THE IMPACT OF WATER AND SEDIMENT QUALITY ON THE HEALTH OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) AND *LABEO ROSAE* (STEINDACHNER, 1894) AT THE PHALABORWA BARRAGE, OLIFANTS RIVER, LIMPOPO PROVINCE

by

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DELARATION

"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 in execution, and that all material contained herein has been duly acknowledged."

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M.L Mahlatji (Ms) **Date:**

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ABSTRACT

Contamination of aquatic ecosystems by agricultural and mining activities, as well as by industrial discharges and urbanization in the catchment of the South African river systems has been the cause of increasing public concern. These activities may result in water pollution. One such river is the Olifants River System in Mpumalanga and Limpopo provinces which is highly impacted.

The main aim of the study was to assess the impact of the water and sediment quality on the health of *Clarias gariepinus* and *Labeo rosae* and human risk when the fish is consumed at the Phalaborwa Barrage in the Lower Olifants River, Limpopo Province. This was achieved by assessing the water and sediment quality of the barrage at the three sampling sites; assessing the condition of the fish and the fish parasites by applying the fish HAI (Health Assessment Index) and PI (Parasite Index); determining the bioaccumulation of selected metals in the muscle tissue of the two fish species; and to determine the Human health risk factor upon consumption of fish contaminated with metals at the barrage.

Ten fish from each fish species were collected seasonally at each site using gill nets of different mesh sizes. Standards methods were followed when testing selected water and sediment constituents. For water quality sampling, the water samples were collected over four seasons (autumn, winter, spring and summer) at three sites (inflow, wall and below wall) from April 2010 to January 2011. Sediment samples were also collected using a Friedlinger mudgrab (225cm^3) at the three sites during winter and summer.

Fish hosts were examined for mobile ectoparasites, weighed and measured. Blood samples were drawn and skin smears were made. Fish were killed, dissected and all external and internal organs were examined as prescribed in the fish health assessment index. The condition factor was determined for each fish population. All parasites were collected, fixed and preserved using standard methods.

The highest water temperature (28.5 ºC) was in spring and lowest in winter (18 ºC). Overall pH was in an alkaline condition as it ranged from 7.4 to 8.7. Most water quality parameters were recorded at higher concentration in spring which includes; water

temperature, pH, conductivity, salinity, TDS, alkalinity, and turbidity. Major ions (cations and anions) recorded were all within the TWQR. Sulphates concentrations were all within the TWQR. Calcium concentration levels exceeded the typical concentration limit in spring but the levels were still within the TWQR for domestic use. Nutrients levels (nitrate, nitrite, sulphate) were very low which indicate oligotrophic conditions. Highest total nitrogen was recorded in spring which is an indicative of eutrophic conditions. Highest phosphorous concentrations were recorded in winter, spring and summer which are an indicative of eutrophic conditions. Noticeable elevated levels were recorded at the inflow in summer which is an indicative of hypertrophic conditions.

The results of the metals concentrations analysed demonstrated that metals within the barrage are present in trace amounts in the water. Analysis of the metal concentrations in water, sediment and fish muscle tissue revealed that the sediment contained the highest concentrations of metals followed by fish tissue and then the water. Metals that were detected in the water column includes; aluminium, antimony, arsenic, barium, boron, iron, manganese, selenium, strontium and tin. Metals that were detected at concentrations above the TWQR for aquatic ecosystem were; aluminium, antimony and selenium.

Arsenic, cadmium and chromium were recorded at concentrations above the detection limit as suggested by CCME. Zinc concentrations were recorded at levels below suggested detection limit. Aluminium, barium, boron, cadmium, chromium, manganese, selenium and strontium were all detected at elevated levels in water and above the TWQR. Among the metals recorded in the water column, significant seasonal variations in concentration were found for aluminium, barium, boron, lead selenium and strontium whereas only boron showed a significant variation between sites.

In comparison to the metals concentrations accumulated in the muscle tissue of both fish species. It was noted that *C. gariepinus* accumulated more metals than *L. rosae*; however *L. rosae* accumulated more metals at elevated concentrations than *C. gariepinus*. The metals that were accumulated at elevated levels in *C. gariepinus* were barium, boron, zinc and selenium. In *L. rosae*, iron, aluminium, strontium, titanium, vanadium and arsenic accumulated at elevated levels. All these metals pose a high risk to fish and human’s health.

The highest population HAI was recorded in summer for both fish species. The lowest HAI value was recorded in autumn for both fish species. Although a high HAI population value was recorded higher in summer for both fish species, in general both fish species from the barrage were in a normal condition. A high number of ectoparasites were recorded in *L. rosae* than in *C. gariepinus* while high numbers of endoparasites were recorded in *C. gariepinus* than *L. rosae*. The dominating ectoparasites for both species were from the class monogenea. Although the prevalence for *Contracaecum sp.* in *C. gariepinus* was 100% in all season, it did not influence the condition of the host. None of the parasites identified from both fish species neither reached alarmingly infection levels nor caused any visible damage to the host.

In conclusion, the water quality at the Phalaborwa Barrage is slightly polluted based on the water quality parameter(phosphorous) presence and recorded of some metals at the inflow that were recorded at elevated levels above the TWQR. Further recommendations are refereed such as extension of this study to all tributaries and to include other fish species in addition to constant monitoring of the impoundment.

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LIST OF ABBREVIATIONS

AEV Acute Effect Value

ATSDR Agency for Toxic Substances and Disease Registry

CEV Chronic Effect Value

CCME Canadian Council of Ministers of the Environment

CSIR Council for Scientific and Industrial Research

DWAF Department of Water Affairs and Forestry

DWA Department of Water Affairs

HAI Health Assessment Index

ICP-OES Inductively coupled plasma - optical emission spectrometry

IPI Inverted Parasite Index

PB Phalaborwa Barrage

PI Parasite Index

SAWQG South African Water Quality Guidelines

SQG's Sediment Quality Guidelines

TWQR Target Water Quality Range

UL University of Limpopo

US-EPA US United States Environmental Protection Agency

WHO World Health Organisation

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Freshwater is the most precious yet a limiting natural resource on earth. Although the earth surface is comprised of 70% of water, only 3% of it is made up of freshwater and it deteriorates mostly due to anthropogenic activities (Davies and Day 1998). Water quality problems caused by pollution is a major concern in the world due to expanding urban settlements, industrial sectors, and commercialized agriculture which contribute to the contamination (pollution) of the aquatic ecosystem (Dallas and Day 2004).

There is a global concern about the progressive pollution of valuable freshwater systems which most organisms, including humans, are reliant upon (Kotze et al. 1999). Water pollution refers to the contamination of the water bodies (e.g. rivers, lakes, oceans, aquifers, and ground water) resulted from anthropogenic activities, this can be harmful to plants and organisms living and /or relying to the water bodies (Heath and Claassen 1999). Aquatic ecosystems are increasingly affected by several anthropogenic impacts, like the elevated levels of nutrients which results in eutrophication, toxic contamination of industrial, agricultural and domestic origin, heat pollution, reaching the water bodies through their catchment area and the atmosphere (Davies and Day 1998).

The pollution of freshwater aquatic systems can be linked to point source discharges (waste water treatment works and industrial effluents) and diffuse surface runoff (agricultural, mining, industrial and urban) (Dallas and Day 2004). The Olifants River is impacted by several anthropogenic activities, particularly mining in the upper catchment, and urbanisation, industrial and agricultural activities in the upper and lower catchments. The major sources in the lower catchment areas of the Olifants River are municipal (treated and untreated sewage waste), mining and industrial discharges (Heath et al. 2010). A vast number of mines and industries, as well as the demographic situation in the catchment of the Olifants River, is an indicative of a possible pollution to the freshwater systems (Stimieet al. 2001). Earlier studies have shown that these activities resulted in the degradation of the Olifants River System

(Ashton and Dabrowski 2011). As a result of these anthropogenic activities, humans and animals as well as other biota, may be exposed to harmful contaminants (Davies and Day 1998, Moss 1998, Dallas and Day 2004).

Modern agriculture, industrialization and urbanisation have negatively affected environmental quality and aquatic systems (du Preezet al. 2003). Water quality is a term used to describe the chemical, physical, and biological characteristics of water, usually in respect to its suitability for an intended purpose .These characteristics are controlled or influenced by substances that are either dissolved or suspended in water (DWAF 1996a). Water quality plays an important role in the wellbeing of all users. Most water quality problems in South Africa are as a result of salinization, enrichment by plant nutrients, microbiological proliferation, particulate sedimentation and silt migration, and acidification (Chapman 1992). Water-borne pollutants (metals, sediment, nutrients, acidic compounds and biocides) are considered to be most important in terms of their impact on the aquatic ecosystems (Adams et al. 1993).

Fish parasites are indicative of many biological aspects of their hosts and they are receiving a lot of attention worldwide from parasite ecologists as potential indicators of environmental quality because of the variety of ways in which they respond to anthropogenic pollution (Sures et al.1999). Ectoparasites are found on the external surfaces such as skin, fins and gills (e.g. gill flukes, anchor worms, fish lice and leeches). Endoparasites are found in the internal tissues such as muscles and organs such as the alimentary canal, liver, and kidney (e.g. trematodes, cestodes, acanthocephalans and nematodes) (Paperna 1996). In addition, the ecto-parasites are exposed to the environment as their hosts whereby it was then assumed that poor water quality will adversely affect ectoparasites to a greater degree than it would to endo-parasites (Avenant-Oldewage 1994).

1.2 RATIONALE OF THE STUDY

Several of the rivers in South Africa are being adversely impacted by contaminated water due to constant increase in anthropogenic activities in the catchments. One of such rivers is the highly impacted Olifants River System in Mpumalanga and Limpopo provinces; a tributary of the trans-boundary Limpopo River System (Ashton 2010). The

Olifants River in the Limpopo province is subsequently receiving pollutants from the upper catchment, which can adversely impact the functions of the aquatic ecosystem in the province. The Olifants River System is the third most polluted river systems in South Africa and has been polluted by anthropogenic activities from agricultural and mining activities, industrial development and urbanisation over the years and contaminated with metals, chemicals and organic pollutants (Heath et al. 2010, Ashton and Dabrowski 2011). There have been reports on major unexplained crocodile and fish kills in the upper sub-catchment (Loskop Dam) and lower sub-catchment (KNP) between 2008 and 2009 which renewed interest in research on water quality problems in the Olifants River System (de Villiers and Mkwelo 2009; van Vuuren 2009; Ashton 2010; Heath et al. 2010; Ashton and Dabrowski 2011).

The communities around the catchment area consume fish from the impoundments, they are potentially at risk to genotoxic, carcinogenic and non-carcinogenic health risks, including developmental, reproductive and chronic systemic effects, through long-term exposure to contaminants that have bio-accumulated in fish tissue (du Preezet al. 2003).

Several studies (system (Seymore et al. 1994, 1995; du Preez et al. 1997; Robinson and Avenant-Oldewage 1997; Avenant-Oldewage and Marx 2000a, b) have been done in the lower catchment (Kruger National Park) on water quality, bioaccumulation and fish health, however, none of the bioaccumulation studies done on the Olifants River linked bioaccumulation of metals in the fish muscle tissues to human health. This study formed part the Water Research Council (WRC) K5/1929 project (Jooste et al. 2013) and VLIR-UOS project of the Department of Biodiversity, University of Limpopo to determine the impact of water and sediment quality on the fish health and fish parasite diversity in the Olifants River, Limpopo province.

1.3 RESEARCH QUESTIONS

1. What is the quality of the water and sediment of the Olifants River at the Phalaborwa Barrage?
2. What is the impact of water and sediment quality on the abundance and infestation rate of parasites at the Phalaborwa Barrage?

3. What is the level of accumulation of metals in the fish muscle tissues at the Phalaborwa Barrage?
4. Are there any health risks to humans upon consumption of fish in that area?
5. What is the fish health of *Clarias gariepinus* and *Labeo rosae* when applying the fish Health Assessment Index (HAI) and Parasite Index (PI)?
6. What is the seasonal abundance and variability of ecto- and endo-parasites and their effects on the two fish species?

1.4 AIM OF THE STUDY

The aim of the study was to assess the impact of water and sediment quality and bio-accumulation of metals on the health and parasites of *Clarias gariepinus* and *Labeo rosae* and to determine the human risk associated with consumption of the fish in the Phalaborwa Barrage in the lower Olifants River in the Limpopo Province

1.5 OBJECTIVES

The aim was achieved by the following objectives:

- To assess the water and sediment quality of the Phalaborwa Barrage by determining the level of physical and chemical substances in the water and sediment at the three sites (inflow, wall and below);
- To determine the concentration levels of selected metals in the muscle tissue of the two fish species and;
- To determine the potential human health risk upon consumption of metal contaminated fish from the barrage;
- To assess the health of *Clarias gariepinus* and *Labeo rosae* by applying the fish Health Assessment Index (HAI) and Parasite Index (PI);
- To determine the seasonal abundance and variability of ecto- and endo-parasites and their effects on the two fish species.

1.6 LITERATURE REVIEW: OLIFANTS RIVER SYSTEM

The Olifants River is one of the rivers that contain high silt loads, salinity and pollutant levels (Grobler et al. 1994). Sediments have been widely used as environmental indicators due to their ability to store contaminants as well as to act as trace contamination sources (US-EPA 2003). Sediment refers to depositional materials with grain sizes from sand, through silt to clay. Fine sediment often contains highest concentrations of contaminants on a dry weight basis because they have a higher relative surface area and thus increased density of sorption sites (US-EPA 2003). Poor cultivation management in the agricultural sector and bad mining practices in the lower sub-catchment have caused extensive soil erosion, resulting in an increase in sediment loads. This has a detrimental effect not only on benthic organisms, but also on the breeding, feeding and refuge areas of fish (van Vuren et al. 1994).

Numerous of South Africa's aquatic ecosystems have been degraded over the past few decades. The Olifants River in Mpumalanga and Limpopo Province is one of the main river systems in South Africa and it has been described as one of the most polluted rivers in Southern Africa, with the Loskop Dam acting as the origin for pollutants from the upper catchment area (Grobler et al. 1994; Ashton and Dabrowski 2011). People living near the Olifants River catchments make use of the river and impoundments in the system for daily living purposes; for example, drinking the water and consuming the fish daily from the Olifants River catchment. The Phalaborwa Barrage has been providing water to the community for drinking, washing, mining and industrial use, fishing and other several activities.

1.7. DESCRIPTION OF THE STUDY AREA

1.7.1 Olifants River System

The Olifants River System is situated in the north east of South Africa and is over 54 570 km^2 in area (Figure 1.1).

The Olifants River System originates just within and east of Gauteng Province and the main stem flows in a northerly direction. Beyond Flag Boshielo Dam it changes direction eastwards, enters the Kruger National Park near Phalaborwa and flows further east, joining with the Letaba River and then Mozambique. The Massingir Dam is just beyond the border in Mozambique border then flows into the Massingir Dam from where it is known as the Rio dos Elefantes. Further downstream the Olifants River joins the Limpopo River before it drains into the Indian Ocean north of the city of Maputo.

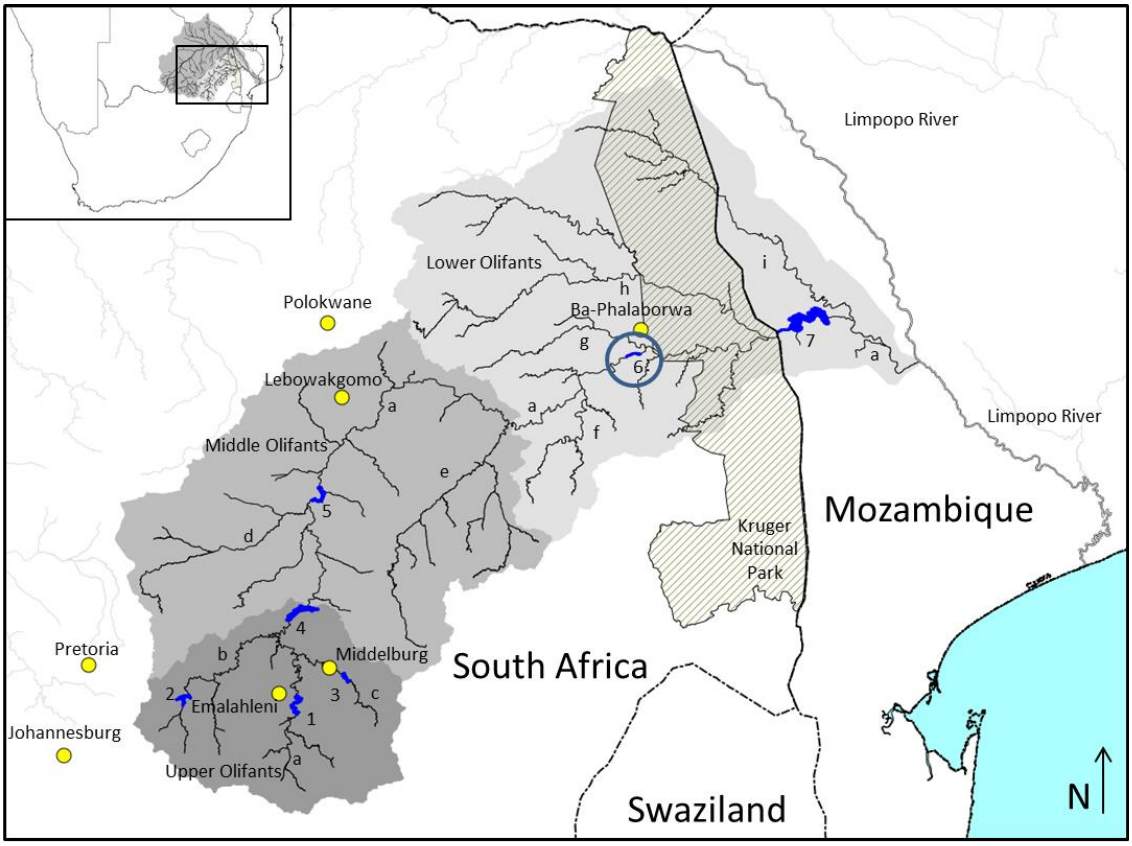

Figure 1.1: Map of the Olifants River system showing the location of major towns, impoundments and tributaries. Major impoundments are depicted by numbers: B) the study site the Phalaborwa Barrage is circled. (Google earth 2013).

The main storage reservoirs in the Olifants River System include; Loskop Dam, Flag Boshielo Dam and the new de Hoop Dam on the Steelpoort River. The high number of mining (coal), and other industrial activities in the upper Olifants, are the main contributors to the poor in-stream and riparian habitat conditions where acid leachate from mines contributing to the poor water quality conditions.

The Olifants River catchment is subdivided into four sub-catchments namely:

The upper sub-catchment form part of the Highveld and is composed of undulating plains and pans, and large open flat area called Springbok Flats. The upper Olifants River is characterised by large scale of mining (coal), coal-fired power generation plants, industries and agriculture (extensive cropping, irrigation and stock farming) activities and several towns and urban centers (Heath et al. 2010; de Villiers and Mkwelo 2009). About 55% of South Africa's electricity from Coal fire and power generation is produced in the highveld which also contribute to the pollution in the aquatic ecosystem (Heath et al. 2010).

The Middle sub-catchment stretches for about 300 km along the Olifants River from below the Loskop Dam (Figure 1.1). The river then flows through the middle veld area of Mpumalanga where it suppliers water to the large irrigation schemes of Groblersdal and Marble Hall before flowing into the Flag Boshielo Dam in the Limpopo Province (Figure 1.1). The second largest irrigation scheme which produces fruits and vegetables for the export market in South Africa is located between the two impoundments.

The middle sub-catchment is characterised by extensive areas of irrigated agricultural and mining activities (platinum, chrome and vanadium) (Claassen et al. 2005). Agricultural pesticides such as organophosphates and carbonates are regularly applied (aerial application) to crops in the Groblersdal and Marble Hall areas and no regulation is taking place with regard to pesticide application in the area (Bollmohr et al. 2008).

The Steelpoort sub-catchment: The Steelpoort sub-catchment consists of the area drained by the Steelpoort River with a surface area of 7 139 km^2. Just close to the Burgersfort town, the Steelpoort River flows north-eastwards through a gorge in the escarpment before joining the middle reaches of the Olifants River. Land use in this sub-catchment consists of small- and medium-scale livestock (dairy and beef cattle) farming and a few small-scale irrigation schemes. There are intensive irrigation and extensive chrome and platinum mining activities in this catchment (de Lange et al. 2003).

Lower Olifants sub-catchment: it stretches from Drakensberg escarpment through the Kruger National Park to the Massingir Dam in Mozambique. It then joins the Limpopo River in Mozambique before it enters the Indian Ocean. This sub-catchment is characterised mostly by agricultural, mining and industrial activities especially around the Phalaborwa town (Heath et al. 2010). There are various mines (gold, platinum, chrome, vanadium, ferro-chrome, copper and phosphate) and numerous smaller urban centres in the Lower Olifants River sub-catchment; the mines use the river as a source of water (Ashton et al. 2001, de Langeet al. 2003). Major industrial and mining operations have developed in the area and have resulted in changes in water quality (physico chemical). Agriculture in the upstream sub-catchment has also contributed to the pollution of the Olifants River resulting in an increases in phosphate and nitrate concentrations thus releasing biocide residues (van Vuren et al. 1994).

1.7.2 Phalaborwa Barrage: Sampling site

Phalaborwa Barrage (24° 4' 12'' S, 31° 8' 43'' E)

Figure 1.2: I- Satellite image of the Phalaborwa Barrage with the three sampling sites (water and sediment); I - inflow, B - wall and C –below wall. LNW – Lepelle Northern Water board (from Google Earth), II- Sluices at the barrage =wall.

The study was done in the Phalaborwa Barrage near Ba-Phalaborwa (Figure 1.2) which is the last impoundment built in the lower Olifants River System, Limpopo Province. The barrage is located about 10 km, South of the town of Ba-Phalaborwa, in the North-eastern part of the Limpopo Province (Figure 1.1). The barrage was constructed in 1959 and it is constructed of a line of 22 sluices with flanking piers to control the flow of water (Figure 1.2b). The water in the barrage is managed by the

Lepelle Northern Water Board (LNW), which supply water to the community and the two mines (phosphate and copper) known as Phalaborwa Industrial Complex (PIC).

The Lepelle Northern Water Board pumps water from the barrage, purify and distribute portable and industrial water to various users in the Phalabowa regions (de Lange et al. 2003). About ± 141 million m^3 per annum of water is extracted from the barrage for domestic, industrial and the surrounding areas. The water level is usually kept almost full, approximately 1.5 m^3/.s. The mean annual runoff is estimated to be 1 201 million m^3 per annum (Lepelle Northern Water 2010). When there is less flow of water into the barrage, supplementary water is released from the Blyderivierspoort Dam (van Vuren *et al.* 1994).

The barrage is located in the Savannah Biome, Lowveld Bioregion and it is bordered by a dense riparian vegetation of the river including indigenous trees, grasses and reeds (Balance et al. 2001). The barrage also provides a habitat for many animals like hippopotami, Nile crocodiles, Nile monitor lizards, water birds with many antelopes and elephants drinking the water and consuming the vegetation. In the State of the Rivers Report, is it reported that the Ecoregion 5.06, upstream of the Phalaborwa Barrage of the Olifants River was in a fair to poor state in terms of in-stream and riparian habitat in the year 2000. The biological indicator of this eco-region reflects a fair state in general (Ballance et al. 2001).

1.8 FISH SPECIES

Two fish species selected for this study were: *C. gariepinus* (Figure 1.3A) and *L. rosae* (Figure 1.3B) (Skelton 2001). The two species were selected because of their abundant availability at the Olifants River, they occupy a variety of habitats, have different feeding habits and feed on diversity of food particles and that they are mostly consumed by humans.

Clarias gariepinus, commonly known as the sharptooth catfish, (Burchell, 1822) belongs to the Order Siluriformes, Suborder Siluroidei and Family Clariidae.

Figure 1.3: Fish species used for the study A: *Clarias gariepinus* (sharptooth catfish) B: *Labeo rosae* (rednose labeo).

Clarias gariepinus is a widely distributed indigenous fish species in the Olifants River in the Limpopo province and throughout Africa.It inhabits calm waters in lakes, streams, rivers, dams; swamps and floodplains, several of which are subjected to seasonal drying, but the presence of the accessory air breathing organs allow the fish to survive during drought periods (Skelton 2001). *Clarias gariepinus* is classified as omnivorous, feeding mainly on aquatic insects, fish and higher plants debris (e.g. fruit) through hunting and scavenging- primary to tertiary consumer trophic levels (Skelton 2001). Because of its wide mouth, it is capable of swallowing relatively large prey whole. It grows fast and is hardy and can tolerate adverse water quality conditions (Jooste et al. 2013).

Labeo rosae (Steindachner, 1894), commonly known as the rednose labeo, belongs to the Order Cypriniformes and Family Cyprinidae. *Labeo rosae* occurs in the northern and north-eastern parts of South Africa (Lowveld reaches of the Limpopo, Incomati and Phongolo River systems). *Labeo rosae* is large in size with a more spindle shaped body, which aids them in free swimming. This fish species generally spawn on newly flooded ground, usually leaving the main river channel to breed in summer (Skelton 2001). Their mouths have a pronounced rostral cap, which covers the upper lip except when feeding. *Labeo rosae* prefers sandy stretches of larger perennial and intermittent rivers. They feed on detritus, algae, and small invertebrates -primary and secondary consumer trophic levels (Skelton 2001).

1.9 LAYOUT OF THE DISSERTATION

Different aspects of this study are discussed in separate chapters.

Chapter 1 contains the introduction of the study, research problem, aim of the study with specific objectives, research questions, outlines the purpose of the study and gives brief literature review. The description of study area, sampling sites and description of the fish species used during this study.

Chapter 2 discusses factors affecting the quality of water and sediment.

