

# **THE DIVERSITY OF AQUATIC INSECTS IN THE TSITSIKAMMA REGION, WITH IMPLICATIONS FOR AQUATIC ECOSYSTEM CONSERVATION**

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

MASTER OF SCIENCE

of

RHODES UNIVERSITY

By

**Terence Andrew Bellingan**

December 2010

## **Abstract**

As a result of research carried out within the last decade to assess the diversity of macroinvertebrates of the Salt River in the Western Cape Province, South Africa, surveys of macroinvertebrates of 20 sites on 11 selected rivers from the same mountain range source were undertaken. This was done to make a preliminary assessment of the conservation status of the rivers of this region.

Aquatic insects from the orders Ephemeroptera, Odonata, Plecoptera, Megaloptera, Trichoptera and the dipteran family Simuliidae were collected using techniques to maximize the number of taxa found. The insects collected were identified to species level where possible. Water physicochemical parameters were recorded at all sites for each sampling trip to characterize these rivers and to establish a set of baseline data for future comparisons. These parameters included measurements made on site and analysis of the concentrations of all the major ions in water samples in the laboratory.

Multivariate analyses including Principle Components Analysis and Canonical Correspondence Analysis were used to reveal patterns within the water physicochemistry and species distributional data. Results include the identification of 123 species from 70 genera and 30 families. A total of 31 species were found to be undescribed, of which 17 were not previously collected and are thus completely new to science. In addition, four of these species could not be placed into any known genus. The analysis of water physicochemistry showed a clear distinction between rivers of Table Mountain sandstone and Bokkeveld shale origin. Downstream effects of anthropogenic influences were discernable too. Distinct macroinvertebrate assemblages were found based upon stream order and water chemistry composition. pH proved to be the most important driver of invertebrate assemblage composition. The high levels of endemism of the macroinvertebrates found within the upper reaches of these rivers and their degree of ecological specialization make these systems a priority for the conservation of aquatic biodiversity on a national and global scale.

*This thesis is dedicated to my parents Andrew and Joy Bellingan, and my brother Jonathan Bellingan.*

## **Declaration**

This thesis has not been submitted to a University other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author unless otherwise stated within the text.

Signed: \_\_\_\_\_ Date: \_\_\_\_\_



## Acknowledgements

I would like to acknowledge the contributions made by the following people and organizations towards this study:

- My supervisor Dr. Ferdinand de Moor and co-supervisor Prof. Martin Villet; to you both I owe an enormous debt of gratitude. Your advice, support and criticism have been invaluable, your enthusiasm and belief in this work a source of inspiration for me. Thank you!
- To the various funders, Cape Nature, Table Mountain Fund, World Wildlife Fund (WWF), The National Research Foundation (NRF) and Natures Valley Trust. Members of the Tsitsikamma Steering Committee: Geoff McIleron, Daniel Cloete, Julie Carlisle, Ian Russel, Onno Huyser, Dana Grobler, Henry Hill, Owen Govender, Karin Kirkman and Andrew Brown are all thanked for their input into this study over the two-year period.
- Nkosinathi Mazungula, Brittany Oliver, Nicholas Riddin, Kekere Soxujwa and Cliff Zingela for their respective help with fieldwork, sorting of samples and identification of material. Thanks for specialist help with identifications to Dr Mike Picker (Plecoptera), Ms Helen Barber-James, Dr Arnold Staniczek, Dr. Michel Sartori (Ephemeroptera) and Dr Benjamin Price (Megaloptera).
- John Simaika, for identifying, and for playing a fundamental part in the collection of, all of the adult Odonata material, and for agreeing to let me present those data here.
- Support from SANParks staff Karina Potgieter and Carol Whitcher for supplying the necessary collecting permits, resources and rangers' services when in the field, particularly the services of Andries Munnik and Albert Maarman who were involved in the majority of the sampling trips. Other rangers included Johnny Johnson, Tryphid Mashala, Charmel Pluim and Piet Handricks; MTO staff for allowing access to areas under their control - Barend Bothma, Tony Scholtz and particularly Graeme Pienaar for providing access to restricted areas and organising accommodation on the Tsitsikamma Hiking Trail. Staff of the Department of Water Affairs and Forestry: Thulani Guzana, Kululwa Mkosana, Portrait Tshatshu, Lorna Ntshebe, Pumza Lubelwana, Thembela Bushula, Elliot Weni, Nkosinathi Tshetu and Xolani Mbandazayo for performing all of the SASS5 protocols and for collecting

samples for water chemistry information; Buster McKenzie, Peter Behr, Brenda Bergé and Jurgen Schmidt for allowing unlimited access across private land to get to the Salt, Matjies and Buffels Rivers. Thanks also go to the owners of the Plateau guesthouse, who waived all costs for the use of their Helipad for ferrying researchers into the mountains.

- To Andrew Bellingan, not only for the use of his vehicle(s) for the duration of the fieldwork over the whole year of sampling, free of charge, but also for loving support and encouragement. Thank you so much Dad! In addition, thank you Gcaaaams! Your wisdom is far beyond mine!

# Contents

|                                                                                                                        |     |
|------------------------------------------------------------------------------------------------------------------------|-----|
| THE DIVERSITY OF AQUATIC INSECTS IN THE TSITSIKAMMA REGION, WITH IMPLICATIONS FOR AQUATIC ECOSYSTEM CONSERVATION ..... | i   |
| Abstract.....                                                                                                          | ii  |
| Declaration.....                                                                                                       | iv  |
| Acknowledgements.....                                                                                                  | v   |
| Contents.....                                                                                                          | vii |
| List of figures.....                                                                                                   | x   |
| List of tables .....                                                                                                   | xiv |
| Chapter 1.....                                                                                                         | 1   |
| General Introduction.....                                                                                              | 1   |
| 1.1 Geography of the study region .....                                                                                | 1   |
| 1.1.1 Geographical characteristics .....                                                                               | 1   |
| 1.1.2 Land use .....                                                                                                   | 2   |
| 1.3 Background literature .....                                                                                        | 3   |
| 1.4 Research objectives .....                                                                                          | 6   |
| 1.4.1 Aims of this study.....                                                                                          | 6   |
| Chapter 2.....                                                                                                         | 8   |
| Materials and methods.....                                                                                             | 8   |
| 2.1 Site Descriptions .....                                                                                            | 8   |
| 2.1.1 Site selection .....                                                                                             | 8   |
| 2.2 Physicochemical parameters .....                                                                                   | 9   |
| 2.2.1 Water temperature data.....                                                                                      | 9   |
| 2.2.2 Environmental and water chemistry data .....                                                                     | 9   |
| 2.2.2.1 On-site measurements.....                                                                                      | 9   |
| 2.2.2.2 Samples for laboratory analysis .....                                                                          | 10  |
| 2.3 Sampling procedure .....                                                                                           | 10  |
| 2.4 Collection of macroinvertebrates .....                                                                             | 11  |
| 2.4.1 Light trapping .....                                                                                             | 12  |
| 2.4.2 Aquatic collecting.....                                                                                          | 12  |
| 2.4.3 Malaise trapping .....                                                                                           | 13  |
| 2.4.4 Aerial and visual collecting.....                                                                                | 13  |
| 2.4.5 SASS5 study .....                                                                                                | 14  |

|                                                               |    |
|---------------------------------------------------------------|----|
| 2.5 Curation of specimens .....                               | 14 |
| 2.5.1 Cataloguing and identification of sampled material..... | 14 |
| 2.6 Analytical methods .....                                  | 15 |
| 2.6.1 Physicochemical data.....                               | 15 |
| 2.6.2 Analysis of macroinvertebrate assemblages .....         | 16 |
| 2.6.2.1 Sampling assessment and species diversity.....        | 16 |
| 2.6.2.2 Multivariate analyses .....                           | 17 |
| Chapter 3.....                                                | 20 |
| Results.....                                                  | 20 |
| 3.1 Physicochemical data.....                                 | 20 |
| 3.1.1 Water temperature.....                                  | 20 |
| 3.1.2 Water chemistry .....                                   | 21 |
| 3.2 Macroinvertebrate assemblages .....                       | 24 |
| 3.2.1 Ephemeroptera .....                                     | 24 |
| 3.2.2 Odonata .....                                           | 25 |
| 3.2.3 Plecoptera .....                                        | 25 |
| 3.2.4 Megaloptera.....                                        | 26 |
| 3.2.5 Trichoptera.....                                        | 26 |
| 3.2.6 Diptera (Simuliidae) .....                              | 27 |
| 3.2.7 Sampling assessment and species diversity.....          | 28 |
| 3.2.8 Multivariate analyses .....                             | 28 |
| Chapter 4.....                                                | 31 |
| Discussion.....                                               | 31 |
| 4.1 Physicochemical data.....                                 | 31 |
| 4.1.1 Water temperature.....                                  | 31 |
| 4.1.2 Water chemistry .....                                   | 32 |
| 4.2 Macroinvertebrate assemblages .....                       | 35 |
| 4.2.1 Ephemeroptera .....                                     | 35 |
| 4.2.2 Odonata .....                                           | 37 |
| 4.2.3 Plecoptera .....                                        | 40 |
| 4.2.4 Megaloptera.....                                        | 41 |
| 4.2.5 Trichoptera.....                                        | 41 |
| 4.2.6 Diptera (Simuliidae) .....                              | 46 |
| 4.2.7 Sampling assessment and species diversity.....          | 47 |

|                                                               |     |
|---------------------------------------------------------------|-----|
| 4.2.8 Multivariate analysis .....                             | 48  |
| Chapter 5.....                                                | 52  |
| Conclusion.....                                               | 52  |
| 5.1 Significance of findings .....                            | 52  |
| 5.1.1 Species diversity.....                                  | 52  |
| 5.1.2 Macroinvertebrate assemblages .....                     | 53  |
| 5.1.3 Sites and rivers .....                                  | 53  |
| 5.1.4 National and international significance .....           | 54  |
| References .....                                              | 56  |
| Figures.....                                                  | 69  |
| Tables .....                                                  | 111 |
| Appendix 1 .....                                              | 139 |
| Detailed site descriptions.....                               | 139 |
| Upper Groot (East) River (33°57'21"S, 23°38'19"E) .....       | 139 |
| Lower Groot (East) River (34°02'05.1"S, 24°12'27.2"E) .....   | 142 |
| Upper Elands River (33°58'17.60"S; 24°3'51.10"E) .....        | 145 |
| Lower Elands River (34°1'2.80"S; 24°3'39.10"E) .....          | 147 |
| Upper Storms River (33°56'57.20"S; 23°54'55.00"E) .....       | 150 |
| Lower Storms River (33°59'19.20"S; 23°55'8.60"E) .....        | 152 |
| Upper Elandsbos River (33°55'56.80"S; 23°46'58.00"E) .....    | 155 |
| Lower Elandsbos River (33°58'0.32"S; 23°46'29.87"E) .....     | 157 |
| Upper Lottering River (33°55'58.60"S; 23°43'45.60"E).....     | 160 |
| Lower Lottering River (33°58'23.90"S; 23°44'50.50"E).....     | 163 |
| Upper Bloukrans River (33°55'4.00"S; 23°38'20.00"E) .....     | 165 |
| Lower Bloukrans River (33°57'21.00"S; 23°38'19.00"E) .....    | 168 |
| Upper Groot (West) River (33°54'45.60"S; 23°34'37.10"E) ..... | 170 |
| Upper Bobbejaans River (33°53'46.50"S; 23°33'19.50"E) .....   | 172 |
| Lower Groot (West) River (33°57'50.50"S; 23°33'30.70"E) ..... | 174 |
| Upper Salt River (33°55'36.20"S; 23°29'23.30"E) .....         | 177 |
| Lower Salt River (33°58'28.30"S; 23°31'19.70"E) .....         | 179 |
| Upper Buffels River (33°58'56.80"S; 23°28'43.90"E) .....      | 181 |
| Upper Matjies River (33°58'50.60"S; 23°27'28.10"E) .....      | 183 |
| Lower Buffels River (33°59'7.90"S; 23°27'48.70"E) .....       | 185 |

## List of figures

Figure 1: Graph of temperature recorded every 2 hours over a year from the Groot (East) River. The data missing from the lower site from the beginning of November onwards is due to the complete cessation of water flow, leaving the data logger exposed to air and sunlight.

Figure 2: Graph of temperature recorded every 2 hours over a year from the Elands River.

Figure 3: Graph of temperature recorded every 2 hours over a year from the Storms River.

Figure 4: Graph of temperature recorded every 2 hours over a year from the Elandsbos River. The abnormal variation recorded during March/April from the lower site is due to the temperature logger being exposed as a result of underestimating the extent to which water levels would recede.

Figure 5: Graph of temperature recorded every 2 hours over a year from the Lottering River.

Figure 6: Graph of temperature recorded every 2 hours over a year from the Bloukrans River. The abnormal variation at the lower site recorded from February to May is due to the logger being removed by members of the public and then replaced again. The consistently high variation in water temperature at the upper site appears to be a natural phenomenon that is accounted for by the positioning of the logger in a pool below a waterfall.

Figure 7: Graph of temperature recorded every 2 hours over a year from the Groot (West) River.

Figure 8: Graph of temperature recorded every 2 hours over a year from the Groot (West) River and its tributary the Bobbejaans River. The comparatively high readings during March/April from the upper Bobbejaans site are due to the temperature logger being exposed as a result of underestimating the extent to which water levels would recede.

Figure 9: Graph of temperature recorded every 2 hours over a year from the Salt River.

Figure 10: Graph of temperature recorded every 2 hours over a year from the Buffels River. The missing data during the last quarter from the lower Buffels site was due to logger malfunction leading to the recordings being irretrievable from the I-Button device.

Figure 11: Graph of temperature recorded every 2 hours over a year from the Buffels River and its tributary the Matjies River. The missing data during the last quarter from the lower Buffels site was due to logger malfunction leading to the recordings being irretrievable from the I-Button device.

Figure 12: Factor plot of the first two Eigenvectors from a PCA of the laboratory analysed water chemistry data from all 20 sampling sites over an annual cycle. The indiscernible cluster of variables on the left includes Sulphate, dissolved Magnesium, Chloride and Sodium.

Figure 13: Ordination plot generated from the first two factors of a PCA of each of the 20 sites, using laboratory analysed water chemistry data collected seasonally/quarterly at each sampling event over a year.

Figure 14: Ordination plot generated from the first two factors of a PCA using laboratory analysed water chemistry data, with the case for the lower Salt River from the winter sampling event treated as an outlier and excluded.

Figure 15: Factor plot the first two Eigenvectors of from a PCA using laboratory analysed water chemistry data, with the case for the lower Salt River from the winter sampling event treated as an outlier and excluded. The indiscernible group of variables on the left includes Sulphate, dissolved Magnesium, Chloride and Sodium.

Figure 16: Seasonally categorised scatter plot of the first two factors of a PCA of laboratory analysed water chemistry data excluding the lower Salt River site for the winter sampling period, cases labelled according to month of collection.

Figure 17: Ordination diagram of the first two factors produced from a PCA using laboratory analysed water chemistry data, with the cases for the lower Salt, upper Matjies and upper and lower Buffels River sites removed for all of the sampling events.

Figure 18: Factor plot of the first two factors produced from a PCA using laboratory analysed water chemistry results, with the cases for the lower Salt, upper Matjies and upper and lower Buffels River sites removed for all of the sampling events. The indiscernible groupings on the right, and in the upper middle area, include Chloride and Dissolved Magnesium and Sulphate and Nitrate/Nitrite respectively.

Figure 19: Seasonally categorised scatter plot of the first two factors of a PCA of laboratory water chemistry results, excluding the lower Salt, upper Matjies and upper and lower Buffels River sites for all of the sampling events. Cases are schematically represented using colour.

Figure 20: Ordination diagram produced from a PCA using on-site water chemistry data from all 20 sites sampled during each sampling event.

Figure 21: Factor plot produced from a PCA using on-site water chemistry data for all 20 sites sampled during each sampling event.

Figure 22: Seasonally categorised scatter plot of the first two factors of a PCA of on-site water chemistry variables. The cases are labelled according to month and schematically represented using colour.

Figure 23: Ordination diagram produced from a PCA using on-site water chemistry data excluding the upper Matjies and upper and lower Buffels River sites for all of the sampling events.

Figure 24: Factor plot produced from a PCA using on-site water chemistry data excluding the upper Matjies and upper and lower Buffels River sites for all of the sampling events.

Figure 25: Seasonally categorised scatter plot of the first two factors of a PCA of on-site water chemistry variables, excluding the upper Matjies and upper and lower Buffels River sites for all of the sampling events. Cases labelled according to month and schematically represented using colour.

Figure 26: Factor plot generated from a PCA of a combination of laboratory analysed and on-site water chemistry data from all 20 sites collected for each sampling event.

Figure 27: Ordination diagram produced from a PCA of a combination of laboratory and on-site water chemistry data from all 20 sites collected for each sampling event.

Figure 28: Factor plot from a PCA using a combination of on-site and laboratory water chemistry results, with the case for the lower Salt River from June/July treated as an outlier and excluded.

Figure 29: Ordination plot generated from a PCA using a combination of on-site and laboratory water chemistry results, with the case for the lower Salt River from the winter sampling period treated as an outlier and excluded.

Figure 30: Categorised scatter plot of seasonal variation produced from the case statistics of a PCA of a combination of on-site and laboratory analysed water chemistry data, with the case for the lower Salt River from the winter sampling period treated as an outlier and excluded. The cases are labelled according to month and schematically represented using colour.

Figure 31: : Factor plot produced from a PCA using a combination of on-site and laboratory analysed water chemistry variables, with the cases for the lower Salt, upper Matjies and upper and lower Buffels River sites treated as outliers and removed for all of the sampling events.

Figure 32: Ordination diagram of river sampling sites produced from a PCA using a combination of on-site and laboratory analysed water chemistry variables, with the cases for the lower Salt, upper Matjies and upper and lower Buffels sites treated as outliers and removed for all of the sampling events.

Figure 33: Seasonally categorised scatter plot of the first two factors of a PCA of a combination of on-site and laboratory analysed water chemistry variables, with the cases for the lower Salt, upper Matjies and upper and lower Buffels River sites treated as outliers and removed for all of the sampling events. The cases are schematically represented using colour.

Figure 34: Sample-based rarefaction curve produced using EstimateS (Colwell 2006). The middle curve represents the number of species expected from the randomizations made from the samples collected in this study. The curves above and below this curve represent the 95% confidence intervals.

Figure 35: Differences in additive species richness, obtained from the diversity partitioning analysis, between the observed and expected diversity for each level within the hierarchical sampling design.  $\alpha_1$ : within site diversity;  $\beta_1$ : between site diversity;  $\beta_2$ : between river reach diversity (upper/lower);  $\beta_3$ : between sampling period diversity.

Figure 36: Ordination plot produce from MDS analysis based upon Bray-Curtis similarity coefficients using all of the species collected, excluding the Odonata, from all 20 sampling sites over a year. Clusters have been overlaid in PRIMER V.6 at resemblance levels of 20, 40 and 60 % similarity.



Figure 37: Ordination plot produced from MDS analysis based upon Bray-Curtis similarity coefficients using all of the species collected, excluding the Odonata, and excluding the sites along the Matjies and Buffels Rivers. Clusters have been overlaid in PRIMER V.6 at resemblance levels of 20, 40 and 60 % similarity.

Figure 38: Ordination triplot produced from the first two axes of a CCA of environmental variables, sites and species data from all sites over a year. Sampling period included in the site labels as autumn (A), spring (S) and summer (D). The eigenvalues for axes one, two and three are 0.489, 0.195 and 0.165 respectively (Species, environmental variable and site abbreviations: - Tables 29, 30 & 33 respectively).

Figure 39: Ordination biplot produced from a DCCA of environmental variables and species data from all sites for autumn, spring and summer sampling seasons. The environmental variable labels have been shifted for easier viewing and interpretation of the plot, particularly those to the left, including Conductivity, Sulphate, Chloride, Organic, Fluoride, Sodium and Mg. These however still correspond to the direction of the arrows (Species and environmental variable abbreviations: - Table 29 & 30 respectively).

Figure 40: Ordination triplot produced from the first two axes of a CCA of environmental variables, site and species data from all sites excluding those along the Matjies and Buffels Rivers. Sampling period included in the site labels as autumn (A), spring (S) and summer (D). The eigenvalues for axes one, two and three are 0.313, 0.171 and 0.142 respectively (Species, environmental variable and site abbreviations: - Tables 29, 30 & 33 respectively).

Figure 41: Ordination biplot produced from a DCCA of environmental variables and species data from all sites for autumn, spring and summer sampling seasons. The environmental variable labels have been shifted for easier viewing and interpretation of the plot, particularly those to the left, including Chloride, Organic, Sodium, Sand, SStones and Mg. These however still correspond to the direction of the arrows (Species and environmental variable abbreviations: - Table 29 & 30 respectively).

Figure 42: Simpson and Shannon-Wiener index values generated from PRIMER V6 for each site, using all of the species collected, excluding the Odonata, from all 20 sampling sites over a year.

Figure 43: A satellite image of the study area showing the rivers in false colour and the sampling sites as red dots along with the respective name assigned to each site.

## List of tables

Table 1: Key to the abbreviations of biotopes sampled during surveying. Principle SASS5 biotopes are underlined.

Table 2: Summary of temperature data recorded from each site using I-Button data loggers.

Table 3: Laboratory analysed water chemistry results recorded over the autumn and winter sampling periods.

Table 4: Laboratory analysed water chemistry results recorded over the spring and summer sampling periods.

Table 5: On-site water chemistry values recorded over the entire sampling period.

Table 6: Eigenvalues from a PCA of all 20 sites using laboratory analysed water chemistry results recorded at each sampling event.

Table 7: Factor coordinates of the first five factors from the PCA of each of the 20 sites, using laboratory analysed water chemistry results recorded at each sampling event.

Table 8: Eigenvalues generated from a PCA using laboratory analysed water chemistry results, with the values for the lower Salt River from the winter sampling period treated as an outlier and excluded.

Table 9: Factor coordinates of the first five factors from a PCA using laboratory analysed water chemistry results, with the value for the lower Salt River from the winter sampling period treated as an outlier and excluded.

Table 10: Eigenvalues generated from a PCA using laboratory analysed water chemistry results, with the lower Salt, upper Matjies and upper and lower Buffels River sites removed, for all four sampling events.

Table 11: Factor coordinates of the first five factors from a PCA using laboratory analysed water chemistry results, with the lower Salt, upper Matjies and upper and lower Buffels River sites removed, for all four sampling events.

Table 12: Eigenvalues generated from a PCA using on-site water chemistry results, pH, Turbidity and Electrical Conductivity.

Table 13: Factor coordinates generated from a PCA of on-site water chemistry results.

Table 14: Eigenvalues generated from a PCA using on-site water chemistry results with the cases for the Matjies and Buffels Rivers excluded.

Table 15: Factor coordinates generated from a PCA of on-site water chemistry results with the cases for the Matjies and Buffels Rivers excluded.

Table 16: Eigenvalues generated from a PCA using a combination of laboratory and on-site water chemistry values for all sites across all sampling events.

Table 17: Factor coordinates of the first five factors generated from a PCA using a combination of laboratory analysed and on-site water chemistry values for all sites across all sampling events.

Table 18: Eigenvalues generated from a PCA using a combination of laboratory analysed and on-site water chemistry values, excluding the case for the lower Salt River site during winter sampling.

Table 19: Factor coordinates generated from a PCA using a combination of laboratory analysed and on-site water chemistry values, excluding the case for the lower Salt River site during winter sampling.

Table 20: Eigenvalues generated from a PCA using a combination of laboratory analysed and on-site water chemistry values, excluding the lower Salt, upper Matjies and upper and lower Buffels River sites, for all four sampling events.

Table 21: Factor coordinates generated from a PCA using a combination of laboratory analysed and on-site water chemistry values, excluding the lower Salt, upper Matjies and upper and lower Buffels River sites, for all four sampling events.

Table 22: Ephemeroptera collected over four sampling events represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

Table 23: Odonata collected over four sampling events represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

Table 24: Plecoptera collected over four sampling events represented by actual abundance, including larvae and adults.. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

Table 25: Megaloptera collected over four sampling events represented by actual abundance, including larvae and adults. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

Table 26: Larval Trichoptera collected over four sampling events represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

Table 27: Adult Trichoptera collected over four sampling events, represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

Table 28: Larval and pupal Simuliidae collected over four sampling events represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

Table 29: List of species and their respective abbreviations used for the canonical and detrended canonical correspondence analyses.

Table 30: Environmental variables used for the canonical and detrended canonical correspondence analyses along with their respective abbreviations.

Table 31: Species diversity results from diversity partitioning analysis.

Table 32: Summary statistics from the canonical and detrended canonical analyses.

Table 33: List of site names and their respective abbreviations grouped over autumn (A), spring (S) and summer (D) sampling periods used in the canonical and detrended canonical correspondence analyses.

Table 34: Summary statistics from the Monte-Carlo permutation tests carried out to test the significance of the environmental variables on the species and site data for the canonical correspondence analyses.

Table 35: A summary of basic site characteristics. See the detailed site descriptions in Appendix 1 for further information.

Table 36: A summary of species data from each site, sorted according to the highest number of species per site

---

## Chapter 1

### General Introduction

---

#### 1.1 Geography of the study region

##### 1.1.1 Geographical characteristics

The Tsitsikamma region receives year-round rainfall, with on average 700-1000mm being recorded per annum (Nel *et al* 2006; Lewis 2010), and falls under the category of Mediterranean climate type (Gasith & Resh 1999). The area lies within the Fynbos Biome (Rutherford *et al* 2006), the smallest but most biologically diverse plant kingdom in the world (Davies & Wishart 2000). Vegetation units include southern afrotemperate forest (Mucina & Geldenhuys 2006), that lines the ravines and riverbeds in the upper catchments and occurs in stands at the base of the mountains, extending onto the plain towards the ocean (Fig. 43). The ridges in general host Tsitsikamma sandstone Fynbos, which is described as “a medium dense, tall proteoid shrubland over a dense moderately tall, ericoid-leaved shrubland – mainly proteoid, restioid, and ericoid fynbos, with fynbos thicket in wetter areas” (Rebelo *et al* 2006).

The rivers of the Tsitsikamma region are generally mild to strongly acid in pH (Harrison & Agnew 1962), and are characteristic of rivers originating from the Fynbos biome in that they are low in nutrients (oligotrophic), relatively unproductive biologically and brown in colour, presumably due to humic acids leached from plant material in the form of tannins and phenolics (King *et al* 1979). It is also postulated that sodium chloride, brought from the Indian Ocean by onshore winds, has a significant effect on the discolouration of these rivers and others that flow along seaward facing slopes of the Cape Fold Mountains (CFM) (King *et al* 1979; Swartz & de Villiers 1998). The rivers of the Tsitsikamma region, including George and Knysna, where large tracts of indigenous forests occur, carry the darkest stained waters in South Africa (Swartz & de Villiers 1998).

The major predictors of the chemical composition of the water in the rivers include the make-up of the underlying geology of the river system; the duration of time the water exists as groundwater; cycles of precipitation and evaporative drying, due to rainfall and evaporation; effects from riparian vegetation; and the exchange of chemicals with the water by the organisms living in the system (Day & King 1995; Day *et al* 1998; Roets *et al* 2008a; Roets *et al* 2008b). The majority of the rivers of the Tsitsikamma region originate from the Table Mountain Sandstone (TMS) from which the

Tsitsikamma Mountain range is formed, along with most of the CFM to the West of the Tsitsikamma (Johnston 2000). At a finer scale, the rivers cut through several geological sequences from the top of the catchments before reaching the ocean. The upper reaches of the rivers transect short stretches of Cedarberg Formation shales, Tchando Formation sandstones and Baviaanskloof Formation sandstones (all of the Table Mountain Group). Nearer to the seashore, Gydo Formation shales of the Bokkeveld Group are encountered (Toerien 1976).

The Tsitsikamma Mountains were formed up as a result of a subduction zone forming along the southern edge of Gondwana during the Carboniferous and Permian eras, which folded the sedimentary rock that had accumulated at the bottom of the Agulhas Sea, approximately 330 million years ago (Johnston 2000; McCarthy & Rubidge 2005). Subsequent to the breakup of Gondwana during the Jurassic and Cretaceous eras, uplift occurred throughout southern Africa, starting 20 million years ago. Two such events are postulated to have occurred. The greatest rise occurred in the eastern half of southern Africa and was accentuated in the coastal areas, causing a tilting of the subcontinent to the West. This had drastic impacts on the climate of the region, causing aridification of the interior and down-cutting of river systems along the east coast which lead to the formation of deep gorges like the Valley of a Thousand Hills in KwaZulu-Natal and the deep valleys encountered along the Tsitsikamma coastline (McCarthy & Rubidge 2005). The scarp that forms the coastal plain between the foothills of the Tsitsikamma Mountains and the Indian Ocean is approximately 180m high because of this uplift, rising to approximately 275m in altitude at the base of the mountains. Formosa peak, the highest point in the Tsitsikamma mountain range, reaches 1 675m above sea level (Toerien 1976). The coastal plateau is a well-defined wave-cut platform, approximately 5km wide in the East and 8km wide in the West, this is a remnant of higher sea levels during the Tertiary prior to the uplift, roughly 70-20 million years ago (Morant & Bickerton 1983).

### **1.1.2 Land use**

According to the Environmental Potential Atlas for South Africa (ENPAT), the majority of the land use for the Tsitsikamma region is for conservation (Fig. 43). The upper catchments for each of the rivers in this study, with the exception of the Matjies and Buffels Rivers, all lie within the Tsitsikamma Forest National Park. Some anthropogenic disturbance does occur within the upper catchments of the Storms, Lottering, Elandsbos and Bloukrans Rivers in the form of the "Tsitsikamma hiking trail" maintained by Mountain to Ocean (MTO) Ecotourism. Huts have been made available to hikers for overnight stays, which include ablution and washing facilities that drain into septic tanks. All of these huts lie far enough from the rivers to be of any major concern. Other impacts within the catchments

include the harvesting of naturally occurring ferns for sale. The catchments of the Matjies and Buffels Rivers contain Kurland Village, which is a substantial settlement, and small agricultural holdings for dairy farming, ecotourism, and small-scale accommodation. Industrial activity occurs within these catchments in the form of the Kurland brick kiln, which manufacture bricks and pavers.

The plateau between the foothills of the Tsitsikamma Mountains and the coast has large areas devoted to the cultivation of pine and gum trees. The main plantations, listed from East to West, include: the Blue Lilies Bush Plantation, which encroaches onto the banks of the Storms and Elands Rivers; the Kleinbos Plantation; the Lottering Plantation, which lines the banks of the Elandsbos and Lottering Rivers; and the Bloukrans Plantation that, unlike the previous plantations, lies in the mountainous catchment of the Bloukrans River. The trees from these plantations, along with the Australian protea, *Hakea sericea* Schrader, have invaded the catchments and in some places have an obviously negative impact on the understory fynbos and indigenous forest scrub (Appendix 1). It is estimated that by the middle of the 21<sup>st</sup> century, the Fynbos biome will be entirely covered by alien vegetation unless urgent steps are taken to control them (van Wilgen *et al* 1996). This appears to be the case for the Tsitsikamma region too. In the vicinity of the Groot (East) River, agricultural activities include dairy farming and the cultivation of proteaceous species for the indigenous flower trade.

### **1.3 Background literature**

The documentation of aquatic macroinvertebrates of the Cape Fold Mountains of the southern Cape was conducted over more than a decade by Barnard (1931-1940), who first described the distinctive fauna that occur in the acidic streams of the rivers originating from the TMS source. Further early studies pertaining to macroinvertebrates of the southern Cape region include those of Scott (1958; 1961), Stuckenberg (1962), Brundin (1966), Harrison & Agnew (1960; 1962), Coetzer (1978; 1986), King (1981; 1983) and King *et al* (1987a; 1987b; 1988).

Harrison & Agnew (1962) carried out the first recorded collection of aquatic invertebrates in the Tsitsikamma region. The motivation for this work was threefold: to determine whether the macroinvertebrate assemblages of the Tsitsikamma region corresponded in their faunal composition to acidic rivers of the southern Cape, compared to findings of then recently completed work on the Great Berg River (Harrison & Elsworth 1958); to explore the association between TMS and the acidity of the rivers, again compared to the Great Berg River; and to investigate the extent of the occurrence of “temperate” species in alkaline streams of the region. The findings of this study aligned with those of Barnard 1931; Barnard 1932; Barnard 1934a; Barnard 1934b & Barnard 1940,

in that, “the fauna is obviously one of very acid streams and these are only found flowing off TMS formations” and, “there is a fauna endemic to the acid streams of the Southern Cape”(Harrison & Agnew 1962). In a later publication by Harrison (1965), it was concluded that the riverine invertebrates of the montane regions in the southern Cape are of an old element that is palaeogenic and palaeo-endemic with Gondwanaland affinities, and are adapted to permanent, cold stenothermal and oligotrophic streams.

Subsequent to these early studies, a wealth of literature has been accumulated on rivers in southern Africa. Through the Water Research Commission, established in 1971 under the Water Research Act (Act No. 34 of 1971), a vast quantity of research has been conducted under the broad field of limnology in South Africa. A very important aspect of this research is the issue of water quality (Dallas *et al* 1998). The formation of the River Health Programme (RHP) focussed attention on the topic of the health of South African riverine ecosystems. Here, the term “health” refers to the ability of the ecosystem to function in way that is comparable to its natural state (Roux *et al* 1999). The suitability of water for use either humans or naturally-occurring organisms can be estimated by the measurement of chemical parameters. However, this is not always suitable as rivers form continua (Reynoldson 1984) and not all disturbances in aquatic systems are chemical in nature (Karr 1991). Thus, the organisms that inhabit an aquatic ecosystem are used to provide direct clues to the health of their habitat (Metcalf-Smith 1994). In South Africa, the first biotic index for determining water quality was developed by Chutter (1972), and the South African Scoring System (SASS) was subsequently developed refined (Chutter 1994; 1998); it is now implemented as version five (Dickens & Graham 2002).

The published literature about the Tsitsikamma region is overwhelmingly marine-orientated (Hanekom *et al* 1989; Tilney *et al* 1996; Cowley *et al* 2002; Faasen & Watts 2007). Work has been done within the Tsitsikamma National Forest Park pertaining to terrestrial reptiles, small mammals and ungulates (Crawford *et al* 1983; Branch & Hanekom 1987; Hanekom & Wilson, 1991) as well as agro-forestry and the treatment of alien invasive plant species (Vlok & Ronde 1989; Louw 1991; Hosking & du Preez 1999). A study in 2002 surveyed some of the rivers within the Tsitsikamma Forest Park for freshwater fish (Russel 2002). The proposed introduction of both Rainbow and Brown Trout into the upper Salt River by a local landowner resulted in environmental impact assessments (EIA) under the Cape Province Nature and Environmental Conservation Ordinance 19 of 1974, administered by the Western Cape Nature Conservation Board. Surveys of the freshwater fish (Bok 2000a, 2000b) and the aquatic macroinvertebrates (Barber-James 2000; de Moor & Barber-James 2001) led to the renewed interest in the macroinvertebrates of the region.



The findings of the ichthyological EIA's included that the upper Salt River and its tributaries have no indigenous fish and that any introduction of alien fish would most likely be detrimental to the macroinvertebrate fauna, as has been found in recent studies in the Cape Floristic Region (Lowe *et al* 2008). However, due to natural waterfall barriers in the river, impacts would be localised to stocking areas as both trout species cannot breed successfully at the low pH levels encountered in the Salt River (Hey 1926; de Moor & Bruton 1988). It was also considered that the absence of any primary freshwater fish in the system played a major role in this river having such a unique and diverse aquatic insect fauna, compared to the findings of Lowe *et al* (2008), where invasive fish species removed indigenous fish species, allowing the proliferation of specific macroinvertebrate groups. In addition, it is also thought that the low pH of some of the Tsitsikamma rivers precludes the production of the carbonate shells of snails, so that these rivers are free of grazing molluscs. The absence of these important herbivores may also be significant in liberating a resource for insect communities (Wiberg-Larsen *et al* 2000; Wichmann *et al* 2004; F. de Moor pers. comm.).

The EIA reports produced from the surveys conducted on the aquatic macroinvertebrates of the Salt River concluded that the aquatic insect fauna found in the Salt River and its tributary, the Wit River, are worthy of conservation (Barber-James 2000; de Moor & Barber-James 2001). The majority of the species found in the Salt River were from families and genera endemic to the southern Cape and South Western Cape region, and these insects show a high degree of ecological specialization, adding further priority to their conservation (Wishart & Day 2002). Important findings included 13 undescribed species, three undescribed genera, and remarkable range extensions of species that were previously considered not to occur in the region. The recommendation of the EIA surveys on the aquatic macroinvertebrates was that no fish, either indigenous or exotic, should be released into this river and that special measures should be taken to preserve the Salt River and its tributaries in the form of a sanctuary for aquatic invertebrates.

The most recent work carried out along the Salt River was at the request of the Natures Valley Trust (NVT). This included the survey of the macroinvertebrates to obtain a more complete coverage of species of macroinvertebrates throughout the annual cycle; and to assess whether any changes in the macroinvertebrate communities had occurred since previous surveys, because several developments had taken place in the catchment and along the riparian zone in the interim period (de Moor *et al* 2004). Of particular concern was the impact of a potentially devastating silt discharge into the Salt River in 2002 that occurred following land-clearing operations in the catchment. This survey resulted in the collection of further undescribed species. It also revealed that the reported sediment discharge, while probably causing immediate impacts, had no discernable long-term

impact on the macroinvertebrate community structure and it was found that sediment-sensitive species were present in large numbers throughout the river. It was also noted that while the Salt River could recover from infrequent 'massive sedimentation events', a continuous increase in sedimentation or more frequent 'massive sedimentation events' could result in a significant deterioration in the conservation status of the river (de Moor & Barber-James 1994, de Moor *et al* 2004).

## **1.4 Research objectives**

### **1.4.1 Aims of this study**

Following the studies carried out on the Salt River and its tributaries discussed above, the question arose as to how unique the invertebrate fauna of this small river was and whether other rivers within the southern Cape quaternary sub-catchment region K (Midgley *et al* 1994), contained similar invertebrate faunas. If this proved to be the case, it would allow a broader-based conservation plan to preserve the biota and ecological conditions in a selection of these rivers. Alternatively, if the rivers were to show sufficiently distinctive components of invertebrate biota - and in particular, limited distributions of populations of endemic species - then a more targeted form of conservation for individual rivers would be required.

One of the recommendations made by de Moor *et al* (2004) was that surveys of more rivers in the southern Cape should be carried out to evaluate the status of the indigenous and endemic invertebrates and any fish fauna found or recorded in these rivers. The aquatic invertebrate biota found there should then also be compared with what is known about the fauna in the rest of the Cape Floristic Region (CFR). This would allow for informed conservation planning prioritizing the selection of rivers to ensure protection of the natural heritage and ecological processes needed to maintain the endemic biota in the rivers of this region. This study grew out of these recommendations, facilitated by a contract with South African National Parks, mediated by the Natures Valley Trust. The terms of reference of this research contract have shaped this thesis to some extent.

The key questions and hypotheses to be addressed during this study are:

1. Which species are found in each of the rivers and what is the relative abundance of each of the species?
2. What patterns of similarity/difference and uniqueness exist in the species assemblages of these rivers?
3. These rivers form clusters of similarity based on the aquatic insect species found.
4. The similarity of taxa between rivers can be explained based on biotic and abiotic factors.

---

## Chapter 2

### Materials and methods

---

#### 2.1 Site Descriptions

##### 2.1.1 Site selection

Two sites were selected along each river, representing upper and lower reaches. Where the river consisted of two major tributaries, a single site below the confluence of the tributaries was chosen. A map of the study area showing the river systems and study sites is provided (Fig. 43) as well as basic site information (Table 35). The selection of sampling sites was undertaken based on two primary factors: accessibility of the site and the characteristics of the site. Accessibility was important for the following reasons: the South African Scoring System protocol version 5 (SASS5) carried out by colleagues from the Department of Water Affairs and Forestry (DWAF) at each site involves the use of cumbersome equipment that does not permit long hikes or rugged terrain (Dickens & Graham 2002). Secondly, it was and still remains the intention of DWAF to use the sites sampled during this study for continual assessment of river health and routine monitoring of water quality, and for that reason relative convenience of access is imperative for repeated visits to the sites. In some instances safety was a pertinent concern, where an overnight stay at a site would have been necessary to reach some of the upper river sampling sites and do all of the required sampling procedures. During periods of drought, the threat of fire presents a tangible safety issue in the CFR that prohibits this.

Regarding characteristics of the sites, the terms of reference for this study required that the sites sampled should be “representative of the river course”. For that reason sites were chosen that were as diverse and heterogeneous, in terms of biotopes, as possible. In some instances, e.g. the Lower Groot (East) River, where the upstream river course was impounded and its flow highly modified, the site was representative of the river in this reach as described in the detailed site descriptions (Appendix 1). This kind of compromise had to be made despite the need to maximize the chances of collecting as many species of aquatic insects as possible along that selected reach of the river.

The process of site selection took place from the 7<sup>th</sup> to the 18<sup>th</sup> of January and from the 10<sup>th</sup> to the 13<sup>th</sup> February 2008. The first trip included visiting all but two of the sites that were set out in the

terms of reference for this study, namely the upper reaches of the Groot (West) and Bobbejaans Rivers. After a day hike into the mountains making up the catchment of the Upper Groot River on the 17<sup>th</sup> of January, a decision was taken by the steering committee that it was not feasible to hike to these sites but rather that they should be reached by helicopter. The second visit during February provided this opportunity and the sites to be sampled along these upper rivers were selected and considered suitable based on availability of safe and reusable landing sites.

The dates of sampling for macroinvertebrates were: 2<sup>nd</sup> to the 10<sup>th</sup> March 2008, representing autumn; 1<sup>st</sup> to the 12<sup>th</sup> July 2008, representing winter; 29<sup>th</sup> September to the 11 October 2008, representing spring; and 16<sup>th</sup> to the 29<sup>th</sup> January 2009, representing summer. At each site, for each sampling trip, the methods described below were followed

## **2.2 Physicochemical parameters**

### **2.2.1 Water temperature data**

Water temperature measurements were considered a priority from the outset of this study, and a significant amount of effort was made to gather these data. The reason for this was to provide a sound set of base-line data to which further studies could be compared. Climastats i-Button data loggers were used to record water temperature. The i-Buttons were placed in watertight plastic bags and thereafter into standard urine sample bottles, ensuring that the logger was kept dry. The bottle was placed in a 50mm diameter, 300mm long galvanised pipe, barred at either end with stainless steel bolts preventing the bottle from falling out. Each pipe was attached to a 6-8kg stone by a stainless steel cable. Each stone had a 12mm hole drilled into it, into which a rawlbolt was inserted and cemented with epoxy glue. The loggers were placed strategically at each site in January 2008 during which time the Climastats i-Buttons were programmed to record temperature at 2 hourly intervals for the next year. The data were retrieved every consecutive trip, at roughly 3 month intervals.

### **2.2.2 Environmental and water chemistry data**

#### **2.2.2.1 On-site measurements**

Along with myself, the team from DWAF undertook the task of gathering on site water quality data [temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS)] using a multiparameter water quality meter (Model: YSI 6920 V2 Sonde; Make: YSI Environmental). The

DWAF team also recorded Dissolved Oxygen (DO) and Turbidity. During the spring survey, it became apparent that the water quality meter was malfunctioning and the DO measurements were too low to be correct and were thus not reliable. The problem persisted for the summer sampling despite a different model of multiparameter water quality meter being used. Additional instruments used for measuring EC, TDS, pH and temperature for comparative purposes included a WTW LF95; EC, TDS and temperature meter and a Cyberscan pH 300 pH and temperature meter.

Physical environmental variables were determined on site by qualitative estimation among researchers. These included flow rate, percentage bottom substrate composition (categorized as: bedrock; boulders; large stones; small stones; gravel; sand; mud; organic material) riparian vegetation composition and average stream width.

#### **2.2.2.2 Samples for laboratory analysis**

Along with assisting in recording on-site water chemistry parameters, the DWAF team also agreed to collect water samples for laboratory evaluated water chemistry analysis. These were collected using 1-litre plastic bottles and stored in a deep freeze in the field laboratory at Petrusville, Storms River Mouth, for later analysis by Talbot & Talbot Laboratories Pietermaritzburg, KwaZulu-Natal. The parameters determined by Talbot & Talbot included Nitrate/Nitrite, Ammonia, Phosphate, Orthophosphate, Total Cadmium, Total Chromium, Total Copper, Total Iron, Total Lead, Total Magnesium, Total Mercury, Total Zinc, Bromide, Chloride, Fluoride, Total Sodium, Sulphate, Sulphide, Turbidity in Nephelometric Turbidity units, Suspended Solids at 105°C and Total Dissolved Solids at 180°C. For some inexplicable reason the same parameters were not recorded consistently and varied between sampling trips. In addition to this, many of the results came back labelled “Insufficient sample”, an example of this most frequently being the Mercury parameter. For the sake of comparison, only water chemistry parameters that were recorded consistently were used for the analysis. These included Ammonia, Chloride, Dissolved Magnesium, Fluoride, Nitrate/Nitrite, Orthophosphate, Sodium, Sulphate, Total Lead, Total Zinc and Total Iron.

### **2.3 Sampling procedure**

The selection of taxa to be identified included insects from the aquatic insect orders Ephemeroptera, Odonata, Plecoptera, Megaloptera and Trichoptera. These groups cover many endemic species that are indicative of ancient lineages (Stuckenberg 1962); include taxa that are either vagile or have restricted dispersal capacity; and cover a diversity of species in the majority of recognized aquatic

functional feeding groups. In addition, the Simuliidae (Diptera) serve as good indicators of the abundance or paucity of particulate organic matter, prevalent flow conditions and water quality. Furthermore, previous studies in the region have focussed on these groups, making this study comparable to previous works (Harrison & Agnew 1962; Barber-James 2000; de Moor & Barber-James 2001; de Moor *et al* 2004).

The sampling of macroinvertebrates was carried out in two ways. Firstly, standardized samples were collected by colleagues from DWAF according to the SASS5 protocol outlined by Dickens & Graham (2002). Each of the principle biotopes (Table 1), were sampled for up to five minutes with a standard SASS net, effectively restricting bias in sampling effort. Instead of returning the sample to the river as is the normal procedure, the samples were preserved.

Secondly, intensive sampling for the collection of invertebrates within any other additional biotopes characteristic of the river reach that were not included in the standardized sampling was undertaken. Additional sampling also attempted to include the activity patterns of the insects e.g. flying adult activity with hand-nets, light and malaise traps. This was carried out to collect any rare taxa that might not be found in the main river course and to obtain a more complete inventory of the species present, but not necessarily the abundance or frequency of occurrence of a species or group at a site. Smaller D-shaped nets, hand nets, forceps and aerial nets were also used to sample additional aquatic biotopes. Intensive sampling overlapped with and often included the principle biotopes targeted by the SASS5 protocol. This provided good quality duplicate specimens that were necessary because insects are often damaged through kicking techniques to dislodge invertebrates and using the larger 1000µm mesh size SASS net. These include caudal lamellae in the Odonata; gills in the Ephemeroptera; fine hairs in the Plecoptera and, occasionally, case material in the Trichoptera. The coarser mesh allows smaller invertebrates to pass through the mesh. For example, the Simuliidae are always underrepresented in SASS5 samples (de Moor pers. comm.).

## **2.4 Collection of macroinvertebrates**

A strict protocol was followed at each site when sampling took place. Each individual biotope sample was assigned a catalogue number in a field notebook immediately after it was collected, with a corresponding number recorded in pencil on a label inserted into the container with the sample. Associated with this catalogue number was a record of all of the relevant details pertaining to the sample, including the date and time, method of collection, photographic record of “up” and “downstream” views of the site and information about the biotope that was sampled.

With the exception of the adult Odonata, which were preserved using acetone to fix their colouration, all samples were placed in 80% ethanol upon collection. In larger samples, this was replaced as soon after the collecting event as possible, as the alcohol can become diluted by body fluids, resulting in softening and deterioration of specimens. Sample bottles ranging in size from 500ml plastic containers to 30ml skirted centrifuge tubes were used for storage, depending on the size and type of sample.

#### **2.4.1 Light trapping**

Light traps were set at each site to collect adult insects, particularly Trichoptera and Megaloptera. Ephemeroptera of the family Caenidae occurred in light traps rather infrequently, but were abundant when they were collected. The light trap apparatus consisted of a sealed 6v battery that was wired to a super-actinic fluorescent bulb. This was suspended, using a suitable dead branch obtained on site, over a circular white tray approximately 4cm deep and 45cm in diameter. The tray was filled with river water to which a few millilitres of dishwashing detergent was added, serving to break the surface tension of the water to drown insects that fall into the water quickly. Unfortunately, the soap does cause hairs and subsequently the coloration to be lost from the wings of Trichoptera, but this is acceptable for the amount and diversity of these insects collected in this fashion. Furthermore, the examination of features used to identify species, male caddis genitalia in particular, are far more easily viewed when specimens are preserved in ethanol as opposed to dried, pinned, material. The light traps were always set up well before dusk and left to run through to the following morning. In that way sampling throughout the night was achieved, and both dusk and dawn flying species that were attracted to light were targeted. On reaching a light trap that had been collecting over-night, the first step was to remove all of the Lepidoptera that tend to clog the tray. Not only are they a nuisance in that they obstruct the collection of the adult aquatic orders, but their scales are shed when they land in the tray, creating a soupy brown mess. These scales were rinsed off in the river by placing the sample in a 250µm-mesh hand-net and carefully letting the water flow through it, removing the scales, which tend to float.

#### **2.4.2 Aquatic collecting**

Aquatic collecting by hand was done to keep the insects in as immaculate a condition as possible for identification and preservation for archiving. In most cases, spring steel “floppy” forceps were used and the insects were collected either from stones or from a 250µm mesh hand-net that was held



downstream from where the stone was removed from the river. The stone and hand-net were both inspected closely for any insects. When wood snags were present in the river, these were removed and the insects and their associated retreats were collected. A biotope that was specifically targeted for its wealth of insect fauna was moss growing in and out of the stream current. Using a D-shaped hygropetric net (mesh size 80µm), moss was thoroughly sampled at each site where it was present by holding the net downstream of the moss and running the fingers between the fronds to unsettle any insects in it. Tufts of moss were preserved with the sampled insects, because many Blackfly and hydroptilid caddis pupal cases are too firmly attached to the moss to be easily removed.

Throughout the survey, each of the principle biotopes common to all sites was thoroughly sampled by hand. Sampling was carried out for at least 90 minutes at each site, with the time allocated to sampling different biotopes being proportional to the relative abundance of the actual biotopes present. Where sites showed a diversity of biotopes in small patches, many of these were sampled and combined. For sites that were homogenous, only a few biotopes were available to be sampled. The more commonly sampled biotopes included stones-in-current and out-of-current; marginal and rooted vegetation-in-current and out-of-current, areas of gravel, sand and mud; and bedrock-in-current (Table 1).

#### **2.4.3 Malaise trapping**

Malaise traps approximately 6 meters in length and 2 meters from the ground were set near to or over the sites where sampling took place and where suitable space could be found to erect them. . The presence of forest was essential for the use of these malaise traps as elevated points for suspending the traps are necessary. The areas where sampling effort was concentrated included the Storms River (upper and lower sites) and Elandsbos River (upper and lower sites). Malaise trapping was used primarily to include diurnal species, and species that are not attracted to light as the nocturnal species that are attracted to light were already targeted through light trapping.

#### **2.4.4 Aerial and visual collecting**

Adult Plecoptera, Trichoptera and Ephemeroptera were collected at each site by sweeping with a 0.08mm mesh hand net or by picking adult insects off substrata by hand. Often adult caddisflies were observed scuttling over rocks during the day, particularly species in the Families Philopotamidae and Glossosomatidae, while in the evening adult Leptoceridae were caught flying just above the water surface or forming mating aggregations. At sites where marginal vegetation

was abundant, sweep netting of the vegetation most often produced adult Plecoptera. The collection of adult Odonata was carried out principally by John Simaika. Adult male dragonflies were counted for one hour along transects at each site; as transect length varied according to site, collection was standardised to an hour of intensive sampling by a two-man team. The duplication of counts was avoided by caging the specimens and releasing them only when the hour of sampling was completed. Identifications were made in the field where possible and voucher specimens were kept from each site. The collection of Odonata was preferably carried out on fine, warm, and windless days to avoid sampling bias as a result of inclement and cold weather.

#### **2.4.5 SASS5 study**

The team from DWAF collected SASS5 samples at all sites and used these to make an assessment of water quality on the basis of the species composition of macroinvertebrates (identified to family level) in the river (Dickens & Graham 2002). SASS5 Samples were also given catalogue numbers and in the laboratory, where invertebrates identified to species level where possible and data were added to the species compliment for each site. All invertebrates collected using this method were preserved and retained as additional collections of representative macroinvertebrates from three major aquatic biotopes sampled at each site on every occasion. The biotopes surveyed included SIC/OC, MVIC/OC (including RMVIC/OC), and GSM samples (Table 1), as described in detail for the SASS5 protocol (Dickens & Graham 2002). The results of this ongoing study are not presented here, but it is mentioned because some of the samples were used here.

### **2.5 Curation of specimens**

#### **2.5.1 Cataloguing and identification of sampled material**

Samples were prioritized according to the manner in which they were collected. The adult light trap samples were sorted first, followed by the especially collected aquatic samples and finally the SASS samples. Each sample was sorted using fine forceps and a Wild M5 dissecting microscope. This process requires the removal of unwanted litter and debris first. In aquatic samples, vegetation matter, stones and gravel pose a hindrance to identifying small nymphs and larvae; for light trap samples non-aquatic orders like moths also needed to be removed to allow identifications to be made easily. An open-ended catalogue system is used where the original field catalogue number is retained for referencing the sample and a letter of the alphabet is added to it each time a new

record for a species is made. Once a specific identification has been reached, the specimens are counted and placed into vials and relabelled, with one species but often many individuals per vial.

The adult Odonata were identified to species on site, producing a comprehensive species list with very little laboratory work. Larval Odonata were identified to species level where possible, but most often only to genus level (Samways & Wilmot, 2003). Adult Trichoptera were identified using expertise and resources in the Albany Museum. Larval Trichoptera were identified to genus or species level (de Moor & Scott 2003) and where possible, species identifications were made from further use of the literature (e.g. Barnard 1934a). Ephemeroptera were identified using expertise and resources from the Albany Museum and species level identifications were obtained for most specimens collected (Barber-James & Lugo-Ortiz 2003). Adult Ephemeroptera were rare and when collected imagoes were all identified to species from resources in the Albany Museum. The majority of the adult and nymphal Plecoptera were sent to Dr. Mike Picker for identification to species, but a few were kept and identified to species (Stevens & Picker 2003). The nymphs could only be identified to genus level with any certainty.

## **2.6 Analytical methods**

### **2.6.1 Physicochemical data**

The temperature and water chemistry data were organised and examined using Microsoft Excel®. In particular, the water temperature data from the i-Button loggers needed to be screened. Most often, the loggers were programmed before being placed into the stream and the data retrieved a while after being removed from the river. Thus, the recordings prior to and after placement in the river had to be removed. Data were easily stitched together as within a two-hour period the temperature of the logger and its housing had equilibrated to that of the stream, making it easy to see when the loggers were placed in the stream. Conversely, it was also simple to observe from the data when the loggers had been removed. Using these data, plots of temperature over time were drawn.

The water chemistry results were tabulated in Microsoft Excel® and analysed using Principle Component Analysis (PCA) in Statistica (Statsoft 2008). Due to the discrepancies in results obtained from Talbot & Talbot Laboratories over the different sampling periods, only parameters that were recorded for each sampling trip were used for the analyses. To use all four sampling trips' worth of data, a column entitled "Month" was added to the data sheet containing cases January/February, March/April, June/July and September/October (Tables 3 and 4). This was done to ascertain whether

the time of year might prove to be influential on the ordination. “Month” was chosen as the grouping variable for the PCA biplots. For the sake of understanding which chemical parameters were similar and different among the different sites along each river, several different PCAs were run. Firstly, a PCA using only the laboratory water chemistry results was run. This resulted in an illegible grouping of data points clustered around the centroid of the ordination axes with one or two points lying further away from the centroid. The outlying data points in a PCA give unusual weights to the remaining points in the central cluster, which would not otherwise account for much variation. By running several analyses using the same data but excluding the extreme values each time, the central cluster of sites over each sampling period, elucidated finer-scale relationships within the data that might otherwise have been characterised only by subsequent axes of the original analysis (Villet pers. comm.).

