

Biodiversity and ecological structures of an African subtropical river and associated floodplain pans

L de Necker

 **orcid.org 0000-0003-3612-9968**

Thesis submitted in fulfilment of the requirements for the degree
Doctor of Philosophy in Environmental Sciences at the North-
West University

Promoter:	Prof NJ Smit
Co-promoter:	Prof LGE Brendonck
Assistant promoter:	Prof JHJ van Vuren

Graduation May 2019

28509633

Table of Content

Acknowledgements	v
Summary	vii
List of Figures	xi
List of Tables	xviii
Abbreviations	xx
Chapter 1: General Introduction	1
1.1 Background.....	1
1.2 Riverine ecosystems and their associated wetlands.....	2
1.3 Assessing ecosystem health and integrity	4
1.3.1 Water quality assessment	4
1.3.2 Aquatic invertebrate assessment	5
1.3.3 Stable isotope assessment.....	5
1.4 Problem statement, hypotheses, aims and objectives:	6
1.4.1 General hypotheses.....	7
1.5 Layout of Thesis	8
Chapter 2: Study Area	9
2.1 Introduction to the Phongolo River	9
2.2 Ecological importance of the lower Phongolo River and Ndumo Game Reserve	12
2.3 Climate and geology	13
2.4 Baseflow from the Pongolapoort Dam.....	14
2.5 Site selection and descriptions	15
2.5.1 The Phongolo River.....	15
2.5.2 Temporary pool.....	19
2.5.3 Nyamithi lake	21
2.5.4 Floodplain pool	25
Chapter 3: Long-term water quality patterns of a flow regulated lowland river	26
3.1 Introduction	26
3.2 Materials and Methods	29
3.2.1 Data collection	29
3.2.2 Laboratory analysis.....	32

3.2.3. <i>Statistical analysis</i>	32
3.3 Results	34
3.3.1 <i>Historical water analysis</i>	34
3.3.2 <i>Present water quality status</i>	42
3.4 Discussion	45
3.5 Conclusions	47
Chapter 4: Assessing the effects of water level fluctuations on aquatic invertebrate communities in a subtropical floodplain pool: a mesocosm assesment	48
4.1 Introduction	48
4.2 Materials and Methods	51
4.2.1 <i>Experimental design and field collection</i>	51
4.2.2 <i>Laboratory analysis</i>	52
4.3 Results	56
4.3.1 <i>Water quality</i>	56
4.3.2 <i>Aquatic invertebrates</i>	59
4.3.3 <i>Zooplankton</i>	65
4.4 Discussion	75
4.5 Conclusion	78
Chapter 5: Ecological assessment of a temporary pool through an atypical drought cycle	79
5.1 Introduction	79
5.2 Materials and Methods	81
5.2.1 <i>Study Area</i>	81
5.2.2 <i>Field collection</i>	82
5.2.2.1 <i>Environmental and biological data</i>	82
5.2.2.2 <i>Stable isotope collection</i>	82
5.2.3 <i>Laboratory analysis</i>	83
5.2.3.1 <i>Environmental and biological analysis</i>	83
5.2.3.2 <i>Stable isotope analysis</i>	83
5.2.4 <i>Statistical analysis</i>	84
5.2.4.1 <i>Environmental and biological data</i>	84
5.2.4.2 <i>Stable isotopes</i>	84
5.3 Results	85

5.3.1 Aquatic invertebrate community	85
5.3.2 Food web and dietary analysis	90
5.4 Discussion	97
5.5 Conclusion	99
Chapter 6: Ecological assessment of a large naturally saline lake through a prolonged drying event	100
6.1 Introduction	100
6.2 Materials and Methods	103
6.2.1 Field collection	103
6.2.2 Laboratory analysis.....	104
6.2.3 Statistical analysis	104
6.2.3.1 Environmental and biological data.....	104
6.2.3.2 Stable isotopes	105
6.3 Results	107
6.3.1 Aquatic invertebrate diversity	107
6.3.2 Food webs and dietary analysis	114
6.4 Discussion	119
6.4.1 Aquatic invertebrate diversity	119
6.4.2 Food webs and dietary analysis	120
6.5 Conclusions	121
Chapter 7: Biodiversity and ecological patterns of the lower Phongolo River	123
7.1 Introduction	123
7.2 Materials and Methods	126
7.2.1 Study area	126
7.2.2 Field sampling.....	126
7.2.3 Laboratory analysis.....	127
7.2.4 Statistical analysis	127
7.2.4.1 Invertebrate diversity	127
7.2.4.2 Stable isotopes	127
7.3 Results	129
7.3.1 Aquatic invertebrates.....	129
7.3.2 Food webs	139
7.3.3 Dietary analysis	144

7.4 Discussion	154
7.4.1 <i>Aquatic invertebrates</i>	154
7.4.2 <i>Stable isotopes</i>	155
7.5 Conclusion:	158
Chapter 8: Conclusions and Recommendations	160
8.1 General remarks	160
8.2 Specific conclusions	160
8.3 Final thoughts	165
8.4 Recommendations	166
Chapter 9: List of references used in the various chapters of this thesis	167
Appendices	189

Acknowledgements

I would like to extend my sincere thanks and gratitude to the following people and organizations who assisted in various ways during the course of this project:

- I would like to thank my three supervisors Prof. Nico Smit, Prof. Luc Brendonck and Prof. Johan van Vuren for all the support and guidance that each of them provided during the course of this project. Your help was invaluable, and I truly appreciate the opportunity provided to me to not only work on such a wonderful project but learn numerous new skills that will make my career in this field successful.
- Prof. Victor Wepener who always had a second to spare for a quick question.
- All my fellow postgraduates at the Water Research Group (WRG), in particular Anrich Kock, Hannes Erasmus, Nico Wolmarans, Dr. Olena Kudlai and Dr. Chris Selbach who assisted me in the field and made all the trips a great deal easier and much more fun.
- A big thank you to Anja Greyling for making all the maps for the various chapters of this thesis.
- The Department of Agriculture, Forestry and Fisheries for providing the data necessary to generate the maps for this thesis.
- The North-West University (NWU) and the WRG for the use of all the necessary equipment and facilities for the project as well as the opportunity of international exposure that was afforded to me.
- The AnimCare Animal Research Ethics Committee at NWU for providing the necessary ethical clearance that made this project possible (Ethics no: NWU-00264-16-A5).
- VLIR-UOS that provided the necessary funding for the project (Project no: ZEIN2013PR396)
- Ezemvelo KZN Wildlife for providing the necessary permits to work in Ndumo Game Reserve (Permit No's: OP 899/2016 and OP 1075/2017).
- Catharine Hanekom and the staff at Ndumo Game Reserve for affording us the opportunity to work in Ndumo Game Reserve.
- Prof. Bernd Sures and Dr. Sonja Zimmerman who afforded me the opportunity to visit their research group at the University of Duisburg-Essen and learn a great deal from many of their postgraduate researchers.
- Dr. Allesandro Manfrin, Jan Lemm and Thomas Meissner at the University of Duisburg-Essen and Dr. David Soto and Dr. Pieter Lemmens at KU Leuven who spent countless patient hours teaching me many of the skills necessary to perform the statistical analyses of this project.
- Prof. Mayumi Ishizuka and Prof. Yoshinori Ikenaka for giving me the opportunity to visit their facilities at the Toxicology laboratory at the Graduate School of Veterinary Medicine, Hokkaido University as well as their organization for the analysis of numerous samples.
- Itchise Pappy at the Toxicology laboratory at the Graduate School of Veterinary Medicine, Hokkaido University for his assistance in analyzing the stable isotope samples.

- Mike Coke for taking the time to proof read the chapters of this thesis and make the necessary editorial changes.
- To my parents (Bets and Pieter de Necker) and my close friends (Lesley-Anne Oosthuizen, Anri Greyling, Imre Krügel and Marissa Venter) for their continued love, support, understanding and encouragement throughout the project. It meant the world to me.
- Finally, a great big thank you to Dr. Ruan Gerber for all your help in the field and in the laboratory, but most of all for your love and support and being there for me during the hardest times.

Summary

Although only recently added to the many existing threats that freshwater ecosystems already face, climate change is one of the most prominent to inland waters in the 21st century. Through extended periods of drought and extreme flooding events, climate change is predicted to severely affect freshwater ecosystems, particularly those that are already exploited by humans. Unfortunately, it is still not well understood exactly how climate change will affect these ecosystems, particularly in semi-arid countries such as South Africa. There is also still a conflict of interest in terms of the needs of aquatic ecosystems and the needs of socio-economic development, particularly in a developing country such as South Africa where growth of industry is necessary for economic growth. Increased development however, also leads to additional pollution of the environment and loss of biodiversity and ecological integrity. Thus, making the conservation and proper management of freshwater ecosystems more difficult. Although progress has been made in South Africa, through the National Water Act of 1998, the protection and restoration of riverine habitats is still somewhat limited due to a continued poor understanding of these ecosystems and their natural functions.

Through construction of dams, irrigation schemes and mismanagement, particularly of flood releases, large floodplain ecosystems in Africa are under threat and the lower Phongolo River and associated floodplain is no exception. Located in northern KwaZulu-Natal, the Phongolo floodplain is South Africa's largest inland floodplain ecosystem and of great economic and socio-ecologic importance. Unfortunately, this ecosystem has been regulated by the Pongolapoort Dam since the 1970's and the upper reaches of the river utilized for anthropogenic activities (mostly irrigation) since at least the 1950's. The river and associated floodplain stretches approximately 80 km downstream of the dam, through the Ndumo Game Reserve (NGR) which is the only protected section of the floodplain, and into Mozambique. The floodplain and NGR are considered a biodiversity hotspot due to the large number of bird, frog and fish species (among other fauna) that are found in this region, many of which are highly dependent on the floodplain and the services it provides. Despite all this, flood releases from the dam are mismanaged, focusing mostly on providing water for socio-economic demands and not the ecological needs of the floodplain.

The Phongolo River ecosystem was quite extensively studied in the 1970's and 1980's after which little research took place until an extensive Water Research Commission research project took place between 2012 and 2014. Unfortunately, a supra-seasonal drought event occurred in South Africa shortly after this project was completed, and rainfall was severely reduced across the country, including the Phongolo region. The last flood release from the Pongolapoort Dam occurred in December 2014 after which only a baseflow has been released in accordance with the "compensation flows" agreement with Mozambique. No documentation is presently available on the possible effects low water flow and drought may have on this ecosystem. In addition, concerns have previously been raised regarding the flooding regime and anthropogenic activities surrounding the area. Thus, the overarching goal of this study was to determine the present environmental state of the lower Phongolo River and associated

floodplain ecosystem and assess the possible effects drought may have on such an ecosystem. In order to achieve this aim, several surveys were conducted between 2016 and 2017 along various types of aquatic habitats found on the floodplain during which time both abiotic and biotic factors were assessed.

For the abiotic assessment, two types of data analysis took place. First, historic water quality data (from the 1970's to present) were obtained from three South African Department of Water and Sanitation (DWS) monitoring sites (gauging stations) within the Phongolo River catchment in order to assess long-term water quality of the Phongolo River, both upstream and downstream of the Pongolapoort Dam and determine potential changes that have occurred since the 1970's. Secondly, additional water samples were collected from three sites on the Phongolo River during the present study (two sites outside NGR and one within) in order to assess the present water quality of the lower Phongolo River during the supra-seasonal drought. With the use of multivariate statistical analyses as well as the Physico-chemical Driver Assessment Index (PAI) various *in situ* and chemical water variables, including salinity and organic nutrients, were assessed.

The key findings of the long-term analysis were that water quality of the upper Phongolo River is affected by numerous anthropogenic activities before it enters the Pongolapoort Dam and has degraded since the 1970's. The primary cause of this is increased organic nutrients (NO_3/NO_2 and PO_4^{3-}) and salinity (conductivity and K) from the large irrigation schemes found in the upper reaches of the river that have grown over the past 40 years. It was further found that the Pongolapoort Dam appears to be trapping nutrient rich sediments which has led to nutrient depleted water entering the lower Phongolo River. This, however, increases as the river flows downstream into NGR through naturally saline groundwater seeping into the river as well as nutrient rich soils and fertilizers. The supra-seasonal drought did not appear to have any significant effect on water quality as the PAI remained similar to that of before the drought.

For the biotic assessment various aspects were analysed. The first of these was an *in situ* field mesocosm experiment that aimed to determine whether length of hydrological connectivity between the Phongolo River and a floodplain wetland would affect aquatic invertebrate communities. Nine mesocosms were set up within NGR next to a floodplain pool selected as the model for the assessment. These mesocosms were separated into three groups for various experimental treatments and water quality and aquatic invertebrate data collected over a five-week period. To prevent physical reduction of aquatic invertebrates these biota were identified and counted as live specimens and placed back into each mesocosm on the day of sampling. The key findings from this assessment were that hydrological connectivity does not affect aquatic invertebrate communities and this was related to the adaptations these biota possess that allow them to resist changes in water quality and water levels. Water quality also changed in a similar manner between the three groups suggesting that a floodplain wetland would need to be flushed with a great deal of water in a short period of time in order to prevent decreasing water quality.

The next biotic assessment was to determine current aquatic invertebrate community structure and aquatic food webs and fish consumer diets, with the use of stable isotope analysis (SIA), in three ecologically important aquatic habitats found in the lower Phongolo region. The first habitat was an

ecologically important and unique temporary wetland located within NGR that was experiencing desynchronised wet/dry cycles. This site is ecologically important as it is the only temporary pool within the Ndumo region that has ever been found to have populations of the annual spotted killifish *Nothobranchius orthonotus*. This site was assessed to determine if certain ecological processes were being disrupted by the out of season drying event induced by the supra-seasonal drought and to determine the diet of the spotted killifish with the use of SIA. Aquatic invertebrate communities and water for select chemical analyses were collected over four surveys, twice before and twice after the site had dried out, and data for food web and dietary analysis collected over two surveys, once before and once after the site had dried out. The aquatic invertebrate community analysis indicated that biological processes remained intact and that changes in the community structure followed the natural changes present in temporary wetlands. This included a decrease in biodiversity as the pool dried up and water quality decreased (largely due to an increase in salinity) and increases after the site had received fresh, low conductivity rainwater. Stable isotope analysis indicated that there was nitrogen enrichment in the food web as all collected food web components had increased nitrogen signatures ($\delta^{15}\text{N}$) after the site had received water. This indicates towards nutrient inputs from anthropogenic activities occurring outside NGR. It was further found that the spotted killifish, which has always exclusively been considered a predator, also feeds on a large amount of plant-based food sources which has never been documented before and are able to shift their diets to include a greater percentage of low-nutrient food sources when necessary. Although there were no apparent long-term adverse effects at the time of analysis, continued nutrient inputs may eventually have negative implications for this aquatic habitat. In addition, an extended shift to low-nutrient plant food sources by the killifish may be the first signs of effects that drought and climate change may have through desynchronization of consumers with their preferential, high-nutrient food sources.

The second habitat that was selected is the ecologically important large saline lake located within NGR. This site is the only naturally saline lake found in the region and was selected as a study site as the water levels were severely reduced during the supra-seasonal drought and water became highly saline as a result of this. In addition, fish for SIA analysis and aquatic invertebrates for diversity analysis were previously sampled at this site in pre-drought conditions (2010 and 2014 respectively) thus providing the present study with data from before the supra-seasonal drought. The site was assessed to determine whether the supra-seasonal drought and accompanying increased salinity had an effect on the aquatic invertebrate community, food web and fish consumer diets present in this site by comparing to the data previously collected. It was found that aquatic invertebrate diversity and abundance was severely affected during the peak of supra-seasonal drought in 2016 with only a few highly tolerant taxa able to survive. Through rainfall and small floods from the Usuthu River at the end of 2016 and beginning of 2017, water became less saline and several fish species and aquatic invertebrate taxa previously found started to return to the lake. Ecosystem resilience was evident at this site since aquatic invertebrate diversity and abundance was able to recover rather quickly after the site received some fresh water and the SIA indicated that fish consumers were able to adapt to the changes in the habitat by switching to generalist feeding behaviour in 2017 compared to a more specialised feeding behaviour evident in 2010.

The final habitat was the lower Phongolo River of which three sections were studied, two of these located in the communal area of Ndumo among anthropogenic activities and one just as the river flows into NGR. In addition to many human activities that take place the lower Phongolo River, the supra-seasonal drought that occurred in the region prevented the release of large floods from the Pongolapoort Dam and only a baseflow was released during the study period. These sites were therefore assessed to determine whether the low baseflow as well as the anthropogenic activities had any effect on the ecology of the river. This was accomplished by studying aquatic invertebrate community structure, aquatic food webs and fish consumer diets of the river. To determine if there was any change in the aquatic invertebrate diversity and community structure results from the present study were compared to those of previous studies on the river and floodplain in 2012 and 2014 respectively. It was found that the supra-seasonal drought did indeed affect the aquatic invertebrate community structure through a loss of rheophilous and pollutant sensitive aquatic invertebrate taxa. However, no loss of the total aquatic invertebrate diversity occurred due to a replacement with more tolerant aquatic invertebrate taxa and frequent taxonomic turnover. In addition, the presence and high abundance of pollutant tolerant aquatic invertebrate taxa within NGR indicated that anthropogenic activities in the surrounding region were most likely affecting the Phongolo River, particularly the section located within the NGR since there was a general increase in nitrogen in the aquatic food webs at all sites. Even though there was nitrogen enrichment in the aquatic food webs as well as a shift towards production of inorganic carbon by autochthonous sources, the food webs remained stable throughout the study period. This suggests that the supra-seasonal drought and anthropogenic activities are most likely not affecting the aquatic food webs. The drought as well as anthropogenic activities did however appear to affect the fish consumers as their diets consisted largely of generalist and opportunistic feeding strategies often found in disturbed ecosystems.

This study found several ecological changes and effects along the lower Phongolo River and its associated wetlands caused by the supra-seasonal drought with its accompanying effects and numerous anthropogenic activities that occur in the region. However, presently these changes and effects are not severe, and the ecosystem appears to be resilient and adaptable with the ability to rapidly recover from disturbances, such as absence of flood releases, anthropogenic activities and drought. It was further found that the lower Phongolo is a resilient ecosystem able to adapt to and recover from disturbances quite rapidly. However, it is quite likely that even this resilient ecosystem will be severely affected if the drought were to continue over an extended period or anthropogenic inputs were to increase, as has been suggested by future predicted climate change and continuously growing human population of this region. It is therefore still imperative that appropriate management and conservation of the region take place. This study also recommends that there is continued monitoring of the lower Phongolo River and ecosystem in order to confirm that recovery from the drought is indeed taking place and that no long-term effects occurred during the supra-seasonal drought.

Keywords: aquatic food webs, biological succession, climate change, diet shift, drought, ecological resilience, floodplain connectivity, salinization, mixing models, water quality index

List of Figures

Chapter 2:

Figure 2.1: Map of the Pongolapoort Dam and lower Phongolo region indicating various irrigation schemes and farms located above the dam and on the floodplain.

Figure 2.2: Annual peak flows of Phongolo River downstream of dam measured from 1983 to 2018. Arrows indicate years of severe drought in 1983 and 2016 (available from <https://www.dwaf.gov.za/Hydrology/Verified/hymain.aspx>. Accessed 14 September 2018).

Figure 2.3: Photographs of typical fish species found in the lower Phongolo River and associated floodplain namely; *Brycinus imberi* (a); *Oreochromis mossambicus* (b); *Hydrocynus vittatus* (c) and *Schilbe intermedius* (d).

Figure 2.4: Monthly peak flows of Phongolo River downstream of Pongolapoort Dam during the present study from 2016 to 2017. (available from <https://www.dwaf.gov.za/Hydrology/Verified/hymain.aspx>. Accessed 14 September 2018).

Figure 2.5: Map of the Ndumo region and Ndumo Game Reserve indicating all sites selected for this study.

Figure 2.6: Photographs of Site 3 located on the lower Phongolo River outside Ndumo Game Reserve indicating the upstream area of the river in 2014 before the weir was built (a); the upstream area in 2016 after the weir was built (b); the downstream area that was sampled in 2016 (c); downstream of the weir indicating water flow (d) and downstream area where local people utilize the river for washing of vehicles (e).

Figure 2.7: Photographs of Site 2 located on the lower Phongolo River outside Ndumo Game Reserve indicating the upstream area of the river and concrete blocks in the stream (a); downstream area of the river with the slower pool and aquatic vegetation (b and c); local women washing clothes in the river (d) and livestock grazing and drinking water from the river (e).

Figure 2.8: Photographs of Site 1 located on the lower Phongolo River within Ndumo Game Reserve indicating the river flowing downstream from right to left into the game reserve (a) and a small boat that local people use for fishing as well as the deforested eastern area of the river within Ndumo Game Reserve that local people use for crop farming (b).

Figure 2.9: Photographs of the temporary pool (Site 4) located within Ndumo Game Reserve during May 2016 (a); August 2016 (b); September 2016 (c); December 2016 (d) and February 2017 (e).

Figure 2.10: Photographs of Nyamithi lake (Site 5) located within Ndumo Game Reserve during September 2014 when the site still received regular water supply from the controlled flood and rainfall (a); May 2016 during the peak of the supra-seasonal drought (b and c) and February 2017 after the site had received some rainwater and a small flood from the Usuthu River (d).

Figure 2.11: Photographs of Nyamithi lake (Site 5) indicating three of the areas namely Site 5.1 (a); Site 5.2 (b) and Site 5.3 (c and d) sampled between 2014 and 2017.

Figure 2.12: Photographs of the outflow of Nyamithi lake (Site 5.4) September 2014 when the site still received regular water supply from the controlled flood and rainfall (a and b) and May 2016 during the peak of the supra-seasonal drought (c and d). This site was also sampled between 2014 and 2017.

Figure 2.13: Photographs of typical aquatic invertebrates that have been found in Nyamithi lake within the Ndumo Game Reserve namely; *Appasus* sp. (a); *Corbicula* sp. (b); *Tarebia granifera* (c) and Ostracoda (d).

Figure 2.14: Photographs of the floodplain pool (Site 6) used as the model for the mesocosm study (see Chapter 4) in February 2014 after the site had received water from the controlled flood and rainfall (a) and December 2016 during the supra-seasonal drought (b).

Chapter 3:

Figure 3.1: Map of lower Phongolo River and floodplain indicating the three gauging stations on the Phongolo River above (Upstream) and below (Downstream 1 and 2) Pongolapoort Dam, as well as the three sampling sites on the lower Phongolo River (Sites 1-3).

Figure 3.2: Monthly peak flows of Phongolo River downstream of Pongolapoort Dam during the present study in 2016-2017. Arrows indicate months during which sampling surveys took place (available from <https://www.dwaf.gov.za/Hydrology/Verified/hymain.aspx>. Accessed 14 September 2018).

Figure 3.3: Line graphs indicating mean ($\pm$ SD) trends in conductivity (a), chlorides (b), calcium (c) and magnesium (d) measured from 1972 to 2017 at the Department of Water and Sanitation gauging stations located above and below Pongolapoort Dam. Arrows indicate years of severe drought. Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

Figure 3.4: Line graphs indicating mean ($\pm$ SD) trends in sodium (a), potassium (b), sulphates (c) and pH (d) measured from 1972 to 2017 at the Department of Water and Sanitation gauging stations located above and below Pongolapoort Dam. Arrows indicate years of severe drought. Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

Figure 3.5: Line graphs indicating mean ($\pm$ SD) trends in ammonium (a), nitrates/nitrates(b) and orthophosphates (c) measured from 1972 to 2017 at the Department of Water and Sanitation gauging stations located above and below Pongolapoort Dam. Arrows indicate years of severe drought. Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

Figure 3.6: Principal Component Analysis (PCA) biplot comparing water quality variables from 1972 to 2017 measured at the Department of Water and Sanitation gauging stations above (red dots) and below (green squares) Pongolapoort Dam. The biplot explains 64.27% of data variation, of which 42.89% is explained by the first axis and 21.38% is explained by the second axis.

Figure 3.7: Principal Component Analysis (PCA) biplot comparing water quality variables from 1981 to 2017 measured at the Department of Water and Sanitation gauging stations below the

Pongolapoort Dam (green dots) and within Ndumo Game Reserve (red squares). The biplot explains 92.76% of data variation, of which 87.27% is explained by the first axis and 5.49% is explained by the second axis.

Figure 3.8: Physico-chemical driver assessment index (PAI) scores with associated Present Ecological Category derived from the three gauging stations located above and below Pongolapoort Dam. Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

Figure 3.9: Principal Component Analysis (PCA) biplot comparing water quality variables measured during the present study on the Phongolo River within (Site 1; red dots) and outside (Site 2; green squares and Site 3; black triangles) Ndumo Game Reserve during the four sampling surveys from 2016 to 2017. The biplot explains 74.97% of data variation, of which 45.11% is explained by the first axis and 29.86% is explained by the second axis.

Chapter 4:

Figure 4.1: Map of Ndumo Game Reserve and floodplain pool used as the model for the experimental field mesocosm experiment.

Figure 4.2: Experimental field mesocosms, with shade netting placed around the experimental area.

Figure 4.3: Dendrogram (a) and nMDS plots (b) of all macroinvertebrate and zooplankton taxa of each mesocosm collected over the five sampling weeks. Last number denotes treatment: 1 – Treatment 1, 2 – Treatment 2, 3 – Treatment 3.

Figure 4.4: Number of taxa (a), number of individuals (b), Margalef's species richness (c) and Pielou's evenness index (d) of all macroinvertebrate taxa collected over the five sampling weeks. Similar superscripts denote significant differences ($p < 0.05$).

Figure 4.5: Shannon-Wiener diversity (a) and Simpson index (b) of all macroinvertebrate taxa collected over the five sampling weeks. Similar superscripts denote significant differences ($p < 0.05$).

Figure 4.6: Number of predators (a), number of gathering collectors (b), number of filter collectors (c) and number of shredders (d) of each functional feeding group over the five sampling weeks. Similar superscripts indicate significant differences ($p < 0.05$).

Figure 4.7: Line graphs depicting changes in zooplankton taxa abundance over the five sampling weeks.

Figure 4.8: Number of taxa (a), number of individuals (b), Margalef's species richness (c) and Pielou's evenness index (d) of all zooplankton taxa collected over the five sampling weeks. Similar superscripts denote significant differences ($p < 0.05$).

Figure 4.9: Shannon-Wiener diversity (a) and Simpson's index (b) of all zooplankton taxa collected over the five sampling weeks. Similar superscripts denote significant differences ($p < 0.05$).

Figure 4.10: Variation partitioning analysis indicating unique and shared contribution of select water quality variables and the five sampling weeks (a) and the unique and shared contribution of select water quality variables and macroinvertebrate biota on zooplankton taxa (b). Asterisks denote level of significance: ** $p < 0.01$, *** $p < 0.001$.

Chapter 5:

Figure 5.1: Map of the study area indicating the location of the selected temporary pool within Ndumo Game Reserve.

Figure 5.2: Number of taxa (a), Number of individuals (b), Margalef's species richness (c), Pielou's evenness index (d), Shannon-Wiener diversity (e) and Simpson's index (f) of all aquatic invertebrate taxa collected from the temporary pool (RP) inside Ndumo Game Reserve during four surveys between 2016 and 2017. RP1 – May 2016; RP2 – August 2016; RP3 – December 2016; RP4 – February 2017.

Figure 5.3: Principle Component Analysis (PCA) biplot of the 30 best fitting aquatic invertebrate taxa (triangles) collected from the temporary pool (RP) inside Ndumo Game Reserve during four surveys between 2016 and 2017 (circles) with water quality variables (arrows) plotted as supplementary variables. The biplot explains 73.13% of data variation of which 45.56% is explained by the first axis and 27.57% is explained by the second axis. RP1 – May 2016; RP2 – August 2016; RP3 – December 2016; RP4 – February 2017.

Figure 5.4: $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values for food sources sampled from the temporary pool (RP) inside Ndumo Game Reserve before and after the drying event. Box plots indicate the 25th, 50th and 75th percentiles, and whiskers the highest and lowest values, excluding the outliers. SPI – Small predatory invertebrates, LPI – Large predatory invertebrates PP – Primary producers. Similar lowercase letters indicate significant differences.

Figure 5.5: $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values for the consumer *Nothobranchius orthonotus* compared before and after the drying event of the temporary pool inside Ndumo Game Reserve. Box plots indicate the 25th, 50th and 75th percentiles, and whiskers the highest and lowest values, excluding the outliers. *Denotes significant differences ($p < 0.01$).

Figure 5.6: Biplot indicating mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the different trophic components sampled from the temporary pool inside Ndumo Game Reserve in May 2016 (before drying event) (a) and February 2017 (after drying event) (b).

Figure 5.7: Relative contribution (%) of food sources to the diets of the consumer, *Nothobranchius orthonotus*, based on Bayesian isotope mixing models (SIAR) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ before (a) and after (b) a drying event the temporary pool inside Ndumo Game Reserve. Plots indicate 95% (middle rectangle), 75% and 25% (external rectangles) credibility intervals. SPI – Small predatory invertebrates, LPI – Large predatory invertebrates, PP – Primary producers

Figure 5.8: Relative contribution (%) of primary producers to the diet of the consumer, *Nothobranchius orthonotus*, based on Bayesian isotope mixing models (SIAR) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ before (a) and after (b) a drying event of the temporary pool inside Ndumo Game Reserve. Plots indicate 95% (middle rectangle), 75% and 25% (external rectangles) credibility intervals.

Chapter 6:

Figure 6.1: Map of Ndumo Game Reserve (NGR) and surrounding region indicating the sampling areas in Nyamithi lake (N1-N4).

Figure 6.2: Dendrogram (a) and nMDS (b) plots of aquatic invertebrate taxa collected during sampling surveys in 2014, 2016 and 2017 in Nyamithi lake within the Ndumo Game Reserve.

Figure 6.3: Canonical Correspondence Analysis (CCA) triplot comparing aquatic invertebrate diversity and select water nutrients variables to all sampling surveys during 2014, 2016 and 2017 in Nyamithi lake within the Ndumo Game Reserve. The biplot explains 45.66% of data variation of which 27.84% is explained by the first axis and 17.84% by the second axis. Only the 30 best fitting aquatic invertebrate taxa and water nutrient variables selected through correlation and forward selection analysis are retained.

Figure 6.4: Comparison of the number of taxa (a), number of individuals (b), Margalef's Species Richness (c), Pielou's Evenness Index (d), Shannon-Wiener diversity (e) and Simpson's Index (f) of aquatic invertebrates collected during the sampling surveys in 2014, 2016 and 2017 in Nyamithi lake within the Ndumo Game Reserve. Lower case letters indicate significant difference ($p < 0.05$).

Figure 6.5: Biplots indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the various food web components collected during the June 2010 (a) and February 2017 (b) sampling surveys in Nyamithi lake within the Ndumo Game Reserve.

Figure 6.6: A biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) of the sampling survey in June 2010 (a) and February 2017 (b) in Nyamithi lake within the Ndumo Game Reserve. Fish consumer data and source data are abbreviated: Fish – fish food sources; c3_plants – C₃ plants; c4_plants – C₄ plants; inv – aquatic invertebrates. Error bars represent 95% confidence intervals.

Chapter 7:

Figure 7.1: Map of Ndumo Game Reserve and surrounding area indicating the three study sites on the Phongolo River.

Figure 7.2: Pie charts indicating the percentage of sensitive (highly sensitive and moderately sensitive) and tolerant (highly and moderately tolerant) aquatic invertebrate families collected during surveys on the Phongolo River in 2012 and 2013 by Smit et al. (2016) (a and b), in 2014 by de Necker (2015) (c and d) and in 2016 – 2017 during the present study (e and f).

Figure 7.3: Principal Component Analysis (PCA) biplot of the 50 best fitting aquatic invertebrate taxa (triangles) collected in the Phongolo River within Ndumo Game Reserve (Site 1) during sampling surveys (circles) with chemical water nutrients (arrows) plotted as supplementary variables. The biplot explains 71.23% of data variation of which 38.49% is explained by the first axis and 32.74% is explained by the second axis. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. Full identities of the abbreviated invertebrate names are presented in Appendix E1.

Figure 7.4: Number of taxa (a), number of individuals (b), Margalef's Species Richness (c) and Pielou's Evenness Index (d) Shannon-Wiener diversity (e) and Simpson's Index (f) of aquatic invertebrates

collected in the Phongolo River within Ndumo Game Reserve (Site 1) during sampling surveys. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017.

Figure 7.5: Principal Component Analysis (PCA) biplot of the 30 best fitting aquatic invertebrate taxa (triangles) collected at the two sampling sites on the Phongolo River outside Ndumo Game Reserve (Site 2 and Site 3) during sampling surveys (circles) with chemical water nutrients (arrows) plotted as supplementary variables. The biplot explains 69.64% of data variation of which 42.47% is explained by the first axis and 27.17% is explained by the second axis. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. Full identities of the abbreviated invertebrate names are presented in Appendix E1.

Figure 7.6: Number of taxa (a), number of individuals (b), Margalef's Species Richness (c), Pielou's Evenness Index (d), Shannon-Wiener diversity (e) and Simpson's Index (f) of aquatic invertebrates collected at the two sampling sites on the Phongolo River outside Ndumo Game Reserve (Sites 2 and 3) during sampling surveys. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. Similar denomination indicates significant differences.

Figure 7.7: Comparative Principal Component Analysis (PCA) biplot of the 30 best fitting aquatic invertebrate taxa (triangles) collected in the Phongolo River within (Site 1) and outside (Sites 2 and 3) Ndumo Game Reserve during sampling surveys (circles) with chemical water nutrients (arrows) plotted as supplementary variables. The biplot explains 37.49% of data variation of which 20.45% is explained by the first axis and 17.04% is explained by the second axis. Full identities of the abbreviated invertebrate names are presented in Appendix E1.

Figure 7.8: Comparison of number of taxa (a), number of individuals (b), Margalef's Species Richness (c), Pielou's Evenness Index (d), Shannon-Wiener diversity (e) and Simpson's Index (f) of aquatic invertebrates collected in the Phongolo River within (Site 1) and outside (Site 2 and Site 3) Ndumo Game Reserve during sampling surveys. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. Similar denomination indicates significant differences.

Figure 7.9: Biplots indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the various food web components collected during September 2016 (a) and February 2017 (b) sampling surveys at Site 1 on Phongolo River within Ndumo Game Reserve. Full identities of the abbreviated sample names are presented in Appendices E3 – E5.

Figure 7.10: Biplots indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the various food web components collected during May 2016 (a) and September 2016 (b) sampling surveys at Site 2 on the Phongolo River outside Ndumo Game Reserve. Full identities of the abbreviated sample names are presented in Appendices E3 – E5.

Figure 7.11: A biplot indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the various food web components collected during February 2017 at Site 2 on the Phongolo River outside Ndumo Game Reserve. Full identities of the abbreviated sample names are presented in Appendices E3 – E5.

Figure 7.12: Biplots indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the various food web components collected during September 2016 (a) and February 2017 (b) sampling surveys at

Site 3 on the Phongolo River outside Ndumo Game Reserve. Full identities of the abbreviated sample names are presented in Appendices E3 – E5.

Figure 7.13: Biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) collected in the Phongolo River within Ndumo Game Reserve (Site 1) collected during the September 2016 (a) and February 2017 (b) sampling surveys. Fish consumer data and source data are abbreviated: Fish – fish food sources; Group A inv – invertebrates with higher nitrogen signature; Group B inv – invertebrates with lower nitrogen signature; Inverts – Aquatic invertebrate families; c3_plant – C_3 plants; c4_plant – C_4 plants. Error bars represent 95% confidence intervals. Taxa included in each source group can be found in Appendix E13 and E14.

Figure 7.14: Biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) of Site 2 collected during the May 2016 (a) and September 2016 (b) sampling surveys. Fish consumer data and source data are abbreviated: Fish – fish food sources; Group A inv – invertebrates with higher nitrogen signature; Group B inv – invertebrates with lower nitrogen signature; Inverts – Aquatic invertebrate families; c3_plant – C_3 plants; c4_plant – C_4 plants. Error bars represent 95% confidence intervals. Taxa included in each source group can be found in Appendix E13 and E14.

Figure 7.15: A biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) of Site 2 collected during the February 2017 sampling survey. Fish consumer data and source data are abbreviated: Fish – fish food sources; Inverts – Aquatic invertebrate families. c3_plant – C_3 plants; c4_plant – C_4 plants. Error bars represent 95% confidence intervals. Taxa included in each source group can be found in Appendix E13 and E14.

Figure 7.16: Biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) of Site 3 collected during the September 2016 (a) and February 2017 (b) sampling surveys. Fish consumer data and source data are abbreviated: Fish – fish food sources; Inverts – Aquatic invertebrate families. c3_plant – C_3 plants; c4_plant – C_4 plants. Error bars represent 95% confidence intervals. Taxa included in each source group can be found in Appendix E13 and E14.

List of Tables

Chapter 3:

Table 3.1: Department of Water and Sanitation gauging station numbers, locations, and years for which water quality data are available for the Phongolo River. All water quality variables for each gauging station are presented in Appendices A1-A3.

Table 3.2: Rating and ecological categories used for implementation of the Physico-chemical Assessment Index (PAI) (Kleynhans and Louw, 2008).

Table 3.3: One-way Analysis of Variance (ANOVA) and chemical water nutrient variables measured at the Department of Water and Sanitation gauging stations from 1972 to 2017. Mean $\pm$ SD, range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

Table 3.4: One-way Analysis of Variance (ANOVA) and chemical water nutrient variables measured during the present study within (Site 1) and outside (Site 2 and Site 3) Ndumo Game Reserve during the four sampling surveys from 2016 to 2017. Mean $\pm$ SD, range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$).

Chapter 4:

Table 4.1: Indication of sampling days and weeks into which days were separated.

Table 4.2: *In situ* water quality variables and Analysis of Variance (ANOVA) of all mesocosms over the five sampling weeks. Mean $\pm$ SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). N-value indicates number of samples per week.

Table 4.3: Chemical water nutrient variables and Analysis of Variance (ANOVA) of all mesocosms over the five sampling weeks. Mean $\pm$ SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). N-value indicates number of samples per week.

Table 4.4: Zooplankton taxa and Analysis of Variance (ANOVA) of zooplankton taxa collected over the five sampling weeks. Mean $\pm$ SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). N-value indicates number of samples per week.

Table 4.5: Pearson correlations between macroinvertebrate functional feeding groups and zooplankton taxa of all mesocosms over the five sampling weeks.

Table 4.6: Pearson correlations between zooplankton taxa and select water quality variables of all mesocosms over the five sampling weeks.

Chapter 5:

Table 5.1: *In situ* and water quality variables collected from the temporary pool (RP) inside Ndumo Game Reserve during all four surveys between 2016 and 2017. RP1 – May 2016; RP2 – August 2016; RP3 – December 2016; RP4 – February 2017.

Table 5.2: Analysis of Variance (ANOVA) summary of linear model results for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of three food source groups and consumers (*Nothobranchius orthonotus*) based on the sampling groups (Groups),

timing before or after the drying event (Timing) and the interaction of these factors of the temporary pool inside Ndumo Game Reserve.

Table 5.3: Tukey's post hoc analysis of significance between the three food source groups of the temporary pool inside Ndumo Game Reserve. SPI – Small predatory invertebrates. LPI – Large predatory invertebrates, PP= Primary producers.

Chapter 6:

Table 6.1: Contribution of various aquatic invertebrate taxa to within group similarities determined through Similarity percentage analysis (SIMPER) analysis. Groups are based on year of sampling (2014, 2016 or 2017) and were determined using dendrogram and nMDS analysis.

Table 6.2: *In situ* and chemical water quality variables collected during the sampling surveys in 2014, 2016 and 2017 from Nyamithi lake within the Ndumo Game Reserve. *Denotes data not available.

Table 6.3: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected in Nyamithi lake within the Ndumo Game Reserve in June 2010. Mean $\pm$ SD (95% Credible interval).

Table 6.4: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected in Nyamithi lake within the Ndumo Game Reserve in February 2017. Mean $\pm$ SD (95% Credible interval).

Chapter 7:

Table 7.1: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected in the Phongolo River within Ndumo Game Reserve (Site 1) in September 2016. Mean $\pm$ SD (95% Credible interval).

Table 7.2: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected in the Phongolo River inside Ndumo Game Reserve in February 2017. Mean $\pm$ SD (95% Credible interval).

Table 7.3: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 2 in May 2016. Mean $\pm$ SD (95% Credible interval).

Table 7.4: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 2 in September 2016. Mean $\pm$ SD (95% Credible interval).

Table 7.5: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 2 in February 2017. Mean $\pm$ SD (95% Credible interval).

Table 7.6: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 3 in September 2016. Mean $\pm$ SD (95% Credible interval).

Table 7.7: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 3 in February 2017. Mean $\pm$ SD (95% Credible interval).

Abbreviations

$\delta^{13}\text{C}$	Carbon stable isotope
$\delta^{15}\text{N}$	Nitrogen stable isotope
$\delta^{15}\text{N}$ component	Mean $\delta^{15}\text{N}$ of each separate food web component
$\delta^{15}\text{N}$ reference	Mean $\delta^{15}\text{N}$ of detritus of each individual
ANOSIM	Analysis of Similarity
ANOVA	Analysis of variance
CCA	Canonical correspondence analysis
DAAD	Deutscher Akademischer Austauschdienst (German Academic Exchange Service)
DDT	Dichlorodiphenyltrichloroethane
DO	Dissolved Oxygen
DO%	Oxygen saturation
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitations
FC	Filtering collector
FFGs	Functional Feeding Groups
GC	Gathering collector
HCl	Hydrochloric acid
IBA	Important bird area
IDP	Integrated Development Plan
IMP	Integrated Management Plant
IRS	Indoor Residual Spraying
LM	Linear models
LPI	Large predatory invertebrates
MCMC	Markov Chain Monte Carlo
Merck KGaA	Merck Kommanditgesellschaft auf Aktien
multipatt	Multi-pattern analysis
NBF	Buffered neutral formalin
NGR	Ndumo Game Reserve
nMDS	non-metric Multidimensional Scaling
NRF	National Research Foundation
NTU	Nephelometric Turbidity Unit
NWU	North-West University
PAI	Physico-Chemical Driver Assessment Index
PCA	Principal Component Analysis
PP	Primary producers
RDA	Redundancy Analysis
SA	South Africa

SIAR	Stable Isotope Analysis in R
SIMPER	Similarity percentage analysis
SPI	Small predatory invertebrates
TDS	Total dissolved solids
TFCA	Transfrontier Conservation Area
TH	Total hardness
TP	Trophic position
TWQG	Target Water Quality Guidelines
UNU-EHS	United Nations University Institute for Environment and Human Security
USA	United States of America
vif	variance inflation factor
WRC	Water Research Commission
WRG	Water Research Group

CHAPTER 1:

General Introduction

1.1 Background

During the past century there has been a considerable decline in the biodiversity of inland waters, with climate change and its effects only recently added to the many existing threats that freshwater ecosystems and associated services face (Aldous et al. 2011; Davidson et al. 2012; UNU-EHS 2016). Although it is clear that these threats, along with predicted climate change, will inevitably further negatively impact aquatic ecosystems, it is still unclear exactly what these impacts will be. Climate change imposes additional stressors on water quality and quantity, predominantly in developing and semi-arid countries, and changes in a variety of ecosystems are already being detected and documented in Africa, particularly southern Africa (Bates et al. 2008). This is largely due to large scale developments such as mining, industry, agriculture and urbanization that are anticipated to grow globally, particularly in developing countries (Nel et al. 2007). Unfortunately, these developments also lead to additional discharges in the form of nutrients, pesticides and numerous other pollutants and pose a serious threat to aquatic resources if not properly managed (Nel et al. 2007). Progress has been made in several countries, including South Africa through the National Water Act of 1998 (DWAF 2004), in the development of environmental strategies aimed at restoring and protecting rivers and their biodiversity. This is still somewhat limited though due to a continued conflict of interest between the needs of aquatic ecosystems (including instream, riparian and river floodplain areas) and the needs of socio-economic developments (including agriculture and industry) (Thoms and Sheldon 2002). In addition, there is also still a poor understanding of the structure and function of riverine ecosystems and environmental flows necessary to sustain their ecological and socio-economic integrity (Cómin and Williams 1994; Arthington et al. 2010). Environmental flow includes not only the quantity, but quality of water necessary to sustain ecological and social functions (Poff et al. 2009). There is thus a need to understand the role of flow in supporting ecological functions such as nutrient control, organic matter processing, food web dynamics and predator-prey interactions (Bond et al. 2008), and how alterations thereof may affect aquatic environments. It is also necessary to establish the relationship between climate, hydrology and biodiversity and to determine water requirements and flow regimes that will not only maintain ecosystem health and support ecological processes but also provide water security for socio-economic purposes (Davidson et al. 2012).

Floods and droughts are considered extreme hydroclimatic events but can also be recurrent features of the natural river flow regime driven by climate and weather patterns and moderated by basin characteristics. Regular fluctuations in river water levels, through predictable floods and droughts, have been recognised as ecologically and economically important (i.e. the flood pulse concept) (Junk et al. 1989; Sparks et al. 1998; Junk 2013) particularly for rivers with adjacent floodplains. However, current human induced climate change already drives changes in average climatic conditions and future climate

change has been predicted to affect not only rainfall variability but the frequency and intensity with which extreme events such as droughts and floods will occur (Bates et al. 2008; Woodward et al. 2012; Mosley 2015). Many freshwater ecosystems are predicted to experience extreme drought-flood hydrological patterns further exaggerated by anthropogenic activities such as flow regulation (Shelton 2009; Garner et al. 2015). These extreme events could have significant effects on freshwater ecosystems and ecological responses as well as socio-economic consequences (Mulholland et al. 1997). The severity of these will depend on the natural conditions and anthropogenic demands of the ecosystem as well as the type of change that is experienced (Caruso 2001; Sprague 2005; Bates et al. 2008).

Low flow drought disturbances are either seasonal or supra-seasonal (White et al. 2012). Seasonal droughts are disturbances in the environment that occur on a regular, predictable basis and, when accompanied by regular floods, can prove to enhance the productivity of an ecosystem, particularly in rivers with extensive associated floodplains (Davidson et al. 2012). Supra-seasonal droughts are thought of as ramps in nature (disturbances that increase in strength and spatial extent over time) and are the result of declined rainfall over an extended period of time, usually several seasons (Lake 2003; Wood and Armitage 2004). Supra-seasonal droughts have complex cascading effects on aquatic ecosystems (Lake 2011; Gaeta et al. 2013), including loss of littoral habitats, change in aquatic food web structure, loss of flow required by rheophilous species and shorter flooding regimes affecting taxa with longer life cycles (Strayer and Findlay 2010; Gaeta et al. 2013; Chessman 2015). These droughts have already increased in frequency and intensity in many regions worldwide, most prominently in semi-arid countries such as Australia and southern Africa (Bond et al. 2008; Chessman 2015; Mosley 2015). Most current knowledge regarding the effects of, and biotic responses to drought arise from studies in temporary wetlands with predictable seasonal droughts. In these habitats, biota have evolved many drought resistant and resilient adaptations (Boulton 2003; Lake 2003; Chaparro et al. 2016). Surprisingly, less has been accomplished on the effects that an unpredictable supra-seasonal drought may have on these habitats. In addition, relatively little is known about the responses of biota to changes in river flow (through reduced rainfall and water availability) and supra-seasonal droughts, particularly in ecosystems associated with regulated rivers (Lake 2003; van Geest et al. 2005; White et al. 2012; Chaparro et al. 2016).

1.2 Riverine ecosystems and their associated wetlands

The flow regime of a river varies between regions and is controlled by both environmental (climate, topography, land cover, soil types and geology) and anthropogenic factors (e.g. alterations to the morphology of the river, water abstraction and the building of dams and diversions) (Piniewski et al. 2017). River floodplains are among the most species rich environments as their hydrological dynamics create a diverse range of habitats and gradients of hydrological connectivity (Ward et al. 1999; Tockner and Stanford 2002). These ecotones are areas of high disturbance, temporal variation and support both terrestrial and aquatic taxa (Junk et al. 1989; Davidson et al. 2012). To maintain ecological integrity and continue to support ecologic and social services, floodplains are dependent on flooding events in order to create seasonal lateral connectivity with a main river channel (Tockner et al. 2002; Paillex et al.

2009). This connectivity may be either permanent or temporary and promotes the exchange of water, nutrient rich sediments and provides nursing areas for fish as well as habitats for a high diversity of biota including macroinvertebrates, fish and macrophytes (Amoros and Bornette 2002; Aarts et al. 2004; Paillex et al. 2007; Rocha et al. 2012). River floodplains also enhance water quality, reduce flooding, and provide nutrient rich soils for crops (Ward and Stanford 1995; Amoros and Bornette 2002; King et al. 2009).

The construction of dams is important to supply water for agriculture, industry and domestic use. However, through controlled flooding regimes, water may be released in a variety of patterns that differ from the natural hydrological regime of a river and thus impacts on the downstream ecosystem (Williams and Wolman 1984; Buytaert et al. 2006; Dube et al. 2016), the most prominent impact being reduced downstream flow (Welcomme 1979; Heath and Plater 2010). If there are channels, streams or floodplain pools dependant on a dammed-up river, these may become either permanent or intermittently dry due to reduced flow (Heath and Plater 2010). Reduced river discharge may result in reduced flood area and environmental flow, leading to variation in abiotic and biotic processes (Tockner and Stanford 2002). These include changes in natural ecological processes such as migration and spawning of fish, nutrient cycling and species richness of aquatic invertebrates (Bunn and Arthington 2002; Heath and Plater 2010). Dams therefore have the potential to severely impact floodplain wetlands where hydrologic and ecological effects of flow regulation cause alterations to the aquatic environment (Heath and Plater 2010). Water abstraction has also become an increasingly common feature in aquatic ecosystems worldwide and can have extensive impacts on ecosystems including longer low-flow conditions and changes in the natural flow regime (White et al. 2012). Döll et al. (2009) found that river flow regime alterations are strongest in semi-arid regions with extensive irrigation as well as regions downstream of large dams. In these regions, river flow alterations can be expected to be a major cause of biodiversity decline.

Many large floodplain systems in Africa are still unstudied and in a relatively natural state, but due to water management strategies such as the construction of dams, irrigation schemes and improperly managed flood release these systems are under severe pressure (Tockner and Stanford 2002). This is certainly the case for the Phongolo floodplain in northern KwaZulu-Natal. This is South Africa's largest inland floodplain system that is under threat due to land use, water abstraction (Smit et al. 2016) and, more recently, a severe supra-seasonal drought (Jozini Local Municipality IDP 2017/18-2021/22, 2017). The floodplain is dependent on water from annual controlled flood releases from the Pongolapoort Dam, completed in 1973, located upstream of the floodplain (Dube et al. 2016). Although guidelines suggesting the volume, frequency, duration and timing of the flood were proposed in the 1970's-1980's (See Chapter 2) these have not been implemented. In addition, at the time of the present study in 2016, South Africa was in the grips of a severe drought, and therefore no floods had been released for two years. These factors may be affecting the aquatic biota of the floodplain as the natural flooding cycle is being disrupted.

1.3 Assessing ecosystem health and integrity

Ecosystem health is difficult to define, although it has been associated with several factors including maintenance of ecological integrity and function, complex food web connectivity and efficient nutrient and energy cycling (UNU-EHS 2016). While there are several chemical, physical and biological means with which to assess the “health” of freshwater ecosystems, it is somewhat challenging to select the most appropriate and relevant indicators. Selection depends on a number of factors, most importantly the end objectives of the study, the ecosystem type and nature of impacts the system experiences. This will decide which indicators will be most likely to detect and diagnose causes of degradation (if present) and guide further management strategies (UNU-EHS 2016).

The use of water quality and aquatic invertebrates as indicators of ecosystem health, particularly in rivers, is well established (Dallas 2000; Dickens and Graham 2002; Simaika and Samways 2011; Helson and Williams 2013; Gerber et al. 2015). These are good indicators as they readily reflect the state of the environment through rapid response, are able to represent impacts of environmental change on various levels and are relatively easy to collect and assess (McGeoch 1998; Simaika and Samways 2011). More recently, stable isotopes and food web analyses have also been used for assessment of ecological integrity. Although these assessments are more labour intensive and do not necessarily reflect rapid responses to changes in the environment, they are quite useful in indicating long term changes and effects (McCann and Rooney 2009; Layman et al. 2012).

1.3.1 Water quality assessment

The impact of drought and possible ecological changes become more uncertain when combined with anthropogenic activities such as overexploitation of resources, water pollution, habitat destruction, water abstraction, dams and regulated discharge (Bond et al. 2008; White et al. 2012). By further reducing flow, droughts have the added effect of causing stagnation of water and concentrating nutrients and pollutants (van Vliet and Zwolsman 2008) and may cause organisms with specific flow requirements or pollution sensitivities to disappear (Leigh 2013). Spot water samples, though useful, only provide a momentary indication of water quality and does not provide clear long-term trends or changes in chemical concentrations (Zhang et al. 2008). It is therefore important to use long-term hydrologic data in order to evaluate natural and anthropogenic induced changes in water quality over time (Amatya and Skaggs 2011). Studies worldwide have made use of long-term data in order to assess changes in river water quality including nitrate fluxes in English rivers (Burt et al. 2011); the hydrology and water quality of pine forest rivers in North Carolina (USA) (Amatya and Skaggs 2011); effects of agriculture on Japanese rivers (Yamazaki et al. 2017) and effects of land use changes on Iranian rivers (Kavian et al. 2018). Water quality data have also been collected across South Africa since the early 1970's. Through the National Chemical Monitoring Programme, managed by the Department of Water and Sanitation (DWS), data from more than 2000 monitoring sites are available on lakes, dams and rivers nationwide (Huizenga et al. 2013). Several studies have made use of these data sets to determine trends in water quality variables across many regions in South Africa (e.g. de Villiers and Thiaart 2007; Bird and Scholes 2012; Huizenga et al. 2013; Lemley et al. 2014). There are, however, no studies on

the long-term water quality of the lower Phongolo River and floodplain and no in-depth assessment has been conducted to determine changes in this ecosystem along with increased human activities.

1.3.2 Aquatic invertebrate assessment

The rate at which an aquatic community can return to equilibrium after a disturbance is a measure of its resilience (Pimm 1984; Brock et al. 2003). In order to assess ecological stability, it is important to determine the ability of a community to resist change and recover after a disturbance as well as species interactions and nutrient flow on an ecosystem level (MacGillivray and Grime 1995; Brock et al. 2003; McMeans et al. 2016). A wide range of organisms (bioindicators) have been suggested and employed to assess aquatic ecosystems. Among the biological communities, macroinvertebrates have proven to be useful indicators to determine the status of freshwater ecosystems, since differences in environmental requirements among taxa produce community characteristics that reflect ecological conditions (Gabriels et al. 2010). Many invertebrates (aquatic and terrestrial) complete either one, several or all of their life stages in the aquatic environment and respond to a broad range of environmental conditions depending on their preferences (e.g. fast or slow flowing water) and sensitivities to various types of pollution (Gerber and Gabriel 2002; Helson and Williams 2013). They are also quite immobile, particularly those that complete all their lifecycles in water, and are constantly in contact with both bottom sediments and the water column which has the potential to expose them to a myriad of stressors (Feio et al. 2007). Because of their direct link to water and their response to environmental stressors, macroinvertebrates and zooplankton are ideal indicators of change in the aquatic environment and are generally used in conjunction with water quality assessments (Miserendino et al. 2011; Helson and Williams 2013; Mereta et al. 2013).

1.3.3 Stable isotope assessment

Assessing the food web of an ecosystem not only provides useful information on ecosystem function but it is also a valuable means to measuring ecosystem resilience and recovery to climate induced changes (Bird et al. 2016). Isotopic signatures can be used to determine the proportion of different food sources ingested by a consumer (Middelburg 2014; Chen et al. 2017; Valladares et al. 2017). This is accomplished by converting the isotopic signatures of the food sources into proportions of a consumer's overall diet (Phillips 2012; Phillips et al. 2014). The advantage of isotopic analysis over stomach and gut content analysis is that this approach determines food sources by a consumer over an extended period of time (Middelburg 2014). This can range from several weeks to months, depending on the organism and type of tissue sampled for analysis (Boecklen et al. 2011; McMeans et al. 2015). Two of the most commonly used isotopes in food web and dietary studies are carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) (Peterson and Fry 1987; Abrantes et al. 2014). Carbon isotopes can be used to indicate the origin of a consumer's food sources as there is very little fractionation during carbon assimilation (between 0-1‰) and the $\delta^{13}\text{C}$ differs between primary producers (Peterson and Fry 1987; Abrantes et al. 2014; Neres-Lima et al. 2017). Nitrogen isotopes are used to determine trophic position of an organism as there is greater trophic fractionation during nitrogen assimilation (approximately 3‰).

Bayesian mixing models (e.g. SIAR and more recently MixSIAR) are useful tools that have become increasingly used to study food webs, consumer diets, energy flow and the effects of various disturbances on ecosystem structure and resilience (e.g. Wantzen et al. 2002; Woodward et al. 2012; Lemmens et al. 2017; Stewart et al. 2017). This is beneficial as it may allow ecologists to detect changes in an organism's diet, whether episodic or seasonal, and possibly back-calculate in order to determine when the diet shift occurred (Phillips and Eldridge 2006; Klaassen et al. 2010; Boecklen et al. 2011) and relate this to possible causes. By incorporating the uncertainties that are associated with using multiple sources and isotopic values, Bayesian mixing models have the potential to provide more robust results than other statistical dietary analyses (Parnell et al. 2010; Valladares et al. 2017).

Stable isotope analysis and Bayesian mixing models have become increasingly used for various aspects of ecological studies worldwide including but not limited to fish diet changes in Brazilian wetlands (Wantzen et al. 2002); the importance of terrestrial sources to stream food webs in Australia (Reid and Thoms 2008); assessment of nitrogen pollution in Spanish estuaries (Baeta et al. 2017); effects of invasive fish on freshwater food webs in Lake Michigan (USA) (Foley et al. 2017); changes in consumer dietary responses in freshwater lakes of New Zealand (Stewart et al. 2017) and dietary changes of spiders in German riparian ecosystems (Manfrin et al. 2018). Although stable isotopes and Bayesian modelling have also been increasingly used in Africa (e.g. Nyunja et al. 2009; Abrantes et al. 2014; Lemmens et al. 2017) and southern Africa, (e.g. Richoux and Froneman 2007; Hill et al. 2015; Bird et al. 2016; Taylor et al. 2017a), none of these studies have determined changes in food webs or consumer diets during an extreme climatic event such as a drought.

1.4 Problem statement, hypotheses, aims and objectives:

Although quite extensively studied in the 1970's to 1980's (e.g. Coke 1970a; Heeg et al. 1980; Heeg and Breen 1982; Rossouw 1985), little research has been conducted on the Phongolo River ecosystem since then and many of these studies either focused on the upper catchments (e.g. DWAF 2008; van der Laan et al. 2012; DWS 2014a) or social aspects (Jaganyi et al. 2009; Lankford et al. 2011). Only recently, through cooperation between North-West University (NWU), KU Leuven (Belgium), University of Johannesburg, University of Zululand and University of Venda, funded by the Water Research Commission (WRC) and VLIR-UOS, has research on the biodiversity and ecology of the lower Phongolo River and its conservation area, Ndumo Game Reserve (NGR), increased again. An extensive WRC research project took place from 2012 to 2014 in order to assess various aspects of the lower Phongolo River and floodplain including the health and diversity of many terrestrial and aquatic biota and a risk assessment analysis of the ecosystem (see Smit et al. 2016). Unfortunately, shortly after this project was completed, a supra-seasonal drought event occurred in South Africa between 2015 and 2017 that severely reduced rainfall in most regions across the country, including northern KwaZulu-Natal and the Phongolo River. The last flood release from the Pongolapoort Dam occurred in December 2014 after which only a baseflow has been released in order to maintain river flow in accordance with the "compensation flows" agreement with Mozambique (DWS 2014a). Based on results obtained from the W4H013 gauging station located below the Pongolapoort Dam (available from: <https://www.dwaf.gov.za/Hydrology/Verified/hymain.aspx>. Accessed 5 May 2018) the overall average

flow per month during the study period was $7.33 \text{ m}^3\text{s}^{-1}$ (data from January 2016 to May 2017; See section 2.4, Chapter 2). This was considerably lower than the average of $26.16 \text{ m}^3\text{s}^{-1}$ flow per month in 2014 before the drought (the average excludes the controlled flooding period of October and November 2014) There is no documentation of the effects that the drought, low baseflow and below average rainfall may be having on the ecosystem. This provided an excellent opportunity to assess the effects of drought on an ecologically important yet severely regulated lowland river ecosystem in South Africa and determine the ecological resilience of the Phongolo ecosystem in the face of climate change. In addition, given the previous concerns raised regarding the water quality and quantity of the region, there is a desperate need to understand the current environmental state of the lower Phongolo River, NGR and associated wetlands.

1.4.1 General hypotheses

- Drought conditions accentuate the poor water quality of the Phongolo River that is already affected by a variety of human induced stressors.
- A lack of flooding events, led on by the supra-seasonal drought, has affected various habitats of the lower Phongolo River and floodplain ecosystem.
- Hydrologic connectivity between the river and a floodplain wetland structures its aquatic invertebrate communities.
- The baseflow released from the Pongolapoort Dam during the supra-seasonal drought is not enough to sustain stable aquatic food webs or aquatic invertebrates communities in the lower Phongolo River.

Specific hypotheses can be found in each of the chapters of the thesis.

Aims:

The main aim of this study was to assess the present status of water quality, aquatic invertebrate diversity, and the food web and fish consumer diets of the lower Phongolo ecosystem. This study further aimed to determine the resilience of the ecosystem by evaluating the responses of these variables to change in flow, water levels and drought.

Objectives:

- Use long-term historic and current water quality data of the middle and lower Phongolo River, multivariate statistics and Physico-Chemical Driver Assessment Index (PAI) analysis to assess the historic and present state of the water quality of the river and how this may have changed over time and through the supra-seasonal drought.
- Make use of *in situ* field mesocosm experiments to determine whether length of hydrological connectivity has any effect on aquatic invertebrate community structure.
- Determine current aquatic invertebrate community structure, aquatic food webs and fish consumer diets in:
 - An ecologically unique temporary wetland of NGR through an out of season drying event.

- The only large naturally saline lake in NGR.
- The lower Phongolo River through the supra-seasonal drought

1.5 Layout of Thesis

This study has been divided into nine chapters

Chapter 1: Background information pertaining to the rationale of the study as well as the set hypotheses, aims and objectives.

Chapter 2: Introduction to the study area and selection of study sites.

Chapter 3: In depth assessment of water quality of the Phongolo River dating back to 1974. Spatial and temporal differences and trends in water quality changes over the past 40 years are analysed and an assessment of water quality of the lower Phongolo River during the supra-seasonal drought is also performed.

Chapter 4: A five-week *in situ* field based mesocosm experiment to determine how varying water levels in a floodplain pool may affect aquatic invertebrate communities.

Chapter 5: An ecological assessment of a unique temporary wetland located within NGR as it went through an out of season drying event. Assessment included analysis of aquatic invertebrate diversity and community structure, food web and fish consumer dietary analysis.

Chapter 6: Analysis of possible changes in aquatic invertebrate biodiversity and changes in the food web and diet of consumers in a naturally saline lake located within NGR and how this may have been affected during the supra-seasonal drought and subsequent increased salinity the site experienced.

Chapter 7: An ecological assessment of the Phongolo River as it flows through the lower reaches of the region and into NGR with the use of aquatic invertebrate diversity, community structure, food web and fish consumer dietary analysis.

Chapter 8: An in-depth discussion of the findings of the study as well as conclusions drawn and recommendations.

Chapter 9: A list of all references used throughout the thesis.

CHAPTER 2:

Study Area

2.1 Introduction to the Phongolo River

The Phongolo River originates in the Wakkerstroom area, Mpumalanga, where it flows steeply eastward, creating the border between Mpumalanga and KwaZulu-Natal (Rossouw 1985; Jaganyi et al. 2009). The river passes just north of Paulpietersburg where the Bivane River flows into it. From here the river flows through a gorge in the Lebombo and Ubombo mountain ranges in northern KwaZulu-Natal, where it is also joined by several small tributaries (Heeg and Breen 1982; DWA 2009). Hereafter, the river flows through the town of Pongolo, where it widens into the intensely cultivated Lowveld area, finally flowing into the Pongolapoort Dam located in Maputaland (Smith et al. 2008; DWA 2009). Conceptualised in the 1950's and completed in 1973, the Pongolapoort Dam (Figure 2.1) was built with the key purpose of supplying enough water to irrigate 40 000 to 50 000ha of cultivated land (mostly sugarcane) in the highly fertile area adjacent to the Phongolo floodplain known as the Makhathini flats (van Vuuren 2009). However, due to the significant price decrease in sugar, among other reasons, only approximately 3 000ha of sugarcane and cotton irrigation has occurred, and the Pongolapoort Dam remains one of the most underutilised impoundments in South Africa (DWA 2009).

The lower Phongolo River and associated floodplain starts below the Pongolapoort Dam in the town of Jozini, stretching approximately 80 km downstream to the confluence of the Phongolo and Usuthu rivers at the international border with Mozambique within the conservation area of Ndumo Game Reserve (NGR; Figure 2.1). It is South Africa's largest riverine floodplain with an area of 120 km² and a width that varies between 0.8 km and 4.8 km (Merron et al. 1993b; Jaganyi et al. 2009). When fully inundated the floodplain covers an approximate area of 10 000ha to 13 000ha and, when flood water has receded, roughly 2200ha of water is contained in the approximately 80-90 floodplain pans (Breen et al. 1978; Heeg and Breen 1982). Approximately 40% of the floodplain lies in South Africa while the remaining 60% is found in Mozambique and forms part of the Rio Maputa River floodplain (Merron et al. 1993a; SA Wetlands Conservation Programme 1996; Dube et al. 2016).

Before the construction of the Pongolapoort Dam, summer flooding inundated the floodplain, creating a mosaic of environments for the aquatic biota of the area and provided a source of food to the local people in the form of fishing. When floods receded, the rich, fertile soils of the floodplain were exposed and cultivated by local inhabitants for a range of crops. Local communities also made use of the adjoining forests and natural resources for a variety of purposes including fuel, construction and grazing area for livestock (van Vuuren 2009). Possible effects and recommended flood release strategies were researched during the planning and construction phase of the dam (e.g. Coke 1970a; Coke 1970b; Heeg et al. 1980; Heeg and Breen 1982). By the time the dam was completed, a detailed flooding regime had been determined that would mimic the natural floods, sustain ecological integrity of the floodplain and continue to provide services to the people in the region with minimal effects on the

irrigation schemes (van Vuuren 2009). For a detailed discussion of the suggested flooding regime see DWS (2014a) and Dube et al. (2015).

However, due to unforeseen circumstances such as the drought of 1981 to 1983, the Domoina cyclone of 1984 (see Rossouw 1985) and lack of funding for continued monitoring, the proposed flooding regime was difficult to implement. At the same time, local people had switched to planting white maize which had the potential for a higher yield, but also needed a longer time to grow than previous crops which meant that the timing of the flooding regime destroyed most of the crops. A new flood release was negotiated which wholly disregarded the original suggested regime and between 1984 and 2005 only 25 controlled flood releases were made from the dam. These occurred at varying times where several years had no release whatsoever while other years had two releases (Figure 2.2). This led to a severe decrease in fish stocks and other aquatic biota as well as a decline in arable floodplain areas as the floods were often so far apart that channels dried up completely (van Vuuren 2009). More than 40 years later there is still no proper management of the dam and its release. The main issue being that no true balance has been found between the timing of the flow releases that will deliver ecological services as well as providing the needed socio-economic services (Dube et al. 2015).

Currently, under normal circumstances, two controlled floods are released from the dam, the largest of which generally occurs in October, while a smaller release occurs around July (Dube et al. 2015; Jozini Local Municipality IDP 2017/18-2021/22, 2017; Figure 2.2). The main goal of this flooding regime is to meet socio-economic demands and, apparently, to fill the wetlands in NGR, although this is generally not achieved. This is largely because the volume and velocity of the flood released from the dam in October is generally not enough to reach many of the wetlands. In order to achieve a maximum flooding event, the controlled flood release should correspond to the natural flooding of the Usuthu River that occurs during the high rainfall season of late November to early December (DWS 2014a). If both rivers flood at the same time, there is a backflow of water into NGR and the wetlands are filled.

Concerns have also been raised regarding not only the effect of the flood releases on the downstream ecosystem, which is still poorly understood, but also the human activities occurring on the floodplain and in the upper reaches of the river's catchment. The lower Phongolo has largely been utilised by small scale subsistence farms for the past few hundred years (DWA 2009; van Vuuren 2009) although larger irrigation schemes have developed in recent years (Smit et al. 2016). Threats to the upper reaches of the Phongolo River include abandoned coal mines in the Paulpietersburg area, and an estimated 199 km² and 480 km² of irrigated and afforested area respectively (DWA 2009). The middle reaches of the Phongolo River are utilised by some 16 000ha of sugarcane and 1000ha of citrus, mango and vegetable farms. Water from the upper Phongolo River is reserved for these various land uses as well as for the Impala Irrigation Scheme in the middle Phongolo (DWA 2009). In addition, the Bivane Dam, located on the Bivane River upstream of its confluence with the Phongolo River was completed in 1995 to provide additional irrigation water to the surrounding sugarcane farms and has also decreased the water inflow into the Pongolapoort Dam (Dube et al. 2015). The lower Phongolo River and Ndumo region is also an endemic intermediate to low-risk malaria area where indoor residual

spraying (IRS) of DDT is employed as a vector control (McHugh et al. 2011; Wepener et al. 2011; Smit et al. 2016).

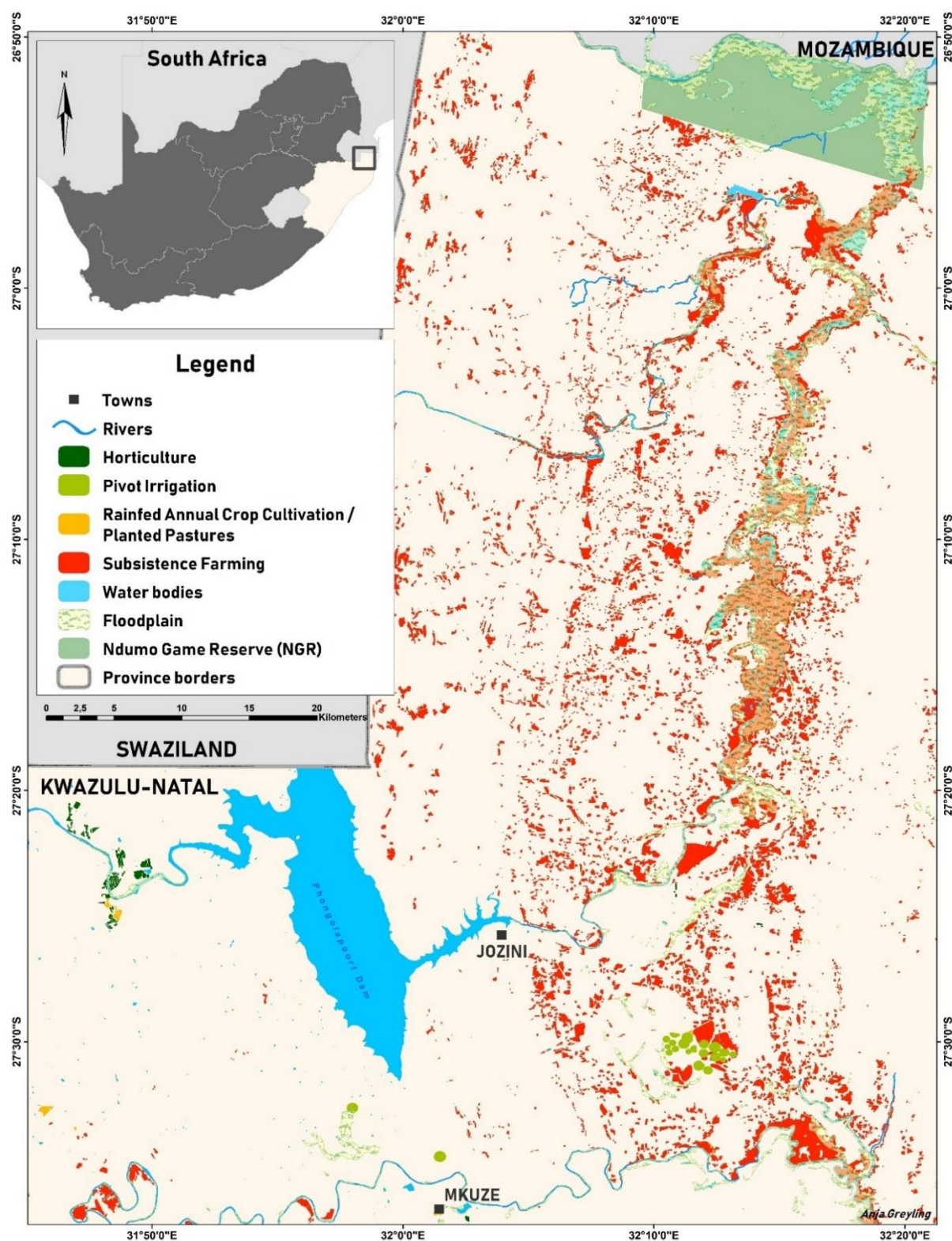


Figure 2.1: Map of the Pongolapoort Dam and lower Phongolo region indicating various irrigation schemes and farms located above the dam and on the floodplain.

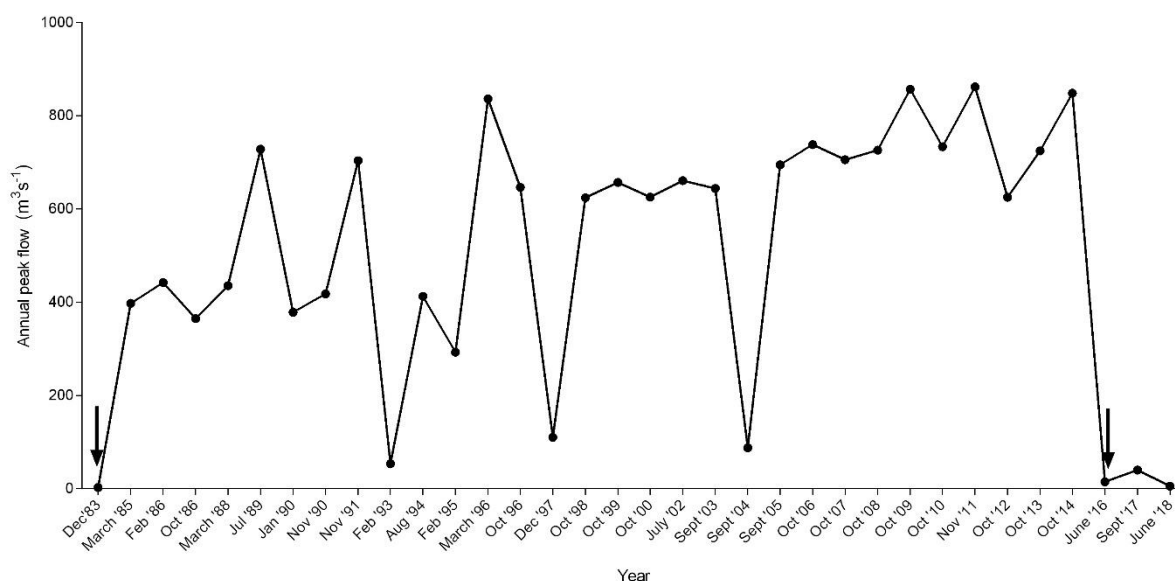


Figure 2.2: Annual peak flows of Phongolo River downstream of dam measured from 1983 to 2018. Arrows indicate years of severe drought in 1983 and 2016 (available from <https://www.dwaf.gov.za/Hydrology/Verified/hymain.aspx>. Accessed 14 September 2018).

2.2 Ecological importance of the lower Phongolo River and Ndumo Game Reserve

Maputaland is a biodiversity hotspot in southern Africa, encompassing an approximate area of 17 000 km² and including parts of southern Mozambique, eastern Swaziland and northern KwaZulu-Natal, South Africa. Parts of Maputaland also fall within the Lubombo Transfrontier Conservation Area (TFCA) initiative between South Africa, Mozambique and Swaziland (Smith et al. 2008). Included in the Maputaland conservation area is the Phongolo floodplain and NGR, the only protected region of the river and floodplain. Many endangered, rare and endemic species are found in the floodplain area, most of which are highly dependent on the river and the numerous temporary, permanent and floodplain associated wetlands (NGR IMP 2009-2013; 2009; Dube et al. 2015). This region supports the most diverse fish fauna in South Africa (44 species; Figure 2.3), most of which do not occur any further south in the country, the third largest population of the Nile crocodile (*Crocodylus niloticus*) (average of 800 individuals) in South Africa, and approximately 39 frog species (Calverly and Downs 2014; Smit et al. 2016). Ndumo Game Reserve, covering an area of 11898 ha, was established in 1924 with the main aim to conserve the hippopotamus (*Hippopotamus amphibius*) as well as the floodplain's remarkable biodiversity. This game reserve is an internationally recognised important bird area (IBA) as it boasts 424 bird species of which 120 are wetland dependant, at least 30 are endangered and five are endemic to Maputaland (NGR IMP 2009-2013, 2009; Smit et al. 2016). In 1997, the game reserve was also declared a Ramsar site under the Wetlands of International Importance convention as it features not only five distinct wetland types (ranging between fresh, brackish and saline and permanent and intermittent rivers, lakes, pools and riparian/gallery forests) but meets six of the Wetlands of International Importance Criteria (NGR IMP 2009-2013, 2009).

Even though NGR is considered such an ecologically important region, it is severely under threat as it is surrounded by both subsistence and commercial agriculture. A large agricultural irrigation scheme also operates upstream of the reserve in its catchment area (SA Wetlands Conservation Programme 1996). In addition, the eastern part of the reserve is utilized by the local community as a farming area and local people fish in the river within NGR and actively abstract water from the river for their crops as well. Very little has been accomplished in terms of research and conservation since the reserve's establishment, and it is unknown whether the water that NGR receives is of a quality and quantity to support and protect this biodiversity hotspot.



Figure 2.3: Photographs of typical fish species found in the lower Phongolo River and associated floodplain namely; *Brycinus imberi* (a); *Oreochromis mossambicus* (b); *Hydrocynus vittatus* (c) and *Schilbe intermedius* (d).

2.3 Climate and geology

KwaZulu-Natal, particularly the northern regions such as where the Phongolo system is located, is characterised by a warm, humid, subtropical climate and experiences two main seasons namely dry winters and wet summers. In the lower Phongolo ecosystem, the winter period generally occurs between May and mid-September and is characterised by temperatures ranging between 16-26°C and little to no rainfall. In the summer period (from end-September to April) temperatures range between 23°C to well above 40°C and high rainfall. The average rainfall along the Lebombo mountains is between 600 to 800mm although most of this does not reach the lower reaches of the Phongolo floodplain. Less detailed weather information for the Ndumo region is available, although the average rainfall is estimated at 638mm, the heaviest of which falls in late summer (Musil et al. 1973; SA Wetlands Conservation Programme 1996; Heath and Plater 2010; Jozini Local Municipality IDP 2017/18-2021/22, 2017). Average annual evaporation for the region is 1660mm with the highest evaporation occurring in winter and early spring (Jozini Local Municipality IDP 2017/18-2021/22, 2017).

The geology and soils of the Phongolo region range from sandy loams to saline calcareous black clay and are part of what makes it such a unique and characteristic ecosystem (Musil et al. 1973; Heeg and Breen 1982). The geology principally consists of marine cretaceous deposits which are overlain by tertiary Pleistocene and Holocene sand deposits (SA Wetlands Conservation Programme 1996; DWS

2014a). Groundwater seepage makes a rather large contribution to the water levels in many of the lakes and wetlands of the floodplain. The marine cretaceous deposits cause naturally saline groundwater in the region and the wetlands that receive groundwater seepage therefore characteristically become quite saline (500 to 5000 $\mu\text{S}\cdot\text{cm}^{-1}$), particularly in the dry winter periods (Heeg et al. 1978; SA Wetlands Conservation Programme 1996). This is particularly true of Nyamithi lake, the largest inland saline lake in NGR. During summer, floods from both the Phongolo River and the Usuthu River, particularly in NGR, flushes out the saline water and replenishes it with fresh, low conductivity water (Heeg et al. 1978).

2.4 Baseflow from the Pongolapoort Dam

Although there was no controlled flood release during the supra-seasonal drought, a monthly baseflow is continuously released (Figure 2.4). As a result of the “compensation flows” agreement with Mozambique, this baseflow is supposed to be no less than 5 m^3s^{-1} (DWS 2014a). Based on results obtained from the W4H013 gauging station located below the Pongolapoort Dam (available from: <https://www.dwaf.gov.za/Hydrology/Verified/hymain.aspx>. Accessed 14 September 2018) the average flow per month during the study period was 7.33 m^3s^{-1} (data from January 2016 to May 2017). However, average flow was higher during 2016 (8.50 m^3s^{-1}) than 2017 (4.54 m^3s^{-1}) (Figures 2.3).

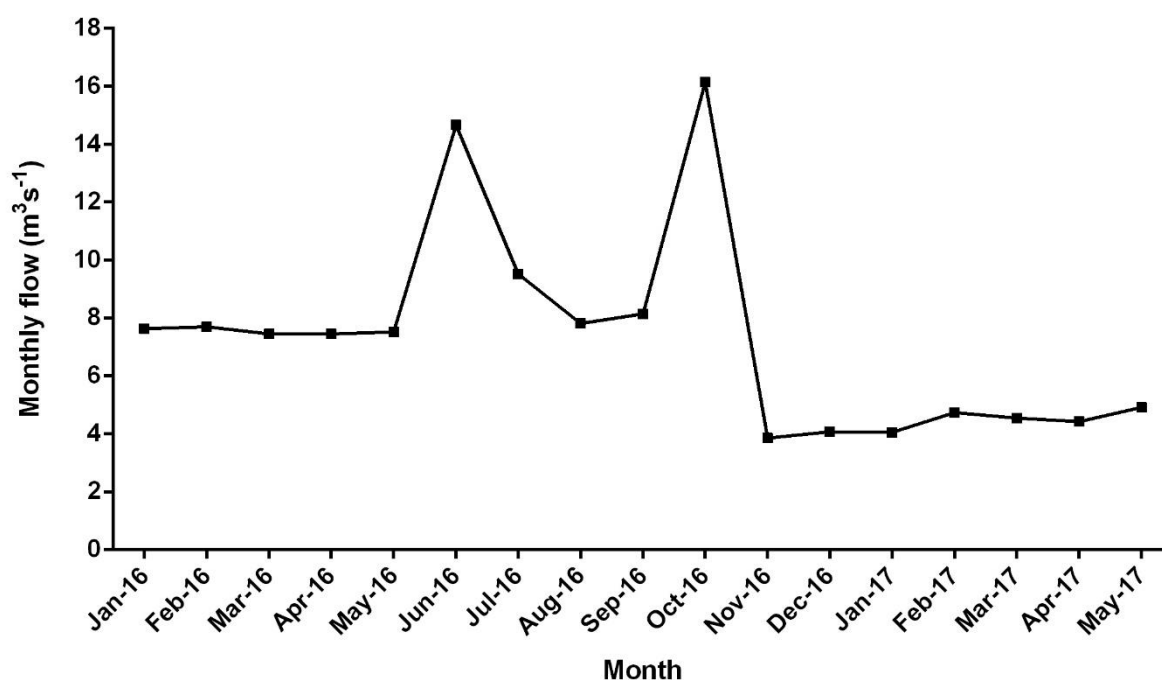


Figure 2.4: Monthly peak flows of Phongolo River downstream of Pongolapoort Dam during the present study from 2016 to 2017. (available from <https://www.dwaf.gov.za/Hydrology/Verified/hymain.aspx>. Accessed 14 September 2018).

2.5 Site selection and descriptions

Six sites were selected for sampling between 2016 and 2017 during the present study (Figure 2.5). Site selection was based on several factors, the most important being accessibility, correspondence with sites previously sampled for research in the area, current impacts and the research questions of the project.

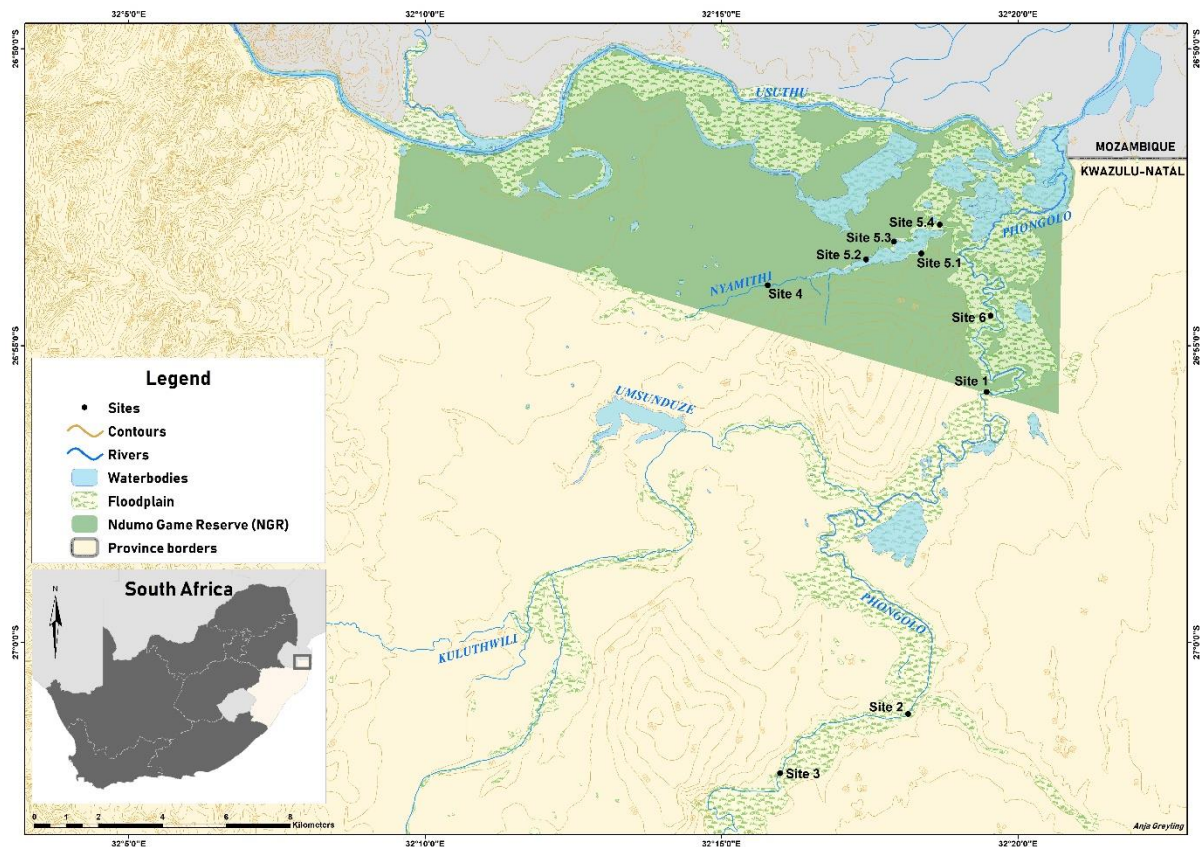


Figure 2.5: Map of the Ndumo region and Ndumo Game Reserve indicating all sites selected for this study.

2.5.1 The Phongolo River

Three sites were selected on the Phongolo River as it flows through the Ndumo region and into NGR. Two of these sites are located outside of the game reserve and one is located just after the river enters the game reserve. The furthest upstream site outside NGR is located approximately 70 km downstream of the Pongolapoort Dam next to a motor vehicle bridge that crosses the Phongolo River (Site 3; Figure 2.56). An unauthorised weir was constructed at this site between 2015 and 2016 in order to dam water up for water abstraction for road building (Figure 2.6a and b). This has created a fast-flowing riffle habitat below the weir in the downstream area of the site (pers. observation, Figure 2.6c and d). Local people also use the downstream area for washing vehicles and laundry (pers. observation; Figure 2.6e). The second site outside NGR (Site 2) is located approximately 4.6 km downstream of Site 3 adjacent to a walkway bridge (Figure 2.7). Concrete blocks are present in the upstream area of the stream, which has created additional slow flowing pools and faster flowing regions (Figure 2.7a). There is a slow-

flowing pool habitat below the bridge with different aquatic plants present (Figure 2.7b and c). This site is also used by local communities to wash clothing and as a watering hole for livestock (pers. observation, Figure 2.7d and e). The site within NGR (Site 1) is located approximately 22 km downstream of Site 2 just as the river enters the southern border of NGR and waterflow is slower here than in the river outside NGR (Figure 2.8). Although this site is located within the game reserve, the eastern part of the game reserve is utilised by the local community as a farming area and local fisherman use small boats to fish on the river inside NGR (pers. observation; Figure 2.8b).

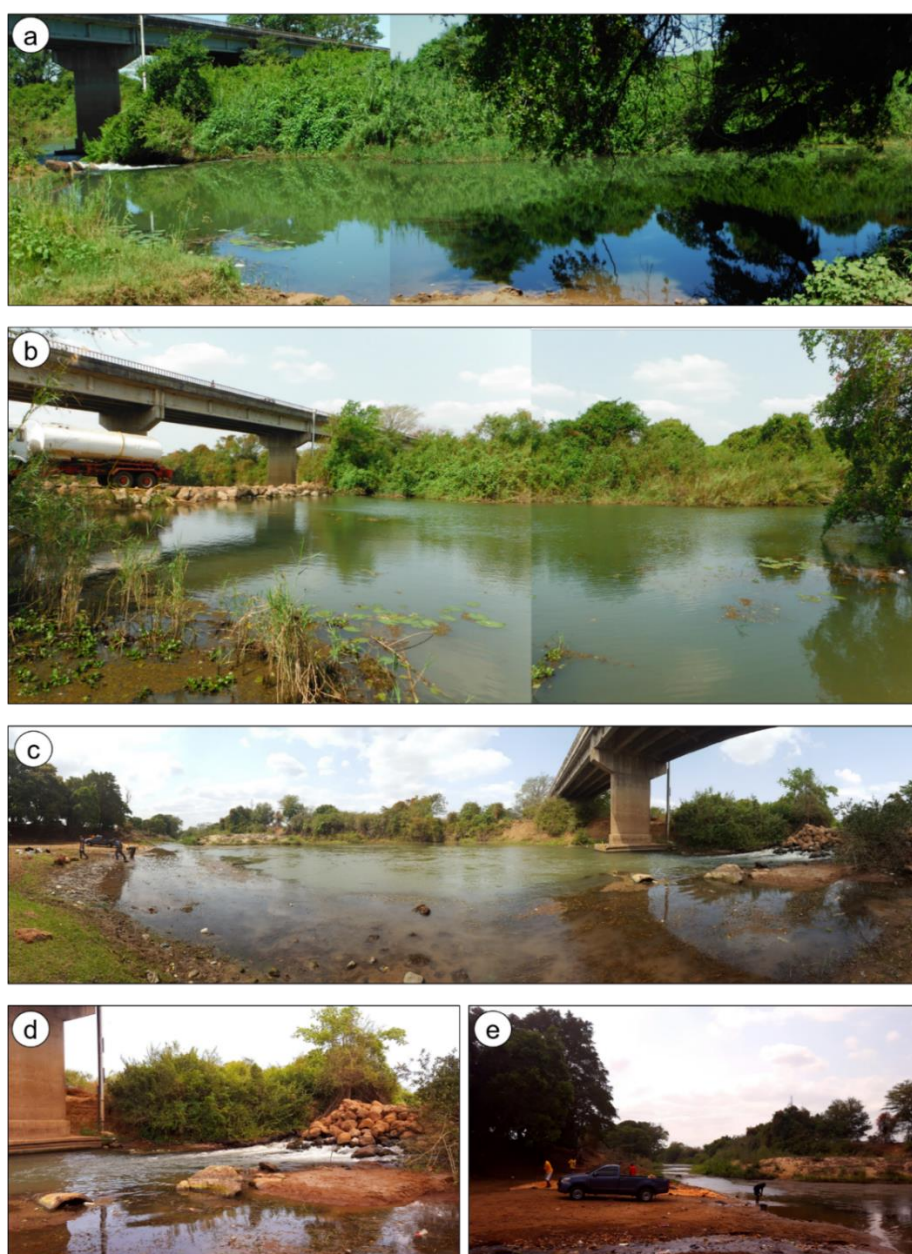


Figure 2.6: Photographs of Site 3 located on the lower Phongolo River outside Ndumo Game Reserve indicating the upstream area of the river in 2014 before the weir was built (a); the upstream area in 2016 after the weir was built (b); the downstream area that was sampled in 2016 (c); downstream of the weir indicating water flow (d) and downstream area where local people utilize the river for washing of vehicles (e).

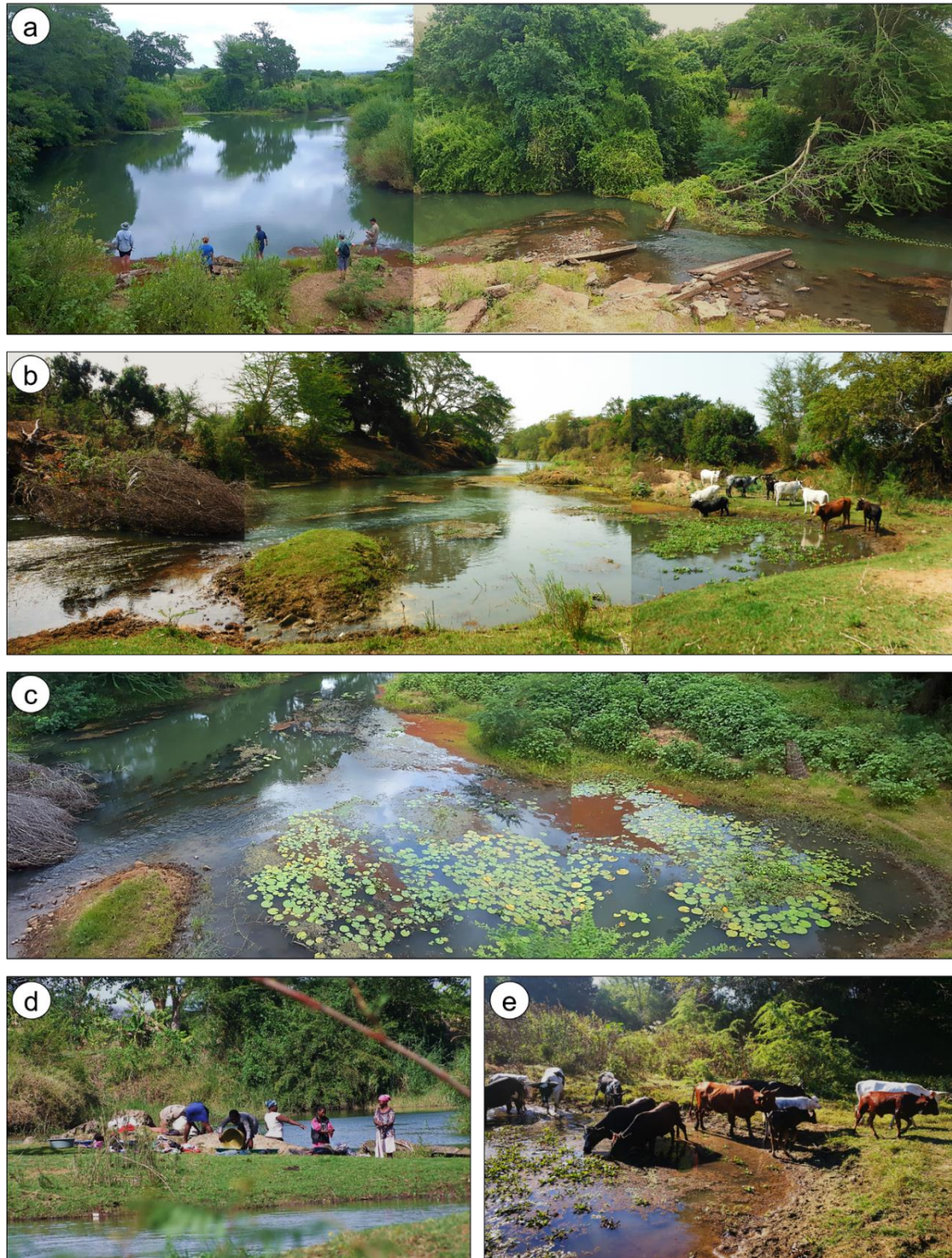


Figure 2.7: Photographs of Site 2 located on the lower Phongolo River outside Ndumo Game Reserve indicating the upstream area of the river and concrete blocks in the stream (a); downstream area of the river with the slower pool and aquatic vegetation (b and c); local women washing clothes in the river (d) and livestock grazing and drinking water from the river (e).