Chapter 3 focuses on the bioaccumulation of metals in the fish muscle tissue and risks associated when contaminated muscle tissue is consumed by humans. The comparison of the level of selected metal accumulation in fish and sediment are discussed here.

Chapter 4 discusses the health and parasite composition by applying the HAI and associated Inverted PI in determining the health and condition factor of *Clarias gariepinus* and *Labeo rosae*.

Chapter 5 provides a short summary of the results, conclusion drawn and recommendations for future studies.

Chapter 6 contains references used in this dissertation. References referred to in separate chapters are included to avoid repetition.

Material and methods of each aspect are included in every relevant chapter.

The African Journal of Aquatic Science referencing method was followed for the dissertation write up. The EndNote™ vX6 Reference Management software (developed by Thomson Reuters) was used for referencing in this dissertation.

CHAPTER 2

WATER AND SEDIMENT QUALITY

2.1 INTRODUCTION

Water quality refers to the physical, chemical, biological and aesthetic properties of water which determines its fitness for a variety of uses and for protecting the health and integrity of aquatic ecosystems' (DWAF 1996a). Water quality is one of the most important factors which influence an aquatic ecosystems integrity, as the distribution of aquatic freshwater organisms is controlled mainly by water quality characteristics, including dissolved oxygen, acidity and nutrient content (Dallas and Day 2004). It is not simple to describe what good water quality or how bad/poor it is without knowing its intended use. The quality of water required for drinking purposes is different from the quality of water irrigation, agricultural or industrial purpose (DWAF 1996a). Therefore, when referring to water quality, certain criteria are followed for various water usages to sustain a healthy ecosystem (DWAF 1996c).

Water quality is determined by the activities in the catchment, the land use and the geology of the area. The Department of Water Affairs (DWA) published a series of South African Water Quality Guidelines (SAWQG's) and strives to maintain South Africa's aquatic ecosystem by maintaining water quality within the Target Water Quality Range (TWQR) (DWAF 1996). There are certain guidelines each for a specific water use; domestic, aquaculture (agriculture) and aquatic ecosystems (DWAF 1996a, DWAF 1996b, DWAF 1996c). The water quality criteria include various water constituents such as Target Water Quality Range (TWQR), the Chronic Effect Value (CEV) and the Acute Effect Value (AEV). The TWQR is the range of concentrations within which no measurable adverse effects are expected on the health of aquatic ecosystems, and should therefore ensure their protection. The CEV is defined as the concentration of a constituent at which there is expected to be a significant probability of measurable chronic effects to up to 5% of the species in the aquatic community. The AEV is defined as that concentration of a constituent above which there is expected to be a significant probability of acute effects to up to 5% of the species in the aquatic community (DWAF 1996c). DWAF (1996c) described "system variables" as the climatic fluctuations influence the physico-chemical parameters which then influence the essential ecosystem process. System variables include all the parameters which regulate essential ecosystem processes and aquatic animal behaviour such as emersion of invertebrates and the spawning and migration of fish (DWAF 1996c).

The water column and sediment layer are in direct contact in aquatic systems. This leads to an exchange of constituents, including metals between these media (Adamset al. 2000). Sediment refers to particulate material that usually lies below water which originates from weathering and erosion of rocks or unconsolidated deposits and is transported by water, or suspended in water (US-EPA 2003). The sediment may accumulate excessive quantities of contaminants that directly and indirectly disrupt the ecosystem, causing a significant contamination and loss of desirable species if the loading of contaminants into the waterways is large enough (US-EPA 1997). This poses a threat to the aquatic environment, because sediments usually contain pollutant concentrations that are far higher than those of the overlying water (Förstner and Salomons 1980, Bervoetset al*.* 1994). Ensuring a good sediment quality is very important to maintain a healthy aquatic ecosystem, which ensures good protection of human health. In this study water and sediment quality were determined in conjunction with fish health, accumulation of metals in fish muscle tissue and human health risk to determine the impact of water constituents have on the health of fish and humans. In addition less research was conducted on sediment quality at the Phalaborwa Barrage.

2.2 METHODOLOGY

2.2.1 Water and sediment sampling and analyses

Water samples were collected in four seasons [autumn (May 2010), winter (July 2010), spring (October 2010) and summer (February 2011)] at three sites (inflow, wall and downstream: below wall). The four seasons were selected to determine seasonal differences. The different study sites were selected to determine the impact of the incoming water from upstream on the quality of the water, sediment and fish health at the barrage. The water samples were collected at a depth of 0.5 m with 1000 ml acid pre-treated propylene bottles (Kartell^{TM}) and immediately refrigerated. The physical-chemical water quality variables which include dissolved oxygen (DO), pH, water temperature, salinity, and electrical conductivity (EC) were determined at each site during the four sampling surveys in *situ* by means of a handheld: YSI model 556 multipara-meter meter. The water samples were analysed for metals and inorganic salts using sequential Inductively Coupled Plasma- Optical Emission Spectrometry (ICP- OES) by an accredited laboratory in (WATERLAB) Pretoria. other water quality parameters: nutrients (total nitrogen and orthophosphate) and ions (chloride; sodium; potassium; calcium; magnesium; fluoride and sulphate); and metals (Ag, Al, As, B, Ba, Be, Bi Cd, Co, Cr, Cu, Fe, Li, Mo, Mn, Ni, Pd, Sb, Se, Si, Sr, Ti, V and Zn) were all analysed at the accredited laboratory. DWAF (1996) and CCME (2012) water quality guidelines were used in this study to evaluate water quality parameters as there are no sediment guidelines for in South Africa.

Sediment samples were collected using a Friedlinger mudgrab (225 cm3) at the three sites (inflow, wall and below wall) during winter and summer. Three to five water and sediment samples were taken and composited then transferred to the 500 ml acid treated propylene sampling bottles and immediately refrigerated prior to laboratory analysis. The sediment samples were dried and digested in nitric and hydrochloric acids before metal analyses at a SANAS accredited laboratory. Metal concentrations were analysed by ICP-OES and recorded as mg/kg dry weight. Sediment were analysed according to Bervoets and Blust (2003).

2.2.2 Results analysis

Water quality results from the chemical laboratory were interpreted and compared to the South African Water Quality Guidelines (SAWQG) (Table 2.1). These results were compared with the TWQR, AEV and CEV for different water uses, where applicable and available. The US Environmental Protection Agency (USEPA) guidelines were also used for metals that did not appear in the South African water quality criteria for aquatic ecosystems. All collected data were analysed using one-way analysis of variance (ANOVA) in statistical package programme, SPSS version 12.0. The mean and standard deviation (calculated using Microsoft Excel) of the respective water quality data and sediment data were calculated for the seasons. A one way ANOVA was performed to determine whether there is any significant difference between samples seasonally and between sites and the difference was considered significant if the p value was less than 0.05.

Table 2.1: Water quality guidelines for aquatic and domestic use

Water Quality Parameters	**Water Quality Guidelines** See footnote for reference
Water temperature ºC	Water temperature should not vary more than 10% from normal (natural) value
Dissolved oxygen (mg/ℓ)	≤5
pH	6.5-9.0
Electrical Conductivity mS/m	No criteria available
TDS (mg/ℓ)	No criteria available
Salinity (‰)	< 0.5 ‰
Turbidity NTU	8 (clear flow) to < 50 NTU (turbid flow)
Alkalinity (mg/ℓ)	No criteria available
Chloride (mg/ℓ)	120
Fluoride (mg/ℓ)	0.75
Sulphate (mg/ℓ)	100
Calcium (mg/ℓ)	<200
Magnesium (mg/ℓ)	<150
Potassium (mg/ℓ)	No criteria available
Sodium (mg/ℓ)	<200
Nitrate (mg/ℓ)	13.00
Total nitrogen (mg/ℓ)	< 0.5 (oligotrophic); > 10 (hypertrophic)
Phosphorous (mg/ℓ)	<0.005 (oligotrophic); >0.25 (hypertrophic)
Water hardness (mg/ℓ)	120-180* $CaCO_3$ hard water
Aluminium	0.001* (pH > 6.5)1
Antimony	0.012
Arsenic	0.011
Barium	0.72
Boron	No guidelines
Cadmium	0.00015 - 0.0041
Chromium	Cr III: 0.012*1
Cobalt	No guidelines
Copper	0.0003 – 0.00141
Iron	Fe vary <10% background concentration.1
Lead	0.0002-0.00121
Manganese	0.181
Nickel	< 0.472
Selenium	0.0021
Silver	No guidelines
Strontium	4.02
Tin	No guidelines
Titanium	No guidelines
Vanadium	No guidelines
Zinc	0.21 0.122

*water hardness: calculated using formula CaCo3 in (mg/ℓ) = (Ca mg/ℓ *2.5) + (Mg mg/ℓ*4.1)

1. DWAF (1996 a, b, c) South African Water Quality Guidelines
2. US-EPA (2012a) – United States Environmental Protection Agency: Water Quality Guidelines – Aquatic Life.

2.3 RESULTS AND DISCUSSION

The recorded seasonal water quality data for the surface water of the Phalaborwa Barrage water at each sampling site are shown in Appendix A. Tables 2.2 to 2.4 are the results of the analyses of the data and were compared water quality guidelines in Table 2.1.The seasonal mean values variation at each sampling site of all the water quality results are drawn in Figures 2.1 to 2.5. Sediment quality data is also shown in Appendix A and Table 2.5. Only the detectable water constituents were included in the data tables and figures.

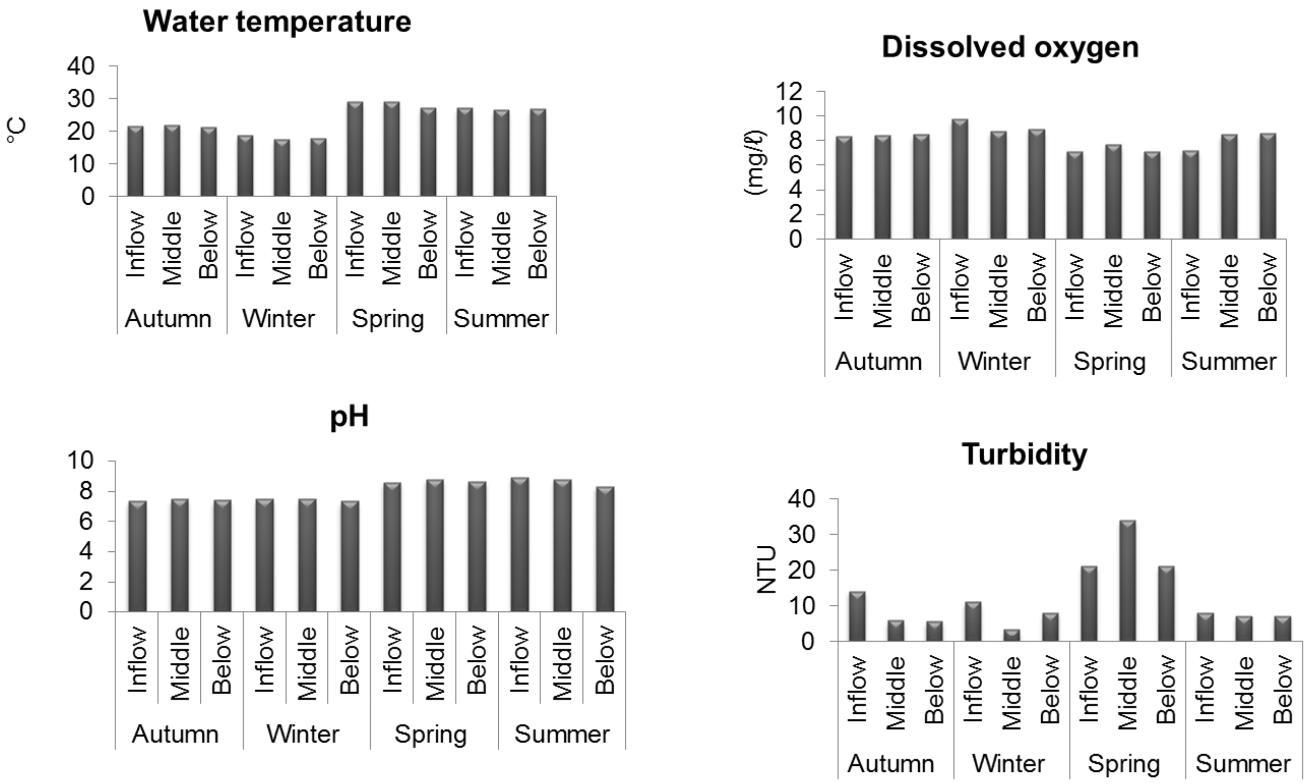

Figure 2.1: Seasonal mean concentrations for water quality data analysis: water temperature, dissolved oxygen, pH and turbidity at three sites at the Phalaborwa Barrage (May 2010- January 2011).

Table 2.2: The seasonal mean concentrations of (with standard deviations) physico-chemical in water (n=4) at the Phalaborwa Barrage (May 2010- January 2011)

	autumn		winter		spring		summer	
Parameters	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Water temperature °C	21.5	±0.4	18.0	±0.7	28.5	±1.2	26.8	±0.3
Dissolved oxygen (mg/ℓ)	8.4	±0.1	9.1	±0.5	7.3	±0.3	8.1	±0.8
Dissolved oxygen (O_2%)	96.3	±1.0	99.0	±3.0	94.5	±5.4	94.8	±1.0
pH	7.3-7.5	±0.1	7.3-7.5	±0.1	8.6-8.8	±0.1	8.3-8.9	±0.3
Conductivity mS/m^{-1}	37.1	±0.1	43.4	±3.8	60.0	±0.6	32.4	±0.0
Salinity ‰	0.2	±0.0	0.2	±0.0	0.3	±0.0	0.2	±0.0
TDS (mg/ℓ)	240.9	±0.4	281.9	±24.4	390.2	±3.9	210.6	±0.0
Alkalinity as $CaCO_3$	64.0	±4.0	104.0	±14.4	142.7	±16.2	74.7	±2.3
Turbidity (NTU)	8.5	±4.8	7.4	±3.9	25.3	±7.5	7.3	±0.6
Water hardness	124	±2	149	±20	177	±6.29	115.67	±7.96

2.3.1 Physico-chemical parameters

Water temperature- the temperature in this study increased seasonally in this order winter, autumn, summer and spring (Table 2.2). The highest mean water temperature was recorded in spring (28.5 ºC ±1.2) and the lowest mean value was recorded in winter (18.0 ºC ±0.7) (Table 2.2). Temperatures measured were all above 25 ºC in spring and summer at all sampling sites (Figure 2.1). The lowest temperature was recorded at the dam wall during winter (17 ºC) and the highest at the inflow and the dam wall during spring (29 ºC) (Appendix A and Figure 2.1). Statistical analysis indicates no significant difference of water temperatures between different seasons ($p>0.001$) and the three sampling sites ($f=0.039$; $df= 3$ $p>0.001$). There is a direct relationship between temperature and dissolved oxygen, thus, any increase in temperature will result in a decrease in dissolved oxygen concentration. This was observed with the high temperature during spring that correlated with decreased dissolved oxygen. The temperatures recorded during this study fell in the range in good condition for both fish species and had thus no adverse effects on the host.

Water temperature could vary resulting from heated industrial effluent discharges, heated return flows, of irrigation water, removal of riparian vegetation cover and inter basin water transfers (DWAF 1996c). Higher temperatures reduce the solubility of dissolved oxygen in water, determines availability of nutrients and toxins, decreasing its concentration and thus its availability to aquatic organisms (DWAF 1996c, Dallas and Day 2004).

Dissolved Oxygen- the highest DO mean value of 9.1±0.5 mg/ℓ was recorded during winter, while the lowest mean value of 7.3 ±0.3 mg/ℓ was recorded during spring (Table 2.2). The highest DO value of 9.74 mg/ℓ was recorded in winter at the inflow and the lowest value of 7.13 mg/ℓ was recorded below the dam wall in spring. The dissolved oxygen concentrations at the inflow were higher during winter season than autumn spring and summer (Figure 2.1; Appendix A). ANOVA statistical analysis indicated that there is no significant difference between sites and seasons ($p>0.05$).

pH- the pH units recorded in this study were acceptable for aquatic ecosystems and ranged between 7.31 below the dam in winter to 8.91 at the inflow in summer (Table 2.2). Thus the pH of the surface water at the Phalaborwa Barrage was near neutral to slightly alkaline (Appendix A). The one-way ANOVA indicated no significant difference of pH units between the three sampling sites ($F=0.10$; $df=2$; $p>0.05$). The pH units in this study might have been affected by elevated levels of TDS and sulphate. In addition the pH of surface water may vary as a result of discharges of effluent (industrial, municipal), runoff, acidic rainfall and microbial activity (DWAF 1996c). The pH units should vary from 6 to 8 (Table 2.1) as suggested by DWAF (1996c). The pH units in this study were within the above-mentioned range except during spring and summer where slightly higher pH was recorded (Figure 2.1). However, the water quality guidelines for domestic use (DWAF 1996a) suggests that the pH of most natural waters lies between 6.5 and 8.5 (Table2.1) because of the geology and geochemistry of underlying rocks and soils. If this is taken into consideration, then the pH in this study are still within the acceptable range.

Turbidity- the highest mean turbidity value was recorded in spring (25.3 ±7.5 NTU) and lowest in summer (7.3 ±0.6NTU) (Table 2.2). There are no target water quality ranges for turbidity for aquatic ecosystems, however aquaculture guidelines indicate that <25NTU is an acceptable turbidity for clear water species (DWAF 1996b). In this study the turbidity values were within TWQR for clear water fish species (<25 NTU) (Table 2.1) for all seasons except in spring (Figure 2.1). The highest turbidity value of 34 NTU was recorded at the dam wall in spring and the lowest value of 0.7 NTU at the dam wall and below the dam in summer (Appendix A and Figure 2.1). There was no significant difference between alkalinity recorded in each site however, seasonal differences were observed ($F= 0.05$, $df=2$ $p>0.05$). The high turbidity value recorded in spring correlates with a study conducted by Ramollo (2008) during 2005 and 2006 in the Phalaborwa Barrage indicating the highest mean value of 40.8 NTU at the barrage.

The effects of high turbidity values include severe aesthetic effects such as appearance, taste and odour; and significant effects on the microbiological quality of the water (DWAF 1996a). This high turbidity values could lead to poor visibility and reduced feeding rates for fishes living in the system. These also prevent sunlight from reaching plants below the surface thus can reduce the rate of photosynthesis, so less oxygen is produced by plants. (Dallas and Day 2004). Geology, anthropogenic activities (industrial, mine discharges, sewage discharge) can contribute significantly to the high or low turbidity levels at a specific site. Turbidity may harm fish and their larvae (Davies and Day 1998).

Total Dissolved Solids (TDS) - the TDS concentrations ranged from 210.6 mg/ℓ to 393.9 mg/ℓ (Appendix A). The highest values were recorded below the dam (310.1 mg/ℓ) and lowest at the dam wall (267.2 mg/ℓ) with no significant difference much among sites ($p>0.05$) (Figure 2.2). Seasonally, the highest TDS concentrations were recorded during spring (390.2 ±3.9 mg/ℓ) with the lowest during summer (210.6 ±0.0 mg/ℓ) (Table 2.2). All recorded TDS values in all surveys were within the TWQR for domestic use (DWAF 1996a) but exceeded TWQR for aquaculture according to DWAF (1996b) (Table 2.1).

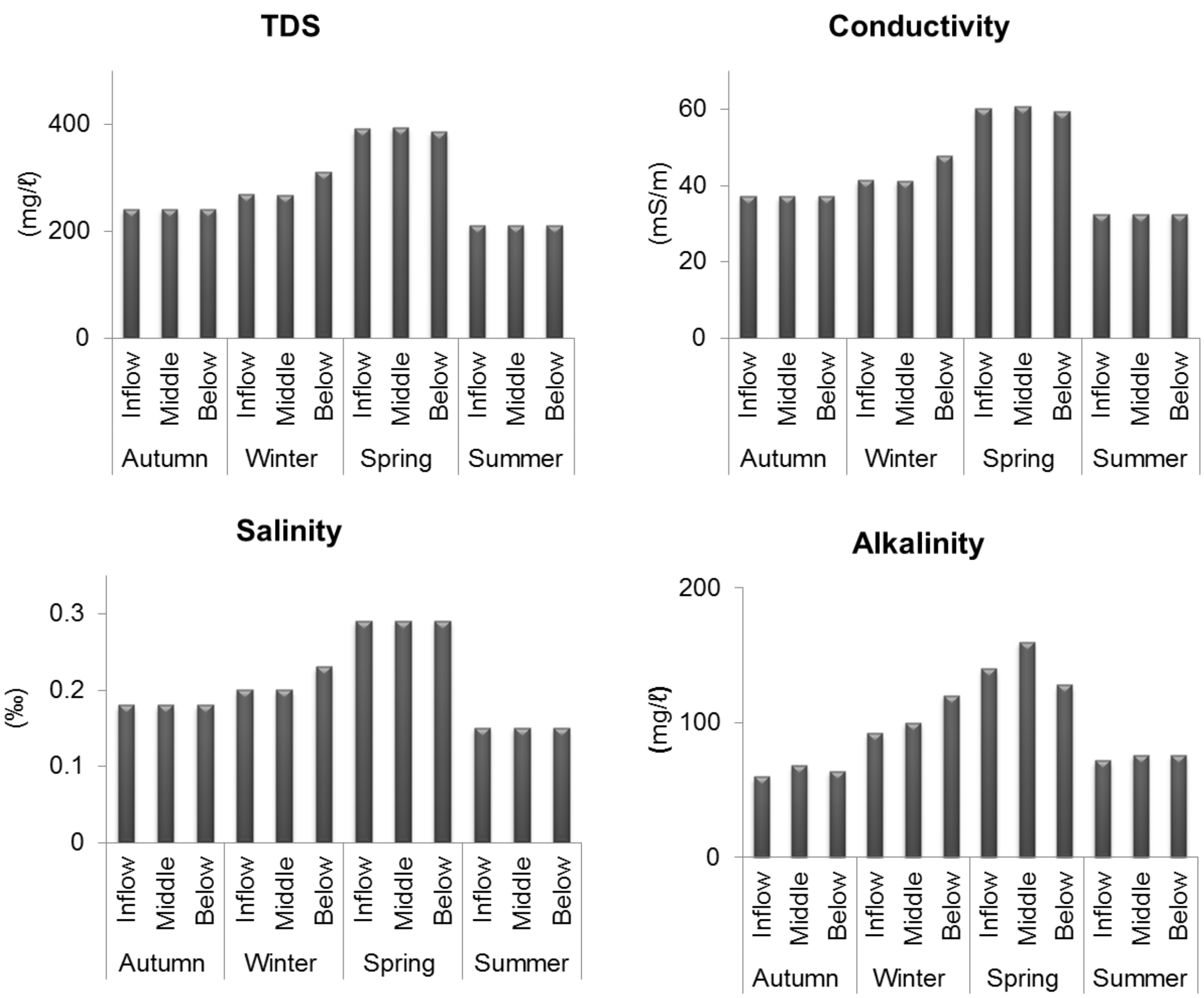

Figure 2.2: Seasonal mean concentrations for water quality data analysis: total dissolved solids, electrical conductivity, salinity, alkalinity and water hardness at three sites at the Phalaborwa Barrage (May 2010- January 2011).

The one-way ANOVA indicated a significant difference in TDS between seasons, $F=121.064$; $df=3$; $p<0.001$. The TDS is directly proportional to EC of water. Naturally, all water contains varying concentrations of TDS as a result of the dissolution of minerals in rocks, geological weathering, atmospheric conditions, soils, and decomposing plant material. However, domestic and industrial discharges and surface runoff from urban and evaporation can also increase the TDS levels. TDS at high or low concentrations may limit growth of organisms and may eventually lead to death. It also reduces penetration of light, smother and clog surfaces (e.g. gills) and absorb nutrients and toxins (Dallas and Day 2004).

Electrical conductivity (EC)- electrical conductivity, along with TDS, serves as a general indicator of change in water quality and affects the taste and freshness of the water (DWAF 1996a).

The highest mean value of EC (60.0 ±0.6 mS/cm) was recorded in spring while the lowest mean value (32.4 ±0.0 mS/cm) was recorded in summer (Figure 2.2). The EC at the Phalaborwa Barrage ranged from 32 mS/cm at all sites in summer to 61 mS/cm at the wall in spring with a mean value of 43 mS/cm (Figure 2.1; Appendix A). The one-way ANOVA for this parameter indicate a significant difference of EC between seasons ($F=121.064$; $df=3$; $p<0.001$). During spring survey the anions (chloride, sulphates and fluoride), cations (calcium, magnesium, potassium and sodium) concentrations were higher than the other seasons (Figure 2.2, Table 2.3).

Salinity- salinity is the saltiness of water (Dallas and Day 2004). Salinity levels varied among seasons, the highest mean salinity value was recorded during spring survey (0.3 ±0.0 ‰) and lowest value was recorded during summer (0.2 ±0.0 ‰) (Figure 2.2, Table 2.2). The salinity of the surface water at the barrage was in the water quality range for freshwater (<0.5‰) (Table 2.1). The highest salinity was recorded in spring at all sites and the lowest in summer also at all sites (Appendix A). Changes in salt concentrations can have adverse effects on aquatic biota, ecological and microbial processes like rates of nutrient cycling and metabolism (Davies and Day 1998).

Alkalinity- the highest mean alkalinity value was recorded in spring (142.7±16.2mg/ℓ) and the lowest mean in autumn (64 ±4.0 mg/ℓ) (Table 2.2). During spring, alkalinity values were significantly higher than the TWQR for aquaculture (20-100 mg/ℓ) (Table 2.1). The alkalinity values for autumn, winter and summer were all within the TWQR for aquaculture (DWAF 1996c). The highest alkalinity was recorded at the wall during spring while the lowest was recorded at the inflow in autumn (Appendix A and Figure 2.2). There was no significant difference between alkalinity recorded in each site however, a significant difference was observed seasonally ($F= 0.05$, $p>0.05$). A low water alkalinity has a low buffering capacity and can be susceptible to alterations in pH, for example from atmospheric, acidic, deposition (Dallas and Day 2004).

