AQUATIC MACROINVERTEBRATE COMMUNITY IN THE WILGE RIVER

By

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ABSTRACT

The Wilge River is one of many adjoining tributaries of the Olifants River located in the Olifants Water Management Area (WMA4) within the Highveld (11) – Lower Level 1 Ecoregion (Dallas, 2007). These river systems experience extreme demand for natural resources, as they flow through heavily utilised economic hubs. They are closely associated with land modification and pollution, primarily mining and industrial-related disturbances and extensive agricultural activities, all of which are the primary cause of impairment to river health.

The primary aim of this study was to study the aquatic macroinvertebrate communities at six monitoring sites along the Wilge River, coupled with two monitoring sites on adjoining tributaries, and to further identify the driving variables that influence these communities both spatially and temporally. Functional Feeding Groups (FFGs) of the aquatic macroinvertebrates and the surrounding land use in the project area was taken into account.

The analysis of *in situ* water quality measured during the period March 2010 to May 2013, clearly illustrated high levels of variation both spatially and temporally. *In situ* water quality was a limiting factor to the aquatic ecosystem from a Dissolved Oxygen (DO) and Percentage Saturation (DO%) perspective. The remaining parameters were within the South African guideline for aquatic ecosystems (DWAF, 1996).

Habitat availability illustrated clear seasonal variation as well, of which the wet season indicated better habitat availability compared to the dry season. The dominant biotopes in the study area were vegetation (VEG) and gravel, sand and mud (GSM). Site WIL04 illustrated the poorest habitat integrity overall primarily attributed to the site's steep incised banks and deep channel which lacks the stones biotope.

The South African Scoring System, *Version 5* (SASS5) results indicated that there was a change in the integrity of the aquatic macroinvertebrate community's in the study area and further illustrated variability both spatially and temporally. It was evident that the aquatic macroinvertebrate communities within the Wilge River and two adjoining tributaries sampled, were generally in a slightly to modified state with moderate variations. The lowest number of taxa, SASS5 and average score per taxon (ASPT) values was recorded at site WIL04 and this was mainly brought about due to changes in flow and habitat availability. The ASPT score ranged from 3.8 at site WIL04 to 7.7 at site WIL02, indicating that the aquatic macroinvertebrate communities were primarily composed of tolerant and moderately tolerant taxa. Of these mostly tolerant taxa, predators and gathering collector populations were the most dominant FFG, with the shredders being the least abundant within the study area.

The Bray-Curtis cluster analysis of the aquatic macroinvertebrate communities clearly illustrated a high level of similarity and seasonal variation among the communities. The high similarity was an indication that similar taxa occurred at the sites within the groups identified. However, in accordance with the Similarity Percentages (SIMPER) analysis, there was no clear indication of dominant taxa. There was however a separation of sites TRI01 and WIL04. This was expected due to differences in the physical stream condition (flow) and other habitats / general biotopes, primarily at site WIL04. Stream bed composition is one of the most important physical factors controlling the structure of freshwater invertebrate communities (Mackay and Eastburn, 1990). The separation and similarity of these two sites were not a consequence of dominant taxa, but rather a consequence of differing water quality, habitat availability and common tolerant taxa driving the system. Inclusive, the seasonal variation illustrated was contributing to the changes in the *in situ* water quality and habitat availability, thus making seasonal variation also a driving variable, in the differences between the sites.

The Redundancy Analysis (RDA) bi-plots indicated, as with the Bray-Curtis similarity matrices and related NMDS plot, that there was a distinct seasonal separation. It further illustrated a clear separation of site TRI01 and WIL04 due to reasons mentioned above. All the environmental variables, with the exception of pH, was identified as significant drivers in the river systems ($p<0.05$). This however varied seasonally. During the wet season, clarity, DO and pH were the significant drivers, while clarity, TDS/EC, percentage saturation and pH were the significant drivers during the dry season ($p<0.05$). These drivers were expected due to possible sources namely intensive agriculture in the project area. The RDA tri-plots further indicated the significant role that the ASPT, SASS5 score and the IHAS played within the aquatic macroinvertebrate community ($p<0.05$). This confirms the importance of habitat as a driving variable in aquatic macroinvertebrate community structures. Consequently, the driving variables in the separation of the sites along the Wilge River and two adjoining tributaries, appear to be a combination of variables (DO, percentage saturation, TDS/EC, clarity and pH), including habitat availability (based on IHAS scores).

To determine the effects and relations between the primary driving changes, to the surrounding land uses in the project area, further multivariate analyses were conducted, which included the FFGs. It was clearly indicated that predators have a negative correlation with the rest of the FFG's which was expected. As the percentage of predators increase at a site, the percentage of the other FFG's decreased. Therefore, there was a large variation and clear changes in the food sources constantly entering into the river system. This is normally related to changes in the land use. However, as the land use is consistent in the study area (agriculture, industrial and mining) the changes in food availability for the aquatic macroinvertebrates may possibly be attributed to seasonal changes. The RDA further illustrated any existing links between the land use and the FFG's, as well as *in situ* water quality. Rural development had minimal influence on the FFG's and had no positive or negative correlation to *in situ* water quality. Medium intensity agriculture was the main driver for predators, but did indicate a negative or positive correlation with *in situ* water quality. Intense agriculture was the driver for scraper/grazers and indicated a positive association with pH and temperature during the wet season, and DO, pH and clarity during the dry season. Infrastructure was highly associated with site WIL01, which was expected due to the railway line over the Wilge River at that site, as well as positively correlated to DO%, EC and TDS. Mining and industry showed a positive correlation with temperature. However, without nutrient data, it was difficult to confirm whether land use was a driving factor or not on the aquatic macroinvertebrate/FFG communities. One aspect is for certain, as there was so much seasonal variation amongst the FFGs, this confirms the above statement that changes in food availability may possibly be attributed to seasonal changes in the food sources and not the land use in the study area.

Overall, it was clearly illustrated that the driving variables in the separation of the sites and the aquatic macroinvertebrate communities and their FFS's, appear to be primarily seasonal variation, *in situ* water quality and habitat availability ($p<0.05$), as opposed to land use or dominant taxon, being the driving change. These driving variables therefore play a crucial role in the complexity of aquatic macroinvertebrate structures.

It is recommended that biomonitoring and management efforts are continued, with the inclusion of organic and inorganic water quality and diatom analysis. The former will provide a clearer view of the type of nutrients entering into the river system in the study area, thus aiding in a better understanding of whether land use is a driving variable affecting the aquatic macroinvertebrate structures. The latter will further improve the understanding of the potential impacts on the water quality within the study area.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

Abbreviations

ASPT	Average Score per Taxa
AMD	Acid Mine Drainage
ANOSIM	Analysis of Similarity
COD	Chemical Oxygen Demand
CSIR	Council for Scientific and Industrial Research
DEAT	Department of Environmental Affairs and Tourism
DO	Dissolved Oxygen
DO%	Percentage Oxygen Saturation
DWAF	Department of Water Affairs and Forestry
EC	Electrical Conductivity
FFG	Functional Feeding Groups
GSM	Gravel, Sand and Mud
IHAS	Integrated Habitat Assessment System, Version 2
MDS	Multidimensional Scaling
mm	Millimeters
mm/a	Millimeters per year
m^3/a	Cubic metres per annum
PCA	Principal Component Analysis
PES	Present Ecological State
RDA	Redundancy Analysis
SASS5	South African Scoring System, Version 5
SIC	Stones-In-Current
SIMPER	Similarity Percentages
SOOC	Stones-Out-Of-Current

SPI	Specific Preference Index
TDS	Total Dissolved Salts
TWQR	Target Water Quality Range
USEPA	United States Environmental Protection Agency
VEG	Vegetation
WMA	Water Management Area
WRC	Water Research Commission

1. INTRODUCTION

South Africa is extremely rich in natural resources – except for water, and in particular running water, which are some of the most degraded ecosystems not only in South Africa, but on earth (Giller and Malmqvist, 1998). This is primarily attributed to streams and rivers being strongly influenced not only by local factors, but also by the landscape through which they flow (Hynes, 1975, Vannote *et al.,* 1980). South Africa is located in a predominantly semi-arid part of the world with an average rainfall of approximately 450 mm per year (mm/a), well below the world average of about 860 mm/a (DWAF, 2004). From a global perspective, South Africa's water resources are scarce and extremely limited. The combined flow of all the rivers within the country amounts to about 49 000 million cubic metres per year (m^3/a), less than half of that of the Zambezi River, the closest large river to South Africa (DWAF, 2004). Increasing demand for water from a social and economic perspective, and decreasing water quality makes careful water management a priority in our country. Catchment land-uses are a major stressor on our freshwater ecosystems, namely:

- Industrial activities, resulting in pollutants and effluents;
- Extensive agricultural activities, the source of fertilizers, pesticides and excessive nutrients;
- Mining activities, contributing to Acid Mine Drainage (AMD) in South Africa, which is reaching a crisis point (DEAT, 2008; Rashleigh *et al.,* 2009); and
- Urbanisation activities, including informal settlements which lack sewage water purification facilities, and as a result of ineffective and insufficient commercial waste water treatment plants due to a lack of financial, administrative and technical support, is another stressor contributing to the impact on freshwater ecosystems.

The Olifants River Catchment area covers approximately 54 400 km^2 and has a total mean annual runoff of 2 400 million cubic meters per year (DWAF, 2001). The Olifants River and some of its tributaries, originates in the Mpumalanga Highveld where they experience extreme demand for natural resources. According to Van Vuuren (2009) and Balance *et al.* (2001) the Olifants River in Mpumalanga is presently one of the most threatened river systems in South Africa. The rivers are associated with land modification and pollution, primarily mining-related disturbances, which is the primary cause of impairment of river health, coupled with industrial activities and extensive agricultural activities (DWAF, 2001). The River flows through Emalahleni, Middelburg, Steelpoort and Phalaborwa, before entering into the Kruger National Park and neighbouring private game reserves (De Villiers and Mkwelo, 2009, Van Zyl *et al.,* 2001). As the Olifants River and its adjoining tributaries are hard-working rivers, flowing through this heavily utilised economic hub, they are classified as highly stressed (DWAF, 2000). In addition, the overall ecological status of the Olifants River in this region has been classified as ‘poor to unacceptable’ (Balance *et al.,* 2001, DWAF, 2000; WRC, 2001). Associated with these activities is high surface run-off, erosion due to stream diversions occurring as a result of agricultural and mining activities, water contamination and ultimately biotic community alteration.

The origin of the Wilge River is near the town Leandra and it is a main river in this part of the Olifants River sub-drainage region situated in Ecoregion 7.02 (DWAF, 2001). The Wilge River flows roughly northwards until it is joined by its main tributary, the Bronkhorstspruit River. The river subsequently flows in a north-easterly direction until its confluence with the Olifants River approximately 12 km upstream of the Loskop Dam wall (DWAF, 2004). With the existing land-use in the Wilge River catchment, namely increasing afforestation, mining, power generation, irrigation, agriculture (main feature of the area), domestic and industrial activities, the river already is under pressure from nutrients and sulphate inputs (De Villiers and Mkwelo, 2009; DWAF, 2004). The in-stream and riparian habitats in this ecoregion illustrates a fair to unacceptable state, with the general condition reflecting a poor status. Biological communities further reflect a fair to unacceptable health (DWAF, 2001). However, a study conducted by De Villiers and Mkwelo, (2009), illustrated that the Loskop Dam monitoring stations, located downstream from the confluence with the Wilge River, recorded a ‘good to fair’ ecological status, despite problems with mine effluent draining into the Wilge River tributary, and frequent fish deaths in the Loskop Dam.

Nonetheless, there is still a concern that the rivers and streams in this study area already contain high sediment (turbidity) and nutrient loads due to the land use in the area. Overgrazing and highly erodible soils is causing severe erosion, resulting in high suspended solids being transported into the Wilge River. Any further increase in sedimentation and erosion may cause a further loss in habitat diversity and quality that will further contribute to impacts on biological communities and integrity. In addition, as power supply, mining and other industrial activities are becoming the economic hub of the study area, this will eventually result in overall cumulative impacts on the Wilge River. With this being said, sites within the Wilge River catchment however, show relatively good water quality in comparison to those in the Olifants River catchment (CSIR, 2010). It is therefore imperative to maintain the ecological integrity of the Wilge River and strive to improve it. Ultimately, in order to manage and conserve the Wilge River, it is essential to understand the catchment and its impeding land uses.

One of the major challenges in water resource management is to identify environmental stressors and understand how these stressors affect aquatic ecosystems (Pan *et al.,* 2004).

This is due to the natural diversity and multiple stressors that are co-occurring. The term stressor(s) refers to variable(s) of anthropogenic landscape changes and confined abiotic stream conditions that reflect human activities (Pan *et al.,* 2004). Various land-uses in the catchment can significantly modify both water chemistry (Johnson *et al.,* 1997) and physical habitat conditions (Roth *et al.,* 1996), which subsequently decrease biological integrity within streams and rivers (Karr and Chu, 1999). As such, aquatic ecosystem conditions are thus a result of a blend of natural (large climatic and topographic variation) (Underwood *et al.,* 2009; Sirami *et al.,* 2010) and anthropogenic factors (rapid urbanisation, agricultural, mining and industrial) (Gopal, 2005; Pavlin *et al.,* 2011; Sirami *et al.,* 2010; Underwood *et al.,* 2009). Alteration in the hydrological regime constitutes the most important case of loss or degradation of habitats in the aquatic ecosystems. The regulation and diversion of river flows by constructing dams and weirs directly affects both upstream and downstream habitats (Poff *et al.,* 1997). Therefore, the loss and degradation of the habitat itself may be the greatest threat to the biodiversity of a region, ultimately resulting from the way we use and manage the catchment area i.e. land use. Therefore, threats to the Wilge River may be primarily coming from anthropogenic activities resulting in biophysical changes in and around the aquatic ecosystems.

An assessment of four years' worth of available *in situ* water quality, habitat availability and aquatic macroinvertebrate data has been conducted on the Wilge River and two adjoining tributaries (Klipspruit and an unnamed tributary of the Klipfonteinspruit) within the Olifants River catchment area.

Biological monitoring, commonly known as “biomonitoring” is the use of biological responses to assess changes in the environment, commonly resulting from anthropogenic sources (Plafkin *et al.,* 1989, Dickens and Graham, 2002). In general biomonitoring involves the use of indicators in the form of individuals, species or communities. Fish, aquatic macroinvertebrates, diatoms and algae are some of the indicators used, although aquatic macroinvertebrates have the longest history of use in biomonitoring programs and the application in South African streams has been well documented (Dickens and Graham, 2002; Plafkin *et al.,* 1989).

Aquatic macroinvertebrates are organisms that are large enough to be seen by the naked eye, yet are small. Different types of macroinvertebrates tolerate different stream conditions and levels of pollution, thus making them ideal indicator species. Depending on the different macroinvertebrates found in a stream, predictions regarding water quality can be made. Different types of aquatic macroinvertebrates include *inter alia*, Ephemeroptera (mayflies), Trichoptera (caddisflies and cased caddisflies), Coleoptera (beetles), Hemiptera (bugs), Diptera (flies), Mollusca (snails) and crustaceans. These communities reflect overall stream condition as they integrate different environmental preferences such as water quality, flow and habitat. As a result, the responding community will provide insight into the presence of pollution in a river system, the amount/intensity of the exposure, and thus provides an indication of the health and integrity of the river system (O'Keeffe and Dickens, 2000). Therefore, aquatic macroinvertebrates form an essential component in assessing riverine ecosystems as they indicate the overall ecological condition (O'Keeffe and Dickens, 2000, Weber *et al.,* 2004).

The benefits of using aquatic macroinvertebrates as indicator species in biomonitoring programs, is that they are abundant in most aquatic habitats and are relatively sedentary, with limited mobility or sessile. Their relatively long life histories (approximately 1 year) allow for the integration of pollution effects over time. Aquatic macroinvertebrate communities are made up of a broad range of species from different trophic levels and tolerances, thus providing information for interpreting cumulative effects (Barbour *et al.,* 1999). Furthermore, as there are a large number of species, different stresses produce different macroinvertebrate communities (Barbour *et al.,* 1999). Therefore, aquatic macroinvertebrates are good indicators of localized disturbances and environmental conditions (Barbour *et al.,* 1999).

There are two universal approaches using macroinvertebrates to conduct biological assessment of rivers and streams (Cummins *et al.,* 2005). One is taxonomic and the other is functional, in other words, "what is it and what does it do"? The taxonomic approach focuses on determining some measures of richness and species diversity, in order to evaluate biodiversity and the sensitivity to changes in water quality parameters (Cummins *et al.,* 2005). The functional approach or functional feeding groups (FFG) classification approach (Cummins and Wilzbach, 1985; Cummins *et al.,* 2005; Rawer-Jost *et al.,* 2000) is based on morphological and behavioural mechanisms by which the macroinvertebrates acquire their food resources (Cummins *et al.,* 2005). Each taxon is categorized based on their mechanisms of obtaining food and the particle size of the food, and not specifically on what they are eating. This makes the technique particularly sensitive to land-use impacts in the watershed, primarily the stream-side (riparian) vegetation that affects the stream/river system flowing through the landscape (Cummins *et al.,* 2005). Five different FFG as per Cummins and Wilzbach, (1985) and Merritt and Cummins, (1996) within the large aquatic macroinvertebrate community have been established. Refer to Table 1 for the description of each macroinvertebrate FFG that was used in this study.

Table 1: Functional feeding groups coupled with their descriptions

FFG	Description
Shredders	Shredders chew conditioned litter or live vascular plant tissue and thus depend on this course, particulate organic matter (CPOM) for their food resources. They are presumed to be more sensitive to perturbation. Examples of shredders include Amphipoda, Potamonautidae, Leptoceridae to name a few (Cummins *et al.,* 2005).
Scrapers (grazers)	The scrapers (grazers) depend upon attached periphyton (i.e., algae and associated flora and fauna) that develops on submerged substrates for their primary food resource (Cummins *et al.,* 2005). Gastropods are common scrapers as well as Hydropsychidae species.
Filter Collectors	The filtering collectors depend upon fine particulate organic matter (FPOM) for their primary food resource which they obtain from the passing water column using constructed silken nets or filtering fans (Cummins *et al.,* 2005). Taxa include *inter alia* Simuliidae and taxa from the order Pelecypoda.
Gathering Collectors	The gathering collectors acquire the FPOM from interstices in the bottom sediments for their primary food resource. These organisms are also called deposit-feeders, as they generally gather fine materials, including plant, animal, and fungal detritus, from the surfaces of substrates (Cummins *et al.,* 2005). Taxa include Baetidae, Caenidae, Polymitarcyidae amongst others.
Predators	Predators are defined as carnivorous that capture and consume live prey (Merritt and Cummins, 1996). All members of the Odonata are predators, as well as Hirundinea, Hydracarina, Perlidae amongst others (Cummins *et al.,* 2005).

With an accumulative awareness towards the health of South African river systems, one should determine whether the Wilge River is being impacted upon by the surrounding land use activities mentioned above. In order to try to understand this, the project will investigate the association between water quality and aquatic macroinvertebrate data collected in the Wilge River and two of its tributaries mentioned above, taking into account their FFG. This will enable our understanding particularly regarding sensitivities towards land-use impacts in the watershed.

This minor dissertation includes six chapters which include the introduction, background to the study area and site descriptions. The methodology and materials contain the various indicators that were assessed and analysed, coupled with the spatial and temporal analysis approach. The fourth chapter is the results and discussion, relating to the *in situ* water quality, habitat and biotic integrity, FFGs and biological, environmental and supplementary statistical analyses. Chapter 5 and 6 includes the conclusion and recommendations and associated references to this study respectively.

1.1. Problem Statement, Aims and Objectives

There are two null hypotheses (H_O) which states that:

- Mining activities, agriculture and industrial activities have a negative impact on the present ecological state (PES) of the Wilge River; and
- *In situ* water quality namely: pH, total dissolved salts (TDS) and dissolved oxygen (DO) has an effect on the macroinvertebrate functional feeding group's structure and their preferences along the Wilge River reach.

The aim of this study was to investigate the macroinvertebrate communities of the Wilge River, taking into consideration seasonality (high and low flows) and macroinvertebrate preferences. In addition, the aim was to consider the correlations between *in situ* water quality and habitat (preferences) and the effect on macroinvertebrate communities.

In order to achieve the above mentioned aims, the specific objectives for the study are as follows:

- Determine the aquatic macroinvertebrate communities at selected sites along the Wilge River;
- Determine the current environmental driver parameters in the Wilge River (i.e. *in-situ* water quality and habitat availability) on a spatial and temporal scale;
- Quantify the driving changes of the Wilge River (i.e. *in situ* water quality) and determine how this was affecting the composition and different FFG's of the instream community structure of macroinvertebrates;
- Determine the link between the primary driving changes to the surrounding land uses in the project area (mining, agriculture and industrial activities);
- Assess the macroinvertebrate preferences in conjunction with the habitat types at selected sites, taking into account seasonality; and
- Identify and determine the primary sources of impacts along the Wilge River within the study area.

2. BACKGROUND TO STUDY AREA AND SITE DESCRIPTIONS

2.1. Study Area and Sampling Sites

The study area for this project was located within the Olifants Water Management Area (WMA4), within quaternary drainage region B20F in the Wilge River catchment. Furthermore, the project falls within the Highveld (11) – Lower Level 1 Ecoregion (Dallas, 2007) and the rivers fall within the lower foothills in the study area. The topography of the region is a gently to moderately undulating landscape of the Highveld plateau. The maximum and minimum elevation of the monitoring sites is 1498m and 1386m at sites WIL01 and WIL05 respectively, a difference of 112m from the most upstream to the most downstream site in the project area. Scattered wetlands occur in the area, coupled with rocky outcrops and ridges which form part of significant landscape features in the wider area. The geology in the Olifants River catchment consists primarily of hard rock formations, with the occurrence of the Bushveld Igneous Complex as the most prominent feature. Rich coal deposits occur in the Upper Olifants Sub-area in the vicinity of Witbank and Middelburg. Soils in this ecoregion are highly erodible. The situation is worsened by intensive cultivation and grazing, which have caused general degradation of land cover. It has further causes the riverbanks to destabilise, undercutting occurs and riverbanks are swept away by floods.

A total of seven sites were monitored, five in the Wilge River, one in the Klipspruit and one in an unnamed tributary of the Klipfonteinspruit, both of which drain in a north westerly direction into the Wilge River. Historical data from 2010 to 2013 was utilised for this study. Sites were selected to represent the receiving environment associated with the surrounding land uses, and potential impacts on the larger Wilge River.

2.1.1 WIL01 – Wilge River

(26° 2'41.46"S, 28°52'2.82"E, Elevation: 1498m)

The site is located in the upper Wilge River Catchment, where the R545 and rail crosses the Wilge River and will serve as the “reference” site on the Wilge River (Figure 1). As the study area is one of the economic hubs for agriculture, no site could be strategically positioned upstream of any areas which are perceived to be impacted by such activities. However, this site is positioned upstream of areas perceived to be impacted by mining and industrial activities. Consequently, site WIL01 will be used to assess the nature, magnitude and relevance of contributing activities/impacts to the effects of mining and industrial activities on the aquatic ecosystems. The site is characterized by all three biotopes, stones-in-current (SIC) and stones-out-of-current (SOOC), gravel, sand and mud (GSM) and vegetation (VEG). The substrate is dominated by sediment, cobbles, as well as artificial substrate in the form of a broken down bridge and old construction debris lying within the channel. This has resulted in some riffle habitat, coupled with the existing cobbles. The riparian and in-stream vegetation is limited, with dispersed in-stream shrubs and reeds, as well as alien invasive vegetation within the riparian zone.

(a) WIL01: Upstream wet season	(b) WIL01: Downstream wet season

Figure 1: Site photograph of site WIL01with (a) showing the artificial substrate in the form of a broken bridge and old construction debris beneath the railway bridge

2.1.2 WIL02 – Wilge River

(25°57'39.31"S, 28°51'3.64"E, Elevation: 1437m)

The site is situated in the Wilge River upstream of industrial activities but downstream of agricultural activities and rural development (Figure 2). The site is characterized by all three biotopes namely SIC, SOOC, GSM and VEG. In addition, site WIL02 is characterized by riffles and rapid habitats with interspersing but extended pools. The substrate is dominated by cobbles, boulders and sediment. Although the banks are steep, the riparian zone is thick with trees, grasses, sedges as well as alien invasive vegetation dominated primarily by the *Populus* species.

(a) WIL02: Upstream

(b) WIL02: Downstream

Figure 2: Site photograph of site WIL02 illustrating no flow conditions and dense riparian vegetation

2.1.3 KLI01 – Klipspruit

(28°50'23.96"E, 28°50'23.96"E, Elevation: 1437m)

The site is located upstream of industrial activities, west of the Wilge River in the Klipspruit, and accounts for any additional impacts entering the system from the south-west (Figure 3). The site is characterized by all three biotopes namely SIC and SOOC, GSM and VEG. In addition, the site consists of riffle habitats with pools further downstream. The substrate is dominated by mud and small cobbles. The riparian vegetation is limited, although there is some overhanging vegetation located in a pool upstream from the riffle area, dominated by grasses and sedges.

(a) KLI01: Upstream

(b) KLI01: Downstream

Figure 3: Site photograph of site KLI01 with (b) illustrating a riffle habitat with SIC

2.1.4 WIL03 – Wilge River

(25°54'7.88"S, 28°51'5.11"E, Elevation: 1400m)

The site is located in the Wilge River and is characterized by all three biotopes namely SIC and SOOC, GSM and VEG (Figure 4). Site WIL03 consists of riffle and rapid habitats with interspersing pools. The substrate is dominated by cobbles, bedrock and sediment (predominantly mud). The riparian zone is thick with grasses, sedges, indigenous trees, as well as alien invasive vegetation dominated primarily by the *Populus* and *Eucalyptus* species. Recently, a road was constructed through site WIL03, consequently altering the flow conditions previously observed at this site (Figure 4c). Due to a lack of appropriate engineering design and construction of the road, the following impacts have occurred at this site:

- The river upstream of this monitoring point is dammed up, due to a farmers road crossing over the river;
- Lack of appropriate culverts constructed beneath the farmers road resulting in limited water flow beneath / through the road in a downstream direction, preventing flow and upstream migration;
- Disturbance of instream and riparian habitat;
- Flow alteration; and
- Siltation.

	
(a) WIL03: Upstream	(b) WIL03: Downstream

(c) Newly constructed farm road over site WIL03 during the dry season survey

(d) Downstream effects as a result of the newly constructed road through the Wilge River. This photo was taken during the dry season

Figure 4: Site photograph of site WIL03 illustrating good riffle habitat (a, b) and the newly constructed road through the Wilge River (c) and the result thereof (d).

2.1.5 WIL04 – Wilge River

(25°52'29.14"S, 28°51'47.27"E, Elevation: 1394m)

This site is located in the Wilge River downstream of agricultural, mining and industrial activities (Figure 5). The site is characterized by two biotopes namely GSM and VEG, during the wet season and only one biotope, GSM during the dry season. The site is characterized by steep, eroded and incised banks, with a deeply eroded channel with limited flow. The substrate is dominated by mud and the riparian zone is dominated by trees, primarily by *Populus* and *Salix* species.

(a) WIL04: Upstream

(b) WIL04: Downstream

Figure 5: Site photograph of site WIL04 illustrating deep eroded channel

2.1.6 TRI01 – Unnamed tributary of the Klipfonteinspruit

(25°53'26.70"S, 28°53'24.97"E, Elevation: 1419m)

This unnamed tributary of the Klipfonteinspruit flows into the Wilge River in a north westerly direction (Figure 6). The site is within the footprint of various industrial and agricultural activities. Site TRI01 is characterized by all three biotopes namely SIC, SOOC (although limited), GSM and limited VEG. In addition, the site shows the characteristic of small riffle habitat with pools further downstream, with the substrate dominated by mud and small cobbles. The riparian vegetation is limited and mainly comprises of grass with severely eroded and undercut banks.

(a) TRI01: Upstream

(b) TRI01: Downstream

Figure 6: Site photograph of site TRI01 illustrating severely eroded and undercut banks

2.1.7 WIL05 – Wilge River

(25°50'40.30"S, 28°52'18.07"E: Elevation: 1386m)

The site is located in the Wilge River downstream of industrial, mining and agricultural activities and characterised by all three biotopes namely SIC, SOOC, GSM and VEG (Figure 7). In addition, the site is characterized by riffle and rapid habitats with interspersing pools. The substrate is dominated by cobbles, bedrock and mud and the riparian zone is thick with grasses and trees, including alien invasive vegetation.

(a) WIL05: Upstream

(b) WIL05: Downstream

Figure 7: Site photograph of site WIL05 with (b) illustrating riffle habitat

A map of the study area showing the location of the aquatic sampling sites is presented in Figure 8.

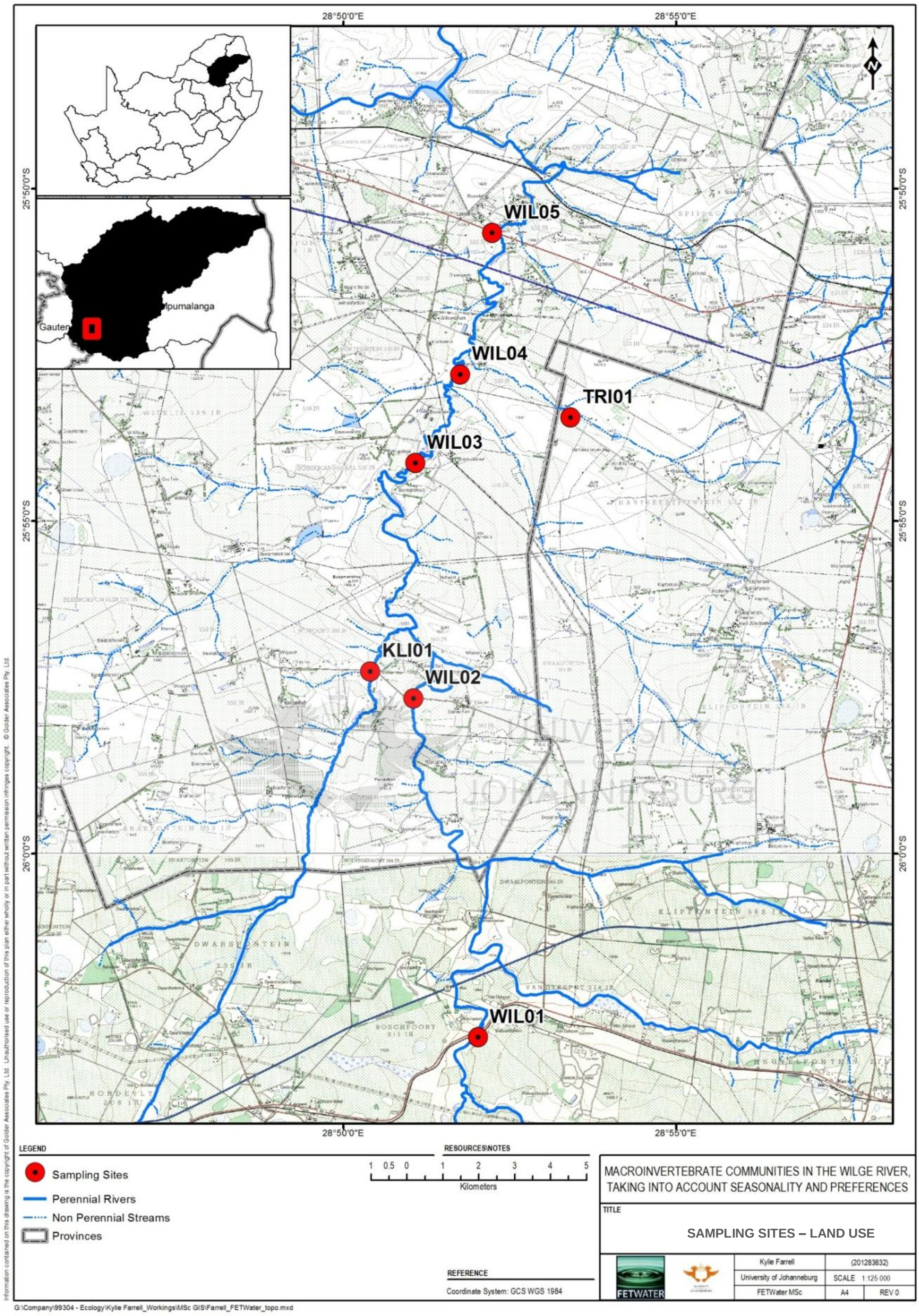


Figure 8: A map of the study area showing the location of the aquatic sampling sites

2.2. Land Use Surrounding Sampling Sites

Catchment determinants such as land use may have direct or indirect effects on the aquatic macroinvertebrate communities (Pan *et al.,* 2004). Therefore, in order to quantify the relationship between land use and biological integrity of stream ecosystems, a land use map was compiled. This aided in the assessment of the surrounding land uses associated with each sampling point, which fundamentally would provide a better understanding of the impacts/stressors at each site and the potential inputs into the Wilge River system. Furthermore, an understanding of the type of land use in the study area and comparing it to the different FFG recorded at each site, will aid in determining the link between the primary driving changes to the surrounding land uses in the project area (mining, agriculture and industrial activities).

A 1 km buffer zone is indicated around each sampling point. This takes into account 1 km upstream of the site, coupled with a 1 km buffer area around selected tributaries, if any, entering into the Wilge River above that monitoring point (Figure 9). The land uses were subsequently superimposed over each buffered area for each sampling point (Figure 10). The agricultural activities were separated into medium and high agricultural activities. Medium agricultural activities included local agricultural activities, cattle grazing, old fields, farmsteads/homesteads, versus high agricultural activities which indicated extensive agricultural activities namely, cattle, pig and chicken farming. Mining land use activities included mining activities and quarries, while industrial land use activities included current construction footprints, as well as existing industrial complexes. Infrastructure included railways and tar and/or gravel roads. In addition to the land use map, further visual observations were made using Google Earth, 2013 as well as ground truthing on the ground while the field surveys were conducted. These additional land uses were included in Table 2.

Table 2: Summary of land uses identified at each sampling point in the study area

Sampling Point	Land use activities
TRI01	• Medium Agriculture • Industrial • Mining
WIL01	• High Agriculture • Pivot Irrigation • Infrastructure

Sampling Point	**Land use activities**
WIL02	• High Agriculture • Pivot Irrigation • Rural Development
KLI01	• High Agriculture • Pivot Irrigation • Rural Development
WIL03	• High Agriculture • Pivot Irrigation • Rural Development • Infrastructure (newly constructed farm road through the Wilge River
WIL04	• High Agriculture • Pivot Irrigation • Industrial • Mining
WIL05	• Medium Agriculture • Pivot Irrigation • Industrial • Mining

Therefore, the study sites are all within this heavily utilised economic hub within the catchment area, as described in the introduction, as the rivers or streams are either flowing through agricultural, industrial or mining activities.

