Aspects of the biological integrity of the rivers flowing into the hyper-eutrophic Roodeplaat Dam: A comparative study

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List of abbreviations and acronyms

ASPT – Average Score Per Taxa

CCME – Canadian Council of Ministers of the Environment

CF – Condition factor

CSI – Cardiosomatic index

CSIR – Council for scientific and industrial research

BOD – Biological oxygen demand

DDT – Dichlorodiphenyltrichloroethane

DCA – Detrended Correspondence Analysis

DEA – Department of Environmental Affairs

DEHP – Bis(2-ethylhexyl) phthalate

DiNP – Diisononyl phthalate

DWAF – Department of Water affairs and Forestry

DWS – Department of Water and Sanitation

EC – Electrical conductivity

EDCs – Endocrine Disrupting Chemicals

EPO – Erythropoietin

FCAs – Focal areas of cellular alterations

FFGs – functional feeding groups

GC-MS – Gas chromatography-mass spectronomy

GIS – geographic information systems

GSH – glutathione

GSI – Gonadosomatic Index

GSM – gravel, sand and mud

H&E – Haemotoxylin and Eosin

HSI – Hepatosomatic index

IHAS – Integrated habitat assessment

LC-MS – Liquid chromatography–mass spectrometry

MD – Mass of moist sediment

MMC – Melanomacrophage centers

MO – mass of organic matter

MPDS – mass of petri-dish + sediment

MP – mass of petri-dish

mS/m – Millisiemens/meter

NAEHMP - National Aquatic Ecosystem Health Monitoring Programme

NFEPA – National Freshwater Ecosystem Priority Areas

NH_3 – Ammonia

NWA – National Water Act

OC pesticides – Organochlorine pesticides

OM – Organic matter content

PAEs – phthalic acid esters

PAHs – Polycyclic aromatic hydrocarbons

PCA – Principal component analyses

PCB – Polychlorinated biphenyl

PE – Phthalate Esters

PES – Present ecological status

PPM – Parts per million

PVC – Polyvinyl chloride

RCA – Reference Condition Approach

REMP – River Eco-status Monitoring Program

RHP – River Health Program

SANAS – South African National Accreditation System

SASS4 - South African Scoring System 4

SASS5 – South African Scoring System 5

SD – Standard deviation

SiO_2 – Silica

SVOCs – Semi-volatile organic compounds

TDS – Total dissolved solids

UAE – United Arab Emirates

USEPA – United States Environmental Protection Agency

UG/L – Microgram/liter

WHO – World Health Organization

WMA – Water management area

WWF – World Wide Fund

WWTP – Waste water treatment plant

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Proverbs 16:3

Commit your actions to the LORD, and your plans will succeed.

Abstract

Eutrophication is an issue of concern for many of South Africa's freshwater sources. Roodeplaat Dam is situated in Pretoria, South Africa, and the catchment is surrounded by a mixture of human activities. The dam, supplied by the three inflowing rivers (Pienaars River, Edendalespruit and Hartbeesspruit, which includes the tributary, the Morelettaspruit) is an important freshwater reservoir as it is used for irrigation purposes, drinking water as well as recreational activities. The eutrophication levels in Roodeplaat Dam has been a cause for concern since the early 1980s and has been highlighted again more recently. The water quality of the three rivers that feed into the Roodeplaat Dam contribute to the eutrophication within the Dam and the surrounding human activities could negatively affect the biotic integrity of these rivers. However, limited research has been done on these rivers with regard to water quality and no information is avaibale on the health of fish inhabiting these rivers. Therefore, the aim of the study was to determine the biotic intergrity of the three inflowing rivers of the Roodeplaat Dam by assessing and comparing aspects of specific physical, chemical and biological parameters. This included the analyses of the water and sediment quality, macro-invertebrate diversity and the health status of fish inhabiting the three rivers. Previously, physical and chemical analyses have been the main focus when determining the quality of water. However, biological response attributes are equally important to assess ecosystem health and therefore, biological monitoring or bio-monitoring has become a crucial part in water quality assessments. The methodology employed for water and sediment quality analyses involved *in-situ* and laboratory analyses. Macro-invertebrate sampling was conducted using the South African Scoring System version 5 (SASS5) and the habitat assessment was performed using the Integrated Habitat Assessment System proposed by Ollis *et al* (2006). Two indicator fish species (*Clarias gariepinus* and *Oreochromis mossambicus*) were selected for necropsy and biometric analyses as well as a semi-quantitaive histopathological assessment of selected target organs based on the protocol by Bernet *et al.* (1999). Microcystin analysis of fish liver tissue was performed using the protocol adapted from Xie and Park (2007). The results were compared both seasonally (high-flow and low-flow), between rivers and in the case of the fish health assessment between species. The water analyses revealed the presence of microcystins and selected metals as well as high levels of nutrients and faecal coliform bacteria in each river. The sediment analyses showed high levels of metals and semi-volatile compounds. The macro-invertebrate diversity showed seasonal differences at each site within each river, the majority of sites were classified as poor and had a lower SASS5 score during the high-flow conditions. The semi-quantitative histopathological

assessment showed that during the low-flow conditions, *C. gariepinus* and *O. mossambicus* collected from Edendalespruit had the highest Fish Index, while *C. gariepinus* from Pienaars had the highest Fish Index during the high-flow conditions. The highest Fish Index value during the high-flow conditions for *O. mossambicus* was seen in Hartbeespruit. The most affected target organ in both species was the liver, during both seasons, and the histological alterations ranged from progressive to regressive changes and inflammation. The overall results showed that *C. gariepinus* had more histological alterations when compared to the *O. mossambicus*. Microcystin analysis of liver samples from each fish species showed a different species response comparing the different microcystin analogues tested for. Microcystin-LR was significantly higher ($p < 0.05$) in *C. gariepinus*, while microcystin-RR and YR was significantly higher ($p < 0.05$) in *O. mossambicus.* Seasonal differences were also observed in *C. gariepinus*. Microcystin-LR was significantly higher during low-flow conditions whereas microcystin-RR and YR were significantly higher during high-flow conditions. There was also a positive correlation seen between the microcystin concentrations and the hepatosomatic index in *C. gariepinus*. Overall, the results showed that each river is polluted and affected by eutrophication to a certain extent, subsequently compromising the biotic intergrity of the system. The macro-invertebrate results showed that most sites were classified as poor in terms of their SASS5 score indicating pollution, this was also indicated in the histological alterations observed in each fish species. Due to the fact that the National Water Act of South Africa states that every person has the right to clean water which is not detrimental to their health, management strategies and recommendations were proposed to improve the quality of water within the three rivers which would ultimately lead to the improvement of water quality within Roodeplaat Dam. The recommendations include regular water quality monitoring, consistent and effective hyacinth clean-up programmes, education of water users on practices which add to the poor quality of the water and most importantly, an integrated water management plan which involves the surrounding communites.

Key words: *Eutrophication, C. gariepinus, O. mossambicus, biotic integrity, microcystins*

Chapter 1: General introduction

1.1. Background Information and problem statement

South Africa is a country rich in natural resources, which play a significant role in the economic development of the country as well as in the livelihoods of her inhabitants (African Development Bank Group 2015). In order to ensure the effective and sustainable use of these natural resources, environmental management laws are put in place by different spheres of government. Environmental management is defined by Barrow (1997) as; *"the process of allocating natural and artificial resources so as to make optimum use of the environment in satisfying basic human needs if possible for an infinite period, and with minimal adverse effects"*. This definition is closely related to the National Environmental Management Act (NEMA) (Republic of South Africa 1998a) which states that, *"Everyone has the right to an environment that is not harmful to his or her health or wellbeing; the State must respect, protect, promote and fulfil the social, economic and environmental rights of everyone and strive to meet the basic needs of previously disadvantaged communities; inequality in the distribution of wealth and resources, and the resultant poverty, are among the important causes as well as the results of environmentally harmful practices"*. Access to clean, safe water is a human right and a basic need, therefore laws and associated regulations have been put in place to ensure that this need is fulfilled. The National Water Act (NWA) (Act No. 36 of 1998) is the core water-related legislation in South Africa, by which all water resources, their uses, their management and distribution is governed. It is based on a number of fundamental principles (Republic of South Africa 1998b). The fifth and sixth fundamental principles mentioned below are particularly important in terms of conservation or protection of natural freshwater resources or river systems:

- *Protection of the quality of water resources is necessary to ensure sustainability in the interests of all water users;*
- *All aspects of water resources need to be managed in an integrated way, and, where appropriate, management functions need to be delegated to a regional or catchment level to enable everyone to participate."*

Bio-monitoring plays an integral part in the protection of freshwater resources and ultimately leads to more effective management plans (Cilliers and Adams 2016). Organisms usually respond to changes in their environments, and these environmental factors are either known as biotic (living) or abiotic (non-living). Abiotic factors in aquatic ecosystems include physical and chemical aspects e.g. temperature, sediment, moisture and organic and inorganic

chemicals whereas biotic factors are the living organisms such as bacteria, macro-invertebrates, amphibians, fish and aquatic bird as well as mammal species and how they interact with each other (Enger and Smith 2006; Muogalo 2009). The above factors can be used to assess the biotic integrity of a river system.

Biotic integrity is defined as *"the ability of a system to generate and to maintain the adaptive biotic components whilst the components progress through natural evolution. In a lotic system such as a freshwater system, biotic integrity is dependent on the flow of the river, energy input, habitat structure, biotic interaction and lastly water quality"* (Karr 1981; Karr *et al*. 1986; Hughes and Gammon 1987; Pinto and Araújo 2007). Physical and chemical analyses of water quality have previously been the main focus when determining the quality of water. However, biological response attributes are equally important to assess ecosystem health and therefore, biological monitoring or bio-monitoring has become a crucial part in water quality assessments (Cranston *et al.* 1996, Gerhardt 1999, Masese *et al.* 2013; Cerveny *et al.* 2016).

Bio-monitoring measures the effect of pollutants on aquatic organisms ranging from tiny organisms such as bacteria to larger organisms such as fish (Lazaridou-Dimitriadou 2002). The use of living organisms in water quality monitoring came about in Europe more than 100 years ago and has since been used in almost every part of the world (Cairns and Pratt 1993; Metcalfe-Smith 1994; Wepener 2008; Szczerbiñska and Gaczyñska 2015; Parmar *et al.* 2016). This is mainly due to the fact that the responses of various biological communities can be monitored in multiple ways to provide an indication of the effects of different pollutants on an ecosystem (Chapman and Jackson 1996). The co-existence and abundance or absence of specific species in certain habitats indicates whether the habitat has been altered.

It is important to note that to achieve optimal results, a bio-monitoring programme should always be used in conjunction with chemical and physical monitoring, as this will determine the presence of contaminants whilst the biological monitoring will determine the response of organisms to these contaminants (Chapman and Jackson 1996; Oertel and Salánki 2003; Masese *et al.* 2013; Cerveny *et al.* 2016). It is also important that an effective aquatic bio-monitoring programme fulfils scientific, economic and social objectives by increasing the understanding of the ecological effects of contaminants, guiding the implementation of cost-effective changes to enhance the quality of the environment and inform the public of the benefits of improved water quality (Loeb and Spacie 1994; Blair and Buytaert 2015).

The water quality of the Roodeplaat Dam has been an environmental concern for a very long time, mainly as a result of nutrient influx (Walmsley and Toerien 1978; Jones and Fred-Lee 1984; Hohls *et al.* 1998; Versfeld and Van Veelen 2007; Marchand 2012). The water quality is considerably poor, in addition to the frequent occurrence of cyanobacteria and water

hyacinths (*Eichhorinia crassipes*). Subsequently, eutrophication and phytoplankton blooms as well as changes to phytoplankton composition are some of the key management issues associated with this dam (Van Ginkel and Silberhauer 2007; DWAF 2008; Konradie and Bernard 2012; Mbiza 2014).

Roodeplaat Dam was constructed in 1959 on the banks of the Pienaars River, 24 km north east of the city of Pretoria and falls within the Crocodile West Marico Water Management Area (Walmsley and Toerien 1978). The dam has a surface area of 3.97 km^2 with a maximum depth of 43 m (van Ginkel *et al*. 2002). It is located within the Nokeng tsa Taemane Local Municipality which falls within the Metsweding District Municipality. Three rivers drain into the dam; the Edendalespruit, Pienaars River and Hartbeesspruit (the latter includes the main first order tributary, the Morelettaspruit) (Walmsley and Toerien 1978). The Roodeplaat Dam catchment has a mixture of different land uses surrounding the dam and the inflowing rivers. It is an important freshwater reservoir as the water is used for irrigation, drinking water as well as recreational activities (Mbiza 2014). Recreational activities on and around the dam include power boating, rowing, angling, picnicking, camping as well as accommodation and conference facilities (DWAF 2008). The areas surrounding the dam house a number of red-listed bird species and habitats for red-listed invertebrates. The Roodeplaat Nature Reserve, located south of the dam, creates a protected area for these species (Vela VKE Consulting Engineers 2008).

The need for current and comprehensive water quality studies on these rivers stems from the existing problem of eutrophication in the dam, as mentioned above. A study done by Mbiza in 2010 and 2012 showed that there was a high potential for algal and plant productivity during both the wet and dry seasons (Walmsley and Toerien 1978; Hohls and Van Ginkel 2004; Lomberg 2010; Mbiza 2014). The dam was classified by the Department of Water and Sanitation in the State of Environment report in 2012 as a hyper-eutrophic system, based on high nutrient concentrations and the fact that the water quality problems within the dam were threatening the health of various organisms which could ultimately lead to limitations on biological activity (Department of Water Affairs 2013). Previous studies (Walmsley and Toerien 1978; Lomberg 2010; Golder associates 2012; Mulders 2015) which attempted to find the cause of the eutrophication within the dam, investigated the effects of the eutrophication on the system, or, focused on the effect of the inflowing rivers on the dam. These studies are briefly summarised below:

A study published in 1978 focused on the chemical composition of the waters flowing into the Roodeplaat Dam, focusing mainly on stretches of the Edendalespruit, Pienaars River and the Hartbeesspruit, including its tributary the Morelettaspruit (also referred to as Moretela or

Moreleta spruit). The results showed that the Pienaars River was the main contributor of water inflow (in terms of volume) and dissolved minerals into the dam (Walmsley and Toerien 1978). This study was the first of a limited number of studies including all three rivers; a similar more detailed water quality study was conducted in 2010 by Lomberg.

Lomberg (2010) focused on seasonal changes with regard to precipitation and the possible effects thereof on water quality of the Roodeplaat Dam drainage basin, from 2006 to 2009; selected physical, chemical and biological parameters of the inflowing rivers were analysed (Lomberg 2010). Only one physical constituent was selected for this study namely total dissolved solids. The chemical constituents tested included pH, inorganic nitrogen, phosphates, sulphates and magnesium. Biological components for this study focused solely on analysing *Escherichia coli* levels. The results showed that there was a positive correlation between the river sites examined and the physical, chemical and biological components analysed within the dam (Lomberg 2010). The biological analysis in Lomberg's research was based on microbial analysis as previously mentioned; however, this was the only analysis related to biological parameters on the rivers flowing into the Roodeplaat Dam, until a study two years later by Golder Associates (2012).

Golder Associates (2012) conducted a situational and gap analysis focusing mainly on Morelettaspruit for the "Moreletta adopt-a-river" project. The gap analysis was done to detect any lacking information on the water quality aspects of the Morelettaspruit which will ultimately be used for the State of the Rivers Report. The gap analysis indicated that very few studies have been done on the riparian habitat of Morelettaspruit itself, several fish species were observed and the Present Ecological State (PES) of the river was classified a "C" (Moderately Modified) in 2011, according to National Freshwater Ecosystem Priority Areas (NFEPA). This was determined by utilizing a range of aquatic assessment tools such as the South African Scoring System version 5 (SASS5), Fish Response Assessment Index (FRAI) and integrated Habitat Assessment System (IHAS). This data is, however, limited and the "Moreletta adopt-a-river" programme is currently not functional (Golder Associates, 2012). Golder (2012) concluded that there is a definite need for a current and comprehensive aquatic monitoring program of all three rivers as well as an in depth biological assessment of the entire system (Golder Associates 2012).

The most recent study on the Hartbeesspruit was done by Mulders (2015). The study focused on the effects of land-use on benthic macro-invertebrates in the upper reaches of the Apies-Pienaar catchment (namely the Hartbeesspruit) as well as surface water chemistry. The results from this study showed that the land-use was not the strongest factor influencing aquatic macro-invertebrates in the Hartbeesspruit but rather the hydrological pathways of

connectivity and stream-flow within the system (Mulders 2015). Aside from the aquatic assessment tools employed by Golder Associates (2012), in-depth biological assessments, especially focusing on fish health, has only been conducted within the Roodeplaat Dam (Hohls and Van Ginkel 2004; Marchand 2009).

The first study related to fish health in the Roodeplaat Dam was conducted by Hohls and Van Ginkel (2004) following a fish kill event. This study mainly focused on a chemical analysis of water samples, measurements of physical water quality parameters and a pathological examination of a single Mozambique Tilapia, *Oreochromis mossambicus*. Macroscopic analysis showed small yellow nodules in the liver, but no visible signs of alterations were seen in any other organ. Toxicity tests conducted on *Poecilia reticulata* showed no signs of toxicity. The water quality analyses showed elevated levels of ionised ammonia and dissolved zinc as well as the presence of cyanobacteria (*Microcystis*). The study suggested the source of contamination to originate from the upstream part of the Pienaars River (Hohls and Van Ginkel 2004).

In 2009 Marchand, a histology-based fish health assessment was done to determine the health status and edibility of two indicator fish species inhabiting the Roodeplaat Dam, namely *Clarias gariepinus* and *O. mossambicus*. Six specific target organs were sampled from each fish (liver, gills, ovaries, testes, heart and kidney) and water and sediment samples were collected to measure the presence and concentrations of endocrine disrupting chemicals (EDCs). Of the 20 *C. gariepinus* sampled, 65% displayed liver abnormalities which ranged from discoloured and fatty livers to livers with nodules. Similar alteration were previously noted in studies where fish had been exposed to microcystins, namely *Microcystis aeruginosa* (Gupta and Guha 2006; Pavagadhi and Balasubramaniana 2013; Sanad *et al.* 2015). However, potential microcystin bioaccumulation in fish tissues were not investigated as part of the study. Out of the 18 *O. mossambicus* sampled, 44% of the male specimens had testicular oocytes (a possible sign of intersex). This condition was previously linked to the presence of EDCs such as organochlorines, polychlorinated biphenols and phthalates (Van Aerle *et al.* 2001; Gercken and Sordyl 2002; Hinck *et al.* 2009; Sun and Tsai 2009). A histopathological analysis found extensive, irreversible alterations mainly within the livers and testes and the bioaccumulation results also showed that consumption of the fish by humans posed a human health risk. The final conclusion suggested that the health effects identified in fish were due to the unsatisfactory ecosystem condition and elevated levels of pollution (Marchand 2009). The study by Marchand (2009) intensified the need for an investigation on the biotic integrity of the inflowing rivers of the Roodeplaat Dam and their constituents, as well as to conduct a histology-based fish health assessment analysis of fish from each of the inflowing rivers to provide a comparative dataset of results.

Therefore, based on the available literature as summarised above, it is clear that there is limited and sporadic research on the inflowing rivers of the Roodeplaat Dam and that there is a need for a recent, comparative study on the biotic integrity of these rivers. The problem statement for this study can therefore be summarized as follows:

- Even though a limited number of studies have investigated specific aspects of water quality of the three rivers flowing into the Roodeplaat Dam, these studies either only investigated one of the three rivers, or, the data is quite dated. Also, in those studies where all three rivers were included, the range of parameters assessed was not comprehensive;
- No information is available on the health of fish inhabiting the downstream reaches of these rivers, nor is there any data available on the possible bioaccumulated levels of microcystins in the fish tissue. Furthermore, to date, fish health has only been investigated in the dam itself.

This study will provide current data on the chemical, physical and biological integrity of the Edendalespruit, Pienaars River and Hartbeesspruit (Morelettaspruit) and a seasonal comparison of these aspects. The results hereof can then be used as baseline data for future bio-monitoring efforts of the three rivers flowing into the Roodeplaat Dam. The results will allow for informed decision-making processes which will aid in the management of this catchment and could also assist in addressing the aims of the fundamental principles of the National Water Act previously mentioned (NWA No.36 of 1998). These aims include protecting the quality of water to achieve sustainable use of water to benefit all users which will ultimately fulfil the basic principles of NEMA (Act No. 107 of 1998).

1.2. Aims and Objectives

The aim of this study was to assess and compare aspects of the biotic integrity of the three rivers (Edendalespruit, Pienaars River and Hartbeesspruit) flowing into the Roodeplaat Dam.

This aim was achieved through the following objectives:

1. Physical, chemical and biological analyses of the water of the selected rivers;
2. Physical and chemical analyses of the sediment of the selected rivers;
3. An assessment of the macro-invertebrate assemblages as an indication of water quality (using South African Scoring System or SASS5) as well as a habitat assessment of the biotopes present within the rivers (using Integrated Habitat Assessment Index or IHAS);

4. An assessment of selected health parameters (target organ histopathology, somatic indices and condition indices) of two fish species, *C. gariepinus* and *O. mossambicus*;
5. A measurement of microcystin levels in the liver tissue of both fish species;
6. An assessment of the result against reference data including water quality guidelines, SASS5 interpretation guidelines and histology index values obtained from fish bred in pollutant-free water.

1.3. Hypotheses and expected results

- Hypothesis 1: *Physical, biological and chemical analyses of the water and sediment of the selected rivers will show elevated levels of pollution*;

- Hypothesis 2: *A comparison of the water and sediment quality between the rivers will indicate that the Pienaars River has the highest level of pollution based on the types and proximity of surrounding human activities*;

- Hypothesis 3: *An assessment of the macro-invertebrate assemblages will yield a low average score per taxon and a low number of taxa present within the rivers indicative of pollution*. Furthermore, the upstream reference sites are expected to have a higher average score per taxon and number of taxa present when compared to the downstream sites. The sites within the Pienaars River are expected to yield the poorest/lowest SASS5 score when compared to sites within Hartbeesspruit and Edendalespruit and based on previous studies the high-flow conditions are expected to have a lower number of taxa present with a lower average score per taxa when compared to the low-flow conditions.

- Hypothesis 4: *The fish health assessment will show a number of toxicant-induced alterations in the target organs of fish analysed, similar to fish from previous studies conducted on the Roodeplaat Dam*. It is also expected that fish collected from Pienaars River will have more toxicant-induced alterations in comparison to the other two rivers due to the surrounding land-use activities including a Wastewater Treatment Plant. It is expected that *C. gariepinus* will show higher histological index values and more organ alterations when compared to *O. mossambicus*. Based on the seasonal algal blooms and the associated production of bio-toxins, seasonal differences are also expected in the level of alterations found in each species within each river.

- Hypothesis 5: *Microcystin will be present at detectable levels in the liver tissue of both fish species*. It is also expected that the microcystin levels in both species will be higher during the high-flow conditions as algal blooms are associated with the warmer, summer months.

- Hypothesis 6: The results of this study will show that the quantitative data for water quality, sediment quality and fish health will be above acceptable guideline and/or reference values for all three rivers.

1.4. Study Design and Thesis Layout

The thesis consists of six chapters and based on the general study design (Figure 1), the focus of each chapter can be summarised as follows:

Chapter 1 (General introduction):

- Chapter 1 provides a general background and literature review of studies that focused on the Roodeplaat Dam and its inflowing rivers and provides the consequent motivation for this study. This chapter also stipulates the aims and objectives of the study and provides a general overview of the study design.

Chapter 2 (Study area):

- Chapter 2 gives a description of the study area, with reference to the surrounding land use activities, natural vegetation, climate, geology and soil types. Each of the three selected rivers is discussed separately and a detailed description is given on the location of the selected study sites within each river.

Chapter 3 (Water and sediment quality):

- Chapter 3 focusses on the analysis of water and sediment quality. A brief introduction is given on eutrophication and its impact on the Roodeplaat Dam. A literature review is presented on the relevant sediment and water pollutants. The methodology employed is described in detail and results obtained for physical, chemical and biological analyses of water and sediment samples are assessed in terms of available water quality guidelines and are compared between seasons and rivers.

Chapter 4 (Aquatic macro-Invertebrates):

- Chapter 4 provides the introduction for biological monitoring in general with a specific focus on macro-invertebrates. The SASS5 bio-monitoring tool is introduced in this section

including a brief description on the uses and advantages of SASS5. A literature review on national and international studies using macro-invertebrates as bio-indicators is presented and the sampling methodology for macroinvertebrate sampling and habitat assessment is explained in detail. The results are discussed and assessed according to specific interpretation guidelines and are compared between seasons and rivers.

Chapter 5 (Fish health):

- Chapter 5 introduces fish as a bio-indicator organism with a brief description of the species chosen. This chapter includes a literature review on national and international studies conducted on the histopathology of selected target organs of fish as well as the use of different somatic indices and the condition index as an indication of fish heath. The methodology followed for the fish necropsy, histological assessment and microcystin bioaccumulation in liver tissue is presented and the results are compared between species, seasons and rivers.

Chapter 6 (General discussion and conclusion):

- Involves an integrated, general discussion and conclusion of the study, summarizing and incorporating the results of the preceding chapters. Recommendations are proposed.

The thesis is presented as separate chapters (in accordance to faculty guidelines) and that has the effect that some repetition may occur in the duplication of references.

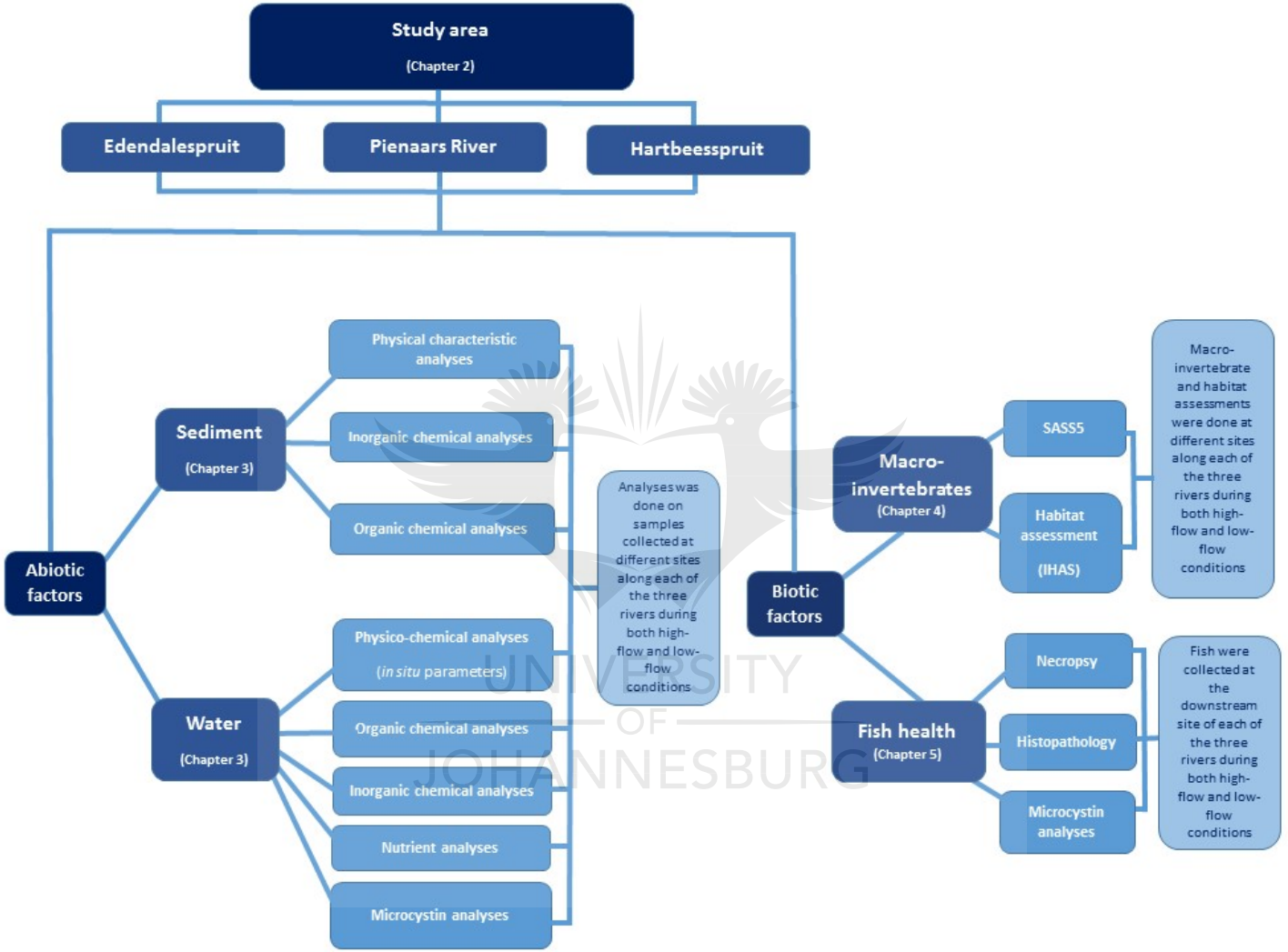

Figure 1: A diagrammatical representation of the study design showing the different abiotic and biotic aspects investigated to assess the biotic integrity of the rivers flowing into the hyper-eutrophic Roodeplaat Dam.

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Chapter 2: Study area

2.1. Introduction

Roodeplaat Dam was constructed in 1959 on the banks of the Pienaars River, 24 km north-east of Pretoria (Walmsley and Toerien 1978). The dam falls within the Crocodile (West) and Marico Water Management Area (WMA) which is predominantly in the North West Province, but with parts in the northern region of Gauteng. The Crocodile and Marico are the two main rivers within this WMA, the Roodeplaat Dam, constitutes one of the largest dams in the Apies-Pienaars sub-management area. The dam has a net capacity of 41.9 x 10.6 m^3, at full capacity covers an area of 396 hectares, and has a maximum depth of 43 m (Steyn *et al.* 1976; Pieterse and Toerien 1978). Currently, the provision of potable water to surrounding urban areas for industrial, commercial and irrigational purposes is the primary use of Roodeplaat Dam. It is also important in terms of conservational and recreational activities such as tourism, accommodation and leisure activities (Vele VKE consultants 2006). There has been an increase in residential and industrial land-use activities over the years and subsequently there is very little natural vegetation left within the area and most of the land-use is dedicated to anthropogenic activities (Lomberg 2010).

There are three rivers flowing into the dam, namely the Edendalespruit, Pienaars River and the Hartbeesspruit, the latter which includes a main tributary, the Morelettaspruit (Walmsley and Toerien 1978; Matshali 2015). Morelettaspruit was included as part of this study due to the volume of water it contributes to the Hartbeesspruit as well as the fact that there is literature available on previous water quality-related studies for this specific tributary. Each river has unique physical features and surrounding human activities, which will be discussed in more depth in following sections of this chapter. Figure 2 shows the location of the Roodeplaat Dam, highlighting the position of each river and the different land-use activities surrounding each river.

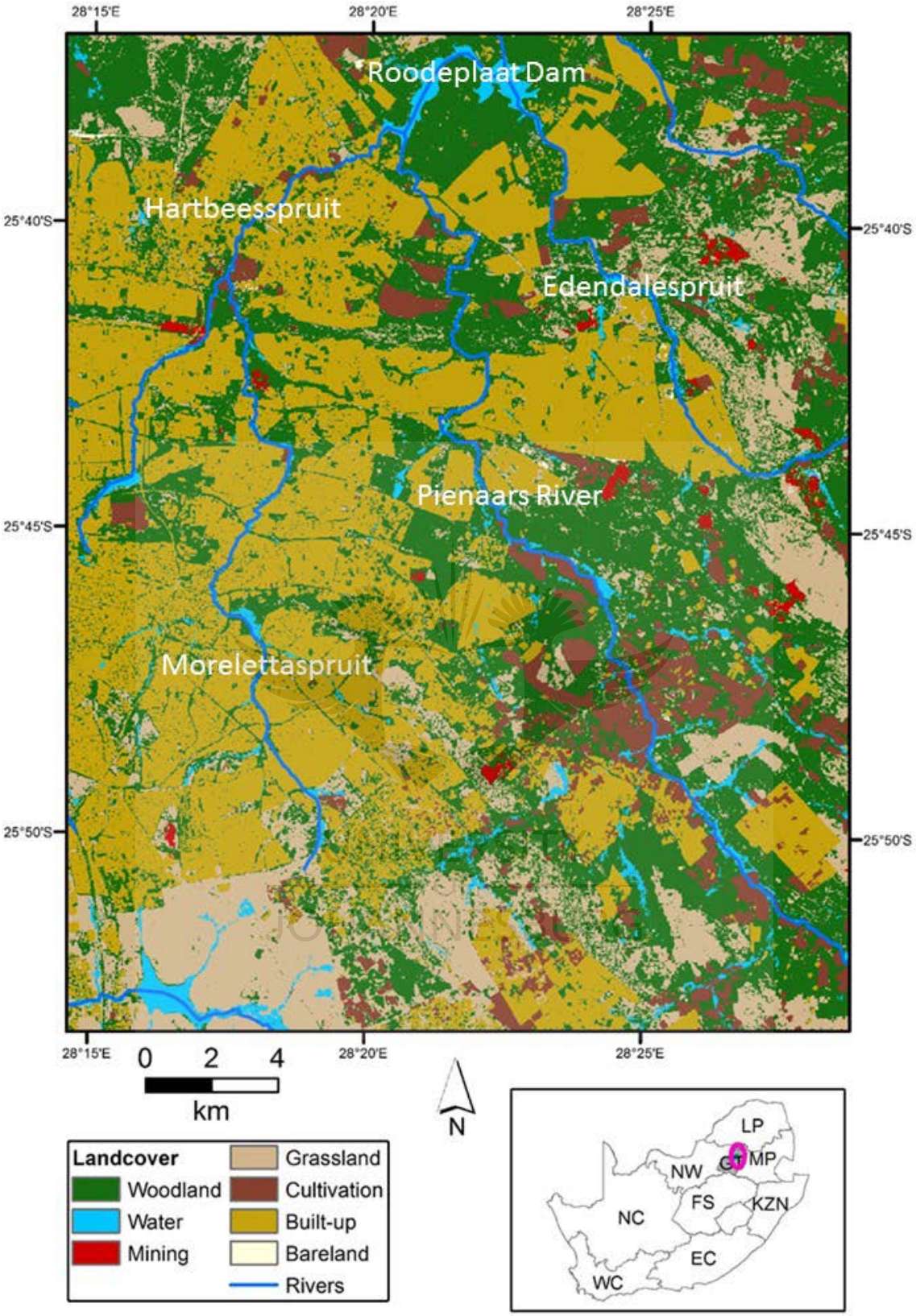

Figure 2: Map showing the location of each river (Edendalespruit, Pienaars River and Hartbeesspruit (with Morelettaspruit)) in relation to the Roodeplaat Dam and the surrounding land cover in the Roodeplaat Dam catchment area. (*Selected sampling sites along each river are indicated but will be discussed in more detail in Section 2.3*

2.2. Climate and Geology

Pretoria has a mean summer rainfall of 537 mm per annum, and an annual precipitation of 600-800 mm. The area is characterised by a moderate dry sub-tropical climate and mean annual temperatures range between 16 – 20 ºC (Mulders 2015). Rainfall and seasonal exposure plays a significant role in the water quality constituents (physical and chemical). It has been found that increased rainfall leads to decreased constituents in water due to dilution (Lomberg 2010).

As previously mentioned, the study area falls within the Crocodile West-Marico Water Management Area (WMA). The geological formations in this WMA are rich in minerals such as Platinum, Gold, Uranium, Iron, Chrome and Manganese (DWAF 2004b). The sub-management area, Apies-Pienaars rivers area, contains dolomite rock which is known to have very high water storage capabilities. Pretoria (City of Tshwane metropolitan municipality) abstracts large quantities of water from these dolomites (DWAF 2004b). It is therefore, important to consider the geological features of an area when conducting water quality analyses, as the major ion chemistry can be explained by the reaction of water with rock and sediment along the watercourse (Gurrieri 1998; Wenchuan and Kelderman 2001; Ohta *et al*. 2005).

2.2.1. Edendalespruit

Edendalespruit is approximately 28 km long, flows from the south-east to the north-west and drains the least amount of water (17%) into Roodeplaat Dam compared to the Hartbeesspruit and the Pienaars River (Swanepoel 1997; Matshali 2015). Edendale lead mine is situated adjacent to Edendalespruit, which previously raised the concern of hazardous concentrations of lead leaching into the river (Glass 2006). There are also two schools located alongside the river, Edendale Primary School and Edendale High School (Glass 2006). The south, north and south-eastern parts of the river is surrounded by small farms and residential dwellings. To the west of the river is Mamelodi Township, which can be classified as a residential area with low-cost housing and dominated by informal settlements. The river is also known to drain the agricultural area on the east side of the dam (Bosnian and Kempster 1985; Swanepoel 1997) and in general, agricultural activities dominate the surrounding area (Lomberg 2010). This river runs south of Franshoek Farm passing through a gap in the Magalies and Leeuwfontein quartzites to drain into the dam on the south-eastern side (DWAF 2000).

2.2.2. Pienaars River

The Pienaars River starts in Mamelodi and forms the catchment basin for Mamelodi Township. It then drains north of Pretoria before entering the Waterberg region near the town of Bela-Bela (RHP 2005). This river joins the Crocodile River just below the confluence of the Crocodile and Elands rivers (DWAF 2004) and this point is located within the Crocodile West Marico Water Management Area, which is in close proximity to the town of Nelspruit. This management area is dominated by mixed bushveld vegetation, varying from dense, short bushveld to more open tree savannah. The Pienaars River drains the largest amount of water into the Roodeplaat Dam (56%) (Swanepoel 1997) and the total length of the river is 46 km (Mothowamodimo 2012; Matshali 2015).

The main land-use activities surrounding the Pienaars River include the Baviaanspoort Waste Water Treatment Plant (WWTP), which is situated on the eastern bank and has a capacity of 40 ML/day. A previous study conducted by Swanepoel (1997) noted that the northern side of the river is surrounded by residential holdings which form part of Mamelodi Township. To the west of the river is irrigated land (Swanepoel 1997). The southern side of the river is populated by a variety of industries (Personal observation). A more recent study conducted by Lomberg (2010) indicated that the main land-use activities has not changed much over a period of ten years in that the two main land-use activities surrounding the Pienaars River is still the Wastewater Treatment Plant (WWTP) as well as the anthropogenic activities associated with the Township of Mamelodi (as previously observed by Swanepoel 1997). Personal observations during the field surveys confirmed this information, furthermore a school was observed in close proximity to the sampling sites in addition to a few industrial businesses.

2.2.3. Hartbeesspruit and Morelettaspruit

The Hartbeesspruit with its main tributary, the Morelettaspruit, drains a portion of the Limpopo water system in the east of Pretoria. Morelettaspruit flows through a breach in the quartzite bridge and is situated within the upper reaches of the Apies-Pienaars sub-management catchment area (Mulders 2015; DEA 2016). The river falls within the Rocky Highveld zone of the grassveld biome and forms a wetland within the Colbyn Wetland Nature Reserve (Grundeling and Marneweck 2000). Hartbeesspruit is pre-dominantly surrounded by residential holdings, from Pretoria East to Pretoria South and Mamelodi. The residential houses range from low-cost to middle- and high-cost. The northern side of the river is surrounded by small farms and nature conservation areas, while the south of the river (upstream) is encircled by industries such as brick manufacturers (Swanepoel 1997). Hartbeespruit drains 23% of the inflowing water into the Roodeplaat Dam.

Morelettaspruit runs through the eastern suburbs of Pretoria and flows through the Moreletta and Faerie Glen nature reserves. The river originates in Moreletapark, Garstfontein and parts of Tweefontein farm and joins up with the main river (Hartbeesspruit) just after Eersterus on the R513 (Regional route in South Africa). The surrounding land uses are predominantly residential with small areas of commercial, industrial and business use (Golder associates 2012).

2.3. Site Selection

Up-, mid- and downstream sites were selected in each river for the sampling of water, sediment and macro-invertebrates as well as for the habitat assessment. The sampling sites were selected based on the available biotopes for macro-invertebrate collection, accessibility for the necessary sampling equipment, the surrounding land-use activities as well as to correspond with previous sampling sites selected by the Department of Water and Sanitation (previously known as Department of Water Affairs) for water quality monitoring. It is important to note that all three rivers are perennial rivers (Matshali 2015) and water levels also influenced the selection of the sampling sites. Biotypes necessary for macroinvertebrate sampling included stones-in-and-out-of-current, gravel, sand and mud as well as vegetation in-and-out of current. The upstream sites (least impacted) in each river also served as reference sites for the macroinvertebrate assessment and water quality analyses.

For the Pienaars River, a total of four sites were selected (P1 (-25.644897; 28.343048), P2 (-25.858554; 28.455832), P3 (-25.745919; 28.372014) and P4 (-25.678603; 28.357253) and for the Hartbeesspruit (and Morelettaspruit), a total of five sites (H1 (25.645433; 28.333719), H2 (-25.720904; 28.261654) H3 (-25.651818; 28.313464) and M1(-25.799684; 28.301661) and M2 (-25.656227; 28.308230) were selected. Only two sites were selected in the Edendalespruit as the upper reaches of the river was found to be dry throughout the study period, therefore only a midstream and a downstream site could be selected (E1 -25.644885; 28.388104 and E2 -25.643206; 28.385210).

Fish collection was done at the downstream sites in each of the rivers (E1, P1 and H1). The downstream site of each river represents the combination of all contaminants from upstream pollution sources before entering the dam. Also, the downstream site of each river was wide enough to launch a boat and release gillnets to enable the collection of an appropriate sample size of large enough fish to conduct a fish health assessment. However, electroshocking to collect fish was done at all sites located in the mid- and upper reaches of the rivers, but in

most cases revealed the absence of the required study species. The selected sites are depicted in Figure 3.

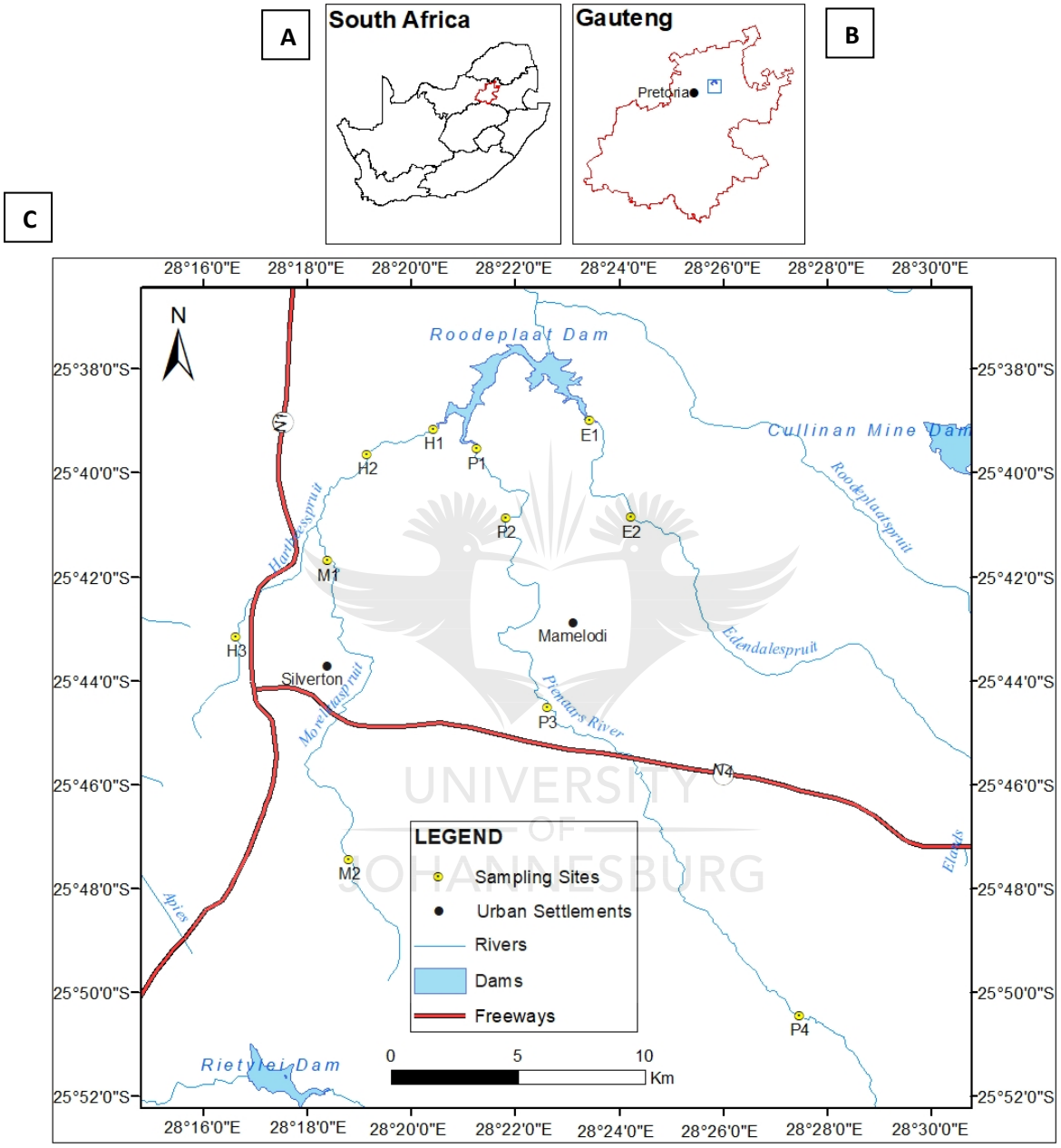

Figure 3: A: Map of South Africa highlighting Pretoria. B: Map of the Province of Gauteng showing Pretoria. C: Map showing the selected sampling sites along Edendalespruit, Pienaars River, Hartbeesspruit and its tributary, Morelettaspruit.

2.4. References

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Chapter 3: Water and sediment quality

3.1 Introduction and background information

South Africa is a water-scarce country and the average rainfall is 410 mm less than the global rainfall of 870 mm per annum. To add to that the rainfall is also unevenly distributed across the different landscapes (Department of Environmental Affairs 2011). Despite this, South Africa's freshwater resources are rich in biodiversity. There are over 200 different types of river ecosystems and almost 800 different types of wetland ecosystems (Department of Environmental Affairs 2011). A large percentage of river ecosystems (60%) are currently threatened while 23% of these ecosystems are critically endangered. It is therefore important that South Africa manage and monitor this irreplaceable resource (Oelofse and Strydom 2010; WWF South Africa and CSIR 2013). The growing population in South Africa is also adding additional stress to freshwater resources as water consumption patterns changes, thus compromising these resources. More stress is added to water resources by factors such as climate change, international obligations and water pollution (Oelofse and Strydom 2010).

Water pollution can be defined as; *"The contamination of water bodies, deeming them less fit for their intended purposes"* (Singh *et al.* 2014). Pollution of the water resources within the country not only limits its uses, but also places additional stress on the economy of the country. This is due to the fact that more money will be spent on the treatment of water in an attempt to restore the water quality, as well as on indirect impacts such as urbanisation and industrialisation leading to further deterioration of water sources (WWAP 2015). According to Oberholster (2013), the main causes for deteriorating water quality in South Africa are salinization, acidification, disease-causing micro-organisms and eutrophication (Oberholster 2013).

3.1.1. Eutrophication

A water body is often described by its trophic status. This term is related to the nutrients and concentrations of phytoplanktonic algae and cyanobacteria (Hutchinson 1969; Carlson 1977; Carlson 1984). The process of eutrophication occurs due to the autotrophic production by primary producers. This production usually consists of internal organic matter and includes photosynthetic plants and algae derived from the nutrients within a dam (Thomas *et al.* 1996). Thus, the eutrophication in any water body can be described at any given time and the trophic status can be classified as; oligotrophic, mesotrophic, eutrophic or hypertrophic (Walmsley 2000).

Oligotrophic systems refer to systems that have a low productivity and low biomass which is usually linked to low concentrations of nutrients (i.e. Nitrogen and Phosphorus). These waterbodies are characterised by their high saturation of oxygen throughout the water column. Mesotrophic lakes are not described in as much detail as oligotrophic or eutrophic lakes, and have often been said to be in a transition between the two trophic states (Thomas *et al.* 1996). There is usually a decrease in oxygen concentrations in the lower layer of water during summer stratification. Eutrophic lakes have high nutrient levels linked to high biomass production, these lakes are characterised by low transparency and very low oxygen levels. Hypertrophic lakes have extremely high nutrient levels associated with biomass production which leads to severely impaired water quality. There is often anoxia or complete loss of oxygen during the summer stratification. Oligotrophic lakes are rich in organic matter such as humus and fulvic acid, which usually comes from external outputs such as fertilizers (Thomas *et al.* 1996). The rate of eutrophication in any water system is accelerated by human activities by means of point and non-point pollution sources such as phosphorus and nitrogen released into aquatic systems (Carpenter *et al.* 1998). These human activities include urbanization, agriculture, forestry and industrial effluents (Carr and Neary 2008).

Eutrophication causes contaminated tap water supplies, degradation of recreational activities and clogging of waterways (Dodds *et al.* 2009; van Ginkel 2002). Cyanobacteria algal blooms, which are also a direct result of severe eutrophication, could ultimately lead to a release of biotoxins (e.g. microcystins), have been known to cause poisoning of domestic animals, wildlife, and even humans since the early 1800s (Francis 1878). Besides the biological effects of eutrophication, it is also known to cause economic losses due to social and ecological responses such as a decrease in tourism and degradation of the aquatic systems (Dodds *et al.* 2009). In South Africa, eutrophication has not been acknowledged as a crisis in general, except in localised water systems such as the Hartebeespoort and Roodeplaat dams where it is recognised as an immense environmental problem (Walmsley 2000; van Ginkel *et al.* 2000; van Ginkel *et al.* 2001a; Oberholster and Ashton 2008; Oloefse and Strydom 2010).

3.1.2. Eutrophication in Roodeplaat Dam

Roodeplaat Dam is classified by the Department of Water and Sanitation (DWS) (previously known as the Department of Water Affairs) as hypertrophic (van Ginkel 2005). A more recent study by Harding (2015) also confirmed the hypereutrophic state of the Dam (Harding 2015). Concerns have been raised about the high levels of eutrophication as the Roodeplaat Dam is an important water system for irrigation, water supply and recreational purposes (Jones and Fred Lee 1984; van Ginkel 2005; DWAF 2008). Enrichment of Roodeplaat Dam has been an ongoing concern since 1978 when Walmsley *et al.* (1978) reported that nutrients within the

sewage effluent (phosphorus and nitrogen) are the main cause of enrichment within the dam. There are two main sewage treatment plants serving the residents within the Roodeplaat catchment; Baviaanspoort works and Zeekoegat works (Walmsley *et al.* 1978; Silberbauer and Esterhuyse 2014). When compared to other impoundments such as Rietvlei and Grootdraai, which are only subjected to occasional cyanobacterial blooms, Roodeplaat Dam and Hartebeespoort Dam are characterised by annual cyanobacterial blooms of fluctuating degrees (Van Ginkel 2005). In 1975, Toerien *et al.* ranked the Roodeplaat Dam as the third most eutrophic water body in South Africa. The dam was compared to 98 other impoundments (Toerien *et al.* 1975). Since then, an eutrophication monitoring plan by the DWS placed Roodeplaat Dam seventh on the list of eight monitored dams including Hartbeespoort and Rietvlei dams (DWS 2014). In 2008, the Department of Water and Sanitation (DWAF 2008) also concluded that the poor water quality within the dam could be as a direct result of the mismanagement of the upstream catchments and poor water quality coming from the three inflowing rivers (Edendalespruit, Pienaars River and Hartbeesspruit) (DWAF 2008). It is therefore imperative to continuously monitor the water quality of the inflowing rivers of the Roodeplaat Dam.

3.1.3. Water quality monitoring

Water quality can be defined as "*the physical, chemical, biological and aesthetic properties of water which are used to determine the fitness of water for different uses and also to protect the health and integrity of ecosystems*" (DWAF 1996a). The effect of human activities on water systems is often gradual and is only detected after extreme changes are noticed. Regular monitoring of chemical, physical and biological parameters is therefore essential to detect changes in the hope of reviving the ecosystem before irreparable damage takes place (Carr and Neary 2008). The functioning of any ecosystem is influenced by abiotic (non-living) and biotic (living) factors. The distribution of organisms is most commonly influenced by the abiotic factors such as light, moisture, temperature, sediment and chemical factors such as salinity and acidity. The biotic factors, are the living organisms and the interactions between them, forming the biological resources (Muoghalo 2015). These factors also influence each other as each living organism can only function optimally in certain environments, based on their tolerance levels. Human activities are known to have significant negative influences on these factors and hence our interactions with the environment need to be monitored and managed (Muoghalo 2015). The National Water Act of South Africa (Act 36 No. of 1998) states that water resource management is solely put into place to achieve the sustainable use of water for the benefit of all users, the constitution has acknowledged that there is a need for the integrated management of all water resources (National Water Act No. 36 of 1998).

A previous study by Hohls (1996) reported that the water quality of ecosystems was previously only managed within the standards related to human health acceptability. This study also implied that water quality monitoring previously prioritized physical and chemical parameters (Hohls 1996). Water quality is not a stagnant condition of a system and it cannot be explained extensively by measuring only these two parameters (Carr and Neary 2008). The structure of surface and underground waters is majorly dependant on geographical, biological and hydrological factors. The quality of water is also, however influenced by human activities resulting in water pollution (Meybeck *et al.* 1996). The extent of water pollution depends on the surrounding activities. More recent studies have shown that the monitoring of biota is now internationally employed to enhance results from physical and chemical analyses. This approach has been proven to be highly informative and has greatly improved the assessment and management of aquatic ecosystems around the world (Salànki *et al*. 2003).

Globally, different water quality monitoring programmes have been developed to address a variety of problems and assess the status of aquatic ecosystems. Freshwater system monitoring in Europe is mainly focused on evaluating the chemical and ecological status of waters and includes an assessment of biological elements such as phytoplankton, macrophytes, phytobenthos, macro-invertebrates and fish. These aspects are monitored frequently and on a wide scale as opposed to the chemical components measurements which are only monitored when priority pollutants are found in the water, sediment or biota (Altenburger *et al.* 2015). The results are then compared to the Environmental Quality Standards as prescribed in the EU directive (EU Directive 2013).

In the United States, the United States Environmental Protection Agency (USEPA) supports different regional programs which thrive on high-quality monitoring at minimal costs, such as the Regional Water Monitoring Program of San Francisco. This program mainly looks at water and sediment quality as well as bioaccumulation of pollutants deemed as having a high priority in the area. The bioaccumulation levels of pollutants are analysed in fish, birds and bivalves (Trowbridge *et al.* 2016). Prior to 2014, China assessed water quality based on the levels of chemical pollutants such as ammonia and the biological oxygen demand (BOD), which was labelled as insufficient. They have since developed a pilot national monitoring program. The program incorporates the hydrology, river morphology, physico-chemical parameters and ecotoxicological aspects. It also includes an assessment of biota such as macro-invertebrates and fish (Wang *et al.* 2014).

In South Africa, until recently, the River Health Program (RHP) has been used to monitor ecological aspects of rivers. This included the monitoring of physical parameters as well as chemical parameters of surface waters. The program was based on the fact that the integrity

or health of the organisms within the river ecosystems will give a direct measure and aid in providing the integrated health of the river as a whole. The program was formalised in 1994 and has since been changed to the River Eco-status Monitoring Program (REMP), which forms part of the National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) (DWS 2016). The National Water Act (NWA 1998) came into effect after the RHP and recognised the fact that it is important to protect the water resources of the country in order to provide services to people who rely on these resources. The protection of water resources can be achieved by implementation of a National Aquatic Ecosystem Health Monitoring Programme. The programme in different provinces was implemented by stakeholders such as DWAF regional offices, provincial departments of the environment (e.g.: Department of Environmental Affairs), conservation agencies, tertiary institutions and the respective municipalities. However, implementation was voluntary and resulted in issues arising with the long-term sustainability (DWS 2016).

The REMP is based on establishment of relative reference conditions which focuses on factors which will determine the most suitable habitat for instream and riparian biota such as the hydrology, geomorphology and physico-chemical conditions. In addition to this, the response of the biota to environmental drivers is also taken into consideration. REMP also determines the ecological status and baseline conditions of a system in order to monitor changes in the future by assessing physical, biological and chemical parameters (DWS 2016).

3.1.4. *In-situ* physico-chemical water quality parameters

Dissolved oxygen

Oxygen levels within aquatic ecosystems directly influences the functioning and survival of aquatic organisms since it is crucial for their respiration. The dissolved oxygen concentration within a system directly indicates the health of the system (DWAF 1996a). The concentration of dissolved oxygen is affected by seasonal variations and biological productivity, and high temperatures resulting in low levels of dissolved oxygen can lead to stressful conditions in aquatic organisms (DWAF 1996a) including fish and macro-invertebrates. This was seen in a study conducted by Sun *et al.* (2012) when crucian carp *Carrasius auratus* was exposed to different levels of nitrite and dissolved oxygen for 30 days. The results showed that low dissolved oxygen levels led to a decreased growth rate, feeding and food conversion efficiency rate, as well as a decrease protein efficiency ratio. The red blood cell count was also significantly lower and signs of oxidative stress was observed. This was associated with decreasing dissolved oxygen conditions coupled with changes in the enzymatic and non-enzymatic antioxidants within the fish liver (Sun *et al.* 2012). In another study the Caddisfly *Clistoronia magnifica,* was exposed to different ranges of dissolved oxygen for 44 days. The

Caddisfly embryos suspended their growth when the oxygen levels decreased and resumed growth as the levels increased (Nebeker *et al.* 1996). In Tokyo Bay, a study was done to determine the range of effects hypoxia can have on benthic macro-organisms from spring to autumn (when hypoxia persists). Hypoxia seemed to act as an obstacle to the recovery of benthic organisms since it causes defaunation and attributes to the failure of settlement in the larval stages (Kodama and Horiguchi 2011).

Eutrophication is known to decrease the dissolved oxygen levels in a system (Carpenter *et al.* 1998). Rixen *et al.* (2010) attempted to determine if dissolved oxygen had a response to eutrophication in the Siak River, a tropical black water, nutrient-poor river in Indonesia (Rixen *et al.* 2010). The dissolved oxygen and dissolved organic matter were measured along the river over 36 hours. The results showed that due to the wastewater discharges being higher during the morning and evening, it increased the ammonium concentrations. Results also showed that increased dissolved organic matter led to an increase in dissolved oxygen, and an increase in waste water treatment discharge led to a decrease in dissolved oxygen (Rixen *et al.* 2010). Fred-lee and Jones (2012) also documented the adverse effects of eutrophication on oxygen levels in fisheries. Their study showed that planktonic algal growth in the surface waters results in a depletion of oxygen which eventually becomes a source of biochemical oxygen demand in the water column. The results emphasised that there is a regressive relationship between nutrient loading and oxygen depletion (Fred-lee and Jones 2012).

In 2000 a pollution incident took place in Roodeplaat Dam, and selected physical and chemical water quality parameters were measured during winter and summer. The results showed anaerobic conditions during the summer sampling survey as a result of enhanced nutrient release from the bottom sediment which in turn produced higher nutrient concentration and higher chlorophyll-*a* concentration as a result of the pollution. The limited number of oxygenated areas in Roodeplaat Dam also highlighted the extent of eutrophication in the dam (Van Ginkel *et al.* 2000).

Total dissolved solids and electrical conductivity

Total dissolved solids (TDS) are a measurement of the total solid compounds present in the water. It is directly proportional to the electrical conductivity (EC). A change in the TDS level can affect the community structure within a system and the adaptation of individual species and even the microbial and ecological processes within certain organisms (DWAF 1996). Electrical conductivity is the ability of water to conduct an electric current. It is also known to reflect the mineral salt content of water. Pure water with no salts will therefore have an EC of 0. It is, furthermore, directly proportional and dependant on the temperature of the water; implying that higher temperatures will result in a higher EC (Environmental Protection Agency

2001). Since the presence of phosphates and nitrates is known to lower the EC in a system, EC has been used previously as a marker for wastewater contamination in freshwater systems (De Sousa *et al.* 2014).