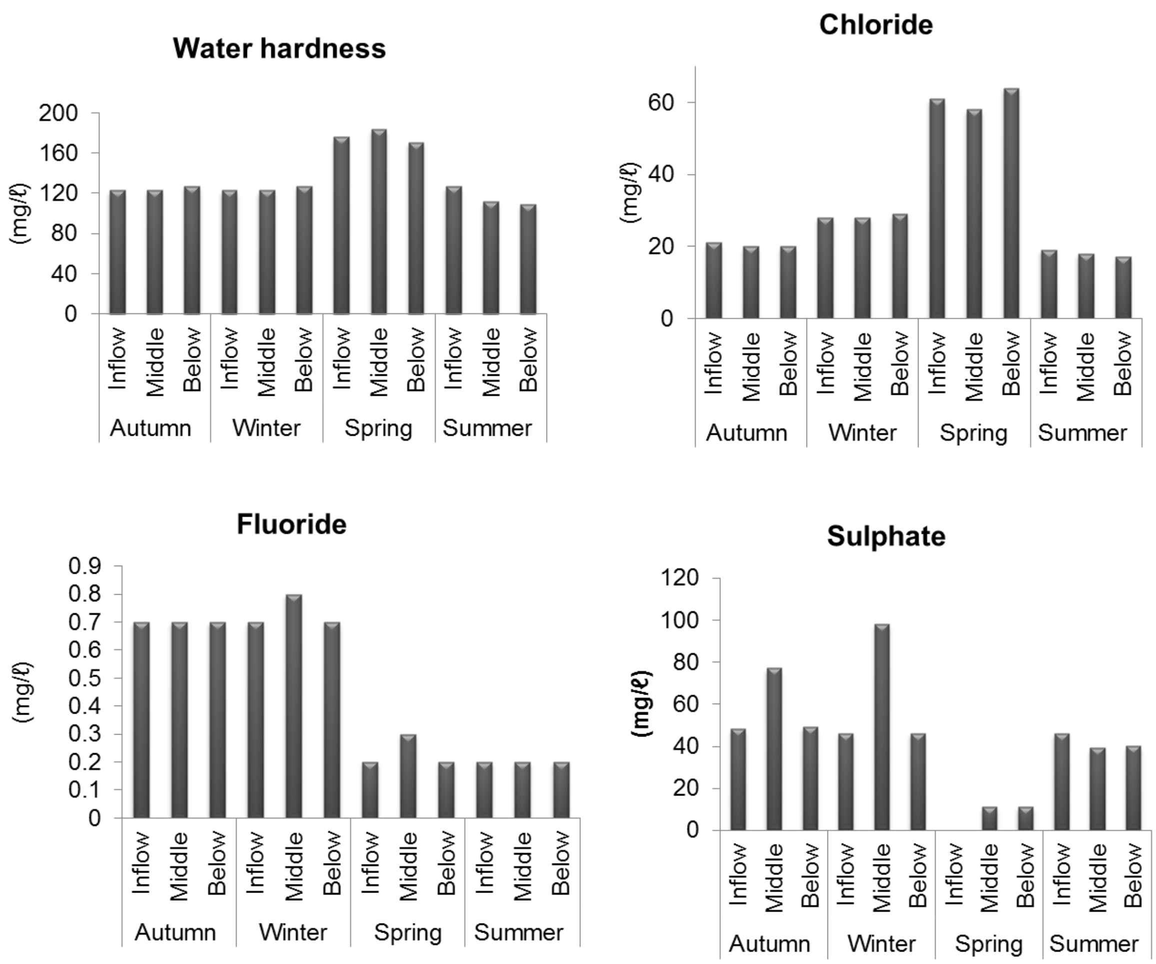

Figure 2.3: Seasonal mean concentrations for water quality data analysis: water hardness; chloride; fluoride and sulphate at the three sites at the Phalaborwa Barrage (May 2010- January 2011).

Water hardness- water hardness refers to the sum of the calcium and magnesium concentrations, expressed as mg/ℓ of calcium carbonate. The results indicate that the water at the Phalaborwa Barrage is moderately hard in summer at the wall and below the dam and became hard during autumn and winter at all sites (Figure 2.3). The lowest mean value was recorded in summer and highest value was recorded in spring at the wall (Table 2.2). The highest mean water hardness value was recorded in spring (177±6.29 mg/ℓ) and the lowest mean in summer (115.67 ±7.96 mg/ℓ) (Table 2.2). Water hardness values between 60 and 120 mg/ℓ calcium carbonate are classified as moderately hard water while the one between 121 and 180 mg/ℓ calcium carbonate are classified as hard water (DWAF 1996a, Dallas and Day 2004) (Table 2.1). The surface water of the barrage during summer at the wall and below the dam wall is considered moderately hard and hard during autumn and winter.

Major ions

Major ions are not toxic at low concentrations; however, they may induce toxic effects on aquatic biota and water users at high concentrations (Dallas and Day 2004).

Table 2.3: The seasonal mean concentrations (with standard deviations) of inorganic nitrogen, phosphorous and major ions (n=4) recorded at the Phalaborwa Barrage (May 2010- January 2011)

Parameters	autumn		winter		spring		summer	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sulphate (mg/ℓ)	58.0	±16.5	63.3	±30.0	7.3	±6.4	41.7	±3.8
Chloride(mg/ℓ)	20.3	±0.6	28.3	±0.6	61.0	±3.0	18.0	±1.0
Fluoride (mg/ℓ)	0.7	±0.0	0.7	±0.1	0.2	±0.1	0.2	±0.0
Calcium(mg/ℓ)	23.0	±0.0	25.7	±5.5	31.3	±2.5	21.7	±3.8
Magnesium (mg/ℓ)	16.3	±0.6	20.7	±1.5	24.0	±0.0	15.0	±0.0
Potassium(mg/ℓ)	3.9	±0.1	3.2	±0.1	4.3	±0.3	2.8	±0.1
Sodium (mg/ℓ)	21.0	±0.0	27.3	±1.2	51.3	±2.3	17.3	±0.6
Nitrate(mg/ℓ)	0.2	±0.0	0.3	±0.1	1.8	±1.6	0.4	±0.3
Nitrite(mg/ℓ)	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
Ammonia (mg/ℓ)	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
Total nitrogen(mg/ℓ)	0.2	±0.0	0.3	±0.1	1.8	±1.6	0.4	±0.3
Phosphorous (mg/ℓ)	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.2	±0.2

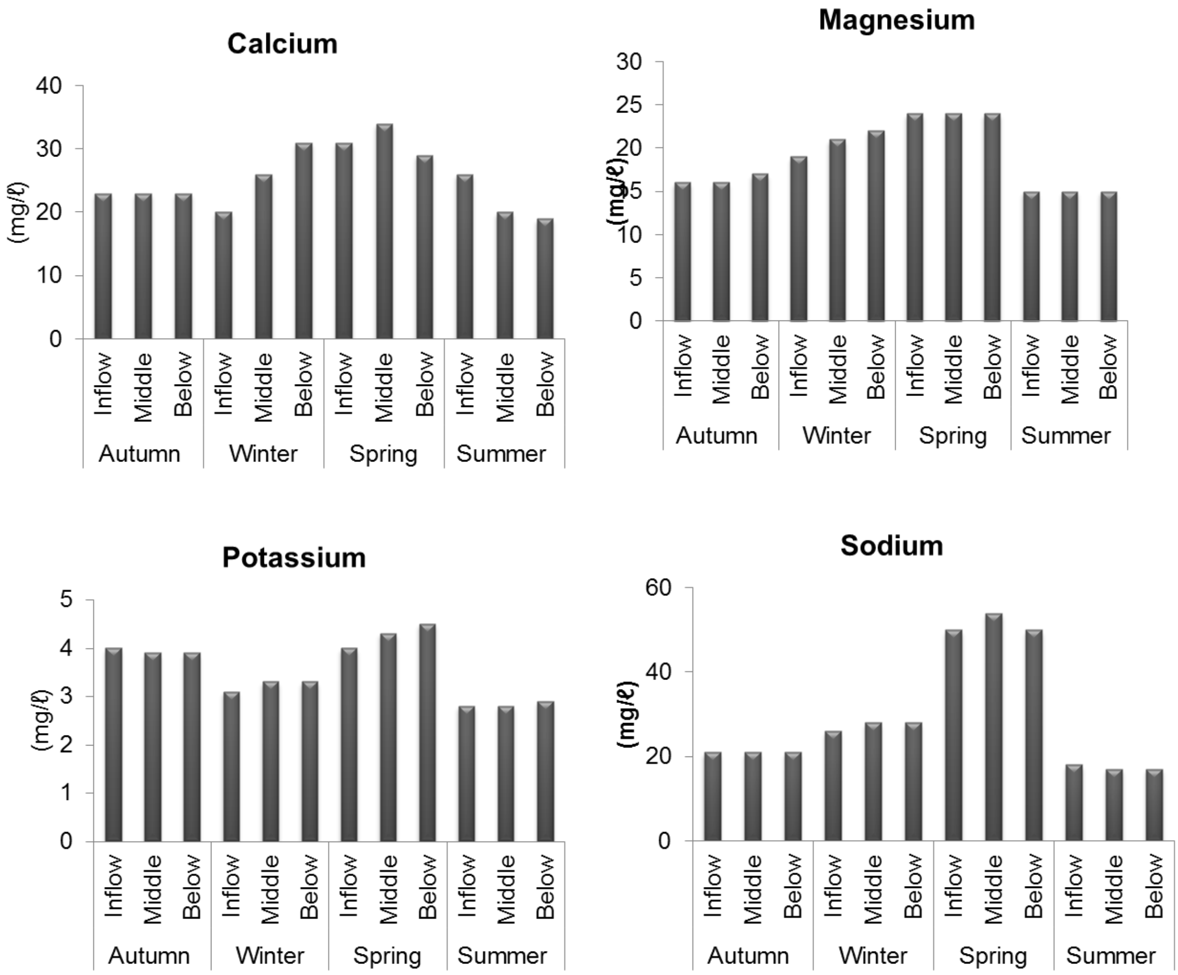

Figure 2.4: Seasonal mean concentrations for water quality data analysis: calcium; magnesium; potassium and sodium at three sites at the Phalaborwa Barrage (May 2010- January 2011).

Anions: Chloride; fluoride and sulphate

Chloride mean concentrations ranged from in summer to in spring (Table 2.3). The highest mean chloride value was recorded in spring (61 ±3.0 mg/ℓ) and the lowest mean in summer (18 ±1.0 mg/ℓ) (Table 2.2). The highest chloride concentrations were recorded below the dam (64 mg/ℓ) in spring with the lowest concentrations below the wall in (14 mg/ℓ) summer but there was no significance difference among sites (Figure 2.3; Appendix A). Chloride levels recorded in this study were all below the detection concentration (limit) of 10 mg/ℓ which showed no concern (Table 2.1). The detection limit is within the TWQR for domestic use but higher than the TWQR and the CEV for aquatic ecosystems (Table 2.1). The SAWQG for Aquaculture indicates that 600 mg/ℓ Cl- is acceptable for freshwater fish species (DWAF 1996c).

Fluoride - the highest mean concentrations were recorded in autumn and winter (0.7± 0.1 mg/ℓ) and the lowest concentrations in spring and summer (0.2 ± 0.1 mg/ℓ) (Table 2.3). The highest concentration was recorded at the wall in winter with the lowest recorded concentration at the inflow and below wall in spring and at all site during summer (Appendix A and Figure 2.3). Although a higher concentration was recorded in winter, it was still acceptable in DWAF (1996c) guidelines. The fluoride values in this study were all within the TWQR, AEV and CEV for aquatic ecosystems (Table 2.1). Fluoride concentrations remained fairly constant during all seasons with values ranging from 0.2 mg/ℓ (spring and summer) to 0.7 mg/ℓ (autumn and winter) (Table 2.2). The concentrations of fluoride at all sites were within the TWQR for all the water quality guidelines as they are all below 0.75 mg/ℓ (DWAF 1996c) (Table 2.1).

Sulphate mean concentrations varied between 7.3 ± 6.4 mg/ℓ in spring to 63.3 ± 30 mg/ℓ in winter during this study (Table 2.3). The highest sulphate concentration was recorded at the dam wall in winter with the lowest concentration below the dam wall in spring (Appendix A and Figure 2.3). The one-way ANOVA indicated that there is no significant difference between seasons ($F= 0.074$; $df =3$; $p>0.05$). Although the typical concentration of sulphate in surface water is 5 mg/ℓ, concentrations of several 100 mg/ℓ may occur where dissolution of sulphate minerals or discharge of sulphate rich effluents from acid mine drainage takes place (DWAF 1996a). There are no SAWQG of sulphate available for aquatic ecosystems. Sulphate concentrations were considerably ten times higher, than the typical concentration but still within the TWQR for domestic use (DWAF 1996a).

The one-way ANOVA indicates no significant difference of all anions in water between the four seasons ($F=0.07$; $df=3$; $p=0.97$).

Cations: Calcium; magnesium; potassium and sodium

Calcium mean concentrations in this study varied from 21.67 ± 3.8 mg/ℓ during summer to 31 mg/ℓ during spring (Table 2.3), however all recorded concentrations fell within the TWQR for domestic use (Table 2.1). The highest calcium concentration was recorded at the dam wall and lowest concentrations were recorded below the dam (Appendix A and Figure 2.4). There are no SAWQE of calcium for aquatic ecosystems. Calcium concentrations at all sites were within the TWQR for domestic use (DWAF 1996a). Calcium is one of the major elements essential for living organisms found as a structural material in bones, teeth, shells and exoskeletons (Dallas and Day 2004). Although calcium is an important element, very little is known about the actual effects of changes in its concentration on aquatic biotas (DWAF 1996a). Calcium is found in various construction materials such as cement, brick lime and concrete. Calcium ions are often the major cations in inland waters, whereby soft waters contain low calcium concentration and hard waters high calcium concentration (DWAF 1996a).

Magnesium mean concentrations varied from 15 ± 0 mg/ℓ in summer to 24 ± 0 mg/ℓ in spring (Table 2.3) and were within the TWQR for domestic use (30 mg/ℓ) (DWAF 1996a) (Table 2.1). The lowest concentrations were recorded at all sites in summer with highest records at all sites in spring (Appendix A and Figure 2.4). The concentration were much higher during winter and spring and higher than the TWQR as suggested by DWAF (1996a), but according to Dallas and Day (2004) magnesium is not considered toxic. Typical concentrations of magnesium in freshwater ecosystems are usually between 4 and 10 mg/ℓ (DWAF 1996 a). The lowest magnesium level recorded during autumn survey was probably due to the dilution effect of the summer rains.

Potassium mean concentrations in this study were within the typical natural occurrence concentration in freshwater (2 to 5 mg/ℓ) and also within the TWQR for domestic use (0-50 mg/ℓ) (Table 2.1). The highest concentration was recorded below the wall and at the wall in spring with the lowest concentration recorded at the inflow and at the wall in summer; however, there was no statistical difference (Appendix A and Figure 2.4). Seasonally highest concentration was recorded during spring (4.3 ±0.3 mg/ℓ) and the lowest concentration was recorded during summer (2.8 ± 0.1 mg/ℓ), however, the concentrations did not vary much among the seasons ($p>0.05$) (Table 2.3). There are no SAWQG available, and its toxic effects to aquatic ecosystems are not known.

Sodium mean concentration in this study varied from 17 ± 0.6 mg/ℓ in summer to 51.3 ± 2.3 mg/ℓ (Table 2.3). The highest sodium concentration was recorded was recorded at all site and the lowest concentration was recorded at the wall and below the wall in summer (Appendix A and Figure 2.4). Low sodium concentrations were recorded throughout the study. They were lower than the TWQR for domestic use (100 – 200 mg/ℓ) (DWAF 1996a). When one way ANOVA was performed on the cations, there was no significant difference between the four seasons (F=0.57; df=3; p=0.64).

Nutrients: inorganic nitrogen and phosphorous

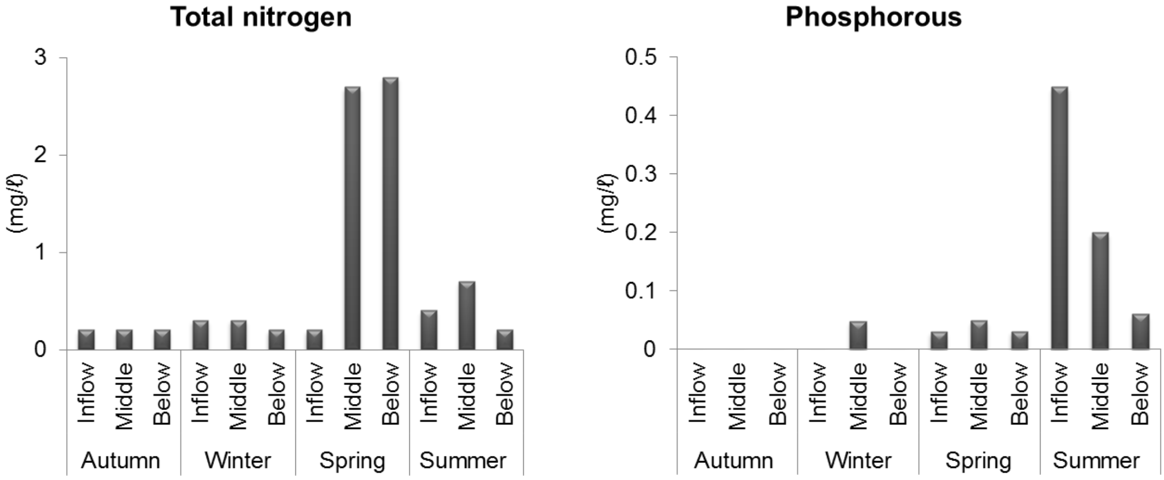

Figure 2.5: Seasonal mean concentrations for water quality data analysis: total nitrogen and phosphorous at three sites at the Phalaborwa Barrage (May 2010-January 2011).

Total inorganic nitrogen

Dallas and Day (2004) suggested the following guidelines for inorganic nitrogen:

- < 0.5 mg/ℓ – Oligotrophic conditions
- 0.5 -2. 5 mg/ℓ – Mesotrophic conditions
- 2.5-10 mg/ℓ – Eutrophic conditions
- >10 mg/ℓ – Hypertrophic conditions

Total nitrogen concentrations remained constant ranging from less than 0.1 to 2.8 mg/ℓ (Appendix A). Nitrite and ammonia were recorded at very low concentrations (<0.2 mg/ℓ) during all seasons at all sampling sites (Table 2.3). Nitrate was detectable throughout the survey with the highest mean concentration of 1.8 ±1.6 mg/ℓ during spring and lowest in autumn with a mean value of 0.2 ±0.0 mg/ℓ (Table 2.3). Elevated nitrogen levels occurred at the wall and below the wall (Appendix A and Figure 2.5). Nitrogen values between 2.5 mg/ℓ and 10 mg/ℓ are considered as eutrophic conditions in which there is low levels of species diversity; highly productive systems, nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms may include species which are toxic to man, livestock and wildlife (DWAF 1996c). High total nitrogen levels (2.5-10 mg/ℓ) were recorded at the wall and below the dam wall in spring (Appendix A and Figure 2.5).

The highest mean total nitrogen concentration recorded in spring is indicative of mesotrophic conditions (DWAF1996c). Nitrogen concentrations recorded during autumn, winter, and summer were all in an oligotrophic conditions (<0.5 mg/ℓ) (Table 2.3).

Inorganic Phosphorous

The South African water quality guidelines for the summer inorganic phosphorous trophic status in aquatic ecosystems (DWAF 1996c, Dallas and Day 2004) are:
< 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

Phosphate concentrations were below the detection level (<0.2 mg/ℓ) during all seasons at all the sampling sites (Appendix A).
Phosphorous (P) concentrations were also very low ranging from non-detectable values (less than <0.025) to 0.450 mg/ℓ (Appendix A and Table 2.3). Seasonally the highest P concentration was recorded in summer (0.24 mg/ℓ) and the lowest concentration was recorded in spring with a value of 0.04 mg/ℓ (Table 2.3). The highest concentration was recorded at the inflow and the lowest at the dam wall (Appendix A and Figure 2.5). One way ANOVA did not indicate any significance difference between seasons in terms of the nutrients: $F=0.785$; $df=3$; $p>0.05$. The highest mean phosphorous value recorded in summer at the Phalaborwa Barrage is an indicative of eutrophic condition and the lowest mean value in spring as an indication of

mesotrophic conditions. Eutrophic conditions were measured in winter, spring and summer at all sites.

2.3.2 Metals and metalloids

Cadmium, Chromium, Cobalt and Copper were recorded at concentrations below detection levels in the water column.

Table 2.4: The seasonal average metals and metalloids variations (with standard deviations) in water: aluminium, antimony: arsenic, barium, boron, iron, manganese, selenium strontium and tin at the Phalaborwa Barrage

Metal	Autumn	Winter	Spring	Summer
Aluminium	*	*	0.64±0.32	*
Aresenic	*	0.02±0.008	*	*
Antimony	*	*	0.04±0.02	*
Barium	0.03±0.00	0.04±0.003	0.05±0.008	*
Boron	*	*	0.19±0.10	*
Iron	0.09±0.03	0.05±0.03	0.41±0.06	*
Manganese	0.04±0.007	0.04±0.007	0.026±0.0	*
Selenium	*	*	0.11±0.06	*
Strontium	*	*	0.17±0.17	0.10±0.03
Tin	*	*	*	0.20±0.02

Aluminium concentrations were only detected during spring at the inflow and wall in the water column. The highest value of 0.67 mg/ℓ was recorded at the inflow, while the lowest of 0.62 mg/ℓ was recorded at the wall (Appendix A). The aluminium concentration recorded during spring at both sites was above the AEV and CEV of 0.15 mg/ℓ and 0.02 mg/ℓ respectively (Table 2.4 and Table 2.1). In this study aluminium was detected at pH levels greater than 6.5 (that is intermediate to alkaline).

Antimony concentration recorded in the water in spring was above the recommended concentration according to CCME and this high concentration could be from high concentrations of metalloids in (CCME 2001). Antimony was recorded only in spring at all sites and the concentration was above CCME recommendation. The high concentration could be due to the high concentration of metal in the sediment (CCME

2001). The highest antimony concentration was recorded at the inflow (0.066 mg/ℓ) and lowest at the wall and below the dam wall (0.03 mg/ℓ) (Appendix A and Table 2.4).

Arsenic- the highest arsenic concentration was recorded at the inflow in summer and lowest below the dam (Appendix A and Table 2.4). Although the arsenic concentration was found to be higher than the TWQR guidelines, the concentration fell within the AEV and CEV range (Table 2.1). Elevated concentrations of arsenic occur where there is pollution from industrial sources, or where geological outcrops of arsenic mineral occur (Dallas and Day 2004).

Barium concentrations varied from 0.03 mg/ℓ in autumn to 0.062 mg/ℓ in spring. The lowest mean concentration value of 0.03 mg/ℓ was recorded in autumn and the highest (0.05 mg/ℓ) in spring (Table 2.4). The highest concentration was recorded at the wall (0.062 mg/ℓ) and lowest at all sites (0.03 mg/ℓ) in autumn (Appendix A).

Boron concentration in this study was only detected during the spring survey with the highest value of 0.257 mg/ℓ recorded at the inflow and lowest value of 0.152 mg/ℓ below the dam (Table 2.4). The boron concentrations at the three sites were less than the concentration limit of 0.5 mg/ℓ (WHO 2006) (Appendix A and Table 2.1).

Iron- the highest mean iron concentration was recorded in spring (0.4 mg/ℓ) and lowest in winter (0.05 mg/ℓ) (Table 2.4). The highest iron concentration was recorded at the inflow in spring and lowest at the wall in winter (Appendix A). The iron concentrations recorded were within the TWQR for aquatic ecosystem but were above the range for the TWQR for domestic use of 0 to 0.1 mg/ℓ. In unpolluted surface water, dissolved iron concentrations range is typically from 0.001 to 0.5 mg/ℓ. Iron is not hazardous to health, but it is considered a secondary or aesthetic contaminant (DWAF 1996c). The elevated levels of iron in spring could be due to agriculture practised along the banks of the Olifants River (Ashton and Dabrowski 2011). Moreover, the concentration of dissolved iron is dependent on pH levels, redox potential, turbidity, suspended matter, aluminium concentrations and the occurrence of several heavy metals, especially manganese. In this study it was noted earlier that all of the above factors were highest in spring.

Manganese concentrations were detected in the water column during autumn (inflow and below the dam), winter (below the dam) and spring (inflow and wall) surveys (Appendix A). All the manganese concentrations recorded throughout the study were below the TWQR of 0.18 mg/ℓ according to the DWAF (1996c) guidelines. The highest manganese concentration was recorded at all sites in spring and lowest at all sites in summer (Appendix A and Table 2.4). Levels of manganese in fresh water typically range from 1 to 200 mg/ℓ and levels of 10 mg/ℓ in acidic groundwater have been reported; higher levels in aerobic waters usually associated with industrial pollution (WHO 2006). Manganese concentrations were lower in the sediment and water samples in the barrage than in the lower Olifants River in the Kruger National Park(Seymore et al. 1994).

Selenium- the selenium concentration was only detected during spring survey with a mean value of 0.1 mg/ℓ at all sites (Table 2.4). All sites recorded a concentration of 0.1 mg/ℓ which is below the AEV, CEV and TWQR for aquatic ecosystems of 0.002 mg/ℓ (Appendix A).