Following this, PCAs of on-site water chemistry were run including the variables pH, electrical conductivity and turbidity, also grouped by month, as these were also collected seasonally over the course of a year. The extreme results were again excluded, to enable discernment of finer scale differences between more similar sites. On-site data were also combined with laboratory analysed data and PCAs were run again excluding outlying sites as described above.

## **2.6.2 Analysis of macroinvertebrate assemblages**

### **2.6.2.1 Sampling assessment and species diversity**

To ascertain the thoroughness of sampling, rarefaction curves were constructed using EstimateS (Colwell 2006). A species by sample incidence matrix was constructed using Microsoft Excel®, including the abundance of each species. The samples used for creating this matrix consisted only of those collected by the intensive methods described above, allowing the organisms to be identified to species level which was not always possible from the SASS5 material. This data was saved in tab-delimited text file format for analysis using EstimateS. Detailed instructions for the layout of the matrix are supplied online by Colwell (2006). Sample-based rarefaction curves with 95% confidence intervals were constructed from this matrix using 999 randomizations when constructing the estimator curves to decrease the effect of sample order, by averaging over randomizations (Colwell 2009). Running many randomizations provides a smoother estimator curve and makes the comparison of curves or indices from the data possible and independent of the order in which samples were collected (Colwell & Coddington 1994, Colwell 2006).

Using PRIMER v6 (Clarke & Gorley 2001) Simpson and Shannon-Weiner index values were generated for each site as a means for making basic comparisons with other studies. These values were plotted using Microsoft Excel®. To get a more intricate measure of  $\alpha$ ,  $\beta$  and  $\gamma$  diversity, Partition 3.0 (Veech & Crist 2009a) was used to analyse data from each of the sites. The Partition package enforces diversity partitioning as a means of dividing the total diversity ( $\gamma$ ) into its constituent parts (Whittaker 1972). Furthermore, using Monte-Carlo permutations, Partition calculates expected values of  $\alpha$  and  $\beta$  diversity, allowing the significance of the observed diversity to be tested (Veech & Crist 2009b). Because the sampling design for this study was hierarchical, the data contained nested levels, the lowest level being that of the biotope from which the sample was collected. Unfortunately, diversity at the lowest level (biotopes within sites) could not be calculated because not all sites contained the same suite of biotopes. The three levels within the data used consisted of the site, the reach of the river (upper/lower) and season. The Bobbejaans and Matjies River sites also had to be removed from the data set because including them left an unbalance in the set of upper and lower sites, as they are tributaries of the Groot (West) and Buffels Rivers respectively and their 'lower' sites would therefore be duplicated in the data set. The data were collated using a pivot table in Microsoft Excel®, and analysed. No sample weighting was used and P-values were based on 1000 individual-based iterations.

#### **2.6.2.2 Multivariate analyses**

Non-metric multidimensional scaling (MDS) was used to analyse the faunal composition of the twenty sampling sites. A second MDS analysis was run excluding the upper and lower Buffels River and the upper Matjies River sites. MDS is a popular tool for examining the relationships of macroinvertebrate communities and a wealth of literature on the subject exists, e.g. Faith & Norris (1989); Boyero & Bailey (2001) and Soldner *et al* (2004). The statistical package PRIMER v6 (Clarke & Gorley 2001) was used for the analyses. The data were log-transformed as large variation existed within the data set, particularly in the abundance of some Caddisfly species such as *Athripsodes bergensis* Scott 1958 and *Chimarra ambulans* Barnard 1934 (Table 27). The data were converted from spreadsheet abundance form into a similarity matrix composed of Bray-Curtis similarity coefficient values. This index is recommended by Clarke & Gorley (2006) and has been shown to be robust in intensive macroinvertebrate studies (Uys 1997). To be confident of the solution obtained, 1000 random restarts were carried out while the minimum stress level was left at the default 0.01.

The relationship between species assemblage and environmental variables was investigated using Canonical correspondence analysis (CCA) and detrended canonical correspondence analysis (DCCA). These analyses were run as a means of direct gradient analysis using the statistical package CANOCO 4.5 (ter Braak & Smilauer 2002a). The CCA produced an ordination plot displaying the “arch effect” often obtained when analysing ecological data. Briefly, the arch effect is due to non-linearity in the data (Podani 2000) indicating the relationships between the species and environment are non-linear (unimodal) and based on strong environmental gradients that cause the species to be distributed unevenly within their habitat or range. The process of detrending the data, as in the DCCA, is to remove this arch effect, to display the gradient in a linear fashion (Podani, 2000) According to Whittaker (1967), the “arch effect” is encountered in ecological ordination whenever the species turnover ( $\beta$  diversity) changes relatively quickly along with environmental gradients.

As no light trapping was conducted during winter of 2008, the environmental variables were excluded for this time period for the CCA and DCCA analyses. The analysis therefore included environmental and species data from autumn and spring of 2008 and summer of 2009. The environmental data used in the CCA and DCCA included both quantitative and qualitative variables. The quantitative variables consisted of the water chemistry parameters measured on-site and those received from Talbot & Talbot laboratories (Table 30). The qualitative variables consisted of estimations of percentage bottom substrate made on site during sampling trips within the categories: bedrock, cobbles, small stones, sand and organic material (Dallas 2007). Marginal riparian vegetation was estimated as a dominance of, or a combination of, palmiet (indigenous macrophytes), alien vegetation or indigenous forest. A review of gradient analyses in general is given by ter Braak & Prentice (1988) and an excellent overview and explanation of the use of canonical correspondence analysis in aquatic ecology is given by ter Braak & Verdonschot (1995).

Inter-species scaling distances were emphasised for the CCA, using biplot scaling as opposed to Hill’s scaling, which were used in previous versions of CANOCO (ter Braak & Smilauer 2002b). The data were log-transformed, for the same reasons given above. The option to down-weight rare species was also chosen, as 28 of the 94 species in the analyses occurred at two or fewer sites. The forward selection of environmental variables was carried out manually and unrestricted Monte-Carlo permutation tests were enforced 1000 times to evaluate the influence of each environmental variable on the data set (ter Braak & Verdonschot 1995). Using permuted Monte-Carlo tests in forward selection, similar to *F*- or *t*- tests in multiple regression, allowed for a p-value for each environmental variable to be calculated and in this way the significance of each variable with respect to the species data could be estimated.

For the DCCA, detrending was carried out by equal width segments, according to the default setting in CANOCO 4.5 (ter Braak 1998; ter Braak & Smilauer 2002b), due to large variation in abundance values between species the species data were log-transformed and the rare species were down-weighted. Tests to evaluate the significance of the first ordination axis and the significance of the canonical axes together were carried out using Monte-Carlo permutations. A thousand unrestricted permutations were run under a reduced model (ter Braak & Smilauer 2002b). The CCA and DCCA were initially carried out using the species and environmental data from all twenty sites and thereafter using only seventeen of the sites, excluding the sites along the upper and lower Buffels and upper Matjies Rivers because of their extreme water quality characteristics.

---

## Chapter 3

### Results

---

#### 3.1 Physicochemical data

##### 3.1.1 Water temperature

Reasonably complete temperature records were obtained for most rivers (Figs 1-11). The exceptions (Figs. 1, 4, 6, 8, 10, 11) were due to several unanticipated events described below. The water levels in the lower Elandsbos and upper Bobbejaans Rivers (Figs. 4 and 8), in January 2008, when the loggers were placed, were unusually high (in excess of bankfull), resulting in an underestimate of the extent to which the water would recede; this led to the loggers being placed where they later became exposed above the river water level and records for March were lost (Fig. 4), and those for both February and March (Fig. 8) were unreliable. The site where the temperature logger was placed in the lower Groot (East) River (Fig. 1) dried up and flow ceased entirely from approximately the 5<sup>th</sup> of January 2009 until the logger was retrieved in March 2009, when flow had still not returned. The data recorded for the summer period of 2008/2009 from the lower Buffels River (Figs. 10 & 11) were lost due to logger malfunction. On more than one occasion, the logger at the lower Bloukrans River (Fig. 6) was removed from the river by members of the public, and then subsequently replaced. From the 16<sup>th</sup> February to the 17<sup>th</sup> of May 2008, the logger was removed and the stone detached, and then the logger and its pipe replaced. On the 1<sup>st</sup> of January 2009, the logger was removed from its housing completely and a note was inserted into the waterproof bag/bottle (along with some unidentifiable plant material). With the exception of the cases mentioned above, on average each data logger collected 4456 temperature recordings over a period of approximately thirteen months.

Several trends are apparent (Figs. 1-11; Table 2). First, there is an annual trend across the year, showing the logical progression of warmer temperatures in summer followed by cooling into winter and then warming through spring to the following summer. Superimposed on this are weekly cycles of temperature variation, correlated to the passing of cold fronts on an irregular basis. For example, a dip in temperature can be observed across every graph (where data are available and have not been lost), on the 25<sup>th</sup> of December 2008, indicating a decrease in temperature of at least 4°C over 4 hours. Another less obvious “cold snap” can be observed over the periods of 15<sup>th</sup>-17<sup>th</sup> March 2008, while a “spike” in warm temperature can be observed over the 20<sup>th</sup>-23<sup>rd</sup> of June 2008. At the finest



scale, daily temperatures cycles are also shown; those of the upper Bloukrans River are extreme, with changes of 6°C being observed during the course of a day/night cycle (Fig. 6).

### **3.1.2 Water chemistry**

The water chemistry data collected over the sampling period and received from Talbot & Talbot laboratories were collated (Tables 3, 4 & 5) and analysed using PCA. The eigenvalues for the PCA (Table 6) using all of the laboratory analysed water chemistry results (Tables 3 & 4) indicate that the first axis accounts for 41.6% of the variation and the first three axes collectively account for 78.9% of the variation within the data. This can be interpreted as three underlying variables accounting for nearly 80% of the variation (Manly 2005). The factor plot and ordination diagrams (Table 7, Figs. 12, 13 & 14) suggest that Nitrate/Nitrite and Orthophosphate are the water chemistry variables responsible for the Lower Salt River site standing out from the other points during the sampling periods of June/July and March/April, having factor coordinates for factor one of 0.029 and 0.107 respectively, and 0.751 and 0.801 for factor two respectively. Sulphate, Dissolved Magnesium, Chloride and Sodium are important in orientating the Matjies and Buffels River systems in the ordination plot over all sampling periods (Figs. 12 & 13) and cause these sites to group together in a staggered manner along the second axis. The concentrations of Total Iron, Total Zinc, Total Lead and Ammonia account for the rest of the sites grouping together very strongly at the origin of the axes (Figs. 12 & 13).

To explore the variation in the rivers other than the outliers distinguished in the initial analysis, a second PCA with a reduced number of samples was run (Tables 8 & 9). Similar to the PCA of all of the data described above, here 41.7% of the variation in the data was accounted for by the first principle component; and 78.8% was accounted for within the first three axes (Tables 8 & 9). The eigenvalues again decreased to below 1.00 upon reaching the fifth axis, which would suggest that any further signal in the data is “noise” (ter Braak & Verdonschot 1995). The ordination diagrams produced from this PCA (Figs. 14 & 16) give a clearer picture of the patterns in water chemistry for these rivers. The readings taken at the Lower Salt River during winter are abnormally high for Orthophosphate and to a lesser degree for Nitrate/Nitrite, compared to other rivers as well as to the same river and site over the rest of the year (Fig. 15). The Matjies and Buffels Rivers, but in particular the Matjies River, have unique water chemistry signatures when compared to the rest of the rivers (Fig. 14). This signature is characterised by an increased concentration of Fluoride, Sulphate, Dissolved Magnesium, Chloride and Sodium, while the remainder of the rivers appear to be poorer in these compounds.

The sites on the upper Bloukrans and the lower Salt Rivers ordinate strongly from the other sites on the second axis (Fig. 14). This is due to elevated levels of Total Iron in the upper Bloukrans River in the winter period, while the lower Salt River had elevated levels of Nitrate/Nitrite and Ammonia during the autumn period (Fig. 15 & 16). There is a trend in the ordination diagram that relates to season across all of the sites (Fig. 15). The Matjies and Buffels River sites illustrate that despite having different water chemistry signatures from the rest of the rivers, the concentrations of the chemicals remain relative to each other over a year. This indicates that there are spatial and temporal patterns of water chemistry variables within these rivers.

The final PCA carried out using the laboratory analysed water chemistry variables excluded the Salt, Matjies and Buffels Rivers, in order to elucidate finer-scale relationships between the remaining rivers. Fewer underlying variables account for the patterns in the data (Tables 10 & 11). Conversely, the variables influence the factors in a more uniform way, suggesting that the rivers selected for this analysis are more similar in their water chemistry than those conducted for the previous analyses (ter Braak & Verdonschot 1995). The first axis in this PCA accounts for 27.1% of the variation while the first three cumulatively account for 69.9%. The majority of the sites form a large, loosely clustered group (Fig. 17) and with the exception of the lower Groot (East) River and Lower Elands River sites, which form a discrete cluster in the fourth quadrant of the plot, no pattern is discernable. Differences in concentration of the combination of Sodium, Chloride, Dissolved Magnesium, and Sulphate and Nitrate/Nitrite compounds are responsible for the Lower Groot (East) River grouping apart over the entire sampling period while the Lower Elands River ordinated out as a result of elevated Nitrate/Nitrite concentrations during autumn (Figs. 18 & 19).

The results from the PCAs conducted on the on-site water chemistry results show similar trends to those from the laboratory analysed water chemistry results. The eigenvalues obtained for the PCA for all of the on-site data for all of the sites are 1.57, 0.94 and 0.48 for the three axes (Table 12). The factor coordinates and factor plot indicate that pH has a strong overall influence on the data, while turbidity and electrical conductivity are important in determining outlier sites (Table 13 & Fig. 21 respectively). The ordination diagram shows that the first axis accounts for 52.4% of the variation in the data and the second axis 31.4% indicating that the Buffels and Matjies Rivers are very different from the rest of the rivers based on the on-site water chemistry parameters (Fig. 20). Unlike the laboratory analysed water chemistry results, the time of year does not appear to have any particular bearing on the pH, turbidity or electrical conductivity in these rivers (Fig. 22).

The final PCA using the on-site water chemistry results was to explore if there were trends amongst the more acidic rivers that grouped together very closely, and thus excluding the Matjies and Buffels

River systems (Figs. 23 & 24; Tables 14 & 15). A seasonally categorised scatter plot ordinated the sites based on water chemistry variables differentiated by sampling period (month) (Fig. 25). The electrical conductivity in the upper Lottering River during autumn was unusually high (Figs. 23 & 25), comparable to the values measured at the upper and lower Buffels River sites over the same period (Table 5). Furthermore, the conductivity for some of the sites including the lower and upper Groot (East), upper Elands, lower Lottering and lower Bloukrans is also high during the winter sampling period. Other than these instances, based on the on-site water chemistry measurements, the majority of the sites clustered with no obvious trends in sampling period or water chemistry variables.

The final analyses of the water chemistry data included running PCAs on a combination of the on-site and laboratory analysed water chemistry results. The first PCA incorporated all of the sites over the entire sampling period; the eigenvalues for the first four axes were 5.53, 1.90, 1.59 and 1.30, after which the remaining ten fall below 1.00 (Table 16). The first axis, dominated by Fluoride, Sodium, Chloride, Dissolved Magnesium, Sulphate and Turbidity accounts for 39.51% of the variation while the second axis along which Nitrate/Nitrite and Orthophosphate are the main contributors only accounts for a further 13.64% (Fig. 26). This can be interpreted as the data indicating evenly distributed amounts of “large” variation (Manly 2005), illustrated by the factor coordinates for the variables on the second axis being close to 1.00 for the first factor while the second factor had scores close to 1.00 for Nitrate/Nitrite and Orthophosphate (Table 17). Thus, the lower Salt River site (for the winter sampling period) and the Matjies River sites lie far from the origin of the axes (Figs. 27).

As done for previous water chemistry analyses, the sites that produced extreme values were excluded systematically, starting with the Lower Salt River site (Tables 18 & 19). A very similar ordination pattern is shown to that produced for the laboratory analysed water chemistry values, with the Matjies and Buffels Rivers grouping separately to the rest of the sites along the first axis while the lower Salt River site for autumn stands out along the second axis (Figs. 28 & 29). There is an annual trend in water chemistry signatures (Fig. 30) but indicating that water chemistry remains relatively similar between rivers relative to the time of year (cf. Fig. 18).

Finally, the results of the PCA using a combination of the on-site and laboratory water chemistry parameters for the overall similar Groot (East), Elands, Storms, Elandsbos, Lottering, Bloukrans, Groot (West) and Bobbejaans Rivers suggest that the water chemistry signature of the Lower Groot (East) River and Lower Elands River sites are dissimilar (Figs. 31 & 32). Sodium, Dissolved Magnesium and Chloride concentrations are important in distinguishing the lower Groot (East) and lower Elands River sites from the rest of the rivers, while Nitrate/Nitrite and Orthophosphate are no longer of any

significance (Fig. 31). Seasonality also appears to have a slight yet discernable effect on the ordination; the data collected during the summer and spring form loose clusters along the second axis while the remainder of the sites are mixed (Fig. 33). Unlike previous analyses, the first six eigenvalues are all larger than 1.00 (Tables 20 & 21). This would indicate that there is not a strong signal within the data, and the differences elucidated by this PCA are only minor. Further evidence for this is that the first axis accounts for only 23.79% of the variation and the second 14.60%, again suggesting a lack of strong underlying variables within the data.

### 3.2 Macroinvertebrate assemblages

In total 71752 aquatic insects, incorporating various life stages from the five aquatic orders and including larvae and pupae from the family Simuliidae were collected and identified. Out of this total, 123 species were identified with certainty from 70 genera and 30 families. A total of 31 species were found to be undescribed and of these 17 were not previously collected and thus new to science (Table 36). Tentatively, four genera were also unidentifiable from available keys and expertise. All species are represented by actual abundance values from their respective site localities (Tables 22 - 28). The unidentifiable species and genera were assigned temporary names, and when informal drawings and descriptions were made of the specimens, the catalogue number from the first sample where the specimen occurred was incorporated into the name, for future reference.

#### 3.2.1 Ephemeroptera

The total number of Mayfly species collected came to 21 from 20 genera and six families (Table 22). The sites containing the highest number of Mayfly species were the upper Groot (East), upper Elandsbos, lower Groot (West) and the upper Salt River sites, each having 11 species, while the Elands River represented the poorest diversity, having two species collected along both the upper and lower reaches. The species common to the majority of the sites sampled were *Pseudocloeon vinosum* Barnard 1932 (Baetidae), occurring at 18 sites; *Baetis harrisoni* Agnew 1978 (Baetidae), occurring at 16 sites; *Lestagella penicillata* (Barnard 1940) (Teloganodidae) and *Castanophlebia calida* Barnard 1932 (Leptophlebiidae), occurring at 15 sites. Unique species included an undescribed species from the genus *Bugillesia* Lugo-Ortiz & McCafferty 1998 (Baetidae), collected from the upper Salt River. *Cheleocloeon excisum* (Barnard 1932) (Baetidae) was collected from the Matjies River alone, while the lower Storms River produced the only specimens of an undescribed species from the genus *Nigrobaetis* Novikova & Kluge 1987 (Baetidae). A single specimen of *Tricorythus discolor*

(Burmeister 1839) (Tricorythidae) was collected from the upper Bloukrans River during autumn, 2008, and was not collected again for the remainder of the sampling year. With the exception of *Lestagella penicillata*, the Teloanodidae species were never abundant when found, with the Upper Salt River producing three of the four undescribed teloganodid species and one undescribed teloganodid genus. The distribution of *Afroptilum sudafricanum* (Lestage 1924) (Baetidae), *Caenis capensis* (Barnard 1932) (Caenidae) and *Adenophlebia auriculata* (Eaton 1884) (Leptophlebiidae) recorded in this study would suggest that the Matjies and Buffels Rivers provide the most suitable environment for these three species. Though they did occur at a few of the other sites, they were not as abundant (Table 22).

### 3.2.2 Odonata

With permission from John Simaika, the adult Odonata that were collected and identified by him and a team of SANParks rangers are documented (Table 23). A total of 31 species were collected from 20 genera, from nine families. The most common species collected were *Orthetrum julia capicola* Kimmins 1957 (Libellulidae) and *Allocnemis leucosticta* Sélys 1863 (Platycnemididae), both occurring at 17 sites, followed by *Pseudagrion furcigerum* (Rambur 1842) (Coenagrionidae) occurring at 14 sites. Three species of Synlestidae were the next most common species, *Chlorolestes conspicuus* Hagen in Sélys 1862, *Chlorolestes umbratus* Hagen in Sélys 1862 and *Ecchlorolestes nylephtha* (Barnard 1937) occurred at 12 sites each. Unique or uncommon species are difficult to distinguish as 14 of the species were collected at two sites or fewer, making nearly half of the total number of species collected “rare” with respect to this study.

### 3.2.3 Plecoptera

A total of seven species of Stonefly from three genera could be identified to species level with certainty (Table 24); adult specimens are ideally necessary for accurate specific identification. Only a single family of Stonefly, of the two that occur in South Africa, has been recorded from the Cape viz. the Notonemouridae. The upper Storms River site contained the highest diversity of Stoneflies, with all three genera collected during this study represented at this site. This included the species *Aphanicerella bifurcata* Barnard 1934, *Aphaniceropsis outeniquae* Barnard 1934, *Aphanicerca capensis* Tillyard 1931 forms “S” and “O” (unique to this site) and *Aphanicerella nigra* Barnard 1934. Forms “S”, “O” and “P” of *Aphanicerca capensis* are considered to be undescribed species (Picker pers. comm.). *Aphanicerca capensis* form “P” was collected from the lower Elandsbos and the upper

and lower Bloukrans River during the spring season and from the upper Groot (West) and upper Elands Rivers in summer.

*Aphanicercopsis* Barnard 1934 was the most widespread genus across this study, occurring in all but one of the 20 sites, followed by *Aphanicercella* Tillyard 1931, collected from 16 sites and finally *Aphanicerca* Tillyard 1931, collected from 13 sites. *Aphanicercella bifurcata* was the most commonly collected species in its identifiable form (10 sites). The nymphs of the genus *Aphanicerca* were found throughout the year along several rivers being more abundant along the lower reaches of rivers.

### 3.2.4 Megaloptera

The Megaloptera were represented by a single family, the Corydalidae, and three identifiable species from three genera during the surveys (Table 25). Very recent work on the Megaloptera of the Western Cape region by Price (2010, unpublished) has resolved many of the problems in identifying these insects to species level. *Platychauiodes woodi* Barnard 1931 was the most commonly collected species, occurring at 13 of the 20 sites sampled. The larvae of *Platychauiodes* Esben-Petersen 1924 remained morphologically variable and were placed together as *Platychauiodes* sp1. *Taeniochauiodes ochraceopennis* Esben-Petersen 1924 was collected in the adult form during autumn of 2008 from the lower Lottering River and again in autumn of 2009 along the upper Bloukrans River while the larvae, distinguishable from the other species, were collected from 10 of the 20 sites. The third species, *Chloroniella peringueyi* Esben-Petersen 1924 was not collected in the adult form in this survey; as *Chloroniella* contains only a single species in southern Africa, the larvae collected from this genus were identified as *C. peringueyi*.

### 3.2.5 Trichoptera

The larval Trichoptera totalled 6740 specimens collected; 25 species were identified from 20 genera in 11 families (Table 26). Not all of the material collected could be identified to species level but the larvae provided valuable information regarding relative abundance and distribution data for the adult species that are “light-trap shy”. During the four surveys, a total of 42,683 specimens of adult Trichoptera were collected, representing 12 families, 21 genera and 47 species (Table 27). From a combination of the data, 17 of the species that were collected can be confirmed as undescribed, excluding two species from the genus *Oecetis* McLachlan 1877 (Leptoceridae) that were repeatedly collected as females and cannot be correlated to any described male species. Furthermore, an

undescribed genus of the family Hydroptilidae (Genus sp TSR152G) was collected in the larval stage from moss in current at the lower Bloukrans River site.

The most common Caddisfly species collected was *Athripsodes bergensis* (Leptoceridae) which occurred at every site sampled in large numbers, with the exception of the Matjies and Buffels River sites, and the Lower Elands River site (Table 26). Other widely distributed species included *Oxyethira velocipes* (Barnard 1934) (Hydroptilidae), *Dolophilodes urceolus* (Barnard 1934) (Philopotamidae), *Leptecho* sp SCR265K (Leptoceridae) and *Barbarochthon brunneum* Barnard 1934 (Barbarochthonidae), each occurring at 15 of the 20 sites, and *Hydroptila cruciata* Ulmer 1912 (Hydroptilidae), *Chimarra ambulans* (Philopotamidae), *Ecnomus similis* Mosely 1932 (Ecnomidae), *Athripsodes prionii* Scott 1958 (Leptoceridae) and *Oecetis modesta* (Barnard 1934) (Leptoceridae) occurring at 14 sites. Of the total 49 species of Caddisfly collected between adult and larvae, 16 were found to occur at two sites or fewer. Some of the unique species that occurred at only a single site include *Orthotrichia* sp SCR164A, Genus sp TSR152G (Hydroptilidae), *Chimarra cereris* Barnard 1934, *Chimarra georgensis* Barnard 1934 (Philopotamidae), *Ecnomus thomasseti* Mosley 1932, *Ecnomus* TSR440G (Ecnomidae), *Dyschimus* TSR28S (Pisuliidae), *Athripsodes* SCR258N, *Leptecho* TSR363H (Leptoceridae), *Petroplax prionii* Barnard 1934 and *Rhoizema montanum* Barnard 1934 (Sericostomatidae). Light trap samples from the upper Bobbejaans River recorded the highest number of adult Trichoptera species (25) followed by the Lower Storms River (21), Upper Elandsbos (19) and Upper and Lower Lottering River (18 and 17, respectively), Upper Salt River (16) and Lower Groot River West and Upper Storms River (15 each).

### 3.2.6 Diptera (Simuliidae)

The Simuliidae (Black flies) were represented by ten species from a single genus (Table 28). One of the species, collected from the lower Bloukrans River was found to be undescribed. A total of 9881 individuals were examined, of which *Simulium vorax* Pomeroy 1922 and *S. merops* de Meillon 1950 were the most common, occurring at 16 and 17 sites respectively and usually in high abundance (Table 28). *S. medusaeforme* Pomeroy 1920, *S. nigritarse* Coquillett 1902 and *S. rutherfordi* de Meillon 1937, each occurred at nine sites. The uncommon species include the previously mentioned *Simulium* TSR152F, found only on the Bloukrans River; *S. bequaerti* Gibbins 1936, found in low numbers at the lower Groot (East) and lower Elands Rivers; *S. dentulosum* Roubaud 1915, found in high numbers at the upper Bloukrans and upper Buffels Rivers. The sites that were the most diverse in Black fly fauna are the lower Groot (East), lower and upper Elands and lower Salt Rivers, each containing five species while the lower Elandsbos River recorded only one species, *S. merops*.

### 3.2.7 Sampling assessment and species diversity

The sampling assessment carried out to ascertain the thoroughness of sampling indicated that sampling effort was insufficient as the ideal asymptote of increasing sample number without an increase in the number of taxa was not reached (Fig. 34). The rarefaction curve slopes towards the asymptote at approximately 80 samples collected but continues to accumulate roughly another 50 species over the next 200 samples. This was however to be expected, as the samples used for the construction of the curves were carried out for the specific purpose of collecting as many species as possible i.e. sampling for maximum diversity, and therefore did not follow the strictly random sampling protocol that the analysis assumes.

Shannon-Wiener and Simpson indices are standard univariate measures of species diversity, where the main aim is to simplify the multivariate complexity of species assemblage data into a single index value (Clarke & Warwick 2001). The values generated from both indices indicate that the upper Matjies, Salt and Elandsbos sites are the most diverse sites while the lower Salt, Groot (West) and Lottering sites are the least diverse (Fig. 42).

The analysis of species diversity, by diversity partitioning, revealed no significant difference between observed species diversity and expected species diversity within sites ( $\alpha$ ) and between sites ( $\beta_1$ ); while significant differences were found between river reach ( $\beta_2$ ) and across sampling period ( $\beta_3$ ) (Fig. 35, Table 31). This would suggest that despite an enormous sampling effort, the full complement of species was not collected from each site, and also that each river reach has its own unique biotic signature.

### 3.2.8 Multivariate analyses

The results from the MDS analysis are presented in the form of a two-dimensional plot where sites are represented as points and the relative distance apart from all of the points on the plot represents the rank dissimilarities of the species compared across the sites, based upon Bray-Curtis index values. The plots are interpreted intuitively, where sites that are grouped closer together have similar species compositions, and thus index values, and sites that are further apart are increasingly dissimilar (Podani 2000). However, clusters were overlaid in PRIMER V6 at 20, 40 and 60% similarity (Figs. 36 & 37). A MDS analysis of all twenty sites provided further indication of the divergence of the Matjies and Buffels River sites from the remaining sites based on their biota, as they grouped together at 40 and 60% similarity. The upper Elands and Storms River sites stand apart from the



remaining sites in similar proportion to the Matjies and Buffels sites however they only group together at 40% similarity. The lower Salt, Elands and Groot (East) River sites group together at 40% similarity. The Lower Groot (East) is however also grouped together with all of the remaining sites at 40 % similarity, and within this grouping, only the lower Groot (West) and upper Bloukrans do not fall within the 60% similarity overlay. The 2-D stress level for this analysis was 0.11, indicating that the pattern can be interpreted with confidence (Fig. 36).

The exclusion of the Matjies and Buffels River sites for a second MDS resulted in the formation of three clusters at a 40% similarity level, consisting of the upper Elands and Storms river sites, the lower Elands and Salt River sites and the remaining sites within the study. As in fig. 36, the lower Groot (East), lower Groot (West) and upper Bloukrans river sites are the only sites that fall outside of the 60% similarity cluster formed by the remaining sites, the lower Storms, lower Bloukrans, upper Salt, upper Groot (East), upper and lower Lottering, upper and lower Elandsbos, the upper Groot (West) and Bobbejaans River sites. The 2-D stress level for this analysis was 0.10 (Fig 37).

The CCA including all sites produced similar results to those obtained from the water chemistry analyses. The eigenvalues from the analysis suggest a strong signal from the data, with the first three canonical axes matching well with the trends within the data, the first axis accounting for 48.9% of the variation and the remaining two a further 36.0 % (Table 32). The species collected primarily from the Buffels and Matjies River sites, *Cheleocloeon excisum* (Barnard 1932) (Baetidae), *Adenophlebia auriculata* (Leptophlebiidae), *Chimarra georgensis* (Philopotamidae) and *Simulium impukane* de Meillon 1936 (Simuliidae), amongst others, are aligned to the water chemistry values that characterise these streams (Fig. 38, Table 33). The environmental variables pH, Sulphate, Chloride, Fluoride, Sodium, Dissolved Magnesium and organic material (as bottom substrate) characterise these two rivers in the analysis, while conductivity, turbidity and Nitrate/Nitrite play less of a significant role. Of the remaining environmental variables, bedrock as the dominant substrate of the upper sites has a strong correlation with many of the species including both species of *Rhoizema* (Sericostomatidae), *Aphanicercella nigra* (Notonemouridae), the undescribed species of *Parecnomina* Kimmins 1957 (Ecnomidae) and the *Dyschimus* Barnard 1934 (Pisuliidae) species, to name a few (Fig. 38). The Monte-Carlo permutation tests indicate that only Total Zinc, pH, dissolved Magnesium, average stream width, organic material, Ammonia, Fluoride, and small stones have a significant ( $p < .001$ ) effect on the data though (Table 34).

The DCCA carried out using data from all twenty sites produced similar results as the CCA. The eigenvalues of the first three axes were 0.489, 0.152 and 0.095, indicating that nearly half the variation within the data can be accounted for by the first canonical axis. Furthermore the p-values

generated from Monte-Carlo permutation tests indicate that the first canonical axes, and the combination of all axes, are significantly correlated to the data (Table 32). The ordination plot shows the same pattern as that of the CCA but more conservatively because of the detrending of data. The lengths of the arrows representing environmental variables are considerably shorter, indicating that they carry less weight in the distinguishing species (ter Braak 1986). The grouping of the species from the more pH-neutral Buffels and Matjies Rivers and the lower Groot (East) River sites is obvious, along with the rest of the water chemistry variables characterising these sites (Fig. 39). There is also an inverse relationship between the influence of the environmental variable “bedrock” and pH.

Running the same CCA and DCCA whilst excluding all data for the Buffels and Matjies Rivers produced less distinctive results. The eigenvalues produced from the first canonical axes of both analyses accounted for less than 35% of the variation (Table 32). The Monte-Carlo permutation tests carried out to assess the significance of the correlation between the axes and the data indicated a strong correlation; P-values of 0.02 were obtained for tests of significance of the first axis and the total axes number (Table 32). The CCA ordination plot produced a circular distribution of species and environmental variables, with the exception of pH and “Bedrock” (Fig. 41). The Monte-Carlo permutation tests carried out in the forward selection of environmental variables process indicate that of the 22 environmental variables included, only the turbidity, Fluoride, marginal vegetation, Orthophosphate, Total Lead and Nitrate/Nitrite do not affect the data significantly (Table 34). The DCCA ordination plot produced similar results to that of the CCA. As in the previous analysis, the lengths of the environmental variable arrows were shorter with respect to the species points than those of the CCA plot (Figs 40 & 41).

---

## Chapter 4

### Discussion

---

#### 4.1 Physicochemical data

##### 4.1.1 Water temperature

The water temperature data collected using I-Button data loggers provided enormous amounts of data. Interesting and unusual findings are discussed. The variation in temperature on a daily basis would suggest that the logger at the upper Bloukrans River site had been exposed to air temperature (viz. figs 4, 6 & 8). This was however impossible as the logger was placed in a deep pool (the deepest pool visible in Plate 33) created from a fissure in the bedrock that drained via a waterfall over the edge of a cliff, thus making it impossible for the logger to be exposed, even if flow stopped completely. The alternative was a faulty logger where data had been corrupted and lost (Figs. 11 & 12). This is equally unlikely, as four different I-Button loggers recorded the same variation. Using a digital temperature meter, a difference of 6°C between the surface of the pool and approximately 1.5m depth was recorded, indicating the change in temperature was not an artefact of sampling. It is thus hypothesized that through the day/night cycle instead of mixing, due to density differences, warm and cool water replace each other (Schlichting & Gersten 2000). This creates a thermocline effect that moves up and down through the water column of the pool, causing the data logger to be exposed to a wide range of temperatures throughout the course of a day/night cycle. Furthermore, due to the major flooding event of November 2007, nearly all of the riparian vegetation upstream of these pools was removed, leaving the shallow river course exposed to direct sunlight. This in turn would cause the extraordinary heating shown at this site, when compared to other shaded sites.

The other rivers are similar in terms of their temperature profile and indicate that the majority of upper river sites tend to show less temperature variation over the year, remaining cooler in the summer and sharing approximately the same temperature as the lower sites during winter.

Several conclusions can be drawn from these temperature data. Firstly, if comparative studies are to be done, it is not as simple as putting a logger into the river under a boulder to prevent it from being washed away. Loggers should be of uniform depth and in areas of similar flow. If a logger is placed downstream from a weir or a large boulder, it will become covered in sediments which may have a

buffering effect leading to inaccurate measurement of water temperature. Furthermore, at sites where the river course is shaded it would be expected that the shade would also have a buffering effect; this can be seen when comparing the upper and lower Storms Rivers or the upper Bobbejaans River and lower Groot River (West) sites (Figs. 3 & 8, respectively). A quantitative analysis of the temperature data would involve the development of a way of characterising the rate of change of temperature at each site, providing a repeatedly measureable variable that can be related to the macroinvertebrate community. Extensive studies to that end have been undertaken (Vannote & Sweeney, 1980; Ritcher *et al* 1996; Rivers-Moore *et al* 2008). Studies have shown the significance of “maximum temperature” as a variable for accounting for increasing aquatic diversity. However, this lies outside of the scope of this thesis, as well as having been carried out on a far greater scale spatially (Jacobsen *et al* 1997). Qualitatively, though, the evidence is clear that each river temperature profile has an upper and lower distinction, and that these differ between reaches of different rivers as well.

#### **4.1.2 Water chemistry**

Multivariate analyses are useful for describing patterns in water chemistry, both for showing similarities between systems and to explore the extent to which the water chemistry variables are responsible for the similarities (Day *et al* 1998). The water chemistry data collected during this study were analysed based upon the type of measurements made; those collected at the site and laboratory analysed water samples. Thereafter, a combination of the data was subjected to the same analyses. The results from both sets of data, and logically the combination of the two, indicate that the chemical signature of the Matjies and Buffels Rivers differ vastly to the rest of the rivers sampled throughout the course of this study. Chloride, Dissolved Magnesium, Nitrate and Nitrite, Orthophosphate, Sodium and Sulphate levels are all comparatively higher, in some instances by orders of magnitude, than the majority of the remaining rivers. Coupled with this are consistently higher pH readings.

Chloride ions have no toxic effects on living systems, except where they contribute to increasing the total dissolved solid (TDS) content of the water and being involved in the ionic, osmotic and water balance of living organisms, they are essential ions (Dallas & Day 2004). The effects of the ions of Magnesium and Sodium on aquatic invertebrates are unknown and negligent respectively, although Sodium is of vital importance as a cation in nerve and muscle function in all organisms (Dallas & Day 2004). According to Day & King (1995), the rivers of the coastal regions of South Africa tend to be dominated by Chloride and Sodium ions, as opposed to Calcium, Magnesium and Bicarbonate ions in

rivers of the Highveld. Sulphate ions occur naturally in streams in concentrations usually lower than Chloride and Sodium, and similar to Sodium they are an essential part of proteins in living systems. Sulphates are generally non-toxic, but in excess, as is the case with acid mine drainage, they can form Sulphuric Acid (Dallas & Day 2004). Orthophosphate and Nitrate/Nitrite are major contributors to the eutrophication of aquatic ecosystems (Strum & Morgan 1996). Nutrients such as Orthophosphate and Nitrate/Nitrite occur in natural systems as particles or detritus as well as in the bodies of aquatic organisms, and enter systems via climatic sources or from the surface geology and land form of the particular catchment (Palmer *et al* 1996; Dallas & Day 2004). Anthropogenic sources of nutrients arise from either point sources, like sewage effluent, industrial discharge and intensive animal enterprises or from diffuse sources including agricultural surface run-off, disturbance of the soil mantle, fertilizers and urban run-off (Dallas & Day 2004). Contamination of aquatic ecosystems by Orthophosphate is most commonly from agricultural fertilizers (Palmer *et al* 1996; Strum & Morgan 1996). Nitrite is rapidly oxidised to Nitrate via microbial activity, while Nitrates in general are the product of the aerobic stabilisation of organic nitrogen (Palmer *et al* 1996; Lewis & Morris 1986). Both compounds are the product of sewage effluents, fertilizers and agricultural runoff (Dallas *et al* 1994).

The Buffels and Matjies catchment is impacted by extensive development in the Kurland area, which includes rural and urban settlements, industrial development, agricultural practices and recreational activities of which equestrian polo is the most important (de Moor *et al* 2004). Impoundments and water abstraction from both the Matjies and the Buffels Rivers also have a serious impact on water levels of these small rivers. During periods of drought, like those experienced over 2009 and up until March of 2010, water ceased flowing entirely, which has dire impacts on species that are not adapted to standing or stagnant water conditions (Hynes 1970). The differences in water chemistry found can also be attributed to the fact that the bottom substrate of the Matjies and Buffels Rivers is composed of soft Gydo Formation shales of the Bokkeveld Group and loamy soil whereas the substrates in the remainder of the rivers in this study are predominately of Table Mountain Sandstone (TMS) Group origin. The chemistry of natural river waters is predominantly determined by the composition of the underlying rock and soil formations, amongst other biotic and abiotic factors (Dallas *et al* 1994). The pH of the Matjies and Buffels rivers was on average neutral, which is most likely due to the buffering effects of Carbonate ions in the rocks and soil, which not only resists changes in pH but also tends to alkalinity (Hynes 1970), while the TMS group soils are leached and poorly buffered. The influence of the underlying geology of the river bed is also apparent in the appearance of the water. The Buffels and Matjies River water is clear unless mud is stirred up, whereas the rest of the rivers' in this study are tannin-stained and humic, increasingly so during

times of rainfall. Changes in pH due to anthropogenic influence are known to have detrimental effects on riverine biota (Guérol *et al* 2000; Braukmann 2001).

The data from the Salt River present the most likely case of nutrient enrichment from an anthropogenic source. In many ways it is a perfect example of a fast-flowing oligotrophic river and it has received the majority of the attention from previous studies for this reason (de Moor *et al* 2000, 2004). The Orthophosphate and Nitrate/Nitrite levels recorded during winter and autumn sampling periods indicate an influx of these compounds other than from a natural source, in which case the levels measured would be consistently high. Furthermore, the fact that only the lower site along the river showed these high concentrations indicates that they do not originate naturally from the source of the river. The high levels of these nutrients could be attributed to sewage effluents, fertilizers or agricultural runoff (Dallas *et al* 1994), and is probably due to agricultural practices and the fertilization of pastures and equestrian Polo fields in the Salt River catchment. The levels of Orthophosphate and Nitrate/Nitrite, despite being relatively elevated compared to other rivers studied as well as levels within this river at other times of the year, still remain well below the South African recommended guideline for the protection of aquatic organisms (Palmer *et al* 1996). However, the organisms that occur in these rivers have evolved under oligotrophic, acidic conditions, and the South African guidelines may not have been designed with such species in mind.

At a finer scale of analysis, once the sites contributing extreme values had been removed, the sites along the lower Groot (East) River and the Lower Elands Rivers were also found to have relatively atypical water chemistry signatures. This could also be attributed to human impacts on the coastal plain between the upper catchment and the lower reaches of the river. While the mountainous catchments of these rivers remain clear of any negative impacts, with the exception of the encroachment of alien invasive plant species that is beyond the scope of this study to measure accurately, the area between the foothills of the mountains and the lowest reaches of the river has extensive human impacts including forestry, agriculture and human settlement. However, the Elandsbos, Lottering, Bloukrans and Storms Rivers share the same anthropogenic impacts, and do not show any signs of degradation based on water chemistry. It is unclear why the lower reaches of these two rivers would be affected and the rest not. The high levels of Total Lead in the upper Bloukrans River (Table 5) are also mysterious.

## 4.2 Macroinvertebrate assemblages

From the outset of sampling, it was obvious that the majority of the sites selected for sampling were significantly affected by flooding during late November, 2007. The short and deeply-incised nature of the Tsitsikamma rivers would suggest that flooding events like this have played an important historical role in shaping the riverine ecosystem. What was taken into account, although it could not be quantified, was that the river systems had all been “reset” and were in a phase of ecological recovery. Unfortunately, measuring the effects of flooding, a topic reviewed by Gasith & Resh (1999), lay outside the scope of this study.

### 4.2.1 Ephemeroptera

The mayfly fauna has a combination of acidobiontic and more northern, tropical elements. While the diversity of species varies between the sites, there are discernable trends. *Baetis harrisoni* and *Pseudocloeon vinosum*, the cosmopolitan species common to most of the sites investigated, are known from rivers across southern Africa (Barber-James & Ortiz 2003). *Afroptilum sudafricanum* also has a wide distribution across Southern Africa. In the southern Cape river systems investigated, its relative abundance indicated a preference for the Matjies and Buffels Rivers (Table 22). Unique species included *Bugillesia* sp, collected only from the Upper Salt River. The only other record of this genus is from the Kruger National Park in northern South Africa (Gattolliat *et al* 2009) and it is otherwise known only from Central and West Africa. The Matjies River also provided the only record of *Cheleocloeon excisum*, but the species is known from many sites around South Africa, from the Western Cape, Eastern Cape and KwaZulu-Natal. The lower Storms River produced a species of *Nigrobaetis*, the closest relative to this being *Nigrobaetis bethunae* Lugo-Ortiz & de Moor 2000 from the Cunene River (Lugo-Ortiz & de Moor 2000); the other species in this genus are known from Sudan (Soldàn 1977), Madagascar and Reunion (Gattolliat 2004). The southern Cape species is certainly undescribed (Barber-James pers. comm.). A species from the genus *Cloeodes* Traver 1938 was collected from a number of sites, but only in low numbers; this was already noted from previous surveys (de Moor & Barber-James 2001; de Moor *et al* 2004), and is also almost certainly an undescribed species. The *Cloeon* Leach 1815 species was found across the length of the sampling area, from the Groot (East) River sites to the Matjies and Buffels Rivers; of the strongly acidic rivers, only the upper Lottering and lower Storms River sites also recorded specimens of this species though. The genus *Cloeon* is in need of revision and it is currently not possible to name species with certainty (Barber-James pers. comm.).

Caenidae were only abundant in the Matjies and Buffels rivers, and only *Caenis capensis* was collected during the survey period. A specimen from the genus *Barnardara* McCafferty & Provonsha 1995 was however collected in April 2004 from Site 4, on the Lower Salt River (de Moor *et al* 2004). The Caenidae are represented in South Africa by three genera, of which *Caenis* Stephens 1835 is by far the most diverse. The nymphs of this genus tend to be found in areas of low or no flow where they feed on fine particulate detritus (Barber-James & Lugo-Ortiz 2003), which would explain their prevalence in the Matjies and Buffels systems and their occurrence in the lower Groot (East) and lower Groot (West) River sites.

The Leptophlebiidae were represented by four identifiable species from four genera. *Aprionyx* Barnard 1932 is known only from southern Africa and contains eight species. Nymphs could not be identified to species level as a systematic revision of the group is needed, linking the life history stages (nymphs and adults). Three adults that were collected from the upper Groot (West) River were identified as *Aprionyx rubicundus* Barnard 1932. Nymphs of this genus prefer fast flowing, clean, mountain streams and are associated with areas of natural forest (Barber-James & Lugo-Ortiz 2003). This is supported by the findings of this study, as *Aprionyx* species was most common at the least disturbed sites where natural vegetation dominated (Table 22). *Castanophlebia calida* was abundant and collected at most sites excluding the lower Salt River site, the Elands River sites and (with the exception of a single individual) the Matjies and Buffels Rivers. *Adenophlebia auriculata* was confined to the Matjies and Buffels Rivers and was recorded only once on the lower Salt River. This would suggest that these species may have allopatric distributions within the Tsitsikamma region. *Adenophlebia peringueyella* Lestage 1924, a species better known from Western Cape rivers (Barnard 1932) was not recorded in any of the rivers in this survey but was recorded from the Salt River in previous surveys (de Moor *et al* 2004). In the Eastern Cape and KwaZulu-Natal, this species is replaced by *A. auriculata* and in some forested areas by *Adenophlebia sylvatica* Crass 1947. It is not clear what factors limit the distribution of *A. peringueyella* further eastwards. The higher pH recorded in the Matjies, Buffels and Lower Salt Rivers may have enabled *A. auriculata* to colonise these rivers. Molecular analysis of the *Adenophlebia* species would help to clarify this diversity, as some of the morphological identification criteria are obscure (Barber-James pers. comm.).

The family Teloganodidae are considered a cold-adapted Gondwanan relict, with other members known from Asia (McCafferty and Wang 1997; Sartori *et al* 2008), Australia and Madagascar (McCafferty & Benstead 2002). In South Africa, the Teloganodidae are known from four genera and five species, all endemic to the South Western Cape (Barber-James & Lugo-Ortiz 2003). This study has resulted in the collection of three further species, currently undescribed. All three were



collected from the Upper Salt River, and two were found at several other sites (Table 22). Two new species of *Nadinetella* McCafferty & Wang 1998 were recorded, one from a number of sites, and one only from the Salt River. A new genus of Teloganodidae was also noted from several rivers. *Lithogloea harrisoni* Barnard 1932 previously recorded on the Upper Salt River was not collected during this survey. *Ephemerellina barnardi* Lestage 1924 was very rare and recorded from upper sections of the Bobbejaans, Elandsbos, Lottering and Groot (East) Rivers. It was previously also recorded in greater abundance along the Upper Salt River during winter (de Moor *et al* 2004). All four known genera of the southern African Teloganodidae are recorded from the rivers flowing off the Tsitsikamma mountains and the upper Salt River records all of these from the present and previous surveys. The surveys undertaken thus far have shown that the Tsitsikamma region is the most species-diverse in Africa regarding this family. When examining the distribution of the teloganodid fauna within this study, it is clear that they are restricted to pristine, acidic rivers. No teloganodid nymphs were recorded from the Matjies and Buffels rivers as well as the Elands River with very few individuals of *L. penicillata* (the most common species collected) being found in the lower Salt, Groot (East) and upper Storms Rivers sites.

A single specimen of *Tricorythus discolor*, was collected from the upper Bloukrans River site. This species is relatively common in the Western Cape, where they can occur in abundance, the nymphs preferring rapidly-flowing and relatively large streams (Barber-James & Lugo-Ortiz 2003). *Tricorythus* species are widespread across Africa, but a revision of the generic placement is needed. *Tricorythus discolor* will need to be placed in a new genus along with a few other close relatives from other parts of southern Africa (Barber-James 2008).

#### 4.2.2 Odonata

The Damselflies (Zygoptera) and Dragonflies (Anisoptera) do not show the distinct distributional patterns amongst these rivers that the Mayflies do (Table 23). The Damselfly families collected during this study included the Coenagrionidae, Lestidae, Platycnemididae, Protoneuridae and Synlestidae. The Coenagrionidae are by far the most species-rich in the Tsitsikamma region, containing seven species from four genera. The widespread species include *Africallagma glaucum* (Burmeister 1839), *Ceriagrion glabrum* (Burmeister 1839), *Ischnura senegalensis* (Rambur 1842), *Pseudagrion hageni hageni* Karsch 1893, *Pseudagrion kersteni* (Gerstäcker 1869) and *Pseudagrion massaicum* Sjöstedt 1909 which occur elsewhere in South Africa and much of Africa; *Pseudagrion furcigerum* is however endemic to the southern Cape region (Tarboton & Tarboton 2005). Of these species, it is the endemic *P. furcigerum* that is most common, occurring at 14 of the 20 sites

surveyed as well as being nearly four times as abundant as any other coenagrionid species and the second most abundant species collected (Table 23).

The species collected from both the families Protoneuridae and Platycnemididae are endemic to South Africa, with the Platycnemididae boasting two endemic genera. *Elatoneura frenulata* (Hagen in Sélys 1860) is restricted to the South Western Cape, with the Tsitsikamma Mountains forming the eastern border of its range. *Allocnemis leucosticta* is distributed from the South Western Cape to the Soutpansberg and was the most commonly collected and widely distributed, species within this study. The lestid damselfly, *Lestes plagiatus* (Burmeister 1839) has a widespread distribution and is known to occur as far South West as Knysna (Tarboton & Tarboton 2005), but was only collected from the upper and lower Groot River (East) sites within this study. What has restricted its distribution further west into the Tsitsikamma region is unclear, although this is most likely an artefact of sampling.

The Synlestidae were represented by four species in this study, three from the genus *Chlorolestes* Sélys 1868 and one from the genus *Ecchlorolestes* Barnard 1937. Out of nine species of synlestid that occur in South Africa, eight are endemic to the CFR, with the majority of these having very restricted ranges (Samways 2008). *Chlorolestes conspicuus* and *C. umbratus* were the most common synlestid species, both occurring at twelve sites but neither species was collected from the Matjies and Buffels Rivers. *C. tessellatus* (Burmeister 1839) was not as common as these species, occurring at only six sites, three of which were the upper and lower Buffels and upper Matjies River sites. This may be due to the tolerance of this species to a wider range of habitat conditions, as seen from its distribution pattern across South Africa from the Cape through to KwaZulu-Natal, while *C. umbratus* and *C. conspicuus* are restricted to the southern Cape and coastal Eastern Cape, and the southern Cape respectively (Tarboton & Tarboton 2005). *Ecchlorolestes nylephtha* is a narrow endemic known primarily from the Tsitsikamma region (Tarboton & Tarboton 2005). During this study it revealed a similar distribution pattern to other endemic species, occurring at twelve sites, further indicating that where the endemic species occur, they are the most common and are restricted to a very specific suite of environmental conditions, which has serious implications for conservation of these endemic species.

The Aeshnidae, represented by three species from two genera, were not abundant anywhere; this is to be expected of large territorial, predatory taxa (Moore 1952; Corbet *et al* 1960; Kormondy 1961). Both *Aeshna minuscula* McLachlan 1896 and *Aeshna subpupillata* McLachlan 1895 are endemic to South Africa, where *Aeshna minuscula* has a more restricted (disjunct) range, occurring in the Drakensberg and the South Western Cape. The remaining Aeshnid species, *Anax speratus* Hagen

1867 is cosmopolitan in distribution and common from the Cape through to central East and West Africa in areas of high rainfall (Tarboton & Tarboton 2002). The two species of Corduliidae, *Syncordulia venator* (Barnard 1933) and *Syncordulia gracilis* (Burmeister 1839) are both South African endemics, the former being a Cape endemic. These species occur sympatrically in the Tsitsikamma region as both were collected from the lower Storms River, a site that was particularly rich in dragonfly species (Table 23). Only one species of Gomphidae was identified during the survey, *Ceratogomphus triceraticus* Balinsky 1963. Collected from the lower Bloukrans River site, this species is very shy and not easily caught (Samways 2008) and could possibly occur elsewhere in the Tsitsikamma region as gomphid nymphs were collected from the upper Lottering River but could not be identified to species.

The Libellulidae collected represented seven genera, *Crocothemis* Brauer 1868, *Nesciothemis* Longfield 1955, *Orthetrum* Newman 1833, *Palpopleura* Rambur 1842, *Sympetrum* Newman 1833, *Tramea* Hagen 1861 and *Trithemis* Brauer 1868. Two species *Crocothemis erythraea* (Brullé 1832) and *Crocothemis sanguinolenta* (Burmeister 1839), are common and are distributed throughout South Africa and extend north into Africa, with *C. erythraea* also extending into Europe (Samways 2008). Despite this, these two species were only collected from a total of five sites, *C. erythraea* from three and *C. sanguinolenta* from two. Their distributions within the study did not overlap though (Table 23). *Nesciothemis farinosa* (Förster 1898) was collected from two sites, and like the *Crocothemis* species, is also a cosmopolitan in its distribution, ranging from Cape Town to southern Arabia, despite being rarely collected during this study. The *Orthetrum* species, consisting of *O. abbotti* Calvert 1892 and *O. julia capicola* showed very different trends in their distributions within this study. *Orthetrum abbotti* was recorded from one individual from a single site, the upper Elands River while *O. julia capicola* was the most widely distributed species, occurring at seventeen sites, along with *Allocnemis leucosticta*. *Orthetrum abbotti*, like all the Libellulidae discussed thus far, is a widespread species while the subspecies *O. julia capicola* is locally endemic to the South Western Cape region, west of Humansdorp, which lies on the far eastern edge of the Tsitsikamma mountains. East of Humansdorp, *O. julia falsum* Longfield 1955 occurs north through to tropical Africa (Tarboton and Tarboton 2002). *Palpopleura jucunda* Rambur 1842 and *Sympetrum fonscolombii* (Sélys 1840) were both only collected from the upper Elands River and lower Groot (West) River respectively. Both species are widespread and common along the south east coast of South Africa and into Africa, with *S. fonscolombii* occurring into Europe and Asia too (Samways 2008). Only a single species of *Tramea*, out of a possible two occurring in South Africa, was collected. *Tramea limbata* Desjardins 1832 is rare in the Highveld, but a common species throughout the coastal areas of South Africa, particularly northern KwaZulu-Natal. This species continues the trend of the cosmopolitan species

being locally rare, as only a single *T. limbata* specimen was collected from a single site, the lower Groot (East) River, a similar situation to *O. abbotti*, *S. fonscolombii* and *P. jucunda*. A total of three species were collected and identified from the genus *Trithemis*, viz. *Trithemis arteriosa* (Burmeister 1839), *Trithemis furva* Karsch 1839 and *Trithemis stictica* (Burmeister 1839). All three species are described as very common throughout South Africa (Samways 2008), but *T. furva* and *T. stictica* occur at fewer than half the sites within this study making these two species exceptions from the general trends described above.

