A COMPARATIVE EVALUATION OF ECOSYSTEM HEALTH OF SELECTED WATER BODIES IN THE LIMPOPO AND OLIFANTS RIVER SYSTEMS USING THE HEALTH ASSESSMENT INDEX AND PARASITE DIVERSITY AS INDICATORS.

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A comparative evaluation of ecosystem health of selected water bodies in the Olifants and Limpopo River Systems using the health assessment index and parasite diversity as indicators

by

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Declaration

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

G.N. Madanire-Moyo **Date**

Student Number:

DEDICATION

This work is dedicated to my husband Jabulani Moyo

and my daughters, Shingirai, Rutendo, Rufaro and Ruvimbo.

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Abstract

South Africa's water resources are limited and scarce in global terms, due to the fact that the country's climate varies from desert to semi-desert in the west to sub-humid along the coastal area. The country is also expected to experience further variability in rainfall, reduced precipitation and increased evaporation as a result of climate change. At the projected population growth and economic development rates, it is unlikely that the projected demand on water resources in South Africa will be sustainable. An additional concern is the declining water quality due to domestic, mining and industrial pollution, and eutrophication as well as salinisation due to agricultural pollution. Thus, aquatic ecosystems must be protected, monitored and managed to ensure sustainable resource use. The aim of the study was to evaluate and compare possible environmental deterioration by analysing fish health and parasite diversity in three dams within the Limpopo and Olifants River Systems by using the fish Health Assessment Index (HAI) and the Inverted Parasite Index (IPI). The intention of the study was to substantiate the theories behind the HAI and IPI in a bid to augment strategies to manage water quality, fish health and aquatic biodiversity.

Seasonal surveys were carried out between April 2008 and April 2010 at three localities. The Luphephe-Nwanedi Dams are in a Nature Reserve located in a rural catchment, the Flag Boshielo Dam in an industrualised and mining catchment whereas the Return Water Dam is located on a platinum mining premise. *Clarias gariepinus* (Burchell, 1822) and *Oreochromis mossambicus* (Peters, 1852) were collected with the aid of gill nets and used as indicator fish species. Fish were examined for external parasites after which they were weighed and measured. Blood was drawn and skin smears were made. The skin smears were examined with a dissecting microscope for the presence of parasites. Fish were killed, dissected and then examined as prescribed in the fish HAI. From the ecto- and endoparasite data collected, infection statistics and ecological parameters were calculated. The HAI values were calculated for each fish species at each sampling site. To verify the results of the HAI, water quality was included in the study.

The nutrients and mining related pollutants of the three dams differed to a great extent and showed a similar increasing trend in the order: Luphephe-Nwanedi Dams < Flag Boshielo Dam < Return water Dam. Our results were consistent with previous work describing Luphephe-Nwanedi Dams as essentially unimpacted and Flag Boshielo Dam as impacted with a combination of mining and agricultural effluents. The results have shown that the Return Water Dam is an extremely polluted site with high levels of nutrients and metals.

Fish health of both species responded similarly to polluted sites although mean population HAI results showed that *C. gariepinus* was more affected in terms of haematocrit necropsy-related alterations. The top six metrics that correlated most to fish health scores were nearly the same for both species (i.e. haematocrit values, inverted ectoparasite index, condition of the kidney, liver, gills and skin). The parasite community of *C*. *gariepinus* comprised 19 metazoan species. Seventeen parasite species were recovered from fish sampled from Luphephe-Nwanedi Dams compared to 11 at Flag Boshielo Dam and four at the Return Water Dam. The parasite community of *O*. *mossambicus* comprised 20 metazoan species. A total of 19 species, 17 species, and 4 species of metazoan parasites from *O*. *mossambicus* were obtained from Luphephe-Nwanedi Dams, Flag Boshielo Dam and the Return Water Dam, respectively. In both fish species, the Shannon Wiener Index, the inverse Simpson Index, equitability and the number of metazoan parasite individuals were highest in fish from Luphephe-Nwanedi Dams.

The results of this study emphasized the negative impacts of urbanization, agricultural and mining activities on the environment. The fish hosts collected in the mining premise supported the poorest and least diverse parasite communities of all sampled sites, with virtual depletion of both heteroxenous and monoxenous species. The Return Water Dam may therefore be regarded as a simulation model for a severely environmentally deteriorated, impoverished habitat, in which all or part of the intermediate hosts have been depleted, enabling the survival of hardy parasite species only. Further studies should address the identification of parasite life stages that are more sensitive to pollutants.

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CHAPTER 1

GENERAL INTRODUCTION AND THESIS OUTLINE

1.1. Introduction

1.1.1. Background and Problem Statement

Freshwater ecosystems are hotspots of diversity because they contain 2.4% of all known species, despite occupying only 0.8% of the terrestrial surface and only representing 0.3% of water of the planet (McAllister *et al*. 1997). These are also among the most altered ecosystems worldwide due to human activities (Malmqvist & Rundle 2002) and have been profoundly altered by industrial, agriculture and urban pollution, water abstraction and regulation, introduction of exotic species, and alteration of riparian habitat and natural hydro morphology (Baron *et al*. 2002; Xenopoulus & Lodge 2006). Economic growth is an ultimate cause of resource balance and biodiversity loss (Mattson & Angermeier 2007). Thus, the importance of monitoring and preserving the aquatic environment cannot be overemphasised, because water provides the life support system for all life forms.

Aquatic monitoring programs in South Africa, as in most countries, used to be based on measuring the physical and chemical variables of a water body (Roux *et al*. 1993). The shortcomings of these methods were that these variables only indicated conditions prevailing during the time of sampling and did not count for the intermittent anthropogenic disturbances of the habitat, and thus could not

predict the overall health of an ecosystem (Roux *et al*. 1993). These measurements were soon supplemented by the identification and quantification of contaminants in living organisms (bioaccumulation monitoring). Both methods only allow the identification of a small number of selected contaminants, without considering complex synergetic and antagonistic reactions of the chemicals in the ecosystem.

Ecotoxicologists generally assess the impacts of pollutants on freshwater fish using standard toxicity tests in the laboratory. Although they provide important information on how organisms respond to environmental stressors, ecotoxicological tests mainly inform about acute and not sub-lethal or chronic effects (Hela *et al*. 2005). Moreover, although polluted sites in nature generally consist of a mixture of pollutants, most ecotoxicological studies focus on exposure and effects of single compounds (Yang 1994). Another limitation of laboratory tests relates to the bioavailability of toxicants: unlike the concentration of toxicants of controlled toxicity tests, physico-chemical properties such as water flow or pH of natural ecosystems may mediate bioavailability (De Zwart 2005). In general, physico-chemical processes (e.g. ionisation, dissolution, precipitation, complexation and partitioning) reduce the concentration of toxicants that is actually experienced by the biota. These processes depend on individual properties of the toxicants and on the abiotic characteristics of the ecosystem (De Zwart 2005). Therefore, although laboratory studies provide invaluable preliminary information on the effects of environment stressors, further studies in

natural habitats are needed to increase ecological realism.

Due to the complexity of natural systems, single parameters do not appropriately reflect the effects of multiple stressors on the integrity of aquatic systems. An adequate set of endpoints is required to determine the biological significance of stress and the underlying cause or mechanistic basis of observed effects (Attrill & Depledge 1997). Therefore, environmental monitoring programs should include a variety of chemical, physical and biological indicators, with each being used in their respective roles as environmental stressors (i.e. xenobiotics), exposure response (i.e. biomarkers) and effects response (i.e. bioindicators) (Xenopoulus & Lodge 2006). Bioindicators have the advantage that they show a long term response (chronic) to intermittent pollution, they respond to all toxicants they are exposed to, and biological assessments are more rapid and comparatively less expensive than chemical analysis (Van der Oost *et al*. 2003).

A wide range of bioindicators and biomarkers are used in aquatic pollution monitoring, spanning from subcellular, organismal to population and community levels (Adams 2002). At the organismal level, fish are widely used as sentinel species to assess environmental health because they have some particular features and advantages as indicators of freshwater ecosystem health (Simon 1999). Fish continually inhabit the receiving water and integrate the chemical, physical, and biological histories of the waters. Most fish species have long life spans (about 2-20 years) and can both reflect long term and current water quality. The sampling frequency needed for trend assessment is less than for

short lived organisms and taxonomy of fish is well-established, enabling professional biologists the ability to reduce laboratory time by identifying most specimens in the field (Simon 1999). Fish have larger ranges and are less affected by natural microhabitat differences than smaller organisms, making them extremely useful for assessing regional and microhabitat differences (Simon 1999). Furthermore, fish are highly visible and valuable components of the aquatic community to the public, making communication easier. In addition, fish themselves provide a habitat for other organisms, such as parasites, which also reflect environmental health on the population and ecosystem level.

Because of the aforementioned features of fish, several approaches have been used over the past years to evaluate the effect of stress on the health of fish populations (Adams *et al*. 1993). An empirical necropsy-based system of organ and tissue indices, to provide a health and condition evaluation for fish populations in the field, was one such approach. This method was originally developed and described by Goede & Barton (1990) and involved a sequence of simple, ordered observations and measurements of external characteristics, internal organs and some blood parameters. The appearance of some vital organs, blood parameters and external aspects, apparently signify whether a population is in equilibrium with its environment or if fish have been challenged (Klemm *et al*. 1992). Although this method provided a health status profile of a fish population, there was no numerical basis for statistical comparison of the entire index with all its variables to another population (Adams *et al*. 1993). Thus,

Adams *et al*. (1993) developed a “quantitative Health Assessment Index” (HAI) based on a modification and refinement of the autopsy-based approach with the objective of minimizing the limitations of the necropsy-based system. The HAI is a quantitative index that allows statistical comparisons to be made between data sets and makes use of post mortem, blood and parasite data. Numerical values are assigned to express the severity of deterioration of fish tissues or extent of parasitism.