Strontium concentrations were only detected during the spring and summer surveys at all sites in water. The highest mean concentration of strontium in water was recorded in spring and the lowest mean concentration was in summer (Table 2.4). The highest strontium concentration was recorded at the wall of the impoundment during spring (0.18 mg/ℓ) and lowest at the wall and below the sluices during summer (0.08 mg/ℓ) (Table 2.4).

Tin concentrations were recorded in summer only at all sites with mean values of 0.19 mg/ℓ (Table 2.4).The highest concentration was recorded at the inflow and at the wall (0.21 mg/ℓ) and lowest below the dam (0.17 mg/ℓ) (Appendix A and Table 2.4). There are no guidelines available for tin.

2.3.3 Metals in sediment

The concentrations of metals in the sediment were higher than those of the water column. More than 50% of the metals that were undetectable in the water column were present in elevated concentrations in the sediment. This is because sediments have been described as the ultimate sink for pollutants, metals and inorganic materials in aquatic systems (Chapman 1992; Bervoets and Blust 2003).

Table 2.5: Seasonal metal concentrations in the sediment (n=6) at the Phalaborwa Barrage in mg/kg dry weight (July 2010 and January 2011)

Metal	Winter 2010		Summer 2011		Sediment Quality Guidelines
mg/kg dry weight (dw)	Mean	SD	Mean	SD	
Aluminium	1883.3	413.88	6000	2835.5	No guidelines
Arsenic	5.5	3.5	-	-	[1]5.9
Antimony	1	1	-	-	No guidelines
Boron	242.3	6.02	42		No guidelines
Barium	354.33	52.3	38.6	39.4	No guidelines
Cadmium	0	0	11	1.4	[1]0.6
Cobalt	12	12.1	14	2	No guidelines
Chromium	80.6	83.4	180	208.09	[1]37.3
Copper	16	18.2	10	2	[1]35.7
Iron	12088	10809	27000	9913.6	No guidelines
Lead	6	2.6	-	-	[1]18
Manganese	378.3	410.4	216	33.2	[1]35.0
Nickel	39.6	43.6	29.3	8.08	No guidelines
Silver	79.3	33.4	-	-	
Strontium	27.3	28.4	24.6	11.01	No guidelines
Titanium	76	33.3	1269.3	633.5	No guidelines
Vanadium	22.667	18.2	136	86.4	No guidelines
Zinc	169.3	15.04	42	46.7	[1]123

Notes
[1]CCME (2012): Canadian Council of Ministers of the Environment: Sediment Quality Guidelines - aquatic life
SD: standard deviation

In sediment 20 metals and metaloids were detected (Table 2.5). All values in the sediment are measured in kilogram dry weight (kg/dw). All the metals are discussed in detail in Chapter 3 (Bioaccumulation). Possible sources of metals are: soil erosion and soil particulates delivered, mining and other anthropogenic activities (agricultural), and waste disposal from large villages and towns (US-EPA 2003).

Aluminium -the highest aluminium concentration (9200 mg/kg) was recorded at the wall during summer and lowest concentration (1568 mg/kg) in winter at the wall (Appendix A). A high mean Al concentration value of 9200 mg/kg was recorded in summer and a low Al mean concentration value of 1800 mg/kg was recorded in winter (Table 2.5).

Antimony concentration was only recorded in winter with a mean value of 1.9 mg/kg (Table 2.5). Highest concentration was recorded below the sluices (2 mg/kg) in the sediment (Appendix A).

Arsenic - a high mean concentration of arsenic in sediment was recorded in winter (5.5 mg/kg) while a low value was recorded in summer (3.5 mg/kg) (Table 2.5). The lowest concentration (2 mg/kg) was recorded in summer at all sites and the highest concentration at the wall in winter (8 mg/kg) (Appendix A).

Barium -a high mean barium concentration (350 mg/kg) was recorded in winter and a low value was recorded in summer (39 mg/kg) (Table 2.5). Barium concentrations varied between sites with a minimum concentration of 1 mg/kg recorded below the sluices summer and a maximum value of 40 mg/kg at the wall in winter (Appendix A).

Boron concentration fluctuated at various sampling sites. A high mean concentration was recorded in winter (242.33 mg/kg) and low in summer (6.02 mg/kg) (Table 2.5). The highest boron concentration was recorded at the wall in winter (248 mg/kg) and lowest (42 mg/kg) in summer (Appendix A).

Cadmium concentrations were recorded only in summer at the inflow and below the dam (Appendix A and Table 2.5). A high cadmium concentration was recorded at the inflow (12 mg/kg) and low concentration was recorded below the sluices (10 mg/kg) (Appendix A).

Cobalt concentrations were only detected in sediment samples. Concentrations for cobalt were constant within sites in all seasons with the highest mean concentration (14 mg/kg) in summer and the lowest mean concentration (12 mg/kg) in winter (Table 2.5). The highest concentration was recorded at the wall (26 mg/kg) in summer and lowest (4 mg/kg) at the inflow in winter (Appendix A).

Chromium concentrations were only detected in sediment samples. A higher chromium mean concentration (Table 2.5) was recorded in summer (180 mg/kg) and lower in winter (81 mg/kg). The lowest concentration value of 30 mg/kg was recorded at the inflow and the highest value of 420 mg/kg was recorded below the wall (Appendix A).

Copper concentrations detected in sediment were high with a value of 37 mg/kg in winter and a low concentration value of 10 mg/kg in summer. The lowest copper concentration was recorded at the inflow in winter (5 mg/kg) and the highest at the wall with a value of 18.19 mg/kg. The highest concentration recorded at the dam is above the sediment guideline as recommended by CCME (935.7 mg/kg) (Table 2.5).

Iron- a higher mean iron concentration was recorded in summer (27000 mg/kg) and the lower in winter (10288 mg/kg) (Table 2.5). The highest iron concentration in sediment was recorded at the inflow in summer (36600 mg/kg). The lowest iron concentration was recorded at the inflow in winter (4600 mg/kg) (Appendix A).

Lead concentrations were undetected during summer at all sites in the sediment (Appendix A). The highest lead concentration was recorded at the wall (9 mg/kg) and lowest at below the dam (4 mg/kg) during winter (Appendix A).

Manganese - a higher mean concentration of manganese in sediment was recorded in winter (378 mg/kg) and lower in summer (216 mg/kg) (Table 2.5). The lowest concentration of 129 mg/kg was recorded at the inflow in winter and the highest concentration of 852 mg/kg was recorded at the wall in winter (Appendix A).

Nickel concentrations were only recorded in the sediment. A higher mean concentration of nickel (39 mg/kg) was recorded in winter and lower in summer (29 mg/kg) (Table 2.5).The highest nickel concentration was recorded at the wall in winter (90 mg/kg) and lowest concentration was recorded at the inflow in winter (12 mg/kg) (Appendix A).

Silver concentrations were only recorded in sediment in winter survey only with a mean concentration of 79 .33 mg/kg which makes perfect sense as silver tends to sink deep into water due to its weight. The highest concentration was recorded at the wall (102 mg/kg) and the lowest concentration was at the inflow (41 mg/kg) (Table 2.5).

Strontium a higher seasonal mean concentration of 27 mg/kg was recorded in winter and a lower concentration of 24. 66 mg/kg was recorded in summer in the sediment (Table 2.5) .The highest strontium concentration (60 mg/kg) was recorded at the wall in winter and the lowest concentration (9 mg/kg) was recorded below the wall in winter.

Tin concentration in the sediment was only detected in summer at the wall (14 mg/kg) (Table 2.5).

Titanium concentration was recorded at higher concentrations in summer (1269 mg/kg) and lower in winter (33. 28 mg/kg) (Table 2.5). The highest concentration was recorded at the wall (1396 mg/kg) and lowest at the inflow in winter (38 mg/kg) (Appendix A).

Vanadium - a higher concentration was recorded in summer (136 mg/Kg) and lower in winter (23 mg/kg) (Table 2.5).The highest concentration was recorded at the inflow in summer (212 mg/kg) and lowest at the inflow in winter(8 mg/kg).

Zinc - a higher mean concentration in the sediment (156 mg/kg) was recorded in winter; and lower in summer (42 mg/kg) (Table 2.5).The highest concentration was recorded at the wall in winter (185 mg/kg) and lowest below the wall in summer (14 mg/kg).The concentration recorded in winter is above the Canadian sediment quality guidelines of 123.0 mg/kg (CCME 2012). High concentration of zinc in the sediment could be from natural processes such as weathering and erosion, and also through industrial activities(DWAF 1996a). A one way ANOVA shows no significant difference of all metal constituents in water between the four seasons ($F=1.62$; $df=1$; $p=0.21$).

2.3 CONCLUSIONS

Water temperatures were normal, with the highest records in spring and lowest in winter. Water temperature values ranged from 18 ºC in winter to 28ºC in spring. Dissolved oxygen concentrations recorded were within the TWQR during all seasons. The pH unitsof the water ranged from 7.4 to 8.7. The highest pH units were recorded in summer and lowest in autumn. The highest alkalinity value was recorded during spring at levels above TWQR for aquaculture water use and lowest in autumn Electric conductivity, turbidity, TDS and salinity were acceptable for aquatic ecosystems.

Water hardness (calcium carbonate), at the Phalaborwa Barrage was found to be moderately hard in autumn and winter at all sites and became harder in spring. The lowest mean value was recorded in summer and highest value was recorded in spring at the dam wall (Figure 2.1). An increase in water hardness was found to decrease the concentrations of metals through the precipitation of insoluble carbonates or calcium carbonate in that it act as a surface for the adsorption of metal ions (Dallas and Day 2004).

The anions were recorded at normal levels and fell within the TWQR. Most cations were recorded at levels within TWQR for aquatic ecosystem. Calcium concentration was very high in spring but the levels were still within TWQR for domestic use. The chloride levels were recorded at levels below detection limit for domestic use but at higher level above CEV and TWQR for aquatic ecosystem. Calcium, magnesium, sodium and potassium did not have the SAWQG for aquatic ecosystems or other guidelines.

The total inorganic nitrogen concentrations were very low, indicative of oligotrophic during autumn, winter, and summer. However, nitrate concentrations during spring were above the TWQR (2.5-10 mg/ℓ) an indicative of eutrophication (DWAF 1996c). Total inorganic P concentrations were above the TWQR (0.025 – 0.25 mg/ℓ), an indicative of eutrophic in winter, spring and summer. Noticeable elevated levels were recorded at the inflow in summer which is an indicative of hypertrophic conditions. Elevated concentrations of nutrients, in this case the total nitrogen and phosphorous may increase the abundance of algae and aquatic plants (DWAF 1996c).Consequently if the phosphorous becomes bioavailable, it will pose potential risk for eutrophication in the barrage.

The metalloids and metals that were detectable in water samples included; aluminium, antimony, arsenic, barium, boron, iron, manganese, selenium, strontium and tin. Metals that were detected in the water column are as follows in decreasing order: Al> Fe> Sr> Sn> B> Ba> Se> Mn> Sb> As. The metalloids and metals recorded above the TWQRs for aquatic ecosystems are aluminium, antimony, arsenic and selenium, an indication that these metals may pose adverse effects to the aquatic ecosystem in barrage if organisms are exposed to these concentrations over a period of time (Dallas and Day 2004). Aluminium was recorded above AEV, indicating that it may have acute effects to the aquatic ecosystem in the barrage. The metals that were within TWQR for aquatic ecosystems were barium, boron, iron and manganese. Several metals, such as cadmium, cobalt, copper, lead, nickel, silver, titanium, vanadium and zinc, the concentrations were below the detection limits of the ICP-OES.

Metals that were detected in the sediment samples are as follows in decreasing order: Fe> Al> Ti>Mn>B> Ba> Cr> Zn> V> Ag> Ni>Sr>Cu> Co>Pb> As> Cd>Sn>Sb> Se. The sediment metal mean concentrations collected at all sampling sites during the two surveys had high levels of aluminium, cadmium, chromium and zinc. However, metal concentrations that were recorded during summer (Al, Sb, Cd, Co, Cr, Fe, Si, Sn, Sr, Ti and V) were higher than the ones recorded in winter (Ag, As, B, Be, Cu, Li, Mn, Pb and Zn). Arsenic, cadmium and chromium were recorded at concentrations above the Canadian sediment quality guidelines (CCME 1999). Zinc concentrations were recorded at levels below suggested guidelines. Aluminium, manganese, cadmium, barium, boron, chromium, selenium and strontium were detected at unacceptable levels in water and sediment.

Generally the water quality at the Phalaborwa Barrage was found to be hard and unsuitable for drinking in its form without any adequate treatments due to elevated levels of water hardness recorded in summer. Eutrophication is a problem in most South African rivers, as a result of high levels of phosphates in urban effluent. Due to elevated (and some unacceptable) levels of metals in the water and sediment it can be concluded that the barrage is polluted with metals (Al, B, Ba, Cd, Cr, Mg, Se and Sr).

CHAPTER 3

BIOACCUMULATION AND HUMAN HEALTH

3.1 INTRODUCTION

Toxic substances to the environment are mostly metals. However, all metals are natural constituents of the environment and are found in varying levels in all ground and surface waters. Some metals are essential, required for the normal metabolism of aquatic organisms, while others are non-essential and play no significant biological roles (Coetzee et al. 2002). Bioaccumulation is the net result of the tendency of living organisms to concentrate chemical substances and accumulate in various tissues and organs. It is a process in which living organisms take up chemicals either directly from the surrounding environment or indirectly through the food chain (Coetzee et al. 2002). Bioconcentration is defined as the accumulation and transfer of substances directly from the surrounding medium (Adams et al. 2000). Among the more important aspects of bioaccumulation is the process of biomagnification, which refers to the concentration of a chemical pollutant increases at each successive level in the food chain (Davies and Day, 1998).

Several studies have been undertaken on bioaccumulation in different organs of the fish in the Olifants River and the Vaal River system (Seymore et al. 1994, 1995; du Preez et al. 1997; Robinson and Avenant-Oldewage 1997; Avenant-Oldewage and Marx 2000a, b; Barker 2006). However, most of these studies were aimed at contributing to the assessment of the health of the aquatic ecosystem under investigation which focused on species and tissue differences in contaminant bioaccumulation as well as the spatial and temporal variation in contaminant concentrations. The health risks to humans when consuming contaminated fish are seldom addressed (Heath et al. 2004).

This chapter deals with bioaccumulation of 20 selected metals in the indicator species *Clarias gariepinus* and *Labeo rosae* as well as health risks in humans consuming contaminated muscle tissue of fish and the correlation between metals in sediment and the fish tissue. Human health risk assessment was performed in order to link the metal accumulation in fish and the effects of those metals on human health if fish was consumed. In fish, metals mostly accumulate in the organs and tissues such as the liver, fat, gills, kidney, skeleton, skin, and to a lesser extend the muscle tissue. The muscle is the portion that is mostly consumed by humans; however, in some cases the whole body is consumed in species where the individual fish are too small to remove the viscera and gills. In this study only the muscle tissue was used to evaluate bioaccumulation of metals in fish because as it forms part of the major edible portion of the fish and the fish muscle is a good indicator in testing risks consumption.

3.2 METHODOLOGY

3.2.1 Fish collection and muscle tissue sampling

The two fish species were collected by means of gill nets with different mesh sizes (30-120 mm). The nets were set at various sites in the barrage depending on the depth, vegetation and substratum. The life fish were kept in large holding tanks filled with dam water until processing. Fish was then sacrificed by severing the spinal cord posterior to the head and dissected on a polyethylene work surface using stainless steel dissecting tools; care was taken to prevent contamination. Ten specimens from each fish species were sampled in each season for bioaccumulation analyses. The two skinless samples of the fish muscle tissue (15-20 g) were collected from each specimen. One sample was wrapped in plastic wrap and the other in aluminium foil. Both were labelled and then stored in a freezer on site. Plastic wrap is ideal for long term storage of the muscle if for metal analysis and also to lessen contamination. After the survey, the fish muscle tissue samples were stored in ultra-deep freezer at -80°C until was analysed.

3.2.2 Laboratory analysis

The frozen muscle tissue samples were sent to a SANAS accredited laboratory, WaterLab (PTY) LTD, in Pretoria. In the laboratory, the muscle tissue samples were dried, digested in nitric acid and hydrochloric acid and analysed to determine the metal content using inductively coupled plasma-optical emission spectrometry (ICP-OES). Inductive coupled plasma optical emission spectrometry (ICP-OES) was used for the detection of metals present in the sediment and muscle tissue samples. The results were corrected for spectral interference between metals and expressed in mg/kg dry weight.

3.2.3 Human health risk assessment

The assessment was done using the methodology outlined by the Environmental Protection Agency of the United States (US-EPA 1997) and the World Health Organization (WHO 2003) as summarised for use in South Africa (Heath et al. 2004). The assessment was carried out to determine the risk of metals in the fish muscle tissue to cause any adverse impact on humans when consuming the fish from the barrage. The human risk assessment was carried out by Dr. Bettina Genthe (CSIR Stellenbosch) for the WRC report of Jooste et al. (2013) using the Risk Assistant™ software package.

3.2.4 Statistical Analysis

All collected data were entered into Microsoft Excel 2010 programme and was later analysed using one-way analysis of variance (ANOVA) in statistical package programme (SPSS version 21). The mean and standard deviation of the metal concentration in the muscle tissue were calculated for each fish species. One-way analysis of variance tests with significance levels of 5% were conducted on each metal to test for differences among the metals in the two fish species and the sediment. A Human Health Risk assessment was carried out to determine whether consumption of fish from Phalaborwa Barrage might result in adverse human health effects. The health risk assessment (Jooste et al. 2013) was carried out according to the methodology as described by the US EPA (1996). For metals that cause non-cancer toxic effects, a Hazard Quotient (HQ) was calculated. The HQ is a value comparing the expected exposure to an exposure that is assumed not to be associated with toxic effects (Jooste et al. 2013). Any HQ less than one is considered to be safe for a lifetime exposure.

3.3 RESULTS AND DISCUSSION

The muscle tissue of each fish species and sediment samples were analysed for the 25 metals but only 20 metals were detected in both fish species muscle tissue and 20 in sediment samples (Table 3.1). Metals such as beryllium, bismuth, molybdenum and wolfram (tungsten) were analysed but are not included in the table because they were below the detection limits, both in the sediment or the muscle tissue of the two fish species. Cadmium was undetected in both fish species; cobalt was only detected in

C. gariepinus and undetected in *L. rosae*, therefore they were excluded in the discussion. Potential human health risks are illustrated in Table 3.2 as HQ. Highlighted cells indicate risks that are considered to be "unacceptable" by the US EPA.

3.3.1 Bioaccumulation in fish muscle tissue

Table 3.1: The mean concentrations of metal and metalloid (mg/kg dw) and the standard deviations in the muscle tissue of *Clarias gariepinus* and *Labeo rosae* (n=15) and sediment (n=6) from the Phalaborwa Barrage (May 2010-January 2011).

Elements Mg/kg dw	*C. gariepinus*		*L. rosae*		Sediment			
	mean	SD	mean	SD	mean	SD	F	P
Ag	1.4	±0.49	1.5	±0.50	79.33	±33.38	0.01	0.92
Al	41.9	±15.23	44.6	±10.06	3941.67	±2892.85	0.12	0.74
As	1.07	±2.18	3.13	±4.33	5.5	±3.53	0.12	0.73
B	246.1	±40.83	184.7	±77.68	196.5	±177.8	0.07	0.8
Ba	310.7	±56.47	217.8	±107.41	181.76	±121.25	0.01	0.94
Cd	0	0	0	±0.00	4.4	±6.07	-	-
Co	0.28	±0.36	0	±0.00	13	±7.87	-	-
Cr	13.4	±1.90	12.3	±2.15	130.33	±151.88	0.01	0.94
Cu	4	±0.67	3.3	±0.78	13	±12.03	0.01	0.94
Fe	92.9	±59.09	101.1	±75.57	19544	±12359.5	0.01	0.91
Mn	3.3	±1.57	2.9	±1.14	297.17	±275.17	0.01	0.91
Ni	1.31	±0.81	0.84	±0.71	34.5	±28.65	0.01	0.91
Pb	1.41	±0.83	1.31	±0.62	6	±2.65	0.01	0.91
Sb	1.92	±1.42	0.95	±0.90	1	±1	0.12	0.73
Se	3.52	±3.00	1.74	±1.70	-	-	0.01	0.92
Sn	1.05	±1.20	0.86	±1.44	3.5	±7	0.01	0.91
Sr	3.2	±2.04	5.7	±4.34	26	±19.29	0.01	0.91
Ti	1.2	±0.42	4.8	±10.41	672.67	±766.96	0.02	0.91
V	0.19	±0.28	3.68	±10.44	79.33	±83.5	0.12	0.73
Zn	193.1	±45.16	157.9	±28.01	105.67	±76.35	0.08	0.77

Aluminium (Al) - a mean Al value of 44.6 mg/kg was recorded in *L. rosae* while 41.9 mg/kg was recorded in *C. gariepinus* (Table 3.1). Aluminium was recorded at higher concentrations in sediment than in the muscle tissues of the two fish species (Table 3.1). The metal concentrations in the muscle tissues of the two fish species were different i.e. *L. rosae* accumulated aluminium at a higher concentration than *C. gariepinus* (Table 3.1). When using one-way ANOVA, all metals showed no significant difference between the two fish species (Table 3.1). The aluminium concentration recorded in this study was found to be higher than the one recorded in the study conducted by Oberholster et al. (2011, 2012). However, levels recorded in this study are in agreement with the study conducted by Crafford (2010) in the Vaal Barrage. *Labeo rosae* accumulated aluminium at elevated concentrations which could be related to the intake of phytoplankton containing higher levels of Al and Fe Oberholster et al. (2012). Oberholster et al. (2012) also stated that aluminium and iron concentrations have the ability to accumulate in algae which then bio magnify in higher trophic level organisms through the food chain which could be the reason why it was detected in higher concentrations in *L. rosae*. Probably the sediment might be the source of the undesirable concentrations of aluminium in the water (Chapter 2). Elevated levels of Ag, As, Ba, B, Ti and Zn were recorded in Phalaborwa Barrage which was found at lower concentration at the Flag Boshielo Dam (Kekana 2013).

Antimony (Sb) - the average antimony concentration in the muscle tissue of *C. gariepinus* (1.92 mg/kg) was double that of *L. rosae* (0.95 mg/kg) (Table 3.1). A mean value of 1 mg/kg of antimony was recorded in sediment (Table 3.1). An elevated concentration of antimony recorded in *C. gariepinus* may be as a result of biomagnification.

Arsenic (As) - a higher mean concentration of As was recorded in *L. rosae* (3.13 mg/kg) than in *C. gariepinus* (1.07 mg/kg) (Table 3.1). A mean concentration of 5.5 mg/kg was recorded in the sediment which is above the concentrations in the muscle tissue. Although arsenic was recorded in low concentration it is very toxic to freshwater and marine aquatic life even in small quantities and it is relatively accessible to aquatic organisms. Arsenic concentration in this study might be from weathering of arsenic containing rocks and industrial activities that utilise arsenic or its compounds (DWAF 1996a). Elevated levels of arsenic in the muscle tissue of *L. rosae* could be due to difference in regulatory ability, behaviour and feeding habit of the two fish species (Kotze et al. 1999).

Barium (Ba) concentrations were more elevated in the muscle tissues of *C. gariepinus* (310.7 mg/kg) than in *L. rosae* (217.8 mg/kg) (Table 3.1). The lower Ba concentration in the sediment samples (181.76 mg/kg) is possibly an indication that biomagnification took place, the species sensibility on the metal uptake from the aquatic environment and species trophic level in the food chain. In addition the type of the food each species consume might have contributed to the elevated levels of barium present in the catfish.

Boron (B) was recorded at higher concentrations in the muscle tissue of *C. gariepinus* (246.1 mg/kg) than in the sediment (196.5 mg/kg) (Table 3.1) but the concentration in the sediment was higher than in *L. rosae* (184.7 mg/kg) (Table 3.1). There was no significant difference between the two fish species. *Clarias gariepinus* is omnivorous scavenger and predator by nature (Skelton 2001), usually feeding at the bottom of the water or in thickly weeded areas, it feeds on anything from benthic organisms, zooplankton, hydrophytes, frogs, seeds, and fruits, to small zooplanktons and fish. The elevated concentrations of boron in *C. gariepinus* could be from the plants as Boron is an essential micronutrient in plants.

Cadmium (Cd) was below detection limit in both the muscle tissues of the two fish species. It was only recorded in sediment samples with a mean concentration value of 11 mg/kg (Table 3.1) which exceeded the Canadian sediment guidelines (0.6 mg/kg) (CCME 2001). Cadmium is found naturally in the earth's crust in association with zinc, lead and copper, thus increase of cadmium could be attributed to the presence of these metals which could be brought by the weathering processes and industrial activities (Dallas and Day 2004). The solubility of cadmium in water is influenced to a large degree by its acidity; suspended or sediment-bound cadmium may dissolve when there is an increase in acidity (CCME 1999). Sediment metal concentrations recorded in this study were found to be lower than the concentration recorded by van Aardt and Erdmann (2004) at the Mooi River catchment. Their study showed Cd concentrations between 20 and 100 mg/g in the kidneys while in this study only the muscle tissue was tested. These findings were found to be in agreement with the work done by Barker (2006) in the Luvuvbu, Shingwedzi, Sabie and Letaba Rivers.

Although Cd was not detected in the muscle tissue of both fish species it is considered toxic to humans and is known to be carcinogen. Metals are non-biodegradable in nature (Wepener et al. 2001) and they tend to bio-accumulate and undergo bio magnification in food chain (James et al. 1998).