As mentioned before, the Roodeplaat catchment area is surrounded by a variety of different land-uses (agriculture, mining, treated wastewater, urbanization etc.) which could possibly affect the conductivity of the water in the selected rivers. The impact of human activities on EC has been highlighted in numerous studies. In Poland, a study was conducted to determine if the surrounding land-uses could possibly impact certain factors of the trophic status of various lakes. The researchers assessed 31 lakes based solely on its economical and recreational value (Sługocki and Czerniawski 2017). Two parameters were chosen, namely; Secchi depth and EC (to simulate the chemical properties of the sites chosen) which showed a significant correlation with the land-uses. The results showed that a high percentage of agricultural activity (with increased nutrient loads) led to higher EC values (Sługocki and Czerniawski 2017). This was similar to the findings of Shabalala *et al.* (2013) where farming as a land-use was assessed in Bonsma Dam in Kwa-Zulu Natal (Shabalala *et al.* 2013). The EC was higher during high-flow conditions due to the rainy season influencing the leaching of nutrients and salts into the water column. The conclusion was that agricultural activities led to an increased EC as it increased the level of Nitrates, Phosphates and Chloride (Shabalala *et al.* 2013).

Mining is one of the land-uses in the Roodeplaat catchment area. Previous studies have shown that lead mining in particular (Edendale mine) has a significant increase in the EC, due to the fact that mine waters and waste from the waters are known to contain high levels of heavy metals (Brogan 2003; Aloh *et al.* 2017).

pH

The pH can be described as the measure of hydrogen ion concentration within water. An increase in the hydrogen ions will lead to a more acidic solution and a decrease will lead to a more basic solution (DWAF 1996). The buffering capacity of water is responsible for the change in pH. In South Africa, most of the freshwater systems have a pH between 6 and 8 although this also changes during different seasons and during day and night. This is directly related to the hydrological cycle (seasonal fluctuations) and photosynthesis (diurnal fluctuations) (DWAF 1996). Industrial activities such as mine drainage and acid precipitation are known to cause acidification of freshwater systems while higher pH values are known to be caused by increased biological activities mainly found within eutrophic systems (DWAF 1996). The pH is thus affected by the temperature, biological activity and concentration of

organic and inorganic ions. It can also affect the toxicity and availability of metals such as ammonium and selenium (DWAF 1996).

Studies have been conducted on by-products of mining, such as Cadmium. Qu *et al.* (2016) determined the toxicity of Cadmium associated with different levels of pH. The aquatic oligochaete *Limnodrilus hoffmeisteri* was exposed to Cadmium under different pH levels and results showed that an increase in the pH values (gradually increased from 6.8 - 8) led to an increase in the toxicity of cadmium and that the cadmium was more detrimental to the antioxidant defence system at a pH of 8 (Qu *et al.* 2016). Similar results were seen in the study conducted by Spurgeon *et al.* (2006) on *Lumbricus rubellus*. The results from these studies suggest that the effects are not species specific.

Temperature

Temperature is known as *"the condition of a body that determines the transfer of heat to or from other bodies"* (DWAF 1996). It plays a crucial role in the rate of chemical reactions such as the metabolic rates of organisms, thus making it one of the most significant factors affecting aquatic organism distribution. The inland waters of South Africa have a temperature range of 5 - 30 ºC as surface waters usually exhibit daily and periodic fluctuations in temperature. Higher temperatures can cause a decrease in the dissolved oxygen within a system thus decreasing its availability for organisms. Unnaturally low temperatures can suppress spawning of fish (DWAF 1996). Since most organisms in freshwater aquatic systems are poikilothermic, they are highly dependent on the temperature and therefore any rapid change can lead to great catastrophes such as mass mortality (DWAF 1996). Many organisms also depend on changes in the temperature as a cue for different phases in their lifecycles such as spawning, migration and emergence (DWAF 1996).

The sub-lethal effects of temperature on aquatic macro-invertebrates were assessed in 2015 by Dallas and Ross-Gillepsie. The study focused mainly on the physiology, metabolism, phenology, reproductive success, behaviour and broad-scale ecological aspects. The following effects were observed with increased temperature (Dallas and Ross- Gillepsie 2015):

- *"Increased growth rates (only up to a species-specific upper limit);*
- *Higher levels of secondary productivity;*
- *Faster growth rates leading to shorter development periods and early emergence cues;*
- *More generations produced within a year;*
- *Unsynchronized emergence periods;*
- *The fecundity rate declines as this directly related to the size of female organisms;*

- *Increase in juvenile mortality rates".*

Of particular importance to this study is the effect of temperature on eutrophication. In Lake Taihu and Lake Kasumigaura, two shallow lakes in China and Japan, Chu *et al.* (2007) investigated whether temperature had an effect on the growth characteristics of two species of microcystins. The results of their study showed an increase in growth rate at higher temperatures implying that temperature is a significant factor affecting the growth rate of algal blooms (Chu *et al.* 2007). The increase of temperature is also known to have an effect on aquatic ecosystems. The rise in temperatures as a result of global warming results in increased fish predation which decreases the zooplankton grazing patterns and an enhanced level of floating plants (Gyllstroom *et al.* 2005; Jeppeson *et al.* 2005; Meerhof *et al.* 2007). Increased temperatures also affects the nutrient processing in an aquatic ecosystem, changes the nutrient loading rate and fish community composition (Feuchtmayr *et al.* 2009).

3.1.5. Nutrients and biological components

The presence of nutrients is required in water bodies. However, elevated levels can be detrimental to the quality of the water. An excess of nutrients can lead to pollution and an increased amount of algae in the water (Minnesota Pollution Control Agency 2008). Phosphorus and nitrogen are known as the primary nutrients, which, in excess can lead to pollution of water bodies. Nitrogen is essential in plant and animal tissue production as it is used to synthesize protein. The atmosphere contains approximately 78% of this gas (DiNitrogen) which mainly cycles through biological processes. In this form it is largely inaccessible and only when it is converted into ammonia (NH_3), it becomes available to primary producers such as plants. Nitrogen exists in a variety of organic and inorganic forms, it, therefore, needs to undergo many transformations in the ecosystem in order to be used by organisms. These transformations include; nitrogen fixation, nitrification, denitrification, aerobic ammonium oxidation and ammonification (Bernhard 2010). This natural nitrogen cycle can be impacted by humans as their activities can lead to an alteration in the earths fixed nitrogen. Nitrification is the process which is used to convert nitrogen to nitrite and then nitrate (Bernard 2010). Nitrate, however, can have negative effects on animal and human health if it occurs in excess. This compound enters aquatic systems by means of Waste Water Treatment systems, animal feedlots, agricultural fertilizers, industrial wastewaters, landfills, and refuse dumps (Minnesota Pollution Control Agency 2008).

Phosphorus is another essential nutrient which converts sunlight into usable energy. It also plays a vital role in cellular growth and reproduction. Naturally, its presence is very rare in water, but lately it is known to occur more often due to human activities in dissolved organic

and inorganic state and often attached to sediment particles (Minnesota Pollution Control Agency 2008). Excessive amounts of phosphates in water can lead to overfertilization, which would eventually lead to excess amounts of aquatic plants which would ultimately result in an overall depletion of oxygen in the water system (Mesner and Gieger 2010). Human activities such as construction, run-off from animal feedlots and fertilizers from farms, allowing animals to overgraze, changing the surrounding land use activities in terms of road or industrial constructions, as well as agricultural activities can lead to elevated amounts of phosphorus entering the system (Mesner and Gieger 2010). It is important to understand that neither phosphorous nor nitrogen alone can be responsible for eutrophication, but the most limiting factor is likely to play the most important role (National Academy Press 2000)

Chlorophyll- *a*

Chlorophyll-*a* is found in all organisms that can photosynthesize oxygenitically and is readily available in cyanobacteria (blue-green algae) (Sheer 2001). The levels of chlorophyll-*a* in any aquatic ecosystem is usually higher in summer when compared to the winter months (Mitchell 1990; Horne and Goldman 1994). The levels of chlorophyll-*a* can be affected by human activities such as destruction of river shorelines and sewage inputs (Mitchell 1990; Horne and Goldman 1994).

Chlorophyll-*a* has been used in numerous studies to indicate eutrophication. Doering *et al.* (2006) investigated whether chlorophyll-*a* responds to nutrient loading and freshwater discharge in Florida, USA at the Franklin Lock and Dam and Caloosahatchee Estuary. The results showed a significant positive correlation between increased chlorophyll-*a* and decreased dissolved oxygen, as well as an increase in nutrient loading and chlorophyll-α. However, further studies are needed to interpret the response of chlorophyll-*a* as an indicator to future changes (Doering *et al.* 2006). In Izma Bay, Turkey, the study looked specifically at nutrient trends and chlorophyll-*a* downstream of a wastewater treatment plant. This was investigated over a period of 1 year on a weekly basis. The physico-chemical parameters (dissolved oxygen, salinity, temperature and pH) were also measured (Sanem *et al.* 2012). A comparison was made between the results obtained before the operation of the wastewater plant. This comparison showed lower levels of chlorophyll-*a* and nitrogen before the operation of the Wastewater treatment plant but no changes in the concentrations of phosphates and phosphorus (Sanem *et al.* 2012).

Faecal coliform bacteria

Faecal coliform bacteria is a type of bacteria found in the faeces of mammals (warm-blooded animal), therefore the levels of faecal coliform in a river can be a reflection of the amount of

sewage discharged into a river (Berg and Metcalfe 1978). The micro-organisms associated with raw sewage being discharged into a system either remain in the system or attach to the fine sediment particles within the water, where they survive for longer periods (Gao *et al.* 2011; Kunkel *et al.* 2013). The presence of this type of bacteria has been linked to the presence of other pathogens within the water. Human beings are usually exposed to these pathogens through cuts, mucus membranes of the body and unhealed abrasions. This has been known to lead to minor illnesses such as diarrhoea, nausea, skin infections such as rashes and ear infections. These health effects are more severe with pathogens such as *Escherichia coli* and *Salmonella* (Washington State Department of Ecology 2005). Besides the diseases caused, high levels of faecal coliforms can also lead to other problems (as a result of the nitrogen and phosphates found in sewage) such as bad odours, negative effects on the surrounding property values and recreational activities and unsightly views (Washington State Department of Ecology 2005).

3.1.6. Inorganic environmental contaminants

Metals are natural compounds of the earth's crust and several of them are necessary for normal growth and development, but almost all of them are toxic to aquatic organisms at certain concentrations (Laws 2000). Due to their destructive effects, heavy metals are one of the most toxic forms of aquatic pollution (Laws 2000). Heavy metals that cause the most severe cases of pollution include Copper, Lead, Mercury, Zinc, Chromium, Cadmium, Magnesium and Iron (Roberts 2001). The sources most likely to cause aquatic metal pollution ranges from industrial discharges to seepage from industrial and mining wastes, however they may also occur naturally within an aquatic system (DWAF 1996; Dallas and Day 2004). A number of studies have been done highlighting the pathological and histological effects on fish that have been exposed to high concentrations of metals (James 1990; Mitsumori *et al.* 1990; Waakles and Rehm 1992; DWAF 1996; Nussey 1998; Batra *et al.* 2004; Wepener *et al.* 2001; Grant 2004; Schmitt *et al.* 2004; Van Dyk 2007). Studies on the bioaccumulation of metals in macro-invertebrates also showed that there were detrimental effects on these organisms, such as a reduction in their diversity and abundance (Hickey and Clements 1998; Mebane 2002.; Beasly and Kneal 2003; Chiba *et al.* 2011).

A link between eutrophication and metal pollution has been investigated before. Cheng-Xui and Jie-Min (2011) assessed the concentration of heavy metals in three eutrophic lakes in China. There was no correlation found between organic matter, nutrients present and metal concentration. However, in the Hai River also in China, contrary results were seen. The study concluded that heavy metal accumulation was associated with eutrophication. This was assessed by looking at filamentous algae and diatoms. The results showed that the ability of

the diatoms to absorb metals decreased in the presence of filamentous algae (Tang *et al.* 2014).

3.1.7. Organic environmental contaminants

Organochlorine pesticides

Organochlorine pesticides (OC pesticides) are chlorinated hydrocarbons agrochemicals which have been widely used in agricultural as well as mosquito, termite and tsetse fly control programs. The most commonly found OC pesticides are DDT, methoxychlor, dieldrin, chlordane, toxaphene, mirex, kepone, lindane, and benzene hexachloride. These pesticides are known to accumulate in the environment and can be inhaled by human beings or ingested by fish and other aquatic organisms which can cause serious health risks such as reproduction and birth defects, dysfunctions within the immune system and cancer (Edwards 1987; Adeyemi 2008; Department of Health and Human Services Centers for Disease Control and Prevention 2009). Studies in various countries highlighted the distribution and effects of OC pesticides in the environment (Dem *et al.* 2007; Wang *et al.* 2007; Imo *et al.* 2007; Ize-Iyamu *et al.* 2007; Darko *et al.* 2008). It is important to monitor the levels of OC pesticides in water and sediment as this will give an indication of the levels of pollution within aquatic systems. The bioaccumulation within aquatic organisms will also shed some light on the metabolization and fate of these pesticides (Kannan *et al.* 1995).

Some chemicals, including some OC pesticides, are also endocrine disrupting (EDCs). These chemicals are anthropogenic, are used in our immediate environment and contain endocrine active substances (Kime *et al.* 1999; Viganò *et al.* 2006; Tabb and Blumberg 2006; Marchand *et al.* 2012). These pollutants are chemically-stable, lipophilic and impervious to microbial, photochemical, chemical and thermal degradation (Miranda *et al.* 2008). They mimic the function of natural hormones and cause disruptions to the endocrine system and obstruct the regular hormonal functions thus leading to complications with the immunological, sexual and neurological development of the affected organism (Cargouet *et al*. 2004; Safe 2005). The most severe effects of endocrine disruptors are most commonly observed in the reproductive organs of exposed animals and includes reduced growth of gonads as well as lower levels of oestrogen and androgen. This has been seen in fish collected in close proximity to wastewater treatment plants and in fish collected close to heavy industrial sites (Pait and Nelson 2002).

A review paper was written in 2010 by Olujimi *et al.* highlighting the need for studies on EDCs such as phenols and phthalates in South Africa, the paper brings to light the fact that South Africa has extensively used chemicals classified as EDCs (Olujimi *et al.* 2010). In developed continents such as Europe and North America, the main source of EDCs in aquatic

ecosystems is sewage and agricultural chemical run-off where-as in developing continents such as Africa, the main cause of EDCs in the aquatic ecosystems is ill-managed industrial and domestic discharge into water systems (Olujimi *et al.* 2010). The study concluded by stating that it is of utmost importance to monitor sites located in close proximity to chemical industries as well as mining and agricultural industries due to the fact that these activities contribute significantly to the availability of EDCs in the ecosystem (Olujimi *et al.* 2010). Olujimi *et al.* (2012) then further went on to conduct a study on the temporal variations of EDCs in selected freshwater systems and wastewater treatment plants. One control site was selected (Kirstenbosch Botanical Garden stream) and 11 priority phenols and 6 phthalate esters (PE) were tested for in five different rivers upstream and downstream from WWTPs. Phthalate esters with low molecular weight were more dominant in the rivers and WWTPs and there was a positive correlation between the levels of the analytes analysed downstream and in the effluent (Olujimi *et al.* 2012).

Phenols

Phenols are used as components of dyes, polymers and drugs, amongst other organic products. They are therefore commonly found in the environment and their presence is associated with the degradation and generation of pesticides and municipal and sewage wastes respectively (Michalowicz and Duda 2007). The toxicity of phenols (like any other chemical) is dependent on the duration and method of exposure as well as the organism type. The toxicity of man-made phenols, however, is a cause for concern to aquatic organisms due to the fact that most phenols are insoluble in water and highly halogenated phenols are particularly more persistent and bioaccumulate. A few of these phenols are active at low concentrations and are known to have endocrine disrupting properties. Phenols are known to biodegrade rapidly in sediment and water (Stewart and Stewart 2008).

Phthalates

Phthalates, which are also known as phthalic acid esters (PAEs), are commonly used in the production of polyvinyl chloride (PVC) base plastics as plasticizers (Hauser and Calafat 2005). The most commonly found route of exposure to humans is ingestion, but once it is ingested it is rapidly metabolized and excreted through faeces or urine. The toxicity of phthalates are based on their structures, this is seen in the case of phthalate monester metabolites which have been linked to testicular toxicity in rats. Other phthalates, however, such as DEHP and DiNP led to adverse effects in liver in rats (Hauser and Calafat 2005). In fish, it has been found to impair female reproduction by affecting significant aspects in the oogenesis process (Carnevali *et al.* 2010).

3.1.8. Sediment quality monitoring

Sediment is important in the transportation of nutrients and contaminants as well as in the elemental cycling within the aquatic environment. Surface erosion is the main source of sediment within surface waters and this is composed of a mineral component. During aquatic monitoring, the sediment can be described as either suspended or deposited, where the deposited sediment is found either on the river bed and as suspended sediment (also known as suspended matter, particulate matter or suspended solids) it is found within the water column (Ongley 1996). River sediment reflects processes such as run-off, soil weathering and atmospheric disposition within the catchment and water column. When environmental conditions change and there is a direct change in the quality of the water, then sediment acts as a source for trace pollutants (Ruether 2009).

Researchers are starting to realise the significance of sediment monitoring and assessment in water quality studies more, although the methods have not been widely accepted or standardized (Stronkhorst *et al.* 2004). Sediment quality monitoring provides useful information on the ecological status of the water body as well as to control the fate and effect of toxicants in the water. It is also a cost-effective tool used in water quality monitoring to give an indication of the level of contaminants, identify areas of concern and provide reference values for future monitoring purposes (Ruether 2009; Carere *et al.* 2012). Different parameters are analysed during sediment monitoring to enhance the quality of the sediment monitoring results. These are grain size, organic content and moisture content.

3.1.8.1 Grain size analysis

The determination of the size of the sediment particles is imperative in order to understand the source, transportation and environmental impact of the sediment (Ongley 1996). This is usually determined by passing the sediment through a series of sieves with sieve sizes ranging from 53 µm – 4000 µm and weighing out the content of each sieve and thereafter calculating the total percentage of each (Ongley 1996). The particle size distribution is known as "*a cumulative frequency distribution or a frequency distribution of relative amounts of particles in a sample within specified size ranges*" (Laswell *et al.* 2006). Studies have shown that there is a direct correlation between sediment grain size and contaminant concentrations. Maslllenikova *et al.* (2012) conducted a study in 2012 to determine whether this was indeed so. The research team sampled sediment from two lakes namely Chebachje and Piketnoye lakes in Russia. The results differed between the two lakes as Chebachje showed an increase in metal concentration with a decrease in particle size while Piketnoye had the highest concentration of metals within the coarse sediment sample (Maslllenikova *et al.* 2012). In the Kruger National Park, South Africa, the physical and chemical characteristics of sediment were

analysed to determine the presence of potential stressors within the system which ultimately lead to the deterioration of the sediment quality (Gerber *et al.* 2015). High levels of metals and OC pesticides were found to be responsible for the reduced sediment quality and these were mainly associated with finer grain sizes (Gerber *et al.* 2015).

However, in the Ell-ren River in Taiwan, a system polluted with heavy metals, a study by Tsai *et al.* (2003) investigated particle size and metal speciation in the river sediment to determine if there were any correlations between the two. The following heavy metals were tested for Copper, Chromium, Zinc, Lead and Nickel. The results showed that the there was no significant correlation between the grain particle sizes and metal speciation concentration (Tsai *et al.* 2003). The results prove why it is essential to investigate grain size analysis as different sites are most likely to yield different results.

3.1.8.2 Organic and moisture content

According to Baldock and Skjemstad (1999), the organic content of sediment consists of all the organic materials found in soils ranging from living organic matter such as plants, dissolved and particulate organic matter to humus and charred organic materials. The functional definition of soil organic matter excludes organic materials larger than 2 mm in size. The main function of soil organic matter is to maximize the water retaining abilities of sediment, decrease the level of water-runoff losses, improve the aeration within the sediment and ultimately produce a better sediment structure (Millar and Turk 1943). Moisture content can be described as the amount of water present in sediment, this measurement is important for sediment productivity (Kalieta 2005), other factors such as sediment temperature, plant growth, transport of nutrients and chemicals as well as groundwater recharge are all dependent on the water content of sediment (Bilskie 2001).

Tansel and Rafiuddin (2016) investigated heavy metal pollution and organic content and whether there was a relationship between these two factors in the Miami River in Florida USA. The accumulation of five metals was tested (Arsenic, Cadmium, Mercury, Zinc and Lead) and the results showed a significant positive correlation between the levels of Mercury, Cadmium and Zinc with organic content. However, Schorer's study conducted from 1993 to 1995 in Germany showed that the organic content does not play a significant role in the absorption of heavy metals. This study was done in an urbanized catchment and the sediment samples were divided into different particle sizes and analysed for the presence of heavy metals. The results did however; show a correlation between the organic content and micro-organic pollutants such as PAHs and PCBs (Schorer 1997).

3.1.8.3 Chemical contaminants in sediment

Anthropogenic activities as a result of urbanization and industrialisation lead to accumulation of contaminants in water and sediment resulting in environmental hazards (Knox and Paller 2013). The majority of these contaminants accumulate in the sediment due to the fact that they do not degrade in water and therefore cling to sediment particles (McCarthy *et al.* 1991). This explains why the concentrations of contaminants are usually higher in sediment in comparison to water samples (Chapman 1992; Bervoets and Blust 2003). The sediment therefore acts as a natural sink and ultimately becomes a source of chemical pollution into the system (Hakanson 1980; Chapman 1992; Bervoets and Blust 2003; Suresh *et al.* 2012).

Various land-use activities are known to increase the concentrations of contaminants in sediment. Smith (2007) conducted a study in the Quesnel River basin in Canada focusing mainly on forestry, mining and agricultural activities and their impact on sediment contamination (Smith 2007). The results were compared to un-impacted sites, which were used as the reference sites. As expected, the results were more elevated in the sites impacted by mining, forestry and agriculture in comparison to the reference sites. However, the study also showed that mining and forestry had a greater impact on sediment contamination (metals and nutrients) in contrast to agriculture (Smith 2007). Conversely, the study by Maanan *et al.* (2014) in Morocco yielded different results. In their study the highest potential for sediment contamination was seen in agricultural areas followed by urban sewage, while traditional land-uses such as oyster farming had the least impact on sediment contamination (Maanan *et al.* 2014).

Sediment contamination is also known to negatively affect the aquatic organisms within a system. Long *et al.* (2002) found a strong correlation between these contaminants and amphipod survival rates and the macro-invertebrate analysis showed that higher concentrations of chemicals in the sediment was associated with fewer species in comparison to the other sites (Long *et al.* 2002). It is therefore important to include sediment chemical analysis as part of aquatic biomonitoring.

3.2. Methodology

3.2.1. *In-situ* physico-chemical water analyses

A water quality assessment was carried out at each site (refer to Chapter 2) within each river during July 2015 (low-flow) and February 2016 (high-flow). Water quality parameters were measured *in situ* using a Eutech PC 450 Handheld meter kit. The following parameters were measured, recorded and analysed:

- pH
- Temperature (ºC)
- Conductivity (µS/cm)
- Total dissolved solids (ppm)
- Dissolved Oxygen (mg/L)
- Dissolved Oxygen (%)

3.3.8.1 Nutrient and biological water analyses

Surface water samples were collected in prepared 1.5 L sterile plastic bottles at each sampling site. The samples for Chlorophyll-*a* were collected in separate plastic bottles, each bottle was covered with aluminium foil. This was done to prevent photo-decomposition through light exposure. All samples were kept on ice prior to analyses. The samples were sent to a SANAS accredited analytical laboratory (WaterLab) in Pretoria, South Africa on the day of collection. The following constituents were tested:

- Free & saline ammonia as N
- Kjeldahl nitrogen
- Nitrite as N
- Nitrate as N
- Total phosphate as P
- Chlorophyll-*a*
- Faecal coliform bacteria / 100 mL
- Microcystin as LR (Samples for microcystin analyses were only collected at the downstream sites where fishes were collected where surface algae was clearly observed)

3.3.8.2 Inorganic water and sediment analyses

An ICP-MS scan was done to determine of concentrations of metals in water and sediment. Samples for metal analyses were collected at all 11 sites (Figure 3). The sample collection was done as explained in section 3.2.2.2 and the analyses were also done at WaterLab in Pretoria, South Africa. For sediment analysis, samples were collected upstream and downstream of each river. Each sample was crushed, split and milled and thereafter digested with ICP-MS. The metals analysed are listed in Table 1.

Table 1: Heavy metals tested for in water and selected sediment samples from Edendalespruit, Pienaars River and Hartbeesspruit using GC-MS

Aluminium	Europium	Mercury	Strontium
Antimony	Gadolinium	Molybdenum	Tantalium
Arsenic	Gallium	Nickel	Terbium
Barium	Germanium	Niobium	Teryllium
Beryllium	Gold	Palladium	Thorium
Bismuth	Hafnium	Phosphorus	Thallium
Boron	Holmium	Platinum	Thulium
Cadmium	Indium	Potassium	Titanium
Calcium	Iridium	Rodium	Uranium
Cerium	Iron	Rubidium	Vanadium
Cesium	Lanthanum	Ruthenium	Ytterbium
Chromium	Lead	Scadium	Yttrium
Cobalt	Lithium	Selenium	Zinc
Copper	Lutetium	Silicon	
Dysprosium	Magnesium	Silver	
Erbium	Manganese	Sodium	

3.3.8.3 Organic water and sediment analyses

Due to the expensive nature of organic analysis, surface water and sediment samples were only collected at the upstream and downstream sites of the three rivers (Figure 3). The water and sediment samples were collected in sterile 1 L glass bottles and 500 mL glass honey jars for analyses at an accredited laboratory (UIS Organic Laboratories) in Pretoria, South Africa. The neck and mouth of each bottle and jar was covered with aluminium foil before the lid was replaced. This was done to prevent contamination from the plastic lid during storage. The samples were refrigerated at 4 °C until they could be analysed through gas chromatography-mass spectrometry (GS-MS). Table 2 summarises the specific chemicals analysed.

Table 2: Specific chemicals analysed during both low-flow and high-flow seasons sampled from the upstream and downstream sampling sites in Edendalespruit, Hartbeesspruit and Pienaars River.

Polychlorinated Biphenols (PCBs)	**Organochlorine (OC) pesticides**
Heptachlorobiphenyls	*4,4'* -DDD
Hexachlorobiphenyls	*4,4'* -DDE
Pentachlorobiphenyls	*4,4'* -DDT
Tetrachlorobiphenyls	Aldrin
Trichlorobiphenyls	Alpha-HCH
Semi-volatile organic compounds (SVOCs)	Alpha-Chlordane
Chlorinated compounds	Beta-HCH
Phthalates	Delta- HCH

Polycyclic aromatic compound	Dieldrin
Phenols	Endosulfan sulfate
2-Chlorophenol	Endrin
2-Nitrophenol	Endrin Ketone
2,4-Dichlorophenol	Gamma-HCH
2,6-Dichlorophenol	Gamma-Chlordane
2-Methylphenol (o-cresol)	Heptachlor
3- and 4-Methylphenol (m+p cresol)	Heptachlor Epoxide Isomer B
2,4 Dimethylphenol	Methoxychlor
2,4,6 Trichlorophenol	Aldrin
2,4,5 Trichlorophenol	
4-Chloro-3-methylphenol	
2,3,4,6 Tetrachlorophenol	
Pentachlorophenol	
Phenol	

3.2.2. Sediment physical analyses

Additional sediment was collected in glass honey jars for physical analyses at the ecotoxicology laboratory at the University of Johannesburg using the standard scientific methods prescribed by American Society for Testing and Materials (ASMT 2001) and United States Environmental Protection Agency (USEPA 2001). All the samples were kept on ice while being transported to the University of Johannesburg; it was then refrigerated at 4 °C until the analysis was performed.

For each sample, 500 g of sediment was removed and dried in an oven for 96 hours at a temperature of 60 °C. This was done to determine the sediment moisture content: Once the samples were removed from the oven and re-weighed, the following calculation was used to determine the moisture content (Standards Association of Australia 1977):

$$\frac{\text{Moist sediment mass (500 g)} - \text{Dry sediment mass}}{\text{Dry sediment mass}} \times 100$$

The organic content was then determined by taking 5 g of sediment and then incinerating it for 6 hours at 600 °C. The samples were weighed for the second time in order to give the percentage organic content for each sample. The following equations were used to determine the organic content of each sediment sample (ASMT 2001): (MD = Mass of moist sediment, MPDS = mass of petri-dish + sediment, MP = mass of petri-dish, MO = mass of organic matter and OM = Organic matter content)

(1) MD = MPDS - MP

(2) MA = MPA - MP

(3) MO = MD - MA

(4) OM = (MO/MD) * 100

The remaining portions of each sample were used to determine the sediment grain size using an Endecott system which uses different sieve sizes (from 53 µm to 4000 µm). The grain size categories (gravel, very coarse sand, coarse sand, medium sand, very fine sand and mud) were thereafter identified using the protocol by Cyrus *et al*. (2000).

3.2.3. Statistical analyses

Multivariate statistical analysis was performed in this study. Principal components analyses (PCA) bi-plots were constructed using Canoco 5 (Version 5.04). PCA is known as a linear response model which evaluate the relation between different sites and possible variables. Two types of scores were used and hence a biplot was created (Šmilauer and Lepš 2014). For PCA, weighted summation is used to indirectly analyse the interactions between variables (Van der Brink *et al.* 2003). This method of analyses was chosen due to the length of the gradient which was relatively short (less than 1.0 SD in most cases) (Van der Brink *et al.* 2003). In PCA biplots, the extent of the variation in the individual response value is indicated by the length of the data arrows. If a case projects further from the 0 in the direction of the arrow head, this means that these cases have above-average values (Šmilauer and Lepš 2014). The opposite applies for corresponding variables projecting further away from 0 in the opposite direction. The angle between the data arrows was taken into consideration, as an angle closer to 0º signifies a positive correlation, while closer to 90º indicates that there is no correlation between variables and finally an angle closer to 180º denotes a negative correlation (Van der Brink *et al.* 2003).

3.2.4. Reference guidelines

The physico-chemical, inorganic and organic water quality data collected was specifically compared to the DWAF water quality guidelines (where available) for domestic use, agricultural use (livestock watering and irrigation), aquatic ecosystems, aquaculture and recreational use (DWAF 1996 a, b, c, d, e). The physical characteristics of the sediment were compared to USEPA (1991) guidelines and the sediment grain size analysed according to Cyrus *et al.* (2001). For the chemical analyses of sediment, the Canadian environmental quality guidelines by the Canadian Council of Ministers of the Environment (CCME 2001) was used.

3.3. Results

3.3.1. Physico-chemical analyses of water

The physico-chemical parameter results are presented in Table 3 and Table 4. During low-flow conditions, site P1 had the highest EC and TDS, whereas site P2 had the lowest levels. The results were similar during high-flow conditions. The concentration of dissolved oxygen was the highest at site H3 and the lowest at site P3 during the low-flow season. The physico-chemical parameters such as conductivity, TDS and pH decreased slightly at site H2 during low-flow conditions and increased slightly at site H2 during the high-flow conditions. For both surveys, site E1 had the highest pH. The pH for most of sites increased during the low-flow conditions compared to high-flow conditions with the exception of the upstream sites (P4, H3 and M2).

Table 3: Physico-chemical water quality parameters measured at selected sites in Edendalespruit, Pienaars River and Hartbeesspruit during July 2015 (low-flow conditions)

PARAMETER	E1	E2	P1	P2	P3	P4	H1	H2	H3	M1	M2
Temperature (°C)	9.8	10.3	12.5	6.4	14.5	9.5	9.5	8.9	13.9	10.7	12.0
Electrical Conductivity (µS/cm)	540.5	629.8	791.2	253.2	711.2	590.9	423.4	550.4	521.6	400.6	474.2
TDS (ppm)	270.1	314.8	395.0	126.2	355.6	295.7	211.6	275.3	260.6	200.4	237.6
Dissolved oxygen (mg/L)	6.9	6.8	7.6	9.6	4.1	9.4	8.7	8.6	10.9	8.8	8.9
Dissolved oxygen (%)	62.3	60.8	70.8	78.6	40.8	82.7	80.5	74.8	104.5	79.7	83.9
pH	8.9	7.8	7.5	7.9	7.7	7.7	8.4	8.2	8.5	7.7	8.0
Time of sample collection	16:20 pm	17:26 pm	8:47 am	12:10 pm	10:15 am	9:45 am	15:45 pm	15:08 pm	17:26 pm	15:15 pm	17:50 pm

Table 4: Physico-chemical water quality parameters measured at selected sites in Edendalespruit, Pienaars River and Hartbeesspruit during February 2016 (high-flow conditions)

PARAMETER	E1	E2	P1	P2	P3	P4	H1	H2	H3	M1	M2
Electrical Conductivity (µS/cm)	481.2	735.9	819.3	246.8	616.9	816.8	423.4	505.8	619.7	297.7	464.0
TDS (ppm)	240.7	367.9	406.9	123.7	308.2	408.2	211.6	253.1	310.0	148.9	232.0
pH	9.0	7.9	7.8	8.5	8.0	7.6	8.2	8.3	7.6	8.2	7.6
Time of sample collection	14:30 pm	10:19 am	9:12 am	8:17 am	13:00 pm	12:30 pm	10:14 am	14:30 pm	15:08 pm	6:50 am	11:11 am

*Temperature and Oxygen could not be recorded during the high-flow condiions due to a mechanical error on the meter.

The results are expressed in the PCA bi-plots (Figure 4 and Figure 5) which were interpreted according to Van der Brink *et al.* (2003), where the groupings confirm that dissolved oxygen during the low-flow conditions was associated with site H3, and high TDS and EC associated with E1, E2 and P1. The bi-plot also shows a positive correlation between dissolved oxygen and pH during the low-flow conditions and a positive correlation between temperature, TDS and EC. Sites within the Pienaars River appeared to be scattered while the sites within Edendalespruit and Hartbeesspruit (with its tributary, the Morelettaspruit) were closely situated near the axis, thus indicating similarities in the physico-chemical values, especially pH.

During high-flow conditions the angle between the TDS and EC and the pH, was approaching 180º thus indicating a negative correlation between the variables. This was also observed during low-flow conditions. During high-flow conditions, the sites within the Pienaars River were more closely associated (with the exception of P2), whereas Edendalespruit was scattered with a weak correlation between the two sites. There was a strong correlation between the downstream sites within the Hartbeesspruit (H1 and H2), and a weak correlation between these two sites and the upstream Hartbeesspruit (H3) and sites within the tributary, the Morelettaspruit (M1 and M2).

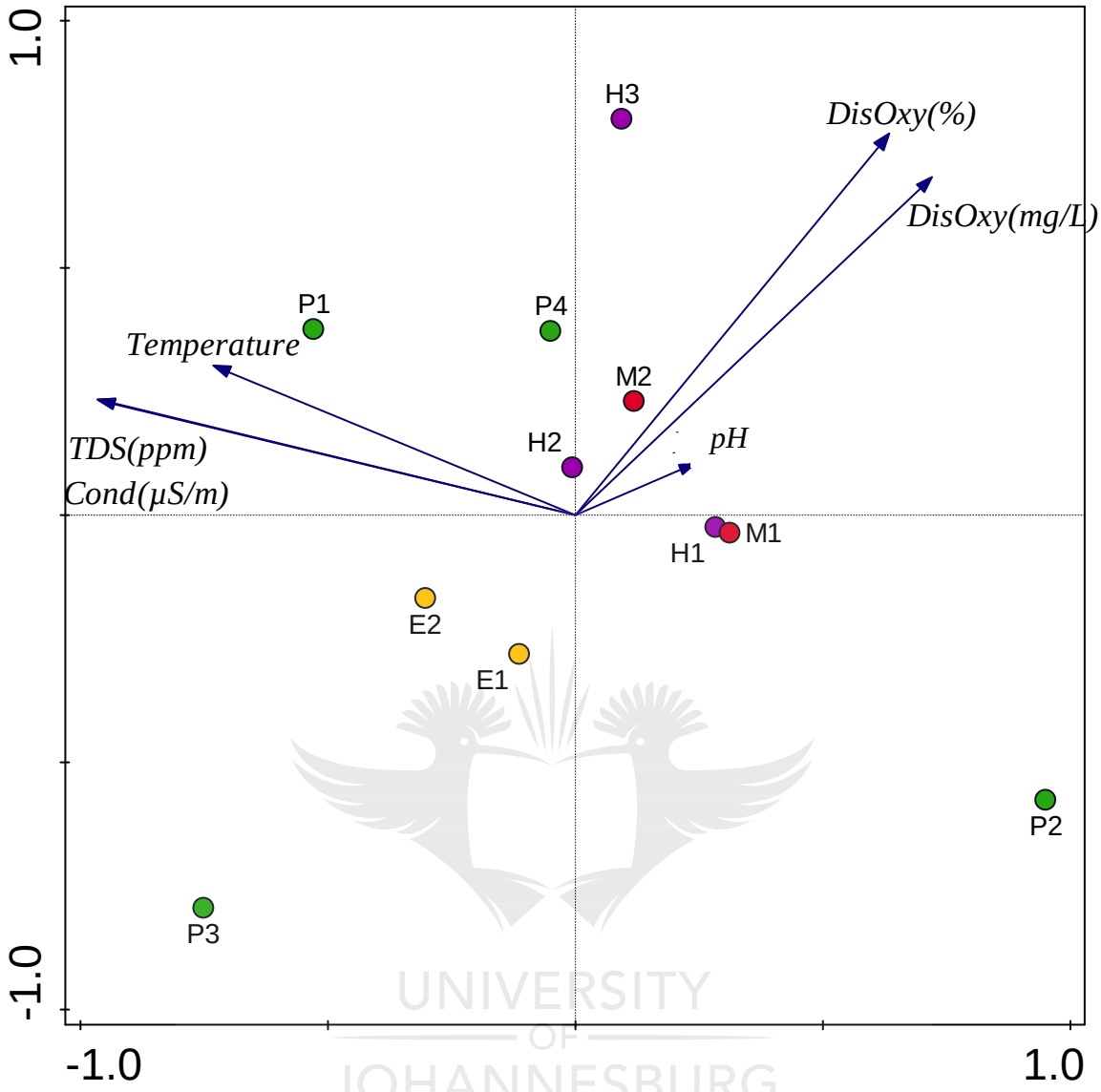

Figure 4: A principal components analysis (PCA) bi-plot showing sampling sites and *in-situ* water quality parameters measured during low-flow conditions at selected sites within Edendalespruit, Pienaars River Hartbeesspruit and its tributary, Morelettaspruit. The bi-plot explains 93.74% of the total variance in data, with 72.87% of the variance explained on the first axis and 20.87% explained on the second axis

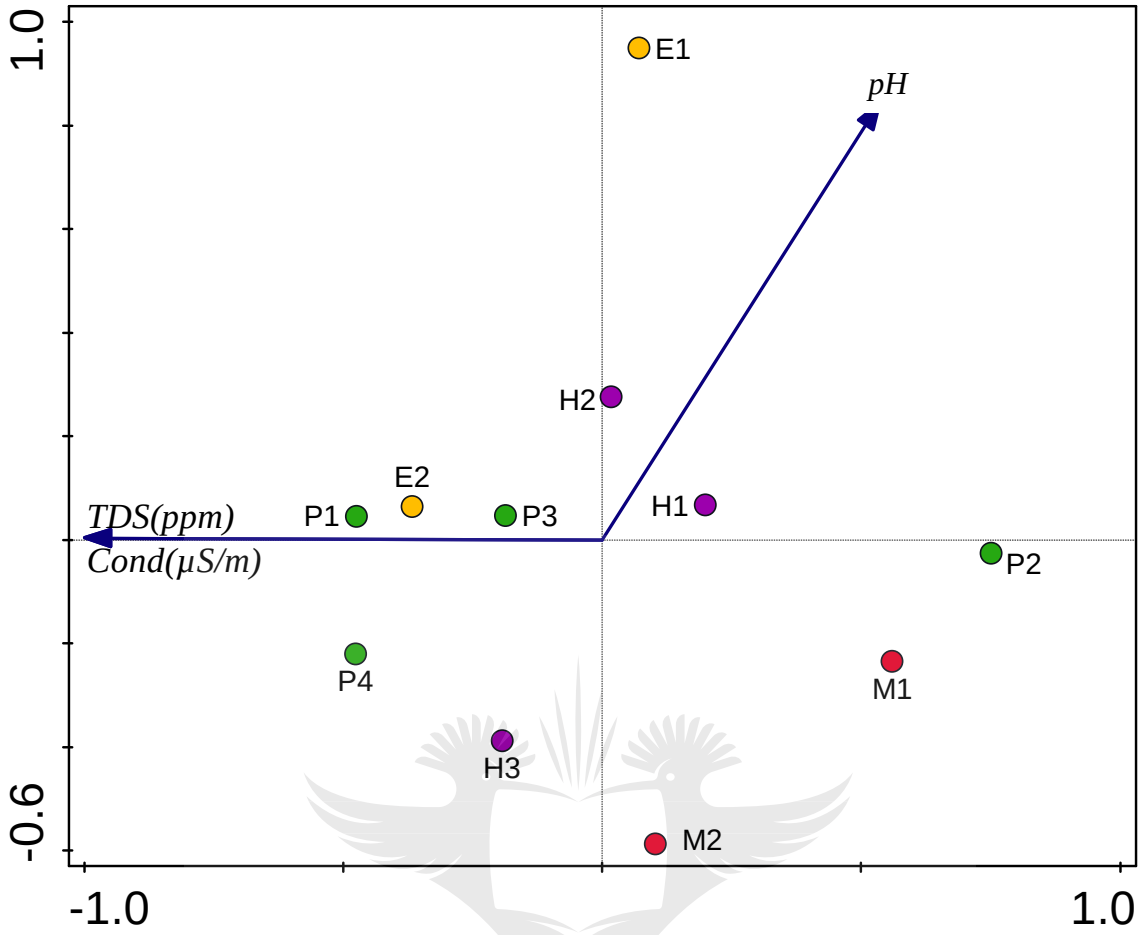

Figure 5: A principal components analysis (PCA) bi-plot showing sampling sites and *in situ* water quality parameters measured during high-flow conditions at selected sites within Edendalespruit, Pienaars River Hartbeesspruit and its tributary, Morelettaspruit.The bi-plot explains 100% of the total variance in data, with 99.43% of the variance explained on the first axis and 0.57% explained on the second axis.

3.3.2. Nutrient and biological analyses

The low-flow results are presented in Table 5 and the high-flow results are presented in Table 6. The corresponding bi-plots are presented in Figure 6 and Figure 7.

Table 5: Nutrient and biological analyses of water samples collected from Edendalespruit, Pienaars River and Hartbeesspruit during July 2015 (low-flow conditions)

	E1	E2	P1	P2	P3	P4	H1	H2	H3	M1	M2
Free & Saline Ammonia as N	<0.2	2.0	0.3	<0.2	<0.2	<0.2	0.7	<0.2	<0.2	<0.2	0.3
Nitrite as N	0.3	0.1	0.7	<0.1	<0.1	0.4	0.1	<0.1	<0.1	<0.1	<0.1
Nitrate as N	1.8	0.5	0.9	0.9	<0.2	<0.2	0.5	0.5	0.5	<0.2	0.5
Kjeldahl Nitrogen	1.4	2.0	6.2	<0.2	<0.2	4.5	0.8	0.3	0.3	0.6	0.3

Total Phosphate as P	0.3	0.9	3.8	<0.2	<0.2	3.6	0.2	0.3	<0.2	<0.2	<0.2
Chlorophyll-*a* in µg/L	20	17	56	1	1	5	8	3	9	15	3
Faecal Coliform Bacteria / 100 mL	60	2700	110000	15	1	140	40	310	100	170	84

Table 6: Nutrient and biological analyses of water samples collected from Edendalespruit, Pienaars River and Hartbeesspruit during February 2016 (high-flow conditions)

	E1	**E2**	**P1**	**P2**	**P3**	**P4**	**H1**	**H2**	**H3**	**M1**	**M2**
Free & Saline Ammonia as N	0.1	14	5.3	<0.1	0.1	24	1.4	0.1	0.1	0.1	0.2
Nitrite as N	0.07	0.1	<0.05	<0.05	<0.05	<0.05	0.3	<0.05	<0.05	<0.05	0.06
Nitrate as N	0.3	0.2	0.1	0.2	0.2	<0.1	0.9	<0.1	0.6	0.1	0.5
Kjeldahl Nitrogen	2.9	14.0	33.0	2.5	0.9	27.0	20.0	2.6	1.4	1.2	2.1
Total Phosphate as P	0.3	0.9	3.6	0.5	<0.2	3.1	0.8	0.4	0.4	0.2	0.3
Chlorophyll-*a* in µg/L	16	143	906	24	9	5	158	54	32	13	46
Faecal Coliform Bacteria / 100 mL	3	65000	>100000	>100000	15	770	330	1600	690	1600	1600

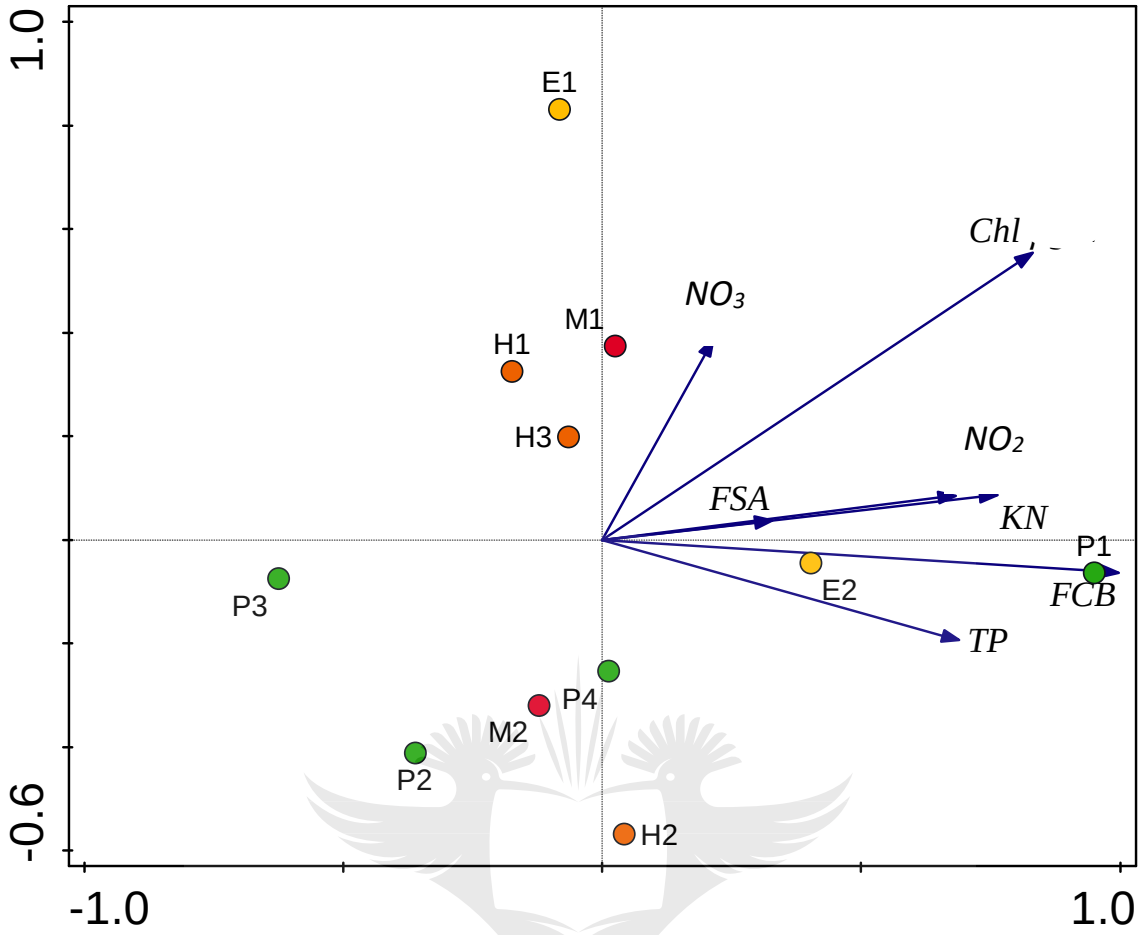

Figure 6: A principal components analysis (PCA) bi-plot showing sampling sites and nutrient measured during low-flow conditions at selected sites within Edendalespruit, Pienaars River Hartbeesspruit and its tributary, Morelettaspruit. The bi-plot explains 98.41% of the total variance in data, with 96.15% of the variance explained on the first axis and 2.25% explained on the second axis. (Chl = chlorophyll; FCB = faecal coliform bacteria; FSA = free & saline ammonia; KN = Kjedahl nitrogen; TP = total phosphorus; NO_2 = nitrite NO_3 = nitrate)

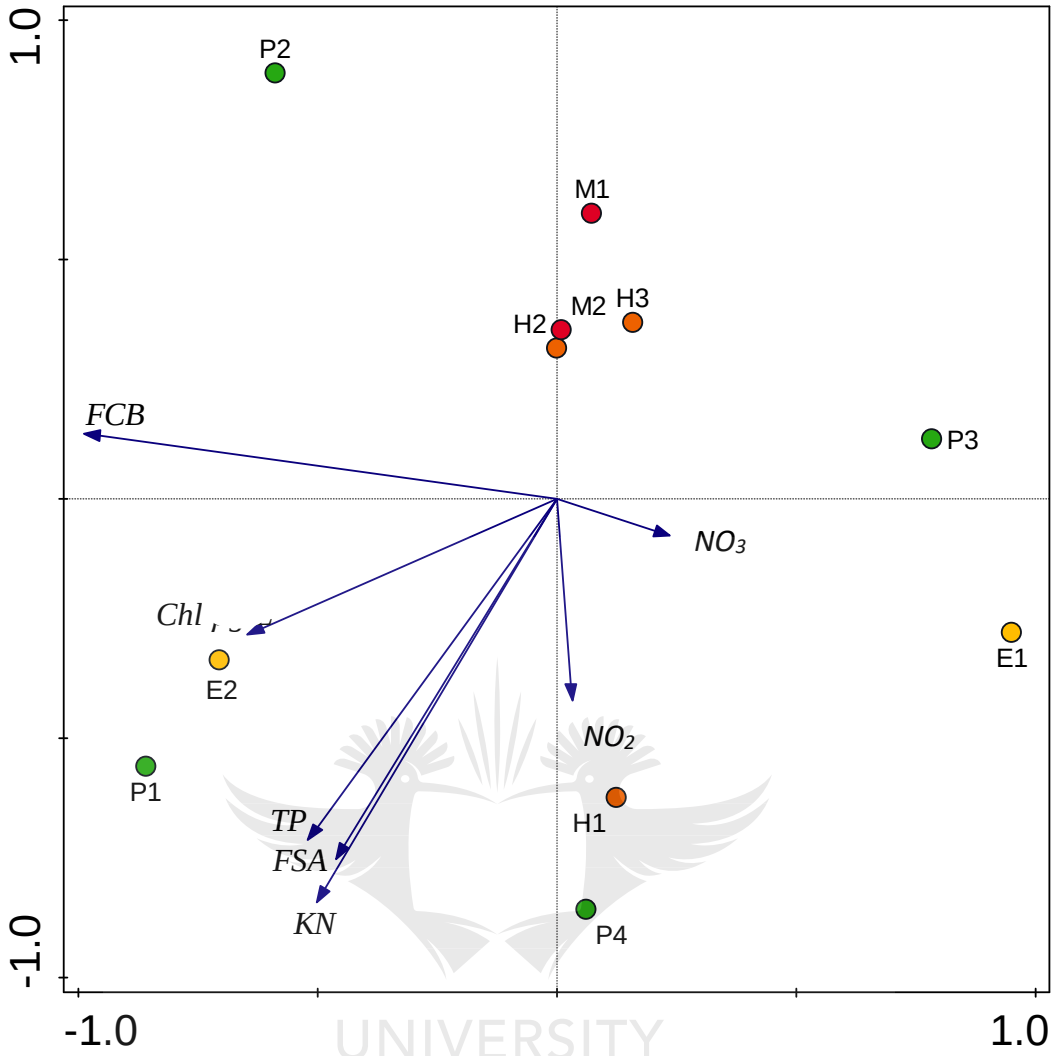

Figure 7: A principal components analysis (PCA) bi-plot showing sampling sites and nutrient measured during high-flow conditions at selected sites within Edendalespruit, Pienaars River Hartbeesspruit and its tributary, Morelettaspruit. The bi-plot explains 98.26% of the total variance in data, with 83.86% of the variance explained on the first axis and 14.4% explained on the second axis. (Chl = chlorophyll; FCB = faecal coliform bacteria; FSA = free & saline ammonia; KN = Kjedahl nitrogen; TP = total phosphorus; NO_2 = nitrite NO_3 = nitrate)

The downstream site within the Edendalespruit had the highest level of nitrate during the low-flow conditions followed by the downstream site within the Pienaars River (P2), which was exactly the same at the site further downstream in the same river (P1). However, during high-flow conditions, the downstream Hartbeesspruit site (H1) had the highest levels of nitrate. The nitrite levels were also fairly low, with the highest level of 0.7 observed at the downstream site within the Pienaars River. The downstream site within Pienaars River (P1) had the highest levels of faecal coliform bacteria during the low-flow conditions, the levels increased drastically during the high-flow season. The downstream site for Pienaars River (P2) which is the closest point to site P1 where fish was collected also had very high levels. The middle reach in Pienaars River (P3) had the lowest levels of faecal coliform bacteria during low-flow conditions

and the second lowest during the high-flow conditions. The Edendalespruit downstream site (E1), had the lowest levels during the high-flow conditions. With the exception of P2 and P3 during the low-flow conditions and E1 and P3 during high-flow conditions, the levels of faecal coliforms observed (>20/ 100 mL) are expected to pose significant risks of transmission of infectious diseases according to DWAF guidelines for domestic use (DWAF 1996a). This risk would occur if humans were to ingest the water for drinking purposes or during recreational activities like skiing and other water sports. Observations at some of the sites sampled revealed livestock ingesting the water. According to the DWAF guidelines (DWAF 1996d) the levels at which significant infections would be seen in livestock are between 1000 – 5000 / 100 mL (in 50% of the samples). Levels above 1000/ 100 mL were observed during the high-flow conditions (sites E2, P1, P2, H1, M1 and M2).

The site downstream Pienaars River also had the highest levels of nitrogen during both seasons with a marked increase during the high-flow conditions. The Edendalespruit upstream, site E2, had highest ammonia levels during low-flow condition as well as the second highest during high-flow conditions. The PCA bi-plot showed a strong positive correlation between the upstream sites of Hartbeesspruit (H2 and H3) and its tributary, sites (M1 and M2). These sites are closely situated and had similar values for the nutrient and bacteria levels during low-flow conditions. The upstream site within Pienaars River showed a strong positive correlation with nitrite and Kjedahl nitrogen whereas P1, the downstream site within the same river showed the same positive correlation as well as a positive correlation with faecal coliform bacteria and ammonia. There was a strong positive correlation between the upstream Edendalespruit (E2) and ammonia and total phosphate concentrations. These values were above average for each river according to (Van der Brink *et al.* 2003; Šmilauer and Lepš 2014).

There was also a strong correlation between this site and chlorophyll-*a*. Similarly, during high-flow conditions, there was a strong positive correlation with P4, P1 and P2 and Kjedahl nitrogen, ammonia and faecal coliform bacteria. The upstream sites within the Hartbeesspruit (H2 and H3) grouped together with the two sites within the tributary showing a negative correlation with nitrite, ammonia and Kjedahl nitrogen. The downstream site in Pienaars River (P1) had the highest chlorophyll-*a* and phosphate levels during both seasons, whereas the lowest chlorophyll-*a* was seen in site P2 and P3 during low-flow conditions and site P3 and P4 during high-flow conditions. The lowest values for phosphate were below detection limits, this was seen in sites P2, P3, H1, M1 and M2 during low-flow conditions and only in P3 during high-flow conditions.

The total phosphate and chlorophyll-*a* in µg/L (overall mean per site) was used in conjunction with the Trophic State Classification Boundaries per DWAF guidelines (Table 7) (Van Ginkel

et al. 2001) to determine the trophic status of each river. The results are presented in Table 8. According to the mean values observed in Table 8, each river was classified as hypertrophic. The highest chlorophyll-a levels were observed at the downstream sites of each of the rivers, with site P1 having the highest level of 481 µg/L. The lowest levels were recorded at site P4 and H2. Interestingly, the highest total phosphate concentrations were detected at sites P1 and P4 in the Pienaars River, with site P1 associated with the highest chlorophyll-*a* concentration, while site P4 had the lowest chlorophyll-*a* concentration.

Table 7: The Trophic State Classification Boundaries per DWAF guidelines (Van Ginkel 2002)

Variable	**Oligotrophic**	**Mesotrophic**	**Eutrophic**	**Hypertrophic**
Total phosphate (mg/L)	<0.015	0.015 – 0.047	0.048-0.013	>0.130
Median Chlorophyll-*a* (µg/L)	0-10	11-20	21-30	>30
% time Chlorophyll-*a* > 30 µg/L	0	<8	8-50	>50

Table 8: The Trophic Status of Edendalespruit, Pienaars River and Hartbeesspruit according to the classification boundaries per DWAF guidelines (Van Ginkel 2002)

Site	**Total Phosphate as P**	**Chlorophyll-*a* in µg/L**	**Trophic Status**
E1	0.15	18	Hypertrophic
E2	0.9	89	Hypertrophic
P1	3.7	509	Hypertrophic
P2	0.35	12.5	Hypertrophic
P3	<0.2	5	Hypertrophic
P4	3.35	5	Hypertrophic
H1	0.5	83	Hypertrophic
H2	0.35	28.5	Hypertrophic
H3	0.2	20.5	Hypertrophic
M1	0.2	14	Hypertrophic
M2	0.25	24.5	Hypertrophic

3.3.3. Microcystin analysis

The results are presented in Table 9. Detectable concentrations of microcystin-LR were only found in the Pienaars River and the Edendalespruit.

Table 9: Results of microcystin analyses conducted at the downstream sites of Edendalespruit, Pienaars River and Hartbeesspruit during low-flow (July 2015) and high-flow (February 2016) conditions

Site	Microcystin as LR (ug/L)	
	low-flow	high-flow
E1	<0.15	0.9
P1	<0.15	1.8
H1	<0.15	<0.15

3.3.4. Organic analyses

During both seasons, no detectable levels of PCBs, SVOCs, phenols or chlorinated pesticides were recorded in any water samples collected. The detection limits for chlorinated pesticides was <1 µg/L, PCBs <0.1 µg/L and SVOCs and phenols <10 µg/L.

3.3.5. Inorganic analyses

The metal analyses results are presented in Table 10 and Table 11 for the low-flow and high-flow conditions respectively.

