ESTABLISHING BIOLOGICAL AND ENVIRONMENTAL DRIVERS THAT INFLUENCE THE HEALTH ASSESSMENT INDEX AS A BIOMONITORING TOOL

BY

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Zoology has not previously been submitted by me for a degree at this or any other University; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

15 November 2018

Phala B.M **Date**

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ABSTRACT

In South Africa, a high anthropogenic demand of water for domestic, recreational, agricultural, urbanisation and industrial purposes has placed enormous pressure on freshwater resources and has led to a decline in water quality. In addition to measuring water quality variables, the health assessment index (HAI) advocated by Avenant-Oldewage and Swanepoel (1993), and modified by Crafford and Avenant-Oldewage (2009) by incorporating the inverted parasite index (IPI), and adapted further by Madanire-Moyo *et al.* (2012) who excluded the white blood cells counts, has been used as a rapid and inexpensive means of assessing and monitoring fish health and, in turn, the state of aquatic ecosystems. However, few studies have evaluated the latter approach in conjunction with other biomonitoring indices to assess the water quality of impoundments. Thus, the aim of this study was twofold. The first was to evaluate the HAI adapted by Madanire-Moyo *et al*. (2012) as a means to establish the health status of *Oreochromis mossambicus* (Peters, 1852) sampled from various impoundments based on a once-off survey. The second was to establish if the adapted HAI, in conjunction with selected biomonitoring indices and environmental variables, could describe monthly and seasonal fluctuations of *O. mossambicus* health.

Once-off surveys were conducted between April and May 2016 at five impoundments. The Luphephe-Nwanedi Dam located in the upper catchment of the Limpopo River System and Rhenosterkop Dam situated in the Elands River, a tributary of the Olifants River System, served as control sites due to little or no anthropogenic activities occurring in their catchment areas. In addition, surveys were conducted at Loskop and Flag Boshielo dams and Phalaborwa Barrage situated in the main stem of the Olifants River for comparison purposes. The latter three impoundments were selected because they vary in water quality and pollution levels. Monthly surveys were carried out at Flag Boshielo Dam from February 2016 to February 2017 to establish if the biomonitoring tools and the quantification of water and sediment quality variables, could describe and predict seasonal fluctuations in the health of *O. mossambicus*.

To this end, water quality variables were measured during each survey. Water and sediment samples were collected for analysis of nutrients and metals. *Oreochromis mossambicus* were collected using gill nets. Fish health was assessed using the adapted HAI that was based on observing parasite burden, haematocrit determination and anomalies in body tissues and organs in conjunction with determining gonad and

hepato-somatic indices, the condition factor, blood glucose levels, microscopy analyses of fish gills and metal concentrations in fish muscle tissues. Water quality in terms of pH, total dissolved solids and electrical conductivity, nutrients and some aqueous and sediment metals exhibited significant differences ($p < 0.05$) between impoundments surveyed with water quality from good to poor being in the order of: Luphephe-Nwanedi Dams **<** Rhenosterkop Dam **<** Loskop Dam **<** Phalaborwa Barrage **<** Flag Boshielo Dam. The HAI scores varied significantly ($p < 0.0001$) between impoundments and substantiated water quality variables results during once off surveys, indicating that the health of fish from Flag Boshielo Dam were most affected when compared to the health of fish surveyed from the other impoundments. Findings from monthly surveys conducted at Flag Boshielo Dam indicated better conditions in terms of water quality variables during a period of high inflow as opposed to periods of low inflow. The HAI scores obtained for fish exhibited significant ($p < 0.0001$) differences between the months surveyed and were found to be in agreement with the water quality findings, indicating that flow regimes and water quality of an impoundment have an impact on fish health.

The condition and somatic indices findings did not seem to be sensitive enough to discriminate between the impoundments during the once off surveys. While during monthly surveys, these indices were significantly ($p < 0.0001$) different between the months, showing to be affected by seasonal fluctuations. Knowledge of the HAI in conjunction with blood glucose levels, gill histopathology and the arithmetic mean thickness of gill epithelium (H_{ar}) best described the health of fish in both the once off and monthly surveys. In conclusion, the findings of this study emphasised the HAI premise that fish from more polluted sites would be more impacted as opposed to less impacted sites, making the HAI adapted by Madanire-Moyo *et al.* (2012) an effective and rapid biomonitoring tool that can be used in the field. Furthermore, this study proved that the HAI can be used either solely or in association with the parasite index (PI) or IPI depending on the objectives of the study, as no pronounced differences were evident when using HAI, HAI with PI and HAI with IPI.

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LIST OF ABBREVIATIONS

ANOVA - Analysis of Variance

AREC – Animal Research and Ethics Committee

BMAA - Beta-N-methylamino-L-amine

BOD - Biological Oxygen Demand

DO - Dissolved Oxygen

DWAF - Department of Water Affairs and Forestry

DWS – Department of Water and Sanitation

EC – Electrical Conductivity

EDCs - Endocrine Disrupting Chemicals

EMU - Electron Microscopy Unit

FBD – Flag Boshielo Dam

GAM - Generalized Additive Model

GSI - Gonadosomatic Index

HAI - Health Assessment Index

HAR - Arithmetic Mean Thickness of Gill Epithelium

HSI - Hepatosomatic Index

ICP-MS – Inductively Coupled Plasma Mass Spectrometry

IPI - Inverted Parasite Index

IUCN - International Union for Conservation of Nature

K – Condition factor

LD – Loskop Dam

LND - Luphephe-Nwanedi Dams

MFI - Mesenteric Fat Index

MT - Metallothionein

PB - Phalaborwa Barrage

PI - Parasite Index

RDA – Redundancy analysis

RND – Rhenosterkop Dam

SANAS – South African National Accreditation System

SAWQG – South African Water Quality Guidelines

SE - Standard Error

SL -Standard length

SMU - Sefako Makgatho Health Sciences University

SPSS – Statistical package for the social sciences

TDS - Total Dissolved Solids

TEM – Transmission electron microscopy

TL – Total length

TWQR - Target Water Quality Range

UNEP – United Nations Environment Programme

USA - United States of America

WHO – World Health Organisation

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 The importance of managing South Africa's water resources

South Africa is a semi-arid country with an average annual rainfall of less than 450 mm, which is approximately half that of the global average of 860 mm (Nomquphu *et al.* 2007; Nare *et al*. 2011). For this reason, South Africa is regarded as the 30^{th} most water stressed country worldwide with an annual freshwater availability of less than 1700 m^3 per capita (Nare *et al.* 2011; Aphane & Vermeulen 2015). Despite the low national precipitation average, the geographical distribution of rainfall is highly variable with the eastern and southern stretches of the country receiving more rain than the north and western regions (Cessford & Burke 2005). This is because of the underlining topography and geomorphology and the country's location in the subtropical region of the Southern Hemisphere between the Atlantic and Indian oceans (UNEP 2008; see Figure 1.1). Given the anticipated increase in population and socio-economic growth, the demand for freshwater in South Africa is envisaged to exceed supply by the year 2025 (Ashton 2002; Nare *et al.* 2011). Therefore, effectively conserving, managing and monitoring of aquatic systems is and continues to be paramount (Annabi *et al*. 2015).

In South Africa, the Limpopo River System plays a pivotal role in providing water for anthropogenic purposes to the north and north-eastern region of the country (Love *et al.* 2010). A major tributary within the Limpopo River System is the Olifants River, which has been documented by Ashton *et al*. (2001) and Dabrowski and De Klerk (2013) to be the most polluted river system in South Africa due to industrial, coal mining and power generating activities in the upper catchment, irrigated agriculture and rural development in the middle reaches and mining and nature conservation practises in the lower region of the system (Heath *et al*. 2010; Dabrowski *et al.* 2014). The continual demand of water in South Africa for the above mentioned purposes (De Klerk *et al.* 2016), has led to a countrywide deterioration in the quality thereof (Oberholster & Ashton 2008).

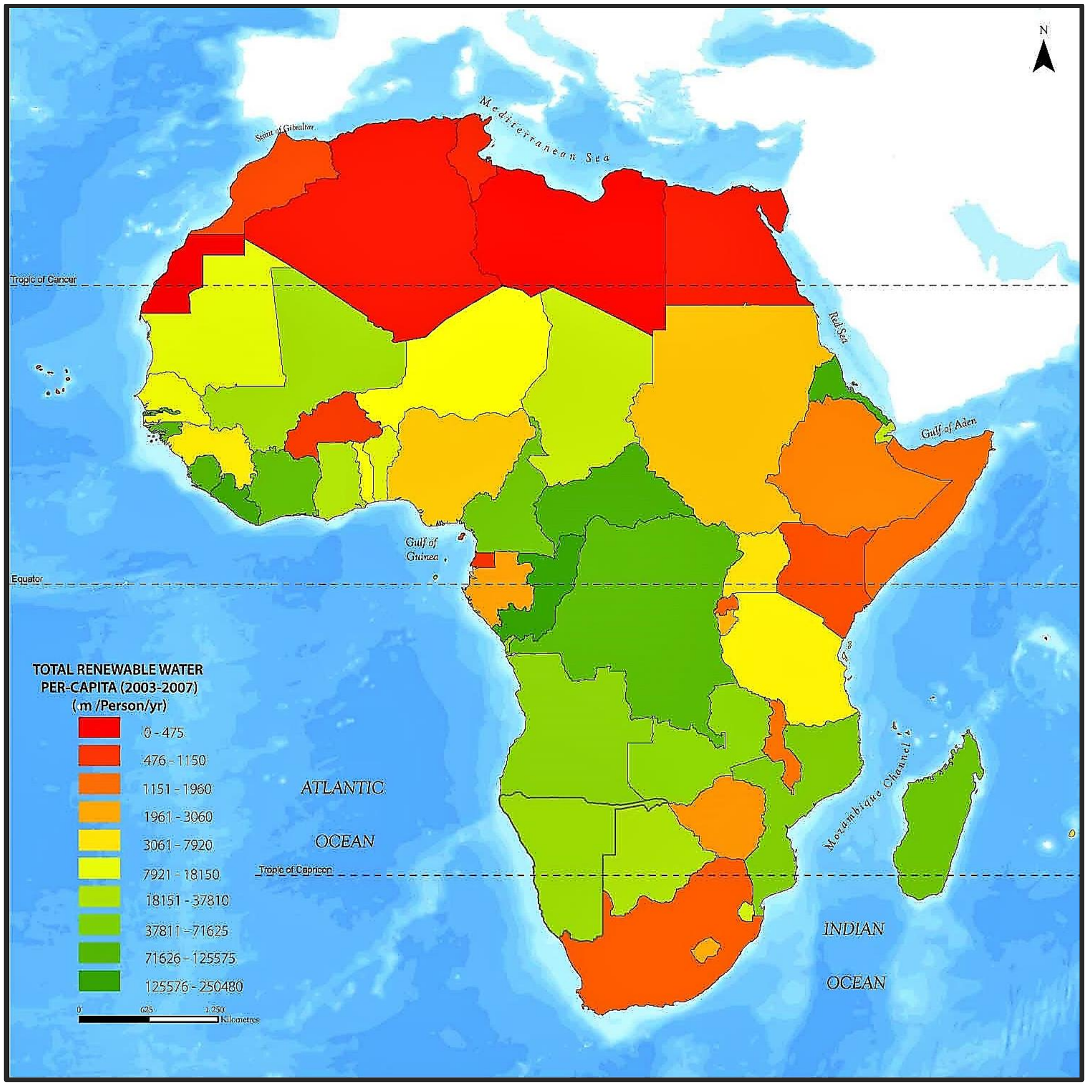

Figure 1.1: A map indicating the total renewable water per-capita for African countries with South Africa shown to be a water stressed country (UNEP 2008).

1.2 Pollution of freshwater systems

Due to underlying geology and geochemical process, metals and metalloids (hereafter referred to as metals) occur naturally in ground and surface waters and play an essential role for the normal functioning and development of aquatic organisms (Dallas & Day 2004). However, natural levels of metals are augmented when released into inland waters from industrial and mining sources with further mobilisation occurring due to acid mine drainage (Strydom *et al.* 2006; Hobbs *et al.* 2008). Metals such as copper (Cu), lead (Pb), mercury (Hg), zinc (Zn) chromium (Cr), cadmium (Cd), manganese (Mn) and iron (Fe) are important micronutrients for the normal function of physiological processes, but if exposure and accumulation levels occur above that of an organism's physiological threshold they then become deleterious to an organism's health (Addo-Bediako *et al*. 2014a; Mahboob *et al.* 2016). Metal toxicity is influenced by variables such as temperature, dissolved oxygen, pH, salinity, water hardness and the presence of mineral and organic suspended solids (Crafford & Avenant-Oldewage 2010; Addo-Bediako *et al.* 2014b). Metals do not degrade in the environment, as a consequence, they accumulate in the body tissues of organisms becoming magnified at higher trophic levels within aquatic systems (Addo-Bediako *et al.* 2014a). The biomagnification of metals higher up the food chain poses a toxicity risk to predatory fish, piscivorous birds, mammals and humans that consume contaminated fish (Jooste *et al*. 2015; Lebepe *et al*. 2016). Metal contaminated fish have been studied by examining accumulation sites such as muscle tissue, gills, liver and intestines (Crafford & Avenant-Oldewage 2010; Addo-Bediako *et al.* 2014a; Jooste *et al.* 2014; Marr *et al.* 2015; Lebepe *et al*. 2016).

Increased delivery of nutrients such as nitrogen (N) and phosphorus (P) from agricultural runoff and effluent from domestic sources and sewage results in the eutrophication of freshwater systems (Codd 2000; De Villiers & Thiart 2007). Eutrophication is generally characterised by an increase in plant biomass and water turbidity that results in poor light penetration, the depletion of dissolved oxygen and the frequent occurrence of cyanobacterial blooms (Oberholster & Ashton 2008). Cyanobacterial blooms produce a wide variety of metabolites such as dermatoxins, hepatoxins and neurotoxins, most of which are toxic to aquatic organisms and humans (Brand *et al.* 2010). In South Africa, a study by Harding (2015) revealed that all cyanobacteria species produce the neurotoxin beta-N-methylamino-L-amine (BMAA). In the same study, Harding (2015) detected cyanobacteria in most water bodies nationwide with traces of the toxin occurring in some molluscs and fish species that are targeted and consumed by humans. Of major concern is that BMAA is a mixed glutamate receptor antagonist that can affect the modulation process of brain development in exposed organisms (Engskog *et al.* 2013).

In addition, effluent discharged by municipal wastewater treatment plants release various endocrine active substances, known as endocrine disrupting chemicals (EDCs), into aquatic systems (Kidd *et al.* 2007). These EDCs disturb the normal function of the endocrine system, interfering with natural hormonal processes and affecting sexual, neurological and immunological development in exposed organisms (Mills & Chichester 2005; Holbech *et al*. 2006). Endocrine disrupting chemicals are easily bioavailable to organisms through absorption via the body surface (skin), gills (respiration) and intake of food and water (Blazer *et al*. 2007).

Abnormalities in fish caused by EDCs include intersex, also referred to as ovotestis, testis-ova or testicular oocytes, whereby both male and female characteristics are present in a fish (Blazer *et al.* 2007). In male fish, the EDCs causes alterations in germ cell development by influencing the production of oocytes in the testis (Soffker & Tyler 2012). Moreover, EDCs such as natural estrogen 17β-estradiol and synthetic estrogen 17α-ethynylestradiol (Nakumura *et al.* 2003), causes feminization whereby the male of a species produces vitellogenin, which is a protein associated with oocyte maturation in females (Lazorchak & Smith 2004). These abnormalities are occasionally visible and can be viewed macroscopically (Blazer *et al.* 2007). In female fish, these chemicals may lead to reduced egg production by interfering with hormonal pathways that regulate reproductive functions (Blazer *et al.* 2007; Vajda *et al.* 2011).

1.3 Responses of organisms to water pollution

Water pollutants induce an array of physiological stress responses in organisms (Scott & Sloman 2004). According to Barton (2002), stress is a general and non-specific response to any factor disturbing an organism's homeostasis. Responses of organisms to pollutants are generally grouped to being that of a primary, secondary and tertiary response (Witeska 2005; Scholz & Mayer 2008) and are summarised in Figure 1.2. A primary response is an acute reaction that occurs at a biochemical level whereby the sympathetic nervous system is stimulated (Barton 2002). During this response, the fight-or-flight mode is triggered whereby two main classes of hormones i.e. catecholamines and corticosteroids are released to prepare the organism to react to pollutants by causing the blood vessels to dilate and the heart rate and blood glucose levels to increase (Barton 2002).

A secondary response is a continuous stimulation of the fight-or-flight mode that occurs at a cellular level and is usually characterised by changes in metabolite levels, haematological features and stress proteins, all of which relate to changes in the natural function of physiological processes such as metabolism, respiration, hydro-mineral balance, immune function and cellular responses (Wendelaar 1997; Barton 2002). Tertiary responses are maladaptive and occur at the organismal level and negatively affect an organism's growth (Watson *et al.* 2012), its resistance to infections from diseases and parasites (Sures 2004; Nachev & Sures 2016), larvae production (Castranova *et al.* 2005), metabolic processes and overall behaviour to the extent that the impact can ultimately lead to death (Barton 2002).

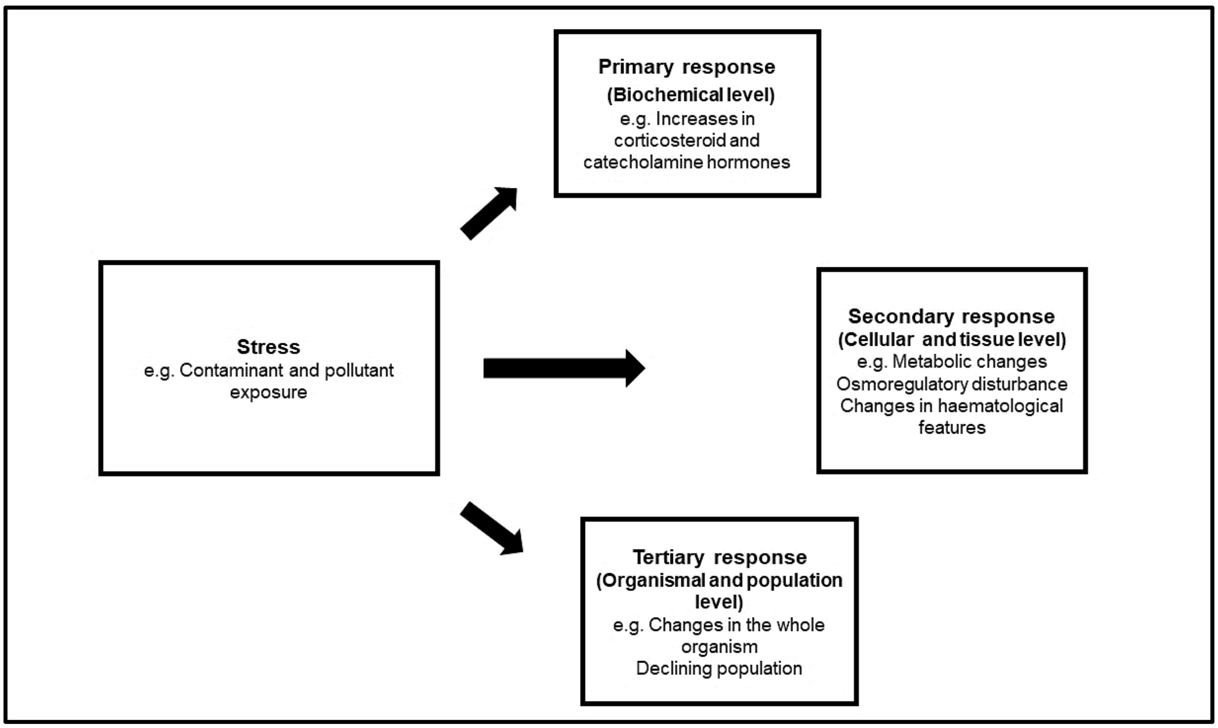

Figure 1.2: A summary of organisms' responses to environmental stressors at molecular, cellular, tissue, organs, organismal and population levels. Adapted from Barton (2002) and Lankadurai *et al.* (2013).

1.4 Monitoring of freshwater systems based on abiotic variables

The abiotic characteristics of water may be influenced by physical, toxic and non-toxic chemical variables (Dabrowski & De Klerk 2013). The physical variables include temperature and turbidity, whereas toxic chemical variables include biocides, EDCs and trace metals while non-toxic chemical variables comprise pH, total dissolved solids (TDS), salinity, conductivity, dissolved oxygen and nutrients i.e. ammonia, ammonium, nitrite, nitrate, phosphate and sulphur (Dallas & Day 2004). As in most countries, several water quality monitoring programmes have been employed over time in South Africa that involve measuring physical and chemical variables *in situ* (Roux *et al.* 1993). However, this type of approach inevitably leads to certain shortcomings. A measure of physical/chemical water quality variables alone is inadequate at providing a full and detailed assessment of the health status of an aquatic system due to the approach being a snapshot of conditions when measurements are recorded (Gerber *et al.* 2017). Another factor constraining abiotic monitoring programme is the fact that such approaches cannot differentiate between pollutants occurring as a result of natural processes from those occurring as a result of anthropogenic factors (Bartram & Ballance 1996).

1.5 Biological monitoring of freshwater systems based on bioindicators

Due to the aforementioned limitations of using physical/chemical water quality variables, implementation of biological monitoring practices has become the preferred means to evaluate water quality and investigate the impact pollutants have on freshwater systems (Nachev & Sures 2016; Parmar *et al.* 2016). In addition to measuring water quality variables, the use of aquatic organisms as bioindicators to assess water quality is preferred since bioindicators are continuously exposed to the surrounding environment i.e. water and sediment (Roux *et al.* 1993; Colin *et al.* 2015). According to Van Gesten and Van Brummele (1996), a bioindicator is an organism that provides information of habitat conditions by its presence, absence and behaviour. Over the years fish have been used successfully as biological indicators of both marine and freshwater systems at the organelle, cell, tissue, organ, organism, population, community and ecosystem level (Scott & Sloman 2004; Wepener *et al.* 2011).

The advantages of using fish as bioindicators are because fish are present in most aquatic systems (Whitfield & Elliot 2002), have long-life spans and slow tissue turnover rates whereby pollutants become integrated into their body tissues and upon analyses can provide a historic record as to the extent of the impact (Adams *et al.* 1993; Gerber *et al.* 2017). Moreover, fish are easily identified, they occupy various trophic levels in an aquatic system, comprise various life forms and have different functional associations (Whitfield & Elliot 2002). Fish also provide a habitat for ichthyoparasites that can also be used to detect the extent of pollution exposure (Al-Niaeem *et al.* 2015). Most of all, fish are of great economic importance as they are the main source of animal protein to a vast number of people globally (Kumar *et al.* 2017).

1.6 Biomonitoring methods using biomarkers

A biomarker is defined as an indicator that can be measured at the molecular, biochemical, cellular, tissue, organismal through to the population and community level as a result of past or present environmental exposure (Adams & Ryon 1994; Annabi *et al.* 2015). Biomarkers can either be of exposure or biomarkers of effect. A biomarker of exposure is indicative of exposure to various chemicals within the system while a biomarker of effect indicates any diverse biochemical, physiological and cellular alteration (Adams 2005; Van Der Oost *et al.* 2003). The use of various enzymes and proteins have been employed as biomarkers in fish toxicology. These include metallothionein (MT) in the cellular metal homeostasis (Ameida *et al*. 2001; Handy *et al.* 2002), cytochrome P450 monooxygenases (Hutchinson *et al.* 2006; Colin *et al.* 2015), acetylcholinesterase (Üner *et al.* 2006; Rakhi *et al.* 2013) and lactate dehydrogenase (Osman 2012). In addition, blood parameters such as red blood cells, haemoglobin, haematocrit and thrombocytes have been used in fish toxicology (Osman 2012). Even though proven to be beneficial, the biomarker approach normally requires expensive equipment and a high degree of specialised skills especially when analysing samples and interpreting the outcome thereof.

The most commonly used indicators to assess fish health in aquatic systems are histopathological assessments (Van Heerden *et al.* 2004a, b), condition and somatic indices (Froese 2006; Freyre *et al.* 2009), haematological parameters and the health assessment index (HAI) (Crafford & Avenant-Oldewage 2009, Madanire-Moyo *et al.* 2012). Although each of these methods have merit for their use, they also have disadvantages (Adams *et al.* 1993; Adams & Greeley 2000).

1.6.1 Histopathological assessments

Histopathology is a biomonitoring method used to determine disease and signs of long term injury in cells, tissues and/or organs (Gerber *et al.* 2017). Histopathological changes are regarded as biomarkers of effect as they assess an organism's exposure to environmental stressors to reveal physiological and biological changes (Marchand *et al.* 2008). Changes observed can be quantified to allow for comparison between sites, species and organs (Van Dyk *et al.* 2009; Da Costa *et al.* 2007). Fish gills are mostly used as these organs are in direct contact with the surrounding medium, making them the main target of infliction when exposed to toxins (Van Heerden *et al.* 2004a; Van Heerden *et al.* 2006). Moreover, fish gills are regarded as a primary site for gas exchange such as acid-base balance and ion regulation and the excretion of metabolic waste products (Biagini *et al.* 2009; Monteiro *et al.* 2009). When fish are exposed to high concentrations of toxic pollutants such as metals, they result in pathological changes such as epithelial necrosis, vasodilation, epithelial lifting, hypertrophy of the respiratory epithelium and hyperplasia in gills (Hughes *et al.* 1978; Wong & Wong 2000). Gonadal histology has also been used in both field and laboratory studies to investigate the effect aquatic toxins have on fish reproduction (Tyler *et al.* 1996; Booth & Weyl 2000; Leino *et al.* 2005). Histological examination of gonads has been used to demonstrate pollutant-induced effects on gametogenesis, vitellogenesis (Booth & Weyl 2000) and morphological alterations (Leino *et al.* 2005).

1.6.2 Condition or somatic indices

Condition indices and the use thereof can be classified as direct and indirect. The direct condition indices are the hepatosomatic index (HSI), gonadosomatic index (GSI) and mesenteric fat index (MFI). These indices provide information on metabolic activities and physiological responses of an organism (Brown & Murphy 2004; Freyre *et al.* 2009). The study of the liver and, in turn, the HSI is associated with liver energetic reserves and metabolic activity of fish (Ighwela *et al.* 2014). Liver is the largest organ that assists with the storing of glycogen and lipids, the production of bile, plasma proteins and removal of foreign substances from the blood (Watson *et al.* 2012; Won *et al.* 2016). Catabolism of mesenteric fat by the liver provides energy for maintenance and reproduction. In female fish, the liver is also responsible for the synthesis of vitellogenin resulting in a decrease in HSI during the pre-spawning period, and an increase in HSI values during the reproduction and spawning periods (Nunes *et al.* 2011). According to Marchand *et al*. (2008) a HSI range of 1 - 2% is considered normal for osteichthyes. In a poor environment, fish usually have a smaller liver (with less energy reserves stored in the liver). The HSI has been reported by Dewi and Prabowo (2017) to decrease in fish exposed to high concentrations of cadmium, lead, mercury and zinc.

Similarly the GSI is an important and sensitive indicator as it reveals the degree by which gonads mature during the breeding season, with female fish undergoing regular cyclical changes in gonadal mass during this period (Satheesh & Kulkarni 2016). An increase in GSI corresponds with the beginning of vitellogenesis, increased body weight and fecundity (Nunes *et al.* 2011). During the pre-spawning period, there exists an inverse relationship between HSI and GSI whereby a decrease in HSI is representative that the stored hepatic compounds are made available for gonadal development (Satheesh & Kulkarni 2016). Gonadosomatic index has been reported to be low in polluted systems. This is because when exposed to estrogenic compounds, the testicular and ovarian growth become inhibited, thus leading to a decrease in GSI (Hassanin *et al.* 2002). Some studies have reported a decrease in GSI as a result of metal pollution (Alhashemi *et al.* 2012; Montenegro & Gonzalez 2012). Although the application of the direct condition indices is a good means for assessing fish health, they should be used in conjunction with biomarkers such as histopathological assessments to further explain the observed morphological changes (Nunes *et al.* 2011).

The indirect condition indices include the condition factor (K), relative condition factor (Kn) and relative weight (Wr) which are calculated according to the following formulae (Froese 2006; Ambily & Nandan 2010):

$$\mathrm{K} = \frac{\mathrm{W} \times 100}{\mathrm{L}^3}$$

Where W is the total body mass (g) of fish and L the total length (cm).

$$\mathrm{Kn} = \frac{\mathrm{W}}{\mathrm{w}}$$

Where W is the total body mass in grams (observed) and w = aL^b (calculated)

$$Wr = \frac{100 \times W}{aL^b}$$

Where, W is the total body mass in grams, L is the standard length, and a and b are the coefficients determined for the length-weight relationship for the species.

These indices provide general information on the overall health of an organism (Freyre *et al.* 2009). A decline in these indices values is usually interpreted as the depletion of energy reserves due to stress and starvation. However, this may not always be true as a decrease in the condition value may be influenced by changes in environmental conditions, a species' habitat, food availability and changes in physiological and morphological demands associated with growth and reproduction (Freyre *et al.* 2009; Watson *et al.* 2012). When determining K and Kn, a value of 1 and higher is indicative of fish that are in good health (Ambily & Nandan 2010; Watson *et al.* 2012) and similarly a value > 100% for Wr (Addo-Bediako *et al.* 2014b). Although the use of indirect indices has merit, they are unsuitable for comparing fish condition for populations from different localities as values can vary according to the sex, size, season and degree of gonad development (Froese 2006).

1.6.3 Health assessment index approach

At the organismal, population and ecological level, the health assessment index (HAI) has been used as a rapid and inexpensive means to successfully investigate the water quality of aquatic systems based on the health of fish populations therein. The HAI is a refinement of the necropsy-based approach that was initially developed in the USA by Goede and Barton (1990) and modified by Adams *et al.* (1993) to provide a quantitative index that scores fish health based on observed body tissue and organ anomalies and parasite burden for specimens sampled from a population in a system (Adams *et al.* 1993). In South Africa, this approach was advocated and applied by Avenant-Oldewage and Swanepoel (1993) to assess the status of various water bodies based on fish health. Marx (1996) expanded the HAI to include the parasite index (PI) that quantifies parasite burden. The sum of all anomalies and parasites reflects the HAI score for a population with high scores indicating poor fish health and poor water quality (Sara *et al*. 2014).

Crafford and Avenant-Oldewage (2009) further refined the HAI by incorporating the inverted parasite index (IPI) into the HAI (see Table 1.1), based on the premise that higher ectoparasite numbers are indicative of better water quality due to them being in direct contact with the surrounding medium and as such, are given a lower HAI score. At highly impacted sites, endoparasite numbers are expected to be higher due to them not being directly exposed to external pollutants and because they are able to take advantage of the host's weakened immune system to reproduce and multiply unabated (Watson *et al.* 2012; Al-Niaeem *et al*. 2015). Thus, high endoparasite numbers contribute to high HAI scores (See Table 1.1).

Table 1.1: The numerical scoring systems used based on the number of ecto- and endoparasites observed with PI being the Parasite Index and IPI the inverted parasite index.

ECTOPARASITES	PI	IPI	ENDOPARASITES	PI
Zero parasites observed	**0**	30	Zero parasites observed	**0**
1 – 10	**10**	20	≤ 100	**10**
11 – 20	**20**	10	101 – 1000	**20**
> 20	**30**	0	> 1000	**30**

Apart from identifying and quantifying parasites, HAI comprises and examines other aspects of an organism's condition. Aspects include the examination of blood constituents (e.g. plasma protein, white blood cell count (WBC%), percentage haematocrit (Hct%), the scoring of anomalies occurring on external extremities such as the eyes, skin, fins, gills and opercula and anomalies associated with internal organs such as spleen, hindgut, kidney, liver, and other variables not included in the final HAI calculation such as the grading of bile colour and mesenteric fat (Watson *et al.* 2012). Examples of anomalies occurring on external extremities include but not limited to exophthalmic eyes, skin aberrations, eroded fins, pale gills and swollen pseudobranch, while those occurring in the internal extremities include liver discolorations, inflamed hindgut etc. (Watson *et al.* 2012). The study by Madanire-Moyo *et al.* (2012) further modified the HAI by excluding blood parameters such as plasma protein determination and white blood cell counts, making this approach more rapid and inexpensive means of assessing fish health in the field. This adapted approach was used in the present study.

The HAI has been successfully tested in various systems to assess fish health and the results of these studies are summarised in Table 1.2. For example, in a study by Van Dyk *et al.* (2009) the health of *Oreochromis andersonii*, *Serranochromis angusticeps, Clarias ngamensis and Clarias gariepinus* from the Okovango Delta were evaluated using the HAI. Despite the system being pristine based on water quality measurements, the HAI scores revealed *O. andersonii* to be the least healthy of the four fish species examined (Van Dyk *et al.* 2009). Histological assessments also corresponded with the HAI findings that *O. andersonii* was the most affected species (Van Dyk *et al.* 2009). In another study, Crafford and Avenant-Oldewage (2009) examined and differentiated Vaal Dam (less polluted) and Vaal River Barrage (polluted) based on the health status of *Clarias gariepinus* using the HAI. Although overall HAI scores established could discriminate between localities, individual estimates varied considerably amongst the specimens examined (Crafford & Avenant-Oldewage 2009).

Similarly, in a study by Madanire-Moyo *et al.* (2012) the health of *C. gariepinus* was evaluated from three sites varying in water quality. Fish were sampled from Luphephe-Nwanedi Dams situated in the Limpopo River System, Flag Boshielo Dam in the Olifants River System and Return Water Dam, which is a reservoir associated with a mining operation near the town of Mokopane in Limpopo Province. Based on water quality variables Madanire-Moyo *et al.* (2012) ascertained Luphephe-Nwanedi Dams to be least impacted of the three dams sampled with Flag Boshielo Dam and the Return Water Dam being moderately to highly polluted systems, respectively. The HAI results revealed that fish collected from the impacted sites were less healthy than those sampled from Luphephe-Nwanedi Dams. In the latter study, HAI scores were also found to vary considerably amongst individuals sampled from each impoundment.

In a study by Watson *et al.* (2012) the health of *C. gariepinus, O. mossambicus and Labeobarbus marequensis* sampled from impoundments and rivers varying in pollution levels during dry and wet/rainy periods was assessed using the HAI approach. Sites located in Loskop (polluted) and Bronkhorstspruit (less polluted) dams and Mamba (polluted) and Balule (less polluted) rivers were sampled. Results revealed no variations in HAI scores between polluted sites during dry periods for each of the species sampled. Low abundance of ectoparasites was recorded during dry/drought conditions and high numbers of endoparasites during the rainy season (Watson *et al.* 2012). No seasonal variation in HAI scores for *C. gariepinus*, *O. mossambicus and L. marequensis* from Loskop and Bronkhorstspruit were reported (Watson *et al.* 2012).

Conversely in a study by Sara *et al.* (2014) that was conducted to evaluate the water quality of Hout River Dam using *Cyprinus carpio, C. gariepinus* and *O. mossambicus* as bioindicators, revealed a low abundance and low seasonal variation of ectoparasites for *O. mossambicus* and *C. gariepinus*. The findings by Sara *et al.* (2014) were contrary to the IPI premise that high ectoparasites are indicative of good water quality for *O. mossambicus* and *C. gariepinus* since the water variables measured in Hout River Dam indicated the systems to be in near pristine conditions (Sara *et al.* 2014).

Table 1.2: Summary of the findings of studies elucidated above. Values in bold indicate scores from moderately polluted sites, while values highlighted in grey indicate scores from polluted sites.

Study site	**HAI scores for *C. gariepinus***	**HAI scores for *O. mossambicus***
Okavango Delta	160	
Vaal Dam	93 - 97	
Vaal River Barrage	115 - 117	
Flag Boshielo Dam	**84**	
Return Water Dam	93	
Luphephe Nwanedi Dams	43	
Hout River Dam	38	41
Bronkhorstspruit Dam		53
Loskop Dam		89
Mamba River		75
Balule River		85

From Table 1.2, previous studies seem to indicate that HAI scores are greatly influenced by parasite load (Crafford & Avenant-Oldewage 2009; Van Dyk *et al.* 2009), seasonality

(Madanire-Moyo *et al.* 2012; Sara *et al.* 2014), water levels (Watson *et al.* 2012), water quality and fish condition (Madanire-Moyo *et al.* 2012; Watson *et al.* 2012; Sara *et al.* 2014), raising the question as to what score is considered to be normal for a given fish species sampled form a pristine, moderately or highly impacted site?

Although the HAI has proven to be an effective biomonitoring tool, studies have revealed the approach to have certain limitations concerning the scoring of anomalies using colour charts since it lends itself to the subjectivity of the investigator. Moreover, studies have shown that the repetitive application of the approach when sampling a single species from the same impoundment does not necessarily yield similar results (Madanire-Moyo *et al.* 2012). Furthermore, necropsy alone may not explain the occurrence of infection or disease in fish and therefore the inclusion of histopathology when establishing the health of fish may further explain some anomalies observed such as the discoloration of gills, liver, kidney and spleen (Sara *et al*. 2014). In terms of metal exposure in fish the HAI is nonspecific at determining the effect of metal pollution (Madanire-Moyo *et al*. 2012). Therefore, evaluating HAI in conjunction with metal concentrations in fish body tissue may reveal the extent metal contamination may impact the health of a species. Additionally, the PI and IPI premise that poor water quality facilitates an increase in endoparasitism in response to a host's weakened immune system needs to be tested further (Sara *et al*. 2014) with regard to metal exposure. For example, despite the fact that endoparasites such as cestodes and acanthocephalans within the intestines of fish are not in direct contact with the external environment, these parasites are able to bioaccumulate metals such as lead, cadmium and selenium (Watson *et al.* 2012) and as a consequence their numbers may not necessarily decrease when the host is exposed to pollutants comprising metals (Watson *et al.* 2012). Due to the limitations expressed in previous studies using the HAI, it may be prudent to use this approach in conjunction with other indices and biomonitoring tools (e.g. histopathological assessments) describing fish health and water quality variables.

1.7 Motivation of the study

In South Africa, few studies have used HAI in conjunction with other biomonitoring indices and tools to assess the water quality of impoundments, especially in the Limpopo and

Olifants River Systems. Hence, this study was designed to evaluate the HAI, in conjunction with other biomonitoring indices and metal concentrations in fish muscle, to better assess and differentiate the water quality of impoundments varying in pollution levels and water quality within the Limpopo River System. The trophic status of the impoundments selected and surveyed was first established based on the data from the Department of Water and Sanitation (DWS 2017).

The Luphephe-Nwanedi Dams, which occur in the upper catchment of the Limpopo River System, were used as the control site because these impoundments were reported by Madanire-Moyo *et al.* (2012) to be relatively unpolluted and are classified by DWS (2017) to be oligotrophic. Similarly, Rhenosterkop Dam situated within the Elands River, which is a tributary of the Olifants River, was chosen as an additional control site based on the assumption that the impoundment was less impacted due to limited land use activities occurring further upstream and because the impoundment was also classified to be oligotrophic by DWS (2017). Currently there is no literature available on the health of fish from this impoundment. The use of these two impoundments allowed for water quality and fish health to be compared with impacted sites within the Olifants River.

Loskop Dam, which is located on the border of the upper and middle catchments of the Olifants River, and Flag Boshielo Dam, which is situated in the middle reaches of the Olifants River (Truter *et al.* 2016) were categorised by DWS (2017) to be eutrophic and oligotrophic respectively and were chosen because they were reported by Botha *et al.* (2011) and Dabrowski *et al.* (2014) to be moderately to highly polluted, respectively. In addition, Phalaborwa Barrage, which is smaller in capacity when compared to Loskop and Flag Boshielo dams, is situated on the fringes where the middle and lower catchment of the Olifants River meet. The system was selected because it is located prior to where the Olifants River meanders into the Kruger National Park. The Barrage was classified to be eutrophic by Addo-Bediako *et al.* (2014b). The latter three impoundments have undergone extensive research in terms of fish health using the HAI (Madanire-Moyo *et al*. 2012; Watson *et al*. 2012) and metal concentration in fish muscle tissues and their impact on human health (Addo-Bediako *et al*. 2014a, 2014b; Jooste *et al*. 2014, 2015; Lebepe *et al*. 2016) with the bulk of the research conducted in Flag Boshielo Dam.