Chromium (Cr) - The highest Cr mean value of 13.4 mg/kg was recorded for *C. gariepinus* and *L. rosae* had a mean value of 12.3 mg/kg (Table 3.1). The Cr mean concentration in the sediment had a value of 130.3 mg/kg which also exceeded the Canadian SQGs of 37.3 mg/kg (CCME 2001). There was no significant difference between the two fish species. The chromium concentrations in muscle tissue of *C. gariepinus* from the Mamba Weir and Balule in the Olifants River, KNP (Avenant-Oldewage and Marx 2000b) were generally higher than those detected in the same fish species within the barrage. Elevated concentrations of chromium in both fish species would definitely pose health risks to humans consuming the fish from the barrage.

Cobalt (Co) was only detected in *C. gariepinus* with a mean value of 0.3 mg/kg (Table 3.1). However, higher cobalt concentration was recorded in the sediment than in *C. gariepinus* with a mean value of 13 mg/kg (Table 3.1).

Copper (Cu) concentrations recorded in *C. gariepinus* (3.9 mg/kg) were slightly higher than that recorded in *L. rosae* (3.4 mg/kg) (Table 3.1). Higher copper concentrations were recorded in the sediment (13 mg/kg) than in the muscle tissues (Table 3.1).

The bioaccumulation pattern of Cu and Zn in different organs/tissues of *C. gariepinus* observed during this study are in agreement with the study done by du Preez et al. (2003); Seymore (1994) and Kotzė et al. (1999) study at the Olifants River found that fish in both the Loskop Dam and below the Phalaborwa Barrage at Mamba Weir (KNP) were exposed to high levels of Cu and Zn which accumulated in liver, gills, skin and muscle of *Oreochromis mossambicus* and *Clarias gariepinus*. Elevated levels of copper, chromium and zinc in humans cause nephritis, anuria and extensive lesions in kidneys.

Iron (Fe) - the iron concentrations were higher in *L. rosae* (101.1 mg/kg) than *C. gariepinus* (93 mg/kg) (Table 3.1). However, the highest Fe concentration was recorded in the sediment (19544 mg/kg) (Table 3.1).

During this study Fe concentration in the muscle tissue of *L. rosae* were higher than those recorded by Crafford (2011) at the Vaal Barrage and Vaal dam. However, Fe levels were recorded at higher concentrations at the Klein Olifants River than those levels recorded in the barrage. Difference in Fe bioaccumulation between the two fish species could be ascribed to differences in feeding habits and behaviour of the two species. *Daphnia pullex* and other planktonic organisms are important food items for younger specimens of *C. gariepinus* (Skelton 2001). The elevated Fe and Zn concentrations could be attributed to effluents from mining and industrial sectors (Kotzè et al. 1999; Coetzee et al. 2002). There is a significant impact on the ecosystem from sediment-associated risk contaminants, they have been found to range from direct effects on benthic communities (Avenant-Oldewage and Marx 2000) to substantial contributions to pollutant loads and effects on upper trophic levels through the food chain. Iron concentrations in this study in the fish muscle tissue were lower than the concentrations in *C. gariepinus* and *L. umbratus* from the Olifants and Klein Olifants River (Coetzee et al. 2002).

Lead (Pb) - both fish species accumulated the same levels of lead into their muscle tissue (1.0 mg/kg) (Figure 3.2 D). Elevated concentrations of lead were recorded in the sediment (6 mg/kg) (Table 3.1). The mean Pb concentrations found in the muscle tissue of the two species were lower than concentrations of this metal found in the muscle tissue of fish in several rivers and dams in the Olifants River catchment by several studies (Seymore et.al.1995; Nussey et al. 1999; du Preez et al. 1997; Watson 1998; Coetzee et al. 2002). These studies found levels of most metals higher in the gills, liver and gonads than the muscle tissue which suggest that primary route of exposure was those organs. Lead presence in this study might be from weathering of sulphide ores and from the boats used in the barrage (DWAF 1996a).

Manganese (Mn) - the mean manganese concentration value in *L. rosae* (2.9 mg/kg) was close to that of *C. gariepinus* (3.3 mg/kg) (Table 3.1). Manganese concentration was elevated in the sediment (297.17 mg/kg).This concentration value was lower than the concentration recorded by Seymore et al. (1995) fish species in the lower Olifants River and in *Labeo umbratus* in the Witbank Damby Nussey et al. (2000). The levels of Mn in the muscle tissue of the two fish species in this study were recorded at lower levels when compared to levels recorded in the muscle tissue of *C. gariepinus* and *L. umbratus* from the Olifants and Klein Olifants River (Coetzee et al. 2002).

Nickel (Ni) - the two fish species recorded same mean concentrations value of 1 mg/kg in their muscle tissue (Table 3.1). The sediment had the highest nickel concentration (34.5 mg/kg) (Table 3.1). Fish are known to accumulate nickel in different tissues when exposed to elevated levels in the environment (Nussey et al. 2000). During this study it was found that *C. gariepinus* accumulated nickel at higher concentration than *L. rosae* although the difference was not significant. Levels of Ni and Pb in the muscle tissue were recorded at higher concentrations at the Phalaborwa Barrage than the levels recorded in the muscle tissue of the fish at the Witbank Dam in Mpumalanga by Nussey et al. (1999; 2000).

Selenium (Se) - an elevated selenium concentration was recorded in *C. gariepinus* (3.52 mg/kg) than in *L. rosae* (1.74 mg/kg) (Table 3.1). Selenium was below detection limit in sediment samples.

Silver (Ag) concentrations were recorded at lower levels in the muscle tissue of *C. gariepinus* (1.4 mg/kg) than in *L. rosae* (2 mg/kg) (Table 3.1). Elevated levels of silver concentrations were recorded in sediment (79.33 mg/kg) as compared to the fish species (Table 3.1).

In sediment higher levels of silver were recorded than in fish muscle tissue and concentrations of these metals can pose adverse health effects to both fish and humans.

Strontium (Sr) mean concentration for *L rosae* was recorded almost twice higher than in *C. gariepinus* with mean values of 6 mg/kg and 3.2 mg/kg respectively (Table 3.1). However, higher strontium concentrations were recorded in sediment than the two fish species with a mean value of 26 mg/kg.

Strontium together with lead accumulates in bony tissues due to their similarities to calcium (Moore and Ramamoorthy 1984). Strontium concentrations in muscle tissue of the two fish species in this study were higher than the Sr concentration in the muscle tissue of yellow fish at the Lower Olifants (Seymore et al. 1995).

Tin (Sn) – constant levels of equal values of tin concentrations were recorded in muscle tissue of the two fish species (Table 3.1). Tin concentrations were recorded at elevated levels in the sediment (3.5 mg/kg) and were lower in the two fish species with a concentration value of 1 mg/kg (Table 3.1). Tin is generally regarded as being relatively immobile in the environment and it binds to the soils and to sediment in water and. Its compounds may also settle out of the water into sediment and may remain unchanged for years. Organic tin compounds may be taken up into the tissues of aquatic animals (WHO 2005).

Titanium (Ti) concentrations were higher in the muscle tissue of *L. rosae* than that of *C. gariepinus* with mean value of 5 mg/kg and 1.2 mg/kg respectively. The Ti mean concentration recorded in the sediment (672.67 mg/kg) was higher than in both fish species (Table 3.1). Titanium is not found unbound to other elements in nature, however, it is the ninth most abundant element in the Earth's crust (0.63% by mass) and is present in most igneous rocks and in sediment derived from them (ATSDR 1997). Some of the titanium compounds may settle out to soil or water. In water, they sink into the bottom sediment. They may remain there for a long time in the soil or sediment. No environmental effects have been reported (Kekana 2013).

Vanadium (V) concentrations were recorded at higher concentrations in *L. rosae* (4 mg/kg) than *C. gariepinus*0.2 mg/kg) (Table 3.1). However, a very high concentration of vanadium was noted in the sediment (79.3 mg/kg) (Table 3.1). The concentration of V in the sediment was recorded higher than the concentration detected in the study done by Watson (1998) at the Olifants River.

Zinc (Zn) concentrations were elevated in muscle tissues of *C. gariepinus* (193 mg/kg) as compared to mean value of 157.9 mg/kg recorded in *L. rosae* (Table 3.1). A low mean concentration value of zinc was recorded in the sediment (105.67 mg/g) (Table 3.1). Zinc biomagnified from the aquatic environment in this case sediment to the muscle tissue of the fish. Levels of Zn in the muscle tissue of *C. gariepinus* from the Upper Olifants (Kotze et al. 1999) and Klein Olifants (Coetzee et al. 2002) were recorded in lower concentrations when compared to those from the barrage. Difference in Zn bioaccumulation between the two fish species could be ascribed to differences in feeding habits and behaviour of the two species. The elevated Zn concentrations could be attributed to effluents from mining and industrial sectors in the Upper Olifants (Kotzè et al. 1999; Coetzee et al. 2002).

3.3.2 Human health risk assessment

Hazard Quotients

The metal bioaccumulation results in this study revealed that the two fish species absorbed metals from their environment. These metals could be transferred to organisms (i.e. Crocodiles, birds) that consume these fish, including humans. Numerous rural communities in the Olifants River catchment rely on fish from the river, and impoundments in the catchment, to supplement their diet, as an important source of protein (Jooste et al. 2013). The risk to human health associated with consuming contaminated fish from the Phalaborwa Barrage impoundment was evaluated based on weekly and daily consumptions of a single 150g fish meal and the results presented in Tables 3.2 for the weekly and the daily consumption. Hazard Quotients were calculated for the metals found in the respective fish species and summed to provide a measure of the potential risk of consuming the fish.

The total hazard quotient (which refers to the measure of levels of concern) was recorded higher in *C. gariepinus* than *L. rosae* on weekly and daily basis consumption (Table 3.2). However, more metals exceeded the Hazard Quotients of 1 (HQs) in *L. rosae* (antimony, arsenic, chromium, and lead). For *C. gariepinus* the unacceptable HQs were: chromium and lead (Table 3.2).

Table 3.2: The contribution of the respective metals to the Hazard Quotient for *Clarias gariepinus* and *Labeo rosae* from the Phalaborwa Barrage; calculated for one fish meal (150 g) consumed on a weekly and daily basis (Adapted from Jooste et al. 2013). The shaded values indicate where HQ value of one was exceeded.

	Clarias gariepinus		***Labeo rosae***	
Harzard Quotients	Weekly consumption	Daily consumption	Weekly consumption	Daily consumption
Aluminium	0	0.02	0	0.02
Antimony	0.34	2.39	0.21	1.44
Arsenic	0.24	1.7	0.66	4.65
Barium	0.11	0.8	0.09	0.62
Boron	0.09	0.63	0.07	0.5
Cadmium	0	0.01	0	0.01
Chromium	0.35	2.46	0.29	2.04
Cobalt	0.05	0.37	0.02	0.12
Copper	0.01	0.05	0.01	0.05
Iron	0.01	0.07	0.01	0.07
Lead	1.98	13.87	1.5	10.48
Manganese	0	0.01	0	0.01
Nickel	0	0.03	0	0.02
Selenium	0.05	0.38	0.03	0.19
Silver	0.02	0.16	0.02	0.15
Strontium	0	0	0	0.01
Tin	0	0	0	0
Vanadium	0	0	0.04	0.26
Zinc	0.05	0.36	0.04	0.3
Total	**3.3**	**23.33**	**2.99**	**20.94**

The HQ values for the daily consumption of fish are seven times those for the weekly consumption. The Total Hazard Quotients for daily consumption for both fish species is higher than the total HQ for weekly consumption (Tables 3.2). For the weekly consumption, both fish species exceeded the recommended HQ of 1 for lead. For daily consumption, both fish species exceeded the recommended HQ for antimony, arsenic, chromium and lead (Table 3.2). The contributions of the metals to the HQ value for *C. gariepinus* were as follows: Pb>Cr>Sb>As>Ba>B>Se>Co>Zn. For *L. rosae* the contributions of the metals to the HQ value were as follows:

Pb>As>Cr>Sb>Ba>B>Zn>V (Table 3.2). Those metals with HQ of 0 are excluded in the table.

The health risks are greater from the consumption of *C. gariepinus* (Total HQ = 26.63) than *Labeo rosae* (Total HQ = 23.93) caused by presence of antimony, arsenic, chromium and lead and to a lesser extent barium, and selenium in the muscle tissues of the two fish species (Table 3.2). Arsenic is an EDM and has the ability to interfere with the nervous system and is highly carcinogenic to humans (ATSDR 2007). A higher hazard quotients ranging from 0.24 to 1.70 for *C. gariepinus* and 0.66 to 4.65 for *L. rosae* were recorded. However, the greatest risk was recorded in antimony, chromium and lead as the hazard quotient value is greater than 1 which is unsafe for a lifetime exposure to humans. Both fish species collected from Phalaborwa Barrage resulted in unacceptably high hazard quotients ranging from 2 to 7 which are considered being safe based on a weekly fish meal.

3.4 CONCLUSIONS

Clarias gariepinus

Metal concentrations in the muscle tissue of *C. gariepinus* in decreasing order were: Ba > B > Zn > Fe > Al > Cr > Cu > Se >Mn > Sr > Sb > Pb > Ag > Ni > Ti > As > Sn > Co > V. The most abundant metal recorded in the muscle tissue of *C. gariepinus* was barium (300.2 mg/kg). Second highest to barium was boron (236.2 mg/kg), followed by zinc (199 mg/kg) and iron (90.1 mg/kg) in descending order. Vanadium was not detected in the muscle tissue of *C. gariepinus*. Similar to *L. rosae*, most metal concentrations were detected below “safe” daily limit for human consumption in both fish species: aluminium, cadmium, cobalt, copper, manganese, nickel, selenium, silver, strontium, tin, vanadium and zinc. Antimony, arsenic, chromium, lead and to a lesser extend barium and boron were the metals that may cause risks because the HQ is above one.

Labeo rosae

Metals accumulated in the muscle tissue of *L. rosae*, in the decreasing order of metal concentrations were: Ba > B > Zn > Fe > Al > Cr >Sr> Ti > V > Cu > Mn> Se > Ag >

Pb> Sb> Sn> Ni > Cd > Co > As.Most of the tested metals concentrations were detected below “safe” daily limit for human consumption in *L. rosae* muscle tissue. However, the HQ calculated values in *L. rosae* for antimony, arsenic; chromium, lead lesser extend barium and boron were recorded at values above one which may cause risks because the HQ is above one.

The hypothesis that metals can accumulate in fish tissues was supported. The two fish species accumulated lower concentrations for most metals as compared to the sediment. The detection of more metals at higher concentration in the sediment is not surprising as the sediment serve as a sink of metal contamination in aquatic ecosystems (Milenkovic et al. 2005). The most abundant metals recorded in sediment samples were aluminium, arsenic, cadmium, cobalt, chromium, copper, iron, manganese, nickel, lead, silver, strontium, tin, titanium and vanadium as compared to metals accumulated in the fish muscle tissue of *C. gariepinus* ; antimony, barium, boron, selenium and zinc. However, barium, boron and zinc, were recorded at lower concentrations in the sediment as compared to concentrations of similar metals in the two fish species.

CHAPTER 4
FISH HEALTH AND PARASITES

4.1. INTRODUCTION

Health Assessment Index

The fish health is assessed by using the Health Assessment Index (HAI) with the Parasite Index (PI) incorporated. The fish Health Assessment Index (HAI) used in this study was developed in the United States of America (USA) as a field necropsy method which is a rapid and inexpensive alternative to more sophisticated approaches for evaluation of fish health and condition (Goede and Barton 1990). When applying the index, a numerical value is awarded to examined fish tissue or organs depending on the degree of stressors-induced abnormalities. The total sum of values awarded is the index value for that fish and the mean for all sampled fish is the index value for that locality. An increase in the index value correlates with decreased water quality (Crafford and Avenant-Oldewage 2009). In the original HAI by Adams et al. (1993), parasites were merely recorded as being present or absent. Adams et al. (1993) then developed the Health Assessment Index (HAI) to minimise those limitations of the necropsy method by rendering its quantitative for statistical analysis and comparisons among data sets (Adams et al. 1993). In the HAI, the index variables are assigned numerical values 0-30; (0, 10, 20 and 30) based on the degree of severity or damage incurred by an organ or tissue from environmental stressors. Avenant-Oldewage and Swanepoel (1993) then suggested the use of fish health studies in South Africa. Subsequently, the fish HAI has been applied and adapted for local conditions, through studies in the Olifants River system (e.g. Avenant-Oldewage et al. 1995; Watson 1998; Marx 1996; Luus-Powell 1997; Jooste et al. 2004 and 2005; Crafford and Avenant-Oldewage 2009; Madanire-Moyo et al. 2012). The HAI has proven to be a simple and inexpensive means of rapidly assessing general fish health in field situations. Although the necropsy method provides a health status profile of a fish population, however, there is no quantitative basis of comparing statistically the entire index with all its variables to another population sample either in time or space.

Parasites

Parasites are present in all ecosystems and form an integral part of every ecosystem (Pietrock and Marcogliese 2003).They are receiving a lot of attention worldwide from parasite ecologists as potential indicators of environmental quality because of the variety of ways in which they respond to anthropogenic pollution (Sures et al. 1999). The presence of parasites weakens the health of the fish and therefore a deteriorated surrounding environment (Avenant-Oldewage 2001).

Parasites were included in the original HAI and were only recorded as absent (value of zero) or present (value of ten or more). It was observed that the endo- and ectoparasites were affected differently by heavy metal pollution and that poor water quality affects ectoparasites to a greater degree than it would as compared to endoparasites. As of this assumption it follows that lower numbers of ectoparasites will correlate with a decrease in water quality (Avenant-Oldewage 1998, Crafford and Avenant-Oldewage 2009). High numbers of endoparasites will thus indicate poorer water quality, and vice versa. This led to the development of a Parasite Index (PI) within the South Africa and tested at the Lower Olifants River in conjunction with the HAI (Marx 1996;Robinson 1996; Luus-Powell 1997). The PI was developed to use as an index to detect pollution effects. It can also reflect the effect of the number of parasites on the HAI i.e. fish health, by differentiating between the numbers of ecto- and endo-parasites and thereafter the inverted PI (IPI) was developed. The inverted parasite index (Table 4.1) is based on the premise that ectoparasites are more directly exposed to the effects of poor water quality than endoparasites (Crafford and Avenant-Oldewage 2009).

4.2 METHODOLOGY

4.2.1 Fish Health Assessment Index (HAI) and associated Parasite Index (PI)

4.2.2 Fieldwork

Four seasonal surveys were conducted at the Phalaborwa Barrage, in May 2010 (autumn), July 2010 (winter), October 2010 (spring) and January 2011 (summer). Ten specimens of each fish species (*C. gariepinus* and *L. rosae*) were collected seasonally. The fish were collected by means of gill nets with different mesh sizes (30-120mm). Nets were set in the late afternoon and left overnight, then collected in the next early morning. Fish were then kept alive in large holding tanks filled with aerated dam water. Fish were processed individually when determining the HAI and PI. Two skin smears were made with a glass slide and examined with the aid of a stereo stereomicroscope (100X magnification). Blood was drawn using a medical syringe from the dorsal aorta into capillary tubes sealed at one end using commercial critoseal clay. Blood collection was done as quickly as possible before the fish died. The haematocrit reading was measured and recorded after centrifugating the capillary tubes in a micro-haematocrit centrifuge (Model: KHT -400) for five minutes at 15 000rpm.

Fish were examined externally by using the revised HAI method (Appendix B)(Heath et al. 2004, Jooste et al. 2004) and all abnormalities on the fins, skin and eyes were recorded on HAI data sheets. Fish were then placed on a polypropylene dissecting board and sacrificed by severing the spinal cord, weighed (in g) and measured (standard and total length in mm). The muscle tissue of each fish species was also scrutinized for encysted parasites. All collected parasites were fixed and preserved (see below). The abundance of all parasites was recorded on field data sheets. The fish was then dissected on the ventral side; all internal organs were examined and assessed using a colour chart developed by Watson (2001),and HAI values were assigned to each organ in the field score sheet as indicated in the revised HAI table (Appendix B). Abnormalities of the internal organs or the presence of parasites were recorded on HAI data sheets.

4.2.3 Calculation of the Health Assessment Index

The original field of all variables from the necropsy-based system were converted to numerical values assigned to the HAI categories (Appendix B). All the variables of the HAI were presented with a value ranging from 0-30, depending on the condition of the organs examined. The HAI value was calculated for each fish within a sample, by summing up the values assigned to the respective HAI categories. The mean HAI for population were calculated seasonally by adding all individual fish health HAI values and dividing it by the total number of fish examined (Appendix B).

4.2.4 Fixing and preserving parasites

Fixing, preservation, whole mount preparation and identification of parasites followed standard procedures used by authors such as Douëllou (1993) for monogeneans, Barson et al. (2008) and Chibwana and Nkwengulila (2010) for digeneans, Anderson (1992) for nematodes, Khalil et al. (1994) for cestodes.

Monogeneans collected from gills and skin were placed in small Petri dishes containing distilled water and then stored in 70% ethanol. The monogeneans specimens from gills were then mounted in glycerine jelly, and sealed with a clear nail varnish and labelled.

Digeneans were placed in 0, 8% saline solution then shaken vigorously to remove excess debris. They were fixed flat between two glass slides in AFA for at least 10 minutes and then preserved in ethanol (70%).

Cestodes were fixed and stored in 10% warm buffered formaldehyde.

Nematodes were fixed in glacial acetic acid and preserved in 70% ethanol.

Copepods were fixed and stored in 70% ethanol.

4.2.5 Laboratory work

Preparation of whole mounts and identification of different parasites were done in the laboratory where specimens were stained either with Horen'sTrichhome™ or Aceto Carmine™ solution depending on availability. If overstrained, they were placed in 2% hydrochloric acid solution. Parasites were cleared in lactophenol or clove oil for 10 minutes or overnight if necessary. Specimens were mounted on pre-cleaned glass slides with Canada balsam or Entellan™ and labelled. Nematodes were cleared with lactophenol and mounted without staining (temporary mount). All parasites were micro-photographed with the aid of a Leica™ stereo microscope or Olympus™ light microscope with an Olympus™ digital camera adapter and a digital camera (C50-50 100X Zoom). The parasites were identified to the highest taxonomic resolution and, where possible, to species level.

4.2.6 Data analysis

Ecological terms used in infestation statistics

There are a variety of terms used by Parasitologists to describe the number of parasites in a host or the number of infected hosts in a sample, such terms include parasite burden; parasite load; level or extent of infection; degree of infection or infection rate. The terminology as recommended by Bush et al. (2001) was adopted for this study.

Parasite prevalence, mean intensities and abundance of ecto- and endoparasites per season for each fish species were calculated as suggested by Margolis et al. (1982).The following indicators were used to determine the infection level:

Prevalence; number of infested individuals of a host divided by the number of hosts examine, expressed in percentage. (Percentage of fish infected);

Mean intensity; total number of a particular parasite species divided by the number of infested hosts (mean number of parasite individuals per infected host);

Abundance; total number of particular parasite species divided by the total number of hosts in a sample (mean number of parasites per infected and non-infected fish).

4.2.7 Parasite index

The ectoparasites and endoparasites are included as separate variables in the HAI tested in South Africa (Luus-Powell 1997, Watson 2001). The eco- and endoparasites were categorised in a table form (Table 4.1).

Table 4.1: The revised Parasite Index (Joosteet al*.* 2004) and Inverted Parasite Index (IPI) (Heathet al*.* 2004).

Ectoparasites	PI	IPI	Endoparasites	PI
0	0	30	0	0
1-10	10	30	<100	10
11-20	20	20	101-1000	20
21-30	30	10	>1000	30
>30	30	0		

4.2.8 Calculating the Condition factor

The condition factor of each fish specimen was calculated using the following formula described by Froese (2006) whereby:

$$CF = \text{weight} \times 10^5 / \text{length}^3$$

The minimum, maximum, and mean condition factor, mean length, mean weight and standard deviation (SD) were statistically determined for each fish species per season to compare condition factors between the various seasons and fish species.

4.3 RESULTS AND DISCUSSION

4.3.1 Health Assessment Index

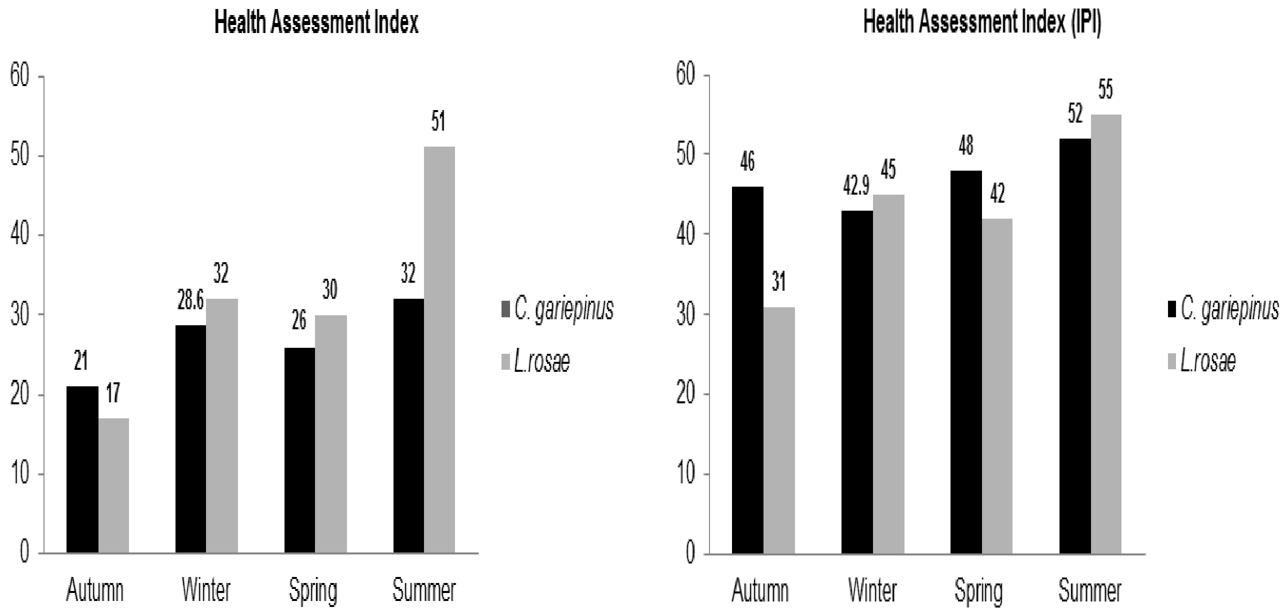

Figure 4.1: Seasonal population Health Assessment Index (HAI) and Inverted Parasite Index (IPI) for *Clarias gariepinus* and *Labeo rosae* at the Phalaborwa Barrage

The two fish species *Clarias gariepinus* and *Labeo rosae* were used in this study as indicator species because they are important angling species, consumed by humans and .they consume different diet. The occurrence of abnormal conditions recorded in this study for the four sampling surveys when conducting the Health Assessment (PI) and HAI (IPI) is outlined in Appendix B.