#### 4.2.3 Plecoptera

The Stonefly fauna collected during this study showed some indication of distributional trends. The upper Matjies and Buffels River sites only recorded nymphs of the genera *Aphanicerella* and *Aphanercopsis*. The lower Salt River site was also found to be remarkably poor in the Plecoptera with only seven nymphs from the genera *Aphanicerca* and *Aphanercopsis* collected during the entire study. Three species morphologically similar to *Aphanicerca capensis* (form O, S and form P) are all considered as valid undescribed species (Picker pers. comm.). The *A. capensis* form P was the more commonly encountered species and during this survey it was recorded only in the Spring season from the lower Elandsbos and the upper and lower Bloukrans Rivers. It was previously also recorded along the Salt River. *Aphanicerca capensis* form S was only recorded from the upper Storms River site, while *Aphanicerca capensis* form O was collected twice, from the upper Salt River site and again the upper Storms River site. The nymphs of *Aphanicerca* spp. that could not be placed into species were found throughout the year along several rivers being more abundant along their lower reaches.

*Aphanicerella bifurcata* was found throughout all seasons at both upper and lower sites on a number of rivers (Table 24). The much rarer species *Aphanicerella nigra* was only collected twice, once from a Malaise trap set along the Upper Storms River site and once from a light trap set along the upper Lottering River site. This species was, however, also previously collected from the Salt River during the Winter season together with *Aphanicerella cassida* Barnard 1934, which was only collected once during this survey from the lower Groot (West) River (Barber-James, 2000; de Moor and Barber-James, 2001). Nymphs of *Aphanicerella* were recorded along most rivers during the surveys. The nymphs of the genus *Aphanercopsis* sp. were the most abundant of all notonemourid species recorded. Adults in this genus confirmed as *Aphanercopsis outeniquae* were collected from the Upper Groot (West) River, Lower Lottering River and Upper Storms River in summer and autumn. They were also collected from the Salt River in a previous survey. The adults collected do

not fit the description of this species perfectly, so it may be that this abundant species represents either a new species or a divergent population of this species.

A significant finding was that not a single Stonefly species was collected from the lower Elands River site. Although only seven species of Notonemouridae have been recorded from the Tsitsikamma rivers, three of these are considered as undescribed species. It is noted that there is a high level of endemism in the Notonemouridae and to ensure survival of species, pristine conditions in rivers where they are found should be preserved. Stevens & Picker (2003) note that *Aphanicercella nigra* and the different forms of *Aphanicercella capensis*, to be described as new species, should be candidates for inclusion in a red data list for Plecoptera. This would hopefully lead to recommendations regarding conservation and preservation of the catchment where these species are found.

#### **4.2.4 Megaloptera**

The distribution of the *Platychauliodes* species recorded in this study suggests that they are the most adaptable species, occurring across the variety of water chemistry conditions found in these rivers. The genus was recorded from all twenty sites within this study, in either larval or adult form (Table 25). The remaining two megalopteran genera, each containing a single species, *Taeniochauliodes ochraceopennis* and *Chloroniella peringueyi* shared similar distributions, with neither species occurring in the Matjies and Buffels, nor the lower Salt, lower Groot (East) and upper or lower Elands River sites.

All three of the above mentioned genera have been recorded in the southern Cape (Barber-James 2000; de Moor & Barber-James 2001; de Moor *et al* 2004). The family was last worked on and had South African species described by Esben-Peterson (1924) and Barnard (1931, 1940). A revision of the South African Megaloptera is currently being undertaken allowing larval and adult stages of these insects to be correlated (Price pers. comm.). Megaloptera are considered a relict group with a restricted distribution in the mountainous regions of the western, southern and eastern Cape, KwaZulu-Natal and Mpumalanga.

#### **4.2.5 Trichoptera**

During the four surveys undertaken, a total of 51 species of Caddisfly were identified, of which 17 are undescribed. This study adds a further seven undescribed species and more material of some of the undescribed species that were collected in previous studies on the Salt River. Four species

collected along the Salt River previously were not recorded during the three surveys of adults conducted during the present study (Barber-James 2000; de Moor & Barber-James 2001; de Moor *et al* 2004). The summer period (January 2009) produced the greatest diversity (38 species) and largest number of Trichoptera specimens collected. The autumn period (April 2008) produced 31 species and 17,418 specimens and the spring period (October 2008) only recorded 21 species, comprising 1184 individuals. The cooler weather in spring undoubtedly influenced the success of light trap collecting with only Chironomidae being collected at the Lower Elands and Upper Groot (East) River during this time. This resulted in these sites recording lower levels of species diversity during spring; however due to sampling taking place throughout the year, and the inclusion of different sampling methods both for aquatic and adult stages, this problem was overcome.

The species of Trichoptera collected are listed under two tables, one for the larvae and another for the adults (Tables 26 & 27). Unlike adult Odonata, which are known to make extensive migrations owing to their good dispersal abilities (Kormondy 1961; Wikelski *et al* 2006), adult Trichoptera are not equipped for long-range dispersal, at least not within the life span of the adult insect. With this in mind, the distributions of the species collected from light trapping can be considered to portray the distribution quite accurately, in terms of residency, of the larvae within streams. Furthermore, the light traps used for collecting adult specimens were not equipped for attracting insects from long distances and yet still produced very large numbers, again suggesting residency of the species collected.

During this study, the family Glossosomatidae was represented by a single species, *Agapetus murinus* (Barnard 1934). This species is endemic to the South Western Cape, and is found in swift flowing mountain streams (Barnard 1934a). Within this study, *A. murinus* was fairly widespread, occurring at twelve sites and absent from the larger slower flowing rivers as well as the Matjies and Buffels Rivers. It was also absent from the upper Bloukrans River site, but this is postulated to result from disturbance due to flooding in November 2007, which modified the upper Bloukrans River tributary drastically by removing marginal vegetation and scouring the bottom substrata. *Agapetus murinus* was later collected approximately one kilometre upstream of the sampling site along the same stretch of river, where natural riparian vegetation still occurred, creating a forest canopy.

Hydroptilidae were collected from the majority of the sites sampled. *Oxyethira velocipes* was the most common species, occurring at fifteen sites, while *Hydroptila cruciata* occurred at fourteen sites. Neither of these species parallel local distributional trends that have been observed in other taxa thus far, as both occur in the Buffels River and in other oligotrophic, humic acid dominated rivers, such as the Bobbejaans River (Table 27). The three remaining species, *Orthotrichia barnardi*

Scott 1963, *Orthotrichia* sp SCR164A (previously collected) and *Genus* sp TSR152G (unplaced genus) are considered to be rare, as only *O. barnardi* was collected from more than one site. Because of the diminutive nature of these micro-caddisflies, it is probable that further species and genera await discovery, especially if a thorough search of hygropetric biotopes is made. The hydroptilid species collected during this study, for which there are distributional data, are all widespread species occurring elsewhere in South Africa (de Moor & Scott 2003).

The most commonly collected species of Philopotamidae was the southern Cape endemic *Dolophilodes urceolus*. This species shares a similar distribution within this study to *A. murinus*, with both species being absent from the same sites (Tables 26 & 27). *Chimarra ambulans*, which occurs in rivers along the South and East coast of South Africa, in hydrobiological regions “A” and “E” (Harrison 1959), was significantly more abundant than any other philopotamid species. The remaining *Chimarra* species, *C. cereris* and *C. georgensis* were collected only once from the upper Bobbejaans and Buffels Rivers, respectively. These species are known from rivers in the southern and eastern Cape, while *C. cereris* is enigmatically also reported from Zimbabwe (de Moor & Scott 2003). It is unclear why these species were so rare during the course of this study. One possibility is that the adults do not come to light traps as readily as the adults of *C. ambulans*, and are therefore not being collected in their identifiable adult stage. It is possible that both species were collected as larvae from other sites but were not identified, though this is unlikely because a thorough examination of the philopotamid larvae was made to look for errors in identification between this family and the similar-looking species from the Ecnomidae and Polycentropodidae.

The Hydropsychidae were distributed across the Tsitsikamma Rivers according to three patterns. *Sciadorus obtusus* Barnard 1934, in general, was collected from the acidic rivers and was not recorded in the Matjies and Buffels Rivers at all. *Macrostemum capense* (Walker 1952) had the most restricted distribution, occurring in the most eastern site, the lower Groot River (East) and then again in the Buffels River. The three *Cheumatopsyche* Wallengren 1891 species were distributed in a more general fashion, all three occurring in the Matjies and Buffels Rivers and in the remaining acidic rivers. It is interesting to note that the lower Groot (East) River produced all three genera of hydropsychid, indicating that this site contains the habitat requirements for species from all three genera. Conditions are not too acidic or too alkaline; with enough SIC biotope (where the two species of *Cheumatopsyche* and *Macrostemum capense* were collected most often) and moss-in-current (MIC) biotope (where *Sciadorus obtusus* was collected from most often) for all four species to occur sympatrically at certain sites.

Two genera of Ecnomidae and only a single genus and species of Polycentropodidae were collected during this survey. The polycentropodid, *Paranyctiophylax* SCR213T, was collected from the lower Bloukrans, Storms and Groot (East) Rivers only. This species, collected from the previous surveys along the Salt River, seems to prefer wider and relatively slower flowing river reaches, as opposed to mountainous streams. According to current literature (de Moor & Scott 2003), the family Polycentropodidae was not recorded from the Tsitsikamma region, or from hydrobiological region “A” (Harrison 1959). The Ecnomidae, represented by six species from this study, only two of which were collected at more than five sites, are family that has not been well documented within South Africa (de Moor & Scott 2003). The species, *Parecnomina* TSR545E and *Parecnomina resima* Morse 1974 were collected predominantly from upper river sites, and follow the already well-established distributional pattern, not occurring in the Matjies and Buffels Rivers, preferring undisturbed and acidic river reaches. The genus *Ecnomus* McLachlan 1864, the most species-diverse ecnomid genus in Southern Africa, recorded four species, of which *Ecnomus similis* was the most common, occurring from the Matjies and Buffels Rivers through to the Groot (East) River. The remaining three species were uncommon and rarely collected. *Ecnomus thomasseti* and *Ecnomus* TSR440G were collected from only the lower Groot (East) River site, along with *E. oppidanus* Barnard 1934, which was also collected from the lower Groot (West), Elands and upper Bobbejaans River sites. Aside from the previously uncollected and thus undescribed species, the *Ecnomus* species are all widespread occurring as far north as Lake Kariba (McLachlan & McLachlan 1971) while the genus *Parecnomina* (the taxonomy of which needs to be re-examined) is endemic to the South Western Cape (de Moor & Scott 2003). This makes the *Parecnomina* species that occur in the Tsitsikamma region important from a conservation perspective.

A single species of Dipseudopsidae, *Dipseudopsis capensis* Walker 1852, was collected from three lower river sites. Intuitively, this is to be expected as *D. capensis* is a burrowing species that prefers areas of sedimentation or deposition within a river course, and the relative dominance of bedrock at many of the upper sites may preclude this species from occupying upper river reaches in the Tsitsikamma Mountains (de Moor pers. comm.). *Dipseudopsis capensis* is widespread throughout Africa, yet another undescribed species and genus of Dipseudopsidae had previously been collected from the Salt River and its tributary, the Hol River, but was unfortunately not collected during this survey (de Moor *et al* 2004). The two most abundant species of Pisuliidae showed a similar distributional pattern to *A. murinus*; as *Dyschimus* TSR28S was collected only from the upper Storms River, it does not contribute meaningfully to overall distributional trends among the macroinvertebrates collected. *Dyschimus collyriifer* Barnard 1934 and *Dyschimus* TSR248F however strengthen the hypothesis that the lower Groot (East), Elands and Matjies and Buffels River sites do



not have the suitable habitat requirements for these species. It should also be noted that all three species of Pisuliidae are endemic to the southern Cape, and two of them have only ever been collected from the Tsitsikamma region.

The southern Cape, or Hydrobiological region “A” (Harrison 1959), records the highest level of endemism within the Leptoceridae in southern Africa with 75% of *Athripsodes* Billberg 1820 species occurring only in the Cape floristic region. The genus *Leptecho* Barnard 1934 is also endemic to the region (de Moor & Scott 2003). Of the total number of leptocerid species collected, not including “*Oecetis* sp” and the “Twisted case *Leptecho*”, which represent unidentifiable yet distinct larvae, only two of the eighteen species are not endemic to the region, *Oecetis modesta* and *Athripsodes harrisoni* (Barnard 1934) (de Moor & Scott 2003). Of the remaining species, it is surprising that *Athripsodes bergensis* is an endemic species as it the most abundant species within the majority of these rivers with the exception of the Matjies and the Buffels Rivers and upper Elands River (Table 27). What makes *A. bergensis* so successful is unclear; this could potentially be a result of this species being at an advantage as many of the rivers are in a phase of recovery, with the flooding during November, 2007, representing a major disturbance event. As this is an endemic species and is not abundant at all sites throughout this study, it cannot be classed as “weedy”. The abundance of this species at particular sites might point to healthy ecosystem functioning, and where it is less abundant or uncommon within the Tsitsikamma region, the opposite could be inferred. The remaining leptocerid species do not show any specific distributional trends that would signify a correlation to water chemistry, disturbance or otherwise; the majority of the leptocerid species occurred at less than half of the sites.

In the case of *Barbarochthon brunneum* only a single adult from the upper Bobbejaans River site was collected. When the occurrence of the larvae of this species are taken into account however, *B. brunneum* was the fourth most common species collected based on relative abundance figures (Tables 26 & 27). What is also immediately obvious when examining the trichopteran data is that *B. brunneum*, like all species of the Mayfly family Teloganodidae for example, does not occur in the Matjies and Buffels Rivers and the lower Elands and Groot (East) River sites. When relative abundance values are taken into account, where a higher abundance suggests the species are more suited to the environment and vice versa, further similar patterns exist where *B. brunneum* and *L. penicillata* both occur in low numbers at the lower Salt, upper Bloukrans and upper Storms River sites. It should also be noted that the collection of *B. brunneum* along the lower Salt River is enigmatic as this species was never previously collected this far downstream of the Salt River despite numerous surveys (Barber-James 2000; de Moor & Baber-James 2001; de Moor *et al* 2004).

The single species of Petrothrincidae, *Petrothrincus demoori* Scott 1993 shares a nearly identical distribution to *B. brunneum*, the difference being that *P. demoori* was not recorded at the lower Salt, lower Bloukrans and lower Storms River sites. The lower Elandsbos and Lottering River sites provided an abundance of specimens of this species though. The reason for this could be due to the fact these are not actually “lower sites”, considering that the upper and lower sites selected on the rivers show quite an altitudinal range overlap as regards upper and lower river site designations (Table 35). It is thus appropriate to bear in mind the altitudinal differences for the sites along each river. Sites on the upper and lower Buffels and Matjies Rivers differed by only 29 metres (61 and 32 masl) whereas the upper and lower Groot (West) River differed by 300m (313 and 13 masl). This was a reflection on the nature of these rivers and the difficulty of getting access to sites containing the variety of biotopes necessary for sampling according to objectives of this survey.

The Sericostomatidae were never abundant in the adult form; the undescribed species *Petroplax* SCR213F occurred at five sites and *Petroplax* TSR447E occurred at three. The third species, *Rhoizema montanum* was only collected from the upper Storms River site. The larvae of *Petroplax* Barnard 1934 were quite common, suggesting that the adults are either “light trap shy” or that they fly only under very specific conditions. The distribution of the sericostomatid genera resembles that of Glossosomatidae, Philopotamidae (*Dolophilodes*), Pisuliidae, Barbarochthonidae and Petrothrincidae, along with the Teloganodidae of the Ephemeroptera.

#### **4.2.6 Diptera (Simuliidae)**

Only the larval and pupal stages of Simuliidae were collected for identification during the course of this study. A total of 39 species are recorded from southern Africa, the rivers of the Tsitsikamma Mountains have produced nine of these (Palmer & de Moor 1998). The larvae of the undescribed *Simulium* species, collected from the Lower Bloukrans and Upper Groot (West) River was also previously collected in the Salt River (de Moor *et al* 2004). The presence of *S. vorax* indicates faster flowing conditions than that of the closely related *S. medusaeforme*. *Simulium dentulosum*, a cascade-loving species, was only recorded from the Upper Buffels and Upper Bloukrans Rivers. *Simulium hessei* Gibbins 1941, a rare species also found in cascades, is a South western Cape endemic (Palmer and de Moor 1998). It was found in very low abundance at four sites. *Simulium merops* is also a South western Cape endemic species that is restricted to cool, acidic waters and its distribution and abundance in samples collected confirms this (Palmer & de Moor 1998). The presence of both of these South western Cape endemic species indicates excellent water quality.

*Simulium bequaerti* is a warm-water species considered absent from the SW Cape. Its occurrence in the lower Elands River and lower Groot (East) River extends its western distribution range.

*Simulium impukane* and *S. rutherfoordi* are widespread mountain stream species found in slow-flowing water. The former species was restricted to the western rivers during these surveys while the latter species was more widely distributed and abundant. *Simulium nigritarse* is one of the most widespread and common species in South Africa and is found under a wide range of flow and water quality conditions. It is considered that this species may comprise a complex of up to 19 separate species that are very difficult to distinguish morphologically (Fain & du Jardin 1983).

#### **4.2.7 Sampling assessment and species diversity**

Despite carrying out intensive sampling to collect as many species from as many biotopes as possible, the sampling assessment curve suggests that the accumulation of uncollected species is still occurring at a steady rate (Fig. 34), because of a combination of factors. First, the inherent design of the sampling procedure: the purposeful search for as many different species as possible, despite being intensive, was not sufficient to collect all of the species that are expected (Chao 2005). Second, the collection of benthic macroinvertebrates that have complex life histories and in some cases strict seasonal preferences in their identifiable adult forms is difficult, and unless the right place is sampled at precisely the right time, species are missed (Colwell & Coddington 2001; Samways *et al* 2010). In many instances light trap samples produced thousands of individuals of a single species while the remaining species were represented by comparatively few individuals. The case of *Barbarochthon* illustrates this point: only a single adult was collected at a single site from light traps throughout the entire survey, while the larvae were common at all but five of the twenty sites (Tables 26 & 27).

The Shannon-Wiener and Simpsons index values obtained must be interpreted with care, as these indices are affected by sampling effort and sample size respectively (Clarke & Warwick 2001). The reason why the lower Salt, Groot (West), Lottering and Storms river sites have diversity index values is because these sites are dominated by very few species within the data set. *Athripsodes bergensis* recorded values of 11200 and 10236 at the lower Lottering and salt sites respectively, while *Chimarra ambulans* and *A. bergensis* dwarf the remaining species in terms of abundance at the lower Storms river site (Table 27). Despite having higher actual species diversity at these lower sites (strictly speaking the number of species recorded at each site) the Matjies and Buffels sites have higher Shannon-Wiener and Simpsons index scores due to having better evenness amongst the

species within the data sets. As the aim of this study was to determine which sites were the most diverse in number of species, little emphasis should be placed on these indices.

The partitioning of total diversity ( $\gamma$ ) into its constituent parts  $\alpha$  and  $\beta$  in hierarchically-scaled data sets allows patterns of local, between-local and between regional diversity to be studied (Lande 1996; Crist *et al* 2003; Magurran 2004). It was originally postulated that the relationship between the various components of diversity was multiplicative, but later proposed that the relationship was additive, where  $\alpha + \beta = \gamma$  (Lande 1996 cited in Samways *et al* 2010). The difference between observed and expected  $\alpha$  diversity indicate that at the level of the site, higher amounts of species diversity are expected, suggesting that further sampling is required, but this difference is not significant (Fig. 35, Table 31). The observed diversity recorded between sites, between rivers reach and between sampling seasons is higher than expected at each level. Though the between-site diversity is not significantly higher, it suggests that each river has a unique signature, and that species composition differs between sites, along the length of a river and naturally across the seasons (Fig. 35 & Table 31). Ecological reasons for the differences in  $\alpha$ -diversity could be due to intraspecific aggregation of species, habitat selection or species having limited dispersal capability (Veech *et al* 2002). A study by Crist *et al* (2003), found similar results in the diversity of forest canopy beetle species sampled at four scales. It was reported that  $\alpha$  species diversity was lower than expected while  $\beta$  diversity levels increased with increasing sampling scale. Diversity partitioning has recently been suggested for use in the field of conservation planning, highlighting the importance of knowing how  $\alpha$  and  $\beta$  diversity is distributed within regional ( $\gamma$ ) diversity (Clarke *et al* 2010; Jost *et al* 2010)

#### **4.2.8 Multivariate analysis**

The non-metric multidimensional scaling analysis (MDS) of all taxa except Odonata at all of the sites produced results that reflect the obvious trends in the data that are visible from a cursory inspection of the species lists, as well as some interesting insights that are not immediately apparent (Figs. 36 & 37). The grouping of river sampling sites on the plot indicate relative similarity of rank coefficient values, in other words, sites that group closely together resemble each other in faunal composition (Clarke & Warwick 2001). The Matjies and Buffels River sites group closely together and nearest to these is the lower Salt River site. This similarity may superficially appear to be as a result of the geographic position of these sites leading to a similarity in taxa, as all four are situated together on the western end of the sampling region (Fig. 43). But, if the water chemistry results are taken into account (Figs. 13, 14 & 27), the grouping of these variables together is a reflection of the aquatic

macroinvertebrates' response to the water quality. A similar case can be made for the lower Elands, lower Groot (East) River and the lower Groot (West) River sites. These sites, along with the lower Salt River site, are true "lower sites" in terms of altitude and represent the slow flowing, depositional stages of the river and are characterised by large pools and few rapids or riffles. It makes sense that these sites would group together as they share similar biotopes and thus fulfil similar species habitat requirements. The upper Elands and upper Storms River sites stand apart from the remaining sites in a similar proportion to the Matjies and Buffels River sites, accompanied by the upper Bloukrans River site which is situated closer towards the main cluster of sites. The interesting similarity between these three outlying sites is that they are all tributaries of the main river course, and were sampled as the main river was either inaccessible or inappropriate. This suggests that that species assemblages vary within the reach of these rivers, and that stream order (Horton 1945, Rowntree & Wadeson 2000), should be taken into account when designating conservation effort. The remaining cluster of variables consists of ten sites, four lower and six upper. These sites are very similar in their faunal makeup and represent the species assemblages that are worth conserving, showing the highest numbers of undescribed species as well abundant numbers of locally endemic species.

The second MDS analysis, removing the Matjies and Buffels River sites to compare more chemically similar sites, showed the same trends as mentioned above (Fig. 37). The tributary sites, the upper Elands, Storms and Bloukrans River sites group apart from the central cluster while the lower Elands, Salt, Groot (East) and Groot (West) River sites are set apart without a particular orientation towards each other. A central cluster containing the same species rich sites as the previous analysis is formed, only slightly less closely clustered. The stress levels of both MDS analyses, 0.11 for the first and 0.1 for the second respectively, indicate that the plots ordinate well, and that useful interpretation of results can be made, however conclusions should be made coupled with the use of alternative techniques (Clarke and Warwick 2001).

The ordination triplots produced from the correspondence analyses (CCA & DCCA) are interpreted as follows: the species points in the diagrams are represented as weighted averages of the axis-scores of the sites that each species occurred at, indicating the relative locations of the 2-dimensional niche space of each species. The environmental variables are represented as lines leading to arrowheads whose coordinates on the plot indicate the correlation of the variable with the axes, the longer the arrow the stronger the correlation with the axis (ter Braak & Verdonschot 1995). A combination of neutral and near neutral pH values, relatively high levels of Sulphate, Magnesium, Fluoride, Chloride and Sodium and relatively elevated conductivity and turbidity

readings are characteristic of the lower Salt, Elands, Groot (East) Rivers and the Matjies and Buffels River sites. The species *S. rutherfordi* (Simuliidae), *O. barnardi* (Hydroptilidae), *P. woodi* (Corydalidae), *C. excisum* (Baetidae), *A. auriculata* (Leptophlebiidae), *S. impukane* (Simuliidae), *A. sudafricanum* (Baetidae), *C. georgensis* (Philopotamidae), *Oecetis* TSR 539K (Leptoceridae), *Oecetis* TSR513B (Leptoceridae), *C. capensis* (Caenidae), *Cheumatopsyche* TSR136E (Hydropsychidae), *Cloeon* sp (Baetidae), *A. harrisoni* (Leptoceridae), *E. similis* (Ecnomidae), *S. dentulosum* (Simuliidae), and *H. cruciata* (Hydroptilidae) share similar niche requirements, being tolerant to the water chemistry characteristics of these sites (Fig. 38). Further ecological relationships are suggested from the CCA relating the to the environmental variables “sand”, “small stones” and to a lesser degree Total Iron and Orthophosphate. *Macrostemum capense* (Hydropsychidae), *S. bequaerti* (Simuliidae), *E. thomasseti* (Ecnomidae) and *E. TSR440G* (Ecnomidae) were all collected from the lower Groot (East) River site which has a predominantly sand and small stone bottom substrate. Completely opposed to this in the CCA ordination diagram is the environmental variable “Bedrock”, representing a predominance of this substrate at a site. The species *Rhoizema* sp (Sericostomatidae), *A. nigra* (Notonemouridae), *R. montanum* (Sericostomatidae), *Dyschimus* TSR28S (Pisuliidae), *A. scramasax* (Leptoceridae) and the *Leptecho* twisted-case sp (Leptoceridae) were collected predominantly from upper sites characterised by primarily bedrock substrate. It is interesting to note though that the environmental variable Bedrock is not significantly correlated to the species data and yet many sites and species are orientated along its gradient on the triplot (Fig. 38).

The “arch effect” that was produced from the CCA was removed by detrended canonical correspondence analysis (DCCA). This method has been criticized as harsh and overzealous in its manipulation of the data (Pielou 1984), and as analogous to “attacking the data with scissors and glue” (Clarke & Warwick 2001). For this reason, conclusions drawn from these analyses are treated with caution. Fortunately, similar results were obtained from the CCA. The major ions mentioned before that separate the Matjies and Buffels Rivers from the remainder are placed together more closely along with conductivity, turbidity, Nitrate/Nitrite, sand, small stones and pH (Fig. 39). The effect of the environmental variables Total Iron and Total Zinc is more pronounced but similar to marginal vegetation, cobbles, average stream width and Orthophosphate, while Total Lead and Ammonia share species with the bedrock dominated sites. The nearly perfectly reverse relationship between bedrock and pH, is due to the fact that the bedrock dominated sites (mostly upper sites) are most often acidic and it is these sites that show the highest diversity of species represented by close clusters of species on the ordination diagrams (Figs. 38 & 39). The DCCA does show a more even distribution of species on the ordination plot, making it easier to interpret. On the left side upper quadrante, the ubiquitous species that occur across many rivers in South Africa and were

repeatedly collected from the Matjies, Buffels and Lower Groot (East) River sites, *C. excisum* (Baetidae), *A. sudafricanum* (Baetidae), *A. auriculata* (Leptophlebiidae), *C. capensis* (Caenidae), *M. capense* (Hydropsychidae), and *S. impukane* (Simuliidae), are represented.

Removing the Matjies and Buffels Rivers site data and repeating the same analyses resulted in a stronger influence from the environmental variables. In the previous CCA of all of the data, eight environmental variables had a statistically significant influence ( $P < 0.05$ ) on the data, whereas the CCA excluding the Matjies and Buffels Rivers' data indicated that fourteen variables had a significant influence on the data (Table 32). The reason for this is that the remaining rivers are more similar in terms of water chemistry and species assemblage and so the differences found when comparing data from each site are attributable to a wider variety of environmental influences. The eigenvalues further support this, only 31.3% of the variation is explained by the first canonical axis of the CCA, with the first three axes accounting for 62.6% of the variation. The significance of environmental variables, pH, sand, Dissolved Magnesium, small stones, Chloride and bedrock are, based upon the F-values, the six most important factors influencing the placement of the species on the ordination plot (Table 34). These variables point to the distinction between the species that occur at the lower Groot (East), Elands, lower Groot (West) and lower Salt Rivers (Fig. 40). This result is further supported by the DCCA carried out excluding the Matjies and Buffels River sites (Fig. 41). Though the eigenvalues generated from the analysis are very similar to those of the CCA excluding the Matjies and Buffels River sites, the canonical axis, and a combination of all the axes, is significantly correlated ( $p \Rightarrow 0.001$ ) to the data (Table 32). The tolerant, widespread species (listed above) occur on the left of the ordination plot along with the environmental variables small stones, sand, average stream width, Chloride, Sodium, Dissolved Magnesium, organic material, turbidity and conductivity. This relationship is not as clear as in the analyses using all of the data.

Carrying out several different multivariate analyses, PCA using the water chemistry data, MDS using species data and canonical correspondence analyses using a combination of both, has produced comparatively similar results. The Matjies and Buffels rivers stand apart from the remaining rivers quite clearly; the lower sites along the Groot (East), Elands, Salt and to a lesser extent Groot (West) Rivers, are also distinct, based on both their water chemistry and faunal composition.

---

## Chapter 5

### Conclusion

---

#### 5.1 Significance of findings

##### 5.1.1 Species diversity

During the course of previous studies in the region (Barber-James 2000; Bok 2000a; 2000b; de Moor & Baber-James 2001; de Moor *et al* 2004), a reasonably thorough inventory of aquatic macroinvertebrate species was compiled, for the Salt River in particular. In addition to what has already been recorded, two further Mayfly species were collected from the Salt River, bringing the total count to 24 species of Ephemeroptera, of which nine are undescribed, two from undescribed genera. No undescribed species of Odonata were collected; however, this survey represents the most comprehensive species list yet for the Odonata for the Tsitsikamma region. A further 18 Dragonfly species were added to the species count for the Tsitsikamma region, totalling 34 species collected to date. All of the Stonefly species that have previously been collected were once again found; in addition to this, a further three species of Plecoptera are added to the list of species for the region, two of which are as yet undescribed. The Megaloptera collected during this study amount to three species, adding a further two species to the last survey in the region (de Moor *et al* 2004). Only four species of previously-collected Trichoptera were not found during this study and 30 were re-collected, leaving a total of 21 species added to the inventory of Caddisfly species for the Tsitsikamma region, including a new species from an unidentifiable genus. An additional eight species and an undescribed species of Simuliidae were collected, producing ten species in total from the Tsitsikamma Rivers.

This study has led to a more comprehensive understanding of the diversity of aquatic insects in this region. As several species that have previously been recorded were not re-collected, and with the abundance of undescribed species and genera collected, there is clearly scope for further discovery. A potential shortcoming facing the collection of insects was cold weather, which resulted in some of the sites being poorly represented, in Trichoptera and Odonata particularly. Only sampling two sites along the length of a river is also prohibitive for the estimation of diversity, as it limits the resolution to which a system can be explored.



### **5.1.2 Macroinvertebrate assemblages**

From the results of the multivariate analyses carried out, it is clear that easily discernable patterns exist in the species assemblages of these rivers. These are attributable to differences in the water chemistry, which is influenced in turn by anthropogenic and natural influences. Similar patterns in species assemblage were found in surveys of acid streams in the western and southern Cape (Agnew & Harrison 1962) and of the Great Berg River (Harrison & Elsworth 1958). The groups of species that are described as “acidobiontic”, those that are endemic to streams associated with the Table Mountain Sandstone system, are distributed similarly within the rivers in this study. The macroinvertebrate assemblages recorded from the rivers of the Tsitsikamma region, despite lying near to the eastern edge of the Table Mountain Sandstone system, show the same patterns as those that occur in the rivers in the rest of the South-Western Cape. It is also noted that the relative abundances of the species recorded may not reflect natural conditions. As sampling took place soon after a very large flooding event, what has been documented could possibly be the ecological recovery phase of the river rather than its natural state (Gasith & Resh 1999, Death 2010).

### **5.1.3 Sites and rivers**

The sites sampled, and the river reach these sites were chosen to represent, show variation in their macroinvertebrate assemblages and water chemistry parameters. First-order streams in the upper catchment and river reaches in the mountain foothills (second and third order reaches) showed dissimilarities in species composition to the lower reaches. The least impacted areas of the river reaches, which lie at higher altitudes, and those that flow through undisturbed indigenous forest, cluster together closely in ordinations, sharing endemic acidobiontic species, while the impacted sites hold more widespread, eurytopic species. Furthermore, where these endemic species occur, in most instances they are the most abundant taxa, further suggesting narrow and highly adapted habitat requirements (de Moor 1992). These findings have a profound bearing on the conservation of these rivers in that the species that are found in the rivers of the Tsitsikamma mountains, the mountains themselves in excess of 300 million years old (McCarthy & Rubidge 2005), are sensitive to changes in water chemistry. Having evolved to cope with very clean, acidic, siltless water of low temperature, the impacts of human development quickly and detrimentally affect species composition. This has been found to be the case along the lower Salt, Elands and Groot (East) Rivers. The underlying shale geology of the Matjies and Buffels Rivers preclude them from realistic comparison to the rivers draining from Table Mountain sandstone. Human impacts through flow modification and nutrient enrichment are discernable in these rivers despite this. The rivers that

have no fish present (Bok 2000a; Bok 2000b; Russel 2002), have also shown the highest diversity of species from the aquatic orders. It has already been suggested that the southern Cape freshwater invertebrate fauna are vulnerable to predation by specialized insectivorous predators such as trout, as their behavioral patterns make them highly visible (de Moor 1992).

Allied to the recommendations of de Moor *et al* (2004), I suggest that special measures be made to conserve the upper catchments of the Storms, Elandsbos, Lottering, Bloukrans, Groot (West), Bobbejaans and Salt Rivers to protect the unique and endemic freshwater invertebrate fauna of the Tsitsikamma region. Areas of indigenous afrotemperate forest should be preserved as a matter of priority because the forests play an important role in the aquatic ecosystem functioning. Alien invasive species like Black Wattle, Pines, Gums and Silky Hakea need to be controlled and the river courses kept free of the detrimental effects of these plants.

#### **5.1.4 National and international significance**

Studies using the Trichoptera (de Moor 1999; de Moor & Scott 2003 and de Moor 2007) to illustrate the patterns in distributional trends of aquatic macroinvertebrates within South Africa have shown the southern Cape region to be the most distinct of all of the 12 hydrobiological regions suggested by Harrison (1959). When comparing the distributional ranges of the trichopteran species from the Salt River, de Moor (2007) found 58% to be Cape endemic species while 21% were wide spread and the remaining 21% were designated as insufficiently studied to be placed into either group. This study supports these findings, as the high number of undescribed species (33% of Trichoptera) and regionally endemic species warrants high conservation priority.

At an international scale, rivers of comparable Mediterranean climate occur in southwestern Australia, California, and the Mediterranean basin (Gasith & Resh 1999). Comparisons between the macroinvertebrate fauna of the Tsitsikamma rivers and rivers from these areas showed that at the family level, each has a unique suite of families endemic to each region while some families are very widespread occurring in almost all regions, e.g. Hydroptilidae. The South African fauna is more similar to northern hemisphere Mediterranean climate region than that of south-western Australia, suggesting active exchange between hemispheres and the possible extinction of shared taxa within the southern hemisphere while the fauna from California and the Mediterranean basin were the most similar (Bonada *et al* 2008). However, the South African and south-western Australian Mediterranean type climate rivers are both depauperate in Ephemeroptera and Plecoptera. Each region has only a single family of Stonefly while three families of Mayfly are represented in south-

western Australia and six in South Africa; California and the Mediterranean basin have ten and eleven families respectively. With the exception of the south-western Australian region, Mediterranean type climates are considered diversity “hot spots” with regards to their fauna and particularly flora.

---

## References

---

- Barber-James, H. M. 2000. Preliminary investigation of the freshwater macroinvertebrates in the Salt River, in relation to the proposed stocking of trout into the upper Salt River, Farm 236, The Craggs. Environmental Impact Assessment Report for Mr Peter Thorpe. Albany Museum, Grahamstown. 18pp.
- Barber-James, H.M. 2008. Chapter 14: A synopsis of the Afrotropical Tricorythidae. In: International Advances in the Ecology, Zoogeography and Systematics of Mayflies and Stoneflies. Hauer, F.R, J. A. Stanford and R. L. Newell (Eds.). University of California Press, Berkeley, California. 187-203pp.
- Barber-James, H.M. & Lugo-Ortiz, C.R. 2003. Chapter 2: Ephemeroptera. In: de Moor, I.J., Day, J.A. & de Moor, F.C. (Eds.) Guides to the freshwater invertebrates of southern Africa. Volume 7: Insecta I. Ephemeroptera, Odonata & Plecoptera. 288pp.
- Barnard, K.H. 1931. The Cape Alder-flies (Neuroptera, Megaloptera). *Transactions of the Royal Society of South Africa*, 19, 169-184.
- Barnard, K.H. 1932. South African May-flies (Ephemeroptera). *Transactions of the Royal Society of South Africa*, 20(3), 201-259.
- Barnard, K.H. 1934a. South African caddis-flies (Trichoptera). *Transactions of the Royal Society of Southern Africa*, 21 (4), 291-394.
- Barnard, K.H. 1934b. South African Stone-flies (Perlaria) with descriptions of new species. *Annals of the South African Museum*, 30(17), 511-548.
- Barnard, K.H. 1940, Additional records and descriptions of new species of South African Alder-flies (Megaloptera), May-flies (Ephemeroptera), Caddis-flies (Trichoptera), Stone-flies (Perlaria) and Dragonflies (Odonata). *Annals of the South African Museum*, 6, 609-61.
- Bok, A. 2000a. Impact of proposed stocking of trout into the upper Salt River, Farm 236, The Craggs. Environmental Impact Assessment Report. Anton Bok & Associates cc. Port Elizabeth. 19pp.
- Bok, A. 2000b. Additional (summer) fish survey of Salt River: addendum to impact of proposed stocking of trout into the upper Salt River, Farm 236, the Craggs. Environmental Impact Assessment Report. Anton Bok & Associates cc. Port Elizabeth. 11pp.

- Bonada, N., Rieradevall, M., Dallas, H.F., Davis, J., Day, J.A., Figueroa, R. Resh, V.H. & Prat, N. 2008. Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. *Freshwater Biology*, 53(4), 772-788.
- Boyero, L. & Bailey, R.C., 2001. Organization of macroinvertebrate communities at a hierarchy of spatial scales in a tropical stream. *Hydrobiologia*, 464(1), 219-225.
- Branch, W. & Hanekom, N., 1987. The herpatofauna of the Tsitsikamma Coastal and Forest National parks. *Koedoe*, 30(1), 49-60.
- Braukmann, U. 2001. Stream acidification in South Germany – chemical and biological assessment methods and trends. *Aquatic Ecology*, 35, 207–232.
- Brundin, L. 1966. Transantarctic relationships and their significance, as evidenced by chironomid midges, with a monograph of the subfamilies Podonominae and Aphroteniinae and the austral Heptagylae. *Kungl. Svenska Vetenskapsakademiens Handlingar*, 11, 1- 472.
- Chao, A. 2005. Species Richness Estimation. In: *Encyclopaedia of Statistical Sciences*. N. Balakrishnan, C. B. Read, & B. Vidakovic (Eds.) New York, Wiley, 7909-7916pp.
- Chutter, F.M. 1972. An empirical biotic index of the quality of water in South African streams and rivers. *Water Research*, 6, 19-30.
- Chutter, F.M. 1994. The rapid biological assessment of stream and river quality by means of the macroinvertebrate community in South Africa. 217-234 pp. In: Classification of rivers and environmental health indicators. Uys, M.C. (Eds.) Proceedings of a joint South African/Australian Workshop. Water Research Commission Report No. TT 63/94, Pretoria, South Africa.
- Chutter, F.M. 1998. Research on the rapid biological assessment of water quality impacts in streams and rivers. Water Research Commission Report No. 422/1/98. Pretoria, South Africa.
- Clarke, A., Mac Nally, R., Bond, N.R. & Lake, P.S. 2010. Conserving macroinvertebrate diversity in headwater streams: The importance of knowing the relative contributions of  $\alpha$  and  $\beta$  diversity. *Diversity and Distributions*, 16(5), 725-736.
- Clarke, K.R. & Gorely, R.N. 2001. PRIMER v6 PRIMER-E Ltd. Plymouth Marine Laboratory, United Kingdom. (Registered to Dr F. de Moor)
- Clarke, K.R. & Gorely, R.N. 2006. PRIMER v6: user manual. PRIMER-E, Plymouth, United Kingdom, 190pp.

- Clarke, K.R. & Warwick, R.M. 2001. Change in marine communities: An approach to statistical analysis and interpretation 2<sup>nd</sup> edition. PRIMER-E, Plymouth, United Kingdom, 172pp.
- Coetzer, A.H. 1978. The invertebrate fauna and biotic index value of water quality of the Great Berg River, Western Cape. *Journal of the Limnological Society of Southern Africa*, 4, 1-7.
- Coetzer, A.H. 1986. Benthic invertebrate communities and the biological assessment of the water quality of the Breede River during 1975 and 1976. *Bontebok*, 5, 42-51.
- Colwell, R.K. 2006. *EstimateS*: Statistical estimation of species richness and shared species from samples. Version 8.2.0. Persistent URL <purl.oclc.org/estimates>
- Colwell, R.K. & Coddington, J. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society (Series B)*, 35, 101-118.
- Corbet, S.P., Longfield, C. & Moore, N.W. 1960. Dragonflies. Collins, St James's Place, London. 260pp.
- Cowley, P.D., Brouwer, S.L. & Tilney, R.L., 2002. The role of the Tsitsikamma National Park in the management of four shore-angling fish along the south-eastern Cape coast of South Africa. *South African Journal of Marine Science*, 24(1), 27-35.
- Crawford, P., Crawford, S. & Crawford, R. 1983. Some observations on cape grey mongooses *Herpestes pulverulentus* in the Tsitsikamma National Parks. *South African Journal of Wildlife Research*, 13(2), 35-40.
- Crist, T.O., Veech, J.A., Gering, J.C. & Summerville, K.S. 2003. Partitioning species diversity across landscapes and regions: a hierarchical analysis of  $\alpha$ ,  $\beta$ , and  $\gamma$  diversity. *The American naturalist*, 162(6), 734-43.
- Dallas, H.F., 2007. The effect of biotope-specific sampling for aquatic macroinvertebrates on reference site classification and the identification of environmental predictors in Mpumalanga, South Africa. *African Journal of Aquatic Science*, 32(2), 165-173.
- Dallas, H.F., Day, J.A. & Reynolds, H.E. 1994. The effects of water quality variables on riverine biotas. Report to the Water Research Commission. Report No. 351/1/94. 230pp.
- Dallas, H.F., Day, J.A. Musibono, D.E. & Day, E.G. 1998. Water quality for aquatic ecosystems: tools for evaluating regional guidelines. Report to the Water Research Commission. Report No. 626/1/98. 240pp.

- Dallas, H.F. & Day, J.A. 2004. The effect of water quality variables on aquatic ecosystems: A review. Report to the Water Research Commission. Report No. TT 224/04. 221pp.
- Davies, B.R. & Wishart, M.J. 2000. River conservation in the countries of the Southern African Development Community (SADC). In: Global perspectives on river conservation: science, policy, and practice. Boon, P.J., Davies, B.R. & Petts, G.E. (Eds.) John Wiley & Sons Ltd, England.
- Day, J.A. & King, J.M. 1995. Geographical patterns, and their origins, in the dominance of major ions in South African rivers. *South African Journal of Science*, 91, 299-306.
- Day, J.A., Dallas, H.F. & Wackernagel, A. 1998. Delineation of management regions for South African rivers based on water chemistry. *Aquatic Ecosystem Health & Management*, 1(2), 183-197.
- Death, R.G. 2010. Disturbance and riverine benthic communities: What has it contributed to general ecological theory? *River Research and Applications*, 26, 15-25.
- de Moor, F.C. 1992. Factors influencing the establishment of aquatic insect invaders. *Transactions of the Royal Society of South Africa*, 48(1), 141-158.
- de Moor, F.C., 1999. The use of Trichoptera to assess biodiversity and conservation status of South African river systems. In: H. Malicky (Ed.) *Proceedings of the IXth International Symposium on Trichoptera*. Chiang Mai. 237 - 244.
- de Moor, F.C. 2007. Regional biogeographical differences in Trichoptera diversity in South Africa : Observed patterns and processes. In: J. Bueno-Soria, R. Barba-Alvarez, & B. Armitage, (Eds.) *Proceedings of the XII<sup>th</sup> International Symposium on Trichoptera*. Ohio: The Caddis Press. 211-218.
- de Moor, F.C. & Barber-James, H.M. 1994. A survey of the freshwater invertebrates of the North-East Cape rivers. Albany Museum Internal Report, 65pp.
- de Moor, F.C., Barber-James H. M, Harrison, A.D. & Lugo-Ortiz, C.R. 2000. The macroinvertebrates of the Cunene River from the Ruacana Falls to the river mouth and assessment of the conservation status of the river. *African Journal of Aquatic Science*, 25(1), 105-122.

- de Moor, F. C. & Barber-James H. M. 2001. Report on the second survey of macroinvertebrates to assess the potential impact of trout stocking in the upper Salt River, The Craggs. Albany Museum Environmental Impact Assessment Report. 32pp.
- de Moor, F.C. & Scott, K.M.F. 2003. Chapter 5: Trichoptera. In: de Moor, I.J., Day, J.H. & de Moor F.C. (Eds). Guides to the Freshwater Invertebrates of southern Africa. Vol. 8: Insecta II: Hemiptera, Megaloptera, Neuroptera, Trichoptera & Lepidoptera. de Moor, I.J., Day, J.H. & de Moor, F.C. (Eds.) 209pp.
- de Moor, F.C., de Moor I.J., James N.P.E. & Barber-James H.M. 2004. An autumn survey of the freshwater macroinvertebrates in the Salt River, southern Cape. Albany Museum Investigational Contract Report. 74pp.
- de Moor, I.J. & Bruton, M.N. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. South Africa, SANSP Report No. 144 NMB, Port Elizabeth, 310pp.
- Dickens, C.W. & Graham, P.M. 2002. The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. *African Journal of Aquatic Science*, 27, 1-10.
- Esben-Petersen, P. 1924. South African Megaloptera. *Annals of the South African Museum*, 19, 151-158.
- Faasen, H. & Watts, S. 2007. Local community reaction to the 'no-take' policy on fishing in the Tsitsikamma National Park, South Africa. *Ecological Economics*, 64(1), 36-46.
- Fain, A. & du Jardin J.P. 1983. The *Simulium nigrিতarse* complex (Diptera: Simuliidae). *Revue de Zoologie Africaine*, 97, 379-452.
- Faith, D. & Norris, R. 1989. Correlation of environmental variables with patterns of distribution and abundance of common and rare freshwater macroinvertebrates. *Biological Conservation*, 50(1-4), 77-98.
- Gasith, A. & Resh, V.H. 1999. Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology and Systematics*, 30, 51-81.
- Gattolliat, J-L., Barber-James, H.M. & Monaghan, M.T. 2009. New species and generic delimitation of the Afrotropical Baetidae (Ephemeroptera) genera *Bugillesia* and *Cheleocloeon*. *Aquatic Insects* 31(3), 167-186.



- Guérol, F., Boudot, J.P., Jacquemin, G., Vein, D., Merlet, D. & Rouiller, J. 2000. Macroinvertebrate community loss as a result of headwater stream acidification in the Vosges Mountains (N-E France). *Biodiversity and Conservation*, 9, 767–783.
- Hanekom, N., Hutchings, L., Joubert, P. A. & Van Der Byl, P. C., 1989. Sea temperature variations in the Tsitsikamma Coastal National Park, South Africa, with notes on the effect of cold conditions on some fish populations. *South African Journal of Marine Science*, 8(1), 145-153.
- Hanekom, N. & Wilson, V., 1991. Blue duiker *Philantomba monticola* densities in the Tsitsikamma National Park and probable factors limiting these populations. *Koedoe*, 34(2), 107-120.
- Harrison, A. D. 1959. General statement on South African hydrobiological regions. Report No. 1, Project 6.8H. Internal report, National Institute for Water Research, CSIR, Pretoria.
- Harrison, A. D. 1965. Geographical distributions of riverine invertebrates in Southern Africa. *Archiv für Hydrobiologie*, 61(3), 387-394.
- Harrison, A.D. & Elsworth, J.F. 1958. Hydrobiological studies in the Great Berg River, Western Cape Province. Part I: General description, chemical studies and main features of the flora and fauna. *Transactions of the Royal Society of South Africa*, 35(3), 125-227.
- Harrison, A.D. & Agnew, J.D. 1960. Exploratory survey of the eastern part of region A (South Western Cape) and region B. National Institute for Water Research, Research Report No 5 (Project 6.8.H. Ref W.6/6/8H).
- Harrison, A.D. & Agnew, J.D. 1962. The distribution of invertebrates endemic to acid streams in the Western and Southern Cape Province. *Annals of the Cape Provincial Museums*, 2, 273-291.
- Hey, S.A. 1926. Fisheries survey 1925-6. Inland Waters, Report No. 1. Department of Mines and Industries, Cape Times Limited. Government Printers.
- Horton, R. E. 1945. Erosional development of streams and their drainage basins: hydro-physical approach to quantitative morphology. *Geological Society of America Bulletin*, 56(3), 275-370.
- Hosking, S. & du Preez, M., 1999. A cost-benefit analysis of removing alien trees in the Tsitsikamma mountain catchment. *South African Journal of Science*, 95, 442-448.
- Hynes, H.B.N. 1970. The ecology of running waters. Liverpool University Press, Liverpool, 555pp.

- Jacobsen, D., Schulze, R. & Encalada, A. 1997. Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude. *Freshwater Biology*, 38, 247-261.
- Johnston, S.T. 2000. The Cape Fold Belt and Syntaxis and the rotated Falkland Islands: dextral transpressional tectonics along the southwest margin of Gondwana. *Journal of African Earth Sciences*, 31(1), 51-63.
- Karr, J.R. 1991. Biological integrity: A long-neglected aspect of water resource management. *Ecological Applications*, 1, 66-84.
- King, J.M. 1981. The distribution of invertebrate communities in a small South African river. *Hydrobiologia*, 83, 43-65.
- King, J.M., 1983. Abundance, biomass and diversity of benthic macro-invertebrates in a western Cape River, South Africa. *Transactions of the Royal Society of South Africa*, 45(1), 11-34.
- King, J.M. Day, J.A. & van der Zel, D.W. 1979 Hydrology and hydrobiology. In: Fynbos ecology: a preliminary synthesis. South African National Scientific Programmes Report Number 40. Council for Scientific and Industrial Research, Pretoria.
- King, J.M., Henshall -Howard , M.P., Day, J.A. & Davies, B.R. 1987a. Leaf-pack dynamics in a southern African mountain stream. *Freshwater Biology*, 18, 325-340.
- King, J.M., Day, J.A., Davies, B.R. & Henshall -Howard , M.P. 1987b Particulate organic matter in a mountain stream in the south-western Cape, South Africa. *Hydrobiologia*, 154, 165-187.
- King, J.M., Day, J.A., Hurley, P.R., Henshall -Howard, M.P. & Davies, B.R. 1988. Macroinvertebrate communities and environment in a southern African mountain stream. *Canadian Journal of Fisheries and Aquatic Sciences*. 45, 2168-2181.
- Kormondy, E.J. 1961. Territoriality and dispersal in Dragonflies (Odonata). *Journal of the New York Entomological Society*, 69(1), 42-52.
- Jost, L., De Vries, P., Walla, T., Greeney, H., Chao, A. & Ricotta, C. 2010. Partitioning diversity for conservation analyses. *Diversity and Distributions*, 16(1), 65-76.
- Lande, R., 1996. Statistics and partitioning of species diversity and similarity among multiple communities. *Oikos*, 76, 5-13.

- Louw, J.H., 1991. The relationship between site characteristics and *Pinus radiata* growth on the Tsitsikamma plateau, South Africa. *South African Journal of Forestry*, 158, 37-45.
- Lowe, S.R., Woodford, D.J., Impson, D.N. & Day, J.A. 2008. The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. *African Journal of Aquatic Science*, 33(1), 51-62.
- Lugo-Ortiz, C.R. & de Moor, F.C. 2000. *Nigrobaetis* Novikova and Kluge (Ephemeroptera: Baetidae): first record and new species from southern Africa with reassignment of one northern African species. *African Entomology*, 8(1), 69-73.
- Lewis, C.A. 2010. Late Quaternary environmental phases in the Eastern Cape and adjacent Plettenberg Bay- Knysna region and Little Karoo, South Africa. *Proceedings of the Geologists Association*. Article in press. 14pp.
- Lewis, W.M. & Morris, D.P. 1986. Toxicity of Nitrite to fish: a review. *Transactions of the American Fisheries Society*, 115, 183-195.
- Magurran, A.E. 2004. Measuring biological diversity. 2<sup>nd</sup> ed. Blackwell Publishing, Oxford. 256pp.
- Manly, B.F.J. 2005. Multivariate Statistical Methods: A Primer. 3<sup>rd</sup> ed. Chapman & Hall/CRC. 214pp.
- McCafferty, W.P. & Wang, T-Q. 1997. Phylogenetic systematics of the family Teloganodidae (Ephemeroptera: Pannota). *Annals of the Cape Provincial Museums*, 19(9), 387-437.
- McCafferty, W. P. & Benstead, J. P. 2002. Cladistic resolution and ecology of the Madagascar genus *Manohyphella* Allen (Ephemeroptera: Teloganodidae). *Annales de Limnologie*, 38, 41–52.
- McCarthy, T. & Rubidge, B. 2005. The story of Earth and life: A southern African perspective on a 4.6-billion-year journey. Struik Publishers, Cape Town, 333pp.
- McLachlan, A.J. & McLachlan, S.M., 1971. Benthic fauna and sediments in the newly created Lake Kariba (Central Africa). *Ecological Society of America*, 52(5), 800-809.
- Metcalf-Smith, J.L. 1994. Biological water-quality assessment of rivers: use of macroinvertebrate communities. In: The Rivers Handbook, Volume 2. Calow, P. & Petts, G. E. (Eds.) Blackwell Science Ltd, Oxford.

- Midgley, D.C., Pitman, W.V. and Middleton, B.J., 1994. The surface water resources of South Africa 1990. In: (1st ed.), *WRC Report 298/6.1/94* vol. 6, Water Research Commission, Pretoria, South Africa.
- Moore, N.W. 1952. On the so-called “territories” of Dragonflies (Odonata-Anisoptera). *Behaviour*, 4(2), 85-100.
- Morant, P.D. & Bickerton, I.B. 1983. Estuaries of the Cape. Part ii: Synopses of available information on individual systems: Groot (Wes) (CMS 23) and Sout (CMS 22). Report No. 19. Pretoria.
- Mucina, L. & Geldenhuys, C.J. 2006. Afrotemperate, subtropical and azonal forests. In: The vegetation of South Africa, Lesotho and Swaziland. Rutherford, M.C. & Mucina, L. (Eds.) *Strelitzia* 19, South African National Biodiversity Institute, Pretoria.
- Nel, J.L., Smith-Adao, L., Roux, D.J., Adams, J., Cambray, J.A., de Moor, F.C., Kleynhans, C.J., Kotze, I., Maree, G., Moolman, J., Schonegevel, L.Y., Smith, R.J., Thirion, C. 2006. Conservation planning for river and estuarine biodiversity in the Fish-to-Tsitsikamma water management area. Water Research Commission Report TT 280/06, Water Research Commission, Pretoria, South Africa
- Palmer, C.G., Goetsch, P.A. & O’Keefe, J.H. 1996. Development of a recirculating artificial stream system to investigate the use of macroinvertebrates as water quality indicators. Report to the Water Research Commission. Report No. 475/1/96. 184pp.
- Palmer, R.W. & de Moor, F.C. 1998. Annotated records of Blackfly (Diptera: Simuliidae) distribution in southern Africa. *African Entomology*, 6, 223–251.
- Podani, J. 2000. Introduction to the exploration of multivariate biological data. Backhuys Publishers, Leiden, Hungary. 407pp.
- Rebelo, A.G., Boucher, C., Helme, N., Mucina, L. & Rutherford, M.C. 2006. Fynbos biome. In: The vegetation of South Africa, Lesotho and Swaziland. Rutherford, M.C. & Mucina, L. (Eds.) *Strelitzia* 19, South African National Biodiversity Institute, Pretoria.
- Reynoldson, T.B. 1984. The utility of benthic invertebrates in water quality monitoring. *Water Quality Bulletin*, 10, 21-28.
- Ritcher, B.D., Baumgartner, J.V., Powell, J. & Braun, D.P. 1996. A method for assessing hydrologic alteration within ecosystems. *Conservation Biology*, 10(4), 1163 - 1174.