Figure 2.8: Photographs of Site 1 located on the lower Phongolo River within Ndumo Game Reserve indicating the river flowing downstream from right to left into the game reserve (a) and a small boat that local people use for fishing as well as the deforested eastern area of the river within Ndumo Game Reserve that local people use for crop farming (b).

2.5.2 Temporary pool

Site 4 is an ecologically unique temporary pool located within NGR (Figure 2.9). Even though approximately 80 floodplain and numerous temporary pools are found within NGR and surrounding region, this is the only site where populations of the annual spotted killifish, *Nothobranchius orthonotus* are found at any given time. In addition, although the site receives water through rainfall and generally dries up between seasons, it is part of a river channel that feeds into the saline Nyamithi lake (Figure 2.1). Due to the severe supra-seasonal drought, the natural wet/dry cycle of this site has been disrupted. This disruption was observed as the site was inundated from May to August 2016 during what is considered the dry period (Figure 2.9a and b) and dry from September to November 2016 when the sites generally receive water from the controlled flood and some rainfall (Figure 2.9c). The site received rainwater in November 2016 and again in December 2016, when a large rainstorm filled the site to overflowing (pers. observation), and it remained filled until February 2017 (Figure 2.9d and e). By May 2017 the site was completely dried up. This site was selected as a study site to determine possible changes in the biodiversity and community structure of aquatic invertebrates of a temporary pool experiencing a supra-seasonal drought as well as the diet composition of the annual killifish under naturally stressed conditions.

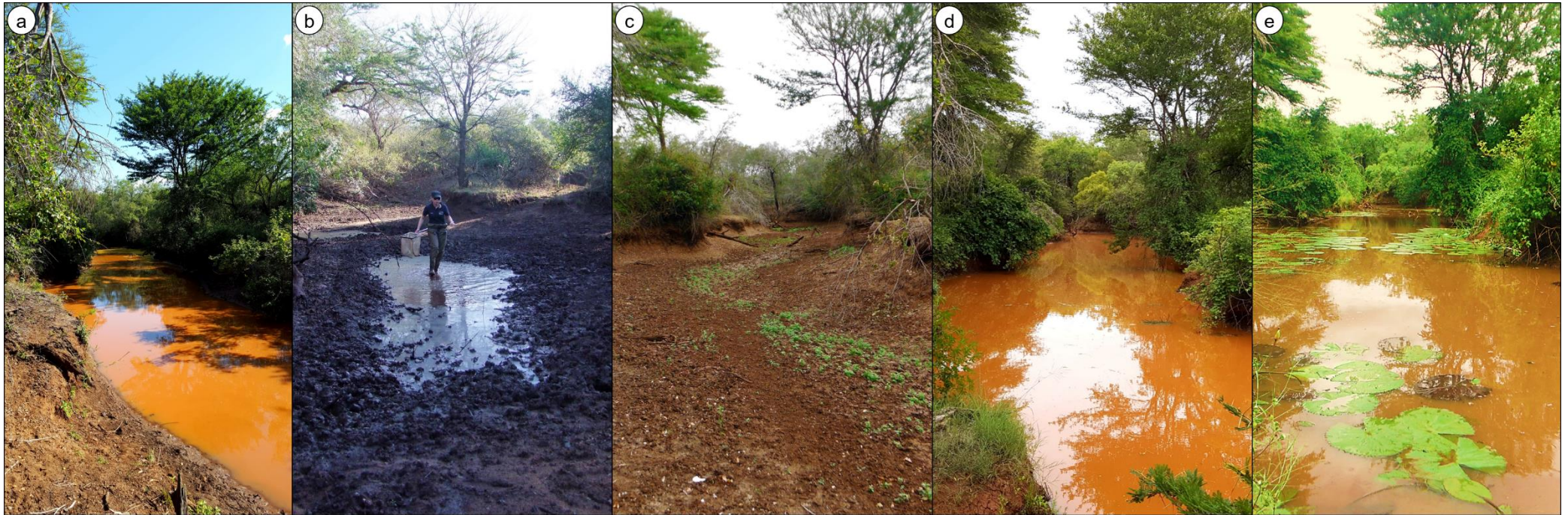


Figure 2.9: Photographs of the temporary pool (Site 4) located within Ndumo Game Reserve during May 2016 (a); August 2016 (b); September 2016 (c); December 2016 (d) and February 2017 (e).

2.5.3 Nyamithi lake

Nyamithi lake (Site 5; Figure 2.10) is one of the largest water bodies within NGR (183.4 ha; Heeg and Breen 1982) and the only naturally saline lake (approximately $5000 \mu\text{S}\cdot\text{cm}^{-1}$) (Heeg and Breen 1982; Seaman et al. 1991). Nyamithi receives water largely from the Phongolo River through the annual controlled flood release in October from the Pongolapoort Dam as well through rainfall during the wet season from its own small catchment and natural floods from the Usuthu River. This site was sampled once in 2010 for food web analysis and has continuously been sampled for aquatic invertebrate community analysis since 2014 when the site was still filled with flood and rainwater (Figure 2.10a). The site became severely dry during 2016 due to the supra-seasonal drought (Figure 2.10b and c). Through some rainfall and floods from the Usuthu River, the site had filled again to at least 60% of its original size by February 2017 (Figure 2.10d). This site was selected for the present study to assess possible long-term changes in the ecology (through aquatic invertebrate community and general food web analysis) and fish consumer diets through the supra-seasonal drought. Four areas were selected in this site for aquatic invertebrate and water sample collection to provide an integrated representation of the site (Site 5.1-5.4) and these areas correlated to those sampled during 2014 (Figure 2.11 and 2.12). Photographs of aquatic invertebrates commonly found in Nyamithi lake are presented in Figure 2.13.

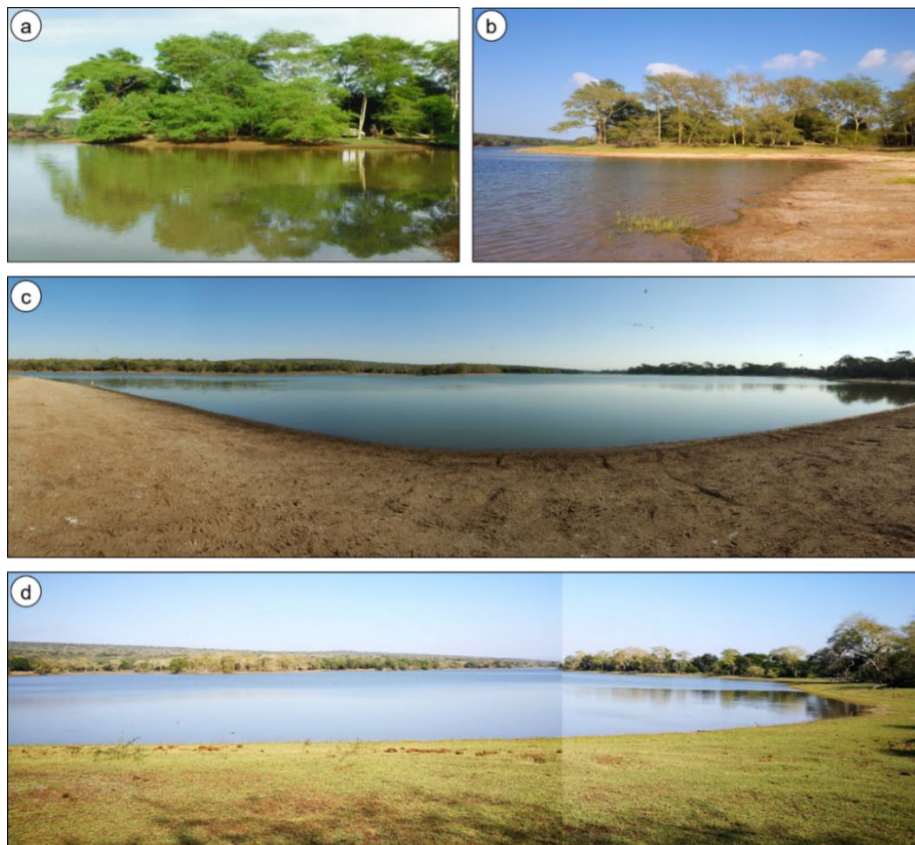


Figure 2.10: Photographs of Nyamithi lake (Site 5) located within Ndumo Game Reserve during September 2014 when the site still received regular water supply from the controlled flood and rainfall (a); May 2016 during the peak of the supra-seasonal drought (b and c) and February 2017 after the site had received some rainwater and a small flood from the Usuthu River (d).

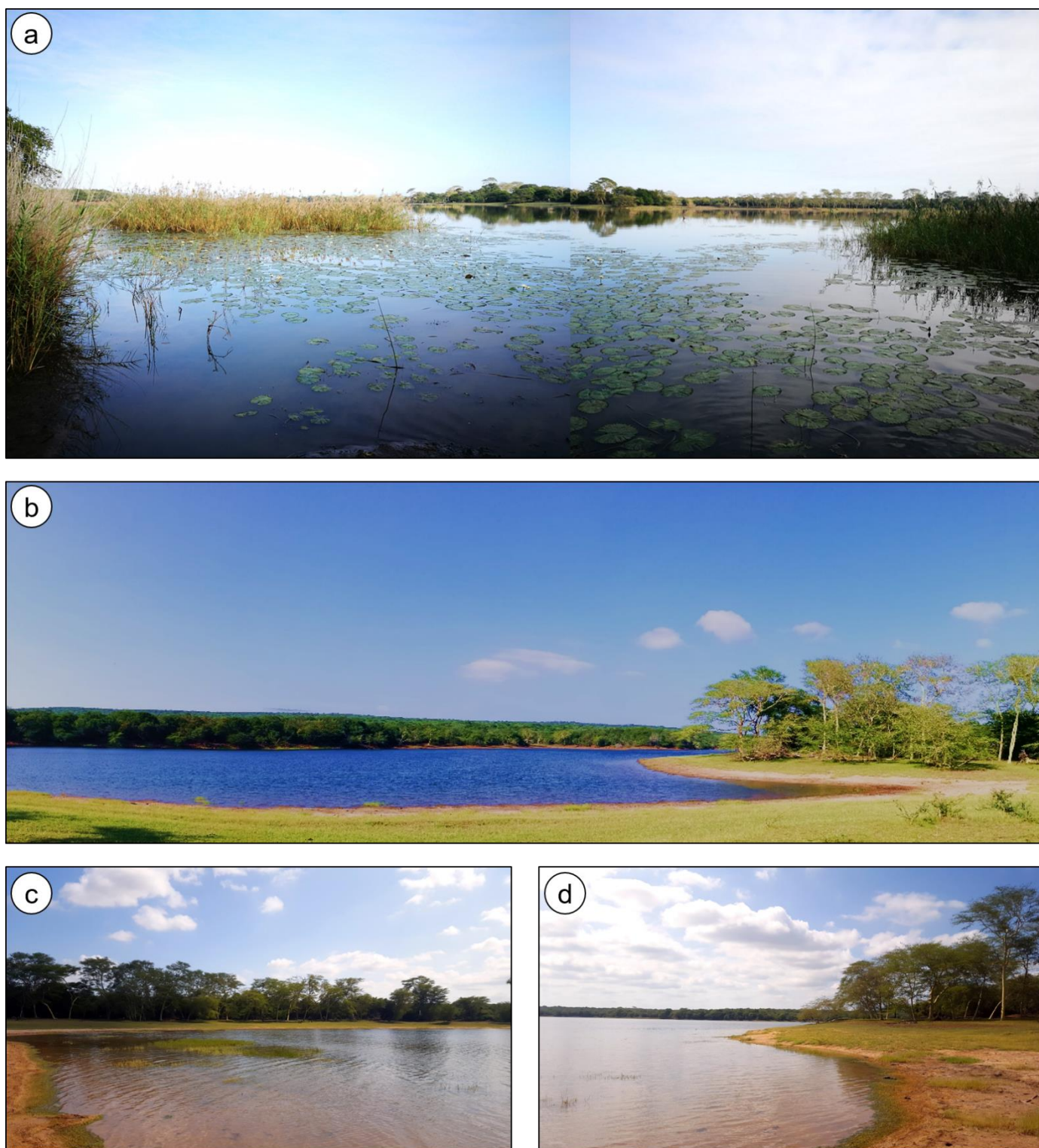


Figure 2.11: Photographs of Nyamithi lake (Site 5) indicating three of the areas namely Site 5.1 (a); Site 5.2 (b) and Site 5.3 (c and d) sampled between 2014 and 2017.

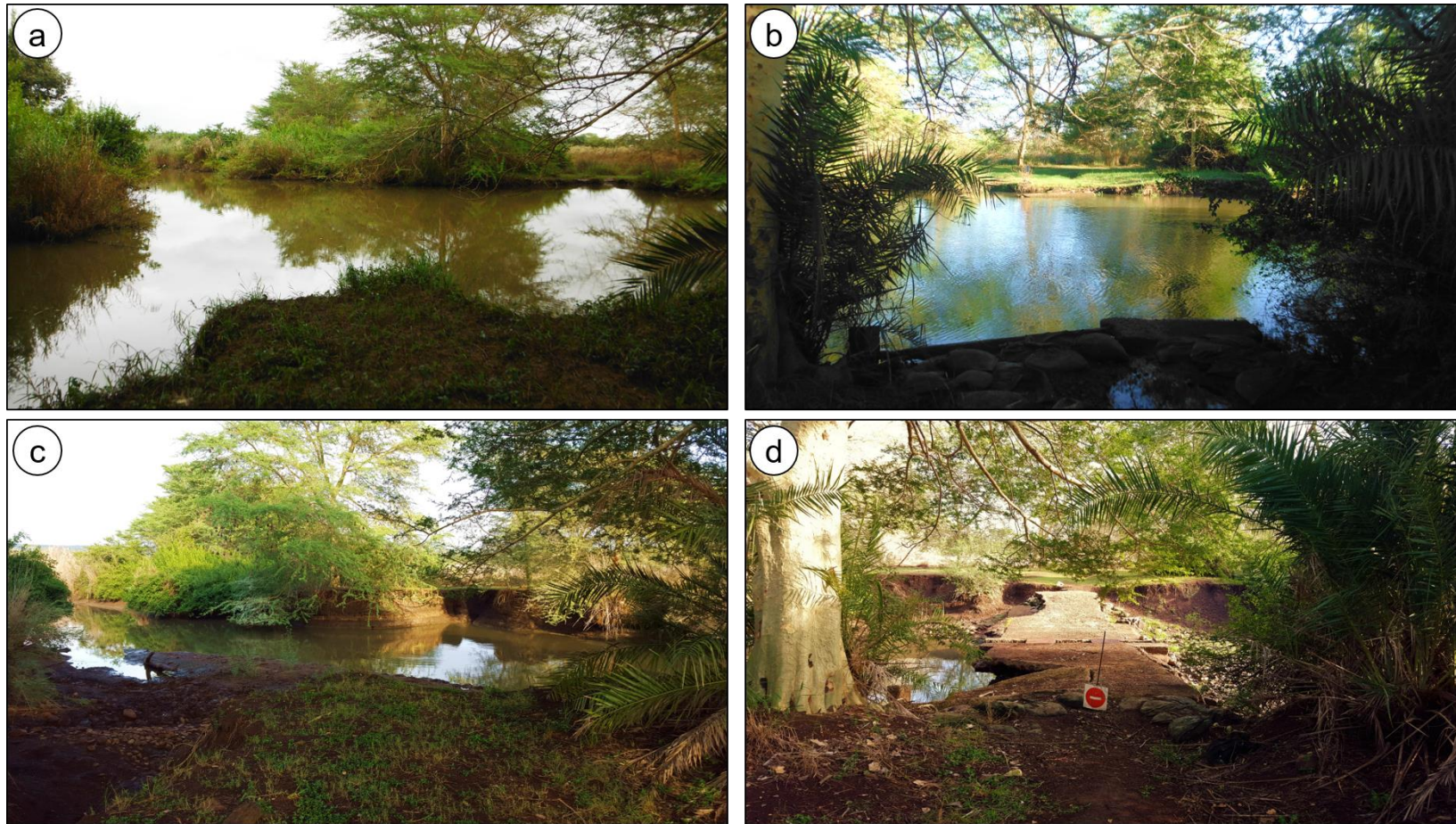


Figure 2.12: Photographs of the outflow of Nyamithi lake (Site 5.4) September 2014 when the site still received regular water supply from the controlled flood and rainfall (a and b) and May 2016 during the peak of the supra-seasonal drought (c and d). This site was also sampled between 2014 and 2017.

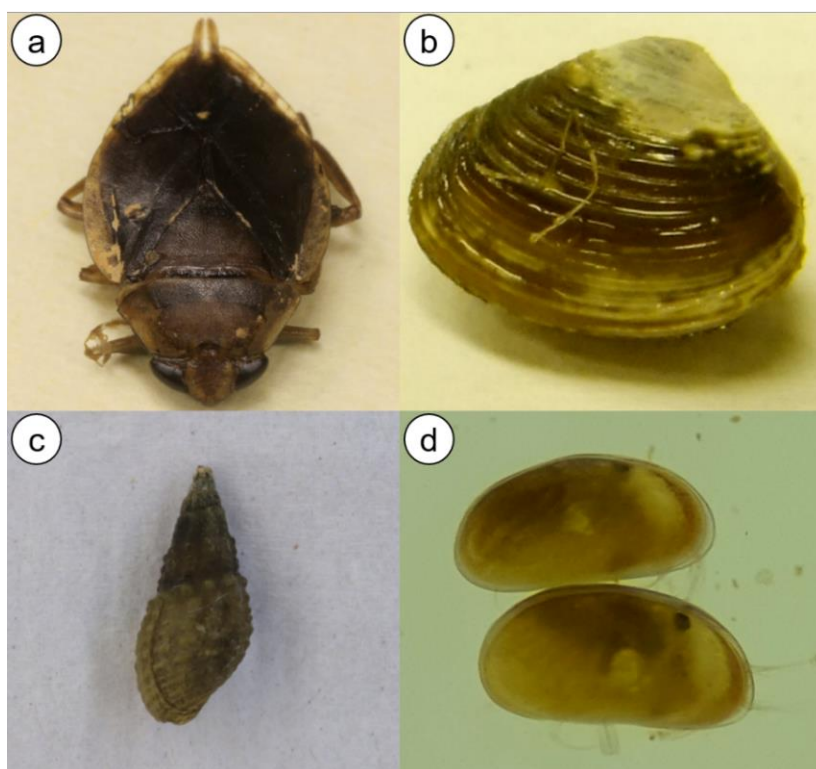


Figure 2.13: Photographs of typical aquatic invertebrates that have been found in Nyamithi lake within the Ndumo Game Reserve namely; *Appasus* sp. (a); *Corbicula* sp. (b); *Tarebia granifera* (c) and Ostracoda (d).

2.5.4 Floodplain pool

A floodplain pool (Site 6) was selected as the model for an *in situ* mesocosm assessment (see Chapter 6). This site is located within NGR approximately 150 m from a channel of the Phongolo River from which it receives water during floods (Figure 2.14). This floodplain pool was used as the model site as it is characteristic of a typical floodplain pool found in NGR study and has been used as a study site in previous research (see de Necker 2015 and Smit et al. 2016; Figure 2.14a) and thus data regarding the aquatic biota of this site are available. The pool last received water from the river in December 2014 and has been dry since mid-2015 (Figure 2.14b).



Figure 2.14: Photographs of the floodplain pool (Site 6) used as the model for the mesocosm study (see Chapter 4) in February 2014 after the site had received water from the controlled flood and rainfall (a) and December 2016 during the supra-seasonal drought (b).

CHAPTER 3:

Long-term water quality patterns of a flow regulated lowland river

3.1 Introduction

It has long been known that there are numerous aspects that cause water quality change and degradation (Khatri and Tyagi 2015). This can result from either natural processes, such as natural high and low flows and weathering of soils and rock, and/or human activities including the construction of dams, the discharge of industrial and domestic wastewater, as well as agricultural drainage (Brainwood et al. 2004; Gerber et al. 2015; Barakat et al. 2016). These phenomena can be exacerbated by climatic conditions such as drought. One of the most prominent effects of drought is reduced flow that places additional stress on freshwater ecosystems already impacted by human activities (Döll et al. 2009; Döll and Zhang 2010).

The negative impacts of dams on the environment, as well as on the biophysical characteristics of a river both downstream and upstream of an impoundment, are well documented (Teodoru and Wehrli 2005; Heath and Plater 2010). These include altering the magnitude and velocity of floods, flows and rates of sedimentation, loss of fertile topsoil, increasing or decreasing nutrient loads downstream, as well as loss of biodiversity (Williams and Wolman 1984; Alcamo et al. 2007; Heath and Plater 2010). The magnitude of these effects may differ between systems, depending on the size of the dam, the purpose for which it was built, the type of river it is located on and the natural variability and function of rivers (Williams and Wolman 1984; Tiemann et al. 2004; Eloisegi et al. 2010). For efficient management, and to minimize impacts on downstream water quality and ecological functions and services (which may support not only environmental but also human health and wellbeing), it is important to maintain the ecological integrity of an ecosystem (Heath and Plater 2010; Amatya and Skaggs 2011; Barakat et al. 2016). One way to assess ecological integrity is the monitoring of spatio-temporal patterns in the abiotic and biotic water quality of a river.

Studies of river basins have revealed how changes in climate, population and economic growth, as well as other socio-economic factors (e.g. agriculture) may lead to significant changes in water use (Alcamo et al. 2007) and thus its ability to sustain healthy ecological and socio-economic functions. Long-term data are essential to establish a baseline of water quality and to evaluate the effects of natural and anthropogenic induced changes in water resources (Amatya and Skaggs 2011). These data sets also indicate long term changes in catchment responses and may contain evidence of rare events (e.g. severe droughts or floods) or rapid shifts in the system's behaviour (Burt 1994; Burt et al. 2010; Howden et al. 2010). In riverine systems associated with floodplains, increased water usage for irrigation reduces the total water supply and groundwater recharge further downstream. This may lead to a drop in the water table, diminishing the extent of the active floodplain, causing salinization and potentially invasion by exotic species (Heath and Plater 2010). Salinization may be further exacerbated through decreased

natural flow and drought, which increases the total dissolved solids (TDS) and conductivity and changes the ionic composition (Barnes et al. 2002; Shanyengana et al. 2004; Singh et al. 2008).

Agricultural activities are associated with both direct and indirect effects on not only surface water but also groundwater quality and is one of the principal causes of water quality degradation, particularly in rural areas (Shabalala et al. 2013; Khatri and Tyagi 2015). Sources of pollution associated with agriculture include livestock, nitrate- and phosphate-rich fertilizers, metals, pathogens, pesticides, irrigation and the removal of natural habitat for farms and plantations (Shabalala et al. 2013; Khatri and Tyagi 2015). These pollutants may cause both acute and long-term degradation and change of water quality and the aquatic community (Shabalala et al. 2013).

South Africa has a well-developed commercial agricultural industry, as well as subsistence agricultural activities predominantly in rural areas (Shabalala et al. 2013), and many of these occur in close proximity to rivers and dams. The Phongolo River is a prime example of this, as the largest part of its catchment is covered by forestry plantations and commercial and subsistence farming (van der Laan et al. 2012). The Pongolapoort Dam was planned and built for the purpose of supplying irrigation water to such activities along its lower reaches (DWS 2014a). The Phongolo River is a cross border river, as it is shared with Mozambique, and is important to both countries (van der Laan et al. 2012). Careful monitoring of water quality over long-term and short-term periods of such cross-border systems is not only ecologically but politically important (Simeonova et al. 2003). Regular monitoring of gauging stations and the completion of a water quality study on the system (DWS 2014b) has resulted in a substantial amount of analytical data available for the Phongolo River dating to the early 1970s.

Water quality may be described by the physical, chemical and microbiological characteristics of a waterbody and is traditionally assessed based on a comparison with existing experimentally determined guidelines (Rajeshwari and Saraswathi 2009) such as the Target Water Quality Guidelines (TWQG) for South African aquatic ecosystems. Although useful, this does not provide a holistic view of spatial, seasonal or historic trends or variations in the studied waterbody (Debels et al. 2005). The use of multivariate approaches and water quality indices allows for the interpretation of complex data sets in order to understand better the quality and ecological status of a studied ecosystem (Gerber et al. 2015). Multivariate analysis enables the discovery of possible drivers that may be influencing water quality and easily identifies and interprets water trends (Alberto et al. 2001; Tomas et al. 2017). Concurrently, the water quality indices ascribe a unitless qualitative value to an aggregate set of measured variables and is based on a set of scientifically determined criteria (Tomas et al. 2017).

Possible impacts of the agricultural developments surrounding Pongolapoort Dam and the upper Phongolo River have been mentioned in previous assessments of the area (e.g. Heeg and Breen, 1982; DWAF 1985; DWA 2009; van der Laan et al. 2012). However, these studies largely focused on the middle and upper reaches of the catchment and there is still a lack of summarizing studies considering long-term analysis of the river itself, particularly its downstream region. This includes determining possible long-term effects of the dam, agricultural activities and droughts on this ecologically and

economically important river. This information is critical for determining sustainability and biodiversity conservation within the ecosystem.

The aim of this chapter is therefore to assess long-term water quality trends of the Phongolo River from the 1970s to 2017, to evaluate whether this has changed, and to determine possible causes. A further aim is to ascertain whether the supra-seasonal drought experienced from 2016 to 2017 had any effects on water quality of the Phongolo River. These aims were achieved with the use of the Physico-chemical Driver Assessment Index (PAI), which is a water quality index, as well as with multivariate statistical analysis. To achieve these aims, the following hypotheses were tested: 1.) Owing to long-term overuse of the Phongolo River, the quality of water entering and being released from the Pongolapoort Dam is poor, as indicated by the Physico-chemical Driver Assessment Index, 2.) The supra-seasonal drought has severely negatively affected the water quality of the lower Phongolo River.

3.2 Materials and Methods

3.2.1 Data collection

Historical data from three South African Department of Water and Sanitation (DWS) monitoring sites (gauging stations) within the Phongolo River catchment were obtained from the DWS National Chemical Monitoring Programme. One of these monitoring sites, W4H006, Phongolo River (Upstream) is located upstream of Pongolapoort Dam, and the remaining two are located downstream of the dam. Station W4H013 (Downstream 1) is located below the dam wall. The final gauging station (W4H009, Downstream 2) is located 80 km downstream of the dam within Ndumo Game Reserve (NGR) (Figure 3.1; Table 3.1). The water quality data assessed included calcium (Ca^{2+}), chloride (Cl^-), conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), potassium (K), magnesium (Mg^{2+}), sodium (Na), ammonium nitrogen ($\text{NH}_4^+\text{-N}$), nitrate nitrogen/nitrite nitrogen ($\text{NO}_3^-\text{-N}/\text{NO}_2^-\text{-N}$), pH, orthophosphate (PO_4^{3-}) and sulphates (SO_4^{2-}). Historical data collected from the DWS database for the three sites are indicated in Table 3.1, along with the years for which data were available, and included two years during which severe droughts occurred (1983 and 2016, respectively). Annual peak flows for the lower Phongolo River, measured at gauging station WH4013 located below the Pongolapoort Dam are presented in section 2.1 (Chapter 2).

Additional water samples were collected during four field collection events in May 2016; August 2016; September 2016; and February 2017, during the supra-seasonal drought. Monthly peak flows of the lower Phongolo River during 2016 to 2017 are shown in Figure 3.2. The months during which field collections took place are indicated by arrows. The first three sampling surveys coincided with times of severe drought (May 2016, August 2016; September 2016) while the final survey took place after increased rainfall occurred in the region (February 2017). A full description of the baseflow released during this study period are presented in section 2.4 (Chapter 2). Field collection took place in the Phongolo River at three sampling sites both within and outside NGR (Figure 3.1). For a full description of the three study sites on the river see section 2.5.1 (Chapter 2).

Physicochemical water parameters, namely pH, temperature ($^{\circ}\text{C}$), dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$), oxygen percentage (%) and conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) were measured *in situ* at each site with the use of an AZ water quality meter (Model 8602 handheld IP67). A single, integrated subsurface water sample from each site was then collected in a prewashed 500 mL polypropylene bottles and frozen until chemical nutrient analysis could take place.

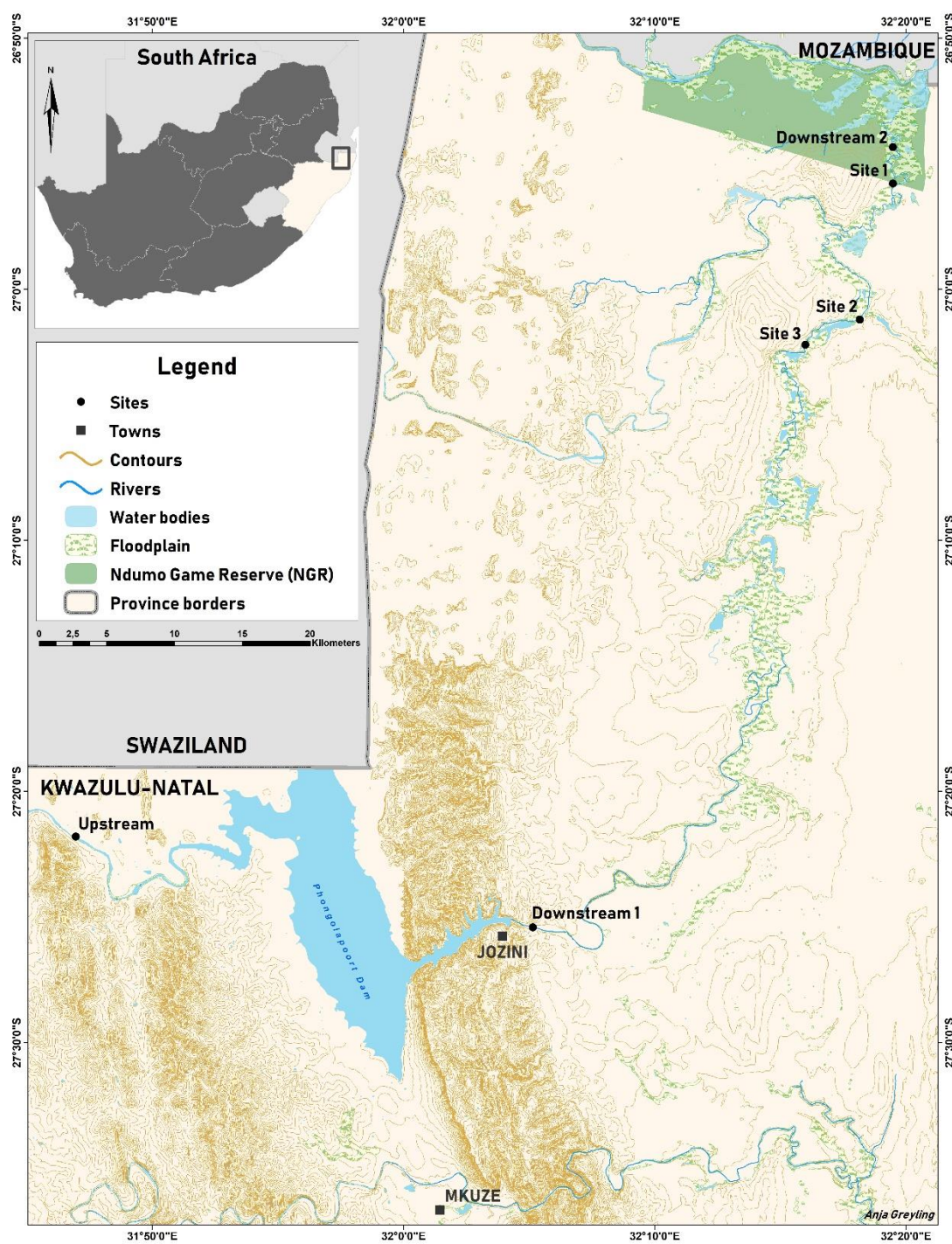


Figure 3.1: Map of lower Phongolo River and floodplain indicating the three gauging stations on the Phongolo River above (Upstream) and below (Downstream 1 and 2) Pongolapoort Dam, as well as the three sampling sites on the lower Phongolo River (Sites 1-3).

Table 3.1: Department of Water and Sanitation gauging station numbers, locations, and years for which water quality data are available for the Phongolo River. All water quality variables for each gauging station are presented in Appendices A1-A3.

River region	DWS gauging station number and location	Years for which historical data are available
Upstream		
(Middle Phongolo River)	W4H006 (Phongolo River at Makanes Drift)	1972–2017
Downstream		
(Lower Phongolo River)	W4H013 (Phongolo River immediately below Pongolapoort Dam)	1984–2017
	W4H009 (Phongolo River within Ndumo Game Reserve)	1980–2017

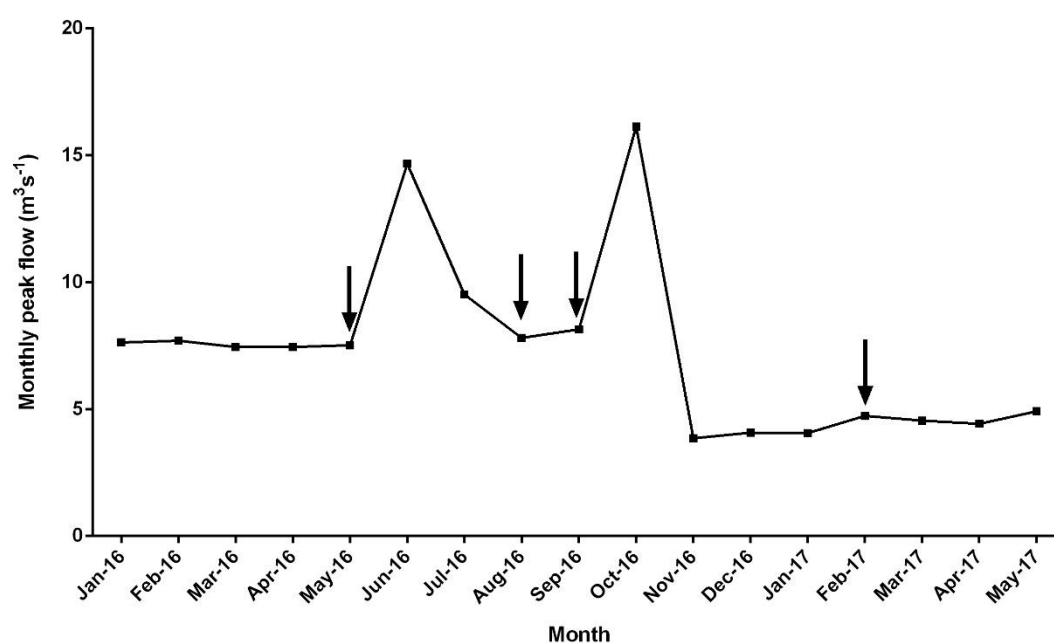


Figure 3.2: Monthly peak flows of Phongolo River downstream of Pongolapoort Dam during the present study in 2016-2017. Arrows indicate months during which sampling surveys took place (available from <https://www.dwaf.gov.za/Hydrology/Verified/hymain.aspx>. Accessed 14 September 2018).

3.2.2 Laboratory analysis

Chemical nutrients were measured in the laboratory using defrosted, unfiltered water samples using appropriate test kits and standard protocols (test kit catalogue numbers in parentheses) with a Merck Spectroquant Pharo 300 Spectrophotometer (Merck KGaA 2014). The nutrients included NO_3^- (NO_3^- -N) (14773), NO_2^- (NO_2^- -N) (14776), SO_4^{2-} (14791), NH_4^+ (NH_4^+ -N) (14752), NH_3 (NH_3 -N), Cl^- , PO_4^{3-} (orthophosphates) (14848) and total hardness (TH) (100961).

3.2.3. Statistical analysis

Two sets of data analysis took place, in line with the study aims. A time series assessment of the chemical water quality of the Phongolo River was done. The water quality data obtained from the DWS database comprised monthly records (1972–2017), and the available data for each variable were averaged to annual records and analysed statistically to determine water quality trends from 1972 to 2017. Line graphs were created to visually assess the general trends and possible changes in water quality from 1972 to 2017 of all three data sets (Upstream, Downstream 1 and Downstream 2). Graphpad Prism 6 was then used to create a one-way Analysis of variance (ANOVA) to determine if there were any significant differences in the water quality variables between the three data sets.

In order to determine possible impacts of the Pongolapoort Dam, the upstream water quality data were compared that of the monitoring point immediately downstream of the dam (Downstream 1). Prior to further analysis, all water quality parameters, with the exception of pH, were log transformed [$y = \log(x+1)$] in order to improve data normality. A Principal Component Analysis (PCA) was created in Canoco v5 to compare the upstream water quality above the dam to that immediately below the dam. A PCA is based on a linear response model which relates a single variable (e.g. water data) to a particular site (or survey). A diagram of the assessed sites is visualised on a two-dimensional basis. Placement of the sites on the diagram signifies either similarities or dissimilarities between the measured variable and sites based on the angle of the variables to one another. Variables at $\leq 90^\circ$ are positively correlated while variables $> 90^\circ$ are negatively correlated (Šmilauer and Lepš 2014). To compare whether water quality differed between the river outside NGR and within the NGR, a second PCA was created to compare the downstream water quality data outside NGR (Downstream 1) to the water quality data within the NGR (Downstream 2).

The Physico-chemical Driver Assessment Index (PAI), a water quality index developed for the South African eco-classification determination process, was applied to determine the present water quality status of the study area. The PAI is a tool to determine the present state of the physical and chemical water quality for a resource unit or a specific site. The PAI makes use of a Microsoft Excel-based weighting and rating framework that allows for the assessment of individual variables, the integration of the variable outcomes and then of the integrated, overall water quality state (Kleynhans et al. 2005). This is based on a multi-criteria decision analysis that considers a range of water quality variables including pH, salts, nutrients, temperature, turbidity, dissolved oxygen and toxicants.

The PAI compares each water quality variable to reference conditions in order to determine the degree to which each variable has changed, as well as the importance of each variable in relation to possible biotic responses it may cause. The result is that each site is assigned an ecological category, ranging from A to F (Table 3.2) (DWAF 2008). Weighting of the physico-chemical variables for the Phongolo River was based on characteristics that are important for lowland rivers (Kleynhans et al. 2008). This analysis was applied to determine the PAI of the three sample sites. In order to determine possible changes in water quality, first the historic PAI of each site was determined using data from the first two to three years of the available data recorded for each site. Then, using data from 2009 to 2014, a second PAI was calculated to determine the water quality before the severe drought of 2016. Finally, using data from 2016 to 2017, a third PAI was calculated to determine whether water quality had changed during the severe drought.

To assess the possible effects of the supra-seasonal drought on the water quality of the Phongolo River, a one-way ANOVA was used to determine if there were any significant differences in the water quality variables between the three sites from 2016 to 2017. A PCA was also created to compare water data collected from each site to one another over the four sampling surveys from 2016 to 2017.

Table 3.2: Rating and ecological categories used for implementation of the Physico-chemical Assessment Index (PAI) (Kleynhans and Louw, 2008).

Ecological category	Description	Score (Percentage of total)
A	Unmodified , natural.	90–100
B	Largely natural with few modifications. A small change in natural habitats and biota may have taken place, but the ecosystem functions are essentially unchanged.	80–89
C	Moderately modified. Loss and change of natural habitat and biota have occurred, but the basic ecosystem functions are still predominantly unchanged.	60–79
D	Largely modified. A large loss of natural habitat, biota and basic ecosystem functions has occurred.	40–59
E	Seriously modified. Extensive loss of natural habitat, biota and basic ecosystem functions.	20–39
F	Critically / Extremely modified. Modifications have reached a critical level and the system has been modified completely, with an almost complete loss of natural habitat and biota. In the worst instances the basic ecosystem functions have been destroyed and the changes are irreversible.	0–19

3.3 Results

3.3.1 Historical water analysis

From 1972 to 2017 all chemical water variables followed a similar trend both upstream and downstream of Pongolapoort Dam, although the upstream site variables fluctuated more than those at the downstream sites (Figure 3.3 – 3.5). Salinity (measured by conductivity, Cl^- , Ca^{2+} , Mg^+ , K and Na) and pH increased at all sites while the monitoring stations downstream of the dam indicated that $\text{NO}_3^-/\text{NO}_2^-$ has decreased over the past approximately five decades. Similar patterns in water quality were apparent between the two severe droughts of 1983 and 2016, as salinity, $\text{NO}_3^-/\text{NO}_2^-$ and PO_4^{3-} were generally highest at the peaks of the droughts (1983 and 2016). The ANOVA analysis indicated that conductivity, Cl^- , Ca^{2+} , Mg^+ , and Na were significantly higher upstream of the dam compared to those downstream (Table 3.3). Conductivity, Cl^- , Ca^{2+} , K and Na were also significantly higher at Downstream 2 compared to Downstream 1. Limited temporal variation is evident on the PCA comparing the upstream and downstream sites between 1972 and 2017 (explaining a total of 64.27% of data variation), as only the sampling surveys of 2016 and 2017 were separated from the other downstream surveys on the second axis (explaining 21.38% of data variation) (Figure 3.6). Distinct spatial variation is apparent on the first axis (explaining 42.89% of data variation) as the upstream surveys separated from the downstream surveys and this is due to significantly higher salinity and pH upstream of Pongolapoort Dam, compared to those at both downstream sites. Spatial separation was also evident on the PCA comparing the two downstream sampling sites (explaining 92.76% of data variation). This was evident on the first axis (explaining 87.27% of data variation) and was due to significantly higher salinity at the site located within NGR compared to that outside (Figure 3.7). Partial temporal variation was also present on the second axis (explaining 5.49%), as the 1980s sampling years at both downstream sampling sites separated somewhat from the remaining sampling years.

The historic PAI % and the ecological category analysis determined that between 1973 and 1986 the Phongolo River water quality was in an unmodified, natural state (A rating) both above and below Pongolapoort Dam (Figure 3.8; Appendix A4). From 2009 to 2014 the river water quality declined to a moderately modified state (C rating). This was due to an increase in all variables, with the exception of nutrients upstream, which decreased. All three sites remained in a moderately modified state during the drought of 2016 and 2017, although the PAI% of the Upstream and Downstream 2 sites decreased during this time. This was attributed to a decrease in pH and salts at the Upstream site and an increase in salts and nutrients at Downstream 2.

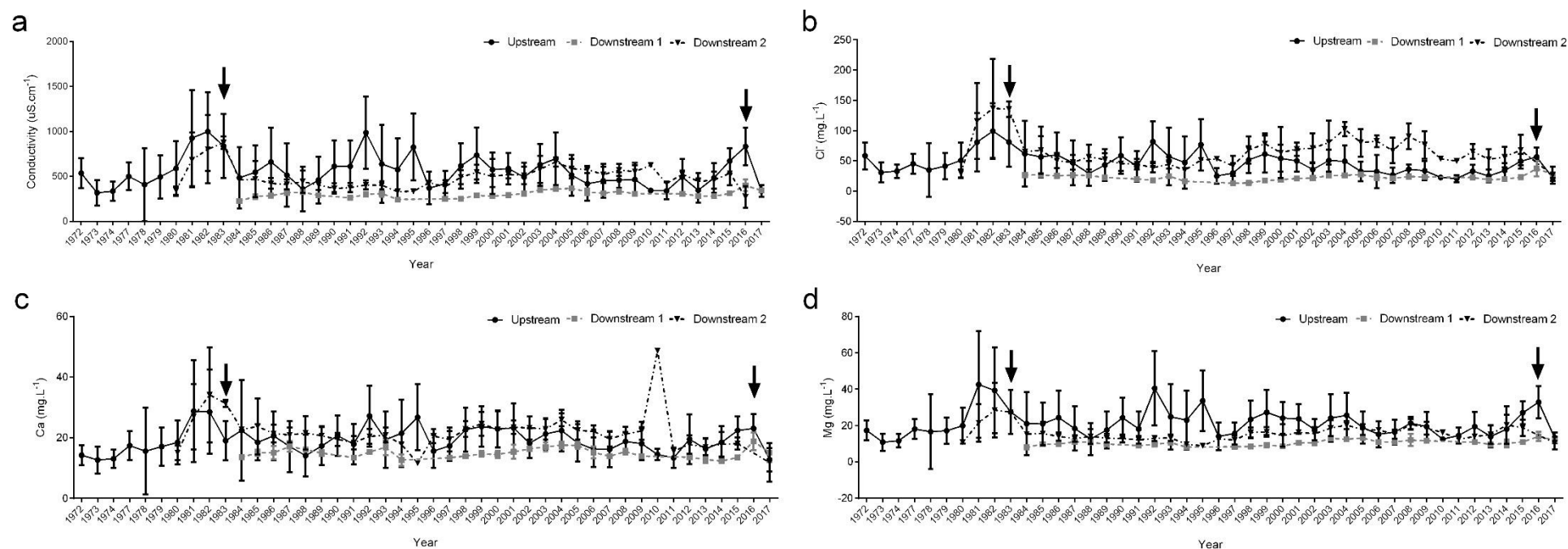


Figure 3.3: Line graphs indicating mean ($\pm\text{SD}$) trends in conductivity (a), chlorides (b), calcium (c) and magnesium (d) measured from 1972 to 2017 at the Department of Water and Sanitation gauging stations located above and below Pongolapoort Dam. Arrows indicate years of severe drought. Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

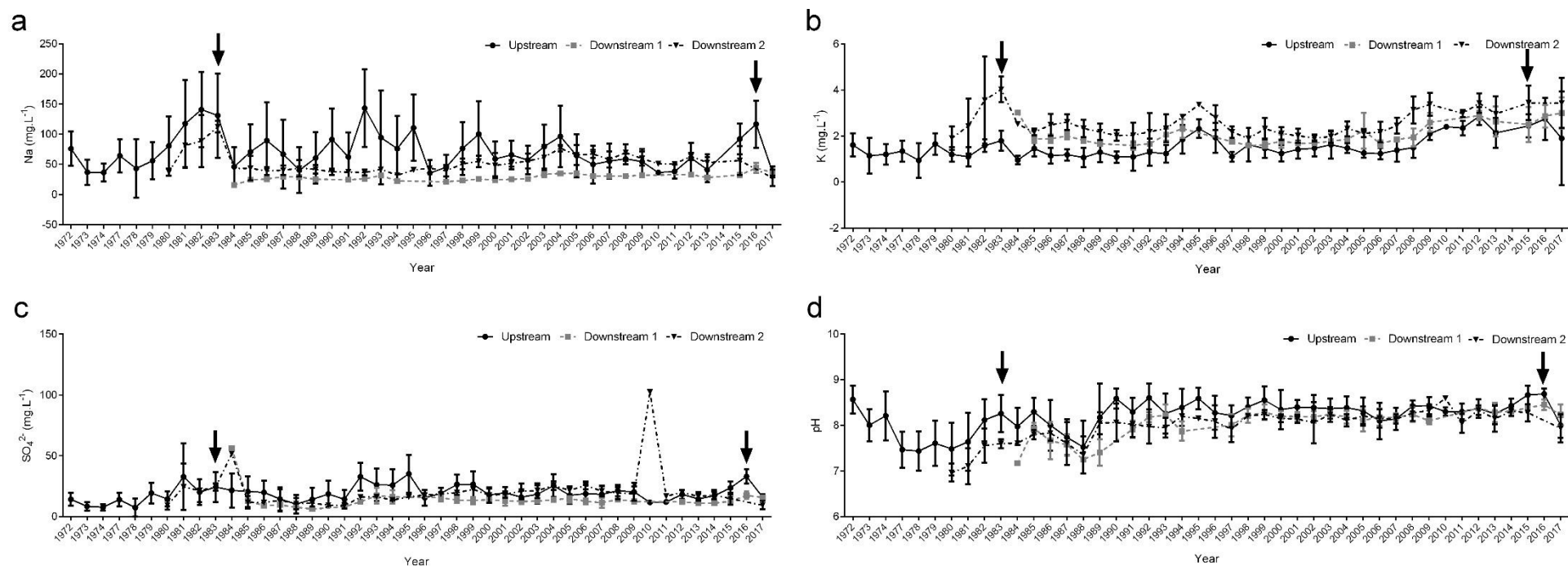


Figure 3.4: Line graphs indicating mean ($\pm$ SD) trends in sodium (a), potassium (b), sulphates (c) and pH (d) measured from 1972 to 2017 at the Department of Water and Sanitation gauging stations located above and below Pongolapoort Dam. Arrows indicate years of severe drought. Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

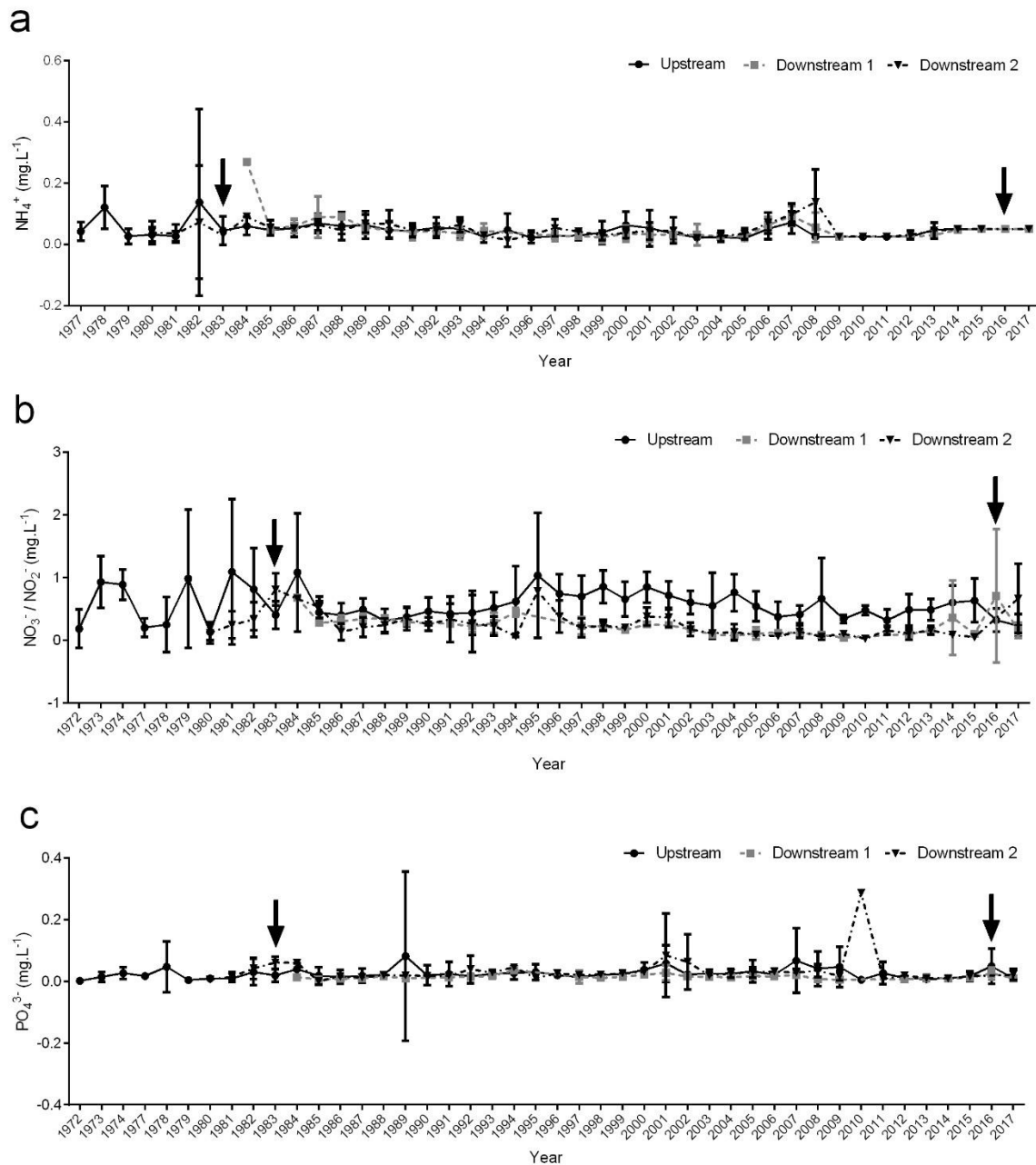


Figure 3.5 Line graphs indicating mean ($\pm$ SD) trends in ammonium (a), nitrates/nitrites(b) and orthophosphates (c) measured from 1972 to 2017 at the Department of Water and Sanitation gauging stations located above and below Pongolapoort Dam. Arrows indicate years of severe drought. Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

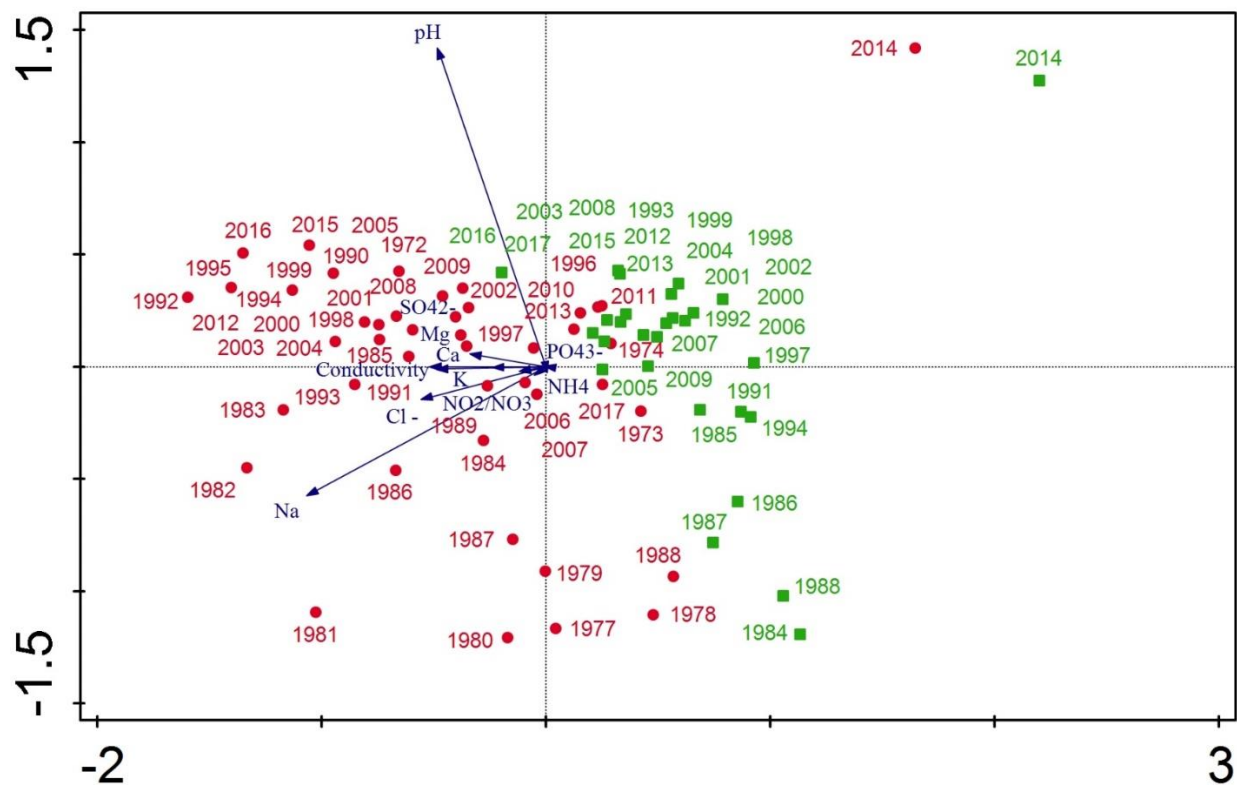


Figure 3.6: Principal Component Analysis (PCA) biplot comparing water quality variables from 1972 to 2017 measured at the Department of Water and Sanitation gauging stations above (red dots) and below (green squares) Pongolapoort Dam. The biplot explains 64.27% of data variation, of which 42.89% is explained by the first axis and 21.38% is explained by the second axis.

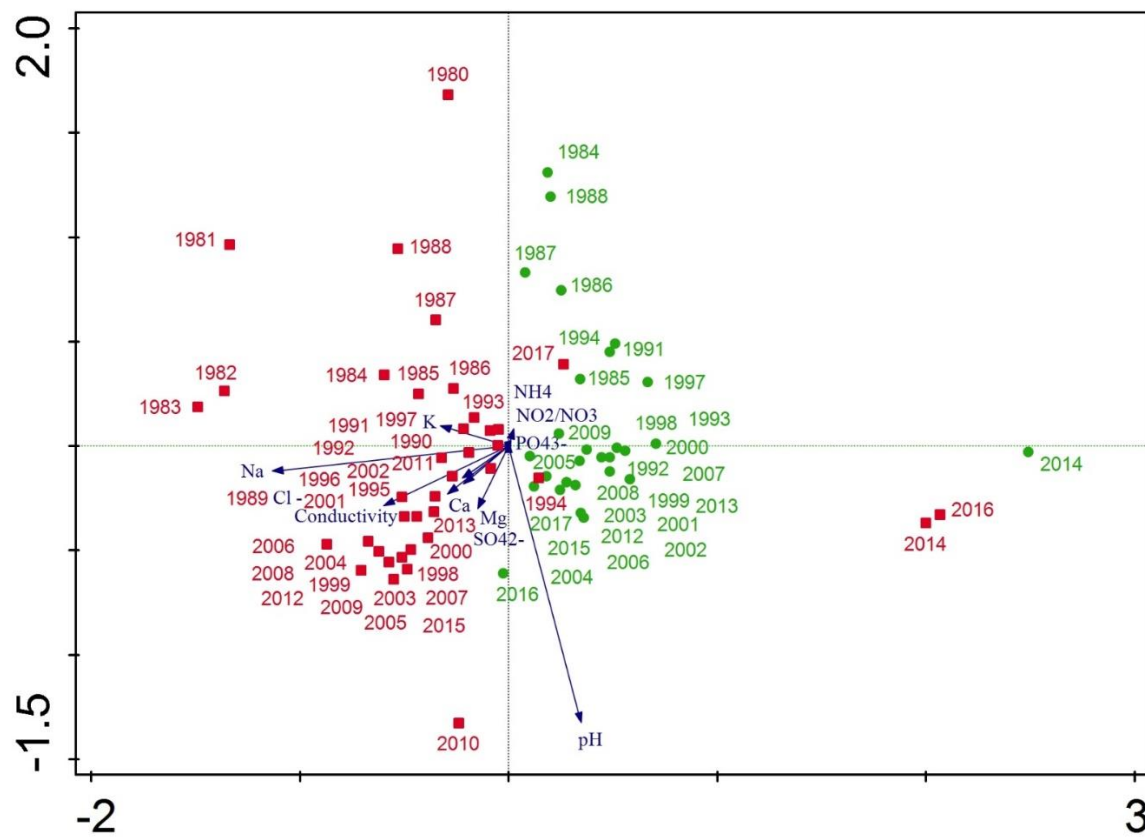


Figure 3.7: Principal Component Analysis (PCA) biplot comparing water quality variables from 1981 to 2017 measured at the Department of Water and Sanitation gauging stations below the Pongolapoort Dam (green dots) and within Ndumo Game Reserve (red squares). The biplot explains 92.76% of data variation, of which 87.27% is explained by the first axis and 5.49% is explained by the second axis.

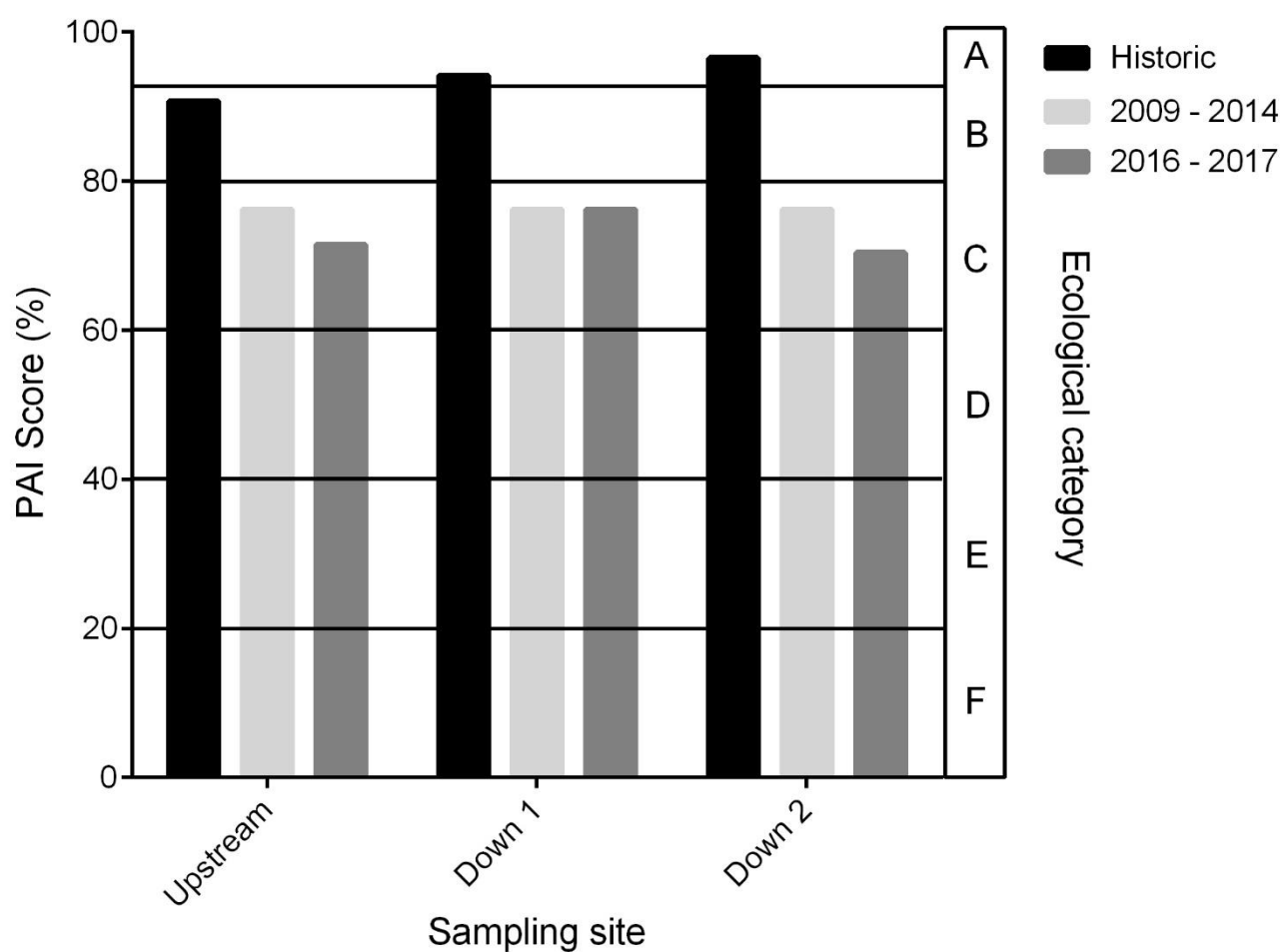


Figure 3.8: Physico-chemical driver assessment index (PAI) scores with associated Present Ecological Category derived from the three gauging stations located above and below Pongolapoort Dam. Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

Table 3.3: One-way Analysis of Variance (ANOVA) and chemical water nutrient variables measured at the Department of Water and Sanitation gauging stations from 1972 to 2017. Mean $\pm$ SD, range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). Upstream – Gauging station located above the dam; Downstream 1 – Gauging station located below dam wall. Downstream 2 – Gauging station located within Ndumo Game Reserve.

Chemical water variables	Upstream	Downstream 1	Downstream 2	ANOVA
Conductivity ($\mu\text{S.cm}^{-1}$)	555.1 $\pm$ 176 ^a (317.7 – 999.4) (n=44)	302.8 $\pm$ 39.44 ^{ab} (227 – 401.3) (n=28)	493 $\pm$ 125.4 ^b (270.3 – 875.5) (n=38)	$p < 0.0001$; F = 30.67
Cl ⁻ (mg.L ⁻¹)	46.63 $\pm$ 18.13 ^{ab} (20.76 – 99.4) (n=44)	22.68 $\pm$ 4.93 ^{ac} (13.17 – 37.1) (n=28)	64.49 $\pm$ 25.81 ^{bc} (26.4 – 136.9) (n=38)	$p < 0.0001$; F = 38.24
SO ₄ ²⁻ (mg.L ⁻¹)	19.26 $\pm$ 6.72 (7.43 – 35.25) (n=43)	14.36 $\pm$ 8.58 (6.45 – 56.2) (n=28)	20.92 $\pm$ 15.74 (8.93 – 102.2) (n=38)	$p = 0.06$; F = 2.95
Ca ²⁺ (mg.L ⁻¹)	19.32 $\pm$ 4.04 ^{ab} (12.67 – 28.77) (n=43)	14.97 $\pm$ 1.65 ^{ac} (12.28 – 18.81) (n=28)	21.81 $\pm$ 6.24 ^{bc} (11.6 – 48.7) (n=38)	$p < 0.0001$; F = 18.3
Mg ⁺ (mg.L ⁻¹)	21.36 $\pm$ 7.49 ^{ab} (10.87 – 42.58) (n=44)	10.52 $\pm$ 1.56 ^{ac} (8.1 – 14.09) (n=28)	15.81 $\pm$ 4.24 ^{bc} (8.9 – 28.54) (n=38)	$p < 0.0001$; F = 34.97
pH (mg.L ⁻¹)	8.21 $\pm$ 0.33 (7.44 – 8.69) (n=44)	8.08 $\pm$ 0.3 ^a (7.17 – 8.45) (n=28)	8 $\pm$ 0.34 ^a (6.96 – 8.59) (n=38)	$p = 0.02$ F = 4.07
NH ₄ ⁺ (mg.L ⁻¹)	0.05 $\pm$ 0.03 (0.02 – 0.14) (n=41)	0.05 $\pm$ 0.05 (0.02 – 0.27) (n=28)	0.05 $\pm$ 0.02 (0.01 – 0.14) (n=38)	$p = 0.82$; F = 0.21
NO ₃ ⁻ /NO ₂ ⁻ (mg.L ⁻¹)	0.56 $\pm$ 0.25 ^{ab} (0.13 – 1.09) (n=44)	0.24 $\pm$ 0.16 ^a (0.04 – 0.71) (n=28)	0.28 $\pm$ 0.26 ^b (0.03 – 1.23) (n=38)	$p < 0.0001$; F = 22.41
PO ₄ ³⁻ (mg.L ⁻¹)	0.03 $\pm$ 0.02 (0.002 – 0.08) (n=44)	0.02 $\pm$ 0.01 ^a (0.02 – 0.04) (n=28)	0.04 $\pm$ 0.07 ^a (0.001 – 0.32) (n=38)	$p = 0.03$; F = 3.81
K (mg.L ⁻¹)	1.56 $\pm$ 0.49 ^{ab} (0.94 – 2.86) (n=43)	2.08 $\pm$ 0.47 ^{ac} (1.59 – 3.02) (n=27)	2.57 $\pm$ 0.58 ^{bc} (1.89 – 4.03) (n=35)	$p < 0.0001$ F = 37.01
Na (mg.L ⁻¹)	71.1 $\pm$ 29.21 ^{ab} (35.53 – 143.4) (n=43)	28.55 $\pm$ 5.78 ^{ac} (15.7 – 44) (n=27)	52.77 $\pm$ 17.35 ^{bc} (27.53 – 112.4) (n=36)	$p < 0.0001$; F = 32.83

3.3.2 Present water quality status

The water quality variables did not differ greatly between Site 2 and Site 3 outside NGR, nor between Site 1 within NGR and the two sites outside NGR (Appendix A5– 8). No spatial differences were evident on the PCA comparing the three sampling sites across the four sampling events from 2016 to 2017 which explains 74.97% of data variation (45.11% explained on the first axis and 29.86% on the second axis) (Figure 3.9). Temporal variation was evident, as each of the sampling surveys separated distinctly from the others. This separation was driven by seasonal changes in the chemical water parameters, as the colder sampling surveys (May and August 2016, austral winter) separated from the warmer sampling surveys (September 2016 and February 2017, austral summer). Salinity (measured by conductivity, Cl, Ca^{2+} , Mg^{+} , K and Na) was positively correlated to the February 2017 surveys, as these variables generally increased from May 2016 to February 2017 both within and outside NGR, although it was slightly higher outside the game reserve. Although the organic nutrients (NO_3^- , NO_2^- , NH_4^+ , NH_3^+ , and PO_4^{3-}) fluctuated between surveys, there was a general increase in organic nutrients from May to September 2016 at Site 1, after which they decreased again in February 2017. Conversely, these variables increased outside NGR on the same time frame. The one-way ANOVA indicated that only NH_4^+ was significantly higher within NGR compared to that outside the reserve (Table 3.4).

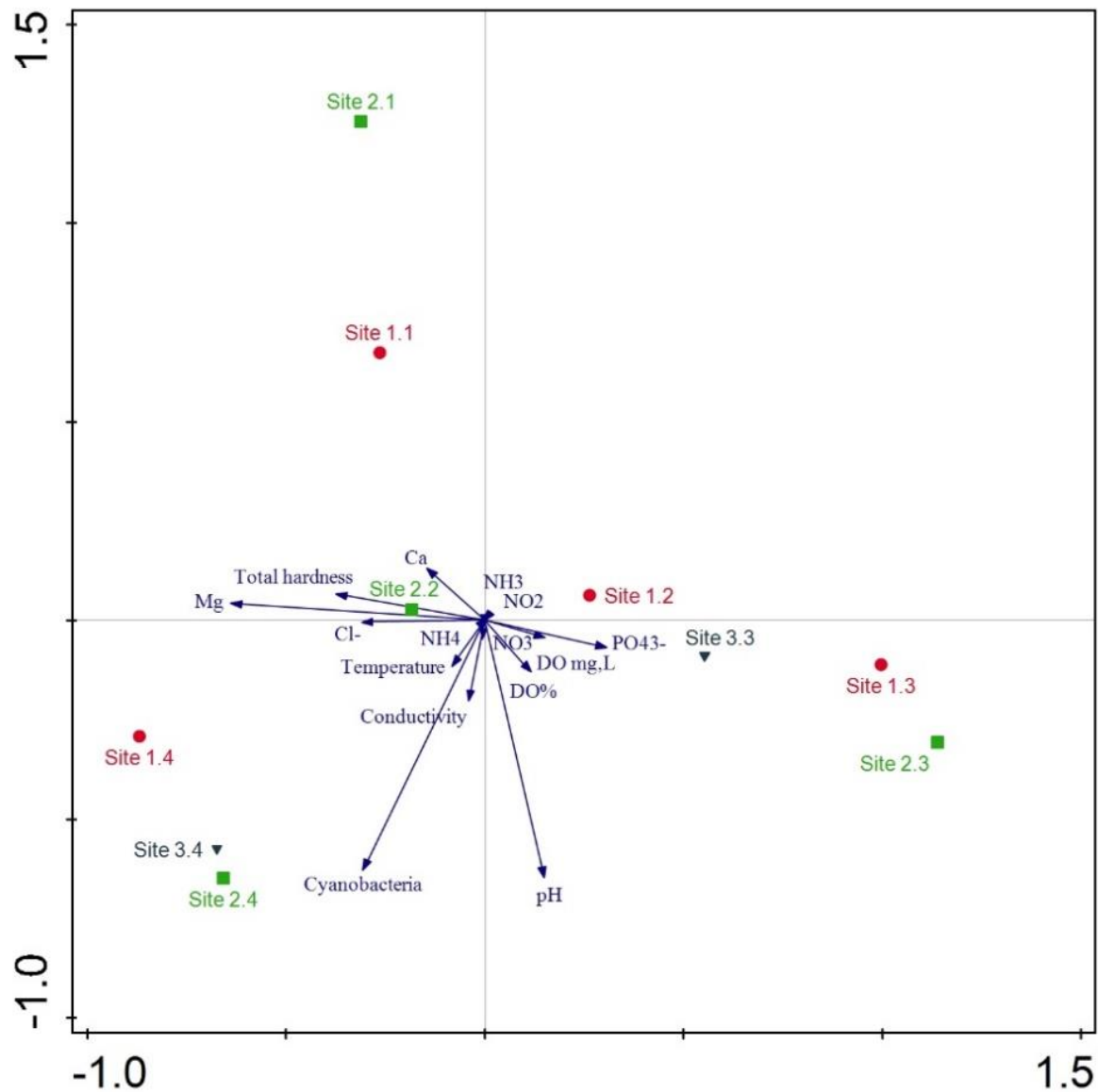


Figure 3.9: Principal Component Analysis (PCA) biplot comparing water quality variables measured during the present study on the Phongolo River within (Site 1; red dots) and outside (Site 2; green squares and Site 3; black triangles) Ndumo Game Reserve during the four sampling surveys from 2016 to 2017. The biplot explains 74.97% of data variation, of which 45.11% is explained by the first axis and 29.86% is explained by the second axis.

Table 3.4: One-way Analysis of Variance (ANOVA) and chemical water nutrient variables measured during the present study within (Site 1) and outside (Site 2 and Site 3) Ndumo Game Reserve during the four sampling surveys from 2016 to 2017. Mean $\pm$ SD, range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$).

Chemical water variables	Site 1 (n=4)	Site 2 (n=4)	Site 3 (n=2)	ANOVA
Temperature ($^{\circ}\text{C}$)	24.04 $\pm$ 4.81 (18– 29.4)	25.08 $\pm$ 7.42 (18.7 – 35.8)	26.00 $\pm$ 5.52 (22.1 – 29.9)	$p = 0.93$; $F = 0.07$
Dissolved Oxygen (mg.L^{-1})	5.87 $\pm$ 2.04 (4.34 – 8.83)	6.71 $\pm$ 2.53 (3.91 – 9.37)	6.51 $\pm$ 2.53 (4.72 – 8.3)	$p = 0.9$; $F = 0.11$
Dissolved Oxygen (%)	71.56 $\pm$ 16.58 (60.15 – 95.8)	77.78 $\pm$ 28.45 (45.3 – 104.4)	79.35 $\pm$ 22.13 (63.7 – 95)	$p = 0.9$; $F = 0.11$
Conductivity ($\mu\text{S.cm}^{-1}$)	529.4 $\pm$ 116.3 (426.8 – 666)	535 $\pm$ 147.8 (403– 735)	618 $\pm$ 91.92 (553 – 683)	$p = 0.71$; $F = 0.36$
pH	8.3 $\pm$ 0.18 (8.07 – 8.48)	8.32 $\pm$ 0.4 (7.79 – 8.65)	8.56 $\pm$ 0.06 (8.52 – 8.6)	$p = 0.57$; $F = 0.61$
Cyanobacteria ($\mu\text{g.L}^{-1}$)	4.92 $\pm$ 3.45 (1.92 – 9.71)	5.20 $\pm$ 4.38 (0.64 – 10.69)	7.49 $\pm$ 5.32 (2.18 – 12.81)	$p = 0.23$; $F = 0.8$
NO_3^- (mg.L^{-1})	1.10 $\pm$ 0.76 (0.5 – 2.2)	0.73 $\pm$ 0.22 (0.5 – 1)	0.43 $\pm$ 0.53 (0.05 – 0.8)	$p = 0.39$; $F = 1.07$
NH_4^+ (mg.L^{-1})	0.07$\pm$0.02^a (0.05 – 0.09)	0.07$\pm$0.01^b (0.06 – 0.09)	0.15$\pm$0.04^{ab} (0.12 – 0.18)	$p = 0.006$; $F = 11.43$
PO_4^{3-} (mg.L^{-1})	0.25 $\pm$ 0.21 (0.03 – 0.47)	0.64 $\pm$ 1.1 (0.03 – 2.28)	0.77 $\pm$ 1.04 (0.03 – 1.5)	$p = 0.72$; $F = 0.34$
SO_4^{2-} (mg.L^{-1})	35.67 $\pm$ 15.14 (25 – 53)	63 $\pm$ 69.31 (21 – 143)	20.5 $\pm$ 10.61 (13 – 28)	$p = 0.59$; $F = 0.58$
Total hardness (mg.L^{-1})	45.08 $\pm$ 20.45 (18 – 67)	44.25 $\pm$ 8.31 (20 – 56)	44 $\pm$ 15.56 (33 – 55)	$p = 0.99$ $F = 0.003$
Mg^+ (mg.L^{-1})	23 $\pm$ 15.6 (4 – 42)	21.75 $\pm$ 12.74 (5 – 35)	25 $\pm$ 15.56 (14 – 36)	$p = 0.97$; $F = 0.03$
Ca^{2+} (mg.L^{-1})	22.08 $\pm$ 5.56 (14 – 26.33)	22.5 $\pm$ 5.75 (15 – 27)	19 $\pm$ 0 (19 – 19)	$p = 0.73$; $F = 0.33$
Cl^- (mg.L^{-1})	48.38 $\pm$ 34.9 (10 – 89)	58 $\pm$ 12.88 (41 – 71)	52.5 $\pm$ 13.44 (43 – 62)	$p = 0.86$; $F = 0.15$

3.4 Discussion

The suite of analyses used to assess the water quality and potential impacts during the past approximately five decades revealed that water quality has become much poorer since the 1970s to the present day. However, the PAI assessment indicated that, historically, the water of the upper Phongolo River was already somewhat impacted before entering Pongolapoort Dam, and was in a poorer state than that below the dam. The upper and middle reaches of the Phongolo River have been subjected to anthropogenic inputs since the 1960s (van Vuuren 2009). The upper reaches are threatened by both functioning and abandoned coal mines near Paulpietersburg, as well as by commercial forestry developments on both the Phongolo and Bivane rivers (DWS 2014b). Mine water often contains high levels of total dissolved solids (TDS), including the dissolved cations calcium, magnesium, sodium and potassium, and anions such as sulphates, chlorides, fluoride, nitrate, bicarbonate and carbonate (Tiwari 2001). In the middle reaches there are approximately 199 km² of mostly sugarcane farms (DWA 2009; van der Laan et al. 2012; Dube et al. 2016), and water from the upper reaches is allocated for forestry, as well as for the Impala Irrigation Scheme in the middle Phongolo River reaches (DWA 2009). In addition, the farms in the region not only abstract water from the river for irrigation, but also make use of nitrogen-rich fertilizers in order to maximize crop yields (Smit et al. 2016).

The water quality of the Phongolo River below the dam has previously been reported as ideal but deteriorating, particularly with regard to increasing salinity (DWA 2009). This is in line with the findings of the present study, as the PAI of the river downstream of the dam has decreased, due largely to higher salinity and nutrients, most markedly further downstream within the NGR protected area. The lower organic nutrient levels, particularly NO₃⁻/NO₂⁻, just downstream of the Pongolapoort Dam than those upstream of the dam, are in contrast with what is generally found with dams that make use of a hypolimnetic discharge (Ward and Stanford 1983; Marshall et al. 2006; González et al. 2013). Dams have been shown to trap silt and nutrients, and thus less nutrients are available below a dam. Since being built, the amount of sediment Pongolapoort Dam carries downstream has decreased (Heath and Plater 2010) and is the most likely cause of low nutrient levels immediately below the Pongolapoort Dam, as also determined by Heeg and Breen (1994) and DWS (2014a). Similarly, in a study on four dams in central Spain by González et al. (2013), lower nutrient concentrations were found in the rivers downstream of the dams than within the dams.

Lowland rivers such as the lower Phongolo River are characterized as regions with distinct floodplains and alluvial, fine-bed channels (Ewart-Smith et al. 2006). The soils of these rivers, particularly those in the wetlands associated with them, are highly fertile due to the fine sediments in the wetlands that are able to absorb a high concentration of nutrients (Nahlik and Mitsch 2008), and are the reason why many farmers plant crops within floodplains. These habitats are therefore highly dependent on receiving nutrient-rich sediment during flooding to replenish the soils (Nahlik and Mitsch 2008). When a dam traps these sediments, as has occurred with Pongolapoort Dam, there is a loss of fertile soil deposits into the floodplain wetlands (Friedl and Wüest 2002; Heath and Plater 2010). Therefore, along with the reduction

of nutrients in the water of the lower Phongolo River, there has most likely also been a loss of nutrient rich soils in the floodplain wetlands associated with the lower Phongolo River. This loss of natural nutrients is often offset through the increased use of fertilizers on farms surrounding an aquatic ecosystem (Humborg et al. 2000), as is the case with the Phongolo system (DWS 2014b). There has been an increase in the number of irrigation schemes, farms, as well as population growth both above and below the dam. A 40% increase in the transformation of the lower Phongolo floodplain's natural vegetation into agricultural land occurred between 1955 and 2003 (Lankford et al. 2011). This included not only small subsistence farms, but also larger commercial cotton and sugarcane farms (Jozini Local Municipality IDP 2017/18-2021/22, 2017). The population has also increased from approximately 40 000 people living around the floodplain in 1982 (Heeg and Breen 1982) to approximately 198 000 people in 2016 (Jozini Local Municipality IDP 2017/18-2021/22, 2017). This has also resulted in increased pesticide use and organic pollution from fertilizers that are used on crops (Jaganyi et al. 2009; DWA 2009; DWS 2014b; Jozini Local Municipality IDP 2017/18-2021/22, 2017) as well as DDT use for malaria vector control, as the lower Phongolo River lies in a low-risk malaria region (Smit et al. 2016). Surface runoff and irrigation return flows from such activities are higher in both potassium and sodium (DWA 1996; van der Laan et al. 2012). These activities are widespread on the lower Phongolo River and are the most likely sources of increased organic nutrients in the lower Phongolo River where it flows into NGR.

During the droughts of 1983 and 2016, flow from the dam was severely reduced. Only a low continuous baseflow was released, as per cross-border agreements with Mozambique (DWS 2014a), and this low flow was accompanied by severe peaks in water salinity and nutrients. The present study also found these variables to increase further from 2016 to 2017. These changes in the water quality are most likely due to a combination of the natural geology of the region, increased agricultural activities and, in the case of the more recent drought, a further reduction in river flow from 2016 to 2017. Cretaceous marine sediments make up a great deal of the region's geology and produces naturally saline groundwater that seeps into the river (Akhurst and Breen 1988; Heath and Plater 2010). The dissolved solids levels in the lower Phongolo are, therefore, naturally slightly higher than most other freshwater river systems, resulting in slightly more saline water within the lower Phongolo River, particularly where it flows into NGR. These dissolved solids levels are particularly high during periods of low flow (Heeg and Breen 1982), and the additional accumulation of ions, increased alkalinity and high salt concentrations occur during extended periods of drought (Tooth and McCarthy 2007). Water quality did diminish during the 2016 drought largely due to increased salinity. However, these changes in water quality were not accompanied by a further decrease in the PAI, which remained fairly similar to that of before the drought. It appears that the baseflow released from the dam was enough to sustain the present quality of water without severe further deterioration. However, when there is regular flow for longer periods of time, and reduced extreme low-flow events, less flushing of the system occurs. This causes accumulation of minerals and leaching of nutrient-rich water (Heath and Plater 2010), which may reduce water quality and the health of the aquatic ecosystem in the long term.

No spatial trends were found in the present water quality status between 2016 and 2017. The sites are situated close to one another and there is no opportunity for the dilution of inputs from regions with high levels of human activity before the water enters the game reserve. Similarly, Gerber et al. (2015) determined that the water quality of rivers within the Kruger National Park was negatively impacted by human activities occurring in the river catchments outside the protected area.

3.5 Conclusions

It is clear that the human activities occurring both above and below the dam have affected the water quality of Phongolo River, since the river's status has decreased from an A to a C rating. This is mostly due to the long-term effects of farming in the region, particularly upstream of the dam, and to continued increases in salinity below the dam. The dam itself also appears to be affecting the water quality of the lower Phongolo River, mostly in the form of decreased nutrients immediately below the dam due to the loss of nutrient-rich sediment. The hypothesis that long-term overuse of the Phongolo River has caused the water entering and being released from the Pongolapoort Dam to be of low quality, as indicated by the Physico-chemical Driver Assessment Index, is therefore accepted. However, the low nutrient levels below the dam are being offset by increased fertilizer use by farmers below the dam, most prominently the larger commercial farms that have emerged beside the lower reaches of the river. Although nutrient levels are at present quite low below the dam, and the farms do not appear to have had any serious negative effects thus far, the upper reaches of the Phongolo River are a prime example of how this may change through long-term exposure. Interestingly, the drought of 2016 did not appear to affect water quality of the Phongolo River significantly, suggesting that the baseflow released from the dam during the drought was sufficient to allow the river to sustain its present state.

The hypothesis that the supra-seasonal drought has severely negatively affected the water quality of the lower Phongolo River is rejected. However, water analyses from 2016 to 2017 downstream of the dam indicated that salinity increased in 2017 when the prevailing drought extended into a second year and the baseflow was further reduced. Assessment of whether further reduction of the baseflow is having any severe effects on the aquatic ecology is therefore necessary, since it is highly likely that salinity would increase further if the drought were to continue. It is also noteworthy that water quality within NGR is clearly being affected by the ongoing human activities alongside the river outside the protected area, which could pose a serious threat to this ecologically important region. This warrants further investigation. Several of these aspects will be assessed in the following chapters of this thesis.

CHAPTER 4:

Assessing the effects of water level fluctuations on aquatic invertebrate communities in a subtropical floodplain pool: a mesocosm assessment.

4.1 Introduction

Climate change drives ecological responses by affecting average climate conditions, as well as altering the frequency and intensity of extreme events such as drought (Easterling et al. 2000; Jentsch and Beierkuhnlein 2008; Chessman 2015). Through climate change, droughts are predicted to increase in severity and length across certain parts of the world, including southern Africa, and this will have a cascading effect on aquatic ecosystems (Bond et al. 2008). There is thus a need for attention from both scientific and management perspectives on drought and its linked consequences such as reduced flooding (Bond et al. 2008). During droughts, water shortage conditions spread throughout the hydrologic cycle, reducing streamflow, groundwater and lake levels. This is called hydrological drought and can be exaggerated by, among other things, water abstraction for human consumption (Mosley 2015).

Floodplain systems depend on seasonal connectivity with a main river channel in order to maintain ecological integrity and support crucial ecologic and social services (Tockner et al. 2002). These include enhancing water quality, reducing flooding, acting as nursing areas for fish and providing habitats to a high diversity of aquatic and terrestrial biota (Ward and Stanford 1995; Amoros and Bornette 2002; King et al. 2009). The duration of hydrologic connectivity of floodplain pools to a river is an important factor controlling the composition of aquatic biota present on the floodplain, as it governs productivity (Jenkins and Boulton 2003). Hydrologic connectivity links areas of floodplain and channel habitats between flood events and is dependent on the distance of the floodplain from the river, topography and the size of the flood (Ward and Stanford 1995; Sheldon et al. 2002; Jenkins and Boulton 2003; Batzer and Boix 2016). Floodplain systems include various types of water bodies, each with a distinct succession, which contributes to the heterogeneity of riverine floodplain systems (Ward et al. 1999). Major threats to the flooding regime of floodplain systems are climate change and river damming. This is particularly true in water-scarce countries where there are conflicting demands for water between consumers and the environment (Pigram 2006; Bond et al. 2008). Water levels of small inland aquatic systems such as pools or inland lakes may be particularly sensitive to climate change because of their greater surface area to volume ratios relative to those of larger aquatic habitats (Gaeta et al. 2013). Due to their small size and dependency on the local environment, pools have often been considered good indicators of environmental change (de Meester et al. 2005). Understanding the effects of flow regulation, and thus length of connectivity and succession, on aquatic biota is key to determining how climate change may

affect these systems in the future. Damming disrupts the natural hydrological regime of a river, reducing discharge and converting the river into a “reservoir river”, where downstream flow may be reduced (Welcomme 1979; Heath and Plater 2010). If there are channels, streams or floodplain pools dependent on a dammed river, these may become either permanently or intermittently dry due to reduced flow (Heath and Plater 2010).

Environmental flow is the quantity and quality of water necessary to sustain ecological and social functions (Poff et al. 2009). Changes in the flow regime may affect natural ecological processes such as migration and spawning of fish, nutrient cycling and species richness of aquatic invertebrates (Bunn and Arthington 2002; Heath and Plater 2010). Reduced river discharge may result in reduced flood area and environmental flow, leading to variation in abiotic and biotic processes (Tockner and Stanford 2002). Dams therefore have the potential to impact floodplain wetlands severely, particularly in semi-arid regions where hydrologic and ecological effects of flow regulation cause alterations to the aquatic environment (Heath and Plater 2010). In rivers that are already affected by drought, regulated river discharge will further reduce flow and will intensify the effects of the drought (White et al. 2012). Drought has complex cascading effects on aquatic ecosystems (Lake 2011; Gaeta et al. 2013), including loss of littoral habitats critical to aquatic food webs, loss of flow required by rheophilous species and shorter flooding regimes affecting taxa with longer life cycles (Strayer and Findlay 2010; Gaeta et al. 2013; Chessman 2015). It is thus important to understand the role of flow in supporting ecological functions such as nutrient control, organic matter processing, food web dynamics and predator-prey interactions (Bond et al. 2008) and how alterations thereof may affect aquatic environments.

Compared to large, permanent water bodies such as lakes and rivers, smaller temporary wetlands are rather simple in their structure (Jocque et al. 2010), and researchers have been mimicking these environments by using mesocosms (de Meester et al. 2005; Pyke 2005). Such experimental field mesocosms allow for a relatively realistic simulation of an aquatic system to determine ecological responses by aquatic organisms to selected variables (environmental or biologic) (Herbst and Blinn 1998; Waterkeyn et al. 2010). Using mesocosms, researchers have studied various aspects of temporary wetlands including assessing rotifer communities (Langley et al. 2001); effects of nutrients on phytoplankton and zooplankton (Beklioglu and Moss 1996); effects of salinity on algae (Herbst and Blinn 1998) and aquatic invertebrates (Waterkeyn et al. 2010) and effects of severe pool drying on aquatic invertebrate taxonomy and traits (Boersma et al. 2014).

Boersma et al. (2014) introduced the “drought resistance hypothesis”, which suggests that taxonomic and trait diversity will not decline or be affected by decreasing water levels in fragmented pools, and the “drought vulnerability hypothesis” which suggests that species richness will gradually decline as water levels fall. Both these hypotheses have merit, since aquatic invertebrates from seasonal aquatic habitats are generally well-adapted to regular and naturally extended periods of low flow or drought (Leigh et al. 2010). However, the resistance and resilience of these biota may be affected if the natural flooding regime is changed by means of prolonged dry seasons, which could cause a decline and reduced recovery of biodiversity (Boulton 2003; Leigh 2013).

Both hydroperiod and hydrologic connectivity have been shown to affect aquatic biota in various ways. Hydroperiod generally affects abundance of aquatic invertebrates (Schneider and Frost 1996; Waterkeyn et al. 2008; Vanschoenwinkel et al. 2009) while hydrologic connectivity changes the successional patterns of aquatic ecosystems (Ward et al. 1999). Research has also considered hydrologic connectivity and its effects on various processes such as aquatic invertebrate post-flood recovery (Boulton et al. 1992); organic matter and nutrients (Tockner et al. 1999; Thoms 2003); aquatic invertebrate composition in dryland rivers (Sheldon et al. 2002); feeding of piscivorous fish (Luz-Agostinho et al. 2009); abiotic and biotic relationships in oxbows (Obolewski 2011); growth of aquatic macrophyte species (Obolewski et al. 2014) and benthic macroinvertebrates under various climatic conditions (Gallardo et al. 2014).

The main aim of this section of the study was to unravel ecological processes in floodplain wetlands by means of *in situ* mesocosms, with the specific focus of determining the temporal changes that take place in water quality and invertebrate communities with respect to variations in hydrological connectivity. To achieve this aim three hypotheses were tested: 1.) Shorter hydrological connectivity of a floodplain pool to a river negatively affects the aquatic macroinvertebrate and zooplankton communities and decreases water quality; 2.) Natural seasonal successional shifts occur in aquatic macroinvertebrate and zooplankton communities over time in a mimicked floodplain pool and 3.) Macroinvertebrate abundance and functional behaviour changes the zooplankton community structure.

4.2 Materials and Methods

4.2.1 Experimental design and field collection

For a full description of the floodplain pool that was selected as the model for this assessment see section 2.5.4 (Chapter 2). Nine mesocosms, each measuring 1.2 m x 1.2 m x 0.6 m, were constructed in the field next to the selected floodplain pool (Figure 4.1) using 3 m x 4 m sections of black plastic sheeting and wire mesh (Figure 4.2). The sheeting was held in place with the use of black irrigation piping and zip ties attached to the corners of each mesocosm. The mesocosms were placed in a grid pattern 1 m apart from one another adjacent to the dry floodplain pool selected for the experiment. Shade netting was placed around the perimeter of the mesocosms to prevent animals from damaging the mesocosms and injuring themselves (Figure 4.2). The top 3 – 10 cm of dry sediment was collected from the most central areas of the selected floodplain pool, as this ensured that the most recent resting eggs of aquatic biota would be collected where the most recent flooding had occurred (Langley et al. 2001; Waterkeyn 2009). The sediment was pooled and placed in each mesocosm to create a 3 cm bottom layer. Water was collected from the Phongolo River adjacent to the floodplain pool by pumping water into a 1000 L water tank. River water was used to flood the mesocosm sediment to simulate natural flooding of the floodplain pool from the river during a controlled flooding event.

The mesocosms were separated into groups of three (T1, T2 and T3) for the various experimental treatments following an adaptation of the methodology set out by Langley et al. (2001). In treatment 1 (T1), three mesocosms (T1.1, T1.2; T1.3) were filled once to 40 cm depth (equating to a volume of 576 L) and left to allow natural evaporation to occur. This would simulate a floodplain pool that received water once from the river during a flooding event through overflow from the river, but which, following inundation, remained isolated from the river. In treatment 2 (T2), three mesocosms (T2.1, T2.2, T2.3) were filled to 40 cm depth and kept at this level for three weeks by adding water regularly, after which they were left for natural evaporation to occur. This would simulate a floodplain pool that was connected to the river during a flooding event, after which the river receded, and the floodplain became isolated. In treatment 3 (T3), three mesocosms (T3.1, T3.2, T3.3) were filled to 40 cm depth and kept at this level throughout the experiment by adding water regularly. This would simulate a floodplain pool that was continuously connected to the river following a flooding event, and which received fresh water continuously by remaining connected to the river. Finally, vegetation growing in the natural floodplain pool was added to each mesocosm to simulate an environment as close to that of a natural floodplain pool as possible.

The experiment took place over a five-week period (as per the methodology of Langley et al. 2001) in November and December 2016, during which time each mesocosm was sampled 11 times (on days 1, 2, 3, 4, 6, 8, 10, 14, 21, 28 and 35). Throughout each sampling event, physicochemical water parameters were measured, and water, macroinvertebrate and zooplankton samples were collected. Samples were collected at the same time each day (9:00am) for consistency purposes. First, physicochemical water parameters were measured *in situ* and a water sample was collected for water nutrient analysis. Methods regarding *in situ* water parameter measurements and water sample

collection can be found in section 3.2.1, Chapter 3. In addition, the concentration of cyanobacteria ($\mu\text{g.L}^{-1}$) was measured *in vivo* with the use of a handheld fluorometer (*AquaFluor*, Turner Design). By submerging a 5 L beaker at various random points in each mesocosm, zooplankton samples were collected by filtering 20 L of water through a 64 μm mesh net and then placing each sample in a 500 mL polypropylene bottle. Lastly, macroinvertebrates were collected by sweeping a standard 30 cm x 30 cm D-frame dip net in each mesocosm 20 times, sampling from all available biotopes (vegetation, open water, deep water and sediment) and placing each sample into a 500 mL polypropylene bottle. All nets were rinsed with clean water between use in each mesocosm so as to prevent cross-contamination. All aquatic invertebrates were kept alive, before being returned to their respective mesocosms, so as to prevent physical reduction in aquatic invertebrate communities.

4.2.2 Laboratory analysis

In the field laboratory, select chemical variables were measured in unfiltered water samples. Methods regarding water sample analysis can be found in section 3.2.2 (Chapter 3). The volume of each live zooplankton sample was measured with a measuring cylinder and recorded. The sample was then mixed, and a homogenous sub-sample of 5 mL removed with the use of a plastic pipette and placed in 70% ethanol. The preserved zooplankton samples were then identified to the lowest possible taxonomic level with the use of a Nikon Model C-LEDS dissection microscope and counted, since live identification of zooplankton was not possible. Using the volume of each live zooplankton sample and the number of individuals of each taxon counted in the preserved sample, the total number of individuals per sample was then calculated. Macroinvertebrates were counted and identified as live specimens in the field laboratory with the use of a Nikon Model C-LEDS dissection microscope and appropriate identification guides (Day et al. 1999; Day et al. 2001a; Day et al. 2001b; Day and de Moor 2002a; Day and de Moor 2002b; Day et al. 2003; de Moor et al. 2003a; de Moor et al. 2003b; Stals and de Moor 2008). Once all aquatic invertebrate samples had been identified and chemical nutrient analysis measured, the samples were placed back into each mesocosm on the day of sampling.