Table 10: Results of metals within Edendalespruit, Pienaars River and Hartbeesspruit above the detection limit (< 0.001) during July 2015 (low-flow conditions) (Concentrations above guideline values are highlighted in orange)

Site	Aluminium (mg/L)	Boron (mg/L)	Barium (mg/L)	Calcium (mg/L)	Iron (mg/L)	Potassium (mg/L)	Magnesium (mg/L)	Manganese (mg/L)	Sodium (mg/L)	Phosphorus (mg/L)	Silicon (mg/L)	Selenium (mg/L)	Titanium (mg/L)
E1	2.13	0.028	0.081	35	6.36	14.5	19	0.376	69	2.38	11.4	0.085	0.056
E2	0.236	0.011	0.093	29	0.511	4.4	27	0.521	39	0.771	9.2	0.159	0.050
P1	0.339	<0.010	0.054	36	0.485	3.1	22	0.077	23	0.030	3.5	0.081	0.039
P2	<0.100	<0.010	0.020	16	0.797	<1.0	14	0.041	7	0.015	8.2	0.049	0.028
P3	0.126	0.014	0.033	40	0.382	1.3	36	0.058	20	0.014	6.9	0.112	0.061
P4	0.218	0.045	0.032	29	0.703	13.5	17	0.098	68	3.24	8.2	0.104	0.049
H1	0.466	0.014	0.047	31	0.800	8.1	19	0.156	45	0.230	6.6	0.079	0.035
H2	0.268	0.013	0.059	30	0.442	2.4	16	0.026	15	0.069	7.5	0.090	0.052
H3	0.131	0.016	0.054	32	0.365	2.4	22	0.035	19	0.019	1.4	0.114	0.054
M1	0.198	<0.010	0.036	28	0.652	1.3	18	0.071	11	0.024	5.2	0.068	0.048
M2	0.104	0.013	0.046	32	0.379	2.3	20	0.047	18	0.044	2.4	0.099	0.052

Table 11: Results of metals within Edendalespruit, Pienaars River and Hartbeesspruit above the detection limit (< 0.001) during February 2016 (high-flow conditions) (Concentrations above guideline values are highlighted in orange).

Site	Aluminium (mg/L)	Boron (mg/L)	Barium (mg/L)	Calcium (mg/L)	Iron (mg/L)	Potassium (mg/L)	Magnesium (mg/L)	Manganese (mg/L)	Sodium (mg/L)	Phosphorus (mg/L)	Silicon (mg/L)	Nickel (mg/L)	Selenium (mg/L)	Titanium (mg/L)
E1	<0.100	0.043	0.045	31	0.145	9.8	15	0.037	49	0.206	5.5	<0.010	0.082	0.026
E2	0.173	0.013	0.124	36	0.596	5.8	30	0.793	53	1.33	11.3	<0.010	0.169	0.038
P1	0.730	0.076	0.052	45	3.31	20	25	0.390	83	3.71	11.5	0.029	0.091	0.034
P2	1.06	<0.010	0.074	21	7.72	1.0	18	0.984	9	0.042	10.9	0.058	0.049	0.021
P3	0.130	0.010	0.084	49	0.433	2.6	42	0.392	20	0.025	9.0	<0.010	0.114	0.040
P4	0.211	0.117	0.035	32	0.584	13.5	19	0.183	72	2.15	8.8	0.013	0.088	0.027
H1	0.530	0.033	0.058	29	1.43	7.1	15	0.128	35	0.329	6.7	0.018	0.072	0.029
H2	1.83	<0.010	0.124	56	3.68	2.5	27	0.894	20	0.052	11.9	0.032	0.094	0.041
H3	0.325	0.016	0.081	35	0.632	3.1	20	0.116	19	0.038	7.4	<0.010	0.099	0.036
M1	0.455	<0.010	0.056	28	1.48	2.0	14	0.235	10	0.027	6.0	0.012	0.060	0.024
M2	0.187	0.015	0.058	35	0.366	2.9	19	0.125	20	0.050	7.2	<0.010	0.080	0.029

Of the 62 metals tested for, only 14 were above the detection limit (< 0.001) namely: Aluminium, Boron, Barium, Calcium, Iron, Potassium, Magnesium, Manganese, Sodium, Phosphorus, Silicon, Selenium, Nickel and Tin. However, Nickel was detected only during high-flow sampling survey. All detected metal concentrations fell within the target water quality ranges for all the required water-use purposes (DWAF guidelines) with the exception of Aluminium and Iron levels during low-flow conditions at the downstream Edendalespruit, site E1. Magnesium concentrations recorded at the middle reach of the Pienaars River site (P3) during both seasons were above the target ranges for all water quality purposes, while Iron concentrations were above the target ranges only during high-flow conditions at site P1 as well as Magnesium at the upstream Edendalespruit, site E2.

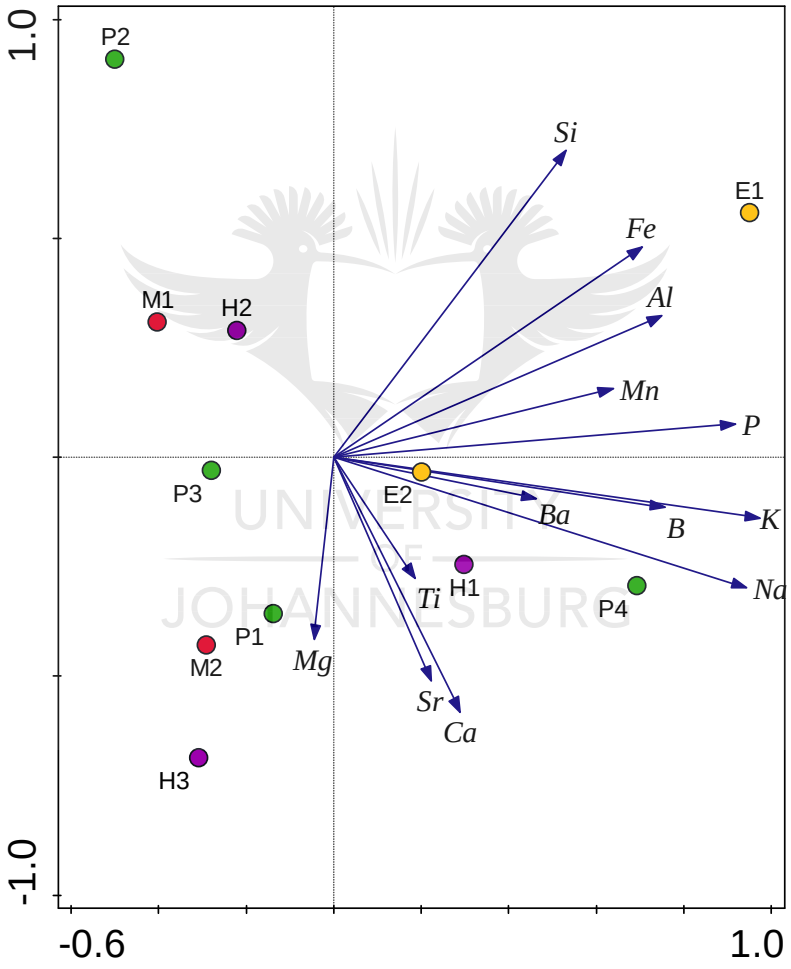

Figure 8: A principal component analysis (PCA) bi-plot showing sampling sites and metals measured during low-flow conditions at selected sites within Edendalespruit, Pienaars River Hartbeesspruit.and its tributary, Morelettaspruit.The bi-plot explains 84.45% of the total variance in data, with 70.85% of the variance explained on the first axis and 13.60% explained on the second axis.

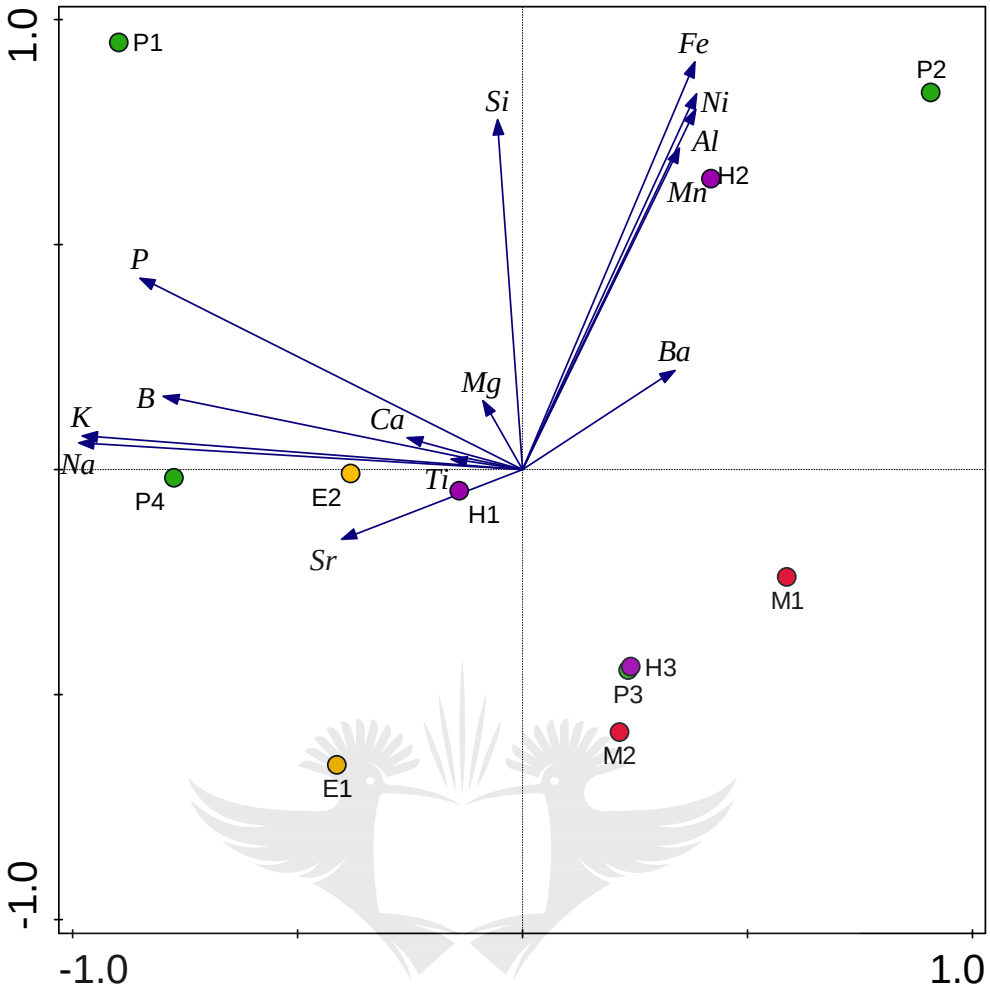

Figure 9: A principal component analysis (PCA) bi-plot showing sampling sites and metals measured during high-flow conditions at selected sites within Edendalespruit, Pienaars River Hartbeesspruit and its tributary, Morelettaspruit. The bi-plot explains 85.75% of the total variance in data, with 61.7% of the variance explained on the first axis and 24.05% explained on the second axis

The PCA bi-plots which provided a spatial and temporal analysis of the results showed that for low-flow conditions 3 main groupings were observed and a variance of 70.85% on the first axis and 13.60% on the second axis. Magnesium was mainly associated with the upstream sites of Hartbeesspruit and Morelettaspruit as well as the downstream and middle reaches of the Pienaars River. Aluminium and Phosphorus were associated with downstream Edendalespruit and upstream Pienaars River. High levels of Potassium, Sodium and Calcium were recorded in Pienaars River and Edendalespruit upstream (E2 and P4) as well as Hartbeesspruit downstream (H1). The high-flow condition analysis showed a total variance of 85.75% with 3 main groupings. The Potassium and Sodium had a big effect on the grouping of Pienaars River and Edendalespruit upstream (E2 and P4). During both seasons the sites within Hartbeesspruit and its tributary Morelettaspruit seem to be closely associated.

3.3.6. Physical analyses of sediment

Physical sediment characteristics of each site were analysed. The characterization for Organic content is summarized in Table 12 and the results of the moisture organic and moisture content are presented in Table 13.

Table 12: Categorisation of the percentage organic content in sediment as defined by USEPA (1991)

Category	Percentage
Very low	<0.05%
Low	0.05-1%
Moderately low	1-2%
Medium	2-4%
High	>4%

Table 13: Organic and moisture content of sediment samples collected at Edendalespruit, Pienaars River and Hartbeesspruit during the low-flow (July 2015) and high-flow (February 2016) conditions

Site	Moisture Content-low-flow (%)	Organic Content-low-flow (%)	Moisture Content-high-flow (%)	Organic Content – high-flow (%)
E1	5.62	2.8	19.33	1.435407
E2	0.3901	7.2	23.03	6.361829
P1	10.02	2.2	29.55	0.867052
P2	8.87	26.2	43.93	18.32947
P3	9.94	3.8	31.14	2.964427
P4	0.6	0.6	26.4	14.61378
H1	12.62	7.31	34.07	5.199307
H2	0.8	24.6	20.2	2.958015
H3	2.62	3.2	18.51	0.683594
M1	1.15	6.6	27.16	7.326733
M2	7.58	2.6	18.37	2.321083

The moisture content of the sediment increased for each river from low-flow to high-flow conditions. The downstream site within the Hartbeesspruit (H1) had the highest moisture content during low-flow conditions and the downstream site within the Pienaars River (P2) during the high-flow conditions. The upstream site within the Pienaars River (P4) and the

upstream site within the Morelettaspruit (M2) had the lowest moisture content during low-flow and high-flow conditions respectively. During the low-flow conditions, the upstream site within the Pienaars River had the lowest percentage of organic content (very low), while all the downstream sites (E1, P1 and H1) had a high organic content along with sites P2, P3 and M2. The sites with low organic content included upstream Edendalespruit (E2) and middle Hartbeesspruit (H2). The downstream Morelettaspruit (M1) had moderately low organic content and the middle Hartbeesspruit H3 medium organic content. During high-flow conditions, the downstream site within the Pienaars River (P1) had a low organic content along with the upstream (H3) site in the Hartbeesspruit.

The downstream site within the Edendalespruit (E1) had a moderately low organic content in contrast to the sediment at the other sampling site within the Edendalespruit (E2), which had a high organic content. The middle site (P3) within the Pienaars River, middle Hartbeesspruit, site H2, and the upstream site in Morelettaspruit was classified as having medium organic content. The sites which contained sediment with high organic content were the upstream and downstream Pienaars River sites (P4 and P2 respectively), the upstream Morelettaspruit (M1) and the downstream site within the Hartbeesspruit (H1). The grain size categories, according to Cyrus et al. (2000) are shown in Table 14 and the distribution of different grain sizes at each sampled site during high-flow and low-flow conditions is displayed in Figure 10 and Figure 11.

Table 14: Sediment grain-size categories (Cyrus *et al.* 2000)

Category	Size
Gravel	>4 000 µm
Very coarse sand	4 000 µm - 2 000 µm
Coarse sand	2 000 µm – 500 µm
Medium sand	500 µm – 212 µm
Very fine sand	212 µm – 53 µm
Mud	<53 µm

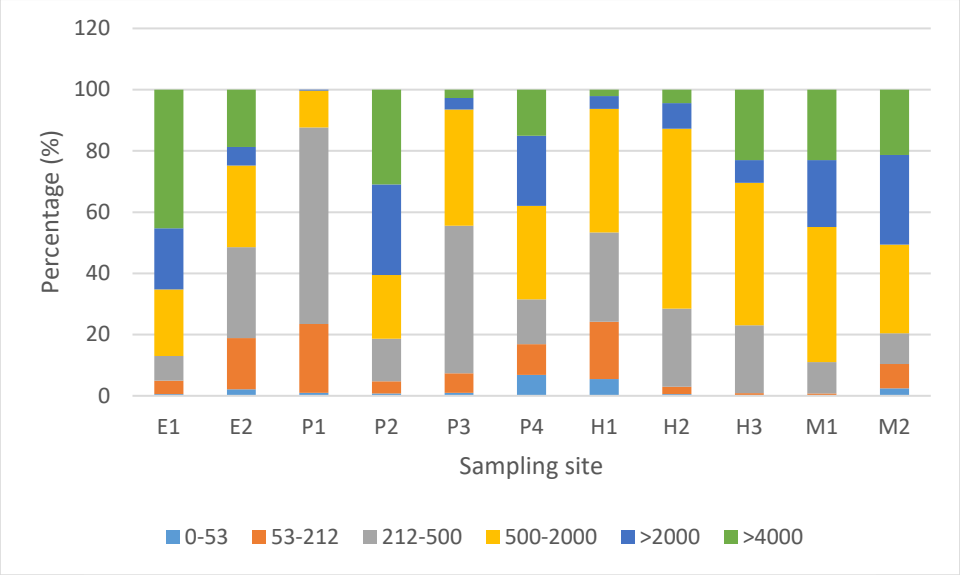

Figure 10: The distribution of the different grain sizes (g) for each site within Edendalespruit, Pienaars River and Hartbeesspruit during the low-flow conditions (July 2015).

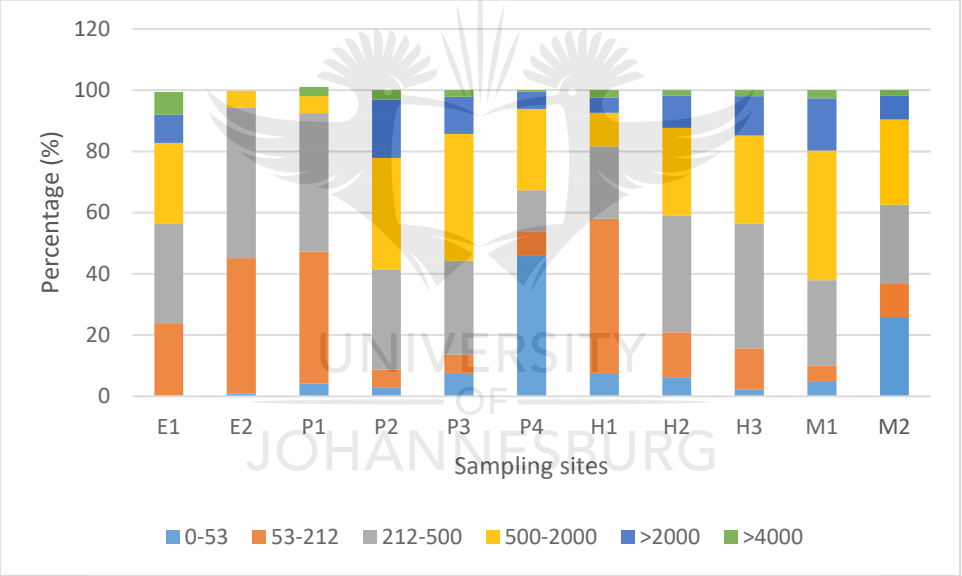

Figure 11: The distribution of the different grain sizes (g) for each site within Edendalespruit, Pienaars River and Hartbeesspruit during the high-flow conditions (February 2016).

In terms of grain-size, the sediment at each site differed, even comparing sites within the same river. The downstream site within the Edendalespruit (E1) was dominated by gravel during the low-flow conditions and medium sand during the high-flow conditions, and the upstream site within the Edendalespruit (E2) was dominated by medium sand during both seasons. The sediment sampled from the downstream site within the Pienaars River (P1) was mainly medium sand during both low-flow and high-flow conditions, whereas P2 was primarily composed of gravel during low-flow conditions and medium sand during high-flow conditions. The sediment sampled from the upstream site in the Pienaars River (P3) was comprised mainly of medium and coarse sand during the low- and high-flow conditions respectively. The

upstream Pienaars River site (P4) was comprised of coarse sand during the low-flow conditions and clay during high-flow conditions. The Hartbeesspruit sites (H1, H2 and H3) were comprised of coarse sand during low-flow conditions, however, during high-flow conditions, the downstream site H1 was mainly comprised of very fine sand and further upstream in the river (H2 and H3) the sediment was comprised of medium sand. The downstream site within the Morelettaspruit (M1) was comprised mainly of coarse sand during both seasons. Site M2 was comprised mainly of very coarse sand during low-flow conditions and medium sand during high-flow conditions.

3.3.6. Organic analyses of sediment

There were no PCBs, chlorinated pesticides and phenols detected at any of the sites. There were, however, a few semi-volatile organic compounds (SVOCs) detected. The results in Figure 12 and Figure 13 are a representation of the SVOCs detected at each site. There were mainly polycyclic aromatic compounds found namely; Phenanthrene, Anthracene, Flouranthene, Pyrene, Crysene, Benzo[a]anthracene, Benzo[k+b]flouranthen and Benzo[a]pyrene. The only phthalate detected was Bis(2-ethylhexyl) phthalate at the upstream sites of Morelettaspruit (M2) and the Pienaars River (P4) respectively.

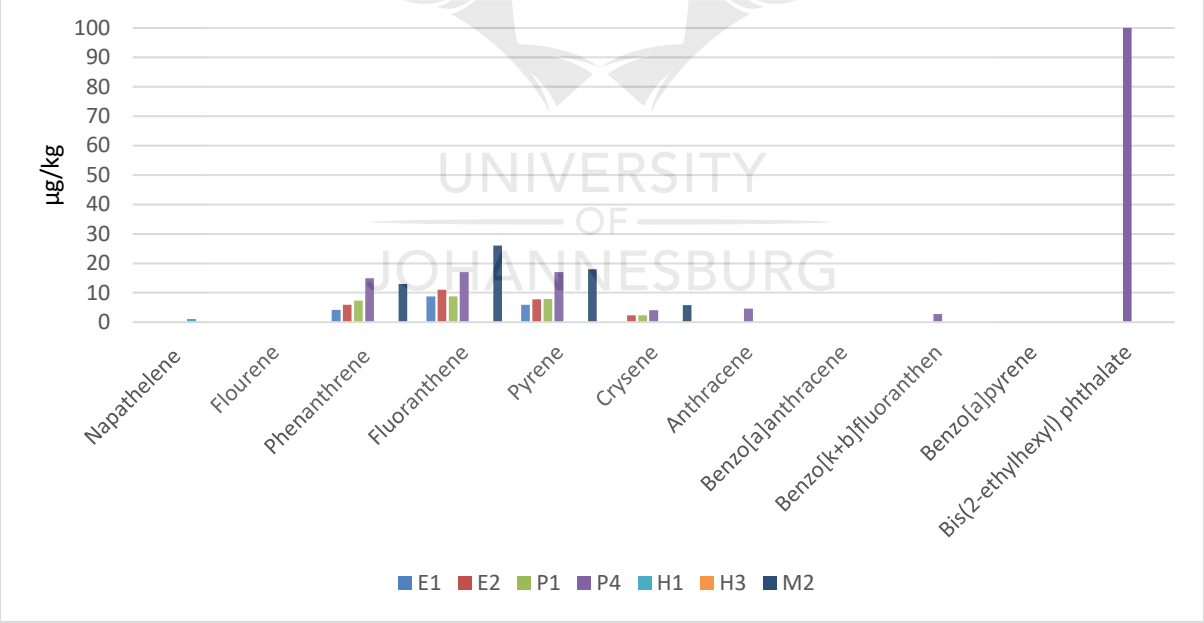

Figure 12: Levels of semi-volatile organic compounds detected within sediment sampled during low-flow (July 2015) conditions at the upstream and downstream sampling site of each river.

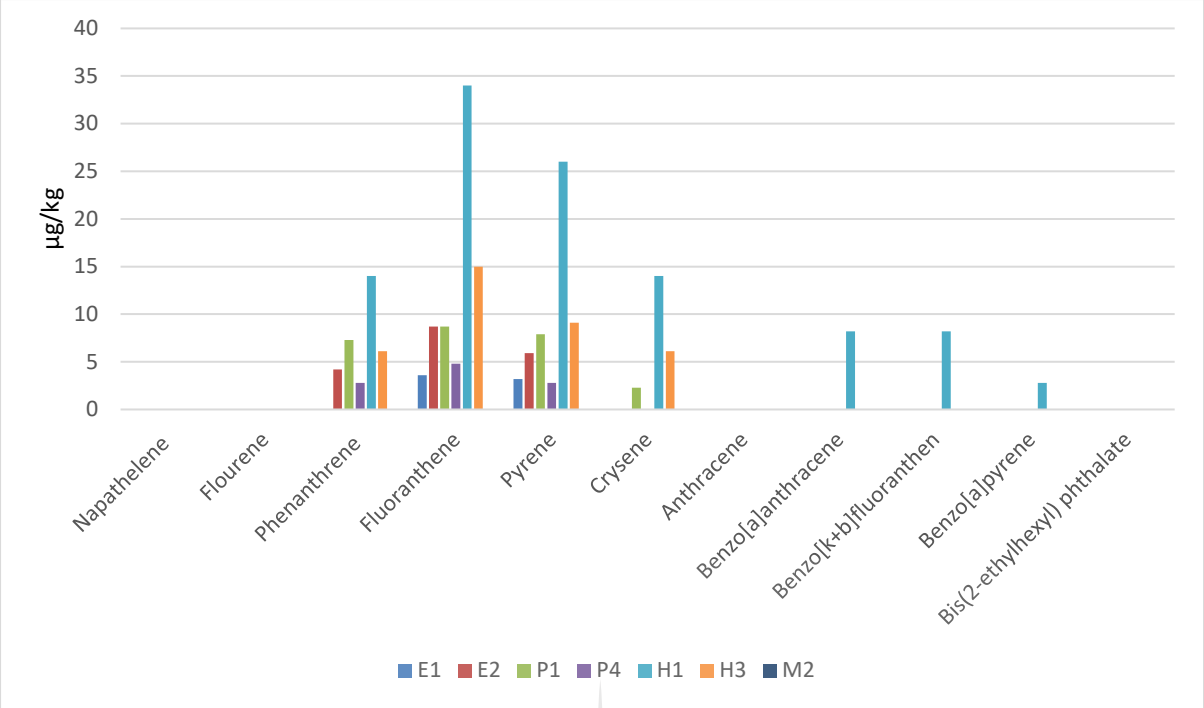

Figure 13: Levels of semi-volatile organic compounds detected within sediment sampled during the high-flow (February 2016) conditions at the upstream and downstream sampling site of each river.

3.3.7. Inorganic analyses of sediment

The results for the metal chemical analyses within sediment samples are presented in Table 15 and Table 16. These tables only list metal concentrations that were above the detection limit. During the low-flow conditions, the highest levels of metal concentrations were observed in the upstream site of Edendalespruit (site E1). This was with the exception of Magnesium and Silicon which was the highest at site P1 and P4 respectively. During the high-flow conditions, again, the highest metal concentrations were observed at site E1, with the exceptions of Calcium and Chromium (highest concentrations at site H1), Manganese, Magnesium and Iron (highest concentrations at site E2) and Silicon (highest concentration at site P1).

Table 15: Metal concentrations detected in sediment samples from Edendalespruit, Pienaars River and Hartbeesspruit during the low-flow conditions (July 2015)

Site	Aluminium (mg/L)	Boron (mg/L)	Barium (mg/L)	Calcium (mg/L)	Chromium (mg/L)	Iron (mg/L)	Potassium (mg/L)	Magnesium (mg/L)	Manganese (mg/L)	Sodium (mg/L)	Phosphorus (mg/L)	Silicon (mg/L)	Titanium (mg/L)
E1	116	0.22	0.598	8	0.521	103	31	13	1.68	5	2.09	736	6.59
E2	169	0.01	1.79	29	0.032	0.082	119	11	2.33	113	4.42	636	25
P1	53	0.029	0.268	15	0.077	136	6.4	23	1.49	4	2.19	742	3.41
P4	101	0.084	0.35	7	0.93	102	15.2	11	1	4	0.001	760	10
H1	81	0.024	0.528	4	0.364	93	24	4	0.644	5	2.66	400	6.65
H3	6.39	0.035	0.327	12	0.874	79	9.6	16	1.22	6	2.11	82	7.22
M1	80	0.374	0.001	13	0.532	126	17.1	28	1.57	7	2.07	742	5.04
M2	63	0.048	0.216	6	0.439	113	11.2	16	1.32	4	3.47	784	5.15

Table 16: Metal concentrations detected in sediment samples from Edendalespruit, Pienaars River and Hartbeesspruit during the high-flow conditions (February 2016)

Site	Aluminium (mg/L)	Boron (mg/L)	Barium (mg/L)	Calcium (mg/L)	Chromium (mg/L)	Iron (mg/L)	Potassium (mg/L)	Magnesium (mg/L)	Manganese (mg/L)	Sodium (mg/L)	Phosphorus (mg/L)	Silicon (mg/L)	Titanium (mg/L)
E1	110	0.039	0.258	2	0.524	163	23	48	2.63	4	3.1	684	5.38
E2	139	0.009	1.3	16	0.028	68	81	3	1.65	107	3.23	671	17
P1	27	0.038	0.147	4	0.524	116	5.7	5.83	0.506	2	2.53	976	4.86
P4	53	0.029	0.268	15	0.077	110	6.4	23	1.49	4	2.19	742	3.41
H1	68	0.035	0.659	19	1.1	123	20	35	1.58	14	2.07	632	4.96
H3	73	0.092	0.226	0	0.346	104	11.8	16	1.35	4	2.07	797	7.5
M1	98	0.085	0.236	3	0.532	112	5.9	18	1.86	3	2.5	698	8.84
M2	103	0.104	0.996	5	0.284	90	29	21	0.894	17	2.95	722	11

The PCA bi-plots constructed for low-flow and high-flow conditions are presented in Figure 14 and Figure 15. The results show a strong correlation between site E2 and Sodium, Barium, Manganese, Potassium, Calcium, Phosphorus and Titanium. The concentrations of Sodium and Potassium at this site were above average at site E2. The upstream and downstream sites within Pienaars River (P1 and P4) showed a strong association with Iron and Boron. This was also seen for both sites within Morelettaspruit (sites M1 and M2) and the downstream site in Hartbeesspruit (site H1). During the high-flow conditions, similar associations were seen in site E2 with the metals Sodium, Barium, Manganese, Potassium, Calcium, Phosphorus and Titanium. There was a strong association seen between H1 and Calcium. During high-flow conditions, site P1 and P4 was associated with Chromium.

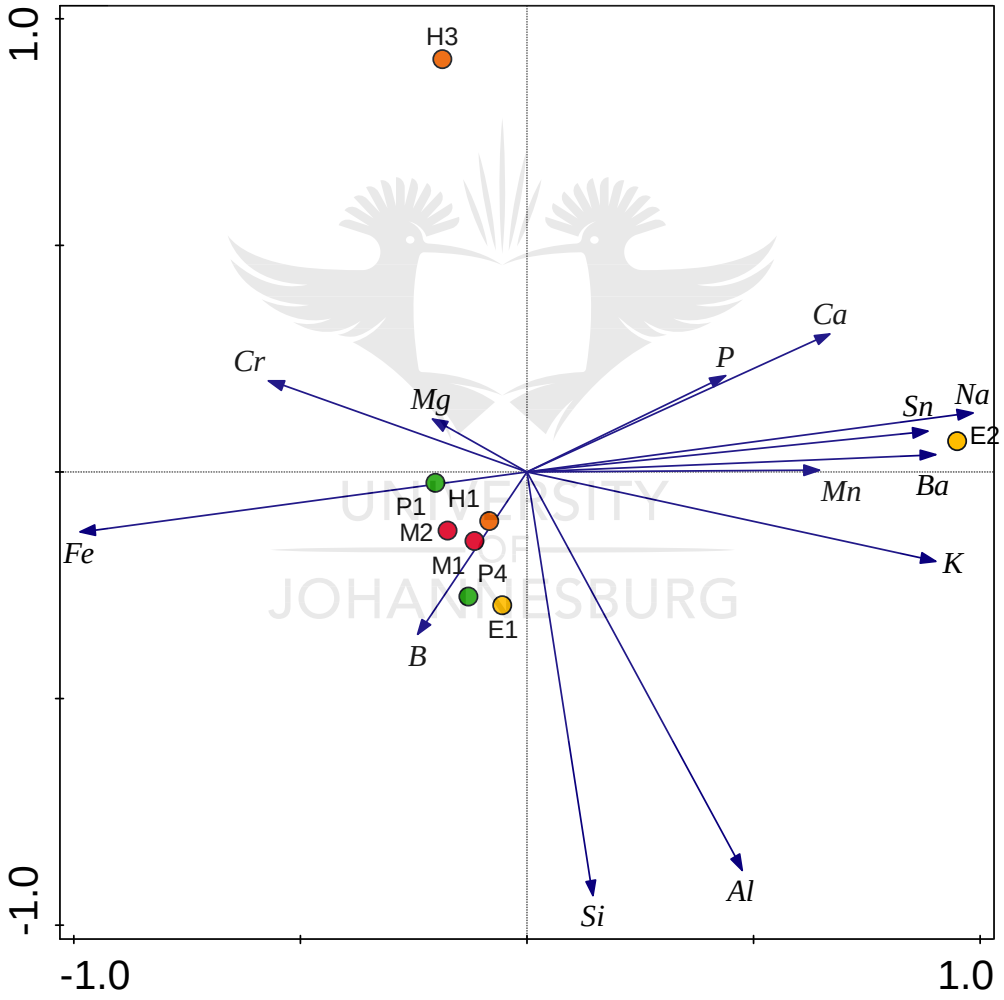

Figure 14: A principal component analysis (PCA) bi-plot showing sampling sites and metals measured in sediment samples during low-flow conditions at selected sites within Edendalespruit, Pienaars River Hartbeesspruit.and its tributary, Morelettaspruit.The bi-plot explains 86.87% of the total variance in data, with 68.24% of the variance explained on the first axis and 51.21% explained on the second axis

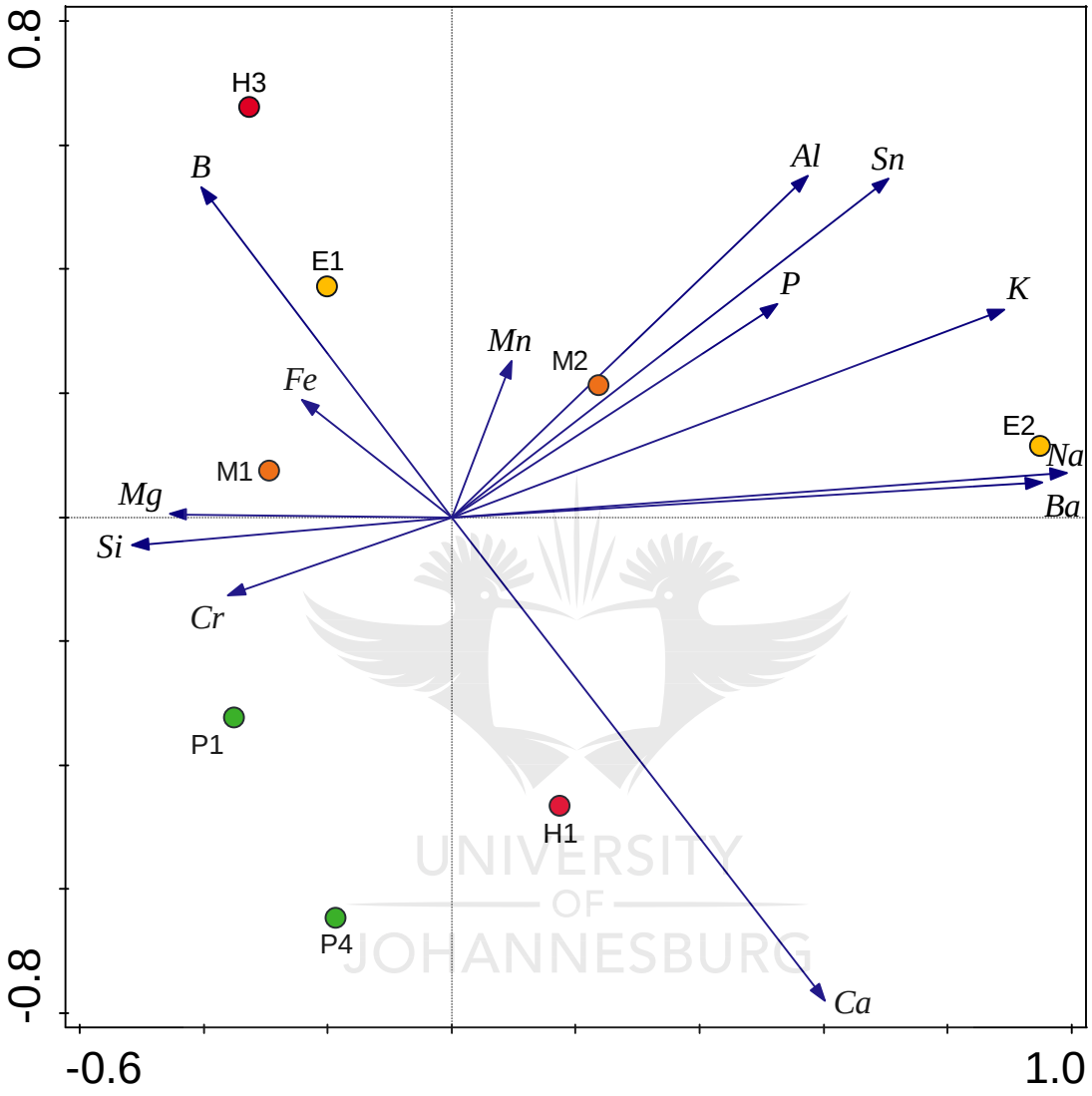

Figure 15: A principal component analysis (PCA) bi-plot showing sampling sites and metals measured in sediment samples during high-flow conditions at selected sites within Edendalespruit, Pienaars River Hartbeesspruit.and its tributary, Morelettaspruit. The bi-plot explains 77.83% of the total variance in data, with 59.28% of the variance explained on the first axis and 33.17% explained on the second axis

3.4. Discussion

The results showed that the sampled sites within the three inflowing rivers were indeed polluted to some extent with a variety of inorganic and organic pollutants. An in-depth review of the results will be discussed below focusing on each river separately.

The physical water quality parameters measured during both seasons at site E1 showed that this site had the highest pH levels, amongst all sampled sites. The pH is important because if it falls outside of the optimum range, it can have lethal effects on the aquatic life within the system. It can also affect the solubility and toxicity of heavy metals and chemicals in the water (Fondriest Environmental 2013). Although there is limited information in the DWAF Water Quality Guidelines for aquatic ecosystems on elevated pH levels and, although, it was fairly high, it still fell between the recommended pH of 6.0 - 9.0 (DWAF 1996, Fondriest Environmental 2013). Due to the high pH, the solubility of metals was expected to be more pronounced, however, this was not the case and most metals tested for fell within the target water quality guidelines (DWAF 1996). The conductivity and total dissolved solids was lower at site E1 when compared to site E2. However, the dissolved oxygen at the sites was similar. It is important to consider the fact that *in-situ* parameters were measured at different times, diurnal fluctuations in pH and oxygen levels have been observed in a number of studies (Morren and Morren 1841; Whitney 1942; Gobler *et al.* 2017). Many studies have shown that the oxygen concentration and pH levels are higher during the late afternoon (Butcher *et al.* 1927; Czezegy 1938).

Edendalespruit is situated downstream of Edendale Mine, which was one of the 7 major silver producing mines in Pretoria and operated between 1890 – 1938 (Glass 2006). It was therefore expected that sites sampled within this river would yield the highest levels of metal concentrations. However, as mentioned before, the metals tested fell within the DWAF target guidelines with the exception of Aluminium and Iron during low-flow conditions in the Edendalespruit downstream site (E1). The Aluminium target range for which no acute effects are in line with the Water Quality Guidelines (DWAF 1996) is 0 – 0.15 mg/L, the level at which there might be adverse effects is 0.15 – 0.5 mg/L. Any concentration above 0.5 mg/L may results in long-term neurotoxic effects if the water is being ingested by humans. These levels are also known to lead to discolouration of water in the presence of iron (the water at E1 was indeed discoloured during both sampling trips). Rondeau *et al.* (2008) conducted an eight-year study to determine if Aluminium in drinking water was a risk in Alzheimer's disease. For this study, 3777 subjects were involved (over the age of 65), 2698 non-demented subjects were analysed and after a test period of 8 years, 253 cases of dementia were identified, 182 of these cases were attributed to Alzheimer's disease. The Aluminium exposure was greater

than 0.1 mg/L and the results proved that Aluminium, specifically found in drinking water is a risk factor for Alzheimer's disease (Rondeau *et al*. 2008).

The target water quality range for Iron according to DWAF (1996) is 0 – 0.1 mg/L, 1 - 10mg/L will have aesthetic effects on the water (taste) and health effects might be seen in young children and sensitive individuals (DWAF 1996). The Iron levels measured at the Edendalespruit downstream site (E1) were 6.36 mg/L during the low-flow conditions. Iron is mainly used in construction materials such as drinking water pipes, and iron oxides are often used in paints and plastics (WHO 1996). The downstream site within Edendalespruit (E1) is situated in between two residential estates, and is surrounded by sparse vegetation, grasslands, fallow cropland and low-density housing (Figure 2.1). Elevated levels of Iron in surface water could also be a result of industrial effluent or domestic waste (Colter and Mahler 2006). Domestic waste generated by residents of the formal and informal houses surrounding this site could possibly lead to the increased Iron levels. Although there is no lethal effects observed in humans after exposure to elevated levels of Iron, elevated Iron levels are known to impact the histology of fish gills (Slaninova *et al*. 2014). The Magnesium levels seen in Edendalespruit (E2) were relatively high and above the target water quality guidelines for domestic water, but no health effects or odours should be observed at these levels, although slight scaling may occur (DWAF 1996). This site is surrounded by fallow cropland and grassland and located downstream of Mamelodi East. The high levels of Magnesium could be as a result of organic compounds supplied by wastewater from activities upstream (Potasznik 2015). This was confirmed by the bacterial analysis.

The nutrient and bacterial analysis showed high levels of faecal coliforms in the upstream site E2. These levels increased during high-flow conditions. The number of colonies per 100 mL increased during the high-flow conditions. This is due to the fact that the rates of biological and chemical processes are dependent on temperature, and although the temperature could not be measured, it is generally assumed that the water temperature is warmer during high-flow (summer) conditions. Higher temperatures also lead to lower oxygen levels which will directly affect the rate of photosynthesis of plants and metabolic rate of micro-organisms (DWAF 1996).

The ammonia levels were also highest during low-flow conditions and second highest during high-flow conditions. There was a strong positive correlation between faecal coliform bacteria and ammonia levels during both seasons. Since the faecal coliform bacteria are found in the faeces of mammals (warm-blooded animals), the levels of faecal coliform in a river is a reflection of the amount of sewage discharged into the river (Berg and Metcalfe 1978). The high levels at this particular site could be as a result of inadequate sewage facilities or as a

result of a lack of adequate sanitary structures in the informal settlement, there was also small-scale livestock farming observed upstream of this site. Faecal coliforms are labelled as indicator organisms as they indicate the presence of pathogens specifically associated with wastewater and / sludge (New Hampshire Department of Environmental Services 2003). Generally, faecal coliform counts are high if there are more than 200 colonies per 100 mL (Flint River Watershed coalition 2011) at this level they are more likely to have increased levels of pathogenic organisms within the water. This was the case for Edendalespruit upstream (site E2) during both seasons.

The microcystin results showed relatively low levels of microcystin-LR in the water of the respective rivers compared to levels detected in Roodeplaat Dam during previous studies. A previous study by van Ginkel *et al.* (2000) measured the microcystin–LR concentrations in the Pienaars River. Results from their study showed levels less than 3 µg/L. The study conducted by Mbiza (2014) in Roodeplaat Dam, showed the total microcystin concentration ranged between 0.3 – 2.5 µg/L between 2011 and 2012 (Mbiza 2014). Mbiza also indicated that in most cases, the concentrations were above 1.5 µg/L, which is above the highest guideline value according to the WHO (1999). These results were similar to the results seen in the Pienaars River during high-flow conditions in the current study (1.8 µg/L). In Roodeplaat Dam, van Ginkel *et al.* (2005) reported the total concentration to be > 10 µg/L from 2003 – 2004 (van Ginkel *et al.* 2005). However, a more recent study in the Roodeplaat Dam indicated alarmingly higher levels; Conradie and Bernard (2012) analysed the microcystin levels in the Roodeplaat Dam over a period of two years. Sampling took place from October 2004 to May 2005 and 70 samples were collected. The maximum concentration was 217 µg/L (Conradie and Bernard 2012). There were no SVOCs, phenols or chlorinated pesticides detected in the water samples from both sites within the Edendalespruit.

Four sites were sampled within the Pienaars River. The downstream site P1 had the highest conductivity during both seasons with the lowest dissolved oxygen and pH during low-flow conditions. Site P1 was situated downstream of the Baviaanskloof WWTP. Wastewater effluents as well as industrial effluents and municipal storm water drainage, usually contain high volumes of dissolved salts. This was confirmed in a study conducted by Morrison *et al.* (2001) who investigated the impacts of a Sewage Treatment Plant on the Keiskamma River. The study focused on pH, electrical conductivity, oxygen and nutrients at selected sites within the river. Samples were collected from three different sites to reflect the different land-uses and their impacts, influent samples were collected from one site at the sewage water plant, effluent samples were collected from discharge point of the sewage treatment plant before it reaches the wetland and the third site after the effluent site within the wetland area (Morrison *et al.* 2001). The site located at the WWTP, site P2, results were compared to the 1984 South

African guidelines (Government Gazette 1984). This showed that the pH in effluent was slightly higher than stipulated in the guidelines (5.5 -7.5). This was not the case for the conductivity, which ranged from 105.0 mS/m and 111.0 mS/m in influent and from 61.0 to 76.0 mS/m in effluent. This was below what was stipulated in the Government Gazette (250 mS/m) (Government Gazette 1984). In a study on a coastal creek in Brazil, the results showed that conductivity was higher downstream from the WWTP effluent discharge, proving that WWTP effluent indeed influences conductivity (Santos *et al.* 2008). The physical water quality parameters of sites upstream from site P2 (sites P3 and P4) fell within the stipulated target levels.

The metal analysis, however, showed that P2 had the highest Iron level (7.65 mg/L) during the high-flow conditions. Site P2 was also associated with Nickel, Aluminium, Manganese and Barium. Oliver and Cosgrove (1975) who also found a wide variety of metals in effluent from wastewater treatment plants in Ontario reported that these metals have a beneficial effect on the treatment system. This is due to the fact that domestic sewage contains essential metal micronutrients. However, problems can occur when the levels become too high. High levels of metals in the effluent can lead to toxicity, which will in turn decrease the efficiency of biological aerobic sewage treatment (Oliver and Cosgrove 1975). Although the levels were high, they were all within the target ranges as specified by DWAF (1996), with the exception of Iron during high-flow conditions.

According to the DWAF guidelines (1996), 1-10 mg/L of Iron will have aesthetic effects on water (taste) and health effects might be seen in young children and sensitive individuals (DWAF 1996). As mentioned previously, Iron is mainly used in construction materials such as drinking water pipes. Iron oxides are often used in paints and plastics (WHO 1996). There is a hardware warehouse 2.5 km away from site P2. In addition to this, there is also a steel and roofing manufacturing company, specialising in roof sheeting (galvanized, zincalume and chromadek), flashings, rainwater goods, zozo huts / garden sheets / steel huts, field toilets and light steel sections in close proximity (less than 2 km away) to the site. These surrounding activities are most likely to be the cause of the high levels of Iron detected at this site.

High levels of Magnesium were found at the site P3. This site is located on Solomon Mahlangu Road in the suburb of Nellmapius. The site also had the highest Calcium level. These two metals are both essential and usually occur together and are responsible for causing water hardness (DWAF 1996). Potasznik and Szymczyk (2015) conducted a study on the Calcium and Magnesium concentrations in surface waters of a river-lake system and found that the concentrations can be influenced by a number of factors including plant cover, soil class and type and geological structure of the catchment. Their study showed that the concentration

levels were also elevated by anthropogenic factors such as agricultural runoff and partially treated municipal wastewater (Potasznik and Szymczyk 2015). Site P2 is surrounded by fallow cropland and high-density housing that could possibly contribute to the elevated levels of Magnesium and Calcium.

There was a strong positive correlation with the faecal coliform bacteria and sites P1 and P2 within the Pienaars River. According to the DWAF guidelines (1996) faecal coliforms should not exceed 1000 coliform per unit (CPU) of 100 mL. The faecal coliform count was extremely high during the high-flow season (>100000) although it was within the limits during the low-flow conditions. The pH was slightly above the limits (5.5 – 7.5). During low-flow conditions it was 7.92 and 8.47 during high-flow conditions (DWAF 1996a). As seen in the study conducted by Kolawole *et al.* (2011), if the water is contaminated by faecal pollution it can have detrimental effects on humans or animals ingesting the water (Kolawole *et al.* 2011). This is due to the fact that ingestion can lead to gastrointestinal distress and waterborne diseases. Residents surrounding the Pienaars River site where fish were collected (P1) alluded that a fair amount of livestock (horses, pigs, dogs etc.) have died after ingesting the water in the river (Personal communication February 2016). The faecal coliform bacteria levels were alarmingly high at this site during both seasons.

Faecal contamination within the rivers can be caused by wildlife, wastewater treatment plants or farming activities. However, the Pienaars River downstream site is in close proximity to the Baviaanspoort wastewater treatment plant which discharges directly into the Pienaars River (at P2). The levels of wastewater constituents are lower in P2 when compared to P1 further downstream which suggests that measures are being put into place to ensure that the discharged water is treated effectively. The contamination at the Pienaars downstream site (P1) could be caused by other surrounding land-uses. The levels for wastewater treatment plant discharge is stipulated by the Department of Water Affairs (2010) in the National Water Act guidelines and it states that the oxygen demand levels should > 65 mg/L, the free and saline ammonia <1.0 mg/L, Nitrate should not exceed 15 mg/L, Total Aluminium > 0.03 mg/L , Total Boron <0.5 mg/L, Cadmium >0.0001 mg/L, Copper >0.002 mg/L, Iron >0.3 mg/L, Lead <0.009 mg/L, Mercury <0.001 mg/L, Selenium <0.008 mg/L, Zinc <0.05 mg/L (Republic of South Africa 1998b).

According to these thresholds, the Iron levels were higher than the acceptable values during the low-flow conditions (0.797 mg/L) and even higher during the high-flow conditions (7.72 mg/L), the Aluminium was also above the limits during the high-flow conditions (1.06 mg/L). The Phosphorus levels in P2 were below the limit, this could be as a result of the implementation of the Phosphate standard in 1998 at the Baviaanspoort and Zeekoegat Water

Care Works (WCW). This was introduced due to the fact that these WCWs were not complying with the standards and because Phosphorus is easier to manage than any other nutrient. This was implemented by Hohls *et al.* (1998) as part of the Eutrophication Management Plan for Roodeplaat Dam.

In Hartbeesspruit, three sites were sampled one of which was a downstream sampling site where fish was collected. The H2 site (middle reaches), which is surrounded by low-density housing and sparse vegetation, had the highest conductivity and total dissolved solids with the lowest pH and dissolved oxygen during the low-flow conditions. This site also had the highest pH during the high-flow. This was also observed in the upstream site H3 during the high-flow conditions. High conductivity and low dissolved oxygen have been seen in a number of studies conducted on urban streams (Almeida *et al*. 2007; Aweng *et. al.* 2011; Nagy *et al.* 2012). The lower dissolved oxygen in urban streams is caused by changes in stream precipitated by urbanizations, whilst, higher conductivity is as a result of increased density of roads (Walsh *et al.* 2005; Herringshaw *et al.* 2011 Morgan *et al.* 2012; Wu *et al.* 2015). Although the metals at both these sites were below target levels, high levels were observed in Calcium, Potassium, Magnesium, Sodium and Silicone levels which might have also attributed to the high conductivity and TDS.

The H3 site which is surrounded by low-and high-density housing had the highest dissolved oxygen amongst all sampled sites during the low-flow conditions, however it still fell within the DWAF target water quality guidelines. According to the water quality guidelines the Target Water Quality Range (TWQR) for aquatic ecosystems is between 80 – 120 % of saturation. The nutrient and bacterial analyses revealed high levels of faecal coliform bacteria, however, this was not higher than that observed at Edendalespruit and Pienaars River. The downstream site within Hartbeesspruit (H1) however, had the highest levels of nitrite, nitrate and Kjeldahl nitrogen during high-flow conditions. Elevated levels of nitrite, nitrate and Kjeldahl nitrogen are usually associated with human impacts (Dubrovsky *et al.* 2010). This site is surrounded by low-density housing, grasslands and sparse vegetation. The site is on the banks of The Blades function venue which claims to be an environmentally friendly venue that does water collection as well as sewage water treatment and have their own organic and herb gardening (Personal communication February 2016). High levels of nitrites and nitrates could be as a result of human inputs such as effluent from treated wastewater from municipal or industrial waste, on-site septic systems, fertilizer and precipitation (Wall 2013). Thus, if The Blades recycles their water back into the river this could possibly explain the results seen. All metals found during both seasons in all three sites were within the normal ranges specified. Interestingly, the sites within Hartbeesspruit and its tributary (Morelettaspruit) were closely associated in terms of

nutrient and bacterial analysis, grouping together during both seasons proving a strong positive correlation between sites.

Morelettaspruit which is a tributary of the Hartbeesspruit had two sampling sites, one upstream and one downstream and there was no fish sampling conducted in this river, in terms of the physical parameters M2 had the lowest pH during the high-flow season. The site was situated within the Derdepoort Nature Reserve and there were gabions on the banks of the river. All metals found during both seasons in both sites were within the normal ranges specified.

Physical characteristics measured for sediment included grain size analysis, soil moisture content and organic content determination. It is important to determine the size of the sediment particles in order to understand the source, transportation and environmental impact of the sediment. The grain sizes differed considerably between sites, rivers and seasons. This might be due to the sampling technique and the fact that sediment was not collected at the same depth during each season due to changes in the flow. The E1 and E2 site was mainly comprised of medium sand with a high organic content which could be the possible explanation for the high levels of metals observed particularly in site E2. According to Rieuwerts *et al.* (2015), factors that influence the bioavailability of metals in sediment are Redox potential, pH, soil texture, clay content and organic matter content (Rieuwerts *et al.* 1998).

The upstream site within the Pienaars River (P4) had the highest amounts of very fine sediment during both seasons and site P1 (downstream) had the highest levels of moisture in sediment during the low-flow as well as the highest organic content during both seasons. The upstream site, site P4 had the lowest organic content. The organic matter of soils also has a direct influence on the formation of sediment structure; this means that it also influences the reactivity, chemical and biological composition (Tisdall and Oades 1982). High levels of organic matter can be used as an indicator of stress, “an overabundance can cause reductions in species richness, abundance, and biomass due to oxygen depletion and build-up of toxic by-products (ammonia and sulphide). This is usually associated with the breakdown of these materials” (Hyland *et al.* 2005). The downstream sites, P1 had P2 (middle reaches) had the highest organic content during both sampling seasons and the reduction in the abundance of macro-invertebrates is very clear, the site had the lowest SASS5 score during the low-flow and amongst the least amount of taxa during the high-flow.

Sediment chemical sample analysis included metal analysis, sites within Edendalespruit had amongst the highest values for Aluminium, Boron, Calcium, Iron, Potassium, Magnesium, Sodium, Phosphorus, Selenium and Titanium. Heavy metals are easily absorbed into sediments which are a sink and secondary source of contamination for aquatic biota. This type

of accumulation is influenced by the pH of the water, concentration of the metals, the chemistry of the river and anthropogenic effects (Edokpayi *et al.* 2016). The concentration of the majority of metals above detection limits (Aluminium, Copper, Iron, Potassium, Silicon and Titanium) in both sediment and water was higher in sediment than in water which is also mainly due to the fact that sediment acts as a natural sink for pollutants (Chapman 1992; Bervoets and Blust 2003).

There are no guidelines developed yet in South Africa for metal concentrations in freshwater sediment, however, a report was written in 2010 by Gordon and Muller in Phase 1 of developing sediment quality guidelines for South Africa. The report specifically focused on the identification of international best practice and applications for South Africa to develop a research and implementation framework. Internationally used approaches were considered; one of which was the Canadian Environmental Quality Guidelines by the Canadian Council of Ministers of the Environment (CCME 2001). Therefore, for the purpose of this study, these guidelines were used to determine acceptable levels of metals concentrations in sediment.

The Iron levels were high at the M1 site during low-flow and even higher during the high-flow at H3. Site M2 had the highest levels of Barium, Copper, Gallium and Lithium during low-flow conditions. During this season, the sediment was mostly comprised of mud with medium organic content. The concentration for Copper in sediment ranged from 5 to 70 mg/kg, however, this value is found to be elevated in sediment near smelters, mining operations, and combustion sources (ATSDR 2004). This site is located downstream of AgriSam Ferro which is a quarry, process crusher, building, garden sand and cement supplier. According to Ashmole and Motloung (2008), the impact of stone mining on the environment should be low. There are no emissions besides those of the diesel-powered earthmoving equipment utilised in its extraction and a small amount of blasting gases. The Gallium concentration in the sediment was high compared to other sites but it still fell within the guideline value of 36 mg/L (Kabata-Pendias 2001). It is usually associated with clay soils (Kabata-Pendias 2001) and site M2 was composed mainly of mud during the low-flow season. The Iron levels were high during the low-flow season at site M1 and during the high-flow season at site H3. This metal is usually found at higher concentrations in water and sediment than any other trace metals (second most abundant metal in the current study). This is due to the fact that it is highly abundant in the earth's crust and found everywhere in aquatic environments (Vuori 1995).

Silicone concentration was the highest when compared to the other metals. During high-flow conditions, the concentration was highest at the site P1. This site was mostly comprised of medium sand during the high-flow season which could explain the high concentrations. There is a silica mine upstream from this site. The Delf Sand Mine is an opencast which specializes

in the mining and beneficiation of an alluvial sand deposit. The mine is located at Donkerhoek near Cullinan and mines the alluvial deposit mainly because of its high silica content (SiO_2 greater than 96%). One of the by-products of the opencast mining is the recovery of high quality plaster sand. The mine is a leading supplier of high content silica sand, this might contribute to the high levels of Silicon at the site.

The sediment organic analysis detected different levels of SVOCs at various sites. These SVOCs were not found in the water samples of the respective sites, only in the sediment samples. PAH concentrations in water are relatively low when compared to concentrations in the bottom sediments due to the fact that they are hydrophobic and therefore when they enter the aquatic environments tend to have a higher affinity for suspended particulates, thus, causing these particles to settle out of the water column into the bottom sediments. (Moore and Ramamoorthy 1984). Naphthalene was only found at the upstream Morelettaspruit site (M2). This is a polycyclic aromatic compound produced by coal, petroleum or tar and used in moth repellents, plastic beer bottles and pesticides. Naphthalene in the environment comes from crude oil, coal tar and forest fires due to the fact that it's a natural combustion product of wood. Humans are mainly exposed to it by means of inhalation through tobacco smoke or pesticides, and very rarely by ingestion (Mason 1995). This site, as mentioned before, was situated within the Derdepoort Resort and in very close proximity to the R513 a very busy main road in Pretoria and since the presence of this PAH is caused by crude oil (petroleum) in the environment, its presence at this site could be due to the high number of vehicles which pass this site every day. According to Environmental Agency (2004) the guideline values for the occurrence of Naphthalene in sediment is based on the percentage of organic content (in this case the percentage is 2.3%) and the surrounding land use. Therefore, for site M2, the guideline value would be 17 mg/kg as it is located within a residential area. The levels detected in the sediment were far lower than this (23 µg/kg). Naphthalene was only found during low-flow conditions. This might be related to the fact that it degrades extensively in soil under aerobic conditions according to the Environmental Agency (2003) (the low-flow conditions dissolved oxygen measurement was 8.94 mg/L). A variety of other PAHs were found during the low-flow season at site M2 which is not unusual, as naphthalene rarely occurs in isolation, this is also known to influence the behaviour (Environmental Agency 2003).

Phenanthrene was found during low-flow conditions at five (E1, P3, M2, F2 and F3) of the seven sites sampled, during the high-flow at five sites (P3, H2, F1, F2 and F3). Morelettaspruit upstream (M2) had the highest levels during low-flow conditions and the downstream site within the Hartbeesspruit had the lowest levels however, during high-flow conditions this site had the highest levels while the E1 site within the Edendalespruit had the lowest levels. This particular PAH occurs naturally in the environment or from anthropogenic sources such as

coal, oil and gas. It is commonly used to manufacture dyes, plastics, pesticides and drugs as well as cholesterol and steroids (ATSDR 1990). The impacts of Phenanthrene were investigated in *Clarias gariepinus* to determine the biomarker responses (Karami *et al.* 2016). They exposed fish to different levels of Phenanthrene (10 and 100 µg/l) for 96 hours and results showed significant changes in the liver as well as brain tissue of the fish. The Hartbeesspruit downstream site (H1) as mentioned before is situated at The Blades function venue. The venue claims to be an environmentally friendly venue that does water collection sewage and water treatment as well as organic and herb gardening (Personal communication). Raw untreated sewage usually contains Pyrene, Phenanthrene and Flouranthrene (Gonzáleza *et al*. 2012), these PAHs were all coincidentally detected at the Hartbeesspruit downstream site (H1) during high-flow conditions, and the concentrations were also considerably high in comparison to the other sites. In treated wastewater these PAHs will be below detection limits (Gonzáleza *et al*. 2012). The Edendalespruit downstream site (E1) is situated within a private residential estate, there is also an art and paint shop located in close proximity to the site where the samples were taken, Phenanthrene is furthermore used in the formulation of printing inks and paints (Weissermel and Arper 1997).

