Assessing the sensitivity of aquatic macroinvertebrates to acid deposition in South African headwater streams

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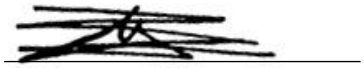
Declaration

I declare that this thesis, submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, is my own, unaided work. This work has not been submitted before for any degree or examination at any other University.



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10 October 2019.

Abstract

GIS was used to identify three study regions in south Africa susceptible to acidification. These regions namely, Mpumalanga (HV), Waterberg (WB) and the south-western Cape (SWC) were identified and categorised to represent high, medium and low acid deposition loads respectively based on the distribution of coalfired power stations and acid sensitive soils and waters. A total of 80 headwater streams representing 84 sampling sites were identified, 21 in Mpumalanga, 33 from the Waterberg and 30 for the south-western Cape and measured for water chemistry. The project tried to identify sites that had no direct human influences on the water quality (i.e. mining and intensive agriculture) within their catchments in order to focus the study on impacts related entirely to atmospheric deposition. Macroinvertebrate samples were collected from a subset (n = 56) of these sites due to habitat suitability, and species response to changes in environmental variables were investigated. Geographically the three study regions differed significantly from each other and this difference was most apparent with the difference in altitude, biomes, ecoregions and species composition.

When compared to the other study regions the Mpumalanga region had very high pH (Mean = 7.24, Minimum = 5.32, Maximum = 9.52) and electrical conductivity (Mean = 180, Minimum = 14, Maximum = 1827) that appeared to be mostly anthropogenically impacted, which meant that a large portion of the streams from this region were unsuitable for the purposes of this study. Furthermore, the region has been subjected to a high degree of environmental degradation attributed to coal mining, agricultural activity and general industrialisation that has led to abstraction from and deterioration of many aquatic ecosystems. During the course of this study the Waterberg experienced a major drought which appears to have influenced species assemblages. The naturally acidic streams of the south-western Cape as well as the overall heterogeneity of the three study regions made comparing species presence/absence across regions unfavourable. Thus, developing a national South African scoring system for acidification became equally implausible. Furthermore, in-depth analysis of species response to seasonal drought from the Waterberg and alkalinity of streams in the Mpumalanga region may have been

feasible had other taxonomic Orders been included. However, for the purposes of this study taxonomic groups primarily associated with acidification were chosen and thus further investigations were only limited to these groups. Nonetheless several mayfly species were found to show acid intolerance or be moderately intolerant.

I have strength for all things in Christ Who empowers me [I am ready for anything and equal to anything through Him Who [a] infuses inner strength into me; I am [b] self-sufficient in Christ's sufficiency].

Philippians 4 vs 13 (AMPC)

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- Norwegian Institute for Water Research (NIVA)
- Czech Geological Survey (CGS)

Overall project

Last but not least, God for always bringing me back to my resting place at His feet.

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Chapter 1 Introduction

While European countries (Hesthagen *et al.*, 2011; Rogora *et al.*, 2013; Lien *et al.*, 1996; Aanes & Bækken, 1993) and North America (Garmo *et al.*, 2014; Skjelkvåle *et al.*, 2005; Brit Lisa Skjelkvåle *et al.*, 2001) are recovering from the acidification effects of the 19th century's coal mining industry, South Africa is only just starting to ask the crucial questions pertaining to coal mining and the potential threat it poses on South Africa's soils and waters, both of which are considered two of the country's most precious commodities instrumental to economic growth and sustainable development (BFAP, 2012; DWA, 2013).

Coal is considered one of the world's most plentiful energy resources and according to BP global the earth currently has enough coal reserves to meet 153 years of global production (BP Global, 2017). The cost-effective nature of coal resources makes them the first point of call in energy production for both first and third world countries and South Africa is no exception. Coal mining has been in existence for more than 150 years in South Africa and currently, more than 77 % of the country's energy demands are met by coal resources (DoE, 2017). In 2006, South Africa was listed as one of the six countries that produced 81.9 % of the total coal extracted in the world and together these countries, namely Australia, China, India, USA, Russia and South Africa account for an estimated 90 % of the total coal reserves in the world (Bian *et al.*, 2010). Eskom which is ranked first in the world as a steam coal user and seventh as an electricity generator, generates about 95 % of the electricity used in South Africa and approximately 45 % of the electricity used in Africa, further affirming the economic value of coal reserves for the country (Department of Energy, 2016). The South African coal reserves are made up of anthracite and bituminous coal (BP, 2017; World Energy Resources, 2013) which are the more mature coal types commonly known as black coal and according to Hancox and Gotz (2014), bituminous is the primary form of coal that is currently being mined in South Africa. Bituminous coal is the same type of coal that dominates the American mining industry and has been associated with the many environmental challenges associated with acid deposition. However, there is evidence to suggest that the coal mined in South Africa has

low sulphur content when compared to that from other countries (Wagner & Hlatshwayo, 2005; Hancox & Gotz, 2014). The differences in the geological period of coal from the Southern hemisphere and that of the Northern hemisphere are said to account for the low concentrations of sulphides, halogens and trace elements reported for the South African coal which was formed during the Permian geological period when compared to the Carboniferous coal of the Northern hemisphere (Wagner & Hlatshwayo, 2005; Hancox & Gotz, 2014). However, a study conducted by Pinetown *et al.* (2007) which investigated the quantitative distribution of minerals in coal from the Witbank and Highveld Coalfields, indicated that minerals in the coal from the Witbank coalfields were dominated by quartz and kaolinite suggesting that they are probably susceptible to acidification (Pinetown *et al.*, 2007). Terrains that are vulnerable to acidification are characterised by bedrock and soils that have low concentrations of weathered minerals (Bohan *et al.*, 1998; White *et al.*, 1999). Both these types of soils and bedrock are present in South Africa (van Tienhoven *et al.*, 1995; Josipovic *et al.*, 2011; Bird, 2011), however due to the paucity of relevant data, the geographic extent and severity of the country's risk to acidification by deposition is not well known.

1.1 Processes that lead to the acidification of freshwater systems

1.1.1 Chemical weathering

Chemical processes taking place in the soils are at the heart of acidification processes driven by anthropogenic activity (Reuss *et al.*, 1987). Soils consist of four main components namely, organic matter, water, air and minerals (sand, silt and clay), (Behan, 1992). The soil texture is determined by the ratio of clay to silt and sand, where sandy soils are characterised by a low clay content and thus are more granular. Soils with a high buffering ability against strong acids are characterised by a high cation exchange capacity (CEC), that is – the ability to store base cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}) sodium (Na^{+}) and potassium (K^{+}). These minerals accumulate in the soil by binding to clay particles primarily and organic matter. Furthermore, soil pore spaces that occupy 30 % - 60 % of the soil are important for storing air and water that is necessary for plant growth and maintaining the water cycle by replenishing streams and recharging aquifers

(Behan, 1992; Marx *et al.*, 1999). Soils with a low buffering capacity are as previously stated vulnerable to acidification. During soil acidification, the acid neutralising capacity of the soil decreases as base cations are depleted by acid deposition associated with SO_4^{2-} and NO_3^- leading to an increase in hydrogen (H^+) ions. This leads to the mobilization of inorganic aluminium (Al^{3+}) and ferric iron (Fe^{3+}) which are toxic to aquatic organisms (Siemion *et al.*, 2014; Bird, 2011; Lu *et al.*, 2014).

Chemical weathering is an important step in soil formation that has gained much attention in environmental sciences because of its ability to impact water quality, stream acidification and nutrient cycling (Middelburg *et al.*, 1988; White & Brantley, 1995). The use of the word weathering implies that the mechanism is dependent on processes associated with the hydrosphere, atmosphere and biosphere (White & Brantley, 1995). Furthermore, chemical weathering is considered to be an acid-base reaction because it involves the neutralisation of acids by solid bases to produce secondary minerals and salts (Roila *et al.*, 1994; Middelburg *et al.*, 1988). The reaction is irreversible and occurs under supply-demand conditions between rocks and their physico-chemical environment. Factors that control the rate and nature of chemical weathering include the type of parent-rock material, topography, climate and biological activity (Middelburg *et al.*, 1988).

Geological formations consist of rock strata with minerals of varying chemical composition (Chigira & Oyama, 2000; Dallas & Day, 2004). The rock strata and the soils formed from it contribute different quantities of these minerals to the waters flowing over or seeping through them (Dallas & Day, 2004). Examples of the most common soils known to have significant impact on stream chemistry based on their chemical composition include calcic soils, which have a high buffering ability against strong acids, organic soils which are known to have low cation exchange capacity as well as lithic soils that have high base content and podzolic soils which are acidic soils that have a pH range of 3.5 – 5 (Fey, 2010b). Rocks such as limestone are rich in calcium carbonate, whereas quartz and kaolinite have slow to intermediate weathering rates respectively (Ulrich, 1986; Kernan, 2010). Acid sensitive waters are generally characterised by slow weathering bedrock such as granite and quartzite as well as young and often poorly buffered podzolic and

organic-rich soils (Kernan, 2010; Fey, 2010b; Ulrich, 1986; Bartram & Ballance, 1996; Hodson & Langan, 1999). These slow weathering rocks are generally not well buffered; consequently, they have a low acid neutralising capacity (ANC) against strong acids that come from the atmosphere in the form of acid deposition. In addition to the above, regions with naturally acidic waters are also vulnerable to acidification because they have low concentration of weathered minerals (Clair *et al.*, 2007; Hogsden & Harding, 2012; Holland *et al.*, 2014; Dallas & Day, 2004). Furthermore, saline waters are said to have a low buffering capacity against strong acids due to their lack of bicarbonate ions (Dallas & Day, 2004). Huizenga (2011) has identified three factors that control surface water chemistry in South Africa, and these include chemical weathering, sulphate contamination, and soil salinisation.

1.1.2 Acid deposition

The burning of fossil fuels leads to an increase in the atmospheric content of greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4) as well as mercury (Hg), sulphur (S) and nitrogen (N) oxides, resulting in atmospheric pollution (Dabrowski *et al.*, 2008; Likens & Bormann, 1974; Rodhe *et al.*, 1981). These gases accumulate in the atmosphere where they react with free radicals to form strong acids which are returned onto the earth's surface as dry and or wet acid deposition, commonly known as acid rain, which often has detrimental effects on the aquatic ecosystem, **Figure 1-1**. Sources of sulphur and nitrogen compounds are diverse, however the effects of stream acidification have been largely attributed to the burning of fossil fuels from coalfired power stations (Akimoto *et al.*, 2006; Baker *et al.*, 1991).

Prior to the deposition of acidic compounds, free radicals (i.e. hydroxyl (OH)) are formed in the atmosphere when ozone (O_3) reacts with water. OH for example may further react with carbon monoxide (CO) to form hydroperoxyl (HO_2) as shown in equation (1) or self-react to form hydrogen peroxide (H_2O_2) (Stockwell *et al.*, 1990).

$OH + CO + M \rightarrow HO_2 + CO_2 + M$ (1)

M; represents non-reactive species such as N_2 or O_2

The free radicals can then react with sulphur dioxide (SO_2) in a cascade of reactions indicated below (equation (2) – (4)) to form sulphuric acid (H_2SO_4) (Stockwell *et al.*, 1990).

$SO_2 + OH + M \rightarrow HOSO_2 + M$ (2)

$HOSO_2 + O_2 \rightarrow SO_3 + HO_2$ (3)

$SO_3 + H_2O \rightarrow H_2SO_4$ (4)

In addition to the above O_3 can also react with NO to form NO_2 as shown in equation (5). The NO_2 than undergoes a series of reactions as demonstrated below leading to the formation of nitric acid HNO_3 (Stockwell *et al.*, 1990).

$NO + O_3 \rightarrow NO_2 + O_2$ (5)

$HO + NO_2 + M \rightarrow HNO_3 + M$ (6)

$NO_2 + O_3 \rightarrow NO_3 + O_2$ (7)

$NO_3 + HCOH \rightarrow COH + HNO_3$ (8)

$NO_3 + NO_2 \rightarrow N_2O_5$ (9)

$N_2O_5 + H_2O \rightarrow 2HNO_3$ (10)

A variety of factors contribute to the type, rate and extent of pollutant formation and they are dependent on the rate of chemical conversion taking place in the atmosphere leading to deposition. For instance, inputs from the atmosphere are characterized by the presence and variation of chemical species as well as their transformation in the form of aerosols or gases that is governed by climatic or meteorological conditions. Furthermore, the macro- and micro-climatic conditions are influenced by the difference in weather conditions, including variations of daily, monthly, and annual temperatures, precipitation distribution, as well as long- and short-range regional air mass transport (Akimoto *et al.*, 2006).

Thus, acidification occurs when poorly buffered soils and waters have been exposed to excessive amounts of acid deposition. When acid rain comes into contact with the soil it leaches away the soil's nutrients and base cations causing the soil to lose its buffering capacity against strong acids. Consequently, acid sensitive waters have low concentrations of bicarbonate, calcium and magnesium ions (Davies & Day, 1998; Desonie, 2007; Kernan, 2010) and a high concentration of SO_4^{2-} and inorganic aluminium which is very toxic to the aquatic ecosystem (Kernan, 2010). High concentrations of dissolved organic carbon (DOC) however are able to complex with toxic aluminium reducing its bioavailability irrespective of pH concentration levels (Schartau *et al.*, 2008a; Laudon *et al.*, 2005; Witters *et al.*, 1990). Low [pH] together with low [DOC] for example can mobilise toxic aluminium. However, low pH and high DOC concentrations on the other hand tend to reduce the toxic effect of aluminium on aquatic fauna, suggesting that DOC concentrations play a crucial role in protecting aquatic invertebrates against toxic aluminum.

Coal mining in South Africa has also been associated with acid mine drainage (Darwall *et al.*, 2009) however this subject will not be covered in the present study as it falls outside the scope of this project. Furthermore, one very important aspect of this project was to try and eliminate confounding factors such as acid mine drainage through targeted selection of headwater streams without catchment industries, so as to try and focus the PhD solely on acid deposition.

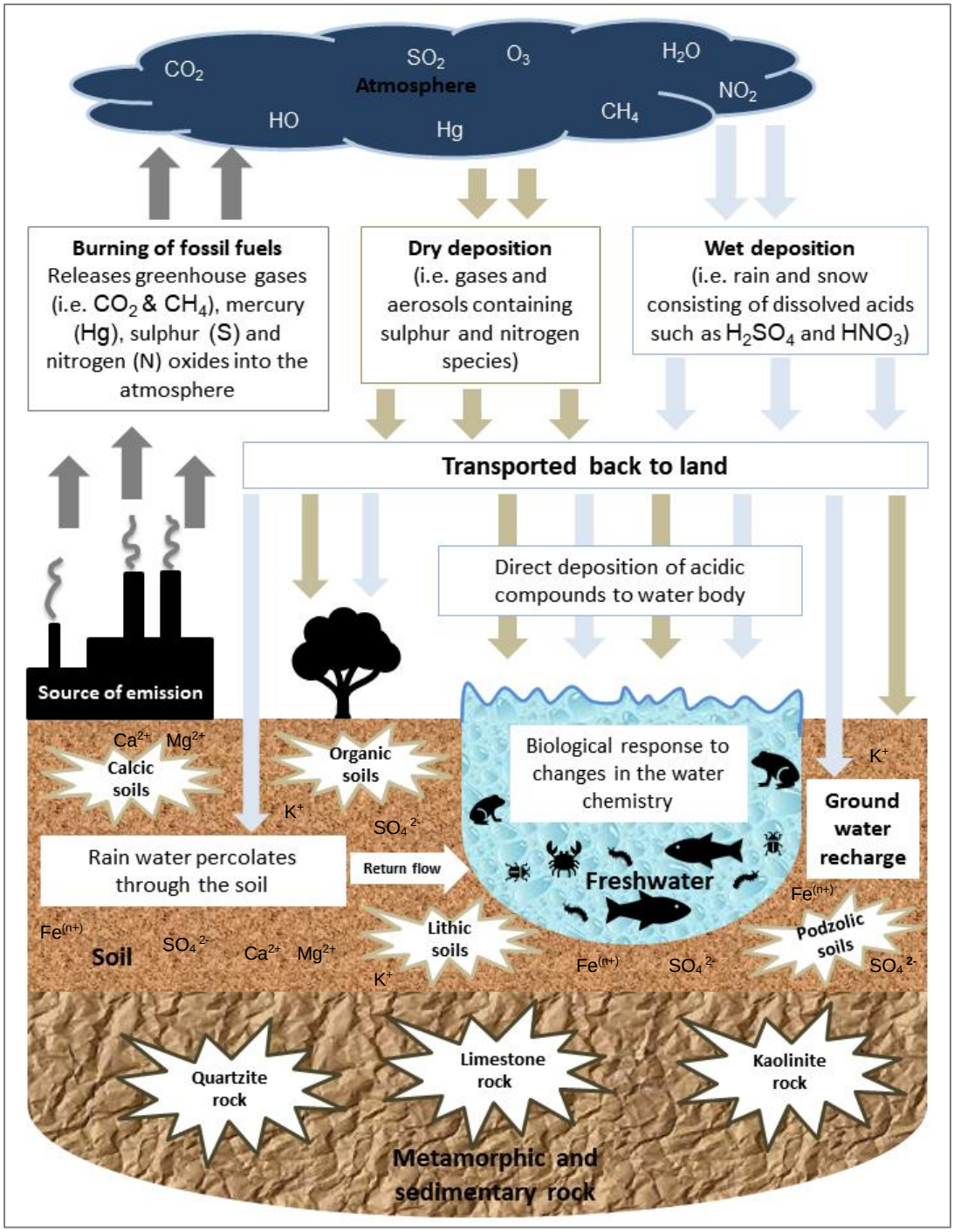


Figure 1-1: Schematic representation of processes that lead to stream acidification and the loss of aquatic fauna. Calcic soils - high buffering ability, organic soils - low cation exchange capacity, lithic soils - high base content and podzolic soils - acidic with pH range 3.5 – 5 (Fey, 2010b). Limestone - rich in calcium carbonate and fast weathering, quartz and Kaolinite are slow weathering to intermediate respectively (Ulrich, 1986; Kernan, 2010). The soil and bedrock weathering rates determine how quickly depleted or leached minerals are replenished, this directly affects base saturation (BS) and cation exchange capacity (CEC) and thus the acidic state of the soils and waters of a catchment.

1.2 Evidence of acid deposition in South Africa

The earliest recorded deposition data for South Africa is from 1985 for the then eastern Transvaal Highveld now known as the Mpumalanga Highveld (Bohm, 1985). Substantial amount of research has been conducted since then try and determine the effects of emissions from coalfired power stations on air quality (Pretorius *et al.*, 2015; Piketh *et al.*, 2002; Pinetown *et al.*, 2007; Freiman & Piketh, 2003; van Tienhoven *et al.*, 1995; Josipovic *et al.*, 2010; Josipovic *et al.*, 2011). The majority of these studies however have focused on the Highveld region (Mpumalanga) of South Africa where approximately 80 % of the countries coalfired power stations are located (Freiman & Piketh, 2003). Furthermore, a study by Freiman and Piketh (2003) has alluded to the transportation of pollutants from the highveld region to other parts of Southern Africa, and similar observations were also made by Bohm (1985). Studies that have surveyed for acid deposition along the coastal regions of the Western Cape Province have concluded that sulphur compounds detected in the rainwater samples were not a sign of acid deposition in the region (King, 1988). Apart from these few reports very limited deposition related research has been conducted in other parts of South Africa.

A study by Josipovic *et al.* (2011) that assessed the critical loads (deposition estimates) of acidic deposition exceedance in South Africa produced total wet and dry acid deposition rates for the Highveld plateau that exceed 100 meq/m^2/yr. Notably, these deposition values are comparable with those said to have caused soil and water acidification in European countries (Posch *et al.*, 2011). Furthermore, these deposition rates have also been associated with major fish kills and the loss of aquatic insects in Europe and North America (Henriksen & Posch, 2001; Battarbee *et al.*, 2014; Mills & Schindler, 1986; Schindler, 1988; Dillon *et al.*, 1984; Siemion *et al.*, 2014), suggesting The findings of Josipovic *et al.* (2011) suggest that deposition rates at the Highveld plateau could exceed the critical load for acidification of acid-sensitive streams and rivers. In addition to the above a few studies in South Africa have assessed how acidic compounds from the atmosphere affect water chemistry (Bird, 2011; Bird & Scholes,

2012), however, there have been no studies that have established how these acidic compounds affect aquatic macroinvertebrates.

Baird (1995) describes acid deposition as a pollution problem that has no respect for state or national boundaries because of its ability to have subsequent effects far downwind from the original source of emissions. The movement of pollutants across state boundaries is termed Long Range Transboundary Air Pollution (LRTAP) (UNECE, 1985; Wettestad, 2002) and what has been deemed the primary cause of acidification in countries such as Norway, Sweden, the Netherlands and the United States. Evidence of LRTAP from the Southern Hemisphere has also been reported by Freiman and Piketh (2003) where the authors illustrated that air from the Highveld region can be transported through several pathways towards the Atlantic Ocean, the South Indian Ocean, the Indian Ocean as well as the equatorial region. The transportation of pollutant across the country and parts of Southern Africa using these pathways suggests that regions with no direct mining activity such as the south-western Cape (to be reviewed in chapter three of the thesis) which is known for its naturally acidic black and white streams are indirectly vulnerable to acidification (Midgley & Schafer, 1992; King, 1988; Dallas & Day, 2004). Furthermore, findings by Freiman and Piketh (2003) indicate the importance of expanding acid deposition related research beyond the Mpumalanga Highveld which has been the primary area of interest for most studies because of the mining activity in the region and the proximity of coalfired power stations to this region.

All things considered acid deposition is a term that has recently received much attention in South Africa over the past 10 years, however its damaging effects on the environment are still very putative (Josipovic *et al.*, 2011; Bird & Scholes, 2012). The Department of Environmental Affairs (DEA) has recognised soil acidification linked to coal mining activity as a major soil degradation problem affecting low income cropping areas in South Africa (DEA, 2015; DEA, 2012; Department of Environmental Affairs (DEA), 2007). The lack of an up to date national data set however, makes it difficult to quantify the level of deterioration that has taken place over the years (DEA, 2006). A report from the Bureau for Food and Agricultural Policy (BFAP) indicated that an estimated 26 % of the high potential arable soils from the Mpumalanga region, which accounts for 46.4 % of the

country's high potential arable soils, has been lost to mining (BFAP, 2012). Furthermore, Bird and Scholes (2012) have observed a significant decrease in the acid neutralising capacity of the soils in this region, suggesting possible acid loading of the soils, or possibly that the acids are being deposited at a much faster rate than the soil is able to buffer them. The findings of Bird and Scholes (2012) echo those of van Tienhoven *et al.* (1995), who indicated that some parts of Mpumalanga have acid sensitive soils and potentially waters, which place them at risk of acidification. The authors went on to suggest that at the time of their study, acidification associated with dry deposition was of greater concern to the country than that from wet deposition (van Tienhoven *et al.*, 1995). Prior to this Bosman and Kempster (1985) conducted a study at the Roodeplaat Dam catchment area where they recorded average rainfall with pH ≤ 4.4 at 9 sampling stations. The authors however highlighted a high acid neutralising capacity in the soils from this region (Bosman & Kempster, 1985). Websites such as the "environment" https://www.environment.co.za/environmental-issues/acid-rain.html (Enviro Editor, 2015; Enviro Editor, 2010), which serve to inform the public on pressing environmental issues as well as "BIZCOMMUNITY" http://www.bizcommunity.com/Article/196/358/91132.html (Agriculture News, 2013), a Marketing and media entity and health24 http://www.health24.com/Lifestyle/Environmental-health/Faqs/Acid-rain-20130312 (Health24, 2011), which is a local online news publisher, have published short articles to raise awareness on the issue of acidification. The growing concerns from local farmers, the community as well as the government, about the lack of up to date information concerning this subject have brought about an urgent need to develop assessment tools to establish and monitor the effects of acid deposition on the environment.

1.3 Acid sensitive soils in South Africa

South African soils have recently been classified into 14 soil types that can either be identified as topsoil or subsurface soils, **Figure 1-2** (Fey, 2010b). The soil classification system applied by Fey (2010) correlates with the one defined by the World Reference Base (WRB) for international classification where soil types are diagnosed based on either the soils morphology or diagnostic horizons (Food and Agriculture Organization of the United Nations., 2006). In this case Fey (2010) used diagnostic horizons as the guiding principle. Of the fourteen soils types three namely, Humic soils, Podzolic soils and Plinthic soils were identified as being acid sensitive soils based on the soil chemistry which was classified as either having low base content, low pH or low cation exchange capacity **Figure 1-2**.

A separate study by Josipovic *et al.* (2011) also made an attempt to classify South African Soils. The main focus for Josipovic *et al.* (2011) however was identifying acid sensitive soils and mapping out their distribution across South Africa. To achieve this the authors categorised all known soil types to represent 5 sensitivity classes (Josipovic *et al.*, 2011). These soils were classified using the soils buffering capacity, where soils with a low buffering ability were characterised by low CEC and BS and allocated soil sensitivity class 1, while soils with the highest buffering ability were placed in class 5.

In this PhD study acid sensitive soils as defined by Josipovic *et al.* (2011) and Fey (2010) were overlaid with each other to produce one soils map, **Figure 1-3**. The purpose of the overlay was to get an overview of the distribution of acid sensitive soils across South Africa. The final map product indicated that the majority of the acid sensitive soils are distributed across the eastern part of South Africa, which has also been associated with high deposition rates as indicated in the previous section (Josipovic *et al.*, 2011).

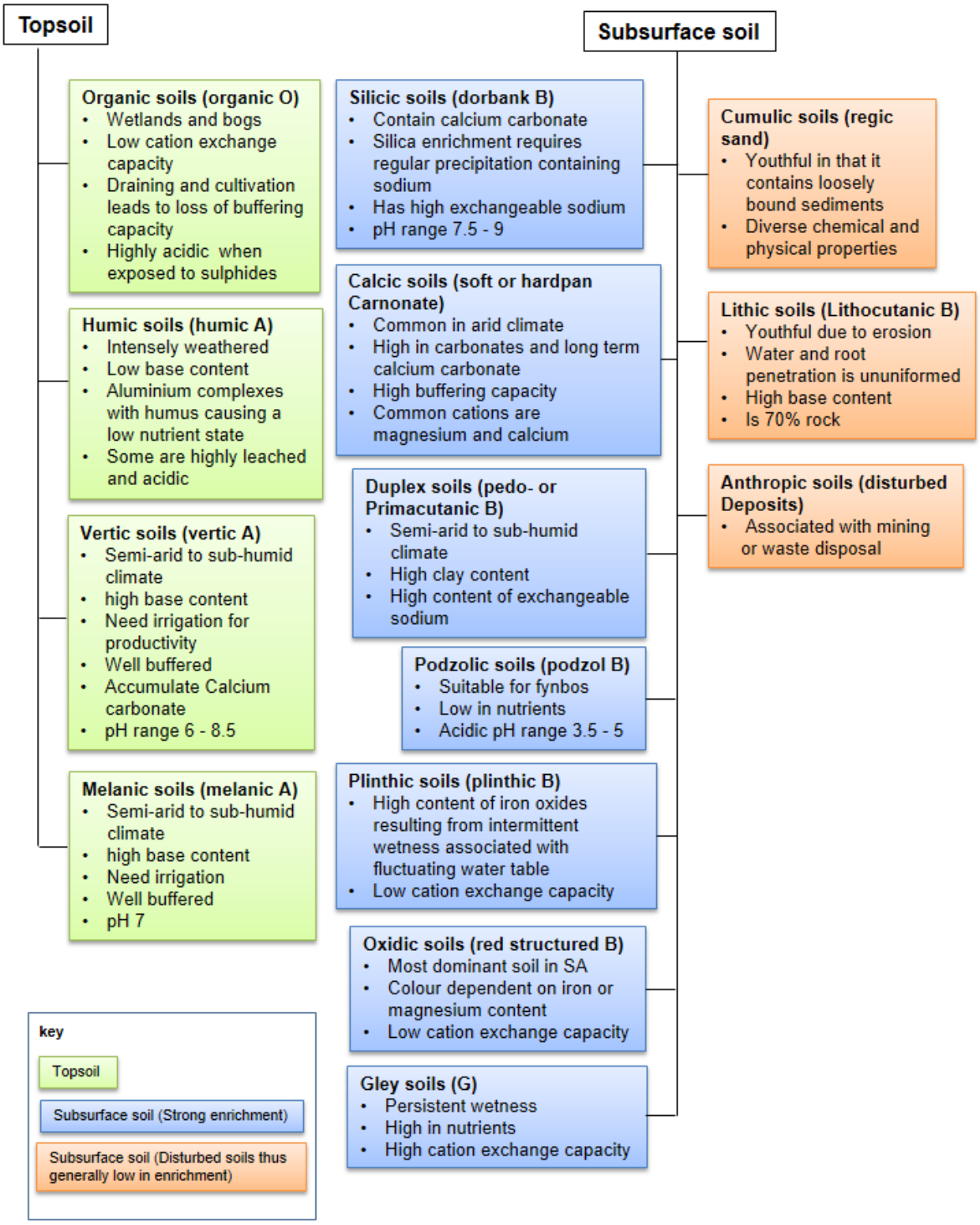


Figure 1-2: Summary of the Fey (2010) soil classification system. http://www.fao.org/nr/land/soils/soil/wrb-soil-maps/classification-key/en/. The figure above represents soils and soil properties which were used to classify acidic and non-acid soils of South Africa.

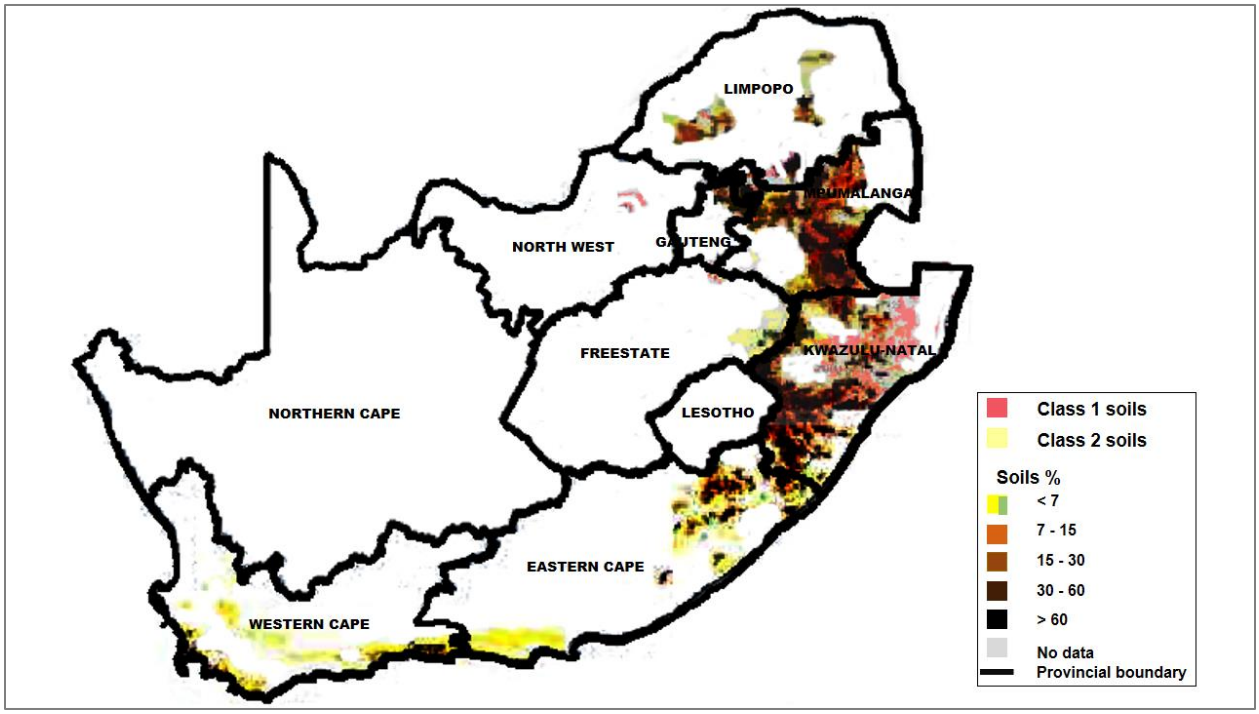


Figure 1-3: Distribution of acid sensitive soils as defined by Fey (2010) and Josipovic *et al.* (2011). The Josipovic *et al.* (2011) soils were expressed as Class 1 and Class 2 soils on the original work while those of Fey (2010) were expressed in percentages. Map not drawn to scale. The purpose of the overlay was to get an overview of the distribution of all previously defined acid sensitive soils across South Africa.

1.4 Naturally acidic waters in South Africa

Naturally acidic streams with pH < 5 have been recorded in the fynbos regions of the Western Cape as well as in the Berg River catchment and Jonkershoek (King, 1988; Britton *et al.*, 1993; Dallas *et al.*, 1995), including various locations along the coastal regions of the Cape Fold Mountains (Silberbauer & King, 1991; Midgley & Schafer, 1992; de Moor & Day, 2013). Furthermore, the Drakensberg escarpment of Mpumalanga (Skoroszewski, 1995), Lake Fundudzi in northern Limpopo (van der Waal, 1997), Lake Benghazi in KwaZulu-Natal (Hart & Appleton, 1997) as well as in the mountains of Lesotho where low pH concentrations have been associated with organic acids from wetland soils (Grobbeiaar & Stegmann, 1987), have been associated with acid sensitive waters that are prone to acidification. A preliminary survey at Verloren Valei Nature Reserve, Mpumalanga also revealed the presence of acid sensitive streams in this part of the Mpumalanga region (Curtis, unpublished data, University of the Witwatersrand).

In addition to the above, several aquatic insects in South Africa have been affiliated with alkaline or acid waterbodies (Harrison & Agnew, 1962; Harrison, 1962) however not many studies have assigned pH tolerance values to these organisms. A study by Oliff (1963) however has associated two mayfly species (*Caenis sp. 3* and *Euthraulus elegans*) and one undescribed chironomid species to slightly acidic and or circumneutral stream conditions of pH 5.2 – 6.8 (Oliff, 1963; Oliff & King, 1964). Many other studies that followed tended to give a general overview of species tolerance (Allanson *et al.*, 1990; de Moor & Day, 2013). The present study however has attempted to define pH tolerance range concentrations for selected species. The aim for establishing such limits was to try and develop an acidification scoring index for South African headwater streams.

1.5 Problem statement and Objectives

In order to further develop and sustain the electrification process in more rural areas of South Africa, the South African Department of Energy together with Eskom (South Africa's main electric utility) have embarked on a project of building two new coal power stations (U.S. Energy Information Administration (eia), 2015; Eskom, 2010). The Medupi power station (4 764 MW) in Limpopo (23°42′00″S 27°33′00″E) and the Kusile (4 800 MW) in Mpumalanga (25°54′59″S 28°55′02″E) were expected to be fully functional by 2019 (Bohlmann *et al.*, 2016) but are still under construction. The addition of new power plants will inevitably increase the current atmospheric emissions of sulphur and nitrogen oxides and the lack of acid deposition and acidification data for South African freshwaters will make it difficult to assess the effects of the two additional power stations on air quality and associated aquatic ecosystems. Thus, there is a need to carry out more extensive research that looks at the physical, chemical and biological state of South Africa's fresh waters relative to acid deposition and acidification, in order to map sensitivity and establish an acidification monitoring system for South African freshwaters. There is currently no locally developed method for assessing acid sensitivity in South Africa. Two studies by van Tienhoven (1995) and Josipovic *et al.* (2011) attempted to do this but the approach applied by both authors was based on European methods. Furthermore, the latter study only looked at acid sensitivity in soils and not in streams as is the case with the present study. As a starting point this PhD study set out to identify regions in South Africa that have acid sensitive soils and waters making them vulnerable to acidification. Findings from European countries and North America suggest a correlation between stream acidification and loss of aquatic fauna. The present study has tried to obtain high-quality water chemistry and biology data to document the current environmental status of acid sensitive streams in three regions of South Africa assumed to represent high, moderate and low deposition loads.

An outline of the NRF project

The current study stems from a broader National Research Foundation (NRF) project that was carried out as a collaboration between the University of the Witwatersrand (WITS), the Norwegian Institute for Water Research (NIVA), the Norwegian Institute for Air Research (NILU) and the University of the North-west in Potchefstroom (NWU). The overall project addresses the theme “Environment” with a specific interest in the effects of air pollution on the aquatic ecosystem as well as predicting future scenarios of climate change and changes in sulphur and nitrogen emissions into the atmosphere. The broad project was broken down into a work plan that was aimed at addressing four major research questions in the form of work packages where two South African institutes, WITS and Potch University, took lead in heading up these projects in collaboration with NIVA and NILU as follows:

Work package one. Sulphur and Nitrogen deposition in three regions of South Africa (lead: NWU. Participants: NILU)

- The aim was to provide good quality data on acid deposition in three regions of South Africa
    - This study was completed, and an article (Mompati *et al*., 2019) has been submitted for publication.
    - Key findings from the study
        - The annual Volume-Weighted Mean (VWM) concentrations of sulphate (SO_4^{2-}) and nitrate (NO_3^-) were highest at sites closest to the coalfired power stations (Mompati *et al*., 2019), article in submission). Deposition fluxes however were highest at Cathedral Peak.

Work package two. **Stream-water chemistry and biology in three acid sensitive regions of South Africa (lead: WITS. Participants: NIVA)**

- **Here the aim was to obtain high-quality water chemistry and biology data to document the current environmental status of acid sensitive streams in three regions of South Africa representing high, moderate and low deposition loads**

Work package three. Calculation and mapping of critical loads and exceedance for the three regions (lead: WITS. Participants: NIVA, NWU and NILU)

- This work package was aimed at determining the critical load of acidity for each of the streams, and the exceedance under present-day deposition
    - The complex differences in the water chemistry between the three study regions did not readily allow the use of the northern hemisphere based critical load models however the work is ongoing.

Work package four. Future threats: air pollution and climate change (lead: NIVA. Participants: NILU, NWU and WITS)

- Here the aim was to assess the threat posed by future climate change and new sources of air pollution to acid sensitive freshwater ecosystems in three regions of South Africa.
    - This particular part of the wider NRF project was not relevant to the work presented in this PhD study however as with work package three, the work is ongoing.

This PhD thesis was specifically aimed at addressing questions under Work package two. However, findings from Work package one, have been referenced in some parts of the thesis.

The key hypothesis

1. Current levels of acid deposition in some parts of South Africa exceed the critical load for acidification of acid-sensitive freshwaters.

2. Regions with acid sensitive soils (Josipovic *et al.*, 2011) may lead to acid sensitive waters across South Africa.

3. Acid sensitive waters with low critical loads may occur in regions with acid sensitive soils

4. Certain groups of indicator species of macroinvertebrates sensitive to acidification may be identified using regional chemical and biological datasets.

5. Evidence of Long Range Transport of Atmospheric pollutants (LRTAP) suggests that regions remote from the industrial source of pollution may still be affected by pollutants through LRTAP depending on the acid state of their soils and waters.

Study Aim

The aim of this PhD study was to collect chemical and biological data from acid sensitive headwater catchments in 3 regions across a deposition gradient to determine the feasibility of identifying national indicator species and the possibility of developing a South African scoring system for acidification.

Objectives

- To use GIS to identify regions of South Africa with potentially acid-sensitive soils and waters and from there to select 3 study regions that represent low, medium and high deposition loads.

- Collect seasonal water chemistry and macroinvertebrate samples from c. 30 streams in each region across a hydrological year

- Conduct taxonomic identification on the macroinvertebrate data to the lowest possible taxonomic level.

- To establish an empirical relationship between species distributions and water chemistry.

- To identify appropriate macroinvertebrate indicator species to develop as a measure of acidity status and to quantify dose-response relationships for use in critical load models and acidification indices.

1.6 Biomonitoring

Following preliminary consultation with local invertebrate specialists a decision was made to focus on four Orders of aquatic insects namely, Ephemeroptera, Diptera, Plecoptera and Trichoptera, which consists of some species that have previously been affiliated with stream acidification (Fjellheim & Raddum, 1992; Fjellheim & Raddum, 1990; Patterson & Morrison, 1993; Wade *et al.*, 1989; Rutt *et al.*, 1990; Murphy *et al.*, 2013). The body size, high degree of species diversity and abundance make aquatic insects ideal candidates for studying changes taking place in the environment, occurring through natural causes or due to anthropogenic activity, as these changes will have direct bearing on species distribution and abundance (Gullan & Cranston, 2014; Thieme, 2005). Though not the main focus in this particular study, diatoms have also been shown to be good indicators of stream acidification (Henriksen & Posch, 2001; Small *et al.*, 2012; Oksanen *et al.*, 1988). For this reason, diatom samples were collected concurrently with macroinvertebrate samples and analysed in an honours project (Lungile Ncube, unpublished Hons dissertation) and this work is due to be published. Overall, deciding on which technique to use is dependent on the questions the investigator is attempting to answer. The present study focused on macroinvertebrates instead of diatoms because they are the most common form of bioassessment tools with a long history of application in various studies. Macroinvertebrate bioassessment assays specific to acidification have been successfully developed and implemented in Europe, Asia and North America (Akimoto *et al.*, 2006; Akimoto, 2003; Zischke *et al.*, 1983; Schartau *et al.*, 2008a; Raddum & Fjellheim, 2003). The paucity of knowledge on invertebrate taxonomy, distributions and ecological tolerances in South Africa provides ample research questions for a PhD research programme.

1.7 Study relevance to Policy decision making

Environmental policies are essential for balancing human requirements for use of the natural environment with biodiversity conservation and maintaining ecosystem services. Consequently, monitoring programmes are designed to give insight into perceived environmental threats, creating room to take necessary precautionary measures and thus are essential to policy makers. For instance, the monitoring of ecological conditions of rivers to inform management, forms part of the key strategic action plans designed to meet the key principles for conserving water resources as defined by the National Water Resource Strategy (DWA 2013). These principles were designed to facilitate water resource protection and ensure that sufficient water is left in the rivers to sustain ecosystem functioning. Furthermore, these principles are aimed at guaranteeing that the freshwater environment remains an asset to economic and social growth (DWA 2013). In this project, stream water quality and biological monitoring has been initiated for streams which are found in and around acid sensitive soils and waters in selected regions of South Africa. If continued, this monitoring may lead to the development of a database for stream water chemistry and macroinvertebrate sampling related to acidification effects from coal power stations which would facilitate future investigations and possibly lead to policy adjustments based on these and subsequent results. Furthermore, these findings could possibly become part of an existing aquatic macroinvertebrate and chemical database that was initiated under the department of water affairs (Fowler *et al.*, 2000; The Freshwater Consulting Group and Soft Craft Systems for DWAF, 2007; Dallas & Day, 2007).

Hewitt and Allott (1999) have highlighted six reasons why monitoring is important and some of these are summarised below. Monitoring enables stakeholders to assess the effects of anthropogenic activity on the environment and to establish a cause and effect relationship between the two. Assessment pertaining to the need for legislative control on emissions and to ensure compliance to emission standards may be initiated and the effectiveness of the legislative control is dependent upon subsequent monitoring. Other reasons for carrying out monitoring are to obtain historical records of environmental

quality and in that way create a data base for future reference (Hewitt & Allott, 1999). In addition to the above the authors highlight that single isolated measurements do not reflect temporal and spatial variations. Thus repeated measurements over long time periods are necessary to achieve sufficient sample density reflecting both temporal and spatial differences (Hewitt & Allott, 1999). For this reason, monitoring of acidification effects needs to be part of the routine monitoring in South Africa and indicator species identified in this study can be incorporated into the River Eco-status Monitoring Programme (REMP) formerly known as the River Health Programme (RHP).

Most of Europe and North America has succeeded in reversing the effects of stream acidification by liming a few areas which were exposed to acidification but for the most part recovery was attained through the reduction of S and N emission, which under the UNECE-CLRTAP used critical load models to establish both the effects of acid deposition on ecosystems as well as to model the benefits of reducing emissions for biological recovery (Yan *et al.*, 2003; Hindar & Wright, 2005; Mcnicol *et al.*, 1995; B L Skjelkvåle *et al.*, 2001; Murphy *et al.*, 2014; Battarbee *et al.*, 2014). These initiatives have also led to the reintroduction of fish species that had disappeared as a result of stream acidification (Larssen *et al.*, 2010). Furthermore, policies have been put in place to ensure that minimal damage to the environment is maintained and that acidified regions have a chance to recover (Curtis *et al.*, 2014; Curtis & Simpson, 2014; Murphy *et al.*, 2014; Stockdale *et al.*, 2014; Larssen *et al.*, 2010). For developing countries on the other hand, it would be ideal to curb challenges associated with water resources and loss of biodiversity by setting and implementing policies that are going to be beneficial in attaining sustainable development, maintaining a healthy aquatic ecosystem and the abatement of air pollution.

1.8 Thesis structure and chapter summaries

This PhD thesis is divided into seven sections representing 10 chapters plus the Appendices.

Section 1

Chapter One — Introduction: This part of the thesis provides context into the wider project, supplies background into the problem statement that leads to the key hypotheses which are followed by the aims and objectives of the thesis. In summary, the introduction touches on coal mining in general and South Africa's dependence on this industry for economic and social development purposes. The adverse effects of coal mining processes that lead to stream acidification and the processes thereof are also covered.

Section 2

Chapter Two — Literature review: Chapter two reviews the use of macroinvertebrates in biomonitoring as well as existing acidification indices. The role of ANC and critical loads in determining stream acidification is also covered.

Section 3

Chapter Three — Defining study regions and sampling sites: Chapter three sets out to address objective one of the thesis which is to use GIS to identify regions of South Africa with acid sensitive soils and waters. Overall, study regions were defined based on the distribution of coalfired power stations and well as on the distribution of acid sensitive soils and waters. The study regions namely Mpumalanga, Waterberg and south-western Cape were then categorised to represent an assumed deposition gradient.

Section 4

Chapter Four	Research approach and methods: Chapter four includes the use of descriptive and analytical statistical approach.

Section 5

Chapter Five to Chapter Eight	Results section: Chapter five includes comparative analysis of the water chemistry data across the three study regions. Chapter six focus on the macroinvertebrate data, whereas Chapter seven and chapter eight cover the south-western Cape and Waterberg study regions respectively. Findings from chapter five and six led to a decision to analyse the respective regions separately. The Mpumalanga region however was not analysed in this manner due to some seasons missing field chemistry data as detailed in appendix 5. Other factors such as not having suitable study sites also led to the exclusion of this region.

Section 6

Chapter Nine	Discussion and study limitations: The discussion is structured in a way that addresses study objectives.

Section 7

Chapter Ten	Conclusion and future work: The conclusion attempts to address to the study hypotheses.

Appendices

Chapter 2 Literature review

Essentially biomonitoring methods are designed to provide a more direct and integrated measure of water quality and its effect on the aquatic ecosystem. The earliest recorded use of biomonitoring techniques in Europe dates back to 1902 where individuals with personal interest on water pollution had advocated the use of biological methods to assess the effects of anthropogenic activity on water quality (Hawkes, 1997). However, it was only in the 1970s that biological methods were introduced formerly for use on a national platform however the adequacy of these methods was called into question (Hawkes, 1997). This led to the development of groups such as the Biological Monitoring Working Party (BMWP) which came up with the BMWP scoring system for macroinvertebrates (Hawkes, 1997). In North America on the other hand legislations such as the Clean Water Act of 1977 were also initiated with the sole purpose of restoring and maintaining the chemical, physical and biological integrity of the United States national waters (Karr & Dudley, 1981; Karr *et al.*, 1986). However, the term 'biological integrity' alone, was hard to define. Therefore efforts to try and restore the integrity of water resources were dominated by non-biological parameters that focused on the chemical and physical properties of the water (i.e. Water quality), under the assumption that maintaining biologically acceptable quantities of selected elements will in turn improve biological integrity (Karr *et al.*, 1986). However, the fault with this approach was that it did not represent a direct measurement of the biological conditions, which led to further a decline of biological integrity (Karr *et al.*, 1986). It became obvious to some researchers that the ability for a water body to maintain a balanced biological community was the best potential indicator of water quality (Karr *et al.*, 1986). These observations led to the development of biological monitoring (biomonitoring) tools. The new approach of prioritising aquatic fauna was deemed too expensive, time consuming and subject to selectivity by many. However, the potential success this new approach presented if applied with care fueled optimism by some that led to the development of biological monitoring indices such as the Index of Biotic Integrity (IBI) (Karr *et al.*, 1986; Karr, 1981) which has since been subject to many improvements (Karr & Chu, 1999). Furthermore, the macroinvertebrate bioassessment approach has since been considered both efficient

and cost effective. In addition to this the aforementioned approach is designed to facilitate rapid assessment of large regions over a short time frame to pinpoint areas of concern worthy of more detailed investigation (Resh *et al.*, 1995). Many of the biomonitoring techniques developed in the 1970s including the BMWP scoring system as well as the Index of Biotic Integrity were designed to address anthropogenic activity related to saprobic systems. However, since then many other biomonitoring methods (Turley *et al.*, 2015; Usseglio-Polatera *et al.*, 2000; Johnson & Wiederholm, 1993; Buss & Salles, 2007; Karr, 1981) including those specific to acidification (Mills & Schindler, 1986; Fjellheim & Raddum, 1990; Murphy *et al.*, 2013) have been developed.

The biomonitoring process includes procedures that can be subdivided into several categories namely behavioral bioassays, toxicity bioassays, bioaccumulation analysis, bioassessment assays as well as studies that focus on fish health (Hohls, 1996; Roux *et al.*, 1993). All these categories require living organisms to determine water quality and each category for example may focus on species response to water contamination, laboratory based investigations, studies that assess species uptake and retention of chemicals, ecological surveys as well as cause and effect assessments respectively (Roux *et al.*, 1993; Hohls, 1996). The present study has used the macroinvertebrate bioassessment approach to investigate differences in the aquatic ecosystem related to acidification. Several studies (Fjellheim & Raddum, 1992; Fjellheim & Raddum, 1990; Patterson & Morrison, 1993; Wade *et al.*, 1989; Rutt *et al.*, 1990; Murphy *et al.*, 2013) have shown that water acidity has a strong effect on species composition, and this has been achieved in part through assessing species presence/absence in relation to pH (Larsen *et al.*, 1996). Other more complex multivariable based methods for assessing water quality have also been adapted by some researchers (Tripole *et al.*, 2008; Murphy *et al.*, 2013).

2.1 The use of macroinvertebrates in biomonitoring

Aquatic macroinvertebrates represent a group of organisms that are widely used in assessing degradation of the aquatic ecosystem related to anthropogenic activity such as urbanisation (Cuffney *et al.*, 2005), industrialisation and agricultural practices (Richards *et al.*, 1997; Odountan & Abou, 2015; Anson *et al.*, 2008; Gullan & Cranston, 2014; Narangarvuu *et al.*, 2014; Hall *et al.*, 1982; Rosenberg, 1992; Sharma & Chowdhary, 2011; Pastuchova, 2006; Márquez *et al.*, 2015; King, 1981; Langton & Casas, 1999). Macroinvertebrates are highly diverse and include species with a wide range of habitat and ecological preferences (Gullan & Cranston, 2014; Sharma & Chowdhary, 2011). Furthermore, macroinvertebrates form an integral part of the aquatic food webs and by breaking down organic matter and consuming dead animals and bacteria they provide nutrients for other aquatic fauna (Bouchard, 2004a; Gullan & Cranston, 2014; Hogsden & Harding, 2012). The lifespan of most aquatic macroinvertebrates consists of two phases, the aquatic phase and the terrestrial phase which has a shorter time span when compared to the former. The aquatic stage is the most important stage for development and can last for a few weeks to several years depending on the type of species and the physico-chemical properties of the water (Merritt & Cummins, 1996). The development phase is divided into several instar stages that vary in number across taxonomic groups (Barber-James *et al.*, 2008; Currie & Adler, 2007; Naiman, 2008). Previous studies have shown that different species display different tolerance levels to a variety of pollutants (Odountan & Abou, 2015; Odume *et al.*, 2012; Hall *et al.*, 1982; Hurford *et al.*, 2010). For instance most Ephemeroptera are known for their low tolerance to pollution and thus are often the first organisms to disappear when a water body is polluted (Fjellheim & Raddum, 1992; Fjellheim & Raddum, 1990; Arimoro *et al.*, 2015; Chapman, 1995), suggesting that a water body that lacks these sensitive taxa, but supports more tolerant taxa, is likely to be polluted (Bouchard, 2004a). Similarly, several studies (Murphy *et al.*, 2013; Fjellheim & Raddum, 1990; Lien *et al.*, 1992) have shown that different taxa respond differently to variations in pH originating from anthropogenic activity or through natural causes. The same species which have been shown to respond in a particular way to organic pollution can have a completely different response to changes related to water pH. However,

species that are able to survive harsh environmental conditions such as acidification tend to be resilient to a number of other adverse changes in the aquatic ecosystem. Indicator species are important in biological monitoring as they tend to respond specifically to a particular type of pollutant when all confounding factors that may influence species presence/absence have been addressed adequately. Thus, the outcome of any biomonitoring procedure would ideally be to identify one or more indicator species that can be used to communicate the presence or the onset of deterioration in an aquatic ecosystem induced by anthropogenic activity to researchers and managers of local aquatic resources alike.

2.1.1 Indicator species

Indicator species are used to monitor changes in the environment that may have detrimental effects on the ecosystem at large (Landres *et al.*, 1988; Dufrêne & Legendre, 1997). The theory behind the use of indicator species is somewhat similar to the species distribution along an environmental gradient concept proposed by Whittaker (Whittaker, 1967; McCune *et al.*, 2002; Whittaker, 1956) where each species is said to have optimum conditions at which it functions best (i.e. environmental niche), and once these conditions move towards being less favourable species number and diversity tend to decline and in some cases species extinction may occur depending on the severity of the problem. Johnson *et al.* (1993) have also defined indicator species in line with the Whittaker concept. Indicator species are used to provide warnings for potential danger where the presence/absence of a particular species can thus be used to indicate environmental conditions that favour or challenge survival of other species in the ecosystem (Siddig *et al.*, 2016; Simberloff, 1998; Zacharias & Roff, 2001; Landres *et al.*, 1988; Dufrêne & Legendre, 1997; Johnson & Wiederholm, 1993). Many authors over the years have reviewed the importance, motivation and criteria for selecting indicator species (Siddig *et al.*, 2016; Zacharias & Roff, 2001; Bakker, 2008; Dufrêne & Legendre, 1997; Dale & Beyeler, 2001). However, no standard guidelines exist as to what constitutes bioindicators (Landres *et al.*, 1988; Siddig *et al.*, 2016; Zacharias & Roff, 2001) but some general characteristics have been proposed over the years by most of the authors who have

published on the subject. The decisions that involve defining and identifying indicator species are often left to the researcher's discretion depending on the study aims and objectives. However, based on previous work, indicator species should ideally be well described (Siddig *et al.*, 2016; Landres *et al.*, 1988), show sensitivity to changes in the environment that is related to catchment attributes by cause and effect (Landres *et al.*, 1988), they must be diverse (Simberloff, 1998), well distributed (i.e. be common to most regions) (Larsen *et al.*, 1996), abundant (Bakker, 2008; Gray & Pearson, 1982; Pearson *et al.*, 1983), and must possess well-defined ecological tolerances (specialists and not generalists) to abiotic factors such as flow rate preferences, temperate, pH etc. that affect species presence/absence, growth rate and reproductive success (Siddig *et al.*, 2016; Landres *et al.*, 1988; Dale & Beyeler, 2001). Therefore, taxa that can tolerate a wide range of environmental conditions do not make good ecological indicators (Landres *et al.*, 1988). For more in depth background reading on the subject, definitions and a comprehensive literature review the reader is referred to Landres *et al*., (1988); Siddig *et al*., (2016); Zacharias and Roff, (2001); as well as Dale and Beyeler, (2001).

2.2 The South African River Eco-status Monitoring Programme (REMP)

The River Eco-status Monitoring Programme (REMP) formerly known as the River Health Programme (RHP), is a national monitoring programme within South Africa that was implemented in 1994 (Eloff *et al.*, 2004). The programme provides useful information that assists in ensuring better management of the South African river systems by conducting biomonitoring surveys and generating information on the general condition of South African rivers and then making that information available to resource managers and the general public. Physical changes that occur within the catchment such as flow alteration, chemical composition and biological characteristics all serve as indicators of the state of the aquatic ecosystem (Eloff *et al.*, 2004). REMP uses species composition and abundance or biological characteristics as its main indicators of river conditions. With REMP a river system can be classified as having natural, good, fair or poor stream condition based on the degree of disturbance observed within the channel. Channelized rivers are identified as being “artificial” because they have been highly modified and bear little or no resemblance to their natural condition and therefore cannot be compared to unmodified streams (Eloff *et al.*, 2004). Several indices are integrated into the REMP and they cover different aspects and factors that directly influence the integrity of the aquatic ecosystem as a whole. The indices include the Index of habitat integrity (IHI), the Fish assemblage integrity index (FAII), the South African scoring system (SASS), the macro-invertebrate response assessment index (MIRAI), the Fish response assessment index (FRAI) as well as the Riparian vegetation index (RVI), to name a few. For the purposes of this study the SASS and the MIRAI are looked at in more detail in the next section.

SASS is a standard bioassessment method that was first developed by the British Biological Monitoring Working Party and was modified to suit South African conditions by Mark Chutter (Dickens & Graham, 2002). The SASS technique is generally used in assessing river health and forms a major component of the REMP. Full details on how the SASS bioassessment is carried out are provided in Dickens and Graham (2002). In summary, the SASS assessment is conducted on site and requires that the

macroinvertebrates be living. During the survey invertebrate samples are collected from different biotopes that represent a range of habitats within the river of interest. Samples from each biotope are kept separate and 15 minutes is allowed for identifying as many species as possible per biotope. The SASS score sheet is then completed according to the findings of the survey. A quality score (QV on the SASS sheet) has been assigned to each taxon and is based on the species vulnerability or resistance to pollution or perturbation. Low scores of 1-3 for example, are assigned to species that are highly resistant to pollution and high scores of 12-15 are indicative of species that are very sensitive to pollution. Hence species that are assigned score values of 14 or 15 are found in pristine waters with a very diverse biological community. For interpretation the SASS score, Number of Taxa (No. Taxa) and the Average Score per Taxa (ASPT) must be calculated. Other factors such as temperature, pH, dissolved oxygen (DO) levels, conductivity, riparian disturbance and instream disturbance play an important role when interpreting the results (Dickens & Graham, 2002). The SASS version 5 (SASS5) is currently the standardised method for biological monitoring that is used throughout South Africa and parts of Southern Africa. However, the SASS5 method was developed to monitor organic pollution primarily and is thus not specifically for pH.

MIRAI is fairly similar to the SASS method and information collected during a SASS survey as well as more detailed information can be used when conducting the MIRAI. The first step to conducting a MIRAI assessment is to determine the Present Ecological State (PES) using the invertebrate data sheets which contain information on taxa with specific flow rate velocity preferences; habitat preferences; water quality preferences as well as the SASS score sheet. The end result for the MIRAI is to derive the Ecological Category (EC) for aquatic invertebrates, which is based on the ability to interpret environmental requirements, preferences and intolerances of invertebrate taxa in an undisturbed river system as well as to be able to interpret their response to changes to their habitat. A more detailed description of the technique can be found in the reference ecoclassification manual for ecostatus determination version 2 (Thirion, 2008).

SASS and MIRAI are highly useful in determining the health of the aquatic ecosystem however both these biomonitoring techniques are more specific to organic pollution. Further assessment is normally required to identify the source of the observed deterioration or change in the aquatic ecosystem. For this reason, the macroinvertebrate indices used for the REMP are not necessarily suitable for the present study which is aimed at determining the effects of acid deposition on the aquatic ecosystem.

Countries previously impacted by acidification have developed acidification scoring systems that give warnings of possible stream acidification by monitoring presence/absence of indicator species. Characteristics of acidified waters include a decline in species abundance along with an increase of acid tolerant taxa such as stoneflies and a decline or absence of sensitive taxa such as mayfly species (Patterson & Morrison, 1993; Lien *et al.*, 1992; Larsen *et al.*, 1996). For example, publications from Europe and North America have shown that loss of bass, crayfish and mayfly species tend to occur at pH 5.5 while snails and frogs generally disappear at pH 6 and 4 respectively (Johnson & Wiederholm, 1993; Mills & Schindler, 1986; Fjellheim & Raddum, 1990; Snucins & Gunn, 2003; US EPA, 2012; US EPA, OAR, OAP, 2017). Therefore, a stream that has all of these organisms is said to be in good environmental condition.

2.3 Acidification indices

Loss of fish populations associated with acidification was first described in the 1960s (Akimoto *et al.*, 2006; Cogbill, 1976; Lien *et al.*, 1996; Lien *et al.*, 1992; Schindler, 1988). However, upon further investigations it was discovered that some macroinvertebrate species disappeared prior the onset of conditions deemed detrimental to fish populations, suggesting that macroinvertebrates could be used as early indicators of acidification (Aanes & Bækken, 1995). Several studies have used ordination techniques and general ecological properties such as species diversity and density to ascertain the effects of stream acidification (Raddum, 1998; Guerold *et al.*, 2000; Zischke *et al.*, 1983; Sandin & Johnson, 2000). Acidified waters are generally characterised by a decline in productivity and species number, increased dominance of acid tolerant taxa such as stoneflies and a decline or absence of sensitive taxa such as mayflies (Patterson & Morrison, 1993). In a study conducted at the Environmental Protection Agency (EPA), Minnesota, Zischke *et al.* (1983) used species densities to assess macroinvertebrate response to acidification. During the study three outdoor experimental channels of different pH were established. The first channel had an ambient pH of 8 and the other two channels were of pH 6 and 5. The results showed that species density and diversity decreased with increasing acidity (Zischke *et al.*, 1983).

Biological indices are based on empirical relationships between measured variables and species assemblages; therefore, developing an efficient scoring system requires accurate macroinvertebrate identification to the lowest possible taxonomic level. For acidification indices species are often grouped according to their tolerance to pH (Aanes & Bækken, 1995), where the presence of certain taxa is associated with a particular pH range. Once the groups are established the taxa are then classified as being acid tolerant or intolerant based on species presence/absence. The different groups are usually assigned numerical “acidification scores” that gauge the degree (i.e. no effect, moderate effects to extreme events) of acidity. The majority of acidification indices are based on species presence/absence and not necessarily on the number of observed sensitive species, thus they require only one accurately identified species representing a specified tolerance level

to classify the site as being impacted or non-impacted (Raddum, 1998; Sandin & Johnson, 2000). Several acidification indices have been developed in Europe and North America and some of these are going to be reviewed in the next section of the thesis. The following indices were chosen for review mainly because they are well known and have been used within their respective nations successfully. The Raddum Index for example is the first well established acidification index which has been adapted for use in many parts of Europe including its place of origin, Norway.

2.3.1 The Raddum Index

The Raddum index, formally known as the Norwegian Acidification Number system is used to determine macroinvertebrate species sensitivity to acidification and was primarily developed for aquatic fauna from Southern and Western Norway (Fjellheim & Raddum, 1990; Aanes & Bækken, 1995; Davey-Bowker, J, Furse *et al.*, 2003). The scoring system assumes a numeric format where a site or watershed is assigned an acidification value (0-1) based on the presence/absence of indicator taxa. During the assessment a score of one is given to species that are highly sensitive to acidity and can only tolerate pH ≥ 5.5. Species that are moderately sensitive to acidity and can tolerance pH ≥ 5.0 are given the score of 0.5. A score value of 0.25 is indicative of highly damaged watersheds that are dominated by acid-tolerant species that can survive a pH ≈ 4.7. Severely damaged waters that have a pH < 4.7 are designated an acidification score of 0. The 1990 (Fjellheim & Raddum, 1990) version of the acidification index is accompanied by a list of taxa that can be readily used for site classification.

Factors that affect the acidification score include calcium levels, pH, Total Organic Carbon (TOC) and the presence of reactive aluminium. Previous studies in Europe and eastern North America have shown that certain species from selected Orders are sensitive to changes in pH (Fjellheim & Raddum, 1990; Zischke *et al.*, 1983). According to the Raddum Index, Plecoptera and Trichoptera taxa are highly tolerant to low pH values and generally have an acidification score of 0. Baetidae on the other hand have an

acidification score of 1, suggesting that they are mainly found in streams with pH ≥ 5.5 (Fjellheim & Raddum, 1990).

2.3.2 The Patterson and Morrison Key

The Patterson and Morrison key (Patterson & Morrison, 1993) is one other method that was developed to assist foresters, rangers and managers of upland ecological reserves in the United Kingdom (UK) to monitor stream acidity. This method is simplified to group indicator species and was designed to readily assist managers in identifying acidic streams by looking at presence/absence of collective taxa. The Patterson and Morrison key uses a decision chart to place forest streams in one of three classes based on the presence/absence of Group 1-3 indicator taxa (**Error! Reference source not found.**). Streams that fall under Class 1 are circumneutral, with a mean pH ≥ 6. Class 2 streams are fairly similar to Class 1 streams, except that they lack group 1 species. Class 3 streams are acidic, and they lack group 1 and 2 taxa. Furthermore, these streams are characterised by a decline in fish population and macroinvertebrate species composition.

Table 2-1: Indicator species and acid tolerance levels for Patterson and Morrison key.

Indicator taxa	Description	Habitat preferences
Group 1 *Gammarus pulex* (shrimp - Crustacea) *Glossosoma* and *Agapetus* species (caddisfly) *Ancylus fluviatilis* (limpet - Mollusc) *Limnaea peregra* (wandering snail - Mollusc) *Asellus aquaticus* (slater - Crustacea)	Acid-intolerant	mean pH ≥ 6 mean water hardness [$CaCO_3$] > 10 mg/l^{-1}
Group 2 *Hydropsyche (*caddisfly*)* Ephemeroptera *Baetis rhodani* *Rhithrogena semicolorata*	Acid-intolerant	mean pH ≥ 5.5
Group 3 Plecoptera Trichoptera Leptophlebiidae (mayfly) Siphlonuridae (mayfly) Dytiscidae (beetles)	Acid-tolerant	mean pH ≤ 4

2.3.3 The Wade Key

The Wade key was designed for upland Wales and involved the collection of macroinvertebrate samples from 104 sites during the spring and summer of 1984 (Wade *et al.*, 1989). Samples were then assigned to one of three abundance categories where 1 = abundance ≤ 9, 2 = abundance ≥ 10 but ≤ 99 and 3 = abundance ≥ 100. These categories were used for the Two-way Indicator Species Analysis (TWINSPAN) classification by following the pseudospecies concept (Wade *et al.*, 1989).

Prior to developing the key, the authors observed that species composition varied between sampling seasons and thus two scoring systems specific to each season were developed. The development of the scoring systems involved three stages of analysis that included classification by TWINSPAN (Hill & Hill, 1979), the use of DECORANA (DCA) (Hill & Gauch, 1980) for ordination analysis and the use of Multiple Discriminant Analysis (MDA) (Frank *et al.*, 1965) to establish empirical relationship between species composition and environmental variables. TWINSPAN classification was cut off at level 2. The 104 sites were categorised to represent 7 site groups reflecting correlation to different environmental variables. Level 1 sites showed the highest correlation to changes in stream pH and aluminium concentrations. *Hydraena gracillis* (Coleoptera) and *Baetis rhodani* (Ephemeroptera) were identified as important level 1 indicator species in both seasons.

2.3.4 The University of Wales System

The University of Wales System (Rutt *et al.*, 1990) is fairly similar to the Wade Key reviewed above and the classification system proposed by Wright *et al.* 1984 (Wade *et al.*, 1989; Wright *et al.*, 1984). Secondary data set from the National Rivers Authority (N.R.A.) and the Scottish River Purification Boards (R.P.B.s) as well as field data collected from 24 second and third order streams of upland Britain, were used to develop this macroinvertebrate classification system. Classification was carried out to family and species level respectively, using TWINSPAN. The indicator taxa identified at species level classification belonged to indicator groups from family level classification, suggesting that family level data alone was sufficient for establishing an empirical relationship between biological and environmental variables. Thus, species level data was excluded from subsequent analyses and final classification was carried out to family level. Sites are assigned to 1 of 4 categories divided according to their tolerance to changes in pH, aluminium concentration and buffering ability, **Figure 2-1**. Overall observations indicated that mean family number per site decreased with increasing acidity.

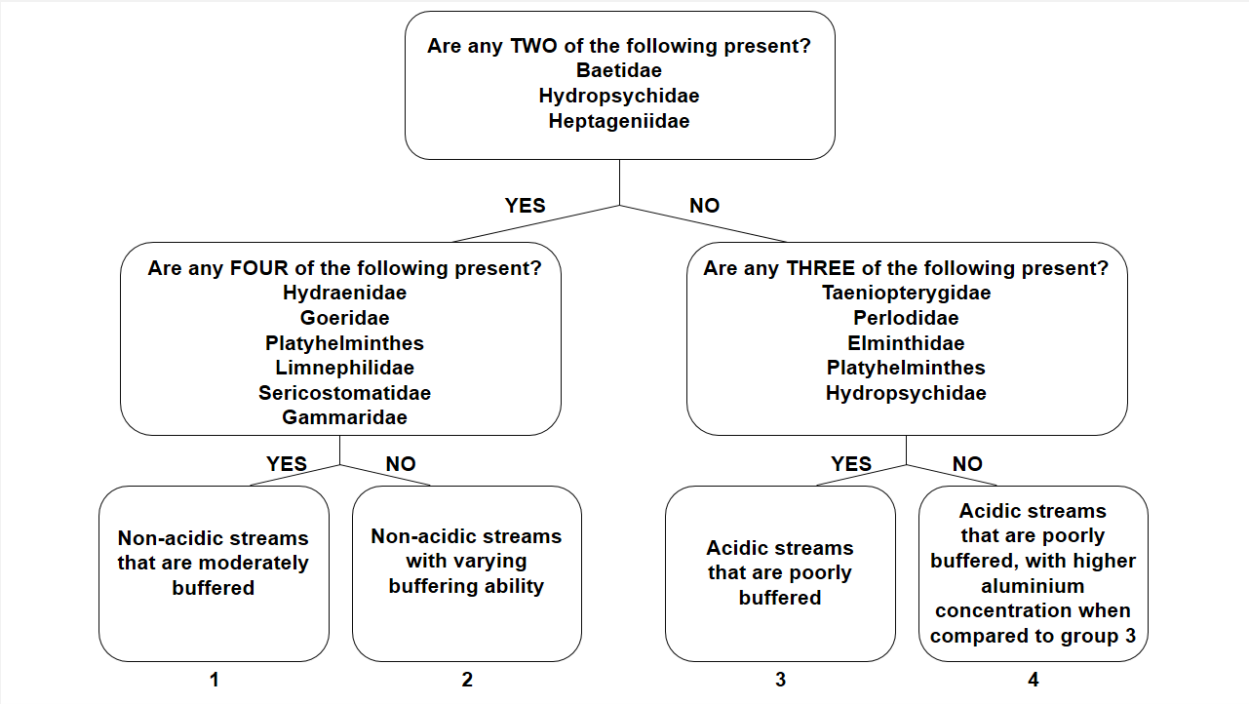


Figure 2-1: The University of Wales classification system with slight modifications. The original system with detailed stream water chemistry specific to each group can be obtained from Rutt *et al*. (1990).

2.3.5 Species-level Acid Water Indicator Community (AWICsp)

The AWIC*sp* scoring system was developed after the UK Centre of Ecology and Hydrology (CEH) was tasked by the UK Environment Agency to develop a scoring system that would serve as a standard method for assessing the extent of damage caused by acidification through the years across England and Wales (Davey-Bowker, J, Furse *et al.*, 2003). The AWIC*sp* scoring system employs a multivariate statistical approach to data exploration by canonical correspondence analysis (CCA) (Murphy *et al.*, 2013). This approach is different from that of other indices reviewed above in that it is not based on predefined species tolerance to acidity established through literature search or laboratory experiments. Instead the authors use corresponding species and field chemistry data for the analysis. During the process forward selection was employed to identify variables with direct effect on species composition. This process was repeated until the addition of a new variable showed no significant impact on the explanatory power of the model. A CCA was then carried out using the selected variables. Using the biplot results obtained from the test dataset utilised to build the model, pH and longitude were the explanatory variables that influenced species distribution along the first axis. A scoring index was then developed by ranking taxa (species scores) along the mean pH gradient. Species response to mean pH varied. Contrary to findings from other studies (Wade *et al.*, 1989; Fjellheim & Raddum, 1990; Patterson & Morrison, 1993), *Baetis rhodani* was distributed across the entire pH gradient, however it was more dominant in circumneutral sites. Index scores from 1 to 9 were then assigned to species based on their relative position along the first axis, where a score value of 1 was indicative of acid-tolerant species and a score value of 9 was assigned to acid-intolerant taxa based on the 10^{th} percentile approach. The final AWICsp scores were then calculated as the average of the scores assigned to each taxon.

The acidification indices reviewed above were all developed for specific regions or with the intention of improving existing methods. Of the five techniques however the Raddum index and the AWIC*sp* scoring system are structured in a way that makes them adaptable for use in other parts of the world (i.e. South Africa). The Patterson and Morrison key on the other hand requires prior knowledge of acid sensitive and tolerant species which is not the case with the aforementioned indices. The Wade key is season specific and was designed after the authors realised that species composition varied between sampling seasons. As with the Patterson and Morrison key making a decision on which seasons to focus on requires prior knowledge or understanding of the acidification patterns that have manifest over the years. The University of Wales system on the other hand pooled together data from existing monitoring programmes within the UK to develop a single classification system. The approaches used in the Patterson and Morrison key, the Wade key as well as in the University of Wales system are not ideal for the present study which is still in its initial phase and has no prior knowledge of the extend of the perceived stream acidification problem for South Africa. Had this knowledge existed we then could have followed in the footsteps of anyone of the aforementioned indices to try and improve existing protocols, however this not the case.

Two indices (AWIC*sp* scoring system and The University of Wales system) have shown that using family and species level data during the development of their scoring systems did not result in different outcomes. Consequently, both studies concluded that family level data alone was sufficient for establishing an empirical relationship between biological and environmental variables. The present study however does not support the use of family level identifications because they tend to ignore valuable information on species diversity (Winston, 1999). Nonetheless, we do acknowledge the attraction of this approach which is more cost effective and efficient when compared to species level identification. However, since species level focused indices such as the Raddum index and the Patterson and Morrison key have been successfully developed and applied over most of Europe it would be better to navigate towards developing similar species level indices from the start and work towards improving them to be more efficient and cost effective as with the Patterson and Morrison key.

Furthermore, as mentioned before acidification indices are primarily based on species presence/absence and not the number of observed acid sensitive species. Therefore, only one accurately identified species representing a specified tolerance level is required to classify streams as being acidified or non-acidified. Despite their many differences all these indices are based on species tolerance to changes in the pH concentrations and the role of the ANC in stream acidification and loss of acid sensitive species.

Since the present study is still in the initial stage of trying to identify an acidification problem within South Africa, taking into account all that goes into developing a scoring system (i.e. species response to varying concentrations to pH and the ANC) this PhD study opted to take the most basic approach to identifying acid sensitive taxa through investing species presence/absence at difference pH concentration range.

2.4 Environmental variables that influence macroinvertebrate assemblage's response to stream acidification

2.4.1 Stream pH

The water pH is influenced by geological and biological activities taking place in a stream. For instance, streams dominated by igneous rock tend to be more alkaline with pH > 7. Furthermore, processes such as photosynthesis, decomposition and leaching of plant organic compounds into the stream may affect pH levels (Davies & Day, 1998). By definition pH is the measurement of the concentration of hydrogen ions (H^+), where pH is expressed as the negative $\log_{10}$ of H^+ ion concentration. This means that a one unit decrease of the pH is indicative of a tenfold increase in hydrogen ions, making pH a direct measure of acidity. The presence of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions favour alkalinity. Waters with 10 mg/l of calcium carbonate are generally considered slightly acidic but are still able to support a significant number of species (Patterson & Morrison, 1993). Most freshwaters ecosystems have a pH of 6 – 8 and low pH values are normally associated with the mobilisation of toxic elements such as the toxic aluminium species Al^{3+} (Davies & Day, 1998). The presence of organic material may lead to further variation in stream acidity (Aanes & Bækken, 1995; Roila *et al.*, 1994). Moderate changes to pH seldom lead to lethal effects however, these subtle changes may result in growth impairment and fecundity among aquatic organisms (Davies & Day, 1998). Many headwater streams serve as nurseries and spawning ground for indigenous fish and in some parts of the world, a pH < 5.6 has been associated with a decline in fish population and a significant change in benthic invertebrate species composition (Patterson & Morrison, 1993).

2.4.2 Acid Neutralising Capacity (ANC)

As mentioned previously, when acid rain comes into contact with the soil it leaches away the soil's nutrients and metals causing the soil to lose its buffering capacity against strong acids. Consequently, acid sensitive waters have low concentrations of bicarbonate, calcium and magnesium ions (Davies & Day, 1998; Desonie, 2007; Kernan, 2010) and high concentration of SO_4^{2-} and inorganic aluminium which are both toxic to the aquatic ecosystem (Kernan, 2010). Poorly buffered soils and waters generally have a low acid neutralising capacity against strong acids that come from the atmosphere in the form of acid deposition. The ANC thus plays an important role in determining stream acidity and for this reason ANC has been shown to be the best correlate with biological response to stream acidification by aquatic fauna and is thus the most important variable when measuring stream acidification (Lien *et al.*, 1992).

Taking into account the wide variety of processes that influence soil and stream water chemistry, because of its composite nature, ANC can be used as an index of the acid-base status in acidified ecosystems (Roila *et al.*, 1994; Aanes & Bækken, 1995).

$$\begin{aligned} ANC &= \Sigma\,[\text{Base cations (BC)}] - \Sigma\,[\text{Strong acid anions (SAA)}] \\ &= [Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}] - [SO_4^{2-} + NO_3^{-} + Cl^{-}] \end{aligned}$$

In this case ANC is expressed in µeq/l, thus, during the calculation all major ions are converted to µeq/l (Lydersen *et al.*, 2004). The ANC will decrease as BC are replaced by SAA, placing aquatic fauna at risk acidification. The Critical limit (ANC_{limit}) refers to the ANC concentration that will lead to loss of acid sensitive taxa. The ANC_{limit} is set by assuming a dose dependent relationship between water chemistry and the biological species population (Fjellheim & Raddum, 1990; Lien *et al.*, 1996). The most commonly used values are 0, 20 or 50 μeql^{-1} and in Norway for example, the ANC_{limit} has been set to 20 μeql^{-1} (Fjellheim & Raddum, 1990; Raddum & Skjelkvåle, 1995). In naturally acidic streams the ANC_{limit} is often lower (0 µeq/l) as the fauna that occupy these streams are well adapted to acidic conditions. For such cases some studies have used both the ANC

and pH to determine the critical limit. Establishing an ANC_{limit} may constitute a useful tool for monitoring acidification.

2.5 Critical loads

The critical loads concept was established for use under the United Nations Economic Commission for Europe (UN/ECE) Convention (Henriksen & Posch, 2001). By definition a critical load is “The highest deposition of acidifying compounds that will not cause chemical changes leading to long term harmful effects of ecosystem structure and function” (Lien *et al.*, 1992) and based on this definition critical loads can be regarded as “deposition estimates”. Similar to the Simple Mass Balance (SMB) model (Sverdrup & De Vries, 1994) the critical loads model considers all the factors associated with the chemical and environmental conditions represented at that site or region which are integrated into the water chemistry of the catchment.

In an ecosystem that has started to deteriorate due to acid deposition, critical loads can help determine how much reduction in deposition (or emissions) is required for recovery at that site. Therefore, critical loads not only assist in determining the sensitivity of an ecosystem, but they are also useful for setting policies that can prevent long term environmental problems. There are three models that are currently used for determining critical loads for surface waters, the Steady-State Water Chemistry model (SSWC), the Diatom model and the First-order Acidity Balance (FAB) model (Curtis, 2002; Henriksen & Posch, 2001). The SSWC model estimates weathering rates based on present day cation exchange capacity and the ‘F-factor’ accounts for base content associated with leaching. The FAB model uses the Simple Mass Balance (SMB) approach which considers the net loss and accumulation of acids, nutrients and base cations in soils and water. Both the SSWC and FAB models employ a dose-response relationship between the pollutant and receptor (Henriksen & Posch, 2001; NADP, 2008). For this study an empirical approach was used to determine the ANC_{limit} for macroinvertebrates of interest. The ANC_{limit} was then used to estimate critical loads under a separate work plan in the wider NRF project.