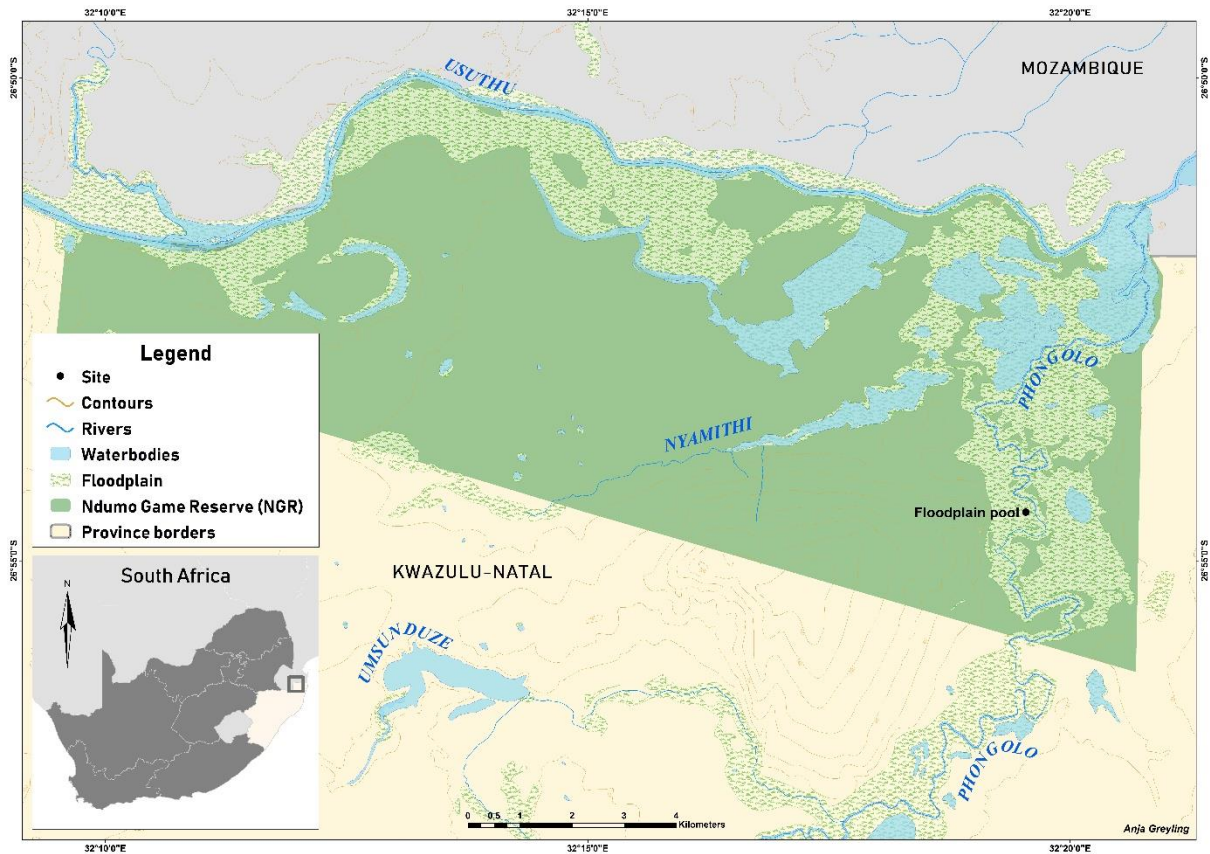


Figure 4.1: Map of Ndumo Game Reserve and floodplain pool used as the model for the experimental field mesocosm experiment.



Figure 4.2: Experimental field mesocosms, with shade netting placed around the experimental area.

4.2.3 Statistical analysis

All environmental data, with the exception of pH, were log transformed [$y = \log(x+1)$] prior to further analysis in order to improve data normality and allow the use of parametric tests. Where necessary, data were tested for normality using the Shapiro-Wilk test for normality. If data were normally distributed, a parametric one-way Analysis of variance (ANOVA) was used to determine if there were any significant differences present whereas if data were non-normally distributed a non-parametric ANOVA (Kruskal-Wallis non-parametric test) was used. Tests for significance (ANOVA or Kruskal Wallis where necessary) were used to determine whether any significant differences were present per treatment group over the sampling days for all water quality variables, macroinvertebrate and zooplankton data, respectively. Negligible differences were present (Appendices B1 - B8), and thus all data points for water quality, macroinvertebrate and zooplankton collected across all three treatments were combined and grouped into sampling weeks (Table 4.1). Tests for significance were then used to determine significant differences in water quality, macroinvertebrate and zooplankton data, respectively, over the sampling weeks. All further statistical analysis was conducted on sampling weeks with all treatments combined. All aquatic invertebrate data, with the exception of the diversity index analysis, were transformed prior to statistical analysis by way of square root ($\sqrt{x}$) in order to reduce the effect of dominant taxa (Clarke and Warwick 2001). Macroinvertebrate and zooplankton data from all sampling days were separated into sampling weeks per treatment (Table 4.1). Using Primer v7, these sampling week groups were then tested for significant differences using Analysis of Similarity (ANOSIM), after which it was visualised using non-metric Multidimensional Scaling (nMDS) plot and a dendrogram (Clarke and Gorley 2006). Multi-level pattern analysis (*multipatt*) was conducted on the zooplankton and macroinvertebrate communities, respectively, over the five sampling weeks for each treatment using the *indicspecies* package for R (R Core Team 2017). This analysis produces a list of species that are associated to particular groups, sites or combinations of these and then determines the statistical significance of these associations (de Cáceres 2013).

Table 4.1: Indication of sampling days and weeks into which days were separated.

Weeks	Week 1	Week 2	Week 3	Week 4	Week 5
Days	Day 1 – 6	Day 8 – 14	Day 21	Day 28	Day 35

All statistical analyses were conducted on lowest invertebrate taxon level. Diversity indices were used to determine species abundance and relationships within the aquatic invertebrate communities (zooplankton and macroinvertebrate communities, respectively) of each week and included: a) Margalef's species richness index (richness), which determines species richness at a particular site by incorporating taxon richness and abundance (Margalef 1968), b) Pielou's evenness index (evenness), which indicates whether there is dominance of individuals in the community at each site (Pielou 1971), c) Shannon-Wiener diversity index (Shannon diversity), which integrates both Margalef and Pielou's indices in order to determine overall diversity of each site (Shannon and Weaver 1963; Clarke and

Warwick 2001) and d) Simpson's index, which determines the probability that two individuals, drawn at random from a community, would belong to the same species. If the probability is high, the diversity is low (Simpson 1949).

Macroinvertebrate families were separated into their various functional feeding groups (FFGs) following Barbour et al. (1999), Merritt et al. (2002) and the guides to invertebrates of southern Africa (section 4.2.2). Significance tests were then used to determine whether there were any significant changes in the various macroinvertebrate FFGs over the sampling period.

In order to determine the unique and shared effects of water quality variables and sampling weeks on zooplankton communities, multiple variation partitioning analysis was applied using the *varpart* function in the *vegan* package for R (Oksanen et al. 2017; R Core Team 2017). Prior to the *varpart* analysis, variance inflation factor (vif) was conducted in the *car* package of R on all the water quality variables (Fox et al. 2017; R Core Team 2017). This test serves to determine collinearity between selected variables, and those variables that are highly correlated (>10) (Zuur et al. 2009) are then excluded from further analysis. The *varpart* analysis indicates the portion of variability that the selected explanatory variables (water quality and sampling weeks, respectively) explain in the response data (zooplankton communities). Variation was partitioned into three components: 1) water quality, to determine the direct effect of the selected water quality variables, 2) sampling weeks, to determine the direct effect of the five sampling weeks and 3) a shared variation between water quality and sampling weeks that would determine the indirect effect of sampling weeks through changes in water quality. The significance of the water quality and sampling weeks partitioning were each then tested with partial redundancy analyses. The shared effect and residual variation not explained cannot be tested for significance (Bocard et al. 2011). Pearson's correlation tests were done in Graphpad Prism 6 in order to determine whether macroinvertebrate FFGs were significantly correlated to any particular zooplankton taxa over the sampling weeks. A qualitative comparison was done between the present study and that by Dube (2015) to assess whether similar aquatic invertebrates present in the model floodplain pool in 2014 were found in the mesocosms. A quantitative analysis was not possible, as aquatic invertebrate sampling and identification differed between the study by Dube (2015) and the present study.

4.3 Results

4.3.1 Water quality

Several specimens of two fish species were found in the three mesocosm treatments (Appendix B9). These fish were observed only at the end of the experiment once the mesocosms were emptied, and it was assumed they entered the mesocosms during initial filling, while pumping water from the river, since they were still small (<10 cm). One *Clarias gariepinus* (African sharptooth catfish; Clariidae) was found in T1.1. Three fish were found in T2; one *Synodontis zambezensis* (brown squeaker; Mochokidae) each in T 2.1 and T 2.2, respectively, and one *C. gariepinus* in T 2.3. Finally, four fish were found in T3, two *C. gariepinus* in T 3.1 and two *S. zambezensis* in T 3.2. Water levels of the mesocosms were measured at the end of the five week period so as to assess how much evaporation took place (Appendix B10). Water levels of mesocosms from T1 were 25.5cm (T1.1), 26.5cm (T1.2) and 23cm (T1.3). Water levels of mesocosms from T3 were 26.5cm (T2.1) and 28cm (T2.2 and T2.3). Water levels of T2 remained at 40cm due to constant refilling.

Two significant differences were found in the water quality variables between T1 and T3 ($p < 0.05$). Temperature was significantly higher in T3 (26.79) in comparison to T1 (25.68; $p = 0.03$; $F = 3.58$), while cyanobacteria were significantly higher in T1 (50.55) than in T3 (37.95; $p = 0.046$; $F = 3.19$) (Appendix B4 and B5). One-way ANOVA of the *in situ* and chemical nutrient water analyses between weeks consisted largely of week 1 that differed from week 2 – week 5 in DO mg.L^{-1} , $\text{DO\%}$, turbidity, cyanobacteria, PO_4^{3-} , NO_3^- and NH_4^+ which all decreased with varying degrees from week 1 to week 5 (Table 4.2 and Table 4.3). Conductivity differed significantly between all five sampling weeks ($p < 0.0001$; $F = 175.4$; Table 4.2), increasing from week 1 to week 5, along with TDS ($p < 0.0001$; $F = 309.6$). Week 1 and week 2, as well as week 1 and week 4, also differed from one another significantly in terms of pH, which decreased slightly over the five weeks ($p < 0.0001$; $F = 10.05$). Ammonia and SO_4^{2-} also decreased significantly from week 1 to week 2 ($p = 0.0009$; $F = 5.13$ and $p = 0.016$; $F = 3.23$, respectively) while Cl⁻ increased significantly after week 1 ($p < 0.0001$; $F = 8.51$). After the vif analysis, water quality variables that were highly correlated were excluded. The excluded variables were TDS, DO mg.L^{-1} , PO_4^{3-} , cyanobacteria and pH. Further analyses were conducted only on the remaining water quality variables.

Table 4.2: *In situ* water quality variables and Analysis of Variance (ANOVA) of all mesocosms over the five sampling weeks. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). N-value indicates number of samples per week.

<i>In situ</i> Water Quality	Time					ANOVA
	Week 1 (n=45)	Week 2 (n=27)	Week 3 (n=9)	Week 4 (n=9)	Week 5 (n=9)	
Dissolved Oxygen (mg.L⁻¹)	7.94 ± 1.59 ^{abcd} (5.63 – 13.45)	4.08±1.1 ^a (1.86 – 5.99)	4.68±0.96 ^b (3.57 – 6.27)	3.16±0.53 ^c (2.17 – 3.69)	3.34±0.71 ^d (2.33 – 4.34)	p<0.0001; F= 62.68
Oxygen Saturation (%)	93.87 ± 15.44 ^{abcd} (64.4 – 125.7)	47.97±12.27 ^a (22.50 – 70.80)	54.81±11.82 ^b (35.6 – 66.8)	39.16±6.00 ^c (26.80 – 44.4)	40.07±6.86 ^d (31 – 52.8)	p<0.0001; F= 82.8
Conductivity (µS.cm⁻¹)	408.2 ± 14.57 ^{abcd} (332 – 432)	420.7±9.77 ^{aefg} (403 – 440)	463.3±10.85 ^{behi} (447 – 484)	491.3±11.32 ^{cfhij} (472 – 506)	555.9±41.22 ^{dgi} (522 – 663)	p<0.0001; F= 175.4
Temperature (°C)	26.51 ± 2.33 (23.7 – 31.3)	25.41±1.18 (23.90 – 28.80)	25.3±0.75 (24.6 – 26.9)	26.88±0.53 (26.2 – 27.7)	26.61±0.42 (25.9 – 27.3)	p= 0.03; F= 2.79
pH	8.08 ± 0.49 ^{ab} (7.53 – 9.24)	7.55±0.14 ^a (7.33 – 7.83)	7.8±0.24 (7.54 – 8.23)	7.7±0.12 ^b (7.49 – 7.84)	7.8±0.17 (7.62 – 8.17)	p<0.0001; F= 10.05
Turbidity (NTU)	337.8 ± 75.69 ^{abcd} (222 – 590)	189.1±39.67 ^{aefg} (120 – 300)	93±12.55 ^{be} (72 – 111)	95.67±20.32 ^{cf} (75 – 141)	100.3±43.89 ^{dg} (24 – 177)	p<0.0001; F= 78.86
TDS (mg.L⁻¹)	284.6±9.55 ^{abcd} (233 – 298)	284.6±9.55 ^a (282 – 308)	322.4±8.02 ^b (312 – 338)	342.1±7.69 ^c (330 – 354)	378.7±6.42 ^d (366 – 387)	p<0.0001; F= 309.6
Cyanobacteria (µg.L⁻¹)	61.44±15.38 ^{abcd} (38.1 – 105.8)	37.27±9.05 ^{aef} (24.73 – 58.73)	27.59±5.7 ^b (19.37 – 36.05)	20.14±6.17 ^{ce} (9.01 – 27.55)	18.4±9.02 ^{df} (7.23 – 34.79)	p<0.0001 F= 48.45

Table 4.3: Chemical water nutrient variables and Analysis of Variance (ANOVA) of all mesocosms over the five sampling weeks. Mean $\pm$ SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). N-value indicates number of samples per week.

Water Nutrients	Time					ANOVA
	Week 1 (n=45)	Week 2 (n=27)	Week 3 (n=9)	Week 4 (n=9)	Week 5 (n=9)	
PO_4^{3-} (mg.L ⁻¹)	0.94 $\pm$ 0.34 ^{abcd} (0.4 – 1.98)	0.36 $\pm$ 0.12 ^a (0.17 – 0.71)	0.16 $\pm$ 0.03 ^b (0.11 – 0.21)	0.25 $\pm$ 0.07 ^c (0.17 – 0.41)	0.33 $\pm$ 0.25 ^d (0.11 – 0.9)	$p < 0.0001$; F= 38.14
NO_3^- (mg.L ⁻¹)	3.52 $\pm$ 1.05 ^{abcd} (2.12 – 7.1)	1.44 $\pm$ 0.49 ^a (0.80 – 2.60)	1.01 $\pm$ 0.15 ^b (0.8 – 1.2)	1.24 $\pm$ 0.42 ^c (0.71 – 1.73)	0.96 $\pm$ 0.24 ^d (0.73 – 1.48)	$p < 0.0001$; F= 53.87
NO_2^- (mg.L ⁻¹)	0.002 $\pm$ 0 ^{abc} (0.002 – 0.002)	0.002 $\pm$ 0 ^{def} (0.002 – 0.002)	0.006 $\pm$ 0.003 ^{adgh} (0.002 – 0.01)	0.015 $\pm$ 0.003 ^{begi} (0.01 – 0.02)	0.02 $\pm$ 0.003 ^{cfhi} (0.02 – 0.03)	$p < 0.0001$ F=623.6
NH_3 (mg.L ⁻¹)	0.03 $\pm$ 0.04 ^a (0 – 0.16)	0.004 $\pm$ 0.005 ^a (0 – 0.01)	0.002 $\pm$ 0.004 (0 – 0.01)	0.002 $\pm$ 0.004 (0 – 0.01)	0.002 $\pm$ 0.004 (0 – 0.01)	$p = 0.0009$; F= 5.13
NH_4^+ (mg.L ⁻¹)	0.43 $\pm$ 0.21 ^{abcd} (0.14 – 1.03)	0.22 $\pm$ 0.82 ^a (0.12 – 0.42)	0.11 $\pm$ 0.04 ^b (0.07 – 0.19)	0.12 $\pm$ 0.05 ^c (0.06 – 0.21)	0.12 $\pm$ 0.06 ^d (0.05 – 0.24)	$p < 0.0001$; F= 19.35
Cl^- (mg.L ⁻¹)	48.16 $\pm$ 38.88 ^{ab} (2.5 – 130)	66.7 $\pm$ 11.67 ^c (40 – 94)	70.67 $\pm$ 4.24 (66 – 81)	84.33 $\pm$ 4.36 ^a (76 – 88)	97.44 $\pm$ 6.69 ^{bc} (84 – 106)	$p < 0.0001$; F= 8.51
SO_4^{2-} (mg.L ⁻¹)	68.13 $\pm$ 60.06 ^a (15 – 249)	36.44 $\pm$ 20.61 ^a (15 – 108)	33.56 $\pm$ 4.3 (28 – 39)	34.67 $\pm$ 4.3 (31 – 43)	59.67 $\pm$ 32.13 (31 – 124)	$p = 0.02$; F= 3.23
Total Hardness (mg.L ⁻¹)	31.49 $\pm$ 19.55 (5 – 55)	42.26 $\pm$ 5.69 (35 – 51)	37.44 $\pm$ 1.24 (36 – 40)	38 $\pm$ 2.69 (33 – 42)	38 $\pm$ 3.39 (31 – 43)	$p = 0.03$; F= 2.72

4.3.2 Aquatic invertebrates

A total of 52 macroinvertebrate taxa were collected during the five-week sampling period, of which 17 were also present in the model floodplain pool studied by Dube (2015) in 2014 (Appendix B11). These shared macroinvertebrate taxa included *Anax* sp., *Anisops* sp. A, *Anopheles* sp., *Caridina nilotica*, *Chironominae*, *Cloeon* and *Procloeon* sp., and *Mesovelgia vittergera*. Several taxa were found during all five sampling weeks, namely *Cloeon* and *Procloeon* sp., *Anisops* sp. A., *Microvelia* sp., *Anopheles* sp., *Hydroglyphus* sp., *Micronecta* sp., *Chironominae*, *Dytiscidae* larvae, *Anax* sp., *Hyphydrus* sp. and *Berosus* sp (Appendix B11). The most abundant taxa found were *Cloeon* and *Procloeon*, with a mean range of 0.44 (week 1) to 63.33 (week 3) individuals found per week. *Spercheus* larvae were found only during week 3, while their adults were found in all but week 3. *Berosus* sp. adults were found throughout the study, but their larvae were found only during weeks 4 and 5. Similarly, *Enochrus* sp. adults were found from week 2 to week 5, while their larvae were found only from week 3 to week 5, and *Hyphydrus* sp. adults were present during all weeks and their larvae from week 2 onwards (Appendix B11).

Based on the aquatic invertebrate data, two groups formed at 35% on the dendrogram, namely Day 1 – 10 and Day 14 – 35 (Figure 4.3a). Two additional groups formed at 53%, namely Day 1 – 6 and Day 8 – 10. Two further groups formed at 63% similarity, namely Day 14 and Day 21 – 35. The nMDS plot indicated the five sampling weeks separating into 5 groups, with some overlap between week 3 to week 5 (Figure 4.3b). The ANOSIM analysis indicated that these groups were different from one another (Global R 0.76) and highly significant (0.1%). Two-way ANOVA analysis of the five-week sampling period (time) and the three various treatments (treatment) indicated that neither treatment nor the interaction between treatment and time had any significant effects on macroinvertebrates or zooplankton, respectively ($p > 0.05$; Appendix B12).

Significant indicator taxa were present in the various treatment mesocosms over the five sampling weeks (Appendices B13 – B15). *Moina micrura* was significantly associated with week 1 and 2 in both T2 and T3, and similarly *Simocephalus serrulatus* was significantly associated with week 2 – week 5 in these two treatments ($p < 0.05$). *Diaphanosoma* sp. and *Mesovelgia vittergera* were significantly associated with week 2 – week 5 and week 3 – week 5, respectively, for all three treatments, while *Anopheles* sp. and *Enochrus* sp. (both adults and larvae) were significantly associated with T1 and T2 during week 2 – week 5 and week 3 – week 5, respectively.

When comparing the three treatments to one another, significant difference ($p < 0.05$) was found only in macroinvertebrate species richness which was higher in T2 (1.87) and T3 (1.84) in comparison to T1 (1.24; $p = 0.004$; $F = 5.94$) (Appendix B6). When comparing the sampling weeks to one another, all of the macroinvertebrate diversity indices increased from week 1 to week 5 with varying degrees (Figure 4.4 and Figure 4.5). Of these indices, three increased significantly from the first to last sampling weeks, namely number of taxa (3 to 13; $p < 0.0001$; $F = 69.13$), species richness (1.19 to 2.63; $p < 0.0001$; $F = 15.1$) and Shannon diversity (0.86 to 1.87; $p < 0.0001$; $F = 16.27$). Number of individuals increased significantly from week 1 (5.93) to week 3 (118.3) after which it decreased again towards week 5 (100.7; $p < 0.0001$;

F=55.3). Evenness and Simpson's index both remained fairly constant ranging from 0.68 – 0.73 and 0.60 – 0.77 respectively.

Four FFGs were found during the study, of which predatory families were the most abundant (37), followed by filtering collectors (7) and gathering collectors (6), while only two shredder families were found during the study (Figure 4.6; Appendix B11). No significant differences were found in macroinvertebrate FFGs between the three treatments (Appendix B16). With regard to sampling weeks, the one-way ANOVA of macroinvertebrate FFGs indicated that the predatory macroinvertebrates increased significantly from week 1 (13.2) to week 5 (232.7; $p < 0.0001$; $F = 20.05$; Appendix B17). Gathering collectors increased significantly from week 1 (1.73) to week 3 (200.3), after which they decreased significantly towards week 5 (58; $p < 0.001$; $F = 37.71$). Filtering collectors increased significantly from week 1 (2.47) to week 2 (26.2) and then decreased towards week 5 (11.33; $p = 0.0002$; $F = 8.13$). Shredders were low in abundance throughout the study and found only from week 1 (2) to week 3 (1).

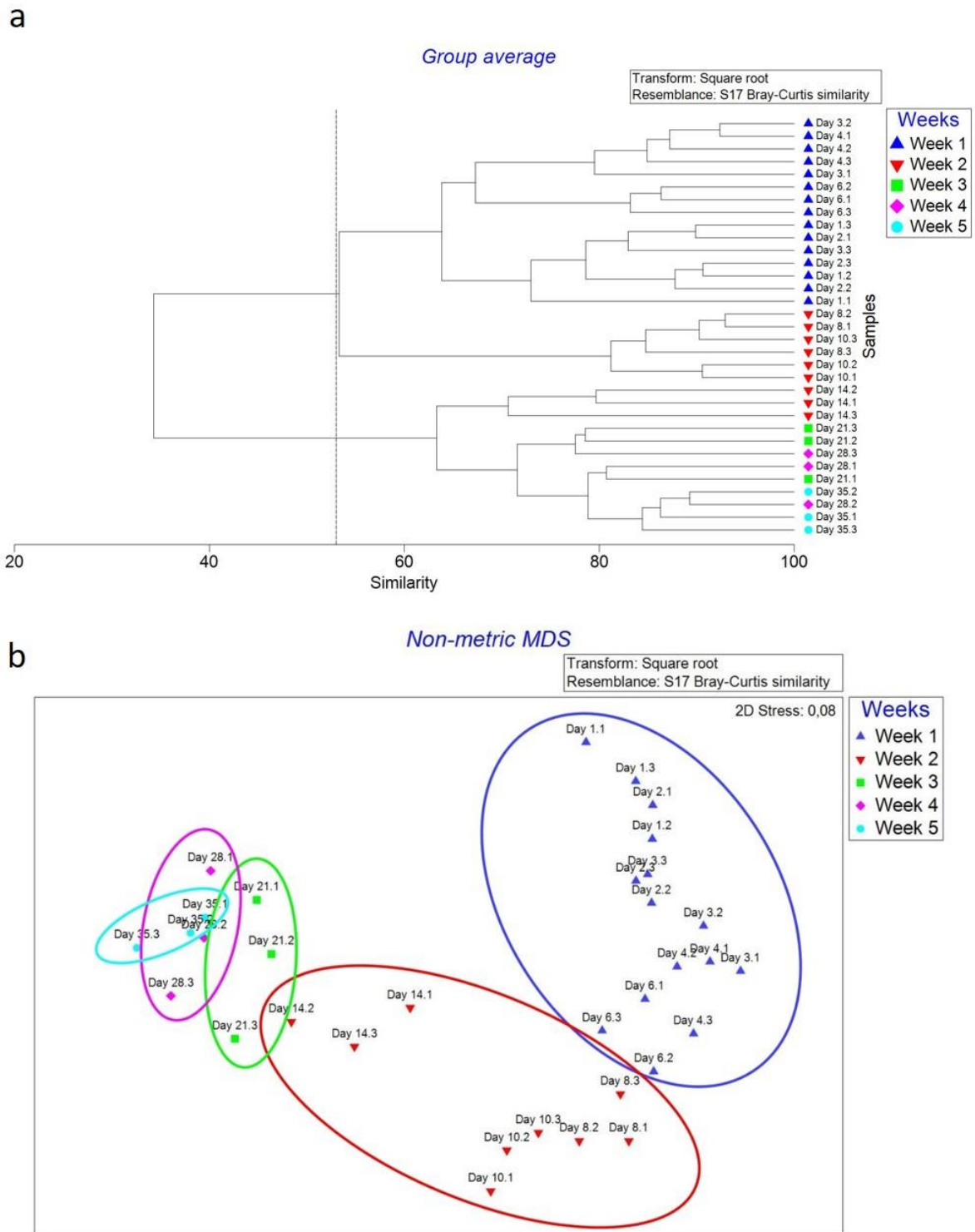


Figure 4.3: Dendrogram (a) and nMDS plots (b) of all macroinvertebrate and zooplankton taxa of each mesocosm collected over the five sampling weeks. Last number denotes treatment: 1 – Treatment 1, 2 – Treatment 2, 3 – Treatment 3.

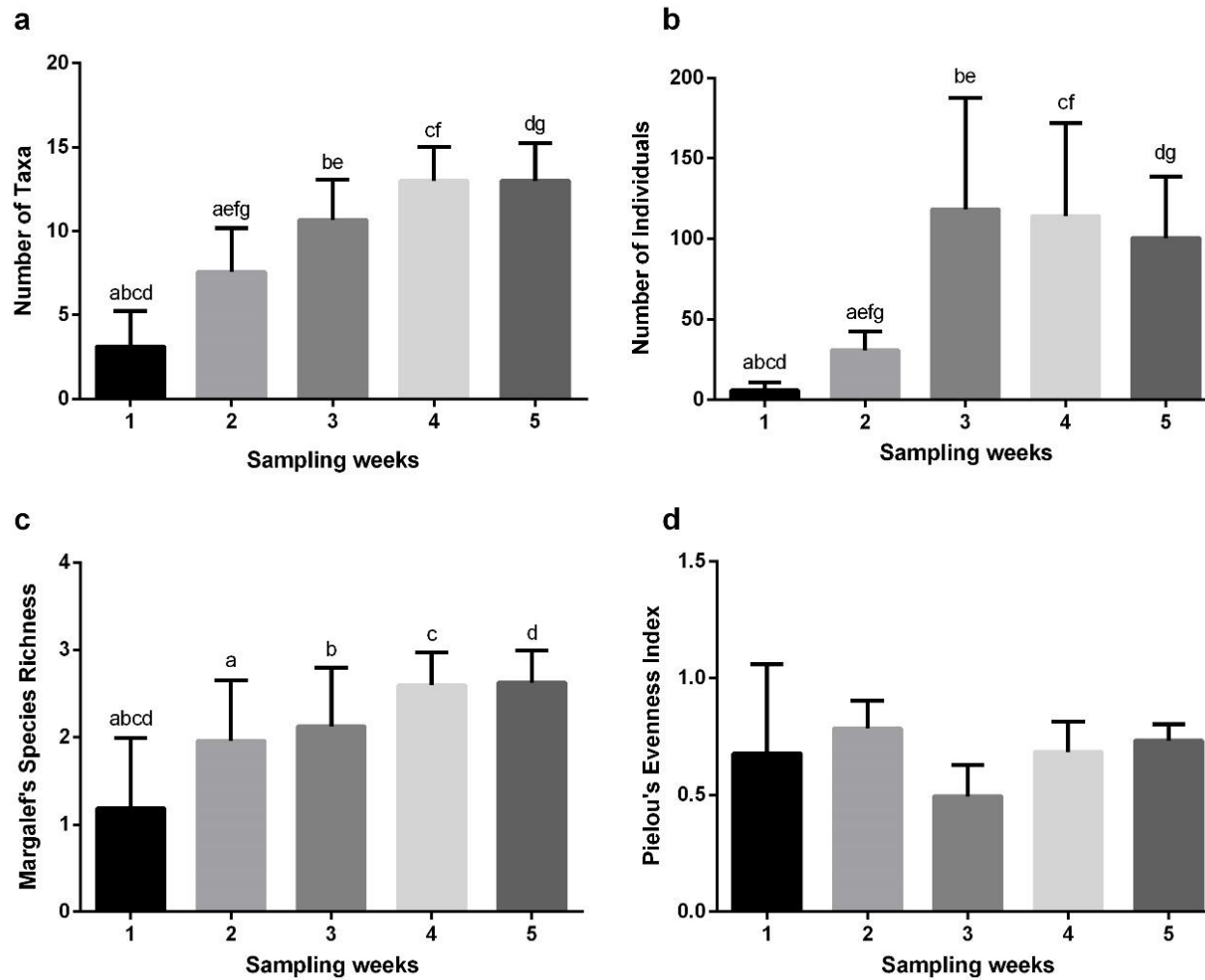


Figure 4.4: Number of taxa (a), number of individuals (b), Margalef's species richness (c) and Pielou's evenness index (d) of all macroinvertebrate taxa collected over the five sampling weeks. Similar superscripts denote significant differences ($p < 0.05$).

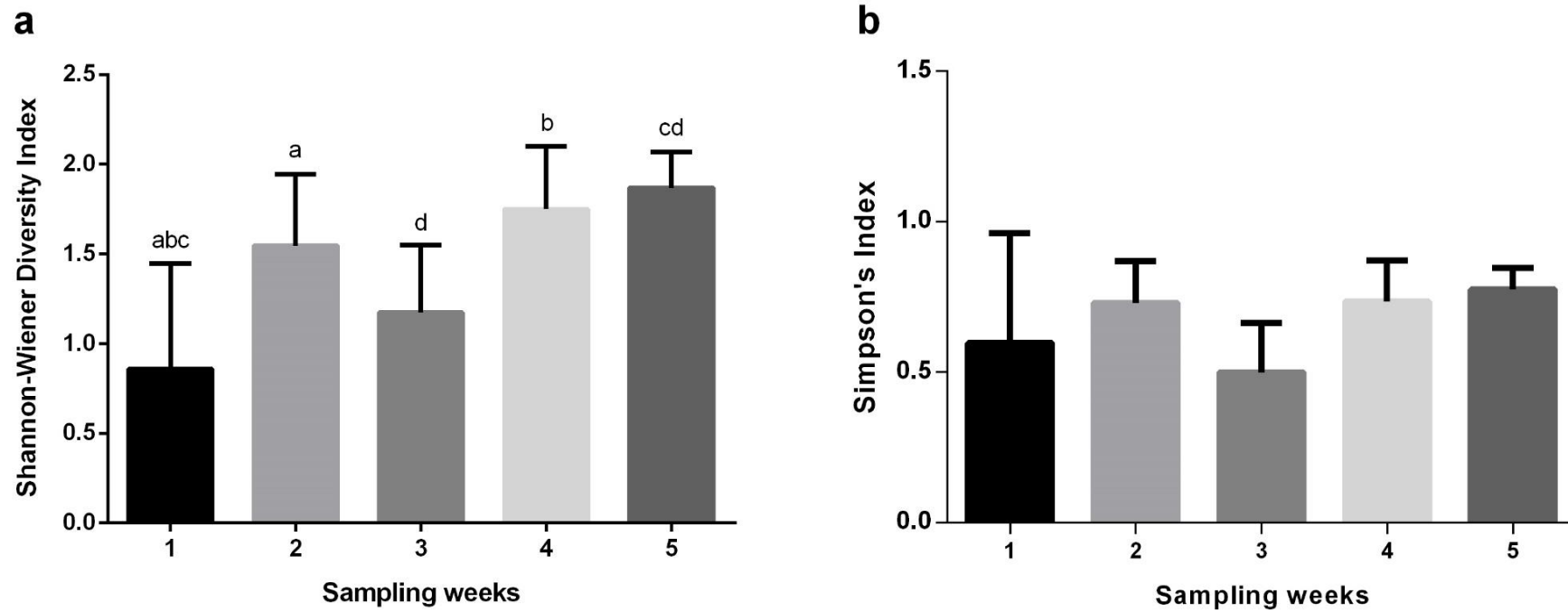


Figure 4.5: Shannon-Wiener diversity (a) and Simpson index (b) of all macroinvertebrate taxa collected over the five sampling weeks. Similar superscripts denote significant differences ($p < 0.05$).

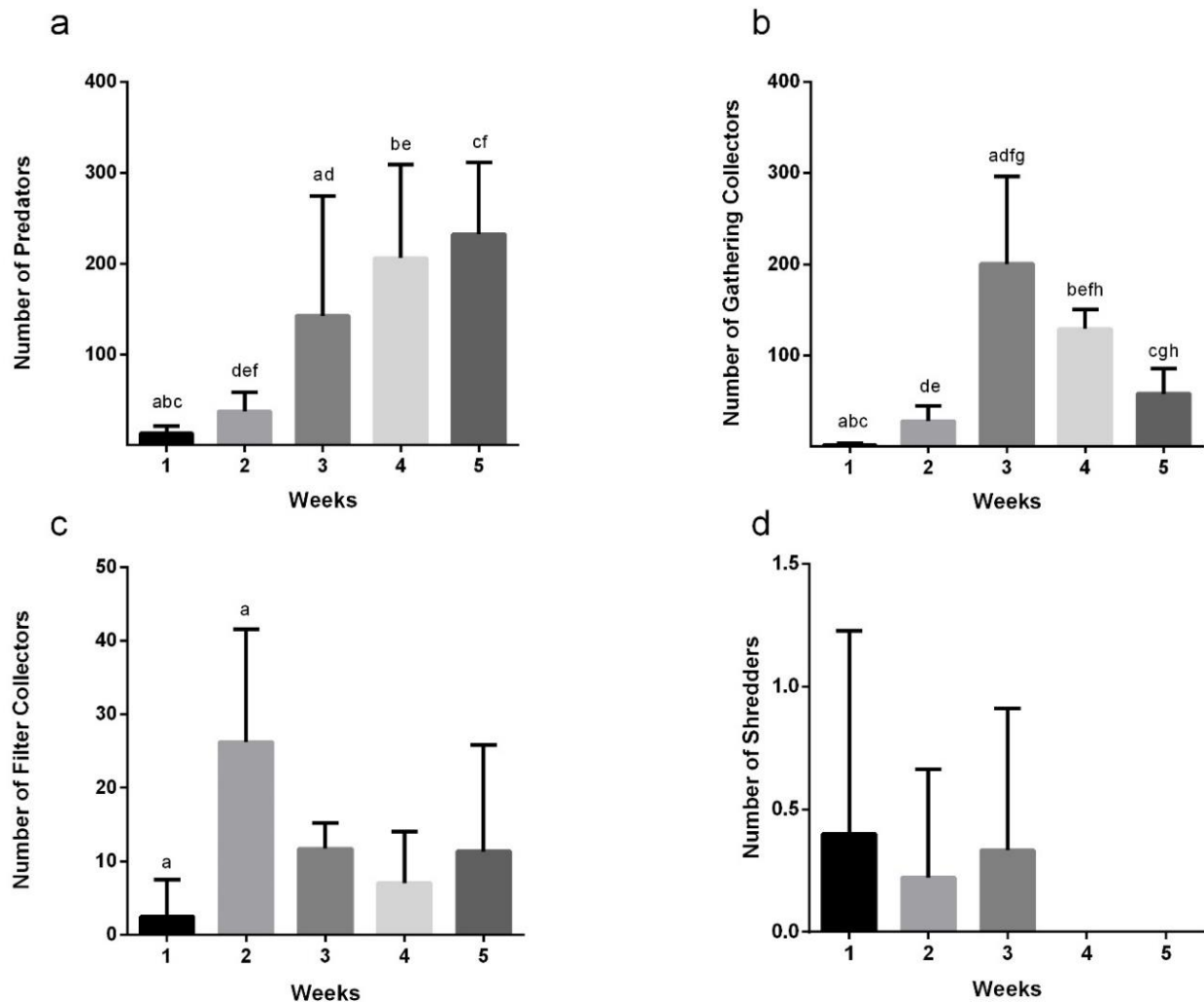


Figure 4.6: Number of predators (a), number of gathering collectors (b), number of filter collectors (c) and number of shredders (d) of each functional feeding group over the five sampling weeks. Similar superscripts indicate significant differences ($p < 0.05$).

4.3.3 Zooplankton

Six zooplankton taxa from six different families were found to have hatched from the resting egg banks during the study, namely *Diaphanosoma* sp. (Sididae), *Thermocyclops* sp. (Cyclopidae), *Moina micrura* (Moinidae), *Metadiaptomus* sp. (Diaptomidae), *Simocephalus serrulatus* (Daphniidae), Darwinulidae and Cyprididae, and all six were present in the three treatments. Three zooplankton taxa were found in 2014 in the model floodplain pool, namely *Alona* sp. (Cladocera), *Diaphanosoma peramatum* (Sididae) and Cyclopoida.

One-way ANOVA of the three treatments indicated that only *Simocephalus serrulatus* differed significantly between T1 and T3 ($p=0.006$; $F=5.35$; Appendix B7). Only two zooplankton genera were found during all five sampling weeks, namely *Diaphanosoma* sp. and *Thermocyclops* sp. The small-bodied cyclopod *Thermocyclops* sp. was also the most abundant taxon found throughout the study, while the larger cyclopod, *Metadiaptomus* sp., was much less abundant throughout the study (Figure 4.7; Appendix B15). The zooplankton ANOVA analysis over the five-week sampling period indicated that three zooplankton taxa increased significantly from the first to the last sampling weeks (Table 4.4). These taxa were *Diaphanosoma* sp. (1.76 to 34.6; $p<0.0001$; $F=32.5$), *Metadiaptomus* sp. (0 to 4.053; $p<0.0001$; $F=7.69$) and Cyprididae (0 to 7.73; $p>0.0001$; $F=15.56$). The remaining taxa showed varying degrees of increase and/or decrease. *Thermocyclops* sp. decreased significantly from the first to last week (58.03 to 29.81; $p<0.0001$; $F=9.1$), *M. micrura* increased from week 1 to week 2 (20.71 to 72.82) and then decreased to 0 in week 5 ($p<0.0001$; $F=30.75$). *Simocephalus serrulatus* increased significantly from week 1 to week 3 (0 to 59.3), but then decreased again towards week 5 (44.58; $p<0.0001$; $F=22.68$), while Darwinulidae were found only during week 2 (2.49; $p=0.0003$; $F=15.56$).

One-way ANOVA of zooplankton diversity between the three treatments indicated that the number of individuals was significantly higher in T1 (8872) in comparison to T3 (4847; $p=0.008$; $F=5.07$) (Appendix B8). In comparison, zooplankton diversity indices across the five sampling weeks generally increased significantly from week 1 to week 5 (Figure 4.8 and Figure 4.9). The number of taxa increased from 2.1 (week 1) to 4.11 (week 5; $p<0.0001$; $F=27.23$), species richness increased from 0.13 (week 1) to 0.38 (week 5; $p<0.0001$; $F=23.06$), evenness increased from 0.39 (week 1) to 0.68 (week 5; $p=0.0004$; $F=5.71$), Shannon diversity increased from 0.29 (week 1) to 0.95 (week 5; $p<0.0001$; $F=26.49$) and Simpson's index increased from 0.17 (week 1) to 0.54 (week 5; $p<0.0001$; $F=22.96$). The number of individuals increased markedly and significantly from week 1 to week 2 (4662 to 10 982, respectively; $p<0.0001$; $F=7.75$), but then decreased again towards week 5 (5809). Pearson correlations between macroinvertebrate FFGs and zooplankton taxa over all sampling weeks indicated that only three significant correlations were found. These correlations were between predators and *Diaphanosoma* sp. ($p=0.03$; $R^2=0.83$), predators and *Thermocyclops* sp. ($p=0.008$; $R^2=0.93$) and filtering collectors and *Metadiaptomus* sp. ($p=0.03$; $R^2=0.83$; Table 4.5).

The first variation partitioning analysis indicated that both selected water quality variables and sampling weeks each had distinct effects on the zooplankton community assemblages. The amount of unique variation each explained by water quality (18%) and sampling weeks (15%) were both highly significant

($p < 0.001$). In addition, these two variables had a 33% combined effect, while 34% was unexplained (Figure 4.10a). Both water quality and the macroinvertebrate community each had distinct effects on the zooplankton community and the amount of unique variation explained by the water quality (9%) and macroinvertebrates (20%) were each highly significant ($p < 0.01$; Figure 4.10b). The shared effect between the two variables was 42% while 29% was unexplained.

Pearson correlations between water quality variables and zooplankton taxa indicated that all the water quality variables used in the variation partitioning analysis were significantly correlated to the zooplankton taxa to varying degrees ($p < 0.05$; Table 4.6). Most prominently, oxygen saturation and conductivity were significantly correlated to all zooplankton except Cyprididae, NO_3^- was correlated to all but *M. micrura* and NO_2^- to all but Darwinulidae. Contrastingly, total hardness (TH) was only correlated to one taxon, namely *M. micrura*.

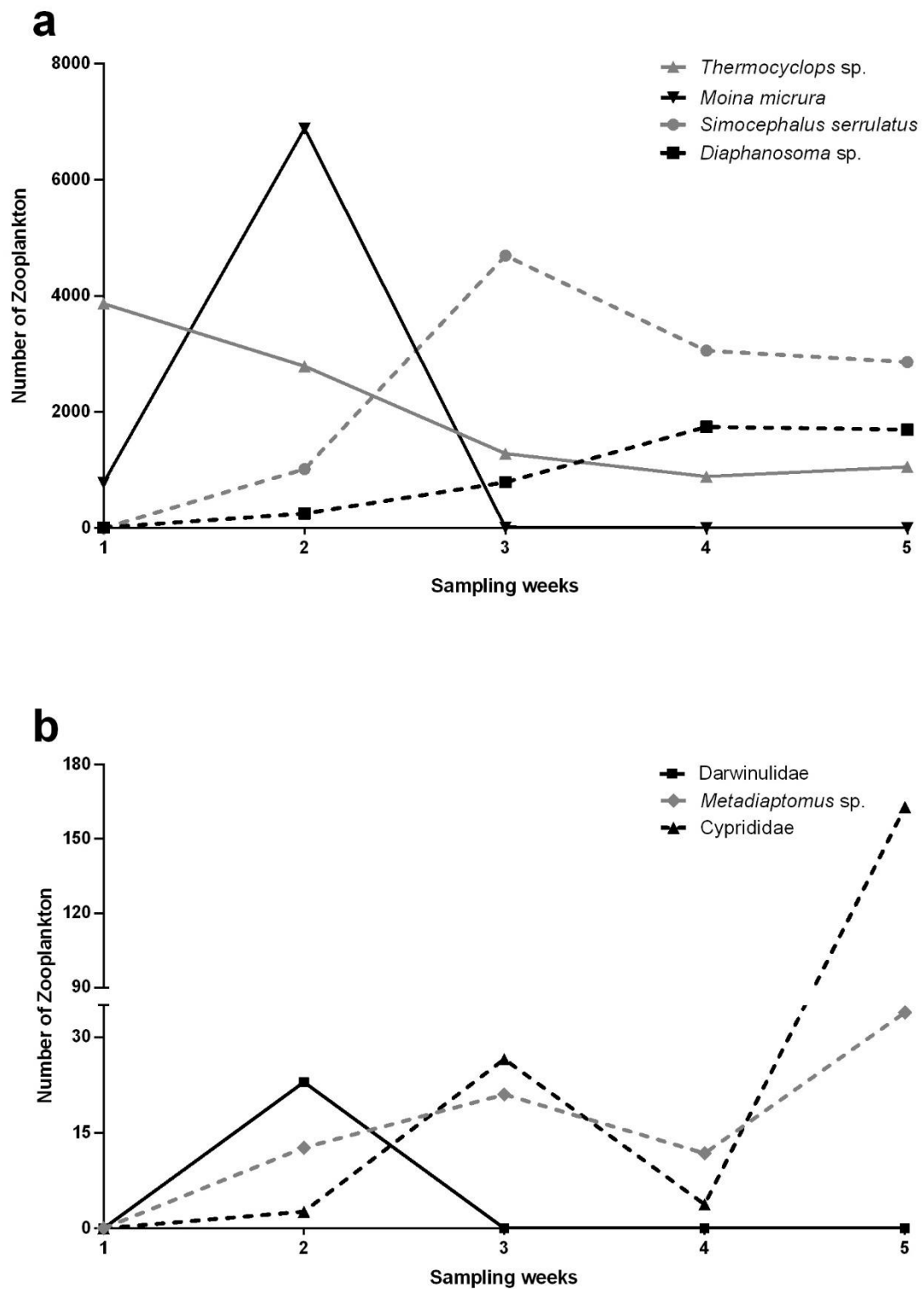


Figure 4.7: Line graphs depicting changes in zooplankton taxa abundance over the five sampling weeks.

Table 4.4: Zooplankton taxa and Analysis of Variance (ANOVA) of zooplankton taxa collected over the five sampling weeks. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). N-value indicates number of samples per week.

Zooplankton	Time					ANOVA
	Week 1 (n=45)	Week 2 (n=27)	Week 3 (n=9)	Week 4 (n=9)	Week 5 (n=9)	
<i>Diaphanosoma</i> sp.	1.76±3.13 ^{abcd} 0 – 11.05	13.88±7.8 ^{ae fg} 0 – 31.75	25.85±11.79 ^{be} 0 – 41.9	36.03±22.4 ^{cf} 0 – 65.44	34.55±23.8 ^{dg} 0 – 69.48	p<0.0001 F= 32.5
<i>Thermocyclops</i> sp.	58.03±22.58 ^{abc} 24.59 – 132.7	49.77±18.06 ^d 18.44 – 78.12	33.93±12.28 ^a 15.13 – 52.15	25.85±15.7 ^{bd} 0 – 49.14	29.81±13.68 ^c 10 – 47.25	p<0.0001 F= 9.10
<i>Moina micrura</i>	20.71±19.03 ^a 0 – 68.6	72.82±40.52 ^{abcd} 13.45 – 148.3	1.58±3.29 ^b 0 – 9.06	0±0 ^c 0 – 0	0±0 ^d 0 – 0	p<0.0001 F= 30.75
<i>Metadiaptomus</i> sp.	0±0 ^a 0 – 0	1.65±3.21 0 – 8.76	3.4±3.28 ^a 0 – 7.75	1.98±2.97 0 – 6.25	4.05±4.43 0 – 12.81	p<0.0001 F= 7.69
<i>Simocephalus serrulatus</i>	0±0 ^{abcd} 0 – 0	21.61±23.91 ^{ae} 0 – 77.77	59.3±36.45 ^{be} 0 – 117.8	43.48±36.24 ^c 0 – 94.44	44.58±31.32 ^d 0 – 95.29	p<0.0001 F= 22.68
Darwinulidae	0±0 ^a 0 – 0	2.42±4.22 ^{abcd} 0 – 11.92	0±0 ^b 0 – 0	0±0 ^c 0 – 0	0±0 ^d 0 – 0	p= 0.0003 F= 5.85
Cyprididae	0±0 ^{ab} 0 – 0	0.44±1.59 ^c 0 – 6	3.80±3.69 ^{ad} 0 – 7.75	0.79±1.89 ^e 0 – 5.66	8.73±9.87 ^{bcde} 0 – 24.19	p<0.0001 F= 15.56

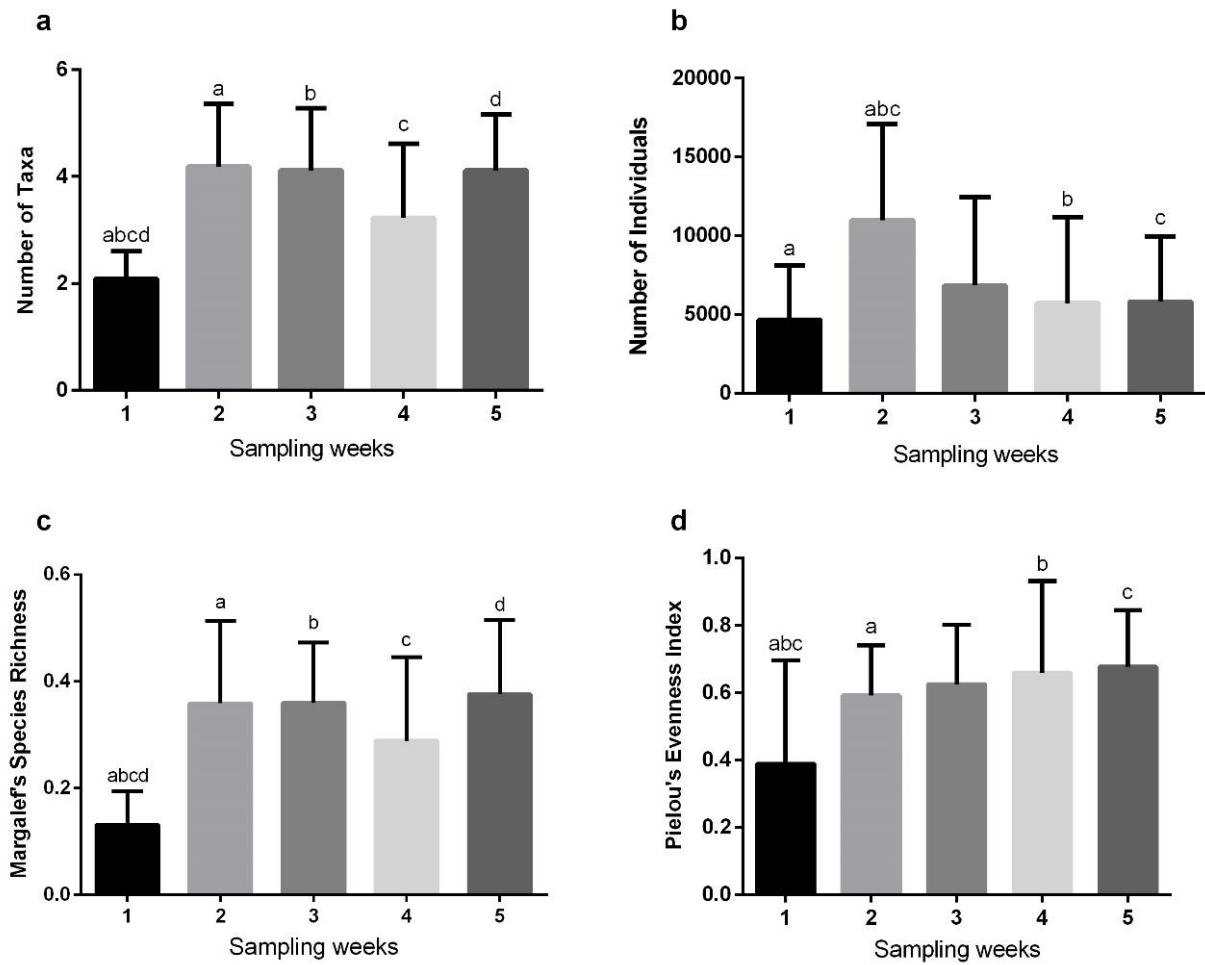


Figure 4.8: Number of taxa (a), number of individuals (b), Margalef's species richness (c) and Pielou's evenness index (d) of all zooplankton taxa collected over the five sampling weeks. Similar superscripts denote significant differences ($p < 0.05$).

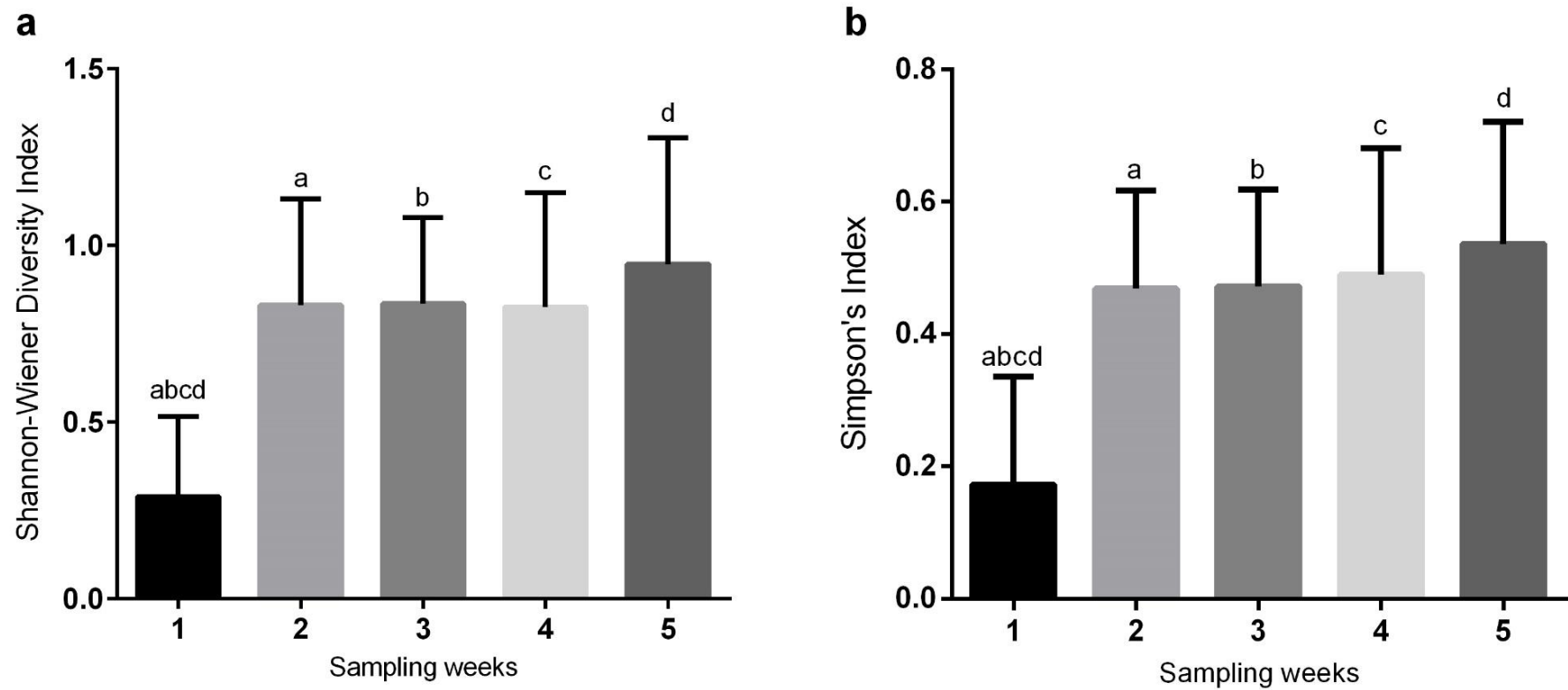
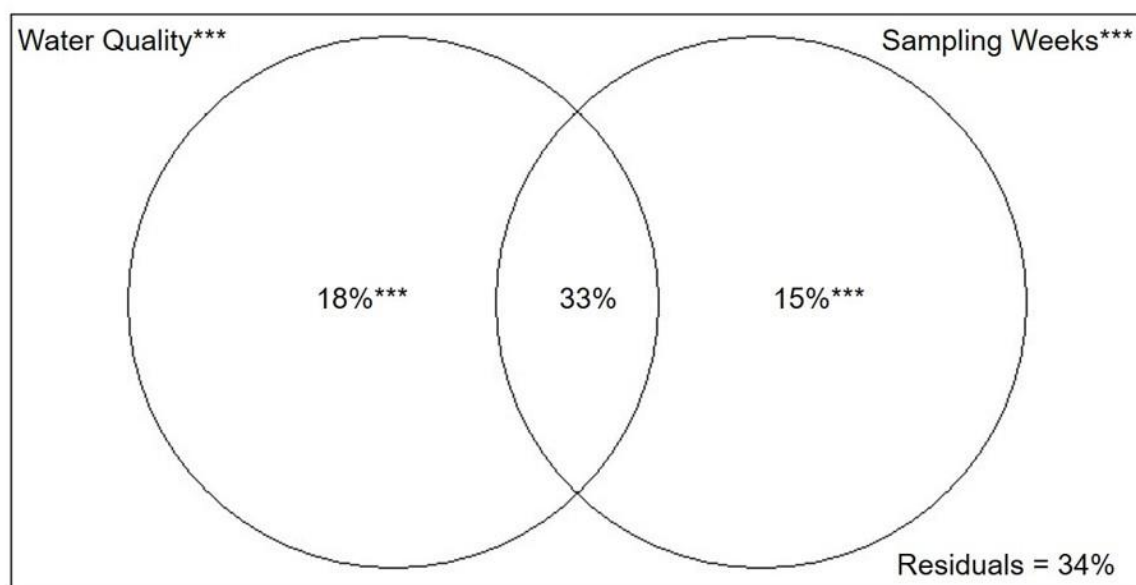


Figure 4.9: Shannon-Wiener diversity (a) and Simpson's index (b) of all zooplankton taxa collected over the five sampling weeks. Similar superscripts denote significant differences ($p < 0.05$).

Table 4.5: Pearson correlations between macroinvertebrate functional feeding groups and zooplankton taxa of all mesocosms over the five sampling weeks.

Family	Predators	Gathering Collector	Filtering Collectors	Shredders
<i>Diaphanosoma</i> sp.	R²= 0.83 p= 0.03	R ² = 0.41 p= 0.24	R ² = 0.13 p= 0.55	R ² = 0.77 p= 0.05
<i>Thermocyclops</i> sp.	R²= 0.93 p= 0.008	R ² = 0.77 p= 0.05	R ² = 0.06 p= 0.69	R = 0.47 p= 0.2
<i>Moina micrura</i>	R ² = 0.61 p= 0.12	R ² = 0.38 p= 0.27	R ² = 0.27 p= 0.37	R ² = 0.24 p= 0.4
<i>Metadiaptomus</i> sp.	R ² = 0.25 p= 0.4	R ² = 0.19 p= 0.46	R²= 0.79 p= 0.045*	R ² = 0.14 p= 0.53
<i>Simocephalus serrulatus</i>	R ² = 0.48 p= 0.2	R ² = 0.7 p= 0.08	R ² = 0.46 p= 0.21	R ² = 0.14 p= 0.53
Darwinulidae	R ² = 0.2 p= 0.45	R ² = 0.07 p= 0.63	R ² = 0.67 p= 0.09	R ² = 0.06 p= 0.7
Cyprididae	R ² = 0.46 p= 0.21	R ² = 0.07 p= 0.66	R ² = 0.07 p= 0.66	R ² = 0.29 p= 0.35

a



b

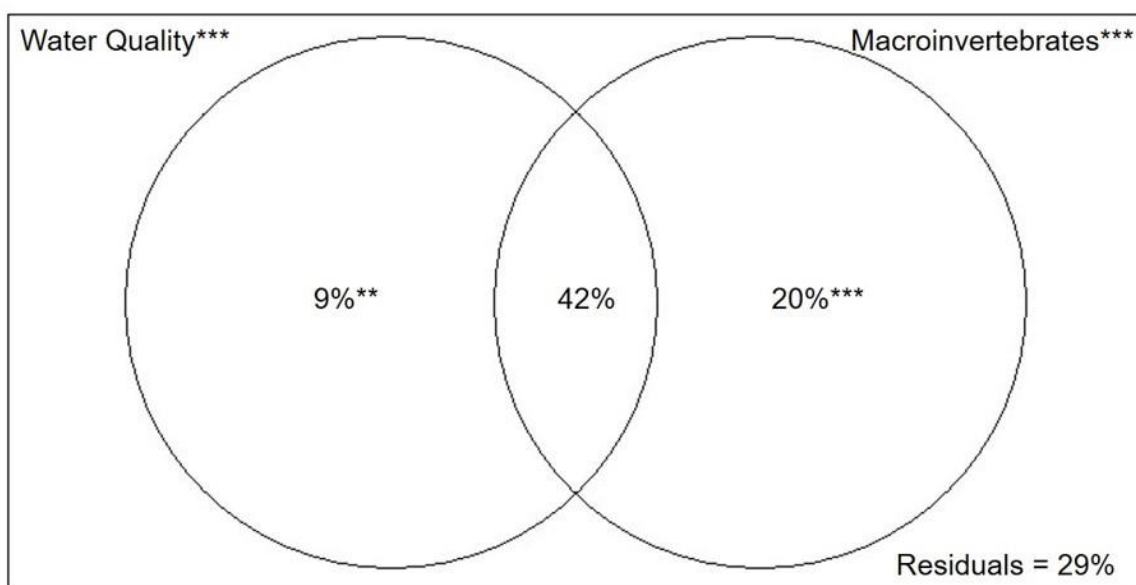


Figure 4.10: Variation partitioning analysis indicating unique and shared contribution of select water quality variables and the five sampling weeks (a) and the unique and shared contribution of select water quality variables and macroinvertebrate biota on zooplankton taxa (b). Asterisks denote level of significance: ** $p < 0.01$, *** $p < 0.001$.

Table 4.6: Pearson correlations between zooplankton taxa and select water quality variables of all mesocosms over the five sampling weeks.

Zooplankton	Water Nutrients					
	Temperature (°C)	Oxygen Saturation (%)	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	NH_4^+ ($\text{mg}\cdot\text{L}^{-1}$)	NH_3 ($\text{mg}\cdot\text{L}^{-1}$)	Chlorophyll a ($\mu\text{g}\cdot\text{L}^{-1}$)
<i>Diaphanosoma</i> sp.	$R^2 = 7.54 \times 10^{-6}$ p=0.98	$R^2 = 0.23$ p<0.0001	$R^2 = 0.36$ p<0.0001	$R^2 = 0.12$ p= 0.0004	$R^2 = 0.04$ p= 0.047	$R^2 = 0.02$ p= 0.19
<i>Thermocyclops</i> sp.	$R^2 = 0.08$ p= 0.005	$R^2 = 0.2142$ p<0.0001	$R^2 = 0.16$ p<0.0001	$R^2 = 0.06$ p= 0.02	$R^2 = 0.12$ p= 0.0006	$R^2 = 0.11$ p= 0.0006
<i>Moina micrura</i>	$R^2 = 0.09$ p= 0.005	$R^2 = 0.06$ p= 0.01	$R^2 = 0.07$ p= 0.01	$R^2 = 0.02$ p= 0.19	$R^2 = 0.01$ p= 0.26	$R^2 = 0.04$ p= 0.06
<i>Metadiaptomus</i> sp.	$R^2 = 0.002$ p= 0.63	$R^2 = 0.06$ p= 0.02	$R^2 = 0.13$ p= 0.0002	$R^2 = 0.10$ p= 0.0013	$R^2 = 0.03$ p= 0.10	$R^2 = 0.02$ p=0.13
<i>Simocephalus</i> <i>serrulatus</i>	$R^2 = 0.008$ p= 0.37	$R^2 = 0.19$ p<0.0001****	$R^2 = 0.18$ p<0.0001	$R^2 = 0.11$ p= 0.0005	$R^2 = 0.04$ p= 0.04	$R^2 = 0.005$ p= 0.51
Darwinulidae	$R^2 = 0.005$ p= 0.47	$R^2 = 0.09$ p= 0.003	$R^2 = 0.0003$ p= 0.86	$R^2 = 0.02$ p= 0.23	$R^2 = 0.01$ p= 0.29	$R^2 = 0.006$ p= 0.46
Cyprididae	$R^2 = 0.004$ p= 0.52	$R^2 = 0.03$ p= 0.09	$R^2 = 0.20$ p<0.0001	$R^2 = 0.04$ p=0.05	$R^2 = 0.009$ p=0.35	$R^2 = 0.004$ p= 0.56

Table 4.6 continued.

Zooplankton	Water Nutrients					
	Total Hardness (mg.L ⁻¹)	NO ₃ ⁻ (mg.L ⁻¹)	NO ₂ ⁻ (mg.L ⁻¹)	SO ₄ ²⁻ (mg.L ⁻¹)	Cl ⁻ (mg.L ⁻¹)	Turbidity (NTU)
<i>Diaphanosoma</i> sp.	R ² = 0.02 p= 0.14	R²= 0.23 p<0.0001	R²= 0.32 p<0.0001	R ² = 0.003 p= 0.61	R²= 0.07 p= 0.009	R²= 0.24 p<0.0001
<i>Thermocyclops</i> sp.	R ² = 0.002 p= 0.68	R²= 0.10 p= 0.002	R²= 0.12 p= 0.0004	R²= 0.15 p<0.0001	R ² = 0.002 p= 0.64	R²= 0.15 p<0.0001
<i>Moina micrura</i>	R²= 0.04 p= 0.04	R ² = 0.007 p= 0.41	R²= 0.07 p= 0.009	R²= 0.08 p= 0.005	R²= 0.04 p= 0.04	R ² = 0.0002 p= 0.88
<i>Metadiaptomus</i> sp.	R ² = 0.02 p= 0.14	R²= 0.15 p<0.0001	R²= 0.14 p= 0.0002	R ² = 0.0005 p= 0.83	R ² = 0.04 p= 0.06	R²= 0.12 p= 0.0004
<i>Simocephalus</i> <i>serrulatus</i>	R ² = 0.02 p= 0.16	R²= 0.27 p<0.0001	R²= 0.07 p= 0.007	R ² = 0.002 p= 0.69	R²= 0.06 p= 0.02	R²= 0.34 p<0.0001
Darwinulidae	R ² = 0.01 p= 0.29	R²= 0.08 p= 0.006	R ² = 0.02 p= 0.19	R ² = 0.003 p= 0.58	R ² = 0.005 p= 0.47	R ² = 0.009 p= 0.36
Cyprididae	R ² = 0.006 p= 0.43	R²= 0.06 p= 0.02	R²= 0.29 p<0.0001	R ² = 0.01 p= 0.31	R ² = 0.02 p= 0.14	R²= 0.058 p= 0.017

4.4 Discussion

In this study, mesocosms were used to test whether treatments mimicking various hydrologic connectivity of wetlands with a river system affect water quality and aquatic invertebrate communities. Few significant shifts were found in the abiotic and biotic factors through hydrologic variability. The lack of effect through these treatments supports the “drought resistance hypothesis” suggested by Boersma et al. (2014) that taxonomic and trait diversity in fragmented pools will remain constant as water levels decline. Several aquatic invertebrate taxa present in the mesocosms during the present study were also found in the model floodplain pool studied by Dube (2015), including similar genera (*Diaphanosoma* sp.) and orders (Cyclopoida) of zooplankton that hatched from the sediments. This indicates that, overall, mesocosms proved to be a useful tool to study the naturally occurring aquatic invertebrate communities of NGR.

Although several macroinvertebrate and zooplankton indicator taxa were significantly associated with each of the three treatments, many of these were shared between the three treatments over similar weeks including *M. micrura*, *Diaphanosoma* sp., *S. serrulatus*, *Mesovelgia vittergera* and *Anopheles* sp. For the larger part, changes in the studied abiotic and biotic variables occurred over time, and not due to hydrologic variability. Similarly, Boersma et al. (2014) conducted a mesocosm study in Arizona, USA, and found no significant difference in aquatic invertebrate taxonomy or functional traits between three drought-severity treatments, even in the presence of changes in water quality. Their study concluded that a local history of drought severity, frequency and duration in a region determines whether aquatic organisms at a given location exhibit traits that allow them to withstand drought disturbance. If local taxa are indeed adapted to drought and the environmental changes that accompany them, pool drying may not cause a loss in taxonomic or trait diversity until all surface water has disappeared. In addition, a mesocosm study by Krabbenhoft et al. (2017) in New Mexico, USA, found that time had the largest impact on aquatic invertebrate assemblages, and that, although highly heterogeneous at the start of the experiment, the aquatic invertebrate assemblages became more homogenous as time continued. They attributed this to successional processes driven by warming and drying of habitats over time. These factors are possibly also why the aquatic invertebrates found during the present study did not appear severely affected by the different hydrologic variabilities, but rather by time. In addition, studies have determined that long hydroperiods have a significant positive effect on aquatic invertebrates (e.g. Schneider and Frost 1996; Studinski and Grubbs 2007; Waterkeyn et al. 2008; Batzer and Boix 2016), but, similar to the present study, that volume and depth do not appear to influence these biota. Possible reasons for this include adaptations that aquatic invertebrates have evolved in order to tolerate hydrologic fluctuations and the stress of drought, such as small body size, short life cycles, rapid growth rate, desiccation resistant eggs and avoidance through active dispersal (Brendonck and Williams 2000; Williams 2006; Batzer and Boix 2016). The most abundant and commonly found macroinvertebrate taxa present during the study consisted largely of small taxa (<10mm) including *Anisops* sp., *Cloeon* and *Proclaeon* sp. and small beetles such as *Hydroglyphus* sp. and *Hyphydrus* sp. (Dytiscidae) and *Berosus* sp. (Hydrophilidae). Similarly, Dube et al. (2016) found these taxa to be the most abundant ones in temporary wetlands of NGR.

These types of invertebrates are cosmopolitan and occur in most aquatic habitats (Stals and de Moor 2008). Since no significant differences were found between the three replicates of each treatment or the three treatment groups, including mesocosms that had fish present and those that did not, and given the low abundance and small size of the fish, it was concluded that fish had no significant impact on the aquatic invertebrate structure in any of the mesocosms and thus any impacts were considered negligible. Studies have also found that fish do not affect aquatic invertebrate diversity or density (both macroinvertebrate and zooplankton) as much in systems where there is abundant refuge vegetation (i.e. aquatic plants) (Gilinsky 1984; Mittelbach 1988) such as was present in the mesocosms (pers. observation; de Necker). In addition, a six-week mesocosm study by Krabbenhoft et al. (2017) found that young-of-year fish did not significantly affect the overall diversity or density of aquatic invertebrates. However, the abundance of small aquatic invertebrate taxa (<10mm) found during the study may be attributed to fish in the mesocosms, as studies have found that larger macroinvertebrate individuals are particularly susceptible to fish predation (Mittelbach 1988; Wellborn et al. 1996; van de Meutter et al. 2006), while there is less evidence for their negative impacts on diversity and abundance.

Most of the macroinvertebrate taxa were air-breathing, non-wintering spring recruits (Wiggins et al. 1980; Batzer and Boix 2016) and the majority were predatory. It has been found that predatory aquatic invertebrates respond positively to drought and reduced discharge, and thus thrive in habitats that have been disconnected from the river (Statzner and Bêche 2010), possibly because prey are easier to catch in enclosed systems. Macroinvertebrate larvae, although present, were not highly abundant. The most likely cause of this was predation on larvae and adults with eggs by predatory macroinvertebrates (Gliwicz and Pijanowska 1989; Ghosh et al. 2005; Batzer and Boix 2016). Many macroinvertebrate taxa are unable to tolerate complete drought and thus avoid it through active migration from a site once it becomes dry (Wiggins et al. 1980). However, these taxa generally have adaptations that enable them to tolerate a wide range of environmental changes, such as low oxygen and increased salinity (Dickens and Graham 2002; Stals and de Moor 2008), as was evident in this study, and was also found in previous research (Pires et al. 2000; van de Meutter et al. 2007; Boersma et al. 2014). Low oxygen levels may have been an important mechanism of the macroinvertebrate community structure, since taxa that are able to breathe atmospheric air often dominate stagnant habitats with low oxygen (Chessman 2015). Although there were no significant observed successional shifts in the macroinvertebrate communities, possible shifts may have been indirectly influenced by changes in the zooplankton communities, as previously found by de Meester et al. (2002) and Waterkeyn et al. (2010).

Zooplankton taxa successfully hatched from resting egg banks in the sediments, indicating that the water was of sufficient quality to stimulate egg hatching (Nielson et al. 2002) and that the mesocosms successfully mimicked a wetland. Continuous shifts were found to occur in the zooplankton communities over the five sampling weeks, as there was cycling from one dominant taxon to the next. This type of succession occurs naturally in aquatic habitats, as found by Eloranta (1982) in a natural lake in Finland, and by Chaparro et al. (2016) in a floodplain lake in Argentina, although in the present study this occurred at a faster rate. It has been observed that succession generally occurs at an increased rate in enclosed or manipulated systems such as mesocosms (Gliwicz and Pijanowska 1989) and could explain the fast turnover rate in zooplankton species and succession in the present study, which would

generally take an entire season to occur in a natural system. Aquatic invertebrates, particularly zooplankton, are affected by a number of variables, including nutrients, temperature, food availability, space and predation (Gliwicz and Pijanowska 1989; Holeck et al. 2008; Waterkeyn et al. 2010; Chaparro et al. 2016). As zooplankton and macroinvertebrate abundance increased with time, concurrent decreases occurred in nutrients (orthophosphates, nitrates, nitrites, ammonium and ammonia) and oxygen concentration. These nutrients are used by aquatic invertebrates and plants as food sources and for biologic processes (Dallas and Day 2004) and, due to the enclosed nature of a pool, with no additional inputs from the environment (e.g. rain or flood water), these nutrients are utilised and not replenished, leading to depletion (Talling 2001). Increasing salinity (measured through conductivity, TDS and Cl⁻) throughout the study was due to evaporation of water, and thus the concentration of salts since, as pools dry out, temperature and conductivity increase and oxygen decreases (Talling 2001; Leigh 2013; Boersma et al. 2014; Batzer and Boix 2016). Evaporation of water also leads to decreased habitat area, causing the concentration of aquatic biota, increasing the intensity of interactions among species and the possible elimination of species through predation or competition for declining food resources, particularly amongst herbivores (Holeck et al. 2008; Boersma et al. 2014).

Most zooplankton taxa that occur in temporary conditions are adapted to exist under continuously fluctuating environmental conditions, whereas certain species are limited by physicochemical parameters such as temperature, dissolved oxygen and salinity. High salinity and temperature have been found to affect aquatic invertebrates negatively, particularly selected zooplankton taxa such as *Moina* sp., *Simocephalus* sp. and *Diaphanosoma* sp., by influencing their hatching, survival, development and reproduction, which causes a cascading effect on other aquatic invertebrates (Rojas et al. 2001; Benider et al. 2002; Waterkeyn et al. 2010). The sudden decrease in *M. micrura* after the first two weeks may be linked to environmental and predatory factors. Studies have found the longevity of *Moina* sp. to decrease from 21 to 7 days, with temperature increases from 15°C to 30°C, respectively, due to accelerated growth that decreases longevity, reduced egg hatching and increased energy use by females for reproduction, leading to premature death of the females (Benider et al. 2002). These species are also more associated with the initial heavy rains of summer (Day et al. 1999), implying that they have a preference for the first cooler fresh water that enters a pond. Similarly, Pagano et al. (2000) conducted a laboratory microcosm experiment and found that *M. micrura* disappeared by day 10 of the experiment.

Diaphanosoma sp. increased gradually over the weeks, as the season moved into warmer summer temperatures. Similar responses to increased temperatures have been found in certain *Diaphanosoma* and *Simocephalus* species, as with *M. micrura* (Benider et al. 2002), although *Diaphanosoma* sp. are also known as “summer genera”, since they appear during the spring to summer successional shift. These taxa are less vulnerable to predation through better escape abilities than the “spring genera” (Gliwicz and Pijanowska 1989). *Diaphanosoma excisum* is also known to appear generally during the heat of summer (Day et al. 1999). Significant correlations between *Thermocyclops* sp. and predatory macroinvertebrates was most likely due to predation by fish and macroinvertebrate predators such as the phantom midge (*Chaoborus microstictus*), which has a preference for copepods (Day et al. 2001a).

Predators generally shift their diet from spring to summer as they grow and mature (Gliwicz and Pijanowska 1989). This “summer shift” may be generalised, based simply on prey density and vulnerability, or selective by targeting only females, eggs, larger individuals or particular species (Gannon and Stemberger 1978; Gliwicz and Pijanowska 1989; Canfield and Jones 1996). Similarly, Pagano et al. (2003) conducted mesocosm experiments and found that *Chaoborus* sp. had a significant negative effect on *Thermocyclops* sp. and *M. micrura*.

4.5 Conclusion

This study aligns with previous research that suggests that aquatic invertebrates possess some degree of tolerance to short-term stress caused by factors such as habitat drying as well as the drought resistance hypothesis that the biology of aquatic invertebrates living in a habitat with frequent droughts will allow them to survive droughts. This was evident as the taxa found during this study did not differ significantly between the three hydrologic treatments. The hypothesis that shorter hydrologic connectivity negatively affects aquatic invertebrates and water quality is thus rejected. The mesocosms proved to mimic successfully the natural ecosystem turnover for the zooplankton community, but not for the macroinvertebrate community. The second hypothesis stating that natural seasonal successional shifts occur in aquatic macroinvertebrate and zooplankton communities over time in a mimicked floodplain pool is therefore accepted with regard to the zooplankton but not the macroinvertebrates. The final hypothesis, that macroinvertebrate abundance and functional habits change zooplankton structure, is accepted as significant correlations were found between macroinvertebrate community structure and their FFGs and zooplankton communities that probably caused some of the shifts seen in the zooplankton communities.

Although the present study found little distinct evidence of the effect of the duration of hydrologic connectivity, predicted future climate change through modified rainfall patterns and more frequent droughts will probably increase drought lengths. In the long term this may affect aquatic biota as there may come a time where not even hardy, drought resistant aquatic invertebrates will be able to complete reproduction and their short lifecycles. This may be particularly true for rivers and floodplains such as the Phongolo system that are already regulated by dams.

CHAPTER 5:

Ecological assessment of a temporary pool through an atypical drought cycle

5.1 Introduction

Regions that naturally undergo seasonal droughts may experience more frequent and lengthier droughts with much warmer water temperatures, higher evaporation and reduced precipitation due to climate change (Bates et al. 2008, Aldous et al. 2011). This, in addition to increasing demand for water as a result of human population growth, is placing a great deal of strain on freshwater aquatic ecosystems (Polunin 2008; Lu et al. 2016; Trochine et al. 2017). While studies have determined the direct effects that drought and climate change may have on specific species and traits, not many have considered the indirect effects (McKee and Atkinson 2000; Lu et al. 2016).

Drought is considered a disturbance to most aquatic environments, particularly those that do not experience recurring droughts. However, many biota that occur in habitats with naturally recurring droughts (drying events) or seasonal wet/dry cycles, such as temporary wetlands and streams, have adaptations that allow them to persist through such an event and may not consider this a disturbance (Brock et al. 2003; Boersma et al. 2014). Some of these adaptations include 1.) desiccation-resistant dormant developmental stages that can survive several dry years (Brendonck and Williams 2000; Brock et al. 2003), 2.) short and rapid life cycles, in order to complete maturation and reproduction before the habitat dries out (Laufer et al. 2009), or 3.) migration to a different water source once the habitat becomes unsuitable (Batzler and Boix 2016).

Many aquatic invertebrates and some specialised fish (e.g. killifish, lungfish) that occur only in temporary habitats (temporary water taxa; Meintjes 1996) employ either one or all of these adaptations in order to survive short inundation periods, and the environmental changes (including increased salinity and water temperature) that accompany the drying of temporary ecosystems (Meintjes 1996). Aquatic invertebrate communities in temporary pools generally consists of a mixture of opportunistic colonisers, such as genera from the families Dytiscidae, Notonectidae and Corixidae, and temporary water taxa, including most zooplankton groups such as Daphniidae, Conchostraca, Notostraca and Anostraca (Meintjes 1996; Waterkeyn et al. 2010). Since the opportunistic taxa generally have no adaptations to drying of a temporary site, they simply disperse to different regions when the site becomes uninhabitable while the temporary water taxa will enter a dormant phase. Thus, there is generally a reduction in aquatic invertebrate diversity and abundance during the drying of a temporary site (Lahr et al. 1999; Villastrigo and García-Criado 2017). Longer dry periods, and the accompanying increased temperatures, may therefore not necessarily influence the temporary water taxa directly. However, extended periods of drought, drought induced changes in the seasonal hydrology (e.g. early or late season drying events) or warmer temperatures may lead to local extinction and loss of biodiversity of species that are susceptible to drought. This includes reducing the resistance and resilience of

temporary water taxa (Suren et al. 2003; Boersma et al. 2014), desynchronization of consumers and food sources, or favouring the growth of nutrient low algae and plants (McMeans et al. 2015). This in turn may indirectly affect the drought-adapted species by changing the quantity and quality of their food source, which may impact on their growth, maturation and fecundity (Krams et al. 2014).

Fish of all feeding guilds are generally able to shift their diet from summer to winter, or from wet to dry seasons, due to natural fluxes in the abundance and distribution of resources (Luz-Agostinho et al. 2009; McMeans et al. 2015; Lu et al. 2016). Other factors causing diet shifts include physiological stress or modified foraging behaviour due to disturbances such as receding water levels due to drought (Lu et al. 2016). In freshwater floodplains, all fish feeding guilds rely on aquatic macrophytes in wet seasons, while in the drier season macrophyte-derived detritus is an important resource as they become more abundant (McMeans et al. 2015).

Stable isotope analysis, particularly carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) has been increasingly used to understand energy flow and food webs in aquatic ecosystems (Bouillon et al. 2011; Post 2002; Parnell et al. 2012; Marchese et al. 2014; Chen et al. 2017; Lemmens et al. 2017). Previous dietary studies have largely made use of stomach or gut content analysis in order to determine a consumer's diet (Balcombe et al. 2005; Laufer et al. 2009; Luz-Agostinho et al. 2009; Lu et al. 2016). As this time-consuming approach only provides a "snapshot" of the organism's diet (Polačik et al. 2014), researchers are now also making use of the isotopic composition of consumers and their probable food sources to draw conclusions about a consumers' integrated diet (Phillips 2012; Phillips et al. 2014).

The eastern African annual killifishes (*Nothobranchius* spp.) are found exclusively in temporary habitats with relatively short hydroperiods and have remarkable life history traits and means of survival under extreme conditions such as drought (Polačik and Reichard 2010; Pinceel et al. 2015; Furness 2016). Due to the type of habitat they live in, annual killifish have adapted an accelerated lifecycle, many reaching sexual maturity within a month (Polačik and Reichard 2010) and possessing very short lifespans for fish (approximately nine weeks to one year) (Wildekamp 2004; Watters 2009; Polačik and Reichard 2010). Once sexually mature, these fish reproduce and deposit eggs in the sediment of the pool before it dries, where the eggs will remain dormant until the pool receives water again (Laufer et al. 2009). Annual killifish are generally not present in aquatic habitats when other larger fish species occur in the same system, with the exception of lungfish (Brendonck, pers. observation). However, juvenile non-annual fish such as *Oreochromis* spp. and *Tilapia* spp. have been found alongside these killifish in temporary pools through accidental introduction following floods during a rainy season (Reichard et al. 2009). Because of their narrow habitat preferences and small distribution range, *Nothobranchius* spp. are rare in southern African aquatic ecosystems, with their populations being under constant threat through destruction of their habitats and anti-malarial and tsetse fly spraying programmes (Skelton 2012).

The lower Phongolo River and floodplain in South Africa is one of the only regions in South Africa where the spotted killifish, *Nothobranchius orthonotus* (Peters 1844), occurs (Skelton 2012; Smit et al. 2016). Numerous floodplain and temporary wetlands are found in this region, all of which play important roles

for the local society and economy (Kok 1980). The Phongolo River and Ndumo region located on the floodplain is also the southernmost distribution for many biota, making it an area of great ecological value (Kok 1980; Smit et al. 2016).

Two previous killifish dietary studies used stomach contents of specimens from *Nothobranchius* spp. in Mozambique (Polačik and Reichard 2010) and *Austrolebias* spp. in South America (Laufer et al. 2009), while only a single study has been conducted on the diet of *Nothobranchius* spp. using stable isotopes (Polačik et al. 2014). None of these studies determined whether the killifish diet changed over time, particularly not during a unique event such as an out of season drying event during severe drought. Furthermore, there is a notable lack of literature regarding killifish *Nothobranchius* spp. in natural systems, particularly *N. orthonotus*.

The main aim of this section of the study was to determine the aquatic invertebrate diversity of a natural temporary pool during disrupted wet/dry cycles as well as the diet of the spotted killifish, *N. orthonotus* from the same temporary pool using stable isotope analysis. Key interests included a) whether the aquatic invertebrate diversity would differ between the sampling surveys, (b) whether possible changes in aquatic invertebrate diversity could be related to changes in chemical water quality, and finally, (c) the relative contributions of various food sources to the diet of this killifish, and whether these change through an out of season drying event. Specific hypotheses for the chapter were: 1.) aquatic invertebrate diversity will differ from before to after a drying event due to changes in chemical water quality; 2.) aquatic invertebrates will contribute the greatest portion to the diet of *N. orthonotus* and 3.) the diet of *N. orthonotus* will change through a drying event due to a greater presence of nutrient-low food sources following the drying event.

5.2 Materials and Methods

5.2.1 Study Area

The study site is located within NGR (Figure 5.1) and receives water primarily through rainfall in the catchment to the south-west outside NGR in the communally-owned region of Ndumo (SA Wetlands Conservation Programme 1996). Rainfall usually occurs from November to March, after which the dry season lasts until October (Lankford et al. 2011; Dube et al. 2015). Although dry habitats are naturally erratic systems, the supra-seasonal drought experienced in South Africa has disrupted the natural wet/dry cycle of the site (see Chapter 2). To cover temporal dynamics in the aquatic invertebrate community assessment, the site was sampled over four surveys. In order to assess the killifish diet, the study was conducted over two seasons. This site was selected as the study site since it is the only site within the lower Phongolo area where these killifish have been found (for a full description of the sampling site see section 2.5.2, Chapter 2). In addition, not only are these fish rare, they have never been found in high abundance within the sampling site. For these reasons, a very low sample size was obtained and no other sampling sites exists to increase replication.

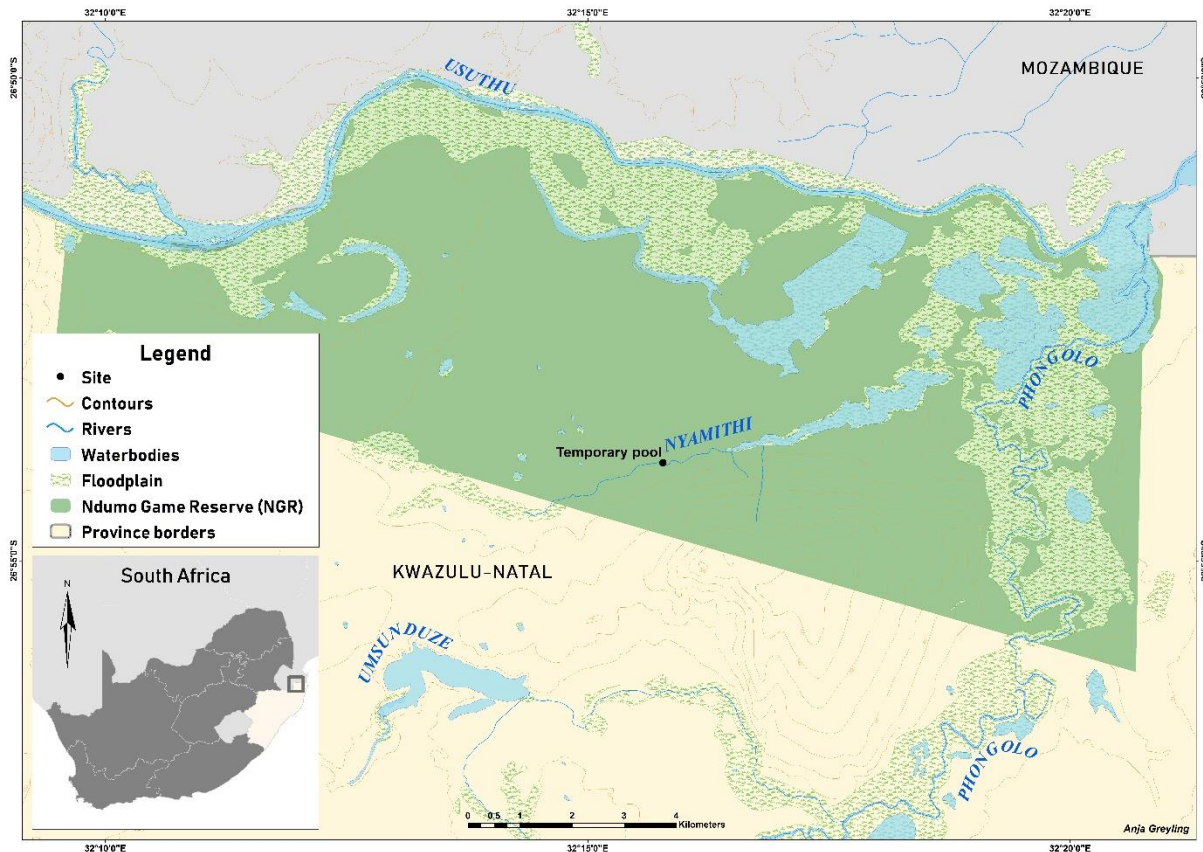


Figure 5.1: Map of the study area indicating the location of the selected temporary pool within Ndumo Game Reserve.

5.2.2 Field collection

Four field collection surveys took place (May 2016; August 2016; December 2016 and February 2017) within NGR. Aquatic invertebrates and water for chemical analyses were collected during all four surveys while stable isotope data was collected only during the first (May 2016) and final (February 2017) survey.

5.2.2.1 Environmental and biological data

Aquatic macroinvertebrates were collected across the entire site from all available biotopes, including submerged vegetation, emergent vegetation, open water and sediment, using a standard 30 cm x 30 cm D-frame dip net. Forty sweeps were taken with the net and the integrated sample then placed in a 500 mL polyethylene honey jar and fixed using 5% buffered neutral formalin (NBF). Zooplankton was collected by filtering 80 L of water collected from different habitats through a 64 µm mesh net and stored in 70% ethanol. Methods regarding *in situ* water parameter measurements and water sample collection can be found in section 3.2.1 (Chapter 3).

5.2.2.2 Stable isotope collection

Two field collection events took place, each over a two-week period. The first collection in May 2016 took place before, and the second collection in February 2017 after the drying of the study site. During

sampling periods all available floating, submerged and marginal aquatic plants were collected and placed in sealable plastic bags. Biofilm was collected by cutting 20 pieces of submerged plants (5 cm each) and placing them in sealable plastic bags with 50 mL of water. The bag was then rubbed vigorously in order to detach biofilm from the stems and leaves. Biofilm consists of a complex mixture of algae, diatoms and other microbes (Roberts et al. 2007; Xu et al. 2012; Marchese et al. 2014). Detritus and leaf litter were collected along the edges of and from the surface of the water and placed in sealable plastic bags. Aquatic invertebrate families were collected using the same method as described above for community analysis. Aquatic invertebrate samples were placed in 1 m x 1 m white plastic trays and four replicates (± 5 g) consisting of multiple individuals from aquatic invertebrate families dominant at the site were picked out and placed in separate 15 mL Falcon tubes. The dominant families collected were Notonectidae, Libellulidae, Coenagrionidae, Nepidae, Belostomatidae and Dytiscidae. Fish, namely *Nothobranchius orthonotus* and *Oreochromis mossambicus* (found only in February 2017), were collected with the use of standard baited 10mm mesh fyke traps submerged at various sections of the site (Hubert et al. 2012). Once captured, fish were identified using keys in Skelton (2012), humanely killed following the ethics approved method of stunning and severing the spinal cord (Ethics No: NWU-00264-16-A5), and total length was measured. Since individuals were small (± 60 mm), specimens were placed in 15 mL Falcon tubes after the head, fins, scales and organs had been removed. In May 2016 five *N. orthonotus* were caught, while in February 2017 four *N. orthonotus* and two *O. mossambicus* were collected. All collected samples (fish, invertebrates and plants) were placed in liquid nitrogen in the field and taken back to the laboratory. All field samples were collected and processed with the necessary permits (Permit No's: OP 899/2016 and OP 1075/2017) and ethical clearance.

5.2.3 Laboratory analysis

5.2.3.1 Environmental and biological analysis

In the laboratory, aquatic invertebrate samples were washed under running tap water to remove the NBF and placed in 70% ethanol. All aquatic macroinvertebrate and zooplankton samples were identified to lowest possible taxonomic level with the use of Nikon Model C-LEDS dissection microscope and with appropriate identification guides (see section 4.2.2, Chapter 4) and counted. Methods regarding water sample analysis can be found in section 3.2.2 (Chapter 3).

5.2.3.2 Stable isotope analysis

In the laboratory all collected samples were oven dried (50°C , 72h) and ground to a fine powder using a sterile pestle and mortar which was cleaned with 10% HCl between each sample preparation. Approximately 1 g of the finely-ground fish and invertebrate samples, respectively, were placed in 2 mL Eppendorf tubes, treated with a 2:1 chloroform: methanol (v:v) solution and placed in the dark at 4°C for 12h for lipid extraction. Once lipid extraction was complete the samples were centrifuged at 1500g for 2 min, the supernatant discarded and the pellet oven dried (50°C for 24h). Each dried sample was weighed (consumers: $1\text{ mg} \pm 0.2\text{ mg}$; primary producers: $5\text{ mg} \pm 0.2\text{ mg}$) (Sartorius MSA6.6S-000-DM

Cubis Micro Balance) and encapsulated in ultrapure tin boats (Elemental Microanalysis 4 x 4 x 11mm) prior to analysis.

Total CO₂ and N₂ content, as well as isotopic ratios (¹³C/¹²C and ¹⁵N/¹⁴N), were analysed for all collected samples through dry combustion using an Elementar elemental analyser coupled to an Isoprime 100 mass spectrometer at Hokkaido University, Japan. Results are presented as δ-values in parts per thousand (‰) relative to atmospheric nitrogen for δ¹⁵N and PeeDee Belemnite for δ¹³C respectively. Quality control was accomplished by analysing replicate measurements of laboratory standards (L-Alanine, SI Science Co., Ltd., Saitama, Japan) for every seven samples with measurement precision of ±0.02‰ for both C and N stable isotope ratios.

All available food sources that were collected were pooled into three biologically meaningful groups, namely: primary producers (all plant species, detritus, leaf litter and biofilm) (PP), small predatory invertebrates (Notonectidae, Libellulidae, Coenagrionidae and Nepidae) (SPI) and large predatory invertebrates (Belostomatidae and Dytiscidae) (LPI). Pooling of multiple sources is recommended when they are logically related, and when within-group isotopic variation is less than between-group variation, providing a more reliable model (Phillips et al. 2005; Phillips et al. 2014; Manfrin et al. 2018).

5.2.4 Statistical analysis

5.2.4.1 Environmental and biological data

All aquatic invertebrate statistical analyses were conducted on lowest taxon level. Diversity indices were conducted on untransformed data and used to determine species abundance and relationships within the aquatic invertebrate communities of each sampling survey. A description of the various diversity indices can be found in section 4.2.3 (Chapter 4). Parametric paired student t-tests were then used to determine if there were significant differences between the surveys before (May 2016 and August 2016) and after (December 2016 and February 2017) the drying event.

