THE IMPACT OF WATER AND SEDIMENT QUALITY ON THE HEALTH OF *OREOCHROMIS MOSSAMBICUS* (PETERS, 1852) AND *SCHILBE INTERMEDIUS* RüPPELL, 1832 AT THE PHALABORWA BARRAGE IN THE OLIFANTS RIVER

by

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DECLARATION

"I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Masters 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."

AF Gohell

Date

ABSTRACT

The Olifants River is presently one of the most threatened river systems in South Africa. The Upper and Middle catchments are heavily impacted by anthropogenic activities, however little is known of the status of the river in the Lower catchment in the Limpopo Province. Thus the aim of this study was to evaluate the impact of water quality on the health of *Oreochromis mossambicus* and *Schilbe intermedius* at the Phalaborwa Barrage. This was achieved through assessing the water (seasonally) and sediment quality (bi-annually) at three sites in the Phalaborwa Barrage (inflow, middle and the wall). Ten specimens of each of the two selected fish species were seasonally collected using gill nets of different mesh sizes from May 2010 to January 2011. The water, sediment and the dorsal muscle tissues from both fish species were analysed for selected metals at an accredited laboratory by means of ICP-OES spectrometry and the human health risk factor upon consumption of fish contaminated with metals was assessed. Fish health was determined at the field laboratory by applying the HAI, PI and IPI. In addition, the condition factor (CF) was determined for the selected fish species during all seasons.

All water constituents with the exception of turbidity during spring (wall), anions (Cl, F and SO_4) and cations (Ca, K, Mg and Na) fell within the target water quality range (TWQR) for aquatic ecosystems throughout the study. Inorganic nitrogen concentrations showed eutrophic (spring) and oligotrophic conditions (autumn, winter and summer), while phosphorus indicated eutrophic (autumn, winter and spring) and hypertrophic (summer) conditions throughout the study. There was no significant difference in anions (F=0.07; df=3; p=0.97), cations (F=0.57; df=3; p=0.64) and nutrients (F=0.79; df=3; p=0.56) detected in water. The metals that were detectable in the water samples were: Al, Fe, Sn, B, Sr, Se, Ba, Mn, Sb and As, while in sediment all metals (Fe, Al, Ti, Mn, Ba, B, Zn, Ag, V, Cr, Ni, Sr, Sn, Co, Cu, Cd, Pb, As and Sb) were detected with the exception of Se. The average metal concentrations detected above the TWQR are; Al, Fe, Se, Sn, Sb and As, in the sediment only Cr and Cd were above suggested quality guidelines. There was no significant difference in metals detected in water (F=0.05;

df=3; p=0.98) and sediment (F=1.62; df=1; p=0.21).

Higher HAI values were recorded for *S. intermedius* than *O. mossambicus,* indicating that *O. mossambicus* was in better health than *S. intermedius.* For *S. intermedius*, parasites contributed the most to the total HAI followed by abnormal liver conditions, haematocrit levels (internal variables) and abnormal gills (external variable). While for *O. mossambicus*, parasites, liver, haematocrit (internal variables), gills, eyes, fins and skin (external variables) contributed to the HAI values. There was no significant difference in HAI values for *O. mossambicus* (F=1.9; df=3; p=0.15) and *S. intermedius* (F=2.58; df=3; p=0.66) throughout the study. The PI and IPI for ectoparasites for both fish species, were higher than the PI for endoparasites. There was a significant difference for PI values of *O. mossambicus* (F=0.07; df=3; p=0.041) and *S. intermedius* (F=3.65; df=3; p=0.019) during the study. *Oreochromis mossambicus* had higher CF values than *S. intermedius* and both fish species had values that indicated good fish.

Metals that were detectable in *O. mossambicus* muscle tissue included: Ba, B, Zn, Fe, Sr, Al, Mn, Cr, Ag, Pb, Ni, Cu, Se, V, Sb, Ti, Sn and Co, while for *S. intermedius* Ba, B, Zn, Fe, Al, Cr, Sr, Mn, Cu, As, Pb, Se, Sn, Ag, Ti, Ni, Sb and Co were recorded. There was no significant difference in bioaccumulation levels between the two fish species (F=1.412; df=3; p=0.250). The human health risk assessment indicated that fish muscle tissues are unsuitable for continuous (weekly/daily) consumption. As some carcinogenic metals i.e. Pb, Cr and Sb for *O. mossambicus* and Pb, As and Cr for *S. intermedius* were detected at HQ levels of more than 50 times the recommended value of one.

In conclusion, the Phalaborwa Barrage is impacted by metals (Al, Fe, Se, Sn, Sb and As) and occasionally with nutrients. The water and sediment quality and metal bioaccumulation have confirmed the latter statement and to a lesser extent the HAI, PI and IPI. The human risk assessment done indicated that continuous consumption of fish by humans, especially *O. mossambicus* may have adverse effects on the health of humans.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Freshwater is a precious and scarce resource on the earth, and life would be non-existent without it. Water is essential for everything on our planet to grow and develop. Unfortunately river systems are continuously undergoing eco-degradation throughout their course of flow due to various anthropogenic stressors e.g. pollution (Heath *et al.* 2010). South Africa's freshwater resources, including rivers, man-made lakes and groundwater, are under increasing pressure from a rising population and a growing economy. Almost all of the country's freshwater resources are protected by legislation, however the water quality of these resources has deteriorated (Ashton *et al.* 2008). In some areas, such as the upper and middle reaches of the Vaal River System, the Mgeni River System, the Crocodile River System (West) and the upper and lower reaches of the Olifants River System, the water quality poses serious health risks to humans and livestock that drink the water (Ashton and Dabrowski 2011). With a growing population and increased urbanization and the apparent failure of most local authorities to successfully treat urban and industrial effluents to appropriate effluent standards, the situation will continue to worsen.

Pollution is defined as the contamination of the environment. It is a serious problem that causes instability, disorder, harm and discomfort to the ecosystem, while a pollutant is something which is present at concentrations in excess of those occurring in natural systems (Jeffries and Mills 1990). The pollution of freshwater resources is considered to be problematic worldwide. Anthropogenic activities, due to continuous development, pollute freshwater ecosystems and oceans. These activities produce excessive levels of nutrients in aquatic ecosystems, resulting in eutrophication (DWAF 1996c).

River systems in South Africa are mostly used for domestic water supply, irrigation, agriculture, aquaculture, mining and industrial production (Ashton 2010; Ashton and Dabrowski 2011). Pollutants of industrial, agricultural and domestic origin, reaching aquatic ecosystems through runoffs, point and non-point pollution are incessantly harming these ecosystems and their associated processes (Richman 1997).

In addition, rapid population growth, industrialization and urbanization in South Africa result in a high concentration of pollutants in the country's surface (Ashton 2010). Natural water resources in South Africa are under constant pressure due to the increasing demand of water for basic needs and consequently affecting the quality of water (e.g. agriculture). Water quality determines and regulates amongst others the health of freshwater aquatic ecosystems as the resource for all users (DWAF 1996c). Water-borne pollutants (metals, sediment, nutrients, acidic compounds and biocides) are considered to be most significant in terms of their impact on aquatic ecosystems (Adams *et al.* 1993). In 1986, the Department of Water Affairs identified polluting substances in the water supply and viewed it as a significant threat to the existence of all South Africa's aquatic resources, including rivers (CSIR 2010).

1.2 OLIFANTS RIVER SYSTEM

The Olifants River (Figure 1.1) flows in a north-easterly direction, eventually flowing through the Kruger National Park (KNP) into the Massingir Dam in Mozambique (thereafter named the *Rio dos Elefantes*) before joining the Limpopo River in Mozambique (Coetzee *et al.* 2002). It is subdivided into nine secondary catchments with 4 sub-catchments: Upper, Middle (upper middle and lower middle), Steelpoort, Lower Olifants sub-catchments (Figure 1.1). The total mean annual runoff of the river is about 2400 million cubic metres per year. The Olifants River and some of its tributaries, notably the Klein Olifants River, Elands River, Wilge River and Bronkhorstspruit, rise in the Highveld grasslands of the Upper Olifants sub-catchment (Coetzee *et al.* 2002). One river, the Ga-Selati, is a major contributor to the poor water quality in the Olifants River below the Phalaborwa Barrage (Heath *et al.* 2010). According to Heath and Claassen (1999), the Olifants River is impacted by mining and agriculture, high levels of certain metals (e.g. aluminium) and a variety of pesticides.

The Olifants River System, especially the Upper Olifants River catchment area, is characterised by intensive mining, agriculture/irrigation and it is the major water use sector, coal power plants producing 60% of the electricity of the country (use the majority of the water for cooling) and industrial activities which have a negative impact on the water quality in the Olifants River System (Coetzee *et al.* 2002). Furthermore, seven towns in the upper catchment negatively impact on the water quality. In the Middle Olifants sub-catchment, heavy overgrazing and dry land cultivation has led to soil erosion, which causes high silt levels in rivers (Heath *et al.* 2010). These high levels of silt result in loss of fish through suffocation (Ashton *et al.* 2001; Ballance *et al.* 2001). According to Ashton *et al.* (2001), there is subsistence farming in the form of livestock farming (cattle, goats and donkeys) throughout the Middle and Lower Olifants sub-catchments.

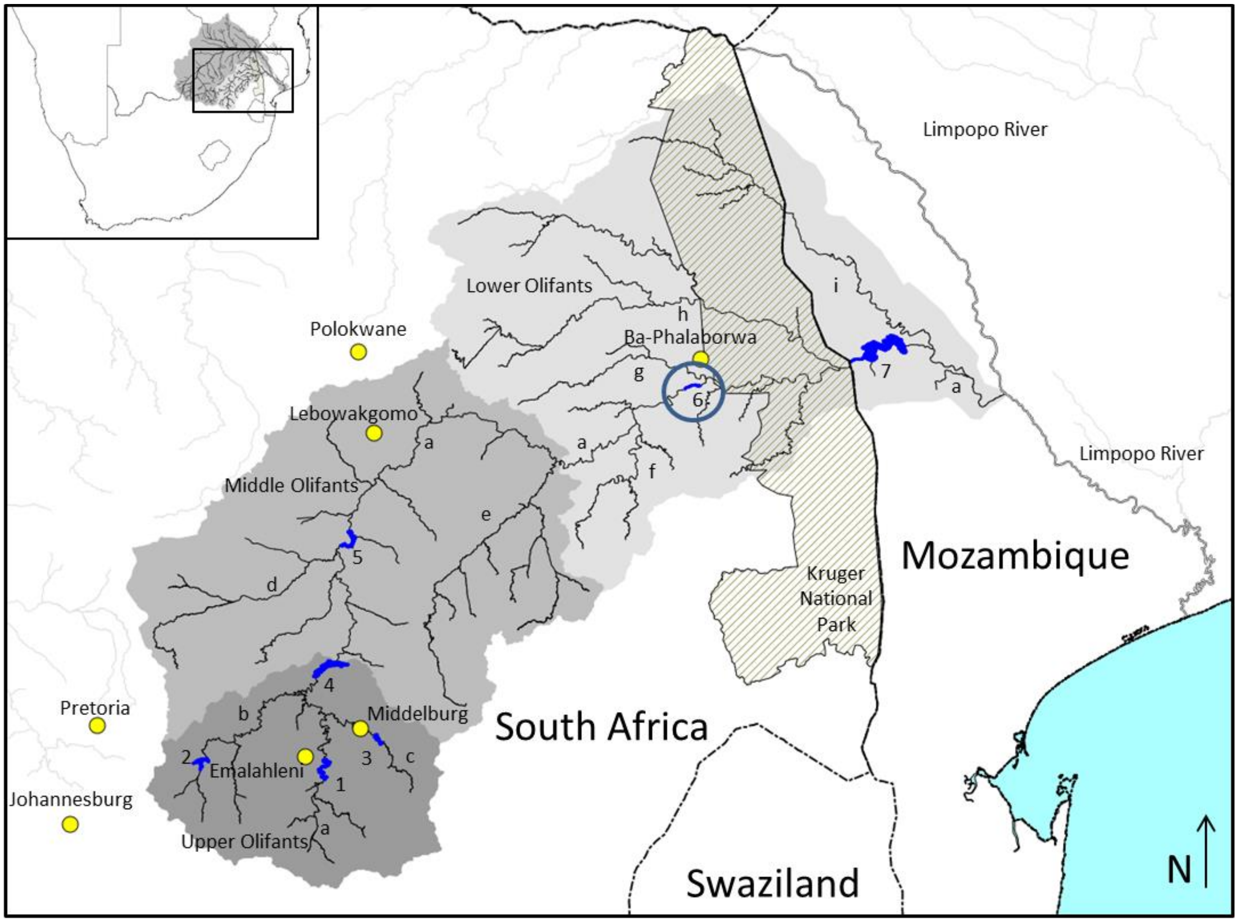

Figure 1.1: Map of the Olifants River system showing the location of major towns, impoundments and tributaries. The study site the Phalaborwa Barrage, 6) is circled. Map by Dr SM Marr.

These anthropogenic activities have resulted in the deterioration of the water quality which is thought to have caused the major crocodile kills in the Olifants River Gorge, KNP (Van Vuuren 2009; Ashton 2010; Heath *et al.* 2010; Ashton and Dabrowski 2011). Post mortem results indicated that the crocodiles died of pansteatitis, a condition caused by the depletion of anti-oxidants in the system, leading to hardening of the body fat (Van Vuuren 2009; Heath *et al.* 2010). The unavailability of these antioxidants (Vitamin E) resulted in the hardening of the crocodile's fat reserves into a rubber-like mass, which is not available for normal metabolism and the animal loses mobility. Pansteatitis has been identified in Nile crocodiles and the sharptooth catfish (*Clarias gariepinus*) in the Olifants and Lower Letaba Rivers in KNP (2008 – 2009) (Huchzermeyer *et al.* 2011; Huchzermeyer 2012). Furthermore, the decline in piscivorous bird (especially heron) populations is a concern, this most likely being linked to the deterioration in the "health" of this river (Myburgh and Botha 2009). Studies of metal bioaccumulation done at the Upper Olifants River as well as the Phalaborwa Barrage, showed that some constituents had bioaccumulated in various organs of selected fish species (Grobler *et al.* 1994; Seymore *et al.* 1994; Van Vuren *et al.* 1999; Ashton *et al.* 2001). The existing evidence in the Olifants River suggests that there is a relationship between the already high and ever increasing levels of water pollution and the sporadic fish kills that emerge mainly during the winter months (Oberholster *et al.* 2010). In turn, the presence of pansteatitis in dead Nile crocodiles and terrapins suggests that this has been caused by the consumption of fish with pansteatitis (Oberholster *et al.* 2010).

According to Heath *et al.* (2010), prior to 1990, most of the research conducted in the Olifants River System focused on biodiversity. Water quality issues were still unnoticed by researchers and only a few studies had direct relationships with water quality. From 1990 to 2002, studies that have been done concentrated on the Upper Olifants River and the KNP. However, studies done on the Lower Olifants River focused on bioaccumulation of selected metals in the Mozambique tilapia (Robinson and Avenant-Oldewage 1997; Kotzè *et al.* 1999) and the sharptooth catfish (Avenant-Oldewage and Marx 2000).

1.3 PHALABORWA BARRAGE (STUDY SITE)

The Phalaborwa Barrage (Figure 1.2) is situated 10 km south of the town of Phalaborwa in the Phalaborwa Local Municipality (location: 24° 4’ 12’’ S, 31° 8’ 43’’ E). The water from the barrage is abstracted from the Olifants River. It was built in 1959 to supply water to the adjacent copper and phosphate mines and was constructed of a line of 22 gates/sluices with flanking piers to control the flow of water (Figure 1.3). The present capacity of the barrage has been reduced by 45% due to siltation (LNW 2010/11). It supplies potable water to Ba-Phalaborwa Municipality, and semi treated/industrial and/or raw water to the mines and other industrial users through networks dedicated to each type of water supplied (Van Vuren *et al.* 1994). The Lepelle Northern Water Board (LNW) treats and purifies water from the barrage for the town of Phalaborwa, the copper and phosphate mines and industrial complex (LNW 2010/11). The water is mainly distributed as potable and as industrial water in various processing plants mainly as transport medium to transport mining residues to the tailing dams and for agriculture. Most of the water supply (domestic and industrial) of Phalaborwa and surrounding areas is extracted from it (± 141 million m^3 per annum). The water level is usually kept almost full with an approximate base inflow of 1.5 m^3/s^{-1} (mean annual runoff under present conditions is estimated to be 1 201 million m^3 per annum).

The barrage has a plant capacity of 150 Ml/day of which 76 Ml/day can be purified to potable standards, having a raw water allocation of 64 000 Ml/annum (175Ml/day). The present consumption is estimated at 47976 Ml/annum (131Ml/day) (LNW 2010/11). When insufficient inflow into the barrage occurs, supplementary water is released from the Blyderivierspoort Dam. During dry seasons the barrage relies on water releases from the Blyde Dam in the Blyde River to boost the flow of the Olifants River downstream in the KNP, and water usage is estimated at 46 million m^3per annum. The Phalaborwa Industrial Complex (PIC) receives water from the Phalaborwa Barrage (LNW 2010/11).

The Phalaborwa Barrage is surrounded by riparian vegetation, consisting of reeds, grasses and indigenous trees. The Phalaborwa Barrage provides habitat for a variety of vertebrates like birds, crocodiles and hippopotami and is a very popular venue for recreational and subsistence fishermen.

Mining and industrial activities at Phalaborwa, outside the western border of the KNP, are a major source of pollution of the Olifants River below the barrage. The low water flow aggravates water quality problems and causes certain aquatic habitats to vanish. High salinity, pollution due to metals and high silt loads are the main concerns for conservation, ultimately contributing to the disappearance of at least 5 fish species from the Lower Olifants River (Van Vuren *et al.* 1994). Protection of aquatic organisms from predators, due to increased turbidity, has led to a decline of scarce species, for instance Pel's fishing owl (*Scotopeli peli*) and tiger fish (*Hydrocynus vittatus*) (Moore *et al.* 1991). High silt loads are generated when sediment-laden releases from the barrage are made, and have been the cause of massive fish kills downstream in the KNP (Van Vuren *et al.* 1994).

Figure1.2: A satellite image of the Phalaborwa Barrage (Google Earth). The three water and sediment sampling sites: A-inflow, B-wall and C-below wall.

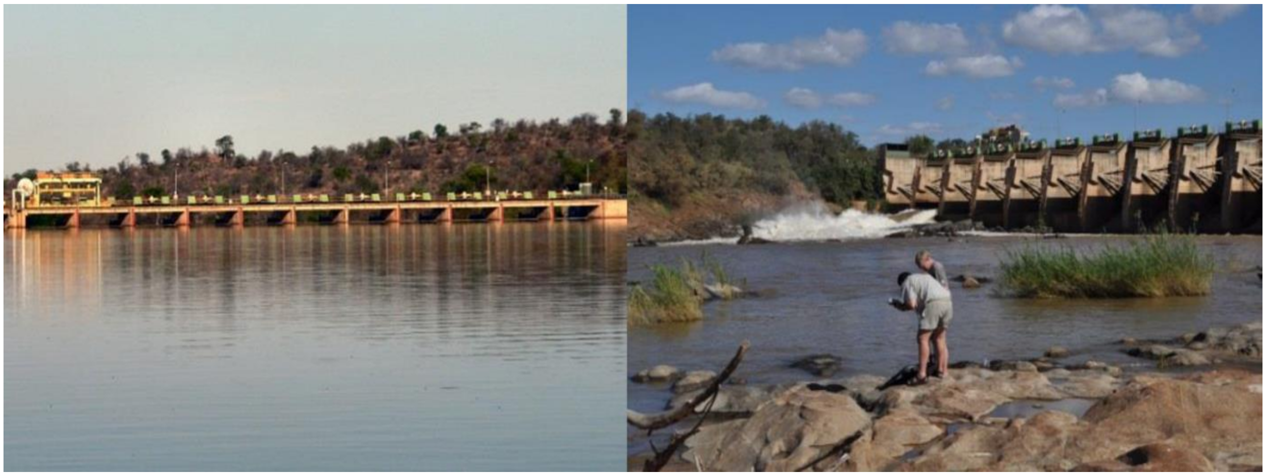

Figure 1.3: The sluices/gates of Phalaborwa Barrage.

1.4 FISH SPECIES

1.4.1 *Oreochromis mossambicus*

Oreochromis mossambicus (Peters, 1852) (Figure 1.4) and *Schilbe intermedius* (Figure 1.5), were chosen because they have different feeding habits, occupying a range of habitats, and different trophic levels in the aquatic ecosystem (Skelton 2001).

Oreochromis mossambicus (Mozambique tilapia) is distributed along the east coastal rivers from the lower Zambezi system south to the Bushmans system, Eastern Cape. This species is furthermore found south of the Phongolo system and is naturally confined to closed estuaries and coastal reaches of rivers (Skelton 2001). The Mozambique tilapia is also found in inland regions and to the south-west and west coastal rivers including the lower Orange and rivers of Namibia. It was introduced to tropical and warm temperate localities throughout the world. The Mozambique tilapia is native to Southern Africa and it is a significant angling fish (Skelton 2001) contributing about 4% of the total tilapia aquaculture production worldwide.

Oreochromis mossambicus is a euryhaline fish species meaning it can tolerate both fresh-waters and saline water (Skelton 2001). This species can survive temperatures between 15°C and 42°C but it prefers warmer waters above 22°C. They are omnivorous, feeding on algae, insects and other invertebrates. They breed in summer, with females raising broods every 3 – 4 weeks (Skelton 2001). The males construct a saucer-shaped nest in the substratum and the female mouth-broods the eggs, larvae and small fry. The juveniles grow rapidly and can breed within their first year, but the growth rate is affected when there is crowding or other adverse effects (Skelton 2001).

Figure 1.4: The Mozambique tilapia – *Oreochromis mossambicus* from the Phalaborwa Barrage.

1.4.2 *Schilbe intermedius*

Schilbe intermedius Rüppell, 1832 (Silver catfish) are widely distributed throughout tropical Africa including the Nile River, West Africa to the Senegal River, and are also found in the Cunene, Okavango and Zambezi systems southwards to the Phongolo in northern Zululand (Skelton 2001). Unlike the Mozambique tilapia this fish species does not contribute to aquaculture production, but it is also a significant angling fish species (Skelton 2001). This species is omnivorous, feeding on insects, snails, plant seeds and fruit (Skelton 2001). It is usually found in turbid waters and is potamodromous (migration within freshwaters only) (Seegers *et al.* 2003). They usually breed during the rainy seasons and may be either single or multiple spawners in different locations. *Schilbe intermedius* usually breeds in slow-flowing rivers and they lay their eggs on aquatic vegetation (Skelton 2001).

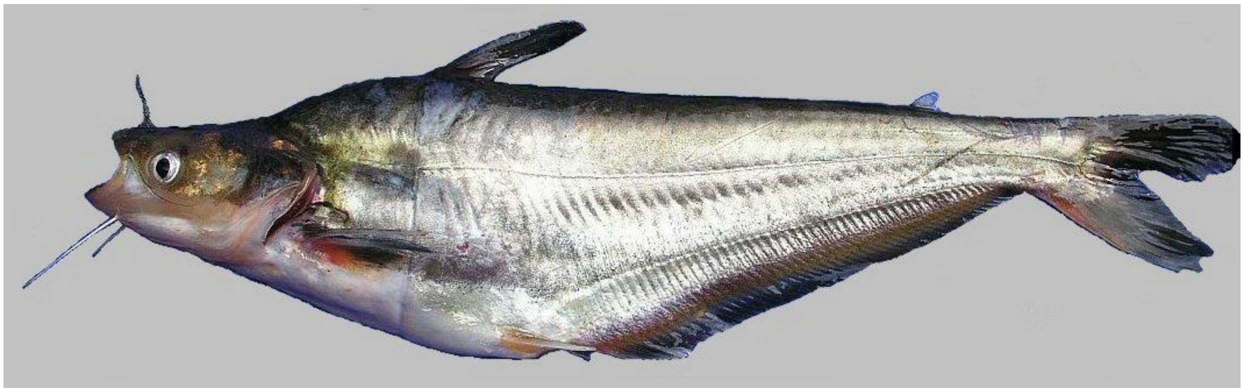

Figure1.5: The silver catfish – *Schilbe intermedius* from the Phalaborwa Barrage.

1.5 PURPOSE OF THE STUDY

The Olifants River is used by people living around the river for daily requirements. Clean water plays a significant role in the wellbeing of all users. The Upper Olifants River catchment area is dominated by mines (coal, platinum, phosphate and copper), coal power plants and industrial and agricultural activities, which have a considerable impact on the Olifants River System. The Phalaborwa Barrage in the Lower Olifants River is the selected site for the study. It is located about 10 km from the Phalaborwa town in the Mopani district on the north-eastern part of the Limpopo Province. The site was selected to verify if the anthropogenic (e.g. mining and agriculture) activities taking place at the upper and middle catchments have adverse effects on the quality of water. Furthermore, little is known about the status of the Olifants River System in the Limpopo Province.

This study formed part of a Water Research Commission (WRC) report:
JOOSTE, A., LUUS-POWELL, W. & ADDO-BEDIAKO, A. (2013). The impact of water and sediment quality on the health of fish and the diversity of fish parasites in two impoundments of the Olifants River, Limpopo province. WRC Project No. K5/1929. Water Research Commission, Pretoria.

1.6 HYPOTHESES

It was hypothesized that;

- The Phalaborwa Barrage is polluted.

- The water and sediment quality have an impact on the health of the two fish species.

- Metals bioaccumulate in fish muscle tissue.

- Fish parasites can be used as bio-indicators of pollution.

1.7 AIM

Aim: To evaluate the impact of water and sediment quality, fish health and parasites and bioaccumulation of metals for *O. mossambicus* and *S. intermedius* and the human health risk at the Phalaborwa Barrage, Olifants River, in the Limpopo Province.

1.8 OBJECTIVES

- To determine the concentration of selected water and sediment constituents at selected sites in the Phalaborwa Barrage.
- To determine and compare the health and condition of *O. mossambicus* and *S. intermedius* by applying the HAI and condition factor protocols.
- To identify and quantify the ecto- and endo-parasites of the two fish species.
- To determine and compare the levels of bioaccumulation of selected metals in muscle tissue of the two fish species.
- To determine the potential health risk to humans upon consumption of fish from the Phalaborwa Barrage.

1.9 RESEARCH QUESTIONS

- What are the concentration levels of selected water and sediment quality constituents at the different sampling sites at the Phalaborwa Barrage? Is this a polluted aquatic ecosystem?
- What is the impact of the water quality on the health of the two fish species?
- Can fish parasites be used as bio-indicators of water pollution?
- What is the concentration level of metals that have bioaccumulated in the muscle tissue of the two fish species?
- What is the human health risk factor upon consumption of fish muscle tissue contaminated with metals?

1.10 THE DISSERTATION IS SUBMITTED AS FOLLOWS:

Chapter 1 Introduces the study, the problem statement and outlines the purpose of the study. The description of sampling sites and study area is discussed in this chapter. A brief description of the fish species used during this study, hypotheses, aim and objectives are included.

Chapter 2 Contains a background on water and sediment quality and the selected water variables. The materials and methods and statistical analysis used are included. The results (graphs and tables) and discussions for four seasons (autumn, winter, spring and summer) are incorporated in this chapter. This is followed by the conclusions on the water and sediment quality at the barrage.

Chapter 3 Focuses on the health and parasite composition of *O. mossambicus* and *S. intermedius* by applying the HAI and the PI. A brief history/development of the HAI and PI is provided. Materials and methods and the statistical analysis which were used are included. Furthermore an extensive discussion on the internal and external variables of fish and their ecto- and endo-parasites are included. The infection statistics of parasites (including prevalence, mean abundance and mean intensity) recorded seasonally are included. This is followed by the conclusions on the health and PI of the fish populations at the barrage.

Chapter 4 Focuses on metal bioaccumulation in the muscle tissue of fish, metals that occur in water adhering to the sediment and the human health risk assessment. Background information, the materials and methods and statistical analysis used are included. A comparison of the level of selected metal accumulation in fish and sediment are discussed followed by the conclusions.

Chapter 5 Summarizes the results obtained and conclusions drawn. Recommendations in the management and conservation of the river are included at the end of the dissertation.

This reference style is written according to the format of the African Journal of Zoology. 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

The term water quality is used to describe the condition of the water including its chemical, physical and biological characteristics usually with respect to its suitability for a particular purpose (i.e. agricultural, domestic, industrial, recreation or fishing); (DWAF 1996c). According to Van Vuren *et al*. (1999), any of the properties of water quality are influenced by substances that are either dissolved or suspended in water or aquatic ecosystems. Furthermore, water quality mainly decreases due to human activities, such as industrial, mining and agricultural activities, sewage spills, urbanization and power plants. Studies in freshwater ecosystems have been of major importance internationally and locally. The latter is as a result of a high demand of water use and contamination by pollutants and if it continues at the current rate there will be a lack of water supply to the population at large (Roux *et al*. 1994). Aquatic organisms such as fish usually suffer when the quality of water deteriorates due to anthropogenic activities (Davies and Day 1998).

In order to use water properly, the former Department of Water Affairs and Forestry (now Department of Water Affairs) has developed guidelines for different categories of water use. The South African Water Quality Guidelines provide scientific and technical information for a particular water quality constituent and describes the potential effects on the health of aquatic ecosystems and fitness of water for other uses (DWAF 1996c). Specific water quality criteria, namely Target Water Quality Range (TWQR), Chronic Effect Value (CEV) and Acute Effect Value (AEV), are included in the South African Water Quality Guideline for Aquatic ecosystems to indicate various categories of fitness of water for a wide range of water use (DWAF 1996c). The TWQR is a management objective derived from quantitative and qualitative criteria. The Department of Water Affairs strives to protect South Africa's water resources by maintaining water quality within the TWQR. The CEV is defined as that 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. While 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). The results of this study were used to compare with available data of the TWQR, CEV and the AEV for the different water constituents.

According to Dallas and Day (2004), human activities affect both quality and quantity of water in aquatic ecosystems. Humans have direct and indirect destructive influences on the aquatic environment and it is usually in the form of sub lethal pollution (Dallas and Day 2004). Pollution may cause water to be undesirable, to have a bad odour, taste and unsuitable for human use. There are two main types of water pollution; non-point and point source pollution, point source pollution occurs when the pollutants are released directly into the aquatic ecosystems (Dallas and Day 2004). Non-point are those which do not enter the aquatic ecosystem directly from a source, the pollutants enter the waters either by run-offs from urban and industrial areas, from mines which the land was left unrecovered (Davies and Day 1998; Heath and Claassen 1999). Non-point pollution is difficult to control because of the way it is discharged (Dallas and Day 2004).