1.8 Hypothesis

The adapted HAI in conjunction with the condition and somatic indices, blood glucose levels, gill microscopy analysis and a knowledge of the metal content in the muscle tissue of fish provides the means to establish the water quality of impoundments.

1.9 AIM AND OBJECTIVES OF THE STUDY

1.9.1 Aim

The aim of the study was twofold. The first was to evaluate the HAI as a means to determine and differentiate the health of *Oreochomis mossambicus* sampled from five various impoundments within the Limpopo River System. The second was to establish if the adapted HAI, in conjunction with other biomonitoring techniques and the quantification of water and sediment quality variables, could better describe and predict seasonal fluctuations in the health of *O. mossambicus* from Flag Boshielo Dam. In order to achieve these aims the following objectives were adopted to:

1.9.2 Objectives

I. Determine the water quality of Luphephe-Nwanedi, Rhenosterkop, Loskop, Flag Boshielo dams and Phalaborwa Barrage based on water quality and the metal content in water and sediment samples collected from these impoundments during a once-off survey.

II. Determine the effects seasonal and fluctuating water levels have on the water quality of Flag Boshielo Dam by conducting monthly surveys and collecting water samples for nutrient and metal content analyses while sediment samples were collected quarterly and analysed for metals.

III. Establish the health of *O. mossambicus* specimens from the five impoundments by implementing the adapted HAI approach in conjunction with the blood glucose levels, K, HSI, GSI, H_{ar} and the quantification of metal tissue content.

IV. Establish changes in the health of *O. mossambicus* specimens collected during monthly surveys from Flag Boshielo Dam based on the adapted HAI approach in conjunction with the blood glucose levels, K, HSI, GSI, H_{ar} and metal content of muscle tissue.

V. Determine which variable(s) and/or method(s) best describe, influence and/or predict the adapted HAI scores established for *O. mossambicus* in this study using a multivariate and statistical modelling approach.

1.10 Dissertation outline

To achieve the aim and objectives of this study, the dissertation was structured as follows:

Chapter one (General introduction and literature review), this chapter eludes to the background, motivation, aims and objectives of the study.

Chapter two (Materials and Methods)

In this chapter, a thorough explanation of when and how the field work was conducted is outlined. This chapter includes a description of the study sites and selected fish species and the materials and methods used to collect fish, water and sediments samples. Furthermore, the techniques applied for laboratory analysis and tissue processing are outlined in this chapter and the calculations of the various indices. A description of the analyses and indices included in the necropsy based health assessment index.

Chapters 3 through to 5 address the research objectives outlined in 1.9.2

Chapter three (Evaluating the water and sediment quality of Luphephe-Nwanedi, Rhenosterkop, Loskop, Flag Boshielo dams and Phalaborwa Barrage)

In this chapter, objective (i) and (ii) are addressed whereby water quality variables are discussed for each impoundment based on land use, climate, topographical and geological conditions of the site. This chapter was divided into two sections whereby the first section addresses the five impoundments based on a once off sampling approach while the second discusses the monthly/seasonal collection of samples from Flag Boshielo Dam.

Chapter four (Evaluating the health of *Oreochromis mossambicus* based on the health assessment indices in conjunction with blood glucose, the K, HSI, GSI, arithmetic thickness of the gill epithelium (H_{ar}) and metal tissue analyses)

In this chapter, various biomonitoring tools i.e. condition factor, hepatosomatic index, gonadosomatic index, metal accumulation in fish muscle, arithmetic thickness of the gill epithelium (H_{ar}), blood glucose levels and the health assessment index were applied to study the effects of water quality on fish. As in chapter 3, this chapter was divided into two sections with the second section focusing solely on results collected from Flag Boshielo Dam.

Chapter five (A multivariate and statistical modelling approach to establish variable(s) and / or method(s) that best describes and predicts the water quality of impoundment based on water and sediment quality and fish health)

For this chapter, various statistical approaches were applied to ascertain the use of the HAI in conjunction with other biomonitoring indices and tools.

Chapter six (General conclusions and recommendations)

In this chapter a general discussion is provided whereby the main findings drawn from the preceding chapters are highlighted. Limitations of the study and recommendations for future research are also discussed.

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CHAPTER 2: MATERIALS AND METHODS

2.1 Introduction

According to Dallas (2013), the criteria commonly used to select reference sites include geology, topography, soils, climate features and land use. In this study, the Luphephe-Nwanedi Dams were selected as a reference site due to these impoundments considered by Madanire-Moyo *et al.* (2012) to be relatively unpolluted based on water quality and fish health. Similarly, Rhenosterkop Dam was selected on the assumption that it was less polluted than the other sites sampled in the main stream of the Olifants River. Due to land use profiling along the river catchment, water and fish health data collected from these impoundments were used to compare between Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage that vary in pollution gradient and are categorised to be impacted by Addo-Bediako *et al.* (2014a,b), Jooste *et al.* (2015) and Lebepe *et al.* (2016) based on water quality and metal bioaccumulation within fish muscle tissues. A once off sampling regime was conducted at Rhenosterkop and Flag Boshielo Dams during April 2016 and at Luphephe-Nwanedi dams, Phalaborwa Barrage and Loskop Dam in May 2016. Conversely, seasonal sampling was conducted at Flag Boshielo Dam during most months from February 2016 to February 2017.

2.2 The Limpopo River System

The Limpopo River System situated in the eastern part of southern Africa, forms the boundary between South Africa, Botswana, Zimbabwe and Mozambique (see Figure 2.1). The origin of the Limpopo River is in South Africa, with the upper Limpopo catchment comprising the Shashe River that defines the South African, Botswana and Zimbabwean boarders (Mwenge Kahinda *et al.* 2016). The region where the Limpopo River forms a boundary between South Africa and Botswana, the Mahalapswe, Lephala, Lotsane, Mogalakwena, Motloutse and Shashe Rivers occur, all originating in Botswana. In the upper catchment within the South African borders, are the Nwanedzi and the Luphephe rivers whose main tributaries feed into the Luphephe-Nwanedi reservoirs (Mwenge Kahinda *et al.* 2016). The region where the Limpopo River basin forms the boundary between South Africa and Zimbabwe makes up the middle reaches of the Limpopo River System, comprising the Mzingwane, Sand and Bubi Rivers as tributaries (Love *et al.*

2010). The lower reaches of the system is where the Limpopo River defines South African and Zimbabwean borders before flowing across and into Mozambique. The main tributaries in this catchment area are the Luvhuvhu, Mwenezi, Olifants, Chokwe and Changane Rivers (Love *et al.* 2010).

2.2.1 Climate, geology, topography and land use in the Limpopo River System

Due to its geographic position, climatic conditions in the Limpopo River System are influenced by prevailing winds such as tropical cyclones that originate over the Indian Ocean (Mwenge Kahinda *et al*. 2016). Air temperatures across the Limpopo River Basin show a marked seasonal cycle, with the hottest temperatures recorded during summer and the lowest during cool, dry winter months (Love *et al.* 2010). Rainfall varies seasonally with the average annual precipitation being 400 mm per annum. Evaporation rates across the Limpopo River are variable, ranging from 3.1 metres per annum in the western and central areas of the basin to 1.7 metres in the cooler mountainous regions in the south-eastern regions of the basin (Ashton *et al.* 2001).

The Limpopo River Basin is dominated by flood plains which are interspersed by low gradient hills, locally incised valleys and medium gradient mountains (Van Der Zaag *et al*. 2010). South African tributaries of the Limpopo River have deep gorges that cut through hills and mountains and are visible as erosional remnants. Soil formations across the Limpopo River reflect the influence of the underlying parent rock, climatic features and biological activity. The dominant sediment types in the basin are moderately deep sandy to sandy-clay (Ashton *et al*. 2001). Land use that occur in the catchment of this system include mining activities in the Waterberg and Soutpansberg areas, as well as agriculture, industrial and conservation activities in other areas of the system.

2.3 The Olifants River System

The Olifants River, which forms the largest sub-basin of the Limpopo River System, originates in the Highveld region of Mpumalanga Province, South Africa (see Figure 2.1). The river flows north-east towards Mozambique where it meets the Letaba River before meandering further eastwards through the Kruger National Park and into Mozambique where it joins and flows into the Limpopo River (Ashton *et al.* 2001; Heath *et al.* 2010). The Olifants River catchment falls within Gauteng, Mpumalanga and Limpopo provinces

(Heath *et al.* 2010). According to Ashton *et al.* (2001), the Olifants River was historically a strong flowing perennial river, but has now become a very weak perennial river with frequent periods of no flow. The upper Olifants River Catchment comprises Witbank, Middleburg, Bronkhorstspruit and Premiere dams upstream of Loskop Dam (Dabrowski *et al.* 2014), whereas the middle reaches of the catchment comprises the region immediately downstream of Loskop, Flag Boshielo and Rhenosterkop dams and Phalaborwa Barrage (Heath *et al*. 2010). Major tributaries in the middle catchment of Olifants River are the Selons, Moses, Bloed and the Elands rivers (Heath *et al*. 2010). The lower catchment comprises the areas below the confluence where the Letaba and Olifants rivers meet and cross the Kruger National Park into Mozambique.

2.3.1 Climate, geology, topography and land use in the Olifants River System

Due to its geographic position, the prevailing winds such as the occurrence of the tropical cyclones from the Indian Ocean influence the climate of the Olifants River System (Ashton *et al.* 2001). Air temperatures across the basin show a marked seasonal cycle, with the hottest temperatures occurring during early summer and low temperatures during cool, dry winter months (De Lange *et al.* 2003). In the catchment within the Highveld, rainfall is highly seasonal, falling predominantly during warmer summer months. The climate in the Olifants River System is described by Ashton *et al.* (2001) as semi-arid and hot with an annual average rainfall of 400 mm but can be as high as 800 t0 900 mm annually on the Mpumalanga Highveld. The geological features of the Olifants River System consist mostly of basic mafic and ultramafic rocks accompanied by extensive areas of acidic and intermediate intrusive rocks. Generally, the valleys of the river tend to be broad and flat-bottomed with channels forged into the underlying substrate (Madanire-Moyo *et al.* 2012). Soil formations across the Olifants River Basin are the strongly influenced by the underlying bedrock. The dominant soil types are moderately deep sandy-clay loams, important for large crop production (Ashton *et al.* 2001).

Land use activities along the Olifants River include mining, industrial, coal fired electric power generation plants with chrome and steel smelters occurring around cities of Witbank and Middleburg in the upper catchment. Along the middle catchment is the intense irrigated agriculture and rural development, while land use in the lower catchment is dominated by mining and nature conservation (Heath *et al.* 2010). Acid mine drainage (Hobbs *et al.* 2008), acid rain, the discharge of industrial effluents and nutrient enrichment from domestic sources (De Villiers & Thiart 2007), habitat destruction and soil erosion has polluted and deteriorated the water quality in this system. As a consequence, the system has received considerable media and scientific attention due to episodic deaths of fish and crocodiles (Ashton 2010, Marr *et al.* 2015), raising concerns about the effectiveness of existing water monitoring and mitigation programmes (De Villers & Mkwelo 2009).

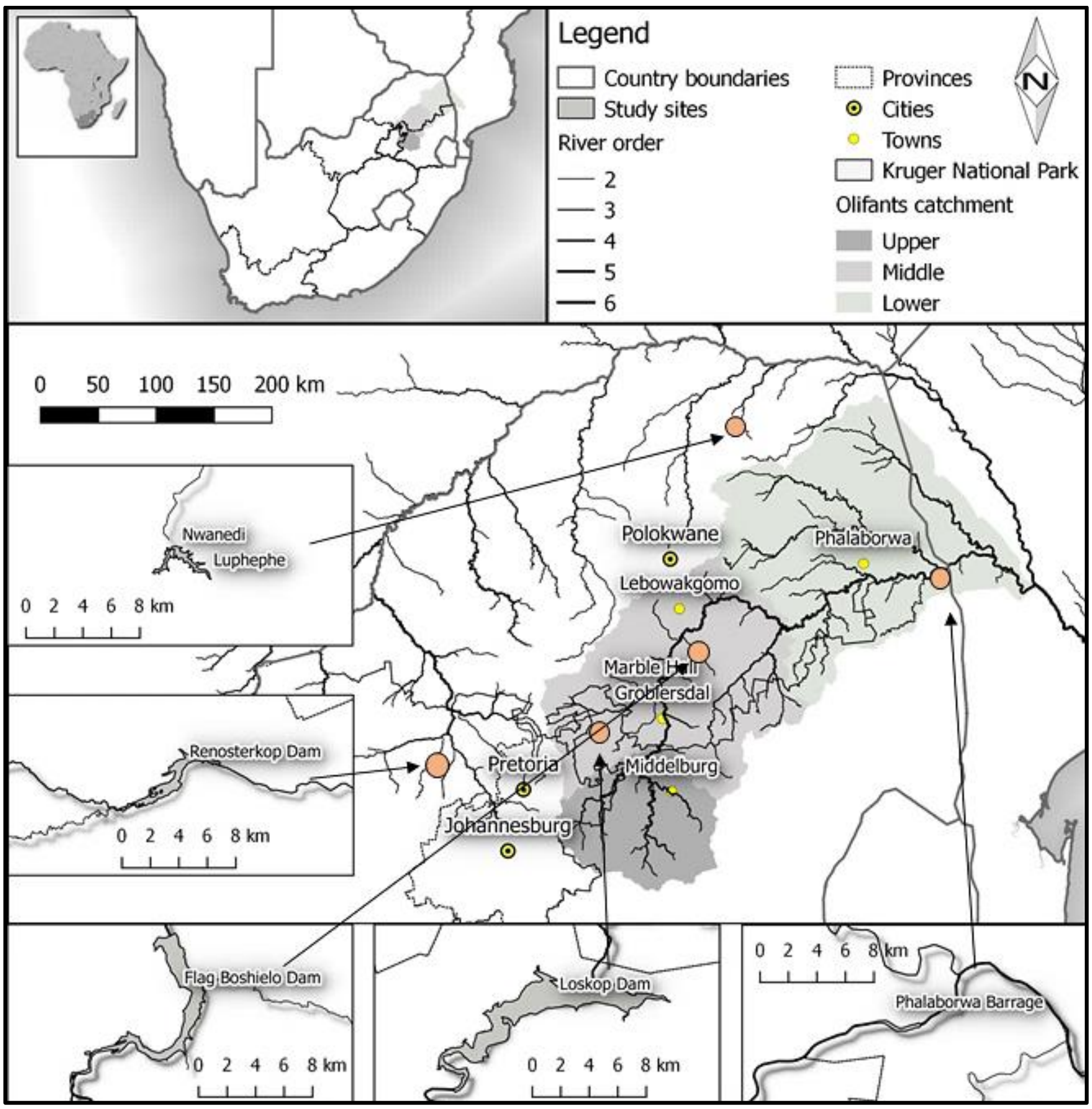

Figure 2.1: A map indicating the location of impoundments within the Limpopo and Olifants River System sampled in the present study.

2.4 Criteria used to select sampling sites

The criteria used to select the sampling sites in this study were adapted from Dallas (2013).

2.4.1 Site one: Luphephe-Nwanedi Dams (22° 37’ 59.99” S: 30° 24’ 17.99” E)

The Luphephe-Nwanedi dams (Figure 2.2) were constructed in 1964 by the Department of Water Affairs. The adjoining dams comprises two lakes connected by a 2.5 m deep channel and are located in a 10,170-ha nature reserve in the foothills of the Venda Mountains within the Vhembe District of Limpopo Province (Madanire-Moyo *et al.* 2012). The Luphephe-Nwanedi dams receive water from the unpolluted Luphephe and Nwanedi mountain streams that join below the dam to form the Nwanedzi River (Madanire-Moyo *et al.* 2012). The Luphephe-Nwanedi dams provide water for irrigation and potable water for nearby communities. These impoundments are considered pristine by Madanire-Moyo *et al.* (2012) based on the fish health and water quality. To date Luphephe-Nwanedi dams have undergone extensive research regarding water quality, parasite diversity and fish health by Madanire-Moyo *et al.* (2010, 2011, 2012).

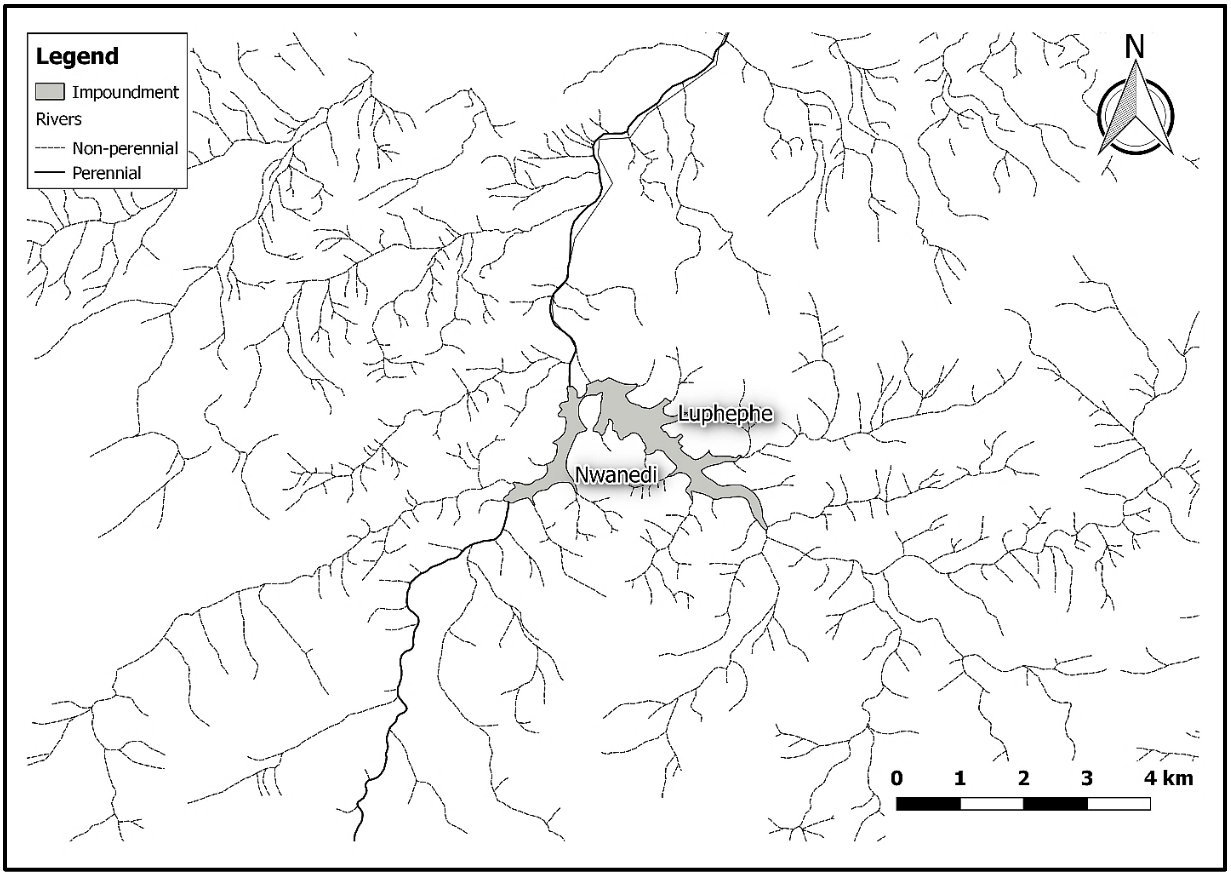

Figure 2.2: A map depicting the size and profile of Luphephe-Nwanedi Dams in the Nwanedi Nature Reserve.

2.2.2 Site two: Loskop Dam (25° 25' 0.59" S: 29° 21' 0.59" E)

Loskop Dam (Figure 2.3) is located within the Loskop Nature Reserve, which is situated approximately 32 km from the town of Groblersdal in Mpumalanga Province (Truter *et al.* 2016). This impoundment was constructed in 1938 by the Department of Water Affairs and in 1979 the wall was raised to its current height of 54 metres (Botha *et al*. 2011). Loskop Dam is of commercial importance supplying water for irrigation to approximately 25,600 ha of agricultural land (Truter *et al.* 2016). Within the past 30 years, this impoundment has experienced a severe decline in the number of Nile crocodiles with several instances of fish kills reported (Ashton 2010) caused by the influx of pollution spills from mines and industries further upstream and from a disease called "pansteatitis" that results in the hardening of the adipose tissue in both crocodiles and fish (Bowden *et al.* 2016).

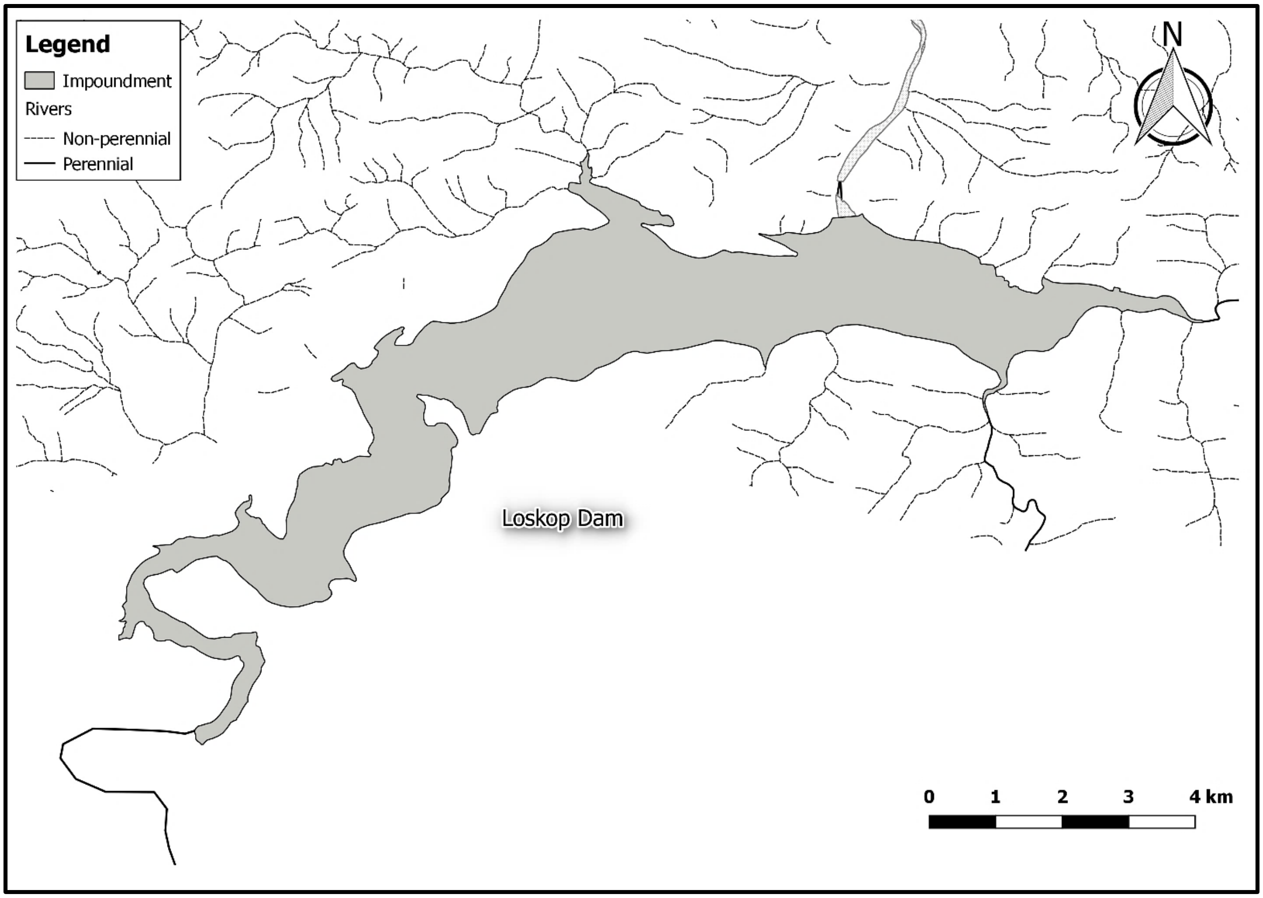

Figure 2.3: The size and profile of Loskop Dam in the upper reaches of the Olifants River.

2.2.3 Site three: Flag Boshielo Dam (24˚ 46' 59.99" S: 29˚ 25' 23.99" E)

Flag Boshielo Dam (Figure 2.4) previously known as Arabie Dam, was constructed in 1987 with the wall raised by a further 5 metres to a height of 30 m in 2005 (Ashton 2010). This impoundment is located downstream of the confluence of the Olifants and Elands rivers. The reservoir is situated about 85 km downstream of Loskop Dam and approximately 25 km north-east from the town of Marble Hall in the extreme north-western corner of Mpumalanga Province (Madanire-Moyo *et al.* 2012). With a mean depth of 8.15 m, the catchment area of the dam covers 4,213 km^2 (Dabrowski *et al.* 2014). Flag Boshielo Dam provides water to neighbouring municipal and rural districts for domestic, agriculture and mining purposes (De Villiers & Mkwelo 2009).

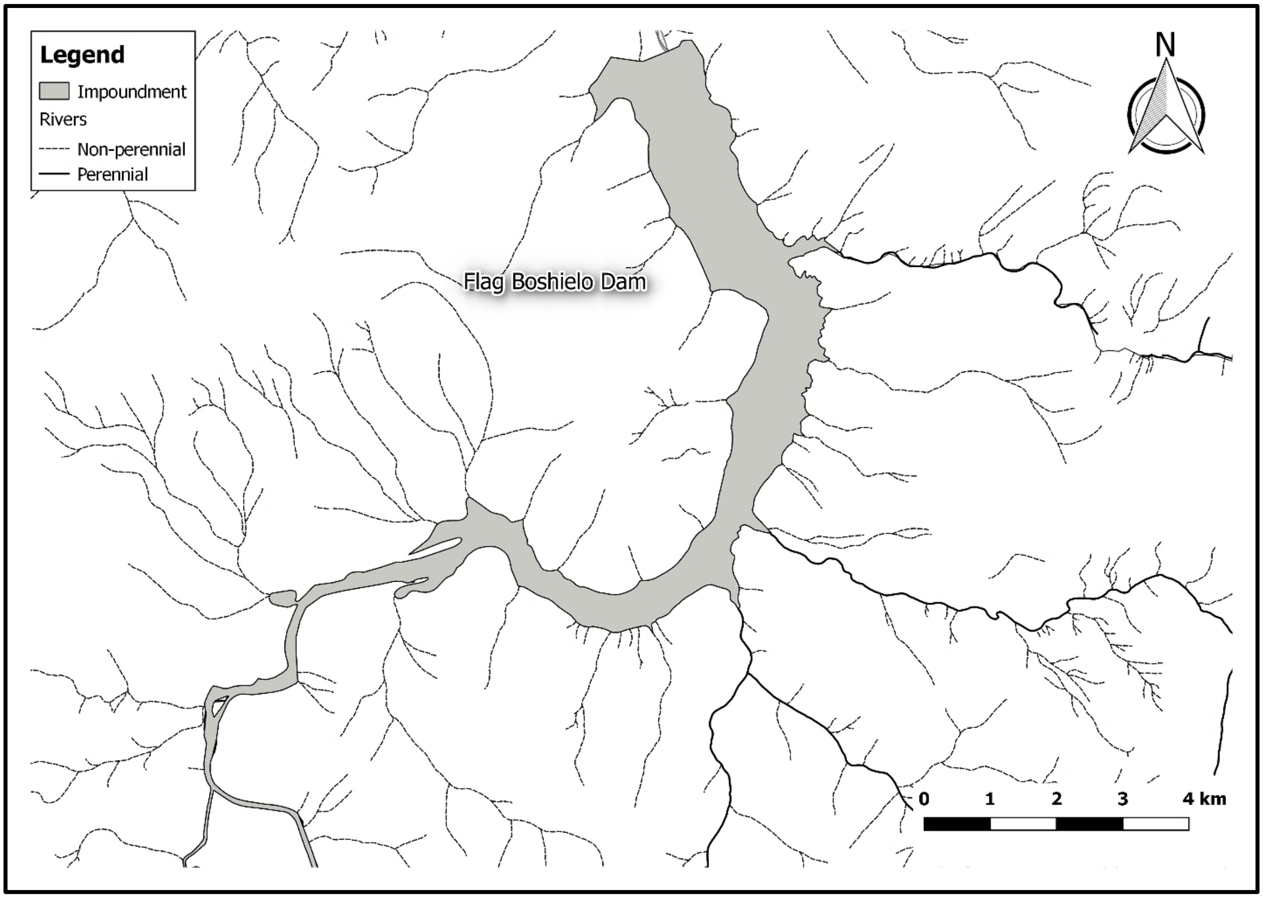

Figure 2.4: A map showing the size and profile of Flag Boshielo Dam and neighbouring perennial and non-perennial rivers that flow into the system.

2.2.4 Site four: Rhenosterkop Dam (25° 06' 16.6" S: 28° 53' 40.7" E)

Rhenosterkop Dam (Figure 2.5) located within the Elands River was constructed in October 1984 (Robarts *et al*. 1992). This impoundment serves as the main storage reservoir of the South Ndebele water supply scheme. At full capacity, Rhenosterkop Dam covers an area of about 3,625 ha and has a maximum depth of 16.5 m, mean depth of 5.6 m and a volume of 205.5 million m^3 (Robarts *et al.* 1992). According to a DWAF (2011) report, no industrial and mining activities occur within the catchment of the Elands River.

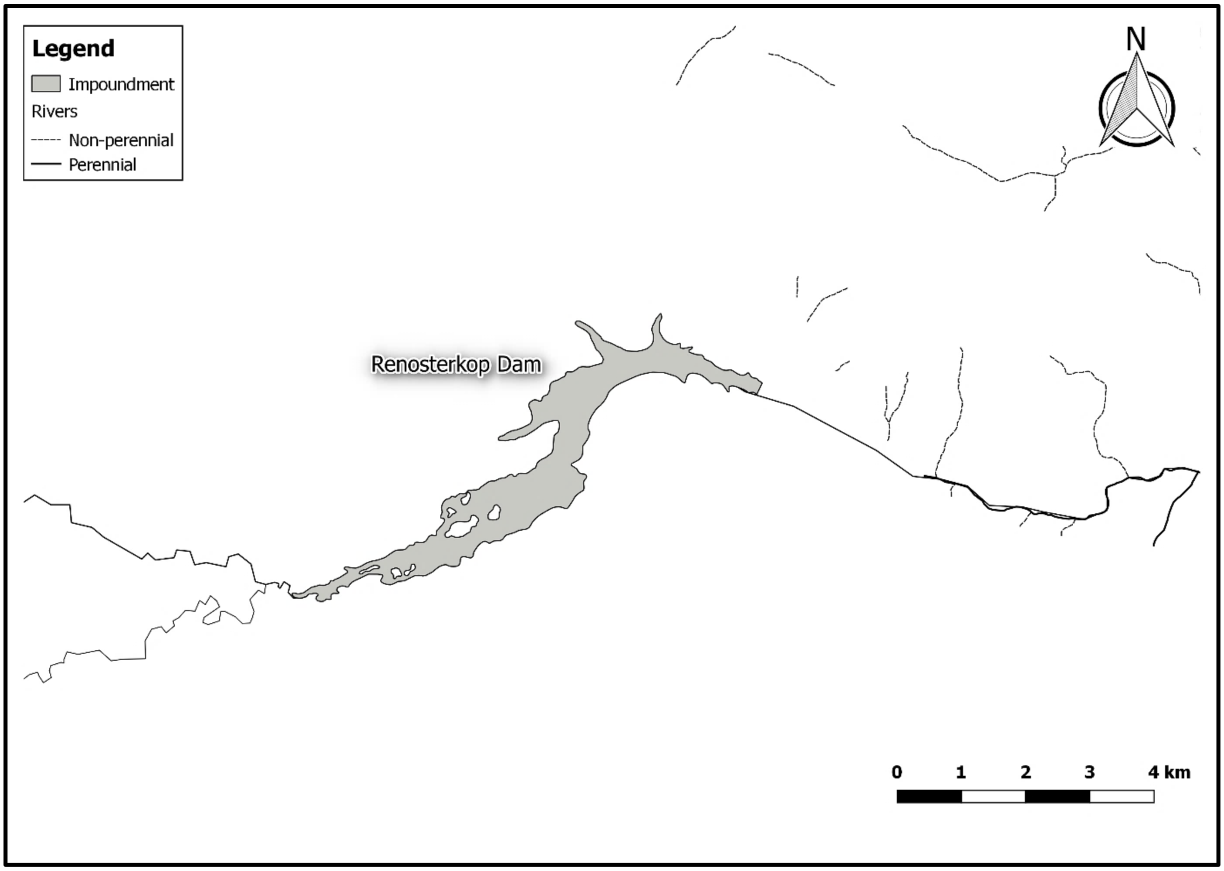

Figure 2.5: The size and profile of Rhenosterkop Dam in the Elands River.

2.2.5 Site five: Phalaborwa Barrage (24°04'10.04" S: 31°08'39.58" E)

The Phalaborwa Barrage (Figure 2.6) was constructed on the main stem of the Olifants River (Jooste *et al.* 2015) and is located near the western boarder of the Kruger National Park approximately 10 km from the town of Phalaborwa in Limpopo Province. This impoundment is located in the Savannah Biome, Lowveld Bioregion and it is bordered by dense riparian vegetation of the river including indigenous trees, grasses and reeds (Ballance *et al.* 2001). Furthermore, this impoundment provides water for the Lepelle Water Board that, in turn, purifies and distributes potable water to various users in the Phalaborwa area. Moreover, the Barrage provides habitat for various birds, crocodiles, water monitors and hippopotami.

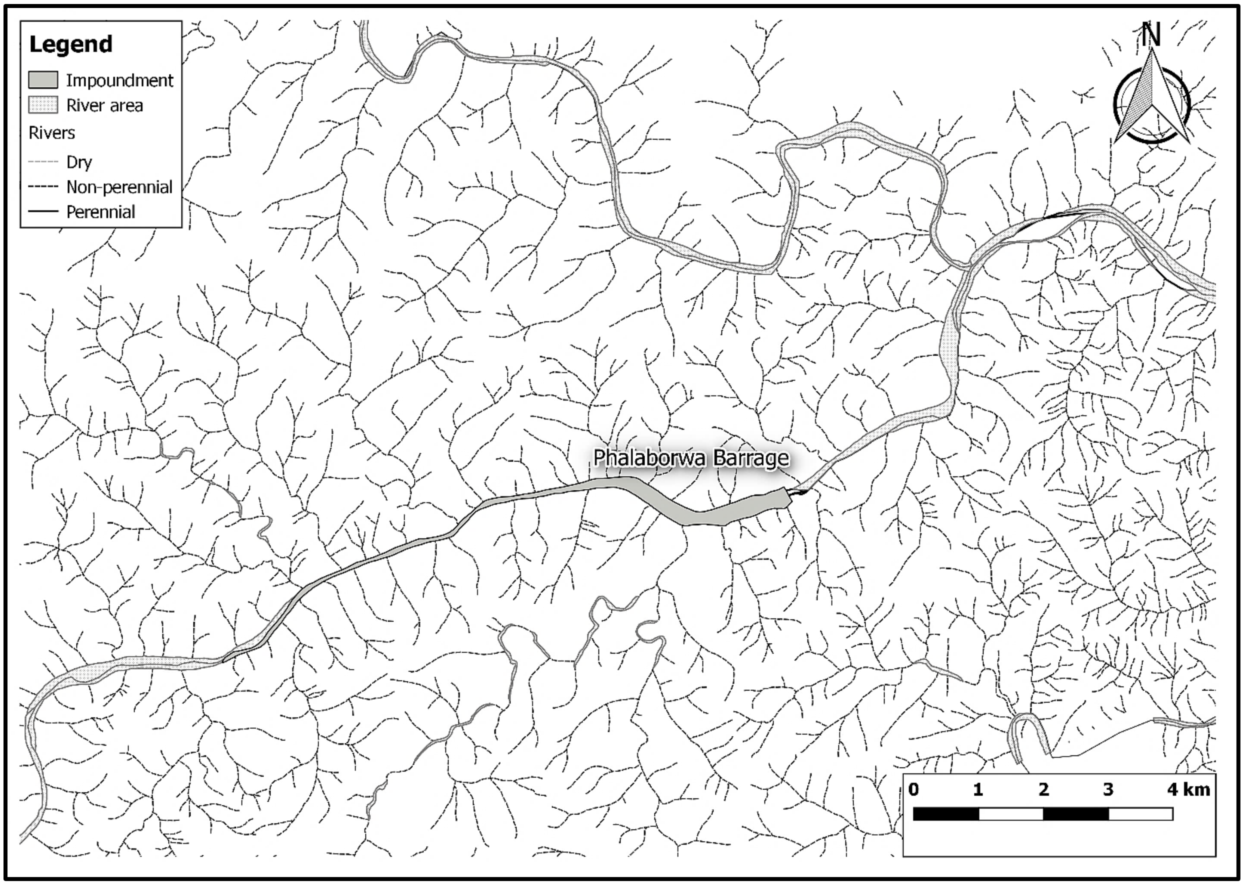

Figure 2.6: The size and profile of Phalaborwa Barrage near the boarder of Kruger National Park.

2.3 Selection of bioindicators

2.3.1 Mozambique Tilapia: *Oreochromis mossambicus* (Peters, 1852)

In this study *Oreochromis mossambicus* was selected as the test organism due to the species being cosmopolitan, with a wide distribution and because this species occurs in each of the above-mentioned impoundments. *Oreochromis mossambicus* is a cichlid mouth brooder native to the coastal rivers of the lower Zambezi to the south as far as the Bushmans System, in the Eastern Cape (Skelton 2001; Figure 2.7). This species has extensively been introduced into the western and south-western flowing rivers of South Africa including the Orange River System and the rivers of the Western Cape (Skelton 2001). *Oreochromis mossambicus* is a euryhaline fish tolerant of fresh and saline water (Skelton 2001) and can grow well in standing waters. This species is also tolerant of a wide range of ecological conditions e.g. can survive temperatures as low as 10°C in brackish or marine waters but prefers warmer temperatures above 22°C to a maximum of 40°C (Froese & Pauly 2010). The Mozambique Tilapia breeds in summer, with females raising a brood every 3 to 4 weeks. Depending on the available resources, juveniles are naturally omnivorous and become herbivorous when they are adults feeding on algae, diatoms, macro-algae, macrophytes and detritus (Skelton 2001; Froese & Pauly 2010). According to the International Union for Conservation of Nature (IUCN), Mozambique Tilapia is listed as near threatened due to this species being frequently hybridised with or by alien tilapia species such as *Oreochromis niloticus* (Welicky *et al.* 2017). Moreover, this species is of socio-economic importance, as it is highly targeted by subsistence fishermen for their livelihoods. Thus, a better understanding of their health and condition is vital (Welicky *et al*. 2017).