Prior to further statistical analysis, aquatic invertebrate data were transformed by way of square root ($\sqrt{}$) in order to reduce the effect of dominant taxa (Clarke and Warwick 2001). Using the *indicspecies* package for R (de Cáceres et al. 2016; R Core Team 2017), *multipatt* analysis was conducted on the aquatic invertebrate communities in order to determine if any particular taxon was significantly associated to a sampling survey. For a full description of the multipattern analysis see section 4.2.3 (Chapter 4). Using Canoco v5, an unconstrained principal component analysis (PCA) was created of the four sampling surveys in order to determine possible differences between surveys in terms of aquatic invertebrate data with water quality variables superimposed as supplementary explanatory variables. All environmental data, with the exception of pH, were log transformed [$y = \log(x+1)$] prior to PCA analysis in order to improve normality of data.

5.2.4.2 Stable isotopes

Differences between food sources and consumer isotopic signatures for δ¹³C and δ¹⁵N were determined using linear models (LM) in the *lm4e* package for R (Bates et al. 2007, R Core Team 2017). Two models

were tested namely a “food source model” and a “consumer model”. Explanatory variables for the food source model were “time” (before or after dry period) and “group” (small predatory invertebrates, large predatory invertebrates and plants) and their interactions. The explanatory variable for the consumer model was “time” while the response variables for all models were $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Analysis of variance (ANOVA) was used to determine statistical significance in food source groups and consumers before and after the drying event respectively. Tukey’s post hoc test was then used to analyse significant pairwise differences within the food groups ($p < 0.05$). To control for inflated false discovery rates, Bonferroni corrected α values were used (Stoline 1981; Armstrong 2014). Distribution of data was assessed for normality using QQ-plots (Wilk and Gnanadesikan 1968; Ghasemi and Zahediasl 2012; Manfrin et al. 2018).

Consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were corrected for trophic fractionation by 1‰ (DeNiro and Epstein 1978; Akamatsu et al. 2004) and 3.37‰ (Taylor et al. 2017a), respectively. Diet composition in consumers was assessed before and after the drying event using stable isotope mixing models from the SIAR package for R (Inger et al. 2010; Parnell and Jackson 2013). Using Bayesian analytical techniques and the isotopic ratios of the food sources and consumers, SIAR provides the most likely percentage contribution of each dietary source to the overall diet of the consumer (Parnell et al. 2010; Boecklen et al. 2011; Marchese et al. 2014; Manfrin et al. 2018). Two models were tested using SIAR, namely the consumer diet before the drying event and the consumer diet following the drying event. These models were single group models, each consisting of a Markov Chains Monte Carlo (MCMC) method for normally-distributed data with a dirichlet mixture mean. The model ran 1000 000 (one million) iterations that thinned by 300 and discarded the first 40 000 iterations, finally producing the most probable dietary proportions with mean, mode and levels of uncertainty, with 95% credibility intervals.

Finally, a graphical representation of the food webs was created in Graphpad Prism 6 for each of the two sampling surveys (May 2016 and February 2017) using the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of all collected sources.

5.3 Results

5.3.1 Aquatic invertebrate community

A total of 65 taxa of aquatic invertebrates were identified of which only three taxa were found throughout all four surveys namely *Anisops* sp. A, Lumbriculidae and *Moina micrura* (see Appendix C1). There was a general increasing trend in the diversity indices from the first to last survey. Diversity was lower before the drying event (May 2016 and August 2016) and greater after the drying event (December 2016 and February 2017) although not significant. May was dominated by *M. micrura* (1720 individuals) and *Thermodiaptomus mixtus* (960 individuals) which led to the lowest evenness (0.37), Shannon diversity (1.14) and Simpson’s diversity (0.60). August had the least number of taxa (16), number of individuals (1689) and species richness (2.02) while December had the greatest diversity overall with the highest number of taxa (35); species richness (4.49), evenness (0.57), Shannon diversity (2.03) and Simpson’s

diversity (0.79) (Figure 5.2). Several taxa were only found after the site had been inundated again including the damselflies *Pseudagrion* sp. and *Enallagma* sp. (Coenagrionidae), predatory diving beetles *Hyphydrus* sp. and *Laccophilus* sp. (Dytiscidae) and small minnow flies (Baetidae) (Appendix C1)

The multipattern analysis indicated that no aquatic invertebrate taxa or families were significantly associated with any particular sampling events. As such, only 30 of the best fitting taxa are shown on the PCA (Figure 5.3). Separation on both axes is associated with differences in the aquatic invertebrate abundances as well as chemical water quality variables and no true temporal differences were present between surveys from before to after the drying event. The December 2016 survey (RP3) separates from all other surveys on the first axis (explaining 45.56% of data variation) and is attributed to a much greater diversity of aquatic invertebrates collected during this survey. Water temperature and several organic nutrients (NO_3^- , NH_3 and PO_4^{3-}) were positively correlated to the December 2016 survey and also highest during this survey (Table 5.1). The August 2016 survey (RP2) separated completely from the other three surveys on the second axis (explaining 27.57% of data variation) largely due to a much lower number of taxa, individuals and taxon richness and a positive correlation with salinity (measured by conductivity, total hardness, Mg^+ and Ca^{2+}) which was highest during this survey (Table 5.1).

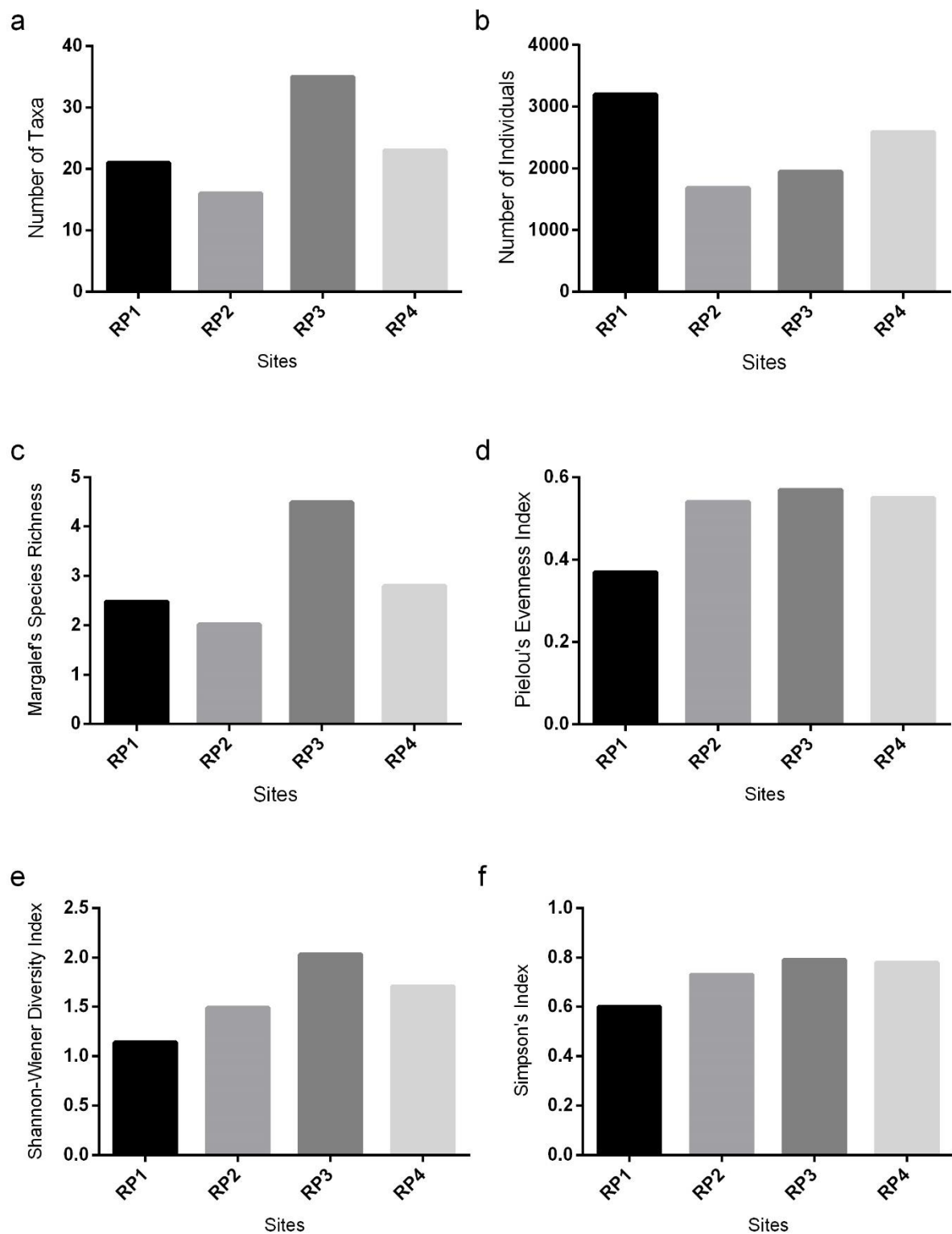


Figure 5.2: Number of taxa (a), Number of individuals (b), Margalef's species richness (c), Pielou's evenness index (d), Shannon-Wiener diversity (e) and Simpson's index (f) of all aquatic invertebrate taxa collected from the temporary pool (RP) inside Ndumo Game Reserve during four surveys between 2016 and 2017. RP1 – May 2016; RP2 – August 2016; RP3 – December 2016; RP4 – February 2017.

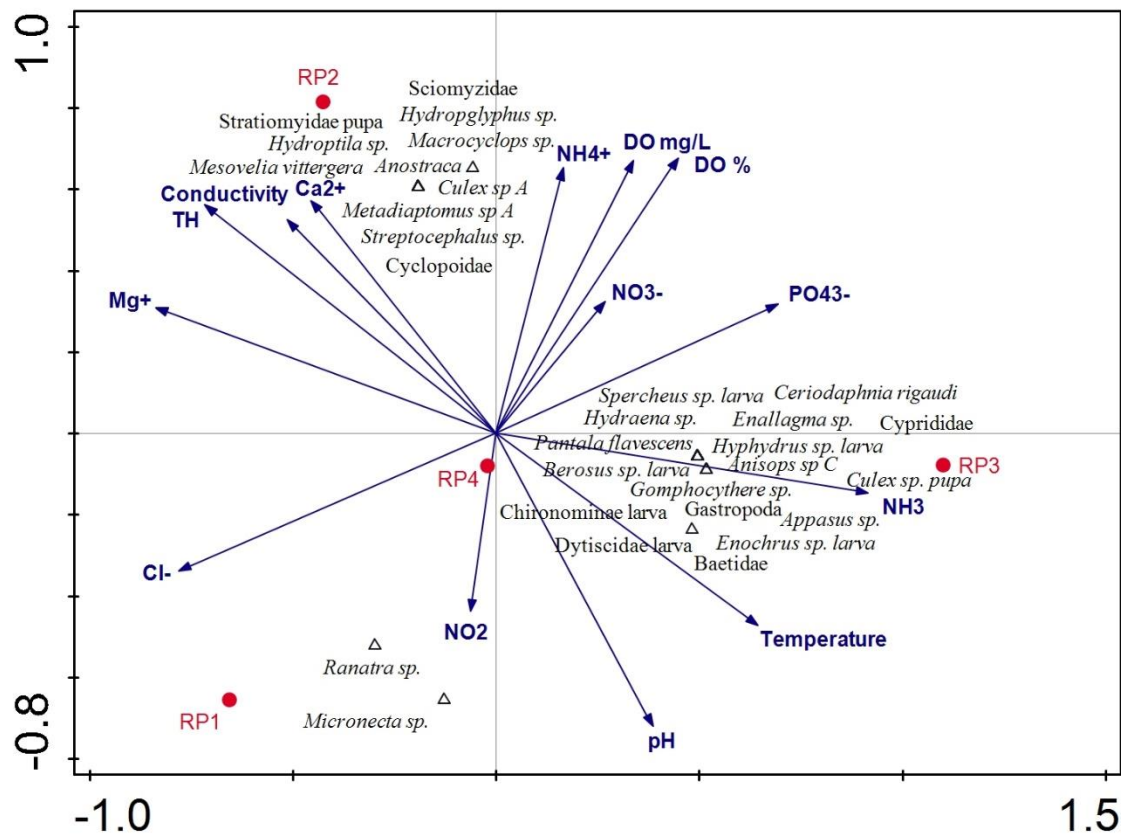


Figure 5.3: Principle Component Analysis (PCA) biplot of the 30 best fitting aquatic invertebrate taxa (triangles) collected from the temporary pool (RP) inside Ndumo Game Reserve during four surveys between 2016 and 2017 (circles) with water quality variables (arrows) plotted as supplementary variables. The biplot explains 73.13% of data variation of which 45.56% is explained by the first axis and 27.57% is explained by the second axis. RP1 – May 2016; RP2 – August 2016; RP3 – December 2016; RP4 – February 2017.

Table 5.1: *In situ* and water quality variables collected from the temporary pool (RP) inside Ndumo Game Reserve during all four surveys between 2016 and 2017. RP1 – May 2016; RP2 – August 2016; RP3 – December 2016; RP4 – February 2017.

Site	RP1	RP2	RP3	RP4
Temperature	21.6	15.3	28.4	29
Dissolved Oxygen (mg.L ⁻¹)	3.96	5.08	4.74	4.92
Oxygen saturation (mg.L ⁻¹)	45.1	64.3	60.2	59.3
Conductivity (µS.cm ⁻¹)	273.2	575	198.1	160.6
pH	7.81	5.65	7.82	7.6
NO ₂ ⁻ (mg.L ⁻¹)	0.03	0.01	0.02	0
NO ₃ ⁻ (mg.L ⁻¹)	0.5	1	1	0
NH ₄ ⁺ (mg.L ⁻¹)	0.27	3.9	1.72	0.08
NH ₃ (mg.L ⁻¹)	0.01	0	0.08	0
PO ₄ ³⁻ (mg.L ⁻¹)	0.10	0.3	0.48	0.04
Total Hardness (mg.L ⁻¹)	33	87	18	33
Mg ⁺ (mg.L ⁻¹)	13	27	3	22
Ca ²⁺ (mg.L ⁻¹)	20	60	15	11
Cl ⁻ (mg.L ⁻¹)	29	10	5	5.7

5.3.2 Food web and dietary analysis

Linear models found significant differences among source groups for both $\delta^{13}\text{C}$ ($p < 0.0001$, $F_{2,71}=15.50$) and $\delta^{15}\text{N}$ ($p < 0.0001$; $F_{2,71}=15.03$) (Figure 5.4; Table 5.2). Timing (before or after the drying event) was also found to have a significant effect on food sources for both $\delta^{13}\text{C}$ ($p < 0.001$; $F_{1,72}=10.68$) and $\delta^{15}\text{N}$ ($p = 0.003$; $F_{1,72}=9.43$). The interaction between groups and timing for both isotopes had no significant effects ($p > 0.05$) (Table 5.2). Analyses also indicated significant differences between PP and SPI groups for both $\delta^{13}\text{C}$ ($p < 0.0001$; $t = 4.14$) and $\delta^{15}\text{N}$ ($p < 0.0001$, $t = -5.37$) while significant differences were only found for $\delta^{13}\text{C}$ between LPI and SPI as well as LPI and PP ($p < 0.0001$; $t = 5.09$ and $p < 0.05$; $t = 2.65$ respectively) (Figure 5.4; Table 5.3). Linear models also revealed that timing had a significant effect on the consumer $\delta^{15}\text{N}$ ($p < 0.01$; $F_{1,7}=14.29$) but none on consumer $\delta^{13}\text{C}$ (Figure 5.5; Table 5.2).

All sources were more $\delta^{13}\text{C}$ depleted after the drying event compared to before, while all but Libellulidae and Notonectidae (SPI group) were $\delta^{15}\text{N}$ enriched after the drying event (Figure 5.6; Appendix C2). Large predators (Dytiscidae and Belostomatidae) became $\delta^{15}\text{N}$ enriched after the drying event, while Belostomatidae had the largest decrease in $\delta^{13}\text{C}$ of all sources after the drying event (Appendix C2). Primary producers were all $\delta^{13}\text{C}$ depleted, with the exception of Poaceae after the drying event, throughout the study with a mean $\delta^{13}\text{C}$ of -26.08‰ before the drying event and -27.25‰ after the drying event. Detritus, leaf litter and biofilm (PP group) were more $\delta^{15}\text{N}$ enriched after the drying event than before, while $\delta^{13}\text{C}$ decreased only marginally (Figure 5.6; Appendix C2). *Nothobranchius orthonotus* $\delta^{13}\text{C}$ changed only marginally after the drying event, while the $\delta^{15}\text{N}$ signatures increased to place these consumers at the top of the food web (Figure 5.6). Two specimens of *O. mossambicus* were found along with *N. orthonotus* after the drying event with isotopic signatures lower than that of the killifish (Figure 5.6).

SIAR analysis indicated that, before the drying event, plant food sources contributed the most toward the diet of *N. orthonotus*, ranging from 17% to 65% while small predators contributed 16% to 63% and large predators 0 to 41% (Figure 5.7; Appendix C3). After the drying event, there was a decrease in the mean amount of small predator contributions (from 41% to 30%), with a large sum of the contributions shifting to plants (from 42% to 49%) and large predators (from 18% to 21%) (Figure 5.7; Appendix C3). The total amount of aquatic invertebrates ingested (SPI and LPI together) contributed 58% and 51% towards the diet of *N. orthonotus* before and after the drying event, respectively. SIAR analysis also indicated that, of all plant material, the major contribution before the drying event was leaf litter (5% to 58%) and biofilm after the drying event (14% to 72%) (Figure 5.8).

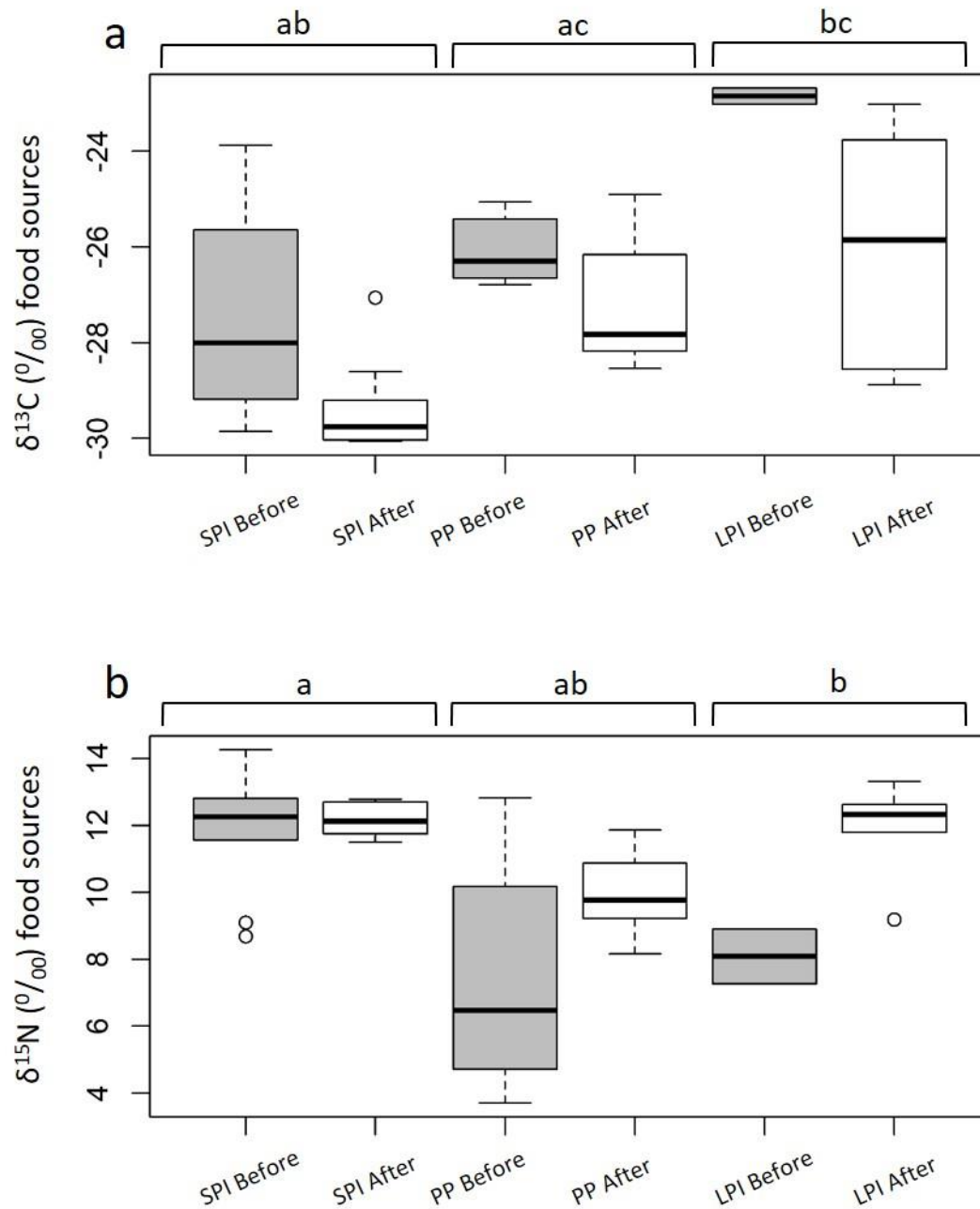


Figure 5.4: $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values for food sources sampled from the temporary pool (RP) inside Ndumo Game Reserve before and after the drying event. Box plots indicate the 25th, 50th and 75th percentiles, and whiskers the highest and lowest values, excluding the outliers. SPI – Small predatory invertebrates, LPI – Large predatory invertebrates PP – Primary producers. Similar lowercase letters indicate significant differences.

Table 5.2: Analysis of Variance (ANOVA) summary of linear model results for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of three food source groups and consumers (*Nothobranchius orthonotus*) based on the sampling groups (Groups), timing before or after the drying event (Timing) and the interaction of these factors of the temporary pool inside Ndumo Game Reserve.

Model	Factors	ANOVA
Sources $\delta^{13}\text{C}$	Groups	$p < 0.0001$ $F_{2,71} = 15.50$
	Time	$p = 0.002$ $F_{1,72} = 10.68$
	Groups x Time	$p = 0.22$ $F_{2,68} = 1.531$
Source $\delta^{15}\text{N}$	Groups	$p = 3.61 \times 10^{-6}$ $F_{2,71} = 15.029$
	Time	$p = 0.003$ $F_{1,72} = 9.434$
	Groups x Time	$p = 0.05$ $F_{2,68} = 3.23$
Consumers $\delta^{13}\text{C}$	Time	$p = 0.32$ $F_{1,7} = 1.16$
Consumers $\delta^{15}\text{N}$	Time	$p = 0.007$ $F_{1,7} = 14.29$

Table 5.3: Tukey's post hoc analysis of significance between the three food source groups of the temporary pool inside Ndumo Game Reserve. SPI – Small predatory invertebrates. LPI – Large predatory invertebrates, PP= Primary producers.

Isotope	Groups	t-value	p value
$\delta^{13}\text{C}$	PP vs SPI	4.14	0.0003
	LPI vs SPI	5.09	8.42×10^{-6}
	LPI vs PP	2.65	0.03
$\delta^{15}\text{N}$	PP vs SPI	-5.37	2.81×10^{-6}
	LPI vs SPI	-1.21	0.70
	LPI vs PP	2.32	0.07

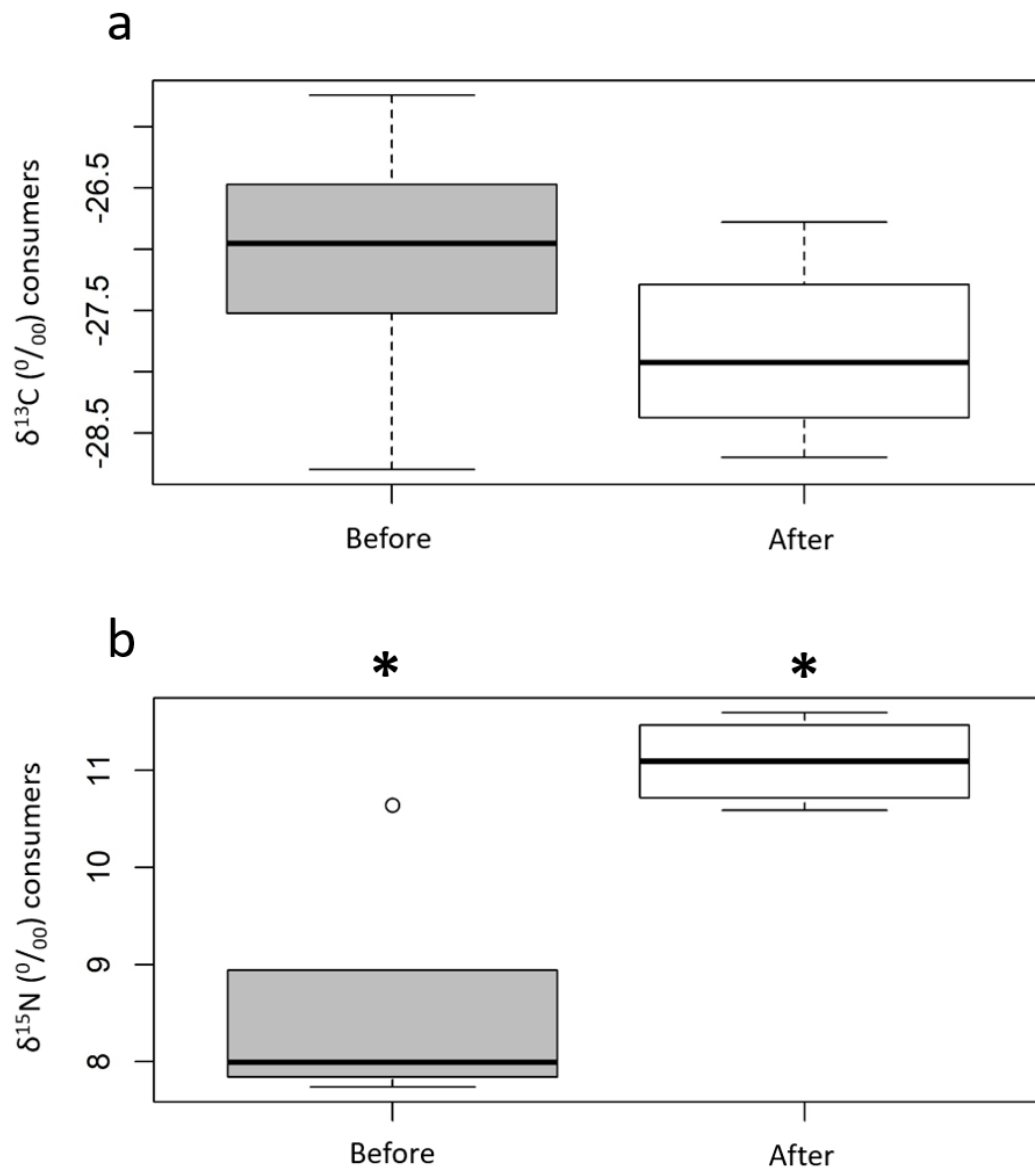


Figure 5.5: $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values for the consumer *Nothobranchius orthonotus* compared before and after the drying event of the temporary pool inside Ndumo Game Reserve. Box plots indicate the 25th, 50th and 75th percentiles, and whiskers the highest and lowest values, excluding the outliers. *Denotes significant differences ($p < 0.01$).

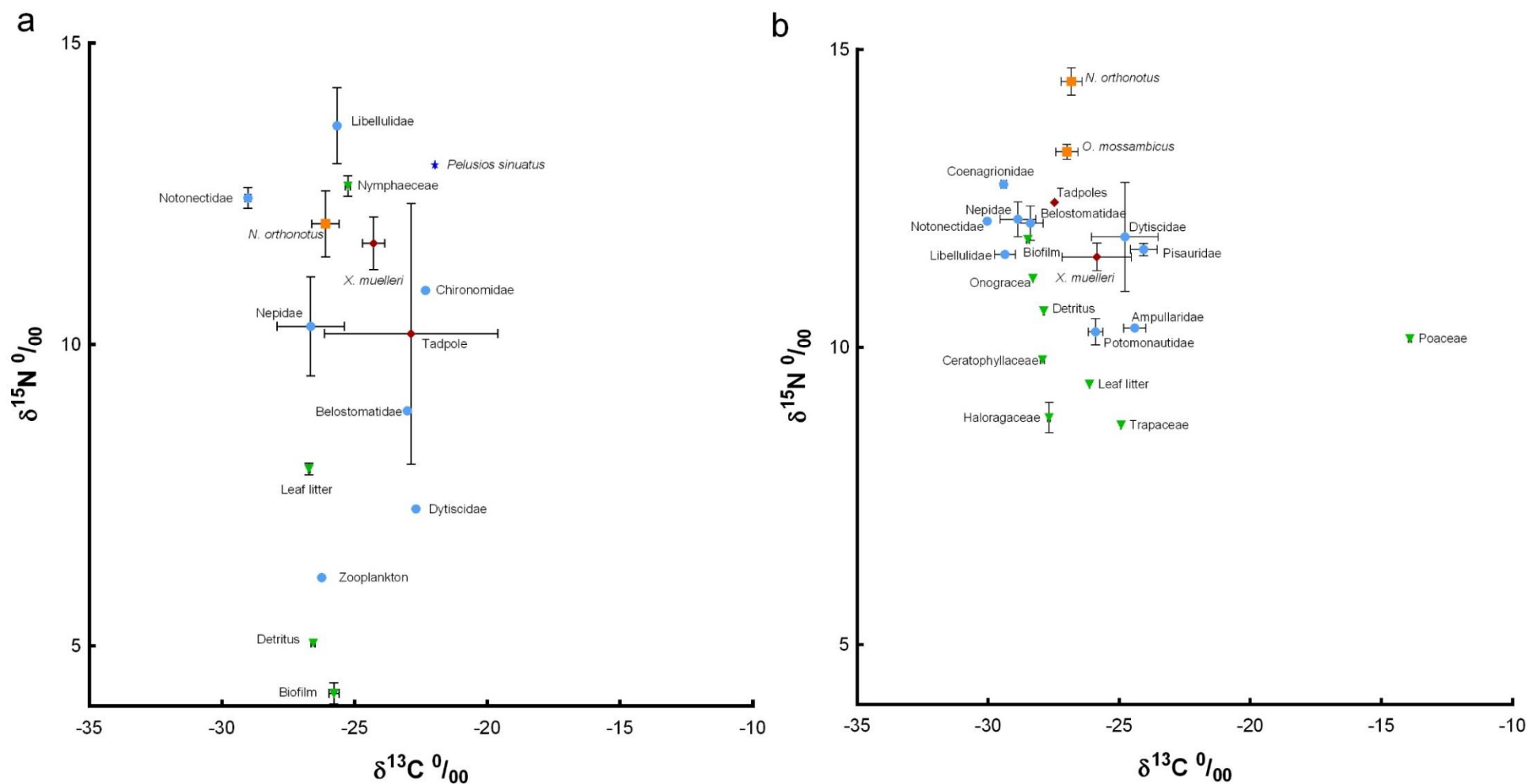


Figure 5.6: Biplot indicating mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the different trophic components sampled from the temporary pool inside Ndumo Game Reserve in May 2016 (before drying event) (a) and February 2017 (after drying event) (b).

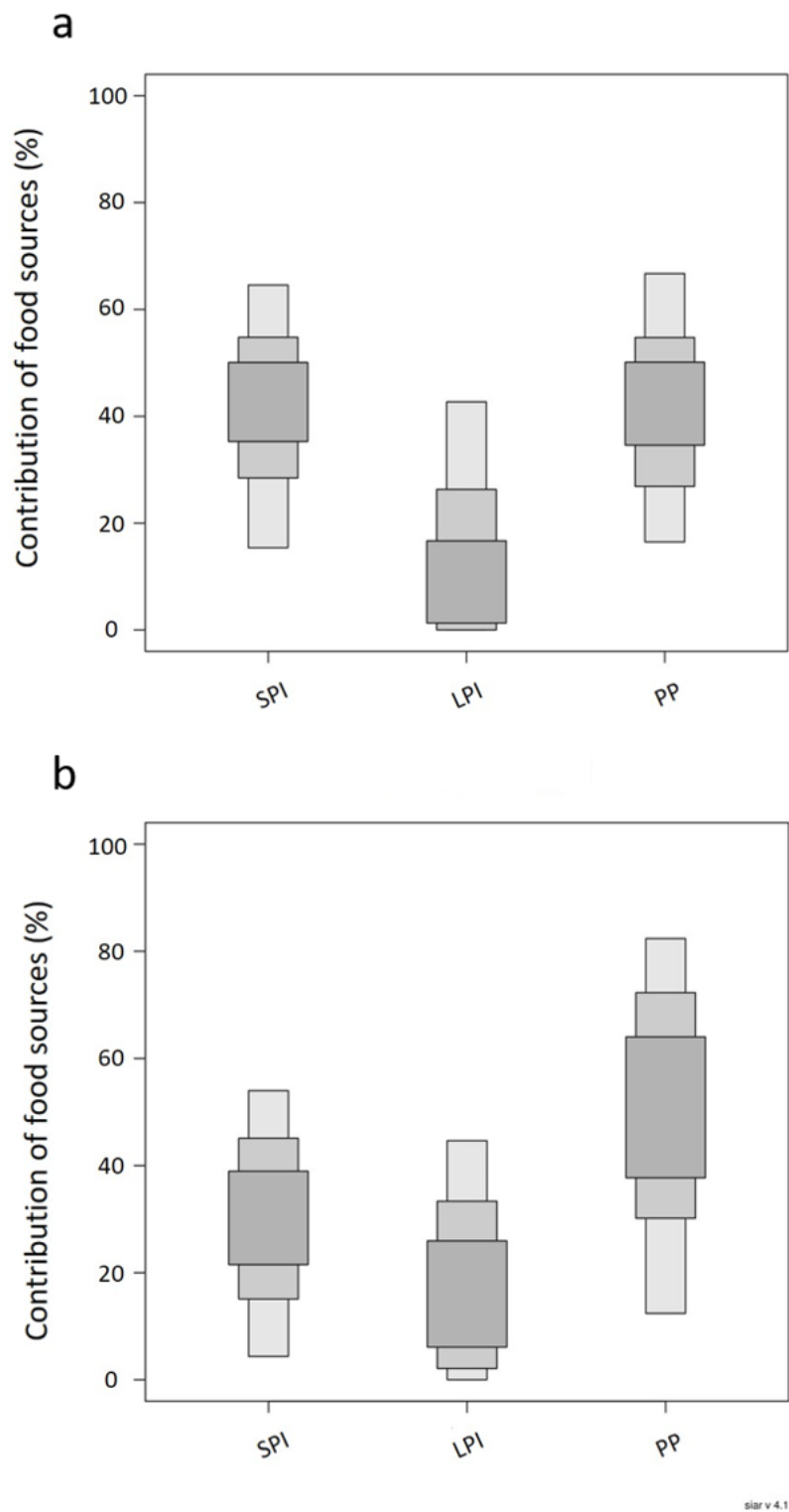


Figure 5.7: Relative contribution (%) of food sources to the diets of the consumer, *Nothobranchius orthonotus*, based on Bayesian isotope mixing models (SIAR) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ before (a) and after (b) a drying event the temporary pool inside Ndumo Game Reserve. Plots indicate 95% (middle rectangle), 75% and 25% (external rectangles) credibility intervals. SPI – Small predatory invertebrates, LPI – Large predatory invertebrates, PP – Primary producers

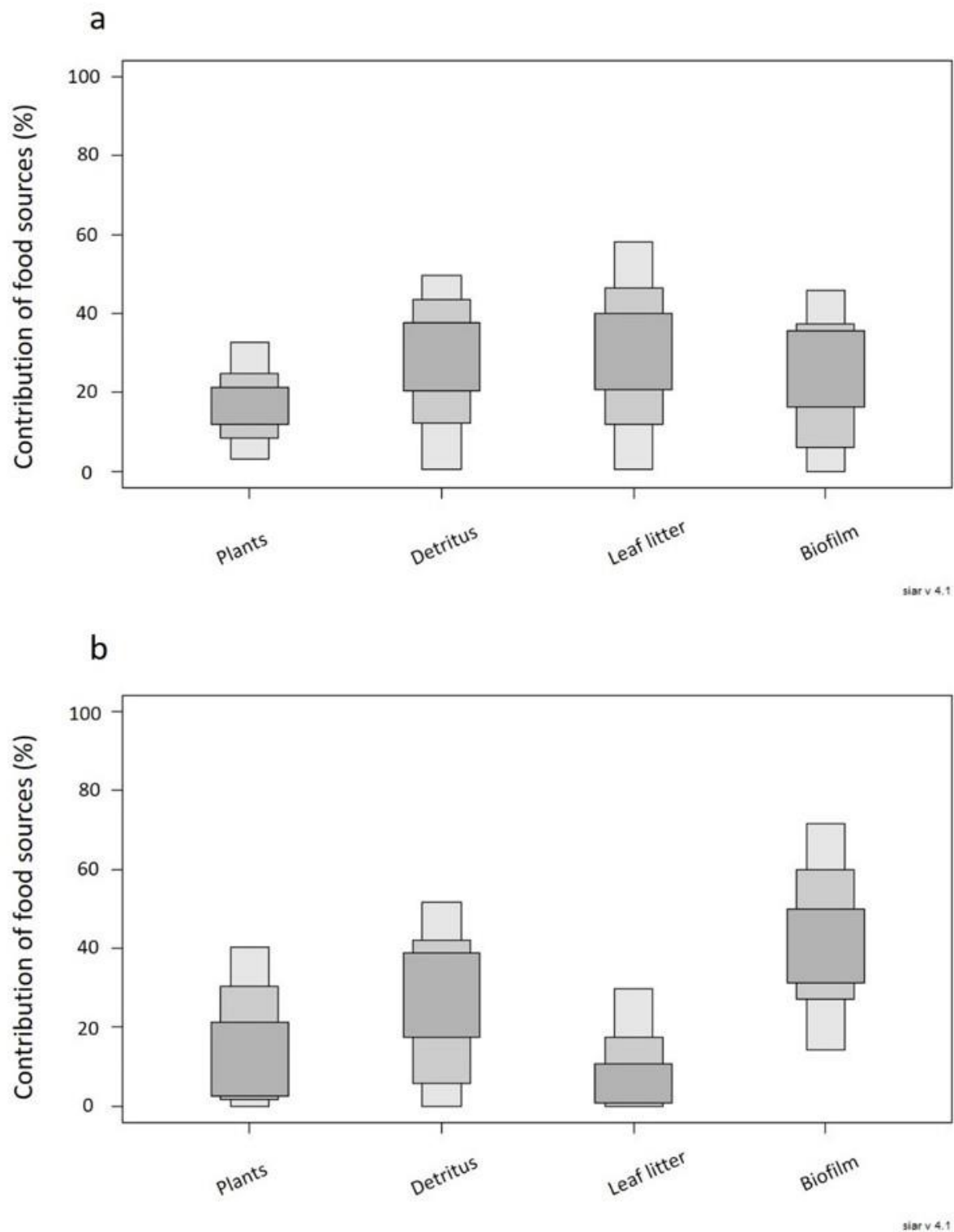


Figure 5.8: Relative contribution (%) of primary producers to the diet of the consumer, *Nothobranchius orthonotus*, based on Bayesian isotope mixing models (SIAR) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ before (a) and after (b) a drying event of the temporary pool inside Ndumo Game Reserve. Plots indicate 95% (middle rectangle), 75% and 25% (external rectangles) credibility intervals.

5.4 Discussion

The lowest aquatic invertebrate diversity, organic nutrients and highest salinity were found during the first two surveys (May 2016 and August 2016) before the pool dried up. After the pool had received rain water again in November 2016, diversity was higher, and the December 2016 survey proved to have the greatest aquatic invertebrate diversity, organic nutrients and lowest salinity. Although no significant shifts in killifish diet before or after the drying event were found, stable isotope analysis and SIAR proved to be useful tools in determining the quality and composition of their diet. The analyses revealed that, although the majority of the diet consisted of aquatic invertebrates, more than half was also composed of low-quality plant material, which had never been recorded before. It was also found that the drying event significantly affected both the food source and consumer isotopic ratios, causing a general enrichment of $\delta^{15}\text{N}$ and a decrease in $\delta^{13}\text{C}$ after the site had dried out.

Reduced precipitation and increased evaporation was the likely cause of increased salinity and reduced taxon diversity in the first two surveys, particularly August 2016. Villastrigo and García-Criado (2017) determined that pond drying in northwestern Spain reduces taxon richness and as a pool dries up, there is concentration of salts leading to increased salinity. This is largely due to the fact that salinity negatively affects aquatic invertebrate species diversity (Timms and Sanders 2002; Brock et al. 2005; Pinder et al. 2005; Waterkeyn et al. 2010). This is particularly true of those taxa that are not permanent residents of temporary pools but rather opportunistic colonisers. Many opportunistic colonisers such as individuals from the families Baetidae, Coenagrionidae, Dytiscidae and Hydrophilidae do not have the necessary adaptations to tolerate changes in water quality and rather actively move away from a site once it becomes intolerable (Vanschoenwinkel et al. 2009). Decreased salinity during December 2016 and February 2017 after the site had received water through rainfall was also the likely reason for higher aquatic invertebrate diversity during this time. Previous studies have also found higher macroinvertebrate diversity and abundance during wetter rainy seasons than during dry seasons (Rocha et al. 2012; Masese et al. 2014) and a decrease in aquatic invertebrate diversity as the dry season ensues. Boix et al. (2004) determined conductivity to be lowest in a Mediterranean pool filled only with rainwater and van de Meutter et al. (2006) also found conductivity to decrease and macroinvertebrate diversity to increase when lakes in the north-eastern parts of Belgium were refilled with low-conductivity rainwater.

The introduction of nitrogen-rich sources through rainfall outside NGR was the most likely cause of the enriched $\delta^{15}\text{N}$ in all consumers and sources after the drying event (February 2017). Although organic nutrients were not exceptionally high during February 2017, it was highest during December 2016 and these nutrients most likely rapidly settled into sediments and were then assimilated by primary producers. Greenway (2010) also found nitrogenous compounds (NO_2^- , NO_3^- , NH_3 , NH_4^+ and PO_4^{3-}) to decrease rapidly in Australian ponds due to particulate settlement. Plants also obtain their nutrients from sediments and then act as the source of nutrients to other fauna (Thiébaud 2008). Currently, both subsistence (crops and livestock) and commercial farming (sugarcane) occurs outside the Reserve (Smit et al. 2016) where farmers are using fertilizers in order to maximize crop yields (Sereda and

Meinhardt 2003; 2005; Smit et al. 2016). A number of rural villages are also located outside NGR (Smit et al. 2016). These farms and settlements lie within the sampling site's catchment area and may contribute to nitrogen loads in the system through fertilizer runoff, raw sewage and animal waste. Many studies have related increased $\delta^{15}\text{N}$ in both fresh and marine aquatic systems to anthropogenic inputs such as fertilizers, animal agriculture and increased human populations into watersheds and wastewater (Cabana and Rasmussen 1996; McClelland et al. 1997; Lake et al. 2001; Jones and Unsworth 2016; Jones et al. 2018).

All primary producers, excluding Poaceae, consistently had $\delta^{13}\text{C}$ values of plants with C_3 photosynthetic pathways (O'Leary 1988; Deegan and Garritt 1997; Marchese et al. 2014). These plant types naturally have low $\delta^{13}\text{C}$ signatures (approximately -28‰) (Peterson and Fry 1987). This, along with the addition of terrestrial and riparian C_3 plant sources (detritus and leaf litter from surrounding trees), caused a cascading effect leading to $\delta^{13}\text{C}$ -depleted higher trophic organisms such as killifish and aquatic invertebrates. Decreases in both carbon and nitrogen isotopic signatures of Notonectidae and Libellulidae after the drying event suggest that these organisms may have received their food sources from different locations. It is possible that the invertebrate prey organisms simply colonized from different water sources with lower $\delta^{15}\text{N}$ than the study site and had not yet integrated the new food sources $\delta^{15}\text{N}$ into their systems. Many aquatic invertebrate taxa are opportunistic or unable to survive drought and so will simply leave a water body when the habitat becomes unsuitable and only return once the environment is suitable again or will simply establish wherever water is available (Barbour et al. 1999; Trzcinski et al. 2016). Libellulid larvae, although unable to fly (de Moor et al. 2003a) were still rather small, suggesting that they had hatched recently and probably still carried the signatures of their parents, which may have been feeding at a different aquatic habitat (i.e. river or wetland) with lower $\delta^{15}\text{N}$ food sources.

The killifish's diet consisted mainly of large and small aquatic invertebrates before and after the drying event. This is in line with what has been found in previous studies in which *N. orthonotus* usually preyed on insects and other aquatic invertebrates (Polačik and Reichard 2010; Skelton 2012; Polačik et al. 2014). These studies, however, relied largely on stomach content analysis (Polačik and Reichard 2010), which does not reveal long-term patterns in the fish diet, or collected only terrestrial and aquatic invertebrates and zooplankton for stable isotope analysis (Polačik et al. 2014), leaving out other possible food sources such as plants. Primary producers formed more than half of the killifish diet, and, although a portion of it may have been inadvertently ingested while foraging for live food (Weis et al. 2001), it is unlikely that it was all accidental ingestion. Studies on other genera of annual killifish in South America such as *Austrolebias viarius*, *A. cheradophilus*, *A. luteoflammulatus*; *A. vanderbergi* and *Cynopoecilus melanotaenia* also confirmed that plant material forms part of these fishes' diets (Laufer et al. 2009; Montaña et al. 2012). In some cases, detritus, algae and diatoms (biofilm) formed the bulk of their diet (Laufer et al. 2009).

It appears that *N. orthonotus* prefers feeding on aquatic invertebrates. However, due to their limited time for growth, reproduction, short lifespan and the variation in food sources between seasons, diet

specialization is unlikely (Polačik and Reichard 2010) and these fish are able to integrate any other food source available, including low-nutrient detrital sources. *Nothobranchius orthonotus* is a top predator in temporary pools. Higher trophic level predators generally have less dietary specialisation than lower trophic level consumers. Such predators respond to seasonal variation by switching their diets to alternate (usually littoral) resources, particularly when pelagic food sources are less abundant (McMeans et al. 2016; Dalu et al. 2017; Stewart et al. 2017). Detritus and other low-quality food sources are important diet substitutes for consumers when preferred resources are absent (McMeans et al. 2015). However, long-term feeding on low-nutrient food sources can indicate a decreased quantity and/or quality of preferential food sources, which may be due to stress from anthropogenic pressures (McMeans et al. 2015). Climate change can affect the timing of the availability of important food sources to consumers, in turn depriving consumers of high-quality food sources if their growth and reproduction cycles are delayed (McMeans et al. 2015).

5.5 Conclusion

Taxon diversity decreased during the first two surveys when there was reduced precipitation and drying of the temporary site. There was also an increase in aquatic invertebrate diversity and water quality from before to after the drying event. This was attributed to reduced salinity from rainwater that filled the pool at the end of November 2016. The first hypothesis that aquatic invertebrate diversity will differ from before to after a drying event due to changes in chemical water quality is accepted. Stable isotopes and Bayesian mixing models successfully produced an extended indication of what the spotted killifish *N. orthonotus* feeds on, and how this may change through a drying event. It was found that these fish are generally predacious, feeding largely on aquatic invertebrates, and thus the second hypothesis that aquatic invertebrates will contribute the greatest portion to the diet of *N. orthonotus* is accepted. However, the analysis also indicated that these killifish feed on a greater variety of food sources than previous studies had revealed, particularly of plant material (up to 65%), which had not been recorded before. In addition, when exposed to a drying event, these fish are able to shift their diet to include a greater percentage of low-nutrient food sources. Significant $\delta^{15}\text{N}$ enrichment was found in all sources and consumers after the drying event, which may reflect anthropogenic inputs from outside NGR through fertilizer runoff from farming, livestock as well as local settlements. Shifts in diet of the killifish towards more plant-based food sources with low $\delta^{13}\text{C}$ may be indicative of the effects of the drought and how continued changes in rainfall patterns through climate change may affect the natural wet/dry cycle of temporary habitats. Global climate change could extend high rainfall, warm temperatures and drought periods, and increase the occurrence of eutrophication, all of which facilitates low-quality food source production. Killifish such as *Nothobranchius* are limited to temporary habitats that have natural wet/dry phases, are adapted to survive extended periods of drought and may not be directly affected by drought and increased temperatures through climate change. However, predicted future temperature and rainfall changes may indirectly affect killifish through later responses in reproduction and growth, possibly preventing them from taking advantage of abundant and nutritious resources when these are available.

CHAPTER 6:

Ecological assessment of a large naturally saline lake through a prolonged drying event

6.1 Introduction

During short-term and predictable droughts aquatic communities generally have predictable shifts in their community composition and rapid recovery (Lake 2003; Bogan and Lytle 2011). However, longer droughts, particularly supra-seasonal droughts that occur over several seasons, have been found to cause extinction of sensitive taxa and slow recovery of select species (e.g. fish and frogs) (Chessman 2015) which has longer term impacts on the ecosystem (Bogan and Lytle 2011). In conjunction with water abstraction, supra-seasonal drought may lead to the depletion of groundwater and lead to a shift of aquatic habitats from perennial to intermittent ecosystems (Bogan and Lytle 2011). To prevent such occurrences, it is important that ecosystems are monitored over long-term periods so as to determine the resistance, resilience and recovery in the face of severe drying events and consequently provide proper management tools (Bogan and Lytle 2011).

Naturally saline aquatic environments are widespread although they tend to be more abundant in arid to semi-arid regions (Hammer 1986; Moore 2016; Karagianni et al. 2018). These ecosystems have several important functions including the provision of essential habitats for migrating shore- and waterbirds (Moore 2016) and acting as biodiversity refugia (O'Connell et al. 2006; Karagianni et al. 2018). Salinity tends to naturally fluctuate in these habitats, usually increasing during the dry season and decreasing in the wet season. This creates a unique environment with an aquatic community composition distinct from other habitats (Williams 2002; Karagianni et al. 2018). Halotolerant (saline tolerant) freshwater biota are usually the most abundant biota but as salinity increases these are replaced with biota only found in saline habitats (Hammer 1986; Karagianni et al. 2018). One of the greatest threats to saline aquatic habitats is hypersalinization (when salinity increases above 50 g.L⁻¹, Hammer 1986) that occurs either due to drought-induced salinity (Ginatullina et al. 2017) through climate change (e.g. supra-seasonal droughts), or secondary salinization (Timms 2005) through anthropogenic activities (Williams 2002) (e.g. water abstraction). This leads to modifications in the hydrology and salinity of these habitats and a resultant loss in biodiversity (Waterkeyn et al. 2008; Waterkeyn et al. 2010; Moore 2016; Wurtsbaugh et al. 2017; Afonina and Tashlykova 2018). Although saline lakes are ecologically important environments in nature, they are not well monitored or protected as it is difficult to quantify their economic importance (Moore 2016).

Resilience in freshwater aquatic biota is driven by their ability to tolerate, acclimatise or avoid environmental changes (James et al. 2003; Ginatullina et al. 2017). Aquatic biota that occur in saline environments or ecosystems that experience frequent disturbances (e.g. droughts or floods), have

evolved specific adaptations to survive in these habitats. Many biota will find refuge in more favourable surrounding habitats when their habitat becomes uninhabitable. Those that are unable to actively disperse must employ other mechanisms such as dormant desiccation resistant life stages and osmoregulation (Brendonck and de Meester 2003; Brock et al. 2003; Nielson et al. 2003). However, even with these strategies, changes in the environment are still unfavourable to a large part of the aquatic community as it consists of biota with varying degrees of tolerance (James et al. 2003). Direct loss of biota and changes in the community structure, food web and consumer diets are among the effects that result from environmental disturbances (e.g. Nielson et al. 2003; Woodward 2009).

The rate at which a community can return to equilibrium after a disturbance is a measure of its resilience (Pimm 1984; Brock et al. 2003) and the ability of a community to resist change and recover after a disturbance indicates ecological stability (MacGillivray and Grime 1995; Brock et al. 2003). In order to assess ecological stability, it is important to not only determine biodiversity and community structure but also the species interaction and nutrient flow in an ecosystem (McMeans et al. 2016). Assessing the food web of an ecosystem not only provides useful information on ecosystem function but is also a valuable means to measuring ecosystem resilience and recovery to climate induced changes (Bird et al. 2016). As discussed in Chapter 5, stable isotope analyses, particularly stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) ratios, and Bayesian mixing models are useful tools that have become increasingly used to study food webs, consumer diets, energy flow and the effects of various disturbances on ecosystem structure, function and resilience (e.g. Wantzen et al. 2002; Woodward et al. 2012; Stewart et al. 2017; Lemmens et al. 2017). It is also possible to monitor whether any changes occur over time and relate this to disturbances in the environment (Phillips 2012).

As introduced in detail in section 2.5.3 (Chapter 2), Nyamithi lake is located within Ndumo Game Reserve (NGR) on the lower Phongolo River floodplain in northern KwaZulu-Natal, South Africa (Figure 6.1). This is the largest inland water body within the game reserve (183.4 ha; Heeg and Breen 1982) and a natural saline lake (approximately $5000 \mu\text{S}\cdot\text{cm}^{-1}$) (Heeg and Breen 1982; Seaman et al. 1991). The whole lower Phongolo River floodplain lies on a marine cretaceous bed which leads to naturally saline groundwater that seeps into Nyamithi lake (Seaman et al. 1991; SA Wetlands Conservation Programme 1996; Welicky et al. 2017). Nyamithi receives water largely from the Phongolo River through an annual controlled flood release in October from the Pongolapoort Dam located approximately 80 km upstream of the game reserve (SA Wetlands Conservation Programme 1996). Additionally, the site receives water through local rainfall during the wet season (November to March) as it has its own catchment, as well as water from natural floods from the Usuthu River. During the drought that South Africa experienced from 2015 to 2017, no large floods were released from the Pongolapoort Dam and the region received below average rainfall (available from <https://www.dwaf.gov.za/Hydrology/Provincial%20Rain/Default.aspx>. Accessed 11 September 2018). Nyamithi lake started drying up (pers. observation) and salinity increased greatly ($>10\,000 \mu\text{S}\cdot\text{cm}^{-1}$). The main aim of this section of the study was therefore to determine whether the supra-seasonal drought and the accompanying increased salinity had any effect on the aquatic invertebrate community and food web structure of Nyamithi lake in comparison to pre-drought conditions. In order to achieve

this aim, the following hypotheses were tested: 1.) The aquatic invertebrate abundance and diversity of Nyamithi lake will be significantly lower during the supra-seasonal drought in comparison to pre-drought conditions; 2) there will be a shift to more resilient and salinity tolerant invertebrate taxa during the supra-seasonal drought; 3.) considerably lower fish diversity and a greater abundance of tolerant fish species will be present in Nyamithi lake during the supra-seasonal drought in comparison to the wet season; 4.) consumer diets will be specialised during the wet season and opportunistic during the supra-seasonal drought and 5.) all above changes are directly or indirectly driven by changes in chemical water composition between the supra-seasonal drought and pre-drought conditions.

6.2 Materials and Methods

A single sampling survey was undertaken in June 2010 and nine surveys between 2014 and 2017. The June 2010 sampling survey took place during the natural dry season before the severe drought when controlled flood releases still occurred. The first survey of 2014 occurred during the natural dry season (September 2014) and the second following a controlled flood release (December 2014). During the supra-seasonal drought in 2016, the site was sampled three times (May, August and December 2016). Four sampling surveys took place in 2017 (February, May, August and November 2017), after the exceptional drought period. The site continuously received water through rainfall and floods from the Usuthu River throughout 2017. Four areas were sampled once during each survey in Nyamithi lake in order to ensure that an integrated sample of all biotopes was collected (Figure 6.1).

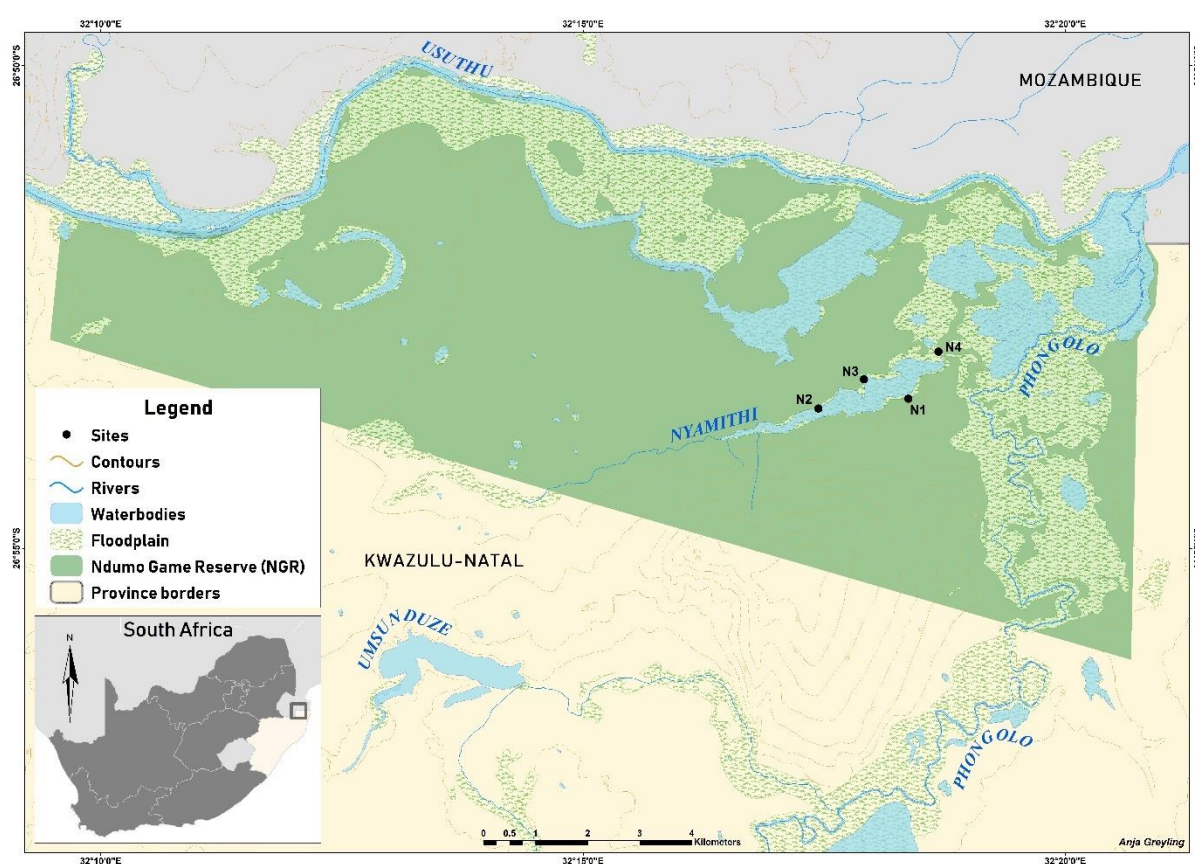


Figure 6.1: Map of Ndumo Game Reserve (NGR) and surrounding region indicating the sampling areas in Nyamithi lake (N1-N4).

6.2.1 Field collection

Aquatic invertebrates (zooplankton and macroinvertebrates) and water for chemical analysis were collected during all sampling surveys in 2014, 2016 and 2017. Methods regarding *in situ* water parameter measurements and water sample collection can be found in section 3.2.1 (Chapter 3). Methods regarding sampling and processing of aquatic invertebrates can be found in section 5.2.2.1 (Chapter 5). Samples for stable isotope analysis were collected only in June 2010 and February 2017. Methods regarding field collection of aquatic invertebrates, plants, biofilm, detritus and leaf litter for

stable isotope analysis as well as storage of samples can be found in section 5.2.2.1 (Chapter 5). For stable isotope analysis, fish were collected from the site with the aid of fyke nets (800 mm hoops) that were placed in the lake early in the morning and serviced every four hours over 12-hour periods, cast-nets and rod and line. Once captured, fish were identified using identification keys in Skelton (2012), humanely killed as discussed in section 5.2.2.2 (Chapter 5) and total length measured. Approximately 5 g of axial muscle tissue was collected from the right side of large individuals (>60 mm) while small specimens were placed in 15 mL Falcon tubes with head, fins, scales and organs removed. All field samples were collected and processed with the necessary permits (Permit No's: OP 899/2016 and OP 1075/2017) and ethical clearance (Ethics No: NWU-00264-16-A5).

6.2.2 Laboratory analysis

Methods regarding water sample analysis can be found in section 3.2.2 (Chapter 3) and methods regarding aquatic invertebrate analysis for biodiversity assessment are presented in section 5.2.3.1 (Chapter 5). Methods regarding stable isotope sample preparation are presented in section 5.2.3.2 (Chapter 5).

6.2.3 Statistical analysis

6.2.3.1 Environmental and biological data

All statistical analyses on the aquatic invertebrate communities were conducted on lowest taxon level. Diversity indices were calculated from untransformed data and diversity assessed for each site and survey using Margalef species richness (richness), Pielou's Evenness index (evenness), Shannon-Wiener diversity index (Shannon diversity) and Simpson's index (for a full description of these indices see section 4.2.3, Chapter 4). Parametric unpaired student t-tests with Welch's correction were then used to determine if there were significant differences between the survey years (2014, 2016 and 2017). Using the *indicspecies* package for R (de Cáceres et al. 2016; R Core Team 2017), *multipatt* analysis was conducted on the aquatic invertebrate communities to determine if any of the invertebrate taxa were associated with a specific sampling survey. For a full description of the multipattern analysis see section 4.2.3 (Chapter 4). A constrained canonical correspondence analysis (CCA) was created of all surveys across the four sampling years from 2014 to 2017 in Canoco v5 in order to determine possible differences between surveys in terms of aquatic invertebrate data, with water quality variables and survey years as explanatory variables. Prior to the CCA analysis all environmental data, with the exception of pH, were log transformed [$y = \log(x+1)$]. The *car* package created for the R environment (Fox et al. 2017; R Core Team 2017) was then used to do a variance inflation factor (vif) analysis on the chemical water quality variables in order to assess possible multicollinearity between variables. Those variables that were too highly correlated to one another (>10) (Zuur et al. 2009) were excluded from the CCA. Forward selection was used during the CCA analysis to determine if any of the remaining chemical water variables significantly explained aquatic invertebrate community structure during each sampling survey. Analysis of Similarity (ANOSIM) was conducted in Primer v7 to test whether aquatic invertebrate diversity and communities were significantly different from one another using the preselected factor "sampling year" and visualised using non-metric Multidimensional Scaling (nMDS)

plot and dendrogram (Clarke and Gorley 2006). Similarity percentage analysis (SIMPER) was used to calculate the percentage (%) that aquatic invertebrate biota contributed to dissimilarities between sampling years.

6.2.3.2 Stable isotopes

The trophic position (TP) of the components of the food webs were calculated using the mean $\delta^{15}\text{N}$ signatures of each component compared to a basal reference using the following equation:

$$\text{TP} = \frac{\delta^{15}\text{N}_{\text{component}} - \delta^{15}\text{N}_{\text{reference}}}{3.37} + 1$$

where $\delta^{15}\text{N}_{\text{component}}$ is the mean $\delta^{15}\text{N}$ of each food web component, $\delta^{15}\text{N}_{\text{reference}}$ is the mean $\delta^{15}\text{N}$ of detritus of each individual system and $3.37^{0/00}$ is the mean trophic enrichment between trophic levels estimated by Taylor et al. (2017a).

A graphical representation of the food web was created in Graphpad Prism 6 for each of the two sampling surveys using the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of all collected sources. A hierarchical Bayesian stable isotope mixing model (MixSIAR; Stock and Semmens 2016) was used to determine the consumer-food source interactions over the two surveys (2010 and 2017) using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios. Potential food sources were grouped into four biologically meaningful groups. These groups were C_3 plants, C_4 plants, aquatic invertebrates and fish. Grouping of multiple food sources is recommended practice if sources are logically related and/or within group isotopic variation is less than between group variation (Phillips et al. 2005; Phillips et al. 2014; Manfrin et al. 2018). Primary producers were separated into C_3 and C_4 plants as these sources have distinct isotopic signatures, particularly for $\delta^{13}\text{C}$. Separation into C_3 and C_4 plants was based on literature review of the various sources as well as the guidelines by Bender (1971) and Smith and Epstein (1971) regarding the average $\delta^{13}\text{C}$ for C_3 and C_4 plants. Biofilm and Poaceae were separated into both C_3 and C_4 categories depending on the $\delta^{13}\text{C}$ signature during a particular survey. The grass family Poaceae includes genera that use both C_3 and C_4 photosynthesis (Aliscioni et al. 2003) and as such was classified as both during this study. Since biofilm consists of a complex mixture of algae, diatoms and other microbes (as mentioned in section 5.2.2.2, Chapter 5) they make use of both C_3 and C_4 photosynthesis (Roberts et al. 2007; Xu et al. 2012; Marchese et al. 2014) and as such can be classified either as C_3 or C_4 plants depending on what its constituents are largely made up of.

A mixing model was created for each survey and evaluated *a priori* to determine whether consumers were positioned within the convex mixing polygon bound by the potential food sources (Smith et al. 2013; Phillips et al. 2014; Valladares et al. 2017; Lemmens et al. 2017). This was achieved by simulating the mixing polygon region of each model using the *sp* and *splancs* packages for the R environment (Smith et al. 2013; R Core Team 2017). Consumer individuals that were located outside the 95% mixing polygon region were excluded from the final mixing model.

Model runs were performed for each survey using varying Markov Chains Monte Carlo (MCMC) parameters until all three MCMC chains of the model converged. Convergence was based on both the Gelman-Rubin and Geweke diagnostics tests (Stock and Semmens 2016) and was only accepted if values were <1.05 for the Gelman-Rubin test and $<5\%$ of values were outside ± 1.96 for the Geweke diagnostic (Stock and Semmens 2016). The MCMC chain length for 2010 was 50 000 with a burn in phase of 25 000 and a thinning of 25. The MCMC chain length for the 2017 mode was 100 000 with a burn in phase of 50 000 and a thinning of 100. Consumers were included as fixed effects, Process only error terms were included in the model and the 95% credible intervals based on individual level variation for each individual consumer. The Bayesian mixing model was run using stable isotope data from all collected fish as consumers (raw data by survey), potential prey groups as food sources (raw data by survey) and a consistent discrimination factor of $1.0\text{‰} \pm 0.25\text{‰}$ for $\delta^{13}\text{C}$ (DeNiro and Epstein 1978; Akamatsu et al. 2004) and $3.37\text{‰} \pm 1.3\text{‰}$ for $\delta^{15}\text{N}$ (Taylor et al. 2017a) was used for all consumers.

6.3 Results

6.3.1 Aquatic invertebrate diversity

A total of 108 aquatic invertebrate taxa from 47 families and three Superfamilies were collected from Nyamithi lake during the surveys of 2014, 2016 and 2017 (Appendix D1). The greatest diversity was found in 2017 with 76 taxa while the lowest diversity was found in 2016 with 23 taxa. Individuals from the families Corixidae (*Agraptocorixa* sp., *Micronecta* sp. and *Sigara* sp.) and Hydrophilidae (*Berosus* sp. and *Hydrophilus senegalensis*) were found in varying abundances throughout all surveys. Individuals from the order Ostracoda (including the Superfamilies Cypridoidea, Cytheroidea and Darwinulidae) were found during all but the August 2016 survey, and were often the most abundant taxon collected per survey with most individuals collected in February 2017 (3471 individuals). Many molluscs were only found during the September and December 2014 surveys and included *Bulinus natalensis* (148 and 1134 individuals, respectively), *B. tropicus* (382 and 266 individuals, respectively) and the invasive snail *Tarebia granifera* (2277 and 321 individuals, respectively). Fewer genera in lower abundance were found during the 2016 and 2017 surveys and included *Cleopatra* sp. (3 individuals in May 2017) *Corbicula* sp. (4 individuals in August 2016; 3 individuals in August 2017) and another invasive snail, *Physa acuta* (93 individuals in May 2017). Several zooplankton taxa were collected only during the 2016 and 2017 surveys. These included Harpacticoida, in highest abundance in August 2016 (810 individuals), and *Diaphanosoma* sp. and *Thermocyclops* sp. both in highest abundance in February 2017 (420 and 960 individuals, respectively). See section 2.5.3 (Chapter 2) for photographs of some of the aquatic invertebrate taxa collected in Nyamithi lake.

Two groups formed on the dendrogram regarding taxa collected during the various surveys (“years”) where 2016 separated from the remaining surveys at approximately 12% similarity (Figure 6.2a). An additional separation occurred between 2014 and 2017 at 24% similarity. At a stress of 0.05 the nMDS indicated a good fit ordination and a similar pattern to the dendrogram was observed where each year separated distinctly from the rest (Figure 6.2b). The most similar groups were September and December 2014 (50.12%), February and May 2017 (43.63%), August and November 2017 (42.57%) and August and December 2016 (42.35%). Using the preselected factor “year”, the ANOSIM indicated significant differences between the groups observed on the dendrogram and nMDS plots (Global R = 0.91; significance level = 0.2%).

Results from the SIMPER analysis indicated that the 2014 surveys were 50.12% similar and the taxa responsible for this similarity were Ostracoda, *T. granifera*, *B. tropicus*, *B. natalensis*, *Agraptocorixa* sp. A, Baetidae and *Enallagma* sp. (Table 6.1). The three surveys in 2016 were 35.55% similar and the taxa responsible for this similarity were *Sigara* sp. and Harpacticoida. Finally, the four surveys in 2017 were 35.43% similar and the representative taxa responsible were Ostracoda, Chironominae, *Hyphydrus* sp. A, *Appasus* sp., *Thermocyclops* sp., *Pseudagrion* sp. and *Micronecta* sp.

The multipattern analysis indicated that no aquatic invertebrate taxa were significantly associated with any particular sampling survey and as such only the 30 best fitting taxa are visualised on the CCA

(Figure 6.3). In addition, after *vif* analysis and forward selection only five chemical water variables (temperature, conductivity, total hardness, PO_4^{3-} and NH_4^+) are indicated on the CCA. Forward selection also identified that none of the chemical water variables had a significant effect on the aquatic invertebrate community composition. The CCA explains 45.66% of data variation on the first two axes and on the first axis (explaining 27.84%) separation is evident between the three sampling years, particularly between 2014 and 2016. This is because most diversity indices were lowest in May 2016, namely number of taxa (5), number of individuals (101), richness (0.87), Shannon diversity (0.66) and Simpson diversity (0.37) (Figure 6.4a-d). These indices were all highest at the end of 2017 as August 2017 had the greatest number of taxa (59) and richness (7.99), while November 2017 had the highest evenness (0.83), Shannon diversity (2.82) and Simpson's index (0.93). The number of taxa, number of individuals and species richness was also significantly lower in 2016 than in 2014 ($p < 0.05$) (Appendix D2). Separation on the second axis (explaining 17.84%) was due to differences in chemical water quality variables between the surveys. A positive correlation of salinity (measured by conductivity, Cl^- and total hardness) with May and August 2016 was because total hardness was highest in May 2016 (1910 mg.L^{-1}) while August 2016 had the highest conductivity ($18\,000 \mu\text{S.cm}^{-1}$) and Cl^- (3280 mg.L^{-1}) (Table 6.2). These surveys were also negatively correlated with temperature as August 2016 had coldest water temperature (17°C). Conductivity was lowest in December 2014 ($3520 \mu\text{S.cm}^{-1}$), while total hardness and Cl^- were lowest in February 2017 (195 mg.L^{-1} and 193 mg.L^{-1} , respectively) and water temperature warmest in November 2017 (33.5°C) (Table 6.2). September 2014 was positively correlated with NH_4^+ because values were highest during this survey (2.2 mg.L^{-1}) while August 2017 had the highest PO_4^{3-} (4.0 mg.L^{-1}) along with the remaining organic nutrients (NO_3^- , NO_2^- and NH_3) and SO_4^{2-} .

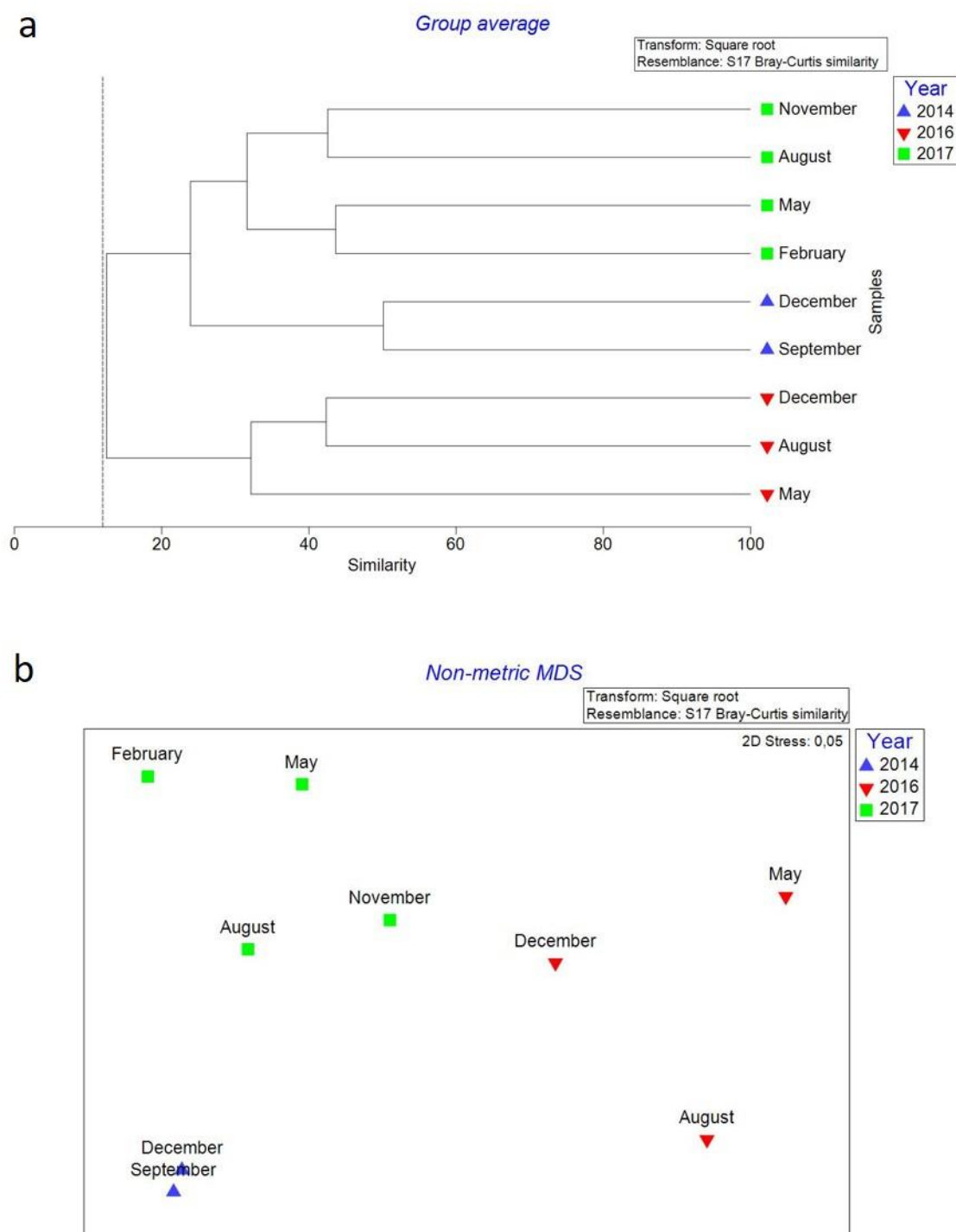


Figure 6.2: Dendrogram (a) and nMDS (b) plots of aquatic invertebrate taxa collected during sampling surveys in 2014, 2016 and 2017 in Nyamithi lake within the Ndumo Game Reserve.

Table 6.1: Contribution of various aquatic invertebrate taxa to within group similarities determined through Similarity percentage analysis (SIMPER) analysis. Groups are based on year of sampling (2014, 2016 or 2017) and were determined using dendrogram and nMDS analysis.

Year	Overall similarity	Species	Average abundance	Average similarity	Percentage contribution (%)	Cumulative contribution (%)
2014	50.12%	Ostracoda	22.23	7.66	15.28	15.28
		<i>Tarebia granifera</i>	32.82	7.57	15.09	30.37
		<i>Bulinus tropicus</i>	17.93	6.89	13.74	44.11
		<i>Bulinus natalensis</i>	22.92	5.14	10.25	54.36
		<i>Agraptocorixa</i> sp. A	14.77	3.8	7.58	61.94
		Baetidae	7.7	3.04	6.07	68.02
		Enallagma sp.	7.41	2.27	4.54	72.55
2016	35.55%	<i>Sigara</i> sp.	12.48	15.44	43.44	43.44
		Harpacticoida	15.5	13.25	37.31	80.75
2017	35.43%	Ostracoda	26.07	9.36	26.42	26.42
		Chironominae	5.54	3.53	9.95	36.38
		<i>Hyphydrus</i> sp. A	7.29	2.98	8.4	44.77
		<i>Appasus</i> sp.	6.62	2.92	8.23	53
		<i>Thermocyclops</i> sp.	12.35	2.74	7.74	60.74
		<i>Pseudagrion</i> sp.	4.01	2.05	5.78	66.52
		<i>Micronecta</i> sp.	2.95	1.4	3.96	70.48

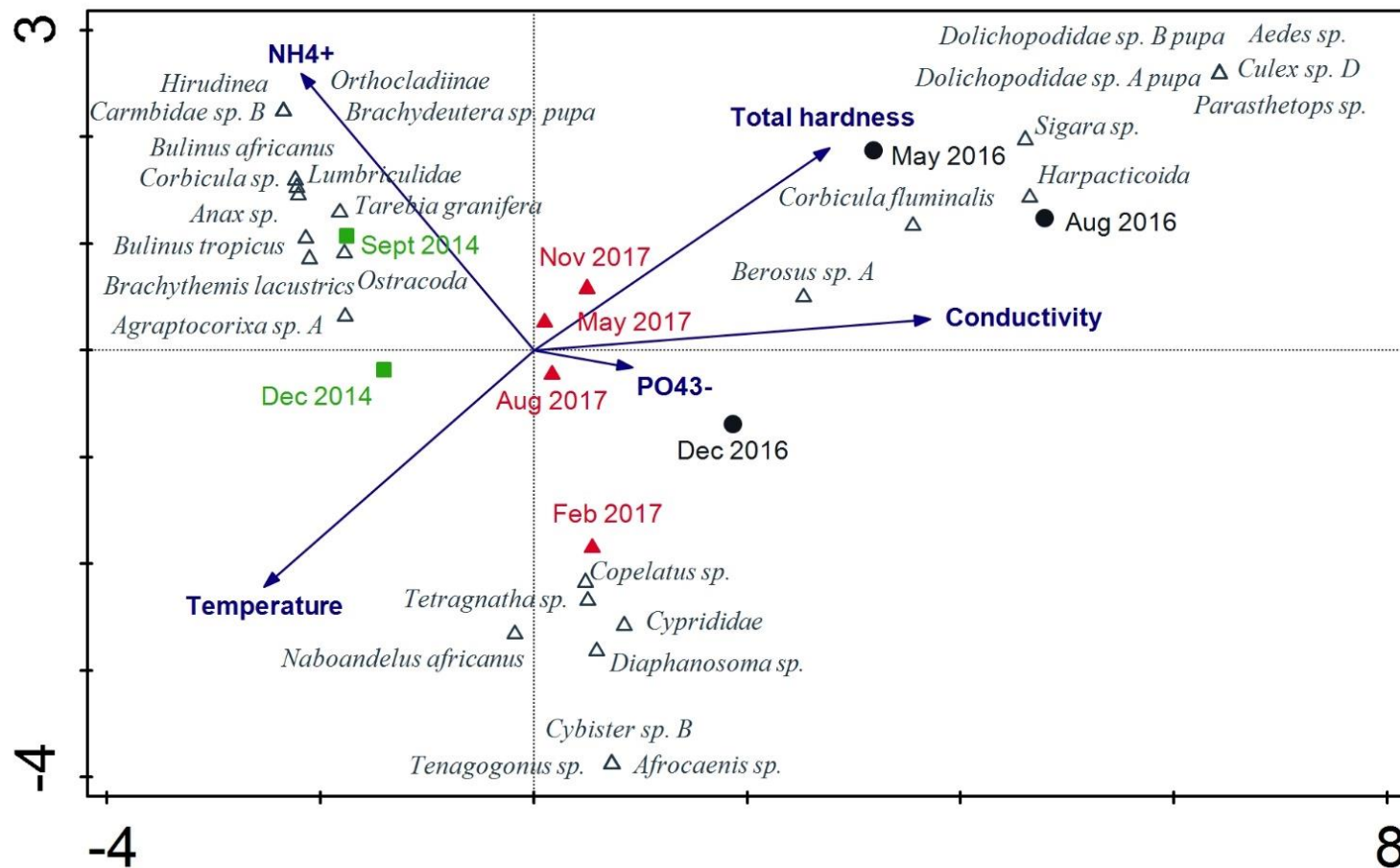


Figure 6.3: Canonical Correspondence Analysis (CCA) triplot comparing aquatic invertebrate diversity and select water nutrients variables to all sampling surveys during 2014, 2016 and 2017 in Nyamithi lake within the Ndumo Game Reserve. The biplot explains 45.66% of data variation of which 27.84% is explained by the first axis and 17.84% by the second axis. Only the 30 best fitting aquatic invertebrate taxa and water nutrient variables selected through correlation and forward selection analysis are retained.

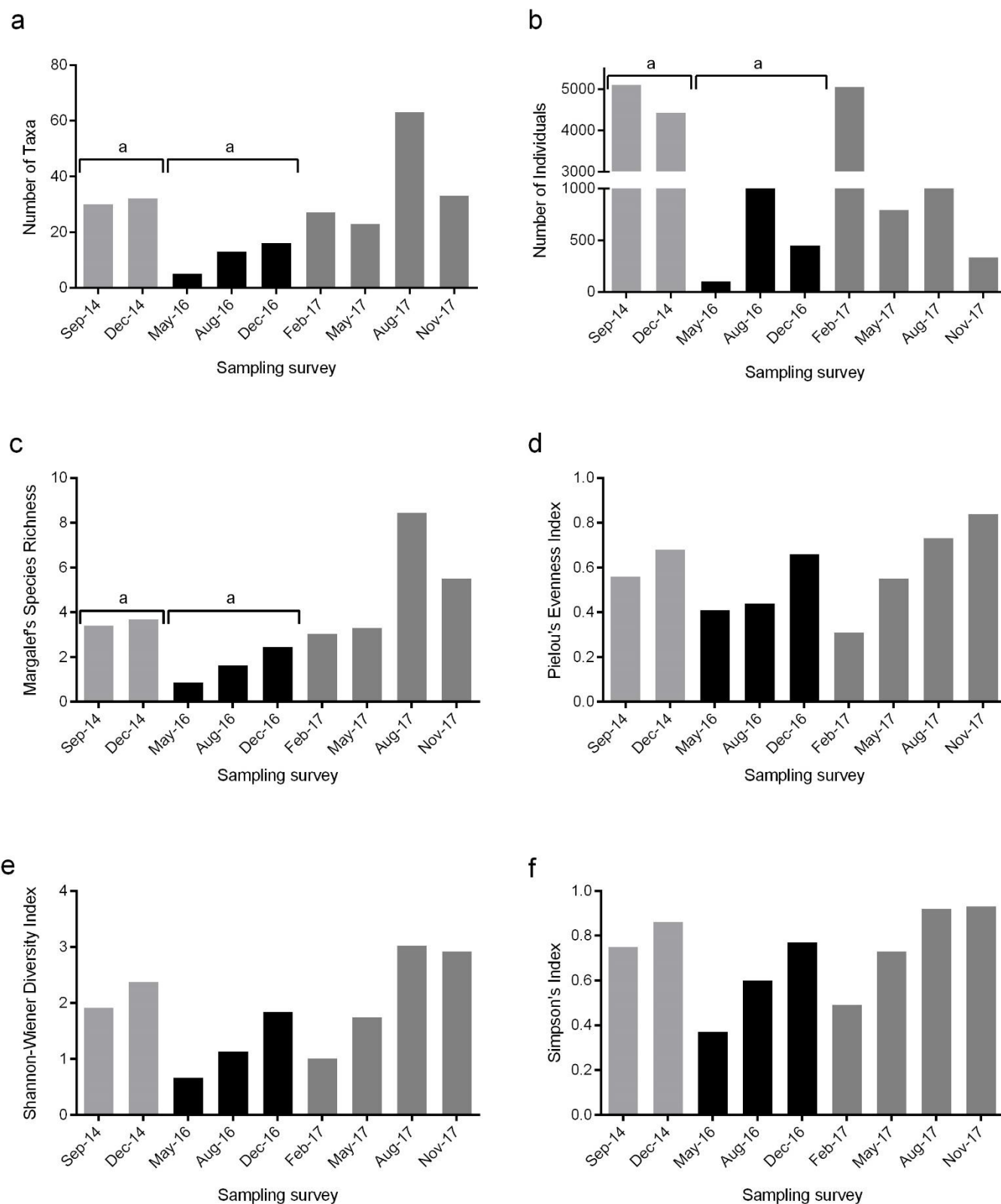


Figure 6.4: Comparison of the number of taxa (a), number of individuals (b), Margalef's Species Richness (c), Pielou's Evenness Index (d), Shannon-Wiener diversity (e) and Simpson's Index (f) of aquatic invertebrates collected during the sampling surveys in 2014, 2016 and 2017 in Nyamithi lake within the Ndumo Game Reserve. Lower case letters indicate significant difference ($p < 0.05$).

Table 6.2: *In situ* and chemical water quality variables collected during the sampling surveys in 2014, 2016 and 2017 from Nyamithi lake within the Ndumo Game Reserve.

*Denotes data not available.

Survey	Sep-14	Dec-14	May-16	Aug-16	Dec-16	Feb-17	May-17	Aug-17	Nov-17
Temperature (°C)	26.1	29.6	23.4	17.6	28.5	29.7	23.8	22.9	33.5
pH	8.8	7.9	8.5	9.3	8.2	8.4	7.1	8.3	8.8
Dissolved Oxygen (mg.L ⁻¹)	5.8	8.3	6.0	10.3	5.5	4.5	1.9	5.3	*
Oxygen Saturation (%)	72.4	105.6	72.4	105.8	70.3	56	19.5	62.5	*
Conductivity (μS.cm ⁻¹)	6150	3520	15323	18000	15565	8428	3570	7663	11090
PO ₄ ³⁻ (mg.L ⁻¹)	0.1	0.2	0.5	0.7	0.3	0.1	0.03	4.0	0.1
NO ₃ ⁻ (mg.L ⁻¹)	2.2	0.4	0.9	1.5	1.0	1.1	1.1	8.2	0.8
NO ₂ ⁻ (mg.L ⁻¹)	0.04	0.04	0.02	0.01	0.04	0	0.02	0.3	*
NH ₄ ⁺ (mg.L ⁻¹)	2.2	0.1	0	0.1	0.2	0.2	0.1	0.5	0.1
NH ₃ (mg.L ⁻¹)	0	0	0.02	0	0.04	0.01	0.01	0.4	*
SO ₄ ²⁻ (mg.L ⁻¹)	313	243	975	*	536	611	45	2283	1320
Cl ⁻ (mg.L ⁻¹)	731	567	300	3280	1068	193	356	1348	1720
Total Hardness (mg.L ⁻¹)	284	234	1910	1130	623	195	188	915	977
Mg ⁺ (mg.L ⁻¹)	79	51	1220	660	355	123	124	333	388
Ca ²⁺ (mg.L ⁻¹)	206	183	690	470	268	71	64	581	645

6.3.2 Food webs and dietary analysis

A total of 14 fish species from nine families were collected in Nyamithi lake during the two surveys of June 2010 and February 2017 (Figure 6.5). Of these, 11 species from all nine families were found during the 2010 survey and 10 species from only seven families during the 2017 survey. Both large (≥ 150 mm) and small (< 150 mm) tigerfish, *Hydrocynus vittatus* (Alestidae) were collected in 2010 with a total of 33 individuals. In 2017, only small *H. vittatus* were collected (10 individuals) and the most abundant species was *Oreochromis mossambicus* (21 individuals) (see section 2.2, Chapter 2 for photographs of some of these fish species).

Three trophic levels were discernible in the food webs of both surveys (Appendix D3). During 2010, the brown squeaker, *Synodontis zambezensis* (Mochokidae), *H. vittatus* (both small and large) were at the top of the food web while the remaining fish, apart from *O. mossambicus*, were a trophic position lower along with most aquatic invertebrates (Athyidae, Hirudinea and Notonectidae). The base of the food web was made up of the primary producers and remaining aquatic invertebrates (Figure 6.5).

In 2017, most fish species were near the top of the food web although the East Coast barb *Enteromius toppini* (Cyprinidae), Hamilton's barb, *E. afrohamiltoni* (Cyprinidae) and *S. zambezensis* were the top predators. *Oreochromis mossambicus* and the rednose Labeo, *Labeo rosae* (Cyprinidae) were a trophic level lower along with aquatic invertebrates and most primary producers and only detritus and biofilm were at the very base of the food web.

The dietary analysis indicated that generally a single food source made the largest contribution (at least 50%) to the consumer diets in 2010, with fewer instances where a mixture of sources was consumed (Figure 6.6a; Table 6.3). The largest percentage of most consumer diets in 2010 consisted of invertebrates (39.4% - 54.8%) except for the red-breasted tilapia, *Coptodon rendalli* (Cichlidae), where C_4 plants made the largest contribution (45.1%). All other sources made smaller contributions ($< 35\%$) and fish made the lowest contribution (2.6% - 12.5%) to all but *H. vittatus*' diet (both large and small) which consumed C_4 plants least (7.1% and 8.6%, respectively). In 2017, the consumer diets consisted of a more varied mixture of food sources, although aquatic invertebrates generally made the largest contribution (38.2% - 47.9% (Figure 6.6b; Table 6.4). In the cases of the African sharptooth catfish, *Clarias gariepinus* (Clariidae), small *H. vittatus* and *O. mossambicus*, C_4 plants made the largest contribution (58.2%, 45.3% and 64.5%, respectively) while fish made the lowest contribution (2.1% - 25.6%) to all but *C. gariepinus* where C_3 plants made the lowest contribution (8.1%).

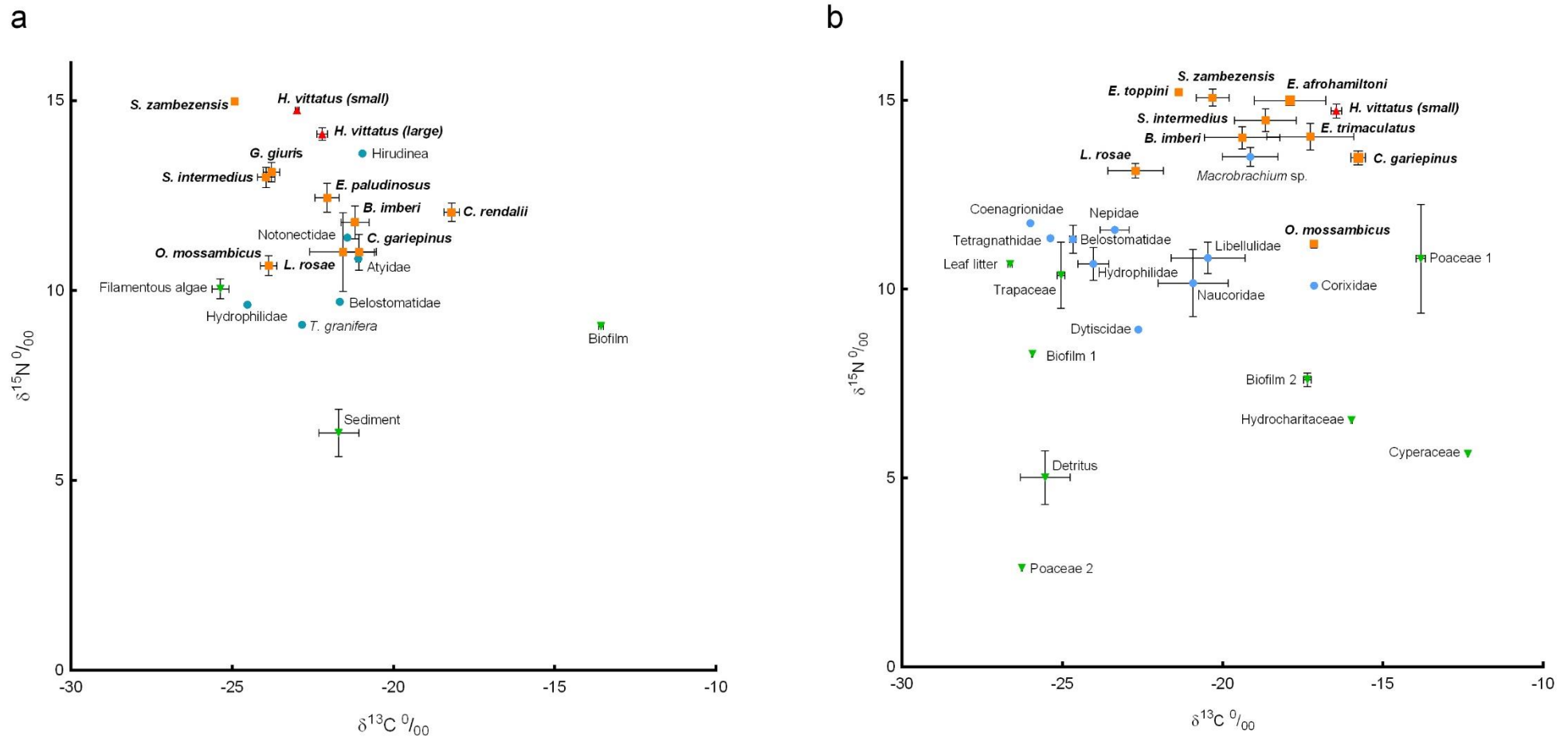


Figure 6.5: Biplots indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (±1 SE) of the various food web components collected during the June 2010 (a) and February 2017 (b) sampling surveys in Nyamithi lake within the Ndumo Game Reserve.

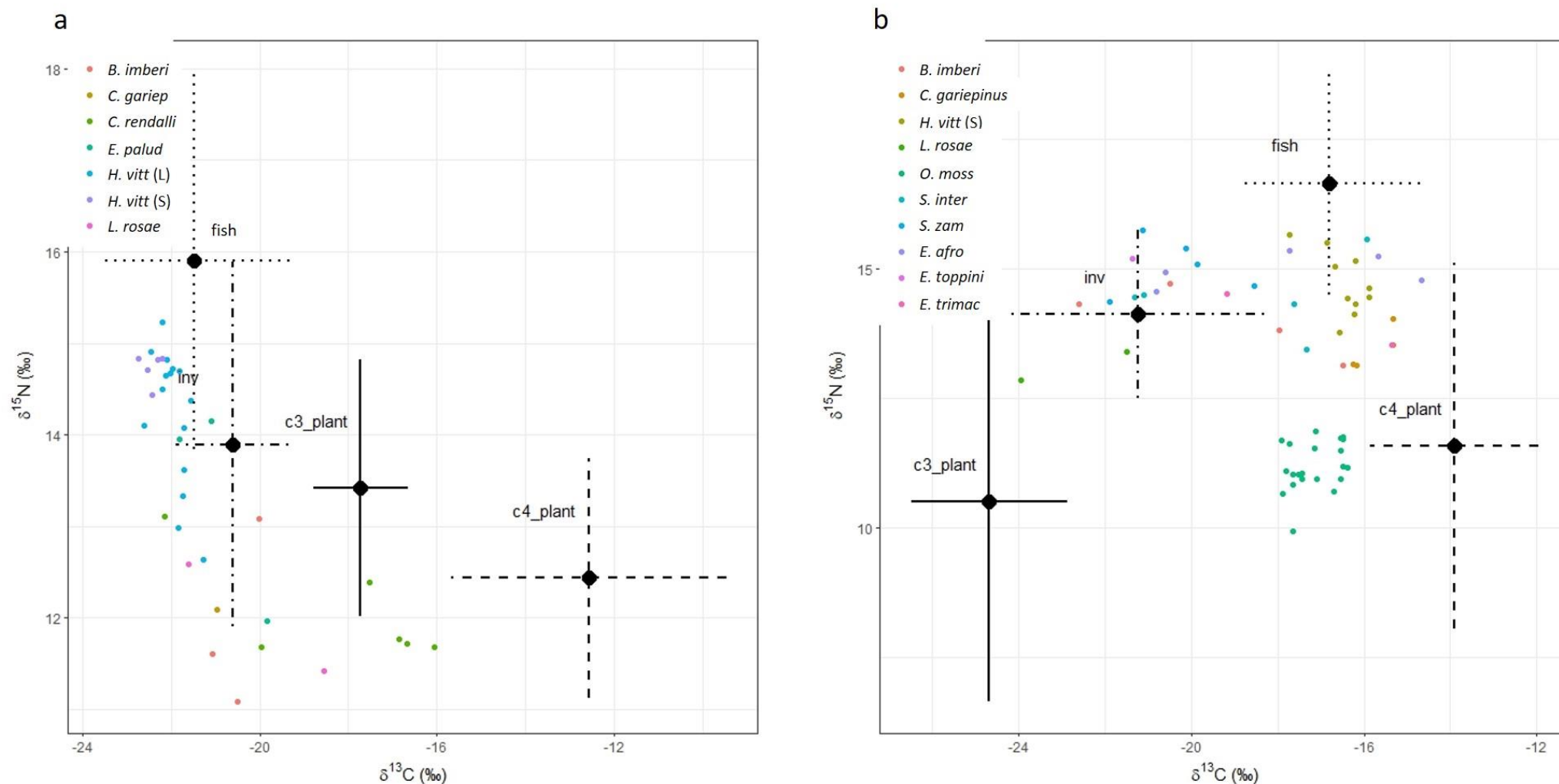


Figure 6.6: A biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) of the sampling survey in June 2010 (a) and February 2017 (b) in Nyamithi lake within the Ndumo Game Reserve. Fish consumer data and source data are abbreviated: Fish – fish food sources; c3_plants – C₃ plants; c4_plants – C₄ plants; inv – aquatic invertebrates. Error bars represent 95% confidence intervals.

