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An assessment of the aquatic macroinvertebrate diversity within the Nyl River Floodplain system, Limpopo, South Africa.

By

Nathan Jay Baker

DISSERTATION

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“*An understanding of the natural world and what’s in it is a source of not only a great curiosity but great fulfilment.*”

—David Attenborough

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SUMMARY

South Africa is a water scarce country with uneven distributions of rainfall both spatially and temporally. With the proposed pressures of climate change, it has been foreseen that within the coming decades, water availability may become a limiting factor for further development in South Africa. Regardless of the usable conversion rate of catchment runoff to riverine surface water flow, most of South Africa's surface water resources are provided by rivers. Due to over exploitation, anthropogenic influences and mismanagement, all river systems within South Africa are impacted to some extent.

Due to a lack of knowledge regarding much of the biota supported by freshwater systems, specifically in semi-arid countries such as South Africa, it is of utmost importance to study freshwater ecosystems to determine the full extent of the biodiversity supported by such systems and how this biodiversity may become affected through anthropogenic impacts and its use as ecological indicators. Macroinvertebrate taxa form much of the global freshwater biodiversity and due to their wide taxonomic diversity, they show adaptations to extremely variable environments, ubiquitous distributions and varying sensitivities to stressors. Therefore, macroinvertebrate taxa are readily used as bioindicator test organisms for many biomonitoring and management studies.

The Nyl and Mogalakwena Rivers are important water sources for the semi-arid Limpopo Province and despite this, they are continually being stressed through increased urbanisation, industrial and mining development as well as intensive agricultural activities. The Nyl River flows through and provides water for the towns of Modimolle (population of >68 513), Mookgophong (population of >35 640) and Mokopane (population of >30 151). The Nyl River provides water resources for the internationally acclaimed Nyl River Floodplain including the RAMSAR accredited Nylsvley Wetland, a recognised biodiversity hot spot. Notwithstanding its importance to aquatic fauna and flora, the Nylsvley Wetland provides suitable habitat and breeding grounds to over 400 avian species, the endangered Roan antelope *Hippotragus equinus* (É. Geoffroy Saint-Hilaire, 1803) and South Africa's only wild rice *Oryza longistaminata* A. Chev. & Roehrich. The wetland also aids in flow attenuation, water retention and water purification during periods of flooding and drought. After the floodplain, the name of the river changes to the Mogalakwena River which becomes an important source of water for many large- and small-scale agricultural and industrial activities established within the Mogalakwena municipality. The Mogalakwena River then flows downstream into the transboundary Limpopo River.

Many facets of the Nyl and Mogalakwena River ecosystem have been characterised, however, information regarding the state (i.e. structure and function) of the macroinvertebrate assemblages is outdated. The study aimed to comprehensively assess the macroinvertebrate community assemblages within the Nyl and Mogalakwena Rivers and their associated wetlands. Additionally, it aimed to identify possible anthropogenic impacts on these rivers by correlating physico-chemical water parameters and sediment characteristics to that of the macroinvertebrate community assemblage distributions.

Ten sites along the Nyl and Mogalakwena Rivers were chosen according to their relative positions to point sources of pollution of the system. Two sampling surveys were conducted, one during the low flow season (July 2016) and one during the high flow season (February 2017) of the system. Macroinvertebrate taxa were collected using 'kick, stir, sweep' methods, fixed using 10 % neutrally buffered formalin and stained using Rose Bengal. In addition to macroinvertebrate sampling, *in situ* water quality parameters were measured and a water and sediment sample were collected from each sampling site and analysed at the University of Johannesburg's laboratories. Laboratory work included the enumeration and identification of macroinvertebrate taxa to the lowest taxonomic level possible using available taxonomic guides. The physical characteristics (organic content and grain size distributions) of the collected sediments were evaluated according to standard United States Environmental Protection Agency (USEPA) methods and the results were used to supplement the water and macroinvertebrate analysis data. Water nutrients were analysed using standard test kits and light spectrophotometry, whereas, water metal contamination was determined using inductively coupled plasma optical emission spectrometry (ICP – OES). Univariate diversity indices, multivariate statistics and correlation analyses were conducted using ComEcoPaC version 1 (Drozd 2010) and Canoco version 5 (Šmilauer & Lepš 2014) and IBM SPSS Statistics 24 software (IBM Corporation, Armonk, NY, USA), respectively.

The water quality at the sites in the upper reaches of the Nyl River was ideal, thereafter deteriorating downstream of the Modimolle wastewater treatment facility (WWTF). Statistical analyses revealed spatial variations between impacted sites and the unimpacted sampling sites for both sampling seasons. These spatial variations were driven by increases to chemical oxygen demand concentrations (decreased dissolved oxygen) and elevated nutrient levels (nitrites, nitrates, ammonia, ammonium, orthophosphates and total nitrogen). The water at the sites adjacent and downstream to

the Modimolle WWTF was strongly correlated to and had higher concentrations of most of the tested nutrients, environmental parameters and metal concentrations, indicating that polluted effluent being discharged from the Modimolle WWTF affected the water quality at these sites. The water quality at the Nylsvley Wetland site (NYL) included a potential recovery, seemingly due to the assimilative ability of wetland environments. The sites within the Mogalakwena and Limpopo Rivers had good water quality, however, drought and flooding during the low flow and high flow sampling surveys, respectively, may have influenced results. Further research during normal flow is needed to validate the findings of this section of river.

The sediment analysis identified varying degrees of modification to the sediments within the Nyl and Mogalakwena Rivers. During low flow conditions, flow obstructions near the source of the Nyl River, influenced organic loads and grain size distributions of the sediment, which in turn, affected macroinvertebrate community assemblage composition, favouring a dominance of gathering collectors such as taxa of the Chironomidae. A site downstream of the Donkerpoort Dam (DPD), included the greatest distribution of varying sediment particle sizes, which may have accounted for the high diversity of macroinvertebrate taxa at this site. The sediments at a sampling site adjacent the Modimolle WWTF (STW) were highly modified, containing high levels of organic content and finer particulate matter. These sediments are compounding the effects of the poor water quality at this site and sites downstream of the Modimolle WWTF, subsequently reducing the overall biodiversity at these sites in favour of tolerant species such as *Tubifex tubifex* (O.F. Müller, 1774). The sediments of the Nylsvley wetland were typical of good wetland habitats, containing a high organic content and finer particulate matter. The Mogalakwena River has been modified by numerous farm dams, which affect water flow and natural sediment movements. Consequently, both benthic and pelagic macroinvertebrate community assemblages were affected.

Univariate diversity indices identified sites upstream of the Modimolle WWTF as having the highest biodiversity of all the sites sampled, subsequently decreasing considerably at sites adjacent and downstream of the Modimolle WWTF and thereafter recovering at the Nylsvley wetland. Multivariate statistical methods conducted using water quality and macroinvertebrate data included clear spatial and temporal variations in the community assemblages of macroinvertebrates within the Nyl and Mogalakwena Rivers. Temporal differences were attributed to seasonality, while spatial differences were attributed to effluent discharge at the Modimolle WWTF. The sites directly influenced by sewage

effluent discharge at the Modimolle WWTF grouped away from all the other sampling sites for both sampling seasons, with the significant drivers for this separation being total nitrogen, nitrites and manganese. There was a substantial decrease in macroinvertebrate diversity and evenness within the reach of the river adjacent to and downstream of the Modimolle WWTF. At these sites, taxa tolerant to low dissolved oxygen levels, increased nitrogenous pollutants and the excess sedimentation were found in high abundances. These taxa included: *Tubifex tubifex*, *Psychoda alternata* Say,1824, *Clogmia albipunctata* (Williston, 1893) and taxa of the Culicidae, Syrphidae and Muscidae. The results of the multivariate analyses were substantiated by means of a Spearman's rank correlation coefficient, which identified significant negative correlations between the diversity indices and many of the tested water parameters, demonstrating that if certain parameters (e.g. nitrites, total nitrogen, orthophosphates and manganese) were to increase in the system, a decrease in species richness, evenness and overall biodiversity would follow. The statistical analyses employed were successful in validating the use of macroinvertebrate community assemblages to discern water quality within the Nyl and Mogalakwena Rivers.

Overall, the Nyl River and its biotic inhabitants are minimally impacted in the upper reaches of the Nyl River, becoming severely impacted by effluent discharge and organic pollutants at the Modimolle WWTF. The Nylsvley wetland appears to be aiding in water purification as the macroinvertebrate community assemblages showed a recovery. Extraordinary flow conditions in the Mogalakwena River for both low flow (drought) and high flow (flooding) sampling seasons would have impacted on water quality, sediment quality and macroinvertebrate community assemblages at the time of sampling. Therefore, additional research is required to gain a clearer understanding of the aquatic integrity of the Mogalakwena River.

This study aided in identifying and describing the macroinvertebrate diversity present within the Nyl and Mogalakwena Rivers and their associated floodplain, thereby adding to the growing body of knowledge regarding South Africa's unique biodiversity. It validated the use of macroinvertebrates as biomonitoring organisms and identified point sources of pollution that are having a severe impact on valuable water resources carried in these rivers. The Nyl and Mogalakwena Rivers are vital water sources in semi-arid Limpopo Province and this study provides another facet to ongoing research on this important catchment.

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Abbreviation	Full Name (abbreviation stands for)
$\mu g.L^{-1}$	Microgram per Litre
µm	Micrometres
$\mu S.cm^{-1}$	Microsiemens per Centimetre
°C	Degrees Celsius
%	Per cent
Al	Aluminium
AMD	Acid Mine Drainage
ASTM	American Society for Testing and Materials
AVEG	Aquatic Vegetation
Ca	Calcium
$CaCO_3^{2-}$	Calcium Carbonate
Cl^-	Chlorides
cm	Centimetres
COD	Chemical Oxygen Demand
CPOM	Course Particulate Organic Matter
Cu	Copper
CWQG	Canadian Water Quality Guidelines
DCA	Detrended Correspondence Analysis
dH_2O	Distilled Water
DFA	Discriminant Function Analysis
DMa	Margalef's Index
DO	Dissolved Oxygen

Abbreviation	Full Name (abbreviation stands for)
DPD	Donkerpoort Dam
DWAF	Department of Water Affairs
EC	Electrical Conductivity
E14	Echo 14 South African Army Outpost
E15	Echo 15 South African Army Outpost
EtOH	Ethanol
F^-	Fluorides
Fe	Iron
FFG	Functional Feeding Group
FPOM	Fine Particulate Organic Matter
g	Grams
GLEN	Glen Alpine Dam
GPS	Global Positioning System
GSM	Gravel, Sand and Mud Biotope
H^+	Hydrogen Ions
H_2SO_4	Sulphuric Acid
H’	Shannon-Wiener Diversity Index
Ha	Hectare
HNO_3	Nitric Acid
ICP – OES	Inductively Coupled Plasma – Optical Emission Spectrometry
J’	Pielou’s Evenness Index
JASP	Jasper
km	Kilometres
KNO	Klein Nyl Oog

Abbreviation	Full Name (abbreviation stands for)
L	Litre
LOD	Limit of Detection
m^3	Cubic metre
m	Metres
Mg^{2+}	Magnesium
$mg.L^{-1}$	Milligram per Litre
ml	Millilitres
mm	Millimetres
Mn	Manganese
MVEG	Marginal Vegetation
NBF	Neutrally Buffered Formalin
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrites
NO_3^-	Nitrates
NYL	Nylsvley Nature Reserve
O_2	Oxygen
OH^-	Hydroxyl Ions
Pb	Lead
PIET	Piet's Farm
PO_4^{3-}	Phosphates
ppb	Parts Per Billion
ppm	Parts Per Million
RDA	Redundancy Analysis

Abbreviation	Full Name (abbreviation stands for)
S	Stones Biotope
SIC	Stones in Current
SRB	Sand Road Bridge
SOOC	Stones Out of Current
SO_4^{2-}	Sulphates
STW	Sewage Treatment Works
TDS	Total Dissolved Salts/Solids
TN	Total Nitrogen
TWQR	Target Water Quality Range
USEPA	United States Environmental Protection Agency
V	Vanadium
VEG	Vegetation Biotope
WWTF	Wastewater Treatment Facility
Zn	Zinc

CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

1.1 FRESHWATER IN A GLOBAL CONTEXT

All life on Earth is dependent on water in one form or another. Of the 0.8 per cent (%) land surface that freshwater ecosystems inundate, they contain a mere 0.1 % of the available water on Earth (Gleick 1996; Jackson et al. 2001). Despite containing a minuscule amount of the available water and associated habitats, freshwater ecosystems contain at least 40 % of fish diversity and about 25 % of the vertebrate diversity globally (Dudgeon et al. 2005). Regardless of species richness and the integral roles these ecosystems play, they are at high risk from anthropogenic influence and according to Sala et al. (2000), are among the most threatened ecosystems on the planet. The biodiversity supported by these freshwater ecosystems provides a plethora of goods and services for human use, including contributing to the world economy (aquaculture and fisheries); being an 'insurance policy' during unforeseen circumstances; storing vast amounts of genetic information; and maintaining various ecosystem services (Pearce 1998; Heal 2000; Covich et al. 2004; Costanza et al. 2014). The services provided by renewable freshwater resources consist of water for consumption, irrigation, industrial production and aquaculture, while channelled water resources provide various benefits involving flood mitigation, electricity production, transportation, as well as habitat availability for important fauna and flora species (Jackson et al. 2001).

1.2 WATER AVAILABILITY IN SOUTH AFRICA

Water is a scarce resource in South Africa which receives an average rainfall of just 500 millimetres (mm) countrywide (DWA 1986). Adding to this scarcity, rainfall is very seasonal and is unevenly distributed across many parts of the country, both spatially and temporally (DWA 1986). With the loom of a warming planet, South Africa's already diminished water resources may become even more depleted in the decades to come (Zucchini & Nenadi 2006). It is proposed that due to South Africa's hydroclimatic characteristics, climate change may ultimately reduce or increase river flow in some regions, and permanently alter the perenniality of many others (Dallas & Rivers-Moore 2014). With proposed changes to South Africa's water availability resulting from climate change, mismanagement and increased population growth, DWA (1986) and Day (2009) have hypothesized that water may become a limiting factor for further development in

South Africa, accounting for more competition between nature and human beings for this vital, dwindling resource. Coupled with the proposed alterations to water availability, Bates et al. (2008) and Dallas & Rivers-Moore (2014) have suggested that global warming may have particularly devastating impacts on freshwater ecosystems by reducing their abilities to fulfil their ecological roles.

According to DWA (1986) and Stuart-Hill et al. (2012), the majority of South Africa's utilised water resources are provided for by rivers and it is estimated that only about 10 % of South Africa's rainfall catchment runoff contributes to river and stream flow. This extremely low conversion rate of rainfall to runoff is attributed to soil inundation and infiltration (DWA 1986; Day 2009). Furthermore, even with such a low rainfall to runoff conversion rate, anthropogenic influence and human impacts (water abstraction, flow obstruction and pollutants) have impacted all South Africa's rivers to some extent (Day 2009). South African rivers have integral roles in freshwater availability as well as commercial and domestic use, however, through mismanagement and poor monitoring, the aquatic integrity of most of these rivers is declining. Therefore, the monitoring of these rivers is pivotal if we are to conserve and retain water resources for future generations.

1.3 RIVERS

Rivers are essential water sources that have important roles in water purification, transport and availability, while simultaneously supporting a large proportion of the world's biodiversity in the form of fauna, flora and microorganisms (Boon 1992; Day 2009). The fauna and flora supported by riverine systems are also of value as they are often used by human beings for food, agriculture and construction materials (Boon 1992; Day 2009).

Like many other aquatic ecosystems, rivers are diverse, multifaceted environments driven by both biotic and abiotic components (Dallas 2004). Because of this complexity, the community compositions of many biotic factors, specifically aquatic macroinvertebrates, represent a high degree of spatial variation across a river's course (Townsend et al. 1997; Dallas 2004). This spatial variation is particularly true for semi-arid environments (e.g. Limpopo Province) (Eekhout et al. 1997). A river's abiotic characteristics such as geomorphology, hydrology and physico-chemical constituents, in turn, determine the variety and abundances of aquatic organisms (Barbour et al. 1999). Considering these abiotic characteristics, the spatial and temporal variability amongst the biotic factors that exists in riverine systems are widely studied and are often used to determine river health

and ecological functionality (Barbour et al. 1999; Dallas 2000; Dickens & Graham 2002). Furthermore, Dallas (2004) describes rivers as being ecosystems that incorporate the characteristics and features from the catchment areas from which they drain. Resulting from this catchment level water accumulation pattern, riverine environments are particularly susceptible to common forms of pollution including: excessive salts, excess nutrients (eutrophication), existence of toxins, metal contaminants and organic waste (usually human faeces) (Day & Davies 1999; Day 2009).

1.4 WETLANDS

In the past, the intrinsic value and ecological importance of wetlands have been overlooked (Ramsar 2013). However, on the 2nd of February 1971, the *Convention on Wetlands of International Importance especially as Waterfowl Habitat* (commonly known as the '*Ramsar Convention*') was held in Ramsar, Iran and provided for the first time forthright and simple requirements for the preservation and sustainable use of these important water sources (Ramsar 2013). Of the 158 contracting countries, South Africa, amongst others, has been a global leader in fulfilling its obligations to sustain its wetlands and through the pioneering legislature (National Water Act 36 of 1998), continues to monitor and ensure that these wetlands remain important areas for conservation and water security (Bird 2010).

According to the National Water Act 36 of 1998 (RSA 1998), a wetland is defined as "*a transitional land type that exhibits characteristics of both aquatic and terrestrial systems. This transitional land is usually inundated with water because of the water table level occurring at or near the surface of the ground, or if it is periodically covered with shallow water. This land typically also supports vegetation types that are adapted to grow in soils and sediments that are submerged or inundated with water.*"

Wetlands are of importance due to the unique roles they have in water purification, flow attenuation, water storage, silt removal and sedimentation capabilities, as well as their ability to reduce the effects of flooding activity (Maltby 1991; Day 2009). Being lentic systems that allow for the deposition of sediments, solids and other pollutants, they are severely affected by hydrological alterations and increased sedimentation (Day 2009). Furthermore, due to this natural sink in which sedimentation and nutrient accumulation occurs, wetlands have some of the most fertile soils and are often utilised and exploited by man for various purposes, which are often irreversible, thereby reducing the ability of

these functional ecosystem hubs and biodiversity hotspots from fulfilling their environmental roles (Day 2009).

1.5 IMPACTS TO FRESHWATER ECOSYSTEMS

In a South African context, the combined effects of anthropogenic activities on aquatic ecosystems include: mining activities and the generation of power (20 % of the water use); cities and towns (< 20 % of the water use through domestic and municipal consumption, refuse disposal, reduced water infiltration and storm water channels); rural areas (< 1 % of the water use through domestic consumption and the lack of sanitation facilities); agricultural and aquaculture activities (61 % of the water use); and recreational activities (Day & Davies 1999; Day 2009). Moreover, Weitjers et al. (2009) identified a significant correlation between the alteration of catchment level land use and freshwater biodiversity loss; they noted that a 6 % loss of freshwater biodiversity would occur if a catchment's land use were modified by as little as 10 %. These catchment level land uses, and their associated impacts are exacerbated by nitrogen deposition, a warming planet and changes in rainfall and runoff patterns which together, are threatening freshwater aquatic ecosystems and the biota which they support. Dudgeon et al. (2005) groups these threats under the following interrelating categories: (i) overexploitation, (ii) water pollution, (iii) flow modification, (iv) destruction of habitat and (v) invasion by exotic species.

1.5.1 OVEREXPLOITATION

Although overexploitation mainly affects larger taxa such as fishes, amphibians, reptiles and birds, freshwater ecosystems can become severely affected if any trophic level of a food web were to be overexploited (Dudgeon et al. 2005). Coupled with the overexploitation of freshwater biodiversity, impacts reducing the amount of available water also cause severe problems to water security and ecosystem health. These impacts are primarily caused by water abstraction, interbasin water transfer, evapotranspiration and excess water use (Day & Davies 1999; Day 2009).

The Limpopo region is facing continued pressure for water security. Development within this already water scarce province has caused a surge in water use which has seen a rise of 4×10^6 cubic metres (m^3) in water demand over the past 70 years. As water demand and resource development within this catchment grows, increased strain is

placed on the rivers, their associated wetlands, the biota which they support and, their ability of these river systems to provide adequate water delivery (Havenga et al. 2007).

1.5.2 FLOW MODIFICATION

Dudgeon et al. (2005) summarize that although modifications to water flow vary in form and severity, they are ubiquitous in freshwater systems and are often most abundant in areas with very inconsistent flow regimes to account for flood control and water storage. Additionally, with threats to global precipitation cycles (increased frequency and severity of droughts and floods) resulting from climate change, Vösömarty et al. (2000) suggest that in the years to come, flow modifications in rivers may intensify.

In the rivers located in the more arid parts of South Africa such as the Limpopo Province, farm dams are a common feature and significantly reduce the availability of water resources (DWA 1986; Day 2009). Although these farm dams have adverse negative effects to the hydrology of the region, they provide water for human utilisation during drier months and have also permitted the expansion of distribution ranges of many lentic, wetland organisms and the water birds that feed on them (Froneman 1997). While modifications to river flow may have positive attributes, these are outweighed by their negative attributes which pose serious threats to the community assemblages of river biota.

1.5.3 WATER POLLUTION

Common forms of water pollution in rivers include salination; increased nutrient loads; eutrophication; toxification; and increases in organic waste seepage (Dallas & Day 2004). Rivers have the ability to dilute considerable amounts of these pollutants, a dilution capacity which is being reduced through modifications to flow and the overexploitation of water resources. In addition to this marked reduction in water flow and subsequent dilution capacities, microorganism abundances (algae, fungi and bacteria) within many systems have shown notable declines, despite their importance in purging systems of harmful pollutants as well as being important food sources for primary consuming organisms such some macroinvertebrates (Day & Davies 1999). Furthermore, impacts resulting from agricultural and industrial activities have also had notable effects on water quality, influencing sedimentation rates, turbidity and introducing pollutants such as "*agrichemicals*" (fertilizers, hormones, antibiotics and biocides) into aquatic systems (Day & Davies 1999; Day 2009).

1.5.4 HABITAT DESTRUCTION

Many aquatic organisms, such as amphibians and macroinvertebrates, require specific habitats in which to live, feed and reproduce (Ehrlich 1988). The habitats that these organisms depend upon are being altered rapidly. Habitat destruction is the main cause of global biodiversity losses (Ehrlich 1988). As the global human population continues to expand, progressively more of the available land masses are being converted for industrial, agricultural and accommodative purposes (Ehrlich 1988). The loss of freshwater biodiversity as a result of habitat transformation or destruction depends upon two key factors, including (i) the number of available habitats that are transformed over time and (ii) the distribution of the species within the specific habitats being transformed (Ehrlich 1988).

Dudgeon et al. (2005) describe that freshwater habitat destruction can be caused by direct or indirect impacts. The direct impacts involved include the canalization of rivers; wetland loss; riparian habitat destruction; or the excavation of river materials such as sand. Alternatively, the indirect impacts can be caused by water abstraction, interbasin water transfer, evapotranspiration, unnatural water quantities and alterations to catchment land use areas (Day & Davies 1999; Dudgeon et al. 2005).

1.5.5 INVASION OF EXOTIC SPECIES

Dudgeon et al. (2005) summarize that the invasion of exotic species compounds the effects of anthropogenically instigated physical and chemical impacts on freshwater ecosystems because of their success in invading and thriving in systems that have been degraded or modified. Through human influence, there have been many significant impacts caused by alien invasive species dramatically affecting ecosystems (e.g. Water Hyacinth *Eichhornia crassipes* (Mart) Solms in many South African rivers). Alien invasive species include faunal, floral, or microbial taxa that have the ability of drastically altering aquatic environments through outcompeting, predating upon and displacing indigenous taxa (Dudgeon et al. 2005). Although many exotic species do not become invasive, the aquarium and ornamental plant trade pose a serious threat to the continuation of invasive exotic species entering aquatic ecosystems (Padilla & Williams 2004; Hulme 2009; Martin & Coetzee 2011).

1.6 BIOLOGICAL ASSESSMENT

Traditionally, the integrity of aquatic ecosystems was monitored solely for human water requirements, however, this was a concept that was deemed short-sighted by Karr & Dudley (1981) and Cairns & Pratt (1993) who proposed that water quality and quantity should be conserved and monitored for all stakeholders – humans, plants and animals alike – for the long-term preservation of these water resources and the ecological services they provide (e.g. drinking water, food and sanitation). The importance of techniques that aid in the holistic assessment and monitoring of aquatic environments have therefore become popular in many developed and undeveloped countries, including South Africa.

Helgen (2002) describes bioassessment as the evaluation of wetland or riverine environments by using collected samples of living organisms for biomonitoring purposes. Where, biological monitoring or biomonitoring, is defined as the use of living organisms to determine whether environments are favourable for their living inhabitants with the intention of assessing the health and the degree to which these systems have been impacted by anthropogenic activities (Cairns & Pratt 1993; Hicks & Nedeau 2000). The living organisms that inhabit these environments and more specifically their community compositions are important for biomonitoring purposes as they can be used to determine the long- or short-term stressors on a system (Bird 2010). In riverine environments, a variety of indicator taxa have been used for bioassessment purposes, these include macrophytes, periphyton and fish. However, according to the literature (Hellawell 1986; Rosenberg & Resh 1993; Ollis et al. 2006; Bird 2010; Buss et al. 2015), the use of macroinvertebrate community assemblages is by far the most successful group of organisms used for the monitoring and assessment of riverine environments.

1.7 MACROINVERTEBRATES AS BIOLOGICAL INDICATORS

The clear majority of global freshwater biodiversity is comprised of macroinvertebrate taxa (Dudgeon et al. 2005; Vlok et al. 2006). These taxa are specifically adapted to life in extremely variable environments where environmental stressors are prevalent. If changes to freshwater environments were to occur, resulting from natural or anthropogenic impact, the macroinvertebrate diversity would be one of the first groups to exhibit changes in their community assemblage. For this reason, the richness and abundances of these organisms along the course of a river often reflect the impacts of flow regime, water quality, hydrology and habitat availability (Vlok et al. 2006).

Of the macroinvertebrate taxa that inhabit freshwater systems, benthic macroinvertebrates are by far the most successfully implemented community assemblages used for the bioassessment of riverine ecosystems (Bird 2010). This is because of their taxonomic diversity, varying degrees of pollution sensitivities, ubiquitous distributions and sedentary nature (Bird 2010). Despite the clear advantages of using benthic macroinvertebrates as monitors of aquatic health, the pollution sensitivities of many of these taxa have not been adequately assessed, specifically in developing countries including South Africa (Ollis et al. 2006).

According to Dickens & Graham (2002) and Vlok et al. (2006), macroinvertebrates play an integral role in both river and wetland health, roles that include: the decomposition of detritus, filtration of water, algal and fungal control (grazing), churning of sediment and providing an abundant food source for higher organisms such as frogs and fish. In addition to forming an important part of aquatic ecosystems, macroinvertebrates are favoured as indicator organisms because of several advantages (summarised by Cairns & Pratt (1993), Ollis et al. (2006) and Bird (2010)), including:

i. They can be collected and analysed using relatively inexpensive equipment;

ii. They are easily collected and identified (at least to family level);

iii. They show variability in their tolerances to different forms of pollution and other impacts;

iv. The general public can relate to them;

v. They exhibit life cycles that are long enough to show temporal changes to an environment and are short enough for recolonisation patterns to be studied if the impacts were to be mitigated, and lastly;

vi. The analysis of community assemblages allows for the determination of river characteristics, habitat quality, water quality as well as provides an indication of the overall health of the freshwater biodiversity of the river being studied.

1.8 PROBLEM STATEMENT

It is well known that global freshwater biodiversity, specifically invertebrate and microbe taxa, remains poorly documented and requires more attention and further research effort and that local endemism might be a large driving factor for species diversity and that freshwater biodiversity may be far greater than originally anticipated (Dudgeon 1999,

2000; Benstead et al. 2003; Strayer et al. 2004; Dudgeon et al. 2005). Therefore, it is imperative that freshwater ecosystems be extensively studied, specifically in semi-arid countries such as South Africa, for the full extent of the biodiversity supported by these systems to be documented and preserved while simultaneously understanding and securing our water resources for future generations.

Recent research that has been conducted on the Nyl River and its associated floodplain includes: wetland management strategies (Vlok et al. 2006); environmental impact assessments (African Environmental Development (AED) 2012); hydrologic and hydraulic modelling (Havenga et al. 2007; Birkhead et al. 2007; Kleynhans et al. 2007); sediment quality (Greenfield et al. 2007; Dahms et al. 2017); metal analyses (Greenfield et al. 2012; Dahms 2016; Musa et al. 2017) and diatom assemblage studies (Musa 2016). Many of these studies have indicated that anthropogenic activities along the course of the Nyl River have severely impacted it. Anthropogenic activities along the Nyl River include mining and agriculture (Greenfield et al. 2007; Greenfield et al. 2010); urban and industrial runoff (Vlok et al. 2006); mismanaged sewage treatment works (Dahms 2016; Musa 2016) and increased population growth. According to AED (2012), proposed platinum mines near the Nyl and Mogalakwena Rivers are also of concern and can harm the future health of these systems and their associated floodplain.

Although the literature has revealed that research is being conducted on this important water source, it is important to note that no comprehensive macroinvertebrate study has yet been carried out and, therefore, the composition of the macroinvertebrate community assemblages within the rivers in question, and more broadly, the Limpopo Province remains largely unknown. A study by Vlok et al. (2006), looked at the sensitivities of various macroinvertebrate taxa to infer water quality, however, the taxa sampled in this study were only identified to family taxonomic level using the South African Scoring System Version 5 (SASS 5) (Dickens & Graham 2002) and this information is now outdated as sampling was conducted in 2001 (15 years prior to the current sampling). Furthermore, no recent literature was found pertaining to the state of the water quality nor macroinvertebrate community assemblages within the Mogalakwena River, thus identifying the need for pioneering research to be conducted on this important river.

To gain a holistic view of the Nyl and Mogalakwena Rivers, and the biodiversity they support, the macroinvertebrate community assemblages, water quality, sediment quality and the aquatic integrity needs to be assessed and studied in more detail.

1.9 HYPOTHESES

Considering the problem statement of this study, the following hypotheses were established:

i. Anthropogenic activities are having negative impacts on the macroinvertebrate community assemblages that inhabit the Nyl and Mogalakwena River systems.

ii. Water quality is continually being affected by increases in nutrient loads as a result of the Modimolle wastewater treatment facility.

iii. Shifts in macroinvertebrate community assemblages reveal that the water quality of the Nyl and Mogalakwena Rivers is declining.

1.10 AIMS AND OBJECTIVES

The intentions behind this study were to provide a comprehensive and holistic evaluation of the aquatic macroinvertebrate community assemblages present within the Nyl and Mogalakwena River system and their associated floodplain to determine the aquatic integrity of these systems. In addition to this, seasonality, water nutrients, sediment characteristics and metal concentrations (water samples) were analysed to correlate any impacts on the macroinvertebrate community assemblages to that of the water quality. The aims of this project were as follows:

i. To conduct a comprehensive assessment of the macroinvertebrate community assemblages along 10 sampling sites along the course of the Nyl and Mogalakwena River system and their surrounding wetlands.

ii. To correlate water nutrients and physico-chemical parameters with that of the macroinvertebrate community assemblages.

iii. To relate sediment characteristics along the course of the Nyl and Mogalakwena River system with that of the collected macroinvertebrate community assemblages.

iv. To identify possible anthropogenic impacts that might be affecting the river and the biota that are dependent on it.

To achieve the aforementioned aims, the following objectives were set:

i. The identification and classification of the aquatic macroinvertebrate taxa present within the system and their respective abundances.

ii. An evaluation of the water quality from the Nyl and Mogalakwena River system by using the collected *in situ* water quality variables and by analysing collected water samples for nutrient loads. Additionally, collected water samples were analysed via ICP – OES to determine the extent of suspended and ionised metals in the water column.

iii. An assessment of collected sediment samples by analysing their physical characteristics including particle size and organic content.

iv. An investigation of the possible point sources of pollution or modifications to the river course that are having a negative effect on water and sediment quality, and therefore, the macroinvertebrate community assemblages that are present there.

1.11 ANTICIPATED OUTPUTS

This research will aid in expanding our knowledge of this important river system, as well as provide a more current data set regarding the state of South Africa's largest ephemeral floodplain and its associated Ramsar wetland, Nylsvley. This study will not only add to the Ramsar database, it will provide, for the first time, a comprehensive list of the aquatic macroinvertebrate taxa that occur within these river systems and perhaps elsewhere in the Limpopo Province.

The conservation and effective management of South Africa's water resources and aquatic biodiversity is essential in ensuring that future generations are afforded the same luxuries regarding water availability and nature's intrinsic value as the current generation enjoys. With this in mind, the results of this study will be communicated to the general public of the towns surrounding the Nyl and Mogalakwena Rivers, with the intention of educating people and informing them of the state of the rivers on which they are reliant.

1.12 CHAPTER OUTLINES

1.12.1 CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Chapter 1 provides a brief background of freshwater environments, water availability and the use of macroinvertebrates as biomonitoring tools. Furthermore, it provides the

justification for this research and identifies the hypotheses, aims, objectives and anticipated outputs of this study.

1.12.2 CHAPTER 2: STUDY AREA AND SITE DESCRIPTIONS

Chapter 2 provides background information regarding the region in which the study was conducted (Nyl and Mogalakwena Rivers in the Limpopo Province) as well as descriptions for each of the selected sampling sites

1.12.3 CHAPTER 3: MATERIALS AND METHODS

Chapter 3 describes, in detail, the various materials and methods that were used to conduct this study. The statistical analysis protocols, quality assurance measures and analysis descriptions have also been presented in the chapter.

1.12.4 CHAPTER 4: WATER QUALITY

In chapter 4, the various aspects of water quality (*in situ* environmental variables, nutrient loads and water metal concentrations) are comprehensively assessed and the results of which, are discussed in detail. These results will be discussed and used as supplementary data in Chapter 6 to help further explain the distributions of macroinvertebrate taxa at each site.

1.12.5 CHAPTER 5: SEDIMENT QUALITY

Chapter 5 addresses the sediment characteristics and profiles at each of the sampling sites with regards to organic content and particle size. These results will be discussed and used as supplementary data in Chapter 6 to help further explain the distributions of macroinvertebrate taxa at each site.

1.12.6 CHAPTER 6: AQUATIC MACROINVERTEBRATE COMMUNITY ASSEMBLAGES

In chapter 6 the results of the univariate and multivariate analyses with respect to macroinvertebrate community assemblages, as well as their spatial and temporal variation along the course of the Nyl and Mogalakwena Rivers are discussed according to the environmental variables (water quality and sediment quality) that may be influencing them.

1.12.7 CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

In chapter 7 the final concluding statements that have emerged from the study as well as any recommendations for future research to be conducted in the area are provided.

1.12.8 CHAPTER 8: REFERENCES

Chapter 8 comprises of a full reference list of the sources cited ‘in text’ throughout this dissertation.

CHAPTER 2 – STUDY AREA & SITE DESCRIPTIONS

2.1 STUDY BACKGROUND

The Nyl River begins on the outskirts of the Waterberg Catchment Area (Limpopo, South Africa) at the confluence of the Klein (small) and Groot (big) Nyl Rivers. The upper reach of the Nyl River has its origins to the west of the town of Modimolle (formerly Nylstroom), subsequently and flows in an easterly direction, through the town of Modimolle. After passing through Modimolle, the Nyl River flows northeasterly, entering the Nyl River floodplain near the town of Mookgophong (formerly Naboomspruit). At the point of inflow, the Nyl River is a well-defined channelled river, however, as it flows through the wetland, the channel becomes less pronounced and disappears completely (Tooth et al. 2002). Towards the Northern end of the floodplain, near the town of Mokopane (formerly Potgietersrus), the main channel of the river reforms and is renamed the Mogalakwena River. The Mogalakwena River continues to flow for approximately 250 km before joining the transboundary Limpopo River.

The Nyl River provides water for the towns of Modimolle (population of >68 513), Mookgopong (population of >35 640) and Mokopane (population of >30 151) (Statistics South Africa 2011). As a result of the river passing through these towns (agricultural and mining lands included) it is impacted by anthropogenic activities (Greenfield et al. 2010). These anthropogenic activities could cause adverse impacts not only to the river itself but also to the biota that are reliant on it. Impacts along the course of the Nyl and Mogalakwena Rivers could also be affecting the aquatic integrity of the Limpopo River, therefore, having cumulative effects on the quantity and quality of water available for neighbouring countries.

The Nyl River floodplain is approximately 16 000 hectares (Ha) in size, making it South Africa's largest ephemeral floodplain (McCarthy et al. 2011). The floodplain is divided up into many privately-owned farms as well as the Nylsvley Nature Reserve which encompasses the Nylsvley Wetland which is of international importance due to its Ramsar accreditation. According to Tarboton (1987), the Nylsvley wetlands provide suitable habitat and breeding grounds for over 400 avian species, including 23 IUCN Red Data species. The author also stated that of the avian species recorded in the floodplain, 102 were waterbird species, with these species incorporating nearly 92% of South Africa's waterbird diversity (Tarboton 1987). The floodplain is also home to over 70 mammalian

species, including the endangered Roan antelope *Hippotragus equinus* (É. Geoffroy Saint-Hilaire, 1803), 58 reptile species, various fish species and over 10 000 insect species (Tarboton 1987). The Nylsvley Nature Reserve is unique in that it is the only place in South Africa that supports the growth of the wild rice *Oryza longistaminata* A. Chev. & Roehrich (see Haskins & Kruger 1997). The floodplain is of further importance due to its water retention and purification properties as well as the aid it provides in the mitigation of the destructive nature of drought and flooding events. Due to the vital ecological roles and massive biodiversity that this floodplain supports, it also adds to the economic structure of the surrounding areas and towns through eco-tourism and water security (Tarboton 1987).