Fluoranthene is a known co-carcinogen, which is a non-carcinogenic chemical of which the carcinogenicity is enhanced by the presence of other carcinogenic chemicals (Irwin 1997). It is often found in fossil fuels and during the combustion of organic matter. It is absorbed by sediment and bioaccumulate in aquatic organisms (Irwin 1997). The upstream site within Morelettaspruit, site M2, had the highest levels during the low-flow conditions. However, there was no Flouranthene detected during the high-flow season. Within the Hartbeesspruit site H1 had the highest levels. The upstream site within the Hartbeesspruit (H3) had the second highest level during the high-flow conditions. There is a lodge (south/north/east/west side of the river) situated on the banks of site H2 where the samples were collected. There are also agricultural activities taking place on the opposite the river. It is important to mention that a small drain was observed located at a bridge that cross the river which was leaking water during both sampling trips. The water was green and foul smelling. Similar to the downstream Hartbeesspruit site (H1), there were high levels of other PAHs (Pyrene, Phenanthrene and Flouranthene, commonly found in untreated sewage), also detected at this site during the high-flow conditions.

Crysene was detected during low-flow conditions at site E2 (upstream), site P1 (downstream), site P3 (middle reaches), site M2 (upstream), sites H1 and at H3 (downstream and upstream respectively). The highest levels were found at site M2 during the low-flow season and site H1 during the high-flow season. Site M2, however, flows towards site H1 (it is located upstream of this site). Crysene is usually formed when petrol, plant and animal matter or waste burns. It

then mixes with dust in the air and is carried into the water and eventually the sediment. Literature shows that high levels of Crysene could be attributed to high volumes of traffic in the area (Banger *et al.* 2010). As mentioned, site M2 is located below the R513.

Anthracene was detected during the low-flow season at sites M2 (upstream) and P3 (middle reaches), however, it was not detected at any sites during the high-flow conditions. Anthracene is a solid organic compound that is insoluble in water and commonly used in insecticides, coating material as well as wood preservatives (McMurry 2004). Benzo[k+b]fluoranthen, Benzo[a]pyrene and Benzo[a]anthracene were all detected during the low-flow season at site M2, however, during the high-flow conditions it was only detected at site H1. The possible explanation for this is that site H1 is located downstream of site M2. The rainy season could possibly have been responsible for the transportation of sediment particles downstream. Each site where Anthracene was detected was mainly composed of mud/clay (M2 and M3) or very fine sand (H1). Yang *et al.* (2011) conducted a study on the Weihe River sediment to determine the levels of Anthracene absorption and found similar results, and the absorption rate in finer particles was 98 – 99%. This is mainly due to the fact that finer sediment particles have a high absorption capacity for Anthracene (Yang *et al.* 2011).

Bis(2-ethylhexyl)phthalate also known as DHEP, is used in the manufacturing of Polyvinyl Chloride (PVC). This Phthalate was detected at sites H2 and P4 during the low-flow season and at site F3 during the high-flow season. DHEP has also been used in paint, printing inks, dyes, sealants, lacquers and rubbers. The land use activities in close proximity to site P4 include a small aerodrome, a few farming properties, a military training base and wedding venues. A few plastic manufacturing companies have been identified upstream of both sites M2 and P3. This could possibly be contributing to the levels of DHEP in the water.

3.5. Conclusion

The results showed evidence of pollution in the three rivers, although no semi-volatile organics was found above the detection limit. These chemicals were present in the sediment implying that they may have been present in the water at some point. The high levels of faecal coliforms at most sites indicate ineffective wastewater treatment mechanisms, uncontrolled agricultural practices and possibly poor sanitation practices. The downstream site within the Pienaars River had the poorest water quality results overall. This is possibly due to the anthropogenic activities upstream in Mamelodi which includes ineffective sewage systems as well as the runoff from the wastewater treatment plant. Each site was unique in its physical sediment characteristics and this could be the reason why the accumulation of pollutants differed at each site. Interestingly, the highest levels of pollutants were recorded at the upstream sites of Edendalespruit, Hartbeesspruit and Morelettaspruit. This was also mainly due to the

surrounding land-use activities which includes formal and informal housing, mining, agricultural practices, as well as a variety of industries. It would be recommended that measures be put in place to minimize the effects of these activities in order to improve the water and sediment quality of the system.

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Chapter 4: Aquatic macro-invertebrates

4.1. Introduction and background information

According to Gerhardt (2011) "*bio-indicators are organisms or communities of organisms which reactions are observed representatively to evaluate a situation, giving clues for the condition of the whole ecosystem*". There are a variety of bio-indicator organisms that can be used for bio-monitoring but the most commonly used organisms are periphyton, fish and macro-invertebrates (Li *et al.* 2010). Aquatic macro-invertebrates are consumers in an aquatic system and feed on bacteria, algae and detritus matter (Lamberti & Resh 1983, Vanni 2002, Díaz Villanueva *et al.* 2012). These organisms form a fundamental part of any ecosystem because they have a moderate intolerance to environmental variability and react strongly to human impacts on aquatic ecosystems (Holt and Miller 2011; Gerhardt 2011).

Macro-invertebrates can give an indication of ecosystem conditions as they occupy the same habitat for a reasonable amount of time, they form a vital part of the river ecosystem as they aid in purifying the water, and they process transported organic matter and serve as a valuable food source for larger aquatic organisms (Carr and Neary 2008, Weber *et al* 2004, O' Keeffe and Dickens 2000, Allan 1995, Skorozjewski and de Moor 1999). Any changes in the aquatic environment will show a difference in the macro-invertebrate family assemblage on both a spatial and temporal scale (Carr and Neary 2008). In addition, benthic invertebrates are recognised widely as significant organisms for bio-assessments as they are visible to the naked eye and therefore easily identified, they also have a very rapid lifecycle (Dickens and Graham 2002). The use of macro-invertebrates in water quality monitoring tends to be cheaper than physical and chemical methods and is often very useful when trying to determine the disturbance events after they have occurred as well as locating pollution sources and reflecting changes in the environment in an integrable manner (Hellawell 1986, Resh *et al.* 1995). Another bio-indicator system which can be used in conjunction with macro-invertebrate diversity indices include saprobic systems and diversity systems. Saprobic systems are based on four zones of gradual self-purification namely; the polysaprobic zone, the α-mesosaprobic zone, the β-mesosaprobic zone, and the lastly; oligosaprobic zone. Each zone is characterised by indicator species, certain chemical conditions as well as the general nature of the bottom of the water body (Friedrich *et al.* 1996).

Various studies around the world have shown the effectiveness of employing macro-invertebrates as bio-indicators. Gratwicke Sharma and Chowdhary (2011) conducted a study on biological indicators of pollution in a central Himalayan river, Tawi. Macro-invertebrates

were used as the bio-indicators and the results showed that there was a significant difference in the macro-invertebrate assemblages caused by the changes in water quality. The study suggested that macro-invertebrates should be employed as biological indicators in the long-term monitoring of the river (Sharma and Chowdary 2011). This was confirmed by Kripa *et al.* (2012) who, in Korraty, India, assessed the suitability of aquatic macro-invertebrates as bio-indicators of river water quality. Their methodology included the use of the Family Biotic Index (FBI), which uses pollution tolerance levels of different taxa. The results showed a deterioration of water quality downstream and good water quality upstream. This was determined by evaluating the abundance and diversity of macro-invertebrate families (Kripa *et al.* 2012). Bae *et al*. (2005) conducted a similar study by using benthic macro-invertebrates in stream monitoring and restoration. The study focused on various case studies within the Han River system in Korea and found that these organisms are very effective in assessing stream restoration and environmental engineering (Bae *et al.* 2005). This was due to the fact that they provide useful information on the suitability of habitats and environmental conditions (Bae *et al.* 2005). The presence of aquatic macro-invertebrates is also associated with the general ecological status of a river as seen in a study conducted in the Erzeni River, Albania. The study confirmed that good water quality was associated with an increased abundance of macro-invertebrates (Keçi *et al*. 2012).

It should be noted that sampling techniques employed in different countries differ only slightly; the principles, results and conclusions are often very similar. The use of aquatic macro-invertebrates in the European countries, with the aim to detect changes in the community structure due to the effects of pollution, is commonly employed based on their tolerance levels (Anagnostopoulou *et al.,* 1994). In Canada, the Canadian Aquatic Biomonitoring Network (CABIN) is used as the national biomonitoring programme. It employs standardized sampling and incorporates the Reference Condition Approach (RCA). Benthic macro-invertebrates are collected and biological assessments are conducted based on their presence, absence and relative abundance (Carter and Pappas 2012). The BMWP (bio-monitoring working party) was established in the UK by Armitage *et al.* (1983). This system evaluates the sensitivity of the macro-invertebrates and assigns a score of 1-10 (10 being most sensitive), where after an Average Score Per Taxa (ASPT) is calculated and the higher the ASPT score, the cleaner the stream (Mason 2002, Armitage *et al*., 1983). This system has also been successfully applied in Brazil, Thailand, Turkey, Spain, Canada, Argentina and Poland (Barton and Metcalfe-Smith 1992, Zamora-Munoz and Alba-Tercedor 1996, Capitulo *et al*. 2001, Mustow, 2002, Czerniawska-Kusza 2005; Zeybek *et al.* 2014; Rodrigues *et al.* 2016). This type of bio-monitoring is a basic, rapid way of assessing the quality of the water and as mentioned before is relatively cheap when compared to other methods such as chemical analysis. In England,

The Trent Biotic Index was developed by Woodiwiss (1964) to assess the pollution in the River Trent. This particular index forms the foundation for many similar types of indices, it is based on the number of defined taxa of benthic invertebrates in relation to the presence of six key organisms sampled at a particular site (Woodiwiss 1964). Studies in other parts of the world have modified the system according to the presence (and abundance) or absence of macro-invertebrates in their specific rivers. These countries did not alter the way the biotopes (also described as a habitat associated with a certain ecological community) were classified, but just simplified the system to make it easier to use within their country (Mustow 2002).

In South Africa, and most parts of Southern Africa, the River Health Program (RHP) was employed and has since been changed into the River Eco-status Monitoring Programme (REMP) in 2016. It forms part of the National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) (DWS 2016). This programme is entirely focused on assessing biological characteristics to determine the health status of rivers. This includes fish and macro-invertebrate communities as well as instream and riparian habitats. The rationale for this programme is that chemical and physical monitoring on its own cannot provide a precise account of the overall condition of an aquatic system (DWAF 2008)

The South African Scoring System 4 (SASS4) method was developed in 1994 by Chutter for macro-invertebrate assessments in river systems. The method has since been refined to version 5 by Dickens and Graham (2002) and is now known as SASS5. This method has been accredited to ISO standards and changes were made to the list of macro-invertebrates included in the identification sheet (Dickens and Graham 2002). For this method, a score for each sampling site in a river is calculated by using the Average Score Per Taxon (ASPT) (based on the sensitivity or tolerance score for each identified taxon), and the number of taxa obtained after sampling from different biotopes including gravel, sand and mud (GSM), stones and vegetation. The SASS5 scoring system formed the backbone of the National River Health Programme and has been recommended to determine flow requirement for a variety of rivers and is also extensively utilised in impact assessments (Dickens and Graham 1998).

4.1.1. The South African Scoring System Version 5

The SASS 5 method was specifically designed for aquatic systems with low-flow hydrology such as rivers and cannot be applied in wetlands, estuaries or impoundments (Dickens and Graham 2002). The SASS5 system is the only standard method available in Africa, however, it is not flawless and challenges have been highlighted by some researchers. In the Western Cape of South Africa, macro-invertebrates were used as bio-indicators to determine their effectiveness in assessing the water quality in the Kleinplaas Dam on the Eerste River (Sweeney 1993). The study showed that although SASS5 is an effective means of determining

the water quality by assessing the macro-invertebrates, it requires some adjustments in terms of including a prediction phase, finer spatial- scale methodologies and greater consideration for the rarity of species. The study took place over two years and showed that the diversity of macro-invertebrates is affected by lack of habitat, sunlight and an increase in salinity which would explain why certain macro-invertebrates are only found during the warmer seasons. During the cooler months, the macro-invertebrates had a decreased metabolic rate which would ultimately affect their reproduction rate, thus decreasing the number of macro-invertebrates when compared to the summer months (Sweeney 1993). Farrell's study on the Wilge River, focusing on seasonal differences between high-flow and low-flow conditions, yielded similar results, implying that seasonal variation was the driving variable in terms of macro-invertebrate diversity variations (Farrell 2014).

In 2012, Odume conducted a study to evaluate macro-invertebrate monitoring along with other eco-toxicological assessments with the focus on the poor environmental water quality in the Swartkops River, located in the Eastern Cape of South Africa. Many urban and industrial areas drain into the river including wastewater effluent discharges, run-off from informal settlements, agricultural lands, surrounding rail and road networks and industrial sites (Odume 2014). The study focused on ecotoxicology, water chemistry, taxonomic and trait-based approaches as well as deformity screening of the macro-invertebrates (Odume 2014). The Chironomidae family was studied in depth and the author focused on their responses to deteriorating water quality and anthropogenic impacts. It was found that the variance in the amount of macro-invertebrates sampled was caused mainly by the effect of the effluent discharged into the river, with the different sampling times having a very small effect on the results (Odume 2014). The study was conducted over a period of 3 years (2009 – 2012) during spring, summer, autumn and winter. Mothololo (2014) did a similar study in Qwa-Qwa in the Free State and found no seasonal variation pattern within the rivers sampled. The ASPT for each river differed and this could be attributed to the localities, geographic factors and anthropogenic effects (Mothololo 2014). Although these studies show contradictory findings regarding seasonal variability, it is still important to include seasonal sampling due to certain factors which are dependent on changing temperatures. These include macro-invertebrate family composition and abundance as well as growth stages, and habitat availability. The latter is an important determining factor in macro-invertebrate assemblages (Sweeney 1993; Cummins *et al.* 2005).

The effect of habitat availability on macro-invertebrate assemblages was shown by Mahlangu (2013) following a study on the Klip River system in Gauteng, South Africa. The ecological status of the system was determined by using various biomonitoring techniques which included fish health and macro-invertebrate biomonitoring. The results of Mahlangu's study

showed low SASS5 and ASPT scores co-incided with the poor water quality. Interestingly, there were no major differences in the community structures of the reference sites (upstream) and other sites further downstream. The reference site, however, had the highest SASS5 and ASPT score. The deterioration in SASS5 and ASPT reflected an alteration in the habitat and anthropogenic activities (Mahlangu 2013).

Ogbeibu and Oribarbor conducted a study in South Nigeria during 2001. Similar to the current study they also investigated three rivers using the upstream, middle reaches and downstream of each river to collect macro-invertebrate samples. Their results showed that the total number of macro-invertebrate taxa was higher at the upstream sites and that river management should be implemented to lessen the impact on the downstream ecology. The researchers used the upstream sites of each river as the ecological reference condition. This viewpoint is also stressed by Dallas (2000) when using the River Health Programme for riverine macro-invertebrates. According to Reynoldson *et al*. (1997) "*The ecological reference condition is the condition representative of a group of the least impacted sites organised by physical, chemical and biological characteristics"*. This is the main reason why for each river assessed in the current study, an upstream site was selected in addition to sites further downstream. Similar trends were reflected in a study done by Mudyezhezha and Ngoshi in Zimbabwe in 2013. The reference site had the highest number of pollution-sensitive families and the downstream sites had the highest numbers of pollution-insensitive families.

There are, two main ways to perform macro-invertebrate assessments, taxonomically and functionally. The taxonomy is the most common way and focuses specifically on the species richness and diversity (e.g. SASS5). This is mainly done to determine sensitivity of species and how they react to changes in water quality (Cummins *et al.* 2005). The other approach incorporates functional feeding groups whereby each taxon is placed in a different feeding group based on their manner of obtaining food as well as the particle size of the food. However, it does not reflect the type of food they eat (Cummins *et al.* 2005).

4.1.2. Functional feeding groups

By assessing functional feeding groups (FFGs), the focus is placed on macro-invertebrates based on their food acquisition behavioural patterns as opposed to their taxonomy (De Moor 1988; McShaffery and McCafferty 1986, 1990). In an aquatic ecosystem, it is expected that a number of different feeding groups co-exist in the same space, occupying the habitat in different proportions (Garmito and Furtado 2009). Due to the fact that functional feeding group compositions are dependent on the morphology and behaviour of species in relation to their manner of obtaining food, this type of assessment can provide important information about the impact of land-uses and ecosystem conditions. For example, agriculture will result in an

increase in phosphate and nitrate run-off which may lead to increased biological activity in the surface waters resulting in algal blooms and an increase in the abundance of grazers/scrapers (Cummins *et al.* 2005). The method considers the basis of food chains and the significance of autrophy and heterophy therein (Merrit *et al.*2002). There are five functional feeding groups and these are presented in Table 17 (Cummins and Wiltzbach 1985; Merrit and Cummins 1996)

Table 18: Functional feeding groups adapted from Cummins and Wiltzbach (1985); Merrit and Cummins (1996)

Scrapers/Grazers *(consume algae and associated material)* E.g. Mayfly larvae, cased caddisfly larvae, pond snail, river limpet	**Shredders** *(which consume leaf litter or other coarse particulate organic matter including wood)* E.g. Freshwater shrimp, the smaller stonefly larvae
Collector-gatherers (*which collect fine particulate organic matter from the stream bottom)* E.g. mayflies, midge larvae, worms	**Collector-filters** (*which collect fine particulate organic matter from the water column using a variety of filters)* E.g. Black fly larvae and net-spinning caseless caddisfly larvae
Predators (*feed on other consumers)* E.g. Dragonfly larvae, damselfly larvae, large stonefly larvae and alderfly larvae	**Others** *(Includes species that are omnivores, or simply do not fit neatly into the other categories)*

Studies have been done to show the effectiveness of FFGs in determining the impact of surrounding land-use activities of a particular river site. Cummins *et al.* (2005) used functional feeding groups in streams and rivers in south Brazil to characterize ecosystem attributes such as the balance between autotrophy (organisms which are able to make their own food) and heterotrophy (organisms which eat other organisms). It also shows the link between different sizes of organic matter and food webs commonly found in-stream. Their study thoroughly explained the different FFGs and focused on different ecosystem parameters. It also interpreted whether organisms were heterotrophic or autotrophic and how useful the FFG method is in terms of evaluating the ecosystem condition. It was concluded that with appropriate and thorough data collection, taking the different seasons into consideration, this method could be extremely suitable when attempting to evaluate the trends in land-use changes in a particular place over a certain number of years. They found that all of the sites sampled were heterotrophic. The majority of the sites had the high levels of suspended organic load as expected. However, the linkage between heterotrophic FFGs with riparian vegetation was lower than expected (Cummins *et al.* 2005). Similar studies however, yielded different results.

Fu *et al.* (2015) conducted a study to determine whether land-use and environmental factors impact the FFGs in Southeast China. The study took place during the low-flow conditions in January 2013 and over 70 sites were sampled. The representative sites for each type of land-use was determined by using geographic information systems (GIS). The collector-gatherers were the dominant FFGs in sites where land-uses such as agriculture and urbanisation were more prominent. There was, conversely, no consistency in the predator distribution in different land-uses (Fu *et al.* 2015). This was confirmed by Farrell (2014), in a study on the association between water quality and the aquatic macro-invertebrates communities in the Wilge River, near the town of Leandra. The study focused on the present in the system taking into consideration their FFGs. Seven sites were monitored and historical data from 2010 - 2013 was used in the study. Sampling was done during both the wet and dry seasons. The results showed that the system was dominated by gathering collectors. This made sense as all habitats sampled had gravel, sand and mud (GSM) sediment. Since agricultural activities are the most dominant land use in the area, which would possibly lead to high levels of nutrients within the river and ultimately eutrophication, the gathering collectors FFG was expected. It was concluded that stressors can co-occur and that it is difficult to attribute biotic communities to certain individual stressors or even specific combinations of them (Farrell 2014).

Walsh (2008) studied specifically the effects of macro-invertebrate community structure responses in agriculturally impacted rivers and considered how this differed when compared to a river with other type of land-uses. The results showed that the agriculturally-impacted sites were dominated by collector-gatherers and collector-filters. This was similar to Farrell's study (2014). There were no other trends in terms of land-uses and FFGs. The study conducted by Ferreira (2008) in the lower Harts/Vaal system which aimed to determine the impact of land-use activities on biological communities (namely; diatoms and macro-invertebrates) and riparian communities, showed that for this particular study, the FFGs could not be used to distinguish different land-uses. However, it proved that fine particulate organic matter was the most abundant food source and that collector-filters, collector-gatherers, predators and scrapers were the most dominant FFG groups (Ferreira 2008). These studies collectively show both the effectiveness and limitations associated with using FFGs to determine the impact between different land-uses. Macro-invertebrates were specifically grouped into different FFGs in the current study to determine whether the impact of land-uses can be determined using this method.

4.1.3. Effects of eutrophication on aquatic macro-invertebrates

As shown in Chapter 3 (Table 7 and Table 8) the rivers flowing into Roodeplaat Dam are in a eutrophic state. Eutrophication affect aquatic biota both negatively and positively. When

nutrient levels increase, there is a visible decrease in the pollution-sensitive species and an increase in the pollution-insensitive species (Zheng and Paul 2007). On the positive side, an increase in benthic algae can provide a better habitat and act as a food source for macro-invertebrates (Dudley *et al.* 1986). Despite changes in the abundance, there are also changes in the composition of macro-invertebrate families (Sabater *et al.*2005), for example, mayflies are abundant when algal biomass is at low levels, whereas the scrapers functional group (caddisflies, freshwater snails, etc.) tend to be more abundant when the nutrient levels are higher as they consume algae (Miltner and Rankin 1998). Oligochaeta-gastropod-dominated assemblages are also more common when there is a change in the abundance of algae and sediment organic carbon concentrations (Chambers *et al.* 2006). Due to this change in assemblages, aquatic macro-invertebrates can also serve as good long-term indicators of eutrophication in a freshwater system (Pan *et al.* 2015).

Pan *et al.* (2015) conducted a study on 20 lakes in the Yangtze floodplain over a period of one year during different seasons. Water quality analysis was conducted with an assessment of the aquatic macro-invertebrate communities. The results showed that as the eutrophication increased within the respective lakes, the abundance of macro-invertebrates decreased. The collector-gatherers (mainly Chironomidae) and predators increased while the scrapers decreased. The study conducted by Mattsen (2009), however, yielded positive and negative aspects of the effect of eutrophication on macro-invertebrates. The study was conducted on Florida Spring-run streams and the aim was to determine the relationship between benthic algae and aquatic macro-invertebrates. There was an elevation in nutrient levels within the springs and streams which eventually lead to increased levels of algae within the system. This study showed that there could be positive and negative aspects to an overabundance of algae on aquatic macro-invertebrates, positive in the sense that increased algae can lead to an increase in macro-invertebrate taxa and richness and negative in the sense that increased algae could result in decreased species evenness and diversity. Where there is an abundance of algae, there would most likely be an increase in scraper macro-invertebrates coupled with a decrease in the Ephemeroptera, Trichoptera and Plecoptera groups (Mattsen 2009).

The effect of nutrient concentrations on the biotic integrity has also been studied by Robertson *et al.* (2006). Low nutrient concentrations showed a wide range of macro-invertebrate families and lower numbers were observed during periods of high nutrient concentrations. It was therefore in the current study expected that lower numbers of macro-invertebrates will occur due to the high levels of nutrients present at selected sites, however there were lower levels of nutrients as seen in Table 5 in Chapter 3.

4.2. Methodology

4.2.1. Macro-invertebrates assessment

The SASS5 method was used to assess the macro-invertebrate communities in the selected rivers. Three biotopes were assessed as the diversity and abundance of aquatic macro-invertebrates depends on the presence of these biotopes: Stones, the first biotope, includes the sampling of stones in-and out-of-current. The second biotope is aquatic vegetation including both aquatic vegetation and marginal vegetation in-and out-of-current. The third biotope is gravel, sand and mud also known as GSM. SASS sites were chosen within each river, including upstream (reference site), mid-stream and downstream (with the exception of E2 – see Chapter 2) based on the availability of biotopes and accessibility. Please refer to Chapter 2 (Site selection). Sampling was done during the low-flow season (July 2015) as well as during the high-flow season (February 2016). Macro-invertebrates were sampled using a SASS net and was done by an accredited SASS5 practitioner (Mrs. Lee-Ann Modley). The SASS net was 30 x 30 cm with a 1 mm mesh and a stout wooden handle.

Standard methodology, as prescribed by Dickens and Graham (2002) was employed. In brief, the stones biotope was sampled for 2 minutes and stones out-of-current as well as bedrock and boulders were sampled for 1 minute. It is important to know that for effective macro-invertebrate sampling with SASS5, at least 2 biotopes need to be present. At some sites, there was no aquatic vegetation. Where it was available, it was sampled by sweeping the net backwards and forwards over the vegetation for a total of 1 m^2. Marginal vegetation in- and out-of-current was sampled in the same manner for a 2 m^2 area. The GSM biotope was sampled for approximately 1 minute. This was done by shuffling or scraping the GSM (Dallas and Day 2007). After each biotope was sampled, the samples were placed into separate trays and identified using the identification guide by Gerber and Gabriel (2002). The identification of macro-invertebrates per tray was done for 15 minutes or less. Each macro-invertebrate specimen was identified to family level. The standard 2008 version of the SASS5 datasheet was completed and the number of taxa and average score per taxa (ASPT) calculated for each site. The River Health Programme: South African Scoring System (SASS) data interpretation guidelines by Dallas (2007), was used to interpret the SASS5 data. These guidelines were developed in 2007 to standardise the method used within South Africa and takes into consideration the spatial variation found within different parts of the country (Dallas 2007). The guidelines were developed using existing data from the River Database and each site was assigned an ecoregion. Biological bands are calculated for each spatial group (reference sites as well as monitoring sites within each ecoregion). A statistical summary is

then provided which gives the mean, minimum and maximum values, and percentiles (which ranges from 22.5th – 90th percentile) for each biological band (Dallas 2007). The "A" biological band is then represented by the 90th percentile which consists of the top 10% of SASS5 scores and ASPT values in any given dataset (usually considered as reference sites) (Dallas 2007). The sites selected for this study falls within the Bushveld Basin Ecological zone and in the Crocodile West Marico Management Area (WMA) which is the "second most populous WMA in the country" (Kleynhans *et al.* 2005).

4.2.2. Statistical analyses

A multi-variate statistical analysis was conducted and a Detrended Correspondence Analysis (DCA) plot was constructed using CANOCO 5 to determine spatial and temporal variation within the macro-invertebrates at each site. DCA plots were constructed for both low-flow and high-flow conditions based on the composition of the macroinvertebrate assemblages within each river. After the SASS 5 score was determined for each site, the sites were then categorized into a class following Dickens and Graham (2002) as seen in Table 18.

Table 18: A description of SASS5 classes according to Dickens and Graham (2002)

SASS CLASS DESCRIPTION	ASPT Score range	Class
Excellent – Unimpaired, community structures and functions comparable to the best situation to be expected. Optimum community structure for stream size and habitat quality.	**5.8-7**	**A**
Very Good – minimally impaired; largely natural with few modifications. A small change in community structure may have taken place but ecosystem functions are still predominantly unchanged.	**5.1-5.7**	**B**
Good – moderately impaired; community structure and function less than the reference condition. Community composition lower than expected due to loss of some sensitive forms. Basic ecosystem functions are still predominantly unchanged.	**4.8-5**	**C**
Fair – largely impaired; fewer families present than expected, due to loss of most intolerant forms. Basic ecosystem functions have changed.	**4.3-4.7**	**D**
Poor – seriously impaired, few aquatic families present, due to loss of most intolerant forms. An extensive loss of basic ecosystem function has occurred.	**0-4.2**	**E/F**

4.2.3. Habitat Assessment

The main aim of the integrated habitat assessment is to assess the major causes of effects on habitat availability and diversity in an ecosystem. The habitat assessment is, therefore, an integral part of the SASS5 assessment as previously mentioned. Habitat influences the abundance and diversity of macro-invertebrates. Three different biotopes (similar to SASS5), stones in- and out-of-current, bedrock, gravel, stones and mud and aquatic vegetation and marginal vegetation (in-and-out of current) were assessed in detail to determine the stream conditions. A score sheet was used to make notes of the various observations. The value of these indices was then calculated as a percentage (Appendix 3). The score sheet was divided into different indices and a rating system was used to describe the habitat quality of each site (McMillan 1998). The IHAS class description displayed in Table 19, was used to describe the (%) obtained for each habitat assessed.

The stones-in-current habitat is divided into six different sections which is assessed on a range from 1-5 to arrive at the final score. These include the total length of broken water in meters (rapids and riffles), total length of submerged stones in current, number of separate stones in current kicked, average size of stones kicked (this would range from gravel to bedrock), the amount of stone surface which was clear (ie. covered/not covered by algae, sediment or silt) and finally the time spent kicking the stones in current was noted (Ollis *et al.* 2006 adapted from McMillan 1998).

The vegetation was divided into 5 different groups, which also had to be assessed on a range of 1-5. The different parameters measured included: length of fringed vegetation sampled which ranged from none to less than 2 m and the amount of aquatic vegetation and or algae sampled which ranged from none to less than 1 m. The location of fringed vegetation sampled also had to be noted. This could either be in a pool, run or mix. Other habitats sampled also had to be rated on a scale from 1 – 5. This included the m^2 of stones out of current sampled (ranging from none to less than 1 m^2), the amount of time taken to sample sand, mud, gravel and bedrock and finally the presence of algae which ranged from none to 2 m^2. The condition of the stream was assessed by looking at factors such as stream width, colour of the water, observation of recent disturbances to the system and any surrounding impacts as well as the percentage of vegetation covering the river banks (Ollis *et al.* 2006 adapted from McMillan 1998).

Table 19: A description of IHAS classes according to Dickens and Graham (2002)

IHAS CLASS DESCRIPTION	IHAS Score (%)	Class
Natural - Unmodified or almost natural conditions; natural biotic template will not be modified. Minimal risk or reduction in habitat availability.	> 70	A
Good - Largely natural with few modifications; only a small risk of modifying the natural biotic template. Risk to the availability of habitat moderate, availability of unique habitats at risk.	61 - 70	B
Adequate/Fair - Modified state; moderate risk of modifying the biotic template occurs. Habitat unavailable to certain aquatic invertebrates.	36 - 60	C
Poor - Largely modified unnatural state; large risk of modifying the biotic template. Natural required habitat generally unavailable to most aquatic invertebrates.	< 35	D

4.3. Results

4.3.1. Macro-invertebrates (SASS5)

The macro-invertebrate assessment showed a variation of diversity in the different rivers and a total of 27 taxa were identified across the 3 rivers. The SASS5 results (Taxa identified, ASPT and SASS5 score) for each river is presented in Table 20 and Table 21. The results per river will be discussed individually below.

Table 20: SASS5 Results for Edendalespruit, Pienaars River and Hartbeesspruit during low-flow conditions (July 2015)

Taxon	Sensitivity score *(Dickens and Graham 2002)*	E2	P2	P3	P4	H2	H3	M1	M2
Ancylidae (Limpets)	6			✓				✓	
Aeshnidae (Hawkers and Emperors)	8				✓				
Atyidae (Freshwater shrimp)	8				✓				
Baetidae >2 species	12	✓		✓					
Baetidae 2 species	6				✓	✓	✓	✓	✓
Baetidae 1 species	4								
Caenidae (Squaregills/Cainflies)	6			✓	✓	✓	✓	✓	✓
Ceratopogonidae (Biting midges)	5	✓		✓				✓	
Chironomidae (Midges)	2	✓	✓	✓		✓	✓	✓	✓
Coenagrionidae (Sprites and blues)	4			✓		✓		✓	
Corixidae* (Water boatmen)	3	✓	✓				✓		
Culicidae* (Mosquitoes)	1		✓		✓		✓		✓
Ecnomidae	8				✓		✓	✓	✓
Hirudinea (Leeches)	3				✓			✓	
Gerridae* (Pond skaters/Water striders)	5				✓				
Gomphidae (Clubtails)	6				✓				✓
Gyrinidae* (Whirligig beetles)	5			✓	✓				
Hydropsychidae 1 sp	4			✓					
Leptophlebiidae (Prongills)	9			✓	✓				
Notonectidae (Backswimmers)	3		✓	✓	✓	✓		✓	
Oligochaeta (Earthworms)	1	✓	✓	✓	✓	✓	✓	✓	✓
Physidae* (Pouch Snails)	3				✓	✓		✓	✓
Psychomyiidae/Xiphocentronidae	8						✓		✓
Potamonautidae* (Crabs)	3	✓							
Simuliidae (Blackflies)	5	✓	✓	✓			✓	✓	✓
Veliidae * (Ripple bugs)	5				✓				
		E2	**P2**	**P3**	**P4**	**H2**	**H3**	**M1**	**M2**
SASS		31	15	62	85	25	43	52	46
Number of Taxa		7	6	12	15	7	10	12	10
ASPT		4.43	2.5	5.17	5.67	3.57	4.3	4.33	4.6
Class		**D**	**E/F**	**B**	**B**	**E/F**	**E/F**	**D**	**D**

*Airbreathers

Table 21: SASS5 Results for Edendalespruit, Pienaars River and Hartbeesspruit during high-flow conditions (February 2016)

Taxon	Sensitivity score *(Dickens and Graham 2002)*	E2	P2	P3	P4	H2	H3	M1	M2
Ancylidae (Limpets)	6			✓		✓		✓	
Aeshnidae (Hawkers and Emperors)	8								
Atyidae (Freshwater shrimp)	8		✓						
Baetidae >2 sp	12								
Baetidae 2 sp	6					✓	✓		
Baetidae 1sp	4	✓	✓	✓				✓	✓
Belostomatidae* (Giant water bugs)	3	✓				✓			
Caenidae (Squaregills/Cainfles)	6		✓				✓		
Ceratopogonidae (Biting midges)	5								
Chironomidae (Midges)	2	✓		✓	✓	✓		✓	✓
Coenagrionidae (Sprites and blues)	4			✓		✓		✓	
Corixidae* (Water boatmen)	3	✓	✓		✓	✓		✓	✓
Culicidae* (Mosquitoes)	1	✓			✓				
Ecnomidae	8						✓		
Hirudinea (Leeches)	3						✓	✓	✓
Hydropsychidae 1 sp	4				✓				
Leptophlebiidae (Prongills)	9								
Libellulidae (Darters / Skimmers)	4			✓					
Notonectidae (Backswimmers)	3					✓		✓	
Oligochaeta (Earthworms)	1	✓	✓	✓		✓			✓
Physidae* (Pouch Snails)	3					✓	✓		
Simuliidae (Blackflies)	5	✓		✓	✓		✓		
SASS		23	22	23	15	31	31	28	13
Number of Taxa		8	5	7	5	9	6	7	5
ASPT		2.88	4.4	3.29	3	3.44	5.17	4	2.6
Class		**E/F**	**E/F**	**E/F**	**E/F**	**E/F**	**B**	**E/F**	**E/F**

*Airbreathers

4.3.1.1. Edendalespruit

As mentioned before, the upper Edendalespruit was the only accessible and appropriate site in terms of available biotopes for SASS5 sampling within this river during both seasons. Site E2 was rated “fair” during low-flow conditions with a total of 7 different taxa as seen in Table 20. The site was largely impaired with fewer families present than expected. This was however not depicted in the IHAS results which showed an IHAS score of 11 for sand, mud and gravel within the river. Although the IHAS score was good indicating that the habitat was largely natural during low-flow conditions, it improved during high-flow conditions as the score increased from a B to an A. The river displayed almost natural conditions with a minimal risk regarding habitat availability. The SASS5 class deteriorated during high-flow conditions, despite the fact that there were a higher number of taxa (8 taxa), the sensitivity score of the sampled taxa was lower and this attributed to the low SASS5 score. The water quality parameters measured *in-situ* showed higher levels of conductivity and total dissolved solids during high-flow conditions (see Table 3 and Table 4).

There was a clear difference in the appearance of the site during the different seasons. During low-flow conditions, there was construction taking place at the site which led to sandy banks and a clearing of vegetation. During high-flow conditions, there was no construction taking place and the vegetation surrounding the river had grown, this is depicted in Figure 16. During the high-flow conditions the site had almost natural conditions with increased vegetation. There was, however, a loss of pollution sensitive taxa. This was unexpected as riparian vegetation is known to improve the water quality and increase the abundance of macro-invertebrates (Segera *et al.* 2012; Fajardo *et al.* 2015). The only possible explanation for this would be that the recent rains in the area before sampling could have increased the stream flow, thus washing organisms downstream. The ASPT, as a function of the SASS5 score, was plotted within the biological bands for the Edendalespruit and is seen in Figure 17. The figure shows that the site fell within the 22.5th percentile (Dallas 2007).

Figure 16: Edendalespruit SASS5 and IHAS sampling sites during low- (A) and high-flow (B) conditions

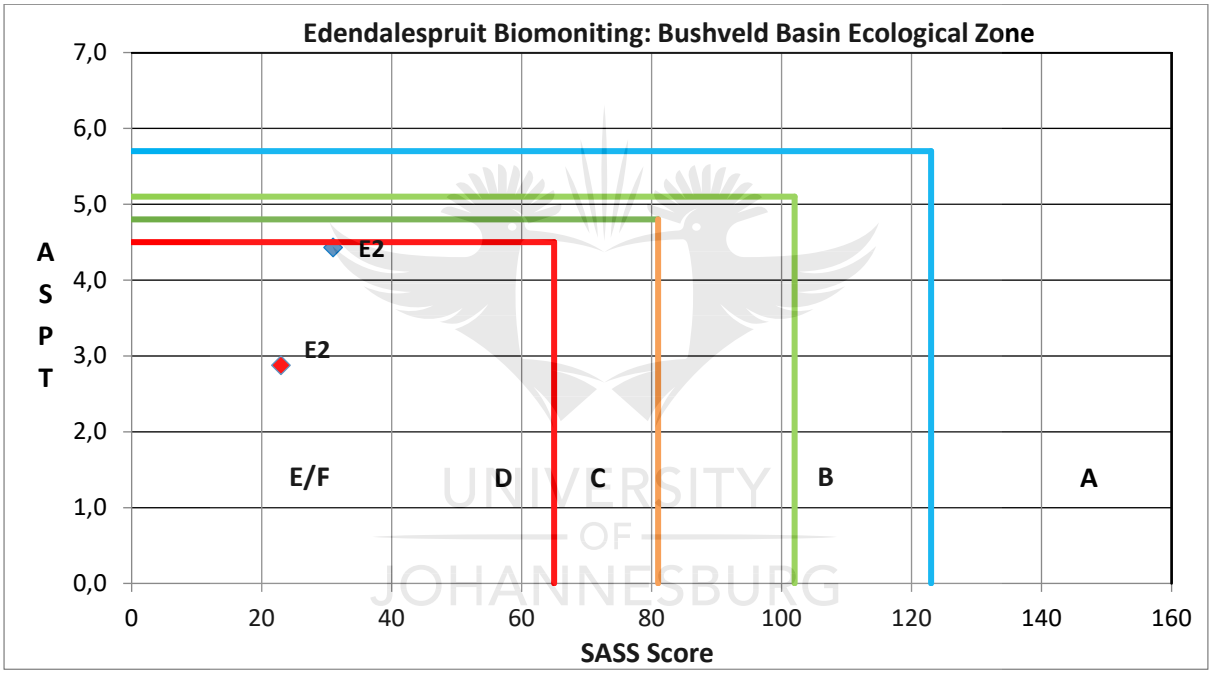

Figure 17: ASPT as a function of the SASS5 score plotted within the biological bands for Edendalespruit during low-flow (July 2015) and high-flow (February 2016) conditions

4.3.1.2. Pienaars River

The downstream reaches of the Pienaars River (site P2) which is in close proximity to the Baviaanspoort wastewater treatment plant had the lowest SASS5 score during low-flow conditions with a score of 2.5 and 6 taxa (Table 20). The number of taxa increased to 8 during the high-flow conditions resulting in an ASPT score of 2.85 (Table 21). The site was still, however, “severely impaired” with a low number of aquatic macro-invertebrates. The site in the middle reaches of the Pienaars River (P3) scored a B during low-flow conditions. The site was “minimally impaired” and was largely natural with very few modifications. The rating for this site dropped during high-flow conditions to an E/F with the number of taxa decreasing from 12 (during the low-flow conditions) to 7. The site in the upper Pienaars River (the

reference site) was rated as a B during low-flow conditions with an abundance of 15 taxa. The SASS5 score and number of taxa dropped to E/F and 5 respectively during the high-flow sampling season. Figure 18 depicts the observations noted, such as the change in flow, vegetation growth and water level at each site during the low- and high-flow conditions. The ASPT as a function of the SASS5 score plotted within the biological bands for all sites sampled within the Pienaars River is seen in Figure 19. The figure shows that the majority of site fell within the 22.5th percentile, two sites (P3 and P4 low-flow) fell within the 90th percentile which represents the top 10% of all SASS5 and ASPT scores in that dataset (Dallas 2007).

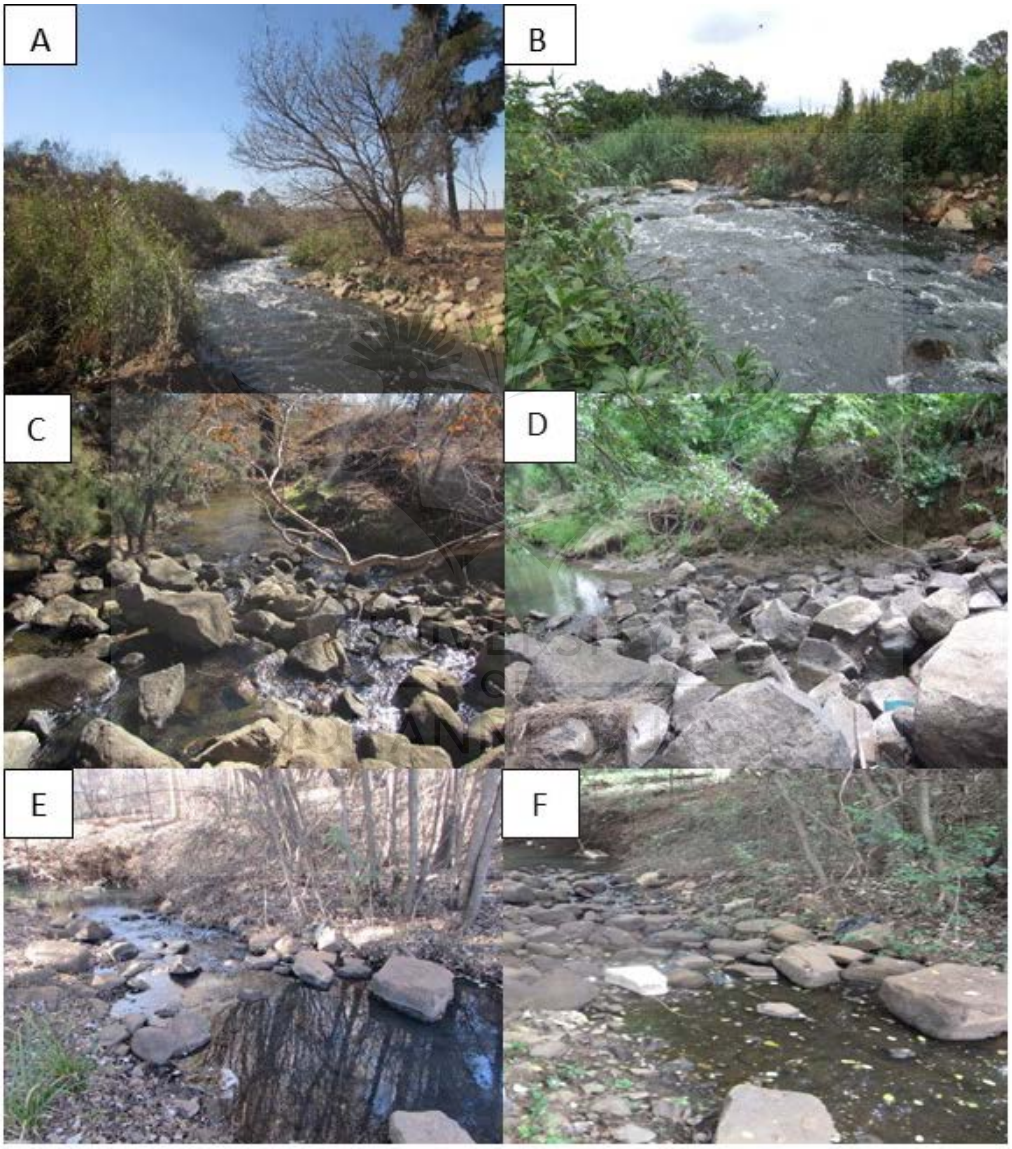

Figure 18: Pienaars River SASS5 and IHAS sampling sites during low- (A = P1, C = P2 and E = P3) and high-flow (B = P1, D = P2, F = P3) conditions.

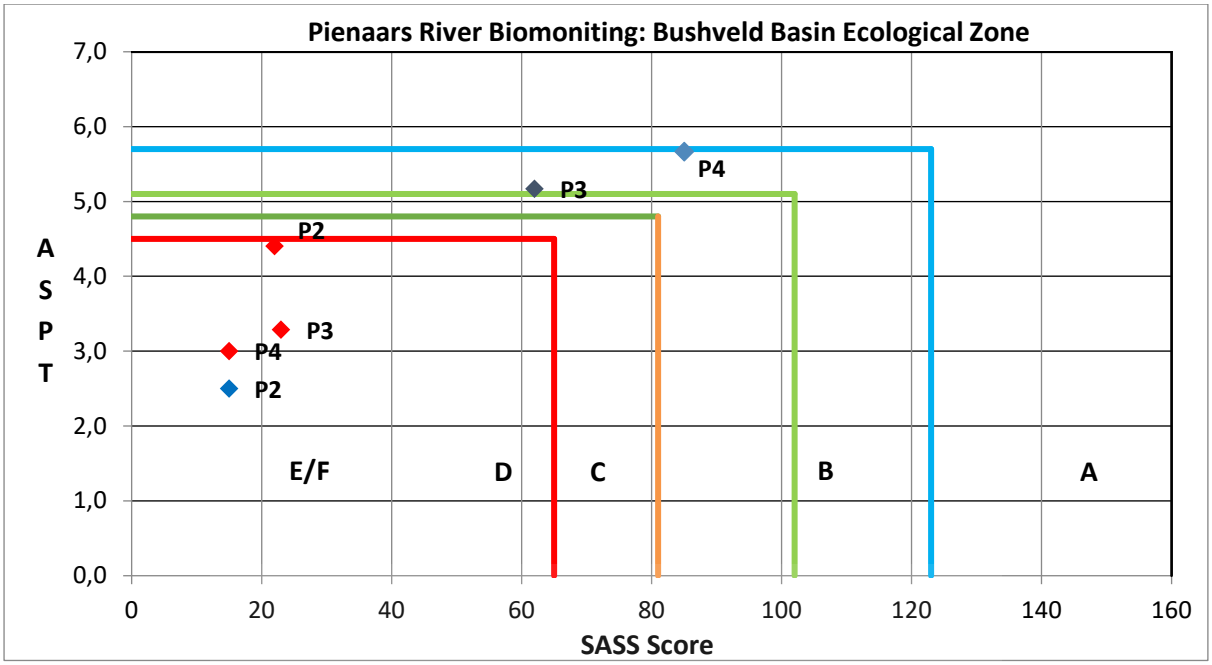

Figure 19: ASPT as a function of the SASS5 score plotted within the biological bands for Pienaars River during low-flow (July 2015) and high-flow (February 2016) conditions

4.3.1.3. Hartbeesspruit and Morelettaspruit

During the low-flow conditions both sites within the Hartbeesspruit were classified as E/F indicating that they were "severely modified". Seven different taxa were sampled at site H2 which is situated in the suburb of Kilner Park (formal housing) and 6 different taxa were sampled at site H3, situated in Kameeldrift East (predominantly small holdings and agricultural activities). The high-flow conditions showed deterioration in conditions of sites H2, however, H3 was rated class B. At this site, 6 pollution-sensitive taxa were found whereas 9 pollution-insensitive taxa was found in H2 placing it into class E/F. The differences in physical characteristics of each site during the different seasons are presented in Figure 20. The figure shows that the downstream site (H2) fell within the 22.5th percentile during both seasons, along with the upstream site H3 during low-flow conditions. During high-flow conditions the upstream site fell within the 67.5th percentile (Dallas 2007).

Figure 20: Hartbeesspruit SASS5 and IHAS sampling sites during low-flow (July 2015) and high-flow (February 2016) conditions (A = H2, B = H3) and high-flow (C = H2, D = H3)

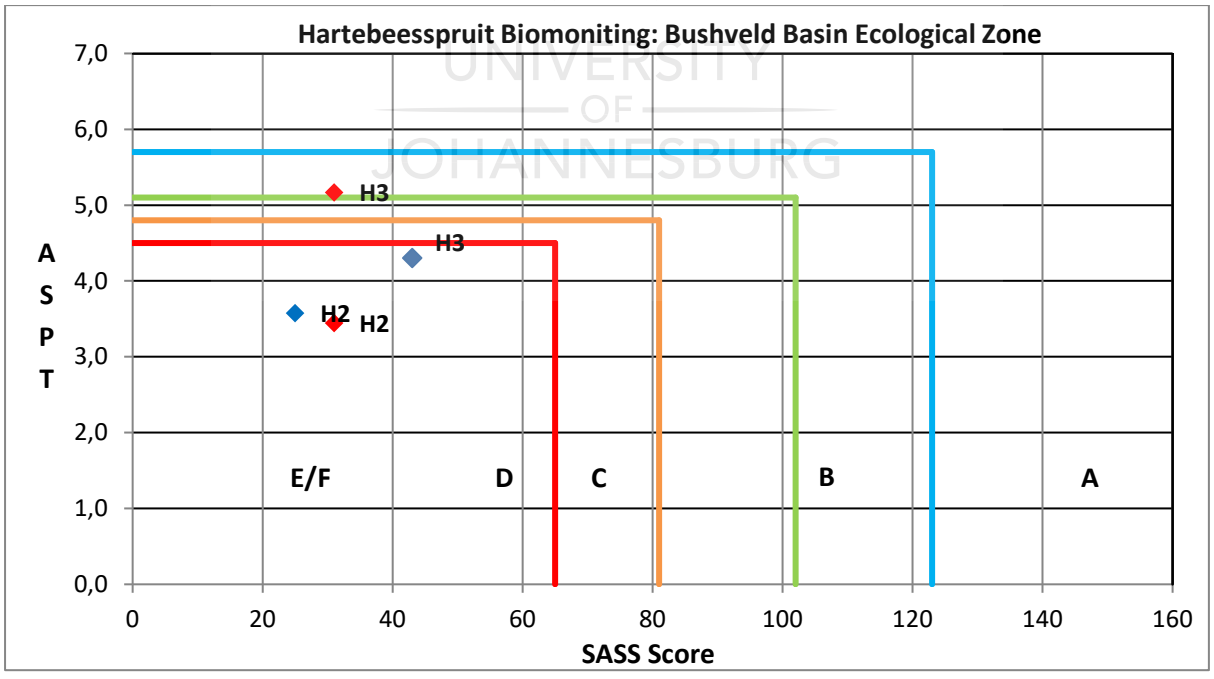

Figure 21: ASPT as a function of the SASS5 score plotted within the biological bands for Hartbeesspruit during low-flow (July 2015) and high-flow (February 2016) conditions During low-flow conditions both M1 and M2 was rated as “fair”, with an ASPT of 4.3 and 4.6 respectively.

The high-flow results showed deterioration as sites dropped from a class D to a class E/F, indicating that there were fewer aquatic macro-invertebrate families present and that the system was “seriously impaired”. Figure 22 is a depiction of the changes over the seasons. Figure 23 shows that the downstream site (M1) fell within the 22.5th percentile during both seasons the upstream site M2 fell within the 22.5th percentile during high-flow conditions and within the 36th percentile during low-flow conditions.

Figure 22: Morelettaspruit SASS5 and IHAS sampling sites during low (A = M1, C = M2) and high-flow (B = M1, D = M2)

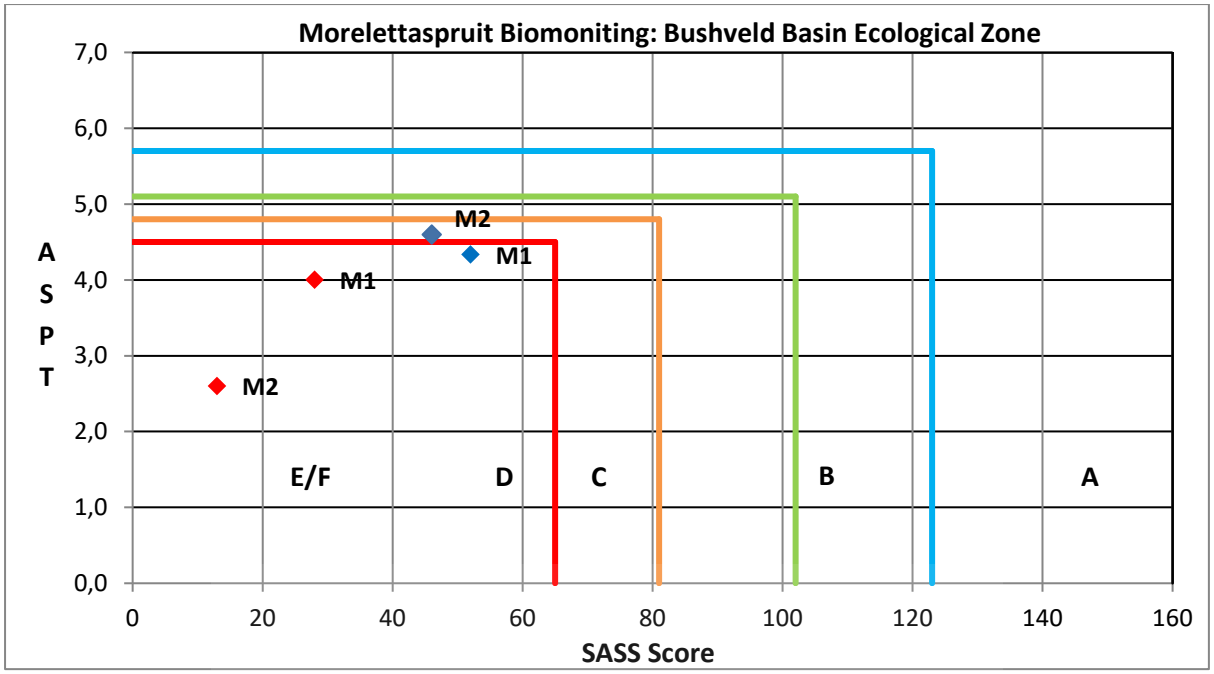

Figure 23: ASPT as a function of the SASS5 score plotted within the biological bands for Morelettaspruit during low-flow (July 2015) and high-flow (February 2016) conditions

The functional feeding groups identified are displayed in Table 22 and Table 23. The results showed that the predator FFG dominated when compared to other FFGs observed. This was the case during both seasons. The shredders were the least abundant, only occurring once during the low-flow conditions in Edendalespruit (site E2).

Table 22: Functional Feeding groups identified within Edendalespruit, Pienaars River and Hartbeesspruit during low-flow conditions (July 2015)

Taxon	E2	P2	P3	P4	H2	H3	M1	M2
Aeshnidae (Hawkers and Emperors)								
Atyidae (Freshwater shrimp)								
Baetidae								
Caenidae (Squaregills/Cainflies)								
Ceratopogonidae (Biting midges)								
Chironomidae (Midges)								
Coenagrionidae (Sprites and blues)								
Corixidae* (Water boatmen)								
Culicidae* (Mosquitoes)								
Ecnomidae								
Hirudinea (Leeches)								
Gerridae* (Pond skaters/Water striders)								
Gomphidae (Clubtails)								
Gyrinidae* (Whirligig beetles)								
Hydropsychidae 1 sp								
Notonectidae (Backswimmers)								
Potamonautidae* (Crabs)								
Simuliidae (Blackflies)								
Veliidae * (Ripple bugs)								

*Airbreathers

Scrapers

Collector gatherers

Shredders

Predators

Collector filter

Table 23: Functional Feeding groups identified within Edendalespruit, Pienaars River and Hartbeesspruit during high-flow conditions (February 2016)

Taxon	E2	P2	P3	P4	H2	H3	M1	M2
Ancylidae (Limpets)								
Aeshnidae (Hawkers and Emperors)								
Atyidae (Freshwater shrimp)								
Baetidae								
Belostomatidae								
Caenidae (Squaregills/Cainflies)								
Ceratopogonidae (Biting midges)								
Chironomidae (Midges)								
Coenagrionidae (Sprites and blues)								
Corixidae* (Water boatmen)								
Culicidae* (Mosquitoes)								
Ecnomidae								
Hirudinea (Leeches)								
Hydropsychidae 1 sp								
Leptophlebiidae (Prongills)								
Libellulidae (Darters / Skimmers)								
Notonectidae (Backswimmers)								
Oligochaeta (Earthworms)								
Physidae* (Pouch Snails)								

***Airbreathers**

Scrapers

Collector gatherers

Shredders

Predators

Collector filters

A seasonal comparison between the ASPT scores is presented in Figure 24. The results showed that the upstream Pienaars River site (P3) had the highest ASPT during the low-flow conditions and the upstream Hartbeesspruit site (H2) had the highest ASPT during the high-flow conditions. The downstream Pienaars River site (P1) had the lowest ASPT during the low-flow conditions. However, the ASPT calculated for the Morelettaspruit during the high-flow conditions was even lower. The majority of sites had a lower ASPT during the high-flow season with the exception of P2 and H3. The upstream sites for the Pienaars River and the Morelettaspruit were both higher during the low-flow conditions. However, the upstream site of Hartbeesspruit was higher during the high-flow season.