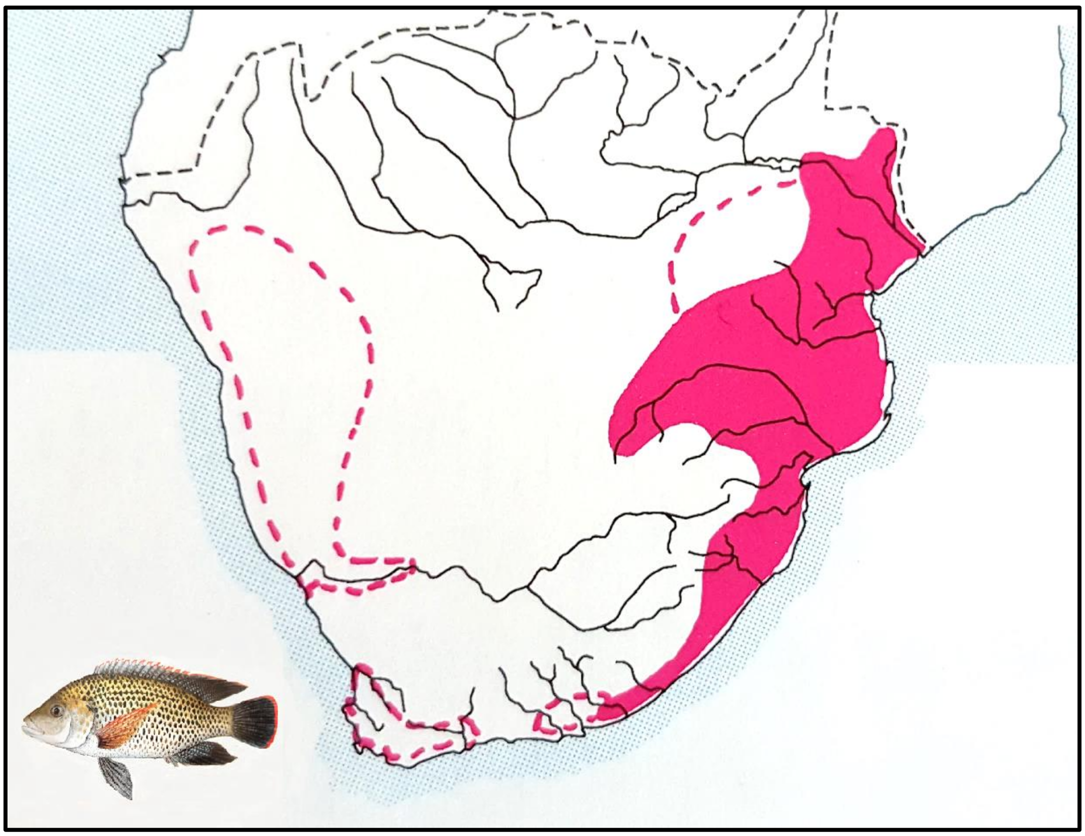

Figure 2.7: A map showing the distribution of *Oreochromis mossambicus* in southern Africa as indicated by the shaded area. Picture adapted from Skelton (2001).

2.4 Measurements of abiotic variables

Water temperature (°C), dissolved oxygen (mg/L), pH, salinity (ppt), total dissolved solids (mg/L) and electric conductivity (EC; μS/cm) were measured *in situ* using a handheld multi parameter meter (YSI model 556 Multi Probe meter). During sampling at the selected study sites, a once-off subsurface measurement of water readings were taken and recorded near the inlet, middle and dam wall. Subsurface water samples for metal analyses were collected in 1 L polypropylene bottles that were pre-treated and cleaned with 10% hydrochloric acid, and stored in a freezer at -20°C. Sediment samples were taken at each site using a mud grab.

When conducting seasonal surveys in Flag Boshielo Dam, three sites representing the riverine, transition and the lacustrine zone were selected whereby subsurface measurements of physico-chemical variables were taken at 1 m intervals to a depth of 2 m in the littoral zone at each of the sites surveyed. Water and sediment samples were collected during April, October, December 2016 and February 2017 and February, April, September and October 2016 respectively. Both water and sediment samples were then sent to WATERLAB, which is a SANAS accredited laboratory (ISO/IEC 17025:2005) in Pretoria, to be analysed for a broad spectrum of metals, non-metals and metalloids as indicated in Appendix A, using inductively coupled plasma mass spectrometry (ICP-MS) analysis. Water nutrients such as chloride, sulphate, fluoride, nitrate, nitrite and ortho-phosphates were also analysed using ICP-MS. In instances when nutrient data for Flag Boshielo Dam and Phalaborwa Barrage were not provided by the laboratory, information was sourced from the DWAF website (DWAF.gov.za/IWQS/WMS/Data/000key.Asp) for water data for these sites pertaining to the month proceeding, the month survey was conducted and the month following the survey to obtain an average.

2.5 Sampling of fish

During each survey, a maximum of 15 specimens of *Oreochromis mossambicus* were collected from each locality using multi-mesh gill nets consisting of five panels, 5 m wide having a 2 m drop with stretch mesh sizes of 44, 60, 75, 100 and 144 mm. The nets were placed and set to soak for approximately 2 hours. On lifting the nets, all fish were carefully removed and immediately placed into holding containers filled with dam water that was well aerated using portable aerator pumps. Fish were then transported in these containers by boat to the field laboratory for processing and examination. On reaching the field laboratory, fish were placed in larger containers filled with fresh, well aerated dam water. All specimens selected were identified using keys provided in Skelton (2001).

2.6. Determining blood glucose levels

To establish the blood glucose levels, fish were processed individually whereby specimens were placed on a dissecting board and blood drawn from the caudal vein at the posterior region of the lateral line using a 5 ml heparinised syringe fitted with a 23 gauge needle (Figure 2.8 A). A drop of blood was placed on a glucose test strip which was then inserted in a hand-held glucometer (Accu-Check ® Active; Figure 2.8 B). Glucose readings were recorded in mmol/L.

2.7 Assessing fish health based on the health assessment index

Application of HAI was conducted using the method described by Adams *et al.* (1993), by implementation of the HAI with PI and the HAI with IPI as evaluated by Crafford and Avenant-Oldewage (2009) and outlined in Heath *et al*. (2004).

2.7.1 Haematocrit determination

Blood was transferred into micro haematocrit tubes that were sealed at one end using commercial Critoseal clay and centrifuged in a haematocrit centrifuge (model KHT 400; Figure 2.8 C) for 5 minutes at 15000 revolutions per minute (rpm). Average haematocrit values were read using the haematocrit reader and expressed as a percentage.

2.7.2 Implementation of the necropsy procedure

The skin, fins and inside the opercula and mouth cavity were visually examined for external fish parasites, with parasites observed, counted and recorded. Skin smears were made by scraping along the length of each side of fish using a microscope slide and the mucus examined for the presence of parasites under a stereo microscope (Model: Leica EZ4). Total body mass in grams (g) was determined using an electronic balance (Model: Salter 235E) while total length (TL) and standard length (SL) were measured and recorded in centimetres (cm) using a measuring board.

Ethical approval (Project number: AREC/07/2017: PG) was obtained from Animal Research and Ethics Committee (AREC), University of Limpopo, thus at all times humane measures were implemented when handling and sacrificing fish. Fish were sacrificed by severing the spinal cord posterior to the brain cavity with a sharp knife or a pair of scissors depending on the fish size. Fish were then dissected (Figure 2.8 D) and the external organs i.e. eyes, skin, fins, gills and opercula and internal organs and tissues i.e. mesenteric fat, liver, spleen, hindgut, kidney and gallbladder examined and values were assigned based on a scoring system using the revised HAI table (Appendix A: Table A2). For example organs were assigned a numerical score based on the degree/extent of anomalies observed (Adams *et al.* 1993, Heath *et al.* 2004). Moreover, internal organs and tissues were assessed using a colour chart (Appendix A:Figure A1) developed by Watson *et al.* (2012). The sex of each specimen was recorded and gonadal stages were categorised according to Brown-Peterson *et al.* (2011), see Table 2.1.

Table 2.1: Macroscopic description of fish at various phases (modified from Brown-Peterson *et al.* 2011).

Phase	**Terminology**
Juvenile (never spawned)	Immature, not possible to visibly distinguish sex
Resting	Sex distinguishable
Developing (ovaries beginning to develop but not ready to spawn)	Maturing, oocytes increase in size and are visible testis increase in size
Ripe (Fish are developmentally and physiologically able to spawn in this cycle)	Mature, late developing, late maturation, late ripening, total maturation, partially spent, fully developed, final oocyte maturation, spawning, gravid ovulated
Regression (cessation of spawning)	Spent, post spawning, recovering
Regenerating (sexually mature, reproductively inactive)	Resting, regressed, recovering, inactive

2.6.2 Determining Parasite index (PI)

Excised gills were transferred to petri dishes containing distilled water and examined for the presence of parasites using a stereo microscope. Similarly, the intestines were removed and examined for endoparasites with the aid of a stereo microscope. Observed ecto- and endoparasites were scored based on the revised HAI Table A2 following Adams *et al.* (1993) and Heath *et al.* (2004).

2.6.3 Determining Inverted parasite index (IPI)

The IPI, as evaluated by Crafford and Avenant-Oldewage (2009), was used to assign numerical values to the number of external parasites. Based on the IPI premise that a higher number of ectoparasites are an indication of good water quality (Crafford & Avenant-Oldewage 2009), ectoparasites were assigned a lower score and the absence or low numbers of ectoparasites were assigned a higher HAI score. The endo- and ectoparasites IPI scores were categorised as presented in Table 1.1.

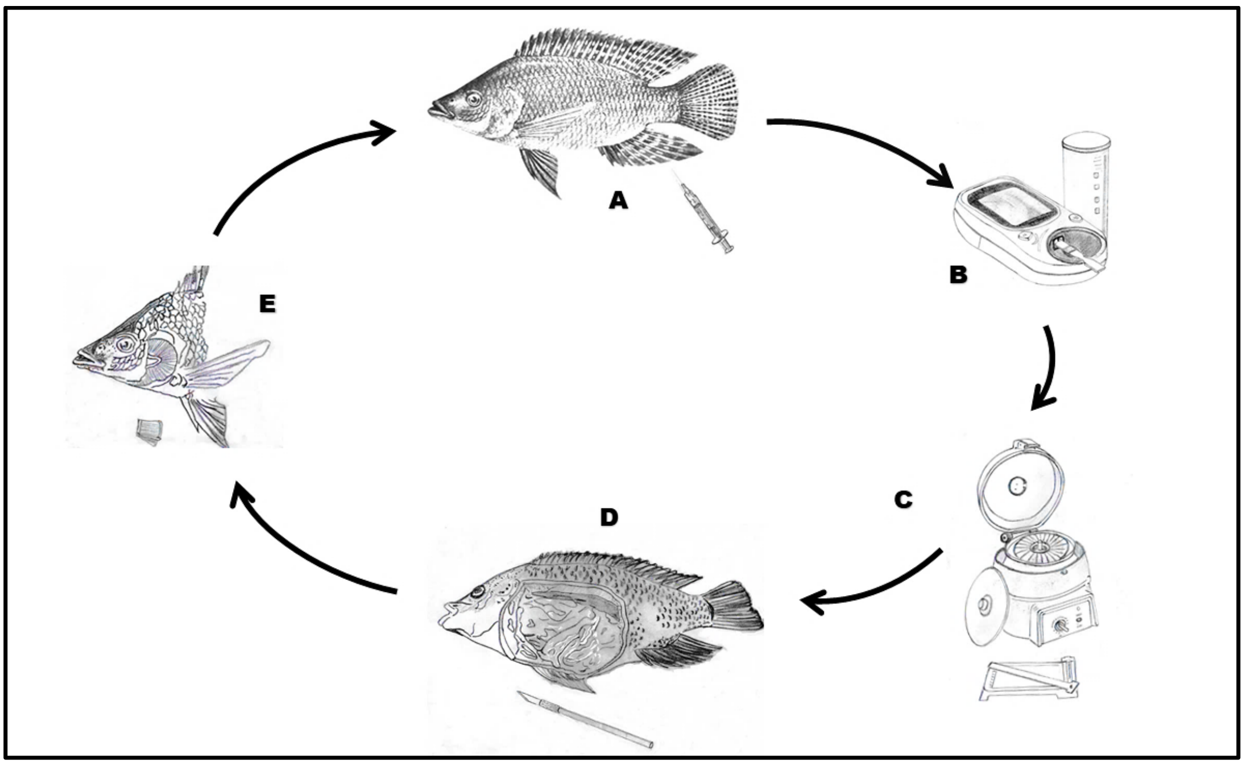

Figure 2.8: Summary of the assessment of fish health. A= Blood drawn from the caudal vein, B = Glucometer used to assess glucose levels based on a drop of blood, C = Haematocrit centrifuge and reader used to assess haematocrit values, D= Fish necropsy, E = Dissection of fish gills for microscopy and parasites.

2.7 Condition and/or somatic indices

To establish information on the overall health of fish sampled, various condition indices were determined.

2.7.1 Condition factor (K)

The condition factor for each fish was calculated using Fulton's condition index (Froese 2006):

$$K = \frac{W \times 100}{L^3}$$

Where W is the total body mass of fish in grams (g) and L the total length in centimetres (cm).

2.7.2 Somatic indices

In addition to total body mass, liver and gonad mass were determined to calculate hepatosomatic (HSI) and gonadosomatic (GSI). These indices were calculated according to the following formulas and expressed as percentages (Freyre *et al*. 2009):

$$HSI = \frac{\text{Liver mass (g)}}{\text{total live mass (g)}} \times 100$$

and

$$GSI = \frac{\text{Gonad mass (g)}}{\text{total live mass (g)}} \times 100$$

2.8 Microscopy analysis of fish gills

To establish the effect of pollutants on fish gill morphology, microscopy analysis was carried out to study pathologies such as epithelial necrosis, vasodilation, epithelial lifting, hypertrophy of the respiratory epithelium and hyperplasia (Hughes *et al.* 1978). The samples were processed at the Electron Microscopy Unit, Sefako Makgatho Health Sciences University (SMU) in Pretoria, South Africa.

2.8.1 Tissue processing of gills

For each specimen, the innermost gills on the left side of the fish were dissected (Figure 2.8 E) and fixed in Karnovsky's fixative (Karnovsky, 1965) for microscopy analysis. The gills were cut into small segments (1 mm^3) while viewed under a stereo microscope (Figure 2.10 B) and were delicately placed into 20 ml vials filled with Millonig's buffer (Millonig, 1961). These sections were then placed in small holding baskets prior to processing. The small baskets containing the cut sections were then placed in an automated tissue processor (Model: Leica EMTP) for a total of 18 hours and 25 minutes (Figure 2.10 C) whereby gill samples cycled through a process of post-fixation, rinsing, dehydration and infiltration. The default protocol and the time sequence used to process these are outlined in Appendix A:Table A3.

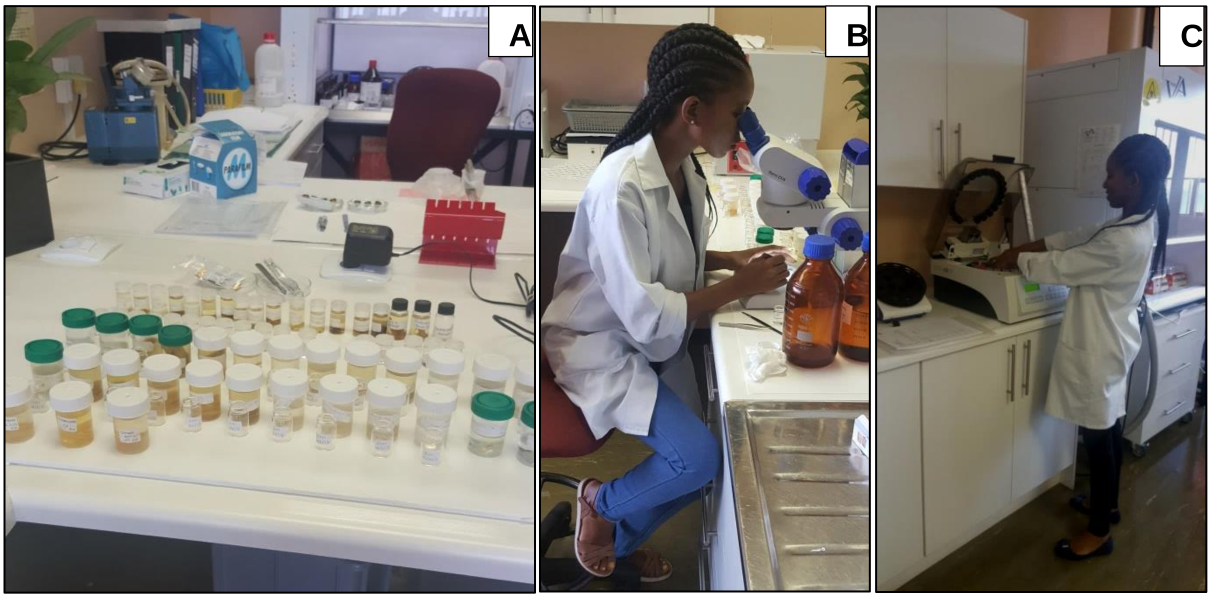

Figure 2.9: Sample preparation. (A) Gills samples fixed in Karnovsky 's fixative. (B) Cutting samples into small pieces with the aid of a stereo microscope. (C) Samples being placed in the processor.

Once processing had lapsed, the samples were removed from the processor and manually placed into sealed apparatus that allowed samples to be infiltrated with 100% epoxy (Model: Epon 812, Electron Microscopy Sciences UK) resin under vacuum for a period of 2 hours. Using an embedding mold (Agar Scientific, UK), samples were embedded in 100% epoxy resin and placed in the oven at 65 – 70°C overnight. Following standard procedures, the blocks were trimmed and semi-thin sagittal sections (1 μm) were cut from the embedded blocks using an ultramicrotome (Model: Leica UC 7, Leica Microsystems) and stained using toluidine blue (Sigma Aldrich, Germany) for viewing by light microscopy. Digital images were taken at 100 times magnification and saved as TIFF files.

2.9.2 Gill morphometric analysis and determination of H_{ar}

The digital images were analysed using a cycloidal grid (Howard and Reed 1998, Grid C1; see Figure A2) as described and used by Van Heerden *et al.* (2004a, b). Point-counting was undertaken whereby structures of the secondary lamellae were quantified (See Figure 2.11) following Van Heerden *et al.* (2004a, b) and Van Heerden *et al.* (2006):

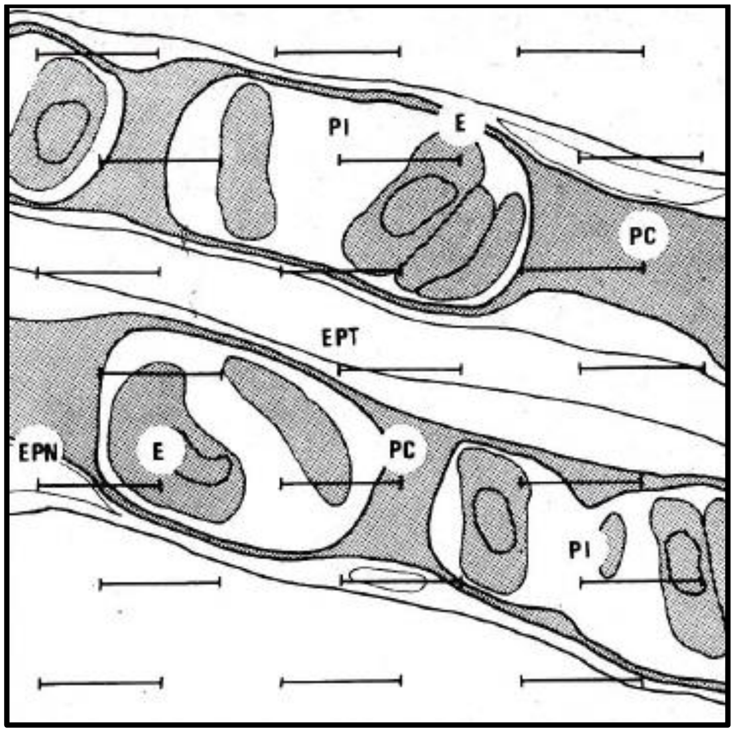

Figure 2.10: Diagram of a section through secondary lamellae with a superimposed grid indicating the points counted. Where E = Erythrocytes in the secondary lamellae, EPT = epithelium covering the secondary lamellae, EPN = Non-tissue (lymphoid) space of the epithelium, PC = Points falling on the pillar cells and PI = plasma in secondary lamellae.

From the epithelium covering the secondary lamellae (EPT), non-tissue (lymphoid) space of the epithelium (EPN) and the intersections between the grid lines and outer surface of the epithelium (I_o) the mean arithmetic thickness of the gill epithelium (H_{ar}) was calculated as:

$$\mathrm{Har} = \frac{(\mathrm{EPT} + \mathrm{EPN}) \times 4.7\mu\mathrm{m}}{2 \times \mathrm{Io}}$$

Where 4.7 µm is the grid constant.

2.10 Establishing metal concentrations in fish muscle tissues

A ±15 g skinless sample of lateral muscle tissue was collected from each fish and placed in ziplock bags and stored at − 80°C prior to metal analysis at the WATERLAB, SANAS accredited (ISO/IEC 17025:2005). Tissue samples were dried and digested according to the methods described by Bervoets and Blust (2003) and analysed for an array of metals and metalloids using ICP-MS (Appendix A:Table A4). However, for comparison purposes, aluminium (Al), arsenic (As), barium (Ba), boron (B), cadmium (Cd), chrome (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), vanadium (V) and zinc (Zn) investigated in fish muscle tissue with regard to human health impacts by Addo-Bediako *et al.* (2014a, b), Jooste *et al.* (2014, 2015), Lebepe *et al.* (2016), Marr *et al.* (2015, 2017) and Sara *et al*. (2017) were selected and reported in this study. All samples were analysed in batches with blanks. Analytical accuracy was determined using certified standards from De Bruyn Spectrometric Solutions (500MUL20-50 STD20) and recoveries were within 10% of the certified values.

2.11 Data analysis

Water measurements were tested for normality and homogeneity of variance using normality plots with Kolmogorov-Smimoff and Shapiro-Wilk tests respectively. For data normally distributed, a one-way Analysis of variance (ANOVA), with Tukey HSD post-hoc test was used to establish the differences between impoundments and surveys. In contrast for non-parametric data, the Kruskal-Wallis test was used. Similarly, biological variables i.e. blood glucose, the HAI, HSI, GSI, arithmetic mean thickness of gill epithelium (H_{ar}) and metal content within fish muscle tissues were tested for normality and homogeneity of variance and a one-way Analysis of variance (ANOVA), with Tukey HSD post-hoc test or Kruskal Wallis analysis applied. Significant differences were ascertained at $p < 0.05$. The results were presented as mean ± standard error (SE) of the mean. All analyses were conducted using Statistical Package of Social Sciences (SPSS software version 25). All graphs were generated using R 3.5.0. statistical software (R Development Core Team 2017).

With regard to the modelling of results, a redundancy analysis (RDA) was performed on environmental variables (i.e. water quality variables) and metal content in water, sediment and fish muscle tissues, together with biological variables such as blood glucose (BGlu), condition factor (K), hepatosomatic index (HSI), gonadosomatic index (GSI), health assessment index (HAI) and arithmetic mean thickness of gill epithelium (H_{ar}). To determine the significance of the environmental variables on the biological variables, results were presented as triplots ordination diagrams. However, at the time of doing these analyses the muscle tissue analyses of metals for fish from Luphephe-Nwanedi Dams (LND) had not been received from the laboratory. Except for LND metal tissue data, rows missing any data for variables tested were omitted from the analysis process. To establish collinearity between variables, bi-plots between sites and biological variables and between sites and environmental variables were generated and the correlation coefficient determined (See Appendix D). Variables sharing a coefficient of $R \geq 0.75$ between sites were removed and excluded from the RDA analyses. To prevent a few high values from unduly influencing the ordination when conducting a redundancy analysis (RDA), using Canoco 4.5 (Canoco software version 4.5: Ter Braak & Prentice 1988) data were log transformed. The Monte-Carlo permutation test set at 999 iterations was used to sequentially select and significantly test each of the environmental variables to establish the maximum extra or reduced-model fit. The same was done when exploring survey data collected from Flag Boshielo Dam. After examining RDA triplot results, the biological and environmental variables closely associated with HAI scores were used to generate a global GLM using R statistical software. This was done based on the flexibility of testing variants used for the global GLM and selecting the best fit model based on the poisson error structure and associated log canonical link function. The best-fit model was determined using the dredge function in R statistical software version 3.5.0. based on the Akaike's information criterion (AIC). When analysing variables that best predict HAI scores between sites, sites were set as a factor using the function in R. For surveys conducted at Flag Boshielo Dam, the months indicative of high and low inflow were set as factors. A Wilcoxon Mann-Whitney test was applied to test for differences between HAI values and tissue metal content recorded between periods of low and high inflow at Flag Boshielo Dam.

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CHAPTER 3: EVALUATING THE WATER AND SEDIMENT QUALITY OF LUPHEPHE-NWANEDI, RHENOSTERKOP, LOSKOP, FLAG BOSHIELO DAMS AND PHALABORWA BARRAGE

3.1 Introduction

Water quality is a term used to define the microbiological, physical, chemical and aesthetic properties of water that makes it suitable to sustain a healthy aquatic ecosystem (DWAF 1996a, b). The composition of inland water bodies is influenced by geological, topographical, climatological and biological factors that occur in the drainage basins and along the main stem of a river (Poff *et al.* 1997; Dallas & Day 2004). Globally, freshwater systems have been adversely affected by land use activities such as urbanisation and wastewater treatment works (WWTWs), agricultural, mining and industrial practices (Crafford & Avenant-Oldewage 2011). The occurrence of pollutants from these sources deteriorate water quality of freshwater ecosystems (Cessford & Burke 2005). The most common pollutants associated with urbanisation include phosphate, ammonia, pesticides, faecal coliforms and bacteria (Bartram & Ballance 1996). Studies by Roberts and Prince (2010) and Carey *et al.* (2011) have confirmed the relationship between settlement growth and a noticeable deterioration in water quality in neighbouring waterbodies utilised by the settlement populace.

In South Africa, infrastructures of most WWTWs are often inadequate to deal with the amount of wastes they receive, and thus the untreated or partially treated wastewater enter inland waters (Oberholster *et al.* 2013; Seanego & Moyo 2013), resulting in the depletion of dissolved oxygen and increased nutrients which in turn lead to eutrophication, cholera outbreaks and major fish kills (Moyo & Mtewa 2002; De Villiers & Thiart 2007). Conversely, agricultural practices such as grazing and vegetation removal may alter water flow dynamics by reducing water infiltration and increasing surface runoff (Stone *et al.* 2005; Strauch *et al.* 2009). Furthermore, the use of fertilisers in agricultural fields may increase nitrogen and phosphorus concentrations which may lead to eutrophication, resulting in the algal blooms and increase in toxic ammonia which may affect the health of aquatic organisms (Paisley *et al.* 2003; De Villiers & Thiart 2007). For example, the use of pesticides and herbicides have been reported by Alexander *et al.* (2007) to affect the reproductive success of fish in aquatic systems. Mining activities are also known to affect water resources through the discharge of mine effluents and seepage from tailings dams which release metals and sulphates (Hobbs *et al.* 2008). Natural levels of metals are augmented when released into inland waters from mining sources with further mobilising occurring due to acid mine drainage (AMD) (Strydom *et al.* 2006). The AMD occurs when sulphide minerals such as iron pyrite and iron disulphide are exposed to air and water, leading to acidic conditions that in turn significantly affect the overall ecological integrity of water systems (Truter *et al.* 2014).

In aquatic systems, the underlying sediment serves as a natural filter whereby contaminants settle on the bottom of the substrate (Osman & Kloas 2010). While determination of the water quality is an indication of the contamination status at the time when the water samples are taken, a measure of sediment quality provides a historical perspective of metal bioaccumulation in the system under investigation (Greenfield *et al.* 2007). Moreover, the fact that sediments contain higher levels of contaminants and are less affected by variation samples thereof, upon analyses, can provide a more stable monitoring platform as opposed to the sole analysis of water (Islam *et al.* 2015). Therefore, it is important to assess the concentrations and distribution of metals in both water and sediment. Historically, in South Africa water quality monitoring resorted to the quantification of physical and chemical variables *in situ*, however, this approach alone is inadequate as it leads to shortcomings as elucidated in Chapter 1.

In South Africa, water quality management is defined as the effort employed to control the physical, chemical and biological characteristics of water to ensure it is suitable for various purposes by the end user (Wepener *et al.* 2000). The Department of Water Affairs and Forestry (DWAF 1996a), Canadian Council of Ministers of the Environment (CCME 2002) and World Health Organisation (WHO 2006) have developed water quality range guidelines considered safe for human consumption and for use in domestic, agricultural, recreational, industrial and aquatic ecosystems.

The aim of this chapter was two-fold. The first was to determine and confirm the water quality of Luphephe-Nwanedi, Rhenosterkop. Loskop, Flag Boshielo dams and Phalaborwa Barrage based on the nutrient and metal content in water and sediment. The second was to determine if Flag Boshielo Dam's water quality fluctuates due to seasonal changes. This was done by analysing water samples taken approximately monthly for nutrients and metals and sediment samples collected on a quarterly basis. The physical/chemical variables and metal levels were compared with guidelines for aquatic ecosystems stipulated by DWAF (1996a), CCME (2002) and WHO (2006).

3.2 Materials and Methods

The methodology used to undertake the collection and analysis of water and sediments samples at each impoundment has been described in Chapter 2.

3.3 Water results from a once off survey of the various impoundments

3.3.1 Water temperature, dissolved oxygen and pH

Although surveys were conducted over a 4 to 5 week period in this study, temperature fluctuated between impoundments, with higher temperatures recorded at Flag Boshielo Dam. Dissolved oxygen (DO) concentrations (mg/L) were adequate and within the specified TWQR, with levels recorded at Rhenosterkop Dam being the highest. However, a significant ($p< 0.05$) difference in DO between impoundments prevailed. Alkaline conditions were recorded in all impoundments with high pH values of 9.28 – 9.88 exceeding the TWQR recorded at Loskop Dam. The pH range at Luphephe-Nwanedi Dams and Phalaborwa Barrage were within the TWQR (Table 3.1).

3.3.2 Total dissolved solids, electrical conductivity and salinity

According to Chapman (1996) and DWAF (1996a), a positive correlation occurs between total dissolved solids (TDS), electrical conductivity (EC) and salinity and this phenomenon was observed in this study. There was a highly significant ($p < 0.0001$) variation in TDS, EC and salinity between the impoundments, with the highest values recorded at Flag Boshielo Dam and lowest at Luphephe-Nwanedi Dams (Table 3.1).

3.3.3 Nutrients and major ions

Levels of ammonium (NH_4^+) were within the specified TWQR, with the highest concentration recorded at Rhenosterkop Dam. These concentrations were not significant ($p > 0.05$) when compared between impoundments**.** Nitrate (NO_3^-) concentrations fluctuated significantly ($p < 0.05$) between impoundments with levels that exceeded TWQRs reported at Rhenosterkop Dam, Flag Boshielo Dam and Phalaborwa Barrage (Table 3.1). Orthophosphates (PO_4^{3-}) concentrations were reported to be high and above the specified TWQR limits for all five impoundments (Table 3.1). Sulphate (SO_4^{2-}) concentrations were highly significant ($p < 0.0001$)

between impoundments, with Loskop Dam having the highest levels that exceeded the TWQR.

3.3.4 Metals and metalloids in water column

Most metal and metalloids concentrations were recorded not to be significantly ($p > 0.05$) different except for Mg, Sr and Zn. Concentrations of Al were reported to be higher than the permissible TWQR in water samples collected from Phalaborwa Barrage. Boron (B) fluctuated within a narrow range of between 0.01 to 0.04 mg/L with higher levels recorded at Rhenosterkop Dam and lowest concentrations occurring at Luphephe-Nwanedi and Flag Boshielo dams. Barium (Ba) exhibited a wide variation of between 0.02 and 0.14 mg/L (Table 3.1), with the highest concentrations recorded at Rhenosterkop Dam. Iron (Fe) concentrations that exceeded the acceptable TWQR limits were recorded at Phalaborwa Barrage. Phosphorus (P) levels were only detected in water samples collected from Flag Boshielo Dam and Phalaborwa Barrage and ranged between 0.025 and 0.25 mg/L. Zinc (Zn) concentrations fluctuated between 0.03 to 0.04 mg/L, with Luphephe-Nwanedi, Loskop, Flag Boshielo dams and Phalaborwa Barrage having concentrations that exceeded the specified TWQR (Table 3.1). Although Ca, K, Mg, Mn, Na, Si, Sr and Ti levels were reported to fluctuate between impoundments, most of these elements occurred within the specified TWQRs.

3.3.5 Metals in sediments

From the sediment samples collected, metal concentrations were found to be significantly ($p > 0.05$) higher when compared to those in water. Sediment samples from Flag Boshielo Dam contained the highest concentrations of most metals, followed by Phalaborwa Barrage and Loskop Dam (see Appendix B, Table B1). Higher concentrations of Cu, La, Na, Pb and Ti were detected in sediment samples from Luphephe-Nwanedi Dams. Due to the underlying substrate of Rhenosterkop Dam, sediment samples were not collected at this impoundment.

Table 3.1: Means ± standard errors (SE) of physico-chemical variables measured at the five impoundments during April and May 2016. Shaded values indicate where the TWQR guidelines have been exceeded.

Variables	Impoundments										
	Luphephe- Nwanedi		Loskop		Flag-Boshielo		Rhenosterkop		Phalaborwa Barrage		TWQR
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	
Temperature(°C)	19.10^{a}	0.00	22.62^{b}	0.59	24.78^{b}	0.68	23.55^{b}	0.74	18.50^{a}	0.31	-
DO (mg/L)	7.63^{a}	0.07	-	-	8.72^{b}	0.83	8.89^{b}	0.28	7.60^{ab}	0.21	6.0 – 9.0
pH	8.13 - 8.20		**9.28 – 9.88**		**9.15 – 9.27**		8.18 – 9.73		8.90 – 9.00		6.6 – 9.0
Electrical conductivity (µS/cm)	141.20^{a}	0.85	540.83^{bc}	31.69	570.17^{c}	0.00	489.9^{bc}	5.58	463.6^{b}	2.27	-
TDS (mg/L)	103.40^{a}	0.40	334.30^{b}	17.44	379.45^{c}	0.00	326.30^{b}	0.00	342.34^{b}	0.59	-
Salinity (ppt)	0.07	0.00	0.26	0.16	0.29	0.00	0.24	0.00	0.26	0.03	-
Ammonium (mg/L)	0.05	0.00	0.07	0.20	0.13	0.84	0.15	0.00	-	-	0.2*
Nitrate (mg/L)	0.21	0.26	0.07	0.22	**0.42**	0.35	**0.36**	0.20	**0.36**	0.04	0.2
Ortho-phosphate (mg/L)	**0.18**	0.08	**0.21**	0.11	**0.67**	0.19	**0.19**	0.03	**0.26**	0.16	0.1*
Sulphate (mg/L)	5.47^{a}	0.23	**142.30^{e}**	4.64	95.35^{d}	1.80	18.21^{b}	0.97	32.99^{c}	2.98	100**
Chloride (mg/L)	35.78^{b}	0.73	21.43^{a}	0.46	43.83^{c}	1.37	55.26^{d}	0.57	-	-	600
Fluoride (mg/L)	0.21^{a}	0.49	0.41^{b}	0.60	**0.80^{c}**	0.65	**1.46^{d}**	0.17	-	-	0.75
Aluminium (mg/L)	<0.001	0.00	<0.001	0.00	<0.001	0.00	0.01	0.00	**0.52**	0.51	0.01
Boron (mg/L)	0.01^{a}	0.00	0.02^{a}	0.00	0.01^{a}	0.00	0.04^{c}	0.00	0.03^{b}	0.00	-
Barium (mg/L)	0.02^{a}	0.00	0.04^{b}	0.01	0.02^{ab}	0.00	0.14^{c}	0.00	0.04^{ab}	0.00	-
Calcium (mg/L)	6.50^{a}	0.50	25.50^{c}	3.59	15.00^{ab}	1.00	20.03^{bc}	0.33	29.00c	1.00	-
Iron (mg/L)	0.03^{a}	0.00	<0.001^{a}	0.00	<0.001a	0.00	0.01^{a}	0.00	**0.41^{b}**	0.06	0.2
Magnesium (mg/L)	4.50^{a}	0.50	16.25^{b}	1.37	15.00b	3.00	11.51	0.01	26.50c	2.50	70
Manganese (mg/L)	<0.001	0.00	0.07	0.00	<0.001	0.00	<0.001	0.00	<0.001	0.00	0.18
Phosphorus (mg/L)	<0.001	0.00	<0.001	0.00	0.02	0.00	<0.001	0.00	0.02	0.01	-
Potassium (mg/L)	1.10	0.20	4.80	0.46	4.40	0.40	13.45	0.04	2.25	0.15	200
Silicon (mg/L)	3.35	0.25	2.20	2.00	5.80bc	0.60	<0.001^{a}	0.00	7.80^{c}	0.20	-
Sodium (mg/L)	11.50 ^{a}	1.50	32.25 ^{a}	5.34	36.50	9.50	61.99	0.17	35.00	2.00	100
Strodium (mg/L)	0.35^{a}	0.00	0.14^{c}	0.01	0.10^{b}	0.01	0.16c	0.00	0.14^{c}	0.00	-
Titanium (mg/L)	0.01^{a}	0.00	0.06^{c}	0.01	0.03^{b}	0.00	<0.001^{a}	0.00	0.07^{c}	0.00	-
Zinc (mg/L)	**0.04^{b}**	0.00	**0.03^{b}**	0.00	**0.03^{b}**	0.00	<0.001^{a}	0.00	**0.04^{b}**	0.00	0.002

Key: SE = standard error, TWQR = target water quality range for South African water quality guidelines for aquatic ecosystems (DWAF 1996a) * = World Health Organisation Guidelines (2006), ** = Canadian Guidelines (2002). Superscripts a, b, c and d indicate the significant differences, with $p < 0.05$.

3.4 Discussion for once off survey of various impoundments

3.4.1 Water temperature, dissolved oxygen and pH

Temperature changes in aquatic systems can be influenced by latitude, altitude, season, the time of day, air circulation, cloud cover and flow depth (DWAF 1996a). In turn, temperature affects physical and chemical processes as well as concentration of nutrients and dissolved solids (Chapman 1996). For example, an increase in water temperature causes a decrease in the solubility of gases such as oxygen (O_2), carbon dioxide (CO_2), nitrogen (N_2) and methane (CH_4) (Palmer *et al.* 2004). Since all the impoundments in this study were surveyed over a 4 to 5 week period, one would expect a slight degree of variability in temperatures recorded due to local climate conditions on the day of sampling, especially given that the localities of these impoundments are within a 400 km radius of each other.

Variations in DO may occur over 24 hour periods due to changes in temperature, salinity, atmospheric pressure, aeration through wind action and the turbulent mixing of the water column and biological processes such as algal photosynthesis and respiration (Chapman 1996). The solubility of oxygen in water is inversely related to temperature, resulting in higher DO concentrations occurring at lower temperatures (Dallas & Day 2004). Although the DO concentrations recorded at all impoundments were within the TWQR limit, higher DO concentrations at Rhenosterkop Dam may be attributed the impoundment's lack of steep embankments and large, flat profile whereby the impoundment's fetch allows for the vast body of water to be exposed to wind action. Wind action in turn allows for water column turnover to occur. Moreover, although chlorophyll levels were not measured in this study, high DO concentrations in Rhenosterkop Dam can also be attributed to increased densities of phytoplankton propagated by the presence of fertilisers and agricultural effluents occurring from commercial farms upstream of the impoundment (De Villiers & Mkwelo 2009).

The pH of aquatic system influences the mobilisation, speciation and toxicity of phosphates, nitrate concentrations and metals (Mmualefe & Torto 2011). The lower the pH, the higher the solubility and increase in heavy metal bioavailability (Waite *et al.* 1984). Alkaline levels recorded at Loskop and Flag Boshielo dams and Phalaborwa Barrage can be because when respiration and decomposition processes release CO_2 by phytoplankton in eutrophic systems, they result in diurnal pH fluctuations of < 6 to > 10 (Dallas & Day 2004). Lebepe *et al*. (2016) stated that the alkali nature of the underlying geology of the Olifants River neutralises the acid mine drainage such that the pH of system water is above 8 in most of the system's catchment. Alkaline pH values at Luphephe-Nwanedi Dams would appear to be influenced by the underlying geology as there is little or no anthropogenic sources occurring at the catchment of these impoundments (Madanire-Moyo *et al.* 2012).