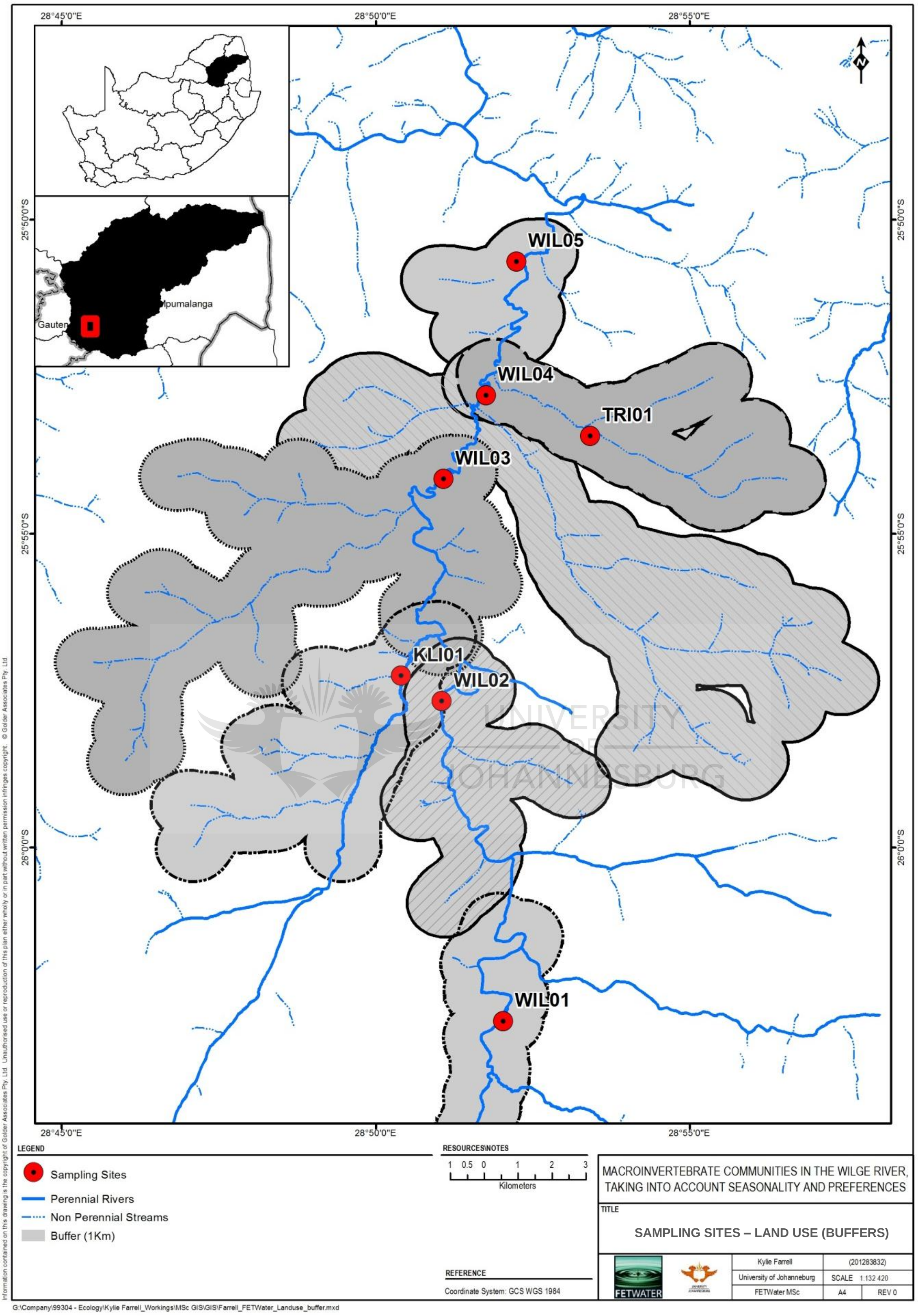


Figure 9: 1 km buffers around each sampling site

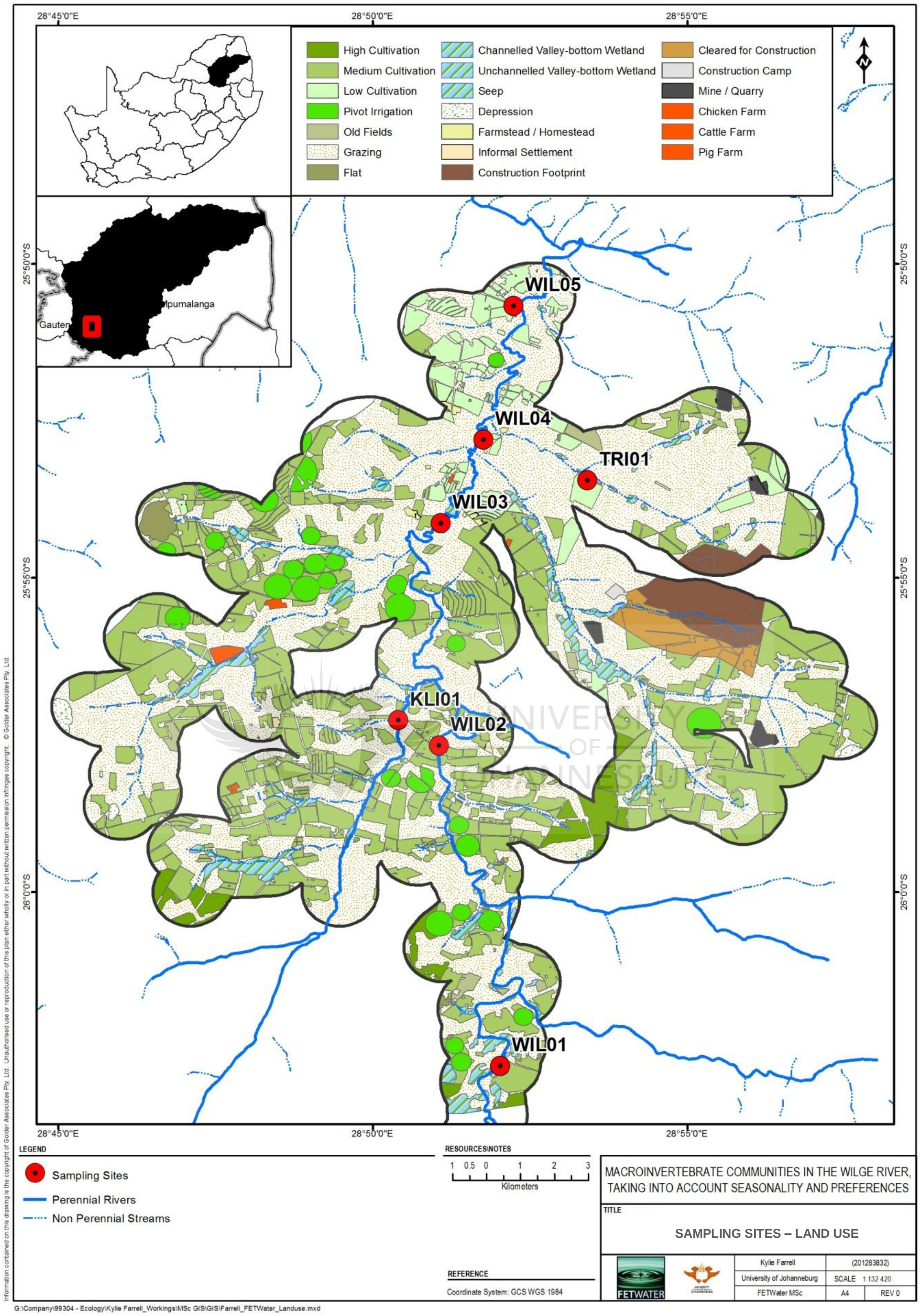


Figure 10: 1 km buffers around each sampling site illustrating land uses upstream of each sampling site

3. MATERIALS AND METHODS

In order to adequately describe the associated aquatic environment, indicators were selected to represent each of the stressor, habitat and response components involved in the aquatic environment. Broad methodologies to characterise these components are described below. These methodologies are generally applied and accepted (DWAF and the United States of Environmental Protection Agency (USEPA)).

- Stressor Indicators: *In situ* water quality.
- Habitat Indicators: Integrated Habitat Assessment System *Version 2* (IHAS).
- Response Indicators: Aquatic macroinvertebrates using the South African Scoring System *Version 5* (SASS5), coupled with recording each taxa's FFG.

Surveys were conducted during both the wet and dry season. Refer to Table 3 which lists the months that were monitored per season. This enabled the assessment of both biological and environmental data on a seasonal scale.

Table 3: Seasonal surveys

Wet season	**Dry season**
March 2010	June 2010
December 2010	September 2010
March 2011	July 2011
November 2011	September 2011
December 2012	August 2012
February 2013	May 2013

3.1. Stressor Indicators

Water quality has a direct influence on aquatic life forms. Although these measurements only provide a "snapshot", the four years' worth of *in situ* water quality may provide valuable insight into the characteristics and interpretation of the sampling sites. Compact hand held instruments were used to record the following water quality parameters:

- pH (Eutech pH Tester);
- Electrical Conductivity (EC) (Eutech ECTester11 Dual Range);
- Dissolved Oxygen (DO) and Percentage Oxygen Saturation (DO%) (Eutech CyberScan DO300);
- Temperature (EutechCyberScan DO300); and
- Clarity (Secci Disk).

The Department of Water Affairs and Forestry (DWAF) published the South African Water Quality Guidelines for Aquatic Ecosystems (Volume 7) in 1996. These guidelines provide target ranges in terms of water quality for protection of aquatic ecosystems. All measured parameters for the sites should be within these target water quality ranges (TWQR). It is these benchmarks that are used to assess the present condition of the river systems and the extent of degradations. Refer to Table 4 below for the TWQR for aquatic water quality. Furthermore, as the DWAF, (1996) guidelines do not provide a TWQR for DO, the median guideline that was used for this study for DO concentration for the protection of aquatic biota, was greater than 5 mg/ℓ (Kempster *et al*., 1980).

Table 4: TWQR as per DWAF, (1996) and Kempster et al., (1980)

Water quality parameter	**TWQR**
pH	6.5 – 9.0
EC	<154.0 mS/m
TDS	<1000 mg/ℓ
DO	>5 mg/ℓ
DO%	80 – 120%
Temperature	5 – 30 °C
Clarity	>25 cm

3.2. Habitat Indicator

Habitat assessment can be defined as the evaluation of the structure of the surrounding physical habitat that influences the quality of the water resource and the condition of the resident aquatic community (Barbour *et al.,* 1996). Habitat quality and availability plays a critical role in the occurrence of aquatic biota. For this reason habitat evaluation is conducted simultaneously with biological evaluations in order to facilitate the interpretation of results.

The IHAS were applied at each sampling site, in order to assess the availability of habitat biotopes for aquatic macroinvertebrates. The IHAS was developed specifically for use with the SASS5 index and rapid biological assessment protocols in South Africa (McMillan, 1998). The index considers sampling habitat and stream characteristics. The sampling habitat is broken down into categories, these being stones-in-current, vegetation and other habitat / general. All of these add up to a possible 100 points (or percentage). It is presently thought that a total IHAS score of over 65% represents good habitat conditions, a score over 55% indicates adequate/fair habitat conditions and anything below 55% is poor (McMillan, 1998) (Table 5).

Table 5: Integrated Habitat Assessment System Scoring Guidelines (Version 2)

IHAS Score	Description
> 65%	Good
55-65%	Adequate/Fair
< 55%	Poor

3.3. Response Indicator

Aquatic macroinvertebrates were sampled using the qualitative kick sampling method called SASS5 (Dickens and Graham, 2002). The SASS5 protocol is a biotic index of the condition of a river or stream, based on the resident macroinvertebrate community, whereby each taxon is allocated a score according to its level of tolerance to river health degradation (Dallas, 1997).

The SASS5 method relied on churning up the substrate with your feet and sweeping a finely meshed SASS net (pore size of 1000 micron), mounted on a 300 mm square frame, over the churned up area. In the SIC habitat (rapids, riffles, runs, etc.) the net is rested on the substrate and the area immediately upstream of the net disturbed by kicking the stones over and against each other to dislodge benthic invertebrates. This is conducted for 2 minutes. The net is also swept under the edge of marginal and aquatic vegetation (VEG) for a distance of 1 - 2 m. Kick samples are collected from areas with gravel, sand and mud (GSM) substrates over a period of 1 minute. Identification of the organisms is made to family level (Thirion et al., 1995; Davies & Day, 1998; Dickens & Graham, 2002; Gerber & Gabriel, 2002).

The endpoint of any biological or ecosystem assessment is a value expressed either in the form of measurements (data collected) or in a more meaningful format by summarising these measurements into one or several index values (Cyrus et al., 2000) The endpoints used for this study were the total SASS score and average score per taxa (ASPT). All sites were scored according to these indices, based on macroinvertebrate diversity.

3.3.1 Biotic Integrity Based on SASS5 Results

Reference conditions reflect the best conditions that can be expected in rivers and streams within a specific area and also reflect natural variation over time. These reference conditions are used as a benchmark against which field data can be compared. Modelled reference conditions for the Highveld Ecoregion were obtained from Dallas (2007) (Table 6).

Table 6: Modelled reference conditions for the Highveld Ecoregion (11) based on SASS5 and ASPT values (Dallas, 2007)

SASS Score	ASPT	Class	Description
>124	>5.6	A	Unimpaired. High diversity of taxa with numerous sensitive taxa
83-124	4.8-5.6	B	Slightly impaired. High diversity of taxa, but with fewer sensitive taxa
60-82	4.6-4.8	C	Moderately impaired. Moderate diversity of taxa
52-59	4.2-4.6	D	Considerably impaired. Mostly tolerant taxa present
30-51	Variable <4.2	E	Severely impaired. Only tolerant taxa present
<30	Variable	F	Critically impaired. A few tolerant taxa present

The FFG approach was used (Cummins and Wilzbach, 1985; Cummins *et al.,* 2005). In most instances, order or family level was sufficient to allow characterization of functional groups (Merritt and Cummins, 1996). As per Table 1, taxa were classified with the following FFG:

- Shredders;
- Scrapers/grazers;
- Filter Collectors;
- Gathering Collectors; and
- Predators.

3.4. Spatial and Temporal Analytical Approach

Two statistical packages were used to analyse the biological and environmental data namely PRIMER-E Ltd (*version* 6) and Canoco (*version* 4.5).

Aquatic macroinvertebrate data was analysed by means of multivariate procedures. This is due to the community-based nature of the data which makes classical univariate assumptions invalid. In contrast to univariate analyses (ANOVA, regression), multivariate procedures consider each taxon to be a variable and the presence/absence of each taxon to be an attribute of a site or time. Subtle changes in community composition across sites, which are generally masked when the characteristics of a site are combined into a single index value, are more likely to be detected by multivariate procedures. Spatial trends in community composition can therefore be displayed by means of multivariate methods of data analyses.

3.4.1 PRIMER-E Ltd (version 6): Displaying community patterns through Cluster Analysis and Non-metric Multi-dimensional Scaling

Bray-Curtis similarity matrices, constructed from the relative abundances of the various aquatic macroinvertebrate taxa recorded spatially and temporally, were subjected to group averaged clustering and two-dimensional non-metric Multidimensional Scaling (MDS) ordinations (Clarke and Warwick, 1994). Both procedures start from a triangular similarity matrix computed between sets of samples. These multivariate techniques attempt to reduce the complexity of the community data by representing relationships between samples in a lower dimension (Cyrus *et al.,* 2000). One-way Analysis of Similarity (ANOSIM) was used to determine the level of the overall differences in the aquatic macroinvertebrate composition among the sampling sites and seasons. It further compares every site to yield a test statistic and a level of significance (Clarke and Green, 1988). To interpret this, the R-statistic value is taken as the degree of similarity between sites and ranges between 1 and -1 (Clarke and Green, 1988). The deviation from zero represents the significance level and a negative R statistic suggests that the similarity across the different sites is higher than those within the sites (Table 7) (Cyrus *et al.,* 2000).

Table 7: Interpretation of statistical significance using R values

***R* value**	**Interpretation**
R = 1	If all replicates within sites are more similar to each other than any other replicates from different sites
R = 0	If the similarities between sites will be the same of average

When the pairwise comparisons in the ANOSIM test detected a significant difference in the aquatic macroinvertebrate compositions spatially and temporally, Similarity Percentages (SIMPER) was used to identify which species typified each of those habitat types. To determine which environmental variables (namely *in situ* water quality and/or land use) were possibly responsible for the various groups, Redundancy Analysis (RDA) and Principal Component Analysis (PCA) was conducted in Canoco version 4.5. All data analysed within Primer was pre-treated and square root transformed.

3.4.1. Canoco Version 4.5: Redundancy Analysis and Principal Component Analysis Approach

When one investigates variation of animal communities across a range of different environmental conditions, one generally finds not only large differences in species composition of the studied communities, but also a certain consistency or predictability of this variation. Canoco version 4.5 was used to compute ordinations of the sampling sites. Multivariate analyses of the study's ecological data were selected as it considered each taxon to be a variable and the presence / absence of each taxon to be an attribute of a site or time period. Subtle changes in community composition across sites, which are generally incognito when the characteristics of a site are combined into a single index value, are more likely to be detected by multivariate procedures. Spatial trends in community composition can therefore be displayed by means of multivariate methods of data analyses.

A principal component analysis (PCA) approach was used to assess the macroinvertebrate communities (Ter Braak and Smilauer, 2004). The PCA is based on a linear response model relating species and environmental variables (van den Brink *et al*., 2003). The results of the ordination is a map of the samples being analysed on a 2-dimensional (2D) bases, where the placements of the samples reflect the (dis)similarities between the samples (Shaw, 2003). In order to determine which water quality variables may be contributing to the structure of the PCA and consequently the structure of the macroinvertebrate communities, various redundancy analyses (RDAs) were conducted.

Following the PCA ordination, the RDA (also a linear response model) included an additional factor which allowed for the selection of the driving variables or environmental data that is intended to be overlaid onto the PCA (Ter Braak and Smilauer, 2004). In this case water quality variables in the study were overlain onto the original PCA. In addition, the RDA further included supplementary data, namely land use, which is further overlaid onto the PCA. The output of the RDA is also a 2D bi-plot which has a matrix of biological or environmental data overlain (tri-plot), in this case water quality and land use. This water quality data is represented by an arrow, where direction, distance and gradient correspond to the correlation between the variables. The approximated correlation is positive when the angle is acute and negative when the angle is larger than 90°. The distance between the sampling sites in the diagram approximates the dissimilarity of the variables as measured by their Euclidean distance (Shaw, 2003). The Monte Carlo permutation tests was conducted in order to test potential relationships ($p<0.05$). All data analysed within Canoco was log transformed and no standardisation.

The following limitations were noted prior to proceeding with the multivariate data analyses. Aquatic macroinvertebrates were not recorded at site WIL04 during the March and June 2010 surveys. Consequently, this site and time period was removed from the aquatic macroinvertebrate dataset, and thus in order to avoid potential anomalies during the analysis procedure, the water quality dataset was also removed for this time and time period. Furthermore, March 2010 was removed from the *in situ* water quality data for the purpose of statistics as the percentage saturation was not measured during that survey.

4. RESULTS AND DISCUSSION

4.1. *In situ* Water Quality

The *in situ* water quality results are presented in Appendix A. This information is important in terms of the interpretation of biological results because of the direct influence water quality has on aquatic life forms. Although these measurements only provide a “snapshot”, there is four years’ worth of data which can provide valuable insight into the characteristics of the sampling sites in question.

It should be noted that this does not constitute the general state of water quality at the sites or streams and does not include chemical water quality analysis, metals or organic contaminants, nutrient or pesticide analysis.

The TWQR as provided by DWAF (1996) is shown for the *in situ* parameters measured in Appendix A. The guideline for DO was obtained from Kempster *et al.,* (1980).

4.1.1 pH

Most fresh waters are usually relatively well buffered and more or less neutral, with a pH range from 6.5 to 8.5, and most are slightly alkaline due to the presence of bicarbonates of the alkali and alkaline earth metals (Bath, 1989). In addition, pH values should not be allowed to vary from the range of data for a specific site and time of day, by more than 0.5 of a pH unit, or greater than 5 %, whichever is the more conservative (DWAF, 1996). The pH of natural waters is determined by geological influences and biotic activities.

Historical and current data, from March 2010 to May 2013 illustrate that the pH values have fluctuated both spatially and temporally and in most instances, have been within the TWQR guidelines for freshwater aquatic ecosystems in South Africa (Figure 11). The pH values recorded have been mostly alkaline, which are common throughout the Highveld catchment. The pH were recorded above the TWQR guideline value (6.5 – 9.0) at all the sites along the

Wilge River, although site WIL05 was border line, during the March 2010 survey (Figure 11). Low pH values were recorded at site TRI01 and WIL03 during the February 2013 and August 2012 surveys respectively (Figure 11). The exceeded and low pH values may have resulted in a range of physiological stresses on the aquatic biota at the time of those surveys. However, the pH subsequently recovered and returned back to its general alkaline trend along the Wilge River following those mentioned surveys (Figure 11).

This trend was also illustrated during the wet and dry season (Figure 12 and Figure 13 respectively). The trend during the dry season illustrated less variability in the data, with least variation occurring at site KLI01, compared to the wet season which illustrated high variation in the pH values (Figure 13).

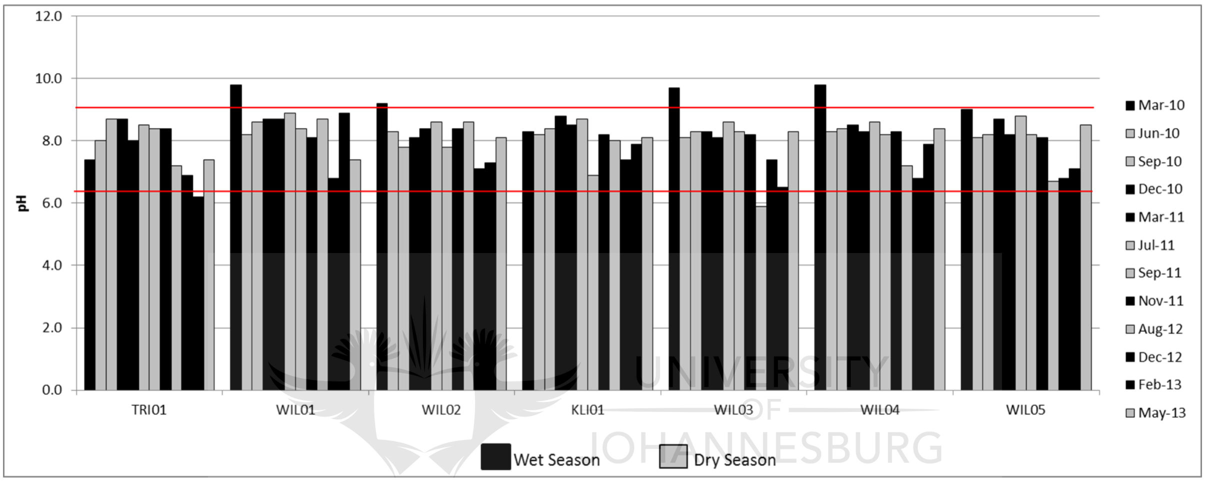


Figure11: Historical and current pH values recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively, red lines indicate guideline values)

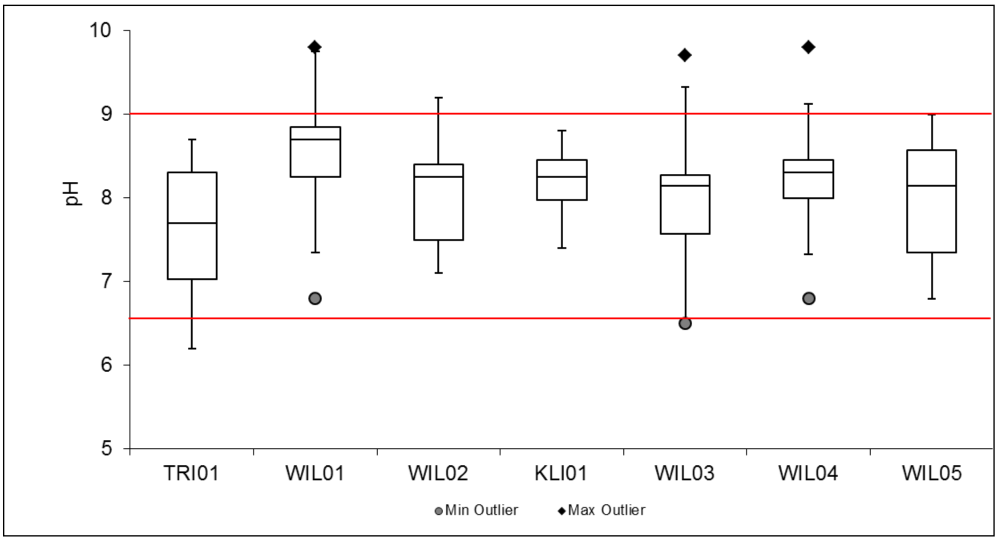


Figure12: A box and whisker plot showing pH levels recorded at the seven monitoring points during the wet season surveys (2010 – 2013) (red lines indicate guideline values, ● Minimum Outlier ◆ Maximum Outlier)

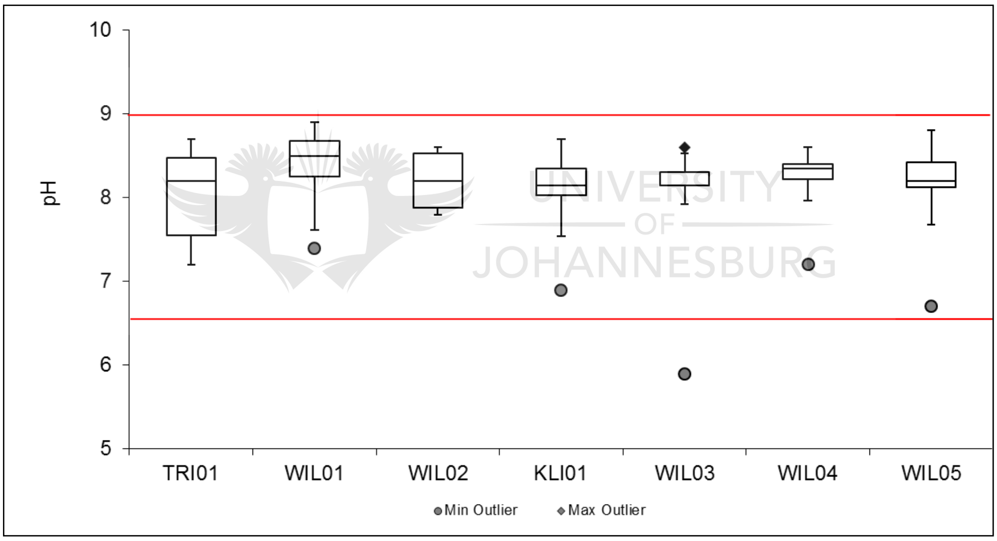


Figure13: A box and whisker plot showing pH levels recorded at the seven monitoring points during the dry season surveys (2010 – 2013) (red lines indicate guideline values, ● Minimum Outlier ◆ Maximum Outlier)

4.1.2 Total Dissolved Salts / Electrical Conductivity

The EC is a measure of the ability of water to conduct an electrical current (DWAF, 1996). This ability is a result of the presence in water of ions such as carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium, all of which carry an electrical charge (DWAF, 1996). Many organic compounds dissolved in water do not dissociate into ions (ionise), and consequently they do not affect the EC (DWAF, 1996). The EC is a rapid and useful surrogate measure of the TDS concentration of waters with a low organic content (DWAF, 1996). For the purpose of interpretation of the biological results collected from the 2010 to 2013 survey, the TDS concentrations were calculated by means of the EC using the following generic equation (DWAF, 1996):

TDS (mg/ℓ) = EC (^{m}S/m at 25 °C) x 6.5

According to Davies and Day (1998), freshwater organisms usually occur at TDS values less than 3000 mg/ℓ. According to the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996) the rate of change of the TDS concentration, and the duration of the change is more important than absolute changes in the TDS concentration. Most of the macroinvertebrate taxa that occur in streams and rivers are sensitive to salinity, with toxic effects likely to occur in sensitive species at salinities > 1000 mg/ℓ (DWAF, 1996). According to the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996; Volume 7) TDS concentrations in South African inland waters should not be changed by > 15% from the natural background values.

Historical and current data collected from March 2010 to May 2013 showed that the TDS concentrations at all sampling sites remained below the TWQR guideline over time and thus was not considered a limiting factor for aquatic biota at these sites (Figure 14). The highest TDS concentrations were recorded at the most upstream site in the Wilge River, site WIL01. The concentrations subsequently decreased in a downstream direction along the Wilge River, where it remained relatively consistent at the two most downstream sites (sites WIL04 and WIL05) (Figure 14). Site TRI01, an unknown tributary of the Klipfonteinspruit which enters the Wilge River further downstream of site WIL04, recorded the lowest TDS concentrations (Figure 14). Therefore, as the TDS concentrations are higher within the Wilge River, this may likely be due to an input of salts along the river.

This trend above was mirrored in Figure 15 (wet season) and Figure 16 (dry season) where site WIL01 indicated the highest TDS concentrations, particularly during the dry season. It further indicated a high variability in the TDS concentrations, compared to the rest of the sites along the Wilge River (Figure 16). As this site is the most upstream site for the project, the high TDS concentrations may be attributed to higher up within the catchment from possible sources namely agricultural activities.

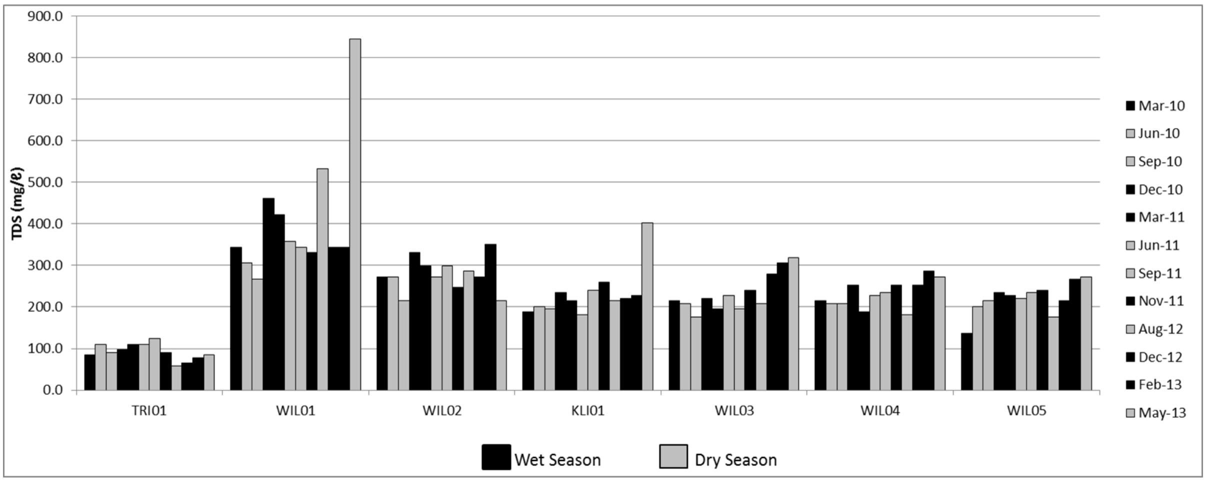


Figure14: Historical and current TDS concentrations recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively)

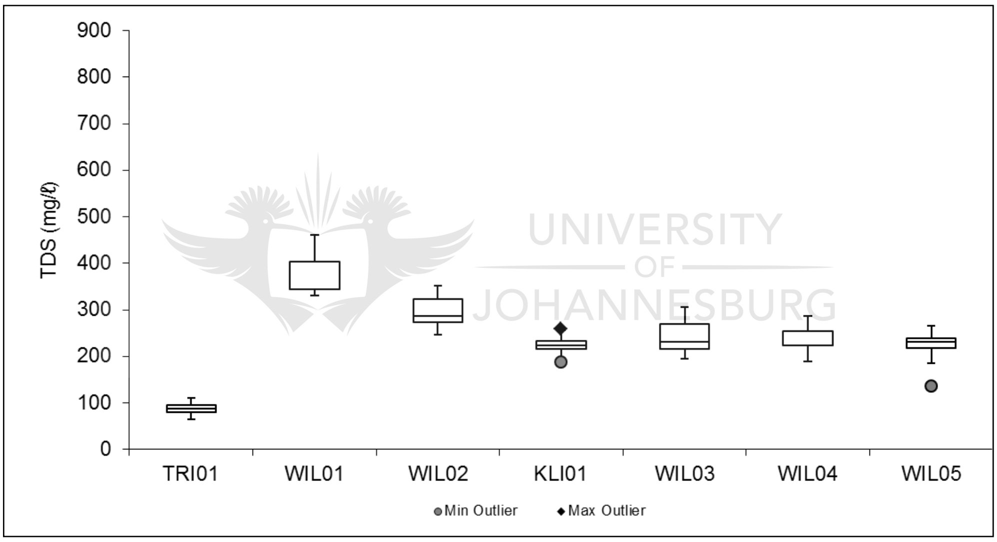


Figure 15: A box and whisker plot showing TDS concentrations recorded at the seven monitoring points during the wet season surveys (2010 – 2013) (● Minimum Outlier ◆ Maximum Outlier)

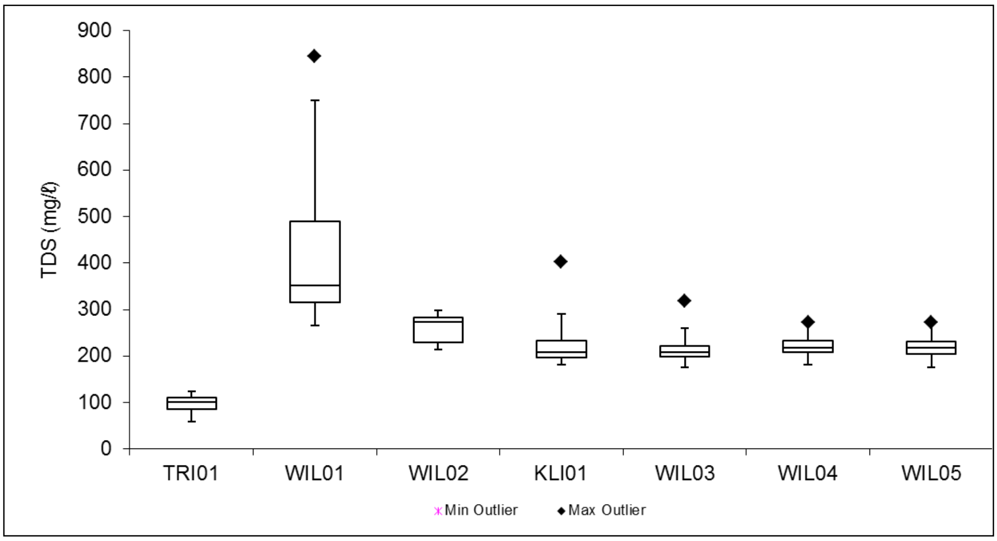


Figure 16: A box and whisker plot showing TDS concentrations recorded at the seven monitoring points during the dry season surveys (2010 – 2013) (◆ Maximum Outlier)

4.1.3 Dissolved Oxygen

The maintenance of adequate Dissolved Oxygen (DO) concentrations is critical for the survival and functioning of the aquatic biota as it is required for the respiration of all aerobic organisms (DWAF, 1996). Therefore, DO concentration provides a useful measure of the health of an ecosystem (DWAF, 1996). The median guideline for DO for the protection of aquatic biota is > 5 mg/ℓ (Kempster *et al.,* 1980).

The DO concentrations clearly illustrate seasonal and temporal variation (Figure 17). Low DO concentrations below 5 mg/ℓ occurred at majority of the sites during the March 2010, November 2011, December 2012 and May 2013 surveys. Between these surveys, the DO concentration either stabilised or increased considerably above the guideline value (Figure 17). Eutrophication is associated with nutrient enrichment which may be a contributing factor to the low DO concentrations through the catchment. It may further be associated with a combination of gradients and habitat. All the monitoring points are located at low gradients, coupled with limited rocky habitats, which functions as an aeration mechanism, thus oxygenating the water. Furthermore, the amount of oxygen dissolved in water is influenced by the aeration rate from the atmosphere, temperature, air pressure and salinity, as well as from the comparative rates of respirations and photosynthesis (Davies and Day, 1998). Consequently, the low DO concentrations observed may have a limiting effect on aquatic biota.

Shown in Figure 18 (wet season) and Figure 19 (dry season), the DO concentrations were typically below the guideline value during the wet season, as opposed to the dry season surveys. In accordance with Davies and Day, (1998), the higher the temperature, the less oxygen is available in water, which thus clarifies this finding. Furthermore, high variability in the DO concentrations was indicated both spatially and seasonally (Figure 18 and Figure 19).