2.2 SITE SELECTION

The study region is in the Limpopo Province, South Africa. Figure 2.1 represents a map of the study area. The map shows the sites at which sampling was undertaken (black triangles) as well the positions of the towns that these rivers provide water for (black dots). Table 2.1 provides supplementary data, indicating the site names and abbreviations, as well as each site's latitudinal and longitudinal Global Positioning System (GPS) coordinates. The sites along the Nyl River were selected according to a previous study conducted by Vlok et al. (2006). These sites were selected due to their relevant position to possible impacts and point sources of pollution. The sites along the Mogalakwena River were selected according to the impacts imposed by agriculture, flow modifications and the various inflowing tributaries. Sampling surveys were undertaken during the South African winter (July 2016 – low rainfall period [low flow]) and the South African summer (February 2017 – high flow period [high flow]).

Table 2.1: Selected study site numbers, site names and site abbreviations as well as each site's associated Southern (latitude) and Eastern (longitude) GPS coordinates.

Site name	Site abbreviation	Latitude	Longitude
Klein Nyl Oog	KNO	-24.695903	28.253307
Donkerpoort Dam	DPD	-24.678008	28.335625
Sewage Treatment Works	STW	-24.706085	28.427777
Jasper	JASP	-24.709702	28.479503
Nylsvley Nature Reserve	NYL	-24.644482	28.696972
Sand Road Bridge	SRB	-23.608263	28.609363

Table 2.1: Continued.

Site name	Site abbreviation	Latitude	Longitude
Glen Alpine Dam	GLEN	-23.191023	28.697663
Piet's Farm	PIET	-22.590188	28.900041
Echo 15 Army Base	E15	-22.457523	28.923920
Echo 14 Army Base	E14	-22.385107	28.970865

2.3 SITE DESCRIPTIONS

2.3.1 KLEIN NYL OOG

Klein Nyl Oog (KNO) (Figures 2.2A, 2.3A) is located on a private farm approximately 2 kilometres (km) downstream of the Klein Nyl River's origin. Agricultural activities in the immediate vicinity of this site include cattle farming and the growing of Lucerne *Medicago sativa* Linn. Further impacts to this site include a man-made weir which obstructs the flow of the river, resulting in increased sedimentation of particulate matter and amplified organic loads upstream of this weir (Figure 2.6A). The aforementioned impacts resulting from this weir allowed for the area above it to be dominated by macrophytes such as *Phragmites* spp. and *Cyperus* spp. (Musa 2016), further restricting the rapid flow characteristic of headwater streams. The geological composition of the area is the major driver for the water quality characteristics of this site. All sampling was carried out below the weir. Biotopes sampled for macroinvertebrates at this site included: shallow fast and slow runs; medium to fast deep runs; shallow to deep rapids; shallow pools with gravel, sand and mud bottoms; submerged aquatic vegetation and marginal vegetation.

2.3.2 DONKERPOORT DAM

Donkerpoort Dam (DPD) (Figures 2.2B, 2.3B) has been identified as the first major modification of the Klein Nyl River and is located about 1 km downstream from KNO. At the outflow point of the impoundment, there is a low, partially degraded farm weir (~ 1.5 metres (m) high). The channel of the river is further modified by concrete pipes located beneath a dirt road bridge, reducing available habitat for fish and macroinvertebrates. The dirt road further adds to the impacts at this site as it provides a source of sedimentation during periods of high rainfall, as well as erosion caused by motor vehicles utilising the road (Figure 2.6B) (Musa 2016). The reach of river between KNO and DPD is impacted by livestock and agricultural farming.

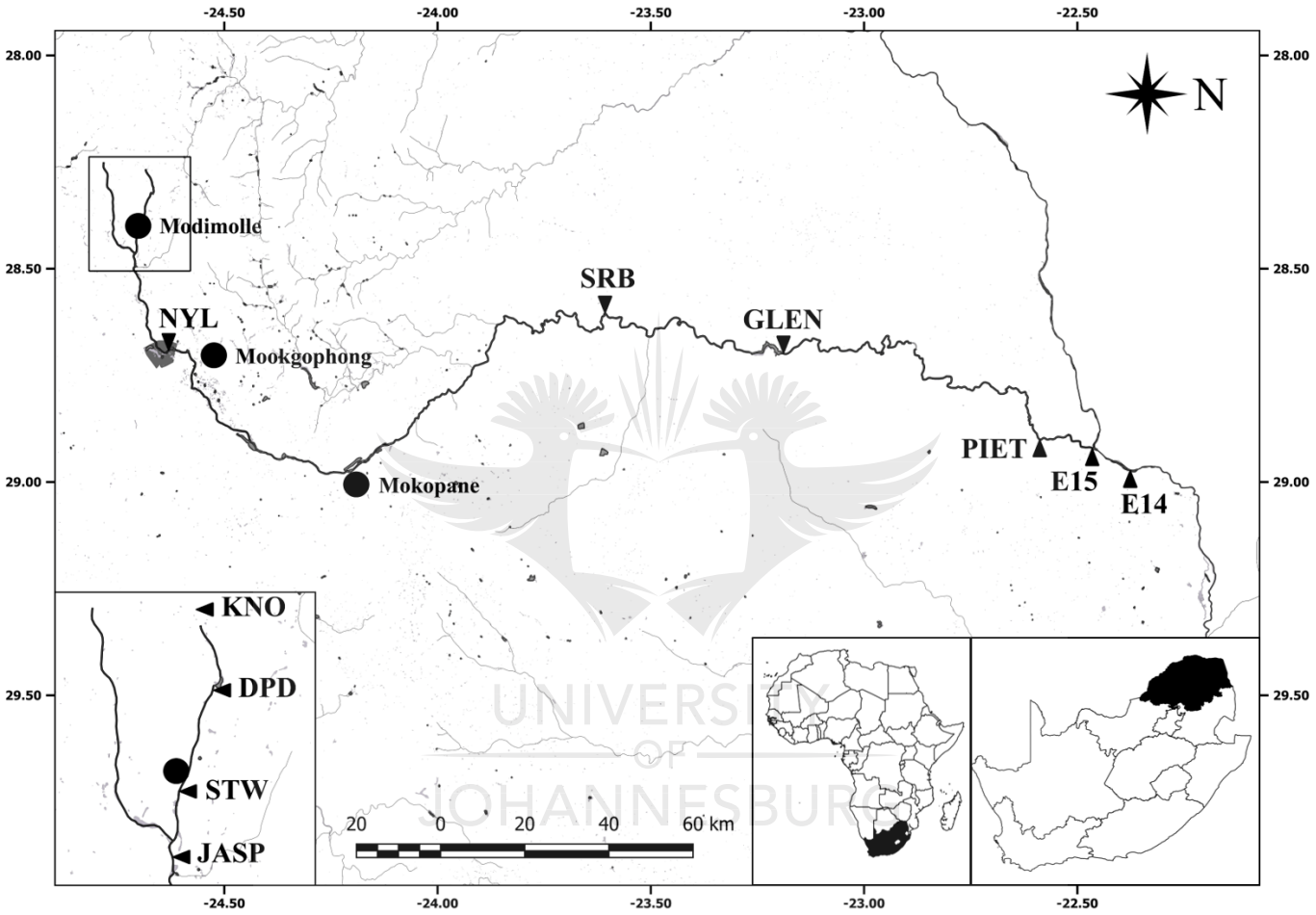

Figure 2.1: Map indicating the study sites along the Nyl and Mogalakwena Rivers (black triangles) as well as the major towns along the river's course (black dots). The study sites along the Nyl River include: Klein Nyl Oog (**KNO**), Donkerpoort Dam (**DPD**), Sewage Treatment Works (**STW**), Jasper (**JASP**) and Nylsvley Nature Reserve (**NYL**). The study sites along the Mogalakwena River include: Sand Road Bridge (**SRB**), Glen Alpine Dam (**GLEN**), Piet's Farm (**PIET**), Echo 15 Army Outpost (**E15**) and Echo 14 Army Outpost (**E14**).

The water at this site had an opaque brown colour, however, patches of clear water were present and the macrophyte dominance at the site was by *Carex austro-africana* (Kük.) and *Cyperus* spp. (Musa 2016; Dahms et al. 2017). The biotopes sampled for macroinvertebrates at this site included: slow deep pools with gravel, sand and mud bottoms; medium to fast shallow runs with gravel, sand and mud bottoms and marginal vegetation.

2.3.3 SEWAGE TREATMENT WORKS

Sewage Treatment Works (STW) (Figures 2.2C, 2.3C) is in the Klein Nyl River and is positioned directly adjacent to the Modimolle wastewater treatment facility (WWTF). During 2015 sampling, Musa (2016) identified that the Modimolle WWTF was completely inoperative and that raw, untreated effluent was being illegally pumped into the river by means of runoff pipelines. At the time of sampling, the site was densely covered in vegetation, with a tree canopy obstructing most sunlight to the water surface. The shallow water column (maximum depth of 30 centimetres (cm)) was completely opaque and grey in colouration, with the surface water exhibiting a layer of floating organic matter (Figure 2.6C). The sediment at this site was black in colour indicating a high amount of organic matter present within the water column. This site had a pungent and unpleasant odour. Aquatic macrophytes at this site were scarce, baring the presence of a few individuals of *Cyperus* spp. and a high abundance of filamentous algae. Raw sewage and discarded sanitary products were present in the river at the time of sampling indicating that untreated effluent was continually being pumped into the Klein Nyl River, despite the recent infrastructural upgrades to this facility in 2016 (DWS 2015). Biotopes sampled for macroinvertebrates at this site included: slow shallow pools with sand and mud bottoms and marginal vegetation.

2.3.4 JASPER

Jasper (JASP) (Figures 2.2D, 2.3D) is situated downstream of the confluence of the Groot and Klein Nyl Rivers and is the first site on the consolidated Nyl River. This site is 8 km downstream of STW and is impacted by STW as well as effluent runoff from the surrounding informal settlement, Phagameng (Vlok et al. 2006). A wetland exists between STW and JASP (Dahms et al. 2017) which may have a purifying effect on the heavily polluted waters coming from the Modimolle WWTF. This site is located under a dirt road bridge, with the channel of the river modified by concrete storm drainpipes. Cattle farms near this site may be putting further pressure on the system through agricultural runoff

and the trampling of the riverbanks. The presence of macrophytes appears to be seasonal, being dominated by filamentous algae in the low flow season (Figure 2.6D) and by sedges, grasses and small isolated islands of reeds in the high flow season. Although the water was considerably clearer than that of STW, sedimentation could be influenced by precipitation runoff or erosion caused by motor vehicles driving on the above road. Biotopes sampled for macroinvertebrates at this site included: medium shallow runs on the left and right banks with gravel, sand and mud bottoms; fast deep runs; marginal vegetation and submerged aquatic vegetation.

2.3.5 NYLSVLEY NATURE RESERVE

Nylsvley Nature Reserve (NYL) (Figures 2.2E, 2.3E) is a Ramsar accredited wetland that is located within the Nylsvley Nature Reserve. The nature reserve serves as breeding grounds and refuge for a variety of avian and mammalian species (Greenfield et al. 2007). Due to the unique characteristics of this nature reserve, it remains a tourism hotspot and supports the local economy through ecotourism. Sampling was conducted at Jacana hide during the high flow season only as water inundation of this wetland is highly seasonal with the site drying up during low flow seasons. The water at this site was clear, with much of its surface being covered with dense mats of emergent and submerged aquatic macrophytes. The substrate was dense and consisted mainly of organic material. The riparian and marginal vegetation at this site was made up of sedges, grasses and reeds. The only biotope sampled for macroinvertebrates at this site was emergent and submerged vegetation in shallow pools.

2.3.6 SAND ROAD BRIDGE

Sand Road Bridge (SRB) (Figures 2.4A, 2.5A) was the first site on the Mogalakwena River. This site was chosen for its relative position to the Sterk River, a major tributary of the Mogalakwena River. It was noted that the Sterk River had a substantial influence on water availability at this site, as the Mogalakwena River's surface water was dry between NYL and SRB. The site is situated near a dirt road bridge, which the river flowed beneath. The site appeared to be highly seasonal with water inundation only noted during the high flow season. Due to the steep gradient variations between the upper and lower Mogalakwena sites, the geomorphology of the river bed had notably changed from being mostly alluvial sediment in the upper Nyl and Mogalakwena River sites to dominantly bedrock in the lower Mogalakwena River sites until the confluence with the Limpopo River (Tooth et al. 2002; McCarthy et al. 2011). The habitats at this site and subsequent

downstream sites may favour differing community assemblages of macroinvertebrates that are better adapted to fast flowing, rocky environments. Flood conditions at the time of high flow sampling made sampling efforts difficult. Therefore, the samples collected at this site may not be an accurate representation of the water quality, sediment composition and macroinvertebrate community assemblages at the time of sampling. Aquatic macrophytes at this site were scarce except for a low density of marginal grasses and sedges. Biotopes sampled for macroinvertebrates at this site included: medium to fast deep runs and marginal vegetation.

2.3.7 GLEN ALPINE DAM

Glen Alpine Dam (GLEN) (Figures 2.4B, 2.5B) was located along the Mogalakwena River, downstream of the 18 900 m^3 irrigation dam. The dam has a massive water retention capacity and is used to provide downstream farms with irrigation water during low flow seasons (Mkhize 2016). Three weeks before low flow sampling, the dam was at 23 % water capacity and due to farmers requiring water, 19 % of the remaining 23 % was released downstream. This release of water three weeks prior to sampling inundated the available biotopes at the site with water, therefore allowing macroinvertebrate communities to establish and be supported, even during drought conditions. The dam wall at this site has a severe effect on the flow regime of the Mogalakwena River with much of the catchment's runoff retained by Glen Alpine Dam (Figure 2.7A). The water at this site was opaque and brown in colour, with possible causes of sedimentation being the low water volume of the dam during the low flow season (higher concentration of suspended particles) and flooding in the high flow season. Macrophytes at this site were scarce except for a few isolated islands of reeds and some grasses on the left and right banks. During high flow sampling, the Mogalakwena River was in flood and although sampling was conducted, the collected samples may not be representative of the water quality, sediment quality or macroinvertebrates. Biotopes sampled for macroinvertebrates at this site included: shallow to deep rapids with rocky bottoms; shallow runs with gravel, sand and mud bottoms and marginal vegetation.

2.3.8 PIET'S FARM

Piet's Farm (PIET) (Figure 2.4C) is situated on the Mogalakwena River adjacent a Sable antelope (*Hippotragus niger* (Harris, 1838)) breeding farm. The farm hosts a considerably long stretch of Mogalakwena River that is severely modified; there were four weirs present within a 1 km reach of the river. Other direct impacts to the site are agricultural runoff from

Lucerne (*M*. *sativa*) plantations. The reach of river between GLEN and PIET is heavily impacted by water abstraction (Figures 2.7B, C), legal and illegal weirs (Figure 2.7D), game farming, agriculture and recreational activities. Due to the high density of farm weirs on this stretch of river, water flow is heavily modified and is greatly reduced (Boroto & Görgens 1999). During low flow sampling, the water released from GLEN (19% of the dam's capacity) did not travel further than PIET, despite being less than 50 km downstream. High flow sampling was conducted during flooding conditions, therefore, the water, sediment and macroinvertebrate samples collected might not be representative of this site at the time of sampling. The geomorphology of the riverbed at this site is almost entirely comprised of bedrock with some shallower, slower flowing pools accumulating gravel, sand and mud (GSM). The water clarity at the site during high flow sampling was low, being attributed to a surge in suspended particles caused by flooding action. The macrophytes at this site were restricted to marginal vegetation (mainly sedges and grasses) and a few isolated islands of reeds, both in the centre of the river and on the banks. Biotopes sampled for macroinvertebrates at this site included: medium to fast deep runs with gravel and sand bottoms, slow shallow to deeps runs with sand and mud bottoms as well as marginal vegetation.

2.3.9 ECHO 15 ARMY OUTPOST

Echo 15 Army Outpost (E15) (Figures 2.4D, 2.5C) is situated about 50 m before the confluence of the Mogalakwena and Limpopo Rivers. It was selected for this study to determine the macroinvertebrate community assemblages present in the Mogalakwena River right before its convergence with the Limpopo River. Access to this stretch of Mogalakwena River was provided by the soldiers at the Echo 15 South African Army Outpost. Impacts between PIET and E15 include extensive agriculture of Lucerne *M*. *sativa* and other crops, livestock grazing pastures and game/hunting farms. Furthermore, at the time of sampling, there were at least three large, partially degraded weirs that could be considerably influencing the flow of the river (Figure 2.7E). This site was noted to be seasonal with regards to surface water flow as both the Limpopo and Mogalakwena Rivers were void of any surface flow during the low flow sampling survey. High flow sampling at this site was conducted during flooding conditions, which may influence the validity of the samples collected at this site regarding their representativeness. The geomorphology of this site mainly consisted of exposed bedrock with patches of sandy-bottomed pools. Macrophytes were limited to grasses and sedges on the banks as well as inundated islands within the river itself. Water clarity was low with flooding activity

being the most likely cause of increased sedimentation within the water column. Biotopes sampled for macroinvertebrates at this site included: medium to slow deep pools with gravel, sand and mud bottoms; fast deep runs with sandy bottoms and marginal vegetation.

2.3.10 ECHO 14 ARMY OUTPOST

Echo 14 Army Outpost (E14) (Figure 2.5D) was situated 10 km downstream of the Mogalakwena-Limpopo River confluence. This site was chosen to determine the state of the Limpopo River regarding the macroinvertebrate community assemblages after adequate mixing of the two rivers. Access to this site was granted by the soldiers stationed at the Echo 14 South African Army Outpost. Game/hunting farms located between E15 and E14 posed minimal impacts to the Limpopo River on the South African side (right bank). Only high flow sampling was conducted at this site as the Limpopo River had no surface flow at the time of the low flow sampling survey. The channel is characterized by having a main channel and various smaller side channels. This site was unique with a well-established riparian zone that contained a high density of large trees and smaller thicket. The aquatic macrophytes at this site consisted of grasses and sedges with these macrophytes being restricted to the margins of the banks. The geomorphology of the riverbed comprised of rapid flowing rocky sections (side channel) and fast flowing alluvial sediment sections (main channel). Although the water was clearer than that of the upstream Mogalakwena River sites, sedimentation was still occurring, and water clarity remained low with the water retaining a light brown colouration. Biotopes sampled for macroinvertebrates included: medium to fast rapids with rocky bottoms; slow to medium runs with rocky bottoms; medium to fast shallow runs with sand and gravel bottoms and marginal vegetation.

Figure 2.2: Photographs of the Nyl River sampling sites during low flow season (July 2016). **A**, Klein Nyl Oog; **B**, Donkerpoort Dam; **C**, Sewage Treatment Works; **D**, Jasper; **E**, Nylsvley Nature Reserve.

Figure 2.3: Photographs of the Nyl River sampling sites during high flow season (February 2017). **A,** Klein Nyl Oog; **B,** Donkerpoort Dam; **C,** Sewage Treatment Works; **D,** Jasper; **E,** Nylsvley Nature Reserve.

Figure 2.4: Photographs of the Mogalakwena River sampling sites during low flow season (July 2016). **A**, Sand Road Bridge; **B,** Glen Alpine Dam; **C**, Piet's Farm; **D,** Echo 15 Army Outpost.

Figure 2.5: Photographs of the Mogalakwena River sampling sites during high flow season (February 2017). **A,** Sand Road Bridge; **B,** Glen Alpine Dam; **C,** Echo 15 Army Outpost; **D,** Echo 14 Army Outpost.

Figure 2.6: Photographs of the impacts observed along the course of the Nyl River. Photographs were taken during the low flow (July 2016) and high flow sampling surveys (February 2017). **A,** Small weir at Klein Nyl Oog; **B,** Extensive dirt road runoff at Donkerpoort Dam; **C,** Organic waste and filamentous algae on the water surface at Sewage Treatment Works; **D,** Filamentous algae at Jasper.

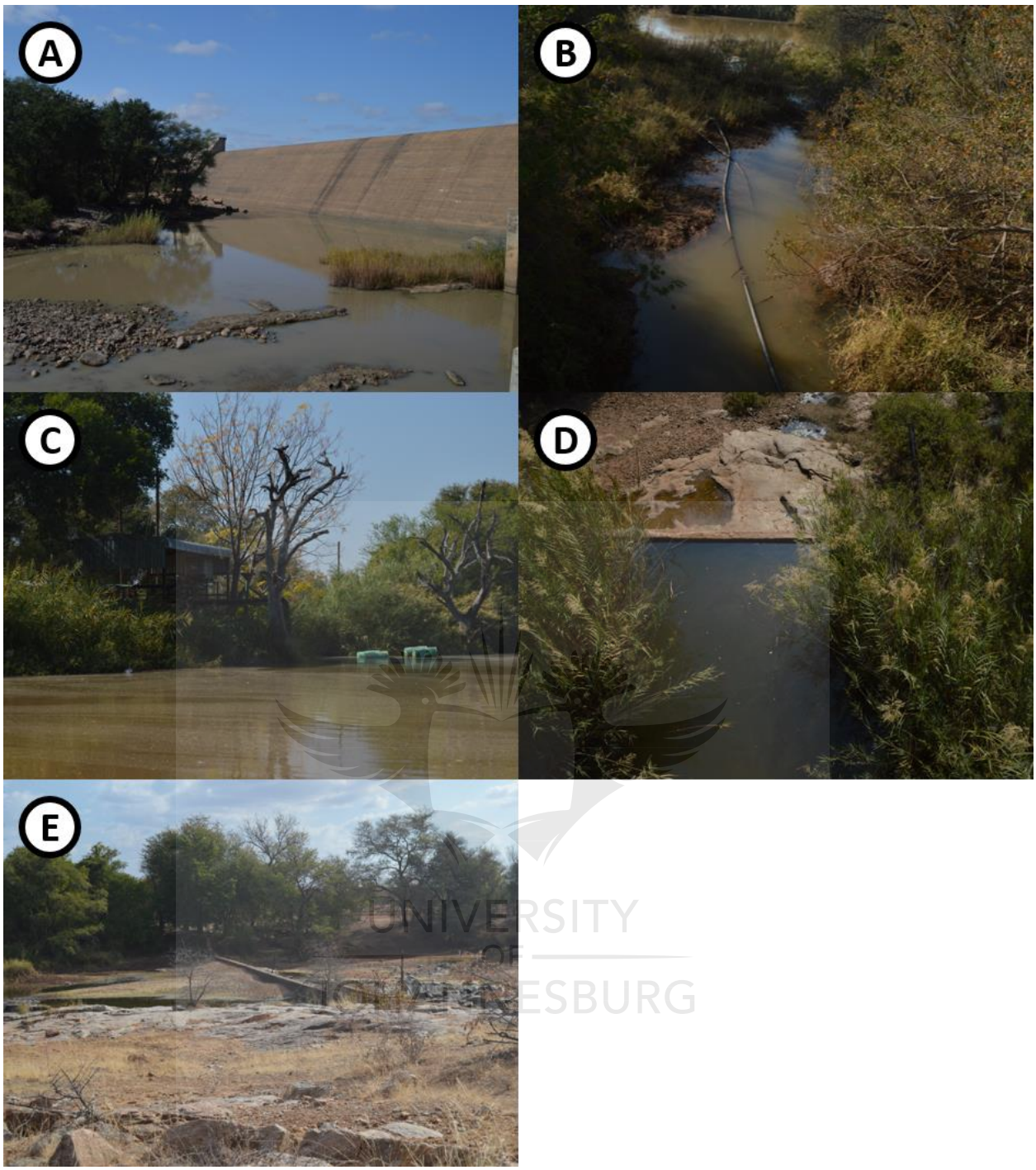

Figure 2.7: Photographs of the impacts observed along the course of the Mogalakwena River. Photographs were taken during the low flow sampling survey (July 2016). **A,** Large dam wall at GLEN; **B,** One of many water abstraction pipes between GLEN and PIET; **C,** A pump house present upstream of a large weir between GLEN and PIET; **D,** One of many small weirs constructed between GLEN and PIET; **E**, A partially degraded dam wall constructed between PIET and E15.

CHAPTER 3 – MATERIALS AND METHODS

3.1 FIELD SAMPLING

3.1.1 WATER

Sampling was conducted in low flow (July 2016) and high flow (February 2017) seasons (Chapter 2). The following *in situ* water quality measurements were taken at each site using a calibrated EUTECH® Multi-Parameter PCTestr^{TM} 35 multi meter: temperature (degrees Celsius - °C), pH, and conductivity (MicroSiemens per centimetre - $\mu S.cm^{-1}$). Following the measurement of the *in situ* water quality parameters, a single water sample was collected from each of the sites. Water samples were collected using 500 millilitre (ml) acid-washed polyethylene bottles. Whilst standing upstream, sample bottles were well rinsed, filled with water and capped below the surface to eliminate the influence of external factors. The bottles were labelled and immediately placed in a field freezer (- 4 °C) to minimise and reduce any microbial action. Samples were kept frozen until further laboratory analysis could be conducted.

3.1.2 SEDIMENT

Each site was assessed with regard to sediment availability and the sediment samples were only collected from flowing sections of the river. Sediment was collected using 1 Litre (L) acid washed polyethylene jars that had been rinsed well with river water. While wearing gloves, the jar was used to scrape and collect the upper 10 cm of the substrate. Following sediment collection, any excess water was drained from the jars before being capped. The jars were labelled, placed in Ziplock bags and stored in a field freezer (-4 °C) to mitigate the effects of any microbial action. The samples were kept frozen until further laboratory analyses.

3.1.3 MACROINVERTEBRATES

At each of the sampling sites, macroinvertebrate communities were collected using a standard 30 x 30 cm, 1 mm mesh size square dip net (Dickens & Graham 2002). Standardised ‘kick-stir-sweep’ methods were used to collect macroinvertebrate communities from all the available biotopes. The sampling efforts for the collection of macroinvertebrate communities as described by Dickens & Graham (2002) were kept constant throughout sampling to remove subjectivity. The biotopes sampled were stones

(S), vegetation (VEG) and gravel, sand and mud (GSM). Where available, the sampling of the stones biotope included stones both in current (SIC) and stones out of current (SOOC). Sampling of the stones biotope was conducted for a maximum of 2 minutes (min), thereafter, the collected samples from both SIC and SOOC were combined to form a single representative stones sample. If available, the consolidated sample from the vegetation biotope included samples collected from marginal vegetation (MVEG) and aquatic vegetation (AVEG). The sampling of vegetation was conducted over a total length of 2 m of marginal and aquatic vegetation. If available, the GSM sample comprised of samples collected from gravel, sand and/or mud in fast, slow or still flowing sections of the river. The sampling of the GSM section was conducted for a maximum of 1 min. Once the macroinvertebrate communities had been collected from their respective biotopes, the nets containing the samples were rinsed using the water from the site and the samples were placed into pre-washed 500 ml polyethylene jars. The net was then inspected to ensure that all the collected macroinvertebrate individuals had been removed and placed in their respective jars. The jars were filled with distilled water (dH_2O), stained using Rose Bengal, labelled and then capped. The net was then carefully rinsed and cleaned before any subsequent sampling commenced. At the next sampling site, the dH_2O and Rose Bengal solution within the jars from the previous site was drained and replaced with a mixture of 10 % Neutrally Buffered Formalin (NBF) and Rose Bengal which preserved and further stained the macroinvertebrate specimens. The samples were then stored until further laboratory analysis.

3.2 LABORATORY ANALYSIS

3.2.1 WATER

3.2.1.1 NUTRIENTS

Unfiltered, frozen water samples were removed from the freezer and allowed to thaw to room temperature (21 °C). The thawed water samples were then analysed by means of a calibrated Merck Spectroquant® Pharo 100 Spectrophotometer (Merck KGaA 2007) using relevant test kits designed to analyse specific nutrients and chemical variables. The nutrients and chemical variables analysed included: acid capacity (total alkalinity – $CaCO_3^{2-}$) [07158]; ammonia (NH_3) [14752]; ammonium (NH_4^+) [14752]; chemical oxygen demand (COD) [01796]; chlorides (Cl^-) [01807]; fluorides (F^-) [14598]; nitrates (NO_3^-) [14773]; nitrites (NO_2^-) [14776]; orthophosphates (PO_4^{3-}) [14848]; sulphates (SO_4^{2-})

[14791]; and total nitrogen (TN). The water samples were pre-treated using a Merck Crack Set 20 test kit [14963] to determinate the total nitrogen and subsequently reanalysed using the nitrate test kit [14773].

3.2.1.2 METAL ANALYSIS

The preparation of the water samples for metal analysis was followed according to the protocols of Dahms et al. (2016). Water samples collected in the field were removed from the freezer and defrosted to room temperature. Once thawed, the water samples were gravity filtered through 0.45 micrometre (µm) pore size Whatman® (Sigma-Aldrich, South Africa) no. 6 filter papers and acidified to a 1 % Nitric Acid (HNO_3) solution using 65 % Suprapur HNO_3. A Perkin Elmer® Spectro Arcos FSH 12 Inductively Coupled Plasma – Optical Emission Spectrometry (ICP – OES) instrument was used to analyse the water samples for the following elements: Aluminium (Al), Copper (Cu), Iron (Fe), Manganese (Mn), Lead (Pb), Vanadium (V) and Zinc (Zn). Final metal concentrations in microgram per Litre ($\mu g.L^{-1}$) were determined using the following equation:

$$Concentration\ (\mu g.L^{-1}) = ICP - OES\ reading\ (mg.L^{-1}) \times 1000$$

Before ICP – OES analysis, calibration was carried out using various matrix matched standards (in parts per million – ppm and parts per billion – ppb) of the following concentrations: 5 ppb, 50 ppb, 100 ppb, 500 ppb, 1 ppm, 5 ppm, 10 ppm, 20 ppm (Dahms et al. 2017). Once the ICP – OES instrument had been calibrated, accurate standard curves were created by removing outliers, correcting curves and recalculating regressions to reduce and avoid the interferences caused by other elements with similar wavelengths (Boss & Fredeen 2004). For quality control purposes, various standards of known concentrations were analysed concurrently in line with the water samples, these standards all produced accurate recoveries.

For elements that were below the limits of detection (LOD) of the ICP – OES instrument, half the detection limit for each element was used as the final concentration in statistical analyses (Dahms et al. n.d.). The LODs for each analysed element are represented in Table 3.1.

Table 3.1: The ICP – OES limits of detection (LOD) for water samples. Values expressed as ug.L^{-1}.

Metal	LOD	Half LOD
Al	17.50	8.75
Cu	2.00	1.00
Fe	4.00	2.00
Mn	2.33	1.17
Pb	22.00	11.00
V	3.00	1.50
Zn	3.00	1.50

3.2.2 SEDIMENT

3.2.2.1 ORGANIC CONTENT

Collected sediment samples were analysed according to the standard protocols developed by the United States Environmental Protection Agency (USEPA 2001) and the American Society for Testing and Materials (ASTM 2000). Sediment samples from each site were removed from the freezer and allowed to completely thaw. From each sediment sample, approximately 300 grams (g) of sediment was removed, put onto glass watch-glasses and placed into a Gallenkamp® oven at 60 °C for 96 hours (hrs) until completely dry. Once dried, 2 g dry weight of sediment was weighed out (accurate to 0.0001 g) and placed into porcelain crucibles. The crucibles were then positioned in a Labcon® type RM4 Muffle Furnace and incinerated at 600 °C for 6 hrs. After incineration, the sediment samples were reweighed to determine the organic content in each sample as a percentage. The organic content percentage was calculated using the following equation:

$$Organic\ content\ (\%) = \left(\frac{Weight\ before\ furnace\ (g) - Weight\ after\ furnace\ (g)}{Weight\ before\ furnace\ (g)}\right) \times 100$$

3.2.2.2 PARTICLE SIZE

Sediment particle size was determined using an Endecott® EFL 2000.1 mechanical sieve system. The sieves were stacked from the largest mesh size at the top to the smallest mesh size at the bottom of the shaker. The various sediment samples were separated by the sieves into the following grain sizes: >4000 µm, >2000 µm, >500 µm, >212 µm, >53 µm and <53 µm. For each sampling site, approximately 100 g of dried sediment was placed into the top sieve (4000 µm) and shaken for 10 min. Once shaken, the constituents

retained by each sieve were reweighed to determine the percentage composition of each grain size. The percentage compositions of each grain size were determined using the following equation:

$$Percentage\ grain\ size\ compostion\ (\%) = \left(\frac{Sieve\ constituents\ (g)}{Total\ sample\ weight\ (g)}\right) \times 100$$

3.2.3 MACROINVERTEBRATES

Macroinvertebrate samples were drained of the 10 % NBF and Rose Bengal solution and washed with running tap water through a 63 µm sieve to remove any formalin residue and fine sediment particles. The samples were then placed in storage trays containing distilled water until identification and enumeration. The macroinvertebrate samples were evaluated using a Zeiss® Stemi DV4 stereoscopic microscope and identified to the lowest taxonomical level possible (majority of the samples were identified to genus level) using the "*Guides to the Freshwater Invertebrates of Southern Africa*" (Day et al. 2001; Day et al. 2002; Day & de Moor 2002a; 2002b; de Moor et al. 2003a; 2003b; Stals & de Moor 2008) and the online databases: World Register of Marine Species (http://www.marinespecies.org), A catalogue of the insects of Southern Africa (http://www.ru.ac.za/media/rhodesuniversity/resources/martin/Insects.html), and BugGuide.Net (https://bugguide.net/node/view/15740). The identified macroinvertebrate taxa were enumerated, stored in 8 ml glass sample vials and fixed using 70 % Ethanol (EtOH).

3.3 STATISTICAL ANALYSIS

3.3.1 WATER AND MACROINVERTEBRATE DATA

To determine spatial and temporal variation that exists between macroinvertebrate community assemblages at the selected sampling sites, various statistical analyses were performed. The macroinvertebrate community assemblage data were square root transformed (√) to allow intermediate and rare taxa to contribute towards the variation between sites while at the same time mitigating the effects of dominant taxa (Clarke & Warwick 2001). Environmental variables such as water nutrients and *in situ* data were log transformed [y = log (x+1)] to account for the effects of dataset skewness (van den Brink et al. 2003). According to Clarke & Warwick (2001), statistical analyses conducted on species-level identifications often produce identical results to that of family-level

identifications, therefore, for consistency, all statistical analyses, excluding the ecological community indices, were conducted at the family-level of the identified macroinvertebrate assemblages. Clarke & Warwick (2001) have indicated that the analyses of higher taxonomical levels are better at detecting pollution gradients compared to that of species-level analysis as many of the statistical programs that are available, find it difficult to compute the large number of zero values that are common in ecological enumeration data.

Multivariate and ordination analyses were conducted using Canoco version 5 (Šmilauer & Lepš 2014). These analyses were used to group and differentiate sampling sites resulting from the environmental variables present at each site (van den Brink et al. 2003; ter Braak & Šmilauer 2012). An unconstrained Detrended Correspondence Analysis (DCA) was created using macroinvertebrate assemblage compositions from each sampling survey to determine site distribution. The macroinvertebrate data was found to be compositional and it contained a high number of zero values, therefore a unimodal DCA analysis was the preferred unconstrained analysis to determine the site distributions. A Principle Component Analysis (PCA) was also conducted to determine site distributions resulting from the environmental variables. Additionally, a constrained ordination in the form of a Redundancy Analysis (RDA) was created by analysing macroinvertebrate community assemblage composition and environmental variables from both sampling surveys to determine site ordination (van den Brink et al. 2003). Monte Carlo permutation testing was used to determine the significance of the ordination created by the RDA analyses (Shaw 2003). According to Šmilauer & Lepš (2014), a DCA and PCA are based on a linear response model that is used to relate sampling sites to that of a single variable (e.g. macroinvertebrate data). Based on similar principles to a PCA, an RDA relates sampling sites to an additional variable (e.g. environmental data). To test which environmental variables contributed the site ordinations the most, an interactive-forward-selection was conducted on all the environmental variables. The interactive-forward selection was used to test the significance ($p \leq 0.05$) of each environmental variable as to their influence on macroinvertebrate and sampling site placement. The environmental variables that were found to be significant were used in further analysis, whereas, those that were not significant were removed from further analysis. Both ordinations generate a two-dimensional graphical diagram that plot samples according to their similarities or dissimilarities to that of the other samples. The similarities and dissimilarities are measured according to the angle that each variable has to another

variable (variables plotting at > 90 ° are negatively correlated to each other and variables that are ≤ 90 ° are positively correlated to each other) (Šmilauer & Lepš 2014). Unconstrained and constrained ordinations were used for analyses as they are complimentary. An unconstrained ordination provides an indication of variability with the macroinvertebrate community assemblage composition alone, whereas, the constrained ordination allows biological variability that is resultant from the environmental variable data to be determined (Lepš & Šmilauer 2003).

As with the environmental variable data, the metal concentrations measured in the water samples from each sampling site were log transformed [y = log (x+1)] to account for the effects of dataset skewness (van den Brink et al. 2003). A constrained analysis in the form of an RDA was created using the macroinvertebrate assemblage data together with the analysed metal concentrations from the water samples to determine site ordination. This was done to determine the biological variability of macroinvertebrate samples that is resultant from the concentrations of metals within the water from each site.

As sampling effort was kept constant at each sampling site, the macroinvertebrate data (species richness and abundance) were found to be normally distributed. ComEcoPaC version 1 (Drozd 2010) was used for the various univariate community ecology indices that were conducted on the macroinvertebrate community assemblage data. The indices that were calculated included: Pielou's Evenness Index (J'), Margalef's Index (DMa) and the Shannon-Weiner Diversity Index (H'). According to Clarke & Warwick (2001), these indices are important as they identify and explain the relationships between the macroinvertebrate community assemblage diversity, richness, evenness and distribution across the sampling sites. Margalef's Index is used to describe species richness (number of taxa) at each sampling site. The DMa is a measurement that determines the total number of individuals (abundance) that make up the total number of species present at that site, by incorporating the total number of species and individuals at a site, producing an index value that is indicative of diversity (Margalef 1968). Pielou's Evenness Index is used to describe how evenly distributed individuals from specific taxa are within the macroinvertebrate community assemblage, thus identifying dominant taxa (many individuals from a single taxonomical group) (Pielou 1971; Pielou 1975; de Necker et al. 2016). Peilou's evenness index ranges from 0 to 1, with 0 suggesting that the assemblage is not evenly distributed and 1 suggesting that there is complete evenness within the assemblage. Lastly, the Shannon-Weiner Diversity Index incorporates both species richness and evenness to produce an index value out of 4.5 for the overall diversity at a

site (the higher the value, the more diverse the macroinvertebrate community) (Shannon-Weaver 1998; Clark & Warwick 2001).