In total, 80 fish were collected from the barrage; 10 fish per season for both *L. rosae* and *C gariepinus* (Appendix B). High HAI values which indicate that the fish contains health problems were recorded in summer for both fish species (Figure 4.1). *Labeo rosae* had the highest HAI value of 51 in summer and *C. gariepinus* had the highest HAI mean value of 32 also in summer (Figure 4.1). Low HAI values, which indicate healthier fish, were recorded in autumn for both fish species (Figure 4.1). *Clarias gariepinus* had a lowest mean value of 21 in autumn and *L. rosae* had a lowest HAI value of 17 in autumn. There was a significant difference ($p<0.05$) of HAI value among the two fish species and also among the seasons. The mean HAI value for *C. gariepinus* at the Phalaborwa Barrage were found to be lower than the mean HAI values recorded at the Vaal Dam and Vaal River Barrage (Crafford and Avenant-Oldewage 2009). The blood haematocrit (Hct) and the PI frequently contributed much to the total HAI (Appendix B). The two fish species appeared to be in overall good condition with only the liver, blood haematocrit and parasites contributing mostly to the HAI. The refined PI was inverted so that the high numbers of ectoparasites would be given lower scores for compatibility with the HAI. For HAI (IPI), lowest HAI (IPI) was recorded in winter for *C. gariepinus* with a mean value of 42. The lowest HAI (IPI) for *L. rosae* was recorded during autumn with a mean value of 31 (Figure 4.1).

External and internal variables

Factors such as size, sex and type of the species may affect or elevate stress response in fish (Adams et al. 1993). The highest weight recorded in this study for C*. gariepinus* is 4843 g while for *L. rosae* is 1030 g (Appendix B) which might be one of the factors that influenced the health of the two fish species. More abnormalities were observed in *C. gariepinus* than in *L. rosae*. The overall abnormalities observed in *C. gariepinus*: aberrations and cysts on the skin; cataracts in the eyes; discoloration and black coloration on the liver and abnormal values of blood haematocrit (HCT).

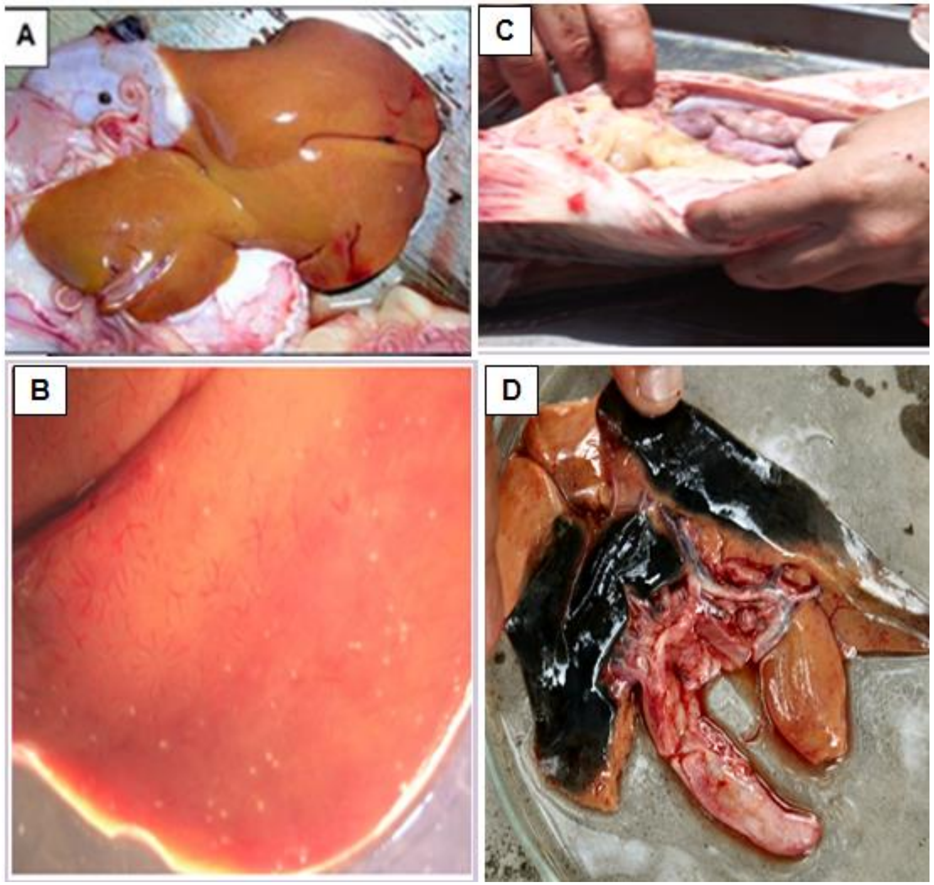

Figure 4.2: Condition (health) of fish at the Phalaborwa Barrage recorded from internal organs of *Clarias gariepinus*. A: Healthy liver; B: white spots on the liver; C: Mesenteric fat in the body cavity; D: Discoloured liver with blackened portions.

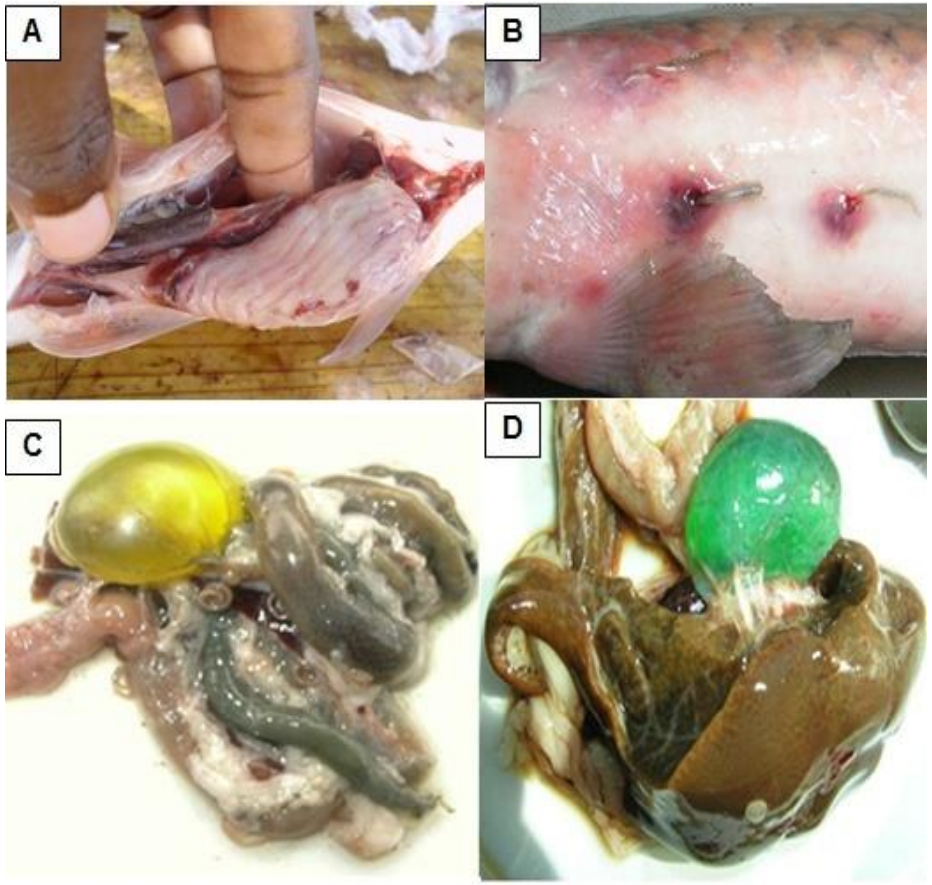

Figure 4.3: A: Condition (health) of fish at the Phalaborwa Barrage recorded from internal organs of *Labeo rosae*; A: Mesentric fat >50%; B: *Lamproglena* sp. on skin of *Labeo rosae*; C: Yellow bile; D: Green bile

In *L. rosae* the abnormalities observed were: skin lesion discolorations on the liver and high HCT values. Presence of both ecto- and endo-parasites contributed more to the total HAI values in both fish species in addition to abnormalities recorded from liver, HCT and skin.

No abnormalities were recorded for the operculum, gill conditions, spleen, kidney, or inflammation of the hindgut in both fish species.

No eye abnormalities were recorded for *L. rosae.* Abnormalities in the eyes were only recorded in *C. gariepinus* in winter in which the eye appeared opaque, probably from the infection of the parasite, *Diplostomum* sp. larvae. This can also be attributed to predation or mechanical injury from the barrage wall, nets or other organism in the water. Although several digenean larvae were retrieved from the eyes during this study but no obvious damage to the eyes was observed, therefore the eyes were recorded as normal with a value of zero.

During this study mild skin aberrations were recorded in both fish species. During winter survey one *L. rosae* showed mild aberration while in *C. gariepinus* aberrations were recorded in summer. No forked or abnormally long or short fins were observed on the fins in both fish species. Several fish exhibited black spots on their skin and fins which might be caused by a digenean larvae (trematode cysts) and they were allocated HAI values from 10-20 (Appendix B and Table 4.1). Except for the presence of these black spots, the fins and skin of most of the fish in this study were normal.

Pseudo-branch: No abnormalities were recorded for the pseudo-branch of *C. gariepinus*.

Discolorations of the liver were recorded in both fish species. For *L. rosae*, reddish to coffee colour was recorded in three livers(Figure 4.2 C and D) during winter and for *C. gariepinus*, one fish had abnormalities in the liver in autumn and six fish had discoloured livers with black spots in summer (Figure 4.2C and D). Liver conditions contributed a great deal to the HAI score in *C. gariepinus*.

When assessing the bile, there was a variation in the colour of the bile between the two fish species (Figure 4.3 C and D). The bile was dark yellow to straw colour for *C. gariepinus*, a yellow to straw colour bile was observed in *L. rosae*. Slight colour changes observed could be attributed to prolonged time the fish spent in the nets before collection. A complete empty gall bladder indicates that the fish have probably eaten recently. Bile colour was not included in the original HAI (Goede and Barton 1990, Adams et al. 1993).

During this study, mesenteric body fat was observed in both fish species. *Clarias gariepinus* were generally fatter (large amount of fat tissue). Larger percentage of fat deposits was recorded in spring and summer than in autumn and winter (Figure 4.3 A). Hard body fat was observed in *C. gariepinus* which could an early sign of pansteatitis (Figure 4.2 C).

Haematocrit values ranging from18 to 29% were recorded in autumn, and spring in *C. gariepinus*. Lowest haematocrit values were recorded in summer (Appendix B). Haematocrit values above normal in *C. gariepinus* were recorded in summer. Haematocrit values that were below normal for *L. rosae* were recorded in autumn, winter and summer and above normal values were recorded in spring. Changes in haematological indices of fish are influenced by the concentration of heavy metals in water and the duration of exposure, both of which can cause reversible and irreversible changes in the homeostatic system of fish (Harper and Wolf 2009).

4.3.2 Parasites

Parasites recorded in this study were grouped into ectoparasites and endoparasites for both fish species. The number of individual parasites collected from each fish species are tabulated in Table 4.2 and 4.3. A total of 18 parasite species (4 monogeneans, 2 copepods, 6 digeneans, 3 cestodes, and 2 nematodes) were collected during the four sampling seasons at the Phalaborwa Barrage. The seasonal infection statistics for the respective fish species are presented in Tables 4.5 and 4.6. Of the 40 fish sampled for each fish species, a total of ten parasites species were recorded from *C. gariepinus* which included seven adults and three larval forms of monogenea, digenea, cestode, nematoda, and copepoda. A total of five metazoan parasites were recorded in *L. rosae* which included three adults and two larval forms of monogenea, digenea, nematoda and copepoda. *Clarias gariepinus* hosted a higher number of parasite diversity than *L. rosae*. This might be due to their different feeding habits, local availability of other parasite species (availability of intermediate and final hosts) and their possibility of colonization (Bushet al. 2001).

Table 4.2: Summary of the number of ectoparasites collected from *Clarias gariepinus* and *Labeo rosae* at the Phalaborwa Barrage.

Ectoparasites		**Site of infection**	**Autumn**	**Winter**	**Spring**	**Summer**
Clarias gariepinus						
Monogenea	*Macrogyrodactylus clarii*	Gills	0	0	0	14
	Quadriacanthus aegypticus	Gills	0	6	2	7
Copepoda	*Lamproglena clariae*	Gills	8	17	16	10
Total			8	23	18	31
Labeo rosae						
Monogenea	*Dactylogyrus pienaari*	Gills	112	4	87	128
	Dogielus sp.	Gills	88	1	0	0
Copepoda	*Lamproglena clariae*	Gills	0	29	0	0
Total			200	34	87	128

Parasite species from the two fish species at the Phalaborwa Barrage

The following parasites were collected from *C. gariepinus*; the ectoparasites included two monogeneans (*Macrogyrodactylus clarii; Quadriacanthus aegypticus*) and one copepod species (*Lamproglena clariae*) collected from the gills (Table 4.2). The ectoparasites collected from *L. rosae* included; two monogeneans (*Dactylogyrus pienaari*; *Dogielus* sp.) and one type of Copepoda species (*Lamproglena* sp.) collected from the gills (Table 4.2).

Table 4.3: The endoparasites collected from *Clarias gariepinus* and *Labeo rosae* at the Phalaborwa Barrage.

Endoparasites		Site of infection	Autumn	Winter	Spring	Summer
Clarias gariepinus						
Digenea						
	Clinostomum sp. Larvae	Branchial cavity	7	4	6	7
	Digenean cysts	Gills	1	0	2	33
	Diplostomum spp. larvae	Eyes, brain	2	1	6	0
	Echinostomum sp. larvae	Gills	0	4	0	0
	Glossidium pedatum (adult)	Intestine	0	1	1	7
Cestoda						
	Larval cestodes	Kidney	1	0	0	0
	Tetracampos ciliotheca (adult)	Stomach lining	1	0	0	0
Nematoda						
	Contracaecum sp. (larvae)	Body cavity	381	149	117	216
	Paracamallanus cyathopharynx (adult)	Intestine	43	54	20	21
Total			**436**	**213**	**152**	**284**
Labeo rosae						
Digenea						
	Nematobothrium sp.	Behind the eyes	13	3	1	0
Cestoda						
	Unidentified cestode larvae	Intestine	0	4	0	0
Nematoda						
	Paracamallanus cyathopharynx (adult)	Intestine	0	20	0	0
Total			**13**	**27**	**1**	**0**

The endoparasites hosted by *C. gariepinus* included five digeneans (*Clinostomum* sp. larvae from branchial cavity, digenean cysts from gills, *Diplostomum* spp. larvae from eyes and brain, *Echinostomum* sp. larvae from gills and *Glossidium pedatum* from the intestines).The cestodes (larval cestodes from the kidneys, *Tetracampos ciliotheca* from stomach lining) and nematodes (*Contracaecum* sp. from the body cavity, *Paracamallanus cyathopharynx* collected from the intestines (Table 4.3). The endoparasites collected from *L. rosae* included one digenean (*Nematobothrium* sp. from the sockets of the fish eyes); cestodes (unknown species) and nematodes (*Paracamallanus cyathopharynx* from the intestine) (Table 4.3). The digenean *Nematobothrium* sp. collected from the eye sockets of *L. rosae* is a new host and locality record (first observed in Flag Boshielo Dam) for South Africa (Kekanaet al. 2011).

The high abundance of monogeneans in *L. rosae* (*Dactylogyrus pienaari* and *Dogielus* sp.) contributed to the elevated number of ectoparasites (PI) (Table 4.2). However these results were unexpected as the quantity of ectoparasites is suspected to be lower in poorer water quality. According to the water quality results (Chapter 2) some water constituents were elevated or above South African and international recommended values. This inconsistency may perhaps be credited to the differential vulnerability of the parasites to the toxicity of different contaminant, the concentration, exposure time and synergistic effects (Marcogliese 2005). Pollutants have dissimilar influences on parasites and may explain the difference in composition of ecto- and endo-parasites at the Phalaborwa Barrage (Khan and Hooper 2007).

4.3.3 Parasites Index

The parasite assemblage of fish can have a potential role as response indicators to environmental stress in relation to the Parasite Index (PI). The ecto- and endo-parasite index value was determined based on the presence of endo- or ecto-parasites in/on the individual fish. The Inverted Parasite Index (IPI) was done in this study to assess the ectoparasites and the revised PI for the endoparasites (Heath et al. 2004). A greater number of parasites were recorded in *C. gariepinus* than *L. rosae* however; *C. gariepinus* exhibited a higher number of endoparasites than ectoparasites while *L. rosae* exhibited a higher number of ectoparasites than endoparasites. High numbers of endoparasites from *C. gariepinus* was due to high number of nematodes while higher number of ectoparasites in *L. rosae* was due to the monogenean.

Table 4.4: Seasonal Parasite Index and Inverted Parasite Index for *Clarias gariepinus* and *Labeo rosae*

Seasons	Autumn	Winter	Spring	Summer
Clarias gariepinus				
N=	9	7	10	14
Ecto PI	5.5	11.4	8	6.4
Ecto Inverted PI (IPI)	35.5	35.7	40	37.1
Endo PI	9	7	10	10
Labeo rosae				
N=	10	10	10	10
Ecto PI	17	8	14	18
Ecto Inverted PI (IPI)	29	32	26	22
Endo PI	7	3	01	22

The lowest ecto-PI recorded for *C. gariepinus* was in autumn (5.5) and the highest ecto PI was recorded in winter with a PI mean value of 11.4 (Table 4.4). The highest ecto IPI was recorded in summer and the lowest was in autumn with IPI mean values of 37.1 and 35.5 respectively (Table 4.4). The lowest PI for endoparasites in *C. gariepinus* was recorded in winter and highest mean PI was in spring and summer with mean endo PI values of 7 and 10 respectively (Table 4.4). The lowest ecto-PI recorded for L*. rosae* was in winter (8) and the highest ecto PI in summer with a PI mean value of 18 (Table 4.4). The highest ecto IPI was recorded in winter and the lowest was in summer with IPI mean values of 32 and 22 respectively (Table 4.4). The lowest PI for endoparasites in *C. gariepinus* was recorded in winter and highest mean PI in summer with mean endo PI values of 3 and 22 respectively (Table 4.4). The high endo PI recorded for *C. gariepinus* was due to elevated numbers of endoparasites (Table 4.3).

4.3.4 Infestation statistics

Infestation statistics (prevalence, mean intensity and abundance) for the individual parasites recorded are presented in Table 4.5 and 4.6.

Table 4.5: The parasite abundance (MA), prevalence (P) and mean intensity (MI) of *Labeo rosae* at Phalaborwa Barrage.

Parasites	Autumn			Winter			Spring			Summer		
	MA	P (%)	MI	MA	P (%)	MI	MA	P (%)	MI	MA		
Ectoparasites												
Monogenea												
Dactylogyrus pienaari	11.2	80	14	0.4	10	4	8.7	100	8.7	12.8	100	12.8
Dogielus sp.	8.8	40	22	-	-	-	-	-	-	-	-	-
Copepoda *Lamproglena* sp.	-	-	-	4.8	50	9.6	-	-	-	-	-	-
Endoparasites Digenea												
Nematobothrium sp.	1.3	70	1.8	0.3	10	3	-	-	-	0.1	1	10
Cestoda												
Cestode larvae	-	-	-	0.4	10	4	-	-	-	-	-	-
Nematoda												
Paracamallanus cyathopharynx	-	-	-	0.4	20	2	-	-	-	-	-	-

Table 4.6: The parasite abundance (MA), prevalence (P) and mean intensity (MI) of *Clarias gariepinus* at Phalaborwa Barrage.

Parasite	**Autumn**			**Winter**			**Spring**			**Summer**		
	MA	P (%)	MI	MA	P (%)	MI	MA	P (%)	MI	MA	P (%)	MI
Ectoparasites												
Monogenea												
Macrogyrodactylus clarii	-	-	-	-	-	-	-	-	-	1.00	14	7
Quadriacanthus aegypticus	-	-	-	0.9	14	6	0.4	20	2	0.5	7	7
Copepoda												
Lamproglena clariae	0.9	55	1.6	2.3	85	2.67	1.4	60	2.3	0.71	29	2.5
Digenea												
*Diplostomum sp.(*larvae)	0.2	11	1	0.1	14	1	0.6	20	3	-	-	-
Clinostomum sp.(larvae)	0.7	22	3.5	0.6	28	2	0.6	30	2	0.5	29	1.75
Glossidium pedatum(adult)	-	-	-	0.1	14	1	0.1	10	1	0.36	21	1.6
Digenean cysts										2.36	14	16.5
Echinostome sp.(larvae)	-	-	-	0.6	14	4	-	-		-	-	-
Endoparasites												
Cestoda												
Tetracampos ciliotheca(adult)	0.1	11	1	-	-	-	-	-	-	-	-	-
larval cestodes	0.2	22	1	-	-	-	0.2	10	2			
Nematoda												
Paracamallanus cyathopharynx	4.7	88	5.38	7.7	100	7.7	2.4	70	3.9	1.5	14	10.5
Contracaecum sp.(larvae)	51.2	100	51.2	21.3	100	21.3	12.1	100	12.1	15.71	100	15.71

Parasites were grouped into ectoparasites or endoparasites (Table 4.2 to 4.6). However not all parasites were identified to genus and species level because one cannot ID larval forms.

The total numbers of ecto- and endo-parasites were used to determine the percentage of hosts infected as the prevalence, mean intensity (the intensity of the infection) and the abundance of each parasite species.

Ectoparasites

Prevalence: The ectoparasites prevalence of both fish species was higher than the endoparasites prevalence (Table 4.5 and 4.6). The highest prevalence was recorded in *L. rosae* for the ectoparasites. The prevalence of ectoparasites in *L. rosae* ranged from 10% in winter to 100% in spring and summer. The prevalence of ectoparasites in *C. gariepinus* ranged from 7% in summer to 85% in winter.

The highest prevalence (100%) for ectoparasites collected from *L. rosae* was in spring and summer while the lowest prevalence was in winter for *Dactylogrus pienaari* with a prevalence value of 10%. In *C. gariepinus*, the highest prevalence recorded for ectoparasites was in winter for *Lamproglena clariae* (85%) while the lowest prevalence was in summer for *Quadriacanthus aegypticus* with a prevalence value of 7% (Table 4.5 and 4.6).

Mean intensity: The mean intensities of ectoparasites in *C. gariepinus* differed seasonally as compared to prevalence of the ectoparasites. Mean intensity ranged from 1.6 during autumn for *Lamproglena clariae* to 7.0 for *Quadriacanthus aegypticus and Macrogyrodactylus clarii* in summer. Mean intensity of ectoparasites for *L. rosae* ranged from 4 for *Dactylogrus pienaari* during winter to 22 during autumn for *Dogielus* sp. (Table 4.5 and 4.6).

Mean abundance: Ectoparasites abundance of 2.3 for *C. gariepinus* was recorded in winter for *Lamproglena clariae* with a low mean value of 0.4 in spring for *Quadriacanthus aegypticus.* For *L. rosae* a high number of abundance for ectoparasites was recorded in summer and a low value of 0.4 in winter for *Dactylogrus pienaari.*

The highest values for ectoparasites in *C. gariepinus* and *L. rosae* were recorded in winter and summer, while lowest value was in spring and winter respectively (Appendix B).

Endoparasites

Prevalence: For endoparasites, both fish species exhibited a slight different seasonal trends compared to those observed for ectoparasites and as well as compared between species. The prevalence of endoparasites for *C. gariepinus* ranged from 10% in spring for *Glossidium pedatum* and larval cestodes to 100% in all seasons for *Contracaecum* sp. and 100% during winter for *Paracamallanus cyathopharynx*. The highest prevalence for *L. rosae* for endoparasites was recorded during autumn for *Nematobothrium* sp. with 70% and lowest during winter and summer for *Nematobothrium* sp. (Table 4.5-4.6).

The highest mean abundance of endoparasites (51.2) in *C. gariepinus* was recorded during autumn and lowest value of 1 was in various seasons for several species. For *L. rosae*, the endoparasites mean abundance ranged from 0.1 to 1.3 for *Nematobothrium* sp. in summer and autumn respectively. Madanire-Moyo and Barson (2010) suggested that parasite species composition and richness in catfish examined in Upper Manyame River in Zimbabwe are influenced by environmental factors such as conductivity, nutrients and dissolved oxygen (Table 4.5-4.6).

The highest mean intensity value of 51.2 for endoparasites in *C. gariepinus* was recorded during autumn and a low value of 1 was recorded in various seasons for several species. For *L. rosae*, mean intensities for endoparasites ranged from 1.8 to 10 for *Nematobothrium* sp. In autumn and summer respectively (Table 4.5-4.6).

Mean abundance: The highest mean value of 51.2 was recorded in autumn for *Contracaecum* sp. and lowest value of 0.1 was recorded in autumn for *Tetracampus*in *C. gariepinus*.For *L. rosae* a higher number of abundance for endoparasites was also recorded in autumn for *Nematobothrium* and a lower value of 0.1 was recorded in summer for the same species.