Table 6.3: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected in Nyamithi lake within the Ndumo Game Reserve in June 2010. Mean±SD (95% Credible interval).

Sources	<i>Brycinus imberi</i>	<i>Clarias gariepinus</i>	<i>Coptodon rendalli</i>	<i>Enteromius paludinosus</i>	<i>Hydrocynus vittatus</i> (large)	<i>Hydrocynus vittatus</i> (small)	<i>Labeo rosae</i>
C ₃ plants	22.9±14.7 (2.4 – 57.0)	22.3±19.8 (0.6 – 72.8)	24.5±26.9 (0.6 – 90.5)	24.2±18.8 (0.8 – 68.2)	19.4±11.1 (1.4 – 42.8)	16.8±12.9 (0.9 – 48.5)	24.6±22.0 (0.7 – 77.2)
C ₄ plants	15.2±9.5 (1.3 – 37.3)	14.1±14.2 (0.4 – 53.1)	45.1±21.4 (0.3 – 76.9)	13.3±11.6 (0.5 – 42.6)	7.1±5.4 (0.6 – 20.6)	8.6±7.5 (0.6 – 28.2)	17.2±15.9 (0.4 – 58.8)
Fish	10.1±4.9 (3.1 – 21.7)	8.8±9.2 (0.5 – 34.4)	2.6±2.0 (0.3 – 7.8)	12.5±9.7 (0.9 – 36.1)	30.5±8.2 (15.2 – 47.0)	35.2±13.9 (6.9 – 62.9)	6.0±5.7 (0.5 – 2.9)
Invertebrates	51.7±12.7 (26.2 – 75.5)	54.8±20.9 (12.5 – 92.1)	27.7±12.5 (4.8 – 52.0)	50.0±18.6 (16.0 – 88.1)	43.0±10.2 (24.0 – 64.0)	39.4±16.3 (11.4 – 76.6)	52.1±20.9 (11.3 – 90.7)

Table 6.4: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected in Nyamithi lake within the Ndumo Game Reserve in February 2017. Mean $\pm$ SD (95% Credible interval).

Sources	<i>Brycinus imberi</i>	<i>Clarias gariepinus</i>	<i>Hydrocynus vittatus</i> (small)	<i>Labeo rosae</i>	<i>Oreochromis mossambicus</i>	<i>Shilbe intermedius</i>	<i>Synodontis zambezensis</i>	<i>Enteromius afrohamiltoni</i>	<i>Enteromius toppini</i>	<i>Enteromius trimaculatus</i>
C ₃ plants	17.3 $\pm$ 7.9 (4.8 – 34.9)	8.1 $\pm$ 5.9 (0.9 – 21.5)	8.0 $\pm$ 4.7 (1.1 – 18.9)	32.2 $\pm$ 23.2 (1.1 – 74.3)	24.2 $\pm$ 5.5 (11.8 – 33.6)	12.0 $\pm$ 8.6 (1.2 – 33.2)	23.0 $\pm$ 13.1 (1.9 – 50.1)	8.8 $\pm$ 6.5 (1.0 – 25.2)	22.2 $\pm$ 17.6 (1.1 – 63.5)	11.3 $\pm$ 8.4 (1.0 – 32.0)
C ₄ plants	29.2 $\pm$ 8.3 (14.2 – 46.4)	58.2 $\pm$ 14.8 (19.1 – 81.5)	45.3 $\pm$ 10.7 (23.1 – 65.1)	12.8 $\pm$ 8.0 (1.8 – 31.3)	64.5 $\pm$ 4.5 (55.2 – 73.3)	23.8 $\pm$ 12.8 (3.5 – 50.3)	16.3 $\pm$ 8.8 (2.9 – 35.1)	24.8 $\pm$ 14.8 (3.3 – 58.8)	18.4 $\pm$ 12.5 (2.1 – 48.8)	34.9 $\pm$ 19.9 (19.9 – 73.4)
Fish	13.6 $\pm$ 6.4 (3.9 – 29.1)	12.0 $\pm$ 10.0 (0.9 – 38.4)	25.6 $\pm$ 10.8 (6.2 – 48.7)	7.7 $\pm$ 6.4 (0.6 – 24.0)	2.1 $\pm$ 1.4 (0.4 – 5.5)	16.3 $\pm$ 12.2 (1.2 – 47.7)	19.3 $\pm$ 11.0 (1.6 – 42.6)	22.6 $\pm$ 16.3 (1.4 – 61.7)	14.6 $\pm$ 12.9 (0.8 – 47.3)	15.7 $\pm$ 14.7 (0.8 – 55.5)
Invertebrates	39.9 $\pm$ 11.7 (15.2 – 61.8)	21.8 $\pm$ 14.3 (3.4 – 59.4)	21.0 $\pm$ 9.8 (4.9 – 42.8)	47.2 $\pm$ 25.3 (4.4 – 89.7)	9.2 $\pm$ 5.8 (1.6 – 24.2)	47.9 $\pm$ 17.3 (11.5 – 80.5)	41.4 $\pm$ 18.1 (7.7 – 77.1)	43.7 $\pm$ 18.6 (7.3 – 79.7)	44.8 $\pm$ 22.4 (5.7 – 86.9)	38.2 $\pm$ 21.7 (4.5 – 83.4)

6.4 Discussion

This section of the study aimed to determine whether supra-seasonal drought and associated changes in chemical water characteristics, particularly salinity, had any effects on aquatic invertebrate community structure, diversity, the aquatic food webs and fish consumption pattern compared to pre-drought conditions in a large saline lake (Nyamithi) in Ndumo Game Reserve (South Africa). Aquatic invertebrate diversity was much lower in 2016 when Nyamithi was at its driest in comparison to pre-drought in 2014. Stable isotope and dietary analysis also indicated shifts in fish diet composition from more specialised during the pre-drought in 2010 to generalist during supra-seasonal drought in 2017.

6.4.1 Aquatic invertebrate diversity

Aquatic invertebrate diversity is naturally low in saline lakes of southern Africa (Seaman et al. 1991) and Nyamithi lake is no exception as even during the wet season surveys (2014) when salinity was at its lowest in the study, aquatic invertebrate diversity and abundance was sparse in comparison to what is present in other wetland sites in NGR (see de Necker 2015 and Dube et al. 2016). Similar findings were reported by Velasco et al. (2006) who collected only 25 invertebrate taxa from 16 families in a Mediterranean saline stream and Senner et al. (2018) who reported lower invertebrate numbers during low lake and high salinity levels in North America. Of the biota collected during the 2014 survey, the invasive snail *Tarebia granifera* and individuals of the order Ostracoda were most dominant. *Tarebia granifera* is known to have a high salinity tolerance which has allowed it to invade not only freshwater systems in South Africa but also estuaries, lagoons and coastal lakes in KwaZulu-Natal (Miranda et al. 2010; Darwall et al. 2011). Ostracods are also present in aquatic environments of varying salinities as they are able to tolerate harsh environmental conditions in both marine and non-marine ecosystems (Day et al. 2001a).

Aquatic invertebrate diversity and abundance was lower in 2016, during the driest period of Nyamithi, than either the 2014 or 2017 surveys, and also coincided with the period of highest lake salinity. Among the few invertebrate taxa found during the 2016 survey were Harpacticoida (Copepoda) of which many genera are estuarine and marine (Day et al. 2001a) as well as genera of the family Corixidae (*Sigara* sp. and *Micronecta* sp.) and Hydrophilidae (*Berosus* sp.) that are known to be hardy, salinity tolerant macroinvertebrates (Williams 1981; Hart et al. 1991). Similarly, Waterkeyn et al. (2008) found copepods and corixids (*Sigara* sp. and *Micronecta* sp.) to survive high salinity in temporary wetlands in France, and Velasco et al. (2006) observed species of the genus *Berosus* in high abundance in a Mediterranean saline stream. Many biota were completely absent during the drought period of 2016, including all molluscs, or in extremely low abundances, such as *Corbicula* sp. of which only 4 individuals were present in August 2016. This is most likely due to their inability to tolerate the severely increased salinity. Some biota, particularly soft-bodied invertebrates such as molluscs, are more sensitive to salinity than others (Hart et al. 1991; Nielson et al. 2003) such as Harpacticoida and corixids. Metzeling (1986) also found freshwater molluscs at salinities up to 2240 mg.L⁻¹ (4480 µS.cm⁻¹) but in lower abundance than at salinities ≤1000 mg.L⁻¹. Once salinity is close to 10 000 mg.L⁻¹ (20 000 µS.cm⁻¹) even those

invertebrates that are able to tolerate increased salinity start to decline and an increase in estuarine and more salinity tolerant biota occurs (Hart et al. 1991; Nielson et al. 2003).

Aquatic invertebrate diversity started increasing again rapidly after the study site received water through a large rainstorm in its local catchment during December of 2016 (pers. observation) when organic nutrients increased, and conductivity also decreased greatly. By the end of 2017, after Nyamithi lake had received small amounts of water through rains and flood water from the Usuthu River throughout the year, aquatic invertebrate biodiversity was greater than during pre-drought conditions in 2014. Similarly, in a lakes study by Ginatullina et al. (2017) in Uzbekistan it was found that zooplankton species richness had increased and surpassed that of pre-disturbance conditions within a year of being affected by increased salinity. Recolonisation largely occurred by active dispersers such as *Anisops* sp., *Appasus* sp. and *Micronecta* sp., most likely from nearby water sources such as the Phongolo and Usuthu Rivers. Several zooplankton taxa were also found during 2017 including Ostracoda and three new taxa not recorded in Nyamithi during the previous surveys of 2014 and 2016 namely *Thermocyclops* sp. (Cyclopidae, Copepoda), *Moina micrura* (Moinidae, Cladocera) and *Diaphanosoma* sp. (Sididae, Cladocera). These taxa most likely hatched from resting egg banks in the sediments, stimulated by the influx of fresh, nutrient rich water through the course of 2017. Recovery after a disturbance occurs rapidly in short-living organisms such as plankton, through resting egg banks, and insects, through recolonisation by active dispersal from local sources (Beketov et al. 2008). Both of these mechanisms are an essential part of recovery of aquatic invertebrates from a disturbance (Brock et al. 2003; Bogan and Lytle 2011).

6.4.2 Food webs and dietary analysis

In the wet season of 2010 a slightly larger abundance of fish species and families were found than during 2017. Continuous monitoring of the site throughout 2016 indicated that only *Oreochromis mossambicus* was able to survive the high salinity period of Nyamithi. All other species of fish were only found again in 2017 after the site received water through flooding from the Usuthu River when salinity also decreased, although *O. mossambicus* was still the most abundant. *Oreochromis mossambicus* is highly tolerant of salinity and found in fresh, brackish, marine and other systems with higher salinity concentrations while the other species of fish are not as tolerant of such high salt concentrations (Skelton 2012).

Both food webs were rather short with only three distinct trophic levels present during both surveys. Long food webs (>4 trophic levels) are not able to persist for extended periods of time in the environment, particularly not in regions where there are often disturbances such as drought and flood and are therefore not common in nature (Borrelli and Ginzburg 2014). Short food webs (≤4 trophic levels) are found most often in the environment as they are generally more stable due to more links between trophic levels, which stabilises the food web and allows for easier recovery from disturbances (Macarthur 1955; Rooney and McCann 2012; Borrelli and Ginzburg 2014).

More specific feeding by several consumers indicates that the environment in 2010 was not under severe stress and that competition for resources was low. If a resource is abundant and there is low

interspecific competition, such as occurs when there are few disturbances in an ecosystem, fish may have more specialised diets (Guedes et al. 2015). Conversely, although fish are generally quite flexible in their diet (McMeans et al. 2015; Taylor et al. 2017b), changes to a more generalised diet typically only occur with increased scarcity of preferential food sources either due to natural or induced (i.e. floods or drought) fluctuations in resources (Gabler and Amundsen 2010; Guedes et al. 2015; Lu et al. 2016). Similarly, research on the piscivorous largemouth bass (*Micropterus salmoides*; Centrarchidae) and Lake trout (*Salvelinus namaycush*; Salmonidae) determined that these species fed on a wide range of prey sizes and trophic levels and switched to feeding on invertebrates only after they had reduced their preferred fish prey (Schindler et al. 1997; Vander Zanden et al. 2000). Additionally, Chavarie et al. (2013) determined that *S. namaycush* were only more specialised when interspecific competition was low. Of the species collected, during the 2010 survey, *H. vittatus* is the only truly specialised feeder as juveniles are invertivores and adults are piscivores (Skelton 2012). However, the dietary analysis indicated that at least 45% of the large *H. vittatus*' diet consisted of aquatic invertebrates and only 30% consisted of fish. Aquatic invertebrates were generally on the same trophic level as most fish, thus had a similar nitrogen signature. These were also the most fed on source by all fish consumers. It is therefore likely that large *H. vittatus* were feeding on fish that had a similar signature to that of aquatic invertebrates, which they had preyed on. A similar result was found by Oliveira et al. (2006) where piscivorous fish had the isotopic signatures of C₃ plants due to predation on fish that preferred feeding on C₃ plants. In 2017, most fish adapted a generalist feeding behaviour with fewer cases where a single food source made up the largest part of their diet. This often occurs in ecosystems undergoing disturbances such as drought or flood due to the loss of preferential food sources (Layman et al. 2007; McMeans et al. 2015; Marklund et al. 2017). Omnivory and generalist feeding behaviour is important during such disturbances as it inhibits the possibility that a single species will exploit the remaining nutrients and become dominant. This stabilises the food web and allows a system to maintain a diverse and variable biological community (Gross et al. 2009; McCann et al. 2005; Rooney et al. 2008; McMeans et al. 2016).

6.5 Conclusions

In this study it was found that aquatic invertebrate diversity, abundance and community structure changed drastically from the wet season to a supra-seasonal drought in a subtropical saline lake in South Africa. The first hypothesis stating that the aquatic invertebrate abundance and diversity of Nyamithi lake will be significantly lower during the supra-seasonal drought in comparison to pre-drought conditions is therefore confirmed. Aquatic invertebrate communities shifted to only highly salinity tolerant taxa during the peak of the supra-seasonal drought. The second hypothesis stating that there will be a shift to more resilient and salinity tolerant invertebrate taxa during the supra-seasonal drought is therefore also accepted. Interestingly, it was also found that the aquatic invertebrates were able to recover rapidly after this unexpected exceptional drying event. Lower fish diversity was also found and only one species, *O. mossambicus*, was able to survive through the driest and highest salinity period in 2016 as it is one of few species with the ability to survive exceedingly high salinity conditions. The third hypothesis that considerably lower fish diversity and a greater abundance of tolerant fish species will

be present in Nyamithi lake during the supra-seasonal drought in comparison to the wet season is also confirmed here. It was also determined that the fish consumer diets switched from a more specialised to a more generalised feeding behaviour, which is frequently observed in disturbed habitats. The fourth hypothesis stating that consumer diets will be specialised during the wet season and opportunistic during the supra-seasonal drought is therefore also accepted. All of these changes in aquatic invertebrate community structure and abundance and fish diversity, abundance, diet and the aquatic food web was largely due to a severe increase in water salinity as a result of the supra-seasonal drought. The final hypothesis stating that all above changes are driven by changes in chemical water composition between the supra-seasonal drought and pre-drought conditions is therefore also confirmed by this study.

Ecosystem resilience was evident in Nyamithi lake since aquatic invertebrate diversity and abundance recovered quickly following the exceptional drying that the site experienced. Similarly, although there was a shift from specialised to generalised feeding strategies by most fish, the food web remained stable and indicates toward a system and biota that are able to adapt to environmental impacts. It is possible that the reason for this ability to adapt stems from the fact that Nyamithi lake undergoes regular disturbances in the form of a controlled flood release. Through years of constant wet/dry cycles, the aquatic biota of this ecosystem have likely adapted to living in a constantly changing environment and are therefore able to survive through, and rapidly recover from, a disturbance such as a supra-seasonal drought. However, even though Nyamithi lake did not experience hypersalinization, this is still a potential risk to the ecosystem. If a drought were to occur over a longer period of time, as is predicted by future climate change, it is quite possible that even the most resilient of taxa would not be able to survive the accompanying environmental changes, including hypersalinization.

CHAPTER 7:

Biodiversity and ecological patterns of the lower Phongolo River

7.1 Introduction

Climate change drives ecological responses through shifts in the average climatic conditions and the frequency and intensity with which extreme events such as droughts occur. These types of events may have acute and complex consequences in aquatic ecosystems and are already evident in many regions, including southern Africa (Lake 2011; Chessman 2015). Low flow drought disturbances are either seasonal or supra-seasonal (White et al. 2012). Seasonal droughts are disturbances in environments that occur on a regular, predictable basis, whereas supra-seasonal droughts are thought of as ramps in nature (disturbances that increase in strength and spatial extent over time) and are the result of rainfall decline over an extended period of time, usually several seasons (Lake 2003; Wood and Armitage 2004). Most current knowledge regarding the effects of, and biotic responses to, drought arise from studies in wetlands with predictable seasonal droughts. In these habitats, biota have evolved many drought-resistant and resilient adaptations (Boulton 2003; Lake 2003; Chaparro et al. 2016). Less is known about the responses of biota to supra-seasonal droughts caused by reduced rainfall and water availability (Lake 2003; Chaparro et al. 2016), particularly in larger riverine habitats. Climate-driven drought places additional stress on freshwater ecosystems already impacted by human activities such as water abstraction and man-made weirs (Döll et al. 2009; Döll and Zhang 2010). When combined with river regulation and water abstraction, the ecological effects of drought and drying become even more uncertain (White et al. 2012).

Humans alter and stress biological systems of rivers by changing the physical habitat, modifying seasonal flow, affecting interactions among stream organisms and contaminating water with pollutants (Karr et al. 2003; Dudgeon et al. 2006; Döll and Zhang 2010). River health is an assessment of how well a river functions in relation to its social and environmental services and is based on an array of criteria depending on both the natural conditions and social background of the system (Liu and Liu 2009). Key aspects that constitute a “healthy” river include the absence of distress measured through various characteristics or indicators, the resilience of the ecosystem in the face of stress and the identification of possible risks such as pollution (Rapport 1989; Norris and Thoms 1999). Common metrics used to determine river health include changes in biodiversity, shifts in species composition due to human influences, food web organization, water quantity and quality, nutrient conditions and inputs, and alterations in flow conditions (Jones 2002; Karr et al. 2003; Norris and Thoms 1999; Liu and Liu 2009; Döll and Zhang 2010). Low-flow alterations to an aquatic environment can affect river health through loss and fragmentation of habitats and vegetation, and reduced flow which leads to a decline in water quality through, among other things, concentration of nutrients and salts (Chessman and Robinson 1987; White et al. 2012). These alterations can then cause loss and extinction of fish and

macroinvertebrate assemblages (Boulton 2003; White et al. 2012), particularly in biota not adapted or resistant to the direct and indirect effects associated with drought.

The lower Phongolo River is regulated by Pongolapoort Dam and, although there was no controlled flood release during the supra-seasonal drought of 2016 and 2017, a monthly baseflow was continuously released. This baseflow is supposed to be no less than $5 \text{ m}^3\text{s}^{-1}$ due to the “compensation flows” agreement with Mozambique (DWS 2014a). The overall average flow per month during the study period was $7.33 \text{ m}^3\text{s}^{-1}$ (data from January 2016 to May 2017). However, flow was higher during 2016 ($8.50 \text{ m}^3\text{s}^{-1}$) than 2017 ($4.54 \text{ m}^3\text{s}^{-1}$) (see section 2.4, Chapter 2). Active water abstraction from the river for irrigation of both subsistence and commercial farms also reduces the volume of water available to the environment. As the baseflow level was determined by downstream human needs it is also not known whether it allows normal ecological processes to occur on the Phongolo River, or to sustain a stable environment in terms of aquatic invertebrate diversity, food web organization, or the diet of higher consumers.

Many aquatic invertebrates have adaptations that provide them with the resistance and resilience to survive drought, including desiccation-resistant life stages or the ability to migrate if a habitat becomes unsuitable (Brendonck and Williams 2000; Chessman 2015). Invertebrates that occur in regions prone to drought or seasonal drying events, particularly those in temporary habitats, generally have these types of adaptation, (see section 5.1, Chapter 5, for examples) (Brendonck and Williams 2000). However, in rivers that are not prone to frequent drying this is not the case. Aquatic invertebrate sensitivity is a measure of taxon or family tolerance to changes in water chemistry (e.g. organic or metal pollution), habitat disturbance (e.g. vegetation removal), flow velocity or preferences for certain types of substrate or biotope (Dallas 2007; Mereta et al. 2013). Biotope availability plays an important role in aquatic invertebrate assemblages, particularly in lotic systems such as rivers, as many aquatic invertebrates have specific habitat or flow requirements and if these are not present, then fewer taxa are likely to be recorded in them (Dallas 2007).

Ecosystem function includes a number of factors such as primary and secondary production, community stability and energy flow through a food web (Woodward 2009). Although there has been an increase in the number of studies demonstrating food web responses to seasonal variation in productivity (Dalu et al. 2017; Hayden et al. 2014; Peralta-Maraver et al. 2017; Stewart et al. 2017.) less is known about food web responses to drought. This is particularly alarming, as there are many ways in which drought can affect food web organization and stability. This includes changes in the abundance of consumers and resources leading to secondary extinction of other biota or resources, loss of links between food web components and reconfiguration or collapse of food web structure (Woodward et al. 2012; Trzcinski et al. 2016).

Effective ways to measure food web structure and stability include assessing the number of trophic transfers or positions (i.e. food chain length) between basal sources and top predators, the connectivity between these levels and the movement of carbon and nitrogen through the food web (Pimm 2002; Abrantes et al. 2014; Ruhí et al. 2016). Although stable food webs are generally rather short (Borrelli

and Ginzburg 2014), most natural ecosystems do not have less than two trophic levels and consist of a diverse range of primary producers, primary consumers and predators (Woodward 2009). Trophic level interactions occur either through direct feeding mechanisms or indirectly through intermediate species, particularly mobile generalist herbivores and detritivores such as fish. These generalists act as important links between trophic levels, transferring energy to higher trophic levels by integrating and switching between both allochthonous and autochthonous food sources (Woodward 2009). This in turn acts as a buffer to disturbances in the environment which increases the stability and resilience of a food web (McCann et al. 2005; Rooney and McCann 2012; McMeans et al. 2016; Stewart et al. 2017). Larger species and those higher in the food web may be particularly vulnerable to disturbances such as drought due to their dependence on the links with lower trophic levels (Ledger and Hildrew 2001; Ledger et al. 2011; Power et al. 2008; Woodward et al. 2012). Species loss can therefore have important consequences in the ecological network by reducing the links between trophic levels (Woodward 2009). During the initial stages of species loss, a reduced effect in the ecological network may not be evident because other species with the same function may occupy the open niche. However, if a particular feeding guild or functionally equivalent species is lost, ecosystem collapse may ensue (Woodward 2009).

Stable isotope analysis is a useful tool in determining trophic relationships, consumer diets, identifying energy sources in food webs, determining trophic niche overlap and constructing a general food web model (Phillips 2012; Abrantes et al. 2014). An organism's tissue reflects the isotopic composition of food that it consumes, due to a consistent trophic enrichment or discrimination between trophic levels. In this way, it can be inferred which trophic level an organism is located on, and can provide information on the assimilated diet of an organism over an extended time period (Valladares et al. 2017). This is in contrast with traditional stomach or gut content analysis that provides only a snapshot of recently-ingested food (Phillips 2012). Isotopic mixing models are used to determine the contribution of food sources to a consumer's diet by converting the isotopic signatures of the food sources into proportions of a consumer's overall diet (Phillips 2012; Phillips et al. 2014; Baeta et al. 2017; Lemmens et al. 2017; Stewart et al. 2017; Valladares et al. 2017).

The main aims of this section were therefore 1.) to determine whether the supra-seasonal drought has any kind of impact on the aquatic invertebrate community structure, food web and diet of consumers and 2.) whether human activities in the Phongolo River catchment have any effect on the assessed aquatic biological activities. Specific hypotheses for this section were as follows. 1.) The supra-seasonal drought will negatively affect the aquatic invertebrate community structure through loss of pollution sensitive and rheophilous genera; 2.) The supra-seasonal drought will affect the food webs through continuous shifts in trophic positions of food web components and loss of distinct trophic levels; 3.) Due to anthropogenic activities occurring in the Phongolo river catchment, organic pollution will be reflected in food web components through nitrogen enrichment; 4) Higher trophic consumers in the Phongolo River are generalist and opportunistic feeders, with large dietary overlaps between species; 5.) The baseflow released from the Pongolapoort Dam is not enough to support a stable food web or aquatic invertebrate community structure in the Phongolo River during a supra-seasonal drought.

7.2 Materials and Methods

7.2.1 Study area

Four two-week field surveys took place, in May 2016; August 2016; September 2016 and February 2017, on the Phongolo River located both within and outside Ndumo Game Reserve (NGR) (Figure 7.1). For a full description of the sites selected for this section of the study see section 2.5.1 (Chapter 2).

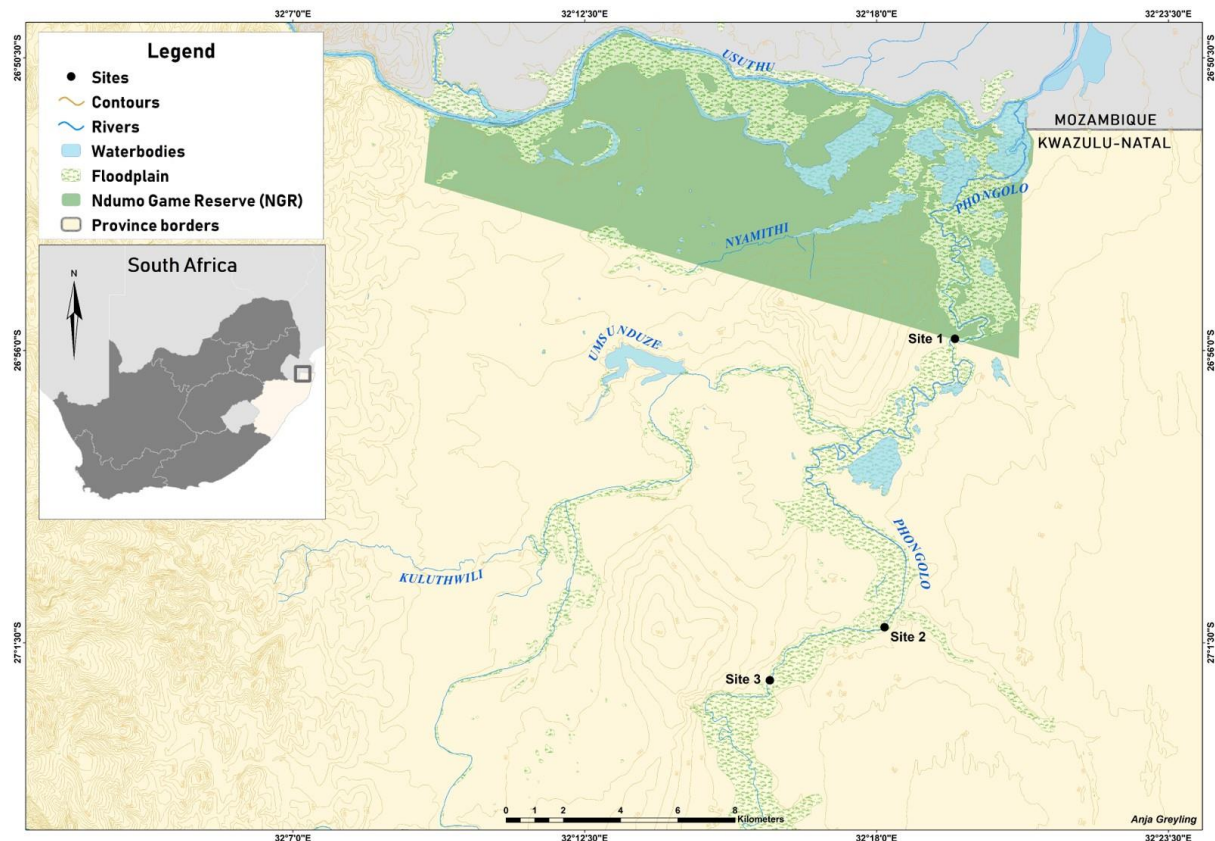


Figure 7.1: Map of Ndumo Game Reserve and surrounding area indicating the three study sites on the Phongolo River.

7.2.2 Field sampling

Aquatic invertebrates (zooplankton and macroinvertebrates) were collected during all four surveys for biological community analysis, but samples for stable isotope analysis were collected only during the first (May 2016), third (September 2016) and last (February 2017) surveys. Methods regarding sampling of aquatic invertebrates for biodiversity assessment can be found in section 5.2.2.1 (Chapter 5). For stable isotope analysis, fish were collected from the river sites using sampling techniques described in section 6.2.1 (Chapter 6). Once captured, fish were identified using identification keys in Skelton (2012), and humanely killed as described in section 5.2.2.2 (Chapter 5). Approximately 5 g of axial muscle tissue was collected from the left side of large (>60 mm) individuals, while small (≤60 mm) specimens with head, fins, scales and organs removed were placed in 15 mL Falcon tubes. Methods regarding

field collection of aquatic invertebrates, plants, biofilm, detritus and leaf litter for stable isotope analysis as well as storage of samples can be found in section 5.2.2.1 (Chapter 5).

7.2.3 Laboratory analysis

Methods regarding aquatic invertebrate analysis for biodiversity assessment can be found in section 5.2.3.1 (Chapter 5). Methods regarding stable isotope sample preparation can be found in section 5.2.3.2 (Chapter 5).

7.2.4 Statistical analysis

7.2.4.1 Invertebrate diversity

Aquatic invertebrate diversity was assessed for each site and survey using Margalef species richness (richness), Pielou's Evenness index (evenness), Shannon-Wiener diversity index (Shannon diversity) and Simpson's index. For full descriptions of these indices see section 4.2.3, Chapter 4. Parametric, unpaired student t-tests were conducted in Graphpad Prism 6 in order to assess possible significant spatial differences in the aquatic invertebrate community diversity between Sites 2 and 3 outside NGR and between Site 1 and the two sites outside NGR. Prior to further analysis, aquatic invertebrate data were transformed by way of square root ($\sqrt{}$) in order to normalise the data and reduce the effect of dominant taxa (Clarke and Warwick 2001). With the *indicspecies* package created for the R environment, multi-level pattern analysis (*multipatt*) was used to determine whether any aquatic invertebrates were significantly associated with a particular sampling site, survey or combination of the two (de Cáceras 2013; de Cáceras et al. 2016; R Core Team 2017). Using Canoco v5, an unconstrained principal component analysis (PCA) was created of Site 1, a comparative PCA of the two sites outside NGR (Site 2 and Site 3) and a comparative PCA of all three sites (Site 1, Site 2 and Site 3), respectively. This was to determine possible differences between surveys in terms of aquatic invertebrate data with water quality variables superimposed as supplementary explanatory variables. Water quality variable analysis and results can be found in section 3.3.2 (Chapter 3).

Pie charts were created to qualitatively compare macroinvertebrate family data collected during the study to samples collected between 2012 and 2013 by Smit et al. (2016) from the Phongolo River, and samples collected in 2014 by de Necker (2015) from floodplain pans in and around NGR. Sensitivity scores were obtained from scores determined by Dickens and Graham (2002). The sensitivity scores were separated into two large categories namely tolerant (sensitivity score 0–7) and sensitive (score 8–15) families. These categories were further separated into families that are highly tolerant (sensitivity score 0–4), moderately tolerant (sensitivity score 5–7), moderately sensitive (sensitivity score 8–12) and highly sensitive (sensitivity score 13–15). Quantitative comparisons were not possible since sampling techniques and identification differed between surveys.

7.2.4.2 Stable isotopes

The trophic position (TP) of the components of the food webs were calculated using the mean $\delta^{15}\text{N}$ signatures of each component compared to a basal reference as explained in section 6.2.2.2 (Chapter 6). Parametric unpaired student t-tests were used to determine if there were any significant changes in

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from the first to last surveys and between sampling sites. Food webs were created in Graphpad Prism 6 for each of the three sites during all sampled surveys using the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of all collected sources. A hierarchical Bayesian stable isotope mixing model (MixSIAR; Stock and Semmens 2016) was used to determine the consumer-food source interactions at each of the sampling sites over the three surveys using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios. Potential food sources were grouped into four biologically meaningful groups: C_3 plants, C_4 plants, aquatic invertebrates and fish. Grouping of multiple food sources is recommended practice if sources are logically related and/or within-group isotopic variation is less than between-group variation (Phillips et al. 2005; Phillips et al. 2014; Manfrin et al. 2018). Aquatic invertebrates were separated into two groups where distinct differences were present in the isotopic signatures, specifically $\delta^{15}\text{N}$. Aquatic invertebrates that were at a higher trophic position were placed under “Group A” and those that were at a lower trophic position were placed under “Group B”. Primary producers were separated into C_3 and C_4 plants as these sources have distinct isotopic signatures, particularly for $\delta^{13}\text{C}$. Detritus, biofilm and Poaceae were separated into both C_3 and C_4 categories depending on the $\delta^{13}\text{C}$ signature during a particular survey as discussed in section 6.2.2.2 (Chapter 6).

A mixing model was created for each of the surveys for each site and evaluated *a priori* (Smith et al. 2013; Phillips et al. 2014; Valladares et al. 2017; Lemmens et al. 2017) as discussed in section 6.2.2.2 (Chapter 6) and consumer individuals that were located outside the 95% mixing polygon region were excluded from the final mixing model. Two consumers were excluded from the Site 1 September 2016 model, one consumer was excluded from the Site 1 February 2017 model, 17 consumers were excluded from the Site 2 May 2016 model, seven individuals were excluded from the Site 2 September 2016 model, one individual was excluded from the Site 2 February 2017 model and three consumers were excluded from the Site 3 September 2016 model,

Model runs were performed for each of the sites and surveys using varying Markov Chains Monte Carlo (MCMC) parameters. The MCMC chain length ranged from 10 000 to 300 000 iterations with a burn-in phase ranging from 5 000 to 200 000 and a thinning range of 5 to 100 and was conducted on each model until all three MCMC chains converged. Convergence was based on both the Gelman-Rubin and Geweke diagnostics tests (Stock and Semmens 2016) (see section 6.2.2.2, Chapter 6). Consumers were included as fixed effects, Process only error terms were included in the model and the 95% credible intervals based on individual level variation for each individual consumer. The Bayesian mixing model was run using stable isotope data from all collected fish as consumers (raw data by site and survey), potential prey groups as food sources (raw data by site and survey) and a consistent discrimination factor of $1.0\text{‰} \pm 0.25\text{‰}$ for $\delta^{13}\text{C}$ (DeNiro and Epstein 1978; Akamatsu et al. 2004) and $3.37\text{‰} \pm 1.3\text{‰}$ for $\delta^{15}\text{N}$ (Taylor et al. 2017a) was used for all consumers.

7.3 Results

7.3.1 Aquatic invertebrates

A total of 131 taxa from 70 families was identified from the Phongolo River both within and outside NGR (Appendix E1). The greatest number of taxa and families were found within NGR (103 taxa from 61 families) compared to outside NGR (82 taxa from 50 families). Forty-three taxa were present only within NGR while 25 taxa were present only outside NGR. May 2016 was the survey with the greatest number of taxa (63) and September 2016 the survey with the least number of taxa (55) overall. Several taxa were found both within and outside NGR, including Libellulidae, *Caridina nilotica* (Atyidae), Lumbriculidae, *Lymnaea natalensis* (Lymnaeidae) and *Physa acuta* (Physidae). *Caridina nilotica* was also the most abundant taxon, with a total of 2187 individuals collected, of which most (680 individuals) were collected in May 2016 at Site 2. Lumbriculidae were most abundant (28 individuals) in August 2016 at Site 1 and *Lymnaea natalensis* and *Physa acuta* were most abundant (36 and 196 individuals, respectively) in February 2017 also at Site 1. The Order Harpacticoida was only collected during May 2016 within NGR while the genera *Larainae* (Elmidae), *Elassoneuria* (Oligoneuriidae), *Chimarra* (Philopotamidae), *Simulium* (Simuliidae) and *Hydropsyche* (Hydropsychidae) were found only outside NGR.

A much greater aquatic invertebrate diversity was found in the present study in comparison to both Smit et al. (2016), who found only 52 families, and the study by de Necker (2015) where only 113 taxa from 49 families were found (Appendix E2). Thirty-eight families were shared between the present study and Smit et al. (2016) while 52 taxa from 39 families were shared between the present study and de Necker (2015). In total, representatives of 27 families were shared between all studies from November 2012 to February 2017 and included Atyidae, Ceratopogonidae, Corixidae, Culicidae, Dytiscidae, Libellulidae, Lumbriculidae, Notonectidae and Thiaridae. Of the families previously recorded in the Phongolo River and floodplain wetlands, 27 families were not found during the present study, including Athericidae, Chlorocyphidae, Ephemeridae, Leptophlebiidae and Tricorythidae (Appendix E2). The abundance of sensitive families decreased from 2012 to 2017 as the greatest percentage of sensitive families were collected by Smit et al. (2016) and the lowest percentage during the present study (Figure 7.2). Of the 23% sensitive families collected by Smit et al. (2016), 6% were highly sensitive (sensitivity score ≥ 13) and 31% moderately sensitive (sensitivity score 8–12) (Figure 7.2a and 7.2b). Only 25% of the families collected by de Necker (2015) were classified as sensitive, and none of these were highly sensitive (Figure 7.2c and 7.2d). The present study also found fewer sensitive families, as only 22% were classified as sensitive, of which only 4% were highly sensitive and 18% moderately sensitive (Figure 7.2e and 7.2f). A much greater percentage (78%) of tolerant families were found during the present study than in the studies by Smit et al. (2016) and de Necker (2015) (63% and 75%, respectively).

The multi-pattern analysis indicated that no aquatic invertebrate taxa or families were significantly associated with any particular sampling events on the river either within or outside NGR. As such, only the best fitting taxa are shown on all the PCA graphs. On the PCA of Site 1, only the 50 best fitting taxa are shown, and this graph explains a total of 71.23% of data variation (Figure 7.3). Separation on both

axes is associated with differences in chemical water parameters as well as aquatic invertebrate abundance and diversity. On the first axis (explaining 38.49% of data variation) temperature is negatively correlated with May and August 2016 (Site 1.1 and Site 1.2 respectively) and positively correlated with September 2016 and February 2017 (Site 1.3 and Site 1.4, respectively). Separation on this axis is also attributed to differences in aquatic invertebrate abundance and diversity as a larger number of taxa were associated with August 2016 than with the other surveys. This is also indicated by the number of taxa (40), species richness (6.9) and Shannon diversity (2.8) that were generally higher during this survey (Figure 7.4). On the second axis (explaining 32.74% of data variation) separation is driven by differences in aquatic invertebrate diversity and abundance, which was much lower in February 2017, and conductivity, which was negatively correlated with May 2016 and August 2016.

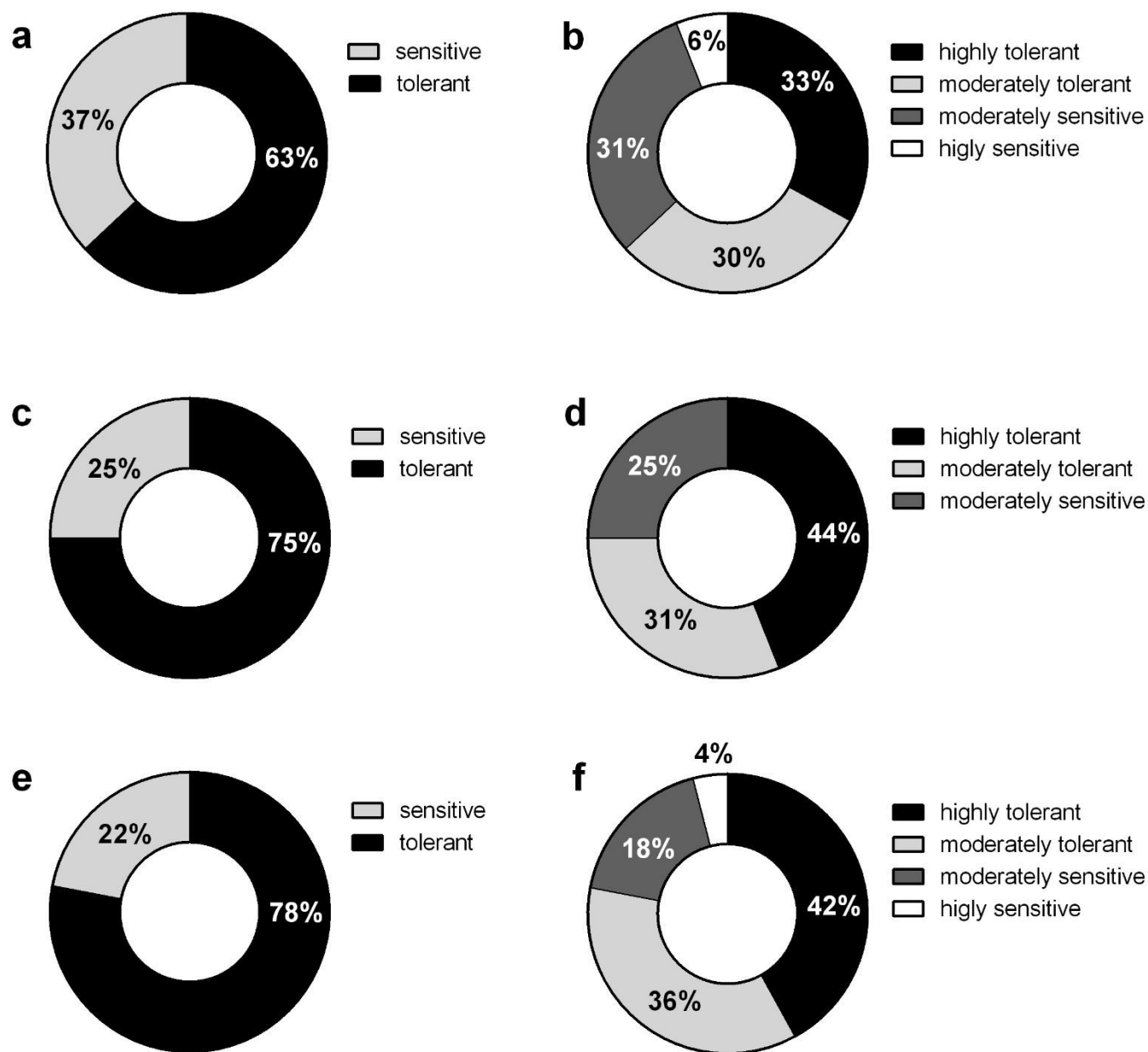


Figure 7.2: Pie charts indicating the percentage of sensitive (highly sensitive and moderately sensitive) and tolerant (highly and moderately tolerant) aquatic invertebrate families collected during surveys on the Phongolo River in 2012 and 2013 by Smit et al. (2016) (a and b), in 2014 by de Necker (2015) (c and d) and in 2016 – 2017 during the present study (e and f).

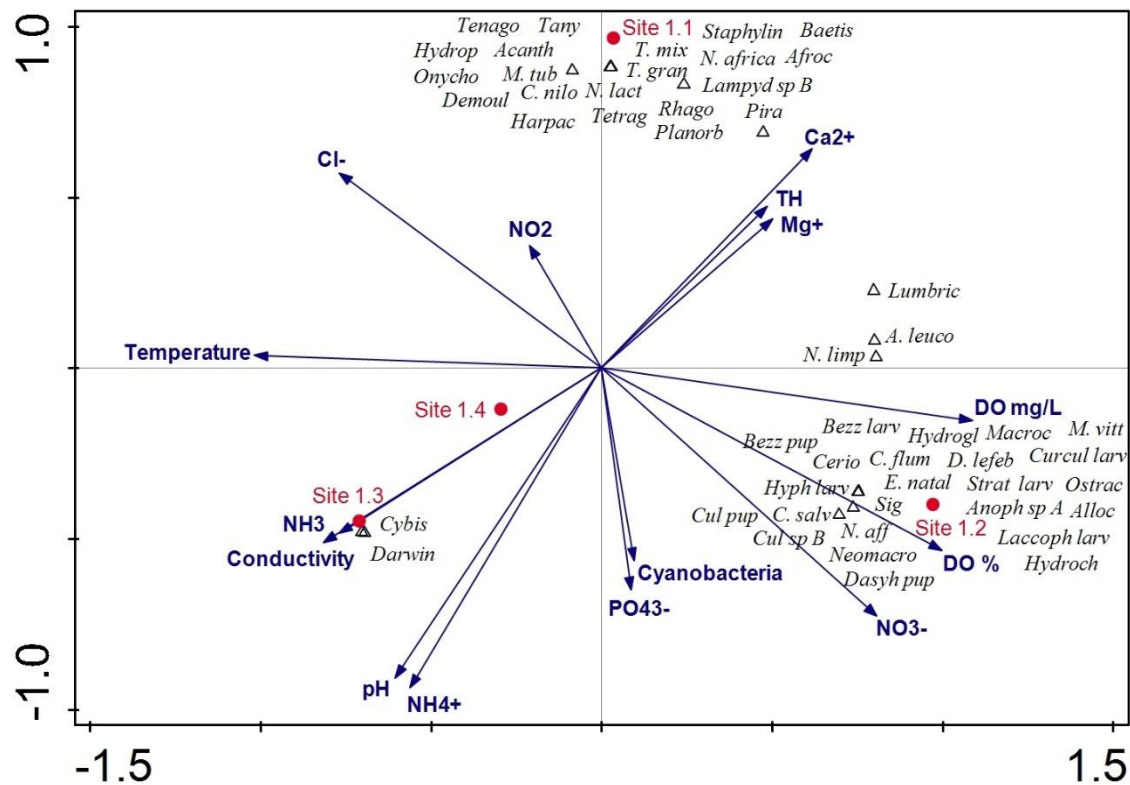


Figure 7.3: Principal Component Analysis (PCA) biplot of the 50 best fitting aquatic invertebrate taxa (triangles) collected in the Phongolo River within Ndumo Game Reserve (Site 1) during sampling surveys (circles) with chemical water nutrients (arrows) plotted as supplementary variables. The biplot explains 71.23% of data variation of which 38.49% is explained by the first axis and 32.74% is explained by the second axis. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. Full identities of the abbreviated invertebrate names are presented in Appendix E1.

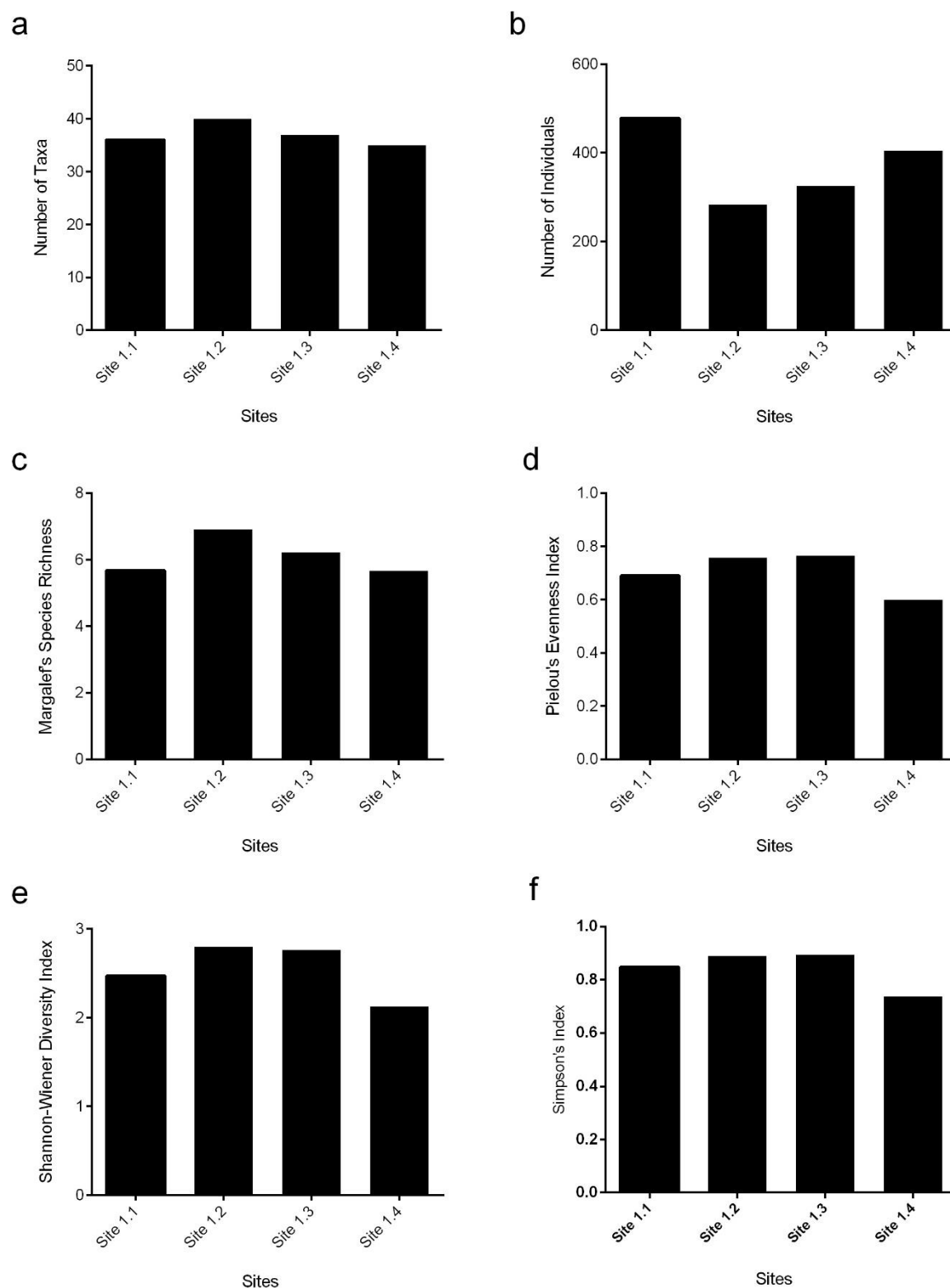


Figure 7.4: Number of taxa (a), number of individuals (b), Margalef's Species Richness (c) and Pielou's Evenness Index (d) Shannon-Wiener diversity (e) and Simpson's Index (f) of aquatic invertebrates collected in the Phongolo River within Ndumo Game Reserve (Site 1) during sampling surveys. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017.

Only 30 of the best-fitting taxa are shown on the PCA comparing the two sites outside NGR (Site 2 and Site 3) and explains a total of 69.64% of data variation (Figure 7.5). Separation on the first axis (explaining 42.47% of data variation) is largely due to a general decrease in diversity from May 2016 (Site 2.1) to February 2017 (Site 2.4) at Site 2 as also indicated by all the invertebrate diversity indices (Figure 7.6). On the second axis (explaining 27.17% of data variation), separation is due to Site 3.4 (February 2017 survey) separating from all other sites. This is attributed to an increase in the number of taxa and individuals at Site 3 from September 2016 (21 and 131, respectively) to February 2017 (27 and 733, respectively) as also indicated by the diversity indices (Figure 7.6). Although diversity was generally higher at Site 3 (Site 3.3 and Site 3.4) than at Site 2 (Site 2.3 and Site 2.4), no true spatial differences were present between these sites as the only significant difference was Simpson's index which was higher at Site 2 than at Site 3 ($p < 0.05$).

The comparative PCA between the river site inside NGR (Site 1) and the two sites outside NGR (Site 2 and Site 3) explains a total of 41.9% and suggests both spatial and temporal variation between sites and surveys. This is largely due to differences in aquatic invertebrate diversity and chemical water nutrients (Figure 7.7). On the first axis (explaining 22.76% of data variation) separation is due to seasonal variation in chemical water parameters, as the colder sampling surveys (May and August 2016, austral winter) separated from the warmer sampling surveys (September 2016 and February 2017, austral summer). Separation on the second axis (explaining 19.14% of data variation) is largely due to higher aquatic invertebrate diversity collected during May 2016 (Site 1.1 and Site 2.1) compared to the other surveys. In addition, Site 1 had significantly higher species richness, evenness, Shannon and Simpson's diversity than either Site 2 or Site 3 outside NGR (Figure 7.8).

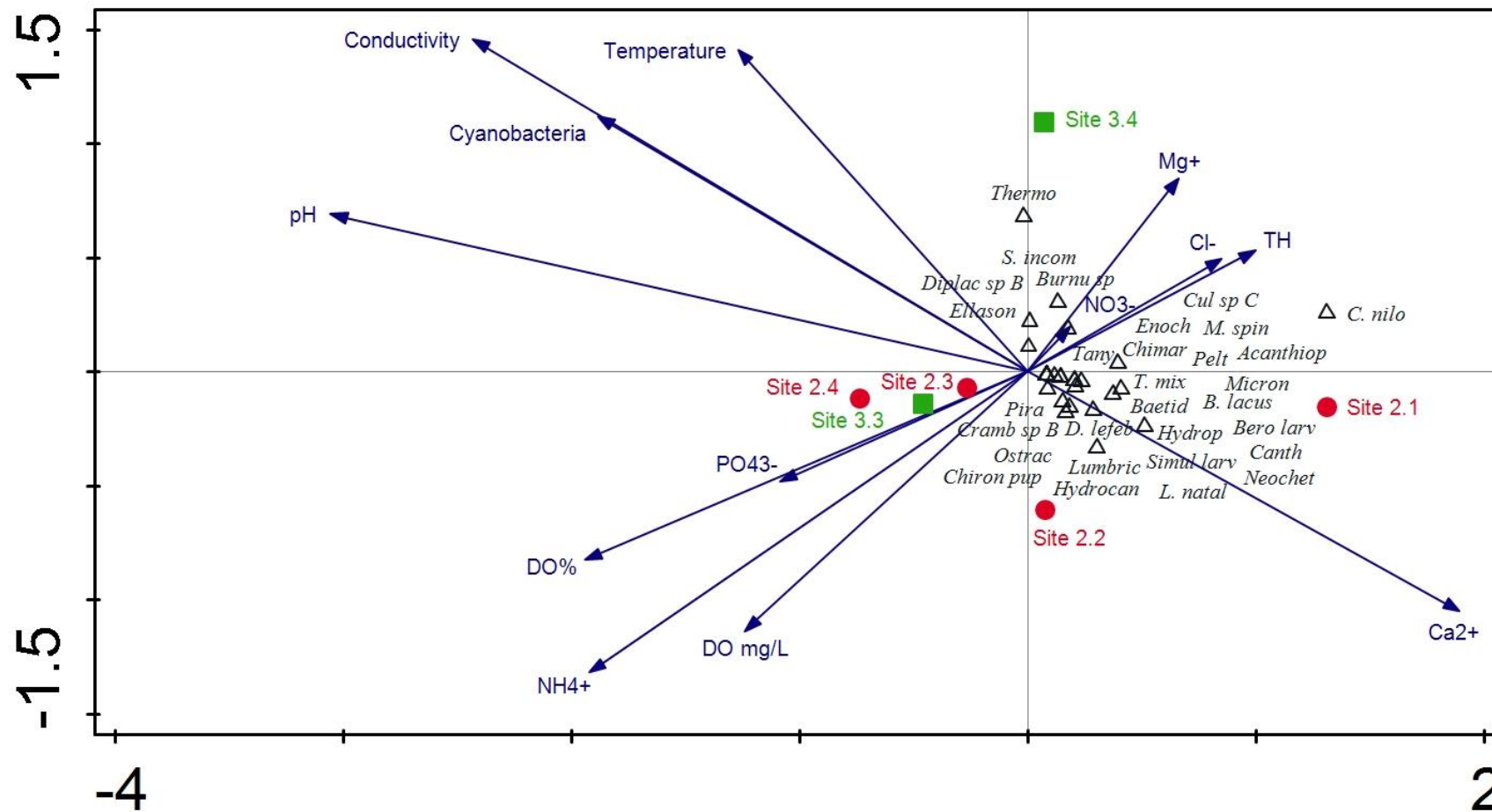


Figure 7.5: Principal Component Analysis (PCA) biplot of the 30 best fitting aquatic invertebrate taxa (triangles) collected at the two sampling sites on the Phongolo River outside Ndumo Game Reserve (Site 2 and Site 3) during sampling surveys (circles) with chemical water nutrients (arrows) plotted as supplementary variables. The biplot explains 69.64% of data variation of which 42.47% is explained by the first axis and 27.17% is explained by the second axis. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. Full identities of the abbreviated invertebrate names are presented in Appendix E1.

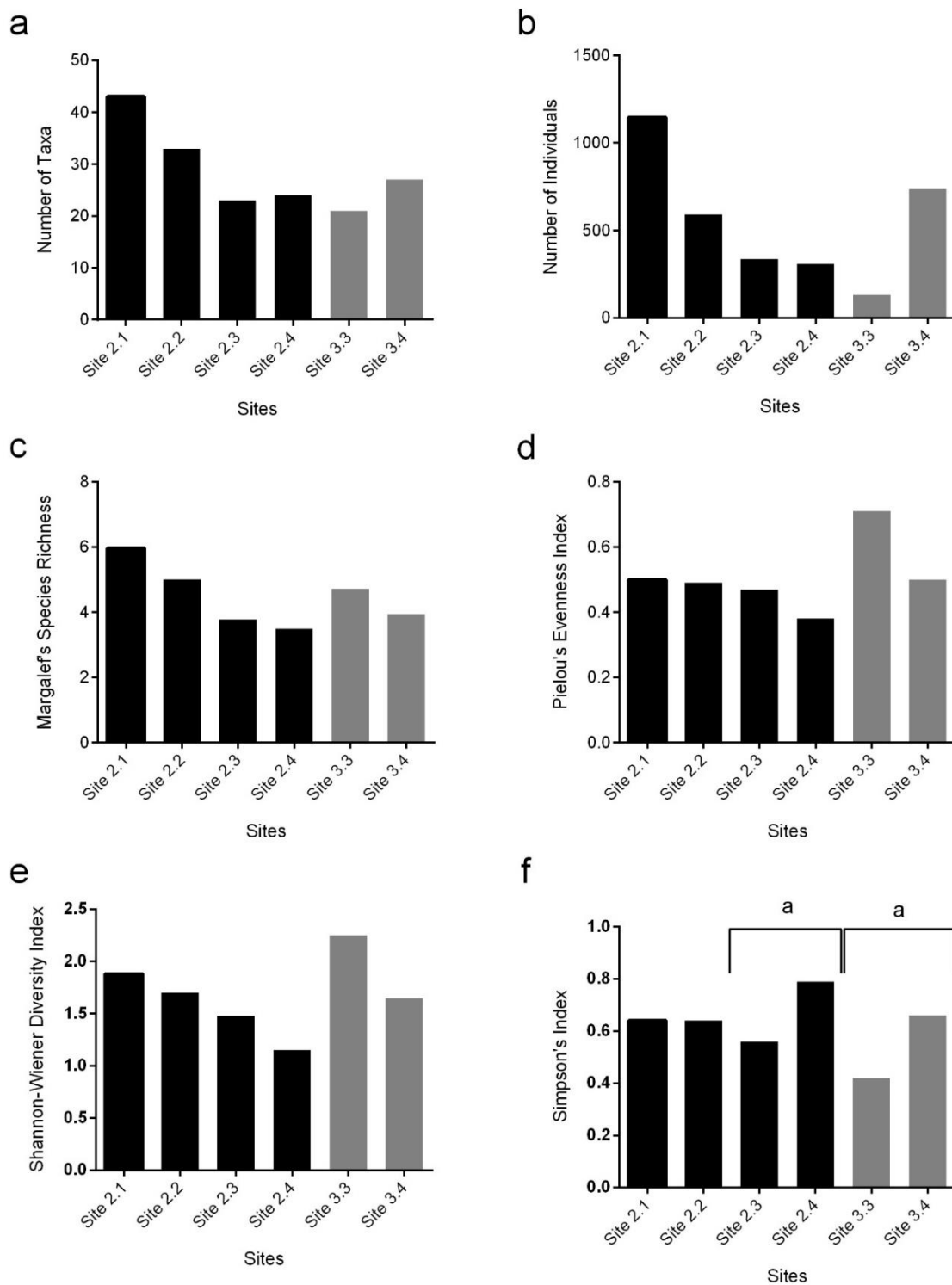


Figure 7.6: Number of taxa (a), number of individuals (b), Margalef's Species Richness (c), Pielou's Evenness Index (d), Shannon-Wiener diversity (e) and Simpson's Index (f) of aquatic invertebrates collected at the two sampling sites on the Phongolo River outside Ndumo Game Reserve (Sites 2 and 3) during sampling surveys. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. Similar denomination indicates significant differences.

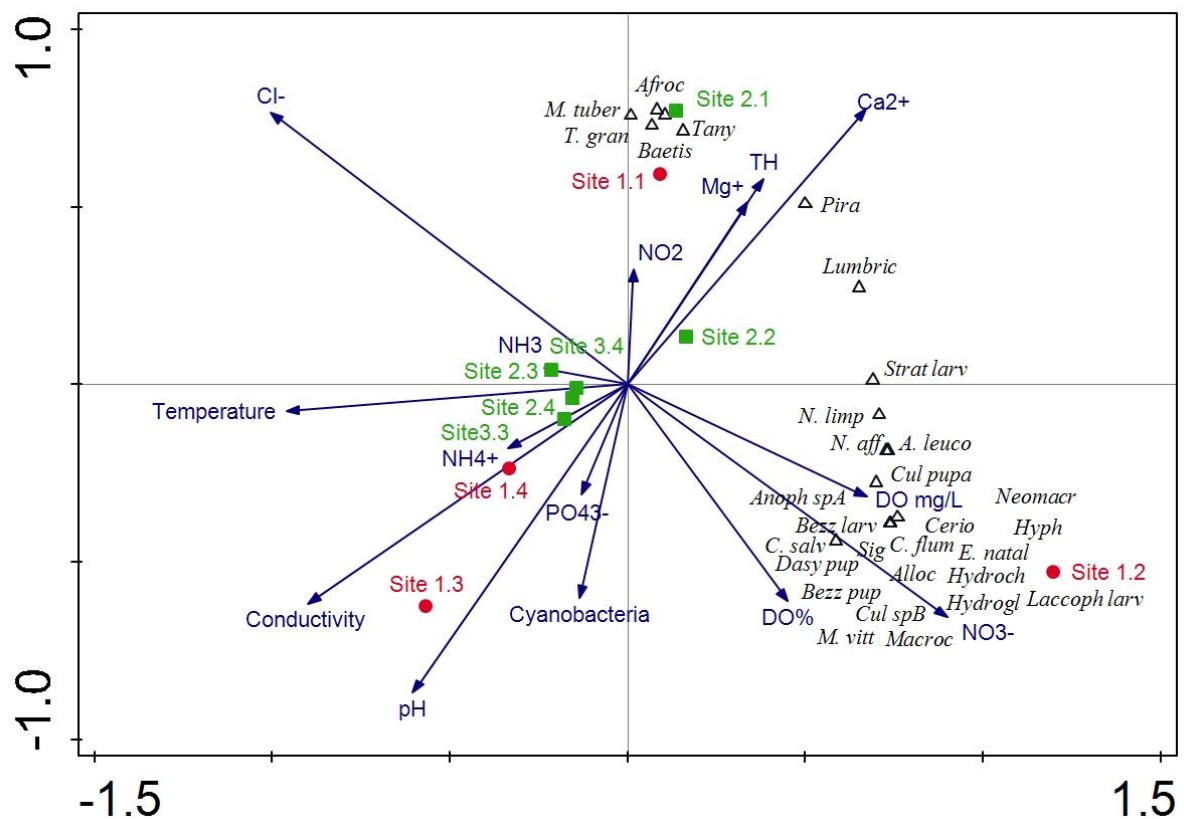


Figure 7.7: Comparative Principal Component Analysis (PCA) biplot of the 30 best fitting aquatic invertebrate taxa (triangles) collected in the Phongolo River within (Site 1) and outside (Sites 2 and 3) Ndumo Game Reserve during sampling surveys (circles) with chemical water nutrients (arrows) plotted as supplementary variables. The biplot explains 37.49% of data variation of which 20.45% is explained by the first axis and 17.04% is explained by the second axis. Full identities of the abbreviated invertebrate names are presented in Appendix E1.

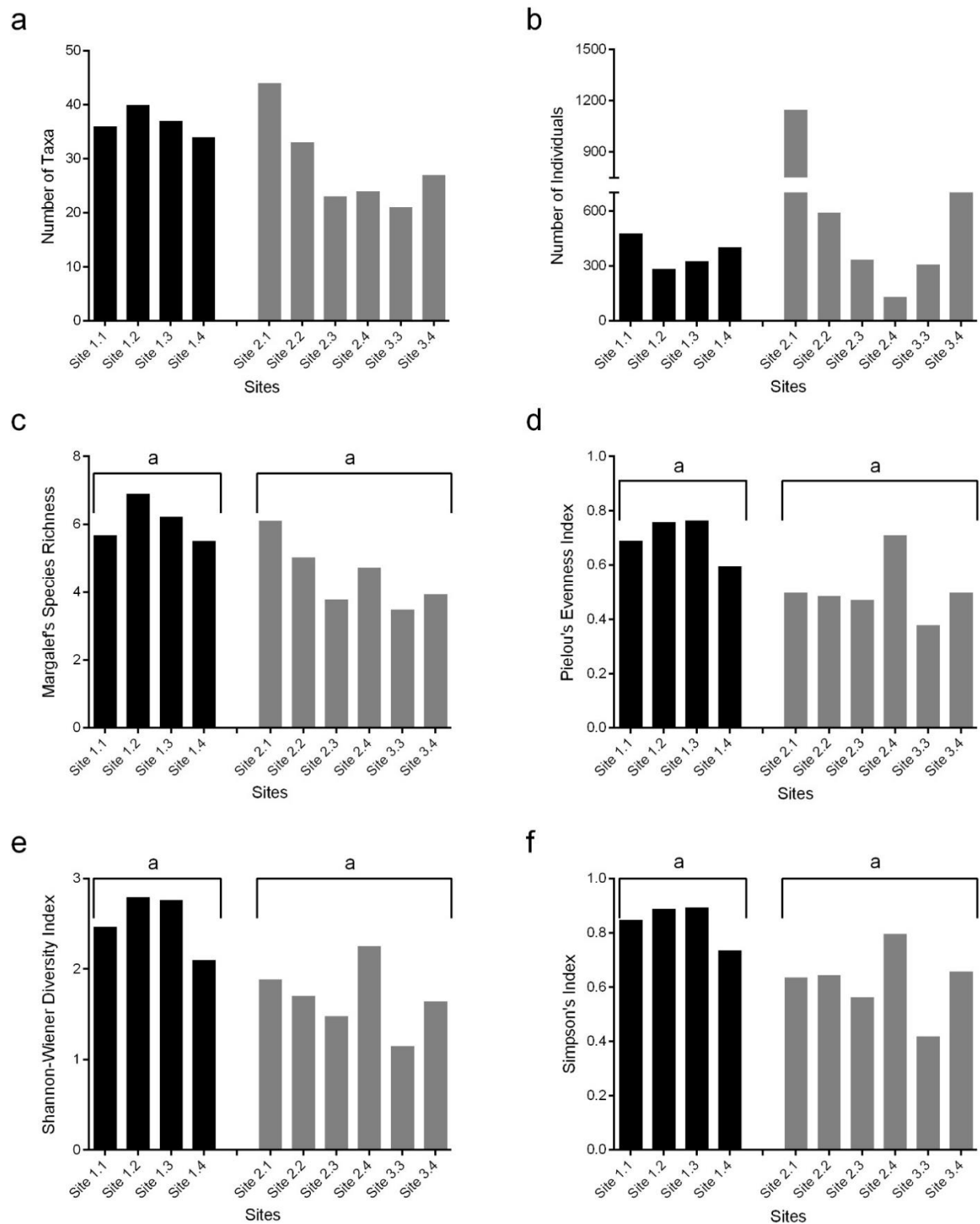


Figure 7.8: Comparison of number of taxa (a), number of individuals (b), Margalef's Species Richness (c), Pielou's Evenness Index (d), Shannon-Wiener diversity (e) and Simpson's Index (f) of aquatic invertebrates collected in the Phongolo River within (Site 1) and outside (Site 2 and Site 3) Ndumo Game Reserve during sampling surveys. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. Similar denomination indicates significant differences.

7.3.2 Food webs

A total of 20 fish species from 10 families was collected in the Phongolo River both within and outside NGR. Of these, 11 species from 8 families were found within NGR and 16 species from 8 families outside NGR (see section 2.2, Chapter 2 for photographs of some of these fish species).

Different trophic levels were discernible on all food webs. Site 1 within NGR had four trophic levels throughout the study, while Sites 2 and 3 outside NGR ranged between three and four trophic levels between seasons (Appendix E3 - E5). A general trend throughout all the food webs was that fish were at or near the top of the food web, while the base of the food web was made up of leaf litter, detritus and biofilm (Figures 7.9 – 7.12). Aquatic invertebrate trophic positions generally depended on feeding strategies. Predatory invertebrates (Dytiscidae, Libellulidae, Gomphidae, Coenagrionidae) were at least one position above other aquatic invertebrates. The southern Churchill *Petrocephalus wesselsi* (Mormyridae) was found to be at the top of the food web at Site 1 during both September 2016 and February 2017 (Figure 7.9a and 7.9b). At Site 2, the Goldsilk seabream *Acanthopagrus berda* (Sparidae) was at the top of the food web in May 2016 together with the tank goby *Glossogobius giuris* (Gobiidae) (Figure 7.10a). The silver robber *Micralestes acutidens* (Alestidae) was at the top of the food web in September 2016 (Figure 7.10b) and *G. giuris* in February 2017 (Figure 7.11). In September 2016 at Site 3 the sawfin suckermouth *Chiloglanis paratus* (Mochokidae) and *A. berda* were at the top of the food web (Figure 7.12a), while predatory aquatic invertebrates (Belostomatidae and Aeshnidae) were at the top of the food web at Site 3 in February 2017, along with fish (Figure 7.12b).

Significant shifts were found in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from the first to last survey at all sites both within and outside NGR (Appendix E6 - E12). Within NGR, fewer distinct shifts were evident in the $\delta^{13}\text{C}$ of food web components while significant enrichment of $\delta^{15}\text{N}$ occurred in all but three components from September 2016 to February 2017 ($p < 0.05$). Outside NGR, $\delta^{13}\text{C}$ generally decreased towards February 2017 survey while $\delta^{15}\text{N}$ was most enriched in September 2016, after which it decreased towards February 2017. Significant differences were also found between Site 2 and Site 3 for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ during September 2016 and February 2017 surveys. In September 2016, $\delta^{13}\text{C}$ was generally lower and $\delta^{15}\text{N}$ higher at Site 3, while in February 2017 $\delta^{13}\text{C}$ was significantly lower at Site 2 and $\delta^{15}\text{N}$ significantly higher at Site 3. The $\delta^{13}\text{C}$ was generally lower outside NGR in September 2016 and higher in February 2017 while the $\delta^{15}\text{N}$ remained highest outside NGR during both these surveys.

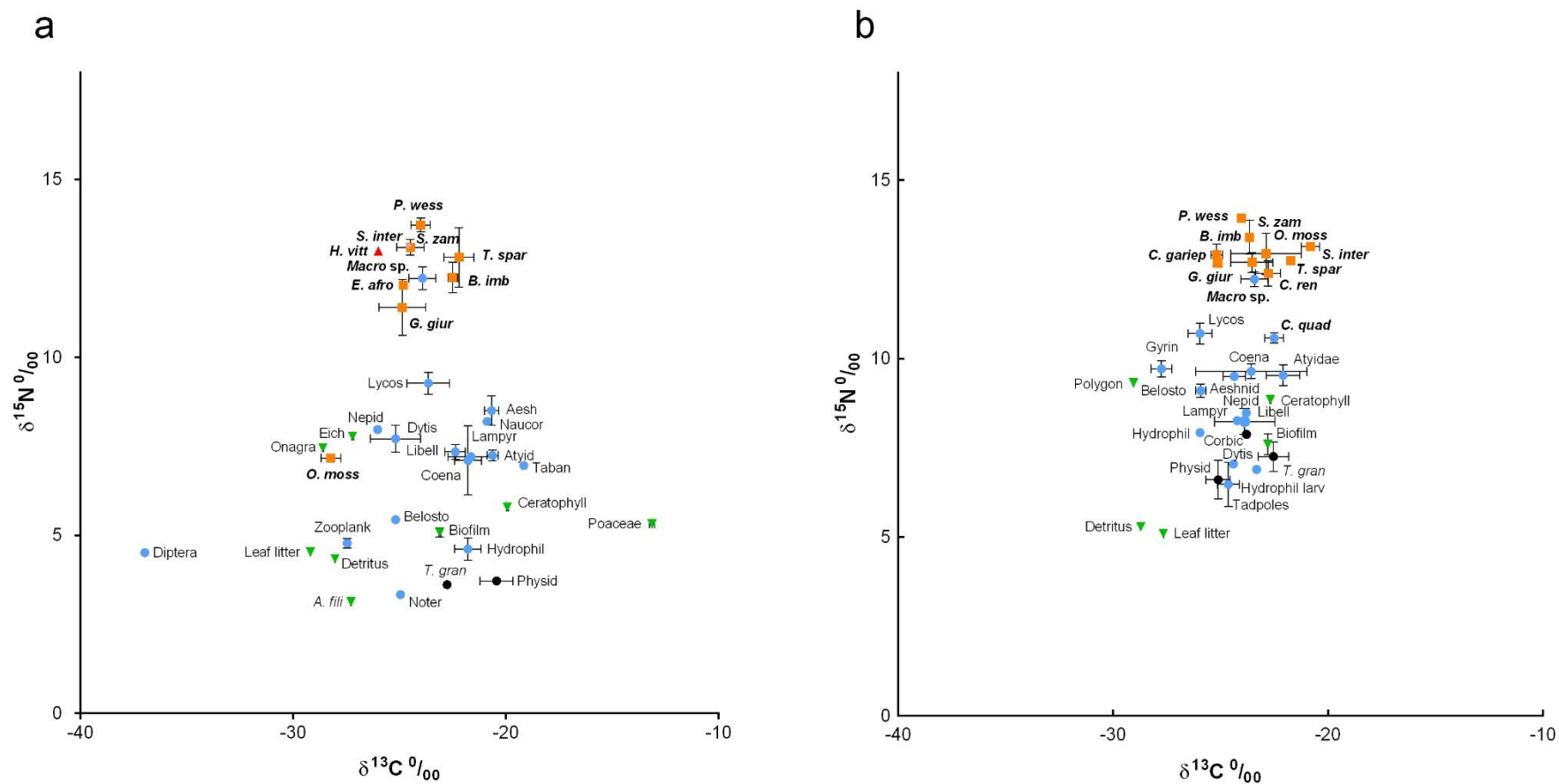


Figure 7.9: Biplots indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (±1 SE) of the various food web components collected during September 2016 (a) and February 2017 (b) sampling surveys at Site 1 on Phongolo River within Ndumo Game Reserve. Full identities of the abbreviated sample names are presented in Appendices E3 – E5.

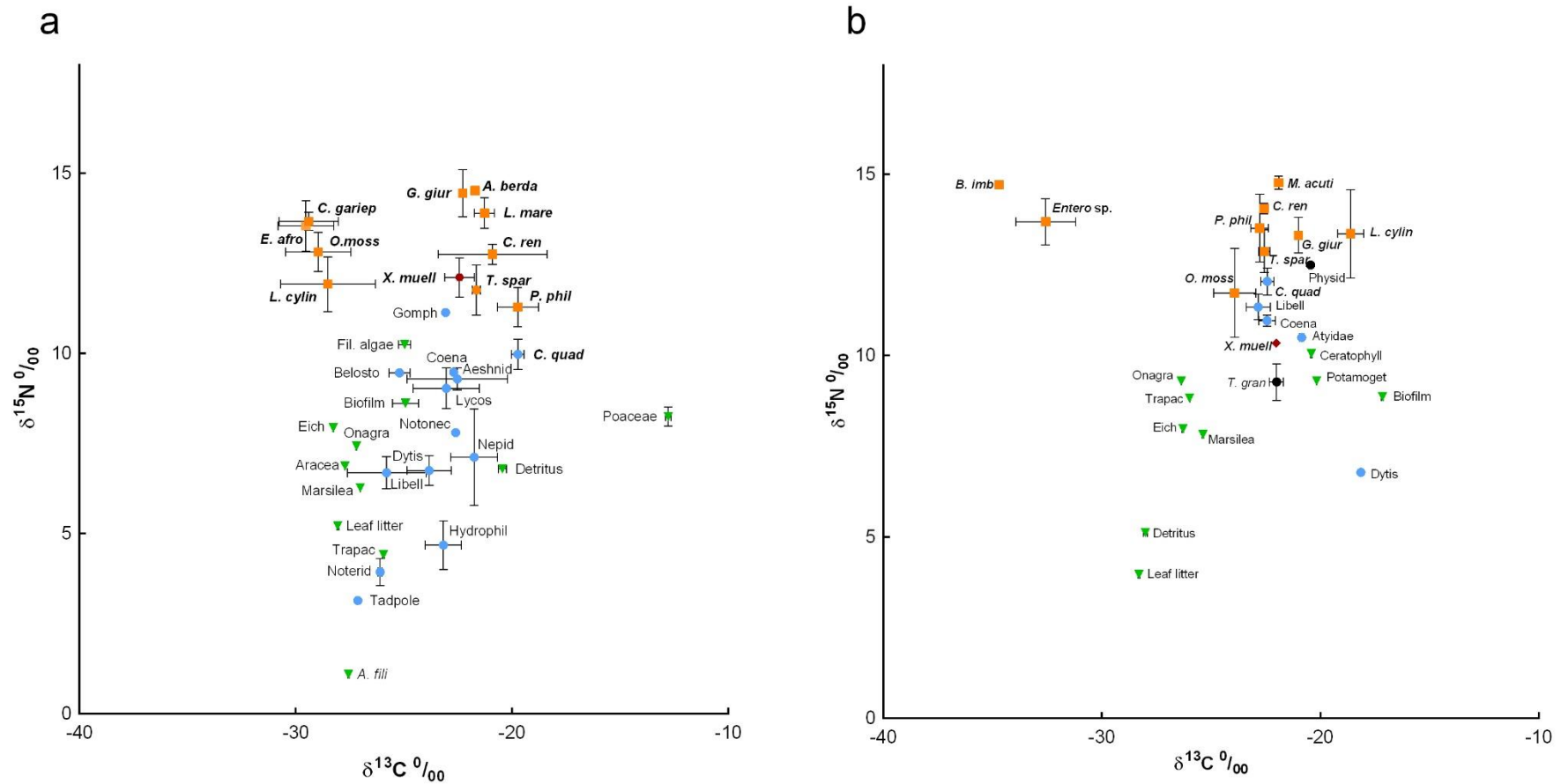


Figure 7.10: Biplots indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (±1 SE) of the various food web components collected during May 2016 (a) and September 2016 (b) sampling surveys at Site 2 on the Phongolo River outside Ndumo Game Reserve. Full identities of the abbreviated sample names are presented in Appendices E3 – E5.

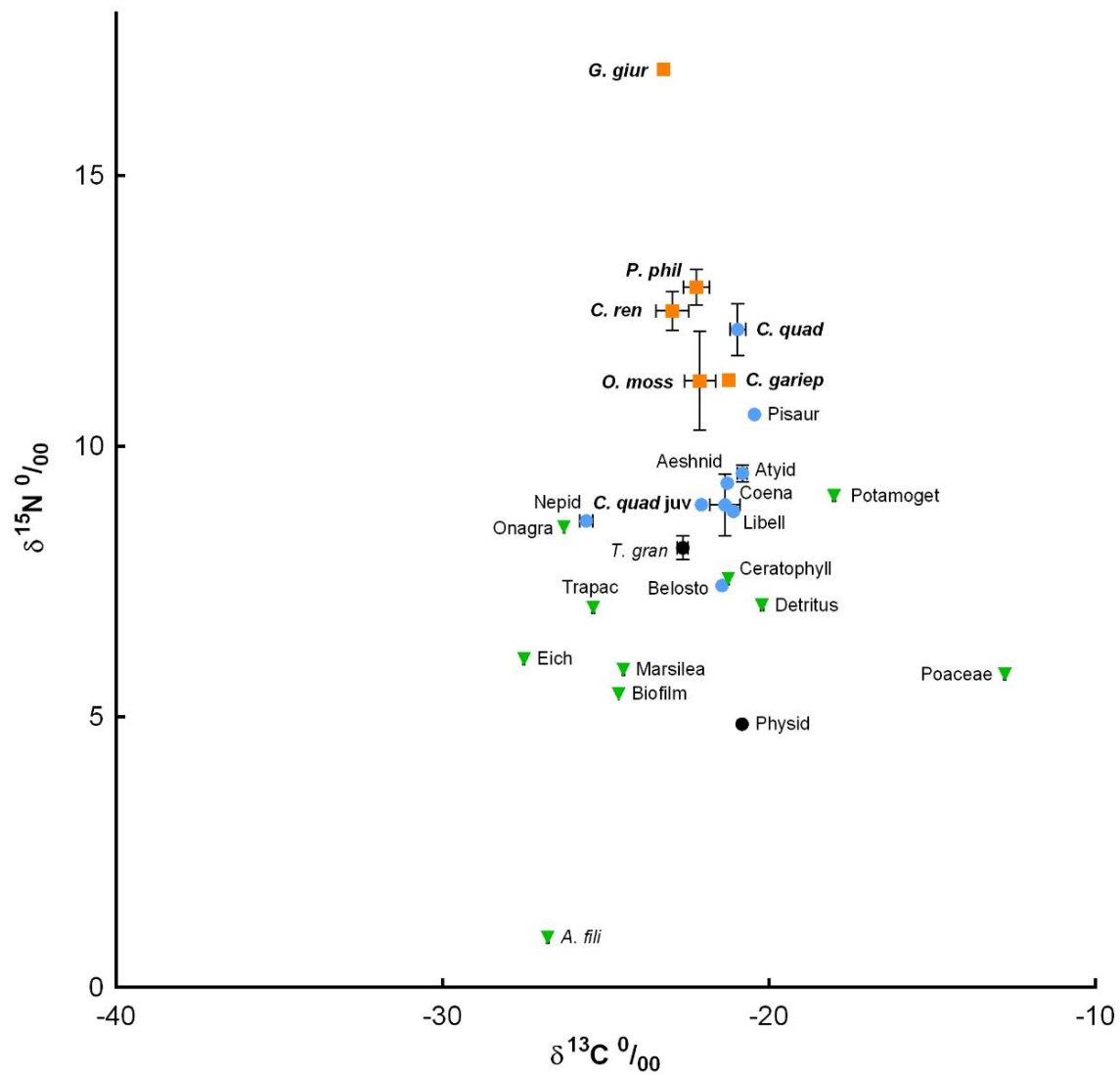


Figure 7.11: A biplot indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the various food web components collected during February 2017 at Site 2 on the Phongolo River outside Ndumo Game Reserve. Full identities of the abbreviated sample names are presented in Appendices E3 – E5.

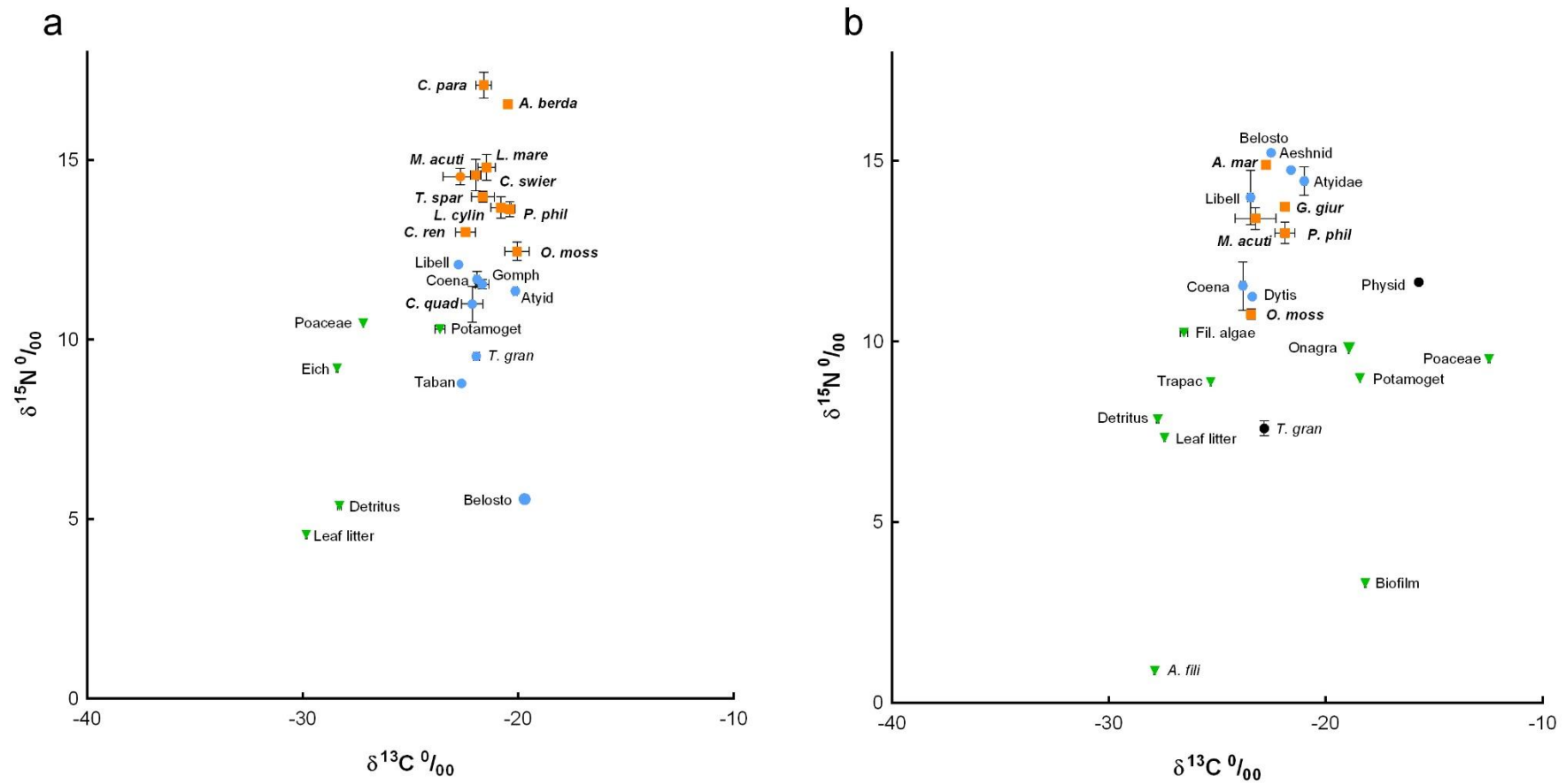


Figure 7.12: Biplots indicating the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures (± 1 SE) of the various food web components collected during September 2016 (a) and February 2017 (b) sampling surveys at Site 3 on the Phongolo River outside Ndumo Game Reserve. Full identities of the abbreviated sample names are presented in Appendices E3 – E5.

7.3.3 Dietary analysis

Within NGR (Site 1), although most consumers generally preyed on an integrated mixture of all food sources in September 2016, C₃ plants made up the largest portion of most consumer diets (32.8% - 61.8%), while all other food sources individually made up a smaller percentage (<30%) (Figure 7.13a; Table 7.1). Two fish species, namely *P. wesselsi* and the banded tilapia *Tilapia sparmanii* (Cichlidae) preyed mainly on fish (36.3% and 29.3%, respectively) while the food source least utilised by all consumers was C₄ plants (2.3% - 10.7%). In February 2017, the isoplot and credible intervals indicated that aquatic invertebrates made up the largest portion of all consumer diets (41.1% - 55.9%) while the remaining food sources were utilised less (<30% individually) (Figure 7.13b; Table 7.2). Both C₃ and C₄ plants were least utilised by all consumers (<15%).

At Site 2, the isoplot and credible intervals indicated that fish were generally the largest contributing food source (27.4% - 49.5%) to the diet of all but two consumers in May 2016 (Figure 7.14a; Table 7.3). The redeyed Labeo, *Labeo cylindricus* (Cyprinidae) and the southern mouthbrooder *Pseudocrenilabrus philander* (Cichlidae) consumed C₄ plants most (39.4% and 32.6% respectively), while *T. sparmanii* consumed C₃ plants most (26.4%). All other food sources made up a smaller integrated part of the consumer diets and generally the least consumed food source was nitrogen low invertebrates (Group B invertebrates) (6.2% - 12.1%). In September 2016, most fish consumed an integrated mixture of all food sources although there appeared to be a preference for both C₃ and C₄ plants (Figure 7.14b; Table 7.4). In particular, *L. cylindricus* consumed C₄ plants most (61.3%±22.4%) and the Mozambique tilapia *Oreochromis mossambicus* (Cichlidae) consumed C₃ plants most (66%±12.5%). Fish was the food source least preyed on by all consumers (<11%). In February 2017, the consumers again ingested a mixture of all food sources although invertebrates were more preyed on (36.8% - 41.3%) by all but *Coptodon rendalii*, which consumed C₃ plants most (44.5%±13.7%) (Figure 7.15; Table 7.5). The two least-consumed food sources were fish and C₄ plants (<15%).

At Site 3, the isoplot and credible intervals indicated that in September 2016 consumer diets were largely made up of an integrated mixture of all food sources, although C₄ plants made the largest contribution to most consumer diets (38.2% - 64.7%) (Figure 7.16a; Table 7.6). *Labeo cylindricus*, *O. mossambicus* and *P. philander* preyed largely on invertebrates (50.4%, 70.9% and 60%, respectively) and fish and C₃ plants were consumed much less (<10%). In February 2017, it was found that consumers ingested an integrated diet of all food sources, although C₃ plants appeared to be most consumed (38.1% - 60.8%) and fish least consumed (8.2% - 15.5%) by all but the giant mottled eel *Anguila marmorata*, which ingested C₄ plants least (17.9%±14.0%) (Figure 7.16b; Table 7.7).

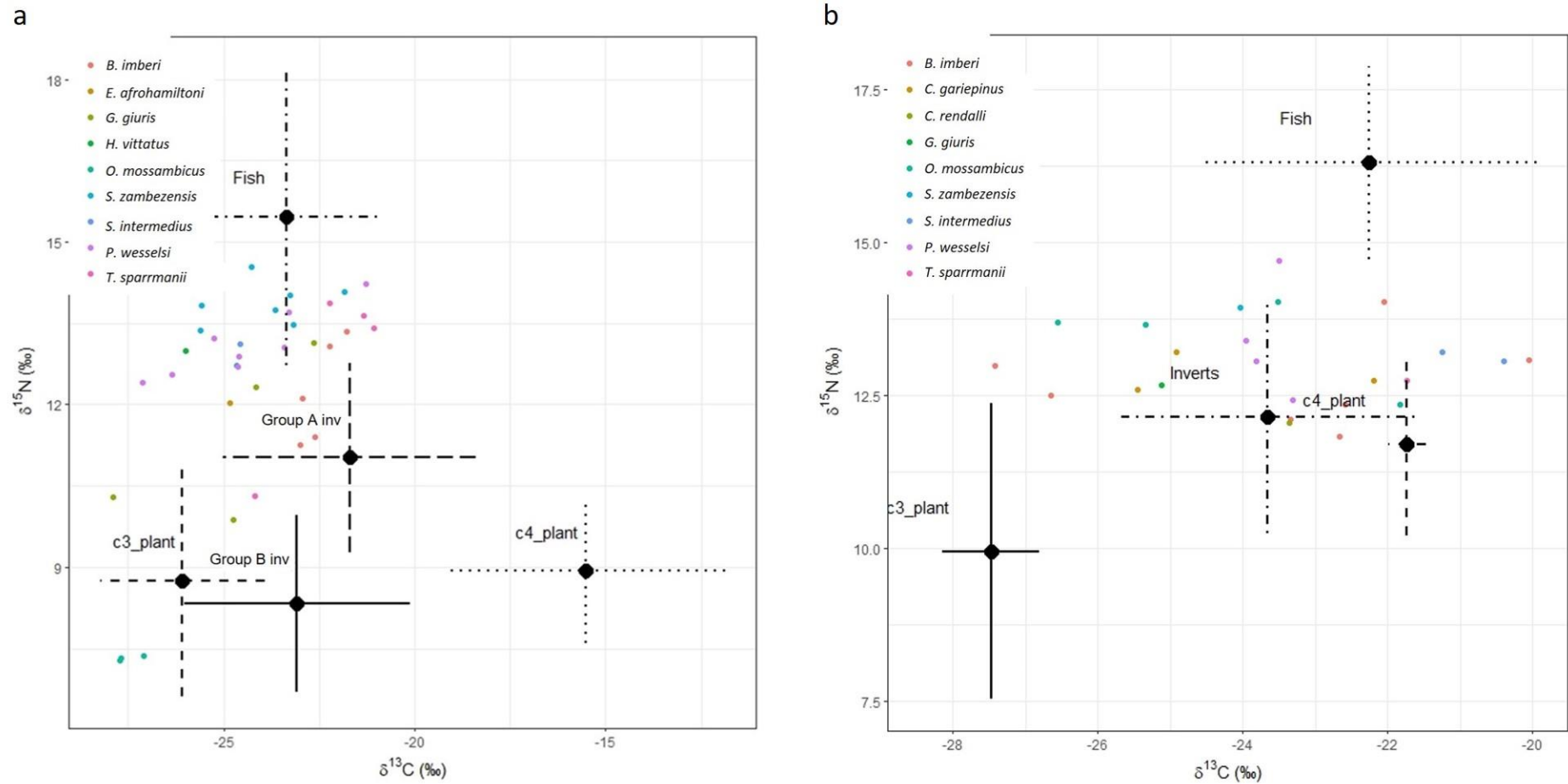


Figure 7.13: Biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) collected in the Phongolo River within Ndumo Game Reserve (Site 1) collected during the September 2016 (a) and February 2017 (b) sampling surveys. Fish consumer data and source data are abbreviated: Fish – fish food sources; Group A inv – invertebrates with higher nitrogen signature; Group B inv – invertebrates with lower nitrogen signature; Inverts – Aquatic invertebrate families; c3_plant – C_3 plants; c4_plant – C_4 plants. Error bars represent 95% confidence intervals. Taxa included in each source group can be found in Appendix E13 and E14.

Table 7.1: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected in the Phongolo River within Ndumo Game Reserve (Site 1) in September 2016. Mean±SD (95% Credible interval).