3.4.2 Total dissolved solids, electrical conductivity and salinity

Total dissolved solids (TDS) is a measure of various inorganic salts dissolved in water (DWAF 1996a) and are known to affect the aesthetic properties of water by increasing turbidity. Natural waters contain various quantities of TDS due to the suspension of mineral rocks, soils and decomposition of plant materials (DWAF 1996a). Moreover, TDS can be attributed to anthropogenic factors such as the discharge of domestic, agricultural and industrial effluents into aquatic bodies (Dallas & Day 2004). Naturally, TDS also occurs during periods of high runoff. But since the surveys were conducted before the rainy season, this was expected not to be a contributing factor. High concentrations of TDS recorded at Flag Boshielo can be attributed to the disposal of agricultural and waste water treatment effluents in the catchment of this impoundment (De Villers & Mkwelo 2009; Heath *et al.* 2010). In turn, low concentrations of TDS recorded at Luphephe-Nwanedi Dams can be attributed to little or no contribution from domestic, agricultural and industrial point sources upstream of the impoundments.

Total dissolved solids readings are directly proportional to those of electrical conductivity (EC); and consequently, EC is routinely used as an estimate of the TDS (DWAF 1996a). Electrical conductivity is the ability of water to conduct an electrical current and is dependent on the concentration of carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium ions (Bartram & Ballance 1996; DWAF 1996a). The more ions present in water, the more electricity can be conducted (Osman & Kloas 2010). Higher and lower EC concentrations recorded for Flag Boshielo and Luphepe-Nwanedi Dams respectively, can be attributed to the same factors governing TDS levels.

Salinity of inland waters can be geological when freely mobile salts leach out of the soil (DWAF 1996a; Bartram & Ballance 1996). Anthropogenic sources that influence salinity concentrations in water bodies include agricultural and urban runoff, mining activities and the burning of fossil fuels (Chapman 1996). High salinity levels recorded at Flag Boshielo Dam are possibly due to agricultural activities taking place at the catchment of this impoundment, while the lowest levels recorded at Luphephe-Nwanedi Dams are possibly attributed to little land use practices within the catchment of these impoundments.

3.4.3 Nutrients and major ions

Natural factors such as weathering, erosion, precipitation and runoff variability influence the concentration of nutrients in water bodies (Dallas & Day 2004). In addition, excessive nutrients may emanate from sewage treatment works, effluents from industrial and agricultural activities as well as atmospheric deposition (De Villiers & Thiart 2007). The major nutrients that contribute to eutrophication of aquatic systems are phosphorus as phosphate ions (PO_4^{3-}), ammonium (NH_4^+) and nitrogen as nitrite (NO_2) and nitrate (NO_3^-) ions (Dallas & Day 2004). The eight ions present in larger quantities in most freshwaters include the cations of sodium (Na^+), potassium (K+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) and the anions such as chloride (Cl^-), sulphate (SO_4^{2-}), bicarbonate (HCO_3^-) and carbonate (CO_3^2) (Jooste *et al*. 2013).

Ammonium (NH_4^+) is the ionised form of ammonia and its toxicity is influenced by concentrations of DO, CO_2, TDS and the presence of metal ions (DWAF 1996a). An increase in pH can result in increased conversion of ammonium to toxic ammonia (Nyathi & Baker 2006), which can be very toxic and fatal to aquatic life, especially fish, and detrimental to the ecological homeostasis of water bodies (Chilundo *et al.* 2008). In this study, NH_4^+ concentrations recorded at all impoundments surveyed were within the recommended TWQR.

Nitrate (NO_3^-) ions are a common form of combined nitrogen found in natural waters that may be biochemically reduced to nitrite by the process of denitrification under anaerobic conditions (Svoboda *et al.* 1993). These ions may enter surface waters through industrial and municipal discharges leaching from waste disposal sites and landfills, as well as agricultural inorganic fertilisers, leading to nitrate concentrations of about 1 to 5 mg/L (Dallas & Day 2004). The main source of NO_3^- in natural waters is derived from the oxidation of plant and animal debris and excretions from humans and animals. Nitrate ion is not held by the soil particles and it is readily leached into the subsoil, draining into underground aquifers (Chapman 1996). According to Dallas and

Day (2004), NO_3^- is not normally toxic, however, high concentrations of above 0.50 mg/L can be toxic.

Dabrowski and De Klerk (2013) described Loskop Dam as the most polluted in the Olifants River due to the various land use activities taking place in the upper catchment of this impoundment. Thus, frequent algal blooms, including cyanobacteria have been reported at Loskop Dam by Oberholster *et al*. (2010; 2012), indicating conditions at Loskop Dam to be eutrophic to hypertrophic. For this reason, elevated levels of NO_3^- were expected. However, this did not occur. It may be assumed that NO_3^- ions were possibly absorbed by phytoplankton and/or deposited into sediment. Nitrate levels that exceeded the TWQRs at Flag Boshielo and Rhenosterkop dams can be related to the discharge of effluents from wastewater treatment plants situated close to and upstream of Flag Boshielo Dam and/or by agricultural runoffs along the Elands and Olifants rivers (De Villiers & Mkwelo 2009). Chapman (1996) stated that the soil in areas with natural vegetation often contain sufficient organic matter, which is a potential source of nitrate due to nitrifying bacterial activity in the soil. This can possibly explain the NO_3^- concentrations recorded above the TWQR at Phalaborwa Barrage, especially since the banks of this water body are flanked by an abundance of riparian vegetation and common reeds (*phragmites australis*) and the possible discharge of effluent from a nearby fertiliser plant.

Orthophosphates (PO_4^{3-}) are generally considered to be a primary nutrient limiting algal and plant growth in freshwaters (De Villiers & Thiart 2007). The PO_4^{3-} concentrations recorded at all the impoundments surveyed were above the TWQR. Similar to NO_3^-, elevated PO_4^{3-} can be attributed to high organic wastes in the water, which are then decomposed by bacteria, resulting in high biological oxygen demand (BOD) occurring within the impoundments. The high PO_4^{3-} concentrations recorded at Flag Boshielo Dam, can be attributed to runoff containing phosphate salts that are leached due to the weathering of rocks and from non-point sources such as drainage from fertilised agricultural lands (Heath *et al.* 2010; Dabrowski *et al.* 2014b).

Sulphate (SO_4^{2-}) is a natural constituent of water that arises primarily due to atmospheric deposition, sulphate mineral dissolution in soils and rock and sulphide mineral oxidation (Dallas & Day 2004). Anthropogenic sources of sulphate include coal mines, power generation plants, phosphate refineries and metallurgical refineries

(Miao *et al.* 2013). Elevated SO_4^{2-} concentrations recorded at Loskop Dam can be related to AMD from current and abandoned coal mines in the upper catchment of the Olifants River System (Dabrowski & De Klerk 2013; Lebepe *et al*. 2016). Since the year 2000, the annual median sulphate concentration at Loskop Dam has exceeded the 100 mg/L threshold specified for aquatic ecosystem health (Jooste *et al.* 2015), with levels expected to rise higher in future. Again, low SO_4^{2-} concentration recorded at Luphephe-Nwanedi Dams can be attributed to the absence of large mines in the catchment area of these impoundments.

Fluoride (F^-) originates from the weathering of fluoride containing minerals and enters surface waters through runoff and groundwater discharge (DWAF 1996a, Chapman 1996). Moreover, fluoride may enter surface waters by liquid and gas emissions from metal and chemical-based manufacturing (Chapman 1996). Due to chrome and steel smelters activities in and around cities of Witbank and Middelburg in the upper catchment of the Olifants River System (Ashton *et al.* 2001), one would expect high influx of fluoride into Loskop Dam. However, this was not observed, instead high fluoride concentration above the specified TWQR was recorded at Rhenosterkop Dam. A possible explanation for the high concentrations recorded can be due to natural processes such as leaching of fluoride in the ground waters or due to wind depositing large quantities of fluoride from factory emissions located in regions neighbouring the catchment of this impoundment.

3.4.4 Metals and metalloids in water column

Metals can enter surface waters through natural processes such as the weathering of igneous and metamorphic rocks and volcanic activity (Dallas & Day 2004). The main anthropogenic sources of metals are domestic sewage, industrial effluents, oil and chemical spills, agricultural runoff, mining and metallurgical activities (Dallas & Day 2004). According to Chapman (1996), more than 50% of total metals present in water are usually adsorbed by suspended particles. Thus, the undetectable concentrations of some metals in this study can be as a result of adsorption and accumulation of metals by suspended solids. There are currently no ideal thresholds available in terms of the TWQR for B, Ba, Na, P, Si, Sr and Ti.

Aluminium (Al) is one of the most common elements in the earth’s crust that is inversely correlated to pH (Crafford & Avenant-Oldewage 2010). Solubility of Al may be mobilised to aquatic environment under acidic (pH < 6) or alkaline (pH > 8) conditions (Mmualefe & Torto 2011). This element occurs primarily as alumina-silicate that are too insoluble to participate in geochemical reactions. Toxicity of Al in fish occurs through respiration where it interferes with the ionic and osmotic balance, leading to hypoxia due to the clogging of interlamellar spaces in fish gills (Dallas & Day 2004; Sara *et al.* 2014). It was expected that high levels of Al would be detected in Loskop and Flag Boshielo dams due to mining and industrial practices in the catchment of these impoundments. However, Al concentrations were below detection limits at these impoundments. It was inferred that Al concentrations may have been deposited into sediments. However, further sampling is necessary to establish if this is true. Elevated levels of Al in surface waters can also be linked to AMD and coal combustion (Svobodova *et al.* 1993). According to Oberholster *et al.* (2012) and Dabrowski *et al.* (2014a), benthic filamentous algae have the ability to accumulate high concentrations of metals. Elevated Al level above the TWQR recorded at Phalaborwa Barrage can be attributed to increased algal blooms, thus, Al concentrations may have been assimilated by phytoplankton in water samples.

Barium (Ba) is a metal that occurs in combination with other elements such as sulphur, oxygen and carbon (USEPA 2008). This metal can react with almost all non-metallic elements, forming poisonous substances that are toxic to organisms in both high and low concentrations (Lenntech 2009). Naturally, Ba originates primarily from natural sources and is present as a trace element in both igneous and sedimentary rocks (WHO 2004). A possible explanation of Ba concentrations detected in Luphephe-Nwanedi and Rhenosterkop dams can be that this metal occurred due to natural processes. Barium is also extensively used in mining, coal and oil combustion. Anthropogenic practices occurring in the upper and middle reaches of the Olifants River Catchment can possibly explain Ba levels recorded at Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage.

Boron (B) is a natural component of freshwaters arising from weathering of parent rocks, soil leaching and volcanic action (Chapman 1996). Anthropogenic sources of B in surface waters include industrial practices, municipal wastewaters, coal-fired electric power generation plants and AMD (Eisler 1990), and can possibly explain B concentrations detected at Loskop and Flag Boshielo dams. In addition, agricultural run-offs can contain B, particularly in areas where it is used to improve crop yields or as a pesticide (Hasenmueller & Criss 2013). Thus, elevated levels of B recorded at Rhenosterkop Dam can be attributed to effluents from agricultural runoff from citrus orchards along the Elands River.

Calcium (Ca) is present in all waters and it is readily dissolved from rocks rich in calcium minerals particularly as carbonates and sulphates (Chapman 1996). Calcium salts together with those of magnesium are responsible for water hardness and are considered buffering nutrients because they can influence pH levels of a water body (Bartram & Ballance 1996). Industrial and agricultural effluents, cement factories and wastewater treatment processes can contribute to Ca enrichment in surface waters (Dallas & Day 2004). Factors that can explain the concentrations recorded for Loskop, Flag-Boshileo and Rhenosterkop dams. Although water hardness was not measured in this study, higher concentrations of Ca in Phalaborwa Barrage is likely due to the hardness of the water or as a result of the mining activities.

Iron (Fe) is the fourth most abundant element that constitute 5% of the earth's crust and is naturally released into surface waters from the weathering of sulphide ores (FeS_2) and igneous, sedimentary and metamorphic rocks (DWAF 1996a, c). Under alkaline or neutral conditions, dissolved Fe decreases while under acidic conditions Fe is highly detectable (Akcil & Koldas 2006). Factors that may explain the Fe levels that were below detection in Loskop and Flag Boshielo dams water samples, and the low levels recorded at Luphephe-Nwanedi and Rhenosterkop dams, due to alkaline conditions that occurred at these impoundments. Anthropogenic sources of Fe also include mining, sewage effluent, landfill leachates and some industrial effluents (DWAF 1996a). Although pH values were also recorded to be alkaline at this impoundment, elevated concentrations of Fe recorded at Phalaborwa Barrage might be due to direct inputs from a phosphate mine (Foskor Ltd) at Phalaborwa and atmospheric deposits of dust containing this metal.

Magnesium (Mg) arises from the weathering of rocks containing ferromagnesium minerals (Chapman 1996). Magnesium plays an important role in energy transfer and it is required by plants to form chlorophyll-*a* (Horne & Goldman 1994). Potential sources of Mg in surface waters include runoff and leaching of fertilisers from agricultural fields and effluents from industrial activities (DWAF 1996b). Concentrations of Mg recorded at all sites were within the TQWR. However, the higher concentration recorded at Phalaborwa Barrage can be attributed to leaching of this metal from the riverbed substrate.

Manganese (Mn) is the eighth most abundant metal in nature and occurs in numerous ores. In aquatic ecosystems, Mn does not occur as a metal but in various salts and mineral forms in association with Fe (DWAF 1996a). Soils, sediments and metamorphic and sedimentary rocks are natural sources of Mn, while industrial effluents are anthropogenic sources (DWAF 1996a; Zaw & Chiswell 1999). In poorly oxygenated waters, Mn increases during the stratification of the water column. Elevated concentrations of Mn recorded at Loskop Dam can be attributed to industrial effluents containing Mn. Although biological oxygen demand (BOD) was not measured in this study, high traces of Mn in this impoundment may have been influenced by the BOD due to this impoundment's eutrophic conditions.

Phosphorus (P) is an essential macronutrient that plays a major role in the building of nucleic acids and use of energy cells in aquatic organisms. Phosphorus is also considered to be a principal nutrient controlling the degree of eutrophication (Dallas & Day 2004). Phosphorus can either be particulate bound or dissolved in water as organic phosphate (PO_4^3), orthophosphate (inorganic and dissolved P), total P (dissolved and particulate) and polyphosphates (Walmsley 2000). Naturally, P is derived mainly through weathering of phosphorus bearing rocks and the decomposition of organic matter (DWAF 1996a). According to Chilundo *et al.* (2008), phosphorus enters surface waters through agricultural runoff and industrial, mining effluents and sewage effluent. The average inorganic P concentrations (in mg/L) used to classify the trophic status of a water body is shown below (DWAF 1996a; Dallas & Day 2004).

< 0.005 mg/L - Oligotrophic conditions
0.005 – 0.025 mg/L - Mesotrophic conditions
0.025 – 0.25 mg/L - Eutrophic conditions
> 0.25 mg/L - Hypertrophic conditions

High concentrations of P recorded at Flag Boshielo Dam and Phalaborwa Barrage of between 0.025 to 0.25 mg/L indicated that these impoundments were eutrophic (Dallas & Day 2004) at the time of sampling. Alternatively, levels detected at Loskop, Luphephe-Nwanedi and Rhenosterkop dams indicated that these impoundments were less impacted in terms of phosphorus. In this case classifying these impoundments to be oligotrophic.

Silicon (Si) exists in water in a dissolved, suspended and colloidal state. It is an essential element for certain aquatic plants and is taken up during cell growth and released during decomposition and decay that can give rise to seasonal fluctuations in aqueous concentrations (Chapman 1996). This element may be discharged into water bodies from industries using siliceous compounds such as glass works (Chapman 1996). Due to high industrial activities occurring in the upper catchment of the Olifants River System, one would expect higher concentrations of Si to be recorded in Loskop Dam. However, this was not detected. High concentrations of Si recorded at Flag Boshielo Dam is possibly a result of decay of certain plants in this impoundment.

Sodium (Na) exists mainly as a bicarbonate occurring in soil at biologically toxic concentrations (Mmualefe & Torto 2011). Common anthropogenic sources of Na include industrial effluents and agricultural runoffs into water bodies. Concentrations of Na recorded at all impoundments fell within permissible TWQR. However, high Na concentrations at Rhenosterkop Dam are possibly due to agricultural runoff into the Elands River.

Strontium (Sr) is a non-radioactive element naturally occurring in rocks, soil, dust, coal and oil (Irwin *et al.* 1997). This element forms about 0.034% of all igneous rock and in the form of the sulphate mineral celestite ($SrSO_4$) and the carbonate strontianite ($SrCO_3$). Strontium compounds are used in making ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, and medicines (Irwin *et al.* 1997). High concentrations of Sr at Luphephe-Nwanedi Dams can be attributed to this metal occurring naturally within the catchment and main stem of this system.

Titanium (Ti) is one of the most abundant chemical elements in the earth's crust present in rocks, soils and bottom sediments of water bodies (Linnik & Zhezherya 2015). Among the most common minerals containing Ti is titanium dioxide (TiO_2) which is a compound widely used in the manufacturing of pigments, production of nanomaterials and as nanotubes in wastewater treatment (Linnik & Zhezherya 2015). Elevated levels of Ti recorded at Phalaborwa Barrage can be explained by the rock formation surrounding this impoundment. Although comparatively lower levels were detected in Loskop and Flag Boshielo dams, these levels can be attributed to wastewater treatment works located within the catchment areas of these two impoundments.

Zinc (Zn) is an essential micro-nutrient for all organisms. It occurs through natural processes such as weathering and in rocks and is readily refined into a pure stable metal (DWAF 1996a). Anthropogenic sources of Zn include industrial wastes, pharmaceuticals, fertilizers and insecticides. Zinc concentrations recorded at the five impoundments were above the TWQR. This can be attributed to natural processes and anthropogenic factors in the catchment of each impoundment.

3.4.5 Metals in sediments

Sediments are regarded as a sink for a wide range of contaminants such as metals concentrations that are far higher than those of the overlying water (De Klerk *et al.* 2016). The physical properties of a sediment such as grain size play an essential role in transportation and sedimentation processes (De Klerk *et al.* 2012). The finer the grain size, the higher the surface area of a specific sediment particle, which increases its ability to adsorb metals (De Klerk *et al*. 2012). According to Wepener and Vermeulen (2005), sediment particle sizes can be classified into the following categories: mud (smaller than 53 µm), fine sand (53 – 212 µm), medium sand (2121 – 500 µm), coarse sand (500 – 2000 µm), very course sand (2000 – 4000 µm) and gravel (larger than 4000 µm). Although sediment particle size was not measured in this study, one can assume that the grain size of sediments from the impoundments surveyed was fine due to high metal concentrations detected. Metals introduced into sediments are enriched directly from geological weathering and retained by clay particles in the soil (Binning & Baird 2001). High traces of Mg detected in sediment samples from Phalaborwa Barrage may be due to a residual build up in the sediment overtime (De Klerk *et al.* 2013).

Elevated levels of Ba, Cd, Co, Er, Fe, Ga, Nb, Ni, P, Pb, Pd, Sm, Sn, U, V, W, Y, Yb and Zn in sediments from Flag Boshielo Dam are likely due to the solubility of these metals and because the residuals of these metals have accumulated in this impoundment over time. Moreover, as water of Loskop Dam flow into Flag Boshielo Dam, most metals precipitates into the sediments as substrates hydroxides (Akcil & Koldas 2006). Similar results were observed in a study by De Klerk et al. (2016), where high concentrations of Al, Fe, As, Fe, Pb, Ni and V were recorded in sediment samples from the upper Olifants River. High levels of As, K, Rb, Sb, Si, Tl and Zr detected in the sediment of Loskop Dam is attributed to AMD, mining and industrial effluents, power generation plants and leachates from landfill sites in the upper catchment. Similarly, the study by De Klerk *et al.* (2013) and De Klerk *et al.* (2016) also showed that the input of acid and polluted water from the associated land use activities in the upper Olifants River was observed to have affected the sediment quality thereof.

Since the catchment area of Luphephe-Nwanedi Dams is dominated by nature conservation areas with little mining and industrial activities occurring, high concentrations of Cu, La, Na, Pb and Ti detected in sediment sampled from these impoundments can be attributed to natural weathering of the underlying substrate.

3.5 Results for monthly surveys conducted at Flag Boshielo Dam

In April 2016, water levels in Flag Boshielo Dam were reported by the Department of Water Affairs (DWA) to be at around the 50% mark, decreasing to approximately 18% in November the same year. Subsequent rainfall during December 2016 and January 2017 in the catchment and surrounding areas raised levels in Flag Boshielo Dam to 43.1%. At the time of writing, the capacity of Flag Boshielo Dam was rated by DWA to be normal to moderately high as opposed to the very low classification received during the previous year. This fluctuation in water levels provided an opportunity to collect samples under a wide range of environmental conditions at Flag Boshielo Dam. Thus, during this study low inflow in terms of water levels into Flag Boshielo Dam occurred during April, May, June, August, September, October and December 2016, while higher levels and inflow occurred during February 2016 and February 2017. The fluctuation in the impoundment's water level during this study is depicted in Figure 3.1.

3.5.1 Water temperature, dissolved oxygen and pH

Seasonal fluctuations in water temperatures showed the expected low winter and high summer temperatures indicative of cold and warm periods respectively (Table 3.2). Dissolved oxygen (DO) concentrations (mg/L) showed no distinct trend, however, mean values recorded in April 2016 were higher than those recorded during the other months surveyed. Concentrations of DO were adequate and fell within the recommended TWQR limits during summer with higher levels above the TWQR recorded during winter. There was a highly significant difference in both temperature and DO between the months surveyed (p < 0.0001). Throughout the study, alkaline conditions persisted (pH 8.03 – 11.68), with higher pH levels recorded above the TWQR limits during warm periods. With regard to water levels, temperature readings were relatively unstable between low and high inflow periods, while DO and pH values were shown to be higher and above the specified TWQR during most months with low inflow as opposed to periods having higher inflow.

3.5.2 Total dissolved solids (TDS), electrical conductivity (EC) and salinity

The average TDS and EC showed a highly significant variation between the months surveyed (p < 0.00001) with TDS values fluctuating between low and high inflow periods. Higher EC values were recorded during the period of low inflow with lower values recorded when dam levels were higher. Similarly, salinity levels increased slightly when inflow and water levels were low (Table 3.2).

3.5.3 Nutrients and major ions

Nutrients concentrations in this impoundment i.e. NH_4^+, NO_3^- and PO_4^{3-} were not significant (p > 0.05) between the months surveyed. Ammonium concentrations fluctuated between high and low inflow periods with levels at times indicating eutrophic conditions during low inflow period. Conversely, NO_3^- concentrations which ranged from 0.33 to 1.44 mg/L were not significant (p > 0.05) between the months surveyed were a standard error could be obtained from the DWA data base. Concentrations of PO_4^{3-} were relatively unstable throughout the sampling period, with high concentrations recorded in June 2016 (Table 3.2).

Although sulphate levels were adequate and within the TWQR limits, they exhibited insignificant (p > 0.05) differences between the months surveyed, with high concentrations recorded in most months with low inflow. Traces of Cl^- and F^- were not significant (p > 0.05) between the months surveyed. Chloride concentrations showed a distinct trend, with high concentrations recorded in September. Fluoride fluctuated with a change in inflow levels with higher concentrations recorded mostly during periods of low inflow. Traces of Cl^- and F^- fell within the permissible TWQR throughout the sampling period.

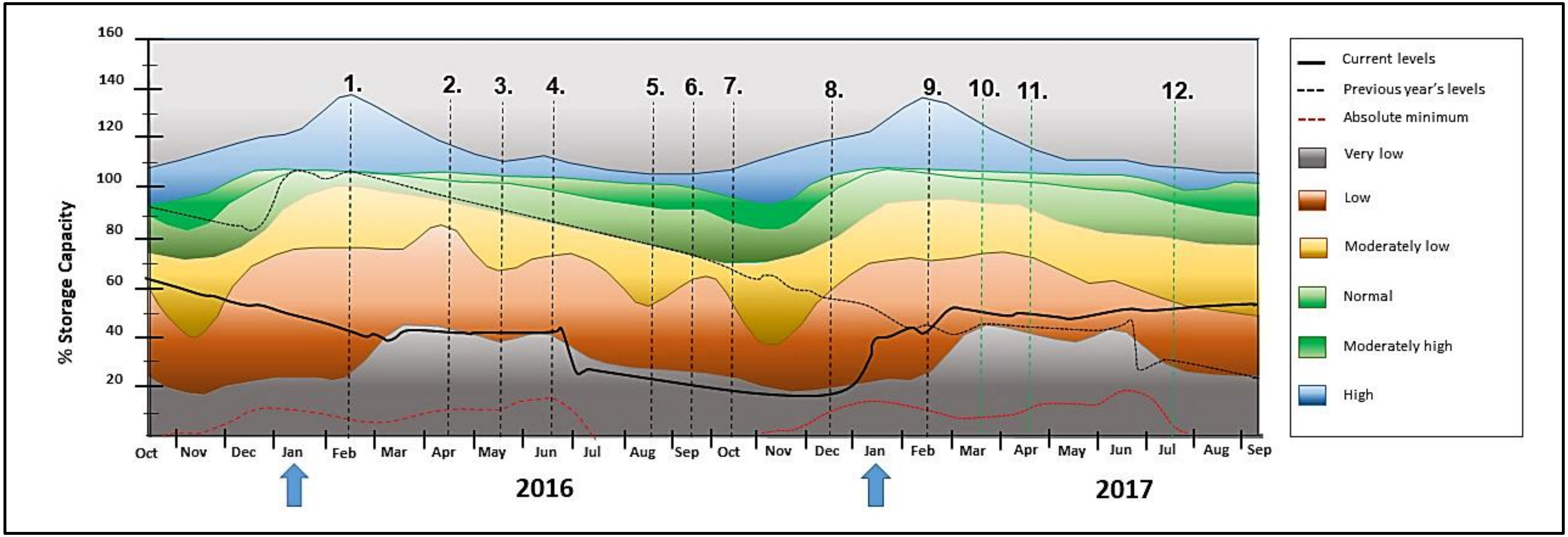

Figure 3.1: The change in storage capacity (%) of Flag Boshielo Dam between October 2016 and September 2017. The period undertaken for this study is indicated by arrows while the various surveys are indicated by dashed black lines and numbers 1 to 9. The continues black line on the graph is an indication of the current storage capacity (%) while the brown dotted line is the previous year's level for the same period. (Source:https://www.dwa.gov.za/hydrology/weekly/percentile.aspx?station = B5R002; Accessed September 2016 and August 2017).

3.5.4 Metals and metalloids in the water column

Total trace metals were not significant ($p > 0.05$) between periods of high and low inflow, with elevated concentrations of most metals recorded during the low inflow period (Appendix B: Table B2). Aluminium fluctuated within a narrow range above the permissible TWQR limits in February 2017, and was recorded to be below detection limits during the other months surveyed. Concentrations of Ca, K, Mg, Na and Sr showed a distinct trend between the months surveyed, with higher levels detected in December compared to the other months surveyed. Iron (Fe) concentrations exceeded the specified TWQR during the low inflow period. Manganese (Mn) concentrations were below detection during the high inflow periods, while in October 2016 Mn levels were recorded to be higher and above the specified TWQR. Similarly, sodium (Na) concentrations were high in October 2016 and exceeded the TWQR. Phosphorus recorded during the period of low inflow indicated oligotrophic conditions with levels recorded during high inflow indicating eutrophic conditions. Concentrations of Si fluctuated, with low levels of this element recorded during October 2016 and high during February 2017. Trace levels of Sr and Ti were detected to be high in October 2016 when compared to other months surveyed. Traces of Zn were above the permissible TWQR except for February 2017 (Figure 3.2).

3.5.5 Metals and metalloids in sediments

In this study, similar to other studies, metal content in sediment were much higher than those in water for the same metals. Most metals except for Eu, Hf, Ho and Tb exhibited significant ($p < 0.05$) differences between the months surveyed. It was recorded that sediment collected during low inflow periods had significantly ($p < 0.05$) higher concentrations of Al, Ba, Ca, Ce, Cs, Dy, Ga, Ge, Er, Eu, Gd, Ho, K, Mg, Mn, Na, Nb, Ni Nd, P, Pb, Pr, Rb, Si, Sm, Sr, Th, U, V and Y (see Appendix B: Table B2). Conversely, Be, Cs, Er, Eu, Ge, Li, Mo, Se, Sn, Tb, U, W and Yb showed higher values with regard to their concentrations during all surveys. Traces of As, B, Be, Co, Cr, Cu, Li, Fe, Hf, La, Sc, Sn, Ti and Zn were detected to be higher during the high inflow period, when compared to other surveys conducted (Figure 3.3).

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Table 3.2: Means and standard errors of physico-chemical variables measured at Flag Boshielo Dam during monthly surveys. Shaded values indicate where the TWQR guidelines have been exceeded. Months highlighted indicate periods of higher water levels and inflow.

Variable	Month																		
	February '16		April '16		May '16		June '16		August '16		September '16		October '16		December '16		February '17		TWQR
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Temperature °C	24.66	0.44	25.55	0.18	23.92	0.91	17.62	0.13	16.12	0.14	20.92	0.11	21.88	0.22	26.53	0.23	26.40	0.00	-
DO (mg/L)	8.40	0.61	**10.93**	0.17	**9.64**	0.68	**9.45**	0.39	7.64	0.13	**10.00**	0.31	5.38	0.55	6.74	0.20	5.28	0.00	6.0 – 9.0
pH	**9.04 – 9.88**		**9.23 – 9.66**		9.04 – 9.68		8.83 - 9.49		8.83 – 9.35		**9.14 – 9.57**		8.53 – 9.04		8.23 – 11.38		8.03 – 9.11		6.6 – 9.0
EC (µS/cm)	570.00	5.55	590.00	3.02	584.33	5.83	528.74	8.28	588.11	36.48	572.56	1.54	612.75	6.78	712.42	13.25	434.30	0.00	-
TDS (mg/L)	379.46	3.63	369.98	0.54	379.47	3.63	404.81	8.74	466.17	27.28	403.00	0.00	422.50	3.39	450.05	6.54	275.60	0.00	-
Salinity (ppt)	0.29	0.00	0.29	0.00	0.29	0.00	0.30	0.00	0.35	0.02	0.30	0.00	0.32	0.00	0.34	0.01	0.20	0.00	-
Ammonium (mg/L)	**1.10**	0.48	0.05	0.00	**0.39**	0.00	0.05	0.00	**0.31**	0.14	0.18	0.00	**0.91**	0.86	0.05	0.00	**1.10**	0.48	0.2*
Nitrate (mg/L)	0.33	0.25	0.05	0.00	0.11	0.00	**0.76**	0.71	**0.92**	0.53	0.05	0.00	**1.45**	0.62	**0.45**	0.00	**0.33**	0.25	0.2
Ortho-phosphates (mg/L)	0.01	0.00	0.08	0.00	0.01	0.00	0.09	0.00	**0.23**	0.19	0.01	0.00	0.03	0.02	0.04	0.00	0.01	0.00	0.1*
Sulphate (mg/L)	65.43	31.92	93.20	0.00	91.40	0.00	98.40	0.00	50.20	47.50	99.00	0.00	49.30	48.00	2.30	0.00	2.30	0.00	100**
Chloride (mg/L)	31.10	10.85	42.60	0.00	43.30	0.00	44.70	3.10	25.75	16.25	48.70	0.00	28.75	19.05	8.40	0.00	-	-	600
Fluoride (mg/L)	0.70	0.10	0.70	0.00	0.67	0.00	0.91	0.01	0.43	0.27	0.44	0.00	0.47	0.22	0.62	0.00	-	-	0.75

Key: SE = standard error, TWQR = target water quality range for South African water quality guidelines for aquatic ecosystems (DWAF 1996a) * = World Health Organisation Guidelines (1984), ** = Canadian Guidelines (2012).

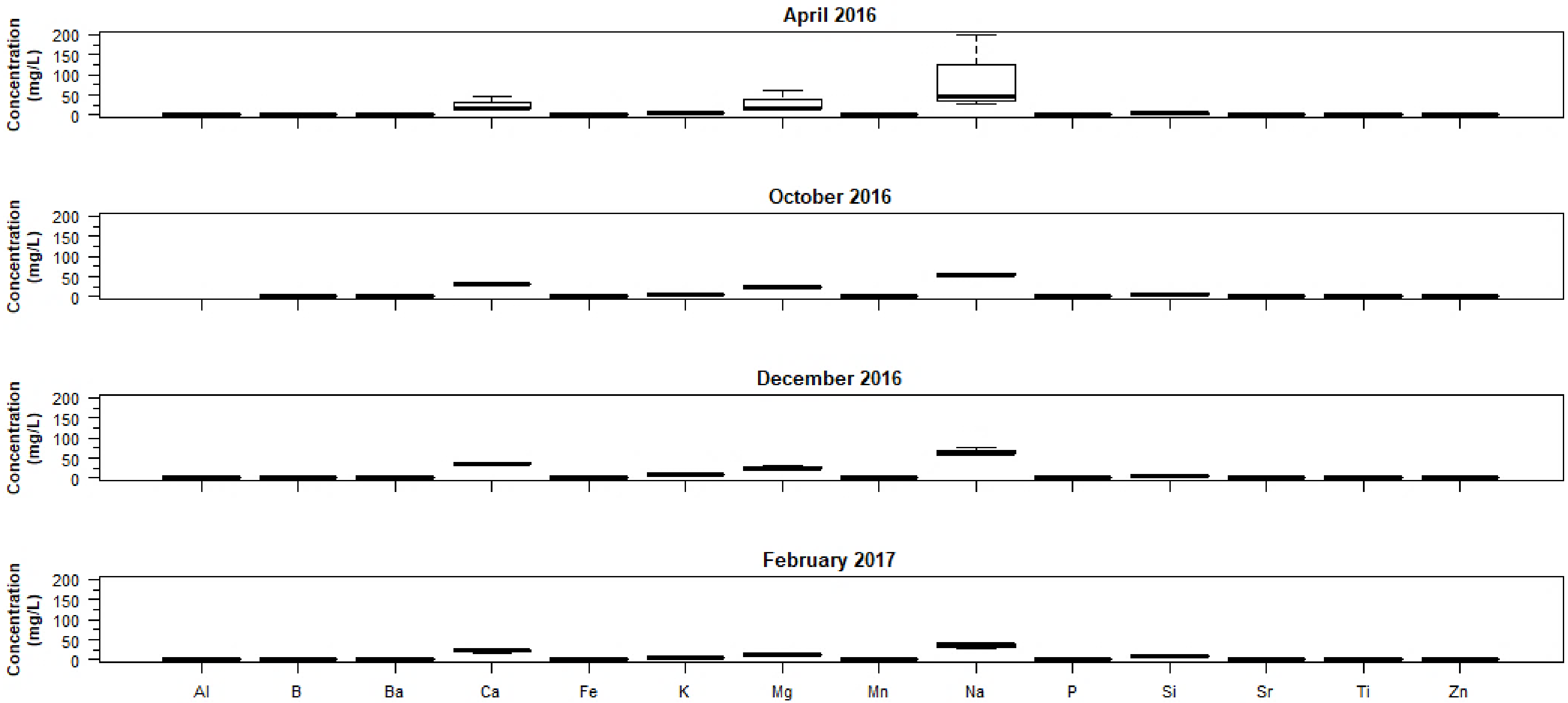

Figure 3.2: Metals detected in water samples collected from Flag Boshielo Dam in April, October, December 2016 and February 2017. For display purposes, values have been transformed using the 4th root.

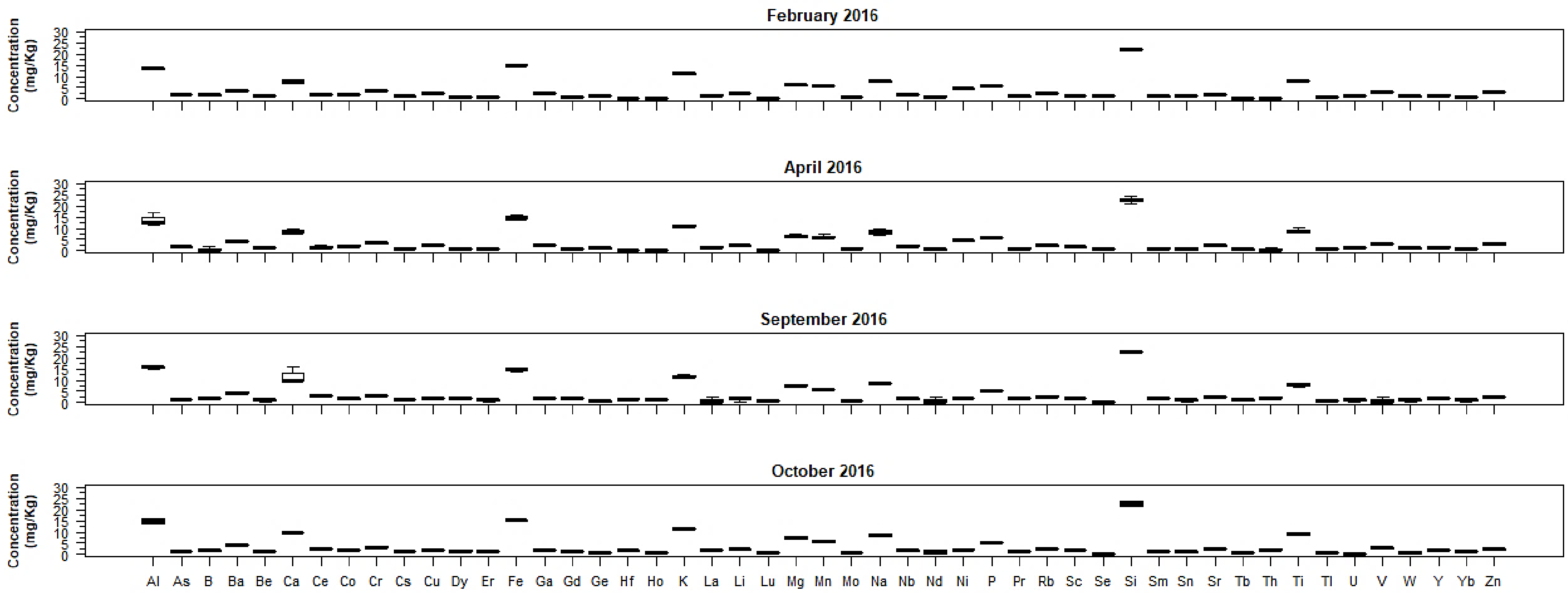

Figure 3.3: Boxplots of metals in sediment samples collected from Flag Boshielo Dam in February, April, September and October 2016. Values have been transformed using the 4th root.

3.6 Discussion for monthly surveys conducted at Flag Boshielo Dam

3.6.1 Water temperature, dissolved oxygen (DO) and pH

Seasonal fluctuations in water temperature exhibited thermal stratification and water mixing during winter. Thermal stratification occurs due to increasing air temperature that exceeds the water temperature (Manasrah *et al.* 2006). Conversely, a decrease in air temperature leads to a loss of buoyancy and causes the density of the surface waters to pass a critical threshold, after which vertical convection occurs and becomes dominant during winter (Manasrah 2002). There existed an inverse relationship between temperature and DO, whereby higher DO concentrations were recorded during colder periods and lower levels during warmer periods. Higher DO concentrations recorded during the colder months can be attributed to shallower waters and a higher turnover of the water column by wind actions in deeper waters. Conversely, lower DO concentrations can be attributed to higher water temperatures that influence mixing of the water column, a decrease in photosynthesis by phytoplankton (Bartram & Ballance 1996) and high biological oxygen demand (BOD) emanating from a high influx of nutrients and high inflow of water into the system. Alkaline conditions recorded during months with low inflow can possibly be attributed to excessive algal blooms, brought about by nutrients enrichment and the utilisation of available CO_2 whereby carbonic acid content is reduced. However, the effect and contribution of phytoplankton remains speculative as phytoplankton concentrations were not measured in this study.