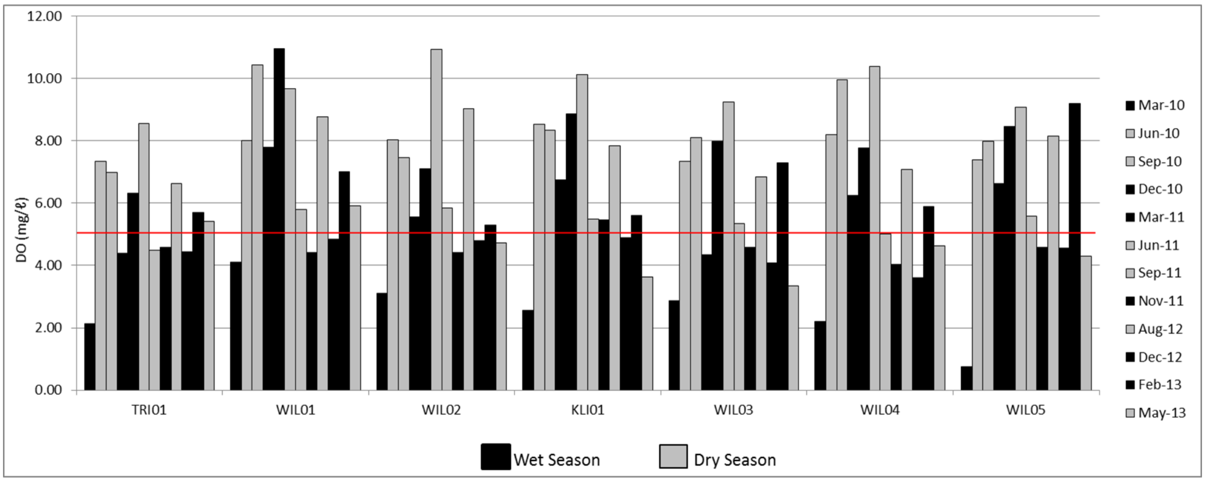


Figure17: Historical and current DO concentrations recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively, red line indicates guideline limit)

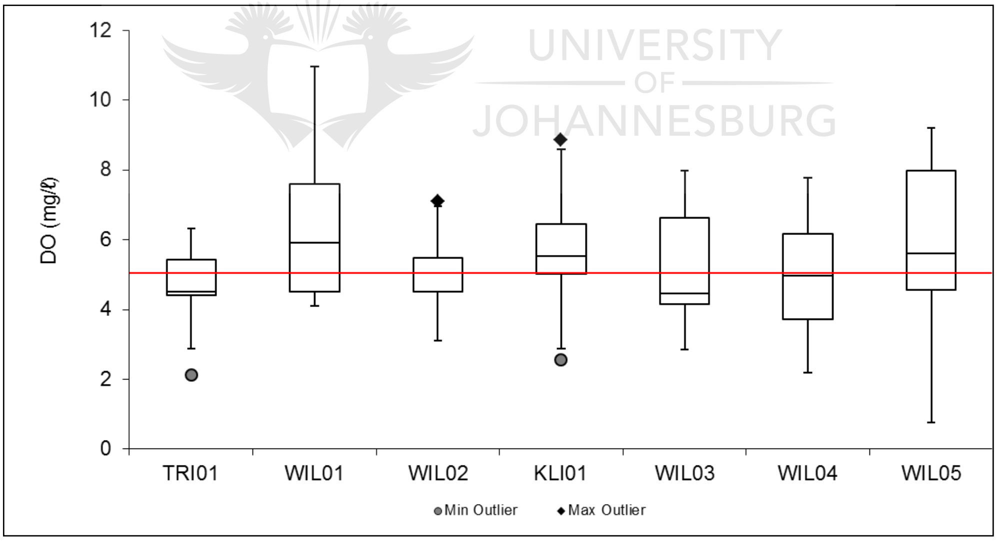


Figure 18: A box and whisker plot showing DO concentrations recorded at the seven monitoring points during the wet season surveys (2010 – 2013) (red line indicates guideline limit, ● Minimum Outlier ◆ Maximum Outlier)

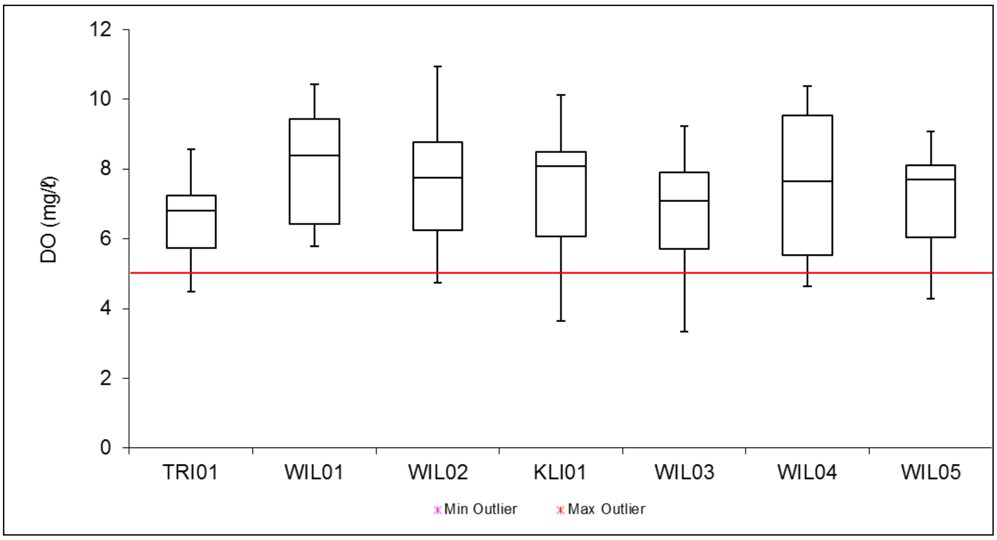


Figure 19: A box and whisker plot showing DO concentrations recorded at the seven monitoring points during the dry season surveys (2010 – 2013) (red line indicates guideline limit)

4.1.4 Percentage Oxygen Saturation (DO%)

Percentage oxygen saturation is the amount of oxygen (O_2) in a litre of water relative to the total amount of oxygen that the water can hold at that temperature. DO levels fluctuate seasonally and diurnally over a 24-hour period and vary with water temperature and altitude. The South African Water Quality Guidelines (DWAF, 1996), state that the TWQR for DO% to protect aquatic biota through most life stagers is 80% - 120%, and that below 40% would be lethal.

Similar to the DO concentrations, the historical data for the percentage saturation collected from March 2010 to May 2013 illustrated high levels of variation both between sites and over time (Figure 20). Although all the sampling sites recorded the percentage saturation to be below and above the guideline values during various surveys, there were no definite trends at any given site or time (Figure 20). Site WIL04 however, recorded low percentage saturation levels, 50% of the monitoring events. This occurred frequently during the wet season and exceeded the guideline value once during the dry season (Figure 20). This may be due to habitat availability namely, a lack of stones habitat and a deeply eroded channel compared to the rest of the sites along the Wilge River. During the August 2012 survey, the percentage saturation levels exceeded the guideline value of 120% at all the sites along the Wilge River and the two adjoining tributaries, with the exception of site WIL03. This exceedance however, recovered in the subsequent wet season monitoring event (December 2012), barring sites WIL03 and WIL04, which fell below the guideline value of 80% (Figure 20). None of the sites recorded the percentage saturation to be below the lethal limit of 40%

(Figure 20). Similar to the DO concentration results, low percentage saturation levels may have a limiting effect on aquatic biota if persistent.

The trend above was mirrored in Figure 21 (wet season) and Figure 22 (dry season). During the wet season, the percentage saturation at site WIL04 fell below the guideline values of 80%, although increased to within the guideline values further downstream at site WIL05 (Figure 21). Conversely during the dry season, the percentage saturation improved at site WIL04 whereby it was measured within the guideline values (Figure 22).

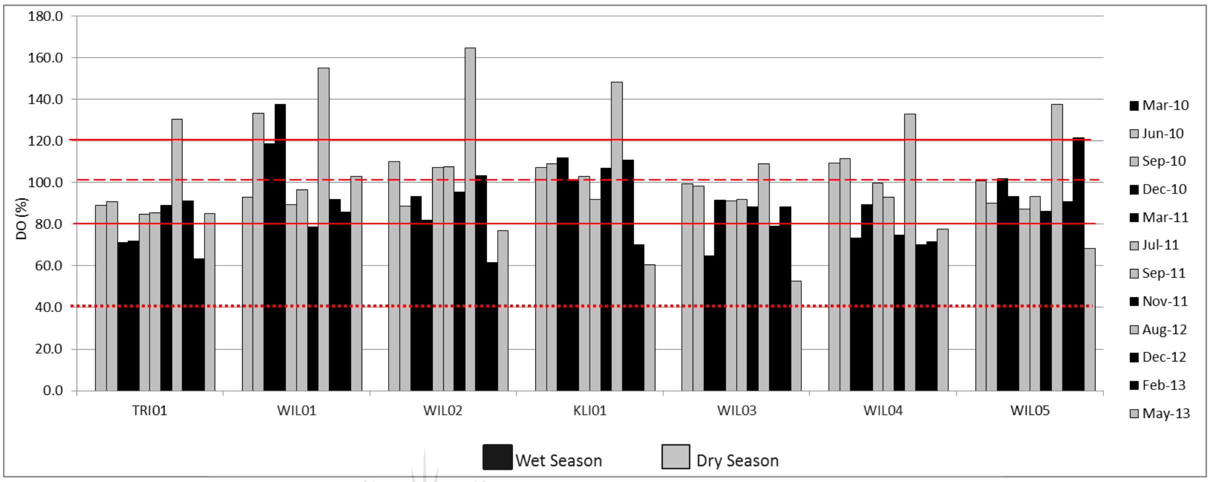


Figure 20: Historical and current DO% recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively, solid red lines indicates target values, dashed line indicates saturation and the dotted line indicates lethal limits). DO% was not recorded during the March 2010 survey.

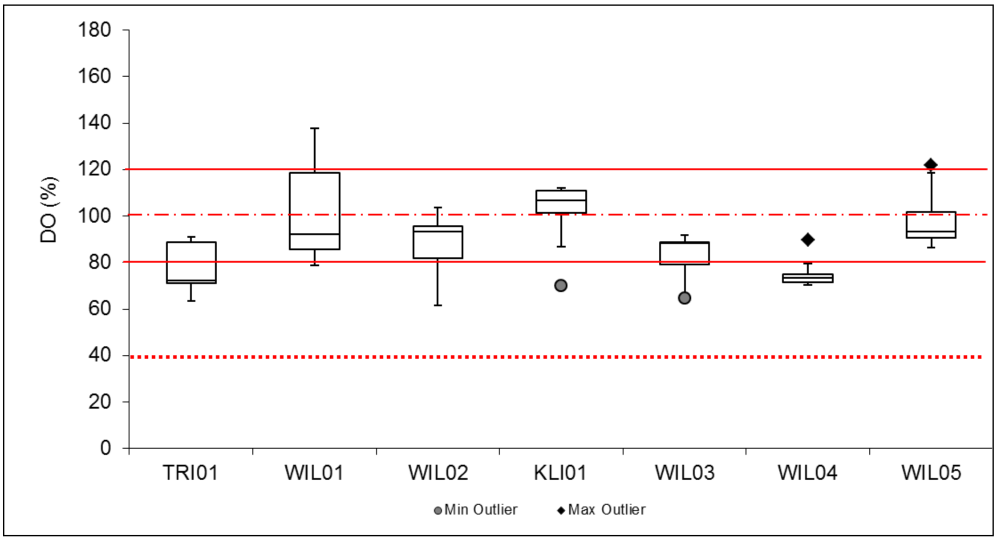


Figure 21: A box and whisker plot showing DO% recorded at the seven monitoring points during the wet season surveys (2010 – 2013) (solid red lines indicates target values, dashed

line indicates saturation and the dotted line indicates lethal limits, ● *Minimum Outlier* ◆ *Maximum Outlier)*

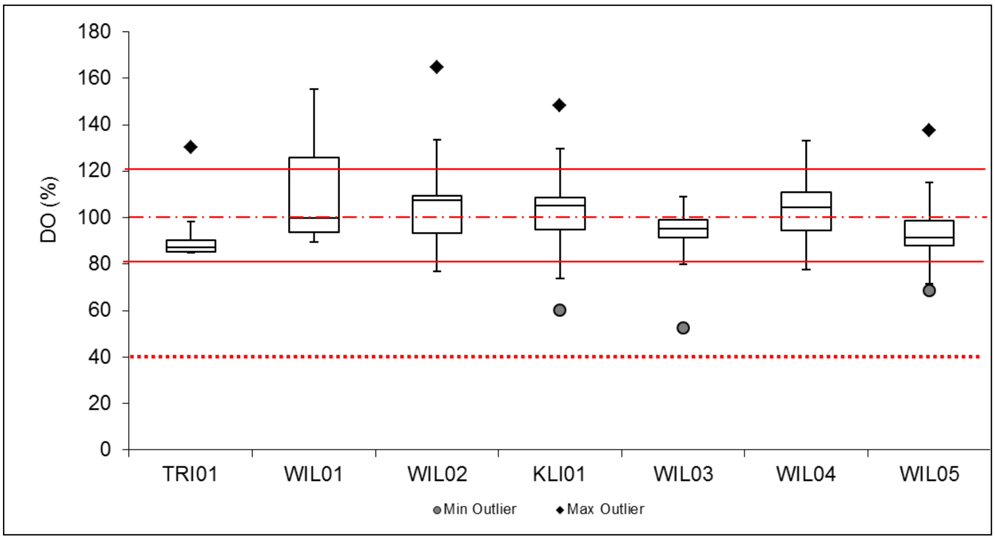


Figure 22: A box and whisker plot showing DO% concentrations recorded at the seven monitoring points during the dry season surveys (2010 – 2013) (solid red lines indicates target values, dashed line indicates saturation and the dotted line indicates lethal limits, ● *Minimum Outlier* ◆ *Maximum Outlier)*

4.1.5 Water Temperature

Water temperature plays an important role in aquatic ecosystems by affecting the rates of chemical reactions and therefore also the metabolic rates of organisms (DWAF, 1996). Temperature affects the rate of development, reproductive periods and emergence time of organisms (DWAF, 2005). Temperature varies with season and the life cycles of many aquatic macroinvertebrates are cued to temperature (DWAF, 2005). The temperatures of inland waters generally range from 5 to 30 degrees Celsius (˚C) (DWAF, 1996).

The water temperatures measured from 2010 – 2013 were considered to be normal for these systems and clearly reflected seasonal variation over time. Overall, the temperature during the high flow conditions ranged from 19.1°C at site WIL01 to 29.4°C at site KLI01 during the March 2010 and December 2012 surveys respectively. During the low flow conditions, the temperature ranged from 8.0°C at site WIL01 to 24.5 ˚C at site WIL04 during the June 2011 and September 2010 surveys respectively (Figure 23). Therefore, temperature was not expected to have a limiting effect on aquatic biota.

The trend in the box and whisker plots reflects the above statement. Figure 24 illustrates higher temperatures during the wet season, while the dry season illustrates lower water temperatures (Figure 24 and Figure 25 respectively).

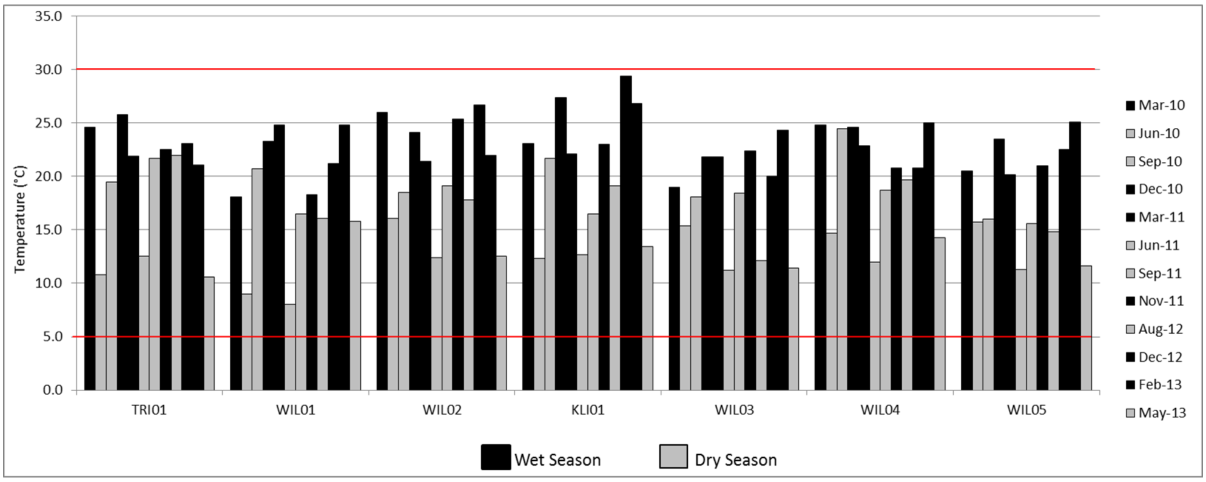


Figure 23: Historical and current water temperature recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively, red lines indicates guideline limit)

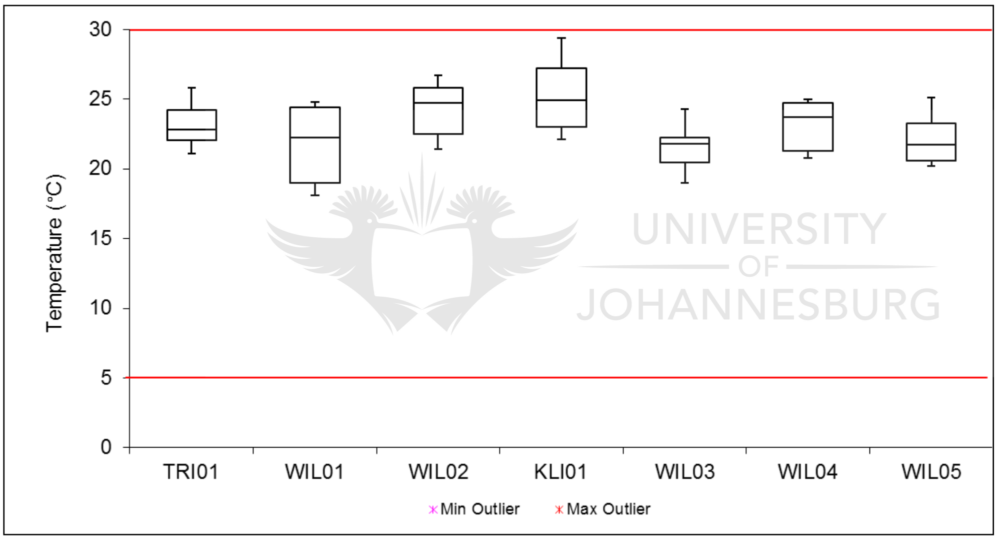


Figure 24: A box and whisker plot showing water temperature recorded at the seven monitoring points during the wet season surveys (2010 – 2013) (solid red lines indicates target values)

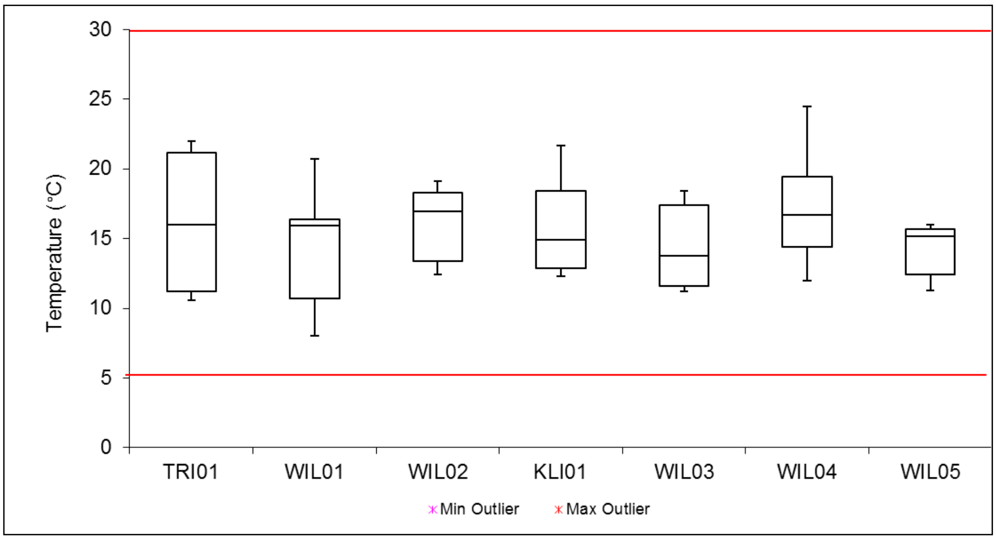


Figure 25: A box and whisker plot showing water temperature recorded at the seven monitoring points during the dry season surveys (2010 – 2013) (solid red lines indicates target values)

4.1.6 Turbidity

Turbidity occurs as a result of 'suspensoids' in the water column. This suspended matter, which may include clay, silt, dissolved organic and inorganic matter, plankton and other microscopic organisms, causes the water to appear turbid (Davies and Day, 1998). Suspended matter causes light to be scattered and absorbed rather than transmitted in straight lines through a water sample and may reduce light penetration, smothers in-stream habitats, interferes with the feeding mechanisms of filter-feeding organisms namely, macroinvertebrates and reduces visibility, thus leading to a reduction in biodiversity and a system which is dominated by a few tolerant species (Davies and Day, 1998).

Historical clarity results illustrated that the clarity levels fluctuated both spatially and seasonally (Figure 26). In most instances, the river or stream indicated the clarity to be 'greater than' the river or streams actual depth (indicated by the red arrows) (Figure 26). Low clarity/high turbidity levels were recorded at site TRI01 (Figure 26). This may be attributed to a combination of activities namely the impoundment upstream from the site, which in itself has excessive turbidity levels and potential run-off from upstream activities. This includes the clearing of land upstream for further industrial activities, consequently resulting in exposed soils. With the lack of and inadequate riparian vegetation at this site, there was no filtration system assisting the river system from a sediment loading perspective. High clarity/low turbidity was recorded at most sites during the dry season. This may be attributed to a lack of run-off due to no rainfall during the dry season, coupled with limited flow transporting sediment downstream. In comparison, turbidity during the wet season was typically high, with cumulative impacts (Figure 10) within the catchment contributing to elevated suspensoids.

This trend was also illustrated in Figure 27 (wet season) and Figure 28 (dry season). A decreasing trend and high variation in clarity was illustrated in a downstream direction along the Wilge River during the wet season (Figure 27). Furthermore during the wet season, the sites situated on the two tributaries indicated the least variation compared to the Wilge River. During the dry season, all the sites with the exception of site TRI01 indicated large variability and high clarity levels (Figure 28).

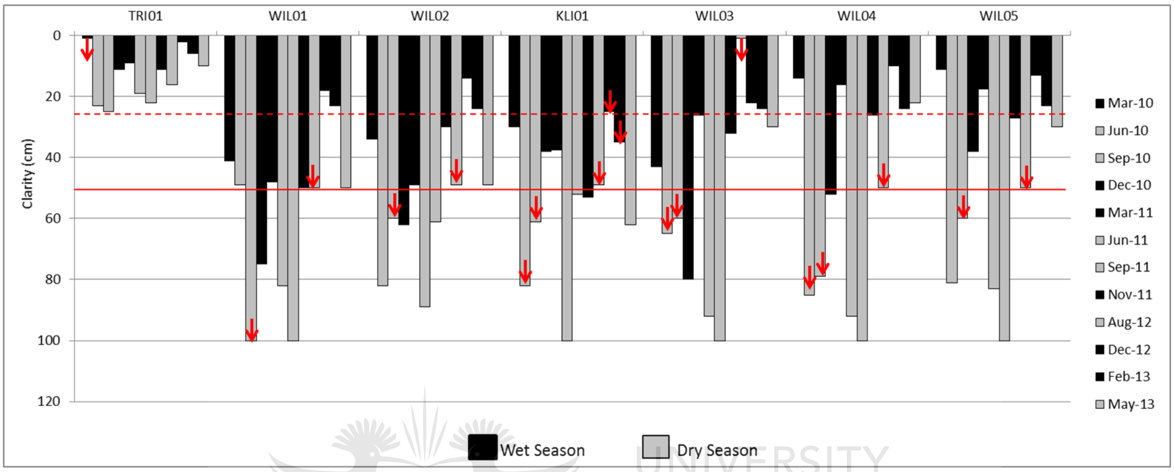


Figure26: Historical and current secchi disk depths recorded at the seven monitoring points as an indication of clarity during the wet and dry season surveys (2010 – 2013) (red dashed line indicates guideline value and solid red line indicates low turbidity, red arrows indicate ‘greater than’ values

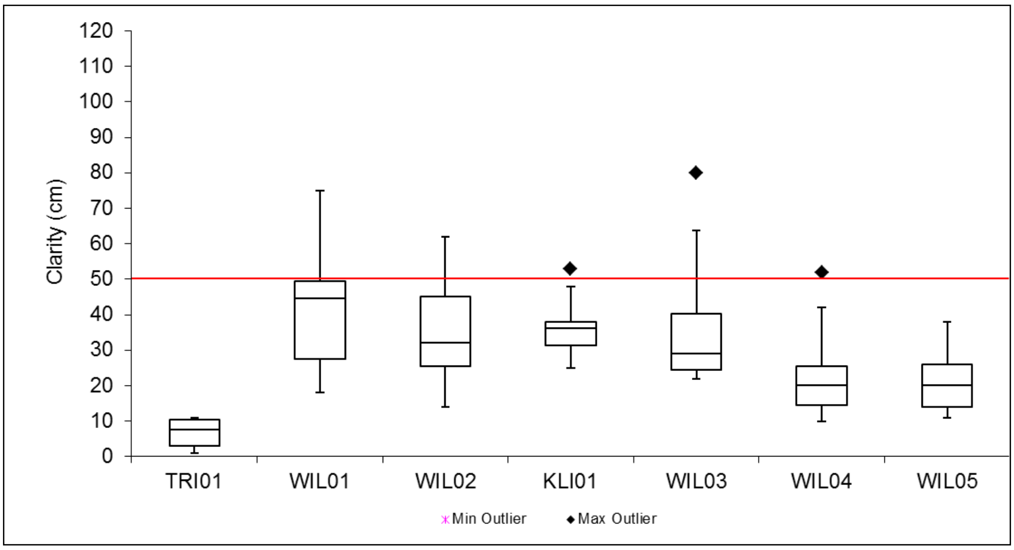


Figure 27: A box and whisker plot showing secchi disk depths recorded at the seven monitoring points as an indication of clarity during the wet season surveys (2010 – 2013) (solid red lines indicates target values, ◆ Maximum Outlier)

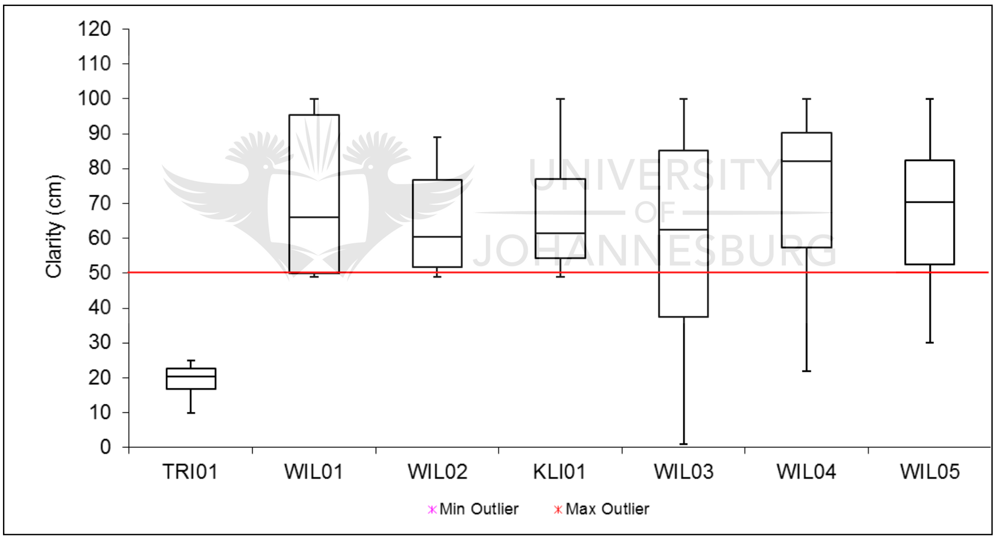


Figure 28: A box and whisker plot showing secchi disk depths recorded at the seven monitoring points as an indication of clarity during the dry season surveys (2010 – 2013) (solid red lines indicates target values)

Therefore overall, the *in situ* water quality measured during the period March 2010 to May 2013, clearly illustrated high levels of variation both spatially and temporally. The pH was generally alkaline, with more variability occurring during the wet season. The TDS concentrations were all below the guideline value of 1000 mg/ℓ. High TDS concentrations were recorded at site WIL01, with high variability during the dry season, but subsequently decreased in a downstream direction along the Wilge River, where it remained relatively consistent at the two most downstream sites. The DO and DO% were the two variables that may have a limiting effect on the aquatic ecosystem in the study area. Both variables fluctuated both seasonally and spatially. The DO concentrations fell mostly below the guideline value of 5 mg/ℓ during the wet season which may be a limiting factor to the aquatic biological communities, diversity and abundances. A similar trend was identified with the DO% during the wet season. From a clarity perspective, a seasonal trend was clearly indicated as during the wet and dry season, the clarity levels were low and high respectively. This was attributed to the high run-off events during the wet season.

4.2. Habitat Integrity

The IHAS *version 2*, was developed by McMillan (1998) for use in conjunction with the SASS5 protocol. The IHAS considers sampling habitat and stream characteristics namely flow, substrate, marginal and in-stream vegetation and general river morphology, all of which aquatic macroinvertebrates depend on for their various lifecycles. The IHAS was employed as an indication of the state of the habitat and used as a tool, to monitor changes both spatially and temporally. The long term IHAS summary scores are provided in Table 8 and Table 9 for the wet and dry season respectively.

Based on the historical IHAS results, habitat availability ranged from good to poor (Table 5, Table 8 and Table 9). During the wet season surveys, habitat availability in the unknown tributary of the Klipfonteinspruit (site TRI01) improved from poor to adequate, although decreased from adequate to poor during the dry season. The site on the Klipspruit (site KLI01) fluctuated from poor to good, with the lowest habitat availability indicated during the dry season (Table 8 and Table 9).This was generally the case within the smaller tributaries when compared to the larger Wilge River, due to lower water levels in the streams, resulting in limited habitat availability, primarily during the dry season (Table 8 and Table 9). The habitat availability in the lower Wilge River sites was variable between March 2010 and May 2013. Sites WIL02, WIL03 and WIL05 had remained good since the December 2012 survey during the wet season, while the habitat availability fluctuated during the dry season (Table 8 and Table 9). Habitat availability at Site WIL01 was predominantly adequate during the wet season, with the exception of the February and August 2012 surveys whereby they decreased to poor (Table 8 and Table 9). This may be related to flow and limited habitat availability at the time those surveys were conducted. Site WIL04 has predominantly poor habitat availability, in comparison to the other sites on the Wilge River. This site is characterised by steep incised banks and a deep channel which lacks the stones biotope. During the dry season, the water level lowers, consequently exposing the undercut banks and resulting in no vegetation in which to sample. Therefore, it was clearly indicated that stream bed composition was one of the most important physical factors controlling the structure of a freshwater invertebrate community (Mackay and Eastburn, 1990). Physical stream condition and other habitats / general biotopes (instream and riparian VEG, GSM) are also important factors to consider.

Table 8: Historical and current IHAS scores from 2010 – 2013 (wet season)

Site	Mar '10	Dec '10	Mar '11	Nov '11	Dec '12	Feb '13
TRI01	46	50	45	50	64	62
WIL01	63	62	63	60	64	49
WIL02	59	58	62	60	86	67
KLI01	66	67	58	51	63	65
WIL03	63	69	41	57	82	73
WIL04		56	46	49	51	45
WIL05	41	61	41	57	82	72

Table 9: Historical and current IHAS scores from 2010 – 2013 (dry season)

Site	Jun '10	Sep '10	Jun '11	Sep '11	Aug'12	May '13
TRI01	67	60	62	45	27	35
WIL01	63	62	65	70	46	55
WIL02	62	61	62	64	14	68
KLI01	70	68	43	53	49	51
WIL03	66	69	72	62	49	71
WIL04		45	40	44	30	21
WIL05	62	70	61	66	54	71

Overall, results illustrated that VEG and GSM were the most dominant biotopes for overall higher IHAS scores within the study area. However, SIC in particular, was the driving habitat at all the sites along the Wilge River, with the exception of site WIL04. Furthermore, the poor habitat availability observed predominantly at site TRI01, WIL04, and during the dry season site KLI01, was largely due to a lack of SIC habitat, incised banks and the habitat was relatively homogenous at those sites (Table 8 and Table 9).

4.3. Aquatic Macroinvertebrate Assessment (Diversity, Functional and Biotic Integrity Approach)

4.3.1 Aquatic Macroinvertebrate Community Assessment

The SASS5 index was designed specifically for the assessment of perennial streams and rivers and is not suitable for assessment of impoundments, isolated pools, wetlands or pans (Dickens and Graham, 2002).

A total of 80 aquatic macroinvertebrate taxa were recorded in the study area over the period 2010 to 2013 (Table 10). The highest number of taxa was recorded at site WIL03, with 30 taxa recorded during the December 2010 survey, and the lowest was at site WIL04, with five

(5) taxa recorded during the March 2011 survey. Refer to Appendix B for the aquatic macroinvertebrate dataset.

The SASS5 scores ranged from 28 at site WIL04 during the March 2011 survey to 155 at site WIL03 during the December 2010 survey (Table 10). The Average Score per Taxon (ASPT) ranged from 3.8 at site WIL04 during the December 2012 survey, to 7.7 at site WIL02 during the March 2010 survey (Table 10). The ASPT values provide an indication of the average tolerance / intolerance of the aquatic macroinvertebrate community at each site (Dickens and Graham, 2002). In this case ASPT values indicated that the macroinvertebrate communities at all the sites are primarily composed of tolerant (1 - 5) and moderately tolerant (6 - 10) taxa (ickens and Graham, 2002).

Table 10: A summary of the number of taxa, SASS5 and ASPT values recorded from March 2010 to May 2013

	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT	# Taxa	SS	ASPT
Site	Mar '10			Jun '10			Sep '10			Dec '10			Mar '11			Jun '11			Sep '11			Nov '11			Aug '12			Dec '12			Feb '13			May '13		
TRI01	12	67	5.6	20	118	3.9	14	81	5.8	17	79	4.6	15	90	6.0	10	63	6.3	17	79	4.6	21	109	5.2	8	37	4.6	15	69	4.6	21	113	5.4	17	91	5.4
WIL01	18	101	5.6	17	99	4.7	19	103	5.4	23	118	5.1	18	97	5.4	17	97	5.7	18	101	5.6	22	115	5.2	19	95	5.0	16	81	5.1	16	95	5.9	21	119	5.7
WIL02	11	85	7.7	22	118	5.4	20	113	5.7	26	138	5.3	22	129	5.9	16	101	6.3	21	126	6.0	21	107	5.1	17	97	5.7	15	84	5.6	18	95	5.3	21	121	5.8
KLI01	13	90	6.9	22	126	4.1	20	106	5.3	21	110	5.2	16	105	6.6	17	111	6.5	20	114	5.7	26	132	5.1	20	121	6.1	21	109	5.2	26	138	5.3	22	120	5.5
WIL03	23	139	6.0	19	114	5.2	20	114	5.7	30	155	5.2	7	47	6.7	15	90	6.0	20	133	6.7	22	112	5.1	15	59	3.9	20	103	5.2	20	108	5.4	20	104	5.2
WIL04	-	-	-	-	-	-	16	90	5.6	13	86	6.6	5	28	5.6	9	56	6.2	14	81	5.8	17	100	5.9	16	88	5.5	9	34	3.8	13	61	4.7	11	62	5.6
WIL05	13	67	5.2	13	96	4.3	20	108	5.4	19	110	5.8	6	30	5.0	14	91	6.5	18	123	6.8	25	143	5.7	23	129	5.6	13	77	5.9	18	109	6.1	25	146	5.8

Taxa: Number of Taxa; SS: SASS5 Score; ASPT: Average Score Per Taxon

Historical data indicated that the number of taxa, the SASS5 and ASPT values at all the sampling sites were variable both spatially and temporally (Figure 29, Figure 32 and Figure 35). Site WIL04 indicated the lowest values and this was supported by the IHAS findings in Table 7 and Table 8, indicating this site to have poor habitat availability in comparison to the other sites along the Wilge River. The low number of taxa and SASS5 score at this site was attributed to inaccessibility to the biotopes at the sampling site as the site is very deep, compared with the upstream site, WIL03 or downstream site WIL05, as well as a lack of SIC. Furthermore, the site is characterised by steep incised banks and a deep eroded channel. Habitat availability affects the structure of freshwater invertebrate communities. Therefore, sites which lack the stones biotope will illustrate sensitive and intolerant taxa, namely Polymitarcyiae (Pale Burrowers), Heptageniidae (Flatheaded mayflies), Plecoptera (Stoneflies), Trichoptera (Caddisflies), which are strongly associated with SIC (Gerber and Gabriel, 2002), will not be recorded, thus ultimately contributing to a lower SASS5 and ASTP score. This was clearly illustrated at site WIL04 and thus indicates the importance of habitat availability, which was influenced by the type of taxa recorded. What could further be attributed to the low number of aquatic macroinvertebrates and thus low SASS5 and ASPT values at site WIL04, may be due to poor water quality. A combination of high turbidity (Figure 27 and Figure 28), low DO and percentage saturation (Figure 17 and Figure 20 respectively) particularly during the wet season (Figure 18 and Figure 21 respectively) may be a causative factor. In addition, the most upstream site on the Wilge River (site WIL01), recorded the second lowest number of taxa, SASS5 and ASPT score along the Wilge River (Figure 29, Figure 32 and Figure 35). Although this site is characterised by all three biotopes, this may therefore be attributed to limited flow, coupled with the highest TDS concentrations recorded along the Wilge River (Figure 12), primarily during the dry season (Figure 14), and seasonally fluctuating percentage saturation (Figure 21 and Figure 22).