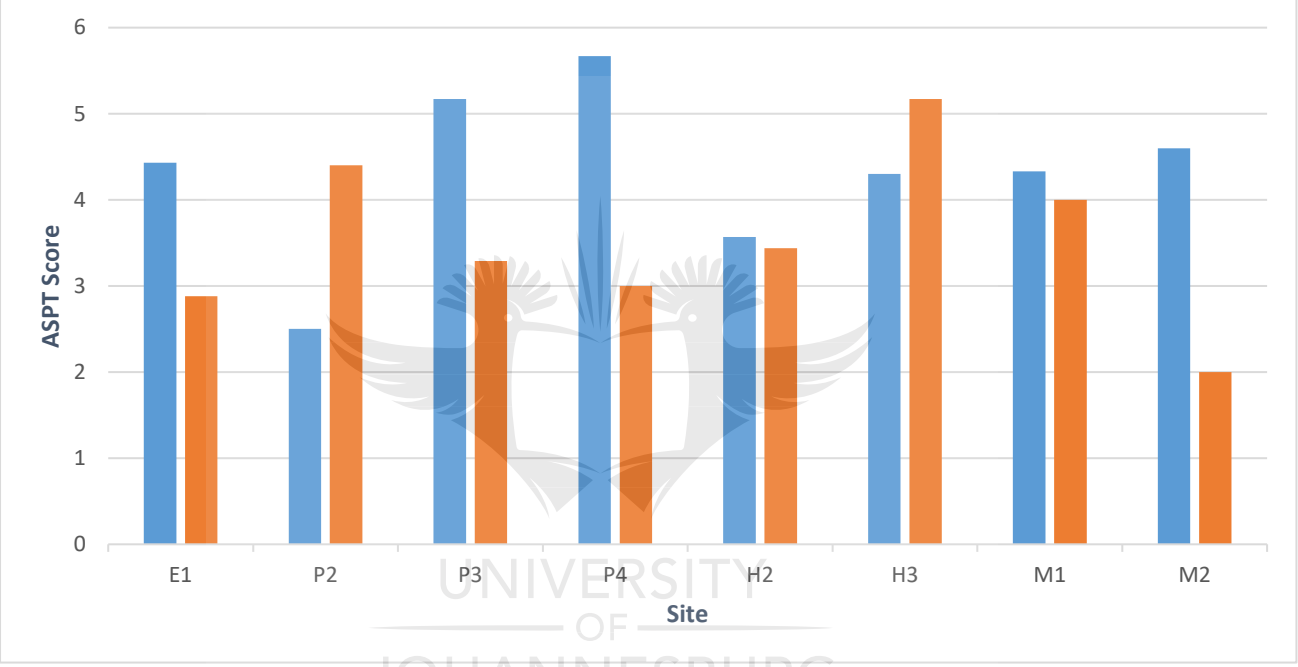

Figure 24: Seasonal comparison of ASPT scores within each sampled river Hartbeesspruit and its tributary Morelettaspruit (low-flow conditions indicated in blue and high-flow conditions indicated in orange)

4.3.2. Statistical Analysis

The DCA plot for low-flow conditions (Figure 25) indicated only one grouping, indicating a strong positive correlation with the majority of sites and macro-invertebrates sampled. The only two sites not clustered with the other sites were located within Pienaars River (sites P2 and P4). During high-flow conditions (Figure 26), there were three groupings observed. The main grouping was observed between the majority of sites (P3, H2, E2, M2, M1, P4 and H3). P2 was an outlier. This site had very low conductivity and TDS whereas the other sites had values in a similar range for these two parameters. P2 is also the only site associated with Atyidae during high-flow conditions.

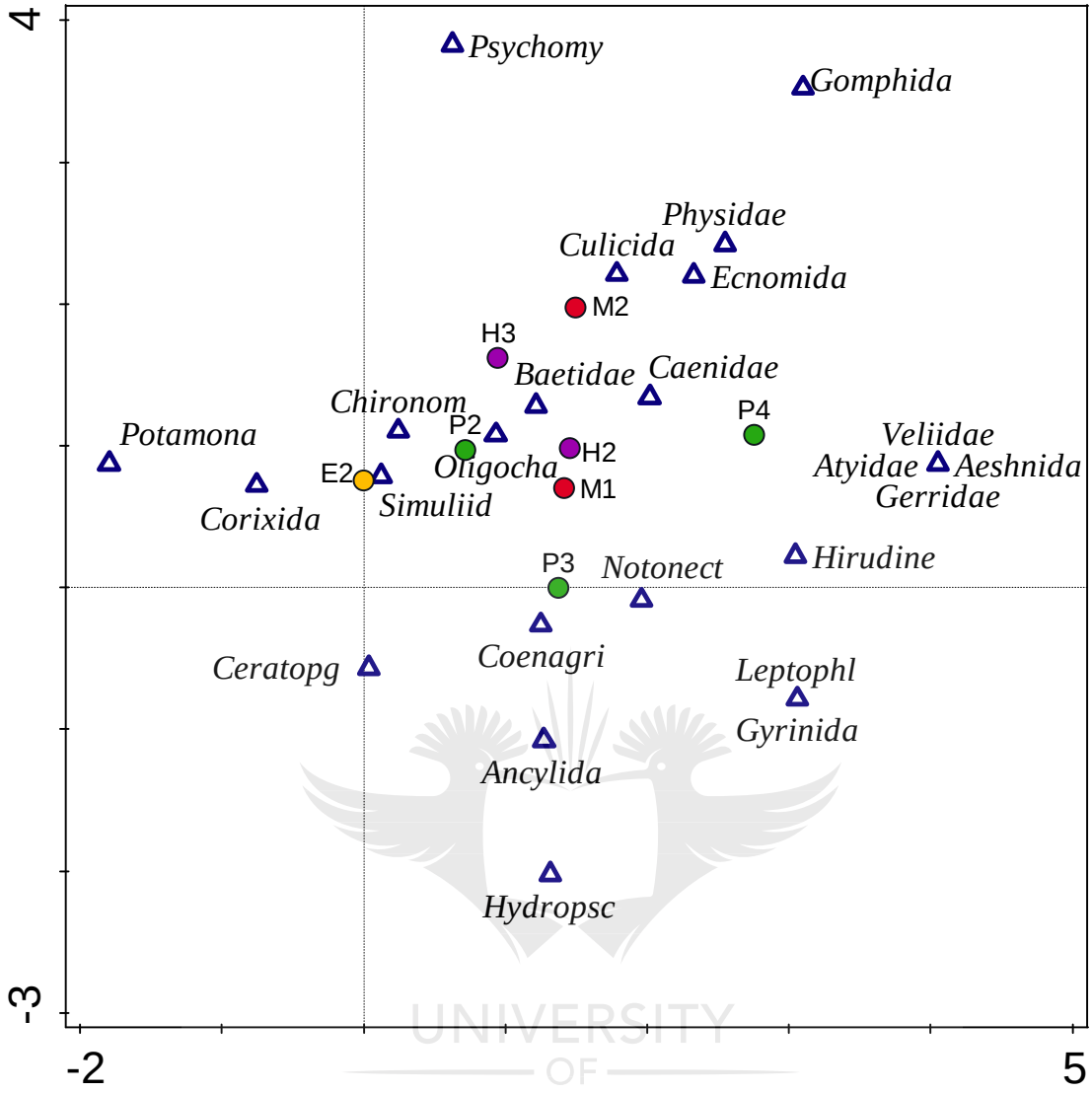

Figure 25: DCA plot based on the composition of the macro-invertebrate assemblages from Edendalespruit (E2), Pienaars River (P2-P4) and Hartbeesspruit (H2-H3) (and its tributary Morelettaspruit M1-M2) during low-flow conditions (July 2015)

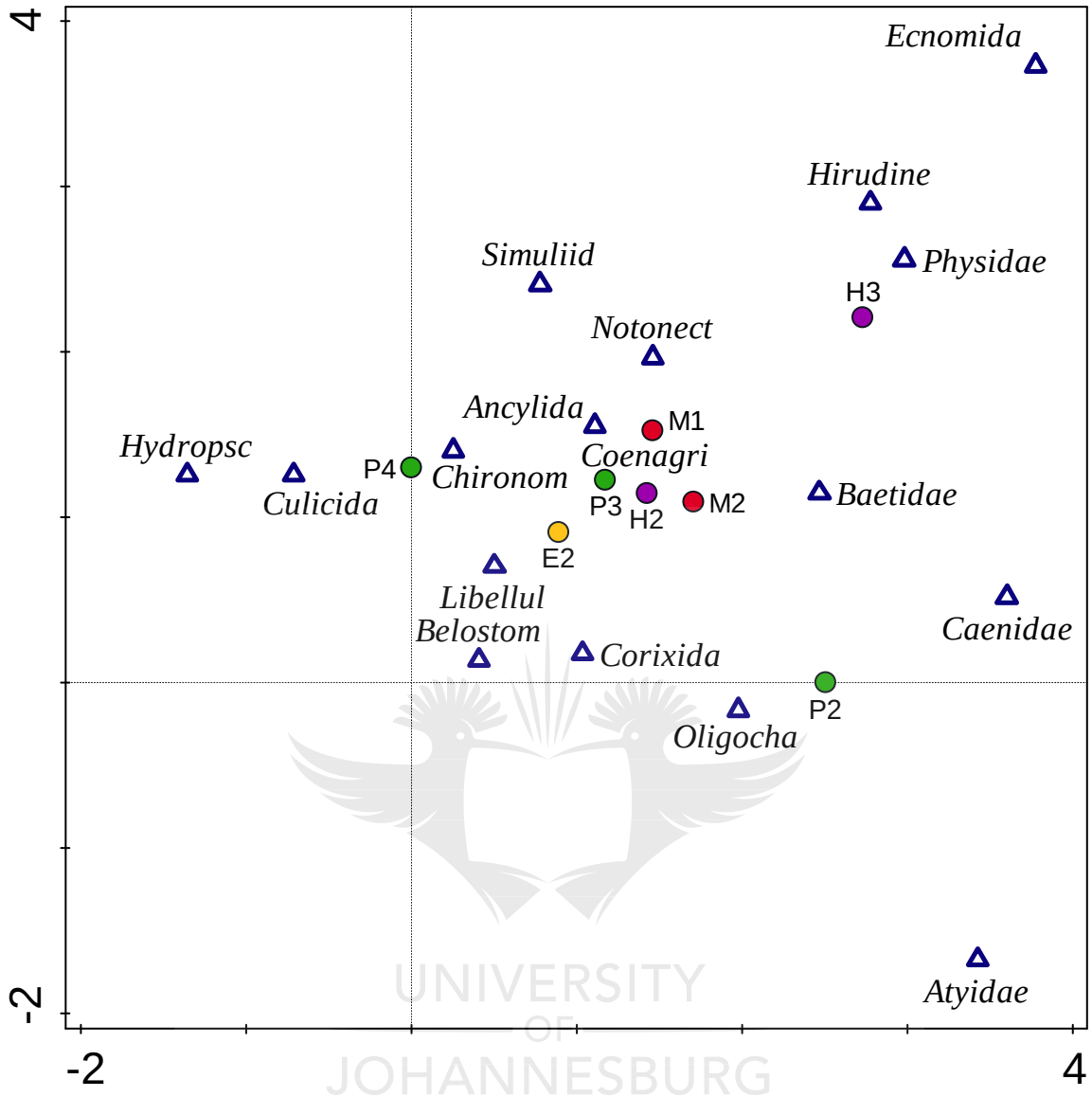

Figure 26: DCA plot based on the composition of the macro-invertebrate assemblages from Edendalespruit (E2), Pienaars River (P2-P4) and Hartbeesspruit (H2-H3) (and its tributary Morelettaspruit M1-M2) during high-flow conditions (February 2016)

4.3.3. Integrated Habitat Assessment Index (IHAS)

The IHAS results for low-flow and high-flow conditions are presented in Table 24 and Table 25 respectively. The results showed that during the low-flow conditions the sites within each river ranged from “largely natural” with few modifications to a “largely modified, unnatural state.” The upstream site within the Pienaars River (P4) and the site within the Edendalespruit (E2), as well as, the downstream site in the Hartbeesspruit (H1), were all rated as “largely natural.” Interestingly, the upstream Hartbeesspruit was the only site rated a “D” implying that the site was “largely modified” and in an unnatural state. Both sites within the Morelettaspruit were rated the same. During the high-flow conditions, the habitat scores increased only in the

Edendalespruit (E2) and the middle reaches of the Pienaars River (P3). The rest of the sites remained the same while the IHAS scores for the downstream site within the Hartbeesspruit and the upstream site within the Morelettaspruit decreased. The Pienaars River upstream sites was rated higher during both seasons whereas in Morelettaspruit only during the low-flow season. The Hartbeesspruit downstream had a higher score during both seasons when compared to the upstream site.

Table 24: Integrated Habitat Assessment System (IHAS) results for Edendalespruit, Pienaars River and Hartbeesspruit during low-flow conditions (July 2015)

IHAS Biotopes assessed	E2	P2	P3	P4	H2	H3	M1	M2
Stones	3	6	6	9	6	3	7	9
Vegetation	6	3	4	4	4	5	6	6
Gravel, sand and mud	11	5	5	7	9	0	5	3
Hand picking/visual observation	0	0	1	1	0	0	0	1
IHAS Score	20	15	16	21	19	8	18	19
Stream conditions	44	33.33	33.33	44.44	42	18	40	40
Total IHAS	**64**	**48**	**49**	**65**	**61**	**26**	**58**	**59**
IHAS Class	**B**	**C**	**C**	**B**	**B**	**D**	**C**	**C**

Table 25: Integrated Habitat Assessment System (IHAS) results for Edendalespruit, Pienaars River and Hartbeesspruit during high-flow conditions (February 2016)

IHAS Biotopes assessed	E2	P2	P3	P4	H2	H3	M1	M2
Stones	6	8	7	6	7	7	7	6
Vegetation	11	5	5	5	4	1	6	3
Gravel, sand and mud	5	7	9	8	3	0	5	0
Hand picking/visual observation	0	0	1	1	0	0	0	1
IHAS Score	22	18	22	20	14	8	18	10
Stream conditions	49	40	47	42	31	18	40	20
Total IHAS	**71**	**60**	**69**	**62**	**45**	**26**	**58**	**30**
IHAS Class	**A**	**C**	**B**	**B**	**C**	**D**	**C**	**D**

Edendalespruit obtained the highest IHAS score when compared to the other sites during both seasons. During low-flow conditions a small section of marginal vegetation out of current (1 - 2 m^2) was available for sampling (Figure 21). This was mostly leafy vegetation, there were no riffles available for sampling but 5 - 10 m^2 of stones-in-current was sampled and six separate stones-in-current with an average size of 2 - 10 cm was kicked for two minutes. No stones-out-of-current was sampled and sand, gravel and mud was each sampled for under a minute and there were less than 2 m^2 of algae observed. During high-flow conditions, the vegetation was more overgrown and visible. There was more instream fringing vegetation available for

sampling and an area of more than 2 m^2 was sampled. More than 1 m of marginal aquatic vegetation was sampled and there was a mix of leafy vegetation and aquatic vegetation.

In the Pienaars River, the downstream site (site P2) showed less than 10 m^2 of submerged stones-in-current and an approximate area of 5 m^2 of riffles was observed. This resulted in 2 – 3 separate stone areas to be kicked. This was done effortlessly as the stone surface was fairly clear. There was approximately 2 m^2 of marginal vegetation observed and less than 1 m aquatic vegetation. Algae was observed on site, however, this was less than 2 m^2. The high-flow observations were similar. However, there were more riffles and the total length of submerged stones was also less. At the upstream site (site P4), there was almost no riffles observed, and the stone surface was clear with only 0 – 2 m^2 of stones submerged. There was approximately 1 – 2 m^2 of marginal vegetation sampled out of current, and algae was only present on some rocks. During the high-flow conditions, 2 - 3 m^2 of riffles was observed and the total length of submerged stones in current was less than 2 - 5 m^2. Between 51 – 75% of the stone surface was clear. However, there were no algae observed at this site.

In Hartbeesspruit, during the low-flow conditions there were no riffles observed on site and the stone surfaces were mostly covered with algae at the downstream site. Although there were marginal vegetation and aquatic vegetation available to sample, this was less than 1 m^2. The following were observed at site H1 during high-flow conditions: 1 - 2 m^2 of riffles and 5 - 10 m^2 of submerged stones-in-current. However, very few stone surfaces were clear. There was approximately 2 m^2 of marginal vegetation observed along the river banks. This allowed for effective sampling out-of-current. Little to no aquatic vegetation was observed on site and there was less than 2 m^2 of algae present. Each biotope was sampled for less than 2 minutes and only some bedrock was sampled.

At the upstream site H2, during the low-flow conditions, 0 - 1 m^2 of a riffle was observed. However, approximately 5 - 10 m of stones was submerged in current. The surface of the stones was mostly covered with algae and there was no aquatic vegetation available for sampling. Approximately 1 - 2 m^2 of marginal vegetation was sampled out-of-current. Similarly, during the high-flow conditions there were no visible riffles and less than 10 m^2 of the stones were submerged in current. For this reason, 4 to 5 separate areas were kicked. There was very little fringing vegetation observed and no aquatic vegetation.

In Morelettaspruit, during the low-flow survey there was approximately 5 m^2 of riffles observed and less than 10 m^2 of the stones were submerged. More than 6 separate stone areas were kicked and most of the stone surfaces were covered with algae. There was more than 2 m^2 of marginal vegetation and approximately 1 m^2 of aquatic vegetation available for sampling. During high-flow conditions, similar conditions were observed, except there was no aquatic

vegetation available to sample and no algae observed. During low-flow conditions at the upstream site of Morelettaspruit, there was more than 5 m^2 of riffles observed onsite. Most of the stones were submerged (>10 m^2) and this led to 4-5 separate areas being kicked. Very little of the stone surface was clear and only 1-2 minutes was spent on the protocol. There was both marginal and aquatic vegetation available for sampling and some algal observed on the rocks. The results for the high-flow conditions were similar except there were no algae observed during high-flow conditions.

4.4. Discussion

The abundance of pollution-sensitive and pollution-insensitive macro-invertebrates can give an indication of the health of the aquatic ecosystem. Aquatic ecosystems with a high number of pollution-insensitive taxa such as Oligochaeta, Hirudinea and Diptera usually have poor water quality. It is also important to note that although these taxa are pollution-insensitive, they can also be found in streams with good water quality. However, pollution-sensitive species are rarely found in poor water quality conditions (Roback and Richardson 1969; Voshell 2002). Taxa from the Ephemeroptera, Plecoptera, and Trichoptera families are known as pollution-sensitive taxa, which often indicate the status of aquatic ecosystems due to the fact that they have very low adaptive capacities. They also occur with taxa from the Gyrinidae, Dytiscidae, Hydrophilidae and Notonectidae families to give a reflection of the ecological and geographical changes within a system as they are often found throughout the year (Voshell 2002).

The overall results showed a decrease in abundance and diversity of macro-invertebrates comparing low-flow and high-flow conditions for most sampling sites. This was expected as aquatic macro-invertebrates are poikilothermic which means that they are more sensitive to changes in the water temperature and therefore temperature of water plays a major role in the structure of aquatic communities (Dallas 2008). All terrestrial and aquatic organisms have an optimal temperature at which normal bodily processes such as growth and reproduction occurs. This is described by Vannote and Sweeney (1980) as the “Optimal Thermal Regime”. Elevated temperatures in aquatic ecosystems increase the respiration and oxygen demand leading to added stress in aquatic organisms. Change in temperature influences abundance, density and distribution of certain species as well as the community composition (Hart 1985; Bell 2006). A few studies have proven that high summer temperatures are responsible for reducing abundance and diversity of certain species (Coutant, 1962 cited by Mann, 1965; Vannote and Sweeney 1980; Hogg *et al*. 1995; Wellborn and Robinson 1996; Hawkings *et al.* 1997). The Seasonal differences observed in macro-invertebrate assemblages in the current study could therefore be a possible result of seasonal temperature variation. However, the expected pattern i.e. reduced abundance during summer temperatures, was not observed at

all sites, but indeed the opposite was found. Therefore, flow should be considered as a determining factor at some sites as opposed to temperature variation.

In the Edendalespruit, the taxa sampled during high-flow conditions were similar to taxa sampled during low-flow conditions (although less abundant), with the exception of two additional macro-invertebrate families namely the Belostomatidae from the Hemiptera order and Coenagrionidae from the order Odonata. All aquatic Hemiptera are predators and the Belostomatidae is known to be a significant pest within fish hatcheries and they prefer the bottom of shallow pools and quiet areas of the river (Gerber and Gabriel 2002). The Odonata family consist of true dragonflies and damselflies such as the Coenagrionidae (also predators) which is commonly found along the edge of the stream within the vegetation (Gerber and Gabriel 2002). The presence of these two predators could be due to the fact that higher temperatures lead to a modification of the predator-prey relationship according to Koler and Rahel (1993). In all three rivers there were taxa present during the high-flow conditions, which were not previously found during low-flow conditions. This was also found in the study conducted by Bredenhand (2005) who evaluated the effectiveness of macro-invertebrates as bio-indicators in the Kleinplaas Dam.

The results statistically supported the fact that the diversity of macro-invertebrates is affected by habitat, sunlight and an increase in salinity, which would explain why certain macro-invertebrates are only found during the warmer seasons (Bredenhand 2005). The macro-invertebrates sampled during high-flow conditions from Edendalespruit consisted of more pollution-insensitive taxa when compared to the low-flow season and this would explain why despite the higher number of taxa, it still received a lower ASPT. According to Chutter (1998), ASPT is more reliable than the SASS5 score to determine the health of good quality rivers because even though there is a poor habitat diversity resulting in less biotic diversity and lower SASS5 score, the organisms present might still have high sensitivity (Chutter 1998). This again, emphasises the importance of habitat assessment.

During low-flow conditions, there was construction taking place at the Edendalespruit site. This resulted in no aquatic vegetation to sample. However, the health of any aquatic ecosystem is impacted by the surrounding activities (in the case of Edendalespruit – mining, informal and formal settlements as well as agricultural activities as mentioned in Chapter 2). Therefore, as mentioned before the composition of functional feeding groups, can be an effective way of determining the impact of surrounding land-uses on a particular river.

Predators were the dominant FFG during both seasons in the Edendalespruit. Where there was an increase in predators there was a visible decrease in taxa from the other FFGs which was seen at all sites. Predators are known to dominate the macro-invertebrate assemblages

in terms of the distribution and abundance of the prey (Likens 2010). The habitat at site E2 (upstream) was dominated by GSM thus it was expected that there would be more collector-gatherers (this was the second dominant FFG during the high-flow). The water quality results (Chapter 3 – Table 5) however, show high levels of faecal coliform bacteria with high levels of total phosphate levels and chlorophyll-*a* levels during low-flow conditions. As a result of these high levels, the site was classified as hypertrophic. The bacteria levels were extremely high during the high-flow conditions and this could possibly explain the presence of collector-gatherers. The fact that the Edendalespruit was classified as hypertrophic led to the expectation that due to the nutrient load in the river, scrapers/grazers would be more abundant as they consume algae and other similar materials (Cummins *et al.* 2005). There were no shredders collected in any of the other sites and only one was found at site E2 during low-flow conditions. This could be due to the season of sampling. The fact that the residential houses located within the estate have manicured lawns which most likely make use of fertilizers as well as anthropogenic activities upstream in Mamelodi could also possibly contribute to the nutrient load at this site. It is important to conduct sampling at a particular time (at the end of the dry season and at the end of the wet season) of the year to get an accurate representation of the FFGs - as was done in this study (Cummins *et al.* 2005). This is especially true with shredders which could be in the early stages of their development and therefore would be most likely to be overlooked due to size and being camouflaged. This could possibly explain their absence during sampling as was previously commented by Cummins *et al.* (2005) in their study.

During both seasons, the PCA plot showed similar macro-invertebrate assemblages within sites E2 and P2. Similar pH levels (slightly alkaline) were observed within these two sites (see Chapter 3) with almost identical levels during low-flow conditions. Peňa and McCabe (2011) confirmed that there is a direct relationship between pH and macro-invertebrate abundances and assemblages (Peňa and McCabe 2011). The downstream site in Pienaars River (P2) was situated on the R513 next to a WWTP and slightly adjacent to a correctional facility. The macro-invertebrate assemblages observed during low-flow conditions for this site further confirmed the impact of land-uses on the presence or absence of certain taxa. The site receives treated sewage effluent from the WWTP and is situated downstream of the Mamelodi township. Although six different taxa were sampled, they were pollution-insensitive taxa thus resulting in a low ASPT. Again, during high-flow conditions less taxa was sampled but the higher sensitivity score led to an increased ASPT. The taxa sampled most frequently at site P2 (downstream) was Chironomidae. This taxon is indicative of poor water quality and usually thrives in organically-enriched habitats and brackish water (Thirion 2007; Arimoro *et al.* 2011). Many studies have shown that urban impacts lead to a reduction in macro-invertebrate

diversity but tend to result in a community dominated by pollution-tolerant taxa such as Oligochaeta, Simuliidae, Chironomidae, Hirudinea and Gastropoda (Davies and Day, 1998; Winter and Duthie, 1998; Paul and Meyer, 2001; Abel, 2002; Robertson, 2006; Silveiria *et al.*, 2006; Makoba *et al.* 2008; Miserendino, *et al.,* 2008; Al-Shami *et al*. 2011; Davies *et al.* 2010) The physico-chemical water quality might also have influenced the dominance of pollution-insensitive taxa. This site had the lowest conductivity and total dissolved solids amongst all the sampling sites in all three rivers during both high-flow and low-flow conditions (as seen in Chapter 3). This was contrary to the results observed in the study by Karaouzas *et al.* (2018), where conductivity and water temperature was highest at the WWTP site when compared to the other sites sampled. However, dissolved oxygen was the lowest and phosphate levels were the highest when compared to the other sites (Karaouzas *et al.* 2018). Site P2 also had the lowest dissolved oxygen levels and highest faecal coliform, chlorophyll-*a* and phosphate levels.

The downstream site of the Pienaars River (P2) is situated in close proximity to the WWTP. As previously mentioned, and it is also downstream of the township of Mamelodi. There was a distinct raw sewage smell at this particular site and the flow was the strongest at this site in comparison to other sites sampled in the Pienaars River during both seasons. The IHAS class stayed constant but there was a large difference in the vegetation which increased during the high-flow conditions, not just within the river, but also within the riparian zone. There were more than 5 m of rapids and riffles, and a majority of the stones were submerged therefore making it very difficult to sample. Although there was aquatic and marginal vegetation available, it was also difficult to sample due to the fast flowing water at this particular site. There was an algal bed observed during both sampling surveys at site P2, this led to the expectation that scrapers would be the most dominant FFG.

Scrapers (mayflies and caddisflies) are known to feed on attached algae from rock and wood surfaces in the river. By eating algae, they play a significant role in keeping it from growing out of control (Awudi and Ajose 2014). Although there were scrapers sampled at this site, they were not the dominant group. The dominant FFG was predators, although this made up 60% of the taxa, collector filters composed the other 40%. There were no other FFGs identified during the low-flow conditions. Water quality results during the low-flow conditions showed high levels of faecal coliform bacteria with even higher levels during the high-flow season. The river had high levels of Chlorophyll-*a* and was classified as hypertrophic during both seasons (Chapter 3 –Table 7). Collector-gatherers increased during the high-flow conditions and were the dominant group. This was however, expected due to the increased levels of faecal coliform bacteria and high levels of chlorophyll-*a*. This FFG is associated with algae, bacteria and faeces (Merrit and Cummins 1996a). The habitat was also dominated by GSM which is the

preferred habitat for collector gatherers. In a study conducted by Fu *et al.* (2015) they showed an increase in collector-gatherers where agriculture and urbanisation were the most dominant land-uses. Miserendino and Pizzolon (2003) and Arimoro (2007) reported similar findings where collector-gatherers are the most dominant group in organically-enriched waters.

The most dominant FFG observed in the middle reaches of the Pienaars River (site P3) was similarly to P2 (downstream). This site is located in Mamelodi. Eight different families were identified and pollutant-insensitive species were dominant. During high-flow conditions, seven families were identified which were also identified during the low-flow conditions. Most families identified were sensitive taxa and indicated good water quality (Dickens and Graham 2002). The results did not reflect the fact that the site was located in the informal settlement of Mamelodi. It was expected that there would be a decreased abundance of macro-invertebrates due to the surrounding land-uses. Informal settlements are known to have inadequate sanitation facilities which could ultimately impact the quality of the water if sewage enters the river as run-off. It is important to mention that this site was located further upstream of the informal settlement in close proximity to the main road in Mamelodi. There was evidence of religious rituals (such as baptism and burning of candles) being performed at this site and an abundance of algae was observed. Although there was high conductivity at this site during low-flow conditions, the nutrient and bacterial levels were the lowest amongst all sites sampled during both seasons. There was not a vast amount of vegetation to sample, and it was mainly out of current, however, there was more than 2 m^2 of algae observed on site. This site was characterized by huge boulders and a very rocky habitat. This could have contributed to the high conductivity as chemical weathering of rocks can increase conductivity (Mwangi 2014). The observed boulders and high conductivity was similar to that of the upstream site of the Pienaars River (P4).

Site P4 (the reference site), was the most pristine site sampled within the Pienaars River and was dominated by pollution-sensitive species. Eight families were identified. However, during the high-flow season, only four families were sampled and pollution-insensitive species were dominant. This was reflected in the enormous difference in the ASPT between seasons. The oligochaetes sampled are known to thrive in extremely polluted systems which are most likely to be organically enriched with very low levels of dissolved oxygen (Bouchard 2004). On the contrary, this site had fairly high dissolved oxygen levels, but, the highest Kjeldahl nitrogen and ammonia were recorded during the high-flow conditions.

The low SASS5 score and ASPT recorded during the high-flow conditions suggest that the downstream site in the Pienaars River (P2) is the most affected by pollutants. This site had the lowest macro-invertebrate diversity, and the surrounding impact of land-use activities such

as the wastewater treatment plant and anthropogenic activities from the township of Mamelodi could explain these results. A similar study was done in Zimbabwe where three sites were chosen within a river impacted by industrial and sewage effluent. The Mazai stream, similarly to the Pienaars River flows into a highly eutrophic dam (Umguza Dam). Two sites were located downstream from the discharge and one site was located upstream. The results showed a diversity of organisms upstream with an abundance of pollution-sensitive taxa such as Hemiptera, Trichoptera, Coleoptera and Odonata. The downstream sites, however, were dominated by pollution-insensitive species such as Chironomidae (Trevor *et al.* 2010). Despite the dominance of pollution-insensitive macro-invertebrates during the high-flow conditions, during the low-flow conditions, sites P2 and P4 had a good diversity of macro-invertebrates when compared to the other sites. According to Galbrand *et al.* (2007), this is indicative of a healthy system. This is due to the fact that diverse families usually require a range of different physical conditions (Galbrand *et al.* 2007).

The downstream site of Hartbeesspruit (site H2) was dominated by predators during the low-flow conditions, as well as during the high-flow conditions, however, even though predators made up 40% of the sample size, 30% of the taxa was scrapers. This could be explained when considering the water quality results which showed the presence of faecal coliforms during the low-flow conditions but extremely high levels during the high-flow conditions. The system was classified as hypertrophic during both seasons. Masese *et al.* (2013) also found that the abundance of scrapers increased during low-flow conditions, they studied the macro-invertebrate functional feeding groups in Kenya. Stones-in-current habitat had the highest score during IHAS which is commonly associated with predators. However, during the high-flow conditions, the GSM habitat had higher scores. This is commonly associated with gathering collectors, which made up the other 30% of the sample. Scrapers are usually associated with non-filamentous algae, micro-flora and fauna as well as faeces and detritus. The habitat assessment showed an abundance of filamentous algae at this particular site and the site was surrounded by small plants and trees.

The downstream site of Hartbeesspruit had an ASPT score of 4.3 with 10 different taxa during the low-flow season. The ASPT increased during the high-flow season even though the number of taxa decreased. The sensitivity scores of the sampled taxa was higher, as was seen in Edendalespruit (E2- upstream). The IHAS results, however, showed deterioration from the low-flow season to the high-flow season at site H2. The site went from largely natural with few modifications to a modified state with habitat unavailable for certain invertebrates as the amount of GSM decreased and the abundance of stones increased. This could be explained by the increased stream flow as a result of the rain which caused the GSM to wash away. This was seen in the FFG assessment, as there were more predators sampled during high-flow

conditions (predators prefer stones) (Cummins *et al*. 2005). The number of faecal coliform bacteria colonies was high during the low-flow period but even higher during the high-flow period. The system was classified as hypertrophic during both seasons. Therefore, filtering collectors and gathering collectors were expected to be the most dominant groups due to the nutrient enrichment at this particular site. However, scrapers were the most abundant group after predators. This site was filled with periphyton which is a dominant food resource for scrapers and could explain their abundance.

At the upstream site of Hartbeesspruit (H3), six different families were sampled but this was dominated by pollution-insensitive taxa during low-flow conditions. The most sensitive taxon found was Caenidae from the order Ephemeroptera. The high-flow results were similar with only 6 families identified and was also dominated by pollution-insensitive families with one pollution-sensitive taxon, Ancylidae. This site was located upstream and hence a higher diversity and abundance of macro-invertebrates were expected. The upstream site (H3) maintained the same IHAS score during low-flow and high-flow conditions and the habitat was largely modified with natural habitat being mostly unavailable to aquatic organisms. This is also depicted in Figure 24 and Figure 25. The physico-chemical parameters such as conductivity, TDS and pH decreased slightly at H2 during the high-flow season and increased slightly at site H3 during the high-flow season as seen in Chapter 3 (Table 3 and Table 4). The sites within Hartbeesspruit are surrounded by urban activities such as formal houses, schools, shopping complexes and guest lodges. A study done in nine metropolitan areas in the United States showed that macro-invertebrate assemblage's response varied in terms of the different types of urbanization. The study was done within common landscape settings such as housing, roads and developed land cover (Cuffney *et al.* 2008). There was a strong correlation present between macro-invertebrate responses and all types of urbanization, with the exception of agricultural. Temperature, however, was one of the strongest influences on macro-invertebrate assemblages and the rate at which the organisms responded to urbanization (Cuffney *et al.* 2008). Besides the routine monitoring by the DWS of the Apies-Pienaars catchment, only one study has been done on one of the rivers chosen for this study, namely the Hartbeesspruit. The study was conducted in 2013 and 5 sampling sites were selected within the catchment. Sampling took place in 6 week intervals and, the results showed that the most dominant factor determining macro-invertebrate communities in the area was the hydrological pathways which influenced the values and concentrations of surface water chemical and physical parameters such as temperature (Mulders 2015). This was confirmed at sites H2 and H3 where an increased temperature lead to the presence of more taxa even though they were pollution-insensitive taxa.

The sites within the Hartbeesspruit's main tributary, the Morelettaspruit, were also classified as hypertrophic. The PCA bi-plot also showed a strong positive correlation between the macro-invertebrate assemblages within both rivers, small differences were observed in the SASS5 scores. Two sites were sampled within Morelettaspruit, although a higher number of taxa were found at M1, the sample consisted mainly of pollution-tolerant taxa and this attributed to the low ASPT score. The upstream site (M2) had less taxa but more pollutant-sensitive taxa such as Ecnomidae and Psychomyiidae from the order Trichoptera. This was expected as the upstream site is associated with pollution-sensitive taxa. ASPT scores for both sites decreased during the high-flow season. Both sites within Morelettaspruit were dominated by predators during both seasons. Scrapers and collector grazers were the only other FFGs present in these rivers. Both sites were characterized by their rocky appearance and the IHAS reflected an abundance of stones. This habitat is commonly associated with predators as was seen in Pienaars River.

Although the physico-chemical parameters varied between sampling sites (Chapter 3 –Table 3 and Table 4), these parameters were assessed against the Target Water Quality Guidelines (TWQG) for aquatic ecosystems (DWAF 1996) and each parameter fell within the Target Water Quality Ranges (TWQR) deeming the water fit for the survival of aquatic organisms.

4.5. Conclusion

When comparing the upstream site of each river to the downstream site; the upstream site of each river proved to be the least impacted site with the highest level of diversity. The downstream sites close to the inflow of Roodeplaat Dam were more impacted with a visible reduction in the diversity and abundance of macro-invertebrate taxa. This was with the exception of Edendalespruit as there was only one site sampled and no comparison could be done within the river. The upstream sites also had more pollution-sensitive taxa and this was most likely due to the fact that there was less human disturbances surrounding these sites. When comparing individual sites, site P2, the downstream Pienaars River site situated downstream of the Wastewater Treatment plant was found to be the most impacted site. Interestingly, an overall comparison of results between the three rivers showed that the Pienaars River (with the exception of P2) had the highest diversity and SASS5 and ASPT scores during the low-flow season and Hartbeesspruit had the highest diversity and ASPT scores during the high-flow season. In terms of FFGs, the predators dominated in the majority of sites sampled which could possibly explain the absence of other FFGs as surrounding land uses such as agriculture and organic run-off could provide the nutrients needed by these FFGs, which in turn provided sufficient food sources for the predators. It is important to mention that there was a wider variety of FFG during the high-flow season when compared to the low-flow season in each river. An overall comparison of macro-invertebrate assemblages

between high-flow and low-flow conditions of the rivers, supported the fact that there was indeed a trend whereby the high-flow SASS5 score was lower when compared to the low-flow conditions, confirming that macro-invertebrate abundance is indeed affected by temperature and flow i.e natural fluctuations. The statistical groupings showed similar assemblages between the macro-invertebrates sampled in Hartbeesspruit and Morelettaspruit during both seasons showing a positive correlation between these sites. This makes sense as the Morelettaspruit is a tributary of the Hartbeesspruit. The same was observed within the Pienaars River with the exception of site P2 during high-flow conditions.

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Chapter 5: Fish Health

5.1 Introduction and background information

Fish have a longer life span when compared to other aquatic organisms. This is one of the main reasons why they are considered to be reliable indicators of changes in any aquatic ecosystem (Roberts 2001). They play a significant role in freshwater ecosystems since some species are at the top of the aquatic food chain and are commonly consumed by humans. This makes them important test organisms for assessing the effects of pollution. Most fish communities respond notably and predictably to a wide range of anthropogenic disturbances such as eutrophication, acidification, pollution and human exploitation (Roberts 2001). As inhabitants of most aquatic ecosystems, they are in constant contact with contaminants which may be present in the water. Fish, therefore, along with other aquatic biota, act as indicators of the overall ecological condition of any aquatic ecosystem. This is the primary reason why they are utilized in monitoring environmental degradation (Roberts 2001; Dallas and Day 2004).

One way of monitoring the effect of environmental pollution in fish is through identifying histological changes in target organs (Stebbing 1985; Swee *et al.* 1997; Van Dyk *et al.* 2012). This comprises of microscopically examining the cells and tissues of an organ and could be semi-quantitatively assessed as seen in the protocol utilised by Bernet *et al.* (1999). Microscopic evaluation is known to efficiently and quickly detect acute and chronic adverse effects in fish (Myers & Fournier 2002; Oliveira Ribeiro *et al.* 2005; Miranda *et al.* 2008). Because of its reliability and effectiveness, analysis of histological changes in different fish tissues has internationally been employed for a number of years in aquatic toxicology to effectively monitor acute and chronic exposure (Wester and Canton 1986; Wester and Canton 1991; Johnson *et al.* 1993; Perry and Laurent 1993; Stentiford *et al.* 2003; Schwaiger *et al.* 2004; Lang *et al.* 2006; Nero *et al.* 2006; Monteiro *et al.* 2008; Van Dyk and Pieterse, 2008; Olarinmoye *et al.* 2009; Van Dyk *et al.* 2009; Sousa *et al.* 2013a, b). When studying the effects of contaminants within an indicator organism, it is important to choose a species which is ecologically relevant to the area.

The indicator species used for this study were the Sharptooth catfish *Clarias gariepinus* (Burchell 1822) and Mozambique tilapia *Oreochromis mossambicus* (Peters 1852). Both these species are indigenous to South Africa. *Clarias gariepinus* is an omnivorous fish which also

preys on other fish whereas *O. mossambicus* feeds only on algae and invertebrates (Skelton 2001).

Clarias gariepinus (Figure 27) is an air-breathing catfish with a scale-less, bony elongated body and a helmet-like head (Skelton 2001). They have long dorsal and anal fins and are usually dark to light brown dorsally with a pale cream to white ventral colour (Skelton 2001). According to Skelton (2001), *C. gariepinus* is one of the fish with the widest distribution in South Africa. They are tolerant of many different habitats but are regarded as a freshwater species (Skelton 2001). *Clarias gariepinus* can tolerate high turbidity and low dissolved oxygen in waters. In pools of drying water, *C. gariepinus* is known to often be the last or only surviving fish (Safriel & Bruton 1984; Van der Waal 1998).

Figure 27: Sharptooth catfish (*Clarias gariepinus*)

Oreochromis mossambicus (Figure 28) is a deep-bodied fish with almost symmetrical, tapered extensions to the rare edge of the single dorsal and anal fins. They occur mainly in coastal regions and are typically found in slow-flowing rivers and streams in both brackish and fresh waters (Froese and Pauly 2007; Luna 2012). Although they are able to live in different salinities, these fish cannot live in waters with temperatures less than 10 °C (Trewewas 1983). The female and non-breeding male is silver grey to pale olive and the breeding male is known to be dark olive to black with white to yellow lower parts of the head (Luna 2012). In tropical waters, they tend to breed throughout the year but in colder waters the breeding season is

significantly shorter (Neil 1966; Bruton and Bolt 1975; De silva and Chandrasoma 1980). It is highly adaptable and therefore regarded as a potential pest (Skelton 2001).

Figure 28: Mozambique Tilapia (*Oreochromis mossambicus*)

5.1.1. Histopathology as a biomarker

Histopathology can provide valuable information on the effects of water quality and environmental stress on selected target organs. This manner of environmental monitoring has proved to be effective in monitoring contaminants in different aquatic habitats ranging from estuarine, to marine and freshwater. A study by Stentiford *et al.* (2003) showed fish affected by different contaminants, including but not limited to, polycyclic aromatic hydrocarbons (PAH). Sampling was done over two seasons to establish seasonal variability. The results showed a wide range of pathologies in each species. The most important conclusion of this study was that histopathology can be used as a reliable and effective tool when monitoring the effects of anthropogenic contaminants in estuaries (Stentiford *et al.* 2003).

A study conducted in marine ecosystems by Giltrap *et al.* (2017) aimed to integrate histopathology with biological effects and contaminant measurements to determine the health of the fish in Ireland (Giltrap *et al.* 2017). The study focused on PAHs and polychlorinated biphenyl (PCB) contamination in four different locations using two different flatfish species. The results showed low levels of PCBs in the fish liver, with fish from three of the four locations selected (Cork, Dublin and Shannon) showing the highest prevalence of lesions in the liver.

This, however, was most likely related to a carcinogenic pathway (Giltrap *et al.* 2017). The overall conclusion of the study highlighted the effectiveness of an integrated response with the inclusion of histopathological biomarkers as a useful tool to evaluate environmental risk (Giltrap *et al.* 2017).

Swee *et al*. (1997) used histopathology as a biomarker to determine the effects of different environmental contaminants on freshwater fish. Five sites were selected, three of which were contaminated and two were reference sites. One of the contaminated sites received effluent containing mixed contaminants from a nuclear weapon facility while another was impacted by kraft mill effluent. The third was contaminated by high levels of PCBs. There were certain pathologies that were common in fish from impacted and non-impacted sites, such as macrophage aggregates (MA), parasites, inflammation, glycogen deficiency, fatty changes in the liver and hyperplasia in the gills. Some pathologies were only found in fish sampled from impacted rivers. This ranged from cholangiomas, to metastatic thyroidal carcinomas in the spleen and hyperplasia of mucous and chloride cells (Swee *et al.* 1997). These lesions were very similar to those seen in fish exposed to specific pollutants in the laboratory (Meyers and Hendricks 1985; Hinton *et al.* 1992; Hinton 1993; Sindermann, 1993). The study suggested that different types of histological alterations can indicate different environmental stressors (Swee *et al.* 1997), however this follow-up studies are needed for comfirmation.

Another example of a similar study was the study conducted by Savaira *et al.* (2015). Thirty farmed Seabass (*Dicentrachus labrax* L.) were assessed to determine the level of histopathological alterations within each fish. The health status of the fish was assessed using the semi-quantitative histological system by Bernet *et al.* (1999). The gills, liver, kidney and intestines were assessed by using the organ index protocol as proposed by Zimmerli *et al.* (2007). Histopathological alterations such as hyperplasia, hyperaemia, hypertrophy and necrosis in the gills were present in 56.7% of the fish. An assessment of the kidney tissue revealed normal structure in 58.7% of the fish. The liver was normal with minimal cases of vacuolization, hypertrophy and hyperaemia. The histology of the intestine was normal. The results of the study concluded that fish histopathology is an effective biomarker due to the fact that it incorporates the effects of both biotic and abiotic aspects on both organ function and fish health (Savaira *et al.* 2015). The above-mentioned studies showed that environmental contaminants can have detrimental effects on the health of fish within a particular aquatic system, these contaminants vary in toxicity and their sources range from natural to anthropogenic. The sampling and analysis of different target organs are usually employed to determine the impacts on the overall health of the fish.

5.1.2. Histology of selected target organs

5.1.2.1 Gill as a target organ

Fish gills are the primary organ for gaseous exchange and ion regulation (Hinton and Laurén 1990). They are a significant target organ in terms of dissolved pollutants. This is mainly because they are continuously in contact with the water and changes in their morphology can be seen because of adaption strategies to maintain their physiological functions when under environmental stress (Jahanbakhshi and Hedayati 2013; Valbone *et al.* 2013; Omar *et al*. 2013; Torres *et al.* 2014). The gills are effective target organs due to their high sensitivity to environmental stress and their response to pollutants present in aquatic ecosystems (Hinton *et al.* 1992). This has been confirmed by many studies ranging from urbanization impacts to impacts as a results of contamination from detergents and metals (Abel and Skidmore 1975; Laurén and McDonald 1985; Verbost *et al.* 1987; Giari *et al.* 2007).

For example, histological, biochemical and ultrastructural features of *Channa punctata* was investigated to determine the effect of selected trace metals (Pandey *et al*. 2008). Fish were exposed to environmentally-relevant concentrations of four essential but toxic metals (Copper, Cadmium, Iron and Nickel) over three different time periods. The results from the light microscopy and scanning electron microscopy showed alterations in the structure of the gills caused by exposure to the metals as well as an alteration in the activities of antioxidant enzymes. The most significant physical alterations seen were deformation of of secondary lamellae, vacuolation and necrosis of filament epithelium (Pandey *et al.* 2008). A similar study was conducted by Abdel-moniem *et al.* (2012), the fish (*Oreochromis niloticus*) were collected from polluted wetlands in Saudi Arabia where water quality results showed elevated levels of heavy metal concentration, with the levels detected for Cadmium and Lead being above the WHO reference values. Histopathological alterations within the gills included hyperplasia, fusing of the lamella and aneurysms (Abdel-moniem *et al.* 2012).

A study by Van Dyk *et al.* (2010) conducted in two urban aquatic systems, in South Africa, reported on the selected impoundments that received effluent from sewage treatment plants and a variety of industries (Van Dyk *et al.* 2010). The results were compared to laboratory-bred fish and alterations were reported for fish from both impoundments. These ranged from circulatory disturbances (e.g. telangiectasia, epithelial lifting and hyperplasia of mucus cells) to structural alterations and regressive changes. The study suggested that elevated levels of pollutants in the selected impoundments could have resulted in the histological changes (Van Dyk *et al.* 2010).

5.1.2.2 Spleen as a target organ

The primary function of the spleen is to remove degenerated and aged red blood cells, it is also responsible for the removal of particulate materials and circulating bacteria from the blood supply. It is known as the primary site of extra-medullary haematopoiesis and for this purpose it contains hematopoietic and lymphoid elements (Suttie 2006). Histological alterations in the spleen can also be used as an indication of polluted environments. Fournie *et al.* (2001) conducted an analysis on the reliability of splenic macrophage aggregates as an indicator of degraded environments. Different species (a total of 983 fish) were sampled from 266 sites. The results showed that fish with splenic macrophage aggregates of high densities (40 mm^2), were found at 16 sites in one fish species. These particular sites were associated with low oxygen levels and sediment contamination leading to an increase in macrophage aggregates. The authors concluded that the study proved that splenic macrophage aggregates are useful indicators of pollution in aquatic habitats (Fournie *et al.* 2001).

Saxena and Saxena (2007) also showed the effect of heavy metal pollution in aquatic systems on lymphoid organs such as the spleen. Fish were exposed for a period of two weeks to water from a polluted system in Ludhiana, Delhi. The water had elevated concentrations of Iron, Manganese, Zinc, Nickel, Chromium, Copper, Lead and Cadmium. Within two weeks, 60% of fish died and histological analysis showed congestion, haemorrhages, lymphocytic infiltration and degenerative changes in the spleen and other organs such as the kidney, gut and liver (Saxena and Saxena 2007).

The histology of the spleen is also affected by the reproductive cycle as seen in the study by Rebok *et al.* (2011). Certain aspects of the salmonid spleen was analysed to determine the effect of the breeding cycle. Samples were collected and processed to be analysed using light microscopy. The results showed a random distribution of melano-macrophage centres surrounded by fibrous capsules. However, during the breeding season there was an increase in the white pulp and decrease in the red pulp when compared to the earlier stages. This, however, seemed to decrease and increase respectively before and after the spawning phases. The researchers concluded that there is a correlation between the sex steroid status and the pulp content levels within the female salmonid spleen (Rebok *et al.* 2011).

5.1.2.3 Liver as a target organ

The fish liver is located ventrally in the general cavity, 80% of the fish liver is made up of hepatocytes (Rappaport *et al.* 1954; Munshi and Datta 1996). The liver performs the following functions: xenobiotic metabolism, excretion, digestion and storage, bile secretion, metabolism

of lipid soluble drugs, synthesis of lipo-protein as well as secretion and urea formation. (Ross *et al.* 1989). Since the liver is a detoxification organ, it is commonly used as a target organ in studies to detect the effects of pollution on fish (Hinton *et al.* 2001). The liver also has a high metal accumulating capacity and in comparison to other organs, is more susceptible to histopathological alterations caused by metals. Liver histopathology was studied in *C. gariepinus* (Van Dyk 2012). The fish were collected from various sites ranging from un-impacted to polluted ecosystems. Fish collected from polluted sites displayed a greater prevalence of toxicopathic non-neoplastic and pre-neoplastic alterations. The conclusion of this study was that the use of liver histopathology in *C. gariepinus* is an effective biomarker for freshwater aquatic pollution (Van Dyk 2012).

Melano-macrophages is a particular type of macrophage known to occur in the parenchyma of fish livers (although they are not restricted to the liver), they are usually concentrated as melano-macrophage centres (MMCs) and size and content is highly dependent on their age, species and health status (Munshi and Dutta 1996). According to Ellis *et al.* (1976) their main function is phagocytic consumption of catabolic products and foreign material. This includes, but is not limited to injected carbon particles. Macrophage centres (with contents varying in amount and size) are quite conspicuous in the *O. mossambicus* liver and are commonly found in the hepatopancreatic tissue (Van Dyk 2006). In *C. gariepinus,* MMCs are commonly used as immunohistological biomarkers as seen in the study conducted by Sayed and Hounes (2016). The MMCs were used as a biomarker for toxicity of silver nanoparticles. A study conducted from 1980 - 1982 focusing on tumorous conditions in the ruffe *Gymnocephalus cernua L.,* suggested that MMCs increased during the development of several histopathological abnormalities of the liver and spleen (Kranz and Peters 1984). Similarly, a study was done on the effect of cadmium chloride on melano-macrophage centres in the liver, spleen and kidney in *O. mossambicus.* The study showed an increase in the MMCs of the exposed fish when compared to the control fish (Suresh 2009).

Although the liver is known as an important organ to assess toxic environmental conditions, it is important to bear in mind that the histopathological results from the liver analysis should be used in conjunction with other measured parameters such as body and organ weight measurements, microscopic observations in other organs and secondary sex characteristics (Wolf and Wheeler 2018).

5.1.2.4 Kidney as a target organ

The kidney is the most important excretory organ in most animals. It also plays a significant role in the maintenance of osmotic balances, haemopoietic and endocrine functions (Groman 1982; Willett *et al.* 1999; Rombout *et al.* 2005). The kidney is not commonly employed as a

reliable indicator organ, possibly due to the fact that there is a lack of biomarkers in kidneys (Hinton *et al.* 1992). Histopathological changes noticed in kidneys include “dilation and inflammation of glomerular blood capillaries, thickening of walls, destruction and fibrosis of the glomerulus, capillary sclerosis, and abnormal proliferation of epithelial cells within the Bowman capsules and glycogen infiltration of tubular epithelium” (Takashima and Hibaya 1995).

5.1.2.5 Heart as a target organ

The fish heart is not commonly regarded as a target organ thus, there is very little literature available on heart histology. However, the heart is known to show signs of deterioration due to metal pollution within a system (Exley 1996; Borges *et al.* 2003). As with every other organ, the macroscopic and microscopic appearance of the heart differs between *O. mossambicus* and *C. gariepinus,* however, the histological structures are fairly similar (Van Dyk 2006). According to Van Dyk (2006), the most commonly observed histopathological changes in the fish heart are muscle necrosis along with inflammation of the cardiac muscle endocardium or epicardium (Van Dyk 2006).

5.1.2.6 Gonads as a target organ

The normal structure of teleosts gonads is paired and elongated (Pieterse 2004). The testes of *O. mossambicus* is creamy white in colour while the testes of *C. gariepinus* are firm and transparent to yellow-white in colour depending on their stage of maturity (Van Dyk and Pieterse 2008). The ovaries of *O. mossambicus* according to Van Dyk (2006) is also paired but saclike in structure and light brown in colour (Van Dyk 2006). *Clarias gariepinus* ovaries are usually larger and lighter in colour when compared to *O. mossambicus* and have fewer spherical eggs (Van Dyk 2006). The appearance for both species depends on the stage of maturity (Pieterse 2004; Van Dyk 2006).

Studies have shown that a variety of endocrine disrupting chemicals and metals are known to target the testes of a number of species such as *C. gariepinus* and *O. mossambicus* (Barnhoorn *et al.* 2004; Pieterse 2004). Pieterse (2004) study exposed *Oreochromis mossambicus* to Copper in the laboratory to determine the levels of histopathological changes (Pieterse 2004). The alterations observed in the testes increased as the concentration of Copper increased (Pieterse 2004). There were no macroscopic abnormalities observed however histological abnormalities ranged from disorganisation of lobules to necrosis, disintegration of sertoli cells and interstitum, detachment of basal membrane and pyknosis (Pieterse 2004).

Another study conducted by Pieterse *et al.* (2010) analysed the histological changes seen in *C. gariepinus* collected from an urban nature reserve in South Africa. The study focused on

the testes and ovaries of this species in order to determine reproductive health effects in males and females. The water and sediment from where the fish were sampled were polluted by agrochemicals as well as industrial activities (Pieterse *et al.* 2010). There were no macroscopic abnormalities in both male and female fish. The histological analysis of the testes showed a number of alterations such as disorganization of the lobules, MMCs, infiltration of mono-nuclear leukocytes as well as testicular oocytes (Pieterse *et al.* 2010). Alterations in the ovarian tissue included structural alterations, MMCs as well as granulomas and seminiferous and ovarian tissue within the same ovary (Pieterse *et al.* 2010). Previous studies have confirmed that these alterations were seen as a result of fish exposed to metals and other contaminants, however the causative agents for these alterations in this particular study were not confirmed (Pieterse *et al.* 2010).

5.1.3. Biometric Indices

Biometric indices are used in fish health studies to determine the organ to body weight ratio. This ratio has commonly been used in stress-related studies and is used as general health indicators of individual organisms (Goede and Barton 1990). Commonly used biometric indices include the Condition factor (CF), Hepatosomatic index (HSI), Splenosomatic index (SSI), Gonadosomatic Index (GSI) and Cardiosomatic index (CSI).

- Condition factor

The CF is defined as the relationship between the length and mass of the fish. A value of 1 will generally indicate that the fish is healthy while a lower factor will indicate that there is a depletion of energy reserves such as stored body fat or glycogen in the liver (Brown *et al.* 1987; Adams *et al.* 1993). This could be a result of changed feeding patterns caused by a variety of stressors (Brown *et al.* 1987). The body shape of the fish also plays a role in the condition factor, i.e. rounded fish such as *O. mossambicus* had a recorded mean CF of 1.67 in a study conducted by Van Dyk in laboratory-bred fish in unpolluted water. The *C. gariepinus* which is longer, dorso-ventrally flattened fish had a mean CF of 0.67 (Van Dyk 2006).

Marchand (2009), conducted a study on *C. gariepinus* from two polluted dams in South Africa, Rietvlei Dam and Marais Dam. Both dams are known to have very high levels of toxicants (Marchand 2009). The condition factor of the fish sampled in this study ranged from 0.6 to 0.9. A similar range was also seen in other studies conducted on wild *C. gariepinus* (Crafford 2000; Watson 2001). Bervoets and Blush (2003) conducted a study in Flanders, Belgium where 14 metal polluted sites were selected and *Gobio gobio* fish tissue were collected for metal analysis. The metal concentration in the tissue correlated with the levels in the water and sediment, however, the condition factor of fish collected from different sites varied and was

not related to the environmental conditions within the river (Bervoets and Blush 2003). Therefore it is important to note that CF can be influenced by natural or seasonal aspects and not only pollution.

Cren (1951) attempted to determine the seasonal relationship between body weight and length with condition factor in Perch (*Perca fluviatilis*). The results showed that there was a significant difference in the CF during different seasons (being higher in summer); however this is directly related to the seasonal changes in gonadal size (Cren 1951).

- Hepatosomatic index

The ratio of liver mass to body mass is determined by the hepatosomatic index (HSI). This index also reflects changes like cellular hyperplasia, hypertrophy or atrophy (Van Dyk 2006). The previously mentioned study by Van Dyk (2006) showed that the normal HSI differs between species, for *O. mossambicus* the mean value for unaffected fish was recorded to be 1.08 and in *C. gariepinus* it was 1.30 (Van Dyk 2006). This index is incredibly important as the liver is the main detoxifying organ of the body.

The hepatosomatic index has been used as a biomarker of sewage pollution in *O. mossambicus*, as seen in the study conducted by Al-Ghais (2013). *Oreochromis mossambicus* from a sewage treatment plant in United Arab Emirates (UAE) and fish raised in a fish farm were compared. Cellular biomarkers, reduced glutathione (GSH) and the hepatosomatic index were tested as biomarkers. The results showed that lower levels of cellular biomarkers in the fish from the sewage treatment plant, and the GSH levels were higher in the liver and muscle tissue in fish from both sites. The HSI was also found to be higher in fish from treated sewage water when compared to the reference fish, thus suggesting that cellular biomarkers, GSH and HSI plays a very important role in identifying the effect that sewage pollution has on the health of fish (Al-Ghais 2013).

- Splenosomatic index

This index is the ratio of spleen mass to body mass ratio. The size of the spleen is known to be a beneficial diagnostic factor due to the fact that the spleen is a haematopoietic organ and any abnormalities such as enlargement or swelling of the spleen could indicate disease or immune system challenges (Goede and Barton 1990). Similarly, according to Payne *et al.* (1978) and Pulsford *et al*. (1995), a smaller spleen could also indicate that the organism has been exposed to organic contaminants (PCBs, PAHs). The SSI mean values of unexposed *C. gariepinus* were recorded as 0.04 and 0.06 for *O. mossambicus* (Van Dyk 2006).

The SSI is also affected by oxygen levels within the aquatic ecosystem. Lai *et al*. (2006) conducted a study on the effect of low oxygen levels and the relationship between

erythropoietin (EPO) levels and SSI levels. Rainbow trout (*Onchorhynchus mykiss*) was reared at the British Columbia University, Vancouver and exposed to normoxic and hypoxic conditions for two months. The SSI dropped after exposure to hypoxic conditions which was related to a drop in the EPO. The study concluded by reporting that fish attempt to counteract hypoxia by increasing red blood cell numbers and the concentration of haemoglobin in the blood, this is achieved by splenic contractions (Lai *et al.* 2006).

- Gonadosomatic index

The gonadal organs response to changes or stresses in the environment can be assessed using the gonadosomatic index (GSI). Since this index gives an indication of the gonadal development and sexual maturity it is affected by the age and gender of fish as well as by specific pollutants (Patnaik *et al*. 1994; Schmitt *et al*. 2004).

The gonadosomatic index is also known to give an indication of reproductive disturbances as was seen in the study conducted on *Gobus niger* in Bizerta lagoon, Tunisia (Loiuz *et al*. 2009). The fish were collected from the lagoon, which is under immense industrial pressure and subject to many anthropogenic disturbances. One reference site was chosen and the fish from the lagoon showed a lower GSI when compared to the fish collected from the reference site. These low GSI values were found to be associated with histopathological changes. Organic pollutants (DDT, PAHs and organotins) were also detected in the sediment collected. There was also a positive correlation between the organic pollutants detected and the histopathological changes observed (Loiuz *et al*. 2009).