Depending on the degree of stressor-induced anomalies, a numerical value is given to examined fish tissues and organs. The index value for that fish is the sum total of values for all examined tissues and organs while the mean calculated for all fish in the sample is the index value for that locality (Crafford & Avenant-Oldewage 2009). Higher index values correlate with decreased water quality, and hence increased stress. Over time, a database is established for detecting trends in the health of a fish population. When a change is observed, more specialised monitoring tools such as chemical analyses can be applied to the problem (Crafford & Avenant-Oldewage 2009).

The HAI is already being used on a continuous basis in North America to determine the effects of pollution on the environment (Chaiyapechara *et al*. 2003). This technique has been tested in the pulp polluted Tennessee River Basin (North Carolina, Tennessee, Alabama, Kentucky), the Hartwell Reservoir (Georgia, South Carolina), which was contaminated with polychlorinated

biphenyls and in the Pigeon River (Tennessee, North Carolina) which received effluents from a bleached craft mill (Adams *et al*. 1993).

In South Africa, the index has been tested and adapted for local conditions through studies on the Olifants River System (Avenant-Oldewage *et al*. 1995; Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001) and on the Vaal River System (Crafford 2000; Groenewald 2000; Bertasso 2004; Crafford & Avenant-Oldewage 2001; 2009). During the study conducted by Marx (1996), the interrelationship between fish health and parasite load was investigated to determine whether parasites should be incorporated into the HAI or used as a separate entity in association with the HAI. Crafford (2000) used four parasite indices, specifically the original parasite index by Adams *et al*. (1993) (distinguished between the presence and absence of parasites), inserted parasite index by Marx (1996) (distinguished between the presence of ectoparasites and endoparasites), refined parasite index by Marx (1996) (distinguished between the number of ectoparasites and endoparasites) and the inverted parasite index (IPI) (Crafford & Avenant-Odewage 2009). The IPI is based on the argument that ectoparasites are more directly exposed to the effects of poor water quality than endoparasites (Crafford & Avenant-Oldewage 2009), therefore relatively few ectoparasites and more endoparasites would be found at a more polluted habitat and *vice versa* at a less polluted site.

Recent studies by the University of Limpopo fish parasitologists (Jooste *et al.* 2003) on the Ga-Selati River have confirmed that parasite communities are

good indicators of environmental stress and biodiversity. To date, this IPI has been successfully applied in the Ga-Selati River (Jooste *et al*. 2005a & b; Luus-Powell *et al*. 2005) using different fish species as indicator organisms.

Taking the before mentioned into account, the purpose of the present study was to determine the effects of anthropogenic activities on fish health and parasite diversity. The HAI, based on the autopsy system as refined by Adams *et al*. (1993) and the IPI as evaluated by Crafford & Avenant-Oldewage (2009), was employed. The study examined the infrapopulation structure of parasites of the sharptooth catfish, *Clarias gariepinus* (Burchell, 1822*)* and the Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852) in relation to water quality. This was done in three dams, namely the Luphephe-Nwanedi Dams, Flag Boshielo Dam and the Return Water Dam of the Limpopo and Olifants River Systems. These dams were selected due to their dissimilar levels of pollution. Selected chemical and physical water parameters were measured to supplement the biological data.

1.2. Motivation for the study

Pollution of freshwater resources in third world countries has reached an alarming stage where it might extend to a point of irreparable damage with irrepairable consequences (Jamil 2001). Thus a major challenge facing humanity for the next century is the proper management of water as a vital resource. Its scarcity, especially in arid and semi-arid regions of the world, calls for fast and

efficient measures to properly manage a water resource and equally distribute it.

South Africa's water resources are extremely scarce in global terms (DWAF 1996), due to the fact that the country's climate varies from desert to semi-desert in the west to sub-humid along the coastal area. The average rainfall of the country is approximately 450 mm per year, which is almost half of the world average rainfall, which is approximately 860 mm per year (NWRS 2002). As a result, South Africa is categorised as a semi-arid country and is also expected to experience further variability in rainfall, reduced precipitation and increased evaporation as a result of climate change.

At the projected population growth and economic development rates, it is unlikely that the projected demand on water resources in South Africa will be sustainable. An additional concern is the declining water quality due to domestic and industrial pollution, and eutrophication and salinisation due to agricultural pollution (Sithole & Murewi 2009). Increases in demand for freshwater are to be anticipated in the domestic, agricultural and industrial sectors, thus water will increasingly become the limiting resource in South Africa. Supply will become a major restriction to the future socio-economic development of the country, in terms of both the amount of water available and the quality of what is available (Sithole & Murewi 2009). It is against this background that this project was carried out. The existing water bodies must be monitored to ensure ecosystem health. The study focussed on identifying the sources and levels of pollution by testing the water quality parameters in three dams of the Limpopo and Olifants

River Systems. The practical implications from the findings of this study are intended for scrutiny by the relevant authorities, which will assist them to develop comprehensive and integrated management practices related to water issues. Sustainable use and management of natural resources is dependent on adequate and accurate current information of the environment. Hence, it is in the interest of this project to assess the extent of alteration and the rate at which changes in water quality and biodiversity are occurring within the three dams.

Recent studies on the Ga-Selati River (Jooste *et al*. 2003, 2005a & b; Luus-Powell *et al*. 2005) recommended that more research be carried out to assess the effectiveness of the revised IPI in different water bodies. This is because a biomonitoring index yielding reliable results in one water body would not necessarily perform the same in a different water body (Lyons *et al*. 1996), hence the adoption of the revised IPI in the current study. Furthermore, despite considerable progress in parasitology in the last decades, major gaps still exist in the knowledge of biology, epizootiology and ecological interactions of fish parasites in South Africa.

The motivation for this study can therefore be summarized as follows:

- Aquatic ecosystems must be protected, monitored and managed to ensure sustainable resource use.
- It is important to apply biomonitoring approaches in order to establish their effectiveness in different systems.

- Fish parasitology (fundamental and applied) is still far from being satisfactory and further research is needed.
- Most of southern Africa remains inadequately sampled and the complete faunistics of dams for this region are still to be discovered.

1.3. Aim, Objectives and Thesis Outline

1.3.1. Aim

The aim of the study was to evaluate and compare possible environmental deterioration by analysing fish health and parasite diversity in three dams within the Limpopo and Olifants River Systems by using the HAI and IPI. The intention of the study was to substantiate the theories behind HAI and the IPI, to augment strategies to manage water quality, fish health and aquatic biodiversity. By documenting the current biodiversity of fish parasite fauna, the study increases the spatial and temporal scale and extends the scope beyond previous studies in fish parasite distribution in southern Africa. It is also hoped that this work will fill some gaps in our knowledge of the epizootiology and ecological interactions of these fascinating invertebrates. To achieve these aims, the following principal objectives were set, and the chapters of this thesis are organized so that each objective could be specifically addressed.

Hypotheses

1. The Health Assessment Index (HAI) and parasite diversity can effectively discriminate three dams of the Limpopo and Olifants River on the basis of water quality.

2. Greater species richness is to be expected in the evolutionary ancient host-parasite systems and in hosts that inhabit their geographic area of origin because they have had time to acquire their helminth fauna (Choudhury & Dick 2000).

3. The sharptooth catfish and the Mozambique tilapia are omnivorous and as such, the diversity of their prey items is bigger, furthering potential parasite transmission.

1.3.2. Objectives

The objectives of the study were to:

1. document temporal and spatial trends in concentrations of water contaminants and to assess contaminant effects on the health of feral fish, *Clarias gariepinus* and *Oreochromis mossambicus*, sampled from three dams of the Limpopo and Olifants River Systems. **(Chapter 3)**.

2. investigate some ecological factors determining community structure of metazoan parasite species of two feral species, *Clarias gariepinus* and

3. *Oreochromis mossambicus*, within the Limpopo and Olifants River Systems. **(Chapters 4 & 5)**.

4. investigate the impact of pollution on the parasite fauna of *Clarias gariepinus* and *Oreochromis mossambicus* inhabiting three dams of the Limpopo and Olifants River Systems. **(Chapter 6)**.

1.3.3. Thesis Outline

The study comprises six chapters and a concluding chapter, and this section presents the highlights therein each chapter. **Chapter 2** describes the study area, with specific reference to the three sampling sites. The fish species, the materials used and the methods followed throughout the study are discussed in this chapter. **Chapters 3 through 6** address the research objectives outlined in 1.3.2., which were originally prepared as individual journal articles to be submitted for publication. Thus, some overlaps in data descriptions are expected. **Chapter 3** provides the results of the HAI and the IPI in determining the health of fish examined. A correlation of water quality data with fish health data as well as a comparison of the health of fish among the three dams of varying degrees of pollution levels is made. Aspects of the community structure and diversity indices for the parasites from *Clarias gariepinus* and *Oreochromis mossambicus* are presented in **Chapters 4 and 5,** respectively. It was in the interest of the study to determine if parasite communities of the two fish species show predictable or stochastic structure and if they do, determine the underlying processes. Results

from these two chapters also add considerably to the distribution and abundance of metazoan parasite infections in the Limpopo and Olifants River Systems, thereby contributing to the biogeography of southern African parasite species. The specific objectives of **Chapter 6** were to compare the diversity and distribution of metazoan parasite fauna along defined pollution gradients and assess the suitability of using parasites as predictors of environmental change. Finally, concluding remarks that highlight the main findings that can be drawn from the preceding chapters are presented, and the main limitations of the study and recommendations for future research are also discussed in **Chapter 7**. A rerence list and an appendix are presented at the end.