As per the box and whisker plots for the number of taxa, all the sites indicate high variability, with the exception of site WIL03, during the wet season and low variability at all the sites during the dry season (Figure 30 and Figure 31 respectively). Furthermore, there was an increase in the number of taxa in both the wet and dry seasons between sites WIL04 and WIL05 (Figure 30 and Figure 31). This was attributed to improved flow at site WIL05 and consequently adequate to good habitat availability at this site, resulting in a greater number of taxa. Historically, the SASS5 scores mirror the above (Figure 33 and Figure 34). In terms of the ASPT, site WIL04 indicated high variability during the wet season compared to the rest of the sites along the Wilge River, while during the dry season, showed the least variation (Figure 36 and Figure 37 respectively). This was due to two biotopes available to sample during the wet season (GSM and VEG) providing more aquatic macroinvertebrate taxa and higher abundances, compared to the dry season, where only one biotope (GSM) is present, contributing to a lower number of species and abundances.

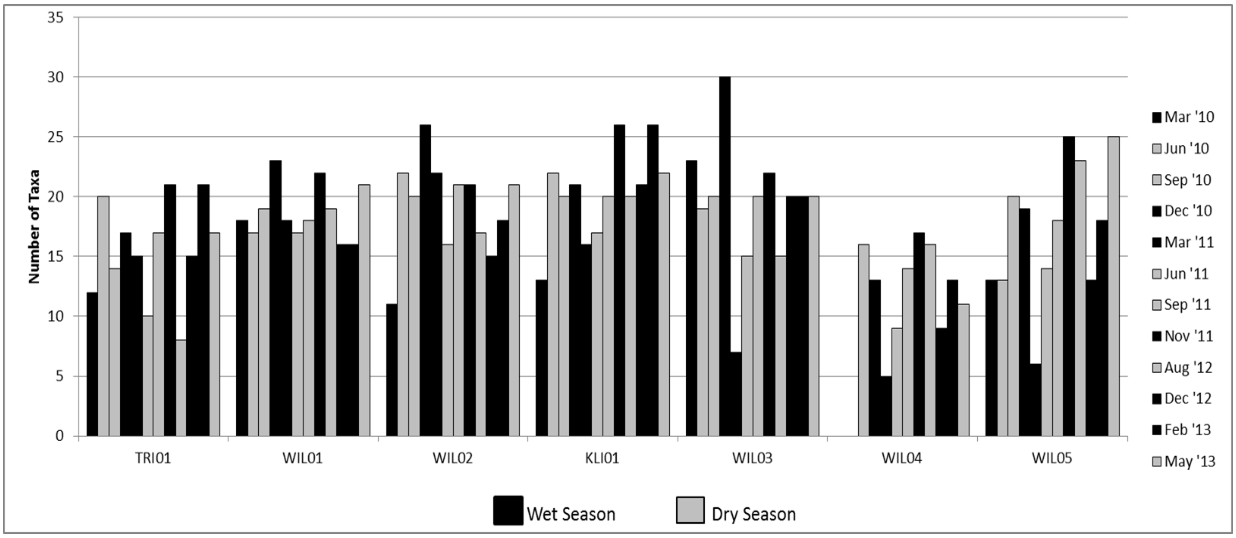


Figure 29: Historical and current number of taxa recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively)

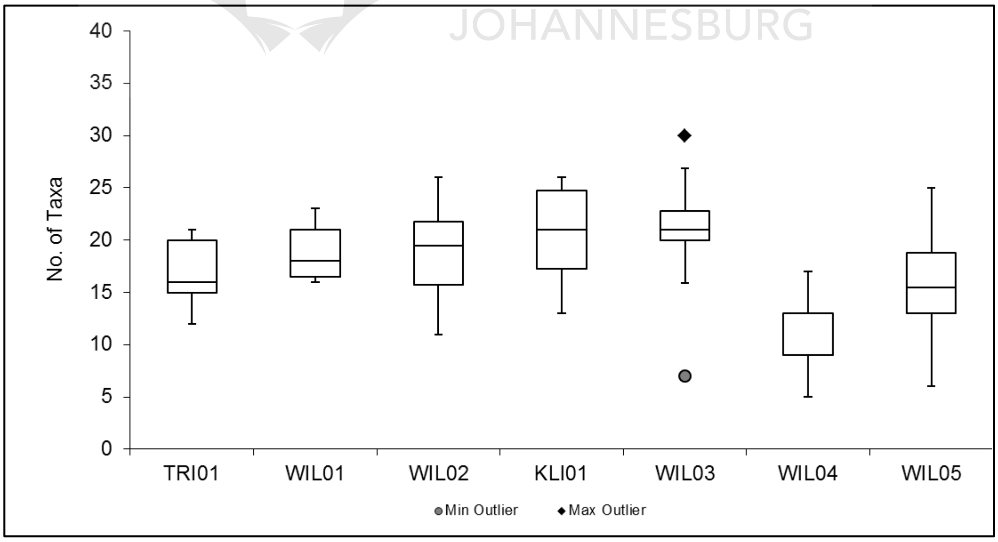


Figure 30: A box and whisker plot showing the number of taxa recorded at the seven monitoring points during the wet season surveys (2010 – 2013) (● Minimum Outlier ◆ Maximum Outlier)

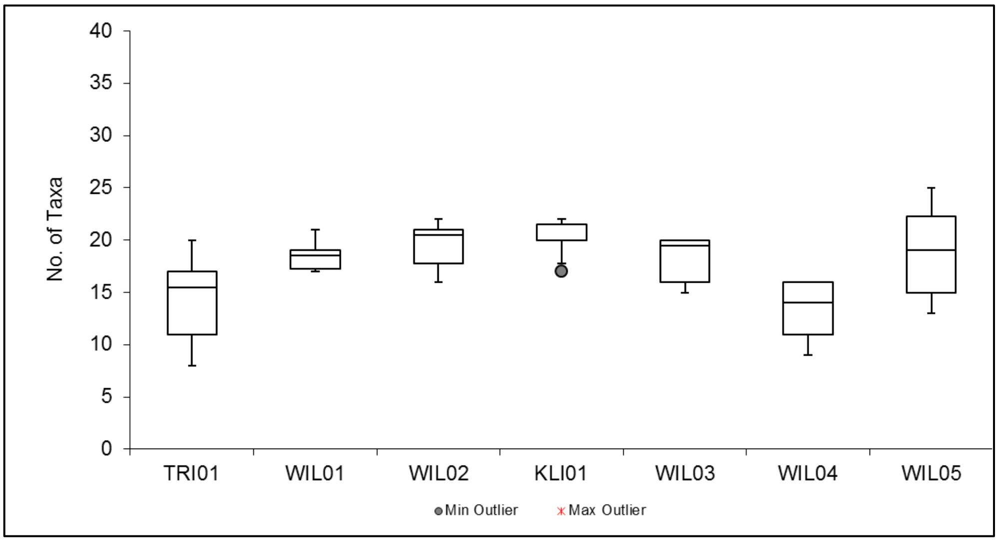


Figure 31: A box and whisker plot showing the number of taxa recorded at the seven monitoring points during the dry season surveys (2010 – 2013) (● Minimum Outlier)

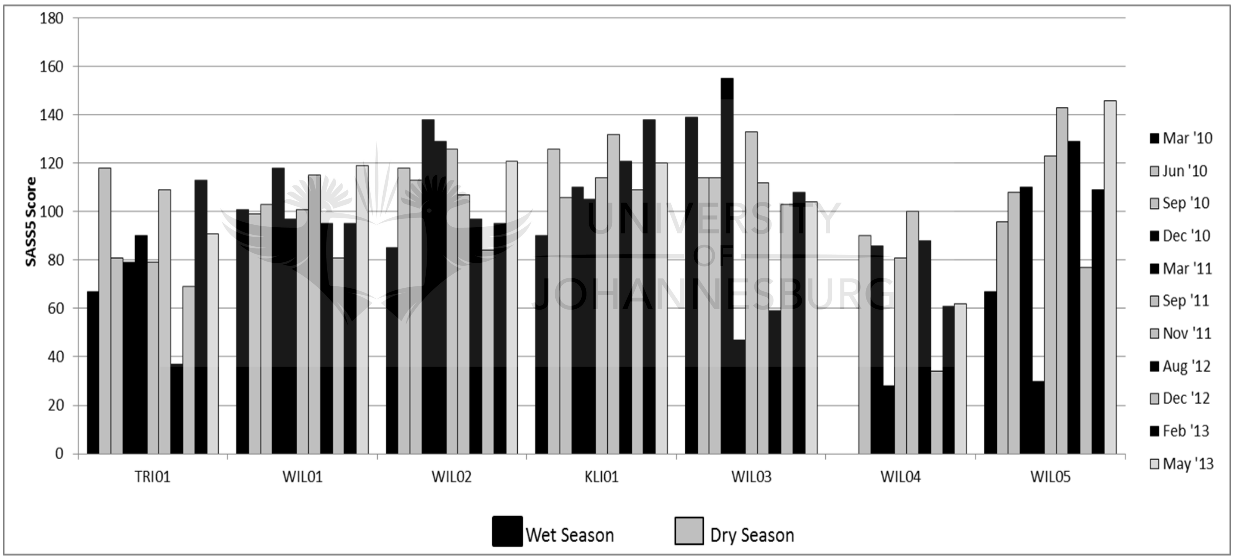


Figure 32: Historical and current SASS5 scores recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively)

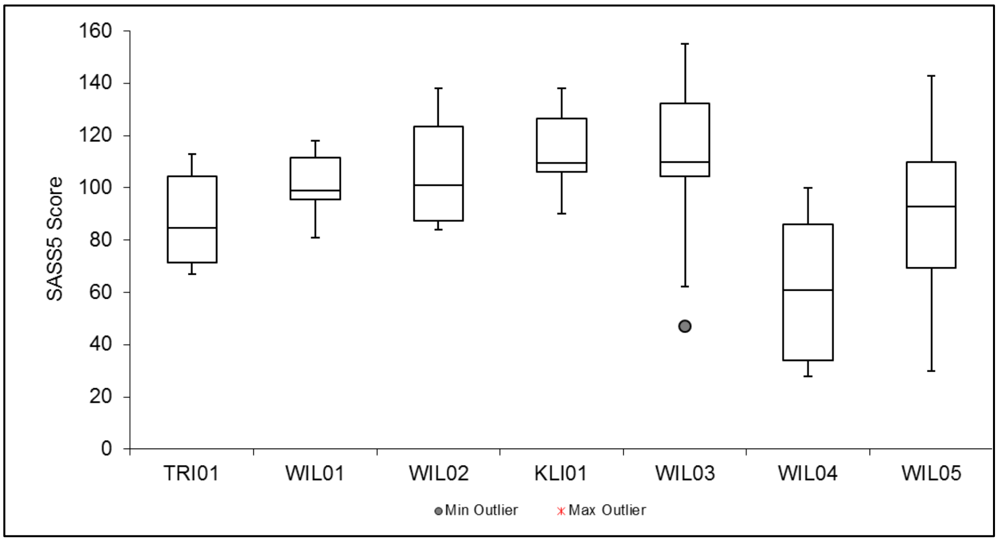


Figure 33: A box and whisker plot showing the SASS5 scores recorded at the seven monitoring points during the wet season surveys (2010 – 2013) (● Minimum Outlier)

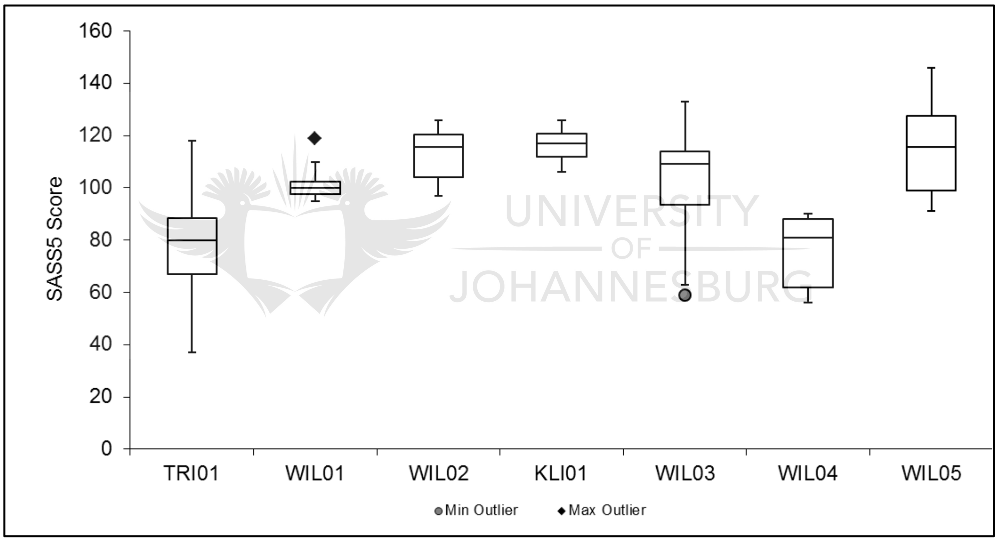


Figure 34: A box and whisker plot showing the SASS5 scores recorded at the seven monitoring points during the dry season surveys (2010 – 2013) (● Minimum Outlier ◆ Maximum Outlier)

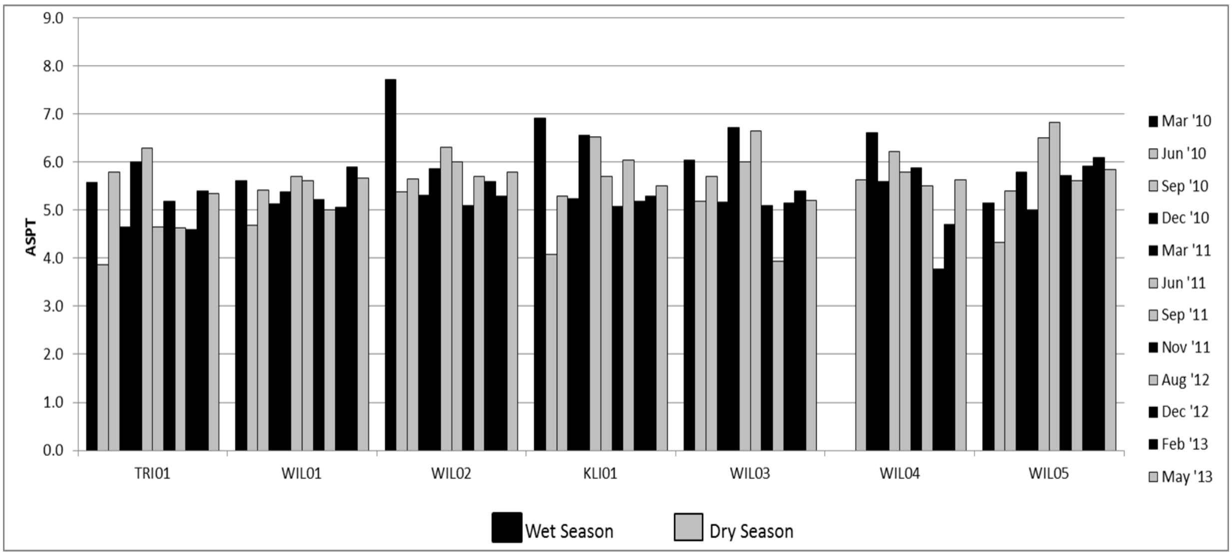


Figure 35: Historical and current ASPT recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively)

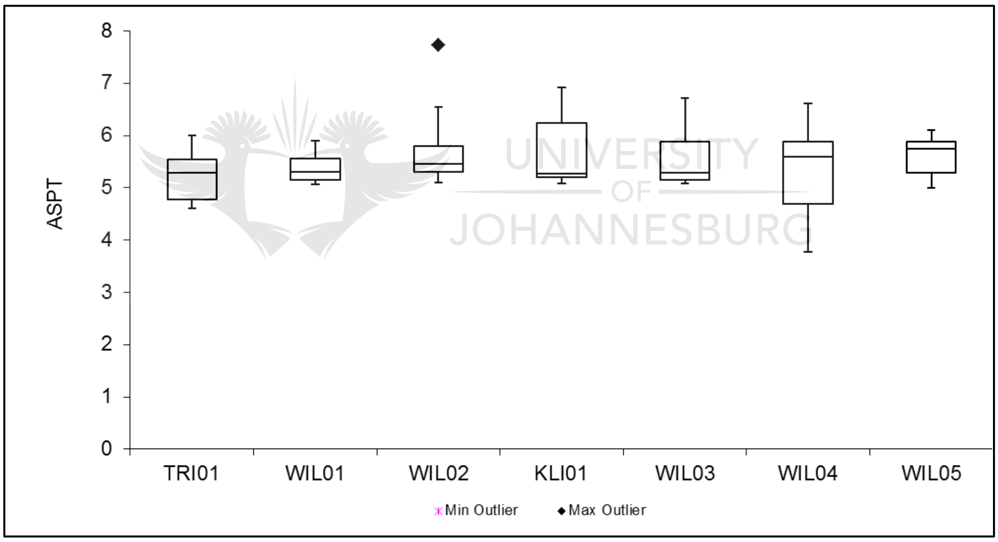


Figure 36: A box and whisker plot showing the ASPT recorded at the seven monitoring points during the wet season surveys (2010 – 2013) (◆ Maximum Outlier)

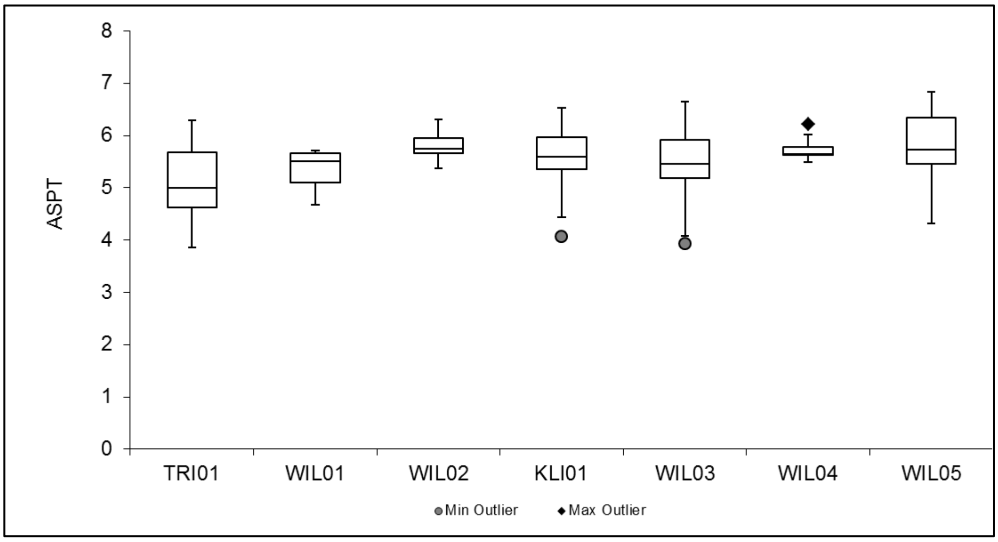


Figure 37: A box and whisker plot showing the ASPT recorded at the seven monitoring points during the dry season surveys (2010 – 2013) (● Minimum Outlier ◆ Maximum Outlier)

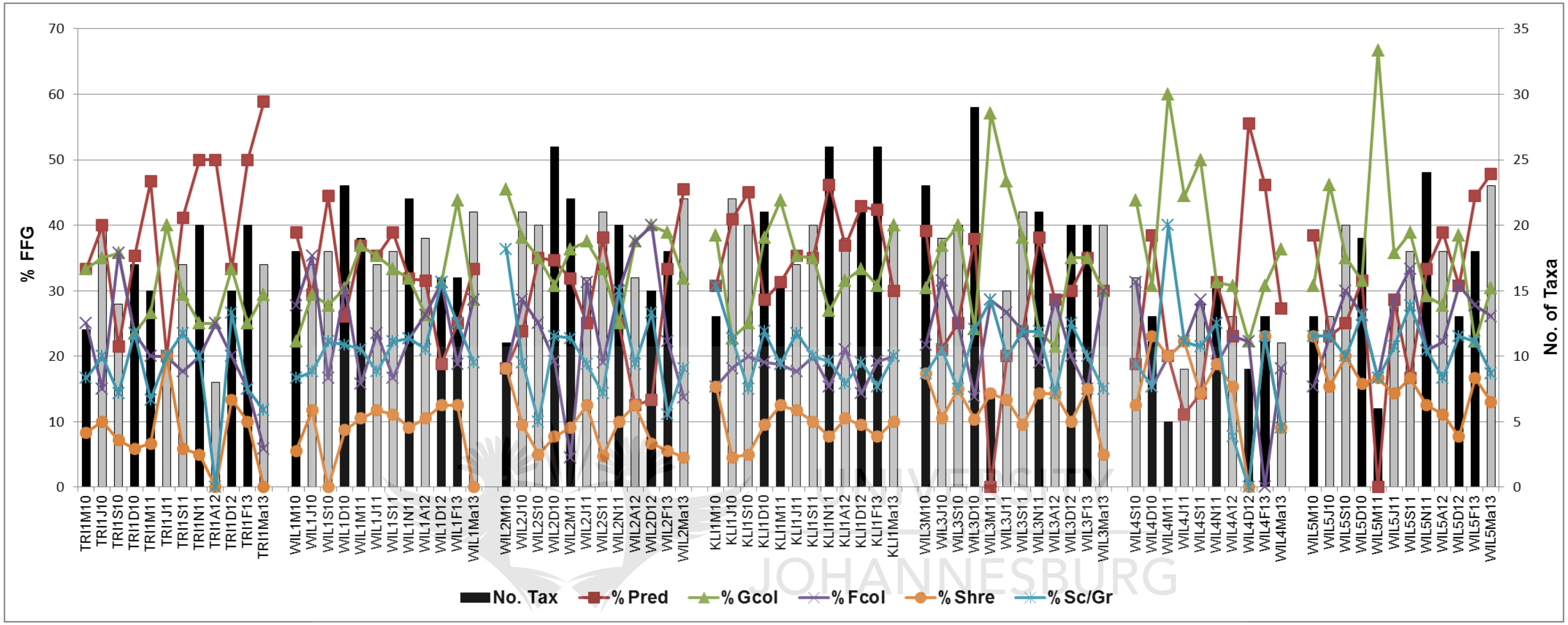
4.3.2 Aquatic Macroinvertebrate Functional Feeding Groups

The taxonomic approach focused on determining some measures of species diversity, in order to evaluate biodiversity and the sensitivity to water quality parameters (Cummins *et al.,* 2005). The FFG classification approach (Cummins and Wilzbach, 1985; Cummins *et al.,* 2005; Rawer-Jost *et al.,* 2000) is based on morphological and behavioural mechanisms by which the macroinvertebrates acquire their food resources (Cummins *et al.,* 2005). Five (5) FFG were identified in this study namely, shredders, scrapers/grazers, filter, gathering collectors and predators. Classifying the different FFG amongst the various aquatic biota will enhance our knowledge regarding land use impacts in the watershed (Cummins *et al.,* 2005). Figure 38 illustrates the number of taxa in conjunction with the percentage contribution of the FFG.

The number of taxa and percentage contribution of the five (5) FFG fluctuated both spatially and seasonally (Figure 38). Figure 38 indicated that predators and gathering collector populations were the most dominant feeding group within the study area, making up 32% and 34% of the total FFGs respectively (Table 11). The filter collectors and scrapers/grazers made up 22% and 20% respectively, with the shredders the least abundant with 11% (Table 11). In terms of the Wilge River reach, gathering collectors dominated the system making up 35 %, followed by predators, 29 %, filter collectors, 23 %, scraper/grazers, 21 % and shredders making up 12% of the FFGs. The gathering collectors feed on FPOM from the stream bottom while predators feed on other consumers (Cummins *et al.,* 2005). Theoretically, this was expected as all the sites have the biotope GSM, thus supporting the gathering collector populations, while some sites included the VEG and SIC biotope, which supports majority of the predator populations. The scraper/grazers, which consume algae and associated material, were expected to be most abundant but were one of the least abundant FFGs (Figure 38). This expectation arises from medium and high agriculture being the most dominant land use in the study area. Therefore, one would expect major nutrient input into the Wilge River system, resulting in eutrophic conditions which would attract taxa within this FFG. However, in degraded rivers, stressors often co-occur but the response of the biotic communities cannot be attributed to individual stressors or specific combinations of them (Cummins *et al., et al.,* 2005; Ormerod *et al.,* 2010). In particular nutrient enrichment and organic pollution are often closely related (Friberg *et al.,* 2009). The shredders, which were the least abundant FFG, chew on litter or live vascular plant tissue and presumed to be more sensitive to perturbation (Cummins *et al.,* 2005) was probable due to the nature of the Wilge River system in this study area and the vast land use in the catchment area (Figure 10). This was further supported by only a few sensitive taxa recorded along these river reaches.

Figure 38 further indicated that when predators are low, the gathering collectors proliferate and *vice versa*. For instance zero predators were recorded at site WIL03 and WIL05 during the March 2011 survey, during which the gathering collectors illustrated a strong component of the FFGs (Figure 38). This evidently indicates that there is a strong relationship between these two FFGs.

Figure 39 to Figure 41 represent the percentage contribution of FFG per site, including standard deviations. Refer to Table 11 which represents the means of the FFG per site for the wet and dry season. Similar to Figure 38, Figure 39 clearly indicates that predators and gathering collectors are the dominant FFG in the study area. However, this further illustrates the seasonal variation and fluctuation in the FFG's. During the wet season, gathering collectors are the dominant FFG with wide variation illustrated by the large standard deviation (Figure 40). This was expected as during the wet season there was more food availability, coupled with enhanced habitat availability. Gathering collectors include taxa such as Baetidae and Caenidae, whose preference is towards moderate flow over rocks and cobbles. This habitat type is present at sites WIL02, WIL03 and WIL05 in the Wilge River, where this FFG was dominant (Figure 40). The dry season indicates a low number of shredders, which was expected due to the process of vegetation die-back during the dry season, resulting in limited course particulate organic matter available for the aquatic macroinvertebrates (Figure 41). Furthermore, only site WIL04 illustrates large variation between the predators and gathering collectors (Figure 41) due to the limited and poor habitat availability at this site, in particular during the dry season where only GSM is available to sample.



The x-axis represents the site name, followed by the month and year of monitoring event. %Pred – percentage of predators, %Gcol – percentage of gathering collectors, %Fcol – percentages of filter collectors, %Shre – percentage of shredders and %Sc/Gr – percentage of scrappers/grazers

Figure 38: Historical and current FFG and number of taxa recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) (dark and grey bars represent the wet and dry season respectively)

Table 11: The means for the different FFG's per site for the wet and dry season

Site	%Pred		%Gcol		%Fcol		%Shre		%Sc/Gr	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY
TRI01	41	39	28	32	21	20	08	07	19	15
WIL01	30	35	33	30	24	25	10	08	23	20
WIL02	26	30	36	36	22	26	10	08	25	17
KLI01	37	37	35	32	17	19	10	09	21	20
WIL03	30	25	34	36	20	28	14	11	23	18
WIL04	34	20	32	35	14	21	14	14	19	15
WIL05	30	30	36	36	23	27	15	15	22	21
Average	**32**		**34**		**22**		**11**		**22**	

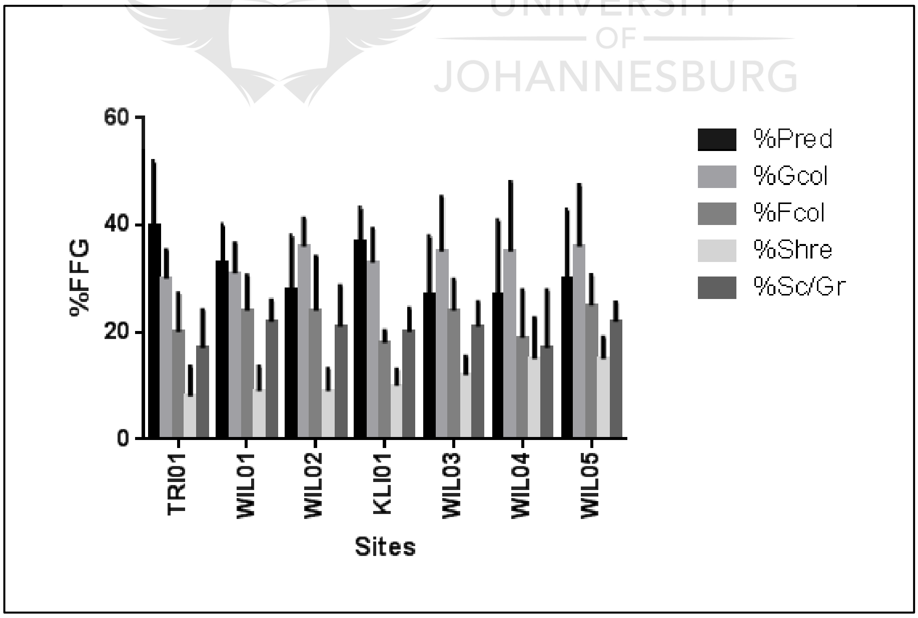


Figure 39: Average % FFG recorded at the seven monitoring points during the wet and dry season surveys (2010 – 2013) including standard deviations (n=83)

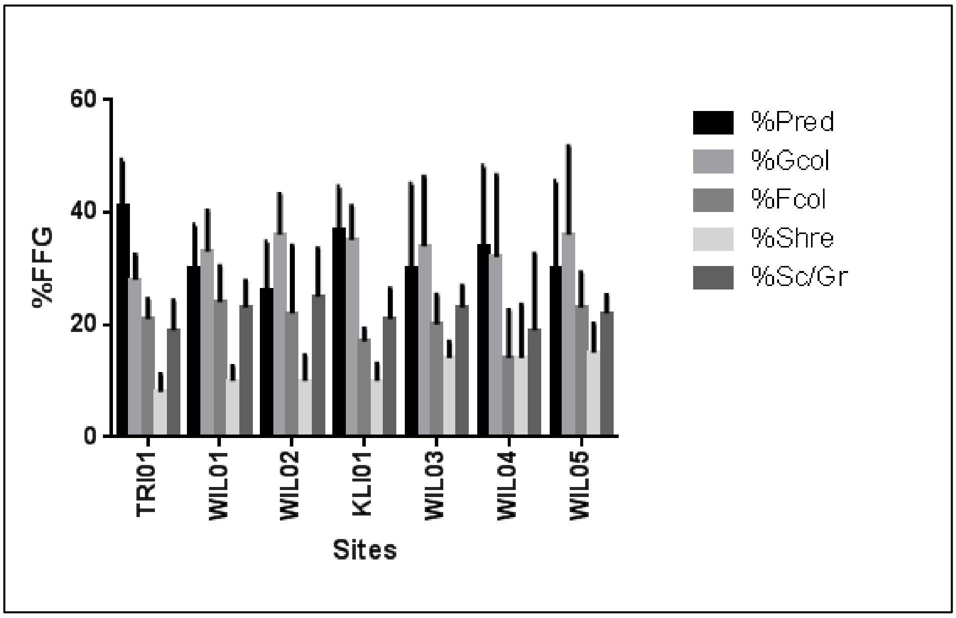


Figure 40: Average % FFG recorded at the seven monitoring points during the wet season survey (2010 – 2013) including standard deviations (n=41)

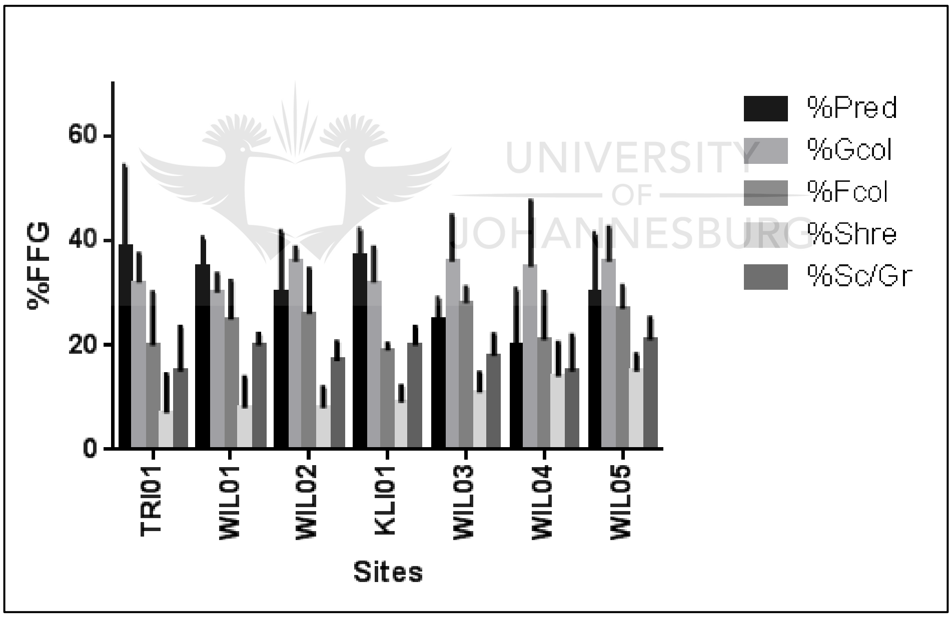


Figure 41: Average % FFG recorded at the seven monitoring points during the dry season survey (2010 – 2013) including standard deviations (n=42)

Overall, the SASS5 results indicate that there was a change in the integrity of the aquatic macroinvertebrate communities in the study area and further illustrated variability both spatially and temporally. It was evident that the aquatic macroinvertebrate communities within the Wilge River and two adjoining tributaries comprises primarily of tolerant and moderately tolerant taxa. Of the mostly tolerant taxa, predators and gathering collector populations were the most dominant feeding group, with the shredders being the least abundant within the study area. This was expected as all the sites have the biotope GSM, thus supporting the gathering collector populations, while some sites included the VEG and SIC biotope, which supports majority of the predator populations. The scraper/grazers, which consume algae and associated material, were expected to be most abundant, but were one of the least abundant FFGs. This expectation arises from medium and high agriculture being the most dominant land use in the study area. Therefore, one would expect major nutrient input into the Wilge River system, resulting in eutrophic conditions and thus attracting taxa within this FFG. However in degraded rivers, stressors often co-occur, but the response of the biotic communities cannot be attributed to individual stressors or specific combinations of them (Cummins *et al.,* 2005; Ormerod *et al.,* 2010).