2.6 Most commonly used indicator groups in biological monitoring

Each river system is very different to the next and their ecosystems respond to regular nutrient and sediment input as well as flow rate (Thirion, 2008). For this reason, each river system supports a particular assemblage of species adapted to a specific flow rate that controls temperature, sediment transport and nutrient load within that river system. Therefore in stream disturbances, sediment input and nutrient load will result in a change in biological community structure that will be reflected through species loss or gain (Thirion, 2008). The type of species that make up a community of invertebrates found within an aquatic ecosystem is influenced by the type of habitat (bedrock, cobbles, vegetation, sand, gravel and mud) found in that stream, the physico-chemical characteristics of the river such as chemical composition (presence-absence of major cations and anions or nutrient load), pH, turbidity, dissolved oxygen (DO), temperature, riparian and in-stream disturbances as well as biological features that influence species survival (Ndaruga *et al.*, 2004; Dickens & Graham, 2002; Geist, 2011). *Simulium metomphalus chutteri* is a known pest species in South Africa and several studies have been conducted previously to try and understand all the different factors that enable this blackfly species to be the most dominant *Simulium* species found in South Africa (de Moor, 1982). A study conducted by Barber-James (2004), investigated the role of increasing turbulence on the feeding ability of *Simulium metomphalus chutteri* and *Simulium nevermannia nigritarse*, and found that cases of high turbulence were more favourable to *Simulium metomphalus chutteri* than they were for *S. nigritarse*. This lack of adaptation to changes in the environment by the *Simulium nevermannia nigritarse* explained its decrease in abundance when compared to *Simulium metomphalus chutteri* (Barber-James, 2004a). The same phenomenon can apply to several other species affected by changes in their natural environment where species failure to adapt may lead to a decrease in species abundance, species migration or an even more drastic response of species extinction. Therefore, species abundance and level of diversity can be interpreted as a window into the state of the aquatic ecosystem.

Patterson and Morrison (1993) point out that during water quality assessment, data obtained from analysing species composition outweighs results obtained solely by analysing water chemistry since water chemistry only gives insight on the chemical composition at the time the sample was taken, while species composition is a reflection of the physical and chemical changes that have taken place in the water body over a longer (months to years) time period. The lifespan of most aquatic insects consists of two phases, the aquatic phase and the terrestrial phase which usually has a shorter time span when compared to the former. The aquatic stage is the most important stage for development and can last for a few weeks to several years depending on the type of species and the physico-chemical properties of the water (Merritt & Cummins, 1996).

Since the beginning of time macroinvertebrates have developed several adaptation strategies that permit them to survive harsh climatic conditions and competitive environments (Merritt & Cummins, 1996; Ross-Gillespie *et al.*, 2018). These adaptations also enable aquatic fauna to take advantage of food that is seasonally available as well as facilitate timed emergence to favourable environmental conditions (Ross-Gillespie *et al.*, 2018). By following these patterns, ecologists are able to study larval growth and catalogue emergence and flight time for adults (Merritt & Cummins, 1996; Ross-Gillespie *et al.*, 2018). However, one of the most important deciding factors for species survival is habitat selection which in most part is a task delegated to the female counterpart, in that, a large portion of habitat selection is dependent on where the female lays her eggs. The timing and habitat choice for oviposition influences life cycles (Merritt & Cummins, 1996). Habitat preferences, taxonomic properties and morphological features of some of the commonly used insect Orders in bioassessment assays are reviewed below. The taxa chosen for review and analysis in this study are taxa that belong to taxonomic groups Ephemeroptera, Plecoptera, Trichoptera, Simuliidae and Chironomidae, that have previously been identified as being acid sensitive or tolerant in other studies (Fjellheim & Raddum, 1992; Rutt *et al.*, 1990; Murphy *et al.*, 2013).

2.6.1 Ephemeroptera (Mayflies)

The origin of Ephemeroptera can be traced back to 290 m years ago (Barber-James *et al.*, 2008). Over 3000 species of Ephemeroptera have been described worldwide in 42 families, and 400 genera of which 61 are only known as fossils (Barber-James et al. 2008; de Moor et al. 2003). Of the known taxa, 12 families, 47 genera and 105 species are found in South Africa (de Moor *et al.*, 2003b; de Moor & Day, 2013). The adult mayfly has a very short life span ranging from a few hours to a maximum of two days that is purely for reproduction purposes (Bouchard, 2004c; Bouchard, 2004d). Thus, the nymph (**Figure 2-2**) stage is the dominant life form for Ephemeroptera (Barber-James *et al.*, 2008). The key identification features include antennae that vary in structure, where some are long and others are short, bead-like, and comb-like or greatly reduced (stump-like) (de Moor *et al.*, 2003b). The antennae are followed by mouth parts and three medial structures that are essential for feeding. The Labrum or upper lip is a median flap-like structure that has a pair of mandibles located just below it. The mandibles are used for crushing, cutting, scraping, and piercing food and these are followed by a pair of maxillae (de Moor *et al.*, 2003b). The hypopharynx lies medial to the above structures which when modified becomes a tongue-like structure. The lower lip is known as the labium. In addition to these the head consists of a pair of eyes or light sensitive ocelli. The three segments of thorax are known as the prothorax, mesothorax and the metathorax and each consists of a pair of jointed legs that terminate with a claw (de Moor *et al.*, 2003b). All the key features mentioned above are essential for species identification and are used to distinguish between taxa of the same family or genera. However the maxilla and labium are unique among species (Lugo-Ortiz *et al.*, 2000; Lugo-Ortiz & McCafferty, 1997) and tend to be the most reliable distinguishing features especially between species of the same genus.

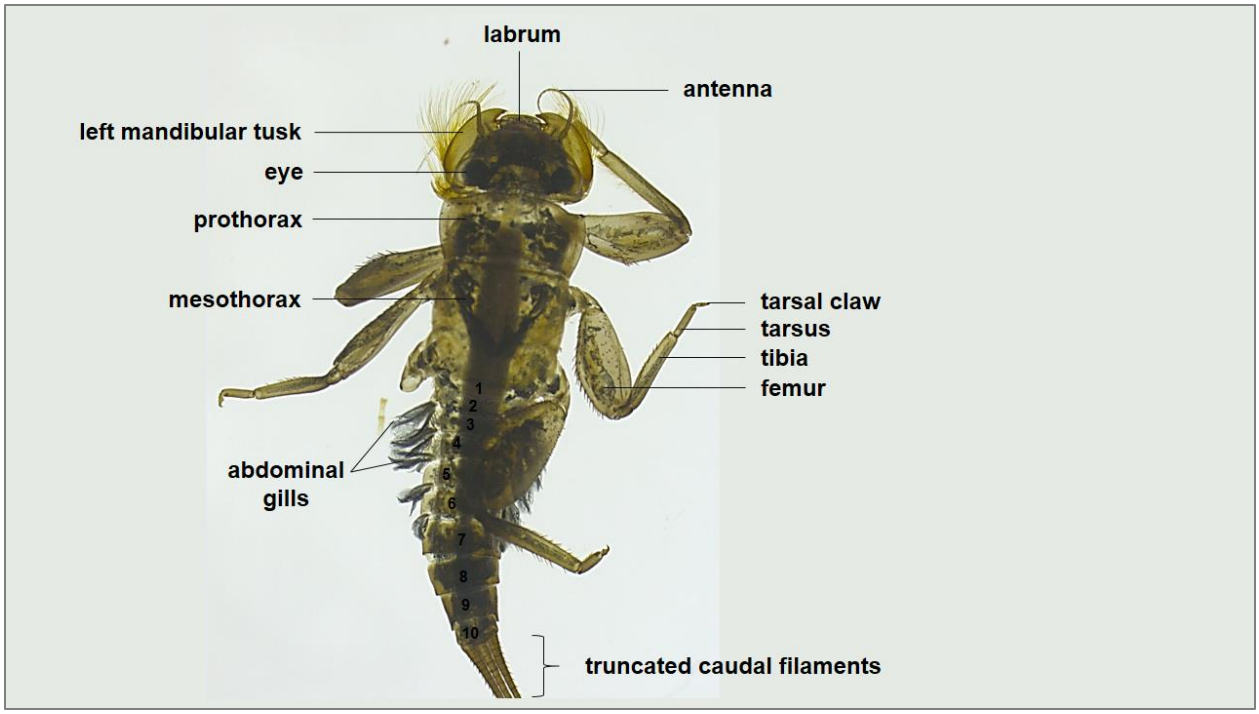


Figure 2-2: Dorsal view of *Euthraulus sp.* nymph, Ephemeroptera.

2.6.2 Plecoptera (Stoneflies)

There are more than 3497 described species of Plecoptera worldwide of which, 33 are said to be found in South Africa (Naiman, 2008; Fochetti & Tierno de Figueroa, 2008). The ecological preferences of stoneflies as well as the reduced flight capacity of the adult form, make them highly endemic (Naiman, 2008; Fochetti & Tierno de Figueroa, 2008). Stonefly larvae are aquatic and as the name may suggest are generally found in riffles, clinging to cobbles, leaf stacks or wood debris. Instances of the highest species diversity in Plecoptera are associated with cold, well oxygenated streams (Bouchard, 2004e). Their ability to survival low thermal conditions give them competitive advantage as most species prefer warmer climate (Merritt & Cummins, 1996). Stoneflies are easily recognisable by several morphological features such as, a pair of claws at the end of each limb, three segmented tarsi, 10 abdominal segments, mouthparts with defined mandibles, two compound eyes, three ocelli, elongate filiform antennae and a pair of cerci as illustrated in **Figure 2-3**. When present, the gills can be located on various body parts

such as the neck, thorax, coxal and abdomen. The life cycle of stoneflies usually lasts for a year or more. However, there are some species that may be bi- or tri-voltine and the nymphs can moult up to 33 times before emerging. Most stoneflies are either shredders or predators, with only a few exceptions of scrapers (Naiman, 2008; Bouchard, 2004e; Merritt & Cummins, 1996).

The Perlidae which is the most widespread in the Northern hemisphere, are the least common stoneflies in Africa. They are a family of predators with an overall large body size of 20 – 50 mm (Bouchard, 2004e). The Nemouridae, also known for their characteristic brown colour, are shredders with a smaller body size of 5 – 20 mm (Bouchard, 2004e).

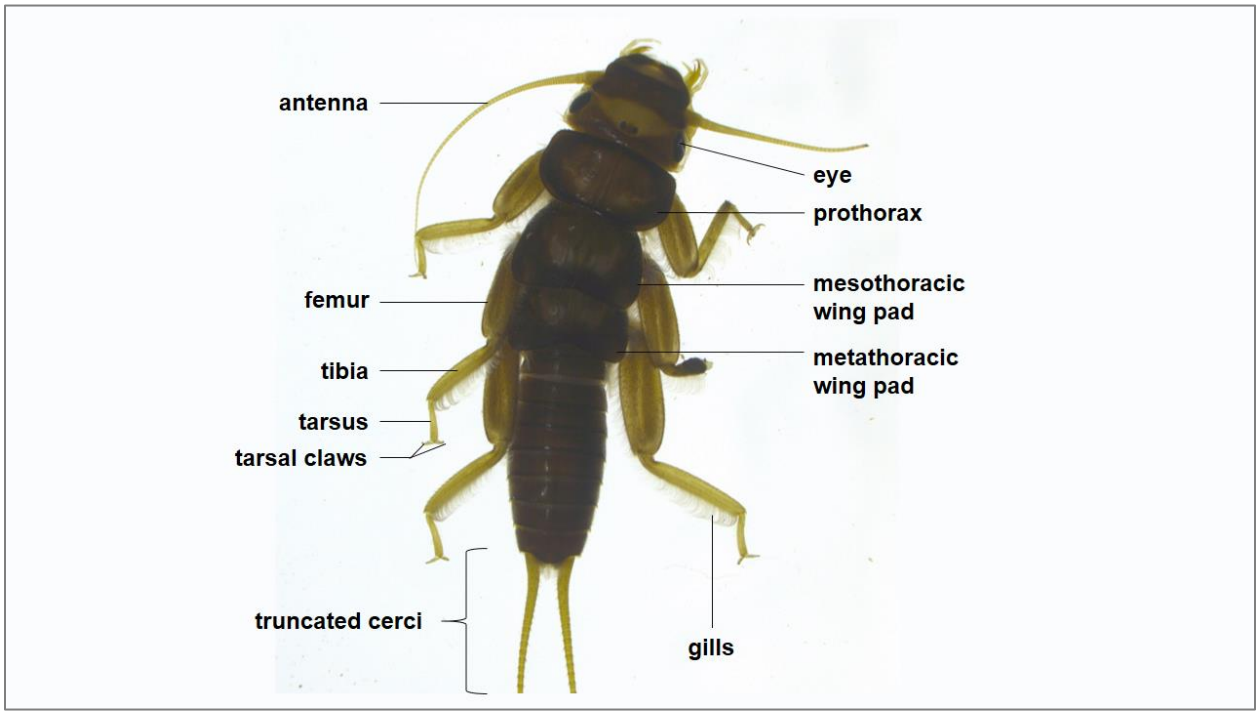


Figure 2-3: Dorsal view of Plecoptera larva, *Neoperla spio*.

2.6.3 Diptera (True flies)

Insects that fall under the order Diptera include, mosquitoes, gnats, midges, houseflies and craneflies. The name Diptera means ‘two wings’, a characteristic feature for adult flies in this order. The size of true flies varies ranging from about 1mm (e.g. midges) to about 70mm (e.g. Robber flies). The larva (grub like, with no legs) of Diptera is very different from the adult form. Similar to the majority of known insects, immature Diptera are very different from their adult forms, in structure, habitat and habitat requirements. The requirements are generally similar for all members of a particular family. For example, larvae of all blackflies (Simuliidae) live in fast flowing rivers for instance while those of brine flies are found in chemically harsh environments (saline waters). Incubation time varies depending on the type of species and can range from a couple of hours, days to months. Maturations or developmental stages move from larva to pupa and then finally the adult form. Pupae can either be entirely inactive or they may be able to swim but they do not feed. The body of both the adult and larva Dipteran is divided into three regions or tagmata, namely, the head; which comprises of a pair of sensory antennae, a pair of mandibles for chewing, a labrum, a pair of maxillae, and a pair of fused second maxillae known as the labium. The adults also contain a thorax that has a pair of jointed legs on each segment of the thorax. The abdomen consists of 11 segments in both adult and larva, but only 9 to 10 are visible to the naked eye.

2.6.3.1 Simuliidae *(Blackflies)*

Blackflies have a worldwide distribution and are known for their pest propensity and the bloodsucking habits of the female adult species to acquire blood essential for egg development. However some species with poorly developed mouth parts are able to develop their eggs without blood (Crosskey, 1962). In some parts of the world, infestations from blackflies have led to reduced tourism, death of wild life and domestic animals and the transmission of diseases (Naiman, 2008; Myburgh, 2002; Currie & Adler, 2007), thus many species of Simuliidae are of economic, veterinary and medical importance (Day *et al.*, 2003). For example *Simulium edwardsellum damnosum* is a

vector for the parasite that causes river blindness, a disease affecting large parts of West Africa (Naiman, 2008; Ugwuanyi *et al.*, 2015; Myburgh & Nevill, 2003; Myburgh, 2002; Post *et al.*, 2011; Crosskey, 1962). There are 214 described species in the Afrotropics from two genera of which the *Simulium* is the most widespread (Day *et al.*, 2003). Blackflies undergo four stages of development that include egg, larva, pupa, and the adult stage and the first three stages of development are aquatic (Day *et al.*, 2003). Generally, simuliids tend to undergo 7 instar stages before maturation. Morphologically, the head capsule is sclerotized and is flanked by a pair of labral/ cephalic fans. A proleg is located ventrally on the lower thorax and consist of a posterior swollen abdomen giving them a vase-like form. The abdominal segment terminates with a posterior circlet of hooks. The last three stages of development mentioned above are necessary for efficient taxonomic identification to species level (Naiman, 2008; Bouchard, 2004b; Kiel, 2001; Currie & Adler, 2007). Under large population densities, blackflies serve as a food source for Plecoptera and other invertebrate species. Simuliids generally feed on fine particulate matter and overwhelming daily inputs of faecal pellets into the water column reaching 429 tonnes have been reported in some parts of the world (Naiman, 2008; Currie & Adler, 2007; Gullan & Cranston, 2014). This further highlights the ecological importance of benthic macroinvertebrate because the inability to process such organic matter could lead to waterborne diseases in poor communities. In medical research blackflies are considered to be the second most important group of insects and for this reason simuliids have received much attentions among taxonomists although there are still significant numbers of species of the world's Simuliidae that remain undiscovered (Naiman, 2008; Currie & Adler, 2007).

The blackfly pest status has been a problem in South Africa since the 1960s and continues to be a major cause for concern among livestock farmers located along the Vaal and Orange River (De Beer & Kappmeier Green, 2012). A network of rivers have been severely impacted by outbreaks over the years and despite efforts to try and control these outbreaks, blackflies remain a nuisance and a major threat to the South African economy (Myburgh & Nevill, 2003; De Beer & Kappmeier Green, 2012). The Simuliidae are grouped into two types of pest species, the mammalian pest species which include

Simulium metomphalus chutteri and *Simulium edwardsellum damnosum*, and the avian pest species which consist of *Simulium meilloniellum adersi* and *Simulium nevermannia nigritarse*. Of the four pest species, *Simulium metomphalus chutteri* is considered the most troublesome and has caused significant damage along the Orange River (Myburgh, 2002; Palmer & Palmer, 1995).

2.6.3.2 Chironomidae *(non-biting midges)*

Chironomidae have a worldwide distribution that includes Antarctica and some oceanic islands (Naiman, 2008; Ferrington, 2007). They occur over a wide range of altitudes and are highly tolerant to changes in temperature and anoxic conditions. Thus, they tend to inhabit most water bodies including mud puddles (Gullan & Cranston, 2014; Entrekin *et al.*, 2007). Because of their ability to withstand a wide range of environmental conditions Chironomidae tend to be the most dominant aquatic invertebrates, both in abundance and species richness (Naiman, 2008). The immature stages of development are primarily associated with the aquatic phase and the adults are terrestrial. Traditionally, species identification has been primarily focused on the adult form and immature stages have been identified with some level of uncertainty across tribes and sometimes species within a genus. However there are 339 genera and 4,147 species that have been described (Naiman, 2008; Ferrington, 2007). Several taxonomists across the globe have made contributions to the keys that are currently available for species identification (Cranston *et al.*, 1995). However, there are limitations to the use of these keys as they are dominated by species from the Palearctic and Nearctic Regions (Naiman, 2008; Cranston *et al.*, 1995; Ferrington, 2007), highlighting the need to have illustrative keys from the Afrotropical Region and other underrepresented Regions (Cranston *et al.*, 1995). In addition to their economic, medical and nutritional value, chironomids also serve as important indicators of habitat degradation (Cranston *et al.*, 1995). For example, a study conducted by Odume *et al.* (2014) demonstrated that small sized chironomids were highly sensitive to water pollution resulting in species loss (Odume *et al.*, 2014). This suggests that Chironomidae size could prove more informative when determining water quality compared to Functional Feeding Groups (FFG) as feeding mechanisms tend to vary

based on food availability. Furthermore, chironomids use a wide range of feeding mechanisms and these differences in feeding modes indicate a wide variety of food preferences, thus making it difficult to associate a particular feeding to a type of species.

2.6.4 Trichoptera (Caddisflies)

Caddisflies have a worldwide distribution and as with Chironomids, species description has been primarily based on adults. Of the 54 known genera, 10 are endemic to South Africa (de Moor *et al.*, 2003a). The larval stage is the dominant phase in the Trichopteran life cycle (**Figure 2-4**). Tolerance to fluctuating levels of dissolved oxygen varies among species of caddisflies and they are thus useful indicators of organic pollution in the aquatic ecosystem (de Moor *et al.*, 2003a). Caddisfly larvae are morphologically distinguishable from those of other insects by the presence of two anal prolegs **Figure 2-4**, each with a single curved claw (de Moor *et al.*, 2003a). Trichopteran species are spread across all functional feeding groups and as with species of mayfly and stoneflies, caddisflies are good indicators of environmental pollution (Bouchard, 2004d).

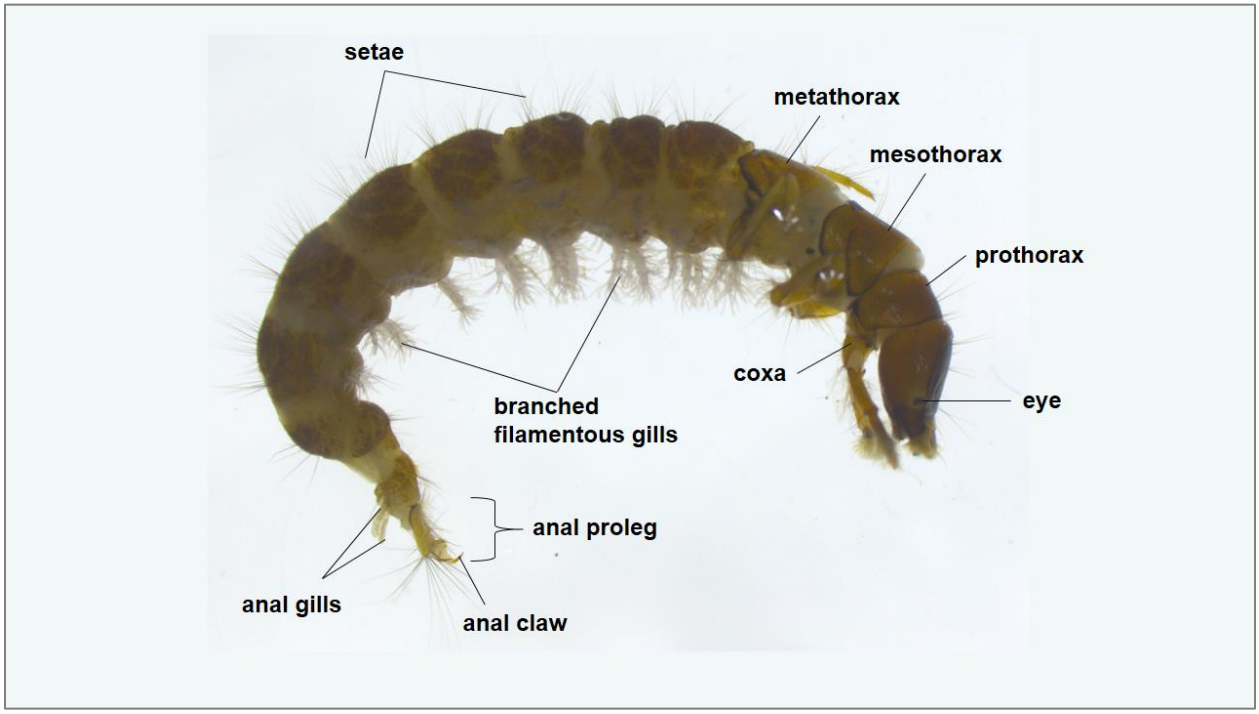


Figure 2-4: Lateral view of Trichopteran larva, *Macrostemum capense*.

The macroinvertebrate bioassessment approach is considered to be both efficient and cost effective and is designed to facilitate rapid assessment of large regions over a short time frame to pinpoint areas of concern worthy of more detailed investigation (Resh *et al.*, 1995). Cummins *et al.* (2005) defines two types of approaches researchers can use to assess changes in freshwater ecosystems. The first is the taxonomic approach, which is suitable for determining biodiversity and species sensitivity to changes in water chemistry. The second is the functional approach which is an approach that uses functional feeding groups to assess the integrity of the aquatic ecosystem (Cummins *et al.*, 2005). In the present study the taxonomic approach was used to establish empirical relationships between macroinvertebrate data and water chemistry, and the choice of method was based on stream properties, species assemblage and abundance.

2.7 Synopsis

The coal mining industry poses a huge threat to the environment. Yet in the context of developing countries, coal resources appear to be the very commodity tied to social and economic growth. It is not feasible to stop industrialisation and socioeconomic development even in light of the adverse effects they pose on the environment. It is however ideal to exercise precautions while attaining progress. Therefore, any country that is largely dependent on coal as an energy source should commit to addressing the environmental challenges associated with coal mining and these tend to vary in severity depending on whether the mine is currently operating or abandoned, the type of mining method used as well as the geological make-up of the mining area (Bian *et al.*, 2010). The deposition data from the Mpumalanga region and the presence of acid sensitive soils and waters in some parts of South Africa seem to suggest that there might be an acidification problem however due to the paucity of relevant data the geographical extent of the problem is unknown. The present study has set out to identify regions of South Africa that are vulnerable to acidification due to their location on acid sensitive soils and presumably waters as well as their close proximity to major sources of acid deposition such as coalfired power stations.

As indicated previously, acidification occurs when sulphur and nitrogen compounds fall onto terrains that have acid sensitive soils and waters. This phenomenon has primarily been associated with the Holarctic region but more recent studies have affiliated acid deposition and stream acidification with developing countries in East Asia (Nagase & Silva, 2007; Bhatti *et al.*, 1992; Wilkening, 2004; Akimoto *et al.*, 2006). In the next section of the work, soils data from the global and national Soil and Terrain (SOTER) digital database were used to identify acid sensitive soils in South Africa.

Chapter 3 Defining study regions and sampling sites

Regions with poorly buffered soils and waters located in and around South Africa were mapped out (**Figure 3-1**) based on literature review (Britton *et al.*, 1993; Silberbauer & King, 1991; Skoroszewski, 1995; Grobbeiaar & Stegmann, 1987). As a result, six potential study areas were identified namely, the Highveld (Mpumalanga), Waterberg (Limpopo), KwaZulu-Natal, the south-western Cape, the Berg River (Western Cape) and Lesotho. The Highveld region in Mpumalanga where 80 % of the Eskom coal power stations are located has the highest reported deposition in South Africa (Josipovic *et al.*, 2011; Josipovic *et al.*, 2010; Freiman & Piketh, 2003; Bohm, 1985). However, the lack of up to date and systematic monitoring of surface waters in this region has left a knowledge gap pertaining to the acidification status of these waters. Moderate deposition has been reported for the Waterberg and KwaZulu-Natal regions (Josipovic *et al.*, 2011) and an increase in emissions is expected for the former upon commissioning of the new coalfired power station (Medupi). The Western Cape is known for having naturally acidic black and white waters (Midgley & Schafer, 1992) however, no deposition data have been reported for this region. Furthermore, because of the lack of coal resources, the Western Cape is presumed to have low deposition rates. Lesotho is a small country located within South Africa and a valuable water supplier to South Africa. Streams from Lesotho are poorly studied albeit, there is evidence to suggest that some wetland streams from this region have acid-sensitive waters (Dunnink *et al.*, 2016).

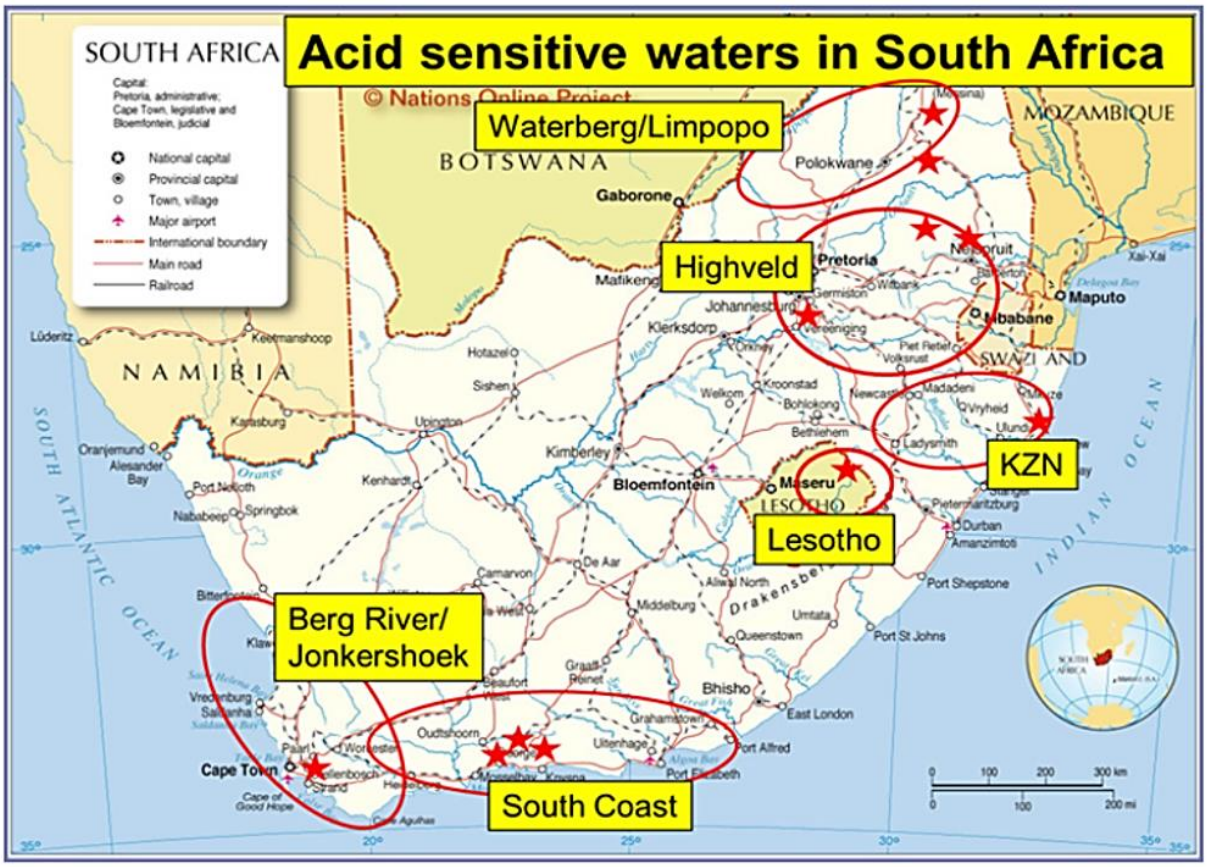


Figure 3-1: Six regions around South Africa associated with high (Mpumalanga), moderate (Waterberg and KwaZulu-Natal) and low acid deposition loads (South Coast, Berg River/ Jonkershoek and Lesotho). The map was assembled based on literature review and streams are represented by the red stars. (**Source:** Prof. C. Curtis – University of the Witwatersrand).

The acid-sensitive regions (**Figure 3-1**) are presumably a reflection of the geology and soil type found in the catchment area. To test this theory streams identified by reviewing literature (**Figure 3-1**) were overlaid onto Humic and Podzolic soils from Fey (2010) (Fey, 2010a; Fey, 2010b). The resulting map (**Figure 3-2**) indicated that the potential study regions were found in and around catchments with soils that have a low buffering ability against strong acids. The final decision was to focus the study on three of the most sensitive regions namely the Highveld of Mpumalanga, the Waterberg and the south-western Cape. These regions were categorised to represent (assumed) high, moderate and low deposition respectively, based on the distribution of coal power stations and acid sensitive soils and waters. Prior to defining sampling points, acid sensitive soils were

identified and classified to represent high, moderate and low sensitivity. Soils showing high sensitivity were then used to identify potential study sites.

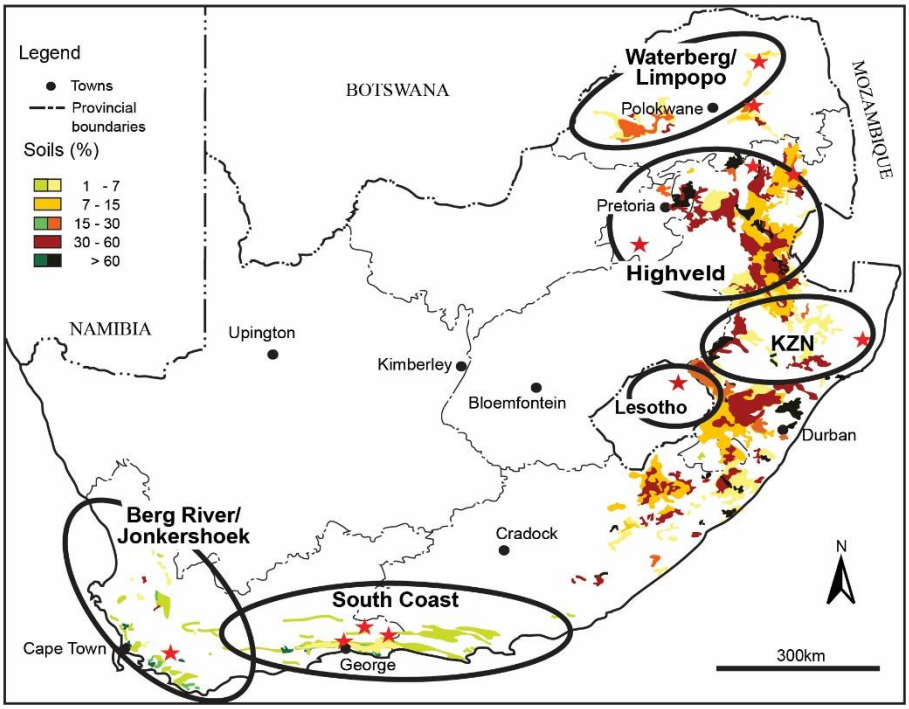


Figure 3-2: Literature based preliminary assessment to determine proximity of sensitive waters (red stars) to sensitive soils. Acid sensitive streams are presumably a reflection of the geology and soil type found in the catchment area. (**Source:** Cartographic unit, University of the Witwatersrand, South Africa).

3.1 The use of geographic information system (GIS) to identify acid sensitive soils

Prior to site selection, soil classification was attempted using various methods described below. The purpose for classifying soils according to their sensitivity to acid deposition was to help guide the site selection process as there are no records of distribution data for acid-sensitive streams in South Africa. Furthermore, no previous studies have investigated the effects of acid deposition on South African headwater streams. Thus, soils and geological data were used as the basis for defining areas with potentially acid sensitive headwater streams across all study regions.

3.1.1 The global and national Soil and Terrain (SOTER) digital database

The SOTER database consists of two soils datasets, one for South Africa (SOTER_ZA) and the other for Southern Africa (SOTERSAF). Both datasets were obtained from the International Soil Reference and Information Centre (ISRIC) website. The database is structured as described in the SOTER manual (2002) (**Figure 3-3**), where the different blocks represent separate relations (tables) found in the database (Tempel, 2002). For the purposes of this study the SoilComponent relation, which among other things, indicates the percentage of the SOTER unit that is occupied by a particular soil type and the RepresentativeHorizon relation that contains information about the soil profile (e.g. Soil horizons) and soil chemistry were used during soil classification. The different fields and attributes represented on each relation are shown in **Figure 3-4**. The relation SoilComponent contains the ISO country code (ISOC), the SOTER unit-ID (SUID) and the Representative profile-ID (PRID). The Representative profile-ID links the SoilComponent relation to the RepresentativeHorizon relation as shown by the arrow (**Figure 3-4**). An example of the SOTERSAF map and the dominant soils legend as used in the FAO Soil map of the World is shown in **Figure 3-5**. The combination of the ISO country and the SOTER unit-ID represent a polygon for a specific dominant soil as defined by the FAO-Unesco Soil Map of the World revised legend (FAO, 1988). For example, ACh is a dominant soil, where AC (Acrisols) is the major soil as defined by FAO 1997 and

h (haplic) is the qualifier or formative element used for naming soil units (**Figure 3-5**). The SOTER_ZA and SOTERSAF datasets are identical however, all classification was carried out using the SOTERSAF dataset because it facilitated easy linkage between the SoilComponent relations and the soil profile relations.

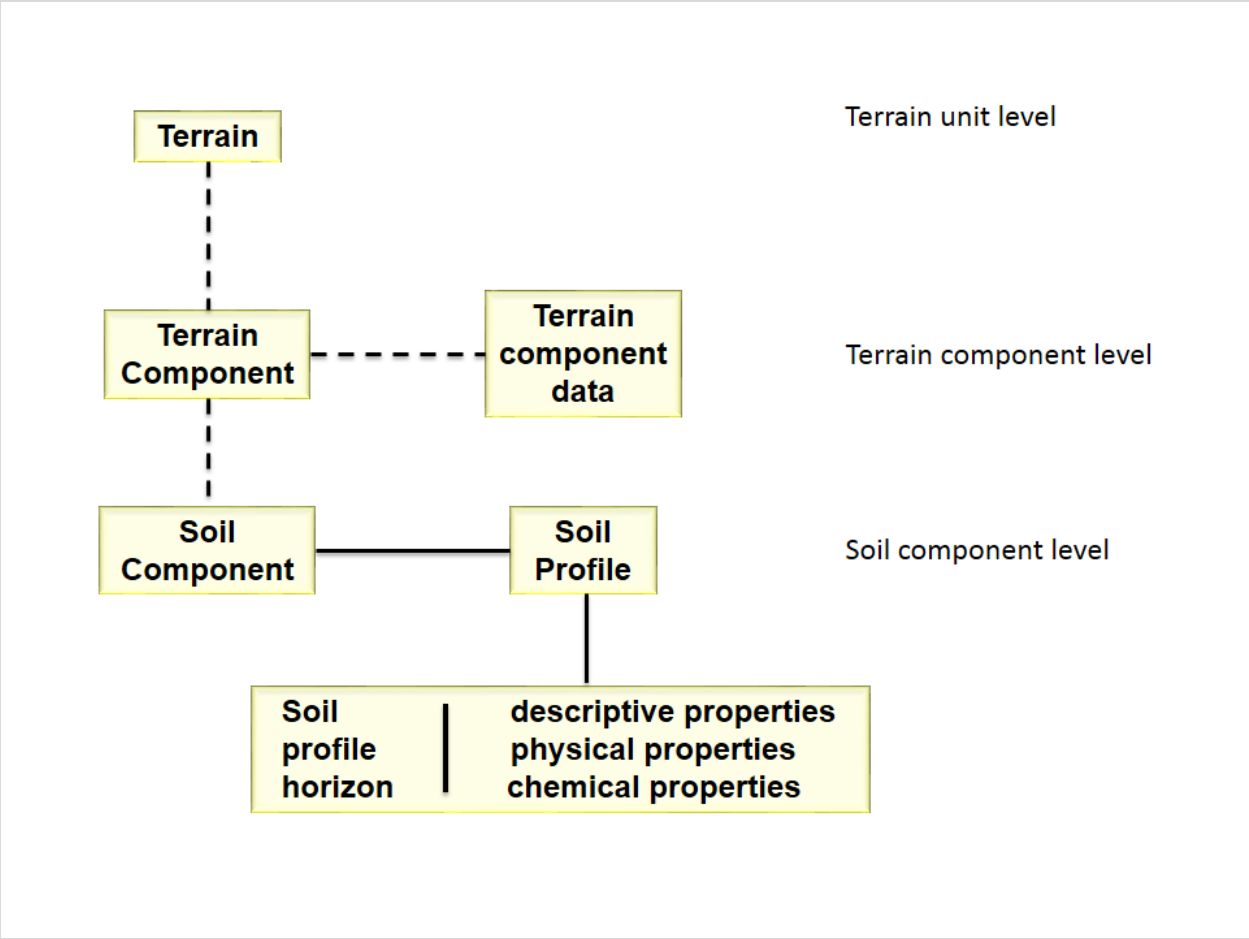
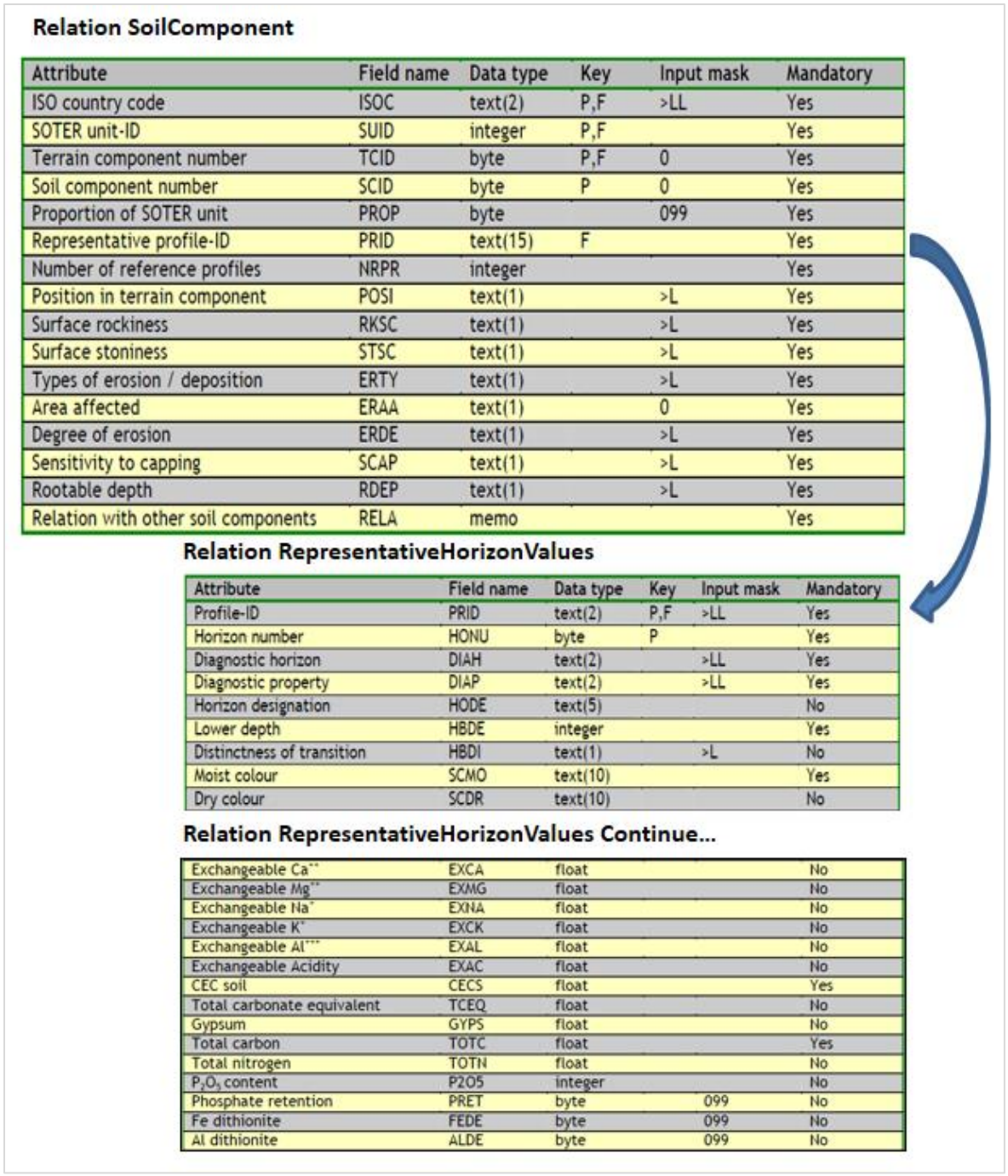


Figure 3-3: A schematic representation of the different relations found in the SOTER database adopted from Tempel (2002) with slight modifications. Examples of the fields found in the Soil component and Soil profile horizon relations are given in **Figure 3-4**.



Relation SoilComponent

Attribute	Field name	Data type	Key	Input mask	Mandatory
ISO country code	ISOC	text(2)	P,F	>LL	Yes
SOTER unit-ID	SUID	integer	P,F		Yes
Terrain component number	TCID	byte	P,F	0	Yes
Soil component number	SCID	byte	P	0	Yes
Proportion of SOTER unit	PROP	byte		099	Yes
Representative profile-ID	PRID	text(15)	F		Yes
Number of reference profiles	NRPR	integer			Yes
Position in terrain component	POSI	text(1)		>L	Yes
Surface rockiness	RKSC	text(1)		>L	Yes
Surface stoniness	STSC	text(1)		>L	Yes
Types of erosion / deposition	ERTY	text(1)		>L	Yes
Area affected	ERAA	text(1)		0	Yes
Degree of erosion	ERDE	text(1)		>L	Yes
Sensitivity to capping	SCAP	text(1)		>L	Yes
Rootable depth	RDEP	text(1)		>L	Yes
Relation with other soil components	RELA	memo			Yes

Relation RepresentativeHorizonValues

Attribute	Field name	Data type	Key	Input mask	Mandatory
Profile-ID	PRID	text(2)	P,F	>LL	Yes
Horizon number	HONU	byte	P		Yes
Diagnostic horizon	DIAH	text(2)		>LL	Yes
Diagnostic property	DIAP	text(2)		>LL	Yes
Horizon designation	HODE	text(5)			No
Lower depth	HBDE	integer			Yes
Distinctness of transition	HBDI	text(1)		>L	No
Moist colour	SCMO	text(10)			Yes
Dry colour	SCDR	text(10)			No

Relation RepresentativeHorizonValues Continue...

Exchangeable Ca^{++}	EXCA	float		No
Exchangeable Mg^{++}	EXMG	float		No
Exchangeable Na^{+}	EXNA	float		No
Exchangeable K^{+}	EXCK	float		No
Exchangeable Al^{+++}	EXAL	float		No
Exchangeable Acidity	EXAC	float		No
CEC soil	CECS	float		Yes
Total carbonate equivalent	TCEQ	float		No
Gypsum	GYPS	float		No
Total carbon	TOTC	float		Yes
Total nitrogen	TOTN	float		No
P_2O_5 content	P2O5	integer		No
Phosphate retention	PRET	byte	099	No
Fe dithionite	FEDE	byte	099	No
Al dithionite	ALDE	byte	099	No

Figure 3-4: Soil component Relation and the Representative Horizon relation. The Soil component relation and the representative horizon relation are linked by the PRID (profile-ID) as indicated by the arrow. In the Soil component relation, the PRID corresponds to its respective SIUD value which together with the ISO country code represents the polygon on the map. The Representative horizon relation contains the descriptive (e.g. horizons number), physical (e.g. moist colour) and chemical (e.g. soils CEC) properties for a given soil profile.

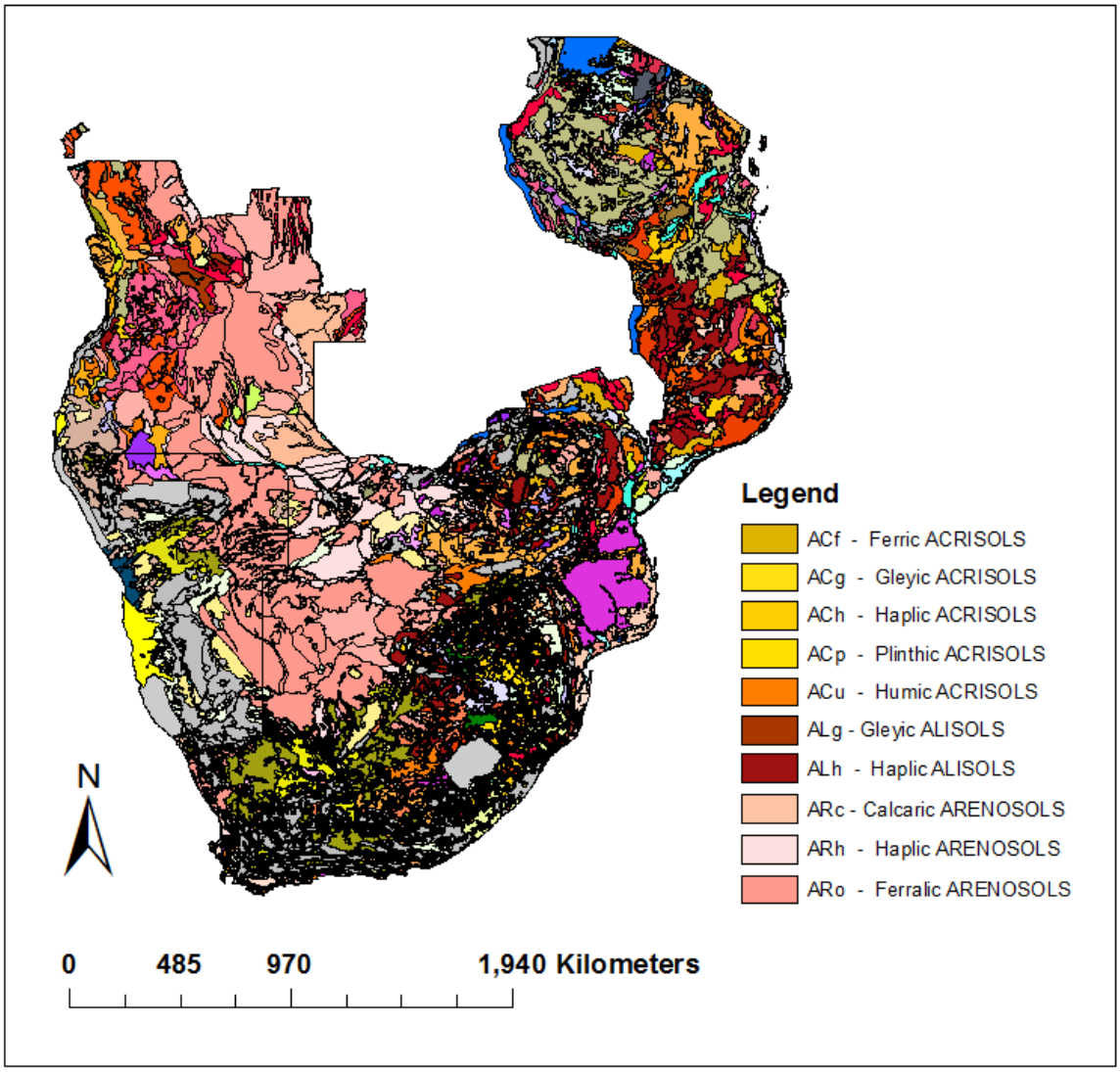


Figure 3-5: SOTERSAF (2003) map for dominant soils. Soil names as described in section 3.1.1 are shown in the Legend.

a) Soil classification using Base saturation (BS) and Cation exchange capacity (CEC) values

Kuylenstierna *et al.* (1995) have proposed a technique for classifying soils according to their sensitivity to acid deposition. During the classification process, soils were categorised into five soil classes ranging in sensitivity, where class 1 contained the most sensitive soils and class 5, the least sensitive based on BS and CEC. A few years later, Cinderby *et al.* (1998) published a paper where they classified acid sensitive soils of the world using the classification system proposed by Kuylenstierna *et al.* (1995). Soil depth and density played a key role during the classification process. Mean BS and CEC values at 50 and 100 cm depth were used to assign sensitivity classes to the soil types as defined by the FAO 1990. In cases where the class sensitivity differed between the two depths the minimum value was assigned to the sensitivity class. The accuracy of this approach was evaluated by the authors upon which they confirmed that the map produced from their study showed patterns and degrees of sensitivity consistent with those from other studies conducted in the UK, and other parts of Europe.

In the present study, the SOTERSAF dataset was used to classify South African soils following the method proposed by Kuylenstierna *et al.* (1995). Soils were assigned sensitivity values (**Table 3-1**) however, unlike the technique employed by Cinderby *et al.* (1998), here, soil depth was disregarded. This was because on the SOTER database the soil depth is presented as rootable depth, where the 50 and 100 metre depth are defined as one categorical variable (**Table 3-2**), instead of separate entities as presented by Cinderby *et al.* (1998). Nonetheless, as in Cinderby *et al.* (1998), some soil profiles (**Table 3-1**) had two different soil classes (rows 12 – 16) and in such instances, likewise, the minimum sensitivity value was assigned to the soil profile. For example, Paraplinthic Arenosols (ARh) soils (rows 12 – 14) were assigned to class 4 and Rhodic Lixisols (LXh) soils (rows 15 – 16) to class 1. Following the above procedure, a soil sensitivity map (**Figure 3-6**) was produced and only class 1 (highly sensitive soils) and class 2 (sensitive soils) soils are represented on the map. The resultant map was exceptionally sparse when compared to maps produced from other studies (Fey, Josipovic and Cinderby) thus, an alternative approach was attempted.

Table 3-1: Assigning sensitivity values to soil profile. The Soil component relation and the representative horizon relation are linked by the PRID (profile-ID). In the Soil component relation, the soil Profile-ID corresponds to its respective SIUD value which together with the ISO country code (**ISOCSUID**) represents the polygon on the map. The Representative horizon relation contains the descriptive (e.g. horizons number), physical (e.g. moist colour) and chemical (e.g. soils CEC) properties for a given soil profile. For the purposes of this study just the chemistry (BS and CEC) properties of the soil are presented on the table below.

	Profile-ID	Soil	Sensitivity Class	BS	CEC	ISOCSUID
1	ZA1	ACh	1	15.0	4.0	744
2	ZA1	ACh	1	8.1	3.7	744
3	ZA1	ACh	1	5.0	4.0	744
4	ZA1003	ACh	1	14.2	10.6	498
5	ZA1003	ACh	1	6.9	7.3	460
6	ZA1003	ACh	1	7.8	5.1	460
7	ZA1004	ACh	1	34.7	7.2	416
8	ZA1004	ACh	1	32.3	6.5	524
9	ZA10423	CMc	5	167.3	9.2	2079
10	ZA10423	CMc	5	237.2	8.7	2360
11	ZA10423	CMc	5	211.4	8.2	2360
12	ZA130	ARh	5	98.2	5.6	1163
13	ZA130	ARh	4	73.9	11.9	1163
14	ZA130	ARh	5	105.1	17.5	1163
15	ZA1817	LXh	1	37.5	5.6	896
16	ZA1817	LXh	2	46.0	4	896

Table 3-2: Rootable depth as defined on the SOTER database.

Code name	Descriptor	Depth
V	Very shallow	< 30 cm
S	Shallow	30 – 50 cm
M	Moderately deep	50 – 100 cm
D	Deep	100 – 150 cm
X	Very deep	≥ 150 cm

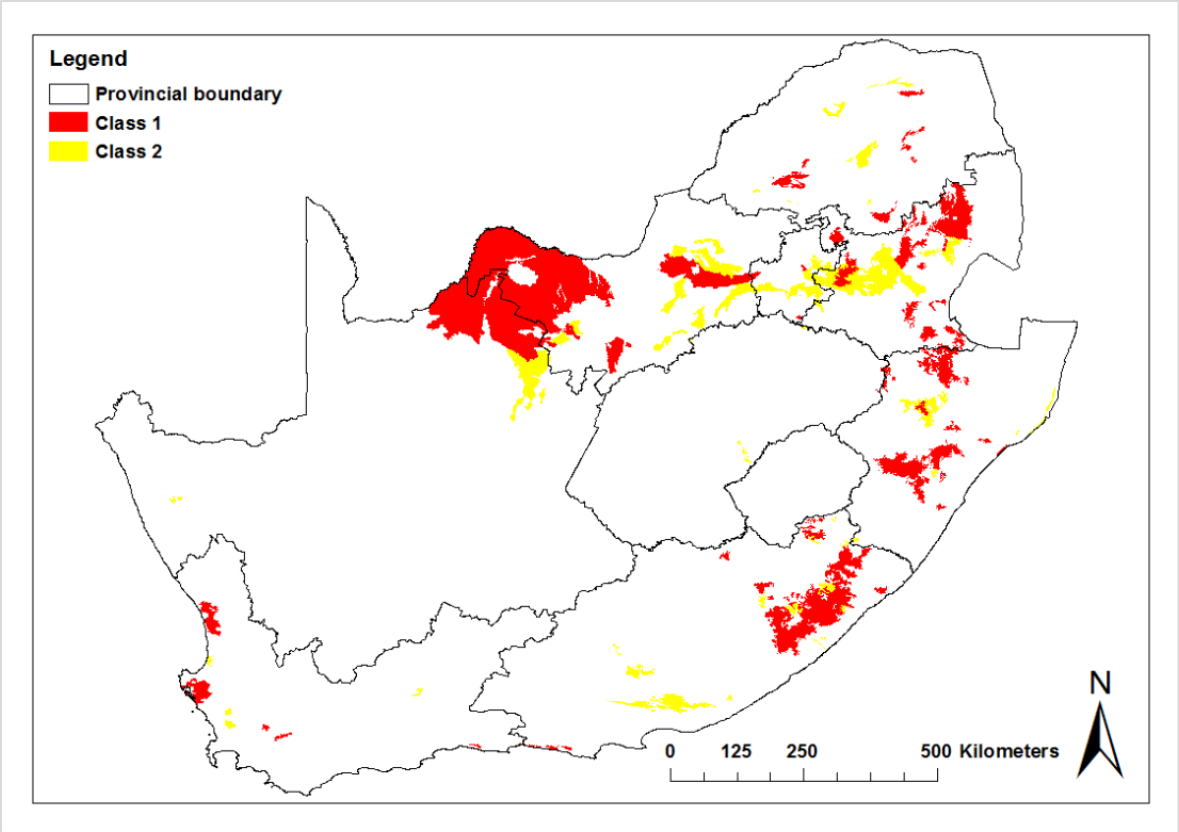


Figure 3-6: Distribution of acid sensitive soils classified using BS and CEC. Only soils representing sensitivity class 1 and 2 from table 3-1 above were plotted on the map.

b) Soil classification using diagnostic horizon and soil properties

One other method for assigning sensitivity classes to soil profiles is by looking at diagnostic horizons. A list of major soils, soil units, diagnostic horizons and diagnostic properties are described in the FAO – Unesco Soils revised legend 1997 manual. Globally soils have been organised into seven master horizons designated H, O, A, E, B, C and R. The H horizon is dominated by organic material and is often the top layer of mineral soils. Whereas the O horizon is found on top of either mineral or organic soils. The A horizon forms below the O horizon and is characterised by the accumulation of humidified organic matter. E horizons are layers of minerals characterised by low silicate clay, iron and aluminium content and high concentration of sand and silt particles. The B horizon is deficient of natural rock structure and is characterised by the illuviation of silicate clay,

iron, aluminium, humus, carbonates, gypsum and silica or a combination of these. The C horizon lacks hard bedrock and is generally not affected by pedogenesis. The R horizon on the other hand consists of hard bedrock such as granite, basalt, quartzite, and indurated limestone or sandstone. A diagnostic horizon consists of physical features that are used to describe and define soil classes. Using these descriptors soil classes were assigned to different soil types. As with the previous section, some soil profiles displayed characteristics that qualified them for more than one soil class, **Table 3-3** (rows 1 – 3 and rows 7 – 9). Rows 1 – 3 indicate that the Calcic Luvisols (LVk) soils have 3 horizons (ABC), with either OC (Ochric) or AR (argic) as the diagnostic horizons. Generally, Luvisols (LV) are soils which have an argic B horizon with a CEC ≥ 24 cmol (+) kg^{-1} clay and a BS ≥ 50 % throughout the B horizon (FAO revised legend 1997 - https://library.wur.nl/WebQuery/wurpubs/451104). The OC horizon is characterised by its very light colour, high chroma and low organic matter content. The argic B horizon is a subsurface horizon that is characterised by a distinctively, high clay content when compared to the overlaying horizon and for an argic B horizon to be considered as diagnostic it should consist of more total clay than the overlaying coarser horizon, in this case OC. Taking all these factors into account and considering that the clay content directly affects BS, the soil profile was assigned to a sensitivity class that corresponded to the argic B horizon, class 5.

Table 3-3: Assigning class sensitivity using diagnostic horizons and diagnostic properties. The Soil component relation and the representative horizon relation are linked by the PRID (profile-ID). In the Soil component relation, the soil Profile-ID corresponds to its respective SIUD value which together with the ISO country code (**ISOCSUID**) represents the polygon on the map. The Representative horizon relation contains the descriptive (e.g. horizons number), physical (e.g. moist colour) and chemical (e.g. soils CEC) properties for a given soil profile. The descriptive, physical and chemical properties of the soil were used during this classification procedure.

	Profile - ID	CEC soil	BS	Sensitivity Class	ISOCSUID	Soil	Horizon number	Horizon designation	Diagnostic horizon	Clay %
1	ZA113	8.2	78.0	3	1461	LVk	1	A	OC	18
2	ZA113	19.3	102.5	5	1461	LVk	2	B	AR	52
3	ZA113	26.8	98.7	5	1461	LVk	3	C		53
4	ZA11948	12.4	13.2	1	2364	FRu	1	A		34
5	ZA11948	9.2	7.9	1	2364	FRu	2	A		33
6	ZA11948	6.5	15.7	1	2364	FRu	3	B	FA	39
7	ZA11952	6.2	65.3	3	2464	PLd	1	A		12
8	ZA11952	6.1	57.8	2	2464	PLd	2	E		11
9	ZA11952	18.5	75.7	4	2464	PLd	3	B		53

Rows 7 – 9 show soil profiles with 3 horizons designated AEB with a different sensitivity class each and no diagnostic horizon (FAO revised legend 1997). Planosols (PL) are soils that have an E horizon showing stagnic properties which are soil properties associated with water saturation and the reduction of iron. Dystric Planosols (PLd) are Planosols that have an Ochric A horizon and a BS < 50 %. Dystric is a formative element for naming dystrophic and infertile soil units with low BS. The presence of the E horizon is a definitive feature for this soil type and thus, the sensitively class corresponding to the E horizon, class 2, was assigned to the overall soil profile. Following these and other soil specific guidelines defined by the FAO-Unesco Soil Map of the World revised legend 1997, a map representing acid sensitive soils of South Africa was produced using diagnostic horizons and diagnostic properties, results not shown. However, as with the map produced in section (a) above, the final map produced in this section of the thesis was sparse and not consistent with other maps produced from previous studies (Fey, 2010a; Josipovic *et al.*, 2011), results not shown. Consequently, a different approach was attempted.

c) Literature-based soil classification

Several soil classification methods have been documented in the literature with each method applicable subject to the amount of soil data that are available for that country or region as well as on the purpose (pedology, agriculture, forestry etc.) for classifying the soils. Soil chemistry data were used for the classification in sections (a) and (b) however, upon careful scrutinisation of the GIS data it was discovered that the chemistry data were missing for about 2/3 of the soils on the SOTER database, suggesting that not all the sensitive soils were represented in the previous sections, which would explain the sparsity of the maps produced previously, and thus an alternative method for classifying soils had to be considered. In the book Soils of South Africa (Fey, 2010b) the author has classified South African soils into 14 soil types representing top-soils or subsurface soils (**Figure 1-2**). Based on the general chemistry and characteristic highlighted for each soil type. Humic soils, Podzolic soils, Plinthic soils and Oxidic Soils were identified as potentially acid sensitive **Figure 1-2**. Examples of each soil type were given, for instance Humic soils include Ferralsols, Acrisols, Luvisols, Cambisols and Umbrisols (Fey, 2010b). Ferralsols, Acrisols, Luvisols, Cambisols, Lixisols and Arenosols were identified as being common across Humic, Plinthic and Oxidic soils. Podzolic soils displayed less variety when compared to the other soil types. A list of formative elements used in naming major soils is given in the FAO-Unesco Soil Map of the world revised legend 1997 manual. Characteristics of all the formative elements listed in the revised legend were used to filter non-sensitive soils based on Fey (2010). Regosols, Arenosols, Podsols, Acrisols and Ferralsols made the final list of acid sensitive soils. These soils were then classified into class 1 or class 2 soils based on the characteristics given on **Table 3-4**, where class 1 soils required at least two of the given characteristics. Regosols for example are formed from unconsolidated material and consist of an ochric or umbric (low base content) A horizon. Eutric Regosols (RGe) have a BS ≥ 50 %, Calcaric Regosols (RGc) are calcareous, Gypiric Regosols (RGy) contain gypsum, Dystric Regosols (RGd) are dystrophic and have a low BS, Umbric Regosols (RGu) have a low base saturation and Gelic Regosols (RGi) have permafrost within 200 cm from the surface. Of the six soil types only RGe, RGc and RGd are found in South Africa, where RGd is the acid sensitive

form and was placed under class 1 based on the classification guidelines highlighted above. Dystric Regosols, Albic Arenosols and Podzols were all grouped under class 1 and the Haplic Acrisols, Ferric Acrisols, Plinthic Acrisols and Ferralsols were all placed under class 2. The soils map produced using this approach gave patterns similar to those observed from previous studies. Furthermore, it was also observed that there was a slight difference in the soil distribution pattern for the maps produced using these three different approaches and thus for efficiency all three maps were combined (**Figure 3-7**) to form a final map that was used for site selection. Other datasets and classification systems were used to compare and validate observed soil patterns, results are included in the appendices.

Table 3-4: Characteristics for defining class 1 and class 2 acid sensitive soils.

Class	Characteristics
Class 1 (extremely sensitive soils)	• Dystrophic soils • Horizons with low base saturation • Soils with albic properties (strong bleaching)
Class 2 (sensitive soils)	• Not strongly Humic • Low base saturation but not necessarily dystrophic

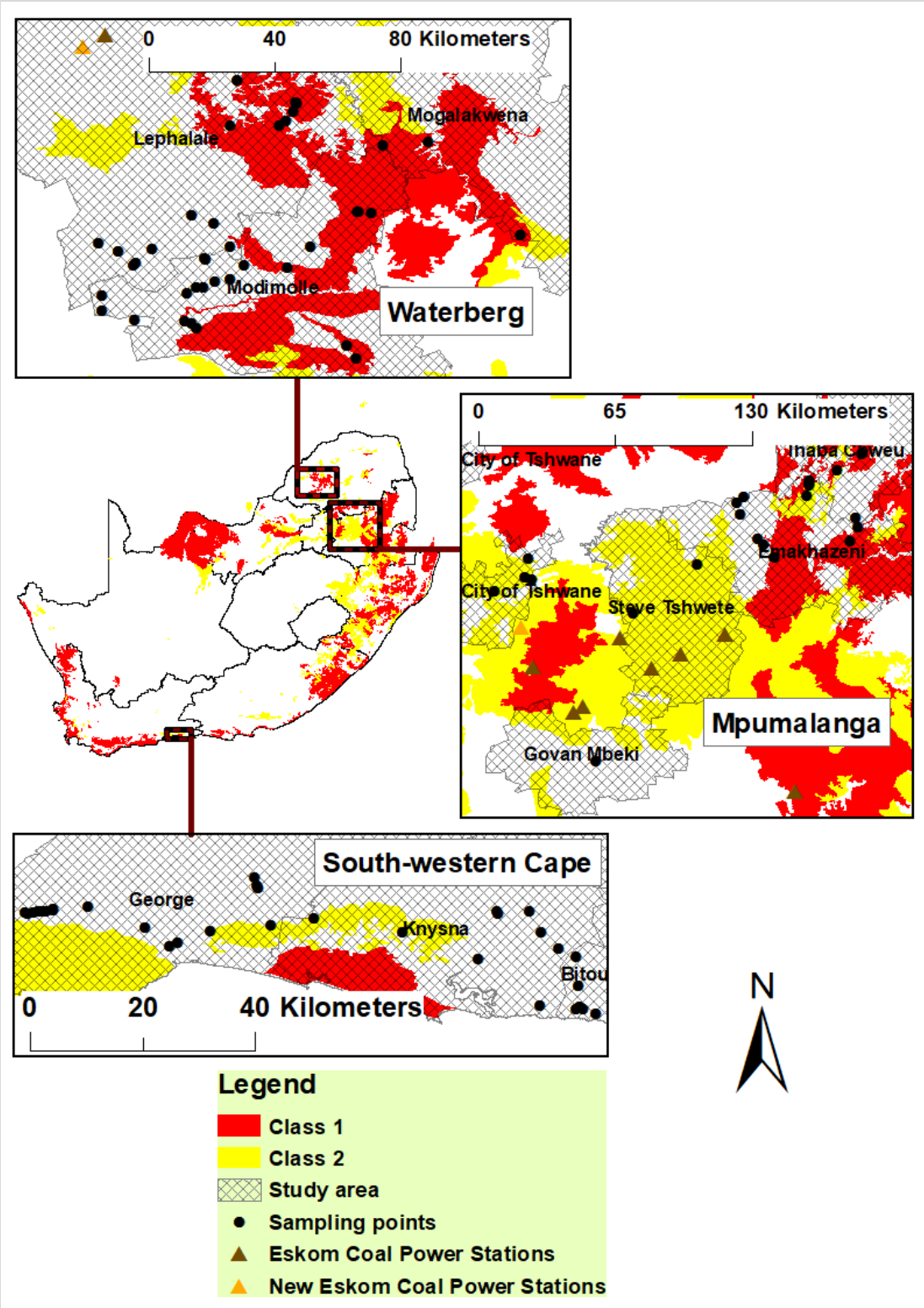


Figure 3-7: Map showing distribution of acid sensitive soils for the Waterberg, south-western Cape and Mpumalanga regions of South Africa. The resultant map is a combination of Soils classified using BS and CEC (**Figure 3-6**), Diagnostic Horizons (**Table 3-3**) and literature review.

3.1.2 National Freshwater Ecosystem Priority Areas (NFEPAs)

There have been various reports addressing the critical state of South Africa's national waters and the threat to biodiversity. In response to these concerns, several freshwater systems in good ecological conditions were identified and designated Freshwater Ecosystem Priority Areas (FEPAs) (Driver *et al.*, 2011). The primary aim for defining these priority areas was to establish the total number of rivers, wetlands and estuaries that exist in the country and to further establish which of these freshwater ecosystems should be kept natural in support of the water resource protection goals of the national water act (Driver *et al.*, 2011; King & Pienaar, 2011; DWA, 2013; Griffin *et al.*, 2014; Nel *et al.*, 2011; Roux & Nel, 2013). The NFEPA project produced several sets of data that can be used as a collective or separately (i.e. river FEPAs, Fish Support Areas and Upstream Management Areas) to address ecological concerns. During the classification process, South African freshwater ecosystems were placed into six (A-F) ecological categories based on habitat integrity and other factors that have direct bearing on water quality. Waterbodies assigned to categories A and B are said to be in good ecological condition because they remain unmodified or mostly natural, while those in C are slightly modified and categories D-F include streams that range from being largely modified to extremely modified. River FEPAs refer to the whole catchment area where streams are assumed to be in good ecological condition. An additional Z category was created for streams with no data or expert opinion. Fish sanctuaries that were slightly modified were identified as Fish Support Areas. Upstream Management Areas were also identified, and these represent sub-quaternary catchments where anthropogenic activity must be regulated to prevent deterioration of downstream river FEPAs and Fish Support Areas (Nel *et al.*, 2011). Each sub-quaternary catchment on the FEPA map has a code that can be used to look up additional information about the river FEPAs, Fish Support Areas and the biodiversity features associated with each (Driver *et al.*, 2011). The FEPA maps are currently the most valuable ecological classification tool that exists in South Africa and they have been used in this study to classify the study sites identified here according to their river conditions as defined on the FEPA manual.

3.2 Site selection

Site selection was based on the guidelines set by Henriksen *et al.* (1992) with slight modifications, i.e. to identify sites with soils that have a low buffering ability and to ensure all sites lack considerable direct human influence within the catchment area. Thus, sampling sites had to be in headwater streams to rule out local sources of pollution such as intensive agriculture, industry as well as dams and reservoirs used for industrial purposes or as receiving bodies for sewage water because these other sources of pollution tend to drown out the signal from deposition (Henriksen *et al.,* 1992). Dams used to generate hydroelectric power and overgrown water bodies with shallow (< 1 m) waters were also excluded (Henriksen *et al.*, 1992).

3.2.1 The Mpumalanga region

The Mpumalanga Highveld (26°12′13″S 28°2′43″E) is a highly industrialised region with well documented concerns relating to poor air quality (Bird, 2011; Josipovic *et al.*, 2011; Piketh, 2016; Garland & Piketh, 2016; Piketh, 2015; Reason *et al.*, 2006) and equally impaired waterbodies such as the Olifants River (Ashton, 2010; de Villiers & Mkwelo, 2009; Coetzee *et al.*, 2002). The open savanna and grasslands of Mpumalanga stem from the climatic changes of the Miocene era associated with drying of the African continent (Osborne 2008, Dupont 2013). Some characteristics of grassland soils include slow decomposition and weathering rates and the ability to store large amounts of carbon (Blair *et al.*, 2014). The grassland biome is highly susceptible to changes in the climate associated with dryness. This directly affects the ecosystem at large and thus climate change remains the number one threat to grasslands (Blair *et al.*, 2014). In addition to the complex biome the Mpumalanga region is subject to extensive mining and agricultural activity, both which are known to lead to soil degradation and the deterioration of the aquatic ecosystem.

Sample collection was primarily set to be conducted exclusively at the Mpumalanga Highveld, however due to the highly industrialised nature of this region, many streams that would have been selected on the basis of the soil sensitivity were unsuitable due to intensive agriculture and mining activity in and around the areas identified as having acid sensitive soils. Hence, a decision was made to look elsewhere and not rely on soil maps, but land use became a key consideration. As a result, site identification was moved east of the Highveld and only seven sites were left in the plateau **Figure 3-8**. The study area extends across parts of the Thaba Chwea municipality, Emakhazeni municipality, the Steve Tshwete municipality, the Govan Mbeki municipality and parts of Gauteng north of Kusile power station. The final list of sampling sites consisted of 22 sites made up of 7 sampling points from the highveld (HV) plateau and 15 from the eastern Highveld (EHV) (**Figure 3-8**). The sampling sites represent streams found in protected areas such as National Parks or conservation sites (**Figure 3-9**A), low impacted areas used primarily for low stock grazing (**Figure 3-9**B) and remote roadside sites (**Figure 3-9**C) associated with a small measure of human related disturbances. Samples collected from "protected areas" will serve as controls as these streams are assumed to have near pristine waters, thus high levels of acidification will presumably be from direct atmospheric deposition. The chemistry and biological data obtained from these sites will be of great conservational value. A complete list of sites sampled during the course of this project and their coordinates is included in the Appendices (Appendix 4). From this point forth throughout the thesis all sampling sites from the Mpumalanga region will be collectively referred to as 'HV' sites.

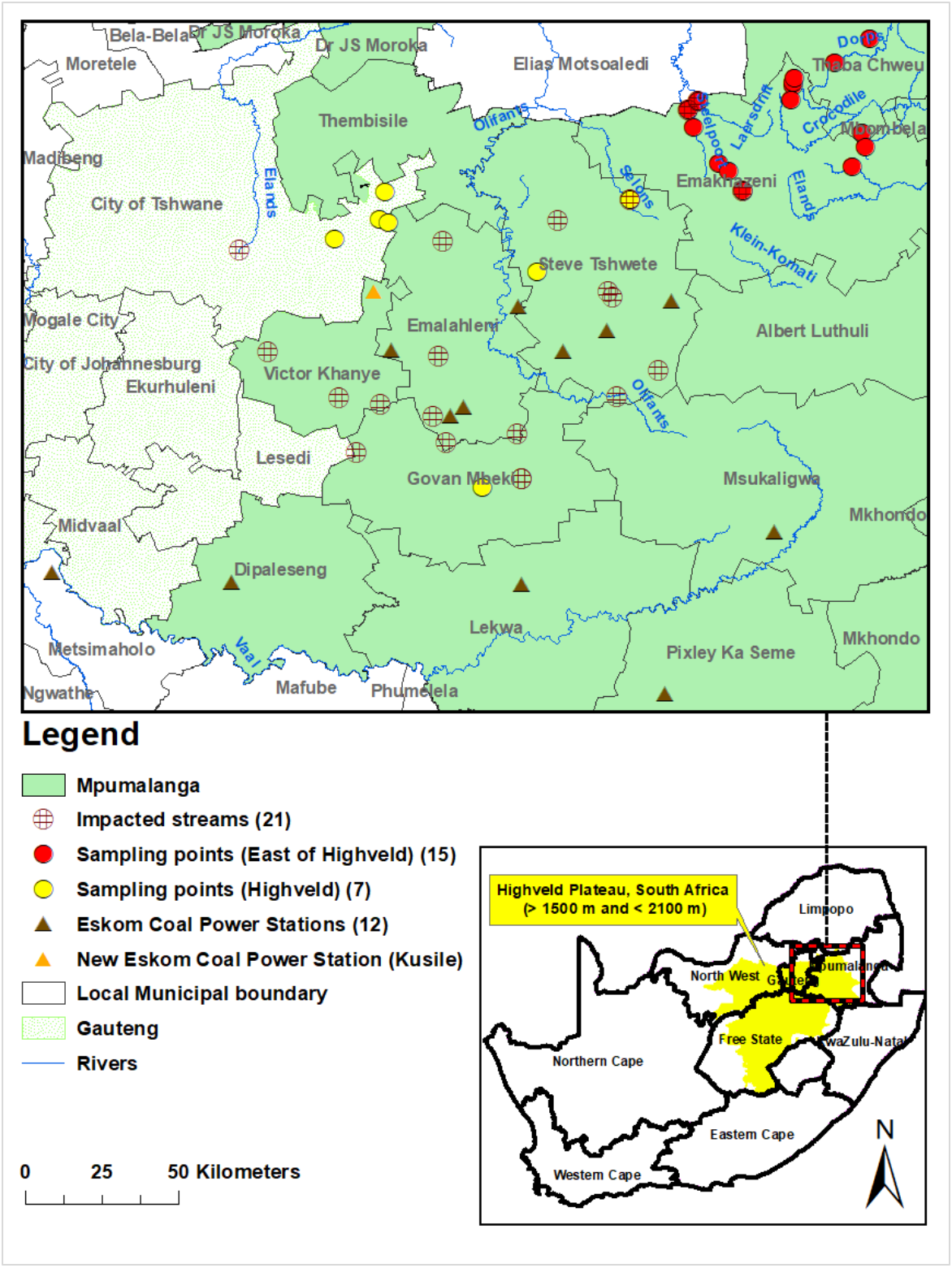


Figure 3-8: Distribution of the Mpumalanga study sites. The plateau raster map was obtained online (https://en.wikipedia.org/wiki/Highveld) and georeferenced onto a South African map. Streams initially selected on the basis of soils but found in the field to have catchments impacted by mining and agricultural activity are indicated in mash circles.

Figure 3-9: Overview of study sites. A – EHV11 (Photo: Prof. Chris Curtis) located at the Verloren Vallei Nature Reserve, Ramsar site. **B** – EHV20 Grazing land surrounded by plantation. **C** – HV23 (Photo: Dr Kari Austnes) remote roadside stream.

To assess habitat integrity and relate headwater streams identified above to conservation goals, sampling points were plotted on FEPA maps (**Figure 3-10** and **Appendices Figure 2**). Several points aligned with or served as tributaries to a number of well-known streams in South Africa **Figure 3-10**. EHV03, and EHV15 (**Figure 3-10**, blue circle) were located on river FEPAs and associated sub-quaternary catchments, suggesting that these sampling points were located on streams that are predominantly natural and of great ecological value. EHV07 and EHV08 were found in Phase 2 FEPA regions (**Figure 3-10**, blue circle). The Phase 2 FEPA status is given to modified streams that are due for rehabilitation however, EHV07 and EHV08 were classified as being in good ecological conditions based on the A and B river conditions that were associated with these sampling points on the FEPA maps. EHV9, EHV13 and EHV16 were identified as Z category streams (**Figure 3-10**, grey circle), a category assigned to rivers with C, D, E or EF

ecological conditions that have no data or expert opinion. Hence macroinvertebrate and chemistry data presented in this study will be the first set of results ever reported for the Kleinspruit (EHV09), Hoppe-se-Spruit (EHV13) and Swartkoppiesspruit (EHV16) (**Figure 3-10**, grey circle). According to the FEPA sub-quaternary catchment codes, EHV12 (**Figure 3-10**, orange circle), located on the Dorpsrivier and EHV13 (**Figure 3-10**, grey circle) are classified as Fish Support Areas for Barbus species 'Ohrigstad'. Furthermore, EHV12 lies on a FEPA wetland ecosystem type region. The Steelpoort sites EHV02 and EHV04 (**Figure 3-10**, orange circle) as well as EHV05 and EHV06 (**Figure 3-10**, turquoise circle) are both located on unnamed streams and are Fish Support Areas that form several FEPA wetland clusters and wetland ecosystem types that are home to *Barbus anoplus* (Chubbyhead Barb) and *Opsaridium peringueyi* (southern barred minnow) fish species. Vegetation type and several geomorphological features were considered during the wetland classification process. Wetland clusters refer to undisturbed wetlands that sit adjacent to each other, facilitating species migration between them. EHV09 and EHV16 (**Figure 3-10**, grey circle) are Upstream Management Areas. The Spookspruit (HV4) and the Trichardspruit (HV23) (**Figure 3-10**, orange circle) from the Highveld plateau were ecologically modified streams reflecting C and EF river conditions respectively, as defined on the FEPA manual (Driver *et al.*, 2011). All the streams that were categorised as being in good ecological condition, modified or part of the Z category are streams that aligned with the 1:500 000 spatial scale used on the FEPA maps and DWS River network (**Figure 3-10**).