Sources	<i>Brycinus imberi</i>	<i>Enteromius afrohamiltoni</i>	<i>Glossogobius giuris</i>	<i>Hydrocynus vittatus</i>	<i>Oreochromis mossambicus</i>	<i>Petrocephalus wesselsi</i>	<i>Schilbe intermedius</i>	<i>Synodontis zambezensis</i>	<i>Tilapia sparrmanii</i>
Group A invertebrates	15.7±10.2 (1.1 – 38.6)	16.1±16.3 (0.4 – 60.2)	14.1±14.9 (0.4 – 56.5)	14±15.3 (0.4 – 58.8)	4±3.9 (0.2 – 14.5)	11.6±8.8 (0.5 – 32.9)	15±15.2 (0.5 – 58.7)	11.4±9.8 (0.4 – 37)	20.7±17.1 (0.5 – 59.4)
Group B invertebrates	21.1±12.1 (1.5 – 46.4)	21.2±18.1 (0.6 – 67.3)	22.8±20.2 (0.4 – 68.3)	18.8±17.5 (0.6 – 61.8)	29±32.6 (0.2 – 90.9)	15.5±10.5 (0.8 – 39.1)	18.4±16.1 (0.5 – 58.6)	17.9±13.4 (0.7 – 47.5)	18±14.1 (0.6 – 51.8)
C₃ plants	32.9±12.3 (9.4 – 56.8)	34.4±21.6 (2.8 – 80.3)	45.3±23.7 (2.9 – 83.6)	36.9±23.7 (2.8 – 83)	61.8±33.5 (1.4 – 95.9)	31.3±13.4 (4.9 – 56.9)	32.8±20.9 (2.9 – 77.9)	36.9±17 (4.4 – 67.4)	21.4±14.1 (2.4 – 54.2)
C₄ plants	7.5±6.2 (0.3 – 22.8)	6.3±7.5 (0.1 – 27.5)	6.8±9.2 (0.1 – 34.4)	4.4±5 (0.1 – 17.7)	5±6.2 (0.1 – 21.7)	2.3±2.6 (0.0 – 9.7)	5.4±5 (0.1 – 27.6)	4.4±4.7 (0.1 – 16.4)	10.7±10.4 (0.1 – 35.8)
Fish	22.8±6.4 (11.6 – 36.1)	22±15.8 (2.2 – 64.2)	13.3±8.3 (2.4 – 33.3)	25.3±17.2 (2.8 – 67.4)	2.9±2.3 (0.4 – 8.4)	36.3±9.1 (20.4 – 55.8)	27.4±16.5 (3.2 – 65.8)	29.5±9.7 (13.4 – 51.5)	29.3±12.4 (9.1 – 57.8)

Table 7.2: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected in the Phongolo River inside Ndumo Game Reserve in February 2017. Mean±SD (95% Credible interval).

Sources	<i>Brycinus imberi</i>	<i>Clarias gariepinus</i>	<i>Coptodon rendalii</i>	<i>Glossogobius giuris</i>	<i>Oreochromis mossambicus</i>	<i>Petrocephalus wesselsi</i>	<i>Schilbe intermedius</i>	<i>Synodontis zambezensis</i>	<i>Tilapia sparrmanii</i>
C₃ plants	9.7±6.4 (0.8 – 25.8)	13.3±11.4 (0.3 – 39.6)	12±11.4 (0.3 – 41.8)	16.1±16.3 (0.2 – 56.8)	10.9±10 (0.2 – 35.8)	11.5±11.5 (0.3 – 41.8)	5.3±5 (0.2 – 29.2)	13±9.8 (0.4 – 34.2)	7.5±7.3 (0.3 – 27.3)
C₄ plants	9.8±6.1 (0.9 – 32.8)	10±8.8 (0.3 – 32.8)	16±16.4 (0.3 – 57.4)	9.7±9.1 (0.3 – 33.5)	7.3±6.7 (0.3 – 25)	11.4±11.4 (0.3 – 42.1)	12.6±13.4 (0.3 – 48.6)	15.8±12.9 (0.5 – 45.3)	15.8±17.6 (0.3 – 65.7)
Fish	24.6±11.7 (6.6 – 51.8)	23.6±14.7 (2 – 53.8)	21±15.1 (1.7 – 58.2)	21.4±16.3 (1.5 – 61.3)	27.5±16.7 (2 – 60.9)	29.7±19.8 (1.8 – 73)	32.1±21.9 (1.7 – 76)	30.1±13.5 (4.3 – 56.1)	26.3±19.5 (1.4 – 72.5)
Invertebrates	55.9±16.3 (12.7 – 79.9)	53.1±21.5 (9.1 – 91.3)	50.9±23.7 (6.6 – 92.6)	52.8±24.7 (5.6 – 93.2)	54.3±22.8 (6.6 – 91.9)	47.3±24.1 (5.4 – 91.9)	50.1±26.3 (4.4 – 92.9)	41.1±19.4 (6.6 – 82.2)	50.3±25.2 (4.7 – 92.9)

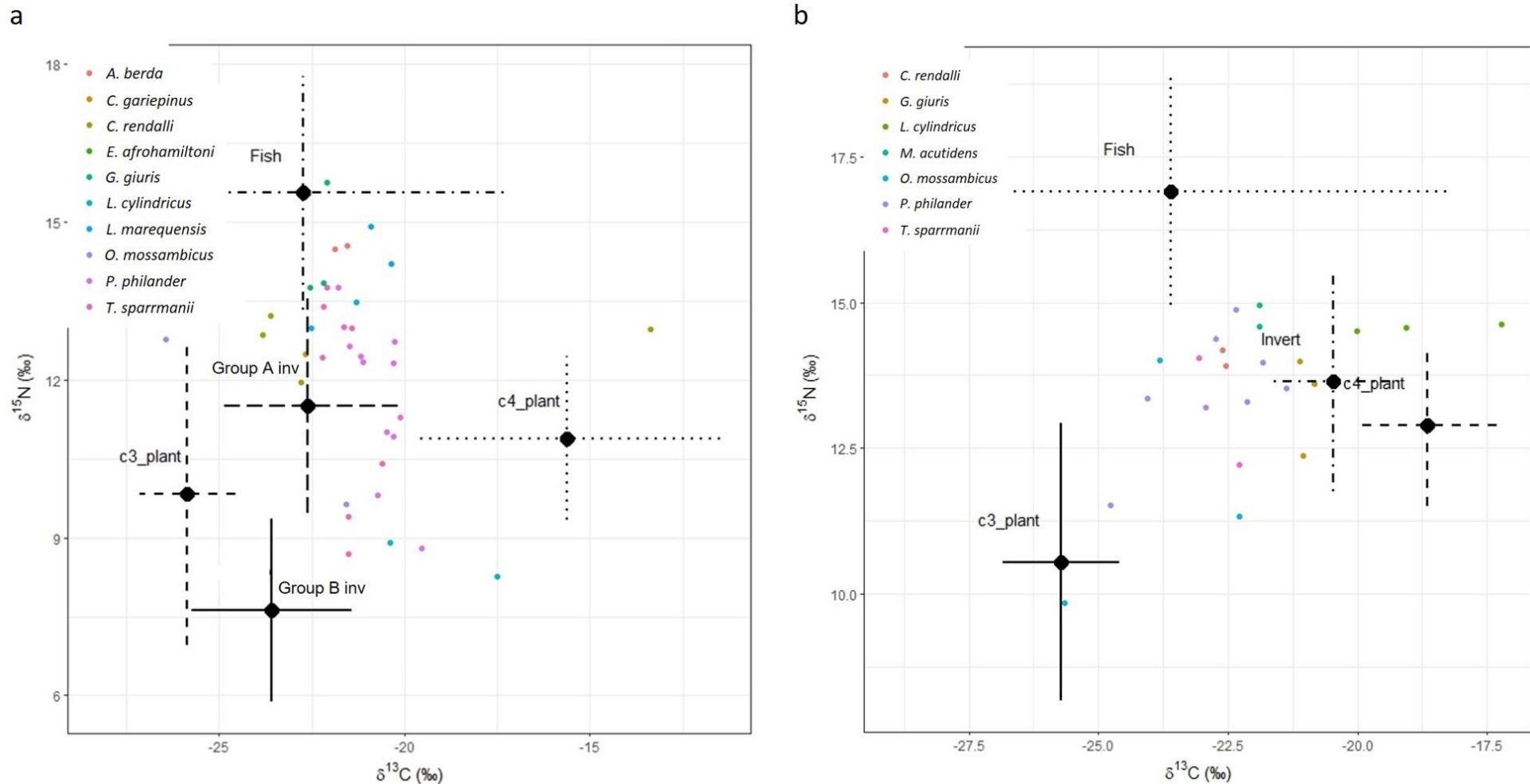


Figure 7.14: Biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) of Site 2 collected during the May 2016 (a) and September 2016 (b) sampling surveys. Fish consumer data and source data are abbreviated: Fish – fish food sources; Group A inv – invertebrates with higher nitrogen signature; Group B inv – invertebrates with lower nitrogen signature; Inverts – Aquatic invertebrate families; c3_plant – C₃ plants; c4_plant – C₄ plants. Error bars represent 95% confidence intervals. Taxa included in each source group can be found in Appendix E13 and E14.

Table 7.3: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 2 in May 2016. Mean±SD (95% Credible interval).

Sources	<i>Acanthopagrus berda</i>	<i>Clarias gariepinus</i>	<i>Coptodon rendalii</i>	<i>Enteromius afrohamiltoni</i>	<i>Glossogobius giuris</i>
Group A invertebrates	17.7±13.7 (0.9 – 51.5)	20.2±19 (0.4 – 69)	16.9±19.7 (0.4 – 75.2)	13.6±17.9 (0.2 – 74.9)	15.6±15.5 (0.4 – 57.5)
Group B invertebrates	9.1±6.2 (0.8 – 25.1)	10.9±11.6 (0.3 – 42.3)	8.6±8.5 (0.2 – 30.4)	6.3±7.3 (0.2 – 26.5)	6.2±5.8 (0.3 – 21.6)
C₃ plants	20.4±12.7 (1.3 – 47.2)	22.7±18.9 (0.5 – 67.7)	9.5±8.2 (0.4 – 30.8)	21.2±23.4 (0.4 – 82.3)	20.4±15.9 (0.6 – 56.7)
C₄ plants	21.5±9.2 (6.1 – 41.1)	18.8±14.9 (1.6 – 56.2)	25.6±21.5 (1.3 – 73.1)	9.4±9 (0.8 – 31.4)	17.4±11.3 (1.5 – 43.4)
Fish	31.3±10.8 (11.6 – 52.9)	27.4±17.3 (3.3 – 69.7)	39.3±21.5 (3.8 – 79.7)	49.5±23.9 (2.7 – 87.2)	40.5±15.7 (10.8 – 72.8)

Table 7.3 continued.

Sources	<i>Labeo cylindricus</i>	<i>Labeobarbus marequensis</i>	<i>Oreochromis mossambicus</i>	<i>Pseudocrenilabrus philander</i>	<i>Tilapia sparrmanii</i>
Group A invertebrates	17±22.9 (0.3 – 82.3)	16.2±14.6 (0.4 – 53.5)	19.8±24.7 (0.2 – 83.4)	22.2±15.9 (0.6 – 55.6)	25.3±20.6 (0.5 – 68.2)
Group B invertebrates	23.1±20.2 (0.2 – 71.3)	6.5±6 (0.2 – 22.3)	9.4±11.3 (0.2 – 40.4)	12.1±9.4 (0.3 – 34.2)	11.2±10.5 (0.3 – 38.5)
C₃ plants	13.2±12.6 (0.4 – 46.7)	17.4±12.7 (0.6 – 45.5)	33.9±30.6 (0.4 – 88.3)	19.1±12.7 (0.7 – 44.9)	26.4±17.4 (0.5 – 56.6)
C₄ plants	39.4±21.8 (1.7 – 81.7)	24.2±13 (2.6 – 51.1)	6.8±6.2 (0.7 – 20)	32.6±9.9 (12.6 – 51.7)	21.9±10.3 (3.2 – 42)
Fish	7±5.8 (0.9 – 21.1)	35.7±12.9 (12.6 – 64.2)	30.1±22.9 (1.9 – 79.1)	14±6.9 (3.2 – 29.5)	15.3±8.8 (3 – 36.9)

Table 7.4: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 2 in September 2016. Mean $\pm$ SD (95% Credible interval).

Sources	<i>Coptodon rendalli</i>	<i>Glossogobius giuris</i>	<i>Labeo cylindricus</i>	<i>Micralestes acutidens</i>	<i>Oreochromis mossambicus</i>	<i>Pseudocrenilabrus philander</i>	<i>Tilapia sparrmanii</i>
C₃ plants	33.8 $\pm$ 9.1 (16.8 – 52.7)	21.3 $\pm$ 9.8 (4.1 – 41.7)	8.2 $\pm$ 5 (1.4 – 19.8)	26.6 $\pm$ 12.9 (4.7 – 55.2)	66 $\pm$ 12.5 (40 – 88.8)	41.8 $\pm$ 8 (26.0 – 56.7)	38.1 $\pm$ 13.1 (11.2 – 64)
C₄ plants	33.8 $\pm$ 12 (11.9 – 58.3)	42.9 $\pm$ 19.1 (6.5 – 77.8)	61.3 $\pm$ 22.4 (6.1 – 90.7)	33.7 $\pm$ 18.1 (3.7 – 72.3)	17.8 $\pm$ 11.6 (2 – 44.6)	27.4 $\pm$ 12.7 (4.4 – 51.7)	29.4 $\pm$ 15.8 (3.6 – 62.5)
Fish	6.7 $\pm$ 4.5 (0.8 – 17.5)	4.7 $\pm$ 4.2 (0.2 – 15.9)	6.2 $\pm$ 6.9 (0.2 – 24.3)	10.2 $\pm$ 8.7 (0.3 – 32.4)	2.8 $\pm$ 2.6 (0.2 – 9.9)	5.4 $\pm$ 3.9 (0.3 – 14.8)	5.4 $\pm$ 5.3 (0.3 – 18.6)
Invertebrates	25.6 $\pm$ 12.4 (5.1 – 53.3)	31.1 $\pm$ 19.8 (2 – 73.6)	24.3 $\pm$ 22.1 (1.1 – 82.2)	29.5 $\pm$ 19.1 (2.2 – 72.6)	13.5 $\pm$ 11.9 (1 – 43.1)	25.4 $\pm$ 14.4 (2.8 – 5)	27.1 $\pm$ 17.7 (1.9 – 65.9)

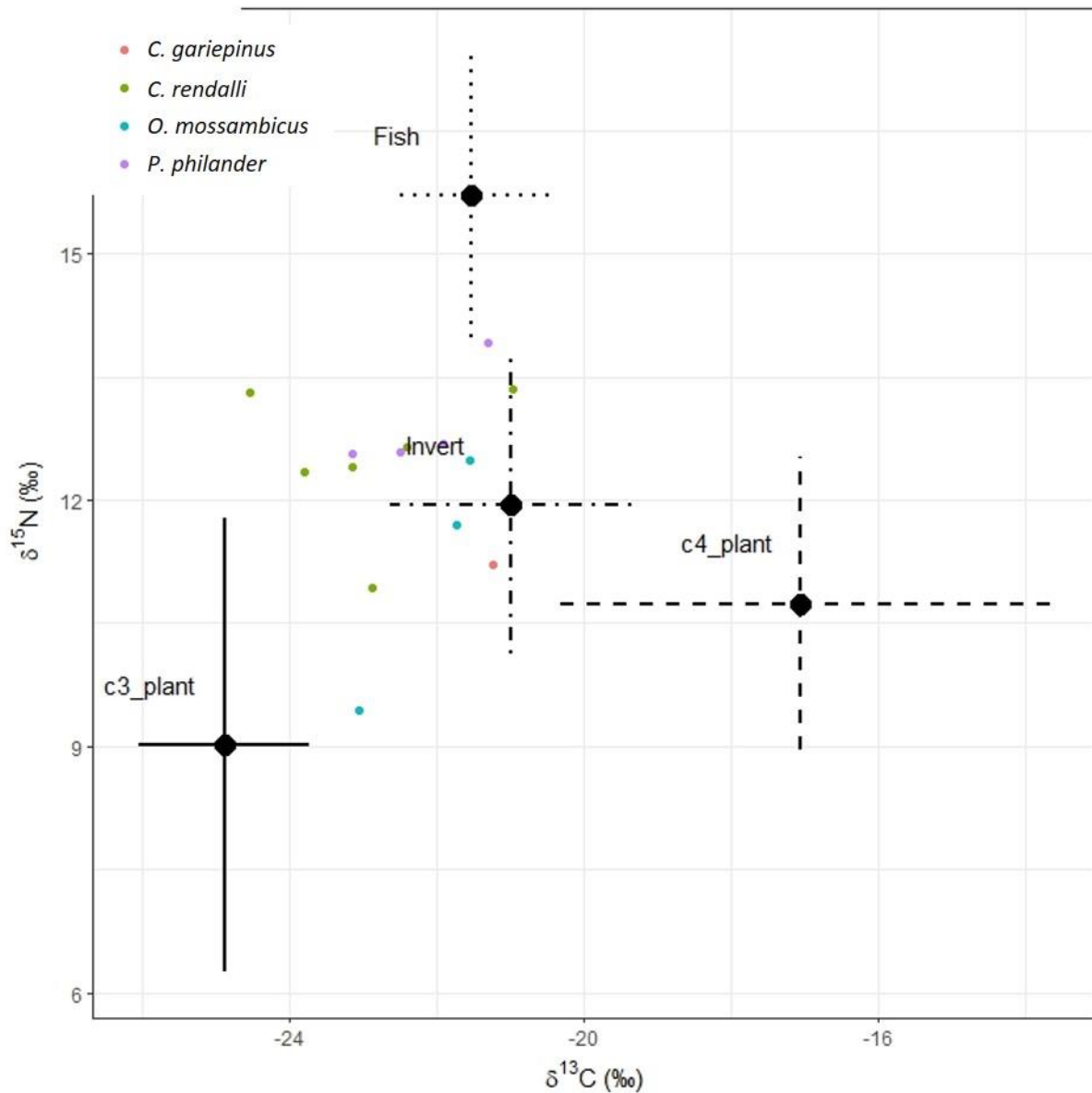


Figure 7.15: A biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) of Site 2 collected during the February 2017 sampling survey. Fish consumer data and source data are abbreviated: Fish – fish food sources; Inverts – Aquatic invertebrate families. c3_plant – C₃ plants; c4_plant – C₄ plants. Error bars represent 95% confidence intervals. Taxa included in each source group can be found in Appendix E13 and E14.

Table 7.5: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 2 in February 2017. Mean $\pm$ SD (95% Credible interval).

Sources	<i>Clarias gariepinus</i>	<i>Coptodon rendalli</i>	<i>Oreochromis mossambicus</i>	<i>Pseudocrenilabrus philander</i>
C ₃ plants	34.6 $\pm$ 13.2 (10.5 – 61.5)	44.5 $\pm$ 13.7 (14.8 – 69)	37.4 $\pm$ 17.6 (4.4– 70.6)	32.8 $\pm$ 14 (6 – 60.8)
C ₄ plants	15.3 $\pm$ 11 (0.9 – 41.4)	9.9 $\pm$ 8.2 (0.4 – 29.4)	14 $\pm$ 12.2 (0.3 – 43.8)	13.1 $\pm$ 10.3 (0.5 – 37.3)
Fish	12.3 $\pm$ 7.9 (1.8 – 31.8)	12 $\pm$ 7.6 (0.9 – 29.7)	7.2 $\pm$ 6.6 (0.5 – 23)	17.3 $\pm$ 11.3 (1.1 – 44)
Invertebrates	37.9 $\pm$ 16.5 (8.9 – 71.4)	33.7 $\pm$ 17.3 (4.9 – 70.1)	41.3 $\pm$ 22.3 (3.5 – 85.6)	36.8 $\pm$ 18.2 (5.4 – 75.5)

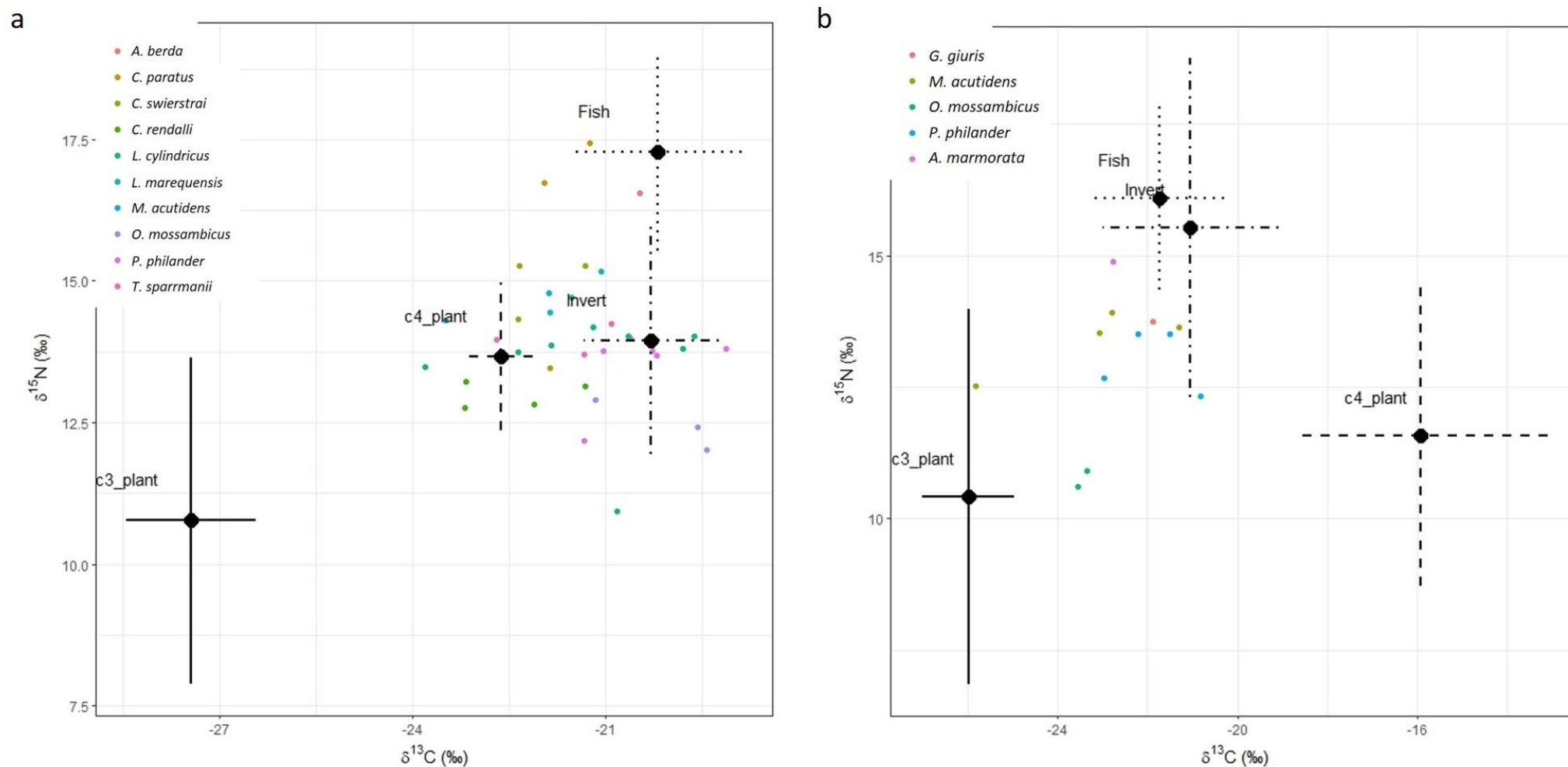


Figure 7.16: Biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of fish consumers (dots) and potential food sources (error bars) of Site 3 collected during the September 2016 (a) and February 2017 (b) sampling surveys. Fish consumer data and source data are abbreviated: Fish – fish food sources; Inverts – Aquatic invertebrate families. c3_plant – C₃ plants; c4_plant – C₄ plants. Error bars represent 95% confidence intervals. Taxa included in each source group can be found in Appendix E13 and E14.

Table 7.6: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 3 in September 2016. Mean±SD (95% Credible interval).

Sources	<i>Acanthopagrus berda</i>	<i>Chiloglanis paratus</i>	<i>Chiloglanis swierstrai</i>	<i>Coptodon rendalli</i>	<i>Labeo cylindricus</i>
C ₃ plants	4.4±3.6 (0.2 – 13.6)	5.3±5.6 (0.1 – 20.6)	5.8±6.1 (0.1 – 22)	7.1±8.2 (0 – 28.6)	3.5±3.8 (0.1 – 13.9)
C ₄ plants	44.5±13.9 (19.4 – 72.2)	38.2±20.1 (5.2 – 78.8)	57.2±19.2 (14.7 – 89.3)	64.7±22.5 (12.8 – 95.6)	42.3±19.6 (7.8 – 85.5)
Fish	8.7±5.6 (1.1 – 22.5)	28.3±16.5 (0.5 – 61.6)	8.2±6.2 (0.4 – 24.2)	2.6±2.5 (0.2 – 9.5)	3.9±3.4 (0.3 – 12.7)
Invertebrates	42.5±12.8 (17.4 – 66.5)	28.1±22.2 (3 – 85.2)	28.8±14.5 (5.4 – 60.5)	25.6±16.4 (2.2 – 63.5)	50.4±17 (10.5 – 79.8)

Table 7.6 continued

Sources	<i>Labeobarbus marequensis</i>	<i>Micralestes acutidens</i>	<i>Oreochromis mossambicus</i>	<i>Pseudocrenilabrus philander</i>	<i>Tilapia sparrmanii</i>
C ₃ plants	4.7±5.3 (0.1 – 18.8)	7.6±8.9 (0.1 – 31.6)	2.6±2.9 (0.1 – 10.9)	2.3±2.3 (0.1 – 8.5)	4.6±5.3 (0.01 – 19.3)
C ₄ plants	48.7±21.3 (8.7 – 88.2)	59.8±23.7 (9.6 – 94.1)	21.9±15.3 (2 – 60.1)	31.7±14.3 (6.4 – 62)	53.2±21.1 (10.5 – 90.9)
Fish	10.9±9.8 (0.4 – 36.1)	7.2±7.2 (0.3 – 25.3)	4.5±4.6 (0.2 – 16.6)	6±5.3 (0.3 – 19.4)	6.2±5.8 (0.3 – 21.4)
Invertebrates	35.8±18.5 (5.3 – 75.8)	25.4±17.9 (2.8 – 69.2)	70.9±15.1 (36.3 – 93.6)	60±12.8 (34 – 84.3)	36±17.7 (4.8 – 73.4)

Table 7.7: MixSIAR dietary results indicating the proportional contribution of the selected potential food sources to the diets of the consumers collected at Site 3 in February 2017. Mean±SD (95% Credible interval).

Sources	<i>Glossogobius giuris</i>	<i>Micralestes acutidens</i>	<i>Oreochromis mossambicus</i>	<i>Pseudocrenilabrus philander</i>	<i>Anguilla marmorata</i>
C ₃ plants	41.5±12.5 (17.6 – 65.8)	48.9±14.9 (16.7 – 74.6)	60.8±16.7 (16.4 – 87.4)	38.1±13.1 (9.7 – 61.9)	38.5±19.1 (5.5 – 77.8)
C ₄ plants	21.3±11.1 (3.2 – 45.4)	15.5±10.3 (1 – 39)	15.9±11.7 (0.9 – 42.4)	24.8±12.5 (2 – 49.3)	17.9±14 (1.2 – 52.5)
Fish	15.5±12 (0.6 – 45.7)	14.7±214.9 (0.3 – 55.7)	8.2±9.1 (0.2 – 31.4)	14.7±14.3 (0.3 – 54.8)	20.1±19.4 (0.3 – 69.2)
Invertebrates	21.7±14.7 (1.4 – 55.5)	20.9±17.4 (0.6 – 61.8)	15.1±16.1 (0.5 – 64.8)	22.4±17.7 (0.8 – 64.4)	23.5±20.1 (0.7 – 72.1)

7.4 Discussion

There are a variety of ways whereby a supra-seasonal drought can affect aquatic ecosystems and their functioning. Direct impacts include reduction of water volume and flow velocity, alterations to habitat and impaired river connectivity. Indirect impacts include changes in water chemistry, alterations to food sources and reduced strength and structure of interspecific interactions (Lake 2003; Woodward 2009). These modifications may lead to loss and extinction of species, changes in community structure and reduced stability of the ecosystem. Both direct and indirect effects were observed in the Phongolo River during the present study, including a decrease in several sensitive aquatic invertebrate families, continuous taxonomic turnover in aquatic invertebrate community structure and changes in carbon isotope signatures of food sources. Significant enrichment of nitrogen isotopes of many food web components also indicates additional stress placed on the Phongolo River in the form of human activities.

7.4.1 Aquatic invertebrates

In this study a decrease in invertebrate biodiversity was found over time, although high diversity was still maintained, particularly in comparison to previous studies of the Phongolo River and others that recorded lower aquatic invertebrate diversity during times of drought and low flow. During low-flow studies on the Little Stour River (England), Wood and Armitage (2004) found an increase in the number of aquatic invertebrate taxa from (42 to 60) as the river discharge increased. Masese et al. (2014) collected only 81 taxa during dry season stream surveys, compared to 93 during wet season surveys in Kenya, and Villastrigo and García-Criado (2017) found only 26 genera in a Brazilian mountain stream experiencing a drought. Differences in aquatic invertebrate community structure in the Phongolo River were also found between the present study and those of previous years (2012, 2013 and 2014, respectively). Although there was a much higher invertebrate diversity found during the present study, a loss in rheophilous macroinvertebrates and those sensitive to changes in water chemistry occurred as representatives of several families previously recorded from the river (e.g. Ephemeridae and Tricorythidae) were not found during the present study. However, the family Ephydriidae (brine flies), and Order Harpacticoida (Copepoda), both of which are highly tolerant to increased salinity (Miliou 1993; Herbst 2001), were found only during the present study. A greater abundance of taxa more commonly associated with lentic systems and slow flowing water, namely Corixidae, Culicidae, Libellulidae and Lumbriculidae, were also found during the present study of which most occurred within NGR. Although drought generally results in loss of lotic species, it may also increase the total number of species found at a site by opening up available habitats to different, sometimes more resistant, taxa (Bogan and Lytle 2007; Bogan et al. 2015).

Of the few rheophilous families found during the present study, including Hydropsychidae, Oligoneuriidae and Philopotamidae, most were present outside NGR. These families are highly flow-dependant and also generally considered to be quite sensitive to changes in water chemistry such as increased organic pollution and salinity (Gerber and Gabriel 2002; Dallas and Day 2007; Chessman 2015). Change in habitat, flow velocity and water chemistry were the likely reasons for the low

abundance of these sensitive families. The two sites outside NGR also have habitat types that are able to support aquatic invertebrates with preferences for faster flowing waters and riffles due to anthropogenic alterations. A recently-constructed weir at the Bridge site (Site 3) and concrete blocks in the stream at the Walkway site (Site 2) (see Study Area) create fast-flowing habitats that would not naturally have occurred at these sites. Conversely, the river within NGR does not have fast-flowing habitats available and generally, particularly at the time of the study, had slower flow (pers. observation). In addition, even though rainfall increased between the first and last surveys, the average monthly baseflow decreased from 2016 to 2017, probably further reducing the ability of the river water to flush the system. When there is reduced streamflow and groundwater storage, such as during drought, concentration of nutrients, pollutants and suspended solids occurs in the water column due to the reduced flushing capacity of the system (Chamier et al. 2012; Mosley 2015). This corresponds with the findings in Chapter 3 regarding deterioration of water quality of the lower Phongolo River during the supra-seasonal drought. Similar findings were also reported by Leigh (2013) from rivers in northern Australia where shifts in taxon diversity from the wet to dry season were largely due to the replacement of rheophilous taxa and those sensitive to changes in water quality by those more associated with still and polluted waters. Chessman (2015) also found that rheophilous species were negatively affected by the Millennium drought in Australia, and Acuña et al. (2005) found that rheophilous taxa were replaced by those more commonly associated with lentic habitats during droughts in the Mediterranean.

Frequent taxonomic replacement was observed from the first to last survey, which led to differences in invertebrate assemblages between sampling surveys. This frequent turnover is probably due to the inability of aquatic invertebrates to cope with the changes in the physical and chemical environment of the river, and not just standard seasonal changes. Invertebrates able to disperse actively (either as larvae or adults) probably moved to different habitats, while local extinction occurred in those unable to disperse actively and were replaced by more resilient taxa as time went on. This is in line with previous research that also found frequent taxonomic turnover and reduced invertebrate diversity in regions with long periods of reduced flow and stream drying (Bogan and Lytle 2011; Leigh 2013). It is also possible that the anthropogenic activities occurring outside NGR had a negative impact on the aquatic invertebrate communities both within and outside NGR. Although biodiversity was greater within NGR compared to outside, the river inside is clearly not well protected from anthropogenic human activities, given the types and abundances of aquatic invertebrates found. Pollutant-tolerant taxa such as *Physa acuta* and Lumbriculidae were dominant in the river inside NGR, and it has been indicated that these types of taxa, particularly snails of the genus *Physa*, flourish in streams that are organically polluted (Al-Shami et al. 2011). This is in agreement with several studies, including Azrina et al. (2006) and Miserendino et al. (2011) that related lower invertebrate biodiversity to river areas under environmental stress due to human activities (e.g. afforestation and pollution), and higher diversity where there were few to no impacts.

7.4.2 Stable isotopes

Nitrogen enrichment occurred from the first to the last survey in many of the food web components, most prominently the primary producers and aquatic invertebrates outside NGR. The carbon signatures

of most basal sources also became more depleted from the first to last survey, particularly outside NGR. With the exception of *L. cylindricus*, *O. mossambicus*, *P. philander* and *T. sparrmanii*, higher food web components did not change as much in their isotopic signatures. Similarly, Bunn and Boon 1993 found little variation in the $\delta^{13}\text{C}$ of fish between taxa and seasons in Australian billabongs. This is also in line with studies that found decreases in the $\delta^{13}\text{C}$ signatures of consumers, particularly the more herbivorous species, during low-flow seasons, when there is reduced water velocity (Finlay et al. 1999; Hicks et al. 2017).

Due to faster isotopic turnover, lower trophic level sources, such as primary producers have greater seasonal variability, while animals that feed at higher trophic positions, such as fish, have slower tissue turnover. Higher trophic consumers will therefore have a slower response to temporal isotopic changes of basal sources (Michener and Lajtha 2007; Post 2002; Middleburg 2014). There is also more variation in aquatic primary producer $\delta^{13}\text{C}$ due to greater physiological diversity in the aquatic macrophytes, as well as larger variation in the concentration and isotopic ratios of dissolved inorganic carbon in systems (Hamilton and Lewis 1992; Benedito-Cecilio et al. 2000; Jackson et al. 2013; Michener and Lajtha 2007). Generally, during dry seasons there is a reduction in allochthonous carbon sources into aquatic habitats and rivers are then more reliant on autochthonous carbon sources produced by primary producers such as algae and phytoplankton. The autochthonous sources are typically more carbon depleted which causes a decrease in the $\delta^{13}\text{C}$ during the drier seasons (Benedito-Cecilio et al. 2000; Marchese et al. 2014). This is in line with the reduced baseflow observed in 2017.

Increased nitrogen in the lower trophic levels of the food web was related to the recent introduction of nitrogen-rich sources into the Phongolo River through runoff from rainfall in the catchment. As previously stated, there are a number of sources for nitrogen inputs surrounding the Phongolo River and increased rainfall in the Ndumo region led to an influx of organic nutrients into the river (see Chapter 3) and subsequent reflection in the food web. This corresponds to the findings in Chapter 5 where nitrogen also increased in the food web of the temporary pool after it had received water from outside NGR. Agricultural activities, through the use of commercial and organic fertilizers and pesticides, often lead to nutrient enrichment in aquatic environments (Dudgeon 2008; Al-Shami et al. 2011). A number of studies have also related nitrogen inputs such as agricultural activities and human wastewater to nitrogen enrichment in food web components (Bunn and Boon 1993; Cabana and Rasmussen 1996; Jones et al. 2018).

The food webs were generally short, consisting of three or four trophic levels, and stable with a wide variety of autochthonous and allochthonous carbon sources forming the base of the food web. Stable food webs are generally short with approximately three trophic levels (producers at the base, intermediate consumers and predators at the top) and found most commonly in nature (Borrelli and Ginzburg 2014). These food webs are able to persist for longer periods of time than food webs with more trophic levels, due to their ability to return to equilibrium more easily after disturbances such as by floods or drought, without loss or extinction of species (Pimm and Lawton 1977; Borrelli and Ginzburg 2014). Although most of the food webs had larger predatory fish consumers at the top, on a few

occasions invertivorous fish (*C. paratus* and *P. wesselsi*) and predatory aquatic invertebrate families (Belostomatidae and Aeshnidae) were at the top of the food webs. Sabo et al. (2010) also found the top predators in large intermittent streams in North America to consist of invertebrates or small-bodied insectivorous fish.

The dietary analysis indicated most consumers to be flexible in their diet, switching between various food sources throughout all sampling surveys. In addition, the fishes most commonly found in the study (*Clarias gariepinus*, *Coptodon rendalli*, *Enteromius afrohamiltoni*, *Labeobarbus marequensis*, *M. acutidens*, *O. mossambicus*, *Synodontis. zambezensis*, *P. philander*, *Schilbe intermedius* and *T. sparmanii*.) are considered generalist feeders with low specificity for food sources (Skelton 2012). Several other species including, *A. berda*, *Chiloglanis swierstrai*, *C. paratus*, *G. giuris*, *Hydrocynus vittatus*, *L. cylindricus*, *P. wesselsi* and *Anguilla marmorata* were not as commonly found. Although these consumers are more specialised feeders (Skelton 2012; Taylor et al. 2017b), the dietary analysis indicated that these and the generalist feeders preyed on a mixture of all available food sources with few instances where a larger percentage (>50%) of more specific food sources were consumed. One such case was *L. cylindricus* (herbivore; Skelton 2012), which mostly consumed nutrient-low plants and algae. Both *A. berda* and *H. vittatus* are considered predators, feeding largely on fish and invertebrates and were present at or near the top of the food webs. However, the dietary analysis indicated that *A. berda* largely consumed C₄ plants during one of the surveys (45%), while *H. vittatus* consumed more C₃ plants (37%). Consumers may incorporate the isotopic signatures of C₃ and C₄ sources not only by direct ingestion, but indirectly through ingestion of insects, invertebrates and molluscs with isotopic signatures of C₃ and C₄ macrophytes (Oliveira et al. 2006). It is therefore likely that both *A. berda* and *H. vittatus* preyed most on fish and invertebrates with the isotopic signatures of C₃ and C₄ plants. Similarly, Oliveira et al. (2006) found that piscivorous fish obtained at least 64% of their carbon signatures from C₃ plants, probably due to a preference for prey that consume C₃ plants. This is an important ability for continuous survival during times of disturbance such as drought or floods. Highly specialised consumers are dependent on only select food sources and are thus more vulnerable to extinction if that particular source disappears (Lu et al. 2016).

Many fish species are flexible in their feeding behaviour, being able to switch their diets seasonally due to natural variation in the abundance and distribution of resources, and to adjust their feeding strategy if their preferred food sources are not available (de Almeida et al. 1997; Merron and Mann 1995; Wantzen et al. 2002; McMeans et al. 2015; Taylor et al. 2017a). This generalist feeding behaviour and continuous shift in food sources may prevent certain resources from becoming dominant, thus acting as a buffer that stabilises the whole food web (McMeans et al. 2015). In comparison to fish from temperate systems, fish from tropical regions also have more herbivorous, detritivorous and omnivorous feeding behaviour (Winemiller and Jepsen 1998). Omnivory is also common among species on higher trophic levels (Thompson et al. 2007) and a large percentage of biota (46% to 87%) in a community will feed on more than one trophic level (Williams and Martinez 2004; Armin and Marquet 2004; Borrelli and Ginzburg 2014).

A larger number of C_3 plants than C_4 plants were found, since the C_3 photosynthesis pathway is the most common, occurring in all trees, most shrubs, herbs and grasses with cool growing seasons while C_4 photosynthesis is less common, mostly occurring in grasses from regions with warm growing seasons (Michener and Lajtha 2007). In contrast to many previous studies (e.g. Forsberg et al. 1993; Jepsen and Winemiller 2007; Jackson et al. 2013; Marchese et al. 2014) the dietary analysis also indicated that C_4 plants were more frequently consumed than C_3 plants, particularly during the September 2016 survey. Most herbivorous and omnivorous aquatic biota do not feed on these sources as they are generally low in nutritional value and poorly digested (Forsberg et al. 1993; Jackson et al. 2013). It is likely that the slow flow of the river during this dry period had an effect on the feeding behaviour of the consumers. Similar results were found by Benedito-Cecilo et al. (2000) who determined that the consumption of C_4 plants was higher during low water times in the Amazon River. Oliveira et al. (2006) also determined that C_3 plant sources were the most important carbon sources to consumers during high and receding water periods but less so during low water periods.

7.5 Conclusion:

When compared to previous studies of the Phongolo River in 2012 – 2014, in the present study there was a loss of rheophilous and pollutant-sensitive aquatic invertebrates. However, this was not reflected by a loss in the total diversity, due to replacement with more tolerant taxa and frequent taxonomic turnover. These changes in aquatic invertebrate community structure were related to both direct and indirect effects of the drought, including reduced flow velocity of the river and changes in the chemical water composition. The first part of the first hypothesis, stating that the supra-seasonal drought will negatively affect the aquatic invertebrate community structure through loss of pollution-sensitive and rheophilous genera, is accepted. However, the second part, stating there will be a decrease in diversity and abundance, is rejected as this was not the case. Depletion of carbon isotopes in many of the basal sources was linked to the climate-driven supra-seasonal drought which drove increased production of dissolved inorganic carbon by autochthonous sources. The food webs remained stable and distinct trophic levels were continuously present throughout the study. The first part of the second hypothesis, stating that supra-seasonal drought will affect the food webs through loss of distinct trophic levels, is rejected. However, the part stating that there will be decreased carbon production by allochthonous sources, is accepted. Based on the abundance and types of aquatic invertebrates found in the river, such as *P. acuta* within NGR, as well as nitrogen enrichment in the lower trophic sources of the food web, it is possible that the Phongolo River is being negatively affected by anthropogenic human activities occurring in the catchment outside the reserve. The third hypothesis, stating that due to anthropogenic activities occurring in the Phongolo River catchment, organic pollution will be reflected in food web components through nitrogen enrichment and in aquatic invertebrate diversity through increased abundance of pollution tolerant taxa, is therefore accepted. Few specialised consumers were found, and most were generalist and opportunistic feeders that fed on an integrated diet of all sources, with overlap between the various consumer diets. The fourth hypothesis, that higher trophic consumers in the Phongolo River are generalist and opportunistic feeders with large dietary overlap between species, is therefore accepted. It is clear from the analysis that the aquatic biota of the Phongolo River

are all fairly resistant to and resilient to changes in the aquatic environment. The baseflow from the Pongolapoort Dam seems to sustain a stable food web, but not a stable aquatic invertebrate community. Thus, the final hypothesis, stating that baseflow released from the Pongolapoort Dam is not enough to support a stable food web or aquatic invertebrate community structure in the Phongolo River during the supra-seasonal drought, is accepted with regard to the food web, but not to the aquatic invertebrate community.

Given the of biota collected, along with the changes seen in the aquatic invertebrate community and the food web, it is possible that the Phongolo River system is still in the initial stages of species loss, as described by Woodward (2009). If the supra-seasonal drought were to continue for much longer it is possible that species loss and ecological disturbances would become apparent.

CHAPTER 8:

Conclusions and Recommendations

8.1 General remarks

Historically the Phongolo River has been utilized extensively by humans in both commercial and small-scale ways. Most prominent is the large-scale irrigation and afforested areas in the upper and middle Phongolo River catchment that have utilized water from the river since the 1960's and was the reason for the construction of the Pongolapoort Dam. During this same period, the local people of the area utilized the Phongolo River floodplain and associated wetlands as fields for their subsistence crops, water from the river and the floods to irrigate these fields as well the vast array of other goods and services the river and floodplain provides (see Chapter 2). Although Ndumo Game Reserve was founded with the chief aim of conserving the ecologically important biota of the area (see Chapter 2), it has not truly been an important aspect for study since its establishment in 1924 and presently the eastern part of the reserve and river is utilized by locals for farming, water abstraction and fishing. In addition, very few studies have considered the ecological effects the numerous anthropogenic activities taking place outside the reserve may have on the abiotic and biotic components of the of the lower Phongolo River and associated floodplains in both the protected and non-protected area. These issues have placed a great deal of pressure on the river and, indirectly, the many unique wetlands that are found in the region. Simultaneously, through the supra-seasonal drought that occurred between 2015 and 2017, the system has experienced further environmental stress through reduced precipitation and no controlled flood releases. It was therefore highly important to determine the present condition of the ecologically important but regulated Phongolo River and floodplain and assess the degree of effect stressors may have had on the various abiotic and biotic components of the studied aquatic environments. The following sections provide concluding remarks on each of the original set objectives of the study.

8.2 Specific conclusions

8.2.1 The first objective of this project entailed assessing historic and present water quality data and determining the Physico-Chemical Driver Assessment Index (PAI) of the middle and lower Phongolo River in order to determine the present water quality of the river and how this may have changed over time and through the drought. The following section highlights findings and the conclusions drawn:

The PAI and long-term water quality analysis of the Phongolo River indicated that the river has historically been affected by the numerous activities that have taken place in the river catchment before it enters the Pongolapoort Dam. Due to an increase in these anthropogenic activities over the years, particularly irrigation schemes in both the upper and lower reaches of the river, as well as construction of the Pongolapoort Dam, the water quality has degraded further since the 1980's.

The causes of degradation included increased organic nutrients ($\text{NO}_3^-/\text{NO}_2^-$ and PO_4^{3-}) as well as increased salinity (conductivity and K) from irrigation return flow and surface water runoff from the nitrogen and phosphate rich fertilizers used by these irrigation schemes. The section of the Phongolo River located below the Pongolapoort Dam was found to consist of significantly lower organic nutrients and salts than upstream of the dam. These nutrients and salts are replenished by the time the river flows into the NGR through both natural and anthropogenic processes. Leaching of organic nutrients from highly fertile soils in the floodplain wetlands and leaching of the naturally saline groundwater into the river are what increases the water salinity and organic nutrients as the river flows through the downstream landscape as observed by the increases in the NGR. These natural processes are being exacerbated by the continuous and ever increasing anthropogenic activities, and, more recently, the supra-seasonal drought through a lack of strong river flow that would under normal circumstances have flushed the river. The Pongolapoort Dam and its discharges, as is the case with most dams globally, has not been properly managed since construction and the possible consequences of this have been noted in previous studies (see Van Vuuren 2009). Heath and Plater (2010) also noted that the dam has reduced the amount of nutrient rich soils that enter the lower floodplain region. Although high organic nutrients are known to have many undesirable effects on an ecosystem, low nutrients can also cause low ecosystem productivity. The local peoples have been making use of the exposed nutrient rich soils in the floodplain wetlands during dry winter periods for many years. This reduces the organic nutrients in the sediments that need to be replenished with nutrient rich sediments during the summer floods, as is the case with the lower Phongolo River. If these wetlands do not receive the replenished nutrients, it reduces the productivity of the soils and arable land, which are important not only to maintain the productivity of the ecosystem but the goods and services the local people rely on. Subsistence farms in the region have increasingly been making use of commercial fertilizers to increase the growth of their crops suggesting that the soils in the floodplains are not as productive as they used to be. The floodplains are likely not being replenished due to decreased nutrient loads from the dam and, more recently, the drought which has reduced the volume and velocity of river water flow that prevented the floodplains from receiving water.

Even though it is a protected area, the quality of water entering the Ndumo Game Reserve borders has also decreased through increased nutrients and water salinity and does not differ from water quality outside the NGR. Nonetheless, these increases in nutrients and salinity in the lower Phongolo region do not appear to have seriously degraded water quality at present and, in an unusual way, may be helping the ecosystem to obtain nutrients that are retained by the Pongolapoort Dam. However, the upper reaches of the Phongolo River are a prime example of how this may change through long term exposure. The supra-seasonal drought was found to further increase water salinity and organic nutrients of the Phongolo River but, interestingly, did not cause a severe degradation of water quality since the PAI of all sites did not decrease compared to before the drought. This suggests that the baseflow released from the Pongolapoort Dam during the drought ($7.33 \text{ m}^3\text{s}^{-1}$) was sufficient to allow the river to sustain its current state. However, during extended periods of low-flow such as a drought, there is accumulation of minerals, increased leaching of nutrients from soils and this may lead to eventual deterioration of water quality. If the drought were to continue, effects may eventually be apparent in the

water quality of the lower Phongolo River. This poses a serious threat to the many unique aquatic habitats reliant on the river for water as well as surface runoff during rainfall.

The hypothesis stating that water quality of the Phongolo River will be degraded due to effects caused by anthropogenic activities and will indicate further degradation due to the recent supra-seasonal drought is accepted with certain limitations. Water quality did decrease further during the supra-seasonal drought and thus this part of the hypothesis is accepted. However, no severe degradation was observed, and this part of the hypothesis is rejected.

8.2.2 The second objective of this study was to make use of in situ field mesocosm experiments to determine if length of hydrological connectivity has any effect on aquatic invertebrate community structure. The following section highlights findings and the conclusions drawn.

The mesocosm assessment found that water quality and quantity and hydrological connectivity did not affect aquatic invertebrates. This is due to the adaptations that these aquatic invertebrates have evolved from living in environments that are constantly exposed to regular and natural periods of low flow and drought and thus do not consider these changes as a disturbance. Thus, as long as the hydroperiod is long enough for aquatic invertebrates to complete the necessary life stages, they will not be affected by reduced water levels and the associated water quality changes. This is in accordance with the “drought resistance hypothesis” suggested by Boersma et al. (2014) which states that aquatic invertebrate taxonomic and trait diversity are resistant to water quality and quantity changes in temporary habitats and do not decline in decreasing water levels. It was also found that the water quality in a simulated floodplain wetland changes and decreases over time regardless of whether the site continues to receive low flows of water or not. This reiterates the results of the water quality analysis of the Phongolo River that water quality will remain in the state it is in and most likely continue to decline when there is a steady low flow for long periods of time. This will most likely only improve if there is high flow that flushes the ecosystem. Although length of hydrologic connectivity of a floodplain pool to a river does not appear to be critical to aquatic invertebrates, this is most likely not true for fish that are known to use floodplain pools for spawning and as nursery sites.

Following these finding, the hypothesis stating that the length of hydrologic connectivity between the Phongolo River and a floodplain wetland structures an aquatic invertebrate community is rejected as there was no evidence of these effects in the mesocosm study.

8.2.3 The third objective was to determine current aquatic invertebrate community structure, aquatic food webs and fish consumer diets in three different types of aquatic habitats found in the lower Phongolo River region.

8.2.3.1 The first habitat is the ecologically unique temporary wetland (Site 4) located in the NGR that experienced out of season drying events during the supra-seasonal drought.

This site relies on rainfall and the subsequent surface runoff that occurs in the catchment, a large part of which lies outside the NGR where many agricultural activities take place. This site is unique as it is not a typical temporary wetland (See Chapter 2) and is the only site in the Ndumo region to have

populations of the annual killifish *N. orthonotus* at any given time. Although the natural wet/dry cycle of the temporary pool has been disrupted (See section 2.5.2, Chapter 2), results indicated that this has not severely affected the aquatic invertebrate communities or their resistance and resilience. This was evidenced by the natural cycle generally observed in temporary pools where there is decreased diversity during drying when the water of the site is also more saline and increased diversity during rewetting when the water is fresher. Even though there was no evidence to suggest the anthropogenic activities occurring outside the NGR and the supra-seasonal drought affected aquatic invertebrates, effects were seen in the food web and diets of organisms. This was evidenced through significant $\delta^{15}\text{N}$ enrichment in all food web sources and consumers after the drying event, reflecting anthropogenic inputs from outside the NRG, this corresponds to the findings in Chapter 3 where enrichment of the aquatic systems was evident as shown by the water quality. Shifts in the diet of the annual killifish towards more plant-based food sources with low carbon signatures is a possible indication of the effects of drought whereby a consumer is desynchronized with its preferential, higher quality food and feeds on less nutritional food sources. Since only spot water samples were collected during each sampling survey it is not surprising that nitrogen enrichment was not reflected in the chemical water analyses as these nutrients likely rapidly settled into the sediments and were utilised by primary producers. The discrepancy between nutrient levels in the water and nutrient levels in the food web further supports the notion that long-term water quality assessment is more reliable than simple spot water assessments which only provide an indication of water quality at the time of sampling. Clearly the adaptations that aquatic invertebrates and annual killifish possess have made them resilient and adaptable to changes in the environment since no severe negative effects were evident during the study. However, predicted future temperature and rainfall changes may affect these biota through later responses in reproduction and growth, possibly preventing them from taking advantage of abundant and nutritious resources when these are available. In addition, although they possess short lifecycles already, climate change may eventually produce hydroperiods too short for even these biota to complete their lifecycles.

8.2.3.2 *The second type of habitat assessed was the large naturally saline lake, Nyamithi, in the NGR (Site 5).*

High evaporation rates along with no flood release for approximately two years from the Pongolapoort Dam and very little rainfall caused the water levels of the lake to severely decrease and concentrate its chemical constituents, most prominently, the salinity. Although naturally saline, this site experienced above normal salinity ($>10\,000\ \mu\text{S}\cdot\text{cm}^{-1}$) during the course of the supra-seasonal drought and had a severe knock on effect on the aquatic diversity and ecology of the site. The five-year assessment of the aquatic invertebrates in this lake found that diversity, abundance and community structure changed markedly from the wet season to the supra-seasonal drought. Only aquatic invertebrates highly adapted to salinity were able to survive in this site during the peak of the drought in 2016. Only a single species of fish, *O. mossambicus*, was able to survive through the highly saline period as it is one of few freshwater species with immense osmoregulatory capabilities allowing them to survive under such immense saline conditions. The site received water through some rainfall in the region and small floods from the Usuthu River at the end of 2016 and beginning of 2017, reducing the salinity and allowing

some fish and aquatic invertebrates to return. Fish consumers also switched their diets from a more specialised during the pre-drought conditions of 2010 to a more generalised feeding behaviour during the supra-seasonal drought as occurs in habitats where there are disturbances present. Ecosystem resilience was evident in Nyamithi lake since aquatic invertebrate diversity and abundance recovered rapidly (within a year) from these environmental changes after the site received water and salinity decreased. Similarly, although there was a shift from specialised to generalised feeding strategies by most fish, the food web remained quite stable and this indicates towards an ecosystem and biota with the ability to adapt to environmental impacts. It is possible that the reason for this ability to adapt stems from the fact that Nyamithi pan undergoes regular disturbances in the form of a controlled flood release. Through years of constant wet/dry cycles, the aquatic biota of this ecosystem have likely adapted to living in a constantly changing environment and are therefore able to survive through, and rapidly recover from, a disturbance such as a supra-seasonal drought. However, if such a drought were to occur over a longer period of time, as is predicted by future climate change, it is quite possible that even the most resilient of taxa would not be able to survive the accompanying environmental changes.

8.2.3.3 The third habitat assessed was the lower Phongolo River along three sections, two sites outside the NGR and one site on the river as it enters the NGR (Sites 1-3).

As was determined by the water quality analysis in Chapter 3, the NGR, particularly the river that flows through it, is affected by anthropogenic activities occurring outside its borders. This study on the aquatic invertebrates supported this as aquatic invertebrate diversity and abundance between the river outside the protected area and the river inside the NGR were similar and did not differ significantly. Although there was a loss of rheophilous and pollutant sensitive aquatic invertebrates, this did not reflect a decrease in the total diversity due to replacement of these taxa with more tolerant taxa and frequent taxonomic turnover. These changes and constant taxonomic turnover was due to various aspects caused by the drought including reduced flow velocity of the river and changes in the chemical water composition (as discussed in Chapter 3). The supra-seasonal drought increased autochthonous production of dissolved inorganic carbon and led to depletion of carbon isotopes in many of the basal sources. Nitrogen enrichment in the lower trophic sources of the food web was observed, most prominently along the region within the NGR, which indicates towards nitrogen rich inputs caused by anthropogenic activities occurring in the catchment outside the reserve. However, neither the drought nor the anthropogenic activities appeared to severely alter the aquatic food webs of the river which remained stable with distinct trophic levels. This did not hold true for consumer diets which consisted largely of generalist and opportunistic feeders that fed on an integrated diet of all sources with an overlap between the various consumer diets. As with the results from Site 5, this points towards a disturbance in the ecosystem. It is clear from the analysis that the aquatic biota of the Phongolo River are relatively resistant and resilient to changes in the aquatic environment. Given the type of biota collected, along with the changes seen in the aquatic invertebrate community and the food web, it is also possible that the Phongolo River system is still in the initial stages of species loss as described by Woodward (2009). If the supra-seasonal drought were to continue for much longer it is possible species loss and ecological disturbances would become apparent.

Following the abovementioned findings of the three study systems, the hypothesis stating that the supra-seasonal drought has disrupted the measured natural ecological processes of the lower Phongolo River and the ecologically unique and important wetlands located within the Ndumo Game Reserve is rejected. Although various aspects that were measured were indeed affected, through aquatic invertebrate taxonomic turnover and consumer diet switching, no severe disruptions were found, and the biota were able to easily adapt and recover from the observed changes. The hypothesis stating that the baseflow released from the Pongolapoort Dam during the supra-seasonal drought is not enough to sustain stable ecological processes on the lower Phongolo River is accepted with certain limitations. The baseflow from the Pongolapoort Dam appears to sustain a stable food web, as it did not change much during the course of the study, but not a stable aquatic invertebrate community as there was constant taxonomic turnover.

8.3 Final thoughts

The lower Phongolo River and associated floodplain is a severely regulated ecosystem and has been affected by numerous agricultural impacts and, during the current study, a severe two-year drought during which time no flood releases occurred. It was therefore quite surprising that no severe effects or significant changes were found in the assessed abiotic and biotic characteristics during the course of this study. It has become clear that the lower Phongolo River is a resilient and adaptable ecosystem with the ability to easily recover from disturbances that have been known to affect the ecology of an ecosystem. It is possible that the reason for this adaptability and resilience stems from the fact that the lower Phongolo ecosystem has always been affected by regular droughts and floods. Before the Pongolapoort Dam was built these were natural occurrences and after 1973 these were mimicked through controlled flood releases from the dam. For several years the flood releases were haphazard and occurred at arbitrary times to serve the agricultural needs of the people. To date, even though these releases now occur at more predictable times (see Chapter 2), they are still asynchronous with the ecological needs of the biota of the region. Thus, in order to survive, the aquatic biota of this ecosystem have adapted to living in this unpredictable environment and built up a tolerance and resilience that enables them to resist changes and recover quite rapidly. However, these adaptations may only allow the aquatic biota to survive in the environment that they have grown used to. Future predicted climate change therefore still poses a serious threat through longer droughts and both less and more intensive floods. A low baseflow as was released during the study period, though able to sustain ecological process, does not allow for flushing of the ecosystem or refilling of wetlands. Thus, during an extended period of drought where there is continuous baseflow release, it is quite likely that water quality would continue to deteriorate, larger areas would dry out, the hydroperiod of temporary wetlands would decrease even further and eventually lead to the inability of even the most resilient biota to adapt and survive. This may be particularly true for rivers and floodplains that are already regulated through dams such as the lower Phongolo River system where a large amount of water is abstracted. In addition, water quality entering the NGR is clearly being affected by anthropogenic activities occurring outside its borders and this is having repercussions on the aquatic biota, although these are currently not of serious concern. Since it is a biodiversity hotspot, wetland of international importance (Ramsar Site)

and a conservation area it is expected that water quality, aquatic invertebrate biodiversity and community structure, food web and consumer diet would be in a better state in the NGR than outside the protected area. However, even though no severe effects were evident along the sites within the NGR, effects were apparent as very few differences were observed between the studied variables inside and outside NGR. Over an extended period of time, and if it is not managed, anthropogenic effects will continue to increase with eventual effects and loss of the unique aquatic biota of the region. It is therefore highly important that effective management strategies are put in place in order to prevent further degradation of this ecologically important ecosystem.

8.4 Recommendations

Future potential research and recommendations were determined during the course of this study

- True “post-drought” research could not yet take place during the present study as no large flood had been released from the Pongolapoort Dam yet. Once the ecosystem has received a suitable flood again, follow up assessments need to take place in order to confirm that recovery is taking place and no lasting effects occurred from the supra-seasonal drought.
- Although there are currently no apparent significant negative effects on the ecological processes of the lower Phongolo with regards to anthropogenic activity, potential future effects need to be further investigated. This is particularly true of the Phongolo River as it supplies water to the Ndumo Game Reserve.
- Further research needs to be undertaken to confirm whether nutrient loading into the protected area of Ndumo Game Reserve is indeed occurring, in order for appropriate management procedures to be put in place.
- Although this study found that length of hydrologic connectivity did not appear to have any effect on aquatic invertebrates, this is likely not true for fish as they will need increased connection times to the river in order to utilize the floodplain to its full potential. Further investigation is needed to determine whether the volume as well as velocity of the controlled flood release from the Pongolapoort Dam allows for long enough hydrologic connectivity that will permit fish to utilise these wetlands successfully.
- An assessment of the potential contribution of the Usuthu River to the ecology of the floodplain needs to take place. This river is an important source of water to several of the wetlands of the NGR, most prominently Nyamithi lake, and is less regulated than the Phongolo River.
- It is highly important that assessments on the effects of climate change continue, with focus on regions that will be most affected by drought, most prominently severely regulated ecosystems such as the lower Phongolo ecosystem, and management strategies need to be put in place to prevent possible future extinction of aquatic biota.

CHAPTER 9:

List of references used in the various chapters of this thesis

Aarts BGW, van den Brink FWB, Nienhuis PH. 2004. Habitat loss as the main cause of the slow recovery of fish faunas of regulated large rivers in Europe: The transversal floodplain gradient. *River Research and Applications* 20: 3-23.

Abrantes KG, Barnett A, Bouillon S. 2014. Stable isotope-based community metrics as a tool to identify patterns in food web structure in east African estuaries. *Functional Ecology* 28: 270-282.

Acuña V, Muñoz G, Omella M, Sabater F and Sabater S. 2005. Drought and postdrought recovery cycles in an intermittent Mediterranean stream: Structural and functional aspects. *Journal of the North American Benthological Society* 24: 919-933.

Afonina EY, Tashlykova NA. 2018. Plankton community and the relationship with the environment in saline lakes of Onon-Torey plain. Northeastern Mongolia. *Saudi Journal of Biological Sciences* 25: 399-408

Akamatsu F, Toda H, Okino T. 2004. Food source of riparian spiders analysed by using stable isotope ratios. *Ecological Research* 19: 655-662.

Akhurst EGJ, Breen CM. 1988. Ionic content as a factor influencing turbidity in two floodplain lakes after a flood. *Hydrobiologia* 160: 19-31.

Alberto WD, Del Pilar DM, Valeria AM, Fabiana PS, Cecilia HA, de Los Ángeles. 2001. Pattern recognition techniques for the evaluation of spatial and temporal variation in water quality. A case study: Suquia River basin (Córdoba-Argentina). *Water Research* 35: 2881-2894.

Alcamo J, Flörke M, Märker M. 2007. Future long-term changes in global water resources driven by socio-economic and climatic changes. *Hydrological Sciences Journal* 52: 247-275.

Aldous A, Fitzsimons J, Richter B, Bach L. 2011. Droughts, floods and freshwater ecosystems: Evaluating climate change impacts and developing adaptations strategies. *Marine and Freshwater Research* 62: 223-231.

Aliscioni SS, Giussani LM, Zuloaga FO, Kellogg EA. 2003. A molecular phylogeny of *Panicum* (Poaceae: Paniceae): Tests of monophyly and phylogenetic placement within the Panicoideae. *American Journal of Botany* 5: 796-821.

Al-Shami SA. CSM Rawi, Ahmad AH, Hamid SA, Nor SAM. 2011. Influence of agricultural, industrial and anthropogenic stresses on the distribution and diversity of macroinvertebrates in Juru River Basin, Penang, Malaysia. *Ecotoxicology and Environmental Safety* 74: 1195-1202.

Amatya DM, Skaggs RW. 2011. Long-term hydrology and water quality of drained pine plantation in North Carolina. *Advances in Forest Hydrology* 54: 2087-2097.

Amoros C, Bornette G .2002. Connectivity and biocomplexity in waterbodies of riverine floodplains. *Freshwater Biology* 47: 761-776.

Armin M, Marquet PA. 2004. Intraguild predation: A widespread interaction related to species biology. *Ecological Letters* 7: 557-564.

Armstrong RA. 2014. When to use the Bonferroni correction. *Ophthalmic and Physiological Optics* 34: 502-508.

- Arthington AH, Naiman RJ, McClain ME, Nilsson C. 2010. Preserving the biodiversity and ecological services of rivers: New challenges and research opportunities. *Freshwater Biology* 55: 1-16.
- Azrina MZ, Yap, CK, Ismail AR, Ismail A, Tan SG. 2006. Anthropogenic impacts on the distribution and biodiversity of benthic macroinvertebrates and water quality of the Langat River, Peninsular Malaysia. *Ecotoxicology and Environmental Safety* 64: 337-347.
- Baeta A, Vieira LR, Lírio AV, Canhoto C, Marques JC, Guilhermino. 2017. Use of stable isotope ratios of fish larvae as indicators to assess diets and patterns of anthropogenic nitrogen pollution in estuarine ecosystems. *Ecological Indicators* 83: 112-121.
- Balcombe SR, Bunn SE, McKenzie-Smith FJ, Davies PM. 2005. Variability of fish diets between dry and flood periods in an arid zone floodplain river. *Journal of Fish Biology* 67: 1552-1567.
- Barakat A, El Baghdadi, Rais J, Brahim A, Slassi M. 2016. Assessment of spatial and seasonal water quality variation of Oum Er Rbia River (Morocco) using multivariate statistical techniques. *International Soil and Water Conservation Research* 4: 284-292.
- Barbour MT, Gerritsen J, Snyder BD, Stribling JB. 1999. Rapid Bioassessment Protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish (2nd edition). Report No. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington D.C.
- Barnes K, Ellery W, Kindness A. 2002. A preliminary analysis of water chemistry of the Mkuze Wetland System, KwaZulu Natal: A mass balance approach. *Water SA* 28: 1-11.
- Bates BC, Kundzewicz ZW, Wu S, Palutikof JP (editors). 2008. Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat, Geneva.
- Bates D, Sarkar D, Bates MD, Matrix L. 2007. The lme4 package. R package version 2. Available from: <https://cran.r-project.org/web/packages/lme4/index.html>. (Accessed on 18 March 2018).
- Batzer D, Boix D. (editors) .2016. Invertebrates in freshwater wetlands: An international perspective on their ecology. Springer, USA.
- Beketov MA, Schäfer RB, Marwitz A, Paschke A, Liess M. 2008. Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: Effect concentrations and recovery dynamics. *Science of the Total Environment* 405: 96-108.
- Beklioglu M, Moss B. 1996. Mesocosm experiment on the interaction of sediment influence, fish predation and aquatic plants with the structure of phytoplankton and zooplankton communities. *Freshwater Biology* 36: 315-325.
- Bender MM. 1971. Variation in the $^{13}\text{C}/^{12}\text{C}$ ratios of plants in relation to the pathway of photosynthetic carbon dioxide fixation. *Phytochemistry* 10: 1239-1244.
- Benedito-Cecilo E, Araujo-Lima CARM, Forsberg BR, Bittencourt MM, Martinelli LC. 2000. Carbon sources of Amazonian fisheries. *Fisheries Management and Ecology* 7: 305-315.
- Benider A, Tifnouti A, Pourriot R. 2002. Growth of *Moina macrocopa* (Straus 1820) (Crustacea, Cladocera): influence of trophic conditions, population density and temperature. *Hydrobiologia* 468: 1-11.
- Bird MS, Perissinotto R, Miranda NAF, Peer N, Raw JL. 2016. Stable isotope analysis of consumer food webs indicates ecosystem recovery following prolonged drought in a subtropical estuarine lake. *African Journal of Marine Science* 38: 411-422.
- Bird TL, Scholes MC. 2012. Using multivariate statistical analysis to assess changes in water chemistry in sections of the Vaal Dam catchment between 1991 and 2008. *Water SA* 35: 647-654
- Bocard D, Gilet F, Legendre P. (editors). 2011. Numerical Ecology with R. Springer, USA.

- Boecklen WJ, Yarnes CT, Cook, BA, James AC. 2011. On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics* 42: 411-440.
- Boersma K, Bogan MT, Henrichs BA, Lytle DA. 2014. Invertebrate assemblages of pools in arid-land streams have high functional redundancy and are resistant to severe drying. *Freshwater Biology* 59: 491-501.
- Bogan MT, Lytle DA. 2007. Seasonal flow variation allows “time-sharing” by disparate aquatic insect communities in montane desert streams. *Freshwater Biology* 52: 290-304.
- Bogan MT, Lytle DA. 2011. Severe drought drives novel community trajectories in desert stream pools. *Freshwater Biology* 56: 2070-2081.
- Bogan MT, Boersma KS, Lytle DA. 2015. Resistance and resilience of invertebrate communities to seasonal suprasedimental drought in arid-land headwater streams. *Freshwater Biology* 60: 2547-2558.
- Boix D, Sala J, Quintana XD, Moreno-Amich R. 2004. Succession of the animal community in a Mediterranean temporary pond. *Journal of the North American Benthological Society* 23: 29-49.
- Bond NR, Lake PS, Arthington AH. 2008. The impacts of drought on freshwater ecosystems: An Australian perspective. *Hydrobiologia* 600: 3-16.
- Borrelli JJ, Ginzburg LR. 2014. Why there are so few trophic levels: Selection against instability explains the pattern. *Food Webs* 1: 10-17.
- Bouillon S, Connolly RM, Gillikin DP. 2011. Use of stable isotopes to understand food webs and ecosystem functioning in estuaries. In: Wolanski E., McLusky DS (editors). *Treatise on estuarine and coastal science*. Academic Press, Waltham, USA. pp 143-173.
- Boulton AJ, HM Valett, Fisher SG. 1992. Spatial distribution and taxonomic composition of the hyperheos of several Sonoran Desert streams. *Archiv für Hydrobiologie* 1: 37-61.
- Boulton AJ. 2003. Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages. *Freshwater Biology* 48: 1173-1185.
- Brainwood MA, Burgin S, Maheshwari B. 2004. Temporal variations in water quality of farm dams: impacts of land use and water sources. *Agricultural Water Management* 70: 151-175.
- Breen CM, Furness HD, Heeg J, Kok J. 1978. Bathymetric studies on the Pongolo river floodplain. *Journal of the Limnological Society of southern Africa* 4: 95-100.
- Brendonck L, de Meester L. 2003. Egg banks in freshwater zooplankton: evolutionary and ecological archives in sediment. In: Vandonk E, Boersma M, Spaak P (editors). *Recent Developments in Fundamental and Applied Plankton Research*. *Hydrobiologia* 491: 65-84.
- Brendonck L, Williams WD. 2000. Biodiversity in wetlands of dry regions (drylands). In: Gopal B, Junk WJ, Davis JA (editors.) *Biodiversity in Wetlands: Assessment, function and conservation*. Backhuys Publishers. Leiden, The Netherlands. pp 181-194.
- Brock MA, Nielsen DL, Shiel RJ, Green JD, Langley JD. 2003. Drought and aquatic community resilience: The role of eggs and seeds in sediments of temporary wetlands. *Freshwater Biology* 48: 1207-1218.
- Brock MA, Nielson DL, Crosslé K. 2005. Changes in biotic communities developing from freshwater wetland sediments under experimental salinity and water regimes. *Freshwater Biology* 50: 1699-1710.
- Bunn SE, Arthington AH. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management* 30: 492-507.
- Bunn SE, Boon PI. 1993. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. *Oecologia* 96: 85-94.

- Burt TP, Howden NJK, Worrall F, Whelan MJ. 2010. Long-term monitoring of river water nitrate: How much data do we need? *Journal of Environmental Monitoring* 12: 71-79.
- Burt TP, Howden NJK, Worrall F, McDonnell JJ. 2011. On the value of long-term, low-frequency water quality sampling: Avoiding throwing the baby out with the bathwater. *Hydrological Processes* 25: 828-830.
- Burt TP. 1994. Long-term study of the natural environment: Perceptive science or mindless monitoring? *Progress in Physical Geography* 18: 475-496.
- Buytaert W, Céleri R, de Bièvre B, Cisneros F, Wyseure G, Deckers J, Hofstede R. 2006. Human impacts on the hydrology of the Andean páramos. *Earth Science Reviews* 79: 53-72.
- Cabana G, Rasmussen JB. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences (USA)*. 93: 10844-10847.
- Calverly PM, Downs CT. 2014. Population status of Nile Crocodiles in Ndumo Game Reserve, KwaZulu-Natal, South Africa (1971-2012). *Herpetologica* 70: 426-438.
- Canfield TJ, Jones JR. 1996. Zooplankton abundance, biomass and size-distribution in selected midwestern waterbodies and relation with trophic state. *Journal of Freshwater Ecology* 11: 171-181.
- Caruso BS. 2001. Regional river flow, water quality, aquatic ecological impacts and recovery from drought. *Hydrological Sciences Journal* 46: 677-699.
- Chamier J, Schachtschneider K, le Matire DC, Ashton PJ, van Wilgen BW. 2012. Impacts of invasive alien plants on water quality, with particular emphasis on South Africa. *Water SA* 38: 345-356.
- Chaparro G, Fontanarrosa MS, O'Farrell I. 2016. Colonization and succession of zooplankton after a drought: Influence of hydrology and free-floating plant dynamics in a floodplain lake. *Wetlands* 36: 85-100.
- Chavarie L, Howland KL, Tonn WM. 2013. Sympatric polymorphism in Lake Trout: the coexistence of multiple shallow-water morphotypes in Great Bear Lake. *Transactions of the American Fisheries Society* 142: 814-823.
- Chen Q, Liu Y, Ho WT, Chan SK, Li QH, Huang JR. 2017. Use of stable isotopes to understand food webs in Macao wetlands. *Wetlands Ecology and Management*. 25: 59-66.
- Chessman BC, Robinson DP. 1987. Some effects of the 1982-83 drought on water quality and macroinvertebrate fauna in the Lower LaTrobe River, Victoria. *Australian Journal of Marine and Freshwater Research* 38: 289-299.
- Chessman BC. 2015. Relationship between lotic macroinvertebrate traits and responses to extreme drought. *Freshwater Biology* 60: 50-63.
- Clarke KR, Gorley RN. 2006. *PRIMER v6. User Manual/Tutorial*. PRIMER-E, Plymouth, UK.
- Clarke KR, Warwick RM. 2001. *Change in marine communities: An approach to statistical analysis and interpretation. Manual for the PRIMER statistical programme*. Natural environment research council, Plymouth, UK.
- Coke M. 1970a. The water requirements of the Pongolo floodplain pas. In: *Water Year 1970, Convention on Water for the Future*, Pretoria, 16-20 November 1970. Department of Water Affairs, Pretoria, South Africa.
- Coke M. 1970b. The status of the Pongolo floodplain pans and the effect of a 1000 cumec flood in December 1970. Unpublished report, Natal Parks, Game and Fish Preservation Board, Pietermaritzburg, South Africa.

- Cómin FA, Williams WD. 1994. Parched continents: Our common future? In: *Limnology Now: A Paradigm of Planetary Problems* (Ed. R. Margalef). Elsevier Science BV Amsterdam, The Netherlands. pp 473-527.
- Dallas H. 2000. Ecological reference condition project: Field Manual. Volume 1: General information, catchment condition, invertebrates and water chemistry. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa.
- Dallas HF, Day JA. 2004. The effects of water quality variables on aquatic ecosystems. WRC Report TT 224/04. Water Research Commission, Pretoria, South Africa.
- Dallas HF. 2007. The influence of biotope availability on macroinvertebrate assemblages in South African rivers: implications for aquatic bioassessment. *Freshwater Biology* 52: 370-380.
- Dallas HF, Day JA. 2007. Natural variation in macroinvertebrate assemblages and the development of a biological banding system for interpreting bioassessment data-a preliminary evaluation using data from upland sites in the south-western Cape, South Africa. *Hydrobiologia* 575: 231-244.
- Dalu T, Wasserman RJ, Froneman PW, Weyl OLF. 2017. Trophic isotopic carbon variation increases with pond's hydroperiods: Evidence from an Austral ephemeral ecosystem. *Scientific Reports* 7: 1-8.
- Darwall W, Smith K, Allen D, Holland R, Harrison I, Brooks E (editors). 2011. *The diversity of life in African freshwaters: Underwater, under threat. An analysis of the status and distribution of freshwater species throughout mainland Africa*. IUCN, Cambridge, UK and Gland, Switzerland.
- Davidson TA, MacKay AW, Wolski P, Mazebedi R, Murray-Hudson, Todd M. 2012. Seasonal and spatial hydrological variability drives aquatic biodiversity in a flood-pulsed, subtropical wetland. *Freshwater Biology* 57: 1253-1265.
- Day JA, de Moor IJ (editors). 2002a. Guides to freshwater invertebrates of southern Africa. Volume 5: Non-Arthropods (The Protozoans, Porifera, Cnidaria, Platyhelminthes, Nemertea, Rotifera, Nematoda, Nematomorpha, Gastrotrichia, Bryozoa, Tardigrada, Polychaeta, Oligochaeta and Hirudinae). WRC Report TT 167/02. Water Research Commission, Pretoria, South Africa.
- Day JA, De Moor IJ (editors). 2002b. Guides to the freshwater invertebrates of Southern Africa. Volume 6: Arachnida and Mollusca (Araneae, Water Mites and Mollusca). WRC Report TT 182/02. Water Research Commission, Pretoria, South Africa.
- Day JA, De Moor IJ, Stewart BA, Louw AE (editors). 2001a. Guides to the Freshwater Invertebrates of Southern Africa. Volume 3: Crustacea II (Ostracoda, Copepoda and Branchiura). WRC Report TT 148/01. Water Research Commission, Pretoria, South Africa.
- Day JA, Harrison AD, De Moor IJ (editors). 2003. Guides to the freshwater invertebrates of Southern Africa. Volume 9: Diptera. WRC Report TT 201/02. Water Research Commission, Pretoria, South Africa.
- Day JA, Stewart BA, De Moor IJ, Louw AE (editors). 2001b. Guides to the Freshwater invertebrates of Southern Africa. Volume 4: Crustacea III (Bathynellacea, Amphipoda, Isopoda, Spelaeogriphacea, Tanaidacea, Decapoda). WRC Report TT 141/01. Water Research Commission, Pretoria, South Africa.
- Day JA, Stewart BA, De Moor IJ, Louw AE. 1999. Guides to the freshwater invertebrates of Southern Africa. Volume 2: Crustacea I (Notostraca, Anostraca, Conchostraca and Cladocera). WRC Report TT 121/00. Water Research Commission, Pretoria, South Africa.
- de Almeida VLL, Hahn SS, de Vazzoler AEAM. 1997. Feeding patterns in five predatory fishes of the high Parana River floodplain (PR Brazil). *Ecology of Freshwater Fish* 6: 123-133.
- de Cáceras M, Jansen F, de Cáceras MM. 2016. Package "indicspecies". R package version 1.7.6. Available from: <https://cran.r-project.org/web/packages/indicspecies/index.html>. (Accessed 18 March 2018).

- de Cáceras M. 2013. How to use the indicpecies package (version 1.7.1). Available from: <https://cran.r-project.org/web/packages/indicpecies/index.html>. (Accessed 18 March 2018).
- de Meester L, Declerk S, Stoks R, Louette G, van de Meutter F, de Bie T, Michels E, Brendonck L. 2005. Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. *Aquatic Conservation: Marine and Freshwater Ecosystems*. 15: 175-725.
- de Meester, Gomez A, Okamura B, Schwenk K. 2002. The monopolization hypothesis and the dispersal-gene flow paradox in aquatic organisms. *Acta Oecologica*. 23: 221-232.
- de Moor IJ, Day JA, de Moor FC (editors). 2003a. Guides to the Freshwater Invertebrates of Southern Africa. Volume 7: Insecta I (Ephemeroptera, Odonata and Plecoptera). WRC Report TT 207/03. Water Research Commission, Pretoria, South Africa.
- de Moor IJ, Day JA, De Moor FC (editors). 2003b. Guides to the Freshwater Invertebrates of Southern Africa. Volume 8: Insecta II (Hemiptera, Megaloptera, Neuroptera, Trichoptera and Lepidoptera). WRC Report TT 214/03. Water Research Commission, Pretoria, South Africa.
- de Necker L. 2015. Macroinvertebrate biodiversity from selected ephemeral and floodplain pans of the Lower Phongolo River. Unpublished MSc thesis. University of Johannesburg.
- de Villiers S, Thiar C. 2007. The nutrient status of South African rivers: Concentrations, trends and fluxes from the 1970s to 2005. *South African Journal of Science* 103: 343-349.
- Debels P, Figueria R, Urrutia Roberta, Barra R, Niell X. 2005. Evaluation of water quality in the Chillán River (Central Chile) using physicochemical parameters and a Modified Water Quality Index. *Environmental Monitoring and Assessment* 110: 301-322.
- Deegan LA, Garritt RH. 1997. Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* 147: 31-47.
- DeNiro MJ, Epstein S. 1978. Influence of diet in the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42: 495-506.
- Department of Water Affairs (DWA). 2009. Adopt-a-River Programme Phase II: Development of an Implementation Plan. Water Resource Quality Situation Assessment. Prepared by H. Hendriks and JN Rossouw for Department of Water Affairs, Pretoria, South Africa.
- Department of Water Affairs and Forestry (DWAF). 1985. The effects of the Domoina floods and releases from the Pongolapoort Dam on the Pongola floodplain. DWAF Report no: B-n3/0704/1. Department of Water Affairs, Pretoria, South Africa.
- Department of Water Affairs and Forestry (DWAF). 1996. South African Water Quality Guidelines (2nd edition). Volume 1: Domestic Use.
- Department of Water Affairs and Forestry (DWAF). 2004. National Water Resource Strategy. Department of Water Affairs and Forestry, Pretoria, South Africa.
- Department of Water Affairs and Forestry (DWAF). 2008. Draft Methods for determining the water quality component of the Ecological Reserve. Department of Water Affairs, Pretoria, South Africa.
- Department of Water and Sanitation (DWS). 2014a. Resource Directed Measures: Reserve determination study of selected surface water and groundwater resources in the Usuthu/Mhlathuze Water Management Area. Pongola Floodplain – EWR Report. Report No: RDM/WMA6/CON/COMP/1213, produced by Tlou Consulting (Pty) Ltd, Pretoria, South Africa.
- Department of Water and Sanitation (DWS). 2014b. Resource Directed Measures: Reserve determination study of selected surface water and groundwater resources in the Usutu/Mhlathuze Water Management Area. River Intermediate EWR – Volume 3: Specialist Reports. Report no: RDM/WMA6/CON/COMP/0813. Report compiled by Tlou Consulting (Pty) Ltd, Pretoria, South Africa.

- Dickens CWS, Graham PM. 2002. The South African Scoring System (SASS) Version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science* 27: 1-10.
- Döll P, Fiedler K, Zhang J. 2009. Global-scale analysis of river flow alterations due to water withdrawals and reservoirs. *Hydrology and Earth System Science* 13: 2413-2432.
- Döll P, Zhang J. 2010. Impact of climate change on freshwater ecosystems: a global-scale analysis of ecologically relevant river flow alterations. *Hydrology and Earth System Science* 14: 783-799.
- Dube T, de Necker L, van Vuren JHJ, Wepener, V, Smit NJ, Brendonck L. 2016. Spatial and temporal variation of invertebrate community structure in flood-controlled tropical floodplain wetlands. *Journal of Freshwater Ecology* 32:1-15.
- Dube T, Wepener V, van Vuren JHJ, Smit N, Brendonck L. 2015. The case for environmental flow determination for the Phongolo River, South Africa. *African Journal of Aquatic Science* 40: 1-8.
- Dube T. 2015. The impact of hydro-ecological variation on aquatic invertebrate communities of South African savanna pans: Towards sustainable management of threatened ecosystems in the Ndumo. Unpublished PhD dissertation. KU Leuven, Belgium.
- Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan CA. 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews* 81: 163-182.
- Dudgeon D. (editor). 2008. *Tropical Stream Ecology*. Elsevier, USA.
- Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO. 2000. Climate extremes: Observations, modelling and impacts. *Science* 289: 2068-2074.
- Eloranta P. 1982. Seasonal succession of phytoplankton in an ice-free pond warmed by a thermal power plant. *Hydrobiologia* 86: 87-91.
- Elosegi A, Díez J, Mutz M. 2010. Effects of hydromorphological integrity on biodiversity and functioning of the river ecosystems. *Global change and River ecosystems*. *Hydrobiologia* 657: 199-215.
- Ewart-Smith J, Ollis D, Day JA, Malan H. 2006. National Wetland Inventory: Development of a Wetland Classification System for South Africa. Report no K8/652. Prepared for: Water Research Commission (WRC) and the South African National Biodiversity Institute (SANBI), Pretoria, South Africa.
- Feio MJ, Almeida SFP, Craveiro SC, Calado AJ. 2007. Diatoms and macroinvertebrates provide consistent and complimentary information on environmental quality. *Hydrobiologia* 169: 247-258.
- Finlay JC, Power ME, Cabana G. 1999. Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. *Limnology and Oceanography* 44: 1198-1203.
- Foley CJ, Henebry ML, Happel A, Bootsma HA, Czesny SJ, Jannssen J, Jude DJ, Rinchar J, Höök TO. 2017. Patterns of integration of invasive round goby (*Neogobius melanostomus*) into a nearshore freshwater food web. *Food Webs* 10: 26-38.
- Forsberg BR, Araujo-Lima CARM, Martinelli LA, Victoria RL, Bonassi JA. 1993. Autotrophic carbon sources for fish of the Central Amazon. *Ecology* 74: 643-652.
- Fox J, Weisberg S, Adler D, Bates D, Baud-Bovy G, Ellison S, Firth D, Friendly M, Gorjanc G, Graves G, Heiberger R, Laboissiere R, Monette G, Murdoch D, Nilsson H, Ogle D, Ripley B, Venables W, Winsemius D, Zeileis A. 2017. Package "car". R package version 2.1.5. Vienna, Austria R Foundation for Statistical Computing. Available from: <https://www.R-project.org/> (Accessed 20 March 2018).
- Friedl G, Wüest A. 2002. Disrupting biogeochemical cycles-Consequences of damming. *Aquatic Sciences* 64: 55-65.
- Furness AI. 2016. The evolution of an annual life cycle in killifish: Adaptation to ephemeral aquatic environments through embryonic diapause. *Biological Reviews* 91: 796-812.

- Gabler HM, Amundsen PE. 2010. Feeding strategies, resource utilisation and potential mechanisms for competitive coexistence of Atlantic salmon and alpine bullhead in a sub-Arctic river. *Aquatic Ecology* 44: 325-336.
- Gabriels W, Lock K, de Pauw N, Goethals PLM. 2010. Multimetric macroinvertebrate index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnologia* 40: 199-207.
- Gaeta JW, Sass GG, Carpenter SR. 2013. Drought-driven lake level decline: Effects on coarse woody habitat and fishes. *Canadian Journal of Fisheries and Aquatic Sciences* 71: 315-325.
- Gallardo B, Dolédec S, Paillex A, Arscott DB, Sheldon F, Zilli F, Méricoux, Castella E, Comín FA. 2014. Response of benthic macroinvertebrates to gradients in hydrological connectivity: A comparison of temperate, subtropical, Mediterranean and semiarid river floodplains. *Freshwater Biology* 59: 630-648.
- Gannon JE, Stemberger RS. 1978. Zooplankton (especially crustaceans and rotifers) as indicators of water quality. *Transactions of the American Microscopical Society* 97: 16-35.
- Garner G, van Loon AF, Prudhomme C, Hannah DM. 2015. Hydroclimatology of extreme river flows. *Freshwater Biology* 60: 2461-2476.
- Gerber A. Gabriel MJM. 2002. Aquatic invertebrates of South African rivers: Field guide. Institute for water quality studies, Department of Water Affairs and Forestry, Pretoria, South Africa.
- Gerber R, Wepener V, Smit NJ. 2015. Application of multivariate statistics and toxicity indices to evaluate the water quality suitability for fish of three rivers in the Kruger National Park, South Africa. *African Journal of Aquatic Science* 3: 247-259.
- Ghasemi A, Zahediasl S. 2012. Normality test for statistical analysis: A guide for non-statisticians. *International Journal of Endocrinology Metabolism* 10: 486-489.
- Ghosh A, Mandal S, Bhattacharjee I, Chandra G. 2005. Biological control of vector mosquitoes by some common exotic fish predators. *Turkish Journal of Biology* 29: 167-171.
- Gilinsky E. 1984. The role of fish predation and spatial heterogeneity in determining benthic community structure. 1984. *Ecology* 65: 455-468.
- Ginatullina E, Atwell L, Saito L. 2017. Resilience and resistance of zooplankton communities to drought-induced salinity in freshwater and saline lakes of Central Asia. *Journal of Arid Environments* 144: 1-11.
- Gliwicz ZM, Pijanowska J. 1989. Chapter 7: The role of predation in zooplankton succession. In: Sommer U (editor). *Plankton Ecology*. Springer, Germany.
- González JM, Mollá S, Roblas N, Descals E, Moya Ó, Casado C. 2013. Small dams decrease leaf litter breakdown rates in Mediterranean mountain streams. *Hydrobiologia* 712: 117-128.
- Greenway M. 2010. Wetlands and Ponds for stormwater treatment in subtropical Australia: Their effectiveness in enhancing biodiversity and improving water quality? *Journal of Contemporary Water Research & Education* 146: 22-38.
- Gross TL, Rudolf L, Levin SA, Dieckmann U. 2009. Generalized models reveal stabilizing factors in food webs. *Science* 325: 747-750.
- Guedes APA, Araújo FG, Pessanha ALM, Mlagre RR. 2015. Partitioning of the feeding niche along spatial, seasonal and size dimensions by the fish community in a tropical Bay in Southeastern Brazil. *Marine Ecology* 36: 38-56.
- Hamilton SK, Lewis WM Jr. 1992. Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. *Geochimica et Cosmochimica Acta* 56: 4237-4246.
- Hammer UT. 1986. Saline lake ecosystems of the world. Volume 59. Kluwer Academic Publishers Group, Dodrecht, The Netherlands.

Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C, Swadling K. 1991. A review of the salt sensitivity of the Australian freshwater biota. *Hydrobiologia* 210: 105-144.

Hayden B, Harrod C, Kimmo K. 2014. Dual fuels: Intra-annual variation in the relative importance of benthic and pelagic resources to maintenance, growth and reproduction in a generalist salmonid fish. *Journal of Animal Ecology* 83: 1501-1512.

Heath SK, Plater AJ. 2010. Records of pan (floodplain wetland) sedimentation as an approach for post-hoc investigation of the hydrological impacts of dam impoundment: The Pongolo River, KwaZulu-Natal. *Water Research* 44: 4226-4240.

Heeg J, Breen C. 1994. Resolution of conflicting values on the Pongola River floodplain. Volume 2. In: Patten B, Jorgenson S, Dumont S. (editors). *Wetlands and shallow continental water bodies*. The Hague: SBP Publishing. pp 303-359.

Heeg J, Breen CM, Colvin PM, Furness HD, Musli CF. 1978. On the dissolved solids of the Pongolo floodplain pans. *Journal of the Limnological Society of southern Africa* 4: 59-64.

Heeg J, Breen CM, Rodgers KH. 1980. The Pongolo floodplain: A unique ecosystem threatened. *Civil Engineer in South Africa* 22: 125-128.

Heeg J, Breen CM. 1982. Man and the Pongolo Floodplain. South African National Scientific Programmes Report no 58. A report of the Committee for Inland Water Ecosystems, National Programme for Environmental Sciences, Cooperative Scientific Programmes. Council for Scientific and Industrial Research, Pretoria, South Africa.

Helson JE, Williams DD. 2013. Development of a macroinvertebrate multimetric index for the assessment of low-land streams in the neotropics. *Ecological Indicators* 29: 167-178.

Herbst DB, Blinn DW. 1998. Experimental mesocosm studies of salinity effects on the benthic algal community of a saline lake. *Journal of Phycology* 34: 772-778.

Herbst DB. 2001. Gradients of salinity stress, environmental stability and water chemistry as a template for defining habitat types and physiological strategies in inland sea waters. *Hydrobiologia* 466: 209-219.

Hicks KA, Loomer HA, Fuzzen MLM, Kleywegt S, Tetreault GR, McMaster ME, Servos MR. 2017. $\delta^{15}\text{N}$ tracks changes in the assimilation of sewage-derived nutrients into a riverine food web before and after major process: Alterations at two municipal wastewater treatment plants. *Ecological Indicators* 72: 747-758.

Hill JM, Jones RW, Hill MP, Weyl OLF. 2015. Comparisons of isotopic niche width of some invasive and indigenous fauna in a South African river. *Freshwater Biology* 60: 893-902.

Holeck KT, Watkins JM, Mills EL, Johannsson O, Millard S, Richardson V, Bowen K. 2008. Spatial and long-term temporal assessment of Lake Ontario water clarity, nutrients, chlorophyll a and zooplankton. *Aquatic Ecosystem Health and Management* 11: 377-391.

Howden NJK, Burt TP, Worral F, Whelan MJ, Bierzoza M. 2010. Nitrate concentrations and fluxes in the River Thames over 140 years (1868-2008): Are increases irreversible? *Hydrological Processes* 23: 2657-2662.

Hubert WA, Pope KL, Dettmers JM. Passive capture techniques. 2012. In: Zale AV, Parrish DL, Sutton TM (editors). *Fisheries techniques* (3rd edition). American Fisheries Society, Bethesda, Maryland.

Huizenga JM, Silberbauer M, Dennis R, Dennis I. 2013. Technical note: An inorganic water chemistry dataset (1972-2011). of rivers, dams and lakes in South Africa. *Water SA* 39: 335-340.

Humborg C, Conley DJ, Rahm L, Wulff F, Cociasu A, Itteko V. 2000. Silicon retention in river basins: Far-reaching effects on biogeochemistry and aquatic food webs in coastal marine environments. *Ambio* 29: 45-50.

- Inger R, Jackson AL, Parnell AC, Bearhop S. 2010. SIAR V4 (Stable Isotope Analysis in R). An Ecologists Guide.
- Jackson AT, Adite A, Roach KA, Winemiller KO. 2013. Primary production, food web structure, and fish yields in constructed natural wetlands in the floodplain of an African river. *Canadian Journal of Fisheries and Aquatic Sciences* 553: 543-553.
- Jaganyi J, Salagae M, Matiwane N. 2009. Integrating floodplain livelihoods into a diverse rural economy by enhancing co-operative management: A case study of the Pongolo floodplain system, South Africa. WRC Report No. 1299/1/08. Water Research Commission, Pretoria, South Africa.
- James KR, Cant B, Ryan T. 2003. Responses of freshwater biota to rising salinity levels and implications for saline water management: A review. *Australian Journal of Botany* 51: 703-713.
- Jenkins KM, Boulton AJ. 2003. Connectivity in a dryland river: Short-term aquatic microinvertebrate recruitment following floodplain inundation. *Ecology* 84: 2708-2723.
- Jentsch A, Beierkuhnlein C. 2008. Research frontiers in climate change: Effects of extreme meteorological events on ecosystems. *Comptes Rendus Geoscience* 340: 621-628.
- Jepsen DB, Winemiller KO. 2007. Basin geochemistry and isotopic ratios of fishes and basal production sources in four neotropical rivers. *Ecology of Freshwater Fish* 16: 267-281.
- Jocque M, Vanschoenwinkel B, Brendonck L. 2010. Freshwater rockpools: A review of habitat characteristics, faunal diversity and conservation value. *Freshwater Biology* 55: 1587-1602.
- Jones B, Cullen-Unsworth L, Unsworth RKF. 2018. Tracking nitrogen source using $\delta^{15}\text{N}$ reveals human and agricultural drivers of seagrass degradation across the British Isles. *Frontiers in Plant Sciences* 9: 133.
- Jones BL, Unsworth RKF. 2016. The perilous state of seagrass in the British Isles. *Royal Society Open Science* 3: 150596.
- Jones G. 2002. Setting environmental flows to sustain a healthy working river. Watershed, Cooperative Research Centre for Freshwater Ecology, Canberra, Australia.
- Jozini Local Municipality Integrated Development Plan (IDP) 2017/18 – 2021/22. 2017. 4th Generation. Jozini Municipality, Bottom Town, Jozini.
- Junk WJ (editor). 2013. The central Amazon floodplain: Ecology of a pulsing system. Springer Science and Business Media, Heidelberg, Germany.
- Junk WJ, Bayley PB, Sparks RE. 1989. The flood pulse concept in River-Floodplain-systems. *Canadian Special Publication of Fisheries and Aquatic Sciences* 106: 110-127.
- Karagianni A, Stamou G, Katsiapi M, Polykarpou P, Dörflinger G, Michaloudi E. 2018. Zooplankton communities in Mediterranean temporary lakes: The case of saline lakes in Cyprus. *Annales de Limnologie-International Journal of Limnology*. 54: 14.
- Karr JD, Showers WJ, Jennings GD. 2003. Low-level nitrate export from confined dairy farming detected in North Carolina streams using $\delta^{15}\text{N}$. *Agriculture, Ecosystems and Environment* 95: 103-110.
- Kavian A, Mohammadi M, Gholami L, Rodrigo-Comino J. 2018. Assessment of the spatiotemporal effects of land use changes on runoff and nitrate loads in the Talar River. *Water* 1: 445.
- Khatri N, Tyagi S. 2015. Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. *Frontiers in Life Science* 8: 23-39.
- King SL, Sharitz RR, Groninger JW, Battaglia LL. 2009. The ecology, restoration, and management of southeastern floodplain ecosystems: A synthesis. *Wetlands* 29: 624-634.