3.6.2 Total dissolved solids (TDS), electrical conductivity (EC) and Salinity

High concentrations of TDS recorded may indicate that routinely measured TDS concentrations of Loskop Dam are a major contributor of dissolved and organic compounds into Flag Boshielo Dam (Dabrowski & De Klerk 2013). Although phytoplankton was not measured in this study, elevated TDS concentrations during summer months were possibly due to the occurrence of phytoplankton blooms which are always associated with lower levels of dissolved oxygen given that most water measurements were taken within a couple of hours after sunrise (Osman & Kloas 2010). Similarly, elevated EC concentrations which indicated electrolyte rich water in this impoundment can be attributed to the discharge of domestic effluents i.e. Motetema sewage treatment plant which is functional and located in one of the tributaries that feed into Flag Boshielo Dam. In addition, during high inflow water containing silt and nutrients may flow into Flag Boshielo Dam from Loskop Dam and the Elands River, thus increasing TDS and EC levels. Salinity showed no significant (p > 0.05) seasonal variation and did not affect changes in water density of Flag Boshielo Dam.

3.6.3 Nutrients and major ions

Concentrations of NH_4^+, NO_3^- and PO_4^{3-} which are commonly associated with sewage input resulted in eutrophication during summer months. This was further supported by alkaline conditions during these months. One of the main point sources of such nutrients is the release of fertilisers from agricultural practices. The runoff containing dissolved nutrients from this type of activities tends to occur intermittently, and thus nutrient concentrations in runoff are often at their highest during high runoff periods when the application of fertilisers and rainfall are high (De Klerk *et al.* 2012). In this study higher levels of NH_4^+ were generally recorded when levels were high. The high levels observed in October can be attributed to water levels decreasing to such a degree that the metabolic waste from the ichtyofauna and biological mass within the system become concentrated. Reasons for the levels of NH_4^+, NO_3^- and PO_4^{3-} recorded during monthly surveys in this study.

Moreover, not all waste water treatment plants in the catchment of Flag Boshielo Dam function optimally, and as a consequence can be contribute to high inputs of effluents rich in nutrient discharged into this impoundment (Dabrowski *et al.* 2014a). Elevated concentrations of these nutrients in surface waters can create high BOD and the accumulation of metabolic products as well as increased algal blooms (Codd 2000; Osman & Kloas 2010). In contrast, low nutrient concentrations can be attributed to high productivity and the depletion of NH_4^+, NO_3^- and PO_4^{3-} (De Villers 2007).

The influence of current and especially abandoned coal mines is reflected by the steady increasing in sulphate concentrations (Oberholster *et al.* 2010). Flag Boshielo Dam is susceptible to the same impacts affecting water quality in the upper catchment, therefore the SO_4^{2-} concentrations recorded are likely to be due to land use activities occurring in the upper Olifants River System. According to Lebepe *et al.* (2016), the annual median of SO_4^{2-} concentrations at Flag Boshielo Dam is approaching the 100 mg/L threshold for aquatic ecosystem health. This is true as in this study SO_4^{2-}

concentrations recorded for April, May, June and September were found to be above 90 mg/L.

Day and King (1995) reported the waters of the Olifants River System to be dominated by Ca, Mg and to a lesser extent, Na and bicarbonate anions. Geological strata are known to contribute to the increase in cations and anions through groundwater discharge. For example, limestone form efficient aquifers that introduce minerals such as Mg, while metamorphic or igneous rocks contribute to Na, K, SO_4^{2-} and Cl^- concentrations (De Klerk *et al.* 2012). Moreover, elevated levels of these ions can be possibly due to reduced absorption by plants and their release from partially uncovered bottom deposits (Potasznik & Szymczyk 2015). This may explain high concentrations of Cl^- recorded during September 2016. High concentrations of these ions can also be attributed to natural processes such as leaching caused by weathering of riverbed substrate. Alternatively, a decrease in water column concentrations of these ions can be due to their uptake and retention by macrophytes and/or because they settle and deposit in the bottom substrate during periods of low inflow only to be re-suspended when water levels increase.

3.6.4 Metals and metalloids in the water column

According to Kotze *et al.* (1999), metal concentrations in water tend to be diluted by higher flows during rainy seasons. This phenomenon, was also observed in this study as low concentrations of metals were recorded during period of high inflow when compared with period of low inflow. Elevated levels of metals recorded during period of low inflow may be an indication that these metals were concentrated in the water as the riverbed dried up when there was no rainfall (De Klerk *et al.* 2012). Since Al was below detection limit in samples collected during April, October and December 2016, it is unlikely that this metal may have been derived from land use activities in the catchment of this impoundment. However, it is necessary to establish whether Al levels recorded in February 2017 can be attributed to non-point contamination upstream or due to leaching caused by the weathering of the riverbed.

3.6.5 Metals and metalloids in sediment

During dry seasons, the rise in temperature and evaporation causes metals to be concentrated in sediment (Davies *et al.* 2006). This phenomenon may explain the high concentrations of metals detected in sediment samples collected during a period of low inflow. Moreover, high concentrations of metals recorded during this period would suggest that there is a residual build-up of metals in sediment over time in Flag Boshielo Dam. Diffusion of contaminants takes place naturally when the concentration in the overlying water is less than that of the underlying water (De Klerk *et al.* 2012). During periods of low rainfall and drought, large areas of previously submerged sediment can be exposed to the atmosphere and become desiccated, whereby nutrients dynamics, microbial communities and the oxidative state of elements are affected (Osman & Kloas 2010; Dabrowski *et al.* 2017). Consequently, the rewetting of sediments can result in acidification and eutrophication (Dabrowski & De Klerk 2013; Dabrowski *et al.* 2017). Therefore, low accumulation of metals during high inflow period suggest that the concentration of metals in the bottom substrate was brought about by changes in water flow.

3.7 Conclusion

During the current study, from the various impoundments surveyed physico-chemical variables such as pH, TDS and EC, nutrients and some aqueous and sediment metals were found to vary the most. Variations recorded were mainly attributed to underlying geology and land use activities within the catchments of the impoundments. These results revealed an increasing trend between the impoundments in terms of good to poor water quality in the order of: Luphephe-Nwanedi Dams **<** Rhenosterkop Dam **<** Loskop Dam **<** Phalaborwa Barrage **<** Flag Boshielo Dam. The Luphephe-Nwanedi Dams were less impacted and found to be oligotrophic in terms of water variables measured, with this study's findings agreeing with those of Madanire-Moyo *et al.* (2012) and the classification by DWS (2017). Conditions at Rhenosterkop Dam were also observed to be oligotrophic, and are in agreement with the DWS (2017) classification of this impoundment. Although Flag Boshielo Dam appeared to be more eutrophic in terms of the abovementioned variables measured, no significant ($p > 0.05$) difference was detected when compared with water quality variables of Loskop Dam and Phalaborwa Barrage. Findings in this study for these impoundments did not agree with classifications provided by the DWS (2017), which classified Loskop Dam to be eutrophic and Flag Boshielo Dam to be oligotrophic. Findings based on phosphorus concentrations revealed Flag Boshielo Dam and Phalaborwa Barrage to be eutrophic and Loskop Dam to be mesotrophic during the time of sampling.

For monthly surveys conducted at Flag Boshielo Dam, it appeared that the physico-chemical variables and metal levels in water and sediment samples fluctuated due to seasonality and a change in water levels. Conditions during low inflow periods were considered to be eutrophic in terms of the nutrients content and the major ions and metals in water and sediment. The degree by which water and sediment conditions influence the health of *Oreochromis mossambicus* collected from these impoundments is examined in the following chapter.

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CHAPTER 4: EVALUATING THE HEALTH OF *OREOCHROMIS MOSSAMBICUS* BASED ON THE HEALTH ASSESSMENT INDICES IN CONJUNCTION WITH BLOOD GLUCOSE, THE K, HSI, GSI, GILL HISTOPATHOLOGY AND METAL TISSUE ANALYSES

4.1 Introduction

Biomonitoring is a method ideal for evaluating water quality and has become fundamental at investigating pollutants and their impact in aquatic ecosystems (Parmar *et al.* 2016; Nachev & Sures 2016). This method involves the use of bioindicators such as fish since they are constantly exposed to the surrounding medium (Bain *et al.* 2000). Biomonitoring is based on the assumption that environmental and anthropogenic impacts will influence changes in the community structure, abundance or diversity of the aquatic biota (Karr & Chu 1998). Furthermore, biomonitoring helps overcome the shortcomings associated with only monitoring the chemical and physical properties of water (Harris & Silveira 1999; Gerber *et al.* 2017). Although a biomonitoring approach has great advantages in evaluating aquatic ecosystems, there are also certain disadvantages that may include, for example, that some methodologies require specialised equipment and expertise to subjectively interpret results (Taylor *et al.* 2007). Therefore, the appropriate test to use is dependent on the aims and objectives of the study and the ecological state of the research area (Resh 2008).

In aquatic systems fish are preferred for use as bioindicators because they are long-lived and occupy various trophic levels (Whitfield & Elliot 2002). As a consequence, fish can assimilate pollution over long periods and upon analyses of body tissue content provide an indication of the extent and impact pollution has at different trophic levels within a system (Whitfield & Elliot 2002; Dalzochio & Gehleng 2016). Advantages of using fish as bioindicators have been highlighted in Chapter 1. Commonly used indicators and biomarkers to assess fish health and, in turn, the water quality of impoundments are haematological parameters, condition and somatic indices, histopathological assessments and the health assessment index (Adams *et al.* 1993; Adams & Greeley 2000; Crafford & Avenant-Oldewage 2009). To determine which indices or a combination thereof best identifies fish health, this chapter comprises two sections as in Chapter 3. The first section focused on evaluating fish health based on once off surveys conducted at Luphephe-Nwanedi, Rhenosterkop, Loskop, Flag Boshielo dams and Phalaborwa Barrage, while the second section focused on evaluating fish health based on monthly surveys at Flag Boshielo Dam. This was achieved by assessing the health of *Oreochromis mossambicus* sampled from the five impoundments using the health assessment indices (HAI) in conjunction with blood glucose levels, the condition factor (K), hepatosomatic index (HSI), gonadosomatic index (GSI), arithmetic mean thickness of gill epithelium (H_{ar}) and metal tissue analyses. The effects of seasonal fluctuations of dam levels, water and sediment quality on *O. mossambicus* from Flag Boshielo Dam were determined using the above-mentioned techniques.

4.2 Materials and methods

The methodology used to assess and analyse fish health is provided in Chapter 2.

4.3 Biological results for once off survey

4.3.1 Blood glucose concentrations

Blood glucose readings taken during the once off survey indicated that glucose levels in fish from the various impoundments were significantly ($p < 0.0001$) different between impoundments, with the highest blood glucose levels recorded for fish sampled from Flag Boshielo Dam and the lowest for specimens from Phalaborwa Barrage (Figure 4.1).

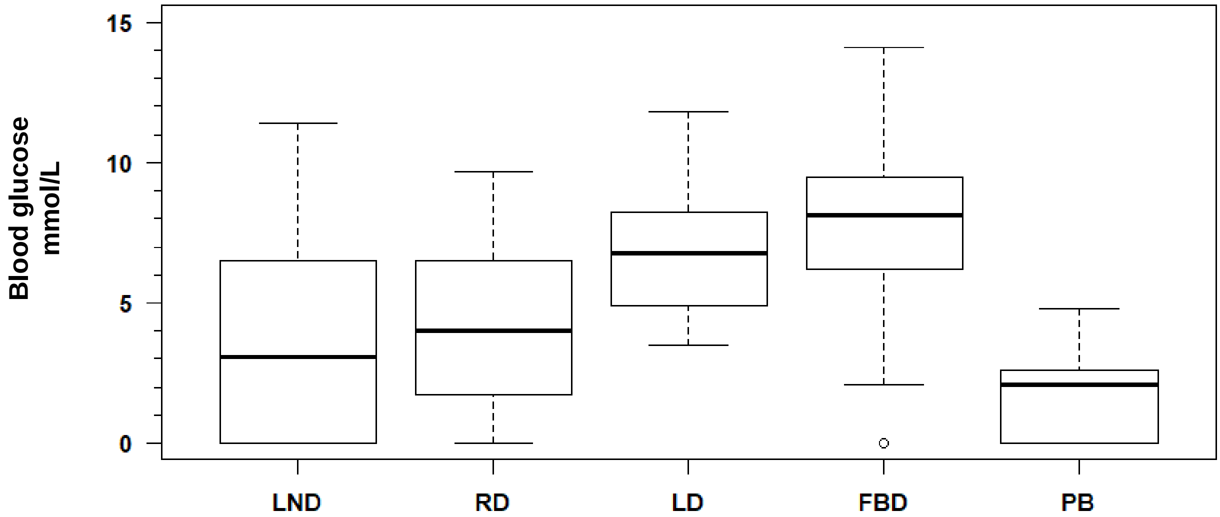

Figure 4.1: Blood glucose concentrations (mmol/L) of fish sampled at Luphephe-Nwanedi (LND), Rhenosterkop (RD), Loskop (LD), Flag Boshielo (FBD) dams and Phalaborwa Barrage (PB) during once off surveys conducted during April and May 2016.

4.3.2 Health Assessment Index

The HAI scores of fish varied significantly (p < 0.0001) between impoundments surveyed, with higher HAI scores recorded for fish collected at Flag Boshielo Dam than the other impoundments surveyed. Similarly, when using HAI with PI and IPI fish health scores varied significantly (p < 0.0001) between the impoundments surveyed (Figure 4.2).

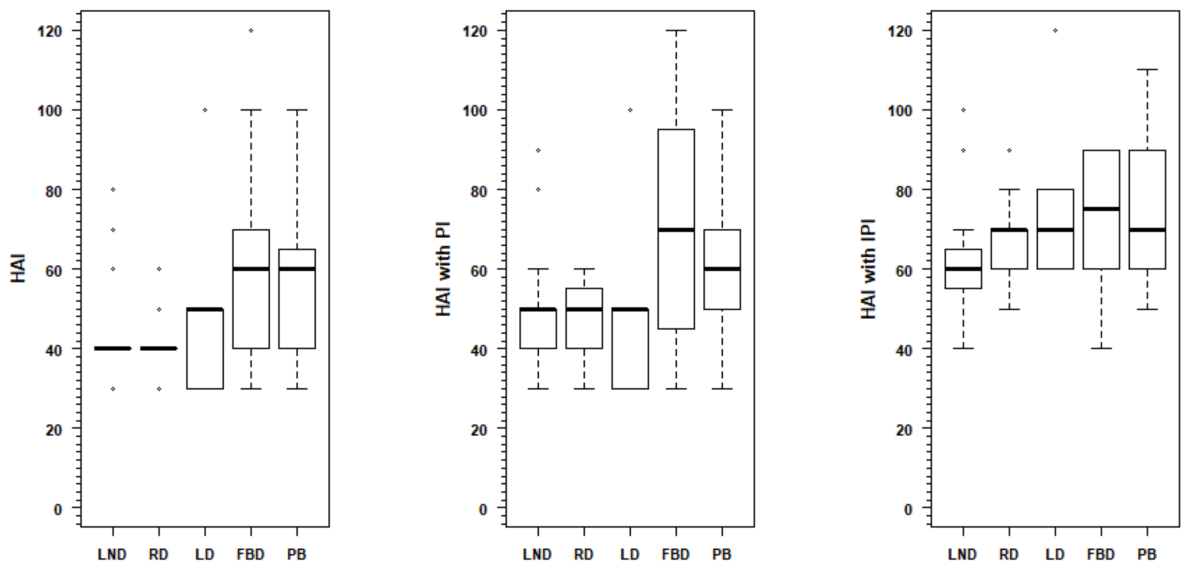

Figure 4.2: Box and whisker plots of HAI, HAI with PI and HAI with IPI scores for fish sampled at Luphephe-Nwanedi (LND), Rhenosterkop (RD), Loskop (LD), Flag Boshielo (FBD) dams and Phalaborwa Barrage (PB) during a once off survey conducted at each impoundment between April and May 2016.

The abnormalities that contributed to the HAI scores for *O. mossambicus* were mostly associated with the fins, skin, eyes, gills, liver, haematocrit and the presence of endo and ectoparasites (Figure 4.3). Eroded fins were prevalent in fish sampled from Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage. Skin aberrations were prevalent in fish from Flag Boshielo Dam. Four percent of fish from Luphephe-Nwanedi Dams were recorded to have eyes that were abnormal, while 10% of fish collected from Loskop and Flag Boshielo dams had signs of bilateral exophthalmos. The most common anomalies observed on the gills included deformed and pale gills in fish sampled from Flag Boshielo Dam and Phalaborwa Barrage. In general, fish sampled from all the five impoundments had dark brown livers, with fish from Loskop Dam having fatty livers with focal discolorations and dark spots. It was observed that 60% of fish from Phalaborwa Barrage had abnormalities that led to higher values when scoring haematocrit readings when compared with fish from the other impoundments. Eighty percent of fish from Rhenostekop Dam were infested with endoparasites when compared with fish from the other impoundments. High ectoparasite numbers were recorded in fish sampled from Luphephe-Nwanedi Dams, while fish from Loskop Dam had the lowest ectoparasite numbers (see Appendix C; Table C1, C2, C3).

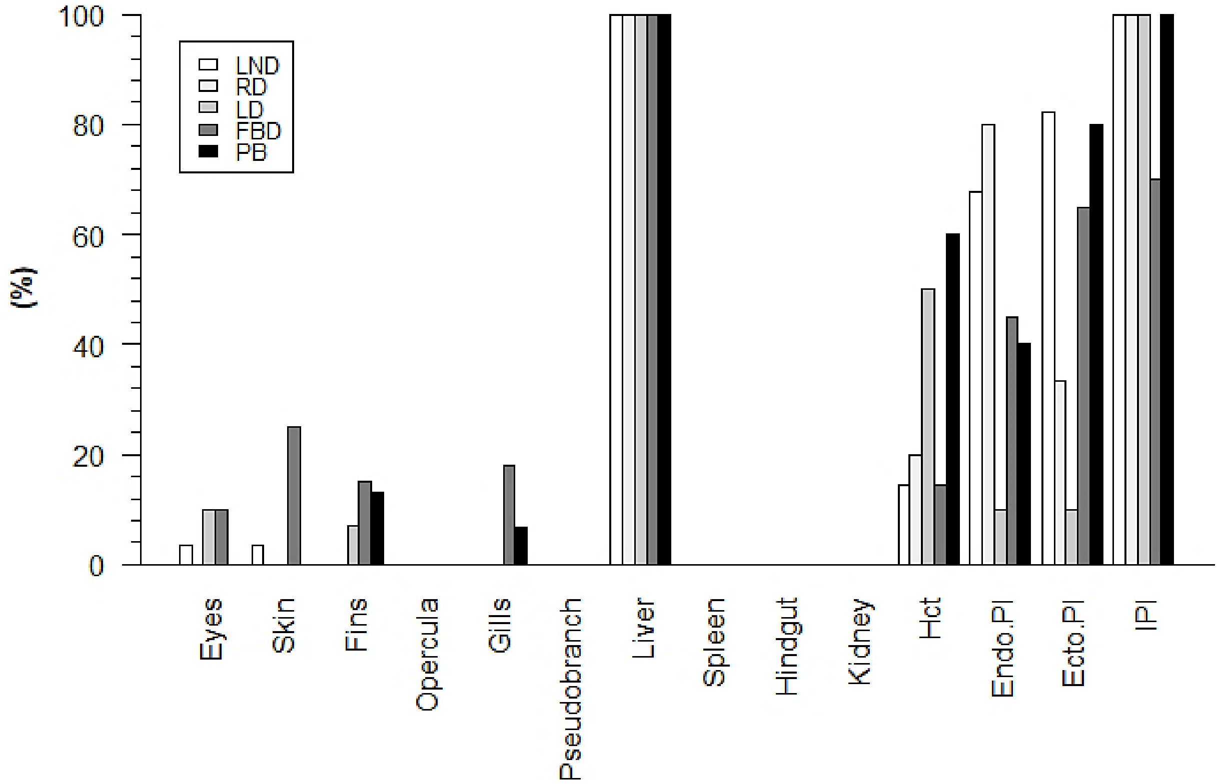

Figure 4.3: Percentage anomalies observed in tissues and organs of fish from Luphephe-Nwanedi (LND), Rhenosterkop (RD), Loskop (LD), Flag Boshielo (FBD) dams and Phalaborwa Barrage (PB) during once off surveys conducted in April and May 2016.

4.3.3 Condition factor (K), hepatosomatic (HSI) and gonadosomatic (GSI) indices

The overall ratio of males to females sampled during the once off survey study at all localities was 60:40 (Table 4.1). The condition factor (K) and gonadosomatic index (GSI) of fish from Loskop Dam were higher and significantly ($p < 0.0001$) different from those sampled from the other impoundments. The mean hepatosomatic index (HSI) was recorded to be higher for specimens sampled from Luphephe-Nwanedi Dams, but not significant ($p > 0.05$) when compared with other impoundments.

Table 4.1: Means ± standard errors of the total length, mass, condition factor (K), hepatosomatic index (HSI) and gonadosomatic index (GSI) of fish caught in Luphephe-Nwanedi (LND), Rhenosterkop (RD), Loskop (LD), Flag Boshielo (FBD) dams and Phalaborwa Barrage (PB) during a once off survey conducted at each impoundment between April and May 2016.

Parameter/metric	Impoundments				
	LND	**RD**	**LD**	**FBD**	**PB**
Sample size	28	15	10	20	15
Sex M:F ratio	68:32	40:60	30:70	70:30	67:33
Body length	25.86±2.19	22.68±1.61	35.38±6.71	27.49±5.42	20.35±0.87
Body mass (g)	285.71±61.54	238.51±45.58	1193.09±614.56	437.96±231.83	132.91±15.19
K	$1.64^{a}\pm0.43$	$2.02^{b}\pm0.03$	$2.39^{c}\pm0.36$	$1.96^{b}\pm0.05$	$1.58^{a}\pm0.05$
HSI	$1.64^{a}\pm0.04$	$1.03^{a}\pm0.08$	$1.62^{b}\pm0.20$	$1.44^{b}\pm0.07$	$0.73^{a}\pm0.06$
GSI	$0.49^{ab}\pm0.11$	$0.16^{a}\pm0.02$	$0.86^{b}\pm0.30$	$0.22^{a}\pm0.04$	$0.19^{a}\pm0.04$

Superscripts ^{a}, ^{b} and ^{c} indicate significant differences, with $p < 0.05$.

4.3.4 Histopathological and morphometrical analysis of gills

Gills of fish from Luphephe-Nwanedi and Rhenosterkop dams showed normal morphology when compared with fish sampled from more polluted sites i.e. Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage. When examining the gill structures using microscopy, it was observed that each gill arch perpendicularly supported many distinct and regular filaments arranged in two rows, with no lesions (Figure 4.4: A, B). The lamellae were covered by squamous epithelium sustained by pillar cells that presented cytoplasm flanges that enclosed the blood spaces. Between the lamellae, filaments examined were lined by a thick stratified epithelium comprising chloride, mucus and pavement cells (Figure 4.4: A, B).

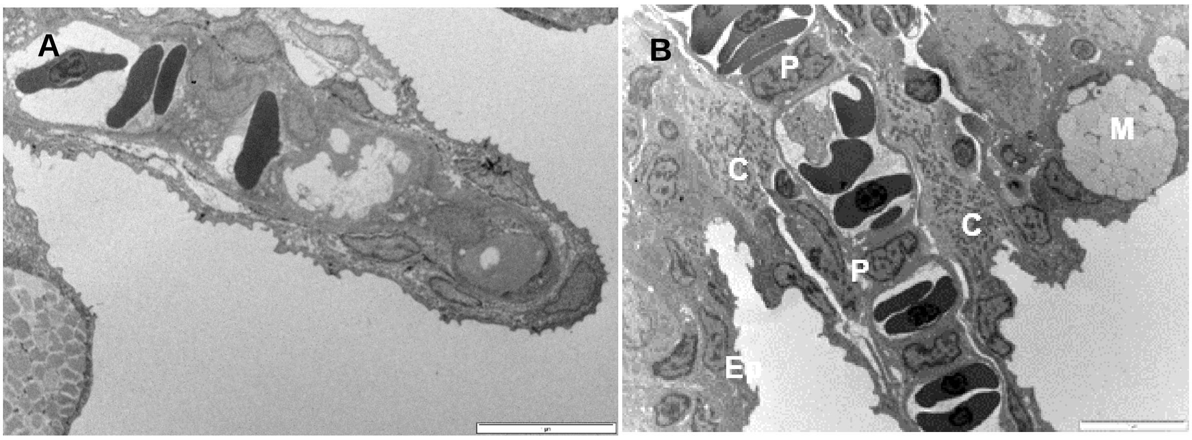

Figure 4.4: Control specimens of *Oreochromis mossambicus* from Luphephe-Nwanedi and Rhenosterkop dams. A = a transmission electron micrograph of sagittal section through secondary lamellae. B = transmission electron micrograph showing blood sinuses with pillar cells (P), chloride cells (C), mucus cells (M) and epithelial cell (Ep).

Histopathological alterations were observed in gills of fish from Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage, with fish from the latter impoundment being severely affected. Pathology observed included the thickening of the epithelium due to hypertrophy of pavement and chloride cells, epithelium detachment (separation between the lamellae and basal membrane) as well as the rupture of pillar cells (Figure: 4.5 A, B).

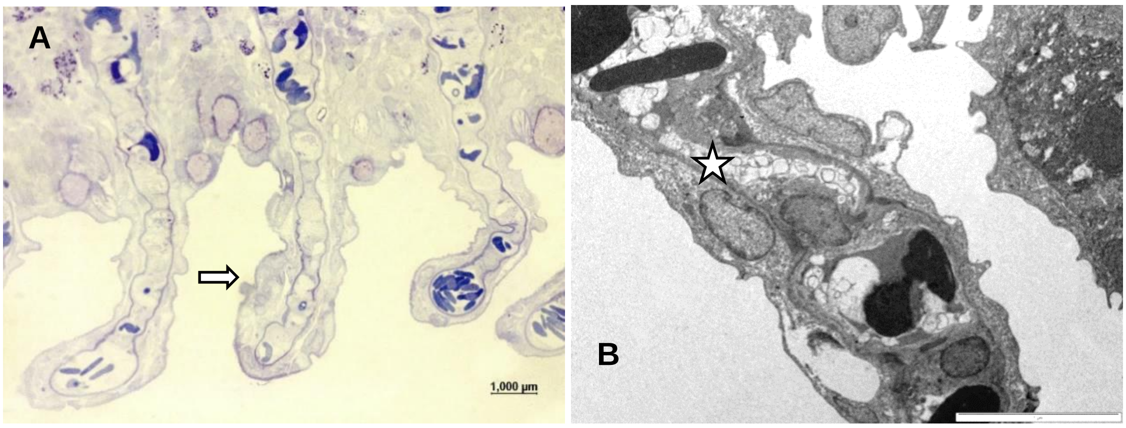

Figure 4.5: Micrographs of sagittal sections through secondary lamellae of *Oreochromis mossambicus* indicative of fish collected from the more polluted sites i.e. Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage. Micrograph using light microscopy (A) and transmission electron microscopy (B) reveal the thickening of the gill epithelium as a result of hypertrophy (arrow) and loss of cellularity (star).

Intercellular oedema (epithelial lifting), necrosis and vasodilation were prevalent in gills of fish from Flag Boshielo Dam and Phalaborwa Barrage (Figure 4.6: A, B, C, D).

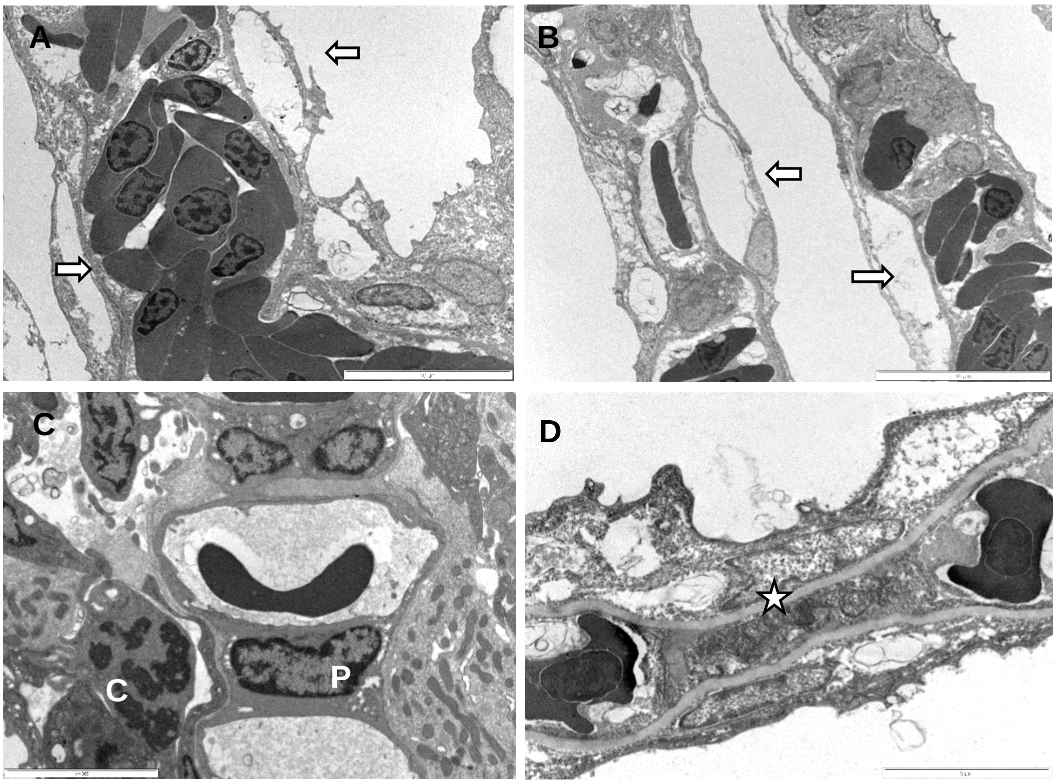

Figure 4.6: Sagittal sections through the secondary lamellae of *Oreochromis mossambicus* from Flag Boshielo Dam and Phalaborwa Barrage viewed using TEM. Micrographs (A) and (B) reveal epithelial lifting as indicated by arrows. Micrograph (C) and (D) reveal necrosis and vasodilation (star) of the secondary lamellae.

Lamellar telangiectasia (aneurysms), deformed gills, hyperplasia of the epithelial and mucous cells and damaged pillar cells were prevalent in the gills of fish from Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage (Figure 4.7: A, B, C, D).

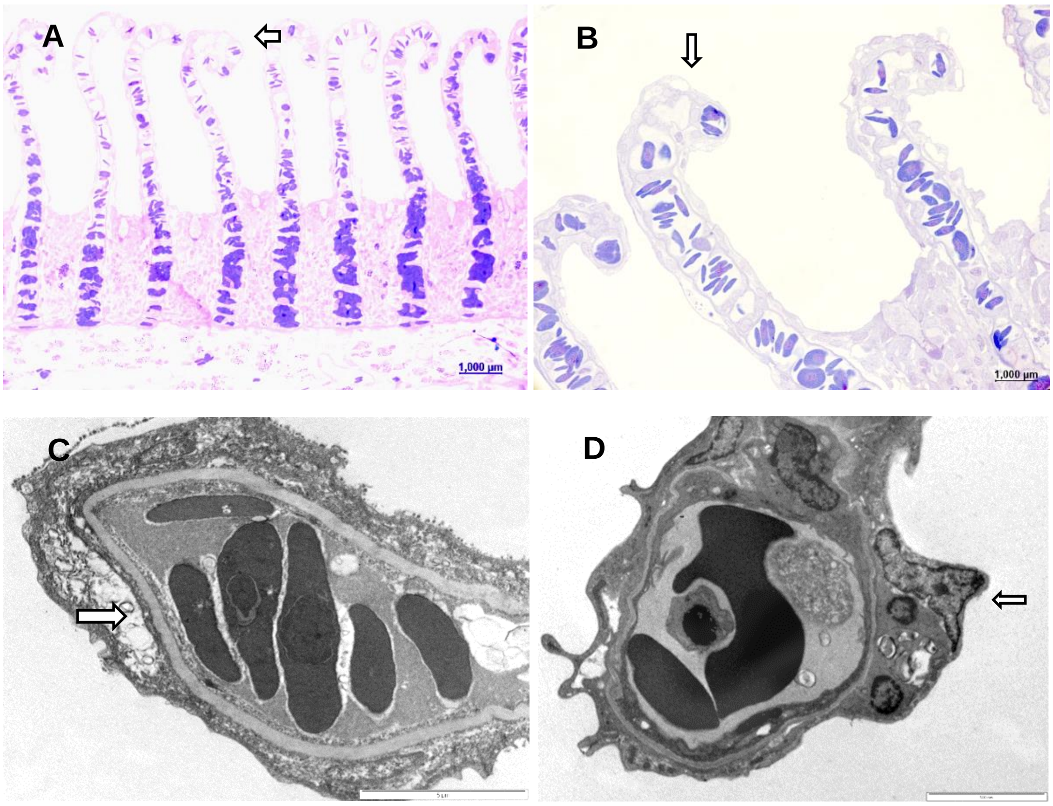

Figure 4.7: Tip of secondary lamellae of *Oreochromis mossambicus* from Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage. Light micrograph (A) and (B) reveal deformed shapes of the gills (club shaping). TEM micrographs (C) and (D) indicate lamellar telangiectasia (aneurysms).

Generally, the arithmetic mean thickness of gill epithelium (H_{ar}) was calculated to be highly significant ($p < 0.0001$) between fish from the various impoundments (Figure 4.8). Although the highest H_{ar} values were recorded for fish from Phalaborwa Barrage, they did not differ significantly ($p > 0.05$) from those calculated for fish from Loskop and Flag Boshielo dams. Conversely, H_{ar} scores for fish from Luphephe-Nwanedi Dams were significantly ($p < 0.05$) lower when compared to gill samples collected from the other impoundments.

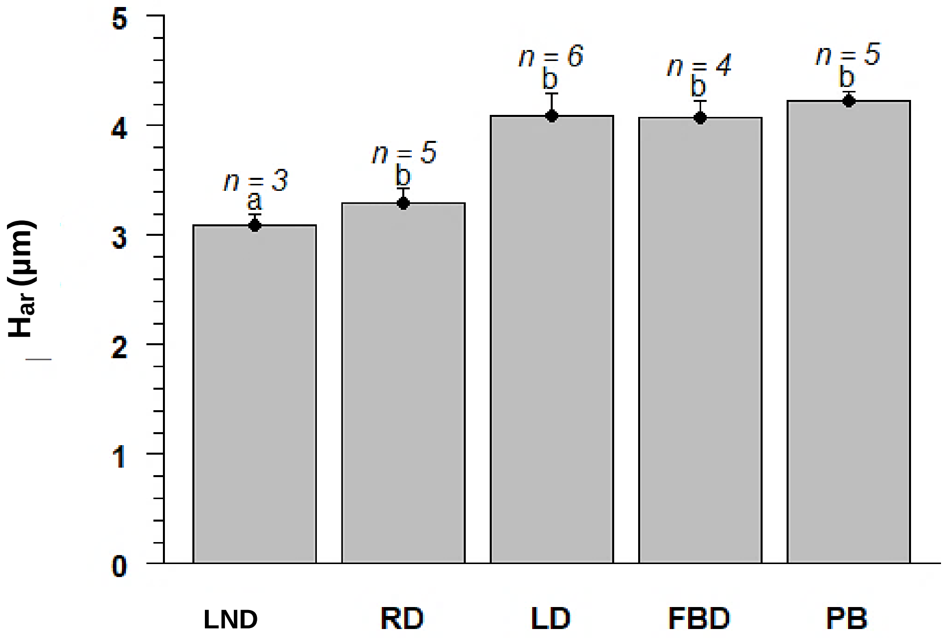

Figure 4.8: Arithmetic mean thickness of gill epithelium (H_{ar}) for fish sampled from Luphephe-Nwanedi (LND), Rhenosterkop (RD), Loskop (LD), Flag Boshielo (FBD) dams and Phalaborwa Barrage (PB) during a once off surveys conducted in April and May 2016.

4.3.5 Metal concentration in fish muscle tissue

At the time of writing, results of muscle tissue metal content for fish from Luphephe-Nwanedi Dams had yet to be received and therefore could not be included in the analyses. Metal accumulation in muscles tissues of fish from the four impoundments were significantly different ($p < 0.05$), with fish from Phalaborwa Barrage accumulating significantly ($p < 0.05$) higher concentrations of Al, Cu, Mn and Zn when compared with specimens collected from the other impoundments. Levels of Ba and Se in muscle tissues of specimens from Rhenosterkop Dam were significantly ($p < 0.05$) higher than those from Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage. Higher concentrations of Cr and Sr were accumulated in muscle tissues of fish from Loskop Dam.

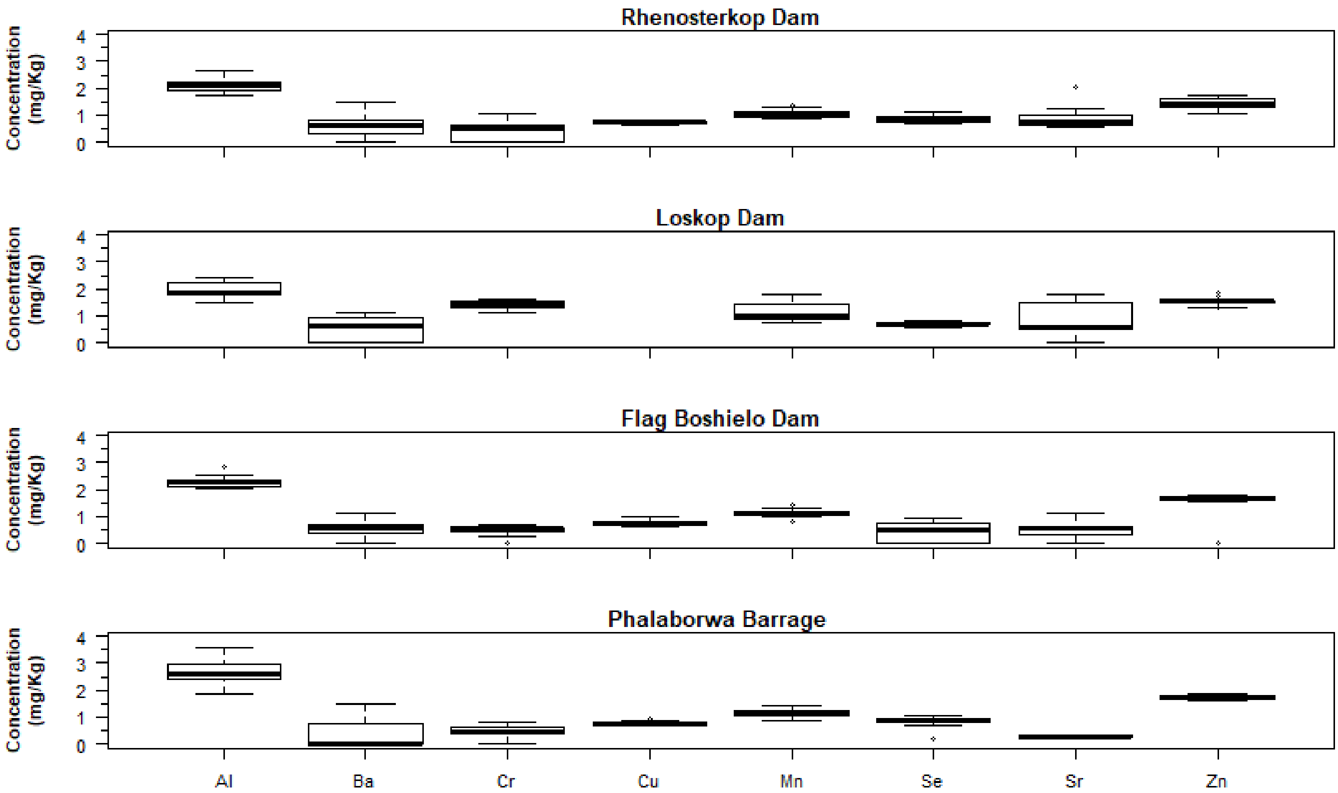

Figure 4.9: Metal concentrations (mg/kg dry weight) detected in muscle tissue of fish from Rhenosterkop, Loskop, Flag Boshielo dams and Phalaborwa Barrage during once off surveys conducted in April and May 2016.