A few of the sampled streams served as tributaries for other sampling sites as well as to some well-known main channels (i.e. Olifants and the Crocodile River). For instance, HV4 (**Figure 3-10**, orange circle) is a tributary to the Olifants River (**Figure 3-10**, red circle), while the Laersdriftspruit (EHV03) (**Figure 3-10**, blue circle) serves as a tributary to the Steelpoort River (**Figure 3-10**, orange circle) and EHV02 lies downstream of EHV04 on the Steelpoort. The Lunskliprivier (EHV07) and the Gemsbokspruit (EHV08) (**Figure 3-10**, blue circle) flow from a river FEPA sub-quaternary catchment where EHV08 flows into EHV07, a tributary of the Crocodile River (**Figure 3-10**, red circle). EHV16 (**Figure 3-10**, grey circle) flows into the Elands (**Figure 3-10**, red circle) which is a NFEPA project

identified free-flowing river of great biodiversity and conservational value that feeds into the Crocodile River (**Figure 3-10**, red circle). EHV13 (**Figure 3-10**, grey circle) flows into EHV12 (**Figure 3-10**, orange circle), a tributary of the Spekboom River which later joins with the Steelpoort. A total of 9 sites (**Figure 3-10**, turquoise circle) were found outside the FEPA stream network, probably due to the large spatial scale of 1:500 000 that was used to project FEPA maps. Similar observations were made with the DWS stream network which shares the same spatial coverage as the FEPA maps, indicating that EHV05, EHV06, EHV10, EHV11, EHV14, EHV17, EHV18, EHV19 and EHV20 (**Figure 3-10**, turquoise circle) all of which are located on unnamed rivers, are very small streams that could only be integrated into a stream network through catchment delineation. Water and macroinvertebrate samples were taken above all mentioned confluent points. A Total of 11 sampling points were found on acid sensitive soils. EHV07, EHV08, EHV11, EHV17 and EHV19 were on class 2 soils while EHV09, EHV10, EHV12, EHV13, EHV15 and EHV16 sat on class 1 soils.

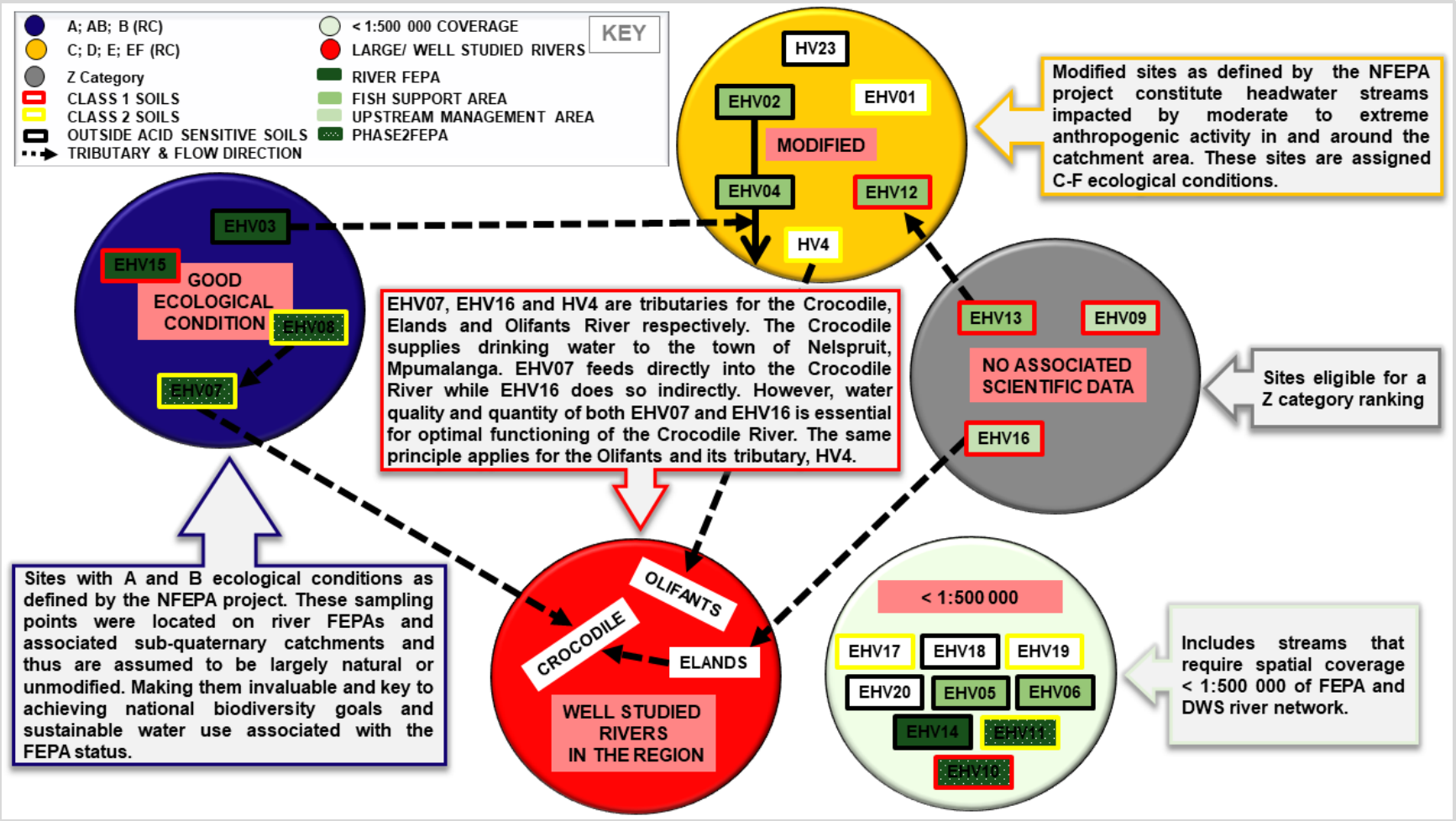


Figure 3-10: Ecological conditions of HV sites. This Figure is colour coded to highlight the unique properties of each site, that includes conservational value based on their location on the FEPA maps as well as with the river channels they flow from or into. EHV09, EHV10, EHV12, EHV13, EHV15 and EHV16 were all located on Class 1 acid sensitive soils, while EHV1, EHV11, EHV17, EHV19 and HV04 were on Class 2 soils.

3.2.2 Waterberg

The Waterberg District forms part of the Waterberg-Bojanala Priority Area (WBPA) that spans Limpopo and North-west Provinces. The WBPA was declared the third air pollution 'hot spot' region in South Africa after the Vaal Triangle Air-shed Priority Area and the Highveld Priority Area (HPA) that were declared National Priority Areas in 2006 and 2007 respectively (Department of Environmental Affairs (DEA), 2007). The Waterberg consists of six local municipalities, namely Bela-Bela, Lephalale, Modimolle, Mogalakwena, Mookgopong and Thabazimbi (**Figure 3-11**). The Lephalale municipality is said to account for 96 % of total emissions from the Waterberg District (DEA, 2014). These high emission rates are attributed to the run-down conditions of the currently running coal power station, Matimba (23° 40′ 6″ S 27° 36′ 38″ E), as well as to inadequate abatement technology. The Waterberg is rich in coal resources and plans of expanding mining to this region are under way (DEA, 2014). The landscape of the Waterberg sites is natural to semi-natural with very limited human disturbances (**Figure 3-12**). In addition to the aims of the present study, the current undisturbed nature of the Waterberg presents an opportunity to assess species distribution and diversity prior to planned industrialisation. Creating species repositories should also form an important part of South Africa's conservation efforts, especially considering the level of industrialisation that is underway.

The Waterberg is dominated by game farms that extract their water for everyday use directly from the source by creating boreholes and dams. Reductions in flow regime are known to have direct impacts on species composition. The Waterberg forms part of the least studied regions in South Africa, consequently macroinvertebrate species from this region are not well described. Furthermore, a study conducted by Huizenga (2011) also indicated that the majority of the South African water quality monitoring stations are concentrated in the south-western and north-eastern parts of the country suggesting that streams from Waterberg region are not well monitored when compared to those from other parts of the country. The few studies that focus on monitoring impacted streams are mostly limited to large rivers such as the Limpopo River, Lephalala, Luvuvhu and the Crocodile, however little or no attention has been given to headwater streams that feed

into some of these large rivers. In instances where macroinvertebrate assessments have been conducted, taxonomic identification is usually done to family level which is an unacceptable approach when addressing environmental concerns affecting species diversity and conservation. Overall the WB and HV share a number of climatic and geographical similarities. Both regions generally experience hot summers that are prone to sporadic heavy rainfall spells in addition to the dry winter season (Thieme, 2005). Furthermore streams from these regions have low content of organic substances due to the lack of vegetation canopy (Thieme, 2005) implying that many sites from these two regions have little or no leaf matter falling into and or accumulating in the streams.

For the purposes of defining the ecological status of the Waterberg sampling sites that is based on known (A; B; C; D; E and EF) river conditions, all sampling points from this region were plotted on FEPA maps (**Appendices Figure 3**) and (**Figure 3-13**) below represents a more inclusive version of these maps. A total of eight sampling points (WG01, WB05, WB07B, WB12, WB16, WB21, WB30 and WB35) representing six headwater streams namely, the Grootfonteinspruit (WG01), Frikkiesloon (WB05 and WB35), the Goud River (WB07B and WB30), the Taaibosspruit (WB12), Sondags River (WB16) and the Malmanies River (WB21), had A and B river conditions that are associated with good ecological conditions (**Figure 3-13** blue circle). However, WB21, WB05 and WB35 were found outside demarcated FEPA regions (i.e. river FEPAs, Fish Support Areas and Upstream Management Area). WB01, WB02, WB22, WB24 and WB26 (**Figure 3-13** orange circle) were identified as modified streams based on previously described river conditions. A total of 20 headwater streams (WB03, WB06, WB07, WB07C, WB09, WB11, WB13, WB14, WB19, WB20, WB23A, WB23B, WB25, WB27, WB31, WB32, WB32A, WB33, WB34 and WG02) (**Figure 3-13** turquoise circle) from this region had spatial coverage less than that used on the FEPA maps (1:500 000), indicating that these sites could only be integrated into a stream network through catchment delineation. The majority (80 %) of these headwater streams (**Figure 3-13** turquoise circle) were found in FEPA regions. For instance, WB06, WB07, WB07C, WB13, WB14 and WB27 (**Figure 3-13** turquoise circle) all sat on river FEPAs and associated sub-quaternary catchments (indicated by dark green coloured square),

implying that these sites have A or B river conditions and are of great conservational value. WB06, WB13, WB14 and WB27 (**Figure 3-13** turquoise circle) specifically sit on sub-quaternary catchment area 414 while WB07 and WB07C are on sub-quaternary catchment 210. Both catchment areas are Fish sanctuaries for Barbus sp. 'Waterberg'. Streams in catchment area 210 have ephemeral FEPA biodiversity features while those of 414 have seasonal to permanent biodiversity features. WB19, WB20, WB23A, WB23B, WB31, WB32 and WB32A (**Figure 3-13** turquoise circle) all sat on the FEPA wetland type of Fish Support Areas for Barbus sp. 'Waterberg'. WB25 also sat on a FEPA wetland type of Upstream Management Area ecosystem. WB33 and WB34 (**Figure 3-13** turquoise circle) which are both located in the Marakele National Park are South African National Park (SANPARKS) sites that have Phase 2 FEPA status, suggesting that these two sites are found on moderately modified streams eligible for rehabilitation. Furthermore, both sampling points are found on demarcated FEPA wetland and wetland cluster regions. Only 24 % (n = 8) of the sampling points (WB03, WB05, WB09, WB11, WB21, WB22, WB35 and WG02) sat outside FEPA regions, further affirming the undisturbed nature of this region and the importance of building species repositories prior to planned industrialisation. These findings also invite the intervention of policy makers to set forth measures that will both benefit the environment and the country moving forward. The Waterberg seems to represent one of the few untouched regions sitting north-west of the highly industrialised Highveld region of South Africa. Overall 10 sampling points (WB01, WB02, WB03, WB07B, WB23A, WB23B, WB24, WB26, WB30 and WB32A) were found on acid sensitive soils (**Figure 3-13**). Several streams served as indirect tributaries for the Limpopo River by first flowing into major channels such as the Lephalala (WB24) or the Mokolo River (**Figure 3-13** red circle). For instance, WB07B and WB30 (**Figure 3-13** blue circle) sit downstream of each other on the Goud River which feeds into the Lephalala River (**Figure 3-13** orange circle), a tributary of the Limpopo River. Similarly, WB01 is a tributary of the Sand River (WB02) (**Figure 3-13** orange circle) in Modimolle which flows into the Mokolo River (**Figure 3-13** red circle), a tributary to the Limpopo River. WB05, WB35 and WG01 (**Figure 3-13** blue circle) are tributaries of the Sterkstroom (**Figure 3-13** red circle) which is in turn a tributary of the Mokolo River.

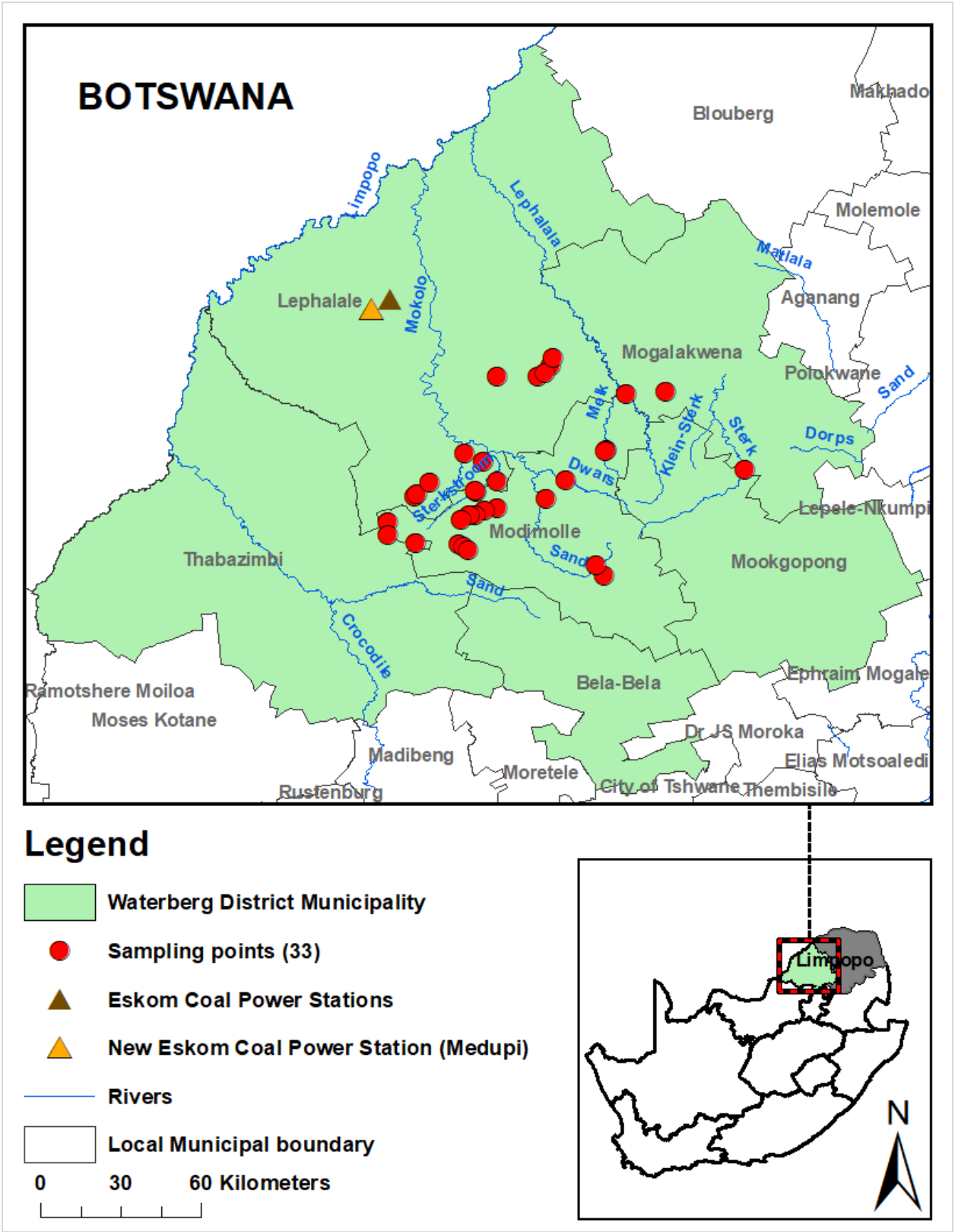


Figure 3-11: Distribution of study sites in the Waterberg.

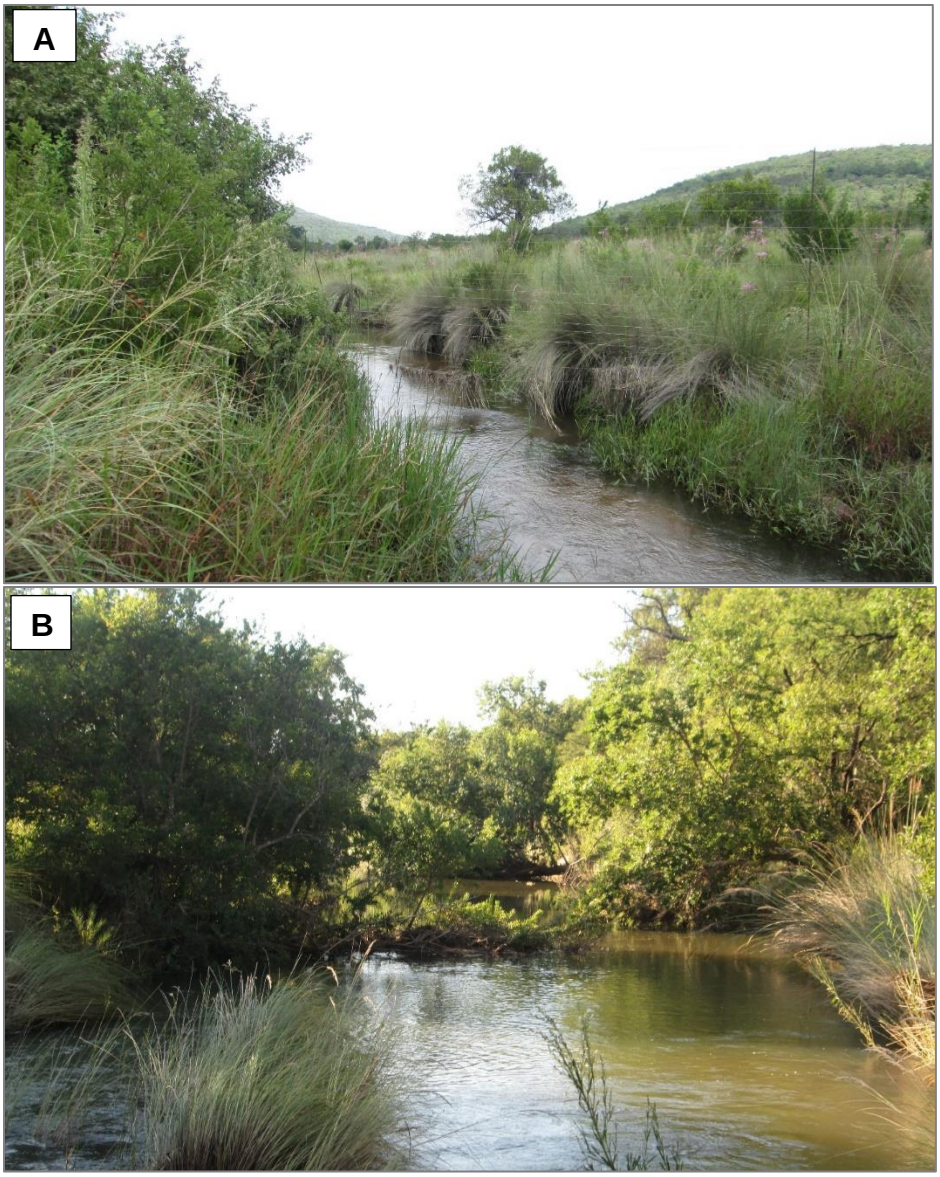


Figure 3-12: Examples of Waterberg study sites. A – WB13, flows into Kololo nature reserve. **B** – WB26, remote roadside stream flowing through a cattle farm.

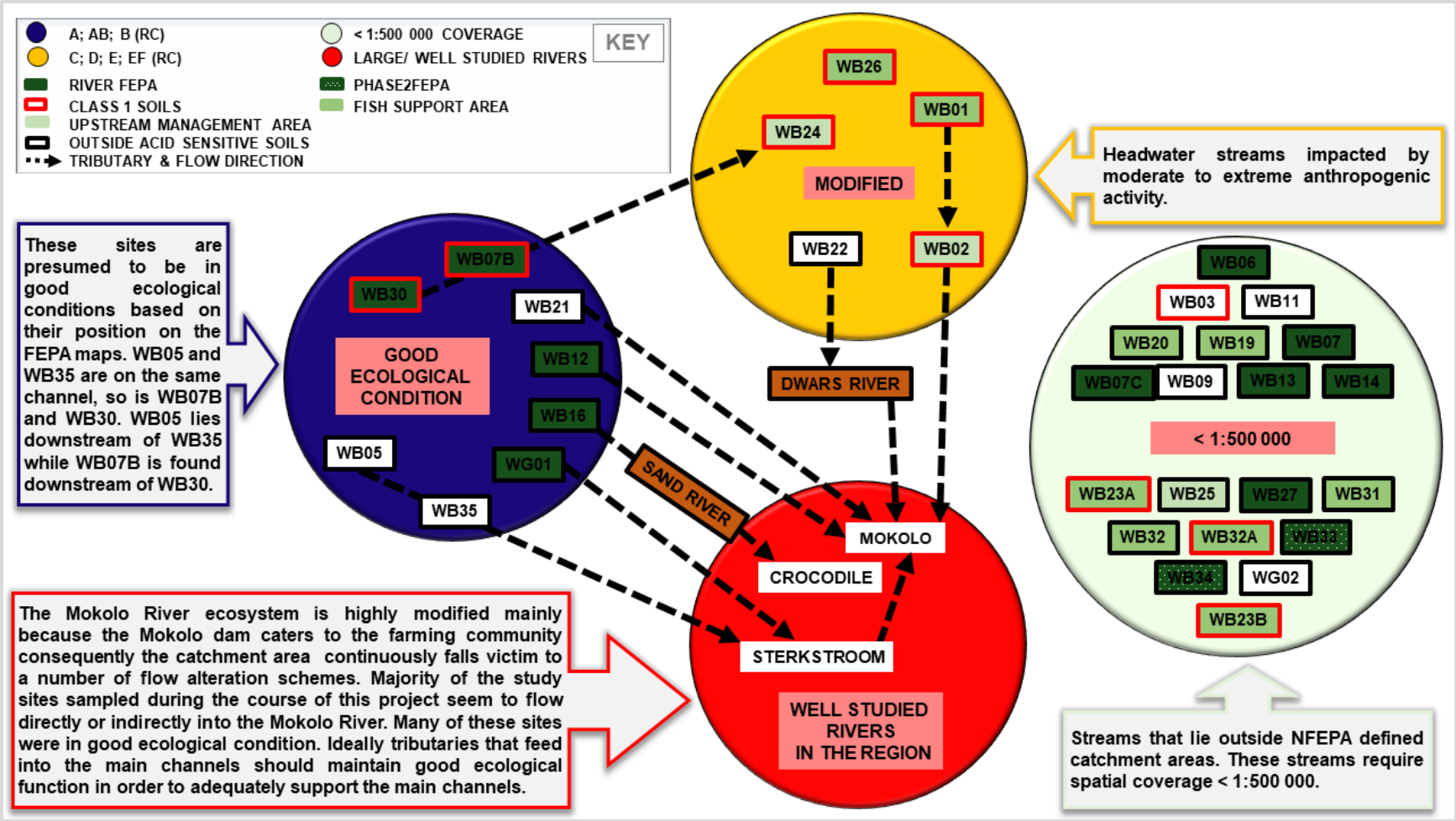


Figure 3-13: Ecological conditions of WB sites. Most of the sites from this region had a spatial scale less than the 1:500 000 of the river FEPAs and DWS stream network. This Figure is colour coded to highlight the unique properties of each site, that includes conservational value based on their location on the FEPA maps as well as with the river channels they flow from or into. WB01, WB02, WB03, WB07B, WB23A, WB23B, WB24,WB26, WB30 and WB32A were all located on class 1 acid sensitive soils.

3.2.3 The south-western Cape (SWC)

The Cape region of South Africa, formerly known as the Cape Province, integrates internationally renowned regions of great biodiversity and conservational value such as Namaqualand and the Cape Floral Kingdom (CFK). According to the National land cover map published on the National Register of Protected Areas and Spatial data report (DEA, 2012), the Cape remains the most natural or undisturbed region in South Africa. The CFK covers a small area yet is considered one of most diverse plant kingdoms in the world that is further associated with a high degree of endemism of some mayfly, caddisfly and stonefly species (Thieme, 2005; de Moor & Day, 2013). Considerable effort has gone into conserving and protecting the biodiversity of this natural asset for economic and recreational value. Consequently, of the three study regions the SWC is the most studied and a large number of described macroinvertebrate species in South Africa are from this region (de Moor & Day, 2013; Woodford *et al.*, 2013; de Moor, 2011; O'Farrell *et al.*, 2015; Eady *et al.*, 2013; Muskett *et al.*, 2016; Odume *et al.*, 2015; Odume *et al.*, 2014). In contrast to the HV and WB study regions that are characterised by hot summers and dry winters, the SWC has a Mediterranean climate that experiences rainfall year round and more so during the winter period (Allanson *et al.*, 1990; Day & King, 1995; Thieme, 2005; de Moor & Day, 2013).

The SWC is without coal mines or coalfired power stations, however this region has naturally acidic streams making it susceptible to LRTAP. Signs of stream acidification reflected through species presence/absence would not only confirm effects of LRTAP to this region but will also imply potential effects of this form of transportation of pollutants in other parts of Southern Africa. Ancient rocks such as those of the Cape fold mountains are associated with low weathering rates and thus streams draining these regions are characterised by low concentrations of minerals and nutrients (de Moor & Day, 2013; Allanson *et al.*, 1990), implying that these streams would generally have a low buffering ability against strong acids. The SWC is well known for its "black and white" waters (**Figure 3-14**). The term 'white waters' refers to the normal clear stream water found in most rivers, while 'black waters' has been the term used to describe the dark coloured

streams that flow through most Fynbos regions. The water colour has been associated with streams flowing from places of high altitude, dissolved organics derived from fynbos vegetation or peat formation (Thieme, 2005; Midgley & Schafer, 1992; Allanson *et al.*, 1990; de Moor & Day, 2013). Furthermore, Midgley and Schafer (1992) noted a correlation between the black water colour and low pH. Similar to the Waterberg, the SWC is highly prone to damming activity that modifies the riverine regime and probably species composition (de Moor & Day, 2013; Allanson *et al.*, 1990). Thus, even in light of all the conservation efforts towards this region the south-western Cape remains under threat to many anthropogenic related activities.

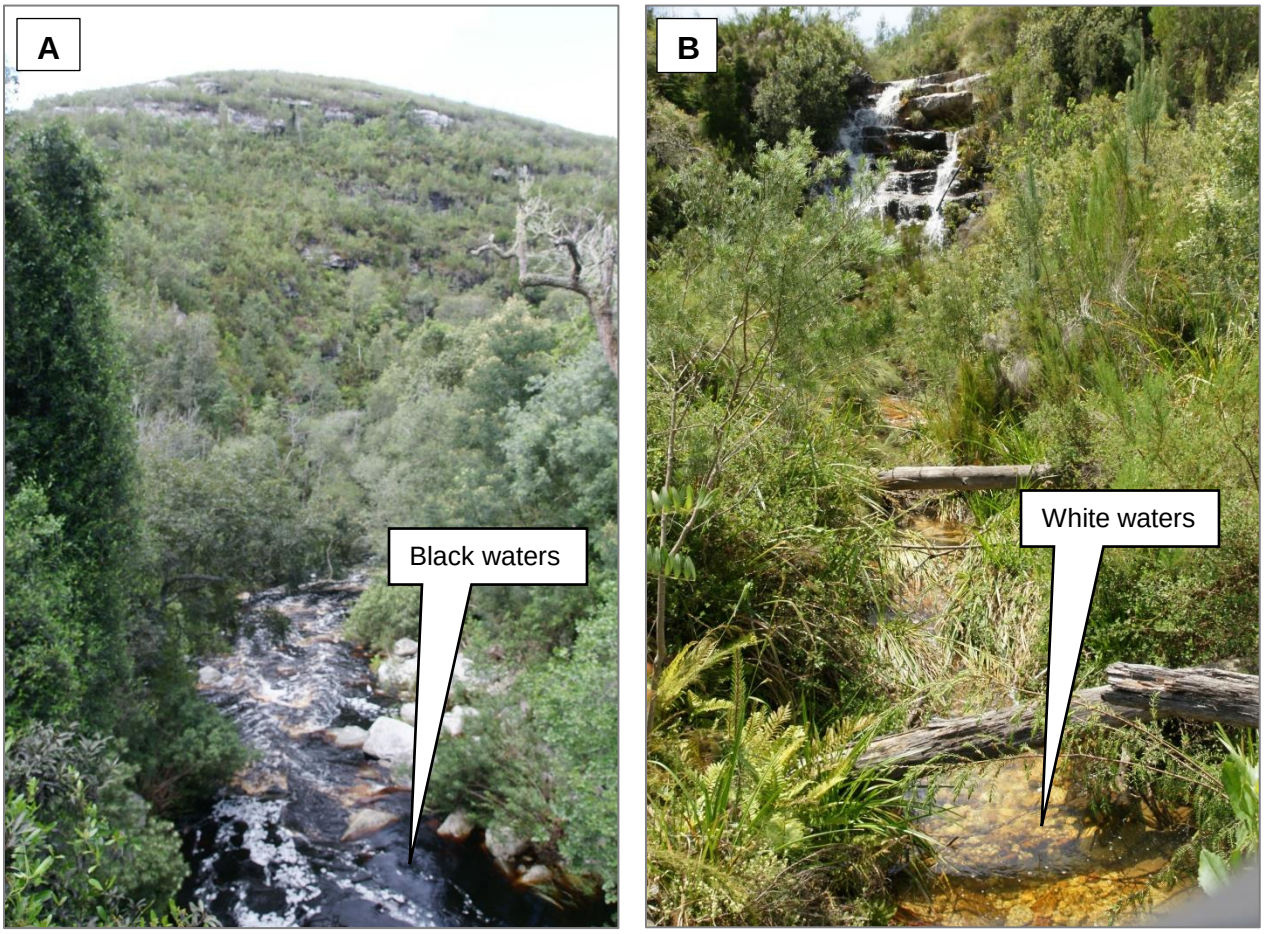


Figure 3-14: Black (i.e. SWC26) and White (i.e. SWC29) waters of the SWC.

A total of 30 sites were identified in the SWC (**Figure 3-15**). These sampling points were distributed along the Garden Route, an area that forms part of the Cape Floral Kingdom. GPS coordinate from the Midgley and Schafer (1992) paper were used to locate these acid sensitive streams (Midgley & Schafer, 1992). The aim was not to sample the exact streams but to include a few as study sites. Thus, some level of overlap can be observed between sites from the present study and those from the Midgley paper (**Figure 3-15**).

Of the 30 sites that were sampled from the SWC fifteen (SWC01, SWC03, SWC16, SWC17, SWC18, SWC19, SWC20, SWC21, SWC24, SWC28, SWC29, SWC30, SWC33, SWC34 and SWC35) were located at the South African National Parks (SANPARKS) in Wilderness (SWC24, SWC28, SWC29, SWC30), Diepwalle (SWC16, SWC17, SWC18, SWC33, SWC34, SWC35) and Harkerville (SWC01, SWC03, SWC19, SWC20, SWC21). SWC24 is a SANPARKS protected site that is located at the Nelson Mandela Metropolitan University (NMMU).

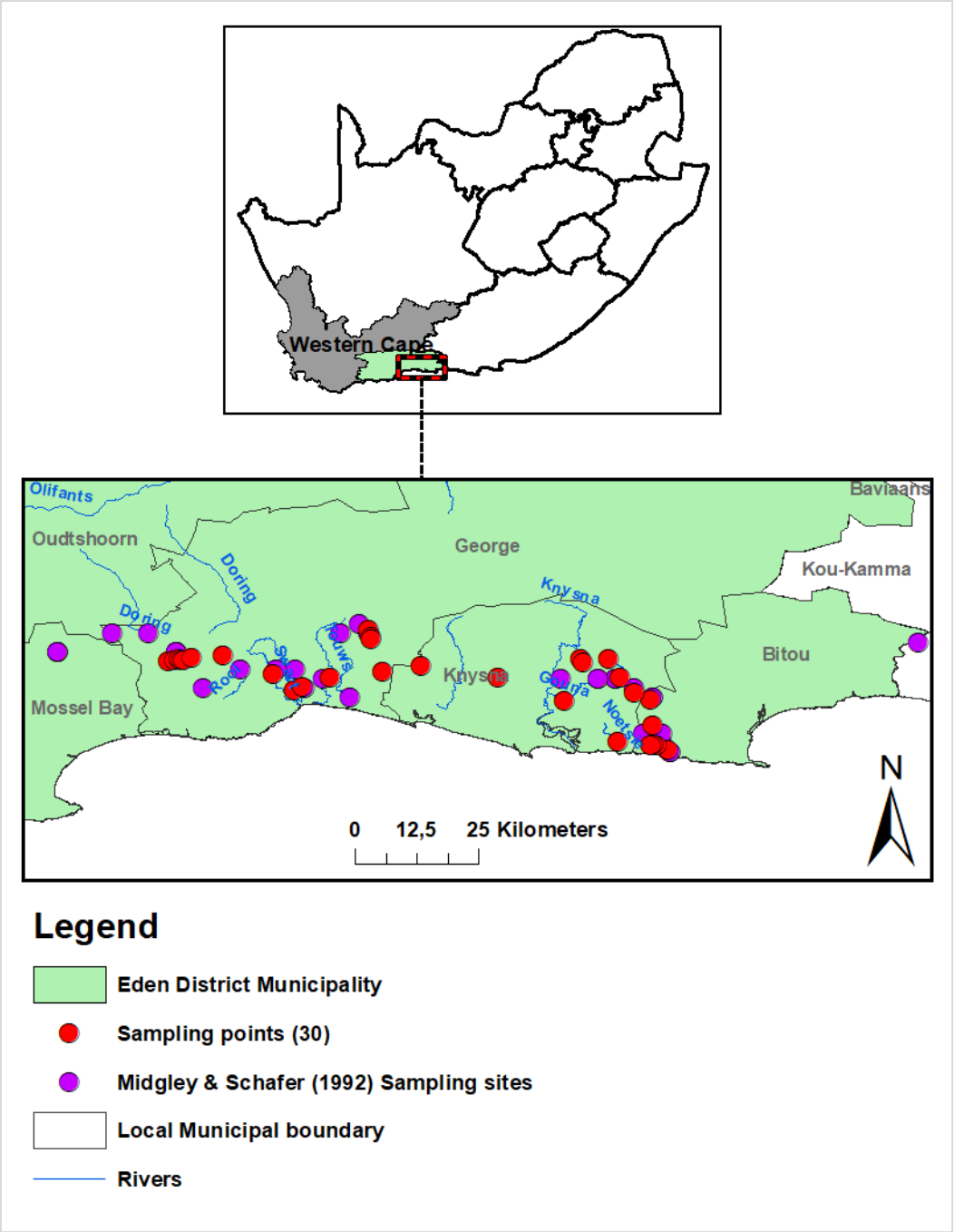


Figure 3-15: Distribution of the SWC sites across the Eden District.

As with the previous sections the SWC sampling points were plotted on FEPA maps (**Appendices Figure 4**). From these maps four distinct stream categories were established (**Figure 3-16**). The first category included sampling points that were considered to be in good ecological condition based on the A or B river conditions described on the FEPA manual. SWC18 and SWC23 (**Figure 3-16**, blue circle) which are both located on the Gouna River in sub-quaternary catchment 9111 are Fish sanctuaries with a FEPA status. This Fish FEPA sub-quaternary catchment has permanent to seasonal biodiversity features and is home to the Cape kurper (*Sandelia capensis*) and the Eastern Cape Redfin (*Pseudobarbus afer cf*. Forest) fish species. SWC18 sits upstream of SWC23 at the Diepwalle National Park whereas SWC23 was sampled from the roadside as it flows behind the Town of Knysna. SWC27 (**Figure 3-16**, blue circle) sits on the Touws River in sub-quaternary catchment 9042 and shares the same catchment properties with 9111 and so does the Homtini River sampling point (SWC36) that is located on sub-quaternary catchment 9016. SWC32 (**Figure 3-16**, blue circle) is found on the Hoekraal River of sub-quaternary catchment 9022 which is also a fish sanctuary as with catchment 9016, 9042 and 9111 but is home to just the *Sandelia capensis* fish species. SWC 25 and SWC37 (**Figure 3-16**, orange circle) which are located on the Kaaimans and Malgas Rivers respectively, are Fish Support Areas with C river conditions. SWC25 sits on sub-quaternary catchment 9065 that has a Wetland FEPA status and is home to the *Pseudobarbus afer cf. Forest* Fish species. SWC37 on the other hand is a Fish Support Area for the Cape galaxias (*Galaxias* slim/ *Galaxias sp. 'zebratus cf.* slim') and *Sandelia capensis* fish species. Two streams SWC22 and SWC24 (**Figure 3-16**, grey circle) were assigned to the Z category which includes streams with zero known scientific data or expert opinion. SWC22 is located on the Noetzie River and was sampled from inside the Pezula Private Estate while SWC24 which sits on the Swart River was sampled from the Nelson Mandela Metropolitan University (NMMU). Both streams lie outside FEPA regions. A total of 21 sampling points (SWC01, SWC03, SWC04, SWC06, SWC07, SWC08, SWC09, SWC14, SWC16, SWC17, SWC19, SWC20, SWC21, SWC26, SWC28, SWC29, SWC30, SWC31, SWC33, SWC34 and SWC35) required a spatial scale less than that used on the FEPA maps or the DWS stream network (**Figure 3-16**, turquoise circle). Of these twenty-one streams, thirteen were

located inside the SANPARKS area. SW01, SWC03, SWC19, SWC20 and SWC21 were all under the Harkerville management areas while SWC16, SWC17, SWC33, SWC34 and SWC35 were under Diepwalle management and SWC28, SWC29 and SWC30 all fell under the Wilderness National Parks management. SWC16, SWC17 and SWC21 (**Figure 3-16**, turquoise circle) were found on river FEPAs and associated sub-quaternary catchments which means that these streams are considered to be in good ecological condition. All these sampling points were located on sub-quaternary catchment 9092 which includes Fish FEPAs, river FEPAs and Wetland FEPAs. The fish sanctuaries support *Galaxias* slim / *Galaxias sp.* '*zebratus cf.* slim', *Pseudobarbus* keurbooms and *Sandelia capensis* fish species. SWC26 (**Figure 3-16**, turquoise circle) was located on sub-quaternary catchment 9065 and thus shares catchment properties with SWC25 (**Figure 3-16**, orange circle). SWC28, SWC29, SWC30, SWC31, SWC33, SWC34 and SWC35 (**Figure 3-16**, turquoise circle) were all located on Upstream Management Areas where the first four streams are on sub-quaternary catchment 9027, a wetland ecosystem. Whereas SWC33 was found on sub-quaternary catchment 9041 while SWC34 and SWC35 were on sub-quaternary catchment 9069. No information is given on the FEPA look up table about the type of FEPA map category and the biodiversity features of these catchment units. Of the thirty sampling points only SWC32 was found on acid sensitive soils.

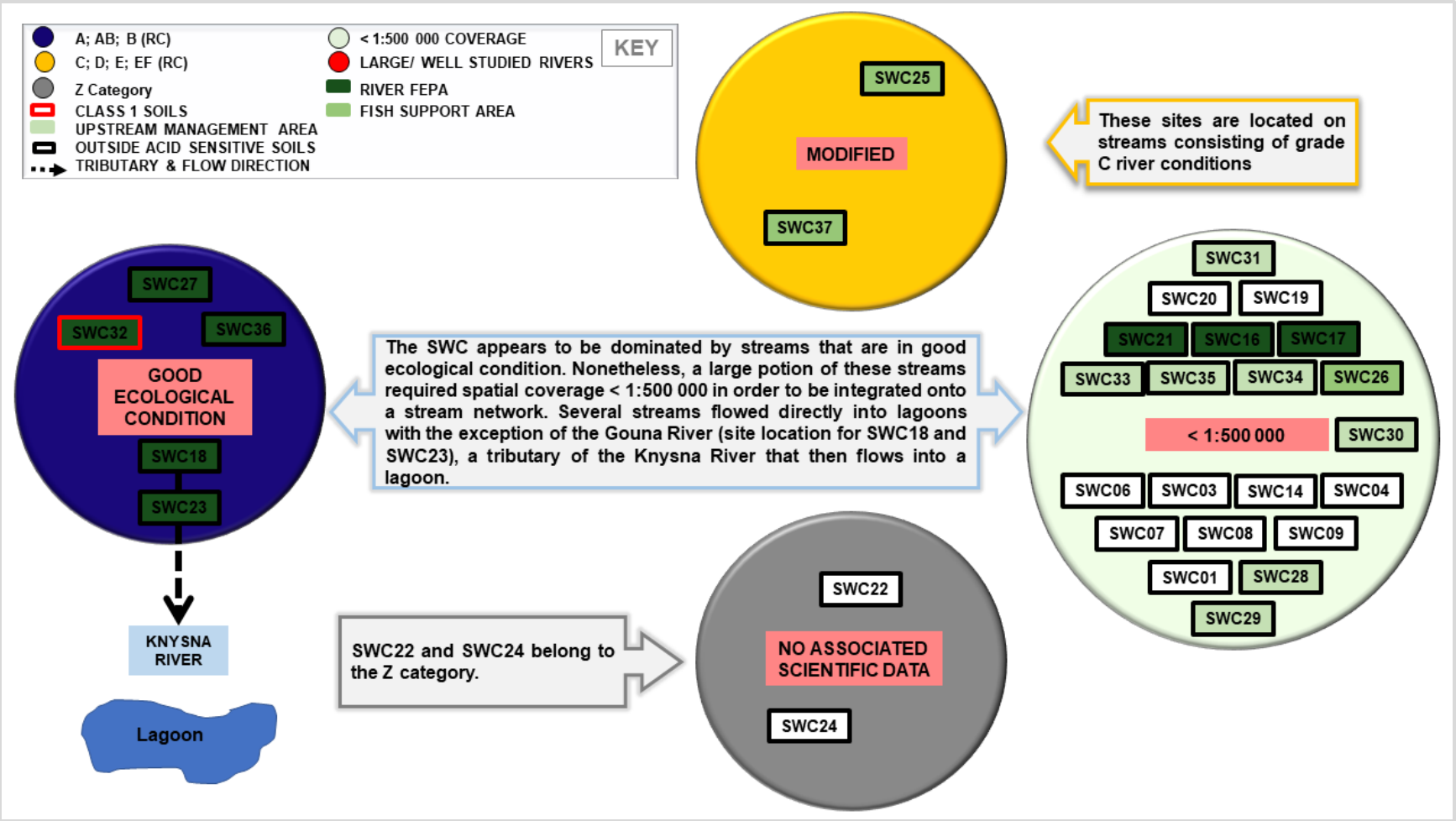


Figure 3-16: Ecological conditions of the SWC streams. This Figure is colour coded to highlight the unique properties of each site, that includes conservational value based on their location on the FEPA maps as well as with the river channels they flow from or into. As with some of the streams from the HV, two streams (SWC22 and SWC24) from this region were identified as Z category streams implying that these two sites do not have any known scientific data or expert opinion on record that is associated with them. Only one site (SWC32) was located on acid sensitive soils.

3.3 Summary

The South African landscape varies considerably resulting in very diverse ecosystems. For instance, the HV sites were distributed across the Highveld and the Eastern Bankenveld ecoregions, while the WB sites were found in the Waterberg and Western Bankenveld ecoregions (**Table 3-5**). All the SWC sites were found on the South Eastern Coastal belt ecoregion (**Table 3-5**), implying a level of homogeneity. However, their location within the CFK which is considered one of most diverse plant kingdoms in the world, associated with a high degree of endemism for some mayfly, caddisfly and stonefly species, suggests there is much variation between the study sites. A level of overlap is observed when comparing ecoregions within the same region, however, when comparing site distribution across study regions there is variation that is most apparent in the altitude, vegetation type and rainfall patterns (**Table 3-5**). The distribution of sampling sites within the same region across different ecoregions highlights a measure of heterogeneity among sites of the same region, implying even greater diversity between the three study regions.

Several soil classification techniques were applied based on data availability to produce the final map of acid sensitive soils (**Figure 3-7**). However, it was landuse patterns, the drought effect and habitat availability that influenced overall site selection more than the distribution of acid sensitive soils or coalfired power stations, particularly for the Waterberg and Mpumalanga regions. For example, upon discovering that streams from the Highveld were impacted by agricultural and mining activity, stream sampling was moved east of the Highveld where most of the sampling points were away from class 1 acid sensitive soils and the Eskom coal power stations. Similarly, due to the drought, site identification for the Waterberg had to be extended to parts of the region that did not necessarily have acid sensitive soils. Consequently, as some sites were drying out, new sites were added. In addition to this, both the Waterberg and Mpumalanga regions had a limited number of habitats suitable for macroinvertebrate sampling and in order to include more invertebrate sites site selection was extended to areas outside acid sensitive soils. Nonetheless 20 % (n = 17) of the sampling points from the SWC, HV and WB were found on class 1 acid sensitive soils.

Table 3-5: Ecoregions

Ecoregions	Highveld	Eastern Bankenveld	Waterberg	Western Bankenveld	South Eastern Coastal Belt
Landscape	Plains, low to moderate relief	Closed hills, Mountains and moderate to high relief	Table lands and Moderate to High relief	Lowlands, hills and moderate to high relief	Closed hills, Mountains and moderate to high relief
Vegetation	Grasslands	Bushveld and mountain grasslands	Bushveld	Bushveld	Fynbos, Mesic Succulent thicket and Afromontane forest
Altitude (m a.m.s.l.)	1100 - 2300	500 - 2300	700 - 900	900 - 1700	0 - 1300
Mean Annual Temperature (°C)	12 - 20	10 - 22	14 – 22	14 – 22	12 - 20
Rain Season	Early to late summer.	Early to mid-summer	Early to mid-summer	Early to mid-summer	All year to very late summer and throughout winter
% of Annual Rain variation	< 20 – 35	< 20 – 34	20 – 35	20 – 35	< 20 – 40

Chapter 4 Research approach and methods

4.1 Collection of water samples and field chemistry data

Stream sampling commenced in September (spring) 2014 and was concluded in October (mid-spring) 2016. Ideally 20 – 30 headwater streams had to be identified per region to measure water chemistry and sample for aquatic macroinvertebrates. Physico-chemical properties of the stream were recorded on a sampling sheet at each site. Water pH, temperature (Temp), conductivity (EC) and dissolved oxygen (DO) levels were measured *in situ* using a calibrated multiprobe Professional Plus (Pro Plus) Yellow Springs Instrument (YSI) metre. Since most of the streams were very shallow (< 0.1 m), the probe was left to settle undisturbed on the riverbed. Water samples were collected using a 500 ml filter rig and filtered through a 0.45 µm (pore size), 47 mm diameter membrane filter (P/N 60173, Whatman™/ GE Healthcare). To prevent premature clogging of the filter membrane from large particles, a grade GF/C coarse (1.2 µm pore size, 47 mm diameter) filter (Cat No. 1822-047, Whatman™/ GE Healthcare) was placed on top of the 0.45 µm membrane. Filtered samples were split into two clean 100 ml bottles pre-soaked in an HCl acid bath and rinsed with deionised water. Samples due for dissolved organic carbon (DOC) analysis at Umgeni Waters were kept at 4 °C (fridge), while samples set for ion chromatography at the University of the North-west (Potch), chemistry department were stored at -20 °C (freezer).

4.2 Lab analysis of water samples

4.2.1 DOC analysis - Umgeni waters

DOC concentrations were analysed at the Umgeni water laboratory on a Tekmar Apollo 9001 TOC Analyser (Metcalf *et al.*, 2014). All instrument and sample settings were carried out as in Metcalf *et al.* 2014.

4.2.2 Ion Chromatograph (IC) - University of the North-west (Potch) chemistry lab analysis

Major ions concentrations were analysed on a Dionex ICS-3000 ion chromatograph (IC) instrument at the NWU chemistry laboratory. The analysis was performed according to Dunnink *et al.* (2016). The Dionex ICS-3000 ion chromatograph machine is equipped with an automatic eluent generator which facilitates the production of high purity ion chromatography (IC) eluents. The presence of an eluent generator eliminates the need to manually prepare eluents from concentrated acids and bases. Potassium hydroxide (KOH) was used as an eluent for anionic while methanesulfonic acid (MSA) was used for cationic analyses. “For the anions an ASRS 300 4 mm suppressor, a 4 mm carbonate removal device (CRD), an AS-15 4 mm analytical column and AG-15 4 mm guard column was installed. The CRD removes carbonates from the eluent stream so that the carbonate peak does not influence the other analytes, thus resulting in higher selectivity. For the cation analyses, a CSRS 300 2 mm suppressor, a CS-16 3 mm analytical column and CG-16 3 mm guard column were used. A five-point calibration was performed for the anions and cations, and in both instances the standards were prepared from certified 1 000 ppm stock solutions manufactured by Spectrascan® and supplied by Industrial Analytical (Pty) Ltd. Calibration-standard concentrations of 10 ppb, 100 ppb, 500 ppb, 1 ppm and 2 ppm were prepared. The calibration was only accepted if the standard deviation of the standard solutions from the calibration line was below 4%. All samples that had concentrations above the highest value used during calibration were diluted to within the calibration range and analysed again” ((Dunnink *et al.*, 2016), page 415, paragraph 3 and 4).

4.3 Macroinvertebrate sampling

Macroinvertebrate sampling for the overall project was scheduled to take place twice during the course of the study, the first trip was planned for the autumn (March to May) of 2015 and the latter for the spring (September to November) of the same year. However, macroinvertebrate samples were collected in the late autumn (May 2015) and late spring (November 2015), with a slight overlap into the winter month (June 2015) for the former. Due to the severe drought experienced in the Waterberg during the spring of 2015 that left 70 % of the sampling sites with no flow (**Figure 4-1)**, additional sampling was conducted for this region in the late summer (Feb 2016). In addition to this, further sampling was conducted in June 2016 in the south-western Cape and the Waterberg to correspond with the water samples collected concurrently for interlaboratory analysis (Appendix 5).

The shallowness of the streams eliminated the option of sampling all aquatic biotopes (i.e. gravel, sand and mud (GSM), vegetation and stony riffles), particularly the vegetation biotope. Thus, for uniform sample collection, macroinvertebrate samples were collected from stony riffles which is the most commonly used biotope in biomonitoring that has been shown to display the highest species diversity and density when compared to the other biotopes. Samples were collected by kick sampling using the standard SASS net with 1 mm mesh on a 30 cm square frame (Dickens & Graham, 2002). Sampling was standardised at 2 mins, for 4 m that was sometimes divided into x 4 square metre spot sampling at 30 secs each, depending on substrate composition. For example, streams dominated by bedrock with few small rocks (< 25 cm) could lead to patchy sampling. All samples collected per site formed a single representative sample for that stream in that season. Samples were collected using the standard bottom up (i.e. starting at bottom of the stream reach and moving up) method. All macroinvertebrate samples were collected by the same person and thus overall sample collection technique is presumed uniform. Macroinvertebrate samples were preserved in 200 ml bottles containing 70 % alcohol.

4.4 Sample sorting and identification

In the laboratory, macroinvertebrate samples were emptied onto a white plastic tray (45 cm wide and 10 cm deep) and separated from debris by hand-picking individuals into sorting trays, using forceps. The organisms were separated by taxonomic Order (i.e. Ephemeroptera, Plecoptera and Trichoptera) or to Family (i.e. Simuliidae and Chironomidae) level. Hand picking stopped when no taxa of interest were visible after the tray had been swirled around and debris parted at least three times to dislodge any remaining organisms. Samples were stored in 10 ml or 50 ml tubes (depending on sample size) containing 70% ethanol for preservation. Larvae were identified to species level where possible using available taxonomic keys and expert advice at the Albany Museum (Grahamstown, South Africa). All specimens were identified under a dissecting microscope except for chironomids, which were mounted on slides according to Ochieng *et al.*, (2008) and identified under a BX63 FOM microscope at the WITS Microscopy and Microanalysis Unit (MMU), using keys described by Armitage *et al.*, (1995) and Day *et al.*, (2003). All macroinvertebrate identifications were verified by experts at the Albany Museum and the Institute for Water Research at Rhodes University.

4.4.1 Standardising macroinvertebrate data

To eliminate any ambiguity, taxa that could only be identified to family level were excluded from the final analysis when the majority of the taxa in the same sample were from the same family and could be identified to genus or species level. However, in cases where the majority of taxa could only be identified to family level with just one or two larvae identifiable to species level, both the species and family level data were kept. The former was excluded because most of the taxa in that family could be identified to the lowest taxonomic level, suggesting that the few unidentifiable individuals probably belong to the bulk sample but lack well-developed morphological features necessary for species level identification. These speculations did not rule out the possibility of new species, however, the data had to be further excluded as species density was too low to include in indicator species analysis. In cases where the majority of the taxa could only be identified to family

level with just a few taxa identifiable to species level, both datasets were kept as this may have been an indication that taxa from that family are generally not well described locally. This was mostly the case with Chironomidae and upon expert advice, identification for this family of aquatic insects was conducted to tribe or genus level.

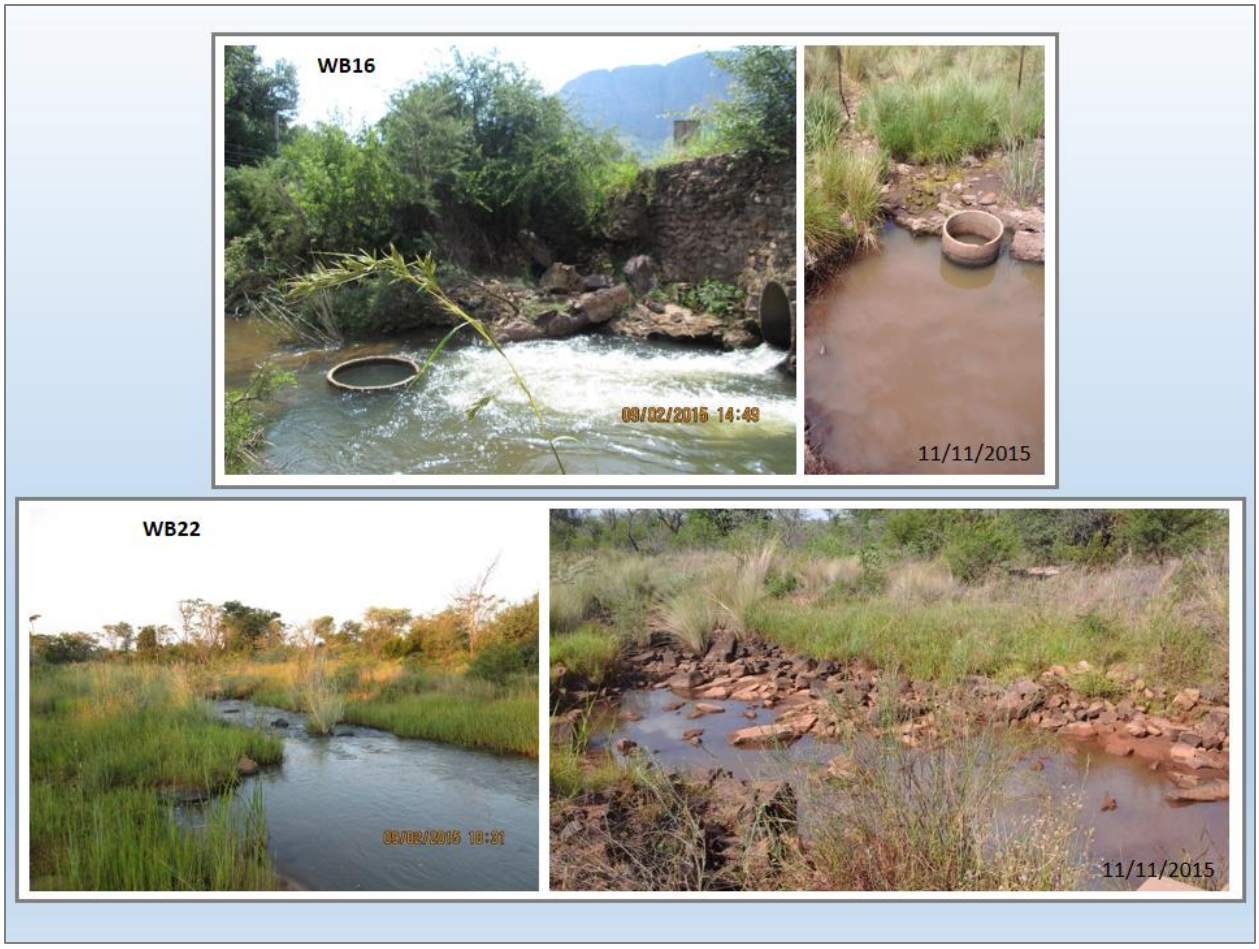


Figure 4-1: An example of sites impacted by the 2015 drought. WB16 (24° 28' 59.988" S 27° 41' 50.604" E) and WB22 (24° 16' 18.840" S 28° 11' 59.064" E)

4.5 An overview of all the data collected during the course of the project

Prior to processing samples for major ion and DOC analysis, sample pH and conductivity were measured. The first set of data received from the University of the North-west was reviewed in August 2015 upon which some discrepancies were discovered. The pH and conductivity measured in the field varied considerably when compared to that measured at the Potch chemistry lab, with unit difference of ± 2 for the pH and ± 6 for the conductivity. Following careful scrutiny of the data other inconsistences such as very high salt concentrations not attributed to sea salt were discovered. These findings raised the need for an interlaboratory comparison. The first batch of intercomparison samples was collected in February 2016 and tested at NIVA. The pH and conductivity measurements from NIVA correlated well with the Wits field data when compared to the NWU measurements. The NIVA salt concentration measurements were high but not as extreme as indicated by the NWU results. These observations suggested possible inconsistences with regard to sample handling and or apparatus settings. Consequently, two additional sampling trips (June 2016 and October 2016) were conducted for the SWC and the Waterberg where duplicate samples were collected, with one batch being taken to the Czech Geological Survey (CGS) lab in Prague and the other to the lab at NWU. The CGS lab was chosen for intercomparison analysis as it has been conducting acidic water related research and contributing data towards the International Cooperative Programme on Assessment and Monitoring of Acidification of Rivers and Lakes (ICP waters) for several decades. Thus, their apparatus, settings and sample handling were considered dependable for the types of streams sampled. ICP waters is an initiative of the CLRTAP that is aimed at promoting globally acceptable monitoring practices for all freshwaters susceptible to acidification (Raddum & Skjelkvåle, 1995). The Mpumalanga region was not a priority at this stage of the project as many of the samples collected from this region seemed to be impacted by agricultural activity. Prior to sample analysis more stringent parameters were set on the NWU apparatus. It is important however to note that the NWU instrument is primarily set to measure rain sample chemistry and not stream water chemistry that generally has much higher major ion and DOC concentrations than rainfall.

This account could in part explain some of the inconsistencies observed from the previous data however it did not explain the difference in the lab pH and conductivity which were measured independently.

All data quality checks and cleaning processes for the chemistry data were carried out by our collaborators at NIVA. The data refining process followed the guidelines set by ICP waters and expert discretion was exercised where necessary. Several tests were conducted on the data and these included the ion balance and conductivity test, assessment of the pH-ANC relationship and the outlier test. The ion balance and conductivity tests were not entirely conclusive due to sample variation across seasons. These variations may have occurred due to error in the calculation or during the water analysis process which may result in there some ions missing. However, the latter seems more plausible especially taking into account all the difficulty experienced while trying to set stringent parameters with the NWU apparatus. Samples that deviated significantly from the expected pH-ANC relationship (i.e. had very low pH (3.6) accompanied by high ANC (156 µeq/l) or more as opposed to a pH of 5.11 and an ANC of 68 µeq/l) were excluded from final analysis. The Grubbs test (Grubbs, 1969) was used to detect outliers for all the major ions including DOC and ANC results across all sampling seasons per region. The test assumes a normal distribution of data where probability of occurrence for a set of observed values is determined relative to sample size. Samples that failed the outlier test were excluded from final analysis. Non-macroinvertebrate sites that passed the outlier test but had only one sample representing either the field chemistry or major ions data were also excluded from final analysis due to insufficient data. As a result, the Mpumalanga region was left with 21 sites while the number of sites for the Waterberg (n = 33) and the south-western Cape (n = 30) remained unchanged resulting in a total of 84 sites distributed across all study regions. New Welgevonden (WG) sites were included in the final analysis. The data presented here for the WG sites is thus preliminary. Macroinvertebrates were sampled twice (June 2015 and November 2015) in the Mpumalanga region, four times (June 2015, November 2015, February 2016 and June 2016) in the Waterberg and three times (June 2015, November 2015 and June 2016) for the South-western Cape. When macroinvertebrate sampling seasons were compared to

the field and lab chemistry data that passed the outlier test and displayed normal pH-ANC relationship, it was discovered that some of the macroinvertebrate sites were missing either the corresponding field or lab chemistry data (**Table 4-1**).

Prior to initiating the data cleaning process, the Mpumalanga region was missing field chemistry data from two sampling seasons GH (Aug – Sept 2015) and I (Nov 2015). The missing data distorted the outcome of all data cleaning attempts for this region and limited the number of corresponding chemistry and macroinvertebrate data. For example, EHV1 had lab chemistry data from four sampling seasons F, GH, I and J representing the Jun 2015, Aug – Sept 2015, Nov 2015 and Feb 2016 sampling seasons respectively. While the field chemistry data was represented by two seasons F and J. The macroinvertebrates were sampled twice at F and I, however all the I samples were missing field chemistry data. HV4 macroinvertebrate samples were missing all corresponding chemistry data due to discrepancies in the expected pH-ANC relationship that resulted in sample exclusion. In addition to this some of the sites were impacted by the drought which further limited the number of seasonal data available for final analysis.

Variation in the chemistry data across sampling seasons for the Waterberg was mainly attributed to the drought, site modification and sites failing some of the data refining tests mentioned above (**Table 4-1**). For example, WB01, a non-invertebrate site was sampled seven times. Of the seven sampling trips major ion data from two seasons (E and GH) failed the ion balance, conductivity and outlier tests. Consequently, the field data from these two seasons were missing corresponding chemistry data which made for incomplete analysis or comparison across seasons for this site. All the WB02 samples passed the outlier test, thus there was complementary field and lab chemistry data at this site. The conditions at WB03 were similar to those of WB01, however, the missing macroinvertebrate data were attributed to the drought and at a later stage to instream disturbances (soil dumping) that led to site modification and habitat degradation, rendering the site unsuitable for further macroinvertebrate sampling. The collection of water samples was continued nevertheless for the purposes of comparing changes in the chemistry data before and after instream disturbances. Furthermore, after the

modification took place WB03 had an orange colour on the streambed that was accompanied by an oily suspension that suggested the possibility of other types of disturbances further upstream. Overall 76 % (n = 25) of the Waterberg sites had complementary seasonal field and lab chemistry data.

Of the three-study regions the SWC was the only region not impacted by the drought. This fact was supported by the homogeneity of the field chemistry data (**Table 4-1**). The four sites (SWC03, SWC04, SWC09 and SWC14) with seven sampling seasons each were located on public roads that did not require a permit prior to sample collection. Thus, these sites were the very first sites to be sampled at this region hence the extra sampling period. The other sites could only be accessed upon acquiring a permit. Three sites (SWC28, SWC29 and SWC30) were missing samples from the GH sampling period due to inaccessible roads from heavy rains. Sites from the SWC had the most inconsistent chemistry data characterised by very high and equally low major ions and DOC values. Consequently, the majority of the major ions data failed the data quality tests due to discrepancies in the pH-ANC relationship among other inconsistences. Site SWC28 was the only site that had corresponding chemistry data for all macroinvertebrate sampling seasons whereas SWC08 had no major ion data corresponding to its one macroinvertebrate sample. Overall 51 % (n = 34) of the sampling seasons from this region had corresponding major ion data.

Focusing solely on macroinvertebrate sites that had corresponding chemistry data reduced the final number of identified species by 30 % (n = 48). Furthermore, the ambiguous chemistry data made it implausible to evaluate how changes in stream chemistry across seasons affected species distribution as major ions data from some seasons had to be excluded, especially for the SWC. For a more detailed review of site evaluation per season the reader is referred to Appendix 5. The Czech data was used as a benchmark for qualitative analysis and results that deviated significantly from the Czech data were excluded from final analysis. To compensate for the large amounts of data lost from the NWU results all Jun 2016 and Oct 2016 samples that failed the data quality check were replaced by the Czech results. Water chemistry along with species

distribution and abundance are influenced by a number geological and landuse patterns and in order to classify and compare sites with similar geographical features the three study regions were analysed separately using methods suited to the type of data available at each region.

Table 4-1: Data correspondence across sampling seasons.

Mpumalanga			
	Number of corresponding seasons		
Site Code	Major ions	Field chemistry	Inverts
EHV01	4 (F,GH,I,J)	2 (F,J)	2 (F,I)
EHV02	4 (F,GH,I,J)	2 (F,J)	2 (F,I)
EHV03	4 (F,GH,I,J)	2 (F,J)	-
EHV04	3 (F,I,K)	2 (F,J)	2 (F,I)
EHV05	2 (F,GH)	1 (F)	1 (I)
EHV06	2 (F,GH)	1 (F)	-
EHV07	4 (F,GH,I,J)	2 (F,J)	1 (I)
EHV08	4 (F,GH,I,J)	2 (F,J)	2 (F,I)
EHV09	2 (GH,I)	2 (F,J)	1 (I)
EHV11	4 (F,GH,I,J)	2 (F,J)	-
EHV12	3 (F,GH,I)	2 (F,J)	1 (I)
EHV13	3 (GH,I,J)	2 (F,J)	1 (I)
EHV14	2 (F,GH)	1 (F)	-
EHV15	4 (F,GH,I,J)	2 (F,J)	1 (I)
EHV16	4 (F,GH,I,J)	2 (F,J)	2 (F,I)
EHV17	3 (CD,F,I)	1 (CD)	-
EHV18	4 (CD,F,I,J)	3 (CD,F,J)	-
EHV19	5 (CD,F,GH,I,J)	3 (CD,F,J)	1 (I)
EHV20	5 (CD,F,GH,I,J)	3 (CD,F,J)	1 (I)
HV23	5 (CD,F,GH,I,J)	2 (CD,J)	-
HV4	1 (CD)	3 (CD,F,J)	2 (F,I)

KEY

SEASON	CODE
Aug - Sept 2014	AB
Nov - Dec 2014	CD
Feb 2015	E
Jun 2015	F
Aug - Sept 2015	GH
Nov 2015	I
Feb 2016	J
Jun 2016	K
Oct 2016	L

Waterberg			
	Number of corresponding seasons		
Site Code	Major ions	Field chemistry	Inverts
WB01	5 (F,I,J,K,L)	7 (E,F,GH,I,J,K,L)	-
WB02	6 (E,F,GH,I,J,K)	6 (E,F,GH,I,J,K)	-
WB03	5 (AB,F,GH,J,K)	7 (CD,E,F,GH,I,J,K)	1 (F)
WB05	8 (AB,E,F,GH,I,J,K,L)	8 (AB,E,F,GH,I,J,K,L)	4 (F,I,J,K)
WB06	8 (AB,E,F,GH,I,J,K,L)	8 (AB,E,F,GH,I,J,K,L)	1 (F)
WB07	2 (AB,E)	2 (AB,E)	-
WB07B	2 (E,GH)	3 (E,F,GH)	-
WB07C	2 (F,GH)	2 (F,GH)	1 (F)
WB09	4 (AB,E,F,GH)	4 (AB,E,F,GH)	-
WB11	3 (AB,F,GH)	3 (AB,F,GH)	-
WB12	3 (F,GH,K)	4 (F,GH,J,K)	3 (F,J,K)
WB13	6 (AB,F,I,J,K,L)	7 (AB,F,GH,I,J,K,L)	4 (F,I,J,K)
WB14	7 (AB,E,F,GH,J,K,L)	8 (AB,E,F,GH,I,J,K,L)	3 (F,I,K)
WB16	6 (AB,E,F,J,K,L)	6 (AB,E,F,J,K,L)	3 (F,J,K)
WB19	3 (AB,E,F)	3 (AB,E,F)	1 (F)
WB20	7 (AB,E,F,GH,J,K,L)	7 (AB,E,F,GH,J,K,L)	-
WB21	8 (AB,E,F,GH,I,J,K,L)	8 (AB,E,F,GH,I,J,K,L)	-
WB22	4 (AB,E,F,GH)	4 (AB,E,F,GH)	1 (F)
WB23A	5 (F,GH,I,J,K)	6 (E,F,GH,I,J,K)	4 (F,I,J,K)
WB23B	5 (F,GH,I,J,K)	5 (F,GH,I,J,K)	-
WB24	8 (AB,E,F,GH,I,J,K,L)	8 (AB,E,F,GH,I,J,K,L)	4 (F,I,J,K)
WB25	1 (E)	3 (AB,E,F)	1 (F)
WB26	8 (AB,E,F,GH,I,J,K,L)	8 (AB,E,F,GH,I,J,K,L)	4 (F,I,J,K)
WB27	5 (E,F,GH,J,K)	5 (E,F,GH,J,K)	-
WB30	7 (E,F,GH,I,J,K,L)	7 (E,F,GH,I,J,K,L)	2 (F,I)
WB31	2 (F,K)	2 (F,K)	-
WB32	2 (F,GH)	2 (F,GH)	-
WB32A	5 (F,GH,I,J,K)	5 (F,GH,J,K,L)	3 (F,J,K)
WB33	2 (GH,J)	2 (GH,J)	1 (J)
WB34	1 (GH)	1 (GH)	-
WB35	1 (L)	1 (L)	-
WG01	1 (L)	1 (L)	-
WG02	1 (L)	1 (L)	-

South-western Cape			
	Number of corresponding seasons		
Site Code	Major ions	Field chemistry	Inverts
SWC01	3 (J,K,L)	6 (F,GH,I,J,K,L)	2 (I,K)
SWC03	3 (J,K,L)	7 (E,F,GH,I,J,K,L)	2 (I,K)
SWC04	2 (J,K)	7 (E,F,GH,I,J,K,L)	-
SWC06	2 (K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC07	3 (F,J,L)	6 (F,GH,I,J,K,L)	-
SWC08	3 (GH,I,L)	6 (F,GH,I,J,K,L)	1 (K)
SWC09	3 (J,K,L)	7 (E,F,GH,I,J,K,L)	-
SWC14	2 (K,L)	7 (E,F,GH,I,J,K,L)	3 (F,I,K)
SWC16	5 (GH,I,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC17	5 (GH,I,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC18	3 (J,K,L)	6 (F,GH,I,J,K,L)	-
SWC19	4 (GH,J,K,L)	6 (F,GH,I,J,K,L)	2 (I,K)
SWC20	4 (GH,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC21	3 (J,K,L)	6 (F,GH,I,J,K,L)	-
SWC22	5 (F,I,J,K,L)	6 (F,GH,I,J,K,L)	2 (I,K)
SWC23	3 (J,K,L)	6 (F,GH,I,J,K,L)	2 (I,K)
SWC24	1 (K)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC25	5 (F,GH,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC26	4 (GH,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC27	5 (GH,I,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC28	5 (F,I,J,K,L)	5 (F,I,J,K,L)	3 (F,I,K)
SWC29	4 (I,J,K,L)	5 (F,I,J,K,L)	3 (F,I,K)
SWC30	4 (I,J,K,L)	5 (F,I,J,K,L)	3 (F,I,K)
SWC31	5 (F,GH,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC32	4 (GH,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC33	4 (GH,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC34	3 (J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC35	4 (GH,J,K,L)	6 (F,GH,I,J,K,L)	2 (F,K)
SWC36	3 (GH,J,K)	6 (F,GH,I,J,K,L)	3 (F,I,K)
SWC37	4 (F,J,K,L)	6 (F,GH,I,J,K,L)	3 (F,I,K)

4.6 Data screening and transformation process

A detailed account of all the data cleaning processes and quality assessment is given in the section 4.5 above as well as in appendices 5. Data reduction however was one major problem encountered during the data cleaning process. Nonetheless final regional (Mean values) and seasonal tables (Appendix 6) were constructed from the remaining raw data and exported to relevant statistical programmes.

R studio was used to visualise outliers and data distribution patterns by means of histograms and quantile plots. This was an important step as data distribution often dictates the type of transformation and modelling techniques to apply to the dataset. Furthermore, understanding distribution patterns reduces the possibility of false positive or obscure results that come in the form of type I and type II errors. The species data was processed on PC-ORD software (Version 7, MjM Software Design, Gleneden Beach, Oregon, USA,) which is specific to ecological data and is thus deemed 'naturally' accommodative to a typical species matrix that is often dominated by zeros. Consequently, standardisation and transformation of the chemistry data was also carried out on PC-ORD. Matrices and data frames generated on PC-ORD were exported as excel files for use on other statistical software. The national (all three study regions combined), regional (x3 matrices of HV, SWC and WB sites) and species data were transformed separately, and different transformation processes were applied to each dataset. The choice of standardisation and transformation method was mostly depended on the type of data values being examined (i.e. decimal fractions, positive or negative values). The process of checking for outliers was carried out as a final step once the data had been transformed. Some outliers can be removed during the data transformation process and thus any outliers that were detected post transformation were deleted. To remove outliers a data cutoff point was chosen and any values that lay beyond that point were excluded from the dataset. In cases where the removal of outliers led to further data reduction problems, alternative non-parametric techniques were employed.

4.6.1 Logarithmic transformation

Data transformation was carried out using the generalised log transformation method instead of the more common log(x) and log(x-1) transformations. This type of log transformation follows the equation:

$$b = \log(x + xmin) - \log(xmin)$$

where $xmin$ represents the smallest positive value in the dataset (McCune & Grace, 2002). The generalised log transformation is ideal for data that has decimal fractions as was the case with the HV and WB sites. Log and square root transformations could not be performed on the south-western Cape dataset as it contained negative values. This also applied to the national dataset.

4.6.2 Relativisation

Relativisation is the process of rescaling the data by row or column properties where the variables are centered by subtracting the Mean and rescaled by dividing with the SD. As a result, all the variables end up with a Mean that is equal to 0 and a variance of 1 (McCune *et al.*, 2002). In this study the national and SWC dataset were relativised by columns following the equation:

$$b_{ij} = \left(x_{ij} - \bar{x}_j\right)/S_j$$

Where x_{ij} is the original data point in row i of column j, while b_{ij} represents the output or adjusted value of x_{ij} and S_j is the standard deviation of column j (McCune *et al.*, 2002).

4.6.3 Macroinvertebrate data

The species data were prepared according to the guidelines given by McCune *et al.*, (2002), whereupon loading the data on PC-ORD, species summary statistics were generated. These summaries give details on data skewness and kurtosis, the total number of sampling sites, the complete number and list of species, the total number of species per site as well as various diversity indices (i.e. Shannon`s diversity index and β-diversity etc.). Rare species (occurring in < 4 sites) were removed by following

recommendations by McCune *et al.* (2002), thereafter the data were standardised through square root transformation prior to conducting similarity and ordination techniques. Species data analysis was centered around three key principles which were to define the distribution of the data (i.e. unimodal, bimodal etc.), to understand different distance measures and when to use them as well as to know how data distribution affects choice of ordination technique.

4.7 Non-parametric rank measurements

Non-parametric tests do not assume normal distribution of data as is the case with parametric tests such as analysis of variance (ANOVA), t-tests and linear regressions. In this study the Kruskall-Wallis non-parametric test (Kruskal & Wallis, 1952) was used to calculate group means. All the test assumptions were met. For example, all sample data were independent, the variables were all continuous and that the groups had equal variance was tested using the Bartlett test which gave a p value > 0.05 indicating homogeneity in the data. This assumption is key to having the Kruskall-Wallis test truly function as equivalent to a one-way ANOVA that calculates group means.

4.8 Species diversity

All three levels of diversity, namely alpha (α), beta (β) and gamma (γ) were determined at each study region using the Whittaker (1972) approach which has been identified (Koleff *et al.*, 2003) as the most widely used method in ecology (Whittaker, 1972). Following the equation:

$$BD = \frac{S_C}{\bar{S}} \quad OR \quad \beta_W = \frac{S_C}{\bar{S}} - 1$$

as reiterated by McCune and Grace (2002), where β_W represents beta diversity, S_C the total number of species (i.e. gamma diversity) and S the average species richness at each site (McCune & Grace, 2002). Here S was obtained from the summary stats generated

from PC-ORD (appendices). The Whittaker (1972) approach was ideal for this study as it does not follow a specific gradient model but instead considers “compositional heterogeneity” (McCune & Grace, 2002).

4.8.1 Alpha, Beta and Gamma diversity

The concept of assessing variation in species composition across spatial scale was first introduced by Whittaker (1972), when he described three independent indices for calculating species diversity namely, α, β and γ diversity (Whittaker, 1972). Alpha diversity, also known as species richness, refers to the total number of species at a sampling site (**Figure 4-2**), while β diversity compares species composition across sites and recounts species that are unique to each locality (**Figure 4-2**). Gamma diversity on the other hand accounts for the total number of species at a regional scale (**Figure 4-2**), simply implying that α and γ diversity are essentially the same measurement differentiated only by spatial scale (Zhang *et al.*, 2014; Harrison *et al.*, 2004; McCune & Grace, 2002; Whittaker, 1972). The pairwise illustration on **Figure 4-2** is the most basic expression of beta diversity. However, there are several other approaches for estimating β diversity that have been tested by different authors (Koleff *et al.*, 2003; Morris *et al.*, 2014; Zhang *et al.*, 2014; Socolar & Gilroy, 2018; Harrison *et al.*, 2004; Anderson *et al.*, 2011; Burkle *et al.*, 2016; Diserud & Odegaard, 2007; Ricotta & Pavoine, 2015; McCune & Grace, 2002; Wilson & Mohler, 1983; Wilson & Shmida, 1984; Økland *et al.*, 1990; Oksanen & Tonteri, 1995; Barwell *et al.*, 2015) that are applicable based on study objectives. For example β diversity can be calculated from Detrended Correspondence Analysis (DCA) (McCune & Grace, 2002; Økland *et al.*, 1990), where a gradient length > 3 is indicative of a unimodal distribution (Palmer & Palmer, 2016; Palmer, 2014). Consequently, β diversity can thus be used to determine the appropriate ordination technique to use on a specific dataset (McCune & Grace, 2002). It is important however to note that there is still much debate among ecologists as to what constitutes β diversity (Koleff *et al.*, 2003; Burkle *et al.*, 2016; Anderson *et al.*, 2011; Barwell *et al.*, 2015; Ulrich & Almeida-Neto, 2012), hence the multitude of concepts and measurements that exist. Nonetheless, species turnover and nestedness are recognised as the key components that define β diversity (McCune &

Grace, 2002; Whittaker, 1972; Anderson *et al.*, 2011; Ulrich & Almeida-Neto, 2012; Staniczenko *et al.*, 2013; Ricotta & Pavoine, 2015).