- Rivers-Moore, N.A., Hughes, D.A. & Mantel, S. 2008. Links between water temperatures, ecological responses and flow rates: A framework for establishing water temperature guidelines for the ecological reserve. Report to the Water Research Commission. Report No. KV 214/08. 57pp.
- Roets, W., Xu, Y., Raitt, L., El-Kahloun, M., Meire, P., Calitz, F., Batelaan, O., Anibas, C., Paridaens, K., Vandenbroucke, T., Verhoest, N. E. C. & Brendonck, L. 2008a. Determining discharges from the Table Mountain Group (TMG) aquifer to wetlands in the Southern Cape, South Africa. *Hydrobiologia*, 607, 175-186.
- Roets, W., Xu, Y., Raitt, L. & Brendonck, L. 2008b. Groundwater discharges to aquatic ecosystems associated with the Table Mountain Group ( TMG ) aquifer : A conceptual model. *Water SA*, 34(1), 77-88.
- Rowntree, K.M. & R.A. Wadeson. 1999. A hierarchical framework for categorising the geomorphology of selected South African rivers. Final report to the Water Research Commission, 334pp.
- Roux, D.J., Kleynhans, C.J., Thirion, C., Hill, L., Engelbrecht, J.S., Deacon, A.R. & Kemper N.P. 1999. Adaptive assessment and management of riverine ecosystems: The Crocodile/Elands River case study. *Water SA*, 25(4), 501-511.
- Russel, I.A. 2002. Freshwater fishes of Tsitsikamma National Park. *Koedoe*, 45(2), 13-17.
- Rutherford, M.C., Mucina, L. & Powrie, L.W. 2006. Biomes and Bioregions of Southern Africa. In: The vegetation of South Africa, Lesotho and Swaziland. Rutherford, M.C. & Mucina, L. (Eds.) *Strelitzia* 19, South African National Biodiversity Institute, Pretoria.
- Samways, M. 2008. Dragonflies and Damselflies of South Africa. Pensoft, Sofia, Moscow. 297pp.
- Samways, M. & Wilmot, B.C. 2003. Chapter 3: Odonata. In: Guides to the Freshwater Invertebrates of Southern Africa. Volume 7: Insecta I. Ephemeroptera, Odonata & Plecoptera. de Moor, I.J., Day, J.A. & de Moor, F.C. (Eds.) 288pp.
- Samways, M., McGeoch, M. & New, T. 2010. Insect Conservation: A Handbook of Approaches and Methods. Wiltshire: Oxford University Press, 441pp.
- Sartori, M., Peters, J.G. & Hubbard, M.D. 2008. A revision of Oriental Teloganodidae (Insecta, Ephemeroptera, Ephemerelloidea). *Zootaxa*, 1957, 1-51.

- Schlichting, H., & Gersten, K. 2000. Boundary-Layer Theory. 8<sup>th</sup> Revised and Enlarged Edition. Springer. 799pp.
- Scott, K.M.F. 1958. Hydrobiological studies of the Great Berg River, western Cape Province. Part 3. The Chironomidae. Transactions of the Royal Society of South Africa. 35(3), 277-298.
- Scott, K.M.F. 1961. Some new caddis flies (Trichoptera) from the Western Cape Province – III. *Annals of the South African Museum*, 46(2), 14-33.
- Soldán, T. 1977. Three new species of mayflies (Ephemeroptera) from the mist oasis of Erkwit, Sudan. *Acta Entomologica Bohemoslovaca* 74(5), 289-294.
- Soldner, M., 2004. Relationship between macroinvertebrate fauna and environmental variables in small streams of the Dominican Republic. *Water Research*, 38(4), 863-874.
- StatSoft, Inc. 2008. STATISTICA (data analysis software system). Version 8.0. [www.statsoft.com](http://www.statsoft.com).
- Stuckenberg, B.R. 1962. The distribution of the montane palaeogenic element in South African invertebrate fauna. *Annals of the Cape Provincial Museum*, 2, 273-291.
- Stevens, D.M. & Picker, M.D. 2003. Chapter 4: Plecoptera In: Guides to the Freshwater Invertebrates of Southern Africa. Volume 7: Insecta I. Ephemeroptera, Odonata & Plecoptera. de Moor, I.J., Day, J.A. & de Moor, F.C. (Eds.) 288pp.
- Strum, W. & Morgan, J.J. 1996. Aquatic chemistry: chemical equilibria and rates in natural waters, 3<sup>rd</sup> Ed. John Wiley & Sons Inc. New York. 1022pp.
- Swartz, C.D. & de Villiers, H.A. 1998. Guidelines for the treatment of Cape coloured waters. Report to the water research commission by the Division of Water Environment and Forestry Technology CSIR, Pretoria. Report No. 534/1/98.
- Tarboton, W. & Tarboton, M. 2002. A fieldguide to the Dragonflies of South Africa. Privately published by authors. 95pp.
- Tarboton, W. & Tarboton, M. 2005. A fieldguide to the Damselflies of South Africa. Privately published by authors. 95pp.
- ter Braak, C. J. F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate analysis. *Ecology*. 67(5): 1167-1179.

- ter Braak, C.J.F. & Prentice, I.C., 1988. A Theory of Gradient Analysis. *Advances in Ecological Research*, 18(03), 271-317.
- ter Braak, C.J.F. & Verdonschot, P.F.M. 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic sciences*. 57(3): 255-289.
- ter Braak, C.J.F. & Smilauer, P. 2002a. canoco for Windows. Version 4.5. Biometris–Plant Research International, Wageningen, the Netherlands. (Registered to Dr F. de Moor).
- ter Braak, C.J.F. & Smilauer, P. 2002b. CANOCO Reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power (Ithaca, NY, USA) 500pp.
- Tilney, R. L., Nelson, G., Radloff, S. E. & Buxton, C. D. 1996. Ichthyoplankton distribution and dispersal in the Tsitsikamma National Park marine reserve, South Africa. *South African Journal of Marine Science*, 17(1), 1-14.
- Toerien, D.K. 1976. Geologie van die Tsitsikammakusstroom. *Koedoe*, 19, 31-41
- Uys, A.C. 1997. The invertebrates of temporary rivers in the Eastern Cape, South Africa. PhD Thesis. Rhodes University. 345pp.
- Vannote, R. & Sweeney, B., 1980. Geographical analysis of thermal equilibria: A conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *The American Naturalist*, 115(5), 667-695.
- Van Wilgen, B.W., Cowling, R.M., & Burgers, C.J. 1996. Valuation of ecosystem services: a case study from the Fynbos, South Africa. *BioScience*, 46, 184-189.
- Veech, J.A. Summerville, K.S., Crist, T.O. & Gering, J.C. 2002. The additive partitioning of species diversity: recent revival of an old idea. *Oikos*, 99(1), 3-9.
- Veech, J.A., & T.O. Crist. 2009a. PARTITION: software for hierarchical partitioning of species diversity, version 3.0. <http://www.users.muohio.edu/cristto/partition.htm>
- Veech, J.A., & T.O. Crist. 2009b. PARTITION 3.0 user's manual. (Unpublished document)
- Vlok, J. & de Ronde, C., 1989. The effect of low-intensity fires on forest floor vegetation in mature *Pinus elliottii* plantations in the Tsitsikamma. *South African Journal of Botany*, 55(1), 11-16.

- Wikelski, M., Moskowitz, D., Adelman, J.S., Cochran, J., Wilcove, D.S. & May, M.L. 2006. Simple rules guide dragonfly migration. *Biology letters*, 2(3), 325-329.
- Whittaker, R.H. 1967. Gradient analysis of vegetation. *Biological Reviews*, 42(2), 207-264.
- Whittaker, R.H. 1972. Evolution and measurement of species diversity. *Taxon*, 21, 213-251.
- Wiberg-Larsen, P., Brodersen, K.P., Birkholm, S., Grénx, P.N. & Skriver, J. 2000. Species richness and assemblage structure of Trichoptera in Danish streams. *Freshwater Biology*, 43, 633-647.
- Wichmann, M.C., Schwager, M. & Jeltsch, F. 2004. Animal species diversity driven by habitat heterogeneity / diversity : the importance of keystone structures. *Journal of Biogeography*, 34, 79-92.
- Wishart M.J. & Day J.A. 2002. Endemism in the freshwater fauna of the south-western Cape, South Africa. *Verhandlungen Internationale Vereinigung Limnologie* ,28, 1-5.



## Figures

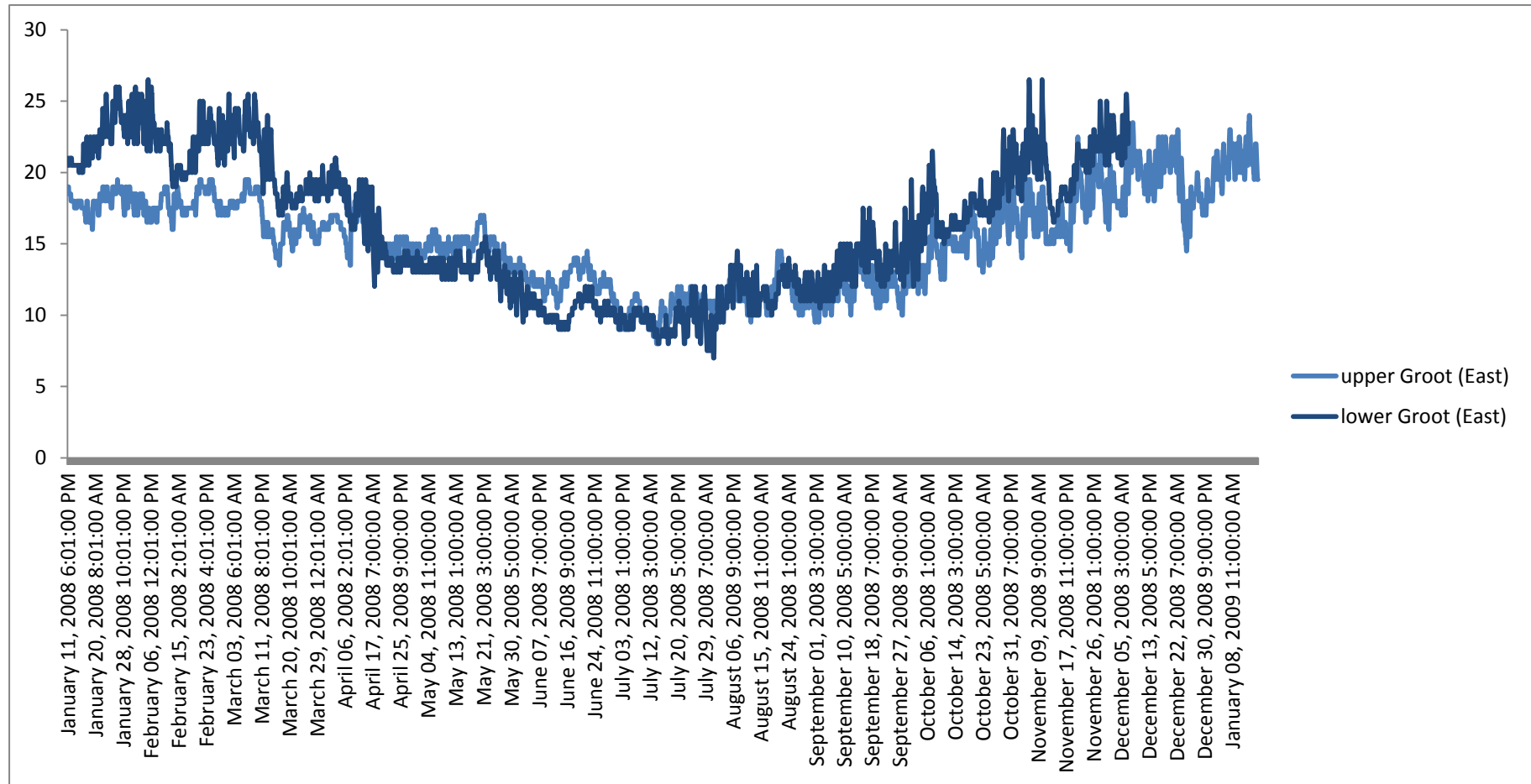


Figure 1: Graph of temperature recorded every 2 hours over a year from the Groot (East) River. The data missing from the lower site from the beginning of November onwards is due to the complete cessation of water flow, leaving the data logger exposed to air and sunlight.

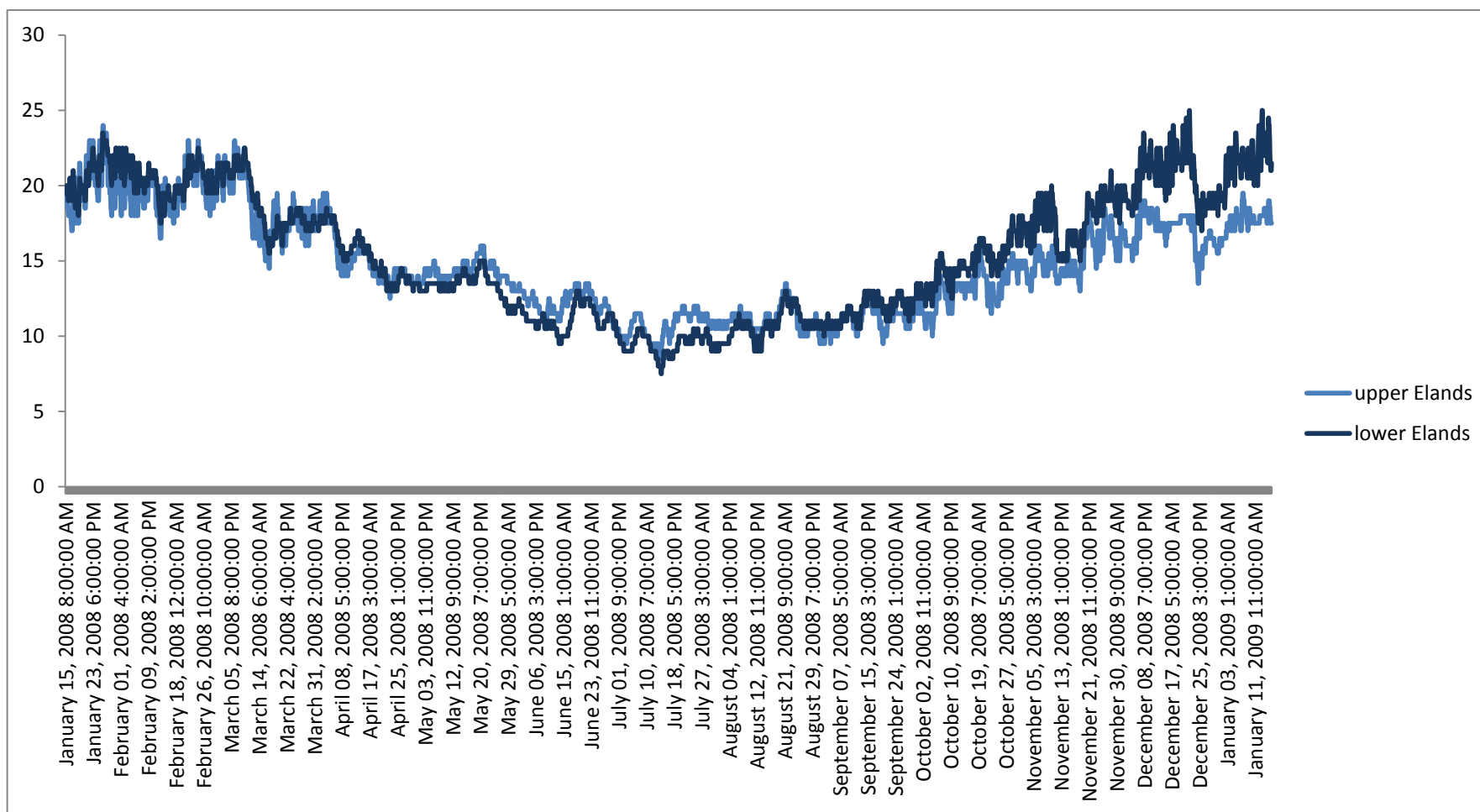


Figure 2: Graph of temperature recorded every 2 hours over a year from the Elands River.

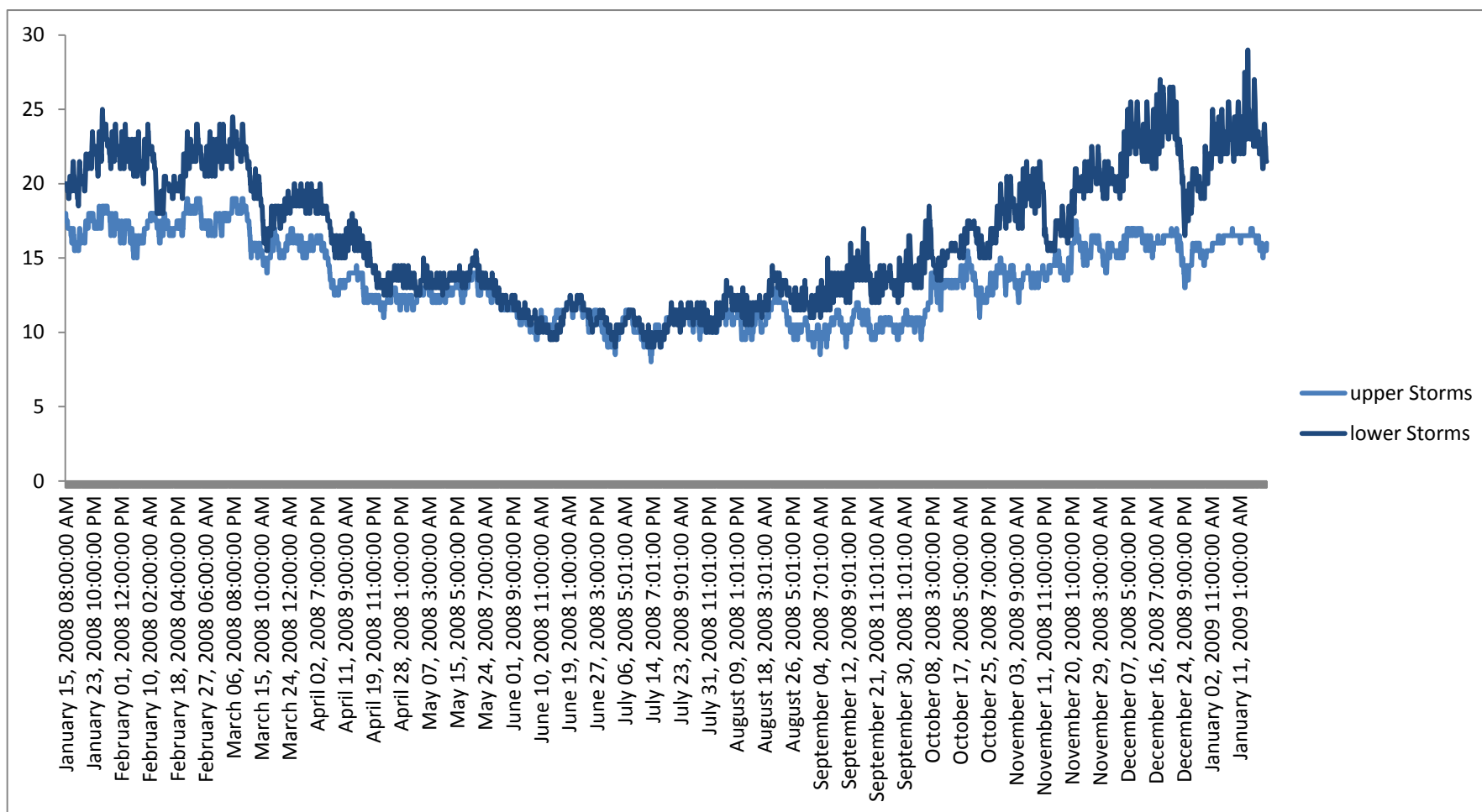


Figure 3: Graph of temperature recorded every 2 hours over a year from the Storms River.

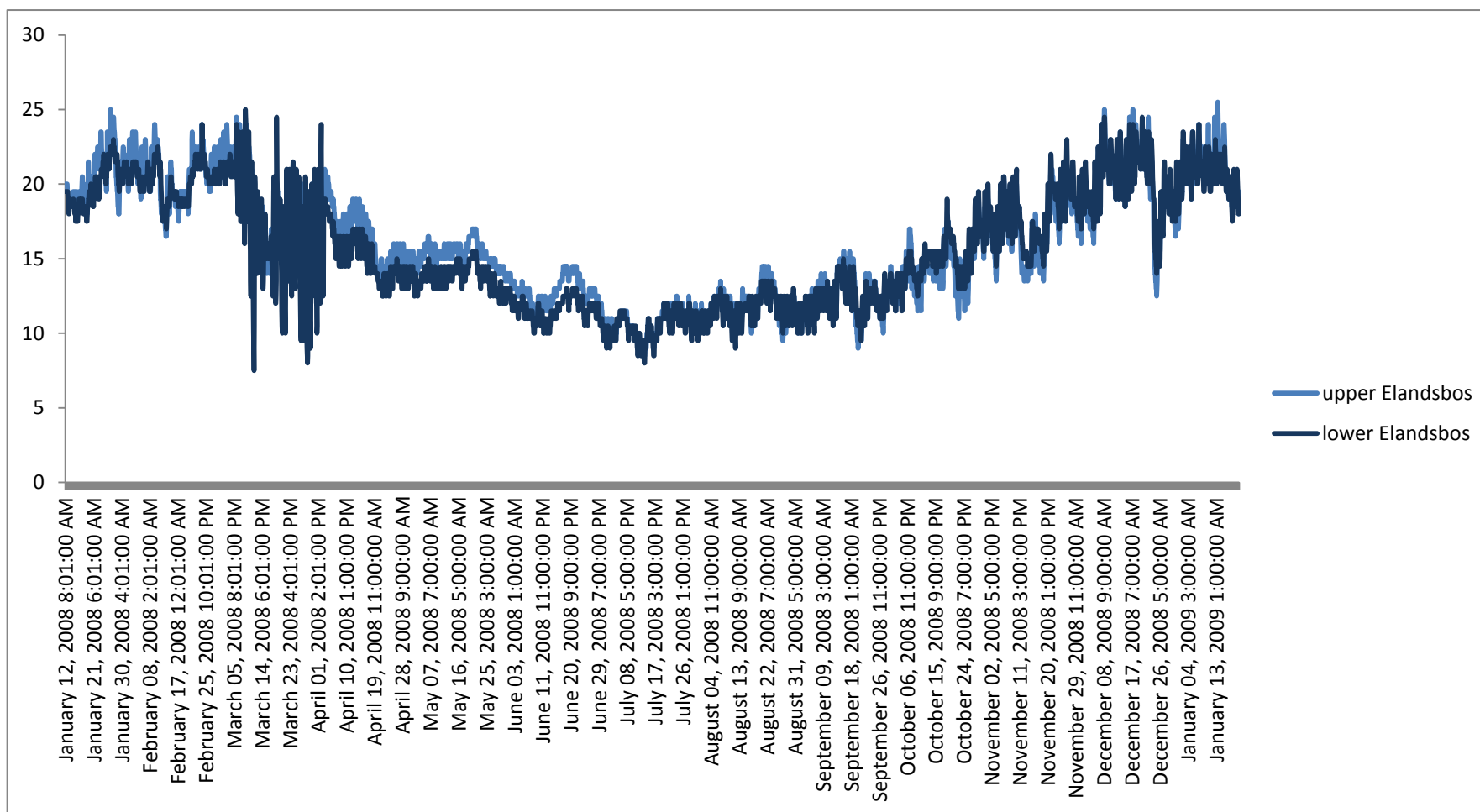


Figure 4: Graph of temperature recorded every 2 hours over a year from the Elandsbos River. The abnormal variation recorded during March/April from the lower site is due to the temperature logger being exposed as a result of underestimating the extent to which water levels would recede.

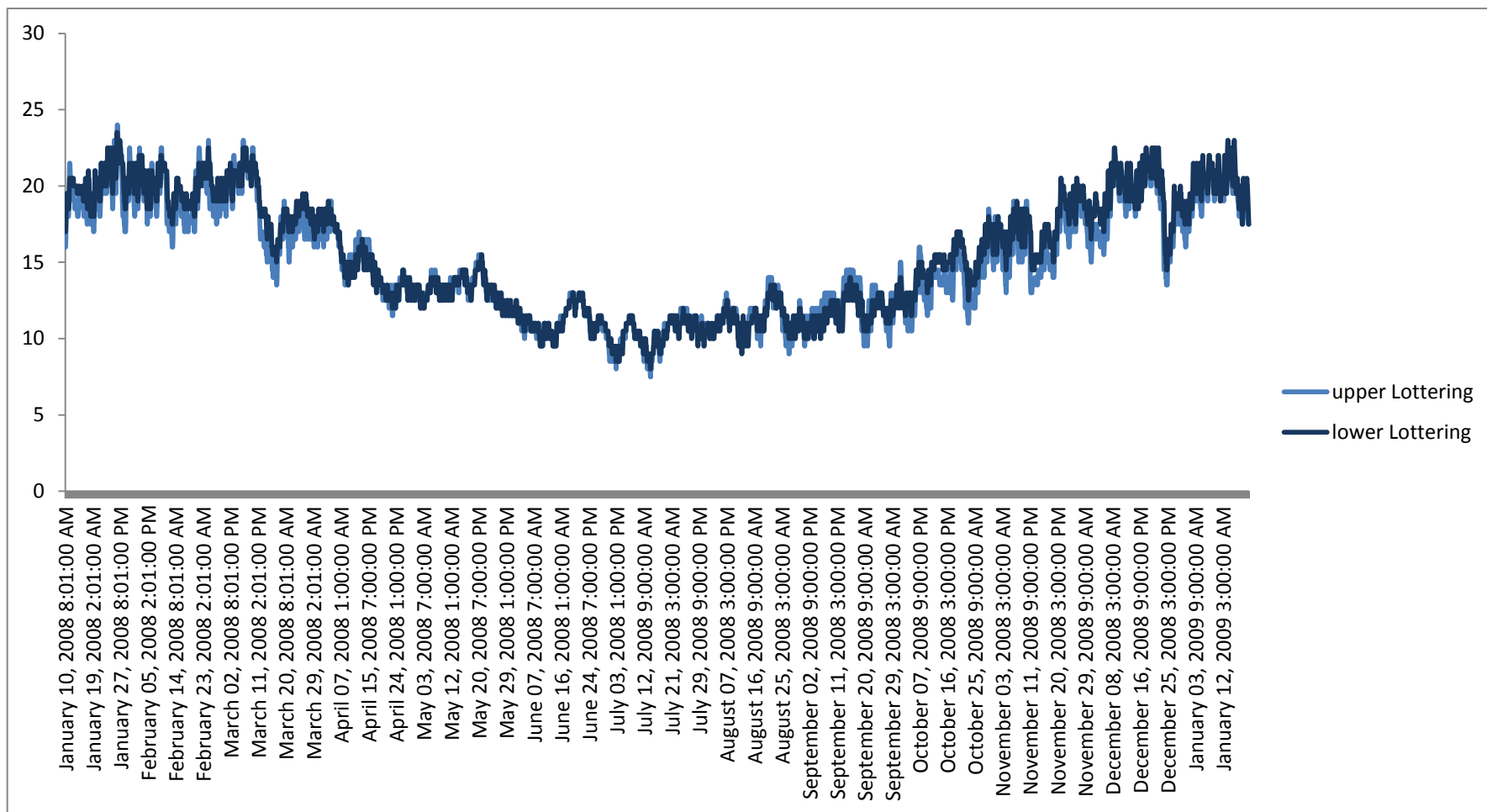


Figure 5: Graph of temperature recorded every 2 hours over a year from the Lottering River.

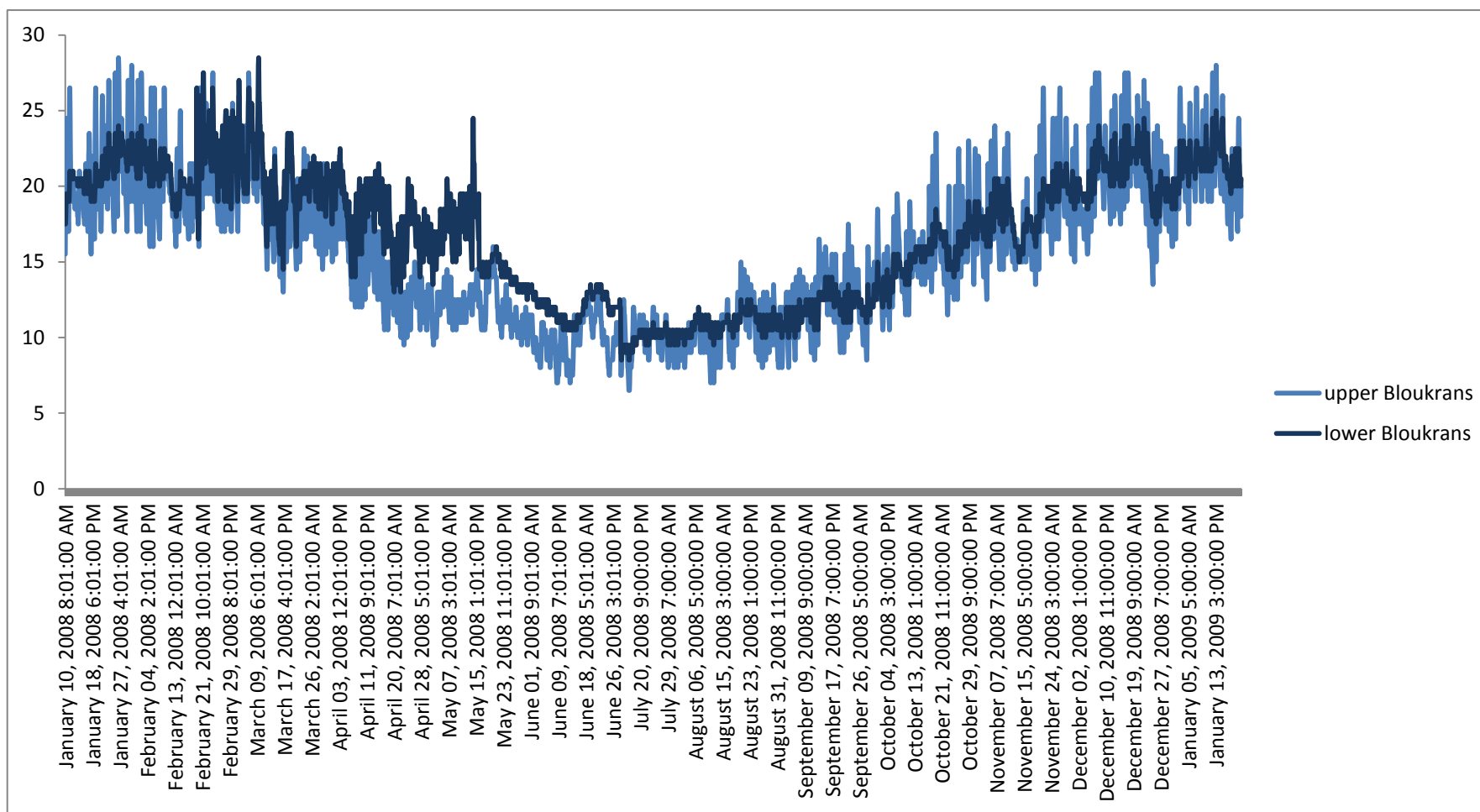


Figure 6: Graph of temperature recorded every 2 hours over a year from the Bloukrans River. The abnormal variation at the lower site recorded from February to May is due to the logger being removed by members of the public and then replaced again. The consistently high variation in water temperature at the upper site appears to be a natural phenomenon that is accounted for by the positioning of the logger in a pool below a waterfall.

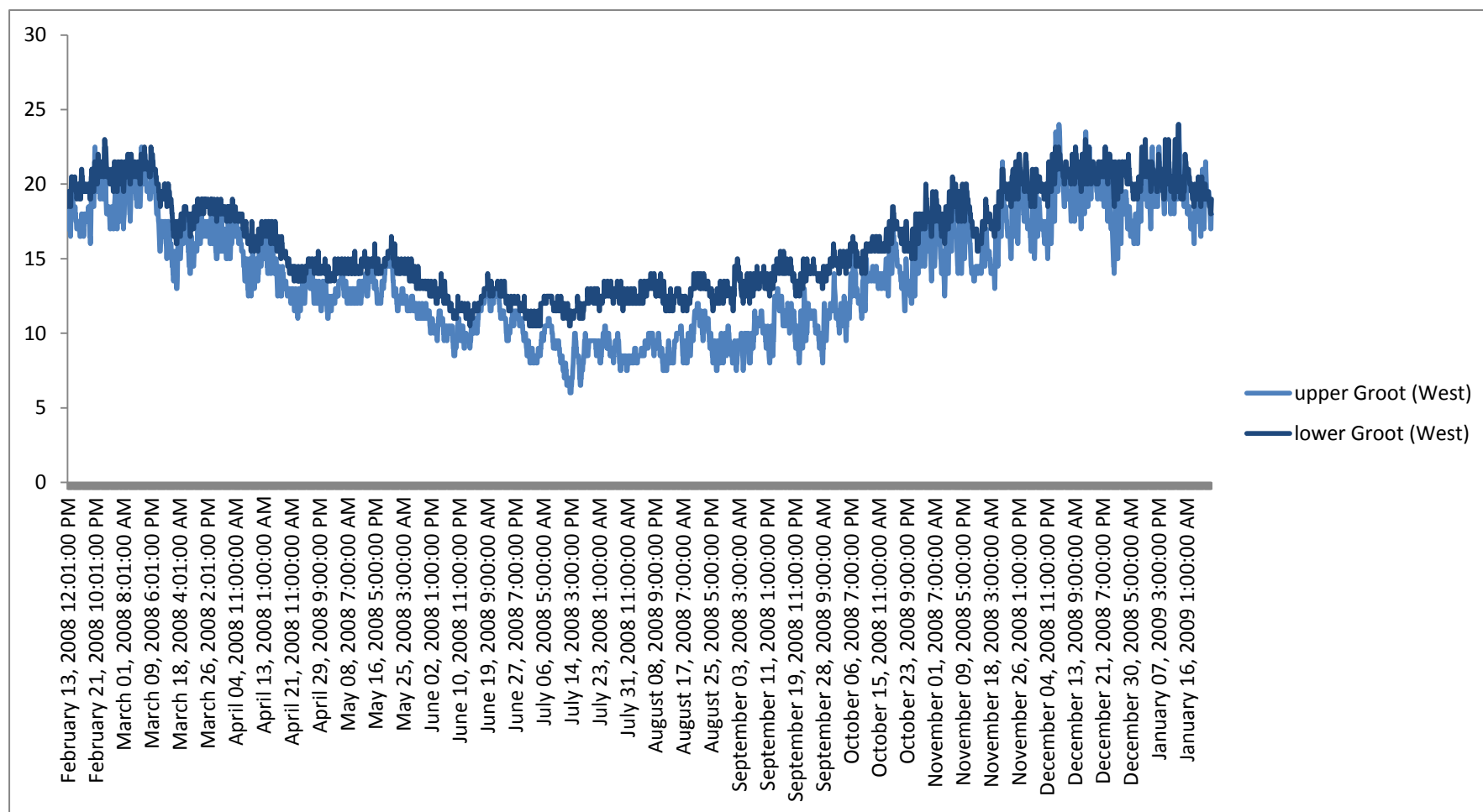


Figure 7: Graph of temperature recorded every 2 hours over a year from the Groot (West) River.

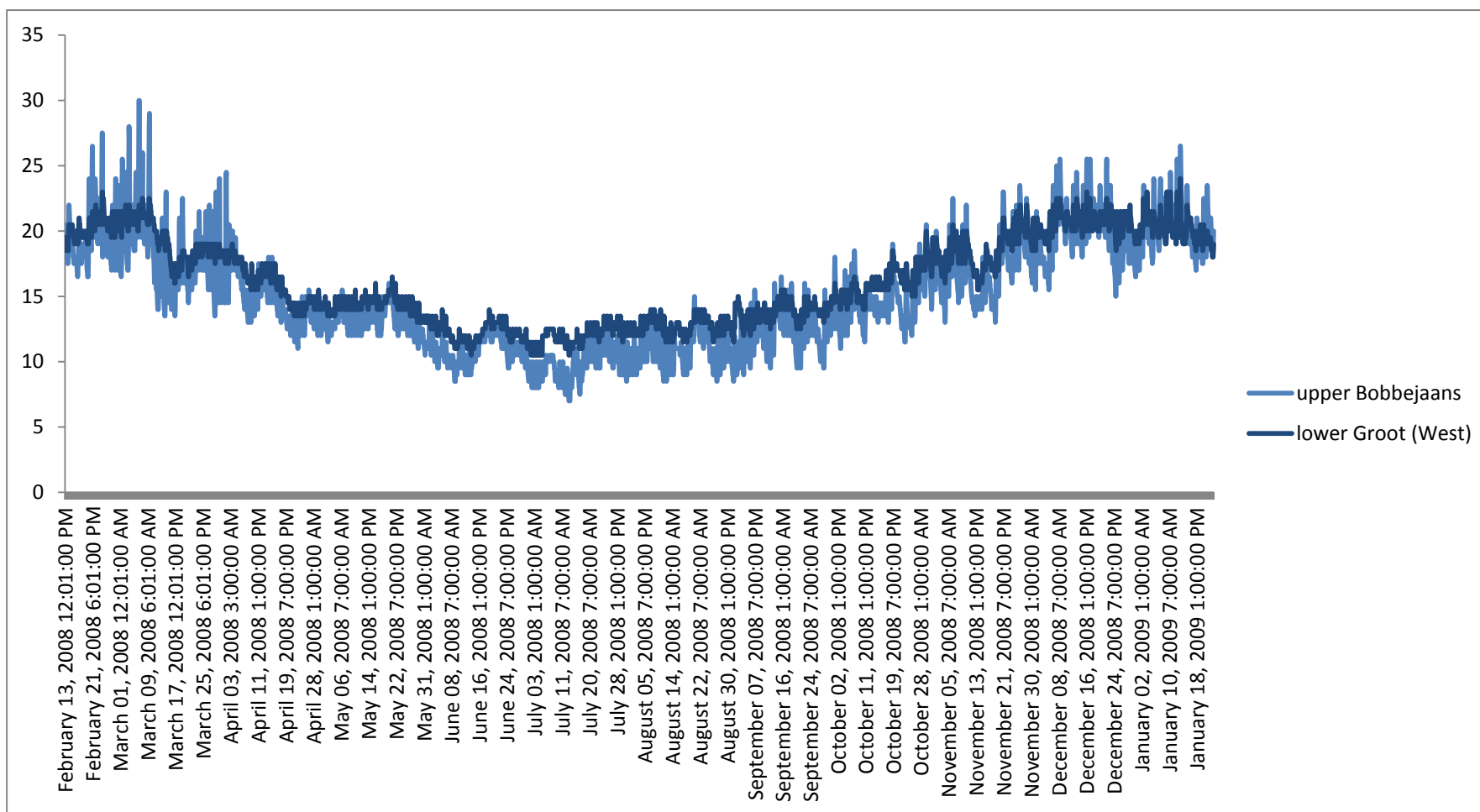


Figure 8: Graph of temperature recorded every 2 hours over a year from the Groot (West) River and its tributary the Bobbejaans River. The comparatively high readings during March/April from the upper Bobbejaans site are due to the temperature logger being exposed as a result of underestimating the extent to which water levels would recede.



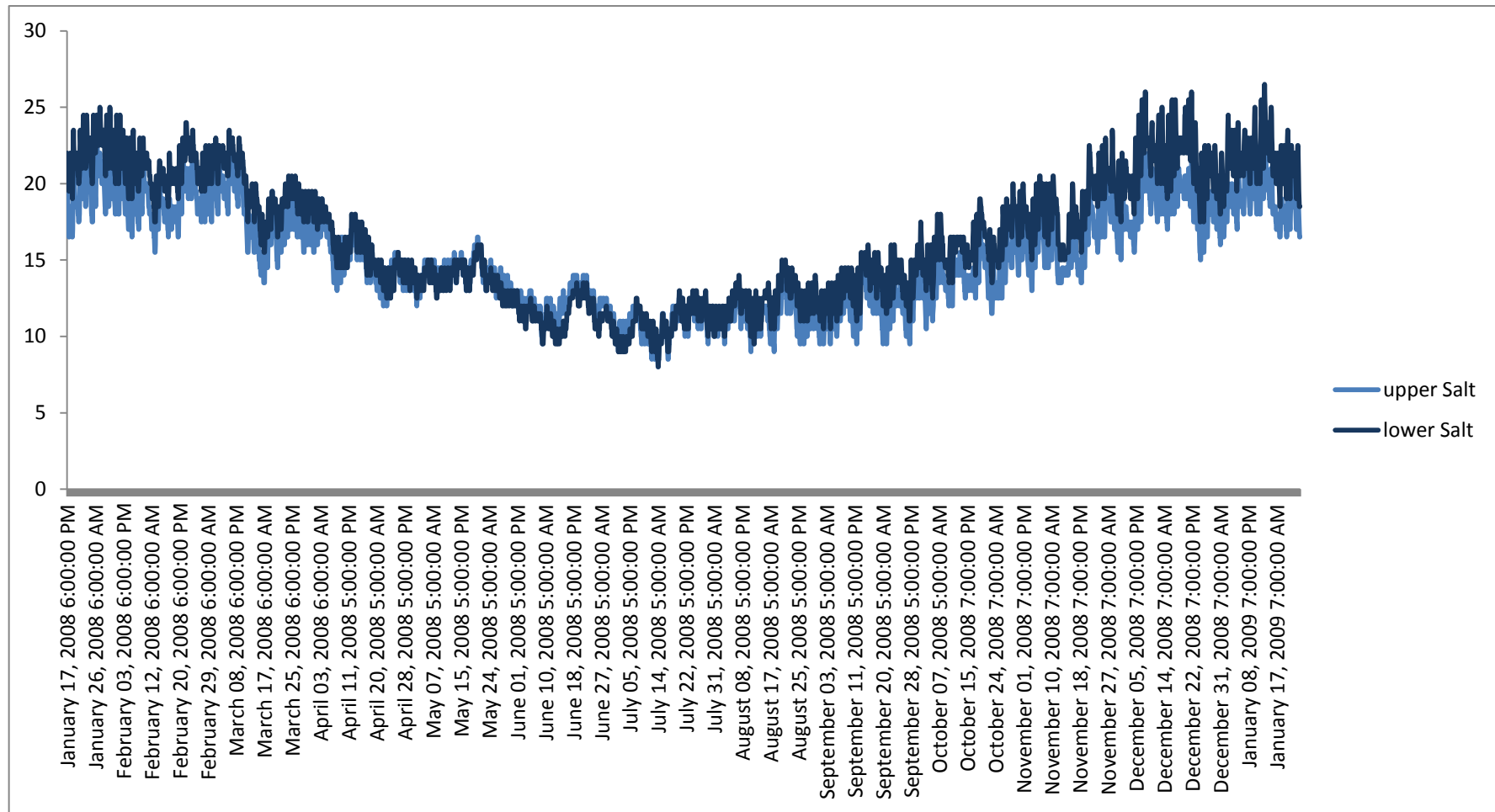


Figure 9: Graph of temperature recorded every 2 hours over a year from the Salt River.

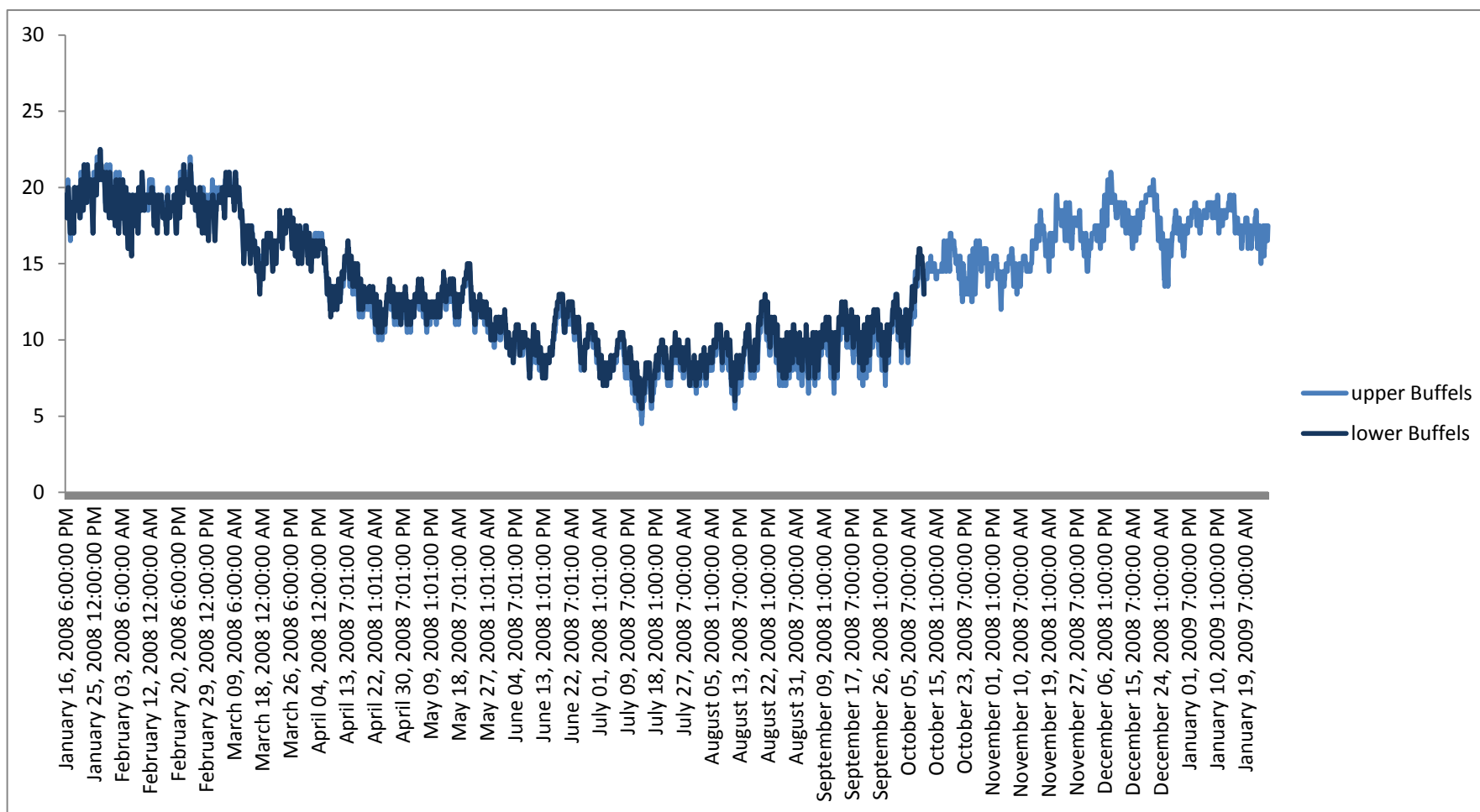


Figure 10: Graph of temperature recorded every 2 hours over a year from the Buffels River. The missing data during the last quarter from the lower Buffels site was due to logger malfunction leading to the recordings being irretrievable from the I-Button device.

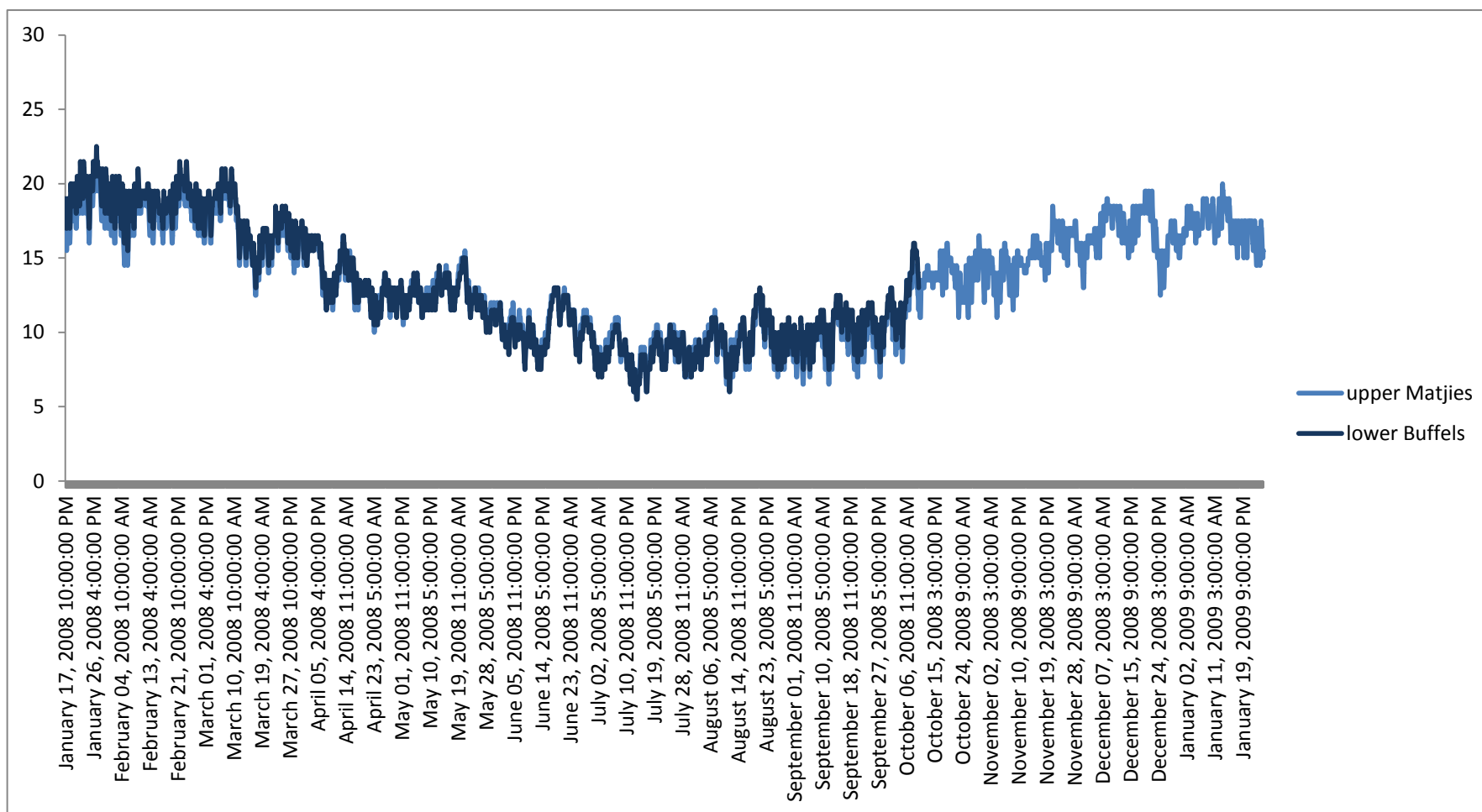


Figure 11: Graph of temperature recorded every 2 hours over a year from the Buffels River and its tributary the Matjies River. The missing data during the last quarter from the lower Buffels site was due to logger malfunction leading to the recordings being irretrievable from the I-Button device.

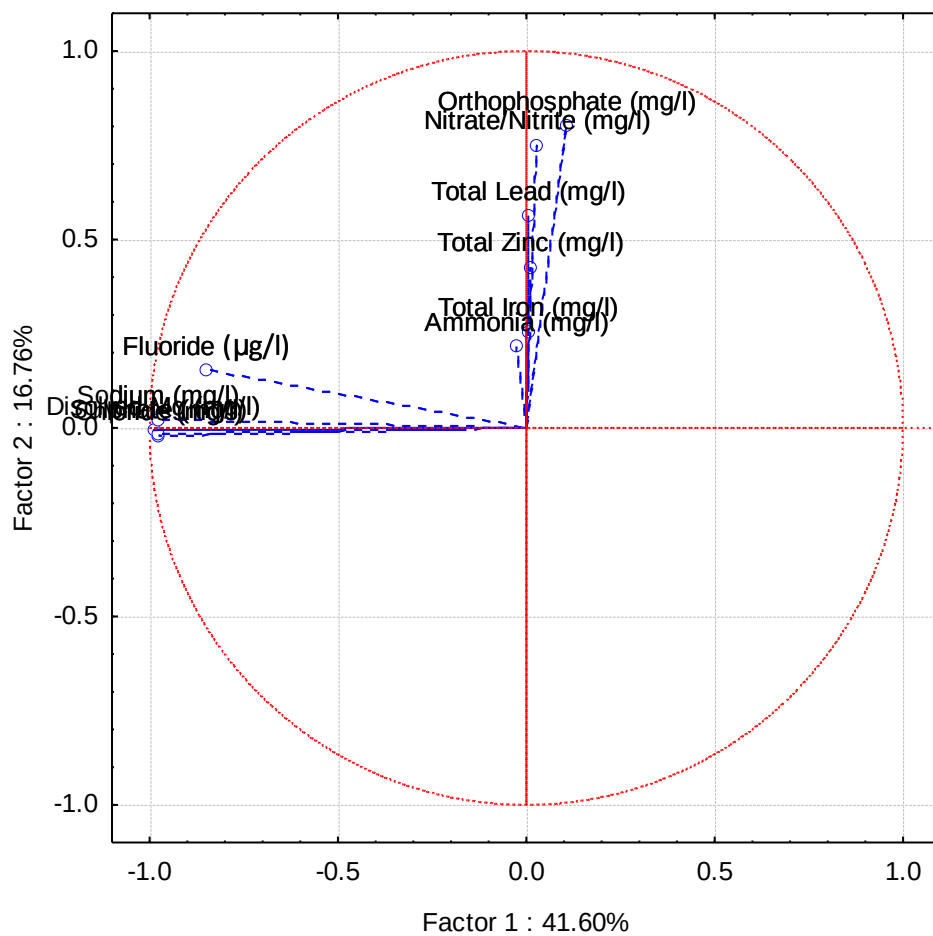


Figure 12: Factor plot of the first two Eigenvectors from a PCA of the laboratory analysed water chemistry data from all 20 sampling sites over an annual cycle. The indiscernible cluster of variables on the left includes Sulphate, dissolved Magnesium, Chloride and Sodium.