5.1.4. Fish health and pollution

Pollutants in aquatic systems can adversely affect the health of fish (Austin 1999). This includes: eutrophication resulting in algal blooms, a natural occurrence that can result in deteriorating water quality, the bioaccumulation of organic and inorganic pollutants in fish tissues; high levels of pollutants that can result in mass mortality, exposure to pollutants can affect the immune system of fish making them more vulnerable to being attacked by pathogens as well as faecal coliform contamination which can potentially result in the development of specific fish diseases (Austin 1999).

5.1.4.1 Organic and Inorganic Pollution

Metal contamination in aquatic environments is usually a consequence of anthropogenic activities ranging from industrial, agricultural, sewage-treated water, urban runoff and domestic waste (Heath 1987; Pinto *et al.* 2003; Sampaio *et al.* 2008). Unnaturally high levels of metals in aquatic systems can lead to oxidative stress which eventually results in cell death in aquatic organisms (Lushchak 2011). Trace metals such as Iron, Copper and Zinc are

important in the normal functioning of cells but when these metals are in excess they could lead to impairment of cellular functions (Stohs and Bagchi 1995).

A study was conducted in 2001 in Nigeria, focusing on the effects of industrial wastewater runoff on fish health. The primary focus area was tannery effluent that is the most polluting of industrial wastewaters. It contains heavy metals such as Arsenic, Chromium, Copper, Iron, Lead and Zinc. *Clarias gariepinus* was selected as the test organism and exposed for eight weeks to lethal concentrations of tannery effluent. The liver, muscle, intestine, gills, bones and skin were removed after dissection. The results showed that the metal accumulation was significantly higher in the liver, possibly due to the detoxification and accumulation role of the liver. The gills and intestine also had high levels. The muscle tissue, however, had the lowest amount of metal accumulation (Gbem *et al.* 2001). The study conducted by Avenant-Oldewage and Marx (2000) confirmed that metal accumulation is commonly found to be the highest in the gills and liver. Bioaccumulation of different metals (Copper, Chromium and Iron) was studied in selected organs (gills, skin and liver) and muscle tissue in *C. gariepinus*. Fish were sampled over four seasons from the Olifants River in the Kruger National Park at two different locations within the river. Chromium had the highest levels within the gills whereas the highest levels of Copper and Iron were found in the liver. The results showed that high levels of contaminants such as metals in aquatic environments are likely to accumulate in the tissues of aquatic organisms. Therefore, the levels need to be monitored to decrease the detrimental effects of these metals on fish health (Avenant-Oldewage and Marx 2000).

In a similar study conducted on *O. niloticus*. The liver, muscle, gills, stomach, intestines, scales and vertebral column were used to determine different levels of Chromium, Copper, Manganese, Nickel, Selenium, Zinc and Cobalt. The liver had the highest levels of Copper and Zinc, whereas the stomach and intestine had the highest levels of Manganese. The results also proved that heavy metal contamination within *O. niloticus* is different within the different stages of fish growth (Rashed 2001). These results are not uncommon as a number of studies globally have shown that metal accumulation is mostly higher in the liver and less significant in the muscle tissue (Canli and Atli 2003; Vinodhini and Narayanan 2008; El-Moselhy *et al.* 2014). Other pollutants are known to not target one organ specifically but focus on negatively affecting an entire system such as the endocrine system, reproductive health, and/ or developmental behaviour, these are commonly known as Endocrine Disrupting Chemicals (Wang and Zhou 2013).

5.1.4.2 Endocrine Disrupting Chemicals (EDCs)

The effects of EDCs on immune systems of fish was documented in 2014 by Bado-Nilles *et al.* who studied at the immunotoxic effects of EDCs with the use of splenic immune cells from

the female three-spined stickleback, *Gasterosteus aculeatus*. Ninety adult female three-spined stickleback were collected and the spleen tissue was removed from each fish. Although further studies need to be conducted on the effects of EDCs on the immune system, this particular study proved that "natural hormones acted as immunostimulants; whilst EDCs acted as immunosuppressants" (Bado-Nilles *et al.* 2014). The effects of EDCs on the reproductive system were further investigated by Zheng *et al.* (2015). The concentrations of phenolic EDCs in Hun River (China) and the incidence of sexual complications such as intersex in the wild crucian Carp (*Carassius carassius)* was assessed. Muscle and gonadal samples were collected and analysed using gas chromatography-mass spectronomy (GS-MS) and light microscopy respectively. The concentrations of EDCs in the muscle tissue correlated with the occurrence of intersex in fish. Based on the low level of sensitivity of the wild crucian carp and the fact that intersex occurred in this species, it is likely to occur in more sensitive species as well. It is also important to note that the concentrations of Bisphenol, Nonylphenol and Octolyphenols were noticeably high downstream of the Sewage Water Plant (Zheng *et al.* 2015).

Similar results were reported in Ibadan, Nigeria. Tilapia species were collected from a freshwater dam which receives discharge from a number of anthropogenic sources, and the endocrine disruptor responses were analysed along with the occurrence of intersex and gonado-histopathological changes (Adeogun *et al.* 2016). The fish were collected between June and September 2014 and a total of 132 fish were sampled. There was a high prevalence of intersex (34.8%) amongst both male and female fish, despite the presence of two pairs of testes and one pair of ovaries. Sediment samples collected showed high levels of contaminants such as heavy metals, phenols and PCBs. The researchers therefore concluded that the introduction of EDCs into the water system by human activities contributes to the intersex condition and is more prevalent in male fish (Adeogun *et al.* 2016).

Although a number of studies have shown that EDCs could be responsible for intersex, Bahamonde *et al.* (2013) questioned whether this is not merely a natural phenomenon in teleost fish. There has been approximately 37 species from 17 different teleost families in over 54 studies with the intersex condition and the fact that fish from reference sites also showed signs of intersex raised questions that perhaps this is a normal occurrence in teleosts (Bahamonde *et al.* 2013). Bahamonde *et al.* (2013) recommended that studies rather focus on how the intersex condition is expressed more in polluted system as compared to reference sites and that researchers take into consideration affected males as well as unaffected males when trying to reach a conclusion. It was also suggested that studies potentially differentiate between various pathways associated with intersex (Bahamonde *et al.* 2013). There are many

different forms of EDCs, some are more common than others. One very common example is organochlorine pesticides (Gore *et al.* 2014).

5.1.4.3 Organochlorine pesticides

Organochlorine pesticides are known to adversely affect the health of aquatic organisms. This has been observed in a number of studies. These impacts ranged from hyperplasia to blood congestion in the secondary lamellae, hypertrophy in gills, pyknotic nuclei, testicular alterations, focal necrosis and hydropic degeneration in liver (Capkin *et al.* 2006; Da Cuna *et al.* 2011). A study was carried out by Satyanarayan *et al.* (2012) to determine the effect of chlorinated hydrocarbon pesticides on the gills, liver, intestine and kidney of the *Cyprinus carpio.* There were different levels of accumulation of each pesticide observed in each organ and results ranged from damaged secondary gill lamellae, thickening in the gill filaments, fatty degeneration and necrosis in the liver to structural abnormalities in the glomeruli of the kidney (Satyanarayan *et al.* 2012). Marcon *et al.* (2015) focused specifically on the effects of organochlorine insecticides on the liver of *Astyanax bimaculatus* (Marcon *et al.* 2015). Fish were exposed to different sublethal concentrations of a particular insecticide. The results, interestingly, showed no morphological changes in the liver as a whole but alterations were observed in localized areas such as nuclear deformities, hypertrophy and degeneration of the hepatic cells (Marcon *et al.* 2015).

In South Africa, Marchand (2008), Marchand *et al.* (2012) and Mooney (2012) found similar results following a histopathological assessment of *C. gariepinus* exposed to different pesticides. This included neoplastic growths in the ovaries of specimens (Marchand 2008; Mooney 2012). Additionally, Marchand *et al.* (2012) found testicular oocytes in *O. mossambicus* inhabiting a system with detectable levels of organochlorine pesticides (Marchand *et al.* 2012).

In rare cases, organochlorine pesticides have little to no effect on the health of fish inhabiting the system. In 2011, McHugh *et al.* collected *Hydrocynus vittatus* from a DDT-infected area and conducted a histology-based fish health assessment. The selected site in Kwa-Zulu Natal is classified as an intermediate to low risk malaria area and is therefore continually treated with DDT for malaria vector control (McHugh *et al.* 2011). The fish species selected for this study has a protected status and is an important food source. The study focused on 3 different organs (liver, gills and kidney) for histopathological analysis. Results showed mild alterations in all the organs analysed and the histology-based fish health assessment protocol indicated that the fish were in a generally healthy state (McHugh *et al.* 2011). Although this study showed that pesticide pollution does not always have an effect on aquatic organisms within a system,

in many cases it may result in eutrophication, which is known to have detrimental effects on aquatic organisms (Shi 2017).

5.1.5. Eutrophication

The impact of eutrophication on aquatic organisms, especially fish, has been studied globally and this ever-growing problem has been known to affect various species in different ways, mostly negative (Smith *et al.* 1999; Verdonschot and Nijboer 2004). Effects ranged from increased haematocrit values to macroscopic and microscopic alterations in target organs (Taylor and Miller 2001; Gupta and Guha 2006; Atencio *et al.* 2008; Pathiratne *et al.* 2010; Marchand *et al.* 2012). Smith *et al.* (1999) also mentioned that some of the effects of eutrophication on lakes and reservoirs include increased production and harvest of fish, a shift in the composition of fish species which is most likely to be in favour of less desirable species and an increase in the number of fish kills in a particular system (Smith *et al.* 1999). Other studies have shown that eutrophication can also lead to an increase in cyprinid species and a decline in salmonid and predatory fishes numbers, as well as a change in the taste of fish caught making it less suitable for human consumption (Colby *et al.* 1972; Nümann 1972; Hartmann and Nümann 1977; Persson 1981, 1985; Persson *et al.*1991; Tammi *et al.* 1999).

Eutrophic systems are also commonly plagued by hepatotoxins such as microcystins which are the main cause of pathologies observed in fish inhabiting these systems (Jia *et al.* 2014). Microcystins are the products of algal blooms, they are toxic and are known to accumulate in fish (Jia *et al.* 2014). They have been implicated in the death of birds, livestock, fish and even wild animals (Carmichael 1994; Kaebernick and Neilan, 2001). Due to the fact that they are hepatotoxins, the liver is known to accumulate the highest levels of (Jai *et al.* 2014).

Jai *et al.* (2014) conducted a study to determine the bioaccumulation of microcystins in four fish species and the risk to humans in Lake Taihu, China. Four regions within Lake Taihu were selected as sampling sites, due to their predisposition to algal blooms. A total of 46 fish from four native species were sampled, these fish were all economically important. The results showed that each analogue of microcystins had different concentrations in the different organs (liver, muscle, kidney and intestine), with the lowest levels seen in the liver which was contrary to results from previous studies (Kotak *et al.* 1995; Best *et al.* 2003; Qui *et al.* 2007; Marie *et al.* 2012; Papadimitriou *et al.* 2012; Woźney *et al.* 2016). The concentrations of microcystins also depended on the species, exposure time and environmental factors such as temperature, pH and nutrient concentrations (Rinehart *et al.* 1994; Harada 1995; Harada *et al.* 1996). A study by Malghaes *et al*. (2003) focused particularly on the bioaccumulation of microcystins in fish and crustaceans in Sepetiba Bay, Brazil. Phytoplankton samples were collected from the water and muscle samples were collected from the fish. The levels of cyanobacteria in the

water were lower than the levels proposed by WHO. In the fish muscle, only 19% of the fish sampled had microcystin levels above the proposed WHO standards (Malghaes *et al*. 2003).

Hepatic abnormalities were seen in a number of studies with fish exposed to microcystins. Pathiratne *et al.* (2010) assessed different biological pollutants and their effects in the hyper-eutrophic Lake Beira in Sri Lanka. Although the lake is prone to a mixture of pollutants, the hepatic abnormalities seen in the test specimen (*O. niloticus)* was said to be as a result of recurrent exposure of fish inhabiting the Lake to PAHs and *Microcystis* blooms (Pathratne *et al.* 2010). In terms of laboratory studies, *C. carpio* was administered microcystins by different pathways (gavage, immersion and intraperitoneal) although the level of necrosis observed was dose-dependent the affected organs were liver, gills and kidneys, with no pathology observed in the heart, spleen or intestines of the *C. carpio* (Carbis 1996). Different target organs are studied when it comes to fish health; this is mainly because different organs will react differently to different toxins (Jorgensen 2010).

Fischer *et al.* (2000) specifically analysed microcystin-LR concentrations in the liver of *Oncorhynchus mykiss*. Results showed histological alterations such as hepatocyte necrosis and morphological changes in the hepatic cells after 48 hours of exposure to microcystin-LR (Fischer *et al.* 2000). Li and Xie (2009) found similar results in the Silver carp *Hypophthalmichthys molitrix* exposed to different concentrations of microcystin -LR, although this species seemed to be more resistant to the microcystin -LR (Li and Xie 2009). However, Schmidt *et al.* (2013) previously mentioned that there may be species differences observed in terms of microcystin content (Schmidt *et al.* 2013). Mitsoura *et al.* (2012) in a study on the effects of microcystin-LR and RR in a cyanobacterial rich lake in Greece, showed histopathological alterations occur mainly in the kidney and liver. These changes included hydropic degeneration and necrosis in organs, as well as focal necrosis, granular glycogen, hepatocyte pyknosis (Mitsoura *et al.* 2012).

Due to the fact that Roodeplaat Dam has been classified as a eutrophic system, a number of studies have been conducted in the system to determine the effect of eutrophication on the fish health.

5.1.6. Previous fish health studies conducted in Roodeplaat Dam

Previous studies on fish health in the Roodeplaat Dam have ranged from studies concentrating solely on Roodeplaat Dam, to comparative studies between other eutrophic systems in South Africa (Marchand *et al.* 2012; Van Dyk *et al.* 2012; Van Dyk 2014; Barnhoorn *et al.* 2015). Although there were no fish health studies conducted in any of the inflowing rivers, these previous studies highlighted the impaired health of fish inhabiting this aquatic system.

The first study that aimed to determine the fish health of *O. mossambicus* in Roodeplaat Dam, was conducted by Hohls and Van Ginkel (2004). This study was necessitated by reported fish kills in the dam. The results showed hepatic alterations and proliferative branchiitis. The researchers suggested that this was as a result of the cyanobacteria, high levels of organic contaminants and low oxygen levels within the dam (Hohls and Van Ginkel 2004). More recently, Marchand *et al.* (2012) studied health-related aspects of *O. mossambicus* and *C. gariepinus*. The study provided baseline data for fish health and bio-monitoring studies on these two indicator species from Roodeplaat Dam. Histopathological changes in *O. mossambicus* and *C. gariepinus* were assessed. Six target organs were selected namely the gills, liver, ovaries, testes, kidney and heart. There were no external macroscopic abnormalities for any of the fish. A macroscopic assessment of the internal organs revealed that 65% of the *C. gariepinus* had liver alterations which included nodules and fatty appearances and two *O. mossambicus* had deformed testes. A histology-based fish health assessment including a semi-quantitative histopathological assessment of the selected organs was done. These alterations differed in severity as well as the type of alterations indentified in the kidney and testes. Histopathological alterations were concentrated in the liver of both species (Marchand *et al.* 2012). However, the mean liver index was 20.89 in *C. gariepinus* and 12.89 in *O. mossambicus*. The oxygen, pH, ammonia, Kjedahl nitrogen, chloride and phosphorus was high, thus raising concerns about the quality of the water. The researchers suggested that the poor water quality resulted to the liver pathology and other histological alterations (Marchand *et al.* 2012).

A comparative study was subsequently conducted using the liver of *C. gariepinus* as a biomarker for aquatic pollution Van Dyk *et al.* (2012). Thirteen polluted and un-impacted sites in South Africa were selected for the study. The aim was to determine whether the health status of the species could reflect the pollution status of the selected aquatic systems. The results revealed CFs, liver indices and hepatosomatic indices which were significantly higher in polluted systems in comparison to un-impacted systems (Van Dyk *et al.* 2012). In addition to this, the results also revealed that fish from Roodeplaat Dam had the highest prevalence of macroscopic abnormalities, amongst the highest prevalence of histological alterations and the highest liver index. A more recent paper reported cholangioma (a neoplastic growth), in the liver of *O. mossambicus* sampled from the Roodeplaat Dam (Van Dyk 2014). This was the first report of this type of condition in the specific species. The presence of cholangioma in fish have been previously associated with parasitic infection and exposure to contaminants (Boorman *et al.* 1997; Blazer *et al.* 2006)

More recently, Barnhoorn *et al.* (2015) assessed the levels of organochlorine pesticides in *C. gariepinus* by sampling fish from Roodeplaat Dam and two other polluted freshwater

impoundments (Rietvlei and Hartebeespoort dams). A health risk assessment was conducted and the muscle tissue was sampled from each fish to test the levels of OC pesticides. One OC pesticide (Dieldrin) was detected above the acceptable risk levels estimated at concentrations for which a cancer risk is likely. The fish collected from Hartebeespoort Dam had no toxic risks. Although the sites are not situated close to areas where DDT is used, the residue was still found within the muscle of fish collected from both impoundment. This could be due to the illegal use of pesticides in the surrounding areas (Barnhoorn *et al.* 2015). The abovementioned studies all reported liver pathology in fish sampled from Roodeplaat Dam, however, no studies have investigated the bioaccumulation of microcystins in fish inhabiting the system.

5.2. Methodology

5.2.1. Fish collection and necropsy

As mentioned in Chapter 2, fish collection was done at the downstream sites of each of the rivers (sites E1, P1 and H1). This was motivated by the fact that the downstream site of each river represents an amalgamation of all contaminants from upstream pollution sources before entering the dam. Also, the downstream site of each river was wide enough to launch a boat and release gillnets to enable the collection of an appropriate sample size of large enough fish to conduct a fish health assessment. However, electroshocking to collect fish was done at all sites located in the mid- and upper reaches of the rivers, but in most cases revealed the absence of the required study species. The fish were collected using gill-nets. Permits were obtained from the Department of Nature Conservation in June 2015 to collect and convey wild animals for scientific purposes (Permit Number: 000176). The fish were then transported in aerated water baths to a nearby area where a field laboratory was set up. Permission was obtained from the respective landowners surrounding the study area to work on their property. Seventy *C. gariepinus* and 61 *O. mossambicus* were collected in total. The availability of fish varied between seasons and hence the sample sizes varied across rivers and between seasons.

Each fish was assessed separately. The first step entailed weighing the fish using a lip-grip scale (g) and measuring the total length (mm). The mass of each fish was recorded with and without the gonads. The following biometric indices were determined for each fish; condition factor (CF), hepatosomatic index (HSI), splenosomatic index (SSI) gonadosomatic index (GSI) and the cardiosomatic index (CSI). Fulton's condition factor was calculated for each fish according to Carlander (1969) as follows:

$$CF = weight\ (g) \times 10^5 / length^3\ (mm)$$

The somatic indices (HSI, SSI, GSI and CSI) were calculated as follows:

Somatic index = organ mass / body mass x 100

The fish were then placed on a clean flat surface where an external examination was done to identify any macroscopic abnormalities. Thereafter, the fish were sedated by covering the eyes with a cloth and then sacrificed by severing the spinal cord anterior to the dorsal fin. This was done in a quick and professional manner to prevent any unnecessary suffering. Ethical clearance was obtained from the Ethics Committee of the Faculty of Science, University of Johannesburg. The gills were removed followed by a ventral incision to expose the internal visceral organs. The liver, heart, spleen, gonads and kidneys were removed and the sex of the fish was recorded.

5.2.2. Histology-based fish health assessment

5.2.2.1 Tissue sampling, processing and histological analysis

A tissue sample was collected from each target organ at the same morphological region to allow for comparative histological analysis. Tissue samples were fixed in 10% neutrally-buffered formalin for 24 hours. Thereafter fixed tissue samples were washed in running tap water for an hour and dehydrated in escalating concentrations of ethanol (30%, 50%, 70%, 80%, 90%, 95%, 100%) for an hour in each concentration. The samples were then transferred into a transitional solvent, Xylene, for 5-30 minutes until they were transparent and then transferred into a second series of Xylene and wax for 5 minutes, where after they were transferred to a 60 °C oven to be infiltrated by wax.

Infiltration of samples entailed placing them into a Paraffin wax and Xylene (50:50 ratio) for 30 minutes and then in a series of pure melted wax in 60 ºC and left over night. The tissue was then embedded into paraffin wax blocks. The embedded samples were left to cool at 4 ºC and then sectioned (5 µm thick) using a Leica RM2125RT microtome. The sectioned wax ribbons were then stretched on an albumin / glycerine and distilled water solution, mounted on glass microscope slides and air dried. There after the dried sections were prepared for light microscopy analysis using standard techniques for Haematoxylin and Eosin staining. The stained sections were then mounted with cover slips using Entellan. Two slides of each organ for each fish specimen were prepared resulting in a total number of 1572 slides. Each slide was analysed using a multi-headed Olympus light microscopy by two assessors to increase the accuracy and objectivity of the results. Light micrographs were produced using an Axioplan 2 microscope at the Spectrum analytical facilities of the University of Johannesburg.

A semi-quantitative histological assessment was done to allow for the statistical comparison between the histological results, based on the protocol by Bernet *et al.* (1999). In brief, each alteration was assigned a score ranging from 0 - 6 where 0 would indicate that the organ remained unchanged, 2 would indicate a mild occurrence, 4 a moderate occurrence with 6 indicating severe occurrence (Bernet *et al.* 1999). The score value was then multiplied by the importance factor of either 1 (minimal pathological importance), 2 (moderate pathological importance), or 3 (marked pathological importance) (Bernet *et al.* 1999). The index values calculated for all organs within a fish were then summed to obtain a total organ index or "Fish Index" per fish.

The scoring scheme designed by Zimmerli *et al.* (2007) was used to evaluate the results. This was adapted by Van Dyk *et al.* (2009) using index results for *C. gariepinus* and *O. mossambicus*. They were classified as follows:

Class 1 (index <10) — normal tissue structure with slight histological alterations

Class 2 (index 10-25) — normal tissue structure with moderate histological alterations

Class 3 (index 26 -35) — pronounced alterations of organ tissue

Class 4 (index >35) — severe alterations of organ tissue

5.3.3 Microcystin Analysis

As shown in Chapter 3, the water quality results confirmed the hypertrophic status of the three rivers as well as the presence of the hepatoxin, microcystin-LR in the water samples. Furthermore, previous literature has shown that fish in this system show a definite hepatic response (Marchand *et al.* 2012; Van Dyk *et al*. 2012). Therefore, liver samples were collected from each fish for microcystin analysis using the protocol adapted from Xie and Park (2007). Liver samples were stored at -20 °C until further analysis could take place. Each sample was cut into small pieces and places into 45 mL plastic tubes. These samples were then labelled accordingly and placed into LabConco FreeZone 2.5 Freeze drier machine. The vacuum collector was set at High Bar at -45 ºC for a period of 72 hours until the samples were completely dry. The dry samples were then removed from the freeze dry machine and grinded into powder form using a pestle and mortar and then stored at -20 ºC. Thereafter, 2 g were weighed of each sample. Samples less than 2 g were weighed to 4 decimals. For these samples, adjustments were made accordingly in the analysis.

Empty tubes were placed in a rack for conditioning at the research facilities of University of South Africa (UNISA), these were then conditioned using 3 mL 100% methanol and then 3 mL

of distilled water. This was followed by adding 20 mL of each sample into the tubes (2 mL at a time), extra care was taken to ensure that samples do not run dry. The samples were then washed with 20 mL methanol. Thereafter each sample was then placed onto a rack and 15 mL (1 mL per minute) of methanol was added. 10 mL of a Butanol, Methanol and distilled water (1:4:5) solution was placed into each weighed sample and the sample was then sonicated in an ELMA S30H sonicator for 3 minutes. The sample was then removed from the sonicator and centrifuged in a Hettich Universal 320R Centrifuge for 20 minutes at 4500 rpm. The supernatant was decanted into a clean 15 mL plastic tube, this process was repeated 3 times per sample. Samples were then stored in -20 ºC until evaporation could take place.

Each sample was evaporated to dryness at the Applied Chemistry laboratory, University of Johannesburg, Doornfontein Campus. Thereafter the samples were stored in the – 20 °C until preparation for microcystin analysis could take place. The dried samples were reconstituted using 100 µL of absolute methanol and thereafter each sample was centrifuged for 15 minutes using an Eppendorf 5702 centrifuge to separate the supernatant. A volume of 70 µL of the supernatant was extracted using a pipette and then placed into a 250 µL polypropylene insert which was held in a 2 mL clear Target DP vial.

A mixed multi standard containing microcystin LR, YR and RR (~5 µg of each in 1 mL of methanol) from Cyano Biotech GmbH (Berlin, Germany). LC-MS grade acetonitrile, methanol and formic acid from Romil (Cambridge, UK) and ultra-purity water of 18.2 MΩ cm-1 was processed with a Milli-Q Q-POD purification system from Millipore (Bedford, MA, USA). Pierce LTQ ESI positive and negative ion calibration solution from Thermo Fisher Scientific (Rockford, IL, USA).

A stock solution of the standard (1 mg/L) was prepared by diluting the standard solution appropriately using methanol. The stock solution was stored in an amber vial at −20 °C. The stock solution was used to prepare calibration solutions by dilution in 50:50 acetronitrile/water. A Thermo Fisher Scientific Q Exactive plus Orbitrap mass spectrometer (Rockford, IL, USA) coupled to a Dionex UltiMate 3000 UHPLC+ focused instrument (Dionex, USA) was used for quantification of the microcystins. The Orbitrap MS was equipped with a heated electrospray interface (HESI) and optimum source conditions compatible with the HPLC flow rate were used (capillary temperature, 290 °C; sheath flow, 50; spray voltage, 3 kV; auxiliary temperature, 400 °C). Analysis was performed in full MS SIM under positive polarity over a scan range from m/z 60 to 1000 with a mass accuracy of <5 ppm. Analysis was done in full MS-SIM with mass resolution of 70 000, Automatic gain control (AGC) was set at 1.0 × 106 with a maximum injection time (IT) of 100 ms. The mass spectrometer was calibrated weekly for mass accuracy using Pierce LTQ ESI positive ion standard.

For the LC separation, a Waters X-Bridge C18 column (4.6 x 75 mm, 3.5 µm particle size) was used. The flow rate was 0.3 mL/min and the column temperature was set at 35 °C. The mobile phase consisted of 0.1% (v/v) formic acid in water (eluent A) and 0.1% (v/v) formic acid in acetonitrile (eluent B). Separation was done using linear gradient elution starting with 2% eluent B and increased to 100 % in 10 min. This composition was held for 4 min and decreased again to 2% in 0.1 min, followed by a re-equilibration time of 3 min (total running time was. 17 min).

Seven calibration levels each were prepared over a concentration range from 25-400 µg/L. Each standard replicate was injected in duplicate. Quantification was performed by external calibration using analyte peak areas. Linear regression analysis was carried out to determine linearity by assessing the linear correlation coefficient r^2. The analytes were identified using their accurate mass and retention time and processing of the data was performed using TraceFinder EFS Software Version 3.2.

5.3.4 Statistical Analysis

Statistical analysis was done in consultation with STATKon at the University of Johannesburg. Frequency tables and summary statistics, including mean, and standard deviation were used to describe distribution of numeric indicators such as fish size, fish index and organ indices. The mean fish index and organ indices was calculated and compared between species, sites and seasons. The Mann-Whitney and independent samples t-test was used to compare the two species and two seasons as these tests compare means of numeric variables for two groups. The t-test was used to determine whether there was a significant difference between the means of two groups and theMann-Whitney U test which is a non-parametric alternative test to the independent sample t-test were both used for accuracy. The Krushkal-Wallis and One way Anova were used to compare the three rivers as these tests compare means of a numeric variable for more than two groups. The equality of variance for the data from the three rivers were calculated using Levene's statistical test which is linked to the One way Anova. Spearman's rho was the test used to determine the linear correlation between histological indicators and other numeric indicators. For all tests, statistical significance was set at $p < 0.05$.

5.4 Results

A total of 131 fish was collected during low-flow conditions in July 2015 and high-flow conditions in February 2016. The sample sizes per river, species and per season is summarised in Table 26.

Table 26: *Clarias gariepinus* and *Oreochromis mossambicus* sampled during low-flow season (2015) and high-flow season (2016) in the Edendalespruit, Pienaars River and Hartbeesspruit.

Fish species	**low-flow (July 2015)**		**Total**	**high-flow (February 2016)**		**Total**
Edendalespruit						
	Males	Females		Males	Females	
Clarias gariepinus	7	4	**11**	2	6	**8**
Oreochromis mossambicus	1	0	**1**	6	18	**24**
Pienaars River						
	Males	Females		Males	Females	
Clarias gariepinus	7	3	**10**	9	12	**21**
Oreochromis mossambicus	1	0	**1**	7	7	**14**
Hartbeesspruit						
	Males	Females		Males	Females	
Clarias gariepinus	6	4	**10**	6	3	**9**
Oreochromis mossambicus	0	0	**0**	13	8	**21**
Total			**33**			**97**

5.4.1 Macroscopic abnormalities

The necropsy results showed various macroscopic abnormalities in both fish species. The percentage prevalence of these abnormalities are presented in Table 27 for low-flow and high-flow conditions respectively. A detailed analysis of the results showed that during low-flow conditions 45% of *C. gariepinus* sampled in Edendalespruit showed liver abnormalities as opposed to 25% during high-flow conditions. Fish from the Pienaars River had a prevalence of 73% liver abnormalities during low-flow conditions and 23% during high-flow conditions, whereas, 80% of the *C. gariepinus* sampled during the low-flow in Hartbeesspruit had macroscopic liver abnormalities. This decreased to 11% during high-flow conditions. The abnormalities included macroscopically-visible nodules which were mostly spherical in shape

and varied in terms of morphological location, size and colour. This was seen in all three rivers during both seasons. There was also distortion of symmetry observed in the liver of a number of *C. gariepinus* (Figure 33 A-E). Interestingly, there were no macroscopic liver abnormalities identified in *O. mossambicus* from Edendalespruit and Pienaars River. However, 19% of the fish sampled in Hartbeesspruit showed macroscopic abnormalities of the liver. A representation of some of the abnormalities can be seen in Figure 29 and Figure 30.

Table 27: Macroscopic abnormalities observed during low-flow conditions (July 2015) and high-flow conditions (February 2016) within Edendalespruit, Pienaars River and Hartbeesspruit

	Clarias gariepinus						***Oreochromis mossambicus***					
Macroscopic observations	**Percentage prevalence %**						**Percentage prevalence %**					
	low-flow			**high-flow**			**low-flow**			**high--flow**		
	Edendale-spruit	Pienaars River	Hartbees-spruit	Edendale- spruit	Pienaars River	Hartbees-spruit	Edendale-spruit	Pienaars River	Hartbees-spruit	Edendale-spruit	Pienaars River	Hartbees-spruit
Nematodes	45	10	50	0	0	0	0	0	0	0	0	5
Pale gills	0	0	0	25	5	11	0	0	0	8	0	5
Nodules in liver	27	20	50	25	18	0	0	0	0	0	0	5
Discoloured liver	18	20	30	0	0	0	0	0	0	0	0	19
Abnormal papilla	0	0	10	0	0	0	0	0	0	0	0	0

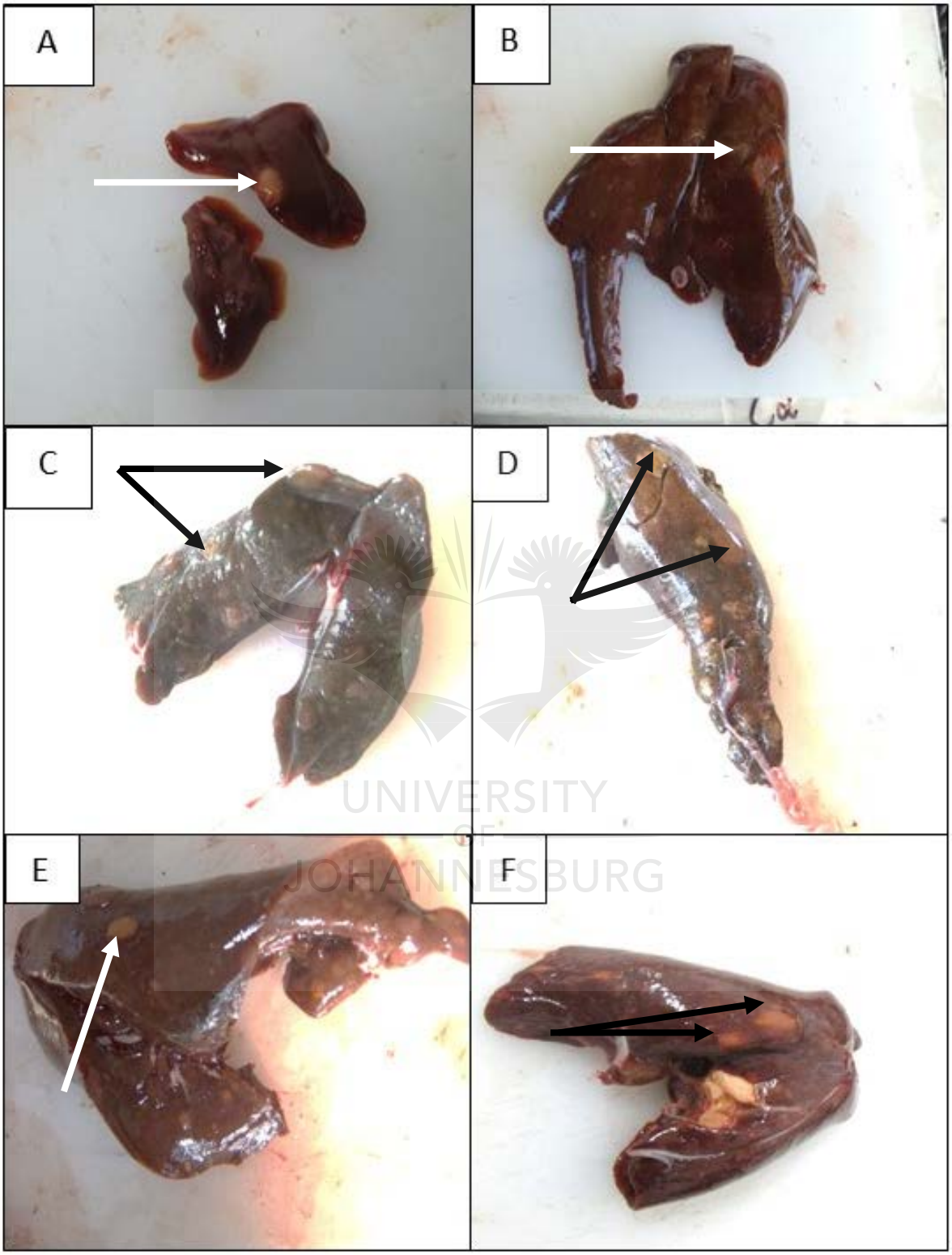

Figure 29: Macroscopic abnormalities observed in the liver of *Clarias gariepinus* during low-flow (July 2015) and high-flow (February 2016) conditions (arrows - nodules)

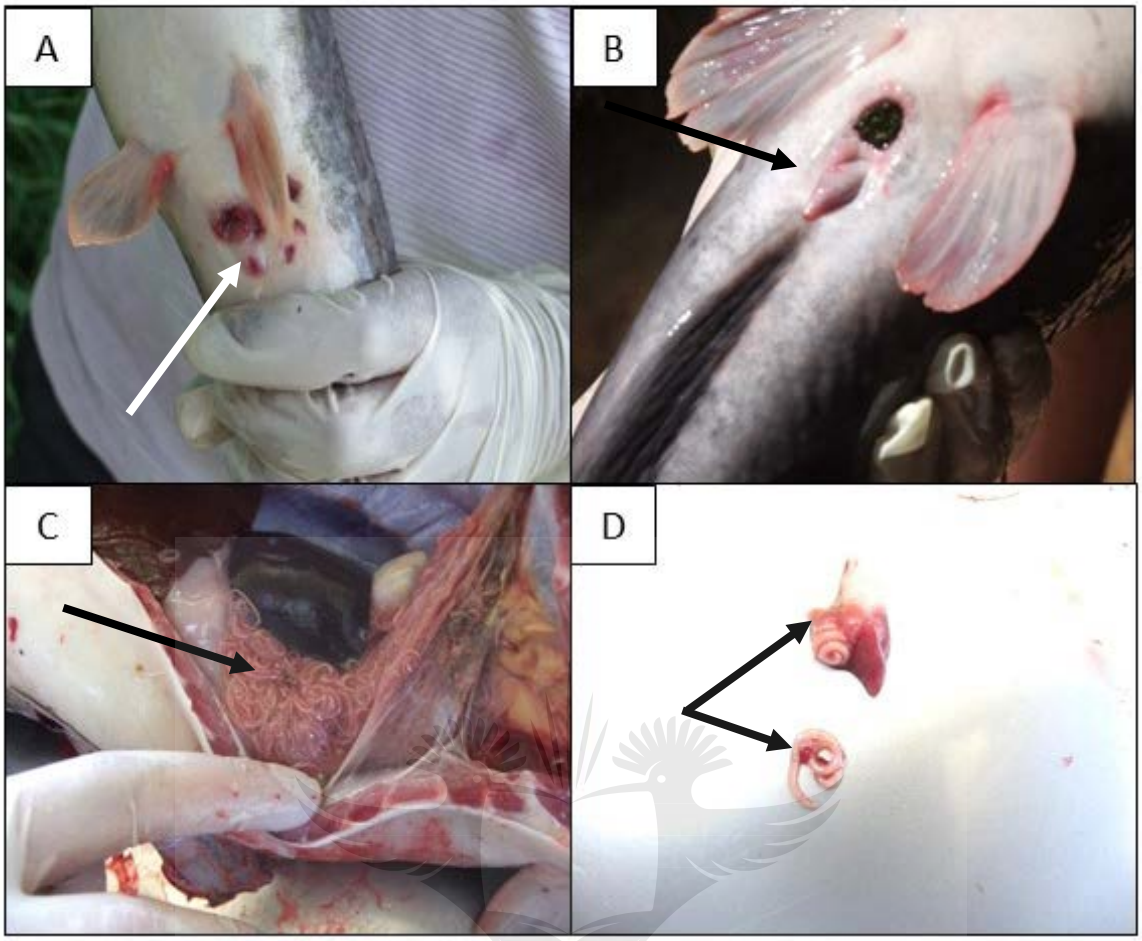

Figure 30: Macroscopic observations during low-flow (July 2015) and high-flow (February 2016) conditions. A- Lesions around the female opening of a *Clarias gariepinus* specimen B- Two papillae visible on the same male *C. gariepinus* specimen C- Presence of nematodes in the visceral cavity of a *C. gariepinus* specimen D- Nematode found in the pericardium of an *Oreochromis mossambicus* specimen.

5.4.2 Biometric indices

The biometric index results are presented below in graphs for comparison between seasons and species. Figure 31 and Figure 32 present the mean CF during both seasons and both species within each river. The *C. gariepinus* sampled from Edendalespruit had the highest mean CF during both seasons, compared to the same species from the other two rivers. However, for *O. mossambicus,* the highest mean CF was seen in fish sampled from Pienaars River during low-flow conditions and Edendalespruit during high-flow conditions. The comparison between low-flow and high-flow conditions showed significant differences ($p < 0.05$) between a number of indices within species during low-flow and high-flow conditions.

This included CF as well as hepatosomatic, splenosomatic and cardiac indices. These indices were all higher during the low-flow conditions.

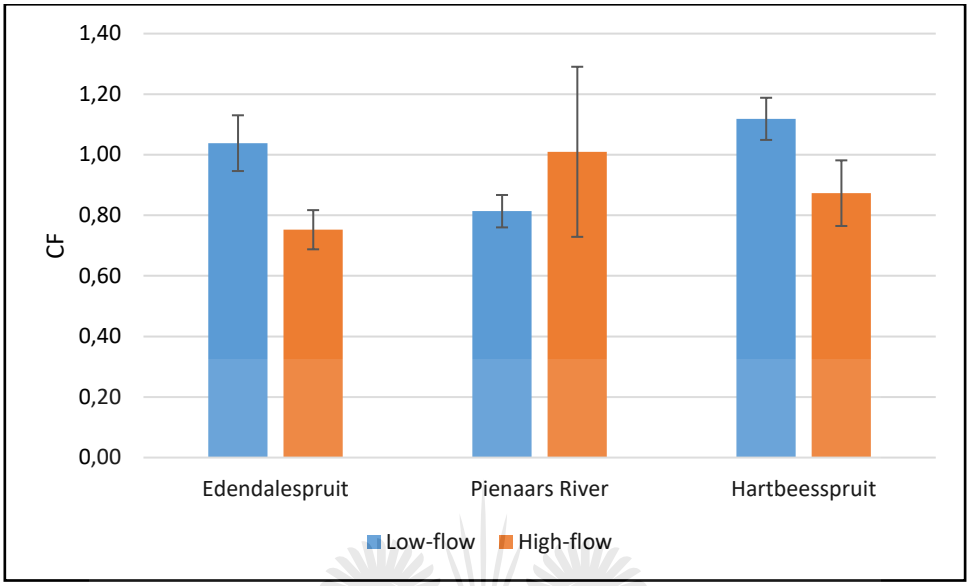

Figure 31: Comparison of mean Condition Factor (CF) of *Clarias gariepinus* between low-flow (July 2015) and high-flow (February 2016) conditions

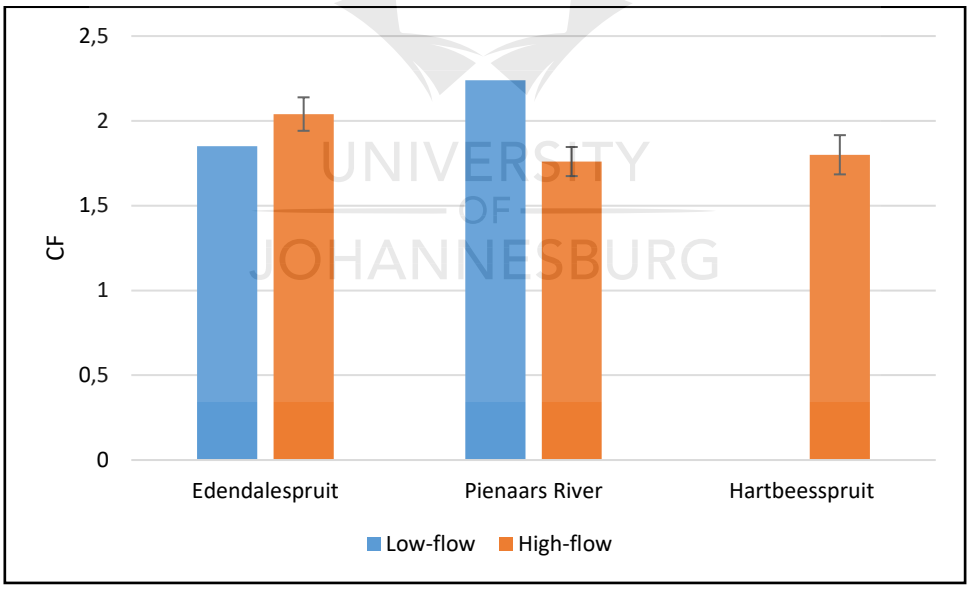

Figure 32: Comparison of mean Condition Factor (CF) of *Oreochromis mossambicus* between low-flow (July 2015) and high-flow (February 2016) conditions

The mean HSI values are presented in Figure 33 and Figure 34. For *C. gariepinus,* fish from Hartbeesspruit had the highest mean HSI value during the low-flow conditions and fish from Pienaars River during the high-flow conditions. This however, these respective values were not significantly different ($p > 0.05$) from the other seasons. There was only one *O. mossambicus* sampled in the Pienaars River and Edendalespruit during low-flow conditions.

Consequently, these values could not be compared. However, the fish sampled in Edendalespruit had the highest mean HSI value. The *O. mossambicus* sampled in Hartbeesspruit during high-flow conditions had the highest mean HSI value. There was a significant difference comparing the HSI of *C. gariepinus* and *O. mossambicus* ($p < 0.05$).

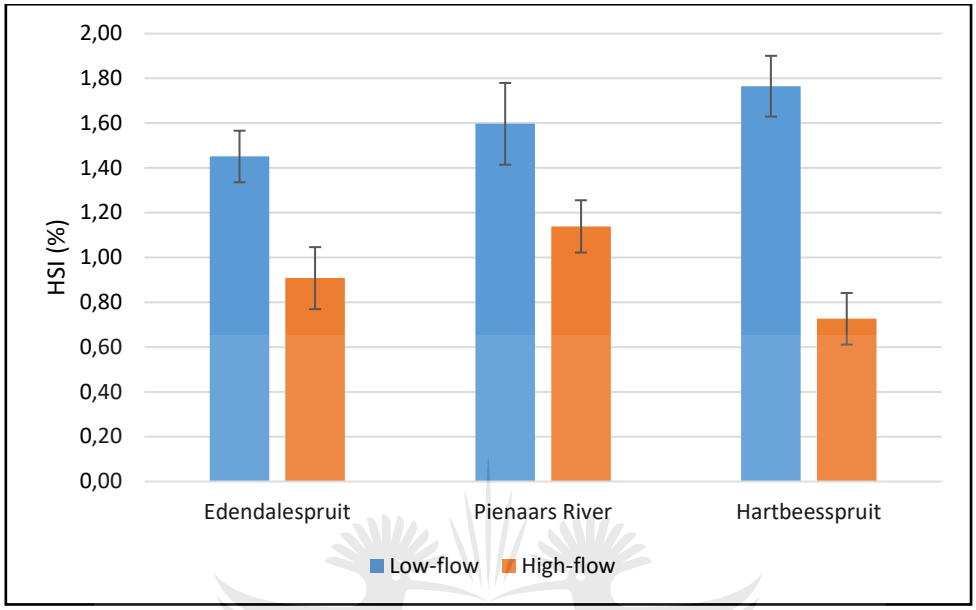

Figure 33: Comparison of Hepatosomatic Index (HSI) of *Clarias gariepinus* during (July 2015) and high-flow (February 2016) conditions

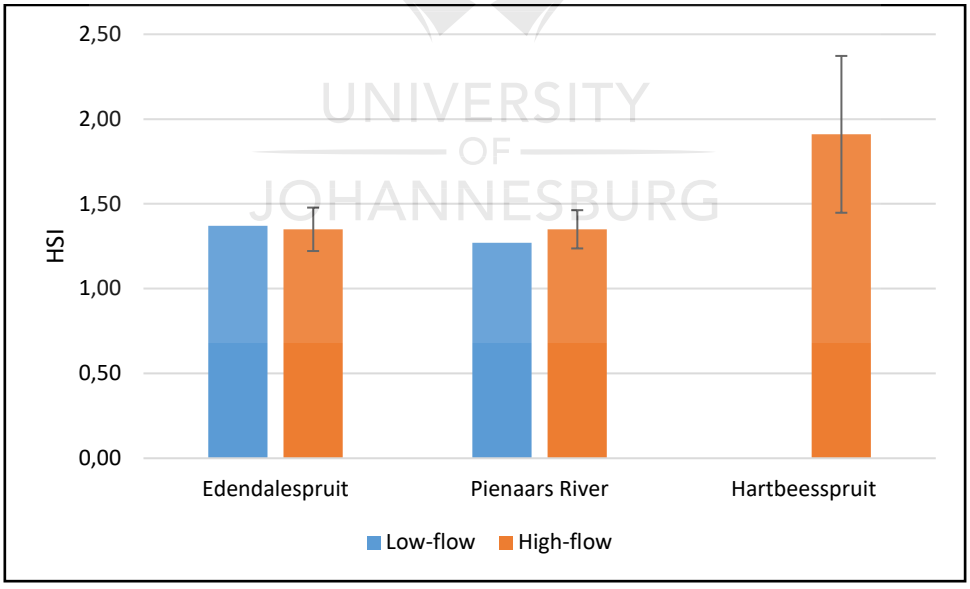

Figure 34: Comparison of Hepatosomatic Index (HSI) of *Oreochromis mossambicus* during (July 2015) and high-flow (February 2016) conditions

The highest mean SSI percentage for *C. gariepinus* and *O. mossambicus* respectively was recorded in Edendalespruit during low-flow conditions. This was not significantly different ($p > 0.05$) when compared to the other two sites. During high-flow conditions, the *C. gariepinus*

sampled from the Pienaars River and *O. mossambicus* from Edendalespruit had the highest mean SSI values respectively. There was a significant difference observed in the SSI in *C. gariepinus* and *O. mossambicus* ($p < 0.05$). This is depicted in Figure 35 and Figure 36.

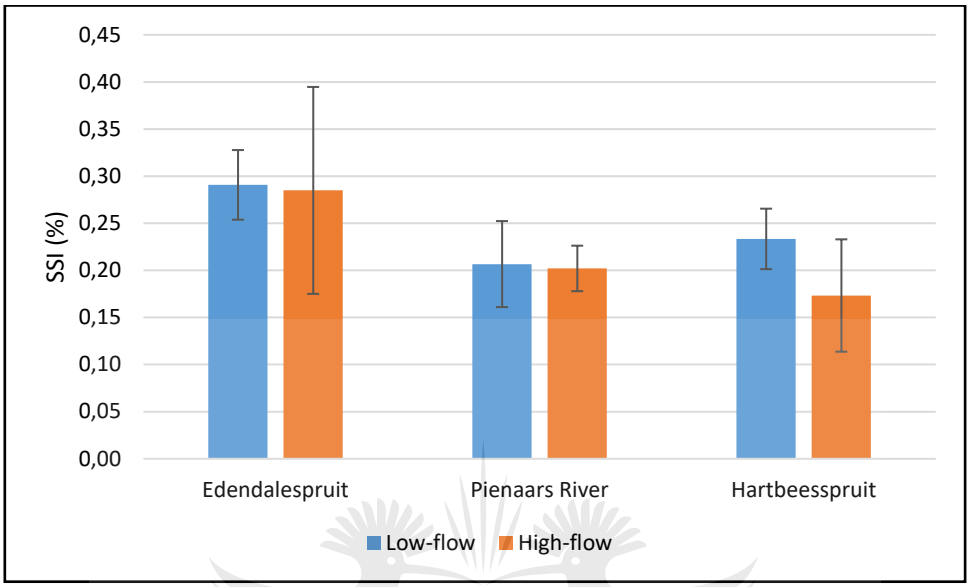

Figure 35: Comparison of Spleno-somatic index (SSI) in *Clarias gariepinus* during (July 2015) and high-flow (February 2016) conditions

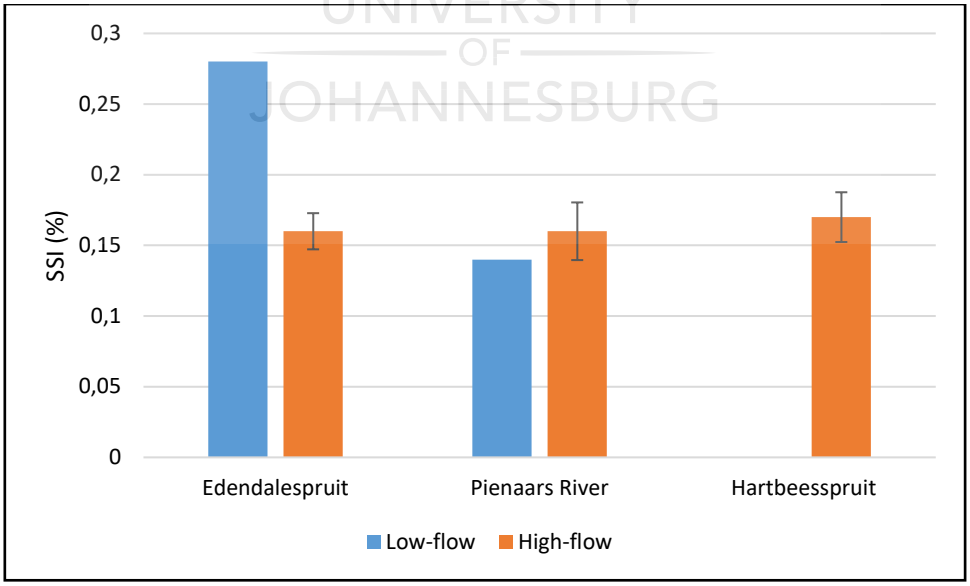

Figure 36: Comparison of Spleno-somatic index (SSI) in *Oreochromis mossambicus* during (July 2015) and high-flow (February 2016) conditions

During high-flow conditions, both species sampled from Pienaars River had the highest mean CSI value when compared to fish from the other two rivers, however this was not significantly different ($p > 0.05$). During the low-flow conditions, *C. gariepinus* from Hartbeesspruit had the highest mean CSI value and during low-flow conditions *O. mossambicus* sampled from Pienaars River and Edendalespruit had the same mean CSI value. During high-flow conditions, *O. mossambicus* sampled from the Pienaars River had the same mean CSI value as Hartbeesspruit. There was a significant difference observed in the CSI in *C. gariepinus* and *O. mossambicus* ($p < 0.05$). The results are presented in Figure 37 and Figure 38.

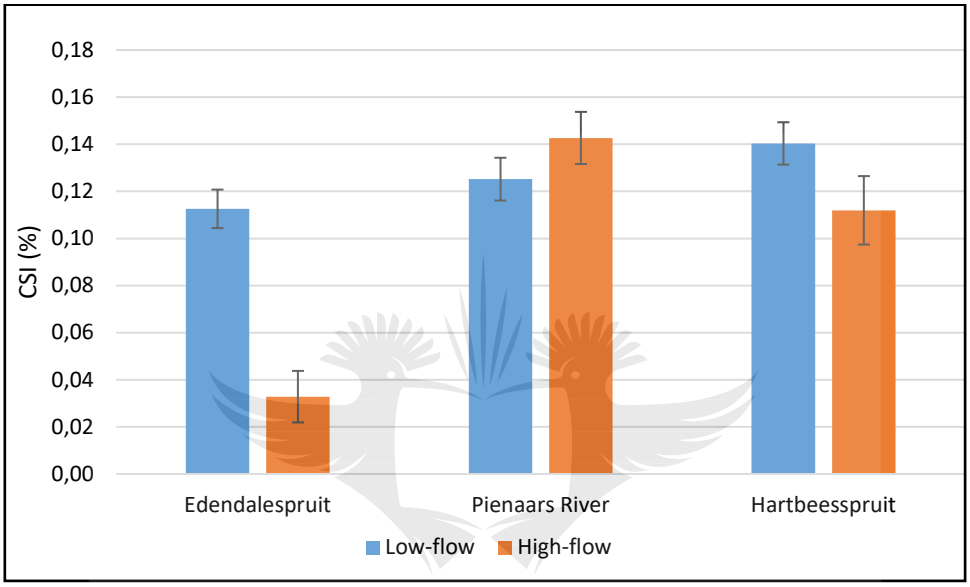

Figure 37: Comparison of the Cardiac Somatic Index (CSI) for *Clarias gariepinus* during (July 2015) and high-flow (February 2016) conditions

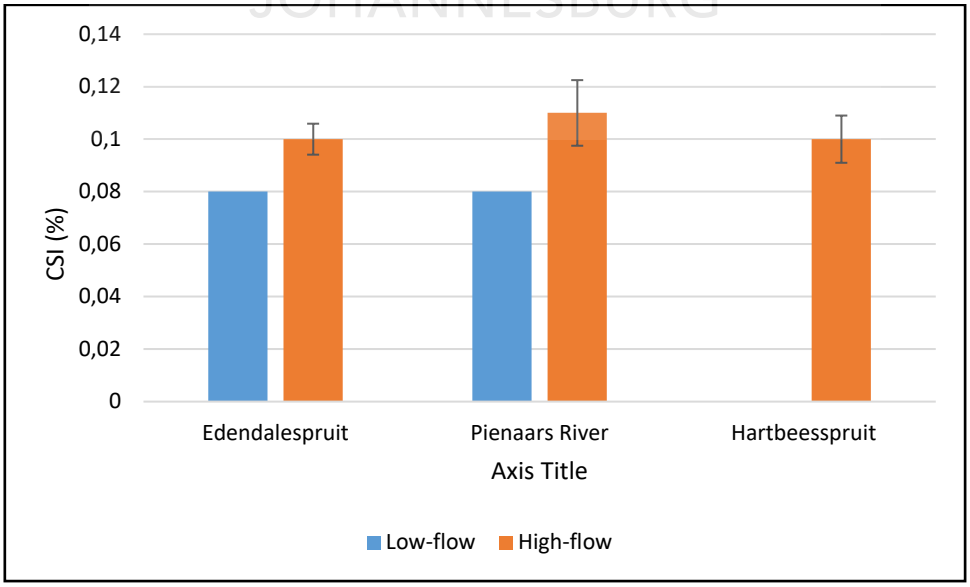

Figure 38: Comparison of the Cardiac Somatic Index (CSI) for *Oreochromis mossambicus* during (July 2015) and high-flow (February 2016) conditions

Figure 39 and Figure 40 show the results for the mean GSI for *C. gariepinus* male fish during both seasons in each river. During low-flow conditions, the males from Hartbeespruit had the highest mean GSI and during high-flow conditions the male fish from Pienaars River had the highest mean GSI. The female *C. gariepinus* sampled from Pienaars River had the highest mean GSI during both seasons. The male *O. mossambicus* had the same GSI during the low-flow in Edendalespruit and Pienaars River. During high-flow conditions, the highest mean GSI was seen in the male *O. mossambicus* from Pienaars River. There were no female *O. mossambicus* sampled during low-flow conditions and the females sampled in Hartbeesspruit during high-flow conditions had the highest mean GSI as seen in Figure 41 and Figure 42.

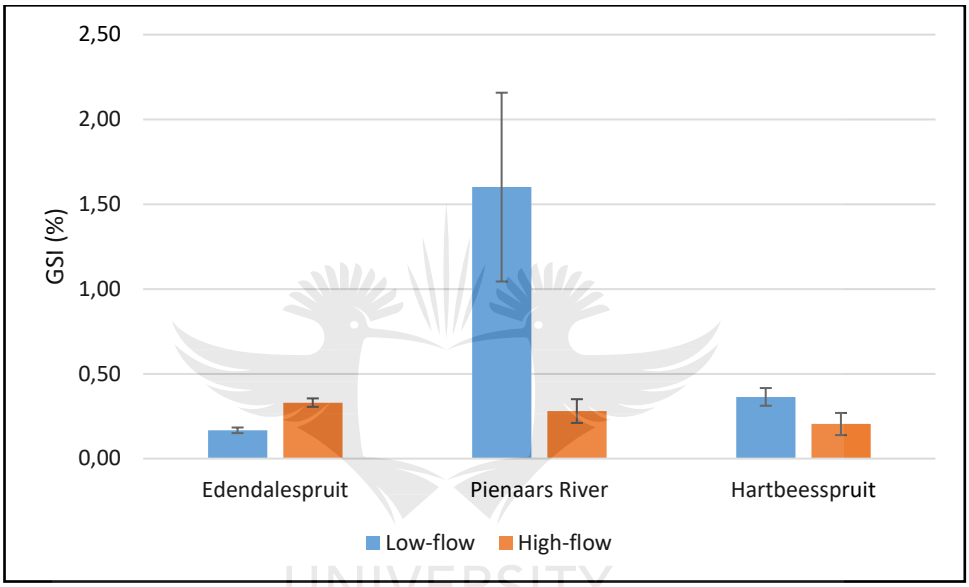

Figure 39: Comparison of the Gonado- Somatic Index (GSI) for *Clarias gariepinus* males during (July 2015) and high-flow (February 2016) conditions.