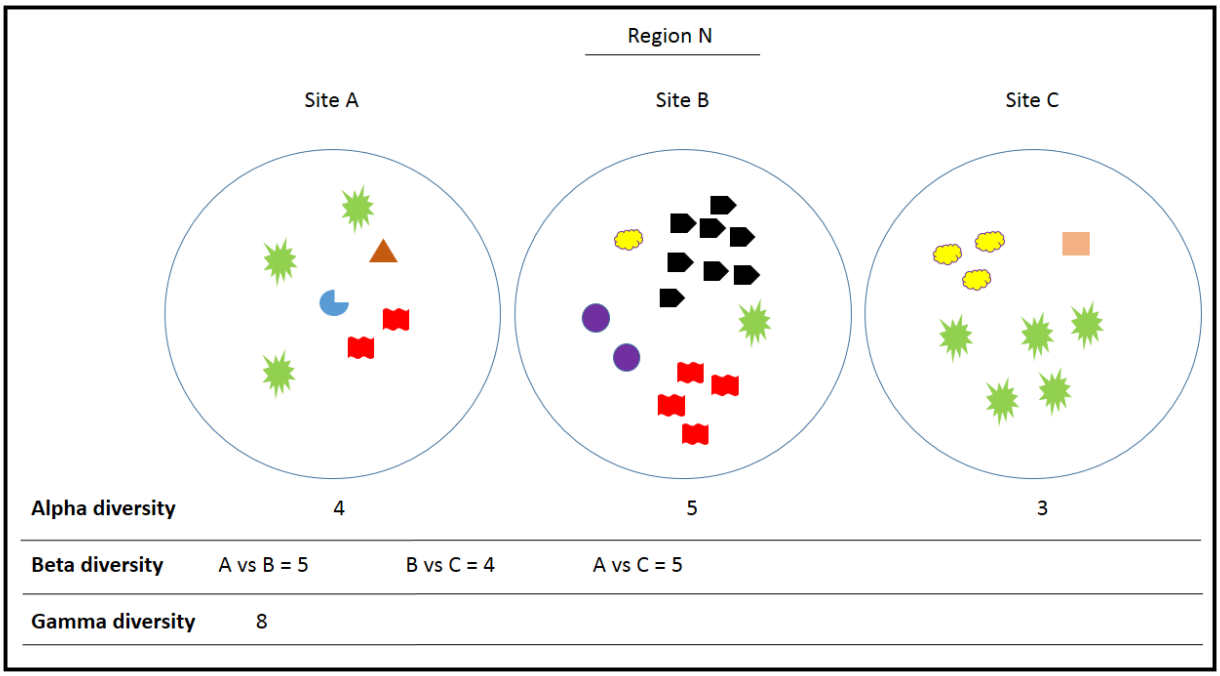


Figure 4-2 Simple representation of α, β, and γ diversity. The blue circles represent study sites A-C. Each coloured shape within each circle represents a single species. Site A for instance has a total of 4 different species that represents the sites alpha diversity and site C for example has 3 species and thus the alpha diversity there is 3. Gamma diversity represents the total number of species found at all study sites which in this case equals 8. Beta diversity is estimated through comparative analysis and represents the total number of species that are unique to both sites. For example, Sites A and B together have a total of 7 species and only two species are common to both sites. However, 2 species are unique to site A and 3 are only found in B, therefore the beta diversity for these two sites equals 5.

4.9 Calculating the median value for the monthly rainfall range estimates from the SWC

Monthly rainfall data were obtained from the South African Weather Services (SAWS) website (http://www.weathersa.co.za/). The data are given in a form of range (i.e. 0 – 10 mm, 10 – 25 mm, 25 – 50 mm etc.) estimates in millimetres (mm) or percentage (%) equivalents. The mm data were used in this study. The decision to use the median values instead of the given range came from observing that the sampling points did not always fall within the same range estimate and thus the rainfall estimation for one dataset could cover a wide range of rainfall patterns that did not reflect field observations (i.e. no stream flow areas). Equally so, extending the range to accommodate all the sampling points led to seasonal overlap. To compensate for these deficiencies the range median values were used. In instances where the sampling sites were distributed across two different monthly rainfall estimations (i.e. 25 – 50 mm and 50 – 100 mm) the two were combined to form one range (25 – 100 mm) and the median was calculated. For the Jun. 2015 sampling season, the sampling sites were distributed across a 25 – 100 mm range which gave a median value of 62.5 mm. The Nov. 2015, Feb. 2015 and Jun. 2016 sampling seasons shared the same range. Sites from the Aug-Sept. 2015 sampling season had a much wider range of 25 – 500 mm which resulted in a median of 262.5 mm. The lowest rainfall (10 – 50 mm) for the SWC came during the Oct. 2016 where the calculated median was 30 mm.

4.10 Indicator Species Analysis (ISA) - the Dufrene-Legendre approach

The Dufrene-Legendre ISA is used to generate an Indicator Value (IndVal) for a particular species based on the species relative abundance and relative frequency of occurrence in the different taxonomic groups of interest (Dufrêne & Legendre, 1997). The Monte Carlo randomisation test is used to test for statistical significance. The results are given in percentages where zero equals no indicator strength and 100 % denotes a perfect indicator species for a particular group (Dufrêne & Legendre, 1997; McCune *et al.*, 2002). Since the technique is based on the relative frequency and relative abundance it means that groups being compared can have species that give a 100 % IndVal for each group. Groups are most commonly defined by environmental variables, experimental treatment or habitat types (McCune *et al.*, 2002). In the case of this present study the three study regions were used to define groups of interest under the habitat type guise, where based on the data at hand we set out to identify indicator species that best represent the typology of the study regions based on their overall abundance and relative frequency. This was an essential step in that if the three study regions are best represented by different species this will in turn reduce the possibility of identifying indicator species on a national scale based on the current dataset. The test was conducted on all recorded species (n = 136) and only species that showed statistical significance were reported on.

4.11 Ordination and classification techniques

The empirical relationship between species data and water chemistry was established by employing classification and ordination techniques. The basis for choosing a particular approach was largely dependent on data availability (see section 4.5 above and appendices 5 for detail) and the distribution (unimodal, bimodal or normal) model (McCune *et al.*, 2002). Consequently, multivariate and univariate techniques were utilised for statistical analysis. As with most species' matrices (McCune *et al.*, 2002) the community data were sparse and largely dominated by zeros as well as rare species.

4.11.1 Classification and Regression Tree (CART) analysis

In this study CART analysis (McCune *et al.*, 2002; Therneau *et al.*, 2018; Milborrow, 2018; Lewis, 2000) was used to identify variables that play a key role in determining differences between sites across study regions. The classification process was carried out using the rpart.plot function in R studio (Therneau *et al.*, 2018; Milborrow, 2018) and the parameters were set as follows:

CART=rpart(Site.Code~., data, method = "class")
rpart.plot(CART, type = 4,extra = 101)
in line with the (Milborrow, 2018) recommendations of Milborrow, (2018).

CART analysis assumes a binary recursive partitioning technique in that during the classification process the root or parent node is split into two groups also known as child nodes that represent either presence/absence or yes/no responses (**Figure 4-3**). The technique is recursive in that as the tree continues to grow in the absence of terminal nodes the child nodes become parent nodes giving rise to other binary nodes.

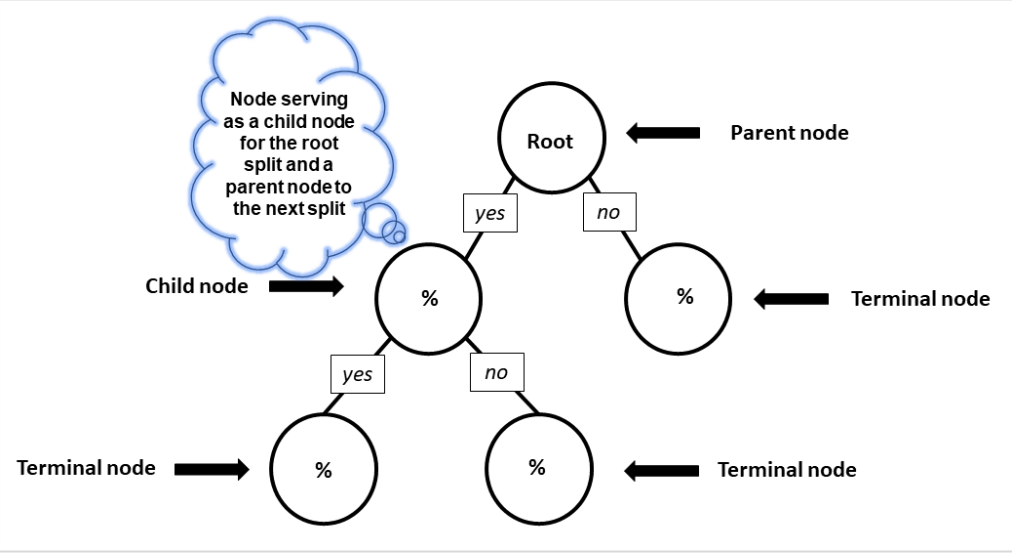


Figure 4-3: An example of the CART analysis decision tree. The percentage (%) represents the number of observations in each node. Decision trees tend to vary according to user defined parameters.

4.11.2 Ordination

An environmental gradient refers to any feature that influences species composition i.e. altitude, soil type, temperature, light intensity and seasonality (Whittaker, 1972). Species tend to occupy different spaces along the gradient and choice of habitat is determined by resource availability, species selection and/or preference. Consequently, gradient length can be used to estimate β-diversity (McCune *et al.*, 2002). Changes in the environmental gradient are said to be simple or complex depending on the number of variables present. More complex gradients are articulated through ordination techniques such as Detrended Correspondence Analysis (DCA), Canonical Correspondence Analysis (CCA) and Non-metric Multidimensional Scaling (NMS), to name a few.

4.11.2.1 Principle component analysis (PCA)

Principle component analysis is the most commonly used technique for defining clusters and establishing their correlation within ordination space. The PCA was conducted on RStudio (Version 1.1.442 – © 2009-2018 RStudio, Inc.) using the ggplot2 and ggfortify packages.

4.11.2.2 Canonical Correspondence Analysis (CCA)

A Canonical Correspondence Analysis (Ter Braak, 1986) was conducted on PC-ORD software version 7 (MJMSoftware Design: https://www.pcord.com/pcordwin.htm) (McCune & Grace, 2002). During the ordination analysis the primary matrix which contains species data is constrained against the secondary matrix that consists of environmental variables. The CCA is very sensitive to background noise and thus some precautionary measures are necessary prior to conducting the analysis (i.e. reducing the number of variables if they exceed number of sampling sites). Prior to performing the analysis species with fewer than four occurrences were removed. For the results, output variables are expressed in vector format where each vector is explained by the axis it sits closest to. The vector length represents the variable that has the most impact on overall

species composition, particularly for the species and sites sitting closest to the explanatory variable. The further the species and sites are from the vector the less correlation there is with the explanatory variable. The Monte Carlo test was used to test for statistical significance.

Chapter 5 Establishing key differences in the physico-chemical properties of the streams in the Mpumalanga (HV), Waterberg (WB) and the southern Cape (SWC) study regions

A total of 80 headwater streams representing 84 sampling points distributed across the HV (n = 21), WB (n = 33) and the SWC (n = 30) were identified. Geographically the three study regions differed from each other and this variation is most apparent in differences in altitude (**Figure 5-1**), ecoregions, biome and climatic zones as highlighted in section **3.2** and **3.3** of the thesis. Stream temperature (Temp), dissolved oxygen (DO), conductivity (EC) and pH, as well as major anion (SO_4^{2-}, NO_3^-, Cl^-, F^- and PO_4^{3-}) and cation (Ca^{2+}, Mg^{2+}, K^+, Na^+ and $NH4^+$) concentrations were measured across all study regions. The acid neutralising capacity was calculated using the equation ANC = Σ BC – Σ SAA as given in section Error! Reference source not found. of the literature review and the role of DOC in stream acidity was assessed. The amount of viable data per site across sampling seasons varied considerably due to discrepancies in the major ions data (See section 4.5 above). However, since the majority of the sites comprised viable field chemistry data, site classification using regression trees was carried out on this dataset.

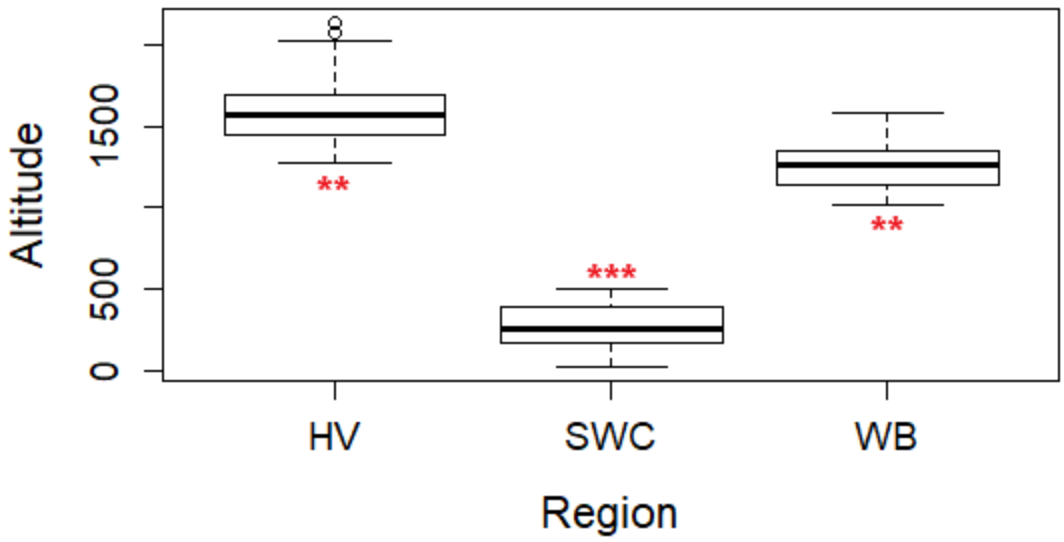


Figure 5-1: The difference in altitude as a reflection of spatial variations between study regions. Streams from the HV region were found at elevation levels > 1200 m from sea level while the SWC had sites sitting as low as 26 m (SWC36) above sea level. The WB sites were in the range of 904 m (WB08) to 1586 m (WB34). Asterisk indicates level of significance (** $p < 0.01$; *** $p < 0.001$). Actual p-values are given in Appendix 7.

5.1 CART analysis

Through CART analysis, environmental variables that play a key role in determining differences between sampling sites across all study regions were identified (**Figure 5-2**). EC and pH were recognized as the most important explanatory variables. In the model the study sites were split into two groups representing EC > or < 68 µS/cm. A majority (n = 31) of the WB sites had EC values that were < 68 µS/cm while the HV (n = 14) and SWC (n = 30) sites were dominated by streams with EC values > 68 µS/cm. Sites with EC values > 68 µS/cm made up 55 % (n = 46) of the overall sample size and more than half (35 %) of these had pH values < 6 whereas 20 % of the remaining sites consisting of HV, WB and SWC sites had pH > 6. Since only 16 out of 55 sites from the HV and WB were classified as having pH > 6 it is safe to assume that some of the streams categorised as having EC < 68 µS/cm consist of sites that have pH < 6. At the end of the classification three terminal nodes were produced and these nodes easily place the streams from each

region into three distinct categories with a small measure of overlap observed mainly between the HV and WB sites **Figure 5-2**.

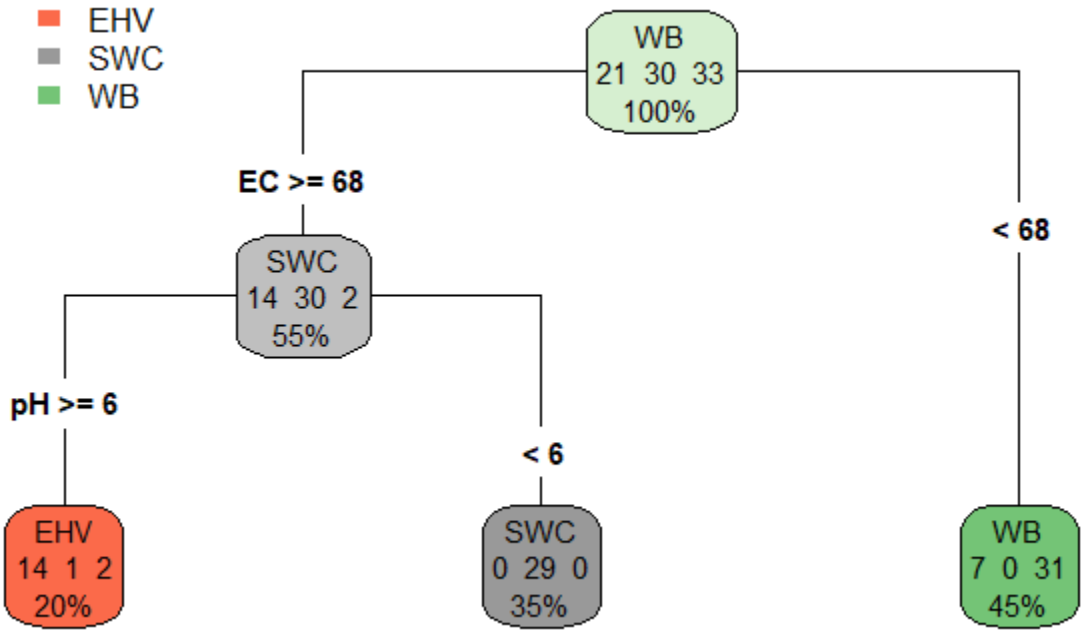


Figure 5-2: CART analysis for the EHV, WB and SWC sampling sites. Classification and regression trees are interpreted in an upside-down tree fashion. The tree root or parent node sits on top of the tree and contains details of the complete (100%) dataset, in our case 84 sampling points representing 21 streams from the HV, 30 in the SWC and 33 from the Waterberg. The study regions are colour coded according to the key given on the top left corner of the figure and the number of sites per region are presented from left to right on the tree nodes following the top down sequence of the key. Here the study regions represent the predicted class or dependent variable. Thus, each node including the root node is colour coded according to the region or predicted class displaying the largest variation when compared to the other classes. Overall the results projected above show a significant difference between study regions that is best explained by the difference in stream pH and conductivity.

5.2 The Kruskal-Wallis non-parametric test

The Kruskal-Wallis test was conducted on all environmental variables and a pairwise comparison using the Dunn (1964) Kruskal-Wallis multiple comparison test revealed major differences in the abiotic properties of the streams regionally. The highest pH concentrations were observed in the HV region (mean pH = 7.24, $p < 0.05$) and the lowest for the SWC (mean pH = 4.88, $p < 0.001$). The Waterberg contained mostly acid to circumneutral streams with a mean pH of 6.21 ($p < 0.05$). Stream conductivity was significantly lower in the Waterberg (mean = 39 µS/cm, $p < 0.001$) when compared to the HV (mean = 180 µS/cm) and the SWC (mean = 161 µS/cm). No statistically significant difference was observed in EC between the HV and SWC sites for the same variable. These EC and pH results echo those obtained from CART analysis. The DO concentrations were not significantly different from each other across all study regions ($p > 0.05$). Streams from the SWC however were generally cooler (mean Temp = 14 °C, $p < 0.01$) while those from the HV (mean Temp = 17 °C) and WB (mean Temp = 19 °C) study regions were typically associated with higher temperatures.

5.2.1 Acidic anion concentrations across study regions

SO_4^{2-} concentrations differed significantly ($p < 0.05$) across all study regions and mean concentrations of 125 µeq/L were observed for the SWC while 1084 µeq/L and 12 µeq/L were detected in the HV and WB respectively (**Figure 5-3**). The Waterberg displayed significantly lower NO_3^- levels (mean = 1 µeq/L, $p < 0.001$) than those in the SWC (mean = 6 µeq/L) and the HV (mean = 12 µeq/L) regions. A comparison of both the latter revealed no statistically significant differences in NO_3^- concentrations between these two regions ($p > 0.05$). Chloride concentrations across all study regions displayed similar patterns to those of SO_4^{2-} where the highest concentrations were observed in the SWC (mean = 1181 µeq/L, $p < 0.001$) when compared to the 132 µeq/L and 119 µeq/L from the HV and Waterberg respectively. Levels of F^- were significantly higher in the HV ($p < 0.001$) when compared to the other study regions, whereas no statistically significant differences were observed for the concentration of PO_4^{3-} ions across all study regions ($p > 0.05$).

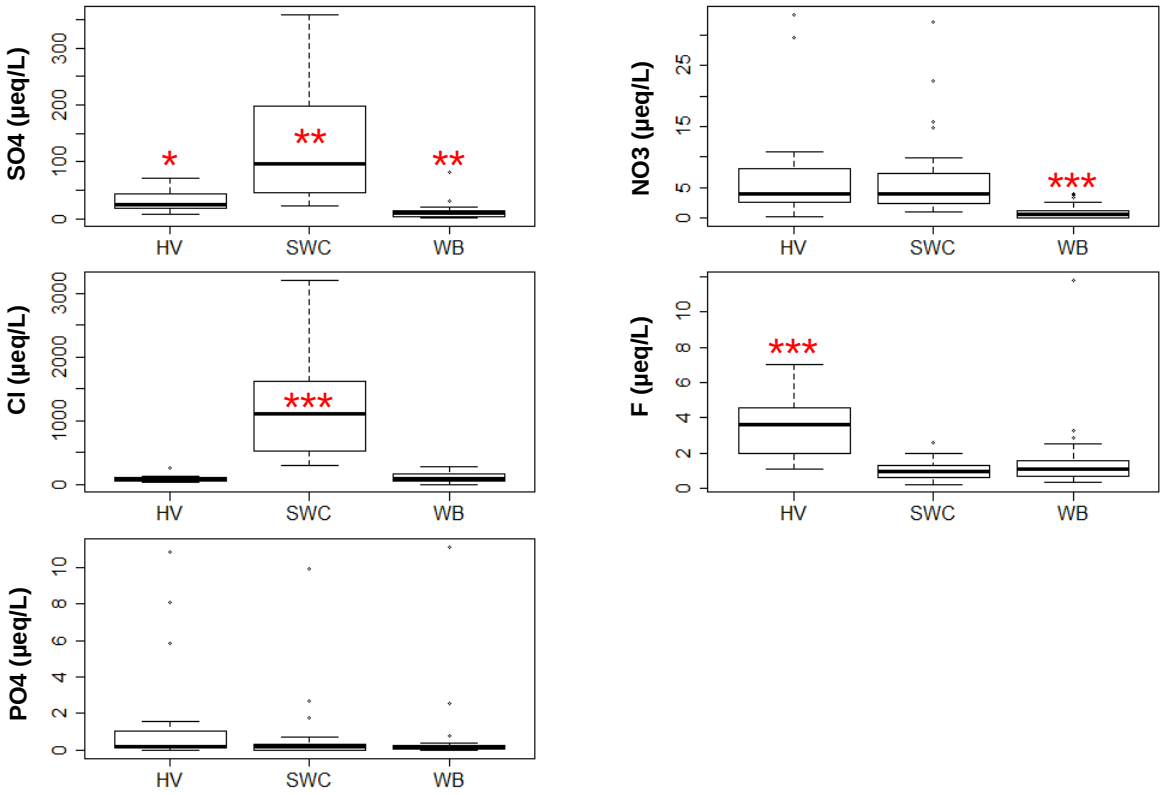


Figure 5-3: Major anion concentrations across the HV, SWC and WB study regions. Boxplots were used to examine major ion concentrations by region. Asterisk indicates level of significance of major ion concentrations across study regions (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Actual p-values are given in Appendix 7. For the boxplots, the box represents the interquartile (25 % - 75 %) range. The line in the middle of the box is the median value for the dataset and the whiskers represent the maximum (top) and minimum (bottom) data values.

Nitrogen (N) saturation occurs when inorganic N inputs exceed biological demands (Curtis, 2002). Mean annual loadings of nitrate across the study regions show evidence of N saturation in 48 % (n = 40) of the study sites (**Table 5-1**). This was reflected in NO_3^- concentrations that exceed 3 µeq/L. The regional NO_3^- mean for the Waterberg was 1 µeq/L (section **5.2.1**) but site specific annual means seem to suggest that 12 % (n = 4) of the sites (WB19, WB23B, WB26 and WB32) from the Waterberg have environmentally harmful NO_3^- concentrations. The N Saturation threshold was interpreted in accordance to the European benchmark as presented in European scientific publications (Curtis *et al.*, 2001). We do however acknowledge that interpreting South African results in terms of European benchmarks is not ideal, especially in the absence of sufficient baseline data from unimpacted regions which are necessary for establishing an appropriate “threshold” value for nitrate in unpolluted South African Streams. However, NO_3^- concentrations > 10 µeq/L are a cause for concern and open room for further investigations.

Table 5-1: Evidence of N Saturation in water samples from the HV, WB and SWC regions of South Africa. Exceedance values (NO_3^- concentrations > 3 µeq/L) are indicated in red.

HV		SWC		WB	
EHV01	41.5	**SWC01**	1.1	**WB01**	0.8
EHV02	57.7	**SWC03**	3.4	**WB02**	1.9
EHV03	8.5	**SWC04**	2.0	**WB03**	1.9
EHV04	24.4	**SWC06**	4.9	**WB05**	0.9
EHV05	2.2	**SWC07**	2.4	**WB06**	0.3
EHV06	2.5	**SWC08**	3.3	**WB07**	0.0
EHV07	6.2	**SWC09**	1.8	**WB07B**	0.1
EHV08	4.0	**SWC14**	9.4	**WB07C**	1.6
EHV09	2.0	**SWC16**	6.9	**WB09**	1.4
EHV11	3.9	**SWC17**	3.8	**WB11**	0.6
EHV12	10.9	**SWC18**	2.5	**WB12**	2.6
EHV13	29.6	**SWC19**	7.2	**WB13**	0.1
EHV14	0.2	**SWC20**	32.1	**WB14**	0.1
EHV15	3.3	**SWC21**	22.5	**WB16**	0.3
EHV16	4.1	**SWC22**	14.7	**WB19**	3.4
EHV17	3.9	**SWC23**	5.5	**WB20**	0.0
EHV18	33.3	**SWC24**	15.8	**WB21**	0.7
EHV19	7.8	**SWC25**	2.4	**WB22**	1.2
EHV20	2.6	**SWC26**	4.0	**WB23A**	1.2
HV23	3.2	**SWC27**	2.1	**WB23B**	3.8
HV4	0.6	**SWC28**	3.6	**WB24**	0.7
		SWC29	5.6	**WB25**	0.2
		SWC30	7.7	**WB26**	4.1
		SWC31	4.0	**WB27**	1.1
		SWC32	3.1	**WB30**	0.1
		SWC33	1.7	**WB31**	0.1
		SWC34	10.0	**WB32**	3.3
		SWC35	4.9	**WB32A**	0.3
		SWC36	3.9	**WB33**	0.0
		SWC37	2.4	**WB34**	0.0
				WB35	1.1
				WG01	0.8
				WG02	0.4

5.2.2 Assessing major cation concentrations across study regions

Significantly high H^+ concentrations were detected in the SWC ($p < 0.001$) when compared to the WB and the HV region (**Figure 5-4**). Calcium was significantly higher (mean = 724 µeq/L, $p < 0.001$) in the HV region when compared to the Waterberg (mean = 90 µeq/L) and the SWC (mean = 77 µeq/L). The Mg^{2+} ion concentrations were significantly lower (mean = 104 µeq/L, $p < 0.001$) in the Waterberg streams when compared to those from the SWC (mean = 246 µeq/L) and the HV (mean = 1005 µeq/L) region whereas no statistically significant differences were detected among the latter for the same variable. Potassium concentrations were highest in the HV (mean = 38 µeq/L, $p < 0.01$) when compared to the SWC (mean = 15 µeq/L) and Waterberg (mean = 11 µeq/L). The SWC also displayed the highest Na^+ ion concentrations (mean = 1004 µeq/L, $p < 0.001$) when compared to the WB (mean = 168 µeq/L) and HV (mean = 375 µeq/L) region, whereas the Waterberg displayed significantly lower NH_4^+ concentrations ($p < 0.001$) when compared to the other two regions.

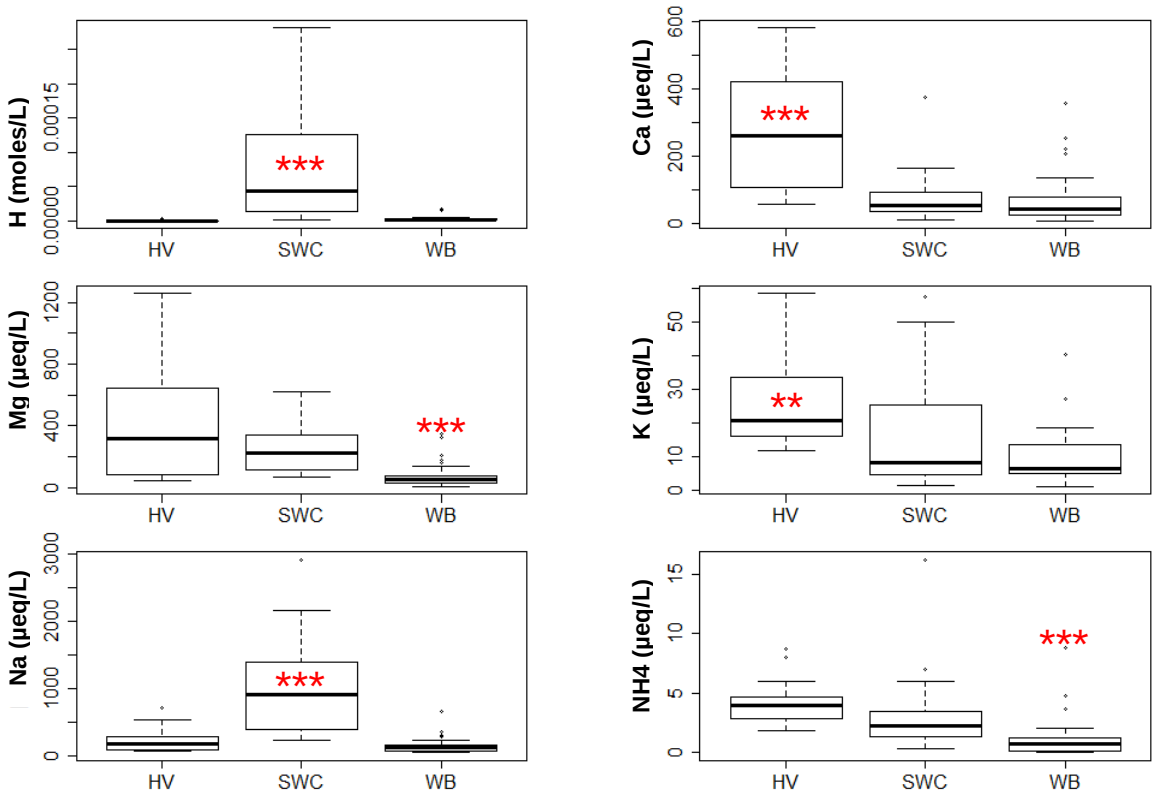


Figure 5-4: Major cation concentrations across the HV, SWC and WB study regions. Asterisk indicates level of significance of major ion concentrations across study regions (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

5.2.3 ANC and DOC concentrations

The acid neutralising capacity was significantly lower (mean 30 µeq/L, $p < 0.001$) in the SWC than in the HV (mean = 914 µeq/L) and the Waterberg (mean = 241 µeq/L) study regions (**Figure 5-5**). Furthermore, DOC concentrations for the SWC were significantly higher ($p < 0.001$) averaging at 13 mg/L when compared to the 3 and 2 mg/L observed for the HV and Waterberg respectively. No statistically significant differences were detected in the DOC between the HV and Waterberg study regions.

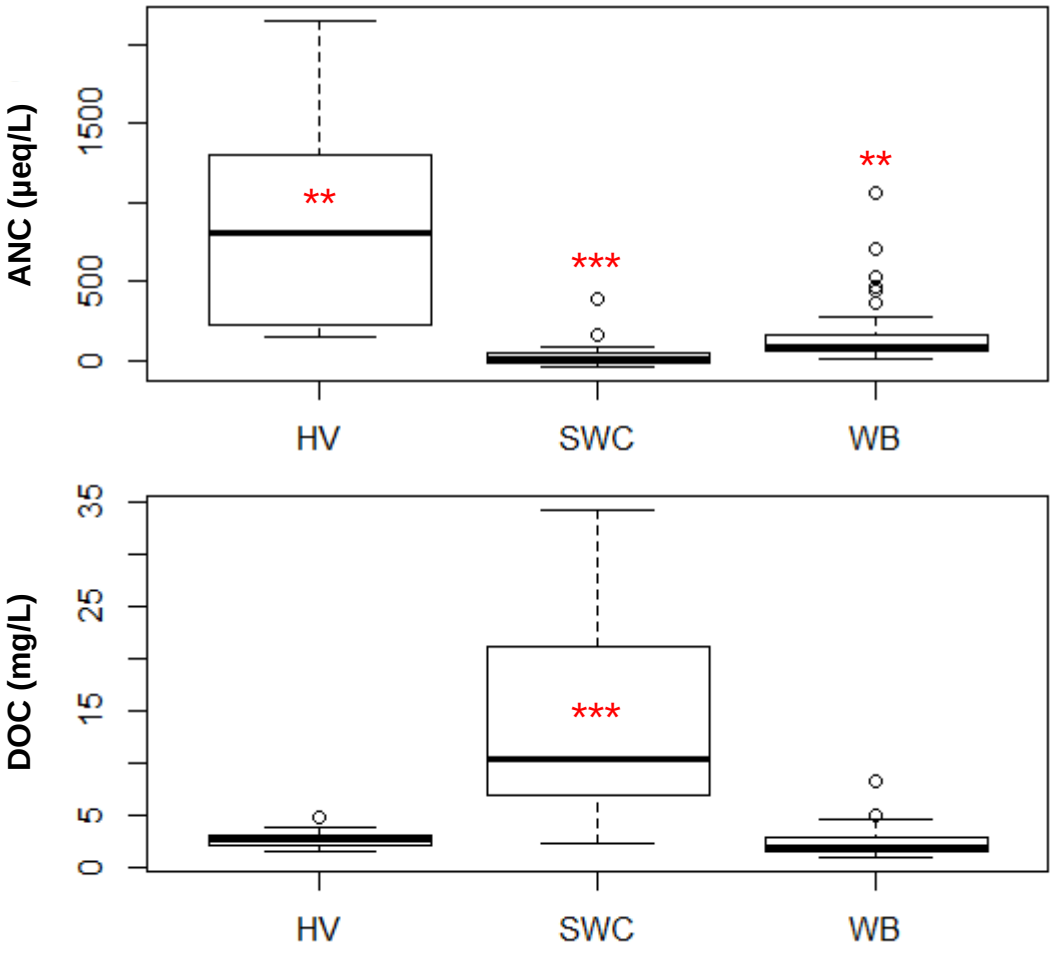


Figure 5-5: Assess the acid neutralising capacity and DOC concentrations across study regions. Asterisk indicates level of significance across study regions (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

5.3 Principle Component Analysis (PCA)

A PCA was performed on the national dataset to reduce data dimensionality to just a few explanatory variables **Figure 5-6:**. A total of thirteen environmental variables (EC, pH, Na^+, NH_4^+; K^+, Mg^{2+}; Ca^{2+},NO_3^-, Cl^-, SO_4^{2-}, F^-, ANC and DOC) were tested and only seven (EC, DOC, Cl^-, Na^+, F^-, ANC and pH) showed significant correlation to a selected number of sites in each region. PC1 accounted for 45.51 % of the variation in the dataset while PC2 represented 28.38 % of the variation. The results indicated that PC1 (45.51 % variance), PC2 (28.38 % variance) and PC3 (11.35 % variance) together accounted for 85 % of the cumulative proportion in the data. Over half (n = 17 of 30 sites) of the SWC sites correlated with Cl^-, Na^+ and DOC concentration and EC, while 43 % (n = 9 of 21 sites) of the Mpumalanga sites and 6 % (n = 2 of 33 sites) of the Waterberg sites were associated with pH, ANC and F^- concentrations. DOC, EC, F^- and to a larger extent pH and ANC concentrations were all correlated to PC2 while [Cl^- and Na^+ ion] were largely correlated to PC1.

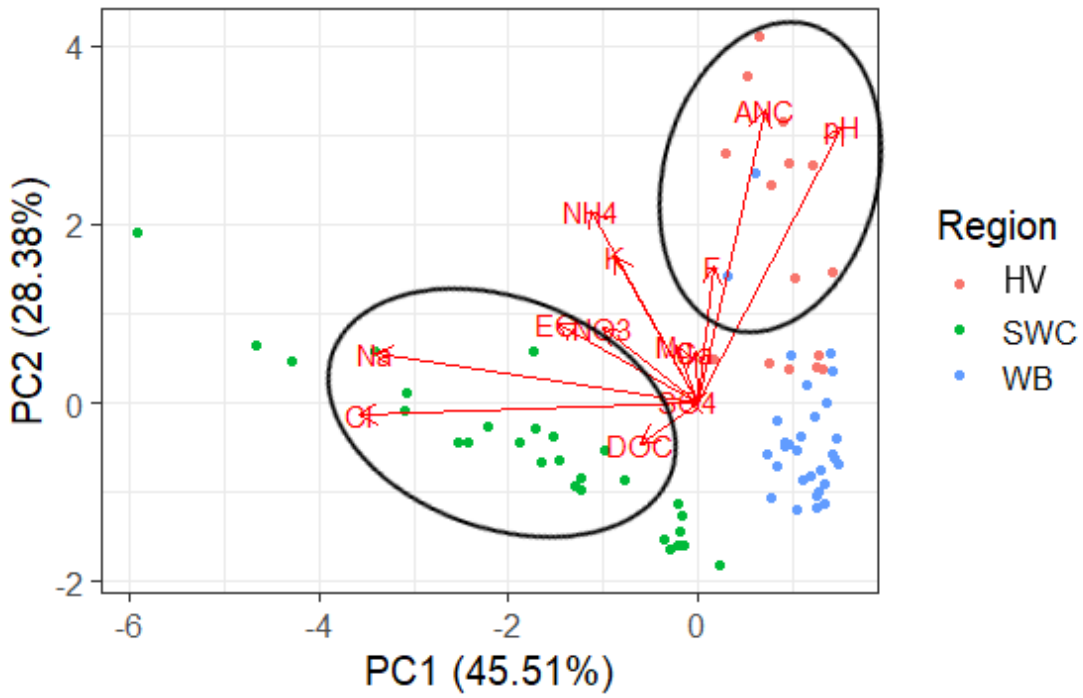


Figure 5-6:PCA on the national dataset. The study sites for the respective regions are indicated in red dots for the Mpumalanga region (HV), green for the south-western Cape (SWC) and blue for the Waterberg (WB). The red arrows represent environmental variables while the ellipses enclose sites and variables that displayed the highest correlation.

Not only do the study regions differ geographically but the chemical variation further accentuates these differences. Overall these PCA results highlight the need to analyse the water chemistry variables from the three study regions separately for adequacy in interpreting research findings. However, as with the CART analysis three distinct clusters that separate the three regions from each other can be seen on the PCA results.

5.4 Dominant marine salts in the SWC samples

Findings from an MSc. project that was carried out under a separate work package of the umbrella project indicated that streams from the SWC were exposed to the lowest levels of acid deposition (Mpho Mompati, unpublished MSc. dissertation, 2018). The work went on to demonstrate that the high SO_4^{2-} concentration detected in the rainwater of the SWC was largely marine. Similarly, in this thesis high stream SO_4^{2-} concentrations were detected for the SWC (**Figure 5-3**) when compared to the other study regions. In light of the findings from the MSc. work this study set out to validate these observations on the stream chemistry. We hypothesised that marine SO_4^{2-} would be correlated to other marine salts i.e. Cl^- and Na^+ and that accumulation of the SO_4^{2-} along with other marine salts would have a much greater effect on the EC than the ANC as marine SO_4^{2-} is not expected to have any adverse effects on the stream pH since it is not associated with acidic deposition (H_2SO_4). To achieve this, streams were categorised according to their distance from sea level using altitude. We anticipated streams from the lower altitudes to have higher sulphate concentrations when compared to those from higher altitudes.

The results revealed a steady gradient where pH, Na^+, Cl^-, SO_4^{2-} and EC concentrations are highest at the lower altitudes compared to altitude > 300 m above sea level (**Figure 5-7**). These observations were true for all the measured variables except for the ANC which showed no statistically significant change across the different altitudes.

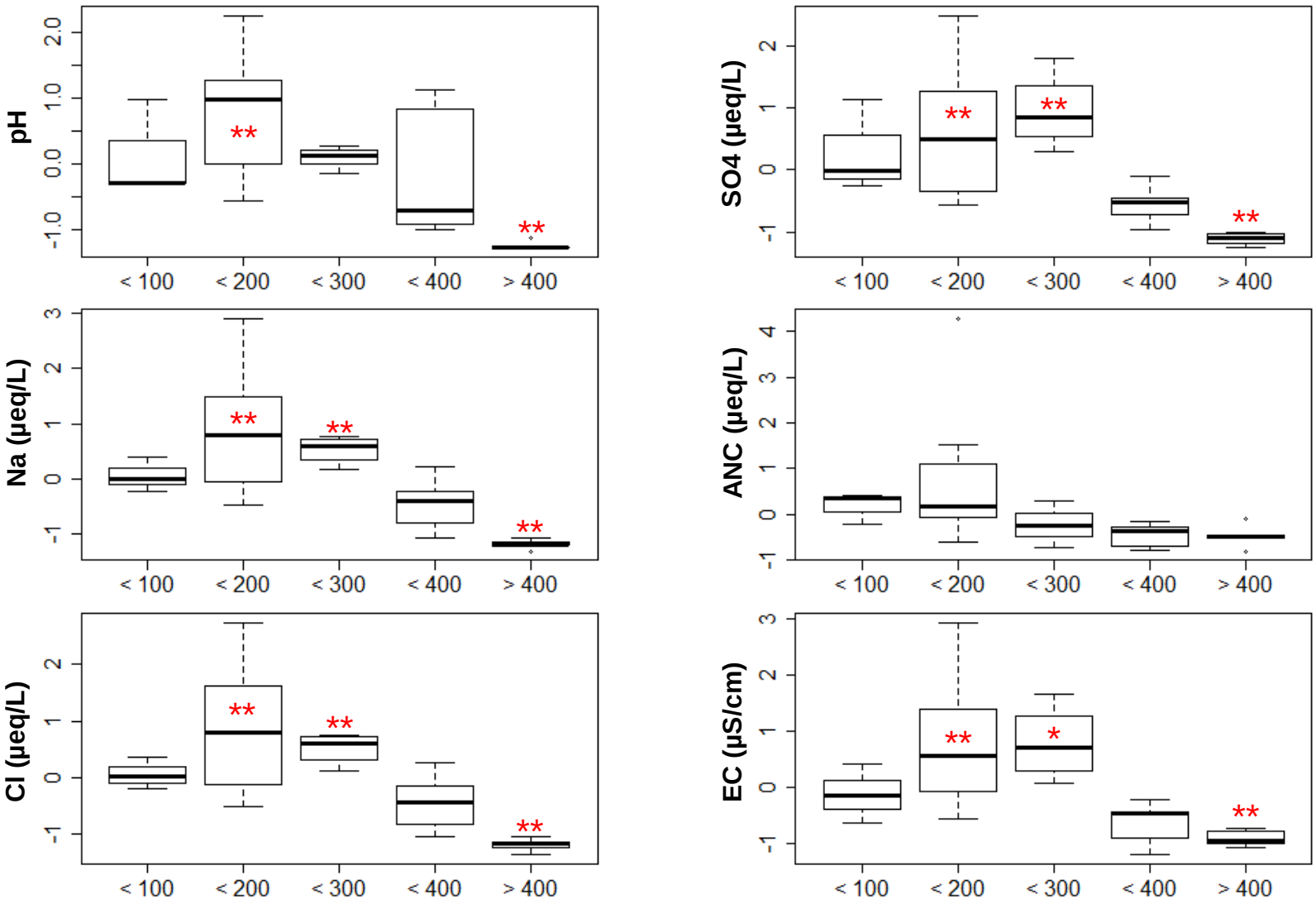


Figure 5-7: Changes in the pH, Na^+, Cl^-, SO_4^{2-}, ANC and EC concentrations along an altitude gradient. This analysis was conducted on the SWC streams exclusively as they displayed the highest non-acid SO_4^{2-} concentrations in the rain chemistry and overall high sea salt concentrations (Na^+ and Cl^-) as demonstrated in the section **5.2.1** and **5.3.** of the thesis.

Correlation analysis on the variables measured above revealed a good correlation between pH and EC (r = 0.67), [Na^+ ion] (r = 0.77), [Cl^- ion] (r = 0.77), [SO_4^{2-} ion] (r = 0.58) and [ANC] (r = 0.65). [Cl^- ion] were highly correlated with [Na^+ ion] (r = 1.00), [SO_4^{2-} ion] (r = 0.90) and EC (r = 0.94). A close correlation was also observed between the [SO_4^{2-} ion] and [Na^+ ion] (r = 0.91) as well as with the SO_4^{2-} and EC (r = 0.89) concentration. In addition to being correlated with pH the [ANC] was well correlated to EC (r = 0.63), [Na^+ ion] (r = 0.67), [Cl^- ion] (r = 0.63), and [SO_4^{2-} ion] (r = 0.60).

5.5 The use of stepwise regression to develop a best-fit model based on the lowest Akaike information criterion (AIC) value system

The aim of this section of the work was not to rule out the PCA results but to facilitate variable reduction. A total of thirteen variables (EC, pH, Na^+, NH_4^+; K^+, Mg^{2+}; Ca^{2+},NO_3^-, Cl^-, SO_4^{2-}, F^-, ANC and DOC) were used to conduct the PCA, where Cl^-, Na^+, DOC, EC, F^-, ANC and pH concentrations displayed the most statistical power. Many of the other variables remained clustered together with no correlation. However we know from **Figure 5-3**, **Figure 5-4** and **Table 5-1** that these variables have displayed a level of variation across the study regions. Thus, four models (**Table 5-2**) were generated for use in the subsequent chapters. Three of the four models were specific to each of the study regions and were aimed at determining region specific changes to the aquatic ecosystem while the fourth model was for the national dataset. The variables included in the national dataset model were based on the CART analysis results where the ANC and pH were identified as the key explanatory variables for defining groups as well as on the observations made in section **5.2** where the selected variables showed statistical differences across study regions. Therefore, variables that seem to be region-specific (i.e. Cl^-, Na^+, DOC and F^-) were excluded from the national dataset. Variables for the south-western Cape were chosen based primarily on the observations made in section **5.2**, where this region displayed the highest SO_4^{2-}, Cl^-, NO_3^- and Na^+ concentrations when compared to the other study regions and the project attempted to address how these variables affect species presence/absence. Variables chosen for the Waterberg were based on a publication by Humphries and Baldwin (2003) which highlights key

characteristics of streams impacted by the drought, namely high EC, nitrogen and temperatures along with low DO and phosphorus (P) concentrations. Streams from the Mpumalanga region were very alkaline and had high [EC] and acid neutralising capacity implying that these three variables were key to understanding species composition from this region. Furthermore, based on the observations made in section **5.2.1** the HV streams also had high F^- ion concentrations, thus this ion was also included as a potential explanatory variable that could be driving species composition. To eliminate prejudice in applying this approach as well as for a more robust approach to analysis a few variables from **Table 5-2** were used to generate automated models presented in **Table 5-3** through stepwise regression.

Table 5-2: Author-defined environmental variables to test against species data. These variables represent key factors that may drive change in species composition and were selected based on the observations made in sections **5.1** and **5.2**

National	SWC	WB	HV
EC; pH; SO_4^{2-}; NO_3^-; ANC	pH; SO_4^{2-}; Cl^-; Na^+; DOC; ANC	ANC; pH; EC; DO; PO_4^{3-}; Temp; NO_3^-	pH; EC;ANC; F^-

The aim of generating these models was to find the best fit model that would be used to explain species composition across study sites. Furthermore, ordination techniques work best when the number of variables being tested is not close to the number of sampling units (McCune *et al.*, 2002). Thus, this model selection process would be especially useful for the HV and WB study regions which had 14 and 17 macroinvertebrate sites each that were going to be tested against 16 environmental variables. Further data adjustments and the removal of outliers could lead to further reduction in these site numbers thus, variable reduction was necessary.

Prior to initiating the stepwise selection process, indicator variables that were going to be used to generate the respective models had to be selected. For this study a few variables from **Table 5-2** were chosen to be indicator variables for the national dataset and the respective regions. These variables were chosen based on the aims and objectives of this study which were to identify streams vulnerable to acidification and thus variables

known to play a role in stream acidity were selected to be indicator variables. Variables that showed dominance in the respective regions were also added. The stepwise selection is a combination of both the forward and backward selection methods. Prior to generating the models shown in **Table 5-3**, the model first had to be defined (method = forward, backward or both) and in our case we wanted to generate a model that used both the forward and backward selection. Thus, the backward method was used to define the full model which contained all the variables of interest and forward selection was used to define the empty model with no variables. The two were then combined for the stepwise selection which involves the addition and removal of variables till the best model with the lowest AIC value is generated. Final models generated for the respective indicator variable are presented in **Table 5-3**. The full model for the national dataset as well as the HV and SWC study regions contained thirteen variables (EC, pH, Na^+, NH_4^+; K^+, Mg^{2+}; Ca^{2+},NO_3^-, Cl^-, SO_4^{2-}, F^-, ANC and DOC). The Waterberg however contained an additional three (temperature, DO and PO_4^{3-}) variables to the thirteen mentioned above. Correlation analysis was conducted on these same variables for the respective regions and the national dataset.

Of the five indicator variables (EC, ANC, pH, SO_4^{2-} and DOC) that were selected for the national dataset the ANC generated a model with the highest number of explanatory variables (Mg^{2+}; Ca^{2+}; Cl^-; Na^+; K^+; NO_3^-; NH_4^+; SO_4^{2-}) (**Table 5-3**). All five models generally varied considerably from each other. The majority of these models however, shared similar variables to the PCA results. Correlation analysis showed a high correlation between the [Cl^- ion] and [Na^+ ion] (r = 0.98) and [SO_4^{2-} ion] (r = 0.94). An equally high correlation was observed between the Na^+ and SO_4^{2-} (r = 0.95) concentrations. The [DOC] however was negatively correlated with pH (r = - 0.61). EC displayed a strong correlation with the [Na^+ ion] (r = 0.89), [Mg^{2+} ion] (r = 0.74), [Cl^- ion] (r = 0.82) and [SO_4^{2-}] (r = 0.86), whereas F^- ion concentration was highly correlated to the pH (r = 0.65), Ca^{2+} (r = 0.76) and the ANC (r = 0.75) concentrations. Equally, the [ANC] was highly correlated to pH (r = 0.74), [Mg^{2+} ion] (r = 0.80) and [Ca^{2+}] (r = 0.91). In addition to being correlated to the variables mentioned above the pH also showed a correlation to [Ca^{2+} ion] (r = 0.67).

Models generated for the SWC using SO_4^{2-} and Cl^- ion concentrations as the indicator variables were identical, suggesting that these two ions respond to the same variables. Furthermore, these ions were highly correlated to each other (r = 0.91). The [Cl^- ion] was also highly correlated to the [Na^+ ion] (r = 1.00), [Mg^{2+} ion] (r = 1.00), EC (r = 0.94), pH (r = 0.79), [K^+ ion] (r = 0.88) and [Ca^{2+} ion] (r = 0.85). Apart from the identical SO_4^{2-} and Cl^- ion models, models generated from the SWC indicator variables varied considerably from each other. Furthermore, the automated system generated models which were either too large or highly reduced and missing key variables for this region such as the Na^+ and Cl^-. From the correlation analysis the pH was well correlated with EC (r = 0.69), Na^+ (r = 0.79), K^+ (r = 0.51), Mg^{2+} (r = 0.80), Ca^{2+} (r = 0.66), Cl^- (r = 0.79), SO_4^{2-} (r = 0.62), F^- (r = 0.75), ANC (r = 0.65) and DOC (r = - 0.49) concentrations. In addition to the Cl^- and pH the SO_4^{2-} was also highly correlated to [Ca^{2+} ion] (r = 0.86), [Mg^{2+} ion] (r = 0.91), [K^+ ion] (r = 0.92), [Na^+ ion] (r = 0.92) and EC (r = 0.90). The [Na^+ ion] was well correlated with the EC (r = 0.94), [K^+ ion] (r = 0.86), [Mg^{2+} ion] (r = 0.99), [Ca^{2+} ion] (r = 0.87), [F^-] (r = 0.84), and the [ANC] (r = 0.67). The [DOC] was not well correlated to all the other variables except for pH. In addition to what has already been mentioned the ANC was also correlated to the EC (r = 0.63), K^+ (r = 0.49), Ca^{2+} (r = 0.88), Mg^{2+} (r = 0.63), Cl^- (r = 0.63), SO_4^{2-} (r = 0.60) and F^- (r = 0.75) ion concentrations, whereas the NO_3^- maintained a moderate correlation to Mg^{2+} (r = 0.42), Na^+ (r = 0.40), Cl^- (r = 0.41) and SO_4^{2-} (r = 0.40) concentrations.

The Waterberg region was highly impacted by the drought and the priority with the reduction was to retain variables that are mostly associated with drought conditions. However, variable reduction could not be achieved without losing some of the key variables of interest. Overall the ANC was highly correlated to the EC, pH and all the base cations (r > 0.70) except for NH_4^+ (r = 0.19).

The Mpumalanga models varied considerably from each other. However, a model could not be generated for NO_3^-, implying that this variable might be affected by other factors such as landuse patterns. Furthermore, correlation analysis revealed that the NO_3^- was poorly correlated to the other variables (r < 0.28).

Table 5-3: Models generated from stepwise regression analysis. These models were designed to explore other factors not covered through the authors approach of analysis that may drive changes in the water chemistry and species composition. Selected variables from **Table 5-2** served as indicator variables (IVs) for generating models for the SWC, WB, HV and national dataset (ND). The question posed was what other factor could be driving changes in the EC, ANC, pH, DOC, DO, SO_4^{-2}, NO_3^-, Cl^- and F^- concentrations?

ND – IVs	EC	ANC	pH	SO_4^{2-}	DOC	PCA	-
	Mg^{2+}; Na^+; DOC	Mg^{2+}; Ca^{2+}; Cl^-; Na^+; K^+; NO_3^-; NH_4^+; SO_4^{2-}	ANC; Cl^-; K^+; DOC; NH_4^+; EC; F^-	Na^+; K^+; pH	pH; EC; Na^+; F^-	Cl^-; Na^+; DOC; EC; F^-; ANC; pH	
SWC – IVs	EC	ANC	pH	SO_4^{2-}	DOC	NO_3^-	Cl^-
	Na^+; DOC; K^+	Ca^{2+}; NO_3^-; SO_4^{2-}; F^-	Mg^{2+}; F^-; NO_3^-; DOC; SO_4^{2-}	Na^+; K^+; Cl^-; Mg^{2+}; ANC; Ca^{2+}; NO_3^-; NH_4^+; pH	pH; F^-; SO_4^{2-}	Mg^{2+}; pH; K^+	Na^+; ANC; SO_4^{2+}; Ca^{2+}; Mg^{2+}; NO_3^-; K^+; NH_4^+; pH
WB – IVs	EC	ANC	pH	SO_4^{2-}	DOC	NO_3^-	DO
	Mg^{2+}; ANC; Ca^{2+}; Cl^-; Na^+; F^-; DOC;DO	Mg^{2+}; Ca^{2+}; Cl^-; Na^+; K^+; NO_3^-; DO; Temp; pH; EC; F^-	ANC; K^+; DO; Temp; EC	Mg^{2+}; ANC; Temp	ANC; K^+; EC; Ca^{2+}; DO	NH_4^+; ANC; SO_4^{2-}; Cl^-; EC	ANC; Temp; pH; DOC; K^+; EC; Ca^{2+}; F^-
HV – IVs	EC	ANC	pH	SO_4^{2-}	DOC	NO_3^-	F^-
	Mg^{2+}; Na^+	EC; SO_4^{-2}; Mg^{+2}; DOC; NH_4^+; Ca^{2+}; Cl^-; Na^+; pH	ANC; EC	Ca^{2+}; Cl^-; K^+; NO_3^-; pH; ANC	K^+; pH; Mg^{2+}; Ca^{2+}; DO; H^+; ANC; Na^+; NO_3^-; Temp; PO_4^{3-}; SO_4^{2-}; F^-	Model could not be improved through adding or subtracting variables	DOC; Cl^-; Na^+;Mg^{2+}; K^+; pH

Chapter 6 The distribution of macroinvertebrate species across the HV, WB and SWC study regions

Macroinvertebrates were collected from a subset (n = 56) of streams due to the lack of suitable habitat in the remainder (section **4.3**, methods); 25 sites were sampled from the SWC, 17 in the WB and 14 from the Highveld region. A total of 164 species were identified from 10955 individuals consisting of 7288 Ephemeroptera nymphs, 1705 Plecoptera, 991 Trichoptera, 440 Simuliidae and 531 Chironomidae. The Ephemeroptera were the most abundant taxon although Chironomidae displayed the highest species richness accounting for 43 % (n = 71) of the total number of recorded species. Regrettably, not all Trichoptera larvae could be identified adequately as the majority of the HV and SWC specimens were covered with silt (**Figure 6-1**). The silting reduced visibility of key identification features that are typically located on the head and thorax. For objectivity and consistency in interpreting the results all Trichoptera related work was excluded from the remainder of the thesis. Exclusion of Trichopteran taxa from the main body of work reduced overall species richness by 7 % (n = 28) which brought the number of species down from 164 to 136. Species abundance was also reduced by 9 % (n = 991 individuals less) leaving a total of 9964 recorded individuals of Ephemeroptera, Plecoptera, Simuliidae and Chironomidae taxa altogether. The number of sampling sites was brought down to 55 because one site (WB19) only had Trichoptera of the chosen candidate indictors. These changes however did not affect the HV and the SWC study regions.

Of the three regions the SWC had the highest number of recorded species (n = 82) identified from 5875 individuals, followed by the Waterberg at 71 species from 1196 individuals while the HV had 61 species identified from 2871 individuals (**Table 6-1**). The Waterberg displayed the highest variability in species composition between sites that was reflected in the slightly higher β-diversity when compared to the SWC and the HV region. The highest number of recorded individuals per site across all study regions were for Mayfly species namely, *Tricorythus reticulatus* (n = 310) for the HV region, *Euthraulus sp.* (n = 227) in the Waterberg and *Lestagella penicillata* (n = 314) for the SWC, representing

the Tricorythidae, Leptophlebiidae and Teloganodidae taxonomic groups respectively. Several taxa (n = 28) fell into the lowest recorded number of individuals category, 93 % of which were chironomids.

Figure 6-1: Silted Trichopteran larvae. The silting was so severe that in most cases it formed clumps on the proleg and around the gills.

Table 6-1: Species diversity across study regions and sampling seasons. β-diversity (highlighted in red) refers to species variation from site to site, while γ-diversity accounts for species richness on a regional scale. α-diversity is given in Appendix 9 as a list of species occurring at each site. The highest number of recorded individuals per species (HNORIPS) and the lowest number of recorded individuals per species (LNORIPS) are included for all regional and seasonal summaries. The South African National Parks (SANPARKS) sites were also evaluated for species diversity. Species names: *Tricorythus reticulatus* (Tr.re.MF), *Euthraulus sp.* (Eu.sp.MF), *Cryptochironomus sp.* (Cryp.sp.CH), *Caenis Type3* (Ca.Sp3.MF), *Lestagella penicillata* (Le.pe.MF), *Castanophlebia calida* (Ca.ca.MF).

	HV	**WB**	**SWC**
Total No. of sites	14	16	25
γ - diversity	**61** (2871)	**71** (1196)	**82** (5875)
β - diversity	4.5	5.1	4.6
LNORIPS	1 (16 *spp.*)	1 (19 *spp.*)	1 (20 *spp.*)
HNORIPS	310 (Tr.re.MF)	227 (Eu.sp.MF)	314 (Le.pe.MF)
SANPARKS Sites	-	**9** Spp. (1 Site)	**72** Spp. (15 Sites)
β - diversity	-	-	3.3
LNORIPS	-	1 (6)	1 (27)
HNORIPS		3 (Cryp.sp.CH)	314 (Le.pe.MF)
June2015 Sites	**36** *Spp.* (6 Sites)	**40** *Spp.* (15 Sites)	**48** (19 Sites)
β - diversity	2.9	6.1	4.5
HNORIPS	178 (Eu.sp.MF)	50 (Eu.sp.MF)	161 (Le.pe.MF)
Nov2015 Sites	**47** *Spp.* (14 Sites)	**20** *Spp.* (7 Sites)	**51** *Spp.* (23 Sites)
β - diversity	4.1	2.7	6.9
HNORIPS	265 (Tr.re.MF)	15 (Ca.Sp3.MF)	102 (Ca.ca.MF)
Feb2016 Sites	-	**38** *Spp*. (9 Sites)	-
β - diversity	-	5.6	-
HNORIPS	-	68 (Eu.sp.MF)	-
June2016 Sites	-	**40** *Spp.* (9 Sites)	*59 Spp*. (25 Sites)
β - diversity	-	4.5	5.8
HNORIPS	-	116 (Eu.sp.MF)	191 (Le.pe.MF)

A total of 16 SANPARKS sites were sampled for macroinvertebrates and 94 % (n = 15) of these were located at the SWC. These sites are of great conservational value as they represent very few presumably pristine streams in South Africa. In addition to this the Marakele National Park site (WB33) for example was only sampled once for macroinvertebrates yet a few undescribed taxa were recorded at the site. Overall the

SWC had a total of 82 recorded species and 88 % (n = 72) of these were present at the SANPARKS sites indicating a high degree of species richness as indicated by the β – diversity value of 3.3.

6.1 Ephemeroptera

A total of 42 mayfly species were recorded in 87 % (n = 48) of the macroinvertebrate sampling sites of which 23 % (n = 11) of these were in the HV region, 29 % (n = 14) from the WB and 48 % (n = 23) in the SWC, accounting for 79 %, 86 % and 92 % of the macroinvertebrate sampling sites in the HV, WB and SWC respectively. The highest number of individuals were recorded at site EHV02 (n = 534) for the HV, WB26 (n = 391) for the Waterberg and SWC16 (n = 646) for the SWC. EHV19 (n = 14) and SWC06 (n = 2) had the lowest number of mayfly species for their respective regions, whereas WB03, WB30 and WB33 all had 1 mayfly species each. Species presence/absence was assessed (**Table 6-2**) to identify species that were common to all three study regions. A total of 11 mayfly species consisting of seven Baetidae (*Afroptilum sudafricanum*, *Baetis harrisoni*, *Cheleocloeon excisum*, *Crassabwa flava*, *Pseudocloeon glaucum*, *Pseudocloeon piscis* and *Pseudocloeon latum)*, two Caenidae (*Caenis Type1* and *Caenis Type2*) as well as two Heptageniidae species of Afronurus (*A. Oliffi*) and Compsoneuria (*C. bequaerti*) were found to be common to all three study regions. These common species are of great importance as they may serve as potential indicator species on the national scale assuming they meet the requirements. From the observations made in this study four mayfly species (*Susua sp.*, *Elassoneuria trimeniana*, *Prosopistoma crassi and Tricorythus reticulatus*) appeared to be restricted geographically to the Mpumalanga Highveld region, while 7 (*Baetis unknown sp2*, *Bugilliesia margaretae*, *Cheleocloeon falcatum*, *Demoreptus unknown sp.*, *Demoulinia crassi*, *Pseudocloeon unknown sp2 and Adenophlebiodes bicolor*) were restricted to the Waterberg and another 7 (*Demoreptus monticola*, *Labiobaetis vinosus*, *Pseudocloeon unknown sp1*, *Aprionyx intermedius*, *Choroterpes sp.*, *Leptophlebiidae sp. and Lestagella penicillata*) seem to be restricted to the south-western Cape. The remaining mayfly species were common in two of the three study regions, where the SWC and WB shared the most species (n = 6), followed by the

HV and WB, n = 4. The HV and SWC had three mayfly species that were common to both regions. Of the three regions the SWC had the highest abundance of mayflies (n = 3817, species richness = 27) followed by the HV at 2662 (species richness = 22) and the Waterberg had 809 individuals representing 28 species.

Table 6-2: Table showing presence/absence of Ephemeroptera species across study regions. Green (+) indicates presence and red (-) absence. Presence/absence summaries are given under the distribution column where three asterisks (***) symbolise species that are present at all three regions, yellow (Region name) highlights endemic or restricted species and species present at two regions are designated HW (HV and WB), SW (SWC and WB) and HS (HV and SWC). Table count summaries are given at the bottom. HV, WB and SWC represent the three study regions as has been the case throughout the text.

Order	Family	Genera	Species	Species code	HV	SWC	WB	Distribution
Ephemeroptera	Baetidae	*Acanthiops*	*Acanthiops varius*	Ac.va.MF	+	-	+	HW
		Afroptilum	*Afroptilum sudafricanum*	Af.su.MF	+	+	+	***
		Baetis	*Baetis harrisoni*	Ba.ha.MF	+	+	+	***
			Baetis unkown sp1	Ba.un.sp1.MF	-	+	+	SW
			Baetis unkown sp2	Ba.un.sp2.MF	-	-	+	WB
		Bugilliesia	*Bugilliesia margaretae*	Bu.ma.MF	-	-	+	WB
		Cheleocloeon	*Cheleocloeon excisum*	Che.ex.MF	+	+	+	***
			Cheleocloeon falcatum	Che.fa.MF	-	-	+	WB
			Cheleocloeon sp.	Che.sp.MF	+	+	-	HS
		Cloeodes	*Cloeodes sp.*	Cl.sp.MF	-	+	+	SW
		Cloeon	*Cloeon unknown sp.*	Cl.un.sp.MF	+	-	+	HW
		Crassabwa	*Crassabwa flava*	Cr.fl.MF	+	+	+	***
		Dabulamanzia	*Dabulamanzia indusii*	Da.in.MF	-	+	+	SW
		Demoreptus	*Demoreptus capensis*	De.ca.MF	-	+	+	SW
			Demoreptus monticola	De.mo.MF	-	+	-	SWC
			Demoreptus unknown sp.	De.un.sp.MF	-	-	+	WB
		Demoulinia	*Demoulinia crassi*	De.cr.MF	-	-	+	WB
		Labiobaetis	*Labiobaetis vinosus*	La.vi.MF	-	+	-	SWC
		Pseudocloeon	*Pseudocloeon glaucum*	Ps.gl.MF	+	+	+	***
			Pseudocloeon piscis	Ps.pi.MF	+	+	+	***
			Pseudocloeon latum	Ps.la.MF	+	+	+	***
			Pseudocloeon unknown sp1	Ps.un.sp1.MF	-	+	-	SWC
			Pseudocloeon unknown sp2	Ps.un.sp2.MF	-	-	+	WB
		Pseudopannota	*Pseudopannota maculosa*	Ps.ma.MF	+	-	+	HW
		Susua	*Susua sp.*	Su.sp.MF	+	-	-	HV
			Total no. of species per region		12	15	20	
			Common species (All regions)		7	7	7	
			Species unique to each region		1	3	6	
			Two region combination species		4	5	7	

Continuation of Table 6-2

Order	Family	Genera	Species	Species code	HV	SWC	WB	Distribution
Ephemeroptera	Caenidae	Caenis	*Caenis Type1*	Ca.sp1.MF	+	+	+	***
			Caenis Type2	Ca.sp2.MF	+	+	+	***
			Caenis Type3	Ca.sp3.MF	-	+	+	SW
	Heptageniidae	Afronurus	*Afronurus oliffi*	Af.ol.MF	+	+	+	***
		Compsoneuria	*Compsoneuria bequaerti*	Co.be.MF	+	+	+	***
	Leptophlebiidae	Adenophlebia	*Adenophlebia auriculata*	Ad.au.MF	+	+	-	HS
			Adenophlebia sp.	Ad.sp.MF	+	+	-	HS
		Adenophlebiodes	*Adenophlebiodes bicolor*	Ad.bi.MF	-	-	+	WB
		Aprionyx	*Aprionyx intermedius*	Ap.in.MF	-	+	-	SWC
		Castanophlebia	*Castanophlebia calida*	Ca.ca.MF	-	+	+	SW
		Choroterpes	*Choroterpes sp.*	Cho.sp.MF	-	+	-	SWC
		Euthraulus	*Euthraulus sp.*	Eu.sp.MF	+	-	+	HW
		unidentified	*Leptophlebiidae sp.*	Le.sp.MF	-	+	-	SWC
	Oligoneuriidae	Elassoneuria	*Elassoneuria trimeniana*	El.tr.MF	+	-	-	HV
Prosopistomatidae		Prosopistoma	*Prosopistoma crassi*	Pr.cr.MF	+	-	-	HV
	Teloganodidae	Lestagella	*Lestagella penicillata*	Le.pe.MF	-	+	-	SWC
	Tricorythidae	Tricorythus	*Tricorythus reticulatus*	Tr.re.MF	+	-	-	HV
			Total no. of species per region		10	12	8	
			Common species (All regions)		4	4	4	
			Species unique to each region		3	4	1	
			Two region combination species		3	4	3	

6.2 Plecoptera

Stonefly species were sampled from 60 % (n = 33) of the macroinvertebrate sites, 30 % (n = 10) of which were divided equally across the HV and Waterberg study regions while the remaining 70 % (n = 23) were in the SWC. These numbers accounted for 36 % (n = 5), 31 % (n = 5) and 92 % of the overall macroinvertebrate sampling sites at the HV, WB and SWC study regions respectively. A total of 11 stonefly taxa consisting of six known and five unknown species were recorded. The highest number of individuals were recorded for the SWC (n = 1641), 35 in the HV and 29 at the Waterberg. Of the 11 species 10 (*Aphanicerca capensis, Aphanicerca capensis, Aphanicerca sp1, Aphanicerca sp2, Balinskycercella sp., Aphanicercella bifurcata, Aphanicercopsis sp., Balinskycercella tugelae, Aphanicercopsis tabularis, Desmonemoura pulchellum, Desmonemoura sp*.) were from the SWC (**Table 6-3**). The highest number of individuals were recorded at EHV16 (n = 22) for the HV, WB13 (n = 18) in the WB and SWC34 (n = 275) for the SWC. While the lowest number (n = 1) were found at EHV02 and EHV12 in the HV region, WB12 (n = 1) for the WB and SWC04 (n = 3) for the SWC.

6.3 Simuliidae

Simuliidae larvae were recorded from 62 % (n = 37) of the macroinvertebrate sites where 11 % (n = 4) of these were located in the HV region, 32 % (n = 12) in the WB and 57 % (n = 21) for the SWC. These values account for 29 %, 75 % and 84 % of the total number of sampling sites at each region respectively. The highest number of recorded individuals in the HV region were for EHV04 (n = 11), WB07C and WB32A for the Waterberg at 55 individuals each and SWC14 (n = 66) for the SWC. Overall 12 Simulium species (*Simulium edwardsellum damnosum, Simulium metomphalus chutteri, Simulium anasolen dentulosum, Simulium pomeroyellum impukane, Simulium nevermannia nigritarse, Simulium nevermannia rutherfoordi, Simulium nevermannia ruficorne, Simulium metomphalus vorax, Simulium sp., Simulium meilloniellum adersi, Simulium pomeroyellum unicornutum, Simulium pomeroyellum alcocki)* were recorded of which four (*Simulium edwardsellum damnosum, Simulium anasolen dentulosum, Simulium*

nevermannia nigritarse, Simulium Nevermannia rutherfoordi) were present at all study regions (**Table 6-4**). The *Simulium metomphalus chutteri, Simulium metomphalus vorax* and *Simulium meilloniellum adersi* species were solely recorded at the SWC and the Waterberg, while the *Simulium pomeroyellum impukane* was recorded at the HV and SWC. Two species (*Simulium nevermannia ruficorne and Simulium sp.)* appeared to be endemic to the SWC while species *Simulium pomeroyellum unicornutum* and *Simulium pomeroyellum alcocki* were recorded only at the Waterberg and HV regions respectively (**Table 6-4**).

6.4 Chironomidae

Chironomids were recorded from 91 % (n = 50) of the macroinvertebrate sites where 24 % (n = 12) of these were found in the HV region, 30 % (n = 15) were in the WB and 46 % (n = 23) in the SWC, accounting for more than 86 % of the sites per region. EHV04 had the highest number of recorded individuals (n = 63) for the HV region while EHV19 and EHV20 both held the lowest recorded number (n = 1) of individuals. The highest number of individuals for the WB were recorded at WB14 (n = 36) and the lowest number (n = 2) at WB22, WB23A and WB25. SWC27 (n = 38) had the highest number for the SWC while SWC08 and SWC32 held the lowest record. Appropriate identification of Chironomidae to species require that the larvae be reared to adults especially since none of the keys used for species identification in South Africa were generated locally. Consequently, none of the chironomids could be confidently identified to species level and upon expert advice identification was conducted to the Genus or Tribe taxonomic level. Of the 71 recorded organisms 15 were endemic to the HV region, 19 to the SWC and 18 for the Waterberg (**Table 6-5**). A total of 10 species (*Lauterborniella sp., Polypedilum sp3, Polypedilum sp4, Polypedilum sp7, Tanytarsus sp2, Orthocladius sp1, Orthocladius sp2, Parametriocnemus sp., Rheocricotopus sp2 and Tokunagaia sp.*) were recorded as present in all three regions while the remaining 9 species were common in two of the three study regions (**Table 6-5**).

Table 6-3: Table showing presence/absence of Plecoptera species across study regions.

Order	Family	Genera	Species	Species code	HV	SWC	WB	Distribution
Plecoptera	Perlidae	*Neoperla*	*Neoperla spio*	Ne.sp.SF	+	-	+	HW
	Notonemouridae	*Aphanicerca*	*Aphanicerca capensis*	Ap.cap.SF	-	+	-	SWC
			Aphanicerca sp1	Ap.sp1.SF	-	+	-	SWC
			Aphanicerca sp2	Ap.sp2.SF	-	+	-	SWC
		Balinskycercella	*Balinskycercella sp.*	Ba.sp.SF	-	+	-	SWC
		Aphanicercella	*Aphanicercella bifurcata*	Ap.bi.SF	-	+	-	SWC
		Aphanicercopsis	*Aphanicercopsis sp.*	Ap.sp.SF	-	+	-	SWC
		Balinskycercella	*Balinskycercella tugelae*	Ba.tu.SF	-	+	-	SWC
		Aphanicercopsis	*Aphanicercopsis tabularis*	Ap.ta.SF	-	+	-	SWC
		Desmonemoura	*Desmonemoura pulchellum*	De.pu.SF	-	+	-	SWC
			Desmonemoura sp.	De.sp.SF	-	+	-	SWC
			Total no. of species per region		1	10	1	
			Common species (All regions)		0	0	0	
			Species unique to each region		0	10	0	
			Two region combination species		1	0	1	

Table 6-4: Table showing presence/absence of Simuliidae species across study regions.

Order	Family	Genera	Species	Species code	HV	SWC	WB	Distribution
Diptera	Simuliidae	*Simulium*	*Simulium edwardsellum damnosum*	Ed.da.BF	+	+	+	***
			Simulium metomphalus chutteri	Me.ch.BF	-	+	+	SW
			Simulium anasolen dentulosum	An.de.BF	+	+	+	***
			Simulium pomeroyellum impukane	Po.im.BF	+	+	-	HS
			Simulium nevermannia nigritarse	Ne.ni.BF	+	+	+	***
			Simulium nevermannia rutherfoordi	Ne.rut.BF	+	+	+	***
			Simulium nevermannia ruficorne	Ne.ruf.BF	-	+	-	SWC
			Simulium metomphalus vorax	Me.vo.BF	-	+	+	SW
			Simulium sp.	Si.sp.BF	-	+	-	SWC
			Simulium meilloniellum adersi	Me.ad.BF	-	+	+	SW
			Simulium pomeroyellum unicornutum	Po.un.BF	-	-	+	WB
			Simulium pomeroyellum alcocki	Po.al.BF	+	-	-	HV
			Total no. of species per region		6	10	8	
			Common species (All regions)		4	4	4	
			Species unique to each region		1	2	1	
			Two region combination species		1	4	3	

Table 6-5: Table showing presence/absence of Chironomidae species across study regions

Family	Subfamily	Tribe	Genera	Species	Species code	HV	SWC	WB	Distribution
Chironomidae	Chironominae			*Chironominae sp1*	Ch.sp1.CH	+	-	-	HV
				Chironominae sp2	Ch.sp2.CH	+	-	-	HV
				Chironominae sp3	Ch.sp3.CH	-	+	-	SWC
				Chironominae sp4	Ch.sp4.CH	-	+	-	SWC
				Chironominae Sp5	Ch.Sp5.CH	-	-	+	WB
	Chironominae	Chironomini	*Baeotendipes*	*Baeotendipes sp.*	Ba.sp.CH	-	-	+	WB
			Chironomus	*Chironomus sp1*	Ch.us.sp1.CH	-	-	+	WB
				Chironomus sp2	Ch.us.sp2.CH	-	-	+	WB
				Chironomus sp3	Ch.us.sp3.CH	-	-	+	WB
			Cryptochironomus	*Cryptochironomus sp.*	Cryp.sp.CH	-	-	+	WB
			Lauterborniella	*Lauterborniella sp.*	Lau.sp.CH	+	+	+	***
			Microtendipes	*Microtendipes sp1*	Mi.t.sp1.CH	-	+	-	SWC
			Microtendipes	*Microtendipes sp2*	Mi.t.sp2.CH	-	+	-	SWC
			Phaenopsectra	*Phaenopsectra sp1*	Ph.sp1.CH	-	+	-	SWC
			Phaenopsectra	*Phaenopsectra sp2*	Ph.sp2.CH	-	+	-	SWC
			Phaenopsectra	*Phaenopsectra sp3*	Ph.sp3.CH	-	+	-	SWC
			Polypedilum	*Polypedilum sp1*	Po.sp1.CH	-	+	+	SW
			Polypedilum	*Polypedilum sp2*	Po.sp2.CH	+	-	-	HV
			Polypedilum	*Polypedilum sp3*	Po.sp3.CH	+	+	+	***
			Polypedilum	*Polypedilum sp4*	Po.sp4.CH	+	+	+	***
			Polypedilum	*Polypedilum sp5*	Po.sp5.CH	-	+	-	SWC
			Polypedilum	*Polypedilum sp6*	Po.sp6.CH	-	+	-	SWC
			Polypedilum	*Polypedilum sp7*	Po.sp7.CH	+	+	+	***
			Stenochironomus	*Stenochironomus sp.*	St.sp.CH	-	+	+	SW
		Tanytarsini	*Cladotanytarsus*	*Cladotanytarsus sp1*	Cl.sp1.CH	+	-	-	HV
				Cladotanytarsus sp2	Cl.sp2.CH	+	-	+	HW
			Micropsectra	*Micropsectra sp1*	Mi.p.sp1.CH	-	+	-	SWC
				Micropsectra sp2	Mi.p.sp2.CH	-	+	-	SWC
			Tanytarsus	*Tanytarsus sp1*	Ta.sp1.CH	-	+	-	SWC
				Tanytarsus sp2	Ta.sp2.CH	+	+	+	***
				Tanytarsus sp3	Ta.sp3.CH	+	-	-	HV
				Tanytarsus sp4	Ta.sp4.CH	+	+	-	HS
			Zavrelia	*Zavrelia sp.*	Za.sp.CH	-	+	-	SWC
			Paratanytarsus	*Paratanytarsus sp.*	Pa.sp.CH	-	+	-	SWC
				Total no. of species per region		12	22	14	
				Common species (All regions)		5	5	5	
				Species unique to each region		5	14	6	
				Two region combination species		2	3	3	

Continuation of Table 6-5

Family	Subfamily	Tribe	Genera	Species	Species code	HV	SWC	WB	Distribution
	Orthocladiinae		*Antillocladius*	*Antillocladius sp.*	An.sp.CH	+	-	-	HV
			Camptocladius	*Camptocladius sp.*	Ca.sp.CH	-	+	-	SWC
			Chaetocladius	*Chaetocladius sp.*	Cha.sp.CH	+	-	-	HV
			Corynoneura	*Corynoneura sp1*	Co.sp1.CH	-	-	+	WB
				Corynoneura sp2	Co.sp2.CH	-	-	+	WB
			Cricotopus	*Cricotopus sp.*	Cric.sp.CH	+	-	-	HV
			Eukiefferiella	*Eukiefferiella sp1*	Eu.sp1.CH	+	+	-	HS
				Eukiefferiella sp2	Eu.sp2.CH	+	-	-	HV
				Eukiefferiella sp3	Eu.sp3.CH	+	-	-	HV
				Eukiefferiella sp4	Eu.sp4.CH	+	-	-	HV
				Eukiefferiella sp5	Eu.sp5.CH	-	+	-	SWC
				Eukiefferiella sp6	Eu.sp6.CH	-	+	-	SWC
			Georthocladius	*Georthocladius sp1*	Ge.sp1.CH	+	-	+	HW
				Georthocladius sp2	Ge.sp2.CH	-	-	+	WB
			Heterotrissocladius	*Heterotrissocladius sp.*	He.sp.CH	-	-	+	WB
			Limnophyes	*Limnophyes sp.*	Lim.sp.CH	+	+	-	HS
				Orthocladiinae sp.	Or.sp.CH	-	-	+	WB
			Orthocladius	*Orthocladius sp1*	Or.sp1.CH	+	+	+	***
				Orthocladius sp2	Or.sp2.CH	+	+	+	***
				Orthocladius sp3	Or.sp3.CH	-	+	-	SWC
				Orthocladius sp4	Or.sp4.CH	-	-	+	WB
			Paracricotopus	*Paracricotopus sp.*	Parac.sp.CH	-	-	+	WB
			Parakiefferiella	*Parakiefferiella sp.*	Parak.sp.CH	-	+	+	SW
			Parametriocnemus	*Parametriocnemus sp.*	Param.sp.CH	+	+	+	***
			Psectrocladius	*Psectrocladius sp1*	Ps.sp1.CH	+	-	-	HV
				Psectrocladius sp2	Ps.sp2.CH	-	+	-	SWC
				Psectrocladius sp3	Ps.sp3.CH	-	-	+	WB
				Psectrocladius sp4	Ps.sp4.CH	-	-	+	WB
			Rheocricotopus	*Rheocricotopus sp1*	Rh.sp1.CH	+	-	-	HV
			Rheocricotopus	*Rheocricotopus sp2*	Rh.sp2.CH	+	+	+	***
			Tokunagaia	*Tokunagaia sp.*	To.sp.CH	+	+	+	***
	Tanypodinae	Coelotanypodini	*Clinotanypus*	*Clinotanypus sp.*	Cl.sp.CH	+	-	+	HW
		Macropelopiini	*Macropelopia*	*Macropelopia sp.*	Ma.sp.CH	+	-	-	HV
		Pentaneurini	*Meropelopia*	*Meropelopia sp.*	Me.sp.CH	-	-	+	WB
			Rheopelopia	*Rheopelopia sp.*	Rh.sp.CH	-	-	+	WB
		Natarsiini	*Natarsia*	*Natarsia sp.*	Na.sp.CH	+	-	-	HV
		Procladiini	*Procladius*	*Procladius sp.*	Pr.sp.CH	-	-	+	WB
				Total no. of species per region		19	13	20	
				Common species (All regions)		5	5	5	
				Species unique to each region		10	5	12	
				Two region combination species		4	3	3	

6.5 Species abundance

The overall sample size was dominated by sparsely distributed and undescribed species, where only 15 % (n = 21) of the 136 recorded species had an abundance > 73 (mean number of individuals across all sites and species) (**Table 6-6**) while 57 % (n = 77) of the remaining species had < 10 individuals each (**Table 6-7**) and the other remaining 28 % (n = 38) represented species with an abundance that was > 10 but < 73 (**Table 6-8**). This group consisted of 36 species. Mayfly species were associated with higher abundance values when compared to other taxa in the group while Chironomidae species generally had lower numbers. Of the 24 species that were recorded as present across all three study regions only 36 % (n = 9) had an abundance > 73.