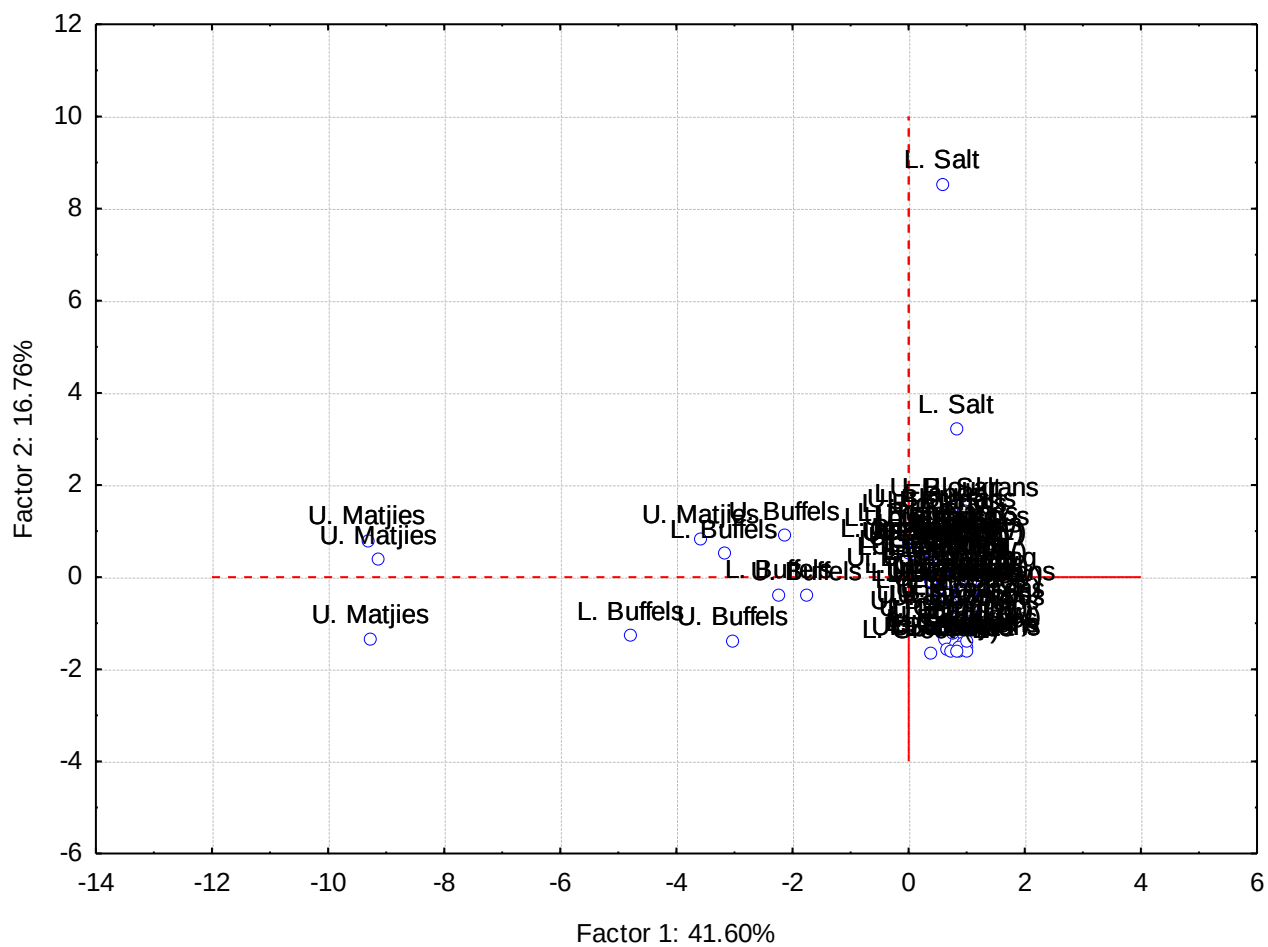


Figure 13: Ordination plot generated from the first two factors of a PCA of each of the 20 sites, using laboratory analysed water chemistry data collected seasonally/quarterly at each sampling event over a year.



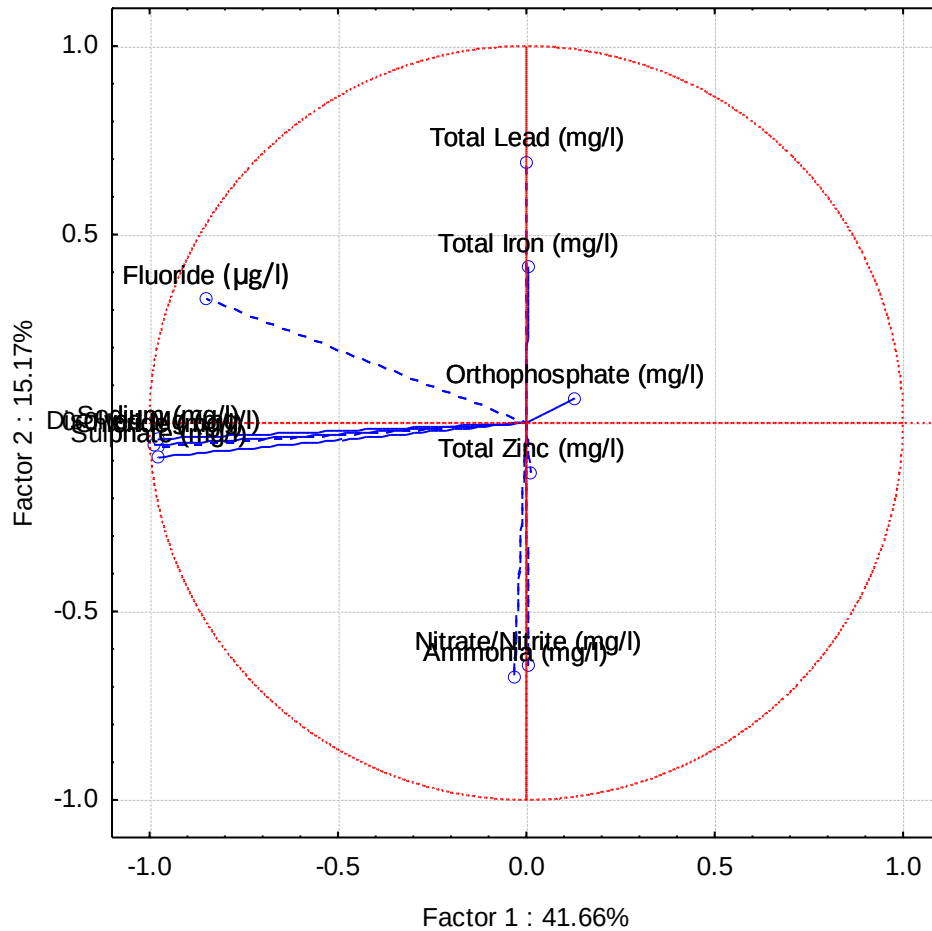


Figure 15: Factor plot the first two Eigenvectors of from a PCA using laboratory analysed water chemistry data, with the case for the lower Salt River from the winter sampling event treated as an outlier and excluded. The indiscernible group of variables on the left includes Sulphate, Dissolved Magnesium, Chloride and Sodium.

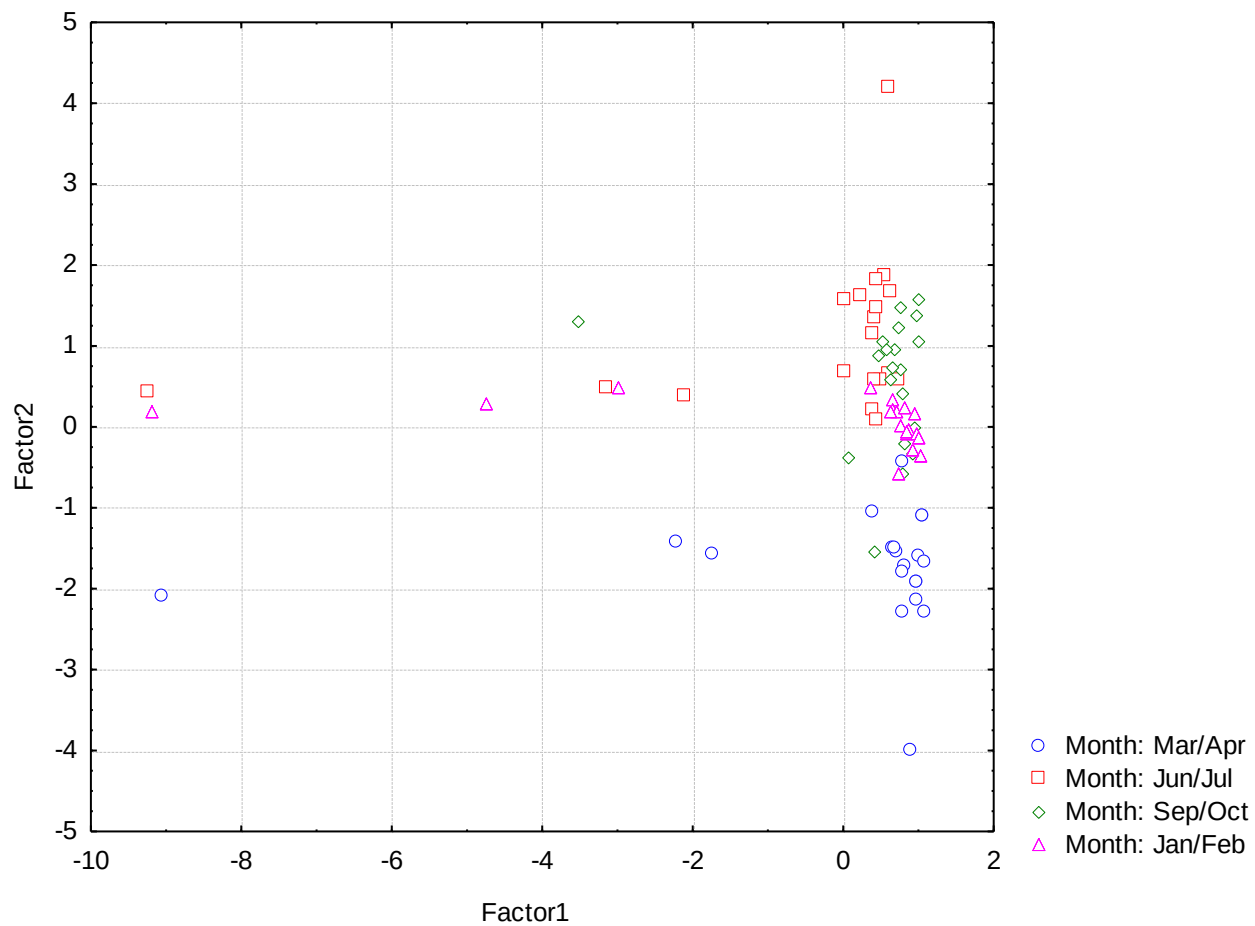


Figure 16: Seasonally categorised scatter plot of the first two factors of a PCA of laboratory analysed water chemistry data excluding the lower Salt River site for the winter sampling period, cases labelled according to month of collection.



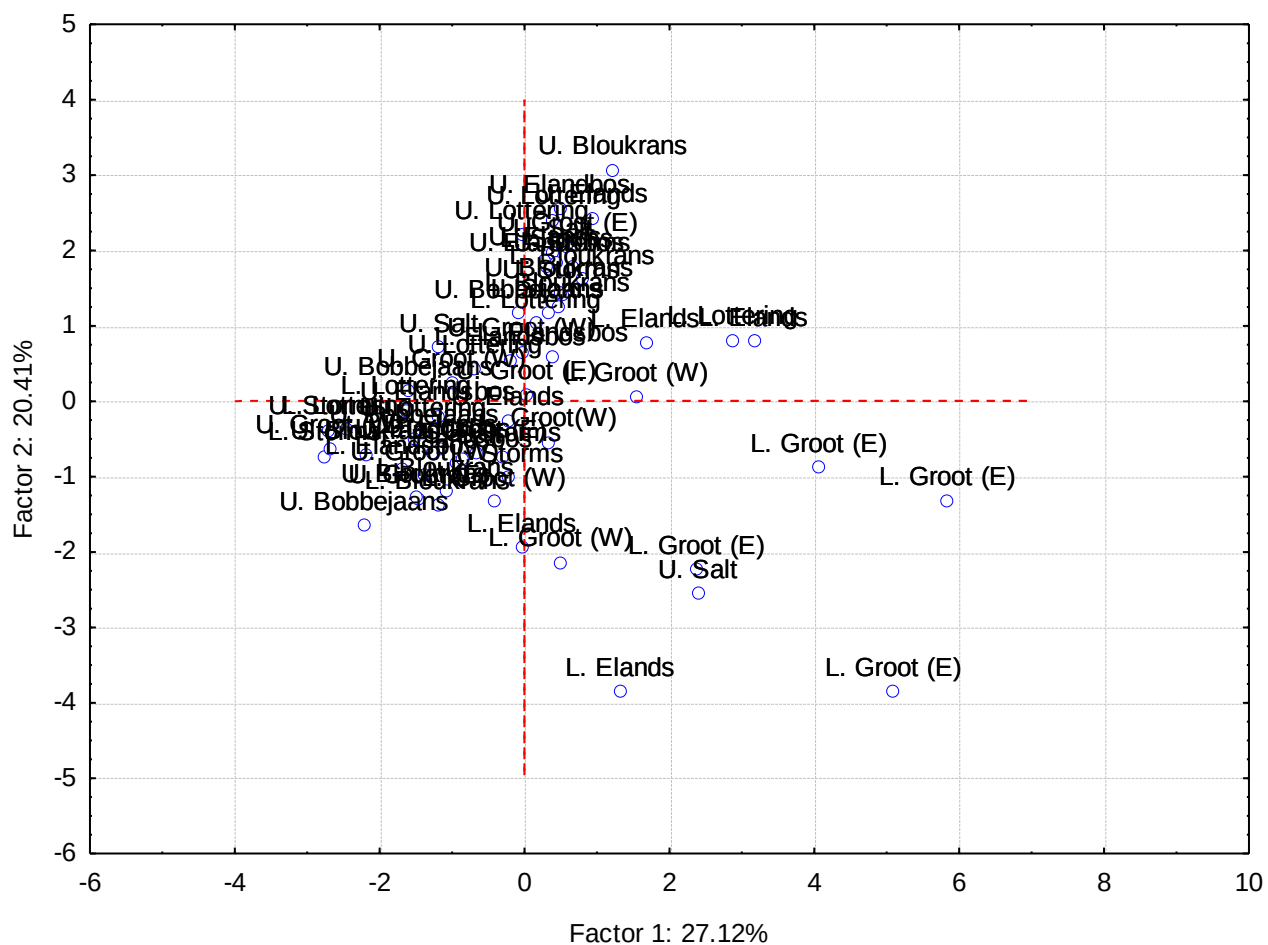


Figure 17: Ordination diagram of the first two factors produced from a PCA using laboratory analysed water chemistry data, with the cases for the lower Salt, upper Matjies and upper and lower Buffels River sites removed for all of the sampling events.

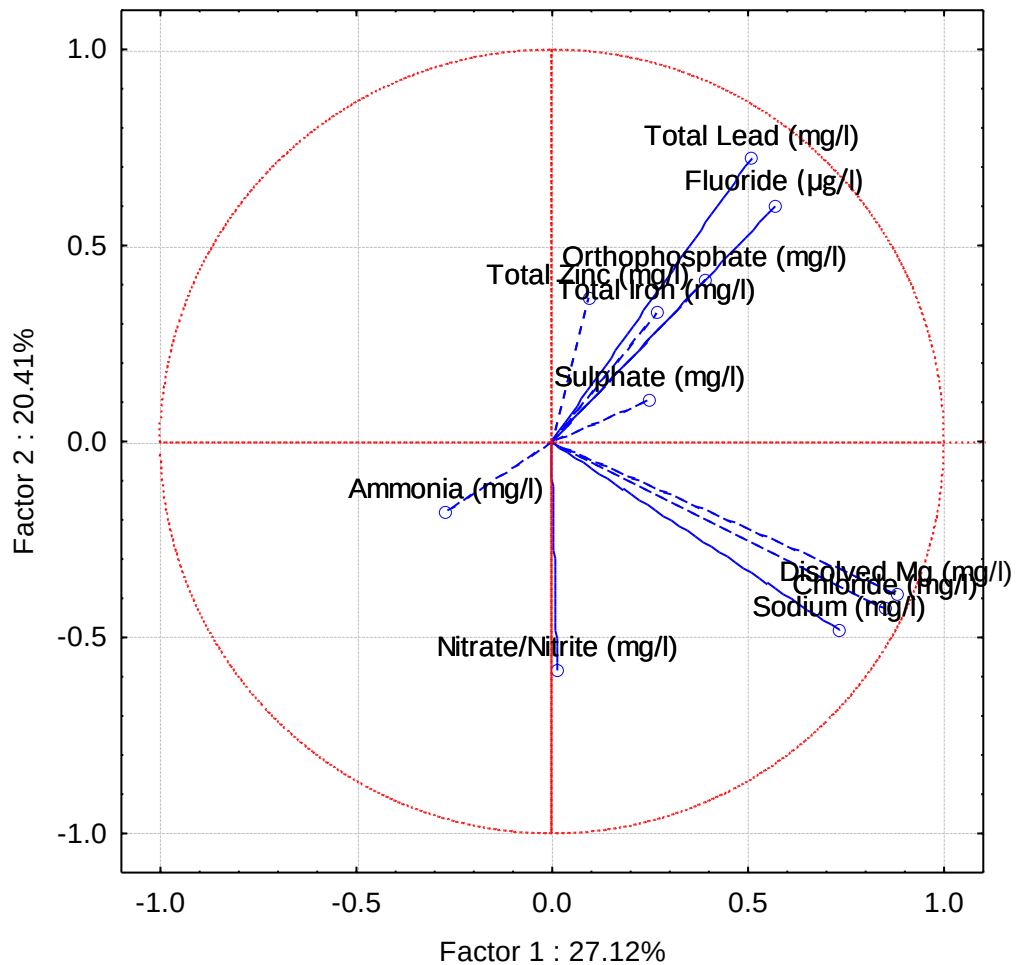


Figure 18: Factor plot of the first two factors produced from a PCA using laboratory analysed water chemistry results, with the cases for the lower Salt, upper Matjies and upper and lower Buffels River sites removed for all of the sampling events. The indiscernible groupings on the right, and in the upper middle area, include Chloride and Dissolved Magnesium and Sulphate and Nitrate/Nitrite respectively.

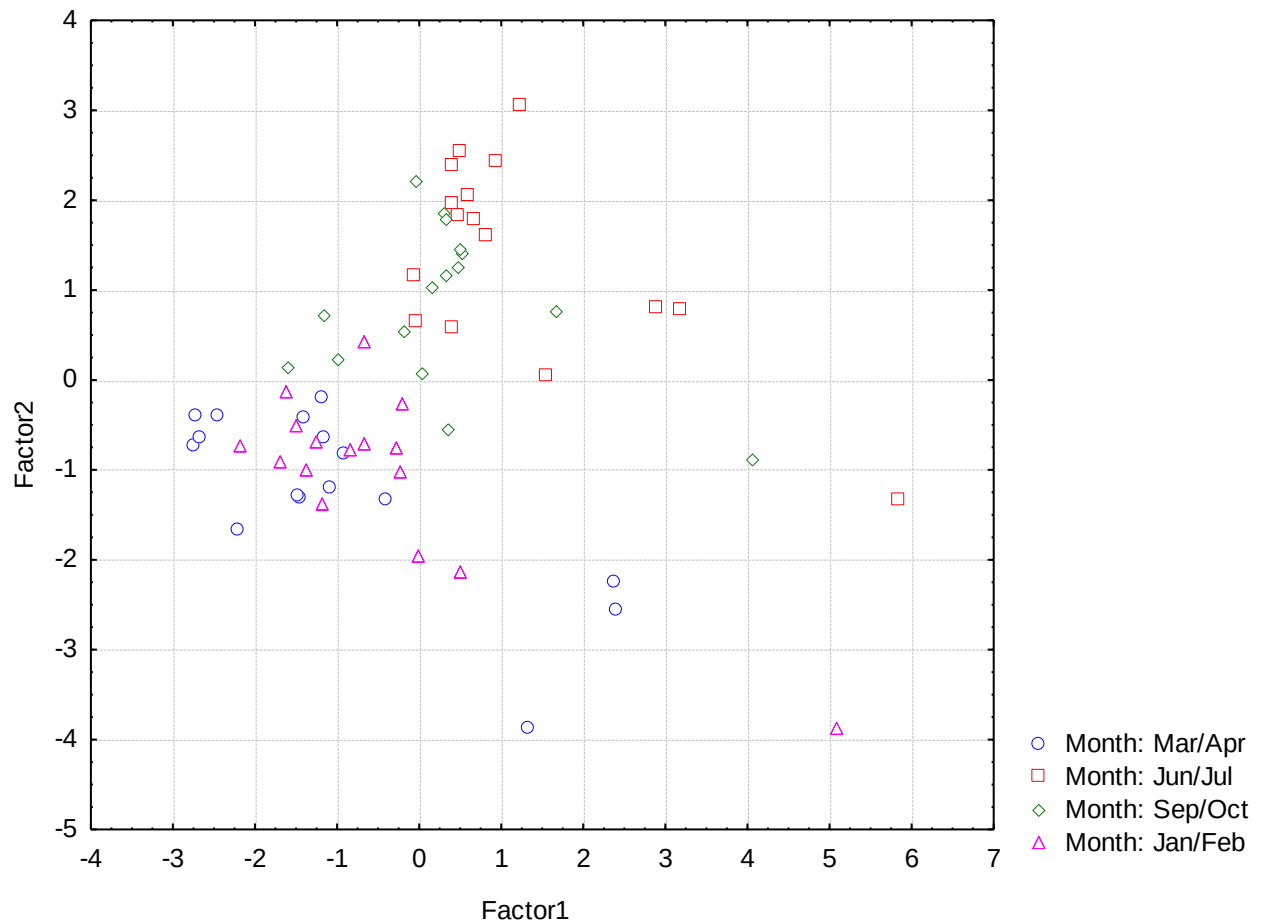


Figure 19: Seasonally categorised scatter plot of the first two factors of a PCA of laboratory water chemistry results, excluding the lower Salt, upper Matjies and upper and lower Buffels River sites for all of the sampling events. Cases are schematically represented using colour.

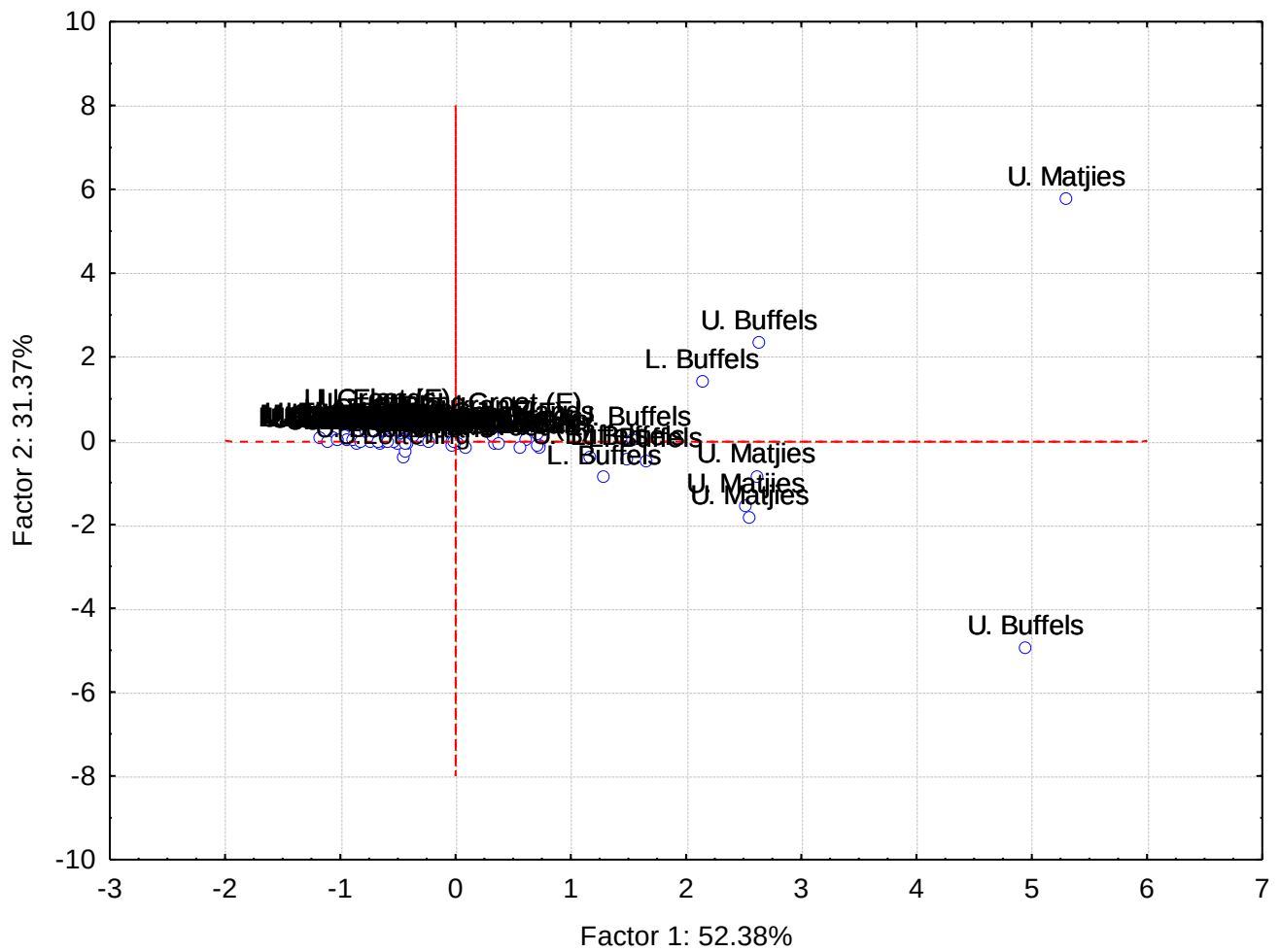


Figure 20: Ordination diagram produced from a PCA using on-site water chemistry data from all 20 sites sampled during each sampling event.

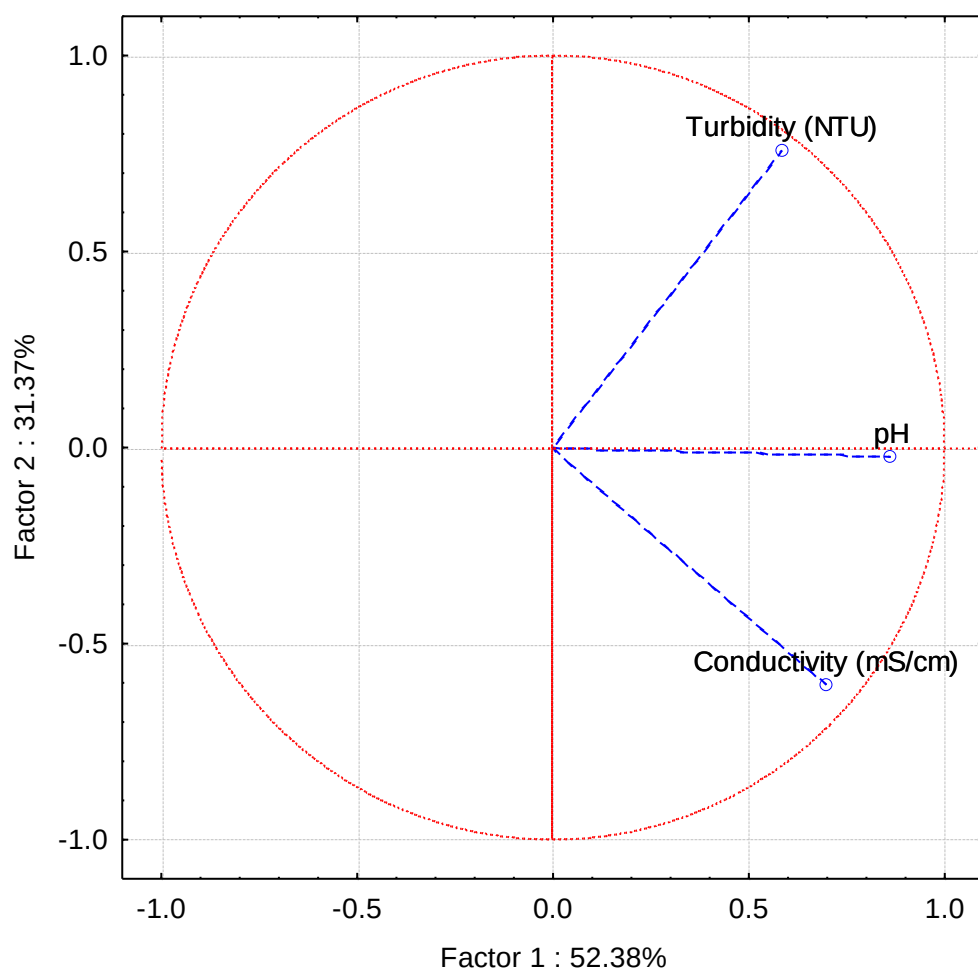


Figure 21: Factor plot produced from a PCA using on-site water chemistry data for all 20 sites sampled during each sampling event.

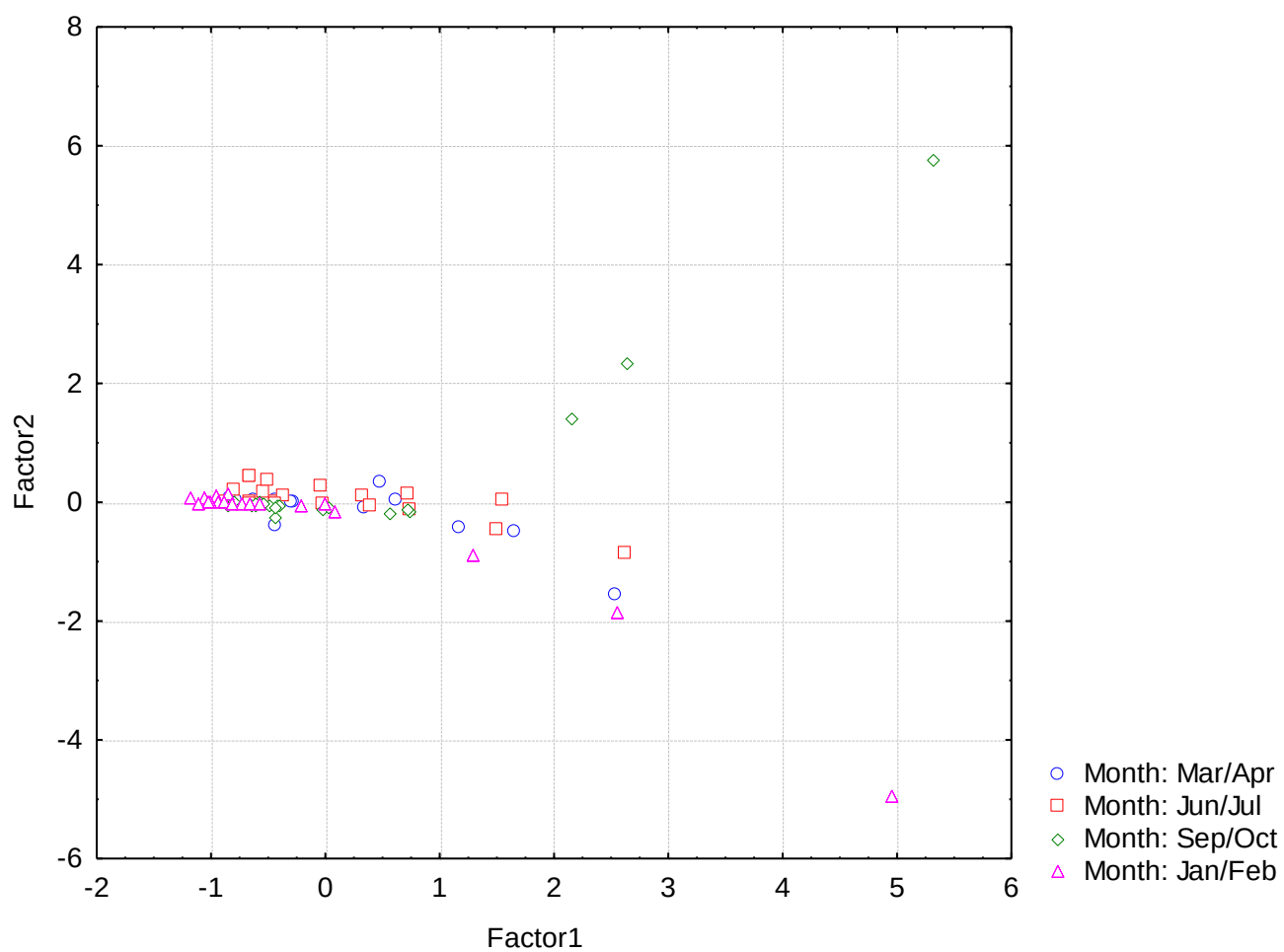


Figure 22: Seasonally categorised scatter plot of the first two factors of a PCA of on-site water chemistry variables. The cases are labelled according to month and schematically represented using colour.

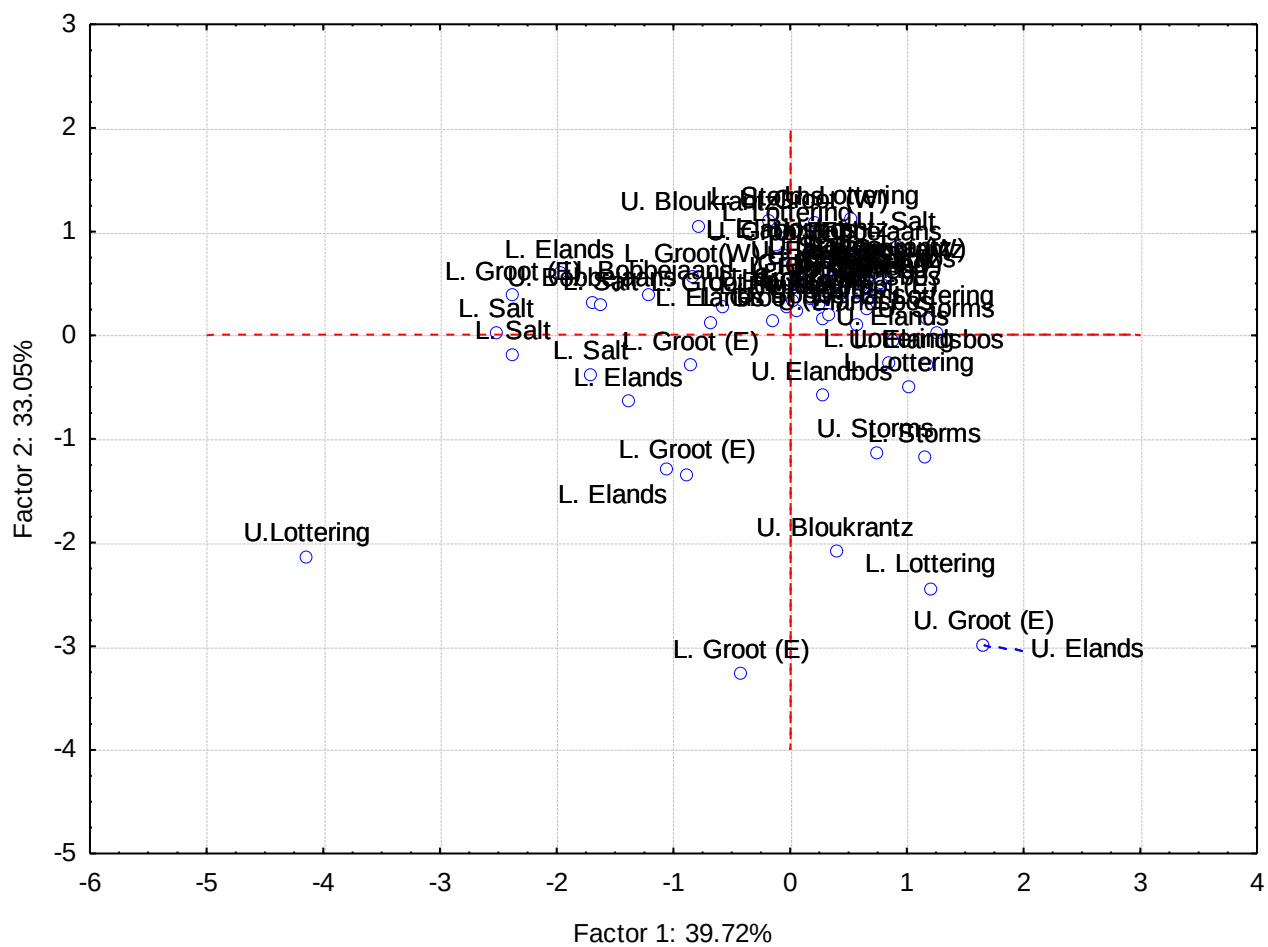


Figure 23: Ordination diagram produced from a PCA using on-site water chemistry data excluding the upper Matjies and upper and lower Buffels River sites for all of the sampling events.

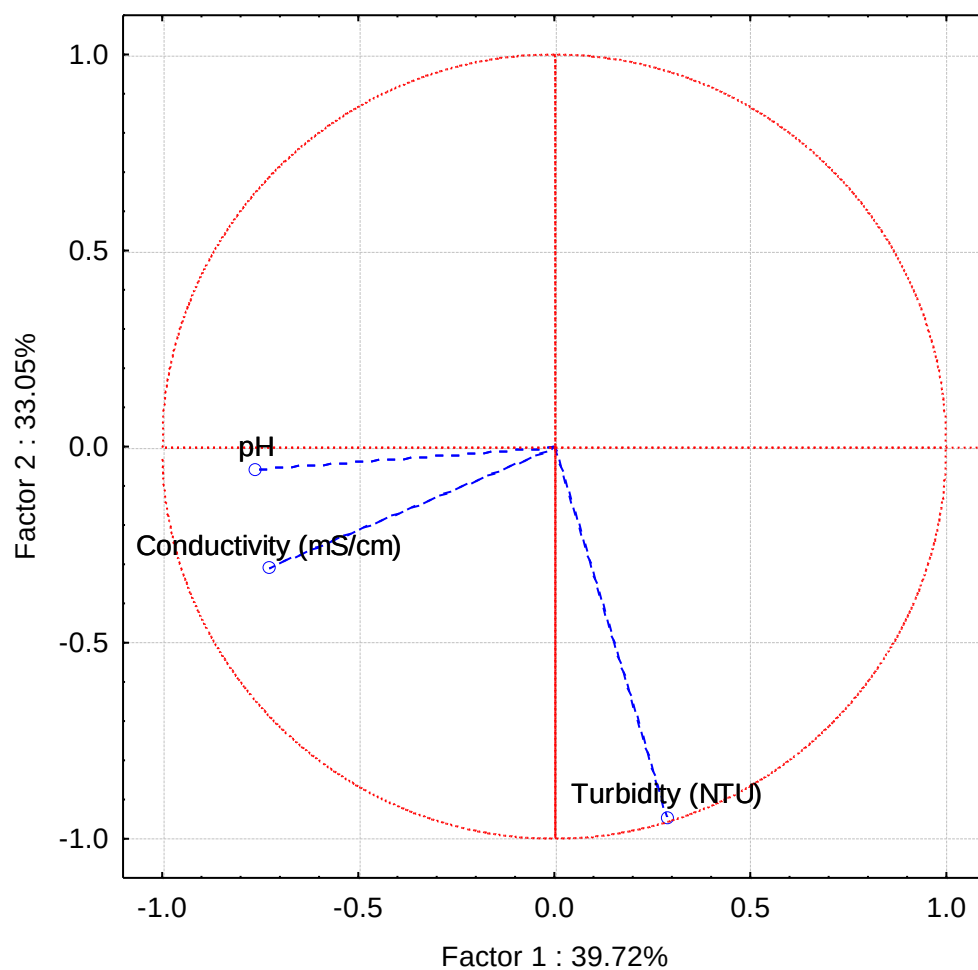


Figure 24: Factor plot produced from a PCA using on-site water chemistry data excluding the upper Matjies and upper and lower Buffels River sites for all of the sampling events.



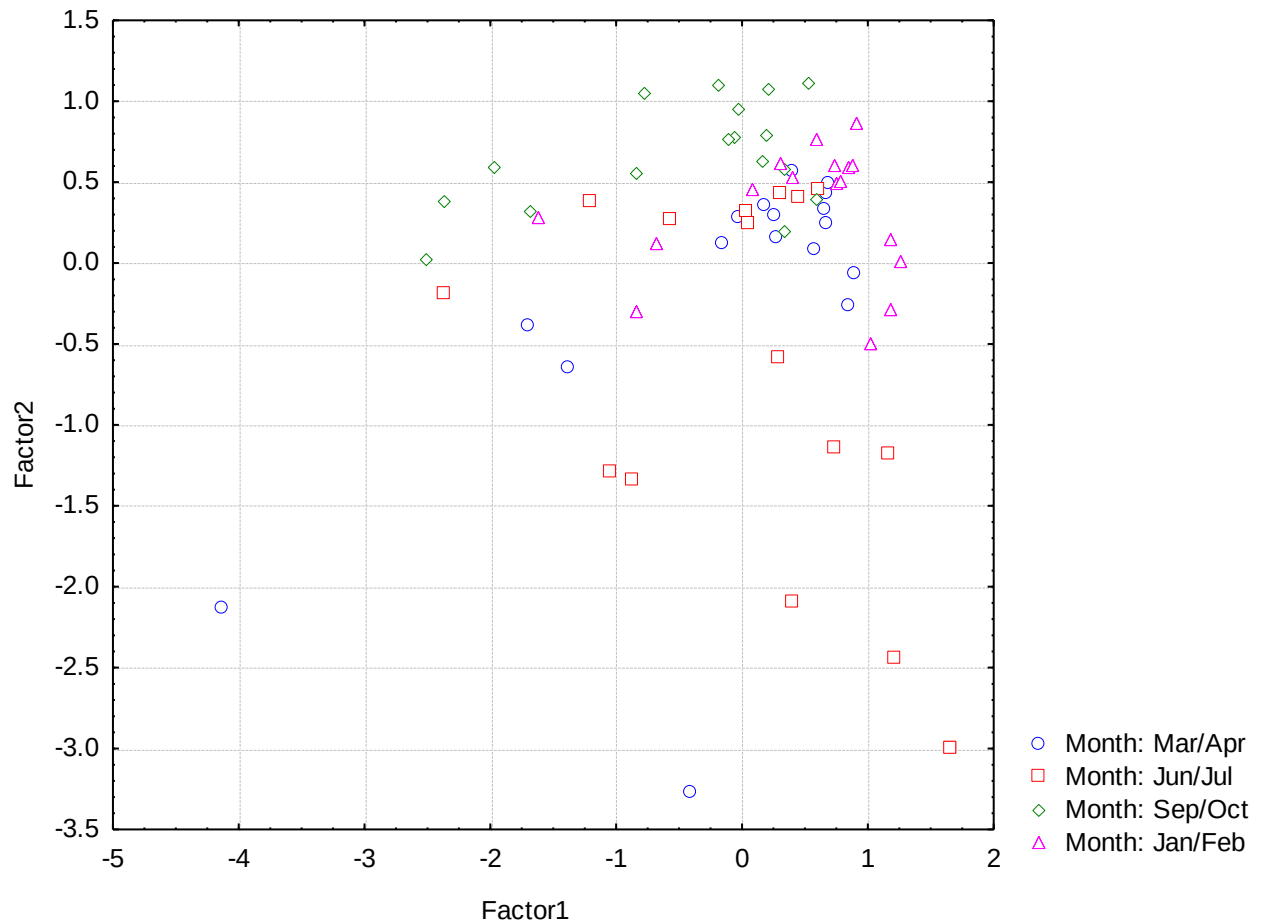


Figure 25: Seasonally categorised scatter plot of the first two factors of a PCA of on-site water chemistry variables, excluding the upper Matjies and upper and lower Buffels River sites for all of the sampling events. Cases labelled according to month and schematically represented using colour.

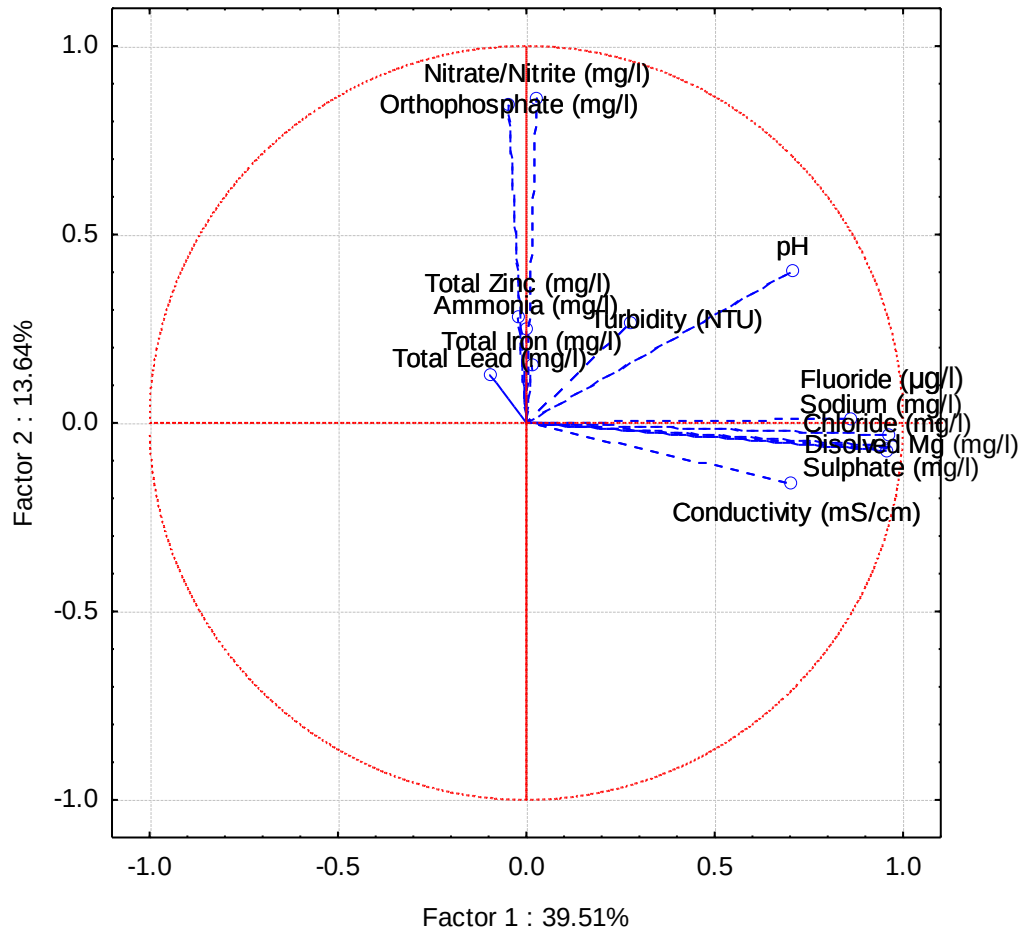


Figure 26: Factor plot generated from a PCA of a combination of laboratory analysed and on-site water chemistry data from all 20 sites collected for each sampling event.

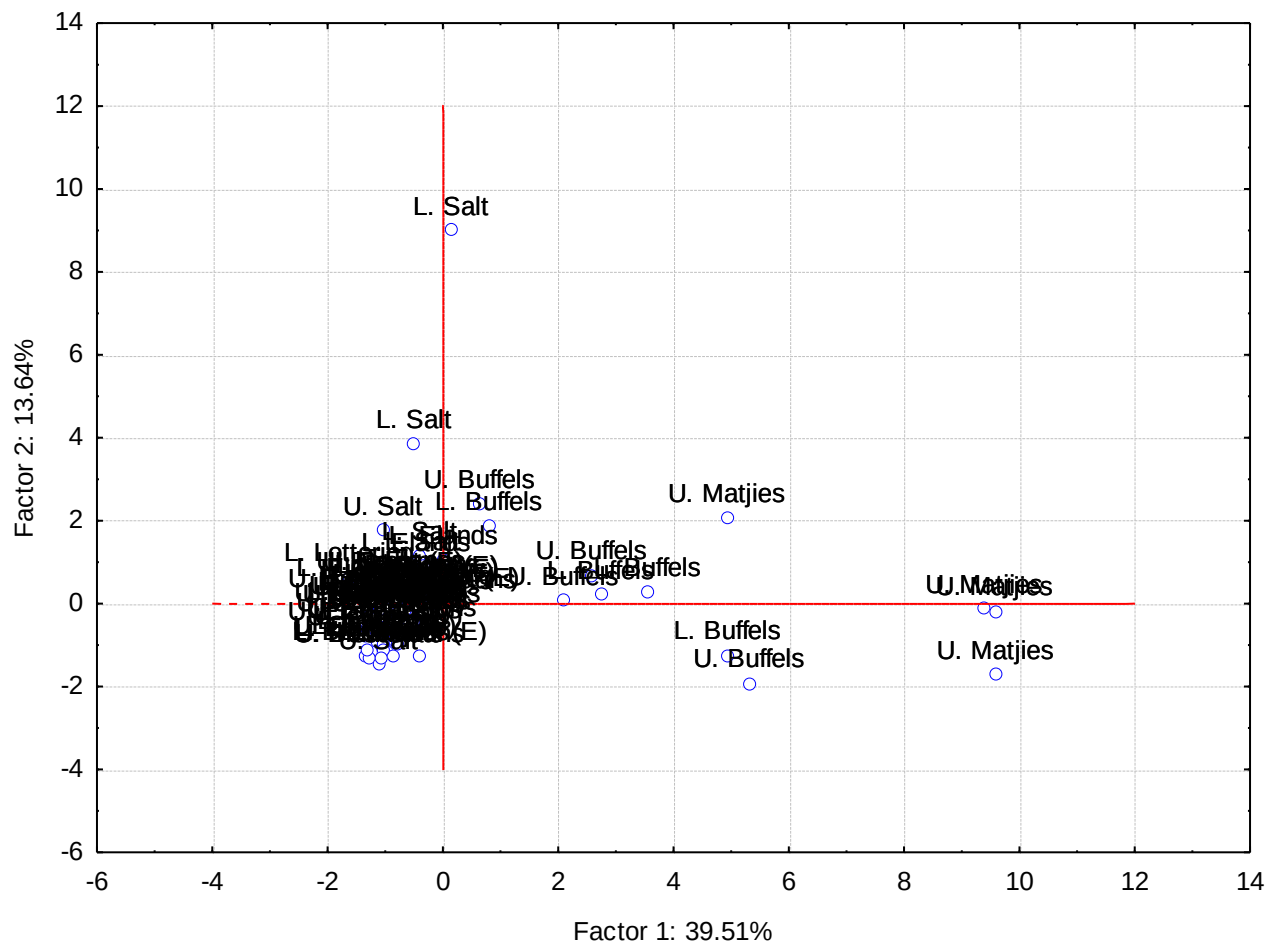


Figure 27: Ordination diagram produced from a PCA of a combination of laboratory and on-site water chemistry data from all 20 sites collected for each sampling event.

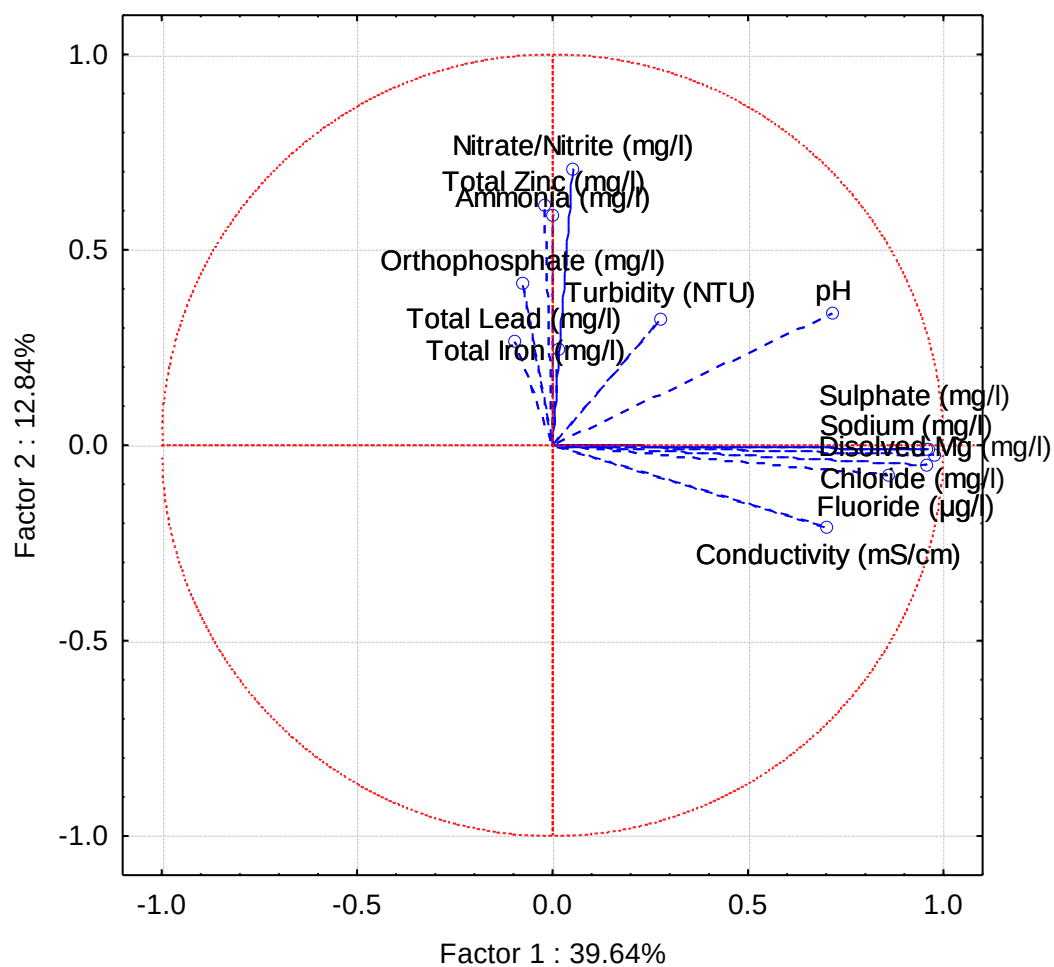


Figure 28: Factor plot from a PCA using a combination of on-site and laboratory water chemistry results, with the case for the lower Salt River from June/July treated as an outlier and excluded.



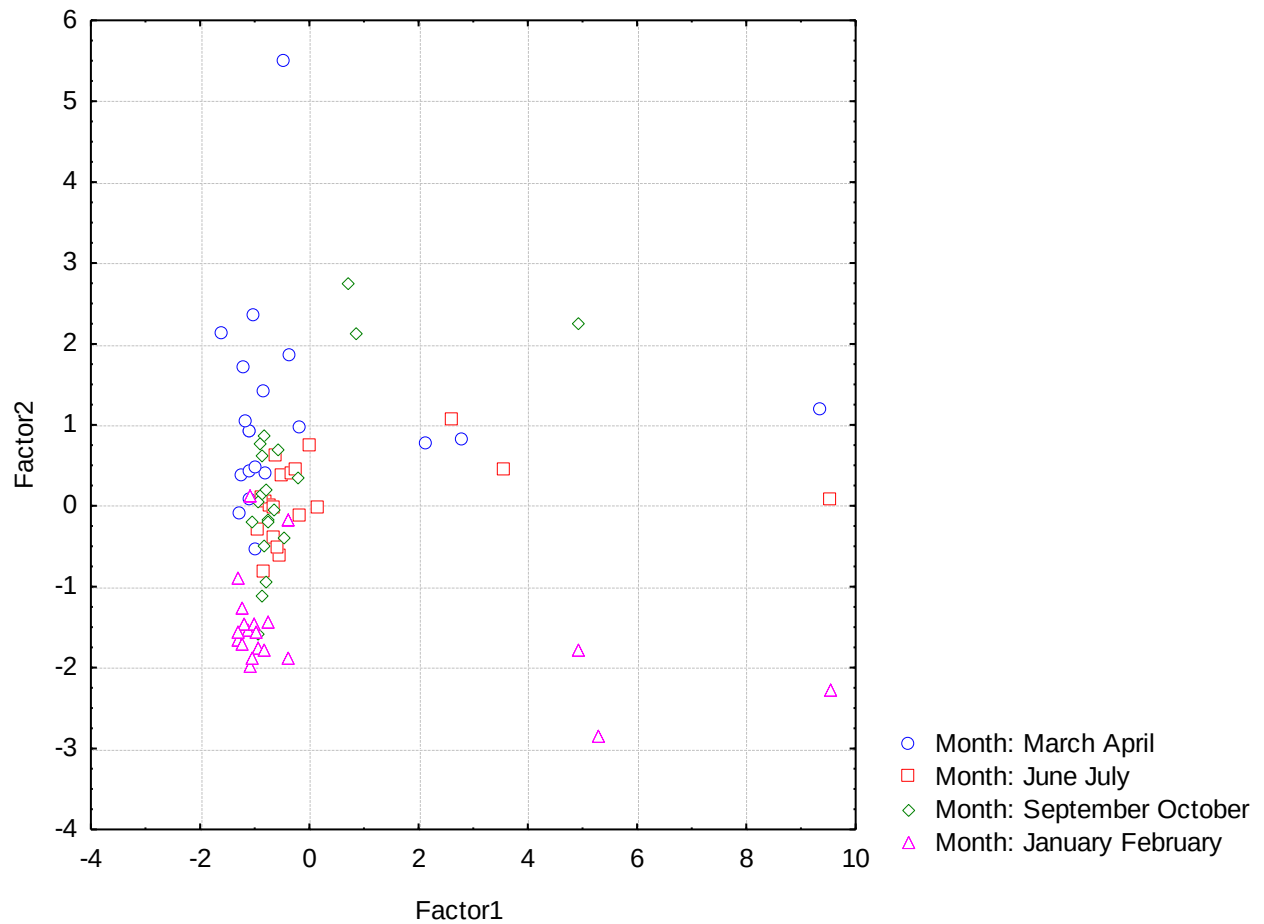


Figure 30: Categorised scatter plot of seasonal variation produced from the case statistics of a PCA of a combination of on-site and laboratory analysed water chemistry data, with the case for the lower Salt River from the winter sampling period treated as an outlier and excluded. The cases are labelled according to month and schematically represented using colour.