Environmental variable data, water metal data and the calculated univariate macroinvertebrate indices (J', DMa and H') were further analysed by means of a Spearman's rank correlation coefficient using IBM's SPSS Statistics 24 software (IBM Corporation, Armonk, NY, USA). The Spearman's rank correlation coefficient was used to determine which univariate macroinvertebrate indices were significantly ($p < 0.05$; $p < 0.01$) positively or negatively correlated to the environmental variables and the metal data, respectively. A Spearman's rank correlation coefficient was used as opposed to a Pearson correlation coefficient because a Kolmogorov-Smirnov test revealed that the data were non-parametric.

3.4 SAMPLING LIMITATIONS

Due to the semi-arid climate within the Limpopo Province, a large moisture deficit exists, making the average rainfall in this region extremely varied (Scholes & Walker 1993). According to Frost (1987) and Higgins et al. (1996), this region also has a distinct 15- to 21-year rain cycle, with much of the rainfall occurring during the summer months. Furthermore, during the years of 2015 and 2016, the Limpopo Province was severely affected by drought, further reducing the water availability for the towns and industries that are dependent on these river systems. Resulting from this high variability in water availability, we were unable to sample all the selected sites during the low flow survey as many were dry of surface water flow and therefore couldn't support the establishment of aquatic macroinvertebrate community assemblages at the time of sampling. Inversely, during the high flow survey, many of the Mogalakwena sites, although inundated with surface water flow, were affected by flooding activities and the samples collected during this period may not be representative of the system at the exact time of sampling. Additionally, more sites were identified in the reach of the Nyl and Mogalakwena River between the sites of NYL and SRB, however, it was found that this reach of the river remained dry for both low flow and high flow sampling surveys, affecting aquatic macroinvertebrate sampling.

While high flow sampling was being conducted, the dissolved oxygen (DO) *in situ* probe malfunctioned and no further DO readings could be taken after KNO. For this reason, obtained DO readings from the low flow survey were omitted from any of the results in

favour of COD which could be analysed from all the collected water samples from both sampling surveys.

CHAPTER 4 – WATER QUALITY

4.1 INTRODUCTION

Day & Davies (1999) describe water quality as "*the overall effects on a user of the physical attributes of, and chemical constituents in, water*". The effects of climate change and direct anthropogenic influence in the forms of increased water temperatures, large precipitation deficits, and longer periods of low flow are said to be having severe impacts to water quality; impacts that may be intensifying many forms of freshwater pollution including: sediment runoff, excess nutrient loads, biocide seepage and thermal pollution (Bates et al. 2008).

In the late 1990's, South Africa proposed and implemented a piece of pioneering legislature that provided, for the first time, legal protection of South Africa's water resources. This legislature is known as the National Water Act 36 of 1998 (NWA 36 of 1998) and allowed aquatic ecosystems to be conserved for human requirements and to ensure that these water resources are preserved in an ecologically sustainable manner. According to the NWA 36 of 1998 (RSA 1998), water quality is defined as "*the quality of all the aspects of water resources including:*

i. The quantity, pattern, timing, water level and assurance of instream flow;
ii. The water quality, including the physical, chemical and biological characteristics of the water;
iii. The character and condition of the instream and riparian habitat; and
iv. The characteristics, condition and distribution of the aquatic biota".

The monitoring of water quality generally involves the analyses of both the physical and the chemical attributes of water. The physical attributes of water include: temperature, pH, oxygen content, turbidity and conductivity, whilst the chemical attributes include: water nutrients, dissolved salts and inorganic substances, and toxicants in the form of trace metals and other pollutants. Once the physical and chemical characteristics of the water have been analysed, they are compared to the South African Water Quality Guidelines for Aquatic Ecosystems and the severity of the impacts to the water are assessed.

According to Vlok et al. (2006), a water body's chemical properties give an indication of the extent to which it is contaminated. This insight into the level of contamination of a

water body is of vital importance as it can be used to assess the suitability of the system in supporting human and animal needs as well as providing an adequate medium for the aquatic biota. Consequently, having an initial understanding of a systems water quality is imperative in identifying the most probable sources of pollution within that system, pollutants that may be having an impact on aquatic biodiversity.

This chapter aims to identify the most probable anthropogenic impacts that are influencing the water quality of the Nyl and Mogalakwena Rivers, both in the physical and chemical aspects.

4.2 RESULTS AND DISCUSSION

Although freshwater ecosystems are dynamic, heterogeneous environments, the South African Target Water Quality Ranges (TWQR) were developed to provide an understanding of the background levels of specific toxicants within a system, allowing collected and analysed water samples to be compared with that of standardised values. The TWQR values for *in situ* and nutrient data are represented by Table 4.1 which provides the TWQR values for the domestic use of water as well as the levels that these variables should be within freshwater aquatic ecosystems.

Table 4.1: The Department of Water Affairs' (DWAF) Target Water Quality Ranges (TWQR) for domestic use and aquatic ecosystems (DWAF 1996a, 1996b). Values are presented as milligram per litre (mg.L^{-1}) unless stated otherwise. **N/A**, not available.

Environmental Variable	TWQR Domestic Use	TWQR Aquatic Ecosystems
Un-ionised Ammonia	0 – 1.0	≤ 0.007
Chemical Oxygen Demand	N/A	N/A
Chloride	0 – 100	N/A
Conductivity (µS.cm^{-1})	0 – 70	Deviation: < 15 %
Fluoride	0 – 1	≤ 0.75
Nitrites	0 – 6	Deviation: < 15 % (Table 4.2)
Nitrates	0 – 6	Deviation: < 15 % (Table 4.2)
pH (logarithmic scale)	6 – 9	Deviation: < 5 %
Phosphates	N/A	Deviation: < 15 % (Table 4.2)
Sulphates	0 – 200	N/A
Temperature (°C)	N/A	Deviation: < 10 %
Total Hardness ($CaCO_3$)	50 – 100	Deviation: < 15%

4.2.1 *IN SITU* WATER QUALITY

Extended data set for *in situ* water quality variables are provided in Appendix A.

4.2.1.1 TEMPERATURE

The temperature of a system plays an integral role in governing the rate at which chemical reactions occur, which in turn determines the metabolic rates of many aquatic organisms (DWAF 1996a; Schmidt-Nielson 1997). Resulting from this important governing role, DWAF (1996a) states that the distribution of aquatic biota may be driven by temperature. Changes to water and air temperatures are often caused by seasonality or daily cycles, with many aquatic organisms using these seasonal or daily cues for physiological and behavioural changes such as reproduction, migration, hatching, emergence and moulting (DWAF 1996a; Schmidt-Nielson 1997). Furthermore, all living organisms, specifically aquatic biota, have optimal temperature ranges in which the health and fitness of the organism is ideal (Schmidt-Nielson 1997). If temperatures were to go beneath or beyond these optimal temperature ranges, specific taxa, unable to cope with such drastic temperature variations, may be lost (Hawkins et al. 1997).

Temperature variations within natural systems can be caused by a variety of factors, including altitude, latitude, the rate of flow and hydrology, climate, vegetation cover and catchment characteristics to name but a few (DWAF 1996a). Moreover, Musa (2016) describes that with an intensification of anthropogenic activity, the temperature of many aquatic ecosystems may be affected, which may, in turn, negatively affect the community assemblages of many aquatic organisms. Anthropogenic activities that can alter the water temperature of aquatic systems include: the discharge of agricultural and industrial effluents, riparian clearing, interbasin water transfers, impoundment discharge, and return flow from irrigation (DWAF 1996a). Coupled with affecting the aquatic biota, changes in temperature can affect the chemical composition of a water body. As temperature increases, the solubility of important dissolved gases (e.g. Oxygen – O_2) within the water column decreases, thus reducing their availability to aquatic biota (DWAF 1996a; Schmidt-Nielson 1997).

The temperatures measured at each sampling site for both sampling surveys are represented in Figure 4.1. For the low flow sampling survey, both KNO and DPD had the lowest recorded temperatures of 11.4 °C, whilst STW showed the highest temperature of 18.5 °C. At GLEN, the temperature reading was 15.6 °C. The higher temperature

recorded at GLEN compared to that of the upstream sites could be attributed to the fact that the Glen Alpine Dam was at 3 % capacity at the time of low flow sampling. The miniscule amount of remaining water, coupled with the lentic nature of the dam, would cause the water to warm faster due to a high surface area to volume ratio.

The high flow sampling survey revealed that E14 had the highest recorded temperature of 27.1 °C, whereas KNO had the lowest temperature of 18.5 °C. The temperature recorded at STW (22.3 °C) was 2.3 °C higher than that of DPD (20 °C), indicating that thermal pollution is taking place between these two sites. Following STW, the river returned to 20.2 °C at JASP only to increase again to 23.8 °C at NYL. The sudden rise in water temperature at NYL could be attributed to the nature of wetland systems which reduce the flow of surface water, causing it to be in contact with direct sunlight for longer periods, thus increasing temperatures. In general, the temperatures recorded during the high flow sampling survey, baring STW, followed a typical river temperature gradient, with the upper reaches being colder in temperature compared to that of the more established, lower reaches.

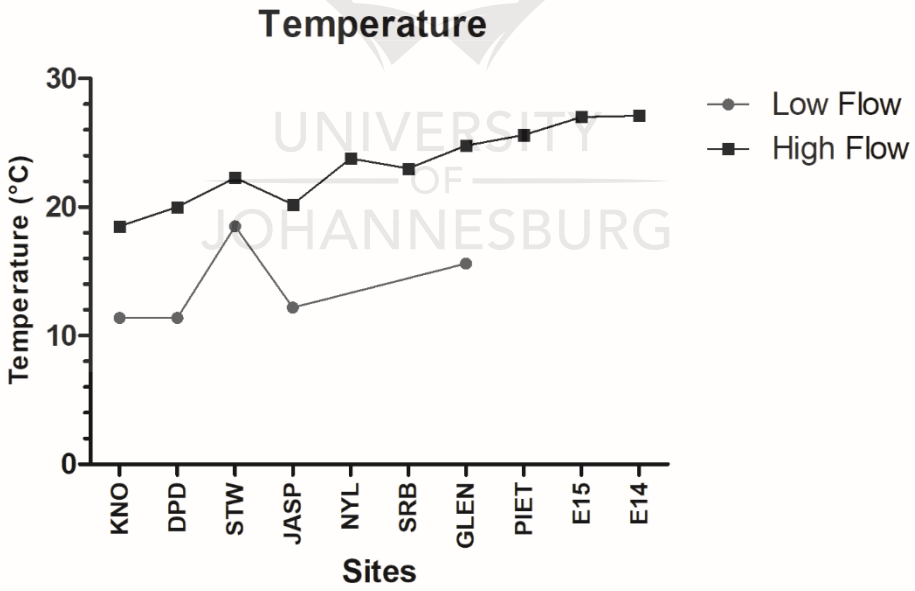

Figure 4.1: *In situ* temperatures recorded at each of the sampling sites for both low flow (July 2016) and high flow (February 2017) sampling surveys.

The high temperatures recorded at STW for both sampling surveys suggest that thermal pollution, arising from the processing of sewage effluent, is causing water temperatures at this site to rise. At the time of low and high flow sampling, the Modimolle WWTF was

not in operation and raw effluent was being pumped into the Nyl River via runoff pipes. This effluent could be influencing the temperatures of the Nyl River, subsequently affecting the viability of the water to sustain specific biota. Furthermore, DWAF (1996a) suggests that increased water temperatures may be attributed to effluent entering the system because of domestic, urban or industrial activities.

4.2.1.2 pH

pH is the measurement of the concentration of hydrogen ions that are present in a solution (DWAF 1996a). This measurement is expressed as:

$$pH = -Log_{10}[H^+]$$

This logarithmic scale ranges from 0 to 14 and is used to determine if a solution is acidic or alkaline. Solutions that contain equal concentrations of hydrogen ions (H^+) and Hydroxyl ions (OH^-) ions are considered electrochemically neutral and have a pH value of 7. If the concentration of either H^+ or OH^- ions were to increase, the pH of a solution would fall below 7 (acidic) and above 7 (alkaline), respectively (DWAF 1996a). Moreover, the rate at which pH is changed is dependent on the buffering capacity of the solution, which, in freshwater systems, is predominantly based on the carbonate-bicarbonate buffering capacity. The carbonate-bicarbonate buffering capacity generally maintains the pH of freshwater systems between 6.4 and 10.3 (DWAF 1996a). Although buffering capacity has a significant effect on the pH of freshwater systems, natural pH variations can also arise from catchment geology and geochemistry (DWAF 1996b). Other factors that can affect the pH of natural waters include: temperature variations, effluent discharge, urban and industrial runoff, microbial activity, natural decay of organic matter, acid mine drainage (AMD) and acid precipitation. Auxiliary effects resulting from variations in pH can cause the alleviation of toxic substances from the surrounding environment and sediments, including trace metals and non-metallic ions (DWAF 1996a). Both the primary and secondary effects emerging from pH variations in natural systems can have devastating impacts to the aquatic biota, as many are adapted to optimal pH levels and specific water conditions.

The pH values measured at each site for both the low flow and high flow sampling surveys are graphically represented in Figure 4.2. The pH values measured during the low flow sampling trip (July 2016) revealed that KNO, the source of the Nyl River, had a pH value of 7, showing that the water at this site was electrochemically neutral. The lowest pH

value of 5.77 was recorded at DPD, whilst STW and JASP both had a pH value of 9.38 which falls above the TWQR (Table 4.1). According to DWAF (1996a), the high pH values recorded at STW and JASP for the low flow sampling trip may be a result of eutrophication in the water. When nutrients are in excess, the resulting eutrophic conditions allow extensive algal blooms to occur. Through the light cycle of photosynthesis, these algal blooms deplete the Carbon Dioxide (CO_2) within the water, thus creating alkaline conditions by skewing the carbonate equilibrium towards carbonic acid species (DWAF 1996a). The high pH value of 10.73 recorded at GLEN for the low flow sampling trip may also be attributed to eutrophication induced increases in pH, as eutrophic conditions are common in lentic systems, especially during droughts.

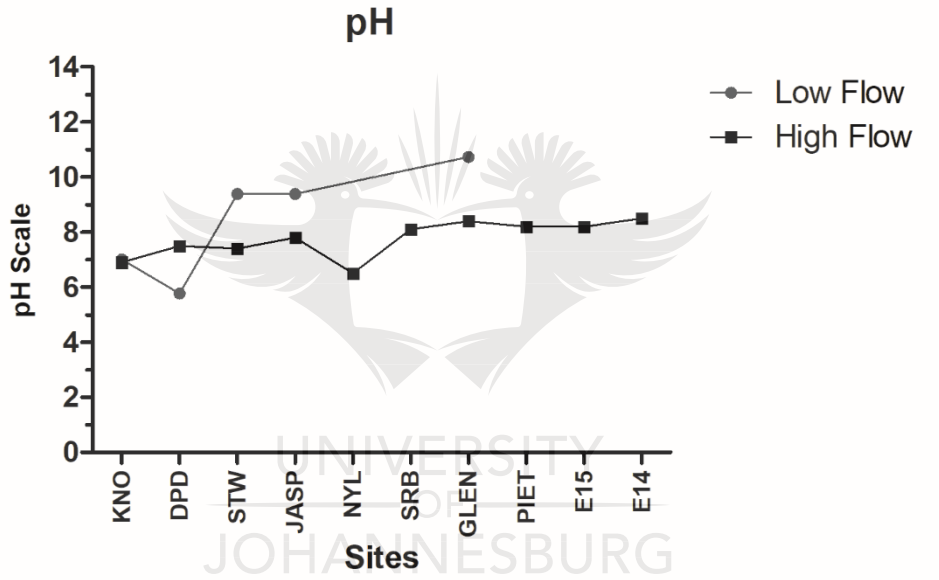

Figure 4.2: *In situ* pH values recorded at each of the sampling sites for both low flow (July 2016) and high flow (February 2017) sampling surveys.

For the high flow sampling survey (February 2017), the pH values recorded from all the sites in both the Nyl and Mogalakwena Rivers were below the TWQR. The pH value recorded at Nyl was the lowest recorded value of 6.5. This opposes the findings of Musa (2016), who reported that the pH values at NYL tend to be higher than that of the rest of the system. The values recorded from the remaining Nyl and Mogalakwena sites show a steady increase in pH from KNO (6.9) to E14 (8.5) which may be attributed to the geology of the region (Figure 4.2). Although the pH values recorded at STW and JASP for the high flow sampling were below the TWQR, the dilution capacity caused by increased

rainfall may have reduced the effects of the of the eutrophic conditions present at these sites.

4.2.1.3 ELECTRICAL CONDUCTIVITY

Water has the ability to conduct an electrical current due to dissolved ions such as carbonates and bicarbonates, calcium, magnesium, sodium, sulphates, nitrates and chlorides (DWAF 1996a; 1996b). The measurement of this conductive ability is called electrical conductivity (EC) and is measured in microsiemens per centimetre ($\mu S.cm^{-1}$). Electrical conductivity is often used to estimate the Total Dissolved Salts/Solids (TDS) of a water source which is defined as the concentration of dissolved ions in water that has an affinity towards the conduction of an electrical charge (DWAF 1996a).

Naturally, both chemical and physical factors can alter the TDS and, in turn, the EC of a system, these factors include: catchment geology, climate, as well the amount of decaying detritus (DWAF 1996a). Because of the lotic nature of rivers, TDS tends to accumulate with the downstream flow as rivers become more established. This accumulative nature is compounded by the effects of evaporation, precipitation and flow modifications (DWAF 1996b). Moreover, anthropogenic activities also pose a serious threat to the EC of freshwater systems and the biota in which they support through urban and industrial runoff, effluent discharge and the return flow agricultural irrigation (DWAF 1996a). Due to the high variability of TDS concentrations in freshwater systems resulting from surrounding geology and other factors, the determination of TWQR for freshwater systems has been difficult to estimate (DWAF 1996a).

Aquatic fauna and flora have adopted a variety of physiological adaptations to be able to survive and thrive in aquatic environments. One mechanism that these organisms have implemented is the ability to maintain homeostasis with that of their surrounding environment by regulating the amount of water and dissolved solids (e.g. ions) in their bodies with that of the surrounding water (DWAF 1996a). Therefore, if variations to EC were to occur, aquatic organisms, specifically juvenile stages, could be seriously affected at an individual or community level (DWAF 1996a). Furthermore, variations to the EC of a system may impact the ecological roles that these organisms provide which may cause it to stop functioning effectively (Vlok et al. 2006).

The results represented by Figure 4.3 show that for both the low and high flow sampling surveys, STW is severely increasing the TDS of the system with the EC readings being

900 µS.cm^{-1} and 597 µS.cm^{-1} respectively. This sudden and excessive increase in TDS at STW can be attributed to the pumping of raw, untreated organic effluent into the Nyl River at the Modimolle WWTF. Although the effluent enters at STW, JASP is also affected by the increase in TDS concentrations showing EC readings of 700 µS.cm^{-1} from the low flow survey and 686 µS.cm^{-1} from the high flow survey. These high EC readings from STW and JASP for both sampling surveys are of great concern to human health and aquatic biota as they deviated from the TWQR by more than 15 % (Table 4.1). Alternatively, the high EC reading from GLEN for the low flow sampling trip is attributed to the drought conditions during sampling. At the time of sampling, Glen Alpine Dam was at 3 % capacity. This minuscule amount of water coupled with increased evaporation potential would increase the concentration of TDS in the water, therefore accounting for higher EC readings.

For the high flow sampling, KNO showed the lowest EC reading of 47 µS.cm^{-1} (Figure 4.3). The low EC reading at NYL (69 µS.cm^{-1}) could be due to the wetland effectively conducting its ecological service of flow reduction, thus allowing for the sedimentation of dissolved and suspended solids.

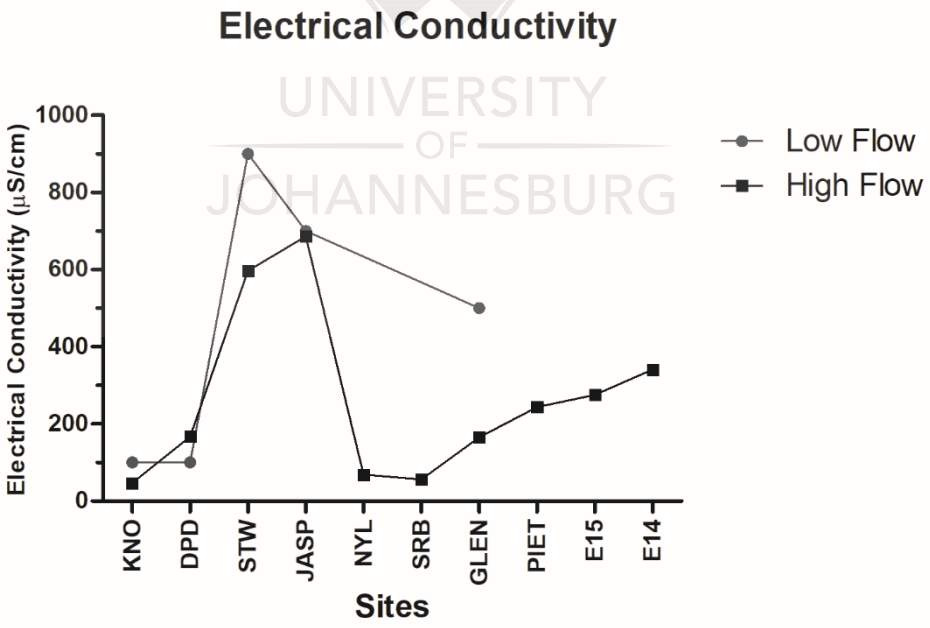

Figure 4.3: *In situ* electrical conductivity readings recorded at each of the sampling sites for both low flow (July 2016) and high flow (February 2017) sampling surveys.

Figure 4.3 shows that the EC reading at SRB was very low (56 µS.cm^{-1}) suggesting that flooding activity during high flow sampling could have influenced the TDS concentration

of the Mogalakwena River via a dilution capacity. SRB is a site very close to the Sterk River, a major tributary of the Mogalakwena River. The Sterk River was in flood at the time of high flow sampling and contributed the majority of surface water flow of the Mogalakwena after the confluence of these two rivers. As the water travelled downstream within the Mogalakwena River channel, the water could become more established, allowing for the stabilisation and gradual increase of TDS towards the sites further downstream of it, towards the Limpopo-Mogalakwena confluence.

4.2.2 NUTRIENTS

Extended data set for water nutrient analysis from both sampling surveys are provided in Appendix A.

4.2.2.1 INORGANIC NITROGEN

The major constituents of inorganic nitrogen in freshwater systems are ammonium (NH_4^+), ammonia (NH_3), nitrites (NO_2^-) and nitrates (NO_3^-) (DWAF 1996a). In this study, only the inorganic species of nitrogen were measured, therefore, any subsequent mention of nitrogen will refer to inorganic nitrogen. In natural systems, NO_2^- and NO_3^- are constantly being converted into one form or the other through the decomposition of NH_3 via the nitrogen cycle. DWAF (1996a) and Vlok et al. (2006) describe that aerobic bacteria *(Nitrobacter* spp. and *Nitromonas* spp.) reduce NH_3 ions, forming NO_2^-. Thereafter, the NO_2^- formed are further reduced into NO_3^-, the most stable positive oxidation state of nitrogen. Inversely, when conditions become anaerobic, common facultative bacteria oxidise NO_3^- ions and form NO_2^-. Figure 4.4 shows the basic equation nitrogen conversion in freshwater environments.

Ammonia and NH_4^+ are nutrients formed by the reduction of inorganic nitrogen through the aerobic or anaerobic processes of organic decay (DWAF 1996a). Although natural sources of ammonia exist (e.g. atmospheric gaseous exchange and soils), these nitrogen species are synonymous with anthropogenic pollution and are commonly associated with eutrophic waters. Anthropogenic activities that significantly influence NH_3 and NH_4^+ concentrations in freshwater systems include fertilizers, effluent discharge, cleaning products and atmospheric deposition.

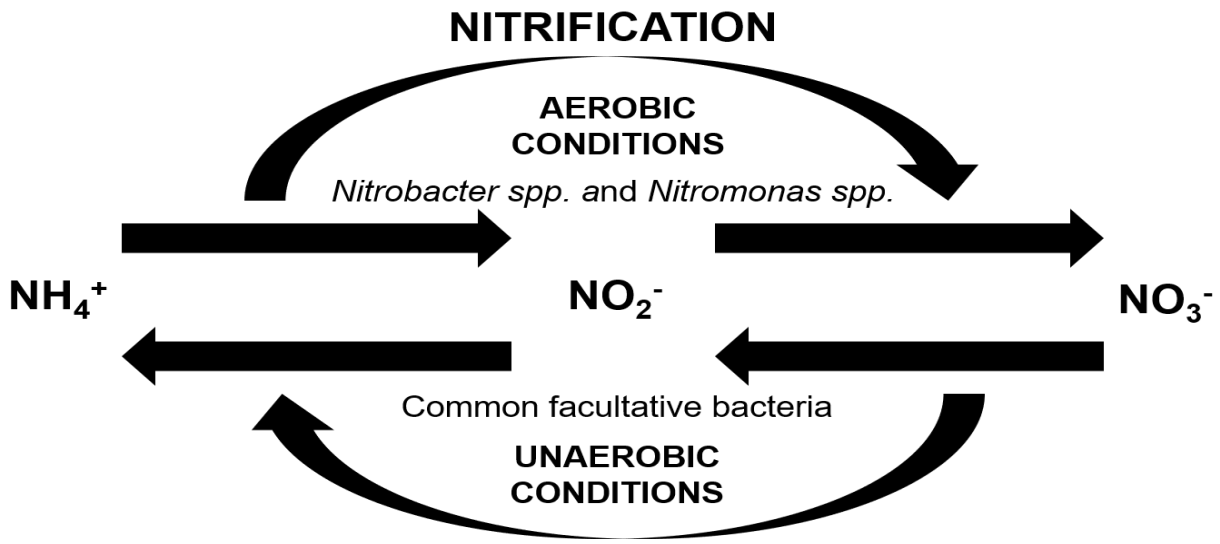

Figure 4.4: A basic equation for the conversion of inorganic nitrogen within freshwater environments [adapted from Vlok et al. (2006)].

Nitrogen is important in maintaining the health of freshwater systems, however, spikes in nitrogen levels, together with other nutrients such as phosphates, may lead to accelerated plant and algal growth, thus influencing water quality through eutrophication. Because inorganic nitrogen is readily absorbed by vegetation and algae, its levels within aquatic environments generally remain low, with high nitrogen levels only rarely occurring (DWAF 1996b). In addition, pH and temperature fluctuations within aquatic systems may influence nitrification and denitrification processes as well as affect the rate of NO_3^- absorption by plants and algae. Nitrogen levels within aquatic systems are highly variable, making it difficult to standardise TWQR for every South African aquatic system. Table 4.2 denotes the nitrogen and phosphorus concentrations that lead to varying degrees of eutrophication in South African aquatic system

The nitrogen levels recorded from each of the sampling sites for both sampling surveys are represented by Figure 4.5A – E. The results from both sampling surveys show that nitrogen levels at KNO and DPD were low, indicating that anthropogenic impacts at these sites are having minimal impacts to inorganic nitrogen loads. Downstream of DPD, the levels of all analysed nitrogen species (NO_2^-, NO_3^-, NH_4^+ and NH_3) from STW and JASP showed considerable spikes in their concentrations, suggesting that nitrogenous pollutants are entering the system. Furthermore, the NH_4^+ and NH_3 levels recorded from STW and JASP were above the TWQR, raising concern for the quality of water in this system for both domestic use and its ability to maintain aquatic integrity (Figure 4.5C, D). DWAF (1996a) states that one of the primary causes of high nitrogen loads in aquatic ecosystems is sewage effluent runoff, which may implicate the Modimolle WWTF as the

principal contributor of nitrogenous pollution within the Nyl River, despite its recent upgrades in 2016 (DWS 2015). Results from the high flow sampling survey show that sites downstream of STW and JASP recover with regards to the nitrogen loads, returning to levels considerably lower than that of the TWQR. The reduction of nitrogenous species concentrations seen at NYL during high flow sampling is attributed to the natural ecosystem services provided by wetlands. Wetlands reduce flow, allow for sedimentation of suspended particles and are generally densely covered with aquatic macrophytes capable of absorbing nitrogen species within a system.

JASP was the site that exhibited the highest NO_2^- concentrations for both the low flow (0.1 $mg.L^{-1}$) and high flow (0.13 $mg.L^{-1}$) sampling surveys (Figure 4.5A). The highest concentrations of NO_3^- were recorded at JASP (4.7 $mg.L^{-1}$) for the low flow survey and both STW and JASP (1.2 $mg.L^{-1}$) for the high flow survey (Figure 4.5B). A possible reason for the higher NO_2^- and NO_3^- concentrations at JASP could be attributed to nutrients flowing downstream from STW and accumulating at JASP. For the low flow sampling survey specifically, NO_2^- and NO_3^- concentrations were considerably higher at JASP compared to STW which was attributed to the nitrogen cycle in which aerobic bacteria reduce the NH_4^+, entering the system in the form of raw nutrients, into NO_2^- and subsequently into NO_3^- as they were carried downstream by river flow. The concentrations of NH_3 and NH_4^+ followed the same trends with STW having the highest concentrations of these nutrients for both sampling surveys. The concentrations of NH_3 at STW for low flow and high flow were 19.9 $mg.L^{-1}$ and 7 $mg.L^{-1}$, respectively, whereas, for NH_4^+, the concentrations were 25.6 $mg.L^{-1}$ and 7 $mg.L^{-1}$, respectively (Figure 4.5C, D). The high values reported by Musa (2016) for NH_4^+ and NH_3 concentrations at STW, further support the fact that the Modimolle WWTF is influencing the aquatic integrity of the Nyl River.

The results of the total nitrogen nutrient test followed the same trends of the nitrogen results (NO_2^-, NO_3^-, NH_4^+, NH_3) discussed previously. Total nitrogen results were low for KNO and DPD, thereafter increasing drastically at STW and JASP. Downstream of JASP, nitrogen levels returned to natural levels and these nutrients stabilised. According to DWAF (1996a), the high levels of nitrogen recorded at STW and JASP are indicative of a hypereutrophic system, whereas, the rest of the system is characteristic of a typical mesotrophic system (Table 4.2).

Table 4.2: The target water quality ranges for both inorganic nitrogen and phosphorus [adapted from DWAF (1996a)]. The classification and effect of each concentration are provided. Inorganic nitrogen concentrations are presented as $mg.L^{-1}$ and inorganic phosphorus concentrations are presented at $\mu g.L^{-1}$.

Average Summer Inorganic Nitrogen Concentrations	Average Summer Inorganic Phosphorus Concentrations	Classification of Water and Effects
< 0.5	< 5	**Oligotrophic conditions**; usually moderate levels of species diversity; usually low productivity systems with rapid nutrient cycling; no nuisance growth of aquatic plants or the presence of blue-green algal blooms.
0.5 – 2.5	5 – 25	**Mesotrophic conditions**; usually high levels of species diversity; usually productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms seldom toxic.
2.5 – 10	25 – 250	**Eutrophic conditions**; usually low levels of species diversity; usually highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms may include species which are toxic to man, livestock and wildlife.
> 10	> 250	**Hypertrophic conditions;** usually very low levels of species diversity; usually very highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae, often including species which are toxic to man, livestock and wildlife.

4.2.2.2 INORGANIC PHOSPHORUS

According to Kempster et al. (1980), phosphorus is one of the most abundant elements on earth, however, in its elemental form, it does not occur in natural environments. In aquatic systems, phosphates are present in the following forms: pyrophosphates, polyphosphates, metaphosphates and orthophosphates (DWAF 1996a). Although each of these species is present within aquatic systems, only orthophosphates (PO_4^{3-}) are biologically available to aquatic biota (DWAF 1996a). For the purposes of this study, only orthophosphates were considered, therefore, further mention of phosphates will relate to orthophosphates. Phosphate is an essential nutrient for all living organisms which,

together with calcium, aids in the formation of bone in higher organisms (Kempster et al. 1980). Although phosphates are non-toxic, their presence, together with other associated pollutants, generally indicates that an aquatic ecosystem is polluted (Kempster et al. 1980). DWAF (1996a) explains that the activities of living organisms, changes in pH and DO content all influence the phosphorus cycle in which phosphate species are synthesised, decomposed or converted into other forms. When PO_4^{3-} are formed through the phosphorus cycle, they become biologically available for aquatic organisms and macrophytes to utilise. In plants and animals, PO_4^{3-} has integral roles in the synthesis of nucleic acids, the regulation of energy within cells and in photosynthetic activities by being converted into cell structures.

DWAF (1996a) and Vlok et al. (2006) state that phosphates occur within aquatic environments because of natural processes or anthropogenic activities. Natural sources of phosphates in aquatic systems include: the natural erosion and weathering of geological structures (e.g. rocks), the decomposition of organic matter and the leaching of salts from soils and sediments. As phosphates within freshwater systems are generally present in very low concentrations, the presence of phosphates in high concentration suggests that pollution is occurring. Anthropogenic activities that increase phosphate concentrations in natural systems include the discharge of domestic and industrial effluents, surface drainage of the catchment, as well as urban and agricultural runoff, particularly fertilizer runoff (DWAF 1996a).

Figure 4.5F indicates the phosphate levels that were present in the Nyl and Mogalakwena River systems during both the low flow and high flow sampling surveys. The highest concentration of PO_4^{3-} during the low flow and high flow sampling surveys were present at STW, these concentrations were 3.52 $mg.L^{-1}$ and 0.81 $mg.L^{-1}$, respectively. Furthermore, the PO_4^{3-} concentrations recorded at STW and JASP (low flow – 0.26 $mg.L^{-1}$ and high flow – 0.13 $mg.L^{-1}$) for both sampling surveys were extremely high, deviating from the TWQR by more than 15 % (Table 4.1). These concentrations are characteristic of a hypertrophic system and the water here poses serious health risks to wildlife, domestic livestock and humans (Table 4.2). These high concentrations are attributed to the organic effluent entering the system at the Modimolle WWTF. Baring STW and JASP, the concentration of PO_4^{3-} within the rest of the Nyl and Mogalakwena River systems remained low and were within the natural phosphate ranges (0.01 – 0.05 $mg.L^{-1}$) as described by DWAF (1996a).

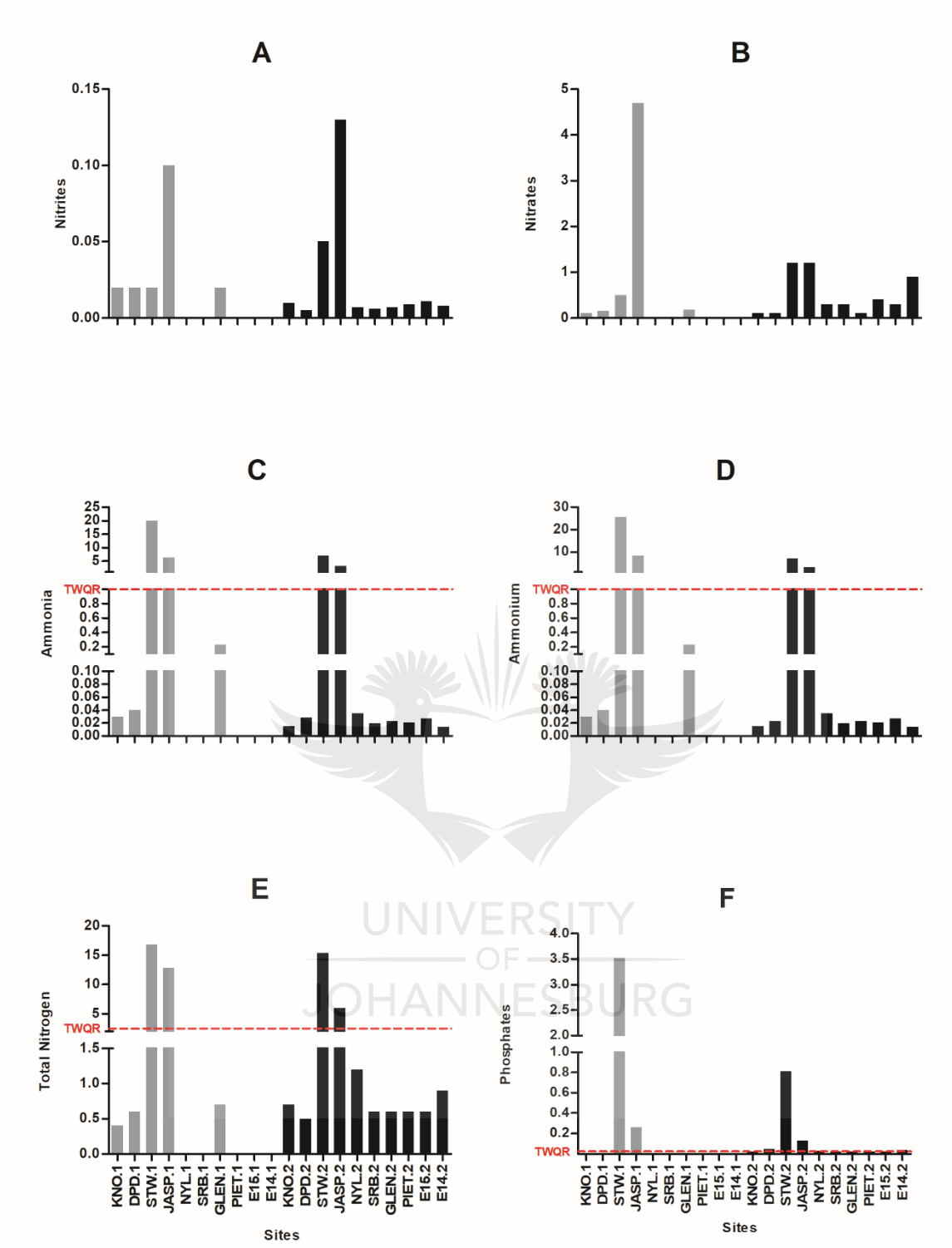

Figure 4.5: Concentration values obtained from the water nutrient analyses of inorganic nitrogen and phosphate species from water samples collected in the Nyl and Mogalakwena Rivers. **A,** Nitrites; **B,** Nitrates; **C,** Ammonia; **D,** Ammonium; **E,** Total Nitrogen; **F,** Orthophosphates. The numeral succeeding the site name denotes sampling season: **1,** low flow (grey bars); **2,** high flow (black bars). The red dotted lines indicate the Target Water Quality Ranges proposed by DWAF (1996a). All concentrations are presented as $mg.L^{-1}$.

