method to count number of individuals' representative of number of species in algal communities. J. Phycol. *32*, 693–196.

Pearsall, W.H., 1932. Phytoplankton in the English Lakes II. Composition of the phytoplankton in relation to dissolved substances. J. Ecol. 2, 241–262.

Platts, W. S., Megaham, W.R., Minshall, H.W., 1983. Methods for evaluating stream, riparian, and biotic conditions. U.S. Forest Service General Technical Report INT-138.

Potapova, M., Charles, D.F., 2002. Benthic diatoms in USA Rivers: distributions along speciation and environmental gradients. J. Biogeogr. 29, 167–187.

Potapova, M., Charles, D.F., 2003. Distribution of benthic diatoms in U.S. Rivers in relation to conductivity and ionic composition. Freshwater Biol. 48, 1311–1328.

Round, F.E., 1991. Diatoms in river water-monitoring studies. J. Appl. Phycol. 3, 129–145.

Saros, J.E., Fritz. S.C., 2000. Nutrients as a link between ionic concentrations composition and diatom distributions in saline lakes. J. Paleolimnol. 23, 449–453.

SPSS Inc., 2007. SPSS Release 16.0.0 for Windows. Polar Engineering and Consulting. SPSS Inc., Chicago (IL).

Strahler, A.N.,1957. Quantitative analysis of watershed geomorphology. Transactions of the American Geophysical Union 38, 913–920.

Tan, X., Ma, P., Xia, X., Zhang, Q., 2014a. Spatial pattern of benthic diatoms and water quality assessment using diatom indices in a subtropical river, China. Clean Soil Air Water 42, 20–28.

Tan, X., Xia, X., Zhao, Q., Zhang, Q., 2014b. Temporal variations of benthic diatom community and its main influencing factors in a subtropical river, China. Environ. Sci. Pollut. R. 21, 434–444.

Taylor, J.C., Harding, W.R., Archibald, C.G.M., 2005. A Methods Manual for the Collection, Preparation and Analysis of Diatom Samples. Version 1.0. WRC Report No. TT 281/07. Water Research Commission, Pretoria.

Taylor, J.C., Harding, W.R., Archibald, C.G.M., 2007. An Illustrated Guide to some Common Diatom Species from South Africa. WRC Report No. TT 282/07. Water Research Commission, Pretoria.

ter Braak, C.F.J., Van Dam, H., 1989. Inferring pH from diatoms: a comparison of old and new calibration methods. Hydrobiologia 178, 209–223.

Ter Braak, C.J.F., Prentice, I.C., 1988. A theory of gradient analysis. Adv. Ecol. Res. 18, 271–313.

ter Braak, C.J.F., Šmilauer, P., 2002. CANOCO Reference Manual and Can Draw for Windows User's Guide: Software for community ordination, version 4.5. Microcomputer Power, Ithaca, New York.

Ter Braak, C.J.F., Verdonschot, P.F.M., 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat. Sci. 37, 130–137.

Van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Neth. J. Aquat. Ecol. 28, 117–133.

Verb, R.G., Vis, M.L., 2000. Comparison of benthic diatom assemblages from streams draining abandoned and reclaimed coal mines and nonimpacted sites. J. N. Am. Benthol. Soc. 19, 274–288.

Waite, I.R., Herlihy, A.T., Larsen, D.P., Klemm, D.J., 2000. Comparing strengths of geographic and non-geographic classifications of stream benthic macroinvertebrates in the Mid-Atlantic Highlands, USA. J. N. Am. Benthol. Soc. 19, 429–441.

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, 579–594.

Zhang, Y., Wang, B., Han, M., Wang, L., 2012. Relationships between the seasonal variations of macroinvertebrates, and land-uses for biomonitoring in the Xitiaoxi River watershed, China. Int. Rev. Hydrobiol. 97, 184–199.

# Chapter 5: Water quality assessment in a small austral temperate river system (Bloukrans River system, South Africa): application of multivariate analysis and diatom indices

* This chapter has been published as: **Mangadze, T**., Taylor, J.C., Froneman, P.W., Dalu, T., 2019. Water quality assessment in a small austral temperate river system (Bloukrans River system, South Africa): application of multivariate analysis and diatom indices. *South African Journal of Botany* 125, 353-359.

**Abstract**: Diatoms are siliceous micro-algae and have been used with increasing frequency as bioindicators in aquatic ecosystems. Typically, European diatom indices have been modified and augmented with data on taxa endemic to and commonly found in other parts of the world. In order to further test the application of diatom indices, water quality and diatom sampling were performed in the Bloukrans River system, Eastern Cape Province of South Africa. Nine sites with contrasting water quality were sampled along the length of the river in February, May and July 2018. Canonical correspondence analysis indicated that differences in diatom community assemblages were best explained by dissolved oxygen, temperature, nitrate, conductivity and phosphate. Diatom-based indices incorporated in OMNIDIA software were also applied to assess the integrity of the water quality as indicated by diatom communities. Several foreign indices (e.g. the trophic diatom index (TDI), biological diatom index (BDI)) and the South African Diatom Index (SADI) were used in the study. The SADI demonstrated that the Bloukrans River was impacted and had significant correlations with water quality variables ($p$ < 0.05). The wider use of the SADI as an indicator of water quality conditions in South African lotic systems is highly recommended.

**Keywords:** Aquatic ecosystems, land-use activities, indicators, biomonitoring, South African Diatom Index (SADI)

## 5.1 Introduction

Throughout the world and particularly the developing world, freshwater ecosystems have experienced a long history of modification by humans (Kilonzo et al., 2014; Munyika et al., 2014) and as a consequence, are recognised as among the most threatened ecosystems (Dudgeon et al., 2006). As rivers continue to be degraded, recent advances in the monitoring of lotic systems have shifted from the measurement of water quality parameters to the use of biotic indicators, which are fast and cost-effective for assessing the effects of environmental stressors (Taylor et al., 2007a; Raburu and Masese, 2012; Bere et al., 2014; Mangadze et al., 2019).

Assessment of water quality using diatoms as bioindicators has gained momentum in South Africa (e.g. Taylor et al., 2007a; Dalu and Froneman, 2016; Mangadze et al., 2017; Musa and Greenfield, 2018) and is now part of the national River Eco-status Monitoring Programme (REMP) since 2016. Diatom-based indices are valuable for monitoring habitats in which it is difficult or inappropriate to use other biological monitoring tools (e.g. indices such as South African Scouring System (SASS) for macroinvertebrates is unsuitable for use in canals and effluent streams). Additionally, diatoms make up the largest proportion of periphyton communities and have specific ecological tolerances, short generation time and preferences, creating a well-adapted biological model for environmental monitoring (Bere and Tundisi, 2011; Bere and Mangadze, 2014). The study of diatoms in South Africa dates back to the 1960s, where most of the research were taxonomic (Giffen, 1963, 1966, 1970; Cholnoky, 1955, 1963, 1965, 1968; Schoeman and Archibald, 1976; Archibald, 1983) but included important diatom ecological studies (Cholnoky, 1968). Studies based on ecological interpretations using quantitative data have subsequently been undertaken and confirmed that diatoms can be used as ecological indicators in freshwater systems (e.g. Bate et al., 2002; De la Rey et al., 2004; Taylor et al., 2005).

Normally, the assessment of biological conditions of streams and rivers has been determined by foreign methods (e.g. Bate et al., 2004; Taylor et al., 2007a, b; Bere et al., 2014), because of lack of information on ecological preferences and tolerances of diatoms in developing countries. However, ecological and floristic differences among regions may contribute to differences in the water quality characteristics of rivers and may lead to variation in diatom taxa composition (Bere, 2016). Endemic diatom species may also occur in different regions, necessitating development of region-specific indices (Harding and Taylor, 2011). This prompted the first attempts to develop the South African Diatom Index (SADI), which incorporates South African endemic species (Harding and Taylor, 2011).

The SADI is based on the same equation as the majority of international diatom indices but it is a modified version of the Specific Pollution Sensitivity Index (SPI), which has the broadest species base of any index currently in use (Harding and Taylor, 2011). However, this index was developed in the northern parts of South Africa and still requires input from other regional studies. Due to varying climatic conditions among different regions of South Africa, there is a need for validation and testing of the SADI in all the country’s river systems to ensure that its scores provide an accurate reflection of the exact type of environmental pollution (Holmes and Taylor, 2015). Thus, the aim of this study was to evaluate the applicability of the SADI and other indices in the Eastern Cape region of South Africa. The results from this study will allow us to ultimately test the efficiency and practical use of the SADI for the south-eastern region of the country.

## 5.2 Materials and methods

### *5.2.1 Study area*

The Bloukrans River system is in the Eastern Cape Province of South Africa (Figure 5.1). The river drains the town of Makhanda (previously Grahamstown) and Belmont Valley farms, flowing in the south-easterly direction. In 2011, the South African National Statistics Agency estimated the population of Makhanda at 67,264 inhabitants. The Bloukrans River catchment area is ~ 230 $km^2$, with a total river length of ~ 40 km. The Bloukrans River is located on the eastern periphery of the Mediterranean rainfall area, i.e. the warm temperate climatic zone, which covers the southern Cape. Mean annual precipitation in the study area is around 600-800 mm, with minimum and maximum air temperatures of 1.5 and 43°C, respectively.

The Bloukrans River is one of the most important rivers in the Eastern Cape Province, supporting diverse activities including cattle and irrigation farming, private game reserves and urban centres. Previous studies indicated that these agricultural and urban activities have contributed to significant soil erosion and nutrient enrichment (Mangadze et al., 2017).

Site selection was undertaken by delineating all forms of degradation in the Bloukrans River catchment and then selecting a stratified random sampling design ($n$ = 9; Figure 5.1) according to Barbour et al. (1999). To capture the impacts of various point and diffuse sources of pollution from agricultural activities, overflowing sewage drains and litter, sites were located both upstream and downstream of the discharge points. A total of nine sites were established, eight along the Bloukrans River and one site on the Kowie River. Sites S3, S4 and S9 were in the forested upper reaches of the system with minimal anthropogenic activities and served as reference sites. Sites S8, S5, S7 and S6 were located in the moderately polluted agricultural area and sites S1 and S2 in the highly polluted lower reaches of the basin, downstream of

Makhanda (Figure 5.1). Sampling was conducted on three separate occasions, in February (hot-wet season), May and July (cool-dry season) in 2018 to capture the two flow extremes typical of the study area.