4.3.3 Biotic Integrity based on SASS5 Results

As per the SASS5 and ASPT values recorded at all the sites over time, the long term Present Ecological State (PES) classes are presented in Table 11 and Table 12 for the wet and dry season respectively. Refer to Table 6 for a description of the PES classes. During the wet season surveys, the PES at the most upstream site on the Wilge River (site WIL01) remained a Class B (slightly impaired) (Table 11). This trend was continued during the dry season at this site (Table 12) and thus illustrates the site to have limited sensitive and intolerant taxa (Dallas, 2007). During the wet season, sites WIL02, WIL03 and WIL05 ranged from a PES Class A (unimpaired) to a PES Class C (moderately impaired), illustrating that all three sites fluctuated with either a high to a moderate diversity of taxa with numerous sensitive taxa (Dallas, 2007). Site WIL04 indicated a PES Class B (slightly impaired) to a PES Class C (moderately impaired), with the exception of a PES Class D (considerably impaired) recorded during the December 2012 survey (Table 11). This was when the number of taxa and SASS5 score was at its lowest (Table 10) and only tolerant taxa were present. The PES however improved to a Class C during the February 2013 survey whereby the number of taxa increased thus influencing the PES (Table 11). The sites along the two tributaries ranged from a PES Class A to C.

During the dry season surveys, all the sites along the Wilge River ranged from a PES Class A to a C, with the most downstream site, WIL05 indicating a PES Class A for majority of the months surveyed (Table 12). Therefore overall, the PES Class as a result of the SASS5 and ASPT data ranged from moderately to unimpaired. Therefore, there was a degree of loss and change of natural habitat and biota occurring in the project area, although the basic ecosystem functions are still predominantly unchanged (Dallas, 2007).

Table 12: Historical and current *PES scores from 2010 – 2013 (wet season)*

Site	Mar'10	Dec'10	Mar'11	Nov '11	Dec'12	Feb '13
TRI01	C	C	B	B	C	B
WIL01	B	B	B	B	B	B
WIL02	B	A	A	B	B	B
KLI01	B	B	A	A	B	B
WIL03	A	A	B	B	B	B
WIL04		B	B	A	D	C
WIL05	C	A	C	A	A	A

Table 13: Historical and current *PES scores from 2010 – 2013 (dry season)*

Site	Jun'10	Sep'10	Jun'11	Sep'11	Aug'12	May '13
TRI01	A	B	C	C	C	B
WIL01	B	B	B	B	B	B
WIL02	B	B	A	A	B	B
KLI01	A	B	A	B	B	B
WIL03	A	B	B	A	D	B
WIL04		B	B	A/B	B	C
WIL05	**A**	B	A	A	A	A

4.4. Biological, Environmental and Supplementary Statistical Analysis (including Functional Feeding Group Approach)

Cluster analysis of the aquatic macroinvertebrate communities recorded at the sites along the Wilge River and two adjoining tributaries, clearly illustrate a high level of similarity (Figure 42). The analysis further illustrated differences between the wet and dry seasons, a clear indication that there was definite seasonal variation among the aquatic macroinvertebrate communities in the study area. The groups identified by the Bray-Curtis cluster analysis and NMDS ordinations, and which illustrate intergroup relationships between taxa, is indicated in Figure 42 to Figure 44. The cluster analysis containing both seasonal data identified 11 groups having two or more sampling sites that were similar, while five sampling sites had no similarity to any of the other sites at a 60% similarity level (Figure 42a). The high similarity was an indication that the aquatic macroinvertebrate community structures are very similar at these sites illustrated in Figure 42a. In addition, they are primarily characterised by tolerant taxa, which are the driving communities within these river systems. There was however a separation of sites TRI01 and WIL04. Site TRI01 was in its own group during some of the wet season months (Group IV) (Figure 42a) while WIL04 was clearly separated, in particular during the March 2011, December 2012 and February 2013 surveys (Figure 42a and Figure 42b). Group IX comprised of all the sites, with the exception of site WIL04, once again indicating a clear separation of this site (Figure 42a). Physical stream condition and other habitats / general biotopes are important factors to consider (Mackay and Eastburn, 1990), and thus this result was expected. The stream bed composition of site WIL04 is dissimilar to the rest of the sites along the Wilge River, with a lack of the stones biotope and during the wet season, the vegetation biotope. Group II and III illustrated a high similarity between sites WIL04 and WIL05 during the surveys conducted in April 2012, and December 2010, November 2011 and February 2013 (Figure 42a and Figure 42b). This was not expected due to the differences in habitat availability between the sites. However, the similarity may be attributed to comparable taxa that were recorded at both sites namely Baetidae, Caenidae and Leptophlebidae. Leptophlebidae are generally found on stones or submerged pieces of wood, while Caenidae prefer slow flowing water with GSM as a habitat (Gerber and Gabriel, 2002). Site WIL04 has a large amount of decomposing material in-stream, i.e. wood as a consequence of de-rooted trees and shrubs, which was contributing to the high organic content of the sediment. The site was further characterised by little to no flow conditions and thus these specifics clarify the presence of selected families at this site. The separation of site TRI01 was primarily attributed to *in situ* water quality compared to the rest of the sites. This site recorded some of the lowest average *in situ* water quality parameters seasonally and temporally, in particular high temperatures (Figure 24 and Figure 25) and high turbidity levels (Figure 27 and Figure 28). This was further explained in the section below in accordance to Figure 45, which illustrates associations between the sites, aquatic macroinvertebrates and the *in situ* water quality parameters. The NMDS plot (Figure 42b) was completed following 17 iterations and showed a stress of 0.25. The stress of less than 0.3 provides potentially useful two dimensional (2D) illustrations (Clarke and Warwick, 1994). The NMDS plot further indicates that there was a separation of sites WIL04 and TRI01, with separate groups during the wet and dry season conditions.

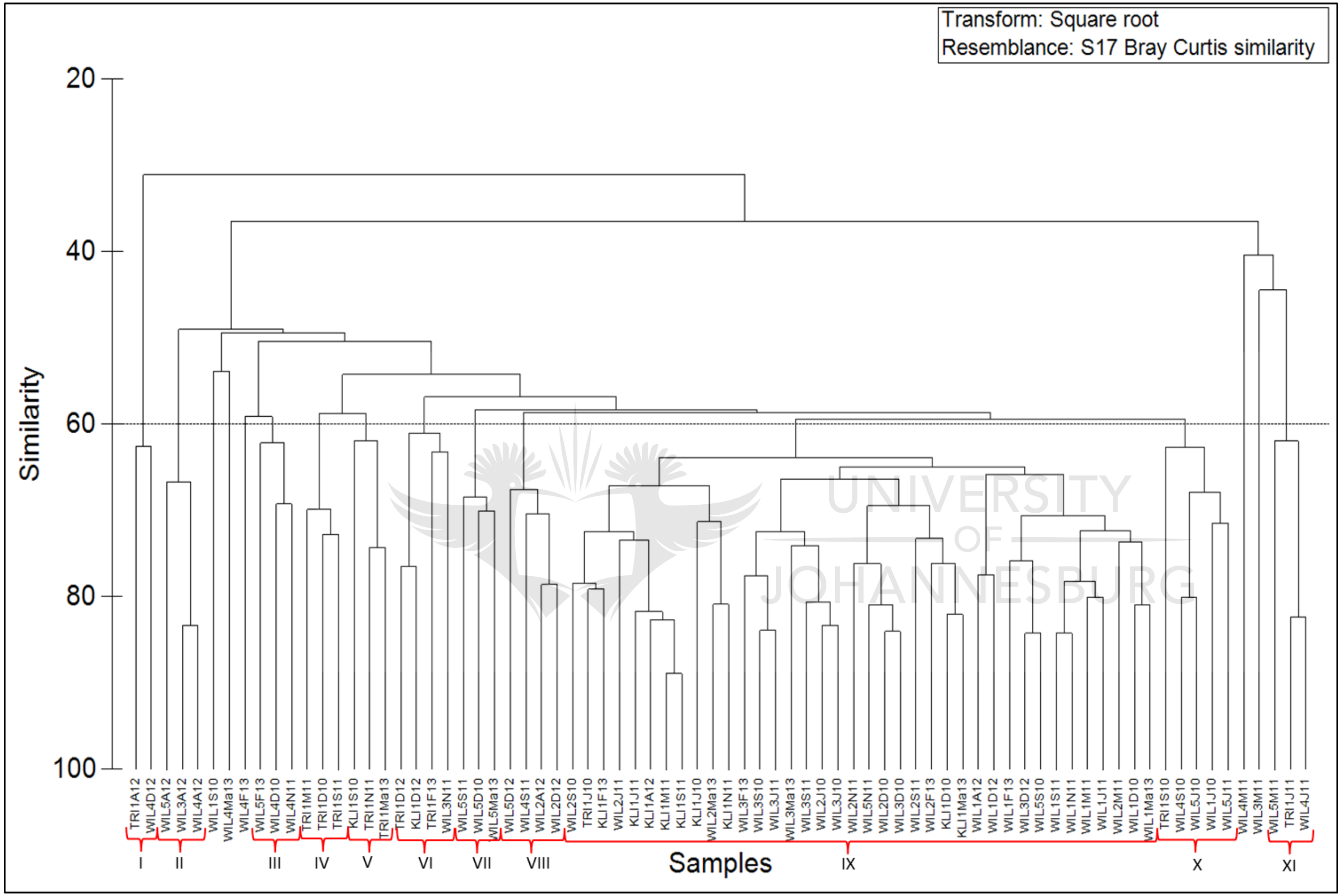


Figure 42a: Bray-Curtis similarity matrix-based cluster analysis of the aquatic macroinvertebrates collected at the sites on the Wilge River and two adjoining tributaries during both wet and dry seasons. Groups were identified with 60% similarity

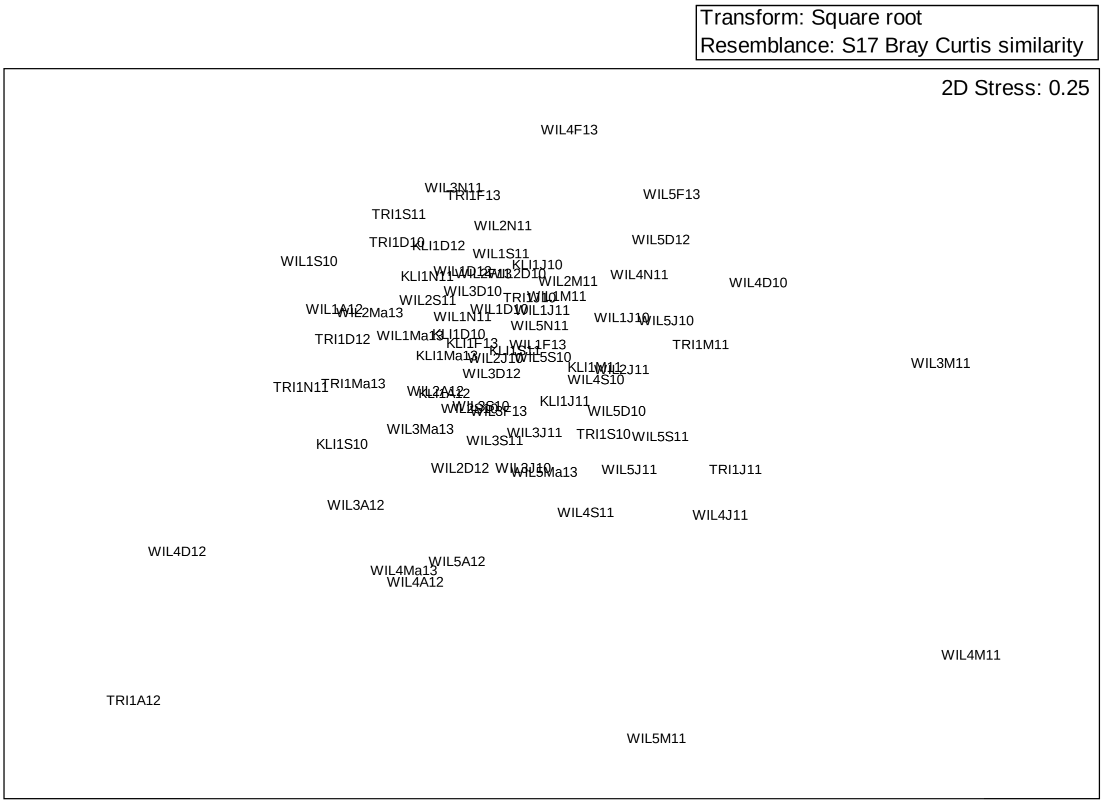


Figure 42b: Bray-Curtis similarity two dimensional representation of the NMDS ordination of the aquatic macroinvertebrates collected at the sites on the Wilge River and two adjoining tributaries during both wet and dry seasons. The NMDS ordination was completed with 17 iterations and showed a stress of 0.25

When considering the community structure of the different seasons separately, the cluster analysis and NMDS plots for wet and dry conditions showed a similar trend with sites WIL04 and TRI01, as well as the similarity between sites WIL04 and WIL05 (Figure 43 and Figure 44). During the wet season, four groups were identified at a 60% similarity level (Figure 43a). Group I illustrated a similarity between sites WIL04 and WIL05. As mentioned above, this result was not expected due to the nature of site WIL04, characterised by a deep channel coupled with steep eroded banks, as opposed to site WIL05 which illustrated good flow conditions coupled with a variety of biotopes. Therefore, the similarity between these two sites may be a consequence of similar taxa recorded at those sites during the wet season, in particular a great abundance of Leptophlebidae at both sites. The same analysis was conducted for the dry season, indicating five groups at a 60% similarity level (Figure 44a). The cluster analysis and NMDS ordinations illustrated similar results to the wet season, primarily the separation of sites TRI01 and WIL04 (Figure 44a and Figure 44b respectively). Group II indicated a high similarity at site WIL05 during the September 2011 and May 2013 survey (Figure 44a and Figure 44b). This was due to high numbers of Caenidae, Heptageniidae, Leptophlebidae and Chironomidae recorded at that site at the time of survey. Group V illustrated a high similarity between sites WIL03, WIL04 and WIL05 during August 2012 (Figure 44a and Figure 44b), primarily due to the Mayfly family, as well as Culicidae. Culicidae prefer pools and any temporary puddles (Gerber and Gabriel, 2002), which are characteristics found at all three sites. It can further be attributed to the dominant GSM biotope between the sites. This result was identified previously with the wet and dry seasons combined (Figure 42a).

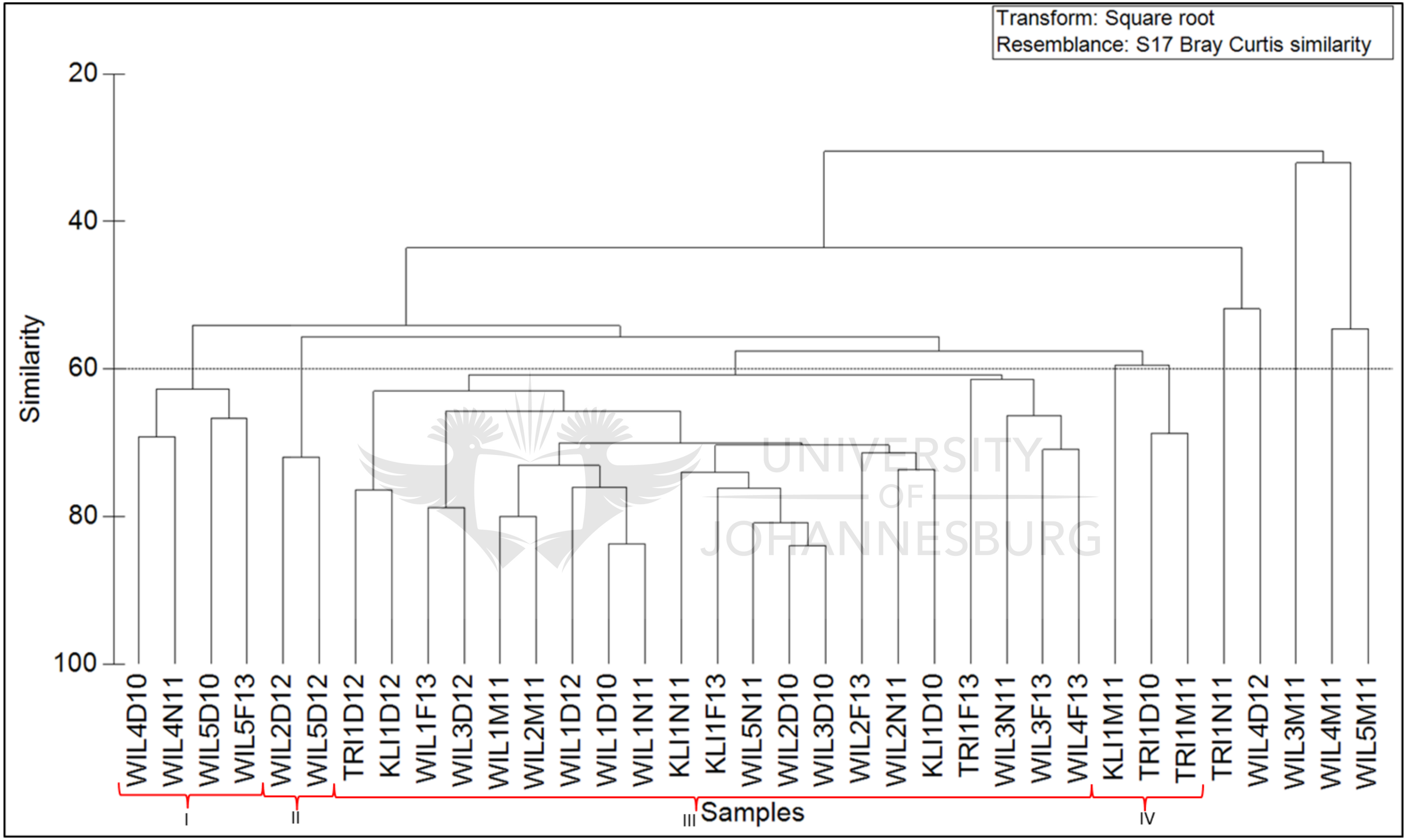


Figure 43a: Bray-Curtis similarity matrix-based cluster analysis of the aquatic macroinvertebrates collected at the sites on the Wilge River and two adjoining tributaries during the wet season. Groups were identified with 60% similarity

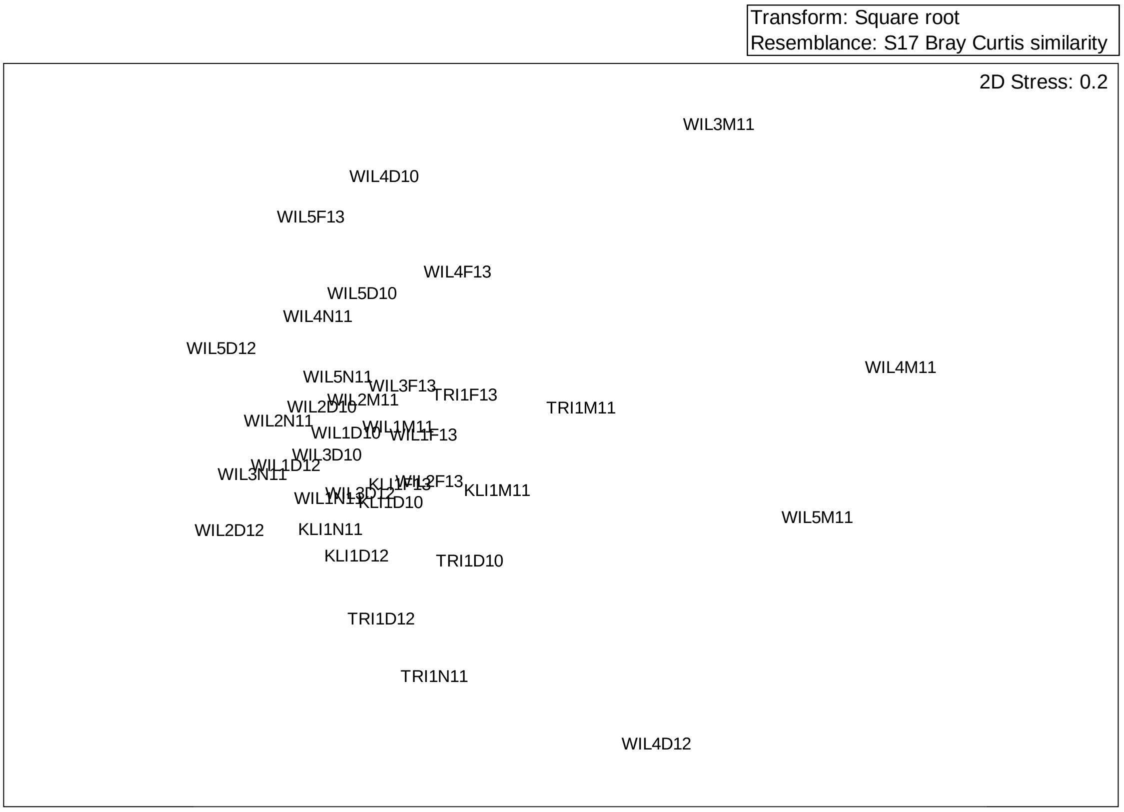


Figure 43b: Bray-Curtis similarity two dimensional representation of the NMDS ordination of the aquatic macroinvertebrates collected at the sites on the Wilge River and two adjoining tributaries during the wet season. The NMDS ordination was completed with 7 iterations and showed a stress of 0.2

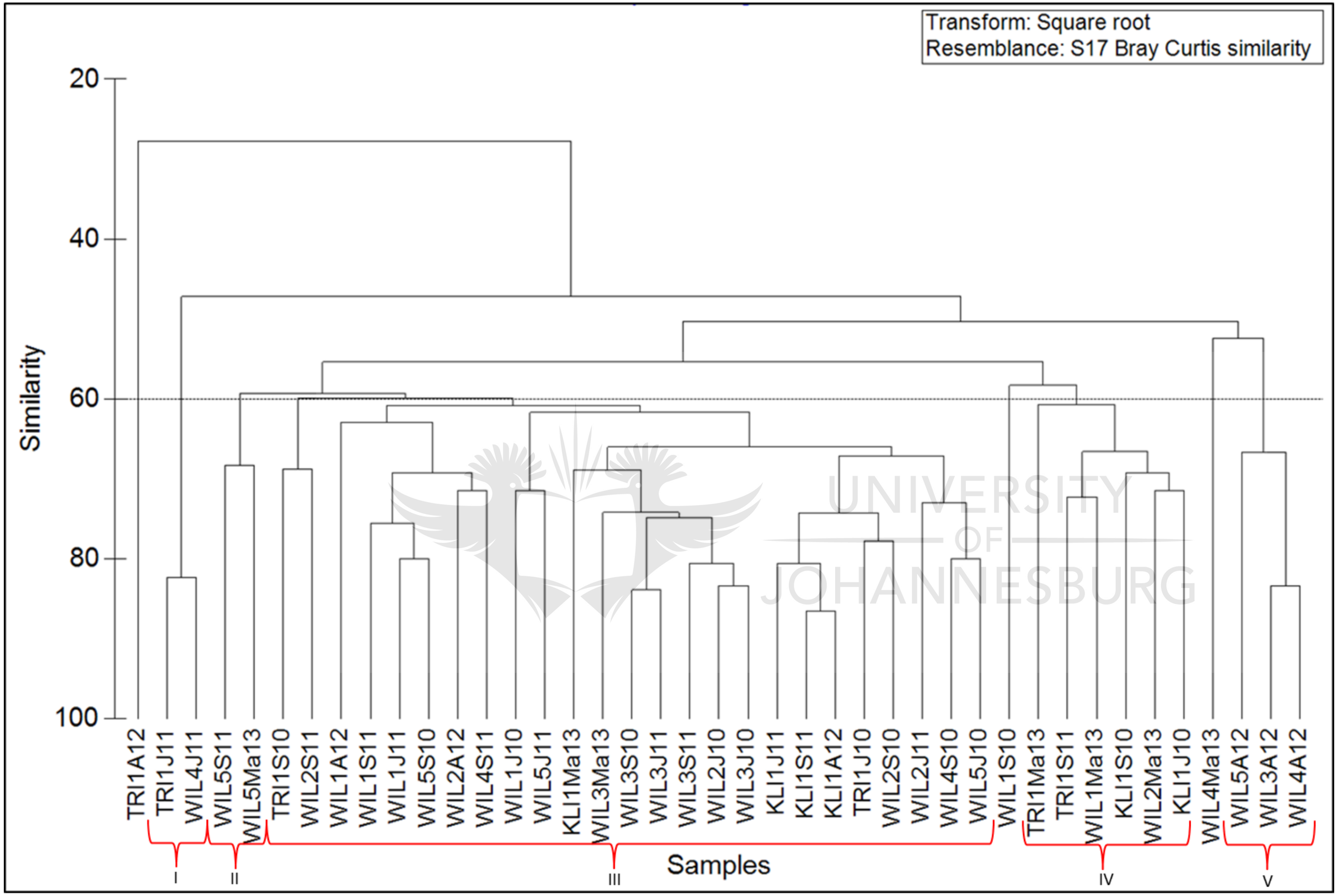


Figure 44a: Bray-Curtis similarity matrix-based cluster analysis of the aquatic macroinvertebrates collected at the sites on the Wilge River and two adjoining tributaries during the dry season. Groups were identified with 60% similarity

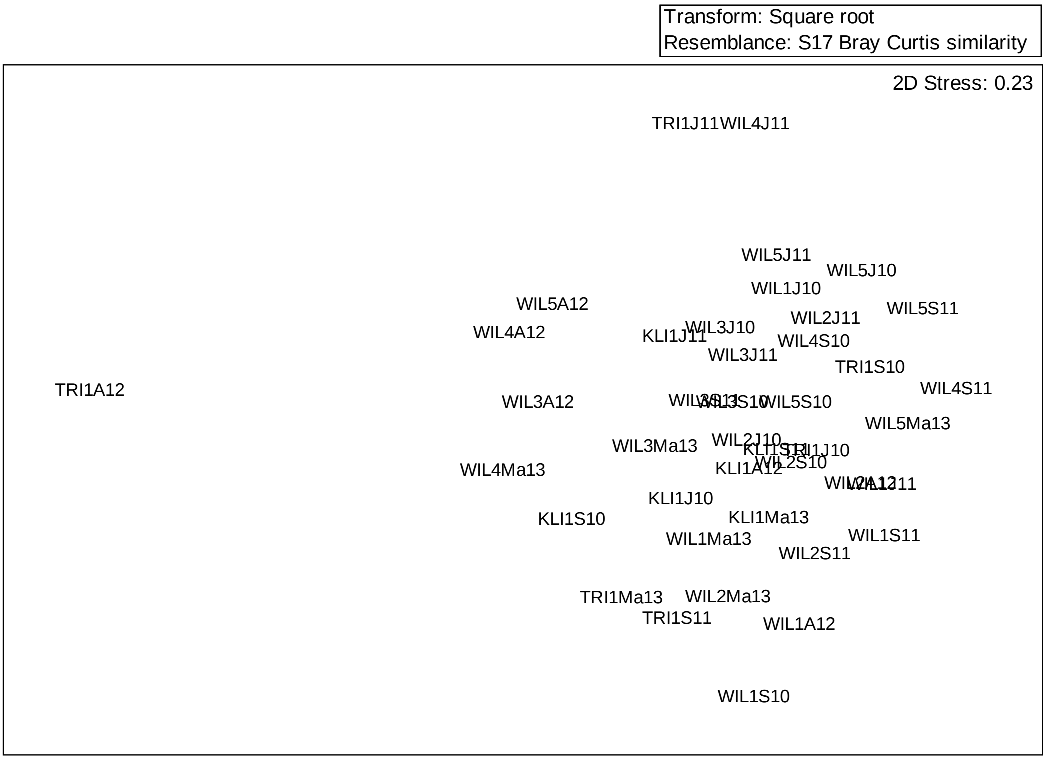


Figure 44b: Bray-Curtis similarity two dimensional representation of the NMDS ordination of the aquatic macroinvertebrates collected at the sites on the Wilge River and two adjoining tributaries during the dry season. The NMDS ordination was completed with 13 iterations and showed a stress of 0.23

Results of a Similarity Percentages – species contribution (SIMPER) analysis were conducted for both the wet and dry seasons and indicated the intergroup relationships between taxa (Table 13). The average similarity between taxa ranged from 62.5% in Group I to 72.22% in Group II. These high similarities were an indication that similar taxa occurred and / or the composition of the groups remained constant, at the sites within the groups. Group I comprised of sites TRI01 and WIL04, although surveyed in the dry and wet season respectively. All the taxa within the group contributed equally at 20% of the abundances, thus clearly indicating no dominant taxa (Table 13). All these taxa are good indicators of pollution taking place at the site. Three of the five taxa were air breathers, reflecting low DO concentrations within the water column at these sites. This was supported by the characteristics of site WIL04 lacking flow conditions, which deters improved DO concentrations, while site TRI01 recorded a low average DO concentration primarily during the wet season. Moreover, the percentage contributions are very low throughout (ranged from 1.53 % to 19.10 %) (Table 14), signifying considerable variability within the groups.

There was a high percentage dissimilarity between Group I with Group II, IV, V, IX and X (Table 14, >50%). This was essentially due to the species composition being consistently different, due to different variables (flow conditions and habitat availability) driving these groupings. Group II comprised the dry season sites that have been grouped together and indicated the highest average similarity at 72.22% however, did not indicate any dominant taxa. There was a high percentage of dissimilarity between Group II with Group I, IV and X (Table 14, >50%). Group III, IV, V, VI, VII, VIII, IX, X and XI showed a high average similarity of 64.51%, 70.82%, 66.03%, 63.99%, 68.95%, 70.32%, 66.29%, 67.38% and 68.72% respectively (Table 13). These groups included both wet and dry season surveys, with the exception of Groups III, IV and VI, which only consisted of data from the wet season (Figure 43a).

Groups showing a high percentage of dissimilarities amongst each other include, Group V and Group III which showed a dissimilarity of 62.94% and Group XI with Group V, VI and VIII which showed a dissimilarity of 62.45%, 60.52% and 60.28% respectively (Table 15). Furthermore, Group I and Group III, VII and VIII showed an 80.9%, 72.8% and 71.8% dissimilarity (Table 15). These dissimilarities are primarily due to a difference in the number of taxa, abundances, composition and further reasons stated above.

Overall, it can be concluded from the multivariate analyses that the separation and similarity of sites mentioned above was not a consequence of dominant taxa, but rather a consequence of differing water quality, habitat availability and common tolerant taxa recorded temporally and seasonally.

Table 14: The contribution of the numerous taxa to the similarity within groups (identified by conducting the SIMPER analysis). The groups were determined using the Bray-Curtis cluster analysis and NMDS

Taxa	**Average Abundance (per site)**	**Average Similarity**	**Contribution (%)**	**Cumulative % contribution**
Group I Average similarity: 62.5				
Caenidae	1.00	12.50	20.00	20.00
Notonectidae*	1.00	12.50	20.00	40.00
Dytiscidae*	1.00	12.50	20.00	60.00
Chironomidae	1.00	12.50	20.00	80.00
Culicidae*	1.00	12.50	20.00	100.00

**Air breathers*

Table 14 continued: The contribution of the numerous taxa to the similarity within groups (identified by conducting the SIMPER analysis). The groups were determined using the Bray-Curtis cluster analysis and NMDS

Taxa	Average Abundance (per site)	Average Similarity	Contribution (%)	Cumulative % contribution
Group II Average similarity: 72.22				
Atyidae	1.00	7.72	10.68	10.68
Caenidae	1.00	7.72	10.68	21.37
Corixidae*	1.00	7.72	10.68	32.05
Dytiscidae*	1.00	7.72	10.68	42.74
Gyrinidae*	1.00	7.72	10.68	53.42
Hydracarina	1.00	7.72	10.68	64.10
Chironomidae	1.00	7.72	10.68	74.79
Culicidae*	1.00	7.72	10.68	85.47
Oligochaeta	0.67	2.78	3.85	89.32
Potamonautidae*	0.67	2.78	3.85	93.16
Group III Average similarity: 64.51				
Atyidae	1.00	7.17	11.11	11.11
Heptageniidae	1.00	7.17	11.11	22.22
Leptophlebiidae	1.00	7.17	11.11	33.33
Belostomatidae*	1.00	7.17	11.11	44.44
Veliidae*	1.00	7.17	11.11	55.56
Elmidae*	1.00	7.17	11.11	66.67
Gyrinidae*	1.00	7.17	11.11	77.78
Tipulidae	1.00	7.17	11.11	88.89
Potamonautidae*	0.67	2.56	3.98	92.86
Group IV Average similarity: 70.82				
Potamonautidae*	1.00	6.25	8.83	8.83
Caenidae	1.00	6.25	8.83	17.66
Leptophlebiidae	1.00	6.25	8.83	26.49
Coenagrionidae	1.00	6.25	8.83	35.33
Gerridae*	1.00	6.25	8.83	44.16
Veliidae*	1.00	6.25	8.83	52.99
Hydracarina	1.00	6.25	8.83	61.82
Gyrinidae*	1.00	6.25	8.83	70.65
Chironomidae	1.00	6.25	8.83	79.48
Simuliidae	1.00	6.25	8.83	88.32
Hydropsychidae	0.67	2.15	3.04	91.35

**Air breathers*

Table 14 continued: The contribution of the numerous taxa to the similarity within groups (identified by conducting the SIMPER analysis). The groups were determined using the Bray-Curtis cluster analysis and NMDS

Taxa	Average Abundance (per site)	Average Similarity	Contribution (%)	Cumulative % contribution
Group V Average similarity: 66.03				
Caenidae	1.00	5.66	8.57	8.57
Leptophlebiidae	1.00	5.66	8.57	17.15
Coenagrionidae	1.00	5.66	8.57	25.72
Corixidae*	1.00	5.66	8.57	34.29
Dytiscidae*	1.00	5.66	8.57	42.87
Gyrinidae*	1.00	5.66	8.57	51.44
Hydropsychidae	1.00	5.66	8.57	60.02
Ceratopogonidae	1.00	5.66	8.57	68.59
Chironomidae	1.00	5.66	8.57	77.16
Heptageniidae	0.67	1.90	2.88	80.05
Gomphidae	0.67	1.90	2.88	82.93
Gerridae*	0.67	1.90	2.88	85.82
Notonectidae*	0.67	1.90	2.88	88.70
Tipulidae	0.67	1.90	2.88	91.59
Group VI Average similarity: 63.99				
Potamonautidae*	1.00	5.59	8.73	8.73
Caenidae	1.00	5.59	8.73	17.46
Leptophlebiidae	1.00	5.59	8.73	26.20
Notonectidae*	1.00	5.59	8.73	34.93
Dytiscidae*	1.00	5.59	8.73	43.66
Chironomidae	1.00	5.59	8.73	52.39
Simuliidae	1.00	5.59	8.73	61.12
Corixidae*	0.75	2.90	4.53	65.65
Oligochaeta	0.75	2.85	4.45	70.10
Coenagrionidae	0.75	2.85	4.45	74.54
Hydracarina	0.75	2.85	4.45	78.99
Elmidae*	0.75	2.85	4.45	83.44
Veliidae*	0.75	2.59	4.04	87.48
Libellulidae	0.50	0.98	1.53	89.01
Hydropsychidae	0.50	0.98	1.53	90.54

**Air breathers*

Table 14 continued: The contribution of the numerous taxa to the similarity within groups (identified by conducting the SIMPER analysis). The groups were determined using the Bray-Curtis cluster analysis and NMDS

Taxa	Average Abundance (per site)	Average Similarity	Contribution (%)	Cumulative % contribution
Group VII Average similarity: 68.95				
Turbellaria	1.00	5.20	7.54	7.54
Atyidae	1.00	5.20	7.54	15.07
Caenidae	1.00	5.20	7.54	22.61
Heptageniidae	1.00	5.20	7.54	30.15
Leptophlebiidae	1.00	5.20	7.54	37.69
Leptophlebiidae	1.00	5.20	7.54	45.22
Elmidae*	1.00	5.20	7.54	52.76
Gyrinidae*	1.00	5.20	7.54	60.30
Ceratopogonidae	1.00	5.20	7.54	67.84
Chironomidae	1.00	5.20	7.54	75.37
Tabanidae	1.00	5.20	7.54	82.91
Corbiculidae	1.00	5.20	7.54	90.45
Group VIII Average similarity: 70.32				
Turbellaria	1.00	7.56	10.76	10.76
Caenidae	1.00	7.56	10.76	21.51
Heptageniidae	1.00	7.56	10.76	32.27
Elmidae*	1.00	7.56	10.76	43.03
Tipulidae	1.00	7.56	10.76	53.79
Corbiculidae	1.00	7.56	10.76	64.54
Leptophlebiidae	0.75	3.95	5.62	70.16
Hydropsychidae	0.75	3.76	5.34	75.51
Dytiscidae*	0.75	3.76	5.34	80.85
Simuliidae	0.75	3.76	5.34	86.20
Oligochaeta	0.75	3.66	5.21	91.40