CHAPTER 2

STUDY AREA, HOST SPECIES, MATERIALS AND METHODS

2.1. Introduction

Pollution of freshwater resources is a serious environmental problem worldwide. As aquatic ecosystems are utilized for agriculture and as urbanization occur, ecosystems accumulate pollutants and the health of resident living organisms is consequently affected by the decrease in water quality. The growing human population and the ever-expanding industrial and mining sector are placing heavy demands on the limited natural resources in both developing and developed countries. In South Africa, the Limpopo and Olifants River Systems are such examples. These two rivers perform a pivotal role in shaping economic prospects of South Africa, as they play a role in power generation, domestic water supply, irrigation, tourism, industrial production, mining and fisheries. However, as in the case of many other inland water bodies, the rivers are gradually undergoing eco-degradation throughout their courses of flow due to various anthropogenic stresses (Ashton *et al*. 2001, Heath *et al*. 2010).

According to the Köppen Classification, the catchment areas of the Limpopo and Olifants Rivers are predominantly semi-arid, dry and hot (FAO 2004; Peel *et al*. 2007). The demand for water throughout these river catchments is high and unevenly spread. Coupled with high evaporation losses from the numerous small dams and larger water supply impoundments, water flow in the

lower reaches of both rivers is usually relatively low. The overall water supply becomes uncertain due to the possibility that global climate change will also have an adverse effect on water availability throughout southern Africa (Ashton *et al*. 2001; IPCC 2001). Thus, the Limpopo and Olifants River Systems were selected for this study. Three sampling sites were selected, namely, Luphephe-Nwanedi Dams (in the Nwanedi Nature Reserve), Flag Boshielo Dam (in the middle of the Olifants River Catchment) and the Return Water Dam (at a platinum mine site).

2.2. Limpopo River Basin

2.2.1. Basin characteristics

The Limpopo River is an international river shared by four countries, Botswana, Mozambique, Zimbabwe and South Africa. The total length of the river is about 1 750 km, located between 20° and 26° south and between 25° and 34° east. With a drainage area of 415 000 km^2, the Limpopo River Basin supports a population of 14 million people, where the poverty average is 52%, and water availability is between 5 to 10 m^3 per person (FAO 2004). The population density in the basin is around 25-50 people per km^2 (Mucina & Rutherford 2006) which makes the Limpopo River Basin one of the densest basins in Africa. The Limpopo River Basin includes an arid area (47%), forest (1%) and wetlands (3%) (Amaral & Sommerhalder 2004).

Topography - Plains are the dominant landform of the basin. These are interspersed by low gradient hills, locally incised valleys and medium gradient mountains. Terrain is thus a principal limiting factor in determining land use options (CGIAR 2003).

Geology - One of the first datable rocks in the world is the Sand River Gneiss in the remote northern extremity of the Limpopo River Basin near the border town of Musina (Fripp 1983; Horrocks 1983). The other candidate for the foundation rocks of the Limpopo River Basin are the Greenstones, which are a combination of volcanic and sedimentary rocks formed about 3.5 billion years ago (Rollinson & Blenkinsop 1995). The Greenstones are notable as being the source of the first commercial gold mines in South African history (Chinoda *et al*. 2009). The third foundation rock of the Limpopo River Basin is the ancient granites, also described as the Fundamental Complex, which forms the very foundation stone of the high South African plateau. The majority of the granite, which provides the characteristic landscape of the African Savannah, belongs to this formation (Watkeys *et al*. 1983; Chinoda *et al*. 2009).

Climate - The climate in the Limpopo River Basin ranges from tropical dry savannah and hot dry steppe to warm and cool temperate (Amaral & Sommerhalder 2004). Climatic data indicates that there is very strong seasonality in the rainfall, with little to no rainfall occurring in the months between May and October. Rainfall varies significantly between years, with an average annual rainfall of 530 mm (Mucina & Rutherford 2006). Rainfall typically occurs in the

form of convective thunderstorms. The short and intense rainy season, with erratic and unreliable rainfall, leads to frequent droughts over the wider Limpopo River Basin (Amaral & Sommerhalder 2004). The evaporation has an average of 1 970 mm ranging from 800 to 2 400 mm/yr, which means a higher evaporation rate than rainfall (Amaral & Sommerhalder 2004).

Hydrology - Along its course the river is joined by eight tributaries such as the main Olifants stream that is also an international tributary, crossing South Africa and Mozambique (Figure 2.1). Where the Limpopo River encounters the Indian Ocean at 25° 15'S, it has a width of 300 meters, partly obstructed by sandbanks (Amaral & Sommerhalder 2004). Although the river is drained by a number of large perennial tributaries, it is not perennial in nature, often experiencing long periods of no surface flow in the stretch which drains through the study area (ARC 2003). These periods of zero surface flow in the river can last for up to eight months per year (FAO 2004) and the river has been known to stop flowing for periods of up to 36 months in recent years (ARC 2003). On major reaches of the Limpopo River, and many of its main tributaries, river flow may occur for 40 days or less in a dry year (ARC 2003).

2.3. Olifants River Basin

2.3.1. Basin characteristics

The Olifants River originates near Bethal in the Highveld of Mpumalanga. The river initially flows northwards before curving in an easterly direction through

the Kruger National Park and into Mozambique where it joins the Limpopo River before discharging into the Indian Ocean (Heath *et al*. 2010). The Olifants River Catchment falls within three provinces viz Gauteng, Mpumalanga and the Limpopo Province (Heath *et al*. 2010).

The upper catchment has large urban centres located in the Emalahleni (Witbank), Steve Tshwete (Middelburg) and also a number of smaller urban centres such as Bronkhorstspruit, Kriel, Hendrina, Kinross and Trichardt (Heath *et al*. 2010). Satellite townships are also associated with most of the mining operations and power stations. Extensive coal mining takes place in the catchment, most of which occurs in the Witbank Coalfields and Highveld Coalfields (Midgley *et al*. 1994). Irrigation farming of diverse crops takes place in various parts of the catchment, the largest of which is the Loskop Dam Irrigation Scheme (De Lange *et al*. 2003).

There are no metropolitan areas situated in the middle catchment but smaller towns like Groblersdal, Marble Hall and Settlers are located in the area. The Western Highveld region, including towns like Siyabuswa and Dennilton is located in the Elands River catchment (De Lange *et al*. 2003). Several rural townships are also located in the area. The major dams in the middle catchment include the Loskop Dam, Flag Boshielo Dam, Rust de Winter Dam, Renosterkop Dam and Rooikraal Dam. Many smaller farm dams are also found in the area (Heath *et al*. 2010). Irrigation farming of diverse crops takes place in various parts of the catchment, the largest of which is the Elands River Irrigation

Scheme. Small mining areas are found in the catchments of Klipspruit, Moses River and Loopspruit as well as the area east of Marble Hall (Heath *et al*. 2010).

Topography – According to Steffen & Kirsten (1991), the catchment can be divided into four zones on the basis of altitude:

1. the mountainous region of the Transvaal Drakensberg in the centre, which divides the catchment in a roughly north-south alignment (1 500 - 2400 m above sea level).
2. the flat plains of the Lowveld in the east (300 - 900 m above sea level)
3. the undulating Highveld in the south (1 200 -1 800 m above sea level) and
4. the undulating Springbok Flats in the west (900 -1 200 m above sea level)

Geology –The geology of the area where the Flag Boshielo Dam was built consist of these Archean granites and also rhyolites that were formed in excess of 2 500 million years ago (De Wit *et al*. 1993). Other rock formations worth noting in the area occur to the west and south of the dam. These formations consist of orthoquartzite, dolomite, gabbro and shale (DWAF 2003).

Climate – According to the South African Weather Services, the Flag Boshielo Dam falls within the Northern Transvaal (now Limpopo Province) climatic zone (Schulze 1994). The climate of the Olifants River Basin is described as semi-arid and hot with an average annual rainfall of 380-700 mm. Thunderstorms are

responsible for most of the rainfall of this region. The South African Weather Services describe the rainy season as starting in November with a peak in January. An important factor is that rainfall is somewhat unreliable and that severe drought conditions occur in about 12% of all years (Schulze 1994).

Hydrology - The main tributaries are the Letaba, Wilge, Elands and Ga-Selati Rivers on the left bank and the Steelpoort, Blyde, Klaserie and Timbavati Rivers on the right bank. The headwaters of these rivers are located along the Highveld Ridge in the Secunda-Bethal area and the rivers then flow in a northerly direction towards Loskop Dam (Heath *et al*. 2010). The Middle Olifants catchment comprises the drainage areas of the Olifants River downstream of Loskop Dam and down to the Flag Boshielo Dam. The Lower Olifants catchment comprises the drainage areas from Flag Boshielo Dam, downstream to the Kruger National Park (Figures 2.1 & 2.4). The river has been known to have zero flow during short periods as it enters the Kruger National Park and a severe drought occurs practically every decade (De Lange *et al*. 2003). The basin is also capable of generating extremely high flows giving rise to devastating floods. During the last floods in February 2000, the flow in the Olifants River peaked at 3,800 m^3/s at its mouth (Midgley *et al*. 1994).

1.4. Sampling sites

From 2008 to 2010 seasonal surveys were carried out in the Limpopo and Olifants River Systems, where catfish and tilapias were collected from three

dams shown in Figure 2.1. Due to the artificial nature of the dams, it was difficult to select a natural pristine site. Sampling in the Luphephe-Nwanedi Dams and the Anglo Platinum Return Water Dam was done seasonally from April 2008 to April 2009 while the Flag Boshielo was sampled seasonally from April 2009 to April 2010.