Klaassen M, Piersma T, Korthals H, Dekinga A, Dietz MW. 2010. Single-point isotope measurements in blood cells and plasma to estimate the time since diet switches. *Functional Ecology* 24: 796–804.

Kleynhans CJ and Louw MD. 2008. River EcoClassification: Manual for EcoStatus determination (Version 2), Module A: EcoClassification and EcoStatus Determination. WRC Report No. TT 329/8, Water Research Commission, Pretoria, South Africa.

Kleynhans CJ, Louw MD, Graham M. 2008. Module G: EcoClassification and EcoStatus determination in River EcoClassification: Index of Habitat Integrity (Section 1: Technical manual) Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC Report no TT 377-08, Water Research Commission, Pretoria, South Africa.

Kleynhans CJ, Louw MD, Thirion C, Rossouw NJ and Rowntree K. 2005. River Ecoclassification: Manual for EcoStatus determination (Version 1). Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC Report No. KV 168/05, Water Research Commission, Pretoria, South Africa.

Kok HM. 1980. Ecological studies of some important fish species of the Pongola floodplain, KwaZulu, South Africa. Unpublished PhD thesis. University of Natal, Pietermaritzburg, South Africa.

Krabbenhof CA, Burdett AS, Turner TF. 2017. Direct and indirect effects of predatory young-of-year fishes in a dryland river food web. *Freshwater Biology* 62: 1410-1421.

Krams I, Kecko S, Kangassalo K, Moore FR, Jankevics E, Inashkina I, Krama T, Lietuvietis V, Meija L, Rantala MJ. 2014. Effects of food quality on trade-offs among growth, immunity and survival in the greater wax moth *Galleria mellonella*. *Insect Science* 22: 431-439.

Lahr J, Diallo AO, Ndour KB, Badji AM, Diouf PS. 1999. Phenology of invertebrates living in a sahelian temporary pond. *Hydrobiologia* 405: 189-205.

Lake JL, McKinney RA, Osterman FA, Pruell RJ, Kiddon J, Ryba SA, Libby AD. 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 870-878.

Lake PS. 2003. Ecological effects of perturbation by drought in flowing waters. *Freshwater Biology* 48: 1161-1172.

Lake PS. 2011. *Drought and Aquatic Ecosystems: Effects and Responses*. Wiley-Blackwell Publishing, USA.

Langley JM, Shiel RJ, Nielsen DL, Green JD. 2001. Hatching from the sediment egg-bank, or aerial dispersing? – The use of mesocosms in assessing rotifer biodiversity. *Hydrobiologia* 466/477: 203-211.

Lankford B, Pringle C, Dickens C, Lewis F, Mander M, Chhotray V, Goulden M, Nxele Z, Quayle L. 2011. Hydrological modelling of water allocation, ecosystem services and poverty alleviation in the Pongola floodplain, South Africa. *Journal of Environmental Planning and Management* 54: 1237-1260.

Laufer G, Arim M, Loureiro M, Piñeiro-Guerra JM, Clavijo-Baquet S, Fagúndez. 2009. Diet of four annual killifishes: An intra and interspecific comparison. *Neotropical Ichthyology* 7: 77-86.

Layman CA, Araujo MS, Boucek R, Harrison E, Jud ZR, Matich P, Hammerschlag-Peyer CM, Rosenblatt AE, Vaudo JJ, Yeager LA, Post D, Bearhop S. 2012. Applying stable isotopes to examine food-web structure: An overview of analytical tools. *Biological Reviews* 87: 542-562.

Layman CA, Quattrochi JP, Peyer CM, Allgeier JE. 2007. Niche width collapse in a resilient top predator following ecosystem collapse. *Ecological Letters* 10: 937-944.

Ledger ME, Edwards FK, Brown LE, Milner AM, Woodward G. 2011. Impact of simulated drought on ecosystem biomass production: An experimental test in stream mesocosms. *Global Change Biology* 17: 2288-2297.

- Ledger ME, Hildew AG. 2001. Recolonization by the benthos of an acid stream following a drought. *Archiv für Hydrobiologia* 152: 1-17.
- Leigh C, Burford M, Sheldon F, Bunn S. 2010. Dynamic stability in dry season food webs within tropical floodplain rivers. *Marine and Freshwater Research* 61: 357-368.
- Leigh C. 2013. Dry-season changes in macroinvertebrate assemblages of highly seasonal rivers: Response to low flow, no flow and antecedent hydrology. *Hydrobiologia* 703: 95-112.
- Lemley DA, Taljaard S, Adams JB, Strydom N. 2014. Nutrient characterisation of river inflow into the estuaries of the Gouritz Water Management Area, South Africa. *Water SA* 40: 687-698.
- Lemmens P, Teffera FE, Wynants M, Govaert L, Deckers J, Bauer H, Woldeyes, Brendonck L, Bouillon S, de Meester L. 2017. Intra- and interspecific niche variation as reconstructed from stable isotopes in two ecologically different Ethiopian Rift Valley lakes. *Functional Ecology* 31:1482-1492.
- Liu C, Liu X. 2009. Healthy river and its indication, criteria and standards. *Journal of Geographical Sciences* 19: 3-11.
- Lu X, Gray C, Brown LE, Ledger ME, Milner AM, Mondragón RJ, Woodward G, Ma A. 2016. Drought rewires the cores of food webs. *Nature Climate Change* 6: 875-878.
- Luz-Agostinho KDG, Agostinho AA, Gomes LC, Júlio-Jr HF, Fugi R. 2009. Effects of flooding regimes on the feeding activity and body condition of piscivorous fish in the Upper Paraná River floodplain. *Brazilian Journal of Biology* 69: 481-490.
- Macarthur R. 1955. Fluctuations of animal populations, and a measure of community stability. *Ecology* 36: 533-536.
- MacGillivray CW, Grime JP. 1995. Testing predictions of the resistance and resilience of vegetation subjected to extreme events. *Functional Ecology* 9: 640-649.
- Manfrin A, Lehmann D, van Grunsven RHA, Larsen S, Syväranta J, Wharton G, Voigt CC, Monaghan MT, Hölker F. 2018. Dietary changes in predators and scavengers in a nocturnally illuminated riparian ecosystem. *Oikos* 1-10.
- Marchese MR, Saigo M, Zilli FL, Capello S, Devercelli M, Montalto L, Paporello G, Wantzen KM. 2014. Food webs of the Paraná River floodplain: Assessing basal sources using stable carbon and nitrogen isotopes. *Limnologia* 46: 22-30.
- Margalef, R. 1968. *Perspectives in Ecological Theory*. University of Chicago Press, Chicago, USA.
- Marklund MHK, Svanbäck, Zha Y, Scharnweber K, Eklöv P. 2017. The influence of habitat accessibility on the dietary and morphological specialisation of an aquatic predator. *Oikos* 127: 160-169.
- Marshall DW, Otto M, Panuska JC, Jaeger SR, Sefton D, Baumberger TR. 2006. Effects of hypolimnetic releases on two impoundments and their receiving streams in southwest Wisconsin. *Lake and Reservoir Management* 22: 223-232.
- Masese FO, Kitaka N, Kipkemboi J, Gettel GM, Irvine K, McMcClain E. 2014. Macroinvertebrate functional feeding groups in Kenyan highland streams: Evidence for a diverse shredder guild. *Freshwater Science* 33: 435-450.
- McCann KS, Rasmussen JB, Umbanhowar J. 2005. The dynamics of spatially coupled food webs. *Ecological Letters* 8: 513-523.
- McCann KS, Rooney N. 2009. The more food webs change, the more they stay the same. *Philosophical Transactions of the Royal Society B* 364: 1789-1801
- McClelland JW, Valiela I, Michener RH. 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. *Limnology and Oceanography* 42: 930-937.

- McGeoch MA. 1998. The selection, testing and application of terrestrial insects as bioindicators. *Biological Reviews* 73: 181-201.
- McHugh KJ, Smit NJ, van Vuren JHJ, van Dyk JC, Bervoets L, Covaci A, Wepener V. 2011. A histology-based fish health assessment of the tigerfish, *Hydrocynus vittatus*, from a DDT-affected area. *Physics and Chemistry of the Earth* 36: 895-904.
- McKee D, Atkinson D. 2000. The influence of climate change scenarios on populations of the mayfly *Cloeon dipterum*. *Hydrobiologia* 441: 55-62.
- McMeans BC, McCann KS, Humphries M, Rooney N, Fisk AT. 2015. Food web structure in temporally-forced ecosystems. *Trends in Ecology & Evolution* 30: 662-672.
- McMeans BC, McCann KS, Tunney TD, Fish AT, Muir AM, Lester N, Shuter B, Rooney N. 2016. The adaptive capacity of lake food webs: From individuals to ecosystems. *Ecological Monographs* 86: 4-19.
- Meintjes S. 1996. Seasonal changes in the invertebrate community of small shallow ephemeral pans at Bain's Vlei, South Africa. *Hydrobiologia* 317: 51-64.
- Merck KGaA (Merck Kommanditgesellschaft auf Aktien). 2014. Spectroquant Pharo 300. Operation Manual.
- Mereta ST, Boets P, de Meester L, Goethals PLM. 2013. Development of a multimetric index based on benthic macroinvertebrates for the assessment of natural wetlands in Southwest Ethiopia. *Ecological Indicators* 29: 510-521.
- Merritt RW, Cummins KW, Berg MB, Novak JA, Higgins MJ, Wessel KJ, Lessard JL. 2002. Development and application of a macroinvertebrate functional-group approach in the bioassessment of remnant river oxbows in southwest Florida. *Journal of the North American Benthological Society* 21: 290-310.
- Merron GS, Bruton M, de Lalouviere PLH. 1993a. Implications of water release from the Pongolapoort Dam for the fish and fishery of the Phongolo floodplain, Zululand. *Southern African Journal of Aquatic Science* 19: 34-49.
- Merron GS, Bruton M, de Lalouviere PLH. 1993b. Changes in fish communities of the Phongolo floodplain, Zululand (South Africa) before, during and after a severe drought. *Regulated Rivers: Research & Management* 8: 335-344.
- Merron GS, Mann BQ. 1995. The reproductive and feeding biology of *Schilbe intermedius* Rüppell in the Okavango Delta, Botswana. *Hydrobiologia* 308: 121-129.
- Metzeling L. 1986. Biological monitoring of the Wimmera, Werribee and Maribyrnong Rivers using freshwater invertebrates. Report No. WQ-5, Rural Water Commission of Victoria, Melbourne.
- Michener R, K Lajtha (editors). 2007. Stable isotopes in ecology and environmental science (2nd edition). Wiley-Blackwell Publishing, USA.
- Middelburg JJ. 2014. Stable isotopes dissect aquatic food webs from the top to the bottom. *Biogeosciences* 11: 2357-2371.
- Miliou H. 1993. Temperature, salinity and light induced variations on larval survival and sex ratio of *Tisbe holothuriae* Humes (Copepoda: Harpacticoida). *Journal of Experimental Marine Biology and Ecology* 173: 95-109.
- Miranda NAF, Perissonotto R, Appleton CC. 2010. Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*. *South African Journal of Science* 106: 55-61.
- Miserendino ML, Casaux R, Archangelsky M, Di Prinzio CY, Brand C, Kutschker AM. 2011. Assessing land-use effects on water quality, in-stream habitat, riparian ecosystems and biodiversity in Patagonian northwest streams. *Science of the Total Environment* 409: 612-624.

- Mittelbach GG. 1988. Competition among refuging sunfishes and effects of fish density on littoral zone invertebrates. *Ecology* 69: 614-623.
- Montaña CG, Schalk CM, Taphorn DC. 2012. First record of Van den Berg's Pearlfish, *Austrolebias vanderbergi* Huber 1995 (Cyprinodontiformes: Rivulidae) in Bolivia with comments on its diet and reproductive biology. *Check List* 8: 589–591.
- Moore JN. 2016. Recent desiccation of Western Great Basin Saline Lakes: Lessons from Lake Abert, Oregon, U.S.A. *Science of the Total Environment* 554-555: 142-154.
- Mosley LM. 2015. Drought impacts on the water quality of freshwater systems; review and integration. *Earth-Science Reviews* 140: 203-214.
- Mulholland PJ, Best GR, Coutant CC, Hornberger GM, Meyer JL, Robinson PJ, Stenberg JR, Turner RE, Vera-Herrera F, Wetzel RG. 1997. Effects of climate change on freshwater ecosystems of the southeastern United States and the gulf coast of Mexico. *Hydrological Processes* 11: 949-790.
- Musil CF, Grunow JO, Bornman CH. 1973. Classification and ordination of aquatic macrophytes in the Pongolo River Pans, Natal. *Bothalia* 11: 181-190.
- Nahlik AM, Mitsch WJ. 2008. The effects of river pulsing on sedimentation and nutrients in created riparian wetlands. *Journal of Environmental Quality* 37: 1634-1643.
- Ndumo Game Reserve: Integrated Management Plan (NGR IMP): 2009-2013. Version 1. 2009. Ezemvelo KZN Wildlife, Pietermaritzburg, South Africa.
- Nel JL, Roux DJ, Maree G, Kleynhans CJ, Moolman J, Reyers B, Rouget M, Cowling RM. 2007. Rivers in peril inside and outside protected areas: A systematic approach to conservation assessment of river ecosystems. *Diversity and Distributions* 13: 341-352.
- Neres-Lima V, Machado-Silva F, Baptista DF, Oliveira RBS, Andrade PM, Oliveira AF, Sasada-Sato CY, Silva-Junior EF, Feijó-Lima R, Angelini R, Camargo PB, Moulton TP. 2017. Allochthonous and autochthonous carbon flows in food webs of tropical forest streams. *Freshwater Biology* 62: 1012-1023.
- Nielson DL, Brock MA, Rees GN, Baldwin DS. 2003. Effects of increasing salinity on freshwater ecosystems in Australia. *Australian Journal of Botany* 51: 655-665.
- Nielson DL, Hillman TJ, Smith FJ, Shiel RJ. 2002. The influence of seasonality and duration of flooding on zooplankton in experimental billabongs. *River Research and Applications* 18: 227-237.
- Norris RH, Thoms MC. 1999. What is river health? *Freshwater Biology* 41: 197-209.
- Nyunja J, Ntiba M, Onyari J, Mavuti K, Soeaert K, Bouillon S. 2009. Carbon sources supporting a diverse fish community in a tropical coastal ecosystem (Gazi Bay, Kenya). *Estuarine, Coastal and Shelf Science* 83: 333-341.
- O'Connel M, Young J, Kingwell R. 2006. The economic value of saltland pastures in a mixed farming system of Western Australia. *Agricultural Systems* 89: 371-389.
- O'Leary MH. 1988. Carbon isotopes in photosynthesis. *BioScience* 38: 328-336.
- Obolewski K, Strzelczak A, Glińska-Lewczuk .2014. Does hydrobiological connectivity affect the composition of macroinvertebrates on *Stratiotes aloides* L. in oxbow lakes? *Ecological Engineering* 66: 77-81.
- Obolewski K. 2011. Macrozoobenthos patterns along environmental gradients and hydrological connectivity of oxbow lakes. *Ecological Engineering* 37: 796-805.
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2017. Package "vegan". Version 2.4.4. Vienna, Austria R Foundation for Statistical Computing. Available from: <https://www.R-project.org/> (Accessed 18 March 2018).

- Oliveira ACB, Soares MGM, Martinelli LA, Moreira MZ. 2006. Carbon sources of fish in an Amazonian floodplain lake. *Aquatic Science* 68: 229-238.
- Pagano M, Koffi MA, Cecchi P, Corbin D, Champalbert G, Saint-Jean L. 2003. An experimental study of the effects of nutrient supply and *Chaoborus* predation on zooplankton communities of a shallow tropical reservoir (Lake Brobo, Côte d'Ivoire). *Freshwater Biology* 48: 1379-1395.
- Pagano M, Saint-Jean L, Arfi R, Bouvy M, Shep H. 2000. Population growth capabilities and regulatory factors in monospecific cultures of the cladocerans *Moina micrura* and *Diaphanosoma excisum* and the copepod *Thermocyclops decipiens* from the Côte d'Ivoire (West Africa). *Aquatic Living Resources* 13: 163-172.
- Paillex A, Castella E, Carron G. 2007. Aquatic macroinvertebrate response along a gradient of lateral connectivity in river floodplain channels. *Journal of the North American Benthological Society* 26: 779-796.
- Paillex A, Dolédec S, Castella E, Méricoux S. 2009. Large river floodplain restoration: Predicting species richness and trait responses to the restoration of hydrological connectivity. *Journal of Applied Ecology* 46: 250-258.
- Parnell A, Jackson A. 2013. siar: Stable Isotope Analysis in R. R package version 4.2. Available from: <https://cran.r-project.org/web/packages/siar/siar.pdf> (Accessed 20 November 2017).
- Parnell AC, Inger R, Bearhop S, Jackson AL. 2010. Source partitioning using stable isotopes: Coping with too much variation. *PLoS One*. 5: e9672.
- Parnell AC, Phillips DL, Bearhop S, Semmens BX, Ward EJ, Moore JW, Jackson AL, Grey J, Kelly DJ, Inger R. 2012. Bayesian stable isotope mixing models. *Environmetrics* 24: 387-399.
- Peralta-Maraver I, Robertson AL, Rezende EL, Lemes da Silva AL, Tonetta D, Lopes M, Petrucio MM. 2017. Winter is coming: Food web structure and seasonality in a subtropical freshwater coastal lake. *Ecology and Evolution* 7: 4534-4542.
- Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18: 293-320.
- Phillips DL, Eldridge PM. 2006. Estimating the timing of diets shifts using stable isotopes. *Oecologia* 142: 195-203.
- Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ. 2014. Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* 92: 823-835.
- Phillips DL, Newsome SD, Gregg JW. 2005. Combining sources in stable isotope mixing models: Alternative methods. *Oecologia*. 144: 520-527.
- Phillips DL. 2012. Converting isotope values to diet composition: The use of mixing models. *Journal of Mammalogy* 93: 342-352.
- Pielou EE. 1971. Measurement of Structure in Animal Communities. *Ecosystem Structure and Function*. In: Wiens JA (editors) *Proceedings of the 31st Annual Biology Colloquium*, Oregon State April 1970. Oregon State University Press, Corvallis, USA.
- Pigram JJ. 2006. *Australia's Water Resources: From use to management*. CSIRO Publishing, Melbourne, Australia.
- Pimm SL, Lawton JH. 1977. The numbers of trophic levels in ecological communities. *Nature* 268: 329-331.
- Pimm SL. 1984. The complexity and stability of ecosystems. *Nature* 307: 321-326.
- Pimm SL. 2002. *Food webs* (2nd edition). The University of Chicago Press, USA.

- Pinceel T, Vanschoenwinkel B, Deckers P, Grégoir A, ver Eecke T, Brendonck L. 2015. Early and late developmental arrest as complementary embryonic bet-hedging strategies in African killifish. *Biological Journal of the Linnean Society* 114: 941-948.
- Pinder MA, Halse SA, McRae JM, Shiel RJ. 2005. Occurrence of aquatic invertebrates of the wheatbelt region of western Australia in relation to salinity. *Hydrobiologia* 543: 1-24.
- Piniewski M, Prudhomme C, Acreman MC, Tylec L, Oglęcki P, Okruszko T. 2017. Responses of fish and invertebrates to floods and droughts in Europe. *Ecohydrology* 10: e1793.
- Pires AM, Cowx IG, Coelho MM. 2000. Benthic macroinvertebrate communities of intermittent streams in the middle reaches of the Guadiana Basin (Portugal). *Hydrobiologia* 435: 167-175.
- Poff NL, Richter BD, Arthington AH, Bunn SE, Naiman RJ, Kendy E, Acreman M, Apse C, Bledsoe BP, Freeman MC, Hendriksen J, Jacobson RB, Kennen JG, Merritt DM, O'Keeffe JH, Olden JD, Rogers K, Tharme RE, Warner A. 2009. The ecological limits of hydrologic alterations (ELOHA): A new framework for developing regional environmental flow standards. *Freshwater Biology* 55 147-170.
- Polačik M, Harrod C, Blažek, Reichard M. 2014. Trophic niche partitioning in communities of African annual fish: Evidence from stable isotopes. *Hydrobiologia* 721: 99-106.
- Polačik M, Reichard M. 2010. Diet overlap among three sympatric African annual killifish species *Nothobranchius* spp. from Mozambique. *Journal of Fish Biology* 77: 754-768.
- Polunin NVC. 2008. *Aquatic Ecosystems: Trends and global prospects*. Cambridge University Press, Cambridge, UK.
- Post DM. 2002. Using stable isotopes to estimate trophic position: Models, methods and assumptions. *Ecology* 83: 703-718.
- Power ME, Parker MS, Dietrich WE. 2008. Seasonal reassembly of a river food web: Floods, droughts and impacts of fish. *Ecological Monographs* 78: 263-282.
- Pyke C. 2005. Interactions between habitat loss and climate change: Implications for fairy shrimp in the Central Valley Ecoregion of California, USA. *Climate Change* 68: 199-218.
- R Core Team. 2017. *R: A language and environment for statistical computing*. Vienna, Austria R Foundation for Statistical Computing. Available from <https://www.R-project.org> (Accessed 15 October 2017).
- Rajeshwari CV, Saraswathi B. 2009. Assessment of water quality of Rivers Tungabhadra and Hundri, India. *Pollution Research* 28: 499-505.
- Rapport DJ. 1989. What constitutes ecosystem health? *Perspectives in Biology and Medicine* 33: 120-132.
- Reichard M, Polačik M, Sedláček O. 2009. Distribution, colour polymorphism and habitat use of the African killifish *Nothobranchius furzeri*, the vertebrate with the shortest life span. *Journal of Fish Biology* 74: 198-212.
- Reid MA, Thoms MC. 2008. Surface flow types, near-bed hydraulics and the distribution of stream macroinvertebrates. *Biogeosciences Discussions* 5: 1175-1204.
- Richoux NB, Froneman PW. 2007. Assessment of spatial variation in carbon utilization by benthic and pelagic invertebrates in a temperate South African estuary using stable isotope signatures. *Estuarine, Coastal and Shelf Science* 71: 545-558.
- Roberts K, Granum E, Leegood RC, Raven JA. 2007. C3 and C4 pathways of photosynthetic carbon assimilation in marine diatoms are under genetic, not environmental, control. *Plant Physiology* 145: 230-235.

- Rocha LG, Medeiros ESF, Andrade HTA. 2012. Influence of flow variability on macroinvertebrate assemblages in an intermittent stream of semi-arid Brazil. *Journal of Arid Environments* 85: 33-40.
- Rojas NET, Marins MA, Rocha O. 2001. The effect of abiotic factors on the hatching of *Moina micrura* Kurz, 1874 (Crustacea: Cladocera) ephippial eggs. *Brazilian Journal of Biology* 61: 371-376.
- Rooney N, McCann KS, Moore JC. 2008. A landscape theory for food web architecture. *Ecology Letters* 11: 867-881.
- Rooney N, McCann KS. 2012. Integrating food web diversity, structure and stability. *Trends in Ecology and Evolution* 27: 40-46.
- Rossouw JN. 1985. The effects of the Domoina floods and releases from the Pongolapoort Dam on the Pongolo floodplain. Report No B-N3/0704/I. Department of Water Affairs, Pretoria, South Africa.
- Ruhí A, Muñoz I, Tornés E, Batalla RJ, Vericat D, Ponsatí L, Acuña V, von Schiller D, Marcé R, Bussi G, Francés F, Sabater S. 2016. Flow regulation increases food-chain length through omnivory mechanisms in a Mediterranean river network. *Freshwater Biology* 61: 1536-1549.
- Sabo JL, Finlay JC, Kennedy T, Post DM. 2010. The role of discharge variation in scaling of drainage area and food chain length in rivers. *Science* 330: 965-967.
- Schindler DE, Hodgson JR, Kitchell JF. 1997. Density-dependent changes in individual foraging specialization of largemouth bass. *Oecologia* 110: 592-600.
- Schneider DW, Frost TM. 1996. Habitat duration and community structure in temporary ponds. *Journal of the North American Benthological Society* 15: 64-86.
- Seaman MT, Ashton PJ, Williams WD. 1991. Inland salt waters of southern Africa. *Hydrobiologia* 210: 75-91.
- Senner NR, Moore JN, Seager ST, Dougill S, Kreuz K, Senner SE. 2018. A salt lake under stress: Relationships among birds, water levels, and invertebrates at a Great Basin saline lake. *Biological Conservation* 220: 320-329.
- Sereda BL, Meinhardt HR. 2003. Insecticide contamination of the water environment in malaria endemic areas of KwaZulu-Natal (South Africa). WRC Report No 1119/1/03, Water Research Commission, Pretoria, South Africa.
- Sereda BL, Meinhardt HR. 2005. Contamination of the water environment in malaria endemic area of KwaZulu-Natal, South Africa by DDT and its metabolites. *Bulletin of Environmental Contamination and Toxicology* 75: 538-545.
- Shabalala AN, Combrinck L, McCrindle R. 2013. Effects of farming activities on seasonal variation of water quality of Bonsma Dam, KwaZulu-Natal. *South African Journal of Science* 109: 1-7.
- Shannon CE, Weaver W. 1963. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, USA.
- Shanyengana ES, Seely MK, Sanderson RD. 2004. Major-ion chemistry and ground water salinization in ephemeral floodplains in some arid regions of Namibia. *Journal of Arid Environments* 57: 71-83.
- Sheldon F, Boulton AJ, Puckridge JT. 2002. Conservation value of variable connectivity: Aquatic invertebrate assemblages of channel and floodplain habitats of a central Australian arid-zone river, Cooper Creek. *Biological Conservation* 103: 13-31.
- Shelton ML. 2009. *Hydroclimatology, Perspectives and Applications*. Cambridge University Press, New York, USA.
- Simaika JP, Samways MJ. 2011. Comparative assessment of indices of freshwater habitat conditions using different invertebrate taxon sets. *Ecological Indicators* 11: 370-378.

- Simeonova P, Simeonov V, Andreev G. 2003. Water quality study of the Struma River Basin, Bulgaria (1989-1998). *Central European Journal of Chemistry* 2: 121-136.
- Simpson, EH. 1949. Measurement of diversity. *Nature* 163: 688.
- Singh AK, Mondal GC, Kumar S, Singh TB, Tewary BK, Sinha A. 2008. Major ion chemistry, weathering processes and water quality assessment in upper catchment of Damodar River basin, India. *Environmental Geology* 54: 745-758.
- Skelton PH. 2012. A complete guide to the freshwater fishes of Southern Africa. Struik Publishers, Cape Town, South Africa.
- Šmilauer P, Lepš J. 2014. *Multivariate Analysis of Ecological Data using Canoco 5*. Cambridge University Press.
- Smit NJ, Vlok W, van Vuren JHJ, du Preez L, van Eeden E, O'Brien GC, Wepener V. 2016. Socio-ecological system management of the Lower Phongolo River and Floodplain using relative risk methodology. WRC Report No 2185/1/16. Water Research Commission, Pretoria, South Africa.
- Smith BN, Epstein S. 1971. Two categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. *Plant Physiology* 47: 380-384.
- Smith JA, Mazumder D, Suthers IM, Taylor MD. 2013. To fit or not to fit: Evaluating stable isotope mixing models using simulated mixing polygons. *Methods in Ecology and Evolution* 4: 612-618.
- Smith RJ, Easton J, Nhancale BA, Armstrong AJ, Cuverwell J, Dlamini SD, Goodman PS, Loffler L, Matthews WS, Monadjem A, Mulqueeny CM, Ngwenya P, Ntumi CP, Soto B, Leader-Williams N. 2008. Designing a Transfrontier conservation landscape for the Maputaland centre of endemism using biodiversity, economic and threat data. *Biological Conservation* 141: 2127-2138.
- South African Wetlands Conservation Programme (SA Wetlands Conservation Programme). 1996. Ndumo Game Reserve, South Africa, information sheet. South African Wetlands Conservation Programme. Document No: 24/21/3/3/15. Department of Environmental Affairs and Tourism, Pretoria.
- Sparks RE, Nelson JC, Yin Y. 1998. Naturalization of the flood regime in regulated rivers. *Bioscience* 48: 706-720.
- Sprague LA. 2005. Drought effects on water quality in the South Platte River Basin, Colorado. *Journal of the American Water Resources Association* 41: 11-24.
- Stals R, de Moor IJ (editors). 2008. *Guides to the Freshwater Invertebrates of Southern Africa Volume 10: Coleoptera*. WRC Report TT 320/07, Water Research Commission, Pretoria, South Africa.
- Statzner B, Bêche LA. 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology* 55: 80-119.
- Stewart SD, Hamilton DP, Baisden WT, Dedual M, Verburg P, Duggan IC, Hicks BJ, Graham BS. 2017. Variable littoral-pelagic coupling as a food-web response to seasonal changes in pelagic primary production. *Freshwater Biology* 62: 2008-2025.
- Stock BC and Semmens BX. 2016. *MixSIAR GUI User Manual*. Version 3.1. Available from: <https://github.com/brianstock/MixSIAR/> (Accessed 10 May 2018).
- Stoline MR. 1981. The status of multiple comparisons: Simultaneous estimation of all pairwise comparisons in one-way ANOVA designs. *The American Statistician* 35:134-141.
- Strayer DL, Findlay SEG. 2010. Ecology of freshwater shore zones. *Aquatic Sciences* 72: 127-163.
- Studinski JM, Grubbs SA. 2007. Environmental factors affecting the distribution of aquatic invertebrates in temporary ponds in Mammoth Cave National Park, Kentucky, USA. *Hydrobiologia* 575: 211-220.

Suren A, Biggs B, Duncan M, Bergley L. 2003. Benthic community dynamics during summer low-flows in two rivers of contrasting enrichment 2. Invertebrates. *New Zealand Journal of Marine and Freshwater Research* 37: 71-83.

Talling JF. 2001. Environmental controls on the functioning of shallow tropical lakes. *Hydrobiologia* 458: 1-8.

Taylor GC, Hill JM, Jackson MC, Peel RA, Weyl OLF. 2017a. Estimating $\delta^{15}\text{N}$ fractionation and adjusting the lipid correction equation using Southern African freshwater fishes. *PLoS ONE* 12: e0178047.

Taylor GC, Weyl OLF, Hill JM, Peel RA, Hay CJ. 2017b. Comparing the fish assemblages and food-web structures of large floodplain rivers. *Freshwater Biology* 62: 1891-1907.

Teodoru C, Wehrli B. 2005. Retention of sediments and nutrients in the Iron Gate I Reservoir on the Danube River. *Biogeochemistry* 76: 539-565.

Thiébaud G. 2008. Phosphorus and aquatic plants. In: White P.J., Hammond J.P. (editors) *The Ecophysiology of Plant-Phosphorus Interactions. Plant Ecophysiology. Volume 7*. Springer, Dordrecht, The Netherlands.

Thompson RM, Hemberg M, Starzomski BM, Shurin JB. 2007. Trophic levels and trophic tangles: The prevalence of omnivory in real food webs. *Ecology* 88: 612-617.

Thoms MC, Sheldon F. 2002. An ecosystem approach for determining environmental water allocations in Australian dryland river systems: The role of geomorphology. *Geomorphology* 47: 153-168.

Thoms MC. 2003. Floodplain-river ecosystems: Lateral connections and the implications of human interference. *Geomorphology* 56: 335-349.

Tiemann JS, Gillette DP, Wildhaber ML, Edds DR. 2004. Effects of lowhead dams on riffle-dwelling fishes and macroinvertebrates in a midwestern river. *Transactions of the American Fisheries Society* 133: 705-717.

Timms BV, Sanders PR. 2002. Biogeography and ecology of Anostraca (Crustacea) in middle Paroo catchment of the Australian arid-zone. *Hydrobiologia* 486: 225-238.

Timms BV. 2005. Salt lakes in Australia: Present problems and prognosis for the future. *Hydrobiologia* 552: 1-15.

Tiwari RK. 2001. Environmental impact of coal mining on water regime and its management. *Water, Air and Soil Pollution* 131: 185-199.

Tockner K, Pennetzer D, Reiner N, Schiemer F, Ward JW. 1999. Hydrological connectivity and the exchange of organic matter and nutrients in a dynamic river-floodplain system (Danube, Austria). *Freshwater Biology* 41: 521-535.

Tockner K, Stanford JA. 2002. Riverine flood plains: Present state and future trends. *Environmental Conservation* 29: 308-330.

Tockner K, Ward JV, Edwards PJ, Kollmann J. 2002. Riverine landscapes: An introduction. *Freshwater Biology* 47: 497-500.

Tomas D, Čurlin M, Marić AS. 2017. Assessing the surface water status in Pannonian ecoregion by the water quality index model. *Ecological Indicators* 79: 182-190.

Tooth S, McCarthy TS. 2007. Wetlands in drylands: Geomorphological and sedimentological characteristics, with emphasis on examples from southern Africa. *Progress in Physical Geography* 31: 3-41.

Trochine C, Guerrieri M, Liboriussen L, Willems P, Lauridsen TL, Søndergaard M, Jeppesen E. 2017. Factors controlling the stable isotope composition and C:N ratio of seston and periphyton in shallow

- lake mesocosms with contrasting nutrient loadings and temperatures. *Freshwater Biology* 62: 1596-1613.
- Trzcinski MK, Srivastava DS, Corbara B, Dézerald O, Leroy C, Carrias JF, Dejean A, Céréghino R. 2016. The effects of food web structure on ecosystem function exceeds those of precipitation. *Journal of Animal Ecology* 85: 1147-1160.
- United Nations University Institute for Environment and Human Security (UNU-EHS). 2016. International water quality guidelines for ecosystems (IWQGES): How to develop guidelines for healthy freshwater ecosystems. A policy oriented approach. Draft for regional consultants.
- Valladares S, Soto DX, Planas M. 2017. Dietary composition of endangered seahorses determined by stable isotope analysis. *Marine and Freshwater Research* 68: 831-839.
- van de Meutter F, de Meester L, Stoks R. 2007. Metacommunity structure of pond macroinvertebrates: Effects of dispersal mode and generation time. *Ecology* 88: 1687-1695.
- van de Meutter F, Stoks R, de Meester L. 2006. Rapid response of macroinvertebrates to drainage management of shallow connected lakes. *Journal of Applied Ecology* 43: 51-60.
- van der Laan M, van Antwerpen R, Bristow KL. 2012. River water quality in the northern sugarcane-producing regions of South Africa and implications for irrigation: A scoping study. *Water SA* 38: 87-96.
- van Geest GJ, Coops H, Roijackers RMM, Buijse AD, Scheffer M. 2005. Succession of aquatic vegetation driven by reduced water-level fluctuations in floodplain lakes. *Journal of Applied Ecology* 42: 251-260.
- van Vliet MTH, Zwolsman JJG. 2008. Impact of summer droughts on the water quality of the Meuse River. *Journal of Hydrology* 353: 1-17.
- van Vuuren L. 2009. Pongolapoort Dam: Development steeped in controversy. *The Water Wheel* 8: 23-27.
- Vander Zanden MJ, Shuter BJ, Lester NP, Rasmussen JB. 2000. Within-and among-population variation in the trophic position of a pelagic predator, lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences* 57: 725-731.
- Vanschoenwinkel B, Hulsmans A, de Roeck E, de Vries C, Seaman M, Brendonck L. 2009. Community structure in temporary freshwater pools: Disentangling the effects of habitat size and hydroregime. *Freshwater Biology* 54: 1487-1500.
- Velasco J, Millán A, Hernández J, Gutiérrez CM, Abellán P, Sánchez D and Ruiz M. 2006. Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. *Saline Systems* 2: 12.
- Villastrigo A, García-Criado F. 2017. Response of a littoral macroinvertebrate assemblage to a persistent hydrological drought in a permanent mountain shallow lake from northwestern Spain. *Annales de Limnologie-International Journal of Limnology* 53: 315-323.
- Wantzen KM, de Arruda Machado F, Voss M, Boriss H, Junk WJ. 2002. Seasonal isotopic shifts in fish of the Pantanal wetland, Brazil. *Aquatic Sciences* 64: 239-251.
- Ward JV, Stanford JA. 1983. The serial discontinuity concept of lotic ecosystems. In: Fontaine, TD, SM Bartell (editors). *Dynamics of lotic ecosystems*. Ann Arbor Science Publishers, Michigan. pp 29-42.
- Ward JV, Stanford JA. 1995. Ecological connectivity in alluvial river ecosystems and its disruption by flow regulation. *Regulated Rivers: Research and Management* 11: 105-119.
- Ward JV, Tockner K, Schiemer F. 1999. Biodiversity of floodplain river ecosystems: Ecotones and connectivity. *Regulated Rivers: Research and Management* 15: 125-139.
- Waterkeyn A, Grillas P, Vanschoenwinkel B, Brendonck L. 2008. Invertebrate community patterns in Mediterranean temporary wetlands along hydroperiod and salinity. *Freshwater Biology* 53: 1808-1822.

- Waterkeyn A, Vanschoenwinkel B, Grillas P, Brendonck L. 2010. Effect of salinity on seasonal community patterns of Mediterranean temporary wetland crustaceans: A mesocosm study. *Limnology and Oceanography* 55: 1712-1722.
- Waterkeyn A. 2009. Community structuring processes in Mediterranean temporary wetlands. Unpublished PhD dissertation, KU Leuven, Belgium.
- Watters BR. 2009. The ecology and distribution of *Nothobranchius* fishes. *Journal of the American Killifish Association* 42: 105-127.
- Weis JS, Samson J, Zhou T, Skurnick J, Weis P. 2001. Prey capture ability of mummichogs (*Fundulus heteroclitus*) as a behavioural biomarker for contaminants in estuarine systems. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 1442-1452.
- Welcomme R. 1979. Fisheries ecology of tropical floodplain rivers. Longman Press, London, UK.
- Welicky RL, De Swardt J, Gerber R, Netherlands EC, Smit NJ. 2017. Drought-associated absence of alien invasive anchorworm, *Lernaea cyprinacea* (Copepoda: Lernaeidae), is related to changes in fish health. *International Journal for Parasitology: Parasites and Wildlife* 6: 430-438.
- Wellborn GA, Skelly DK, Werner EE. 1996. Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics* 27: 337-363.
- Wepener V, van Dyk JC, Bervoets L, O'Brien G, Covaci A, Cloete Y. 2011. An assessment of the influence of multiple stressors on the Vaal River, South Africa. *Physics and Chemistry of the Earth* 36: 949-962.
- White HL, Nichols SJ, Robinson WA, Norris RH. 2012. More for less. a study of environmental flows during droughts in two Australian rivers. *Freshwater Biology* 57: 858-873.
- Wiggins GB, Mackay RJ, Smith IM. 1980. Evolutionary and ecological strategies of animals in annual temporary pools. *Archiv für Hydrobiologie* 58: 97-206.
- Wildekamp RH. 2004. A world of killies: Atlas of the oviparous cyprinodontiform fishes of the world. Volume 4. Elyria, American Killifish Association.
- Wilk MB, Gnanadesikan R. 1968. Probability plotting methods for the analysis of data. *Biometrika* 55: 1-17.
- Williams DD. 2006. The biology of temporary waters. Oxford University Press, New York, USA.
- Williams GP, Wolman MG. 1984. Downstream effects of dams on alluvial rivers. Geological Survey Professional Paper 1286. US Government Printing Office, Washington, DC, USA.
- Williams RJ, Martinez ND. 2004. Limits to trophic levels and omnivory in complex food webs: Theory and data. *American Naturalist* 163: 458-468.
- Williams WD. 1981. The limnology of saline lakes in Western Victoria: A review of some recent studies. *Hydrobiologia* 82: 233-259.
- Williams WD. 2002. Environmental threats to salt lakes and the likely status of inland saline ecosystems in 2025. *Environmental Conservation* 29: 154-167.
- Winemiller KO, Jepsen DB. 1998. Effects of seasonality and fish movement on tropical river food webs. *Journal of Fish Biology* 53: 267-296.
- Wood PJ, Armitage PD. 2004. The response of the macroinvertebrate community to low-flow variability and supra-seasonal drought within a groundwater dominated stream. *Archiv für Hydrobiologia* 161: 1-20.
- Woodward G, Brown, LE, Edwards FK, Hudson LN, Milner AM, Reuman DC, Ledger ME. 2012. Climate change impacts in multispecies systems: Drought alters food web size structure in a field experiment.

Philosophical Transactions of The Royal Society of London Series B: Biological Sciences 367: 2990-2997.

Woodward G. 2009. Biodiversity, ecosystem functioning and food webs in fresh waters: Assembling the jigsaw puzzle. *Freshwater Biology* 54: 2171-2187.

Wurtsbaugh WA, Miller C, Null SE, DeRose RJ, Wilcock P, Hahnenberger M, Howe F, Moore J. 2017. Decline of the world's saline lakes. *Nature Geoscience* 11: 816-821.

Xu J, Fan X, Zhang X, Xu D, Mou S, Cao S, Zheng Z, Miao J, Ye N. 2012. Evidence of coexistence of C3 and C4 photosynthetic pathways in a green-tide-forming alga, *Ulva prolifera*. *PLoS ONE* 7: e37438.

Yamazaki Y, Muneoka T, Okazawa H, Kimura M, Tsuji O. 2017. Influence of the agricultural land agglomeration to the nutrients of the river water in the Tokachi River basin. *Paddy and Water Environments* 15: 277-290.

Zhang Z, Hibberd A, Zhou JL. 2008. Analysis of emerging contaminants in sewage effluent and river water: Comparison between spot and passive sampling. *Analytica Chimica Acta*. 607: 37-44.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. Mixed effects models and extensions in ecology with R. Springer, New York, USA.

Appendices

Appendix A

Appendix A1: Annual water quality variables of the Phongolo River obtained from gauging station W4H006, upstream of Pongolapoort Dam from 1972 – 2017 and used in the Physico-chemical Assessment Index (PAI). *Denotes data not available.

Year	Conductivity ($\mu\text{S.cm}^{-1}$)	pH	Na (mg.L^{-1})	Mg ⁺ (mg.L^{-1})	Ca ²⁺ (mg.L^{-1})	Cl ⁻ (mg.L^{-1})	NO ₃ ⁻ /NO ₂ ⁻ (mg.L^{-1})	SO ₄ ²⁻ (mg.L^{-1})	PO ₄ ³⁻ (mg.L^{-1})	K (mg.L^{-1})	NH ₄ ⁺ (mg.L^{-1})
1972	536.21	8.57	75.94	17.35	14.22	58.39	0.18	14.15	0	1.61	*
1973	317.68	8	36.92	10.87	12.67	30.85	0.93	8.43	0.02	1.15	*
1974	335.1	8.21	36.44	11.68	13.06	33.09	0.89	7.73	0.03	1.2	*
1977	500.36	7.47	64.08	18.1	17.4	45.16	0.2	13.95	0.02	1.34	0.04
1978	409.59	7.44	43.28	16.64	15.58	35.11	0.25	7.43	0.05	0.94	0.12
1979	496	7.61	55.93	17.17	17.1	41.4	0.98	19.33	0	1.66	0.03
1980	589.35	7.48	80.63	19.94	18.44	50.83	0.13	14.44	0.01	1.2	0.03
1981	926.22	7.64	117.48	42.58	28.77	80.96	1.09	32.82	0.01	1.1	0.03
1982	999.44	8.12	140.89	39.32	28.56	99.4	0.81	20.07	0.03	1.58	0.14
1983	838.7	8.26	130.8	27.5	19.04	81.16	0.41	24.26	0.02	1.8	0.05
1984	485	7.97	45.9	21.07	22.43	61.8	1.08	21.53	0.04	0.96	0.06
1985	546.5	8.3	70.64	21.01	18.48	56.75	0.44	20.61	0.02	1.46	0.05
1986	662.03	8	89.66	24.4	20.64	60.01	0.4	19.95	0.01	1.15	0.05
1987	514.54	7.74	66.94	18.41	17.14	46.69	0.49	14.51	0.02	1.19	0.07
1988	359.21	7.52	40.25	12.68	14.07	29.14	0.31	10.23	0.02	1.07	0.06
1989	460.15	8.17	60.6	17.57	17.27	43.13	0.37	14.19	0.08	1.3	0.06
1990	611.96	8.59	91.27	24.32	20.7	58.72	0.46	18.59	0.02	1.09	0.05
1991	499.83	8.29	62.16	18.16	17.85	41.01	0.42	14.31	0.02	1.09	0.04
1992	986.59	8.6	143.37	40.51	27.16	81.86	0.44	32.8	0.02	1.3	0.06
1993	638.38	8.26	94.51	24.81	19.37	57.24	0.52	26.15	0.02	1.23	0.05
1994	574.62	8.39	75.91	23.02	21.5	47.39	0.62	25.83	0.03	1.85	0.03
1995	826.7	8.59	110.64	33.69	26.77	76.68	1.03	35.25	0.03	2.32	0.05
1996	370.25	8.28	35.53	14.08	15.65	25.1	0.74	15.4	0.02	1.93	0.02
1997	417.4	8.21	45.17	15.67	17.32	29.77	0.7	19.17	0.01	1.11	0.03
1998	617.18	8.4	76.24	23.38	22.65	51.78	0.86	26.39	0.02	1.63	0.03
1999	737.08	8.55	100.06	27.28	23.7	61.39	0.66	26.54	0.02	1.46	0.04

Appendix A1 continued

Year	Conductivity ($\mu\text{S.cm}^{-1}$)	pH	Na (mg.L^{-1})	Mg ⁺ (mg.L^{-1})	Ca ²⁺ (mg.L^{-1})	Cl ⁻ (mg.L^{-1})	NO ₃ ⁻ /NO ₂ ⁻ (mg.L^{-1})	SO ₄ ²⁻ (mg.L^{-1})	PO ₄ ³⁻ (mg.L^{-1})	K (mg.L^{-1})	NH ₄ ⁺ (mg.L^{-1})
2000	574.55	8.34	59.04	24.12	22.95	54.18	0.85	17.89	0.04	1.25	0.06
2001	587.31	8.4	65.58	23.75	23.24	50.04	0.72	19.55	0.06	1.42	0.05
2002	493.42	8.39	57.01	17.74	18.23	35.74	0.6	16	0.02	1.47	0.03
2003	629.75	8.36	79.41	24.13	21.25	50.55	0.55	18.32	0.03	1.62	0.02
2004	698.3	8.38	96.45	25.66	22.38	49.41	0.76	25.52	0.02	1.47	0.02
2005	513.8	8.32	64.27	18.93	18.29	32.66	0.54	17.49	0.03	1.26	0.02
2006	419	8.1	49.84	15.21	16.23	32.5	0.37	18.87	0.02	1.24	0.05
2007	451.38	8.14	55.24	16.46	16.17	26.39	0.41	18.25	0.07	1.38	0.1
2008	459.43	8.42	59.22	21.32	18.77	36.28	0.66	21.26	0.04	1.5	0.05
2009	465.6	8.43	54.48	19.45	18.05	33.94	0.34	20.31	0.05	2.1	0.03
2010	343	8.3	36.34	12.57	14.48	22.69	0.48	11.75	0.01	2.4	0.03
2011	340.05	8.3	38	14.56	13.39	20.76	0.32	12.04	0.03	2.35	0.03
2012	494.68	8.37	60.75	19.46	19.51	33.07	0.49	18.27	0.01	2.86	0.03
2013	345.6	8.25	40.99	14.01	16.33	25.13	0.49	14.6	0.01	2.14	0.03
2014	483.67	8.43	*	18.07	18.46	34.02	0.6	17.16	0.01	*	0.05
2015	674.02	8.67	92.03	27.02	22.38	49.41	0.63	23.59	0.02	2.44	0.05
2016	833	8.69	116.66	32.86	23.1	56.4	0.32	33.06	0.05	2.74	0.05
2017	359.6	8.08	40.66	13.3	13.72	23.62	0.24	16	0.01	1.82	0.05

Appendix A2: Annual water quality variables of the Phongolo River obtained from gauging station W4H013 downstream of Pongolapoort Dam from 1984 – 2017 and used in the Physico-chemical Assessment Index (PAI). *Denotes data not available.

Year	Conductivity ($\mu\text{S.cm}^{-1}$)	pH	Na (mg.L^{-1})	Mg ⁺ (mg.L^{-1})	Ca ²⁺ (mg.L^{-1})	Cl ⁻ (mg.L^{-1})	NO ₃ ⁻ /NO ₂ ⁻ (mg.L^{-1})	SO ₄ ²⁻ (mg.L^{-1})	PO ₄ ³⁻ (mg.L^{-1})	K (mg.L^{-1})	NH ₄ ⁺ (mg.L^{-1})
1984	227	7.17	15.7	8.1	13.6	26.7	0.68	56.2	0.01	3.02	0.27
1985	276.71	7.93	24.27	9.86	15.03	26.8	0.28	13	0.01	1.88	0.05
1986	285.82	7.67	25.53	9.88	15.11	25.26	0.3	9.02	0.01	1.88	0.06
1987	317.56	7.57	29.51	11.13	17.09	26.63	0.34	9.71	0.01	2.01	0.09
1988	293.3	7.39	25.67	9.91	15.07	22.95	0.3	6.45	0.01	1.68	0.06
1991	262.6	7.91	24.44	9.08	13.48	20.22	0.26	8.08	0.01	1.59	0.04
1992	301.09	8.18	25.84	9.68	15.32	18	0.22	12.21	0.02	1.67	0.04
1993	309.78	8.22	31.51	10.67	16.9	24.93	0.28	17.19	0.02	2.06	0.04
1994	244.8	7.87	22.6	8.18	12.64	16.24	0.45	16.22	0.04	2.32	0.04
1997	249.63	8.01	21.25	8.43	13.4	13.17	0.23	15.51	0.01	1.76	0.03
1998	252.78	8.21	22.91	8.5	13.96	13.76	0.23	13.54	0.01	1.59	0.03
1999	288.35	8.29	25.62	9.28	14.65	17.51	0.17	13.23	0.01	1.62	0.02
2000	279.96	8.19	24.02	8.75	14.5	18.98	0.25	13.91	0.02	1.78	0.04
2001	293.08	8.19	25.22	10.54	15.39	21.03	0.24	13.05	0.03	1.68	0.03
2002	309.69	8.19	26.08	10.44	16.33	21.73	0.18	12.56	0.02	1.69	0.03
2003	350.33	8.24	33.42	12.92	16.98	25.62	0.09	12.71	0.01	1.78	0.03
2004	360.67	8.19	34.93	12.51	17.58	26.07	0.07	13.88	0.01	1.87	0.03
2005	367.13	8.11	35.17	13.15	17.46	27.68	0.11	14.89	0.02	2.24	0.03
2006	328.05	8.18	30.62	11	15.14	23.01	0.12	12.57	0.02	1.66	0.06

Appendix A2 continued

Year	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	pH	Na ($\text{mg}\cdot\text{L}^{-1}$)	Mg ⁺ ($\text{mg}\cdot\text{L}^{-1}$)	Ca ²⁺ ($\text{mg}\cdot\text{L}^{-1}$)	Cl ⁻ ($\text{mg}\cdot\text{L}^{-1}$)	NO ₃ ⁻ /NO ₂ ⁻ ($\text{mg}\cdot\text{L}^{-1}$)	SO ₄ ²⁻ ($\text{mg}\cdot\text{L}^{-1}$)	PO ₄ ³⁻ ($\text{mg}\cdot\text{L}^{-1}$)	K ($\text{mg}\cdot\text{L}^{-1}$)	NH ₄ ⁺ ($\text{mg}\cdot\text{L}^{-1}$)
2007	313.71	8.17	30.28	11.1	13.95	21.05	0.12	11.41	0.02	1.85	0.09
2008	332.63	8.24	30.35	12.03	15.41	24.04	0.09	14.08	0.01	1.95	0.06
2009	309	8.1	32	11.67	13.92	23.72	0.04	12.56	0.01	2.6	0.03
2012	309.04	8.38	33.02	10.85	13.57	22.51	0.1	12.1	0.01	2.89	0.03
2013	282.43	8.28	28.02	9.65	12.83	19.03	0.15	11.08	0.01	2.63	0.03
2014	279.01	8.31	*	10.23	12.28	19.92	0.36	11.17	0.01	*	0.05
2015	310.78	8.38	32.36	11.02	13.52	23.19	0.1	12.54	0.01	2.51	0.05
2016	401.25	8.45	44	14.09	18.81	37.1	0.71	17.49	0.03	2.87	0.05
2017	342	8.23	36.4	11.93	15.1	28.23	0.16	15.7	0.01	3	0.05

Appendix A3: Annual water quality variables of the Phongolo River obtained from gauging station W4H009 downstream of Pongolapoort Dam within the Ndumo Game Reserve from 1980 – 2017, and used in the Physico-chemical Assessment Index (PAI). *Denotes data not available.

Year	Conductivity ($\mu\text{S.cm}^{-1}$)	pH	Na (mg.L^{-1})	Mg ⁺ (mg.L^{-1})	Ca ²⁺ (mg.L^{-1})	Cl ⁻ (mg.L^{-1})	NO ₃ ⁻ /NO ₂ ⁻ (mg.L^{-1})	SO ₄ ²⁻ (mg.L^{-1})	PO ₄ ³⁻ (mg.L^{-1})	K (mg.L^{-1})	NH ₄ ⁺ (mg.L^{-1})
1980	342.2	6.96	37.17	10.81	15.07	29.51	0.12	9.44	0.01	1.91	0.04
1981	684.89	7.11	81.44	21.52	26.96	115.89	0.25	24.41	0.01	2.44	0.04
1982	801.5	7.56	88.61	28.54	34.14	136.91	0.33	21.24	0.04	3.56	0.07
1983	875.5	7.6	112.4	27.45	31.35	135.5	0.81	24.45	0.06	4.03	0.03
1984	461	7.61	43.1	15.3	22.8	65.2	0.66	51.7	0.06	2.52	0.09
1985	469	7.8	43.45	15.7	23.7	67.7	0.53	11.75	0	2.18	0.06
1986	422.63	7.83	39.16	14.04	21.45	50.41	0.13	11.41	0.02	2.49	0.05
1987	411.57	7.6	40.17	12.89	20.89	47.51	0.21	13.39	0.01	2.61	0.06
1988	441.89	7.35	43.76	14.06	21.6	56.7	0.24	10.66	0.02	2.33	0.05
1989	404	8.05	41.62	13.14	20.67	52.14	0.34	11.08	0.02	2.19	0.07
1990	365.92	8.06	37.35	12.89	19.46	45.63	0.26	9.44	0.02	2.03	0.07
1991	376.08	8.02	37.23	12.11	18.44	44.88	0.34	8.93	0.02	2.04	0.05
1992	404.92	7.97	36.39	13.06	20.44	38.72	0.26	16.06	0.04	2.19	0.05
1993	402	7.95	41.63	13.23	20.85	44.41	0.23	16.33	0.03	2.33	0.06
1994	332	8.2	31.9	9.8	17.8	34.6	0.06	13.6	0.05	2.84	0.03
1995	335	8.15	40.6	8.9	11.6	51.3	0.78	16.2	0.01	3.37	0.01
1996	430	8.08	43.7	12.6	20.57	53.43	0.38	17.77	0.03	2.79	0.03
1997	385.44	7.92	39.28	12.13	19.48	41.84	0.2	18.67	0.02	2.18	0.05
1998	491.75	8.21	50.78	16.23	22.88	67.98	0.24	19.63	0.02	1.9	0.04
1999	546.71	8.24	54.81	16.51	24.62	78.45	0.2	22.49	0.03	2.32	0.03
2000	498.75	8.15	48.29	14.52	22.76	63.67	0.38	17.65	0.03	2.11	0.04

Appendix A3 continued

Year	Conductivity ($\mu\text{S.cm}^{-1}$)	pH	Na (mg.L^{-1})	Mg ⁺ (mg.L^{-1})	Ca ²⁺ (mg.L^{-1})	Cl ⁻ (mg.L^{-1})	NO ₃ ⁻ /NO ₂ ⁻ (mg.L^{-1})	SO ₄ ²⁻ (mg.L^{-1})	PO ₄ ³⁻ (mg.L^{-1})	K (mg.L^{-1})	NH ₄ ⁺ (mg.L^{-1})
2001	522.5	8.14	51.64	16.34	23.49	69.54	0.36	18.27	0.08	2.01	0.04
2002	511.67	8.06	55.44	15.55	23.28	70.81	0.17	20.67	0.06	1.92	0.05
2003	579.75	8.24	61.54	18.61	22.88	80.56	0.11	21.39	0.02	1.98	0.02
2004	661.64	8.11	76.77	20.27	25.92	102.36	0.13	25.58	0.03	2.32	0.03
2005	586.82	8.2	65.5	17.78	22.44	80.15	0.08	21.54	0.03	2.12	0.04
2006	577.1	8.15	67.17	17.6	22.13	82.7	0.07	25.53	0.03	2.17	0.07
2007	520.22	8.14	57.97	15.42	19.61	67.06	0.14	20.95	0.03	2.46	0.1
2008	570.5	8.27	69.5	20.82	21.23	90.82	0.05	19.32	0.03	3.14	0.14
2009	554.4	8.33	59.62	18.62	22.28	76.74	0.1	18.83	0.02	3.38	0.03
2010	624	8.59	50.96	16.22	48.7	53.61	0.03	102.19	0.29	*	0.03
2011	421.33	8.04	50.75	12.55	15.59	49.83	0.16	16.81	0.01	2.98	0.03
2012	525	8.33	63.21	14.03	17.74	65.75	0.11	19.98	0.02	3.43	0.03
2013	445.02	8.14	52.96	15.04	16.98	52.77	0.16	16.62	0.01	2.96	0.05
2014	459.41	8.31	*	20.33	17.87	56.39	0.09	17.85	0.01	*	0.05
2015	536.33	8.28	56.32	18.84	18.11	68.01	0.05	14.79	0.02	3.44	0.05
2016	483.92	8.27	*	16.67	21.11	34.83	1.23	39	0.32	*	0.07
2017	270.33	7.93	27.53	10.5	11.83	26.4	0.67	9.4	0.02	3.43	0.05

Appendix A4: Summary of the final scores allocated to water quality variables, and the final physico-chemical driver assessment index (PAI) scores, with associated Present Ecological Category derived from the three gauging stations located above and below Pongolapoort Dam. Upstream – Gauging station located above the dam; Down 1 – Gauging station located below dam wall. Down 2 – Gauging station located within Ndumo Game Reserve. Values 0 – 5 indicate amount of change from reference conditions. 0 – No change, 1 – Small change, 2 – Moderate, 3 – Large, 4 – Serious, 5 – Extreme.

Site	Years assessed	Physico-Chemical Metrics								Present ecological category
		pH	Salts	Nutrients	Temperature	Turbidity	Oxygen	Toxics	PAI (%)	
Upstream	Historic (1973 - 1974)	1	0	2	0	0	0	0	90.75	A
	2009 - 2014	1	1	1	1.5	1.5	1.5	1	76.24	C
	2016 - 2017	2	2	1	1.5	1.5	1.5	1	71.51	C
Downstream 1	Historic (1984 - 1986)	1	0	1	0	0	0	0	94.19	A
	2009 - 2014	1	1	1	1.5	1.5	1.5	1	76.24	C
	2016 - 2017	1	1	1.5	1.5	1.5	1.5	1	76.24	C
Downstream 2	Historic (1980 - 1981)	0	0	1	0	0	0	0	96.56	A
	2009 - 2014	1	1	1	1.5	1.5	1.5	1	76.24	C
	2016 - 2017	1	2	2	1.5	1.5	1.5	1	70.43	C

Appendix A5: *In situ* water quality variables measured at Site 1 located within Ndumo Game Reserve during the four sampling surveys conducted from 2016 to 2017. Numbers indicate sampling survey. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017.

Date	Temperature (°C)	Dissolved Oxygen (mg.L ⁻¹)	Dissolved Oxygen (%)	Conductivity (µS.cm ⁻¹)	pH	Cyanobacteria (µg.L ⁻¹)
Site 1.1	22.95	5.57	60.15	427	8.07	1.92
Site 1.2	18	8.83	95.8	439	8.26	5.05
Site 1.3	25.8	4.76	61.6	586	8.48	3
Site 1.4	29.4	4.34	68.7	666	8.39	9.71

Appendix A6: Chemical water nutrient variables measured at Site 1 located within Ndumo Game Reserve during the four sampling surveys conducted from 2016 to 2017. Numbers indicate sampling survey. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. *Denotes data not available.

Date	NO ₃ ⁻ (mg.L ⁻¹)	NO ₂ ⁻ (mg.L ⁻¹)	NH ₄ ⁺ (mg.L ⁻¹)	NH ₃ (mg.L ⁻¹)	PO ₄ ³⁻ (mg.L ⁻¹)	SO ₄ ²⁻ (mg.L ⁻¹)	Total hardness (mg.L ⁻¹)	Mg ⁺ (mg.L ⁻¹)	Ca ²⁺ (mg.L ⁻¹)	Cl ⁻ (mg.L ⁻¹)
Site 1.1	0.5	0.02	0.05	<0.01	0.11	25	51.33	25	26.33	63.5
Site 1.2	2.2	0.01	0.07	<0.01	0.37	*	44	21	23	10
Site 1.3	1	0.02	0.09	0.02	0.47	53	18	4	14	31
Site 1.4	0.7	<0.01	0.08	<0.01	0.03	29	67	42	25	89

Appendix A7: *In situ* water quality variables measured at Site 2 and Site 3 located outside Ndumo Game Reserve during the four sampling surveys conducted from 2016 to 2017. Numbers indicate sampling survey. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017.

Date	Temperature (°C)	Dissolved Oxygen (mg.L ⁻¹)	Dissolved Oxygen (%)	Conductivity (µS.cm ⁻¹)	pH	Cyanobacteria (µg.L ⁻¹)
Site 2.1	22.6	3.91	45.3	403	7.79	0.64
Site 2.2	18.7	9.37	98.7	447	8.23	6.5
Site 2.3	23.2	9.22	104.4	555	8.61	2.98
Site 2.4	35.8	4.33	62.7	735	8.65	10.7
Site 3.3	22.1	8.3	95	553	8.60	2.18
Site 3.4	29.9	4.72	63.7	683	8.52	12.81

Appendix A8: Chemical water nutrient variables measured at Site 2 and Site 3 located within Ndumo Game Reserve during the four sampling surveys conducted from 2016 to 2017. Numbers indicate sampling survey. 1 – May 2016; 2 – August 2016; 3 – September 2016; 4 – February 2017. *Denotes data not available.

Date	NO ₃ ⁻ (mg.L ⁻¹)	NO ₂ ⁻ (mg.L ⁻¹)	NH ₄ ⁺ (mg.L ⁻¹)	NH ₃ (mg.L ⁻¹)	PO ₄ ³⁻ (mg.L ⁻¹)	SO ₄ ²⁻ (mg.L ⁻¹)	Total hardness (mg.L ⁻¹)	Mg ⁺ (mg.L ⁻¹)	Ca ²⁺ (mg.L ⁻¹)	Cl ⁻ (mg.L ⁻¹)
Site 2.1	0.5	0.02	0.07	<0.01	0.1	25	54	27	27	64
Site 2.2	1	0.01	0.09	<0.01	0.13	*	47	20	27	56
Site 2.3	0.6	0.02	0.07	0.01	2.28	21	20	5	15	41
Site 2.4	0.8	<0.01	0.11	<0.01	0.03	143	56	35	21	71
Site 3.3	<0.05	0.02	0.12	0.02	1.5	13	33	14	19	43
Site 3.4	0.8	<0.01	0.06	0.01	0.03	28	55	36	19	62

Appendix B

Appendix B1: Diversity indices and Analysis of Variance (ANOVA) of invertebrate taxa collected in Treatment 1. Mean $\pm$ SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p<0.05$).

Diversity Indices	Category			ANOVA
	Treatment 1.1	Treatment 1.2	Treatment 1.3	
Number of Taxa	9.93 $\pm$ 7.01 (2–27)	11.29 $\pm$ 5.99 (2–16)	13 $\pm$ 7.46 (3–30)	p= 0.5 F= 0.71
Number of Individuals	14940 $\pm$ 3811 (1428–52583)	14444 $\pm$ 3182 (826–37384)	12646 $\pm$ 10557 (1683–38020)	p= 0.88 F= 0.13
Margalef's Species Richness	0.94 $\pm$ 0.7 (0.14–2.5)	1.1 $\pm$ 0.61 (0.15–2.14)	1.27 $\pm$ 0.7 (0.26–2.75)	p= 0.42 F= 0.88
Pielou's Evenness Index	0.27 $\pm$ 0.13 (0.01–0.49)	0.3 $\pm$ 0.1 (0.13–0.47)	0.21 $\pm$ 0.13 (0.04–0.49)	p= 0.13 F= 2.19
Shannon-Wiener Diversity Index	0.62 $\pm$ 0.38 (0.01–1.15)	0.69 $\pm$ 0.35 (0.23–1.19)	0.55 $\pm$ 0.38 (0.05–1.23)	p= 0.59 F= 0.54
Simpson's Index	0.35 $\pm$ 0.22 (0.002–0.65)	0.38 $\pm$ 0.2 (0.1–0.64)	0.3 $\pm$ 0.22 (0.01–0.68)	p= 0.61 F= 0.5

Appendix B2: Diversity indices and Analysis of Variance (ANOVA) of invertebrate taxa collected in Treatment 2. Mean $\pm$ SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p<0.05$). * Indicates non-parametric ANOVA was used.

Diversity Indices	Category			ANOVA
	Treatment 2.1	Treatment 2.2	Treatment 2.3	
Number of Taxa	10.43 $\pm$ 1.69 (2–24)	11.57 $\pm$ 1.75 (2–24)	12.86 $\pm$ 6 (4–23)	p= 0.6 F= 0.52
Number of Individuals *	11066 $\pm$ 10009 (2700–38330)	9530 $\pm$ 9179 (911–29760)	10813 $\pm$ 9860 (2422–35901)	p= 0.84 Chi - square= 0.35
Margalef's Species Richness	1.03 $\pm$ 0.68 (0.12–2.37)	1.23 $\pm$ 0.8 (0.14–2.54)	1.31 $\pm$ 0.63 (0.36–2.25)	p= 0.56 F= 0.6
Pielou's Evenness Index	0.26 $\pm$ 0.1 (0.07–0.4)	0.31 $\pm$ 0.16 (0.01–0.6)	0.27 $\pm$ 0.15 (0.02–0.46)	p= 0.64 F= 0.45
Shannon-Wiener Diversity Index	0.6 $\pm$ 0.32 (0.06–1.06)	0.78 $\pm$ 0.5 (0.01–1.74)	0.72 $\pm$ 0.45 (0.03–1.33)	p= 0.53 F= 0.65
Simpson's Index	0.32 $\pm$ 0.18 (0.02–0.59)	0.41 $\pm$ 0.24 (0.002–0.76)	0.40 $\pm$ 0.25 (0.01–0.67)	p= 0.55 F= 0.62

Appendix B3: Diversity indices and Analysis of Variance (ANOVA) of invertebrate taxa collected in Treatment 3. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). * Indicates non-parametric ANOVA was used.

Diversity Indices	Category			ANOVA
	Treatment 3.1	Treatment 3.2	Treatment 3.3	
Number of Taxa	11.21±5.59 (3–23)	12.36±7.32 (1–25)	12.71±5.78 (3–23)	p= 0.81 F= 0.22
Number of Individuals*	9345±8459 (1201–28670)	6994±6138 (717–20128)	6725±8557 (209–28834)	p= 0.26 Chi-square = 2.71
Margalef's Species Richness	1.15±0.58 (0.28–0.60)	1.34±0.9 (0–2.89)	1.50±0.77 (0.27–2.99)	p= 0.47 F= 0.78
Pielou's Evenness Index	0.3±0.16 (0.01–0.63)	0.33±0.15 (0–0.54)	0.32±0.14 (0.14–0.61)	p= 0.86 F= 0.15
Shannon-Wiener Diversity Index	0.75±0.46 (0.01–1.62)	0.86±0.51 (0–1.6)	0.82±0.45 (0.16–1.68)	p= 0.82 F= 0.2
Simpson's Index	0.43±0.24 (0.003–0.78)	0.45±0.23 (0–0.73)	0.42±0.2 (0.07–0.77)	p= 0.94 F= 0.07

Appendix B4: *In situ* water quality variables and Analysis of Variance (ANOVA) of the three mesocosm treatments. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$).

<i>In situ</i> Water Quality	Category			ANOVA
	Treatment 1	Treatment 2	Treatment 3	
Dissolved Oxygen (mg.L ⁻¹)	5.91±2.77 (2.03–13.45)	5.65±2.48 (2.46–10.88)	5.66±1.99 (1.86–9.98)	p= 0.88 F= 0.13
Oxygen Saturation (%)	67.45±29.08 (23.6–125.7)	67.69±28.85 (32.4–118.5)	68.67±24.68 (22.5–119.3)	p= 0.98 F= 0.02
Conductivity (µS.cm ⁻¹)	434.7±57.41 (332–663)	438.8±42.62 (400–548)	439.4±46 (396–555)	p= 0.92 F= 0.09
Temperature (°C)	25.68±1.82^a (23.7–31.3)	25.96±1.65 (23.9–3)	26.79±1.79^a (23.7–30.2)	p= 0.03 F= 3.58
pH	7.84±0.41 (7.4–9.24)	7.86±0.44 (7.36–9.2)	7.86±0.41 (7.33–9.15)	p= 0.96 F= 0.01
TDS (mg.L ⁻¹)	301.1±31.4 (233–382)	305.6±29.72 (280–385)	305.9±31.92 (278–387)	p= 0.78 F=0.25
Turbidity (NTU)	243.6±131.5 (66–580)	236.8±126.7 (24–590)	213.8±93.57 (75–390)	p= 0.57 F= 0.58
Cyanobacteria (µg.L ⁻¹)	50.55±22.27^a (11.23–105.8)	43.81±21.94 (7.23–96.07)	37.95±16.01^a (8.33–65.17)	p= 0.05 F= 3.19

Appendix B5: Chemical water nutrient variables and Analysis of Variance (ANOVA) of the three mesocosm treatments. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences (p<0.05).

Water Nutrients	Category			ANOVA
	Treatment 1	Treatment 2	Treatment 3	
PO_4^{3-} (mg.L ⁻¹)	0.66±0.42 (0.13–0.9)	0.57±0.43 (0.11–1.98)	0.54±0.37 (0.11–1.41)	p= 0.4 F= 0.76
NO_3^- (mg.L ⁻¹)	2.24±1.49 (1.8–7.1)	2.21±1.25 (1.7–5.04)	2.4±1.4 (2.18–5.4)	p= 0.84 F= 0.17
NO_2^- (mg.L ⁻¹)	0.005±0.006 (0.002–0.02)	0.006±0.007 (0.002–0.03)	0.006±0.008 (0.002–0.03)	p= 0.65 F= 0.43
NH_3 (mg.L ⁻¹)	0.02±0.03 (0–0.15)	0.02±0.03 (0–0.16)	0.01±0.03 (0–0.13)	p= 0.94 F= 0.06
NH_4^+ (mg.L ⁻¹)	0.32±0.24 (0.05–1.03)	0.28±0.19 (0.05–0.69)	0.26±0.18 (0.06–0.68)	p= 0.38 F= 0.98
Cl^- (mg.L ⁻¹)	63.76±32.28 (2.5–130)	64.88±31.22 (2.5–104)	60.45±31.33 (2.5–106)	p= 0.84 F= 0.17
SO_4^{2-} (mg.L ⁻¹)	60.82±53.08 (17–245)	53.82±50.88 (15–249)	42.97±27.65 (16–145)	p= 0.28 F= 1.3
Total Hardness (mg.L ⁻¹)	33.42±15.01 (5–54)	36.21±14.85 (5–55)	38.82±12.67 (5–55)	p= 0.31 F= 1.19

Appendix B6: Diversity indices and Analysis of Variance (ANOVA) of macroinvertebrate taxa collected in the three mesocosm treatments. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences (p<0.05). * Indicates non-parametric ANOVA was used.

Diversity Indices	Category			ANOVA
	Treatment 1	Treatment 2	Treatment 3	
Number of Taxa	6.52±4.64 (0–18)	6.73±4.52 (0–15)	7.21±4.28 (0–16)	p= 0.81 F= 0.21
Number of Individuals	42.48±53.54 (0–210)	36.48±43.36 (0–188)	45±61.63 (0–269)	p= 0.8 F= 0.22
Margalef's Species Richness	1.24±0.98^{ab} (0–3.25)	1.87±0.88^a (0–3.25)	1.84±0.87^b (0–3.21)	p= 0.004 F= 5.94
Pielou's Evenness Index*	0.72±0.25 (0–1)	0.7±0.3 (0–1)	0.69±0.27 (0–1)	p= 0.83 Chi - square= 0.38
Shannon-Wiener Diversity Index*	11.03±0.57 (0–2.07)	1.27±0.66 (0–2.24)	1.28±0.61 (0–2.1)	p= 0.05 Chi - square= 5.82
Simpson's Index*	0.58±0.26 (0–1)	0.66±0.3 (0–1)	0.66±0.27 (0–1)	p= 0.13 Chi - square= 4.12

Appendix B7: Zooplankton taxa and Analysis of Variance (ANOVA) assessment of zooplankton taxa across all three mesocosm treatments. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). * Indicates non-parametric ANOVA was used.

Zooplankton	Category			ANOVA
	Treatment 1	Treatment 2	Treatment 3	
<i>Diaphanosoma</i> sp. *	731±1230 (0–4283)	392±875.2 (0–4828)	254.3±546.3 (0–2255)	p= 0.43 Chi - square= 1.69
<i>Thermocyclops</i> sp. *	3279±3548 (160–17612)	2796±2229 (100–9936)	2361±1890 (0–7300)	p= 0.56 Chi - square= 1.15
<i>Moina micrura</i> *	2512±5271 (0–21988)	2378±4299 (0–14715)	1813±3133 (0–12074)	p= 0.98 Chi - square= 0.02
<i>Metadiaptomus</i> sp. *	5.76±14.69 (0–61)	10.45±22.09 (0–77)	12.33±32.13 (0–164)	p= 0.71 Chi - square= 0.68
<i>Simocephalus serrulatus</i> *	2339±3864 ^a (0–13872)	1015±1741 (0–6048)	373.4±788.5 ^a (0–3248)	p= 0.40 Chi - square= 1.85
Darwinulidae *	3.21±18.45 (0–106)	6.91±22.24 (0–82)	8.67±29.61 (0–142)	p= 0.57 Chi - square= 1.14
Cyprididae *	2.73±10.67 (0–54)	27.36±90.19 (0–408)	24.73±103.7 (0–585)	p= 0.86 Chi - square= 0.30

Appendix B8: Diversity indices and Analysis of Variance (ANOVA) of zooplankton taxa collected in the three mesocosm treatments. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$). * Indicates non-parametric ANOVA was used.

Diversity Indices	Category			ANOVA
	Treatment 1	Treatment 2	Treatment 3	
Number of Taxa	3.06±1.25 (1–6)	3.27±1.35 (1–7)	3.06±1.48 (0–7)	p= 0.77 Chi - square= 5.82
Number of Individuals	8872±6183 ^a (826–24485)	6626±4702 (835–18945)	4847±4377 ^a (0–18112)	p= 0.008 Chi - square= 5.82
Margalef's Species Richness	0.21±0.12 (0–0.53)	0.27±0.17 (0–0.71)	0.26±0.17 (0–0.74)	p= 0.13 F= 2.1
Pielou's Evenness Index	0.57±0.27 (0–1)	0.51±0.29 (0–1)	0.57±0.26 (0–0.95)	p= 0.52 F= 0.67
Shannon-Wiener Diversity Index	0.56±0.31 (0–1.18)	0.6±.39 (0–1.40)	0.64±0.43 (0–1.53)	p= 0.68 F= 0.39
Simpson's Index	0.34±0.19 (0–0.68)	0.34±0.23 (0–0.72)	0.38±0.23 (0–0.76)	p= 0.67 F= 0.41

Appendix B9: Fish present on the last day of sampling in the each of the replicates of the three mesocosm treatments. T1 – Treatment 1; T2 – Treatment 2; T3 – Treatment 3.

Mesocosm	Fish species	
	<i>Clarias gariepinus</i>	<i>Synodontis zambezensis</i>
T 1.1	1	None
T 1.2	None	None
T 1.3	None	None
T 2.1	None	1
T 2.2	None	1
T2.3	1	None
T 3.1	2	None
T 3.2	None	2
T 3.3	None	None

Appendix B10: Water levels measured in each of the replicates of the three mesocosm treatments on the first day of sampling (Day 1) and the final day of sampling (Day 35). T1 – Treatment 1; T2 – Treatment 2; T3 – Treatment 3.

Mesocosm	Water level on Day 1	Water level on Day 35
T 1.1	40 cm	25.5 cm
T 1.2	40 cm	26.5cm
T 1.3	40 cm	23 cm
T 2.1	40 cm	26.5 cm
T 2.2	40 cm	28 cm
T2.3	40 cm	28 cm
T 3.1	40 cm	40 cm
T 3.2	40 cm	40 cm
T 3.3	40 cm	40 cm

Appendix B11: Detailed list of aquatic invertebrates, families and functional feeding groups collected from all three hydrologic treatments across all sampling days. T1 – Treatment 1, T2 – Treatment 2, T3 – Treatment 3. * Denotes corresponding taxa found by Dube (2015). FC – Filter collector, GC – Gathering collector.

Taxon	Family	Functional feeding group	Day 1			Day 2			Day 3		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Anax</i> sp.*	Aeshnidae	Predator	0	0	0	0	0	0	0	0	0
<i>Caridina nilotica</i> *	Atyidae	FC	0	0	0	0	0	0	0	0	0
<i>Cloeon</i> and <i>Procloeon</i> sp. *	Baetidae	GC	0	0	0	1	0	0	0	1	1
<i>Lethocerus niloticus</i>	Belostomatidae	Predator	0	0	0	0	0	0	0	0	0
<i>Atractides</i> sp.	Calopterygidae	Predator	0	0	0	0	0	1	0	0	0
<i>Chaoborus microstictus</i>	Chaoboridae	Predator	0	0	0	0	0	0	0	1	0
Chironominae *	Chironomidae	GC	0	0	0	0	0	0	1	0	0
Orthocladinae	Chironomidae	GC	0	0	0	0	0	0	0	0	0
<i>Diplacodes</i> sp.	Coenagrionidae	Predator	0	0	0	0	0	0	0	0	0
<i>Pseudagrion</i> sp.	Coenagrionidae	Predator	0	0	0	0	0	0	0	0	0
<i>Phaon iridipennis</i>	Coenagrionidae	Predator	0	0	0	1	0	0	1	0	0
<i>Micronecta</i> sp.	Corixidae	Predator	0	1	2	0	1	3	0	2	1
<i>Anopheles</i> sp. *	Culicidae	FC	0	0	0	0	0	0	0	0	0
<i>Culex</i> sp.	Culicidae	FC	0	0	0	0	0	0	0	0	0
<i>Thermocyclops</i> sp.	Cyclopidae	FC	4103	9108	5152	5472	12404	8819	33074	19086	6990
Cyprididae	Cyprididae	FC	0	0	0	0	0	0	0	0	0
<i>Simocephalus serrulatus</i>	Daphniidae	FC	0	0	0	0	0	0	0	0	0
Darwinulidae	Darwinulidae	FC	0	0	0	0	0	0	0	0	0
<i>Metadiaptomus</i> sp.	Diaptomidae	FC	0	0	0	0	0	0	0	0	0
<i>Laccophilus</i> sp. *	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Laccophilus</i> sp. larvae	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 1			Day 2			Day 3		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Cybister</i> sp. A	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Laccotrephes</i> sp.	Dytiscidae	Predator	1	0	0	1	0	0	1	0	0
<i>Rhantaticus congestus</i>	Dytiscidae	Predator	0	1	0	0	1	1	0	0	1
<i>Eretes</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hyphydrus</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	1	0	0
<i>Hydroglyphus</i> sp.	Dytiscidae	Predator	0	0	0	2	0	1	0	2	4
<i>Hyphydrus</i> sp. larvae	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Rhantus</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hydaticus</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Dytiscidae</i> larvae*	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
Ephydriidae	Ephydriidae	FC	0	0	0	0	0	0	0	0	0
<i>Tenagogonus</i> sp.	Gerridae	Predator	0	0	0	0	0	0	0	0	0
<i>Gerris swakopensis</i>	Gerridae	Predator	0	0	1	0	0	1	0	0	1
<i>Peltodytes</i> sp. larvae	Haliplidae	FC	0	0	0	0	0	0	0	0	0
<i>Hydrachna</i> * sp.	Hydrachnidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hydraena</i> sp.	Hydraenidae	GC	0	0	0	0	0	0	0	0	0
<i>Hydrometra</i> sp.	Hydrometridae	Predator	0	0	0	0	0	0	0	0	0
<i>Berosus</i> sp. larvae	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hydrophilus senegalensis</i> *	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0
<i>Laccobius</i> sp. *	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0
<i>Enochrus</i> sp. larvae	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 1			Day 2			Day 3		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Enochrus</i> sp. *	Hydrophilidae	GC	0	0	0	0	0	0	0	0	0
<i>Berosus</i> sp. *	Hydrophilidae	GC	0	0	0	0	0	0	0	0	0
<i>Athripsodes harrisoni</i>	Leptoceridae	Shredder	0	0	0	0	0	0	0	0	0
<i>Leptocerus</i> sp.	Leptoceridae	Shredder	0	0	0	2	0	0	2	0	0
<i>Polpopleura lucio</i>	Libellulidae	Predator	0	0	0	0	0	0	0	0	0
<i>Pirata</i> sp.	Lycosidae	Predator	0	0	0	0	0	0	0	0	0
<i>Mesovelia vittergera</i> *	Mesoveliidae	Predator	0	0	0	0	0	0	0	0	0
<i>Moina micrura</i>	Moinidae	FC	0	21	60	202	374	343	1349	626	509
<i>Canthydrus</i> sp. *	Noteridae	Predator	0	0	0	0	0	0	0	0	0
<i>Anisops</i> sp. A *	Notonectidae	Predator	0	1	0	0	2	3	0	1	0
<i>Anisops</i> sp. B	Notonectidae	Predator	0	0	0	0	0	0	0	0	0
<i>Enithares</i> sp.	Notonectidae	Predator	0	0	0	0	0	0	0	0	0
<i>Plea</i> sp.	Pleidae	Predator	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> sp. * [§]	Sididae	FC	97	32	0	0	124	62	56	0	0
<i>Spercheus</i> sp. larvae	Spercheidae	FC	0	0	0	0	0	0	0	0	0
<i>Spercheus</i> sp.	Spercheidae	FC	0	0	0	1	1	0	0	1	0
<i>Microvelia</i> sp.	Veliidae	Predator	0	0	0	0	0	0	15	15	15

[§] Dube (2015) identified *Diaphanosoma peramatum*.

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 4			Day 6			Day 8		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Anax</i> sp.*	Aeshnidae	Predator	0	0	0	0	0	2	0	3	3
<i>Caridina nilotica</i> *	Atyidae	FC	0	0	0	0	0	1	0	0	0
<i>Cloeon</i> and <i>Procloeon</i> sp. *	Baetidae	GC	0	2	2	5	4	4	6	13	0
<i>Lethocerus niloticus</i>	Belostomatidae	Predator	0	0	0	0	0	0	0	0	0
<i>Atractides</i> sp.	Calopterygidae	Predator	0	0	0	0	0	0	0	0	0
<i>Chaoborus microstictus</i>	Chaoboridae	Predator	1	3	0	1	0	0	1	0	1
Chironominae*	Chironomidae	GC	0	0	0	1	0	0	2	9	2
Orthocladinae	Chironomidae	GC	0	0	0	0	1	0	0	0	0
<i>Diplacodes</i> sp.	Coenagrionidae	Predator	0	0	0	0	0	0	0	0	0
<i>Pseudagrion</i> sp.	Coenagrionidae	Predator	0	0	0	0	0	0	0	0	0
<i>Phaon iridipennis</i>	Coenagrionidae	Predator	0	0	0	0	0	0	0	0	0
<i>Micronecta</i> sp.	Corixidae	Predator	0	0	2	1	2	1	1	5	3
<i>Anopheles</i> sp. *	Culicidae	FC	0	0	0	9	4	14	51	14	46
<i>Culex</i> sp.	Culicidae	FC	0	0	0	0	0	0	0	0	0
<i>Thermocyclops</i> sp.	Cyclopidae	FC	19585	13044	18740	6788	6785	4836	12817	12076	11225
Cyprididae	Cyprididae	FC	0	0	0	0	0	0	0	0	0
<i>Simocephalus serrulatus</i>	Daphniidae	FC	0	0	0	0	0	0	0	26	25
Darwinulidae	Darwinulidae	FC	0	0	0	0	0	0	0	0	0
<i>Metadiaptomus</i> sp.	Diaptomidae	FC	0	0	0	0	0	0	0	77	27
<i>Laccophilus</i> sp. *	Dytiscidae	Predator	0	0	0	0	0	1	0	0	0
<i>Laccophilus</i> sp. larvae	Dytiscidae	Predator	0	0	1	0	0	0	0	0	0

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 4			Day 6			Day 8		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Cybister</i> sp. A	Dytiscidae	Predator	0	0	0	0	0	0	0	0	1
<i>Laccotrephes</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Rhantaticus congestus</i>	Dytiscidae	Predator	0	0	1	0	1	1	0	0	0
<i>Eretes</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hyphydrus</i> sp.	Dytiscidae	Predator	1	0	0	0	0	0	0	0	0
<i>Hydroglyphus</i> sp.	Dytiscidae	Predator	8	6	7	10	5	9	2	3	8
<i>Hyphydrus</i> sp. larvae	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Rhantus</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hydaticus</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0
<i>Dytiscidae</i> larvae*	Dytiscidae	Predator	0	0	0	0	1	0	0	1	0
Ephydriidae	Ephydriidae	FC	0	0	0	1	2	0	0	0	0
<i>Tenagogonus</i> sp.	Gerridae	Predator	0	0	0	0	0	0	0	0	0
<i>Gerris swakopensis</i>	Gerridae	Predator	0	1	0	0	1	0	0	1	0
<i>Peltodytes</i> sp. larvae	Haliplidae	FC	0	0	0	0	0	0	0	0	0
<i>Hydrachna</i> * sp.	Hydrachnidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hydraena</i> sp.	Hydraenidae	GC	0	0	0	0	0	0	0	0	0
<i>Hydrometra</i> sp.	Hydrometridae	Predator	0	0	0	0	0	0	0	0	0
<i>Berosus</i> sp. larvae	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hydrophilus senegalensis</i> *	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0
<i>Laccobius</i> sp. *	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0
<i>Enochrus</i> sp. larvae	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 4			Day 6			Day 8		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Enochrus</i> sp. *	Hydrophilidae	GC	0	0	0	0	0	0	1	0	1
<i>Berosus</i> sp. *	Hydrophilidae	GC	0	0	0	1	1	1	0	1	0
<i>Athripsodes harrisoni</i>	Leptoceridae	Shredder	0	0	0	0	0	0	1	0	0
<i>Leptocerus</i> sp.	Leptoceridae	Shredder	0	0	0	0	0	2	0	0	0
<i>Polpopleura lucio</i>	Libellulidae	Predator	0	0	0	0	0	0	0	0	0
<i>Pirata</i> sp.	Lycosidae	Predator	0	0	0	0	1	1	0	0	0
<i>Mesovelgia vittergera</i> *	Mesoveliidae	Predator	0	0	0	0	0	0	0	0	0
<i>Moina micrura</i>	Moinidae	FC	1697	2109	6485	4361	10872	6227	30512	29602	16143
<i>Canthydrus</i> sp. *	Noteridae	Predator	0	0	0	0	0	0	0	0	0
<i>Anisops</i> sp. A *	Notonectidae	Predator	0	0	4	0	0	1	1	0	1
<i>Anisops</i> sp. B	Notonectidae	Predator	1	0	1	0	0	0	0	0	0
<i>Enithares</i> sp.	Notonectidae	Predator	0	0	0	0	0	0	0	0	0
<i>Plea</i> sp.	Pleidae	Predator	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> sp. * [§]	Sididae	FC	0	0	0	0	0	198	269	713	105
<i>Spercheus</i> sp. larvae	Spercheidae	FC	0	0	0	0	0	0	0	0	0
<i>Spercheus</i> sp.	Spercheidae	FC	0	0	0	2	0	1	2	1	1
<i>Microvelia</i> sp.	Veliidae	Predator	4	8	9	5	10	0	5	3	5

[§] Dube (2015) identified *Diaphanosoma peramatum*

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 10			Day 14			Day 21		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Anax</i> sp.*	Aeshnidae	Predator	0	2	2	0	2	0	0	1	3
<i>Caridina nilotica</i> *	Atyidae	FC	0	0	0	0	0	0	0	0	1
<i>Cloeon</i> and <i>Procloeon</i> sp.*	Baetidae	GC	15	29	18	26	51	20	214	273	92
<i>Lethocerus niloticus</i>	Belostomatidae	Predator	0	0	0	0	0	0	0	0	0
<i>Atractides</i> sp.	Calopterygidae	Predator	0	0	0	0	0	0	0	0	0
<i>Chaoborus microstictus</i>	Chaoboridae	Predator	0	13	0	0	0	0	0	0	0
Chironominae *	Chironomidae	GC	17	7	11	0	4	0	4	1	2
Orthocladinae	Chironomidae	GC	0	0	0	0	0	0	0	0	0
<i>Diplacodes</i> sp.	Coenagrionidae	Predator	0	0	0	0	0	0	0	0	0
<i>Pseudagrion</i> sp.	Coenagrionidae	Predator	0	0	0	0	0	0	0	0	0
<i>Phaon iridipennis</i>	Coenagrionidae	Predator	0	0	0	0	0	0	0	0	0
<i>Micronecta</i> sp.	Corixidae	Predator	2	2	3	0	2	0	1	4	2
<i>Anopheles</i> sp.*	Culicidae	FC	30	14	21	31	7	14	10	12	4
<i>Culex</i> sp.	Culicidae	FC	0	0	0	0	0	0	0	0	3
<i>Thermocyclops</i> sp.	Cyclopidae*	FC	4689	8536	12226	9049	1995	2738	5136	4150	2283
Cyprididae	Cyprididae	FC	0	0	0	0	35	36	85	94	60
<i>Simocephalus serrulatus</i>	Daphniidae	FC	686	1146	1456	11560	11071	1510	31756	7445	3073
Darwinulidae	Darwinulidae	FC	0	0	0	106	228	286	0	0	0
<i>Metadiaptomus</i> sp.	Diaptomidae	FC	0	0	0	96	70	72	27	94	68
<i>Laccophilus</i> sp.*	Dytiscidae	Predator	0	0	0	0	0	0	0	1	3
<i>Laccophilus</i> sp. larvae	Dytiscidae	Predator	0	0	0	0	0	0	0	0	0

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 10			Day 14			Day 21		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Cybister</i> sp. A	Dytiscidae	Predator	0	0	0	0	0	0	4	2	0
<i>Laccotrephes</i> sp.	Dytiscidae	Predator	0	0	0	1	0	0	0	0	0
<i>Rhantaticus congestus</i>	Dytiscidae	Predator	1	2	1	1	0	0	0	0	0
<i>Eretes</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0	0	0	1
<i>Hyphydrus</i> sp.	Dytiscidae	Predator	1	1	1	0	0	1	0	0	3
<i>Hydroglyphus</i> sp.	Dytiscidae	Predator	13	6	3	8	4	2	6	5	3
<i>Hyphydrus</i> sp. larvae	Dytiscidae	Predator	1	1	0	0	16	0	2	8	0
<i>Rhantus</i> sp.	Dytiscidae	Predator	1	1	1	0	0	0	1	1	0
<i>Hydaticus</i> sp.	Dytiscidae	Predator	0	0	0	0	0	1	0	0	0
<i>Dytiscidae</i> larvae*	Dytiscidae	Predator	0	2	0	0	3	5	4	0	0
Ephydriidae	Ephydriidae	FC	0	0	0	0	0	0	0	0	0
<i>Tenagogonus</i> sp.	Gerridae	Predator	0	0	0	0	1	0	0	0	0
<i>Gerris swakopensis</i>	Gerridae	Predator	0	2	0	0	1	0	0	4	2
<i>Peltodytes</i> sp. larvae	Haliplidae	FC	0	0	0	0	0	2	0	0	0
<i>Hydrachna</i> * sp.	Hydrachnidae	Predator	0	0	0	0	0	0	0	0	0
<i>Hydraena</i> sp.	Hydraenidae	GC	0	0	0	0	0	0	0	0	0
<i>Hydrometra</i> sp.	Hydrometridae	Predator	4	7	2	2	4	3	1	3	1
<i>Berosus</i> sp. larvae	Hydrophilidae	Predator	0	0	0	0	0	0	1	1	1
<i>Hydrophilus senegalensis</i> *	Hydrophilidae	Predator	0	0	1	2	0	7	0	1	1
<i>Laccobius</i> sp.*	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	0
<i>Enochrus</i> sp. larvae	Hydrophilidae	Predator	0	0	0	0	0	0	2	3	0

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 10			Day 14			Day 21		
			T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Enochrus</i> sp. *	Hydrophilidae	GC	0	0	2	2	3	7	2	10	1
<i>Berosus</i> sp. *	Hydrophilidae	GC	0	0	2	0	2	2	0	1	1
<i>Athripsodes harrisoni</i>	Leptoceridae	Shredder	0	0	0	0	0	0	0	0	0
<i>Leptocerus</i> sp.	Leptoceridae	Shredder	0	0	0	0	1	0	0	0	1
<i>Polpopleura lucio</i>	Libellulidae	Predator	0	0	0	0	0	0	0	0	0
<i>Pirata</i> sp.	Lycosidae	Predator	0	0	0	0	0	0	0	1	0
<i>Mesovelia vittergera</i> *	Mesoveliidae	Predator	0	0	0	0	0	4	24	12	2
<i>Moina micrura</i>	Moinidae	FC	40948	33477	29132	3725	1399	930	109	0	0
<i>Canthydrus</i> sp. *	Noteridae	Predator	0	0	0	0	0	0	0	0	0
<i>Anisops</i> sp. A *	Notonectidae	Predator	1	1	6	0	1	29	1	1	253
<i>Anisops</i> sp. B	Notonectidae	Predator	0	0	0	0	0	0	0	0	0
<i>Enithares</i> sp.	Notonectidae	Predator	0	0	0	0	0	0	0	0	0
<i>Plea</i> sp.	Pleidae	Predator	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> sp. * [§]	Sididae	FC	1694	1499	400	711	462	930	3819	1835	1471
<i>Spercheus</i> sp. larvae	Spercheidae	FC	0	0	0	0	0	0	5	0	0
<i>Spercheus</i> sp.	Spercheidae	FC	0	0	0	0	2	0	0	0	0
<i>Microvelia</i> sp.	Veliidae	Predator	39	12	10	19	4	20	25	13	20

[§] Dube (2015) identified *Diaphanosoma peramatum*

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 28			Day 35		
			T1	T2	T3	T1	T2	T3
<i>Anax</i> sp.*	Aeshnidae	Predator	0	1	1	0	4	3
<i>Caridina nilotica</i> *	Atyidae	FC	0	0	0	0	0	0
<i>Cloeon</i> and <i>Procloeon</i> sp. *	Baetidae	GC	130	97	96	31	85	34
<i>Lethocerus niloticus</i>	Belostomatidae	Predator	0	0	0	1	0	0
<i>Atractides</i> sp.	Calopterygidae	Predator	0	0	0	1	0	0
<i>Chaoborus microstictus</i>	Chaoboridae	Predator	0	0	0	0	0	0
Chironominae*	Chironomidae	GC	12	1	10	0	1	0
Orthocladinae	Chironomidae	GC	0	0	0	0	0	0
<i>Diplacodes</i> sp.	Coenagrionidae	Predator	0	5	1	8	5	4
<i>Pseudagrion</i> sp.	Coenagrionidae	Predator	0	0	0	0	3	0
<i>Phaon iridipennis</i>	Coenagrionidae	Predator	0	0	0	0	0	0
<i>Micronecta</i> sp.	Corixidae	Predator	24	11	14	29	15	22
<i>Anopheles</i> sp. *	Culicidae	FC	5	2	3	1	2	2
<i>Culex</i> sp.	Culicidae	FC	9	0	1	2	26	0
<i>Thermocyclops</i> sp.	Cyclopidae	FC	4356	2215	1414	3140	2860	3495
Cyprididae	Cyprididae	FC	2	32	0	3	742	720
<i>Simocephalus serrulatus</i>	Daphniidae	FC	21129	3712	2681	12055	10108	3576
Darwinulidae	Darwinulidae	FC	0	0	0	0	0	0
<i>Metadiaptomus</i> sp.	Diaptomidae	FC	0	71	35	67	33	205
<i>Laccophilus</i> sp. *	Dytiscidae	Predator	0	2	1	0	0	2
<i>Laccophilus</i> sp. larvae	Dytiscidae	Predator	0	0	0	0	0	0

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 28			Day 35		
			T1	T2	T3	T1	T2	T3
<i>Cybister</i> sp. A	Dytiscidae	Predator	0	0	0	0	0	0
<i>Laccotrephes</i> sp.	Dytiscidae	Predator	0	0	0	0	0	0
<i>Rhantaticus congestus</i>	Dytiscidae	Predator	0	0	0	0	0	0
<i>Eretes</i> sp.	Dytiscidae	Predator	4	0	0	0	0	1
<i>Hyphydrus</i> sp.	Dytiscidae	Predator	0	1	4	2	2	4
<i>Hydroglyphus</i> sp.	Dytiscidae	Predator	17	12	5	15	6	10
<i>Hyphydrus</i> sp. larvae	Dytiscidae	Predator	63	2	0	5	3	0
<i>Rhantus</i> sp.	Dytiscidae	Predator	1	2	0	0	2	1
<i>Hydaticus</i> sp.	Dytiscidae	Predator	1	0	1	2	0	1
<i>Dytiscidae</i> larvae*	Dytiscidae	Predator	2	1	3	6	0	3
Ephydridae	Ephydridae	FC	0	0	0	0	0	0
<i>Tenagobius</i> sp.	Gerridae	Predator	0	0	0	0	0	0
<i>Gerris swakopensis</i>	Gerridae	Predator	0	0	0	1	2	0
<i>Peltodytes</i> sp. larvae	Halipidae	FC	0	0	0	0	0	0
<i>Hydrachna</i> * sp.	Hydrachnidae	Predator	0	0	0	2	0	0
<i>Hydraena</i> sp.	Hydraenidae	GC	5	13	6	5	3	4
<i>Hydrometra</i> sp.	Hydrometridae	Predator	0	0	1	0	0	0
<i>Berosus</i> sp. larvae	Hydrophilidae	Predator	2	0	1	0	0	0
<i>Hydrophilus senegalensis</i> *	Hydrophilidae	Predator	0	0	1	4	0	0
<i>Laccobius</i> sp. *	Hydrophilidae	Predator	1	5	2	0	0	0
<i>Enochrus</i> sp. larvae	Hydrophilidae	Predator	10	1	3	25	2	1

Appendix B11 continued

Taxon	Family	Functional feeding group	Day 28			Day 35		
			T1	T2	T3	T1	T2	T3
<i>Enochrus</i> sp. *	Hydrophilidae	GC	6	5	3	7	1	2
<i>Berosus</i> sp. *	Hydrophilidae	GC	1	1	1	1	0	0
<i>Athripsodes harrisoni</i>	Leptoceridae	Shredder	0	0	0	0	0	0
<i>Leptocerus</i> sp.	Leptoceridae	Shredder	0	0	0	0	0	0
<i>Polpopleura lucio</i>	Libellulidae	Predator	1	0	0	0	0	0
<i>Pirata</i> sp.	Lycosidae	Predator	0	0	0	0	0	0
<i>Mesovelia vittergera</i> *	Mesoveliidae	Predator	40	23	17	130	32	47
<i>Moina micrura</i>	Moinidae	FC	0	0	0	0	0	0
<i>Canthydrus</i> sp. *	Noteridae	Predator	0	1	0	0	3	0
<i>Anisops</i> sp. A *	Notonectidae	Predator	1	9	231	39	53	114
<i>Anisops</i> sp. B	Notonectidae	Predator	0	0	0	0	0	0
<i>Enithares</i> sp.	Notonectidae	Predator	0	0	0	0	3	1
<i>Plea</i> sp.	Pleidae	Predator	0	0	1	0	0	1
<i>Diaphanosoma</i> sp. * [§]	Sididae	FC	11662	3259	777	5815	5011	4447
<i>Spercheus</i> sp. larvae	Spercheidae	FC	0	0	0	0	0	0
<i>Spercheus</i> sp.	Spercheidae	FC	1	0	0	1	0	0
<i>Microvelia</i> sp.	Veliidae	Predator	30	32	26	40	18	20

[§]Dube (2015) identified *Diaphanosoma peramatum*

Appendix B12: Two-way Analysis of Variance (ANOVA) of the effects of the five sampling weeks (Time), the three mesocosm treatments (Treatment) and their interactions on macroinvertebrate and zooplankton communities respectively.