Metal contamination contributes largely to water pollution and it is a major source of toxic pollution. Metals are dangerous because they cannot be destroyed by biological degradation and they accumulate with ease in the environment and have adverse effects to the aquatic ecosystem (Buermann *et al.* 1995). Heavy metals such as lead, copper, cadmium, zinc and iron are the ones which are ranking as major polluting chemicals in developed and developing countries (Buermann *et al.* 1995). However, metals are required for normal functioning of the aquatic ecosystem. Thus they have an important role, however there are ranges set by DWAF (1996c) of which the metals should not exceed or be below tolerable ranges (van Vuren *et al.* 1999). The ionic form is usually the form that causes mortalities rather than the complex metal compounds (Heath and Claassen 1999). All species have tolerance limits within which they may be able to survive, however the limits vary from species to species. Consequently, the increase of metal concentrations in water will result in adverse effects, such as: reduced ecosystem functioning, reduction in diversity, introduction or loss of key species (Dallas and Day 2004).

Sediment quality

Sediment forms an important part of the aquatic environment, and help to determine the overall assessment of metals in aquatic life (Jumbe and Nandini 2009). Direct discharge of contaminants increase the concentration of trace elements in aquatic systems, thus most are adsorbed on sediment (Sinicrope *et al.* 1992). Furthermore, pathogens, nutrients, metals, and organic chemicals are also adsorbed onto both inorganic and organic materials that eventually settle in depositional areas (Burton 2002). Sediment is known to capture hydrophobic chemical pollutants which enter the water, then slowly release the contaminants back into the water (Chapman 1996). Thus, in most aquatic systems, the concentrations of metals in sediment are far greater than the concentrations dissolved in the water column. Once contaminants are bound to a particle surface, they become less likely to be bio-transformed and desorption is usually very slow; therefore, adsorbed contaminants will reside for long periods in the sediment (Burton 2002). However, sediment play an important role in physico-chemical and ecological dynamics and any change in toxic concentrations of metal concentrations on the sediment will affect the natural aquatic life support systems (Jumbe and Nandini 2009). Although the true extent of sediment contamination is not known, it is apparent that huge quantities of sediments in industrialized countries are contaminated with metal and organic chemicals at levels that pose risks both to aquatic life and to humans consuming aquatic organisms (Burton 2002). Consequently, ensuring a good sediment quality is very important to maintain a healthy aquatic ecosystem, which ensures good protection of human health plus aquatic life.

Furthermore, in this study, water and sediment quality was examined in conjunction with fish health, bioaccumulation and human health risk to determine the effects that water constituents have on the health of fish and humans upon consumption of fish from the Phalaborwa Barrage (Chapter 4).

2.2 MATERIAL AND METHODS

Field work

Water sampling and analysis

Seasonal (autumn, winter, spring and summer) water samples were collected from three sites in the barrage: inflow, wall/sluices and below the wall (sluices), from May 2010 to January 2011. Water samples were collected in acid pre-treated propylene bottles, immediately refrigerated and frozen for chemical analyses. In addition a handheld multi parameter instrument (YSI Model 554 Datalogger with a 4 m Multi Probe System) was used to determine the electrical conductivity (EC), salinity, pH, dissolved oxygen and water temperature *in situ* at the sampling sites. The frozen samples were sent to an accredited laborabory WaterLab (PTY) LTD in Pretoria for analysis of metals and inorganic salts using Inductively Coupled Plasma Optical Spectrometry (ICP-OES). Alkalinity, tubidity nutrients, sulphate chloride, fluoride were also determined by Waterlab

Sediment sampling and analysis

Sediment samples were taken bi-annually using a Friedlinger mud grab at all sampling sites. Three to five samples were taken and composited before being transferred into 500 ml acid pre-treated propylene sampling bottles. The samples were frozen for storage prior to analysis at WaterLab (PTY) LTD. Sediment samples were dried at 60°C for 48 hours, then a subsample of 5 g was digested in a 4:1 mixture of concentrated nitric acid (HNO_3) and hydrochloric acid (HCl). Metals and metalloids were determined by using ICP-OES spectrometry, method according to Bervoets and Blust 2003. The ICP-OES spectrophotometer was set to scan sequentially for all 52 elements rather than for a limited set of elements. The spectral lines were examined to ensure that there was no interference between elements. The element concentrations were determined using the ICP-OES and reported as mg/kg dry weight.

Statistical analysis

The mean and standard deviation of the water chemistry, water and sediment metal concentrations were calculated. A one-way ANOVA was performed to determine whether there was seasonal variance for the water chemistry and metals in the water and sediment using SPSS VERSION 21 (2012).

Analysis of data

Where applicable, the water quality results from the chemical laboratory were compared with the TWQR, AEV and CEV of the South African water quality guidelines for different water uses developed by DWAF (1996c). Other international literature such as the United States Environmental Protection Agency (US-EPA) and the Canadian environmental water guidelines (CCME) were used for further comparison. The Canadian sediment quality guidelines were used for sediment quality (CCME 1999) as there are no guidelines yet in South Africa.

2.3 RESULTS AND DISCUSSION

2.3.1 Physico-chemical Water Quality

Physico-chemical system variables are important in the regulation of essential processes in the ecosystem (e.g. migration and spawning). Aquatic organisms are adapted to natural seasonal changes. If there are any sudden changes in the normal functioning of these cycles, the entire ecosystem will be disrupted because the physiological and ecological functions will be affected (DWAF 1996c).

The seasonal water quality data for the Phalaborwa Barrage are summarized in Appendix Table 1. The average values for the general water quality variables were compared with the water quality guidelines in Table 2.1, with an exception of water pH where a range was given. Only the detectable water constituents are in the data tables. The seasonal average values (not all) are presented graphically in Figure 2.1.

Water temperature

The highest water temperature value (29.2°C) was recorded at the inflow during spring, while lowest value (17.42°C) was recorded at the wall/sluices during winter (Appendix Table 1; Figure 2.1). The highest mean value of water temperature (28°C) was recorded during the spring season at the wall/sluices of the barrage, while the lowest value (18°C) was recorded during winter at the wall/sluices (Table 2.1; Figure 2.1).

Oreochromis mossambicus, according to Skelton (2001) can survive in temperatures ranging from 15°C to 42°C. The temperatures of inland waters in South Africa range from 5 – 30°C (DWAF 1996c), and all recorded water temperatures fell within suggested ranges during the study.

Table 2.1: Average seasonal physico-chemical values for the water at the Phalaborwa Barrage (May 2010 – January 2011), n=12.

Water quality parameters	Autumn	SD	Winter	SD	Spring	SD	Summer	SD	Water Quality Guidelines
Water temp. °C	21.55	±0.4	18	±0.7	28.47	±1.2	26.77	±0.3	Temperature should not vary more than 10% from normal (natural) value[2]
Dissolved oxygen (mg/ℓ)	8.44	±0.1	9.13	±0.5	7.32	±0.3	8.1	±0.8	*
Dissolved oxygen (%)	96.32	±1.0	99.04	±3.0	94.49	±5.4	94.82	±1.0	80% – 120% of saturation[2]
pH	7.33 – 7.46		7.31 – 7.46		8.57 – 8.76		8.29 8.91	-	Should not vary by > 5%[2]
Conductivity (EC) mS/m-1	37.07	±0.1	43.37	±3.8	60.03	±0.6	32.4	±0.0	No criteria available
Salinity ‰	0.18	±0.0	0.21	±0.0	0.29	±0.0	0.15	±0.0	0.05‰ or < 0.5‰[2]
TDS mg/ℓ	240.93	±0.4	281.88	±24.4	390.22	±3.9	210.6	±0.0	TDS should not change by >15% from normal cycles[2]
Alkalinity as CaCO3 (mg/ℓ)	64	±4.0	104	14.4	142.67	±16.2	74.67	±2.3	No criteria available
Turbidity in NTU	8.5	±4.8	7.43	±3.9	25.33	±7.5	7.3	±0.6	<25

Notes:
1 - (DWAF 1996a) – South African Water Quality Guidelines: Volume1: Domestic use.
2 - (DWAF 1996c) – South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems.
2 - (BC-EPD 2006) – British Columbia Environmental Protection Division: Water Quality Guidelines
3 - *- No guidelines available

The temperature of water varies with seasons and specific times of the day, thus temperature is constantly changing. When temperature increases it affects a lot of processes, like the amount of oxygen dissolving in water, toxicity of some substances, physiological processes, and it causes stress for aquatic organisms, it also affects the development of aquatic organism (Dallas and Day 2004). The water quality guidelines for aquatic ecosystems in South Africa specify a TWQR whereby water temperature should not be allowed to vary from the background daily average water considered to be normal for that specific site and time of day, by >2°C or > 10%.

Dissolved oxygen

The highest DO value (9.74 mg/ℓ) was recorded at the inflow during winter, while lowest value (7.13 mg/ℓ) was recorded below the wall in spring (Appendix Table 1; Figure 2.1).

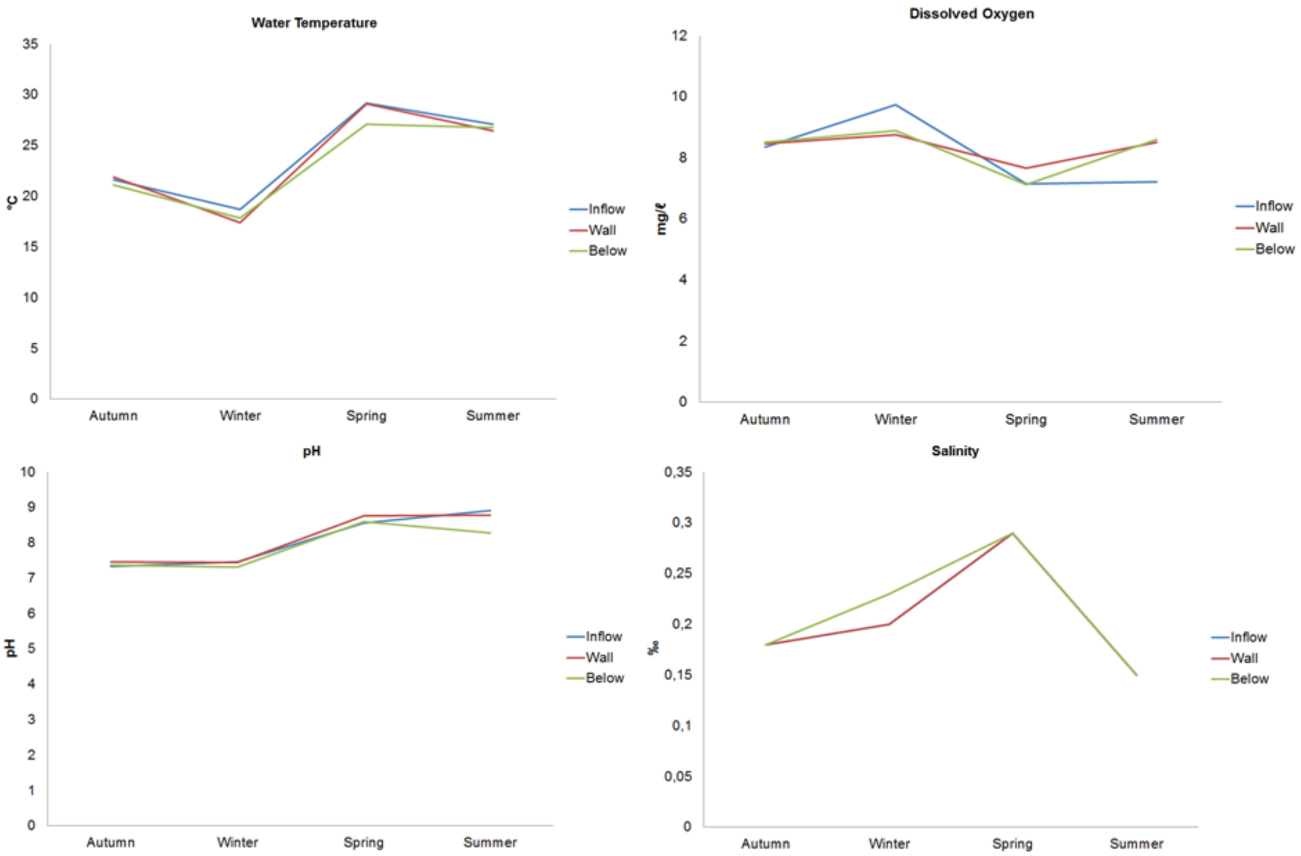

Figure 2.1: The seasonal variation of: water temperature, dissolved oxygen, pH and salinity at three sites at the Phalaborwa Barrage (May 2010 – January 2011), n= 3 per season.

The highest mean value of DO (9.13 mg/ℓ) was recorded during winter, while the lowest mean value (7.32 mg/ℓ) was recorded during spring (Figure 2.1). Water samples for all surveys were taken in the mornings, thus high values during winter were expected because cold water has higher oxygen concentration. While in warm water oxygen is lower thus the decrease during spring. Concentrations of DO vary at different times of the day due to processes like photosynthesis, respiration and changes in temperature (Dallas and Day 2004). Concentrations below 5 mg/ℓ may have adverse effects on the endurance and performance of biological communities, and below 2 mg/ℓ may result in the mortality of most fish (DWAF 1996c). The mean DO concentrations recorded during this study were all above 5 mg/ℓ, thus does not have any adverse effects in the performance and endurance of the biological communities.

Dissolved oxygen levels need to be maintained as oxygen is vital for the survival of aquatic organisms like fish which depend on it for respiration (Chapman 1996). Factors that cause an increase in DO are atmospheric re-aeration, increasing atmospheric pressure, decreasing salinity and temperature, respiration of aquatic biota and decomposition by micro-organisms (Dallas and Day 2004).

pH

The pH values ranged from 7.31 – 8.91, from neutral to slightly alkaline (Appendix Table 1). The lowest pH value was recorded below the wall during winter, and higher pH values were recorded at the inflow during summer (Appendix Table1; Figure 2.1). According to Heath *et al*. (2010), the pH of the Phalaborwa Barrage in the Olifants River neutral to slightly alkaline, the findings in this study were similar with their results. The pH values recorded are within suggested values by DWAF (1996c). The somewhat high pH values recorded in this study may have been increased by biological activity more especially during warmer temperatures.

The pH is largely determined by atmospheric deposition of acid forming substances, human activities (mine drainage, industrial effluents and acid precipitation) and geological processes (Dallas and Day 2004). Small changes in pH are seldom lethal, but can cause other adverse effects like a reduction in growth rate and fecundity due to physiological stress (Dallas and Day 2004).

Salinity

Salinity levels varied from 0.15‰ – 0.29‰ (Appendix Table 1; Figure 2.1). The highest mean value (0.29‰) was recorded during spring while the lowest mean value (0.15‰) was in summer (Table 2.1). The salinity of the surface water fell within the limit for freshwater (<0.5‰) (DWAF 1996c). Evaporation might have contributed to the higher salinity throughout spring, before the summer rains.

Salinity refers to the saltiness of water; and changes in salt concentrations can have adverse effects on aquatic biota such as ecological and microbial processes (Davies and Day 1998; Dallas and Day 2004). Some fish are tolerant to high salinities are defined as euryhaline (e.g. *O. mossambicus*) while those tolerating only limited ranges are referred to as stenohaline (e.g. *S. intermedius*) (Skelton 2001).

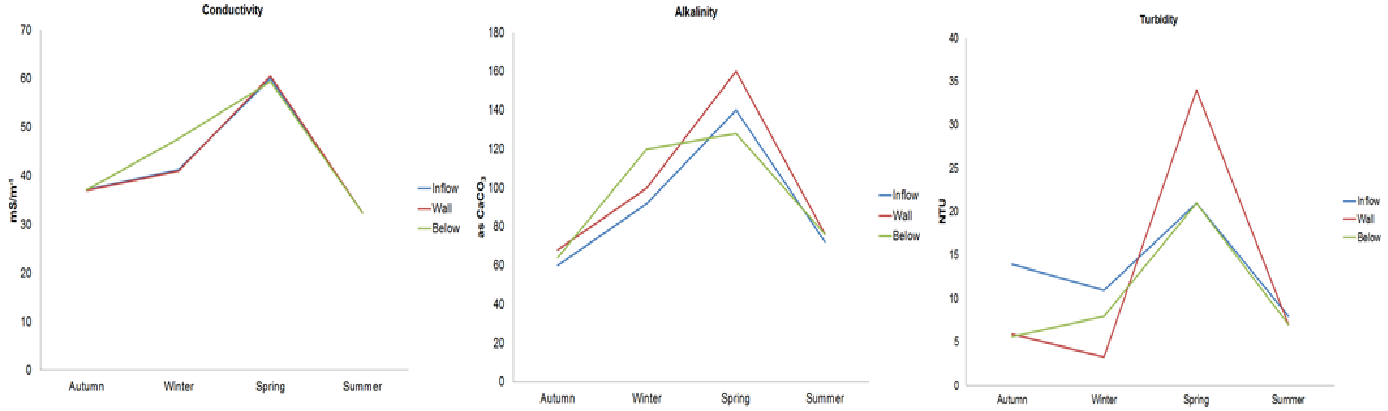

Figure 2.2: The seasonal variation of: electrical conductivity, alkalinity and turbidity at three sites at the Phalaborwa Barrage (May 2010 – January 2011), n= 3 per season.

Electrical conductivity

The highest EC value (60.6 mS/m^{-1}) was recorded at the wall/sluices during spring, while lowest value (32.4 mS/m^{-1}) was recorded at all sites during summer (Appendix Table 1; Figure 2.2). The highest mean value of EC (60.03 mS/m^{-1}) was recorded during spring while the lowest mean value (32.4 mS/m^{-1}) was recorded in summer (Table 2.1). The increase of EC during spring and summer may be due to agricultural activities.

The ability to conduct the electrical current is because of the presence of certain ions like anions and cations because they all carry an electric charge (DWAF 1996c). Thus, the greater the EC in the water the greater the number of various ions present in the water (Dallas and Day 2004). During the spring survey both anions (chloride, sulphates and fluoride) and cations (calcium, magnesium, potassium and sodium) had higher concentrations compared to the other seasons. The one-way ANOVA showed that the EC had a significant variance between seasons (F= 121.06; df= 3; p<0.001).

Alkalinity

Alkalinity concentrations ranged from 60 – 160 mg/ℓ at Phalaborwa Barrage. The highest concentration was recorded in spring (wall/sluices) and the lowest was in winter (inflow) (Appendix Table 1; Figure 2.2). The highest mean value of Alkalinity (142.67

mg/ℓ) was recorded during spring and the lowest (64 mg/ℓ) was recorded during winter. It is no surprise that alkalinity was elevated during spring as it is a known phenomenon that photosynthesis increases when it gets warmer and according to DWAF (1996c), however there is limited information available on the effects of alkalinity.

Turbidity

The highest TDS value (34 NTU) was recorded at the wall/sluices during spring, while lowest value of 7 NTU was recorded two sites (at the wall/sluices and below the wall) during summer (Appendix Table 1; Figure 2.2). The highest mean value of turbidity was recorded during spring (25.33 NTU) and the lowest mean value was recorded during summer (7.3 NTU) (Figure 2.2). Of the turbidity levels recorded three of the four seasons, (autumn, winter and summer) were within the TWQR of aquaculture guidelines for clear water fish species (<25NTU) (DWAF 1996b), with an exception of a spring value (25.33 NTU) recorded at the wall/sluices which had a value above the TWQR. Values ranging between 35 – 40 NTU may have a negative impact of fish that use sight as a hunting mechanism. The elevated turbidity levels during spring (rainy season) may have been caused by rain.

Department of Water Affairs and Forestry (1996b) refers to turbidity as the “cloudiness” of water by suspended particles and is measured in nephelometric turbidity units (NTU). Suspended particles can clog fish gills, reduce growth rates and decrease resistance to diseases (Davies and Day 1998).

Total Dissolved Solids

The highest Total dissolved solids (TDS) value (393.9 mg/ℓ) was recorded at the wall/sluices during spring, while lowest value (210.6 mg/ℓ) was recorded at all sites during summer (Appendix Table 1). The highest mean value of TDS (390.2 mg/ℓ) was recorded during spring and the lowest (281.9 mg/ℓ) was recorded during winter (Table 2.1). Neither of the seasons had readings above the DWAF guidelines for domestic use of 450 mg/ℓ (DWAF 1996a).

Total dissolved solids are defined as the total quantity of dissolved material, organic and inorganic, ionized and unionized, in a water sample (Dallas and Day 2004). They can be increased by domestic and industrial discharges and surface runoff from urban and agricultural areas. Extremely high TDS concentrations may affect the growth of organisms and may eventually lead to death (DWAF 1996c).

2.3.2 Major ions

The seasonal major ions data for the Phalaborwa Barrage are summarized in the Appendix Table 1. Figures 2.3 and 2.4 indicate the seasonal variation in the mean of Anions: chloride, fluoride and sulphate and Cations: calcium, magnesium, potassium and sodium (May 2010 – January 2011).

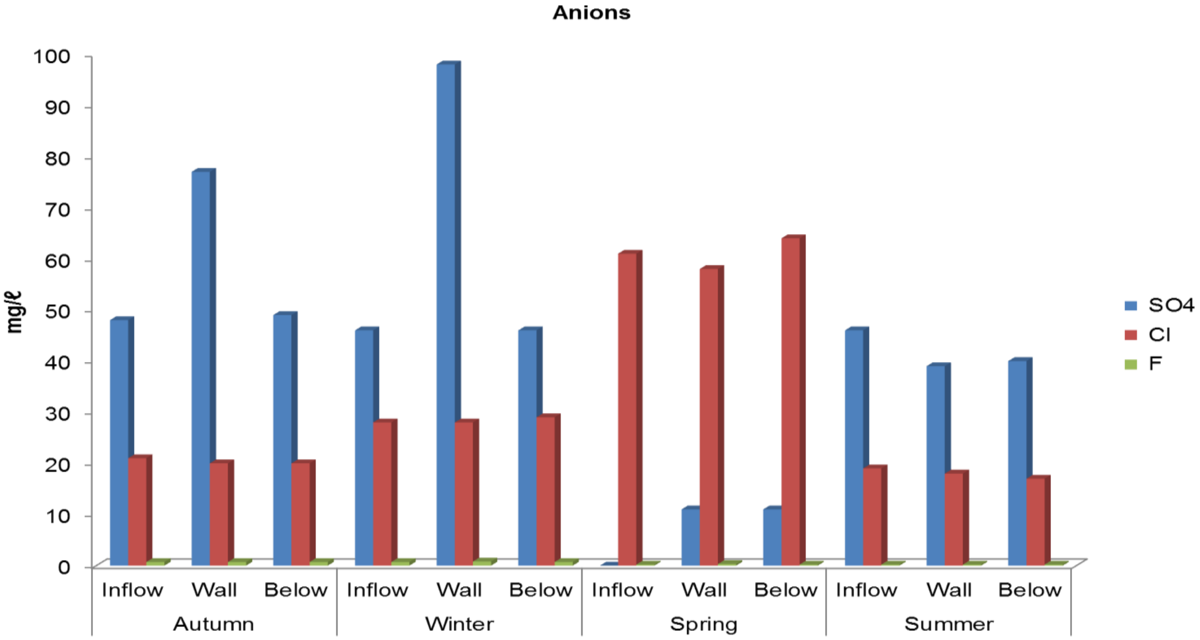

Figure 2.3: The seasonal variation in anion concentrations (SO_4, Cl & F) recorded at the Phalaborwa Barrage (May 2010 – January 2011), n= 3 per season.

Anions: Sulphate, Chloride and fluoride

Sulphate

The highest sulphate value (98 mg/ℓ) was recorded above the wall/sluices during winter, while lowest value (11 mg/ℓ) recorded was above and below the wall/sluices during spring (Appendix Table 1; Figure 2.3). The sulphate levels recorded may indicate natural concentrations as they were in acceptable levels according to values suggested by DWAF (1996a).

Sources of sulphates are usually mines and industries (DWAF 1996a). If sulphate concentrations are increased, they form sulphuric acid, thus affecting aquatic biota, in humans it results in diarrhea (DWAF 1996; Dallas and Day 2004). There is no TWQR available for sulphate in aquatic ecosystems, however, TWQR for domestic use is between 0 – 200 mg/ℓ (DWAF 1996a).

Chloride

The highest chloride value (64 mg/ℓ) was recorded below the wall/sluices during spring, while the lowest value (17 mg/ℓ) was recorded during summer below the wall (Appendix Table 1; Figure 2.3). Most fish can tolerate chloride concentrations of up to 600 mg/ℓ in terms of aquaculture conditions (DWAF 1996b), thus the chloride levels recorded during this study are of no concern as they were below 600 mg/ℓ.

Chloride ions facilitate ionic, osmotic and water balance of body fluids thus important. They are generally not toxic to organisms, but in high concentrations, they can make water unfit for drinking. Chloride ions have no toxic effects on living systems, except if they increase the TDS. Canadian guidelines for chloride were followed with values below 120 mg/ℓ considered to be acceptable (CCME 2012)

Fluoride

The highest fluoride value (0.8 mg/ℓ) was recorded at the wall/sluices during winter, while lowest value (0.2 mg/ℓ) was recorded during spring at two sites (inflow and below the wall/sluices) and summer at all sites (Appendix Table 1; Figure 2. 3). Fluoride is toxic at concentrations above 2.54 mg/ℓ (DWAF 1996c). The fluoride levels at the barrage were in acceptable levels according to values suggested by DWAF (1996c)

Fluoride is often used in insecticides, wood preservatives, chemical industries, water treatment, glass and enamel manufacturing. Exposures over a long period cause fluorosis (DWAF 1996c). The TWQR for aquatic ecosystems is 0.75 – 1.5 mg/ℓ, the CEV is 1.5 mg/ℓ and the AEV is 2.54 mg/ℓ (DWAF 1996c).

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

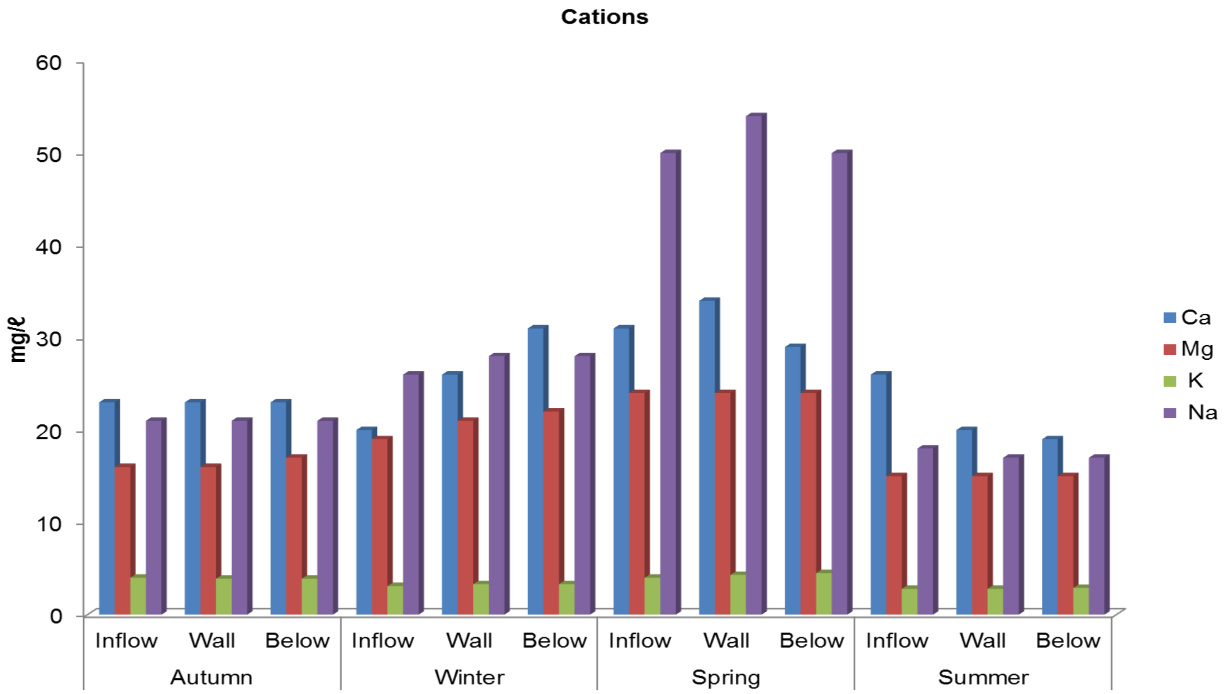

Figure 2.4: The seasonal variation in cation concentrations (Ca, Mg, K & Na) recorded at the Phalaborwa Barrage (May 2010 – January 2011), n= 3 per season.

Calcium

The highest calcium value (34 mg/ℓ) was recorded above the wall/sluices during spring, while the lowest value (19 mg/ℓ) was recorded during summer (below the wall/sluices) (Appendix Table 1; Figure 2.4). The calcium levels at the barrage were in acceptable levels according to values suggested by DWAF (1996c) Table 2.1.

Calcium is one of the major components that cause the hardness of water together with magnesium, therefore the waters that contain a high levels of calcium tend to be hard while the ones that contain less calcium the water is soft (Dallas and Day 2004). There are no TWQR available for calcium for freshwater aquatic ecosystems. According to DWAF (1996a), calcium levels up to 250 mg/ℓ are acceptable for aquatic biota.

Magnesium

The highest magnesium value (24 mg/ℓ) was recorded at all sites during spring, while lowest value (15 mg/ℓ) was recorded at all sampling sites during summer (Appendix

Table 1; Figure 2.4). All the values recorded were below the TWQR range of 50 – 70 mg/ℓ as suggested by DWAF (1996c).

Magnesium is an essential element, it occurs primarily from weathering of rocks containing ferro-magnesium minerals (DWAF 1996c). The solubility of magnesium in water is determined by the pH and the carbonates in the water. Little is known about the effects of magnesium on organisms (Dallas and Day 2004).

Potassium

The highest potassium value (4.0 mg/ℓ) was recorded at the inflow during autumn and spring, while lowest value (2.8 mg/ℓ) was recorded at the inflow and wall/sluices during summer (Appendix Table 1; Figure 2.4). The potassium levels were within the TWQR for domestic use (DWAF 1996a).

The concentrations of potassium in natural water are increased by agricultural runoffs and domestic waste (DWAF 1996a). Sometimes potassium acts as a nutrient and can limit plant growth. There are no TWQR for potassium available for aquatic ecosystems, however the TWQR for domestic use is 0 – 50 mg/ℓ (DWAF 1996a).