2.4.1. Site one: Luphephe-Nwanedi Dams ($22^{o}39.492`S$, $30^{o}25.342`E$)

The Luphephe-Nwanedi Dams, constructed in 1964 by the then Department of Water Affairs, are situated at the foothills of the Soutpansberg. The twin-dams have a surface area of approximately 220 ha and are connected by a 2.5 m deep channel (Figure 2.2). In 1979, the dams and their surrounding area of 10 170 ha have been proclaimed a Nature Reserve (Figure 2.2). The twin dams receive water from the relatively unpolluted Luphephe and Nwanedi mountain streams, both which join to form the Nwanedzi River, a small distance below the dam walls (Figures 2.2 & 2.3A & B). Due to the absence of intensive agricultural, industrial and mining activities in close proximity of the dam, it was for the purpose of the study, the best choice as a reference site since it contains relatively little pollution (Oberholster *et al*. 2009). This therefore allowed for effective comparisons with the other more polluted sites.

2.4.2. Site two: Flag Boshielo Dam ($24^{o}49.057`S$, $29^{o}24.509`E$)

The Flag Boshielo Dam is situated about 25 km north-east of the town of Marble Hall in the extreme north-western corner of South Africa's Mpumalanga Province (Clark 1997). The dam is about 200 km north of Pretoria and about the same distance south east of Polokwane (Figure 2.1). Other local towns of interest in the area of Flag Boshielo Dam are Groblersdal and Middleburg (Figure 2.4). Construction of the dam was completed in 1987 (Clark 1997).

Several large impoundments situated upstream of Flag Boshielo Dam have a big influence on the water level of this dam. The largest of these are Loskop Dam, about 85 km upstream in the Olifants River, and also the Mkhombo Dam (Rhenosterkop Dam), about 70 km upstream in Elands River (Figure 2.4). The confluence of the Elands River with the Olifants River forms an important landmark at the inlet to the dam. Several other small non-perennial streams also feed the dam but only for short periods of high rainfall. The western shore of the dam forms part of the Schuinsdraai Nature Reserve, a 9 037 ha Provincial Nature Reserve.

The Flag Boshielo Dam is situated in the middle region of the Olifants River. This area contains the highest number of people in the catchment areas of the three selected sites, most who live in rural settlements (Figure 2.4). Along the Olifants River there are commercial and subsistence agriculture as well as numerous point and diffuse sources of industrial pollution (Heath & Claassen 1999; Figure 2.4). Over the past few years, the Olifants River has been systematically impaired because of an increase in agricultural and mining

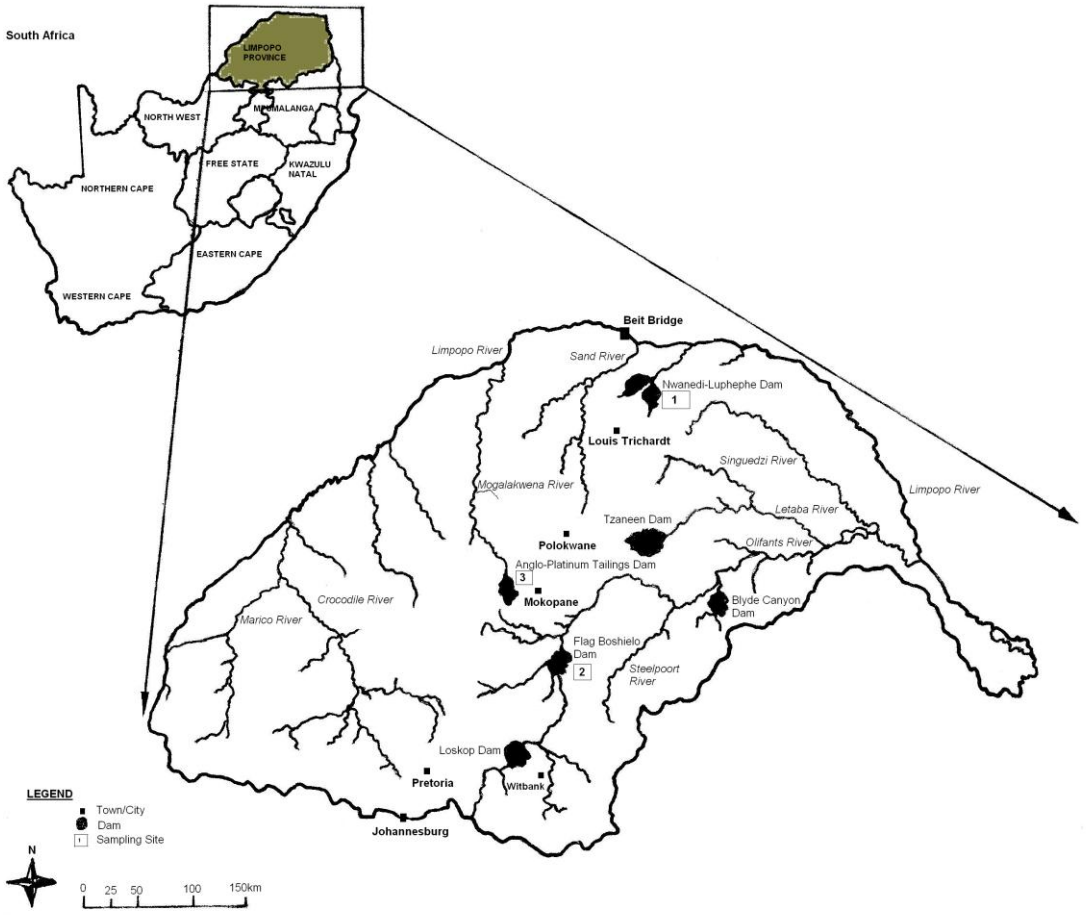


Figure 2.1: Sketch map of Limpopo River and its catchment showing sampling sites. 1 = Luphephe-Nwanedi Dams. 2 = Flag Boshielo Dam and 3 = Anglo Platinum Return Water Dam.

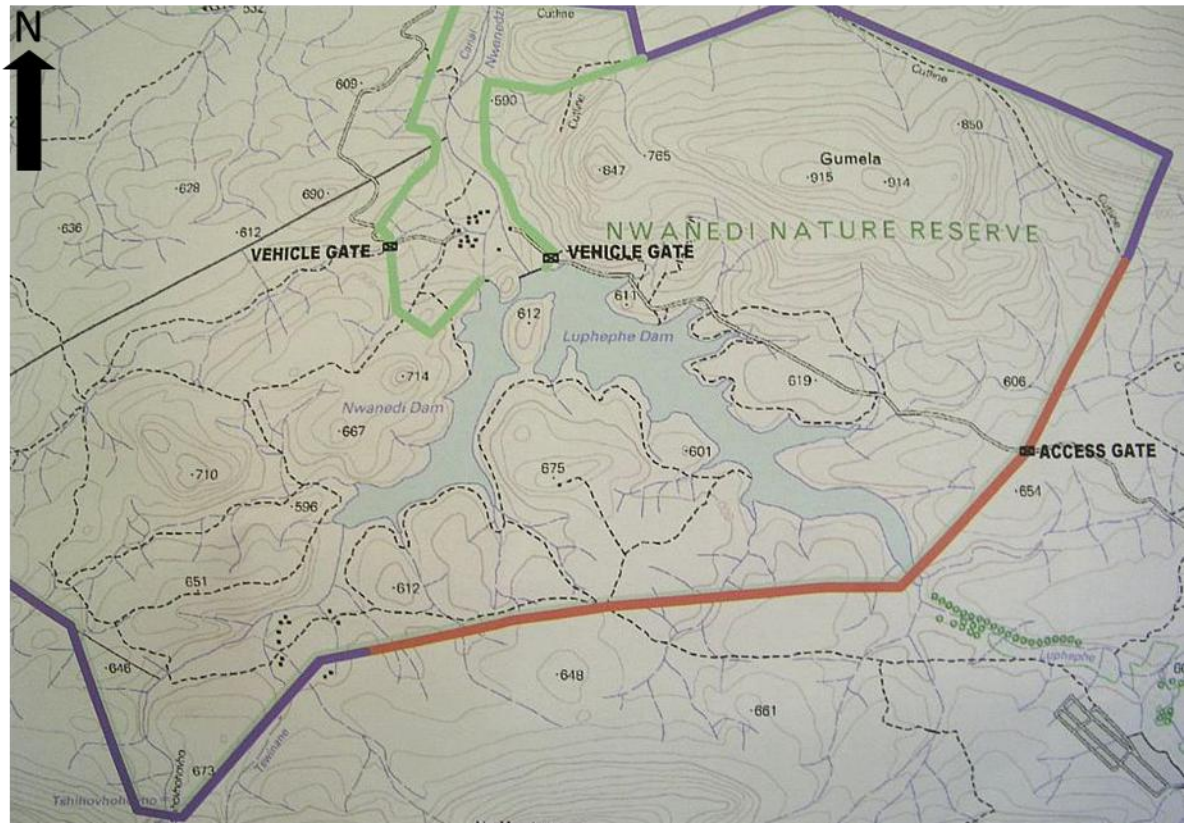


Figure 2.2: Luphephe-Nwanedi Dams in the Nwanedi Nature Reserve. (Source: http://www.dwaf.gov.za)