Factor	Significance	
	Macroinvertebrates	Zooplankton
Time	p< 0.001; F= 3.62	p= 0.02; F= 1.61
Treatment	p= 0.71 F= 0.34	p= 0.67; F= 0.40
Time x Treatment	p= 0.99 F= 0.51	p> 0.99; F= 0.37

Appendix B13: Multi-level pattern analysis of macroinvertebrate and zooplankton communities collected from Treatment 1 over the five sampling weeks.

Time	Treatment 1	
	Taxon	p value
Week 2,3,4, and 5	<i>Diaphanosoma</i> sp.	0.02
	<i>Cloeon</i> and <i>Procloeon</i> sp.	0.01
	<i>Anopheles</i> sp.	0.04
	<i>Hydroglyphus</i> sp.	0.04
Week 3, 4 and 5	Cyprididae	0.01
	<i>Simocephalus serrulatus</i>	0.04
	<i>Mesovelia vittergera</i>	0.02
	<i>Enochrus</i> sp. larva	0.02
	<i>Enochrus</i> sp.	0.04
	Dytiscidae larva	0.02
	<i>Hyphydrus</i> sp. larva	0.01

Appendix B14: Multi- level pattern analysis of macroinvertebrate and zooplankton communities collected from Treatment 2 over the five sampling weeks.

Time	Treatment 2	
	Taxon	p value
Week 1 and 2	<i>Moina micrura</i>	0.01
Week 2 and 4	Dytiscidae larva	0.03
Week 3 and 4	<i>Laccophilus</i> sp.	0.05
Week 2,3 and 5	<i>Gerris swakopensis</i>	0.03
Week 3, 4 and 5	<i>Mesovelia vittergera</i>	0.01
	<i>Enochrus</i> sp. larva	0.01
	<i>Enochrus</i> sp.	0.04
	<i>Rhantus</i> sp.	0.03
Week 2,3,4, and 5	<i>Simocephalus serrulatus</i>	0.01
	<i>Diaphanosoma</i> sp.	0.02
	<i>Anax</i> sp.	0.01
	Chironominae	0.01
	<i>Anopheles</i> sp.	0.02

Appendix B15: Multi-level pattern analysis of macroinvertebrate and zooplankton communities collected from Treatment 3 over the five sampling weeks.

Time	Treatment 3	
	Taxon	p-value
Week 1 and 2	<i>Moina micrura</i>	0.01
Week 3 and 4	<i>Berosus</i> sp. larva	0.05
	<i>Culex</i> sp.	0.05
Week 3, 4 and 5	Cyprididae	0.03
	<i>Mesovelia vittergera</i>	0.04
	<i>Laccophilus</i> sp.	0.02
	<i>Hyphydrus</i> sp.	0.02
Week 2,3,4, and 5	<i>Simocephalus serrulatus</i>	0.008
	<i>Diaphanosoma</i> sp.	0.02
	<i>Enochrus</i> sp.	0.008
	<i>Microvelia</i> sp.	0.04

Appendix B16: Macroinvertebrate functional feeding groups and one-way Analysis of Variance (ANOVA) assessments of each functional feeding group in each of the three mesocosm treatments. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$).

Functional Feeding Groups	Category			ANOVA
	Treatment 1	Treatment 2	Treatment 3	
Predators	62.73±99.41	48.82±46.4	95.55±121.9	p= 0.5
	(1–310)	(3–153)	(3–313)	F= 0.71
Gathering Collectors	41.82±74.48	54.91±85.62	34.27±40.75	p= 0.78
	(0–220)	(0–285)	(0–116)	F= 0.25
Filter Collectors	14.18±18.8	11±11.33	7.82±9.83	p= 0.57
	(0–53)	(0–30)	(0–31)	F= 0.58
Shredders	0.09±0.3	0.18±0.6	0.55±0.82	p= 0.2
	(0–1)	(0–2)	(0–2)	F= 1.7

Appendix B17: Macroinvertebrate functional feeding groups and one-way Analysis of Variance (ANOVA) assessments of each functional feeding group over the five sampling weeks. Mean±SD and range in parentheses. Within rows: common superscripts indicate significant differences ($p < 0.05$).

Functional Feeding Groups	Category					ANOVA
	Week 1	Week 2	Week 3	Week 4	Week 5	
Predators	13.2±8.22	37.33±21.17	142.7±132	206±102.8	232.7±78.53	p< 0.0001
	(1–25)	(10–72)	(61–295)	(108–313)	(153–310)	F= 20.05
Gathering Collectors	1.73±0.61	28.11±16.36	200.3±96.02	129±21.66	58±27.78	p< 0.0001
	(0–7)	(3–60)	(96–285)	(116–154)	(40–90)	F= 37.71
Filter Collectors	2.47±4.98	26.22±15.37	11.67±3.51	7±7	11.33±14.47	p= 0.0002
	(0–16)	(9–53)	(8–15)	(2–15)	(2–28)	F= 8.13
Shredders	0.4±0.83	0.22±0.44	0.33±0.58	0±0	0±0	p= 0.79
	(0–2)	(0–1)	(0–1)	(0–0)	(0–0)	F= 0.43

Appendix C

Appendix C1: Detailed list of aquatic invertebrates, abbreviations and families collected from the temporary pool (RP) inside Ndumo Game Reserve during all four surveys between 2016 and 2017. RP1 – May 2016; RP2 – August 2016; RP3 – December 2016; RP4 – February 2017.

Name	Family	RP 1	RP 2	RP 3	RP 4
<i>Anax</i> sp.	Aeshnidae	0	0	0	1
Anostraca	Anostraca	0	660	0	0
Baetidae	Baetidae	2	0	28	2
<i>Cloeon</i> and <i>Procloeon</i>	Baetidae	0	0	0	1
<i>Appasus</i> sp.	Belostomatidae	0	0	5	1
Chironominae	Chironomidae	0	0	43	9
Tanypodinae	Chironomidae	9	0	0	0
<i>Eurycercus</i> sp.	Chydoridae	0	0	15	460
<i>Enallagma</i> sp.	Coenagrionidae	0	0	1	0
<i>Pseudagrion</i> sp.	Coenagrionidae	0	0	5	9
<i>Micronecta</i> sp.	Corixidae	13	0	2	3
<i>Sigara</i> sp.	Corixidae	0	2	2	0
Crambidae sp. A larvae	Crambidae	0	0	0	1
<i>Culex</i> sp. A	Culicidae	0	427	35	7
<i>Culex</i> sp. pupa	Culicidae	0	0	28	0
<i>Culex</i> sp. B	Culicidae	1	0	0	0
Cyclopoidae	Cyclopidae	0	165	0	0
<i>Macrocylops</i> sp.	Cyclopidae	0	1	0	0
<i>Thermocyclops</i> sp.	Cyclopidae	0	0	450	400
Cyprididae sp. A	Cyprididae	0	0	30	0
Cyprididae sp. B	Cyprididae	0	0	0	920
<i>Cyzicus</i> sp. A	Cyzicidae	0	0	5	0
<i>Cyzicus</i> sp. B	Cyzicidae	3	0	0	0
<i>Ceriodaphnia rigaudi</i>	Daphniidae	0	0	357	0
<i>Daphnia obtuse</i>	Daphniidae	4	0	0	0
<i>Simocephalus serrulatus</i>	Daphniidae	0	0	682	437
<i>Metadiaptomus</i> sp. A	Diaptomidae	0	312	0	0
<i>Metadiaptomus</i> sp. B	Diaptomidae	0	0	45	0
<i>Thermodiaptomus mixtus</i>	Diaptomidae	960	2	0	0
<i>Tropodiaptomus</i> sp.	Diaptomidae	7	0	0	0
Dytiscidae sp. B larvae	Dytiscidae	0	0	0	2
Dytiscidae sp. A larvae	Dytiscidae	0	0	9	0
<i>Hydroglyphus</i> sp.	Dytiscidae	0	2	0	0
<i>Hyphydrus</i> sp. A larvae	Dytiscidae	0	0	3	0
<i>Laccophilus</i> sp. larvae	Dytiscidae	0	0	0	2

Appendix C1 continued

Name	Family	RP 1	RP 2	RP 3	RP 4
<i>Laccophilus</i> sp.	Dytiscidae	0	0	3	0
<i>Laccotrephes</i> sp.	Dytiscidae	4	0	0	0
Ephyridae larvae	Ephyridae	0	0	14	0
Gastropoda	Gastropoda	0	0	5	0
<i>Gerris swakopensis</i>	Gerridae	1	0	0	0
Hydrachnellae	Hydrachnidae	0	0	3	4
<i>Hydraena</i> sp.	Hydraenidae	0	0	1	0
<i>Berosus</i> sp. larvae	Hydrophilidae	0	0	1	0
<i>Enochrus</i> sp. larvae	Hydrophilidae	0	0	3	0
<i>Hydrophilus senegalensis</i>	Hydrophilidae	0	0	4	0
<i>Hydrophilus</i> sp. larvae	Hydrophilidae	0	0	22	0
<i>Hydroptila</i> sp.	Hydroptilidae	0	3	0	0
<i>Bradinopyga cornuta</i>	Libellulidae	0	0	0	10
<i>Pantala flavescens</i>	Libellulidae	0	0	1	0
<i>Gomphocythere</i> sp.	Limnocytheridae	0	0	4	0
Lumbriculidae	Lumbriculidae	420	8	7	16
<i>Mesovelia vittergera</i>	Mesoveliidae	0	1	0	0
<i>Moina micrura</i>	Moinidae	1720	15	40	4
Nematoda	Nematoda	13	0	0	0
<i>Barborophilus afzelii</i>	Nepidae	2	0	0	0
<i>Ranatra</i> sp.	Nepidae	2	0	0	1
<i>Canthydrus</i> sp.	Noteridae	0	0	0	1
<i>Anisops</i> sp. A	Notonectidae	24	1	68	285
<i>Anisops</i> sp. B	Notonectidae	0	0	5	0
<i>Nychia limpida</i>	Notonectidae	1	0	0	0
Ostracoda	Ostracoda	5	6	0	0
<i>Bulinus forskalii</i>	Planorbidae	2	1	0	13
<i>Plea</i> sp.	Pleidae	0	0	3	3
Sciomyzidae larvae	Sciomyzidae	0	1	0	0
<i>Diaphanosoma</i> sp.	Sididae	3	0	20	0
<i>Spercheus</i> sp. larvae	Spercheidae	0	0	3	0
Stratiomyidae pupa	Stratiomyidae	0	54	0	0
<i>Streptocephalus</i> sp.	Streptocephalidae	0	28	0	0
<i>Conosia</i> sp. pupa	Tipulidae	1	0	0	0

Appendix C2: Names, number of replicates (N) and mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($\pm$ SD) for both food sources and consumers before and after a drying event of the temporary pool inside Ndumo Game Reserve.

Components		Time	N	Mean $\delta^{13}\text{C}$ ($\pm$ SD)	Mean $\delta^{15}\text{N}$ ($\pm$ SD)
Small predatory invertebrates	Nepidae	Before	4	-26.66 ($\pm$ 2.19)	10.3 ($\pm$ 1.42)
		After	4	-28.87 ($\pm$ 1.18)	12.15 ($\pm$ 0.51)
	Libellulidae	Before	2	-25.66 ($\pm$ 0.05)	13.63 ($\pm$ 0.63)
		After	2	-29.36 ($\pm$ 0.4)	11.56 ($\pm$ 0.06)
	Coenagrionidae	After	3	-29.41 ($\pm$ 0.14)	12.73 ($\pm$ 0.04)
	Notonectidae	Before	4	-29.02 ($\pm$ 0.2)	12.43 ($\pm$ 0.3)
After		4	-30.04 ($\pm$ 0.02)	12.12 ($\pm$ 0.05)	
Large predatory invertebrates	Belostomatidae	Before	1	-23.02 ($\pm$ 0)	8.90 ($\pm$ 0)
		After	2	-28.39 ($\pm$ 0.49)	12.08 ($\pm$ 0.29)
	Dytiscidae	Before	1	-22.69 ($\pm$ 0)	7.27 ($\pm$ 0)
		After	4	-24.79 ($\pm$ 2.2)	11.85 ($\pm$ 1.58)
Plants	Nymphaeaceae	Before	4	-25.25 ($\pm$ 0.15)	12.63 ($\pm$ 0.29)
	Onogracea	After	4	-28.3 ($\pm$ 0.03)	11.16 ($\pm$ 0.03)
	Poaceae	After	4	-13.91 ($\pm$ 0.1)	10.15 ($\pm$ 0.02)
	Ceratophyllaceae	After	4	-27.94 ($\pm$ 0.14)	9.79 ($\pm$ 0.04)
	Trapaceae	After	4	-24.94 ($\pm$ 0.03)	8.69 ($\pm$ 0.02)
	Haloragaceae	After	4	-27.68 ($\pm$ 0.08)	8.82 ($\pm$ 0.44)
	Detritus	Before	4	-26.57 ($\pm$ 0.13)	5.05 ($\pm$ 0.05)
		After	4	-27.88 ($\pm$ 0.05)	10.61 ($\pm$ 0.02)
	Leaf litter	Before	4	-26.72 ($\pm$ 0.06)	7.94 ($\pm$ 0.17)
		After	4	-26.14 ($\pm$ 0.02)	9.37 ($\pm$ 0.03)
	Biofilm	Before	4	-25.78 ($\pm$ 0.02)	4.21 ($\pm$ 0.3)
		After	3	-28.49 ($\pm$ 0.08)	11.81 ($\pm$ 0.04)
Fish	<i>Nothobranchius orthonotus</i>	Before	5	-26.1 ($\pm$ 1.03)	12 ($\pm$ 1.09)
		After	4	-26.83 ($\pm$ 0.69)	14.46 ($\pm$ 0.4)
	<i>Oreochromis mossambicus</i>	After	2	-27($\pm$ 0.42)	13.28 ($\pm$ 0.13)

Appendix C3: Relative contribution of selected food sources obtained from SIAR analysis to the diet of *Nothobranchius orthonotus* before and after a drying event of the temporary pool inside Ndumo Game Reserve.

Food Source	Time	Minimum (5%)	Mode	Mean	Maximum (95%)
Small predatory invertebrates	Before	0.16	0.42	0.41	0.63
	After	0.04	0.28	0.3	0.54
Large predatory invertebrates	Before	0	0.05	0.17	0.41
	After	0	0.14	0.21	0.45
Plants	Before	0.17	0.43	0.42	0.65
	After	0.12	0.46	0.49	0.82

Appendix D

Appendix D1: Detailed list of the diversity and abundance of aquatic invertebrates collected in Nyamithi lake within Ndumo Game Reserve during sampling surveys in 2014, 2016 and 2017. FC- Filter collector, GC – Gathering collector.

Species Name	Family	Functional feeding group	Sep-14	Dec-14	May-16	Aug-16	Dec-16	Feb-17	May-17	Aug-17	Nov-17
<i>Anax</i> sp.	Aeshnidae	Predator	54	10	0	0	0	0	0	0	0
<i>Burnupia</i> sp	Ancylidae	Scraper	0	11	0	0	0	0	0	0	0
<i>Caridina nilotica</i>	Atyidae	FC	3	66	0	0	0	0	0	34	34
Baetidae	Baetidae	GC	0	0	0	0	0	6	2	1	0
<i>Cloeon</i> and <i>Procloeon</i> sp	Baetidae	GC	52	67	0	0	4	0	0	0	0
<i>Appasus</i>	Belostomatidae	Predator	22	118	0	0	1	33	10	184	16
<i>Lethocerus niloticus</i>	Belostomatidae	Predator	0	16	0	0	0	0	0	0	0
<i>Afrocaenis</i> sp.	Caenidae	GC	0	0	0	0	0	3	0	0	0
<i>Bezzia</i> sp (larvae)	Ceratopogonidae	Predator	1	12	0	0	0	0	2	0	2
<i>Bezzia</i> sp. (pupae)	Ceratopogonidae	None	0	0	1	0	0	0	0	1	1
Ceratopogonidae	Ceratopogonidae	Predator	0	0	0	0	0	0	0	8	0
Chironominae (larvae)	Chironomidae	GC	14	40	0	3	4	70	20	19	15
Chironominae (pupae)	Chironomidae	NONE	0	0	0	3	0	1	0	4	5
Tanypodinae	Chironomidae	Predator	0	896	0	0	0	0	0	67	0
<i>Ceragrion</i> sp.	Coenagrionidae	Predator	0	0	0	0	0	0	0	8	0
Coenagrionidae	Coenagrionidae	Predator	0	0	0	0	0	3	0	48	1
<i>Enallagma</i> sp.	Coenagrionidae	Predator	29	89	0	0	13	3	8	0	3
<i>Pseudagrion</i> sp A	Coenagrionidae	Predator	0	0	0	0	0	31	7	34	4
<i>Corbicula fluminalis</i>	Corbiculidae	Scraper	0	0	0	4	0	0	0	3	0
<i>Corbicula</i> sp.	Corbiculidae	Scraper	47	5	0	0	0	0	0	0	0
<i>Agraptocorixa</i> sp A	Corixidae	Predator	81	422	0	0	0	0	0	14	0
Corixidae larvae	Corixidae	Predator	0	0	0	0	0	0	0	0	0

Appendix D1 continued

Species Name	Family	Functional feeding group	Sep-14	Dec-14	May-16	Aug-16	Dec-16	Feb-17	May-17	Aug-17	Nov-17
<i>Micronecta</i> sp.	Corixidae	Predator	0	0	0	0	59	7	1	25	10
<i>Sigara</i> sp	Corixidae	Predator	0	0	78	464	50	0	0	2	15
Crambidae sp A (larvae)	Crambidae	Shredder	0	0	0	0	0	0	0	1	0
Crambidae sp B (larvae)	Crambidae	Shredder	1	0	0	0	0	0	0	0	0
<i>Aedes</i> sp	Culicidae	FC	0	0	0	161	0	0	0	0	0
<i>Anopheles</i> sp A	Culicidae	FC	0	4	0	0	0	0	0	0	0
<i>Anopheles</i> sp B	Culicidae	FC	12	0	0	1	0	0	0	0	0
<i>Culex</i> sp A	Culicidae	FC	0	0	0	0	1	1	0	1	0
<i>Culex</i> sp D	Culicidae	FC	0	0	0	1	0	0	0	0	0
<i>Malaya</i> sp.	Culicidae	FC	0	1	0	0	0	0	0	0	0
Curculionidae	Curculionidae	Shredder	0	0	0	0	0	0	0	0	1
<i>Neohydromus affinis</i>	Curculionidae	Shredder	0	0	0	0	0	0	0	1	0
<i>Pseudobagous longulus</i>	Curculionidae	Shredder	0	1	0	0	0	0	2	1	0
<i>Thermocyclops</i> sp.	Cyclopidae	FC	0	0	0	0	0	960	208	16	0
Cyprididae	Cyprididae	FC	0	0	1	0	77	3458	0	261	46
<i>Ceriodaphnia</i> sp.	Daphniidae	FC	0	0	0	0	0	0	0	0	0
Darwinulidae	Darwinulidae	FC	0	0	0	0	0	13	332	82	0
<i>Thermodiaptomus mixtus</i>	Diaptomidae	FC	0	0	0	0	0	0	1	0	0
Dolichopodidae sp A (pupa)	Dolichopodidae	NONE	0	0	0	1	0	0	0	0	0
Dolichopodidae sp B (pupa)	Dolichopodidae	NONE	0	0	0	1	0	0	0	0	0
<i>Hydaticus</i> sp	Dytiscidae	Predator	0	0	0	0	0	0	0	0	3
<i>Hydroglyphus</i>	Dytiscidae	Predator	0	0	0	1	2	0	0	8	1
<i>Copelatus</i> sp	Dytiscidae	Predator	0	0	0	0	0	1	0	1	0
<i>Cybister</i> sp A	Dytiscidae	Predator	0	0	0	0	0	0	0	0	4
<i>Cybister</i> sp B	Dytiscidae	Predator	0	0	0	0	0	2	0	0	0

Appendix D1 continued

Species Name	Family	Functional feeding group	Sep-14	Dec-14	May-16	Aug-16	Dec-16	Feb-17	May-17	Aug-17	Nov-17
<i>Hydrovatus</i> sp A	Dytiscidae	Predator	0	0	0	0	0	0	0	1	0
<i>Hyphydrus</i> sp A	Dytiscidae	Predator	0	0	0	0	0	0	107	169	34
<i>Hyphydrus</i> sp B	Dytiscidae	Predator	0	5	0	0	0	0	0	0	0
<i>Laccophilus</i> sp	Dytiscidae	Predator	0	2	0	0	0	0	0	11	3
<i>Laccotrephes</i>	Dytiscidae	Predator	0	0	0	0	0	0	0	3	0
<i>Brachydeutera</i> sp. (pupa)	Ephyridae	NONE	11	0	0	0	0	0	0	0	0
<i>Naboandelus africanus</i>	Gerridae	Predator	0	2	0	0	0	7	0	0	0
<i>Neogerris severini</i>	Gerridae	Predator	0	0	0	0	0	0	0	8	0
<i>Tenagogonus</i> sp.	Gerridae	Predator	0	0	0	0	0	4	0	0	0
<i>Ceratogomphus</i> sp.	Gomphidae	Predator	0	0	0	0	0	0	0	1	0
Gomphidae	Gomphidae	Predator	0	0	0	0	0	0	0	4	0
<i>Ictinogomphus ferox</i>	Gomphidae	Predator	0	1	0	0	0	0	1	2	2
<i>Paragomphus</i> sp.	Gomphidae	Predator	0	0	0	0	0	0	0	2	0
<i>Gyrinus</i> sp.	Gyrinidae	Predator	0	0	0	0	0	0	2	0	0
Harpacticoida	Harpacticoida	FC	0	0	20	810	184	0	12	1	0
Hirudinea	Hirudinea	Predator	1	0	0	0	0	0	0	0	0
Hydrachnellae	Hydrachnidae	Predator	3	26	0	0	0	0	0	0	0
Hydraenidae larvae	Hydraenidae	GC	0	0	0	0	0	0	0	1	0
<i>Parasthetops</i> sp.	Hydraenidae	GC	0	0	0	1	0	0	0	0	0
<i>Hydrochus</i> sp A	Hydrochidae	Shredder	0	0	0	0	0	0	0	1	0
<i>Berosus</i> (larvae)	Hydrophilidae	Predator	23	15	0	0	26	3	1	20	20
<i>Berosus</i> sp.	Hydrophilidae	GC	0	0	1	56	16	1	0	95	13
<i>Enochrus</i> larvae	Hydrophilidae	Predator	0	0	0	0	0	1	0	2	0
<i>Hydrophilus senegalensis</i>	Hydrophilidae	GC	2	11	0	0	0	0	12	47	1
<i>Laccobius</i> sp.	Hydrophilidae	Predator	0	0	0	0	0	0	0	0	2

Appendix D1 continued

Species Name	Family	Functional feeding group	Sep-14	Dec-14	May-16	Aug-16	Dec-16	Feb-17	May-17	Aug-17	Nov-17
<i>Brachythemis lacustris</i>	Libellulidae	Predator	4	5	0	0	0	0	0	0	0
<i>Brachythemis leucosticta</i>	Libellulidae	Predator	0	0	0	0	0	0	0	12	27
<i>Orthetrum</i> sp	Libellulidae	Predator	0	0	0	0	0	0	2	0	0
<i>Pantala flavescens</i>	Libellulidae	Predator	0	0	0	0	9	0	0	4	0
<i>Tetrathemis pollenii</i>	Libellulidae	Predator	0	0	0	0	0	0	0	4	0
<i>Trithemis</i> sp.	Libellulidae	Predator	0	0	0	0	0	0	2	0	0
Lumbriculidae	Lumbriculidae	GC	173	9	0	0	0	2	0	0	2
<i>Pirata</i> sp.	Lycosidae	Predator	0	0	0	0	1	2	1	6	0
<i>Lymnaea natalensis</i>	Lymnaeidae	Scraper	0	0	0	0	0	0	0	1	0
<i>Mesovelia vittergera</i>	Mesoveliidae	Predator	0	0	0	0	0	12	0	17	2
<i>Moina micrura</i>	Moinidae	FC	0	0	0	0	1	0	17	3	0
<i>Laccocoris</i> sp.	Naucoridae	Predator	0	0	0	0	4	0	0	92	13
<i>Macrocoris</i> sp.	Naucoridae	Predator	0	0	0	0	0	0	0	1	0
<i>Naucoris obscuratus</i>	Naucoridae	Predator	0	0	0	0	0	0	0	2	0
<i>Ranatra</i> sp.	Nepidae	Predator	0	5	0	0	0	0	0	7	1
<i>Canthydrus</i> sp	Noteridae	Predator	2	0	0	0	0	0	0	15	4
<i>Anisops</i> sp A	Notonectidae	Predator	16	46	0	0	0	2	0	8	21
<i>Anisops</i> sp C	Notonectidae	Predator	0	0	0	0	0	0	0	0	0
<i>Enithares</i> sp.	Notonectidae	Predator	0	128	0	0	0	0	0	0	0
<i>Nychia limpida</i>	Notonectidae	Predator	0	40	0	0	0	0	0	0	25
Orthocladinae	Orthocladinae	GC	10	0	0	0	0	0	0	0	0
Ostracoda	Ostracoda	FC	693	329	0	0	0	0	0	72	0
<i>Physa acuta</i>	Physidae	Scraper	314	0	0	0	0	0	0	93	0
<i>Bulinus africanus</i>	Planorbidae	Scraper	24	0	0	0	0	0	0	0	0

Appendix D1 continued

Species Name	Family	Functional feeding group	Sep-14	Dec-14	May-16	Aug-16	Dec-16	Feb-17	May-17	Aug-17	Nov-17
<i>Bulinus depressus</i>	Planorbidae	Scraper	0	0	0	0	0	0	0	0	1
<i>Bulinus natalensis</i>	Planorbidae	Scraper	148	1134	0	0	0	0	0	0	0
<i>Bulinus tropicus</i>	Planorbidae	Scraper	382	266	0	0	0	0	0	0	0
Planorbidae	Planorbidae	Scraper	0	0	0	0	0	0	0	1	0
<i>Allocnemis leucosticta</i>	Platynemididae	Predator	7	0	0	0	0	1	0	4	0
<i>Plea</i> sp.	Pleidae	Predator	0	0	0	0	0	0	0	1	3
Psychodidae larvae	Psychodidae	GC	0	0	0	0	0	0	0	1	0
Scirtidae larva	Scirtidae	GC	0	0	0	0	0	0	0	3	0
<i>Diaphanosoma</i> sp.	Sididae	FC	0	0	0	0	0	420	41	0	0
Tabanidae larvae	Tabanidae	Predator	0	0	0	0	0	0	0	4	0
<i>Tetragnatha</i> sp.	Tetragnathidae	Predator	0	0	0	0	0	3	0	2	0
<i>Cleopatra</i> sp.	Thiaridae	Scraper	17	0	0	0	0	0	3	0	0
<i>Tarebia granifera</i>	Thiaridae	Scraper	2277	321	0	0	0	0	0	0	0
Tipulidae larvae	Tipulidae	Predator	0	0	0	0	0	0	0	1	0

Appendix D2: Parametric unpaired t-tests with Welch's correction comparing all diversity indices of aquatic invertebrates collected at Nyamithi lake within Ndumo Game Reserve between the sampling years of 2014, 2016 and 2017.

Index	2014 vs 2016	2014 vs 2017	2016 vs 2017
Number of taxa	p = 0.01, t = 5.97	p = 0.73; t = 0.37	p = 0.07; t = 2.54
Number of individuals	p = 0.01; t = 7.94	p = 0.12; t = 2.16	p = 0.34; t = 1.08
Margalef's species richness	p = 0.03; t = 4.36	p = 0.40; t = 0.96	p = 0.07; t = 2.51
Pielou's evenness	p = 0.48; t = 0.8	p = 0.99; t = 0.02	p = 0.55; t = 0.64
Shannon-Wiener diversity	p = 0.14; t = 2	p = 0.93; t = 0.09	p = 0.17; t = 1.62
Simpson's diversity	p = 0.26; t = 1.35	p = 0.92; t = 0.12	p = 0.31; t = 1.14

Appendix D3: Names, abbreviations (in parentheses), number of replicates, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures ($\pm\text{SE}$) and trophic position of various food web components collected along Nyamithi lake inside Ndumo Game Reserve during the June 2010 and February 2017 sampling surveys.

Components	September 2016				February 2017			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic position	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Consumers								
<i>Brycinus imberi</i> (<i>B. imberi</i>)	10	-21.19 (± 0.53)	11.79 (± 0.43)	1.81	4	-19.39 (± 1.17)	14.01 (± 0.29)	2.83
<i>Clarias gariepinus</i> (<i>C. gariepinus</i>)	4	-21.07 (± 0.49)	11.01 (± 0.47)	1.58	4	-15.77 (± 0.22)	13.47 (± 0.18)	2.67
<i>Coptodon rendalli</i> (<i>C. rendalli</i>)	6	-18.2 (± 0.96)	12.06 (± 0.24)	1.89				
<i>Enteromius afrohamiltoni</i> (<i>E. afrohamiltoni</i>)					5	-17.9 (± 1.12)	14.99 (± 0.13)	3.12
<i>Enteromius paludinosus</i> (<i>E. paludinosus</i>)	8	-22.06 (± 0.59)	12.44 (± 0.38)	2				
<i>Enteromius toppini</i> (<i>E. toppini</i>)					1	-21.37 (± 0)	15.21 (± 0)	3.19
<i>Enteromius trimaculatus</i> (<i>E. trimaculatus</i>)					2	-17.26 (± 1.35)	14.03 (± 0.35)	2.84
<i>Glossogobius giuris</i> (<i>G. giuris</i>)	7	-23.79 (± 0.26)	13.12 (± 0.26)	2.2				
<i>Hydrocynus vittatus</i> (<i>H. vittatus</i>) (large)	20	-22.21 (± 0.12)	14.12 (± 0.16)	2.5				
<i>Hydrocynus vittatus</i> (small)	13	-23 (± 0.14)	14.75 (± 0.06)	2.69	10	-16.46 (± 0.16)	14.72 (± 0.18)	3.04
<i>Labeo rosae</i> (<i>L. rosae</i>)	3	-21.57 (± 1.74)	11.01 (± 1.04)	1.58	2	-22.72 (± 0.86)	13.13 (± 0.19)	2.57
<i>Oreochromis mossambicus</i> (<i>O. mossambicus</i>)	10	-23.87 (± 0.41)	10.65 (± 0.26)	1.47	21	-17.15 (± 0.12)	11.19 (± 0.1)	2
<i>Shilbe intermedius</i> (<i>S. intermedius</i>)	13	-23.95 (± 0.28)	12.98 (± 0.27)	2.16	5	-18.66 (± 0.97)	14.46 (± 0.3)	2.97
<i>Synodontis zambezensis</i> (<i>S. zambezensis</i>)	1	-24.93 (± 0)	14.98 (± 0)	2.76	5	-20.31 (± 0.51)	15.06 (± 0.22)	3.15
Invertebrates								
Atyidae	2	-21.09 (± 0.08)	10.84 (± 0.07)	1.53				
Belostomatidae	1	-21.67 (± 0)	9.7 (± 0)	1.19	2	-24.67 (± 0.09)	11.32 (± 0.38)	2.03
Coenagrionidae					1	-26 (± 0)	11.74 (± 0)	2.16
Corixidae					2	-17.15 (± 0.004)	10.09 (± 0.02)	1.67
Dytiscidae					1	-22.63 (± 0)	8.93 (± 0)	1.33

Appendix D3 continued

Components	September 2016				February 2017			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic position	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Invertebrates								
Hirudinea	1	-20.97 (± 0)	13.6 (± 0)	2.35				
Hydrophilidae	1	-24.53 (± 0)	9.62 (± 0)	1.17	2	-24.04 (± 0.48)	10.67 (± 0.43)	1.84
Libellulidae					3	-20.46 (± 1.16)	10.82 (± 0.42)	1.89
<i>Macrobrachium</i> sp.					2	-19.14 (± 0.87)	13.5 (± 0.25)	2.68
Naucoridae					2	-20.92 (± 1.09)	10.16 (± 0.88)	1.69
Nepidae					1	-23.37 (± 0.45)	11.56 (± 0.05)	2.11
Notonectidae	1	-21.43 (± 0)	11.39 (± 0)	1.69				
<i>Tarebia granifera</i> (<i>T. granifera</i>)	1	-12.84 (± 0.34)	9.1 (± 0.03)	1.01				
Tetragrathidae					1	-25.37 (± 0)	11.34 (± 0)	2.04
Primary Producers								
Biofilm 1	5	-13.57 (± 1.38)	9.06 (± 0.08)	1	3	-25.93 (± 0.02)	8.29 (± 0.01)	1.14
Biofilm 2					6	-17.36 (± 0.12)	7.6 (± 0.17)	0.93
Cyperaceaea					4	-12.35 (± 0.01)	5.63 (± 0.02)	0.35
Detritus					12	-25.54 (± 0.77)	5.01 (± 0.71)	0.16
Filamentous algae	4	-15.36 (± 0.52)	10.04 (± 0.26)	1.29				
Hydrocharitaceae					4	-15.98 (± 0.03)	6.53 (± 0.03)	0.62
Leaf litter					4	-26.63 (± 0.07)	10.66 (± 0.02)	1.84
Poaceae 1					8	-13.82 (± 0.14)	10.8 (± 1.43)	1.88
Poaceae 2					4	-26.27 (± 0.01)	2.62 (± 0.02)	0
Sediment	4	-21.7 (± 1.08)	6.25 (± 0.62)	0.17				
Trapaceae					8	-25.04 (± 0.12)	10.37 (± 0.87)	1.75

Appendix E

Appendix E1: Detailed list of aquatic invertebrates, abbreviations (in parentheses), and families collected during surveys along the Phongolo River within Ndumo Game Reserve (Site 1) and outside Ndumo Game Reserve (Sites 2 and 3).

Name	Family	May 2016		August 2016		September 2016			February 2017		
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
<i>Anax</i> sp.	Aeshnidae	1	9	0	2	4	4	0	6	1	0
<i>Burnupia</i> sp. (Burnu)	Ancylidae	7	4	0	0	0	0	0	14	0	4
<i>Caridina nilotica</i> (C. nilo)	Atyidae	127	680	12	330	12	217	234	51	56	395
<i>Acanthiops</i> sp. (Acanthiop)	Baetidae	18	6	0	0	0	0	0	0	0	0
Baetidae (Baetid)	Baetidae	0	51	14	22	2	7	15	0	5	13
<i>Baetis</i> sp.	Baetidae	12	8	0	10	0	0	0	0	0	0
<i>Cloeon</i> and <i>Procloeon</i> sp. (Clo&Pro)	Baetidae	0	2	0	3	5	0	0	0	1	160
<i>Demoulinia</i> sp. (Demoul)	Baetidae	9	0	0	2	0	0	0	0	0	0
<i>Appasus</i> sp. (Appas)	Belostomatidae	2	0	0	1	0	0	0	1	2	3
<i>Afrocaenis</i> sp. (Afroc)	Caenidae	2	2	0	1	0	0	0	0	0	1
Calanoida (Calano)	Calanoida	0	0	0	6	0	1	7	0	0	0
<i>Bezzia</i> sp. (Bezz)	Ceratopogonidae	0	0	2	0	0	0	0	0	0	0
<i>Dasyhelea</i> sp. pupa (Dasy pup)	Ceratopogonidae	0	0	1	0	0	0	0	0	0	0
Chironominae (Chiron)	Chironomidae	0	1	15	6	83	3	6	4	0	8
Tanypodinae larvae (Tany)	Chironomidae	3	3	0	0	0	0	0	0	0	0
Chydoridae (Chydo)	Chydoridae	0	0	0	0	27	0	0	0	0	0
<i>Ceriagrion</i> sp. (Ceriag)	Coenagrionidae	0	0	0	0	0	0	0	0	1	2
Coenagrionidae (Coenagrionid)	Coenagrionidae	0	0	0	0	0	0	0	0	4	17
<i>Enallagma</i> sp. (Enall)	Coenagrionidae	0	0	7	7	0	0	0	9	0	0
<i>Pseudagrion</i> sp. A (Pseuda spA)	Coenagrionidae	29	26	4	0	17	40	0	3	6	15
<i>Pseudagrion</i> sp B (Pseuda spB)	Coenagrionidae	0	0	0	0	0	0	12	0	4	0

Appendix E1 continued

Name	Family	May 2016		August 2016		September 2016			February 2017		
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
<i>Corbicula fluminalis</i> (C. flum)	Corbiculidae	0	0	1	0	0	0	0	0	0	0
<i>Corbicula</i> sp. (Corbic)	Corbiculidae	13	0	0	0	10	0	1	0	0	0
<i>Micronecta</i> sp. (Micron)	Corixidae	1	1	1	0	1	0	0	0	0	0
<i>Sigara</i> sp. (Sig)	Corixidae	0	0	1	0	0	0	0	0	0	0
Crambidae sp. A larva (Cramb spA)	Crambidae	0	0	0	0	1	1	0	17	3	2
Crambidae sp. B larva (Carmb spB)	Crambidae	0	6	0	3	0	0	1	0	0	0
<i>Anopheles</i> sp. A (Anoph spA)	Culicidae	0	0	1	0	0	0	0	0	0	0
<i>Anopheles</i> sp. B (Anoph spB)	Culicidae	0	0	0	1	0	0	0	0	0	0
<i>Culex</i> sp. A (Cul spA)	Culicidae	0	0	2	1	12	0	0	0	0	0
<i>Culex</i> sp. B (Cul spB)	Culicidae	0	0	2	0	0	0	0	0	0	0
<i>Culex</i> sp. C (Cul spC)	Culicidae	0	1	0	0	0	0	0	0	0	0
<i>Mansonia</i> sp. (Mans)	Culicidae	0	0	0	0	1	0	0	0	0	0
Curculionidae larvae (Curcu larv)	Curculionidae	0	6	79	120	0	0	0	0	3	0
<i>Cyrtobagous salviniae</i> (C. salv)	Curculionidae	0	0	6	0	0	1	0	1	0	0
<i>Neochetina</i> sp. (Neochet)	Curculionidae	0	3	4	0	0	0	0	1	0	0
<i>Neohydromus affinis</i> (N. aff)	Curculionidae	0	2	19	5	0	0	0	1	0	0
<i>Neohydronomus pulchellus</i> (N. pulch)	Curculionidae	0	0	0	0	0	0	1	0	0	0
<i>Stenopelmus rufinasus</i> (S. rufi)	Curculionidae	0	0	0	0	0	0	0	3	0	0
Cyclopoidae (Cyclop)	Cyclopidae	0	0	0	1	0	0	0	0	0	0
<i>Thermocyclops</i> sp. (Thermo)	Cyclopidae	0	0	0	0	47	0	0	0	1	40
Cyprididae (Cyprid)	Cyprididae	0	0	0	0	7	0	0	0	0	0
Cytheroidea (Cyther)	Cytheroidea	0	0	0	0	0	0	0	1	0	0
<i>Ceriodaphnia</i> sp. (Ceriod)	Daphniidae	0	0	30	1	0	0	0	0	0	0

Appendix E1 continued

Name	Family	May 2016		August 2016		September 2016			February 2017		
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
<i>Simocephalus serrulatus</i> (S. serru)	Daphniidae	0	0	0	0	11	0	0	0	0	0
Darwinulidae (Darwin)	Darwinulidae	0	0	0	0	7	0	0	3	1	0
<i>Thermodiaptomus mixtus</i> (T. mix)	Diaptomidae	2	24	0	0	0	0	0	0	0	0
<i>Hydroglyphus</i> sp. (Hydrogl)	Dytiscidae	0	0	1	0	0	0	0	0	0	0
<i>Cybister</i> sp. (Cyb sp.)	Dytiscidae	0	0	0	0	4	0	0	1	0	0
Dytiscidae larvae (Dytis larv)	Dytiscidae	0	0	0	0	1	0	0	0	0	0
<i>Hyphydrus</i> sp. larva (Hyph larv)	Dytiscidae	0	0	1	0	0	0	0	0	0	0
<i>Laccophilus</i> sp. (Laccoph)	Dytiscidae	3	0	1	0	0	0	0	2	0	0
Elminae larva (Elmin larv)	Elmidae	0	6	0	0	1	0	4	2	0	8
<i>Larainae</i> sp. larva (Larain larv)	Elmidae	0	0	0	0	0	0	2	0	0	1
<i>Brachydeutera</i> sp. pupa (Brachyd pup)	Ephydriidae	0	0	0	0	0	0	0	5	0	0
Ephydriidae larvae (Ephyd larv)	Ephydriidae	0	0	0	0	0	0	0	1	1	1
<i>Eurymetra natalensis</i> (E. natal)	Gerridae	0	0	2	0	0	0	0	0	0	0
<i>Naboandelus africanus</i> (N. afric)	Gerridae	2	0	0	0	0	0	0	0	0	0
<i>Tenagogonus</i> sp. (Tenago)	Gerridae	2	0	0	0	0	0	0	0	0	0
<i>Ceratogomphus</i> sp. (Ceratog)	Gomphidae	0	0	0	0	0	0	3	0	0	0
<i>Microgomphus mozambicensis</i> (M. mozam)	Gomphidae	0	6	0	1	1	6	0	0	0	0
<i>Onychogomphus</i> sp. (Onycho)	Gomphidae	2	0	0	0	0	0	0	0	0	0
<i>Aulonogyrus</i> sp. (Aulon)	Gyrinidae	0	0	0	0	2	0	0	0	0	0
<i>Orectogyrus</i> sp. (Orecto)	Gyrinidae	23	0	8	3	0	0	0	0	0	0
<i>Peltodytes</i> sp. (Pelt)	Haliplidae	0	1	0	0	0	0	0	1	0	0
Harpacticoida (Harpac)	Harpacticoida	3	0	0	0	0	0	0	0	0	0

Appendix E1 continued

Name	Family	May 2016		August 2016		September 2016			February 2017		
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
<i>Afronurus</i> sp. (Afronu)	Heptageniidae	2	2	1	0	0	0	0	0	1	3
Hirudinae (Hirud)	Hirudinea	0	0	0	0	0	0	0	3	0	0
Hydrachnellae (Hydrach)	Hydrachnidae	0	0	0	0	1	0	0	0	0	0
<i>Hydrochus</i> sp. (Hydroc)	Hydrochidae	0	0	1	0	0	0	0	0	0	0
<i>Allocotocerus</i> sp. (Alloc)	Hydrophilidae	0	0	5	0	0	0	0	0	0	0
<i>Amphiops</i> sp. (Amphi)	Hydrophilidae	0	0	0	0	0	0	0	2	0	0
<i>Berosus</i> sp. larvae (Bero larv)	Hydrophilidae	0	1	0	0	0	0	0	0	0	0
<i>Enochrus</i> sp. larvae (Enoch larv)	Hydrophilidae	0	1	0	0	4	0	1	6	0	0
<i>Hydrophilus</i> sp. larvae (Hydroph larv)	Hydrophilidae	0	0	0	1	0	0	0	0	0	0
<i>Laccobius</i> sp. (Laccob)	Hydrophilidae	0	1	0	2	7	0	0	2	0	0
<i>Hydropsyche</i> sp. (Hydrops)	Hydropsychidae	0	0	0	0	0	1	0	0	0	0
<i>Hydroptila</i> sp. (Hydrop)	Hydroptilidae	2	57	0	14	0	8	1	0	2	2
Lampyridae sp. A larva (Lampy spA)	Lampyridae	0	0	0	0	0	0	0	3	0	0
Lampyridae sp. B larva (Lampy spB)	Lampyridae	1	0	0	0	0	1	0	0	0	1
<i>Brachythemis leucosticta</i> (B. leucost)	Libellulidae	0	2	0	0	0	0	0	0	0	0
<i>Bradinopyga cornuta</i> (B. corn)	Libellulidae	0	0	0	0	1	2	0	0	0	2
<i>Diplacodes lefebvrii</i> (D. lefeb)	Libellulidae	0	11	5	4	0	0	0	0	0	0
<i>Diplacodes luminalus</i> (D lumin)	Libellulidae	0	0	0	0	0	0	4	0	0	0
<i>Diplacodes</i> sp. (Diplac)	Libellulidae	0	0	0	0	0	0	0	6	0	4
<i>Olpogastra</i> sp.(Olpo)	Libellulidae	0	0	0	0	1	0	1	0	0	0
<i>Orthetrum</i> sp. (Orthet)	Libellulidae	2	0	0	2	3	0	0	0	0	0
<i>Tetrathemis polleni</i> (T. poll)	Libellulidae	0	0	0	0	17	0	0	0	0	0

Appendix E1 continued

Name	Family	May 2016		August 2016		September 2016			February 2017		
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
<i>Trithemis</i> sp. (Trith)	Libellulidae	0	0	0	0	0	0	1	0	2	0
<i>Gomphocythere</i> sp. (Gompho)	Limnocytheridae	0	0	0	0	0	0	0	1	0	0
Lumbriculidae (Lumbric)	Lumbriculidae	15	18	28	17	1	2	0	3	1	0
<i>Pirata</i> sp. (Pira)	Lycosidae	9	3	3	3	0	0	0	0	0	0
<i>Lymnaea natalensis</i> (L. natal)	Lymnaeidae	6	9	5	3	3	2	1	36	0	1
<i>Macrothrix spinose</i> (M. spin)	Macrothricidae	0	6	0	0	0	0	0	0	0	0
<i>Mesovelgia vittergera</i> (M. vitt)	Mesoveliidae	0	0	1	0	0	0	0	0	0	0
Naididae (Naid)	Naididae	0	0	0	0	1	1	0	0	0	0
<i>Laccocoris</i> sp. (Laccoc)	Naucoridae	0	0	0	0	0	3	0	0	1	0
<i>Macrocoris</i> (Macro)	Naucoridae	0	0	1	0	0	0	0	0	0	0
<i>Naucoris obscuratus</i> (N. obsc)	Naucoridae	0	1	0	0	0	0	1	7	0	2
<i>Ranatra</i> sp. (Rana)	Nepidae	0	0	0	0	1	0	1	0	0	0
<i>Canthydrus</i> sp. (Canth)	Noteridae	0	1	0	0	0	0	0	2	0	0
<i>Hydrocanthus</i> sp. (Hydrocan)	Noteridae	0	1	0	0	0	0	0	0	0	0
<i>Enithares</i> sp. (Enith)	Notonectidae	0	0	0	1	0	0	0	0	0	0
<i>Anisops</i> sp. (Anis)	Notonectidae	0	1	0	0	14	1	0	0	3	0
<i>Notonecta lactitans</i> (N. lacti)	Notonectidae	1	0	0	0	0	0	0	0	0	0
<i>Nychia limpida</i> (N. limp)	Notonectidae	1	0	4	2	0	0	0	0	0	0
<i>Elassoneuria</i> sp. (Elasson)	Oligoneuriidae	0	0	0	0	0	0	0	0	0	1
Ostracoda (Ostrac)	Ostracoda	0	6	1	4	0	1	1	0	0	0
<i>Chimarra</i> sp. (Chim)	Philopotamidae	0	22	0	0	0	0	0	0	0	1
<i>Physa acuta</i> (P. acuta)	Physidae	12	22	3	10	11	13	10	196	13	17
<i>Biomphalaria pfeifferi</i> (B. pfei)	Planorbidae	0	0	0	0	0	0	0	3	0	0

Appendix E1 continued

Name	Family	May 2016		August 2016		September 2016			February 2017		
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
<i>Gyraulus costulatus</i> (G. cost)	Planorbidae	0	0	0	0	2	0	0	0	0	0
Planorbidae (Planorb)	Planorbidae	2	0	0	0	0	0	0	0	0	0
<i>Allocnemis leucisticta</i> (A.leuc)	Platycnemididae	1	0	5	0	0	0	0	0	0	0
<i>Plea</i> sp.	Pleidae	0	0	0	2	1	0	0	0	0	0
Sciomyzidae larvae (Sciom larv)	Sciomyzidae	0	0	0	0	0	4	0	0	0	0
Scirtidae (Scirt)	Scirtidae	0	0	0	0	0	1	0	0	0	0
<i>Simulium</i> sp. larva (Simul larv)	Simulidae	0	8	0	0	0	0	0	0	0	0
<i>Sphaerium incomitatum</i> (S. incom)	Sphaeridae	0	2	3	0	0	0	0	5	0	9
Staphylindae larvae (Staph larv)	Staphylinidae	1	0	0	0	0	0	0	0	0	0
Stratiomyidae larva (Stratio)	Stratiomyidae	0	1	2	0	0	0	0	0	0	0
Syrphidae larva (Syrph)	Syrphidae	0	0	0	0	1	0	0	0	0	0
Tabanidae larvae (Taban)	Tabanidae	0	0	0	0	0	0	0	0	1	0
<i>Tetragnatha</i> sp. (Tetrag)	Tetragnathidae	1	0	0	0	0	0	0	0	0	0
<i>Melanoides tuberculata</i> (M. tuber)	Thiaridae	21	62	0	0	0	0	0	0	12	0
<i>Tarebia granifera</i> (T. gran)	Thiaridae	126	60	0	0	0	15	0	0	6	20
<i>Neomacrocoris</i> sp. (Neomacr)	Veliidae	0	0	1	0	0	0	0	0	0	0
<i>Rhagovelia</i> sp. (Rhago)	Veliidae	14	0	1	0	0	0	0	0	0	0

Appendix E2: Aquatic invertebrate family diversity and sensitivity scores collected during surveys along the Phongolo River in 2012 – 2013 by Smit et al (2016), in 2014 by de Necker (2015) and in 2016 – 2017 during the present study. Sensitivity scores determined by Dickens and Graham (2002). * denotes that there is no known sensitivity score.

Family	Sensitivity score	Smit et al. 2016	de Necker 2015	Present study
Aeshnidae (Hawkers and Emperors)	8	x	x	x
Ampullariidae (Apple snails)	*		x	
Ancylidae (Limpets)	6	x	x	x
Athericidae (Snipe flies)	10	x		
Atyidae (Shrimps)	8	x	x	x
Baetidae (>2 sp.) (Mayflies)	12	x	x	x
Belostomatidae (Giant water bugs)	3	x	x	x
Caenidae (Squaregills)	6	x	x	x
Calanoida	*			x
Ceratopogonidae (Biting midges)	5	x	x	x
Chironomidae (Midges)	2	x	x	x
Chlorocyphidae (Jewels)	10	x		
Chydoridae	*			x
Coenagrionidae (Sprites and blues)	4	x	x	x
Corbiculidae (Clams)	5	x		x
Cordulidae (Cruisers)	8	x		
Corixidae (Water boatmen)	3	x	x	x
Crambidae (aquatic caterpillars)	12	x	x	x
Culicidae (Mosquitoes)	1	x	x	x
Curculionidae (weevils)	*		x	x
Daphniidae (water fleas)	*		x	x
Diaptomidae (micro-crustacea)	*			x
Dolichopodidae (Long legged flies)	*		x	
Dytiscidae (Predacious diving beetles)	5	x	x	x
Ecnomidae (Caddisflies)	8	x		
Elmidae/Dryopidae (Riffle beetles)	8	x		x
Ephemeridae (Mayflies)	15	x		
Ephydriidae (shore flies/brine flies)	3		x	x
Gerridae (Water striders)	5	x	x	x
Gomphidae (Clubtails)	6	x	x	x
Gyrinidae (Whirligig beetles)	5	x		x
Haliplidae (Crawling water beetles)	5			x
Harpacticoida	*			x
Heptageniidae (Flathead mayflies)	13	x		x
Hirudinea (Leeches)	3	x	x	x
Hydracarina (Water mites)	8	x	x	x

Appendix E2 continued

Family	Sensitivity score	Smit et al. 2016	de Necker 2015	Present study
Hydraenidae (Minute moss beetles)	8		x	
Hydrochidae (Elongated water scavenger beetles)	*		x	x
Hydrometridae (Water measurers)	6	x		
Hydrophilidae (Water scavenger beetles)	5	x	x	x
Hydropsychidae (1 sp.) (Caddisflies)	4			x
Hydropsychidae (>2 sp.) (Caddisflies)	12	x		
Hydroptilidae (Cased caddisflies)	6	x		x
Lampyridae	*		x	x
Leptoceridae (Cased caddisflies)	6	x		
Leptophlebiidae (Prongills)	9	x		
Lestidae (Emerald damselflies/Spreadwings)	8	x		
Libellulidae (Darters/Skimmers)	4	x	x	x
Limnichidae (Marsh-loving beetles)	10		x	
Limnocytheridae	*			x
Lumbriculidae (Oligochaeta)	1	x	x	x
Lycosidae (Wolf spiders)	*			x
Lymnaeidae (Pond snails)	3		x	x
Macrothricidae	*			x
Mesoveliidae (Ripple bugs)	*		x	x
Moinidae	*			x
Muscidae (House/Stable flies)	1		x	
Naididae (Nematodes)	*			x
Naucoridae (Creeping water bugs)	7	x	x	x
Nepidae (Water scorpions)	3		x	x
Neritidae (Snail)	*		x	
Noteridae (Diving beetles)	5		x	x
Notonectidae (Backswimmers)	3	x	x	x
Oligoneuriidae (Brushlegged mayflies)	15	x		x
Ostracoda	*		x	x
Philopotamidae (Caddisflies)	10	x		x
Physidae (Pouch snails)	3	x		x
Planorbidae (Orb snails)	3	x	x	x
Platycnemididae (Stream Damselflies)	10		x	x
Pleidae (Pygmy backswimmers)	4	x	x	x
Potamonautidae (Crabs)	3	x		
Psychomyiidae (Moth flies)	1	x		
Sciomyzidae	*			x
Scirtidae (Marsh beetles)	*		x	x

Appendix E2 continued

Family	Sensitivity score	Smit et al. 2016	de Necker 2015	Present study
Sididae	*			X
Simuliidae (Blackflies)	5	X		X
Spercheidae	*		X	
Sphaeriidae (Pill clams)	3	X		X
Staphylinidae (Rove beetles)	*			X
Stratiomyidae (Soldier flies)	*		X	X
Synlestidae (Sylphs)	8	X		
Syrphidae (Rat tailed maggots)	1			X
Tabanidae (Horse flies)	5	X	X	X
Teloganodidae (Spiny crawlers)	12		X	
Tetragnathidae (water associated spiders)	*			X
Thiaridae (Snails)	3	X	X	X
Tricorythidae (Stout crawlers)	9	X		
Triopsidae (Tadpole shrimp)	*		X	
Turbellaria (Flatworms)	3	X		
Veliidae (Ripple bugs)	5	X		X
Viviparidae (Snails)	5		X	
Total		52	49	66

Appendix E3: Names, abbreviations (in parentheses), number of replicates, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures ($\pm\text{SE}$) and trophic position of various food web components collected along the Phongolo River at Site 1 inside Ndumo Game Reserve during the September 2016 and February 2017 sampling surveys.

Components	September 2016				February 2017			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic position	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Consumers								
<i>Brycinus imberi</i> (B. imb)	5	-22.51 (± 0.23)	12.24 (± 0.43)	3.34	7	-23.54 (± 0.99)	12.69 (± 0.28)	3.2
<i>Clarias gariepinus</i> (C. gariep)	*				2	-25.18 (± 0.27)	12.90 (± 0.31)	3.26
<i>Enteromius afrohamiltoni</i> (E. afro)	1	-24.83 (± 0)	12.03 (± 0)	3.28				
<i>Coptodon rendalii</i> (C. rend)	*				2	-22.78 (± 0.35)	12.39 (± 0.35)	3.11
<i>Glossogobius giuris</i> (G. giur)	4	-24.86 (± 1.1)	11.41 (± 0.78)	3.1	1	-25.13 (± 0)	12.67 (± 0)	3.19
<i>Hydrocynus vittatus</i> (H. vitt)	1	-26.00 (± 0)	12.99 (± 0)	3.57	*	*	*	*
<i>Oreochromis mossambicus</i> (O. moss)	5	-28.23 (± 0.48)	7.17 (± 0.11)	1.84	5	-22.88 (± 1.64)	12.93 (± 0.57)	3.27
<i>Petrocephalus wesselsi</i> (P. wess)	8	-24.01 (± 0.46)	13.72 (± 0.19)	3.78	1	-24.03 (± 0)	13.93 (± 0)	3.56
<i>Schilbe intermedius</i> (S. inter)	1	-24.57 (± 0)	13.12 (± 0)	3.6	2	-20.81 (± 0.42)	13.14 (± 0.08)	3.33
<i>Synodontis zambezensis</i> (S. zam)	8	-24.49 (± 0.65)	13.09 (± 0.22)	3.6	4	-23.65 (± 0.15)	13.40 (± 0.48)	3.4
<i>Tilapia sparmanii</i> (T. spar)	4	-22.2 (± 0.71)	12.81 (± 0.83)	3.51	1	-21.74 (± 0)	12.74 (± 0)	3.21
Invertebrates								
Aeshnidae (Aesh)	4	-20.67 (± 0.34)	8.51 (± 0.42)	2.23	2	-24.36 (± 0.52)	9.50 (± 0)	2.25
Atyidae (Atyid)	6	-20.63 (± 0.26)	7.24 (± 0.15)	1.86	4	-22.09 (± 0.78)	9.53 (± 0.29)	2.26
Belostomatidae (Belosto)	1	-25.19 (± 0)	5.44 (± 0)	1.32	5	-25.92 (± 0.24)	9.10 (± 0.18)	2.13
<i>Cherax quadricarinatus</i> (C. quad)					3	-22.5 (± 0.43)	10.57 (± 0.14)	2.57
Coenagrionidae (Coena)	4	-27.78 (± 0.63)	7.11 (± 0.97)	1.82	2	-23.57 (± 2.59)	9.64 (± 0.2)	2.29
Corbiculidae (Corbic)					1	-23.79 (± 0)	7.88 (± 0)	1.77
Diptera	1	-36.97 (± 0)	4.52 (± 0)	1.05				
Dytiscidae (Dytis)	5	-25.19 (± 1.17)	7.71 (± 0.38)	2	1	-24.4 (± 0)	7.05 (± 0)	1.52
Gyrinidae (Gyrin)					4	-27.75 (± 0.48)	9.71 (± 0.23)	2.31

Appendix E3 continued

Components	September 2016				February 2017			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic position	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Invertebrates								
Hydrophilidae (Hydrophil)	3	-21.79 (± 0.61)	4.61 (± 0.32)	1.08	1	-25.95 (± 0)	7.93 (± 0)	1.78
Hydrophilidae larvae (Hydrophil larv)					1	-23.32 (± 0)	6.89 (± 0)	1.48
Lampyridae (Lampyr)	2	-21.65 (± 1.08)	7.21 (± 0.08)	1.85	1	-24.22 (± 0)	8.26 (± 0)	1.88
Libellulidae (Libell)	6	-22.38 (± 0.48)	7.34 (± 0.21)	1.89	3	-23.88 (± 1.4)	8.24 (± 0.36)	1.87
Lycosidae (Lycos)	4	-23.65 (± 1)	9.28 (± 0.3)	2.46	5	-25.96 (± 0.56)	10.70 (± 0.29)	2.6
<i>Macrobrachium</i> sp. (<i>Macro</i> sp.)	10	-23.63 (± 0.63)	12.22 (± 0.32)	3.34	5	-23.42 (± 0.63)	12.22 (± 0.21)	3.06
Naucoridae (Naucor)	1	-20.89 (± 0)	8.2 (± 0)	2.14				
Nepidae (Nepid)	1	-26.03 (± 0)	7.97 (± 0)	2.08	1	-23.80 (± 0)	8.48 (± 0)	1.94
Noteridae (Noter)	1	-24.96 (± 0)	3.34 (± 0)	0.7				
Physidae (Physid)	2	-20.44 (± 0.78)	3.72 (± 0.02)	1.27	2	-25.11 (± 0.57)	6.61 (± 0.54)	1.48
Tabanidae (Taban)	1	-19.15 (± 0)	6.96 (± 0)	1.86				
<i>Tarebia granifera</i> (<i>T. gran</i>)	1	-22.77 (± 0)	3.61 (± 0)	1.78	4	-22.54 (± 0.71)	6.48 (± 0.62)	1.96
Tadpoles					4	-24.63 (± 0.51)	7.26 (± 0.41)	1.35
Primary producers								
<i>Azolla filiculoides</i> (<i>A. fili</i>)	4	-27.29 (± 0.02)	3.14 (± 0.11)	0.64				
Biofilm	5	-23.11 (± 0.05)	5.08 (± 0.13)	1.21	3	-22.80 (± 0.02)	7.60 (± 0.29)	1.68
Ceratophyllaceae (Ceratophyll)	4	-19.93 (± 0.05)	5.79 (± 0.05)	1.43	4	-22.68 (± 0.02)	8.85 (± 0.01)	2.06
Detritus	4	-28.03 (± 0.02)	4.35 (± 0.03)	1	4	-28.72 (± 0.02)	5.29 (± 0.03)	1
<i>Eichhornia crassipes</i> (<i>Eich</i>)	4	-27.21 (± 0.04)	7.78 (± 0.02)	2.02				
Leaf litter	4	-29.19 (± 0.03)	4.53 (± 0.03)	1.06	4	-27.66 (± 0.02)	5.10 (± 0.02)	0.94
Onogracea (Onogra)	4	-28.61 (± 0.04)	7.45 (± 0.01)	1.92				
Poaceae	4	-13.13 (± 0.11)	5.32 (± 0.09)	1.29				
Polygonaceae (Polygon)					4	-29.05 (± 0.01)	9.32 (± 0.02)	2.2
Zooplankton (Zooplank)	4	-27.45 (± 0.09)	4.78 (± 0.13)	1.13				

Appendix E4: Names, abbreviations (in parentheses), number of replicates, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures ($\pm\text{SE}$) and trophic position of various food web components collected along the Phongolo River at Site 2 outside Ndumo Game Reserve during the May 2016, September 2016 and February 2017 sampling surveys.

Components	May 2016				September 2016			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic position	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Consumers								
<i>Acanthopagrus berda</i>	2	-21.7 (± 0.17)	14.52 (± 0.03)	3.29				
<i>Brycinus imberi</i>					4	-34.7 (± 0.04)	14.71 (± 0.05)	3.85
<i>Clarias gariepinus</i>	6	-29.4 (± 1.37)	13.66 (± 0.25)	3.04				
<i>Coptodon rendalii</i>	4	-20.9 (± 2.52)	12.75 (± 0.28)	2.77	2	-22.57 (± 0.03)	14.05 (± 0.14)	3.65
<i>Enteromius</i> sp. (<i>Entero</i> sp.)					2	-32.57 (± 1.37)	13.69 (± 0.64)	3.54
<i>Enteromius afrohamiltoni</i> (<i>E. afro</i>)	2	-29.53 (± 1.28)	13.54 (± 0.7)	3				
<i>Glossogobius giuris</i>	3	-22.27 (± 0.14)	14.45 (± 0.65)	3.27	3	-21 (± 0.08)	13.31 (± 0.49)	3.43
<i>Labeo cylindricus</i>	8	-28.51 (± 2.2)	11.92 (± 0.75)	2.52	4	-18.6 (± 0.6)	13.36 (± 1.21)	3.44
<i>Labeo marequensis</i>	4	-21.27 (± 0.46)	13.89 (± 0.42)	3.11				
<i>Micralestes acutidens</i>					2	-21.9 (± 0)	14.76 (± 0.18)	3.86
<i>Oreochromis mossambicus</i>	7	-28.95 (± 1.51)	12.82 (± 0.54)	2.79	3	-23.92 (± 0.98)	11.72 (± 1.22)	2.96
<i>Pseudocrenilabrus philander</i>	12	-19.73 (± 0.95)	11.28 (± 0.54)	2.33	8	-22.77 (± 0.4)	13.51 (± 0.94)	3.49
<i>Tilapia sparmanii</i>	8	-21.65 (± 0.19)	11.76 (± 0.7)	2.47	3	-22.56 (± 0.25)	12.87 (± 0.59)	3.3
<i>Xenopus muelleri</i> (<i>X. muell</i>)	4	-22.42 (± 0.69)	12.11 (± 0.54)	2.58	1	-22.02 (± 0)	10.34 (± 0)	2.55
Invertebrates								
Aeshnidae	3	-22.53 (± 2.32)	9.29 (± 0.31)	1.74				
Atyidae					7	-20.85 (± 0.10)	10.5 (± 0.07)	2.6
Belostomatidae	2	-25.2 (± 0.5)	9.46 (± 0.11)	1.79				
<i>Cherax quadricarinatus</i>	7	-19.73 (± 0.29)	9.98 (± 0.42)	1.94	11	-22.43 (± 0.29)	12.04 (± 0.37)	3.05
Coenagrionidae	1	-22.7 (± 0)	9.48 (± 0)	1.8	2	-22.44 (± 0.38)	10.96 (± 0.16)	2.73
Dytiscidae	4	-23.83 (± 1.02)	6.75 (± 0.41)	0.99	1	-18.14 (± 0)	6.78 (± 0)	1.49
Gomphidae	1	-23.07 (± 0)	11.14 (± 0)	2.29				

Appendix E4 continued

Components	May 2016				September 2016			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic position	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Invertebrates								
Hydrophilidae	4	-23.17 (± 0.84)	4.68 (± 0.68)	0.37				
Libellulidae	3	-25.8 (± 1.82)	6.69 (± 0.45)	0.97	3	-22.84 (± 0.55)	11.34 (± 0.36)	2.85
Lycosidae	2	-23.04 (± 1.52)	9.03 (± 0.57)	1.66				
Nepidae	2	-21.75 (± 1.07)	7.12 (± 1.33)	1.1				
Noteridae	2	-26.29 (± 0.09)	3.93 (± 0.38)	0.15				
Notonectidae (Notonec)	1	-22.6 (± 0)	7.80 (± 0)	1.3				
Physidae					1	-20.44 (± 0)	12.49 (± 0)	3.19
Tadpoles	1	-27.13 (± 0)	3.14 (± 0)	0				
<i>Tarebia granifera</i>					5	-22 (± 0.32)	9.27 (± 0.5)	2.23
Primary producers								
Araceae	4	-27.72 (± 0.01)	6.88 (± 0.01)	1.02				
<i>Azolla filiculoides</i>	4	-27.56 (± 0.03)	1.1 (± 0.1)	0				
Biofilm	4	-24.92 (± 0.6)	8.61 (± 0.09)	1.54	2	-17.15 (± 0.04)	8.87 (± 0.01)	2.11
Ceratophyllaceae					4	-20.4 (± 0.03)	10.05 (± 0.01)	2.46
Detritus	4	-20.44 (± 0.18)	6.8 (± 0.04)	1	4	-28 (± 0.1)	5.12 (± 0.05)	1
<i>Eichhornia crassipes</i>	4	-28.26 (± 0.02)	7.94 (± 0.01)	1.34	4	-26.28 (± 0.03)	7.99 (± 0.04)	1.85
Filamentous algae	4	-24.96 (± 0.28)	10.24 (± 0.11)	2.02				
Leaf litter	4	-28.06 (± 0.04)	5.22 (± 0.12)	0.53	4	-28.3 (± 0.04)	3.97 (± 0.06)	0.66
<i>Marsilea ephippiocarpa</i> (<i>Marsilea</i>)	4	-27.02 (± 0.02)	6.27 (± 0.07)	0.84	4	-25.37 (± 0.03)	7.82 (± 0.02)	1.8
Onograceae	4	-27.19 (± 0.01)	7.43 (± 0.12)	1.19	4	-26.36 (± 0.02)	9.3 (± 0.01)	2.24
Poaceae	4	-12.78(± 0.12)	8.24 (± 0.26)	1.43				
Potamogetonaceae					4	-20.16 (± 0.01)	9.3 (± 0.02)	2.24
Trapaceae	4	-25.94 (± 0.04)	4.42 (± 0.02)	0.29	4	-25.95 (± 0.01)	8.83 (± 0.03)	2.1

Appendix E4 continued

Components	February 2017			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Consumers				
<i>Clarias gariepinus</i>	1	-21.23 (± 0)	11.22 (± 0)	2.23
<i>Coptodon rendalii</i>	6	-22.95 (± 0.5)	12.50 (± 0.36)	2.61
<i>Glossogobius giuris</i>	1	-23.22 (± 0)	16.96 (± 0)	3.94
<i>Oreochromis mossambicus</i>	3	-22.11 (± 0.47)	11.21 (± 0.91)	2.23
<i>Pseudocrenilabrus philander</i>	4	-22.22 (± 0.39)	12.94 (± 0.33)	2.74
Invertebrates				
Aeshnidae	1	-21.27 (± 0)	9.31 (± 0)	1.67
Atyidae	3	-20.82 (± 0.14)	9.5 (± 0.15)	1.72
Belostomatidae	1	-21.43 (± 0)	7.42 (± 0)	1.11
<i>Cherax quadricarinatus</i>	6	-20.95 (± 0.24)	12.15 (± 0.48)	2.51
<i>Cherax quadricarinatus</i> juveniles (<i>Cherax</i> juv)	1	-22.07 (± 0)	8.92 (± 0)	1.55
Coenagrionidae	2	-21.34 (± 0.47)	8.91 (± 0.57)	1.55
Libellulidae	1	-21.09 (± 0)	8.80 (± 0)	1.52
Nepidae	2	-25.59 (± 0.19)	8.62 (± 0.04)	1.46
Physidae	1	-20.82 (± 0)	4.87 (± 0)	0.35
Pisauridae (Pisaur)	1	-20.45 (± 0)	10.59 (± 0)	2.05
<i>Tarebia granifera</i>	4	-22.63 (± 0.16)	8.12 (± 0.21)	1.32
Primary producers				
<i>Azolla filiculoides</i>	4	-26.77 (± 0.03)	0.92 (± 0.04)	0
Biofilm	3	-24.6 (± 0.02)	5.42 (± 0.04)	0.5
Ceratophyllaceae	4	-21.24 (± 0.02)	7.55 (± 0.03)	1.15
Detritus	4	-20.21 (± 0.05)	7.06 (± 0.04)	1
<i>Eichhornia crassipes</i>	4	-27.5 (± 0.03)	6.07 (± 0.03)	0.71
<i>Marsilea ephippiocarpa</i>	4	-24.46 (± 0.03)	5.87 (± 0.1)	0.65
Onograceae	4	-26.28 (± 0.02)	8.5 (± 0.05)	1.43
Poaceae	4	-12.77 (± 0.02)	5.78 (± 0.05)	0.62
Potamogetonaceae	4	-18 (± 0.04)	9.08 (± 0.04)	1.6
Trapaceae	4	-25.38 (± 0.04)	7.02 (± 0.02)	0.99

Appendix E5: Names, abbreviations (in parentheses), number of replicates, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures ($\pm\text{SE}$) and trophic position of various food web components collected along the Phongolo River at Site 3 outside Ndumo Game Reserve during the September 2016 and February 2017 sampling surveys.

Components	September 2016				February 2017			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic position	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Consumers								
<i>Anguila marmorata</i> (A. marmo)					1	-22.76 (± 0)	14.89 (± 0)	3.09
<i>Acanthopagrus berda</i> (A. berda)	1	-20.47 (± 0)	16.56 (± 0.36)	4.32				
<i>Chiloglanis paratus</i> (C. para)	2	-21.6 (± 0.36)	17.08 (± 0.36)	4.48				
<i>Coptodon rendalii</i>	4	-22.44 (± 0.45)	10.99 (± 0.5)	3.26				
<i>Chiloglanis swierstrai</i> (C. swier)	4	-21.96 (± 0.25)	14.58 (± 0.44)	3.73				
<i>Glossogobius giuris</i>					1	-21.87 (± 0)	13.74 (± 0)	2.75
<i>Labeo cylindricus</i> (L. cylin)	11	-20.79 (± 0.48)	13.68 (± 0.3)	3.47				
<i>Labeo marequensis</i> (L. mare)	2	-21.46 (± 0.39)	14.8 (± 0.36)	3.8				
<i>Micralestes acutidens</i> (M. acuti)	2	-22.68 (± 0.81)	14.54 (± 0.24)	3.72	4	-23.24 (± 0.94)	13.41 (± 0.3)	2.65
<i>Oreochromis mossambicus</i>	3	-20.05 (± 0.56)	12.45 (± 0.25)	3.1	2	-23.44 (± 0.09)	10.76 (± 0.15)	1.86
<i>Pseudocrenilabrus philander</i> (P. phil)	8	-20.38 (± 0.23)	13.64 (± 0.21)	3.45	4	-21.88 (± 0.46)	13.01 (± 0.3)	2.53
<i>Tilapia sparmanii</i>	3	-21.64 (± 0.54)	13.98 (± 0.16)	3.55				
Invertebrates								
Aeshnidae					2	-21.6 (± 0.06)	14.75 (± 0.11)	3.05
Atyidae	7	-20.13 (± 0.1)	11.36 (± 0.06)	2.78	4	-20.99 (± 0.09)	14.44 (± 0.39)	2.96
Belostomatidae	1	-19.7 (± 0)	5.56 (± 0)	1.06	1	-22.52 (± 0)	15.22 (± 0)	3.19
<i>Cherax quadricarinatus</i>	9	-22.13 (± 1.38)	10.99 (± 1.42)	2.67				
Coenagrionidae	2	-21.91 (± 0.11)	11.68 (± 0.22)	2.87	2	-23.82 (± 0.11)	11.54 (± 0.67)	2.1
Dytiscidae					1	-23.39 (± 0)	11.24 (± 0)	2.01
Gomphidae (Gomph)	4	-21.67 (± 0.32)	11.54 (± 0.14)	2.83				
Libellulidae	1	-22.77 (± 0)	12.09 (± 0)	2.99	2	-23.46 (± 0.13)	13.99 (± 0.75)	2.82
Physidae					1	-15.7 (± 0)	11.65 (± 0)	2.13

Appendix E5 continued

Components	September 2016				February 2017			
	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic position	Number of replicates	Mean $\delta^{13}\text{C}$ ($\pm\text{SE}$)	Mean $\delta^{15}\text{N}$ ($\pm\text{SE}$)	Trophic Position
Invertebrates								
Tabanidae	1	-22.62 (± 0)	8.78 (± 0)	2.01				
<i>Tarebia granifera</i>	6	-21.94 (± 0.14)	9.54 (± 0.11)	2.24	4	-22.83 (± 0.03)	7.6 (± 0.21)	0.93
Primary producers								
<i>Azolla filiculoides</i>					4	-27.88 (± 0.04)	0.89 (± 0.02)	0
Biofilm					4	-18.17 (± 0.04)	3.31 (± 0.02)	0
Detritus	4	-28.29 (± 0.09)	5.37 (± 0.12)	1	4	-27.74 (± 0.06)	7.85 (± 0.04)	1
<i>Eichhornia crassipes</i>	4	-28.4 (± 0.04)	9.2 (± 0.05)	2.14				
Filamentous algae (Fil. algae)					4	-26.54 (± 0.17)	10.25 (± 0.02)	1.71
Leaf litter	4	-29.84 (± 0.04)	4.57 (± 0.08)	0.76	4	-27.43 (± 0.03)	7.34 (± 0.04)	0.85
Onograceae					4	-18.93 (± 0.02)	9.82 (± 0.03)	1.58
Poaceae	4	-27.19 (± 0.02)	10.46 (± 0.04)	2.51	4	-12.46 (± 0.04)	9.52 (± 0.01)	1.5
Potamogetonaceae (Potamoget)	4	-23.62 (± 0.22)	10.29 (± 0.03)	2.46	4	-18.42 (± 0.02)	8.99 (± 0.04)	1.34
Trapaceae (Trapac)					4	-25.30 (± 0.02)	8.89 (± 0.03)	1.31

Appendix E6: Parametric unpaired t-test result and $\delta^{13}\text{C}$ and of $\delta^{15}\text{N}$ isotopic signatures of shared food web components collected along the Phongolo River at Site 1 inside Ndumo Game Reserve during the September 2016 and February 2017 sampling surveys.

Source	$\delta^{13}\text{C}$ p value	$\delta^{15}\text{N}$ p value
Aeshnidae	p= 0.03; t= 5.88	p= 0.1; t= 2.37
Atyidae	p= 0.16; t= 1.77	p= 0.001; t= 6.99
<i>Brycinus imberi</i>	p= 0.29; t= 1.19	p= 0.9; t= 0.13
Biofilm	p= 0.003; t= 5.89	p= 0.005; t= 7.89
Ceratophyllaceae	p<0.0001; t= 48.74	p<0.0001; t= 58.35
Coenagrionidae	p= 0.61; t= 0.67	p= 0.08; t= 2.56
Detritus	p<0.0001; t= 23.49	p<0.0001; t= 19.98
Leaf litter	p<0.0001; t= 37.13	p<0.0001; t= 14.34
Libellulidae	p= 0.4; t= 1.01	p<0.0001; t= 139.6
Lycosidae	p= 0.1; t= 2.02	p= 0.01; t= 3.41
<i>Oreochromis mossambicus</i>	p= 0.03; t= 3.13	p= 0.0004; t= 9.89
<i>Synodontis zambezensis</i>	p= 0.25; t= 1.26	p= 0.59; t= 0.58

Appendix E7: Parametric unpaired t-test result and $\delta^{13}\text{C}$ and of $\delta^{15}\text{N}$ isotopic signatures of shared food web components collected along the Phongolo River at Site 2 outside Ndumo Game Reserve during the May 2016, September 2016 and February 2017 sampling surveys. 1 – May compared to September; 2 – May compared to February; 3 – September compared to February.

Source	$\delta^{13}\text{C}$ p value	$\delta^{15}\text{N}$ p value
Atyidae	p=0.87	p=0.01
<i>Azolla filiculoides</i>	p<0.0001	p=0.19
	¹ p= 0.0009	¹ p=0.08
Biofilm	² p=0.63	² p<0.0001
	³ p=0.001	³ p=0.0001
	¹ p<0.0001	¹ p=0.002
<i>Cherax quadricarinatus</i>	² p=0.01	² p=0.01
	³ p=0.001	³ p=0.86
	¹ p=0.55	¹ p=0.01
<i>Coptodon rendalli</i>	² p=0.48	² p=0.6
	³ p=0.48	³ p=0.01
Ceratophyllaceae	p<0.0001	p<0.0001
Coenagrionidae	p=0.29	p=0.02
	¹ p<0.0001	¹ p<0.0001
Detritus	² p=0.29	² p=0.002
	³ p<0.0001	³ p<0.0001
	¹ p<0.0001	¹ p=0.36
<i>Eichornia</i> sp.	² p<0.0001	² p<0.0001
	³ p<0.0001	³ p<0.0001
<i>Glossogobius giuris</i>	p= 0.003	p=0.24
<i>Labeo cylindricus</i>	p=0.003	p= 0.36
Leaf litter	p=0.004	p=0.0004
Libellulidae	p=0.24	p=0.002
	¹ p<0.0001	¹ p<0.0001
<i>Marsilea</i> sp.	² p<0.0001	² p=0.02
	³ p<0.0001	³ p=0.0002
Nepidae	p=0.16	p=0.46
	¹ p=0.02	¹ p=0.48
<i>Oreochromis mossambicus</i>	² p=0.003	² p=0.21
	³ p=0.2	³ p=0.75
	¹ p<0.0001	¹ p=0.0005
Onagraceae	² p<0.0001	² p=0.001
	³ p=0.04	³ p=0.0002
	¹ p=0.01	¹ p=0.003
<i>Pseudocrenilabrus philander</i>	² p=0.03	² p=0.02
	³ p=0.35	³ p=0.26
Poaceae	p>0.999	p=0.002
Potamogetonaceae	p<0.0001	p=0.004
<i>Tarebia granifera</i>	p=0.13	p=0.09
<i>Tilapia sparrmanii</i>	p=0.04	p=0.26
	¹ p=0.49	¹ p<0.0001
Trapaceae	² p=0.0001	² p<0.0001
	³ p=0.0008	³ p<0.0001

Appendix E8: Parametric unpaired t-test result and $\delta^{13}\text{C}$ and of $\delta^{15}\text{N}$ isotopic signatures of shared food web components collected along the Phongolo River at Site 3 outside Ndumo Game Reserve during the September 2016 and February 2017 sampling surveys.

Source	$\delta^{13}\text{C}$ p value	$\delta^{15}\text{N}$ p value
Atyidae	p=0.0002	p=0.004
Coenagrionidae	p=0.01	p=0.87
Detritus	p=0.004	p<0.0001
Leaf litter	p<0.0001	p<0.0001
<i>Micralestes acutidens</i>	p=0.68	p=0.047
<i>Oreochromis mossambicus</i>	p=0.02	p=0.01
<i>Pseudocrenilabrus philander</i>	p=0.04	p=0.13
Poaceae	p<0.0001	p<0.0001
Potamogetonaceae	p=0.0001	p<0.0001
<i>Tarebia granifera</i>	p=0.001	p=0.001

Appendix E9: Parametric unpaired t-test result and $\delta^{13}\text{C}$ and of $\delta^{15}\text{N}$ isotopic signatures of shared food web components collected along the Phongolo River outside (Sites 2 and 3) Ndumo Game Reserve during the September 2016 sampling survey.

Source	$\delta^{13}\text{C}$ p value	$\delta^{15}\text{N}$ p value
Atyidae	p=0.0002	p<0.0001
<i>Cherax quadricarinatus</i>	p=0.61	p=0.11
<i>Coptodon rendalii</i>	p=0.8	p=0.02
Coenagrionidae	p=0.38	p=0.13
Detritus	p=0.08	p=0.14
<i>Eichornia</i> sp.	p<0.0001	p<0.0001
<i>Labeo cylindricus</i>	p=0.024	p=0.81
Leaf litter	p<0.0001	p=0.002
<i>Micralestes acutidens</i>	p=0.51	p=0.54
<i>Oreochromis mossambicus</i>	p=0.038	p=0.61
<i>Pseudocrenilabrus philander</i>	p=0.0003	p=0.77
Potamogetonaceae	p=0.0005	p<0.0001
<i>Tarebia granifera</i>	p=0.88	p=0.64
Trapaceae	p=0.22	p=0.2

Appendix E10: Parametric unpaired t-test result and $\delta^{13}\text{C}$ and of $\delta^{15}\text{N}$ isotopic signatures of shared food web components collected along the Phongolo River outside (Sites 2 and 3) Ndumo Game Reserve during the February 2017 sampling survey.

Source	$\delta^{13}\text{C}$ p value	$\delta^{15}\text{N}$ p value
Atyidae	p=0.37	p=0.0004
<i>Azolla filiculoides</i>	p<0.0001	p=0.45
Biofilm	p<0.0001	p<0.0001
Coenagrionidae	p=0.15	p=0.13
Detritus	p<0.0001	p<0.0001
<i>Oreochromis mossambicus</i>	p=0.1	p=0.93
Onograceae	p<0.0001	p<0.0001
<i>Pseudocrenilabrus philander</i>	p=0.6	p=0.88
Poaceae	p=0.0017	p<0.0001
Potamogetonaceae	p=0.0006	p=0.16
<i>Tarebia granifera</i>	p=0.31	p=0.13
Trapaceae	p=0.18	p<0.0001

Appendix E11: Parametric unpaired t-test result and $\delta^{13}\text{C}$ and of $\delta^{15}\text{N}$ isotopic signatures of shared food web components collected along the Phongolo River within (Site 1) and outside (Sites 2 and 3) Ndumo Game Reserve during the September 2016 sampling survey. 1 – River inside compared to Walkway; 2 – River inside compared to Bridge outside.

Source	$\delta^{13}\text{C}$ p value	$\delta^{15}\text{N}$ p value
Atyidae	¹ p=0.46	¹ p<0.0001
	² p=0.12	² p<0.0001
<i>Brycinus imberi</i>	¹ p<0.0001	¹ p=0.0042
Biofilm	¹ p<0.0001	¹ p<0.0001
Ceratophyllaceae	¹ p=0.83	¹ p=0.12
Coenagrionidae	¹ p=0.42	¹ p=0.03
	² p=0.85	² p=0.02
Detritus	¹ p=0.83	¹ p<0.0001
	² p=0.06	² p=0.002
<i>Eichornia</i> sp.	¹ p<0.0001	¹ p=0.008
	² p<0.0001	² p<0.0001
<i>Glossogobius giuris</i>	¹ p=0.04	¹ p=0.1
Leaf litter	¹ p<0.0001	¹ p=0.0007
	² p<0.0001	² p=0.73
Libellulidae	¹ p=0.55	¹ p=0.0012
<i>Oreochromis mossambicus</i>	¹ p=0.03	¹ p=0.06
	² p=0.0001	² p=0.0005
Onograceae	¹ p<0.0001	¹ p<0.0001
Poaceae	² p<0.0001	² p<0.0001
<i>Tilapia sparrmanii</i>	¹ p=0.66	¹ p=0.95
	² p=0.55	² p=0.26

Appendix E12: Parametric unpaired t-test result and $\delta^{13}\text{C}$ and of $\delta^{15}\text{N}$ isotopic signatures of shared food web components collected along the Phongolo River within (Site 1) and outside (Sites 2 and 3) Ndumo Game Reserve during the February 2017 sampling survey. 1 – River inside compared to Walkway; 2 – River inside compared to Bridge outside.

Source	$\delta^{13}\text{C}$ p value	$\delta^{15}\text{N}$ p value
Aeshnidae	² p=0.12	² p=0.01
Atyidae	¹ p=0.2	¹ p=0.93
	² p=0.25	² p<0.0001
Biofilm	¹ p<0.0001	¹ p=0.02
	² p<0.0001	² p=0.004
<i>Cherax quadricarinatus</i>	¹ p=0.046	¹ p=0.02
<i>Coptodon rendalii</i>	¹ p=0.84	¹ p=0.84
Ceratophyllaceae	¹ p<0.0001	¹ p<0.0001
Coenagrionidae	¹ p=0.54	¹ p=0.13
	² p=0.94	² p=0.19
Detritus	¹ p<0.0001	¹ p<0.0001
	² p=0.0001	² p<0.0001
Leaf litter	¹ p=0.002	¹ p<0.0001
Libellulidae	² p=0.8	² p=0.04
<i>Macrobrachium</i> sp.	¹ p=0.04	¹ p=0.03
<i>Oreochromis mossambicus</i>	¹ p=0.67	¹ p=0.19
	² p=0.75	² p=0.02
<i>Tarebia granifera</i>	¹ p=0.9	¹ p=0.24
	² p=0.71	² p=0.02

Appendix E13: Sources collected for stable isotope analysis with fishes grouped taxonomically and by feeding strategy. Potential prey is noted according to Marshall 2011; Skelton 2012 and Taylor et al 2017.

Family	Genera	Feeding strategy	Prey
Alestidae	<i>Brycinus imberi</i>	Insectivore/Invertivore	Aquatic insects, terrestrial insects, zooplankton
	<i>Hydrocynus vittatus</i>	Juveniles – Invertivore	Juveniles – Aquatic invertebrates
		Adults - Piscivore	Adults – Fish
	<i>Micralestes acutidens</i>	Insectivore/Omnivore	Terrestrial insects, zooplankton
Anguillidae (eel)	<i>Anguila marmorata</i>	Predator	Crabs, frogs and fish
Cichlidae (tilapias)	<i>Coptodon rendalli</i>	Herbivore/Omnivore	Algae, detritus, invertebrates, small fish
	<i>Oreochromis mossambicus</i>	Detritivore/Omnivore	Detritus, zooplankton, phytoplankton, larval fish and eggs
	<i>Pseudocrenilabrus philander</i>	Benthivore/Omnivore	Crustaceans, molluscs, aquatic insects, seeds, fish
	<i>Tilapia sparrmanii</i>	Herbivore/Omnivore	Algae, detritus, invertebrates, small fish
Clariidae (catfish)	<i>Clarias gariepinus</i>	Predator/Omnivore/ Scavenger	Aquatic invertebrates, birds, crustaceans, fish, frogs, plant materials, reptiles, small mammals, snails, terrestrial insects, zooplankton
Cyprinidae (barbs)	<i>Enteromius afrohamiltoni</i> , <i>Enteromius</i> sp.,	Invertivore/Omnivore	Algae, aquatic invertebrates, crustaceans, detritus, plant material, periphyton, terrestrial insects, seeds, shrimp, small fish, zooplankton
Cyprinidae (labeos)	<i>Labeo cylindricus</i>	Herbivore	Aufwuchs, various forms of algae (epilithic, epiphytic and filamentous algae), periphyton
	<i>Labeobarbus marequensis</i>	Omnivore	Algae, aquatic invertebrate larvae, freshwater molluscs, small fish, snails, terrestrial insects

Appendix E13 continued

Family	Genera	Feeding strategy	Prey
Gobiidae	<i>Glossogobius giuris</i>	Juveniles – Benthic invertivore	Juveniles – Bottom living invertebrates
		Adults – Predators	Adults – Fish and tadpoles
Mochokidae	<i>Chiloglanis paratus</i>	Invertivore	Small invertebrates
	<i>Chiloglanis swierstrai</i>		
	<i>Synodontis zambezensis</i>	Benthic invertivore/Scavenger	Detritus, plant matter, small insects, molluscs, Molluscs, crustaceans, aquatic insects, small fish, detritus, algae
Mormyridae	<i>Petrocephalus wesselsi</i>	Invertivore	Aquatic invertebrates, insect larvae
Schilbeidae (catfish)	<i>Schilbe intermedius</i>	Omnivore	fish, insects, shrimp, snails, plant seeds, fruit
Sparidae	<i>Acanthopagrus berda</i>	Predator	Aquatic invertebrates, molluscs, shrimp, crabs and small fish

Appendix E14: Food source groupings and lower classification of all primary producers and aquatic invertebrates collected for dietary analysis during the surveys of the Phongolo River during the May 2016, September 2016 and February 2017 sampling surveys. A – Aquatic invertebrates classified under “Group A” invertebrates; B – Aquatic invertebrates classified under “Group B” invertebrates

Food source group	Lower classification	Genera
C ₃ plants	Araceae	<i>Colocasia esculenta</i> (Taro)
	Biofilm	
	Detritus	
	Filamentous algae	<i>Cladophora</i> sp.
	Onagraceae	
	Poaceae	Species 2
	Pontederiaceae	<i>Eichhornia crassipes</i> (water hyacinth)
	Polygonaceae	
	Salviniaceae (water ferns)	<i>Azolla filiculoides</i> (red water fern); <i>Marsilea ephippiocarpa</i> (water clover)
	Trapaceae	<i>Trapa</i> sp. (Water chestnut)
C ₄ plants	Biofilm	
	Ceratophyllaceae	
	Detritus	
	Leaf litter	
	Poaceae	Species 1
	Potamogetonaceae	<i>Potamogeton</i> sp.
Invertebrates	Aeshnidae (A)	
	Atyidae	<i>Caridina nilotica</i>
	Belostomatidae (B)	
	Coenagrionidae (A)	
	Corbiculidae	<i>Corbicula</i> sp.
	Diptera (B) ¹	
	Dytiscidae (A)	
	Gomphidae (A)	
	Gyrinidae	
	Hydrophilidae (B)	
	Lampyridae (A)	
	Libellulidae (A)	
	Lycosidae (A)	
	Naucoridae (A)	
	Nepidae (A)	
	Noteridae (A)	
	Notonectidae (A)	
	Physidae (B)	<i>Physa acuta</i>
	Pisauridae	
	Tabanidae (A)	
	Tadpoles (B)	
	Thiaridae (B)	<i>Tarebia granifera</i>
	Zooplankton (B)	