**Air breathers*

Table 14 continued: The contribution of the numerous taxa to the similarity within groups (identified by conducting the SIMPER analysis). The groups were determined using the Bray-Curtis cluster analysis and NMDS

Taxa	Average Abundance (per site)	Average Similarity	Contribution (%)	Cumulative % contribution
Group IX Average similarity: 66.29				
Chironomidae	0.97	5.09	7.69	7.69
Caenidae	0.97	5.08	7.66	15.35
Leptophlebiidae	0.95	4.80	7.24	22.59
Dytiscidae*	0.95	4.77	7.20	29.79
Coenagrionidae	0.89	4.26	6.43	36.21
Simuliidae	0.84	3.80	5.73	41.95
Potamonautidae*	0.84	3.75	5.66	47.60
Gyrinidae*	0.82	3.52	5.31	52.91
Heptageniidae	0.79	3.36	5.07	57.99
Elmidae*	0.79	3.34	5.04	63.02
Turbellaria	0.79	3.27	4.93	67.95
Oligochaeta	0.76	3.06	4.62	72.58
Ceratopogonidae	0.76	3.00	4.53	77.10
Tricorythidae	0.71	2.64	3.99	81.09
Corixidae*	0.68	2.40	3.61	84.70
Tipulidae	0.58	1.70	2.57	87.27
Corbiculidae	0.55	1.59	2.40	89.67
Veliidae*	0.50	1.21	1.83	91.50
Group X Average similarity: 67.38				
Caenidae	1.00	7.60	11.29	11.29
Heptageniidae	1.00	7.60	11.29	22.57
Leptophlebiidae	1.00	7.60	11.29	33.86
Coenagrionidae	1.00	7.60	11.29	45.15
Gyrinidae*	1.00	7.60	11.29	56.43
Simuliidae	1.00	7.60	11.29	67.72
Potamonautidae*	0.80	4.55	6.75	74.47
Elmidae*	0.80	4.55	6.75	81.22
Corbiculidae	0.80	4.55	6.75	87.97
Chironomidae	0.80	4.37	6.48	94.45

**Air breathers*

Table 14 continued: The contribution of the numerous taxa to the similarity within groups (identified by conducting the SIMPER analysis). The groups were determined using the Bray-Curtis cluster analysis and NMDS

Taxa	**Average Abundance (per site)**	**Average Similarity**	**Contribution (%)**	**Cumulative % contribution**
Group XI Average similarity: 68.72				
Atyidae	1.00	13.13	19.10	19.10
Caenidae	1.00	13.13	19.10	38.21
Leptophlebiidae	1.00	13.13	19.10	57.31
Chironomidae	1.00	13.13	19.10	76.41
Baetidae	0.67	4.44	6.47	82.88
Potamonautidae*	0.67	3.92	5.71	88.59
Heptageniidae	0.67	3.92	5.71	94.29

**Air breathers*

Table 15: Percentage of dissimilarity between the groups

Groups	Percentage of dissimilarity (%)	Groups	Percentage of dissimilarity (%)
I and II	58.55	IX and I	68.18
I and III	80.9	IX and II	48.39
I and VI	62.16	IX and III	47.9
I and VII	72.88	IX and V	44.17
I and VIII	71.81	IX and VI	40.89
II and III	58.08	IX and VII	41.48
II and VII	55.04	IX and VIII	40.84
III and VII	42.74	IX and X	40.58
IV and I	68.49	IX and XI	58.54
IV and II	57.02	IX and IV	43.42
IV and III	56.83	X and I	75.91
IV and VIII	58.01	X and II	54.46
IV and V	41.28	X and III	44.1
IV and VI	43.2	X and IV	45.24
IV and VII	55.01	X and V	52.61
IV and XI	51.53	X and VI	51.03
V and I	57.32	X and VII	41.7
V and II	48.5	X and VIII	45.17
V and III	62.94	X and XI	52.16
V and VI	45.34	XI and II	52.66
V and VII	52.22	XI and III	52.94
V and VIII	54.81	XI and V	62.45
VI and II	55.69	XI and VI	60.52

Table 15 continues: Percentage of dissimilarity between the groups

Groups	Percentage of dissimilarity (%)		Groups	Percentage of dissimilarity (%)
VI and III	51.15		XI and VII	56.22
VI and VII	53.03		XI and VIII	60.28
VI and VIII	47.7		VIII and III	50.33
VIII and II	54.65		VIII and VII	43.79

With the purpose to provide insight to the source for the spatial groups of sites identified in the NMDS ordination, an Analysis of Similarities (ANOSIM) was conducted. This was applied from the original similarity matrix for the aquatic macroinvertebrates recorded from the sites along the Wilge River and two adjoining tributaries during both the wet and dry seasons. This approach compares all the sites in the study temporally with the aim to yield a test statistic and level of significance (Clark and Warwick, 2001). Refer to Table 7 which illustrates the R value which is the degree of similarity between the sites. The closer the R value is to 1, the more significant differences are between the sites (Clark and Warwick, 2001). The results of the ANOSIM test conducted on the groups, determined by the Bray-Curtis similarity matrix derived cluster analysis and NMDS ordination, indicated that there was a significant difference ($p<0.05$; global R statistic = 0.718) between most of the groupings consisting of the sites during both the wet and dry seasons. This was further an indication of the possible seasonal variation within the Wilge River and two adjoining tributaries in the study area. Therefore, seasonal variation may further be a contributing factor to the changes in the environmental conditions, namely *in situ* water quality and habitat availability. Therefore, seasonal variation, *in situ* water quality and habitat availability are all driving variables in the differences between the groupings.

In order to further assess the correlations between the aquatic macroinvertebrate data, environmental variables and land use data, a multivariate analysis was conducted. In both the PCA and RDA bi-or-tri-plots, each arrow points in the direction of the steepest increase of values for the corresponding variable. The angles between the arrows indicate the degree of correlation between the variables: the approximated correlation is positive when the angle is acute and negative when the angle is larger than 90 degrees. The length of the arrow is a measure of fit for the variables. The distance between the sampling sites in the diagram approximates the dissimilarity of their water quality variables as measured by their Euclidean distance (Ter Braak and Smilauer, 2004). It must be noted that data from June 2010 to May 2013 was used. Data from March 2010 was excluded due to the fact that the *in situ* water quality, DO% was not measured during that survey. Furthermore, it must be noted that sample site WIL3A12 was an outlier and consequently excluded in the analyses. This may be attributed to the low pH that was recorded at the site during the survey (pH=5.9).

A RDA was completed for both wet and dry seasons to indicate any correlations among the sites along the Wilge River and two adjoining tributaries (Figure 45). Furthermore, this was conducted for both the wet and dry seasons, based on the aquatic macroinvertebrate communities with *in situ* water quality superimposed (Figure 46 and Figure 47). The RDA bi-plots for both the wet and dry season data indicate, as with the Bray-Curtis similarity matrices and related NMDS plots (Figure 42a and Figure 42b respectively), that there was a distinct separation of wet and dry conditions.

In terms of the sample sites, the RDA bi-plot illustrated a clear separation of site TRI01 (Figure 45) (similar to the Primer analyses that were carried out). In accordance to the bi-plot, this site further illustrated a high dissimilarity with the other sites in the study. Site TRI01 was closely associated with aquatic macroinvertebrate taxa namely *inter alia* Baetidae, Leptophlebidae*,* Naucoridae, Lestidae, Nepidae, Notonectidae and Dixidae, all of which prefer low to medium flow velocities, a characteristic of this site (Gerber and Gabriel, 2002). Furthermore, Naucoridae, Notonectidae and Nepidae are air breathing taxa, thus indicating low DO concentrations in the water column. This was confirmed by Figure 18 and Figure 19 which illustrated the lowest average DO concentration recorded at this site seasonally. The site was associated with high temperatures but negatively associated with turbidity (Figure 45). Both of these findings were expected as the site was characterised by low water levels contributing to higher water temperatures. Furthermore, the site measured some of the highest turbidity levels (low clarity) in the study area (Figure 27 and Figure 28) which may be attributed from the upstream dam, which contains high sedimentation loads, thus contributing to raised sediment levels moving in a downstream direction, primarily during the wet season, as well as the surrounding exposed soils in the area. Therefore, this site was clearly driven by water quality.

The remaining sites are distributed on the tri-plot, although small clusters of sites WIL03, WIL04 and WIL05 occur, illustrating a high similarity to each other. This was likewise indicated on the NMDS ordination (Figure 44b). Nonetheless, they do not show a positive or negative correlation to any of the environmental variables i.e. *in-situ* water quality (Figure45). Figure 46 and Figure 47 reflects the correlation among the sites during the wet and dry seasons respectively. Both seasons, site TRI01 was grouped together of which during the wet season, the site was not correlated with any of the environmental variables (Figure 46), as opposed to the dry season, where the site had a positive correlation with temperature, DO and percentage saturation (Figure 47). From a DO concentration perspective, according to Davies and Day, (1998), the higher the temperature, the less oxygen is available in water. This finding was expected, as the DO concentration recorded at site TRI01 during the dry season exceeded the concentrations recorded during the wet season. Overall, with exception of site TRI01, there was clear seasonal and temporal variation amongst the sites based on the aquatic macroinvertebrate communities and environmental variables (Figure 46 and Figure 47).

In order to identify which environmental variable were significant drivers among the sampling sites, a Monte Carlo Permutation Test was conducted. The results indicated that all the environmental variables, with the exception of pH, was identified as significant drivers in the river systems ($p<0.05$). During the wet season (Figure 46), it was identified that clarity, DO and pH were the significant drivers, while during the dry season (Figure 47), clarity, TDS/EC, percentage saturation and pH were the significant drivers ($p<0.05$). These drivers were expected due to possible sources namely intensive agriculture in the project area (Figure 3). The alkaline pH seems to be the general trend within the Highveld area, as can be noted over time (Figure 11).

The RDA bi-plot (Figure 45) further indicates the preferences of the aquatic macroinvertebrate communities towards environmental variables. Chironomidae, Ceratopogonidae, Gomphidae, Oligochaeta, Ancylidae and Elmidae amongst others, were all highly correlated with high TDS concentrations and low temperature. This was expected as they all have low sensitivity scores and thus are tolerant to poor water quality. Coenagrionidae, Caenidae, Simulidae and Velidae were highly associated with percentage saturation, but negatively correlated to low pH levels and DO concentrations (Figure 45).

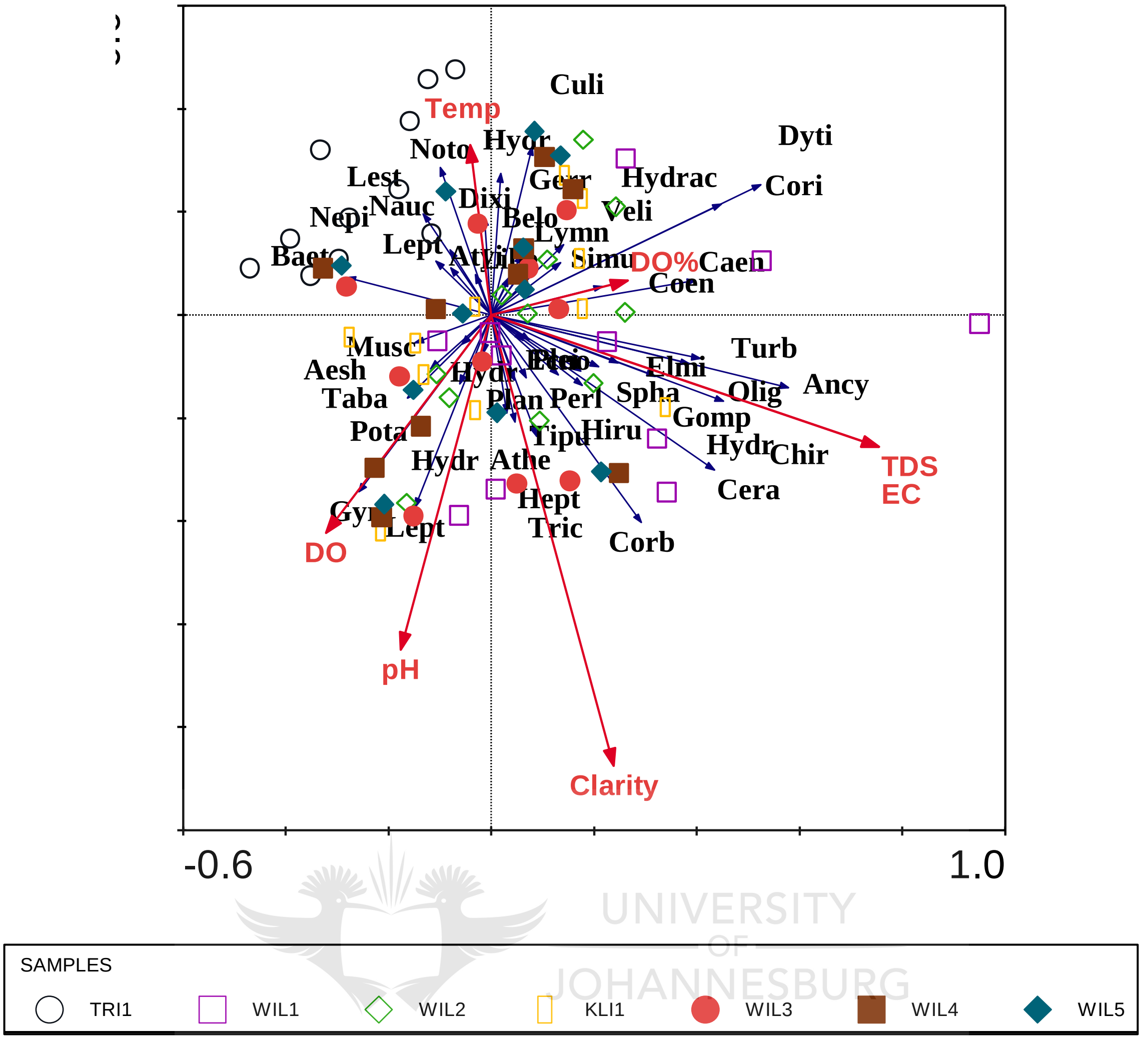


Figure 45: RDA plot showing the correlation among the sites along the Wilge River and two adjoining tributaries during both the wet and dry seasons based on the aquatic macroinvertebrate communities with environmental variables (in situ water quality) superimposed. This tri-plot represents 63.8% of the variation in the data, where 42.0 % is displayed on the first axis, while an additional 21.8 % by the second axis.

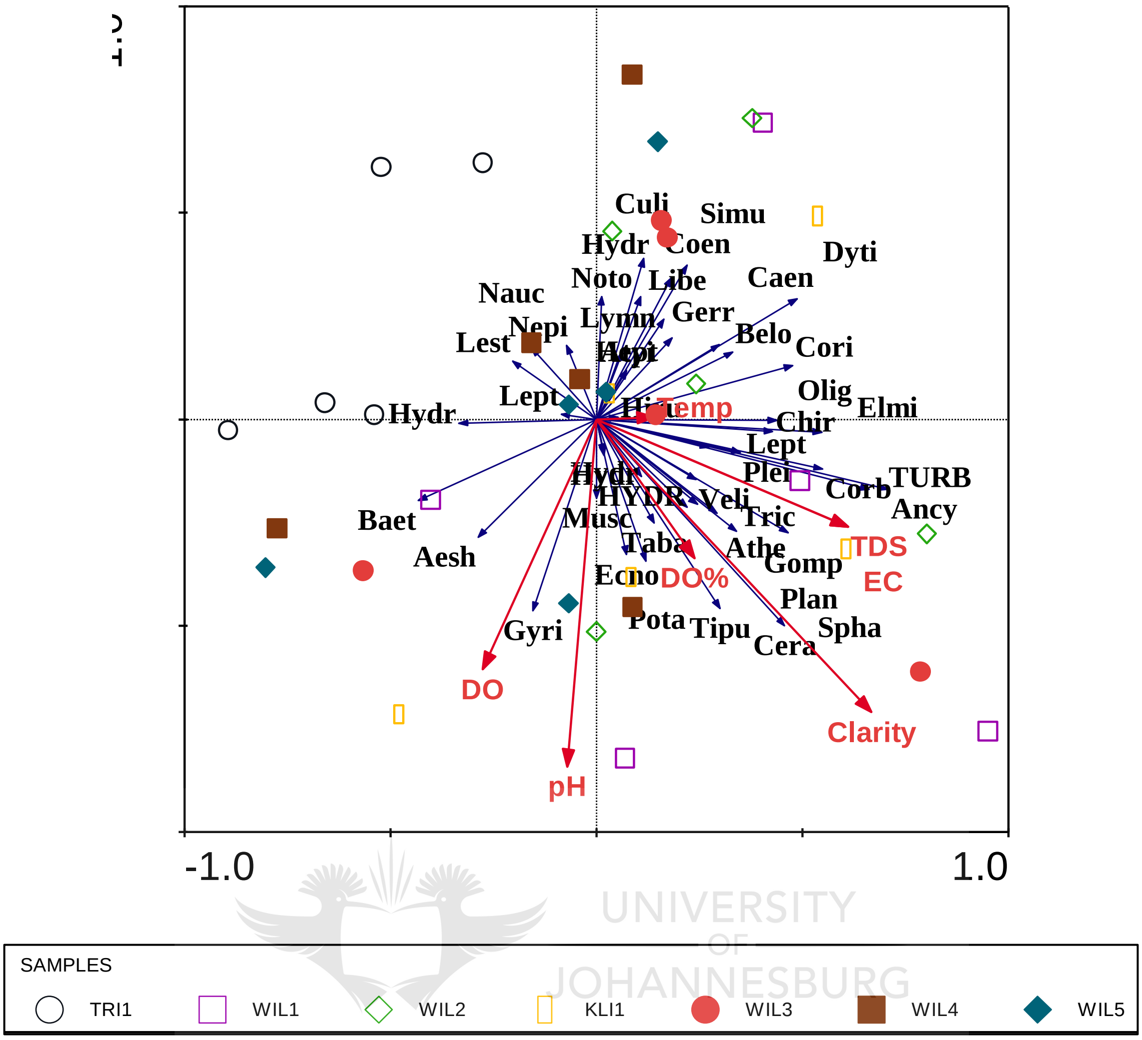


Figure 46: RDA plot showing the correlation among the sites along the Wilge River and two adjoining tributaries during the wet season based on the aquatic macroinvertebrate communities with environmental variables (in situ water quality) superimposed. This tri-plot represents 66.4% of the variation in the data, where 46.2 % is displayed on the first axis, while an additional 20.2 % by the second axis.

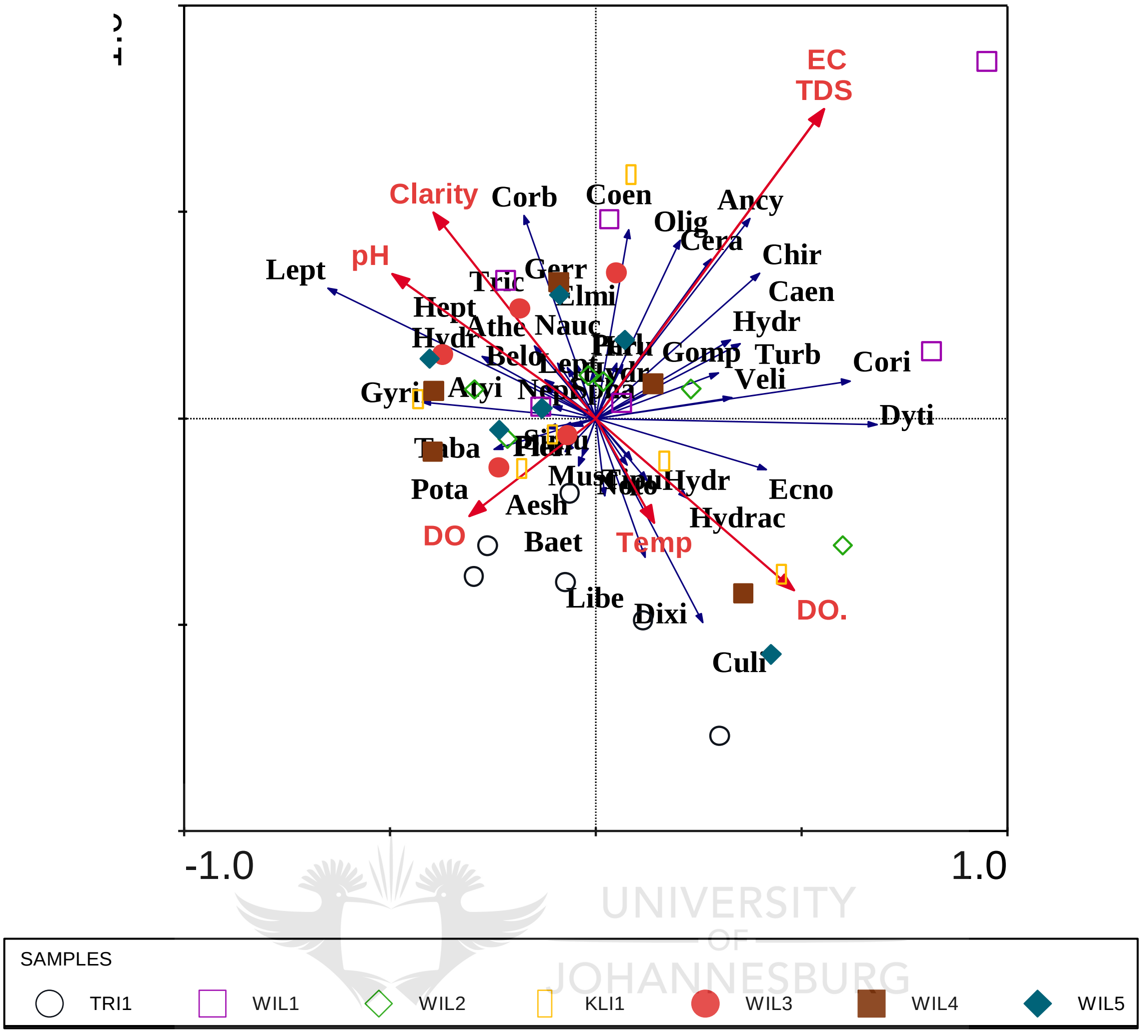


Figure 47: RDA plot showing the correlation among the sites along the Wilge River and two adjoining tributaries during the dry season based on the aquatic macroinvertebrate communities with environmental variables (in situ water quality) superimposed. This tri-plot represents 68.0% of the variation in the data, where 43.7 % is displayed on the first axis, while an additional 24.3 % by the second axis.

Figure 48 to Figure 50 indicates the relationship between the SASS5 and ASPT scores and habitat availability (IHAS), further illustrating the importance of habitat as a driving variable in aquatic macroinvertebrate community structure. A RDA was completed for both wet and dry seasons for the sites along the Wilge River and two adjoining tributaries, coupled with *in situ* water quality (Figure 48 to Figure 50). Figure 48 indicates the separation of sites TRI01 and WIL04 from the rest of the sites. The separation of site WIL04 is habitat related. As mentioned above, stream bed composition is one of the most important physical factors controlling the structure of freshwater invertebrate communities (Mackay and Eastburn, 1990). Physical stream condition and other habitats / general biotopes are also important factors to consider. Consequently, site WIL04 is characterised by steep incised banks and a deep channel. Furthermore, GSM is the only biotope that can be sampled during the dry season, due to vegetation die-back and lowered water levels resulting in exposed eroded river banks. Consequently, these factors contribute to lower aquatic macroinverbrate diversity at this site. During the wet season (Figure 49), site WIL01 was positively associated with environmental variables such as temperature, percentage saturation, pH and IHAS. It was identified following the Monte Carlo Permutation Test that all *in situ* water quality parameter's, with the exception of pH, as well as the ASPT and SASS5 score and the IHAS, were significant drivers for the river systems spatially ($p<0.05$) (Figure 48). However, during the wet season (Figure 49), only TDS/EC, pH and the SASS5 and ASPT values showed to be significant drivers ($p<0.05$), whilst during the dry season, TDS/EC, clarity, percentage saturation, SASS5 score and IHAS were the significant drivers ($p<0.05$, Figure 50). Therefore, the driving variables in the separation of the sites along the Wilge River and two adjoining tributaries, appear to be a combination of variables (DO, percentage saturation, TDS/EC, clarity and pH) and including habitat availability (based on IHAS scores) (Table 7 and Table 8).

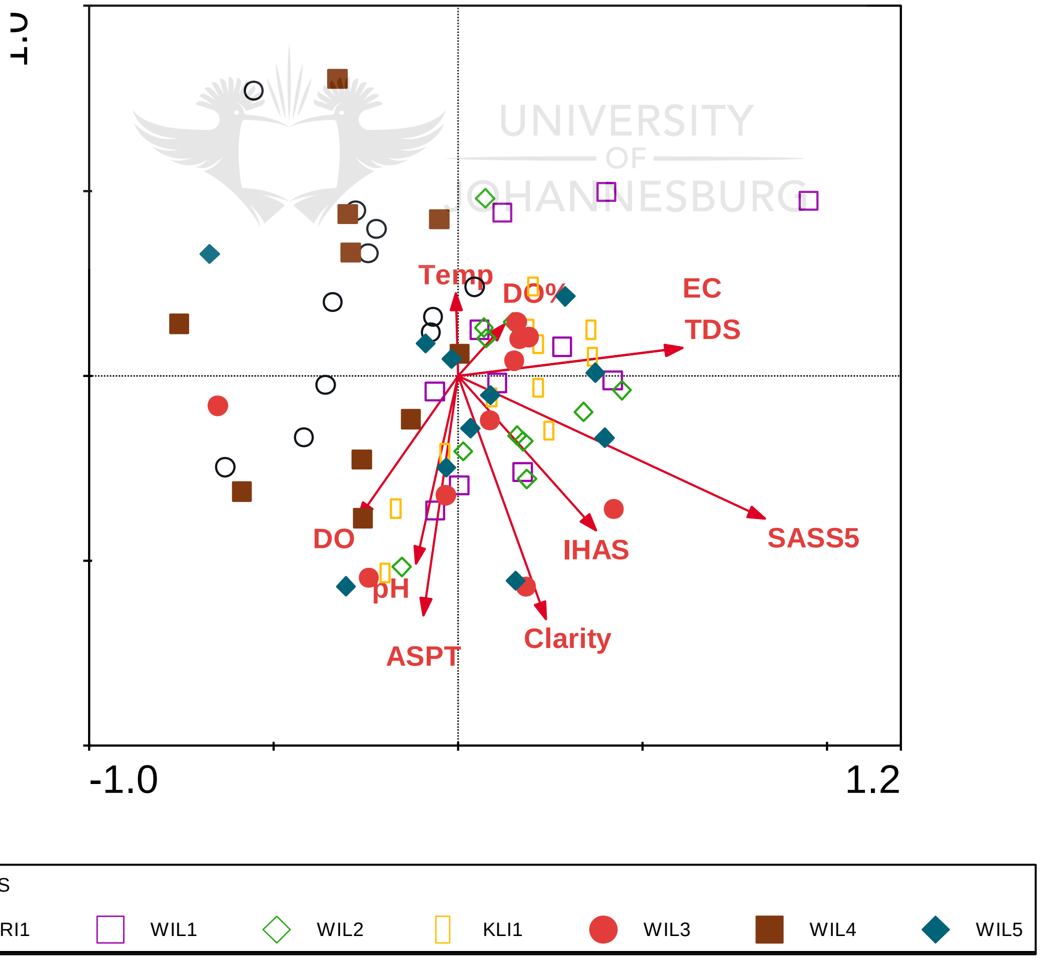


Figure 48: RDA plot showing the correlation among sites on the Wilge River and adjoining tributaries during high and low flow based on invertebrate communities with variables superimposed, including habitat, SASS5 and ASPT values. This bi-plot describes 58.7% of the variation in the data, where 39.9% is displayed on the first axis, while 18.8% is displayed on the second axis.

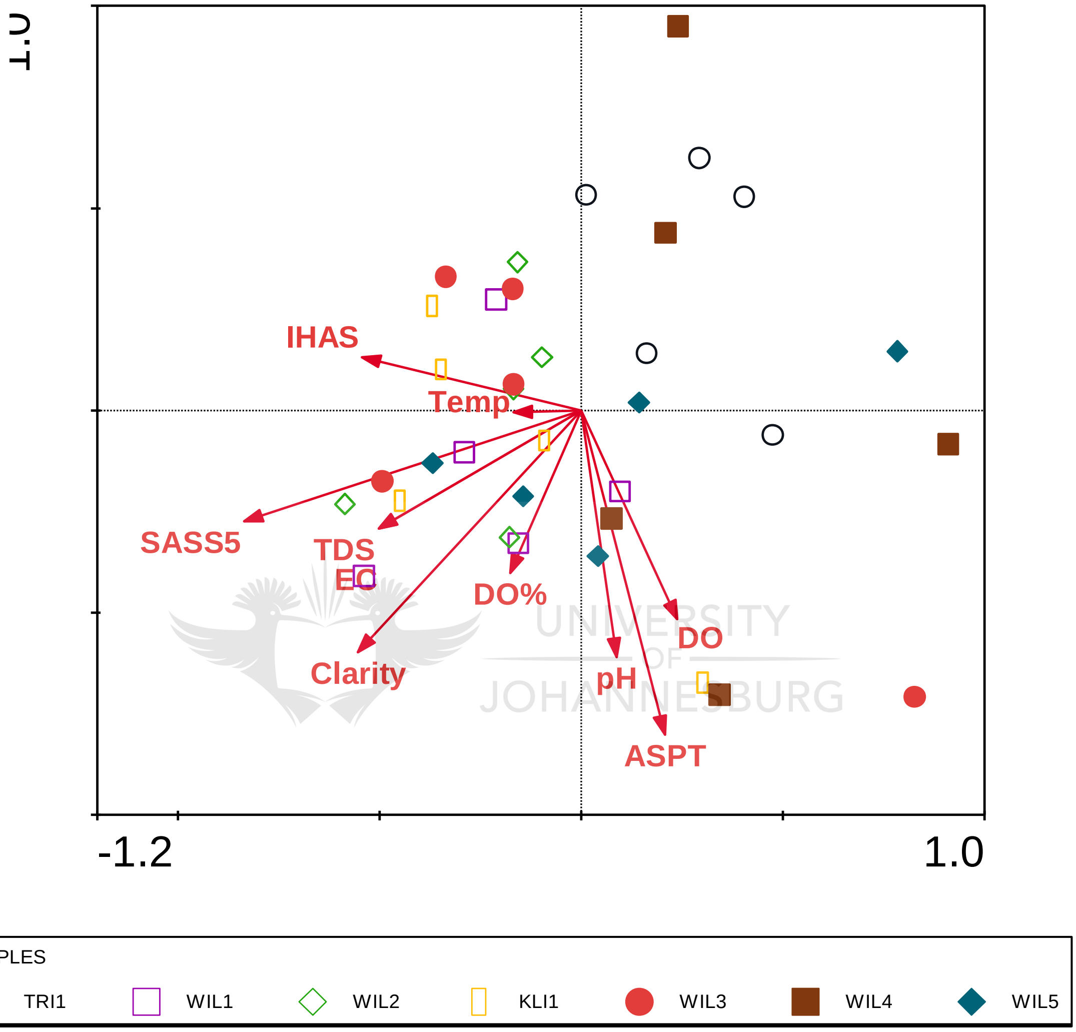


Figure 49: RDA plot showing the correlation among sites on the Wilge River and adjoining tributaries during the wet season based on invertebrate communities with variables superimposed, including habitat, SASS5 and ASPT values. This bi-plot describes 56.1% of the variation in the data, where 40.2% is displayed on the first axis, while 15.9% is displayed on the second axis.

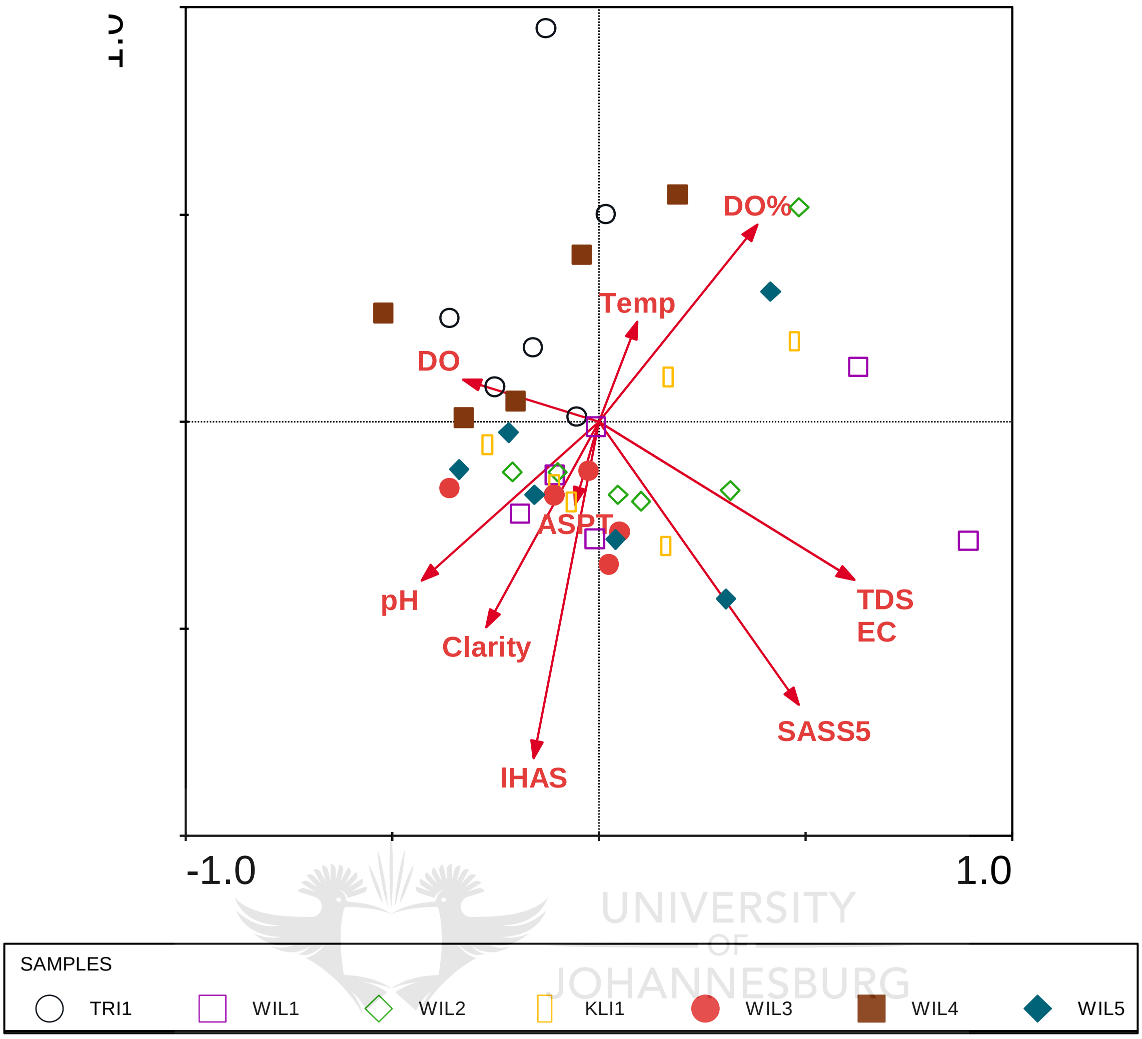


Figure 50: RDA plot showing the correlation among sites on the Wilge River and adjoining tributaries during the dry season based on invertebrate communities with variables superimposed, including habitat, SASS5 and ASPT values. This bi-plot describes 56.2% of the variation in the data, where 32.9% is displayed on the first axis, while 23.3% is displayed on the second axis.