Sodium

The highest sodium value (54 mg/ℓ) was recorded above the wall/sluices during spring, while lowest value (17 mg/ℓ) was recorded above and below the wall/sluices the wall during summer (Appendix Table 1; Figure 2.4). Very low concentrations of sodium were recorded throughout the study, lower than the TWQR for domestic use (100 – 200 mg/ℓ) (DWAF 1996a).

The levels of sodium in surface waters are generally low in areas of high rainfall and high in arid areas with low mean annual precipitation. Elevated sodium concentrations can result in nausea and vomiting (DWAF 1996a).

The one-way ANOVA showed no significant difference of all cations in water between the four seasons (F=0.57; df=3; p=0.64).

2.3.3 Nutrients: Inorganic nitrogen and inorganic phosphorus

Nutrients are generally not toxic, however they stimulate eutrophication if present in excess (DWAF 1996c). Criteria are given as narrative, numerical or ranges for constituents such as inorganic nitrogen (nitrate, nitrite, and ammonium) and inorganic phosphorus (ortho-phosphates).

Inorganic nitrogen

Total nitrogen is the sum of nitrate, nitrite, ammonia and ammonium. Higher total inorganic nitrogen levels were recorded during spring than in all other seasons (Appendix Table 1). The values recorded indicated eutrophic conditions according to Dallas and Day (2004), with the highest mean value of 2.75 mg/ℓ (Figure 2.3). The trophic status for three seasons (autumn, winter and summer) at the Phalaborwa Barrage can be classified as oligotrophic with respect to nitrogen. There are agricultural activities close to the barrage, rain may therefore have resulted in runoff of fertilizers which caused eutrophic conditions during spring. The nitrite and ammonia levels recorded for all four seasons were below the detection levels (Appendix Table 1).

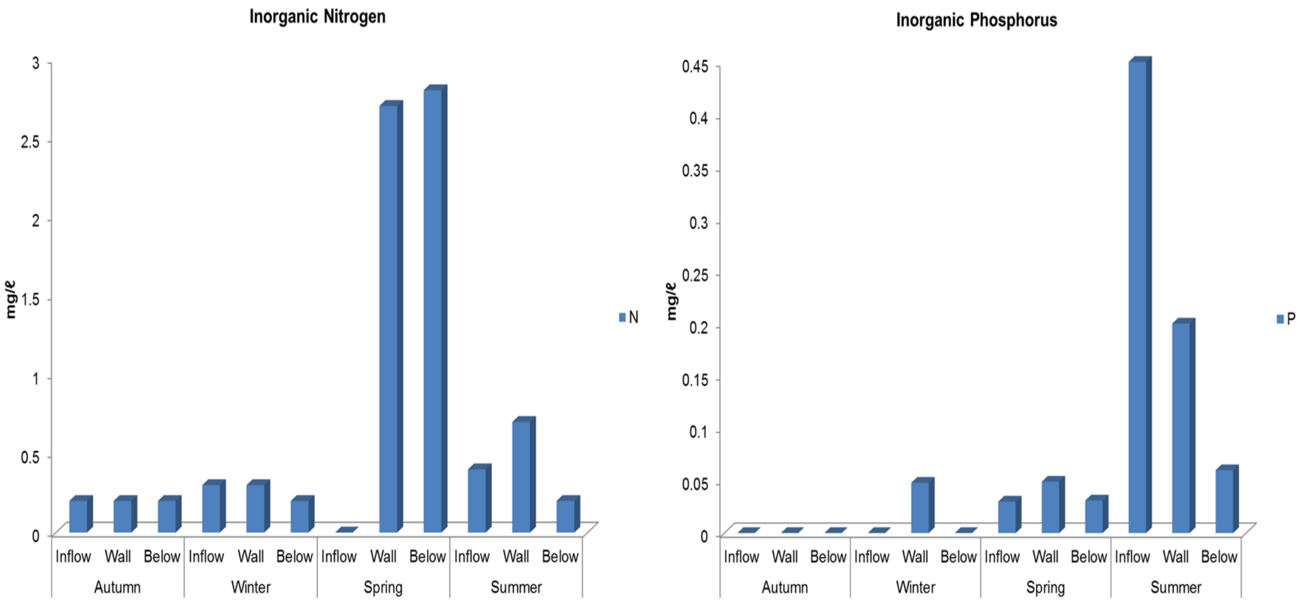

Figure 2.5: The seasonal variation in inorganic nitrogen and inorganic phosphorus concentrations from the Phalaborwa Barrage (May 2010 – January 2011), n= 3 per season.

Inorganic nitrogen refers to all major inorganic nitrogen components (NH_3, NH_4^+, NO_3^- and NO_2^-) and is of concern because of its ability to stimulate aquatic plant growth and algae. Aquatic organisms are most sensitive to toxic concentrations of NH_3 (DWAF

1996c). Nitrate is the more stable of the two forms and is usually far more abundant in the aquatic environment (DWAF 1996c).

Nitrite is naturally, however human activities (industrial metals, dyes, sewage effluents, aquaculture and agriculture) can increase its concentrations in aquatic ecosystems. Higher nitrite levels result in acute anoxia, mortality and loss of equilibrium whereas lower levels result in lower productivity, activity, growth and poor health (Dallas and Day 2004). The TWQR for nitrite in aquaculture is between 0.0625 – 0.25 mg/ℓ. Furthermore this range is considered safe for freshwater fish (DWAF 1996b).

Ammonia is commonly associated with sewage and industrial effluents and it may occur free or in an un-ionized form (NH_3) or as ammonium (NH_4^+). Ammonium has little to no toxicity but it contributes to eutrophication. The acute effects of NH_3 (in fish) are loss of equilibrium, increased breathing rate, cardiac output and oxygen intake, in extreme conditions coma and death may occur. The TWQR for un-ionized NH_3 in the aquatic ecosystem is 0.007 mg/ℓ; CEV is 0.015 mg/ℓ and the AEV is 0.1 mg/ℓ (DWAF 1996c).

Nitrates are abundant in surface waters (< 0.1 mg/ℓ N) because of the photosynthetic activity of the plants in the water (Dallas and Day 2004). Other sources of nitrates are fertilizers, agricultural runoff, etc. It is toxic in humans at high concentrations, as NO_3 binds to fetal haemoglobin to form methaemoglobin (Dallas and Day 2004). Nitrates stimulate the growth of algal blooms and some are toxic to man, animals (livestock) and cause bad odours in the waters (DWAF 1996a). There are no guidelines for NO_3 in aquatic ecosystems, thus TWQR for domestic use (0 – 6 mg/ℓ) and aquaculture (300 mg/ℓ) was used (DWAF 1996b).

The total inorganic nitrogen concentration levels (mg/ℓ) of a water body are used to classify it into a trophic status. The following guidelines were suggested by DWAF (1996c) and Dallas and Day (2004):

- < 0.5 mg/ℓ N-Oligotrophic conditions
- 0.5 – 2.5 mg/ℓ N-Mesotrophic conditions
- 2.5 – 10 mg/ℓ N-Eutrophic conditions
- >10 mg/ℓ N-Hypertrophic conditions

Nitrate

The highest NO_3 value was recorded below the wall (2.8 mg/ℓ) during spring, while lowest value (0.2 mg/ℓ) was recorded at all sites in autumn and one site (below the wall) in winter and summer (Appendix Table 1). The highest mean value of 2.75 mg/ℓ was recorded during the spring survey, while the lowest mean was recorded during autumn at 0.2 mg/ℓ (Figure 2.3). The highest values were recorded during a rainy season (spring) therefore elevated NO_3 values in this study may have been caused by agricultural runoffs.

Inorganic phosphorus

The phosphate (PO_4) concentrations were below detection limit in the surface waters at the Phalaborwa Barrage (Appendix Table 1) but the total inorganic phosphorus were measured in the ICP-OES scan. The highest phosphorus value was recorded at the inflow (0.45 mg/ℓ) during summer, while lowest value (0.03 mg/ℓ) was recorded at the inflow in spring (Appendix Table 1). The highest mean value of phosphorus was recorded during summer (0.24 mg/ℓ) and the lowest during spring (0.04 mg/ℓ) (Figure 2.3). The three seasons (winter, spring and summer) had eutrophic conditions at all three sites. However, in summer, the inflow experienced a hypertrophic condition. Excess nutrient residues can result in toxic algal blooms which can be toxic to fish, clogging their gills thus decreasing respiration (DWAF 1996c).

A One-way ANOVA did not show any significant variance of total inorganic nitrogen and phosphorus during the four seasons (F=0.79; df=3; p=56).

Phosphorus in the sediment

The highest phosphorus concentration (346 mg/kg) in the sediment was detected at the wall/sluices in winter, while the lowest value (86 mg/kg) was recorded below the wall during summer (Appendix Table 3). The overall average for phosphorus in sediment was 167 mg/kg (Table 2.3). Sedimentation and absorption of phosphorus on sediment particles results in a slow, but continuous, loss from the water column (CCME 2004). Sediment, therefore, act as a sink for phosphorus during low-flow periods, but may release phosphorus to the water column under high-flow and/or anoxic conditions (CCME 1999).

Inorganic phosphates such as orthophosphates (H_2PO_4 and HPO_4) are the only inorganic forms of phosphorus which can be directly used by aquatic biota. Orthophosphates are mined to obtain phosphorus for use in agriculture and industry and are common ones which cause eutrophication in the aquatic ecosystems (Dallas and Day 2004). Phosphate naturally enters the water by weathering of rocks and leaching of phosphate rocks as well as decomposition of organic matter (Correl 1998). According to Dallas and Day (2004), human activities can increase the concentration of phosphorus through point and non-point source pollution.

The South African water quality guidelines (DWAF 1996c) recommended the following total inorganic phosphorus (TP) ranges to establish the trophic status of a water body:

- <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

It is a serious concern when nutrient over-enrichment occurs as it decreases the level of DO within the water. This depletion of DO may in turn result in death and elimination of aerobic benthic organisms, in severe cases, fish kills (Rabalais *et al.* 1996).

2.3.4 Metal concentrations in water and sediment

The average water column and sediment metal concentration data for the Phalaborwa Barrage are summarized in Table 2.2 and Table 2.3. Full seasonal data presented in Table 2 (Appendix). The metals and metalloids not detected were beryllium (Be), bismuth (Bi), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), molybdenum (Mo), nickel (Ni), lead (Pb), Lead (Pb), titanium (Ti), vanadium (V) and zinc (Zn). They were included in the analyses but were below the lower detection levels of the laboratory's equipment in all the water samples and were therefore were omitted from the tables and discussion.

In water

Aluminium was recorded in the water column only during the spring survey at two sites (Appendix Table 2) at an average concentration of 0.643 mg/ℓ (Table 2.2). A higher concentration of Al was recorded at the inflow with a value of 0.669 mg/ℓ while a lower value was recorded at the wall/sluices with a value of 0.617 mg/ℓ.

The values exceeded the TWQR and the AEV guidelines by DWAF (1996c), CCME (2012) and US-EPA (2001). When compared to a study done by Oberholster *et al*. (2010) at Loskop Dam, the Al concentrations in this study were higher.

Table 2.2: The mean seasonal metal concentrations (mg/ℓ) of the water column at the Phalaborwa Barrage (May 2010 – January 2011). The shaded values indicate where the guidelines were exceeded (n= 3 per season).

Metal	Autumn	SD	Winter	SD	Spring	SD	Summer	SD	Water Quality Guidelines
Aluminium (Al)	*	*	*		0.64	±0.32	*	*	0.001** (pH>6.5)^{1}; 0.1^{3}
Arsenic (As)	*	*	0.02	±0.0085	*	*	*	*	0.01^{1};0.005^{3};0.01^{4}
Antimony (Sb)	*	*	*	*	0.04	±0.02	*	*	0.01^{4}
Boron (B)	*	*	*	*	0.19	±0.10	*	*	1.5^{3};1.2^{2}
Barium (Ba)	0.03	±0.0006	0.04	±0.003	0.05	±0.008	*	*	0.7^{4}
Iron (Fe)	0.09	±0.03	0.05	±0.03	0.41	±0.061	*	*	Fe vary <10% background conc. ^{1} 0.3^{3}
Manganese (Mn)	0.04	±0.0078	0.04	*	0.026	±0	*	*	0.18***^{1};<1.3^{2}
Selenium (Se)	*	*	*	*	0.11	±0.06	*	*	0.002^{1}; 0.001^{3}
Tin (Sn)	*	*	*	*	*	*	0.20	±0.023	No criteria- only for organo-tin
Strontium (Sr)	*	*	*	*	0.17	±0.172	0.10	±0.035	4.0^{4}

Notes:
*– Below detection levels.
** – pH dependent.
*** – Water Hardness dependent.
References:
1 – (DWAF 1996c) South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems.
2 – (BC-EPD 2006), – British Columbia Environmental Protection Division: Water Quality Guidelines.
3 – CCME (2012) – Canadian Council of Ministers of the Environment: Water Quality Guidelines – Aquatic Life.
4 – (US-EPA 2001) – United States Environmental Protection Agency: Water Quality Guidelines – Aquatic Life.

These Al concentrations are thus of great concern as they have exceeded the Acute Effect Values for aquatic ecosystems (DWAF 1996a), if they persist even for a short while or at a high frequency they can cause death and disappearance of sensitive species immediately.

Arsenic was detected in the water during winter only at one site (below the wall) with a value of 0.02 mg/ℓ (Appendix Table 2; Table 2.2). In a study done by Jooste *et al*. (2013), in the Flag Boshielo Dam, they recorded lower As concentrations in the water than those in this study. Arsenic was above the CEV values suggested by DWAF (1996c), CCME (2012) and US-EPA (2001).

Table 2.3: The mean concentrations of phosphorus and metals in sediment collected (n=6) from the Phalaborwa Barrage (July 2010 and January 2011). The shaded values indicate where the guidelines were exceeded, n=3 for two seasons.

Parameters (mg/kg)	Average	SD	Sediment quality guidelines
Phosphorus	167	±54.01	No guidelines
Aluminium	3942	±2893	No guidelines
Arsenic	5.5	±4	5.9 mg/kg (dry weight)
Antimony	1.5	±1	No guidelines
Boron	182	±178	No guidelines
Barium	194	±100	No guidelines
Cadmium	11	±6	0.6 mg/kg (dry weight)
Chromium	45	±8	37.3 mg/kg (dry weight)
Cobalt	13	±52	No guidelines
Copper	13	±12	35.7 mg/kg (dry weight)
Iron	19544	±12360	No guidelines
Lead	6	±3	35.0 mg/kg
Manganese	297	±275	No guidelines
Nickel	35	±29	No guidelines
Silver	79	±33	No guidelines
Strontium	17	±19	No guidelines
Tin	14	±7	No guidelines
Titanium	673	±767	No guidelines
Vanadium	79	±84	No guidelines
Zinc	106	±76	123.0 mg/kg (dry weight)

*CCME (1999): Canadian Council of Ministers of the Environment: Sediment Quality Guidelines- aquatic life.

Antimony was recorded in the water during the winter survey only at all the sites (Appendix Table 2) with a mean value of 0.04 mg/ℓ (Table 2.2). A higher value was recorded at the inflow with a value of 0.07 mg/ℓ, while a lower value of 0.032 mg/ℓ was recorded at the wall/sluices. When compared to a study done by Jooste *et al.* (2013) at Flag Boshielo Dam, the mean Sb concentration in this study was higher. The values recorded in the water column at the barrage for Sb were above the TWQR of 0.01 mg/l (US-EPA 2001).

Boron concentrations were only detected in the water samples during the spring survey at all the sites (Appendix Table 2). The mean value was 0.194 mg/ℓ (Table 2.2). A higher value was recorded at the inflow while a lower value was recorded below the wall with values of 0.257 mg/ℓ and 0.152 mg/ℓ respectively (Appendix Table 2). The mean concentration from Flag Boshielo Dam (Jooste *et al*. 2013) was lower compared this study. The values recorded at all the sites were below the suggested guidelines by the BC-EPD (2006) and the Canadian guidelines CCME (2012).

Barium was recorded in the water during autumn and spring (all sites) and winter (two sites) (Appendix Table 2). The highest Ba value (0.062 mg/ℓ) was recorded at the wall/sluices during spring, while lowest value (0.032 mg/ℓ) at the inflow during autumn (Appendix Table 2). Mean concentrations of 0.03 mg/ℓ (autumn), 0.04 mg/ℓ (winter) and 0.05 mg/ℓ (spring) were recorded (Table 2.2). The mean concentration of Ba was higher (0.053 mg/ℓ) from Flag Boshielo Dam compared to this study (Jooste *et al*. 2013). The values detected were below the guideline value suggested by the US-EPA (2001).

Iron concentrations were detected during autumn (all sites), winter and spring (two sites) in the water (Appendix Table 2). The highest Fe concentration in the water column was recorded during spring at the inflow with a value of 0.449 mg/ℓ, while the lowest during winter at the inflow with a value of 0.034 mg/ℓ. The highest mean value (0.41 mg/ℓ) was recorded during spring while the lowest mean value (0.05 mg/ℓ) in winter (Table 2.2). When compared to a study done by Jooste *et al*. (2010) at Flag Boshielo Dam, the mean Fe concentration in this study was lower with an exception during spring (0.41 mg/ℓ). Iron concentrations recorded in this study were above guidelines by DWAF (1996c) and Canadian guidelines (CCME 2012).

Manganese was detected during three seasons (autumn, winter and spring) (Appendix Table 2) of sampling, the highest mean concentration was recorded during winter with a value of 0.038 mg/ℓ while the lowest mean was during spring with a value of 0.026 mg/ℓ (Table 2.2). Manganese was only recorded at two sites in autumn (inflow and below wall), one site during winter (below wall) and two sites during spring (inflow and at the wall/sluices). The highest Mn concentration was below the wall/sluices (0.043 mg/ℓ) in autumn and the lowest during spring at the inflow and at the wall/sluices (Appendix

Table 2). Mean Mn concentration of was lower (0.027 mg/ℓ) from Flag Boshielo Dam compared to this study with an exception during spring (Jooste *et al*. 2013) All the values recorded were below the suggested water quality guidelines (DWAF 1996c; BC-EPD 2006).

Selenium concentrations were only detected during spring in the water at all sampling sites, at an average value of 0.11 mg/ℓ (Table 2.2). The highest concentration of Se was recorded at the inflow while the lowest value was recorded at the wall/sluices with values of 0.123 mg/ℓ and 0.1 mg/ℓ respectively (Appendix Table 2). Selenium concentrations in the water were above the South African aquatic ecosystems guidelines (DWAF 1996c).

Strontium was only detected in spring and summer at all sampling sites in the water (Appendix Table 2). The highest mean value for strontium was recorded during spring while the lowest recorded in summer with values of 0.172 mg/ℓ and 0.1 mg/ℓ respectively (Table 2.2). The highest value recorded was 0.18 mg/ℓ at the wall/sluices during spring while the lowest (0.08 mg/ℓ) were at the wall/sluices and below the wall (Appendix Table 2). The mean concentration from Flag Boshielo Dam (Jooste *et al.* 2013) was found to be lower (0.15 mg/ℓ) compared this study. The concentrations of Sr in the water column were below the suggested water quality guidelines of the United States (US-EPA 2001).

Tin was only detected during the summer survey in the water at all the sampling sites, with an average value of 0.19 mg/ℓ (Table 2.2). Highest tin concentration was recorded at the inflow and at the wall/sluices (0.21 mg/ℓ) and the lowest (0.17 mg/ℓ) below the wall (Appendix Table 2). When compared to a study done by Jooste *et al*. (2013) at Flag Boshielo Dam, the mean Sn concentration in this study was higher. There are no criteria for Sn: only for organo-tins.

In sediment

Aluminium was recorded at all the sampling sites during the two seasons (Appendix Table 3), with an average of 3942 mg/kg dry weight (Table 2.3). The highest Al value was recorded at the wall/sluices during summer, while the lowest value was detected during winter with values of 9200 mg/kg and 1568 mg/kg respectively (Appendix Table 3).

Arsenic was detected at two sites during winter with an overall mean value of 5.5 mg/kg dry weight (Table 2.3). The highest value was detected above the wall/sluices and the lowest at the inflow with values of 8 mg/kg and 3 mg/kg respectively (Appendix Table 3). Only the As concentration level at the wall/sluices was above the suggested guidelines by the Canadian sediment quality guidelines (CCME 1999).

Antimony was recorded during winter only at two sites with a mean of 1.5 mg/kg dry weight (Table 2.3). A higher value was detected below the wall with a value of 2 mg/kg while a lower value was recorded at the inflow with a value of 1 mg/kg (Appendix Table 3). There are no guidelines available for Sb concentrations in sediment.

Boron was recorded during two surveys at all sites in winter and one site in summer (Appendix Table 3). The mean value was 182 mg/kg dry weight (Table 2.3). The highest value was recorded during winter above the wall/sluices and the lowest value in summer at the inflow with values of 248 mg/kg and 42 mg/kg respectively. There are no guidelines for B in sediment (Appendix Table 3).

Barium concentrations were detected during both sampling seasons at all the sites, with an overall mean value of 194 mg/kg dry weight (Table 2.3). The highest concentration detected was 414 mg/kg during winter at the wall/sluices, while the lowest concentration was detected during summer with a value of 2 mg/kg at the wall/sluices (Appendix Table 3). There are no guidelines available for Ba in sediment.

Cadmium was detected during the summer survey at the inflow and below the wall (Appendix Table 2). The mean concentration value was 11 mg/kg dry weight (Table 2.3). A higher value was detected at the inflow with a value of 12 mg/kg while a lower value was detected below the wall with a value of 10 mg/kg (Appendix Table 3). The Cd levels were over ten times the Canadian sediment quality guidelines by the CCME (1999).

Chromium was detected during two seasons at all sites, with a mean value of 45 mg/kg (Table 2.3), a higher value was detected during winter below the wall with a value of 177 mg/kg, while a lower value was detected at the inflow with a value of 3 mg/kg

(Appendix Table 3). The concentrations recorded for Cr were above the suggested Canadian quality sediment guidelines (CCME 1999) with an exception of the values recorded at the inflow and below the wall during winter.

Cobalt was detected during the two seasons at all sampling sites with a mean value of 13 mg/kg dry weight (Table 2.3). The highest value recorded for Co was 26 mg/kg during winter at the wall/sluices, while the lowest value was recorded at the inflow during winter with a value of 4 mg/kg (Appendix Table 3). There are no Co guidelines available for sediment in aquatic ecosystem (see Chapter 4 for more detailed effects).

Copper was detected during the two seasons at all the sampling sites with a mean value of 13 mg/kg (Table 2.3). The highest and lowest concentrations detected were recorded during winter with values of 37 mg/kg and 5 mg/kg respectively (Appendix Table 3). The recorded Cu values were below Canadian sediment quality guidelines (CCME 1999).

Iron was recorded for both the surveys conducted, with a mean value of 19544 mg/kg (Table 2.3). A much higher value was detected during summer at the inflow with a value of 36600 mg/kg, while a lower value during winter at the inflow with a value of 4600 mg/kg (Appendix Table 3). There are no aquatic guidelines available for Fe in sediment

Nickel was recorded during two seasons at all the sampling sites with a mean value of 35 mg/kg dry weight (Table 2.3). The highest and lowest concentrations detected were recorded during winter above the wall/sluices and at the inflow with values of 90 mg/kg and 12 mg/kg respectively (Appendix Table 3). There are no sediment quality guidelines for Ni

Lead it was detected only during winter at all the sampling sites, with a mean value of 6 mg/kg dry weight (Table 2.3). The highest concentration was recorded at the wall/sluices and the lowest below the wall with values of 9 mg/kg and 4 mg/kg respectively (Appendix Table 3). The Pb concentrations were below the Canadian sediment guidelines (CCME 1999). Regardless of the origin of Pb in sediment, aquatic organisms may be adversely affected by exposure to elevated levels (CCME 1999).

Manganese was detected at all sites during sampling seasons with an overall average of 297 mg/kg dry weight (Table 2.3). The highest and lowest values were recorded during winter at the wall/sluices (852 mg/kg) and at the inflow/sluices (129 mg/kg) (Appendix Table 3). There are no guidelines for Mn in sediment.

Selenium was below detectable levels.

Silver was detected during winter at all the sampling sites with a mean value of 79 mg/kg (Table 2.3). The highest value recorded for Ag was 102 mg/kg at the wall/sluices while a lower value of 41 mg/kg was recorded at the inflow (Appendix Table 3). There are no guidelines available for Ag in water and/or sediment.

Strontium was detected during both surveys at an average value of 17 mg/kg dry weight (Table 2.3). The highest value was recorded during summer at the wall with a value of 36 mg/kg, while the lowest during winter at the wall/sluices with a value of 6 mg/kg (Appendix Table 3). There are no sediment quality guidelines for strontium.

Tin was detected in summer at one site above the wall/sluices with a value of 14 mg/kg (Appendix Table 3). There are no sediment quality guidelines available for Sn.

Titanium had a mean value of 673 mg/kg dry weight (Table 2.3). The highest concentration was detected during summer at the inflow (1830 mg/kg), while the lowest (38 mg/kg) during winter at the inflow (Appendix Table 3). There are no guidelines available for Ti in sediment.

Vanadium was only detected during the two seasons at all sampling sites, with a mean value of 79 mg/kg dry weight (Table 2.3). The highest concentration value recorded was 212 mg/kg during summer at the inflow, while the lowest value was at the inflow during winter with a value of 8 mg/kg (Appendix Table 3). There are no guidelines available for vanadium in sediment.

Zinc was detected for both seasons at all sampling sites with an average value of 106 mg/kg dry weight (Table 2.3). The highest value was detected during winter at the wall/sluices with a value of 185 mg/kg while the lowest value was detected during summer below the wall with a value of 14 mg/kg (Appendix Table 3). The values recorded during winter at all sampling sites were above the suggested Canadian sediment quality guidelines (CCME 1999), while the concentrations recorded in summer were within the guidelines.

A one-way ANOVA shows no significant difference of all metal constituents in water between the four seasons (F=0.05; df=3; p=0.98) as well as sediment during the two sampling seasons (F=1.62; df=1; p=0.21).

See Chapter four (4) for detailed effects of metals on aquatic organisms and humans.

2.4 SUMMARY OF THE WATER AND SEDIMENT QUALITY

System variables

System variables (pH, water temperature and dissolved oxygen) at the barrage were within the TWQR for aquatic ecosystems suggested by DWAF (1996c). Throughout the study the barrage showed pH values that were alkaline, and the high pH values may be attributed to TDS and sulphate concentrations. As expected winter had the lowest temperatures however the highest temperatures were recorded during spring. The lowest DO concentration was recorded during spring while the highest values were during winter which is known to be normal, as DO is higher in colder waters than in warmer waters. Dissolved Oxygen concentrations were at acceptable levels (above 5 mg/ℓ) according to DWAF (1996c). The turbidity during the four seasons was within the value suggested by DWAF for aquaculture of <25 NTU (DWAF 1996b) except during winter at the wall/sluices having a value of 34 NTU, which is above the recommended <25 NTU. The TDS, salinity and EC had higher values during spring and lower values in summer, however they were all within TWQRs and acceptable for aquatic ecosystems and adaptable for euryhaline and stenohaline fish species.

Major ions

The anions, fluoride and sulphate values were higher in winter than the other seasons, while chloride was higher in spring. The cations (calcium, magnesium, potassium and sodium), were all higher in spring than the rest of the seasons. All the values were within the TWQR values as suggested by DWAF (1996c) for aquatic ecosystems and domestic use. The concentrations detected may have been due to weathering of rocks while sulphates concentrations were probably increased by mining activities taking place upstream.

Nutrients

Total inorganic nitrogen (nitrite, nitrate and ammonia) levels were elevated in spring and eutrophic conditions were recorded, while oligotrophic conditions were recorded during the other seasons (autumn, winter and summer). Total inorganic phosphorus (ortho-Phosphate and phosphorus) values were much higher in summer than during the other three seasons; eutrophic conditions were recorded for phosphorus during three seasons and a hypertrophic condition during the summer at the inflow. The elevated levels caused eutrophication conditions at the sampling sites, which could result in an increase in algal growth and aquatic macrophytes. When considering mean concentration values for both total nitrogen and phosphorus for the four surveys, depending on the season, for total nitrogen the barrage experienced oligotrophic, (autumn, winter and summer) and eutrophic conditions (spring), while total phosphorus showed eutrophic conditions (winter, spring and summer).

Metals

The concentrations of metals varied significantly during each season. Aluminium was detected during the spring season only, the values were above the AEV as suggested by DWAF (1996c) and the CCME (2012). Antimony was detected during spring at all the sites, all recorded values exceeded the suggested aquatic ecosystem guideline by US-EPA (2001). During the study, the As concentrations were recorded during winter, the values were above the TWQR, however lower than the CEV suggested by DWAF (1996a), US-EPA (2001) and CCME (2012). Barium was recorded during the four seasons but the values were below the guidelines suggested by US-EPA (2001). Boron was detected only during the spring survey, and the values were below the guidelines suggested by CCME (2012) and BC-EPD (2006). Iron and Mn were recorded during three seasons (autumn, winter and spring), Fe concentrations were above suggested water quality guidelines while Mn concentration levels were within the TWQR suggested by DWAF (1996a), CCME (2012) and US-EPA (2001). Selenium was only detected during spring and the values were above the suggested TWQR for aquatic ecosystems by DWAF (1996a). Strontium was detected in spring and summer but the concentrations were below the water quality guidelines suggested by US-EPA (2001), whereas Sn was detected in summer only.

In the sediment samples the only metals above the suggested guidelines by the CCME (1999) were; Cd and Cr, while Cu was within the suggested guidelines except above the wall/sluices during summer. Zinc concentrations exceeded the suggested guidelines during the winter season, while during summer all concentrations recorded were within the guidelines.

2.5 CONCLUSIONS

Water temperatures were within normal ranges and DO levels were within the TWQR throughout the study. The pH levels were alkaline during the study which may be due to increased biological activity. Turbidity levels recorded during this study were within the TWQR for clear water fish species (<25NTU) except during the spring survey at the wall/sluices. The TDS, salinity and EC, were within TWQRs and acceptable for aquatic ecosystems and suitable for euryhaline and stenohaline fish species. Nitrogen showed eutrophic and oligotrophic conditions, while phosphorus showed eutrophic and hypertrophic and throughout the study.

The levels of all anions were low. Chloride concentration was within the TWQR for aquaculture and fluoride concentration was within the TWQR for aquatic ecosystems while sulphate was in the acceptable level for domestic use (DWAF 1996a). All the cation concentration levels were within the TWQRs for domestic use, irrigation and livestock watering, the slightly elevated levels can be due to geological formations.