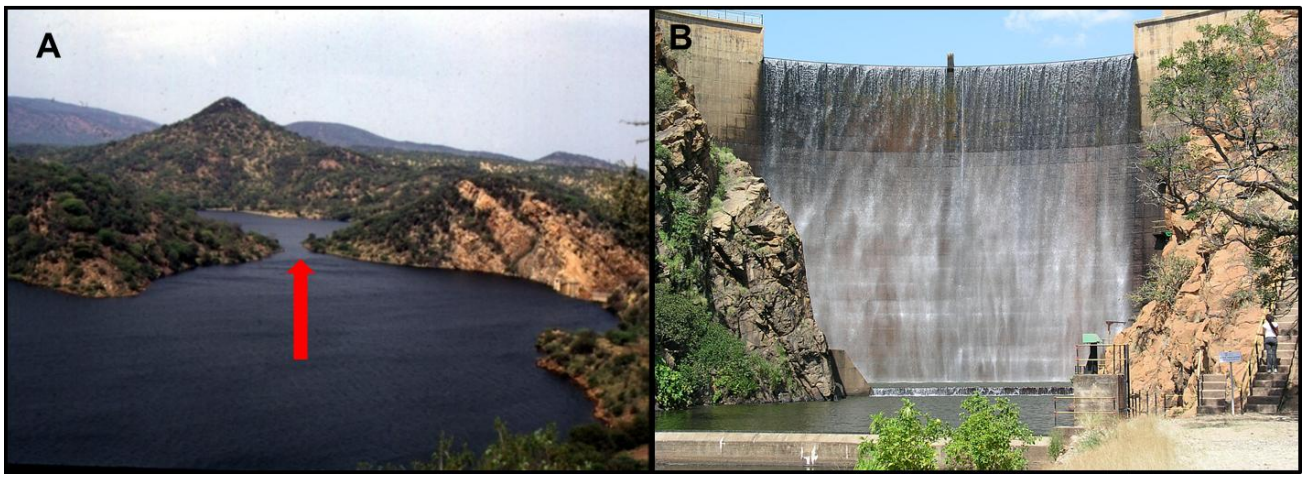


Figure 2.3: A = Channel linking the Luphephe-Nwanedi Dams (arrowed). B = Luphephe Dam overflowing, April 2008.

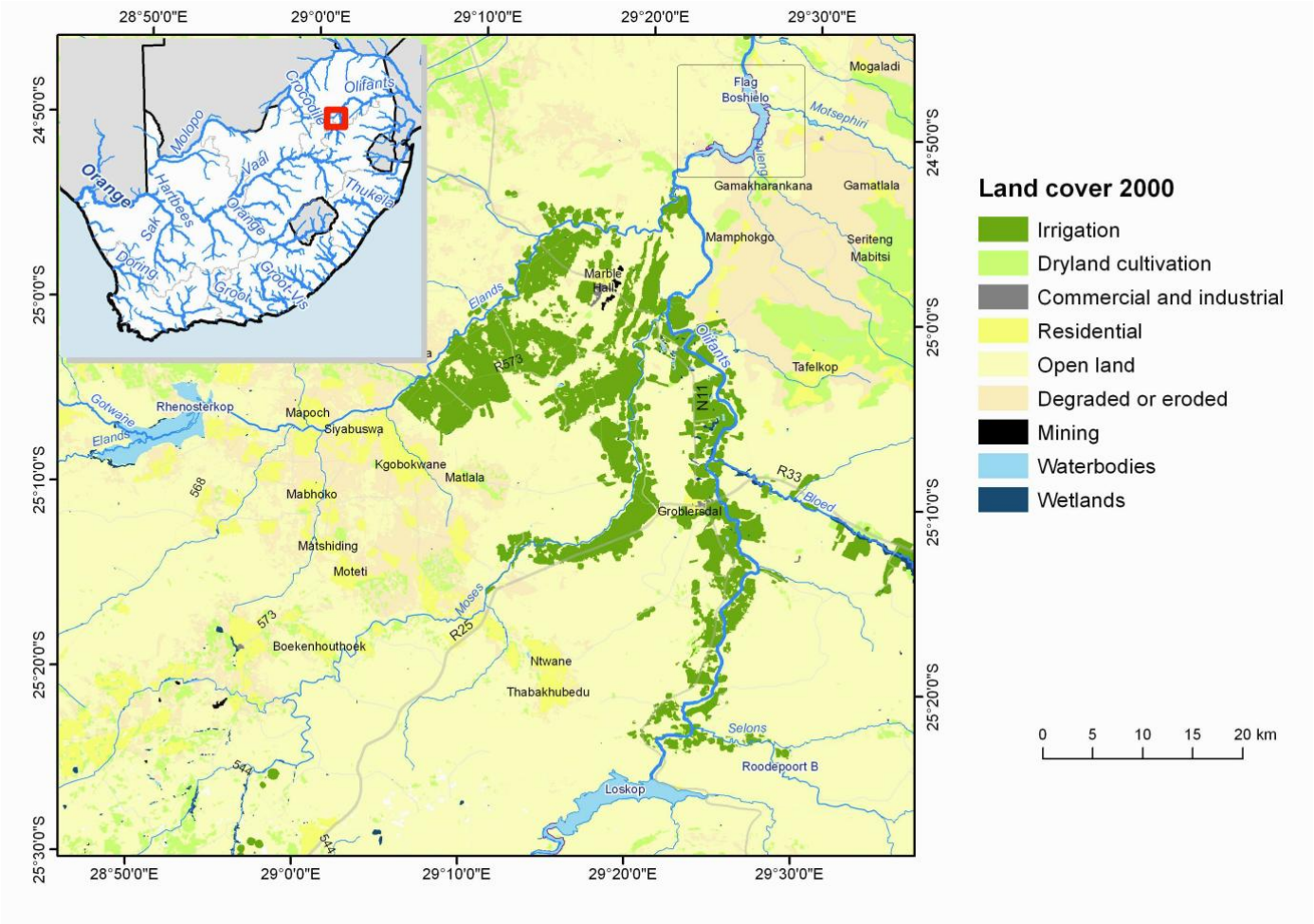


Figure 2.4: Map showing the catchment areas, rivers and urban/industrial developments around the Flag Boshielo Dam, Olifants River System. (Source: http://www.dwaf.gov.za)

activities, industrial development and urbanization. This river system is often described as one of the most polluted systems in South Africa and has been described as “The Battered River” (Van Vuren *et al.* 1999).

The existing dam is a composite structure and comprises a 770 m long embankment with a 455 m long roller compacted concrete gravity section across the riverbank (DWAF 2003; Figure 2.5). The dam has a central overflow spillway section with a four metre high and a 200 m long earth embankment on the right bank which acts as an emergency break-section to protect the dam in case of extreme floods (DWAF 2003). At full supply level, the shoreline of the Flag Boshielo Dam has a length of 65 km, a full supply height of 817 masl and a net storage capacity of 100 million m^3. The dam has a total catchment area of 23 712 km^2 (DWAF 2003).

Figure 2.5: Flag Boshielo Dam with outflow into the Olifants River.

2.4.3. Site three: The Anglo Platinum Return Water Dam (23^{o}59.622`S, 29$^{o}$24.509`E)

The Anglo Platinum Limited mine is situated in the Mogalakwena River sub-catchment of the Limpopo River. This sub-catchment consists of the area drained by the Mogalakwena River and its tributary streams, notably the Nyl River in the upper reaches (Figure 2.1). The mine uses sewage effluent from Mokopane's wastewater treatment plant as well as water from Doorndraai Dam (pipeline operated by the Lepelle Northern Water Board) for processing water in the mining operations. The water from the mine's processing plants are pumped to the tailings dam from where it overflows into the Return Water Dam (Figure 2.6). This site is thus severely polluted.

Figure 2.6: The Anglo Platinum Mine Return Water Dam.

2.5. Fish Species

The sharptooth catfish, *Clarias gariepinus* (Figure 2.7) and the Mozambique tilapia, *Oreochromis mossambicus* (Figure 2.8) were chosen as the model fish species. These two fish species are hardy species, occurring even in the most polluted waters. However, tissue and organ anomalies resulting from environmental stress can be observed in these two fish species (Ramollo 2008; Crafford & Avenant-Oldewage 2009). This makes them good indicators of chronic environmental stress, enabling them to reflect cumulative effects of both past and recent water quality conditions. The sharptooth catfish is probably the most widely distributed fish in Africa, inhabiting tropical swamps, rivers, and dams, some of which are subjected to seasonal drying (Skelton 2001). It is tolerant to low oxygen concentrations and can endure desiccation due to the possession of accessory air breathing organ (pseudobranch). *Clarias gariepinus* is omnivorous, utilizing various kinds of food resources available in their habitats.

Oreochromis mossambicus naturally occurs along the eastern coast of Africa, in the lower Zambezi and its tributaries and eastward-flowing rivers and coastal lagoons southward to the Bushman's River, South Africa (Skelton 2001). This species is tolerant of fresh, brackish, marine waters and even higher salinities such as sea water (Skelton 2001). This tilapia survives lower temperatures below 15^{o}C) in brackish or marine waters but prefers warmer water temperatures (above 22^{o}C). In general, it feeds on algae, especially diatoms and

Figure 2.7: The sharptooth catfish*, Clarias gariepinus* (Burchell, 1822).

Figure 2.8: The Mozambique tilapia*, Oreochromis mossambicus* (Peters, 1852).

detritus (Skelton 2001). Although Mozambique tilapias are opportunistic feeders, the juveniles are mostly omnivorous, while adults mainly feed on detritus (Kím *et al*. 2002). According to IUCN, it is listed as “Near Threatened” on its Red Data List of Threatened Species and is likely to become locally extinct (Cambray & Swartz 2007), due to hybridization and competition with the introduced Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758). This hybridization may disrupt its genetic adaptations to resist drought, capacity to survive and reproduce in seawater and to resist temperatures as low as 11°C (Moralee *et al*. 2000).