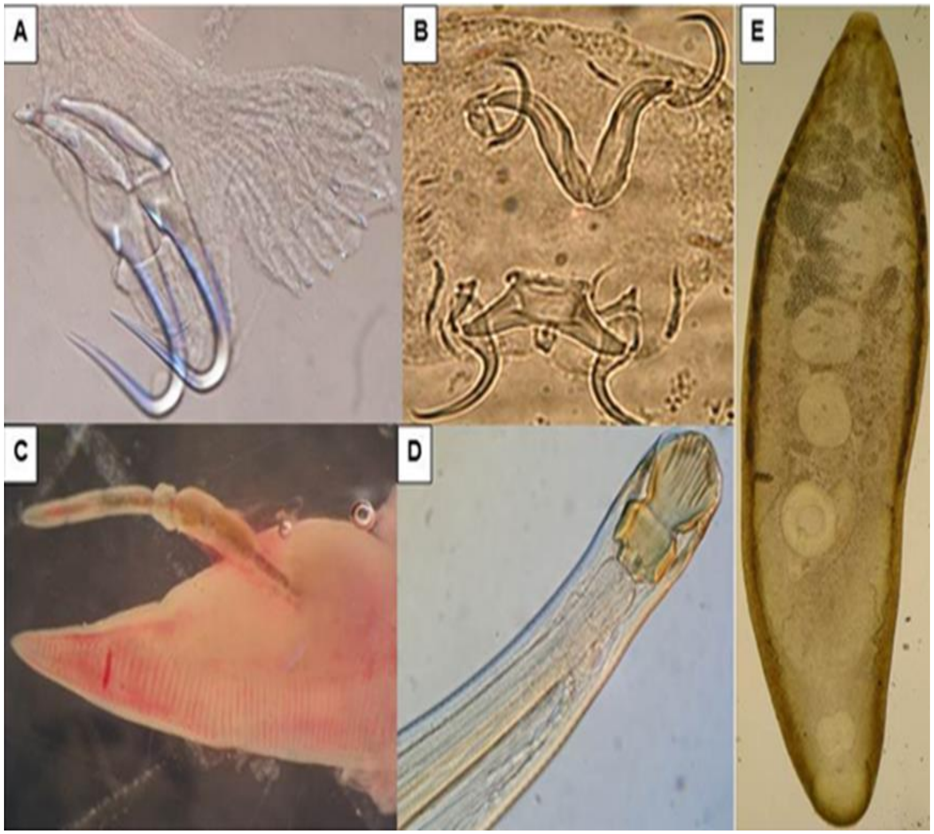

Figure 4.4: A-E ecto and endo-parasites recorded from the gills of C*. gariepinus* A: *Macrogyrodactylus clarii*; and B: *Quadriacanthus aegypticus*; C: *Lamproglena clariae* attached on the gill filament; endo-parasites from intestines D: *Paracamallanus cyathopharynx*; endo-parasite from the stomach lining E: *Glossidium pedatum* (adult)

Figure 4.5: A-D ecto and endo-parasites recorded from gills of *Labeo rosae* A: *Dactylogyrus pienaari* B: *D. pienaari* copulatory organ; C: *Lamproglena*sp. attached on the skin; D: *Lamproglena sp*.; endo-parasite collected from eye socket E: *Nematobothrium* sp.

Ectoparasites (monogenea and copepoda)

Monogenea: The monogeneans are ectoparasites found in both freshwater and marine. They can act as both ectoparasites and endoparasites (Luus-Powell 2004).They have a direct life cycle that predominantly occurs on the gills and skin of the fish receiving nourishment from the blood, mucus and skin cells while as endoparasites they are found in the stomach (Poulin 2002). Monogeneans can result in fish mortalities in both aquaculture and natural environment (Luus-Powell 2004). They can also decrease respiratory and growth rate which can affect the productivity (economy)(Paperna 1996).

Monogenea during this study were mostly from the gills of both fish species. Two monogenean species were recorded in this study from the gills of *C. gariepinus* namely; *Macrogyrodactylus clarii* described byGussev (1961) quoted by (El-Naggar and Serag 1987) and *Quadriacanthus aegypticus* (Figure 4.4 A-B). *Macrogyrodactylus clarii* was detected only in summer with prevalence of 14%. *Quadriacanthus aegypticus* was detected in winter, spring and summer. In *C. gariepinus*, the highest mean abundance was 1.0 for *Macrogyrodactylus clarii* in summer and one for *Quadriacanthus aegypticus* in winter while the lowest mean abundance was recorded in spring for *Quadriacanthus aegypticus* with a mean value of 0.4 (Table 4.4).

Two monogenean species were collected from gill filaments of *L. rosae*: *Dactylogyrus pienaari* and *Dogielus*sp. *Dactylogyrus pienaari* occurred in all seasons while *Dogielus* sp only occurred in autumn. The highest monogenea mean abundance for *L. rosae* was recorded for *Dactylogyrus pienaari* in summer with a mean value of 12.8 and 100% prevalence while the lowest mean abundance of 0.4 was recorded in winter for *Dactylogyrus pienaari* (Table 4.4). *Dactylogyrus* in a highly diversified group within the monogenean gill parasites with more than 900 nominal species that are highly host specific to freshwater fish of the family Cyprinidae (Gibson et al. 1996). None of the monogeneans from both fish species had any negative impact to the host. Monogenean has been found to be very sensitive to pollution. Seasons have a significant influence on the abundance of monogeneans. Sex and size of a fish do not have any significant influence on the abundance and intensity of monogeneans however size was a significant factor for prevalence, this concept was evident in this study (Avenant-Oldewage 2001).

Copepoda: Most copepods have several free living copepodid stages. The adult females are mostly parasitic whereas the males are usually smaller and free living, although dwarf parasitic male forms attached to the female exist. Copepods parasitizing the gills and skin of fish are serious pests and thus are of commercial importance in both freshwater and marine fish farms. *Lamproglena* (described by Fryer 1956) is a copepod found on the gills of *C. gariepinus*. They cause extensive gill tissue proliferation, which may interfere with respiration (Marx and Avenant-Oldewage 1996).

The copepods recorded in this study include, a new species, i.e. *Lamproglena sp*. (Figure 4.5 C and E) collected from the gills and skin of *L. rosae*. *Lamproglena clariae* (Figure 4.4 C) was recorded from the gills of *C. gariepinus* during all seasons with the highest mean abundance and prevalence of 85% during winter survey (Table 4.5). Higher numbers of *Lamproglena sp*. was recorded in winter with a prevalence of 50% for *L. rosae* (Table 4.5).

Endoparasites

Digenea: the digeneans are a diverse group of parasites with respect to their hosts and their sites/habitats. They cause serious and fatal diseases in several animals including humans. Digeneans are important fish parasites with fishes serving both as intermediate host and final hosts (flukes).

Five different digeneans were collected from *C. gariepinus*. *Clinostomum* larvae collected from branchial cavity were recorded in high numbers during autumn and summer with a mean intensity of 3.5 and in lower numbers in winter. *Diplostomum.* larvae were collected from eyes and were recorded in high numbers in spring and lower in winter (Table 4.3).

Echinostomum larvae collected from the gills during winter with a mean intensity of four. Adult digeneans (*Glossidium pedatum)* collected from the intestine with a mean intensity of 1.6 during the summer survey and it was recorded in lower numbers during winter and spring (Figure 4.4 E, Table 4.3).

Only one digenean larva was collected from *L. rosae*. The *Nematobothrium* sp was collected from the eye sockets (Figure 4.5 E). *Nematobothrium* sp was recorded in high numbers and prevalence in autumn and in low numbers in spring.

Cestoda: the tapeworms (cestodes) are all endoparasites with adults infecting the intestine of freshwater fishes and larvae found in/on different internal organs. The life cycle of cestodes involves more than one intermediate host, including planktonic copepods, molluscs and fish. Some cestodes develop into adult stages in piscivorours birds, are important for parasites in that they can disseminate the eggs over long distances, making it difficult to control the spread of infections between different catchments (Madanire-Moyo et al. 2012b). An adult cestode (T*etracampos ciliotheca*) and larval cestode were collected from the stomach lining and kidneys respectively (Table 4.2).

Cestode larvae were collected from the kidneys of *C. gariepinus* in high numbers during autumn and low numbers during spring with the highest prevalence of 22% in autumn. Unidentified larval cestodes were collected from *L. rosae* in high numbers in autumn and low numbers in spring.

The Nematodes are also known as the round-worms are the most significant metazoan parasites associated with human and domestic animal infections; however, most nematodes are free living and found in a wide variety of aquatic and terrestrial habitats. Most species' life cycle involves several larval stages, some of which can be free living. Some parasitic nematodes are however viviparous, e.g. *Procamallanus* spp. Only a few researchers have published their work on nematode parasites in the country, and these include Prudhoe and Hussey (1977), Mashego (1982), Mashego and Saayman (1981), Boomker (1982, 1994), Mokgalong (1996), Barson and Avenant-Oldewage 2006, Luus-Powell 1997, 2005, Madanire-Moyo et al. 2011, 2012).

The nematodes recorded during this study included *Contracaecum* sp. larva collected from the body cavity of *C. gariepinus* with a high mean abundance and intensity of 51.2 in autumn. *Contracaecum* sp. (larvae) were collected from the body cavity (Table 4.2 and 4.5; Figure 4.4). The high infection rate from *Contracaecum* sp. however has no apparent effect on the health of the fish or appears to affect the condition of the host negatively, it only farms part of its life cycle before it emerges into birds into adult form. Infection of this metazoan parasite in the catfish may originate from the consumption of intermediate host, a crustacean or may be related to a lateral transfer of these larvae.

Paracamallanus cyathopharynx was collected from the intestine of both fish species and detected in higher numbers in *C. gariepinus* in winter and in lower numbers in summer. *Paracamallanus cyathopharynx* were collected from the intestines of both fish species. *Contracaecum* larvae have been recorded from *C. gariepinus* and other fish species from several water bodies in South Africa (Whitfield and Heeg 1977; Mashego and Saayman 1981; Boomker 1982, 1994; Saayman et al. 1991; Madanire-Moyo et al. 2010), Zimbabwe (Chishawa 1991; Barson 2004; Barson and Marshall 2004), and East Africa (Aloo 2001). It is a cosmopolitan parasite of fish-eating birds and mammals (Anderson 1992) and can reach alarming intensities without affecting the condition of the host (Mashego and Saayman 1981; Boomker 1982; Paperna 1996), an adaptation that probably ensures that the larvae survive to reach the final host without killing the intermediate host.

4.3.5 The Condition Factor

The condition factor (CF) is a general indicator of fish health, by correlating the fish body mass in grams to its length in centimetres. The index is often used in aquaculture; to monitor feeding intensity, age and growth rates in fish (Anene 2005). In the current study a different approach was used where the weight (in grams) and length (total length in millimetres) of the fish was used to calculate the CF instead of using centimeters (Williams 2000).

The condition factor of a fish is classified as ideal when its value is 1.00 9Bervoets and Blust 2003). However this depends on the fish species sampled as weight and length relationship differ between fishes and may also vary due to geographic location (Bervoets and Blust 2003). From the sample size of 80 specimens from both fish species, in spring and summer the mean condition factor for *C gariepinus* was higher than the calculated mean CF value during autumn and winter (Figure 4.6).

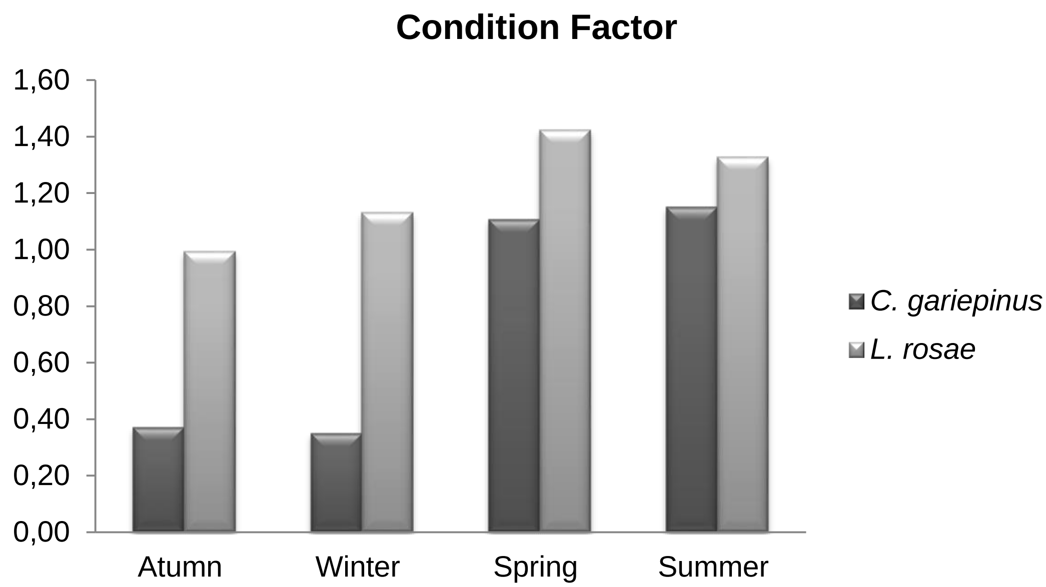

Figure 4.6: Seasonal condition factor in both fish species at the Phalaborwa Barrage

The mean CF value for *L. rosae* in summer, spring and winter was higher than the mean CF value calculated in autumn (Figure 4.6). The highest mean value calculated for *L. rosae* was in spring with a mean CF value of 1.42 and lowest in autumn with a mean CF value of 0.99. The highest CF mean value (1.15) calculated for *C. gariepinus* was in summer and lowest in winter with a mean CF value of 0.35. Since *L. rosae* spawn in summer, their adult stage is expected in spring hence there is a higher condition factor in spring compared to other seasons and lowered metabolism in winter. Lower Condition Factor values for both fish species were calculated for autumn and winter when more juveniles were prominent. The condition factor is not always constant for a species or population over time and it is usually influenced by both biotic and abiotic factors such as feeding regime, breeding activity, food availability and metabolic rate (Froese 2006). In addition, Anene (2005) states that the fish with lower condition factors are recorded for relatively large sizes, whereas relatively higher condition factors are recorded for smaller fish, the latter statement was observed in this study. The CF values calculated in this study were found to be higher than the CF values calculated in the Vaal Dam and Vaal River Barrage (Crafford and Avenant-Oldewage 2009).

4.4 CONCLUSIONS

Health Assessment Index and Parasite Index

In terms of HAI, both fish species recorded a mean population values ranging from 17 to 51 which indicated to be an intermediate impacted site. The Health Assessment Index (HAI) values of the two fish species differed significantly with higher values recorded for *C. gariepinus* than *L. rosae*. Higher HAI values were recorded for *L. rosae* than *C. gariepinus* during winter, spring and summer which are an indication that *L. rosae* was more affected by parasites. The highest population mean HAI value was recorded in summer for both fish species and the lowest HAI value was recorded in autumn for both fish species. The Endo-PI was higher than Ecto-PI for *C. gariepinus*. The opposite was observed for *L. rosae* in which the Ecto-PI was higher than the Endo-PI in all seasons except summer. No abnormalities in fins, kidneys, opercules, hindgut, mesentery fat, bile and spleens were observed during the study.

All the fish at the barrage appear to be healthy, with no visible abnormalities except a few fishes (n=25) which showed mild skin aberration, discolorations on the liver, below and above normal haematocrit values of some fish (mostly in *C. gariepinus*). Eyes, skin, fins and opercula were found to be normal for both fish species at the Phalaborwa Barrage, the liver condition and haematocrit values contributed mostly to the HAI values. Liver discoloration, HCT values, and parasites contributed to higher HAI values in *C. gariepinus*. The haematocrit values were one of the most affected variables, showing differences among species.

A greater number of parasites were recorded in *C. gariepinus* than *L. rosae* however; *C. gariepinus* exhibited a higher number of endoparasites than ectoparasites while *L. rosae* exhibited a higher number of ectoparasites than endoparasites. High numbers of endoparasites from *C. gariepinus* was due to high number of nematodes while higher number of ectoparasites in *L. rosae* was mainly due to the monogenean group of parasites. It can therefore be concluded that ectoparasites in *L. rosae* contributed to the elevated HAI value.

High numbers of ectoparasites were recorded in *L. rosae* than in *C. gariepinus* while higher numbers of endoparasites were recorded in *C. gariepinus* than *L. rosae*. The dominating ectoparasites for both species were from the class monogenea. Although the prevalence for *Contracaecum sp.* in *C. gariepinus* was 100% in all seasons, it did not influence the condition of the host. The parasites identified from both fish species neither reached alarmingly infection levels nor caused any visible damage to the host. One digenean parasite (*Nematobothrium sp*.) found in the orbit of *L. rosae* is a new record for South Africa (and possibly a new species) which was first identified at the Flag Boshielo Dam. Fish health (including parasite burden) is important as freshwater fish is consumed (especially in rural areas) by humans on a regular basis. The digenean larvae, including *Clinostomum* and the digenean cysts were observed from the gills and the muscle.

The highest prevalence was recorded in *L. rosae* for the ectoparasites group. The highest prevalence for ectoparasites collected from *L. rosae* was in spring and summer while the lowest prevalence was recorded in winter for *Dactylogrus pienaari.* In *C. gariepinus*, the highest prevalence recorded for ectoparasites was in winter for *Lamproglena clariae* while the lowest prevalence was recorded in summer for *Quadriacanthus aegypticus*. The highest mean intensities for ectoparasites in *C. gariepinus* were recorded in summer for *Quadriacanthus aegypticus and Macrogyrodactylus clarii* and lowest *in* autumn for *Lamproglena clariae*. The highest mean intensity for ectoparasites for *L. rosae* was recorded in winter for *Dactylogrus pienaari* and lowest in autumn for *Dogielus* sp.

Higher numbers for C. gariepinus were recorded in winter for *Lamproglena clariae* and low numbers of ecto-parasites were recorded in spring for *Quadriacanthus aegypticus.* For *L. rosae* highest numbers of ectoparasites were recorded in summer and low numbers of ectoparasites were recorded in winter for *Dactylogrus pienaari.* Highest number of ectoparasites in *C. gariepinus* and *L. rosae* were recorded in winter and summer, while the lowest number of parasites was recorded in spring and winter respectively.

For endoparasites, both fish species exhibited a slight different seasonal trends compared to those observed for ectoparasites and as well as compared between species. The lowest prevalence of endoparasites for *C. gariepinus* was recorded in spring for

Glossidium pedatum and larval cestodes in all seasons and the highest prevalence was recorded in all seasons for *Contracaecum* sp. The highest prevalence for *L. rosae* for endoparasites was recorded during autumn for *Nematobothrium* sp and lowest during winter and summer for *Nematobothrium* sp*.*

The same trend for mean intensity of endoparasites was observed for both fish species. The highest mean abundance of endoparasites in *C. gariepinus* was recorded during autumn. For *L. rosae*, the lowest endoparasites mean abundance was recorded in summer and autumn for *Nematobothrium* sp. The highest mean intensity value for endoparasites in *C. gariepinus* was recorded during autumn. For *L. rosae*, mean intensities for endoparasites ranged from 1.8 to 10 for *Nematobothrium* sp in autumn and summer respectively.

Condition Factor

When comparing the two fish species, *L. rosae* had slightly higher condition factor values in all seasons than *C. gariepinus* which also correlated with the HAI values calculated for each fish species. The condition or wellness of *L. rosae* was also better than that of *C. gariepinus* during all seasons. Furthermore, the condition factor findings correlate with the HAI values of both fish species whereby *C. gariepinus* had highest parasite numbers and highest HAI values, of which both might have impacted on the overall condition of its health as compared to *L. rosae*. *Labeo rosae* showed opposite results to *C. gariepinus*; less ectoparasites, lower HAI values and condition factor.

CHAPTER 5

GENERAL CONCLUSIONS AND FUTURE RECOMMENDATIONS

The aim of the study was to assess/identify, if any, the impact of the water and sediment quality (chapter 2) on the health of *C. gariepinus* and *L. rosae* and parasites collected from both species by applying the fish Health Assessment Index, to evaluate the diversity of parasites of the two fish species (chapter 4), to determine the levels of metal bio-accumulation of selected toxic metal constituents on the muscle tissue and the associated risk to human health when consuming contaminated fish (chapter 3), at the Phalaborwa Barrage in the Olifants River in the Limpopo province. To achieve the aim of the study, the following research questions of the research project (as mentioned in chapter 1) in terms of conclusions are discussed below.

Water quality

What is the quality of the water at the Phalaborwa Barrage?

Water quality at the barrage during the four sampling seasons was slightly polluted with high turbidity, TDS and EC levels. Water temperature was recorded in normal concentrations, with highest records in spring and lowest in winter. Water temperature values ranged from 18 ºC in winter to 28ºC in spring. Dissolved oxygen concentrations recorded were within the TWQR during all seasons. The pH of the water varied moderately hard to hard and alkaline with pH ranging from 7.4 to 8.7. The highest pH units were recorded in summer and lowest in autumn. The highest alkalinity value was recorded during spring and lowest in autumn and the elevated concentration was found to be higher than the TWQR for aquaculture water use. Electric conductivity, turbidity, TDS and salinity values were acceptable for aquatic ecosystems.

Water hardness (calcium carbonate), at the Phalaborwa Barrage was moderately hard in summer and hard in autumn and winter. An increases in water hardness have been found to decrease the concentrations of metals through the precipitation of insoluble carbonates or calcium carbonate in that it act as a surface for the adsorption of metal ions (Dallas and Day 2004).

The anions were recorded at normal levels and fell within the TWQR. Most cations were recorded at levels within TWQR for aquatic ecosystem. Calcium concentration levels exceeded the typical concentration limit in spring but the levels were still within TWQR for domestic use. The chloride levels were recorded at levels below detection limit for domestic use but at higher level above CEV and TWQR for aquatic ecosystem. Calcium, magnesium, sodium and potassium do not have the SAWQG for aquatic ecosystems.

The total nitrogen concentrations were very low, indicative of oligotrophic during autumn, winter, and summer. However, nitrate concentrations during spring were above the TWQR (2.5-10 mg/ℓ) an indicative of eutrophication. The phosphorous concentrations were above the TWQR (0.025 – 0.25 mg/ℓ), an indicative of eutrophic in winter, spring and summer. Noticeable elevated levels were recorded at the inflow in summer which is an indicative of hypertrophic conditions. Elevated concentrations of nutrients, in this case the total nitrogen and phosphorous may increase the abundance of algae and aquatic plants (DWAF 1996c).Consequently if the phosphorous becomes bioavailable, it will pose potential risk for eutrophication in the barrage.

The metalloids and metals that were detectable in water samples included; aluminium, antimony, arsenic, barium, boron, iron, manganese, selenium, strontium and tin. The metalloids and metals recorded above the TWQRs for aquatic ecosystems are aluminium, antimony, arsenic and selenium, an indication that these metals may pose adverse effects to the aquatic life in the barrage if organisms are exposed to these concentrations over a period of time. Aluminium was recorded above AEV, indicating that it may have acute effects to the aquatic ecosystem in the barrage. The elements that were within TWQR for aquatic ecosystems were barium, boron, iron and manganese. Metals that were detected in the water column are as follows in decreasing order: Al> Fe> Sr> Sn> B> Ba> Se> Mn> Sb> As. Several metals, such as cadmium, cobalt, copper, lead, nickel, silver, titanium, vanadium and zinc, the concentrations measured were less than the detectable concentration limit for the metal analysis.

Metals detected in the sediment samples are as follows in decreasing order: Fe> Al> Ti> Mn> B> Ba> Cr> Zn> V> Ag> Ni> Sr> Cu> Co> Pb> As> Cd> Sn> Sb> Se. The sediment metal mean concentrations collected at all sampling sites during the two surveys had high levels of aluminium, cadmium, chromium and zinc. However, metal concentrations that were recorded during summer (Al, Sb, Cd, Co, Cr, Fe, Si, Sn, Sr, Ti and V) were higher than the ones recorded in winter (Ag, As, B, Be, Cu, Li, Mn, Pb and Zn). Arsenic, cadmium and chromium were recorded at concentrations above the detection limit (CCME). Zinc concentrations were recorded at levels below suggested detection limit. Aluminium, barium, boron, cadmium, chromium, manganese, selenium and strontium were detected at elevated levels in water and sediment.

Generally the water quality at the Phalaborwa Barrage was found to be hard and unsuitable for drinking in its form without any adequate treatments due to the level of hardness recorded in summer. Eutrophication is a problem in most South African rivers, as a result of high levels of phosphates in urban effluent. The source of metals and some of the elevated physical-chemical variables may be the water coming from upstream given the human activities happening in the catchments area.

What is the level of accumulation of metals in the fish muscle tissue and human health risks upon consumption of the fish at the Phalaborwa Barrage?

Both fish species sampled at the barrage accumulated metals at elevated concentrations. All the metals detected in the sediment also accumulated in the fish muscle tissue except cadmium in both fish species and cobalt in *C. gariepinus*. The highest concentration levels of aluminium, boron, barium, iron and zinc were recorded in the two fish species and sediment samples however barium, boron and zinc concentrations in the sediment are much lower than those of the two fish species. The metals and other elements that have accumulated at elevated levels in *C. gariepinus* were barium, boron, zinc and selenium. In the muscle tissue of *L. rosae*, iron, aluminium, strontium, titanium, vanadium and arsenic were present at unacceptable levels. More metals were accumulated in *C. gariepinus* than *L. rosae*; however *L. rosae* accumulated more metals at elevated concentrations than *C. gariepinus*. Both fish species and sediment samples showed elevated levels of B, Ba, Fe and Zn. Although As, Cd, Cr, Ni and V were detected in fairly low concentrations they can be toxic even in small concentrations (Dallas and Day 2004).