The metals that were detectable in the water samples were; Al, As, Ba, B, Fe, Mn, Sb, Se, Sr and Sn. Aluminium and arsenic are elements which have carcinogenic properties (IARC 2012) and are highly sensitive to pH and temperature changes, and since they are above the suggested TWQR they should be closely monitored as they can cause irreversible damage to the aquatic ecosystem. While in sediment all the metals and metalloids were detected with the exception of Se. In the water column, the metals and metalloids that were detected above the TWQR for aquatic ecosystems suggested by

DWAF (1996c), CCME (2012) and US-EPA (2001) were; Al, As, Fe, Sb and Se. Barium, B, Mn and Sr were within suggested water quality guidelines. In the sediment only Cd and Cr were above the Canadian sediment quality guidelines (CCME 1999). Furthermore, copper concentrations were within the Canadian sediment quality guidelines except during winter at the wall/sluices.

The hypothesis that the Phalaborwa Barrage is polluted due to the various anthropogenic activities taking place above the barrage and the Lower Olifants was confirmed in this study, supported by the water and sediment data. In addition, it has provided an indication of the status of the water and sediment quality from the Phalaborwa Barrage. The water from the Phalaborwa Barrage is mainly used for industrial, agricultural and domestic purposes around the Phalaborwa area. Most of the higher concentrations were recorded at the inflow, suggesting that most of the pollutants are from upstream of the barrage as it is more impacted. Although some concentrations were elevated most of the water and sediment constituents were within the TWQRs. This could be due to the dilution effect from the Blyde River. Nevertheless, metals recorded above the TWQRs should be monitored closely as some of them are carcinogenic (see Chapter 4), they can pose health effects for both aquatic biota and humans.

CHAPTER 3

FISH HEALTH ASSESSMENT INDEX AND PARASITE INDEX

3.1 INTRODUCTION

Chronic exposure of aquatic organisms (e.g. fish) to stressful pollutants have an impact on the immune, endocrine, metabolic and the nervous system, which are important for the normal/proper functioning of an organism (Handy *et al.* 2003). Morphological, physiological and genetic changes in organisms have been linked to certain environmental stressors, and these changes can be used as indicators of adverse environmental conditions (Kotzè *et al.* 1999). The adverse changes that occur in organisms are caused by pollutants; these pollutants are mostly found in food sources (biomagnification) and the surrounding environment (bio-concentration). Fish are close to the top of the aquatic food web and are usually used as bioindicators of aquatic ecosystems. Furthermore, they are easy to catch and identify and common in most river systems. Nevertheless, different species have different tolerances to pollution.

Certain responses to pollution are visible with the naked eye, but some can only be observed through microscopic examination of tissues such as the skin, gills, liver and the gut. Adverse effects of pollutants on gills include secretion of excess mucus, hemorrhage and oedema. In addition, on the skin the following may be visible: lesions, inflammation, ulcers, dermal ulcerations, epithelial erosion, fin rot and hypo-secretion of mucus (Harper and Wolf 2009). According to Harper and Wolf (2009), liver size, coloration and lipidosis can also indicate stress conditions in fish. Monitoring metals is important for the health of the organisms, the potential impact on humans (by consuming contaminated fish) and as indicators of metal accumulation in a system (Heath *et al.* 2004). Thus, fish is suitable for the evaluation of toxic effects by determining the accumulation of pollutants in their tissues, as they are sensitive to environmental changes. According to van Dyk (2003), early toxic effects of pollution may be evident on fish tissues before significant changes can be identified in fish behavior.

There are numerous methods that have been used over the past years to assess the health of fish however most of them are expensive and cannot be applied to field studies. Goede and Barton (1990) and Goede (1992) developed an empirical necropsy-based system of organ and tissue indices to provide a fish health and condition assessment for fish populations as an alternative. The method was later improved by Adams *et al.* (1993), developing a quantitative Health Assessment Index (HAI), with the intention to reduce limitations of the necropsy-based system. Depending on the degree of abnormalities, numerical values are assigned to fish tissues and organs assessed. The values of all fish are summed for an index value for that locality. When the index increases it indicates poor water quality, when it decreases it shows better water quality (Watson *et al.* 2012).

In South Africa, the HAI has been widely used in various studies on the Olifants River System (Avenant-Oldewage *et al.* 1995; Heath *et al.* 2004; Madanire-Moyo *et al.* 2012a, Jooste *et al*. 2013). The fish species that were used include *Clarias gariepinus*, *Oreochromis mossambicus* and *Labeo* species (Marx 1996; Robinson 1996; Watson 2001; Luus-Powell 1997; Ramollo 2008; Watson *et al.* 2012, Jooste *et al*. 2013). The HAI was also tested in the Vaal River System on *C. gariepinus* (Crafford and Avenant-Oldewage 2009). The HAI was used to test the impact of mine effluents on the health of fish in the lower Ga-Selati River (Limpopo Province) with *C. gariepinus* and *O. mossambicus* as indicator species (Jooste *et al.* 2004). It should be noted that the exact cause of pollution cannot be determined by using the HAI, however it can be used in assessing the health of fish and consequently the health status of the aquatic ecosystem (Heath *et al.* 2004).

Human activities have adverse effects on the aquatic environment thus affecting fish health ultimately resulting in parasitic infections and diseases (Poulin 1992). Fish parasites reflect the habits of the fish and their interactions, they indicate various biological aspects of their hosts, such as diet and migration (Williams *et al.* 1992). They can give a good indication of the status of the environment they reside in (Marcogliese and Cone 1997). Therefore they are complementary to fish health assessment as indicators of dysfunctional ecosystems. There has been an increasing interest in fish parasites as indicators (Sures 2001) and how they respond to pollution. Furthermore, many papers have been published that concentrate on the relationship between pollution and parasitism in the aquatic environment (MacKenzie *et al.* 1995; Sures *et al.* 1999; Sures 2001; Madanire-Moyo *et al*. 2012b).

3.2 HEALTH ASSESSMENT INDEX

The fish Health Assessment Index (HAI) used was developed in the United States of America as a necropsy-based condition assessment (Goede and Barton 1990) and quantified by Adams *et al.* (1993). The HAI is based on the assumption that fish in a physiological good condition and are not experiencing strain from the environment will have organs and other body structures in good condition. Biochemical and physiological alterations in the environment, if severe or prolonged, will lead to adverse effects such as changes in external and internal organs and blood chemistry (Hinton and Laurén 1990; Roberts 2001). When normal appearances start changing it is considered as an indication of problems within the fish population (Adams *et al*. 1993). The HAI assesses the external condition of fish (any abnormalities of the skin, fins, opercules and eyes) as well as internal organs by assigning values based on the abnormality or damage observed, i.e. abnormal conditions can assume values of 10, 20 or 30 depending on the severity of the conditions (Appendix Table 4) (Adams *et al.* 1993). Watson (2001), developed a colour chart to limit the subjective nature of colour assessments done during evaluation of the liver, bile colour and spleen.

The aim of this chapter was to evaluate the health and condition of fish by applying the HAI and condition factor on two fish species in the Lower Olifants River System.

The HAI was selected for this study and applied on *Oreochromis mossambicus* (Mozambique tilapia) and *Schilbe intermedius* (Silver catfish) from the Phalaborwa Barrage.

3.3 PARASITE INDEX (PI)

Parasites are an indigenous component of the food chain (Landsberg *et al.* 1998). They are important as they form an integral part in the biology of fish. Parasites may not harm fish, however when they occur in large numbers they cause adverse effects on the host and may even result in death (Ewald 1995). They are divided into ectoparasites and endoparasites, and they are assessed according to the hypothesis that ectoparasites are more susceptible to pollution than endoparasites as they are directly exposed to polluted water, depending on the type of pollution (Avenant-Oldewage 1998; Luus-Powell *et al.* 2005; Watson *et al.* 2012). Parasites were included in the original fish HAI (Adams *et al.* 1993) but recorded as absent (given a value of zero) or present (given a value of ten). Throughout fish health studies on the Olifants River, it was clear that endo- and ectoparasites were affected differently by metal pollution, as a result it lead to the development of a Parasite Index (PI) within the South African HAI protocol (Marx 1996; Robinson 1996; Luus-Powell 1997). The PI has been refined to differentiate between the number of ecto- and endoparasites, usually the numbers of endoparasites are higher in a single fish than ectoparasites. Furthermore, the inverted PI (IPI) was developed based on the premise that the count of ectoparasites (being more directly exposed to pollutants) adds to a higher HAI thus their numbers may reflect fish health and water quality (Crafford and Avenant-Oldewage 2009).

3.4 MATERIALS AND METHODS

3.4.1 Health Assessment Index

Ten fish of each species were collected seasonally from the Phalaborwa Barrage by means of gill nets. On the boat, visible parasites from the external surface of fish were removed and placed in glass vials containing water from the barrage. The fish were transported to the field laboratory in Phalaborwa and transferred to large aerated holding tanks containing dam water to minimize stress. One fish was selected at a time to assess the health with the aid of the HAI. Firstly, skin smears were made from the fish using glass slides, and checked for ectoparasites using a stereo microscope. Blood was drawn from each specimen using a medical syringe and was used to fill two capillary tubes; one end of the capillary tube was plugged using commercial Critoseal^{TM}

clay. The blood samples were centrifuged in a haematocrit centrifuge (Model: KHT-400) for approximately five minutes separating the blood in plasma and red blood cells to obtain the haematocrit (Hct) reading. The Hct was measured by a haematocrit reader and recorded. The fish were weighed and measured for total and standard length. The fish were then examined externally using the HAI field score sheet (Appendix Table 4) and data was recorded on HAI data sheets. Fish were sacrificed by severing the spinal cord prior to dissection. The fish were dissected; the gills, eyes and the gut of the fish were removed and placed in separate petri-dishes containing distilled water. All internal organs (liver, spleen, kidneys, haematocrit and hindgut) were assessed with the aid of a colour chart developed by Watson (2001) and values were assigned to each organ according to the HAI field score sheet (Jooste *et al.* 2004).

Calculation of the Health Assessment Index

Variables of the HAI are presented with a value ranging from 0 – 30, depending on the condition of the organs assessed (Appendix Table 4). If there were any abnormalities found in the internal and external variables they were given a value (Appendix Table 4). The HAI for a sample population was calculated by adding all individual HAI values and dividing it by the total number of fish examined, the standard deviation was calculated as proposed by Adams *et al.* (1993).

3.4.2 Parasites

As mentioned previously, immediately after the fish were removed from the gill nets, macroscopic examinations for mobile ectoparasites were done on the boat. The parasites found were recorded and kept in small glass containers filled with water from the site for further identification at the temporary field laboratory. Furthermore the fish from which the parasites were retrieved from were marked and placed in holding tanks filled with water from the sampling site. At the field laboratory, skin smears by means of glass slides were done from each fish and scrutinized for ectoparasites (monogeneans and copepods), after the fish were sacrificed, opened ventrally, the body cavity and mesenteries were examined for parasites.

The gills, eyes, swim bladder and urinary bladder were placed in separate petri-dishes containing distilled water, while the gut (alimentary canal and associated organs) was placed in petri-dishes containing saline solution and examined individually for parasites. Muscles were also scrutinized for encysted parasites.

Fixation and preservation of parasites

Monogeneans were fixed in alcohol formalin acetic acid (AFA), mounted individually on glass microscope slides using glycerine jelly and some preserved in 70% ethanol. 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 70% ethanol. Cestodes were fixed and stored in 4% warm buffered formaldehyde. Nematodes were fixed in glacial acetic acid and preserved in 70% ethanol. Copepods were fixed and preserved in 70% ethanol for further identification.

Preparation of whole mounts and identification of different parasites were done in the laboratory where specimens were stained either with Horen's Trichhome™ or Aceto Carmine™ solution. When overstained, they were placed in 2% hydrochloric acid (HCl) water 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 labeled. Nematodes were cleared with lactophenol and mounted without staining (temporary mount). All parasites were micrographed with the aid of a Wild™ stereo microscope or Olympus™ compound microscope.

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 terms which were used for this study were suggested by the American Society of Parasitologists (Bush *et al.* 2001), the terms include prevalence (expressed in percentages), mean intensity and mean abundance where:

Prevalence = number of infected individuals of a host species divided by the number of hosts examined, expressed in percentage,

Mean intensity = total number of a particular parasite species divided by the number of infected hosts,

Mean abundance (relative density) = total number of a particular parasite species divided by the number of hosts in a sample.

3.4.3 Parasite Index and Inverted Parasite Index

Endoparasites are usually found in larger numbers than ectoparasites and more than 1000 nematode larvae can be observed in a single host. Endo- and ectoparasites were categorized as shown in Table 3.1. The revised Parasite Index (PI) was introduced to distinguish more between the numbers of ecto- and endoparasites (Crafford and Avenant-Oldewage 2009). The inverted parasite index is based on the principle that ectoparasites are directly exposed than endoparasites to the water, and that some ectoparasites will be found if the water is in good condition (Crafford and Avenant-Oldewage 2009). Thus, good water quality associates with low HAI values. However, there is an inconsistency found in the refined parasite index classification as higher numbers of ectoparasites are given increasing numerical scores, resulting in an increase in the HAI value. Higher numbers of ectoparasites, nevertheless, suggest better water quality and should be given a lower score for this correlation to be reflected in the HAI value.

Table 3.1: The revised Parasite Index and Inverted Parasite Index (modified from Crafford and Avenant-Oldewage 2009 and Jooste *et al.* 2004).

Ectoparasites			Endoparasites	
Number	PI	IPI	Number	PI
0	0	30	0	0
1 – 10	10	30	<100	10
11 – 20	20	20	101 – 1000	20
>20	30	0	>1000	30

3.4.4 Condition Factor

The Condition Factor (CF) is a method used to indicate fish condition by correlating the fish body mass to its length. It is a method which is usually used in aquaculture, mainly for monitoring feeding intensity, age and growth rates (Anene 2005). The CF allows comparison for the condition of individual fish within a population, or individual fish from different populations and/or two or more populations from different localities. On the other hand the CF can be used as an index of the productivity of water (Barnham and Baxter 1998).

Calculation of the Condition Factor

The population condition factors of the two fish species were calculated according to Heath *et al*. (2004), where:

$CF = W \times 10^5 / L^3$

(W = weight in g; L = standard length in mm)

The standard deviation for each sample was calculated as proposed by Adams *et al*. (1993).

3.5 RESULTS AND DISCUSSION

3.5.1 Health Assessment Index

Fish in their aquatic habitats are naturally subjected to frequent stressors and their health can reflect the quality of water they live in. The stressors usually include unfavorable or unpredictable temperatures, high water velocities and sediment loads, low DO concentrations and limited food accessibility (Matthews 1998). If contamination of water is severe, adverse effects may be observed on fish, resulting in death (Hinton and Laurén 1990).

The lowest HAI value, signifying good quality water, was recorded at the Phalaborwa Barrage during autumn. The lowest HAI for the two fish species was 26 (Figure 3.1A). High HAI values are indicative of fish with health problems, highest index values were recorded during winter for *O. mossambicus* and *S. intermedius* with values 49 and 54 respectively (Figure 3.1A). Abnormalities observed throughout the study in *O.*

mossambicus were recorded for the gills, skin, liver and Hct, while for *S. intermedius* were for the skin, gills, liver and blood (Appendix Table 5 & Table 6).

The major contributor to the HAI value was parasites for both fish species followed by the gills and liver (Appendix Table 5 & Table 6). For the HAI, incorporated with the IPI, the lowest index values were recorded during autumn for *O. mossambicus* and *S. intermedius* with values of 44 and 36 respectively (Figure 3.1B). The highest index value of 67 was recorded for *O. mossambicus* while 65 was recorded for *S. intermedius* during winter (Figure 3.1B).

The maximum weight for *O. mossambicus* was 235.2 g (during winter 2010) (Appendix Table 5b) while for *S. intermedius* a maximum value of 587 g (during autumn 2010) (Appendix Table 6a) were recorded during the study. According to Adams *et al.* (1993) factors such as size, species and sex may manipulate the natural inconsistency of stress responses in fish. The factors mentioned in the latter statement might have had an influence on the health condition of the two fish species from the Phalaborwa Barrage. There was no significant difference in HAI values for *O. mossambicus* (F=1.9; df=3; p=0.15) and *S. intermedius* (F=2.58; df=3; p=0.66) throughout the study.

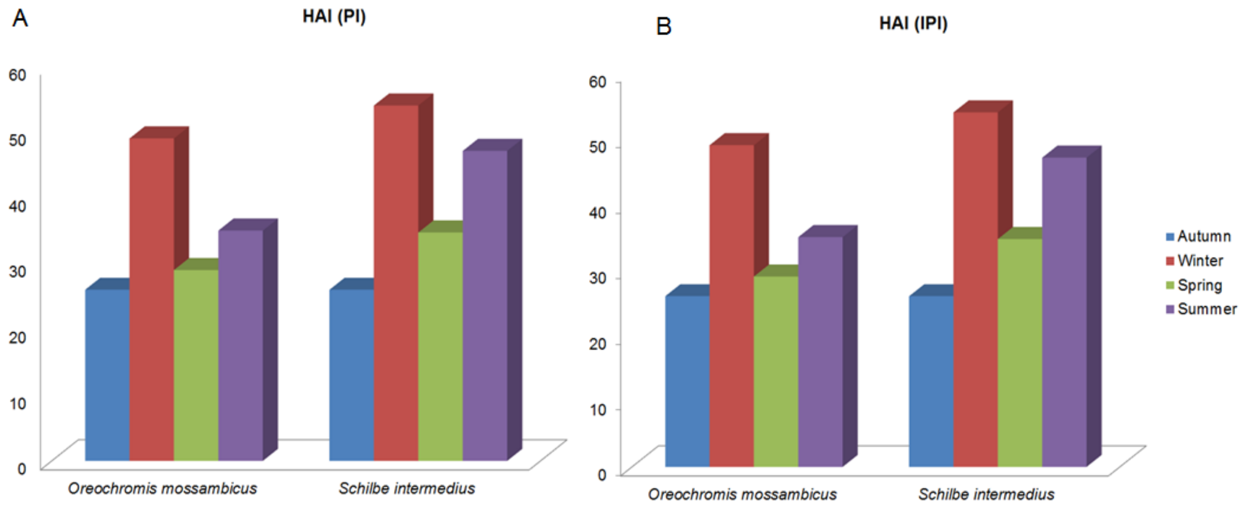

Figure 3.1: Seasonal Health Assessment Index values for *Oreochromis mossambicus* and *Schilbe intermedius* at the Phalaborwa Barrage. A revised Parasite Index **A** and **B** Inverted Parasite index (May 2010 – January 2011).

3.5.1.1 External variables

Fins and skin

According to Reichenbach-Klinke (1973), inflamed fins may be as a result of fin rot, metabolic disturbances, obstructed circulation and piscine tuberculosis. Fins can also be affected or damaged by the quality of water, predation, bacterial infections and parasites (Austin and Austin 1999).

The skin is directly in contact with water pollutants; nonetheless it operates as a protection medium (Roberts 2001). It is covered with a mucous cuticle that functions primarily to protect against erosion, or harmful substances and to be slippery to escape predation (Bowser 1999). The skin is particularly significant for the fish to maintain proper osmoregulation processes (Roberts 2001). Adams *et al.* (1993) stated that skin abnormalities are rated as mild aberrations, moderate aberrations and severe aberrations.

In *O. mossambicus*, during the autumn and spring surveys, a few fish had eroded fins and a HAI value of 10 was allocated to these fish (Appendix Table 5; Figure 3.2 C). During the four surveys most of the fish exhibited black spots on the skin and fins which are caused by a digenean larvae (metacercaria/trematode cysts) (Figure 3.2 E). Thus, except for the eroded fins and trematode cyst recorded in some of the fish, the fins and skin of most of the fish during the surveys were normal (Appendix Tables 5). While for *S. intermedius* no skin abnormalities were observed with the exception of three fish during the winter season with mild skin aberrations (Appendix Table 6).

Eyes

The eyes of fish can indicate their physical condition, such as starvation, chlorine poisoning, harsh conditions in winter, as this result in depressed eyes (Goede and Barton 1990). According to a study done by Karvonen *et al.* (2004), a heavy load of parasites can result in cataracts or the cloudiness of the eyes. Cataracts cause fish to be more vulnerable to predation as their vision is impaired (Seppälä *et al.* 2006).

The eyes of both fish species examined during this study were normal, with the exception of a cataract which was observed in one specimen of *O. mossambicus*

(Appendix Table 5; Figure 3.2 F). The cataract was most likely caused by the presence of a digenean larva (*Diplostomum* sp.) in the eye, however predation or mechanical injury cannot be excluded (Roberts 2001).

Gills and opercules

Gills function as multipurpose organs (Heath 1995). They are directly involved in a variety of functions which include osmoregulation, respiratory gas exchange, nitrogenous waste excretion and acid-base balance.

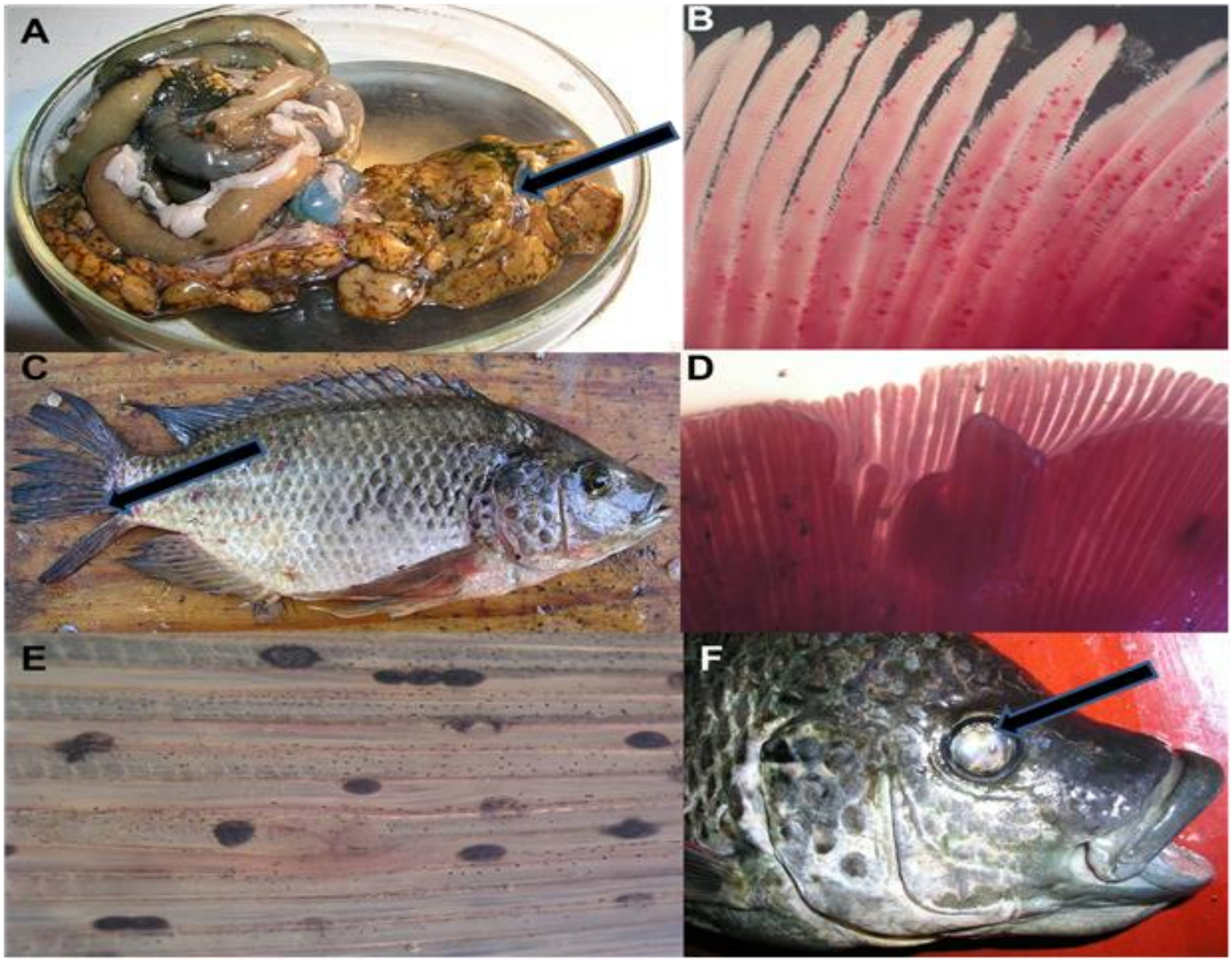

Figure 3.2: A – E. Abnormalities recorded from the external surface and internal organs of *Oreochromis mossambicus* from Phalaborwa Barrage (May 2010 – January 2011). **A**. Focal discoloration of liver. **B**. Bloody marks on gill filaments. **C**. Eroded fin. **D**. Deformed gills. **E**. Black cysts (caused by digenean larvae). **F**. Opaque eye.

Gills are of importance, because they are in direct contact with water and thus a close ionic regulation and metal uptake occur in the gills (Coetzee *et al.* 2002; Hubbard 2005). They are sensitive indicators of environmental pressure, together with exposure to harmful compounds found in aquatic ecosystems in consequence of anthropogenic activities (Hinton and Laurén 1990). According to Versteeg and Giesy (1986), metal contamination on fish gills can result in hyperplasia, hypertrophy of mucous cells, necrosis and fusion on gill filaments.

Abnormalities of gills for both fish species (all four seasons) were observed during this study (Figure 3.2 B & D). *Oreochromis mossambicus* had the highest incidence of abnormal gill conditions, the abnormalities included: pale, frayed and clubbed gills, while for *S. intermedius* comprised of pale and clubbed gills. This may be as a result of high concentrations of some metals which exceeded suggested guidelines (See Chapter 2). The operculum of fish is a hard bony flap which covers and protects the gills. It usually divides the head and the body in most fish (Zapata *et al.* 1996). Opercules were normal for both fish species for all survey.

3.5.1.2 Internal variables

Liver

The liver plays a major role in detoxification (Gonzálezi *et al.* 1993), thus it is vital in metabolism and excretion of toxic substances. Histological changes may take place in the liver if exposed to toxic substances or responding to stressors in the environment, therefore it can be used as a biomarker to indicate prior exposure to pollutants (Hinton and Laurén 1990; Van Dyk *et al*. 2009). In a study conducted by Van Dyk (2003), different types of fish species were exposed to different toxicants, resulting in histopathological changes in the livers.

The liver is able to degrade toxic substances, however if the concentrations are too high, it may result in abnormalities (Goede 1992). An abnormal liver may have fatty deposits on it, a tan color and a presence of liver nodules. In the autumn and summer surveys, liver anomalies were observed in some *O. mossambicus* (Appendix Table 5) and for *S. intermedius* abnormalities were observed during all the surveys (Appendix

Table 6; Figure 3.2 A). Anomalies of both fish include focal discoloration and fatty deposits and were allocated a value of 30 for both fish species.

Spleen

The spleen is red, black and granular it is considered to be normal, while if enlarged with nodules or grey color it is considered to be abnormal (Goede 1992). When some fish experience stress conditions, histological changes, jointly with increased vacuolation of hemoblasts and swelling, can be anticipated in the splenic blood cells (Peters and Schwarzer 1985). In a study conducted by Adams *et al.* (1993), anomalies observed were as a result of enlargement of the spleen and some due to the presence of nodules, swelling may possibly signify diseases and/or bacterial infection. No abnormalities were observed for the spleen during the four surveys for both the fish species (Appendix Tables 5 & 6)

Kidneys

Kidneys are excretory organs (Goede and Barton 1990), and in freshwater fish the kidney excretes water and conserves the ions. The abnormalities in kidney that are likely to occur include: presence of parasites, granular appearance and nodules (Bowser 1999). According to Goede and Barton (1990), swollen kidneys are a common sign of pathology. In a study conducted by Adams *et al.* (1993), abnormalities were due to swelling which was most likely caused by a parasitic infection. In the four surveys conducted none of the abnormalities mentioned were observed for both fish species.

Haematocrit

Haematocrit values reflect the percentage of red blood cells in the blood volume (Schuett *et al.* 1997). The haematocrit values vary amongst individuals with higher values (>30%) indicating that the population may be under stress while lower values (<30%) indicate the presence of diseases (Goede and Barton 1990). Haematocrit values vary depending on the physiological state and wellbeing of the individual fish (Jawad *et al.* 2004).

In *O. mossambicus,* a lower haematocrit value was recorded in winter with a mean value of 28%, while a slightly higher mean value was recorded in spring (29%)

(Appendix Table 5). No haematocrit readings could be taken for autumn and summer, because sufficient amount of blood could not be drawn due to the size of the fish. For *S. intermedius*, the lowest mean value was recorded during the winter survey (20%), while the highest mean value was recorded during spring (48.5%) (Appendix Table 6).

Some of the haematocrit values recorded for *S. intermedius* and *O. mossambicus* were abnormally low with an exception of the 48.5% recorded for *S. intermedius*. The latter may be attributed to the high numbers of ectoparasites recorded. It is assumed that low values are indicative the presence of disease (Goede and Barton 1990). In this study, lower haematocrit readings recorded for some fish, especially *S. intermedius*, which is may be due to a high number of parasites.

Hindgut

The hindgut is found between the midgut and the anus. It is where the final stages of digestion and absorption take place before excretion (Sivadas 2005). The cells in the intestine shrink during periods of starvation, spawning and migration in some fish species. According to Jooste *et al.* (2004), inflammation or redness may be caused by presence of parasites or poisoning.

No fish exhibited any abnormalities such as inflammation of the hindgut, thus the hindgut of all fish during the four surveys of this study did not affect the HAI score.

3.5.2 Parasite Index

The presence of fish parasites can serve as an indicative tool of ecosystem health (Sures 2001). Thus the PI and the IPI are used as useful because parasites are good indicators of the quality of water (Avenant-Oldewage 1998). Ectoparasites are sensitive to changes in the environment, therefore when water is polluted they decrease in numbers but when the water quality is good they increase in numbers (Avenant-Oldewage 1998; Luus-Powell *et al.* 2005). On the other hand endoparasites increase in polluted water, this may be as a result that they are not in direct contact with the water. However, the number of ecto- and endoparasites fluctuate not only due to pollution but also to low oxygen levels and increasing temperatures. Thus endo- and ectoparasites were incorporated as separate variables in the PI tested in South Africa, as a result of a variety of response that the parasites might exhibit when exposed to pollutants (Marx 1996; Robinson and Avenant-Oldewage 1996; Luus-Powell 1997; Watson 2001).