4.2.2.3 SULPHATES

The oxyanion of sulphur (+ VI oxidative state) is known as sulphate (SO_4^{2-}) (DWAF 1996b). According to Kempster et al. (1980), SO_4^{2-} are harmless to humans and animals in low concentrations. If the concentrations were to increase and SO_4^{2-} are converted into more harmful forms (e.g. Sulphuric Acid – H_2SO_4), effects on human and animal health as well as damage to infrastructure (e.g. bridges) could occur (Kempster et al. 1980; DWAF 1996b; Dallas & Day 2004). The dissolution of rocks and soils, particularly calcium sulphate, is the primary contributor to natural concentrations of SO_4^{2-} in aquatic systems (DWAF 1996b). Anthropogenic sources of SO_4^{2-} in aquatic systems include: acid precipitation, atmospheric deposition (fossil fuel combustion), agricultural runoff (fertilizers), AMD (conversion of SO_4^{2-} to H_2SO_4) and other industrial processes that utilise H_2SO_4 or other sulphates species (e.g. tanneries) (DWAF 1996a; van der Welle et al. 2008).

Figure 4.6A is a graphical representation of the SO_4^{2-} concentrations in water samples collected from the Nyl and Mogalakwena Rivers for both sampling surveys. The concentrations of SO_4^{2-} for both sampling surveys ranged from 27 $mg.L^{-1}$ at KNO (low flow) to 86 $mg.L^{-1}$ at JASP (low flow). Although SO_4^{2-} concentrations fluctuated between sites for both sampling surveys, all the analysed SO_4^{2-} concentrations were below the TWQR proposed for domestic use (DWAF 1996b). Vlok et al. (2006) describe that the TWQR for domestic use can be suitably used for aquatic organisms as no TWQR exist for freshwater aquatic systems. Furthermore, DWAF (1996b) states that sulphates tend to accumulate in water bodies, with concentrations gradually increasing as water flows downstream. This accumulation pattern may account for the increased SO_4^{2-} concentration of 69 $mg.L^{-1}$ at E14 during the high flow survey.

4.2.2.4 CHLORIDES

Chlorine, in its natural form, does not occur in aquatic environments (DWAF 1996a). Hutchinson (1975) states that chlorine in aquatic environments occurs as chlorides (Cl^-), an anion of elemental chlorine. Chlorides are highly water soluble and are common in aquatic environments in varying concentrations (DWAF 1996b). Natural sources of Cl^- result from the binding of chlorine to most elements (Vlok et al. 2006). According to Kempster et al. (1980), Cl^- is a common form of industrial and domestic pollutants; pollutants such as irrigation backflows, sewage and effluent discharge and industrial processes can influence the Cl^- content of surface waters (DWAF 1996b). The effect Cl^-

has on aquatic organisms is dependent on the ability of these organisms' mechanisms to regulate Cl^- loads; plants are particularly susceptible to high Cl^- concentrations in water (Kempster et al. 1980). Secondary effects caused by increased Cl^- concentrations in water could arise from their interactions with metals, which tend to accelerate oxidative and corrosive actions (Vlok et al. 2006).

The Cl^- concentration values recorded from all sites for both sampling surveys are represented in Figure 4.6B. No TWQR exist for aquatic systems, however, all recorded Cl^- concentrations were below the TWQR for domestic use (Table 4.1) (DWAF 1996b). The highest Cl^- concentrations were recorded at JASP (87.79 $mg.L^{-1}$) and STW (62.19 $mg.L^{-1}$) for the low flow sampling survey. These high Cl^- concentrations as compared to KNO and DPD suggest that pollutants are affecting Cl^- loads in the system at these sites. Increased Cl^- concentrations at STW and JASP can be attributed to raw sewage effluent entering the system at the Modimolle WWTF, a form of surface water pollution that is known to increase Cl^- loads (DWAF 1996b). Although the Cl^- concentrations at JASP and STW are higher than those of the upstream and subsequent downstream sites, these concentrations alone pose no threat to the health of living systems, except if they lead to increases in the TDS of the system (Dallas & Day 2004). The Cl^- concentration of 59.79 $mg.L^{-1}$ recorded at GLEN for the low flow survey is most probably the result of evaporative water loss during the 2016 drought which causes an accumulative effect of nutrients in the remaining water.

4.2.2.5 FLUORIDES

Fluoride (F^-), an anion of fluorine is the primary form of elemental fluorine in natural environments (DWAF 1996a). Resulting from the high electron affinity of F^-, it can interact with almost every element (DWAF 1996b). Due to this affinity, F^- commonly reacts with calcium, potassium and phosphate ions in water to form insoluble complexes (DWAF 1996a). Environmental conditions and water chemistry also affect the toxicity of F^- within aquatic systems; increases in temperature lead to increased F^- toxicity, whereas, increases in total hardness reduce the toxicity of F^- (DWAF 1996a). Naturally, F^- in freshwater sources arise because of the surrounding geology (e.g. sedimentary and igneous rocks), however, as anthropogenic activities continue to pollute aquatic systems, F^- levels are increasing. Anthropogenic sources of F^- include: insecticide usage and manufacturing; industrial processes and water treatment for consumption (DWAF 1996a). In mammals, low concentrations of F^- have essential roles in the formation of bone and

enamel strengthening in teeth, however, in high concentrations can lead to fluorosis (calcification of bones and ligaments).

The results from the low flow and high flow sampling surveys represented in Figure 4.6C indicate that F^- concentrations from each of the sampling sites were below the TWQR of 0.75 $mg.L^{-1}$. Fluoride concentrations in general, were higher for the high flow sampling survey, with PIET showing the highest F^- value concentration of 0.648 $mg.L^{-1}$. The lands surrounding PIET were extensively modified for agricultural purposes, which in periods of increased rainfall, become more productive due to accelerated rates of plant growth. The insecticides used on plant pests during productive seasons may be influencing F^- concentrations at PIET through agricultural runoff (DWAF 1996a).

4.2.2.6 CHEMICAL OXYGEN DEMAND

According to DWAF (1996c), chemical oxygen demand (COD) is defined as "*the amount of oxygen required to oxidise all the organic matter that is susceptible to oxidation by a strong chemical oxidant*". Put more simply, the COD provides an estimation on the amount of organic matter that is present in a water source and the ability of this organic matter to deplete DO (DWAF 1996a, 1996c). Within aquatic systems, organic matter exists in two forms, namely: autochthonous organic matter (originates within the water body itself) and allochthonous organic matter (originates external to the water body). Allochthonous organic matter is resultant from anthropogenic influences such as agricultural activities and urban and industrial effluent runoff. Dissolved oxygen, an extremely important environmental variable for aquatic organisms is highly dependent upon the presence of this organic, and thus oxidisable matter within the water body (DWAF 1996c). Therefore, as organic wastes accumulate in the surface waters of aquatic systems (increased COD), the DO of the system becomes depleted, reducing the viability of the water to support the biota that is dependent upon it (DWAF 1996a). Additional to daily fluctuations, DO concentrations in aquatic systems are influenced by factors such as temperature and air pressure (DWAF 1996c).

Previous studies conducted by Vlok et al. (2006), Dahms (2016) and Musa (2016) have identified that organic loads within the Nyl River system are being severely influenced by organic effluent runoff from the Modimolle WWTF. Additionally, Zhao et al. (2004) describe that the COD method is preferred for water bodies that are heavily polluted with

organic matter. For these reasons, the COD method was used to assess the oxygen demand of the organics entering the Nyl and Mogalakwena River systems.

The COD of the water sampled from each site for both sampling surveys are represented in Figure 4.6D. For the low flow sampling survey, the COD measurements at KNO and DPD were 4.6 mg $O_2.L^{-1}$ and 9.2 mg $O_2.L^{-1}$, respectively, whereas COD at STW and JASP were considerably higher, 52.8 mg $O_2.L^{-1}$ and 20.4 mg $O_2.L^{-1}$, respectively. Despite the seemingly high COD values at STW and JASP compared to that of KNO and DPD, they are indicative of waters that have low to very low organic contamination (Van Damme et al. 2008).

As compared to the low flow survey, KNO showed a COD value of 33.9 mg $O_2.L^{-1}$ for the high flow survey, indicating that the organic content at this site had increased, possibly because of the stemming of flow caused by the weir. Thereafter, there was a decrease in COD at DPD (11.6 mg $O_2.L^{-1}$), followed by an increase in COD of 44.6 mg $O_2.L^{-1}$ and 31.6 mg $O_2.L^{-1}$ at STW and JASP, respectively. At NYL for the high flow survey, the COD value measured was 52.8 mg $O_2.L^{-1}$, suggesting an increase in organic matter. Wetlands tend to have high densities of aquatic vegetation which is continually growing and being replaced by new shoots, the result of which causes detritus build-up (allochthonous organic matter) within the water body, therefore leading to amplified levels of organic content and increased COD values. Thereafter, the COD values for the Mogalakwena River sites ranged from 7.3 mg $O_2.L^{-1}$ at SRB to 14.3 mg $O_2.L^{-1}$ at GLEN and E15.

4.2.2.7 TOTAL ALKALINITY

According to Polling (1999), the total alkalinity is a measurement of the dissolved bases within a water source which has roles in the buffering capacity of water (rate of change of pH) and is determined by the number of dissolved carbonate species present. Alkalinity is measured in terms of calcium carbonate ($CaCO_3$) and is therefore closely related to total hardness which is calculated according to the summation of calcium (Ca) and magnesium (Mg) concentrations and is expressed as mg.L^{-1} of $CaCO_3$ (DWAF 1996a; 1996b). The alkalinity and thus the total hardness of aquatic systems is naturally influenced by the surrounding geology, specifically, minerals containing Ca and Mg (DWAF 1996b). DWAF (1996b) describes that hardness can be resultant from bicarbonate salts (Ca and Mg) or non-bicarbonate salts (NO_3^-, SO_4^{2-} and Cl^-), of which, the non-bicarbonate salts do not easily precipitate out of the water column. Furthermore,

Bell (1976) states that with an increase in alkalinity and therefore total hardness, the toxicity of many metals is reduced. Table 4.3 adapted from Vlok et al. (2006), represents the classification classes of water hardness based upon on determined $CaCO_3$ concentration ranges.

Table 4.3: Classification of water hardness based upon calcium carbonate ($CaCO_3$) concentrations [adapted from Vlok et al. (2006)]. All $CaCO_3$ concentrations are presented as $mg.L^{-1}$.

Concentration of $CaCO_3$ ($mg.L^{-1}$)	Classification of Water Hardness
< 60	Soft
60 – 119	Medium
120 – 179	Hard
> 180	Very hard

Considering both sampling surveys, the median $CaCO_3$ values measured at the sites along the Nyl River and the sites along the Mogalakwena River were 50 $mg.L^{-1}$ and 75 $mg.L^{-1}$, respectively. These values indicate that for the most part, the hardness of the water in Nyl River is soft in nature and the hardness of the water in the Mogalakwena River is medium in nature (Table 4.3). A study by Vlok et al. (2006) showed a similar result with regards to the water hardness of the Nyl River, reporting a median $CaCO_3$ value of 35 $mg.L^{-1}$. The minimum $CaCO_3$ concentration of 11 $mg.L^{-1}$ was recorded at KNO for the high flow season, whereas a maximum $CaCO_3$ concentration of 183 $mg.L^{-1}$ was recorded at JASP during the high flow survey (Figure 4.6E). The concentrations of $CaCO_3$ at STW for both low flow (176 $mg.L^{-1}$) and high flow (165 $mg.L^{-1}$) sampling surveys were indicative of hard waters. According to Ashton et al. (2001) and Magalies Water (2013), the extensive usage and unreliable supplies of water within the Nyl-Mogalakwena catchment area have resulted in high water demands, specifically within the towns of Modimolle and Mookgopong, that cannot be sustained by the current supply. Therefore, the Department of Water Affairs (DWAF) have implemented a water transfer scheme that transfers potable waters originating in the Apies and Pienaars Rivers via a pipeline from the Klipdrift and Temba WWTFs in the Tswane Municipality District to the towns of Modimolle and Bela-Bela (Magalies Water 2013). This interbasin transfer of water may be increasing the hardness of the water at STW and JASP, as the water originating in the Apies and Pienaars Rivers may differ in $CaCO_3$ concentrations. Baring the hard water at

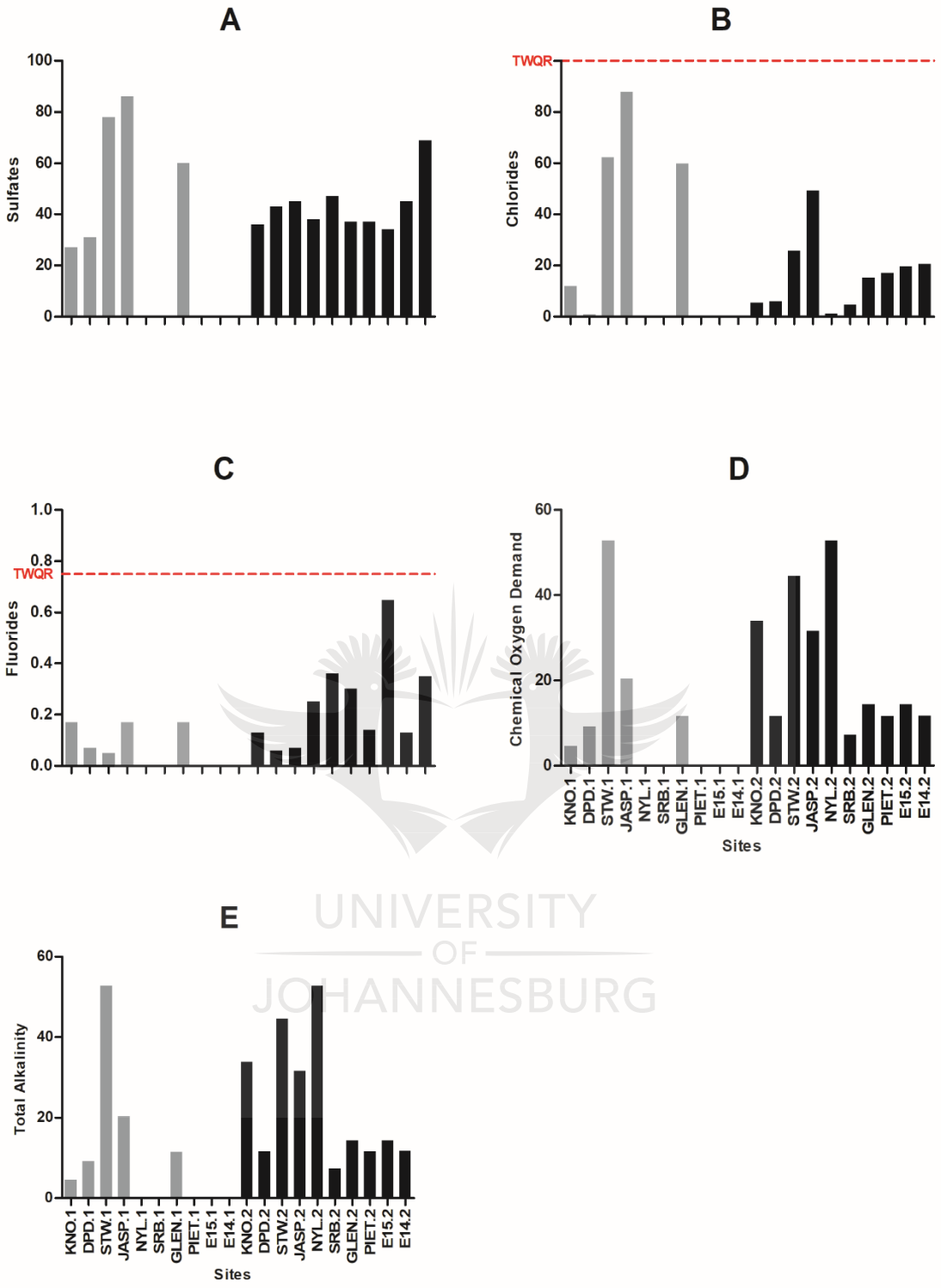

Figure 4.6: Concentration values obtained from the water nutrient analyses of water samples collected in the Nyl and Mogalakwena Rivers. **A,** Sulphates; **B,** Chlorides; **C,** Fluorides; **D,** Magnesium; **E,** Calcium; **F,** Total Alkalinity. The numeral succeeding the site name denotes sampling season: **1,** low flow (grey bars); **2,** high flow (black bars). The red dotted lines indicate the Target Water Quality Ranges proposed by DWAF (1996a; 1996b). All concentrations are presented as $mg.L^{-1}$.

STW and JASP, the overall soft to medium nature of the water at the other sampling sites could increase the speciation and toxicity of metals within the water column, which may further impact the aquatic integrity of the Nyl and Mogalakwena Rivers.

4.2.3 STATISTICAL ANALYSIS OF WATER QUALITY

Figures 4.7 and 4.8 represent the results of the spatial and temporal analysis of the water quality and environmental variable data. To determine whether any temporal variations in water quality and environmental variables existed between the sampling sites, the results from the two sampling seasons were first analysed separately (Figure 4.7A, B).

The Principal Component Analysis (PCA) of the low flow results explained 95.97 % of the total variation, 75.78 % on the first axis and 20.19 % on the second axis (Figure 4.7A). These results identified that there were three distinct groupings of sampling sites. The site groupings were as follows: **group 1,** the relatively unimpacted sites close to the source of the Nyl River, KNO and DPD; **group 2,** STW and JASP which are affected by sewage runoff; and **group 3,** GLEN groups by itself as it was separated from JASP by 240 km of dry riverbed and at the time of low flow sampling, it was significantly affected by drought conditions.

Nitrogenous nutrients (NO_2^-, NO_3^-, NH_3, NH_4^+, TN), PO_4^{3-} and COD are the variables with the strongest positive correlations to group 2 (STW and JASP) accounting for the ordination of these sites away from the others. JASP had a very strong positive correlation with NO_2^-, suggesting that anaerobic conditions between STW and JASP, possibly resulting from eutrophication, are increasing NO_2^- concentrations by oxidising the NO_3^- entering the system at the Modimolle WWTF (DWAF 1996a). The separation of group 3 (GLEN) from the other site groupings was attributed to the high pH, Cl^- and SO_4^{2-} concentrations at this site. The sites in group 1 (KNO and DPD) showed negative correlations with most of the water quality and environmental variable levels, accounting for their ordination away from that of the other site groupings.

The PCA conducted on the high flow data (Figure 4.7B) showed that 90.91 % of the total variation was explained on the first two axes, with 73.28 % on the first and 17.63 % on the second. Good rains during the high flow season allowed for more sites along the Nyl and Mogalakwena Rivers to be sampled, yet, the PCA of the high flow data showed similar site groupings. The site groupings were as follows: **group 1,** KNO, DPD and NYL which showed signs of minimal anthropogenic impacts to water quality; **group 2,** STW

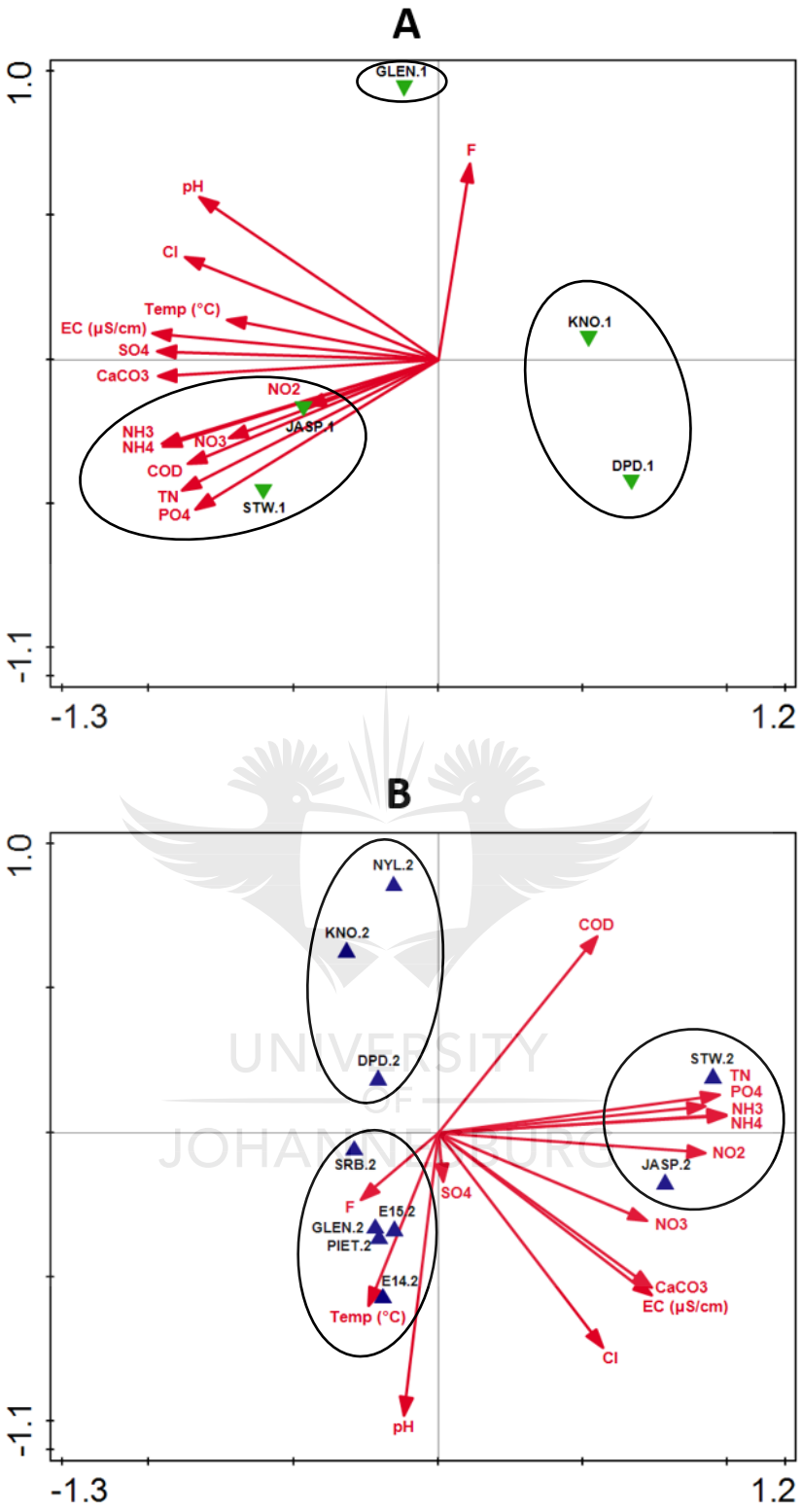

Figure 4.7: Unconstrained Principle Component Analysis bi-plots indicating the water quality variables collected from sampling sites along the Nyl and Mogalakwena Rivers for **A,** the low flow sampling season and **B,** the high flow sampling season. The numeral succeeding the site name denotes sampling season: **1,** low flow (green downward triangles); **2,** high flow (blue upward triangles). The red arrows indicate the individual water quality variables.

and JASP that showed high concentrations of various water nutrients; and **group 3,** SRB, GLEN, PIET, E15 and E14, the sites along the Mogalakwena River.

The similar site groupings between low flow and high flow sampling seasons show that there are definitive spatial trends that exist within the Nyl and Mogalakwena River systems. Temperature, pH and F^- are the driving factors contributing to the ordination of the sites in group 2 (SRB, GLEN, PIET, E15 and E14). This is to be expected due to the relationship between temperature and pH, higher water temperatures lead to increases in pH values (DWAF 1996a). Like the results of the low flow season, STW and JASP had the highest concentrations of most of the water quality variables tested and these sites showed strong positive correlations to nitrogenous nutrients and PO_4^{3-}. The sites in group 1 (KNO, DPD and NYL) are grouped together as they had low concentrations of many of the water quality variables tested, more specifically, they showed strong negative correlations to Cl^-, EC and $CaCO_3$.

Figure 4.8 contains the combined results of the tested water quality variables for both sampling surveys. The combined analysis explains 86.94 % of the total variation with 66.72 % of the variance being explained on the first axis and a further 20.22 % of variation on the second axis. Spatial differences between the sites are again evident, with STW and JASP for both sampling surveys plotting away from the other sampling sites. It is evident that COD is the driving variable leading to the ordination of STW and JASP for the high flow season, whereas the ordination of STW for the low flow season is resultant from strong positive correlations to nitrogenous nutrients and PO_4^{3-}. The strong correlations of these variables indicate that nutrients are entering the system at STW, most likely through effluent runoff and that the reduced flow during the dry season allowed these nutrients to settle, thus severely affecting the water quality at the site.

Excluding STW and JASP, the site ordination from the combined assessment shows a more complex grouping structure. Again, we can see that the sites sampled during high flow season, except KNO and DPD, show strong positive correlations to temperature and F^- content. According to DWAF (1996a), F^- shows a directly proportional relationship with temperature, meaning that as temperature increases, so too would F^- concentrations. Vertical separation of the sites indicates that temporal variations in water quality exist within the Nyl and Mogalakwena River system.

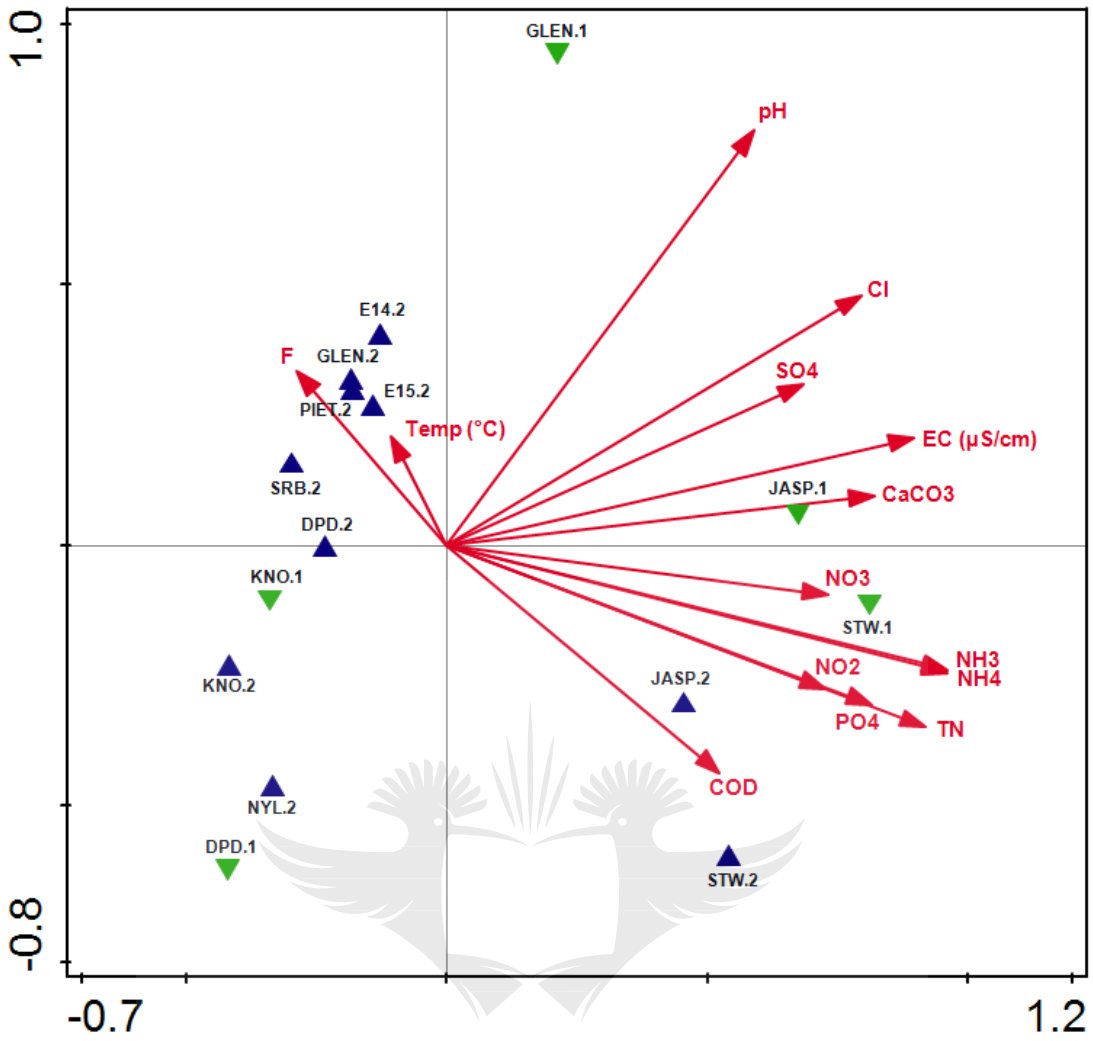

Figure 4.8: An unconstrained Principle Component Analysis bi-plot indicating the combined results of the water quality variables collected from sampling sites along the Nyl and Mogalakwena Rivers for both the low flow and high flow sampling seasons. The red arrows indicate the individual water variables. The numeral succeeding the site name denotes sampling season: **1,** low flow (green downward triangles); **2,** high flow (blue upward triangles). The red arrows indicate the individual water quality variables.

The ordination of GLEN during the low flow season suggests that water quality at this site was unique compared to the other sampling sites. This could be attributed to a severe drought during low flow sampling which reduced volume the of water within Glen Alpine Dam down to 3 %. This reduction in water volume may have caused the remaining water to become concentrated with nutrients and other pollutants, therefore, driving it away from the other sites in the combined PCA. As before, KNO and DPD for both sampling surveys showed negative correlations to almost all the analysed water variables, indicating that the water quality at these sites has been minimally impacted.

4.2.4 METALS

Galvin (1996) explains that trace elements, in specific concentrations, are extremely important for the health and effective metabolic functioning of all living organisms. Naturally, the surface and groundwater of aquatic ecosystems contain these important elements in low concentrations, however, if the concentrations of these trace elements were to increase, the organisms that inhabit these systems could accumulate these elements and be negatively affected (Nussey et al. 1999). Depending on the biological importance, concentration and toxicity of these metals, their effects on aquatic organisms vary. According to Robinson and Avenant-Oldewage (1997), the two factors adding to the detrimental impacts these metals have on aquatic organisms are (i) the inability of these metals to degrade within aquatic environments and (ii) the tendency of these metals to accumulate within aquatic organisms, therefore persisting in aquatic environments.

When metals enter aquatic environments either through natural (weathering or decomposition processes) or anthropogenic sources (mining, industrial activities or various other pollutants), they interact with other water constituents to reach stability (Vlok et al. 2006). The surrounding water quality (e.g. temperature and pH) influences the toxicity of the metals as well as their interactions with the other water constituents. Once a metal has reached stability, its toxicity may further be influenced by other pollutants in the water or by the life stage and overall susceptibility of the organism to the toxicant (Hellawell 1986).

The metal concentrations reported in this section are total metal concentrations within the samples analysed and not the dissolved concentrations. Furthermore, there was no differentiation between metal species that may be more toxic than others (e.g. hexahydrate aluminium). The TWQR values for trace metals are well documented for human consumption and domestic use in South Africa, however, the TWQR for aquatic ecosystems remain vague, making it difficult to fully assess the severity of these pollutants in a system. For this reason, the South African TWQR, as well as the Canadian Water Quality Guidelines (CWQG) for trace metals, are represented in Table 4.4 (DWAF 1996a, 1996b; CCME 1999). The TWQR and CWQG set out by the DWAF (1996a, 1996b) and the CCME (1999) (Table 4.4) are used to determine the optimal concentrations of these metals in aquatic systems for the ideal health of the aquatic biota; if concentrations were to fall below the TWQR, organisms would be vulnerable to

diseases and if the concentrations were to exceed the TWQR, organisms could experience toxicity symptoms (du Preez et al. 1997).

Overall, water metal concentrations were higher during the high flow than during the flow, with the exception of Mn and Pb. An extended data set for metal analysis on the water collected from both sampling surveys is provided in Appendix B.

Table 4.4: Target Water Quality Ranges (TWQR) for the concentrations of trace metals in water for domestic use (DWAF 1996b), aquatic ecosystems (DWAF 1996a) and Canadian Water Quality Guidelines (CCME 1999). All values are presented as $\mu g.L^{-1}$. **N/A**, not available.

Element	TWQR – Domestic Use	TWQR – Aquatic Ecosystems	CWQG
Aluminium	0 - 150	≤ 10	100
Copper	0 - 1000	≤ 0.3 (soft water)	2
Iron	0 - 100	Deviation: < 10 %	300
Manganese	0 - 50	180	N/A
Lead	0 - 10	≤ 0.5 (soft water)	1
Vanadium	0 - 100	N/A	N/A
Zinc	0 - 3000	≤ 2	30

4.2.4.1 ALUMINIUM

Aluminium is one of the most common elements in the Earth's crust and so, is readily found within aquatic ecosystems in varying concentrations (DWAF 1996a). The pH of water is the determining factor for the solubility of Al (DWAF 1996a; Vlok et al. 2006; Dahms 2016). The toxicity of Al to aquatic organisms is dependent upon its solubility. With decreases in pH, Al occurs in the toxic, soluble form of hexahydrate species, whereas when pH increases, Al occurs as hydroxide complexes which are less soluble and biologically unavailable (DWAF 1996a). Anthropogenic sources that either increase Al concentrations or toxicity in aquatic systems include: AMD, acid precipitation, atmospheric deposition (fossil fuel combustion), industrial processes and the purification of drinking water (flocculants). DWAF (1996a) describes that high concentrations of toxic, bioavailable Al species coupled with reductions in pH levels in aquatic ecosystems, could cause deleterious harm to aquatic organisms. The myriad of effects on aquatic organisms suggested by the authors includes: influences on respiration through mucus coagulation around the gills; interference of calcium protein regulation and calcium metabolisms in

various organs and the brain; and changes to sodium ion exchange and osmotic balance, which could cause neuromuscular dysfunction.

The concentrations of Al ranged from below detection at most of the sampling sites for both sampling seasons to 54.804 $\mu g.L^{-1}$ at GLEN for the high flow season (Figure 4.9A). The below detection results obtained from many of the sites can be attributed to the alkaline conditions of the river system at the time of sampling. When pH conditions are alkaline, Al becomes biologically unavailable and dissolved concentrations are reduced (Dahms 2016). Of the concentrations that were above detection, the source of the Nyl River (KNO) had the lowest Al concentration of 20.494 $\mu g.L^{-1}$ during the low flow season. STW for the low flow sampling survey and the sites along the Mogalakwena River for the high flow survey, except E15, had Al concentrations higher than the TWQR (Table 4.4), showing Al toxicity of some form. The results of this study were considerably lower than those reported by Vlok et al. (2006) and Dahms (2016) who reported Al concentrations ranging from 18 $\mu g.L^{-1}$ to 2089 $\mu g.L^{-1}$ and 25.1495 to 367.4903 $\mu g.L^{-1}$, respectively.

4.2.4.2 COPPER

Copper is one of the most abundant metals on earth and is ubiquitously used for a plethora of human activities. Naturally, Cu occurs in most aquatic systems, however, due to its extensive use, the USEPA has deemed it potentially hazardous (DWAF 1996a). In aquatic ecosystems, Cu occurs in three oxidation states, namely: Cu^{+}, Cu^{2+} and Cu^{3+}, however, the forms of Cu that are of most concern are its monovalent (Cu^{+}) and hydroxide ($CuOH^{+}$) forms. When the pH of a system is reduced, Cu dissociates into its divalent form (Cu^{2+}), which can be removed from the system by binding to organic matter or by being absorbed by particulates (Robinson & Avenant-Oldewage 1997). Inversely, when pH is increased, Cu tends to precipitate out, becoming biologically unavailable (Grosell et al. 1997). Furthermore, Cu toxicity is influenced by the chemical properties of water including dissolved constituents (e.g. Zn, Ca), reductions in TH ($CaCO_3$) and the reduction of DO concentrations (DWAF 1996a).

The oxidation of sediments is the major contributor to natural Cu concentrations, however, Cu concentrations within the environment are drastically increased through anthropogenic activities (Nussey et al. 1999). According to Nussey et al. (1999) and Vlok et al (2006), Cu concentrations within the Nyl River system are influenced by sewage runoff, agricultural runoff (fungicides and pesticides), aquatic algaecides and fertilizer

manufacturing. High concentrations of Cu in aquatic ecosystems can have detrimental effects on the health and success of aquatic organisms. DWAF (1996a) states that despite severe effects to higher organisms such as fish, macroinvertebrate community assemblages have been found to be altered when Cu is present in high concentrations. Furthermore, author states that the early life stages of these macroinvertebrate taxa show a higher sensitivity to Cu pollution compared to that of adults, which may have effects on the recruitment, species richness and abundances of these organisms.

The Cu concentrations measured at sites along the Nyl and Mogalakwena Rivers ranged from 2.065 $\mu g.L^{-1}$ at SRB for the high flow having the lowest concentration to the highest concentration of 8.392 $\mu g.L^{-1}$ at E14. For the most part, Figure 4.9B indicates that Cu concentrations at each of the sampling sites were above the TWQR and CWQG, suggesting that the Nyl and Mogalakwena Rivers have naturally high levels of Cu. The decline in Cu levels measured at SRB may be resultant from flooding activity during high flow sampling, where flood waters could dilute the Cu concentrations in the water. The Cu concentrations measured during the high flow season were higher in comparison to the low flow season. Although the results of this study appear to recommend seasonality as a cause of variations to Cu concentrations, similar results from Vlok et al. (2006) suggest that Cu concentrations within the Nyl River are related on a temporal scale instead.

4.2.4.3 IRON

Iron is an abundant element that is common in aquatic ecosystems in two oxidation states, namely: divalent ferrous iron (F^{2+}) and trivalent ferric iron (Fe^{3+}) (DWAF 1996a). The concentrations and species of Fe in aquatic environments are dependent upon interactions with DO, pH and organic and inorganic matter (DWAF 1996a). At lower pH, Fe will occur as either Fe^{2+} in oxygenated waters or as Fe^{3+} in anoxic conditions. Furthermore, interactions between microorganisms and sediments may also influence the oxidation states and concentrations of Fe within the environment (DWAF 1996a). According to Train (1979), natural surface waters contain varying concentrations of Fe depending on the surrounding geology of the region. Iron can enter aquatic ecosystems via the dissolution of rocks (surrounding geology) or through anthropogenic impacts such as industrial effluents and steel production (Galvin 1996). According to Vlok et al. (2006), the major source of Fe contamination within the Nyl River arises from sewage runoff at the Modimolle WWTF.