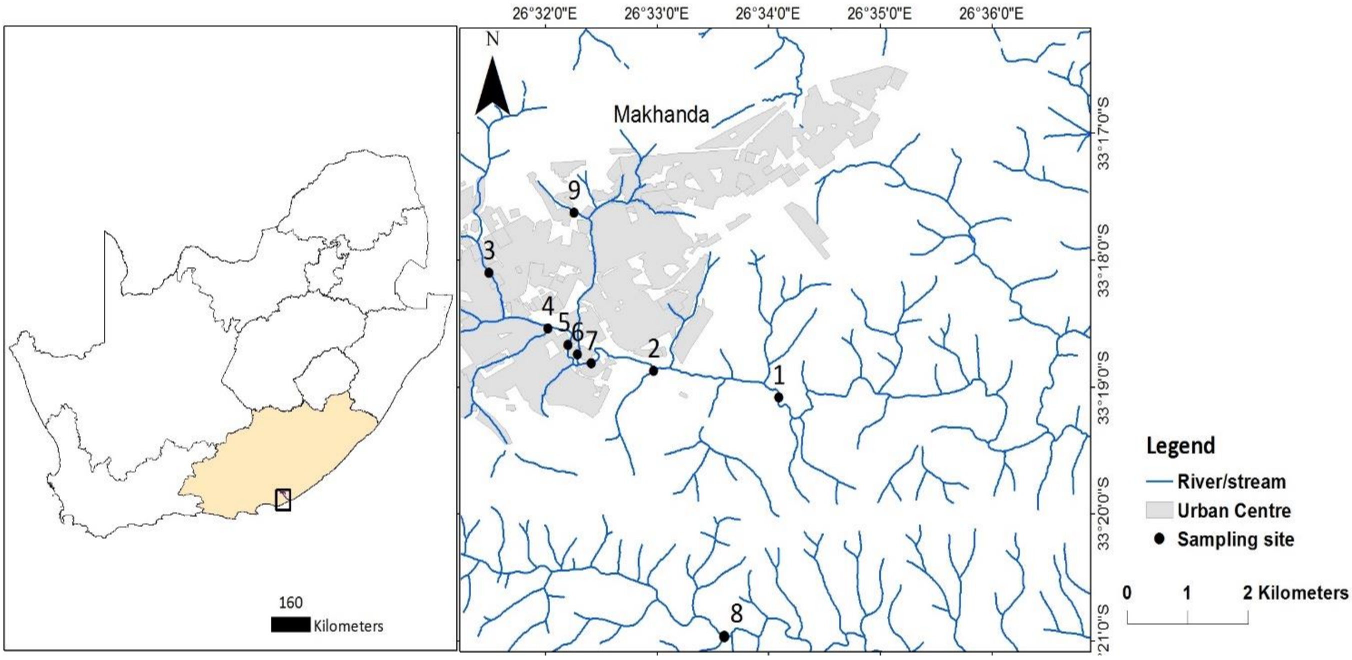


**Figure 5.1:** The location of the study area and sampling sites.

### *5.2.2 Water quality variables*

Water quality variables (e.g. conductivity, pH, salinity and temperature) were measured *in situ* using a portable multi-parameter probe (PCTestr 35, Eutech/Oakton Instruments, Singapore). Dissolved Oxygen (DO) and turbidity were determined using a DO meter (DO 850045, Per Scientific, Taiwan) and turbidity meter (AL250T-IR, Aqualytic, Germany), respectively. Water samples (500 mL) were also collected at each site following standard methods (APHA, 1988) and transported to the laboratory within three hours for analysis of nutrients (phosphate and nitrate). Nutrient concentrations, employed as proxies for land-use activities, were determined by using a multi-parameter bench photometer (HI 83203, Hanna Instruments Inc., Rhode Island) within 12 h of collection according to the methods described in Dalu et al. (2016).

### *5.2.3 Biological samples*

Epilithic diatoms were sampled randomly from each sampling site (Barbour et al., 1999; Potopova et al., 2005) and a surface area of 25 $cm^2$ for each riffle was brushed with a toothbrush and pooled to form a single sample. Detailed sampling and collection procedures are described by Taylor (2005). The algal suspension from each sample was emptied into a labelled plastic container and preserved in Lugol's iodine for microscopic examination in the laboratory. Diatoms were acid-cleaned using the potassium permanganate/hydrochloric acid method by Taylor et al. (2005). A minimum of 300 valves per sample was counted at 1000× magnification using an Olympus CX light microscope. Diatom identification to species or genus level was done using guides by Taylor et al. (2007c) and the current accepted nomenclature was checked with AlgaeBase (Guiry and Guiry, 2017).

### *5.2.4 Data analysis*

Diatom community data were entered into OMNIDIA version 5.3 (Lecointe et al., 1993) software which incorporated the following indices: the South African Diatom Index (SADI), based on the French Specific Pollution Sensitivity Index (SPI; Harding and Taylor, 2011), Biological Diatom Index (BDI) which includes 14 water quality parameters (Debenest et al., 2008), Percentage Pollution-Tolerant Valves (%PTV) which reflects organic pollution and forms part of the UK Trophic Diatom Index (TDI) (Kelly and Whitton, 1995), Eutrophication/Pollution Index (EPI; Dell'Uomo, 1996), Trophic Index (TI; Rott et al., 1999), Watanabe index (WAT; Watanabe et al., 1986), Commission of Economical Community Index (CEC; Descy and Coste, 1991) and Biological Index of Water Quality (BIWQ; Lobo et al., 2004). With the exception of the CEC, SADI, TDI and WAT, all the indices are based on the formula by Zelinka and Marvan (1961). Pearson correlation analysis was performed to determine the relationship between diatom indices and the environmental variables using SPSS 16.0 for Windows software (SPSS Inc, 2007).

Two-way analysis of variance (ANOVA) with Turkey's post-hoc HSD tests were used to compare means of water quality variables among sampling sites and periods after testing for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Diatom relative abundances (%) were then analysed using multivariate data analysis to assess variations in assemblage composition among the different sites. Preliminary detrended correspondence analysis (DCA) was applied to determine whether linear or unimodal analysis ordination methods should be used. The DCA indicated that the gradient was >3 standard deviation units (i.e. 4.2), indicating that the use of unimodal ordination techniques was more appropriate (Ter Braak and Verdonschot, 1995). Thus, canonical correspondence analysis (CCA) was used to assess the relationships between water quality parameters and benthic diatom communities from the different sites. Preliminary CCA selected a subset of water quality parameters that were significant with relation to diatom species distribution patterns and removed variables that had a variance inflation factor above 20 from the analysis (Ter Braak and Smilauer, 2012). Rare species were down weighted in the CCA analysis by including the relative abundance of diatom taxa that were present in a minimum of four samples and had a relative abundance of >1% in at least one sample. The CCA was performed using CANOCO version 5.1 (Ter Braak and Smilauer, 2012). Univariate analysis was conducted using SPSS version 16 (SPSS Inc, 2007).

## 5.3 Results

### *5.3.1 Water quality*

The values of the physico-chemical variables recorded in the Bloukrans River system during this study are summarised in Table 5.1. No significant differences ($F_{(1.61)} = 1.279$, $p = 0.18$) were observed in mean environmental variables between the three sampling periods. The differences in temperature and pH observed were not statistically significant between the sampling sites. Nitrate, conductivity, salinity and phosphate were significantly higher (ANOVA, $p < 0.05$) at sites S1 and S2, which were affected by overflowing sewage effluent from Makhanda as well as dumped garbage/refuse in the streams compared to the rest of the sites. Dissolved oxygen concentrations were significantly lower at sites S1 and S2 compared to the rest of the sites, with sites S3 and S9 having significantly higher (ANOVA, $p < 0.05$) concentrations compared to the rest of the sites.

### *5.3.2 Diatom community composition*

During the entire study, 118 diatom species representing 36 genera were identified. The mean diatom frequency of occurrence for the dominant taxa across the study area is highlighted in Appendix B.1, Table S2. The composition of the diatom assemblages varied both spatially and temporally (Figure 5.2). The first four CCA axes explained 70.9% of the total variance in the community due to measured environmental variables (Table 5.2). The first two axes explained44.8 % and 17.9%, respectively, of the variance among sites in the diatom assemblage data. Monte Carlo unrestricted permutation test indicated that axes 1 and 2 were statistically significant ($p < 0.05$).

**Table 5.1:** Means (± SD) of water quality variables recorded at all the sites (S1-S9) in February, May and July 2018. Different letters indicate significant differences obtained through Turkey's post hoc comparison test (Tukey's HSD, $p < 0.05$).

| Parameter | Site | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 |
| Temperature(°C) | 15.61±3.68 | 16.95±2.87 | 15.98±2.12 | 15.93±2.41 | 13±5.37 | 17.28±4.81 | 15.43 ± 4.26 | 16.23±1.99 | 16.03±2.1 |
| Conductivity (µS cm$^{-1}$) | 1848.77±668.04$^{a}$ | 1993.77±174.89$^{a}$ | 704.8±299.63$^{c}$ | 602±353.21$^{c}$ | 1098.11±618.34$^{b}$ | 1522.77±256.71$^{a}$ | 942.33±1025.98$^{b}$ | 830.88±130.23$^{c}$ | 704.66±291.49$^{c}$ |
| DO (mg L$^{-1}$) | 4.42±1.92$^{b}$ | 4.71±2.19$^{b}$ | 6.53±2.75$^{a}$ | 6.84±2.59$^{a}$ | 5.81± 0.86$^{b}$ | 3.86±3.3$^{b}$ | 4.79±2.71$^{b}$ | 4.71±2.4$^{b}$ | 6.14±2.3$^{a}$ |
| pH | 8.23±0.12 | 8.35±0.09 | 7.48±0.54 | 7.93±0.11 | 7.82±0.69 | 7.86±0.1 | 7.55±0.31 | 7.48±0.97 | 7.22±0.19 |
| Salinity (ppt) | 872.11±279.28$^{a}$ | 1000.88±86.5$^{a}$ | 368.77±81.32$^{b}$ | 374.33±51.79$^{b}$ | 422.55±380.77$^{b}$ | 758.22±136$^{b}$ | 536.33±466.26$^{b}$ | 337±111.3$^{b}$ | 201.11±29.23$^{c}$ |
| Nitrate (mg L$^{-1}$) | 114±49.8$^{a}$ | 143.95±63.41$^{a}$ | 6.38±6.29$^{c}$ | 7.6±1$^{c}$ | 20.45±32$^{b}$ | 18.08±21.49$^{b}$ | 18.71±29.61$^{b}$ | 24.21±32.96$^{b}$ | 1.56±1.06$^{c}$ |
| Phosphate (mg L$^{-1}$) | 6.29±3.85$^{a}$ | 3.61±1.83$^{a}$ | 1.83±1.55$^{b}$ | 1.7±1.41$^{b}$ | 1.66±1.96$^{b}$ | 4.68±3.64$^{a}$ | 2.83±2.1$^{b}$ | 1.04±1.2$^{b}$ | 1.1±0.72$^{b}$ |

Phosphate, nitrate and conductivity were positively associated with the first and second axes, while DO and temperature were negatively associated with the first axis (Figure 5.2). The CCA axes 1 and 2 separated the sites into three groupings. The first grouping was comprised of the less impacted agricultural sites S3, S4 and S9 with good to medium water quality (Figure 5.2) that were negatively associated with the first axis (Figure 5.2). These sites were associated with higher DO and temperature levels and had low phosphate and nitrate concentrations. This grouping was characterised by species such as *Encyonema minutum* (Kützing) Grunow, *Fragilaria tenera* (W Smith) Lange-Bertalot, *Epithemia sorex* Kützing, *Pinnularia divergens* W. Smith. However, the second grouping of sites S8, S5, S6 and S7 were the most variable in their diatom composition and occurred across the entire ordination space. (Figure 5.2). Diatoms occurring in these sites were therefore predominantly a subset of those occurring in all the other different land use sites. The third grouping consisted of very poor water quality downstream of the urban-agricultural sites S1 and S2 that were positively associated with the first and second axes (Figure 5.2). These sampling sites were associated with high nutrient concentrations and conductivity levels. Sites S1 and S2 were associated with *Gomphonema parvulum* (Kützing) Kützing, *Nitzschia palea* (Kützing) W. Smith, *Navicula radiosa* Kützing, *Rhopalodia gibba* (Ehrenberg) O. Müller and *Trybionella apiculata* Gregory (Figure 5.2).