The only ectoparasite groups recorded for *O. mossambicus* and *S. intermedius* were monogeneans from the gills and copepods from the skin and gills (Table 3.3). Endoparasites recorded from *O. mossambicus* were monogeneans, digeneans, cestodes and nematodes whilst for *S. intermedius* digeneans, cestodes and nematodes were recorded.

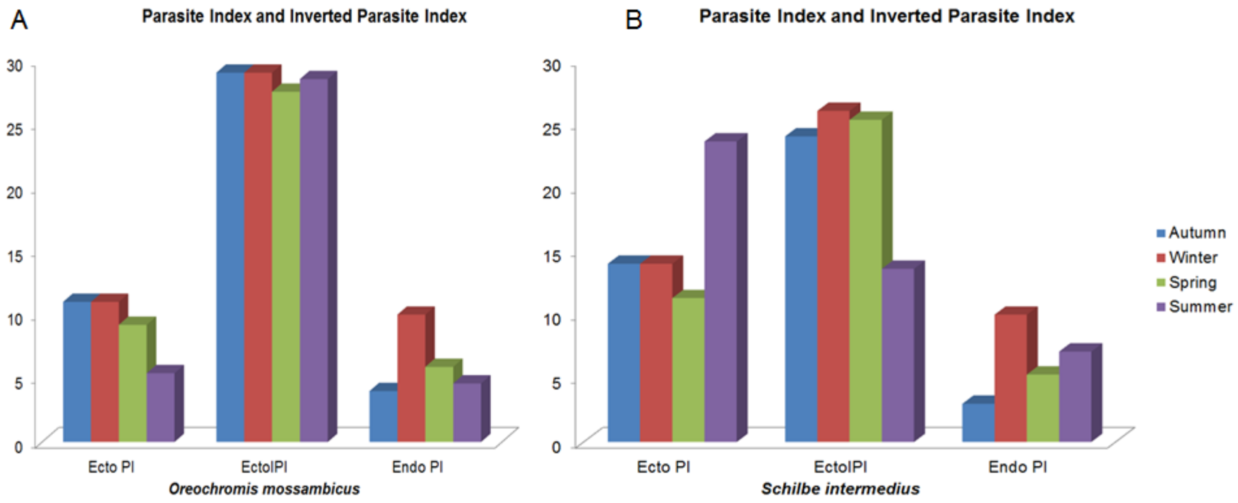

Figure 3.3: Seasonal Parasite Index (PI) and Inverted Parasite Index (IPI) of *Oreochromis mossambicus* **A.** and *Schilbe intermedius* **B.** at the Phalaborwa Barrage (May 2010 – January 2011).

The minimum PI for ectoparasites of *O. mossambicus* was 5.4 recorded during summer, while for *S. intermedius* was 14 during autumn and winter (Figure 3.3). The maximum PI for ectoparasites in *O. mossambicus* was 11 during autumn and winter, while for *S. intermedius* was 23.6 during summer. Higher ectoparasite PI values were recorded for both *O. mossambicus* and *S. intermedius.* High numbers of ectoparasite values indicate good quality water. For ectoparasite IPI, higher index values were recorded for *O. mossambicus* with values of 29 during autumn and winter, while a lower value was recorded for S*. intermedius* during summer with a value of 13.6 (Figure 3.3), indicating acceptable water quality. The minimum endoparasite PI for *O. mossambicus* was 4, while for *S. intermedius* was 3, both values were recorded in autumn. The maximum endoparasite PI for both fish species was recorded during winter with a value of 10 (Figure 3.3). There was significant difference for PI values of *O. mossambicus* (F=0.07; df=3; p=0.041) and *S. intermedius* (F=3.65; df=3; p=0.019) during the study.

3.5.3 Parasites

A total of 19 parasites species (7 monogeneans, 6 digeneans, 3 nematodes, 2 cestodes and 2 copepods) were found during four surveys at the Phalaborwa Barrage. Twenty four of which were adult stages infecting the skin, gills and the digestive tract. Fish from the barrage are potential intermediate hosts for at least 10 parasite species whose life cycles are completed when fishes are eaten, typically by fish-eating birds, but also reptiles (e.g. crocodiles).

Metazoan parasites from fish species from Phalaborwa Barrage

A diverse range of parasites (13 adults and 6 larval forms) were recorded for the two fish species from Phalaborwa Barrage. The seasonal infection statistics for the respective fish species are presented in Tables 3.2 to 3.3.

The monogenean *Enterogyrus* sp. (*O. mossambicus*) and the copepod *Ergasilus* sp. (S. intermedius) are new species, host and locality record for South Africa (Luus-Powell *et al.* 2011). The parasites recovered from each fish species are important as they form an integral part in their biology and are an indigenous component of the food chain (Landsberg *et al*. 1998). According to Marcogliese and Cone (1997), most parasites are host specific, but how specific the parasite is depends on the type of parasite or the stage of its life cycle. Hosts are important for the completion of the parasites life cycle, and if the parasite enters a wrong host, they may cause serious damage or even kill the host (Williams *et al.*1992).

Table 3.2: The parasite mean abundance, prevalence and mean intensity infestation statistics of *O. mossambicus* at the Phalaborwa Barrage (May 2010 – January 2011).

	Autumn			Winter			Spring			Summer		
Parasite	M A	P (%)	M I	M A	P (%)	M I	M A	P (%)	M I	M A	P (%)	M I
Ectoparasites												
Monogenea												
Cichlidogyrus halli	0.2	10	2	4.2	100	4.2	0.25	25	1	0	0	0
Cichlidogyrus sclerosus	5.5	100	5.5	0.6	60	1	0.9	38	2.3	7.3	100	7.3
Scutogyrus longicornis	0	0	0	0.1	10	1	0	0	0	0	0	0
Copepoda												
Lernaea cyprinacea	0.1	10	1	0	0	0	0	0	0	0	0	0
Endoparasites												
Monogenea												
Enterogyrus sp.	0.1	10	1	0	0	0	0.75	37.5	2	0	0	0
Digenea												
Digenean cysts (Blackspot)	0.2	10	2	0.2	10	2	2.3	66.6	3.5	1.8	50	3.6
Digenean larvae (unidentified)	0	0	0	0	0	0	0.25	25	0.7	0.2	20	1
Diplostomum sp.	0	0	0	0	0	0	0.125	12.5	1	0	0	0
Clinostomum sp.	0.3	10	3	0.2	10	2	0.25	25	1	0	0	0
Euclinostomum sp.	0	0	0	0	0	0	0	0	0	0.75	50	1.5
Cestoda												
Gryporhynchid larvae	0	0	0	4.1	80	5.	0.5	25	2	.25	25	1.2
Nematoda												
Contracaecum sp. (larvae)	0	0	0	0.2	20	1	0	0	0	0.60	50	1.2

Notes:
1- M A– Mean Abundance.
2- P– Prevalence.
3- M I– Mean

Table 3.3: The parasite mean abundance, prevalence and mean intensity infestation statistics of *S. intermedius* at the Phalaborwa Barrage (May 2010 – January 2011).

		Autumn			Winter			Spring			Summer	
Parasite	M A	P (%)	M I	M A	P (%)	M I	M A	P (%)	M I	M A	P (%)	M I
Ectoparasites												
Monogenea												
Schilbetrema quadricornis	22.7	90	25.2	22.5	90	25	13.3	73.3	18.2	20	82	23
Schilbetrema sp. A	2.9	40	7.25	0	0	0	0	0	0	7.9	43	18
Schilbetrema sp. B	0	0	0	0	0	0	0	0	0	3.6	14	25
Copepoda												
Ergasilus sp.	0	0	0	0	0	0	0.07	6.7	1	0	0	0
Endoparasites												
Digenea												
Digenean larvae (unidentified)	0	0	0	0	0	0	0.3	6.7	5	0.07	7.14	1
Cestoda												
Unidentified larvae	0	0	0	0	0	0	0	0	0	0.07	7.14	1
Nematoda												
Contracaecum sp. (larvae)	0.8	20	4	0.8	20	4	1.5	46.7	3.3	1.93	14.3	3.38
Paracamallanus cyathopharynx	0.2	10	2	0	0	0	0.2	13	1.5	0.07	7.14	1

Notes:
1- M A– Mean Abundance.
2- P– Prevalence.
3- M I– Mean

Infestation statistics

Infestation statistics for the individual parasites recorded are presented in Tables 3.2 and 3.3. However not all parasites were identified to genus and species level as the study focused on the presence and number of ecto- and endoparasites of the two fish species and their impact on the health of the fish using the PI.

The infestation statistics showed that the prevalence for ectoparasite of both fish species was higher than the prevalence for endoparasites (Tables 3.2 & 3.3). For *O. mossambicus* the highest monogenean (ecotoparasites) prevalence recorded was 100% (Table 3.2). A prevalence of 100% was recorded for *Cichlidogyrus sclerosus* and *Cichlidogyrus halli* in autumn and winter. While for *S. intermedius* the highest prevalence value of 90% was recorded for *Schilbetrema quadricornis* in autumn and winter. The lowest ectoparasite prevalence value for *O. mossambicus* was 10% for *C. halli* (monogean) and *Lernaea cyprinacea* (copepod) in winter and *Scutogyrus longicornis* during autumn (Table 3.2). The lowest ectoparasite (*Ergasilus* sp.- copepod) and endoparasite (digenean larvae) prevalence value for *S. intermedius* was 6.7% during spring.

Ectoparasites

Monogenea

Monogeneans, are known as parasitic flukes and belong to the phylum Platyhelminthes. There are two types of monogeneans, those found on the gills and skin (ectoparasites) and those in the stomach (endoparasites). In natural conditions they do not cause any harm to the host unless the host is under stress (Luus-Powell 2004). However, they can result in secondary infections which cause injuries to gills leading to fusion and hyperplasia of gill tissues and blockage of respiratory surfaces due to large numbers thus decreasing the efficiency of respiration.

Two species of *Cichlidogyrus* and a species of *Scutogyrus* were recorded from the gill filaments of *O. mossambicus* during this study (Appendix Table 7; Figures 3.4 A & B). *Cichlidogyrus halli* was recorded during three seasons (autumn, winter and spring)

(Appendix Table 7), while *C. sclerosus* was recorded during all seasons and *Scutogyrus longicornis* was recorded only in winter (Appendix Table 7).

Figure 3.4: Monogenean ectoparasites recorded from the gills of *Oreochromis mossambicus* and *Schilbe intermedius.* **A.** & **B.** *Cichlidogyrus* sp. collected from *Oreochromis mossambicus*; **C.** & **D.** *Schilbetremma* sp. collected from *Schilbe intermedius* (May 2010 – January 2011). Photos C and D by Mr WJ Smit.

Monogeneans recovered from fish are mostly ectoparasites with the exception of *Enterogyrus* sp which is found in the stomach of fish was recorded for two seasons (Appendix Table 7). The highest mean abundance value recorded for *O. mossambicus* was 0.5 in spring while the lowest value of 0.1 was recorded during autumn (Table 3.2).

Three species of *Schilbetrema* were recorded from the gill filaments of *S. intermedius* during this study (Appendix Table 8). *Schilbetrema quadricornis* was recovered from the gills throughout the four sampling seasons while *Schilbetrema* sp. (Figure 3.4 A) was recorded for two seasons only (autumn and summer) and *Schilbetrema* sp. (Figure 3.4 B) was recorded during summer (Appendix Table 8) (Figures 3.4 C & D).

A higher number of monogeneans were recorded for *S. intermedius* than *O. mossambicus*, in particular for *Schilbetrema quadricornis* from *S. intermedius,* the abundance was high during all the sampling seasons. In a study conducted by Madanire-Moyo *et al.* (2012b), it was mentioned that seasons have a significant influence on the abundance of monogeneans. Cultured fish are kept in a confined conditions and parasites can lower the production/growth rate or even kill the fish, whereas wild fish have lower monogenean intensities which usually have heavy parasite burdens thus resulting in epizootics, (Madanire-Moyo *et al.* 2011). According to Madanire-Moyo *et al.* (2012b), even the smallest host examined was infected which suggest that even young and immature fish get infected with gill parasites when they start schooling with larger fish. In this study the latter statement was true especially for *O. mossambicus* as most of the fish caught at the barrage were usually young. The abundance of monogeneans during winter and spring was low and this may be due to the slightly elevated salinity in the water or lower water temperatures (Chapter 2). According to Luus-Powell *et al.* (2006), salt treatment is a method commonly used in for monogenean infections in the cultured fish industry.

Copepoda

Parasitic copepods (Crustacea) are usually found on gills, skin, eyes, fins and the mouth of fish feeding on mucus, and fish tissue resulting in wounds which can cause secondary infections (Bush *et al.* 2001; Barson *et al.* 2008). The damage they cause has adverse effects on the function and survival of its host (Paperna 1996).

Two copepod species were recorded from the two fish species (Tables 3.2 & 3.3; Figures 3.5). *Lernaea cyprinacea* was recorded from the skin of *O. mossambicus* (Figure 3.5 A)*,* while *Ergasilus* sp. was recorded from the gills of *S. intermedius* (Figure 3.5 B).

During breeding *O. mossambicus* are concentrated more in littoral zones (Barson *et al.* 2008). Therefore, it would have been more likely for it to be infected by the larval copepodites of *L. cyprinacea*, which prefer warm waters (Paperna 1996) in the littoral zone. *Lernaea cyprinacea* was recorded once during the autumn survey while for *Ergasilus* sp. was recorded only during spring (Appendix Tables 7 & 8). *Lernaea cyprinacea,* are equipped with antlers that anchor the parasite in the tissues of fish, the attachment is very pathogenic by its nature (Khalifah 1976). *Ergasilus* sp. attaches to fish gills using its second antennae to hold the gill filaments tightly resulting in tissue damage and obstruct blood flow (Piasecki *et al*. 2004). There were no visible effects caused by the recorded copepods. *Lernaea cyprinacea* has been of great concern to fish culturists, and reports have been made on its impact on the host (Barson *et al.* 2008). The two copepods are two of three genera known to cause serious harm in fish culture, they can cause mortalities to their hosts (Paperna 1996). The mean abundance value for *L. cyprinacea* (0.1) was higher than that of *Ergasilus* sp. (0.07) (Table 3.2). Both copepods are not host specific however (Paperna 1996; Barson *et al.* 2008).

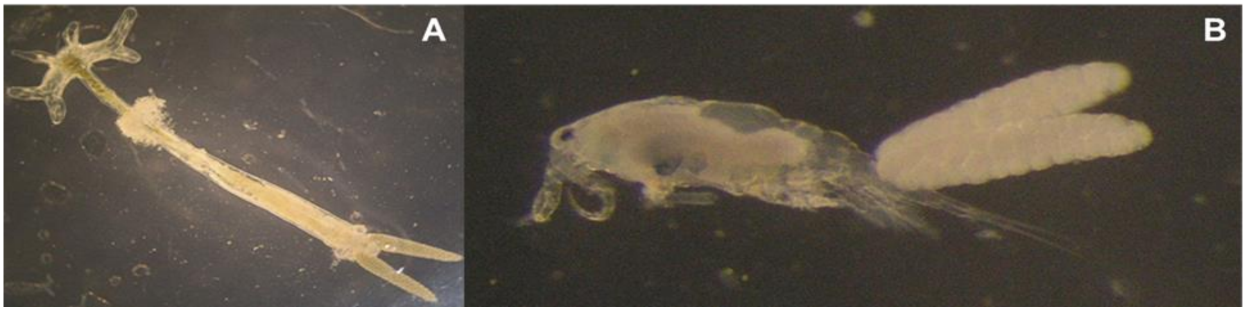

Figure 3.5: Copepod ectoparasites recorded from *Oreochromis mossambicus* and *Schilbe intermedius* at the Phalaborwa Barrage (May 2010 and October 2011). **A.** *Lernaea cyprinacea* removed from the skin of *Oreochromis mossambicus.* **B.** *Ergasilus* sp. from the gills of *Schilbe intermedius.*

The lowest ectoparasite mean intensity value recorded for *O. mossambicus* was 1 for *cyprinacea* during autumn (Table 3.2). While for *S. intermedius* a low mean intensity value of 1 was recorded for *Ergasilus* sp. during spring (Table 3.3).

There was no significance in PI values for both fish species (F=1.939; p=0.125).

Endoparasites

The highest prevalence of 100% was recorded for endoparasites for *O. mossambicus* during winter for gryporynchid larvae, while the highest prevalence value recorded for *S. intermedius* was 46.7% during spring for *Contracaecum* sp. The lowest value for *O.*

mossambicus is 12.5% for *Diplostomum* sp. during spring, whilst for *S. intermedius* was 6.7% for digenean larvae during spring (Table 3.3).

The highest endoparasite mean intensity for *O. mossambicus* was recorded during the summer survey with a value of 5.13 for gryporynchid larvae, whereas for *S. intermedius* a value of five (5) was recorded for digenean larvae during spring (Tables 3.2 and 3.3). The lowest endoparasite mean intensity value recorded for *O. mossambicus* was one (1) for *Enterogyrus* sp*.* during autumn, *Clinostomum* sp. and *Diplostomum* sp. during spring and *Contracaecum* sp. during winter (Table 3.2). For *S. intermedius* the lowest endoparasite mean intensity value recorded was one (1) for: digenean larvae, an unidentified cestode larva and the adult nematode *Paracamallanus cyathopharynx* during summer (Table 3.3).

Digenea

They are generally acknowledged as flukes, and are usually not host specific, they require a snail as an intermediate host (Brusca and Brusca 2003; Paperna 1996). According to Bartoli and Boudouresque (2007), digeneans can infect fish either in the adult form or as larvae. Several freshwater fish studies done in Africa have recorded numerous metacercarial infections (Khalil 1969; Mashego 1982; Britz *et al.* 1985; Mashego and Saayman 1989; Paperna 1996; Luus-Powell 2004; Madanire-Moyo *et al.* 2012b). Crocodiles, piscivorous birds, water lizards and rarely humans are the definitive hosts of digenean parasites found in fish.

Digeneans recorded from *O. mossambicus* include digenean larvae (from the gills, fins and skin) during all surveys, *Diplostomum* sp. (from the eye) during spring, *Clinostomum* sp., from the body cavity and branchial cavity (autumn, winter and spring) and *Euclinostomum* sp. from the muscle tissue (summer) (Appendix Table 7; Figure 3.6). While from *S. intermedius* only digenean larvae from the body cavity were recorded during spring and summer (Appendix Table 8). The highest digenean mean abundance for *O. mossambicus* was 2.3 and for *S. intermedius* was 0.3 for digenean larvae during spring (Tables 3.2 & 3.3). The lowest mean abundance for *O. mossambicus* was 0.2 for *Clinostomum* sp. (during winter) (Table 3.2) and digenean larvae (unidentified) during autumn and winter (Table 3.2), while for *S. intermedius* the lowest recorded was 0.07 for unidentified digenean larvae during summer (Table 3.3).

A high number of digenean larvae in the eye can disturb the movement of fish, thus it is more likely to be captured by predators (Bartoli and Boudouresque 2007). *Diplostomum* sp. (Figure 3.6 D) can cause a range of disease symptoms (lens cataract and growth reduction) which may result in fish mortality (Owen *et al.* 1993).

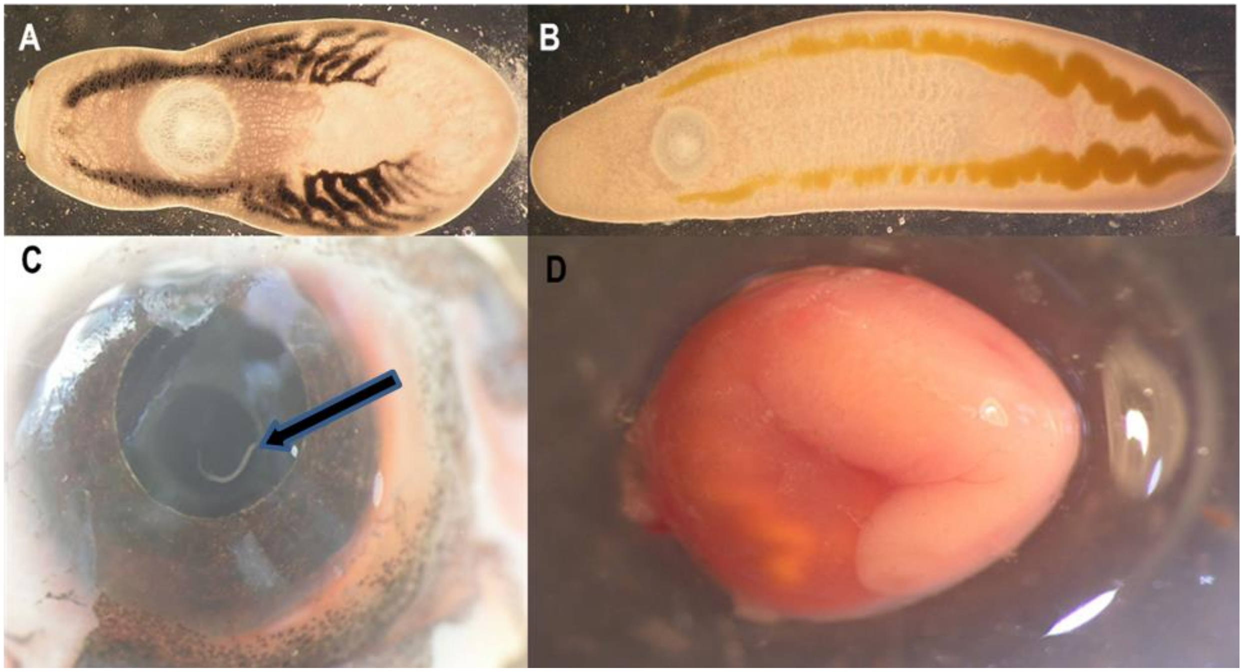

Figure 3.6: Digenean endoparasites recorded from *Oreochromis mossambicus* and *Schilbe intermedius* at the Phalaborwa Barrage (May 2010 – January 2011). **A**. *Euclinostomum* sp. removed from the muscle tissue. **B**. *Clinostomum* sp. **C**. *Diplostomum* sp. in the eye. **D**. Encysted *Clinostomum* sp. from the body and branchial cavity.

A cataract was recorded in this study during spring in the eye of one *O. mossambicus*, which may have been caused by *Diplostomum* sp.. Infestations by *Clinostomum* sp. are not harmful to the host though a mass mortality of fish at a fish station in South Africa was attributed to this parasite (Britz *et al.* 1985). Furthermore *Euclinostomum* sp. (Figure 3.6A) cysts found in the muscle tissue makes the fish unappealing to some consumers, thus fish should be well cooked to avoid human infections. Digenean metacercariae recorded from *O. mossambicus* were encysted on the fins, gills and skin of the fish. The cyst is black in color and according to Mashego and Saayman (1989) the black color is due to dentritic pigmentation on the cyst wall and it is responsible for the black spot on the fish and therefore frequently referred to as ‘Black spot’.

Cestoda

Cestodes or tapeworms are also (like the Monogenea, Digenea) part of the flatworms belonging to phylum Platyhelminthes (Brusca and Brusca 2003). Large numbers can result in adverse effects such as a reduction in growth and affect the reproductive success of fishes.

Cestodes recorded from *O. mossambicus* included gryporynchid cestodes larvae from the outer wall of the small intestine (autumn, winter and spring) (Appendix Table 7; Figure 3.7).

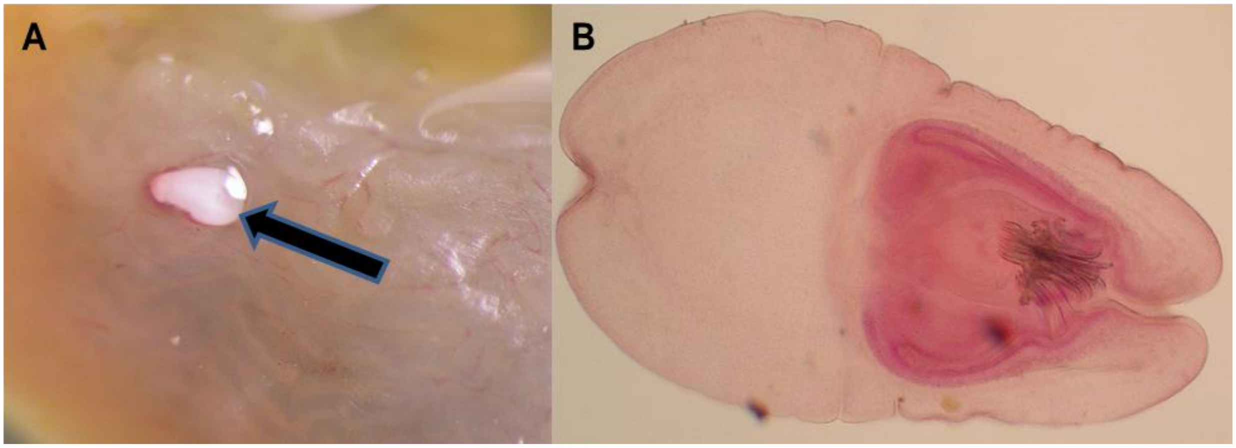

Figure 3.7: Cestode larvae from *Oreochromis mossambicus* at the Phalaborwa Barrage (July 2010 – January 2011). **A.** encysted & **B.** gryporynchid larvae from the small intestine.

While from *S. intermedius* only an unidentified cestode larvae was recorded during summer (Appendix Table 8). The highest cestode mean abundance for *O. mossambicus* was 5.15 in summer and for *S. intermedius* was 0.07 in summer (Table 3.3). The lowest mean abundance for *O. mossambicus* was 0.33 during the spring survey. A higher number of cestode parasites were recorded for *O. mossambicus* than *S. intermedius* (Table 3.2). According to Madanire-Moyo *et al.* (2012b), gryporynchid cestode larvae are evidently associated with pollution. The first intermediate hosts of several cestodes are frequently oligochaetes, which are able to tolerate organic pollution because they feed on organic particles in the sediment (Sibley *et al.* 2000).

Nematoda

Nematodes are commonly known as roundworms and they can affect all freshwater and brackish water fish. Heavier infections are usually observed in predatory fish (Paperna 1996; Brusca and Brusca 2003). Nematodes (both adults and larval stages) can be found in many parts of the fish, including the alimentary canal, swim bladder, the skin or fins and the muscle.

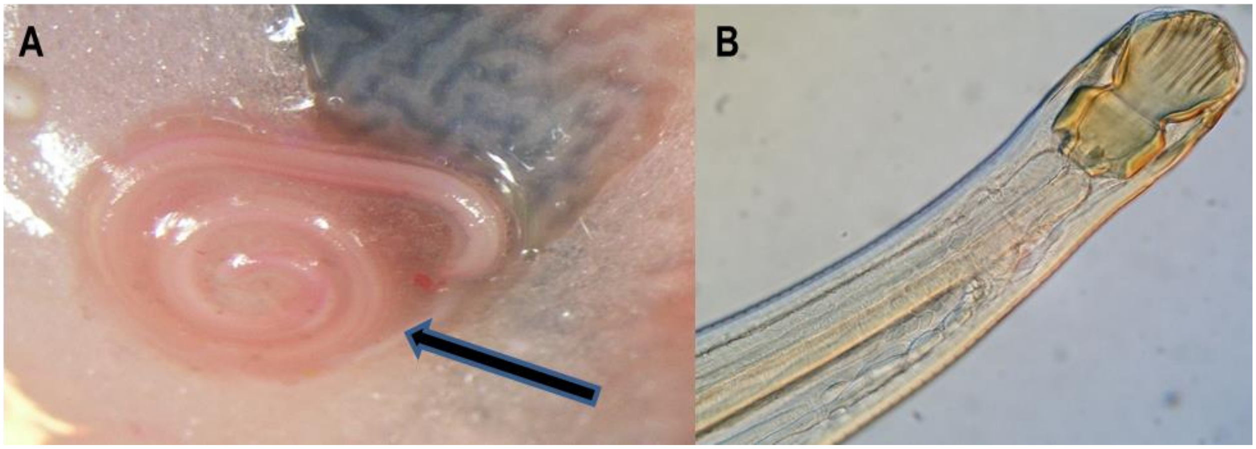

Figure 3.8: Nematode endoparasites recorded from *Oreochromis mossambicus* and *Schilbe intermedius* at the Phalaborwa Barrage (May 2010 – January 2011). **A**. *Contracaecum* sp. recorded from the body cavity of both the fish species. **B**. *Paracamallanus cyathopharynx.*

Nematodes recorded from *O. mossambicus* (winter and summer) and *S. intermedius* (all seasons) included *Contracaecum* sp. from the body cavity (Appendix Tables 7 & 8; Figure 3.8A). *Paracamallanus cyathopharynx* an adult nematode (Figure 3.8B) was found in the intestine of *S. intermedius* (during autumn, spring and summer). The highest nematode mean abundance was recorded for *Contracaecum* larvae from *S. intermedius* during summer with a value of 1.93 (Table 3.2), while the lowest value recorded was 0.07 for *P. cyathopharynx* in *S. intermedius* (Table 3.2).

According to Paperna (1996), neither encysted nor free *Contracaecum* species can severely affect fish. However inflammation around encysted larvae is localized. In addition, infestation resulting from extensive inflammation and fibrosis are usually observed only in larger fish with no impact on the body condition.

Large (200 – 350 g) tilapia can accommodate up to 12 worms (Paperna 1996), however parasites recorded in *O. mossambicus* were fewer in numbers. Infestations by camallanids (*P. cyathopharynx* and *Procamallanus laevionchus*) are abundant and intense (up to 20), predominantly in the stomach of *Clarias* spp. and in other catfish (e.g. *S. intermedius*) (Paperna 1996). According to Smit and Luus-Powell (2012), temperature is considered to be an important factor affecting the presence or seasonal variations of parasites. Some parasitic groups decrease in variation as the months get hotter (spring – summer), meaning there are more parasites in winter than warmer months (Khidr 1990). In this study there was little variation in parasite groups recorded for both fish species because parasites were present throughout the study period.

The high abundance of monogeneans on *O. mossambicus* (*Cichlidogyrus* spp.) and *S. intermedius* (*Schilbetrema* spp.) contributed to the high ectoparasite PI and IPI values recorded. However these results were unexpected as the quantity of ectoparasites is suspected to be lower in poorer water quality while in this study they were higher especially for *S. intermedius*. According to the water quality results (Chapter 2) some water constituents were above the South African and international water quality guidelines. This inconsistency may be attributed to the differential vulnerability of the parasites to the toxicity of different contaminants, the concentration, exposure time and synergistic effects (Marcogliese 2005).