All living organisms require Fe for respiratory purposes, in particular, its essential roles in respiratory enzymes and pigments (e.g. haemoglobin) (Galvin 1996). Due to these important roles in metabolic functioning, limited toxicity and reduced bioavailability in aquatic systems, Fe has been classified as a 'non-critical' element (DWAF 1996a).

The Fe concentrations measured at each of the sampling sites for both sampling surveys are indicated in Figure 4.9C. All measured Fe concentrations were below the CWQG of 300 $\mu g.L^{-1}$ (Table 4.4), however, the concentrations measured at STW for the low flow survey (175.882 $\mu g.L^{-1}$) and at KNO for the high flow survey (255.097 $\mu g.L^{-1}$) deviated from the other sampling sites by more than 10 % (Table 4.4), which may indicate Fe contamination of some form. The high concentrations at KNO for this study opposes those reported by Dahms (2016), which, for their study, was the site with the lowest Fe concentrations of 34.8587 $\mu g.L^{-1}$. Although STW (low flow) and KNO (high flow) showed deviations greater than the TWQR, these levels pose no threat to the aquatic biota due to limited toxicity and bioavailability of Fe species (DWAF 1996a).

4.2.4.4 MANGANESE

The absorption of NO_3^- and enzymatic processes within plants, animals and microorganisms are dependent upon Mn (DWAF 1996a). Within aquatic systems, elemental Mn is absent but commonly occurs within salts and minerals containing Fe. Manganese can occur as soluble manganous (Mn^{2+}) but is easily oxidised to its insoluble form, manganic (Mn^{4+}). Although essential to fauna and flora, high concentrations of Mn becomes toxic to aquatic organisms (DAWF 1996a). Galvin (1996) describes that DO has a large influence on Mn levels within aquatic systems; for the most part, if DO concentrations were to increase, Mn concentrations would decrease and vice versa. The author explains that this process occurs through the oxidation of Mn species which causes them precipitate out of the water column, becoming biologically unavailable. Furthermore, the toxicity of Mn is influenced by increases in pH levels, where ionic Mn species become more prevalent when pH is reduced (Hellawell, 1986).

In aquatic ecosystems, Mn enters through the dissolution of soils, sediments and the surrounding geology, however, AMD and industrial processes such as the chemical industry (e.g. glass and paint production), the fertilizer industry and the steel industry (e.g. battery production), are the major contributors to high Mn concentrations in aquatic environments (DWAF 1996a).

Manganese concentrations ranged from 2.545 µg.L^{-1} at NYL for the high flow to 128.801 µg.L^{-1} at STW for the high flow (Figure 4.9D). Not considering STW for the high flow season, Mn concentrations for the low flow season were higher compared to those of the high flow season. This opposes the study by Vlok et al. (2006) which reported that Mn levels were higher in the high flow periods than low flow periods. Moreover, the Mn concentrations measured by Vlok et al. (2006) were significantly higher than the concentrations measured in this study. This could suggest that Mn contamination in the Nyl River has seen an improvement. Furthermore, it is important to note that Fe and Mn have a close association (Dahms 2016) and therefore show a similar trend in measured concentrations. Despite the elevated Mn concentrations measured at STW, all the sampling sites showed Mn levels lower than the TWQR of 180 µg.L^{-1}, suggesting that these concentrations are not of concern and pose no threat to aquatic health (DWAF 1996a).

4.2.4.5 LEAD

Lead is an abundant, non-essential element that, according to USEPA, is toxic and easily accessible to the organisms inhabiting aquatic environments (DWAF 1996a). It can exist in four main oxidations states, however, in its most toxic divalent form (Pb^{2+}), it readily bio-accumulates in aquatic organisms (DWAF 1996a). Interactions of Pb with the ambient environment are influenced by pH levels. As pH is reduced, Pb^{2+} becomes more biologically available, which in turn increases its uptake and accumulation within aquatic organisms (DWAF 1996a). Although Pb^{2+} is readily accumulated in aquatic organisms, DWAF (1996a) suggests that it does not biomagnify through natural food webs. The toxicity of Pb in aquatic systems is largely dependent upon water hardness, DO, organic material and the life stage of the organism (Hellawell 1986; DWAF 1996a; Vlok et al. 2006). Toxicity of Pb is increased with a decrease in DO and $CaCO_3$ concentrations. Not much information is available on the effects of Pb on aquatic invertebrates, however, its acute and chronic effects on vertebrates are well documented.

Within natural aquatic environments, Pb concentrations are facilitated by the weathering of sulphide ores (e.g. galena), interactions with suspended sediments and organic ligands (DWAF 1996a). According to Vlok et al. (2006), the low solubility of Pb in aquatic environments restricts Pb pollution to localised areas around the points of discharge. Sources of Pb pollution include atmospheric deposition, urban and industrial runoff, wastewater runoff, mining activities and the combustion of fossil fuels (DWAF 1996a).

Most of the sites sampled during both low flow and high flow seasons had Pb concentrations below detection and therefore showed values of 0 $\mu g.L^{-1}$ (Figure 4.9E). The results of the low flow sampling survey indicated that Pb concentrations at STW and GLEN were 53.282 $\mu g.L^{-1}$ and 26.888 $\mu g.L^{-1}$, respectively. JASP with a Pb concentration of 24.561 $\mu g.L^{-1}$, was the only site during high flow sampling that had concentrations above detection. Each of the three measured concentrations were above the TWQR and CWQG, indicating Pb contamination at these sites. The pumping of raw sewage effluent into the Nyl River at the Modimolle WWTF is the probable cause for Pb contamination at STW for the low flow season and JASP for the high flow season. During high flow, the amount of water in the river had increased. This increased flow would carry pollutants entering the system at STW downstream to JASP, where the wetlands reduce flow and allow for the sedimentation. The high Pb concentration at GLEN for the low flow season is attributed to abnormally high concentrations of pollutants in the remaining 3 % of the water within Glen Alpine Dam.

4.2.4.6 VANADIUM

Vanadium has essential roles in the growth of plants and has also been suggested to be an import micronutrient for the human metabolism, however, when present in high concentrations, it becomes toxic (Kempster et al. 1980). Vanadium has a variety of oxidation states, of which, the most common form in surface waters is vanadium pentoxide (V_2O_5) (Byerrum et al. 1974). The solubility, biological availability and toxicity of V within aquatic environments is influenced by interactions with pH, DO and particulate matter (e.g. sulphur and chlorides) (ATSDR 2012). Furthermore, DWAF (1996b) states that V commonly interacts with other elements such as chromium and iron. In natural surface waters, V concentrations can range from 0.04 $\mu g.L^{-1}$ to 220 $\mu g.L^{-1}$ (region geology dependent) and concentrations tend to increase with a decrease in pH (ATSDR 2012).

Vanadium enters aquatic environments through natural sources such as the erosion of land surfaces, atmospheric deposition, precipitation and leaching from the natural geology (ATSDR 2012). Anthropogenic sources of V in aquatic environments include domestic sewage runoff, fertilizer usage and agricultural runoff, the burning of fossil fuels

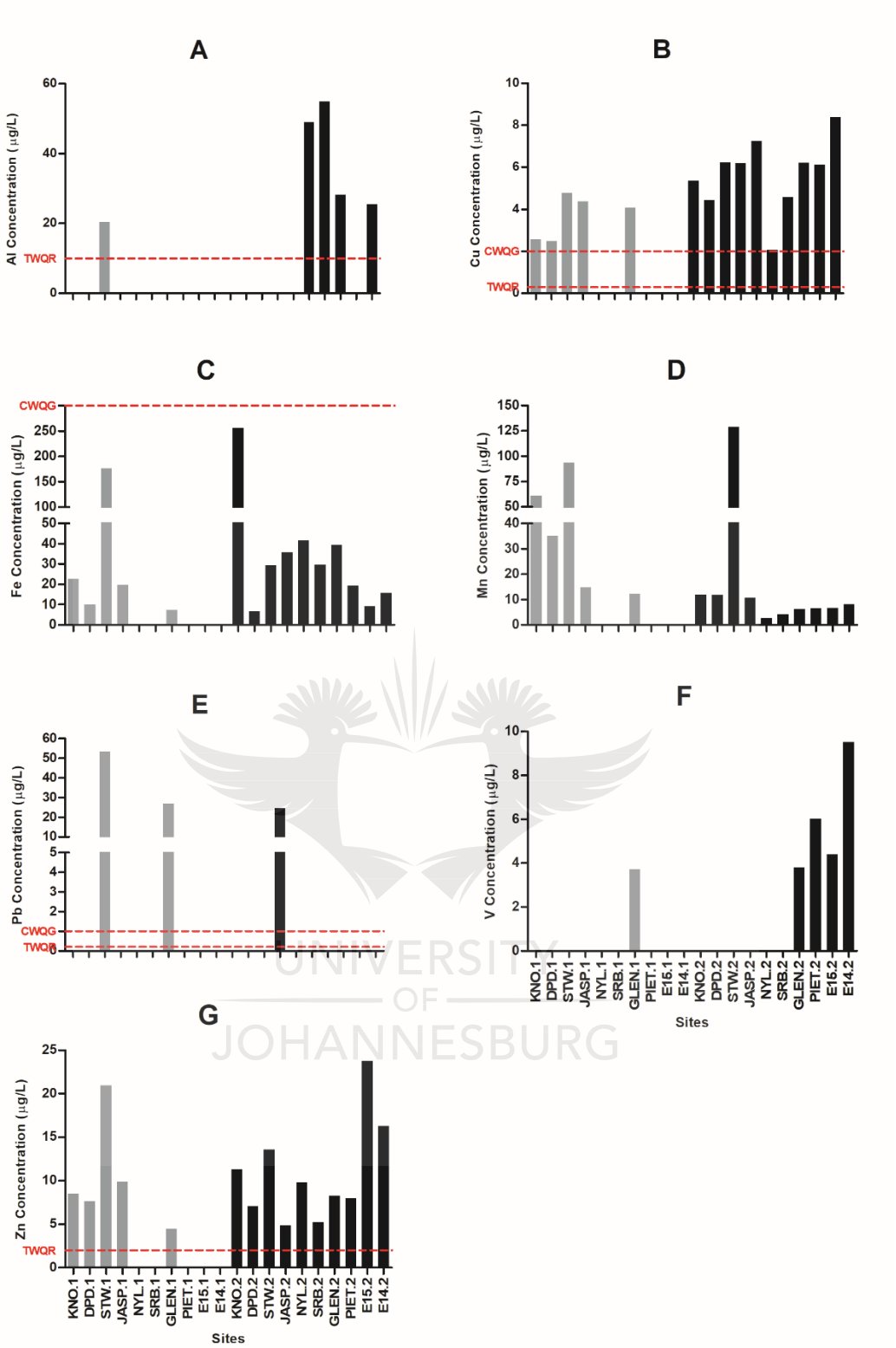

Figure 4.9: Metal concentrations obtained from the water samples collected in the Nyl and Mogalakwena Rivers. **A,** Aluminium; **B,** Copper; **C,** Iron; **D,** Manganese; **E,** Lead; **F,** Vanadium; **G,** Zinc. The numeral succeeding the site name denotes sampling season: **1,** low flow (grey bars); **2,** high flow (black bars). The red dotted lines indicate the Target Water Quality Ranges proposed by DWAF (1996a) and the Canadian Water Quality Guidelines (CCME 1999). All concentrations are presented as $\mu g.L^{-1}$.

and industrial processes (e.g. oil refining and mining activities) (Byerrum et al. 1974; ATSDR 2012).

Vanadium concentrations at all the sites along the Nyl River for both sampling surveys were below detection (0 $\mu g.L^{-1}$), whereas, the sites along the Mogalakwena River for both sampling surveys, except SRB, had V concentrations ranging from 3.723 $\mu g.L^{-1}$ at GLEN (low flow) to 9.508 $\mu g.L^{-1}$ at E15 (high flow) (Figure 4.9F). Considering that only the Mogalakwena sites showed measurable V concentrations, it would suggest that the underlying geology of the Mogalakwena River is rich in V, therefore, natural V concentrations within the surrounding waters would also be elevated. This is supported by the fact that a myriad of mining activities, including Vanadium mining, are being undertaken around the Mogalakwena River and its major tributaries (e.g. the Sterk River) (Ashton et al. 2001). Tentative TWQR of V exist for the domestic use of water, however, little is known about the effects of V on aquatic systems and no TWQR for these systems have yet been developed. Therefore, elevated V concentrations in the Mogalakwena River are not of concern to the health of the aquatic biota as they are resultant from the surrounding, natural geology.

4.2.4.7 ZINC

Zinc is a micronutrient that is essential to all life as it creates the active sites in a variety of metalloenzymes (DWAF 1996a). In water, two oxidation states of Zn occur, namely: its elemental form (Zn) and as divalent Zn (Zn^{2+}). Galvin (1996) states that Zn in water is usually detected as ionic, colloidal or inorganic compounds, therefore, Zn concentrations in water are generally quite low, below 10 $\mu g.L^{-1}$. Although Zn is essential for life, even at low concentrations, Zn^{2+} becomes toxic to aquatic organisms (DWAF 1996a). The toxicity of Zn is highly influenced by a variety of different reactions. When the hardness of water increases, Zn toxicity is reduced, whereas, in soft waters, Cu reacts with Zn, increasing its toxicity. A similar rise in Zn toxicity is seen when pH levels and DO concentrations are reduced (DWAF 1996a).

Naturally, Zn occurs in rocks and other geological structures and can enter aquatic environments via erosion, weathering or through the leaching of sediments and soils (Fisher 2011). Concentrations of Zn in aquatic systems are predominantly caused by the dissolution of minerals and the decomposition of organic matter. Zinc is a common anthropogenic pollutant that arises from industrial activities, including: pharmaceutical

manufacturing, the use and development of biocides and the galvanisation of metals (DWAF 1996a; Kempster et al. 1996).

Zinc concentrations ranged between 4.473 $\mu g.L^{-1}$ at GLEN for the low flow season to 23.573 $\mu g.L^{-1}$ at E15 for the high flow season (Figure 4.9G). Average Zn concentrations were higher during the high flow season compared to the low flow season, which could indicate that increased volumes and flow rates of water influenced Zn concentrations in the system. The concentrations of Zn measured at all the sampling sites were above the TWQR of 2 $\mu g.L^{-1}$, and for the most part, excluding STW (low flow) and E15 (high flow), the concentrations remained relatively stable. Considering that KNO, the source of the Nyl River, had Zn concentrations of 8.506 $\mu g.L^{-1}$ and 11.333 $\mu g.L^{-1}$ for the low flow and high flow seasons, respectively, the assumption would be that Zn levels within this system are naturally high and are therefore not of concern to the aquatic biota. Although the Zn concentrations reported by Dahms (2016) were a lot higher than those reported in this study, similar spatial trends in the patterns of accumulation of Zn at sites along the Nyl River were observed.

4.3 CONCLUSIONS

The water analyses have provided a comprehensive understanding of the water quality in both the Nyl and Mogalakwena Rivers. When considering the results in a holistic manner, they clearly show that the Modimolle WWTF is having a severe impact on the water quality of the Nyl River. The water quality of the sites upstream of the Modimolle WWTF (KNO and DPD) is minimally impacted; the levels of all the tested parameters, including metal concentrations, appear to be natural. The minimally impacted waters of KNO and DPD become severely polluted at STW through sewage effluent runoff. The runoff and pumping of raw sewage into the Nyl River seems to be the principal contributor to elevated levels of all the tested nutrients and metal concentrations within the Nyl River system, subsequently affecting the downstream water quality at JASP. During periods of inundation, the Nylsvley wetland appears to be fulfilling its natural ecosystem service of purifying the water coming from the upstream sites (STW and JASP), because most of the parameters measured at NYL, were present in low concentrations.

The conditions within the Mogalakwena River were highly variable between low flow and high flow sampling surveys. During low flow sampling, most of the Mogalakwena River had little to no surface water flow and drought conditions made sampling efforts difficult.

The site downstream of the Glen Alpine Dam (GLEN), was the only site that was sampled during the low flow season, showing high concentrations for only two of the measured parameters, namely Cl^- and Pb. These concentrations could be attributed to the extensive water usage and evaporative loss during the drought conditions, where the volume of water within the dam was reduced to a mere 3 %. Coupled with a reduction in water volume, persistent nutrients and metals may have become concentrated, increasing the levels of these substances in the water at this site. During high flow sampling, the sites along the Mogalakwena River were found to be relatively stable with regards to each of the analysed parameters, except F^-. The increased levels of F^- at PIET could have arisen from insecticide usage in the agricultural lands located between GLEN and PIET. The levels of the remaining parameters were attributed to natural background levels, however, flooding conditions at the time of sampling may have had a dilution effect, reducing the concentrations of these nutrients and metals within the water column. The results of the low flow and high flow sampling surveys may not accurately represent the water quality in the Mogalakwena River at the time of sampling. Therefore, to precisely assess the water quality of the Mogalakwena River, further sampling efforts are required during normal flow conditions.

CHAPTER 5 – SEDIMENT QUALITY

5.1 INTRODUCTION

According to Chapman (1996), sediment (fine particulate organic matter [FPOM] and course particulate organic matter [CPOM]) relates to particles that are greater than 0.45 µm in size. Sediments in aquatic systems are the result of the physical and chemical weathering of rocks alongside erosive mechanisms that facilitate the transferal and deposition of soil and sediment particles from their point sources into aquatic systems (Chapman 1996). When in aquatic systems, sediments are suspended and are transported downstream, subsequently becoming categorized according to particle size (Chapman 1996). While being transported, suspended sediment particles act as attachment sites for many forms of contaminants such as metals and organic matter. Therefore, the sediments and attached contaminants are carried downstream, eventually reaching an ultimate settling point (sink), where accumulation occurs (Chapman 1996; USEPA 2001; Gerber et al. 2015). Due to the long lifespans of contaminants within these sinks, the sediments may act as a constant source of pollution to the surrounding aquatic environment (Filgueiras et al. 2004). Thus, the health of sediments is of utmost importance for the maintenance of aquatic integrity in many aquatic ecosystems (Pejman et al. 2015).

Sediment is a vital constituent within aquatic ecosystems. It provides environmental services to many forms of aquatic biota in the form of habitat availability, food resources and areas for the spawning and rearing of young macroinvertebrates (USEPA 2001; Ferreira 2010). The characteristics of sediments are important for both toxicity and aquatic biodiversity. Groffman & Bohlen (1999) and Ferreira (2010) state that the two sediment characteristics of most importance are organic content and grain (particle) size.

Firstly, the distribution of grain sizes is ultimately indicative of the physical characteristics of the sediment, which, in turn, is the determining factor for the composition of biological community assemblages (PSEP 1986; Wenter & van Vuren 1997). Grain size can give an indication of the diversity of benthic macroinvertebrates. Through sediment transportation, the grain size of sediments are seperated into various sizes; areas with coarser particles are generally indicative of low benthic diversity, whereas, areas with finer particles typically exhibit a higher diversity of benthic organisms (Malherbe 2006).

Secondly, the Coastal Research Unit of Zululand (CRUZ) (2000) state that the productivity of a system can be determined by measuring the organic content present within its sediments, where organic content is defined as the quantity of dead organic material that is available for benthic aquatic macroinvertebrates to utilise. Di Toro et al. (1991) describes that the non-ionic organic chemical bioavailability of contaminants within a system is directly impacted by the amount of oxidisable organic material within the sediment. In this way, the bioavailability of these toxicants (e.g. metals) can be influenced by the total organic content of the sediment. Benthic aquatic macroinvertebrates are in direct contact with the sediment, therefore, these organisms show adverse effects to sediment contamination. Aquatic pollution arising from sediment contamination often yields chronic implications, as long-term exposures can cause impacts to the growth, behaviour and reproduction of many aquatic biota (Chen & Chen 1999).

The aim of this chapter is to assess the quality of the sediments collected from the Nyl and Mogalakwena River Systems in terms of organic content and grain size distributions.

5.2 RESULTS

The physical characteristics of the sediment samples collected from sites along the Nyl and Mogalakwena Rivers were analysed according to the methods described in Chapter 3. The determined organic content and grain size distributions were converted to a percentage proportion of the total sediment sample and were then compared with the classification systems proposed by Cyrus et al. (2000) and USEPA (1991) for organic content (Table 5.1) and grain size distributions (Table 5.2), respectively.

Table 5.1: The classification of organic content percentages as described by USEPA (1991).

Organic Content (%)	Classification
< 0.05	Very low
0.05 – 1	Low
1 – 2	Moderately low
2 – 4	Medium
> 4	High

Table 5.2: The classification used by Cyrus et al. (2000) for the analysis of sediment grain size.

Grain Size (µm)	Classification
> 4000	Gravel
2000 – 4000	Very coarse sand
500 – 2000	Coarse sand
212 – 500	Medium sand
53 – 212	Very fine sand
< 53	Mud

The organic content of the sediments collected during the low flow season ranged from 2 % to 24.38 % as can be seen in Table 5.3. The sediments collected at KNO and STW were indicative of sediments with high organic content percentages (Table 5.1), having an organic percentage composition of 24.38 % and 10 %, respectively. The sites DPD, JASP and GLEN sampled during the low flow season had medium percentages of organic content, containing 2 %, 2.5 % and 2.5 %, respectively.

Table 5.3: Physical characteristics (organic content and grain size distribution) of sediment sampled at sites along the Nyl and Mogalakwena Rivers during the low flow sampling season. The numeral succeeding the site name denotes sampling season: **1,** low flow (July 2016). All values are presented as percentage (%).

Site Name	Organic content	Grain sizes					
		Gravel	Very coarse sand	Coarse sand	Medium sand	Fine sand	Mud
KNO.1	24.38	0.01	0.13	14.91	31.38	38.69	14.88
DPD.1	2.00	33.11	9.64	16.57	29.22	10.87	0.59
STW.1	10.00	0.64	0.64	9.21	41.73	43.51	4.27
JASP.1	2.50	10.52	4.47	17.83	57.45	9.37	0.36
GLEN.1	2.50	1.44	2.39	38.41	42.21	14.31	1.23

The results of the grain size distributions of the sediments collected during the low flow season are presented in Table 5.3. These results indicate that the grain size of the sediment collected at KNO was dominated by medium and fine sands as more than 80 % of the sample was represented by these grain sizes. At DPD, no dominant sediment fractions were identified, as all grain size categories apart from mud (< 53 µm), ranged

between 9.64 % and 33.11 %, indicating a heterogeneous distribution of sediment particles of varying sizes. As with organic content, KNO and STW showed similar grain size distributions; STW was dominated by medium and fine sands, with these fractions contributing relatively equal percentages of 41.73 % and 43.51 %, respectively. The grain size distributions at JASP were dominated by medium sand which constituted 57.45 % of the sediment sample. The grain size of the sediments collected from GLEN are distributed between coarse sand (38.41 %), medium sand (42.21 %) and find sand (14.31 %), constituting a combined percentage of 94.93 %. The most underrepresented sediment fractions for the low flow sampling survey were gravel, very coarse sand and mud, as many of the collected sediments from the sampled sites contained very low percentages of these fractions. Overall, the sediments sampled in the low flow season consisted mainly of sands with coarse, medium and fine grain sizes.

The organic content of the sediments collected during the high flow season predominantly ranged from 0 % – 2.49 %, with the exception of sites STW and NYL, which, correspondingly had high organic contents of 5.97 % and 18.41 % (Table 5.4). The organic content at KNO dropped considerably between the sampling seasons from 24.38 % (high organic content) in the low flow season to 2 % (medium percentage of organic content) in the high flow season. The organic content classification at DPD and STW remained the same between both sampling seasons, having medium and high percentages of organic content, respectively. The organic content at JASP and GLEN had been reduced from medium organic percentages in the low flow season to moderately low organic content percentages in the high flow. The sediment collected from sites on the Mogalakwena River had organic content percentages ranging from low (0.05 – 1 %) to medium (2 – 4 %), with the sediment collected from E14 on the Limpopo River containing 0 % organic content.

The grain sizes of the sediments collected from sampling sites during the high flow season followed similar trends to those collected in the low flow season. These results are tabulated in Table 5.4. Like with the organic content percentage, the sediment composition at KNO had shifted from predominantly medium and fine sands to being dominated by 72.95 % of medium sand. Grain size distributions at DPD and STW and JASP remained constant between low and high flow season. At NYL, the sediment showed to be evenly distributed, with grain size distributions ranging from 3.69 % gravel to 32.19 % fine sand. It is important to note, however, that the larger gravel particles (gravel and very coarse sand) present at NYL consisted of larger pieces of organic matter,

characteristic of densely vegetated wetland areas such as the Nylsvley wetland (Ferreira 2010). The sediments from the sampling sites located on the Mogalakwena and Limpopo Rivers (SRB – E14) were generally dominated by coarse sands (500 – 2000 µm) with less coarse sands being dominant at SRB and E15 which showed compositions of 61.48 % and 43.80 % of medium sand, respectively. The sampling sites GLEN (53.9 %), E15 (85 %) and E14 (57.63 %) contained sediments dominated by the coarse sand fraction. As in the low flow season, the most underrepresented sediment fractions in sediment sampled from the high flow season were gravel, very coarse sand and mud. Overall, the sediments from sites in the Nyl River consisted mostly of medium to fine sand grains, whereas, the sediments in the Mogalakwena and Limpopo Rivers consisted mainly of coarse and medium sand grains.

Table 5.4: Physical characteristics (organic content and grain size distribution) of sediment sampled at sites along the Nyl and Mogalakwena Rivers during the high flow sampling season. The numeral succeeding the site name denotes sampling season: **2,** high flow (February 2017). All values are presented as percentage (%).

Site Name	Organic content	Grain sizes					
		Gravel	Very coarse sand	Coarse sand	Medium sand	Fine sand	Mud
KNO.2	2.00	1.16	0.77	20.46	72.95	4.56	0.10
DPD.2	2.49	30.13	12.03	15.22	33.42	9.12	0.08
STW.2	5.97	0.64	1.24	21.37	35.43	34.53	6.79
JASP.2	1.49	1.03	1.38	7.36	57.79	31.49	0.95
NYL.2	18.41	3.69	6.10	25.10	24.52	32.19	8.41
SRB.2	2.00	0.00	0.05	7.34	61.48	29.83	1.30
GLEN.2	1.50	0.64	4.61	53.90	35.71	5.06	0.09
PIET.2	0.50	4.56	6.45	85.00	3.77	0.11	0.11
E15.2	2.49	0.09	0.87	24.47	43.80	25.22	5.55
E14.2	0.00	0.12	1.52	57.63	36.33	4.29	0.12

5.3 DISCUSSION

The results of the above analyses revealed that sediments within the Nyl River for both low and high flow sampling surveys contained moderately low to medium percentages of organic matter. According to CRUZ (2000), the amount of organic content within aquatic

sediments is a measure of the systems productivity with a higher organic content signifying a higher productivity. Therefore, the productivity of the Nyl River, for the most part, can be considered to be moderately low to medium. During the low flow season, the sediments collected from KNO were identified as having a high percentage of organic content (24.38 %). The possible reason for this may be the obstruction of flow caused by a man-made weir present at the site (see Chapter 2). Moreover, Musa (2016) described that the area above and around the weir was dominated by aquatic macrophytes such as *Phragmites* spp. and *Cyperus* spp. which may be introducing additional organic matter into the river at this site. A reduction of flow caused by low precipitation during the low flow season in conjunction with the weir, would have created a sink where organic matter and finer particulate matter could settle, thus changing the sediment characteristics to favour benthic macroinvertebrates such as species of the Chironomidae. This corresponds with the invertebrate analysis, whereby the taxa sampled at KNO were dominated by a large number of Chironomidae individuals. Taxa within the family Chironomidae are gathering collectors that feed on detritus (dead organic material), the main constituent of the organic content in river sediments (Chapman 1996). During the high flow season, the waters above the weir were not seen to be stagnant, and thus natural churning of the sediments by flow would have transported sediments and organic material downstream. This is supported by the macroinvertebrate data, where a temporal shift in macroinvertebrate community assemblages between low flow and high flow was noted (see Chapter 6).

The organic content at DPD for both sampling surveys was deemed to be moderate and there were no grain size fractions that appeared to be dominant. The moderate productivity and variety of different benthic habitats could be a factor accounting for DPD having the highest macroinvertebrate diversity for both sampling surveys. During the high flow season, increased precipitation had caused considerable sediment deposition at the site from the dirt road above. Although this sedimentation didn't influence the grain size distributions at this site, it reduced the abundance of macroinvertebrate individuals at this site considerably, most likely due to smothering or habitat loss or both (Hart 1985; Dudgeon et al. 2005).

Considering the relationship between organic content and river productivity, the productivity of the Nyl River at STW was high, subsequently decreasing to a moderately low to medium productivity at JASP. The sediments at STW consisted predominantly of medium and fine sands and a low percentage of mud, whereas the sediments at JASP

consisted mainly of medium-grained sand. The water quality results in Chapter 4 indicate that STW is severely impacted by sewage effluent discharge entering from the Modimolle WWTF. This effluent, rich in organic pollutants (nitrogenous pollutants and phosphates), is suspended in the water column, turning the water grey in colour. Furthermore, these pollutants are transported downstream by river flow to JASP, subsequently reducing the macroinvertebrate diversity at this site. As these pollutants are transported, they bind to sediment particles and eventually settle out of the water column, accumulating within the sediment on the river bed (Chapman 1996; USEPA 2001; Dallas & Day 2004). According to Dallas & Day (2004), smaller particles have a larger surface area to volume ratio, and therefore have a larger area for the attachment of contaminants. The finer grain sizes and the high organic content of the sediment at STW for both the low and high flow seasons is attributed to effluent discharge at STW and could be a source of sustained pollution, even if effluent discharge is halted. Moreover, the high organic content and small grain size of these sediments negatively affect benthic and pelagic macroinvertebrate assemblages and more tolerant taxa such as *Tubifex tubifex* (O.F. Müller, 1774) and species of the Syrphidae and Chironomidae are favoured. After STW, the physical characteristics of the sediment at JASP seem to return to a state like those of the sites above the Modimolle WWTF. This recovery in sediment characteristics may be attributed to the wetlands that are present between STW and JASP. The vegetation within these wetlands may cause particulate matter to settle out of the water column by reducing river flow and trapping any suspended particulate and organic matter (Davies & Day 1999). Although these wetlands appear to be aiding in reducing the downstream transportation of contaminated particulate matter, they may be compounding the toxicity of many water pollutants, including nitrogen.

The organic content of the sediments collected at NYL was high, indicating that the Nylsvley wetland is a productive system. This was expected as wetlands usually contain higher organic contents than lentic systems due to their increased vegetation and lack of flow (Ferreira 2010; Dyamond 2017). Furthermore, the sediments at NYL were seen to have the highest percentage of mud (< 53 µm), which according to Dallas & Day (2004) is typical wetland environments because they exhibit reduced flow and have fewer biotopes as compared to riverine habitats. The macroinvertebrate community assemblages at NYL showed a recovery when compared to the upstream sites (STW and JASP), suggesting that the wetland is fulfilling its environmental service of ‘filtering’

contaminants from the water. The taxa identified from NYL were those better adapted to survival in lotic systems.

The productivity of the Mogalakwena River was low to moderate with the sediment grain sizes being predominantly coarse and medium sand. As described in Chapter 2, the steep gradient variations along the course of the Mogalakwena River have altered the river bed from being primarily alluvial-based, as in the Nyl River, to predominantly bedrock in the Mogalakwena River (Tooth et al. 2002; McCarthy et al. 2011). This change in river geomorphology may account for the coarser grain sizes that were present in the Mogalakwena River. At the time of high flow sampling, the Mogalakwena River was in a state of flood, which may have altered the sediment composition at the sampling sites. Dallas & Day (2004) describe that flooding activity resuspends sediment particles, transporting them downstream. This disturbance and transport of sediment coupled with increased river flow would have had an affect on macroinvertbrate community assemblages at the sampling sites, therefore, these assemblages would not be representative of the river at the time of high flow sampling. In addition to flooding activity, Boroto & Görgens (1999) state that the Mogalakwena River is highly modified, containing "*several hundred small farm dams*" that are used for domestic and agricultural purposes. Furthermore, the authors describe that the reductions in surface water flow caused by these farm dams have become common in the Mogalakwena River system, thereby modifying natural sediment movements and macroinvertebrate community assemblages.

5.4 CONCLUSION

The sediment quality within the Nyl River shows varying degrees of modification. At the origin of the Nyl River (KNO) during low flow conditions, flow obstructions in the form of a man-made weir is affecting organic loads into the system and therefore the sediment composition, however, during high flow periods, organic content is reduced, and sediment quality is restored. At DPD, the medium productivity and even distribution of varying particle sizes may be aiding in providing habitat for a greater diversity of macroinvertebrate taxa. After DPD, the sediment becomes highly modified by effluent discharge from the Modimolle WWTF. At NYL, the sediments were found to have higher percentages of organic content and mud particles. This was expected, however, as wetlands contain a higher density of vegetation and the lack of flow allows fine particles to settle out of the water column. According to Boroto & Görgens (1999), the Mogalakwena River is in a highly modified state due to several hundred farm dams along

its course. This was not quite reflected by the sediment analyses which found that the sediments contained low to medium organic content percentages and with grain size distributions of predominantly coarser and medium sands. Nevertheless, flow modifications may be severely impacting the sediments and therefore benthic macroinvertebrate communities within the Mogalakwena River, however, due to flooding conditions at the time of sampling, further research during normal flow is required to validate these claims. Sediment quality is an important aspect in maintaining aquatic integrity. The modified state of the sediments within the Nyl and Mogalakwena Rivers has already had negative implications to aquatic biotic communities and should be monitored frequently as to hinder any further impacts to the water quality and the aquatic health of these important water systems.

CHAPTER 6 – AQUATIC MACROINVERTEBRATE COMMUNITY ASSEMBLAGES

6.1 INTRODUCTION

One of the key components of the NWA 36 of 1998 (RSA 1998) is the protection and sustainable use of the reserve, which, according to RSA (1998) is defined as "*the quality and quantity of water required to satisfy the basic human needs, and to protect aquatic ecosystems, in order to secure ecologically sustainable development and use of the relevant water resources*". Monitoring techniques form an important part of maintaining the reserve and aid determining the ecological state of water resources so that mitigation measures can be suggested and implemented (Malherbe 2010; Ollis et al. 2006).

Traditionally, the monitoring of water quality in South Africa was limited to the analysis of the physical and chemical properties of periodically collected water samples. This form of analysis, however, was deemed insufficient for the complete assessment of aquatic integrity, as it does not consider the auxiliary effects chemicals could have on the aquatic biota, spatial and temporal gradients and the in-stream chemical interactions that may lead to heightened toxicity or speciation (Ten Bink & Woudstra 1991; Karr & Chu 1998; Oberholster et al. 2008). Additionally, Roux et al. (1993) state that the chemical monitoring of water alone does not account for anthropogenic impacts such as habitat alterations, habitat destruction and flow modifications. Therefore, the monitoring of biological communities within river systems is a useful tool, as organisms integrate the effects and interactions of all of the aforementioned variables (Karr & Dudley 1981; Roux et al. 1993; Barbour et al. 1999).

There have been a variety of organism groups that have been used for biological monitoring purposes (e.g. bacteria, diatoms, algae and fish), however, macroinvertebrates remain the most widely utilised assemblage (Dallas & Day 2004). For the bioassessment of riverine environments, the aquatic macroinvertebrates that are often used are those that inhabit riverbeds (varying substrate types) or vegetation (submerged or emergent), these include insects and their larvae, molluscs, crustaceans and worms (Allan 1995). The fact that aquatic macroinvertebrate taxa are short-term indicators of water quality (they are directly influenced by changes to the chemical and physical properties of their surrounding environments) is one of the primary purposes for using them in biological monitoring studies. Although macroinvertebrate taxa are

commonly used as biomonitoring organisms, the identification of these organisms to lower taxonomical levels remains difficult as their taxonomy, specifically in South Africa, is poorly documented.

According to Allan & Castillo (2007), de Necker (2016) and Moyo & Richoux (2017), most biological assessments using macroinvertebrate community assemblages are used in one of two ways: (i) taxonomic assessments – the determination of overall biodiversity using species richness and abundance; and (ii) functional assessments – the determination of environmental conditions using biological traits. One form of functional assessments is the determination of the feeding strategies (Functional Feeding Groups – FFG) that macroinvertebrates within an assemblage utilise (Cummins et al. 2005). Macroinvertebrate taxa represent various trophic levels within aquatic ecosystems, from detritus feeders to top predators. Therefore, the different feeding strategies that these macroinvertebrate taxa utilise can be used to evaluate and assess ecological processes at an ecosystem level to get a better understanding of the effects anthropogenic activities are having on that particular system (Barbour et al. 1999; Merrit et al. 1999; de Necker 2016).

Statistical tests in the form of univariate (diversity indices) and multivariate (ordination plots) analyses are used to simplify the patterns of change within the macroinvertebrate community assemblages as they are usually difficult to observe and describe due to their complexity (matrices are too large as they include species and sample data). Moreover, the graphical representations that these statistical methods produce allow relationships between macroinvertebrate community assemblage data and changes in environmental variable data to be observed (Clarke & Warwick 1994).

The aim of this chapter was to evaluate and determine the composition of the macroinvertebrate community assemblages within the Nyl and Mogalakwena Rivers. Uni- and multivariate analyses were used for the spatial and temporal investigation, which will aid in the identification of anthropogenic activities (point source and non-point source pollutants) that are impacting the macroinvertebrate community assemblages within these rivers.

6.2 RESULTS AND DISCUSSION

6.2.1 GENERAL MACROINVERTEBRATE BIODIVERSITY

The collected macroinvertebrate individuals were identified to the lowest taxonomic level possible using relevant taxonomical guides and electronic databases (Day et al. 2001; Day et al. 2002; Day & de Moor 2002a, 2002b; de Moor et al. 2003a, 2003b; Stals & de Moor 2008; World Register of Marine Species (WoRMS); A catalogue of the insects of Southern Africa; BugGuide.Net), however, a lack of knowledge regarding the taxonomy of most South African macroinvertebrates species made the identification of all the samples to species level difficult. Therefore, in this chapter, the word 'taxa' will be used as a broad term describing the various taxonomic levels to which the macroinvertebrate samples were identified.