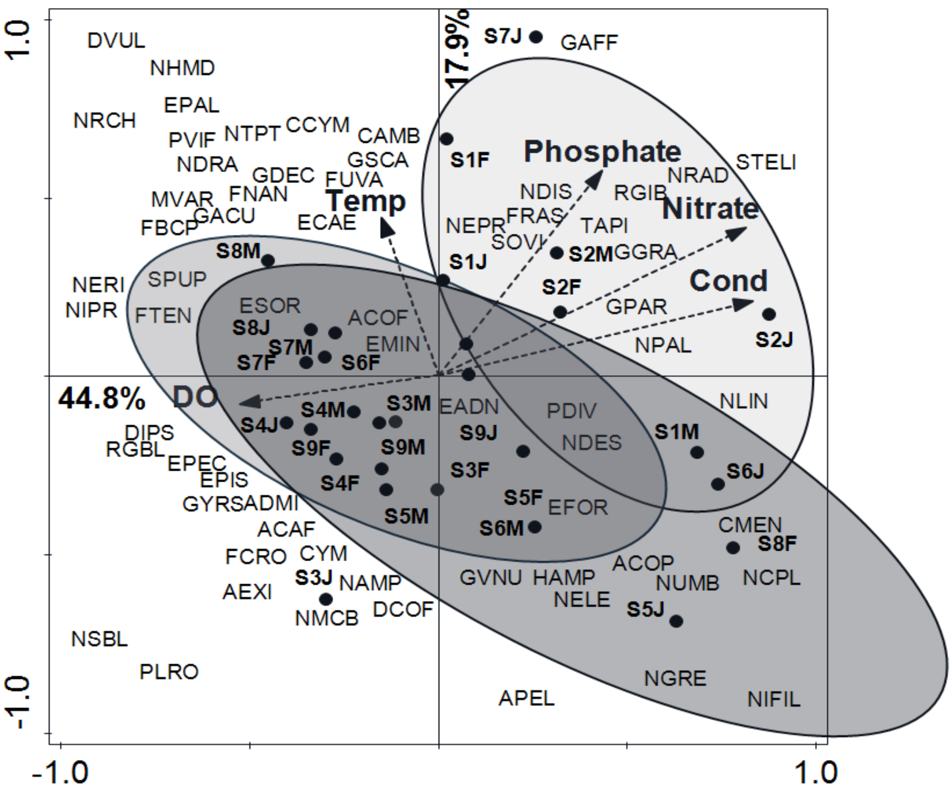


**Figure 5.2:** Ordination tri-plot based on canonical correspondence analysis (CCA) showing effects of five water quality variables (DO, temperature, nitrate, conductivity and phosphate) on diatom species composition in nine sampling sites during three sampling periods (S1F site sampled in February, S1M site sampled in May, S1J site sampled in July). Abbreviations: Temp – temperature, Cond – conductivity. Taxa codes correspond to those in Appendix B.1, Table S2.

**Table 5.2:** Results of the CCA showing the most dominant diatom species composition at nine sites with respect to five environmental variables (DO, temperature, nitrate, conductivity and phosphate).

| Statistic | Axis 1 | Axis 2 | Axis 3 | Axis 4 |
|---|---|---|---|---|
| Eigenvalues | 0.42 | 0.27 | 0.21 | 0.19 |
| Explained variation (cumulative) | 8.77 | 14.61 | 19.06 | 23.15 |
| Pseudo-canonical correlation | 0.94 | 0.92 | 0.91 | 0.93 |
| Explained fitted variation (cumulative) | 26.85 | 44.75 | 58.38 | 70.9 |

### *5.3.3 Diatom indices*

Of the 118 diatom species identified, only 107 species were listed in OMNIDIA. This is because the rare taxa were not included when these indices were developed (Lobo et al., 2004). The less impacted sites S3, S4 and S9 had high SADI scores recorded on all sampling occasions indicating moderate to good water quality (Table 5.3). Low SADI scores were recorded for sites S1 (7.47 ± 3.99) and S2 (7.2 ± 4.34), indicating a general deterioration in water quality. The BDI scores were also close to those of SADI, showing the same general trend in water quality (Table 5.3). The %PTV of most sites (i.e. S1, S2, S5, S7, S8 and S6) was also above the 20% limit indicating the presence of organic pollutants (Table 5.3).

Correlation analysis indicated significant correlations ($p < 0.05$) between water quality variables and diatom index scores, ranging from $R^2 = 0.21$ to $R^2 = 0.87$. Temperature, salinity, pH and nitrate did not correlate significantly with the WAT index (Table 5.4). Correlations between the SADI scores and water quality variables was generally significantly higher ($p < 0.05$) compared to the correlations between some foreign index scores such as WAT, BIWQ, TI and CEC and the measured water quality variables (Table 5.4).

**Table 5.3:** Means (± SD) of diatom index scores recorded for the Bloukrans River system. Abbreviations: EPI – Eutrophication/Pollution Index, BDI – Biological Diatom Index, TI – Trophic Index, WAT – Watanabe Index, SADI – South African Diatom Index, CEC – Commission of Economical Community Index, TDI – Trophic Diatom Index, % PTV – Percentage Pollution-Tolerant Valves, BIWQ – Biological Index of Water Quality Trophic Index.

| **Indices** | **Sites** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** | **S7** | **S8** | **S9** |
| WAT | 10.27 ± 0.57 | 10.73 ± 0.31 | 11.73 ± 1.03 | 10.63 ± 0.21 | 10.63 ± 1.68 | 10.3 ± 0.95 | 10.03 ± 0.06 | 10.53 ± 0.4 | 9.9 ± 1.01 |
| TDI | 5.63 ± 1.4 | 6.8 ± 2.72 | 7.6 ± 1.87 | 12.4 ± 3.37 | 7.9 ± 3.72 | 5.6 ± 1.35 | 5.37 ± 2.22 | 13.13 ± 4.4 | 9.33 ± 3 |
| %PTV | 46.37 ± 31.17 | 39.83 ± 34.55 | 15.63 ± 7.21 | 9.27 ± 7.61 | 36.5 ± 34.38 | 26.13 ± 14.88 | 32.33 ± 28.39 | 38.87 ± 22.42 | 13.1 ± 3.82 |
| CEC | 3.37 ± 5.83 | 2.3 ± 3.98 | 3.77 ± 6.52 | 4.83 ± 8.37 | 5.6 ± 9.7 | 4.57 ± 7.91 | 0 | 13.43 ± 4.22 | 9.47 ± 8.29 |
| SADI | 7.47 ± 3.99 | 7.2 ± 4.34 | 13.3 ± 0.87 | 15.43 ± 1.88 | 9.37 ± 6.27 | 9.77 ± 4.88 | 9.53 ± 3.72 | 11.9 ± 3.3 | 12.33 ± 1.16 |
| BDI | 7.83 ± 4.03 | 9.23 ± 5 | 12.67 ± 1.48 | 16.37 ± 2.12 | 10.47 ± 6.99 | 8.9 ± 3.91 | 9.97 ± 4.15 | 14.43 ± 3.98 | 13.17 ± 0.25 |
| EPI-D | 8 ± 2.71 | 9.6 ± 3.85 | 13.17 ± 0.67 | 13.47 ± 2.89 | 9.1 ± 4.16 | 7.8 ± 3.65 | 9.23 ± 3.76 | 12.37 ± 3.61 | 12.17 ± 1.4 |
| BIWQ | 11.97 ± 7.81 | 12.17 ± 4.62 | 9.13 ± 5.45 | 7.13 ± 4.19 | 14.27 ± 2.49 | 14.03 ± 1.76 | 11 ± 2.41 | 13.4 ± 2.82 | 8.9 ± 4.51 |
| TI | 4.87 ± 2.9 | 6.3 ± 4.2 | 8.07 ± 3.59 | 10.17 ± 3.62 | 6.43 ± 3.1 | 4.63 ± 2.41 | 6.03 ± 3.57 | 9.43 ± 4.24 | 8.07 ± 1.85 |
| Diversity | 3.66 ± 0.3 | 3.55 ± 0.38 | 3.66 ± 0.49 | 3.92 ± 0.09 | 3.76 ± 0.68 | 4.12 ± 0.58 | 3.11 ± 0.89 | 3.79 ± 0.19 | 3.68 ± 0.4 |
| Evenness | 0.57 ± 0.08 | 0.53 ± 0.06 | 0.54 ± 0.07 | 0.58 ± 0.02 | 0.56 ± 0.1 | 0.61 ± 0.09 | 0.46 ± 0.13 | 0.57 ± 0.03 | 0.55 ± 0.06 |

**Table 5.4:** Pearson correlation coefficients between measured environmental variables and diatom index scores at nine sites in the Bloukrans River system. Abbreviations: EPI – Eutrophication/Pollution Index, BDI – Biological Diatom Index, CEC – Commission of Economical Community Index, TI – Trophic Index, WAT – Watanabe Index, SADI – South African Diatom Index, TDI – Trophic Diatom Index, % PTV – Percentage Pollution-Tolerant Valves, BIWQ – Biological Index of Water Quality Trophic Index. Numerical values indicate significant correlations at $p \leq 0.05$.

| | Temperature | Conductivity | DO | pH | Salinity | Nitrate | Phosphate |
|---|---|---|---|---|---|---|---|
| **WAT** | – | -0.34 | 0.42 | – | – | – | -0.39 |
| **TDI** | -0.31 | -0.62 | 0.49 | -0.33 | -0.63 | -0.37 | -0.71 |
| **%PTV** | -0.45 | 0.66 | -0.40 | 0.34 | 0.51 | 0.58 | 0.53 |
| **CEC** | 0.23 | -0.39 | 0.31 | -0.49 | -0.56 | -0.33 | -0.53 |
| **SADI** | 0.55 | -0.81 | 0.78 | -0.47 | -0.78 | -0.63 | -0.79 |
| **BDI** | -0.41 | -0.83 | 0.72 | -0.50 | -0.71 | -0.60 | -0.70 |
| **EPI-D** | 0.59 | -0.78 | 0.79 | -0.52 | -0.73 | -0.48 | -0.78 |
| **BIWQ** | -0.35 | 0.55 | -0.76 | 0.36 | 0.43 | 0.29 | 0.33 |
| **TI** | 0.41 | -0.76 | 0.72 | -0.44 | -0.72 | -0.45 | -0.70 |

## 5.4 Discussion

### *5.4.1 Diatom community composition in relation to water quality variables*

Patterns of diatom assemblages reflected the anthropogenic impacts along the Bloukrans River system. Water quality variables, such as DO, temperature, nitrate, conductivity and phosphate were associated with land-use patterns, and further linked diatom community responses with anthropogenic impacts. Numerous studies have demonstrated that diatom communities vary along nutrient gradients (e.g. Bere and Tundisi, 2011; Dalu et al., 2017b). This was reflected in the CCA results that indicated distinct differences in diatom assemblages in the Bloukrans River sites. Sites S3, S4 and S9 had relatively good water quality and were characterised by species such as *Encyonema minutum*, *Fragilaria tenera*, *Epithemia sorex*, *Pinnularia divergens*. These species are commonly recorded in less polluted waters with lower ionic strength and conductivity (Lowe, 1974; Taylor et al., 2007b). Temperature was also associated with changes in diatom composition in these sampling sites. Pan et al. (1996) observed that temperature is an important driver of metabolic activities in benthic diatoms in rivers and streams. By contrast, sites S1 and S2 with poor water quality were characterised by species such as *Gomphonema parvulum*, *Nitzschia palea*, *Navicula radiosa*, *Rhopalodia gibba* and *Trybionella apiculata*, all of which are indicators of water with high nutrient concentrations and organic pollution, high electrical conductivity, low oxygenated water and low percentage canopy cover (Bere and Tundisi, 2009; Kalyoncu and Şerbetci, 2013). CCA can be a useful tool to identify groups of reference sites with which monitoring sites of different land-use categories can be compared (Rosenberg and Resh, 1993, Mueller et al., 2014; Mangadze et al., 2015). However, model development of multivariate techniques can be challenging and there is an uncertainty in their predictions. Thus, it is essential to use multivariate methods in combination with biotic indices for evaluating the stream health (Reynoldson et al., 1997).