4.4 Biological results for monthly survey undertaken at Flag Boshielo Dam

During this study, low inflow in terms of water levels into Flag Boshielo Dam occurred during April, May, June, August, September, October and December 2016, while high inflow occurred during February 2016 and February 2017. When testing for significant differences between high and low inflow, data collected during months having high inflow were pooled and similarly data associated with months indicative of low inflow were pooled and tested.

4.4.1 Blood glucose concentrations

Blood glucose levels of fish sampled varied significantly ($p < 0.0001$) between the months surveyed, with specimens collected during April having higher glucose levels as opposed to fish collected during other months. The lowest mean blood glucose concentration of 3.59 mmol/L was recorded for fish collected in August 2016 (Figure 4.10).

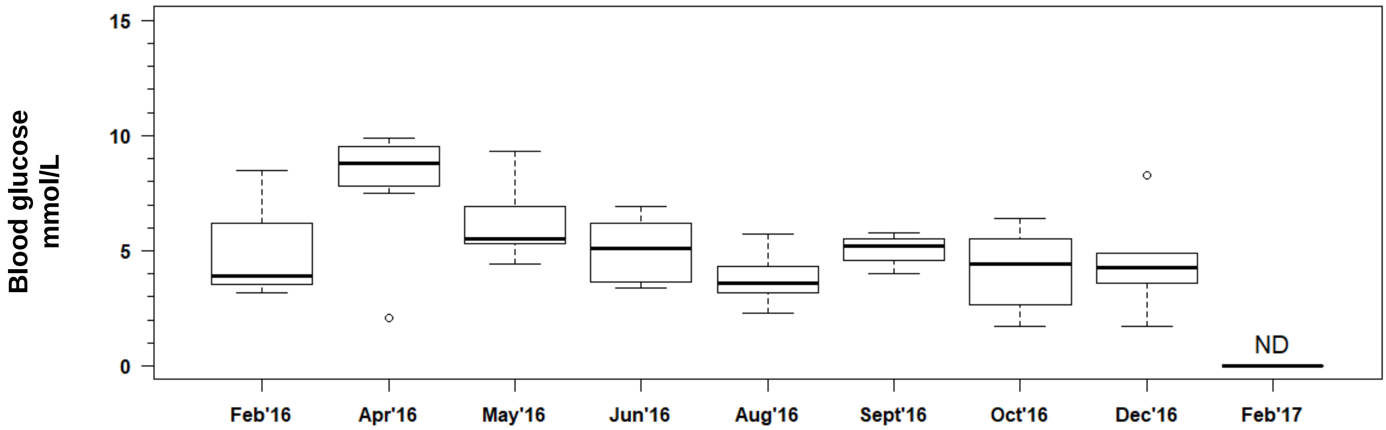

Figure 4.10: Blood glucose concentrations (mmol/L) recorded for *Oreochromis mossambicus* sampled from Flag Boshielo Dam from February 2016 to February 2017. ND denotes no data available.

4.4.2 Health Assessment Index (HAI)

Higher HAI scores were recorded during low inflow periods. Monthly variation between HAI, HAI with PI and HAI with IPI scores for *O. mossambicus* were significant (p < 0.0001). Indices scores revealed that *O. mossambicus* sampled in April, June and September 2016 were more affected in terms of necropsy-related anomalies and haematocrit values when compared to other months surveyed. No significant differences (p > 0.05) occurred between indices of HAI with PI and HAI with IPI (Figure 4.11).

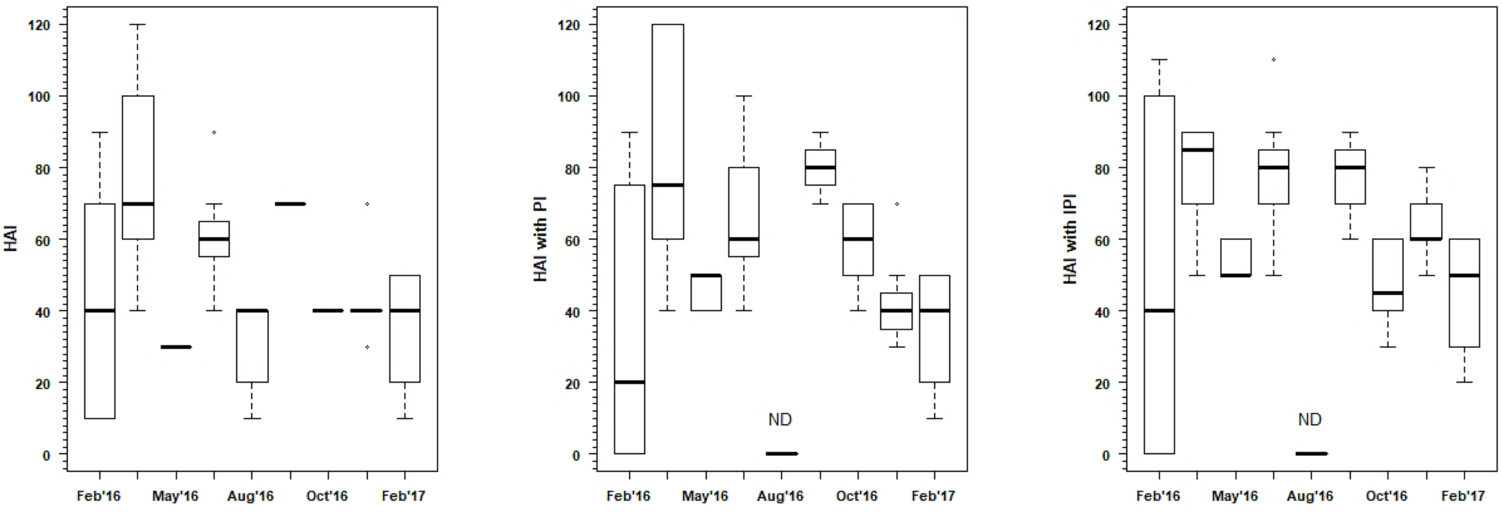

Figure 4.11: Box and whisker plots of HAI, HAI with PI and HAI with IPI scores for *Oreochromis mossambicus* sampled from Flag Boshielo Dam in February, April, May, June, August, September, October, December 2016 and February 2017. ND denotes no data available.

Abnormal eyes, eroded fins, pale gills, dark brown livers, abnormal haematocrit readings and high endoparasite numbers were responsible for the HAI scores during periods of low inflow, while skin lesions and ectoparasite numbers contributed to the HAI scores recorded during the high inflow period (Figure 4.12).

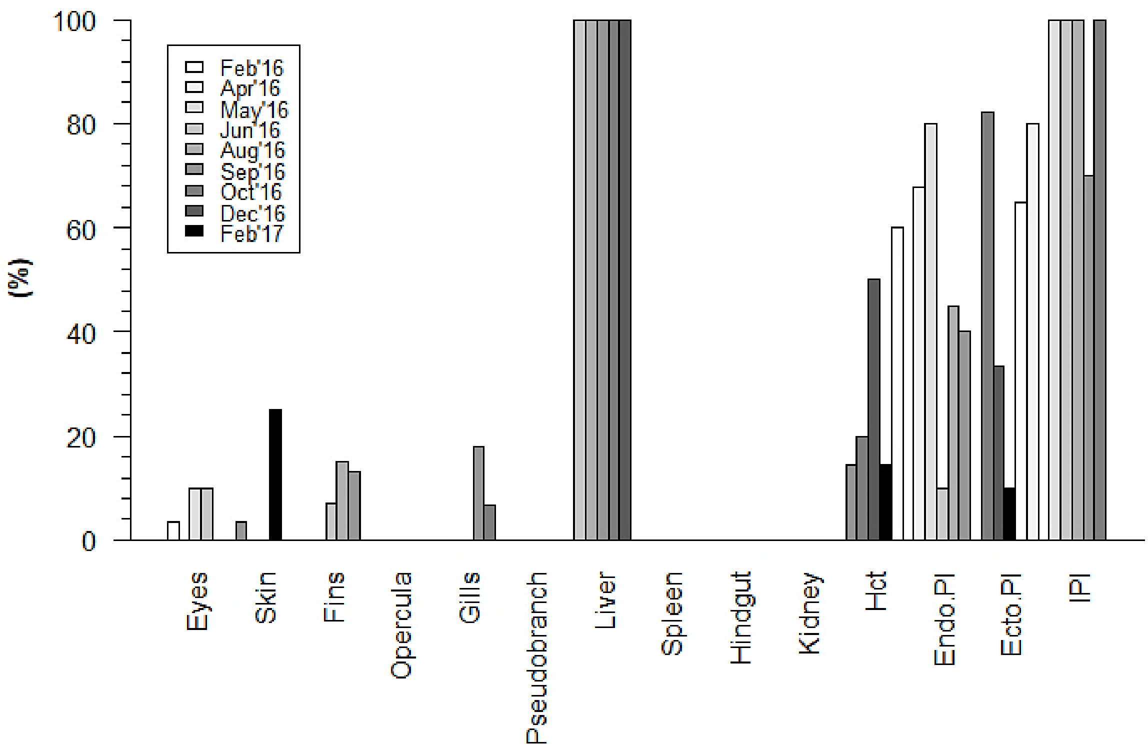

Figure 4.12: Percentage anomalies observed in tissues and organs of *Oreochromis mossambicus* sampled from Flag Boshielo Dam during February, April, May, June, August, September, October, December 2016 and February 2017.

4.4.3 Condition factor (K), hepatic (HSI) and gonadosomatic (GSI) indices

Variations exhibited between K, HSI and GSI values were highly significant ($p < 0.0001$) between the months surveyed. Fish collected during all months were recorded to be in good health in terms of K values, with values ranging from 1 – 2. However, higher K values were recorded for fish sampled during low inflow periods. High HSI and GSI values were recorded during most months representing a period of low inflow, with fish collected in August 2016 having the highest values of these indices (Figure 4.13). Most fish collected in February 2017 were sub-adults and as a consequence the GSI could not be determined.

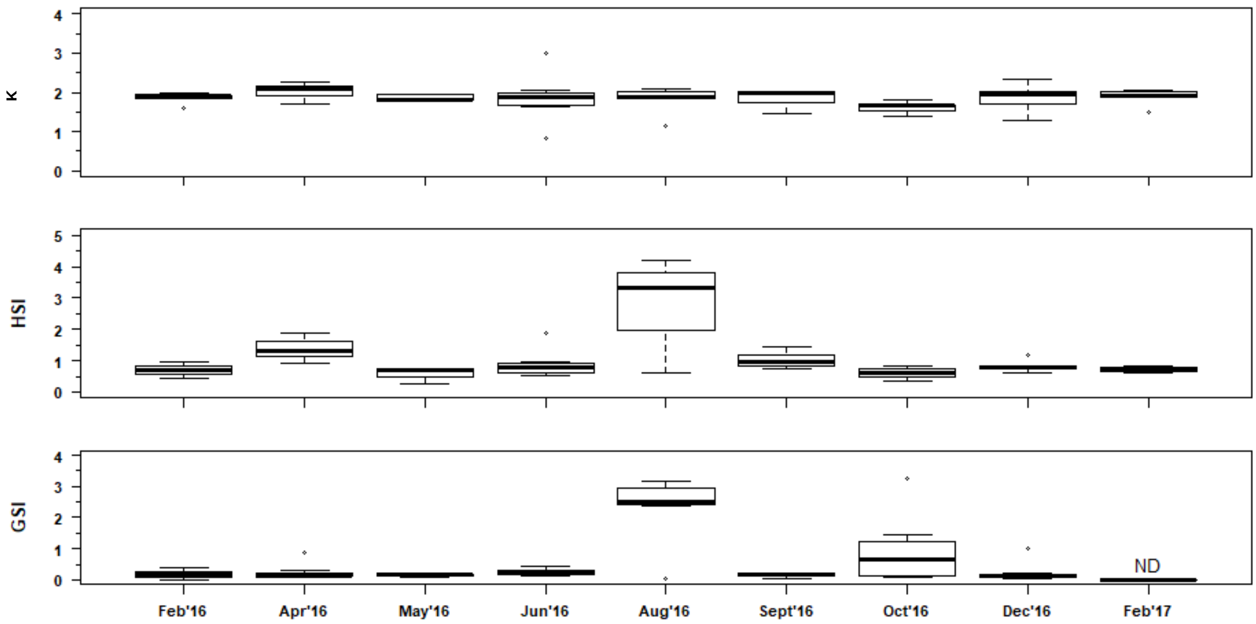

Figure 4.13: Condition factor (K), hepatosomatic index (HSI) and gonadosomatic index (GSI) of fish sampled at Flag Boshielo Dam during February, April, May, June, August, September, October, December 2016 and February 2017. ND denotes no data available.

4.4.4 Histopathological and morphometrical analysis of gills

Oreochromis mossambicus gills sampled in September 2016 were more affected and exhibited the highest H_{ar} when compared to other months sampled. The gills of specimens sampled in August 2016 were less affected and had the lowest H_{ar} (Figure 4.14). Pathology observed in gills of most fish included deformed lamellar, desquamation of the epithelial layer, thickening of the gill epithelium as a result of hypertrophy occurring in pavement and chloride cells. The monthly variation in H_{ar} of fish sampled were highly significant ($p < 0.0001$) between the months surveyed.

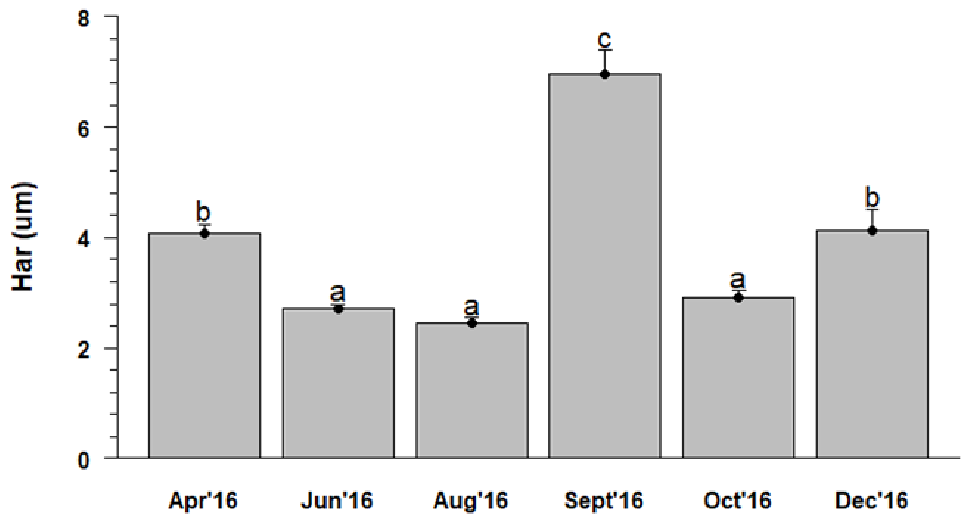

Figure 4.14: Arithmetic mean thickness of gill epithelium (H_{ar}) of *Oreochromis mossambicus* sampled from Flag Boshielo Dam during April, June, August, September, October and December 2016. Superscripts ^{a}, ^{b} and ^{c} indicate the significant differences.

4.4.5 Metal concentration in fish muscle tissue

Except for Cr, Pb, Se and Sr, concentrations of metals in fish muscle tissues surveyed from Flag Boshielo Dam varied significantly ($p < 0.05$) between surveys conducted. High levels of Al, As, Co, Cr, Cu, Mn, Mo, Ni, V and Zn were recorded in specimens during the low inflow period when compared to the high inflow period (Figure 4.15). Conversely, high concentrations of Ba, Hg, Se, Sr and Pb were detected in muscle tissues of fish sampled during the high inflow period.

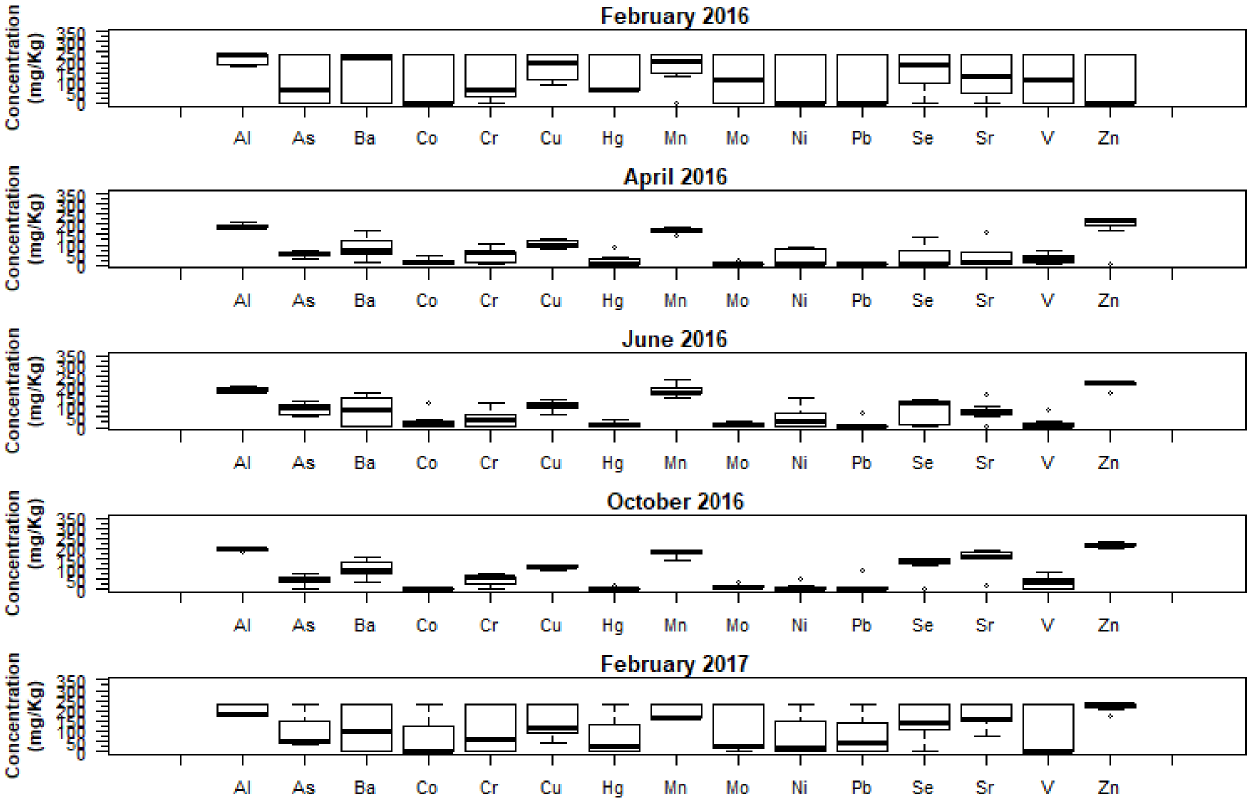

Figure 4.15: Metal concentrations (mg/kg dry weight) detected in muscle tissues of *Oreochromis mossambicus* sampled from Flag Boshielo Dam during monthly surveys.

4.5 Discussion for the once off and monthly surveys

4.5.1 Blood glucose levels

According to Carefood (1994), blood glucose concentrations in fish may be affected by a variety of environmental stressors and changes in water quality. Elevated blood glucose levels can be attributed to enhanced catecholamines that act directly on the liver to stimulate glycogenolysis which results in the mobilisation of blood glucose during stressful events (Silbergeld 1974). High blood glucose levels recorded at Flag Boshielo Dam during the once off survey can be attributed to high concentrations of metals detected in sediment that the fish may have been exposed to through ingestion of benthic organisms and detritus. For example, metal concentration in *Synodontis zambezensis* is inferred by Sara *et al.* (2017) to be due to this species habitat and feeding preference. The findings in this study seems to agree with those by Witeska (2005) which showed that short or long term exposures of fish to various metals i.e. Pb, Cd and Zn induces stress and, in turn, can lead to elevated glucose concentrations. Moreover, similar findings were observed in the study by Vosyliene

(1999) where high levels of glucose were recorded for common carp, silver carp and rainbow trout after exposure to copper.

With regard to monthly surveys at Flag Boshielo Dam, elevated blood glucose levels in fish sampled during April 2016 were probably due to high concentrations of sulphate (SO_4^{2-}), chloride (Cl^-) and fluoride (F^-) recorded during this period. Similar observation has been made by Diwan *et al.* (1979) after exposing *Mystilus edulis* to industrial effluents containing these ions. Lower blood glucose levels recorded for fish sampled in August 2016 might have been due to increased glucose oxidation to meet the higher energy demands warranted during chronic exposure as reported for *Glossogobius giuris* by Venkataramana *et al.* (2005). Martinez-Porchas *et al.* (2009) stated that previous exposure to stress conditions should be considered when measuring blood glucose levels, because these are a source of error when the fish has been acclimatised to certain stress factors in aquatic systems. Studies by Barton *et al.* (2003) and Barton *et al.* (2010) showed elevated blood glucose levels occurring in fish due to transporting and handling. Therefore, further investigations are required to determine if the procedure of handling and the transportation of fish applied in this study had an impact on blood glucose levels.

4.5.2 Health assessment index

The HAI is a systematic method used to identify external and internal anomalies of fish in a field necropsy (Schmitt *et al.* 2004). According to Goede and Barton (1990), when fish are exposed to elevated levels of contaminants, tissue and organ function change in order to maintain homeostasis, with the physiological response in fish from polluted environment being more affected. A low HAI score is indicative of good water quality (Adams *et al.* 1993; Crafford & Avenant-Oldewage 2009). The HAI results obtained during once off survey suggested that fish from impacted sites, i.e. Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage had poorer health scores than those collected from less polluted sites, i.e. Luphephe-Nwanedi and Rhenosterkop dams. Although Luphephe-Nwanedi and Rhenosterkop dams were selected to be control sites the HAI scores recorded for fish from these impoundments were considerably higher than a mean HAI score of 41 recorded for the same species in Hout River Dam by Sara *et al.* (2014). The authors of this study considered Hout River Dam to be in pristine state due to little rural and urban development within the catchment. Conversely the HAI scores recorded at Flag Boshielo and Loskop dams were higher than the means of 53 and 75 recorded for the same fish species at Bronkhorstspruit Dam (unpolluted) and Mamba River (polluted) respectively by Watson *et al.* (2012). Results in this present study seemed to indicate that the HAI scores were greatly influenced by the predominance of abnormal gills, liver, fins, skin and eyes most affected.

Previous findings on fish health indicate that HAI scores were greatly influenced by parasite loads (Crafford & Avenant-Oldewage 2009; Van Dyk *et al.* 2009), seasonality (Madanire-Moyo *et al.* 2012a; Sara *et al.* 2014) and water levels (Watson *et al.* 2012). According to Kotze *et al.* (1999), during high flow periods, pollutants existing in the water column become diluted, and as a consequence fish are exposed to less pollutants as compared to during a period of low inflow. Hence it can be inferred that the low HAI scores recorded during months with high inflow are attributed to a dilution effect and the reduction of exposure to metals in the water column and vice versa.

I. Haematocrit (Hct %)

According to Heath *et al.* (2004) haematocrit scores of between 30% and 45% are considered normal in a healthy fish. High haematocrit can result from acute stress, gill damage or impaired osmoregulation (Barton *et al.* 1985: Sara *et al.* 2014). Short-term exposure to low concentrations of metals can also induce an increase in haematocrit (Madanire-Moyo *et al.* 2012a). Fish stress reaction causes an osmotic imbalance and changes in regulatory system of ionic interchange which can diminish blood pH and increase erythrocytes volume and subsequently haematocrit (Rios *et al.* 2002). Metal pollution recorded in Phalaborwa Barrage, evident from the observations of pale gills and a high H_{ar} values, might have contributed to the high recorded Hct% values for fish from this impoundment.

Similarly, abnormal Hct% values recorded during period of low inflow period at Flag Boshielo Dam can be attributed to the exposure to high concentrations of some metals recorded during this period. The study by Watson *et al.* (2012) revealed similar findings whereby heavy metal pollution in the Olifants River System, were associated with low haematocrit levels in fish assessed.

II. Eyes, skin, fins and gills

In the present study, bilateral exophthalmus was more prevalent in fish sampled from Loskop and Flag Boshielo dams when compared to specimens collected from the other impoundments. This probably resulted from the bioconcentration of pollutants, parasitic infection or predation. According to Reichenbach-Klinke (1973), bacterial or parasitic infection of the eye may result in lesions of the peri-orbital tissue. Moreover, eye flukes such as metacercariae of larval digenean parasites are known to induce cataracts due to metabolic excretions and mechanical structures of the lens (Shariff *et al.* 1980). In turn, cataracts may play a role in impairing the vison of fish and increasing their susceptibility to avian predation (Seppälä *et al.* 2006) that, in turn, can induce higher stress levels in fish.

The parasitic copepod, *Learnea cyprinacea* was observed on the skin of fish from Flag Boshielo Dam, causing a severe inflammatory response at the attached site. Fins of fish from Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage were haemorrhagic and eroded. According to Pelis and McCornick (2003), fin abnormalities may be attributed to overcrowding, water quality, feed type, bacterial infection and handling of fins. These factors can explain fin abnormalities recorded for fish from these three localities.

Moreover, gills abnormalities observed in fish from Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage can be attributed to pollution levels of these impoundments. The mechanism of dissolved metals toxicity can be related to interference of ionic and osmotic balance and respiratory problems resulting in coagulation of mucous on the gills (Madanire-Moyo *et al.* 2012a). Furthermore, environmental contaminants and parasitic infections affect the physiology of the gills resulting in anaemia (Watson *et al.* 2012). Factors that can explain abnormalities observed for gills of fish from these three localities.

In the study by Watson *et al.* (2012), similar abnormalities were recorded for the same fish species from different localities. Blindness was prevalent in fish sampled from the polluted localities, i.e. Mamba River and Loskop Dam. In addition, skin lesions caused by *L. cyprinaceae* and deformation of fin rays were recorded for the same fish species during drought periods at the same localities (Watson *et al.* 2012). The same study further recorded gills of *O. mossambicus* to be abnormal due to parasitic infections and hyperplasia.

III. Liver

Liver is the main detoxification organ and is responsible for excreting xenobiotic chemicals and metals, thus it is a target organ for xenobiotic substances (Won *et al.* 2016). Abnormalities noted in different fish from the five impoundments included fatty liver, focal discolorations and liver discolorations, with livers of fish from Loskop Dam being the most heavily affected. Fatty liver is a very common pathological state attributed to excessive accumulation of fat in cellular cytoplasm which may be related to dietary intake (Madanire-Moyo *et al.* 2012a). Accumulation of fat on the liver can also result from the inability of fish to convert the fat stored in hepatocytes to phospholipids (Runnells *et al.* 1965). This can explain the fatty livers of fish observed at Loskop Dam. Focal discolorations observed in livers of fish from Loskop Dam may have possibly been induced by focal infections, inflammation or necrosis. Most fish livers from all the impoundments surveyed, including those of fish sampled during monthly surveys appeared to have discolorations. This can be attributed to inadequate food intake or chronic stress from the breakdown of hepatic glycogen and potential hepatic carcinogens, mycotoxins and metals, causing rapture of metaplastic ducts (Watson *et al.* 2012). However, one cannot conclude on the macroscopic observations made, thus further histopathological assessments are needed to explain and support the necropsy findings in this study.

IV. Ectoparasites

Ectoparasites have been used in aquatic ecosystems as bioindicators of deteriorating water quality especially with regard to pollutants (Khan & Billard 2007). The study by Madanire-Moyo and Barson (2010) showed that there is a link between environmental conditions and parasitism. A relationship between parasites and pollutants depend on the type of life cycle exhibited by the parasite and the concentration of pollutants involved (Madanire-Moyo *et al.* 2012b). For metazoan parasites with indirect lifecycles, environmental conditions need to be favourable for both the intermediate and final host as well as for the free-living stages of the parasites (Dzikowski *et al.* 2003), while for metazoans with direct life cycle (i.e. monogeneans), are in direct with the contact with the surrounding medium, thus poor water quality may adversely affect their numbers and diversity (Pietrock & Marcogliese 2003). High ectoparasite numbers recorded at Luphephe-Nwanedi Dams were consistent with the IPI premise that more ectoparasites are indicative of better water quality. However, high ectoparasites numbers recorded in fish sampled from Phalaborwa Barrage and Flag Boshielo Dam can be explained by virtue of the fact that certain pollution conditions favours the propagation of parasites by excluding their natural predators as stated by Moller (1987).

During monthly surveys at Flag Boshielo Dam, ectoparasite numbers were lower during low flow period and higher during the high inflow period. These results seemed to agree with Watson *et al.* (2012), who recorded high parasite numbers during flood period. According to Avenant-Oldewage (1998), ectoparasite diversity and abundance tend to decrease when water quality is poor and heavily polluted. This reason may provide an explanation as to the findings observed in the present study. However, Jooste *et al.* (2004) expressed that a knowledge of the pollution type and the host's biology is important when evaluating and interpreting observed parasites.

V. Endoparasites

Unlike ectoparasites, endoparasites are generally not in direct contact with aqueous pollutants and are therefore able to take advantage of a host's weakened immune system to proliferate and possibly propagate (Sures 2004). According to Nachev and Sures (2009), variations in endoparasite numbers in polluted localities is species dependent. In the present study, high endoparasite numbers were recorded at less impacted sites (Luphephe-Nwanedi and Rhenosterkop Dams) as opposed to more polluted sites (Loskop Dam, Flag Boshielo Dam and Phalaborwa Barrage). This finding seems to be in contrary with findings by Sures (2004), who stated that endoparasites take advantage of the weakened immune system of the host. A possible explanation with regard to the findings observed in this study can be that in impacted sites, some endoparasites species are less likely to complete their life cycles either due to direct adverse effects that have an impact on a parasite's free-living stages or as an indirect consequence of the elimination of their intermediate hosts (Madanire-Moyo *et al.* 2012a). Moreover, Watson *et al.* (2012) stated that endoparasites will not necessarily decrease when exposed to metals in an aquatic environment because some parasites have a high capacity to bioaccumulate metals such as lead, cadmium and selenium occurring in the intestines of their final hosts (Watson *et al*. 2012). Therefore, it appears that the premise of endoparasites taking advantage of a hosts weakened immune system needs to be investigated further for local conditions.

4.5.3 Condition factor (K), hepatosomatic (HSI) and gonadosomatic (GSI) indices

a) Condition factor (K)

Condition factor (K) is the measurement of the general health condition used to compare growth conditions of fish and indicate environmental quality (Sara *et al.* 2014; Satheesh & Kulkarni 2016). Knowledge of the condition factor, calculated by ratio of body weight to body length, assumes heavier fish with respect to its mass is healthier. This is because the fish has the potential to utilise body tissue nutrients such as fat and protein when under environmental and physiological stress making a K value of one and higher, indicative of good health (Freyre *et al.* 2009; Watson *et al.* 2012). Differences in K have been interpreted as a measure of histological events such as fat reservation, gonadal development and adaptation to the environment (Zin *et al*. 2011). Overall K values of *O. mossambicus* collected from all the impoundments surveyed in this study were above one. Due to differences in the water quality of the various impoundments surveyed, fish from more polluted sites were expected to have recorded K values of less than one. However, this was not the case. It was inferred that in more impacted sites, whereby anthropogenic sources of organic pollutants lead to conditions of eutrophication and in turn higher algae productivity (Madanire-Moyo *et al* 2012a), provided a source of food for *O. mossambicus* as this species is able to feed on algae and detritus (Skelton 2001; Welicky *et al.* 2017). The food and energy intake by *O. mossambicus* may facilitate the species to have sufficient reserves to offset the negative effects that metal contamination and poor water quality can induce. This can possibly be an explanation for the higher fish condition scores recorded at Loskop Dam.

With regard to monthly surveys conducted at Flag Boshielo Dam, the K values were above one. Although this species is tolerant to a wide range of temperatures (8 - 35°C), with breeding occurring at > 18°C (Sara *et al.* 2014), seasonal variations of K were to be expected. However, this was not observed in this study, suggesting that seasonal variations did not influence the K of fish sampled. Similar findings whereby K of *O. mossambicus* were not affected by seasonal changes were observed in studies by

Sara *et al*. (2014) and Watson *et al*. (2012). For example, Sara *et al.* (2014) obtained a mean K value 2.0 for fish sampled at Hout River Dam. Conversely, Watson *et al.* (2012) recorded a mean K value of 2.4 for the same species from Loskop Dam. Hence possible reasons for little variation in K scores reported here are because there appears not to be drastic changes in seasonal and environmental conditions occurring within Flag Boshielo Dam and because it can be assumed that given the size of the dam that there is sufficient food and resources available for the species to sustain and maintain its condition.

b) Hepatosomatic index (HSI)

Hepatosomatic index (HSI) is associated with liver energetic reserves and metabolic activity and has been reported to decrease in fish exposed to pollutants (Craig *et al.* 2000). Although the liver is not the main organ for energy storage, it is associated with fish condition and feeding activity, playing a role in nutrient metabolism linked to food abundance and warm temperatures (Nunes *et al.* 2011). The HSI values recorded for fish sampled during the once off survey appeared not to be affected by the water quality of the impoundments as these values fell within the range of 1 - 2%. Values stipulated by Marchand *et al*. (2008) to be normal for osteichthyes. However, variations in HSI scores can be attributed to food availability in the different impoundments and differences in water quality.

Surveys investigating fish HSI at Flag Boshielo Dam were influenced by seasonal variation. Variations in HSI are normally attributed to energy storage for reproduction (Da Costa & Araujo 2003). According to Hismayasari *et al.* (2015), an increase in HSI is related to the increase of ovary maturation level and gametogenesis due to the development of oocyte in fish is associated with hepatic synthesis of vitellogenin, which is a protein associated with oocyte maturation (Singh & Srivastava 2015). This can explain the high HSI recorded in fish sampled during low inflow period. The low HSI recorded for fish sampled during February 2016 was probably caused by the decrease in energy that was used for the development of ovaries. A finding assumed to be a response to pre- and postspawning conditions whereby O. *mossambicus* during most months of low inflow prepare and sustain themselves in order to breed when conditions become favourable that will allow for the survival of larvae and fry.

c) Gonadosomatic index (GSI)

Gonadosomatic index (GSI) of fish has been widely used to indicate maturity of fish, periodicity of spawning and to predict the breeding season (Nunes *et al.* 2011). The index is generally indicative of reproductive success whereby index scores increase during the peak period of maturity and spawning only to decline abruptly after spawning (Jan & Ahmed 2016). Higher GSI values recorded for fish from Loskop Dam was probably because the fish were in a reproductive state. Conversely, lower GSI values recorded for fish at Phalaborwa Barrage may be because gonads of fish were affected by the high metal content that were detected in water samples of this impoundment. Hassanin *et al.* (2002) stated that, when exposed to endocrine disrupting chemicals in water, testicular and ovarian growth are inhibited, leading to a decrease in GSI. This reason can possibly be reason for the low GSI values recorded for fish from Phalaborwa Barrage. However, further investigations need to be conducted in order to ascertain whether the GSI in this impoundment were affected by water quality or by the virtue of the fact that the fish sampled were found to be spent.

Based on the GSI values observed during monthly surveys at Flag Boshielo Dam, the reproductive cycle of *O. mossambicus* can be divided into two phases i.e. pre-spawning and spawning phase. Pre-spawning phase which is also referred to as mature phase occurred in fish sampled during April, May, June and August 2016 where the high HSI values recorded are suggestive of stored hepatic compounds being made available for gonadal development (Satheesh & Kulkarni 2016). During this phase, ovaries were observed to be enlarged, yellow in colour and with large number of spherical ova visible. The spawning phase occurred during September, October and December 2016. During this period, the ovaries of fish were very enlarged, occupying the entire body cavity. Numerous translucent eggs were observed and at times fry were found in stomachs of some fish sampled during this period.

4.4.4 Histopathological and morphometrical analysis of gills

Fish gills are multifunctional organs that are essential for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion (Javed & Usmari 2015). In addition, gills are considered as primary target organs to contaminants in water (Rudnicki *et al.* 2009). Gills of fish sampled from Phalaborwa Barrage were found to be more affected when compared with those from the other impoundments surveyed.

The surface of gills contains specialised ion-transporting chloride cells and have a net negative charge which results in a high affinity for cationic metals (Gensemer & Playle 1999). Thus, an increase in waterborne metals can interfere with normal gill function. Elevated levels of Al and Fe above the TWQR were recorded in the water samples taken from Phalaborwa Barrage. These metals were probably the cause of major histomorphological changes such as epithelium lifting, whereby gas exchange capabilities of the gills of fish from Phalaborwa Barrage were decreased due to high H_{ar} and reduction of interlamellar space which impedes water flow across respiratory surface. These findings were similar to those in a study by Au *et al.* (2004), who observed edema of gill lamella which caused respiratory stress in fish. However, further investigations are required to determine evidence of gas exchange restriction at high H_{ar}. The study by Van Heerden *et al.* (2006) showed gill damage and high H_{ar} in fish exposed to high concentrations of copper (Cu), which exceeded the TWQR for levels detected in Boskop and Klerkskraal dams. In addition a study by Rudnicki *et al.* (2009) found epithelial detachment, hyperplasia and hypertrophy of the cells to occur for fish exposed to pesticides.

Changes such as thickening of the epithelium due to hypertrophy of pavement and chloride cells, epithelium detachment or lamellar telangiectasia, hyperplasia and aneurysm observed in the present study are known to be usual gill lacerations in response to many other chemicals in water (Hassanfnezhad *et al.* 2014). These changes are examples of compensatory responses which generally result in increased distance between water and the blood as a means to act as a natural barrier against entering contaminants (Van Heerden *et al.* 2006). However, increased chloride cell turnover due to hypertrophy can result in increased necrotic and apoptotic cell numbers (Wong & Wong 2000).

For surveys conducted at Flag Boshielo Dam, gills of fish sampled during the low inflow period were adversely affected when compared to the high inflow period. This is possibly due to AMD and other pollutants from anthropogenic activities taking place in the catchment of this impoundment which results in mobilisation of metals from sediments into the water column (Addo-Bediako *et al.* 2014a). Fish may have been exposed to these metals through ingestion or via respiration. According to Kotze *et al.* (1999), gill tissues can act as a depot, where the uptake of metals significantly exceeds the elimination resulting in the accumulation thereof. The highest H_{ar} for fish sampled in September 2016 could possibly be an indication of a high accumulation of metals in the gills. However, further analyses of metal content in fish gills are required to confirm their impact on gill morphology.

4.5.5 Metal concentration in fish muscle tissues

Concentrations and uptake of metals in fish is subject to environmental and species-specific biological factors as well as chemical and physical states of metals (Coetzee *et al.* 2002). Furthermore, metal bioaccumulation in fish can be influenced by seasonality due to fluctuations in exposure routes i.e. diet and solution, as well as aqueous concentration and bioavailability (Luoma & Rainbow 2005). Not all metals are hazardous and toxic to fish. Some are important micronutrients for the normal function of physiological processes, however, if exposure and accumulation levels occur above that of the fish's physiological threshold, metals become deleterious to the health of the fish (Mahboob *et al.* 2016). Only those metals that were detected in muscle tissues of fish from all the impoundments were discussed in this study.

Acid mine drainage, prevalent in the upper catchment mobilises metals from the sediment and bedrock in the Olifants River (Ashton & Dabrowski 2011). This phenomenon is assumed to be the main driver for the increase of metal concentrations in fish muscle tissues from this system (Jooste *et al.* 2014). Variations in metals accumulated by *O. mossambicus* from the impoundments surveyed in this study can be attributed to differences in metal concentrations detected in water and sediment. However, support of the association between metal bioaccumulation in muscle tissues and metals detected in water and sediment need to be established. In a study by Addo-Bediako *et al.* (2014a) and Jooste *et al.* (2014), no association was recorded between metals detected in water and sediment samples, and those detected in fish muscle tissues. This is because fish bioaccumulate metals from their immediate environment over time as opposed to levels that can fluctuate in the water depending on physical variables such as flow rate, water volume, the source and nature of the pollution including chemical properties such as water pH and water hardness (Goswami *et al.* 2016). High concentrations of Al, Cu, Mn, and Zn recorded in muscle tissues of fish from Phalaborwa Barrage may indicate chronic exposure to these metals when compared to fish from other impoundments surveyed. These findings were consistent with those in a study by Kotze *et al.* (1999), who also found evidence of Cu and Zn bioaccumulation in tissues of *O. mossambicus* from Loskop Dam. Toxicity may develop if excessive levels of metals are absorbed and assimilated (Watanabe *et al.* 1997). Conversely, in a study by Mashifane and Moyo (2014) acute toxicity of Cu, Pb and Fe determined by 24 – 96 h LC_{50} showed *O. mossambicus* fry to be more sensitive to metals than fingerlings. Thus, toxicity studies need to be implemented to determine the sensitivity of fish to metals.