Table 6-6: List of species that displayed the highest abundance. Anything above average (> 73) was considered to be abundant. Species that were common to all study regions (HV, WB, SWC) are highlighted in red. Species of mayfly (MF), stonefly (SF), blackfly (BF) and chironomids (CH) are indicated in brackets

Species name	No. of Individuals	No. of Sites
Lestagella penicillata (MF)	1683	21
Euthraulus sp. (MF)	1150	17
Tricorythus reticulatus (MF)	1023	9
Aphanicerca capensis (SF)	853	20
Castanophlebia calida (MF)	847	18
Adenophlebia auriculata (MF)	449	14
Baetis harrisoni (MF)	339	18
Aphanicerca sp1 (SF)	327	17
Balinskycercella tugelae (SF)	214	22
Afroptilum sudafricanum (MF)	214	20
Caenis Type1 (MF)	205	21
Aprionyx intermedius (MF)	203	7
Balinskycercella sp. (SF)	165	10
Pseudocloeon glaucum (MF)	130	19
Parametriocnemus sp. (CH)	130	23
Afronurus oliffi (MF)	116	12
Simulium anasolen dentulosum (BF)	105	9
Leptophlebiidae sp. (MF)	105	6
Simulium edwardsellum damnosum (BF)	96	22
Pseudopannota maculosa (MF)	95	11
Simulium nevermannia rutherfoordi (BF)	93	15

Table 6-7: List of species with < 10 individuals each. This group was mostly dominated by Chironomid species. Species that were common to all study regions (HV, WB, SWC) are highlighted in red. Species of mayfly (MF), stonefly (SF), blackfly (BF) and chironomids (CH) are indicated in brackets.

Species name	No. of Individuals	No. of Sites
Pseudocloeon latum (MF)	8	1
Pseudocloeon unknown sp1 (MF)	8	2
Baetis unkown sp2 (MF)	7	3
Demoreptus capensis (MF)	7	2
Demoreptus unknown sp.(MF)	7	3
Choroterpes sp. (MF)	6	4
Bugilliesia margaretae (MF)	6	3
Acanthiops varius (MF)	6	1
Adenophlebiodes bicolor (MF)	6	1
Susua sp. (MF)	5	3
Aphanicercella bifurcata (SF)	5	3
Aphanicercopsis sp. (SF)	5	3
Desmonemoura pulchellum (SF)	5	1
Desmonemoura sp. (SF)	4	1
Simulium pomeroyellum impukane (BF)	4	2
Simulium sp. (BF)	4	2
Simulium pomeroyellum unicornutum (BF)	4	3
Simulium pomeroyellum alcocki (BF)	3	2
Baeotendipes sp. (CH)	3	2
Chironominae sp1 (CH)	3	1
Chironominae sp2 (CH)	3	2
Chironominae sp3 (CH)	3	1
Chironomimae sp4 (CH)	3	3
Chironominae Sp5 (CH)	3	2
Chironomus sp2 (CH)	3	2
Chironomus sp3 (CH)	3	2
Cladotanytarsus sp1 (CH)	3	2
Cladotanytarsus sp2 (CH)	3	1
Cryptochironomus sp. (CH)	3	1
Micropsectra sp1 (CH)	2	1
Micropsectra sp2 (CH)	2	2
Phaenopsectra sp1 (CH)	2	1
Phaenopsectra sp2 (CH)	2	1
Phaenopsectra sp3 (CH)	2	2
Polypedilum sp2 (CH)	2	1
Polypedilum sp5 (CH)	2	2
Polypedilum sp6 (CH)	2	2
Stenochironomus sp. (CH)	2	2
Tanytarsus sp1 (CH)	2	2

Tanytarsus sp2 (CH)	2	1
Tanytarsus sp3 (CH)	2	2
Tanytarsus sp4 (CH)	2	1
Zavrelia sp. (CH)	2	1
Paratanytarsus sp. (CH)	2	1
Microtendipes sp2 (CH)	2	1
Antillocladius sp. (CH)	2	1
Camptocladius sp. (CH)	2	1
Chaetocladius sp. (CH)	2	1
Corynoneura sp1 (CH)	2	1
Corynoneura sp2 (CH)	1	1
Cricotopus sp. (CH)	1	1
Eukiefferiella sp1 (CH)	1	1
Eukiefferiella sp2 (CH)	1	1
Eukiefferiella sp3 (CH)	1	1
Eukiefferiella sp4 (CH)	1	1
Eukiefferiella sp5 (CH)	1	1
Eukiefferiella sp6 (CH)	1	1
Georthocladius sp1 (CH)	1	1
Georthocladius sp2 (CH)	1	1
Heterotrissocladius sp. (CH)	1	1
Limnophyes sp. (CH)	1	1
Orthocladiinae sp. (CH)	1	1
Orthocladius sp3 (CH)	1	1
Orthocladius sp4 (CH)	1	1
Paracricotopus sp. (CH)	1	1
Parakiefferiella sp. (CH)	1	1
Psectrocladius sp1 (CH)	1	1
Psectrocladius sp2 (CH)	1	1
Psectrocladius sp3 (CH)	1	1
Psectrocladius sp4 (CH)	1	1
Rheocricotopus sp1 (CH)	1	1
Clinotanypus sp. (CH)	1	1
Macropelopia sp. (CH)	1	1
Meropelopia sp. (CH)	1	1
Natarsia sp. (CH)	1	1
Procladius sp. (CH)	1	1
Rheopelopia sp. (CH)	1	1

Table 6-8: List of macroinvertebrate species with > 10 but < 73 individuals each. Species that were common to all study regions (HV, WB, SWC) are highlighted in red. Species of mayfly (MF), stonefly (SF), blackfly (BF) and chironomids (CH) are indicated in brackets.

Species name	No. of Individuals	No. of Sites
Labiobaetis vinosus (MF)	69	4
Dabulamanzia indusii (MF)	69	4
Cheleocloeon excisum (MF)	64	10
Cheleocloeon sp. (MF)	60	11
Cloeodes sp. (MF)	55	4
Caenis Type2 (MF)	54	12
Caenis Type3 (MF)	53	10
Pseudocloeon piscis (MF)	51	8
Compsoneuria bequaerti (MF)	41	14
Orthocladius sp2 (CH)	41	14
Crassabwa flava (MF)	37	3
Baetis unkown sp1 (MF)	37	8
Demoreptus monticola (MF)	36	14
Adenophlebia sp. (MF)	35	6
Pseudocloeon unknown sp2 (MF)	33	12
Cheleocloeon falcatum (MF)	32	8
Demoulinia crassi (MF)	31	16
Cloeon unknown sp. (MF)	29	8
Prosopistoma crassi (MF)	29	6
Elassoneuria trimeniana (MF)	28	2
Neoperla spio (SF)	25	6
Aphanicerca sp2 (SF)	25	7
Aphanicercopsis tabularis (SF)	24	3
Simulium metomphalus chutteri (BF)	24	6
Simulium nevermannia nigritarse (BF)	23	13
Simulium nevermannia ruficorne (BF)	22	5
Simulium metomphalus vorax (BF)	21	7
Simulium meilloniellum adersi (BF)	21	14
Rheocricotopus sp2 (CH)	21	14
Chironomus sp1 (CH)	20	9
Lauterborniella sp. (CH)	18	4
Microtendipes sp1 (CH)	18	4
Polypedilum sp1 (CH)	18	11
Polypedilum sp3 (CH)	17	8
Polypedilum sp4 (CH)	17	2
Polypedilum sp7 (CH)	16	7
Orthocladius sp1 (CH)	16	9
Tokunagaia sp. (CH)	11	5

Species that displayed the highest abundance, for example *Lestagella penicillata* (n = 1683), *Euthraulus sp.* (n = 1150), *Tricorythus reticulatus* (n = 1023), *Aphanicerca capensis* (n = 853), *Castanophlebia calida* (n = 847) and *Adenophlebia auriculata* (n = 449) were all restricted geographically. Species abundance plays a crucial role when trying to identify indicator species, as rare and sparsely distributed species do not make good biological indicators because other unknown factors may be contributing towards their scarcity in the field.

6.6 Identifying potential indicator species

As a first step towards identifying indicator species, macroinvertebrates were plotted against a pH gradient to determine species response to changes in pH. The requirement was that all evaluated species had to have been recorded more than once in order to define minimum and maximum pH range for species presence and thus inverts that did not meet this requirement were excluded from the analysis. Thereafter properties of potential indicator species were refined following guidelines set by Gray & Pearson, (1982); Pearson *et al.*, (1983) and Larsen *et al.*, (1996).

6.6.1 Ephemeroptera

Of the 42 recorded mayfly species, 35 were detected at several sites across sampling seasons. These 35 species were plotted against a pH gradient to establish species presence/absence against changes in the pH. *Tricorythus reticulatus* (Tr.re.MF) exhibited a high affinity for alkaline streams in the range of pH 7.4 – 8.2 (**Figure 6-2**). *Cloeon unknown sp.* (Cl.un.sp.MF), *Caenis Type3* (Ca.sp3.MF) species, *Cheleocloeon sp.* (Che.sp.MF), *Adenophlebiodes bicolor* (Ad.bi.MF), *Demoulinia crassi* (De.cr.MF), *Pseudocloeon unknown sp2* (Ps.un.sp2.MF) and *Pseudocloeon piscis* (Ps.pi.MF) all displayed a preference for circumneutral (pH > 6) to alkaline (pH ≥ 7.9) streams. *Adenophlebiodes bicolor*, however, exhibited a much narrower pH range (6.1 – 6.5) that restricted it mainly to circumneutral streams. *Caenis Type2* (Ca.sp2.MF) together with *Pseudocloeon latum* (Ps.la.MF) and *Baetis unknown sp1* (Ba.un.sp1.MF) were found at

a pH ≥ 5.6. Their tolerance to alkaline conditions however varied between species. *Pseudopannota maculosa* (Ps.ma.MF) had a pH tolerance between 5.4 and 8.2 while *Cloeodes sp.* (Cl.sp.MF), *Dabulamanzia indusii* (Da.in.MF), *Crassabwa flava* (Cr.fl.MF), *Cheleocloeon falcatum* (Che.fa.MF) and *Baetis harrisoni* (Ba.ha.MF) all occurred between pH 5 and 8. *Demoreptus monticola* (De.mo.MF) occurred only in the very narrow pH range of 4.8 to 5.6. *Demoreptus capensis* (De.ca.MF) also displayed a similarly narrow pH range of 4.8 to 5.8. *Compsoneuria bequaerti* (Co.be.MF) and *Afronurus oliffi* (Af.ol.MF) both had a much wider pH range (4.8 – 8.2) when compared to all other taxa mentioned prior. *Leptophlebiidae sp.* (Le.sp.MF) were mostly found in waters with pH 4.6 to 6.1 and *Pseudocloeon unknown sp1* (Ps.un.sp1.MF) were not far behind at 4.5 to 6.1. *Adenophlebia sp.* (Ad.sp.MF) displayed a tolerance for both acidic and alkaline waters with a pH range of 4.3 to 8 while a slightly less but similar tolerance range (4.2 – 7.4) was observed for the *Adenophlebia auriculata* (Ad.au.MF) species. *Pseudocloeon glaucum* (Ps.gl.MF) could survive even more acidic conditions at pH 3.9 and alkaline streams of pH 7.9. *Aprionyx intermedius* (Ap.in.MF) had a pH range of 3.8 to 6.1 while *Choroterpes sp.* (Cho.sp.MF) species were found at a 3.6 – 6.2 pH range. *Cheleocloeon excisum* (Che.ex.MF), *Afroptilum sudafricanum* (Af.su.MF) and *Castanophlebia calida* (Ca.ca.MF) species could all survive acidic conditions ≥ 3.6 with varying alkalinity of pH 8.1, 8.2 and 6.6 respectively. *Labiobaetis vinosus* (La.vi.MF) and *Lestagella penicillata* (Le.pe.MF) species had corresponding tolerance to pH of 3.1 – 6.1 (**Figure 6-2**).

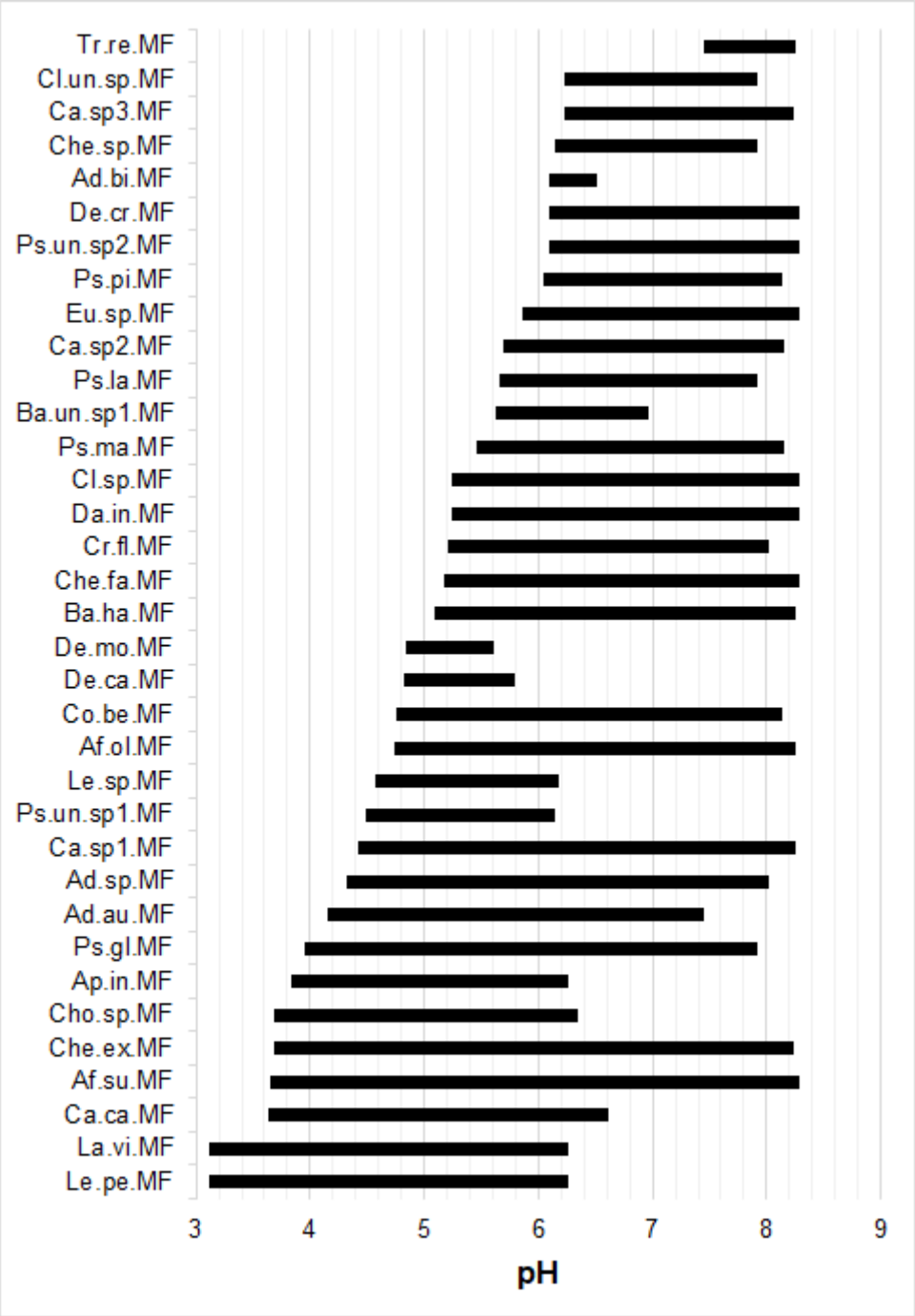


Figure 6-2: Ephemeroptera species pH range of occurrence in the present study.

6.6.2 Plecoptera

All recorded stonefly species (n = 11) were plotted against the pH gradient to determine species presence/absence in relation to variation in pH. Stoneflies are known for their high tolerance to acidic conditions (Lien *et al.*, 1992; Schartau *et al.*, 2008a; Raddum & Skjelkvåle, 1995; Fjellheim & Raddum, 1992; Larssen *et al.*, 2010) however this was apparently not the case with some of the species recorded in this study (**Figure 6-3**). *Neoperla spio* (Ne.sp.SF) was found in streams that were within a range of pH 5.4 – 8. *Aphanicercella bifurcata* (Ap.bi.SF) was the only species from the SWC that preferred less acidic conditions of pH 4.4 to 5.2 when compared to *Aphanicerca capensis* (Ap.cap.SF), *Aphanicerca sp1* (Ap.sp1.SF), *Aphanicerca sp2* (Ap.sp2.SF), *Aphanicercopsis tabularis* (Ap.ta.SF) and *Balinskycercella tugelae* (Ba.tu.SF) which were all found at pH ≥ 3.2. Furthermore, all these species displayed a wide range of pH tolerance. However, the *Aphanicercopsis tabularis* species was not detected at pH > 4.6 (**Figure 6-3**).

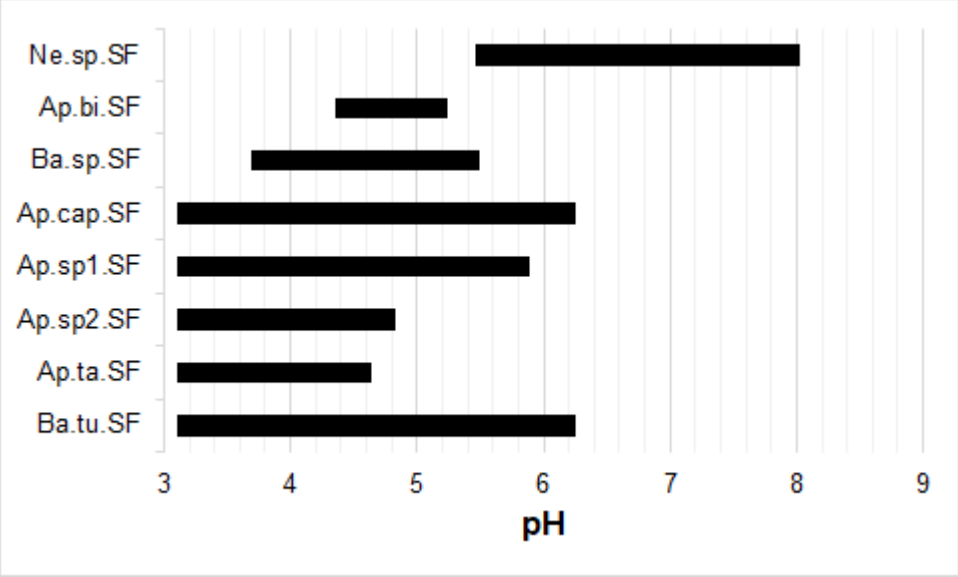


Figure 6-3: Plecoptera species pH range of occurrence in the present study.

6.6.3 Simuliidae

From the pH gradient the Simulium species could be divided into three groups (**Figure 6-4**). The first group included two species (*Simulium meilloniellum adersi, Simulium pomeroyellum unicornutum*) that were recorded at pH ≥ 5.8. The second group consisted of another two species (*Simulium metomphalus chutteri* and *Simulium metomphalus vorax*) which were found at pH ≥ 4.4 whereas the third group included species of *Simulium pomeroyellum impukane, Simulium nevermannia nigritarse, Simulium nevermannia rutherfoordi, Simulium nevermannia ruficorne, Simulium nevermannia rutherfoordi, Simulium edwardsellum damnosum* and *Simulium anasolen dentulosum* that were found in streams with a pH < 4. However as was observed for the stoneflies, blackflies that were found at a pH < 4 were also found in circumneutral to alkaline streams (**Figure 6-4**).

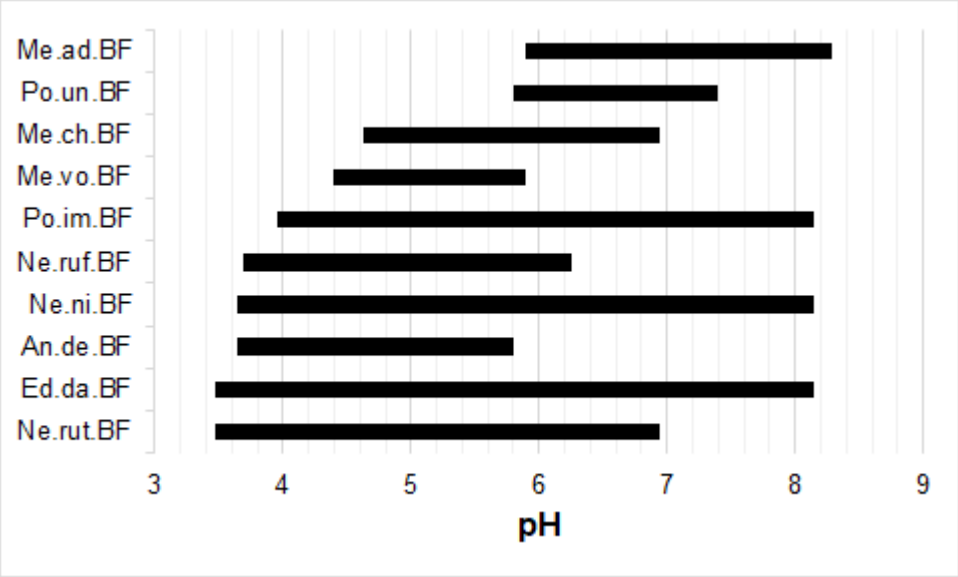


Figure 6-4: Simuliidae species pH range of occurrence in the present study.

6.6.4 Chironomidae

Chironomidae species (n = 29) that had been recorded multiple times across sampling seasons were plotted against a pH gradient (**Figure 6-5**). Of the 29 species *Cladotanytarsus sp2* (Cl.sp2.CH) commonly found in alkaline streams of pH 7 – 8.8. The *Baeotendipes sp.* (Ba.sp.CH) was found mostly in circumneutral to alkaline headwater streams (pH = 6.7 – 7.4). Equally so the *Georthocladius sp1* (Ge.sp1.CH) also showed a high preference for circumneutral to alkaline streams (pH = 6.7 – 8.4), while the *Heterotrissocladius sp.* (He.sp.CH) had a very narrow pH range of 6.4 to 6.6. *Orthocladiinae sp.* (Or.sp.CH) were found at pH 6.3 – 7.1 while species of *Eukiefferiella sp6* (Eu.sp6.CH) and *Stenochironomus sp.* (St.sp.CH) were recorded at an extremely narrow range of pH 6.3 to 6.4 for the former and 6.1 for the later. The *Psectrocladius sp3* (Ps.sp3.CH) and *Chironomus sp1* (Ch.us.sp1.CH) species were found at pH ≥ 6 to pH ≥ 7.4, while *Orthocladius sp2* (Or.sp2.CH) and *Limnophyes sp.* (Lim.sp.CH) species had a much wider range at pH ≤ 5.8 to pH ≥ 8.6. The *Tanytarsus sp1* (Ta.sp1.CH) also had a very narrow pH range of 5.7 to 6. Whereas *Orthocladius sp1* (Or.sp1.CH) and *Polypedilum sp4* (Po.sp4.CH) species could be detected at a slightly lower pH of 5.6 and maximums of 6.6 and 6.2 respectively. *Polypedilum sp5* (Po.sp5.CH) was found at a slightly lower pH range (pH = 5.3 to 6.1) when compared to the two species mentioned previously. *Polypedilum sp4* (Po.sp4.CH) and *Lauterborniella sp.* (Lau.sp.CH) species had a much wider pH range (≥ 5.1 to ≥ 8.6) when compared to all the other species mentioned prior. Similar observations were made for *Rheocricotopus sp2* (Rh.sp2.CH) and *Tokunagaia sp.* (To.sp.CH) where the latter had a notably wide pH range of 4.4 to 8.6. Both the *Parametriocnemus sp.* (Param.sp.CH) and *Microtendipes sp1* (Mi.t.sp1.CH) species were also detected at pH 4.4. The remaining 7 species were all detected at a pH <4.4. Chironomidae species are known for their tolerance to a wide range of environmental conditions including very acidic conditions, however none of the chironomids recorded in this study were found at pH < 4 (**Figure 6-5**).

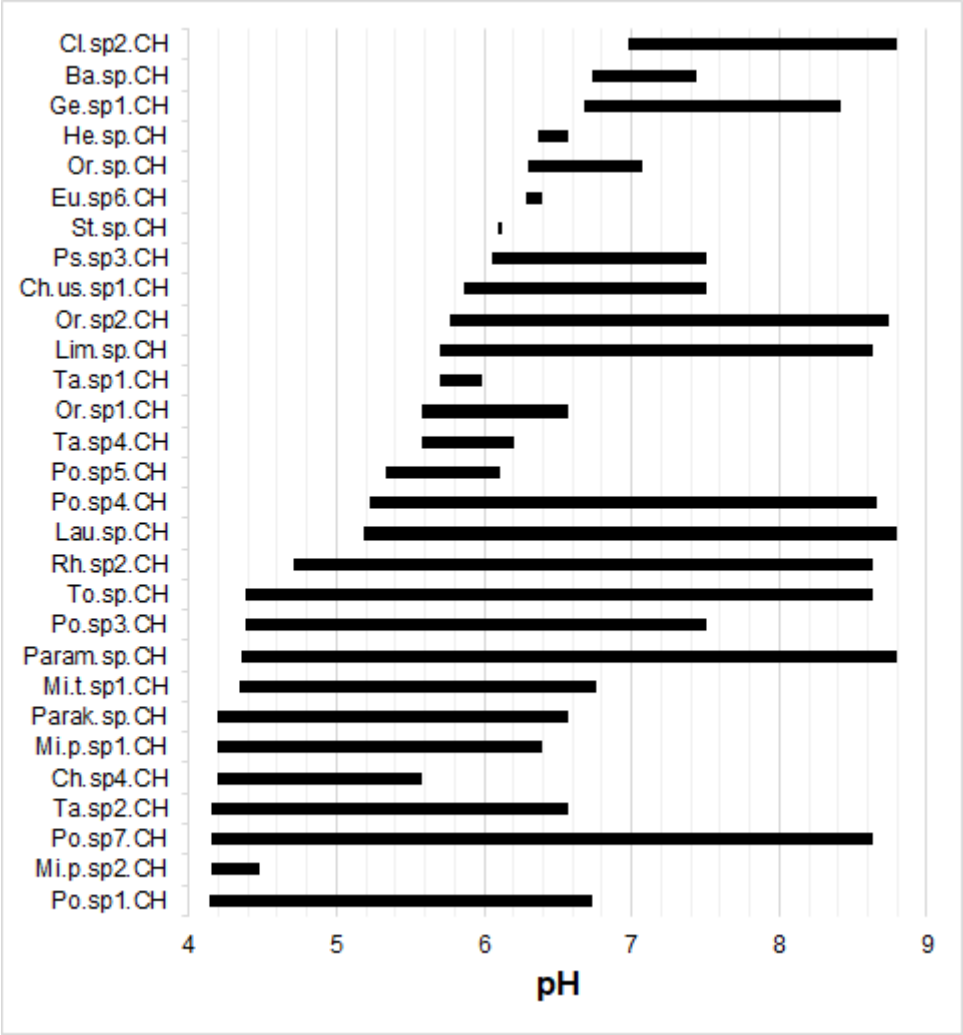


Figure 6-5:Chironomidae species pH range of occurrence in present study.

6.6.5 An overview of macroinvertebrate species that were recorded at the Mpumalanga, Waterberg and south-western Cape study regions – Common species

Overall a total of 25 species of aquatic insects were recorded at all three study regions. This number was made up of 11 mayfly species (*Afroptilum sudafricanum*, *Baetis harrisoni*, *Cheleocloeon excisum*, *Crassabwa flava*, *Pseudocloeon glaucum*, *Pseudocloeon piscis*, *Pseudocloeon latum*, *Caenis Type1*, *Caenis Type2*, *Afronurus oliffi* and *Compsoneuria bequaerti*), 10 Chironomidae species (*Lauterborniella sp.*, *Polypedilum sp3*, *Polypedilum sp4*, *Polypedilum sp7*, *Tanytarsus sp2*, *Orthocladius sp1*, *Orthocladius sp2*, *Parametriocnemus sp.*, *Rheocricotopus sp2 and Tokunagaia sp*.) and 4 blackfly species (*Simulium edwardsellum damnosum*, *Simulium anasolen dentulosum*, *Simulium nevermannia nigritarse* and *Simulium nevermannia rutherfoordi*). When plotted against the pH gradient, the majority of these species were associated with a wide range of pH (**Figure 6-6**) concentrations, making it difficult to adequately define sensitive groups. Three mayfly species (*Pseudocloeon piscis, Caenis Type2* and *Pseudocloeon latum*) however were found at a pH > 5.6 and one undescribed chironomid species (*Orthocladius sp1*) was recorded at pH > 5.0. According to the Raddum acidification index, species that are highly sensitive to acidity can only tolerate pH ≥ 5.5 while moderately sensitive species can tolerate pH ≥ 5.0 (Raddum, 1993; Raddum & Fjellheim, 1993), suggesting that these four species could serve as potential indicator species on a national scale (**Figure 6-6**). The Simuliidae species *Simulium anasolen dentulosum* was generally recorded in streams with pH < 6. Presumably the presence of this blackfly species and the absence of all four potential indicator species mentioned above could be an indication of stream acidification. For ease of understanding categorisation of potential indicator species, Raddum's definition of tolerant and intolerant species will be applied throughout the process of interpreting the results.

McCune *et al.* (2002) have suggested that depending on the purposes of the study one should generally consider deleting rare and sparsely distributed species that occur in less than 5 % of the sampling units (McCune *et al.*, 2002). In this study a total of 55 sites were

sampled for macroinvertebrates and in adhering to the recommendations above (McCune *et al.*, 2002) species with fewer than 3 occurrences would be considered rare. However, taking into account the major geographical differences of the HV, WB and SWC study regions this number was set at 7 % in order to reduce the influence of geographical differences on species composition. Thus, species with fewer than 4 occurrences were deemed uncommon and deleted to reduce background noise in the data. The majority (n = 24) of the 25 species recorded across the study regions occurred on four or fewer occasions. Based on the aforementioned number of occurrences the four potential indicator species mentioned above (i.e. *Pseudocloeon piscis*, *Caenis Type2*, *Pseudocloeon latum* and *Orthocladius sp1*), as well as *Simulium anasolen dentulosum* did not fall into the category of rare species but their distribution patterns were very low. A total of 9 species (*Afroptilum sudafricanum*, *Baetis harrisoni*, *Pseudocloeon glaucum*, *Caenis Type1*, *Afronurus oliffi*, *Parametriocnemus sp.*, *Simulium edwardsellum damnosum*, *Simulium anasolen dentulosum*, and *Simulium nevermannia rutherfoordi*) had an abundance > 73. However, this high abundance seems to correspond with the species tolerance over a wide range of ecological conditions, many of which appear to be driven by geographical differences as some regions exhibited much higher abundances. Had the 5 % rule been applied on a regional basis this would have been equated to at least one site per region which would be at least three sites on a national scale and thus the estimations above will remain unchanged both on a regional or national scale.

A study by Pearson and Gray (1982 Part I,1983 Part II) categorised species abundances into 12 classes that could be used to identify indicator species. Class I represented (=) species with 1individual per species, Class II = 2-3 individuals per species, Class III = 4-7, Class IV = 8-15, Class V = 16-31, Class VI = 32-63, Class VII = 64-127, Class VIII = 128-255, Class IX = 265-511 individuals, Class X = 512-1023 individuals while Class XI represented 1024-2047 individuals and Class XII = 2048-4094 individuals per species (Gray & Pearson, 1982; Pearson *et al.*, 1983). The authors went on to explain that species with abundances that fell into Class V and VI made ideal indicator species, while species that fall on the far extremes (very low or very high) of these groups did not make good indicator species as many other factors may be driving these abundances. Thus, species

with an intermediate abundance of 16 to 63 individuals were considered good indicator species as changes in these numbers may be easily detected and related to catchment properties.

This same principle was applied in this study. However, in light of the geographical differences that exist between the study regions the 16-63 individuals pre-requisite was applied regionally and not on a national scale. Consequently, none of four species dubbed potential indicator species met these requirement as they all had regional abundances that were < 16 individuals per species. In fact, only five (*Baetis harrisoni*, *Caenis Type1*, *Parametriocnemus sp.*, *Afroptilum sudafricanum and Simulium edwardsellum damnosum*) of the 25 common species met those requirements. The *Simulium edwardsellum damnosum* had one less individual for the Mpumalanga region but was included in the species list above, nonetheless. Of the five species listed above *Baetis harrisoni* was the only one that could be considered a potential indicator species as it displayed moderate sensitivity by being present only in streams with pH > 5.0. The one disadvantage of using this species as an indicator species is its tolerance to pH ≥ 8.0 as this pH range is usually associated with streams impacted by anthropogenic activity. However, for the purposes of establishing tolerance to pH and ANC limit this species might prove a useful candidate.

Larsen *et al.* (1996) conducted a study that looked at the role of species abundance and site number (distribution) when identifying suitable indicator species. Overall species abundances varied considerably, however from a distribution point of view the authors generally focused on species that occurred in at least 50 % of the study sites. The *Baetis harrisoni* did not meet these site requirements as it was only recorded in 33 % (n = 18 of 55) of the study sites, which accounted for 24 % (n = 6 of 25) of the sites in the SWC, 23,5 % (n = 4 of 17) in the WB and 57 % (n = 8 of 14) of sites from the HV region. To meet these requirements the *Baetis harrisoni* should have been recorded in at least 12.5 sites in the SWC and 8.5 in the WB.

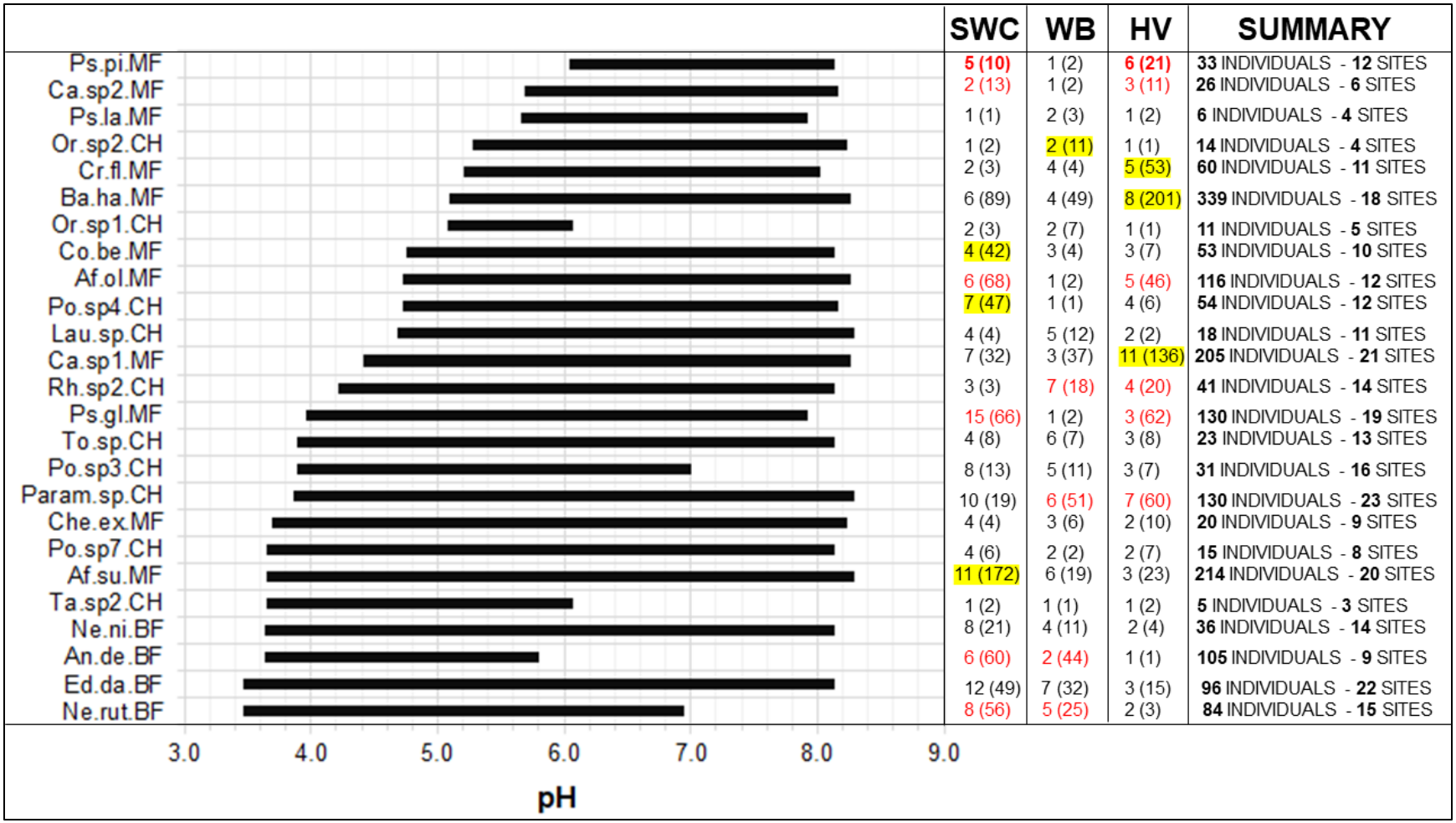


Figure 6-6: Ranking of common species according to their apparent tolerance to pH as estimated here through range of occurrence. Species abundance is given in brackets and the number of occurrences (number of sites) is indicated to the left of abundance values. Values highlighted in red indicate two regions that displayed the highest abundance while the yellow highlight represents a region that had the highest abundance when compared to the other two regions. Overall species abundance and distribution varied considerably across study regions.

6.6.5.1 Species responses to environmental variables

Macroinvertebrate response to changes in the water chemistry was assessed and a total of thirteen variables (EC, pH, Na^+, NH_4^+; K^+, Mg^{2+}; Ca^{2+}, NO_3^-, Cl^-, SO_4^{2-}, F^-, ANC and DOC) were tested on the species data. The ordination results indicated that the first axis explained 9.5 % of variance while the second and third axes explained 3.5 % and 2.9 % of variance respectively. The pH (r = 0.78) and ANC (r = 0.57) explained most of the variation in axis 1. *Baetis harrisoni* (Ba.ha.MF), *Cheleocloeon excisum* (Che.ex.MF), *Parametriocnemus sp*. (Param.sp.CH), *Afronurus oliffi* (Af.ol.MF) and *Lauterborniella sp*. (Lau.sp.CH) species were all associated with the first axis (**Figure 6-7**). *Compsoneuria bequaerti* (Co.be.MF) and *Pseudocloeon glaucum* (Ps.gl.MF) species corresponded well with the Ca^{2+} ion while Mg^{2+} showed correspondence to *Polypedilum sp4* (Po.sp4.CH) species. *Polypedilum sp3* (Po.sp3.CH) was closely associated with the SO_4^{2-} ion. The Eigenvalue (0.309) for the first axis is higher than the predicted mean (0.210) from the Monte Carlo randomisation test (p = 0.018) therefore we reject the null hypothesis of no relationship between species data and the environmental variables.

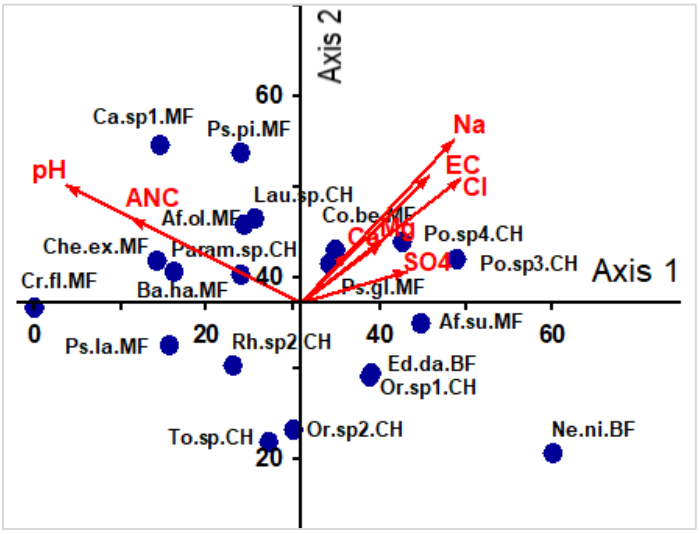


Figure 6-7: Canonical Correspondence Analysis (CCA) conducted on macroinvertebrate species that were common to the HV, WB and SWC study regions. Axis scores are weighted averages of species scores. These scores have been centered and standardized to unit variance and the axes scaled to optimize representation of species.

6.7 Indicator Species Analysis (ISA) using the Dufrene-Legendre approach

The Indicator Species Analysis (ISA) test was used to generate Indicator Value (IndVal) for the 136 taxa recorded in this PhD study. The technique is based on the species relative abundance and relative frequency of occurrence in the different taxonomic groups of interest (Dufrêne & Legendre, 1997). Since the technique is based on the relative frequency and relative abundance it means that groups being compared can have species that give a 100 % IndVal for each group.

The results show that based on the relative abundance and relative frequency of all 136 species recorded in this study the HV, WB and SWC study regions all comprise different indicator species. Three species (*Parametriocnemus sp.*, *Afroptilum sudafricanum and Simulium edwardsellum damnosum*) that showed high abundance values across study regions (section **6.6.5**) did not make the list for any of the study regions. This is probably due to the high mean values generated during the randomisation test which in turn gave nonsignificant p-values (**Table 6-9**).

Statistical significance is based on the difference between observed values and the mean of the values generated during the randomisation test and weighted by the standard deviation (Dufrêne & Legendre, 1997). Based on these findings and those from section **6.6.5** national indicator species could not be identified and thus an ANC limit based on macroinvertebrate species could not be generated neither.

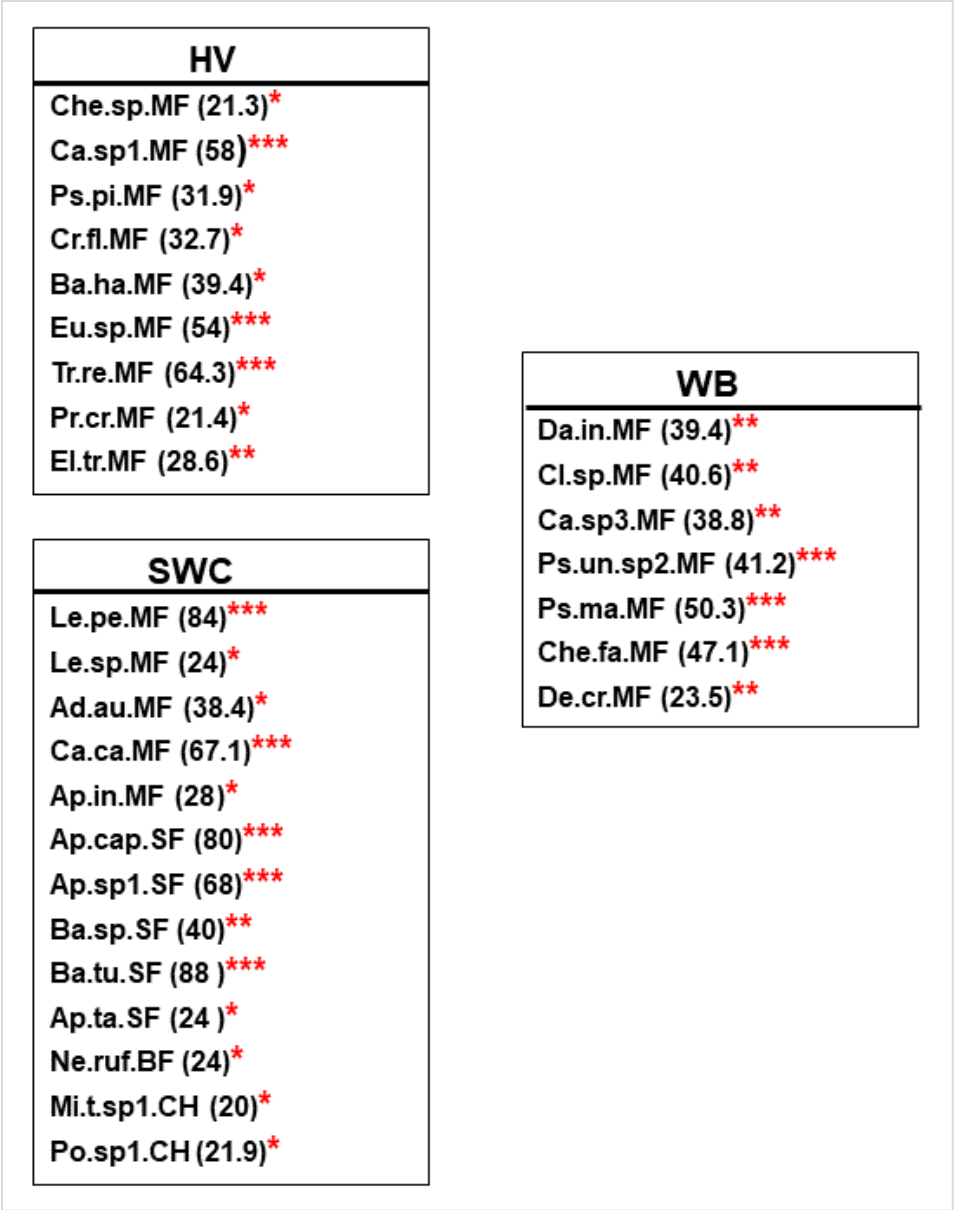


Figure 6-8: A list of indicator species for the respective study regions generated using the Dufrene-Legendre ISA approach. The indicator value (IndVal) percentage which indicates each species' faithfulness to a particular group based on relative abundance and relative frequency is given in brackets. The asterisk represents the level of significance(* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Overall the SWC had the largest number of indicator species with the highest IndVals. No indicators were shared by the study regions. The overall low yield of perfect (IndVal = 100 %) indicator scores further accentuates the low species abundance results shown on section **6.5.**

Table 6-9: Table demonstrating statistical basis for choosing indicator species. This table was generated to show the IndVal against the mean, standard deviation (SD) and p-values generated from the Monte Carlo test for the species that did not make the indicator species list versus those that made the list but had low IndVal percentages and those that had high IndVals for the respective regions. 4999 permutations were used to test for statistical significance. The mean and SD of the randomisation test values act as the main qualifiers for species significance and group allocation.

Species	IndVal (%)	Mean	SD	p-value
Parametriocnemus sp.	26.6	24.7	7.14	0.32
Afroptilum sudafricanum	31.4	21.5	6.40	0.08
S. edwardsellum damnosum	19.1	21.8	5.72	0.61
Baetis harrisoni	39.4	19.9	6.28	0.01
Caenis Type1	58.0	23.2	7.04	0.0008
Tricorythus reticulatus	64.3	13.1	5.56	0.0002
Pseudopannota maculosa	50.3	14.7	5.74	0.0004
Lestagella penicillata	84	22.2	6.48	0.0002

6.8 Defining potential indicator species for stream acidification on a regional scale

The indicator species generated from the ISA were plotted against a pH gradient to determine species response to changes in stream pH on a regional scale. Down scaling the species identification required that more stringent parameters be set. For instance, species had to have been recorded in at least 80 % of the sampling sites instead of 50 % as previously stated in section **6.6.5** for them to be considered good indicators regionally. Thus, potential species from the HV had to be present at 11 of the 14 macroinvertebrate sites, 13 for the Waterberg and 20 for the SWC.

Only one species (*Caenis Type1*) from the Mpumalanga region met the proposed site requirements (**Figure 6-9**). However, based on species tolerance to pH this particular mayfly could not serve as an ideal indicator for stream acidification as it displayed a high tolerance to a wide range of pH concentrations. Nonetheless three species (*Cheleocloeon sp.*, *Pseudocloeon piscis* and *Euthraulus sp.*) found at pH > 5.5 could be classified as intolerant to low pH whereas *Crassabwa flava* and *Baetis harrisoni*, which were only recorded at pH > 5.0, could be considered moderately intolerant. The majority of these

species however had low distribution rates that were accompanied by equally low ISA indicator values, implying that overall relative abundance and relative frequency was too low for them to qualify as perfect (IndVal = 100 %) indicator species regionally. Similar observations were made for the Waterberg (**Figure 6-10**). The SWC was mostly dominated by acid tolerant aquatic insects (**Figure 6-11**).

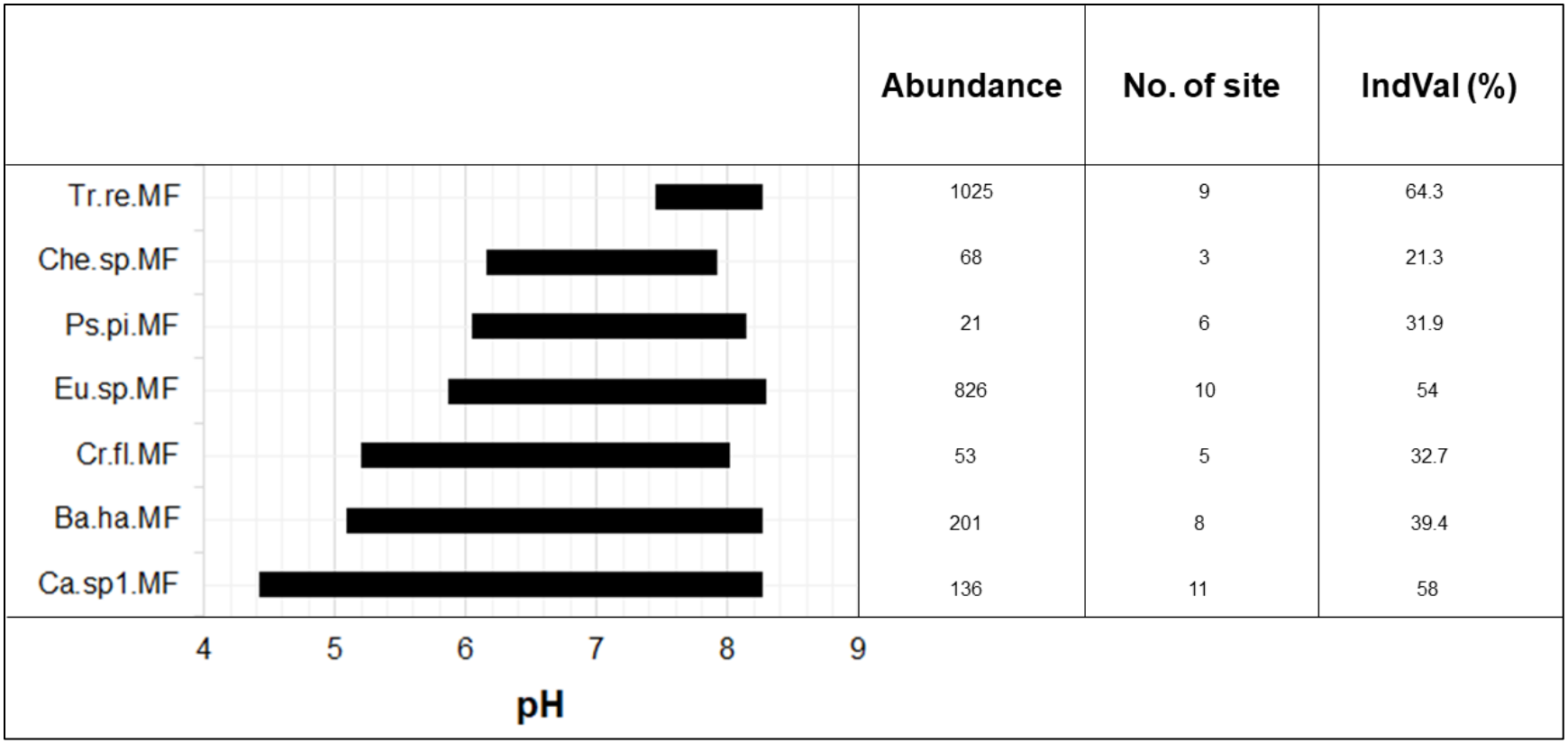


Figure 6-9: Ranking of Mpumalanga ISA defined indicator species according to their tolerance to pH. Indicator species identified in section 6.7 were plotted against a pH gradient to determine species response to changes in the stream pH. A total of 9 mayfly species were identified for the HV however two species namely, *Prosopistoma crassi* (Pr.cr.MF) and *Elassoneuria trimeniana* (El.tr.MF) were not included on the plot due to problems with missing field chemistry data as detailed in the appendices (Appendix 6). Individual species abundance, number (No.) of sites species were recorded from and indicator values are given on the left side of the graph.

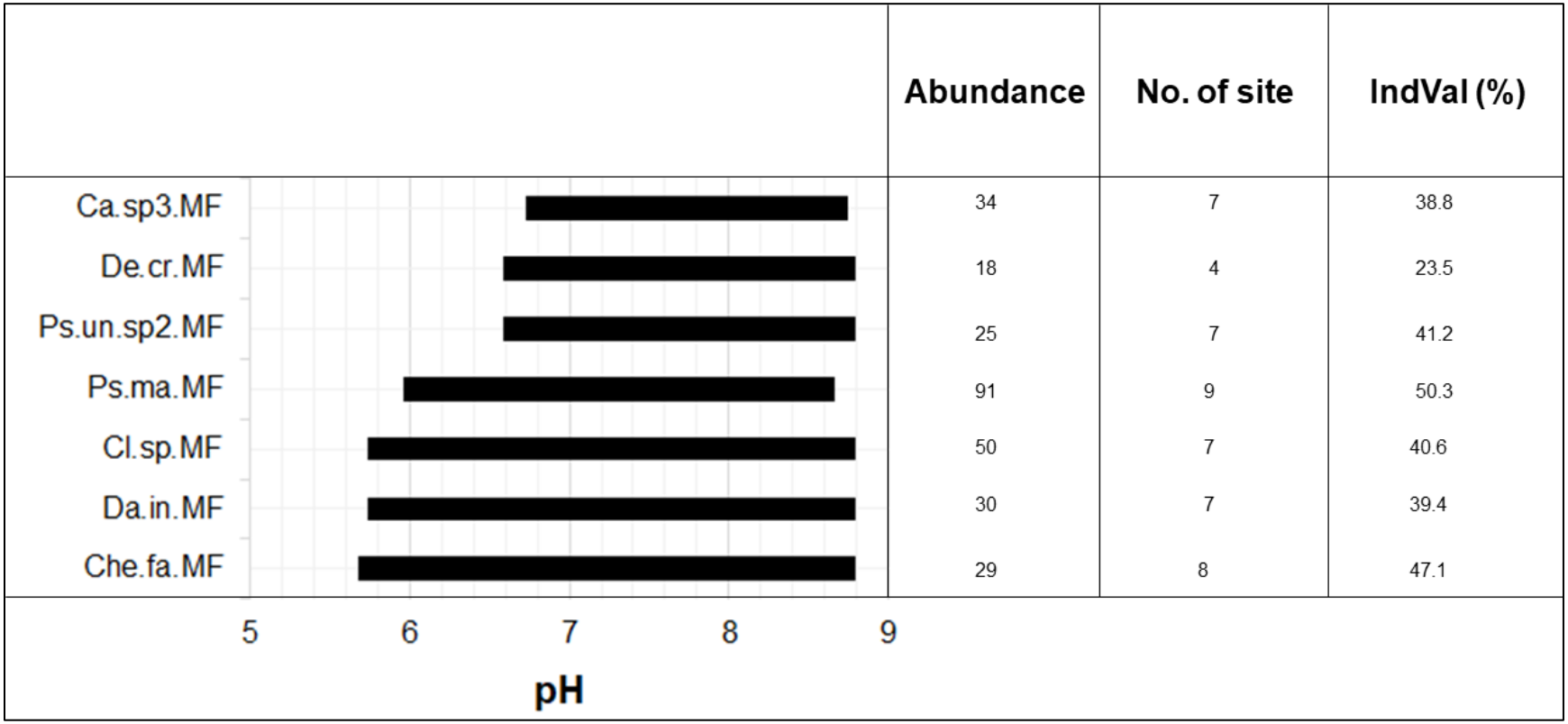


Figure 6-10: Ranking of Waterberg ISA defined indicator species according to their tolerance to stream pH. Indicator species identified in section 6.7 were plotted against a pH gradient to determine species response to changes in the stream pH. A total of 7 mayfly species were identified for the Waterberg. Individual species abundance, number (no.) of sites species were recorded from and indicator values (IndVal) are given on the left side of the graph.

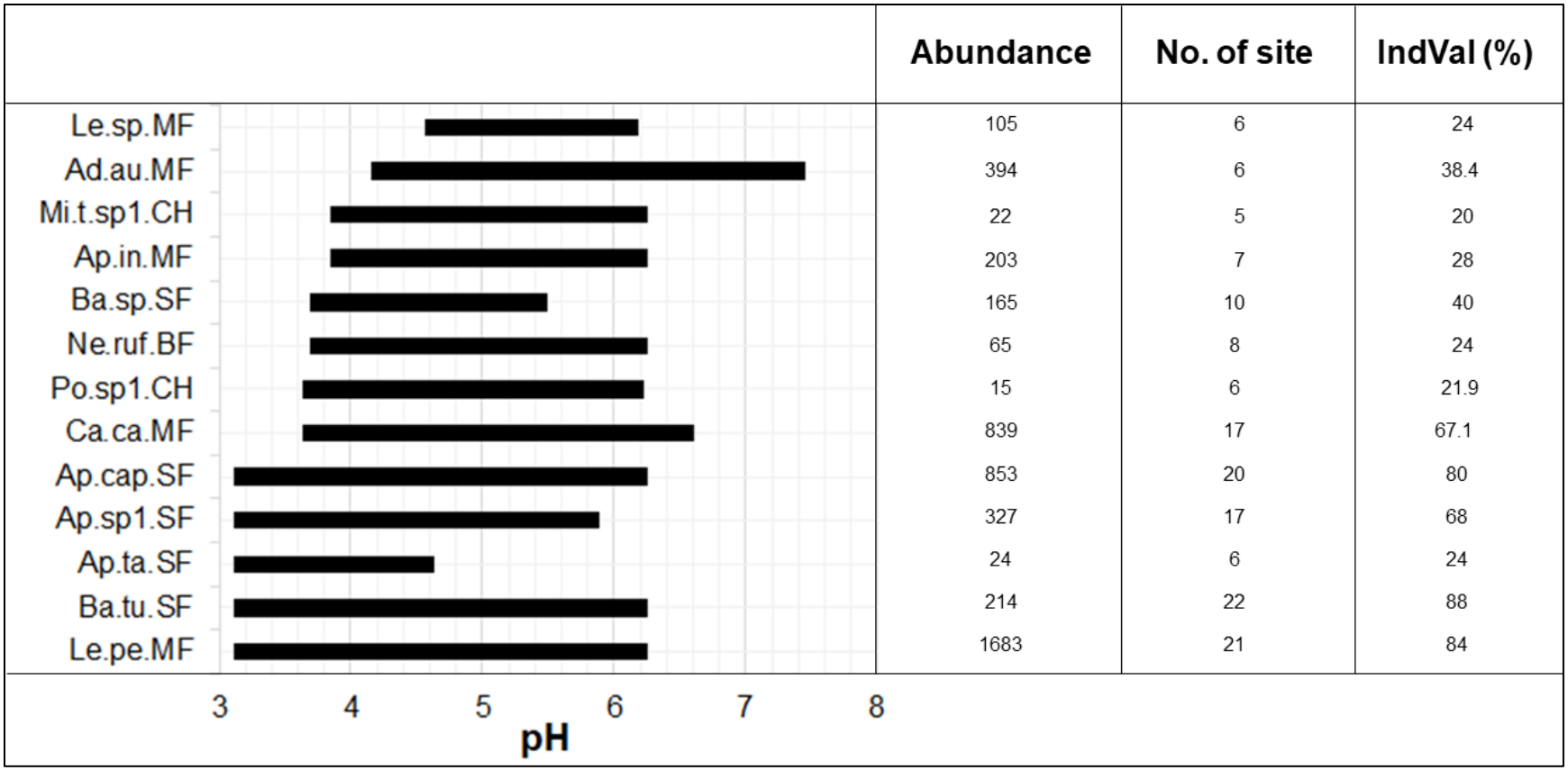


Figure 6-11: Ranking of south-western Cape ISA defined indicator species against the pH gradient. Indicator species identified in section 6.7 were plotted against a pH gradient to determine species response to changes in the stream pH. A total of 5 mayfly species, 2 Chironomidae, 1 blackfly and 5 stonefly species were identified for the SWC. Individual species abundance, number (No.) of sites species were recorded from and IndVal are given on the left side of the graph.

Table 6-10: List of species that can be categorised as being acid tolerant or intolerant. Based on the data presented in this study species under column one may not survive pH < 5.5 while those listed on the second column could not survive pH < 5.0. The third column represents species which can only tolerate p ≥ 4.2. However, the basis of this categorisation needs further review as these are quite acid conditions already and the questions to ask is what range tolerant species would fall on? Equally so based on the literature a pH < 4 leads to fish kill (Lien *et al.*, 1992) and thus column three species represent acid conditions that still favour fish survival. Biogeographic restrictions have not been taken into account. This Table rather functions as an overview of observations made in the study. For species distributions information the reader is referred to sections Error! Reference source not found. - **6.4**.

Intolerant species (pH ≥ 5.5)	**Moderately intolerant species (pH ≥ 5.0)**	**Moderately tolerant (pH ≥ 4.2)**	**Tolerant species ?**
Cl.un.sp.MF	Ps.ma.MF	Ap.bi.SF	
Ca.sp3.MF	Cl.sp.MF	Mi.p.sp2.CH	
Che.sp.MF	Da.in.MF	Le.sp.MF	
Ad.bi.MF	Cr.fl.MF		
De.cr.MF	Che.fa.MF		
Ps.un.sp2.MF	Ba.ha.MF		
Ps.pi.MF	Po.sp5.CH		
Eu.sp.MF			
Ca.sp2.MF			
Ps.la.MF			
Ba.un.sp1.MF			
Ne.sp.SF			
Me.ad.BF			
Po.un.BF			
Cl.sp2.CH			
Ba.sp.CH			
Ge.sp1.CH			
He.sp.CH			
Or.sp.CH			
Eu.sp6.CH			
St.sp.CH			
Ps.sp3.CH			
Ch.us.sp1.CH			
Or.sp2.CH			
Lim.sp.CH			
Ta.sp1.CH			
Or.sp1.CH			
Ta.sp4.CH			

Chapter 7 Assessing the role of soils, geology, landcover and water chemistry in defining species composition in the south-western Cape (SWC) region of South Africa

The SWC falls under Region four (Temperate acid waters of the Cape Fold montane region) of the five Limnological Regions of Southern Africa (Allanson *et al.*, 1990). These streams are characterised by temperate acid waters that drain the ancient rocks of the Cape fold mountain (Allanson *et al.*, 1990; de Moor & Day, 2013). Other characteristics associated particularly with the SWC are listed on **Table 7-1** below. Streams from the SWC are naturally acidic due to the presence of organic acids attributed to humic soils and plant organic matter (Midgley & Schafer, 1992). The presence of dissolved organic matter gives the water a dark brown colour and due to the contrasting colour between the streams in this region the SWC has become known for its black and white waters. Fey (2010) described organic and humic soils as soils that have a low content of base cations and are highly acidic when exposed to sulphur (Fey, 2010a). Of the three study regions the SWC exhibited the highest concentration of acid anions and the lowest concentration of base cations and an equally low acid neutralising capacity. Indigenous forests and fynbos are the dominant landcover for the region. In addition to the above the south-western Cape is well known for its high endemism (de Moor & Day, 2013). Based on field observations and literature search (Midgley & Schafer, 1992; Allanson *et al.*, 1990; Harrison, 1962) black waters are generally more acidic than white headwater streams. However, upon conducting the Welch Two Sample t-test on several environmental variables (EC, pH, Na^+, NH_4^+; K^+, Mg^{2+}; Ca^{2+},NO_3^-, Cl^-, SO_4^{2-}, F^-, ANC and DOC) no statistically significant differences were observed ($p > 0.05$) between the black and white streams for all the other variables measured in this study except the DOC concentrations ($p < 0.001$) (**Figure 7-1**).

Table 7-1: A summary of some key characteristic of the streams from the south-western Cape.

Characteristic feature	Description	Reference
Limnological region	Falls under region 4 of the five Limnological Regions of Southern Africa	(Allanson *et al.*, 1990)
Dominant landcover	Fynbos and indigenous forest	(Allanson *et al.*, 1990; Midgley & Schafer, 1992; de Moor & Day, 2013; Rivers-Moore *et al.*, 2018)
Geology	Quartzitic rocks, sandstones	(Allanson *et al.*, 1990; de Moor & Day, 2013)
Streams	Oligotrophic streams with black and white waters	(Allanson *et al.*, 1990; Day & King, 1995; de Moor & Day, 2013)
Na^+ and Cl^-	Dominant ions in stream waters	(Silberbauer & King, 1991; Day & King, 1995)
Conductivity	High estimations (100 mSm^{-1})	(Day & King, 1995)
Elevated DOC concentrations	Peat stained waters due to high concentrations of dissolved organic compounds	(Harrison & Agnew, 1962; Harrison, 1962; Midgley & Schafer, 1992; Allanson *et al.*, 1990; de Moor & Day, 2013)
Endemic Ephemeroptera, genus	*Adenophlebia, Aprionyx, Castanophlebia, Choroterpes* and selected *Euthraulus*	(Harrison, 1962; Allanson *et al.*, 1990; de Moor & Day, 2013)
Endemic Plecoptera, genus	*Aphanicerca*, *Aphanicercella* and *Aphanicercopsis*	(Harrison, 1962; Allanson *et al.*, 1990; de Moor & Day, 2013)

The DOC concentration is a major distinguishing feature between black and white streams. For example, some European countries have classified regions dominated by organic compounds by using the DOC concentration where streams with DOC > 5 mg/L are generally characterised by a dark brown colour whereas clear waters have been associated with DOC concentrations < 5 mg/L (McFarland *et al.*, 2010).

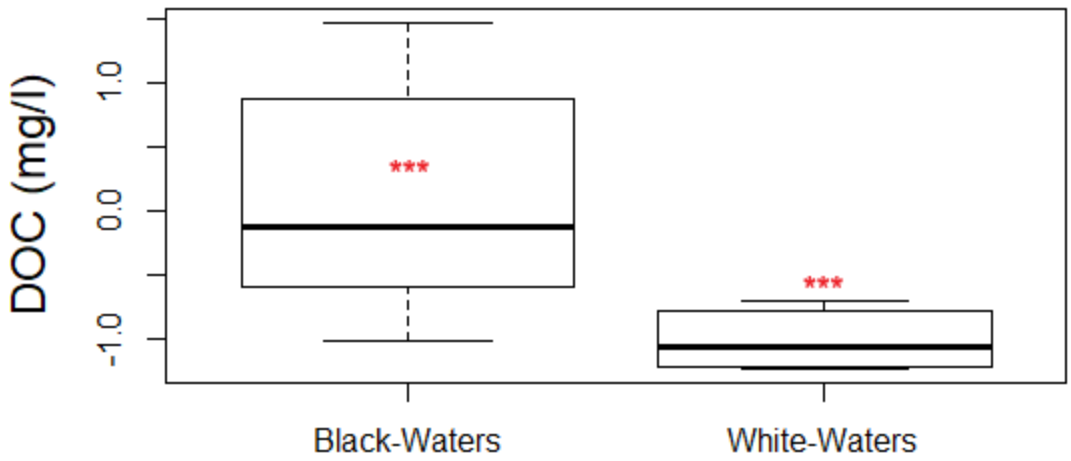


Figure 7-1: DOC concentrations for black and white streams from the SWC study region. The number of white water streams was far less (n = 8) than that of black waters (n = 17). However, statistical summaries revealed a significant difference in the average (Mean), minimum (Min) and maximum (Max) values (i.e. white waters had Mean = 5.6 mg/l, Min 2.2 mg/l and Max = 11.9 while black waters had higher values with Mean = 16.5, Min = 4.1 and Max = 34.1) for DOC. Nonetheless black waters are generally known to be the dominant stream type in the region (Allanson *et al.*, 1990; Midgley & Schafer, 1992; Harrison, 1962). The asterisk indicates level of significance (*** $p < 0.001$).

From a South African context however, during the course of this study DOC concentrations for the SWC varied considerably with seasons in both black (SWC03, SWC04, SWC06, SWC07, SWC08, SWC09, SWC14, SWC16, SWC18, SWC19, SWC20, SWC21, SWC23, SWC24, SWC25, SWC26, SWC27, SWC31, SWC32, SWC35, SWC36 and SWC37) and white (SWC01, SWC17, SWC22, SWC28, SWC29, SWC30, SWC33, SWC34) headwater streams. Thus, a DOC indicator value aimed at distinguishing between black and white headwater streams could not be estimated. Increasing DOC concentrations however seem to correspond to rainfall events (**Figure 7-2**).

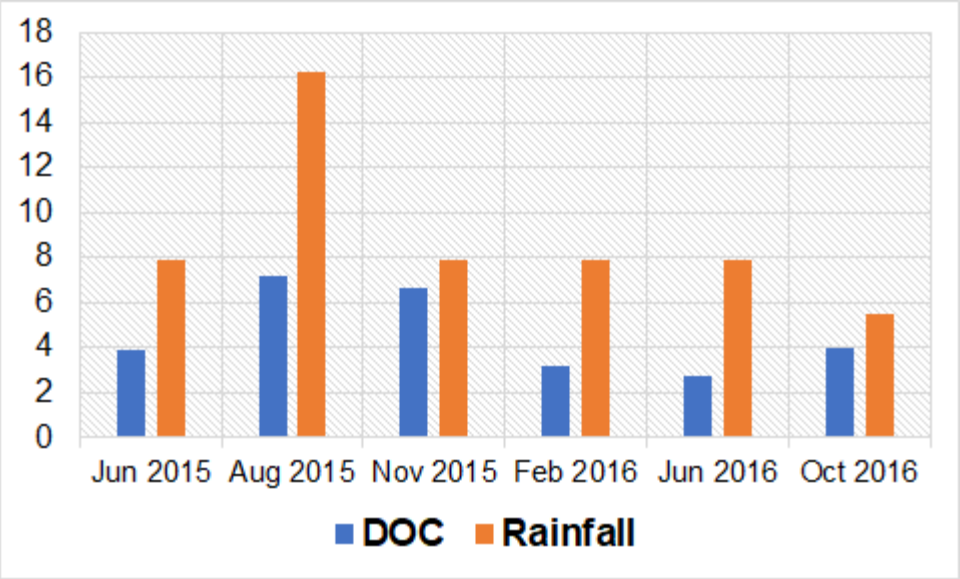


Figure 7-2: Graph showing relationship between DOC (mg/l) and rainfall patterns in the south-western Cape. The DOC concentration was plotted against the median values from monthly rainfall (mm).

A study by Harrison and Agnew (1962) categorised streams from the SWC into two stream categories that included peat stained acid streams (pH = 5.0 – 5.9) and clear water streams which were designated pH 6.0 – 6.9 (Harrison, 1962; Harrison & Agnew, 1962). These two groups of streams were further distinguished from each other by the type of species that inhabit them. Since then several studies (Midgley & Schafer, 1992; de Moor & Day, 2013), including findings from this thesis have reported pH values that are much lower than those of Harrison and Agnew. In light of this the present study set out to categorise streams from the SWC to represent five groups of streams that included waterbodies with pH ≤ 4.0, pH ≥ 4.1 but ≤ 4.5, pH ≥ 4.6 but ≤ 4.9, pH ≥ 5.0 but ≤ 5.4 and those with pH ≥ 5.5 but ≤ 5.9. The aim of this exercise was to relate these stream categories to soil, geology and landcover data in order to evaluate how these features affect stream chemistry and species composition. The landcover dataset was obtained from the Garden Route National Park (GRNP) while the geological data was obtained from the SOTER database. Catchment delineation was carried out prior to assigning soils data, landcover and geological features to study sites. Catchment delineation was outsourced, and the results were verified and edited by the project head (Prof. Curtis, University of the Witwatersrand). Dominant landcover (Fynbos/ rehabilitation areas;

Indigenous Forest/ Rehabilitation zone; Plantation; Build-up/ Forest Village/ Roads/ Infrastructure/ Pasture and Wetland/Water regions) within the catchment area, geological features (Nardouw, Peninsula, Kaaimans, Cape granite, Quaternary and Enon), Lithic leptosol soils (ZA3065, ZA3163 and ZA3177) and acid neutralising capacity were tested. The PCA results showed that different pH grouping variables corresponded to a wide range of soil, geological and landcover features (**Figure 7-3**). For instance, ZA3177 soils and Indig Forest were both closely associated with study sites that belonged to three different pH categories (pH ≥ 4.1 but ≤ 4.5, pH ≥ 4.6 but ≤ 4.9, pH ≥ 5.0 but ≤ 5.4). Therefore, no one pH range showed preference to a particular land feature over the other. The overall results however have demonstrated that majority of the sites with pH ≤ 4.0 were largely associated with ZA3065 soils, Fynbos/rehab landcover and Peninsula rock formation. One site with pH ≤ 4.0 corresponded with Nardouw and plantation features. The first Principle Component (PC1) of the ordination results explained 46.29 % variance in the data, PC2 explained 18.96 % of the variance and PC3 explained 14.99 % of the variance, together they accounted for 80.24 % of the cumulative proportion of the data. Fynbos/rehab (r = 0.42), Peninsula (r = 0.36), ZA3065 (r = 0.35) and ZA3163 (r = 0.25) were all positively correlated to PC1. Whereas Indig Forest (r = - 0.42), quaternary (r = - 0.31) rock and ZA3177 soils (r = - 0.43) were all negatively correlated to PC1. Plantation (r = 0.53) and Nardouw (r = 0.54) were both positively correlated to PC2. None of the white headwater streams had pH ≤ 4.0.

Acid neutralising capacity plays an important role in buffering stream acidity. This process is dependent on base cations since low base cation concentrations will lead to a low acid neutralising capacity. Findings from this thesis have so far demonstrated that the SWC has low base cation concentrations that are accompanied by a low acid neutralising capacity. Streams that have an ANC > 200 µeq/l are said to be insensitive to acidification, whereas those with an ANC < 50 µeq/l are deemed highly sensitive to acidification, while an ANC < 0 µeq/l has been associated with chronically acidic streams (USEPA, 2009; US EPA, OAR, OAP, 2017; US EPA, 2012; Langhelle, 2000). In the current study streams from the SWC were classified into five ANC categories (ANC < 0 µeq/l, ANC < 20 µeq/l, ANC < 50 µeq/l, ANC < 100 µeq/l and ANC < 200 µeq/l) and a PCA was carried out to

assess how these different ANC categories relate to soil, geology and landcover data. The results displayed distribution patterns similar to those of pH where ANC concentrations could not be attributed solely to a particular geology, soil and landuse pattern. PC1 explained 50.91 % variance, PC2 20.42 % and PC3 11.17 % and together they accounted for 82.5 % of the cumulative proportion of the data.

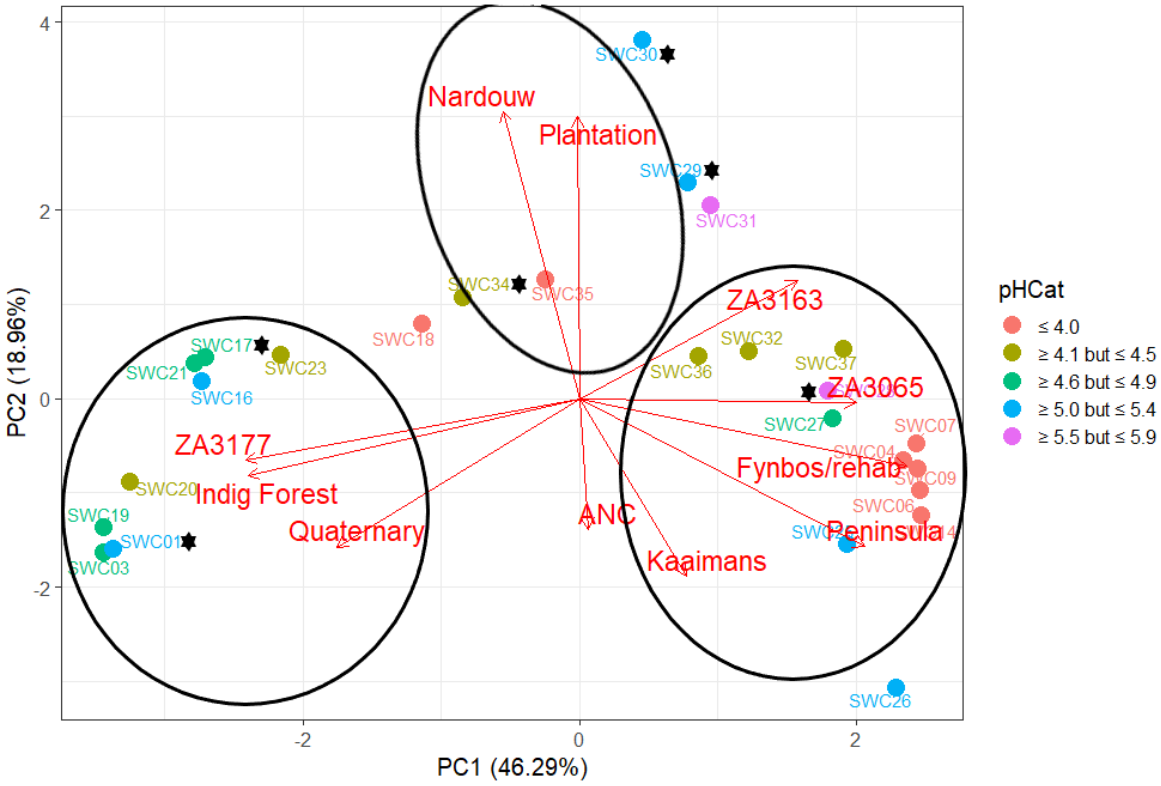


Figure 7-3: pH Categories (pHCat) for the SWC sampling sites. Ordination analysis aimed at relating stream pH to soil, geological and landcover data. To achieve this, streams from the SWC were classified into five pH categories which were plotted against the ANC, landcover (Fynbos/ rehabilitation areas, Indigenous Forest/ Rehabilitation zone, Plantation, Build-up/ Forest Village/ Roads/ Infrastructure/ Pasture and Wetland/Water regions), geology (Nardouw, Peninsula, Kaaimans, Cape granite, Quaternary and Enon) and Soils (ZA3065, ZA3163 and ZA3177) data. One site (SWC22) had a pH > 6.0 which obscured the whole plot and thus was excluded from final analysis. The loadings (ANC, landcover, soil and geology) are represented by red arrows. The black circles represent site clusters and their associated features. Black stars represent white water sites. Each cluster appears to accommodate sites with a wide range of pH values. Decreasing the number of groups to three (i.e. group 1 = pH ≤ 4.0, group 2 = ≥ 4.1 but ≤ 4.9, group 3 = ≥ 5.0 but ≤ 5.9) or two (i.e. group 1 = pH ≤ 4.9, group 2 = ≥ 5.0 but ≤ 5.9) did not yield a better result.