2.6. Water Quality Parameters

Surface water temperature, dissolved oxygen content, salinity, pH, conductivity and total dissolved solids were determined *in situ* using a handheld YSI multi parameter instrument. Subsurface water samples were collected at all sampling sites and put in acid treated sampling bottles. Samples were frozen immediately and taken to an accredited laboratory for analyses of selected water parameters. The following parameters were of interest for this study: turbidity, dissolved nutrients (orthophosphate, nitrites and nitrates), anions (chloride, fluoride and sulphates), non-toxic constituents (total dissolved solids (TDS)) and toxic constituents (selected metals i.e. aluminium, copper, iron, lead, manganese, nickel and zinc).

2.6.1. Target Water Quality Range

Water quality guidelines provide an objective means for judging the quality needed to maintain a particular environmental value. The South African Guidelines for the Protection of Aquatic Ecosystems lists the recommended Target Water Quality Range (TWQR) for most water constituents (DWAF 1996). The TWQR is the range of concentrations or levels within which no measurable adverse effects are expected on the health of aquatic ecosystems (DWAF 1996). TWQR is a management objective (rather than a water quality criterion) derived from quantitative and qualitative criteria. As a matter of policy, the former Department of Water Affairs and Forestry strives to protect South Africa's water resources by maintaining water quality within the TWQR (DWAF 1996). The results obtained for water quality analyses during this study were compared with the TWQR for aquatic ecosystems where applicable and available.

2.7. Sampling of Fish and Parasites

2.7.1. Field sampling

During each seasonal survey approximately 20 host specimens of each of the selected host species were collected at each locality using multi-mesh gill nets of various mesh sizes (30 – 120 mm stretch mesh sizes). Sampling in the Luphephe-Nwanedi Dams and the Anglo Platinum Return Water Dam was done seasonally in April 2008 (autumn), July 2008 (winter), October 2008 (spring) and

January 2009 (summer). Sampling in the Flag Boshielo Dam was carried out in April 2009 (autumn), July 2009 (winter), October 2009 (spring) and January 2010 (summer). Gill nets of stretched mesh sizes 30–110 mm were set out in the late afternoon, left overnight and collected early in the morning. As the fish were removed from the gill nets (Figure 2.9A), they were checked for mobile external parasites (body surface, gill cavity and buccal cavity) (Figure 2.9B). The external parasites found were removed using a brush, placed in sampling bottles containing dam water and recorded. The fish from which parasites were obtained were marked using a tagging gun and plastic tags and the tag number recorded. The two species were placed in separate holder tanks (Figure 2.10) for further examination at the field laboratory.

Fish were killed by severing the spinal cord, covering its eyes with a damp cloth. Skin smears were made by holding fish firmly on the head and scrapping the skin on both sides with glass slides. The slides were scrutinized for monogeneans with the aid of a stereomicroscope. Blood samples were collected immediately after, by placing a fish horizontally on a dissection board, inserting a collar needle just below the lateral line, fitting a vacutainer containing ethylene-diamine-tetra acetic acid (EDTA) anticoagulant solution (for *C*. *gariepinus*) or Heparin (for *O*. *mossambicus*), which drew blood by suction.

Capillary tubes were three-quarter filled with blood and plugged at one end using commercial critoseal clay. These blood samples were centrifuged in a Heraeus-christ centrifuge for five minutes at 15 000 revolutions per minute. A

haematocrit reader (Figure 2.11) was used to determine the haematocrit value for each fish by expressing the amount of red and white blood cells as a percentage of the total measurement. The standard length (SL in mm), weight and sex of each fish specimen were recorded.

Figure 2.9: Field work in the Flag Boshielo Dam. A = collecting fish from gill nets. B = examining the buccal cavity of *Clarias gariepinus* for ectoparasites.

Figure 2.10: Aerated tanks as used during field sampling.

2.8. The Health Assessment Index and Parasite Index

Examination of external organs and tissues (gills, fins, skin, eyes and opercula) and internal organs and tissues (mesenteric fat, hindgut, kidney, liver, bile and spleen) was performed as described by Heath *et al*. (2003) and Jooste *et al*. (2005b) and recorded on HAI data sheets adapted from Adams *et al*. (1993). All internal organs were assessed with the help of a colour chart developed by Watson (2001) (Figure 2.12).

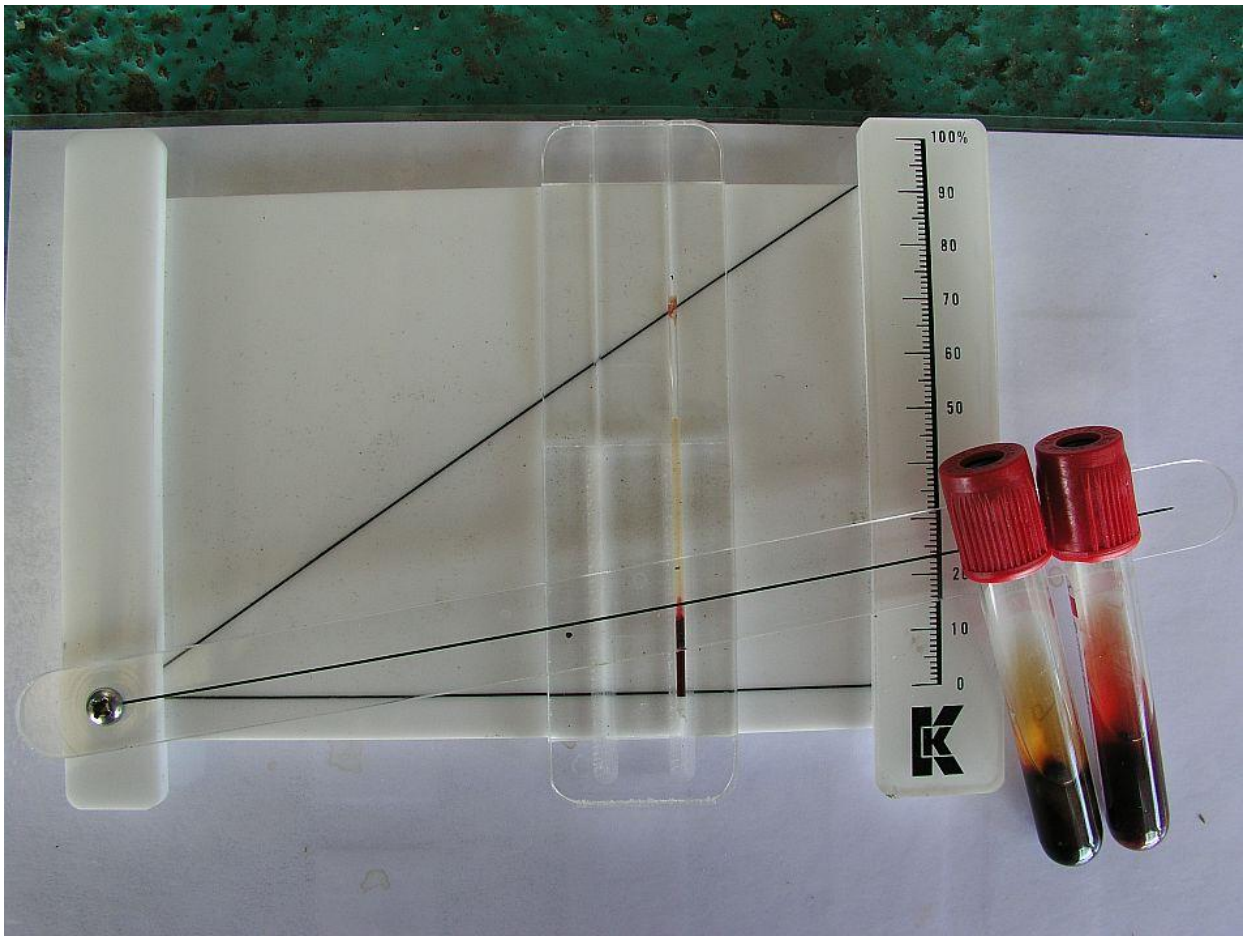


Figure 2.11: The haematocrit reader with capillary tube and blood samples.

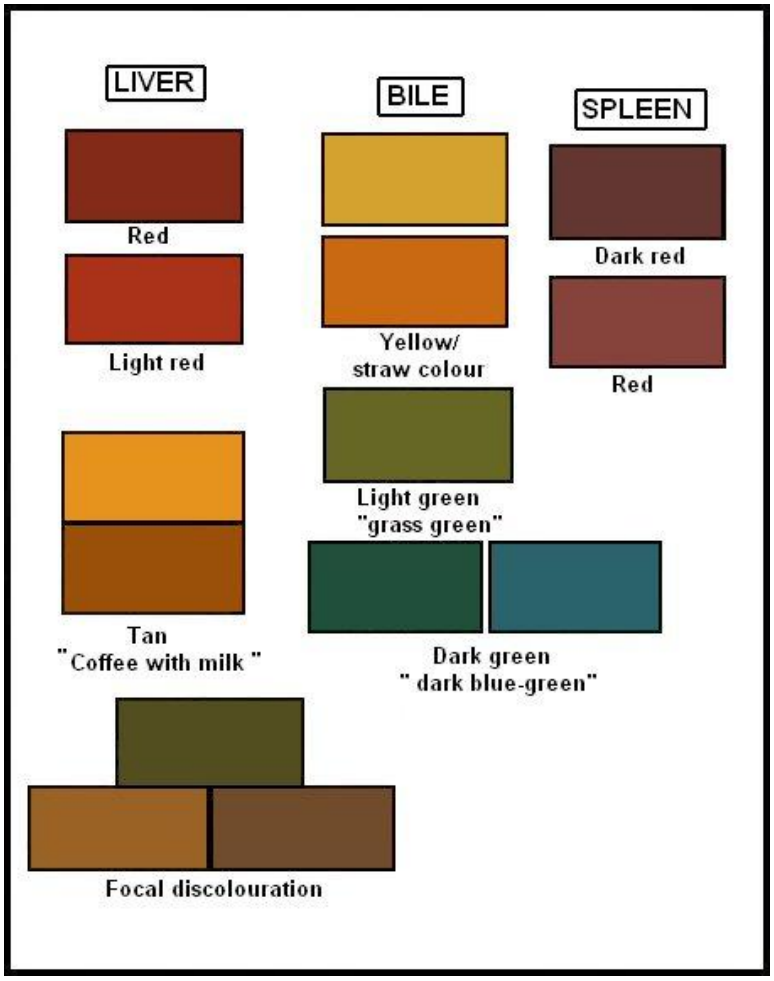


Figure 2.12: Colour chart used to compare the colour of liver, bile and spleen (Source: Watson 2001).