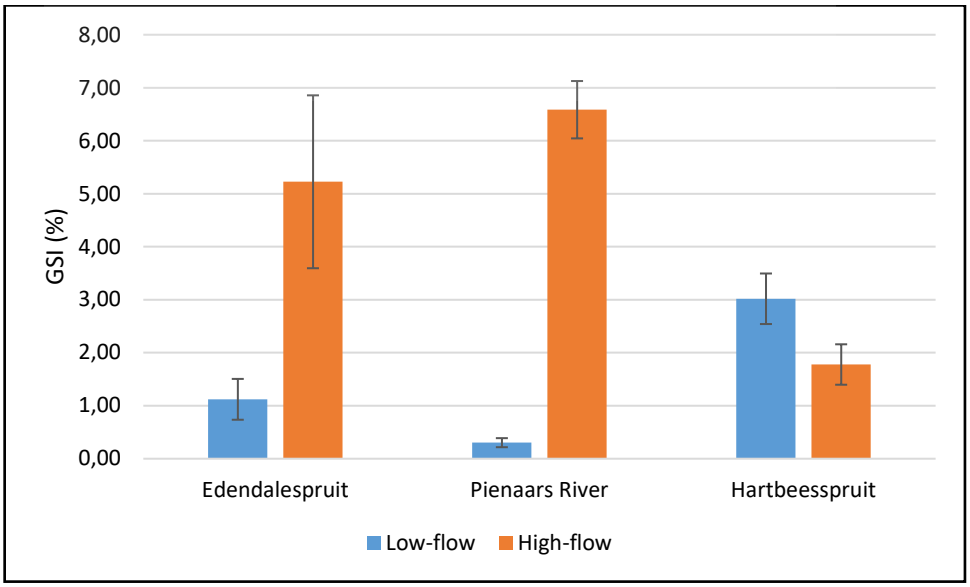

Figure 40: Comparison of the Gonado-Somatic Index (GSI) for *Clarias gariepinus* females during (July 2015) and high-flow (February 2016) conditions

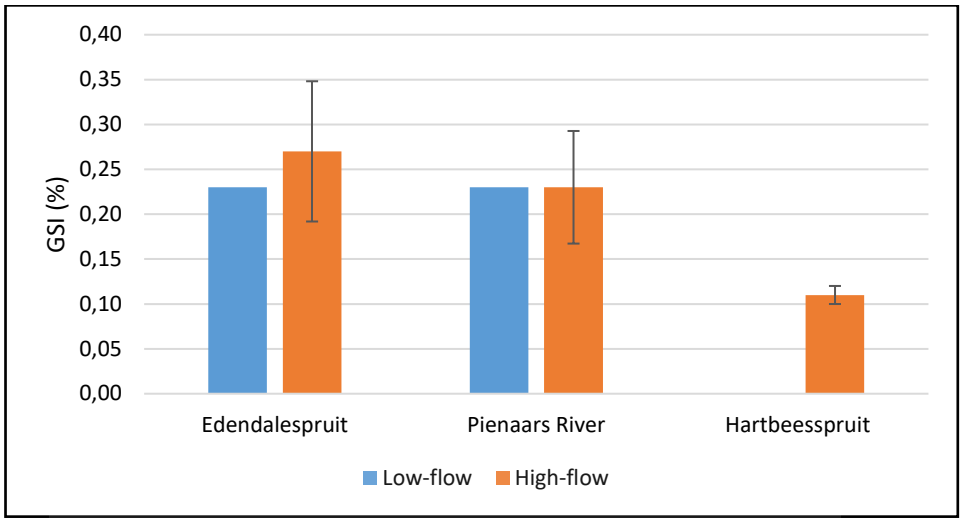

Figure 41: Comparison of the Gonado- Somatic Index (GSI) for *Oreochromis mossambicus* males during (July 2015) and high-flow (February 2016) conditions

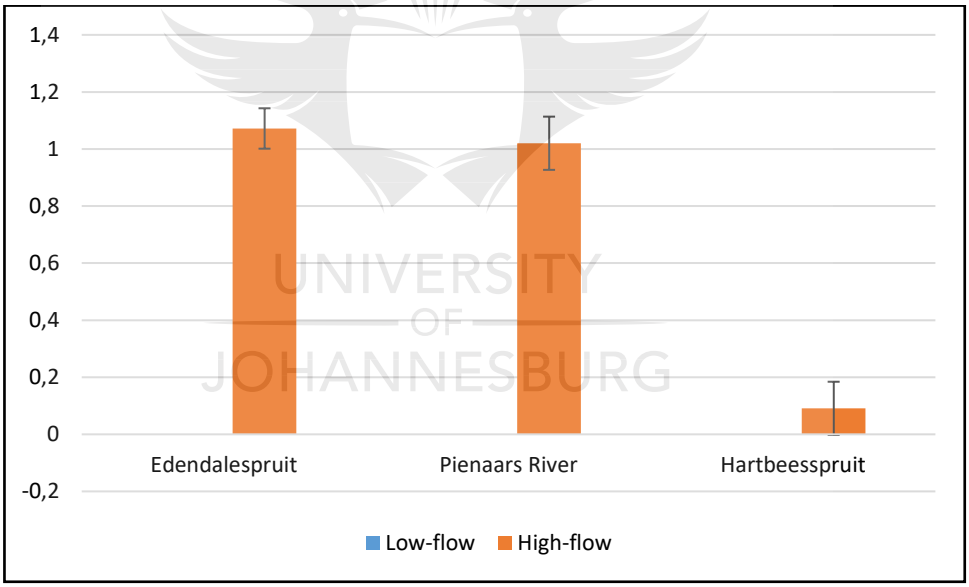

Figure 42: Comparison of the Gonado- Somatic Index (GSI) for *Oreochromis mossambicus* females during (July 2015) and high-flow (February 2016) conditions

Table 28: Biometric indices of *Clarias gariepinus* sampled from Edendalespruit, Pienaars River and Hartbeesspruit during low-flow (July 2015) and high-flow (February 2016) conditions (Mean values and standard deviation presented per survey)

Clarias gariepinus															
	Body Mass	**Mass - Gon**	**Total Length**	**Liver Mass**	**Spleen Mass**	**Gonad Mass (M)**	**Gonad Mass (F)**	**Heart Mass**	**CF**	**CF (N)**	**HSI**	**SSI**	**GSI (M)**	**GSI (F)**	**CSI**
	g	**g**	**mm**	**g**	**g**	**g**	**g**	**g**			**%**	**%**	**%**	**%**	**%**
Edendalespruit (low-flow)															
Mean	3681.82	3657.38	702.27	55.53	10.82	5.06	3.33	2.66	0.31	0.31	0.42	0.14	0.17	1.12	0.03
Std Dev	1831.65	1805.62	143.53	35.14	7.75	4.08	56.74	1.09	1.85	1.85	1.37	0.28	0.05	0.77	0.08
Edendalespruit (high-flow)															
Mean	2641.25	2132.37	667.5	21.09	6.16	18.69	109.22	3.71	0.75	0.58	0.91	0.29	0.33	5.22	0.13
Std Dev	2040.96	2112.02	176.89	14.896	6.98	8.09	108.02	3.63	0.18	0.25	0.44	0.35	0.05	3.99	0.01
Hartbeesspruit (low-flow)															
Mean	3218.18	3172.99	719.09	52.11	7.58	25.59	97.45	4.15	0.81	0.80	1.59	0.21	0.92	3.41	0.13
Std Dev	1557.45	1563.26	101.24	25.34	7.63	37.20	4.08	2.40	0.18	0.18	0.61	0.15	1.65	0.84	0.03
Hartbeesspruit (high-flow)															
Mean	2345.90	2255.91	665.45	27.17	5.41	9.72	145.58	3.60	0.99	0.96	1.76	0.33	0.75	6.69	0.23
Std Dev	1166.47	1164.88	119.42	14.07	4.67	7.89	67.13	2.24	1.33	1.33	3.05	0.65	1.47	1.83	0.42
Pienaars River (low-flow)															
Mean	3284.00	3224.70	647.00	54.25	7.70	11.48	91.17	4.23	1.12	1.097	1.76	0.23	0.36	3.01	0.14
Std Dev	2070.35	2069.08	89.20	25.09	6.15	5.35	52.56	1.68	0.22	0.22	0.43	0.10	0.10	1.17	0.03
Pienaars River (high-flow)															
Mean	2660.00	2643.09	653.33	22.44	6.56	8.57	33.58	3.49	0.87	0.86	0.73	0.17	0.20	1.78	0.11
Std Dev	1701.15	1696.82	171.04	22.72	11.06	10.02	30.02	3.57	0.16	0.15	0.35	0.18	0.16	0.66	0.04

CF - Condition factor
CF(N) - Calculated without gonads
HSI - Hepatosomatic index
SSI - Splenosomatic index
GSI - Gonadosomatic index
CSI - Cardiosomatic index

Table 29: Biometric indices of *Oreochromis mossambicus* sampled from Edendalespruit, Pienaars River and Hartbeesspruit during low-flow (July 2015) and high-flow (February 2016) conditions (Mean values and standard deviation presented per survey)

Oreochromis mossambicus															
	Body Mass	**Mass - Gon**	**Total Length**	**Liver Mass**	**Spleen Mass**	**Gonad Mass (M)**	**Gonad Mass (F)**	**Heart Mass**	**CF**	**CF (N)**	**HSI**	**SSI**	**GSI (M)**	**GSI (F)**	**CSI**
	g	**g**	**mm**	**g**	**g**	**g**	**g**	**g**			**%**	**%**	**%**	**%**	**%**
Edendalespruit (high-flow)															
Mean	918.75	910.59	356.00	11.88	1.43	2.44	10.06	0.92	2.04	2.02	1.35	0.16	0.28	1.07	0.10
Std Dev	224.35051	221.47	22.46	5.00	0.62	1.62	8.07	0.27	0.48	0.48	0.63	0.06	0.19	0.64	0.03
Pienaars (high-flow)															
Mean	500	497.07	299.29	6.39	0.86	2.39	3.46	0.51	1.76	1.75	1.35	0.16	0.49	0.87	0.11
Std Dev	244.95	244.68	40.71	3.23	0.66	2.31	2.29	0.29	0.32	0.33	0.42	0.08	0.71	0.63	0.05
Hartbeesspruit (high-flow)															
Mean	792.73	788.73	348.33	17.57	1.35	3.03	5.59	0.76	1.79	1.79	1.91	0.17	0.32	1.48	0.10
Std Dev	395.06	393.87	47.49	37.75	0.96	2.46	2.99	0.49	0.53	0.53	2.12	0.08	0.15	2.29	0.041

CF - Condition factor
CF(N) - Calculated without gonads
HSI - Hepatosomatic index
SSI - Splenosomatic index
GSI - Gonadosomatic index
CSI - Cardiosomatic index

5.4.3 Histopathology results

5.5.3.1 Gills

The percentage prevalence of histological alterations observed in each species is presented in Table 30. The most common histological alterations found in the gills of *C. gariepinus* during both seasons within each river was circulatory changes such as telangiectasia, regressive changes such as secondary lamella branching and vacuolation. Focal gill epithelial cell hyperplasia was only found in fish from Edendalespruit during both the low-flow and high-flow survey. Fish from Edendalespruit had the lowest mean gill histology index, whereas the highest mean gill histology index was seen in fish from Pienaars River. There was a significant difference ($p < 0.05$) between these two sites. Examples of the different types of histological alterations seen in the gills of *C. gariepinus* and *O. mossambicus* are presented in Figure 45 and Figure 48 respectively.

5.5.3.2 Liver

A variety of histological alterations were observed in the livers of both *C. gariepinus* (Figure 43 and Figure 44) and *O. mossambicus* (Figure 46). The alterations observed ranged from regressive to progressive and inflammatory changes. Eight different types of FCAs (Focal areas of cellular alterations) were also observed in *C. gariepinus*. There was, however, a larger variety of FCAs observed in *C. gariepinus* and a higher percentage prevalence observed in this species, this resulted in a higher mean liver histology index. However, the sample size for each species should be considered as values were presented as percentages and not frequency. The mean liver histology index differed significantly ($p < 0.05$) between the two species. The livers of fish from Pienaars River collected during both seasons were more affected according to the results presented in Table 34. The liver histology mean index was significantly higher ($p < 0.05$) in fish from Pienaars River in comparison to fish from Hartbeesspruit, which had the lowest mean liver histology index. A larger number of histological changes were observed in the liver during low-flow conditions, the liver histology index was significantly higher ($p < 0.05$) compared to high-flow conditions.

5.5.4.2 Spleen

The histological alterations observed in spleen tissue for both species collected during both seasons included melano-macrophage centers (some encapsulated) and infiltration in the form of mononuclear leukocytes. Encapsulated melano-macrophage centers were especially noticeable in *O. mossambicus* during the high-flow season in Edendalespruit. The SSI showed

no significant differences between species or male and female specimens ($p > 0.05$). The spleen histology index was slightly higher during the low-flow conditions. The difference was statistically significant ($p < 0.05$). Examples of the different types of histological alterations seen in the spleens of *C. gariepinus* and *O. mossambicus* are presented in Figure 45 and Figure 48 respectively.

5.5.4.3 Heart

A semi-quantitative histological assessment of the heart tissue of both species showed that reaction patterns was either regressive or inflammatory responses. Common observations were melano-macrophages, vacuolation and myocarditis. Fish from the Pienaars River had the highest mean heart histology index and fish from the Hartbeesspruit had lowest mean heart histology index. There was also a significant difference ($p < 0.05$) found when comparing the mean values for the heart histology index between all *C. gariepinus* and all *O. mossambicus*. *Clarias gariepinus* had the highest values of the two species. The male fish from each river across both seasons had higher mean heart histology indices, this was significantly different ($p < 0.05$) when compared to the female fish.

5.5.4.4 Kidney

The alterations observed in the kidneys was most commonly observed in the interstitial tissue for both species. The alterations ranged from structural to intercellular deposits and infiltration in the form of mono-nuclear leukocytes. There were no statistically significant differences ($p > 0.05$) for the mean kidney histology indices comparing seasons, sites, species or sex. Examples of the different types of histological alterations seen in the kidneys of *C. gariepinus* and *O. mossambicus* are presented in Figure 45 and Figure 48 respectively.

5.5.4.5 Gonads

The histological alterations in the gonads from both species for each river during both seasons were similar. Melano-macrophage centers were observed in the interstitial tissue of both ovaries and testes and the highest percentage prevalence was seen in *C. gariepinus* and testes from *O. mossambicus* from Hartbeesspruit. There were no testicular oocytes found in *C. gariepinus,* this phenomenon was only observed in *O. mossambicus* sampled from Hartbeesspruit. The ovaries of most females sampled were in the mature stages of oogenesis. Examples of the different types of histological alterations seen in the gonads of *C. gariepinus* and *O. mossambicus* is presented in Figure 45 and Figure 47 respectively.

Table 30: Histological alterations observed in the each target organ of *Clarias gariepinus* and *Oreochromis mossambicus* during low-flow (July 2015) and high-flow (February 2016) conditions in Edendalespruit, Pienaars River and Hartbeesspruit.

		Clarias gariepinus						*Oreochromis mossambicus*					
Gills													
Functional Unit	**Alterations**	**Edendalespruit**		**Pienaars River**		**Hartbeesspruit**		**Edendalespruit**		**Pienaars River**		**Hartbeesspruit**	
		Low Flow	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**
Circulatory Changes													
	Aneurysm/Haemor. e.g. telangiectasia	58%	63%	36%	36%	50%	60%	0%	69.50%	0%	43%	*n/a	76%
Regressive Changes													
Epithelium	**Structural alterations (e.g. sec lam branching)**	58%	75%	55%	36%	20%	30%	0%	26%	0%	7%	*n/a	14%
	Plasma alterations (e.g. vacuolation)	8%	12.5%	0%	0%	0%	0%	0%	0%	0%	0%	*n/a	0%
Progressive Changes													
Epithelium	**Hypertrophy**	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	*n/a	0%
	Hyperplasia	8%	12.5%	0%	0%	0%	0%	0%	26%	0%	7%	*n/a	14%
Mucous cells	**Hypertrophy**	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	*n/a	0%
Liver													
		Edendalespruit		**Pienaars River**		**Hartbeesspruit**		**Edendalespruit**		**Pienaars River**		**Hartbeesspruit**	
		Low Flow	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**
Regressive changes													
Hepatocytes	**Intracelluar deposits**	0%	25%	9%	4.5%	10%	0%	0%	35%	0%	0%	*n/a	38%
	Hepato-nuclear pleomorphism/ chromatin clearing	0%	37.5%	45%	18%	20%	20%	0%	22%	0%	0%	*n/a	19%
	Hepatocellular pleomorphism	0%	0%	0%	14%	0%	0%	0%	0%	0%	0%	*n/a	0%
	Steatosis	36%	25%	45%	27%	50%	0%	0%	8%	0%	7%	*n/a	19%
	Vacuolation other than steatosis	0%	12.5%	0%	0%	0%	40%	0%	0%	0%	0%	*n/a	0%
	Melano-macrophage centers (Light gold granular)	45%	0%	45%	27%	0%	0%	0%	30%	0%	29%	*n/a	43%

	(Light gold vacuolated)	0%	50%	0%	9%	0%	0%	0%	0%	0%	0%	*n/a	0%
	(Dark brown solid large)	36%	25%	45%	36%	70%	0%	0%	0%	0%	0%	*n/a	0%
Progressive Changes													
	Hepatocyte hypertrophy: Hydropic change	0%	0%	0%	0%	0%	80%	0%	0%	0%	0%	*n/a	0%
	Hypertrophy: Steatosis	0%	0%	9%	5%	0%	0%	0%	0%	0%	0%	*n/a	0%
	Wall proliferation/ Fibrosis	0%	0%	0%	27%	0%	0%	0%	0%	0%	0%	*n/a	0%
Inflammatory Changes													
	Infiltration (Mononuclear leukocytes)	18%	0%	0%	27%	10%	10%	0%	0%	0%	0%	*n/a	0%
	Granulomatosis	0%	0%	0%	5%	10%	0%	0%	0%	0%	0%	*n/a	0%
Foci of cellular alteration (FCA) or liver nodules													
Hepatocytes	**Clear cell foci**	55%	25%	55%	18%	0%	0%	0%	0%	0%	0%	*n/a	0%
	Vacuolated foci	18%	25%	18%	27%	10%	0%	0%	0%	0%	0%	*n/a	0%
	Eosinophilic foci	55%	12.5%	9%	9%	0%	0%	0%	0%	0%	0%	*n/a	0%
	Basophilic foci	0%	0%	9%	0%	10%	0%	0%	0%	0%	0%	*n/a	0%
	Mixed foci	36%	0%	0%	9%	10%	0%	0%	0%	0%	0%	*n/a	0%
	Necrotic foci	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	*n/a	0%
	Hypertrophic foci	0%	0%	18%	18%	0%	0%	0%	0%	0%	0%	*n/a	0%
	Granular foci	0%	12.5%	0%	0%	0%	0%	0%	0%	0%	0%	*n/a	0%
Spleen													
		Edendalespruit		**Pienaars River**		**Hartbeesspruit**		**Edendalespruit**		**Pienaars River**		**Hartbeesspruit**	
		Low Flow	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**
Regressive Changes													
	Intercellular deposits (eg. **Melano-macrophage centers**)	100%	12.5%	73%	52%	60%	75%	0%	66%	0%	57%	*n/a	64%
Heart													
		Edendalespruit		**Pienaars River**		**Hartbeesspruit**		**Edendalespruit**		**Pienaars River**		**Hartbeesspruit**	
		Low Flow	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**
Regressive Changes													

Atrium	(eg. Melano-macrophage centers)	0%	0%	0%	0%	0%	0%	0%	30%	0%	7%	*n/a	0%
	Vacuolation	0%	0%	0%	23%	0%	11%	0%	0%	0%		*n/a	0%
Ventricle	(Vacuolation) -myocardium	50%	0%	0%	0%	0%	34%	0%	17%	0%	36%	*n/a	5%
	(Vacuolation) - epicardium	0%	0%	0%	0%	0%	0%	0%	0%	0%	21%	*n/a	10%
Inflammatory Changes													
Atrium	(eg. myocarditis)	50%	0%	10%	0%	50%	22%	0%	13%	0%	0%	*n/a	10%
Kidney													
		Edendalespruit		**Pienaars River**		**Hartbeesspruit**		**Edendalespruit**		**Pienaars River**		**Hartbeesspruit**	
		Low Flow	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**
Regressive Changes													
	Intercellular deposits (eg Melano-macrophage centers)	36%	50%	50%	19%	80%	89%	0%	46%	0%	64%	*n/a	33%
Inflammatory Changes													
	Infiltration (eg MNL)	0%	12.5%	0%	9.5%	0%	44%	0%	0%	0%	0%	*n/a	24%
Gonads (Ovaries)													
		Low Flow	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**
Regressive Changes													
	Melano-macrophage centers	0%	0%	100%	0%	100%	100%	*n/a	33%	*n/a	0%	*n/a	4%
Gonads (Testis)													
		Edendalespruit		**Pienaars River**		**Hartbeesspruit**		**Edendalespruit**		**Pienaars River**		**Hartbeesspruit**	
		Low Flow	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**	**Low Flow**	**High Flow**
Regressive Changes													
	Melano-macrophage centers	10%	0%	30%	13%	50%	20%	5%	0%	0%	0%	*n/a	17%
Intersex													
	Testicular oocytes	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	*n/a	8%

*n/a - No *O. mossambicus* was sampled at this site

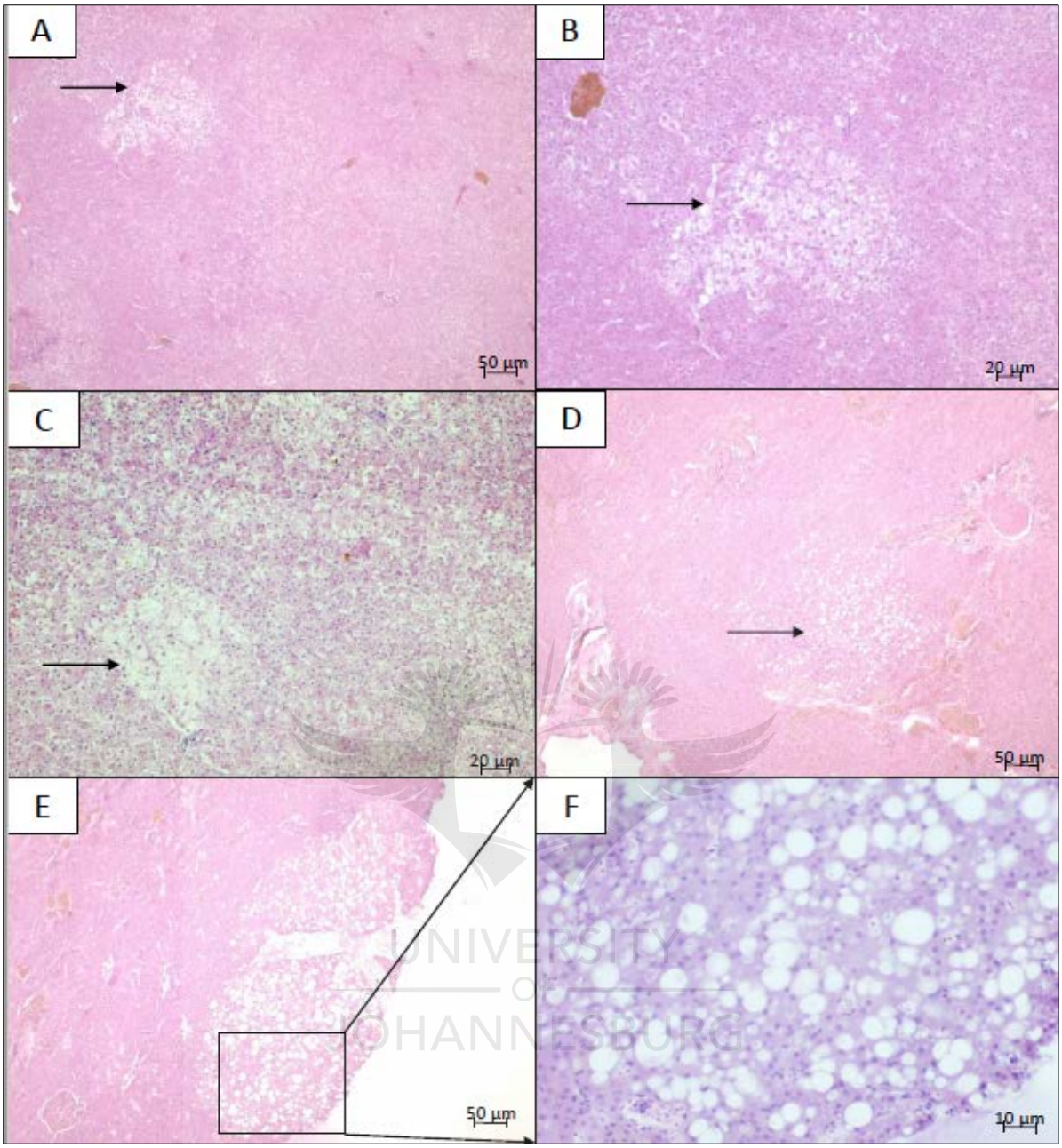

Figure 43: Light micrographs of the liver of *Clarias gariepinus* from Edendalespruit, Pienaars River and Hartbeesspruit (H&E): A - C – Focal areas of hepatocyte hypertrophy; D – F - Macrovesicular steatosis (arrow) at different magnifications

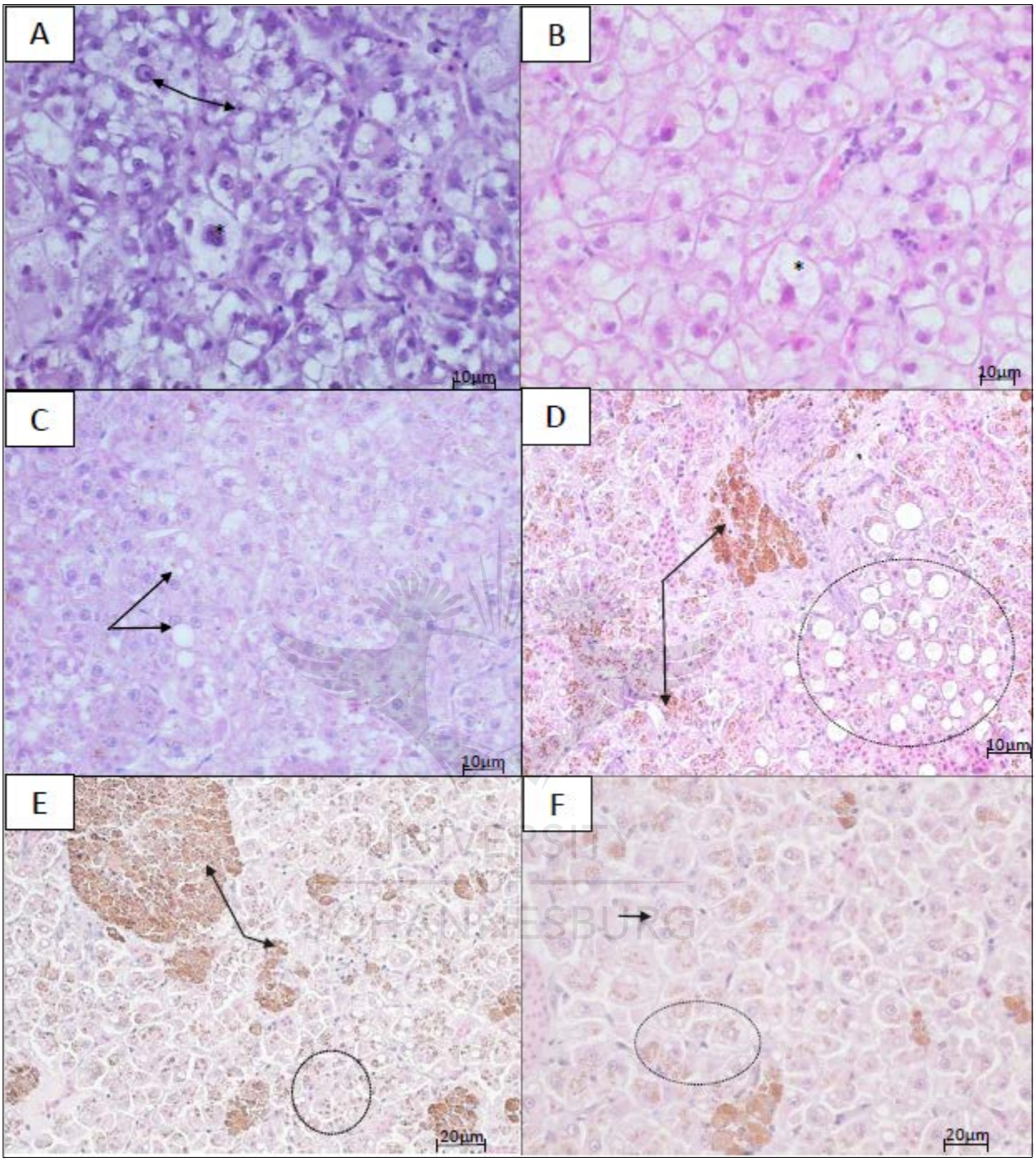

Figure 44: Light micrographs of the liver of *Clarias gariepinus* from Edendalespruit, Pienaars River and Hartbeesspruit (H&E): A – Nuclear pleomorphism (arrows); B- Hypertrophy of hepatocytes (asterix); C - Macrovesicular steatosis; D - steatosis (circle), melano-macrophage centers (MMCs) (arrows) and; E - Intracellular deposits (circle) and MMCs (arrows); F – Intracellular deposits (circle and arrow).

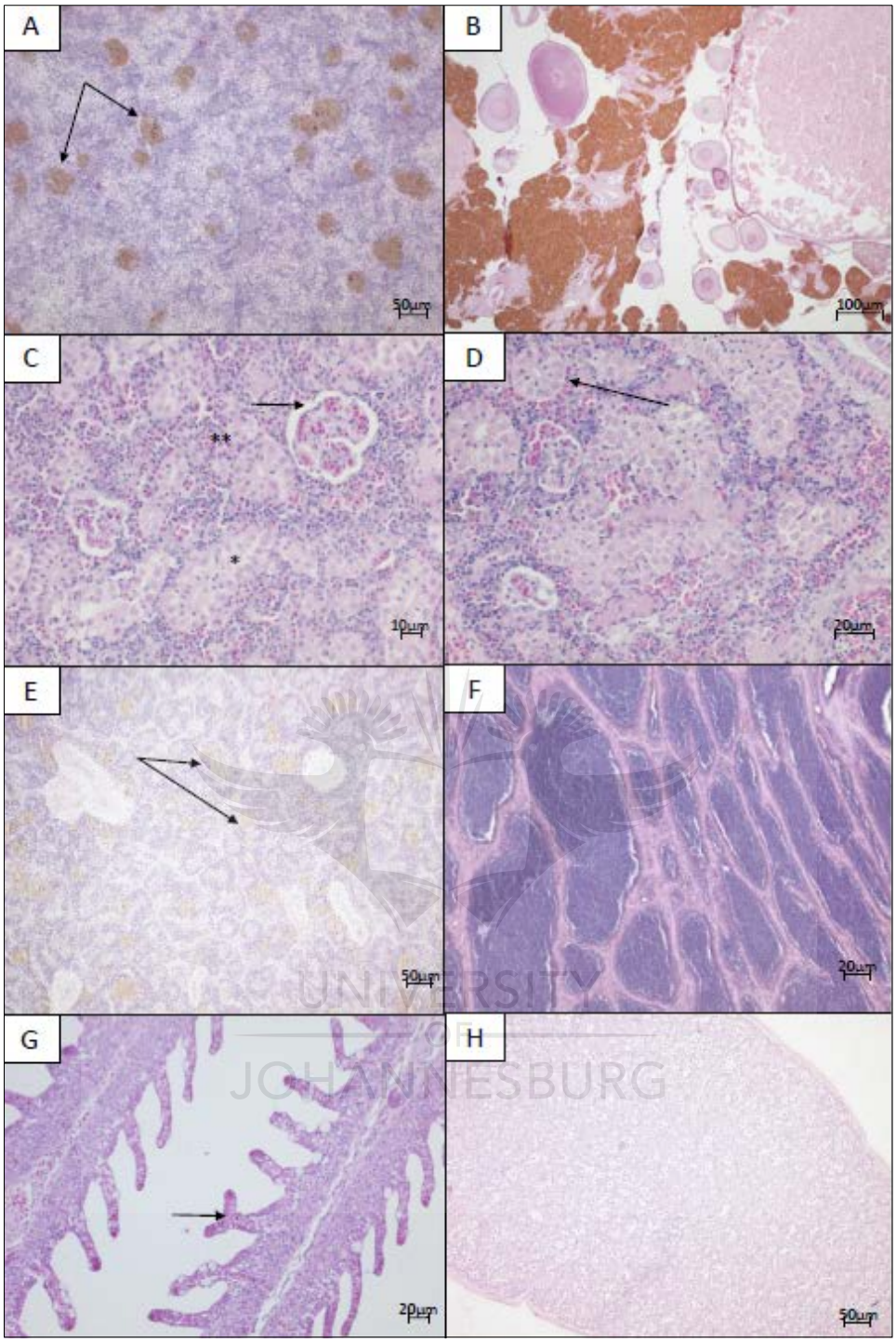

Figure 45: Light micrographs of the spleen, kidney, testis and kidney of *Clarias gariepinus* from Edendalespruit, Pienaars River and Hartbeesspruit (H&E): A – Increase in melano-macrophage centers in the spleen (arrows); B – Increase in melano-macrophage centers in the ovaries; C – Cross section of the kidney showing the glomerulus (arrow) a longitudinal section through the proximal tubule (*) and hematopoietic tissue (**) D – Cross section through the kidney showing a cross section through a renal tubule (arrow); E – Increase in melano-macrophage centers in the kidney (arrows); F – Mature spermatozoa in the testis; G – Mucous cells (arrows) and branching of the secondary lamella (*); H – longitudinal section through an immature testis

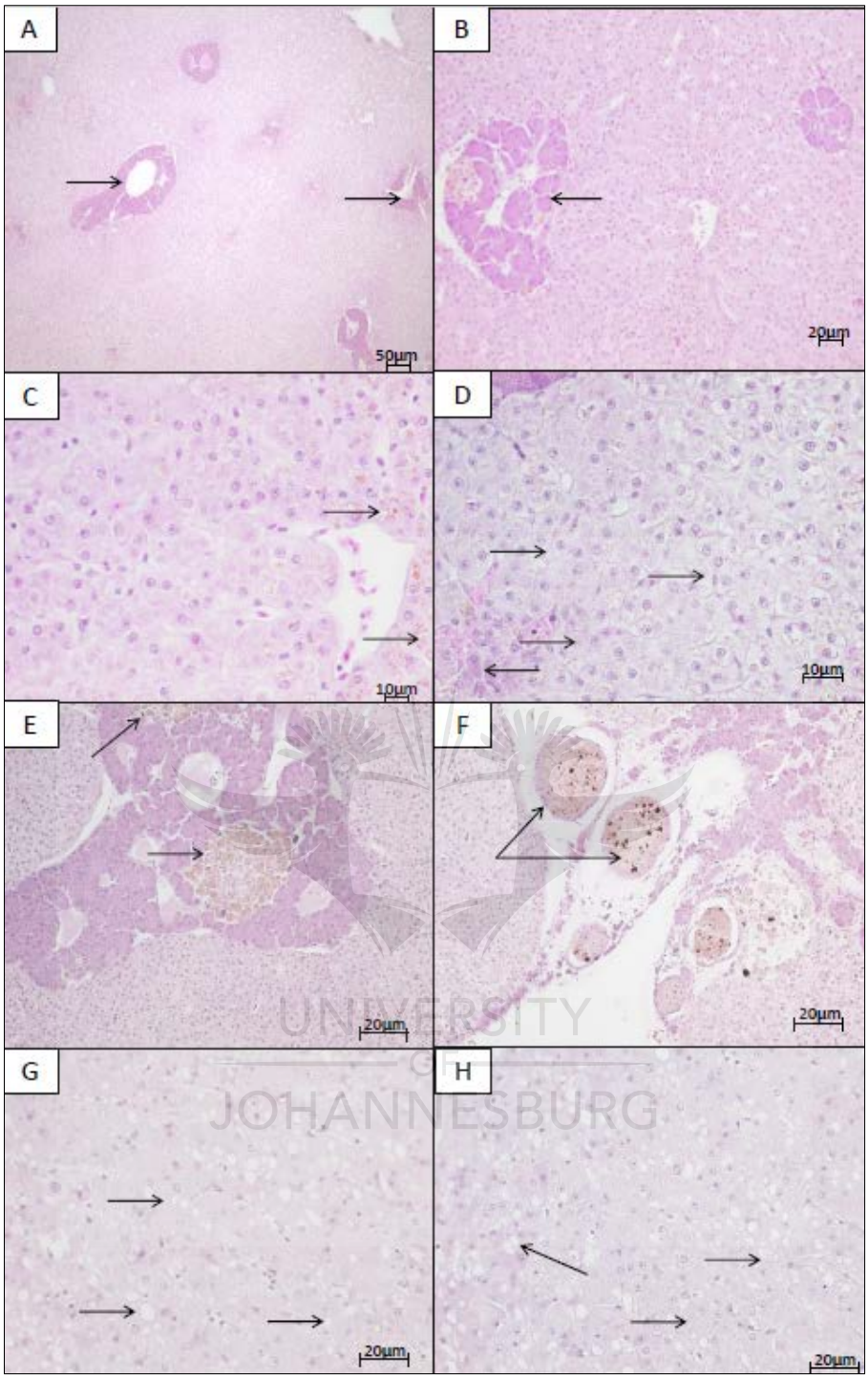

Figure 46: Light micrographs of the liver of *Oreochromis mossambicus* from Edendalespruit, Pienaars River and Hartbeesspruit (H&E): A - B – Low magnification view showing hepatopancreatic tissue (arrows) located throughout the liver tissue; C - D – Intracellular deposits visible within hepatocytes (arrows); E - F – Melano-macrophage centers (arrows); G – H – Steatosis (lipid vacuoles visible in the liver tissue (arrows).

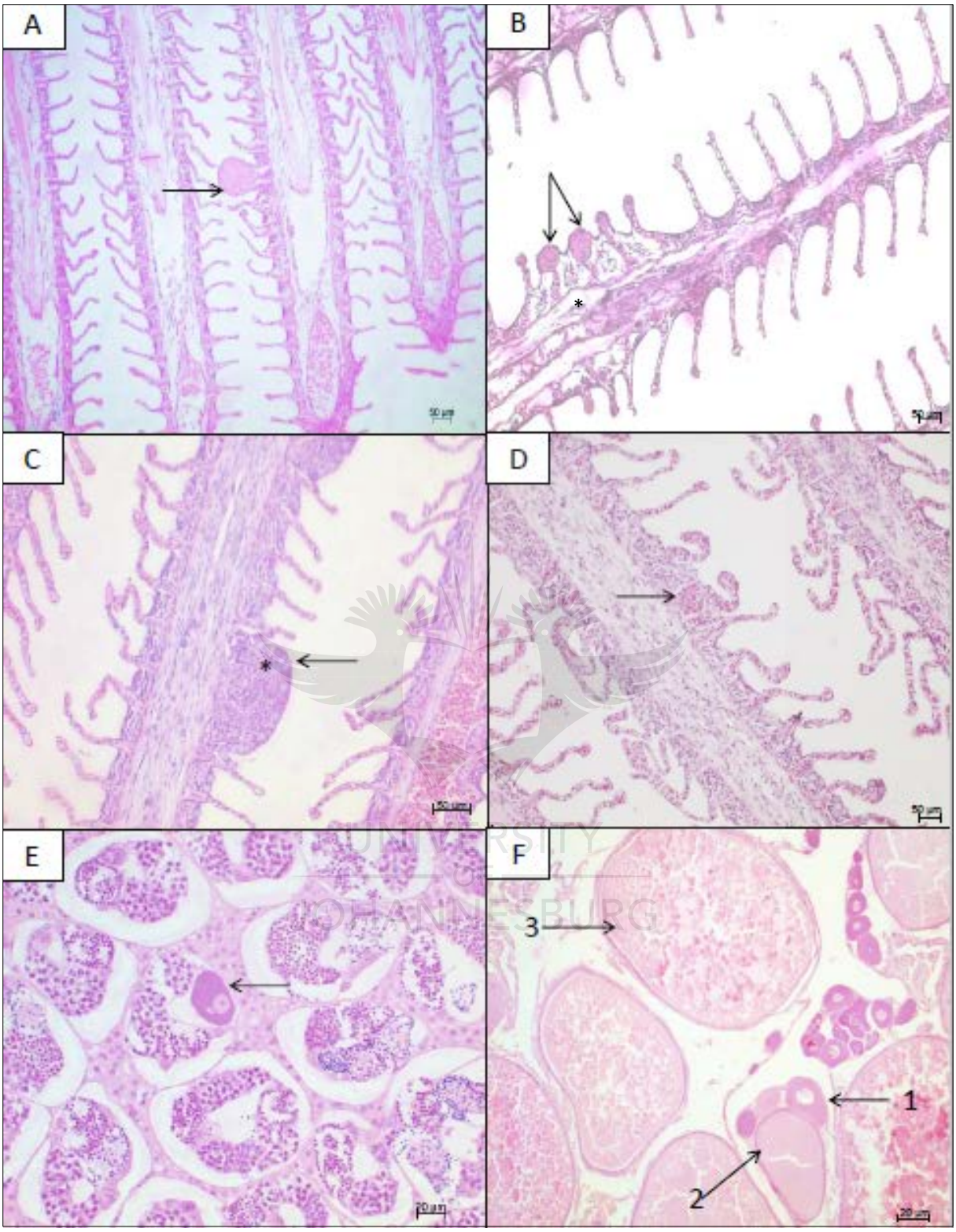

Figure 47: Light micrographs of the gills and gonads of *Oreochromis mossambicus* from Edendalespruit, Pienaars River and Hartbeesspruit (H&E). A - B – Telangiectasia (aneurysm) visible in the distal region of the secondary lamellae (arrows); lifting of primary epithelium (asterix) C – Focal epithelial (*) and mucus cell (arrow) hyperplasia visible between secondary lamellae; D – Aneurysm visible in the proximal region of a secondary lamella; E – Testicular oocyte (arrow); F – Ovarian tissue showing various stages of oocyte development 1 = primary

oocyte (perinuclear stage), 2 = cortical alveolar stage, 3 = mature oocyte filled with yolk globules

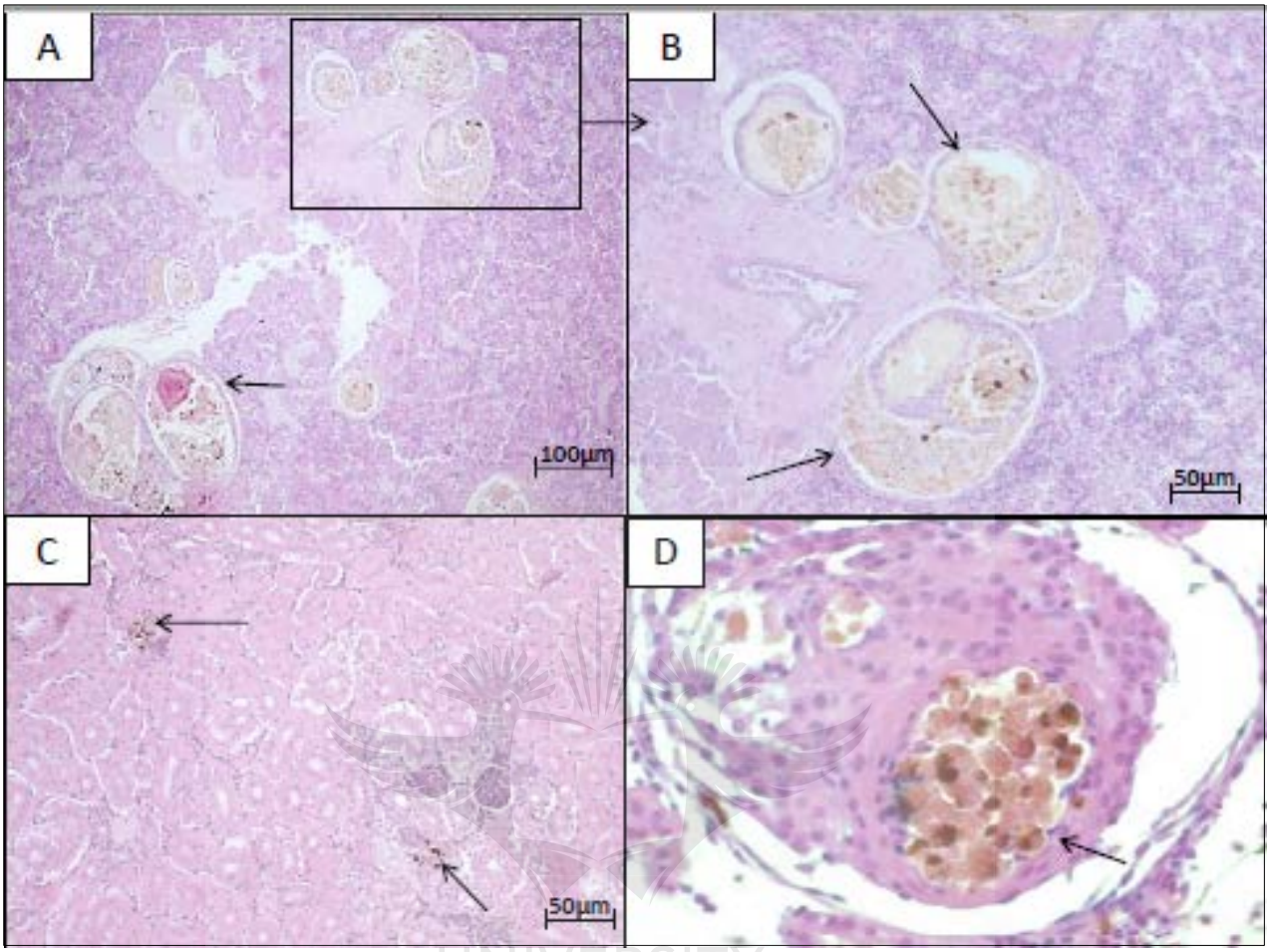

Figure 48: Light micrographs of the spleen and Kidney of *Oreochromis mossambicus* from Edendalespruit, Pienaars River and Hartbeesspruit (H&E): A - B – Encapsulated melano-macrophage centers (arrows) in the spleen; C - D – Melano-macrophage (arrows) visible in the kidney

5.4.4 Semi-quantitative histological assessment

The results (mean organ indices and standard deviations) are displayed in Table 31. The results from Marchand (2012) for the Roodeplaat Dam and from Van Dyk (2006) for laboratory-bred fish from the same species are also presented (Table 32). An overview of the results showed that the mean fish index for all *C. gariepinus* for all three rivers during both seasons fell within Class 2, indicating normal tissue structure with moderate histological alterations. For *O. mossambicus* the fish indices also fell within Class 2 for fish from each river with the exception of the fish Pienaars River which were classified as a Class 1, indicating normal tissue structure with slight histological alterations. These results are presented in Figure 51 and Figure 52.

Table 31: Statistics presenting the mean organ indices and fish indices for *Clarias gariepinus* sampled from Edendalespruit, Pienaars River and Hartbeesspruit during low-flow (July 2015) and high-flow (February 2016) conditions

Organ Indices	Edendalespruit		Pienaars River		Hartbeesspruit		**Roodeplaat Dam values (Marchand 2012)**	**Reference values (Van Dyk 2006)**
	low-flow	high-flow	low-flow	high-flow	low-flow	high-flow		
Gill index	3.27 ± 2.45	3.28 ± 1.96	1.25 ± 0.00	1.71 ± 0.00	1.4 ± 0.82	1.78 ± 0.76	12.20 ± 4.10	0.70 ± 0.98
Liver index	12.72 ± 5.68	12 ± 10.81	8 ± 6.59	12.00 ± 7.46	9.6 ± 11.31	6.22 ± 4.78	20.89 ± 8.59	9.07 ± 6.07
Spleen index	4.72 ± 1.62	0.5 ± 0.00	3.82 ± 1.04	1.52 ± 1.64	2.8 ± 0.00	3.56 ± 1.03	n/a	n/a
Heart index	2.36 ± 1.97	1 ± 1.15	0.5 ± 0.00	3.52 ± 0.75	1.8 ± 1.10	2.22 ± 1.03	4.20 ± 4.15	0.00 ± 0.00
Kidney index	1.27 ± 1.91	1.5 ± 2.00	1.75 ± 0.00	0.57 ± 0.89	2.2 ± 1.49	3.55 ± 1.67	8.90 ± 2.79	3.20 ± 1.64
Ovary index	0.00 ± 0.00	0 ±0.00	1.00 ± 1.15	0.95 ± 1.14	1 ± 0.00	0.66 ± 0.00	4.40 ± 3.75	0.00 ± 0.00
Testes index	0.28 ± 0.00	0 ± 0.00	1.00 ± 2.83	0.09 ± 0.00	0 ± 0.00	0.22 ± 0.00	2.20 ± 2.90	0.36 ± 1.21
Mean fish index	24.54 ± 7.38	18.24 ± 13.63	15.81 ± 9.98	20.38 ± 7.89	18.8 ± 13.10	18.22 ± 6.51	49.90 ± 10.44	13.10 ± 6.03

Table 32: Statistics presenting the mean organ indices and fish indices for *Oreochromis mossambicus* sampled from Edendalespruit, Pienaars River and Hartbeesspruit during low-flow (July 2015) and high-flow (February 2016) conditions

Organ Indices	Edendalespruit		Pienaars River		Hartbeesspruit		Roodeplaat Dam values (Marchand 2012)	Reference values (Van Dyk 2006)
	low-flow	high-flow	low-flow	high-flow	low-flow	high-flow		
Gill index	2.00**	2.58 ± 1.63	0.00**	1.28 ± 0.82	n/a*	2.54 ± 2.14	10.89 ± 3.95	0.00 ± 0.00
Liver index	4.00**	2.83 ± 1.92	4.00**	1 ± 1.10	n/a*	3.81 ± 2.59	12.89 ± 5.83	8.20 ± 3.17
Spleen index	0.00**	2.33 ± 1.60	0.00**	2.57 ± 1.77	n/a*	2.38 ± 1.29	n/a	n/a
Heart index	0.00**	1.42 ± 3.24	2.00**	1.14 ± 1.79	n/a*	0.57 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Kidney index	4.00**	1.25 ± 1.35	0.00**	1.86 ± 1.35	n/a*	1.19 ± 2.03	8.56 ± 7.51	3.00 ± 2.29
Testes index	0.00**	0.08 ± 0.00	0.00**	0 ± 0.00	n/a*	1.14 ± 0.00	13.00 ± 6.50	0.00 ± 0.00
Ovary index	0.00	0.08 ± 0.00	0.00	0 ± 0.00	n/a*	0.00 ± 0.00	1.80 ± 1.99	0.67 ± 1.00
Mean fish index	0.00**	10.58 ± 0.00	0.00**	7.85 ± 4.87	n/a*	12.38 ± 7.12	39.11 ± 12.06	11.50 ± 3.95

n/a*= No *Oreochromis mossambicus* sampled
**Values for the single *O. Mossambicus* sampled

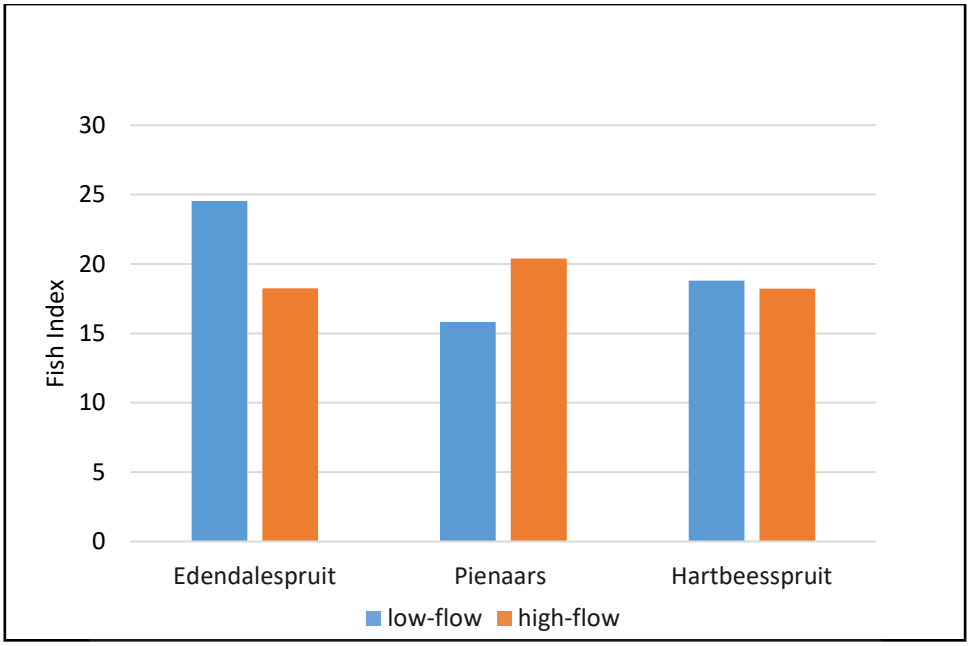

Figure 49: A comparison between Fish Indices for *Clarias Gariepinus* each sampled river during (July 2015) and high-flow (February 2016) conditions

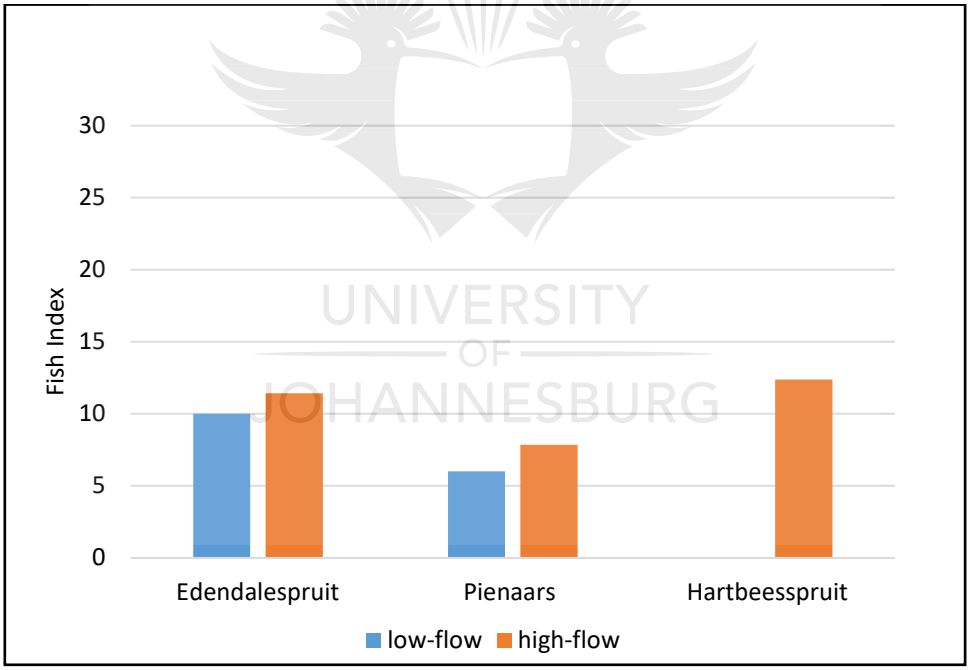

Figure 50: A comparison between Fish Indices for *Oreochromis mossambicus* each sampled river during (July 2015) and high-flow (February 2016) conditions

5.5.5 Microcystin results

Some of the liver samples were compromised during the preparation for microcystin analysis, however, this was less than 15 % of the total sample size. The results are shown in Table 33. The microcystin results showed a visible difference in terms of species. The mean microcystin-LR concentration was significantly higher ($p < 0.05$) in *C. gariepinus*, whereas mean

microcystin- YR and -RR concentrations were significantly higher ($p < 0.05$) in *O. mossambicus*. In *C. gariepinus,* the mean microcystin-YR and -RR concentrations were significantly lower ($p < 0.05$) when compared to microcystin-LR in the same species. The microcystin concentrations in *C. gariepinus* liver was compared within each river, between seasons as well as between male and female fish. The following observations were noted: microcystin-YR concentrations in Hartbeesspruit were significantly higher when compared to Pienaars River and Edendalespruit. For *C. gariepinus,* the microcystin-LR and -RR concentrations were significantly higher ($p < 0.05$) in Edendalespruit when compared to the Pienaars River and Hartbeesspruit. There was a moderate negative correlation ($r = 0.343$) observed between the microcystin-LR and fish index in Edendalespruit and in Hartbeesspruit there was a strong positive correlation observed between the HSI and microcystin-RR and –YR ($r = 0.513$). When comparing the concentrations of microcystins between the male and female *C. gariepinus* there were no significant differences observed ($p > 0.05$). There was, however, a moderate positive correlation between microcystin-LR and HSI in males ($r = 0.317$) and microcystin-RR and HSI in females ($r = 0.05$). There was also a moderate positive correlation ($r = 0.374$) between the liver index and microcystin-YR in female *C. gariepinus*. Microcystin-LR was significantly higher ($p < 0.05$) in both males and females when compared to the other two analogues of microcystins. A seasonal comparison between *C. gariepinus* collected during high-flow conditions and low-flow conditions showed that microcystin-LR was higher during the low-flow conditions whereas microcystin-RR was higher during the high-flow conditions. A moderate positive correlation ($r = 0.397$) was observed between the HSI and microcystin-LR during the low-flow conditions. Due to the two samples from the low-flow conditions both being compromised, no seasonal comparison between seasons was possible in *O. mossambicus*. A statistical analysis of microcystin concentrations in *O. mossambicus* showed that microcystin-RR concentrations were significantly higher ($p < 0.05$) in Edendalespruit when compared to Pienaars River and Hartbeesspruit. Microcystin-RR was also significantly higher ($p < 0.05$) in females when compared to microcystin-LR and microcystin-YR.