To determine the effects and relations between the primary driving changes, to the surrounding land uses in the project area, further multivariate analyses were conducted. A RDA was completed showing the dissimilarity among sites along the Wilge River and two adjoining tributaries based on aquatic macroinvertebrate FFG's (scraper/grazers, gathering collectors, shredders, filter collectors and predators) with *in situ* water quality and the surrounding land uses, as identified from Figure 3 and Table 2 (Figure 51 to 53). Similarly to the above multivariate analyses, there was a separation of sites TRI01, the unknown tributary on the Klipfonteinspruit. Furthermore, the separation of site WIL01 on the Wilge River was also identified. Sites WIL03 and WIL05, although scattered randomly throughout the bi-plot, are closely associated with each other (Figure 51). This was attributed to similar habitat availability and flow conditions at these two sampling sites. The bi-plot illustrates the primary FFGs within the system, to be a combination of predators, gathering, filter collectors and shredders (Figure 51). It further indicated that predators have a negative correlation with the rest of the FFG's which was expected. As the percentage of predators increase at a site, the percentage of the other FFG's decreased. This was expected as predators capture and consume live prey (Merritt and Cummins, 1996) namely, all members of the Odonata, as well as Hydracarina and Perlidae amongst others (Cummins *et al.,* 2005). This was verified as site TRI01 was the only site where Perlidae was recorded and up to five of the Odonata taxa, all of which are predators (Figure 51). Similar results were identified during the wet season (Figure 52). Furthermore during the wet season, scraper/grazers showed a positive association with temperature change however, no correlation was identified during the dry season (Figure 52 and Figure 53 respectively). Scraper/grazers depend upon attached periphyton that develops on submerged substrates for their primary food resource (Cummins *et al.,* 2005). Therefore, there was a variation and clear changes in the food sources constantly entering into the river system primarily. This is normally related to changes in the land use however, as the land use is consistent in the study area (agriculture, industrial and mining) the changes in food availability for the aquatic macroinvertebrates may possibly be attributed to seasonal changes. Site WIL01 was positively associated with filter collectors. This FFG is dependent on fine particulate organic matter as their primary food resource which they obtain from the passing water column as this site. It was expected that shredders would be more abundant at this site, as this site was the only site with in-stream aquatic vegetation, which would contribute to the course particulate organic matter that this FFG depend on for their food resource. However, this was not the case with the exception of the survey conducted in February 2013 when the site was positively associated to shredders (Figure 51).

The RDA further illustrates any existing links between the land use and the FFG's, as well as *in situ* water quality (Figure 51 to Figure 53). Rural development spatially (Figure 51) and during the wet season (Figure 52) and dry season (Figure 53) had the least impact in the study area and minimal influence on the FFG's and further had no positive or negative correlation to *in situ* water quality (Figure 51 to Figure 53). Medium agriculture was the main driver for predators (Figure 51), but did not illustrate any positive or negative correlation towards *in situ* water quality spatially (Figure 51) or during the wet (Figure 52) and dry season (Figure 53). High agriculture (intensive cultivation) was the driver for scraper/grazers (Figure 51) and indicated a positive association with pH and temperature during the wet season (Figure 51) and DO, pH and clarity during the dry season (Figure 53). This was expected as it comes down to food source. In terms of the predators, medium agricultural activities provide the nutrients required by the rest of the FFG, which in turn are preyed upon by the predators. High agriculture (intensive cultivation) further provides the nutrients to the river systems, resulting in algae formations that are consumed by scraper/grazers. However, this was not the case seasonally (Figure 52 and Figure 53). In the wet season, industrial and mining activities were the drivers for increased predators and high agriculture was the driver for scraper/grazers and shredders (Figure 52). Conversely during the dry season, industrial and mining activities did not have an influence on predators (Figure 53). Consequently, the predator FFG may possibly be influenced by water quality or habitat availability variables, which incidentally shows some correlation with the presence of industrial and mining activities (Figure 53). Furthermore during the dry season, industry and mining, was the driver for scraper/grazers, shredders and gathering collectors (Figure 53). The diversity of shredders, scrapers and predators has been observed to be lower in urbanized than in undisturbed streams (Brinkman, 2007). However, agricultural and urban environments produce habitats, which support specific aquatic macroinvertabrate taxa, in particular Diptera (Culicidae and Chronomidae) (Brinkman, 2007), of which in this particular study, were highly observed. Furthermore, infrastructure was highly associated with site WIL01, which was expected due to the railway line over the Wilge River at that site, as well as positively correlated to percentage saturation, EC and TDS (Figure 51). Mining and industry showed a positive association with temperature.

Nevertheless, without having chemical water quality data (nutrients), it was difficult to confirm whether land use was a driving factor or not on the FFG communities in this study. However, there is validated evidence within the literature that confirms that land use have numerous effects on physical-chemical conditions in rivers and streams, with many ramifications for biological communities (Cooper *et al.,* 2012). Furthermore, many studies, including those from med-ecosystems, have shown that land use affects the growth, development, reproduction, and behaviour of individual organisms and the diversity, composition, abundance and biomass of biological communities (Cooper *et al.,* 2012). Therefore, considering organic and inorganic data going forward in future studies, would be recommended in order to further evaluate land use as a driving factor on the aquatic macroinvertebrate communities in this study area. However one aspect is for certain, that there was considerable seasonal variation amongst the FFGs, which may confirm that changes in food availability may possibly be further attributed to seasonal changes in the food sources, ultimately altering the FFG communities and structures. The seasonal changes in the food sources may be attributed to *inter alia,* increased runoff events which may alter hydrologic characteristics of aquatic systems, affecting ecosystem productivity and food availability during the wet season. Vegetation die-back, which occurs during the dry season, results in lowered food availability for those taxa dependent upon the vegetation biotope, both for habitat and as a food source. Furthermore, higher water temperatures, during the wet season, increases the rate of microbial activity and consequently the rate of decomposition of organic material, resulting in less food being available for invertebrates (Meyer and Edwards, 1990).

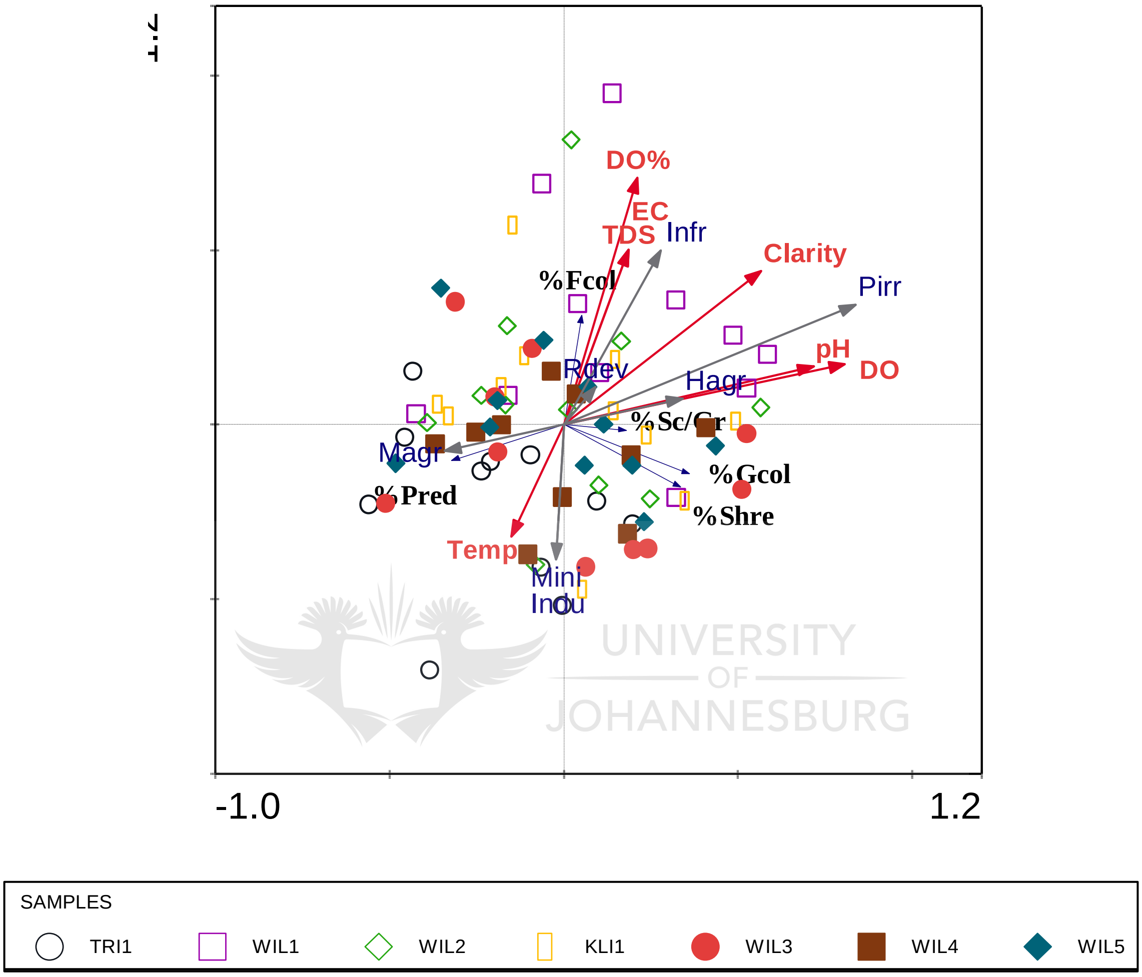


Figure 51: RDA plot showing the correlation among sites along the Wilge River and two adjoining tributaries based on aquatic macroinvertebrate FFG's with supplementary data (land use) and variables (in situ water quality) superimposed during both wet and dry seasons. This tri-plot represents 70.5% of the variation in the data, where 69.8% is displayed on the first axis and an additional 0.7 % by the second axis.

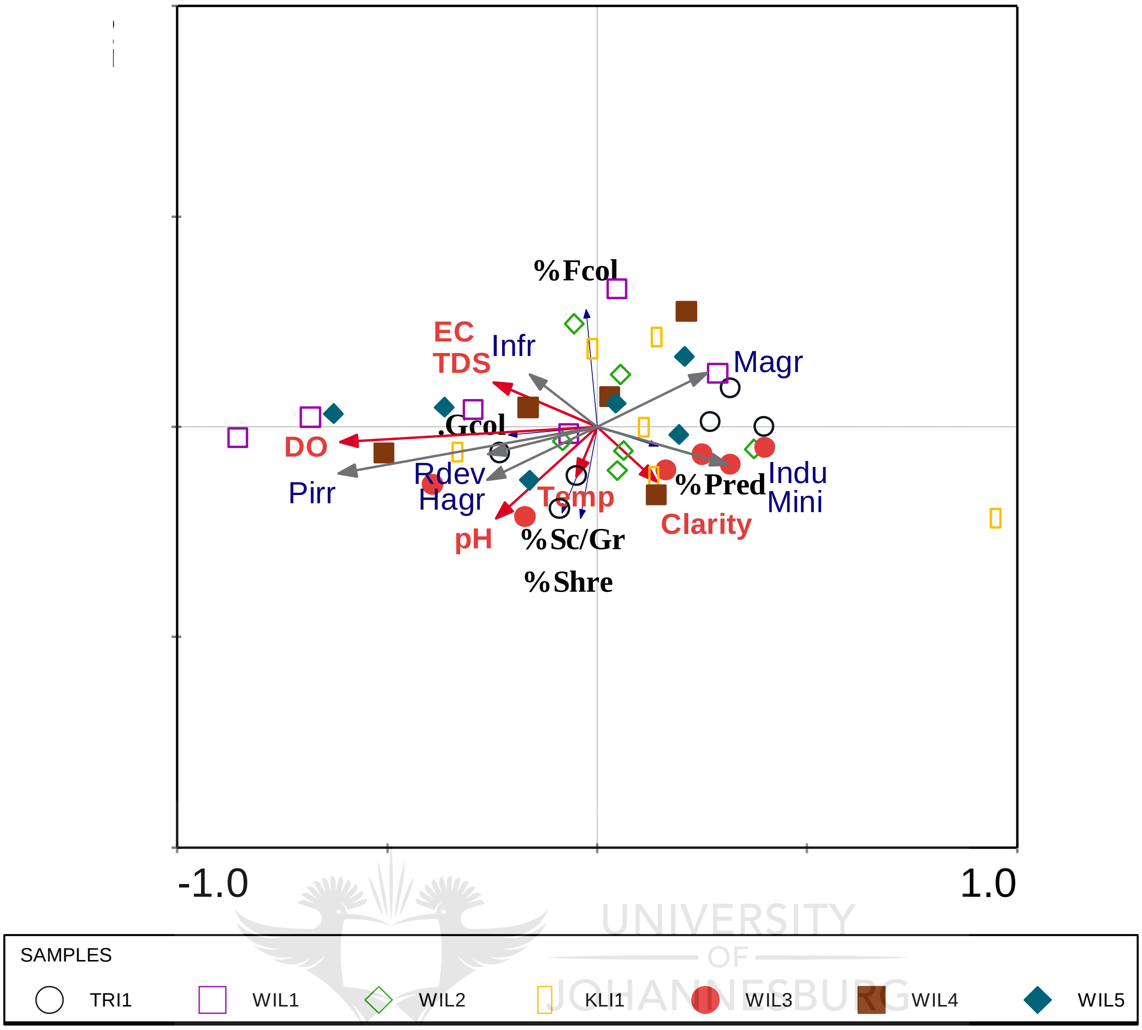


Figure 52: RDA plot showing the correlation among sites along the Wilge River and two adjoining tributaries based on aquatic macroinvertebrate FFG's with supplementary data (land use) and variables (in situ water quality) superimposed during the wet season. This tri-plot represents 70.5% of the variation in the data, where 69.8% is displayed on the first axis and an additional 0.7 % by the second axis.

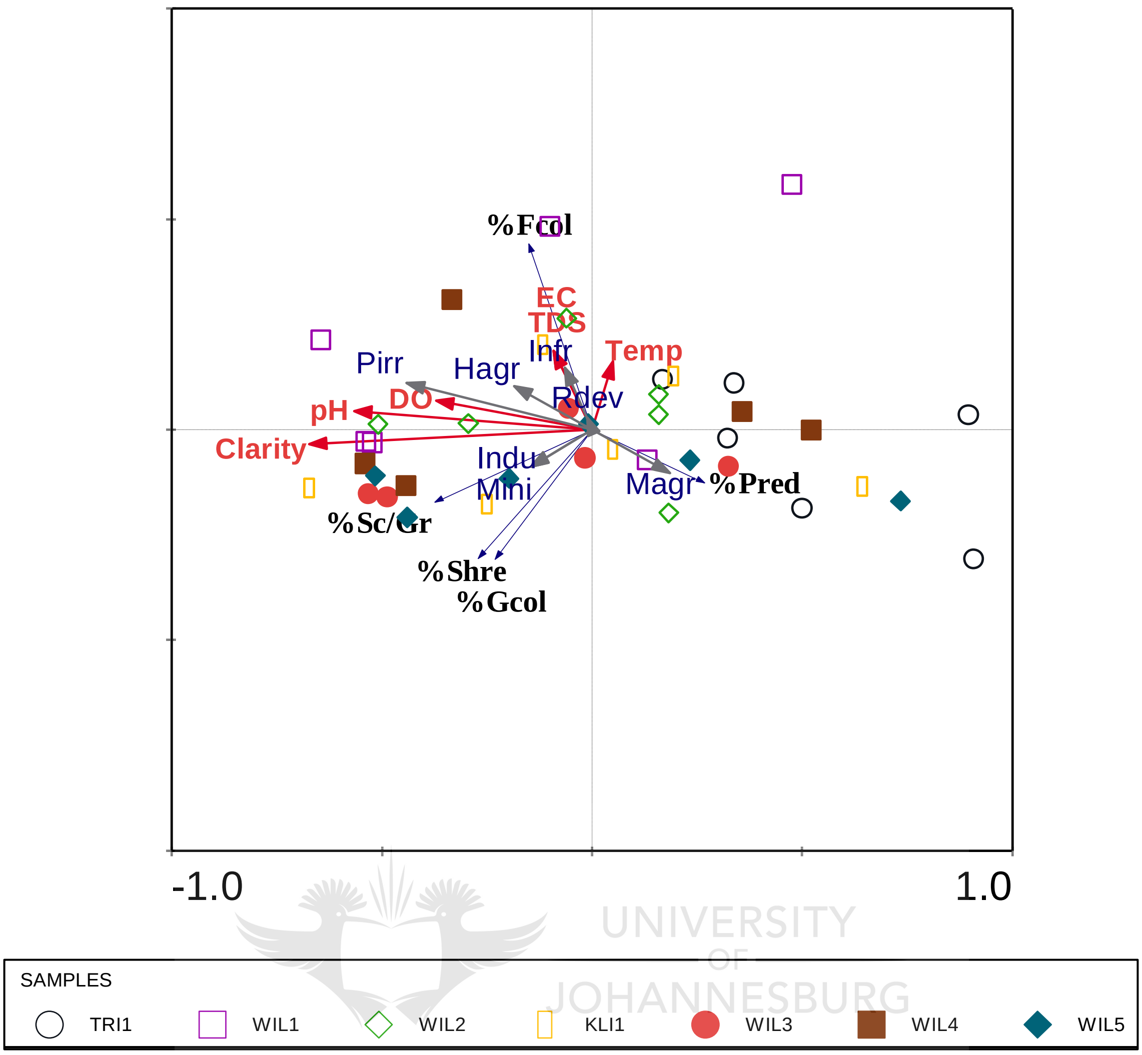


Figure 53: RDA plot showing the correlation among sites along the Wilge River and two adjoining tributaries based on aquatic macroinvertebrate FFG's with supplementary data (land use) and variables (in situ water quality) superimposed during the dry season. This tri-plot represents 70.8% of the variation in the data, where 67.4% is displayed on the first axis and an additional 3.4 % by the second axis.

Overall, it was clearly illustrated that the driving variables in the separation of the sites along the Wilge River and the two adjoining tributaries and the aquatic macroinvertebrate communities and their FFS's appear to be primarily *in situ* water quality, habitat availability ($p<0.05$) and natural seasonal variation, as opposed to land use or dominant taxa, being the driving change. These variables are forever changing seasonally, whereas land use is constant and this elucidates the wide seasonal variation in both the aquatic macroinvertebrate communities and their FFG's. However, this is not to say that land use is not a driving factor for the changes in the aquatic macroinvertebrate assemblage's and FFG structures. Land use changes have numerous effects on physical-chemical conditions in streams and rivers, with many ramifications for biological communities. Numerous studies, including those from med-ecosystems, have illustrated that land use changes have an effect on the growth, reproduction, development and behaviour of individual organisms and the diversity, abundance, composition and biomass of biological communities (Cooper *et al.,* 2012). It has been illustrated that aquatic macroinvertebrate communities have shown numerous responses to land use changes (Waite *et al.,* 2010; Cheimonopoulou *et al.,* 2011 and Mazor*et al.,* 2011). Species richness, density and biomass of many aquatic macroinvertebrates namely the Ephemeroptera, Plecoptera and Trichoptera (EPT), decline whereas those of some Dipterans (e.g. Chironomidae, Muscidae) and Annelidae namely, Oligochaeta and Hirudinea increase, with increasing catchment development (Dallas and Day, 2007; Song *et al*., 2009; Waite *et al.,* 2010). Furthermore, studies in med-regions including South Africa, have observed light livestock grazing or abandoned farmlands, can result in the loss of sensitive groups namely Plecoptera (Brinkman, 2007 and Mazor*et et al.,* 2011). This study can confirm this statement as such taxa was not observed in this study area. As the aquatic macroinvertebrates in most med-regions consist of many endemic species which are vulnerable to environmental changes, land use alterations can have large effects on local biodiversity (Bonada *et al.,* 2004). This could be varied with organic and inorganic water quality to provide a clearer view of the type of nutrients within the system in the study area.

5. CONCLUSION AND RECOMMENDATIONS

The objectives set out for this study as illustrated in Section 1.1 have been reached. The aquatic macroinvertebrate communities, coupled with their FFG's were identified at five sites along the Wilge River, as well as two additional sites on two adjoining tributaries. The aquatic macroinvertebrate communities that were identified were expected and typical of the conditions within the study area.

The analysis of *in situ* water quality measured during the period March 2010 to May 2013, clearly illustrated high levels of variation both spatially and temporally. *In situ* water quality was a limiting factor to the aquatic ecosystem from a Dissolved Oxygen (DO) and Percentage Saturation (DO%) perspective. The remaining parameters were within the South African guideline for aquatic ecosystems (DWAF, 1996).

Habitat availability played a crucial role in the complexity of aquatic macroinvertebrate structures, due to their preferences and dependence towards certain habitats. Habitat availability illustrated clear seasonal variation, of which the wet season indicated better habitat availability compared to the dry season. The dominant biotopes in the study area were VEG and GSM. Site WIL04 illustrated the poorest habitat integrity overall. This was attributed to the site’s steep incised banks and deep channel which lacks stones biotope. During the dry season the water level was lower, thus exposing the undercut banks resulting in a lack of VEG.

The SASS5 results indicate that there was a change in the integrity of the aquatic macroinvertebrate communities in the study area and further illustrated variability both spatially and temporally. It was evident that the aquatic macroinvertebrate communities within the Wilge River and two adjoining tributaries sampled, are generally in a modified state with moderate variations. The lowest number of taxa, SASS5 and ASPT values were recorded at site WIL04. It appears that the modifications were brought about largely due to changes in flow conditions and habitat availability, compared to sites WIL03 and WIL05, located upstream and downstream of this site respectively. The ASPT score ranged from 3.8 at site WIL04 to 7.7 at site WIL02, indicating that the aquatic macroinvertebrate communities are primarily composed of tolerant and moderately tolerant taxa. The low diversity recorded at site WIL04 corresponds to the findings of the IHAS index, where the habitat integrity at this site was predominantly poor (<55 %) during both the wet and dry seasons. Of these mostly tolerant taxa, predators and gathering collector populations were the most dominant feeding group, with the shredders being the least abundant within the study area. This was expected as all the sites have the biotope GSM, thus supporting the gathering collector populations, while some sites included the VEG and SIC biotope, which supports majority of the predator populations. The scraper/grazers, which consume algae and associated material, were expected to be most abundant, but were one of the least abundant FFGs. This expectation arises from medium and high agriculture being the most dominant land use in the study area. Therefore, one would expect major nutrient input into the Wilge River system, resulting in eutrophic conditions and thus attracting taxa within this FFG. The fact that scrapers/grazers were not abundant can be seen as an indication of low nutrient inputs and thus relatively good *in situ* water quality. The relatively high SASS5 and ASPT values and PES throughout the study area further testify to this. However in rivers, stressors often co-occur, but the response of the biotic communities cannot be attributed to individual stressors or specific combinations of them.

Consequently, SASS5 has proven to be an effective tool when assessing the ecological integrity of the aquatic macroinvertebrate communities in the study area. However, assessing the quality and quantity of habitat availability, has proven to be ineffective, despite the trend identified at site WIL04. Consequently, it may have an impact on the confidence in the interpretation of the SASS5 results. As habitat is one of the major factors affecting aquatic macroinvertebrate communities, coupled with water quality, it was important to illustrate the effects of the changes in the aquatic macroinvertebrate communities, by using statistical measures. Consequently, multivariate statistics have proven to be more effective in this matter and thus were conducted by assessing the relation between communities, including their FFG, *in situ* water quality, habitat availability and land use.

The Bray-Curtis cluster analysis of the aquatic macroinvertebrate communities clearly illustrated a high level of similarity and definite seasonal variation among the communities. The high similarity was an indication that similar taxa occurred at the sites within the groups identified. However, in accordance with the SIMPER analysis, there was no clear indication of dominant taxa. There was however a separation of sites TRI01 and WIL04. Stream bed composition was one of the most important physical factors controlling the structure of freshwater macroinvertebrate communities (Mackay and Eastburn, 1990). The separation and similarity of these two sites were not a consequence of dominant taxa, but rather a consequence of differing water quality, habitat availability and common tolerant taxa driving the system. Inclusive, the seasonal variation illustrated was contributing to the changes in the *in situ* water quality and habitat availability, thus making seasonal variation also a driving variable, in the differences between the sites.

The RDA bi-plots for both the wet and dry season data indicate, as with the Bray-Curtis similarity matrices and related NMDS plots, that there was a distinct separation of wet and dry conditions. It further illustrated a clear separation of site TRI01 and WIL04 due to reasons mentioned above. It was indicated that all the environmental variables, with the exception of pH, was identified as significant drivers in the river systems ($p<0.05$). This however varied seasonally. During the wet season, clarity, DO and pH were the significant drivers, while clarity, TDS/EC, percentage saturation and pH were the significant drivers during the dry season ($p<0.05$). These drivers were expected due to possible sources namely intensive agriculture in the project area. The RDA tri-plots further indicated the significant role that the ASPT, SASS5 score and the IHAS played within the aquatic macroinvertebrate community ($p<0.05$). This confirms the importance of habitat as a driving variable in aquatic macroinvertebrate community structures. Consequently, the driving variables in the separation of the sites along the Wilge River and two adjoining tributaries, appear to be a combination of variables (DO, percentage saturation, TDS/EC, clarity and pH), including habitat availability (based on IHAS scores).

To determine the effects and relations between the primary driving changes to the surrounding land uses in the project area, it clearly indicated that predators have a negative correlation with the rest of the FFG's which was expected. As the percentage of predators increased at a site, the percentage of the other FFG's decreased. Therefore, there was a large variation and clear changes in the food sources constantly entering into the river system. This is normally related to changes in the land use. However, as the land use is consistent in the study area (agriculture, industrial and mining) the changes in food availability for the aquatic macroinvertebrates may possibly be attributed to seasonal changes.

Without having chemical data (nutrients), it was difficult to confirm whether land use was a driving factor or not on the aquatic macroinvertebrate /FFG communities. However one aspect was for certain, as there was so much seasonal variation amongst the FFGs, this confirms the above statement that changes in food availability may possibly be attributed to seasonal changes in the food sources and not the land use in the study area.

Overall, it was clearly illustrated that the driving variables in the separation of the sites and the aquatic macroinvertebrate communities and their FFS's appear to be primarily seasonal variation, *in situ* water quality and habitat availability ($p<0.05$), as opposed to land use or dominant taxa, being the driving change. These driving variables therefore play a crucial role in the complexity of aquatic macroinvertebrate structures. Therefore, one of two of the null hypotheses has been disproven, namely that mining activities, agriculture and industrial activities have a negative impact on the PES of the Wilge River. However, this may be attributed to a lack of information from an organic and inorganic water quality perspective. This data would provide information regarding the type of nutrients entering the river system in the study area, which may be linked to land use.

Consequently, the following are recommended going forward:

- Biomonitoring and management efforts in the study area be continued, considering the temporal and spatial variation in the physical, chemical and biological characteristics of the streams and rivers;
- Organic and inorganic water quality should be incorporated in the biomonitoring program. This will provide a clearer view of the type of nutrients entering into the river system in the study area, thus aiding in a better understanding whether land use was a driving variable affecting the aquatic macroinvertebrate structures; and
- Diatom analysis should be considered as an additional aquatic index to be conducted in the study area. This index will improve the understanding of the potential impacts on the water quality within the study area. Diatoms are a major group of algae contained in periphyton (Barbour *et al.,* 1999). Periphyton consist of micro-organisms attached to substrate, their characteristics are affected by physical, chemical, and biological disturbances that occur within stream reaches during the time in which the

assemblage developed (Barbour *et al.,* 1999). Periphyton communities are primary producers and are an important foundation of many aquatic food chains (Barbour *et al.,* 1999). The Specific Preference Index (SPI) in the diatom index has been illustrated by De la Rey, *et al.,* (2004), to be more sensitive in evaluating chemical parameters, as well as changes in EC and Chemical Oxygen Demand (COD), resulting in the SPI giving a good reflection of general water quality. The advantages of diatom analysis include the following:

- The cell wall, also called a frustule, can persist in the environment long after the organisms have died. This supports accurate historical determinations of what conditions used to be like (De la Rey *et al.,* 2004);
- Up to 70% of changes in water quality can be reflected in diatom communities;
- Diatom communities change in response to average water quality changes and not just major changes;
- They occur in all types of aquatic ecosystems and are not washed away easily;
- Diatom indices can be measured in any water system even after flow has stopped;
- Methodologies can be compared worldwide due to the cosmopolitan nature of many diatom species; and
- Diatoms can be used in conjunction with other indices to determine and monitor water quality.

6. REFERENCES

BALANCE, A, HILL, L, ROUX, D, SILBERBAUER, M and STRYDOM, W (2001) *State of the Rivers Report: Crocodile, Sabie- Sand and Olifants River Systems.* Resource Quality Services, DWAF, Pretoria, South Africa (www.csir.co.za/rhp).

BATH, AJ (1989) *EC and pH measurements. Specifications on field instrument and sampling procedure.* Internal Report, Institute for Water Quality Research, Department of Water Affairs and Forestry, Pretoria, South Africa.

BARBOUR, MT, GERRITSEN, J and WHITE, JS (1996) *Development of the stream condition index (SCI) for Florida Department of Environmental Protection*, Tallahassee, Florida.

BARBOUR, MT, GERRITSEN, J, SNYDER, BD, and STRIBLING, JB (1999) *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition.* EPA 841-B-99-002.U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

BONADA, N, ZAMORA-MUÑOZ, C, RIERADEVALL, M and PRAT, N (2004) *Trichoptera (Insecta) collected in Mediterranean river basins of the Iberian Peninsula: taxonomic remarks and notes on ecology.* Graellsia 60: 41-69.

BRINKMAN, J (2007) *Influences of human disturbance and natural physical and chemical variables on biological community structure in streams an southern coastal Santa Barbara Country, California, and an index of biological integrity.* M.A. Thesis, University of California, Santa Barbara, California, USA.

CHEIMONOPOULOU, MT, BOBORI, DC, THEOCHAROPOULOS, I and LAZARIDOU, M (2011) *Assessing ecological water quality with macroinvertebrates and fish: a case study from a small Mediterranean river.* Environmental Management 47: 279-290.

CLARKE, KR, and GREEN, RH (1988) *Statistical Design and Analysis for 'biological effects' study.* Marine Ecology Progress Series 46: 213-226.

CLARKE, KR, and WARWICK, RM (1994) *Change in Marine Communities: An approach to Statistical analysis and interpretation*. Unpublished Manual for PRIMER statistical programme. Natural Environment Research Council, United Kingdom.

COOPER, SD, LAKE, PS, SABATER, S, MELACK, JM and SABO, JL (2012) The effects of land use changes on streams and rivers in Mediterranean climates. Hydrobiologia DOI 10.1007/s10750-012-1333-4.

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH (CSIR) (2010).*Can the Olifants River Still be Saved?* Press Release, 23 April 2010. CSIR.

CUMMINS, KW and WILZBACH, MA (1985) *Field procedures for the analysis of functional feeding groups in stream ecosystems.* Appalachian Environmental Laboratory, Contribution No. 1611, University of Maryland, Frostburg, Maryland.

CUMMINS, KW, MERRITT, RW and ANDRADE, PCN (2005) *The use of invertebrate functional feeding groups to characterize ecosystem attributes in selected streams and rivers in south Brazil.* Studies on Neotropical Fauna and Environment 40 (1):69-89.

DALLAS, HF (1997) *A preliminary evaluation of aspects of SASS (South African Scoring System) for the rapid bioassessment of water in rivers with particular reference to the incorporation of SASS in a national biomonitoring programme.* South African Journal of Aquatic Science 23: 79-94.

DALLAS, HF (2007) *River Health Programme: South African Scoring System (SASS) Data Interpretation Guidelines.* Report produced for the Department of Water Affairs and Forestry (Resource Quality Services) and the Institute of Natural Resources.

DALLAS, HF and DAY, JA (2007) *Natural variation in macroinvertebrate assemblages and the development of biological banding system for interpreting bioassessment data – a preliminary evaluation using data from upland sites in the south-western Cape, South Africa.* Hydrobiologia 575: 231-244.

DAVIES, B and DAY, J (1998) *Vanishing Waters.* University of Cape Town Press.

DE LA REY, PA, TAYLOR, JC, VAN RENSBURG, A and VOSLOO, A (2004) *Determining the possible application value of diatoms as indicators of general water quality: A comparison with SASS5.* Water SA 30(3): 325-332.

DE VILLIERS, S and MKWELO, ST (2009) *Has monitoring failed the Olifants River, Mpumalanga?* Water SA 35(5): 671-676.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (1996) *South African Water Quality Guidelines. Volume 7: Aquatic Ecosystems.*

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (2000) *Olifants River Ecological Water Requirements Assessment, Ecological Management Class:* Technical Input. Final Draft. Report No PB 000-00-5499, Department of Water Affairs and Forestry, Pretoria.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (2001) *State of the Rivers Report, Crocodile, Sabie/Sand and Olifants River Systems.*

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (2004) *National Water Resource Strategy "Our Blue Print for Survival".* First Edition.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (2005) *River Ecoclassification: Manual for Ecostatus Determination.* First Draft for Training Purposes. Department of Water Affairs and Forestry.

DEPARTMENT OF ENVIRONMENTAL AFFAIRS AND TOURISM (DEAT) (2008) *A National Framework for Sustainable Development in South Africa.*Department of Environmental Affairs and Tourism, Pretoria.

DICKENS, C. and GRAHAM, M (2002) *South African Scoring System (SASS) version 5: Rapid Assessment Method for Rivers,* Umgeni Water.

FRIBERG, N, SKRIVER, J, LARSEN, SE, PEDERSEN, ML and BUFFAGNI, A (2009) *Stream macroinvertebrate occurrence along gradients in organic pollution and eutrophication*. Freshwater Biology 55: 1405-1419.

GERBER, A and GABRIEL, MJM (2002) *Aquatic Invertebrates of South African Rivers Field Guide. Institute for Water Quality Studies*. Department of Water Affairs and Forestry. 150pp.

GILLER, PS and MALMQVIST, B (1998) *The biology of streams and rivers.* Biology of Habitats. Oxford University Press, New York.

GOPAL, B (2005) *Does inland aquatic biodiversity have a future in Asian developing countries.* Hydrobiologia 542: 69-75.

HYNES, HBN (1975) *The stream and its valley. Verhandlungen Internationale Vereinigungfür Theoretische und Angewandte.* Limnologie 19: 1-15.

JOHNSON, BL, RICHARDS, C, HOST GE and ARTHUR, JW (1997) *Landscape influences on water chemistry in Midwestern stream ecosystems.* Freshwater Biology 37: 193-208.

KARR, JR and CHU, EW (1999) *Restoring life in running waters: better biological monitoring.* Island Press, Washington, D.C.

KEMPSTER, PL, HATTINGH, WAJ and VAN VLIET, HR (1980) *Summarized water quality criteria.* Department of Water Affairs, forestry and environmental Conservation, Pretoria. Technical Report No TR 108. 45pp.

MAZORET, RD, GILLETT, DJ, SCHIFF, K, RITTER, K and STEIN, ED (2011) *Ecological conditions of watersheds in coastal Southern California: summary of the storm water monitoring coalition's stream monitoring program first year (2009*). Southern California coastal water research project, Costa Mesa, California, USA. Technical Report 639.

MCMILLAN, PH (1998) *An Invertebrate Habitat Assessment System (IHASv2), for the Rapid Biological Assessment of Rivers and Streams*. A CSIR research project, number ENV – P-I 98132 for the Water Resource Management Program, CSIR.Ii + 44p.

MACKAY, N and EASTBURN, D (1990) *The Murray. Imprint Limited, Brisbane, Australia. 363pp. From Gerber, A. and Gabriel, M.J.M. 2002. Aquatic Invertebrates of South African Rivers Field Guide.* Institute for Water Quality Studies. Department of Water Affairs and Forestry. 150pp.

MERRITT, RW and CUMMINS, KW (1996) *Trophic relations of macroinvertebrates.* In: Hauer FR, Lamberti GA, editors. Methods in stream ecology. Academic Press: San Diego, p 453-474.

MEYER, JL and EDWARDS, RT *(*1990) *"Ecosystem Metabolism and Turnover of Organic Carbon along a Blackwater River Continuum*." Ecology 60:1 pp: 255-1,269.

O'KEEFFE, J. and DICKENS, C (2000) *Aquatic Invertebrates. In King JM, Tharme RE and De Villiers MS. (editors) Environmental Flow Assessments for Rivers: Manual for the Building Block Methodology.* Water Research Commission Report No. 576/1/98. pp: 231-244.

ORMEROD, SJ, DOBSON, M, HILDREW, AG and TOWNSEND, CR (2010) Multiple stressors in freshwater ecosystems. Freshwater Biology 55: 1-4.