A total of 190 taxa from 74 families and 20 orders were identified from the sampling sites along the Nyl and Mogalakwena Rivers for both sampling surveys. Due to the difficulty in species-level identification, the total number of taxa collected for this study may, in fact, be higher, as many of the macroinvertebrate samples could only be identified to the order, family or genus level. Table 6.1 provides a detailed taxonomic list of all the identified taxa, including their abundances and the localities in which they were sampled.

The dominant orders of macroinvertebrates for the combined low and high flow sampling seasons were Diptera and Coleoptera containing 14 families each, while the most diverse family was Libellulidae in which 13 taxa were identified. The most commonly occurring families were Chironomidae and Baetidae, being present at 93.3 % and 86.7 % of all sampling sites for both sampling seasons, respectively. Of the 74 families identified in this study, 67 were identified during the low flow season and 60 during the high flow season. From the total number of families, seven were represented by a single individual: Hydrachnidae, Mesoveliidae, Pleidae, Chaoboridae, Hydrochidae, Dryopidae and Chrysomelidae.

Figure 6.1A and B show graphical representations of the total number of taxa (species/ taxonomic richness) and the total number of individuals of each taxon (abundance) collected from the sampling sites for both sampling surveys, respectively. In Figure 6.1A, a trend is evident, that shows the total number of taxa within the Nyl River is high in the upper reaches at KNO and DPD, thereafter decreasing at STW and JASP. Sites KNO and DPD have a higher number of taxa as they are close to the source of the Nyl River, where the water is relatively unimpacted by anthropogenic activities. Furthermore, an

extra biotope in the form of SIC was sampled at KNO for both sampling seasons which would have increased the number of taxa identified at this site. After STW and JASP for the high flow sampling season, the macroinvertebrate community assemblages show a recovery at NYL. Wetlands attenuate the flow of rivers and streams, thereby forming lentic systems (Musa 2016). The macroinvertebrate community assemblages identified from NYL showed a shift towards taxa that were more suited and better adapted to lentic environments such as the Nylsvley Wetlands. The number of taxa identified from GLEN in the high flow season (11 taxa) was considerably lower than in the low flow season (45 taxa). The reduction in the number of taxa at GLEN was attributed to flooding activity during the time of high flow sampling. This flooding activity also accounted for the reduced number of taxa at other sites along the course of the Mogalakwena River, including SRB, PIET and E15. During flood conditions, a large volume of water flows through the channel of a river and apart from the obvious mechanical removal and subsequent washing of the macroinvertebrates downstream, floods can also affect macroinvertebrate assemblages by (i) disturbing the available habitat, (ii) changing food supplies and (iii) altering the chemical and physical factors of the water (Rader et al. 2008).

For the most part, the total abundance of individuals collected (Figure 6.1B) in the low season is higher than those collected in the high flow season. The sites STW and JASP were the only sites where the high flow abundances exceeded that of the low flow abundances. Inversely to the total number of identified taxa (Figure 6.1 A), there is an increase of the abundance of macroinvertebrates at STW and JASP compared to KNO and DPD, however, this was due to the presence of the dominant species *Tubifex tubifex* (O.F. Müller, 1774) (Naididae) which had a combined abundance of 11 578 individuals. There was a considerable decrease in the abundance of macroinvertebrate taxa at DPD during the high flow season compared to the low flow season. Increased precipitation washed sediment from the sand road above the site into the river, this sediment was deposited into the main channel and the flow of the river was reduced. The reduction of flow and the modifications to habitats caused by this sedimentation is the main attribute to the reduction of abundances and taxa richness at this site (Figure 6.1A, B) (see Chapter 5). A student T-test revealed that the number of taxa sampled at GLEN for the high flow season was significantly lower ($p < 0.05$) than the number sampled during the low flow season, with flooding activity during high flow sampling being the most probable cause of this reduction in taxa abundances. After PIET for the high flow season, the abundances of macroinvertebrate taxa at the sites E15 and E14 increased. However, the

macroinvertebrate community assemblages and diversity at these sites would most likely be altered when flow and water conditions return to normal (Rader et al. 2008).

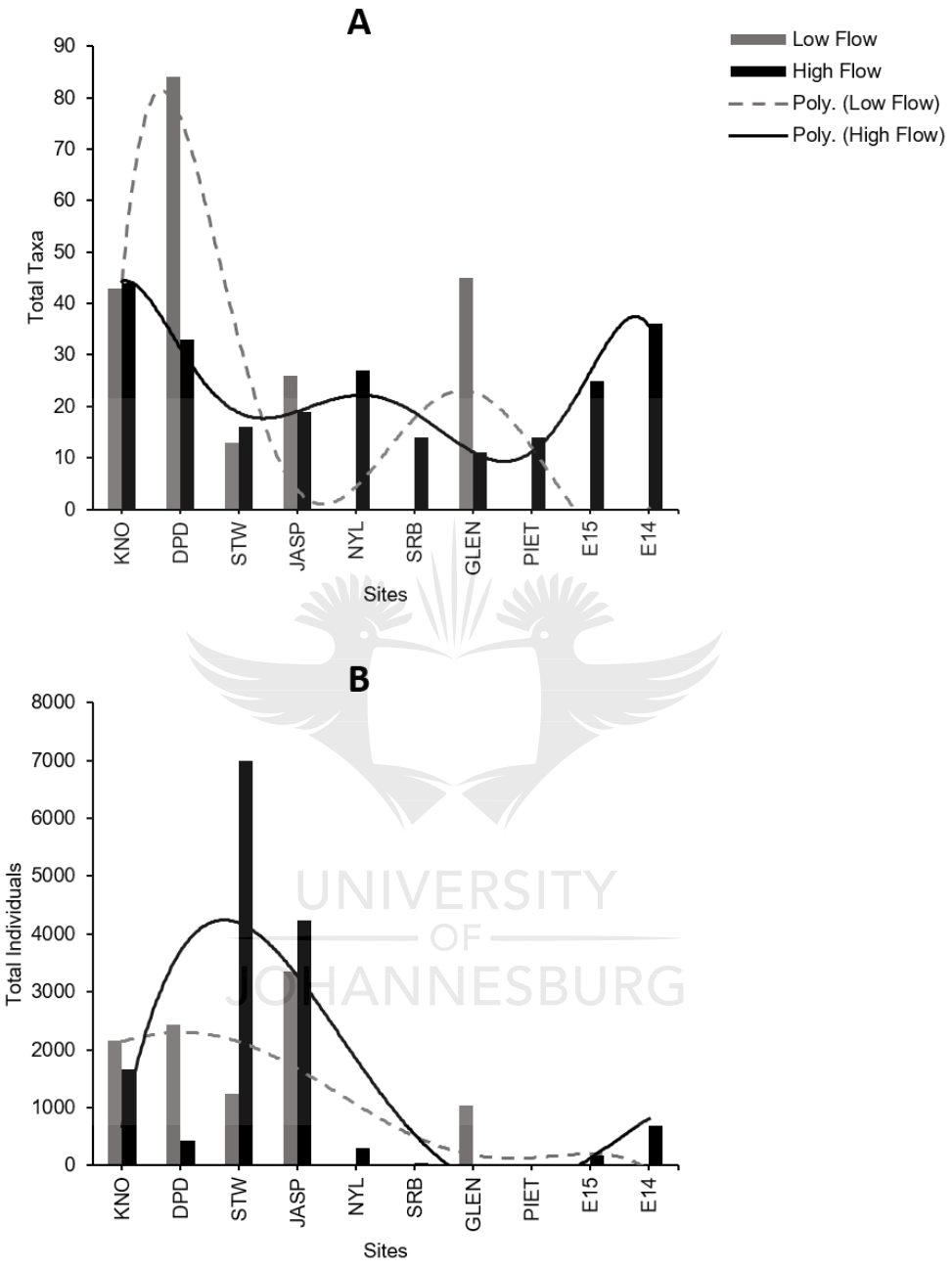

Figure 6.1: Graphical representations of the macroinvertebrate taxa collected in the Nyl and Mogalakwena Rivers during low flow (July 2016) and high flow (February 2017) sampling seasons. **A,** the total number of taxa (taxa richness); **B,** the total number of individuals (abundance). The polynomial trend lines are used to determine if any trends between sites are visible with regards to the number of taxa and number of individuals collected from each site.

Table 6.1: Macroinvertebrate taxa identified from all the sampling sites for both sampling surveys (July 2016 and February 2017). The taxonomy of the macroinvertebrates was followed according to the "*Guides to the Freshwater Invertebrates of Southern Africa*" (Day et al. 2001; Day et al. 2002; Day & de Moor 2002a, 2002b; de Moor et al. 2003a, 2003b; Stals & de Moor 2008) and the online databases: World Register of Marine Species (http://www.marinespecies.org), A catalogue of the insects of Southern Africa (http://www.ru.ac.za/media/rhodesuniversity/resources/martin/Insects.html), and BugGuide.Net (https://bugguide.net/node/view/15740).

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
Phylum: Cnidaria				
Hydrozoa/ Hydroidolina	Anthoathecata	Hydridae Dana, 1846	*Hydra oligactis* Pallas, 1766	GLEN.1 (188)
Phylum: Annelida				
Clitellata/ Oligochaeta	Haplotaxida	Naididae Ehrenberg, 1828	*Branchiura sowerbyi* Beddard, 1892	KNO.1 (13); GLEN.1 (12); KNO.2 (35); E15.2 (4); E14.2 (2)
			Tubifex tubifex (O.F. Müller, 1774)	STW.1 (608); JASP.1 (2120); STW.2 (5345); JASP.2 (3505)
		Lumbricidae Claus, 1876	Lumbricidae gen. spp.	KNO.1 (285); DPD.1 (148); GLEN.1 (126); KNO.2 (1263); DPD.2 (15); NYL.2 (8); SRB.2 (4); GLEN.2 (12); PIET.2 (1); E14.2 (7); E15.2 (32)
	Lumbriculida	Lumbriculidae Vejdovský, 1884	Lumbriculidae gen. spp.	DPD.1 (8); STW.1 (3); JASP.1 (24); GLEN.1 (26); STW.2 (114); JASP.2 (1)
Clitellata/ Hirudinea	Rhynchobdellida	Glossiphoniidae Vaillant, 1890	*Helobdella conifera* (Moore, 1933)	JASP.1 (1); JASP.2 (27); NYL.2 (1)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
			Helobdella stagnalis (Linnaeus, 1758)	JASP.1 (573); GLEN.1 (20); KNO.2 (3); JASP.2 (338); SRB.2 (7); E15.2 (1); E14.2 (1)
	Arhynchobdellida	Salifidae Johansson, 1910	*Barbronia* sp.	GLEN.1 (1); JASP.2 (9)
Phylum: Mollusca				
Gastropoda/ Prosobranchia	Neotaenioglossa	Thiariidae Gill, 1871 (1823)	*Melanoides tuberculata* (O. F. Müller, 1774)	GLEN.1 (10); E15.2 (39); E14.2 (105)
			Tarebia granifera (Lamarck, 1816)	E14.2 (14)
Gastropoda/ Heterobranchia	Hygrophila	Bulinidae P. Fischer & Crosse, 1880	*Bulinus africanus* (Krauss, 1848)	DPD.1 (5); DPD.2 (61)
			Bulinus depressus Haas, 1936	KNO.1 (3); DPD.1 (3); GLEN.1 (1); KNO.2 (1); DPD.2 (38); NYL.2 (1)
			Bulinus forskalii (Ehrenberg, 1831)	NYL.2 (2); PIET.2 (1); E15.2 (5)
		Planorbidae Rafinesque, 1815	*Biomphalaria pfeifferi* (Kraus, 1848)	KNO.2 (3)
			Ferrissia sp.	DPD.1 (6), GLEN.1 (9); DPD.2 (1); PIET.2 (1); E15.2 (1)
			Gyraulus costulatus (Krauss, 1848)	DPD.1 (19); GLEN.1 (7); DPD.2 (18); STW.2 (1)
		Physidae Fitzinger, 1833	*Physella acuta* (Draparnaud, 1805)	STW.1 (1); JASP.1 (98); JASP.2 (299); NYL.2 (1)
		Lymnaeidae	*Pseudosuccinea columella* (Say, 1817)	KNO.2 (10)
			Radix natalensis (Krauss, 1848)	KNO.1 (3); DPD.1 (26); GLEN.1 (2); DPD.2 (72)
Bivalvia/ Heterodonta	Venerida	Corbiculidae Gray, 1840	*Corbicula fluminalis* (O. F. Müller, 1774)	E15.2 (1); E14.2 (2)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
		Sphaeriidae Deshayes, 1855 (1820)	Sphaeriidae gen. spp.	GLEN.1 (1); KNO.2 (6)
Phylum: Arthropoda/ Subphylum: Crustacea				
Malacostraca/ Eumalacostraca	Decapoda	Potamonautidae Bott, 1970	*Potamonautes unispinus* Stewart & Cook, 1998	DPD.1 (2); GLEN.1(1); KNO.2 (4); DPD.2 (1)
		Atyidae De Haan, 1849	*Caridina nilotica* (Roux, 1833)	DPD.1 (16); GLEN.1 (4); DPD.2 (7); GLEN.2 (1); PIET.2 (2)
Phylum: Arthropoda/ Subphylum: Chelicerata				
Arachnida/ Acari	Sarcoptiformes/ Oribatida	Hydrozetidae Grandjean, 1954	*Hydrozetes* sp.	DPD.1 (7)
	Trombidiformes/ Prostigmata	Hydrachnidae Leach, 1815	*Hydrachna* sp.*	DPD.1 (1)
	Araneae/ Opisthothelae	Tetragnathidae Menge, 1866	*Tetragnatha boydi* Pickard-Cambridge, 1898	KNO.1 (1); DPD.1 (1); GLEN.1 (1); KNO.2 (3); DPD.2 (4); E15.2 (4); E14.2 (5)
		Lycosidae Sundevall, 1833	*Pirata oneilli* (Purcell, 1903)	KNO.1 (1); STW.1 (1); KNO.2 (2); DPD.2 (1); JASP.2 (5); GLEN.2 (2); PIET.2 (1); E14.2 (1)
			Pirata trepidus Roewer, 1960	STW.1 (2); STW.2 (1)
		Pisauridae Simon, 1890	*Nilus massajae* (Pavesi, 1883)	E14.2 (2)
Phylum: Arthropoda/ Subphylum: Hexapoda				
Entognatha/ Collembola	Entomobryomorpha		(two morphotypes identified to the order level)	JASP.1 (6); PIET.2 (1)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
	Odonata/ Zygoptera	Lestidae Calvert, 1901	*Lestes* sp.	KNO.1 (1); DPD.1 (6); KNO.2 (2)
		Platycnemididae Tillyard, 1917	*Allocnemis leucosticte* Sélys, 1863	KNO.2 (6); DPD.2 (7); SRB.2 (2)
			Elattoneura glauca (Sélys, 1860)	GLEN.1 (3)
		Coenagrionidae Kirby, 1890	*Agriocnemis pinheyi* Balinsky, 1963	KNO.1 (2); DPD.1 (5)
			Ceriagrion glabrum (Burnmeister, 1839)	KNO.1 (7)
			Enallagma sp.	GLEN.1 (4)
			Ischnura senegalensis (Rambur, 1842)	E14.2 (1)
			Pseudagrion sp.	KNO.1 (6); DPD.1 (21); DPD.2 (12); NYL.2 (2); PIET.2 (5); E15.2 (7)
			Teinobasis sp.	KNO.1 (3); DPD.1 (16)
		Chlorocyphidae Cowley, 1937	Chlorocyphidae gen. spp.	GLEN.1 (2)
	Odonata/ Anisoptera	Gomphidae Rambur, 1842	*Lestinogomphus angustus* Martin, 1911	DPD.1 (1); GLEN.1 (1)
			Onychogomphus supinus supinus Selys, 1854	KNO.1 (4); DPD.1 (24); GLEN.1 (3); DPD.2 (1); SRB.2 (1); E15.2 (5); E14.2 (12)
			Paragomphus genei (Selys, 1841)	DPD.1 (40)
		Aeshnidae Rambur, 1842	*Anax imperator* Leach, 1815	DPD.1 (1); KNO.2 (1); DPD.2 (1); NYL.2 (3)
		Libellulidae Rambur, 1842	*Atoconeura biordinata biordinata* Karsch, 1899 *	SRB.2 (1)
			Bradinopyga cornuta Ris, 1911	DPD.1 (19); NYL.2 (49)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
			Diplacodes lefebvrii (Rambur, 1842)	KNO.2 (3); NYL.2 (35)
			Macrodiplax cora (Kaup in Brauer, 1867) *	E15.2 (3)
			Olpogastra lugubris (Karsch, 1895)	E14.2 (1)
			Orthetrum caffrum caffrum Burmeister, 1839	DPD.1 (1); STW.1 (1); KNO.2 (4); SRB.2 (2)
			Rhyothemis semihyalina semihyalina (Desjardins, 1832)	DPD.1 (3); GLEN.1 (4)
			Tetrathemis spp. *	KNO.1 (1); DPD.1 (8); DPD.2 (6)
			Tramea basilaris (Palisot de Beauvois, 1817) *	NYL.2 (3)
			Trithemis dorsalis (Rambur, 1842)	KNO.1 (5); DPD.1 (22); NYL.2 (1)
			Tholymis tillarga (Fabricus, 1798) *	E15.2 (1)
			Zygonyx sp.	E14.2 (16)
			Libellulidae gen. sp.	PIET.2 (1)
	Hemiptera/ Sternorrhyncha	Aphididae Latreille, 1802	*Rhopalosiphum nymphaeae* (Linnaeus, 1761)	DPD.1 (6); JASP.1 (1); GLEN.1 (3); JASP.2 (1)
	Hemiptera/ Heteroptera	Mesoveliidae Douglas & Scott, 1867	*Mesovelia vittigera* Horváth, 1895	GLEN.1 (1)
		Gerridae Leach, 1815	*Eurymetra natalensis* (Distant, 1903)	DPD.1 (1); E15.2 (1)
			Gerris sp.	NYL.2 (1)
			Limnogonus sp.	KNO.2 (2)
			Tenagogonus sp.	KNO.1 (1)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
		Corixidae Leach, 1815	*Micronecta* sp.	KNO.1 (2); GLEN.1 (10); KNO.2 (1); NYL.2 (1)
			Sigara sp.	KNO.1 (1)
			Agraptocorixa sp.	KNO.2 (1)
			Trichocorixa sp. *	NYL.2 (4)
		Notonectidae Latreille, 1802	*Anisops varia* Fieber, 1851	KNO.1 (4); NYL.2 (70)
		Pleidae Fieber, 1851	*Plea pullula* Stål, 1855	JASP.1 (1)
		Naucoridae Leach, 1815	*Laccocoris limigenus* Stål, 1865	DPD.1 (8); GLEN.1 (2); DPD.2 (1); GLEN.2 (3); E15.2 (1); E14.2 (2)
			Naucoris obscuratus Montandon, 1913	KNO.2 (1)
		Belostomatidae Leach, 1815	*Appasus* sp.	DPD.1 (1); DPD.2 (2); PIET.2 (1); E15.2 (1)
	Trichoptera/ Spicipalpia	Hydroptilidae Stephens, 1836	*Hydroptila* sp. 1	KNO.1 (8); DPD.1 (128); KNO.2 (6)
			Hydroptila sp. 2	DPD.1 (2)
			Oxythira velocipes (Barnard, 1934)	DPD.1 (10); KNO.2 (1)
			Tricholeichiton sp.	DPD.1 (12)
			Orthotrichia sp.	DPD.1 (1)
			Catoxyethira sp.	DPD.1 (32)
	Trichoptera/ Annulipalpia	Hydropsychidae Curtis, 1835	*Cheumatopsyche afra* (Mosely, 1935)	KNO.1 (3); DPD.1 (13); E14.2 (8)
			Cheumatopsyche thomasseti (Ulmer, 1931)	GLEN.1 (34)
			Hydropsyche longifurca Kimmins, 1957	DPD.1 (12)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
			Macrosternum capense (Walker, 1852)	DPD.1 (1)
		Ecnomidae Ulmer, 1903	*Ecnomus thomasseti* Mosely, 1932	KNO.1 (2); DPD.1 (10); GLEN.1 (9); DPD.2 (1); E14.2 (2)
	Trichoptera/ Brevitentoria	Leptoceridae Leach, 1815	*Athripsodes* sp. 1	DPD.1 (4)
			Athripsodes sp. 2	DPD.1 (6); E14.2 (1)
			Oecetis sp. 1	DPD.1 (28); GLEN.1 (2); KNO.2 (13); DPD.2 (7)
			Oecetis sp. 2	E14.2 (3)
			Setodes sp. 1	DPD.1 (2)
			Setodes sp. 2	DPD.1 (6)
			Trianodes sp.	E14.2 (1)
			Trichosetodes sp.	DPD.1 (2)
			Leptocerinae sp. 1	DPD.1 (1)
	Lepidoptera	Crambidae Latreille, 1810	Schoenobiinae gen. sp.	KNO.2 (7); PIET.2 (1); E15.2 (7)
	Diptera/ Nematocera	Tipulidae Latreille, 1802	*Conosia* sp.	DPD.2 (1)
			Dicranomyia capicola Alexander, 1921	DPD.1 (3); JASP.1 (1); JASP.2 (1)
			Dicranomyia tipulipes Karsch, 1886	KNO.2 (1)
			Gonomyia sp.	DPD.1 (3); DPD.2 (1)
			Limnophila sp.	SRB.2 (8)
			Tipula pomposa Bergroth, 1888	KNO.1 (1)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
		Psychodidae Newman, 1834	*Clogmia albipunctata* (Williston, 1893)	STW.1 (3); JASP.1 (12); STW.2 (5); JASP.2 (1)
			Psychoda alternata Say, 1824	STW.1 (358); JASP.1 (3); STW.2 (18)
		Dixidae Schiner, 1868	*Dixa bicolor* Wood, 1933	KNO.1 (2); KNO.2 (1)
		Chaoboridae Newman, 1834	*Chaoborus microstictus* Edwards, 1930	GLEN.1 (1)
		Ceratopogonidae Newman, 1834	*Atrichopogon* sp.	E15.2 (1)
			Bezzia sp.	KNO.1 (18); DPD.1 (10); GLEN.1 (3); KNO.2 (16); DPD.2 (3); NYL.2 (5)
			Culicoides sp. 1	KNO.1 (15); DPD.1 (10); JASP.1 (4); GLEN.1 (28); KNO.2 (35); DPD.2 (2); E15.2 (2)
			Culicoides sp. 2	KNO.1 (117); DPD.1 (2); JASP.1 (3); GLEN.1 (1)
			Dasyhelea sp.	DPD.1 (1)
			Forcipomyia sp.	NYL.2 (1)
			Leptoconops sp.	DPD.1 (1)
			Ceratopogonidae gen. sp.	DPD.1 (2)
		Culicidae Meigen, 1818	*Aedes* sp.	STW.2 (48)
			Anopheles sp. 1	KNO.1 (4); DPD.1 (13)
			Anopheles sp. 2	DPD.1 (1)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
			Culex sp. 1	STW.1 (15); STW.2 (955); JASP.2 (1); NYL.2 (2)
			Culex sp. 2	STW.2 (292)
			Mimomyia sp.	GLEN.1 (1)
		Chironomidae Macquart, 1838	Chironominae gen. spp.	KNO.1 (492); DPD.1 (683); STW.1 (188); JASP.1 (252); GLEN.1 (290); KNO.2 (23); DPD.2 (70); STW.2 (8); JASP.2 (10); NYL.2 (44); SRB.2 (7); GLEN.2 (1); E15.2 (38); E14.2 (44)
			Chironomidae gen. sp.	DPD.1 (2)
			Orthocladiinae gen. spp.	KNO.1 (2); DPD.1 (5); JASP.1 (29); KNO.2 (23); E15.2 (1); E14.2 (1)
			Tanypodinae gen. spp.	KNO.1 (1093); DPD.1 (108); GLEN.1 (7); KNO.2 (59); DPD.2 (2); STW.2 (5); NYL.2 (16); E14.2 (1)
		Simuliidae Newman, 1834	*Simulium (Edwardsellum) damnosum* Theobald, 1903)	E14.2 (2)
			Simulium (Freemanellum) hirsutilateris de Meillon, 1937	DPD.1 (20)
			Simulium (Meilloniellum) adersi Pomeroy, 1922	JASP.1 (13); KNO.2 (3); DPD.2 (41); E14.2 (21)
			Simulium (Metomphallus) bovis de Meillon, 1930	E14.2 (54)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
			Simulium (Metomphallus) medusaeforme Pomeroy, 1920	KNO.2 (6)
			Simulium (Nevermannia) nigritarse Coquillett, 1902	DPD.1 (57); JASP.1 (183); KNO.2 (23)
			Simulium (Nevermannia) sp.	GLEN.1 (1); KNO.2 (27); DPD.2 (21)
			Simulium (Pomeroyellum) alcocki Pomeroy, 1922	KNO.1 (22); KNO.2 (17)
			Simulium (Pomeroyellum) mcmahoni de Meillon, 1940	DPD.1 (35)
	Diptera/ Brachycera	Tabanidae Latreille, 1802	*Haematopota* sp.	DPD.1 (3); NYL.2 (2)
			Tabanus sp.	KNO.1 (1); DPD.1 (10); JASP.1 (2); GLEN.1 (6)
		Stratiomyidae Latreille, 1802	*Stratiomys sp.*	KNO.1 (1); DPD.1 (1); JASP.2 (1)
		Dolichopodidae Latreille, 1809	Dolichopodidae gen. spp.	DPD.1 (2)
		Ephydridae Zetterstedt, 1837	Ephydridae gen. spp.	JASP.1 (2); STW.2 (41); JASP.2 (4)
		Muscidae Latreille, 1802	Muscidae gen. spp.	KNO.1 (3); STW.1 (27); JASP.1 (27); GLEN.1 (1); KNO.2 (1); STW.2 (31); JASP.2 (8); SRB.2 (1); GLEN.2 (2); E15.2 (3); E14.2 (2)
		Syrphidae Latreille, 1802	Syrphidae gen. spp.	STW.1 (39); KNO.2 (1); STW.2 (122)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
	Coleoptera/ Adephaga	Gyrinidae Latreille, 1802	*Aulonogyrus* sp.	KNO.1 (1); DPD.1 (5)
			Orectogyrus sp.	DPD.1 (9)
		Haliplidae Aubé, 1836	Haliplidae gen. sp.	GLEN.1 (2)
		Noteridae C.G. Thomson, 1860	*Canthydrus* sp.	NYL.2 (3)
			Hydrocanthus klarae Gschwendtner, 1930	KNO.1 (1)
		Dytiscidae Leach, 1815	*Copelatus* sp.	JASP.1 (1)
			Hydroglyphus sp.	JASP.1 (1)
			Hydrovatus sp.	DPD.1 (2); E14.2 (1)
			Peschetius ultimus Biström & Nilsson, 2003	PIET.2 (1)
			Rhantus concolorans (Wallengren, 1881)	DPD.1 (2); NYL.2 (1)
			Uvarus sp.	DPD.1 (3); DPD.2 (2)
			Dytiscidae gen sp.	KNO.2 (1); JASP.2 (6); GLEN.2 (1); E14.2 (1)
	Coleoptera/ Polyphaga	Aphodiidae Leach, 1815	*Rhyssemus* sp.	SRB.2 (1); GLEN.2 (2)
		Hydrochidae Thomson, 1859	*Hydrochus amrishi* (Makhan, 1998)	DPD.1 (1)
		Hydrophilidae Latreille, 1802	*Allocotocerus* sp.	DPD.1 (3); STW.2 (1)
			Amphiops sp.	DPD.2 (1)
			Berosus sp.	NYL.2 (1); E15.2 (2)
			Chasmogenus patrizii (Balfour-Browne, 1948)	KNO.1 (2)
			Crenitis excusa Hebauer, 1994	KNO.1 (2); DPD.1 (1); STW.2 (1)

Table 6.1: Continued.

Class/ Subclass	Order/ Suborder	Family	Species	Locality (number of individuals)
			Enochrus sp.	DPD.1 (4); DPD.2 (3); JASP.2 (4)
			Helochares sp.	KNO.1 (1); GLEN.1 (1)
			Hydrochara sp.	GLEN.1 (1); GLEN.2 (1)
			Laccobius sp.	JASP.1 (2); KNO.2 (1); NYL.2 (2)
			Paracymus sp.	DPD.2 (3)
			Hydrophilidae gen. sp.	KNO.2 (2); JASP.2 (3)
		Hydraenidae Mulsant, 1844	*Hydraena cooperi* Balfour-Browne, 1954	JASP.1 (1)
			Ochthebius andronius d'Orchymont, 1948	JASP.1 (1)
		Scirtidae Fleming, 1821	Scirtidae gen. sp.	JASP.1 (2)
		Elmidae Curtis, 1830	Elmidae gen. sp.	KNO.2 (1); E14.2 (1)
		Dryopidae Billberg, 1820	Dryopidae gen. sp.	GLEN.2 (1)
		Limnichidae Erichson, 1846	Limnichidae gen. sp.*	PIET.2 (2)
		Chrysomelidae Latreille, 1802	Chrysomelidae gen sp.	DPD.1 (1)
		Curculionidae Latreille, 1802	*Cyrtobagous salviniae* Calder & Sands, 1985	GLEN.1 (1)
			Pseudobagous longulus (Gyllenhal, 1836)	KNO.1 (1); NYL.2 (3)

* species reported from the Limpopo Province for the first time.

6.2.2 FUNCTIONAL FEEDING GROUPS

The identified macroinvertebrate taxa were separated into Functional Feeding Groups (FFGs) according to the classifications proposed by Barbour et al. (1999), Merritt et al. (2002), Cummins et al. (2005) and online databases such as BugGuide.Net. A detailed list of the macroinvertebrate families with their corresponding FFGs is provided in Appendix C. The classification system used in the following section along with the categorisation and descriptions of the various FFGs and the food resources that each group utilises is explained in Table 6.2.

Table 6.2: The classification and description of the various Functional Feeding Groups (FFG) including the food resources that each group utilises [adapted from Merritt & Cummins (1996)].

Functional Feeding Group	Code	Feeding mechanisms	Main food resources	Food particle size range (mm)
Shredders	SH	Chew litter, live vascular plant tissue or gouge wood	Coarse particulate matter – decomposing vascular plants	> 1.0
Filtering collectors	FC	Suspension feeders – filter and ingest suspended particles in the water column	Fine particulate matter – detritus particles, faeces, bacteria and algae	0.01 – 1.0
Gathering collectors	GC	Deposit feeders – feed on sediments or loose particulate matter from areas where decomposition takes place	Fine particulate matter – detritus particles, faeces, bacteria and algae	0.05 – 1.0
Scrapers	SC	Graze off the surfaces of rocks, wood or stemmed aquatic plants	Periphyton – non-filamentous algae, detritus, microbes and faeces	0.01 – 1.0
Predators	PR	Ingest the body fluids of captured or engulfed prey or tissue	Prey – living animal tissue	>0.5

Across all sites for both sampling seasons, gathering collectors were the most abundant FFG (Figures 6.2A, 6.3B), consisting mainly of taxa from the Chironomidae (Chironomids) and Naididae (Oligochaetes). According to Prat & Ward (1994), the abundances of oligochaetes and chironomids within lotic systems increase when elevated levels of organic pollutants are present. Furthermore, in a study by Oberholster et al. (2008) on

the Hennops River and its associated wetlands (Gauteng, South Africa), the authors reported that there was a significant correlation between increased conductivity and high densities of the gathering collectors FFG. The current study shows a similar correlation, with the abundances of gathering collector organisms rising at STW and JASP, as conductivity levels increased (see Chapter 4). Moreover, the high abundances and percentage compositions of the gathering collectors FFG at the other sites may have arisen from modifications of flow caused by man-made weirs and dam walls. The weirs present upstream and downstream of STW and JASP may cause sinks to form where detritus and other organic matter can accumulate, thereby creating abundant food sources for gathering collector organisms to thrive and expand in numbers.

The filtering collector FFG were represented at all of the sampling sites for both sampling seasons, except GLEN (high flow) and PIET (high flow) (Figures 6.2A, 6.3A). The highest abundances of filtering collectors were found at STW (Culicidae) and E14 (Oligoneuriidae) during the high flow season, respectively, whereas, the lowest abundances of filtering collectors were present at JASP, NYL, SRB and E15 for the high flow season. In the study by Oberholster et al. (2008) on wetlands fed by the Hennops River, a correlation was found between filtering collectors and vascular plants, however, the results of the current study indicate that there is no correlation in the Nyl and Mogalakwena Rivers as filtering collectors were not found in high abundances at many of the sites, including NYL and JASP which, during the high flow season, contained high densities of emergent, submerged and marginal macrophytes. The high abundances of filtering collectors found at STW and E15 were sampled from the GSM and SIC biotopes, respectively. At STW (high flow), the sediments were found to contain a high percentage of organic content (see Chapter 5). Therefore, the high organic levels, as well as the reduced flow of the river at STW, may account for the high densities of filtering collectors at this site. In contrary to STW, the sediments at E14 contained no organic content, with the dominant filtering collector taxon being *Elattoneura* sp. (Oligoneuriidae) which were sampled from the SIC biotope. The high abundance of *Elattoneura* sp. at E14 was attributed to fast flowing conditions within the sampled biotope, which alleviates particulate matter from the surrounding environment, suspending it in the water column. The high density of suspended particles can then be filtered from the water column by organisms that have attached themselves to stones and other submerged objects, organisms such as *Elattoneura* sp. Furthermore, *Elattoneura* sp. is considered to be highly sensitive (Dickens & Graham 2002). Low COD values recorded at E14 (see

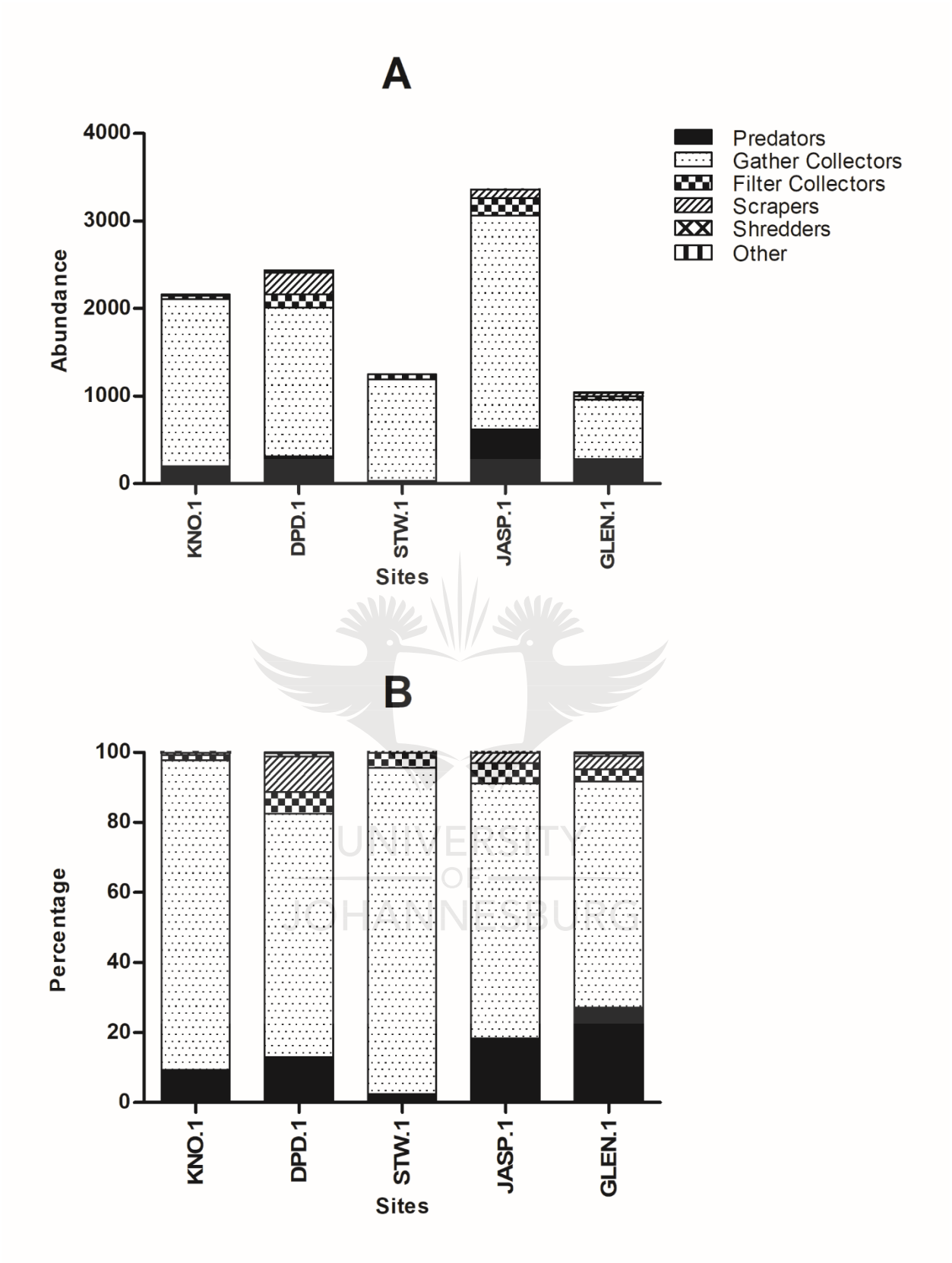

Figure 6.2: The Functional Feeding Groups (FFG) of the macroinvertebrate taxa collected from all sites during low flow sampling season. **A,** Abundance and **B,** Percentage contribution of each FFG. The number succeeding the site name denotes the sampling season: **1,** low flow (July 2016).