Although metals were not measured in other organs such as gonads, gills and liver, low metal concentrations in muscle tissues of fish from Flag Boshielo Dam can possibly be because more metals may have accumulated in these organs. Studies by Canli and Atli (2003) have shown that various fish species accumulate metals at different rates and levels and that different metals accumulate differently within the same species of fish. In fish, gender differences in metal accumulation seem to occur only in gonads and during certain stages of the reproductive cycle (e.g. spawning). For example, Zn is deposited in gonads during gonadal development and lost during spawning (Seymore *et al.* 1996). The gills are a good reflection of concentrations of metals in the water, while liver concentrations represent storage of metals (Arain *et al.* 2008). Metals absorbed through the gills or across the intestinal wall are distributed through circulation bound to transport proteins where they may be utilised for essential life functions or be detoxified by binding to the protein, metallothionein (Olssen *et al.* 1998). Therefore, it is imperative to consider various organs when investigating metal bioaccumulation in fish.

Temporal variation of metal levels in fish is usually ascribed to the variation in climatic conditions such as rainfall and fluctuation in pollutants input into a system (Kotze *et al.* 1999). High concentrations of metals recorded in specimens sampled during a period of low inflow can be attributed to concentrated levels of these metals in the water which became bioavailable to fish through their body surface, respiration and intake of contaminated food and water (Coetzee *et al.* 2002). Lower levels of metals detected in muscle tissues of specimens sampled during the high inflow was consistent with most trends often described in the literature for the relation between water flow and metal concentrations in fish, because water levels of metals tend to be diluted during periods of high rainfall and inflow (Seymore *et al.* 1995). High concentrations of Ba, Hg, Pb, Se and Sr recorded during this period can be due to the influx of these metals from land sources in the catchment of Flag Boshielo Dam during high flow periods. Besides the direct uptake of aqueous metals from the environment, *O. mossambicus* feeds on filamentous green algae, which can results in the intake and accumulation of high metal concentrations (Oberholster *et al.* 2008; Froese & Pauly 2010). Moreover, the pH in the stomach of *O. mossambicus* decreases significantly from 6 to as low as 2.9 after feeding commences (Kotze *et al.* 1999) resulting in metal contaminants becoming more bioavailable during the ingestion, digestion and assimilation process (Kotze *et al*. 1999). In Flag Boshielo Dam, biomagnification of metals is also a possibility that requires further investigations whereby the uptake of metals and their subsequent release in various food sources and trophic levels can be determined.

4.4.6 Conclusion

In this study the variables that best indicated the health of fish from the various impoundments surveyed appeared to be the HAI with PI and IPI as well as blood glucose concentrations and arithmetic mean thickness of gill epithelium (H_{ar}). Compared to fish sampled from more impacted sites such as Loskop and Flag Boshielo dams, the health of fish from Luphephe-Nwanedi Dams were observed to be less impacted based on the aforementioned variables, followed by fish from Rhenosterkop Dam. Haematocrit values, ectoparasites and abnormal gills, eyes and liver contributed the most to HAI scores that provided the best overall assessment of fish condition. No significant difference was observed between HAI, HAI with PI and HAI with IPI scores. Findings in this study further confirmed the HAI premise, that higher HAI would be recorded for fish sampled from more polluted localities than those from less polluted localities. Findings recorded during monthly surveys at Flag Boshielo Dam indicated that during periods of high inflow the fish were in better health in terms of HAI, blood glucose levels and H_{ar} values as opposed to fish sampled during the period of low inflow. The findings reported in this study indicate that the flow regime and water levels have a clear impact on the water quality and the health of fish from this impoundment. Overall, biomarkers that best described fish health and could discriminate between the various impoundments, based on the findings in Chapter 3, are the HAI, blood glucose levels and H_{ar}.

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CHAPTER 5: A MULTIVARIATE AND MODELLING APPROACH TO ESTABLISH VARIABLE(S) THAT BEST DESCRIBE AND PREDICT THE HEALTH OF *OREOCHROMIS MOSSAMBICUS*

5.1 Introduction

In the last three decades, there has been an enormous expansion of statistical tools available to ecologists to look for trends and associations between environmental drivers and biological factors (Mcardle & Anderson 2001). A short list of available statistical techniques includes linear regression, generalised linear modelling (GLM), generalised additive modelling (GAM), regression and classification trees, survival analysis, neural networks and multivariate methods (Zuur *et al.* 2010). Multivariate methods include techniques such as principal component analysis (PCA), canonical correlation correspondence analysis (CCA), factor analysis (FA) and redundancy analysis (RDA). Moreover, multivariate analysis account for interacting variables on a spatio-temporal scale that can guide management strategies for complex systems (Pinto & Maheswari 2011). Application of these approaches offer a better understanding of water quality and possible sources that affect the system under investigation by identifying hidden relations between variables and reducing complex datasets to a small number of factors without information loss (Jha *et al.* 2014; Wang *et al.* 2017). Each of these techniques have advantages and disadvantages. For example, for some methods, a single outlier may determine and influence the results and conclusion. Conversely, heterogeneity may cause errors in linear regression, analysis of variance models and certain multivariate methods (Hurlbert 1984).

In ecotoxicology, multivariate analyses have been used to exhibit variations in community composition among sites and to relate these variations to measured chemical stress (Van Den Brink *et al.* 2003). Whereas Tavakol *et al.* (2015) used GLM's to predict biological and environmental variables that influence parasite infestation of *Contracaecum* spp. in *Oreochromis mossambicus* collected from various water bodies in Limpopo Province. In this study, RDA and predictive models were used to establish which biological and environmental variables influenced the Health Assessment Index (HAI) scores established for *O. mossambicus*.

5.2 Data analysis

The methodology used to model variables that are strongly associated and/or that best predict and influence HAI scores for the two approaches used are described in Chapter 2 paragraph 2.11.

5.3 Results and discussion for a once off survey at various impoundments

After testing for collinearity, a total of 13 biological and 26 environmental variables recorded between sites for the once off survey were used to establish associations between the various variables and HAI scores using RDA. Of these variables, 13 were in close proximity to the HAI vector in the RDA plot and were included to generate a global model in R. Similarly, a total of 13 biological and 17 environmental variables were used to test for collinearity for variables measured during multiple surveys conducted at Flag Boshielo Dam. Of these, a total of 15 were used to generate a global GLM.

5.3.1 Multivariate analysis of biological and environmental variables from the once off survey conducted at various impoundments.

Figure 5.1 shows the ordination diagram of RDA for variables measured during a once off survey. The first axis (λ_1 = 0.95) displays the relationship between environmental with biological variables and the second axis the residual variation (λ_2 = 0.03). Axis 1, 2 and 3 accounted for 95.6% of the variation (Table 5.1). With regard to the HAI scores a positive and strong association with aqueous aluminium (Al) and sediment concentrations of Al, calcium (Ca) and magnesium (Mg), especially with regard to fish collected from Flag Boshielo Dam and Phalaborwa Barrage was observed. A weak association between HAI and $H_{ar,}$ and between water pH and dissolved oxygen (DO) is observed. In contrast HAI scores were negatively correlated with blood glucose levels and water temperature and sediment potassium levels. Conversely, there was no association between biological variables HSI, GSI and muscle tissue levels of titanium (TI) and strontium (Sr). Similarly, with regard to environmental variables no association occurred between HAI scores and aqueous levels of potassium (K) and levels of zinc (Zn), phosphate (P) and Ti in sediment.

Table 5.1: Eigenvalues of the correlation matrix between health and environmental variables established using Redundancy analysis (RDA) for the once off survey.

Total Variance	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.953	0.029	0.002	0.001
Species-environment correlations	1.000	1.000	1.000	1.000
Cumulative percentage variance of species data	95.3	98.2	99.3	100.0
Cumulative percentage variance of species-environment relation	95.3	98.2	99.3	100.0

None of the biological or environmental variables were closely associated with the Luphephe-Nwanedi Dam site. Biological variables such as HSI, GSI and selenium (Se) muscle tissue levels were strongly associated with fish from Loskop Dam. In contrast water K levels were the variable most associated with the Rhenosterkop Dam site. The biological variable H_{ar} was strongly associated with pH and water magnesium levels.

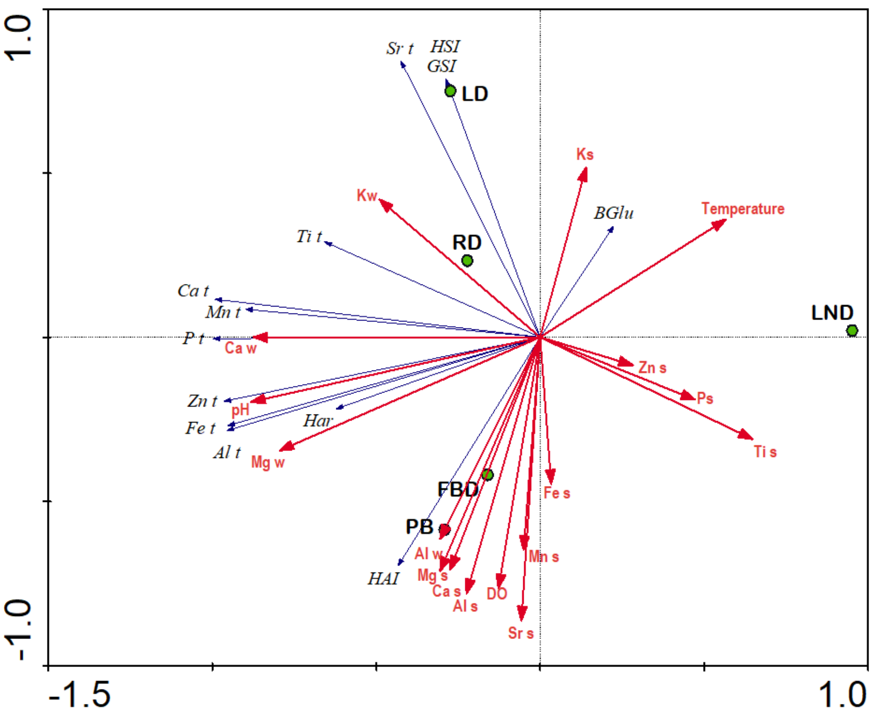

Figure 5.1: A triplot depicting the superposition of ordination of the redundancy analysis (RDA) conducted between biological and environmental variables associated with *Oreochromis mossambicus* sampled from Luphephe-Nwanedi (LND), Rhenosterkop (RD), Loskop (LD), Flag Boshielo (FBD) dams and Phalaborwa Barrage (PB). With biological parameters health assessment index (HAI), hepatosomatic index (HSI), gonadosomatic index (GSI) and blood glucose (BGlu) and various muscle tissue

metals indicated by subscript *t*. Water and sediment metals are indicated by subscript *w* and *s*, respectively.

From Figure 5.1 environmental variables Al_w, Mg_w, Mg_s, Ca_s, Al_s, DO, pH, biological variables that included Al_t, H_{ar}, GSI and blood glucose (BGlu) in addition to PO_4 (and indication of eutrophication) and K (an indication of fish condition) were selected and used to generate a global GLM. Manual backward selection was applied and the dredge function used to establish the variables that best predicted HAI scores. The variables that provided the best fit-model are provided in Table 5.2.

Table 5.2: Degrees of freedom (*df*), *F*-value and *p*-value of variables used to predict the health assessment index (HAI) and to test for significant differences using a generalised linear model for *Oreochromis mossambicus* collected from five different impoundments during a once-off survey.

Variable	*df*	*F*	*p*
Aluminium muscle tissue content (Al_t)	27	2.05	*
Blood glucose (BGlu)	27	2.25	ns
Fish condition (K)	27	0.06	ns
Water pH (pH)	14	2.44	ns
Mass x total length (Mass: TL)	27	1.23	ns
Sites	4	1.63	ns

* $p \leq 0.05$, (non-significant), ns = $p > 0.05$ (significant)

Of the variables tested, Al_t from specimens collected from the various sites had a weak but significant influence ($p < 0.01$) on the predictability and outcome of the model. The model was tested by fitting observed HAI scores against those predicted (Figure 5.2) to yield an $R^2 = 0.91$. The Q-Q plot and residual testing of this model are provided in the Appendix D. From Figure 5.2, the predictive model fits the observed data well with the exception that low HAI scores of 30 – 40 are at times being over estimated with higher HAI scores of 50 – 80 being underestimated. An artefact possibly influenced by 9% variance that the model fails to consider or incorporate. When removing the environmental and biological variables associated with Luphephe-Nwanedi Dam the model decreases to provide an $R^2 = 0.71$.

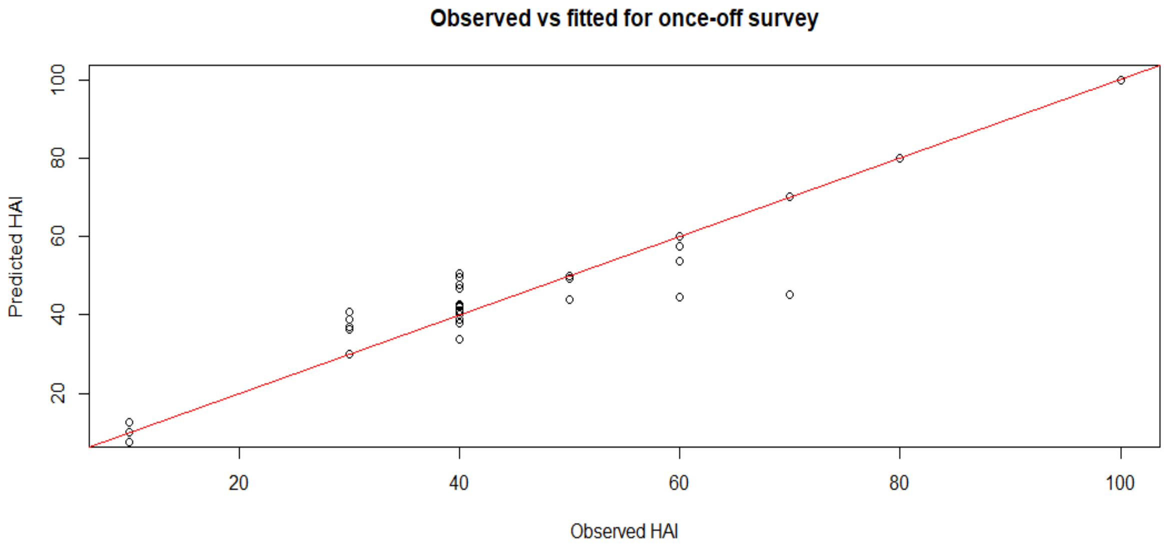

Figure 5.2: Observed vs fitted data of the model used to predict which variables best predict and effect HAI scores for *Oreochromis mossambicus* collected from the five localities.

5.3.2 Multivariate analysis of biological and environmental variables from surveys conducted at Flag Boshielo Dam.

The ordination diagram with the first axis (λ_1 = 57) displaying the relation of environmental with biological parameters for surveys done at Flag Boshielo Dam with the second axis indicative of the residual variation (λ_2 = 34) (see Figure 5.3). Axis 1, 2 and 3 account for 97.0% of the variation (Table 5.3). Variables strongly associated with the HAI scores were H_{ar} and to a lesser extent with GSI, aqueous iron (Fe), sodium (Na) and Mg in sediment. Variables that were collected during the 2nd, 3rd and 4th survey when water levels and inflow were low in Flag Boshielo Dam.

Table 5.3: Eigenvalues of the correlation matrix between health and environmental variables established using Redundancy analysis (RDA) for surveys conducted at Flag Boshielo Dam.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.566	0.337	0.059	0.037
Species-environment correlations	1.000	1.000	1.000	1.000
Cumulative percentage variance of species data	56.6	90.4	96.3	100.0
Cumulative percentage variance of species-environment relation	56.6	90.4	96.3	100.0

Fish condition (K) was at the centroid of the RDA plot. A weak association was observed between HAI and HSI, tissue concentrations of Zn and manganese (Mn) and blood glucose levels. Similarly, a weak association occurred between HSI and H_{ar}. Environmental variables NH_4^+, pH and water conductivity was strongly associated with the fifth survey when water levels and inflow were at the lowest.

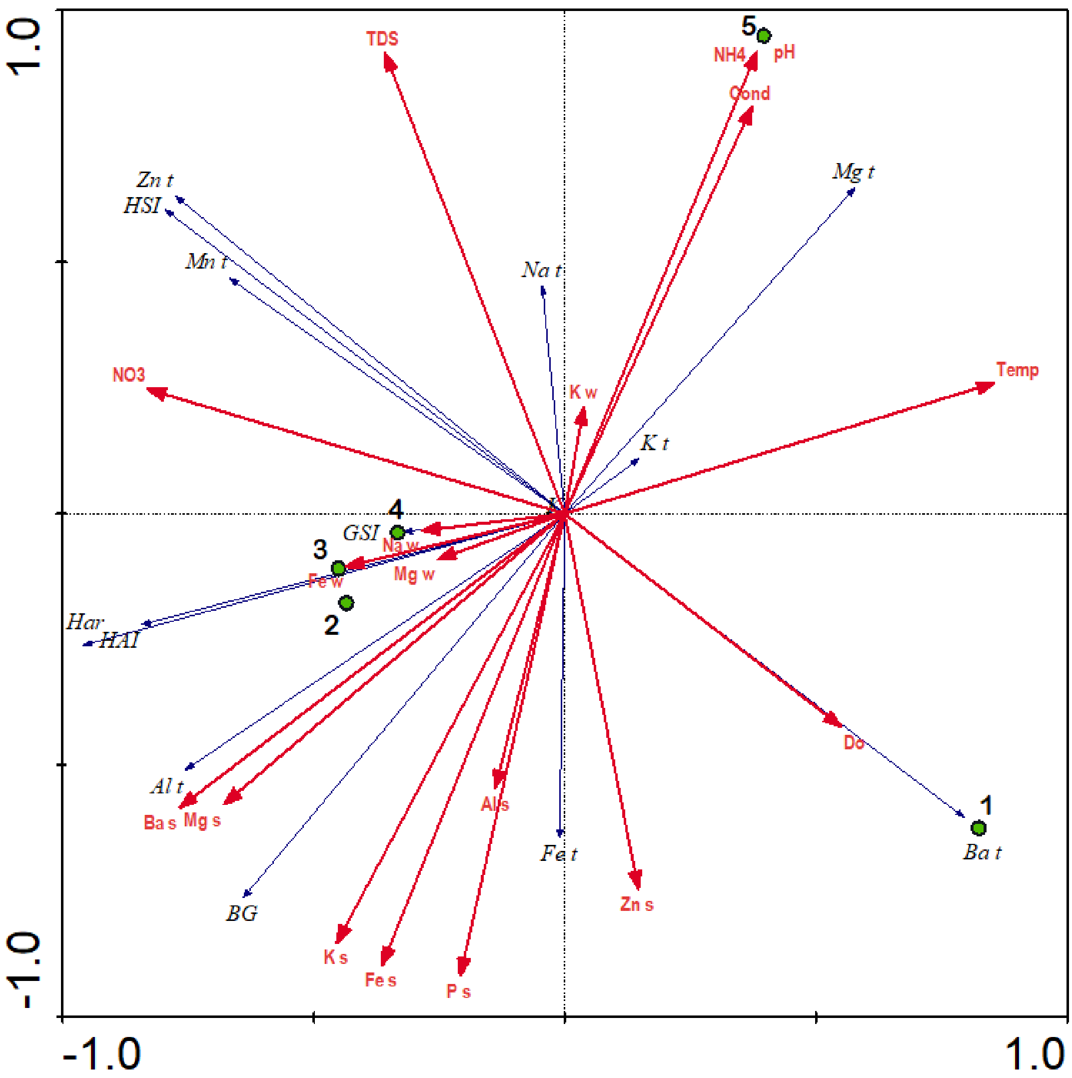

Figure 5.3: A triplot depicting the superposition of ordination of the redundancy analysis (RDA) of biological and environmental variables associated with monthly surveys with the health of *Oreochromis mossambicus* from Flag Boshielo Dam. With biological parameters health assessment index (HAI), hepatosomatic index (HSI), gonadosomatic index (GSI) and blood glucose (BGlu) and various muscle tissue metals indicated by subscript *t*. Water and sediment metals are indicated by subscript *w* and *s*, respectively. Numeral's 1, 2, 3, 4 and 5 indicate fixed variables February '16, April '16, May '16, June' 16 and October'16.

From Figure 5.3 environmental variables Al_s, K_s, P_s, Fe_w, Fe_s, Na_w, Mg_w, Mg_s, Ba_s, PO_4 and biological variables that included Al_t, H_{ar}, GSI, K and blood glucose (BGlu) were selected and used to generate a global GLM. The month surveyed was set as categorical using the as factor function in R. The variables that provided the best fit-model are provided in Table 5.4.

Table 5.4: Degrees of freedom (df), F-value and p-value of variables used to predict the health assessment index (HAI) and to test for significant differences using a generalised linear model for *Oreochromis mossambicus* collected during periods of high and low inflow from Flag Boshielo Dam.

Variable	*df*	*F*	*p*
Aluminium muscle tissue content (Al_t)	59	5.31	*
Blood glucose (BGlu)	50	1.48	ns
Fish condition (K)	59	1.48	ns
Gonadal somatic index GSI	50	1.36	ns
H_{ar}	36	1.36	ns
Flow levels	0	-	-

* $p \geq 0.05$, (non-significant), ns = $p < 0.05$ (significant)

Of the variables tested, Al_t had a weak but significant ($p < 0.01$) influence on the predictability and outcome of the model. To test the model observed HAI scores were fitted against those predicted (Figure 5.4). An $R^2 = 0.96$ was achieved. The Q-Q plot and residual testing of this model are provided in the Appendix D. Again, the over or underestimation of HAI scores can be attributed to 4% variance that the model fails to consider.

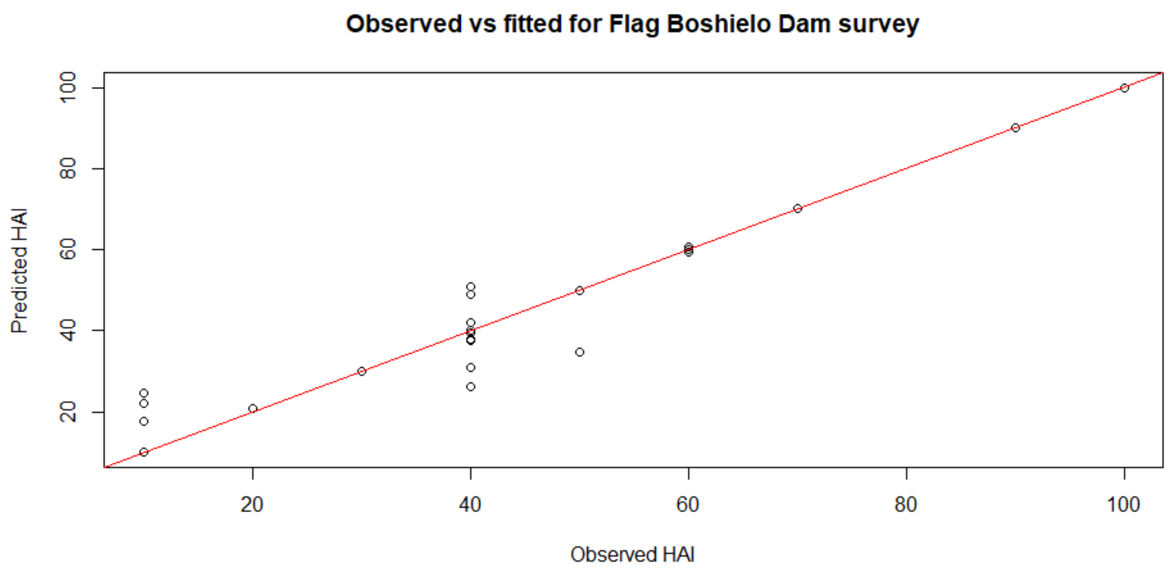

Figure 5.4: Observed vs fitted data for the model used to predict which variables best predict and effect HAI scores for *Oreochromis mossambicus* collected from Flag Boshielo Dam.

5.4 Discussion and conclusion

From both the once-off and monthly trials fish condition (K) was at the centroid of the RDA plots. This can be attributed to K fluctuating little between impoundments and monthly surveys conducted for *O. mossambicus*. An indication that K remains a crude and crucial indicator of fish health. From applying the a GLM to variables collected using both sampling techniques it appears that K, muscle tissue content of aluminium (Al*t*), blood glucose and H_{ar} provide a good estimate of HAI scores and possibly play an influential role to some degree on fish health. According to Mmualefe & Torto 2011, the solubility of Al increases during the alkaline and acidic conditions. Given the alkaline pH range that were recorded in this study, the chemical speciation of Al would appear to predominantly have been in the form of relatively non-toxic Al hydroxides $Al(OH)_3$ and $Al(OH)_4^-$ as opposed to the highly toxic Al^{3+} which predominates during acidic conditions (Dabrowski *et al.* 2014).

The influence of variables such as body mass and total length recorded in the once off survey and used in the GLM, and GSI data recorded on HAI scores during the pre- and post-spawning period of fish surveyed monthly in Flag Boshielo Dam are intuitive. Nonetheless, indicators such as Al_t and blood glucose and their application to better determine HAI scores require further investigation. However, unless a rapid and inexpensive assay to determine Al_t becomes available, the practical application of this variable in the field remains elusive since Al_t content observed this study could be region specific.

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CHAPTER 6: GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. Summary of findings

The findings of this study addressed the question raised in Chapter 1 as to what HAI scores are considered normal or abnormal for *Oreochromis mossambicus* sampled from a pristine, moderately polluted and polluted site. From the findings in this study, the answer would appear to be more complex as the water quality of an impoundment varies due to environmental factors and the influx of pollutants that, in turn, will affect the health of fish populations therein. In addition, this study substantiated the suitability of *O. mossambicus* as a model organism for biological monitoring, as this species provided a better understanding of the impacts of pollutants from various impoundments.

To meet the objectives set, the results of this study were discussed in relation to water quality, land use, climatic, topographic and geological factors associated with the sites surveyed. For surveys conducted between impoundments, water temperature and dissolved oxygen varied significantly ($p < 0.05$). Total dissolved oxygen, EC and salinity exhibited a positive correlation with each other and were highly significant ($p < 0.0001$) between the impoundments. Concentrations of PO_4^{-3} exceeded the target water quality range (TWQR) limits for all impoundments and SO_4^{-2} were highly significant, with Loskop Dam having concentrations that exceeded the TWQR limits. Metal levels in both water and sediments fluctuated between impoundments with Flag Boshielo Dam exhibiting high concentrations when compared with the other impoundments. Based on these variables, the state of impoundments in terms of good to poor water quality were revealed in the order of; Luphephe-Nwanedi Dams **<** Rhenosterkop Dam **<** Loskop Dam **<** Phalaborwa Barrage **<** Flag Boshielo Dam, categorising Luphepe-Nwanedi and Rhenoserkop dams as oligotrophic, Loskop Dam as mesotrophic and Phalaborwa Barrage and Flag Boshielo Dam as eutrophic. These findings are inconsistent with the water quality reported for these impoundment by the DWS (2017). The discrepancy can be attributed to possible defences between the period when samples were taken by DWS (2017) and this study. Furthermore, this study showed that seasonality and water flow regimes have an impact on the water quality of an impoundment and in turn the health of fish. For seasonal surveys Flag Boshielo Dam was found to fluctuate from mesotrophic conditions, during a period of high inflow, to eutrophic conditions when water inflow was low and aqueous nutrient loads and metal concentrations high.

With regard to biomarkers and indicators used, blood glucose levels were highly significant ($p < 0.0001$) between impoundments with the highest concentrations recorded for fish at Flag Boshielo Dam, confirming the water results. Thus, blood glucose as a biomarker in conjunction with health assessment index (HAI) scores seemed to be sensitive at evaluating the health of fish and, in turn, the water quality of the impoundments surveyed. However, since elevated blood glucose levels can occur in fish due to transporting and handling, further investigations are required to determine if the procedure of handling and transporting of fish had an impact on blood glucose levels. The HAI with the associated parasite index (PI) and inverted parasite index (IPI) were able to assess the health of fish and discriminate between the water quality of various impoundments and were in line with what the water quality variables revealed. Thus, this biomonitoring method based on fish health was able to discriminate between the water quality of the impoundments terms of good to poor water quality in the order of: Luphephe-Nwanedi Dams **<** Rhenosterkop Dam **<** Loskop Dam **<** Phalaborwa Barrage **<** Flag Boshielo Dam. The premise that a higher HAI score is an indicative of poorer water quality was emphasised and occurred in this study. The condition and somatic indices did not seem to be affected by the water quality of impoundments surveyed as these indicated that all fish sampled were generally in good health. An explanation is because these indices are not suitable for comparisons among fish populations as values can vary with the sex, size, season and degree of maturity (Froese 2006) as observed in fish sampled during monthly surveys at Flag Boshielo Dam. Thus, association between reproduction health and water quality needs to be investigated further. Differences in gill histopathology and arithmetic mean thickness of gill epithelium (H_{ar}) were recorded to be highly significant ($p < 0.0001$), with fish from Phalaborwa Barrage being most affected. These findings correlate with high aqueous aluminium (Al) and iron (Fe) levels detected above the TWQR at Phalaborwa Barrage, followed by levels recorded for the same metals at Flag Boshielo, Loskop, Rhenosterkop and Luphephe-Nwanedi dams.

Certain metals were found to be high in muscle tissues of fish sampled from the various impoundments. Converse to determining metal concentrations in water, knowledge of metal content in fish muscle tissue did not seem to discriminate between impoundments in terms of water quality. The reason can be due to the fact that fish bioaccumulate metals from their immediate environment over time. Moreover, monthly surveys indicated that metal content in body tissue can vary seasonally and with fluctuating water levels. Therefore, determination of metal bioaccumulation should be done seasonally and/or at the biological organisation level e.g. using blood, various tissues and/or organ samples.

In this study the biomarkers that best described fish health and, in turn, the status of an impoundment were the HAI with the associated PI and IPI, blood glucose levels, gill histopathology and H_{ar} values. Since there was little difference in what HAI, HAI with PI and HAI with IPI scores revealed, the HAI approach, whether used solely or in association with PI and IPI remains a rapid and inexpensive means for evaluating fish health and the water quality of aquatic systems. However, in conjunction with the HAI approach, future studies should consider measuring blood glucose as the addition of this rapid and easy assessment and can help define health results further.

6.2 Future research and recommendations

With regard to the implementation of the HAI, in this study only a few parasites were identified to species level. Observations of parasite burden would, however, seem to indicate that various groups and species of parasites differ in their response to water contaminants. This requires further investigation whereby experiments can be run in controlled laboratory conditions to test the susceptibility of parasites to contaminants. With regard to liver discolorations, further studies should be done to establish a scoring system relevant for *O. mossambicus* that allows for a finer scoring system e.g. 0, 10, 20 and 30 can be used to classify between a healthy and diseased liver. Furthermore, each fish species needs to have its own unique colour chart and scoring system based on the biology and feeding preference to refine the scoring system associated with liver colour. Currently when using the colour chart to score liver colour, when brown, the liver is given a score of 30 since it is considered to be unhealthy and a score of zero when observed to be red, based on the assumption that it is a healthy liver. No scores are provided in-between for any shade other than brown and red. However, due to the feeding preference of *O. mossambicus*, a species that is able to consume blue-green algae that is toxic to most aquatic animals, brown livers in adults may be perfectly normal and healthy. It would, therefore, be of interest to do feeding trials using fry, juveniles and adults to assess if any changes occur in liver colours based on dietary intake.

Since HAI, HAI with PI and HAI with IPI yielded no significant differences when assessing fish health, these indices can be used individually, depending on the objectives of the study and research question. For example, Adams *et al.* (1993) found that the scoring of ecto- and endoparasites by the mere observance of their presence or absence allowed for the differentiation between impacted and less impacted sites, while Crafford and Avenant-Oldewage (2009), Watson *et al.* (2012) and Madanire-Moyo *et al.* (2012) identified parasites to species level and used the HAI with PI and IPI to differentiate between impacted sites. A comparative study should be conducted between the original HAI by Adams *et al.* (1993), Crafford and Avenant-Oldewage (2009) and the adapted version by Madanire-Moyo *et al.* (2012), to establish if there are significant differences between HAI scores when determining fish health.

Although the biological parameters evaluated in this study have merit for use as biomonitoring tools they, except for K, require that the test organism be sacrificed. The use of the metabolomics approach, which requires only a drop of blood to obtain data, may provide a suitable alternative. In this study blood drops using a Whatman 901 filter card were collected from each specimen sampled. These blood samples should be analysed to determine which metabolites may be more suitable at determining fish health and/or may complement HAI results reported here.

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APPENDIX A

Table A 1: Fish health variables with assigned characters showing the norm and deviation from the norm in the necropsy based system (adapted from Adams *et al.* 1993).

Variables	Variable condition	Original field designation	Substituted value for the HAI
External variables			
Length	Total length in millimetres	mm	-
Weight	Weight in grams	G	-
Eyes	Normal	N	0
	Exophthalmia	E1/E2	30
	Haemorrhagic	H1/H2	30
	Blind	B1/B2	30
	Missing	M1/M2	30
	Other	OT	30
Fins	No active erosion or previous erosion healed over	0	0
	Mild active erosion with no bleeding	1	10
	Severe active erosion with haemorrhage / secondary infection	2	20
Skin	Normal, no aberrations	0	0
	Mild skin aberrations – “black spot” < 50	1	10
	Moderate skin aberrations – “black spot” > 50	2	20
	Severe skin aberrations	3	30
Opercules	Normal/no shortening	0	0
	Mild/slight shortening	1	10
	Severe shortening	2	20
Gills	Normal	N	0
	Frayed	F	30
	Clubbed	C	30
	Marginate	M	30
	Pale	P	30
	Other	OT	30
Pseudobranch	Normal	N	0
	Swollen	S	30
	Lithic	L	30
	Swollen and lithic	P	30
	Inflamed	I	30
	Other	OT	30
Thymus[a]	No haemorrhage	0	0
	Mild haemorrhage	1	10
	Moderate haemorrhage	2	20
	Severe haemorrhage	3	30
Internal variables (necropsy)			
Mesenteric fat	(Internal body fat expressed regarding amount present)		
	None	0	-
	Little, where less than 50% of each cecum is covered	1	-
	50% of each cecum is covered	2	-
	More than 50% of each cecum is covered	3	-
	Caeca are completely covered by large amount of fat	4	-

Table A 1 continued….

Variables	Variable condition		Original field designation	Substituted value for the HAI
Spleen	Black		B	0
	Red		R	0
	Granular		G	0
	Nodular		NO	30
	Enlarge		E	30
	Other		OT	30
Hindgut	Normal, no inflammation or reddening		0	0
	Slight inflammation or reddening		1	10
	Moderate inflammation or reddening		2	20
	Severe inflammation or reddening		3	30
Kidney	Normal		N	0
	Swollen		S	30
	Mottled		M	30
	Granular		G	30
	Urolithic		U	30
	Other		OT	30
Liver	Red		A	0
	Light red		B	30
	"Fatty" liver, "coffee with cream" colour		C	30
	Nodules in liver		D	30
	Focal discolouration		E	30
	General discolouration		F	30
	Other		OT	30
Bile	Yellow or straw colour, bladder empty or partially full		0	-
	Yellow or straw colour, bladder full, distended		1	-
	Light green to "grass" green		2	-
	Dark green to dark blue-green		3	-
Blood (haematocrit)	Normal range		30-45%	0
	Above normal range		>45%	10
	Below normal range		19-29%	20
	Below normal range		<18%	30
Parasites	No observed parasites		0	0
	Few observed parasites		1	10
*Endoparasites[b]	No observed endoparasites		0	0
	Observed endoparasites	< 100	0	10
		101 -1000	1	20
		> 1000	3	30
*Ectoparasites[b]	No observed ectoparasites		0	0
	Observed ectoparasites	1 – 10	1	10
		11 – 20	2	20
		> 20	3	30

a - no values were assigned to these values in the original HAI
b - Refinement of the HAI, variables inserted during previous studies

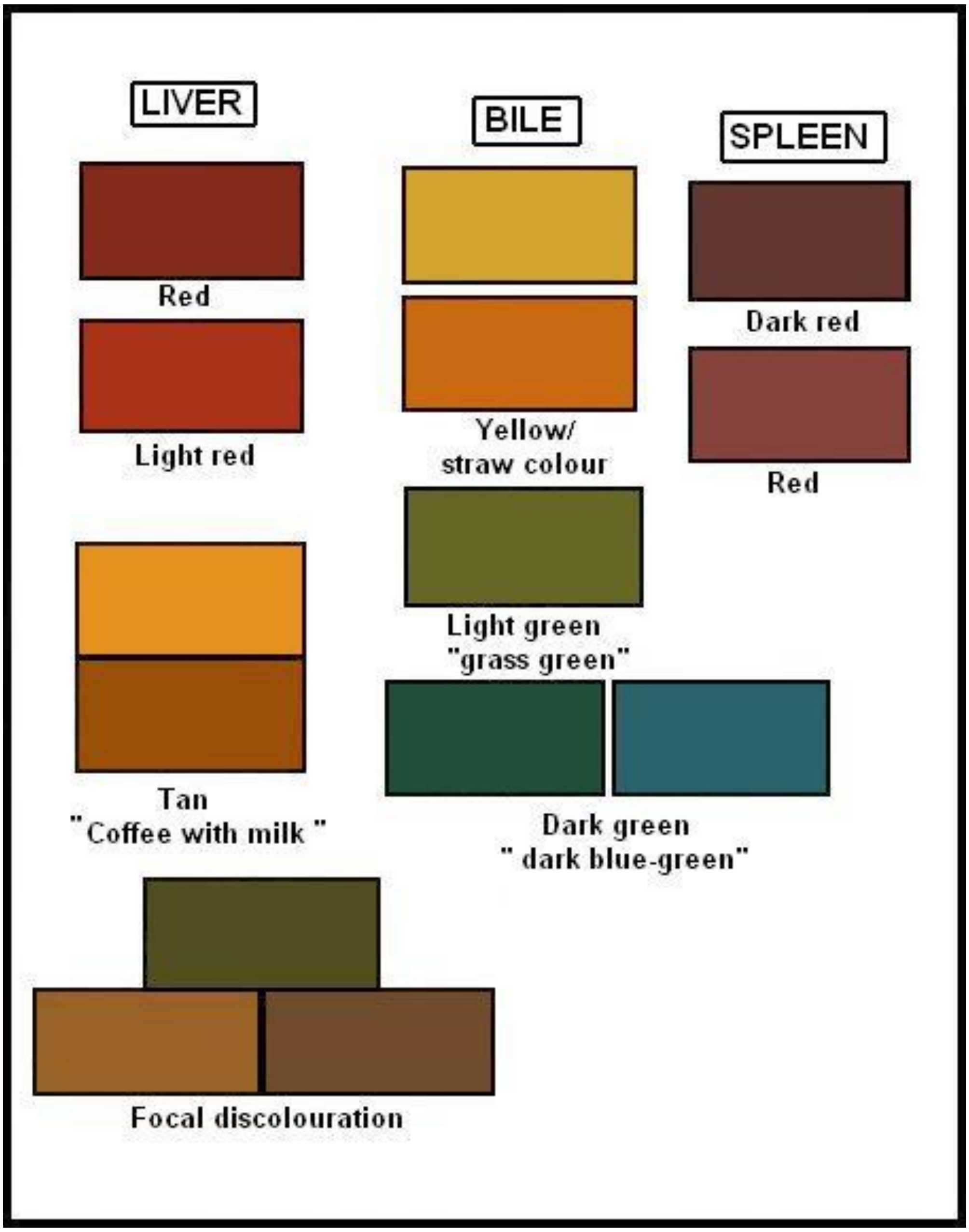


Figure A1: Colour chart used to compare the colour of liver, bile and spleen (Source: Watson *et al.* 2012).