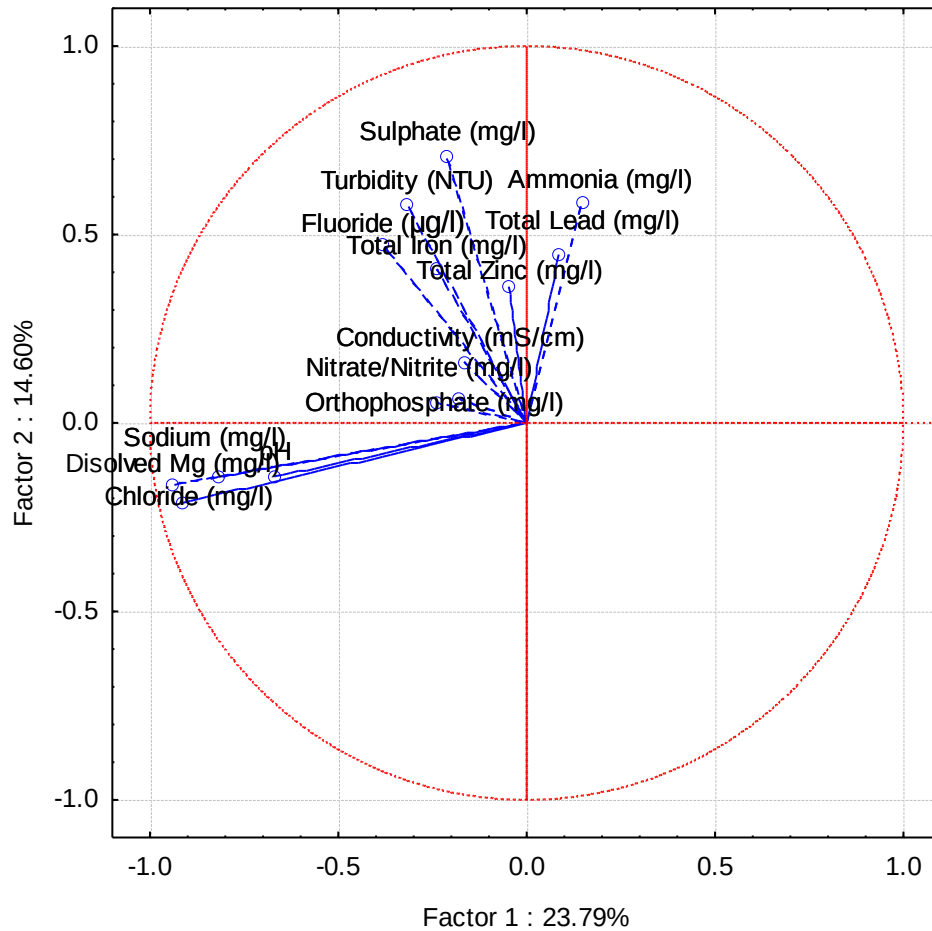


Figure 31: : Factor plot produced from a PCA using a combination of on-site and laboratory analysed water chemistry variables, with the cases for the lower Salt, upper Matjies and upper and lower Buffels River sites treated as outliers and removed for all of the sampling events.





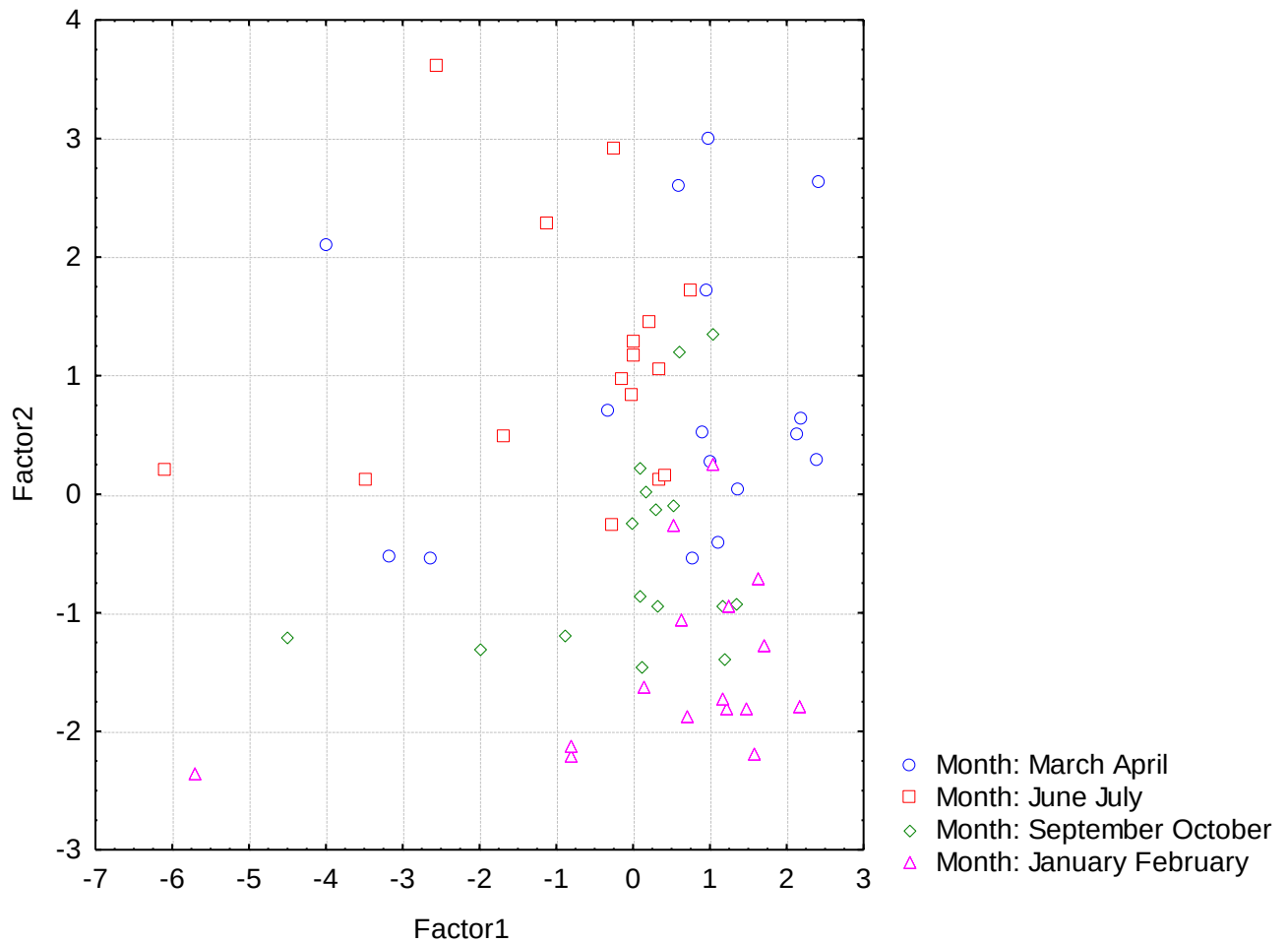


Figure 33: Seasonally categorised scatter plot of the first two factors of a PCA of a combination of on-site and laboratory analysed water chemistry variables, with the cases for the lower Salt, upper Matjies and upper and lower Buffels River sites treated as outliers and removed for all of the sampling events. The cases are schematically represented using colour.

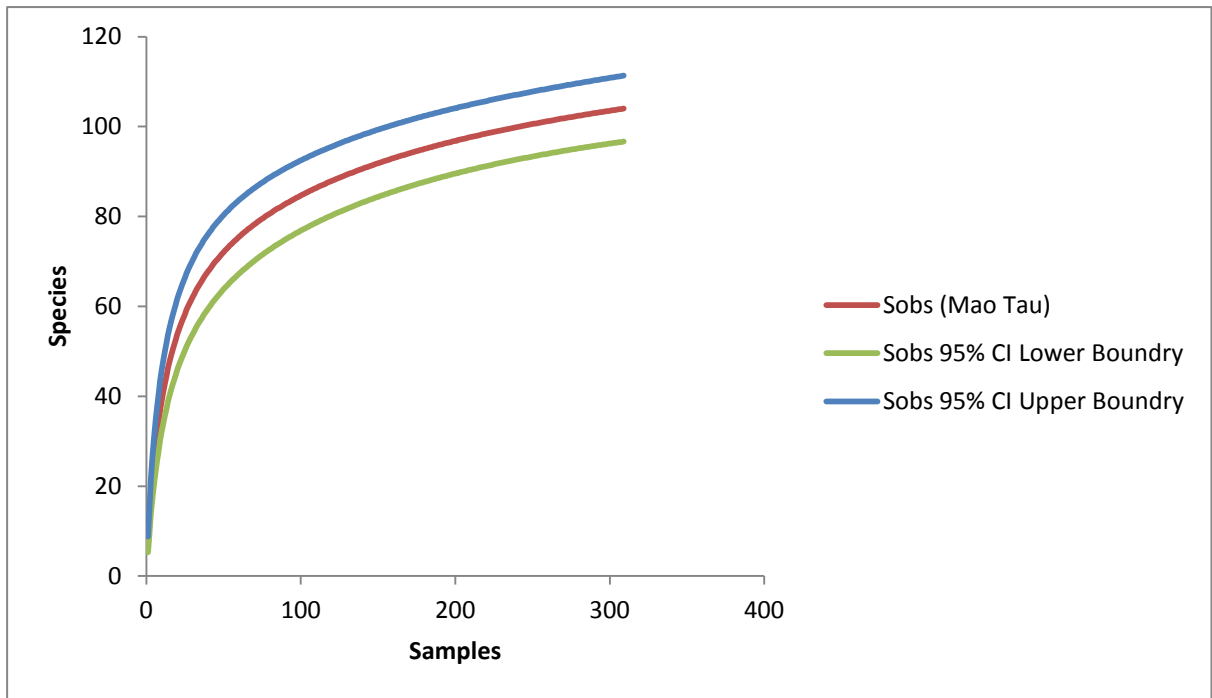


Figure 34: Sample-based rarefaction curve produced using EstimateS (Colwell 2006). The middle curve represents the number of species expected from the randomizations made from the samples collected in this study. The curves above and below this curve represent the 95% confidence intervals.

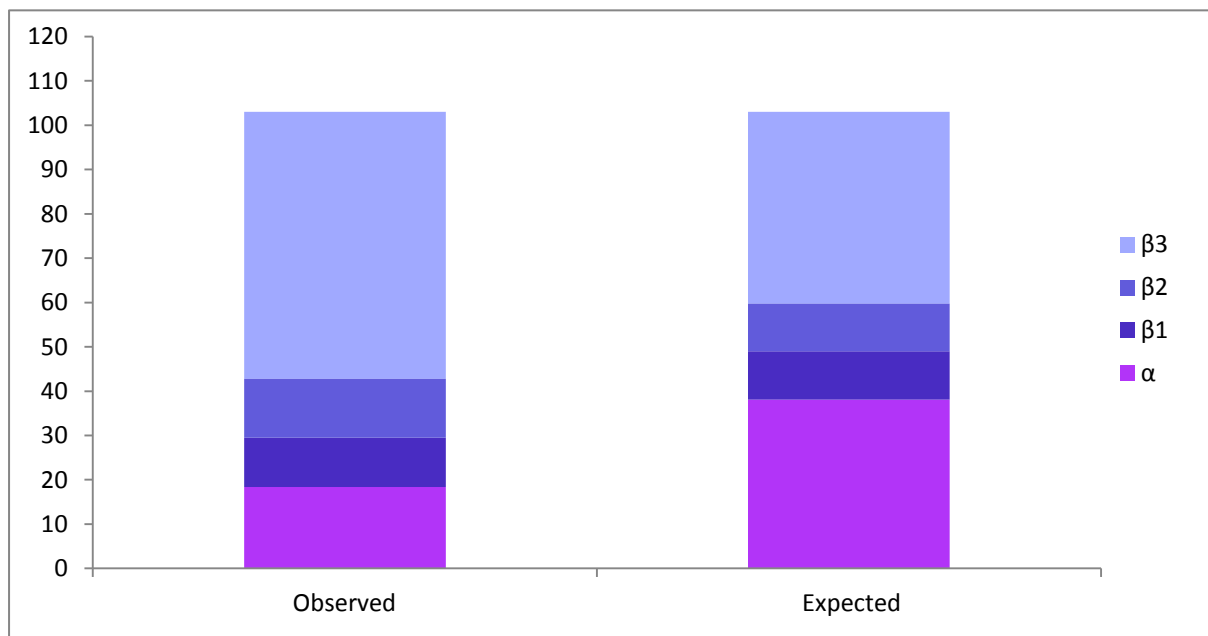


Figure 35: Differences in additive species richness, obtained from the diversity partitioning analysis, between the observed and expected diversity for each level within the hierarchical sampling design.  $\alpha_1$ : within site diversity;  $\beta_1$ : between site diversity;  $\beta_2$ : between river reach diversity (upper/lower);  $\beta_3$ : between sampling period diversity.

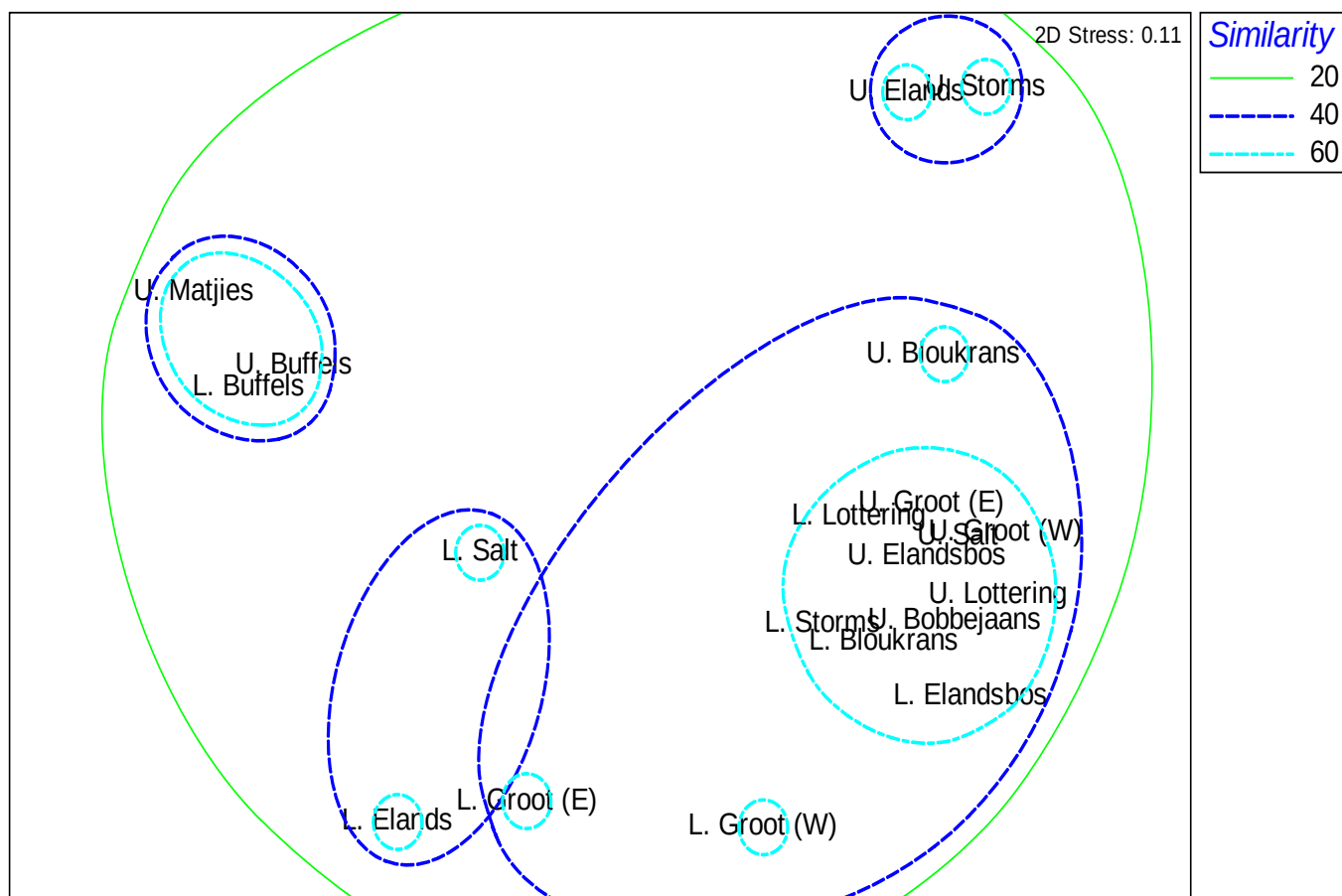


Figure 36: Ordination plot produce from MDS analysis based upon Bray-Curtis similarity coefficients using all of the species collected, excluding the Odonata, from all 20 sampling sites over a year. Clusters have been overlaid in PRIMER V.6 at resemblance levels of 20, 40 and 60 % similarity.

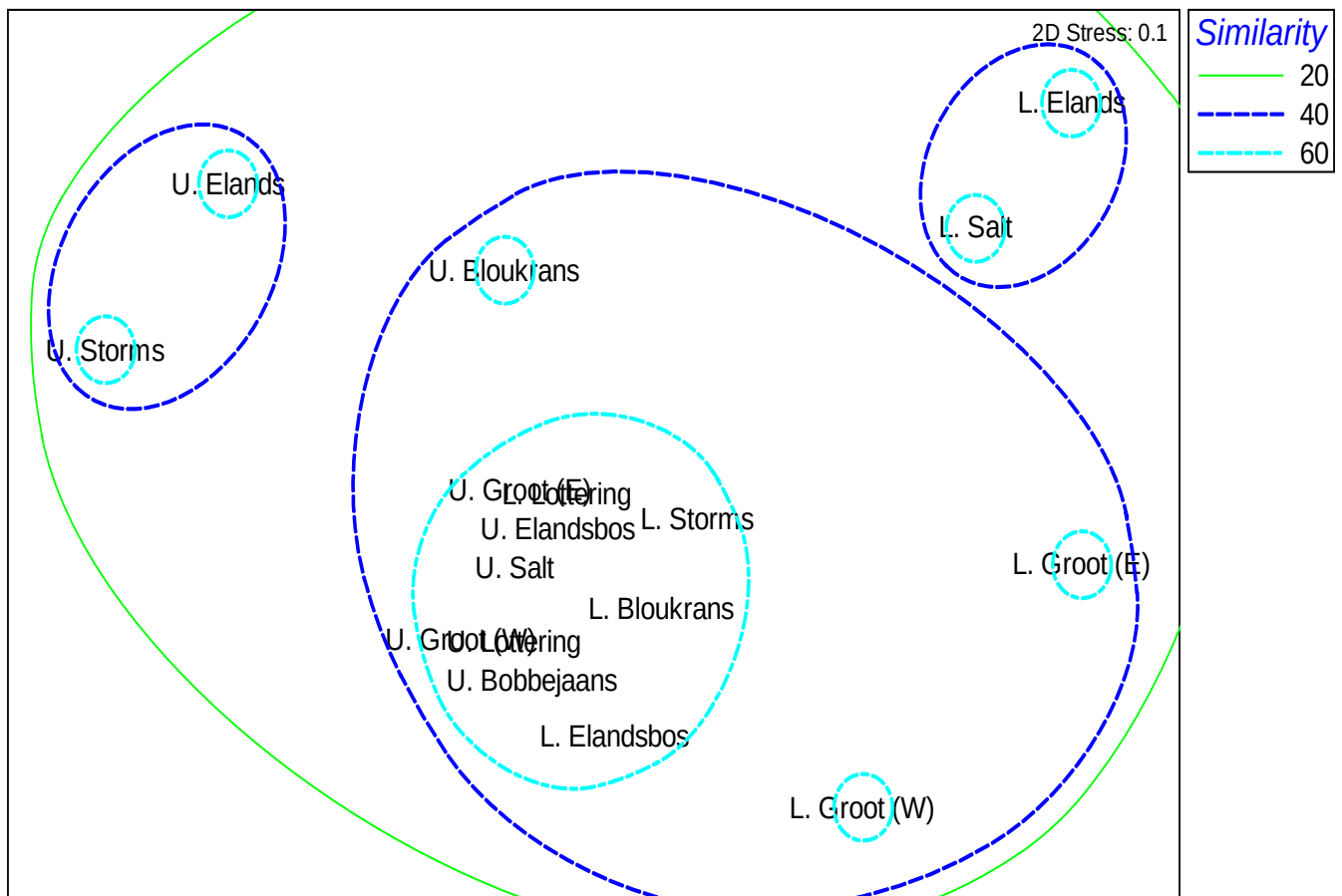


Figure 37: Ordination plot produced from MDS analysis based upon Bray-Curtis similarity coefficients using all of the species collected, excluding the Odonata, and excluding the sites along the Matjies and Buffels Rivers. Clusters have been overlaid in PRIMER V.6 at resemblance levels of 20, 40 and 60 % similarity.



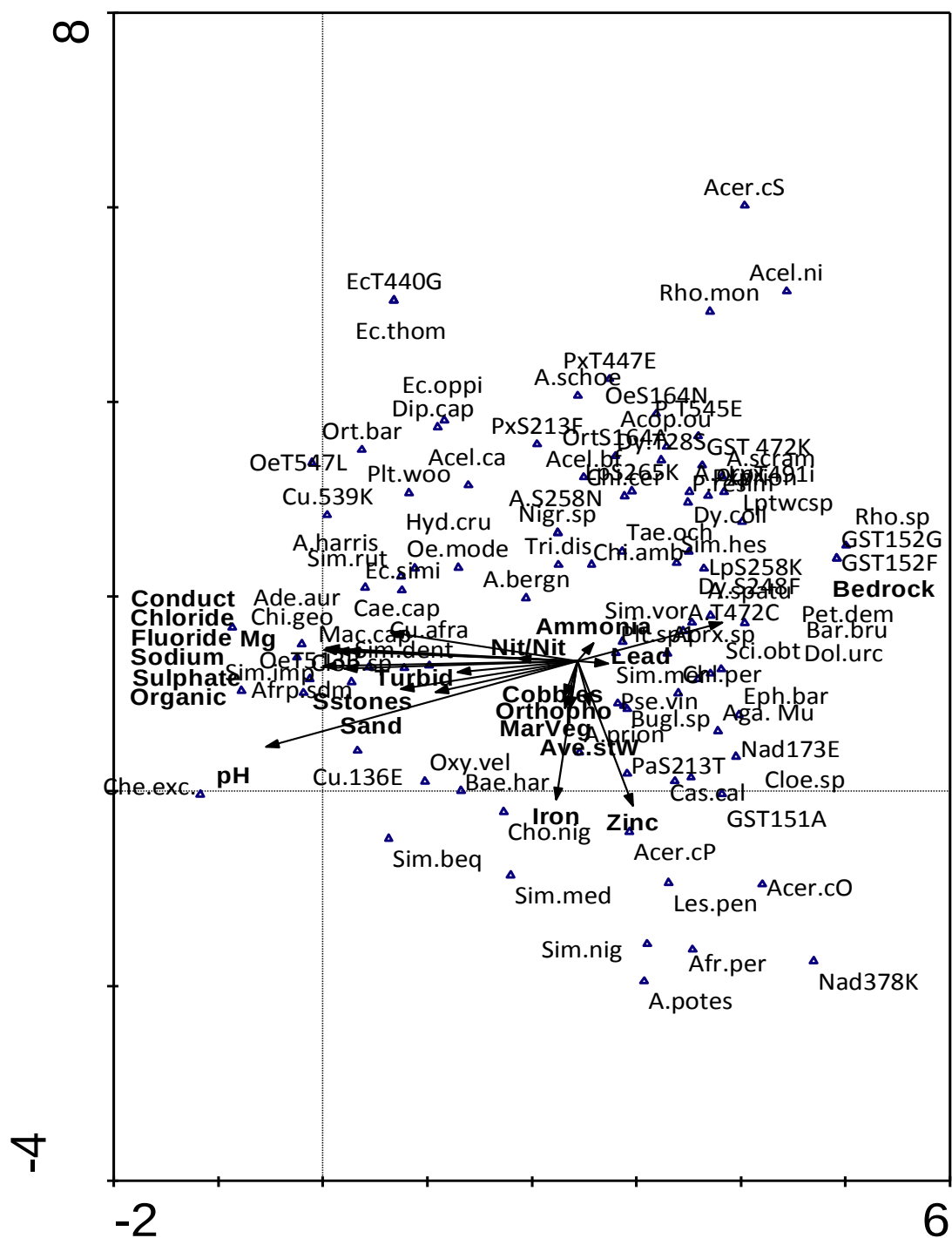


Figure 39: Ordination biplot produced from a DCCA of environmental variables and species data from all sites for Autumn, Spring and Summer sampling seasons. The environmental variable labels have been shifted for easier viewing and interpretation of the plot, particularly those to the left, including Conductivity, Sulphate, Chloride, Organic, Fluoride, Sodium and Mg. These however still correspond to the direction of the arrows (Species and environmental variable abbreviations: - Table 29 & 30 respectively).



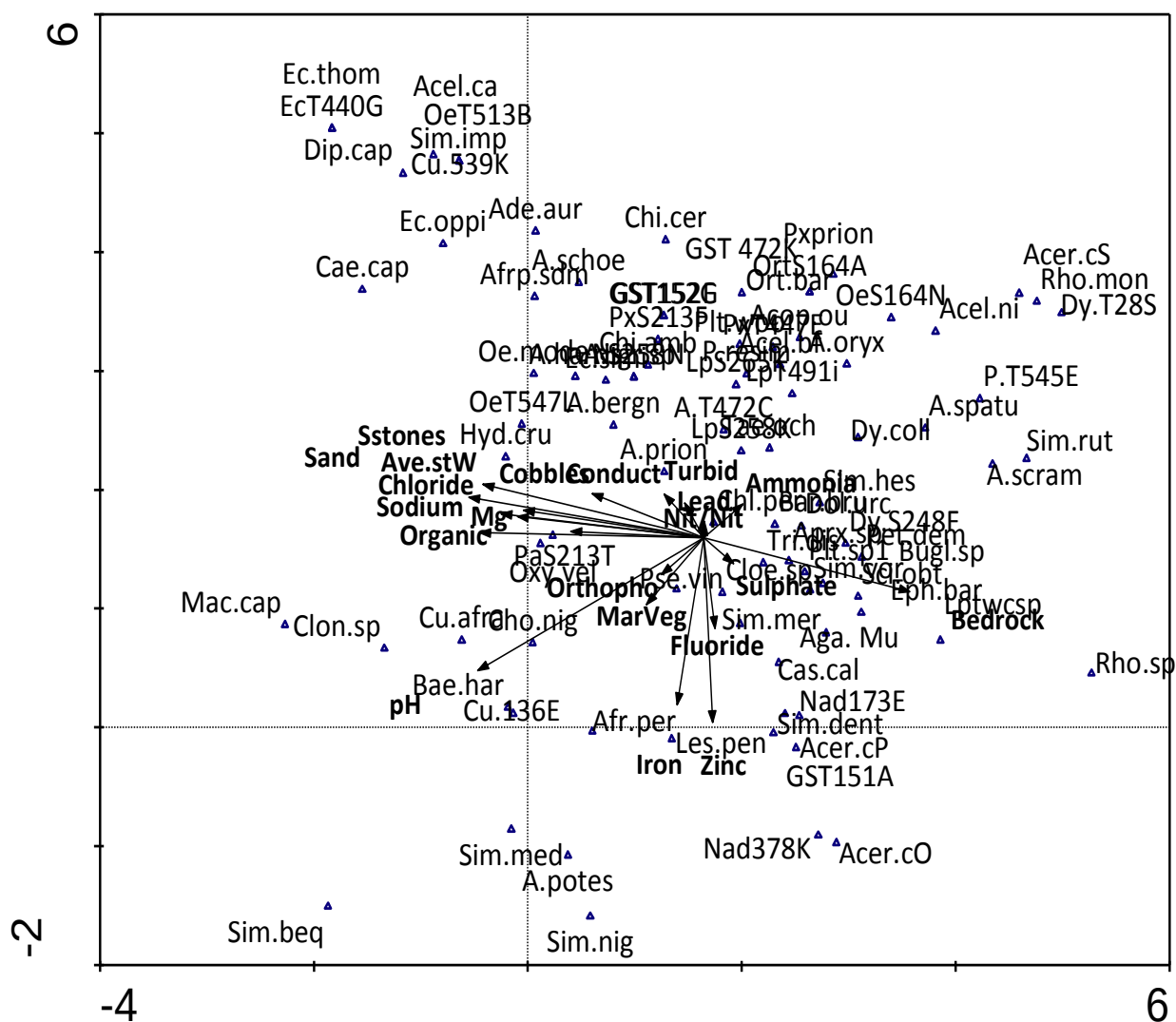


Figure 41: Ordination biplot produced from a DCCA of environmental variables and species data from all sites for Autumn, Spring and Summer sampling seasons. The environmental variable labels have been shifted for easier viewing and interpretation of the plot, particularly those to the left, including Chloride, Organic, Sodium, Sand, SStones and Mg. These however still correspond to the direction of the arrows (Species and environmental variable abbreviations: - Table 29 & 30 respectively).



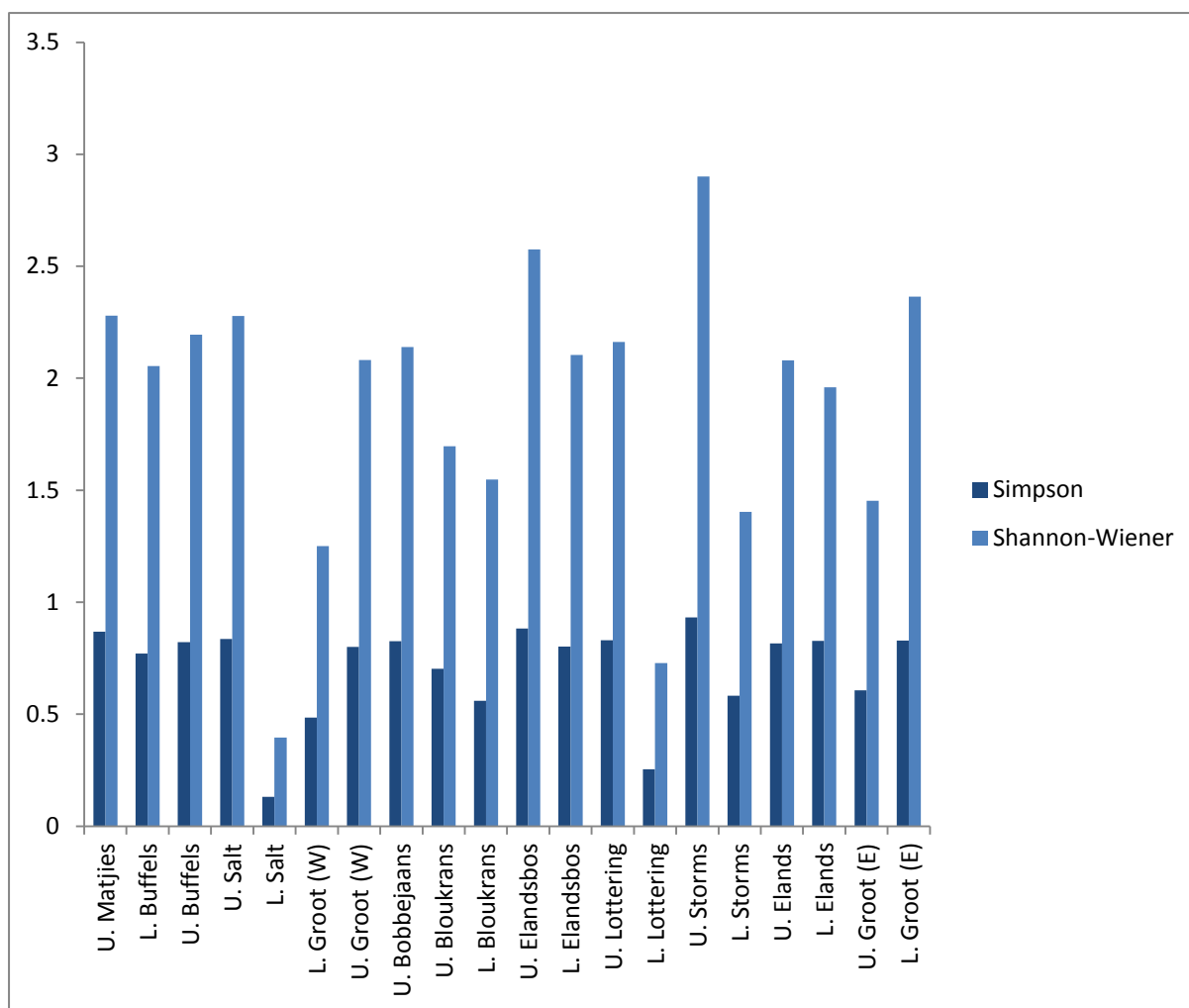


Figure 42: Simpson and Shannon-Wiener index values generated from PRIMER V6 for each site, using all of the species collected, excluding the Odonata, from all 20 sampling sites over a year.



Figure 43: A satellite image of the study area showing the rivers in false colour and the sampling sites as red dots along with the respective name assigned to each site.

---

## Tables

---

Table 1: Key to the abbreviations of biotopes sampled during surveying. Principle SASS5 biotopes are underlined.

| <b>Biotope</b> | <b>Description</b>                      | <b>Biotope</b> | <b>Description</b>                        |
|----------------|-----------------------------------------|----------------|-------------------------------------------|
| BRIC           | Bedrock in current                      | <u>MVIC</u>    | <u>Marginal vegetation in current</u>     |
| BROC           | Bedrock out of current                  | <u>MVOC</u>    | <u>Marginal vegetation out of current</u> |
| DRIFT          | Drift net sample                        | (OC)           | Suffix for out-of current biotope         |
| FNW            | Flying near water                       | RAM            | Rooted aquatic macrophytes                |
| <u>GSM</u>     | <u>Gravel, sand and mud</u>             | RIC            | Roots in current                          |
| HYG            | Hygropetric splash zone or waterfall    | ROC            | Roots out of current                      |
| (IC)           | Suffix for in-current biotope           | SED            | Sediment sample                           |
| LIGHT          | Light trap sample                       | SEEP           | Groundwater seepage                       |
| LPIC           | Leaf pack or leaf litter in current     | SNAG           | Log jam, submerged wood                   |
| LPOC           | Leaf pack or leaf litter out of current | SED            | Sediment sample                           |
| MALAISE        | Malaise trap set                        | <u>SIC</u>     | <u>Stones in current</u>                  |
| MIC            | Moss in current                         | <u>SOC</u>     | <u>Stones out of current</u>              |
| MOC            | Moss out of current                     | SOP            | Surface of pool                           |
| MUD            | Mud sample                              |                |                                           |

---

Table 2: Summary of temperature data recorded from each site using I-Button data loggers.

| Locality (Upper/Lower) | Min (°C) | Max (°C) | Range (°C) | Mean with stdv (°C) | Comments                       |
|------------------------|----------|----------|------------|---------------------|--------------------------------|
| U. Groot (E)           | 8.0      | 24.0     | 16.0       | 15.2 ± 3.2          |                                |
| L. Groot (E)           | 7.0      | 26.5     | 19.5       | 16.0 ± 4.8          | Logger found exposed           |
| U. Elands              | 8.5      | 24.0     | 15.5       | 14.8 ± 3.3          |                                |
| L. Elands              | 7.5      | 25.0     | 17.5       | 15.4 ± 4.2          |                                |
| U. Storms              | 8.0      | 19.0     | 11.0       | 13.5 ± 2.7          |                                |
| L. Storms              | 9.0      | 25.0     | 20.0       | 16.4 ± 4.4          |                                |
| U. Lottering           | 7.5      | 24.0     | 16.5       | 15.0 ± 3.6          |                                |
| L. Lottering           | 8.0      | 23.5     | 15.4       | 15.4 ± 3.8          |                                |
| U. Elandsbos           | 8.5      | 25.5     | 17.0       | 16.1 ± 3.8          |                                |
| L. Elandsbos           | 7.5      | 25.0     | 17.5       | 15.7 ± 3.9          | Logger found exposed           |
| U. Bloukrans           | 5.5      | 28.5     | 23.0       | 15.4 ± 4.9          |                                |
| L. Bloukrans           | 8.5      | 25.0     | 16.5       | 15.9 ± 4.4          | Logger removed, later replaced |
| U. Groot (W)           | 6.0      | 24.0     | 18.0       | 14.0 ± 4.0          |                                |
| L. Groot (W)           | 10.5     | 24.0     | 13.5       | 16.4 ± 3.5          |                                |
| U. Bobbejaans          | 7.0      | 26.5     | 19.5       | 14.3 ± 3.9          | Logger found exposed           |
| U. Salt                | 8.0      | 23.0     | 15.0       | 15.2 ± 3.3          |                                |
| L. Salt                | 8.0      | 26.5     | 18.5       | 16.3 ± 4.2          |                                |
| U. Buffels             | 4.5      | 22.5     | 18.0       | 13.8 ± 4.2          |                                |
| L. Buffels             | 5.5      | 22.5     | 17.0       | 12.9 ± 4.0          | Logger malfunctioned           |
| U. Matjies             | 5.5      | 20.5     | 15.0       | 13.5 ± 3.5          |                                |

Table 3: Laboratory analysed water chemistry results recorded over the autumn and winter sampling periods.

| Site          | Month   | Ammonia (mg/l) | Chloride (mg/l) | Dissolved Magnesium (mg/l) | Fluoride ( µg/l) | Nitrate/Nitrite (mg/l) | Orthophosphate (mg/l) | Sodium (mg/l) | Sulphate (mg/l) | Total Lead (mg/l) | Total Zinc (mg/l) | Total Iron (mg/l) |
|---------------|---------|----------------|-----------------|----------------------------|------------------|------------------------|-----------------------|---------------|-----------------|-------------------|-------------------|-------------------|
| U. Storms     | Mar/Apr | 0.38           | 4               | 0.2                        | 110              | 0.51                   | 0.007                 | 5.70          | 9.4             | 0.04              | 0.040             | 0.34              |
| L. Storms     | Mar/Apr | 0.36           | 4               | 0.2                        | 100              | 0.57                   | 0.002                 | 7.10          | 8.9             | 0.04              | 0.040             | 0.43              |
| U. Bobbejaans | Mar/Apr | 0.40           | 14              | 0.9                        | 50               | 0.56                   | 0.001                 | 12.0          | 7.4             | 0.04              | 0.040             | 0.27              |
| U. Groot (W)  | Mar/Apr | 0.29           | 5               | 0.2                        | 90               | 0.54                   | 0.002                 | 6.20          | 10.4            | 0.04              | 0.040             | 0.27              |
| L. Elandsbos  | Mar/Apr | 0.34           | 16              | 1.2                        | 180              | 0.48                   | 0.001                 | 15.0          | 15.5            | 0.04              | 0.040             | 0.46              |
| L. Lottering  | Mar/Apr | 0.31           | 4               | 0.2                        | 70               | 0.53                   | 0.010                 | 8.20          | 10.7            | 0.04              | 0.050             | 0.43              |
| L. Bloukrans  | Mar/Apr | 0.32           | 20              | 1.2                        | 130              | 0.52                   | 0.004                 | 14.0          | 13.4            | 0.04              | 0.040             | 0.16              |
| U. Lottering  | Mar/Apr | 0.34           | 11              | 0.9                        | 170              | 0.49                   | 0.003                 | 10.0          | 18.5            | 0.04              | 0.040             | 0.43              |
| U. Elandsbos  | Mar/Apr | 0.18           | 14              | 1.0                        | 150              | 0.31                   | 0.002                 | 12.0          | 13.8            | 0.04              | 0.040             | 0.31              |
| L. Groot (E)  | Mar/Apr | 0.30           | 47              | 3.7                        | 220              | 0.52                   | 0.001                 | 29.0          | 17.0            | 0.04              | 0.040             | 0.97              |
| U. Groot (E)  | Mar/Apr | 0.23           | 18              | 1.2                        | 49               | 0.43                   | 0.001                 | 14.0          | 10.0            | 0.04              | 0.040             | 0.38              |
| L. Elands     | Mar/Apr | 0.22           | 41              | 3.1                        | 110              | 1.08                   | 0.005                 | 28.0          | 10.5            | 0.04              | 0.040             | 0.40              |
| U. Elands     | Mar/Apr | 0.33           | 15              | 1.1                        | 170              | 0.50                   | 0.001                 | 11.0          | 18.1            | 0.04              | 0.040             | 0.50              |
| L. Groot (W)  | Mar/Apr | 0.32           | 28              | 1.4                        | 130              | 0.55                   | 0.001                 | 19.0          | 12.1            | 0.04              | 0.070             | 0.43              |
| U. Bloukrans  | Mar/Apr | 0.23           | 17              | 1.2                        | 49               | 0.43                   | 0.001                 | 14.0          | 10.0            | 0.04              | 0.040             | 0.38              |
| U. Salt       | Mar/Apr | 0.22           | 41              | 3.1                        | 110              | 1.08                   | 0.050                 | 28.0          | 10.5            | 0.04              | 0.040             | 0.40              |
| L. Salt       | Mar/Apr | 0.38           | 77              | 4.8                        | 90               | 1.46                   | 0.070                 | 47.0          | 17.0            | 0.04              | 0.080             | 0.26              |
| U. Buffels    | Mar/Apr | 0.29           | 360             | 48.                        | 220              | 0.55                   | 0.001                 | 152.0         | 91.4            | 0.04              | 0.040             | 0.47              |
| U. Matjies    | Mar/Apr | 0.35           | 1270            | 151.0                      | 530              | 0.42                   | 0.005                 | 500.0         | 406.0           | 0.04              | 0.080             | 0.25              |
| L. Buffels    | Mar/Apr | 0.31           | 390             | 49.0                       | 370              | 0.51                   | 0.001                 | 159.0         | 87.7            | 0.04              | 0.040             | 0.26              |
| U. Salt       | Jun/Jul | 0.19           | 16              | 1.2                        | 280              | 0.23                   | 0.037                 | 12.0          | 7.2             | 0.06              | 0.040             | 0.30              |
| L. Bloukrans  | Jun/Jul | 0.27           | 21              | 1.3                        | 290              | 0.38                   | 0.024                 | 14.0          | 11.0            | 0.08              | 0.040             | 0.38              |
| U. Groot (E)  | Jun/Jul | 0.12           | 18              | 1.4                        | 280              | 0.18                   | 0.019                 | 12.0          | 7.6             | 0.08              | 0.030             | 0.50              |
| L. Groot (E)  | Jun/Jul | 0.16           | 72              | 4.8                        | 360              | 0.43                   | 0.011                 | 41.0          | 12.2            | 0.08              | 0.020             | 1.10              |
| U. Elands     | Jun/Jul | 0.17           | 18              | 1.2                        | 370              | 0.23                   | 0.015                 | 11.0          | 16.6            | 0.08              | 0.030             | 0.50              |
| L. Elands     | Jun/Jul | 0.09           | 35              | 2.8                        | 320              | 0.56                   | 0.037                 | 24.0          | 9.6             | 0.08              | 0.030             | 0.50              |
| U. Storms     | Jun/Jul | 0.13           | 18              | 1.4                        | 300              | 0.30                   | 0.014                 | 13.0          | 9.3             | 0.08              | 0.040             | 0.40              |
| L. Storms     | Jun/Jul | 0.13           | 19              | 1.4                        | 230              | 0.24                   | 0.011                 | 12.0          | 9.9             | 0.08              | 0.050             | 0.91              |
| U. Elandsbos  | Jun/Jul | 0.12           | 15              | 1.2                        | 300              | 0.20                   | 0.015                 | 8.0           | 16.0            | 0.08              | 0.040             | 0.59              |
| U. Lottering  | Jun/Jul | 0.19           | 14              | 1.0                        | 310              | 0.30                   | 0.015                 | 9.0           | 17.2            | 0.08              | 0.050             | 0.46              |
| U. Bloukrans  | Jun/Jul | 0.08           | 12              | 1.8                        | 260              | 0.16                   | 0.009                 | 12.0          | 6.1             | 0.07              | 0.030             | 5.40              |
| L. Groot (W)  | Jun/Jul | 0.20           | 34              | 2.1                        | 280              | 0.45                   | 0.013                 | 20.0          | 13.5            | 0.06              | 0.040             | 0.62              |
| L. Lottering  | Jun/Jul | 0.20           | 30              | 2.8                        | 390              | 0.51                   | 0.014                 | 22.0          | 24.6            | 0.07              | 0.040             | 0.80              |
| L. Elandsbos  | Jun/Jul | 0.26           | 18              | 1.5                        | 280              | 0.43                   | 0.015                 | 14.0          | 15.8            | 0.06              | 0.020             | 0.66              |
| L. Salt       | Jun/Jul | 0.21           | 85              | 5.8                        | 270              | 4.47                   | 0.136                 | 48.0          | 14.2            | 0.08              | 0.030             | 0.34              |
| U. Buffels    | Jun/Jul | 0.20           | 312             | 52.0                       | 410              | 0.57                   | 0.016                 | 150.0         | 84.0            | 0.07              | 0.040             | 0.80              |
| L. Buffels    | Jun/Jul | 0.15           | 387             | 68.0                       | 560              | 0.55                   | 0.012                 | 194.0         | 98.1            | 0.06              | 0.040             | 0.66              |
| U. Matjies    | Jun/Jul | 0.20           | 1035            | 158.0                      | 760              | 0.50                   | 0.014                 | 730.0         | 265.0           | 0.07              | 0.030             | 0.56              |
| U. Bobbejaans | Jun/Jul | 0.19           | 18              | 1.3                        | 320              | 0.31                   | 0.012                 | 10.0          | 6.6             | 0.06              | 0.030             | 0.40              |
| U. Groot (W)  | Jun/Jul | 0.22           | 18              | 1.4                        | 180              | 0.44                   | 0.011                 | 12.0          | 10.5            | 0.08              | 0.030             | 0.47              |

Table 4: Laboratory analysed water chemistry results recorded over the spring and summer sampling periods.

| Site          | Month   | Ammonia (mg/l) | Chloride (mg/l) | Dissolved Mg (mg/l) | Fluoride ( µg/l) | Nitrate/Nitrite (mg/l) | Orthophosphate (mg/l) | Sodium (mg/l) | Sulphate (mg/l) | Total Lead (mg/l) | Total Zinc (mg/l) | Total Iron (mg/l) |
|---------------|---------|----------------|-----------------|---------------------|------------------|------------------------|-----------------------|---------------|-----------------|-------------------|-------------------|-------------------|
| L. Salt       | Sep/Oct | 0.08           | 11              | 2.0                 | 180              | 0.02                   | 0.075                 | 21.0          | 7.4             | 0.04              | 0.0100            | 0.16              |
| U. Buffels    | Sep/Oct | 0.07           | 58              | 4.7                 | 250              | 1.39                   | 0.033                 | 35.0          | 18.3            | 0.04              | 0.010             | 0.68              |
| U. Matjies    | Sep/Oct | 0.07           | 482             | 55.0                | 560              | 0.47                   | 0.025                 | 256.0         | 132.0           | 0.06              | 0.040             | 1.18              |
| L. Buffels    | Sep/Oct | 0.07           | 75              | 7.0                 | 320              | 1.04                   | 0.028                 | 43.0          | 23.0            | 0.05              | 0.030             | 0.90              |
| U. Groot (W)  | Sep/Oct | 0.07           | 13              | 0.9                 | 190              | 0.27                   | 0.016                 | 10.0          | 8.7             | 0.04              | 0.009             | 0.16              |
| U. Bobbejaans | Sep/Oct | 0.19           | 10              | 0.6                 | 180              | 0.26                   | 0.015                 | 9.7           | 6.3             | 0.04              | 0.010             | 0.13              |
| L. Elandsbos  | Sep/Oct | 0.07           | 18              | 1.3                 | 190              | 0.27                   | 0.016                 | 13.0          | 10.0            | 0.05              | 0.030             | 0.34              |
| L. Lottering  | Sep/Oct | 0.07           | 15              | 1.2                 | 230              | 0.42                   | 0.016                 | 13.0          | 12.5            | 0.06              | 0.050             | 0.40              |
| L. Bloukrans  | Sep/Oct | 0.13           | 20              | 1.4                 | 200              | 0.35                   | 0.015                 | 14.0          | 9.3             | 0.07              | 0.050             | 1.19              |
| U. Groot (E)  | Sep/Oct | 0.20           | 21              | 1.4                 | 180              | 0.49                   | 0.021                 | 16.0          | 8.3             | 0.05              | 0.060             | 0.30              |
| L. Storms     | Sep/Oct | 0.07           | 21              | 1.4                 | 230              | 0.29                   | 0.016                 | 14.0          | 6.8             | 0.06              | 0.060             | 0.40              |
| U. Storms     | Sep/Oct | 0.14           | 19              | 1.3                 | 300              | 0.36                   | 0.015                 | 14.0          | 8.2             | 0.07              | 0.050             | 0.28              |
| U. Lottering  | Sep/Oct | 0.07           | 10              | 0.7                 | 260              | 0.25                   | 0.015                 | 8.8           | 22.1            | 0.06              | 0.060             | 0.38              |
| L. Groot (E)  | Sep/Oct | 0.07           | 82              | 6.0                 | 200              | 0.46                   | 0.016                 | 4.9           | 12.6            | 0.07              | 0.050             | 0.51              |
| L. Elands     | Sep/Oct | 0.07           | 34              | 2.6                 | 90               | 0.17                   | 0.016                 | 23.0          | 8.3             | 0.08              | 0.090             | 0.26              |
| U. Elands     | Sep/Oct | 0.25           | 17              | 1.3                 | 230              | 0.30                   | 0.016                 | 12.0          | 12.2            | 0.08              | 0.070             | 0.39              |
| U. Elandsbos  | Sep/Oct | 0.07           | 14              | 1.3                 | 190              | 0.30                   | 0.014                 | 14.0          | 9.5             | 0.08              | 0.070             | 0.46              |
| U. Bloukrans  | Sep/Oct | 0.09           | 20              | 1.2                 | 130              | 0.45                   | 0.021                 | 15.0          | 3.3             | 0.01              | 0.060             | 0.27              |
| L. Groot(W)   | Sep/Oct | 0.15           | 31              | 1.8                 | 120              | 0.27                   | 0.017                 | 21.0          | 7.3             | 0.04              | 0.060             | 0.24              |
| U. Salt       | Sep/Oct | 0.10           | 15              | 0.9                 | 140              | 0.21                   | 0.014                 | 10.0          | 5.5             | 0.04              | 0.070             | 0.17              |
| U. Groot (E)  | Jan/Feb | 0.07           | 23              | 1.4                 | 120              | 0.31                   | 0.015                 | 13.0          | 6.6             | 0.04              | 0.010             | 0.09              |
| U. Elands     | Jan/Feb | 0.07           | 20              | 1.6                 | 200              | 0.31                   | 0.008                 | 12.0          | 16.7            | 0.04              | 0.009             | 0.23              |
| L. Groot (E)  | Jan/Feb | 0.07           | 79              | 5.9                 | 200              | 0.22                   | 0.001                 | 45.0          | 10.2            | 0.04              | 0.009             | 0.24              |
| L. Elands     | Jan/Feb | 0.07           | 21              | 2.8                 | 110              | 0.37                   | 0.001                 | 21.0          | 6.7             | 0.04              | 0.009             | 0.05              |
| U. Storms     | Jan/Feb | 0.07           | 23              | 1.5                 | 160              | 0.47                   | 0.019                 | 13.0          | 8.7             | 0.04              | 0.010             | 0.11              |
| L. Storms     | Jan/Feb | 0.07           | 26              | 1.6                 | 200              | 0.33                   | 0.001                 | 17.0          | 7.8             | 0.04              | 0.010             | 0.11              |
| U. Lottering  | Jan/Feb | 0.16           | 15              | 1.1                 | 210              | 0.41                   | 0.017                 | 11.0          | 9.1             | 0.04              | 0.060             | 0.14              |
| U. Elandsbos  | Jan/Feb | 0.11           | 22              | 1.0                 | 90               | 0.38                   | 0.010                 | 10.0          | 7.5             | 0.04              | 0.030             | 0.11              |
| L. Elandsbos  | Jan/Feb | 0.10           | 13              | 1.0                 | 120              | 0.44                   | 0.002                 | 8.7           | 8.3             | 0.04              | 0.010             | 0.13              |
| L. Lottering  | Jan/Feb | 0.07           | 9               | 0.6                 | 200              | 0.44                   | 0.008                 | 7.3           | 7.0             | 0.04              | 0.010             | 0.10              |
| L. Bloukrans  | Jan/Feb | 0.07           | 11              | 1.6                 | 80               | 0.40                   | 0.003                 | 16.0          | 7.8             | 0.04              | 0.010             | 0.09              |
| L. Groot (W)  | Jan/Feb | 0.07           | 37              | 2.3                 | 110              | 0.39                   | 0.004                 | 23.0          | 8.1             | 0.04              | 0.009             | 0.08              |
| U. Bloukrans  | Jan/Feb | 0.07           | 5               | 0.8                 | 110              | 0.39                   | 0.001                 | 8.5           | 5.4             | 0.04              | 0.009             | 0.04              |
| U. Salt       | Jan/Feb | 0.07           | 20              | 1.4                 | 190              | 0.32                   | 0.001                 | 13.0          | 6.3             | 0.04              | 0.010             | 0.01              |
| L. Salt       | Jan/Feb | 0.07           | 93              | 5.9                 | 180              | 0.39                   | 0.063                 | 49.0          | 11.9            | 0.04              | 0.009             | 0.01              |
| U. Buffels    | Jan/Feb | 0.07           | 387             | 52.0                | 520              | 0.31                   | 0.001                 | 189.0         | 123.0           | 0.04              | 0.010             | 0.04              |
| U. Matjies    | Jan/Feb | 0.07           | 1588            | 134.0               | 700              | 0.29                   | 0.001                 | 536.0         | 295.0           | 0.04              | 0.010             | 0.01              |
| L. Buffels    | Jan/Feb | 0.07           | 610             | 87.0                | 590              | 0.37                   | 0.001                 | 268.0         | 178.0           | 0.04              | 0.010             | 0.03              |
| U. Bobbejaans | Jan/Feb | 0.07           | 19              | 0.8                 | 170              | 0.29                   | 0.003                 | 9.6           | 2.2             | 0.04              | 0.009             | 0.03              |
| U. Groot (W)  | Jan/Feb | 0.07           | 18              | 1.0                 | 100              | 0.41                   | 0.008                 | 11.0          | 6.6             | 0.04              | 0.009             | 0.04              |

Table 5: On-site water chemistry values recorded over the entire sampling period.