Levels of antimony, arsenic, chromium and lead in both fish species were present in concentrations that would be expected to cause both health and toxic effects to humans if contaminated muscle tissue is consumed due to the calculated hazard quotients ranging from close to double to twenty times the safe dose (Jooste et al. 2013). Thus, to a certain extent the metals that accumulated in both fish species were in un-acceptable levels. The health of both fish species seem to be negatively impacted by the pollutants and if the fish is consumed it will result in health risk such as cancer in humans.

The high metal concentrations detected in the muscle might indicate long-term (chronic) exposure of the fish to these metals thus unacceptable to human consumption. Water quality in the Phalaborwa Barrage is compromised by pollution events from upstream of the barrage, mainly attributed to mining and industrial activities. In addition water quality monitoring in the Olifants River indicates that the quality of the water has deteriorated since the 1970s as a result of industrial, mining and agricultural activities (van Vuren et al. 1994; de Villiers and Mkwelo, 2009). Therefore, it is likely that the fish in the Phalaborwa Barrage are exposed to a wide range of contaminants and likely a more concentrated mix of pollutants. If fish were to be caught and eaten on a weekly basis from Phalaborwa Barrage one would expect adverse health effects based on an excess exposure to a number of different elements. An overall comparison between the two fish species indicated that, *C. gariepinus* accumulated more metals in terms of numbers however *L. rosae* accumulated metals at higher concentrations than *C. gariepinus*. This may be due to differences in their feeding habits and difference in metal uptake (Oberholster et al. 2012). Tertiary trophic level consumers, like *C. gariepinus*, are more exposed to metals through biomagnification. On the other hand, *L. rosae* normally feed on detritus, algae and small invertebrates (Skelton 2001) and are therefore primary and secondary trophic level consumers. Primary and secondary trophic level consumers like the rednose labeo are less exposed to biomagnification than tertiary consumers.

Both fish species and sediment samples showed elevated levels of B, Ba, Fe and Zn. Although As, Cd, Cr, Ni and V were detected in fairly lower concentrations they can be toxic even in small concentrations (Dallas and Day 2004). Levels of antimony, arsenic, chromium and nickel were present in concentrations that would be expected to cause both toxic effects due to elevated hazard quotients ranging from close to double to twenty times the safe dose. In every 1000 people one to four people could be at risk of developing cancer (US-EPA 2003). The result implies that the metal content of the sediment has a stronger influence on the metal content of the muscle tissue than that of the water column at the Phalaborwa Barrage. But they could have biomagnified through the food chain. In conclusion, the findings of this study indicated that the two fish species accumulated some metals at unacceptable levels in their muscle tissue which may pose a health risk to humans if consumed on a weekly basis. It is therefore seems that the Phalaborwa Barrage can be regarded as impacted by possibly effluents containing metals and agricultural run-off.

What is the impact of water quality on fish health and their parasites?

Fish health

Groenewald (2000) suggested that the HAI should be used when comparing different seasons at the same locality using the same sampling species, this was applied in this study. With regard to the impact of water and sediment quality on the health of the two fish species sampled, both fish species seemed to be fairly healthy in terms of their health assessment index and the condition factor. Both fish species recorded a mean population values ranging from 17 in autumn to 51 in summer for *L. rosae* while for *C. gariepinus* the mean HAI ranged from 21 in autumn to 32 in summer. The two fish species appear to be in overall good health despite the fact that *Clarias gariepinus* had a high HAI due to the nematode larvae abundance. The highest HAI was recorded in summer for both fish species. The lowest HAI value was recorded in autumn for both fish species. Although a high HAI population value was recorded for both fish species, in general both fish species from the barrage were in a normal condition even in summer. Elevated HAI values in both fish species resulted from the presence of parasites, followed by the liver, haematocrit (Internal variables) and the skin (external variable). The Endo-PI was higher than Ecto-PI for *C. gariepinus* due to high number of nematodes recorded. The opposite was observed for *L. rosae* in which the Ecto-PI was higher than the Endo-PI in all seasons except summer. No abnormalities in fins, kidneys, opercules, hindgut, mesentery fat, bile and spleen were observed during the study. However, several abnomalities were observed in the skin, liver and haematocrit readings. The liver exhibited a greater frequency of abnormality in almost all the *C. gariepinus* sampled in this study. The highest

Condition Factor was recorded in spring and summer for *C gariepinus* and lowest in winter. The highest CF value for *L. rosae* was recorded in spring and lowest in autumn.

Parasites

A greater number of parasites were recorded in *C. gariepinus* than *L. rosae* however; *C. gariepinus* hosted a higher number of endoparasites than ectoparasites while *L. rosae* exhibited a higher number of ectoparasites than endoparasites. The high endoparasite PI of C*. gariepinus* was due to high numbers of nematode larvae in the body cavity. The high number of ectoparasites in *L. rosae* was due to the monogenean parasites. It's concluded that ectoparasite PI in *L. rosae* contributed to the elevated HAI value because ecto PI is based on very low numbers. From the study, the hypothesis that the number of ectoparasites will be higher in less polluted water and the number of endoparasites will be lower was better supported for *L. rosae* than in *C. gariepinus* which recorded more endoparasites than ectoparasites mainly due to elevated number of nematode larvae. The PI for endoparasites was higher for *C. gariepinus* during autumn, spring and summer, and for *L. rosae* more endoparasites were recorded in summer.

The dominating ectoparasites for both species were from the Platyhelminthes class monogenea. Of the 40 fish sampled for each fish species, a total of ten parasites species were recorded from *C. gariepinus* which included seven adults and three larval forms of monogenea, digenea, cestode, nematoda, and copepoda. A total of five metazoan parasites were recorded in *L. rosae* which included three adults and two larval forms of monogenea, digenea, nematoda and copepoda. *Clarias gariepinus* hosted a higher number of parasite diversity than *L. rosae*. Although the prevalence for *Contracaecum sp.* in *C. gariepinus* was 100% in all seasons, it did not influence the condition of the host. None of the parasites identified from both fish species neither reached alarmingly infection levels nor caused any visible damage to the host. One digenean parasite (*Nematobothrium sp*.) found in the orbit of *L. rosae* is a new record for South Africa (and possibly a new species) which was first identified at the Flag Boshielo Dam (Jooste et al. 2013). Fish health (including parasite burden) is important as freshwater fish is consumed (especially in rural areas) by humans on a regular basis. The digenean larvae, including

Clinostomum and the digenean cysts from the gills and muscle, are of zoonotic importance and these larvae have the potential to develop in humans (Jooste et al. 2013).

In conclusion, the fish are in a fair to good health due to their ability to adapt well in their environment.

RECOMMENDATIONS FOR FUTURE STUDIES

Water and sediment quality determined and the bio-assessment of the fish health should be extended to all the tributaries of the Olifants River in the lowveld. Continued monitoring will also serve as an early warning system and thus ensure that preventative measures be taken in good time to maintain the integrity of the Lower Olifants sub-catchment. .

Two of the objectives of this study were to determine the (a) bio-accumulation levels, (b) the risk factor for human health upon consuming contaminated fish were all accomplished. This should be extended to all other fish species in the Lower Olifants sub-catchment.

Bioaccumulation of metals should include the liver, gills and skin because it's where most accumulation of metals occur. The results of the levels of bioaccumulation of metals in the muscle tissue of the two fish species should be incorporated in the database on the Olifants River in the Limpopo Province.

In addition most metals as in this study have not been tested before at the barrage. It is therefore important that the barrage be continuously monitored by sampling the water and sediment for quality analysis as well as the fish tissues on a regular basis.

It is suggested that there should be continuous monitoring programme in collaboration with the Department of Water Affairs in order to determine the source of pollution to the barrage and the impact it has upon the biological component and the aquatic ecosystem as a whole. Future studies should also look at the primary and secondary trophic levels and the top predators e.g., metal bioaccumulation in crocodiles and fish eating birds for biomagnification in the food chain.

Continuous assessment and monitoring of persistent organic pollutants (POPS) together with metals in the Olifants River should be included in future studies. The findings of the bioaccumulation and human health risk assessment clearly indicate that the situation at the impoundment is sufficiently dire that there is a need to begin addressing the potential long-term health risks associated with human consumption of fish from the Olifants River, and possibly extending it to the other impoundments/tributaries and to other fish species in the Olifants River System, including those in Mozambique.

CHAPTER 6

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APPENDICES

APPENDIX A: WATER AND SEDIMENT QUALITY

Appendix A 1.1: The seasonal water quality constituents at the three sampling sites of the Phalaborwa Barrage

Water constituent	Autumn (May 2010)			Winter (July 2010)			Spring (October 2010)			Summer (February 2011)		
	Inflow	Wall	Below	Inflow	Wall	Below	Inflow	Wall	Below	Inflow	Wall	Below
Water temperature °C	21.63	21.9	21.11	18.73	17.42	17.84	29.2	29.10	27.11	27.10	26.45	26.77
Dissolved oxygen (mg/ℓ)	8.35	8.46	8.51	9.74	8.75	8.9	7.15	7.67	7.13	7.21	8.51	8.59
Dissolved oxygen (O_2%)	95.36	96.23	97.37	102.56	97.36	97.2	96.11	98.94	88.43	94.42	95.93	94.12
pH	7.33	7.46	7.38	7.46	7.45	7.31	8.57	8.76	8.59	8.91	8.79	8.29
Conductivity (EC) mS/m	37.1	37.0	37.1	41.3	41.1	47.7	60.1	60.6	59.4	32.4	32.4	32.4
Salinity ‰	0.18	0.18	0.18	0.2	0.2	0.23	0.29	0.29	0.29	0.15	0.15	0.15
TDS (mg/ℓ)	241.15	240.5	241.15	268.45	267.15	310.05	390.65	393.9	386.1	210.6	210.6	210.6
Alkalinity as $CaCO_3$	60	68	64	92	100	120	140	160	128	72	76	76
Turbidity NTU	14	5.9	5.6	11	3.3	8.0	21	34	21	8	7	7
Nitrate (mg/ℓ NO_3^-)	0.2	0.2	0.2	0.3	0.3	0.2	<0.2	2.7	2.8	0.4	0.7	0.2
Nitrite (mg/ℓ NO_2^-)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.2	<0.2	<0.2
Ammonia(mg/ℓ NH_3^+)	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Ortho-Phosphate (mg/ℓ PO_4^{3-})	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Phosphorous (mg/ℓ P)	<0.025	<0.025	<0.025	<0.025	0.048	<0.025	0.03	0.049	0.031	0.45	0.20	0.06
Sulphate (mg/ℓ SO_4^{2-})	48	77	49	46	98	46	<5	11	11	46	39	40
Chloride (mg/ℓ Cl^-)	21	20	20	28	28	29	61	58	64	19	18	17
Fluoride (mg/ℓ F^-)	0.7	0.7	0.7	0.7	0.8	0.7	0.2	0.3	0.2	0.2	0.2	0.2
Calcium (mg/ℓCa)	23	23	23	20	26	31	31	34	29	26	20	19
Magnesium (mg/ℓ Mg)	16	16	17	19	21	22	24	24	24	15	15	15
Potassium (mg/ℓ K)	4.0	3.9	3.9	3.1	3.3	3.3	4.0	4.3	4.5	2.8	2.8	2.9
Sodium (mg/ℓ Na)	21	21	21	26	28	28	50	54	50	18	17	17

Appendix A 1.2: Seasonal metal concentrations in the water at the three sampling sites at the Phalaborwa Barrage

Metals in mg/ℓ	Autumn (May 2010)			Winter (July 2010)			Spring (October 2010)			Summer (February 2011)		
	Inflow	Wall	Below	Inflow	Inflow	Wall	Below	Inflow	Inflow	Wall	Below	Inflow
Aluminium (Al)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.669	0.617	<0.1	<0.100	<0.100	<0.100
Arsenic (As)	<0.01	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	<0.01	<0.01	<0.130	<0.010	<0.010
Antimony (Sb)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.066	0.032	0.036	<0.010	<0.010	<0.010
Boron (B)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	0.257	0.173	0.152	<0.025	<0.025	<0.025
Barium (Ba)	0.032	0.033	0.033	<0.025	0.037	0.041	0.056	0.062	0.046	<0.025	<0.025	<0.025
Beryllium (Be)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Bismuth (Bi)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	0.025	0.029	<0.025	<0.090	<0.140	<0.025
Cadmium (Cd)	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cobalt (Co)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Chromium (Cr)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Copper (Cu)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Iron (Fe)	0.095	0.065	0.131	<0.025	0.034	0.073	0.449	0.363	<0.025	<0.070	<0.025	<0.025
Manganese (Mn)	0.032	<0.025	0.043	<0.025	<0.025	0.038	0.026	0.026	<0.025	<0.025	<0.025	<0.025
Molybdenum (Mo)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Nickel (Ni)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Lead (Pb)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.020	<0.020	<0.020
Selenium (Se)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.123	0.1	0.115	<0.020	<0.020	<0.020
Silver (Ag)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Tin (Sn)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	0.210	0.210	0.170
Strontium (Sr)	-	-	-	-	-	-	0.171	0.18	0.165	0.140	0.080	0.080
Titanium (Ti)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Vanadium (V)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Zinc (Zn)	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025

Appendix A 1.3: Seasonal mean concentrations in sediment at the Phalaborwa barrage per site

Metals	Winter (July 2010)			Summer (February 2011)				
mg/kg	Inflow	Wall	Below	Inflow	Wall	Below	Mean	SD
Phosphorous	107	346	127	194	142	86	167.0	±95.0
Aluminium	1730	1568	2352	5000	9200	3800	3941.7	±2892.9
Arsenic	3	8	0	0	0	0	1.8	±3.3
Antimony	1	0	2	0	0	0	0.5	±0.8
Boron	236	248	243	42	0	0	128.2	±126.1
Barium	316	414	333	84	2	12	193.5	±181.5
Cadmium	0	0	0	12	0	10	3.7	±5.7
Cobalt	4	26	6	16	12	14	13.0	±7.9
Chromium	3	177	35	5	7	42	44.8	±66.8
Copper	5	37	6	10	12	8	13.0	±12.0
Iron	4600	2448	7184	3660	1680	2760	1954	±12359
Lead	5	9	4	0	0	0	3.0	±3.7
Manganese	129	852	154	202	254	192	297.2	±275.2
Nickel	12	90	17	28	38	22	34.5	±28.6
Silver	41	102	95	0	0	0	39.7	±48.3
Strontium	13	6	9	24	36	14	17.0	±11.1
Tin	0	0	0	0	14	0	2.3	±5.7
Titanium	38	100	90	1830	582	1396	672.7	±767.0
Vanadium	8	43	17	212	42	154	79.3	±83.5
Zinc	155	185	168	96	16	14	105.7	±76.4

APPENDIX B: FISH HEALTH ASSESSMENT INDEX PARASITES

Appendix B 1.1: Seasonal values of fish HAI variables for *Clarias gariepinus* at the Phalaborwa Barrage

Fish #	Length cm		Mass g	Sex	Eyes	Skin	Fins	Oper-cula	Gills	Liver	Spleen	Hind gut	Kid-ney	Blood Hct (%)	Ecto-PI	Ecto IPI	Endo-PI	Total HAI	HAI IPI
	SL	TL																	
AUTUMN																			
1	69	78.52	502	M	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
2	29	34.6	363.2	M	0	0	0	0	0	0	0	0	0	0	0	10	10	40	30
3	28.5	32.9	383.9	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
4	667	738	3822	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
5	550	635	1531	M	0	0	0	0	0	0	0	0	0	20	10	10	40	60	30
6	362	416	4843	F	0	0	0	0	0	0	0	0	0	0	0	10	10	40	30
7	243	268	1159	M	0	0	0	0	0	0	0	0	0	0	0	10	10	40	30
8	670	759	4566	M	0	0	0	0	0	30	0	0	0	0	10	10	50	70	30
9	553	614	1612	M	0	0	0	0	0	0	0	0	0	0	0	10	10	40	30
																	Mean	21	45.5
WINTER																			
1	310	342	291.5	F	30	0	0	0	0	0	0	0	0	0	10	10	50	70	30
2	320	368	378.7	M	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
3	730	835	4630	F	0	0	0	0	0	0	0	0	0	20	10	10	40	60	30
4	390	432	567	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
5	670	748	3298	F	0	0	0	0	0	0	0	0	0	0	20	10	30	30	20
6	710	815	4610	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
7	387	429	564	M	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
8	44	49	939	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
9	27.8	31.3	247.2	M	0	0	0	0	0	0	0	0	0	0	30	10	40	20	10
10	30	34	334	M	0	0	0	0	0	0	0	0	0	0	30	10	40	20	10
																	Mean	30	40

Appendix B 1.1: Continued

Fish #	Length cm		Mass g	Sex	Eyes	Skin	Fins	Oper-cula	Gills	Liver	Spleen	Hind gut	Kid-ney	Blood Hct (%)	Ecto-PI	Ecto IPI	Endo-PI	Total HAI	HAI IPI
	SL	TL																	
SPRING																			
1	21.2	342	126.2	M	0	0	0	0	0	0	0	0	0	20	10	10	40	60	30
2	17	22	74.3	M	0	0	0	0	0	0	0	0	0	0	0	10	10	40	30
3	52.5	62.8	1758	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
4	27	29.3	160.6	M	0	0	0	0	0	0	0	0	0	20	10	10	40	60	30
5	37	41.4	721.3	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
6	46.8	53	1602.1	M	0	0	0	0	0	0	0	0	0	20	10	10	40	40	30
7	42.1	48.3	782	F	0	0	0	0	0	0	0	0	0	0	0	10	10	40	30
8	51.3	58.1	1608.7	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
9	51.2	57.8	1594.2	F	0	0	0	0	0	0	0	0	0	20	10	10	40	60	30
10	40.8	47	782.7	F	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
															Mean		26	46	
SUMMER																			
1	34.5	40.2	545.6	F	0	0	0	0	0	0	0	0	0	10	30	10	50	30	10
2	20.8	23.5	95	M	0	0	0	0	0	0	0	0	0	10	20	10	40	40	20
3	44.5	50	1159.3	M	0	0	0	0	0	0	0	0	0	0	0	10	10	40	30
4	33.6	38.4	564.5	M	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
5	36	42	572.3	M	0	0	0	0	0	0	0	0	0	10	0	10	20	50	30
6	35.6	40.8	593.7	M	0	0	0	0	0	30	0	0	0	0	0	10	40	70	30
7	48	54.5	1150.8	F	0	10	0	0	0	30	0	0	0	0	0	10	50	80	30
8	32.3	36.5	436.3	M	0	0	0	0	0	30	0	0	0	0	0	10	40	70	30
9	33.4	38	429.1	M	0	0	0	0	0	30	0	0	0	0	0	10	40	70	30
10	32	36	360.1	F	0	0	0	0	0	30	0	0	0	0	0	10	40	70	30
11	38.5	43.2	588.1	M	0	0	0	0	0	0	0	0	0	0	20	10	30	30	20
12	45	52	1050.3	M	0	0	0	0	0	0	0	0	0	0	10	10	20	40	30
13	35.6	41.4	553.4	F	0	0	0	0	0	30	0	0	0	0	0	10	40	70	30
14	27.4	31.2	279.6	F	0	0	0	0	0	0	0	0	0	0	0	10	10	40	30
															Mean	32	52		

Appendix B 1.2: Health Assessment Index (HAI) of *Labeo rosae* from Phalaborwa Barrage.

Fish	Length			Mass	Sex	Eyes	Skin	Fins	Oper-cula	Gills	Liver	Spleen	Hind gut	Kidneys	Hct	Ecto-PI	Endo-PI	HAI	HAI IPI	IPI
	SL	FL	TL																	
AUTUMN																				
1	24	26	30	315.6	M	0	0	0	0	0	0	0	0	0	0	30	10	0	10	10
2	11.5	10.5	13.5	22.2	BABY	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
3	22.5	25	29	246.2	M	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
4	27	29	33	388	M	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
5	29	27	34.5	453.9	M	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
6	18	20	24	122.2	F	0	0	0	0	0	0	0	0	0	0	20	20	0	10	20
7	15	16	19	66.3	F	0	0	0	0	0	0	0	0	0	20	10	30	0	30	50
8	20.5	22.5	26	181	M	0	0	0	0	0	0	0	0	0	10	0	30	10	20	40
9	31	33	37.4	566.9	F	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
10	20	22	26	124	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
																Mean		14	31	
WINTER																				
1	23	25	29	272	F	0	0	0	0	0	30	0	0	0	0	10	30	10	50	70
2	27	29	33	468	F	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
3	28	31	36	435	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	40
4	26	28	33	427	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
5	31	26	34	332	F	0	0	0	0	0	30	0	0	0	0	10	30	10	50	70
6	24	26	30	260	F	0	0	0	0	0	30	0	0	0	0	10	30	10	50	70
7	25	27	31	462	F	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
8	31	23	34	330	M	0	0	0	0	0	0	0	0	0	0	20	20	10	20	30
9	24	26	31	425.1	M	0	0	0	0	0	0	0	0	0	0	20	20	0	20	20
10	27	29	34	449	F	0	30	0	0	0	0	0	0	0	0	30	10	0	60	40
																	Mean	31	35	

Appendix B1.2 continues

	Length			Mass	Sex	Eyes	Skin	Fins	Oper-cula	Gills	Liver	Spleen	Hind gut	Kidneys	Hct	Ecto-PI	Endo-PI	HAI	HAI IPI	IPI
	SL	FL	TL																	
SPRING																				
1	30.2	33.5	36.9	714.4	M	0	0	0	0	0	0	0	0	0	0	10	30	0	10	1
2	28.7	30.5	35	520.1	F	0	0	0	0	0	0	0	0	0	10	20	20	0	30	2
3	33	37.2	40.2	1030	F	0	0	0	0	0	0	0	0	0	0	10	30	10	20	3
4	26.6	32.2	33.5	520	F	0	0	0	0	0	0	0	0	0	0	20	20	0	20	4
5	20	22	25	272.5	M	0	0	0	0	0	0	0	0	0	20	20	20	0	40	5
6	23.2	26.5	289	M	M	0	0	0	0	0	0	0	0	0	0	10	30	30	40	60
7	22.5	24.8	29.2	275.2	M	0	0	0	0	0	0	0	0	0	30	10	30	0	40	7
8	22.3	24.2	27.9	317.5	F	0	0	0	0	0	0	0	0	0	30	20	20	0	50	8
9	20.5	22.5	26	232.7	M	0	0	0	0	0	0	0	0	0	30	10	30	0	40	9
10	20.3	22.9	25.4	265.4	M	0	0	0	0	0	0	0	0	0	30	10	30	0	40	10
																Mean		30	42	
SUMMER																				
1	22	24.2	27	260.9	M	0	0	0	0	0	0	0	0	0	20	20	20	20	60	60
2	20	22.3	25	195.9	M	0	0	0	0	0	0	0	0	0	0	20	20	20	40	40
3	22	24	27.2	271.8	M	0	0	0	0	0	0	0	0	0	0	20	20	20	40	40
4	20.2	22.4	25.5	226.5	F	0	0	0	0	0	0	0	0	0	0	30	10	10	40	20
5	19.8	21.6	25.2	216.2	F	0	0	0	0	0	0	0	0	0	0	20	20	20	40	40
6	21	23.2	26.5	261.3	M	0	0	0	0	0	0	0	0	0	0	10	30	30	40	60
7	15.5	17	19.5	83.7	F	0	0	0	0	0	0	0	0	0	0	20	20	20	40	40
8	15	16.5	18.9	82.5	M	0	0	0	0	0	0	0	0	0	30	10	30	30	70	90
9	18.7	19.2	21.2	259.3	F	0	0	0	0	0	0	0	0	0	30	10	30	30	70	90
10	21.6	23.9	27.2	275.5	M	0	0	0	0	0	0	0	0	0	30	20	20	20	70	70
																Mean		**51**	**55**	

Appendix B 1.3: Fish Health Assessment Index (HAI) variables with assigned characters showing the norm and deviation from the norm in the necropsy based system (adapted from Adams et al. 1993; revised by Heath et al. 2004a; Jooste et al. 2004)

Variables	Variable condition External variables	Original field designation	Substituted value for the HAI
Eyes	Normal Exopthalmia Haemorrhagic Blind Missing Other	N E1/E2 H1/H2 B1/B2 M1/M2 OT	0 30 30 30 30 30
Fins	No active erosion or previous erosion healed over Mild active erosion with no bleeding Severe active erosion with haemorrhage / secondary infection	0 1 2	0 10 20
Skin[a]	Normal, no aberrations Mild skin aberrations – “black spot” < 50 Moderate skin aberrations – “black spot” > 50 Severe skin aberrations	0 1 2 3	0 10 20 30
Opercules	Normal/no shortening Mild/slight shortening Severe shortening	0 1 2	0 10 20
Gills	Normal Frayed Clubbed Marginate Pale Other	N F C M P OT	0 30 30 30 30 30
Spleen	Black Red Granular Nodular Enlarge Other	B R G NO E OT	0 0 0 30 30 30
Hindgut	Normal, no inflammation or reddening Slight inflammation or reddening Moderate inflammation or reddening Severe inflammation or reddening	0 1 2 3	0 10 20 30
Kidney	Normal Swollen Mottled Granular Urolithic Other	N S M G U OT	0 30 30 30 30 30
Liver	Red Light red “Fatty” liver, “coffee with cream” colour Nodules in liver Focal discolouration General discolouration Other	A B C D E F OT	0 30 30 30 30 30 30
Blood (haematocrit)	Normal range Above normal range Below normal range Below normal range	30-45% >45% 19-29% <18%	0 10 20 30
Parasites *Endoparasites[b]	No observed endoparasites Observed endoparasites < 100 101 -1000 > 1000	0 0 1 3	0 10 20 30
*Ectoparasites[b]	No observed ectoparasites Observed ectoparasites 1 - 10 11 - 20 > 20	0 1 2 3	0 10 20 30

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