The gills of the fish were removed, placed in Petri dishes containing dam water and examined for copepod and monogenean parasites using a stereomicroscope with transmitted light (Figures 2.13A & B). The fish were then opened ventrally and the body cavity (Figure 2.14) and mesenteries were examined for metazoan parasites. Designated characters were assigned to the organs as indicated in the revised HAI (Table 2.1). Portions of the skin between

the lateral line and dorsal fin of each fish were peeled off with forceps and fillets of muscle tissue were cut and examined for encysted parasitic forms. The liver, spleen, gall bladder and kidneys of each fish were also examined for parasites or cysts (Figures 2.13A & B).

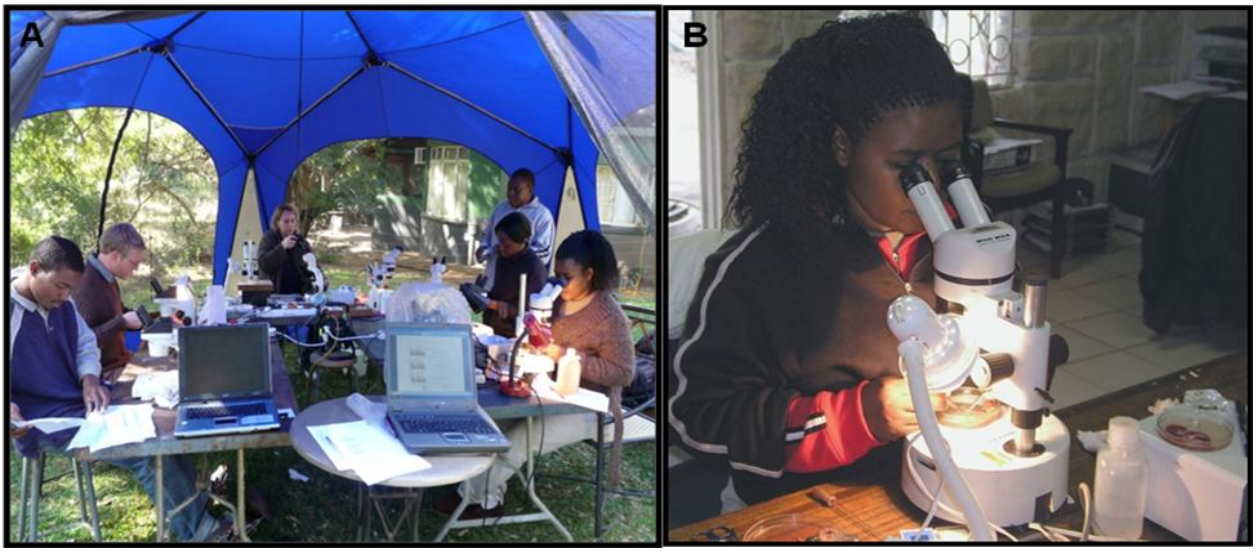


Figure 2.13: Searching for parasites in the field laboratory. A = Luphephe-Nwanedi Dams B = Anglo Platinum Mine Return Water Dam.

Figure 2.14: Fish dissection during field work.

Table 2.1: Fish health variables with assigned characters showing the norm and deviation from the norm in the necropsy based system (adapted from Adams *et al*. 1993).

Variables	Variable condition	Original field designation	Substituted value for the HAI
External variables			
Length	Total length in millimetres	mm	-
Weight	Weight in gram	g	-
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	Normal, no aberrations	0	0
	Mild skin aberrations	1	10
	Moderate skin aberrations	2	20
	Severe skin aberrations	3	30
Opercules	Normal/no shortening	0	0
	Mild/slight shortening	1	10
	Severe shortening	2	20
Gills	Normal	N	0
	Frayed	F	30
	Clubbed	C	30
	Marginate	M	30
	Pale	P	30
	Other	OT	30
Pseudobranch	Normal	N	0
	Swollen	S	30
	Lithic	L	30
	Swollen and lithic	P	30
	Inflamed	I	30
	Other	OT	30
Thymus[a]	No haemorrhage	0	0
	Mild haemorrhage	1	10
	Moderate haemorrhage	2	20
	Severe haemorrhage	3	30
	Swollen	S	30
	Mottled	M	30
	Granular	G	30
	Urolithic	U	30
	Other	OT	30

Table 2.1: (Continued).

Variables	Variable condition	Original field designation	Substituted value for the HAI
Internal variables (necropsy)			
Mesenteric fat[a]	(Internal body fat expressed with regard to amount present)		
	None	0	-
	Little, where less than 50% of each cecum is covered	1	-
	50% of each cecum is covered	2	-
	More than 50% of each cecum is covered	3	-
	Cecae are completely covered by large amount of fat	4	-
Spleen	Black	B	0
	Red	R	0
	Granular	G	0
	Nodular	NO	30
	Enlarge	E	30
	Other	OT	30
Hindgut	Normal, no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal	N	0
	Swollen	S	30
	Mottled	M	30
	Granular	G	30
	Urolithic	U	30
	Other	OT	30
Liver	Red	A	0
	Light red	B	30
	“Fatty” liver, “coffee with cream” colour	C	30
	Nodules in liver	D	30
	Focal discolouration	E	30
	General discolouration	F	30
	Other	OT	30
Bile[a]	Yellow or straw colour, bladder empty or partially full	0	-
	Yellow or straw colour, bladder full, distended	1	-
	Light green to “grass” green	2	-
	Dark green to dark blue-green	3	-
Blood (haematocrit)	Normal range	30-45%	0
	Above normal range	>45%	10
	Below normal range	19-29%	20
	Below normal range	<18%	30
Parasites	No observed parasites	0	0
	Few observed parasites	1	10
Endoparasites[b]	No observed endoparasites	0	0
	Observed endoparasites < 100	0	10
	100 -500	1	20
	> 500	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

2.8.1. Inverted Parasite Index (IPI)

The Inverted Parasite Index (IPI) evaluated by Crafford & Avenant-Oldewage (2009) was used to assign numerical values to the number of ecto- and endoparasites. The IPI is based on the premise that ectoparasites are more directly exposed than endoparasites to the effects of water quality (Crafford & Avenant-Oldewage 2009). It follows that more ectoparasites are to be found in good water quality environments. Since large numbers of ectoparasites are indicative of good water quality, they should be given a lower score because good water quality correlates with low HAI values. Thus, the absence or low numbers of ectoparasites are indicative of poor water quality, and are therefore given a higher score. Conversely, large numbers of endoparasites have a higher HAI score. Therefore endo- and ectoparasites were categorized as presented in Table 2.2.

Table 2.2: Numerical scoring system in use with the Inverted Parasite Index (IPI).

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

2.8.2. Fixation and preservation of parasites

Monogeneans collected from the skin were placed in a small Petri dish with dam water and fixed by adding hot (±70^{o}C) alcohol-formalin-acetic acid (AFA) fixative and stored in 70% ethanol. Unstained specimens used for measurement of the hamuli (anchors) and marginal hooks were mounted either in a mixture of ammonium picrate-glycerine (GAP) or in glycerine jelly under slight coverslip pressure, while slightly heated over an open flame. The preparations were sealed with clear nail vanish. Monogeneans collected from the gills were fixed and preserved in 4% formalin.

Digeneans were placed in saline solution and shaken vigorously from time to time to dislodge debris, fixed in hot AFA for approximately 10 minutes and preserved in 70% ethanol to which 5% glycerine was added.

Nematodes were carefully removed from the body cavity or stomach (nematodes in the stomach attached firmly when disturbed therefore brushing them with a fine brush helped release their firm hold) and fixed in glacial acetic acid for approximately 2 minutes (they uncoiled and stretched) and preserved in 70% ethanol with 2% glycerine added.

Cestodes were first cleared in 0.8% saline solution and then relaxed by swirling them in a small volume of dam water in a sample bottle. After muscle fatigue sets in, specimens were fixed by quickly adding buffered formalin while

still being swirled. Cestodes were removed from the buffered formalin solution after 10 minutes (to prevent the contained acetic acid from dissolving the calcareous corpuscles) and preserved in 70% ethanol.

Copepods from the skin and gills were kept alive in dam water. Excess mucus and debris were removed from the parasite with the aid of a fine brush. The copepods were fixed by adding 70% ethanol to the water in small quantities over a period of approximately one hour, where after they were stored in 70% ethanol.