### *5.4.2 Diatom-based indices*

Diatom-based indices simplify the complicated ecology of lotic systems as stream health can be assessed by simple calculation of one metric (Ollis et al., 2006). The present results indicate that sites S3, S4 and S9 were relatively un-impacted by organic contamination (%PTV values below 20) (Table 5.3). A marked increase in %PTV index score (%PTV values >50) at sites S1 and S2. The increase in %PTV index scores in the Bloukrans River is a useful threshold of potential concern for management and conservation of lotic systems. The BDI, EPI and SADI also showed the same results. Site S1 had the lowest water quality, with all SADI values being <10 (Table 5.3). The significant correlations between diatom index scores and water quality variables of rivers recorded also indicate that diatom indices can be reliably employed to assess water quality. Taylor (2004) found strong correlations between diatom indices and water quality variables (conductivity and nutrients) when re-analysing diatom samples from the Jukskei-Crocodile River system in South Africa. Other studies have also found indices developed in Europe, America and Australasia to be applicable in assessing the ecological conditions of river ecosystems in sub-Saharan Africa (Walsh and Wepener, 2009; Bere et al., 2014; Dalu et al., 2016).

Over the last decade, efforts have been devoted to designing a more effective use of diatoms as monitoring and assessment tools of water quality and ecological health for South Africa (Morales et al., 2013; Wetzel et al., 2013). Taylor (2007b) and most recently Dalu and Froneman (2016) highlighted potential limitations of using foreign based indices locally as there may be region-specific taxa that are absent in the indices reference list. Several common and abundant diatom taxa, many of which have only been recently described, are not incorporated into these indices (Harding and Taylor, 2011). It is worth noting that the BDI was not accurate in Austrian conditions as the index was developed from data collected from the French national monitoring network, which was mainly focused on monitoring water quality impacts (Rott et al., 2003).

For that reason, the SADI has been designed to be cost effective (i.e. diatom data are easy to collect and analyse) and appropriate for assessment of water quality and general river conditions in southern Africa (Harding and Taylor, 2011). Based on studies by Taylor (2004), the SPI produced good results and was the most reliable diatom index used under South African conditions. This was also true in this study where SADI had strong significant correlations with all water quality variables considered. The index clearly indicates that wastewater from municipalities and agricultural practises were key contributors to deterioration of water quality in the Bloukrans River system. The SADI has been validated and confirmed for a limited number of ecoregions in South Africa (Harding and Taylor, 2011; Holmes and Taylor, 2015), therefore there is a need for continuous modification and validation in the other ecoregions in order for SADI to qualify as a national biotic index. Further studies from other ecoregions are required as some taxa might have been omitted in this version of SADI. In conclusion, these results support wider use of the SADI as an indicator of water quality conditions in South African river systems.

## 5.5 References


APHA., 1988. Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health association, Washington.

Archibald, R.E.M., 1983. The diatoms of the Sundays and Great Fish rivers in the Eastern Cape Province of South Africa. Bibliotheca Diamotologica. J Cramer, Vaduz, Germany. 431 pp.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: algal, benthic macroinvertebrates and fish (2nd ed.). Washington DC: EPA 841-B-99-002, US Environmental Protection Agency, Office of Water.

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

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

Bere, T., Tundisi, J.G., 2009. Weighted average regression and calibration of conductivity and pH of benthic diatoms in streams influenced by urban pollution – Sao Carlos/SP Brazil. Acta Limnol. Bras. 21, 317-325.

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, 93–102.

Bere, T., Mangadze, T., 2014. Diatom communities in streams draining urban areas, community structure in relation to environmental variables. J. Trop. Ecol. 55, 271–281.

Bere, T., 2016. Challenges of diatom-based biological monitoring and assessment of streams in developing countries. Environ. Sci. Pollut. R. 6, 5477–5486.

Bere, T., Mangadze, T., Mwedzi, T., 2014. The application and testing of diatom-based indices of water quality assessment in the Chinhoyi Town, Zimbabwe. Water SA 3, 503–511.

Cholnoky, B.J., 1955. Diatomeen aus salzhaltigen Binnengewässern der westlichen Kaap-Provinz in Südafrika. Ber. Deutsche Bot. Gesellsch 68, 11–23.

Cholnoky, B.J., 1963. Beiträge zur Kenntnis des marinen Litorals von Südafrika. Bot. Mar 5, 38–83.

Cholnoky, B.J., 1965. Preliminary report on the deposits and recent diatom flora of the St. Lucia Lagoon. Unpublished report. National Institute for Water Research, CSIR, Pretoria. File W 9/4/3/4 Typescript. 13 pp.

Cholnoky, B.J., 1968. Die Diatomeenassoziationen der Santa-LuciaLagune in Natal (Südafrika). Bot. Mar. Vol. XI Supplement 1–121. De Greuyter and Co., Hamburg. DAY JH (ed.) (1981) Estuarine Ecology with Particular Reference to Southern Africa. AA Balkema. Cape Town.

Dalu, T., Froneman, P.W., 2016. Diatom-based water quality monitoring in southern Africa, challenges and future prospects. Water SA 42, 551–559.

Dalu, T., Bere, T., Froneman, P.W., 2016. Assessment of water quality based on diatom indices in a small temperate river system: a case of the Kowie River, South Africa. Water SA 42, 183-193.

Dalu, T., Wasserman, R.J., Magoro, M., Mwedzi, T., Froneman, P.W., Weyl, O. L. F., 2017b. Variation partitioning of benthic diatom community matrices: Effect of multiple variables on benthic diatom communities in an Austral temperate river system. Sci. Total Environ. 601–602,73–82.

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.

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. Aquat. Toxicol. 88, 88–94.

Dell'Uomo, A., 1996. Assessment of water quality of an Apennine river as a pilot study. In Whitton, B. A. and E. Rott (eds), Use of Algae for Monitoring Rivers II. Institut fű̈r Botanik, Universitát Innsbruck, Innsbruck 65–73.

Descy, J.P., Coste, M., 1991. A test of methods for assessing water quality based on diatoms. Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie 24, 2112–2116.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowle, D.J., Lėvěque, C., Naiman, R.J., Prieur-richard, A.H., Soto, D., Tiassny, M.L.J.S., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163–182.

Guiry, M.D., Guiry, G.M., 2017. AlgaeBase, Worldwide electronic publication. Galway: National University of Ireland, Retrieved from http://www.algaebase.org.

Giffen, M.H., 1963. Contributions to the diatom flora of South Africa. I. Diatoms of the estuaries of the Eastern Cape Province. Hydrobiologia 21, 201–265.

Giffen, M.H., 1966. Contributions to the diatom flora of South Africa. 11. Diatoms from the Hogsback region of the Amatola Mountains, Eastern Cape Province South Africa. Beihefte z. Nova Hedwegia Bd. 21, 123–150.

Giffen, M.H., 1970. New and interesting marine and littoral diatoms from Sea Point, near Cape Town, South Africa. Bot. Mar 13, 87–99.

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. WRC Report No 1707/1/11 Water Research Commission, Pretoria.

Holmes, M., Taylor, J.C., 2015. Diatoms as water quality indicators in the upper reaches of the Great Fish River, Eastern Cape, South Africa. Afr J Aquat Sci. 40, 321–338.

Kalyoncu, H., Şerbetci, B., 2013. Applicability of diatom-based water quality assessment indices in Dari stream, Isparta - Turkey. World Acad. Sci. Eng. Technol. 7, 188–196.

Kelly, M. G., Whitton, B.A., 1995. The trophic diatom index: a new index for monitoring eutrophication in rivers. J. Appl. Phycol. 7, 433–444.

Kilonzo, F., Masese, F.O., Van Griensven, A., Willy, B., Joy, O., Lens, P.N.L., 2014. Spatial-temporal variability in water quality and macroinvertebrate assemblages in the Upper Mara River basin, Kenya. Phys. Chem. Earth 67, 93-104.

Lecointe, C., Coste, M., Prygiel, J., 1993. ‘‘Omnidia’’: software for taxonomy, calculation of diatom indices and inventories management. Hydrobiology 269, 509–513.

Lobo, E.A., Callegaro, V.L., Hermany, G., Bes, D., Wetzel, C.E., Oliveira, M.A., 2004. Use of epilithic diatoms as bioindicator from lotic systems in southern Brazil, with special emphasis on eutrophication. Acta Limnol. Bras. 16, 25–40.

Lowe, R.L., 1974. Environmental requirements and pollution tolerance of freshwater diatoms. United States Environmental Protection Agency, Cincinnati, Ohio.EPA-670/4– 74–005.

Mangadze, T., Dalu, T., Froneman, P.W., 2019. Biological monitoring in southern Africa: A review of the current status, challenges and future prospects. Sci. Total Environ. 648, 1492–1499.

Mangadze, T., Wasserman, R.J., Dalu, T., 2017. Use of diatom communities as indicators of conductivity and ionic composition in a small Austral temperate river system. Water, Air and Soil Pollut. 228, 428–439.

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.

Morales, E.A., Guerrero, J.M., Wetzel, C.E., Sala, S., Ector, L., 2013. Unravelling the identity of Fragilaria pinnata Ehrenberg and Staurosira pinnata Ehrenberg: research in progress on a convoluted story. Cryptogamie Algol 34, 89–102.

Mueller, M., Pander, J., Geist, J., 2014. A new tool for assessment and monitoring of community and ecosystem change based on multivariate abundance data integration from different taxonomic groups. Environ. Syst. Res. 3, 12.

Munyika, S., Kongo, V., Kimwaga, R., 2014. River Health Assessment Using Macroinvertebrates and Water Quality Parameters: A Case of the Orange River in Namibia. Phys. Chem. Earth 76-78, 140-148.

Musa, R., Greenfield, R., 2018. Use of diatom indices to categorise impacts on and recovery of a floodplain system in South Africa.  Afr. J. Aquat. Sci. 43, 59-69

Ollis, D.J., Boucher, C., Dallas, H.F., Esler, K., 2006. Preliminary testing of the integrated habitat assessment system (IHAS) for aquatic macroinvertebrates. South Afr. J. Aquat. Sci. 31, 1-14.