Table A 2: Preparation procedure in the automated processor (Model: LEICA EMTP, Leica Microsystems)

Process	Vial	Chemical	Time
Buffer	1	Millonig's buffer	1x15 minutes
	2	Millonig's buffer	1x15 minutes
Post fixative	3	Osmium tetroxide (OsO_4)	1x1 hour
Buffer	4	Millonig's buffer	1x15 minutes
Rinse	5	Distilled water	1x15 minutes
	6	Distilled water	1x15 minutes
enblock staining	7	Uranyl acetate (UrAc)	1x40 minutes
Rinse	8	Distilled water	1x15 minutes
	9	Distilled water	1x15 minutes
Dehydration		Ethanol	
	10	50% EtOH	1x20 minutes
	11	70% EtOH	1x20 minutes
	12	80% EtOH	1x20 minutes
	13	90% EtOH	1x20 minutes
	14	95% EtOH	1x20 minutes
	15	100% EtOH	1x20 minutes
	16	100% EtOH	1x20 minutes
Infiltration		Propylene oxide	
	17	100%	1x20 minutes
	18	100%	1x20 minutes
Resin infiltration		Epoxy resin: propylene oxide	
	19	1:3	1x1 hour
	20	1:1	1x8 hours
	21	3:1	1x1 hour

APPENDIX B

Table B1: Metals and metalloids detected in sediment samples from Luphephe-Nwanedi, Loskop, Flag Boshielo dams and Phalaborwa Barrage.

Metals	Impoundment			
	LND	LD	FBD	PB
Ag	NA	0.0	0.0	1.0
Al	27972.0	27436.0	79353.0	79269.0
As	10.0	7.4	15.0	1.3
Au	NA	0.0	0.0	0.2
B	14.0	6.0	15.0	0.0
Ba	149.0	110.0	268.0	148.0
Be	3.0	1.8	4.5	0.0
Bi	0.0	0.0	0.1	0.3
Ca	2122.0	294.0	4471.0	28575.0
Cd	NA	0.0	0.2	0.0
Ce	15.0	6.5	32.0	30.0
Co	17.0	8.2	24.0	19.0
Cr	136.0	18.0	146.0	382.0
Cs	1.4	2.0	2.0	2.5
Cu	60.0	14.0	56.0	23.0
Dy	0.8	0.5	1.8	3.7
Er	0.5	0.3	1.2	0.0
Eu	NA	0.0	0.3	0.7
Fe	45426.0	48963.0	64389.0	59981.0
Ga	34.0	25.0	67.0	10.0
Gd	1.0	0.5	2.1	4.1
Ge	1.6	1.1	2.4	0.6
Hf	NA	0.0	0.0	1.9
Hg	NA	0.0	0.0	0.0
Ho	NA	0.0	0.4	0.6
In	NA	0.0	0.1	0.0
Ir	NA	0.0	0.0	0.0
K	16009.0	28503.0	14812.0	6015.0
La	7.7	2.5	15.0	0.0
Li	32.0	14.0	41.0	28.0
Lu	NA	0.0	0.2	0.2
Mg	1104.0	588.0	3568.0	18743.0
Mn	883.0	536.0	2815.0	1214.0
Mo	0.8	1.0	2.6	0.1
Na	3912.0	1170.0	2333.0	2203.0
Nb	22.0	12.0	31.0	2.2
Nd	1.1	0.0	0.0	12.0
Ni	308.0	293.0	744.0	79.0
Os	0.0	0.0	0.0	0.0
P	961.0	612.0	1140.0	323.0

Table B1 continued….

Metals	Impoundment			
	LND	LD	FBD	PB
Pb	21.0	17.0	21.0	13.0
Pd	0.0	0.0	0.0	0.0
Pr	1.6	0.6	3.3	3.2
Pt	0.0	0.0	0.0	0.0
Rb	27.0	75.0	49.0	23.0
Rh	0.0	0.0	0.0	0.0
Ru	0.0	0.0	0.0	0.0
Sb	0.0	2.8	0.8	0.0
Sc	4.7	4.1	11.0	25.0
Se	0.0	0.0	1.2	0.5
Si	229474.0	331285.0	197699.0	187070.0
Sm	1.5	0.5	4.1	2.9
Sn	1.5	0.0	5.6	0.9
Sr	14.0	2.4	19.0	40.0
Ta	NA	0.0	0.0	0.0
Tb	NA	0.0	0.4	0.5
Te	NA	0.0	0.0	0.0
Th	NA	0.0	4.5	8.3
Ti	4806.0	2258.0	4211.0	2195.0
Tl	0.5	0.9	0.4	0.6
Tm	NA	0.0	0.2	0.3
U	2.8	2.8	3.4	1.6
V	83.0	18.0	114.0	96.0
W	1.4	0.6	1.6	0.4
Y	7.8	4.3	20.0	6.5
Yb	0.5	0.3	1.0	0.0
Zn	91.0	79.0	148.0	17.0
Zr	202.0	329.0	171.0	18.0

Table B2: Metals and metalloids detected in water samples from Flag Boshielo Dam during monthly surveys.

Month	Al	B	Ba	Ca	Fe	Ga	K	Mg	Mn	Na	P	Si	Sr	Ti	Zn
Apr-16	NA	0.010	0.027	14	NA	NA	4.0	12	NA	27	NA	5.2	0.087	0.030	0.033
Apr-16	NA	0.011	0.022	16	NA	NA	4.8	18	NA	46	0.015	6.4	0.110	0.033	0.035
Oct-16	NA	0.020	0.042	33	0.065	NA	6.6	24	0.056	57	0.065	7.0	0.195	0.068	0.042
Oct-16	NA	0.027	0.076	33	0.453	0.013	5.4	23	0.189	52	0.092	6.4	0.196	0.073	0.043
Oct-16	0.315	0.074	0.219	45	1.92	0.033	7.0	61	0.749	201	0.267	1.0	0.352	0.103	0.042
Dec-16	NA	0.033	0.026	31	0.035	0.001	6.9	25	0.028	62	NA	6.9	0.139	0.026	0.043
Dec-16	0.101	0.051	0.057	40	0.285	0.003	7.3	31	0.063	78	NA	6.0	0.155	0.035	0.094
Dec-16	NA	0.033	0.093	36	0.078	0.005	7.2	25	0.093	63	0.262	6.4	0.143	0.030	0.027
Feb-17	0.014	0.031	0.026	23	NA	0.002	5.3	15	NA	41	0.016	8.3	0.102	0.039	0.441
Feb-17	0.874	0.017	0.059	18	1.14	0.005	5.0	10	0.035	27	0.060	10.3	0.066	0.032	0.189
Feb-17	0.114	0.027	0.047	23	0.173	0.003	5.3	12	NA	37	NA	8.4	0.095	0.022	0.080

Table B3: Metals and metalloids detected sediment samples from Flag Boshielo Dam during monthly surveys.

Month	Ag	Al	As	Au	B	Ba	Be	Bi	Ca	Cd	Ce	Co	Cr	Cs	Cu
Feb-16	0.000	36220	10	0.00	1.83	133	3.13	0.000	1989	0.000	18	21	149	1.07	50
Feb-16	0.00	30323	11	0.000	20	188	2.86	0.000	4956	0.000	7.69	19	125	1.37	37
Apr-16	0.000	79353	15	0.000	15	268	4.48	0.106	4471	0.176	32	24	146	2.02	56
Apr-16	0.000	17806	8.57	0.000	0.000	226	2.13	0.000	3441	0.000	5.19	22	145	1.10	42
Apr-16	0.000	27101	2.58	0.000	0.000	348	0.751	0.000	9165	0.000	4.86	12	83	0.833	14
Sep-16	0.000	50712	3.27	0.000	11	492	1.80	0.162	64212	0.000	67	3.98	49	2.14	7.28
Sep-16	0.000	60404	3.28	0.000	8.18	333	2.59	0.201	8347	0.000	74	7.13	76	3.24	9.10
Sep-16	0.000	65280	2.87	0.000	12	328	0.000	0.294	9382	0.000	86	9.56	102	3.37	14
Oct-16	0.000	67278	4.55	0.007	17	300	2.79	0.000	9135	0.000	42	11	126	3.27	18
Oct-16	0.000	34920	1.52	0.000	5.48	323	1.30	0.087	8603	0.000	32	7.28	64	1.19	6.78

Table B3 continued….

Month	Dy	Er	Eu	Fe	Ga	Gd	Ge	Hf	Hg	Ho	In	Ir	K	La	Li
Feb-16	0.929	0.494	0.000	53921	33	1.02	1.77	0.000	0.000	0.000	0.000	0.000	13725	8.58	35
Feb-16	0.605	0.320	0.000	45906	37	0.620	1.59	0.000	0.000	0.000	0.000	0.000	18096	4.39	26
Apr-16	1.82	1.18	0.290	64389	67	2.08	2.39	0.000	0.000	0.398	0.104	0.000	14812	15	41
Apr-16	0.434	0.307	0.207	44112	37	0.475	1.30	0.000	0.000	0.000	0.000	0.000	12921	2.98	25
Apr-16	0.696	0.498	0.207	34463	40	0.533	1.00	0.000	0.000	0.000	0.000	0.000	17892	1.91	9.42
Sep-16	7.65	0.000	1.13	32936	15	7.97	0.346	4.82	0.000	1.33	0.000	0.000	18457	0.000	0.000
Sep-16	10	5.44	0.972	47911	15	9.54	0.565	7.61	0.000	1.85	0.000	0.000	23729	34	22
Sep-16	12	5.25	1.11	58422	16	12	0.000	5.76	0.000	1.87	0.000	0.000	17574	0.000	31
Oct-16	7.08	3.63	0.639	59630	19	6.46	0.610	5.77	0.000	1.15	0.000	0.000	15964	19	41
Oct-16	5.46	2.91	0.722	50988	13	4.94	0.309	7.94	0.000	0.984	0.000	0.000	18433	16	11

Table B3 continued……

Month	Lu	Mg	Mn	Mo	Na	Nb	Nd	Ni	Os	P	Pb	Pd	Pr	Pt	Rb
Feb-16	0.000	1956	1243	0.888	3275	18	1.03	485	0.000	1074	22	0.000	1.91	0.000	28
Feb-16	0.000	1363	1319	0.914	4487	20	1.29	295	0.000	1050	20	0.000	0.978	0.000	23
Apr-16	0.157	3568	2815	2.63	2333	31	0.000	744	0.000	1140	21	0.029	3.34	0.000	49
Apr-16	0.000	1111	1225	0.712	4971	17	0.893	434	0.000	928	18	0.000	0.795	0.000	12
Apr-16	0.000	1580	709	0.311	8152	12	0.333	221	0.000	1439	7.49	0.000	0.578	0.000	28
Sep-16	0.460	3056	890	0.089	6349	4.69	0.000	9.54	0.000	599	25	0.000	7.00	0.000	41
Sep-16	0.655	3511	1137	0.272	6118	8.66	0.000	15	0.000	706	26	0.000	7.81	0.000	66
Sep-16	0.611	4063	1194	0.359	5079	7.64	33	20	0.000	757	35	0.000	9.08	0.000	48
Oct-16	0.387	4162	1449	0.432	3824	8.95	16	28	0.000	795	38	0.000	4.37	0.000	30
Oct-16	0.343	2556	865	0.215	7333	6.34	0.000	8.61	0.000	874	16	0.000	3.52	0.000	39

Table B3 continued……

Month	Rh	Ru	Sb	Sc	Se	Si	Sm	Sn	Sr	Ta	Tb	Te	Th	Ti	Tl
Feb-16	0.000	0.000	0.000	6.39	2.13	231565	1.58	1.55	12	0.000	0.000	0.000	0.000	4516	0.502
Feb-16	0.000	0.000	0.000	5.51	2.27	248459	0.892	1.32	20	0.000	0.000	0.000	0.00	4391	0.512
Apr-16	0.000	0.000	0.792	11	1.22	197699	4.08	5.57	19	0.000	0.354	0.000	4.51	4211	0.406
Apr-16	0.000	0.000	0.000	6.22	0.437	260840	0.683	0.616	25	0.000	0.000	0.000	0.00	5230	0.449
Apr-16	0.000	0.000	0.000	6.60	0.452	337108	0.607	0.000	57	0.000	0.149	0.000	0.000	11051	0.313
Sep-16	0.000	0.000	0.134	7.96	0.000	253199	6.46	1.47	56	0.126	0.941	0.000	13	2190	0.000
Sep-16	0.000	0.000	0.289	10	0.000	277598	7.33	0.000	27	0.599	1.21	0.000	21	4628	0.856
Sep-16	0.000	0.000	0.000	15	0.000	276878	8.33	2.26	26	0.469	1.37	0.000	23	5149	0.916
Oct-16	0.000	0.000	0.383	15	0.000	210080	4.55	2.47	25	0.805	0.843	0.000	27	4958	0.910
Oct-16	0.000	0.000	0.000	9.78	0.000	318925	3.34	0.931	31	0.284	0.579	0.000	9.65	9386	0.643

Table B3 continued……

Month	Tm	U	V	W	Y	Yb	Zn
Feb-16	0.000	2.85	90	1.43	7.27	0.475	109
Feb-16	0.000	2.72	72	0.990	4.26	0.350	76
Apr-16	0.162	3.36	114	1.60	20	0.950	148
Apr-16	0.000	2.23	80	1.30	2.88	0.334	94
Apr-16	0.000	1.58	56	0.275	3.93	0.533	29
Sep-16	0.640	0.000	0.000	0.000	14	3.85	90
Sep-16	0.917	5.38	46	1.59	22	5.47	20
Sep-16	0.863	5.39	0.000	1.84	22	0.000	31
Oct-16	0.572	0.000	79	0.000	13	3.37	38
Oct-16	0.485	0.000	53	1.24	12	3.12	13

APPENDIX C

Table C1: Health assessment index of *Oreochromis mossambicus* from Luphephe-Nwanedi dams during May 2016

Fish NO	SL (cm)	TL (cm)	Mass (g)	sex	eyes	skin	fins	operculum	gills	psedo-branch	liver	spleen	hindgut	kidney	Bile	Hct %	Parasites	HAI	Ecto PI	Endo PI	HAI/PI	Ecto IPI	Endo IPI	HAI with IPI
1	23.8	28.8	357.4	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
2	18.4	23	191.2	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	20	10	60	10	10	50
3	21.7	26.1	285	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
4	16.5	20.6	125.5	F	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	0	40	20	0	50
5	20.9	24.8	243.8	F	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
6	23.4	28.8	278.1	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	20	0	50	10	0	40
7	21.1	25.3	272.4	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	0	40	20	0	50
8	22	26.2	294.3	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
9	22.9	27.3	308.4	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
10	24.2	29.3	301.2	M	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60
11	21.4	25.9	293.7	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	0	40	20	0	50
12	25	29.9	326	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	0	10	40	30	10	70
13	20.3	24.8	234.9	M	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
14	19.9	23.3	240.3		0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	10	40	30	10	70
15	19.4	23.4	213.9	F	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
16	21.6	26.1	327	F	0	20	0	0	0	0	30	0	0	0	0	20	10	80	10	10	90	20	10	100
17	22.2	26.5	310.9	f	0	0	0	0	0	0	30	0	0	0	0	20	10	60	10	0	60	20	0	70
18	20.1	24.4	263.9	F	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60

Table C1 continued…

19	22.3	26.5	287.5	M4	30	0	0	0	0	0	30	0	0	0	0	0	10	70	10	10	80	20	10	90
20	21.2	24.5	259.5	M3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
21	22.6	26.7	355.8	M4	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
22	24.2	27.8	325.6	F2	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	0	50
23	22.2	26.2	337.3	M3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	0	40	20	0	50
24	23.5	26.9	416.4	M4	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
25	20.2	24	270.3	M2	0	0	0	0	0	0	30	0	0	0	0	20	10	60	10	0	60	20	0	70
26	19.2	22.5	183.5	F2	0	0	0	0	0	0	30	0	0	0	0	20	10	60	0	10	60	30	10	90
27	22.3	26.5	347.3	M4	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
28	23.5	28.1	348.8	M3	0	0	0	0	0	0	30	0	0	0	0	0	0	30	10	10	50	20	10	60

Table C2: Health assessment index of *Oreochromis mossambicus* from Rhenosterkop Dam during April 2016

Fish NO	SL (cm)	TL (cm)	Mass	sex	eyes	skin	fins	operculum	gills	psedo-b	liver	spleen	hindgut	kidney	Bile	Hct %	Parasites	HAI	Ecto PI	Endo PI	HAI/PI	Ecto IPI	Endo IPI	HAI with IPI
1	18.9	22.8	248.6	M2	0	0	0	0	0	0	30	0	0	0	0	20	10	60	0	10	60	30	10	90
2	19.2	23.5	287.9	F3	0	0	0	0	0	0	30	0	0	0	0	20	10	60	0	10	60	30	10	90
3	19.7	24	242.1	F3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	0	10	40	30	10	70
4	17	20.4	183.6	M2	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60
5	20	24.6	315.6	M2	0	0	0	0	0	0	30	0	0	0	0	0	10	40	0	10	40	30	10	70
6	18.7	22.5	225.3	F3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	0	10	40	30	10	70
7	20.3	24.5	285.7	F3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	0	10	40	30	10	70
8	19	22.3	229.9	M2	0	0	0	0	0	0	30	0	0	0	0	20	0	50	0	0	50	30	0	80
9	18.7	22.3	227.9	M2	0	0	0	0	0	0	30	0	0	0	0	0	10	40	0	10	40	30	10	70
10	18.4	21.2	198.9	M2	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
11	19.3	23.5	251.9	F4	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60
12	19.5	24.8	284.8	F4	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
13	15.5	19	138.5	F1	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
14	18.5	22.9	249.6	F4	0	0	0	0	0	0	30	0	0	0	0	0	10	40	20	10	60	10	10	50
15	18.5	21.9	207.3	F3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	20	10	60	10	10	50

Table C3: Health assessment index of *Oreochromis mossambicus* from Loskop Dam during May 2016

Fish NO	SL (cm)	TL (cm)	Mass	sex	eyes	skin	fins	operculum	gills	psedo-branch	liver	spleen	hindgut	kidney	Bile	Hct %	Parasites	HAI	Ecto PI	Endo PI	HAI/PI	Ecto IPI	Endo IPI	HAI with IPI
1	26.4	31.2	702.9	F4	0	0	0	0	0	0	30	0	0	0	0	20	0	50	0	0	50	30	0	80
2	32.3	38.6	1336.5	F5	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60
3	35.4	42.5	1827.1	M2	30	0	10	0	0	0	30	0	0	0	0	20	10	100	0	10	100	20	10	120
4	24.9	30.9	658.5	F3	0	0	10	0	0	0	30	0	0	0	0	0	10	50	10	0	50	20	0	60
5	25.1	30.8	680.3	F3	0	0	0	0	0	0	30	0	0	0	0	20	0	50	0	0	50	30	0	80
6	31.9	38.1	1271.3	F5	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60
7	31.3	35.4	1509.6	M3	0	0	0	0	0	0	30	0	0	0	0	20	0	50	0	0	50	30	0	80
8	45.3	47	2326.3	M2	0	0	0	0	0	0	30	0	0	0	0	20	0	50	0	0	50	30	0	80
9	33.8	39.3	1310.4	F4	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60
10	20.1	24	308	M1	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60

Table C4: Health assessment index of *Oreochromis mossambicus* from Flag Boshielo Dam during May 2016

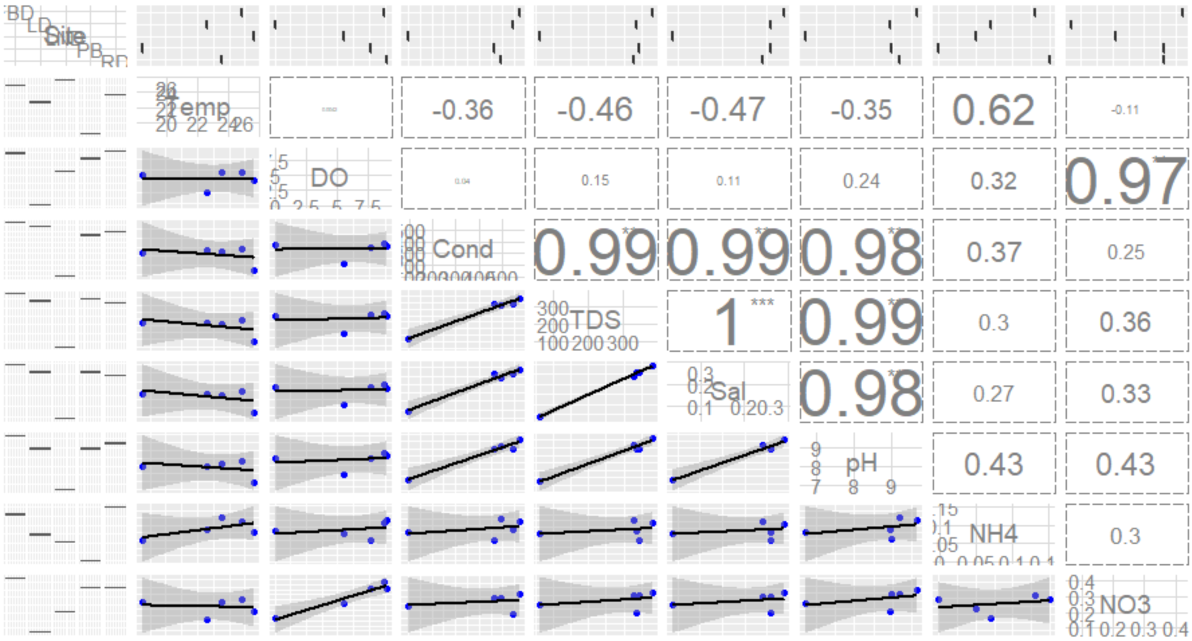
Fish NO	SL (cm)	TL (cm)	Mass	sex	eyes	skin	fins	operculum	gills	psedo-branch	liver	spleen	hindgut	kidney	Bile	Hct %	Parasites	HAI	Ecto PI	Endo PI	HAI/PI	Ecto IPI	Endo IPI	HAI with IPI
1	20.25	25	301.6	M3	0	0	0	0	0	0	30	0	0	0	0	20	10	60	20	0	70	10	0	60
2	25.1	29.5	451.2	M4	0	0	0	0	0	0	30	0	0	0	0	20	10	60	10	10	70	20	10	80
3	22.5	27.1	428.7	M4	0	0	0	0	30	0	30	0	0	0	0	30	10	100	30	0	120	0	0	90
4	21.5	26	346.2	M4	0	0	0	0	0	0	30	0	0	0	0	0	10	40	30	10	70	0	10	40
5	13.1	15.5	77.6	F3	0	0	0	0	0	0	30	0	0	0	0	30	10	70	30	10	100	0	10	70
6	17.5	21.8	186.4	M2	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	0	40	20	0	50
7	31.7	37.2	875.3	M3	0	0	0	0	30	0	30	0	0	0	0	0	10	70	10	0	70	20	0	80
8	32.9	39.1	906.6	M4	0	0	0	0	30	0	30	0	0	0	0	0	0	60	0	0	60	30	0	90
9	30.3	37.1	963.3	M4	30	0	0	0	0	0	30	0	0	0	0	0	0	60	0	0	60	30	0	90
10	22.9	27.5	425.6	M3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	0	10	40	30	10	70
11	22	26.1	401.5	F4	0	0	0	0	0	0	30	0	0	0	0	0	10	40	0	10	40	30	10	70
12	23.9	28	484.9	M3	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60
13	21	25.5	361.8	F3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	0	40	20	0	50
14	19.9	24	279.8	F4	0	20	0	0	0	0	30	0	0	0	0	0	10	60	30	10	90	0	10	60
15	19.8	23.9	283.4	F3	0	20	10	0	0	0	30	0	0	0	0	0	10	70	10	10	80	20	10	90
16	22.3	26.6	269.61	M2	0	20	0	0	0	0	30	0	0	0	0	0	10	60	10	10	70	20	10	80
17	20.7	24.5	299.8	F4	0	20	10	0	30	0	30	0	0	0	0	0	10	100	30	0	120	0	0	90
18	22.7	27.3	423.6	M2	0	20	20	0	0	0	30	0	0	0	0	0	10	80	30	0	100	0	0	70
19	23.7	28.2	474.6	M4	30	20	0	0	30	0	30	0	0	0	0	0	10	120	0	10	120	30	10	150
20	25.4	29.9	518.4	M3	0	20	0	0	0	0	30	0	0	0	0	0	0	50	0	0	50	30	0	80

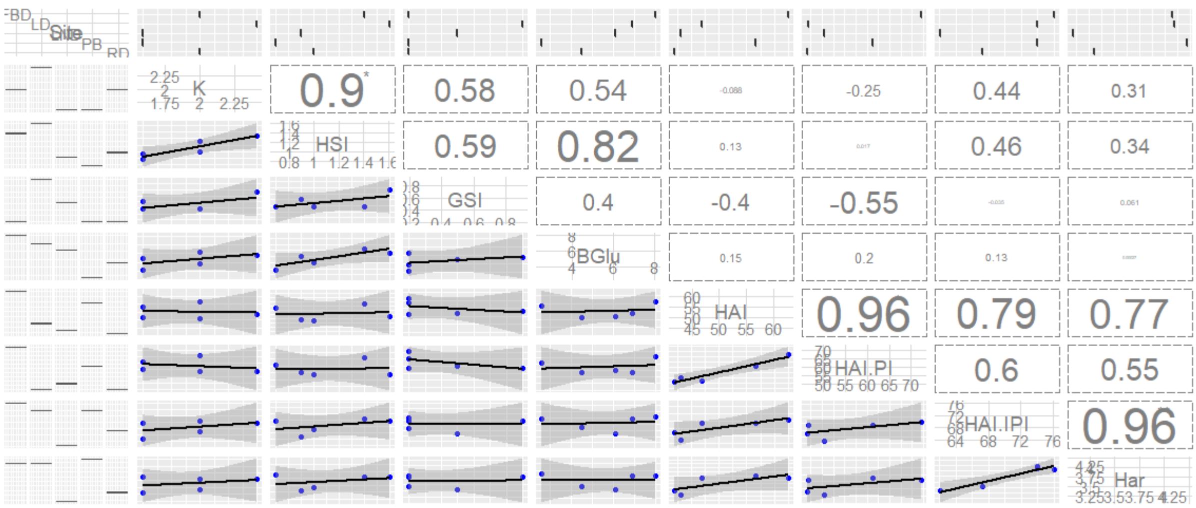
Table C5: Health assessment index of *Oreochromis mossambicus* from Phalaborwa Barrage during May 2016

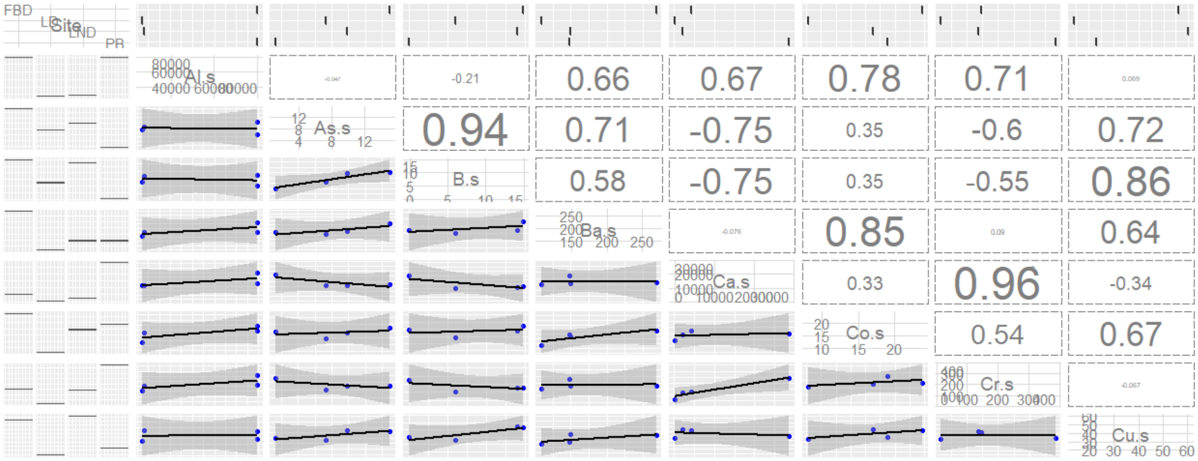
NO	SL (cm)	TL (cm)	Mass	Sex	eyes	skin	fins	operculum	gills	psedo-branch	liver	spleen	hindgut	kidney	Bile	Hct %	Parasites	HAI	Ecto PI	Endo PI	HAI/PI	Ecto IPI	Endo IPI	HAI with IPI
1	17.4	20.7	149	M2	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	0	40	20	0	50
2	16.4	20.2	115.9	M2	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
3	17.3	20.9	158.9	M3	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	0	40	20	0	50
4	17.1	19.7	103.3	F2	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
5	17.2	21	150.3	M2	0	0	0	0	0	0	30	0	0	0	0	0	0	30	0	0	30	30	0	60
6	17.3	20.5	153.8	M1	0	0	0	0	0	0	30	0	0	0	0	0	10	40	10	10	50	20	10	60
7	17.3	20.6	133.7	M1	0	0	0	0	0	0	30	0	0	0	0	20	10	60	10	0	60	20	0	70
8	18.3	22.2	134.1	F3	0	0	0	0	30	0	30	0	0	0	0	30	10	100	10	0	100	20	0	110
9	15.7	19.5	131.2	M2	0	0	0	0	0	0	30	0	0	0	0	30	10	70	10	0	70	20	0	80
10	16.3	19.2	118.4	F3	0	0	0	0	0	0	30	0	0	0	0	20	10	60	20	0	70	10	0	60
11	16.5	20.1	132.2	F3	0	0	0	0	0	0	30	0	0	0	0	30	10	70	10	10	80	20	10	90
12	16.7	20.3	126.2	M1	0	0	10	0	0	0	30	0	0	0	0	30	10	80	10	10	90	20	10	100
13	16.5	19.4	121.6	F3	0	0	0	0	0	0	30	0	0	0	0	30	0	60	0	0	60	30	0	90
14	16.1	19.3	132	M1	0	0	10	0	0	0	30	0	0	0	0	20	0	60	0	0	60	30	0	90
15	17.5	21.6	133	M3	0	0	0	0	0	0	30	0	0	0	0	20	10	60	10	10	70	20	10	80

APPENDIX D

Generilised linear models used to predict the variables that best predict and affect the HAI scores for *Oreochromis mossambicus* from the various impoundments during once off and monthly surveys.



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0.67
0.78
0.71
0.94
0.71
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0.35
-0.6
0.72
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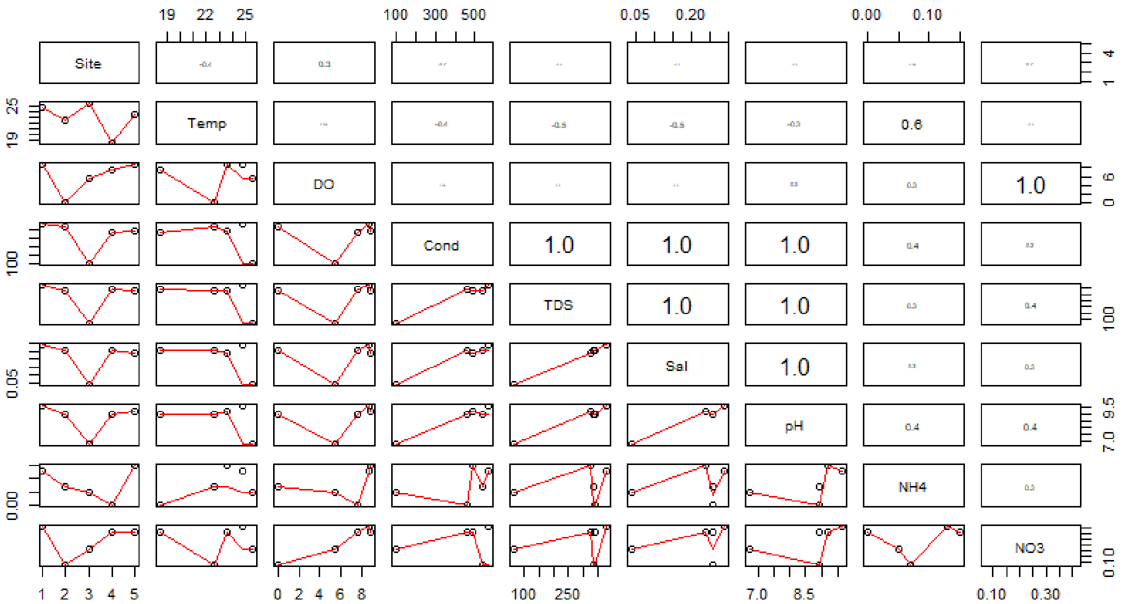
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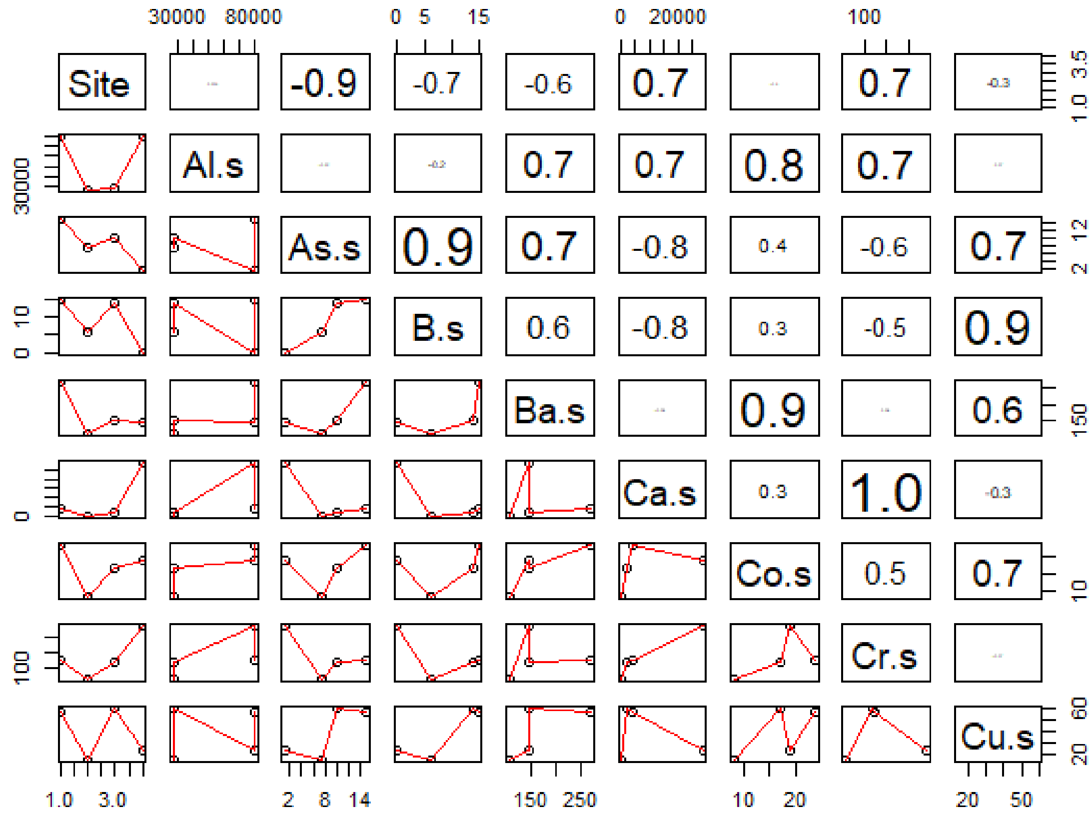
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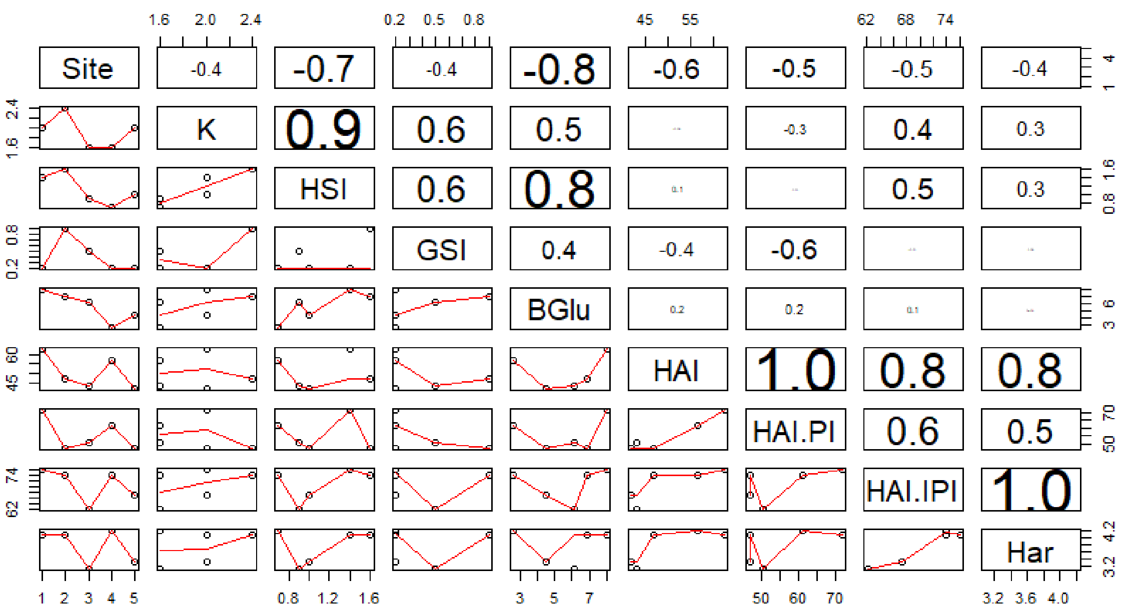
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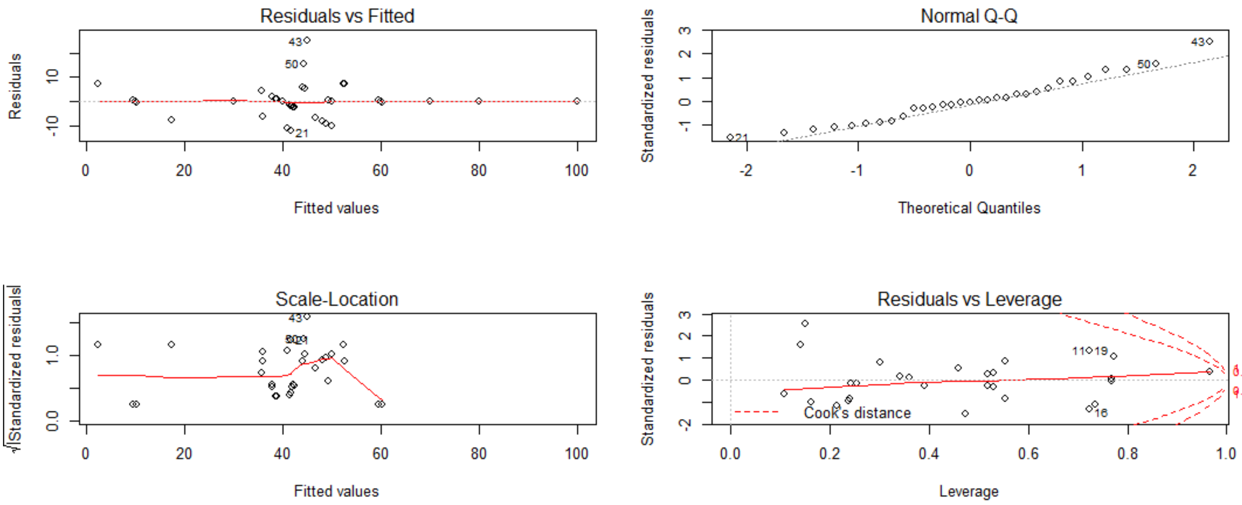
Har


Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Scale-Location
√|Standardized residuals|
Fitted values
Residuals vs Leverage
Standardized residuals
Cook's distance
Leverage