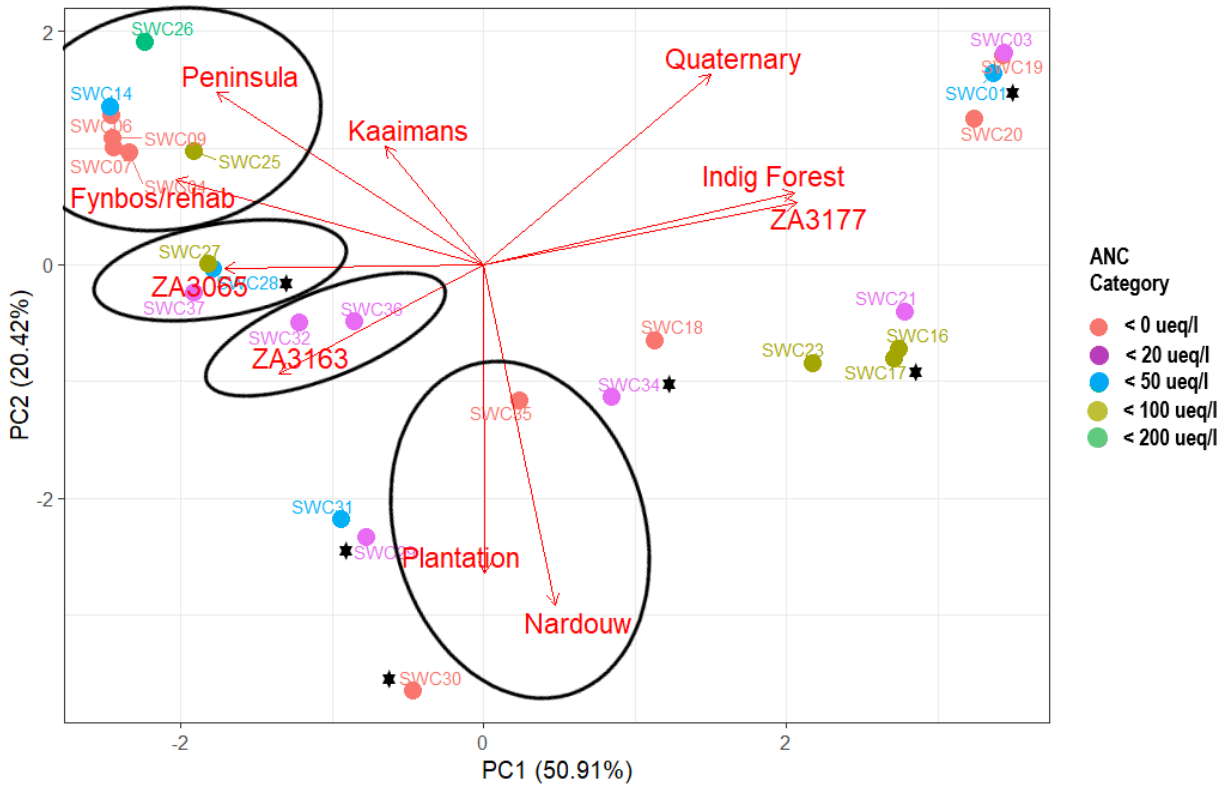


Figure 7-4: PCA showing relationship between the ANC, study sites, soils, landcover and geology in the SWC. None of the white headwater stream sites (indicated by a black star) had ANC < 0 µeq/l.

7.1 Species composition

Aquatic insects that inhabit naturally acidic streams are generally well adapted to the acid conditions resulting in very high species composition that is expressed through species richness and abundance. Furthermore, naturally acidic streams tend to have higher species richness when compared to non-acid streams (Petrin *et al.*, 2007). In some instances, however this high variation in species composition and abundance may be deceptive as it could potentially camouflage anthropogenic variations in the water chemistry.

The first section of this chapter has highlighted key characteristics of the SWC water bodies one of which is the black and white headwater streams. Species comparison was conducted between these stream types to establish difference in species composition. A total of 23 species were restricted to black headwater streams and 17 for the white water streams (**Table 7-2**) and together they accounted for 49 % (n = 40 of 82 species) of the total number of species recorded at the south-western Cape. The remaining species (n = 42) were found in both stream types with some species (*Cheleocloeon excisum*, *Caenis Type1*, *Pseudocloeon piscis*, *Leptophlebiidae sp.*, *Adenophlebia auriculata*, *Aprionyx intermedius*, *Choroterpes sp.*, *Aphanicerca capensis*, *Balinskycercella sp.*, *Aphanicercella bifurcata*, *Balinskycercella tugelae*, *Chironomimae sp4*, *Microtendipes sp1*, *Polypedilum sp3*, *Polypedilum sp5*, *Polypedilum sp7*, *Parakiefferiella sp.*, *Parametriocnemus sp.* and *Rheopelopia sp.*) showing greater preference for white water streams based on relative abundance percentages and other species (*Compsoneuria bequaerti*, *Afronurus oliffi*, *Baetis harrisoni*, *Aphanicerca sp2*, *Aphanicercopsis tabularis*, *Simulium edwardsellum damnosum*, *Simulium anasolen dentulosum*, *Polypedilum sp1*, *Polypedilum sp4*, *Tanytarsus sp1* and *Tokunagaia sp.*) having higher preference for black water streams. A few species (*Lestagella penicillata*, *Pseudocloeon glaucum*, *Afroptilum sudafricanum*, *Crassabwa flava*, *Baetis unkown sp1*, *Castanophlebia calida*, *Aphanicerca sp1*, *Simulium metomphalus chutteri*, *Simulium nevermannia nigritarse*, *Simulium nevermannia rutherfoordi*, *Simulium nevermannia ruficorne* and *Simulium metomphalus vorax*) were equally distributed between the two stream types.

Table 7-2: List of species from the SWC that are restricted to either black or white headwater streams. Beta diversity is given on the last row of the Table.

Black waters	White waters
La.vi.MF	Che.sp.MF
Da.in.MF	Ca.sp2.MF
Cl.sp.MF	Ca.sp3.MF
Ps.la.MF	Ps.sp.MF
De.ca.MF	De.me.MF
Ad.sp.MF	Ap.sp.SF
De.sp.SF	De.pu.SF
Po.im.BF	Si.sp.BF
Ch.sp3.CH	Me.ad.BF
Lau.sp.CH	Mi.p.sp1.CH
Mi.p.sp2.CH	Ph.sp1.CH
Po.sp6.CH	Ph.sp2.CH
Ta.sp2.CH	Ph.sp3.CH
Ta.sp4.CH	St.sp.CH
Pa.sp.CH	Za.sp.CH
Mi.t.sp2.CH	Eu.sp5.CH
Ca.sp.CH	Lim.sp.CH
Eu.sp1.CH	
Eu.sp6.CH	
Or.sp1.CH	
Or.sp2.CH	
Or.sp3.CH	
Ps.sp2.CH	
3.8	**2.5**

Species composition was also assessed for the different ANC categories presented on (**Figure 7-4**) following the same process as above which was to identify species unique to each category. The results revealed that according to the records made in this study streams with ANC < 0 µeq/l were mostly dominated by Chironomidae (**Table 7-3**). All the taxa identified as potential indicator species (section **6.7**, **Figure 6-8**) for the SWC were found in both black and white headwater streams across a wide range of ANC values (**Table 7-4**). The majority of mayfly species occurred in white waters that had an ANC > 50 µeq/l and < 100 µeq/l.

Table 7-3: List of species which according to the observations made in this study are found exclusively in streams with ANC < 0 µeq/l, < 20 µeq/l, < 50 µeq/l, < 100 µeq/l and < 200 µeq/l. Species abundance is given in brackets.

< 0 µeq/l	< 20 µeq/l	< 50 µeq/l	< 100 µeq/l	< 200 µeq/l
Po.sp6.CH (1)	Ad.sp.MF (20)	Cr.fl.MF (60)	Da.in.MF (32)	Che.sp.MF (69)
St.sp.CH (2)	Ap.sp.SF (8)	De.pu.SF (3)	Ps.la.MF (6)	Cl.sp.MF (51)
Ta.sp2.CH (5)	Ch.sp3.CH (1)	Si.sp.BF (2)	De.ca.MF (3)	Ca.sp3.MF (37)
Ta.sp4.CH (3)	Ph.sp3.CH (1)	Eu.sp6.CH (6)	De.sp.SF (1)	Ph.sp2.CH (1)
Mi.t.sp2.CH (1)	Za.sp.CH (1)	Lim.sp.CH (3)	Ph.sp1.CH (1)	
Ca.sp.CH (1)			Pa.sp.CH (1)	
Eu.sp1.CH (2)			Or.sp2.CH (14)	
Eu.sp5.CH (1)			Or.sp3.CH (1)	
Ps.sp2.CH (1)				

Table 7-4: Indicator species for the SWC as reported in section 6.7. Indicator species relative abundance percentages are presented below. The red highlight indicates stream conditions that the respective species had a high affinity for.

Species	White waters	Black waters	< 0 µeq/l	< 20 µeq/l	< 50 µeq/l	< 100 µeq/l	< 200 µeq/l
Le.pe.MF	41	59	15	6	16	51	12
Le.sp.MF	66	34	1	11	5	47	36
Ad.au.MF	63	37	31	8	6	55	1
Ca.ca.MF	48	52	4	3	27	49	17
Ap.in.MF	68	32	0	1	40	56	3
Ap.cap.SF	63	37	46	21	26	7	1
Ap.sp1.SF	49	51	32	43	7	11	8
Ba.sp.SF	62	38	13	40	26	22	0
Ba.tu.SF	67	33	12	35	15	30	8
Ap.ta.SF	16	84	70	26	0	4	0
Ne.ruf.BF	53	47	0	35	29	25	10
Mi.t.sp1.CH	88	12	36	0	52	0	12
Po.sp1.CH	35	65	58	14	14	13	0

7.2 Species response to environmental variables

Ordination analysis (**Figure 7-5**) was conducted on seventeen environmental variables (Altitude, Fynbos/rehab, Indig Forest, Plantation, Nardouw, Peninsula, Kaaimans, Quaternary, ZA3065, ZA3163, ZA3177, pH, Na^+, Cl^-, SO_4^{2-}, ANC and DOC) representing landcover, soils, geology and water chemistry datasets. However, the output results showed no relationship between macroinvertebrates and the environmental variables ($p > 0.05$). A second analysis was then conducted on twelve water chemistry variables (pH, Na^+, NH_4^+; K^+, Mg^{2+}; Ca^{2+},NO_3^-, Cl^-, SO_4^{2-}, F^-, ANC and DOC). The ordination results indicated that the first axis explained 32.9 % of variance while the second and third axis explained 14.0 % and 10.2 % of variance respectively and together, they accounted for 57.0 % of cumulative percentage. The [pH] ($r = 0.83$), [Na^+] ($r = 0.463$), [Mg^{2+}] ($r = 0.48$), [Ca^{2+}] ($r = 0.47$), [Cl^-] ($r = 0.46$), [F^-] ($r = 0.58$), [ANC] ($r = 0.54$) and [DOC] ($r = - 0.47$) explained most of the variation on axis 2, whereas Na^+ ($r = - 0.495$), K^+ ($r = - 0.479$), [Mg^{2+}] ($r = - 0.424$), [NO_3^-] ($r = - 0.546$), [Cl^-] ($r = - 0.515$) and [SO_4^{2-}] ($r = - 0.365$) explained most of the variation on axis 3. *Polypedilum sp1*, *Simulium edwardsellum damnosum*, *Tokunagaia sp.* and *Simulium nevermannia nigritarse* corresponded with DOC concentrations while the [K^+] was closely related to *Balinskycercella sp.* The ANC and pH corresponded to *Adenophlebia auriculata* and *Cheleocloeon excisum* species, while *Pseudocloeon glaucum*, *Afroptilum sudafricanum* and *Polypedilum sp7* were all closely associated with Na^+, Mg^{2+}; Ca^{2+}, Cl^-, SO_4^{2-} and F^- concentrations. The Eigenvalue (0.550) for the first axis is higher than the predicted mean (0.220) from the Monte Carlo randomisation test $p = 0.0140$ implying a statistically significant relationship between species data and environmental variables.

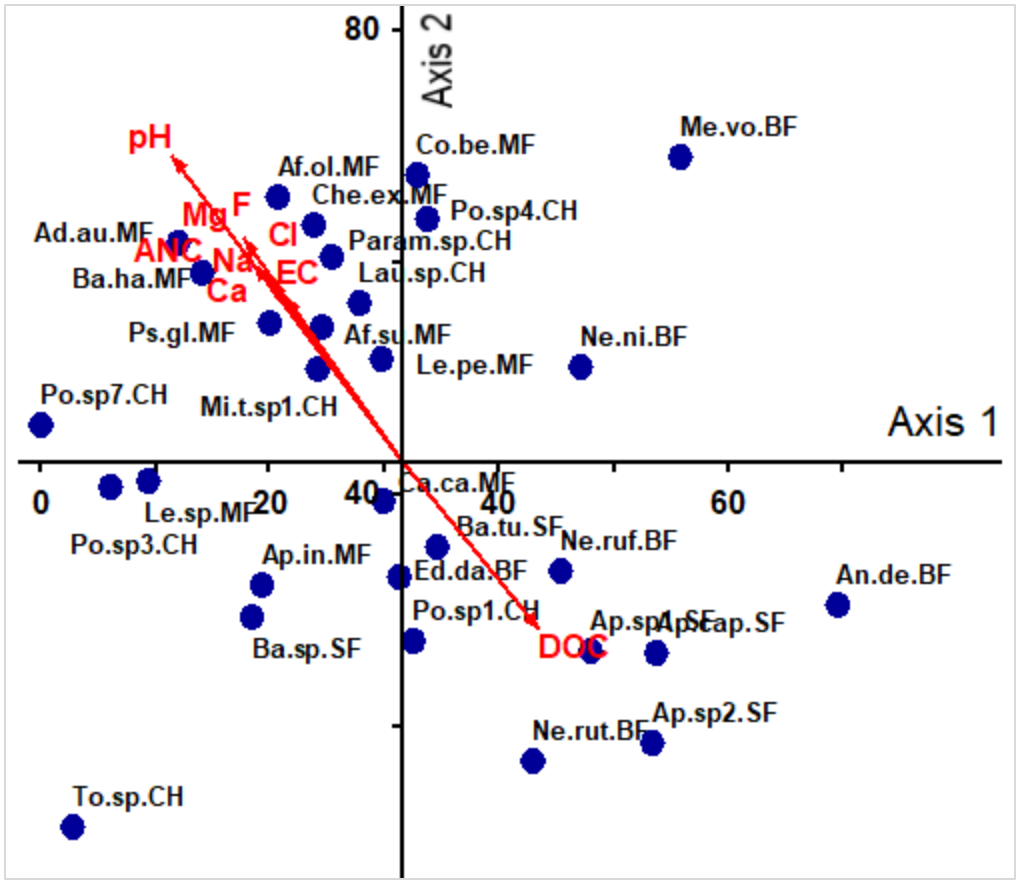


Figure 7-5: Canonical Correspondence Analysis (CCA) showing correspondence between species data and the water chemistry. Axis scores are weighted averages of species scores. These scores have been centered and standardized to unit variance and the axes scaled to optimize representation of species.

7.3 Key findings

Understanding water chemistry is key to understanding the compositions of aquatic invertebrate species assemblages. The water chemistry for the SWC is very complicated however this chapter set out to highlight key characteristics for these headwater streams. Based on the findings from this study and other publications by various authors (Allanson *et al.*, 1990; Midgley & Schafer, 1992; Dallas & Day, 2004; Harrison, 1962; King, 1988), streams from the SWC are characterised by low base cations, low ANC, high marine salt concentrations and fluctuating DOC concentrations. Overall analysis in this chapter varied considerably however potential indicator species identified in the previous chapters remain ideal candidates because of their distribution across an ANC range and their presence in both black and white headwater streams.

Chapter 8 The Waterberg region

8.1 Flow regimes

Flow regimes tend to vary across seasons and are dependent on rainfall events and the percolation rate of groundwater that replenishes the stream. Seasonal and permanent streams generally share the same hydrological characteristics throughout the wet season and are only distinguishable from each other during prolonged droughts where the former ceases to flow (Hughes, 2000). Key characteristics of water bodies in drought-stricken regions include low stream flow, high water temperatures, high EC and low DO concentrations (Churchel & Batzer, 2006; Reznickova *et al.*, 2007; Boulton, 2003; Humphries & Baldwin, 2003; Lake, 2003). Due to the large number of sites that were impacted by the drought, streams from the Waterberg were chosen to represent what appeared to be permanent, seasonal and ephemeral streams. The stream classification was based purely on the observations made in this study which were superimposed on literature based characteristics for such waterbodies (Hughes, 2000; Lake, 2011; Churchel & Batzer, 2006). Streams that flowed continuously for the duration of the project (i.e. WB05, WB06 and WB14) were classified as perennial, while those that went dry at the peak of the drought (November 2015) and recovered at the first rainfall event (February 2016) were designated as seasonal streams (i.e. WB12, WB16 and WB32A). Streams that remained dry after the rain events were classified as ephemeral streams (i.e. WB22 and WB25). Ephemeral streams tend to respond to major storm events (Hughes, 2000). There was no major storm even during the course of this project and thus none of the ephemeral streams identified in this study flowed for the remainder of the study. Theoretically no major differences were expected in the chemistry data across these categories however, we anticipated a difference in species composition as studies have shown that perennial and non-perennial streams tend to differ in species composition (Churchel & Batzer, 2006). To test the theory of no significant difference between environmental variables the Kruskall-Wallis test was conducted. The results revealed no statistical difference for all the environmental variables (Temperature, DO, EC, pH, Na^+, NH_4^+; K^+, Mg^{2+}; Ca^{2+},NO_3^-, Cl^-, SO_4^{2-}, F^-, DOC, ANC and PO_4^{3-}) tested ($p >$

0.05) except for Cl^- which was significantly lower in the seasonal streams ($p < 0.05$) than in perennial and ephemeral streams.

The Kruskal-Wallis test was also conducted on the June 2015 to October 2016 sampling seasons to test for seasonal variation of selected environmental variables across sampling sites. The results showed that the temperature, DO, NH_4^-, PO_4^{-3}, ANC and DOC concentrations (**Figure 8-1**) varied considerably across seasons. The temperature was highest in the summer (Feb-2016) and during the drought period (Nov-2015). The Aug/Sept-2015 and Oct-2016 campaigns were not macroinvertebrate sampling seasons but were included in this section of the work to establish a general pattern of disparity for the environmental variables. Temperatures would be naturally low in June because it is in winter. However, overall increases in the temperature corresponded to a decrease in dissolved oxygen. NH_4^- and PO_4^{-3} concentrations were highest during the June 2015 sampling period. The ANC concentrations were highest in November 2015 during the peak of the drought where more than half of the sampling sites had dried out. Statistically significant ($p < 0.05$) changes in DOC concentrations were detected over the June 2015, November 2015 and February 2016 sampling seasons. Correlation analysis gave an overall low correlation ($r \leq 0.65$) among variables, where the DOC was positively correlated to the ANC ($r = 0.58$) and NH_4^- ($r = 0.65$). A positive correlation was also observed between DO and NH_4^- ($r = 0.60$) as well as with the former and PO_4^{-3} ($r = 0.54$).

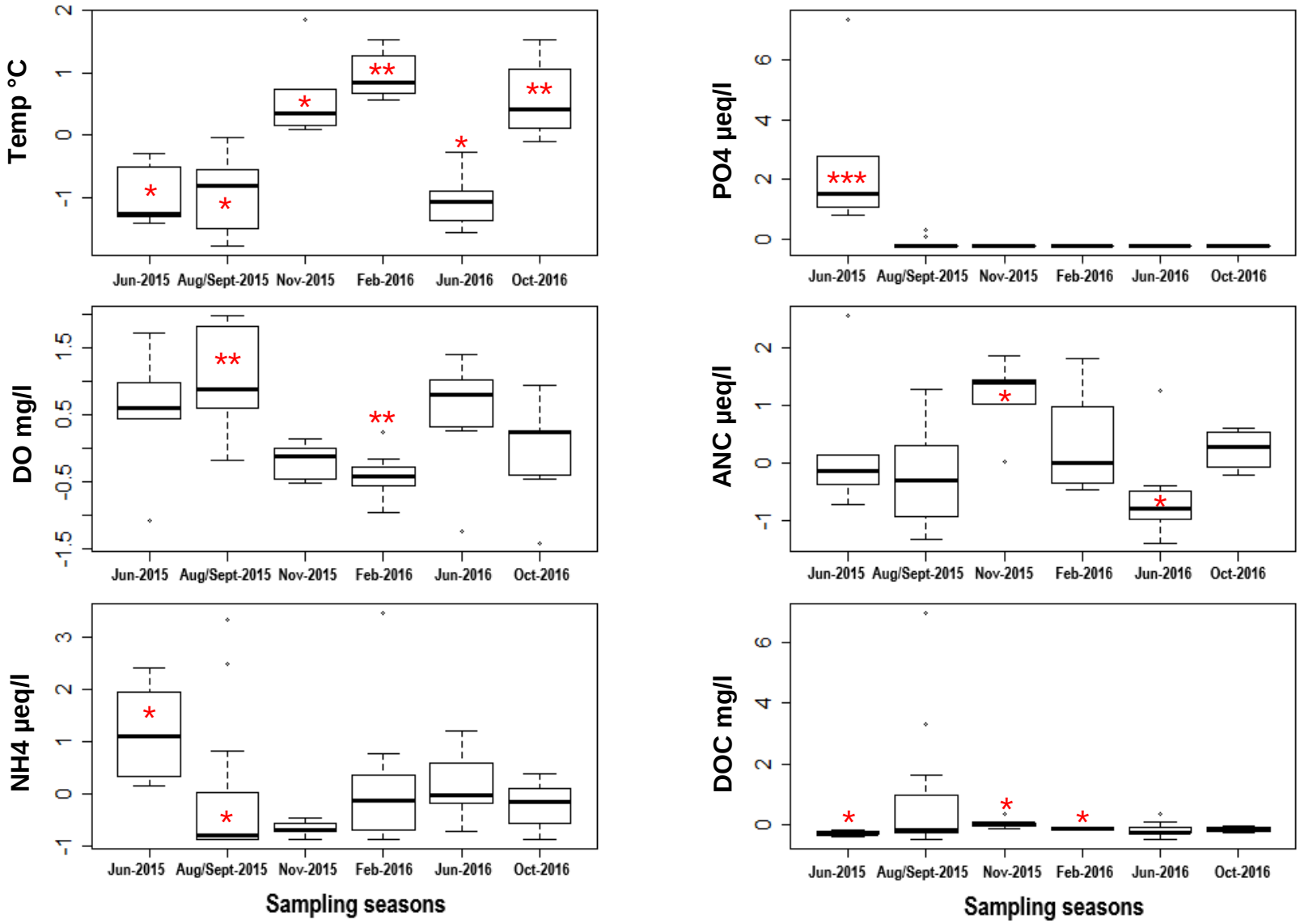


Figure 8-1: Seasonal variation of abiotic factors. Only variables that showed statistical significance are reported on. The asterisk indicates level of significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

8.2 Assessing species composition across stream categories and sampling seasons.

Previous publications (Straka *et al.*, 2019; von Schiller *et al.*, 2016; Stubbington *et al.*, 2016) have shown that perennial and non-perennial streams tend to house different species. For this study the ISA test was conducted on the perennial, seasonal and ephemeral streams to establish which species were unique to each category and which were common to all three categories. The results showed that chironomids were the dominant taxa across all stream categories (**Table 8-1**), while species that had a more ubiquitous distribution displayed a level of species variation. Column five (**Table 8-1**) is mostly dominated by species that are common to perennial and seasonal streams. Only one species was common to perennial and ephemeral streams. The latter contained a further two species, *Cloeon unknown sp*. (Cl.un.sp.MF) and *Simulium metomphalus chutteri* (Me.ch.BF) that were also found in seasonal streams. *Cloeon unknown sp*. and *Simulium metomphalus chutteri* are able to evade desiccation by persisting in temporary pools (Churchel & Batzer, 2006; Barber-James, 2004b), either burying their eggs in sediment or by diapausing (Palmer & Moor, 1998; Churchel & Batzer, 2006) and thus tend to recolonise rapidly following dry conditions. This seems to be the case based on the observations made in this study (**Figure 8-2**), where the abundance of both *Cloeon unknown sp*. and *Simulium metomphalus chutteri* more than doubled after rainfall events. These response patterns do not only suggest that the macroinvertebrates in this region have become well adapted to these environmental conditions but also that some of the streams in the Waterberg dry out by fragmentation where a stream will have puddles and no flow. β-diversity was highest for perennial streams (β-diversity = 3.0) and lowest (β-diversity = 1.0) for the species common to all stream categories. Ephemeral streams showed a slightly higher variation in species composition (β-diversity = 2.0) across study sites than seasonal streams (β-diversity = 1.7), highlighting the conservation value of these intermittent streams.

Table 8-1: List of species recorded in perennial (P), seasonal (S) and ephemeral (E) streams of the Waterberg that were categorised according to the observations made in this study. β-diversity for each group of species is given on the last row of the Table in bold, where perennial streams had overall high β-diversity of 3.0 while of the two nonperennial stream categories the ephemeral streams had a higher β-diversity at 2.0. The species highlighted in red represent indicator species that were identified in section **6.7** (**Figure 6-8**) of the thesis. Many of these are found across all stream categories or in perennial streams.

Perennial (P)	Seasonal (S)	Ephemeral (E)	Common species (P, S, E)	Other
Che.ex.MF	Ps.gl.MF	Ba.un.sp2.MF	Da.in.MF	Cl.sp.MF (P & S)
Ca.sp2.MF	Ps.pi.MF	De.un.sp.MF	Co.be.MF	Ca.sp1.MF (P & S)
Ps.la.MF	Bu.ma.MF	Co.sp2.CH	Cr.fl.MF	Ca.sp3.MF (P & S)
Af.ol.MF	Ba.sp.CH	Parac.sp.CH	Ba.un.sp1.MF	Af.su.MF (P & S)
De.ca.MF	Ch.us.sp2.CH		Eu.sp.MF	Ba.ha.MF (P & S)
Ca.ca.MF	Ch.us.sp3.CH		Ps.un.sp2.MF	An.de.BF (P & S)
Ac.va.MF	Cryp.sp.CH		Ps.ma.MF	Ne.ni.BF (P & S)
Ad.bi.MF	Po.sp1.CH		Che.fa.MF	Me.ad.BF (P & S)
Me.vo.BF	Po.sp7.CH		De.cr.MF	Po.sp3.CH (P & S)
Po.un.BF	St.sp.CH		Ne.sp.SF	Rh.sp2.CH (P & S)
Ch.Sp5.CH	Ps.sp4.CH		Ed.da.BF	Ch.us.sp1.CH (P & E)
Cl.sp2.CH	Pr.sp.CH		Ne.rut.BF	Cl.un.sp.MF (S & E)
Po.sp4.CH			Lau.sp.CH	Me.ch.BF (S & E)
Ta.sp2.CH			Param.sp.CH	
Co.sp1.CH			To.sp.CH	
Ge.sp1.CH				
Ge.sp2.CH				
He.sp.CH				
Or.sp.CH				
Or.sp1.CH				
Or.sp2.CH				
Or.sp4.CH				
Parak.sp.CH				
Ps.sp3.CH				
Cl.sp.CH				
Me.sp.CH				
Rh.sp.CH				
3.0	**1.7**	**2.0**	**1.0**	**2.4**

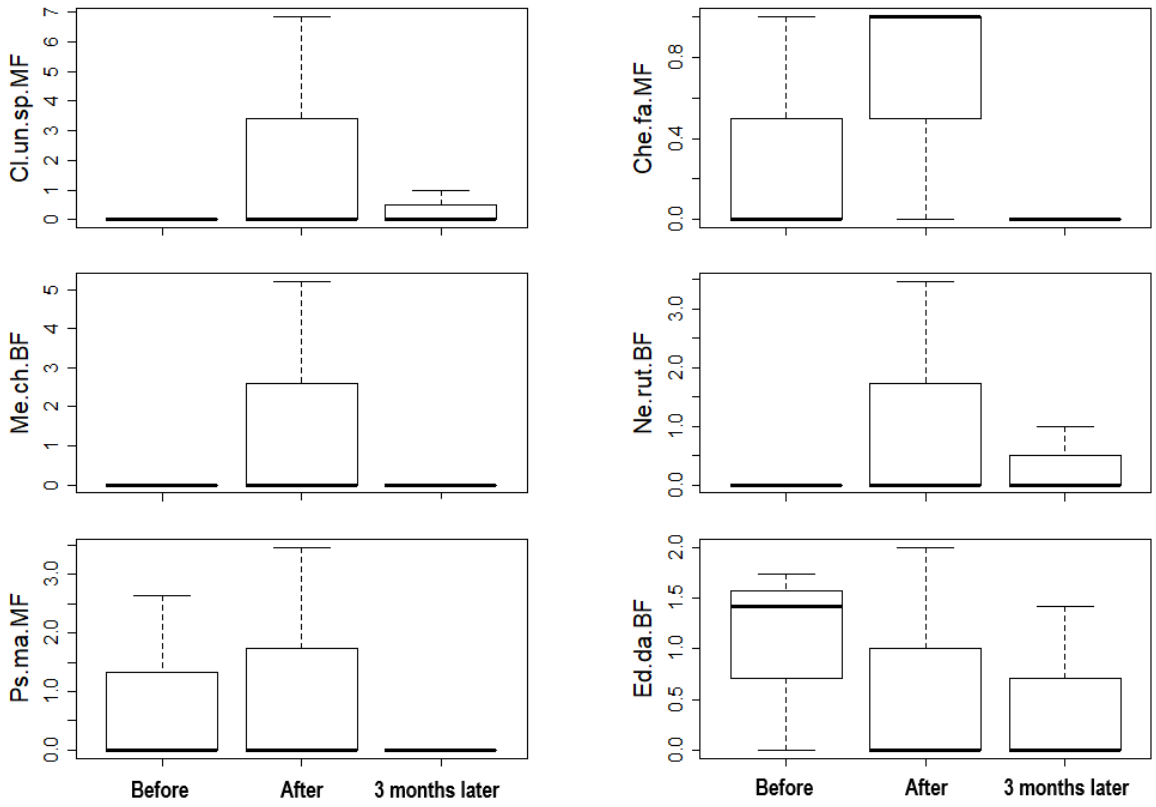


Figure 8-2: Boxplot representation of species recovery rate from the drought.
The results presented here are from WB12, WB16 and WB32A study sites that had undergone the November 2015 drought. Macroinvertebrates were collected 3 months (June 2015) before the drought set in. At this stage species abundance varied considerably where some species had overall low species count and others with slightly higher abundances. By November 2015 these streams were completely dry. Macroinvertebrates were sampled two weeks after a brief rainfall event (Feb 2016) and species number for Cl.un.sp.MF, Me.ch.BF and Ne.rut.BF had increased dramatically. However, by June 2016 (3 months later) these species abundances had dropped back down towards the initial numbers. Statistical significance was not tested on this dataset due to the large skewness in the data that is represented by the heavily one-sided whiskers and the small sample size (3 sites).

8.3 Key findings

Of the sixteen sites from the Waterberg, nine were considered perennial, four seasonal and three were ephemeral. This section of the work was aimed highlighting the key distinguishing features of streams from the Waterberg compared to those from the Mpumalanga and SWC. Three stream types that carry unique fauna have been identified. Species composition between the intermittent and perennial streams differed greatly. These differences highlighted the need to identify indicator species that are able to withstand all these different streams conditions and not species that are associated with just one type of waterbody. Species richness was much lower in the ephemeral streams.

A total of 71 species consisting of mayfly (n = 28), chironomid (n = 34), stonefly (n = 1) and blackfly (n = 8) immatures were recorded in the Waterberg. Of these, 27 species were restricted to perennial streams, 12 to seasonal and 4 to ephemeral streams (**Table 8-1**). The remaining 28 species consisted of 13 taxa that were common to just two of the three stream categories and 15 which were common to all three categories. Indicator species identified in section **6.7** (**Figure 6-8**) were mostly recorded across all three stream categories or in at least two (perennial and seasonal) of the three. Similar observations were made when species composition was compared across sampling seasons. Where all the indicator species identified in section **6.7** (**Figure 6-8**) were common to all sampling seasons. These observations highlight the importance of understanding flow regions and their influence on species presence/absence especially when working towards identifying indicators for monitoring stream conditions. Ideally indicator species should be ubiquitous or at least be found in perennial streams.

Two species, namely *Cloeon unknown sp*. and *Simulium metomphalus chutteri*, were recorded exclusively in non-perennial (seasonal and ephemeral) streams. *Cloeon* species, (Barber-James, 2015) are mainly associated with temporary pools or ponds, while *Simulium metomphalus chutteri* is known to bury its eggs in sediment, or to undergo diapause during unfavourable conditions and recolonise at the first sign of rain, implying

that streams from the Waterberg dry by fragmentation where only some parts of the stream dry out while some regions along the streambed remain as pools.

Table 8-2: List of species that were restricted to the respective sampling seasons and those that were common across all sampling seasons. The species highlighted in red represent indicator species that were identified in section **6.7** (**Figure 6-8**) of the thesis.

Jun-2015	Aug/Sept-2015	Feb-2016	Jun-2016	Recorded throughout the seasons - common
Che.ex.MF	Me.sp.CH	Ca.sp2.MF	De.ca.MF	Da.in.MF
Ps.pi.MF		Ps.gl.MF	Ca.ca.MF	Cl.sp.MF
Af.ol.MF		Bu.ma.MF	An.de.BF	Ca.sp1.MF
Ba.un.sp2.MF		Ac.va.MF	Po.un.BF	Ca.sp3.MF
De.un.sp.MF		Ba.sp.CH	Ch.Sp5.CH	Eu.sp.MF
Ad.bi.MF		Ch.us.sp2.CH	St.sp.CH	Ps.un.sp2.MF
Ta.sp2.CH		Ch.us.sp3.CH	Co.sp1.CH	Ps.ma.MF
Co.sp2.CH		Cryp.sp.CH	Ge.sp2.CH	Che.fa.MF
Parac.sp.CH		Po.sp1.CH	Or.sp4.CH	Lau.sp.CH
Parak.sp.CH		Po.sp4.CH	Cl.sp.CH	Param.sp.CH
		Ge.sp1.CH	Rh.sp.CH	
		Ps.sp4.CH		
		Pr.sp.CH		

Chapter 9 Discussion

The present study has focused on pollutants that originate from a regional source, which at the national scale may be considered a point source, i.e. coalfired power stations. Point source pollutants are known for their non-uniform distribution in that the concentration levels tend to vary at different points of the system be it the atmosphere or a water body, suggesting that the degree of mixing is unknown and thus a preliminary survey is necessary to determine sampling sites (Hewitt & Allott, 1999). There are thousands of streams in South Africa and trying to sample each one would have not been feasible; therefore, a decision was made to focus on three of the potentially most sensitive regions in South Africa, namely the Mpumalanga, Waterberg and south-western Cape study regions. As a starting point, national scale spatial datasets has been used to identify acid sensitive soils in South Africa, in conjunction with previous studies demonstrating the presence of naturally acidic (and hence acid sensitive) streams in the south-western Cape. Using the GIS approach, the site selection process was reduced to just regions which have been identified as having acid sensitive soils and presumably acid sensitive waters. After making contact with the lead author we found that data from the Josipovic *et al.* (2011) paper were unavailable for use in the present study. Thus, in order to construct maps similar to those of Josipovic *et al.* (2011) which show the distribution of acid sensitive soils across South Africa, soils data had to be obtained from the global and national Soil and Terrain (SOTER) digital database. This database however had an incomplete soil chemistry dataset. Therefore, other classification methods had to be employed in order to map the spatial distribution of acid sensitive soils (Section 3.1.1). Once the soils maps were constructed, they were then used to locate potentially acid sensitive waters. For the south-western Cape however the distribution of naturally acidic streams rather than acid sensitive soils was used to identify sampling sites.

9.1 Study site profiling

Mountain streams are thought to be pristine, unpolluted ecosystems because of their remote location and for this reason they have been used as early-warning indicators of changes in the climate and atmospheric chemical composition (Rogora *et al.*, 2013). In this PhD, sample collection was focused on headwater streams. Many potential study sites however found in and around acid sensitive soils were impacted by the drought, mining or agricultural activity and thus site identification in many parts of the Waterberg and Mpumalanga regions respectively had to be moved outside of areas mapped to have acid sensitive soils. Overall, very few Mpumalanga sites were located in areas that were not impacted by agricultural or other human activity. Furthermore, due to unforeseen circumstances as detailed in Appendix 5, the majority of the macroinvertebrate data from this region were missing corresponding field chemistry data for two sampling seasons (Appendix 5). Having a majority of the sites impacted by agricultural activity together with the missing field chemistry data made the Mpumalanga region unsuitable for further analysis beyond what has been presented in chapter 5 and 6 of the thesis, which give a general overview of the water chemistry and macroinvertebrate data respectively for the three study regions.

In contrast to the Mpumalanga region, a majority of the sites in the Waterberg were located on game farms and nature reserves and represented a wide range of natural to semi natural aquatic ecosystems with undisturbed catchments. However, the drought that corresponded with our sampling seasons made it difficult to identify sampling sites found in and around acid sensitive soils with no other confounding factors or variables that may obscure the effects related to acid deposition.

Several studies have shown that atmospheric pollution is a major problem in areas where coalfired power stations are concentrated. However, evidence of possible long range transportation of pollutants meant that the study sites had to be extended beyond the region where the coalfired power stations are concentrated. This led to us focusing also on the south-western Cape which has naturally acidic waters. One may argue that

streams from the south-western Cape are very different from those in the northern parts of South Africa and may question our inclusion of the south-western Cape. However, the south-western Cape was intended to serve as a background for deposition, especially since previous studies have shown that it has acid sensitive waters with a low buffering ability against strong acids. Thus, what the present study intended to do was to identify species that occur in the south-western Cape as well as in other parts of the country. Based on our literature search there has been no ecotoxicity work anywhere in the country that has looked at the effects of stream acidity on macroinvertebrates anywhere in South Africa.

A study by Oliff (1963) however has associated some mayfly species (*Caenis sp. 3* and *Euthraulus elegans*) and one undescribed chironomid to slightly acidic and or circumneutral stream conditions of pH 5.2 – 6.8 (Oliff, 1963; Oliff & King, 1964). However other studies prior to that (Harrison & Agnew, 1962; Harrison, 1962) and several that followed tended to give a general overview of species tolerance (Allanson *et al.*, 1990; de Moor & Day, 2013). The present study however has attempted to define potential pH tolerance ranges for selected species. Furthermore, to factor out all confounding factors and variables the study had to include a large number of sites that cover key national spatial gradients (i.e. in deposition) and that included sites assumed to be impacted and unimpacted by acidification. However, fundamentally the problem faced by the present study was finding suitable study regions that had high deposition, sensitive soils and geology and no other landuse. The Mpumalanga region would have been ideal had much of the region not been subject to intensive agriculture and other activities such as mining.

Water samples and macroinvertebrate data were collected quarterly and bi-seasonally respectively, across the Mpumalanga, Waterberg and the south-western Cape study regions. The scarcity of literature related to stream acidification in South Africa meant that assumptions around the reference conditions, site selection as well as results interpretation be based on observations from other parts of the world.

9.2 Water chemistry data

Salinisation is said to be a common feature amongst arid regions and South Africa is no exception (Mensah *et al.*, 2015). South African freshwaters are generally associated with high salinity that is primarily attributed to sea salts at the coastal regions. Other instigators include saline soils, irrigation, removal of natural vegetation and overall low rain fall and high evaporation rates during which some major ions (i.e. Ca^{2+} and SO_4^{2-}) are known to crystalize out of solution while Na^+ and Cl^- stay in solution (de Moor & Day, 2013; Dallas & Day, 2004; King & Pienaar, 2011; Kefford *et al.*, 2005). Huizenga *et al.* (2011) indicated that South African waters consist of streams that drain catchment geologies with high weathering rates, some with chloride salinisation and others with high sulphur concentrations. In a different study the same authors also demonstrate that the Mpumalanga and Limpopo catchment areas are dominated by slow weathering minerals while those from the coastal regions have high chloride and sulphate concentrations (Huizenga *et al.*, 2013). The observations made by these authors are consistent with those from this study where the south-western Cape was shown to have high sodium, chloride and sulphate concentrations that are not attributed to acid deposition.

CART analysis suggests significant differences between the study regions that are best explained by the differences in stream pH and conductivity. Streams from the Waterberg had significantly lower conductivity than those from the south-western Cape and Mpumalanga regions. The south-western Cape and Mpumalanga were best distinguished by the difference in pH where the south-western Cape streams had significantly lower stream pH concentrations when compared to those from the Mpumalanga region. The Waterberg seems to be the least impacted by acidification followed by the south-western Cape however this can only be verified through applying critical loads which are the best way to distinguish natural from anthropogenic acidity. The Mpumalanga region had the highest concentration of acidic compounds however this region also had the highest concentration of basic ions matched by an overall high ANC concentration, when compared to the sample streams in the other two provinces. The lower DOC values generally observed for the Mpumalanga and the Waterberg regions can primarily be

attributed to the lack of riparian vegetation from these regions, while high DOC concentrations for the south-western Cape were attributed to the fynbos vegetation which has been shown (Midgley & Schafer, 1992) to be linked to highly coloured leachates.

Nitrogen concentrations suggest evidence of some degree of N Saturation across all study regions, though local background data are absent.

9.3 Macroinvertebrate data

When working with ecological data, species diversity, richness and evenness are important terms to understand as each one represents an important ecological aspect. All three are presented here and several conclusions about this dataset may be deduced from observing these features individually. For instance, variation in beta diversity across study regions highlights a need for region specific studies and biological conservation efforts. Whittaker (1972) suggests that a low species diversity is indicative of disturbed environments. McCune *et al.* (2002) considers beta diversity values that are < 1 as low and those > 5 as high. Following this logic, it can be concluded that none of the study regions from this project exhibited disturbed environmental conditions based on the beta diversity values obtained after observing Ephemeroptera, Plecoptera, Simuliidae and Chironomidae data. Here beta diversity assumes that all the variables that could influence species presence/absence for these taxonomic groups, including how species relate to each other, have been considered and that the present compositional heterogeneity is a true reflection of the landscape or hyperspace that these species occupy. This is important for this particular group of aquatic insects as they tend to give valuable information about habitat integrity and here beta-diversity is telling us just how much information we can get out of them i.e. data quality and also the current state of the environment at each region. Acidified waters are generally recognised by shifts in community structure where acid sensitive taxa are replaced by acid tolerant species (Schartau *et al.*, 2008). Based on the recovery patterns of taxon in the Waterberg after the drought it is possible that species diversity and distribution for the Waterberg is a reflection of species adaptation to prolonged drought periods that are characteristic for

the region. These observations were supported by comparing species composition from the late-autumn 2015 and summer 2016 (**Figure 8-2**) where the latter was dominated by *Cloeon* species that are primarily associated with temporary pools (Barber-James, 2015). The species recovery rate shown by the Waterberg streams suggests that streams from this region are prone to seasonal droughts which has resulted in species adaptation to these harsh environmental conditions by utilising refugia. This adaptation strategy is characterised by the high level of species resistance and resilience against adverse conditions. However, these tentative observations can only be confirmed by subsequent studies of species composition pre- and post-drought as well as by observing species recovery patterns.

Most Chironomidae are able to survive a wide range of harsh environmental conditions including acidic conditions, however based on the observations made in this study some chironomids appear to be less tolerant to acidic conditions than some stonefly and mayfly species recorded here. *Pseudocloeon glaucum* is found throughout Southern Africa and in some parts of East Africa and possesses wide ecological tolerance (Lugo-Ortiz *et al.*, 2000), a property that is evident in the species wide tolerance of changes in pH.

The tendency of adult stoneflies to remain in one locality (i.e. very limited dispersal abilities) together with their habitat preferences make many of them narrowly endemic, a trait consistent with the observations made in this study where Perlidae and Notonemouridae species were restricted geographically (**Table 6-3**), with the Perlidae occurring at higher altitudes.

The Simuliidae are grouped into two types of pest species, the mammalian pest species which include *Simulium metomphalus chutteri* and *Simulium edwardsellum damnosum*, and the avian pest species which consist of *Simulium meilloniellum adersi* and *Simulium nevermannia nigritarse*. Of the four pest species, *Simulium metomphalus chutteri* is considered the most troublesome and has caused significant damage along the Orange River. All four pest species known to South Africa were detected in the samples collected during the course of the project.

Of the four species of Teloganodidae described in the south-western Cape (de Moor & Day, 2013), *Lestagella penicillata* was the only one sampled throughout the course of this study at 84 % (n = 21) of the sites from this region. This particular species also showed the highest abundance of all species sampled in this project.

9.4 Indicator species

There are a variety of factors that need to be considered when relating species presence/absence to a set of environmental variables and these require in depth knowledge of the study region (i.e. the influence of ecoregions, seasonality, common and uncommon species distribution patterns), including the advantages and disadvantages of selected sampling techniques and choice of biotope. For an effective indicator species both impacted and unimpacted ecosystems should be analysed so as to get a better idea of where particular taxa or taxon is likely to occur or be absent. One way in which to achieve this is by collecting a vast number of samples representing some spatial gradients which is what we have attempted to do with this PhD study as a national survey was not possible. In this study we have attempted to identify indicator species for acidification. Many of the species that could survive extremely acidic conditions of pH < 4 could survive a wide range of changes in pH and thus were not suitable for the purposes of this study. The identification of indicator species is based on the assumption that each taxon is said to have optimum conditions at which it functions best (i.e. environmental niche), and once these conditions move towards being less favourable species number and diversity tend to decline and in some cases species extinction may occur depending on the severity of the problem. Furthermore, indicator species must possess well-defined ecological tolerance (specialists and not generalists) to abiotic factors such as flow rate preferences, temperature, pH etc. Species however that can tolerate a wide range of environmental conditions do not make good ecological indicators. Countries previously impacted by acidification have developed acidification scoring systems that give warnings of possible stream acidification by monitoring presence/absence of indicator species. Characteristics of acidified waters include a decline in species abundance along with an increase of acid tolerant taxa such as stoneflies and a decline or absence of sensitive

taxa such as mayfly species. For example, publications from Europe and North America have shown that loss of bass, crayfish and mayfly species tend to occur at pH 5.5 while snails and frogs generally disappear at pH 6 and 4 respectively.

This section of the work was set to establish an empirical relationship between the aquatic fauna and measured environmental variables linked to acidification. This is an essential first step to evaluating the feasibility of developing a national scoring system for acidification that will encompass enormously diverse ecosystems such as those presented in this study. Species composition responds to water chemistry and too much variation in the water chemistry across study regions would imply notable variation in species composition as well. With the species data we asked what are the key factors driving species composition and what is their potential impact on our ability to develop a scoring system or identify indicator species on a national or regional scale? Both water chemistry and species composition varied considerably across the study regions and these differences did not justify continuing with a collective analysis of the three regions. Other confounding factors other than chemistry, included climatic gradients (summer vs winter or year-round rainfall zones, droughts in the Waterberg), altitude gradients (temperature), biomes (forest vs grassland, fynbos) and indeed landuse (i.e. agriculture and mining in otherwise sensitive areas of the Highveld) led to major challenges in identifying national scale indicators. Thus, in chapters 7 and 8 the south-western Cape and the Waterberg were reviewed separately focusing mostly on the key features that distinguish the two regions from each other. Prior to reaching this decision several potential indicator species had been identified (**Table 6-10**) however much work still needs to go into fully developing a suitable acidification scoring system. Moderately to fully tolerant species were a challenge to define on a regional and national scale as species that showed tolerance to these conditions also showed tolerance to a wide range of pH concentrations. Furthermore, species from the south-western Cape are naturally adapted to acid conditions and thus laboratory experiments would probably be more ideal in defining such groups. However, the results presented in this thesis have set the foundations for such work.

The majority of acidification indices are based on species presence/absence and not necessarily on the number of observed sensitive species, thus they require only one accurately identified species representing a specified tolerance level to classify the site as being impacted or non-impacted. For this study the ANC critical limit could not be determined for the national dataset due to the lack of suitable indicators.

9.5 Study limitations

Many of the difficulties experienced during the course of this study were related to species identifications. All Chironomidae taxa could only be identified to tribe or genus level with certainty. According to experts at the Albany Museum and Rhodes University, Trichoptera, Chironomidae and Simuliidae larvae require that sampled organisms be reared to adult forms for accurate taxonomic identification. For this reason, some Trichoptera, Chironomidae and Simuliidae larvae were not readily identifiable. In the case of Simuliidae however the pupal stage is important for accurate species identification. In addition to the above the size of sampling net may have compromised sampling of smaller macroinvertebrates such as chironomids that are less than 1 mm in size.

Another major limitation to this PhD study was the lack of comprehensive species level data. This is a global problem however the techniques that are currently applied in South Africa (i.e. Family level identifications) further exacerbate this problem. On the surface there seems to be a large amount of macroinvertebrate work taking place but in actual fact the techniques applied create huge limitations to this and future studies if taxonomic identification is left at family level. Furthermore, such identification is of very limited value in biodiversity and conservation studies.

This point counts as both a limitation and recommendation for future work. There is a large amount of knowledge that exists in the form of unpublished reports and some that can only be acquired through conversation with experts in the field. Equally so there is a major gap in the literature that exists not because the work has not been done but because it was never published, or projects were left incomplete. A concerted and

coordinated effort needs to be made to pull together all this research in order to fill some of the gaps in the literature. All these reports should ideally be archived at one institution (i.e. The South African Environmental Observation Network (SAEON), the Albany museum or the Water Research Commission (WRC)) and should be a starting point for anyone conducting research to avoid repetition or overlap and to build comprehensive repositories that follow chronological order. Furthermore, nongovernmental organisations conducting similar research (i.e. water and invertebrate related) should thus be required to share any of their research findings with the relevant institutes. In addition to this, private institutes should also be requested to run their project proposals through the relevant institutes to allow record keeping and for a more structured approach to maintain research archives and species repositories and databases. As much as it is the researchers' job to pull together reports, in the case of species data, species repositories and historical records will go a long way in helping the researcher along in addressing relevant questions that will contribute towards building comprehensive repositories in the future. The limiting factor here is that information is mostly scattered. However, we do acknowledge and appreciate efforts from our local scientists (i.e. Dallas HF, Janssens MP and Day JA) who have made strides towards correcting these setbacks, yet there is still some work be done. Setting standards (i.e. an ideal taxonomic level for species identification and standard environmental variables that must be measured) may prove useful in building and maintaining consistency in the type of information provided on the respective databases.

Last but not least we recognise that a key limitation for this study is that it is not a laboratory based testing of species tolerances but rather an empirical study. The lack of spatial data and water quality data to go alongside species distribution information is essential but would ideally be corroborated by ecotoxicity and lab based studies. Furthermore, we collected as much seasonal water chemistry quality as possible, but we acknowledge that species tolerances can only be established through experimental laboratory setups.

Chapter 10 Conclusions

Environmental gradients including soil, geology, climate and biome variation across the study regions was reflected in both the chemistry data and species composition. The streams sampled from the respective study regions fall into three distinct categories, where the Mpumalanga is dominated by circumneutral to alkaline streams with most catchments subject to agricultural or other human related activities, while the south-western Cape and Waterberg are dominated by acid as well as acid to circumneutral streams respectively with a small measure of overlap in some of the Waterberg and Mpumalanga sites. The large number of endemic taxa and the presence of rare and undescribed species did not favour the development of a national bioassessment index for acidification. However the regional indicator species identified in section **6.7** (**Figure 6-8**) appear to be good candidates as the majority of them were found in all the stream types for the respective regions (i.e. perennial streams for the Waterberg and both black and white headwater streams for the south-western Cape). Nonetheless more work still needs to be done to fully establish these indices. The present study however has laid the foundations to facilitate such work through identifying rare and specialised species that can be ruled out as useful indicator species.

More work needs to go into defining acid tolerant and intolerant species for South Africa and the key challenge will be to try and identify species that are solely restricted to the acid conditions. The Waterberg appears to be home to many sensitive species, but because of the drought that took place during the course of this study, it was difficult to assess the factors affecting species presence/absence. The sparseness in community data and water chemistry may have reflected the effect of the drought rather than the actual species and chemistry composition. However, a large number of undescribed species were found in the Waterberg and this can be primarily attributed to the area being understudied. Rather than to use these results to make inferences about the larger population it might be worthwhile to rather use these findings to guide future work. Furthermore, the setbacks encountered during the course of this study did not hinder the novelty of the findings from this study. Separately the study regions gave invaluable

information concerning local species composition, highlighting the importance of small-scale conservation efforts rather than regional attempts. In addition to this much of the research conducted on freshwater insects normally leads to family level species identification. With the present study however species identification was carried out to the lowest possible taxonomic level.

Streams from the Mpumalanga region are well buffered against acidification, whether naturally or through agricultural activities, and this is evident in the high acid neutralising capacity. However, this does not nullify the adverse effect of the coalfired power stations on other regions with less buffered soils through long range transport of atmospheric pollutants.

Important empirical data on the distributions of a large number of species across pH gradients have been collected, providing useful suggestions for candidate indicator species which could be further tested through laboratory studies. Some river FEPAs and Fish support Areas have acid-sensitive soils, making them vulnerable to acid deposition. Furthermore, in regions of higher deposition it is entirely possible that distributions of certain macroinvertebrate species may have been impacted – including possible impacts or perhaps even loss of species not yet identified. Taking into account the paucity of monitoring data and the lack of species repositories for South African macroinvertebrate data, all findings from this study are invaluable.

One of the key hypotheses set forth by the present study stated that the current levels of acid deposition in some parts of South Africa exceed the critical load for acidification of acid sensitive freshwaters. As it stands the present study was not able to test this hypothesis as no apparent relationship could be established between the acid deposition data which was analysed under a separate work package (Mompati *et al*. (2019), article in submission) and the stream chemistry data from this PhD study. Mompati *et al*. (2019) did conclude however that the annual Volume-Weighted Mean (VWM) concentrations of sulphate (SO_4^{2-}) and nitrate (NO_3^-) were highest at sites closest to the coalfired power stations. Based on the deposition data produced by Josipovic *et al.* (2011), we had also

hypothesised that there is a deposition gradient that exists where the Mpumalanga Highveld appears to have the highest deposition rates followed by the Waterberg and KwaZulu-Natal. These assumptions will be presented better in forthcoming papers that will investigate the effects of acid deposition on stream chemistry, which is another essential step in the development of locally relevant critical loads or similar acidification models. The present study however has focused entirely on collecting empirical data to develop the biological components and progress towards a nationally or regionally relevant ANClimit or pH threshold value for selected taxa. From the soils maps we were able to show that some regions of South Africa do have acid sensitive soils. Very low ANC values were recorded in the south-western Cape and parts of the Waterberg indicating that streams from these two regions have very little buffering ability against strong acids and thus are vulnerable to acidification. The challenge with the Mpumalanga region however was identifying catchments lacking major human disturbances. Nonetheless very high mean ANC concentration were recorded for the Mpumalanga region (mean = 914 µeq/L) suggesting that the Mpumalanga region has a high acid neutralising capacity against strong acids from the atmosphere. Based on the low ANC values the south-western Cape (mean ANC = 30 µeq/L) appears to be the most vulnerable to acid deposition. However, based on the findings from Mompati *et al*. (2019), deposition is very low in this region. Furthermore, very high DOC concentrations which leads to low pH, would afford some protection against acidification by strong acid deposition with some buffering and complexation with toxic Aluminium. Therefore, further acidification of the naturally acid streams of the south-western Cape is unlikely for the foreseeable future.

10.1 Recommendations and future research

1. One of the key aims of this study was to develop a national scoring system for immature aquatic insects in acidic streams. This however was not achieved partly because of the variation in species composition across study regions as well as due to the drought experienced in the Waterberg. However, since this was the first study of this nature in South Africa, there was not much groundwork set in place for developing a scoring system. For instance, we had initially hypothesised that the HV would have the most acidic streams based on the location of the coal fired power stations and the meteorological data. However, this was not the case as streams from this region are well buffered, with ANC > 200 µeq/l, which may be due at least partly to intensive agricultural and mining activities in the region. Here we recommend a follow up study where macroinvertebrates will be collected once a month for one year. The aim for such sampling frequency would be to collect as much invert data as possible that is reflective of a variety of changes in the water chemistry that can be related to species data.

2. As many as the sites sampled in this project for the Mpumalanga region showed signs of being well buffered it might be worthwhile to extend sampling to other parts of this region and increase efforts to identify sites without catchment disturbances through more intensive GIS screening of land use.

3. The samples that were collected from the Waterberg and Mpumalanga regions were dominated by rare and sparsely distributed mayfly species namely, *Demoulinia crassi*, *Pseudocloeon piscis*, *Pseudopannota maculosa* and *Crassabwa flava*. DNA barcoding is a technique that has recently been adopted by entomologists for building species specific databases. For future work we propose that DNA barcoding be performed on some of the species collected in this study. The present study only analysed selected species groups, however, many other species were collected and sorted but not analysed. This data would be sufficient for a master's project and possibly two honours projects. Considering

the variable nature of the South African climate it might be worthwhile to try and develop a scoring system for acidification in a controlled environment such as a laboratory.

4. When working with macroinvertebrate species it might be useful to invest in a sonicator to dislodge silt from silt covered organisms. Furthermore, it is important to use 90 or 95 % alcohol for species preservation as lower concentrations may be diluted down by water from the sample, which may lead to the accumulation of bacteria around the specimen that may distort species identification and lead to loss of material as was the case with some of the samples from this study.

5. Even though it appears as though there isn't an acidification problem in South African streams, the atmospheric deposition data are a cause for concern and therefore it will be worthwhile to incorporate stream water acidification into the South African stream monitoring programme for long term monitoring, as most studies have indicated that lack of understanding on the subject is mainly due to the lack of historical data. This could be achieved by focusing initially on key selected streams and broadened with time.

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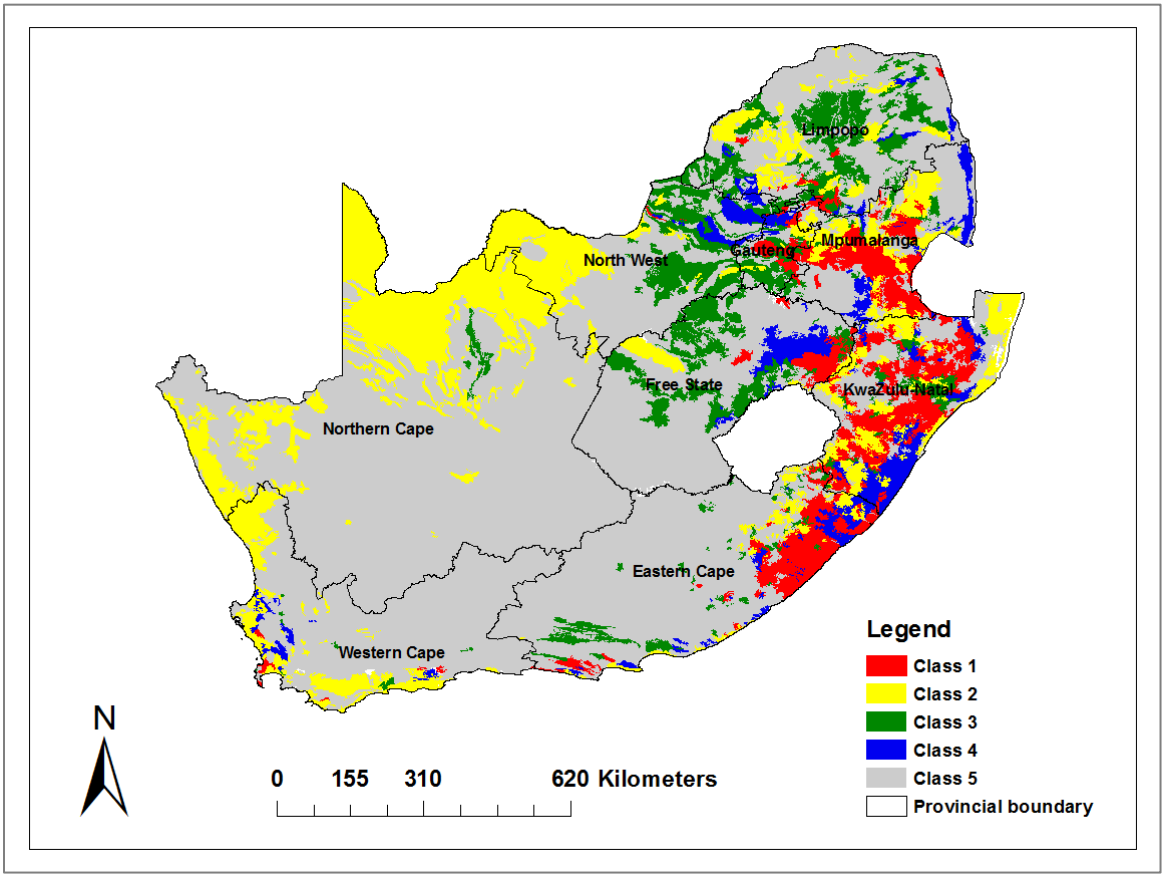
Appendices

Appendix 1: Soil classification using Cinderby *et al.* (1998) assigned classes

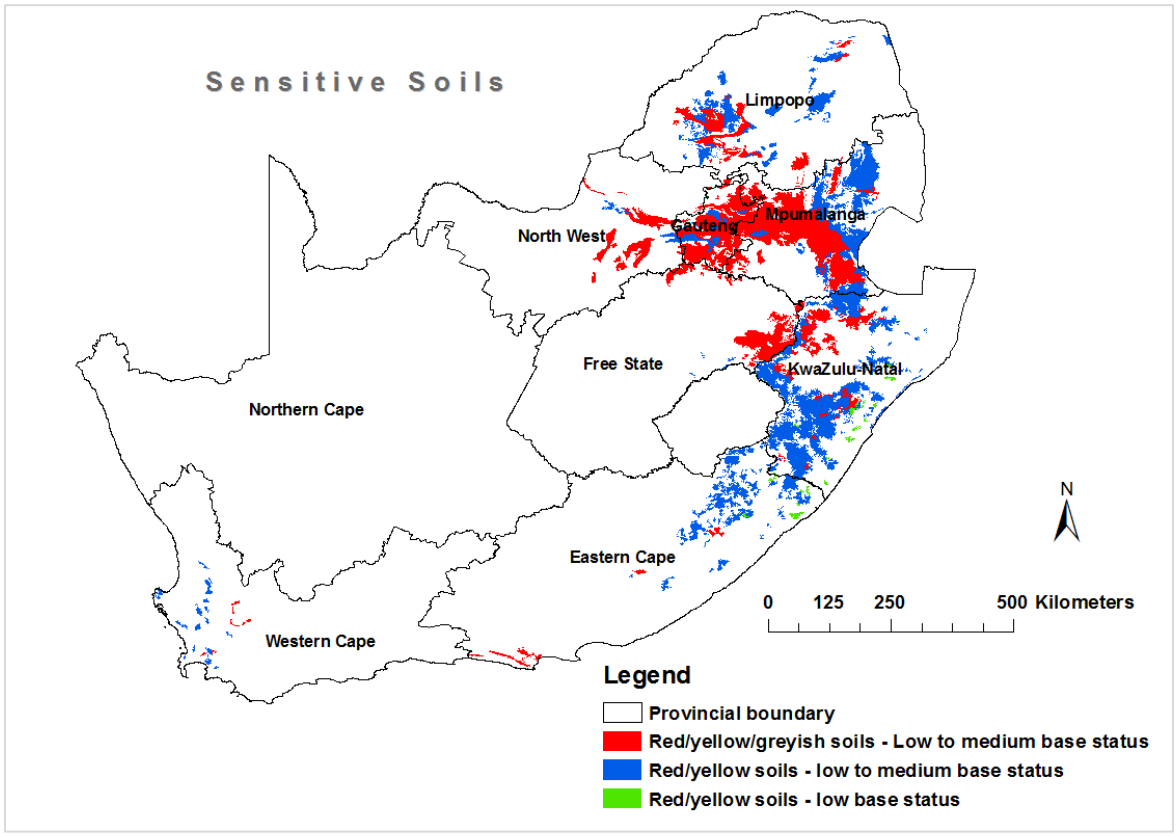
The Cinderby *et al.* (1998) classification process (reviewed in chapter 3 section **3.1.1 a)** resulted in the classification of the world's soils. During which soils found throughout the world were listed and designated classes 1-5. Using this list of soils and their respective classes, South African soils were classified by simply assigning sensitivity classes as given by Cinderby *et al.* (1998) to soils found on the SORTER database (**Appendices Figure 1**). As with the other maps produced from previous studies and the one produced using classification methods applied during the present study, acid sensitive soils were mostly concentrated in the eastern part of the country (**Appendices Figure 1**).



Appendices Figure 1: Class 1 – 5 of South African soils classified using sensitivity values as assigned by Cinderby et al. (1998).

Appendix 2: Generalised soils map generated from the ARC dataset

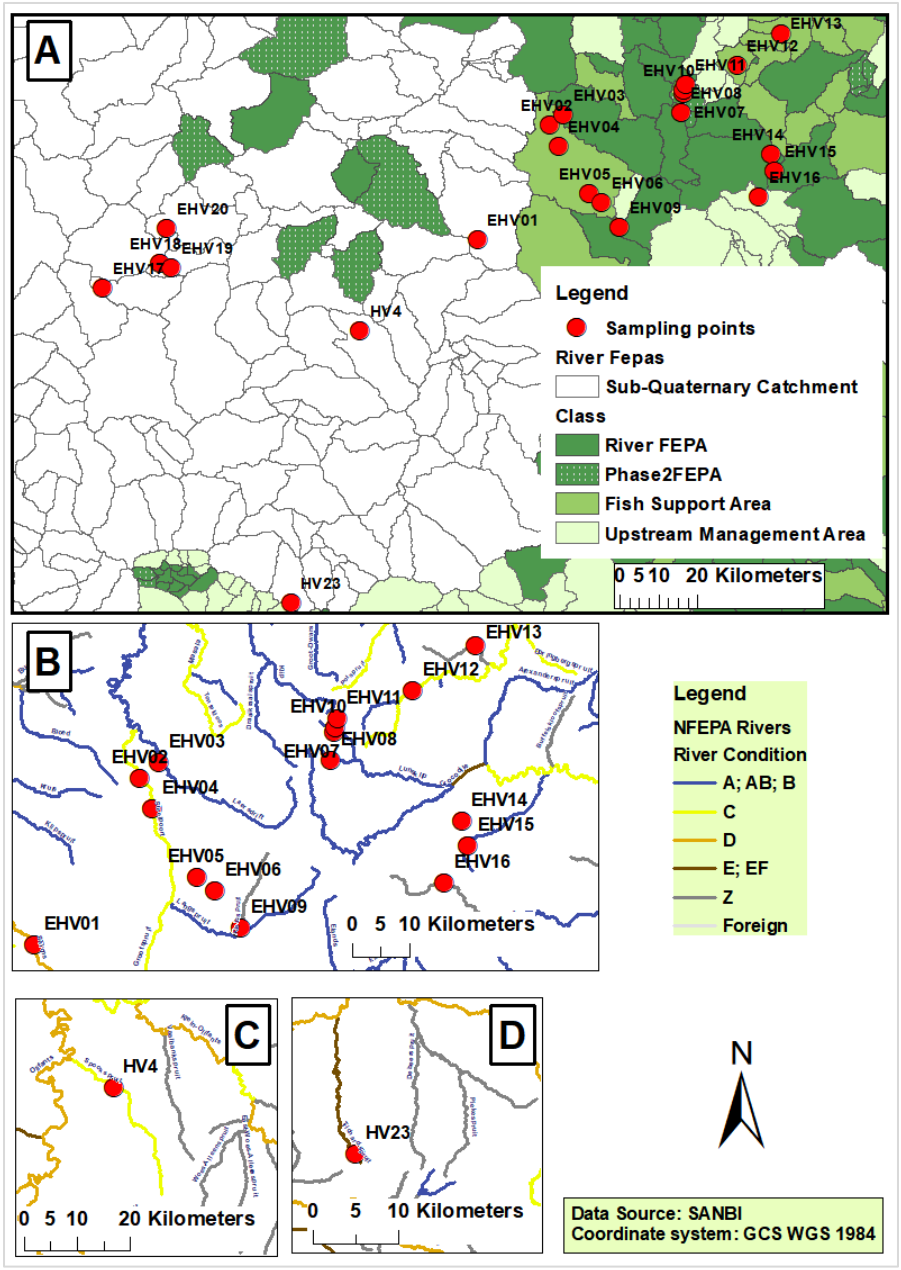
An AGIS (Agriculture Geo-referenced information system) comprehensive Atlas of South Africa with generalised soil patterns obtained from the Agricultural Research Council (ARC) website was used to construct a map of acid sensitive soils in South Africa. For their classification the ARC used soil information obtained from the national land type survey published in 1974 and 1977 (ARC). From this dataset, 28 broad soil patterns were identified and classified into 19 generalised soil patterns according to their pedogenesis and land use capability. The 19 classes were grouped further into 9 soil types based on the soil texture and base cation content. The most acid sensitive of these nine soil types were used to generate an acid sensitive soils map (**Appendices Figure**).



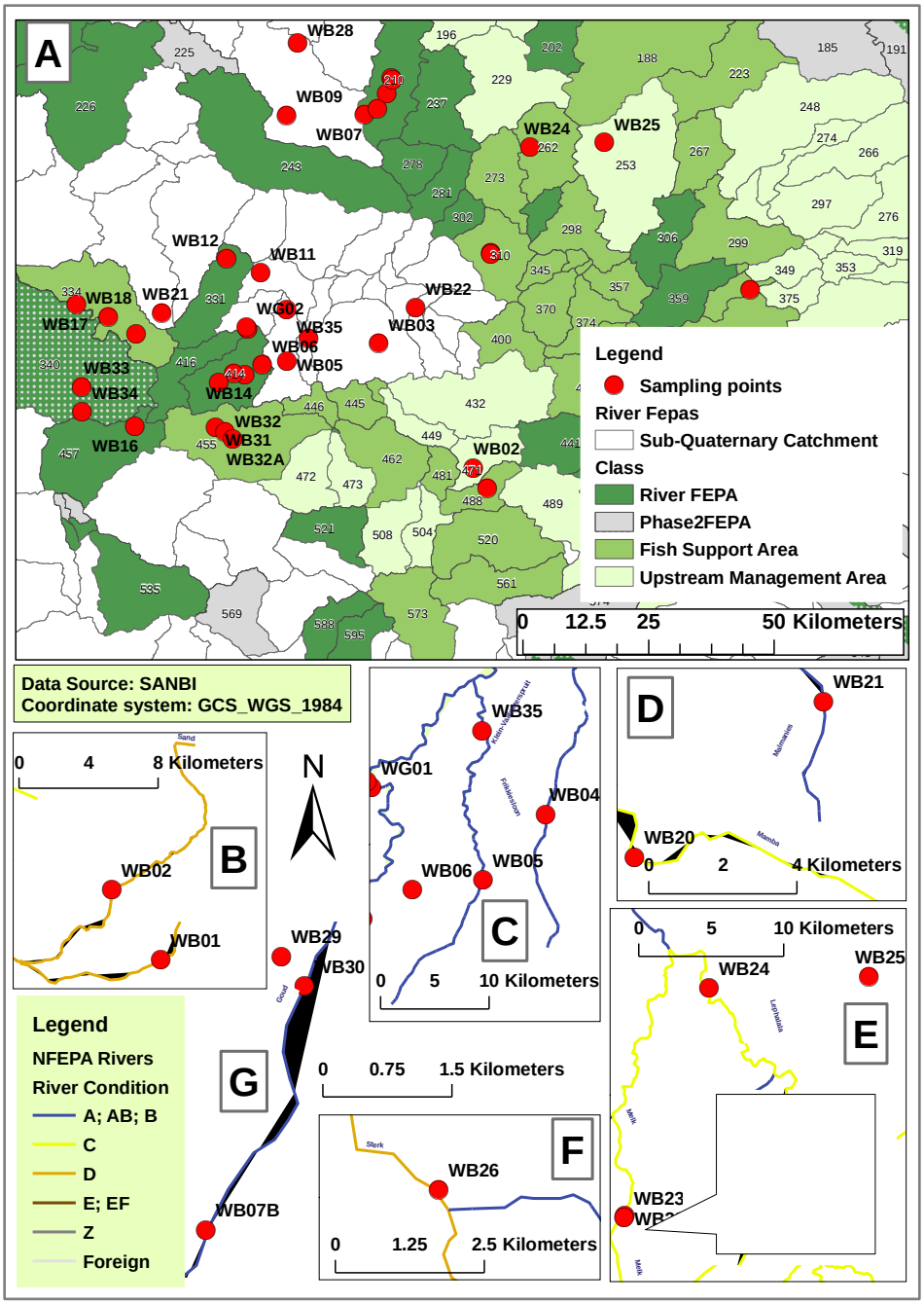
Appendices Figure 2: Map showing the distribution of soils with low to medium base status over South Africa. Although this classification approach was relatively different to those reviewed and applied during the present study, the acid sensitive soils distribution patterns were quite similar.

Appendix 3: Representation of the study sites on FEPA maps

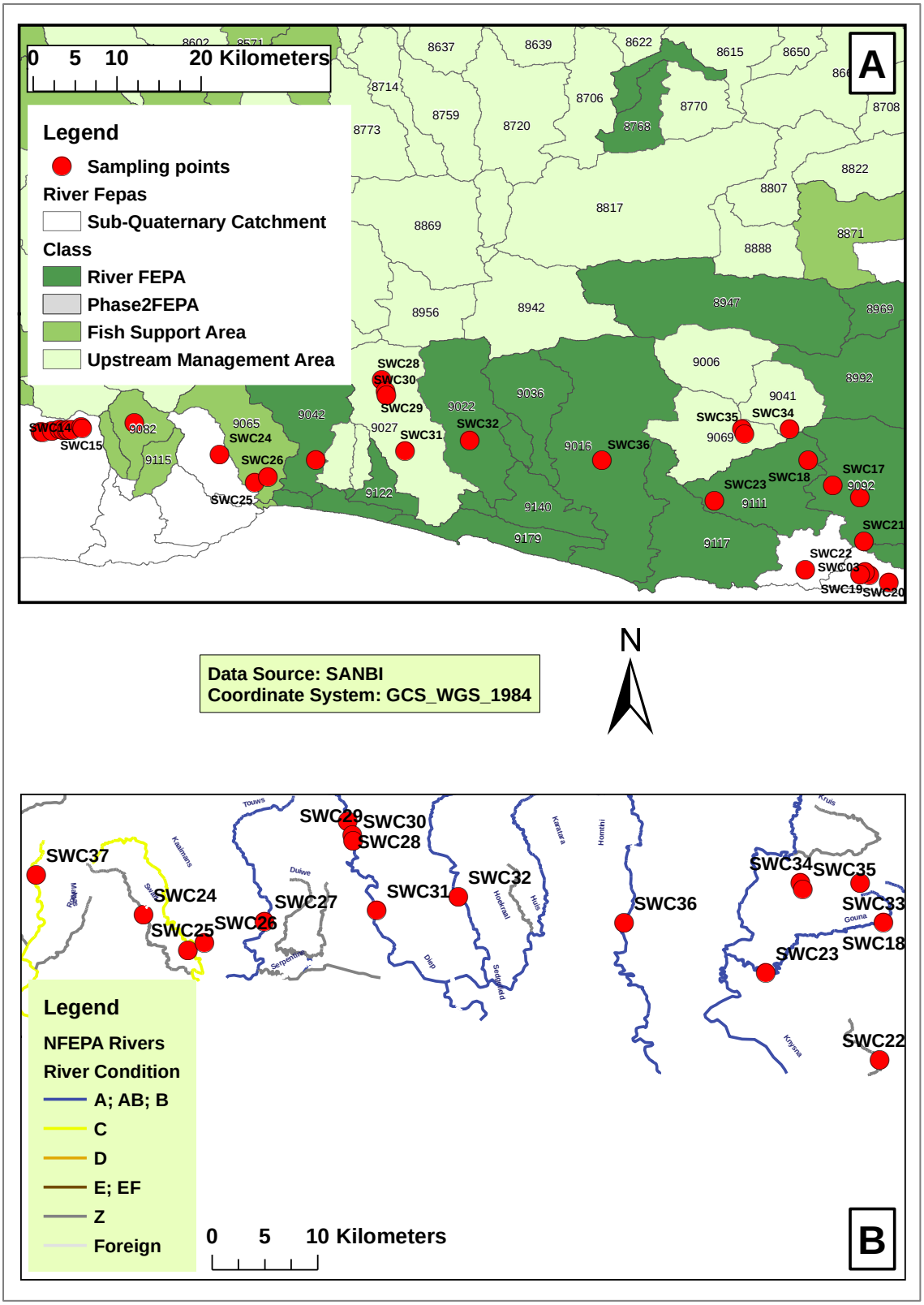
In an effort to gauge the conservational value of the sampling sites sampling points were plotted on FEPA maps (**Appendices Figure 2** to **Appendices Figure 4**) and majority of the Study sites including those from the highly impacted Mpumalanga region, were found in and around FEPA regions.



Appendices Figure 2: The position of the HV sampling sites relative to river FEPAs. **A** – River FEPAs, Fish Support Areas and Upstream Management Areas with their associated sub-quaternary catchments. **B** – NFEPA project stream conditions of selected EHV sites. **C-D** – Stream conditions for two HV sites located on the Highveld Plateau.



Appendices Figure 3: The position of the WB sampling sites relative to river FEPAs. **A** – River FEPAs, Fish Support Areas and Upstream Management Areas with their associated sub-quaternary catchments. **B-G** – Stream conditions of selected WB sites.



Appendices Figure 4: The position of the SWC sampling sites relative to river FEPAs. The SWC was dominated by streams in good ecological conditions.

Appendix 4: Complete list of sites sampled during the study

Appendices Table 1: Complete list of sites. Green - invert sites. Blue - sites where only water samples were collected. Red - sites excluded from the overall project due to habitat unsuitability, site modifications or drought that occurred during the course of the study. English translations for some of the stream names are given in quotation marks.