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. J. N. Am. Benthol. Soc. 15, 481-495.

Potapova, M., Coles, J.F., Giddings, E.M.P., Zappia, H., 2005. A comparison of the influences of urbanization in contrasting environmental settings on stream benthic algal assemblages. Pp 333–359 in Brown, L.R., Gray, R.H., Hughes, R.M., Meador, M.R., editors. Effects of urbanization on stream ecosystems. American Fisheries Society, Symposium 47, Bethesda, Maryland.

Raburu, P.O., Masese, F.O., 2012. A fish-based index for assessing ecological integrity of riverine ecosystems in Lake Victoria Basin. River Res. Appl. 28, 23–38.

Reynoldson, T.B., Norris, R.H., Resh, V.H., Day, K.E., Rosenberg, D.M., 1997. The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. J. N. Am. Benthol. Soc. 16, 833-852.

Rosenberg, D.M., Resh, V.H., 1993. Freshwater Bio-monitoring and Benthic Macroinvertebrates. Chapman and Hall, New York, USA.

Rott, E., Pipp, E., Pfister, P., 2003. Diatom methods developed for river quality assessment in Austria and a cross-check against numerical trophic indication methods used in Europe. Algological Studies 110, 91–115.

Rott, E., Pipp, E., Pfister, P., van Dam, H., Ortler, K., Binder, N., Pall, K., 1999. Indikationslisten für Aufwuchsalgen in o¨ sterreichischen Fliessgewa¨ ssern. Teil 2: Trophieindikation (sowie geochemische Pra¨ferenzen; taxonomische und toxikologische Anmerkungen). Wasserwirtschaftskataster herasgegeben vom Bundesministerium f. Land- u.Forstwirtschaft, Wien. ISBN 3-85 174-25-4: 248 pp.

Schoeman, F.R., Archibald, R.E.M., 1976. The diatom flora of Southern Africa. Special Report WAT 50. CSIR, Pretoria.

SPSS Inc., 2007. SPSS Release 16.0.0 for Windows. Polar Engineering and Consulting. Chicago (IL): SPSS Inc.

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

Taylor, J.C., Janse van Vuurenand, 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., Prygiel, J., Vosloo, A., Pieter, A., Rey, D., Ransburg, L.V., 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. Hydrobiologia 592, 455–464.

Taylor, J.C., Harding, W.R., Archibald, C.G.M., 2007c. An Illustrated Guide to Some Common Diatom Species from South Africa. WRC Report No TT 282/07. Water Research Commission, Pretoria, South Africa.

Taylor, J.C., Harding, W.R., Archibald, C.G.M., Van Rensburg, L., 2005. 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, 237–246.

Ter Braak, C.J.F., Šmilauer, P., 2012. Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, USA, 496 pp.

Ter Braak, C.J.F., Verdonschot, P.F.M., 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat. Sci. 37, 130-137.

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, 579–594.

Watanabe, T., Asai, K., Houki, A., 1986. Numerical estimation of organic pollution of flowing waters by using the epilithic diatom assemblage – Diatom Assemblage Index (DIApo). Sci. Total Environ. 55, 209–218.

Wetzel, C.E., Martínez-Carreras, N., Hlúbiková, D., Hoffmann,L., Pfister,L., Ector, L., 2013. New combinations and type analysis of Chamaepinnularia species (Bacillariophyceae) from aerial habitats. Cryptogam. Algol 34, 149-168.

Zelinka, M., Marvan, P., 1961. Zur prasisiering der biologischen klassifikation der reinheot fliessender gewa¨sser. Archive Hydrobiol. 57, 389–407.

# Chapter 6: Summary and Conclusions

## 6.1 Summary

The major aim of the study was to employ effective assessment biomonitoring methods using diatoms and macroinvertebrate functional groups to characterize the urban and agricultural impacts in the Bloukrans River system in the Eastern Cape Province of South Africa. Biomonitoring programmes in South Africa have traditionally made use of aquatic bioindicator organisms such as fish, riparian vegetation and macroinvertebrates to assess ecosystem health (Department of Water Affairs and Forestry, 2000). Biomonitoring techniques using autotrophic organisms such as diatoms have also been recently included in bioassessments since they occur in a wider variety of waters and are particularly useful as early warning indicators to changes in nutrient levels (CCME, 2006). The results of the current study indicate that diatom community structure provide insights in the water quality whilst macroinvertebrate functional groups reflect the level of ecological disturbance including loss of riparian vegetation and eutrophication as a result of anthropogenic activities. This was unexpected as previous studies suggested that the macroinvertebrate functional approach is more suitable and rapid for characterising ecosystem conditions (Miserendino and Masi, 2010), detecting land-use change (Zhang et al., 2010; Fierro et al., 2017) and evaluating river health (Masese et al., 2014). These results suggest that the two biological indicators were complementary tools for detecting changes in the water quality and ecological status of the Bloukrans River in the Eastern Cape province of South Africa.

The Bloukrans River system formed the focal point of the investigation because it includes all major land-use categories (agricultural, urban, open canopy, forest sites and private game reserves) within its catchment area.  Moreover, the system has been the subject of previous investigations (e.g. Muskett et al., 2016; Odume and Mgada, 2016; Dalu et al., 2017b, c; Nhiwatiwa et al., 2017), which showed strong evidence that the ecological integrity of the river has been compromised. The purpose of this chapter is to further discuss on each of the approaches applied in this study and propose for the integration of functional-based approaches into existing biomonitoring programmes in South Africa.

## 6.2 Incorporation of functional based approach into the existing taxonomically based biomonitoring approach in South Africa

The use of functional approaches (life forms and ecological guilds) has gained popularity in ecological assessments of aquatic systems over the last decade (Cummins et al., 2005; Miserendino and Masi, 2010; Masese et al., 2014; Fierro et al., 2017; Moyo and Richoux, 2017). Results of Chapter 3 showed that the functional approach provides insights into the mechanisms that structure the macroinvertebrate communities in the Bloukrans River system. The taxon richness within all functional feeding groups was lowest in stream order 2/3, highly impacted sites. Collectors-gatherers were by far the most abundant FFG, having their lowest relative abundance in less impacted streams, increased abundance in stream order 1 (moderately impacted), and very high abundance in the highly impacted sites. Undoubtedly, this stems from the ubiquitous abundance of fine and course detritus during both the summer and winter season which could in all likelihood be attributed by increased urbanisation and poor wastewater treatment within Makhanda area. By contrast, the abundances of shredders and scrapers were very low even in less impacted stream order 1 sites; however, abundances decreased further along the land-use gradient. Limited presence of shredders can be probably be attributed to the sampling period of the present study as temperatures were continuously high therefore there was no leaf shedding thus supporting microbial growth and related degradation of coarse organic matter. These results are similar to patterns reported for other streams in tropical rivers, where sampling was done in the dry season, when low-order streams generally have peak collector-gatherer abundances and limited shredder abundances (Stoaks and Kondratieff, 2014; Fierro et al., 2017). Winterbourn et al. (1981) also observed an absence or paucity of shredders from many forested mountain streams in New Zealand and proposed that the variability and unpredictability of high flows were important causative factors leading to leaf litter retention. From the results of the Linear mixed effects model (LMM), various chemical (e.g. phosphate and TDS) and site-specific (e.g. canopy cover and channel width) environmental parameters best explained the changes in functional feeding group structures in this study. Likewise, variables related to land-use activities and river size have also been found to be important in structuring the distribution of macroinvertebrate FFGs in other tropical (Thompson and Towsend, 2004; Moyo and Richoux, 2017) and temperate rivers (Schmera et al. 2013; Heino et al. 2014).

The FFG ratios in this study also demonstrated that all the sites were dominated by heterotrophic conditions, displayed lower expected linkage with riparian input and stable substrates were limited. Additionally, the feeding interactions (P/P ratio) were not clearly observed at all the stream order categories in this study. However, these FFG ratios require additional tests and possible modifications. Merritt et al. (2002) used thresholds in the interpretation of the FFG ratios and assessment of ecosystem function and concluded that it was impossible to transpose the thresholds from another region directly into a mid-European context. Thus, threshold values for the ecosystem attributes should be re-examined for the austral temperate river systems. Overall, there was a clear change in the functional composition of streams along the human-impact gradient. It is worth noting that these results were broadly in agreement with the diatom biomonitoring data (see Chapter 4 and 5) in this study.

### 6.3 Potentials, challenges and prospects for the South African Diatom Index (SADI) as a bioassessment method for South African rivers.

Throughout the world, diatom indices have gained popularity as a tool to provide cost effective and integrated reflection of water quality in both freshwater and brackish systems (Prygiel et al., 2002; Hering et al., 2006; Li et al., 2010; Lavoie et al., 2014; Tang et al., 2017). Diatom-based bio-monitoring methods are also routinely employed in monitoring programmes (REMP) in South Africa (e.g. de la Rey et al., 2004; Harding et al., 2005; Taylor et al., 2007; Janse van Vuuren and Taylor, 2015; Dalu et al., 2017b). Results of Chapter 4 highlight the advantages of using diatoms in assessing impacts of urban pollution on aquatic ecosystems. The study demonstrated the responsiveness of diatoms along gradients of conductivity and major ions, thereby improving our understanding of how diatoms are distributed in South Africa's water habitats with respect to ionic strength and composition and how these communities are likely to respond to anthropogenic activities such as nutrient enrichment.

Several diatom-based indices have been developed in the northern hemisphere, many of which can be used in several regions, including South Africa, as they are based on the ecology of widely distributed or cosmopolitan diatom taxa (e.g. Kelly and Whitton, 1995; Dell'Uomo, 1996; Lobo et al., 2004; Debenest et al., 2008). However, based on the results of Chapter 5, the SADI produced good results and was the most reliable diatom index considered since it included indicator and tolerance values endemic diatoms of South Africa. Thus, SADI can likely be used with confidence in other ecoregions across the country and is expected to detect disturbance and changes in river systems. The continuous collection of diatom samples from additional eco-regions in South Africa for water quality evaluation should, however, be complimented with sound taxonomical investigations of these samples to continually update and add to the knowledge of South Africa's diatom flora (Dalu and Froneman, 2016).

Since the main finding of Chapters 5 advocates for the use of SADI in biomonitoring and bioassessment programs, consideration should be given to emerging scientific advances in the index calibration and scoring criteria. Concerns have been raised about the lack of expertise by non-specialists in diatom identification (Kociolek and Stoermer, 2001; Dalu and Froneman 2016). Moreover, there are also several sources of uncertainty in the pathway from sample collection to data interpretation, one of which is the process of identification and enumeration of the species. Mann (2010) demonstrated the robustness of using alternative techniques such as molecular approaches (DNA-based methods) to unlock taxonomic information in a form that can be used for broad ecological assessments. DNA barcoding stands out to be one of the potential techniques which can help with accurate identification of species up to taxa level. Other studies have also demonstrated the accuracy and effectiveness of DNA-based methods as biomonitoring tools for diatom and macroinvertebrates communities (Hajibabaei et al., 2008; Vasselon et al., 2017). Nonetheless, Dalu and Froneman (2016) argue that the traditional identification techniques will still be required in the foreseeable future in the identification of new species in Africa due to absence of diatom experts and lack of necessary resources (e.g. identification guides).