| Site          | Month   | pH  | Conductivity (mS/cm) | Turbidity (NTU) | Site          | Month   | pH  | Conductivity (mS/cm) | Turbidity (NTU) |
|---------------|---------|-----|----------------------|-----------------|---------------|---------|-----|----------------------|-----------------|
| U. Storms     | Mar/Apr | 5.1 | 0.088                | 1.0             | L. Salt       | Sep/Oct | 6.8 | 0.319                | 0.7             |
| L. Storms     | Mar/Apr | 5.1 | 0.075                | 1.1             | U. Buffels    | Sep/Oct | 7.4 | 0.319                | 24.3            |
| U. Bobbejaans | Mar/Apr | 5.2 | 0.056                | 1.3             | U. Matjies    | Sep/Oct | 7.4 | 1.574                | 60.9            |
| U. Groot (W)  | Mar/Apr | 4.7 | 0.065                | 1.0             | L. Buffels    | Sep/Oct | 7.5 | 0.298                | 15.5            |
| L. Elandsbos  | Mar/Apr | 4.7 | 0.074                | 1.1             | U. Groot (W)  | Sep/Oct | 5.0 | 0.060                | 0.2             |
| L. Lottering  | Mar/Apr | 4.6 | 0.090                | 1.8             | U. Bobbejaans | Sep/Oct | 5.1 | 0.500                | 0.1             |
| L. Bloukrans  | Mar/Apr | 4.6 | 0.080                | 0.9             | L. Elandsbos  | Sep/Oct | 5.4 | 0.070                | 0.5             |
| U. Lottering  | Mar/Apr | 4.6 | 1.300                | 1.3             | L. Lottering  | Sep/Oct | 5.2 | 0.073                | 0.3             |
| U. Elandsbos  | Mar/Apr | 4.9 | 0.063                | 1.4             | L. Bloukrans  | Sep/Oct | 5.0 | 0.079                | 0.5             |
| L. Groot (E)  | Mar/Apr | 6.1 | 0.216                | 5.0             | U. Groot (E)  | Sep/Oct | 5.3 | 0.082                | 0.5             |
| U. Groot (E)  | Mar/Apr | 4.7 | 0.076                | 1.2             | L. Storms     | Sep/Oct | 5.4 | 0.078                | 0.1             |
| L. Elands     | Mar/Apr | 6.6 | 0.155                | 1.9             | U. Storms     | Sep/Oct | 5.0 | 0.086                | 1.2             |
| U. Elands     | Mar/Apr | 4.6 | 0.069                | 1.6             | U. Lottering  | Sep/Oct | 4.7 | 0.053                | 0.2             |
| L. Groot (W)  | Mar/Apr | 5.4 | 0.107                | 1.2             | L. Groot (E)  | Sep/Oct | 6.6 | 0.310                | 0.3             |
| U. Bloukrans  | Mar/Apr | 5.4 | 0.070                | 1.1             | L. Elands     | Sep/Oct | 6.9 | 0.142                | 0.4             |
| U. Salt       | Mar/Apr | 4.9 | 0.072                | 0.8             | U. Elands     | Sep/Oct | 4.7 | 0.083                | 1.0             |
| L. Salt       | Mar/Apr | 6.2 | 0.300                | 1.3             | U. Elandsbos  | Sep/Oct | 5.0 | 0.062                | 0.8             |
| U. Buffels    | Mar/Apr | 6.9 | 1.260                | 1.7             | U. Bloukrans  | Sep/Oct | 5.9 | 0.075                | 0.1             |
| U. Matjies    | Mar/Apr | 7.5 | 4.178                | 1.2             | L. Groot(W)   | Sep/Oct | 5.9 | 0.119                | 0.6             |
| L. Buffels    | Mar/Apr | 7.6 | 1.317                | 1.3             | U. Salt       | Sep/Oct | 5.1 | 0.075                | 0.7             |
| U. Salt       | Jun/Jul | 4.9 | 0.069                | 1.0             | U. Groot (E)  | Jan/Feb | 4.8 | 0.094                | 0.8             |
| L. Bloukrans  | Jun/Jul | 5.2 | 0.100                | 1.1             | U. Elands     | Jan/Feb | 4.5 | 0.087                | 0.9             |
| U. Groot (E)  | Jun/Jul | 4.5 | 0.100                | 5.1             | L. Groot (E)  | Jan/Feb | 5.4 | 0.286                | 1.3             |
| L. Groot (E)  | Jun/Jul | 6.1 | 0.200                | 2.7             | L. Elands     | Jan/Feb | 5.8 | 0.133                | 1.1             |
| U. Elands     | Jun/Jul | 4.5 | 0.100                | 5.1             | U. Storms     | Jan/Feb | 4.1 | 0.094                | 1.5             |
| L. Elands     | Jun/Jul | 6.7 | 0.100                | 2.8             | L. Storms     | Jan/Feb | 4.9 | 0.089                | 0.7             |
| U. Storms     | Jun/Jul | 4.9 | 0.100                | 2.8             | U. Lottering  | Jan/Feb | 4.3 | 0.060                | 1.4             |
| L. Storms     | Jun/Jul | 4.5 | 0.100                | 2.9             | U. Elandsbos  | Jan/Feb | 4.4 | 0.064                | 1.9             |
| U. Elandsbos  | Jun/Jul | 5.2 | 0.100                | 2.1             | L. Elandsbos  | Jan/Feb | 4.5 | 0.080                | 0.9             |
| U. Lottering  | Jun/Jul | 4.6 | 0.100                | 0.9             | L. Lottering  | Jan/Feb | 4.5 | 0.089                | 2.1             |
| U. Bloukrans  | Jun/Jul | 5.5 | 0.100                | 3.9             | L. Bloukrans  | Jan/Feb | 4.4 | 0.084                | 0.8             |
| L. Groot (W)  | Jun/Jul | 5.8 | 0.100                | 1.0             | L. Groot (W)  | Jan/Feb | 5.0 | 0.124                | 0.8             |
| L. Lottering  | Jun/Jul | 4.8 | 0.100                | 4.4             | U. Bloukrans  | Jan/Feb | 4.6 | 0.064                | 0.8             |
| L. Elandsbos  | Jun/Jul | 4.9 | 0.100                | 0.9             | U. Salt       | Jan/Feb | 4.3 | 0.074                | 0.5             |
| L. Salt       | Jun/Jul | 6.8 | 0.300                | 1.0             | L. Salt       | Jan/Feb | 5.9 | 0.307                | 0.5             |
| U. Buffels    | Jun/Jul | 7.1 | 1.200                | 5.7             | U. Buffels    | Jan/Feb | 6.7 | 13.250               | 0.8             |
| L. Buffels    | Jun/Jul | 7.2 | 1.500                | 2.3             | U. Matjies    | Jan/Feb | 7.3 | 4.810                | 0.6             |
| U. Matjies    | Jun/Jul | 7.4 | 3.700                | 6.2             | L. Buffels    | Jan/Feb | 6.7 | 2.260                | 0.6             |
| U. Bobbejaans | Jun/Jul | 6.4 | 0.100                | 0.8             | U. Bobbejaans | Jan/Feb | 4.7 | 0.063                | 0.6             |
| U. Groot (W)  | Jun/Jul | 5.2 | 0.100                | 1.0             | U. Groot (W)  | Jan/Feb | 4.4 | 0.076                | 0.8             |

Table 6: Eigenvalues from a PCA of all 20 sites using laboratory analysed water chemistry results recorded at each sampling event.

| Value number | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|--------------|------------|------------------|-----------------------|--------------|
| 1            | 4.576489   | 41.60444         | 4.57649               | 41.6044      |
| 2            | 1.843538   | 16.75944         | 6.42003               | 58.3639      |
| 3            | 1.466202   | 13.32911         | 7.88623               | 71.6930      |
| 4            | 1.419436   | 12.90396         | 9.30566               | 84.5970      |
| 5            | 0.802277   | 7.29342          | 10.10794              | 91.8904      |
| 6            | 0.412766   | 3.75241          | 10.52071              | 95.6428      |
| 7            | 0.235441   | 2.14037          | 10.75615              | 97.7832      |
| 8            | 0.145567   | 1.32333          | 10.90171              | 99.1065      |
| 9            | 0.059112   | 0.53739          | 10.96083              | 99.6439      |
| 10           | 0.029970   | 0.27245          | 10.99080              | 99.9163      |

Table 7: Factor coordinates of the first five factors from the PCA of each of the 20 sites, using laboratory analysed water chemistry results recorded at each sampling event.

| Variables              | Factor 1  | Factor 2  | Factor 3  | Factor 4  | Factor 5  |
|------------------------|-----------|-----------|-----------|-----------|-----------|
| Ammonia (mg/l)         | -0.025856 | 0.215755  | -0.146535 | 0.861237  | 0.231008  |
| Chloride (mg/l)        | -0.976121 | -0.020251 | -0.078071 | 0.026483  | -0.001092 |
| Dissolved Mg (mg/l)    | -0.990490 | -0.006184 | -0.044473 | 0.040338  | 0.005519  |
| Fluoride (µg/l)        | -0.848733 | 0.153394  | 0.215323  | -0.261536 | -0.024879 |
| Nitrate/Nitrite (mg/l) | 0.028658  | 0.751333  | -0.539433 | -0.059435 | 0.187124  |
| Orthophosphate (mg/l)  | 0.107182  | 0.801416  | -0.357245 | -0.299979 | -0.060181 |
| Sodium (mg/l)          | -0.977864 | 0.022009  | -0.054133 | 0.007823  | 0.009548  |
| Sulphate (mg/l)        | -0.976160 | -0.015327 | -0.050149 | 0.100708  | -0.001993 |
| Total Lead (mg/l)      | 0.005503  | 0.564268  | 0.652568  | -0.215983 | -0.318614 |
| Total Zinc (mg/l)      | 0.011186  | 0.426998  | 0.373042  | 0.669852  | -0.325883 |
| Total Iron (mg/l)      | 0.004426  | 0.254549  | 0.633427  | -0.088906 | 0.708388  |



Table 8: Eigenvalues generated from a PCA using laboratory analysed water chemistry results, with the values for the lower Salt River from the winter sampling period treated as an outlier and excluded.

| Value number | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|--------------|------------|------------------|-----------------------|--------------|
| 1            | 4.582449   | 41.65863         | 4.58245               | 41.6586      |
| 2            | 1.668893   | 15.17176         | 6.25134               | 56.8304      |
| 3            | 1.628244   | 14.80222         | 7.87959               | 71.6326      |
| 4            | 1.163334   | 10.57576         | 9.04292               | 82.2084      |
| 5            | 0.874483   | 7.94984          | 9.91740               | 90.1582      |
| 6            | 0.455765   | 4.14332          | 10.37317              | 94.3015      |
| 7            | 0.385083   | 3.50075          | 10.75825              | 97.8023      |
| 8            | 0.145223   | 1.32021          | 10.90347              | 99.1225      |
| 9            | 0.057449   | 0.52227          | 10.96092              | 99.6448      |
| 10           | 0.029865   | 0.27150          | 10.99079              | 99.9163      |

Table 9: Factor coordinates of the first five factors from a PCA using laboratory analysed water chemistry results, with the value for the lower Salt River from the winter sampling period treated as an outlier and excluded.

| Variables              | Factor 1  | Factor 2  | Factor 3  | Factor 4  | Factor 5  |
|------------------------|-----------|-----------|-----------|-----------|-----------|
| Ammonia (mg/l)         | -0.030021 | -0.673937 | 0.481149  | -0.361721 | 0.048108  |
| Chloride (mg/l)        | -0.976272 | -0.065143 | -0.039827 | 0.011065  | -0.022222 |
| Dissolved Mg (mg/l)    | -0.990539 | -0.056736 | -0.003433 | 0.003630  | -0.008796 |
| Fluoride (µg/l)        | -0.849281 | 0.329555  | 0.086345  | 0.109479  | 0.050941  |
| Nitrate/Nitrite (mg/l) | 0.004576  | -0.644946 | 0.307070  | 0.465587  | 0.322488  |
| Orthophosphate (mg/l)  | 0.128505  | 0.065479  | 0.335821  | 0.850886  | -0.150115 |
| Sodium (mg/l)          | -0.977799 | -0.043772 | 0.001390  | 0.047642  | 0.000233  |
| Sulphate (mg/l)        | -0.976310 | -0.092245 | 0.011443  | -0.036222 | -0.034473 |
| Total Lead (mg/l)      | 0.001748  | 0.693328  | 0.573980  | -0.016566 | -0.189684 |
| Total Zinc (mg/l)      | 0.012725  | -0.132027 | 0.813100  | -0.239623 | -0.365612 |
| Total Iron (mg/l)      | 0.004855  | 0.412105  | 0.435776  | -0.135339 | 0.756061  |

Table 10: Eigenvalues generated from a PCA using laboratory analysed water chemistry results, with the lower Salt, upper Matjies and upper and lower Buffels River sites removed, for all four sampling events.

| Value number | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|--------------|------------|------------------|-----------------------|--------------|
| 1            | 2.983028   | 27.11844         | 2.98303               | 27.1184      |
| 2            | 2.245056   | 20.40960         | 5.22808               | 47.5280      |
| 3            | 1.765148   | 16.04680         | 6.99323               | 63.5748      |
| 4            | 1.161778   | 10.56162         | 8.15501               | 74.1365      |
| 5            | 0.907098   | 8.24635          | 9.06211               | 82.3828      |
| 6            | 0.764291   | 6.94810          | 9.82640               | 89.3309      |
| 7            | 0.394187   | 3.58352          | 10.22059              | 92.9144      |
| 8            | 0.323533   | 2.94121          | 10.54412              | 95.8556      |
| 9            | 0.269608   | 2.45098          | 10.81373              | 98.3066      |
| 10           | 0.156366   | 1.42151          | 10.97009              | 99.7281      |

Table 11: Factor coordinates of the first five factors from a PCA using laboratory analysed water chemistry results, with the lower Salt, upper Matjies and upper and lower Buffels River sites removed, for all four sampling events.

| Variables              | Factor 1  | Factor 2  | Factor 3  | Factor 4  | Factor 5  |
|------------------------|-----------|-----------|-----------|-----------|-----------|
| Ammonia (mg/l)         | -0.270445 | -0.179848 | 0.833473  | -0.107560 | 0.083910  |
| Chloride (mg/l)        | 0.850804  | -0.424812 | -0.063503 | 0.025141  | 0.014436  |
| Dissolved Mg (mg/l)    | 0.878384  | -0.392405 | -0.074993 | -0.047116 | 0.057163  |
| Fluoride (µg/l)        | 0.567358  | 0.602464  | 0.059429  | -0.271226 | -0.322006 |
| Nitrate/Nitrite (mg/l) | 0.014474  | -0.583785 | 0.546061  | 0.308001  | 0.002493  |
| Orthophosphate (mg/l)  | 0.390653  | 0.412782  | 0.062211  | 0.657896  | -0.147999 |
| Sodium (mg/l)          | 0.733622  | -0.480499 | 0.008181  | 0.010150  | 0.079125  |
| Sulphate (mg/l)        | 0.249631  | 0.106598  | 0.653413  | -0.462229 | -0.405744 |
| Total Lead (mg/l)      | 0.509797  | 0.721227  | 0.075324  | 0.126091  | 0.052353  |
| Total Zinc (mg/l)      | 0.092656  | 0.366729  | 0.563078  | 0.386184  | 0.302076  |
| Total Iron (mg/l)      | 0.265996  | 0.327422  | 0.074057  | -0.409030 | 0.711410  |

Table 12: Eigenvalues generated from a PCA using on-site water chemistry results, pH, Turbidity and Electrical Conductivity.

|   | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|---|------------|------------------|-----------------------|--------------|
| 1 | 1.571491   | 52.38302         | 1.571491              | 52.4         |
| 2 | 0.941073   | 31.36911         | 2.512564              | 83.8         |
| 3 | 0.487436   | 16.24787         | 3.000000              | 100.0        |

Table 13: Factor coordinates generated from a PCA of on-site water chemistry results.

| Variables            | Factor 1 | Factor 2  | Factor 3  |
|----------------------|----------|-----------|-----------|
| pH                   | 0.861760 | -0.024020 | 0.506747  |
| Conductivity (mS/cm) | 0.698100 | -0.604902 | -0.383081 |
| Turbidity (NTU)      | 0.584394 | 0.758018  | -0.289643 |

Table 14: Eigenvalues generated from a PCA using on-site water chemistry results with the cases for the Matjies and Buffels Rivers excluded.

|   | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|---|------------|------------------|-----------------------|--------------|
| 1 | 1.191462   | 39.71538         | 1.191462              | 39.71538     |
| 2 | 0.991639   | 33.05462         | 2.183100              | 72.77001     |
| 3 | 0.816900   | 27.22999         | 3.000000              | 100.00000    |

Table 15: Factor coordinates generated from a PCA of on-site water chemistry results with the cases for the Matjies and Buffels Rivers excluded.

| Variables            | Factor 1  | Factor 2  | Factor 3  |
|----------------------|-----------|-----------|-----------|
| pH                   | -0.761848 | -0.060029 | 0.644968  |
| Conductivity (mS/cm) | -0.726854 | -0.310717 | -0.612485 |
| Turbidity (NTU)      | 0.287632  | -0.944188 | 0.160554  |

Table 16: Eigenvalues generated from a PCA using a combination of laboratory and on-site water chemistry values for all sites across all sampling events.

| Value number | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|--------------|------------|------------------|-----------------------|--------------|
| 1            | 5.531712   | 39.51223         | 5.53171               | 39.5122      |
| 2            | 1.909599   | 13.63999         | 7.44131               | 53.1522      |
| 3            | 1.594082   | 11.38630         | 9.03539               | 64.5385      |
| 4            | 1.304249   | 9.31606          | 10.33964              | 73.8546      |
| 5            | 0.877494   | 6.26782          | 11.21714              | 80.1224      |
| 6            | 0.710821   | 5.07729          | 11.92796              | 85.1997      |
| 7            | 0.634141   | 4.52958          | 12.56210              | 89.7293      |
| 8            | 0.522028   | 3.72877          | 13.08413              | 93.4580      |
| 9            | 0.362915   | 2.59225          | 13.44704              | 96.0503      |
| 10           | 0.259741   | 1.85529          | 13.70678              | 97.9056      |
| 11           | 0.198684   | 1.41917          | 13.90547              | 99.3248      |
| 12           | 0.058865   | 0.42046          | 13.96433              | 99.7452      |
| 13           | 0.029401   | 0.21000          | 13.99373              | 99.9552      |
| 14           | 0.006269   | 0.04478          | 14.00000              | 100.0000     |

Table 17: Factor coordinates of the first five factors generated from a PCA using a combination of laboratory analysed and on-site water chemistry values for all sites across all sampling events.

| Variables              | Factor 1  | Factor 2  | Factor 3  | Factor 4  | Factor 5  |
|------------------------|-----------|-----------|-----------|-----------|-----------|
| Ammonia (mg/l)         | -0.001464 | 0.248172  | -0.788587 | 0.138144  | 0.127495  |
| Chloride (mg/l)        | 0.958505  | -0.066639 | -0.074248 | 0.090011  | -0.005028 |
| Dissolved Mg (mg/l)    | 0.974836  | -0.062406 | -0.094823 | 0.065254  | 0.000047  |
| Fluoride (µg/l)        | 0.859021  | 0.009771  | 0.124212  | -0.182962 | -0.106764 |
| Nitrate/Nitrite (mg/l) | 0.028142  | 0.863033  | 0.103616  | 0.278978  | -0.088553 |
| Orthophosphate (mg/l)  | -0.046362 | 0.844339  | 0.276852  | 0.191991  | -0.077479 |
| Sodium (mg/l)          | 0.962470  | -0.031378 | -0.063795 | 0.047700  | -0.020564 |
| Sulphate (mg/l)        | 0.957811  | -0.071759 | -0.142100 | 0.070274  | 0.017147  |
| Total Lead (mg/l)      | -0.097383 | 0.127109  | -0.483033 | -0.259998 | -0.802711 |
| Total Zinc (mg/l)      | -0.022750 | 0.279495  | -0.703679 | -0.145066 | 0.368258  |
| Total Iron (mg/l)      | 0.014026  | 0.152399  | -0.029644 | -0.797095 | 0.115930  |
| pH                     | 0.706814  | 0.403904  | 0.107238  | -0.099128 | 0.152500  |
| Conductivity (mS/cm)   | 0.701872  | -0.158405 | 0.062935  | 0.160275  | -0.130231 |
| Turbidity (NTU)        | 0.275057  | 0.264540  | 0.292932  | -0.598255 | 0.040494  |

Table 18: Eigenvalues generated from a PCA using a combination of laboratory analysed and on-site water chemistry values, excluding the case for the lower Salt River site during winter sampling.

| Value number | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|--------------|------------|------------------|-----------------------|--------------|
| 1            | 5.550080   | 39.64343         | 5.55008               | 39.6434      |
| 2            | 1.798214   | 12.84439         | 7.34829               | 52.4878      |
| 3            | 1.579072   | 11.27908         | 8.92737               | 63.7669      |
| 4            | 1.211795   | 8.65568          | 10.13916              | 72.4226      |
| 5            | 0.870910   | 6.22078          | 11.01007              | 78.6434      |
| 6            | 0.835332   | 5.96666          | 11.84540              | 84.6100      |
| 7            | 0.621475   | 4.43910          | 12.46688              | 89.0491      |
| 8            | 0.519399   | 3.70999          | 12.98628              | 92.7591      |
| 9            | 0.405564   | 2.89689          | 13.39184              | 95.6560      |
| 10           | 0.296405   | 2.11718          | 13.68825              | 97.7732      |
| 11           | 0.217843   | 1.55602          | 13.90609              | 99.3292      |
| 12           | 0.058252   | 0.41609          | 13.96434              | 99.7453      |
| 13           | 0.029383   | 0.20988          | 13.99372              | 99.9552      |
| 14           | 0.006277   | 0.04483          | 14.00000              | 100.0000     |

Table 19: Factor coordinates generated from a PCA using a combination of laboratory analysed and on-site water chemistry values, excluding the case for the lower Salt River site during winter sampling.

| Variables              | Factor 1  | Factor 2  | Factor 3  | Factor 4  | Factor 5  |
|------------------------|-----------|-----------|-----------|-----------|-----------|
| Ammonia (mg/l)         | 0.002330  | 0.590145  | -0.635388 | 0.105437  | 0.254622  |
| Chloride (mg/l)        | 0.958504  | -0.048826 | -0.116729 | 0.043216  | -0.025404 |
| Dissolved Mg (mg/l)    | 0.975311  | -0.023563 | -0.121315 | 0.025082  | -0.018761 |
| Fluoride (µg/l)        | 0.857566  | -0.076959 | 0.146094  | -0.215825 | -0.095567 |
| Nitrate/Nitrite (mg/l) | 0.053775  | 0.706369  | 0.177601  | 0.413526  | 0.371298  |
| Orthophosphate (mg/l)  | -0.074490 | 0.414045  | 0.541446  | 0.248302  | -0.590500 |
| Sodium (mg/l)          | 0.962350  | -0.010238 | -0.078087 | 0.019107  | -0.020206 |
| Sulphate (mg/l)        | 0.958445  | -0.010264 | -0.165561 | 0.023367  | -0.017523 |
| Total Lead (mg/l)      | -0.097458 | 0.264406  | -0.356397 | -0.493038 | -0.230570 |
| Total Zinc (mg/l)      | -0.021677 | 0.615842  | -0.389654 | -0.120679 | -0.372404 |
| Total Iron (mg/l)      | 0.014397  | 0.244609  | 0.232167  | -0.758665 | 0.173068  |
| pH                     | 0.715749  | 0.337167  | 0.296700  | 0.071121  | -0.049926 |
| Conductivity (mS/cm)   | 0.701899  | -0.210429 | -0.070433 | 0.083156  | 0.027157  |
| Turbidity (NTU)        | 0.276380  | 0.321734  | 0.584560  | -0.269881 | 0.289222  |

Table 20: Eigenvalues generated from a PCA using a combination of laboratory analysed and on-site water chemistry values, excluding the lower Salt, upper Matjies and upper and lower Buffels River sites, for all four sampling events.

| Value number | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|--------------|------------|------------------|-----------------------|--------------|
| 1            | 3.330315   | 23.78796         | 3.33031               | 23.7880      |
| 2            | 2.043714   | 14.59796         | 5.37403               | 38.3859      |
| 3            | 1.875260   | 13.39472         | 7.24929               | 51.7806      |
| 4            | 1.461894   | 10.44210         | 8.71118               | 62.2227      |
| 5            | 1.069048   | 7.63606          | 9.78023               | 69.8588      |
| 6            | 1.007925   | 7.19946          | 10.78816              | 77.0583      |
| 7            | 0.864197   | 6.17284          | 11.65235              | 83.2311      |
| 8            | 0.712254   | 5.08753          | 12.36461              | 88.3186      |
| 9            | 0.459583   | 3.28273          | 12.82419              | 91.6014      |
| 10           | 0.419842   | 2.99887          | 13.24403              | 94.6002      |
| 11           | 0.319008   | 2.27863          | 13.56304              | 96.8789      |
| 12           | 0.249532   | 1.78237          | 13.81257              | 98.6612      |
| 13           | 0.159128   | 1.13663          | 13.97170              | 99.7979      |
| 14           | 0.028300   | 0.20214          | 14.00000              | 100.0000     |

Table 21: Factor coordinates generated from a PCA using a combination of laboratory analysed and on-site water chemistry values, excluding the lower Salt, upper Matjies and upper and lower Buffels River sites, for all four sampling events.

| Variables              | Factor 1  | Factor 2  | Factor 3  | Factor 4  | Factor 5  |
|------------------------|-----------|-----------|-----------|-----------|-----------|
| Ammonia (mg/l)         | 0.146156  | 0.582854  | 0.667176  | -0.023431 | 0.070588  |
| Chloride (mg/l)        | -0.913391 | -0.211136 | 0.100667  | -0.060094 | -0.045017 |
| Dissolved Mg (mg/l)    | -0.939989 | -0.165396 | 0.045535  | -0.100696 | 0.005677  |
| Fluoride (µg/l)        | -0.381584 | 0.473533  | -0.617409 | 0.037772  | -0.301241 |
| Nitrate/Nitrite (mg/l) | -0.179845 | 0.066381  | 0.750061  | 0.035895  | 0.056705  |
| Orthophosphate (mg/l)  | -0.240441 | 0.054991  | -0.266428 | 0.646742  | -0.350535 |
| Sodium (mg/l)          | -0.818222 | -0.141044 | 0.163211  | -0.102423 | 0.107480  |
| Sulphate (mg/l)        | -0.210571 | 0.709120  | 0.129261  | -0.203907 | -0.388934 |
| Total Lead (mg/l)      | 0.084120  | 0.447475  | 0.123144  | 0.245878  | 0.305158  |
| Total Zinc (mg/l)      | -0.046252 | 0.361347  | 0.198826  | 0.716974  | -0.059625 |
| Total Iron (mg/l)      | -0.239414 | 0.407715  | -0.354338 | -0.012199 | 0.540895  |
| pH                     | -0.667842 | -0.140879 | 0.158523  | 0.381579  | 0.169030  |
| Conductivity (mS/cm)   | -0.165690 | 0.162407  | 0.254769  | -0.383262 | -0.435330 |
| Turbidity (NTU)        | -0.318592 | 0.578268  | -0.299206 | -0.327697 | 0.273901  |

Table 22: Ephemeroptera collected over four sampling events represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

| EPHEMEROPTERA                      | U. Matjies | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|------------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>Baetidae</b>                    |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Afroptilum sudafricanum</i> *** | 28         | 49         | 53         |         | 7       |              |              |               |              | 4            |              |              |              | 2            |           |           |           |           |              |              |
| <i>Baetis harrisoni</i> ***        | 4          | 4          | 11         | 4       | 11      | 5            | 1            | 10            | 1            | 162          | 5            |              | 1            |              |           | 48        |           | 73        | 3            | 53           |
| <i>Bugillesia sp.*</i>             |            |            |            | 1       |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Cheleocloeon excisum</i> **     | 22         |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Cloeodes sp.*</i>               |            |            |            | 217     | 1       | 13           | 34           | 14            |              | 3            | 120          | 31           | 13           | 1            |           |           |           |           | 1            |              |
| <i>Nigrobaetis sp.*</i>            |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           | 1         |           |           |              |              |
| <i>Pseudocloeon vinosum</i> ***    | 1          |            |            | 789     | 53      | 8            | 104          | 208           | 108          | 341          | 239          | 119          | 472          | 542          | 3         | 347       | 18        | 146       | 823          | 16           |
| <i>Cloeon sp.**</i>                | 1          | 4          | 2          |         |         |              |              |               |              |              |              |              | 9            |              |           | 1         |           |           | 2            | 35           |
| <b>Caenidae</b>                    |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Caenis capensis</i> **          | 50         | 466        | 27         |         |         | 1            |              |               |              |              |              |              |              |              |           |           |           |           |              | 26           |
| <b>Heptageniidae</b>               |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Afronurus peringueyi</i> ***    |            |            |            |         |         | 4            |              | 1             |              | 43           | 1            | 9            |              |              |           | 2         |           |           | 1            |              |
| <b>Leptophlebiidae</b>             |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Aprionyx sp.**</i>              |            |            |            | 11      |         | 1            | 2            | 10            | 25           | 2            | 15           | 5            |              |              | 6         | 3         |           |           | 6            |              |
| <i>Aprionyx rubicundus</i> **      |            |            |            |         |         |              | 3            |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Castanophlebia calida</i> **    |            |            | 1          | 137     |         | 7            | 109          | 29            | 3            | 234          | 70           | 58           | 38           | 69           | 31        | 26        |           |           | 55           | 2            |
| <i>Choroterpes nigrescens</i> **   |            |            | 6          | 12      | 1       | 75           |              |               | 2            | 171          | 2            | 42           | 12           | 12           |           | 48        | 2         |           |              | 113          |
| <i>Adenophlebia auriculata</i> **  | 48         | 36         | 55         |         | 1       |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <b>Teloganodidae</b>               |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Ephemerellina barnardi</i> *    |            |            |            |         |         |              |              | 7             |              |              | 1            |              | 1            |              |           |           |           |           | 1            |              |
| <i>Lestagella penicillata</i> **   |            |            |            | 89      | 1       | 13           | 20           | 1             | 7            | 308          | 62           | 131          | 6            | 45           | 1         | 75        |           |           | 92           | 8            |
| <i>Genus sp TSR151A</i> *          |            |            |            | 43      |         |              |              | 3             | 7            | 10           | 22           |              |              | 1            |           |           |           |           | 6            |              |
| <i>Genus sp TSR 472K</i> *         |            |            |            |         |         | 1            | 1            |               |              |              |              |              | 1            |              |           |           |           |           |              |              |
| <i>Nadinetella sp. TSR173E</i> *   |            |            |            | 1       |         | 1            |              | 13            | 1            |              | 25           |              | 19           |              |           |           |           |           | 34           |              |
| <i>Nadinetella sp .TSR378K</i> *   |            |            |            | 1       |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <b>Tricorythidae</b>               |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Tricorythus discolor</i> ***    |            |            |            |         |         |              |              |               | 1            |              |              |              |              |              |           |           |           |           |              |              |

Table 23: Odonata collected over four sampling events represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

| ODONATA                              | U. Matjies | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|--------------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>Aeshnidae</b>                     |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Aeshna minuscula</i> ***          |            |            |            |         |         | 1            |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Aeshna subpupillata</i> ***       |            |            | 1          |         |         |              |              |               | 1            | 1            |              |              | 2            |              |           | 1         |           |           |              |              |
| <i>Anax speratus</i> ***             |            |            |            |         |         |              |              | 1             | 1            | 1            |              |              | 2            | 2            |           | 1         | 2         |           |              |              |
| <b>Corduliidae</b>                   |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Syncordulia gracilis</i> **       |            |            |            |         | 1       |              |              |               |              |              |              |              |              |              |           | 3         |           |           |              |              |
| <i>Syncordulia venator</i> *         |            |            |            |         |         |              |              |               | 1            |              |              |              |              |              |           | 1         |           |           |              |              |
| <b>Coenagrionidae</b>                |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Africallagma glaucum</i> ***      |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              | 1            |
| <i>Ceriagrion glabrum</i> ***        | 3          | 3          | 3          | 4       |         | 1            |              |               |              |              | 1            | 2            |              |              |           | 5         | 15        |           | 1            | 2            |
| <i>Ischnura senegalensis</i> ***     |            |            |            |         |         | 8            |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Pseudagrion furcigerum</i> *      |            |            |            | 8       | 8       | 14           | 12           | 19            | 5            | 55           |              |              | 13           | 29           |           | 31        | 18        | 16        | 1            | 5            |
| <i>Pseudagrion hageni hageni</i> **  |            | 3          | 3          |         | 5       | 9            |              |               |              |              |              | 12           |              |              |           | 10        | 6         |           |              | 10           |
| <i>Pseudagrion kersteni</i> ***      |            |            |            | 1       |         |              |              |               |              | 1            |              |              |              |              |           |           |           |           |              |              |
| <i>Pseudagrion massaicum</i> ***     |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              | 1            |
| <b>Gomphidae</b>                     |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Ceratogomphus triceraticus</i> *  |            |            |            |         |         |              |              |               |              | 1            |              |              |              |              |           |           |           |           |              |              |
| <b>Lestidae</b>                      |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Lestes plagiatus</i> ***          |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           | 1            | 3            |
| <b>Libellulidae</b>                  |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Crocothemis erythraea</i> ***     |            |            |            | 1       |         |              |              |               |              |              |              |              |              |              |           | 2         | 2         |           |              |              |
| <i>Crocothemis sanguinolenta</i> *** |            |            |            |         |         |              |              | 2             | 1            |              |              |              |              |              |           |           |           |           |              |              |
| <i>Nesiothemis farinosa</i> ***      |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           | 6         |           |           |              | 10           |
| <i>Orthetrum abbotti</i> ***         |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           | 1         |           |              |              |
| <i>Orthetrum julia capicola</i> ***  | 1          | 1          | 3          | 5       | 6       | 16           |              | 2             | 2            | 5            | 1            | 3            | 4            | 5            |           | 7         | 10        | 8         |              | 7            |
| <i>Palpopleura jucunda</i> ***       |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           | 1         |           |              |              |
| <i>Sympetrum fonscolombii</i> ***    |            |            |            |         |         | 13           |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Tramea limbata</i> ***            |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              | 1            |
| <i>Trithemis arteriosa</i> ***       |            |            |            | 1       | 3       | 4            |              | 2             |              | 5            | 1            |              |              |              |           | 11        | 6         |           |              | 16           |
| <i>Trithemis furva</i> ***           |            | 1          | 1          |         |         | 8            |              |               |              |              |              |              |              |              |           |           |           | 1         |              |              |



|                                    | U. Matjies | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|------------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>ODONATA</b>                     |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Trithemis stictica</i> ***      |            |            | 2          |         |         | 11           |              | 1             |              |              |              | 1            |              |              |           | 6         | 6         |           |              | 20           |
| <b>Platycnemididae</b>             |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Allocnemis leucosticta</i> **   | 20         | 15         | 6          | 12      | 7       | 8            | 6            | 3             | 10           | 60           | 10           | 10           |              | 8            |           | 20        | 30        | 13        | 10           |              |
| <b>Protoneuridae</b>               |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Elattonneura frenulata</i> *    |            |            |            |         |         | 3            | 1            | 2             |              |              |              | 1            | 1            |              |           | 6         | 10        |           | 5            | 10           |
| <b>Synlestidae</b>                 |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Chlorolestes conspicuus</i> *   |            |            |            | 4       |         |              | 5            | 3             | 5            | 1            | 2            |              | 5            | 3            | 5         |           | 2         | 1         | 3            |              |
| <i>Chlorolestes tessellatus</i> ** | 7          | 8          | 6          |         | 6       |              |              |               | 8            |              |              | 19           |              |              |           |           |           |           |              |              |
| <i>Chlorolestes umbratus</i> **    |            |            |            | 8       | 10      | 7            | 3            | 22            | 5            | 16           |              | 1            |              |              |           | 9         | 10        |           | 1            | 7            |
| <i>Ecchlorolestes nylephtha</i> *  |            | 1          |            | 19      |         | 4            | 4            |               | 3            |              | 14           |              | 4            | 13           | 29        |           | 8         | 22        | 7            |              |

Table 24: Plecoptera collected over four sampling events represented by actual abundance, including larvae and adults.. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

| PLECOPTERA                          | U. Matjies | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|-------------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>Notonemouridae</b>               |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Aphanicerca capensis</i> form O* |            |            |            | 2       |         |              |              |               |              |              |              |              |              |              | 1         |           |           |           |              |              |
| <i>Aphanicerca capensis</i> form P* |            |            |            |         |         |              | 2            |               | 4            | 1            |              | 1            |              |              |           |           | 1         |           |              |              |
| <i>Aphanicerca capensis</i> form S* |            |            |            |         |         |              |              |               |              |              |              |              |              |              | 2         |           |           |           |              |              |
| <i>Aphanicerca</i> sp               |            |            |            | 55      | 6       |              | 60           |               | 1            | 31           |              | 12           | 2            | 87           | 68        | 2         | 7         |           | 35           | 3            |
| <i>Aphanicercella cassida</i> **    |            |            |            |         |         | 10           |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Aphanicercella bifurcata</i> *   |            | 3          |            | 3       |         | 8            | 6            | 1             | 1            | 3            | 12           | 1            | 3            | 1            | 70        | 1         | 1         |           |              |              |
| <i>Aphanicercella nigra</i> **      |            |            |            |         |         |              |              |               |              |              |              |              | 9            |              | 25        |           |           |           |              |              |
| <i>Aphanicercella</i> sp            | 8          | 12         | 1          | 10      |         | 4            |              | 9             | 3            | 20           | 1            | 3            | 1            |              | 3         | 1         | 4         |           | 17           | 7            |
| <i>Aphaniceropsis outeniquae</i> *  |            |            |            |         |         | 2            | 5            | 1             |              |              |              |              | 1            | 1            | 3         |           |           |           | 6            |              |
| <i>Aphaniceropsis</i> sp            | 27         | 13         | 12         | 266     | 1       | 3            | 25           | 3             | 24           | 36           | 456          | 14           | 31           | 96           | 358       | 10        | 161       |           | 79           | 7            |

Table 25: Megaloptera collected over four sampling events represented by actual abundance, including larvae and adults. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

| MEGALOPTERA                               | U. Matjies | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|-------------------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>Corydalidae</b>                        |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Chloroniella peringueyi</i> *          |            |            |            | 2       |         | 1            | 2            | 2             |              | 4            | 4            | 1            | 1            | 3            |           | 5         |           |           | 8            |              |
| <i>Platychauliodes</i> sp1*               | 1          | 6          | 3          | 11      |         | 4            | 3            | 3             | 14           | 11           | 11           | 3            | 7            | 14           | 17        | 7         | 29        |           | 7            | 1            |
| <i>Platychauliodes woodi</i> *            | 4          | 6          | 4          | 3       | 1       |              | 9            | 6             |              | 3            | 1            |              |              | 2            | 2         |           | 1         | 1         |              |              |
| <i>Taeniochauliodes ochraceopennis</i> ** |            |            |            | 5       |         | 6            | 1            | 6             | 6            |              | 2            |              | 1            | 1            | 5         |           |           |           | 1            |              |



|                                   | U. Matijes | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|-----------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>TRICHOPTERA</b>                |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Athripsodes harrisoni</i> **   |            |            |            |         |         | 10           |              | 35            |              | 5            |              | 5            | 11           | 1            |           |           |           |           | 2            | 19           |
| <i>Athripsodes schoenobates</i> * |            |            |            |         |         | 1            |              |               |              |              |              |              | 11           |              |           |           |           |           |              |              |
| <i>Athripsodes bergensis</i> *    |            |            |            | 42      | 60      | 183          | 2            | 12            | 32           | 76           | 86           | 64           | 3            |              |           | 4         |           | 1         | 1            | 282          |
| <i>Oecetis sp</i>                 |            |            |            | 42      | 8       | 21           | 11           | 5             | 17           | 40           | 2            |              | 19           |              | 5         | 32        | 57        | 3         | 3            | 124          |
| <i>Leptecho twisted case sp.*</i> |            |            |            |         |         | 1            |              | 1             |              | 1            |              |              |              |              | 82        |           | 21        |           | 6            |              |
| <b>Barbarochthonidae</b>          |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Barbarochthon brunneum</i> *   |            |            |            | 63      | 1       | 143          | 58           | 222           | 7            | 152          | 184          | 14           | 704          | 62           | 4         | 15        | 43        |           | 35           |              |
| <b>Petrothrincidae</b>            |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Petrothrincus demoori</i> *    |            |            |            | 10      |         | 1            | 3            | 1             | 2            |              | 12           | 2            | 29           | 4            | 5         |           | 59        |           | 27           |              |
| <b>Sericostomatidae</b>           |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Petroplax sp.*</i>             |            |            |            | 1       |         |              |              | 2             |              | 2            | 1            | 1            | 2            | 3            | 2         | 1         | 1         |           | 2            | 1            |
| <i>Rhoizema sp.*</i>              |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           | 3         |           |              |              |

Table 27: Adult Trichoptera collected over four sampling events, represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

|                                   | U. Matjies | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|-----------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>TRICHOPTERA</b>                |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <b>Glossosomatidae</b>            |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Agapetus murinus</i> *         |            |            |            | 2       |         |              | 8            | 1             |              |              | 1            | 15           |              | 4            | 1         |           |           |           |              |              |
| <b>Hydroptilidae</b>              |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Hydroptila cruciata</i> ***    | 1          | 6          | 12         |         | 11      | 5            | 3            | 1             |              |              | 1            | 3            |              | 4            |           | 2         |           | 335       | 2            | 1            |
| <i>Orthotrichia barnardi</i> **   |            | 1          |            |         |         |              |              | 5             |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Orthotrichia SCR164A</i> *     |            |            |            |         |         |              |              | 1             |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Oxyethira velocipes</i> **     |            | 15         | 22         | 5       | 49      | 9            |              | 4             | 2            | 1            | 10           | 2            |              | 5            |           | 10        |           | 1         | 2            |              |
| <b>Philopotamidae</b>             |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Chimarra ambulans</i> **       |            | 1          | 3          | 27      | 153     | 6            | 242          | 620           |              | 239          | 70           | 411          | 165          | 9            |           | 1099      |           |           | 4            |              |
| <i>Chimarra cereris</i> ***       |            |            |            |         |         |              |              | 1             |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Chimarra georgensis</i> **     |            |            | 1          |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Dolophilodes urceolus</i> *    |            |            |            | 19      | 1       | 2            | 38           | 23            | 15           | 26           | 17           |              | 3            | 9            | 27        | 2         | 24        |           | 1            |              |
| <b>Hydropsychidae</b>             |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Cheumatopsyche afra</i> ***    | 3          | 9          |            |         | 21      | 1            |              |               | 2            | 2            |              |              |              |              |           | 219       |           | 78        |              | 6            |
| <i>Cheumatopsyche TSR539K</i> *   | 4          | 17         | 34         |         | 83      |              |              |               |              |              |              | 4            |              |              | 1         |           |           |           |              |              |
| <i>Macrostemum capense</i> ***    |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              | 6            |
| <i>Sciadurus obtusus</i> *        |            |            |            | 2       |         |              |              | 1             | 2            |              | 3            |              |              |              | 3         |           |           |           |              |              |
| <b>Ecnomidae</b>                  |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Ecnomus oppidanus</i> **       |            |            |            |         |         | 1            |              | 3             |              |              |              |              |              |              |           |           |           | 1         |              | 6            |
| <i>Ecnomus similis</i> ***        | 15         | 67         | 22         | 12      | 1       | 21           | 9            | 13            |              | 10           |              |              | 3            |              |           | 5         | 1         |           | 1            | 13           |
| <i>Ecnomus thomasseti</i> ***     |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              | 7            |
| <i>Ecnomus TSR440G</i> *          |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              | 5            |
| <i>Parecnomina resima</i> *       |            |            |            | 13      |         |              | 34           | 29            | 2            | 38           | 29           | 24           | 104          | 99           |           | 13        |           |           | 20           |              |
| <i>Parecnomina TSR545E</i> *      |            |            |            |         |         |              |              | 1             | 1            |              |              |              | 1            |              | 10        |           | 1         |           |              |              |
| <b>Polycentropodidae</b>          |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Paranyctiophylax SCR213T</i> * |            |            |            |         |         |              |              |               |              | 78           |              |              |              |              |           | 9         |           |           |              |              |
| <b>Dipseudopsidae</b>             |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Dipseudopsis capensis</i> ***  |            |            |            |         | 1       |              |              |               |              | 1            |              |              |              |              |           |           |           |           |              | 2            |
| <b>Pisuliidae</b>                 |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Dyschimus collyriifer</i> *    |            |            |            | 5       |         |              | 19           | 9             | 1            |              | 1            |              | 1            | 1            | 13        | 2         | 13        |           | 1            |              |

|                                 | U. Matijes | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|---------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>TRICHOPTERA</b>              |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Dyschimus TSR28S*</i>        |            |            |            |         |         |              |              |               |              |              |              |              |              |              | 2         |           |           |           |              |              |
| <i>Dyschimus SCR248F*</i>       |            |            |            | 7       |         |              |              |               | 6            |              | 3            | 5            | 9            | 2            |           | 1         | 2         |           |              |              |
| <b>Leptoceridae</b>             |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Athripsodes bergensis*</i>   | 5          | 12         | 11         | 420     | 10236   | 1160         | 621          | 524           | 311          | 4244         | 354          | 436          | 896          | 11200        | 35        | 3744      | 1         | 140       | 56           | 190          |
| <i>Athripsodes harrisoni***</i> | 1          | 12         | 7          |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Athripsodes oryx*</i>        |            |            |            |         |         |              | 29           | 1             | 4            | 4            | 2            |              | 1            |              | 34        | 2         |           |           |              |              |
| <i>Athripsodes potes*</i>       |            |            |            |         |         |              |              |               |              |              | 1            |              |              |              |           | 8         |           |           |              |              |
| <i>Athripsodes prionii*</i>     |            | 3          |            | 8       |         | 9            | 6            |               |              |              | 11           | 15           | 2            | 3            |           | 1         |           |           |              | 2            |
| <i>Athripsodes scramasax*</i>   |            |            |            | 1       |         |              |              |               | 1            |              |              |              | 3            | 2            | 83        |           | 169       |           | 1            |              |
| <i>Athripsodes spatula*</i>     |            |            |            |         |         |              |              | 22            |              |              |              |              | 8            |              |           |           |           |           |              |              |
| <i>Athripsodes SCR258N*</i>     |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           | 1         |           |           |              |              |
| <i>Athripsodes TSR472C*</i>     |            |            |            | 1       |         | 6            |              | 3             |              |              | 20           | 12           | 12           | 15           |           | 1         |           |           | 1            | 2            |
| <i>Leptecho TSR491i*</i>        |            |            |            |         |         |              |              | 1             |              |              |              |              | 2            | 1            |           |           |           |           |              |              |
| <i>Leptecho TSR363H*</i>        |            |            |            |         |         |              |              |               |              |              | 1            |              |              |              |           |           |           |           |              |              |
| <i>Leptecho SCR258K*</i>        |            |            |            |         |         | 4            |              |               |              |              | 153          | 3            | 13           | 14           |           |           |           |           | 123          |              |
| <i>Leptecho SCR265K*</i>        |            | 2          |            | 75      |         |              | 727          | 442           | 7            | 204          | 30           | 2            | 59           | 225          |           | 9         | 3         | 1         | 32           |              |
| <i>Oecetis modesta**</i>        | 2          | 4          | 12         | 5       | 18      | 4            |              | 4             |              | 29           | 2            |              | 1            | 1            | 1         | 14        |           | 1         |              | 5            |
| <i>Oecetis SCR164N*</i>         |            | 3          |            | 1       |         | 1            | 2            | 1             | 6            |              |              |              |              |              | 11        |           |           |           |              |              |
| <i>Oecetis TSR513B*</i>         | 5          |            |            |         |         | 2            |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Oecetis TSR547L*</i>         |            | 1          | 2          |         |         |              |              |               |              |              |              |              |              |              |           | 1         |           |           |              |              |
| <b>Barbarochthonidae</b>        |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Barbarochthon brunneum*</i>  |            |            |            |         |         |              |              | 1             |              |              |              |              |              |              |           |           |           |           |              |              |
| <b>Petrothrincidae</b>          |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Petrothrincus demoori*</i>   |            |            |            |         |         |              | 6            | 3             |              |              | 4            | 2            | 26           | 54           | 7         |           |           |           |              |              |
| <b>Sericostomatidae</b>         |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Petroplax prionii*</i>       |            |            |            |         |         |              | 4            |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Petroplax SCR213F*</i>       |            |            |            |         |         |              |              | 9             |              |              |              |              |              |              |           | 1         | 1         |           | 2            | 1            |
| <i>Petroplax TSR447E*</i>       |            |            |            |         |         | 1            |              |               |              |              |              |              |              |              | 2         | 1         |           |           |              |              |
| <i>Rhoizema montanum*</i>       |            |            |            |         |         |              |              |               |              |              |              |              |              |              | 9         |           |           |           |              |              |

Table 28: Larval and pupal Simuliidae collected over four sampling events represented by actual abundance. S-W Cape endemic\* R.S.A endemic\*\* Afrotropical\*\*\*

| Diptera                          | U. Matjies | L. Buffels | U. Buffels | U. Salt | L. Salt | L. Groot (W) | U. Groot (W) | U. Bobbejaans | U. Bloukrans | L. Bloukrans | U. Elandsbos | L. Elandsbos | U. Lottering | L. Lottering | U. Storms | L. Storms | U. Elands | L. Elands | U. Groot (E) | L. Groot (E) |
|----------------------------------|------------|------------|------------|---------|---------|--------------|--------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|-----------|-----------|-----------|--------------|--------------|
| <b>Simuliidae</b>                |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Simulium bequaerti</i> ***    |            |            |            |         |         |              |              |               |              |              |              |              |              |              |           |           |           | 1         |              | 5            |
| <i>Simulium dentulosum</i> ***   |            |            | 97         |         |         |              |              |               | 178          |              |              |              |              |              |           |           |           |           |              |              |
| <i>Simulium hessei</i> *         |            |            |            | 1       |         |              |              | 1             |              | 1            |              |              |              |              |           |           |           |           | 12           |              |
| <i>Simulium impukane</i> ***     | 46         | 89         | 121        |         | 8       | 6            |              |               |              |              |              |              |              |              |           |           |           |           |              |              |
| <i>Simulium medusaeforme</i> *** |            |            |            |         | 4       | 186          | 1            |               |              |              | 155          |              |              | 2            |           | 58        | 27        | 342       |              | 44           |
| <i>Simulium merops</i> **        | 8          |            |            | 126     | 232     |              | 33           | 18            | 68           | 33           | 243          | 16           | 278          | 103          | 46        | 161       | 39        | 8         | 305          | 3            |
| <i>Simulium nigrirtarse</i> ***  |            |            |            | 1       | 2       |              |              |               | 1            |              | 159          |              |              |              | 57        | 4         | 21        | 38        |              | 2            |
| <i>Simulium rutherfordi</i> ***  | 89         | 112        | 432        |         | 27      | 1            |              |               |              |              |              |              | 2            | 15           | 66        |           | 325       |           |              |              |
| <i>Simulium vorax</i> ***        |            | 4          | 257        | 255     |         |              | 10           | 21            | 784          | 63           | 743          |              | 185          | 242          | 19        | 37        | 364       | 5         | 2406         | 18           |
| <i>Simulium TSR152F</i> *        |            |            |            |         |         |              |              |               |              | 14           |              |              |              |              |           |           |           |           |              |              |



Table 29: List of species and their respective abbreviations used for the canonical and detrended canonical correspondence analyses.

| Species name                       | Abbrev.  | Species name                   | Abbrev.  | Species name                     | Abbrev.  | Species name                          | Abbrev.  |
|------------------------------------|----------|--------------------------------|----------|----------------------------------|----------|---------------------------------------|----------|
| <i>Adenophlebia auriculata</i>     | Ade.aur  | <i>Bugillesia</i> sp.          | Bugl.sp. | <i>Genus</i> sp. TSR152F         | GST152F  | <i>Genus</i> sp. TSR 472K             | GST 472K |
| <i>Afronurus peringueyi</i>        | Afr.per  | <i>Caenis capensis</i>         | Cae.cap  | <i>Genus</i> sp. TSR152G         | GST152G  | <i>Genus</i> sp. TSR151A              | GST151A  |
| <i>Afroptilum sudafricanum</i>     | Afrp.sdm | <i>Castanophlebia calida</i>   | Cas.cal  | <i>Hydroptila cruciata</i>       | Hyd.cru  | <i>Petroplax</i> SCR213F              | PxS213F  |
| <i>Agapetus murinus</i>            | Aga. Mu  | <i>Cheleocloeon excisum</i>    | Che.exc. | <i>Leptecho</i> SCR258K          | LpS258K  | <i>Petroplax</i> TSR447E              | PxT447E  |
| <i>Aphanicerca capensis</i> form O | Acer.cO  | <i>Cheumatopsyche afra</i>     | Cu.afra  | <i>Leptecho</i> SCR265K          | LpS265K  | <i>Petrothrincus demoori</i>          | Pet.dem  |
| <i>Aphanicerca capensis</i> form P | Acer.cP  | <i>Cheumatopsyche</i> TSR136E  | Cu.136E  | <i>Leptecho</i> TSR363H          | LpT363H  | <i>Platychauiodes</i> sp.1            | Plt.sp.1 |
| <i>Aphanicerca capensis</i> form S | Acer.cS  | <i>Cheumatopsyche</i> TSR539K  | Cu.539K  | <i>Leptecho</i> TSR491i          | LpT491i  | <i>Platychauiodes</i> woodi           | Plt.woo  |
| <i>Aphanicerella cassida</i>       | Acel.ca  | <i>Chimarra ambulans</i>       | Chi.amb  | <i>Leptecho</i> twisted case sp. | Lptwcsp. | <i>Pseudocloeon vinosum</i>           | Pse.vin  |
| <i>Aphanicerella bifurcata</i>     | Acel.bf  | <i>Chimarra cereris</i>        | Chi.cer  | <i>Lestagella penicillata</i>    | Les.pen  | <i>Rhoizema montanum</i>              | Rho.mon  |
| <i>Aphanicerella nigra</i>         | Acel.ni  | <i>Chimarra georgensis</i>     | Chi.geo  | <i>Macrostemum capense</i>       | Mac.cap  | <i>Rhoizema</i> sp.                   | Rho.sp.  |
| <i>Aphaniceropsis outeniquae</i>   | Acop.ou  | <i>Chloroniella peringueyi</i> | Chl.per  | <i>Nadinetella</i> sp. TSR173E   | Nad173E  | <i>Sciadorus obtusus</i>              | Sci.obt  |
| <i>Aprionyx</i> sp.                | Aprx.sp. | <i>Choroterpes nigrescens</i>  | Cho.nig  | <i>Nadinetella</i> sp. TSR378K   | Nad378K  | <i>Simulium bequaerti</i>             | Sim.beq  |
| <i>Athripsodes bergensis</i>       | A.bergn  | <i>Cloeodes</i> sp.            | Cloe.sp. | <i>Nigrobaetis</i> sp.           | Nigr.sp. | <i>Simulium dentulosum</i>            | Sim.dent |
| <i>Athripsodes harrisoni</i>       | A.harris | <i>Cloeon</i> sp.              | Clon.sp. | <i>Oecetis modesta</i>           | Oe.mode  | <i>Simulium hessei</i>                | Sim.hes  |
| <i>Athripsodes oryx</i>            | A.oryx   | <i>Dipseudopsis capensis</i>   | Dip.cap  | <i>Oecetis</i> SCR164N           | OeS164N  | <i>Simulium impukane</i>              | Sim.imp  |
| <i>Athripsodes potes</i>           | A.potes  | <i>Dolophilodes urceolus</i>   | Dol.urc  | <i>Oecetis</i> TSR513B           | OeT513B  | <i>Simulium medusaeforme</i>          | Sim.med  |
| <i>Athripsodes prionii</i>         | A.prion  | <i>Dyschimus collyrifer</i>    | Dy.coll  | <i>Oecetis</i> TSR547L           | OeT547L  | <i>Simulium merops</i>                | Sim.mer  |
| <i>Athripsodes schoenobates</i>    | A.schoe  | <i>Dyschimus</i> SCR248F       | Dy.S248F | <i>Orthotrichia barnardi</i>     | Ort.bar  | <i>Simulium nigrিতarse</i>            | Sim.nig  |
| <i>Athripsodes</i> SCR258N         | A.S258N  | <i>Dyschimus</i> TSR28S        | Dy.T28S  | <i>Orthotrichia</i> SCR164A      | OrtS164A | <i>Simulium rutherfordi</i>           | Sim.rut  |
| <i>Athripsodes scramasax</i>       | A.scram  | <i>Ecnomus oppidanus</i>       | Ec.oppi  | <i>Oxyethira velocipes</i>       | Oxy.vel  | <i>Simulium vorax</i>                 | Sim.vor  |
| <i>Athripsodes spatula</i>         | A.sp.atu | <i>Ecnomus similis</i>         | Ec.simi  | <i>Paranyctiophylax</i> SCR213T  | PaS213T  | <i>Taeniochauiodes ochraceopennis</i> | Tae.och  |
| <i>Athripsodes</i> TSR472C         | A.T472C  | <i>Ecnomus thomasseti</i>      | Ec.thom  | <i>Parecnomina resima</i>        | P.resim  | <i>Tricorythus discolor</i>           | Tri.dis  |
| <i>Baetis harrisoni</i>            | Bae.har  | <i>Ecnomus</i> TSR440G         | EcT440G  | <i>Parecnomina</i> TSR545E       | P.T545E  |                                       |          |
| <i>Barbarochthon brunneum</i>      | Bar.bru  | <i>Ephemerellina barnardi</i>  | Eph.bar  | <i>Petroplax prionii</i>         | Pxpri    |                                       |          |