Parasites are known to be more diverse in good quality water as well as their intermediate and final hosts than in polluted water (Marcogliese 2005), but a decrease in species richness and diversity in the helminth parasite communities of fishes can be as a result of water pollution. This usually affects ectoparasite species that are in direct contact with organic and inorganic pollutants (Pietrock and Marcogliese 2003; Madanire-Moyo *et al.* 2012b). However, according to Sibley *et al.* (2000), pollutants have dissimilar influences on parasites, which may be the explanation to the difference in composition of ecto- and endoparasites at the Phalaborwa Barrage during winter and spring surveys. Monogenean parasites are sensitive to salinity (Möller 1987), and during winter and spring in this study and some of the monogeneans were lower during those seasons. Thus clearly indicating that the parasite species composition and richness in freshwater fish are influenced by environmental stressors, however the response may be absent if the pollution level is moderate (Madanire-Moyo *et al.* 2010).

3.5.4 Condition factor

In fisheries science, the condition factor (CF) compares the health, condition of a fish and it is based on the hypothesis that heavier fish of a given length are in healthier conditions (Anene 2005). This index can be used to monitor the feeding intensity, age and growth rates in fish (Oni *et al.* 1983). The condition factor declines with the increase in length thus manipulating the reproductive succession in fish (Welcomme 1979). It is powerfully influenced by both biotic and abiotic environmental surroundings plus it can be used as an index to evaluate the status of the habitat in which fish live (Oni *et al.* 1983).

Several studies done by Saliu (2001), Lizama and Ambrósio (2002), Welcomme (1979), Dadzie and Wangila (1980) and Oni *et al.* (1983) focused on the determination of changes in condition factor with season, fish length, sex and/or reproductive status of fish. The length-weight relationship of fish is an essential fishery management tool. Its significance is pronounced in estimating the standard weight at a given length group and in assessing the relative condition of the fish population (Bolger and Connolly 1989). A value of 0.80 designate a tremendously deprived fish, while a value of 1.60 signifies a fair fish (Barnham and Baxter 1998).

The highest mean CF (3.63±0.57; 3.63±1.55) was recorded in *O. mossambicus* (winter and spring), while the lowest value (1.09±0.26) was observed in *S. intermedius* (spring) (Table 3.4 and Figure 3.9).

Table 3.4: The mean population Condition Factor values calculated during the four seasons for the two fish species at the Phalaborwa Barrage. Mean (CF) and standard deviation (SD).

Seasons	*Oreochromis mossambicus*		*Schilbe intermedius*	
	CF	SD	CF	SD
Autumn	2.96	±0.58	1.24	±0.39
Winter	3.63	±0.57	1.44	±1.08
Spring	3.63	±1.55	1.09	±0.26
Summer	1.96	±1.07	1.31	±0.21

The results for *O. mossambicus* were always above 1.60 which indicates that the fish were in a fair condition. For *S. intermedius* all the condition factor values recorded were below 1.60 however above 0.80 during this study (Table 3.4 and Figure 3.9) indicating a slightly fair condition.

According to Ahmed *et al*. (2011), in some species from a reproductive point of view, the highest CF values are reached, thus the elevated values in this study during winter and spring for *O. mossambicus* may have been as a result of breeding. A decline in the CF was observed during summer for *O. mossambicus*, most likely demonstrating an adjustment in feeding activity.

The mean values (2.13 – 2.37) recorded by Watson *et al.* (2012) for *O. mossambicus* at Loskop Dam were lower than those in this study with the exception of summer. Despite the differences noted in this study, Oni *et al.* (1983) observed that condition factor is not always constant for a species or population over time and possibly will be influenced by both biotic and abiotic factors such as feeding regime and state of gonad development (Saliu 2001). According to Saliu (2001), the general trend is that 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 similar to the results in this study, thus the fish at the Phalaborwa Barrage showed to be in good condition.

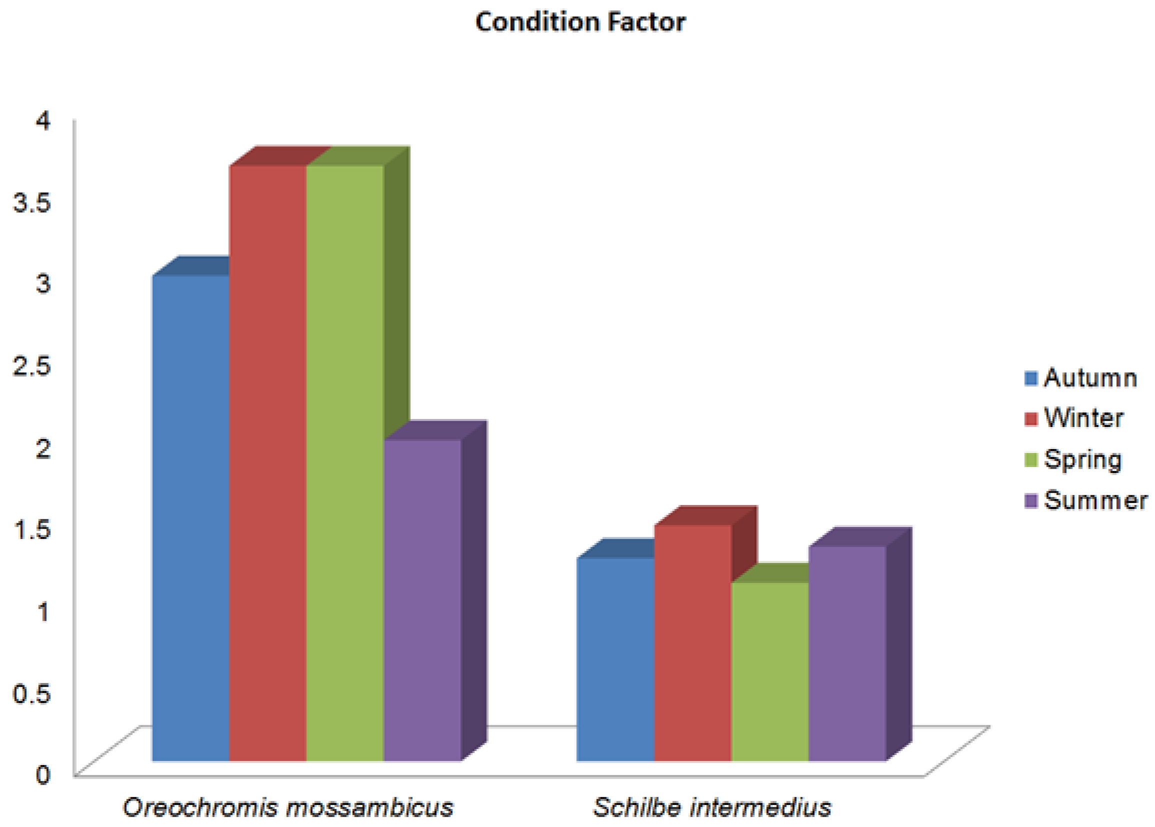

Figure 3.9: Seasonal Condition Factor for *Oreochromis mossambicus* and *Schilbe intermedius* at the Phalaborwa Barrage (May 2010 – January 2011).

3.6 CONCLUSIONS

Health Assessment Index

The HAI has demonstrated to be a straight forward and economical means of quickly assessing fish wellbeing in the field (Adams *et al.* 1993). It was not designed to be problem-solving in character, but to grant a first level assessment of the wellbeing of a fish population. All aquatic ecosystems are different in terms of water quality and fish species, thus the HAI tests differently in dissimilar aquatic ecosystems, depending on fish species used and the nature of stressors (Jooste *et al.* 2004). Higher HAI values were recorded for *S. intermedius* with the highest value of 54 during winter (Figure 3.1A), which indicates that *S. intermedius* was more affected by stressors than *O. mossambicus*. This trend was observed throughout the study, lower HAI values were recorded for *O. mossambicus* and the lowest HAI value was recorded during autumn

(24). According to Schmitt *et al.* (2005), a population HAI value of ≤20 designate an unimpacted or moderately impacted site, a value of >50 indicate intermediate sites and lastly values of >70 signify heavily impacted sites. When interpreting the results and comparing them to those mentioned by Schmitt *et al.* (2005), all sites indicated to be intermediate impacted sites during this study. One-way ANOVA indicated that there was no significant difference in HAI values for *O. mossambicus* (F=1.9; df=3; p=0.15) and *S. intermedius* (F=2.58; df=3; p=0.66) throughout the study.

In *S. intermedius,* parasites contributed the most to the total HAI followed by the abnormal condition of liver, haematocrit (internal variables) and gills (external variable), this was different to those found for *O. mossambicus.* The variables that increased the HAI for *O. mossambicus*, besides the parasites, include internal variables (liver and haematocrit) and external variables (gills, eyes, fins and skin). No abnormalities of the kidney, opercules and spleen were observed during the study. All the fish at the barrage appear to be in an acceptable physical form, not having any noticeable abnormal health effects, with the exception of some fins, skin, liver and low haematocrit value of some fish (mostly *O. mossambicus*).

The HAI, like any other method, has limitations. The investigator may understand observations differently, moreover this may manipulate end results of the HAI. However, it is important to highlight that the HAI was a successful method in the evaluation of fish health for this study at the Phalaborwa Barrage, as it provided a clear health status for the two fish species sampled. Nevertheless, biological data can never under any circumstance replace chemical and physical data and *vice versa*, though they can be used in close association with one another. The HAI will be more significant if it is coupled with histopathological studies (Hinton and Laurén 1990).

Parasite Index

In an aquatic environment, the use of fish parasites as bioindicators of contamination has been confirmed to be particularly appropriate due to their capacity of bioconcentration (Sures *et al.* 1999; Sures 2001; Sures and Reimann 2003). Ectoparasites decrease in diversity and abundance when water is significantly contaminated (Avenant-Oldewage 1998). The latter statement was not supported by the findings in this study. The number of endoparasites, however are more likely to increase in polluted waters, which might be attributed to a lowered immune system. However, that was not the case in this study as ectoparasites recorded for *O. mossambicus* and *S. intermedius* were higher than endoparasites (with an exception for *O. mossambicus* during summer).

The PI and IPI for ectoparasites were mostly higher than the PI for endoparasites. The PI for endoparasites was higher for *O. mossambicus* during autumn and spring, and for *S. intermedius* during winter and summer. To some extent the barrage was impacted and the numbers of endoparasites were expected to be higher than ectoparasites. Furthermore, some ectoparasites (especially the monogeneans, *Cichlidogyrus* spp. and *Schilbetrema* spp.) were recorded almost throughout the study for both fish species. Consequently it appears that parasites vary in the response to different water constituents therefore more intense studies are required to make a more concrete conclusion. Further studies need to focus on using parasites as biological indicators of pollution. One-way ANOVA showed that there was statistically significant difference for PI values of *O. mossambicus* (F=0.07; df=3; p=0.041) and *S. intermedius* (F=3.65; df=3; p=0.019) during the study.

Condition Factor

Oreochromis mossambicus had CF values which indicated that the fish is in a good condition throughout the surveys with mean values ranging from 1.96 – 3.63. While for *S. intermedius* all the CF values recorded ranged between 1.09 – 1.44, indicating a fair condition. There were no values below 1 recorded, thus none of the values recorded indicated deprived fish.

CHAPTER 4

BIOACCUMULATION OF METALS AND HUMAN HEALTH RISK ASSESSMENT

4.1 INTRODUCTION

Bioaccumulation is described as ‘the net result of uptake, transformation, and elimination of a substance in an organism as a result of waterborne exposure’ (OECD 2001). Bioconcentration is referred to as ‘the accumulation and transfer of substances via the food chain, resulting in an increase of internal concentrations in organisms on higher levels of the trophic chain (Adams *et al.* 2000). There is an increasing awareness of potential hazards by metals associated with mining in freshwater ecosystems (Du Preez *et al*. 2003). The metal’s natural concentrations in the environment in the past two decades have amplified due to sources such as mining, industrial and agricultural activities and geochemical structure (Heath and Claassen 1999; Sprocati *et al.* 2006; Singh *et al.* 2007). These sources of pollution disturb the physiochemical characteristics of water, sediment and biological components, species richness and diversity eventually altering the ecosystem compositions (Dallas and Day 2004; Al-Rawi 2005; Mantovi *et al.* 2005; Singh *et al.* 2007). Increased contaminants in freshwater systems have further resulted in decreased numbers of fish, Nile crocodiles, terrapins and fish eagles (Myburgh and Botha 2009; Botha *et al.* 2011).

Metals are natural constituents in the aquatic environment and some are needed for metabolic process thus they are essential, whereas others are non-essential playing no significant biological roles (Coetzee *et al.* 2002). Metal contamination of aquatic systems and the adverse effects it has on humans and aquatic organisms have been a widely recognized problem. Over the years, the then Department of Water Affairs and Forestry (DWAF) discovered that South Africa has problems with contamination of its aquatic ecosystems (CSIR 2010). Most metal constituents are adsorbed and trapped in the sediment, thus eliminating pollutants from the water column decreasing the toxicity to aquatic organisms. As a result sediment is known to be a sink for pollutants therefore poses the highest risk to the aquatic environment (Wepener and Vermeulen 2006). Usually the constituents bound to the sediment are assimilated by benthic organisms which are subjected to predation, thus pollutants are transferred throughout the food web which is a process known as biomagnification also known as bio-amplification (Anderson 1990; Davies and Day 1998).

To achieve an absolutely dependable assessment of contamination in the aquatic ecosystem, chemical and physical monitoring should be coupled with biological monitoring, as living organisms can provide an excellent indication of the water quality (Grayman *et al.* 2001). Several studies of fish species have been performed in the Olifants River in terms of bioaccumulation: *Oreochromis mossambicus, Schilbe intermedius* and *Clarias gariepinus* (Grobler 1994), *O. mossambicus* and *C. gariepinus* (Van Vuren *et al.* 1994); *Labeobarbus marequensis* (Seymore *et al.* 1995); *O. mossambicus* (Robinson and Avenant-Oldewage 1997), *O. mossambicus* and *C. gariepinus* (Kotzè *et al.* 1999), *Labeo umbratus* (Nussey *et al.* 2000) and *C. gariepinus* by Avenant-Oldewage and Marx (2000).

According to Du Preez *et al.* (2003), literature on the occurrence of pollutants in fish from South African freshwater systems indicate that surveys performed were to investigate chemical contaminants only. The majority of studies thus far only described the species and tissue differences in bioaccumulation, health of the aquatic ecosystems and the spatial and temporal variation in contaminant concentrations, however risks to humans when consuming contaminated fish were hardly ever addressed.

This chapter thus deals with bioaccumulation, comparison of 20 metals in the dorsal-lateral muscle tissue of *Oreochromis mossambicus* (Mozambique tilapia) and *Schilbe intermedius* (Silver catfish) and the human health risk assessment upon consumption of contaminated fish. The data were used to establish which fish species contained higher concentrations of the 20 metals, and how the accumulated metals affect human health. This study focused on the muscle tissue only, as it can be used as an indicator tissue in pollution monitoring of most heavy metals (Wepener 1997), and it is the portion regularly consumed by humans (Du Preez *et al*. 2003). In this study the protocol by Heath *et al.* (2004) was followed where only fish muscle tissues were tested for bioaccumulation of metals.

4.2 MATERIAL AND METHODS

Fish were collected by means of gill nets of different mesh sizes. The nets were placed at various sites at the barrage depending on the depth and vegetation. The collected fish were kept in large holding tanks filled with water from the barrage. Fish were sacrificed by severing the spinal cord behind the head (Chapter 3), they were dissected on polyethylene work surface using stainless steel dissecting tools, special care was taken to prevent contamination. A skinless sample of the muscle tissue (± 50 g) was collected from five fish per survey from each of the two fish species (*O. mossambicus* and *S. intermedius*), then individually wrapped in aluminium foil, labelled, and frozen on site. The samples were stored in a -80°C freezer at the Department of Biodiversity, University of Limpopo, prior to analysis for metal content.

Laboratory Analyses

The frozen muscle tissue samples were sent to the accredited laboratory in Pretoria for chemical analyses of metals. The muscle tissue samples were dried at 60°C for 48 hours, weighed, digested in concentrated nitric acid and hydrochloric acid (4:1) according to Bervoets and Blust (2003). The samples were analysed to determine the metal content using sequential inductively coupled plasma - optical emission spectrometry (ICP-OES) with the results corrected for spectral interference between metals. The results were expressed in mg/kg dry weight. The following metals were selected for the analysis: aluminium, arsenic, antimony, barium, beryllium, boron, bismuth, cadmium, cobalt, chromium, copper, iron, lead, manganese, molybdenum, nickel, selenium, silver, strontium, tin, titanium, vanadium, tungsten (wolfram) and zinc. Of these, beryllium, bismuth, molybdenum and tungsten (wolfram) were below the detection level of the apparatus for the fish muscle tissue samples thus not considered further in the dissertation.

Human health risk assessment

The assessment was carried out according to 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 by Heath *et al*. (2004b).

The hazard quotients for each metal are summed to calculate the hazard quotient for the fish species. Those greater than 1 indicate an unacceptably high risk to human health for weekly consumption of the fish species, however Hazard Quotients less than 1 are considered to be safe for a lifetime exposure. The values used in this study follow those recommended by the US-EPA for an impoverished rural community reliant on fish as an additional protein source such as those utilising the fish from the Phalaborwa Barrage. The average metal concentration in the muscle tissues for the respective fish species from the barrage was used in the health risk assessment (Table 4.1). Reference Dose levels developed by the US-EPA were used for the evaluation of the Hazard Quotients (US-EPA 2012).

Human health risk assessment procedure was conducted according to the protocol described by 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) using the Risk Assistant™ software package for the WRC K5/1929 report of Jooste *et al*. (2013).

4.3 RESULTS AND DISCUSSION

Table 4.1 summarises 20 metal concentrations recorded from muscle tissues of *O. mossambicus* and *S. intermedius* and sediment. Metals such as beryllium, bismuth, molybdenum and tungsten (wolfram) (Appendix Table 9 & 10) were analysed but not included in table because their values were below detection limits.

Aluminium

The highest aluminum concentration (111 mg/kg) was recorded for *S. intermedius* while lowest value (7 mg/kg) was recorded for *O. mossambicus* (Appendix Tables 9 & 10). A mean concentration value of 60 mg/kg was recorded for *O. mossambicus,* 55 mg/kg for *S. intermedius* and 3941.67 mg/kg in sediment. Aluminium was higher in sediment than the muscle tissues of the fish (Table 4.1). Even though Al was recorded at concentrations above the TWQR in water and elevated in the sediment, it seems to be not biologically available as it did not bioaccumulate much in the muscle tissue. The Hazard Quotients (HQ) calculations were below one for daily consumption of *O. mossambicus* (0.03) and *S. intermedius* (0.03) (Table 4.2). The Al levels do not pose health effects on humans on consumption.

Table 4.1: The average concentration of metals in the muscle tissue of *Oreochromis mossambicus* (n=6) and *Schilbe intermedius* (n=15) and sediment (n=6) from Phalaborwa Barrage (May 2010 – January 2011).

Metals mg/kg (dry weight)	*Oreochromis mossambicus*		*Schilbe Intermedius*		Sediment	
	Average	SD	Average	SD	Average	SD
Aluminium	60	±33.09	55	±17.26	3941.67	±2892.85
Arsenic	*	*	6	±4.09	5.5	±3.53
Antimony	1.75	±0.96	1.5	±0.74	1	±1
Barium	298	±30.38	320	±74.05	196.5	±177.8
Boron	246	±18.47	257	±50.88	181.76	±121.25
Cadmium	*	*	*	*	4.4	±6.07
Chromium	14	±1.22	15	±1.52	130.33	±151.88
Cobalt	1	±0	1	±0.41	13	±7.87
Copper	5	±0.82	4	±1.41	13	±12.03
Iron	126	±27.35	112	±46.20	19544	±12359.5
Lead	6	±7.96	3.2	±5.82	6	±2.65
Manganese	15	±17.01	4	±4.50	297.17	±275.17
Nickel	5	±8.91	1	±0.4	34.5	±28.65
Selenium	2.4	±2.10	4.1	±2.72	*	*
Silver	5	±8.00	2	±0.49	79.33	±33.38
Strontium	75	±70.50	8.21	±18.17	26	±19.29
Tin	1.5	±0.70	4.6	±2. 07	3.5	±7
Titanium	2	±1.55	1.53	±0.72	672.67	±766.96
Vanadium	5.5	±3. 53	*	*	79.33	±83.5
Zinc	214	±15.08	209	±41.90	105.67	±76.35

* Below detection level

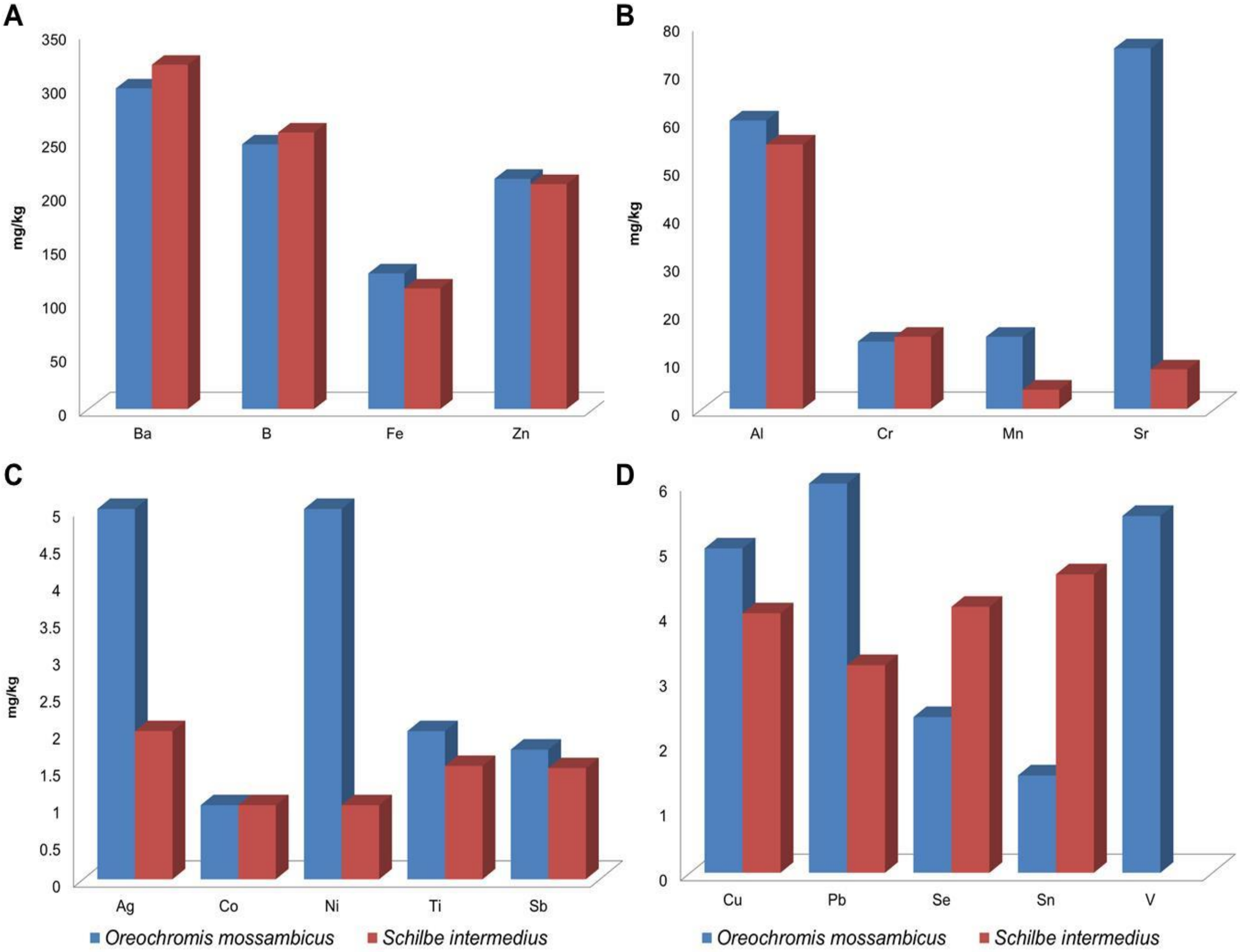

Figure 4.1: **A – D**. Indicate average concentration of metals, expressed in mg/kg dry weight, in the muscle tissue of *Oreochromis mossambicus* and *Schilbe intermedius* from Phalaborwa Barrage.

Aluminium is the most abundant element in the earth's crust (ATSDR 2008a). Anthropogenic sources of Al are from air emissions, waste water effluents, acid mine drainage, mining, industrial productions, coal-fired power plants (ATSDR 2008a). Solubility of Al is pH dependent (Dallas and Day 2004). Soluble ionized Al is toxic to fish, it can cause mucus build up on gills affecting its respiration ability while its complexes have been linked to fish-kills (Dallas and Day 2004).

According to ATSDR (2008a), Al can affect the nervous system in humans. The International Agency for Research on Cancer (IARC) (IARC 2012) decided that Al was carcinogenic to humans, therefore classified it in group 1: sufficient evidence in humans or sufficient evidence in animals and strong mechanistic data in humans inadequate in humans and limited in animals.

Table 4.2: The Hazard Quotients, based on one fish meal (150 g) eaten on a weekly and daily basis, calculated for metals found in the muscle tissue of two fish species from the Phalaborwa Barrage (adapted from Jooste *et al*. 2013). The shaded values indicate where HQ value of one was exceeded.

Metals	Weekly HQ		Daily HQ	
	Oreochromis mossambicus	*Schilbe intermedius*	*Oreochromis mossambicus*	*Schilbe intermedius*
Aluminium	*	*	0.03	0.03
Antimony	0.23	0.11	1.59	0.79
Arsenic	*	0.95	0.05	6.63
Barium	0.11	0.12	0.80	0.87
Boron	0.09	0.10	0.66	0.70
Cadmium	*	*	0.03	*
Chromium	0.35	0.38	2.42	2.69
Cobalt	0.07	0.06	0.50	0.40
Copper	0.01	0.01	0.07	0.06
Iron	0.01	0.01	0.10	0.09
Lead	6.14	4.06	42.95	28.40
Manganese	0.01	*	0.06	0.02
Nickel	0.02	*	0.13	0.02
Selenium	0.03	0.04	0.22	0.27
Silver	0.08	0.03	0.56	0.19
Strontium	0.01	*	0.07	0.01
Vanadium	0.03	*	0.21	0.02
Zinc	0.05	0.05	0.38	0.38
Total HQ	**7.26**	**5.94**	**50.82**	**41.56**

Antimony

The highest antimony concentration of 3 mg/kg dry weight was recorded for *O. mossambicus* while the lowest value was 1 mg/kg recorded for both fish species (Appendix Tables 9 & 10). The mean concentration for antimony was higher for *O. mossambicus* compared to *S. intermedius*, with values of 1.75 mg/kg and 1.5 mg/kg respectively (Table 4.1; Figure 4.1). The mean value recorded for sediment was 1 mg/kg (Table 4.1).

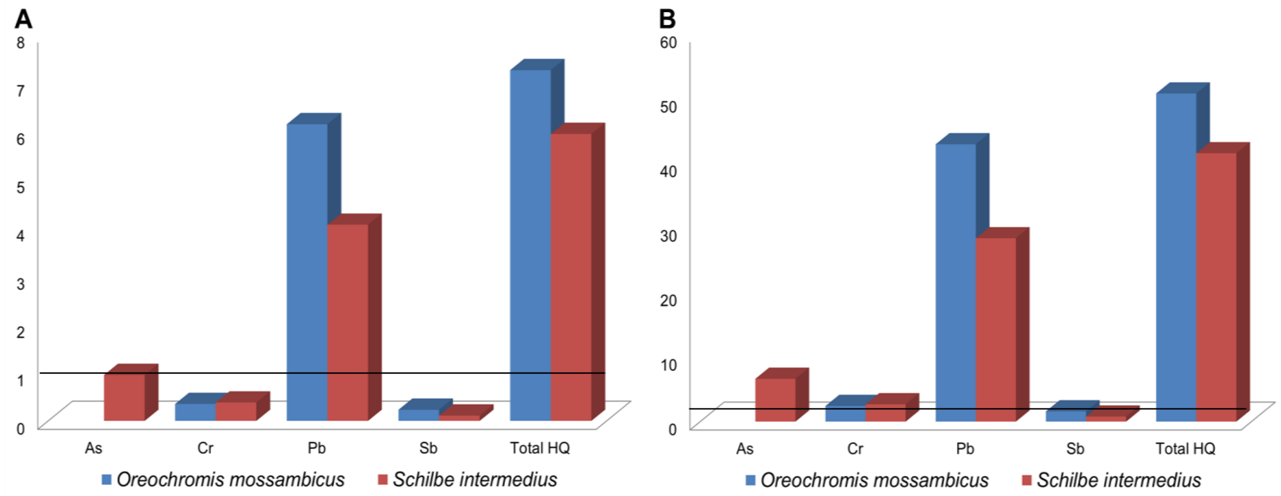

Figure 4.2: The Hazard Quotients for the four highest metals, based on the consumption of one weekly (A) and daily (B) fish meal of 150g from the muscle tissue of *Oreochromis mossambicus* and *Schilbe intermedius* from Phalaborwa Barrage (adapted from Jooste *et al*. 2013). The solid line indicates the acceptable level for long-term exposure (HQ= 1).

Hazard Quotients calculations for *O. mossambicus* exceeded one for daily consumption only (1.59), indicating that health effects may occur upon consumption of this fish (Table 4.2). While values below one were calculated for *O. mossambicus* (0.23) and *S. intermedius* (0.11) for weekly consumption (Table 4.2) and daily (0.79) for *S. intermedius* only (Table 4.2; Figure 4.2**)**.

Antimony naturally exists in the earth's crust (ATSDR 1992). Sources of Sb in water ecosystems are from coal combustion, industries, mines, agricultural run-offs, non-ferrous metal productions (like copper) and urban effluents (ATSDR 1992). Its effects are similar to those of Arsenic (ATSDR 1992). Antimony also has beneficial effects in medicine, it can be used to treat parasite infections in humans. According to the ATSDR (1992), elevated levels can irritate eyes and lungs, can cause heart and lung problems, stomach pain, diarrhea, vomiting and stomach ulcers. Nonetheless it is not classified as a human carcinogen (ATSDR 1992).

Arsenic

Arsenic concentrations for *O. mossambicus* were below detection limit, while for *S. intermedius* the highest and lowest values were 15 mg/kg and 4 mg/kg respectively. (Appendix Table 10). The mean concentration value of arsenic (As) for *S. intermedius*

(6 mg/kg) was higher than that of the sediment (5.5 mg/kg) (Table 4.1; Figure 4.1). The As recorded in the muscle tissue of *S. intermedius* had an unacceptable high HQ value of 6.63 for daily consumption, while for weekly consumption it had a value below one (Table 4.2; Figure 4.2).