For the improvement in efficiency, accuracy and reliability in the use of SADI, the following is recommended: 1) Endemic diatoms should be further investigated and studied as they may be valuable indicators of water quality; 2) Training of diatom taxonomists for the purposes of quality assurance; 3) Developing an integrated approach that combines morphological, molecular and ecological aspects for monitoring purposes; and finally 4) Expanding the available identification guides or keys to include species from other regions.

## 6.4 Conclusions and recommendations

In addition to the scientific knowledge on biomonitoring techniques using diatoms and macroinvertebrate functional groups, this study highlights the importance of an integrated approach to monitoring environmental water quality. Chapter 3 provided a detailed view of how FFGs change along a land-use gradient and further highlighted the advantages of including functional approaches in freshwater biomonitoring in South Africa. This research also demonstrated that benthic diatoms are excellent for biomonitoring and reflect changes in water quality due to changes in catchment land-use (Chapter 4). Although several of the foreign diatom-indices considered where applicable to the study area, the study highlights the importance of using the SADI which is modified to include South Africa's endemic species. Overall, the two biomonitoring assessment methods employed during the study indicate that the Bloukrans River system is ecologically degraded, particularly in the downstream regions of the city of Makhanda. The poor ecological condition reflects the poor management of water plant, failure on the upkeep of infrastructure (burst water pipes and sewage plant) and the loss of riparian vegetation due to encroachment through urbanisation. These results were broadly in agreement with previous studies conducted within the system (see for example Muskett et al., 2016; Odume and Mgada, 2016; Dalu et al., 2017b, c; Nhiwatiwa et al., 2017). Therefore, it is proposed that the two biological indicators techniques should, be used as complementary techniques in the biomonitoring of rivers and streams in South Africa.

In a broader context, this study highlights the effectiveness of using biomonitoring tools to assess the ecological integrity of the Bloukrans River system and to identify the drivers of the ecosystem degradation. To increase the efficacy of these methods, more in-depth studies are recommended on the following aspects:

- To improve taxonomic based approaches currently used in South Africa, it is recommended that the effectiveness of the functional approaches be further studied under varying water quality and quantity (flow rates) conditions, with a view of refining the existing method. Clarification of ecological requirements of the macroinvertebrate functional groups from South Africa is also required to develop a regional FFG classification system (Farrel et al., 2015).

- Due to the combined impacts of variables (eutrophication, ionic composition, organic and metal pollution) in the environment, there is need for validation of the observed effects of variables with experimental manipulations performed under controlled conditions. Laboratory mesocosm experiments (integrating the interactive effects of several factors such as nutrient availability, light intensity, eutrophication, ionic strength, organic and metal pollution) that better resemble natural/field conditions must be done to enable improved accuracy in the extrapolations from laboratory bioassays to responses in the natural systems. The mesocosm experiments will also be vital in developing improved water quality guidelines for organic and metal pollution which could aid water quality managers, engineers and consultants in evaluating the impact of organic and metal pollution on the lotic environments.

- There is need for further studies on South Africa's endemic diatom species and their ecological requirements so that these can be incorporated in a modified SADI scoring system. This will ensure that SADI scores provide an accurate reflection of the exact type of environmental pollution.
- Due to lack of expertise by non-specialists in diatom identification, there is need to adopt new paradigms in diatom-based biomonitoring such as molecular approaches (DNA-based methods). The DNA based method allows improved efficiency and reduced analytical error through automation and standardisation. Additionally, the development of DNA metabarcoding allows to investigate prokaryote and eukaryote biodiversity present in environmental samples (Creer, 2010).

## 6.5 References


Bere, T., Tundisi, J.G., 2010. Biological monitoring of lotic ecosystems: the role of diatoms. Brazilian Journal of Biology 70, 493-502.

Berthon, V., Bouchez, A., Rimet, F., 2011. Using diatom lifeforms and ecological guilds to assess organic pollution and trophic level in rivers: a case study of rivers in south eastern France. Hydrobiologia 673, 259–271.

CCME., 2006. Developing biocriteria as a water quality assessment tool in Canada: scoping assessment. PN 1350.

Creer, S., 2010. Second-generation sequencing derived insights into the temporal biodiversity dynamics of freshwater protists. Mol. Ecol. 19, 2829–2831.

Cummins, K.W., Merritt, R.W., Andrade, P.C.N., 2005. The use of invertebrate functional groups to characterize the ecosystem attributes in selected streams and rivers in south Brazil. Stud. Neotrop. Fauna Environ. 40, 69–89.

Dalu, T., Froneman, P.W., 2016. Diatom-based water quality monitoring in southern Africa, challenges and future prospects. Water SA 42, 551–559.

Dalu, T., Wasserman, R.J., Tonkin, J.D., Alexander, M.E., Dalu, M.T., Motitsoe, S.N., Manungo, K.I., Bepe, O., Dube, T., 2017b. Assessing drivers of benthic macroinvertebrate community structure in African highland streams: an exploration using multivariate analysis. Sci. Total Environ. 601, 1340–1348.

Dalu, T., Wasserman, R.J., Tonkin, J.D., Mwedzi, T., Magoro, M., Weyl, O.L.F., 2017c. Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macroinvertebrate communities in an austral South African stream. Sci.Total Environ. 607–608, 317–325.

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. Aquat. Toxic. 88, 88–94.

Dell'Uomo, A., 1996. Assessment of water quality of an Apennine river as a pilot study. In Whitton, B. A. and E. Rott (eds), Use of Algae for Monitoring Rivers II. Institut fűr Botanik, Universitát Innsbruck, Innsbruck 65–73.

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.

Department of Water Affairs and Forestry (DWAF)., 2000. National Biomonitoring Programme for Riverine Ecosystems: Ecological Reference Condition Project: Field-manual. General Information, Catchment Condition, Invertebrates and Water Chemistry. NBP Report Series No. 10. IWQS, Department of Water Affairs and Forestry, Pretoria, South Africa.

Farrell, K., Van Vuren, J.H.H., Ferreira,M., 2015. Do aquatic macroinvertebrate communities respond to land-use effects in the Wilge River, Mpumalanga, South Africa? Afr. J. Aquat. Sci. 40, 165-173.

Fierro, P., Bertrán, C., Tapia, J., Hauenstein, E., Peña-Cortés, F., Vergara, C., Cerna, C., Vargas-Chacoff, L., 2017. Effects of local land-use on riparian vegetation, water quality, and the functional organization of macroinvertebrate assemblages. Sci. Total Environ. 609, 724–734

Harding, W.R., Archibald, C.G.M., Taylor, J., 2005. Diatomology in South African biomonitoring: the South African Diatom Collection. Afr. J. Aquat. Sci. 30, 221–221.

Hajibabaei, M.M., Shokralla, S., Zhou, X., Singer, G.A.C., Baird, D.J., 2011. Environmental barcoding: a nextgeneration sequencing approach for biomonitoring applications using river benthos. PloS one 6, e17497.

Heino, J., Ilmonen, J., Paasivirta, L., 2014. Continuous variation of macroinvertebrate communities along environmental gradients in northern streams. Boreal Environ. Res. 19, 21–38.

Hering, D., Johnson, R.K., Kramm, S., Schmutz, S., Szoszkiewicz, K., Verdonschot, P.F.M., 2006. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: a comparative metric-based analysis of organism response to stress. Freshw. Biol. 51, 1757–1785.

Janse Van Vuuren, S., Taylor, J.C., 2015. Changes in the algal composition and water quality of the Sundays River, Karoo, South Africa, from source to estuary. Afr. Aquat. Sci. 40, 339–357.

Kelly, M. G., Whitton, B.A., 1995. The trophic diatom index: a new index for monitoring eutrophication in rivers. J Appl Phycol. 7, 433–444.

Kelly, M.G., 1998. Use of community-based indices to monitor eutrophication in European rivers. Environ. Conserv. 25, 22-29

Kociolek, J.P., Stoermer, E.F., 2001. Taxonomy and ecology: A marriage of necessity. Diatom Res. 16, 433–442.

Lavoie, I., Campeau, S., Zugicdrakulic, N., Winter, J.G.,Fortin, C., 2014. Using diatoms to monitor stream biological integrity in Eastern Canada: An overview of 10 years of index development and ongoing challenges. Sci. Total Environ. 475, 187–200.

Li, Y.M., Ferguson, D., Wang, Y.F., Li, C.S., 2010. Paleoenvironmental inferences from diatom assemblages of the middle Miocene Shanwang Formation, Shandong, China. J Paleolimnol. 43,799–814.

Lobo, E.A., Callegaro, V.L., Hermany, G., Bes, D., Wetzel, C.E., Oliveira, M.A., 2004. Use of epilithic diatoms as bioindicator from lotic systems in southern Brazil, with special emphasis on eutrophication. Acta. Limnol. Bras. 16, 25–40.

Mann, D.G., 2010. Discovering diatom species: is a long history of disagreements about species-level taxonomy now at an end? Plant Ecol. Evol. 143, 251–264.

Masese, F.O., Kitaka, N., Kipkemboi, J., Gette, G.M., Irvine, K., McClain, M.E., 2014. Macroinvertebrate functional feeding groups in Kenyan highland streams: evidence for a diverse shredder guild. Nigeria. J. Aquat. Ecol. 5, 67–98.

Miserendino, M.L., Masi, C.I., 2010. The effects of land use on environmental features and functional organization of macroinvertebrate communities in Patagonian low order streams. Ecol. Indic. 10, 311–319.

Muskett, P.C., Hill, J.M.,Weyl, P.S.R., 2016. Macroinvertebrate communities associated with duckweed (Lemnaceae) in two Eastern Cape rivers, South Africa. Afr. Aqua. Sci. 41, 481-488.

Merritt, R.W., Cummins, K.W., Berg, M.B., Novak, J.A., Higgins, M.J., Wessell, K.J., Lessard, J.L., 2002. Development and application of a macroinvertebrate functional group approach to the bioassessment of remnant river oxbows in southwest Florida. J. N. Am. Benthol. Soc. 21, 290–310.

Moyo, S., Richoux, N.B., 2017. Macroinvertebrate Functional Organisation Along the Longitudinal Gradient of an Austral Temperate River. J. Afr. Zool. 52,125–136.

Nhiwatiwa, T., Sithole, T., Dalu, T., 2017. Nhiwatiwa, T., Sithole, T., Dalu, T., 2017. Assessment of river quality in a subtropical Austral river system, a combined approach using benthic diatoms and macroinvertebrates. Appl Water Sci. 7, 4785–4792.

Odume, O. N., Mgaba, N., 2016. Statistical analysis of macroinvertebrate community assemblage structure in relation to river health assessment of an urban river, Eastern Cape, South Africa. Aquat. Ecosyst. Health Manag. 4988, 420– 430.