Table 30: Environmental variables used for the canonical and detrended canonical correspondence analyses along with their respective abbreviations.

| Water Chemistry parameter | Abbreviation | Water Chemistry parameter | Abbreviation |
|---------------------------|--------------|---------------------------|--------------|
| Ammonia (mg/l)            | Ammonia      | pH                        | pH           |
| Chloride (mg/l)           | Chloride     | Conductivity (mS/cm)      | Conduct      |
| Dissolved Mg (mg/l)       | Mg           | Turbidity (NTU)           | Turbid       |
| Fluoride ( $\mu$ g/l)     | Fluoride     | Average stream width (m)  | Ave.stW      |
| Nitrate/Nitrite (mg/l)    | Nit/Nit      | Marginal vegetation       | MarVeg       |
| Orthophosphate (mg/l)     | Orthopho     | Bedrock                   | Bedrock      |
| Sodium (mg/l)             | Sodium       | Cobbles                   | Cobbles      |
| Sulphate (mg/l)           | Sulphate     | Small stones              | Sstones      |
| Total Lead (mg/l)         | Lead         | Sand                      | Sand         |
| Total Zinc (mg/l)         | Zinc         | Organic material          | Organic      |
| Total Iron (mg/l)         | Iron         |                           |              |

Table 31: Species diversity results from diversity partitioning analysis.

|                                 | Additive Richness (Observed diversity) | Expected diversity | p-Value |
|---------------------------------|----------------------------------------|--------------------|---------|
| Within site ( $\alpha$ 1)       | 18.39                                  | 38.06              | 1.000   |
| Between site( $\beta$ 1)        | 11.11                                  | 10.92              | 0.311   |
| Between river reach( $\beta$ 2) | 13.28                                  | 10.8               | 0.001   |
| Between season ( $\beta$ 3)     | 60.22                                  | 43.22              | 0.001   |

Table 32: Summary statistics from the canonical and detrended canonical analyses.

|                             | CCA<br>Eigenvalues |                                | DCCA<br>Eigenvalues |                                |
|-----------------------------|--------------------|--------------------------------|---------------------|--------------------------------|
|                             | All sites          | Excluding Matjies &<br>Buffels | All sites           | Excluding Matjies &<br>Buffels |
| Axis 1                      | 0.489              | 0.313                          | 0.493               | 0.313                          |
| Axis 2                      | 0.195              | 0.171                          | 0.151               | 0.161                          |
| Axis 3                      | 0.165              | 0.142                          | 0.086               | 0.094                          |
|                             |                    |                                | DCCA p-Values       | DCCA p-Values                  |
| Significance of<br>axis 1   | -                  | -                              | 0.001               | 0.001                          |
| Significance of all<br>axes | -                  | -                              | 0.001               | 0.001                          |

Table 33: List of site names and their respective abbreviations grouped over Autumn (A), Spring (S) and Summer (D) sampling periods used in the canonical and detrended canonical correspondence analyses.

| Site name        | Abbreviation |
|------------------|--------------|
| Lower Bloukrans  | L.Blkra      |
| Lower Buffels    | L.Buffe      |
| Lower Elands     | L.Eland      |
| Lower Elandsbos  | L.Elbos      |
| Lower Groot (E)  | L.GroE       |
| Lower Groot (W)  | L.GroW       |
| Lower Lottering  | L.Lotte      |
| Lower Salt       | L.Salt       |
| Lower Storms     | L.Storm      |
| Upper Bloukrans  | U.Blkra      |
| Upper Bobbejaans | U.Bobbe      |
| Upper Buffels    | U.Buffe      |
| Upper Elands     | U.Eland      |
| Upper Elandsbos  | U.Elbos      |
| Upper Groot (E)  | U.GroE       |
| Upper Groot (W)  | U.GroW       |
| Upper Lottering  | U.Lotte      |
| Upper Matjies    | U.Matji      |
| Upper Salt       | U.Salt       |
| Upper Storms     | U.Storm      |

Table 34: Summary statistics from the Monte-Carlo permutation tests carried out to test the significance of the environmental variables on the species and site data for the canonical correspondence analyses.

| Water chemistry parameter | F - Value<br>from CCA<br>of data<br>from all<br>sites | p-Value<br>estimate<br>from CCA<br>of data<br>from all<br>sites | Water chemistry parameter | F - Value<br>from CCA<br>of data<br>with sites<br>excluded | p-Value<br>estimate<br>from CCA<br>of data<br>with sites<br>excluded |
|---------------------------|-------------------------------------------------------|-----------------------------------------------------------------|---------------------------|------------------------------------------------------------|----------------------------------------------------------------------|
| pH                        | 8.985                                                 | 0.001                                                           | pH                        | 4.386                                                      | 0.001                                                                |
| Total Zinc                | 3.314                                                 | 0.001                                                           | Sand                      | 4.023                                                      | 0.001                                                                |
| Dissolved Mg              | 2.882                                                 | 0.001                                                           | Dissolved Mg              | 3.902                                                      | 0.001                                                                |
| Average stream width      | 2.716                                                 | 0.001                                                           | Small stones              | 3.76                                                       | 0.001                                                                |
| Ammonia                   | 2.348                                                 | 0.001                                                           | Chloride                  | 3.368                                                      | 0.001                                                                |
| Organic                   | 1.95                                                  | 0.001                                                           | Bedrock                   | 3.192                                                      | 0.001                                                                |
| Fluoride                  | 1.63                                                  | 0.012                                                           | Average stream width      | 2.493                                                      | 0.001                                                                |
| Small stones              | 1.614                                                 | 0.013                                                           | Total Zinc                | 2.255                                                      | 0.001                                                                |
| Total Iron                | 1.428                                                 | 0.0819                                                          | Sodium                    | 2.732                                                      | 0.002                                                                |
| Cobbles                   | 1.299                                                 | 0.1269                                                          | Organic                   | 2.466                                                      | 0.002                                                                |
| Marginal vegetation       | 1.524                                                 | 0.21                                                            | Total Iron                | 2.172                                                      | 0.002                                                                |
| Total Lead                | 1.152                                                 | 0.2537                                                          | Ammonia                   | 1.792                                                      | 0.008                                                                |
| Orthophosphate            | 1.072                                                 | 0.3546                                                          | Cobbles                   | 1.601                                                      | 0.025                                                                |
| Chloride                  | 1.021                                                 | 0.4086                                                          | Sulphate                  | 1.502                                                      | 0.047                                                                |
| Sodium                    | 0.948                                                 | 0.4845                                                          | Turbidity                 | 1.348                                                      | 0.1379                                                               |
| Turbidity                 | 0.955                                                 | 0.4935                                                          | Fluoride                  | 1.116                                                      | 0.2587                                                               |
| Sulphate                  | 0.923                                                 | 0.5445                                                          | Marginal vegetation       | 1.065                                                      | 0.3417                                                               |
| Conductivity              | 0.865                                                 | 0.6094                                                          | Orthophosphate            | 0.949                                                      | 0.5165                                                               |
| Nitrate/Nitrite           | 0.692                                                 | 0.8881                                                          | Conductivity              | 0.692                                                      | 0.7802                                                               |
| Bedrock                   | 0.674                                                 | 0.9071                                                          | Total Lead                | 0.66                                                       | 0.851                                                                |
| Sand                      | 0.674                                                 | 0.9101                                                          | Nitrate/Nitrite           | 0.707                                                      | 0.8641                                                               |

Table 35: A summary of basic site characteristics. See the detailed site descriptions in Appendix 1 for further information.

| Site along river         | Latitude (South) | Longitude (East) | Altitude | Average stream width (m) |
|--------------------------|------------------|------------------|----------|--------------------------|
| Lower Groot (East) River | 34°02'05.1"      | 24°12'27.2"      | 14       | 7                        |
| Upper Groot (East) River | 33°58'21.2"      | 24°07'16.1"      | 275      | 3                        |
| Upper Elands River       | 33°58'17.6"      | 24°03'51.1"      | 313      | 1                        |
| Lower Elands River       | 34°01'02.8"      | 24°03'39.1"      | 56       | 4                        |
| Lower Storms River       | 33°59'19.2"      | 23°55'08.6"      | 65       | 14                       |
| Upper Storms River       | 33°56'57.2"      | 23°54'55.0"      | 285      | 2                        |
| Upper Elandsbos River    | 33°55'56.8"      | 23°46'58.0"      | 254      | 3                        |
| Lower Elandsbos River    | 33°58'00.8"      | 23°46'29.7"      | 215      | 5                        |
| Lower Lottering River    | 33°58'23.9"      | 23°44'50.5"      | 218      | 5                        |
| Upper Lottering River    | 33°55'58.6"      | 23°43'45.6"      | 267      | 7                        |
| Upper Bloukrans river    | 33°55'04"        | 23°38'20"        | 276      | 2                        |
| Lower Bloukrans River    | 33°57'21"        | 23°38'19"        | 40       | 8                        |
| Upper Groot (West) River | 33°54'45.6"      | 23°34'32.6"      | 313      | 5                        |
| Lower Groot (West) River | 33°57'58.3"      | 23°33'32.5"      | 14       | 14                       |
| Upper Bobbejaans River   | 33°53'46.5"      | 23°33'19.5"      | 414      | 3                        |
| Lower Salt River         | 33°58'28.3"      | 23°31'19.7"      | 47       | 5                        |
| Upper Salt River         | 33°55'36.2"      | 23°29'23.3"      | 265      | 4                        |
| Upper Buffels River      | 33°58'56.8"      | 23°28'43.9"      | 61       | 2                        |
| Lower Buffels River      | 33°59'07.9"      | 23°27'48.7"      | 32       | 2                        |
| Upper Matjies River      | 33°58'50.6"      | 23°27'28.1"      | 51       | 1                        |

Table 36: Summary of species data from each site, sorted according to the highest number of species per site

| Site          | Total no. of species | No. of S-W Cape<br>endemic sp. | No. of undescribed sp. |
|---------------|----------------------|--------------------------------|------------------------|
| U. Bobbejaans | 63                   | 32                             | 9                      |
| L. Storms     | 63                   | 26                             | 11                     |
| L. Groot (W)  | 59                   | 22                             | 7                      |
| U. Salt       | 58                   | 29                             | 9                      |
| L. Bloukrans  | 58                   | 25                             | 6                      |
| U. Lottering  | 57                   | 31                             | 9                      |
| U. Groot (E)  | 56                   | 27                             | 7                      |
| U. Elandsbos  | 54                   | 27                             | 8                      |
| L. Lottering  | 52                   | 26                             | 7                      |
| U. Bloukrans  | 50                   | 22                             | 7                      |
| L. Groot (E)  | 50                   | 10                             | 5                      |
| U. Elands     | 48                   | 21                             | 6                      |
| U. Groot (W)  | 45                   | 23                             | 4                      |
| L. Elandsbos  | 43                   | 19                             | 6                      |
| U. Storms     | 43                   | 25                             | 6                      |
| L. Salt       | 38                   | 9                              | 0                      |
| L. Buffels    | 37                   | 9                              | 3                      |
| U. Buffels    | 37                   | 5                              | 1                      |
| U. Matjies    | 29                   | 5                              | 1                      |
| L. Elands     | 23                   | 4                              | 1                      |

---

## Appendix 1

---

### Detailed site descriptions

#### Upper Groot (East) River (33°57'21"S, 23°38'19"E)

*Introductory notes:* This site is situated in the foothills of the Eastern Tsitsikamma Mountains, approximately 1.6 kilometers from their base where the river leaves the mountains, at an altitude of 275 meters above sea level. Access is via an MTO forestry road that is used to service a water abstraction pipeline running parallel to the river, approximately 15 meters above the western bank.

*River channel:* The sampling site was approximately 18 meters in length. A chute of 1 meter in height, at the upstream end, flows into a waist-deep pool (where the data logger was placed). This is followed by another chute leading to a riffle of approximately 5 meters in length and 3 meters wide that flows over bedrock into another waist-deep pool densely lined with Palmiet (Plates 1 & 2 respectively). Further downstream, conditions remained the same, with slow-flowing deep pools lined with homogenous Palmiet (*Prionum serratum*: Juncaceae). The bottom substrate consists of bedrock, small boulders, cobbles, stones and gravel (Plate 3). Flow rate at this site remained low throughout sampling; however, when site selection was taking place in January 2008, the river was higher than that measured at any other time when we visited the site (Plate 1).

*Riparian vegetation:* The river course at this site has very little canopy and the riparian vegetation primarily consists of Palmiet and other indigenous plant species. Twenty to thirty meters further up the banks, alien trees such as *Eucalyptus* spp (Myrtaceae) and Black Wattle (*Acacia mearnsii*: Fabaceae) are the dominant vegetation type. The eastern bank, up to approximately 3 meters from the river's edge, is devoid of vegetation with the exception of moss, and is best described as a root bank (Plate 4). Palmiet was scoured from this area as a result of the flooding that occurred during November of 2007, which left bedrock and root-bound soil exposed. The western bank at the site is covered with indigenous vegetation, consisting mostly of woody bush and a few ferns (Plate 5).





Plate 1: Upstream view of upper Groot (East) River showing the two chutes and the sampling area. Water flow was high and colour strongly humic. Photo: F. C. de Moor, 11 January 2008.



Plate 2: Downstream view of the upper Groot (East) River. The stream narrows and is nearly overgrown by Palmiet before opening into a wide pool that has isolated outcrops of bedrock breaking the surface. Photo: T. A. Bellingan, 2 July 2008.





Plate 3: Upstream view of the upper Groot (East) River, showing the heterogeneous nature of bottom substrate found at this site. The water colour was clearer when the water level was lower, with the converse occurring during increased water volumes; c.f. Plate 1. Photo: T. A. Bellingan, 16 January 2009.



Plate 4: Eastern bank of upper Groot (East) River showing part of the exposed root bank where Palmiet was uprooted after flooding during November 2007. Photo: F. C. de Moor, 11 January 2008.





Plate 5: A section of the western bank of the upper Groot (East) River; vegetation is fairly dense, only shading the river in the late afternoon. Photo: T. A. Bellingan, 16 January 2009.

**Lower Groot (East) River (34°02'05.1"S, 24°12'27.2"E)**

*Introductory notes:* The site sampled on the lower section of the Groot (East) River lies approximately 6.5 kilometers from the river mouth, at an altitude of 14 meters above sea level. This site is reached by MTO forestry roads, through several pine plantations. It is situated immediately downstream of a causeway crossing the river that is approximately 66 meters long and 6 meters wide. The length of river sampled lay along either side of this causeway, as it provided the best biotopes for the collection of macroinvertebrates.

*River channel:* At this point the river channel is approximately 60 meters wide and is almost entirely choked with Palmiet and exotic vegetation, leaving only relatively small sections flowing between, and underneath, the plants. The main flow is along the left side of the riverbed (Plate 6) and, after flowing under the causeway, flows parallel along it, across the river bed, and then downstream along the right hand bank (Plate 7). Some of the water escapes this route and flows underneath the Palmiet. This pattern is obviously dependant on the amount of water flowing in the river at the time, but was the case for each of the sampling surveys, with the exception of the survey in January 2009 when no water was



flowing at all, leaving the data logger — that was placed under one of the pipes — exposed. As is characteristic of the lower reaches of these rivers, large pools are common, broken up by weirs and shallow riffles over cobbles, stones, gravel and sand. At times, a filamentous alga was also common at this site (Plate 8). The water colour was darkly stained by tannins and humic acid during each sampling event.

*Riparian vegetation:* Both upper banks of the Groot River at this site are characterized by pine forest. A short distance upstream and downstream of the causeway on the left hand bank there is a patch of indigenous forest that reaches down to the river's edge. This extends along the bank for approximately 200m before meeting another pine plantation. Associated with the pine forest, and vegetation choking the river bed itself, are alien invasive species such as Black Wattle, Bugweed (*Solanum mauritianum*: Solanaceae) and Kikuyu grass (*Pennisetum clandestinum*: Poaceae) (Plate 6, 7).



Plate 6: An upstream view of the "main channel" of water flowing along the left bank of the lower Groot (East) River. Note the abundant alien vegetation within the river course, resulting in the diversion of the water flow. Photo: T. A. Bellingan, 7 April 2008.





Plate 7: Downstream view of the lower Groot (East) River flowing along the length of the causeway.  
Photo: F. C. de Moor, 10 January 2008.



Plate 8: Filamentous algae is sometimes prevalent at the lower Groot (East) River; bottom substrate is clearly illustrated. A fair amount of wood debris is also present, providing an additional biotope that was sampled. Photo: T. A. Bellingan, 4 October 2009.



**Upper Elands River (33°58'17.60"S; 24°3'51.10"E)**

*Introductory notes:* After assessing the main course of the Elands River, it was decided that the area with reasonable access was too homogeneous and lacked the necessary biotopes to meet the requirements of this study. Instead, a tributary of the Elands River was sampled. As with the other sites, access is via MTO forestry roads through plantations and into the mountains over a poorly-maintained, and what appeared to be rarely-used, upper catchment access road. The altitude of this site was recorded as 313 meters above sea level, making it the second highest site sampled. The tributary drains underneath the access road, through a makeshift causeway, making it very convenient for sampling.

*River channel:* The area sampled began upstream of a deep pool below a waterfall of roughly 3 meters in height; biotopes included splash and hygropetric zones as well as “potholes” in the bedrock filled with stones. The pool, approximately 8 meters in length and 5 metres in breadth, provided small amounts of marginal vegetation and stones out of current (Plate 9). Below the pool the stream forms a run over some bedrock that weaves between boulders, and over cobbles and stones, with small amounts of gravel and some coarse sand (Plate 10). The total length of the sampling site along the river was approximately 21 meters.

*Riparian vegetation:* This site was unique in that the left bank was completely overgrown with alien *Eucalyptus* spp trees while the right bank seemed to be pristine indigenous forest. Both vegetation “types” extended down to the banks of the river (Plate 11). The site is shaded for most of the day with only patches of sunlight reaching the river bed. This is due to the canopy formed by mature *Eucalyptus* spp trees. The exotic vegetation has a negative effect on the system due to plant debris falling into the river. This material is not broken down naturally and thus chokes river flow and possibly alters water chemistry as well.





Plate 9: Upstream view of the pool sampled below the waterfall along the tributary of the upper Elands River. Photo: T. A. Bellingan, 4 October 2008.



Plate 10: Downstream view of the upper Elands River showing the pool below the waterfall. Photo: T. A. Bellingan, 16 January 2009.





Plate 11: Upstream view of the riparian vegetation, with emphasis on the left bank (on right of picture). Note the lack of undergrowth and the amount of dead plant matter accumulating on the soil compared to that of the right bank. Photo: T. A. Bellingan, 16 January 2009.

#### **Lower Elands River (34°1'2.80"S; 24°3'39.10"E)**

*Introductory notes:* The Elands River is reached via the Robbehoek MTO forestry road, through a large tract of indigenous forest followed by immature pine plantation. This site is situated within a comparatively deep valley at the end of a forestry cul-de-sac. Care must be taken in getting to this site as the steep slopes can prove treacherous, especially when wet. The altitude at this site is 56 meters above sea level and approximately 6 kilometers from the river mouth.

*River channel:* We were fortunate to be able to sample from a riffle flowing over some bedrock, small boulders, cobbles and stones that provided “in current” biotopes (Plate 12). The Elands River along this section is characterized by large, deep pools (8-12 meters wide and in excess of 30 meters long) alongside steep cliffs (Plate 13). These pools are lined with vegetation, due to Palmiet being plentiful (Plate 14). Beyond this small riffle, the site was almost devoid of useful riverine sampling biotopes. The sampling site incorporated a stretch of river of approximately 12 meters long.



*Riparian vegetation:* The steep banks along this reach of the Elands River are lined with indigenous forest, up until the tops of the steep valley. Along the left bank, the natural vegetation only extends up to the edge of the river valley, after which pine forest plantation ensues. The river valley is wide enough to prevent complete shading by the forest canopy; however, due to its deep incision, only a few hours of direct sunlight reach the river below.



Plate 12: The riffle sampled along the lower reaches of the Elands River. Photo: T. A. Bellingan, 4 October 2008.





Plate 13: Downstream view of the lower Elands River showing the large slow-flowing pool and steep banks with forest along the river margin. Photo: F. C.de Moor, 14 January 2008.



Plate 14: Upstream view of the lower Elands River showing a small chute below a large forest- and Palmiet-lined pool. Photo: T. A. Bellingan, 4 October 2008.



**Upper Storms River (33°56'57.20"S; 23°54'55.00"E)**

*Introductory notes:* This site lies on a tributary of the Storms river, as was the case for the upper Elands River site. The site is situated within a tract of indigenous forest, called Sleepkloof, along the Tsitsikamma Hiking trail approximately 700 meters from an overnight rest hut. It is reached via a SANParks road that is used to service the hiking trail hut, off of the N2 freeway. It lies at an altitude of 285 meters above sea level. The main river is less than 1 kilometer along the tributary's course from the area that we sampled, although due to the presence of waterfalls and other steep areas reaching the main river course is not safely possible (Plate 15).

*River channel:* The tributary is a small stream that reduced to little more than a trickle during the dry period that coincided with summer sampling in January of 2009. The stream weaves in between boulders and over bedrock, which make up a large proportion of the stream bed; small riffles over sand and stones are also present (Plate 16). Because of the rocky nature of the stream bed, very little true marginal vegetation biotope is present at this site. A large, three-tiered waterfall is found at the lower end of the sampling site, providing hygropetric and splash zones as well a pool at its base with "out of current" biotope (Plate 17). The pool is approximately 3 meters long, 5 meters wide and 1.5 meters deep. This site is complex in nature, as the stream is slow flowing, with only indigenous vegetation and small seeps, providing good habitat for insects. The entire sampling site, including the waterfall, is roughly 35 meters in length.

*Riparian vegetation:* As mentioned above, the site lies within indigenous forest and is completely surrounded by dense natural vegetation with no alien plant species observed. A canopy completely covers this site, shading it throughout the day. The stream banks are made up of root mats, moss and decaying leaf litter.



Plate 15: Downstream view of pools below the waterfall, which is approximately 10 meters in height. The vegetation surrounding the the river, and sometimes growing from within the river bed, can be seen. Photo: T. A. Bellingan, 19 January 2008.



Plate 16: Upstream view of part of the area sampled along the upper Storms River; the predominantly bedrock substrate is visible. Photo: F. C. de Moor, 14 January 2008.

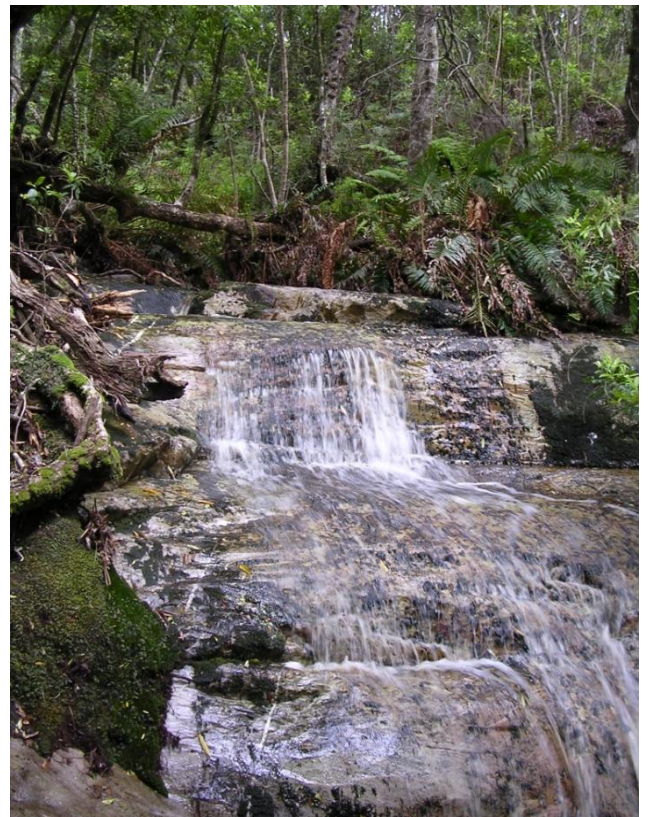


Plate 17: Upstream view above the waterfall showing the bedrock and moss in current biotopes. Photo: F. C. de Moor, 14 January 2008.



**Lower Storms River (33°59'19.20"S; 23°55'8.60"E)**

*Introductory notes:* This site lies at an altitude of 65 meters above sea level and, as the river winds, is situated approximately 4.8 kilometers from the river mouth. It is conveniently placed in the vicinity of the low water bridge that crosses the Storms River. Sampling took place both downstream and upstream of the bridge.

*River channel:* The river bed at this site is approximately 27 meters wide, making it the widest of rivers sampled in this study. The river winds through vegetation growing in the riverbed, until it is dammed up by the bridge (Plate 18). As sampling was carried out during the year, it was noted that this area became inundated with filamentous algae. The concrete structure of the bridge added several advantages to the site: a place to conceal the data logger safely; a place for the collection of adult insects as they seek refuge under its shaded parts; “bedrock in current biotope” is provided by the concrete foundation, with moss anchored to it. Further below the bridge, deeper riffles over large stones are found as well as marginal vegetation biotopes, due to the abundance of Palmiet (Plate 19). Upstream of the bridge is a deep, large pool, roughly 70 meters long and 12 meters at its widest (Plate 18) and beyond this is a shallow riffle over rounded cobbles, perfect for sampling the stones-in-current biotope (Plate 20). From the riffle, sampled upstream of the bridge to downstream of the bridge, the overall length of the sampling site is approximately 180 meters in length.



Plate 18: An upstream view from the bridge along the lower Storms River showing the impounding effect of the bridge and the "islands of vegetation". Photo: T. A. Bellingan, 3 October 2008.

*Riparian vegetation:* As mentioned above, there is an abundance of Palmiet lining the river channel, as well as other woody vegetation and grasses growing in the riverbed forming "islands" of vegetation (Plate 20). The majority of vegetation surrounding this site is indigenous forest, coming down all the way to the river's edge on the cliff-like left bank, above and below the bridge. On the right bank downstream of the bridge, the same pattern of vegetation is seen, being forested up to the river bank. Upstream of the bridge, the road winds along the river bank up and out of the river valley resulting in a slight modification of the vegetation as a result of the change in soil composition with the construction of the road.





Plate 19: Downstream view from the bridge along the lower Storms River showing the bridge foundation and deeper riffle as well as an abundance of Palmiet along the river's edge. Photo: T. A. Bellingan, 3 October 2008.



Plate 20: Lower Storms River: Upstream view of the shallow riffle sampled, approximately 150 meters upstream of the bridge. Photo: T. A. Bellingan, 3 October 2008.



**Upper Elandsbos River (33°55'56.80"S; 23°46'58.00"E)**

*Introductory notes:* This site is arguably the most difficult to sample in terms of convenience of access. The road follows what is called “Mangold se pad”, which is taken to get to within half an hour’s walking distance of the site; a small tributary of the Elandsbos River is crossed, draining from “Heuningkloof”. In addition to samples taken at the main river site, light traps were set up at this tributary, downstream of the stone causeway, as it was convenient and presented an opportunity for further sampling within the catchment. The road forms part of the Tsitsikamma Hiking Trail; however, at a certain point the trail must be abandoned and a descent into the valley through fire-devastated Pine trees and thick Fynbos is made. The altitude of the river at this site is 254 meters above sea level.

*River channel:* The river is not more than 2 meters across at its widest point; at its deepest, it reaches roughly 1.3 meters and, for the most part, it meanders between bedrock outcrops over which shallower water flows. The sampling area extends approximately 40 meters along the length of the river. On average, the stream is quite deep with a few shallow riffles (Plates 21 & 22). The bottom substrate consists of rounded cobbles, stones and Palmiet root banks. A large clear pool is situated on the left bank, which overflows through Palmiet into the main river, forming a marshy hygropetric seep (Plate 23).

*Riparian vegetation:* The stream at the site, as well as above and below the site, is lined with dense Palmiet up to the water’s edge (Plates 22 & 22). Further up the banks, low-growing woody vegetation and ferns are found. Some invasive species, particularly Pine trees, are found, but most of these are dead as a result of a relatively recent fire; succession is, however, expected and inevitable. Thick *Restio*-dominated Fynbos is found further up the banks, making reaching and leaving the river difficult.



Plate 21: Downstream view of the upper Elandsbos River showing exposed bedrock and riparian Palmiet that forms dense root mats below the waters' surface. Photo: T. A. Bellingan, 6 October 2008.



Plate 22: Upstream view of the Upper Elandsbos River, the only riffle being just visible behind the dense Palmiet. Dead pine trees can be seen in the background. Photo: T. A. Bellingan, 6 October 2008.





Plate 23: Upper Elandsbos River: a large, clear-water pool on the left bank of the river; this proved very useful for sampling Odonata. Photo: F. C. de Moor, 10 January 2008.

#### **Lower Elandsbos River (33°58'0.32"S; 23°46'29.87"E)**

*Introductory notes:* Due to the cascading, incised nature of the Elandsbos River valley downstream (South) of the R102, and a lack of biotopes that could be sampled, this site was chosen despite lying at an altitude of 215m above sea level. The site is approximately 850 meters from the R102 along an MTO forestry road, making it easily accessible. Felling of the mature pine plantation along the right bank during autumn of 2009 resulted in a pine tree falling across the river, modifying flow considerably. A rainfall event later in the year dislodged the tree that had blocked the channel and flow returned to 'normal'. At the lower end of the site, on the right hand bank, a seep draining from the plantation trickled into the river, creating a soggy patch along the bank that resulted in a few stones being covered with filamentous algae. After clear-felling of the pine plantation, however, this increased to a small spring, resulting in a far larger area being covered by the black algae (Plate 24).

*River channel:* The width of the channel at this site varies from 2 - 10 meters, with a section of bedrock protruding from the right bank, providing a refuge for the temperature data



logger and a pool nearly 2 meters deep alongside it. The total length of the sampling site was approximately 80 meters. The bottom substrate of this site consists of rounded cobbles and sand, which meant that the depth and shape of the river changed considerably when flooding events caused movement of the substrate material. Nevertheless, for the entire sampling period, shallow riffles over the cobbles were present and provided stones-in-current biotope (Plate 25). A small island of Palmiet, protected by the bedrock outcrop, provided a marginal vegetation biotope (Plate 26).



Plate 24: The lower Elandsbos River showing the seep staining the white cobbles black, as a result of algal growth and detritus settling between the stones. The indigenous forest is seen on the far (left) bank. Photo: T. A. Bellingan, 20 January 2009.

*Riparian vegetation:* The site lies on the margin between a large tract of indigenous forest and MTO pine plantations. At the upper end of the site, the river bank on the right side is mostly indigenous trees with few aliens present (Plate 27). Further downstream, the transition to pine forest begins. The left bank is made up entirely of indigenous forest and approximately 50 meters below the site it also becomes pine plantation. Some Palmiet is present along the water's edge.





Plate 25: View of the shallow riffle at the bottom end of the lower Elandsbos River sampling site, below a large shallow pool. Photo: T. A. Bellingan, 20 January 2009.



Plate 26: Lower Elandsbos River: upstream view of a large shallow pool formed due to cobble and stone movements. The island of Palmiet can be seen. Photo: T. A. Bellingan, 20 January 2009.





Plate 27: Upstream view from the upper reach of the Lower Elandsbos River sampling site. Indigenous forest on both banks and clean white cobbled substrate are illustrated. Photo: T. A. Bellingan, 20 January 2009.

#### **Upper Lottering River (33°55'58.60"S; 23°43'45.60"E)**

*Introductory notes:* This site lies at an altitude of 267 meters above sea level and approximately 1.2 kilometers into the foothills of the Tsitsikamma Mountains in an area called “Noorman se bos”. It is situated above and below a concrete causeway built across the Lottering River, making it a particularly convenient site to sample.

*River channel:* The natural, unmodified part of the river is narrow, approximately 1.5 meters in width. Due to the construction of the causeway, however, a large pool of approximately 9 meters in width has formed. This is littered with boulders, large stones and cobbles. Upstream of the pool the bottom substrate is mostly bedrock, while the left hand consists of a root bank, of roughly 7 meters long, below the water surface (Plate 28). In the middle of this large pool is an island of vegetation above a large root mat fixed into clay-like soil (Plate 29). Downstream of the causeway, the stream is shallow and forms a riffle over large and



small stones, as it leaves the pipes under the causeway. The river then meanders through Palmiet into a shaded run (Plate 30). The entire sampling site, including the width of the causeway, is approximately 60 meters in length.



Plate 28: The Lottering River upstream of the large pool formed by the causeway. The upper end of the large Palmiet formed root bank (referred to in the text) can be seen in the lower right corner of the plate. Photo: F. C. de Moor, 3 April 2008.

*Riparian vegetation:* The vegetation along this site is disturbed and invaded by alien trees; this could be due to the impact of the road descending into the valley above the left bank of the river. This area of the Lottering catchment appears to have higher numbers of invasive plant species than in any of the other upper sites selected. Downstream of the causeway, on the right bank, large Black Wattle trees shade the riverbed. Wattle saplings occur in abundance amongst indigenous woody bush on both left and right banks above the causeway. Palmiet lines the river on both banks, occurring in greater abundance than at any other plant (Plates 29 & 30).





Plate 29: Upstream view of the upper Lottering River, taken from the middle of the causeway. Photo: T. A. Bellingan, 4 July 2008.



Plate30: Downstream view from the causeway crossing the upper Lottering River. The DWAF team are preparing to sample the small riffle below the pipes under the road. Photo; T. A. Bellingan, 4 July 2008.



**Lower Lottering River (33°58'23.90"S; 23°44'50.50"E)**

*Introductory notes:* The geographical situation regarding this site is very similar to that of the lower Elandsbos River site. The Lottering River is also deeply incised to within a few hundred meters of the N2 freeway and R102 roads and no easy access could be gained to the “altitudinal” lower reaches of this river. The site is reached via a path from the R102 road, approximately 175 meters upstream of the bridge over the Lottering River. It lies at an altitude of 218 meters above sea level and roughly 4 kilometres from the river mouth. The river bends to an east/west orientation when heading upstream of the bridge, so that it is nearly parallel with the road. Sampling took place along this section of river.

*River channel:* The river channel is approximately 6 meters wide; depth estimates by visual means are difficult because of the tannin colour of the water, but the channel may reach a maximum depth of several meters. The substrate predominantly consists of large and small stones and cobbles, with the larger stones usually holding tufts of moss. A small chute, overgrown with woody riparian vegetation (Plate 31) marks the top end of the sampled area. The river forms a shallow pool below this chute, broken up by riffles over the stony substrate and ending in a large deep pool. This follows the bend of the river for at least 75 meters onwards towards the R102 bridge (Plate 32). The length of the sampling site, from the chute to the lower end of the pool, is approximately 45 meters.

*Riparian vegetation:* Possibly due to its proximity to a national road, the banks of the Lottering River are infested with alien vegetation, nearly exclusively Black Wattle. Palmiet also lines the banks of the river and forms small islands of vegetation within the stream bed. Areas that were swept clean of Palmiet during heavy rainfall events had been colonised by grasses (Plate 32).





Plate 31: Upstream view of the lower Lottering River showing the small chute in the top centre, followed by deeper, slower-flowing riffles over cobbles. The branches of a pine tree can be seen in the top left of the plate. Photo: T. A. Bellingan, 24 January 2009.



Plate 32: Downstream view of the lower section of the lower Lottering River sampling site, ending in a large pool that forms a bend in the river that runs towards the R102 Bridge. A small section of the riffle sampled can be seen in the lower left corner of the plate. Photo: T. Bellingan, 24 January 2009.



### **Upper Bloukrans River (33°55'4.00"S; 23°38'20.00"E)**

*Introductory notes:* This site is comparable to the Elandsbos River site in terms of distance and access. At an altitude of 276 meters, it is situated well into the mountains. A key must be obtained from either the Bloukrans or Storms River Village SANParks forestry stations to access the MTO/ SANParks road, turning right into the forest off the R102 at the top of the Bloukrans pass. A fairly large tract of indigenous forest must be passed through (Platbos), followed by MTO pine plantation, before a rest-hut on the Tsitsikamma hiking trail is reached. A small tributary flowing from “Heksekloof” is crossed in the plantation section of the drive to the site; this was sampled as an additional site in the catchment, using light traps only. From the Bloukrans hut a steep section of the hiking trail must be followed to get to the site along the river. The river sampled was a tributary of the Bloukrans River, as the main river was far too incised and inaccessible to get to safely (Plate33).

*River channel:* The river channel showed very high levels of natural modification from a recent flooding event, undoubtedly the heavy rains that fell during November of 2007. The channel had been scoured clean of any vegetation growing within it, and along the banks, large amounts of debris could be seen (Plate 34). Working upstream from the top of a very high cliff-waterfall, the site starts with a pool approximately 3 meters long and 4 meters wide. Upstream of this is a waterfall of approximately 4 meters in height and above this a pool of 4 meters wide and 7 meters long. Above this, another pool is connected by a chute over bedrock, between large stones. Further upstream of this pool are a series of small pools with shallow riffles between them. The substrate along this site varies from boulders in the pools with sand along their edges, to stones and cobbles in the riffles and is dominated by bedrock, allowing for the formation of the chutes and waterfalls (Plate 33 & 34).

*Riparian vegetation:* As mentioned above, the riparian vegetation along the stream has been highly modified by a recent flood event. Upon visiting the site for the first time, what vegetation that remained alive stood at least 2 meters up the banks, and consisted of thick woody Fynbos and Keurboom. As the sampling proceeded, the riparian vegetation was seen to encroach back down the banks and recovery was evident (Plate 35). It should be mentioned that the invasive Australian protea *Hackea sericia* (Proteaceae) has begun to encroach unchecked from the surrounding catchment.





Plate 33: Aerial view of the upper Bloukrans River tributary from the balcony of the hiking trail hut. A researcher, seated left-of-centre above the top chute, can be used for scale. Photo: F.C. de Moor, 27 March 2009.





Plate 34: Upstream view of the upper Bloukrans River tributary showing stones- in-current biotope. Marginal vegetation biotope was sparse due to flood damage, with the exception of the pools downstream. Photo: F. C. de Moor, 8 January 2008.



Plate 35: Upstream view of the pools at the lower end of the upper Bloukrans River tributary, taken from above the large waterfall seen in Plate 33. Photo: T. A. Bellingan, 23 January 2009.



**Lower Bloukrans River (33°57'21.00"S; 23°38'19.00"E)**

*Introductory notes:* The area sampled was in the region of 100 meters upstream of a gauging weir, where the R102 crosses the Bloukrans River forming the boundary between the Eastern and Western Cape Provinces. It lies deep within the river valley at an elevation of 40 meters above sea level, approximately 800 meters upstream of the confluence with the Vark River.

*River channel:* The riverbed is approximately 15 meters wide and is composed of large, angular, slab-like boulders that divert the river into many smaller channels that flow underneath and around them (Plate 36). The substrate within these channels is composed of small cobbles, stones and gravel. A large pool is formed due to the weir upstream of which the majority of sampling took place, mostly within the riffles and pools that formed as a result of bedrock intrusions from the right bank. The total length of the sampling site was approximately 75 meters. Some scrap metal debris was also found in the river channel at this site.



Plate 36: Downstream view of the lower Bloukrans River sampling area; the R102 road and large pool leading to the weir can be seen in the top centre of the plate. Photo: F.C. de Moor; 21 January 2009.



*Riparian vegetation:* The Bloukrans pass winds through a section of indigenous forest called the Rugbos, which stretches the entire length of the pass. The riparian vegetation is thus all indigenous with very few exceptions. The R102 road that lies in close proximity to the right bank has caused some modification of the vegetation and surrounding landscape. The left bank, for most of the site, is a cliff above which the forest grows. Closer to the riverbed, Palmiet fringes the river edges, forming good marginal vegetation biotope. This only occurs along the right bank, due to the rocky nature of the left bank (Plate 37).



Plate 37: Upstream view of the lower Bloukrans River. Dense, natural forest lines the riparian zone of the river. The jagged nature of boulders in the riverbed and the very low flow of the river at this time are illustrated. Photo: F. C. de Moor, 21 January 2009.

**Upper Groot (West) River (33°54'45.60"S; 23°34'37.10"E)**

*Introductory notes:* This is the first of two sites that are reached by helicopter; no roads or hiking trails existed that allow access to the river at this altitude. Vehicles and excess equipment were left at the Plateau Guest House along the R102 between the Bloukrans and Groot (West) River passes while sampling took place. A helipad, owned by the guest house, can be used to ferry teams to the site. Obviously, the use of a helicopter makes getting into the mountains far easier, thus allowing researchers to get high up into the catchment, provided a suitable place could be found to land the helicopter. The altitude at this site is 313 meters above sea level.

*River channel:* The River along this upper section winds considerably and is already incised into a fairly steep valley, the climb from the helicopter landing site to the riverbed being approximately 75 meters. The riverbed where sampling took place is approximately 9 meters wide with the stream flowing over most of its breadth between boulders and large stones, providing stones-in-current biotopes in slower flowing water (Plate 38). A larger, more swift-flowing, channel occurred along the left bank as a reasonably large piece of flat bedrock was exposed, providing little water resistance. Not far upstream of the area that was sampled, a large pool, of roughly 1.5 meters depth, stretched around the bend of the river, providing marginal vegetation biotope (Plate 39). The area sampled stretched approximately 55 meters along the length of the river.

*Riparian vegetation:* This site is situated within pristine natural forest. Although pine trees have colonized the upper slopes of the mountains and down to the valley edges, no alien vegetation was observed near the river course. The sampling site is almost entirely shaded by forest canopy, with only odd patches of direct sunlight reaching the riverbed. Forest trees and woody understory growth arise from the water's edge on the right bank while the left bank is lined with large tree ferns, *Cyathea* sp. (Cyatheaceae), followed by a steep slope covered with grass, Restionaceae and thick stands of Keurboom (*Virgilia oroboides*: Fabaceae) higher up.





Plate 38: Upstream view of the upper Groot (West) River, dense woody vegetation can be seen lining the river banks. Photo: T. A. Bellingan, 12 July 2008.



Plate 39: Downstream view of the upper Groot (West) River. The tree ferns, referred to in the text, are visible on the left of the plate as well as the slab of bedrock promoting faster water flow. Photo: T. A. Bellingan, 12 July 2008.



**Upper Bobbejaans River (33°53'46.50"S; 23°33'19.50"E)**

*Introductory notes:* This site is the second of the two sites reached by helicopter. At an altitude of 414 meters above sea level, it lies higher than any other site sampled during this study. Despite this, the river course is incised and has begun undulating in its flow. The valley that one has to hike down to get to this site, even though deeper, is not as steep and densely vegetated as that of the Upper Groot (West) (Plate 40).

*River channel:* The river channel at the site is approximately 4 meters wide and, in places, up to 1.5 meters deep. The upstream part of this site starts with a small chute, above which a long riffle (roughly 12 meters long) flows over large stones and boulders, leading to a pool (Plate 41). The pool then drains over some smaller cobbles and stones, with the edges of the riffle lined with gravel and sand in places. This riffle continues for approximately 3 meters before the channel of flow is split by a large boulder with a butterfly pattern on it (Plate 42). The riffle then turns to a large chute into another deep pool, where the temperature data logger was positioned under some small boulders. The length of the area sampled at this site was approximately 60 meters

*Riparian vegetation:* Palmiet lines the stream margins in abundance, serving to stabilize the soil by forming root banks and providing excellent marginal vegetation biotope for sampling. Further up the banks, flood damage from the summer rains of 2007 is visible, with the woody vegetation being broken and flattened by the flood waters. Succession by smaller proteaceous shrubs is taking place on both the left and right banks while the woody vegetation recovers. Further up the banks, burnt pine trees and proteas are common amongst thick superabundant Restio grass.





Plate 40: An aerial view of the upper Bobbejaans River, taken from the helicopter while descending to land. The area sampled lies between the two pools visible in the centre of the photograph. Photo: T. A. Bellingan, 10 October 2008.



Plate 41: Upstream view of the upper Bobbejaans River from the left bank, illustrating a long riffle leading to the chute (bottom left) and a large pool that is visible on the right. Photo: T. A. Bellingan, 10 October 2008.





Plate 42: Downstream view of the upper Bobbejaans River, taken from the left bank. The boulder with the butterfly shape on it, referred to in text, can be seen to the left of the SANParks ranger, Albert Maarman. Photo: T. A. Bellingan, 10 October 2008.

#### **Lower Groot (West) River (33°57'50.50"S; 23°33'30.70"E)**

*Introductory notes:* This site lies only 14 meters above sea level and approximately 2.7 kilometers from the river mouth, making it the lowest site sampled as well as the closest to its mouth and the ocean. Access to the sampling site is via a private road to the Natures Valley pump and water purification station. The area sampled lies approximately 400 meters upstream of the pump house. The river should be crossed and the right bank used to reach the site, as the left bank is densely vegetated and steep in places.

*River channel:* The river channel is typical of the lower reaches of a river. The river bed is wide (up to 18 meters in places) with large slow-flowing pools separated by shallow riffles over cobbles, stones, sand and mud in places (Plate 43). The pools generally contain large amounts of decaying leaf packs, collected on the river bottom, and can be in excess of 2 meters deep. The site that was sampled was approximately 50 meters in length.



*Riparian vegetation:* The site lies within natural forest that would appear to be particularly old, given the size of the Yellowwood trees growing on the river banks. The forest canopy extends over the river and, due to with the height of the trees, casts a large shadow early, and late, in the day; however, due to the large width of the river, a high quantity of light still penetrates to the riverbed at midday. The immediate banks are covered in grass that overhangs into the water on the edges of the pools (Plates 44 & 45).



Plate 43: An upstream view of the lower Groot (West) River showing the large pools and shallow riffles found at this site. Photo: T. A. Bellingan, 7 October 2008.





Plate 44: Lower Groot (West) River: Upstream view from the right bank, showing a researcher collecting by hand from the 'stones-in-current' biotope. Photo: T. A. Bellingan, 7 October 2008.



Plate 45: Downstream view of the lower Groot (West) River. Volunteers are shown next to a large slow-flowing pool lined with marginal vegetation. Photo: T. A. Bellingan, 7 October 2008.

**Upper Salt River (33°55'36.20"S; 23°29'23.30"E)**

*Introductory notes:* The site along the upper Salt River was chosen as per the terms of reference for this study, viz. in order to match previous studies in the region as closely as possible. Access to the site is via the Kurland Estate, from the N2 freeway. The altitude at this site is 265 meters above sea level.

*River channel:* The river channel at this site, as with many other sites, has been highly modified by the flooding event of November 2007, making it nearly unrecognizable from the descriptions of previous studies. The area sampled stretched for approximately 85 meters above an impounded area resulting from the weir, from where an inter-basin transfer furrow arises. The bottom substrate at this site is comprised of a combination of bedrock, a few large boulders, cobbles, and gravel between the stones. During the sampling period, the water was clear and the larger stones were covered with a moss, *Fissidens plumosus* Hornsch (Fissidentaceae), and two liverwort species, *Riccardia* sp. (Aneauraceae) and *Frullania* sp. (Jubulaceae) (Plate 46). The river channel is approximately 4 meters wide and, on average, not more than 40 centimeters deep.

*Riparian vegetation:* Vegetation along the banks of the upper Salt River consists of thick woody bush along the edges with a few ferns making their way to the waters' edge. Further upstream, indigenous forest shades out and replaces much of this vegetation. Small tussocks of Palmiet can be found growing within the river course, although this is not as common as that found at some other sites (Plate 47). Higher up the banks, exotic species - *Eucalyptus spp*, on the left bank, and Pines trees on the right - overshadow the majority of indigenous species (Plate 48).





Plate 46: Detail of the slow-flowing riffle shown in Plate 48, at the upper Salt River; the diverse bottom substrate can be seen, along with liverworts and mosses growing on the larger stones in the bottom right corner of the plate. Photo: T. Bellingan, 24 January 2009.



Plate 47: Upstream view from the sampling area: Upper Salt River. The dense woody marginal vegetation is shown as well as a naturally-formed pool. Photo: T. A. Bellingan, 24 January 2009.





Plate 48: A downstream view of the upper Salt River showing a slow-flowing riffle. The large *Eucalyptus spp* trees that have invaded the left bank are visible in the background. Photo: T. A. Bellingan; 24<sup>th</sup> January 2009.

#### **Lower Salt River (33°58'28.30"S; 23°31'19.70"E)**

*Introductory notes:* This site lies in the de Vasselot section of the Tsitsikamma National Park and is reached via a hiking trail off the R102 between Natures Valley and Kurland. For convenience sake, a large portion through the Fynbos plateau can be crossed by vehicle if a key for the chain “gate” can be obtained from SANParks at the de Vasselot rest camp station. The road, however, only goes to the edge of the valley and the river below must still be reached by foot. The site corresponds to the lower site used in previous studies on the Salt River. The site lies at an elevation of 47 meters above sea level, and is typical of a lower, depositional, stage of a river.

*River channel:* Along the section of river sampled (approximately 65 meters), the riverbed is roughly 15 meters at its widest point. At base flow, however, the stream channel only covers approximately half, or less, of this width in the form of slow-flowing pools separated

by wide, shallow, riffles. The bottom substrate in the pools consists primarily of sand covered with leaf litter and other detritus while the riffles wind between large stones and over cobbles, pebbles and gravel (Plate 49). Towards the edges of the streambed, the substrate turns to fine sand with large amounts of leaf litter and wood debris. The streambed at this site has also been considerably modified by the floods of November 2007.



Plate 49: An upstream view of a shallow riffle within the sampling area along the lower Salt River. The dense forest that lines the river along its lower reaches is evident, casting a significant amount of shade over the riverbed. Photo: T. Bellingan, 24 January 2009.

*Riparian vegetation:* As mentioned previously, this site lies within the boundaries of the National Park, and thus, within pristine natural forest. The river is lined with tall mature forest trees on both banks that cast a large amount of shade over the stream bed, because of this sunlight only reaches the river bed for a few hours either side of midday (Plate 50).





Plate 50: A downstream view of the lower Salt River. A large pool forms beyond the farthest riffle shown. The sandy substrate of the pools is also illustrated. Photo: T. Bellingan, 24 January 2009.

**Upper Buffels River (33°58'56.80"S; 23°28'43.90"E)**

*Introductory notes:* The site on the upper Buffels River corresponds to the upper reaches of the river in that it is near to the source of the river even though it lies at the relatively low altitude of 61 meters above sea level. The Buffels River, along with its tributary the Matjies River, does not originate from the Tsitsikamma Mountains as with the other rivers in this study. The upper reaches lie a few kilometers from the foothills of the Tsitsikamma Mountains, on the inland end of the piedmont plain that separates the mountains from the Indian Ocean. The site is reached via the private property of Brenda Bergé, owner of Bracken Fern farm.

*River channel:* The riverbed at the sampling sites is roughly 4 meters wide, while the stream channel can be reduced to less than a meter wide, depending on flow conditions (Plate 51). Several pools are found downstream of the sampling area, formed by bedrock outcrops.



One of these contains a pump for water extraction. Small chutes, formed between these pools are usually lined with Moss. A stretch of approximately 35 meters of river was sampled at this site. The geology of the Buffels River is very different to that of the other rivers in this study; the riverbed is composed of soft, dark-coloured, shale as opposed to the hard, whitish, table mountain sandstone/quartzite that is found in every other river surveyed. This gives the water a muddy, dirty, appearance when disturbed (Plate 52).

*Riparian vegetation:* Grasses and Palmiet grow along the banks of the river forming a thick green carpet of vegetation covering the clay-like soil up to a few meters from the water's edge. From there on, indigenous forest lines the rivers and extends up to the tops of the valleys. This provides a reasonably thick canopy; however, enough light penetrates to allow understory marginal vegetation to flourish (Plate 51).



Plate 51: An upstream view of the sampling site along the upper Buffels River. Flow rate at this time was particularly low, as the photo illustrates; the river was reduced to a trickle along the riffles. Photo: T. Bellingan, 10 March 2008.





Plate 52: A Downstream view of the upper Buffels River showing the area below the sampling site. The brown water colour is illustrated as well as pools formed as a result of bedrock slabs. Many Eastern Cape Redfin Minnows (*Pseudobarbus afer*) could be found here. Photo: T. Bellingan, 10 March 2008.

#### **Upper Matjies River (33°58'50.60"S; 23°27'28.10"E)**

*Introductory notes:* The sampling site on the upper Matjies River is situated at an elevation of 51 meters above sea level, i.e. ten meters lower than the sampling site on the upper Buffels River. Access to the Matjies River site was via the property of Mr. Jürgen Schmidt, down a logging path (sleep pad) that follows on from the driveway of his homestead. Caution must be advised when using this path as the combination of a steep gradient and leaf litter makes conditions under foot very slippery, especially when wet.

*River channel:* The river channel at this site is very narrow, approximately 2 meters at its widest, with the small chutes between and over bedrock narrowing to as little as 30 centimeters at base flow. For most of the sampling period, not more than a trickle was present (Plate 53). Sampling took place between a series of small pools and shallow riffles, roughly 30 meters downstream of the confluence of another tributary of the Matjies. The



water is clear, unless the bottom is disturbed, in which case clouds of fine sediment are produced. The bottom substrate consists of bedrock, large stones, pebbles and mud (Plate 54). The bottoms of the pools are layered with leaf debris and a fine, brown alga was present on all available surfaces. The sampling site was approximately 50 meters in length.

*Riparian vegetation:* As mentioned before, the stream is heavily shaded by natural forest that grows down to the banks, excluding nearly all other understory growth. Where marginal vegetation was found, it was sparse due to the heavily-shaded nature of the stream; some shrubbery was found growing along the upper banks, but this was not nearly as dense as the Palmiet found at some other rivers in this survey (Plate 53).



Plate 53: An upstream view from the middle of the sampling area along the upper Matjies River. Water flow at the time of the photograph was low, as was customary for this site. The sparsely-vegetated banks are also illustrated. Photo: T. Bellingan, 10 April 2008.





Plate 54: A downstream view of the upper Matjies River sampling site; the clear water in pools formed by bedrock intrusions can be seen. Some volunteer collectors are included in the foreground. Photo: T. Bellingan, 10 April 2008.

#### **Lower Buffels River (33°59'7.90"S; 23°27'48.70"E)**

*Introductory notes:* The area sampled along the lower Buffels River lies approximately 32 meters above sea level and nearly 3.2 kilometers from the river mouth. It is the second of two confluence sites. This site is particularly difficult to reach: to hike directly to the site, it is important to locate the start of the old “sleep pad”, or logging path, after which is easy to follow. Alternatively, the site can be accessed via the Upper Matjies site, which may be simpler, but more treacherous, as hiking downstream along the river has resulted in a few injuries from falling during the slippery conditions.

*River channel:* The river channel is approximately 7 meters wide with the majority of the stream bed surface area being covered by slow-moving or still water. The width varies in places where bedrock breaks the surface of the stream (Plate 55). The sampled area is below a large shallow pool that stretches at least 10 meters upstream. Immediately



downstream of the pool, a rocky outcrop forms a riffle, of roughly 4 meters in length, that is littered with large stones and cobbles, making for an excellent “in current” biotope. This is followed by another pool, which is in turn followed by a second riffle (Plate 56). The total length of the sampling site is roughly 85 meters. The margins of the river channel, for most of their length along the sampling site, are composed of clay-like soil held together by the marginal vegetation, rather than rock-like margins as seen at most other sites. The water colour was clear except if the stream bed was disturbed, resulting in clouds of murky sediment, or during times of flooding (Plate 57).

*Riparian vegetation:* The entire length of the sampling site is lined with indigenous forest that casts a large amount of shade over the site. However, due to the breadth of the stream, enough light still penetrates to allow for good cover of the banks by shrubs and grasses (Plate 55). No alien plant species were observed at this site, or any of the other sites sampled along the Buffels river system.



Plate 55: Upstream view from the upper end of the lower Buffels River sampling site showing a large pool that precedes the riffle where the majority of the in-current sampling took place. Photo: T. A. Bellingan, 26 January 2009.





Plate56: Downstream view of the lower Buffels River with a riffle in the foreground, followed by a pool and a second riffle. Photo: T. A. Bellingan, 10 April 2008.



Plate 57: Downstream view of the lower Buffels River sampling site after heavy rainfall that resulted in the river rising nearly half a meter from base flow. Photo: T. A. Bellingan, 9 October 2008.