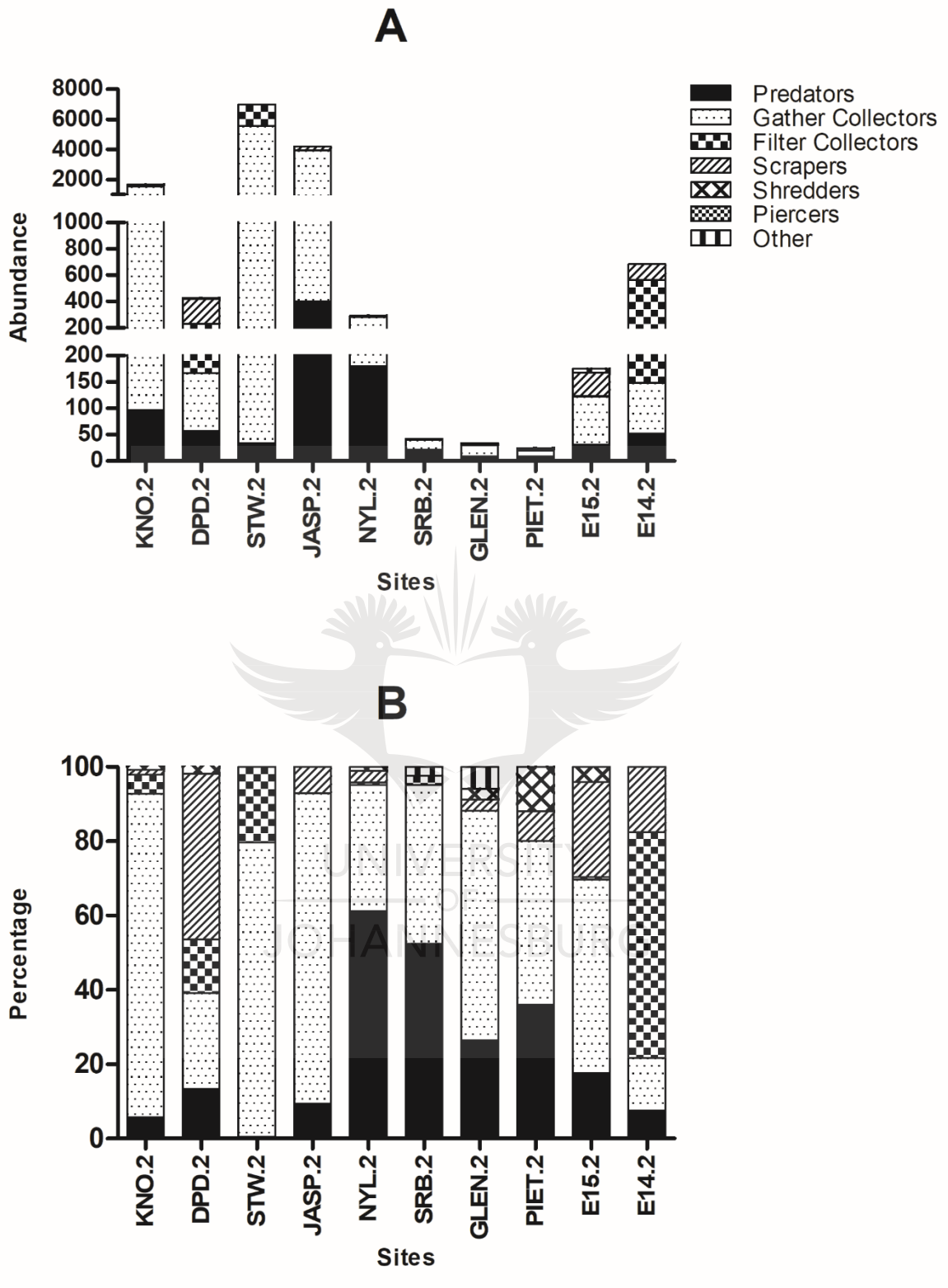

Figure 6.3: The Functional Feeding Groups (FFG) of the macroinvertebrate taxa collected from all sites during high flow sampling season. **A,** Abundance and **B,** Percentage contribution of each FFG. The number succeeding the site name denotes the sampling season: **2,** high flow (February 2017).

Chapter 4) suggest that *Elattoneura* sp. require a high level of DO, of which, is typically reduced when organic content within the water is high (DWAF 1996c).

Predators were present at each of the sampling sites for both low flow and high flow seasons, however, they did not constitute a large percentage composition at any of the sites except at NYL (high flow season). At NYL, the macroinvertebrate community assemblage had shifted from being dominated by the gathering collector FFG to a community assemblage where the dominant FFG was predators (Libellulidae) (61.22 %). The current study showed a similar result to the research conducted by de Necker (2016) and Dyamond (2017) on ephemeral and floodplain pans respectively. The authors' results showed that the macroinvertebrate community assemblages within wetland systems were dominated by the predator FFG. One reason for this may be that predators generally show life-history strategies that are better adapted to extended and long-term survival in temporary systems (De Roeck 2008). Due to flooding activity in the Mogalakwena River at the time of high flow sampling, the seemingly high predator percentage compositions (Figure 6.3B) observed at SRB (52.38 %), GLEN (26.47 %) and PIET (36.00 %) may not be accurate representations of the macroinvertebrate community FFGs at these sites at the time of sampling.

Baring SRB, scrapers were found at each of the sampled sites for both the low flow and high flow seasons. The taxa that made up the scapers FFG for the Nyl and Mogalakwena Rivers consisted of Molluscs (Thiariidae, Bulinidae, Planorbidae, Physidae and Lymnaeidae), Ephemeropterans (Heptageniidae), Hemipterans (Corixidae) and Coleopterans (Elmidae, Dryopidae and Hydroptilidae). The site with the highest abundance of scrapers was JASP during high flow sampling with the dominant scraper at this site being *Physella acuta* (Draparnaud, 1805). Due to their feeding behaviours, scrapers tend to feed on algae and other microbes that are attached to the surfaces of stemmed plants (Merritt & Cummins 1996) (Table 6.2). During the high flow sampling season, JASP was densely covered in emergent and marginal vegetation (see Chapter 2), providing the ideal habitat and ample food resources for the occurrence of high abundances of scrapers such as molluscs. Furthermore, *Physella acuta* is a tolerant species being able to thrive in conditions of poor quality, hence its common name 'Sewage Snail' (Griffiths et al. 2015). The tolerance to poor water quality allows this species to occur in densities of up to 3000 / m^2 where other, more sensitive taxa cannot survive (Day & de Moor 2002). A scraper of concern is *Tarebia granifera* (Lamarck, 1816) which has been classified in the National Environmental Management: Biodiversity Act

No. 10 of 2004 (NEMBA) (RSA 2004) as a category 1b invasive species, requiring control through an invasive species management programme. It is unclear whether the identification of *Tarebia granifera* is indeed accurate, as differentiation from *Melanoides tuberculata* (O. F. Müller, 1774) is difficult, but with known occurrences in the Limpopo River catchment downstream of E14 (Dyamond 2017), the evidence suggests that their distribution may be spreading upstream and therefore, the displacement of other, native aquatic snail species may occur (Griffiths et al. 2015). Considering all sites and sample seasons, DPD in the high flow season had the highest percentage composition of scrapers (44.50 %) (Figure 6.3B). This 44.5 % scraper composition at DPD was diverse and comprised of *Bulinus africanus* (Krauss, 1848) (32.10 %), *Bulinus depressus* Haas, 1936 (20.00 %), *Ferrissia* sp. (0.53 %), *Gyraulus costulatus* (Krauss, 1848) (9.47 %) and *Radix natalensis* (Krauss, 1848) (37.89 %). Compared to the sampling of DPD during the low flow season, the percentage of scrapers drastically increased. The attenuation of flow caused by excess sedimentation during the high flow season could possibly account for the sudden increase in the scraper FFG. When sedimentation occurs, habitats can be altered by (i) providing more substrate for aquatic macrophytes to grow, (ii) altering or destroying suitable habitats for certain taxa, or (iii) by displacing certain taxa, allowing others to fill previously unavailable niches.

Excluding plant piercers represented by *Rhopalosiphum nymphaeae* (Linnaeus, 1761) (Aphididae) and dung feeders represented by *Rhyssemus* sp. (Aphodiidae), the least abundant FFG was the shredders, however, this was expected as Cummins et al. (2005) describes that the shredders are generally the least common FFG, often being under-represented due to their small size and camouflaging abilities.

There were no sampling sites across either of the sampling seasons that showed an even distribution of FFGs, however, DPD during the high flow season showed the greatest diversity of FFG by percentage: gathering collectors (25.76 %), filtering collectors (14.52 %), predators (13.35 %), scrapers (44.50 %) and shredders (1.87 %). The site that showed the lowest diversity with regards to FFG was STW (low flow season) as its percentage composition was comprised of 93.11 % gathering collectors, mostly represented by *Tubifex tubifex.*

6.2.3 UNIVARIATE STATISTICS

The results of the Margalef's species richness index (DMa) are represented in Figure 6.4A. In general, the sites upstream of the Modimolle WWTF, KNO and DPD, showed high species richness for both the low flow and high flow seasons. For the low flow season, the sites STW and JASP had drastically reduced species richness compared to the upstream sites, with the species richness only recuperating to higher levels at GLEN. The high flow results show a similar trend in the decrease of species richness at STW and JASP, however, at NYL, the species richness had risen and returned to levels comparable to those of DPD and KNO. The sites along the Mogalakwena River for the high flow sampling survey showed similar species richness levels compared to the sites near the source of the Nyl River (KNO and DPD), however, this data may not be representative, specifically at SRB, GLEN and PIET, due to the flooding activity at the time of sampling. A low number of species and individuals were identified from these sites, suggesting that the Margalef's index score may not be accurately representing the macroinvertebrate diversity at these sites at the time of sampling. The sites E15 and E14 (high flow season) showed a higher species richness compared to that of the other Mogalakwena River sites, and the Margalef's index score is perhaps more representative of the species at these sites as they were more established and were less influenced by flooding activity at the time of sampling. The sampling of DPD during the low flow season yielded the highest species richness, with all other sites for both sampling seasons having fewer species than DPD.

The results of Pielou's Evenness Index (J') can be seen in Figure 6.4B. The J' revealed that the taxa at KNO are not evenly distributed, with its evenness being lower than that of DPD. The macroinvertebrate community assemblages at KNO for the low and high flow sampling seasons were dominated by the Chironomidae (subfamilies Chironominae and Tanypodinae) (Table 6.1). According to Dickens & Graham (2002), many of the taxa belonging to the Chironomidae are tolerant to poor water quality, however, the results seen in Chapter 4 indicate that the water quality at KNO was not poor. A possible explanation for the low evenness at KNO may be due to its relative position to the source of the Nyl River. Water arising from underground sources and natural springs lack essential nutrients and minerals that aid in supporting diverse communities of sensitive macroinvertebrate taxa, and thus more tolerant taxa are favoured. The calculated J' scores for both sampling seasons is higher at DPD compared to STW and JASP.

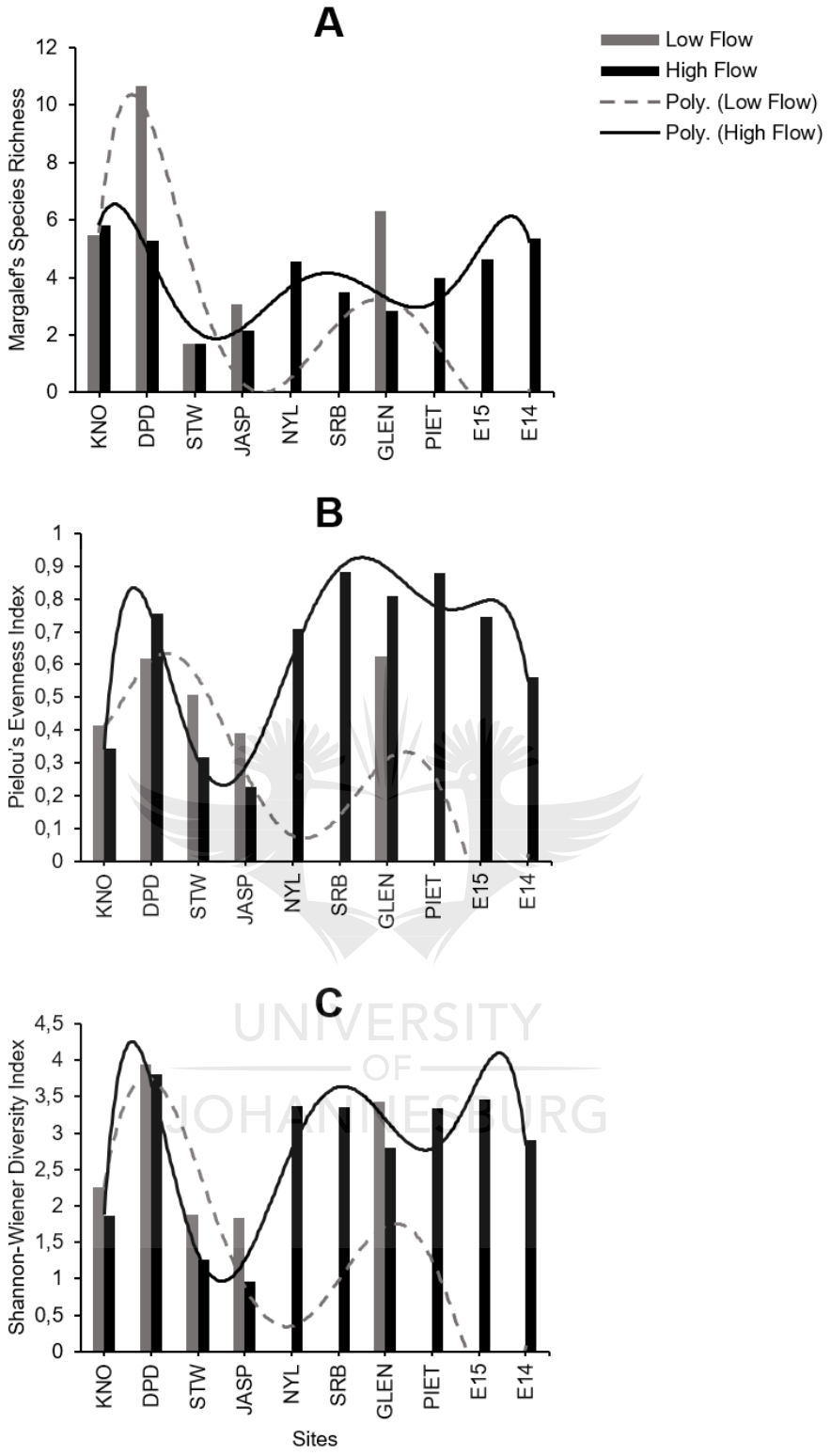

Figure 6.4: Graphical representations of the results of the univariate diversity indices for the macroinvertebrate taxa collected in the Nyl and Mogalakwena Rivers during low flow (July 2016) and high flow (February 2017) sampling seasons. **A,** Margalef's Species Richness Index; **B,** Pielou's Evenness Index; **C,** Shannon-Wiener Diversity Index. The polynomial trend lines are used to determine if any trends in species richness, evenness and overall biodiversity between sites are visible.

The low index scores at STW and JASP for both sampling seasons are indicative of sites with low species richness and high abundances of dominant taxa, namely Chironominae gen spp., *Helobdella stagnalis* (Linnaeus, 1758), *Tubifex tubifex* and *Psychoda alternata* Say, 1824. These two sites were also the sites that showed the poorest water quality throughout the study, with elevated values for many of the tested nutrients and metals. The remaining sampling sites along the Nyl and Mogalakwena Rivers for both sampling surveys showed that the calculated J' scores were high and that the distribution of taxa at these sites was relatively even, therefore no particularly dominant taxa were present.

Figure 6.4C displays a graphical representation of the results of the Shannon-Wiener Diversity Index (H') for both the low flow and high flow sampling seasons. Overall, the results of the H' showed trends similar to those seen in Figure 6.4B, with STW and JASP both having a low diversity and the other sampling sites, except KNO, all having high diversity. The H' scores at KNO (low flow, 2.25; high flow, 1.87) were lower compared to those from DPD (low flow, 3.94; high flow, 3.81). The low diversity results obtained at KNO for both sampling surveys were comparable to the findings of Malherbe et al. (2010) who reported low diversity scores at a reference site along the Mvoti River, KwaZulu Natal. Although the results of the study by Malherbe et al. (2010) were reported from a river in a different ecoregion, they may further support the reasoning mentioned previously regarding the low evenness and diversity at KNO. The low flow and high flow diversity at DPD were the highest recorded in the study. Minimal impacts and ample suitable habitat for various FFGs of macroinvertebrates could have accounted for the high diversity at this site. The dense vegetation at NYL which acts as a 'filter', reducing the concentrations of pollutants entering the system could have provided suitable habitat and abundant food resources for many macroinvertebrate taxa, accounting for the high H' score (3.37) at this site during the high flow season. The diversity scores at the sites along the Mogalakwena River for both low flow and high flow were generally high (mean H' score of 3.22), suggesting a high richness and distribution of species at these sites. As discussed previously, the index scores at SRB, GLEN and PIET may not be representative of the macroinvertebrate community assemblages at these sites due to flooding activity during high flow sampling.

6.2.4 MULTIVARIATE STATISTICS

The DCA bi-plot represented in Figure 6.5 shows the distribution of macroinvertebrate families at each of the sampling sites for both sampling seasons and explains a total of

35.68 % of the variation, 25.30 % on the first axis and 10.38 % on the second axis. A separation of the sampling sites along a spatial gradient can be seen with the majority of the sites grouping away from STW and JASP for both sampling seasons. This separation could be attributed to the high levels of many of the measured environmental variables at the sites STW and JASP (low flow and high flow) (see Chapter 4). The reason for the separation of E14 away from the other sampling sites could be due to the fact that E14 was the only site sampled on the Limpopo River, therefore, the macroinvertebrate community composition at this site was different to those within the Nyl and Mogalakwena River systems. No temporal trends were evident from the DCA as the major drivers for separation were the family abundances at STW and JASP.

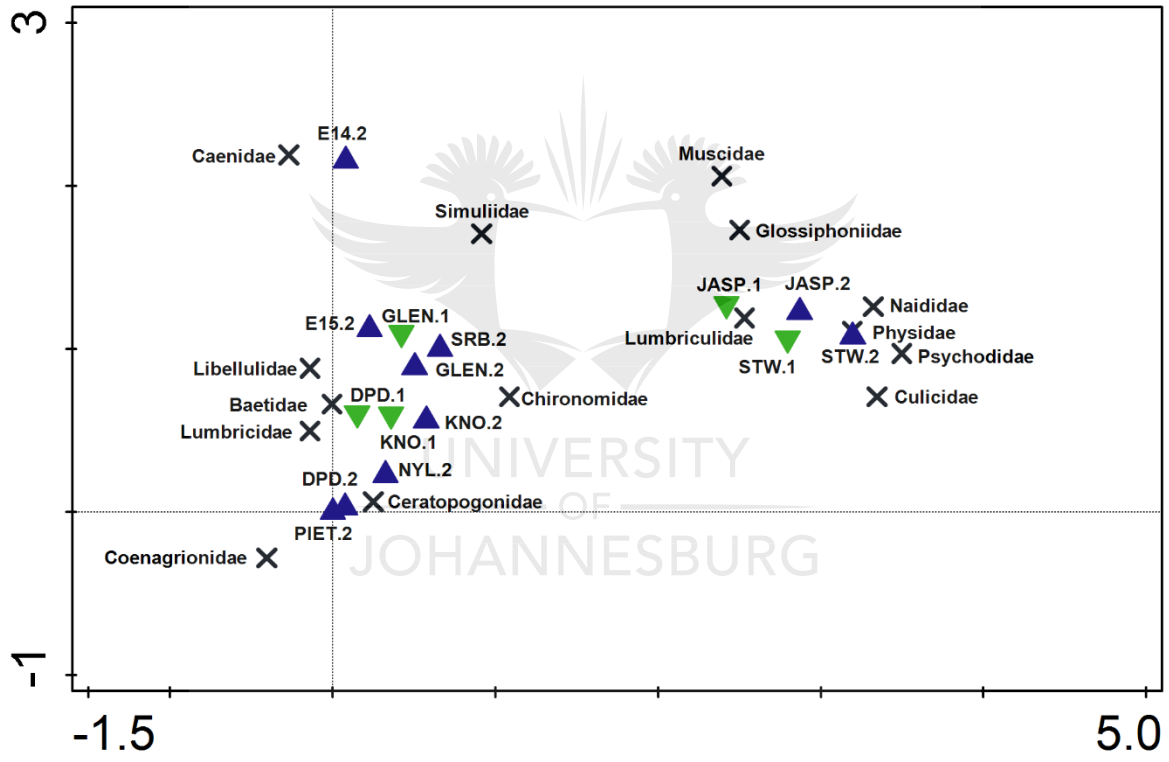

Figure 6.5: An unconstrained Detrended Correspondence Analysis (DCA) bi-plot summarising the patterns of macroinvertebrate family composition variation across the sampling sites for both the low flow and high flow seasons. Only the macroinvertebrate families with the largest weighted values (highest relative abundances) are represented in this DCA. The numeral succeeding the site name denotes sampling season: **1,** low flow (green downward triangles); **2,** high flow (blue upward triangles). The black crosses indicate the positions of the macroinvertebrate families relative to the sampling sites.

According to Godfrey (1978), Dickens & Graham (2002) and Oberholster et al. (2008), the families that showed strong positive correlations with STW and JASP, namely Muscidae, Glossiphonidae, Naididae, Lumbriculidae, Physidae, Psychodidae and Culicidae, are also families that show a high tolerance to poor water quality conditions. The DCA represented in Figure 6.5 only displays 15 out of the total 74 macroinvertebrate families identified from this study; these 15 families are the families that had the highest weighting on site ordinations due to their relative abundances and distributions across the sites (Lepš & Šmilauer 2003). The remaining families that were not displayed in the DCA correlated more with the sites that showed high diversity index scores, namely: KNO, DPD, NYL and GLEN.

The closer the allignment of a macroinvertebrate family to a specific site, the stronger the linear relationship between the family and that site, generally inferring that the family has its highest abundance at the site to which it plots to closest (Lepš & Šmilauer 2003). The Libellulidae, Baetidae, Lumbriculidae, Ceratopogonidae and Coenagrionidae showed low correlations to both STW and JASP, suggesting that the taxa within these families had low abundances at these sites and were therefore more strongly correlated to the other sampling sites, namely: KNO, DPD, NYL, SRB, GLEN, PIET and E15. Furthermore, according to Dickens & Graham (2002), the taxa within these families show a higher sensitivity to poor water quality which relates to the results indicated in Chapter 4. Taxa from the Simuliidae were found in high abundances from KNO, DPD and JASP (low flow) and E14 (high flow), accounting for its ordination between these sites. According to Oberholster et al. (2008), taxa within the Simuliidae require standing, submerged substrates (rocks, wood, stems of vascular plants) for attachment. The sites DPD and JASP contained sparsely distributed larger rocks and stones, ideal for the attachment of Simulidae larvae, whereas at KNO (low flow) and E14 (high flow), the sampling of an extra biotope in the form of SIC, may have accounted for the increased Simulidae abundances at these sites (Vlok et al. 2006). Taxa from the family Chironomidae were present at all the sampling sites for both sampling seasons baring PIET (high flow). Therefore, the ordination of the family Chironomidae is relatively central to the ordination of the sampling sites, being more closely correlated with KNO because their high abundances, most likely due to the increased organic content in the sediments at this site (see Chapter 5). Despite not plotting particularly close to the NYL site marker, the high abundances of the taxa from the Libellulidae (88 individuals) is a major driver for the separation of STW and JASP away from the other sampling sites. Taxa from the

Libellulidae are predators and as discussed previously, predators generally show life-history strategies better suited for the long-term survival in wetland environments (De Roeck 2008). Their life-history traits and the abundant food resources within wetland systems may account for increased abundances and diversity of the Libellulidae taxa at NYL.

The constrained RDA bi-plots represented by Figures 6.6 and 6.7 were used to identify the differences, if any, between the sampling sites and the influences imposed by the measured environmental variables, including metals, on the grouping of the sites based on the macroinvertebrate data. In short, the RDA bi-plots further explain the ordination of the macroinvertebrates and sites (Figure 6.5) by introducing the levels of environmental variables measured at each site. Temporal variation was tested using the combined data from the low flow and high flow sampling surveys. Although the macroinvertebrate data are not visible, the ordination of sites in these RDA bi-plots focuses on the abundances of all the macroinvertebrate families collected throughout this study (Figures 6.6, 6.7). Musa & Greenfield (2018) provides an interpretation of the results presented in these ordinations. The author describes that a positive correlation is observed when the angle between the response variable (sampling site – distribution was based on macroinvertebrate data) and the apex of the driver (environmental variable) is less than 90 ° (> 90°) and that the strength of the positive correlation can be determined by the proximity of the response variable to that of the driver apex. Inversely, a negative correlation is observed when the angle between the driver apex and the response variable is greater than 90 ° (< 90 °). Put simply, the RDA bi-plots are divided into four quadrants, response variables plotting within the same quadrant of a driver show a positive correlation to that driver, whereas, response variables plotting in the quadrant that opposes that drivers are negatively correlated to it.

The first two axes of the RDA bi-plot represented in Figure 6.6 explain 72.99 % of the site variation based on the macroinvertebrate data and its relationship to the environmental variables. Axis one explained 58.32 %, clearly separating the impacted sampling sites, namely STW and JASP, from the relatively unimpacted sampling sites, namely KNO, DPD, NYL, SRB, GLEN, PIET, E15 and E14. Separation along axis two explained a further 14.67 % of the data variation, with this variation being attributed to temporal differences between sampling seasons. Despite the high explained variance, the Monte Carlo permutation test on the first axis and other conical axes revealed that the ordination in Figure 6.6 was insignificant (p = 1.00).

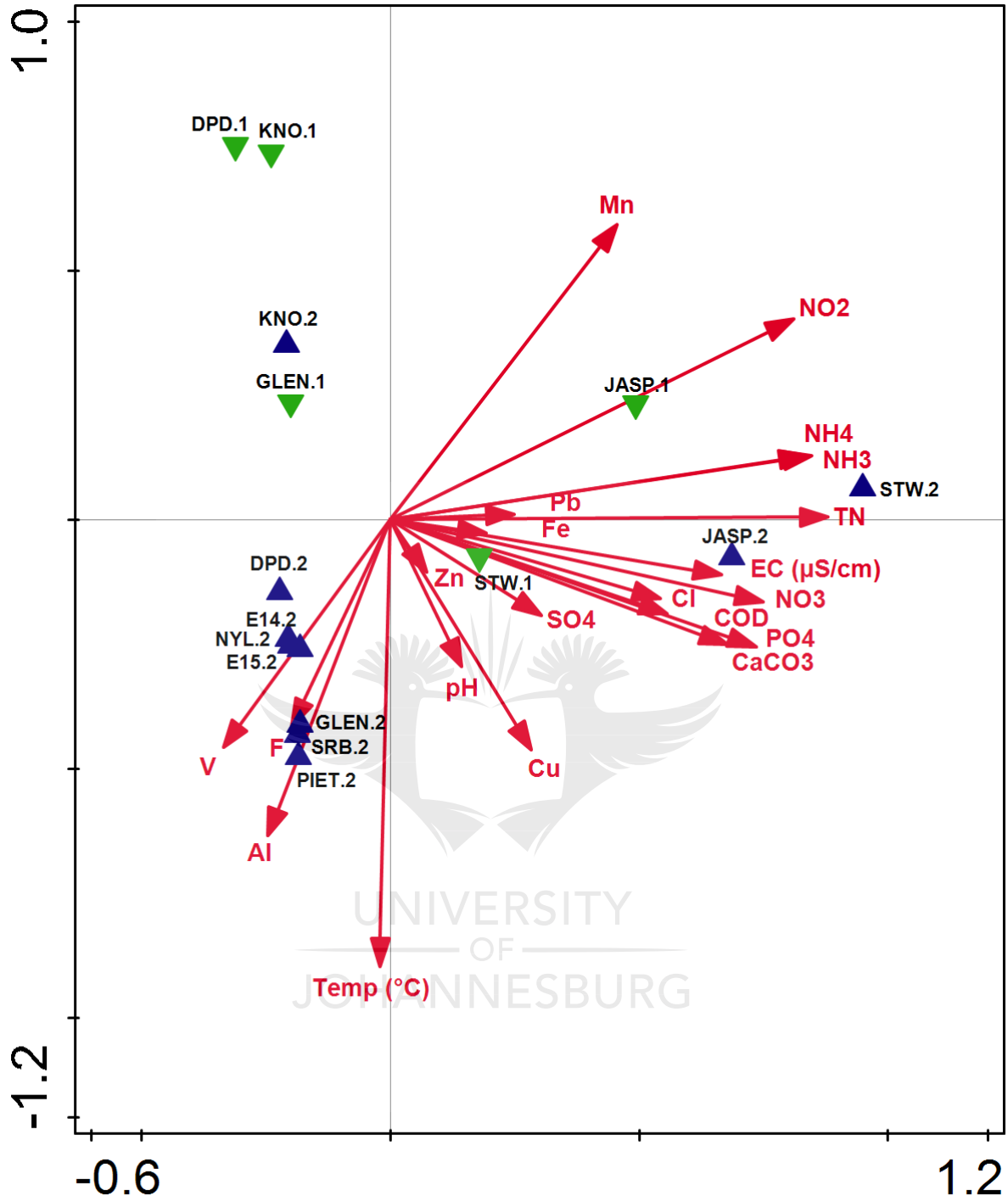

Figure 6.6: A constrained Redundancy Analysis bi-plot indicating the distribution of the sampling sites and the water quality variables (including metal concentrations) by considering the collected macroinvertebrate data for the combined low flow and high flow sampling seasons. The numeral succeeding the site name denotes sampling season: **1,** low flow (green downward triangles); **2,** high flow (blue upward triangles). The red arrows indicate the individual water quality variables.

The sites sampled during the high flow season, not including KNO, STW and JASP, grouped together and away from the low flow sites, indicating noticeable temporal differences between the low flow and high flow data (Figure 6.6). This variation was expected due to the different times of year that sampling was undertaken; low flow sampling was conducted in July 2016 (South African winter) and high flow was conducted in February 2017 (South African summer). During the high flow season, KNO showed the lowest recorded *in situ* water temperatures (18.5 °C). The colder groundwaters erupting from subterranean sources at the headwaters of the Klein Nyl River would have accounted for the colder temperatures and thus the grouping of this site with those sampled during the low flow season. Spatial similarities accounted for the grouping of KNO (low and high flow), DPD (low flow) and GLEN (low flow) (group 1) and for the grouping of the other high flow sites, namely: DPD, NYL, SRB, GLEN, PIET, E15 and E14 (group 2). Additionally, Figure 6.6 distinctly shows spatial differences between groups 1 and 2 and the sites STW and JASP, which formed an isolated grouping (group 3).

The faunal assemblages at the sites within group 1, located in the first quadrant, show strong negative correlations to all the measured environmental variables except Mn, where a slight positive correlation can be observed. The assemblages of the sites in group 1 are indicative of macroinvertebrate communities that contain sensitive taxa, taxa that are associated with softer waters with high concentrations of DO (low COD values) and lower pH levels. Furthermore, these taxa occur in waters which contain reduced levels of nutrients (TN, NO_2^-, NO_3^-, NH_3, NH_4^+, PO_4^{3-}) and other environmental variables, including Cl^-, SO_4^{2-}, EC, Zn and Cu. DPD (low flow) and GLEN (low flow) within group 1 consequently had the highest diversities of macroinvertebrate taxa across all sites, respectively, being comprised of mostly sensitive taxa such as: *Hydra oligactis* Pallas, 1766, *Afronus* sp., *Povilla adusta* Navás, 1912, *Elattoneura glauca* (Sélys, 1860) and *Chaoborus microstictus* Edwards, 1930. Therefore, considering the relatively unimpacted nature and good water quality at the sites plotted within group 1, it was expected that the diversity and abundances of the vast majority of taxa identified in this study would be highest at these sites.

The faunal structures contained in group 2 (quadrant 3), show strong positive correlations with temperature, F^-, V and Al, weak positive correlations with increased pH, Zn and Cu, and negative correlations to the remaining drivers. The faunal assemblages at these sites include taxa from the Belostomatidae, Notonectidae, Oligoneuriidae, Thiariidae,

Dryopidae, Corbiculidae, Limnichidae and Aphodiidae. Although group 2 is correlating positively with that of F^-, V and Al, the possibility of these elements affecting the macroinvertebrate assemblages is small. The water quality analysis (see Chapter 4) provides evidence to suggest that the underlying geology of the Mogalakwena River contains naturally high concentrations of V and Al, thus the surface waters would show the same elevated trend (Ashton et al. 2001; Dahms 2016). Natural or anthropogenic sources could account for the increased F^- levels within the Mogalakwena River, however, without further sampling and research effort during normal flow conditions, the effect these levels have on the aquatic biota within these systems is unknown. However, according to Dickens & Graham (2002), members of the Oligoneuriidae are extremely sensitive to changes in water quality. During high flow sampling, individuals of *Elassoneuria* sp. (Oligoneuriidae) were present within the Mogalakwena River at SRB and the Limpopo River at E14. The occurrence of such a sensitive taxon would suggest that the water within the Mogalakwena and Limpopo Rivers was of good quality, despite being affected by flooding activity and elevated F^- concentrations during the high flow sampling survey, this, however, requires validation.

The results of the water quality analysis (see Chapter 4) indicated that the water quality at the sites within group 3 (quadrants 2 and 4) was poor. The increased levels of nutrients (TN, NO_2^-, NO_3^-, NH_3, NH_4^+ and PO_4^{3-}), alkalinity and water hardness (increased pH and $CaCO_3^{2-}$ concentrations), COD (reduced DO) and other environmental variables (Cl^-, Mn, Pb, Fe, Zn and Cu) exhibited at these sites, are indicative of severe organic pollution, which subsequently reduces DO concentrations, therefore, favouring highly tolerant, air-breathing macroinvertebrate taxa (DWAF 1996a, 1996c; Vlok et al. 2006; Oberholster 2008). Figure 6.6 shows similar trends in site distributions to that of the water quality results (see Chapter 4), despite incorporating the superimposed macroinvertebrate abundance data. The macroinvertebrate community composition at these sites favour biotic assemblages that are tolerant to poor quality, a result shared by Vlok et al. (2006) and Musa & Greenfield (2018) in studies conducted on the same system. The faunal assemblages of group 3 show strong positive correlations to the environmental variables mentioned above and consequently include tolerant species such as *Helobdella stagnalis*, *Tubifex tubifex*, *Psychoda alternata*, *Clogmia albipunctata* Say, 1824, and species of the Syrphidae. Many authors (e.g. Hynes 1960; Godfrey 1978; Vlok et al. 2006; Oberholster et al. 2008) regard these taxa as having particularly high tolerances to elevated organic loads, reduced DO levels and generally poor water quality. Moreover,

Ingram et al. (1966) explicitly explain that when organic pollution occurs, a decrease in the faunal diversity follows, with a subsequent increase in the abundances of the taxa that remain due to less competition and more abundant food resources. The conditions described by Ingram et al. (1966) reflect the conditions to that of STW, thus poor water quality is having a severely negative influence on the macroinvertebrate community assemblages at this site, and JASP downstream.

Interactive forward selection of the environmental variables was conducted on the RDA bi-plot presented in Figure 6.7 to determine which of the measured environmental variables had a significant ($p \leq 0.05$) relationship with the macroinvertebrate community assemblage data. The first two axes of this ordination accounted for 89.46 % of the explained fitted variation, with 71.69 % explained by the first axis and 17.77 % explained by the second axis. The Monte Carlo permutation test in conjunction with the forward selection identified that the combined explained variation of the selected significant ($p \leq 0.05$) environmental variables accounted for 74.56 % of the total variation, these variables were: TN (45.7 %), temperature (12.9 %), NO_2^- (9.5 %) and Mn (6.4 %). The spatial and temporal trends identified in Figure 6.6 were once again evident in Figure 6.7, with temporal separation occurring between the sites sampled in different seasons and spatial variation separating STW and JASP (low flow and high flow) away from the other sampling sites.

The temporal separation of the sampling sites was attributed to the increased water temperatures during high flow sampling (summer) compared to low flow sampling (winter). The temperature of water within riverine ecosystems is influenced by the amount of solar radiation capable of heating the warming the water. The quantity of solar radiation reaching the water can be affected by a variety of factors (e.g. climate, geology, water volume, flow, vegetation cover), thus anthropogenic alterations to any of these factors will impact the thermal regime of a river (Dallas & Day 2004). Due to the poikilothermic (cannot regulate body heat) nature of the aquatic macroinvertebrate taxa that inhabit these riverine systems, they are completely susceptible and are dependent upon temperature variations for behavioural, metabolic, reproductive, developmental and feeding cues (Dallas & Day 2004). Therefore, the seasonal temperature fluctuations between low flow and high flow seasons (Figure 6.7) may influence the macroinvertebrate community assemblages present at each of the sampling sites. As seen from the combined water quality PCA in Chapter 4 (Figure 4.8), temperature and COD are negatively correlated, meaning that as temperatures increase, DO concentrations are

reduced and vice versa. This may account for the lower macroinvertebrate diversity during the high flow season as compared to the low flow season and may also account for the changes to community structures between seasons. Each organism is specifically adapted to an optimal temperature range, some of which show a high sensitivity to thermal variation (stenothermal organisms) and others being tolerant to thermal fluctuations (eurythermal) (Schmidt-Nielson 1997). According to Kemp et al. (2014), organisms that inhabit waters with high temperatures (reduced DO concentrations) generally show adaptations to their respiratory systems. The highly sensitive *Elassoneuria* sp. found at SRB and E14 during the high flow sampling season provides an example of such an adaptation; *Elassoneuria* sp. have large gills (Gillies 1974; de Moor et al. 2003a), ideal for the absorption of DO from the surrounding environment. Changes to temperatures could, therefore, affect macroinvertebrate community assemblage composition, depending on the level and length of the experienced fluctuation (Dallas & Day 2004).