Prygiel, J., Carpentier, P., Almeida, S., Coste, M., Druart, J.C., Ector, L., Guillard, D, Honeré, M.A., Iserentant, R., Ledeganck, P., Lalanne-cassou, C., Lesniak, C., Mercier, I., Moncaut, P., Nazart, M., Nouchet, N., Peres, F., Peeters, V., Rimet, F., Rumeau, A., Sabaters, Straub, F., Torrisi, M., Tudesque, L., Van Der Vijver, B., Vidal, H., Vizi Net, J., Zydek, N., 2002. Determination of

the biological diatom index (IBD NF T 90-354): Results of an inter-comparison exercise. J. Appl. Phycol. 14, 27-3.
Schmera, D., Erős, T., Heino, J., 2013. Habitat filtering determines spatial variation of macroinvertebrate community traits in northern headwater streams. Community Ecol. 14, 77–88.
Sonneman, J. A., Walsh,C.J., Breen, P.F., Sharpe, A.K., 2001. Effects of urbanization on streams of the Melbourne region, Victoria, Australia. II. Benthic diatom communities. Freshw. Biol. 46, 553–565.
Stoaks, R.D., Kondratieff, B.C., 2014. The aquatic macroinvertebrates of a first order Colorado, USA Front Range stream: What could the biodiversity have been before irrigated agriculture? J Kansas. Entomol. Soc. 87, 47-65.
Tang, T., Tang, T., Tan, L., Gu, Y., Jiang, W., Cai, Q., 2017. Identifying community thresholds for lotic benthic diatoms in response to human disturbance. Sci. Rep. 7, 4134.
Taylor, J.C., Prygiel, J., Vosloo, A., Pieter, A., Rey, D., Ransburg, L.V., 2007a. 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 592, 455-464.
Thompson, R.M., Towsend, C.R., 2004. Land use influences on New Zealand stream communities' effects on species composition, functional organization, and food-web structure. N. Zeal. J. Mar. Fresh. 38, 595–608.
Tomanova, S., Goitia, E., Helešic, J., 2006. Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. Hydrobiologia 556, 251–264.
Vasselon V., Rimet F., Tapolczai K., Bouchez A., 2017. Assessing ecological status with diatoms DNA metabarcoding: Scaling-up on a WFD monitoring network (Mayotte island, France). Ecol. Indic. 82, 1–12.
Winterbourn, M. J.; Rounick, J. S.; Cowie, B. 1981. Are New Zealand stream ecosystems really different? New Zeal. J. Mar. Fresh. 15, 321–32.
Zhang, Y., Dudgeon, D., Cheng, D., Thoe, W., Fok, L., Wang, Z., Lee, J.H.W., 2010. Impacts of land use and water quality on macroinvertebrate communities in the Pearl River drainage basin, China. Hydrobiologia 652,71–88.

## APPENDICES

**Appendix A.1:** The distribution of most frequently occurring diatoms. Abbreviations: * < 1%, ** > 1 to 4.9%, *** > 5 to 9.9% and **** >10%.

**Table S1:** The distribution of most frequently occurring diatoms. Abbreviations: * < 1%, ** > 1 to 4.9%, *** > 5 to 9.9% and **** >10%.

| Species | Code | Diatom frequency for sites | | | |
|---|---|---|---|---|---|
| | | Stream Order 1 | | Stream Order 2/3 | |
| | | Winter | Summer | Winter | Summer |
| *Achnanthes standeri* | Asta | * | * | **** | * |
| *Achnanthidium affine* | Aaff | * | * | * | ** |
| *Achnanthidium crassum* | Acra | ** | * | * | * |
| *Achnanthidium exiguum* | Aexi | ** | ** | * | * |
| *Achnanthidium minutissum* | Amin | * | * | * | * |
| *Adlafia bryophila* | Abry | ** | ** | * | * |
| *Amphipleura pellucida* | Apel | ** | ** | * | * |
| *Amphora coffeaeformis* | Acof | * | ** | * | * |
| *Amphora copulata* | Acop | * | * | * | ** |
| *Amphora pediculus* | Aped | * | * | * | ** |
| *Amphora* sp. | Amp | * | ** | * | * |
| *Cocconeis engelbrechtii* | Ceng | * | * | * | * |
| *Craticula ambigua* | Camb | ** | * | * | * |
| *Cyclostephanos dubius* | Cdub | * | * | * | * |
| *Cyclotella meneghianana* | Cmen | * | * | * | ** |
| *Cyclotella ocellata* | Cocel | * | * | *** | *** |
| *Cyclotella* sp. | Cyc | ** | ** | ** | *** |
| *Cymbella cymbiformis* | Ccym | * | * | * | ** |
| *Cymbella kappii* | Ckap | ** | * | * | * |
| *Cymbella* sp. | Cym | * | ** | * | * |
| *Diadesmis confervacea* | Dcon | * | * | * | ** |
| *Diatoma vulgaris* | Dvul | * | * | *** | *** |
| *Diploneis* sp. | Dip | ** | * | * | ** |
| *Encyonema caespitosum* | Ecae | * | ** | * | * |
| *Encyonema minutum* | Emin | * | ** | * | ** |
| *Entomoneis paludosa* | Epal | * | * | * | * |
| *Epithemia adnata* | Eadn | * | * | * | ** |
| *Epithemia sorex* | Esor | ** | * | * | * |
| *Epithemia* sp. | Epi | ** | ** | * | ** |
| *Eunotia formica* | Efor | * | * | *** | *** |
| *Eunotia minor* | Emin | * | * | ** | ** |
| *Eunotia pectinalis* var. *undulata* | Epec | ** | * | * | ** |
| *Fragilaria biceps* | Fbic | ** | * | * | * |

| | | | | | |
|---|---|---|---|---|---|
| *Fragilaria crotonensis* | Fcro | * | * | * | * |
| *Fragilaria nanana* | Fnan | * | * | * | * |
| *Fragilaria* sp. | Fra | * | ** | * | * |
| *Fragilaria tenera* | Ften | ** | ** | * | * |
| *Fragilaria ulna* | Fuln | ** | * | *** | ** |
| *Fragilaria ulna* var. *acus* | Fuva | ** | * | ** | ** |
| *Geissleria decussis* | Gdec | * | ** | * | * |
| *Gomphonema acuminatum* | Gacu | * | ** | * | * |
| *Gomphonema affine* | Gaff | * | ** | * | * |
| *Gomphonema gracile* | Ggra | * | * | * | ** |
| *Gomphonema Lagenula* | Glag | * | * | * | * |
| *Gomphonema loticullum* | Glot | * | * | * | * |
| *Gomphonema parvulum* | Gpar | * | * | *** | *** |
| *Gomphonema venusta* | Gven | * | * | * | ** |
| *Gyrosigma scalproides* | Gsca | * | * | * | * |
| *Gyrosigma* sp. | Gyr | * | ** | * | ** |
| *Hantzschia amphioxys* | Hamp | ** | ** | * | ** |
| *Hantzschia distinctepunctata* | Hdis | ** | * | ** | ** |
| *Hippodonta capitata* | Hcap | ** | * | * | * |
| *Melosira varians* | Mvar | * | ** | * | ** |
| *Navicula antonii* | Nant | * | ** | * | * |
| *Navicula arvensis* var. *maior* | Narv | * | * | * | * |
| *Navicula capitatoradiata* | Ncap | ** | * | * | * |
| *Navicula cryptocephala* | Ncry | * | * | * | ** |
| *Navicula cryptotonella* | Ncrp | * | * | * | * |
| *Navicula erifuga* | Neri | * | * | * | ** |
| *Navicula gregaria* | Ngre | ** | * | * | ** |
| *Navicula heimansoides* | Nhei | ** | ** | * | * |
| *Navicula microrhombus* | Nmic | * | ** | * | * |
| *Navicula radiosa* | Nrad | * | ** | * | * |
| *Navicula reichardtiana* | Nrei | ** | ** | * | * |
| *Navicula rhynchocephala* | Nrhy | ** | * | * | * |
| *Navicula riediana* | Nrie | * | * | * | * |
| *Navicula* sp. 1 | Nav1 | ** | *** | ** | *** |
| *Navicula* sp. 2 | Nav2 | ** | * | * | * |
| *Navicula* sp. 3 | Nav3 | * | ** | * | ** |
| *Navicula* sp. 4 | Nav4 | ** | * | * | ** |
| *Navicula* sp. 5 | Nav5 | ** | * | * | * |
| *Navicula subrhynchocephala* | Nsub | ** | ** | ** | * |
| *Navicula tripunctata* | Ntri | ** | ** | * | * |
| *Navicula trivalis* | Ntrv | * | * | * | ** |
| *Navicula veneta* | Nven | * | * | * | ** |
| *Navicula zanonii* | Nzan | ** | ** | ** | ** |
| *Nedium productum* | Npro | * | ** | * | * |

| | | | | | |
|---|---|---|---|---|---|
| *Nitszchia desertorum* | Nides | ** | * | * | * |
| *Nitzschia agnewii* | Niagn | * | ** | * | * |
| *Nitzschia amphibia* | Niamp | * | * | * | * |
| *Nitzschia archiblii* | Niarc | * | ** | * | * |
| *Nitzschia capitellata* | Nicap | * | ** | * | * |
| *Nitzschia communis* | Nicom | * | * | ** | * |
| *Nitzschia dissipita* var. *media* | Nidis | * | ** | * | * |
| *Nitzschia dravellensis* | Nidra | ** | * | * | * |
| *Nitzschia elegantula* | Nele | ** | * | * | * |
| *Nitzschia filiformis* | Nifil | * | ** | * | * |
| *Nitzschia gracilis* | Nigra | ** | * | * | * |
| *Nitzschia liebertruthii* | Nilie | * | ** | * | ** |
| *Nitzschia linearis* | Nilin | * | * | * | * |
| *Nitzschia obtusa* var. *kutzii* | Niobt | * | ** | * | ** |
| *Nitzschia palea* | Nipal | ** | * | *** | *** |
| *Nitzschia pura* | Nipur | * | ** | * | * |
| *Nitzschia sigma* | Nisig | * | ** | * | * |
| *Nitzschia* sp. 1 | Nit1 | * | ** | ** | * |
| *Nitzschia* sp. 2 | Nit2 | * | ** | ** | ** |
| *Nitzschia* sp. 3 | Nit3 | * | ** | * | * |
| *Nitzschia* sp. 4 | Nit4 | * | ** | * | * |
| *Nitzschia* sp. 5 | Nit5 | * | ** | * | ** |
| *Nitzschia sublinearis* | Nisub | * | * | * | * |
| *Nitzschia umbonata* | Nium | ** | * | * | * |
| *Pinnularia acrosphaeria* | Pacr | * | * | * | ** |
| *Pinnularia divergens* | Pdiv | ** | ** | * | * |
| *Pinnularia* sp. | Pinn | ** | * | * | ** |
| *Pinnularia viridoformis* | Pvir | * | * | * | * |
| *Placoneis dicephala* | Pldic | ** | * | * | * |
| *Planothidium rostratum* | Plros | ** | * | * | ** |
| *Rhopalodia gibba* | Rgib | * | * | * | ** |
| *Rhopalodia gibberula* | Rgibe | * | * | * | ** |
| *Sellaphora pupula* | Spup | ** | * | * | ** |
| *Sellaphora* sp. | Sel | ** | * | * | * |
| *Staurosira elliptica* | Stell | * | * | *** | *** |
| *Stenopterobia delicatissima* | Stdel | ** | * | * | ** |
| *Suriella ovalis* | Suova | ** | *** | ** | *** |
| *Surirella* sp. | Sur | ** | ** | ** | *** |
| *Trybionella apiculata* | Tapi | * | * | *** | *** |
| *Trybionella debilis* | Tdeb | * | ** | *** | * |
| *Trybionella gracilis* | Tgra | * | * | * | * |

**Appendix B.1:** The distribution of most frequently occurring diatoms. Abbreviations: * < 1 %, ** > 1 to 4.9 %, *** > 5 to 9.9 % and **** >10 %.