ID	Region	Site Code	Name	Latitude	Longitude	Altitude (m)
1	Highveld	HV1	Selons River	-25.646849	29.669429	1600
2	Highveld	HV4	Spookspruit	-25.858629	29.397260	1483
3	Highveld	HV6	Klein Olifants River	-26.147253	29.752932	1630
4	Highveld	HV7	Woestalleenspruit	-25.930499	29.618806	1561
5	Highveld	HV8	Coetzerspruit	-25.915454	29.606304	1574
6	Highveld	HV9	Kleinspruit	-25.619944	29.998575	1834
7	Highveld	HV10	Klipspruit	-25.767825	29.121031	1429
8	Highveld	HV12	Steelpoort River	-25.380667	29.838614	1447
9	Highveld	HV13	Laersdrift Spruit	-25.356438	29.867502	1461
10	Highveld	HV14	Keeromspruit	-25.707020	29.456762	1416
11	Highveld	HV17	Klippoortjiespruit	-26.104008	29.108467	1541
12	Highveld	HV18	Bronkhorstspruit	-26.226240	28.816077	1579
13	Highveld	HV19	Rietspruit	-26.279892	29.091633	1593
14	Highveld	HV20	Piekespruit	-26.462578	29.352302	1599
15	Highveld	HV21	Viskuile	-26.462578	29.352302	1599
16	Highveld	HV22	Olifants River	-26.221828	29.630608	1614
17	Highveld	HV23	Trichardspruit	-26.488469	29.237099	1609
18	Highveld	HV24	Steenkoolspruit	-26.331760	29.337994	1568
19	Highveld	HV25	Kromdraaispruit	-26.246478	28.937917	1560
20	Highveld	HV26	Wilge Spruit	-26.388239	28.868158	1669
21	Highveld	HV28	Dwars-in-die-Weg Spruit	-26.358239	29.130432	1575
22	Highveld	HV29	Koffiespruit	-26.091074	28.606399	1549
23	Highveld	HV30	Elands River	-25.792742	28.523168	1507
24	Highveld	EHV01	Selonsrivier "Oleander River"	-25.646700	29.669417	1600
25	Highveld	EHV02	Steelpoort River	-25.382350	29.837633	1442
26	Highveld	EHV03	Laersdriftspruit	-25.357650	29.867383	1461
27	Highveld	EHV04	Steelpoort River	-25.431983	29.856517	1464
28	Highveld	EHV05	Unnamed Stream	-25.540267	29.928100	1579
29	Highveld	EHV06	Unnamed Stream	-25.560931	29.956597	1688
30	Highveld	EHV07	Lunskliprivier	-25.310653	30.146625	2077
31	Highveld	EHV08	Gemsbokspruit	-25.354697	30.140533	2026
32	Highveld	EHV09	Kleinspruit "Little Stream"	-25.619950	29.998583	1833
33	Highveld	EHV10	Unnamed Stream	-25.302833	30.147417	2135
34	Highveld	EHV11	Unnamed Stream	-25.288314	30.150783	2131
35	Highveld	EHV12	Dorpsrivier "Town's River"	-25.243336	30.270508	1623
36	Highveld	EHV13	Hoppe-se-Spruit "Hoppe's Stream"	-25.172394	30.371717	1555
37	Highveld	EHV14	Unnamed Stream	-25.450236	30.348681	1314
38	Highveld	EHV15	Buffelskloofspruit "Buffalo Gorge Stream"	-25.489903	30.357856	1530
39	Highveld	EHV16	Swartkoppiespruit "Black Hill Stream"	-25.549572	30.321347	1595
40	Highveld	EHV17	Unnamed Stream	-25.760042	28.801475	1412
41	Highveld	EHV18	Unnamed Stream	-25.702620	28.934010	1390
42	Highveld	EHV19	Unnamed Stream	-25.711823	28.960703	1326
43	Highveld	EHV20	Unnamed Stream	-25.622865	28.949124	1282
44	Waterberg	WB01	Unnamed Stream (Modimolle R33)	-24.5941	28.32816	1371
45	Waterberg	WB02	Sand River (Modimolle R33)	-24.55784	28.30291	1366

46	Waterberg	WB03	Unnamed Stream (Modimolle R33)	-24.33503	28.13356	1174
47	Waterberg	WB04	Klein-Vaalwaterspruit (Bakkerspas)	-24.3264	28.00811	1301
48	Waterberg	WB05	Frikkiesloon (Bakkerspas)	-24.36704	27.96916	1317
49	Waterberg	WB06	Unnamed Stream (Bakkerspas)	-24.3731	27.92506	1386
50	Waterberg	WB07	Unnamed Stream (N of Vaalwater)	-23.92552	28.10809	1262
51	Waterberg	WB07B	Goud River (N of Vaalwater)	-23.88863	28.14829	1146
52	Waterberg	WB07C	Unnamed Stream (N of Vaalwater)	-23.91462	28.13099	1197
53	Waterberg	WB08	Unnamed Stream (East of Lephalale)	-23.7126	28.11732	904
54	Waterberg	WB09	Unnamed Stream (Lephalale-Vaalwater)	-23.92708	27.96854	1100
55	Waterberg	WB10	Unnamed Stream (East of Lephalale)	-23.76953	27.98989	1031
56	Waterberg	WB11	Unnamed Stream (NW of Vaalwater R517)	-24.2085	27.92246	1075
57	Waterberg	WB12	Taaibosspruit (NW of Vaalwater R517)	-24.18336	27.8612	1015
58	Waterberg	WB13	Unnamed Stream (Bakkerspas)	-24.39118	27.89438	1374
59	Waterberg	WB14	Unnamed Stream (Bakkerspas)	-24.38895	27.87451	1397
60	Waterberg	WB15	Unnamed Stream (Bakkerspas)	-24.48467	27.81714	1417
61	Waterberg	WB16	Sondags River (Bakkerspas-Marakele road)	-24.48333	27.69739	1325
62	Waterberg	WB17	Unnamed Stream (Drains Marakele)	-24.2651	27.59267	1052
63	Waterberg	WB18	Unnamed Stream (Drains Marakele)	-24.28768	27.64985	1177
64	Waterberg	WB19	Unnamed Stream (Drains Marakele)	-24.32584	27.6941	1282
65	Waterberg	WB20	Unnamed Stream (Drains Marakele)	-24.31864	27.69922	1260
66	Waterberg	WB21	Malmanies River (Drains Marakele)	-24.28068	27.74528	1315
67	Waterberg	WB22	Jim se Loop River (NE of Vaalwater)	-24.27186	28.19981	1220
68	Waterberg	WB23	Melk River (NE of Vaalwater)	-24.1779	28.37403	1374
69	Waterberg	WB23A	Unnamed (African Explorer, NE of Vaalwater)	-24.17285	28.33482	1335
70	Waterberg	WB23B	Unnamed (African Explorer, NE of Vaalwater)	-24.1746	28.33431	1330
71	Waterberg	WB24	Lephalala River (NE of Vaalwater)	-23.98421	28.40468	1156
72	Waterberg	WB25	Mokopane River	-23.97516	28.53716	1073
73	Waterberg	WB26	Sterk River (Mokopane)	-24.23869	28.79875	1139
74	Waterberg	WB27	Unnamed Stream (Bakkerspas)	-24.40524	27.84774	1474
75	Waterberg	WB28	Unnamed Stream (East of Lephalale)	-23.79766	27.9885	1076
76	Waterberg	WB29	Unnamed Stream (N of Vaalwater)	-23.86006	28.15617	1116
77	Waterberg	WB30	Goud River (N of Vaalwater)	-23.86315	28.15858	1111
78	Waterberg	WB31	Unnamed Stream (Rankins Pass)	-24.48573	27.84122	1368
79	Waterberg	WB32	Unnamed Stream (Rankins Pass)	-24.49362	27.8585	1365
80	Waterberg	WB32A	Unnamed Stream (Rankins Pass)	-24.50649	27.87245	1278
81	Waterberg	WB33	Unnamed Stream (Marakele National Park (NP))	-24.413	27.6021	1271
82	Waterberg	WB34	Unnamed Stream (Marakele NP)	-24.457372	27.602236	1586
83	Waterberg	WB35	Frikkiesloon (Welgevonden East Gate road)	-24.27458	27.96859	1135
84	Waterberg	WG01	Grootfonteinspruit (Welgevonden Game Reserve)	-24.3097	27.89966	1197
85	Waterberg	WG02	Unnamed Stream (Welgevonden Game Reserve)	-24.3058	27.89698	1180
86	SW Cape	SWC01	Harkerville	-34.07834	23.22738	187
87	SW Cape	SWC03	Harkerville	-34.07066	23.20661	202
88	SW Cape	SWC04	George	-33.91671	22.31796	436
89	SW Cape	SWC05	George	-33.91765	22.32042	430
90	SW Cape	SWC06	George	-33.91615	22.32998	422
91	SW Cape	SWC07	George	-33.91431	22.33789	434
92	SW Cape	SWC08	George	-33.91529	22.34197	444
93	SW Cape	SWC09	George	-33.91520	22.34570	444
94	SW Cape	SWC10	George	-33.91467	22.34768	446
95	SW Cape	SWC11	George	-33.91498	22.35096	459
96	SW Cape	SWC12	George	-33.91475	22.35269	464
97	SW Cape	SWC13	George	-33.91489	22.35636	454
98	SW Cape	SWC14	George	-33.91174	22.36131	443
99	SW Cape	SWC15	George	-33.91337	22.36243	437

100	SW Cape	SWC16	Diepwalle	-33.98756	23.19597	186
101	SW Cape	SWC17	Diepwalle	-33.97445	23.16725	267
102	SW Cape	SWC18	Diepwalle	-33.94753	23.14084	361
103	SW Cape	SWC19	Harkerville	-34.06729	23.20152	207
104	SW Cape	SWC20	Harkerville	-34.07049	23.19643	182
105	SW Cape	SWC21	Harkerville	-34.0344	23.20046	248
106	SW Cape	SWC22	Pezula	-34.06491	23.1377	166
107	SW Cape	SWC23	Knysna	-33.99073	23.04033	60
108	SW Cape	SWC24	NMMU Campus	-33.9413	22.5097	212
109	SW Cape	SWC25	7 Passes	-33.97133	22.54741	55
110	SW Cape	SWC26	7 Passes	-33.96503	22.56159	142
111	SW Cape	SWC27	7 Passes	-33.94692	22.61294	125
112	SW Cape	SWC28	Bergplaas	-33.86143	22.68385	329
113	SW Cape	SWC29	Bergplaas	-33.87321	22.68785	301
114	SW Cape	SWC30	Bergplaas	-33.87810	22.68845	302
115	SW Cape	SWC31	7 Passes	-33.93743	22.70860	174
116	SW Cape	SWC32	7 Passes	-33.92578	22.77816	147
117	SW Cape	SWC33	Diepwalle	-33.91397	23.12092	506
118	SW Cape	SWC34	Diepwalle	-33.91391	23.06993	385
119	SW Cape	SWC35	Diepwalle	-33.91909	23.07232	342
120	SW Cape	SWC36	Homtini Pass	-33.94783	22.91945	26
121	SW Cape	SWC37	Montagu Pass	-33.9072	22.41823	330

Appendix 5: A review of viable chemistry data per sampling season across all study regions

A total of 9 sampling expeditions took place between Aug 2014 and Oct 2016. The project set out to identify 20 – 30 headwater streams per study region, an objective that was achieved by June 2015, making this at the time the first comprehensive dataset of the project going forward. Thus, samples collected between Aug 2014 and Feb 2015 were considered to be preliminary data. However, the drought and data cleaning process led to a huge reduction in viable data and the "preliminary data" became part of the official data to be used during final analysis. These changes offset the amount of comparable data across study region however it increased the amount of available chemistry data per site to accommodate statistical analysis. This section is aimed at reviewing the amount of data that was available per season after the data cleaning process. This information is essential as it influenced study approach and the type of techniques applied during analysis. All data cleaning processes were carried out after all sample collection had been concluded.

1) 2014 to 2015 sampling seasons

The amount of data available for comparative analysis across seasons and study regions was reduced drastically due to the large number of samples that failed the data cleaning process and on account of the drought. A total of 34 streams were sampled in the HV region during the Nov-Dec 2014 sampling season. Majority (n = 28) of these sites were impacted by agricultural activity and only six sites remained suitable to the purposes of this study. The impacted streams were excluded from the overall project. The remaining sites namely, EHV17, EHV18, EHV19, EHV20, HV23 and HV4 all passed the data cleaning process and consisted of corresponding field chemistry and major ions data (**Appendices Table 2**). More sites were added during the Jun 2015 sampling period which brought the number of HV sites to 22. However, after the data refining process only 21 sites remained for the overall project. Of the 21 sites EHV09, EHV13 and HV4 all failed the outlier test whereas EHV17 and HV23 passed the same test but were missing field

chemistry data (**Appendices Table 2**). During the Aug-Sept 2015 sampling season water samples were collected from 19 streams. Two sites, EHV17 and HV4 that would have brought the number of sampled sites to 21 during this season as well were dry. Nevertheless, all 19 sites were missing field chemistry data due to difficulties encountered during the course of the project that led to one member leaving the project and withholding field notes taken collectively while in the field. Of the 19 sites EHV04 and EHV18 failed the outlier test. None of the HV samples from this season could be used in comparative studies because of the missing field chemistry data. Furthermore, these samples were excluded from all seasonal and regional mean calculations (**Appendices Table 2**). As with the previous season samples collected during the Nov 2015 sampling period were missing field chemistry data. A few sites were impacted by the drought which brought the number of sampled sites down to 18. From the 18 sites HV4 was the only sample that failed the data quality check (**Appendices Table 2**).

Twenty-seven streams were sampled in the WB during the Aug-Sept 2014 sampling spree. After reviewing the data and conducting data quality checks only 20 sampling points remained (**Appendices Table 3**), of which 15 had corresponding field chemistry and major ion data. WB01 and WB02 were not sent for ion chromatography due to human error consequently these samples were missing major ions data. WB07B, WB12 and WB25 all failed the data quality assessment due to the presence of outliers and/ or obscure pH-ANC relationship. WB sites were revisited in Feb 2015 and new sites were added. This brought the number of sampling sites in this region to 24 (**Appendices Table 3**). Twenty of the 24 streams passed the data quality tests and from these only 18 sites had corresponding field chemistry and major ions data. Additional WB sites (WB31, WB32 and WB32A) were discovered during the Jun 2015 sampling period (**Appendices Table 3**). Of the 27 streams sampled over this period WB03, WB07B and WB25 all failed the outlier test. The remaining sites all had corresponding field chemistry and major ions data. At this point in the project a few streams from the WB had been excluded from the overall study due to seasonality or site modifications. Equally so new sites located inside the Marakele National Park were added to the project during the Aug-Sept 2015 sampling session (**Appendices Table 3**).

A total of 26 sites were sampled in this season and only two sites (WB01 and WB13) failed the outlier test. WB16 had missing data and the remaining sites all had corresponding chemistry data. The impact of the drought was most pronounced during the Nov 2015 sampling season (**Appendices Table 3**). A total of 13 sites were sampled. WB03 and WB14 failed the data quality check due to an ambiguous pH-ANC relationship and the presence of outliers.

Appendices Table 2: Seasonal summaries for the HV region. Y - samples that Passed the Data Cleaning Process (PDCP) and had corresponding field chemistry and major ions data, also referred to as corresponding chemistry data (CCD). N - samples that failed the data cleaning process and thus could not be used in comparative studies across seasons or regions.

Season	Nov-Dec 2014		Jun 2015		Aug-Sept 2015		Nov 2015	
Site code	PDCP	CCD	PDCP	CCD	PDCP	CCD	PDCP	CCD
EHV01	-	-	Y	Y	Y	N	Y	N
EHV02	-	-	Y	Y	Y	N	Y	N
EHV03	-	-	Y	Y	Y	N	Y	N
EHV04	-	-	Y	Y	N	N	Y	N
EHV05	-	-	Y	Y	Y	N	Dry	
EHV06	-	-	Y	Y	Y	N	Dry	
EHV07	-	-	Y	Y	Y	N	Y	N
EHV08	-	-	Y	Y	Y	N	Y	N
EHV09	-	-	N	N	Y	N	Y	N
EHV11	-	-	Y	Y	Y	N	Y	N
EHV12	-	-	Y	Y	Y	N	Y	N
EHV13	-	-	N	N	Y	N	Y	N
EHV14	-	-	Y	Y	Y	N	Dry	
EHV15	-	-	Y	Y	Y	N	Y	N
EHV16	-	-	Y	Y	Y	N	Y	N
EHV17	Y	Y	Y	N	Dry		Y	N
EHV18	Y	Y	Y	Y	N	N	Y	N
EHV19	Y	Y	Y	Y	Y	N	Y	N
EHV20	Y	Y	Y	Y	Y	N	Y	N
HV23	Y	Y	Y	N	Y	N	Y	N
HV4	Y	Y	N	N	Dry		N	N

Appendices Table 3: Seasonal summaries for the WB. N - missing samples. Y - samples that Passed the Data Cleaning Process (PDCP) and had corresponding field chemistry and major ions data, also referred to as corresponding chemistry data (CCD). N - samples that failed the data cleaning process and thus could not be used in comparative studies across seasons or regions.

	Aug-Sept 2014		Feb 2015		Jun 2015		Aug-Sept 2015		Nov 2015	
Sampled	**PDCP**	**CCD**	**PDCP**	**CCD**	**PDCP**	**CCD**	**PDCP**	**CCD**	**PDCP**	**CCD**
WB01	N	N	N	N	Y	Y	N	N	Y	Y
WB02	N	N	Y	Y	Y	Y	Y	Y	Y	Y
WB03	Y	Y	Y	Y	N	N	Y	Y	N	N
WB05	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
WB06	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
WB07	Y	Y	Y	Y	Dry					
WB07B	N	N	Y	Y	N	N	Y	Y	Dry	
WB07C					Y	Y	Y	Y	Dry	
WB09	Y	Y	Y	Y	Y	Y	Y	Y	Dry	
WB11	Y	Y	-	-	Y	Y	Y	Y	Dry	
WB12	N	N	N	N	Y	Y	Y	Y	Dry	
WB13	Y	Y	N	N	Y	Y	N	N	Y	Y
WB14	Y	Y	Y	Y	Y	Y	Y	Y	N	N
WB16	Y	Y	Y	Y	Y	Y	N	N	Dry	
WB19	Y	Y	Y	Y	Y	Y	Dry			
WB20	Y	Y	Y	Y	Y	Y	Y	Y	Dry	
WB21	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
WB22	Y	Y	Y	Y	Y	Y	Y	Y	Dry	
WB23A					Y	Y	Y	Y	Y	Y
WB23B					Y	Y	Y	Y	Y	Y
WB24	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
WB25	N	N	Y	Y	N	N	Dry			
WB26	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
WB27			Y	Y	Y	Y	Y	Y	Dry	
WB30			Y	Y	Y	Y	Y	Y	Y	Y
WB31					Y	Y	Dry			
WB32					Y	Y	Y	Y	Dry	
WB32A					Y	Y	Y	Y	Dry	
WB33							Y	Y	Dry	
WB34							Y	Y	Dry	
WB35										
WG01										
WG02										

The first sampling expedition to the SWC was conducted over the late summer (Feb 2015) where water samples were collected from thirteen streams (**Appendices Table 4**). Twelve of these were found in George and one site was located in Knysna. The George streams were highly compacted therefore streams that were less than 1 m in width and sat at close proximity (< 200 m) to larger streams were excluded from further sampling. These adjustments brought the number of sampled streams down to seven. All seven sites failed the data quality check due to ambiguous pH-ANC data (**Appendices Table 4**), leaving this season with just the field chemistry data. New sites were added during the Jun 2015 sampling session which brought the final number of study sites to 30. Of the 30 streams sampled in this season only 6 (SWC07, SWC22, SWC25, SWC28, SWC31 and SWC37) passed the outlier test for major ions. Overall very few SWC sites had corresponding major ion chemistry data and this offset comparison studies across seasons and study regions (**Appendices Table 5**).However, all 30 SWC sites had full field chemistry data.

The first batch of macroinvertebrate samples was collected during the Jun 2015 sampling period however since the majority of the SWC sites failed the data quality tests many of these macroinvertebrate samples were left without corresponding major ions data. When compared to the other regions the SWC had the least number of sites that passed the data quality check over the 2014-2015 sampling period.

Appendices Table 4: Seasonal summaries for the SWC. N - samples that failed the data cleaning process and thus could not be used in comparative studies across seasons or regions. Y - samples that Passed the Data Cleaning Process (PDCP) and had corresponding field chemistry and major ions data or corresponding chemistry data (CCD).

	Feb 2015		Jun 2015		Aug-Sept 2015		Nov 2015	
Sampled	**PDCP**	**CCD**	**PDCP**	**CCD**	**PDCP**	**CCD**	**PDCP**	**CCD**
SWC01	-	-	N	N	N	N	N	N
SWC03	N	N	N	N	N	N	N	N
SWC04	N	N	N	N	N	N	N	N
SWC06	N	N	N	N	N	N	N	N
SWC07	N	N	Y	Y	N	N	N	N
SWC08	N	N	N	N	Y	Y	Y	Y
SWC09	N	N	N	N	N	N	N	N
SWC14	N	N	N	N	N	N	N	N
SWC16			N	N	N	N	Y	Y
SWC17			N	N	Y	Y	Y	Y
SWC18			N	N	N	N	N	N
SWC19			N	N	Y	Y	N	N
SWC20			N	N	Y	Y	N	N
SWC21			N	N	N	N	N	N
SWC22			Y	Y	N	N	Y	Y
SWC23			N	N	N	N	N	N
SWC24			N	N	N	N	N	N
SWC25			Y	Y	Y	Y	N	N
SWC26			N	N	Y	Y	N	N
SWC27			N	N	Y	Y	Y	Y
SWC28			Y	Y	-	-	Y	Y
SWC29			N	N	-	-	Y	Y
SWC30			N	N	-	-	Y	Y
SWC31			Y	Y	Y	Y	N	N
SWC32			N	N	Y	Y	N	N
SWC33			N	N	Y	Y	N	N
SWC34			N	N	N	N	N	N
SWC35			N	N	Y	Y	N	N
SWC36			N	N	Y	Y	N	N
SWC37			Y	Y	N	N	N	N

Appendices Table 5: Summary of the Jun 2015 sampling period. Y - samples that Passed the Data Cleaning Process (PDCP) and had corresponding field chemistry and major ions data, also referred to as corresponding chemistry data (CCD). N - samples that failed the data cleaning process and thus could not be used in comparative studies across seasons or regions.

WB			SWC			HV		
Sampled	PDCP	CCD	Sampled	PDCP	CCD	Sampled	PDCP	CCD
WB01	Y	Y	SWC01	N	N	EHV01	Y	Y
WB02	Y	Y	SWC03	N	N	EHV02	Y	Y
WB03	N	N	SWC04	N	N	EHV03	Y	Y
WB05	Y	Y	SWC06	N	N	EHV04	Y	Y
WB06	Y	Y	SWC07	Y	Y	EHV05	Y	Y
WB07B	N	N	SWC08	N	N	EHV06	Y	Y
WB07C	Y	Y	SWC09	N	N	EHV07	Y	Y
WB09	Y	Y	SWC14	N	N	EHV08	Y	Y
WB11	Y	Y	SWC16	N	N	EHV09	N	N
WB12	Y	Y	SWC17	N	N	EHV10	Y	Y
WB13	Y	Y	SWC18	N	N	EHV11	Y	Y
WB14	Y	Y	SWC19	N	N	EHV12	Y	Y
WB16	Y	Y	SWC20	N	N	EHV13	N	N
WB19	Y	Y	SWC21	N	N	EHV14	Y	Y
WB20	Y	Y	SWC22	Y	Y	EHV15	Y	Y
WB21	Y	Y	SWC23	N	N	EHV16	Y	Y
WB22	Y	Y	SWC24	N	N	EHV17	Y	N
WB23A	Y	Y	SWC25	Y	Y	EHV18	Y	Y
WB23B	Y	Y	SWC26	N	N	EHV19	Y	Y
WB24	Y	Y	SWC27	N	N	EHV20	Y	Y
WB25	N	N	SWC28	Y	Y	HV23	Y	N
WB26	Y	Y	SWC29	N	N	HV4	N	N
WB27	Y	Y	SWC30	N	N			
WB30	Y	Y	SWC31	Y	Y			
WB31	Y	Y	SWC32	N	N			
WB32	Y	Y	SWC33	N	N			
WB32A	Y	Y	SWC34	N	N			
			SWC35	N	N			
			SWC36	N	N			
			SWC37	Y	Y			

2) Feb 2016

Some of the WB streams that stopped flowing the previous year (Nov 2015) had recovered from the drought (**Appendices Table 6**). A total of 19 sites were sampled in this region and WB12 was the only site that failed the data quality test due to an erroneous pH-ANC relationship. A total of 28 streams were sampled from the SWC and two streams that would have brought the number of samples to 30 had dried out. Of the 28 sites SWC06, SWC14 and SWC24 failed the ion balance and outlier tests. The remaining sites

had corresponding field and major ions data. From the 17 sites sampled at the HV EHV09, EHV12 and HV4 were the only sites that failed the data quality check. The remaining streams had viable field and major ions data. It is important to note that these results were the first set of results produced from the Potch lab after setting stringent parameters prior sample analysis. Furthermore, this season represents the only season where all three study regions had substantial field and major ions data.

Appendices Table 6: Summary of the Feb 2016 sampling period. Y - samples that Passed the Data Cleaning Process (PDCP) and had corresponding field chemistry and major ions data, also referred to as corresponding chemistry data (CCD). N - samples that failed the data cleaning process and thus could not be used in comparative studies across seasons or regions.

WB			SWC			HV		
Sampled	**PDCP**	**CCD**	**Sampled**	**PDCP**	**CCD**	**Sampled**	**PDCP**	**CCD**
WB01	Y	Y	SWC01	Y	Y	EHV01	Y	Y
WB02	Y	Y	SWC03	Y	Y	EHV02	Y	Y
WB03	Y	Y	SWC04	Y	Y	EHV03	Y	Y
WB05	Y	Y	SWC06	N	N	EHV04	Y	Y
WB06	Y	Y	SWC07	Y	Y	EHV07	Y	Y
WB12	N	N	SWC09	Y	Y	EHV08	Y	Y
WB13	Y	Y	SWC14	N	N	EHV09	N	N
WB14	Y	Y	SWC16	Y	Y	EHV11	Y	Y
WB16	Y	Y	SWC17	Y	Y	EHV12	N	N
WB20	Y	Y	SWC18	Y	Y	EHV13	Y	Y
WB21	Y	Y	SWC19	Y	Y	EHV15	Y	Y
WB23A	Y	Y	SWC20	Y	Y	EHV16	Y	Y
WB23B	Y	Y	SWC21	Y	Y	EHV18	Y	Y
WB24	Y	Y	SWC22	Y	Y	EHV19	Y	Y
WB26	Y	Y	SWC23	Y	Y	EHV20	Y	Y
WB27	Y	Y	SWC24	N	N	HV23	Y	Y
WB30	Y	Y	SWC25	Y	Y	HV4	N	N
WB32A	Y	Y	SWC26	Y	Y			
WB33	Y	Y	SWC27	Y	Y			
			SWC28	Y	Y			
			SWC29	Y	Y			
			SWC30	Y	Y			
			SWC31	Y	Y			
			SWC32	Y	Y			
			SWC33	Y	Y			
			SWC34	Y	Y			
			SWC35	Y	Y			
			SWC36	Y	Y			
			SWC37	Y	Y			

3) Jun 2016

At this stage of the project the Mpumalanga streams were not essential to the key objectives of this project as these sites proved to be impacted by agricultural activity and had two seasons that were missing a full set of field chemistry data. Thus, water and macroinvertebrate samples were only collected from the WB and the SWC. Furthermore, water samples collected here were the first batch of samples to be taken to the Czech Republic for interlaboratory comparison. A total of 48 samples representing 29 streams from the SWC and 19 from the WB were collected (**Appendices Table 7**). One site from the SWC and 14 from the WB had not recovered from the drought. Of the 48 samples collected only one SWC site failed the data quality check. The remaining samples had a full set of field and major ions data. Furthermore, this was the first set of data where Fe and Al concentrations were measured.

Appendices Table 7: Summary of the Jun 2016 sampling period. Y - samples that Passed the Data Cleaning Process (PDCP) and had corresponding field chemistry and major ions data, also referred to as corresponding chemistry data (CCD). N - samples that failed the data cleaning process and thus could not be used in comparative studies across seasons or regions.

WB			SWC		
Sampled	**PDCP**	**CCD**	**Sampled**	**PDCP**	**CCD**
WB01	Y	Y	SWC01	Y	Y
WB02	Y	Y	SWC03	Y	Y
WB03	Y	Y	SWC04	Y	Y
WB05	Y	Y	SWC06	Y	Y
WB06	Y	Y	SWC08	N	N
WB12	Y	Y	SWC09	Y	Y
WB13	Y	Y	SWC14	Y	Y
WB14	Y	Y	SWC16	Y	Y
WB16	Y	Y	SWC17	Y	Y
WB20	Y	Y	SWC18	Y	Y
WB21	Y	Y	SWC19	Y	Y
WB23A	Y	Y	SWC20	Y	Y
WB23B	Y	Y	SWC21	Y	Y
WB24	Y	Y	SWC22	Y	Y
WB26	Y	Y	SWC23	Y	Y
WB27	Y	Y	SWC24	Y	Y
WB30	Y	Y	SWC25	Y	Y
WB31	Y	Y	SWC26	Y	Y
WB32A	Y	Y	SWC27	Y	Y
			SWC28	Y	Y
			SWC29	Y	Y
			SWC30	Y	Y
			SWC31	Y	Y
			SWC32	Y	Y
			SWC33	Y	Y
			SWC34	Y	Y
			SWC35	Y	Y
			SWC36	Y	Y
			SWC37	Y	Y

4) Oct 2016

As with the previous season, water samples were only collected from the WB and the SWC. All the SWC streams had recovered from the drought which meant all 30 streams from this region were sampled (**Appendices Table 8**). Of the 30 sites three failed the data quality check while the remaining sites had a full set of field chemistry and major ions data. Some of the Waterberg streams had dried out again and three new sites were

added which brought the overall number of sampled streams to 15. All 15 sites had a full set of field and major ions data (**Appendices Table 8**).

Appendices Table 8: Summary of the Oct 2016 sampling period. Y - samples that Passed the Data Cleaning Process (PDCP) and had corresponding chemistry data (CCD). N - samples that failed the data cleaning process and thus could not be used in comparative studies across seasons or regions.

WB			SWC		
Sampled	**PDCP**	**CCD**	**Sampled**	**PDCP**	**CCD**
WB01	Y	Y	SWC01	Y	Y
WB05	Y	Y	SWC03	Y	Y
WB06	Y	Y	SWC04	N	N
WB13	Y	Y	SWC06	Y	Y
WB14	Y	Y	SWC07	Y	Y
WB16	Y	Y	SWC08	Y	Y
WB20	Y	Y	SWC09	Y	Y
WB21	Y	Y	SWC14	Y	Y
WB24	Y	Y	SWC16	Y	Y
WB26	Y	Y	SWC17	Y	Y
WB30	Y	Y	SWC18	Y	Y
WB32A	Y	Y	SWC19	Y	Y
WB35	Y	Y	SWC20	Y	Y
WG01	Y	Y	SWC21	Y	Y
WG02	Y	Y	SWC22	Y	Y
			SWC23	Y	Y
			SWC24	N	N
			SWC25	Y	Y
			SWC26	Y	Y
			SWC27	Y	Y
			SWC28	Y	Y
			SWC29	Y	Y
			SWC30	Y	Y
			SWC31	Y	Y
			SWC32	Y	Y
			SWC33	Y	Y
			SWC34	Y	Y
			SWC35	Y	Y
			SWC36	N	N
			SWC37	Y	Y

Appendix 6: Final data tables

		Temperature				DO %				Conductivity				pH				H			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
EHV01	2.0	10.4	24.2	17.3	9.8	69.7	107.0	88.4	26.4	84.6	143.6	114.1	41.7	7.9	8.0	8.0	0.1	9.5E-09	1.2E-08	1.1E-08	1.7E-09
EHV02	2.0	13.2	22.1	17.7	6.3	74.7	109.4	92.1	24.5	165.1	240.1	202.6	53.0	7.9	8.3	8.1	0.3	5.5E-09	1.4E-08	9.6E-09	5.9E-09
EHV03	2.0	13.7	22.7	18.2	6.4	96.0	108.8	102.4	9.0	90.0	109.0	99.5	13.4	7.6	8.1	7.9	0.4	7.4E-09	2.4E-08	1.6E-08	1.2E-08
EHV04	2.0	12.6	22.5	17.6	7.0	64.0	108.8	86.4	31.7	150.0	189.8	169.9	28.1	7.6	8.1	7.8	0.4	7.2E-09	2.8E-08	1.8E-08	1.5E-08
EHV05	1.0	10.8	10.8	10.8	NA	75.6	75.6	75.6	NA	314.1	314.1	314.1	NA	8.1	8.1	8.1	NA	7.9E-09	7.9E-09	7.9E-09	NA
EHV06	1.0	11.3	11.3	11.3	NA	69.4	69.4	69.4	NA	144.7	144.7	144.7	NA	7.6	7.6	7.6	NA	2.3E-08	2.3E-08	2.3E-08	NA
EHV07	2.0	11.9	18.3	15.1	4.5	68.7	101.9	85.3	23.5	14.1	21.3	17.7	5.1	7.0	7.4	7.2	0.3	3.8E-08	1.0E-07	6.9E-08	4.4E-08
EHV08	2.0	12.1	17.6	14.9	3.9	79.5	105.9	92.7	18.7	19.8	24.4	22.1	3.3	7.1	7.5	7.3	0.3	3.5E-08	8.5E-08	6.0E-08	3.5E-08
EHV09	2.0	13.1	20.1	16.6	4.9	67.9	101.9	84.9	24.0	26.3	36.3	31.3	7.1	6.9	7.5	7.2	0.4	3.5E-08	1.3E-07	8.2E-08	6.6E-08
EHV10	1.0	15.1	15.1	15.1	NA	92.1	92.1	92.1	NA	15.3	15.3	15.3	NA	5.9	5.9	5.9	NA	1.3E-06	1.3E-06	1.3E-06	NA
EHV11	2.0	9.3	17.6	13.5	5.9	54.3	78.3	66.3	17.0	18.3	53.3	35.8	24.7	6.1	6.3	6.2	0.2	4.6E-07	7.8E-07	6.2E-07	2.3E-07
EHV12	2.0	12.3	19.9	16.1	5.4	65.3	103.2	84.3	26.8	116.4	192.5	154.5	53.8	7.9	8.2	8.0	0.2	6.6E-09	1.4E-08	1.0E-08	5.3E-09
EHV13	2.0	13.3	19.5	16.4	4.4	59.7	94.7	77.2	24.7	79.0	140.2	109.6	43.3	7.5	7.9	7.7	0.3	1.2E-08	3.2E-08	2.2E-08	1.5E-08
EHV14	1.0	16.4	16.4	16.4	NA	89.9	89.9	89.9	NA	207.2	207.2	207.2	NA	7.9	7.9	7.9	NA	1.2E-08	1.2E-08	1.2E-08	NA
EHV15	2.0	16.6	21.1	18.9	3.2	65.6	105.0	85.3	27.9	146.3	180.3	163.3	24.0	7.8	8.2	8.0	0.3	5.9E-09	1.6E-08	1.1E-08	7.3E-09
EHV16	2.0	14.9	19.2	17.1	3.0	62.2	108.9	85.6	33.0	56.3	83.5	69.9	19.2	7.4	8.0	7.7	0.4	9.5E-09	3.9E-08	2.4E-08	2.1E-08
EHV17	1.0	20.2	20.2	20.2	NA	62.0	62.0	62.0	NA	56.0	56.0	56.0	NA	6.2	6.2	6.2	NA	5.9E-07	5.9E-07	5.9E-07	NA
EHV18	3.0	18.0	20.4	19.1	1.2	55.5	80.0	67.8	12.3	19.0	22.7	21.4	2.1	5.3	5.7	5.5	0.2	1.9E-06	5.0E-06	3.4E-06	1.6E-06
EHV19	3.0	12.1	27.3	20.4	7.7	103.0	103.2	103.1	0.1	17.0	55.4	32.4	20.3	5.4	7.0	6.1	0.8	1.1E-07	3.8E-06	1.6E-06	1.9E-06
EHV20	3.0	13.4	30.5	22.7	8.7	104.0	106.7	105.4	1.4	93.0	158.0	119.3	34.2	7.0	7.6	7.3	0.3	2.8E-08	1.0E-07	6.1E-08	3.8E-08
HV23	2.0	20.8	22.8	21.8	1.4	110.1	110.1	110.1	NA	218.0	321.0	269.5	72.8	7.9	9.5	8.7	1.1	3.0E-10	1.2E-08	6.0E-09	8.1E-09
HV4	3.0	9.6	22.2	16.5	6.4	87.9	92.2	90.1	2.2	973.0	1827.0	1420.0	428.4	5.7	7.0	6.1	0.7	1.0E-07	2.0E-06	1.3E-06	1.1E-06

		Na				NH4				K				Mg				Ca			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
EHV01	4	401.6	502.7	450.4	42.6	3.3	19.7	11.7	7.7	23.3	99.9	63.7	31.4	286.6	512.8	386.5	104.5	194.3	421.3	326.4	95.9
EHV02	4	440.3	710.8	544.4	123.2	2.6	18.5	7.2	7.6	30.3	55.5	40.0	10.9	540.1	739.0	626.1	92.4	295.6	1202.8	624.8	422.2
EHV03	4	202.1	355.4	285.3	63.2	2.1	13.0	5.0	5.4	24.9	28.8	26.9	2.0	339.0	807.1	559.6	195.6	320.8	1108.8	545.4	376.9
EHV04	3	341.5	467.5	425.3	72.6	3.6	6.6	5.3	1.5	18.7	30.2	24.7	5.8	556.4	772.6	663.3	108.1	406.6	1352.9	926.2	479.9
EHV05	2	695.1	754.5	724.8	41.9	3.1	5.5	4.3	1.7	15.1	17.2	16.1	1.5	1108.3	1407.1	1257.7	211.3	456.1	512.0	484.0	39.5
EHV06	2	105.1	337.5	221.3	164.3	0.7	6.3	3.5	4.0	22.3	26.2	24.2	2.7	143.6	256.5	200.0	79.8	134.3	217.6	176.0	58.9
EHV07	4	56.8	88.4	72.4	13.5	0.4	6.8	3.0	2.8	12.2	19.6	14.5	3.5	56.4	108.2	79.4	25.1	61.6	136.3	81.5	36.6
EHV08	4	79.2	140.8	102.7	27.2	0.3	5.5	3.2	2.3	8.6	27.7	15.8	8.7	70.9	94.2	80.5	10.0	74.0	133.4	98.0	25.4
EHV09	2	60.4	101.0	80.7	28.7	0.3	5.0	2.6	3.3	11.7	22.1	16.9	7.4	69.4	97.5	83.4	19.8	80.3	89.9	85.1	6.7
EHV10	1	94.5	94.5	94.5	NA	7.2	7.2	7.2	NA	23.7	23.7	23.7	NA	52.0	52.0	52.0	NA	57.5	57.5	57.5	NA
EHV11	4	85.0	112.0	99.6	12.8	0.8	8.4	4.0	3.4	12.3	33.0	19.8	9.1	53.7	205.3	128.3	79.3	38.3	208.6	124.2	85.5
EHV12	3	138.5	167.5	148.6	16.4	4.3	8.7	6.0	2.4	21.2	56.4	36.4	18.1	750.0	1285.3	979.7	275.6	260.0	488.4	363.7	115.7
EHV13	3	210.0	254.4	227.0	24.0	0.0	4.1	1.8	2.1	16.4	44.4	31.8	14.2	549.1	803.2	651.1	134.3	288.0	423.2	359.7	68.0
EHV14	2	457.2	628.9	543.0	121.4	2.1	15.3	8.7	9.3	19.6	21.8	20.7	1.6	879.8	1148.4	1014.1	189.9	368.8	656.1	512.5	203.1
EHV15	4	260.1	364.8	306.1	52.0	0.1	13.4	3.9	6.4	5.1	15.9	11.7	4.6	458.5	741.3	643.2	125.8	351.0	945.6	583.0	254.3
EHV16	4	149.1	289.2	191.4	65.6	0.7	5.0	2.1	2.0	11.3	16.7	14.2	2.2	345.0	489.3	410.0	63.9	187.2	384.0	262.6	84.6
EHV17	3	208.7	331.1	264.3	61.9	0.0	15.3	6.4	8.0	21.2	69.3	50.2	25.5	61.3	144.0	112.2	44.5	65.6	151.2	118.9	46.5
EHV18	4	87.2	110.2	100.2	9.8	0.4	7.2	4.2	3.0	22.5	40.9	35.6	8.8	31.9	45.9	40.2	6.5	40.5	68.8	56.4	11.7
EHV19	5	87.7	159.7	121.0	29.7	0.2	5.3	2.3	2.0	20.4	61.3	46.4	17.7	45.1	156.4	85.1	44.1	57.4	195.9	117.3	63.2
EHV20	5	270.0	623.9	402.0	149.5	0.2	18.5	8.0	7.8	41.1	78.5	58.5	16.8	255.6	392.5	314.8	49.9	216.7	428.7	313.3	89.1
HV23	5	617.2	823.7	740.6	77.9	0.0	14.0	5.0	5.9	23.0	110.6	75.3	37.2	557.2	1171.1	939.8	250.9	417.8	1161.7	834.6	293.1
HV4	1	1828.6	1828.6	1828.6	NA	4.8	4.8	4.8	NA	162.8	162.8	162.8	NA	11845.1	11845.1	11845.1	NA	8204.4	8204.4	8204.4	NA

		NO3				Cl				SO4				F				PO4			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
EHV01	4	19.9	71.4	41.5	21.7	141.2	241.8	171.7	47.5	25.5	283.9	133.8	108.7	7.8	9.3	8.7	0.6	0.0	1.1	0.6	0.5
EHV02	4	41.9	80.7	57.7	17.7	167.7	395.7	278.2	100.6	140.1	279.5	191.4	61.3	2.2	6.6	4.3	1.9	0.0	1.5	0.7	0.7
EHV03	4	4.1	13.1	8.5	4.5	98.6	142.2	122.2	18.5	37.2	63.7	48.8	11.1	2.9	4.8	4.0	0.8	0.0	0.7	0.2	0.3
EHV04	3	14.6	30.5	24.4	8.5	162.0	193.6	179.0	15.9	82.0	184.5	129.6	51.6	3.9	6.6	5.1	1.4	0.0	0.7	0.2	0.4
EHV05	2	0.2	4.2	2.2	2.8	233.2	288.6	260.9	39.2	71.4	72.0	71.7	0.4	2.5	3.3	2.9	0.6	0.0	0.7	0.3	0.5
EHV06	2	0.1	4.9	2.5	3.4	71.6	83.0	77.3	8.1	13.9	33.0	23.4	13.5	2.6	5.0	3.8	1.7	0.4	2.8	1.6	1.7
EHV07	4	1.7	10.4	6.2	3.7	32.5	66.9	45.6	15.3	3.7	30.9	15.3	11.8	0.5	1.8	1.2	0.5	0.0	0.6	0.3	0.3
EHV08	4	0.1	7.8	4.0	3.2	31.2	95.8	53.0	29.0	6.1	58.8	21.4	25.1	1.4	1.6	1.6	0.1	0.0	23.4	5.8	11.7
EHV09	2	1.3	2.7	2.0	1.0	27.9	76.9	52.4	34.7	4.4	29.0	16.7	17.4	1.0	2.6	1.8	1.1	0.0	0.0	0.0	0.0
EHV10	1	13.1	13.1	13.1	NA	77.1	77.1	77.1	NA	7.4	7.4	7.4	NA	1.2	1.2	1.2	NA	1.0	1.0	1.0	NA
EHV11	4	1.8	7.5	3.9	2.5	22.3	69.0	40.8	20.0	2.8	47.2	20.4	18.9	1.8	2.6	2.1	0.4	0.0	0.5	0.2	0.2
EHV12	3	6.2	18.1	10.9	6.4	77.0	152.9	103.4	42.8	28.0	92.4	49.8	36.9	3.5	3.8	3.6	0.2	0.0	0.4	0.1	0.2
EHV13	3	21.7	39.4	29.6	9.0	102.6	117.8	111.2	7.8	22.1	30.9	25.4	4.8	4.4	5.7	4.9	0.7	0.0	0.0	0.0	0.0
EHV14	2	0.2	0.2	0.2	0.0	87.6	124.2	105.9	25.9	28.8	44.9	36.9	11.4	6.7	7.3	7.0	0.4	0.0	1.0	0.5	0.7
EHV15	4	0.2	5.5	3.3	2.2	91.4	104.7	96.7	6.1	26.0	40.5	31.9	6.2	4.4	7.0	5.5	1.1	0.0	42.7	10.8	21.3
EHV16	4	0.1	7.5	4.1	3.1	37.6	49.4	41.9	5.1	15.8	19.7	17.8	2.0	4.1	5.2	4.4	0.5	0.0	32.2	8.0	16.1
EHV17	3	0.7	7.4	3.9	3.4	97.9	224.2	178.2	69.8	29.6	268.0	117.8	130.8	1.6	2.8	2.1	0.6	0.0	1.0	0.5	0.5
EHV18	4	27.8	36.1	33.3	3.9	27.5	39.4	35.8	5.6	5.3	13.3	8.4	3.8	0.6	1.6	1.1	0.5	0.0	0.7	0.2	0.4
EHV19	5	2.9	13.2	7.8	3.8	34.9	110.5	79.2	33.7	8.8	113.8	36.7	44.2	1.9	2.7	2.4	0.4	0.0	0.4	0.1	0.2
EHV20	5	0.6	5.5	2.6	2.3	80.9	224.2	137.3	55.3	36.5	147.6	66.1	46.5	0.1	6.9	4.8	2.8	0.0	0.6	0.2	0.3
HV23	5	0.3	6.5	3.2	2.7	234.2	358.1	312.3	50.0	438.9	728.7	569.2	113.3	10.7	13.5	12.1	1.0	0.0	30.5	8.4	13.3
HV4	1	0.6	0.6	0.6	NA	281.6	281.6	281.6	NA	21132.5	21132.5	21132.5	NA	10.0	10.0	10.0	NA	2.2	2.2	2.2	NA

		ANC				DOC			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD
EHV01	4	703.9	1039.4	880.1	172.1	3.3	7.5	5.5	1.7
EHV02	4	926.8	1947.6	1308.0	446.3	3.1	4.7	3.6	0.8
EHV03	4	771.9	2081.0	1237.9	581.4	2.6	2.7	2.7	0.1
EHV04	3	1158.6	2317.7	1706.5	582.1	2.8	10.3	6.0	3.8
EHV05	2	2031.7	2264.0	2147.9	164.3	1.6	2.5	2.0	0.6
EHV06	2	284.4	752.1	518.3	330.7	2.9	4.5	3.7	1.2
EHV07	4	133.7	252.3	180.7	50.8	0.8	3.3	1.9	1.0
EHV08	4	178.8	283.4	218.6	47.2	1.3	3.3	2.1	0.8
EHV09	2	186.7	203.1	194.9	11.6	1.4	4.9	3.1	2.5
EHV10	1	130.0	130.0	130.0	NA	1.4	1.4	1.4	NA
EHV11	4	142.5	480.3	306.7	182.0	2.7	2.9	2.8	0.1
EHV12	3	1085.2	1683.5	1364.2	301.2	1.4	6.3	3.2	2.7
EHV13	3	1004.1	1169.0	1103.3	87.4	2.1	3.9	3.1	0.9
EHV14	2	1608.8	2285.9	1947.3	478.8	1.7	2.9	2.3	0.9
EHV15	4	1177.0	1788.7	1412.2	289.1	1.7	2.3	2.0	0.3
EHV16	4	631.5	1110.9	814.4	208.3	0.9	2.3	1.5	0.6
EHV17	3	97.7	344.3	245.7	130.5	3.0	5.1	4.1	1.1
EHV18	4	125.6	182.3	154.9	23.2	0.5	6.1	2.5	2.6
EHV19	5	112.6	452.7	246.2	143.8	3.2	7.1	4.8	1.6
EHV20	5	747.2	1008.2	882.7	94.0	1.9	4.3	2.8	0.9
HV23	5	1077.8	2247.0	1705.6	457.3	6.9	15.2	10.7	3.0
HV4	1	626.0	626.0	626.0	NA	2.7	2.7	2.7	NA

		Temperature				DO %				Conductivity				pH				H			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
WB01	7.0	15.3	27.7	20.5	4.8	82.5	178.9	110.4	32.8	47.6	116.2	65.9	25.5	6.7	7.8	7.1	0.4	1.6E-08	1.9E-07	9.7E-08	6.4E-08
WB02	6.0	11.7	22.3	17.2	4.8	26.7	124.3	68.3	31.6	83.0	317.0	212.5	85.8	6.8	7.1	7.0	0.1	7.6E-08	1.4E-07	1.1E-07	2.4E-08
WB03	7.0	12.5	24.8	17.3	5.2	17.3	99.4	62.2	34.9	28.8	128.4	50.9	36.7	5.7	6.3	6.0	0.2	5.2E-07	2.1E-06	1.3E-06	6.6E-07
WB04	2.0	16.2	18.6	17.4	1.7	105.8	109.0	107.4	2.3	23.0	27.4	25.2	3.1	5.3	5.6	5.4	0.2	2.8E-06	5.2E-06	4.0E-06	1.7E-06
WB05	8.0	11.3	22.5	17.0	4.5	37.3	127.9	99.2	28.5	10.0	20.4	14.0	4.0	5.4	6.8	6.1	0.5	1.7E-07	4.1E-06	1.3E-06	1.3E-06
WB06	8.0	12.0	24.4	18.4	4.3	43.4	130.0	93.1	26.3	11.0	16.7	13.0	1.8	5.3	6.3	5.7	0.4	4.7E-07	5.1E-06	2.7E-06	1.9E-06
WB07	2.0	19.1	27.9	23.5	6.2	40.3	89.0	64.7	34.4	35.0	41.0	38.0	4.2	4.6	5.3	5.0	0.6	4.6E-06	2.8E-05	1.6E-05	1.6E-05
WB07B	3.0	15.2	27.7	20.8	6.4	36.1	116.8	84.0	42.4	23.4	28.2	25.2	2.6	5.6	6.1	5.9	0.2	7.6E-07	2.3E-06	1.5E-06	7.7E-07
WB07C	2.0	15.4	17.1	16.3	1.2	92.0	104.0	98.0	8.5	25.0	25.3	25.2	0.2	4.6	5.2	4.9	0.4	7.1E-06	2.3E-05	1.5E-05	1.2E-05
WB09	4.0	9.5	25.6	15.9	6.9	48.6	117.6	95.4	32.5	27.6	42.9	33.2	6.8	5.5	6.3	5.8	0.3	5.5E-07	3.3E-06	1.9E-06	1.2E-06
WB11	3.0	16.7	20.6	18.5	2.0	42.0	103.6	71.1	30.9	20.6	26.9	22.9	3.5	5.5	6.2	5.7	0.4	5.9E-07	3.5E-06	2.3E-06	1.5E-06
WB12	4.0	15.0	26.0	19.2	4.8	75.3	107.0	91.1	14.1	12.5	36.3	22.0	10.2	5.2	6.1	5.7	0.4	7.6E-07	6.6E-06	2.7E-06	2.7E-06
WB13	7.0	11.3	25.0	17.5	5.2	68.4	131.6	90.3	22.8	10.0	14.6	12.3	1.8	5.5	6.6	6.0	0.3	2.5E-07	3.2E-06	1.4E-06	9.3E-07
WB14	8.0	10.5	23.4	17.6	5.5	36.7	130.5	91.7	30.4	8.9	17.4	10.9	2.9	5.0	6.2	5.5	0.3	6.8E-07	1.0E-05	3.8E-06	2.7E-06
WB16	6.0	13.1	25.3	19.2	4.7	55.6	109.0	87.2	19.8	17.3	72.9	45.4	21.5	6.0	7.0	6.6	0.4	1.1E-07	1.1E-06	3.8E-07	3.9E-07
WB17	1.0	24.9	24.9	24.9	NA	80.0	80.0	80.0	NA	120.0	120.0	120.0	NA	6.2	6.2	6.2	NA	6.5E-07	6.5E-07	6.5E-07	NA
WB18	1.0	23.2	23.2	23.2	NA	96.0	96.0	96.0	NA	35.1	35.1	35.1	NA	5.1	5.1	5.1	NA	7.8E-06	7.8E-06	7.8E-06	NA
WB19	3.0	20.3	23.2	22.2	1.6	40.9	109.0	78.0	34.4	19.1	30.5	25.2	5.7	5.3	5.5	5.4	0.1	3.0E-06	5.4E-06	3.9E-06	1.3E-06
WB20	7.0	11.6	27.4	20.2	5.6	31.4	113.0	81.5	33.4	9.4	23.4	13.6	4.7	5.3	6.3	5.9	0.3	4.7E-07	4.8E-06	1.7E-06	1.5E-06
WB21	8.0	14.4	24.3	19.7	3.0	34.7	99.1	62.9	25.7	18.0	60.3	30.0	14.1	5.2	5.9	5.6	0.2	1.4E-06	5.9E-06	3.0E-06	1.8E-06
WB22	4.0	15.5	27.7	19.7	5.6	55.1	119.0	89.2	26.2	30.2	48.5	38.3	7.6	6.6	7.1	6.8	0.2	8.5E-08	2.5E-07	1.7E-07	8.8E-08
WB23	1.0	16.2	16.2	16.2	NA	75.0	75.0	75.0	NA	26.6	26.6	26.6	NA	6.2	6.2	6.2	NA	7.1E-07	7.1E-07	7.1E-07	NA
WB23A	6.0	8.1	29.0	20.9	8.0	46.8	115.0	80.3	24.9	30.0	48.7	38.3	7.7	6.0	6.6	6.3	0.2	2.8E-07	1.0E-06	6.1E-07	2.9E-07
WB23B	5.0	9.0	22.4	16.5	5.4	60.0	108.2	88.4	19.8	36.7	68.8	51.5	13.5	6.4	6.9	6.6	0.2	1.3E-07	4.3E-07	2.8E-07	1.1E-07
WB24	8.0	13.6	28.4	21.0	5.9	31.8	115.6	84.8	26.3	22.0	42.6	30.4	8.9	6.1	7.5	6.8	0.5	3.4E-08	7.4E-07	2.7E-07	3.0E-07
WB25	3.0	17.7	27.9	21.6	5.5	19.4	38.8	27.7	10.0	28.6	33.9	31.2	2.7	4.4	5.6	5.1	0.6	2.8E-06	4.5E-05	1.7E-05	2.4E-05
WB26	8.0	12.3	28.2	22.3	5.8	9.1	130.0	88.4	40.2	82.8	202.3	148.7	42.7	6.8	8.3	7.7	0.6	5.1E-09	1.7E-07	4.0E-08	5.6E-08
WB27	5.0	13.1	25.7	18.3	5.5	29.5	90.0	69.9	24.1	7.8	12.7	9.9	2.4	4.8	6.5	5.6	0.6	3.3E-07	1.6E-05	5.3E-06	6.4E-06
WB28	1.0	21.2	21.2	21.2	NA	53.1	53.1	53.1	NA	31.0	31.0	31.0	NA	5.3	5.3	5.3	NA	4.8E-06	4.8E-06	4.8E-06	NA
WB29	1.0	24.9	24.9	24.9	NA	39.1	39.1	39.1	NA	23.7	23.7	23.7	NA	5.1	5.1	5.1	NA	8.5E-06	8.5E-06	8.5E-06	NA
WB30	7.0	13.3	24.4	19.6	4.2	0.5	98.1	44.7	36.7	11.4	144.5	50.2	45.4	5.6	7.2	6.0	0.6	5.8E-08	2.8E-06	1.4E-06	9.2E-07
WB31	2.0	9.1	10.6	9.9	1.1	75.0	80.4	77.7	3.8	59.0	72.6	65.8	9.7	6.1	6.8	6.4	0.5	1.6E-07	8.5E-07	5.0E-07	4.9E-07
WB32	2.0	11.5	15.5	13.5	2.8	102.0	137.7	119.9	25.2	13.9	15.8	14.9	1.3	6.3	7.1	6.7	0.6	7.2E-08	4.8E-07	2.8E-07	2.9E-07
WB32A	5.0	12.6	23.2	16.6	5.0	57.2	153.9	102.2	34.9	44.7	75.3	55.2	12.3	6.6	7.4	7.0	0.3	3.6E-08	2.5E-07	1.3E-07	8.0E-08
WB33	2.0	16.2	27.1	21.7	7.7	71.3	71.8	71.6	0.4	19.9	25.6	22.8	4.0	6.2	6.6	6.4	0.3	2.5E-07	5.9E-07	4.2E-07	2.4E-07
WB34	1.0	13.4	13.4	13.4	NA	84.0	84.0	84.0	NA	11.7	11.7	11.7	NA	5.6	5.6	5.6	NA	2.7E-06	2.7E-06	2.7E-06	NA
WB35	1.0	25.7	25.7	25.7	NA	78.6	78.6	78.6	NA	24.4	24.4	24.4	NA	6.0	6.0	6.0	NA	1.0E-06	1.0E-06	1.0E-06	NA
WG01	1.0	24.6	24.6	24.6	NA	101.0	101.0	101.0	NA	14.5	14.5	14.5	NA	6.5	6.5	6.5	NA	3.0E-07	3.0E-07	3.0E-07	NA
WG02		25.3	25.3	25.3	NA	104.0	104.0	104.0	NA	15.7	15.7	15.7	NA	6.5	6.5	6.5	NA	3.5E-07	3.5E-07	3.5E-07	NA

		Na				NH4				K				Mg				Ca			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
WB01	5	83.5	211.8	129.3	53.6	0.1	3.7	0.8	1.6	9.6	42.6	18.6	13.9	134.1	184.0	164.4	18.9	164.4	266.7	221.1	41.3
WB02	6	230.9	1033.4	667.7	263.1	0.0	1.6	0.6	0.6	27.7	56.9	38.2	9.9	271.2	1413.1	889.6	431.0	287.1	1343.2	687.9	417.8
WB03	5	65.3	194.1	157.0	53.9	0.0	2.5	1.1	1.3	2.8	8.5	5.0	2.5	38.5	130.3	82.7	36.3	30.4	71.6	50.1	19.4
WB04	2	116.2	119.5	117.9	2.4	0.0	0.2	0.1	0.1	7.9	10.5	9.2	1.9	62.9	67.1	65.0	2.9	33.1	44.5	38.8	8.1
WB05	8	59.9	102.8	87.7	13.5	0.0	3.5	0.6	1.2	2.7	10.9	5.1	2.6	15.1	48.7	28.9	12.1	10.5	47.7	26.4	12.9
WB06	8	55.2	84.5	70.5	10.2	0.0	0.6	0.2	0.2	2.6	8.9	4.8	2.3	27.2	39.1	32.7	5.0	12.3	56.8	26.8	15.3
WB07	2	156.1	172.9	164.5	11.9	0.0	0.1	0.1	0.1	3.3	8.9	6.1	3.9	41.3	49.8	45.6	6.0	20.8	31.3	26.1	7.4
WB07B	2	129.9	134.7	132.3	3.4	0.2	0.3	0.3	0.1	5.6	6.5	6.0	0.7	46.3	71.3	58.8	17.7	30.8	52.8	41.8	15.5
WB07C	2	127.0	129.5	128.2	1.7	0.7	3.5	2.1	2.0	3.5	6.0	4.8	1.7	48.7	57.3	53.0	6.1	20.5	35.9	28.2	10.9
WB09	4	226.1	473.2	302.7	115.2	0.0	5.5	1.4	2.7	6.0	7.8	6.8	0.9	53.7	65.7	58.1	5.5	25.7	43.9	35.1	7.9
WB11	3	90.5	94.0	91.9	1.8	0.0	0.4	0.2	0.2	10.1	13.8	11.4	2.1	46.2	61.8	52.0	8.5	21.2	50.5	34.7	14.8
WB12	3	53.8	133.8	94.2	40.0	2.5	6.4	4.8	2.0	4.9	15.5	9.9	5.3	29.6	45.1	38.5	8.0	33.0	56.5	45.9	12.0
WB13	6	67.2	92.6	75.5	9.0	0.0	0.5	0.2	0.2	2.2	6.2	3.7	1.7	12.5	24.2	19.0	4.1	12.1	26.4	20.0	4.8
WB14	7	38.2	125.6	72.2	27.6	0.0	0.4	0.1	0.1	1.1	10.2	2.9	3.2	6.9	30.6	14.3	8.0	4.0	21.1	11.3	6.6
WB14B	1	59.4	59.4	59.4	NA	0.1	0.1	0.1	NA	0.8	0.8	0.8	NA	9.5	9.5	9.5	NA	7.0	7.0	7.0	NA
WB16	6	48.2	270.8	160.5	74.0	0.0	3.5	0.7	1.4	1.7	14.7	7.3	4.9	41.4	222.4	135.1	71.6	51.1	350.2	208.1	114.5
WB17	1	289.3	289.3	289.3	NA	0.9	0.9	0.9	NA	28.4	28.4	28.4	NA	287.3	287.3	287.3	NA	485.3	485.3	485.3	NA
WB18	1	177.7	177.7	177.7	NA	0.4	0.4	0.4	NA	12.9	12.9	12.9	NA	64.6	64.6	64.6	NA	37.1	37.1	37.1	NA
WB19	3	93.0	141.2	116.2	24.2	0.2	4.3	1.6	2.3	11.3	24.3	17.9	6.5	22.9	54.6	39.9	16.0	18.1	57.5	36.3	19.9
WB20	7	48.6	96.8	68.6	15.3	0.0	0.8	0.2	0.3	3.0	9.2	5.3	2.5	20.2	30.1	24.5	3.6	12.1	27.1	18.2	5.4
WB21	8	84.6	101.8	92.5	5.6	1.7	10.3	3.7	2.8	3.6	9.8	6.4	1.9	27.1	43.4	34.1	5.2	27.5	56.6	40.6	9.5
WB22	4	118.7	310.1	216.5	80.6	0.2	3.8	1.2	1.8	10.5	27.4	17.7	7.3	70.3	126.1	105.6	24.4	46.6	86.8	75.7	19.4
WB23	1	137.7	137.7	137.7	NA	0.3	0.3	0.3	NA	3.9	3.9	3.9	NA	92.0	92.0	92.0	NA	34.5	34.5	34.5	NA
WB23A	5	160.4	364.1	229.9	86.1	0.0	3.6	0.8	1.6	5.5	23.3	11.2	7.0	55.1	125.7	74.6	29.1	40.9	77.2	53.7	14.2
WB23B	5	169.9	378.2	288.5	94.5	0.0	22.6	8.8	9.1	11.2	42.5	27.2	13.6	110.3	225.1	174.2	50.1	58.2	148.7	102.8	39.5
WB24	8	96.4	171.5	136.7	25.0	0.0	3.9	0.9	1.3	7.9	24.8	14.3	6.5	55.7	99.6	70.1	16.3	40.4	80.4	57.6	14.4
WB25	1	120.2	120.2	120.2	NA	0.0	0.0	0.0	NA	5.0	5.0	5.0	NA	68.8	68.8	68.8	NA	43.9	43.9	43.9	NA
WB26	8	311.8	952.1	668.4	227.5	0.1	3.9	1.2	1.2	28.5	50.4	40.4	7.6	205.9	583.5	351.9	128.4	227.9	458.1	356.4	73.4
WB27	5	48.1	63.8	57.8	6.1	0.0	3.3	0.7	1.4	1.7	3.8	2.6	1.0	5.0	23.6	12.6	7.2	6.3	29.2	15.4	9.4
WB28	1	168.3	168.3	168.3	NA	0.1	0.1	0.1	NA	11.9	11.9	11.9	NA	42.4	42.4	42.4	NA	18.2	18.2	18.2	NA
WB29	1	105.5	105.5	105.5	NA	0.1	0.1	0.1	NA	7.4	7.4	7.4	NA	31.1	31.1	31.1	NA	13.4	13.4	13.4	NA
WB30	7	123.1	289.1	199.5	68.1	0.4	1.9	1.0	0.6	5.6	36.1	10.6	11.2	44.9	152.9	70.2	38.3	31.4	232.2	81.7	69.1
WB31	2	289.4	425.8	357.6	96.5	1.1	1.9	1.5	0.5	16.7	16.8	16.7	0.1	319.7	333.6	326.7	9.8	32.1	237.0	134.5	144.9
WB32	2	79.3	79.3	79.3	0.0	0.2	0.3	0.3	0.1	1.3	7.6	4.4	4.4	42.8	57.6	50.2	10.5	24.8	145.5	85.1	85.4
WB32A	5	99.4	246.3	159.5	54.9	0.0	1.0	0.4	0.4	7.0	21.1	12.4	5.7	155.9	255.4	204.3	38.2	203.6	336.9	254.4	54.0
WB33	2	26.7	81.5	54.1	38.7	0.0	0.1	0.0	0.0	0.6	8.5	4.6	5.6	12.9	69.0	40.9	39.7	19.9	45.7	32.8	18.2
WB34	1	56.8	56.8	56.8	NA	0.0	0.0	0.0	NA	0.9	0.9	0.9	NA	5.2	5.2	5.2	NA	8.5	8.5	8.5	NA
WB35	1	129.8	129.8	129.8	NA	1.3	1.3	1.3	NA	16.0	16.0	16.0	NA	51.2	51.2	51.2	NA	62.5	62.5	62.5	NA
WG01	1	87.5	87.5	87.5	NA	0.2	0.2	0.2	NA	5.2	5.2	5.2	NA	21.4	21.4	21.4	NA	21.7	21.7	21.7	NA
WG02	1	81.8	81.8	81.8	NA	0.0	0.0	0.0	NA	5.7	5.7	5.7	NA	24.7	24.7	24.7	NA	28.0	28.0	28.0	NA

		NO3				Cl				SO4				F				PO4			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
WB01	5	0.0	4.1	0.8	1.8	39.6	118.5	58.3	33.9	5.5	22.9	10.0	7.4	2.8	3.7	3.3	0.4	0.0	0.5	0.1	0.2
WB02	6	0.0	9.4	1.9	3.7	117.8	291.7	214.5	58.1	13.2	58.4	32.5	18.4	11.8	46.7	31.9	12.0	0.0	67.6	11.3	27.6
WB03	5	0.0	6.3	1.9	2.6	60.1	187.4	146.3	49.7	0.8	5.1	2.9	1.7	1.1	2.2	1.6	0.5	0.0	0.0	0.0	0.0
WB04	2	23.6	35.5	29.5	8.4	151.3	162.9	157.1	8.2	1.0	2.7	1.8	1.2	1.3	1.6	1.5	0.2	0.0	0.0	0.0	0.0
WB05	8	0.1	3.9	0.9	1.2	44.8	101.7	76.5	16.8	1.9	7.6	4.5	1.9	0.4	1.1	0.7	0.3	0.0	0.7	0.1	0.2
WB06	8	0.0	1.3	0.3	0.5	50.4	86.3	69.1	14.0	1.1	7.4	3.4	2.0	0.4	1.1	0.6	0.2	0.0	2.7	0.3	1.0
WB07	2	0.0	0.0	0.0	0.0	211.9	220.6	216.2	6.1	9.8	15.7	12.7	4.2	0.7	1.0	0.9	0.2	0.0	0.0	0.0	0.0
WB07B	2	0.1	0.2	0.1	0.0	145.3	194.1	169.7	34.5	7.5	11.8	9.6	3.1	0.9	1.0	0.9	0.1	0.0	0.1	0.1	0.1
WB07C	2	0.0	3.3	1.6	2.3	176.1	176.7	176.4	0.4	13.8	18.9	16.3	3.6	0.7	0.8	0.7	0.1	0.0	0.3	0.1	0.2
WB09	4	0.0	4.9	1.4	2.3	242.6	300.9	272.8	25.2	7.4	14.0	9.9	3.2	1.2	2.0	1.6	0.3	0.0	1.2	0.3	0.6
WB11	3	0.2	1.5	0.6	0.8	77.0	95.4	87.3	9.3	2.6	3.7	3.1	0.6	1.0	1.2	1.1	0.1	0.0	1.1	0.4	0.6
WB12	3	0.4	4.2	2.6	2.0	53.3	121.4	85.8	34.1	8.0	14.2	11.4	3.1	1.0	1.2	1.1	0.1	0.0	0.5	0.3	0.3
WB13	6	0.0	0.1	0.1	0.0	55.9	101.6	68.6	16.8	2.8	7.8	4.0	1.9	0.4	0.6	0.5	0.1	0.0	0.5	0.1	0.2
WB14	7	0.0	0.4	0.1	0.2	18.6	162.3	60.9	46.8	0.4	9.1	3.0	3.7	0.3	0.7	0.4	0.1	0.0	0.4	0.1	0.1
WB14B	1	0.0	0.0	0.0	NA	42.9	42.9	42.9	NA	1.7	1.7	1.7	NA	0.4	0.4	0.4	NA	0.0	0.0	0.0	NA
WB16	6	0.0	1.7	0.3	0.7	11.3	133.7	47.5	44.2	5.3	39.7	20.1	13.0	0.6	1.8	1.3	0.5	0.0	0.9	0.1	0.4
WB17	1	0.3	0.3	0.3	NA	310.5	310.5	310.5	NA	9.0	9.0	9.0	NA	2.7	2.7	2.7	NA	0.0	0.0	0.0	NA
WB18	1	0.3	0.3	0.3	NA	198.1	198.1	198.1	NA	5.2	5.2	5.2	NA	0.8	0.8	0.8	NA	0.0	0.0	0.0	NA
WB19	3	1.3	4.5	3.4	1.7	95.7	174.8	140.9	40.8	9.4	19.9	16.1	5.9	0.5	0.6	0.6	0.0	0.0	0.5	0.2	0.3
WB20	7	0.0	0.1	0.0	0.0	34.2	84.5	57.8	16.9	5.6	18.4	9.9	4.4	0.5	1.2	0.7	0.2	0.0	0.6	0.1	0.2
WB21	8	0.1	2.8	0.7	0.9	89.6	127.6	101.2	12.4	1.5	8.3	3.6	2.4	0.8	1.2	1.0	0.1	0.0	0.7	0.1	0.3
WB22	4	0.0	4.6	1.2	2.2	81.0	153.5	125.6	33.6	7.2	20.7	13.0	6.1	1.3	3.9	2.6	1.1	0.0	1.1	0.3	0.5
WB23	1	0.4	0.4	0.4	NA	158.1	158.1	158.1	NA	2.7	2.7	2.7	NA	0.7	0.7	0.7	NA	0.0	0.0	0.0	NA
WB23A	5	0.0	4.5	1.2	1.9	190.6	506.1	265.5	135.2	0.3	26.1	7.0	11.1	0.8	1.7	1.2	0.3	0.0	0.6	0.1	0.3
WB23B	5	0.2	10.5	3.8	4.5	150.2	294.2	213.9	71.5	0.7	15.1	4.9	6.1	2.0	3.8	2.9	0.8	0.0	11.4	2.6	5.0
WB24	8	0.0	4.7	0.7	1.6	103.0	185.9	143.8	28.9	5.5	18.4	11.0	4.2	1.0	2.3	1.6	0.6	0.0	1.0	0.1	0.4
WB25	1	0.2	0.2	0.2	NA	127.4	127.4	127.4	NA	7.6	7.6	7.6	NA	0.5	0.5	0.5	NA	0.0	0.0	0.0	NA
WB26	8	0.2	9.9	4.1	4.2	144.9	362.6	265.9	75.9	41.9	152.1	82.6	39.4	0.4	15.3	11.8	4.8	0.0	0.4	0.0	0.1
WB27	5	0.0	5.1	1.1	2.2	32.7	57.1	47.1	8.9	0.5	7.8	4.8	3.0	0.2	0.6	0.3	0.1	0.0	1.4	0.3	0.6
WB28	1	0.0	0.0	0.0	NA	221.9	221.9	221.9	NA	14.7	14.7	14.7	NA	1.2	1.2	1.2	NA	0.0	0.0	0.0	NA
WB29	1	0.0	0.0	0.0	NA	141.0	141.0	141.0	NA	18.9	18.9	18.9	NA	0.4	0.4	0.4	NA	0.0	0.0	0.0	NA
WB30	7	0.0	0.4	0.1	0.1	143.4	234.5	189.9	32.4	2.1	22.4	9.6	6.9	0.8	2.5	1.3	0.6	0.0	0.4	0.1	0.1
WB31	2	0.1	0.1	0.1	0.0	62.3	135.9	99.1	52.0	19.6	40.2	29.9	14.5	2.0	2.0	2.0	0.0	0.0	0.6	0.3	0.4
WB32	2	0.0	6.7	3.3	4.7	41.3	45.7	43.5	3.1	0.8	0.8	0.8	0.0	0.4	0.5	0.4	0.0	0.0	22.3	11.1	15.7
WB32A	5	0.1	0.7	0.3	0.2	58.1	102.1	84.4	17.1	7.0	38.2	16.9	13.2	1.7	2.5	1.9	0.3	0.0	4.0	0.8	1.8
WB33	2	0.0	0.0	0.0	0.0	4.4	77.3	40.9	51.6	12.4	15.8	14.1	2.4	0.6	1.1	0.8	0.3	0.0	0.2	0.1	0.1
WB34	1	0.0	0.0	0.0	NA	4.2	4.2	4.2	NA	0.6	0.6	0.6	NA	0.7	0.7	0.7	NA	0.0	0.0	0.0	NA
WB35	1	1.1	1.1	1.1	NA	94.8	94.8	94.8	NA	5.0	5.0	5.0	NA	1.5	1.5	1.5	NA	0.0	0.0	0.0	NA
WG01	1	0.8	0.8	0.8	NA	65.3	65.3	65.3	NA	3.4	3.4	3.4	NA	1.4	1.4	1.4	NA	0.0	0.0	0.0	NA
WG02	1	0.4	0.4	0.4	NA	62.2	62.2	62.2	NA	4.3	4.3	4.3	NA	1.1	1.1	1.1	NA	0.0	0.0	0.0	NA

		ANC				DOC			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD
WB01	5	373.4	523.5	464.3	56.0	1.9	4.1	2.9	1.0
WB02	6	714.0	3263.8	2034.4	998.4	1.9	2.8	2.2	0.4
WB03	5	75.3	198.0	143.6	55.5	1.2	8.4	3.9	2.7
WB04	2	38.9	45.9	42.4	4.9	NA	NA	NA	NA
WB05	8	40.7	113.1	66.1	24.9	1.2	2.2	1.8	0.4
WB06	8	34.8	99.3	62.0	23.2	1.0	3.0	1.8	0.7
WB07	2	-6.1	32.6	13.2	27.3	NA	NA	NA	NA
WB07B	2	55.3	63.6	59.4	5.9	2.7	6.5	4.6	2.7
WB07C	2	18.4	21.3	19.8	2.0	0.9	1.1	1.0	0.2
WB09	4	13.3	286.1	118.7	117.1	1.5	2.7	2.0	0.5
WB11	3	75.2	133.5	99.0	30.6	0.6	2.0	1.3	0.7
WB12	3	75.9	107.2	88.7	16.4	0.9	1.9	1.4	0.5
WB13	6	30.8	58.8	45.5	10.7	0.9	2.4	1.6	0.5
WB14	7	16.9	58.1	36.7	14.3	1.6	4.6	2.5	1.0
WB14B	1	32.1	32.1	32.1	NA	2.9	2.9	2.9	NA
WB16	6	95.9	808.8	443.0	263.5	1.7	5.6	3.3	1.4
WB17	1	770.3	770.3	770.3	NA	NA	NA	NA	NA
WB18	1	88.7	88.7	88.7	NA	NA	NA	NA	NA
WB19	3	39.0	68.9	50.0	16.5	0.8	1.3	1.0	0.2
WB20	7	40.9	57.2	49.0	6.7	1.0	2.4	1.7	0.5
WB21	8	47.8	95.2	68.2	16.1	1.1	2.2	1.6	0.3
WB22	4	150.0	375.7	275.7	93.4	2.7	2.9	2.8	0.1
WB23	1	106.8	106.8	106.8	NA	NA	NA	NA	NA
WB23A	5	56.9	157.6	95.8	37.2	1.7	3.9	2.6	0.9
WB23B	5	201.5	460.9	370.0	101.1	2.6	4.4	3.3	0.7
WB24	8	91.4	172.6	123.2	33.6	1.5	2.9	2.5	0.4
WB25	1	102.6	102.6	102.6	NA	1.3	1.3	1.3	NA
WB26	8	683.7	1403.2	1064.6	272.4	2.9	3.5	3.2	0.2
WB27	5	24.8	50.5	35.5	11.3	0.6	1.6	1.1	0.4
WB28	1	4.3	4.3	4.3	NA	1.7	1.7	1.7	NA
WB29	1	-2.6	-2.6	-2.6	NA	1.4	1.4	1.4	NA
WB30	7	27.1	502.3	162.4	163.4	1.3	4.0	2.4	1.0
WB31	2	686.6	726.2	706.4	28.0	3.9	6.2	5.0	1.7
WB32	2	101.3	241.7	171.5	99.3	1.1	1.4	1.3	0.2
WB32A	5	359.8	670.0	529.0	118.4	2.3	3.2	2.8	0.4
WB33	2	43.3	111.5	77.4	48.2	0.1	2.3	1.2	1.6
WB34	1	66.6	66.6	66.6	NA	8.2	8.2	8.2	NA
WB35	1	158.7	158.7	158.7	NA	2.1	2.1	2.1	NA
WG01	1	66.4	66.4	66.4	NA	2.0	2.0	2.0	NA
WG02	1	73.5	73.5	73.5	NA	1.9	1.9	1.9	NA