The spatial variation of the macroinvertebrate taxa within the Nyl and Mogalakwena Rivers was strongly driven by elevated nitrogen (TN and NO_2^-) concentrations entering the Nyl River at the Modimolle WWTF (STW) (Figure 6.7). Baring a few tolerant taxa present at STW and JASP, the macroinvertebrate assemblages identified in this study showed negative correlations to TN and NO_2^-. Nitrogen has essential roles within all aquatic ecosystems, however, in high concentrations, it becomes toxic to aquatic biota and is one of the primary causes of eutrophic conditions (Dallas & Day 2004). Many studies (e.g. Vlok et al. 2006; Dahms 2016; Musa & Greenfield 2018) have implicated the Modimolle WWTF as a point source of organic, nitrogenous based pollution as it was found to pump raw, untreated sewage effluent into the Nyl River at STW. The sewage effluent entering the system at STW is rich in organic matter and NH_3 and NH_4^+ species that subsequently decomposes, being converted into NO_2^- and NO_3^- through the nitrogen cycle (see Chapter 4) (DWAF 1996a; Vlok et al. 2006). The COD concentrations at STW were amongst the highest recorded for this study. The measurement of high COD concentrations infers the presence of elevated levels of organic matter, particularly allochthonous organic matter, arising from sewage effluent runoff at this site (DWAF 1996c). The oxidation of the organic matter reduces the levels of DO in the water column, thus creating anaerobic conditions. The conversion of NH_4^+ to other nitrogen species is dependent upon the type of facultative bacteria present. Under anaerobic conditions, as is the case at STW, *Nitrobacter* spp. and

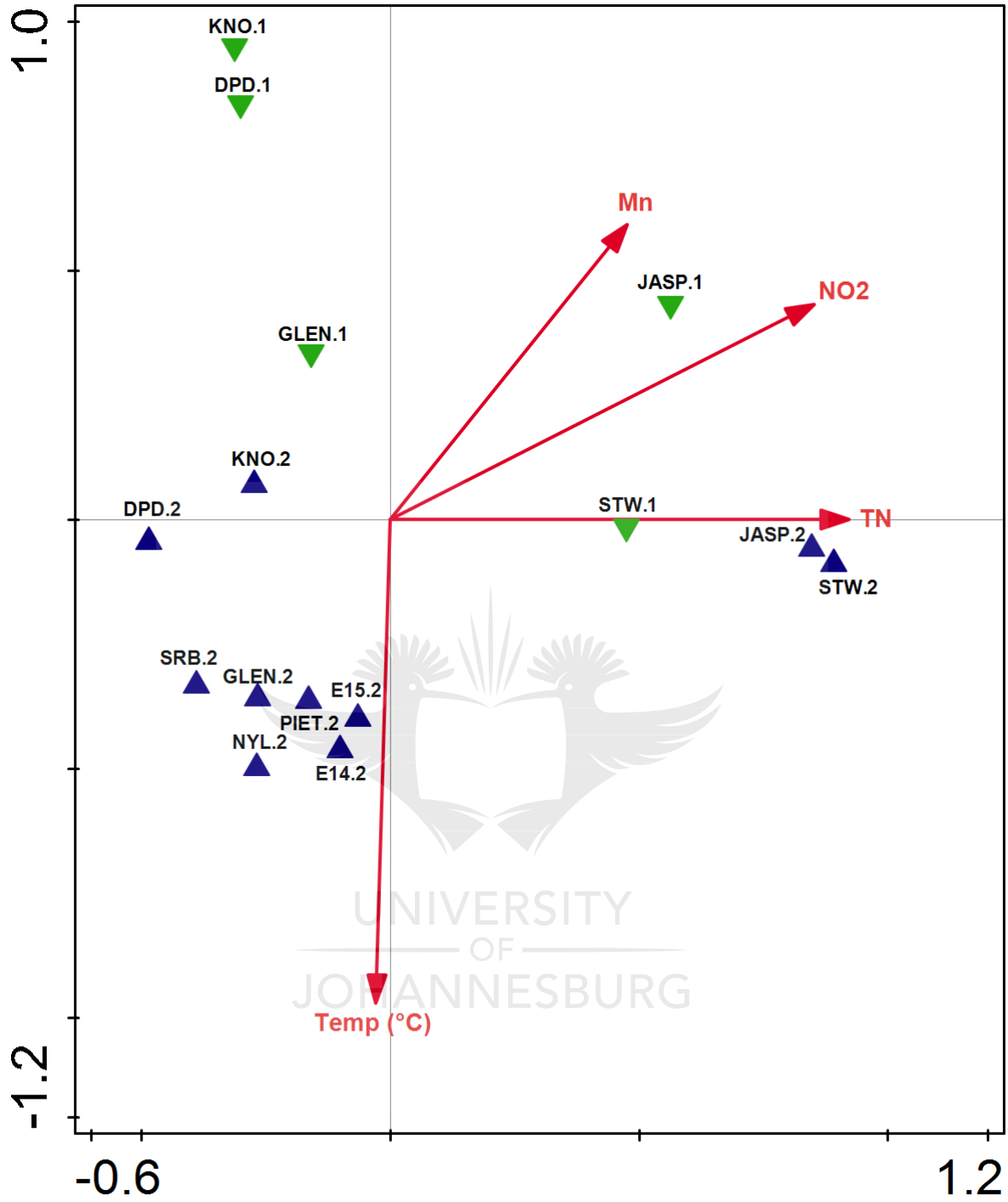

Figure 6.7: A constrained Redundancy Analysis bi-plot with forward selection indicating the distribution of the sampling sites and the significant ($p \leq 0.05$) water quality variables (including metal concentrations) by considering the collected macroinvertebrate data for the combined low flow and high flow sampling seasons. The numeral succeeding the site name denotes sampling season: **1,** low flow (green downward triangles); **2,** high flow (blue upward triangles). The red arrows indicate the individual water quality variables.

Nitromonas spp. facilitate the oxidation of NO_3^- forming NO_2^-, a species of nitrogen that is particularly toxic to aquatic organisms (DWAF 1996a; Dallas & Day 2004). Due to the lotic nature of rivers, impacts upstream often have deleterious effects on water quality and biotic integrity at subsequent downstream sites. This is the case at JASP, which showed an overall biodiversity score (H') lower than that of STW, the major point source of pollution in the Nyl River. Although wetland systems act as natural 'filters' for pollutants entering aquatic systems, the high density of aquatic and emergent vegetations (higher organic content) deplete DO concentrations, and therefore, these systems tend to show DO concentrations lower than that of lotic systems (Rai & Munchi 1979; Hamilton et al.1995; Davies & Day 1999; Dodds 2002). Consequently, the wetlands located between STW and JASP may be compounding the elevated NO_2^- concentrations at JASP (low flow) on account of the relatively low DO concentrations (high COD values) characteristic of these types of environments.

In conjunction with elevated nitrogen levels, the water at STW (low and high flow) contained the highest concentration of Mn, with the likely source being the effluent pumped into the system at this site. Galvin (1996) states that Mn and DO are inversely correlated, meaning that as DO within a system decreases, Mn concentrations increase. The high COD (low DO) readings at STW for both the low flow and high flow surveys may account for these elevated Mn levels. Although the levels of Mn were within ranges proposed by the TWQR for aquatic ecosystems (DWAF 1996a), heightened Mn concentrations may be having both direct and indirect impacts on the water quality and thus, the aquatic biota at STW and subsequent downstream sites such as JASP. According to DWAF (1996a), direct impacts of high Mn concentrations in aquatic ecosystems are due to its toxicity, with studies showing that Mn toxicity can disrupt the central nervous system of various aquatic fauna. Indirect impacts caused by elevated Mn levels include the alteration to nitrogen assimilation patterns of aquatic vegetation. Aquatic vegetation readily absorbs nutrients (specifically nitrogenous based nutrients) from the environment, a process that is important in the reduction of nutrient burdens in aquatic systems (DWAF 1996a). Manganese levels within the water column may influence this assimilation ability, therefore, the concentration of various nitrogen species within the aquatic system may subsequently be affected. If plants cannot perform the environmental service of removing nutrients from the water column, elevated total nitrogen levels, as is evident STW and JASP, may occur.

6.2.5 SPEARMAN'S RANK CORRELATION COEFFICIENT

A Spearman's rank correlation between the determined values of the environmental variables (including water metal concentrations) and the obtained values from the various diversity indices is presented in table 6.3. According to Dyamond (2017), the strength of linear relationships between environmental variables and the derived index values of the various diversity indices (DMa, J' and H') can be determined using correlations such as the Spearman's rank correlation. In short, if a significantly positive correlation is observed between an environmental variable and an index value, an increase in the environmental variable would result in an increase of the index value (e.g. Al and J'). Inversely, if a significantly negative correlation is observed, an increase in the value of an environmental variable would see a decrease in the index score (e.g. PO_4^{3-} and H').

These results show that DMa is significantly negatively correlated to EC, NO_3^-, TN and COD at a 95 % confidence level ($p \leq 0.05$) and to PO_4^{3-} and $CaCO_3^{2-}$ at a 99 % confidence level ($p \leq 0.01$), meaning that if these environmental variables were to increase, there would be a subsequent decrease in species richness. The correlation between J' and the environmental variables revealed that evenness was negatively correlated to TN at a 95 % confidence level and to NO_2^- and Mn at a 99 % confidence level. Moreover, a significantly positive correlation ($p \leq 0.05$) was observed between J' and Al. Therefore, with an increase of TN, NO_2^- and Mn, the presence of dominant taxa, often tolerant to these nutrients, would emerge. These results are identical to those presented in RDA bi-lot in Figure 6.7, which identified that sensitive taxa are strongly negatively correlated with these variables in particular. The H' index takes into consideration both the DMa and J' to give an overall understanding of the biodiversity at a site. No positive correlations existed between the environmental variables and H', however, H' was found to be significantly correlated to NO_2^-, TN, PO_4^{3-}, Cl^-, $CaCO_3^{2-}$ and Fe at a confidence level of 95 %. This indicates that a decrease in overall species diversity would occur if any of these environmental variables were to increase within the system.

The negative correlations of many of the environmental variables to species richness, evenness and overall biodiversity further support the trends observed in the RDA bi-plots with regards to water quality and macroinvertebrate community assemblage composition. These results indicate that the environmental variables that show significant negative correlations to overall biodiversity are present in elevated levels at STW and JASP, and consequently, the faunal assemblages at these sites show decreased diversity and

increased evenness; these taxa are tolerant to high nutrient loads and were present in very high abundances.

Table 6.3: A Spearman's rank correlation coefficient between the measured environmental variables (including water metal concentrations) and the various macroinvertebrate diversity index values obtained. Values shown with superscript asterisk symbols indicate that significant correlations exist. ***,** correlation is significant at $p \leq 0.05$ (dark grey bars); ****,** correlation is significant at $p \leq 0.01$ (light grey bars).

	Margalef's Index	Pielou's Evenness Index	Shannon-Wiener Index
Temp (°C)	-0.292	0.444	0.093
pH	-0.301	0.145	-0.129
EC ($\mu S.cm^{-1}$)	-0.524*	-0.406	-0.406
NO_2^-	-0.195	-0.788**	-0.559*
NO_3^-	-0.594*	-0.380	-0.496
NH_3	-0.418	-0.500	-0.300
NH_4^+	-0.422	-0.495	-0.313
TN	-0.552*	-0.590*	-0.601*
PO_4^{3-}	-0.802**	-0.335	-0.543*
SO_4^{2-}	-0.345	-0.184	-0.150
Cl^-	-0.475	-0.421	-0.529*
F^-	0.048	0.241	-0.043
COD	-0.568*	-0.444	-0.507
$CaCO_3^{2-}$	-0.661**	-0.425	-0.546*
Al	-0.347	0.572*	-0.006
Cu	-0.300	-0.214	-0.289
Fe	-0.482	-0.300	-0.621*
Mn	0.014	-0.675**	-0.332
Pb	-0.240	-0.281	-0.209
V	0.147	0.434	0.262
Zn	-0.214	-0.264	-0.243

6.3 CONCLUSION

The ecological indices (DMa, J', H') conducted on the macroinvertebrate community assemblages showed a decreased trend in species richness, evenness and overall biodiversity from the upper reaches of the Nyl River which contained the site (DPD) with the highest species richness and most diverse FFG composition, to the downstream sites,

STW and JASP, which were heavily impacted by allochthonous organic pollutants entering the system at the Modimolle WWTF. The macroinvertebrate community assemblages at these sites consisted of a small number of gathering and filtering collector taxa, including *Tubifex tubif*ex and Culicidae species which show a high tolerance to extreme levels of organic pollution. These taxa which were found in large abundance, are capable of exploiting the copious amounts of rich, organic food resources without any real competition or the risk of predation. At NYL, the ecological service of 'filtering' pollutants from the water column provided by the Nylsvley wetland, allowed for a recovery of the macroinvertebrate communities with regards to diversity, showing a shift in assemblage composition, favouring predators and taxa better adapted to life in lotic ecosystems. Extraordinary flow conditions in the Mogalakwena River during low flow (drought conditions) and high flow (flooding activity) made the accurate determination of the macroinvertebrate community assemblage compositions within this river difficult, therefore, further research is needed during normal flow conditions. In spite of these extraordinary flow conditions, the presence of highly sensitive species such as *Elassoneuria* sp. and *Hydra oligactis* at sites along the Mogalakwena and Limpopo Rivers, suggest that the water within these systems is of good quality.

The results of the various statistical analyses indicate that the Modimolle WWTF, despite recent upgrades to the facility in 2016 (DWS 2015), continues to impact the aquatic integrity of the Nyl River through the discharge of allochthonous organic matter, thereby increasing organic nutrient loads, and consequently, decreasing macroinvertebrate diversity within the system at STW and JASP. Forward selection and a Monte Carlo permutation testing in RDA bi-plots identified the following environmental variables: TN, NO_2^- and Mn, as significant ($p \leq 0.05$) drivers of spatial variation between the macroinvertebrate community assemblages at each sampling site for both sampling seasons. Total nitrogen was attributed to be the dominant factor in the alteration of macroinvertebrate community assemblages at the sites. The interaction of inorganic nitrogen with DO and elements such as Mn may be compounding the effects of organic pollution entering the system by facilitating the formation of toxic species such as NO_2^- and inhibiting the uptake of NO_3^- by the surrounding vegetation, respectively. Additionally, temperature was found to be a major driver of temporal variation between the macroinvertebrate community assemblages present at each sampling site. The temporal variation was attributed to seasonal differences in surface water temperatures during the low flow (winter) and high flow (summer) sampling seasons, which may be altering the

macroinvertebrate community assemblages according to their thermal tolerances, however, further research on this required.

The RDA bi-plot analyses were able to distinguish between impacted and non-impacted sites above and below the Modimolle WWTF. The changes to macroinvertebrate community assemblages can be attributed to the sewage effluent discharge, increased organic loads, and subsequent reductions to DO availability caused by the Modimolle WWTF. This is a result shared by the Spearman's rank correlation coefficient that identified macroinvertebrate diversity and evenness are significantly negatively correlated to increases in a variety of environmental variables including: EC, NO_2^-, NO_3^-, TN, PO_4^{3-}, Cl^- COD, $CaCO_3^{2-}$, Fe and Mn, all of which are directly or indirectly associated with sewage effluent runoff and organic pollution. Therefore, the results of the RDA bi-plots and the Spearman's rank correlation coefficient validate the use of macroinvertebrate community assemblages to discern water quality within the Nyl and Mogalakwena Rivers.

CHAPTER 7 – CONCLUSIONS AND RECCOMENDATIONS

7.1 CONCLUDING REMARKS

The Nyl and Mogalakwena Rivers are important water reservoirs for the semi-arid Limpopo Province. The Nyl River is the primary source of domestic water for the towns of Modimolle, Mookgophong and Mokopane. In addition to supporting domestic requirements, the Nyl River flows into and inundates the internationally acclaimed Nyl River floodplain which encompasses the Ramsar accredited Nylsvley Wetlands. These wetlands are a biodiversity hot spot, providing habitat and refugia for a multitude of aquatic and terrestrial fauna and flora, including native and migratory avian species (Tarboton 1987). At the lower end of the floodplain, the Mogalakwena River forms, eventually becoming a tributary of the transboundary Limpopo River (Tooth et al. 2002). The Mogalakwena River has an integral role in providing water for the extensive agricultural activities and the plethora of industrial and mining activities along its banks. Therefore, the economic and ecological importance of these rivers and the floodplain in which they inundate, underpins the necessity to increase monitoring and mitigation efforts to preserve aquatic integrity and ensure the water resources are being utilised in a sustainable manner.

The water quality in the upper reaches of the Nyl River (sites KNO and DPD) was found to be mostly natural, with elevated levels of some metals (e.g. Mn and Fe) being attributed to geo-chemical processes. The sediments, however, appear to be more impacted. The sediments collected from KNO during the low flow contained a high organic content and were found to consist mainly of finer particulate matter. This was attributed to a man-made weir present at the site, which would have stemmed the flow and created a sink for particulate and organic matter to settle out of the water column, allowing species of the Chironomidae to dominate. After the rains of the high flow season, organic loads were reduced, and the sediments became coarser. Sedimentation at DPD during the high flow season significantly altered habitat availability for aquatic fauna, which resulted in a decline in macroinvertebrate richness and abundance. Despite the identified impacts, the macroinvertebrate communities at these sites were, for the most part, natural and included both tolerant and sensitive taxa. The diversity indices revealed that community compositions between KNO and DPD differed, with DPD showing a higher diversity and evenness of taxa. This was expected however, as ground water emerging from

subterranean sources at KNO, lacks essential minerals and nutrients that are necessary in supporting a wide diversity of taxa.

Despite its recent infrastructural and capacity upgrades completed in 2016 (DWS 2015), the Modimolle WWTF was found to be the primary contributor of allochthonous nutrients entering the Nyl River system downstream of DPD, therefore leading to a significant decline in aquatic integrity. The results showed that the sewage effluent discharge was impacting the sediment composition, water quality and macroinvertebrate community assemblages at STW and subsequent downstream sites such as JASP. The sediment composition had changed from being predominantly comprised of coarser and medium sands in the upper reaches of the Nyl River to finer particulate matter at STW. Suspended particulate matter is known to accumulate contaminants, eventually settling out of the water column into sediments (Chapman 1996, USEPA 2001, Gerber et al. 2015). Therefore, contaminants within sediments may be compounding the effects of the pollutants entering the system and may act as a continual source of contamination, even if effluent discharge is hindered (Filgueiras et al. 2004). In addition, the water quality at STW and JASP showed a steep decline, with the presence of heightened levels of many of the tested parameters. At STW, severe organic pollution was evident, highlighted by elevated nutrients (NO_2^-, NO_3^-, NH_4^+, NH_3, TN and PO_4^{3-}), EC and COD (depleted DO concentrations). Although the sediment composition at JASP had returned to conditions like those upstream of the Modimolle WWTF, the water quality analyses revealed that the levels of many of the tested water variables were higher at JASP than STW. This is a result reflected by the diversity indices which identified JASP as having a lower biodiversity score. An increase in water hardness was also observed, which may have arisen due to an interbasin water transfer scheme between the Apies and Pienaars Rivers in the Gauteng Province, South Africa (Magalies Water 2013). These impacts were the determining drivers for the macroinvertebrate community assemblages at STW and JASP, with only a few, highly tolerant taxa being present in high abundances. These taxa included: *Tubifex tubifex*, *Psychoda alternata*, *Clogmia albipunctata* and members of the Syrphidae, Culicidae and Chironomidae.

The decreased levels of most of the tested variables at NYL indicate that the wetland is acting as a ‘filter’, purifying the waters coming from the polluted upstream sites, therefore allowing macroinvertebrate community assemblages to recover. The sediments were determined to be natural of wetland systems, consisting of fine particulate matter and high organics (Dallas & Day 2004; Ferreira 2010; Dyamond 2017). A shift in the

macroinvertebrate community assemblages was also noted, with the taxa present at this site being better adapted to life-histories in lotic systems (De Roeck 2008). These taxa included members of the Libellulidae and Notonectidae.

The Mogalakwena River has been noted to be highly modified by several hundred farm dams of all shapes and sizes (Boroto & Görgens 1999). These modifications to the river are not only having impacts to downstream flow and water availability, but to the biotic habitat integrity and natural processes such as sediment mobility. The river bed had changed from being mostly alluvial based substratum in the Nyl River to mostly bedrock in the Mogalakwena River (Tooth et al. 2002; McCarthy et al. 2011). This alteration in river geomorphology may have accounted for the coarser grain sizes of the sediments and therefore the reduced diversity of benthic macroinvertebrates within the Mogalakwena River. The overall water quality of the Mogalakwena River was determined to be good, with only F^-, Al and V showing elevated concentrations. Despite the elevated concentrations of these elements, they may be of little concern due to the occurrence of highly sensitive taxa such as *Hydra oligactis* and *Elassoneuria* sp. Drought and flooding conditions during low flow and high flow sampling surveys, respectively, would have influenced the water quality, sediment quality and macroinvertebrate community assemblages within the Mogalakwena River at the time of sampling. Therefore, further sampling during normal flow conditions is needed to validate the obtained results.

The multivariate analyses conducted on the macroinvertebrate data for low flow and high flow sampling seasons revealed significant ($p < 0.05$) spatial and temporal differences between the sampling sites. The temporal differences were driven by temperature, separating the sites by the season in which they were sampled. The spatial variation between sites were significantly driven by TN, NO_2^- and Mn, all of which are associated with allochthonous pollutants entering the Nyl River system at STW. Nitrogenous pollutants readily interact with the surrounding water (DWAF 1996a, 1996b; Dallas & Day 2004). Anaerobic conditions caused by the oxidation of organic matter facilitates the formation of toxic NO_2^- species, which may further be compounded by high concentrations of Mn which inhibits the assimilation of NO_3^- by aquatic vegetation (DWAF 1996a; Dallas & Day 2004). The RDA bi-plots were capable of distinguishing between sites upstream and downstream of the Modimolle WWTF, which is seriously impacting water and sediment quality, thereby reducing the diversity of macroinvertebrate community assemblages in the Nyl River. This is a result shared by a Spearman's rank

correlation coefficient which identified significant negative correlations between the diversity indices and many tested environmental and water parameters.

The hypotheses of this study were as follows:

i. Anthropogenic activities are having negative impacts on the macroinvertebrate community assemblages that inhabit the Nyl and Mogalakwena River systems.

This hypothesis is accepted. The results of this study indicate that anthropogenic activities such as flow modifications, sewage effluent discharge and possibly interbasin water transfer schemes (Magalies Water 2013) are having negative impacts to the quality and quantity of water as well as to the quality of sediments within the Nyl and Mogalakwena Rivers. The quality of water and sediment is of utmost importance in maintaining aquatic integrity, therefore, any alterations to these aspects are subsequently reflected by changes to the macroinvertebrate community assemblage that inhabit these rivers.

ii. Water quality is continually being affected by increases in nutrient loads as a result of the Modimolle wastewater treatment facility.

This hypothesis is accepted. The results show that despite recent upgrades to the Modimolle WWTF in 2016 (DWS 2015), it continues to discharge sewage effluent into the Nyl River at STW. The results of the water quality analysis revealed that water at STW and JASP contained, amongst others, extremely elevated levels of nutrients, leading to the classification of these sites as being hypereutrophic.

iii. Macroinvertebrate community assemblages reveal that the water quality of the Nyl and Mogalakwena Rivers is declining.

This hypothesis is accepted. The results of the RDA bi-plot analyses and the Spearman's rank correlation coefficient revealed that the species richness, evenness and overall biodiversity of macroinvertebrate community assemblages showed significantly ($p < 0.05$) negative correlations to many of the tested water and environmental parameters (e.g. TN, NO_2^-, Mn), thereby validating the use of macroinvertebrate community assemblages as a tool to discern water quality within the Nyl and Mogalakwena Rivers.

7.2 RECOMMENDATIONS

As this was the first comprehensive macroinvertebrate study on the Nyl and Mogalakwena River systems, it is suggested that a follow-up study be conducted to address gaps in the current literature. Follow-up research should be undertaken over a longer duration, possibly over many seasons and should be carried out during normal river flow conditions. It should take into account the various tributaries of the Nyl and Mogalakwena Rivers to determine the extent of their impact to the water quality, sediment quality and macroinvertebrate assemblages, and should also include sampling of the Limpopo River above and below the Mogalakwena confluence. Furthermore, Barbour et al. 1999 describes that habitat assessments are a necessity in macroinvertebrate community assemblage studies, therefore, a comprehensive habitat assessment should also be conducted on these river systems.

Previous studies by Vlok et al. (2006), Dahms (2016) and Musa (2016) on the Nyl River system recommended further research be conducted regarding water quality, metal contamination as well as fish, amphibian and macroinvertebrate assemblages within the combined Nyl and Mogalakwena Rivers. Although the current study has addressed aspects of these recommendations, further research on higher trophic levels are recommended, including fish and amphibian assemblages. Additionally, physiological analyses such as biomarker and toxicity testing would be beneficial to pinpoint exactly how organisms such as fish, amphibians, diatoms and macroinvertebrates may be responding to changes to environmental conditions and water quality.

Due to the multiple urban, industrial and agricultural stressors on the aquatic integrity of the Nyl and Mogalakwena Rivers, monitoring efforts should continue and be intensified, specifically in the light of the many established and proposed mining activities in the vicinity of the Nyl and Mogalakwena catchment areas (Ashton et al. 2001, AED 2012, Dahms 2016).

Lastly, it is suggested that the "*Guides to the Freshwater Invertebrates of Southern Africa*", where possible, should be updated to incorporate more current research. Furthermore, we recommend a revision of the identification keys for some of the key groups of invertebrates (e.g. Hemiptera) as many of the keys are only useful for family level identification.

CHAPTER 8 – REFERENCES

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APPENDIX A – WATER QUALITY PARAMETERS

Table A.1: *In situ* water quality variables and nutrients measured at each sampling sites for the low flow sampling survey. The numeral succeeding the site name denotes sampling season: **1,** low flow (July 2016). All values presented as $mg.L^{-1}$, unless stated otherwise.

SITE NAME	TEMP (°C)	pH	EC ($\mu S.cm^{-1}$)	NO_2^-	NO_3^-	NH_3^+	NH_4^+	TN	PO_4^{3-}	SO_4^{2-}	Cl^-	F^-	COD	$CACO_3^{2-}$
KNO.1	11.4	7.00	100	0.020	0.10	0.030	0.03	0.4	0.005	27	11.84	0.170	4.6	39
DPD.1	11.4	5.77	100	0.020	0.15	0.040	0.04	0.6	0.005	31	0.79	0.070	9.2	33
STW.1	18.5	9.38	900	0.020	0.50	19.900	25.60	16.8	3.520	78	62.19	0.050	52.8	176
JASP.1	12.2	9.38	700	0.100	4.70	6.360	8.20	12.8	0.260	86	87.79	0.170	20.4	93
GLEN.1	15.6	10.73	500	0.020	0.18	0.230	0.23	0.7	0.005	60	59.79	0.170	11.5	78

Table A.2: *In situ* water quality variables and nutrients measured at each sampling sites for the high flow sampling survey. The numeral succeeding the site name denotes sampling season: **2,** high flow (February 2017). All values presented as $mg.L^{-1}$, unless stated otherwise.

SITE NAME	TEMP (°C)	pH	EC ($\mu S.cm^{-1}$)	NO_2^-	NO_3^-	NH_3^+	NH_4^+	TN	PO_4^{3-}	SO_4^{2-}	Cl^-	F^-	COD	$CACO_3^{2-}$
KNO.2	18.5	6.90	47	0.010	0.10	0.015	0.015	0.7	0.020	36	5.43	0.130	33.9	11
DPD.2	20.0	7.50	167	0.005	0.10	0.028	0.023	0.5	0.050	43	6.07	0.060	11.6	50
STW.2	22.3	7.40	597	0.050	1.20	7.000	7.000	15.3	0.810	45	25.73	0.070	44.6	165
JASP.2	20.2	7.80	686	0.130	1.20	3.200	3.200	6.0	0.130	38	49.33	0.250	31.6	183
NYL.2	23.8	6.50	69	0.007	0.30	0.035	0.035	1.2	0.031	47	1.25	0.360	52.8	35
SRB.2	23.0	8.10	56	0.006	0.30	0.02	0.020	0.6	0.022	37	4.79	0.300	7.3	27
GLEN.2	24.8	8.40	165	0.007	0.10	0.023	0.023	0.6	0.024	37	15.39	0.140	14.3	80
PIET.2	25.6	8.20	244	0.009	0.40	0.021	0.021	0.6	0.020	34	17.23	0.648	11.6	57
E15.2	27.0	8.20	275	0.011	0.30	0.027	0.027	0.6	0.020	45	19.83	0.130	14.3	72
E14.2	27.1	8.50	341	0.008	0.90	0.014	0.014	0.9	0.040	69	20.73	0.350	11.7	88

APPENDIX B – METAL CONCENTRATIONS

Table B.1: Concentrations of metals detected in water samples collected from sites along the Nyl and Mogalakwena Rivers during the low flow sampling period. The numeral succeeding the site name denotes sampling season: **1,** low flow (July 2016). All metal concentrations are presented in $\mu g.L^{-1}$. The abbreviation **BD** is used for metals that were below the detection.

SITE NAME	Al	Cu	Fe	Mn	Pb	V	Zn
KNO.1	BD	2.550	22.530	60.905	BD	BD	8.506
DPD.1	BD	2.478	9.828	35.067	BD	BD	7.648
STW.1	20.494	4.769	175.882	93.675	53.282	BD	20.944
JASP.1	BD	4.382	19.574	14.641	BD	BD	9.856
GLEN.1	BD	4.070	7.236	12.212	26.888	3.723	4.473

Table B.2: Concentrations of metals detected in water samples collected from sites along the Nyl and Mogalakwena Rivers during the high flow sampling period. The numeral succeeding the site name denotes sampling season: **2,** high flow (February 2017). All metal concentrations are presented in $\mu g.L^{-1}$. The abbreviation **BD** is used for metals that were below the detection.

SITE NAME	Al	Cu	Fe	Mn	Pb	V	Zn
KNO.2	BD	5.347	255.097	11.870	BD	BD	11.333
DPD.2	BD	4.437	6.526	11.790	BD	BD	7.091
STW.2	BD	6.230	29.448	128.801	BD	BD	13.594
JASP.2	BD	6.191	35.910	10.650	24.561	BD	4.862
NYL.2	BD	7.259	41.699	2.545	BD	BD	9.823
SRB.2	49.068	2.065	29.486	4.084	BD	BD	5.211
GLEN.2	54.804	4.569	39.374	6.058	BD	3.807	8.279
PIET.2	28.227	6.225	19.213	6.393	BD	6.014	8.001
E15.2	BD	6.110	9.106	6.699	BD	4.385	23.753
E14.2	25.527	8.392	15.639	8.008	BD	9.508	16.262

APPENDIX C – FUNCTIONAL FEEDING GROUPS

Table C.1: A list of the macroinvertebrate families with their associated Functional Feeding Groups (FFG) collected at various sites along the Nyl and Mogalakwena Rivers during the low flow sampling season. The numeral succeeding the site name denotes sampling season: **1,** low flow (July 2016).

Family	FFG	Code	KNO.1	DPD.1	STW.1	JASP.1	GLEN.1
Hydridae	Predator	PR					188
Naididae	Gathering collector	GC	13		608	2120	12
Lumbricidae	Gathering collector	GC	285	148			126
Lumbriculidae	Gathering collector	GC		8	3	24	26
Glossiphoniidae	Predator	PR				574	20
Salifidae	Predator	PR					1
Thiariidae	Scraper	SC					10
Bulinidae	Scraper	SC	3	8			1
Planorbidae	Scraper	SC		25			16
Physidae	Scraper	SC			1	98	
Lymnaeidae	Scraper	SC	3	26			2
Sphaeriidae	Filtering collector	FC					1
Potamonautidae	Shredder	SH		2			1
Atyidae	Shredder	SH		16			4
Hydrozetidae	Predator	PR		7			
Hydrachnidae	Predator	PR		1			
Tetragnathidae	Predator	PR	1	1			1
Lycosidae	Predator	PR	1		3		
Pisauridae	Predator	PR				6	
Baetidae	Gathering collector	GC	23	559	2		136
Heptageniidae	Scraper	SC		2			
Caenidae	Gathering collector	GC	1	46			76

Table C.1: Continued.

Family	FFG	Code	KNO.1	DPD.1	STW.1	JASP.1	GLEN.1
Polymitarcyidae	Gathering collector	GC		2			2
Leptophlebiidae	Gathering collector	GC		130			
Lestidae	Predator	PR	1	6			
Platycnemididae	Predator	PR					3
Coenagrionidae	Predator	PR	18	42			4
Chlorocyphidae	Predator	PR					2
Gomphidae	Predator	PR	4	65			4
Aeshnidae	Predator	PR		1			
Libellulidae	Predator	PR	6	53	1		4
Aphididae	Plant piercer	PRC		6		1	3
Mesoveliidae	Predator	PR					1
Gerridae	Predator	PR	1	1			
Corixidae	Scraper	SC	3				10
Notonectidae	Predator	PR	4				
Pleidae	Predator	PR				1	
Naucoridae	Predator	PR		8			2
Belostomatidae	Predator	PR		1			
Hydroptilidae	Scraper	SC	8	175			
Hydroptilidae – Oxythira	Predator	PR		10			
Hydropsychidae	Filtering collector	FC	3	26			34
Ecnomidae	Predator	PR	2	10			9
Leptoceridae	Predator	PR		49			2
Tipulidae	Predator	PR	1	6		1	

Table C.1: Continued.

Family	FFG	Code	KNO.1	DPD.1	STW.1	JASP.1	GLEN.1
Psychodidae	Gathering collector	GC			361	15	
Dixidae	Filtering collector	2					2
Chaoboridae	Predator	PR				1	
Ceratopogonidae	Predator	PR	150	26		7	32
Culicidae	Filtering collector	FC	4	14	15		1
Chironomidae	Gathering collector	GC	1587	798	188	281	297
Simuliidae	Gathering collector	GC	22	112		196	1
Tabanidae	Predator	PR	1	13		2	6
Stratiomyidae	Gathering collector	GC	1	1			
Dolichopodidae	Predator	PR		2			
Ephydridae	Gathering collector	GC				2	
Muscidae	Predator	PR	3		27	27	1
Syrphidae	Filtering collector	FC			39		
Gyrinidae	Predator	PR	1	14			
Haliplidae	Shredder	SH					2
Noteridae	Predator	PR	1				
Dytiscidae	Predator	PR		7		2	
Hydrochidae	Shredder	SH		1			
Hydrophilidae	Predator	PR	5	8		2	2
Hydraenidae	Predator	PR				2	
Scirtidae	Gathering collector	GC				2	
Chrysomelidae	Shredder	SH		1			
Curculionidae	Shredder	SH	1				1

Table C.2: A list of the macroinvertebrate families with their associated Functional Feeding Groups (FFG) collected at various sites along the Nyl and Mogalakwena Rivers during the high flow sampling season. The numeral succeeding the site name denotes sampling season: **2,** high flow (February 2017).

Family	FFG	Code	KNO.2	DPD.2	STW.2	JASP.2	NYL.2	SRB.2	GLEN.2	PIET.2	E15.2	E14.2
Naididae	Gathering collector	GC	35		5345	3505					4	2
Lumbricidae	Gathering collector	GC	1263	15			8	4	12	1	32	7
Lumbriculidae	Gathering collector	GC			114	1						
Glossiphoniidae	Predator	PR	3			365	1	7			1	1
Salifidae	Predator	PR				9						
Thiariidae	Scraper	SC									39	119
Bulinidae	Scraper	SC	1	99			3			1	5	
Planorbidae	Scraper	SC	3	19	1					1	1	
Physidae	Scraper	SC				299	1					
Lymnaeidae	Scraper	SC	10	72								
Corbiculidae	Filtering collector	FC									1	2
Sphaeriidae	Filtering collector	FC	6									
Potamonautidae	Shredder	SH	4	1								
Atyidae	Shredder	SH		7					1	2		
Tetragnathidae	Predator	PR	3	4							4	5
Lycosidae	Predator	PR	2	1	1	5			2	1		1
Pisauridae	Predator	PR								1		2
Baetidae	Gathering collector	GC	42	18		4	32	7	8	7	7	28
Oligoneuriidae	Filtering collector	FC						1				328
Caenidae	Gathering collector	GC	4							1	8	5
Polymitarcyidae	Gathering collector	GC		4								
Leptophlebiidae	Gathering collector	GC										7

Table C.2: Continued.

Family	FFG	Code	KNO.2	DPD.2	STW.2	JASP.2	NYL.2	SRB.2	GLEN.2	PIET.2	E15.2	E14.2
Lestidae	Predator	PR	2									
Platycnemididae	Predator	PR	6	7				2				
Coenagrionidae	Predator	PR		12			2			5	7	1
Gomphidae	Predator	PR		1				1			5	12
Aeshnidae	Predator	PR	1	1			3					
Libellulidae	Predator	PR	7	6			88	3		1	4	17
Aphididae	Plant piercer	PRC				1						
Gerridae	Predator	PR	2				1				1	
Corixidae	Scrapers	SC	2				5					
Notonectidae	Predator	PR					70					
Naucoridae	Predator	PR	1	1					3		1	2
Belostomatidae	Predator	PR		2						1	1	
Hydroptilidae	Scraper	SC	6									
Hydroptilidae – Oxythira	Predator	PR	1									
Hydropsychidae	Filtering collector	FC										8
Ecnomidae	Predator	PR		1								2
Leptoceridae	Predator	PR	13	7								5
Crambidae	Shredder	SH	7							1	7	
Tipulidae	Predator	PR	1	2		1		8				
Psychodidae	Gathering collector	GC			23	1						
Dixidae	Filtering collector	FC	1									
Ceratopogonidae	Predator	PR	51	5			6				2	
Ceratopogonidae – Atrichopogon	Gathering collector	GC									1	

Table C.2: Continued.

Family	FFG	Code	KNO.2	DPD.2	STW.2	JASP.2	NYL.2	SRB.2	GLEN.2	PIET.2	E15.2	E14.2
Culicidae	Filtering collector	FC			1295	1	2					
Chironomidae	Gathering collector	GC	105	72	13	10	60	7	1		39	46
Simuliidae	Filtering collector	FC	76	62								77
Tabanidae	Predator	PR					2					
Stratiomyidae	Gathering collector	GC				1						
Ephydridae	Gathering collector	GC			41	4						
Muscidae	Predator	PR	1		31	8		1	2		3	2
Syrphidae	Filtering collectors	FC	1		122							
Noteridae	Predator	PR					3					
Dytiscidae	Predator	PR	1	2		6	1		1	1		2
Aphodiidae	Feeds on dung	-						1	2			
Hydrophilidae	Predator	PR	3	7	2	7	3		1		2	
Elmidae	Scraper	SC	1									1
Dryopidae	Scraper	SC							1			
Limnichidae	Gathering collector	GC								2		
Curculionidae	Shredder	SH					3					