**Table S2:** The distribution of most frequently occurring diatoms. Abbreviations: * < 1 %, ** > 1 to 4.9 %, *** > 5 to 9.9 % and **** >10 %.

| | | Diatom frequency for sites | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | Codes | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 |
| *Achnanthes standeri* Cholnoky | ASTD | | | | | | * | | *** | |
| *Achnanthidium affine* (Grunow) Czarnecki | ACAF | | | | **** | | | | **** | |
| *Achnanthidium crassum* (Hustedt) Potapova and Ponader | ADCR | | **** | | | **** | ** | | | |
| *Achnathidium exiguum* (Grunow) Czarnecki | AEXI | | | | | *** | *** | | | **** |
| *Achnanthidium minutissimum* (Kützing) Czarnecki | ADMI | | | | | *** | *** | | *** | *** |
| *Amphipleura pellucida* Kützing | APEL | | | | **** | | | | | **** |
| *Amphora coffeaeformis* (Agardh) Kützing | ACOF | *** | | *** | *** | *** | | | | **** |
| *Amphora copulata* (Kützing) Schoeman and Archibald | ACOP | | | **** | **** | | **** | | | |
| *Amphora pediculus* (Kützing) Grunow | APED | | | **** | | **** | **** | | | |
| *Amphora* sp. | Amp | **** | *** | ** | ** | | *** | | | |
| *Cocconeis engelbrechtii* Cholnoky | CENG | | | | | | * | | | |
| *Craticula ambigua* (Ehrenberg) D.G. Mann | CAMB | | * | | | | | | | |
| *Cyclostephanos dubius* (Fricke) Round | CDUB | | | | * | | | | | |
| *Cyclotella meneghianana* (Kützing) | CMEN | | | | **** | **** | **** | **** | | |
| *Cyclotella ocellata* Pantocsek | COCE | | | *** | **** | | **** | * | **** | |
| *Cyclotella* sp. | Cyc | | | *** | *** | **** | | **** | | **** |
| *Cymbella cymbiformis* Agardh | CCYM | | | | | | **** | | | |
| *Cymbella kappii* (Cholnoky) Cholnoky | CKAP | *** | | | | **** | *** | | | **** |
| *Cymbella* sp.1 | CYM | | | | | **** | | | **** | |
| *Diadesmis confervacea* Kützing | DCOF | | | ** | | ** | | | **** | **** |
| *Diatoma vulgaris* Bory | DVUL | | | **** | *** | | | | | ** |
| *Diploneis* sp. | DIPS | | | | | **** | | | **** | **** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Encyonema caespitosum* Kützing | ECAE | **** | | *** | | | | | | |
| *Encyonema minutum* (Kützing) Grunow | EMIN | **** | | | **** | **** | | | *** | |
| *Entomoneis paludosa* (W.Smith) Reimer | EPAL | | | | **** | | | | | |
| *Epithemia adnata* (Kützing) Brébisson | EADN | | *** | **** | | | | | | |
| *Epithemia sorex* Kützing | ESOR | | | | **** | | | | | |
| *Epithemia* sp. | EPIS | | | | **** | **** | | | | |
| *Eunotia formica* Ehrenberg | EFOR | | | **** | | *** | | | **** | |
| *Eunotia minor* (Kützing) Grunow | EMIN | | | | | ** | | | **** | ** |
| *Eunotia pectinalis* var. undulata (Dyllwyn) Rabenhorst | EPEC | | | | | | | *** | **** | **** |
| *Fragilaria biceps* (Kützing) Lange-Bertalot | FBCP | | | | | | **** | | | **** |
| *Fragilaria crotonensis* Kitton | FCRO | | | | | | **** | | * | **** |
| *Fragilaria nanana* Lange-Bertalot | FNAN | | | | **** | | **** | *** | | |
| *Fragilaria* sp. | FRAS | **** | ** | **** | | | | | ** | |
| *Fragilaria tenera* (W.Smith) Lange-Bertalot | FTEN | | | ** | *** | | | | | **** |
| *Fragilaria ulna* (Nitzsch.) Lange-Bertalot | FULN | | | | **** | | | | **** | |
| *Fragilaria ulna var. acus* (Kützing) Lange-Bertalot | FUVA | | | | **** | **** | **** | | **** | |
| *Geissleria decussis* (Oestrup) Lange-Bertalot and Metzeltin | GDEC | * | | | **** | | *** | | | **** |
| *Gomphonema acuminatum* Ehrenberg | GACU | | | | ** | | | **** | *** | |
| *Gomphonema affine* Kützing | GAFF | **** | **** | | | | | | | |
| *Gomphonema gracile* Ehrenberg | GGRA | **** | | | | | | | | |
| *Gomphonema lagenula* Kützing | GLGN | **** | * | | * | | **** | *** | ** | |
| *Gomphonema parvulum* (Kützing) Kützing | GPAR | **** | **** | ** | * | | * | | | * |
| *Gomphonema venusta* Passy, Kociolek and Lowe | GVNU | ** | *** | *** | | | **** | | *** | |
| *Gyrosigma scalproides* (Rabenhorst)Cleve | GSCA | | **** | **** | *** | | ** | | ** | ** |
| *Gyrosigma* sp. | GYRS | | | **** | * | | | | | **** |
| *Hantzschia amphioxys* (Ehrenberg) Grunow | HAMP | | ** | | | *** | **** | **** | | ** |
| *Hantzschia distinctepunctata* Hustedt | HDIS | **** | **** | | | | | | | |
| *Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin and Witkowski | HCAP | | | | **** | | **** | | | |
| *Melosira varians* Agardh | MVAR | | | | | | **** | | **** | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Navicula antonii* Lange-Bertalot | NANT | | | | **** | **** | *** | | | |
| *Navicula arvensis* Hustedt | NARV | | | **** | | | *** | | | |
| *Navicula capitatoradiata* Germain | NCPR | **** | | ** | *** | *** | | | | |
| *Navicula cryptocephala* Kützing | NCRY | | | | | | | | *** | **** |
| *Navicula cryptotonella* Lange-Bertalot | NCTE | *** | *** | **** | | | **** | ** | | ** |
| *Navicula erifuga* Lange-Bertalot | NERI | | | **** | **** | | | | | |
| *Navicula gregaria* Donkin | NGRE | | | | **** | **** | **** | | **** | |
| *Navicula heimansoides* Lange-Bertalot | NHMD | *** | | | **** | *** | | | | **** |
| *Navicula microrhombus* (Cholnoky) Schoeman and Archibald | NMCB | | | | | **** | | | | |
| *Navicula radiosa* Kützing | NRAD | | | **** | **** | * | | **** | **** | |
| *Navicula reichardtiana* Lange-Bertalot | NRCH | *** | | **** | *** | | | *** | | |
| *Navicula rhynchocephala* Kützing | NRHY | | **** | | **** | *** | | | | |
| *Navicula riediana* Lange-Bertalot and Rumrich | NRIE | ** | ** | | **** | ** | **** | **** | *** | ** |
| *Navicula* sp. 1 | NASP | | | | * | **** | **** | | | |
| *Navicula* sp. 2 | NVSD | | | *** | **** | **** | *** | | | |
| *Navicula subrhynchocephala* Hustedt | NSRH | | **** | **** | | | | | | |
| *Navicula tripunctata* (O. Müller) Bory | NTPT | | *** | **** | | | | | *** | **** |
| *Navicula veneta* Kützing | NVEN | | | | **** | | **** | | *** | |
| *Nedium productum* (W.M.Smith) Cleve | NEPR | | *** | **** | **** | | **** | | | **** |
| *Nitszchia desertorum* Hustedt | NDES | **** | **** | | | **** | *** | *** | | ** |
| *Nitzschia agnewii* Cholnoky | NAGW | | | *** | | **** | **** | **** | | **** |
| *Nitzschia amphibia* Grunow | NAMP | | | | | | **** | **** | | |
| *Nitzschia archibaldii* Lange-Bertalot | NIAR | | **** | | | **** | | | | |
| *Nitzschia capitellata* Hustedt | NCPL | **** | *** | *** | *** | *** | **** | **** | | |
| *Nitzschia communis* Rabenhorst | NCOM | **** | **** | | | **** | *** | | | |
| *Nitzschia dissipita* (Kützing) Grunow | NDIS | | **** | | | | | | | |
| *Nitzschia dravellensis* Coste and Ricard | NDRA | **** | | **** | | | | | | **** |
| *Nitzschia elegantula* Grunow | NELE | **** | *** | | | ** | **** | **** | | *** |
| *Nitzschia filiformis* (W.M.Smith) Van Heurck | NIFIL | **** | | **** | *** | *** | | | **** | |
| *Nitzschia gracilis* Hantzsch | NIGR | | *** | | *** | | *** | **** | *** | **** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Nitzschia liebertruthii* var.*major* Grunow | NILM | | **** | | *** | | *** | *** | *** | |
| *Nitzschia linearis* (Agardh) W.Smith | NLIN | ** | *** | *** | **** | **** | **** | ** | *** | |
| *Nitzschia palea* (Kützing) W.Smith | NPAL | **** | **** | * | * | ** | *** | ** | ** | |
| *Nitzschia pura* Hustedt | NIPR | *** | *** | ** | *** | *** | | **** | | ** |
| *Nitzschia sigma* (Kützing) W.Smith | NSIG | *** | | ** | *** | **** | *** | **** | **** | |
| *Nitzschia* sp. 1 | NIS1 | * | **** | | *** | | *** | | | |
| *Nitzschia* sp. 2 | NIS2 | | | | | ** | | **** | **** | ** |
| *Pinnularia divergens* W.M.Smith | PDIV | | | *** | **** | **** | *** | | * | **** |
| *Pinnularia* sp. | PINS | | | ** | ** | | | | *** | **** |
| *Pinnularia viridoformis* Krammer | PVIF | * | | *** | **** | | | | **** | |
| *Placoneis dicephala* (W.Smith) Mereschkowsky | PDIC | | ** | | | **** | | | | |
| *Planothidium rostratum* (Hustedt) Lange-Bertalot | PLRO | | | **** | | *** | | | | **** |
| *Rhopalodia gibba* (Ehrenberg) O.Muller | RGIB | *** | *** | **** | *** | | | | | |
| *Rhopalodia gibberula* (Ehrenberg) O.Muller | RGBL | *** | *** | ** | *** | | | ** | **** | |
| *Sellaphora pupula* (Kützing) Mereschkowksy | SPUP | | | | | | * | | | |
| *Staurosira elliptica* (Schumann) Williams and Round | STELI | **** | *** | | *** | *** | | | | |
| *Stenopterobia delicatissima* (Lewis) Brébisson | STDE | | | | *** | | | | **** | |
| *Suriella ovalis* Brébisson | SOVI | **** | *** | * | **** | | | | | |
| *Surirella* sp. | SURS | | | | * | | | | | **** |
| *Trybionella apiculata* Gregory | TAPI | *** | **** | | | | | | | |
| *Trybionella debilis* Arnott | TDEB | | | | | | | | *** | **** |
| *Trybionella gracilis* W. Smith | TGRL | | | | | | | | **** | |