PAN, Y, HERLIHY, A, KAUFMANN, P, WIGINGTON, J, VAN SICKLE, J and MOSER, T (2004) *Linkages among land-use, water quality, physical habitat conditions and lotic diatom assemblages: A multi-spatial scale assessment*. Hydrobiologia 515: 59-73.

PAVLIN, M, BIRK, S and HERING, D (2011) *The role of land use, nutrients, and other stressors in shaping benthic invertebrate assemblages in Slovenian rivers*. Hydrobiologia 678: 137-153.

PLAFKIN, JL, BARBOUR, MT, PORTER, KD, GROSS, SK and HUGHES, RM (1989) *Rapid Assessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish*. EPA: Washington, D.C. Rosenberg, D.M., V. H. Resh (eds). 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall: New York, NY.

POFF, N, LEROY, JD, ALLAN, MB, BAIN, JR, KARR, KL, PRESTEGAARD, BD, RICHER, R, SPARKS, E and STROMBERG, JC (1997) *The natural flow regime: a paradigm for river conservation and restoration*. Bioscience 47: 769–784.

RASHLEIGH, B, HARDWICK, D and ROUX, D (2009). *Fish Assemblage Patterns as a Tool to Aid Conservation in the Olifants River Catchment (East), South Africa*. Water Research Commission, Pretoria, South Africa, 35(4): 517-524.

RAWER-JOST, C, BOHMER, J, BLANK, J and RAHMAN, H (2000) *Macroinvertebrate functional feeding group methods in ecological assessment.* Hydrobiologia 422-423 (0): 225-232.

ROTH, NE, ALLAN, JD and ERICKSON, DL (1996) *Landscape influences on stream biotic integrity assessed at multiple spatial scales*. Landscape Ecology 11: 141-156.

SHAW, PJA (2003) *Multivariate statistics for the environmental sciences*. London: Arnold Publishers.

SIRAMI, C, NESPOULOUS, A, CHEYLAN, JP, MARTY, P, HVENEGAARD, GT, GENIEZ, P, SCHATZ, B and MARTIN, JL (2010) L*ong term anthropogenic and ecological dynamics of a Mediterranean landscape: impacts on multiple taxa*. Landscape and Urban Planning 96: 214–223.

SONG, MY, LEPRIEUR, F, THOMAS, A, LEK-ANG, S, CHON, TS and LEK, S (2009) *Impact of agricultural land use on aquatic insect assemblages in the Garonne river catchment (SW France).* Aquatic Ecology 43: 999-1009.

TER BRAAK, CJ and SMILAUER, F (2004) *Canoco for Windows version 4.5.*© 1997–2004. Wageningen: Biometrics – Plant Research International.

THIRION, CA, MOCKE, A and WOEST, R (1995) *Biological monitoring of streams and rivers using SASS4. A Users Manual.* Internal Report No. N 000/00REQ/1195.

Institute for Water Quality Studies. Department of Water Affairs and Forestry, Pretoria, South Africa.

UNDERWOOD, EC, VIERS, JH, KLAUSMEYER, KR, COX, RL and SHAW, MR (2009) *Threats and biodiversity in the Mediterranean biome*. Diversity and Distributions 15: 188–197.

VAN DEN BRINK, PJ, VAN DEN BRINK, NW and TER BRAAK, CJF (2003) *Multivariate analysis of ecotoxicological data using ordination: demonstration of utility on the basis of various examples.* Australian Journal of Ecotoxicology. 9, 141–156.

VAN VUUREN, L (2009) *Experts unite to save abused river from extinction.* The Water Wheel Jan/Feb 14-17.

VAN ZYL, HC, MAREE, PJ, VAN NIEKERK, AM, VAN TONDER, GJ and NAIDOO, C (2001) *Collection, treatment and re-use of mine water in the Olifants River catchment.* The South African Institute of Mining and Metallurgy 101 (1):41-46.

VANNOTE, RL, MINSHALL, GW, CUMMINS, KW, SEDELL, JR and CUSHING, CE (1980) *The river continuum concept.* Canadian Journal of Fisheries and Aquatic Sciences 37: 130-137.

WAITE, IR, BROWN, LR, KENNEN, JG, MAY, JT, CUFFNEY, TF, ORLANDO, JL and JONES, KA (2010) *Comparison of water-shed disturbance predictive models for stream benthic macroinverbrates for three distinct ecoregions in western US.* Ecological Indicators 10: 1125-1136.

WATER RESEARCH COMMISION (WRC) (2001) *State of the Rivers Report, Crocodile, Sabie-Sand and Olifants River Systems.* Institute for Water Quality Studies, Department of Water Affairs and Forestry.

WEBER, NS, BOOKER, DJ, DUNBAR, MJ, IBBOTSON, AT, and WHEATER, HS (2004) *Modelling stream invertebrate drift using particle tracking. IAHR Congress Proceedings. Fifth International Symposium on Ecohydraulics*. Aquatic Habitats: Analysis and Restoration. September 12-17, 2004, Madrid, Spain.

APPENDIX A

Water Quality

	TWQR	TRI1M10	TRI1J10	TRI1S10	TRI1D10	TRI1M11	TRI1J11	TRI1S11	TRI1N11	TRI1A12	TRI1D12	TRI1F13	TRI1Ma13	WIL1M10	WIL1J10	WIL1S10	WIL1D10	WIL1M11	WIL1J11	WIL1S11	WIL1N11	WIL1A12	WIL1D12	WIL1F13	WIL1Ma13
pH	**6.5 - 9.0**	7.4	8.0	8.7	8.7	8.0	8.5	8.4	8.4	7.2	6.9	6.2	7.4	9.8	8.2	8.6	8.7	8.7	8.9	8.4	8.1	8.7	6.8	8.9	7.4
DO	**>5 (mg/ℓ)**	2.1	7.3	7.0	4.4	6.3	8.6	4.5	4.6	6.6	4.4	5.7	5.4	4.1	8.0	10.4	7.8	11.0	9.7	5.8	4.4	8.8	4.8	7.0	5.9
DO%	**80 -120 %**	-	89.2	90.8	71.3	72.1	84.9	85.6	88.9	130.3	91.2	63.5	85.1	-	92.9	133.5	118.6	137.7	89.5	96.6	78.7	155.2	92.1	85.8	103.1
Temp	**5 - 30 ˚C**	24.6	10.8	19.5	25.8	21.9	12.5	21.7	22.5	22.0	23.1	21.1	10.6	18.1	9.0	20.7	23.3	24.8	8.0	16.5	18.3	16.1	21.2	24.8	15.8
EC	**<154 (mS/m)**	13.0	17.0	14.0	15.0	17.0	17.0	19.0	14.0	9.0	10.0	12.0	13.0	53.0	47.0	41.0	71.0	65.0	55.0	53.0	51.0	82.0	53.0	53.0	130.0
TDS	**<1000 (mg/ℓ)**	84.5	110.5	91.0	97.5	110.5	110.5	123.5	91.0	58.5	65.0	78.0	84.5	344.5	305.5	266.5	461.5	422.5	357.5	344.5	331.5	533.0	344.5	344.5	845.0
Clarity	**>25 cm**	1.0	23.0	25.0	11.0	9.0	19.0	22.0	11.0	16.0	2.0	6.0	10.0	41.0	49.0	100.0	75.0	48.0	82.0	100.0	50.0	50.0	18.0	23.0	50.0
	TWQR	**WIL2M10**	**WIL2J10**	**WIL2S10**	**WIL2D10**	**WIL2M11**	**WIL2J11**	**WIL2S11**	**WIL2N11**	**WIL2A12**	**WIL2D12**	**WIL2F13**	**WIL2Ma13**	**KLI1M10**	**KLI1J10**	**KLI1S10**	**KLI1D10**	**KLI1M11**	**KLI1J11**	**KLI1S11**	**KLI1N11**	**KLI1A12**	**KLI1D12**	**KLI1F13**	**KLI1Ma13**
pH	**6.5 - 9.0**	9.2	8.3	7.8	8.1	8.4	8.6	7.8	8.4	8.6	7.1	7.3	8.1	8.3	8.2	8.4	8.8	8.5	8.7	6.9	8.2	8.0	7.4	7.9	8.1
DO	**>5 (mg/ℓ)**	3.1	8.0	7.5	5.6	7.1	10.9	5.9	4.4	9.0	4.8	5.3	4.7	2.6	8.5	8.3	6.7	8.9	10.1	5.5	5.5	7.9	4.9	5.6	3.6
DO%	**80 -120 %**	-	110.1	88.8	93.3	81.9	107.3	107.5	95.6	164.8	103.5	61.5	77.1	-	107.4	109.1	112.0	101.2	102.9	92.0	106.8	148.4	110.9	70.2	60.4
Temp	**5 - 30 ˚C**	26.0	16.1	18.5	24.1	21.4	12.4	19.1	25.4	17.8	26.7	22.0	12.5	23.1	12.3	21.7	27.4	22.1	12.7	16.5	23.0	19.1	29.4	26.8	13.4
EC	**<154 (mS/m)**	42.0	42.0	33.0	51.0	46.0	42.0	46.0	38.0	44.0	42.0	54.0	33.0	29.0	31.0	30.0	36.0	33.0	28.0	37.0	40.0	33.0	34.0	35.0	62.0
TDS	**<1000 (mg/ℓ)**	273.0	273.0	214.5	331.5	299.0	273.0	299.0	247.0	286.0	273.0	351.0	214.5	188.5	201.5	195.0	234.0	214.5	182.0	240.5	260.0	214.5	221.0	227.5	403.0
Clarity	**>25 cm**	34.0	82.0	60.0	62.0	49.0	89.0	61.0	30.0	49.0	14.0	24.0	49.0	30.0	82.0	61.0	38.0	37.5	100.0	52.0	53.0	49.0	25.0	35.0	62.0
	TWQR	**WIL3M10**	**WIL3J10**	**WIL3S10**	**WIL3D10**	**WIL3M11**	**WIL3J11**	**WIL3S11**	**WIL3N11**	**WIL3A12**	**WIL3D12**	**WIL3F13**	**WIL3Ma13**	**WIL4S10**	**WIL4D10**	**WIL4M11**	**WIL4J11**	**WIL4S11**	**WIL4N11**	**WIL4A12**	**WIL4D12**	**WIL4F13**	**WIL4Ma13**		
pH	**6.5 - 9.0**	9.7	8.1	8.3	8.3	8.1	8.6	8.3	8.2	5.9	7.4	6.5	8.3	8.4	8.5	8.3	8.6	8.2	8.3	7.2	6.8	7.9	8.4		
DO	**>5 (mg/ℓ)**	2.9	7.3	8.1	4.3	8.0	9.2	5.4	4.6	6.8	4.1	7.3	3.3	10.0	6.3	7.8	10.4	5.0	4.0	7.1	3.6	5.9	4.6		
DO%	**80 -120 %**	-	99.4	98.5	64.7	91.7	91.2	92.1	88.3	109.1	79.1	88.4	52.7	111.7	73.4	89.6	99.8	92.9	74.9	133.1	70.2	71.7	77.7		
Temp	**5 - 30 ˚C**	19.0	15.4	18.1	21.8	21.8	11.2	18.4	22.4	12.1	20.0	24.3	11.4	24.5	24.6	22.9	12.0	18.7	20.8	19.7	20.8	25.0	14.3		
EC	**<154 (mS/m)**	33.0	32.0	27.0	34.0	30.0	35.0	30.0	37.0	34.0	43.0	47.0	49.0	32.0	39.0	29.0	35.0	36.0	39.0	28.0	39.0	44.0	42.0		
TDS	**<1000 (mg/ℓ)**	214.5	208.0	175.5	221.0	195.0	227.5	195.0	240.5	208.0	279.5	305.5	318.5	208.0	253.5	188.5	227.5	234.0	253.5	182.0	253.5	286.0	273.0		
Clarity	**>25 cm**	43.0	65.0	60.0	80.0	26.0	92.0	100.0	32.0	1.0	22.0	24.0	30.0	79.0	52.0	16.0	92.0	100.0	26.0	50.0	10.0	24.0	22.0		
	TWQR	**WIL5M10**	**WIL5J10**	**WIL5S10**	**WIL5D10**	**WIL5M11**	**WIL5J11**	**WIL5S11**	**WIL5N11**	**WIL5A12**	**WIL5D12**	**WIL5F13**	**WIL5Ma13**												
pH	**6.5 - 9.0**	9.0	8.1	8.2	8.7	8.2	8.8	8.2	8.1	6.7	6.8	7.1	8.5												
DO	**>5 (mg/ℓ)**	0.8	7.4	8.0	6.6	8.5	9.1	5.6	4.6	8.2	4.6	9.2	4.3												
DO%	**80 -120 %**	-	100.7	90.0	101.8	93.5	87.3	93.2	86.3	137.5	90.7	121.7	68.5												
Temp	**5 - 30 ˚C**	20.5	15.7	16.0	23.5	20.2	11.3	15.6	21.0	14.8	22.5	25.1	11.6												
EC	**<154 (mS/m)**	21.0	31.0	33.0	36.0	35.0	34.0	36.0	37.0	27.0	33.0	41.0	42.0												
TDS	**<1000 (mg/ℓ)**	136.5	201.5	214.5	234.0	227.5	221.0	234.0	240.5	175.5	214.5	266.5	273.0												
Clarity	**>25 cm**	11.0	81.0	60.0	38.0	17.5	83.0	100.0	27.0	50.0	13.0	23.0	30.0												

APPENDIX B

Aquatic Macroinvertebrates

Taxon	QV	Mar-10							Jun-10							Sep-10						
		TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05
TURBELLARIA (Flatworms)	3	1				A			A	1	A	1	A			1		C		B		B
ANNELIDA																						
Oligochaeta (Earthworms)	1					A			A		B		A				A	A		B		A
Hirudinea (Leeches)	3	A	A														A					
CRUSTACEA																						
Amphipoda (Scuds)	13																					
Potamonautidae* (Crabs)	3	A	A	A	A	1		1	A	A	A	1			A	A		A		A	A	1
Atyidae (Freshwater Shrimps)	8					A		B					OBS							A		A
HYDRACARINA (Mites)	8															A						
PLECOPTERA (Stoneflies)																						
Perlidae	12																					
EPHEMEROPTERA (Mayflies)																						
Baetidae 1sp	4																					
Baetidae 2 sp	6	B						B								B			B	B	B	C
Baetidae > 2 sp	12		C	C	C	C			B	C	C	C	C		C		B	B				
Caenidae (Squaregills/Cainfles)	6	A	B		A	B		B	B	A	B	A	A		B	A		B	A	B	A	B
Heptageniidae (Flatheaded mayflies)	13	1	A	B	A	B			1	B	B	B	A		A	A	A	A	A	A	B	A
Leptophlebiidae (Prongills)	9	B	A	B	B	B		A	B	B	B	B	B		B	B	A	B	B	C	B	B
Tricorythidae (Stout Crawlers)	9			A		B			1		B	A	B		1			A		B	A	
ODONATA (Dragonflies & Damselflies)																						
Chlorocyphidae (Jewels)	10																					
Coenagrionidae (Sprites and blues)	4		A		B	B		A	A	A	1	B	B		A	A	B	A	A	A	A	A
Lestidae (Emerald Damselflies/Spreadwings)	8																					
Aeshnidae (Hawkers & Emperors)	8					1												A	A			
Corduliidae (Cruisers)	8																					
Gomphidae (Clubtails)	6				1				1								A	1		A		
Libellulidae (Darters/Skimmers)	4					1											A		1			
HEMIPTERA (Bugs)																						
Belostomatidae* (Giant water bugs)	3		OBS		A	A		1				A					A				A	
Corixidae* (Water boatmen)	3								A		A	A	1				A		B			
Gerridae* (Pond skaters/Water striders)	5		OBS			A			OBS	1												A
Hydrometridae* (Water measurers)	6																					
Naucoridae* (Creeping water bugs)	7												1									
Nepidae* (Water scorpions)	3		1			1		OBS											1			
Notonectidae* (Backswimmers)	3	1																		A		
Pleidae* (Pygmy backswimmers)	4											A							A			
Veliidae/M...veliidae* (Ripple bugs)	5	1	B	1		A		A	1	A		1										A
TRICHOPTERA (Caddisflies)																						
Ecnomidae	8		1													1						
Hydropsychidae 1 sp	4							A				B				A			A			B
Hydropsychidae 2 sp	6	B	B	B	A				B	B	B		B				B	B			B	
Hydropsychidae > 2 sp	12					C									B					B		
Cased caddis:																						
Hydroptilidae	6																		A			
Leptoceridae	6					A		1														A
COLEOPTERA (Beetles)																						
Dytiscidae/Noteridae* (Diving beetles)	5				B	1			A		A	A					A	1	B	A		A
Elmidae/Dryopidae* (Riffle beetles)	8			A	A	A			1	1	A		A		A					A	A	A
Gyrinidae* (Whirligig beetles)	5		A	1		1		OBS	A	A	A	A	A		1	A	A	B	A	B	B	B
Hydraenidae* (Minute moss beetles)	8											1					A		A			
Hydrophilidae* (Water scavenger beetles)	5	1								A	A	B	1						A			
Psephenidae (Water Pennies)	10				A							A										
DIPTERA (Flies)																						
Athericidae (Snipe flies)	10			1																		
Ceratopogonidae (Biting midges)	5							A			A					A	A	A	A	B	A	A
Chironomidae (Midges)	2		A			A				B	B	B	A			A	B	B	A	B	B	B
Culicidae* (Mosquitoes)	1																	1	1	1	A	
Dixidae* (Dixid midge)	10																					
Muscidae (House flies, Stable flies)	1																					
Simuliidae (Blackflies)	5	B	A	A	A	A			B	C	B	B	B		B	B		A	A	A	A	1
Syrphidae* (Rat tailed maggots)	1																					
Tabanidae (Horse flies)	5								A									A				
Tipulidae (Crane flies)	5								1		A	A			1			A				
GASTROPODA (Snails)																						
Ancylidae (Limpets)	6		A														A					
Lymnaeidae* (Pond snails)	3																					
Physidae* (Pouch snails)	3																					
Planorbinae* (Orb snails)	3											1										
PELECYPODA (Bivalvles)																						
Corbiculidae (Clams)	5		B							C	B		B		B		B				B	B
Sphaeriidae (Pill clams)	3									A	1		1									
Total SASS Score		**67**	**101**	**85**	**90**	**139**		**67**	**99**	**118**	**126**	**58**	**115**		**96**	**81**	**103**	**113**	**106**	**114**	**90**	**108**
Total No. Of Taxa		**12**	**18**	**11**	**13**	**23**		**13**	**20**	**17**	**22**	**22**	**19**		**13**	**14**	**19**	**20**	**20**	**20**	**16**	**20**
Total ASPT		**5.6**	**5.6**	**7.7**	**6.9**	**6.0**		**5.2**	**5.0**	**6.9**	**5.7**	**2.6**	**6.1**		**7.4**	**5.79**	**5.42**	**5.65**	**5.30**	**5.70**	**5.63**	**5.40**

		Dec-10							Mar-11							Jun-11						
Taxon	QV	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05
TURBELLARIA (Flatworms)	3		B	A	A	B		A									A	A		A		
ANNELIDA																						
Oligochaeta (Earthworms)	1	A	B	B	A	A				1	A				1		A			1		
Hirudinea (Leeches)	3																					
CRUSTACEA																						
Amphipoda (Scuds)	13																					
Potamonautidae* (Crabs)	3	A	A	A	A	A	A		A	B	A	A	A			1	A	A	1		A	
Atyidae (Freshwater Shrimps)	8					A	OBS	OBS							1	1				A	1	1
HYDRACARINA (Mites)	8				1																	
PLECOPTERA (Stoneflies)																						
Perlidae	12																					
EPHEMEROPTERA (Mayflies)																						
Baetidae 1sp	4	A			A				A	A		1	A	1	A			1			A	
Baetidae 2 sp	6	B	B	B	B	B		A	B	A	B	B				B	B	C	A	C	B	B
Baetidae > 2 sp	12						B				B	B					C		B			A
Caenidae (Squaregills/Cainfles)	6	B	B	B	B	B		B	B	A	B	A		1	A	A		B	B	B	A	B
Heptageniidae (Flatheaded mayflies)	13		A	A	A		A	A	A	A	A	A	B			A	A	B	A	A	B	A
Leptophlebiidae (Prongills)	9	A	A	C	C	B	A	C	A	A	B	B	B	1	A	B	B	A	B	B	C	C
Tricorythidae (Stout Crawlers)	9			C	C	1					A	B	A					A	A	A		
ODONATA (Dragonflies & Damselflies)																						
Chlorocyphidae (Jewels)	10																					
Coenagrionidae (Sprites and blues)	4	B	B	B	A	A		B	A	A	1						A			A		A
Lestidae (Emerald Damselflies/Spreadwings)	8																					
Aeshnidae (Hawkers & Emperors)	8							1	A		A	A							A			
Corduliidae (Cruisers)	8					A													A			
Gomphidae (Clubtails)	6		1			A						A					1					
Libellulidae (Darters/Skimmers)	4																		1			
HEMIPTERA (Bugs)																						
Belostomatidae* (Giant water bugs)	3		A	A	A	A	1	1		A	1			1								
Corixidae* (Water boatmen)	3	A	C	B	A	B		A											A			
Gerridae* (Pond skaters/Water striders)	5	OBS		A		OBS			1	1							1					
Hydrometridae* (Water measurers)	6																					
Naucoridae* (Creeping water bugs)	7																					
Nepidae* (Water scorpions)	3																					
Notonectidae* (Backswimmers)	3	A			A	A																
Pleidae* (Pygmy backswimmers)	4			A																		
Veliidae/M...veliidae* (Ripple bugs)	5	A	B	A		A	A	A	1	A	A					A						
TRICHOPTERA (Caddisflies)																						
Ecnomidae	8	A	A				A				A											
Hydropsychidae 1 sp	4	A	A						A	A	A	B	B				B		A		A	B
Hydropsychidae 2 sp	6				B			B	A	B	A					B	B		B	A		
Hydropsychidae > 2 sp	12			C														B				
Cased caddis:																						
Hydroptilidae	6																					
Leptoceridae	6							1						1								
COLEOPTERA (Beetles)																						
Dytiscidae/Noteridae* (Diving beetles)	5	1	A	1	A	A				A	A	A					A	1	1	A		
Elmidae/Dryopidae* (Riffle beetles)	8		A	B	A	A	A	A		A	B	B					1	A	A	A		A
Gyrinidae* (Whirligig beetles)	5	A	A	A		OBS	A	A	A	B	A	B				B	B	A	A	1	A	B
Hydraenidae* (Minute moss beetles)	8								A													
Hydrophilidae* (Water scavenger beetles)	5																					A
Psephenidae (Water Pennies)	10																					
DIPTERA (Flies)																						
Athericidae (Snipe flies)	10					A																
Ceratopogonidae (Biting midges)	5		B	A	A	B		A		A		A					B					
Chironomidae (Midges)	2	A	B	B	A	B	1	B	A	B	1	A			A	A	B	A	A	B	A	B
Culicidae* (Mosquitoes)	1																					
Dixidae* (Dixid midge)	10																					
Muscidae (House flies, Stable flies)	1										A											
Simuliidae (Blackflies)	5	A	A		A	B			A		A	A						A	A	A		B
Syrphidae* (Rat tailed maggots)	1																					
Tabanidae (Horse flies)	5			A		A	A	1	A									1				1
Tipulidae (Crane flies)	5			A	A	A	A			A	A	B					1	1	B			
GASTROPODA (Snails)																						
Ancylidae (Limpets)	6		A	B		A				1	1											
Lymnaeidae* (Pond snails)	3																					
Physidae* (Pouch snails)	3					B																
Planorbinae* (Orb snails)	3	1		A	A	A																
PELECYPODA (Bivalvles)																						
Corbiculidae (Clams)	5		C	B		A		1		B	A		A				C	A				B
Sphaeriidae (Pill clams)	3		B	B																		
Total SASS Score		**79**	**118**	**138**	**110**	**155**	**86**	**110**	**90**	**97**	**129**	**105**	**47**	**28**	**30**	**63**	**97**	**101**	**111**	**90**	**56**	**91**
Total No. Of Taxa		**17**	**23**	**26**	**21**	**30**	**13**	**19**	**15**	**18**	**22**	**16**	**7**	**5**	**6**	**10**	**17**	**16**	**17**	**15**	**9**	**14**
Total ASPT		**4.6**	**5.1**	**5.3**	**5.2**	**5.2**	**6.6**	**5.8**	**6.0**	**5.4**	**5.9**	**6.6**	**6.7**	**5.6**	**5.0**	**6.30**	**5.71**	**6.31**	**6.53**	**6.00**	**6.22**	**6.50**

Taxon	QV	Sep-11							Nov-11							Aug-12						
		TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05
TURBELLARIA (Flatworms)	3			A	A	A	1	A		1	A	B	B	A	A		A	A	C	B		B
ANNELIDA																						
Oligochaeta (Earthworms)	1		A	A			1		A	A		1	A				1	A		A	1	
Hirudinea (Leeches)	3	1											1									
CRUSTACEA																						
Amphipoda (Scuds)	13								A													
Potamonautidae* (Crabs)	3	A	1	1	1					A	1	A	1	A	A		1	1	A	A	1	
Atyidae (Freshwater Shrimps)	8					1	1	1					A	1	A					1	1	A
HYDRACARINA (Mites)	8		1	B						1		A					1					B
PLECOPTERA (Stoneflies)																						
Perlidae	12			1				1														
EPHEMEROPTERA (Mayflies)																						
Baetidae 1sp	4				1			1														
Baetidae 2 sp	6	B	B	A	C	A	C	B	B	B	B	B		B	A	B	B			B	C	
Baetidae > 2 sp	12		B	B		C		C					B					C	B			C
Caenidae (Squaregills/Cainfles)	6	A	B	A	B	B	C	B	B	A	A	B	A	A	A	A	C	B	1	B	C	C
Heptageniidae (Flatheaded mayflies)	13			A	1	A	C	A		A				B	A			B	A		B	B
Leptophlebiidae (Prongills)	9	1	A	C	B	B	C	B	1	B	B	A	B	C	B							
Tricorythidae (Stout Crawlers)	9			B	B	B		B			B	B			B				A			1
ODONATA (Dragonflies & Damselflies)																						
Chlorocyphidae (Jewels)	10																					
Coenagrionidae (Sprites and blues)	4	1	B	A	A	A			A	A	A			1	B		A		A	B		
Lestidae (Emerald Damselflies/Spreadwings)	8								1				1									
Aeshnidae (Hawkers & Emperors)	8				B				1			1							1			
Corduliidae (Cruisers)	8																					
Gomphidae (Clubtails)	6		A		A		A		1	1		A					A		A			
Libellulidae (Darters/Skimmers)	4															1			1			1
HEMIPTERA (Bugs)																						
Belostomatidae* (Giant water bugs)	3			1							1	A	1	1	A							
Corixidae* (Water boatmen)	3	1			1	1			B	C	1	A	B		B		C	B	B	B	1	B
Gerridae* (Pond skaters/Water striders)	5	1							A			1										
Hydrometridae* (Water measurers)	6			1																		
Naucoridae* (Creeping water bugs)	7																					
Nepidae* (Water scorpions)	3												1		1							
Notonectidae* (Backswimmers)	3			A					A	A		A	1			1						
Pleidae* (Pygmy backswimmers)	4											A			A							
Veliidae/M...veliidae* (Ripple bugs)	5	1	A		B					1	B	A	A	1	A							A
TRICHOPTERA (Caddisflies)																						
Ecnomidae	8																	A			1	
Hydropsychidae 1 sp	4	A	1		A		B	A	1	B		A						A				
Hydropsychidae 2 sp	6		B	B		B					B		B	B	B		B		B	B		C
Hydropsychidae > 2 sp	12																					
Cased caddis:																						
Hydroptilidae	6																					
Leptoceridae	6							1			1											
COLEOPTERA (Beetles)																						
Dytiscidae/Noteridae* (Diving beetles)	5	1	A	A	B	A			1	A	1	B	A		A	A	C	B	B	A	B	B
Elmidae/Dryopidae* (Riffle beetles)	8		1		A	C	A	B		A		B	B	B	B		A	A	A			B
Gyrinidae* (Whirligig beetles)	5	A	B	1	1	1		B	B			1		A	A				A	1	1	A
Hydraenidae* (Minute moss beetles)	8	1															1					
Hydrophilidae* (Water scavenger beetles)	5					A			1			1					B			A	1	A
Psephenidae (Water Pennies)	10																					
DIPTERA (Flies)																						
Athericidae (Snipe flies)	10					1																
Ceratopogonidae (Biting midges)	5	1	B	1	B	A	B	B	1	A	1	1			B		A	B	B			
Chironomidae (Midges)	2	A	B	B	B	B	B	B	A	B	A	B	A		A	A	B	C	C	B	B	B
Culicidae* (Mosquitoes)	1								1							B				C	C	C
Dixidae* (Dixid midge)	10					1										A						
Muscidae (House flies, Stable flies)	1	1																				
Simuliidae (Blackflies)	5	A		A	A	B		A		A	1	A	B	A	B		B	B	B			
Syrphidae* (Rat tailed maggots)	1																					B
Tabanidae (Horse flies)	5							1							A							
Tipulidae (Crane flies)	5		1		B		1		1	A	B	A	1	1				B	A			
GASTROPODA (Snails)																						
Ancylidae (Limpets)	6		B	A				1		A	A	A	1	1	A		1					
Lymnaeidae* (Pond snails)	3																					
Physidae* (Pouch snails)	3																					
Planorbinae* (Orb snails)	3										1											
PELECYPODA (Bivalvles)																						
Corbiculidae (Clams)	5		A			A	B	A		A	A				A		1	1				
Sphaeriidae (Pill clams)	3																					
Total SASS Score		**79**	**101**	**126**	**114**	**133**	**81**	**123**	**109**	**122**	**107**	**132**	**122**	**100**	**143**	**37**	**95**	**88**	**112**	**58**	**66**	**103**
Total No. Of Taxa		**17**	**18**	**21**	**20**	**20**	**14**	**18**	**21**	**22**	**21**	**26**	**22**	**17**	**25**	**8**	**19**	**16**	**19**	**14**	**13**	**18**
Total ASPT		**4.65**	**5.61**	**6.00**	**5.70**	**6.65**	**5.79**	**6.83**	**5.2**	**5.6**	**5.1**	**5.1**	**5.1**	**5.9**	**5.7**	**4.6**	**5.0**	**5.5**	**5.9**	**4.1**	**5.1**	**5.7**

		Dec-12							Feb-13							May-13						
Taxon	QV	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05	TRI01	WIL01	WIL02	KLI01	WIL03	WIL04	WIL05
TURBELLARIA (Flatworms)	3		A	A		1		1				A	A				B		A	B		A
ANNELIDA																						
Oligochaeta (Earthworms)	1	A	B	A	A	1				B	A	1	A	1			B	A	B	B		
Hirudinea (Leeches)	3																1					1
CRUSTACEA																						
Amphipoda (Scuds)	13																					
Potamonautidae* (Crabs)	3	1	A		A				A	B	B	A	1	1				1	A			
Atyidae (Freshwater Shrimps)	8					B							B	B	C					A	A	A
HYDRACARINA (Mites)	8					1				A												
PLECOPTERA (Stoneflies)																						
Perlidae	12																					
EPHEMEROPTERA (Mayflies)																						
Baetidae 1sp	4						1					A		B		B		A	1			
Baetidae 2 sp	6	B			C	C		C	B		C	B	B				C		B	C	B	C
Baetidae > 2 sp	12		B	C						C		B			B			C	B			B
Caenidae (Squaregills/Cainfles)	6	C	C	C	B	B	1	B	1	B	B	B	A			B	C	B	B	A	B	C
Heptageniidae (Flatheaded mayflies)	13			B	B	A		A	B	A	B	A	B		B	1	A		B		A	B
Leptophlebiidae (Prongills)	9	B	B	B	B	A		B	B	B	B	B	A	1	B	A	A	B	A	B		B
Tricorythidae (Stout Crawlers)	9										B	B	A					B	A	A		A
ODONATA (Dragonflies & Damselflies)																						
Chlorocyphidae (Jewels)	10																					A
Coenagrionidae (Sprites and blues)	4	B	C		B	B	B		B	B	B	B	A	B	B	A	B	A	B	B	1	B
Lestidae (Emerald Damselflies/Spreadwings)	8								1													
Aeshnidae (Hawkers & Emperors)	8											1	1					1				
Corduliidae (Cruisers)	8																					
Gomphidae (Clubtails)	6						A									1		B				1
Libellulidae (Darters/Skimmers)	4	1			A																	1
HEMIPTERA (Bugs)																						
Belostomatidae* (Giant water bugs)	3		A				B	1	A		B		B	A	B				1			A
Corixidae* (Water boatmen)	3	1	C	C		B			B	1		C	B	1		C	C	B	C	B	A	
Gerridae* (Pond skaters/Water striders)	5				B	C					A	A				1			A			
Hydrometridae* (Water measurers)	6	1			A				1							1						
Naucoridae* (Creeping water bugs)	7								1						1					1		
Nepidae* (Water scorpions)	3								1			1	1	A	1							1
Notonectidae* (Backswimmers)	3	B			A		1		B		B	B	A			B		A	1	A		
Pleidae* (Pygmy backswimmers)	4				1							1						A				
Veliidae/M...veliidae* (Ripple bugs)	5				A	A		A	B	B	1	A		B	B	A	B					
TRICHOPTERA (Caddisflies)																						
Ecnomidae	8																1					
Hydropsychidae 1 sp	4	A			B	B		B									1	1				1
Hydropsychidae 2 sp	6		B	B						B	B	B	B		B		A	A		A		B
Hydropsychidae > 2 sp	12																B		B	B		
Cased caddis:																						
Hydroptilidae	6																					
Leptoceridae	6								1						B							A
COLEOPTERA (Beetles)																						
Dytiscidae/Noteridae* (Diving beetles)	5	A	C	1	B	B	B	1	1		A	B	A	A	1	1	B	B	1	B	A	B
Elmidae/Dryopidae* (Riffle beetles)	8	1	B	B	B	A		A		1		A	B	B	B				A			A
Gyrinidae* (Whirligig beetles)	5					1			1	1		1	A	A	A	B	A	A		B		A
Hydraenidae* (Minute moss beetles)	8																1	A				
Hydrophilidae* (Water scavenger beetles)	5															1	A	1	A			
Psephenidae (Water Pennies)	10																					
DIPTERA (Flies)																						
Athericidae (Snipe flies)	10																					
Ceratopogonidae (Biting midges)	5				1	1			1	1	1	A				A	B	A	A	B	1	A
Chironomidae (Midges)	2	1	B	A	C	B	1		B	B	B	C	B		B	A	C	B	B	C	1	C
Culicidae* (Mosquitoes)	1			B			A					1								1		1
Dixidae* (Dixid midge)	10																					
Muscidae (House flies, Stable flies)	1																					
Simuliidae (Blackflies)	5	B	B	C	B	1		B	B	B	B	A					1	A		C		
Syrphidae* (Rat tailed maggots)	1																					
Tabanidae (Horse flies)	5											1									1	A
Tipulidae (Crane flies)	5			1	1			1				A			1	1		A		1		1
GASTROPODA (Snails)																						
Ancylidae (Limpets)	6		A		A												B	1				
Lymnaeidae* (Pond snails)	3					1																
Physidae* (Pouch snails)	3																					
Planorbinae* (Orb snails)	3																					
PELECYPODA (Bivalvles)																						
Corbiculidae (Clams)	5		B	B				A			B				1		A		B	A	1	B
Sphaeriidae (Pill clams)	3														1							
Total SASS Score		**69**	**81**	**84**	**109**	**108**	**34**	**77**	**113**	**95**	**95**	**138**	**108**	**61**	**109**	**91**	**119**	**120**	**121**	**104**	**62**	146
Total No. Of Taxa		**15**	**16**	**15**	**21**	**20**	**9**	**13**	**21**	**16**	**18**	**26**	**20**	**13**	**18**	**17**	**21**	**22**	**21**	**20**	**11**	25
Total ASPT		**4.6**	**5.1**	**5.6**	**5.2**	**5.4**	**3.8**	**5.9**	**5.4**	**5.9**	**5.3**	**5.3**	**5.4**	**4.7**	**6.1**	**5.35**	**5.67**	**5.45**	**5.76**	**5.20**	**5.64**	**5.84**