		Temperature				DO %				Conductivity				pH				H			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
SWC01	6.0	11.4	18.2	14.2	2.3	46.3	96.0	80.7	18.0	156.0	369.1	277.6	72.7	4.6	5.9	5.4	0.5	1.3E-06	2.6E-05	7.4E-06	9.9E-06
SWC03	7.0	11.0	18.6	14.6	2.8	50.3	92.5	71.8	12.9	139.1	559.0	298.4	129.9	4.2	5.3	4.8	0.5	4.9E-06	6.6E-05	2.3E-05	2.4E-05
SWC04	7.0	7.9	17.0	12.4	3.7	64.1	109.0	90.5	18.6	62.8	157.9	87.0	32.4	3.5	4.1	3.9	0.2	7.2E-05	3.5E-04	1.5E-04	9.2E-05
SWC05	1.0	16.6	430.0	16.6	NA	99.3	99.3	99.3	NA	200.6	200.6	200.6	NA	3.8	3.8	3.8	NA	1.7E-04	1.7E-04	1.7E-04	NA
SWC06	6.0	7.6	15.9	11.1	2.9	59.8	111.5	96.5	19.9	62.8	177.5	95.7	41.2	3.4	4.0	3.8	0.2	9.5E-05	4.5E-04	2.0E-04	1.3E-04
SWC07	6.0	9.2	21.7	14.0	4.5	47.3	101.8	85.0	20.6	55.7	203.1	112.4	56.4	3.5	4.0	3.8	0.2	1.0E-04	3.5E-04	1.8E-04	8.9E-05
SWC08	6.0	8.8	16.8	12.4	2.8	71.6	103.0	86.0	12.3	57.5	206.8	108.2	53.4	3.5	4.0	3.8	0.2	1.0E-04	3.2E-04	1.8E-04	7.9E-05
SWC09	7.0	8.4	18.7	12.9	3.5	54.4	105.9	83.8	17.3	63.2	235.8	108.4	58.1	3.5	4.0	3.8	0.2	1.0E-04	3.3E-04	1.8E-04	8.0E-05
SWC10	1.0	15.5	446.0	15.5	NA	105.4	105.4	105.4	NA	226.5	226.5	226.5	NA	3.7	3.7	3.7	NA	2.1E-04	2.1E-04	2.1E-04	NA
SWC11	1.0	16.1	459.0	16.1	NA	97.4	97.4	97.4	NA	262.3	262.3	262.3	NA	3.7	3.7	3.7	NA	2.2E-04	2.2E-04	2.2E-04	NA
SWC14	7.0	8.0	23.5	12.9	5.2	73.2	120.9	95.7	17.4	63.4	189.5	91.5	44.9	3.5	4.0	3.8	0.2	1.0E-04	3.5E-04	1.7E-04	9.2E-05
SWC15	1.0	15.6	437.0	15.6	NA	107.1	107.1	107.1	NA	170.0	170.0	170.0	NA	3.8	3.8	3.8	NA	1.7E-04	1.7E-04	1.7E-04	NA
SWC16	6.0	8.4	18.4	12.2	3.6	55.4	102.0	82.9	18.0	139.8	270.5	194.5	43.5	4.8	5.7	5.4	0.3	1.9E-06	1.5E-05	5.3E-06	4.9E-06
SWC17	6.0	8.4	18.0	12.3	3.5	52.3	98.0	81.4	15.8	133.9	241.5	174.9	36.6	4.4	5.1	4.8	0.3	7.8E-06	3.6E-05	2.0E-05	1.2E-05
SWC18	6.0	8.4	16.7	12.1	3.1	81.1	122.0	100.3	16.8	94.1	129.7	108.6	12.9	3.7	4.4	4.0	0.3	4.2E-05	2.2E-04	1.1E-04	6.9E-05
SWC19	6.0	10.9	17.8	13.8	2.4	46.4	92.0	74.5	16.5	137.0	313.4	237.4	58.4	4.1	5.1	4.6	0.4	8.3E-06	8.5E-05	3.7E-05	3.0E-05
SWC20	6.0	11.0	19.7	14.3	3.3	38.1	96.0	68.1	18.7	127.2	430.2	279.1	99.5	4.0	5.0	4.5	0.4	9.1E-06	1.1E-04	4.3E-05	3.6E-05
SWC21	6.0	8.6	17.6	12.4	3.3	76.6	94.8	82.3	6.6	129.6	294.0	209.8	53.1	4.5	5.3	4.9	0.3	4.7E-06	3.0E-05	1.6E-05	9.6E-06
SWC22	6.0	8.9	19.0	13.8	3.5	45.6	121.8	88.2	24.6	270.1	521.0	397.7	82.8	6.0	6.6	6.3	0.2	2.3E-07	1.0E-06	5.7E-07	2.9E-07
SWC23	6.0	9.0	22.7	14.7	5.2	51.4	103.9	89.2	20.1	121.7	284.8	201.4	53.7	4.1	5.3	4.5	0.5	5.4E-06	8.5E-05	4.5E-05	3.3E-05
SWC24	6.0	8.6	19.5	13.6	3.7	85.9	114.1	101.6	9.4	64.7	93.4	78.7	9.6	3.1	3.9	3.7	0.3	1.2E-04	7.8E-04	2.8E-04	2.7E-04
SWC25	6.0	9.5	21.6	14.8	4.8	49.5	103.6	92.6	21.3	108.2	211.4	157.4	42.7	4.3	6.2	5.4	0.8	6.6E-07	5.0E-05	1.4E-05	2.0E-05
SWC26	6.0	9.5	20.0	14.3	4.1	51.3	109.5	92.4	22.2	121.8	311.4	214.4	72.0	4.4	6.3	5.4	0.8	5.5E-07	4.4E-05	1.4E-05	1.8E-05
SWC27	6.0	10.1	22.0	15.4	4.3	59.8	106.3	93.1	16.8	103.4	174.8	134.7	24.7	4.6	5.5	4.9	0.4	3.2E-06	2.8E-05	1.6E-05	9.6E-06
SWC28	5.0	9.6	18.1	13.1	3.3	56.6	120.0	92.0	23.1	118.7	167.4	133.0	19.7	5.2	5.7	5.5	0.2	2.1E-06	6.3E-06	3.4E-06	1.8E-06
SWC29	5.0	9.3	20.1	14.7	4.3	62.2	105.0	87.3	15.8	103.2	203.9	152.8	36.3	5.1	5.7	5.4	0.2	2.0E-06	7.2E-06	4.1E-06	2.4E-06
SWC30	5.0	9.3	19.8	14.6	4.6	52.4	103.0	90.0	21.2	115.3	176.9	136.2	25.1	4.8	5.6	5.2	0.3	2.5E-06	1.4E-05	7.2E-06	5.0E-06
SWC31	6.0	10.5	22.9	15.7	4.9	59.9	98.7	86.5	15.9	148.9	289.6	210.0	51.3	5.6	6.4	5.8	0.3	4.1E-07	2.7E-06	1.7E-06	8.3E-07
SWC32	6.0	9.5	21.1	14.0	4.1	50.6	98.0	88.1	18.6	90.2	182.1	126.7	31.1	3.9	4.7	4.3	0.3	2.1E-05	1.4E-04	6.5E-05	4.4E-05
SWC33	6.0	9.5	17.2	12.6	2.9	52.2	104.8	85.6	18.2	58.9	91.0	75.7	10.6	3.8	4.6	4.3	0.3	2.6E-05	1.4E-04	6.2E-05	4.5E-05
SWC34	6.0	9.1	17.8	12.7	3.2	71.0	107.2	87.5	13.2	109.8	172.6	134.7	20.7	3.7	4.5	4.2	0.3	3.3E-05	2.0E-04	8.4E-05	6.9E-05
SWC35	6.0	8.2	18.3	12.3	4.0	78.9	111.9	95.4	12.4	80.2	109.9	89.9	10.6	3.5	4.3	4.0	0.3	5.2E-05	3.4E-04	1.3E-04	1.1E-04
SWC36	6.0	9.9	22.3	14.4	4.5	51.5	99.8	83.7	18.5	77.6	174.8	120.2	34.3	4.1	5.1	4.5	0.4	8.1E-06	8.1E-05	4.4E-05	2.8E-05
SWC37	6.0	9.0	19.7	13.6	3.7	50.2	102.0	82.9	18.9	55.1	98.1	76.8	13.9	3.7	4.4	4.1	0.3	4.0E-05	2.2E-04	9.5E-05	7.2E-05

		Na				NH4				K				Mg				Ca			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
SWC01	3	1980.6	2370.6	2156.8	197.7	2.1	4.8	3.0	1.5	42.3	76.2	57.4	17.3	383.2	585.1	492.0	101.8	128.3	161.7	144.6	16.7
SWC03	3	298.4	2154.5	1397.4	974.2	0.0	3.5	1.4	1.9	5.1	39.3	27.7	19.6	74.6	517.3	339.0	233.6	17.2	121.0	82.2	56.7
SWC04	2	71.0	418.3	244.7	245.6	0.1	0.5	0.3	0.2	0.9	3.0	2.0	1.5	25.9	108.7	67.3	58.5	10.2	12.8	11.5	1.9
SWC06	2	330.4	473.4	401.9	101.2	0.6	1.5	1.0	0.6	3.8	5.3	4.6	1.1	100.0	131.9	115.9	22.5	22.7	47.1	34.9	17.3
SWC07	3	204.1	370.9	313.3	94.6	0.5	3.1	2.0	1.3	0.4	3.3	2.2	1.6	60.9	130.6	106.5	39.5	3.5	46.2	29.0	22.6
SWC08	3	170.4	367.9	252.4	102.9	1.7	12.3	5.8	5.6	0.5	2.0	1.4	0.8	57.6	131.1	86.1	39.4	6.2	36.5	26.3	17.4
SWC09	3	249.1	410.5	353.1	90.2	1.0	2.7	2.1	1.0	1.6	3.4	2.3	1.0	72.4	134.8	104.7	31.3	13.5	29.6	19.6	8.7
SWC14	2	311.5	330.2	320.9	13.2	0.7	2.2	1.5	1.1	7.0	8.5	7.8	1.0	94.3	99.1	96.7	3.4	30.9	51.3	41.1	14.5
SWC16	5	812.3	1657.4	1170.5	320.6	0.0	2.0	1.0	0.8	12.6	32.4	25.2	9.3	178.7	389.6	267.2	79.3	55.8	146.4	100.6	34.2
SWC17	5	581.2	1483.3	1179.8	380.4	0.9	4.3	2.3	1.5	10.1	50.3	25.9	14.8	143.6	334.3	271.4	83.2	45.8	120.1	88.6	28.3
SWC18	3	233.6	761.2	494.5	263.9	0.0	1.5	1.0	0.9	3.6	15.6	8.8	6.1	56.6	175.3	123.7	60.8	16.3	52.4	39.6	20.2
SWC19	4	330.9	2042.2	1512.7	793.8	0.1	8.9	4.3	4.0	6.0	57.3	35.5	21.4	94.6	505.9	337.4	185.5	18.8	119.2	84.3	44.6
SWC20	4	1460.5	2265.9	1896.0	338.9	0.9	20.4	7.0	9.0	15.9	49.3	31.2	13.9	344.4	587.8	458.3	111.9	82.7	154.2	118.5	32.3
SWC21	3	1310.5	1722.4	1558.0	218.2	4.9	7.5	6.0	1.4	23.9	51.4	35.8	14.1	343.6	438.8	398.9	49.5	112.1	150.3	130.6	19.1
SWC22	5	2153.8	3225.0	2916.6	456.8	0.0	8.9	4.6	3.6	35.2	78.1	49.9	16.5	390.6	752.1	623.7	148.7	307.4	402.7	375.8	39.0
SWC23	3	1116.3	1689.5	1331.0	312.4	0.7	4.8	3.2	2.1	20.7	22.8	21.5	1.2	268.0	394.3	318.5	66.9	155.7	176.1	163.9	10.7
SWC24	1	379.9	379.9	379.9	NA	1.2	1.2	1.2	NA	4.4	4.4	4.4	NA	102.0	102.0	102.0	NA	23.5	23.5	23.5	NA
SWC25	5	461.2	1293.9	1069.7	346.1	0.0	6.8	2.8	2.7	5.0	15.4	11.4	4.0	150.9	374.3	305.2	90.0	54.7	114.5	93.7	23.1
SWC26	4	1106.2	2224.0	1748.7	468.0	2.9	4.6	3.5	0.8	11.8	24.6	18.8	5.4	273.9	515.9	409.1	101.0	106.4	168.3	144.0	26.7
SWC27	5	191.3	1097.0	780.5	353.3	0.2	3.6	1.8	1.3	1.4	9.9	6.9	3.3	64.5	273.2	197.0	83.6	15.5	82.9	52.5	24.3
SWC28	5	464.5	1063.3	881.9	237.6	0.0	4.0	2.2	1.9	2.3	7.7	5.9	2.2	134.9	279.5	224.3	61.9	30.3	83.7	62.2	22.1
SWC29	4	916.5	1394.4	1220.3	210.0	0.0	2.6	1.2	1.4	1.6	3.4	2.6	0.8	179.9	358.7	293.3	78.1	22.7	53.1	33.4	13.5
SWC30	4	782.1	1077.8	973.0	138.2	0.2	2.1	1.0	0.9	1.7	5.7	3.4	1.7	199.1	303.9	263.3	46.5	19.8	55.1	34.3	15.0
SWC31	5	771.8	1654.6	1415.3	364.1	0.0	6.4	1.8	2.8	4.0	9.5	7.7	2.3	219.7	428.8	357.6	82.2	48.5	107.1	86.0	22.4
SWC32	4	651.0	1072.1	907.8	200.2	0.1	20.0	5.5	9.7	2.9	6.3	5.0	1.6	147.8	258.2	219.3	50.4	24.2	64.0	41.7	16.5
SWC33	4	209.7	560.5	449.4	161.3	0.7	8.5	3.4	3.7	0.5	3.1	1.8	1.2	51.3	160.7	120.4	47.7	12.7	49.4	30.5	15.5
SWC34	3	618.9	987.7	828.1	189.3	0.2	6.1	2.5	3.1	5.3	10.3	8.3	2.7	145.0	252.8	214.5	60.3	30.5	73.6	52.3	21.6
SWC35	4	471.1	898.4	641.1	185.0	3.1	50.6	16.2	23.0	4.3	23.4	9.9	9.1	119.4	152.2	143.3	16.0	25.3	70.8	44.5	20.6
SWC36	3	842.2	1049.9	937.0	105.0	0.0	5.2	1.8	2.9	10.4	15.4	13.1	2.5	198.7	252.3	220.2	28.3	60.8	101.5	79.7	20.5
SWC37	4	165.6	515.9	400.9	159.5	0.1	3.8	2.6	1.7	1.7	9.0	6.6	3.3	48.0	142.5	115.3	45.0	15.4	77.4	52.1	27.0

		NO3				Cl				SO4				F				PO4			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
SWC01	3	0.0	1.7	1.1	1.0	2276.9	2863.5	2530.8	301.1	226.6	353.2	281.6	64.9	1.2	1.5	1.3	0.2	0.0	0.0	0.0	0.0
SWC03	3	0.4	6.2	3.4	2.9	343.0	2501.5	1622.5	1133.6	41.5	299.7	206.4	143.2	0.4	2.0	1.4	0.9	0.0	0.0	0.0	0.0
SWC04	2	0.0	4.0	2.0	2.8	80.0	528.0	304.0	316.8	9.4	49.8	29.6	28.5	0.3	0.8	0.6	0.4	0.0	0.0	0.0	0.0
SWC06	2	3.7	6.2	4.9	1.8	419.2	620.2	519.7	142.1	35.2	39.9	37.6	3.3	0.7	1.0	0.9	0.2	0.0	0.0	0.0	0.0
SWC07	3	0.1	3.9	2.4	2.1	325.3	500.0	439.9	99.3	13.1	67.2	42.0	27.2	0.4	1.1	0.8	0.4	0.0	0.5	0.2	0.3
SWC08	3	0.1	5.5	3.3	2.9	225.5	506.3	326.3	156.3	15.0	41.1	27.8	13.1	0.3	0.7	0.5	0.2	0.0	1.1	0.7	0.6
SWC09	3	0.0	4.1	1.8	2.1	294.5	573.4	461.8	147.6	20.5	23.9	22.2	1.7	0.0	0.8	0.5	0.4	0.0	0.0	0.0	0.0
SWC14	2	8.9	9.9	9.4	0.7	362.7	411.7	387.2	34.6	43.0	46.7	44.8	2.6	0.7	1.0	0.9	0.2	0.9	4.5	2.7	2.5
SWC16	5	3.5	10.7	6.9	3.5	816.8	1961.6	1303.8	431.3	98.4	239.1	163.5	58.7	0.9	1.5	1.2	0.2	0.0	0.0	0.0	0.0
SWC17	5	1.0	7.7	3.8	2.5	667.8	1732.9	1343.9	456.1	68.0	264.4	160.9	69.6	0.0	1.6	1.0	0.7	0.0	0.0	0.0	0.0
SWC18	3	0.0	6.9	2.5	3.9	282.9	959.9	601.3	340.3	33.1	164.0	94.7	65.8	0.0	0.5	0.2	0.3	0.0	0.5	0.2	0.3
SWC19	4	1.3	10.7	7.2	4.3	394.5	2385.3	1772.3	925.0	51.3	318.8	218.3	126.5	0.5	1.6	1.3	0.6	0.0	0.5	0.1	0.3
SWC20	4	14.6	65.7	32.1	22.9	1799.1	2780.5	2292.9	419.1	130.9	279.3	197.0	64.5	0.8	1.3	1.1	0.2	0.0	0.6	0.2	0.3
SWC21	3	10.0	38.5	22.5	14.5	1473.8	2029.7	1791.1	286.2	260.2	330.0	297.0	35.1	1.6	1.7	1.7	0.1	0.0	0.5	0.2	0.3
SWC22	5	5.1	35.8	14.7	12.4	2198.5	3671.7	3204.5	597.8	263.1	460.2	358.9	73.1	2.3	3.1	2.6	0.3	0.0	46.2	9.9	20.3
SWC23	3	4.4	6.3	5.5	1.0	1224.1	1994.6	1523.9	412.7	190.7	307.6	237.8	61.7	1.0	1.2	1.1	0.1	0.0	0.8	0.3	0.5
SWC24	1	15.8	15.8	15.8	NA	477.3	477.3	477.3	NA	44.2	44.2	44.2	NA	0.8	0.8	0.8	NA	1.8	1.8	1.8	NA
SWC25	5	0.0	5.6	2.4	2.3	557.5	1537.5	1281.6	409.8	49.7	222.7	134.2	62.0	0.6	1.7	1.4	0.5	0.0	0.6	0.2	0.3
SWC26	4	0.0	7.4	4.0	3.0	1231.2	2509.2	1939.7	532.3	118.8	285.9	216.7	70.5	1.3	2.6	2.0	0.7	0.0	0.9	0.3	0.4
SWC27	5	0.0	5.2	2.1	2.8	223.4	1313.3	898.1	428.1	18.4	175.2	84.9	57.4	0.0	1.3	0.7	0.6	0.0	0.7	0.2	0.3
SWC28	5	0.1	8.9	3.6	3.6	577.1	1291.1	1091.8	295.8	25.8	93.7	58.5	24.0	1.0	1.4	1.2	0.2	0.0	1.1	0.5	0.5
SWC29	4	3.1	6.6	5.6	1.7	1123.2	1648.7	1443.2	224.8	61.3	153.7	89.1	43.5	0.7	1.1	0.9	0.1	0.0	0.6	0.1	0.3
SWC30	4	1.7	17.2	7.7	6.9	994.9	1346.6	1211.4	151.2	55.6	140.3	82.7	39.3	0.7	0.9	0.8	0.1	0.0	1.4	0.3	0.7
SWC31	5	0.4	7.1	4.0	2.5	961.1	1990.0	1705.1	421.4	55.8	185.4	112.9	46.4	1.4	1.7	1.6	0.1	0.0	1.4	0.4	0.6
SWC32	4	0.5	6.8	3.1	2.7	735.2	1274.7	1056.4	254.9	52.3	154.6	96.2	42.6	0.9	1.4	1.2	0.2	0.0	0.1	0.0	0.0
SWC33	4	0.4	4.6	1.7	1.9	262.1	684.0	545.4	194.8	19.6	52.7	37.7	13.9	0.2	0.5	0.4	0.1	0.0	0.0	0.0	0.0
SWC34	3	1.1	25.9	10.0	13.8	691.2	1195.5	960.6	253.9	69.3	190.1	126.2	60.7	0.4	0.7	0.6	0.2	0.0	0.8	0.3	0.5
SWC35	4	0.0	10.7	4.9	4.4	546.4	1065.2	759.7	227.4	64.4	161.8	95.7	44.8	0.0	0.6	0.4	0.3	0.0	1.2	0.3	0.6
SWC36	3	1.7	6.2	3.9	2.3	1030.9	1287.1	1119.0	145.6	98.2	137.5	111.8	22.3	0.9	1.3	1.1	0.2	0.0	0.3	0.1	0.2
SWC37	4	0.0	5.8	2.4	2.8	211.0	696.9	521.7	216.3	22.6	62.1	48.6	18.0	0.0	1.0	0.6	0.5	0.0	0.6	0.3	0.3

		ANC				DOC			
Site Code	N	Min	Max	Mean	SD	Min	Max	Mean	SD
SWC01	3	24.5	58.2	37.3	18.3	10.4	14.2	11.9	2.0
SWC03	3	-4.2	36.0	14.1	20.4	6.3	9.5	7.9	1.6
SWC04	2	-38.8	18.6	-10.1	40.6	15.4	31.5	23.4	11.4
SWC06	2	-31.9	22.2	-4.8	38.3	18.2	34.3	26.3	11.4
SWC07	3	-69.6	-10.6	-33.4	31.7	7.4	33.1	21.0	12.9
SWC08	3	-27.5	69.4	8.8	52.9	26.0	45.6	34.1	10.2
SWC09	3	-75.3	34.3	-6.1	60.2	16.2	33.1	25.8	8.7
SWC14	2	-3.3	53.4	25.0	40.1	17.6	26.4	22.0	6.2
SWC16	5	25.1	154.3	89.2	57.2	4.6	26.8	10.8	9.1
SWC17	5	17.3	103.0	57.2	31.6	4.9	9.1	6.9	1.8
SWC18	3	-47.0	-6.2	-31.9	22.3	6.1	17.1	9.7	6.4
SWC19	4	-111.2	16.3	-27.9	57.5	6.2	30.2	13.3	11.4
SWC20	4	-57.6	4.5	-17.8	29.0	2.5	21.5	11.4	7.9
SWC21	3	5.5	18.9	12.7	6.8	4.8	9.6	7.2	2.4
SWC22	5	226.7	682.4	387.9	175.9	6.7	10.6	8.3	1.6
SWC23	3	50.8	83.5	67.8	16.4	4.9	34.2	15.5	16.2
SWC24	1	-27.5	-27.5	-27.5	NA	32.9	32.9	32.9	NA
SWC25	5	42.9	99.0	61.8	22.2	4.3	49.8	17.8	18.6
SWC26	4	140.9	187.2	160.3	22.9	8.5	47.5	21.2	17.8
SWC27	5	-0.2	136.6	51.8	51.5	1.4	5.3	4.1	1.6
SWC28	5	-5.4	72.4	20.4	32.1	2.4	4.2	3.3	0.7
SWC29	4	-82.5	159.7	11.9	103.7	2.2	2.4	2.3	0.1
SWC30	4	-80.4	16.2	-27.8	44.4	1.8	2.5	2.2	0.3
SWC31	5	26.6	71.4	44.7	21.9	2.6	61.3	15.3	25.7
SWC32	4	5.8	37.9	18.2	14.5	4.7	14.7	8.8	4.3
SWC33	4	-27.3	62.9	17.4	42.0	2.7	6.0	4.0	1.5
SWC34	3	-14.1	38.1	6.4	27.8	3.5	10.2	6.2	3.5
SWC35	4	-73.3	69.1	-21.3	62.4	3.7	22.6	9.7	8.8
SWC36	3	-9.5	64.5	15.3	42.7	6.1	9.5	7.4	1.8
SWC37	4	-38.8	65.1	2.1	44.5	3.1	9.8	6.3	3.2

Appendix 7: Kruskal-Wallis rank sum test results

Dunn (1964) Kruskal-Wallis multiple comparison. p-values adjusted (adj) with the Bonferroni method. Non-significant values are highlighted in yellow.

Difference in temperature across study regions

Kruskal-Wallis chi-squared = 38.636, df = 2, p-value = 4.077e-09

Comparison	***Z***	***P.unadj***	***P.adj***
1 HV - SWC	2.935711	3.327843e-03	9.983530e-03
2 HV - WB	-2.070911	3.836715e-02	1.151014e-01
3 SWC - WB	-6.202965	5.540902e-10	1.662271e-09

Difference in DO across study regions

Kruskal-Wallis chi-squared = 0.63792, df = 2, p-value = 0.7269

Comparison	***Z***	***P.unadj***	***P.adj***
1 HV - SWC	-0.64553547	0.5185802	1
2 HV - WB	-0.08734928	0.9303939	1
3 SWC - WB	0.69571270	0.4866088	1

Difference in EC across study regions

Kruskal-Wallis chi-squared = 43.092, df = 2, p-value = 4.392e-10

Comparison	***Z***	***P.unadj***	***P.adj***
1 HV - SWC	-2.209870	2.711420e-02	8.134261e-02
2 HV - WB	3.079059	2.076555e-03	6.229666e-03
3 SWC - WB	6.541116	6.106151e-11	1.831845e-10

Difference in pH across study regions

Kruskal-Wallis chi-squared = 52.703, df = 2, p-value = 3.595e-12

Comparison	***Z***	***P.unadj***	***P.adj***
1 HV - SWC	6.810693	9.712970e-12	2.913891e-11
2 HV - WB	2.731129	6.311783e-03	1.893535e-02

3 SWC - WB	-5.111960	3.188328e-07	9.564983e-07

Difference in H across study regions

Kruskal-Wallis chi-squared = 51.997, df = 2, p-value = 5.117e-12

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	-6.770743	1.281227e-11	3.843682e-11
2 HV - WB	-2.728386	6.364501e-03	1.909350e-02
3 SWC - WB	5.065625	4.070616e-07	1.221185e-06

Difference in Na across study regions

Kruskal-Wallis chi-squared = 48.343, df = 2, p-value = 3.18e-11

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	-4.089747	4.318442e-05	1.295533e-04
2 HV - WB	1.399896	1.615445e-01	4.846335e-01
3 SWC - WB	6.812752	9.574887e-12	2.872466e-11

Difference in NH4 across study regions

Kruskal-Wallis chi-squared = 33.725, df = 2, p-value = 4.751e-08

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	1.763215	7.786418e-02	2.335925e-01
2 HV - WB	5.276837	1.314327e-07	3.942981e-07
3 SWC - WB	4.303389	1.682049e-05	5.046146e-05

Difference in K across study regions

Kruskal-Wallis chi-squared = 16.431, df = 2, p-value = 0.0002704

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	3.2489238	0.0011584252	0.0034752756
2 HV - WB	3.9801738	0.0000688649	0.0002065947
3 SWC - WB	0.8580631	0.3908576489	1.0000000000

Difference in Mg across study regions

Kruskal-Wallis chi-squared = 33.47, df = 2, p-value = 5.396e-08

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	0.2921248	7.701912e-01	1.000000e+00
2 HV - WB	4.4666320	7.946066e-06	2.383820e-05
3 SWC - WB	5.1363140	2.801794e-07	8.405381e-07

Difference in Ca across study regions

Kruskal-Wallis chi-squared = 22.564, df = 2, p-value = 1.26e-05

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	3.722260	1.974479e-04	5.923438e-04
2 HV - WB	4.688521	2.751863e-06	8.255590e-06
3 SWC - WB	1.141268	2.537584e-01	7.612751e-01

Difference in NO3 across study regions

Kruskal-Wallis chi-squared = 38.818, df = 2, p-value = 3.722e-09

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	0.05892014	9.530157e-01	1.000000e+00
2 HV - WB	4.64366036	3.422901e-06	1.026870e-05
3 SWC - WB	5.64447752	1.656836e-08	4.970509e-08

Difference in Cl across study regions

Kruskal-Wallis chi-squared = 54.801, df = 2, p-value = 1.259e-12

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	-5.9508644	2.667300e-09	8.001900e-09
2 HV - WB	-0.7553009	4.500684e-01	1.000000e+00
3 SWC - WB	6.4748997	9.487496e-11	2.846249e-10

Difference in SO4 across study regions

Kruskal-Wallis chi-squared = 55.037, df = 2, p-value = 1.119e-12

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	-2.841205	4.494340e-03	1.348302e-02
2 HV - WB	3.150125	1.632004e-03	4.896013e-03
3 SWC - WB	7.414218	1.223447e-13	3.670342e-13

Difference in F across study regions

Kruskal-Wallis chi-squared = 23.949, df = 2, p-value = 6.303e-06

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	4.7321740	2.221279e-06	6.663836e-06
2 HV - WB	4.1098217	3.959647e-05	1.187894e-04
3 SWC - WB	-0.8279706	4.076872e-01	1.000000e+00

Difference in PO4 across study regions

Kruskal-Wallis chi-squared = 3.7405, df = 2, p-value = 0.1541

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	1.226504	0.2200089	0.6600268
2 HV - WB	1.931387	0.0534352	0.1603056
3 SWC - WB	0.851949	0.3942424	1.0000000

Difference in ANC across study regions

Kruskal-Wallis chi-squared = 46.71, df = 2, p-value = 7.197e-11

Comparison	*Z*	*P.unadj*	*P.adj*
1 HV - SWC	6.596366	4.213582e-11	1.264075e-10
2 HV - WB	3.122684	1.792098e-03	5.376295e-03
3 SWC - WB	-4.363137	1.282106e-05	3.846318e-05

Difference in DOC across study regions

Kruskal-Wallis chi-squared = 40.638, df = 2, p-value = 1.498e-09

Comparison	***Z***	***P.unadj***	***P.adj***
1 HV - SWC	-4.2157428	2.489574e-05	7.468721e-05
2 HV - WB	0.6934389	4.880342e-01	1.000000e+00
3 SWC - WB	6.0996655	1.062906e-09	3.188719e-09

Difference in Altitude across study regions

Kruskal-Wallis chi-squared = 73.665, df = 2, p-value < 2.2e-16

Comparison	Z	P.unadj	P.adj
1 HV - SWC	8.408652	4.147512e-17	1.244254e-16
2 HV - WB	3.740179	1.838891e-04	5.516672e-04
3 SWC - WB	-5.612467	1.994620e-08	5.983861e-08

Appendix 8: Species recorded at the Albany Museum General (Gen) cataloguing book

Several species were discovered during this project and a few of these were added to the Albany Museum Gen book. Details are given below. For consistency and convenience, the records entered here are as recorded at the Albany Museum.

GEN 2508
WB16 – Waterberg (Limpopo), on route to Marakele via Bakker's Pass
Date: 10 February 2016
Coordinates: -24.48333 S; 27.69739 E
Collected by: Londiwe Khuzwayo
Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2508A
Ephemeroptera
Cloeon sp.

GEN 2509

WB22 – Waterberg (Limpopo), Dwarsrivier

Date: 10 February 2015

Coordinates: -24.27186 S; 28.19981 E

Collected by: Londiwe Khuzwayo

Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2509A

Ephemeroptera

Demoreptus sp.

Picture not available

GEN 2510

WB26 – Waterberg (Limpopo), Sterkrivier

Date: 01 June 2015

Coordinates: -24.23869 S; 28.79875 E

Collected by: Londiwe Khuzwayo

Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2510A

Ephemeroptera

Cheleocloeon Falcatum (?)

GEN 2511

WB26 – Waterberg (Limpopo), Sterkrivier

Date: 08 February 2016

Coordinates: -24.23869 S; 28.79875 E

Collected by: Londiwe Khuzwayo

Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2511A

Ephemeroptera

Pseudocloeon sp.

GEN 2512

EHV16 – Eastern Highveld (Mpumalanga), Swartkoppiespruit

Date: 23 May 2015

Coordinates: -25.54957 S; 30.32135 E

Collected by: Londiwe Khuzwayo

Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2512A

Ephemeroptera

Susua sp.

GEN 2514

EHV9 – Eastern Highveld, Mpumalanga

Date: 22 May 2015

Coordinates: -25.36200 S; 29.99858 E

Collected by: Londiwe Khuzwayo

Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2514A

Ephemeroptera

Prosopistoma Crassi

GEN 2515

SWC16 – South-western Cape, Diepwalle (Kleineiland Rivier)

Date: 04 November 2015

Coordinates: -33.9877 S; 23.19638 E

Collected by: Londiwe Khuzwayo

Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2515A

Ephemeroptera

Baetis (unknown)

GEN 2515B

Ephemeroptera

Demoreptus capensis

GEN 2516

SWC28 – South-western Cape, Bergplaas (Klienplat)

Date: 05 November 2015

Coordinates: -33.8615 S; 22.68382 E

Collected by: Londiwe Khuzwayo

Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2516A

Ephemeroptera

Demoreptus monticola

GEN 2517

SWC26 – South-western Cape, Silwer Rivier

Date: 05 Nov 2015

Coordinates: -33.965 S; 22.56153 E

Collected by: Londiwe Khuzwayo

Determined by: Londiwe Khuzwayo and Dr Helen James

GEN 2517A

Ephemeroptera

Cloeodes sp. (unknown)

Appendix 9: Full species list per site: α-diversity

Matrix contents: Total number of sites (Sample units) – 56 Sample units
Species number – 164 Species

Sample Unit	Species code	Species name	α-diversity
HV4	Ne.ni.BF	*Simulium nevermannia nigritarse*	3
	Ch.sp2.CH	*Chironominae sp2*	1
	Po.sp3.CH	*Polypedilum sp3*	5
	Po.sp7.CH	*Polypedilum sp7*	1
	Ta.sp4.CH	*Tanytarsus sp4*	1
	Ps.sp1.CH	*Psectrocladius sp1*	2
EHV01	Che.ex.MF	*Cheleocloeon excisum*	8
	Che.sp.MF	*Cheleocloeon sp.*	62
	Ca.sp1.MF	*Caenis Type1*	50
	Ps.gl.MF	*Pseudocloeon glaucum*	57
	Ps.pi.MF	*Pseudocloeon piscis*	1
	Ps.la.MF	*Pseudocloeon latum*	2
	Af.su.MF	*Afroptilum sudafricanum*	1
	Co.be.MF	*Compsoneuria bequaerti*	2
	Cr.fl.MF	*Crassabwa flava*	11
	Ba.ha.MF	*Baetis harrisoni*	47
	Eu.sp.MF	*Euthraulus sp.*	255
	Cl.un.sp.MF	*Cloeon unknown sp.*	5
	Tr.re.MF	*Tricorythus reticulatus*	5
	Po.sp3.CH	*Polypedilum sp3*	1
	Cric.sp.CH	*Cricotopus sp.*	2
	Ge.sp1.CH	*Georthocladius sp1*	1
	Param.sp.CH	*Parametriocnemus sp.*	4
	To.sp.CH	*Tokunagaia sp.*	1
	Na.sp.CH	*Natarsia sp.*	2
EHV02	Ca.sp1.MF	*Caenis Type1*	22
	Ps.pi.MF	*Pseudocloeon piscis*	1
	Af.ol.MF	*Afronurus oliffi*	1
	Ba.ha.MF	*Baetis harrisoni*	30
	Eu.sp.MF	*Euthraulus sp.*	162
	Tr.re.MF	*Tricorythus reticulatus*	310
	Pr.cr.MF	*Prosopistoma crassi*	8
	Ne.sp.SF	*Neoperla spio*	1
	Cl.sp1.CH	*Cladotanytarsus sp1*	1

	Po.sp3.CH	*Polypedilum sp3*	1
	Param.sp.CH	*Parametriocnemus sp.*	1
EHV04	Ca.sp1.MF	*Caenis Type1*	39
	Ps.gl.MF	*Pseudocloeon glaucum*	4
	Ps.pi.MF	*Pseudocloeon piscis*	3
	Co.be.MF	*Compsoneuria bequaerti*	1
	Ba.ha.MF	*Baetis harrisoni*	74
	Eu.sp.MF	*Euthraulus sp.*	98
	Tr.re.MF	*Tricorythus reticulatus*	103
	Pr.cr.MF	*Prosopistoma crassi*	5
	Ne.sp.SF	*Neoperla spio*	8
	Ed.da.BF	*Simulium edwardsellum damnosum*	9
	Po.im.BF	*Simulium pomeroyellum impukane*	1
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Cl.sp2.CH	*Cladotanytarsus sp2*	1
	Lau.sp.CH	*Lauterborniella sp.*	1
	Po.sp2.CH	*Polypedilum sp2*	1
	Po.sp4.CH	*Polypedilum sp4*	2
	Po.sp7.CH	*Polypedilum sp7*	2
	Cha.sp.CH	*Chaetocladius sp.*	2
	Eu.sp2.CH	*Eukiefferiella sp2*	2
	Eu.sp3.CH	*Eukiefferiella sp3*	2
	Eu.sp4.CH	*Eukiefferiella sp4*	1
	Lim.sp.CH	*Limnophyes sp.*	2
	Param.sp.CH	*Parametriocnemus sp.*	28
	Rh.sp1.CH	*Rheocricotopus sp1*	1
	Rh.sp2.CH	*Rheocricotopus sp2*	13
	To.sp.CH	*Tokunagaia sp.*	4
	Cl.sp.CH	*Clinotanypus sp.*	1
EHV05	Ca.sp1.MF	*Caenis Type1*	4
	Ca.sp2.MF	*Caenis Type2*	7
	Ba.ha.MF	*Baetis harrisoni*	11
	Eu.sp.MF	*Euthraulus sp.*	17
	Tr.re.MF	*Tricorythus reticulatus*	73
EHV07	Ca.sp1.MF	*Caenis Type1*	10
	Ps.pi.MF	*Pseudocloeon piscis*	7
	Cl.sp1.CH	*Cladotanytarsus sp1*	1
	Po.sp4.CH	*Polypedilum sp4*	1
	Po.sp7.CH	*Polypedilum sp7*	5
	Param.sp.CH	*Parametriocnemus sp.*	1
	An.sp.CH	*Antillocladius sp.*	1

EHV08	Ca.sp1.MF	*Caenis Type1*	2
	Ca.sp2.MF	*Caenis Type2*	3
	Af.su.MF	*Afroptilum sudafricanum*	11
	Ba.ha.MF	*Baetis harrisoni*	8
	Ad.au.MF	*Adenophlebia auriculata*	54
	Eu.sp.MF	*Euthraulus sp.*	188
	Tr.re.MF	*Tricorythus reticulatus*	81
	El.tr.MF	*Elassoneuria trimeniana*	26
	Lausp.CH	*Lauterborniella sp.*	1
	Po.sp4.CH	*Polypedilum sp4*	1
	Or.sp1.CH	*Orthocladius sp1*	1
	Param.sp.CH	*Parametriocnemus sp.*	1
EHV09	Ca.sp1.MF	*Caenis Type1*	3
	Ca.sp2.MF	*Caenis Type2*	1
	Ps.pi.MF	*Pseudocloeon piscis*	7
	Af.su.MF	*Afroptilum sudafricanum*	11
	Af.ol.MF	*Afronurus oliffi*	2
	Cr.fl.MF	*Crassabwa flava*	3
	Ba.ha.MF	*Baetis harrisoni*	24
	Ad.au.MF	*Adenophlebia auriculata*	1
	Eu.sp.MF	*Euthraulus sp.*	5
	Ac.va.MF	*Acanthiops varius*	2
	Tr.re.MF	*Tricorythus reticulatus*	10
	El.tr.MF	*Elassoneuria trimeniana*	11
	Ta.sp2.CH	*Tanytarsus sp2*	2
	Ta.sp3.CH	*Tanytarsus sp3*	1
	Cric.sp.CH	*Cricotopus sp.*	1
	Eu.sp1.CH	*Eukiefferiella sp1*	1
	Param.sp.CH	*Parametriocnemus sp.*	24
	Rh.sp2.CH	*Rheocricotopus sp2*	2
	To.sp.CH	*Tokunagaia sp.*	3
EHV12	Ca.sp1.MF	*Caenis Type1*	1
	Af.ol.MF	*Afronurus oliffi*	7
	Ba.ha.MF	*Baetis harrisoni*	4
	Eu.sp.MF	*Euthraulus sp.*	51
	Tr.re.MF	*Tricorythus reticulatus*	127
	El.tr.MF	*Elassoneuria trimeniana*	25
	Ne.sp.SF	*Neoperla spio*	1
	Ed.da.BF	*Simulium edwardsellum damnosum*	1
	Me.ch.BF	*Simulium metomphalus chutteri*	2
	An.de.BF	*Simulium anasolen dentulosum*	1

	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	2
	Po.al.BF	*Simulium pomeroyellum alcocki*	2
	Rh.sp2.CH	*Rheocricotopus sp2*	2
EHV13	Che.ex.MF	*Cheleocloeon excisum*	2
	Che.sp.MF	*Cheleocloeon sp.*	4
	Ca.sp1.MF	*Caenis Type1*	3
	Ps.gl.MF	*Pseudocloeon glaucum*	1
	Af.ol.MF	*Afronurus oliffi*	1
	Cr.fl.MF	*Crassabwa flava*	5
	Ba.ha.MF	*Baetis harrisoni*	3
	Eu.sp.MF	*Euthraulus sp.*	13
	Tr.re.MF	*Tricorythus reticulatus*	37
	Pr.cr.MF	*Prosopistoma crassi*	11
	Ne.sp.SF	*Neoperla spio*	3
EHV15	Po.sp4.CH	*Polypedilum sp4*	2
	Param.sp.CH	*Parametriocnemus sp.*	1
	Rh.sp2.CH	*Rheocricotopus sp2*	3
EHV16	Ca_sp1_MF	*Caenis Type1*	1
	Ps.pi.MF	*Pseudocloeon piscis*	2
	Co.be.MF	*Compsoneuria bequaerti*	4
	Af.ol.MF	*Afronurus oliffi*	35
	Cr.fl.MF	*Crassabwa flava*	32
	Ad.sp.MF	*Adenophlebia sp.*	12
	Eu.sp.MF	*Euthraulus sp.*	30
	Ps.ma.MF	*Pseudopannota maculosa*	2
	Tr.re.MF	*Tricorythus reticulatus*	277
	El.tr.MF	*Elassoneuria trimeniana*	7
	Su.sp.MF	*Susua sp.*	2
	Ne.sp.SF	*Neoperla spio*	22
	Ed.da.BF	*Simulium edwardsellum damnosum*	5
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	1
	Ch.sp1.CH	*Chironominae sp1*	1
	Cl.sp.CH	*Clinotanypus sp.*	1
EHV19	Che.sp.MF	*Cheleocloeon sp.*	2
	Ca.sp1.MF	*Caenis Type1*	1
	Cr.fl.MF	*Crassabwa flava*	2
	Eu.sp.MF	*Euthraulus sp.*	7
	Ps.ma.MF	*Pseudopannota maculosa*	2
	Or.sp2.CH	*Orthocladius sp2*	1
EHV20	Ma.sp.CH	*Macropelopia sp.*	1
WB03	Da.in.MF	*Dabulamanzia indusii*	1

	Ma.ca.CF	*Macrostemum capense*	24
	Ch.sp1.CF	*Cheumatopsyche sp1*	38
	Param.sp.CH	*Parametriocnemus sp.*	3
	To.sp.CH	*Tokunagaia sp.*	1
WB05	Che.ex.MF	*Cheleocloeon excisum*	1
	Cl.sp.MF	*Cloeodes sp.*	2
	Ca.sp3.MF	*Caenis Type3*	1
	Ps.la.MF	*Pseudocloeon latum*	1
	Ba.ha.MF	*Baetis harrisoni*	1
	De.ca.MF	*Demoreptus capensis*	1
	Eu.sp.MF	*Euthraulus sp.*	1
	Ps.un.sp2.MF	*Pseudocloeon unknown sp2*	1
	Ps.ma.MF	*Pseudopannota maculosa*	21
	Che.fa.MF	*Cheleocloeon falcatum*	6
	De.cr.MF	*Demoulinia crassi*	1
	Ne.sp.SF	*Neoperla spio*	5
	Ed.da.BF	*Simulium edwardsellum damnosum*	2
	An.de.BF	*Simulium anasolen dentulosum*	1
	Me.vo.BF	*Simulium metomphalus vorax*	1
	Po.un.BF	*Simulium pomeroyellum unicornutum*	1
	Ma.ca.CF	*Macrostemum capense*	4
	Ch.sp1.CF	*Cheumatopsyche sp1*	77
	Ec.th.CF	*Ecnomus thomasseti*	1
	Sc.ob.CF	*Sciadorus obtusus*	3
	Ch.Sp5.CH	*Chironominae Sp5*	1
	Co.sp1.CH	*Corynoneura sp1*	1
	Ge.sp1.CH	*Georthocladius sp1*	2
	Or.sp.CH	*Orthocladiinae sp.*	3
	Or.sp1.CH	*Orthocladius sp1*	4
WB06	Ad.bi.MF	*Adenophlebiodes bicolor*	1
WB07C	Me.ch.BF	*Simulium metomphalus chutteri*	1
	Ec.th.CF	*Ecnomus thomasseti*	2
	Ec.op.CF	*Ecnomus oppidamus*	2
	Ec.ki.CF	*Ecnomus kimminsi*	2
	Co.sp2.CH	*Corynoneura sp2*	1
	Parac.sp.CH	*Paracricotopus sp.*	3
WB12	Da.in.MF	*Dabulamanzia indusii*	2
	Ps.pi.MF	*Pseudocloeon piscis*	2
	Cr.fl.MF	*Crassabwa flava*	1
	Ba.un.sp1.MF	*Baetis unkown sp1*	1
	Ps.ma.MF	*Pseudopannota maculosa*	7

	Che.fa.MF	*Cheleocloeon falcatum*	2
	Ne.sp.SF	*Neoperla spio*	1
	Ed.da.BF	*Simulium edwardsellum damnosum*	3
	An.de.BF	*Simulium anasolen dentulosum*	43
	Ne.ni.BF	*Simulium nevermannia nigritarse*	8
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	1
	Ma.ca.CF	*Macrostemum capense*	4
	Ch.sp1.CF	*Cheumatopsyche sp1*	6
	Ph.ch.sp.CF	*Philopotamidae chimarra sp.*	1
	Le.sp.CF	*Leptocerid sp.*	3
	un.c.CF	*unidentified case*	1
	St.sp.CH	*Stenochironomus sp.*	1
	Ps.sp4.CH	*Psectrocladius sp4*	6
	To.sp.CH	*Tokunagaia sp.*	1
WB13	Da.in.MF	*Dabulamanzia indusii*	4
	Cl.sp.MF	*Cloeodes sp.*	3
	Ca.sp3.MF	*Caenis Type3*	2
	Af.su.MF	*Afroptilum sudafricanum*	1
	Cr.fl.MF	*Crassabwa flava*	1
	Ba.ha.MF	*Baetis harrisoni*	7
	Ba.un.sp1_MF	*Baetis unkown sp1*	3
	Ca.ca.MF	*Castanophlebia calida*	8
	Eu.sp.MF	*Euthraulus sp.*	15
	Ps.un.sp2.MF	*Pseudocloeon unknown sp2*	1
	Ps.ma.MF	*Pseudopannota maculosa*	4
	Che.fa.MF	*Cheleocloeon falcatum*	4
	Ac.va.MF	*Acanthiops varius*	2
	Ne.sp.SF	*Neoperla spio*	18
	Ed.da.BF	*Simulium edwardsellum damnosum*	2
	Ch.sp1.CF	*Cheumatopsyche sp1*	6
	Ch.sp2.CF	*Cheumatopsyche sp2*	40
	Ch.sp3.CF	*Cheumatopsyche sp3*	34
	Ph.ch.sp.CF	*Philopotamidae chimarra sp.*	1
	Hy.cr.CF	*Hydroptila cruciata*	1
	Or.sp.CF	*Orthotrichia sp.*	1
	Ta.sp2.CH	*Tanytarsus sp2*	1
	He.sp.CH	*Heterotrissocladius sp.*	5
	Or.sp1.CH	*Orthocladius sp1*	3
	Or.sp2.CH	*Orthocladius sp2*	10
	Parak.sp.CH	*Parakiefferiella sp.*	1
	Param.sp.CH	*Parametriocnemus sp.*	8

	Rh.sp2.CH	*Rheocricotopus sp2*	5
	To.sp.CH	*Tokunagaia sp.*	2
WB14	Af.su.MF	*Afroptilum sudafricanum*	1
	Ps.ma.MF	*Pseudopannota maculosa*	2
	Che.fa.MF	*Cheleocloeon falcatum*	1
	Ne.sp.SF	*Neoperla spio*	2
	Ed.da.BF	*Simulium edwardsellum damnosum*	1
	Me.vo.BF	*Simulium metomphalus vorax*	7
	Ma.ca.CF	*Macrostemum capense*	19
	Ch.sp1.CF	*Cheumatopsyche sp1*	345
	Ph.ch.sp.CF	*Philopotamidae chimarra sp.*	2
	Di.me.CF	*Diplectronella medialis*	22
	Oe.mo.CF	*Oecetis modesta*	1
	Le.sp.CF	*Leptocerid sp.*	1
	Tr.sp.c.CF	*Trichosetodes sp. case*	1
	Le.c.CF	*Leptocerid case*	1
	Ch.c.CF	*Cheumatopsyche case*	6
	Di.c.CF	*Diplectronella case*	2
	Lau.sp.CH	*Lauterborniella sp.*	1
	Param.sp.CH	*Parametriocnemus sp.*	27
	Rh.sp2.CH	*Rheocricotopus sp2*	7
	To.sp.CH	*Tokunagaia sp.*	1
WB16	Da.in.MF	*Dabulamanzia indusii*	3
	Cl.sp.MF	*Cloeodes sp.*	1
	Ca.sp3.MF	*Caenis Type3*	6
	Ps.gl.MF	*Pseudocloeon glaucum*	2
	Af.su.MF	*Afroptilum sudafricanum*	12
	Ba.un.sp1.MF	*Baetis unkown sp1*	2
	Eu.sp.MF	*Euthraulus sp.*	11
	De.cr.MF	*Demoulinia crassi*	12
	Cl.un.sp.MF	*Cloeon unknown sp.*	48
	At.sp.CF	*Athripsodes sp*	3
	Or.sp.CF	*Orthotrichia sp.*	2
	Po.sp3.CH	*Polypedilum sp3*	4
	Po.sp7.CH	*Polypedilum sp7*	1
	Rh.sp2.CH	*Rheocricotopus sp2*	1
WB19	Ec.sp.CF	*Ecnomus sp.*	2
	At.sp.CF	*Athripsodes sp*	1
	At.sc.CF	*Athripsodes sand cases*	1
WB22	Da.in.MF	*Dabulamanzia indusii*	2
	Co.be.MF	*Compsoneuria bequaerti*	2

	Cr.fl.MF	*Crassabwa flava*	1
	Ba.un.sp1.MF	*Baetis unkown sp1*	1
	Ba.un.sp2.MF	*Baetis unkown sp2*	1
	De.un.sp.MF	*Demoreptus unknown sp.*	4
	Eu.sp.MF	*Euthraulus sp.*	50
	Ps.un.sp2.MF	*Pseudocloeon unknown sp2*	1
	Ps.ma.MF	*Pseudopannota maculosa*	5
	Che.fa.MF	*Cheleocloeon falcatum*	1
	De.cr.MF	*Demoulinia crassi*	1
	Cl.un.sp.MF	*Cloeon unknown sp.*	1
	Ne.sp.SF	*Neoperla spio*	3
	Ed.da.BF	*Simulium edwardsellum damnosum*	8
	Ma.ca.CF	*Macrostemum capense*	53
	Ch.sp1.CF	*Cheumatopsyche sp1*	19
	Ch.sp2.CF	*Cheumatopsyche sp2*	3
	Ch.sp3.CF	*Cheumatopsyche sp3*	7
	Ch.sp4.CF	*Cheumatopsyche sp4*	2
	Ch.sp5.CF	*Cheumatopsyche sp5*	2
	Hy.cr.CF	*Hydroptila cruciata*	1
	At.sp.CF	*Athripsodes sp*	1
	Lau.sp.CH	*Lauterborniella sp.*	1
	To.sp.CH	*Tokunagaia sp.*	1
WB23A	Da.in.MF	*Dabulamanzia indusii*	1
	Cl.sp.MF	*Cloeodes sp.*	4
	Ca.sp1.MF	*Caenis Type1*	3
	Ca.sp3.MF	*Caenis Type3*	16
	Ps.la.MF	*Pseudocloeon latum*	2
	Af.su.MF	*Afroptilum sudafricanum*	2
	Ba.ha.MF	*Baetis harrisoni*	35
	Ps.un.sp2.MF	*Pseudocloeon unknown sp2*	3
	Ps.ma.MF	*Pseudopannota maculosa*	10
	Che.fa.MF	*Cheleocloeon falcatum*	3
	Ed.da.BF	*Simulium edwardsellum damnosum*	8
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	4
	Ma.ca.CF	*Macrostemum capense*	12
	Ch.sp1.CF	*Cheumatopsyche sp1*	102
	Tr.sp.CF	*Trichosetodes sp.*	2
	Ch.th.CF	*Cheumatopsyche thomasseti*	1
	Cl.sp2.CH	*Cladotanytarsus sp2*	2
WB24	Cl.sp.MF	*Cloeodes sp.*	2

	Ca.sp3.MF	*Caenis Type3*	1
	Eu.sp.MF	*Euthraulus sp.*	3
	Ps.un.sp2.MF	*Pseudocloeon unknown sp2*	1
	Ps.ma.MF	*Pseudopannota maculosa*	1
	Ad.bi.MF	*Adenophlebiodes bicolor*	1
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	2
	Ma.ca.CF	*Macrostemum capense*	4
	Ch.sp1.CF	*Cheumatopsyche sp1*	13
	Ch.sp2.CF	*Cheumatopsyche sp2*	2
	Ph.ch.sp.CF	*Philopotamidae chimarra sp.*	1
	Le.na.CF	*Leptonema natalense*	1
	Ti.sp.CF	*Tinodes sp.*	2
	Ch.us.sp1.CH	*Chironomus sp1*	16
	Lau.sp.CH	*Lauterborniella sp.*	1
	Po.sp3.CH	*Polypedilum sp3*	1
	Ge.sp2.CH	*Georthocladius sp2*	2
	Ps.sp3.CH	*Psectrocladius sp3*	7
	Rh.sp2.CH	*Rheocricotopus sp2*	1
	Cl.sp.CH	*Clinotanypus sp.*	2
	Rh.sp.CH	*Rheopelopia sp.*	2
WB25	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	6
	Ma.ca.CF	*Macrostemum capense*	1
	Ec.sp.CF	*Ecnomus sp.*	3
	Ch.us.sp1.CH	*Chironomus sp1*	1
	Param.sp.CH	*Parametriocnemus sp.*	1
WB26	Da.in.MF	*Dabulamanzia indusii*	17
	Che.ex.MF	*Cheleocloeon excisum*	4
	Cl.sp.MF	*Cloeodes sp.*	36
	Ca.sp1.MF	*Caenis Type1*	32
	Ca.sp2.MF	*Caenis Type2*	2
	Ca.sp3.MF	*Caenis Type3*	7
	Af.su.MF	*Afroptilum sudafricanum*	2
	Co.be.MF	*Compsoneuria bequaerti*	1
	Af.ol.MF	*Afronurus oliffi*	2
	Eu.sp.MF	*Euthraulus sp.*	227
	Ps.un.sp2.MF	*Pseudocloeon unknown sp2*	17
	Ps.ma.MF	*Pseudopannota maculosa*	29
	Che.fa.MF	*Cheleocloeon falcatum*	11
	De.cr.MF	*Demoulinia crassi*	4
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Me.ad.BF	*Simulium meilloniellum adersi*	1

	Po.un.BF	*Simulium pomeroyellum unicornutum*	1
	Ch.sp1.CF	*Cheumatopsyche sp1*	47
	Ph.ch.sp.CF	*Philopotamidae chimarra sp.*	1
	Ch.th.CF	*Cheumatopsyche thomasseti*	1
	Hy.cr.CF	*Hydroptila cruciata*	15
	Le.sp.CF	*Leptocerid sp.*	1
	Cl.sp2.CH	*Cladotanytarsus sp2*	2
	Lau.sp.CH	*Lauterborniella sp.*	5
	Po.sp4.CH	*Polypedilum sp4*	1
	Or.sp2.CH	*Orthocladius sp2*	1
	Or.sp4.CH	*Orthocladius sp4*	2
	Param.sp.CH	*Parametriocnemus sp.*	9
	Rh.sp2.CH	*Rheocricotopus sp2*	1
	Me.sp.CH	*Meropelopia sp.*	1
WB30	Che.ex.MF	*Cheleocloeon excisum*	1
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Me.vo.BF	*Simulium metomphalus vorax*	4
	Ma.ca.CF	*Macrostemum capense*	18
	Ph.ch.sp.CF	*Philopotamidae chimarra sp.*	3
	Ec.th.CF	*Ecnomus thomasseti*	3
	Le.sp.CF	*Leptocerid sp.*	1
	Po.sp3.CH	*Polypedilum sp3*	2
	Ps.sp3.CH	*Psectrocladius sp3*	1
	Rh.sp2.CH	*Rheocricotopus sp2*	2
WB32A	Cl.sp.MF	*Cloeodes sp.*	2
	Ca.sp1.MF	*Caenis Type1*	2
	Ca.sp3.MF	*Caenis Type3*	1
	Af.su.MF	*Afroptilum sudafricanum*	1
	Co.be.MF	*Compsoneuria bequaerti*	1
	Cr.fl.MF	*Crassabwa flava*	1
	Ba.ha.MF	*Baetis harrisoni*	6
	Ba.un.sp1.MF	*Baetis unkown sp1*	1
	Eu.sp.MF	*Euthraulus sp.*	17
	Ps.un.sp2.MF	*Pseudocloeon unknown sp2*	1
	Ps.ma.MF	*Pseudopannota maculosa*	12
	Che.fa.MF	*Cheleocloeon falcatum*	1
	Bu.ma.MF	*Bugilliesia margaretae*	2
	Ed.da.BF	*Simulium edwardsellum damnosum*	8
	Me.ch.BF	*Simulium metomphalus chutteri*	27
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	12
	Me.ad.BF	*Simulium meilloniellum adersi*	8

	Ch.sp1.CF	*Cheumatopsyche sp1*	2
	Ph.ch.sp.CF	*Philopotamidae chimarra sp.*	1
	Ch.th.CF	*Cheumatopsyche thomasseti*	6
	Ba.sp.CH	*Baeotendipes sp.*	3
	Lau.sp.CH	*Lauterborniella sp.*	4
	Po.sp3.CH	*Polypedilum sp3*	3
	Param.sp.CH	*Parametriocnemus sp.*	3
	Rh.sp2.CH	*Rheocricotopus sp2*	1
	Tosp.CH	*Tokunagaia sp.*	1
WB33	Cl.un.sp.MF	*Cloeon unknown sp.*	1
	Ba.sp.CH	*Baeotendipes sp.*	1
	Ch.us.sp2.CH	*Chironomus sp2*	1
	Ch.us.sp3.CH	*Chironomus sp3*	1
	Cryp.sp.CH	*Cryptochironomus sp.*	3
	Po.sp1.CH	*Polypedilum sp1*	1
	Po.sp3.CH	*Polypedilum sp3*	1
	Po.sp7.CH	*Polypedilum sp7*	1
	Pr.sp.CH	*Procladius sp.*	2
SWC01	Ps.gl.MF	*Pseudocloeon glaucum*	4
	Ap.cap.SF	*Aphanicerca capensis*	79
	Ap.sp1.SF	*Aphanicerca sp1*	66
	Ba.tu.SF	*Balinskycercella tugelae*	9
	Ap.ta.SF	*Aphanicercopsis tabularis*	1
	Me.vo.BF	*Simulium metomphalus vorax*	3
	Si.sp.BF	*Simulium sp.*	2
	Me.ad.BF	*Simulium meilloniellum adersi*	1
	Mi.p.sp1.CH	*Micropsectra sp1*	1
	Param.sp.CH	*Parametriocnemus sp.*	2
SWC04	Ba.sp.SF	*Balinskycercella sp.*	1
	Ba.tu.SF	*Balinskycercella tugelae*	2
SWC06	Le.pe.MF	*Lestagella penicillata*	1
	Ca.ca.MF	*Castanophlebia calida*	1
	Ap.cap.SF	*Aphanicerca capensis*	97
	Ap.sp1.SF	*Aphanicerca sp1*	79
	Ba.tu.SF	*Balinskycercella tugelae*	6
	Ap.ta.SF	*Aphanicercopsis tabularis*	3
	Ed.da.BF	*Simulium edwardsellum damnosum*	2
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	9
	Mi.p.sp2.CH	*Micropsectra sp2*	1
	Po.sp1.CH	*Polypedilum sp1*	3
	Po.sp3.CH	*Polypedilum sp3*	1

	Po.sp7.CH	*Polypedilum sp7*	3
	Ta.sp2.CH	*Tanytarsus sp2*	2
	Ca.sp.CH	*Camptocladius sp.*	1
	Eu.sp1.CH	*Eukiefferiella sp1*	1
	Param.sp.CH	*Parametriocnemus sp.*	1
	To.sp.CH	*Tokunagaia sp.*	5
SWC08	Ch.sp3.CH	*Chironominae sp3*	1
SWC14	Le.pe.MF	*Lestagella penicillata*	72
	Ca.ca.MF	*Castanophlebia calida*	24
	Ap.cap.SF	*Aphanicerca capensis*	192
	Ap.sp1.SF	*Aphanicerca sp1*	2
	Ba.tu.SF	*Balinskycercella tugelae*	4
	Ap.ta.SF	*Aphanicercopsis tabularis*	13
	Ed.da.BF	*Simulium edwardsellum damnosum*	1
	An.de.BF	*Simulium anasolen dentulosum*	34
	Ne.ni.BF	*Simulium nevermannia nigritarse*	8
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	23
	Mi.p.sp2.CH	*Micropsectra sp2*	1
	Po.sp1.CH	*Polypedilum sp1*	6
SWC16	Le.pe.MF	*Lestagella penicillata*	314
	La.vi.MF	*Labiobaetis vinosus*	2
	Che.ex.MF	*Cheleocloeon excisum*	1
	Ps.gl.MF	*Pseudocloeon glaucum*	5
	Ps.la.MF	*Pseudocloeon latum*	1
	Af.su.MF	*Afroptilum sudafricanum*	26
	Af.ol.MF	*Afronurus oliffi*	1
	Ba.ha.MF	*Baetis harrisoni*	8
	Ba.un.sp1.MF	*Baetis unkown sp1*	3
	De.ca.MF	*Demoreptus capensis*	2
	Ad.au.MF	*Adenophlebia auriculata*	189
	Ca.ca.MF	*Castanophlebia calida*	93
	Ap.in.MF	*Aprionyx intermedius*	1
	Ap.cap.SF	*Aphanicerca capensis*	1
	Ap.sp1.SF	*Aphanicerca sp1*	21
	Ap.sp2.SF	*Aphanicerca sp2*	10
	Ba.sp.SF	*Balinskycercella sp.*	1
	Ba.tu.SF	*Balinskycercella tugelae*	6
	De.sp.SF	*Desmonemoura sp.*	1
	Ed.da.BF	*Simulium edwardsellum damnosum*	8
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	5
	Ne.ruf.BF	*Simulium nevermannia ruficorne*	2

	Po.sp1.CH	*Polypedilum sp1*	2
	Po.sp3.CH	*Polypedilum sp3*	3
	Po.sp4.CH	*Polypedilum sp4*	1
	Po.sp5.CH	*Polypedilum sp5*	1
	To.sp.CH	*Tokunagaia sp.*	1
SWC17	Le.pe.MF	*Lestagella penicillata*	37
	Ca.sp1.MF	*Caenis Type1*	1
	Ps.gl.MF	*Pseudocloeon glaucum*	3
	Af.su.MF	*Afroptilum sudafricanum*	16
	Le.sp.MF	*Leptophlebiidae sp.*	21
	Ad.au.MF	*Adenophlebia auriculata*	25
	Ca.ca.MF	*Castanophlebia calida*	102
	Ap.in.MF	*Aprionyx intermedius*	6
	Ap.cap.SF	*Aphanicerca capensis*	5
	Ap.sp1.SF	*Aphanicerca sp1*	6
	Ba.sp.SF	*Balinskycercella sp.*	28
	Ba.tu.SF	*Balinskycercella tugelae*	21
	Ap.ta.SF	*Aphanicercopsis tabularis*	1
	Ed.da.BF	*Simulium edwardsellum damnosum*	3
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	3
	Ph.sp1.CH	*Phaenopsectra sp1*	1
	Po.sp3.CH	*Polypedilum sp3*	3
	To.sp.CH	*Tokunagaia sp.*	1
SWC19	Ca.sp1.MF	*Caenis Type1*	1
	Ps.gl.MF	*Pseudocloeon glaucum*	4
	Ps.pi.MF	*Pseudocloeon piscis*	2
	Ap.cap.SF	*Aphanicerca capensis*	1
	Ap.sp1.SF	*Aphanicerca sp1*	4
	Ba.tu.SF	*Balinskycercella tugelae*	20
	An.de.BF	*Simulium anasolen dentulosum*	14
	Ch.sp4.CH	*Chironominae sp4*	1
	Lau.sp.CH	*Lauterborniella sp.*	1
	Po.sp6.CH	*Polypedilum sp6*	1
	Ta.sp4.CH	*Tanytarsus sp4*	2
	Mi.t.sp2.CH	*Microtendipes sp2*	1
	Or.sp1.CH	*Orthocladius sp1*	2
	Ps.sp2.CH	*Psectrocladius sp2*	1
SWC20	Le.pe.MF	*Lestagella penicillata*	15
	Ps.gl.MF	*Pseudocloeon glaucum*	5
	Af.su.MF	*Afroptilum sudafricanum*	29
	Ap.cap.SF	*Aphanicerca capensis*	8

	Ap.sp1.SF	*Aphanicerca sp1*	2
	Ba.sp.SF	*Balinskycercella sp.*	73
	Ba.tu.SF	*Balinskycercella tugelae*	10
	Ed.da.BF	*Simulium edwardsellum damnosum*	2
	Po.im.BF	*Simulium pomeroyellum impukane*	2
	Ne.ruf.BF	*Simulium nevermannia ruficorne*	11
	Lau.sp.CH	*Lauterborniella sp.*	1
	Param.sp.CH	*Parametriocnemus sp.*	2
	Rh.sp2.CH	*Rheocricotopus sp2*	1
SWC22	Le.pe.MF	*Lestagella penicillata*	18
	Che.sp.MF	*Cheleocloeon sp.*	1
	Ca.sp1.MF	*Caenis Type1*	13
	Ca.sp2.MF	*Caenis Type2*	8
	Ca.sp3.MF	*Caenis Type3*	3
	Ps.gl.MF	*Pseudocloeon glaucum*	1
	Ps.pi.MF	*Pseudocloeon piscis*	3
	Ps.un.sp1.MF	*Pseudocloeon unknown sp1*	2
	Cho.sp.MF	*Choroterpes sp.*	1
	Me.ch.BF	*Simulium metomphalus chutteri*	1
	Me.ad.BF	*Simulium meilloniellum adersi*	4
	Ph.sp2.CH	*Phaenopsectra sp2*	1
	Po.sp7.CH	*Polypedilum sp7*	1
	Param.sp.CH	*Parametriocnemus sp.*	2
SWC23	Le.pe.MF	*Lestagella penicillata*	3
	Ps.gl.MF	*Pseudocloeon glaucum*	1
	Ca.ca.MF	*Castanophlebia calida*	10
	Cho.sp.MF	*Choroterpes sp.*	3
	Ap.bi.SF	*Aphanicercella bifurcata*	1
	Ba.tu.SF	*Balinskycercella tugelae*	7
	Or.sp2.CH	*Orthocladius sp2*	2
SWC24	Le.pe.MF	*Lestagella penicillata*	18
	La.vi.MF	*Labiobaetis vinosus*	9
	Ca.ca.MF	*Castanophlebia calida*	5
	Ap.cap.SF	*Aphanicerca capensis*	69
	Ap.sp1.SF	*Aphanicerca sp1*	75
	Ap.sp2.SF	*Aphanicerca sp2*	4
	Ba.tu.SF	*Balinskycercella tugelae*	10
	Ap.ta.SF	*Aphanicercopsis tabularis*	4
	An.de.BF	*Simulium anasolen dentulosum*	1
	Ne.ruf.BF	*Simulium nevermannia ruficorne*	1
	Mi.p.sp2.CH	*Micropsectra sp2*	1

	Parak.sp.CH	*Parakiefferiella sp.*	3
	Param.sp.CH	*Parametriocnemus sp.*	1
SWC25	Le.pe.MF	*Lestagella penicillata*	290
	Da.in.MF	*Dabulamanzia indusii*	2
	Ps.gl.MF	*Pseudocloeon glaucum*	18
	Co.be.MF	*Compsoneuria bequaerti*	3
	Af.ol.MF	*Afronurus oliffi*	34
	Ba.ha.MF	*Baetis harrisoni*	24
	Le.sp.MF	*Leptophlebiidae sp.*	37
	Ad.au.MF	*Adenophlebia auriculata*	3
	Ca.ca.MF	*Castanophlebia calida*	53
	Ap.in.MF	*Aprionyx intermedius*	92
	Ap.cap.SF	*Aphanicerca capensis*	14
	Ap.bi.SF	*Aphanicercella bifurcata*	2
	Ba.tu.SF	*Balinskycercella tugelae*	2
	Ed.da.BF	*Simulium edwardsellum damnosum*	7
	Po.sp4.CH	*Polypedilum sp4*	7
SWC26	Le.pe.MF	*Lestagella penicillata*	65
	La.vi.MF	*Labiobaetis vinosus*	26
	Che.ex.MF	*Cheleocloeon excisum*	1
	Cl.sp.MF	*Cloeodes sp.*	1
	Ps.gl.MF	*Pseudocloeon glaucum*	1
	Af.su.MF	*Afroptilum sudafricanum*	2
	Af.ol.MF	*Afronurus oliffi*	1
	Ba.ha.MF	*Baetis harrisoni*	5
	Ba.un.sp1.MF	*Baetis unkown sp1*	3
	Le.sp.MF	*Leptophlebiidae sp.*	18
	Ad.au.MF	*Adenophlebia auriculata*	1
	Ca.ca.MF	*Castanophlebia calida*	58
	Ap.in.MF	*Aprionyx intermedius*	2
	Ap.cap.SF	*Aphanicerca capensis*	4
	Ap.sp1.SF	*Aphanicerca sp1*	10
	Ba.tu.SF	*Balinskycercella tugelae*	6
	Ne.ruf.BF	*Simulium nevermannia ruficorne*	1
	Lau.sp.CH	*Lauterborniella sp.*	1
	Mi.t.sp1.CH	*Microtendipes sp1*	1
	Po.sp3.CH	*Polypedilum sp3*	2
	Po.sp4.CH	*Polypedilum sp4*	2
	Param.sp.CH	*Parametriocnemus sp.*	2
SWC27	Le.pe.MF	*Lestagella penicillata*	222
	Ps.gl.MF	*Pseudocloeon glaucum*	4

	Af.su.MF	*Afroptilum sudafricanum*	2
	Co.be.MF	*Compsoneuria bequaerti*	36
	Af.ol.MF	*Afronurus oliffi*	21
	Ba.ha.MF	*Baetis harrisoni*	2
	Ad.au.MF	*Adenophlebia auriculata*	1
	Ca.ca.MF	*Castanophlebia calida*	149
	Ap.cap.SF	*Aphanicerca capensis*	33
	Ap.sp1.SF	*Aphanicerca sp1*	5
	Ba.tu.SF	*Balinskycercella tugelae*	20
	Ed.da.BF	*Simulium edwardsellum damnosum*	2
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	4
	Ne.ruf.BF	*Simulium nevermannia ruficorne*	4
	Po.sp4.CH	*Polypedilum sp4*	29
	Ta.sp1.CH	*Tanytarsus sp1*	6
	Pa.sp.CH	*Paratanytarsus sp.*	1
	Or.sp1.CH	*Orthocladius sp1*	1
	Or.sp3.CH	*Orthocladius sp3*	1
SWC28	Le.pe.MF	*Lestagella penicillata*	128
	Che.ex.MF	*Cheleocloeon excisum*	1
	Ca.sp1.MF	*Caenis Type1*	3
	Ps.gl.MF	*Pseudocloeon glaucum*	10
	Ps.pi.MF	*Pseudocloeon piscis*	2
	Af.su.MF	*Afroptilum sudafricanum*	26
	Co.be.MF	*Compsoneuria bequaerti*	1
	Af.ol.MF	*Afronurus oliffi*	2
	Cr.fl.MF	*Crassabwa flava*	1
	Ba.ha.MF	*Baetis harrisoni*	7
	Ba.un.sp1.MF	*Baetis unkown sp1*	3
	De.mo.MF	*Demoreptus monticola*	15
	Le.sp.MF	*Leptophlebiidae sp.*	1
	Ad.au.MF	*Adenophlebia auriculata*	84
	Ap.cap.SF	*Aphanicerca capensis*	1
	Ap.sp1.SF	*Aphanicerca sp1*	8
	Ba.sp.SF	*Balinskycercella sp.*	14
	Ba.tu.SF	*Balinskycercella tugelae*	4
	De.pu.SF	*Desmonemoura pulchellum*	3
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Mi.t.sp1.CH	*Microtendipes sp1*	6
	Po.sp3.CH	*Polypedilum sp3*	1
	Po.sp5.CH	*Polypedilum sp5*	1
	Po.sp7.CH	*Polypedilum sp7*	1

	Ta.sp1.CH	*Tanytarsus sp1*	1
	Lim.sp.CH	*Limnophyes sp.*	1
	Param.sp.CH	*Parametriocnemus sp.*	2
SWC29	Le.pe.MF	*Lestagella penicillata*	2
	Ca.sp1.MF	*Caenis Type1*	5
	Ca.sp2.MF	*Caenis Type2*	4
	Ps.pi.MF	*Pseudocloeon piscis*	1
	Ad.au.MF	*Adenophlebia auriculata*	21
	Ca.ca.MF	*Castanophlebia calida*	7
	Ap.cap.SF	*Aphanicerca capensis*	3
	Ba.sp.SF	*Balinskycercella sp.*	5
	Ap.bi.SF	*Aphanicercella bifurcata*	3
	Ba.tu.SF	*Balinskycercella tugelae*	25
	Po.sp1.CH	*Polypedilum sp1*	2
	Po.sp3.CH	*Polypedilum sp3*	1
	Param.sp.CH	*Parametriocnemus sp.*	4
SWC30	Le.pe.MF	*Lestagella penicillata*	106
	Af.su.MF	*Afroptilum sudafricanum*	13
	De.mo.MF	*Demoreptus monticola*	13
	Le.sp.MF	*Leptophlebiidae sp.*	19
	Ad.au.MF	*Adenophlebia auriculata*	45
	Ca.ca.MF	*Castanophlebia calida*	31
	Ap.in.MF	*Aprionyx intermedius*	3
	Ap.cap.SF	*Aphanicerca capensis*	21
	Ap.sp1.SF	*Aphanicerca sp1*	17
	Ba.tu.SF	*Balinskycercella tugelae*	38
	Ed.da.BF	*Simulium edwardsellum damnosum*	4
	Me.ch.BF	*Simulium metomphalus chutteri*	1
	An.de.BF	*Simulium anasolen dentulosum*	1
	Ne.ni.BF	*Simulium nevermannia nigritarse*	4
	Po.sp4.CH	*Polypedilum sp4*	3
	St.sp.CH	*Stenochironomus sp.*	1
	Eu.sp5.CH	*Eukiefferiella sp5*	1
	Param.sp.CH	*Parametriocnemus sp.*	2
SWC31	Le.pe.MF	*Lestagella penicillata*	4
	Ca.sp1.MF	*Caenis Type1*	3
	Ps.gl.MF	*Pseudocloeon glaucum*	3
	Ps.pi.MF	*Pseudocloeon piscis*	2
	Af.su.MF	*Afroptilum sudafricanum*	5
	Co.be.MF	*Compsoneuria bequaerti*	2
	Af.ol.MF	*Afronurus oliffi*	9

	Cr.fl.MF	*Crassabwa flava*	2
	Ad.au.MF	*Adenophlebia auriculata*	13
	Ap.cap.SF	*Aphanicerca capensis*	14
	Ba.tu.SF	*Balinskycercella tugelae*	1
	Ed.da.BF	*Simulium edwardsellum damnosum*	1
	Me.ch.BF	*Simulium metomphalus chutteri*	3
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Me.vo.BF	*Simulium metomphalus vorax*	4
	Lau.sp.CH	*Lauterborniella sp.*	1
	Po.sp1.CH	*Polypedilum sp1*	1
	Po.sp3.CH	*Polypedilum sp3*	1
	Po.sp4.CH	*Polypedilum sp4*	1
	Eu.sp6.CH	*Eukiefferiella sp6*	6
	Param.sp.CH	*Parametriocnemus sp.*	1
	To.sp.CH	*Tokunagaia sp.*	1
SWC32	Le.pe.MF	*Lestagella penicillata*	46
	Ca.sp1.MF	*Caenis Type1*	6
	Ps.gl.MF	*Pseudocloeon glaucum*	2
	Af.su.MF	*Afroptilum sudafricanum*	2
	Ad.au.MF	*Adenophlebia auriculata*	7
	Ca.ca.MF	*Castanophlebia calida*	15
	Ap.cap.SF	*Aphanicerca capensis*	8
	Ap.sp1.SF	*Aphanicerca sp1*	8
	Ba.tu.SF	*Balinskycercella tugelae*	4
	Ed.da.BF	*Simulium edwardsellum damnosum*	1
	Po.im.BF	*Simulium pomeroyellum impukane*	2
	Mi.t.sp1.CH	*Microtendipes sp1*	1
SWC33	Le.pe.MF	*Lestagella penicillata*	49
	Le.sp.MF	*Leptophlebiidae sp.*	9
	Ca.ca.MF	*Castanophlebia calida*	84
	Ap.in.MF	*Aprionyx intermedius*	92
	Ap.cap.SF	*Aphanicerca capensis*	30
	Ap.sp1.SF	*Aphanicerca sp1*	6
	Ap.sp2.SF	*Aphanicerca sp2*	1
	Ba.sp.SF	*Balinskycercella sp.*	4
	An.de.BF	*Simulium anasolen dentulosum*	1
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	9
	Mi.t.sp1.CH	*Microtendipes sp1*	11
	Po.sp1.CH	*Polypedilum sp1*	1
	Po.sp7.CH	*Polypedilum sp7*	1

	Rh.sp2.CH	*Rheocricotopus sp2*	1
SWC34	Le.pe.MF	*Lestagella penicillata*	81
	Che.ex.MF	*Cheleocloeon excisum*	1
	Ps.un.sp1.MF	*Pseudocloeon unknown sp1*	1
	Ca.ca.MF	*Castanophlebia calida*	33
	Cho.sp.MF	*Choroterpes sp.*	3
	Ap.cap.SF	*Aphanicerca capensis*	238
	Ba.sp.SF	*Balinskycercella sp.*	21
	Ap.sp.SF	*Aphanicercopsis sp.*	8
	Ba.tu.SF	*Balinskycercella tugelae*	8
	Ed.da.BF	*Simulium edwardsellum damnosum*	3
	An.de.BF	*Simulium anasolen dentulosum*	9
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	11
	Ne.ruf.BF	*Simulium nevermannia ruficorne*	10
	Ch.sp4.CH	*Chironominae sp4*	2
	Mi.p.sp1.CH	*Micropsectra sp1*	1
	Ph.sp3.CH	*Phaenopsectra sp3*	1
	Po.sp3.CH	*Polypedilum sp3*	1
	Za.sp.CH	*Zavrelia sp.*	1
	Parak.sp.CH	*Parakiefferiella sp.*	3
	Rh.sp2.CH	*Rheocricotopus sp2*	1
SWC35	Le.pe.MF	*Lestagella penicillata*	2
	Ca.ca.MF	*Castanophlebia calida*	2
	Ap.cap.SF	*Aphanicerca capensis*	31
	Ap.sp1.SF	*Aphanicerca sp1*	4
	Ap.sp2.SF	*Aphanicerca sp2*	3
	Ba.tu.SF	*Balinskycercella tugelae*	8
	Ap.ta.SF	*Aphanicercopsis tabularis*	2
	Ed.da.BF	*Simulium edwardsellum damnosum*	5
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Ne.rut.BF	*Simulium nevermannia rutherfoordi*	1
SWC36	Le.pe.MF	*Lestagella penicillata*	164
	Ps.gl.MF	*Pseudocloeon glaucum*	4
	Af.su.MF	*Afroptilum sudafricanum*	5
	Ba.ha.MF	*Baetis harrisoni*	43
	Ad.au.MF	*Adenophlebia auriculata*	2
	Ad.sp.MF	*Adenophlebia sp.*	1
	Ca.ca.MF	*Castanophlebia calida*	117
	Ap.cap.SF	*Aphanicerca capensis*	4
	Ap.sp1.SF	*Aphanicerca sp1*	9
	Ba.sp.SF	*Balinskycercella sp.*	4

	Ba.tu.SF	*Balinskycercella tugelae*	1
	Ne.ni.BF	*Simulium nevermannia nigritarse*	4
	Me.vo.BF	*Simulium metomphalus vorax*	1
	Po.sp4.CH	*Polypedilum sp4*	4
SWC37	Le.pe.MF	*Lestagella penicillata*	46
	Ps.gl.MF	*Pseudocloeon glaucum*	1
	Af.su.MF	*Afroptilum sudafricanum*	46
	Ad.au.MF	*Adenophlebia auriculata*	3
	Ca.ca.MF	*Castanophlebia calida*	55
	Ap.in.MF	*Aprionyx intermedius*	7
	Ap.sp1.SF	*Aphanicerca sp1*	5
	Ba.sp.SF	*Balinskycercella sp.*	14
	Ba.tu.SF	*Balinskycercella tugelae*	2
	Ne.ni.BF	*Simulium nevermannia nigritarse*	1
	Me.vo.BF	*Simulium metomphalus vorax*	1
	Mi.t.sp1.CH	*Microtendipes sp1*	3

Appendix 10: Water and Macroinvertebrate sampling sheet

<table>
<tr><td colspan="3">Province/Town:</td><td colspan="2">Width (m):</td><td rowspan="15">Notes</td></tr>
<tr><td colspan="3">Site Code:</td><td colspan="2">Depth cm/m:</td></tr>
<tr><td colspan="3">Date:</td><td colspan="2">Flow:</td></tr>
<tr><td colspan="3">Time:</td><td colspan="2">Turbidity:</td></tr>
<tr><td colspan="2" rowspan="2">Grid Reference Lat: S
Long: E</td><td rowspan="2"></td><td colspan="2">Colour:</td></tr>
<tr><td colspan="2">Riparian Disturbance:</td></tr>
<tr><td colspan="3">Altitude (m):</td><td colspan="2">Instream Disturbance:</td></tr>
<tr><td colspan="3">YSI Measurements:</td><td rowspan="2">Access</td><td>YES</td></tr>
<tr><td>Temperature</td><td></td><td>NO</td></tr>
<tr><td>DO %</td><td></td><td colspan="2" rowspan="5">Details for contact person to gain access:</td></tr>
<tr><td>DO mg/L</td><td></td></tr>
<tr><td>Conductivity</td><td></td></tr>
<tr><td>pH</td><td></td></tr>
<tr><td colspan="3">2x Water samples (mL) filtered:</td></tr>
<tr><td rowspan="2">Invertebrate site</td><td>YES</td><td rowspan="2">Photographs</td><td>YES</td><td rowspan="2">Weather Conditions:</td></tr>
<tr><td>NO</td><td>NO</td></tr>
<tr><td colspan="3" rowspan="3">Site Description:</td><td colspan="2" rowspan="3">Photo Details:</td><td rowspan="2">Diatom sample</td><td>YES</td></tr>
<tr><td>NO</td></tr>
<tr><td colspan="2">River name:</td></tr>
<tr><td colspan="3"></td><td colspan="2">Name of Sampler:</td></tr>
</table>