Table 33: Microcystin-LR, YR and RR concentration levels in *Clarias gariepinus* and *Oreochromis mossambicus* liver tissue sampled from Edendalespruit, Pienaars River and Hartbeesspruit during low-flow (July 2015) and high-flow (February 2016) conditions

Clarias gariepinus (low-flow)											
	Edendalespruit			**Pienaars River**				**Hartbeesspruit**			
Fish Number	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**	**Fish Number**	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**	**Fish Number**	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**
1	0	0	0	**1**	0.509875	0	0	**1**	0.021938	0	0.00325
2	0	0	0.05625	**2**	4.347	0	0	**2**	1.085	0.247688	0
3	0	0	0.011757	**3**	2.278625	0	0	**3**	1.077875	0	0
4	0.1383	0	0.017871	**4**	1.066313	0	0	**4**	1.5745	0	0
5	0.6549	0.26	0.002938	**5**	1.235375	0	0	**5**	0.157438	0	0
6	0.1279	0	0.00625	**6**	8.262368	0	0	**6**	1.457438	0	0
7	0	0	0	**7**	0	0	0	**7**	0	0	0
8	0	0	0	**8**	0	0	0	**8**	0	0	1.407625
9	0	0	0	**9**	3.802313	0	0.014313	**9**	0	0	0
10	0	0	0.00775	**10**	2.586063	0	0	**10**	0	0	0
11	0	0	0								
Mean Values	**0.0837**	**0.0236**	**0.0102**		**2.4088**	**0**	**0.0014**		**0.5374**	**0.0248**	**0.1411**
	Clarias gariepinus (high-flow)										
	Edendalespruit			**Pienaars River**				**Hartbeesspruit**			
Fish Number	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**	**Fish Number**	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**	**Fish Number**	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**
1	0	0	0	**1**	2.06525	0	0	**2**	0	0	0
2	0	0	0	**2**	0	0	0	**3**	0	0.290188	0
3	0	0	0.158188	**3**	0	0	0	**4**	0	0.200063	0
4	0	0	0	**4**	0	0	0.006813	**5**	0	0.219375	0
5	0	0	0.158188	**5**	0	0	0	**6**	0	0.096813	0.00675
6	0	0	0	**6**	0	0	0	**7**	0	0.133981	0.012571

7	0	0.5592	0	**7**	0	0	0	**8**	0	0.471938	0
8	0.2048	1.5396	0	**8**	0	0	0	**9**	0.20475	0.09825	0.012688
				9	3.28975	0	0	**10**	0.14725	0	0.011688
				10	1.321688	0	0				
				11	1.436688	0	0				
				12	0	0	0				
				13	0.05925	0	0				
				14	0.202	0	0				
				15	0.455375	0	0				
				16	0	0	0				
				17	0	0	0.004563				
				18	0	0	0				
				19	0	0	0				
				20	0	0	0.005438				
				21	0	0	0				
Mean Values	**0.0256**	**0.2624**	**0.0396**		**0.4205**	**0**	**0.0008**		**0.0391**	**0.1679**	**0.0049**
Oreochromis mossambicus (high-flow)											
	Edendalespruit			**Pienaars River**				**Hartbeesspruit**			
Fish Number	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**	**Fish Number**	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**	**Fish Number**	**Microcystin-LR (pg/mg)**	**Microcystin-YR (pg/mg)**	**Microcystin-RR (pg/mg)**
1	0	0	0.063375	**1**	1.0865	0	0	**1**	0	0	0.017938
2	0	0	0.063375	**2**	0	0.10625	0	**3**	0	0	0.001938
3	0	0	0.023763	**3**	0.219	0	0	**5**	0.076148	0	0
4	0	0	0.462875	**4**	0	0	0	**7**	0	0.523111	0
5	0	0.243125	0.356375	**5**	0	0.138375	0	**8**	0	0.064096	0.017726
6	0	0.100991	0.824037	**6**	0	0.198313	0	**9**	0	0	0.049651
7	0	0	0.0114	**7**	0	0	0	**10**	0	0	0.029643
8	0	0	0	**10**	0	0	0.044	**11**	0	0	0.043696
9	0	0	0.02625	**11**	0	0	0.007938	**12**	0	0	0

10	0	0	0.30725	**13**	0	0.058	0.007938	**13**	0	0.656031	0.026838
11		0	0.621875					**14**	0	0	0
13	0	0.118954	0.086757					**15**	0	0	0.004125
15	0	0.06625	1.0235					**19**	0	0	0.087522
16	0	0	0					**20**	0	0	0.01875
17	0	0.344938	0.258188					**21**	0	0	0.017938
19	0	0	0.847875								
20	0	0	0.563375								
21	0	0	0.075257								
Mean Values	**0**	**0.0486**	**0.3119**		**0.1306**	**0.0501**	**0.0060**		**0.0051**	**0.0829**	**0.0211**

5.5 Discussion

5.5.1 Macroscopic abnormalitieS

Most of the macroscopic abnormalities observed during both seasons within each river were found in *C. gariepinus* and these were predominantly in the liver. There were, however, other abnormalities observed in organs such as the gills, spleen and gonads. Previous studies conducted in Roodeplaat Dam also showed severe macroscopic abnormalities in the liver of *C. gariepinus*. These organs were noted to be discoloured (red, green, yellow or black) with large fatty nodules (Van Dyk *et al.* 2012) which was similar to the livers of fish sampled as part of this current study. The nodules identified in fish from Roodeplaat Dam was found to be more fatty in nature. This was confirmed in the histological analysis which showed a higher percentage prevalence of steatosis, this was seen mainly in fish from Hartbeesspruit. Macroscopic liver nodules have been found to reflect histological alterations such as inflammation, necrosis and a variety of FCAs associated with different types of hypertrophy. These types of alterations have been suggested to be associated with fish exposed to microcystins (Gupta and Guha 2006). This was indeed true as different concentrations of different analogues of microcystins were found in both species.

The only abnormalities observed in *O. mossambicus* in Roodeplaat Dam in a previous study by Marchand *et al.* (2012) were deformed testes and structural alterations of the kidney. In the current study, macroscopic abnormalities such as abnormal papillae were noted only in *C. gariepinus.* This, however, has been seen before in previous studies. Kruger *et al.* (2013), conducted a study in Rietvlei and Marias dams using the urogenital papillae as a biomarker of aquatic pollution. This macroscopic abnormality was compared to the GSI and the urogenital papillae length index. The researchers assumed that the abnormal papillae (which was seen in 16.49% of the males collected) was an indication of intersex (Kruger *et al.* 2013). The histological assessment showed that only 62.50% of the fish suspected to have intersex, did indeed have the condition. In their study, the mesenteric fat which was also tested, showed high levels of the EDC, Nonyphenol. Abnormal papillae in *C. gariepinus* were also observed by Bengu *et al.* (2017). Although, more than 80% of fish sampled had macroscopic abnormalities of the papillae, not all of these fish showed histological signs of intersex. There were also some fish which did not display macroscopic abnormalities but still had intersex (Bengu *et al.* 2017). Similar to the study conducted by Kruger *et al.* (2013), EDCs were found in the water samples. However, these were not at very high levels. There were, however, high levels of faecal coliforms and certain SVOCs detected. From these studies it is clear that

abnormally-shaped papilla does not necessarily indicate intersex. However in the current study the *C. gariepinus* displayed abnormal urogenital papillae (two papillae observed on the same fish) with no intersex but testicular oocytes were observed in *O. mossambicus* collected from the same river (Hartbeesspruit). Interestingly, although no detectable levels of EDCs was seen in water samples from the Hartbeesspruit, the sediment showed the highest levels of SVOCs during both seasons in this river and its tributary Morelettaspruit.

Pale gills were seen in both species sampled from Edendalespruit and Hartbeesspruit. Although this could be as a result of the gill nets, extra care was taken in handling the fish in gill nets. The results showed that most of the fish with pale gills were found in Edendalespruit which corresponds with the high prevalence of gill epithelial hyperplasia in fish from this site. This alteration has been associated with an increase in the surfactant layer that is responsible for protecting the gill lamella (Temmink *et al.* 1983). Hyperplasia could be the possible cause of the pale gills observed. This is due to the fact that this alteration decreases the oxygen distance between water and blood resulting in hypoxia (Skidmore and Towell 1972; Cerqureira and Fernendas 2002).

Biometric Indices

Previous studies have shown that the CF for *C. gariepinus* ranges from 0.6 – 0.9 (Marchand 2006; Van Dyk 2006; Crafford and Avenant-Oldewage 2009). This was the case during low-flow as well as the high-flow conditions in the current study. According to Van Dyk (2006), the CF for laboratory-bred *O. mossambicus* is 1.67. During low-flow conditions both the *O. mossambicus* sampled had a higher CF. This was significantly different ($p < 0.05$) when compared to *C. gariepinus*. It is important to note that morphometric differences need to be considered when comparing the CF between species. Sara *et al*. (2013) conducted a study to determine the ecological status of The Hout River Dam in the Limpopo Province of South Africa and found that *O. mossambicus* had the highest CF values between the three species compared (*C. gariepinus, O. mossambicus* and *C. carpio*) (Sara *et al.* 2013). They concluded that *O. mossambicus* had higher values due to the fact that they are filter feeders and can therefore make use of the natural productivity of phytoplankton and zooplankton throughout the year (Sara *et al.* 2013). The CF is affected by a change in body shape of the fish as a result of a change in feeding habits and environmental stressors such as metal contamination, depletion of energy reserves or change in seasons (Keyombe *et al.* 2015).

The study by Htun-han (1978), showed a higher CF in summer, related to gonad size. Sara *et al.* (2013) had similar results but concluded that it was because of a depletion of energy reserves during low-flow conditions (stored liver glycogen or body fat) (Adams *et al.* 1993).

This is contrary to this particular study, the mean body mass of the fish during the low-flow conditions was significantly higher when compared to the high-flow conditions. There were no significant differences observed in the mean masses of gonads but generally the mean mass was higher during the high-flow conditions in each river with the exception of Pienaars River, this corresponds with Htun-han (1978). There did not seem to be an association between the fish with higher CF and the presence of mesenteric fat in fish from the current study.

The CF of the female *C. gariepinus* was lower than that of males during the low-flow conditions. This could be due to the fact that females utilized their metabolic energy for body maintenance and egg laying (Keyombe *et al.* 2015). Seasonal changes could also be as a result of food availability, a wide range of environmental factors and gonadal development (Pope and Willis 1996). This means that the GSI can directly affect the CF. For this reason, the mean CF was calculated with and without the inclusion of gonad mass. There was, however, a negligible and non-significant difference observed comparing these values. The higher CF also possibly meant that the males were in a better condition physically during low-flow conditions when compared to the females. In general, heavier fish are assumed to be in better health as an increased body weight could be as a result of food availability and metabolic rate (Bolger and Connoly 1989; Keyombe *et al.* 2015). For example, the study conducted by Keyombe *et al*. (2015) in Lake Naivasha, Kenya, suggested that *C. gariepinus* with a CF of <1 indicated that the fish were not doing well in the lake which may be due to the food availability (Keyombe *et al.* 2015). In the current study the highest CFs were seen in both species collected from the Pienaars River and the Hartbeesspruit. The study by Bagenal and Tesch (1978) reported that fish sampled from the most polluted sites had the highest values which could be indicative of the high productivity at the site due to effluent from mines, municipal treatment plants and agricultural activities (Bagenal and Tesch 1978). This could possibly also apply to the Hartbeesspruit (agricultural activities) and the Pienaars River (municipal treatment plants). When taking into consideration the fact that the availability of food could possibly be reflected in the CF (Keyombe *et al.* 2015), it is important to mention that the inflow of the Hartbeesspruit and Pienaars River are in closer proximity to each other as opposed to the inflow of Edendalespruit. Due to the unique shape of the dam and location of the rivers, this could mean that there is more food available on the eastern side of the dam in these two rivers.

With regard to the HSI, there were a few deviations observed in each river. The HSI was significantly ($p < 0.05$) higher during the low-flow conditions when compared to the high-flow conditions for both species. A decrease in the HSI has been seen in studies of fish exposed to a variety of pesticides. This may be as a result of the regressive histological and physiological alterations caused by exposure to toxicants within the water (Jordaan *et al.* 2013;

Saravanan *et al.* 2016; Nnadi *et al.* 2018). However, there were no pesticides detected in the water or sediment sampled at any of the sites during the current study.

The *C. gariepinus* sampled from Hartbeesspruit had the highest HSI during the low-flow season. This was significantly higher when compared to fish from Pienaars River which had the lowest HSI of the three sites. This was expected due to the fact that the highest percentage prevalence of macroscopic nodules were observed in fish from this site and since the nodular growths signifies an increase in hepatocytes it will therefore increase the HSI. A low HSI in sites receiving sewage effluent, such as the site in the Pienaars River, has been observed in other studies as well (Ma *et al.* 2005; Sadekarpawar and Parikh 2013). According to the researchers, this lower HSI was as a result of the sub-lethal toxicity of the sewage effluent (Ma *et al.* 2005; Sadekarpawar and Parikh 2013; Araújoa 2018). Hartbeesspruit had the highest HSI for *O. mossambicus,* this site also had the highest percentage prevalence of macroscopic nodules in the liver. During the high-flow conditions, both species sampled from Hartbeesspruit had the highest percentage prevalence of steatosis. This resulted in increased hepatocyte size associated with steatosis i.e. the excessive accumulation of lipids in the hepatocytes (Feist *et al.* 2004). The increased liver size could also be associated with sewage effluents discharge (Gallaway *et al*. 2003). There were also positive correlations seen between microcystin-LR, YR and RR concentrations and the HSI when comparing sites, seasons and sex in *C. gariepinus*. Another study have shown an increase in hepatocytes as a result of microcystin exposure (Malbrouck and Kestemont 2006).

Due to the livers' role in vitellogenesis, changes in the HSI and GSI may be linked (Scott and Pankhurst 1992). Vitellogenesis is known as yolk formation through nutrients being deposited into the oocytes. The liver also plays an important role in nutrient metabolism and synthesis of vitellogen in females (Wootton 1998; Lubzens *et al.* 2010), thus resulting in a correlation between these two indices. This correlation has been seen in a number of studies (Kyomo 1988; Nunes *et al.* 2011; Singh and Srivastava 2015). However, in the current study there was no correlation seen between GSI and HSI.

The study conducted by Van Dyk (2006) indicated that the mean GSI reference values calculated for sexually mature *C. gariepinus* ranges between 0.17 – 0.59 for males and 0.63 - 12.41 for females (Van Dyk 2006). The male mean GSI for each river was low but fell within the reference value ranges during both seasons. Although one should consider the sexually maturity of the gonads and seasonal variation when comparing GSI values, the decreased GSI can also be as a result of the fish being exposed to different environmental pollutants. This has been seen in a number of studies (Friedman *et al.* 1996; Schmitt and Dethloff 2000, Pieterse 2004; Ackermann 2008; Marchand *et al.* 2008; Bengu *et al.* 2017). The GSI for

females was higher within each river during both seasons (there was no statistical difference between the two seasons). According to Bruton (1979), gonadal maturation is associated with increased water temperature and spawning season. For *C. gariepinus* this period is between is between September and March (Bruton 1979). The sampling season was during February 2016 and thus during the spawning season. Mature females with bigger ovaries therefore resulted in a higher mean GSI. The GSI between males and females was not compared due to the fact that they are completely different in structure, shape and size. Akpaniteaku (2012) conducted a study to determine if there was a relationship between the size of *C. gariepinus* spawners and their ovaries. The fish were obtained from the lower Niger River, and placed in groups according to their maturity levels. There was a positive correlation between the length of the females and the size of their ovaries and the gonadosomatic index for mature females went up to 10.35% (Akpaniteaku 2012).

The GSI reference ranges as indicated by Van Dyk (2006) for laboratory-bred *O. mossambicus* is 0 – 2 for males and 0 – 4 for females. Fish from each river sampled during both seasons fell within the reference value ranges. There were no females sampled during the low-flow conditions. This could possibly be due to the fact that spawning takes place in higher temperatures ranging from 18 – 25 °C (James and Bruton 1992), thus resulting in an absence of female fish to sample. *Oreochromis mossambicus* breeds in nests in the shallow water, in summer the males guard the nests and females carry the young fish in the mouths and cannot feed. This may play a role in the difference in CF. The testicular histology was assessed in a DDT- sprayed area by Marchand *et al.* (2008) in *C. gariepinus* and *O. mossambicus.* The results showed that exposure to toxicants in the aquatic environment can affect the GSI. The mean GSI was lower in *O. mossambicus* with testicular oocytes. This was also seen in a study conducted by Jobling *et al.* (1998) where fish were exposed to sewage effluent. In the current study only one sampled *O. mossambicus* displayed testicular oocytes. As mentioned in Chapter 3, a function venue which treats sewage water on site is located on the banks of Hartbeesspruit where the fish were collected. This correlates with the study by Jobling *et al.* (1998) where fish were also exposed to sewage effluent. The observation in a single fish is, however, not conclusive.

The splenosomatic index (SSI) was above the reference value ranges proposed by Van Dyk (2006) (0.01 - 0.04). This was seen during both seasons for both sexes of *C. gariepinus* in each river, and it was significantly higher during the low-flow conditions. The biggest spleens were seen amongst the *C. gariepinus* sampled during the high-flow conditions. However, the fish was the smallest within in the sampled group. Alterations in the environment can lead to an increased volume of the spleen. This type of adaptation insures that the organ maintains its functions (Lizama *et al.* 2006).

Histopathology

The number of histological alterations in *C. gariepinus* varied amongst organs, however most of the alterations were seen within the gills and the livers. Gill pathology ranged from circulatory to regressive and progressive changes. The most common pathologies seen were aneurysms (e.g. telangiectasia), structural branching (e.g. secondary lamella branching), plasma alterations (e.g. vacuolation) and epithelial hyperplasia. Numerous studies have been conducted on the structural changes of fish gills as a result of toxicants exposure. However, a literature review revealed that these changes have been more commonly reported after lethal and not sub-lethal exposure to contaminants (Mallat 1985). The gills of fish are also known to be sensitive primary target organs for a wide range of stressors including metals (Laurén and McDonald 1985, Verbost *et al*. 1987, Hinton *et al*. 1992, Garcia-Santos *et al.* 2007). The percentage prevalence for the observed alterations was the highest in fish from Edendalespruit during both seasons. The mean gill index observed during the low-flow conditions for *C. gariepinus* sampled in the Edendalespruit was the highest amongst all sampling sites during both seasons. During the low-flow conditions, circulatory and regressive changes were the most common (58%) in fish sampled from Edendalespruit. Circulatory changes are most commonly associated with pathological conditions of the blood and tissue flow. Telangiectasia is usually linked to a disintegrated pillar cell system (Takashima and Hibiya 1995). This phenomenon is fairly common in studies related to acute, chronic, lethal and chronic sub-lethal effects of chemical and physical irritants (Mallat 1985). According to Wolf et al. 2015, Telangiectasia is often considered as a consequence of sampling and rather a non-pathological condition (Wolf *et al.* 2015).

Similar results were seen in a study conducted in the Tamis River, Romania and Serbia (Lujić *et al.* 2013). A histological assessment of the gills was conducted to determine if it can be used as an indicator of water pollution. The selected river was prone to anthropogenic impacts such as ill-maintained irrigation systems, sewage effluents and livestock farms amongst others. The results also showed progressive changes in the gill histology such as hyperplasia and hypertrophy. Circulatory disturbances such as telangiectasia were also observed. The researchers concluded that the histological alterations seen were mainly caused by elevated levels of Iron, Chloride and Copper in the water as a result of the surrounding anthropogenic activities. In the current study there were no detectable levels of Copper and Chloride found in any of sites sampled, however the Iron levels in Edendalespruit was higher than the recommended guidelines which could possibly explain the gill pathologies observed. Similar results were seen in a number of studies where elevated Iron levels impacted the histology of fish gills (Peuranen *et al.* 1994; Dalzell and MacFarlane 1999; Slaninova *et al.* 2014).

Although regressive changes such as structural alterations were observed in fish from each river, the percentage prevalence was higher in Edendalespruit during both seasons. Structural alterations such as secondary lamella branching had a high percentage prevalence in fish from Edendalespruit. This was also seen in another study where Brown trout were exposed to sewage effluent (Bernet *et al.* 2004). As previously mentioned, the Edendalespruit site was situated between two formal housing estates (Chapter 2) and the water quality results (Chapter 3) showed that although this site had the highest pH and the highest Nitrate levels, it had the lowest faecal coliform bacteria levels during the high-flow season and second lowest during the low-flow season.

Vacuolation was only observed in *C. gariepinus* from Edendalespruit. During both seasons, progressive changes in the epithelial cells (hyperplasia) of the gills were only found in *C. gariepinus* from Edendalespruit. The percentage prevalence was high when compared to the other alterations found in fish at this site. Hyperplasia is a defence mechanism which has also previously been linked to metal pollution (Alazemi *et al*. 1996; Camargo *et al*. 2007; Garcia-Santos *et al*. 2007; Mohamed 2009). The study conducted by Van Dyk *et al.* (2009) focused specifically on the histological responses in the gills of *C. gariepinus* in two polluted urban impoundments (Marais and Rietvlei dams). Hyperplasia was also found. This study reported that this could be as a result of micro-organism infection, ectoparasites, metals or ammonia (Hugget *et al* 1992). The Edendalespruit is in close proximity of the abandoned Edendale mine (Chapter 2). The link between hyperplasia and metal contamination could be explained by the location of the site. This site also had strong positive correlations with Silicon, Iron, Aluminium, Magnesium and Potassium (Chapter 3 – Figure 8).

The liver pathology in *C. gariepinus* ranged from a variety of dispersed or diffused lesions to different types of foci of cellular alteration (FCA). There were no circulatory disturbances observed in any of the fish sampled. Disturbances were mainly regressive or progressive in nature. Overall the highest number of different pathologies was observed in *C. gariepinus* sampled in the Pienaars River. The most dominant were regressive changes, which imply that the changes were more regenerative. Intracellular deposits were found in fish collected from all three rivers. However, it was not seen in fish from Edendalespruit during the low-flow conditions and Hartbeesspruit during the high-flow conditions. The highest percentage prevalence was recorded in Edendalespruit (25%) during the high-flow conditions.

Intracellular deposits and melano-macrophage centres are commonly-found histological alterations in *C. gariepinus* from polluted and reference sites so it has been suggested that it might be a normal histological feature linked to normal physiological processes within the species (Haaparanta *et al.* 1996; Van Dyk *et al.* 2012). Melano-macrophages are a particular

type of macrophage which is known to occur in the parenchyma of fish livers, they are usually concentrated as melano-macrophage centres and their size and content is highly dependent on the age, species and health status of fish (Munshi and Dutta 1996). According to Ellis *et al.* (1976) “melano-macrophages is phagocytic in nature.” Melano-macrophages were the most common histological alterations observed in each river during both seasons, the highest percentage prevalence was observed in Hartbeesspruit during both seasons. They differed in appearance and ranged from light-gold vacuolated to light-gold granulated and dark solid large. In a study conducted by Van Dyk *et al.* (2012), liver samples were sampled from *C. gariepinus* from Roodeplaat Dam. The liver tissue was stained to determine the content within the MMCs observed and the sections stained positive for hemosiderin (Perls Prussian blue) (Van Dyk *et al.* 2012).

Hepatocellular and nuclear pleomorphism was also fairly common alterations found during both seasons in each river with the exception of the low-flow survey for Edendalespruit. The percentage prevalence was found to be the highest in fish from the Pienaars River during the low-flow conditions. Steatosis was also observed in livers from all *C. gariepinus* and although the percentage prevalence was highest during the low-flow survey in Hartbeesspruit, it was not observed at all during the high-flow survey. Vacuolation other than steatosis (most likely glycogen) was found during the high-flow survey with a high percentage prevalence in Edendalespruit and Hartbeesspruit. This alteration was also noted in fish from the Edendalespruit during the low-flow conditions, but the percentage prevalence was lower. Steatosis is known as excess lipid accumulation in the hepatocytes (Wolf and Wolfe 2005). The study conducted by Van Dyk *et al.* (2012) observed this alteration in *C. gariepinus* from reference sites as well as polluted sites and as the percentage prevalence was higher in the polluted sites, they concluded that it could possibly be an indication of toxicant exposure. Previous laboratory studies have showed similar alterations in fish exposed to microcystins (Fischer *et al.* 2000; Xie and Li 2009).

Foci of cellular alterations (FCA) were prevalent in the livers of fish from each river during both seasons, with a variety of FCAs observed. FCAs are aggregated hepatocytes which appear different from the other surrounding hepatic parenchyma due to their morphology and staining characteristics (Feist *et al.* 2004). The varieties observed included clear cell, vacuolated, eosinophilic, basophilic, mixed, necrotic, hypertrophic and granular foci. Van Dyk *et al.* (2012) also identified a variation in cytoplasmic appearance in *C. gariepinus* collected from Roodeplaat Dam. These ranged from clear to granular, dense, clumped and mixed. The most prevalent cytoplasmic characteristics seen were clumped, basophilic, possibly due to proliferation of the endoplasmic reticulum (Feist *et al.* 2004; Van Dyk *et al.* 2012). In the current study, clear cell cytoplasm and eosinophilic cytoplasm were most prevalent. This is mostly

likely due to proliferation of the smooth endoplasmic reticulum (Bannasch 1997; Feist *et al.* 2004). Clear cell foci were common in fish livers sampled during the low-flow season in Edendalespruit and Pienaars River as more than half the livers sampled displayed these characteristics. This indicates an increase of glycogen storage within these cells (Feist *et al.* 2004). The percentage prevalence of foci was higher and more frequently observed in Pienaars River and the least common in fish Hartbeesspruit. Granular foci and necrotic foci were only observed in fish from the Edendalespruit and Pienaars River.

The most common alteration observed in the ovaries and testes of both species was MMCs. The highest percentage prevalence was seen in Hartbeesspruit. Testicular oocytes were observed in 23% of the *O. mossambicus* males sampled only at Hartbeesspruit, but was absent in the *C. gariepinus* males. This phenomenon was also observed in 2009 by Marchand in the study conducted on Roodeplaat Dam where 44% of the *O. mossambicus* males had testicular oocytes and no *C. gariepinus* were affected. In Bulawayo, Teta *et al.* (2018), found similar results in peri-urban water bodies. The study focused mainly on the effects of EDCs on wild fish. Sewage-treatment plants, one urban stream and dams which have been polluted by a variety of effluents were selected as sampling sites, in addition to one pristine dam (Teta *et al.* 2018). The results showed that 50% of *O. mossambicus* sampled had testicular oocytes, and a 0% prevalence in the *C. gariepinus* (Teta *et al.* 2018). The percentage prevalence of this phenomenon in the current study was relatively low when compared to Teta *et al*. (2018) and Marchand (2009). Although these studies show that this phenomenon could possibly be species specific, the first study with histological evidence of intersex in a species inhabiting a South African water source was found in *C. gariepinus* (Barnhoorn *et al.* 2004). The study was conducted in Rietvlei Dam (also hypereutrophic) in South Africa, and the condition was expressed in 20% of the males (Barnhoorn *et al.* 2004). More recently, Bengu *et al*. (2017) conducted a study on *C. gariepinus* sampled from Orlando Dam and the Klipspruit wetland system in Soweto, south of Johannesburg in South Africa. The results showed 13.6% and 50% of intersex-related alterations in fish from Klipspruit wetland system and the Orlando Dam respectively (Bengu *et al.* 2017). The water quality results of this particular study also showed alarmingly high levels of faecal coliforms and metal concentrations, whereas the sediment samples had detectable levels of EDC's (Bengu *et al.* 2017).

These findings support previous studies which have shown that this condition is common in fish that have been exposed to EDCs (Kime *et al.* 1999; Cheek *et al.* 2001; Zaroogian *et al.* 2001; Jobling and Tyler 2003; Barnhoorn *et al.* 2004, 2010; Marchand *et al.* 2008; Pieterse *et al.* 2010; Blazer *et al.* 2012). The most severe effects of endocrine disruptors have been seen in fish collected in close proximity to wastewater treatment plants whereas as the effects seen in fish collected close to heavy industrial sites were more linked to reproductive abnormalities

such as a reduced growth of gonads as well as lower levels of estrogen and androgen (Pait and Nelson 2002). Although there were no polychlorinated bi-phenols, chlorinated pesticides and phthalates detected in the water and sediment of any sites there was an exception, with levels of Bis (2-ethylhexyl) phthalate in the sediment at the upstream sites of Morelettaspruit (M2) and Pienaars River (P3). Marchand (2009) also found no EDCs above the detection limit in water samples from Roodeplaat Dam and therefore concluded that testicular oocytes in *O. mossambicus* was rather due to exposure during foetal development or an exposure to low concentrations (undetectable) at chronic levels (Damstra *et al.* 2004).

The most common histological alteration found in the spleen and kidney of both species was melano-macrophage centres. The prevalence of this alteration in *O. mossambicus* was the highest in the Edendalespruit during both seasons. The highest prevalence of this alteration in *C. gariepinus* was recorded in Hartbeesspruit during the high-flow conditions. Previous studies have linked the occurrence of melano-macrophage centers in the spleen to low dissolved oxygen levels, sediment pollution and heavy metal pollution (Fournie *et al.* 2001; Saxena and Saxena 2007). Edendalespruit had the lowest dissolved oxygen during the low-flow conditions and as mentioned previously, a strong correlation with a variety of metals.

As seen in Chapter 3, the levels of microcystins in water samples collected from each river was either low or below the detection limits, different concentrations of different analogues were however found in the liver tissue of both species. This phenomenon was also previously seen by Schmidt *et al.* (2013) where the concentrations of microcystins was low in the water sampled but higher concentrations were present in the organs sampled from fish. Microcystin concentrations differed amongst species which is most likely due to the different feeding habits. Omnivorous fish are more exposed to the cyanobacterial blooms compared to phytoplanktivorous species (Jai *et al.* 2014). The two indicator species sampled in this study are both omnivorous species, however, *C. gariepinus* is known to also consume other fish while the diet of *O. mosssambicus* is mainly comprised of macro-invertebrates and plants. Microcystin-LR had significantly higher concentrations in *C. gariepinus* when compared to the concentrations observed in *O. mossambicus.* Similar results were obtained in a study conducted by Jai *et al.* (2014). The study focused on the bioaccumulation in four different species of carp (Jai *et al.* 2014). Microcystin- LR, the most commonly found and most toxic microcystin (Fischer *et al.* 2000; Fournie and Courtney 2002; Nchabeleng *et al.* 2014), was found mostly in one species, indicating species difference in bioaccumulation (Jai *et al.* 2014*)*.

Bioaccumulation of microcystin-LR is also higher in edible tissue of fish (Razaitabar *et al.* 2017) and since *C. gariepinus* feeds on other species, the presence of microcystins in the fish consumed could contribute to the elevated levels in *C. gariepinus*. This analogue is also

usually present as a result of ingesting contaminated water (Zurawell *et al.* 2005; Pearson *et al*. 2010; Niedermeyer *et al.* 2014). *Clarias gariepinus* is a bottom feeder but known to come up to the surface of the water to gulp air which could result in ingestion of contaminated water where dead algae is found, hence explaining the elevated concentration of microcystin-LR in this specific species. The findings from this current study were contrary to the study conducted by Zwede *et al.* (2018). Three different fish species namely; *O. niloticus, C. gariepinus* and *Cyprinus carpio* were sampled in Koha reservoir, Ethiopia over six months. The liver and muscle tissue of each fish was analysed and microcystins above the detection limit were more commonly presently in the liver tissue. In terms of concentrations, microcystin-LR had higher concentrations when compared to microcystins-RR and YR. In terms of species differences, *O. niloticus* had the highest concentrations of microcystin-LR followed by *C. carpio* while *C. gariepinus* had the lowest levels (Zwede *et al.* 2018).

The seasonal differences observed in each river could possibly be attributed to the detoxification due to the higher temperatures as discussed by WHO (1999) and Shimizu *et al.* (2013). Algal blooms are influenced by the light intensity, Nitrogen: Phosphorus ratio and the distribution of temperature (Van Liere and Mur 1980; Schreurs 1992; Robarts and Zohary 1987). However, the concentration of microcystins is usually coupled with low pH levels as previous studies have shown that microcystins can even resist boiling (Harada *et al.* 1995; WHO 1999). In *C. gariepinus,* the microcystin-LR concentrations were significantly higher during low-flow conditions, whereas, the microcystin-RR and YR was significantly higher during high-flow conditions. Many studies have shown that cyanobacterial blooms are influenced by temperature and that an increase in temperature means an increase in algal blooms (Xue *et al.* 2017; Preece *et al.* 2017; Pham *et al.* 2017). Microcystin-LR, however, is very stable in water and according to Kotak *et al.* (1995) has a negative correlation with temperature and Nitrate concentration (Kotak *et al.* 1995). Kotak *et al.* (1995) also found a positive correlation between pH, phosphorus and chlorophyll-*a* with microcystin-LR concentrations. It was therefore expected that in this current study, microcystin-LR concentrations would be higher during the high-flow conditions as there was an increase in pH, Phosphorus and chlorophyll-*a*. However, this was not the case. Xie *et al.* (2016) conducted a study to determine the effect of environmental factors on the concentrations of microcystin-LR and YR and found that temperature was not a factor in the varying concentrations but that the concentrations were more influenced by the Phosphorus and Nitrate concentrations (Xie *et al.* 2016). As there was an increase in microcystin-YR in the current study, this could indicate a positive correlation between Phosphorus and Nitrate and microcystin-YR. This could also be true for Microcystin-RR as this analogue is known to have similar properties when compared to Microcystin-YR (Butler *et al.* 2009).

A comparison of microcystin concentrations between sites showed that microcystin-RR and LR concentrations were significantly higher in Edendalespruit when compared to Pienaars River and Hartbeesspruit. This was interesting as most studies have confirmed that microcystin-LR is the most commonly found microcystin. Mbukwa *et al.* (2012) also found microcystin-RR to be the most common microcystin in water samples collected in Hartbeespoort Dam, however, no tissue samples were analysed (Mbukwa *et al.* 2012). The concentrations of microcystin-YR were significantly higher in Hartbeesspruit when compared to Pienaars River and Edendalespruit. Interestingly, this site had the highest Phosphorus and Nitrate during the high-flow conditions, further suggesting that this analogue is influenced by these nutrient concentrations.

5.6 Conclusion

Based on the results and discussion presented above, the general conclusion is that in terms of fish indices, *C. gariepinus* was the most affected fish between the two species, this was also seen in the study conducted in Roodeplaat Dam by Marchand *et al.* (2012). Both species sampled in Edendalespruit were the most affected during the low-flow conditions and only in *O. mossambicus* could one clearly see that the fish collected during the high-flow were more affected. The histological results highlighted the fact that the livers of *C. gariepinus* was the most affected organ and fish from the Pienaars River was most severely affected by histological alterations in the liver; there was a significant difference between the liver pathology of the two sampled species. The liver is the main detoxification organ and the high prevalence of histological alterations in *C. gariepinus* could imply that this species is more severely affected by pollution within the system, which is most likely as a result of its feeding habits and breathing behaviour. There was a significant difference seen only in the liver histology and heart histology when comparing the two species. Histological alterations in other organs were overall more prevalent in Hartbeesspruit, this could possibly be the effects of the sewage water being treated on site in close proximity to the site where fish was sampled. Testicular oocytes were only observed in the *O. mossambicus* sampled from this site so this could also related to the waste water discharge released at the site. Microcystin results showed significant differences in the concentrations of the different analogues which could possibly be due to the different and unique diets of each species. The fish health results indicate that there is a definite health response in the three rivers and that there is a need for regular monitoring and a rehabilitation plan to decrease the pollution within these systems.

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Chapter 6: General conclusions and recommendations

6.1 General conclusion

In 2008, the Department of Water and Sanitation (previously known as Department of Water Affairs and Forestry), released a resource management plan (RMP) for Roodeplaat Dam. The report was compiled with the input of community members, government officials and stakeholders from various business sectors (DWAF 2008). The aim of the RMP was to meet the objectives of the National Water Act which includes protecting the water resource, reduction of pollution and degradation as well as access to the water (DWAF 2008). The report highlighted the main problems experienced in, and around Roodeplaat Dam. This ranged from issues such as unlawful use of the water surface, erosion and sedimentation, to ecological degradation as a direct result of user activities (DWAF 2008). The department also acknowledged the dire situation concerning the water quality, highlighting the occurrence of water hyacinths (*Eichhorinia crassipes*) and cyanobacteria as a result of the algal blooms (DWAF 2008). The department attributed the water quality problems in the dam to surrounding land-uses, discharge from two wastewater treatment plants (Zeekoegat and Baviaanspoort) and the recirculation of nutrients from the bottom sediments (DWAF 2008). DWAF (2008) also concluded that the poor water quality within the dam could be as a direct result of mismanagement of upstream catchments and poor water quality entering from Edendalespruit, Pienaars River and Hartbeesspruit (DWAF 2008). One of the action plans indicated in the report to improve the water quality within the dam, was to investigate the status of Roodeplaat Dam and its associated ecosystems, in the hope of developing a comprehensive set of baseline data for future monitoring purposes (DWAF 2008). This emphasizes the contribution of the current study in providing this much needed background information which may contribute towards improving the management and monitoring strategies of Roodeplaat Dam.

This current study is the first comprehensive attempt to investigate various aspects related to the biotic integrity of these three rivers, specifically with regard to fish health. These type of studies are important to assist in establishing effective management practices in the catchment and to encourage more realistic efforts towards the conservation of the water systems and aquatic life within it (Silberbauer and Esterhuyse 2014). Although this has been acknowledged and the

Department of Water and Sanitation currently conducts fortnightly water quality monitoring within the dam, unfortunately, no long-term strategy was developed to improve the water quality (DWAF 2008).

It is important to acknowledge that ecosystem components cannot be analysed independently when attempting to determine the biotic integrity of a system. Aquatic ecosystems are complex systems where both biotic and abiotic factors interact, and cannot be assessed without integrating each component. This chapter will integrate the results and draw conclusions from each chapter to evaluate the biotic integrity of the system and recommend possible monitoring and mitigation measures to assist in the management of this system.

The key findings from the preceding chapters are summarised below:

- According to the water quality results, only three of the 64 metals were detected above the target water quality range at different sites during both seasons;
- Faecal coliform bacteria was detected at each site during both seasons, however, these levels increased considerably during the high-flow season;
- Most of the sampling sites within all three rivers were classified as hypertrophic based on the Trophic State Classification Boundaries per DWAF guidelines (Van Ginkel 2002)
- Each river showed traceable levels of metals and semi-volatile organic compounds in the sediment samples;
- The physical characteristics of the sediment varied between rivers and between sites within rivers. The organic and moisture content also differed considerably amongst seasons, rivers and sites;
- Most of the sampling sites were classified as ”fair” or “poor” (seriously or largely impaired) according to the macro-invertebrate assessment;
- The majority of macro-invertebrates sampled from each site had low sensitivity scores indicating that the systems were dominated by pollution-tolerant species, even though, according to the habitat assessment, the majority of the sites were classified as “good”;
- There is a clear response evident in fish from all three rivers: *Clarias gariepinus* was found to be the most affected species in terms of macroscopic and microscopic tissue alterations, especially with regard to liver alterations. This different response in species was consistent with the findings of a fish health assessment in Roodeplaat Dam (Marchand 2009). The analysis showed microcystin-LR, RR and YR levels in the liver

tissue, which could be a possible causative agent of the liver alterations identified. Differences in pathology were seen in terms of species, seasons and sites.

Chapter 3 provided an overview of the water and sediment analyses and showed that although there were pollutants such as metals, nutrients and bacteria present in each river, the majority of these contaminants were below the target water quality guidelines as stipulated by DWAF (DWAF 1996 a, b, c, d, e). Some exceptions included specific metals e.g. Iron, Magnesium and Aluminium which were detected at levels above the target water quality ranges in the water of both the Edendalespruit and the Pienaars River. These metals, when present at levels above the target water quality ranges do not lead to any health effects in humans but do affect the aesthetic (taste and smell) quality of the water (DWAF 1996a). In terms of the effect on aquatic biota, studies have shown that Aluminium exposure in fish can be linked, for example, to gill damage as well as oxidative stress in the lymphocytes (Galar-Martinez *et al.* 2010). Both fish species sampled from Edendalespruit did indeed have the highest percentage of circulatory alterations as well as regressive and progressive changes in the gill tissue when compared to fish from the other two rivers. Aluminium is toxic to invertebrates and are therefore frequently used as a pesticide. The high Aluminium concentration may therefore have an effect on the macroinvertebrates.

The Magnesium: Calcium ratio is used to determine the total hardness of water, which is an important factor in fish culture. Studies have shown that an imbalance in this ratio can be toxic to some species (Brown and Lynam 1981; Shi *et al.* 1995; Wang *et al.* 2002; Silva *et al.* 2003). Bijvelds *et al.* (1996) concluded that *O. mossambicus* is less sensitive to disturbances of tissue mineral balance when compared to other fish species. Very soft (0-10 mg/L) or very hard water (300 mg/L and above) can, however, influence hatching success of *C. gariepinus* (Molokwu and Okpokwasili 2002).

High levels of pesticides were expected in the water due to the different agricultural practices in the areas surrounding the rivers. However, there were no pesticides detected in the water but there were elevated levels of selected SVOCs detected in the sediment of different sites of each river. This is due to the fact that PAHs usually settle out of the water column and accumulate in the sediment (Moore and Ramamoorthy 1984). The SVOCs were present in higher percentages in the Hartbeesspruit and its tributary the Morelettaspruit during both seasons when compared to the other rivers. The presence of these SVOCs were directly linked to the surrounding land-uses such as agricultural practices, discharge of raw untreated sewage and close proximity to roads and manufacturing industries (Mason 1995; Weissermel and Arper 1997; McMurry 2004).

The nutrient and bacterial analyses showed that there is indeed a need for concern. Very high levels of faecal coliform bacteria were observed in the Pienaars River (specifically the site where fish was sampled) and high levels were also seen in the site adjacent to the wastewater treatment plant during the high-flow season (site P2). The faecal coliform count increased at each site during high-flow conditions which could possibly have compromised the biotic integrity resulting in a decreased abundance in macro-invertebrates and increase in fish pathologies as reported in other studies (Minor 2007; Midgley *et al.* 2006). The nutrient levels were high in all three rivers which explain the eutrophic status of the receiving impoundment, the Roodeplaat Dam. The first hypothesis stating that the physical, biological and chemical analyses of the water and sediment of the three rivers will show elevated levels of pollution was therefore accepted.

The second hypothesis was formulated based on the comparison of the water and sediment quality between the rivers. It was expected that the results will indicate that the Pienaars River has the highest level of pollution. Based on the results from this particular study, this hypothesis was rejected as levels of pollutants were detected in all three rivers. Edendalespruit had more metals above the target water quality range during the low-flow conditions. This river also had the highest levels for a variety of metals present in the sediment. The highest levels of SVOCs were seen in the Hartbeesspruit and its tributary the Morelettaspruit. This could possibly be a direct result of the surrounding land-use activities such as a busy main road (R513) and a function venue which treats sewage water on site.

The map highlighting the land-use activities surrounding each river (Chapter 2) showed a variety of potential impacts. This results in several possible sources of pollution, with Edendalespruit being impacted primarily by formal settlements and mining activities and the Pienaars River being primarily impacted by a wastewater treatment plant. The Pienaars River is also impacted by anthropogenic effects from informal settlements near the upper reaches. The water quality results from the sampling sites downstream in the Pienaars River showed the highest chlorophyll-*a*, phosphate and faecal coliform levels as well as high conductivity and the lowest level of dissolved oxygen. Monitoring chlorophyll-*a* is important as this can indicate the algal growth within a system, as high chlorophyll-*a* levels are associated with high levels of nutrients (coincidentally this site was covered in algae during the sample collection during both seasons). High levels of nutrients in a system, such as Nitrogen and Phosphorus, are pollution indicators of man-made sources such as fertilizer runoff, ill-functioning WWTPs or a septic system leak. These results could indicate that perhaps the wastewater treatment plant situated near the Pienaars River

(Baviaanspoort) has a detrimental effect on the water even though it complies with the wastewater effluent guidelines (Department of Water Affairs 2010). There were high levels of Silicon in the Pienaars River sediment but other than that the results showed that all metals and SVOCs tested for in the sediment were below the detection limit (where applicable).

In Chapter 4, assessing the macro-invertebrate assemblages, it was expected that, based on previous studies, the high-flow conditions will yield a lower number of taxa and a lower average score per taxa when compared to the low-flow conditions (Coutant, 1962 cited by Mann, 1965; Vannote and Sweeney 1980; Hogg *et al*., 1995; Wellborn and Robinson 1996; Hawkins *et al.* 1997). Surprisingly, this was not the case as there was no set pattern observed in the macro-invertebrate results: During the high-flow conditions, each site either deteriorated when compared to the low-flow conditions or remained within the same ecological class following the guidelines (E/F) (Dickens and Graham 2002). This was with the exception of the upstream reaches of the Hartbeesspruit where the SASS5 score increased during the high-flow conditions. Results for all sites sampled (with the exception of upper reaches of Hartbeesspruit) during the low-flow conditions revealed that the upstream, reference sites, had a higher average scores per taxa taxon (ASPT) resulting in a better ecological class. However, during the high-flow conditions, the opposite was seen in all three rivers. The SASS5 results are directly related to the biotic factors which includes the quality of the water at a particular site as well as the availability of habitats for certain macro-invertebrates (Chutter 1994, 1998; McMillan 1998; Dickens and Graham 2002).

The integrated habitat assessment (IHAS) results showed that the condition of the habitat at all sites improved during the high-flow conditions especially in terms of the riparian vegetation, with the exception of the upstream Morelettaspruit site. This was due to the fact that only one biotope was available. However, previous studies have shown that regardless of the available habitat, high-flow conditions such as increased temperature reduces the abundance of certain species (Hawkins *et al.* 1997); this was confirmed by the current study. The upstream or reference sites for each river were expected to have a higher average score per taxon and a high number of different taxa present when compared to the downstream sites. The Pienaars River was expected to yield the poorest SASS5 results when compared to sites within the Hartbeesspruit and the Edendalespruit. The macro-invertebrate assessment also showed that the Pienaars River downstream had the lowest SASS5 score as well as the lowest number of taxa. Overall, a comparison of results between rivers showed that the Pienaars River (with the exception of P1)

had the highest diversity, SASS5 and ASPT scores during the low-flow season and Hartbeesspruit had the highest diversity and ASPT scores during the high-flow season.

A comparison of the SASS5 results showed that the system is “fairly” to “seriously impaired” (Dickens and Graham 2002) and there are very few aquatic macro-invertebrate taxa. There has definitely been an impact with regard to basic ecosystem function. The third hypothesis, based on the assumption that an assessment of the macro-invertebrate assemblages will yield a low average score per taxon and a low number of taxa, was therefore accepted for most of the sites sampled.

A larger number of fish was collected during the high-flow season for both species even though the same sampling methodology was employed. The spawning season for *C. gariepinus* is September to March and the high-flow sampling survey took place during February after heavy rains which could explain the larger sample size, as fish were more active (Bruton 1979). During the lower water temperatures and low-flow conditions (July - winter), both species are generally less active. Spawning in *O. mossambicus* is also regulated by temperature (Bruton and Bolt 1975). They are, however, highly adaptable but cannot live in temperatures less than 10 ºC (Trewewas 1983). Thus, when assessing the biotic integrity of an aquatic ecosystem it is equally important to consider the natural variations in ecosystem dynamics. This was the primary motivation for the seasonal surveys and there were indeed seasonal variations observed. Fish were sampled close to the inflow of the Roodeplaat Dam, in all three rivers. Prior to the sampling survey, an attempt to collect fish through electroshocking in the upper reaches of the rivers took place. This, however, proved to be unsuccessful as at most of the sites fish were absent, especially at the upstream sites. The reason for this could be due to the depth of the water in the upper reaches, all of the upstream sites were shallow. The only species found in the upper reaches of the Hartbeesspruit and Edendalespruit was *Labeobarbus aeneus.*

However, the total number of *C. gariepinus* and *O. mossambicus* sampled during both seasons implied that there is still a good number of fish present in the lower reaches of the rivers near the inflow of the dam, despite the pollution levels. Scheinin *et al.* (2011) found that an increase in vegetation in a system will have a positive effect on the abundance of fish in the system (Scheinin *et al.* 2011). In this current study, there was an overgrowth of water hyacinths observed at each site which could explain the abundance of fish. This is due to the fact that the fish eat the roots of

the water hyacinth and it is also used as a nursery for smaller fish (Gopal 1987; Kateregga and Sternerb 2007).

The fish health assessment focused on histopathology of selected target organs preceded by a general necropsy to identify microscopic and macroscopic abnormalities respectively. This approach was based on many studies that have shown that fish exhibit toxicant-induced lesions following exposure to pollutants and this can affect the histology of target organs (Bernet *et al.* 1999; Schmitt *et al.* 2006 Marchand 2009; Marchand *et al.* 2012; Pieterse *et al.* 2015; Savaira *et al.* 2015). There was a definite health response in fish sampled in all three rivers. The fish health assessment showed a number of macroscopic and microscopic alterations in the organs analysed. These alterations were similar to results of previous studies conducted on the same species in Roodeplaat Dam (Van Dyk *et al.* 2012; Marchand *et al.* 2012). Marchand conducted a study on both species; focusing on six target organs. The results showed liver abnormalities in *C. gariepinus* and testicular oocytes in the *O. mossambicus* males. The results from her study also showed that *C. gariepinus* had more macroscopic organ alterations when compared to *O. mossambicus*. Similar results were expected in fish inhabiting the inflowing rivers. This was indeed the case. *Clarias gariepinus* showed high percentages of macroscopic abnormalities, particularly in the liver, whereas there were none observed in the *O. mossambicus*. The histological results showed that fish indices for *C. gariepinus* were also higher during both seasons when compared to *O. mossambicus*. The similarity in the results compared to the previous study in the dam could be explained by the obvious fact that the water in the dam originates from the inflowing rivers, hence the type of pollutants are expected to be similar. Also, the fact that fish can migrate within the larger catchment area should be considered. However, studies have shown that *Clarias gariepinus* do not usually have significant migrational movement and usually stay within 1 km of their habitats (Willoughby and Tweddle 1978). The western and eastern section of the dam is separated by the Roodeplaat Nature Reserve and is only linked by a narrow section in the north (Figure 3). Considering the variation of land-use activities surrounding the upper reaches of the three rivers, it is possible that the fish in the downstream reaches of the Pienaars River and the Hartbeesspruit, which flows into the western side of the dam, might show a different health response compared to the fish in the downstream reaches of the Edendalespruit, which flows into the eastern side of the dam.

A key impact on this system's biotic integrity is the close proximity of the wastewater treatment plant to the Pienaars River. It was therefore expected that fish collected from this site will have

more toxicant-induced alterations compared to fish from the other two rivers (Liney *et al.* 2006; Fontaínhas-Fernandes *et al.* 2008; Marchand *et al.* 2012). This was indeed the case. However, based on the water quality analysis, the wastewater treatment plant is being well managed as all constituents released are below the values stipulated in the National Water Act (section 21 (f) and (h)) for treated wastewater in South Africa (Republic of South Africa 1998). The surrounding land-uses play a major role in the quality of the water and ultimately the health of the fish.

The highest fish index was observed in the Pienaars River, thus, the fish collected from this site were most affected. The fish health results show a number of alterations that could well be as a result of long-term exposure (the age of fish were not determined as part of this study), however the circulatory, progressive and regressive changes observed in the fish are mainly of minimal or moderate pathological importance and can be reversed if the stressor is neutralized as also previously reported by Bernet *et al.* (1999).

Previous studies have confirmed that seasonal differences are also expected in the level of alterations found in different fish species (Robarts *et al.* 1987; Amrani *et al.* 2003; Wiedner *et al.* 2007; Lehman *et al.* 2008; Marchand 2009; Conradie and Barnard 2012; Wells *et al.* 2015; Visser *et al.* 2016). It is also important to bear in mind that when we compare environmental conditions and findings between high and low flow, we cannot conclude that it was the flow rate that caused the results as it may also be the season or a combination of both. The percentage prevalence of macroscopic abnormalities seen in *C. gariepinus* was higher during the low-flow season in Pienaars River and Hartbeesspruit. However, the Edendalespruit had the highest fish index during the low-flow conditions. This was based on alterations identified in all the organs assessed. Statistically, there was a significant difference in the fish indices comparing the high-flow and low-flow seasons ($p < 0.05$), there was also a significant difference between the fish species comparing the three rivers. This difference in response could be linked to the different behavioural characteristics of the two species. *Clarias gariepinus* is an air-breather that gulps air at the water surface (Bruton 1979) where the dead algae are usually found. This increases their chances of exposure to biotoxins such as microcystins, which is a hepatotoxin released by the blue-green algae (cyanobacteria) (Butler *et al.* 2009). This ingestion of surface algae could possibly be the explanation for the more severe liver pathology observed in *C .gariepinus*.

Detectable levels of microcystins were found in the water in Edendalespruit and Pienaars River during high-flow conditions. Surprisingly, the Pienaars River had the highest level of microcystins,

as well as a lower macro-invertebrate ASPT and increased nutrients and bacteria when compared to Hartbeesspruit during the high-flow conditions. Bioaccumulation of microcystin concentrations in the liver tissue of the sampled fish showed significant differences between species, sites and seasons. However, the highest concentrations of microcystins were seen in Edendalespruit. In terms of species differences, microcystin-LR levels were significantly higher in *C. gariepinus,* whereas microcystin-RR and YR concentrations were significantly higher in the *O. mossambicus* specimens. This difference is most-likely as a result of the different feeding habits of the two species, as explained before. Seasonally, microcystin-LR concentrations was significantly higher during the low-flow conditions. The presence of this particular analogue is not dependant on temperature but positive correlations have been seen between pH, phosphorus and chlorophyll-*a* (Kotak *et al.* 1995). The fact that, in this current study, pH, phosphorus and chlorophyll-*a* levels were higher during the high-flow conditions, indicate that these factors did not influence the concentrations of microcystin-LR in the current study. In terms of microcystin-LR and YR, a study has shown that temperature was not a factor in the varying concentrations but that the concentrations were more influenced by the Phosphorus and Nitrate concentrations (Xie *et al.* 2016). As previously observed in Chapter 3, Phosphorus and Nitrate concentrations increased during the high-flow conditions, and since the previous study by Kontak *et al.* (1995) showed the negative correlation with these nutrients and Microcystin-LR, the results from study could indicate a positive correlation between Phosphorus and Nitrate and microcystin-YR. This could possibly be the same for Microcystin-RR, as this analogue is known to have similar properties when compared to microcystin-YR (Butler *et al.* 2009). Positive correlations were observed between the HSI and the different concentrations of the different analogues of microcystins detected. This was also reported in previous studies which showed an increase in HSI and liver pathology such as enlarged hepatocytes and necrosis after microcystin exposure (Soong *et al.* 1994; Best *et al.* 2003; Malbrouck and Kenstemont 2006).

As mentioned in Chapter 1, the definition of biotic integrity is “the ability of a system to generate and to maintain the adaptive biotic components, this depends on a variety of factors such as the flow of the river, habitat structure, biotic interaction and water quality”. According to this definition the biotic integrity of the three inflowing rivers of the Roodeplaat Dam is being partially maintained as at most of the sites, the habitat is intact based on the various biotopes present and there are biotic interactions, however, the species diversity has clearly been compromised in terms of macro-invertebrates and the health of fish is affected. The water quality is also compromised. It is therefore obvious, based on the findings of this study, that the biotic integrity of the three rivers

flowing into the Roodeplaat Dam is compromised. A monitoring plan needs to be developed which focuses on all three rivers as a complete system.

6.2 Management and Mitigation

In South Africa there is specific legislation in place to aid in protecting the quality of water resources in order to ensure sustainability for all water users. It also ensure that all aspects of water resources need to be managed in an integrated way, and, where appropriate, management functions need to be delegated to a regional or catchment level to enable everyone to participate. This can only take place once the quality of the water resource is known in order to move towards the integrated management of any water resource (Republic of South Africa 1998). Guidelines as prescribed by the Department of Water and Sanitation are used as a common basis in South Africa from which to derive water quality objectives. This set of guidelines is known as an "essential requirement" which assists and equips all role players to act accordingly in order to maintain the fitness of water, its intended uses and ultimately to protect the health of aquatic ecosystems in the country (DWAF 1996).

This study on the three rivers flowing into the Roodeplaat Dam brought to light a number of issues regarding the biotic and abiotic factors within these systems. In order to develop a monitoring plan for the three rivers flowing into the Roodeplaat Dam, the National Aquatic Ecosystem Biomonitoring Programme can be used as a basis. This programme looks at biotic factors such as fish, macro-invertebrates and riparian vegetation and abiotic factors such as the geomorphology, hydrology, water quality and habitat (Fowler *et al.* 2000). The information provided in this study can be considered as baseline data to enhance future studies and contribute to future monitoring programmes in an attempt to improve the status of the inflowing rivers and ultimately the receiving dam.

In terms of environmental management, the bio-monitoring tools employed in this study were effective in terms of determining the extent of pollution in the system. Although the exact sources of the water pollution could not be identified, the various land-uses surrounding the different sites play a major role by releasing pollutants linked to activities such as farming, wastewater discharge, urbanization, mining and industrialization. From the results of this study it is clear that each river is polluted and plans to remediate the system would need to be employed at each site assessed.

The first step in remediation would be to address the root of the problem. The city of Tshwane is plagued by issues such as improper sanitation. Many disadvantaged communities suffer as there is inadequate national financing available to efficiently resolve common issues such as sanitation backlogs. This might be as a direct result of poor infrastructure due to ill-devised designs and their implementations, bulk infrastructure and lack of water user education (Chauke 2017). The main issue with improper sanitation as seen in the informal settlements upstream from the selected sampling sites, is the fact that it leads to an influx of raw sewage into the aquatic system. This has a direct effect on the biotic integrity as discussed.

It is also important for government departments to enforce existing legislation in order to improve the water quality of the Roodeplaat Dam and the inflowing rivers. Based on the water quality results, the Baviaanspoort wastewater treatment plant is being maintained according to the prescribed guidelines. However, there is a biotic response downstream of the wastewater treatment plant to the treated sewage. This implies that more stringent measures need to be put in place to improve the quality of the water in the downstream reaches. It is also important to enforce the existing legislation regarding the discharge of mining effluent into water sources. Although the Edendale mine is an abandoned mine and the AgriSam mine is known to have little to no environmental impacts, it is still important to measure and monitor the effluent from these two mines. This should correlate with what is stipulated in the Government Notice (GN) No. R. 704 which list specific requirements for mine-water discharges to ensure that water resources are protected (Republic of South Africa 1998).

The overpopulation of water hyacinths in the Roodeplaat Dam and at the inlets of each river is another management issue which needs to be addressed. This has been acknowledged by the Department of Water and Sanitation (DWAF 2008). It is important to address this problem before it grows exponentially as seen in the hypertrophic Hartebeespoort Dam, which is currently covered with hyacinths (EnviroEditor 2017). Once the problem reaches this extent, it will be more difficult to remediate. It is imperative to develop a consistent monitoring plan for selected points within the rivers. The major obstacle in commissioning an effective monitoring plan, is the implementation of such a plan. Effective implementation cannot be achieved without sufficient finances and budget constraints remain an unresolved issue in South Africa. Poor financial management of funds and non-payment for services provided by the government in certain areas is the leading cause of financial challenges faced in the city (Chauke 2017). Due to the fact that the overgrowth of blue-green algae is the cause of Microcystins in the system, addressing this problem would automatically deal with the Microcystin issue. A number of mechanisms have been

proposed for resource limited areas such as sedimentation and coagulation; ozonation and ultraviolet disinfection; activated carbon; chlorination and permanganate as well as nanofiltration and ultrafiltration methods (Roegner *et al.* 2013). But ultimately, prevention is better than cure, it would be easier to prevent contamination by Microcystins by putting in mechanisms to prevent the occurrence instead of attempting to control the situation after it has contaminated the water.

In order to maintain the improved water quality after effective remediation, it is important to educate all water-users and specifically the community members about the detrimental effects of their every day practices on the quality of the water. This includes washing clothes with detergents in the river, unsanitary practices, the use of fertilizers by formal settlements and small farm holdings as well as littering. As mentioned, in order for continuous monitoring to be effective, funds will be required and this is a major obstacle. It is therefore, the responsibility of community members in the surrounding areas to assist to improve the quality of the water by changing their habits and supporting the government efforts to provide clean and safe water. As seen with the RMP, management plans are usually commissioned and developed by the local government, but are very rarely implemented. In cases like these, the community plays an important role in protecting the biotic integrity of the water resources surrounding them.

Furthermore, the biotic integrity of the inflowing rivers need to be protected and managed effectively as it has a direct influence on the biotic integrity of the Roodeplaat Dam. The Roodeplaat Dam is an essential water source as it is an important freshwater reservoir, used for irrigation as well as recreational activities. The recreational activities in and around the dam include power boating, rowing, angling, picnicking, camping as well as accommodation and conference facilities. In addition, the areas surrounding the dam house a number of red-listed bird species and habitats for red-listed invertebrates. The dam is also the primary drinking source for animals in the neighbouring Roodeplaat Nature Reserve. Tourism is also affected by the aesthetic properties of an aquatic ecosystem and it is therefore important to monitor and maintain favourable aesthetic quality to continue receiving economic gain from tourism in and around Roodeplaat Dam.

6.3 Recommendations

The following recommendations are proposed to aid in more effective management of Roodeplaat Dam and its inflowing rivers despite the financial constraints:

- Quarterly monitoring of selected sites should be done to determine changes in water and sediment quality. This should not only include *in-situ* measurements but levels of inorganic and organic compounds should also routinely be monitored;
- Implementation and/or continuation of a project which focuses solely on Hyacinth clean-up within the Roodeplaat Dam and the inflowing rivers;
- Develop programmes which focus solely on educating the surrounding communities and industries. Due to the fact that poor water quality and unhealthy fish unsuitable for consumption are a direct result of the practices of surrounding land users, these programs should provide information on how to limit practices which increase pollution. This will assist in increasing awareness which is a key aspect in management of a water resource.
- Community engagement to determine and change the perceptions of community members regarding the water quality of the rivers and to develop a community-based water management plan. This will enhance the sense of responsibility to protect water resources within local community.
- Further research should include an analysis of stomach content of both species to determine if it is indeed the feeding habits which leads to increased pathology in the liver.

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