Arsenic is a natural occurring element extensively dispersed in the earth's crust. Sources of As include; herbicides, fertilizers and insecticides, industrial practices, mining and coal burning (ATSDR 2007a). Arsenic is one of the four Endocrine Disruptive Metal (EDM) among cadmium, mercury and lead. They have the ability to interfere with the nervous system and reproduction thus threatening their survival (Bornman *et al.* 2009). Fish can accumulate As; most of which is in organic form and is less harmful. While in humans ingestion of inorganic As can escalate the risk of skin, liver, bladder and lung cancer. Inorganic As is highly carcinogenic to humans (ATSDR 2007a). The IARC (IARC 2012), classified As and inorganic As compounds in group 1: sufficient evidence in humans or sufficient evidence in animals and strong mechanistic data in humans inadequate in humans and limited in animals.

Barium

The highest (459 mg/kg) and lowest (237 mg/kg) barium (Ba) concentrations were recorded for *S. intermedius* (Appendix Table 10). The muscle tissues had higher mean barium (Ba) concentration values compared to sediment with mean values of 320 mg/kg for *S. intermedius* and 298 for mg/kg *O. mossambicus* and 196.5 mg/kg for sediment (Table 4.1). Barium levels recorded for both fish species had calculated HQ values of below one (Table 4.2), thus no adverse effects for Ba are expected upon human consumption of the two fish species.

Barium occurs in nature only in ores containing mixtures of elements (ATSDR 2007b). Excess Ba enters the environment through mining, insecticides, rodenticides, herbicides, refining, and production of Ba compounds and burning of coal and oil (ATSDR 2007b). Fish can accumulate Ba, while in humans it can cause gastrointestinal disturbances and muscular weakness, paralysis, vomiting, diarrhoea, bleeding of the kidneys, affecting the central nervous system and possibly death, however it is not carcinogenic (Dallas and Day 2004; ATSDR 2007b).

Boron

The highest and lowest boron concentration values were recorded for *S. intermedius* with values of 330 mg/kg and 186 mg/kg respectively (Appendix Table 10). Higher mean B concentration values were recorded in the two fish species than in the sediment with values of 257 mg/kg for *S. intermedius*, 246 mg/kg for *O. mossambicus* (Figure 4.1) and 181.76 mg/kg for sediment (Table 4.1). Boron levels recorded for both fish species had HQ values of below one (Table 4.2), therefore adverse effects for B may not be expected upon human consumption of the two studied fish species.

Boron is natural occurring, usually found in sediment and sedimentary rock formations and rarely exists in elemental form (ATSDR 2010). Human activities such as agricultural, mining and industrial activities, glass product manufacture and sewage discharges increases B in the environment (US-EPA 2008). It is an essential micronutrient in plants, however large amounts of B over a short period can be toxic to humans. Boron can affect the stomach, intestine, liver, kidney, and brain and may lead to death in humans, however it is not a known human carcinogen (ATSDR 2010).

Cadmium

Cadmium concentrations were below detection limits for both fish species though it was detected in the sediment (Table 4.1). The sediment had a mean value of 4.4 mg/kg. Cadmium is the only metal which was not detected in any of the two fish species during this study (Appendix Tables 9 &10). The cadmium HQ value calculated for *O. mossambicus* for daily consumption was below one and below detection levels for weekly consumption. For *S. intermedius* values were below detection levels for both weekly and daily consumption (Table 4.2). Therefore adverse effects for Cd may not be expected upon human consumption of the muscle tissue of the two fish species.

Cadmium is a natural element in the earth's crust (ATSDR 2008b). The main anthropogenic sources for Cd are mining, use of fertilizers, fossil fuel and waste combustion (ATSDR 2008b). Cadmium is highly toxic to freshwater aquatic life (ATSDR 2008b). According to ATSDR (2008b) and Bornman *et al.* (2009), Cd is an EDM, affecting the estrogenic hormones and are highly destructive to aquatic life. Exposure to lower levels of Cd in air, food, or water can result in a build-up in kidneys and may result in kidney disease (ATSDR 2008b). The International Agency for Research on Cancer (IARC 2012) classified Cd and Cd compounds in group 1: sufficient evidence in humans and limited evidence in animals.

Cobalt

An equivalent value of 1 mg/kg was recorded for Cobalt for the two fish species (Appendix Tables 9 & 10). Lower mean Co concentrations were recorded in the fish muscle tissue than the sediment. *Oreochromis mossambicus* and *S. intermedius* had average concentrations of 1 mg/kg (Figure 4.1) and 13 mg/kg for sediment (Table 4.1). Given the HQ values below one it can be assumed that no Co toxicity can be expected upon human consumption of the muscle tissue (Table 4.2).

Cobalt is a naturally occurring element found in water, animals, rocks, soil and plants (ATSDR 2004a). According to ATSDR (2004a), it enters the environment through natural sources as well as burning of coal, oil or the manufacture of cobalt alloys. It can be both beneficial (part of vitamin B12) and harmful to humans. Adverse effects that may arise include; diarrhea, dermatitis, vomiting, , kidney, lung and heart effects, coma and may result in death (ATSDR 2004a). Some Co can be carcinogenic according to the International Agency for Research on Cancer (IARC 2012) and ATSDR (2004a).

Chromium

The highest concentration for chromium was recorded for *S. intermedius*, while the lowest value was recorded for *O. mossambicus* with values of 18 mg/kg and 12 mg/kg respectively (Appendix Tables 9 & 10). The muscle tissues had lower Cr concentrations than in the sediment with mean values of 14 mg/kg in *O. mossambicus;* 15 mg/kg in *S. intermedius* (Figure 4.1) and 130.33 mg/kg in the sediment (Table 4.1). The HQ values for Cr were above one for daily consumption for both fish species, *S. intermedius* had a slightly higher value (2.69) compared to *O. mossambicus* (2.42) (Table 4.2; Figure 4.2).

The latter indicate possible risks upon consumption of the muscle tissues of the two fish. On a daily basis HQ values were below one for weekly consumption (Table 4.2) of the muscle tissue.

Chromium is a naturally occurring element found in organisms and the environment (ATSDR 2008c). The main sources of Cr are industries, mines, power plants and cooling towers (CCME 1999). It is an essential trace nutrient needed in small amounts for carbohydrate and lipid metabolism. Low Cr concentrations have been reported to temporarily reduce the growth phase for young fish, and chromium compounds are classified as human carcinogens (DWAF 1996c; ATSDR 2008c). Metallic Cr and Cr (III) compounds falls under group 3: inadequate in humans and limited in animals (IARC 2012). While Cr (VI) compounds are in group 1: sufficient evidence in humans/animals and strong mechanistic data in humans (IARC 2012).

Copper

The highest and lowest copper values were recorded for *S. intermedius* with values 8 mg/kg and 3 mg/kg respectively (Appendix Table 9 & 10). *Oreochromis mossambicus* had a higher Cu mean value of 5 mg/kg compared to the 4 mg/kg in *S. intermedius* (Figure 4.1), while 13 mg/kg was recorded for sediment (Table 4.1). The HQ values for Cu were below one thus for both weekly and daily it can be assumed that Cu toxicity can be expected upon consumption of the muscle tissue of the two studied fish (Table 4.2).

Copper is one of the world's widely used metals, occurring naturally in most waters (DWAF 1996c). Anthropogenic sources include sewage systems, fungicides and pesticides, mining, industries, coal-burning, iron- and steel-producing industries (DWAF 1996c). Invertebrates are adversely affected by elevated Cu concentrations, however, it is an essential element in animals (including humans) (DWAF 1996c; ATSDR 2004b). High doses can damage the liver and kidneys and can even result in death, however it is not carcinogenic (ATSDR 2004b).

Iron

The highest and lowest iron values were recorded for *S. intermedius* with values of 174 mg/kg and 47 mg/kg respectively (Appendix Table 9 & 10). A higher mean Fe concentration (126 mg/kg) was recorded for *O. mossambicus* compared to *S. intermedius* (112 mg/kg) (Table 4.1; Figure 4.1). Iron toxicity may not be expected upon consumption of *O. mossambicus* and *S. intermedius* muscle tissues because the HQ values were below one (Table 4.2).

Iron is the fourth most abundant element in the earth's crust (DWAF 1996c). It is an essential micronutrient in all organisms (Dallas and Day 2004). Anthropogenic sources of Fe are; mining agricultural activities and sewage effluents (Phippen *et al.* 2008). Iron can be toxic at high concentrations and it can excessively accumulate in fish and may result in reduction of normal functioning of organisms (Wepener *et al.* 1992; Dallas and Day 2004). Ferric oxide [Fe(III)]; Fe-dextrin complex and Fe sorbitol-citric acid complex are placed in group 3: Inadequate in humans and limited in animals (IARC 2012). While Fe-dextran complex is classified in group 2B: limited evidence in humans and less than sufficient evidence in animals (IARC 2012).

Manganese

The highest concentration of Mn was recorded for *O. mossambicus* with a value of 40 mg/kg and the lowest value was 2 mg/kg for both fish species (Appendix Table 9 & 10). Muscle tissues had lower mean concentrations of Mn than sediment, 15 mg/kg for *O. mossambicus*, 4 mg/kg for *S. intermedius* (Figure 4.1) and 297.17 mg/kg for the sediment (Table 4.1). With HQ values below one, it can be assumed that no Mn adverse effects can be expected upon human consumption of the muscle tissue (Table 4.2).

Manganese is a natural occurring metal that is found in numerous types of rocks (ATSDR 2008d). Sediment, soils and sedimentary rocks are natural sources of Mn, while industrial discharges, fertilizers, paint, dyes, glass and ceramic industries account as anthropogenic sources (Dallas and Day 2004). Manganese is an essential nutrient and small amounts are required everyday as part of a diet (ATSDR 2008d). However in larger amounts, it can cause a poisoning syndrome in mammals, with neurological damage which is sometimes irreversible (Dallas and Day 2004). Manganese cannot cause cancer because there is insufficient data (ATSDR 2008d).

Nickel

The highest concentration of nickel was recorded for *O. mossambicus* with a value of 23 mg/kg and the lowest value of 1 mg/kg for both fish species (Appendix Table 9 & 10). Nickel mean concentrations for muscle tissues were lower than the sediment. *Oreochromis mossambicus* had a mean value of 5 mg/kg, 1 mg/kg in *S. intermedius* (Figure 4.1) and 34.5 mg/kg for the sediment (Table 4.1). The Hazard Quotient values for Ni were below one (Table 4.2), thus no Ni toxicity is expected.

Nickel is an abundant natural element, it is released into the atmosphere by windblown dust, volcanoes, burning of fuel oil and industries involved in Ni refining, steel production and other Ni alloy production (ATSDR 2005a). Nickel is toxic in high and very low concentrations (Dallas and Day 2004). Nickel deficiency affects growth, dermatitis and haemoglobin (ATSDR 2005a). The IARC (2012) mentioned that some Ni compounds are carcinogenic to humans. It categorized Ni metals and alloys in group 2B (possibly carcinogenic to humans) (IARC 2012) and Ni compounds in group 1 (carcinogenic to humans) (IARC 2012).

Lead

The highest and lowest lead concentrations were recorded for *S. intermedius* with values 24 mg/kg and 1 mg/kg (Appendix Table 10). Lead mean concentrations were lower for muscle tissues than sediment. The muscle tissue of *O. mossambicus* had a mean value of 6 mg/kg, 3.2 mg/kg in *S. intermedius* and 6 mg/kg for the sediment (Table 4.1The Hazard Quotients for Pb were extremely high for both fish species especially for daily consumption of *O. mossambicus* (42.95) (Table 4.2; Figure 4.2). Therefore consumption of fish can cause adverse effects in humans.

Lead is a natural occurring metal found in the earth's crust in relatively small amounts. Most of the Pb concentrations are from anthropogenic activities such as burning fossil fuels, wastewater discharge and mining activities (ATSDR 2007c). Low Pb concentration in fish leads to adverse effects such as a formation of a film of coagulated mucous over gills and the whole body (DWAF 1996c). In humans it mainly affects the nervous system, brain damage and even death (ATSDR 2007c). The ATSDR (2007c) listed Pb as a probable human carcinogen and as an endocrine disruptor chemical/metal (EDM) affecting the reproductive hormones. The IARC has determined that inorganic Pb and Pb compounds are probably carcinogenic to humans (Group 2A), however there is little evidence whether organic Pb compounds will cause cancer.

Selenium

The highest concentration of selenium was recorded for *S. intermedius* with a value of 7 mg/kg while the lowest value of 1 mg/kg was recorded for both fish species (Appendix Table 9 & 10). *Oreochromis mossambicus* had a slightly lower mean value than *S. intermedius*, 2.4 mg/kg and 4.1 mg/kg respectively while sediment concentrations were below detection limit (Table 4.1). The Hazard Quotient values for Se were below one (Table 4.2), thus no Se toxicity is expected.

Selenium is a natural occurring mineral element widely distributed in nature (ATSDR 2003). Sources of Se are both natural and anthropogenic; weathering of rocks may result in low levels in water, while anthropogenic sources are from coal and oil burning (ATSDR 2003). Toxicity effects of Se in fish are; changes in feeding patterns, pathological changes, hematological changes and even death. Selenium is an essential micronutrient required at low levels. People exposed to acute levels of Se have reported; damage to nervous system, irritation and rashes however it is not a known human carcinogen (ATSDR 2003). Selenium and its compounds have been classified in group 3: inadequate in humans and limited in animals (IARC 2012).

Silver

The highest value recorded for silver was 21 mg/kg for *O. mossambicus*, while the lowest value (1 mg/kg) was recorded for both fish species (Appendix Table 9 & 10). *Oreochromis mossambicus* had a mean value of 5 mg/kg, 2 mg/kg in *S. intermedius*

(Figure 4.1) and 79.33 mg/kg in the sediment (Table 4.1). The Hazard Quotient values for Ag were below one (Table 4.2), thus no Ag toxicity is expected.

Silver is a rather rare metal that occurs naturally in the earth's crust (ATSDR 1990; Dallas and Day 2004). It occurs in several oxidation states, however ionic Ag is more toxic to aquatic organisms. In freshwater, Ag is commonly from geological sources, the anthropogenic sources are: mining and industrial activities, waste water and sewage effluents. Silver is toxic in low doses to freshwater fish (Dallas and Day 2004). Consumption of large doses of Ag nitrate can cause corrosive damage to the gastrointestinal tract, diarrhea, vomiting, shock, argyria and even death (ATSDR 1990). The IARC (2012) did not classify Ag or its compounds as carcinogens.

Tin

Highest tin concentration (7 mg/kg) was recorded for *S. intermedius* while the lowest (1 mg/kg) was recorded for *O. mossambicus* (Appendix Table 9 & 10). *Oreochromis mossambicus* had a mean value of 1.5 mg/kg, 4.6mg/kg in *S. intermedius* (Figure 4.1) and 3.5 mg/kg in the sediment (Table 4.1). No Hazard Quotient values for Sn were calculated (Table 4.2).

Tin is a natural occurring element in the earth's crust (ATSDR 2005b). It is released into the environment by both natural processes and human activities, such as mining, coal and oil combustion (ATSDR 2005b). Organic Sn compounds can accumulate in the central nervous system where, in very high concentrations, they have similar effects to those of As. Neither IARC (2012) nor ATSDR (2005b) has classified Sn or inorganic Sn as human carcinogens (ATSDR 2005b).

Strontium

The highest concentration recorded for strontium was 175 mg/kg for *O. mossambicus* while the lowest value of 2 mg/kg was recorded for *S. intermedius* at the inflow (Appendix Table 9 &10). *Oreochromis mossambicus* had a mean value of 75 mg/kg, 8.21 mg/kg for *S. intermedius* (Figure 4.1) and 26 mg/kg in the sediment (Table 4.1).

The Hazard Quotient values for Sr were below one (Table 4.2), thus no Sr toxicity is expected.

Strontium is a natural occurring element. The discarding of coal and incinerator ash and industrial wastes may increase Sr concentrations in the environment, some of it accumulates in fish, vegetables and livestock (ATSDR 2004c). Low levels of stable Sr has not been shown to affect adult health with an exception of stable Sr chromate (ATSDR 2004c). Furthermore, the only Sr compound that may result in cancer is Sr chromate, however it is due to Cr not Sr. High levels of radioactive Sr can damage bone marrow, cause anaemia and prevent the blood from clotting normally. The IARC has concluded radioactive Sr as a human carcinogen because it emits beta radiation (ATSDR 2004c).

Titanium

Highest titanium concentration was recorded for *O. mossambicus* with a value of 4 mg/kg, while the lowest value of 1 mg/kg was recorded for both fish species (Appendix Table 9 & 10). *Oreochromis mossambicus* had a mean value of 2 mg/kg, 1.53 mg/kg in *S. intermedius* (Figure 4.1) and 672.67 mg/kg in the sediment (Table 4.1).

Titanium is the seventh-most abundant metal in the earth’s crust and is always bonded to other elements in nature (ATSDR 1997). The problematic one is Ti tetrachloride which is not found naturally. It is primarily from air emissions and when it comes into contact with water, it rapidly forms hydrochloric acid and Ti oxide. It is very corrosive and can cause burns. Titanium compounds can cause irritation to the skin, eyes, lungs eventually leading to death, however there is no evidence that chronic exposure to Ti can cause cancer (ATSDR 1997).

Vanadium

Vanadium concentrations were only recorded for *O. mossambicus* for two fish (8 mg/kg and 3 mg/kg), while for *S. intermedius* were below detection limit (Appendix Table 9 & 10). The mean V concentration value for *O. mossambicus* was 5.5 mg/kg and 79.33

mg/kg in the sediment. Given the HQ values below one it can be assumed that no V toxicity can be expected upon human consumption of the muscle tissue (Table 4.2).

Vanadium and its compounds can be found in the earth's crust and rocks (ATSDR 2009). It mainly enters the environment from natural sources (continental dust, volcanic emissions and marine aerosols) and from anthropogenic sources such as burning of fuel oils and coal. Clinical signs of V toxicity included respiratory distress, mucosal irritation, diarrhea, necrosis of liver cells and cloudy swelling of renal tubules and paralysis of the legs (US-EPA 1992). Vanadium is not classified as a human carcinogen (ATSDR 2009). The IARC (2012) has classified V pentoxide as possible carcinogen to humans and placed it in group 2B.

Zinc

The highest (249 mg/kg) and lowest (161 mg/kg) zinc concentrations were recorded for *S. intermedius* (Appendix Table 10). A mean value of 214 mg/kg was recorded for *O. mossambicus*, 209 mg/kg for *S. intermedius* (Figure 4.1) and 105.67 mg/kg for sediment (Table 4.1) thus implying biomagnification took place. The Hazard Quotients for Zn were below one, it can be assumed that no Zn toxicity can be expected upon human consumption of the muscle tissue (Table 4.2).

Zinc is one of the most common elements in the earth's crust. Natural sources of Zn include weathering and erosion from rocks, industrial wastes, fertilizers and insecticides (DWAF 1996c). Zinc is an essential micronutrient for all organisms (Dallas and Day 2004). It can cause mortality in fish, it forms insoluble compounds in the mucus covering the gills therefore impairing the respiratory function, oedema and liver necrosis (DWAF 1996c; Jackson 2005). In humans it can cause stomach cramps and vomiting and anemia It is however not identified nor classified as a human carcinogen by the IARC (2012).

Discussion of the results

Bioaccumulation

In general, the levels of metals obtained in this study were somewhat lower in the muscle tissues than the sediment however with a few exceptions (Ba, B, Zn, Sb and Se). Thus the barrage could be acting as a sink for sediment/silt and metals from the middle catchment and the lower catchment upstream of the barrage (Buermann *et al*. 1995). Results from the sediment analysis suggest that the metals were settling in the sediment at the Phalaborwa Barrage as there appeared to be some metals which were elevated. This can be attributed to a build-up of metals coming from the Upper catchment of the Olifants River and accumulating in the lower catchment. The Phalaborwa Barrage is a heavily silted location with a build-up of fine sediment rich in organic matter (Van Vuren *et al.* 1994; Buermann *et al.* 1995). Thus with low flow seasons (low rainfall and no flushing) there is bound to be a continuous immobilization of metals in the sediment. Higher metal concentrations were detected in *O. mossambicus* than *S. intermedius*, this may be as a result of dissimilar food particles they feed on. The fish species accumulated all metals detected in sediment with the exception of Se.

In this study the bioaccumulation of Al in the two fish species was elevated and differed slightly. In a study done by Jooste *et al*. (2013), in the Flag Boshielo Dam, they recorded almost similar Al concentrations in muscle tissues to those recorded in this study. Unacceptable high concentrations of As were recorded in *S. intermedius* and the sediment only. Arsenic concentration for S. intermedius was higher than in sediment which may indicate that the fish species may have biomagnified the metal from its food sources. Arsenic has been classified as a carcinogen by US-EPA (2007), regular consumption of contaminated fish may result in adverse health problems. According to DWAF (1996c), humans are more sensitive to As than aquatic organisms, studies indicate that it is slowly excreted from the human body hence more time for absorption in different tissues (Kapaj *et al.* 2006). Concentration of As (0.7 mg/kg) was lower in *S. intermedius* from Flag Boshielo Dam (Kekana 2013) compared to this study.

Furthermore the slightly elevated As concentration may be as a result of weathering of rocks or agricultural activities as it is present in fertilizers and pesticides (DWAF 1996c).

The mean concentrations for Ba and B were higher than all the other metals detected in the muscle tissues of the two fish species, however *S. intermedius* had higher levels than *O. mossambicus*. Concentrations in *S. intermedius* from Flag Boshielo Dam (Kekana 2013) were found to be lower for Ba (3.3 mg/kg) and B (121 mg/kg) compared to this study.The high Ba and B concentrations may have been affected by weathering of rocks, anthropogenic activities in the upper and middle catchments. It can be concluded that *O. mossambicus* accumulated these metals (Ba and B) from the detritus and algae (food-chain) in the water, since B was recorded at lower concentration levels in the water and sediment (see Chapter 2). The findings are similar to the study by Emiroğlu *et al.* (2010), where they determined B concentrations in water, sediment and biotic samples (amphibians bivalves, crustaceans, fish, gastropod, hirudineans, insect larvae, snakes and plants) and the fish species, accumulated more B compared to all other biota sampled.

Cadmium levels were below detection limits in the muscle tissues, however present in the sediment. This implies that Cd accumulated in the sediment however it was not bioavailable to the fish. However this may change over time as Cd has the potential to accumulate in fish tissues as seen in the study on *L. rosae* (0.3 mg/kg) by Kekana (2013) at Flag Boshielo Dam. For now Cd does not pose any major problem in fish at Phalaborwa Barrage, however further monitoring and studies of this metal is essential due to its high toxicity.

Chromium, Cu, Fe and Mn in this study were higher than those recorded in a study by Robinson and Avenant-Oldewage (1997), where they tested bioaccumulation levels of metals of some organs and tissues of *O. mossambicus* from the Lower Olifants River. These metals can originate from mining, sewage effluents in the upper, middle and lower catchment areas.

Nickel was detected in low concentrations especially for *S. intermedius*, the results were lower compared to the work done by Nussey *et al.* (2000) in the tissues of *Labeo* *umbratus* (moggel) from the Witbank Dam where the highest Ni value was 114.51 mg/kg.

Comparison to the results by Kekana (2013) who studied metal bioaccumulation in muscle tissues of *S. intermedius* and *L. rosae* revealed similar concentrations of 3.2 mg/kg and 2.5 mg/kg for Ni.

Strontium was considerably higher in muscle tissues of *O. mossambicus c*ompared to *S. intermedius*. Seymore *et al.* (1995), observed a trend of bioaccumulation of Mn, Pb and Sr in various organs of *Barbus marequensis* (yellow fish), now *Labeobarbus marequensis*, from the Lower Olifants River. Strontium and Mn concentrations recorded in this study for *O. mossambicus* were higher than some recorded by Seymore *et al.* (1995), while for *S. intermedius* Mn, Pb and Sr concentrations were lower. The highest mean Mn, Pb and Sr concentrations recorded for *L. marequensis* by Seymore *et al.* (1995) were 12 mg/kg, 36 mg/kg and 60 mg/kg dry weight respectively. The Phalaborwa Barrage was one of their selected study sites, thus this implies that the concentrations of Sr and Mn have increased over the years indicating that more pollution may have taken place. Strontium concentrations recorded may be from Loskop Dam as it acts as a basin for heavy metals from mining activities at the upper catchment (Grobler *et al*. 1994), while Mn may be from fertilizers and industrial discharges.

Higher mean concentrations of Zn were recorded in the muscle tissues of both fish species, this was however not in accordance with the concentrations recorded by Kotzè *et al.* (1999). They recorded values below 100 mg/kg dry weight in the muscle tissues of *O. mossambicus* and *C. gariepinus* from the Lower Olifants River catchment. The high concentrations of Zn may be as a result of agricultural activities around catchment or natural sources such as weathering of rocks.

Low concentrations of the following metals; Co, Sb, Sn, Ti, Ag and Se were recorded in the muscle tissues of both fish species with the exception of V which was recorded for *O. mossambicus* only. The levels of above mentioned metals during this study could be due to the low levels of these metals in the natural environment.

Recorded differences in the bioconcentration of metals in the two fish species in this study may be as a result of metabolic, physiological and behavioural adaptations of fish at the Phalaborwa Barrage. Younger *O. mossambicus* normally feeds on algae, detritus, and diatoms while larger ones feed on insects and other invertebrates (Skelton 2001). Detritus, algae and insects are usually suspended in the water, therefore, increasing its potential to accumulate most of the metals found in the water than in the sediment. *Schilbe intermedius*, on the other hand, is more active at night (Skelton 2001). It is known to be an opportunistic predator, its diet mainly consist of fish, aquatic insects larvae, terrestrial insects, aquatic insects and crustaceans (Winemiller and Kelso-Winemiller 1996).

Omnivorous fish accumulate more metals than other fish with different feeding habits (Mathis and Cummings 1973). This could be the reason why *O. mossambicus* accumulated higher metal concentrations than *S. intermedius* in this study. Notably high levels of Ba, B and Zn were recorded for *O. mossambicus*, this may be due to the size differences as concentrations were generally lower with increasing fish size. The current data and those of previous studies (Coetzee *et al.* 2002), point out that the size of fish must be considered, especially when dealing with juveniles compared to adult fish. According to Coetzee *et al.* (2002), there is a correlation between the bioaccumulation of metal concentrations and the lengths of the fish.

Human Health Risk Assessment

The metal bioaccumulation results in this study indicate that the selected fish species are absorbing metals from their environment. The metals will be conveyed to organisms that consume these fish, including humans. The Olifants River catchment is surrounded by mostly rural communities which depend on fish from the river and impoundments in the catchment as a food source to supplement their diet (source of protein).

Human health risk is predicted by evaluating the inherent ability of a chemical to cause adverse effects (Heath *et al.* 2004). Risk assessment is the process of quantifying the probability of a damaging effect to individuals or populations from certain human activities. The use of specific chemicals, or the operations of specific facilities (power plants) is not allowed unless it can be shown that they do not augment the risk of fatality or illness above a specific threshold (Merrill 1997). Certain metals, viz, Pb, As and Cr for *S. intermedius* and Pb, Cr and Sb for *O. mossambicus* detected in the muscle tissues of the selected fish species were at levels where people consuming fish from the barrage place themselves at serious health risks (e.g. cancer). A risk assessment is done if the concentration levels of toxic constituents are above AEV and some selected metals (Pb, As, Cr and Sb) for this study were above toxic levels thus it was done. Health effects associated with the ingestion of fish containing metals that may cause human health risks are discussed in more detail under each metal above. The health risk posed to children of the community would be extensively higher than that of adults because children weigh less than adults (US-EPA 1997).

Lead, As, Cr and Sb Levels were at concentrations that would be expected to cause both toxic effects (with hazard quotients ranging from close to double to twenty times the safe dose). As well as carcinogenic risks of between 1 – 4 people in 1,000 having the chance of developing cancer at the concentrations of Pb, As and Cr measured in the fish (Jooste *et al*. 2013). The risk to human health linked with consuming contaminated fish from the Phalaborwa Barrage impoundment was evaluated based on weekly and daily consumptions of a single 150 g fish meal (Jooste *et al.* 2013). Hazard Quotients were calculated for metals found in selected fish species and summed to provide a measure of the potential risk of consuming the fish (Jooste *et al.* 2013). According to Jooste *et al.* (2013), HQ values for the daily consumption of fish are seven times higher than those for weekly consumption. The Phalaborwa Barrage exceeded the recommended HQ for Pb for the weekly consumption of the two species. For the daily consumption selected fish species from the barrage exceeded the recommended HQ for Pb (exception *S. intermedius*), As (exception *O. mossambicus*), Cr and Sb (Jooste *et al*. 2013). According to Jooste *et al*. (2013), of the species considered, evidence from surveys and fish markets in the Olifants River catchment suggests that *O. mossambicus* is favoured among rural communities which is of great concern because the fish recorded elevated Total HQ at the barrage especially for Pb .

The Total HQ value for the two species from Phalaborwa Barrage exceeded the recommended value of one, the highest was recorded for *O. mossambicus* based on both weekly and daily consumption (Table 4.2). This is of great concern because as mentioned above this species is one of the most favoured among the rural community.

This study further illustrated how assessing environmental levels of metals by means of fish can be applied in ecological effects assessments. *Schilbe intermedius* is one of the fish species found to be more abundant at the Phalaborwa Barrage than *O. mossambicus*. Furthermore, it was the most useful bioindicator species because there was always sufficient supply of it during this study while *O. mossambicus* was always found in lesser numbers thus making sampling difficult. The use of *S. intermedius* is recommended because it was abundant and produced consistent results.

4.4 SUMMARY

The majority of the metals (Al, Ag, Ba, B, Co, Cu, Cr, Fe, Ni, Mn, Pb, Sb, Se, Sn, Sr, Ti and Zn) were detected in all the fish samples collected in the Phalaborwa Barrage (Table 4.1). Arsenic was below detection limits in *O. mossambicus*, V in *S. intermedius* and Cd for both fish. Generally, the two fish species sampled had lower concentrations for most metals as compared to sediment (Table 4.1).

Accumulation of metals in *O. mossambicus* followed the decreasing order:

Ba> B> Zn> Fe> Sr> Al> Mn > Cr > Ag > Pb > Ni > Cu > Se > V > Sb > T i> Sn > Co.

For *S. intermedius*: Ba > B > Zn > Fe > Al > Cr > Sr > Mn> Cu > As> Pb > Se > Sn > Ag > Ti > Ni > Sb > Co (Table 4.1). Concentrations of Al, Ba, B, Co, Cu, Cr, Fe, Pb, Sb, Se and Zn were almost the same in the muscle tissues of both fish species. The following metals were higher in *O. mossambicus* than *S. intermedius* Zn> Fe> Sr> Al> Mn> Pb> V> Ni> Ag> Cu> Ti> Sb. While Ba> B> Cr> Sn> Se were higher in *S. intermedius* than *O. mossambicus* (Table 4.1). There was no significant difference in bioaccumulation levels between the two fish species at the Phalaborwa Barrage (F=1.412; p=0.250).

Results from the human health risk assessment for both fish species indicate that certain metals have HQ values where adverse effects may arise, especially for *O. mossambicus.*

4.5 CONCLUSIONS

Human actions such as agricultural and mining activities and sewage discharges in the catchment area of the Olifants River contributed to the rise of metal concentrations in the fish muscle tissues and sediment. However activities mentioned in the latter statement may not be the only sources of the escalating metal concentrations. Activities upstream such as poor agricultural management and bad mining activities may have contributed to some of the elevated metals and sediment loads (Heath *et al*. 2010) and have detrimental effects on aquatic organisms (e.g. fish). Consequently, monitoring in the Olifants River indicates that the quality of water has deteriorated since the 1970s as a result of industrial, mining and agricultural activities (De Villiers and Mkwelo 2009). Therefore, it is likely that the distribution pattern of fish in the river inflow of the barrage exposes them to a more concentrated mix of pollutants than they would experience in the main body of the barrage where pollutants would be more diluted.

The hypothesis that metals bioaccumulate in aquatic biota like fish was supported in this study as each fish species showed a high level of some metals at some point during this study. It was however noted that metal concentrations in *O. mossambicus* were generally higher than in *S. intermedius* this may be as a result of their different feeding habits (diet). The human health risk assessments available in the Water Research Commission (WRC) report by Jooste *et al.* (2013) recommends that fish from the barrage are unsuitable for continuous weekly/daily consumption of the fish as some metals such as Pb, Cr and Sb for *O. mossambicus* and Pb, As and Cr for *S. intermedius* may result in serious adverse human health effects such as cancer. Furthermore effective monitoring programs should be considered to evaluate whether evidence of long-term health effects of the metals identified in this study can be isolated in the rural communities consuming fish from the Phalaborwa Barrage.

CHAPTER 5

GENERAL SUMMARY AND CONCLUSION

Pollution has been a great concern in the Olifants River System over the past decade. The state of the Olifants River System is deteriorating due to the various anthropogenic activities in the Upper Catchment (Van Vuuren 2009; Ashton and Dabrowski 2011). It has experienced massive fish and crocodile mortalities, particularly in the upper and lower catchment and it is under constant pressure from both demand and supply (Heath *et al.* 2010; Van Vuuren 2010).

In the present study, all physico-chemical system variables were within the target water quality range (TWQR) for aquatic ecosystems suggested by the DWAF guidelines (DWAF 1996c) with an exception of turbidity during spring. The anions such as chloride, fluoride and sulphate and cations (Ca, Mg, K and Na) were all within the TWQR for aquatic ecosystem (DWAF 1996c) and domestic use (DWAF 1996a) at the barrage. Elevated levels of nutrients (nitrate, nitrite, sulphate and phosphate) were recorded at the barrage. In the present study, eutrophic conditions were recorded for nitrate during spring and for phosphorus during three seasons (winter, spring and summer) and hypertrophic conditions at the inflow during summer.

The toxic water constituents (Al, As, Ba, B, Fe, Sb, Se, Sn and Sr) differed notably during the sampling seasons. The mean concentrations of Al, As Se and Sb were above the TWQR for aquatic ecosystems, whereas those of Ba, B, Mn, Fe and Sr were within the suggested TWQR for aquatic ecosystems (DWAF 1996c; US-EPA 2001; BC-EPD 2006; CCME 2012).

The quality of water at the barrage is much better compared to the Flag Boshielo and Loskop Dams (Oberholster *et al.* 2010; Jooste *et al.* 2013).

The toxic constituents; Al, Ba, B, Cr, Fe, Mn, Ti and Zn were at elevated levels in the sediment. There are no guidelines for Al, Ba, B, Fe, Mn and Ti. However guidelines for Cd, Cr and Zn (CCME 1999), and the values recorded during this study for Cd and Cr were above the suggested guidelines value while for Zn they were below the suggested value. The sources of metals above the TWQRs are believed to be mainly from the agricultural activities around and above the catchment and water flowing in from the polluted upper Olifants catchment. Even though some metals were high, most of the water constituents were within the mentioned TWQRs. This could be due to the dilution effect from the Blyde River, however this may have no effect on the sediment quality.

The hypothesis that metals adhere to sediment was confirmed in this study and it can be concluded that the water and sediment at the Phalaborwa Barrage is moderately polluted. Frequent flushing of the sluices and supply of water from the Blyde River should be done constantly so that the effects can be reduced to some level. On the other hand, with increasing demand for water, pile up of silt and increased pollution in the upper catchments this will be difficult to achieve. In conclusion, monitoring of the Olifants River System should continue as the situation may worsen with time.

Based on the HAI, *O. mossambicus* was more affected during winter (with higher HAI) while *S. intermedius* was more affected during summer (compared to other seasons). Lower HAI mean values were recorded in autumn and spring for both fish species which is what was expected when compared with the water quality data. Parasite infestation, liver discolorations, eye defects, deformed gills, skin inflammations, fin defects and blood parameter values contributed to elevated HAI values during this study. With the exception of the liver condition, the health of the fish species was not much impaired during this study. The fish species appear to be in acceptable physical form with no obvious health related problems with the exception of the liver abnormalities. No abnormalities of opercules, hindgut, kidneys, and spleen were observed during the four sampling seasons.

The condition factor did not differ much between the two fish species. The factors ranged from 1.09 to 3.63. Condition factor of 1.60 indicate an excellent condition (trophy class fish) while 0.80 indicate extremely poor fish condition with a big head and narrow, thin body (Barnham & Baxter 1998).

The hypothesis that the number of ecto-parasites will be lesser in polluted water and the number of endo-parasites will be higher was not supported for both the fish species during the sampling seasons. Thus it can be concluded that the original evidence of the

IPI, the number of ecto-parasites increases with improving water quality, was not well supported by this study.

The monogeneans (ecto-parasites) were recorded in higher numbers than any other group. Ecto-parasite numbers were always higher than endo-parasites for both the fish species with the exception of spring and summer in *O. mossambicus.* This further supports the statement that ecto-parasite numbers are higher in less impacted waters. Copepods were also present on both fish species.

Digeneans and cestodes were recorded in higher numbers for *O. mossambicus* than *S. intermedius* with the exception of nematodes which were present in *S. intermedius* only.

The parasitic infestation statistics (mean abundance, prevalence and mean intensity) from *O. mossambicus* and S. *intermedius* differed seasonally during this study. Parasites recorded during this study showed to be site specific with monogeneans always recorded from the gills and stomach lining (*O. mossambicus*), copepods always from the skin (*O. mossambicus*) and gills (*S. intermedius*). For *O. mossambicus,* digenean larvae were collected from the skin, gills, small intestine, fins, eyes, branchial body cavity and muscle (depending on the species), while for *S. intermedius* they were found in the body cavity only. Cestode larvae were recorded from the outer lining of the small intestine for *O. mossambicus*, while for *S. intermedius* an unidentified cestode larvae was recorded from the body cavity. Nematodes were found in the body cavity (larvae) for *O. mossambicus* while for *S. intermedius* they were found in the intestine (adult) and body cavity (larvae).

Higher numbers of monogeneans were recorded for *S. intermedius* than *O. mossambicus* during this study. Nevertheless, large numbers of monogeneans is not uncommon in freshwater fish. However, the prevalence and mean abundance of all parasites recorded during this study was relatively low and there is no reason for concern at this stage. The hypothesis that fish parasites can be used as bio-indicators of pollution was not well supported during this study, as ecto-parasites were higher than endo-parasites for both fish species, it is said endo-parasites are higher than ecto-parasites in polluted water (depending on the type of pollution).

All the metals detected in the sediment were found in the muscle tissues with the exception of Cd. Barium, B and Zn were the metals with the highest concentrations. The two Endocrine Disruptive Compounds detected at the barrage were As and Pb which can affect the normal functioning of the endocrine system.

Metals detected in the sediment had higher concentrations compared to those in the muscle tissues with a few exceptions (Ba, B, Sr and Zn).

The following metals were higher in *O. mossambicus* than *S. intermedius* Zn> Fe> Sr> Al> Mn> Pb> V> Ni> Ag> Cu> Ti> Sb and Ba> B> Cr> Sn> Se were higher in *S. intermedius* than *O. mossambicus* (Table 4.1). The metal bioaccumulation results in this study show that the selected fish species are absorbing metals from their environment. The metals would be conveyed to organisms that consume these fish (humans). The human health risk assessments suggest that the fish from the Phalaborwa Barrage are not suitable for continuous human consumption as some of the metals were at levels where the recommended HQ level of one was exceeded. The Hazard Quotients recommended for Pb for weekly consumption was exceeded for both fish species. The HQ values based on daily consumption for *O. mossambicus* (Pb, Cr and Sb) and *S. intermedius* (Pb, Cr and As) as a result are expected to cause adverse health effects (some carcinogenic) in humans may specifically in children upon consumption of these muscle tissues (Jooste *et al*. 2013). Higher Total HQ values were recorded for *O. mossambicus* based on both weekly and daily consumption.

The heavy metal concentrations recorded in water, sediment and muscle tissues of selected fish are of great concern, Therefore, the incorporation of HAI, PI and IPI bioaccumulation can provide a more expanded view of the ecological state of a river system and/or catchment as a whole.

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APPENDIX

Table 1: The seasonal water quality constituents at the three sampling sites of the Phalaborwa Barrage.

Water constituent	May 2010			July 2010			October 2010			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^{-1}	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
Phosphorus (mg/ℓ P)	<0.025	<0.025	<0.025	<0.025	0.048	<0.025	0.03	0.049	0.031	0.450	0.200	0.060
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
Sulphur (mg/ℓ S)	24	24	24	20	23	21	12	11	13	23	18	18

Table 2: Seasonal metal concentrations in the water column at the three sampling sites at the Phalaborwa Barrage.

Metals in mg/ℓ	May 2010			July 2010			October 2010			February 2011		
	Inflow	Wall	Below	Inflow	Wall	Below	Inflow	Wall	Below	Inflow	Wall	Below
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

Table3: Phosphorus and metal concentrations in the sediment at the three sampling sites in the Phalaborwa Barrage.

Metals mg/kg	Winter 2010			Summer 2011				
	Inflow	Wall	Below	Inflow	Wall	Below	Mean	SD
Phosphorus	107	346	127	194	142	86	167	±95
Aluminium	1730	1568	2352	5000	9200	3800	3942	±2893
Arsenic	3	8	*	*	*	*	5.5	±4
Antimony	1	*	2	*	*	*	1.5	±1
Boron	236	248	243	42	*	*	182	±178
Barium	316	414	333	84	2	12	194	±100
Cadmium	*	*	*	12	*	10	11	±6
Cobalt	4	26	6	16	12	14	13	±52
Chromium	3	177	35	5	7	42	45	±8
Copper	5	37	6	10	12	8	13	±12
Iron	4600	24480	7184	36600	16800	27600	19544	±12360
Lead	5	9	4	*	*	*	6	±3
Manganese	129	852	154	202	254	192	297	±275
Nickel	12	90	17	28	38	22	35	±29
Silver	41	102	95	*	*	*	79	±33
Strontium	13	6	9	24	36	14	17	±19
Tin	*	*	*	*	14	*	14	±7
Titanium	38	100	90	1830	582	1396	673	±767
Vanadium	8	43	17	212	42	154	79	±84
Zinc	155	185	168	96	16	14	106	±76

*Below detection level

Table 4: Fish health variables with assigned characters showing the norm and deviation from the norm in the necropsy based system (after Adams *et al.* 1994).

Variables	Variable condition	Original field designation	Substituted value for the HAI
	External variables		
Eyes	Normal	N	0
	Exopthalmia	E1/E2	30
	Haemorrhagic	H1/H2	30
	Blind	B1/B2	30
	Missing	M1/M2	30
	Other	OT	30
Fins	No active erosion or previous erosion healed over	0	0
	Mild active erosion with no bleeding	1	10
	Severe active erosion with haemorrhage / secondary infection	2	20
Skin[a]	Normal, no aberrations	0	0
	Mild skin aberrations – “black spot” < 50	1	10
	Moderate skin aberrations – “black spot” > 50	2	20
	Severe skin aberrations	3	30
Opercules	Normal/no shortening	0	0
	Mild/slight shortening	1	10
	Severe shortening	2	20
Gills	Normal	N	0
	Frayed	F	30
	Clubbed	C	30
	Marginate	M	30
	Pale	P	30
	Other	OT	30
Spleen	Black	B	0
	Red	R	0
	Granular	G	0
	Nodular	NO	30
	Enlarge	E	30
	Other	OT	30
Hindgut	Normal, no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal	N	0
	Swollen	S	30
	Mottled	M	30
	Granular	G	30
	Urolithic	U	30
	Other	OT	30

Table 4 continued

Variables	Variable condition **Internal variables**	Original field designation	Substituted value for the HAI
Liver	Red	A	0
	Light red	B	30
	“Fatty” liver, “coffee with cream” colour	C	30
	Nodules in liver	D	30
	Focal discolouration	E	30
	General discolouration	F	30
	Other	OT	30
Blood (haematocrit)	Normal range	30-45%	0
	Above normal range	>45%	10
	Below normal range	19-29%	20
	Below normal range	<18%	30
Parasites			
*Endoparasites[b]	No observed endoparasites	0	0
	Observed endoparasites< 100	0	10
	101 -1000	1	20
	> 1000	3	30
*Ectoparasites[b]	No observed ectoparasites	0	0
	Observed ectoparasites 1 - 10	1	10
	11 - 20	2	20
	> 20	3	30

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

Table 5: Health Assessment Index (HAI) of *Oreochromis mossambicus* 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	EctoIPI	Endo-PI	Total HAI	HAI IPI
	SL	**TL**																	
AUTUMN (a)																			
1	10.5	13.5	43.2	M	0	0	0	0	30	0	0	0	0	0	10	30	10	50	70
2	10.5	14.6	43.6	M	0	0	0	0	30	0	0	0	0	0	10	30	0	50	70
3	16	19.1	121.9	M	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
4	10	12	26.8	M	0	0	10	0	0	0	0	0	0	0	10	30	10	30	50
5	12	15	39.3	M	0	0	0	0	0	30	0	0	0	0	20	20	0	50	50
6	7.9	9.5	11	M	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
7	4.2	5.8	2.2	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
8	4.5	6	2.5	M	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
9	4.4	5.8	2.2	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
10	4.3	5.7	2.9	M	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
														Total	**110**	**290**	**40**	**260**	**440**
														Mean	**11**	**29**	**4**	**26**	**44**
WINTER (b)																			
1	18	22	235.2	M	0	0	0	0	30	0	0	0	0	0	10	30	10	50	70
2	20	23	213	F	0	10	0	0	0	0	0	0	0	20	20	20	10	60	60
3	17.6	22	189	M	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
4	18.3	22.5	215.5	M	0	10	0	0	30	0	0	0	0	0	10	30	10	60	80
5	13	16.2	104.8	M	0	0	0	0	30	0	0	0	0	0	10	30	10	50	70
6	18	22	197	F	0	0	0	0	30	0	0	0	0	0	10	30	10	50	70
7	18	22	197	F	0	10	0	0	30	0	0	0	0	0	10	30	10	60	80
8	15.8	18.4	132.3	F	0	0	0	0	30	0	0	0	0	0	10	30	10	50	70
9	14.3	17.1	121.2	M	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
10	17.6	21.9	198.5	M	0	0	0	0	30	0	0	0	0	20	10	30	10	70	90
														Total	**110**	**290**	**100**	**490**	**670**
														Mean	**11**	**29**	**10**	**49**	**67**

Table 5 continued

SPRING (c)																			
1	14.5	17.5	101	M	0	0	0	0	30	0	0	0	0	0	20	20	10	50	60
2	13.8	16.7	100	M	0	0	0	0	0	0	0	0	0	20	20	20	10	50	50
3	11	13.3	45.7	M	0	0	0	0	30	0	0	0	0	0	20	20	10	60	60
4	14	16.6	60.5	F	0	0	10	0	30	0	0	0	0	0	10	30	10	60	70
5	14	16.5	100	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
6	11.2	13.8	24.5	M	0	0	0	0	0	0	0	0	0	0	0	30	0	0	30
7	10	12.8	78.7	M	30	0	0	0	0	0	0	0	0	0	10	30	10	50	70
8	11.2	14.1	52.8	M	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
9	17.2	13.8	110.5	M	0	0	0	0	30	0	0	0	0	0	10	30	10	50	70
10	15.1	18.2	121.8	M	0	0	0	0	0	0	0	0	0	0	0	30	10	10	30
11	14.3	16.8	107.8	M	0	0	0	0	0	0	0	0	0	0	0	30	0	0	30
12	13.3	15.1	105	M	0	0	0	0	0	0	0	0	0	0	0	30	0	0	30
														Total	**110**	**330**	**70**	**350**	**560**
														Mean	**9.1**	**27.5**	**5.8**	**329**	**47**
SUMMER (d)																			
1	13	9.5	11.4	M	0	0	0	0	30	30	0	0	0	0	20	20	**10**	**90**	**90**
2	13	13	40	M	0	0	0	0	30	0	0	0	0	0	10	30	**10**	**50**	**70**
3	14	13	40	M	0	0	0	0	30	0	0	0	0	0	10	30	**10**	**50**	**70**
4	17.6	14	42	M	0	0	0	0	30	0	0	0	0	0	20	20	**10**	**60**	**60**
5	18.3	17.6	118	M	0	0	0	0	30	0	0	0	0	20	10	30	**10**	**70**	**90**
6	19.3	18.3	124.8	M	0	0	0	0	0	0	0	0	0	0	0	30	**0**	**0**	**30**
7	18.9	19.3	123.9	F	0	0	0	0	0	0	0	0	0	0	0	30	**0**	**0**	**30**
8	17.9	18.9	113.7	M	0	0	0	0	0	0	0	0	0	20	0	30	**0**	**20**	**30**
9	19.1	17.9	109.6	M	0	0	0	0	0	0	0	0	0	20	0	30	**0**	**20**	**30**
10	17.5	19.1	107.3	M	0	0	0	0	0	0	0	0	0	20	0	30	**10**	**30**	**30**
11	17	17.5	114.3	M	0	0	0	0	0	0	0	0	0	20	0	30	**0**	**20**	**30**
12	16	17	102	F	0	0	0	0	0	0	0	0	0	20	0	30	**0**	**20**	**30**
13	13	16	110	F	0	0	0	0	0	0	0	0	0	20	0	30	**0**	**20**	**30**
														Total	**70**	**370**	**60**	**450**	**620**
														Mean	**5.3**	**28.5**	**4.6**	**35**	**48**

Table 6: Health Assessment Index (HAI) of *Schilbe intermedius* at the Phalaborwa Barrage.

Fish #	Length cm		Mass g	Sex	Eyes	Skin	Fins	Oper-cula	Gills	Liver	Spleen	Hind gut	Kid-ney	BloodHct (%)	Ecto-PI	Ecto-IPI	Endo-PI	Total HAI	HAIIPI
	SL	**TL**																	
AUTUMN (a)																			
1	34	38.5	587	F	0	0	0	0	30	30	0	0	0	0	20	20	10	90	90
2	19.4	21.6	82.8	M	0	0	0	0	0	0	0	0	0	0	20	20	0	20	20
3	12	13.5	21.3	F	0	0	0	0	0	0	0	0	0	0	30	10	0	30	10
4	28	33	344.6	M	0	0	0	0	0	0	0	0	0	0	0	30	0	0	30
5	17.9	21.1	72.2	M	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
6	22	25	124	M	0	0	0	0	0	30	0	0	0	0	10	30	0	40	60
7	19	22	83	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
8	26.9	31.3	320	F	0	0	0	0	0	0	0	0	0	0	30	10	0	30	10
9	19.8	22.3	84.7	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
10	31	36	445	F	0	0	0	0	0	0	0	0	0	0	0	30	10	10	40
														Total	**140**	**240**	**30**	**260**	**360**
														Mean	**14**	**24**	**3**	**26**	**36**
WINTER (b)																			
1	25.5	28.5	185	F	0	0	0	0	0	30	0	0	0	20	10	30	10	70	90
2	28.5	33	379	F	0	10	0	0	0	0	0	0	0	0	20	20	10	40	40
3	19	22	79	M	0	0	0	0	0	0	0	0	0	0	20	20	10	30	30
4	31	37.5	504	F	0	10	0	0	0	30	0	0	0	20	10	30	10	80	90
5	34	38.5	587	F	0	0	0	0	0	0	0	0	0	20	10	30	10	40	60
6	17.9	23	72.2	M	0	0	0	0	30	30	0	0	0	0	10	30	10	80	100
7	31.5	35.5	410	F	0	10	0	0	0	30	0	0	0	0	10	30	10	60	80
8	29.1	36.5	430	F	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
9	22	25.5	131	F	0	0	0	0	30	0	0	0	0	0	20	20	10	60	60
10	28	32	289	F	0	0	0	0	30	0	0	0	0	0	20	20	10	60	60
														Total	**140**	**260**	**100**	**540**	**650**
														Mean	**40**	**26**	**10**	**54**	**65**
SPRING (c)																			
1	17	21.2	66.2	M	0	0	0	0	30	30	0	0	0	0	0	30	10	70	100
2	17.5	21.4	64.9	M	0	0	0	0	0	0	0	0	0	20	20	20	0	40	40

Table 6 continued

3	18.2	21	70	F	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
4	20	24.5	94	F	0	0	0	0	0	30	0	0	0	20	10	30	0	60	80
5	14	16.9	26.6	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
6	13	15	23.2	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
7	23.3	28.4	157	F	0	0	0	0	0	0	0	0	0	0	10	30	0	10	40
8	20.6	24.5	99.6	F	0	0	0	0	0	0	0	0	0	0	20	20	0	20	20
9	20.6	24.5	99.6	F	0	0	0	0	30	0	0	0	0	0	30	10	10	70	70
10	31	36.6	352	F	0	0	0	0	0	30	0	0	0	20	0	30	10	60	90
11	28	34	288.7	F	0	0	0	0	0	30	0	0	0	10	30	10	10	80	60
12	19	22.5	77	M	0	0	0	0	0	0	0	0	0	0	0	20	10	10	30
13	28	32	255.6	F	0	0	0	0	0	0	0	0	0	0	10	30	10	20	40
14	13.9	15.8	25	M	0	0	0	0	0	0	0	0	0	10	10	30	10	30	50
15	13	15	25	F	0	0	0	0	0	0	0	0	0	10	0	30	0	10	40
														Total	**170**	**380**	**80**	**520**	**760**
														Mean	**11.3**	**25.3**	**5.3**	**34.7**	**50.7**
SUMMER (d)																			
1	19	22	108.2	M	0	0	0	0	0	30	0	0	0	0	30	10	10	70	50
2	19	22	103	F	0	0	0	0	0	0	0	0	0	0	20	20	10	30	30
3	17	20.5	63.1	M	0	0	0	0	30	30	0	0	0	0	30	10	10	100	80
4	22.5	26.3	155.4	F	0	0	0	0	30	30	0	0	0	0	20	20	10	90	90
5	19	22.5	72	M	0	0	0	0	0	0	0	0	0	10	30	10	10	50	30
6	16	19.5	55.2	M	0	0	0	0	0	0	0	0	0	0	10	30	0	10	30
7	19	23	104	F	0	0	0	0	0	0	0	0	0	0	30	10	10	40	20
8	22.4	26	136	M	0	0	0	0	0	0	0	0	0	0	20	20	0	20	20
9	21	24	135	F	0	0	0	0	0	0	0	0	0	0	20	20	0	20	20
10	14	16.5	30.6	M	0	0	0	0	0	0	0	0	0	0	30	10	10	40	20
11	17	21	74	M	0	0	0	0	0	30	0	0	0	0	0	0	0	30	30
12	19	21	70	M	0	0	0	0	0	30	0	0	0	0	30	10	10	70	50
13	21	23	132	F	0	0	0	0	0	0	0	0	0	0	30	10	10	40	20
14	26	29	270	F	0	0	0	0	0	0	0	0	0	0	30	10	10	40	20
														Total	**330**	**190**	**100**	**650**	**510**
														Mean	**23.6**	**13.6**	**7.1**	**47.1**	**36.4**

Table 7: Total numbers of ectoparasites and endoparasites retrieved from *Oreochromis mossambicus* at the Phalaborwa Barrage.

Parasite	Site of infection	Autumn (2010)	Winter (2010)	Spring (2010)	Summer (2011)
Ectoparasites					
Monogenea					
Cichlidogyrus halli	Gills	2	42	2	*
C. sclerosus	Gills	55	7	7	29
Scutogyrus longicornis	Gills	*	1	*	*
Copepoda					
Lernaea cyprinacea	Skin	1	*	*	*
	Total	**58**	**50**	**9**	**29**
Endoparasites					
Monogenea					
Enterogyrus sp.	Stomach lining	1	*	6	*
Digenea					
Diplostomum sp.	Eyes	*	*	1	*
Clinostomum sp.	Body cavity & branchial cavity	3	2	3	*
Euclinostomum sp.	Muscle	*	*	*	3
Digenean larva (Black spot)	Gills, fins & skin	2	2	28	18
Cestoda					
Cestode larvae	Small intestine	*	31	4	67
Nematoda					
Contracaecum sp.	Body cavity	*	2	*	6
	Total	**6**	**37**	**42**	**94**

Notes:
*– No present

Table 8: Total numbers of ectoparasites and endoparasites retrieved from *Schilbe intermedius* at the Phalaborwa Barrage (May 2010-January 2011).

Parasite	Site of infection	Autumn (2010)	Winter (2010)	Spring (2010)	Summer (2011)
Ectoparasites					
Monogenea					
Schilbetrema quadricornis	Gills	216	225	200	300
Schilbetrema sp. A	Gills	4	*	*	120
Schilbetrema sp. B	Gills	*	*	*	20
Copepoda					
Ergasilus sp.	Gills	*	*	1	*
	Total	**220**	**225**	**201**	**440**

Table 8 continued

Endoparasites						
Digenea						
Digenean larvae	Body cavity		*	*	5	*
Cestoda						
Unidentified larvae	Body cavity		*	*	*	1
Nematoda						
Contracaecum sp.	Body cavity		8	8	17	27
Paracamallanus cyathopharynx	Intestine		1	1	3	1
		Total	**9**	**9**	**25**	**31**

Notes:
*– No present

Table 9: Metal concentrations in the muscle tissue of *Oreochromis mossambicus* at the Phalaborwa Barrage (May 2010- January 2011).

Metals (mg/kg)	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Mean	SD
Aluminium	70	59	110	59	52	7	60	±33
Antimony	2	*	1	3	1	*	1.75	±1
Arsenic	*	*	*	*	*	*	*	*
Barium	347	318	300	287	267	271	298	±30
Beryllium	*	*	*	*	*	*	*	*
Bismuth	*	*	*	*	*	*	*	*
Boron	274	249	235	256	242	220	246	±18
Cadmium	*	*	*	*	*	*	*	*
Cobalt	*	1	1	*	*	*	1	±1
Chromium	14	12	14	14	15	12	14	±1
Copper	4	4	6	5	5	4	5	±1
Iron	102	153	158	97	139	106	126	±27
Manganese	3	40	34	8	2	5	15	±17
Molybdenum	*	*	*	*	*	*	*	*
Nickel	1	23	1	1	2	1	5	±9
Lead	*	2	2	2	2	21	6	±8
Selenium	2	1	6	1	2	*	2.4	±2
Silver	5	1	21	1	1	1	5	±8
Tin	*	*	*	1	2	*	1.5	±1
Tungsten (wolfram)	*	*	*	*	*	*	*	*
Strontium	4	52	175	59	13	147	75	±71
Titanium	4	*	*	1	1	*	2	±2

Table 9 continued

Metals (mg/kg)	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Mean	SD
Vanadium	*	3	8	*	*	*	5.5	±3
Zinc	204	192	219	232	211	228	214	±15

* Below detection level

Table 10: Metal concentrations in the muscle tissue of *Schilbe intermedius* at the Phalaborwa Barrage (May 2010-January 2011).

Metals (mg/kg)	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Mean	SD
Aluminium	47	55	52	45	42	57	53	37	51	57	48	111	48	48	69	55	±17
Antimony	*	1	1	*	2	*	*	2	*	*	*	*	*	*	1	1.75	±1
Arsenic	*	*	4	*	*	*	5	*	*	15	4	7	4	4	5	6	±4
Barium	326	331	377	309	231	354	365	209	237	415	238	387	279	279	459	320	±74
Beryllium	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Bismuth	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Boron	252	260	297	242	199	279	297	186	198	330	219	304	221	221	351	257	±51
Cadmium	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cobalt	*	*	*	*	*	*	*	*	*	1	*	*	1	1	*	1	±0.4
Chromium	16	15	15	16	14	17	15	14	14	14	14	13	18	18	15	15	±2
Copper	4	3	5	4	4	5	6	3	4	3	8	3	3	3	4	4	±1
Iron	100	186	47	87	74	144	174	139	135	81	135	173	57	57	88	112	±46
Manganese	2	3	3	5	5	3	3	2	2	2	3	20	5	5	2	4	±4
Molybdenum	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Nickel	2	1	1	1	1	1	1	1	*	1	*	1	*	*	1	1	±0.4
Lead	24	1	1	2	1	3	1	1	1	2	1	4	2	2	2	3.2	±6
Selenium	4	3	2	3	3	7	1	7	7	*	*	*	*	*	*	4.1	±2.3
Silver	1	2	2	2	1	2	2	1	2	2	2	2	1	1	2	2	±0.5
Tin	7	*	3	2	*	6	*	*	5	*	*	*	*	*	*	4.6	±3
Tungsten (wolfram)	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Strontium	2	2	4	2	2	3	*	2	2	3	3	73	7	7	3	8.21	±18
Titanium	1	2	1	2	1	2	*	1	2	2	2	*	1	1	2	1.53	±1

Table 10 continued

Metals (mg/kg)	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Mean	SD
Vanadium	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Zinc	217	247	251	207	163	226	246	156	162	249	161	236	167	167	275	209	±42

* Below detection level