2.8.3. Preparation of whole mounts of parasites

Monogeneans, digeneans and cestodes used for body measurements and detailed anatomical studies were stained in Horen's Trichome, counterstained in acetocarmaine and mounted in Canada balsam or Entellan. Nematodes were cleared for examination in lactophenol. Some specimens were examined as temporary mounts in lactoglycerol. Copepods were cleaned and cleared in lactophenol and studied using the wooden slide technique (Humes & Gooding 1964). All measurements of identified parasites were made with a calibrated ocular micrometer for an Olympus microscope and drawings were made with the aid of an Olympus Drawing tube attachment (U-DA).

2.8.4. Parasite identification

The parasites were identified based on their morphology and using drawings. Observations, drawings and measurements were made using an Olympus BX50 microscope, fitted with a drawing tube and a digital camera. Identifications were based on parasite descriptions in the literature: Parasite collections were identified according to various authors: Ergens (1973); Kritsky & Kulo (1988); Douëllou & Chishawa (1995); Khalil & Mashego (1998) for monogeneans; Barson *et al*. (2008); Chibwana & Nkwengulila (2010) for digeneans; Anderson (1992) for nematodes; Kuchta *et al*. (2008) and Scholz *et al*. (2009) for cestodes; Marx & Avenant-Oldewage (1996) for copepods; Avenant-Oldewage & Knight (1994) and Avenant *et al*. (1989) for crustaceans.

2.8.5. Calculation of the condition factor (K) and Health Assessment Index (HAI)

The numerical values used to classify recorded abnormalities were adopted as demonstrated in the user manual developed by Avenant-Oldewage *et al*. 1995. Additional variables made by Marx (1996) and slight modifications by Watson (2001) were adhered to. Original field designations of all variables from the autopsy based system were substituted with comparable numerical values into the HAI. Variables of the HAI are presented with a value ranging from 0-30, depending on the condition of the organs tested. Any abnormalities in the eyes, gills, pseudobranch, kidney, liver and spleen are given values of 30. For the

other variables (skin and fins), abnormalities are rated 10, 20 or 30 depending on the degree of abnormality, with the greatest abnormality ranked as 30.

To calculate an index value for each fish within a sample, numerical values for all variables were summed. The HAI for sample populations were calculated by adding all individual fish health index values and dividing it by the total number of fish examined. The standard deviation for each sample was calculated as proposed by Adams *et al*. (1993):

$$SD = \frac{\sum_{i-1}^{N} (V_i - X)^2}{N - 1}$$

where: N = number of fish per site; X = average index for each site and V_i = index value for fish i. The coefficient of variation (CV) was calculated as proposed by Adams *et al*. (1993): CV = 100 x SD/X, where: SD = standard deviation and X = average index for each site.

The condition factor (K) of fish, based on the analysis of length-weight data, indicates the condition of the fish in a habitat. The K was determined for the different fish populations to ascertain any differences in health of the fish between the different sampling sites. The condition factor for each fish was calculated using the formula: $K = 100\ W/L^3$, where: W = weight in g and L = standard length in millimetres (Bagenal & Tesch 1978).

2.9. Data analyses

Prevalence, intensity of infection and abundance were calculated and used as defined in Bush *et al*. (2001). The Shannon Wiener's index (H') and Brillouin's index were calculated as measures of community diversity (Magurran 1988) and the terms diversity and diverse were used to reflect the values of these diversity indices. For comparisons of most of the variables within the dams, regression analysis and analysis of variance (ANOVA) were used with suitable transformations where necessary. Multivariate analyses (e.g. ordination; Generalised Linear Models (GLM); cluster analyses) were used for comparisons among the three dams. Statistical software such as SPSS, STATISTICA, PRIMER 5 and CANOCO were used for these analyses.

CHAPTER 3

A COMPARATIVE ASSESSMENT OF THE HEALTH STATUS OF FERAL POPULATIONS OF *CLARIAS GARIEPINUS* AND *OREOCHROMIS MOSSAMBICUS* FROM THREE DAMS OF THE LIMPOPO AND OLIFANTS RIVER SYSTEMS USING THE FISH HEALTH ASSESSMENT INDEX PROTOCOL.

3.1. Introduction

Effective management strategies for aquatic ecosystems are developing in many countries (Barbour *et al*. 1999) and the paradigm is changing from the chemical-based to biological approach (Davis & Simon 1995). During the last several decades, water quality has been frequently evaluated by chemical monitoring such as nutrients, biochemical oxygen demand, and hazard chemicals. However, health assessments of aquatic ecosystems, based on various types of aquatic taxa, have been a hot central issue for effective water quality monitoring and this approach has been considered as a surrogate for achieving the goal of ecological integrity in aquatic ecosystems (Hunsaker & Levine 1995). In fact, Caims (1990) pointed out that simple chemical measurements may not detect an integrative health condition of water environments due to dynamic spatial and temporal variations. Thus, one approach to determine whether stress is occurring in an organism or population would be to compare biochemical events with indices that measure the health of the whole animal or population.

In aquatic ecosystems, fish are generally regarded as representative indicators of overall system health. Because of their position in the food chain, fish integrate the effects of many biotic and abiotic variables acting on the system and reflect secondary impacts of stress mediated through the food chain (Karr 1981). Thus, fish have been used successfully as a comparative animal model to generate data about the state of affairs in various environmental studies. Some of the commonly used approaches for qualitatively assessing fish health are age, growth analysis, the liver somatic index (LSI) and the condition factor (Adams *et al*. 1993). Other quantitative methods have used multiple tissue weights and blood chemistry (Karr 1995). Each of these sets of health measures has its own set of advantages, depending on the objectives of the particular study, but most of them cannot be rapidly and inexpensively applied to field studies. As a rapid and inexpensive alternative to these more sophisticated approaches for evaluating fish health and condition, Goede & Barton (1990) developed a field necropsy method that provides a health profile of fish based on the percentage anomalies observed in the tissues and organs of individuals sampled from a population.

A modification of the method of Goede & Barton (1990) was developed to numerically quantify variables of feral fish health (Adams *et al*. 1993). In this approach, index variables are assigned numerical values based on the degree of severity or damage caused by environmental stressors. Organs that are grossly examined include: eyes, skin, fins, opercules, gills, liver, spleen, hindgut, kidneys

and pseudobranchs. Other variables include the presence of parasites, as well as the values of haematocrit, leukocrit and plasma proteins. With the exception of the plasma protein and leukocrit measurements, every other variable can be obtained by field necropsy. All values are equally weighted and range from zero (no effect) to 30 (severely affected).

The Health Assessment Index (HAI) has been used in a wide range of waterways and river basins throughout the United States (North Carolina, Tennessee, Alabama and Kentucky) as well as reservoirs in Georgia and South Carolina (Adams *et al*. 1993). In South Africa, Avenant-Oldewage and Swanepoel (1993) suggested that fish be used for ecosystem health studies. Thereafter, The HAI was incorporated in the "Field Biosurveys and Integrated Ecological Assessment" by the Department of Water Affairs and Forestry (Killian 1996; Killian *et al*. 1997). This biomonitoring tool has been used successfully in the Olifants River System (Avenant-Oldewage *et al*. 1995; Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001; Ramollo 2008); in the Vaal River System (Crafford 2000; Groenewald 2000; Crafford & Avenant-Oldewage 2001; 2009; Bertasso 2004) and in the Ga-Selati River (Jooste *et al*. 2003; 2005a & b; Luus-Powell *et al*. 2005) using various fish species as indicator organisms and variations of the Parasite Index (discussed below).

The fish HAI, developed by Adams *et al*. (1993) made use of organs, blood and parasites as evaluative parameters. Parasites were seen as an indication of disease - an indication of deteriorated condition and therefore only

their presence or absence was recorded in the original HAI. Avenant-Oldewage *et al*. (1995) recommended the use of fish ecto- and endoparasites as an indicator of environmental health. Studies conducted by some authors (Khan & Thulin 1991; Overstreet 1993) supported the hypothesis that parasite communities are good indicators of environmental stress and biodiversity. Subsequent studies carried out in South Africa (Marx 1996; Robinson 1996; Luus-Powell 1997; Crafford 2000; Watson 2001; Jooste *et al*. 2003; 2005a & b; Luus-Powell *et al*. 2005; Ramollo 2008; Crafford & Avenant-Oldewage 2009) went a step further than Adams *et al*. (1993) and incorporated ecto- and endoparasites as separate variables in the HAI.

Crafford (2000) and Crafford & Avenant-Oldewage (2009) used four parasite indices, namely the original parasite index by Adams *et al*. (1993) (distinguished between the presence and absence of parasites), inserted Parasite Index by Marx (1996) (distinguished between the presence and absence of ecto- and endoparasites), refined parasite index by Marx (1996) (distinguished between the number of ecto- and endoparasites) and the inverted Parasite Index (IPI). The IPI is based on the premise that ectoparasites are more directly exposed than endoparasites to the effects of water quality. It follows that more ectoparasites are to be found in good water quality environments. Since large numbers of ectoparasites are indicative of good water quality, they should be given a lower score because good water quality correlates with low HAI values. It follows that absence or low numbers of ectoparasites are indicative of poor water

quality, and are thus given a higher score. Therefore endo- and ectoparasites were categorized as presented in Table 2.2.

3.2. Materials and Methods

Individual specimens of two commercially important feral fish species, *Clarias gariepinus* and *Oreochromis mossambicus* were sampled seasonally using gill nets of different stretched mesh sizes (30-110 mm) from three dams of the Limpopo and Olifants River Systems from April 2009 to January 2010. Description of the sampling sites as well as a detailed account of the sampling procedure and the examination of fish and parasites is given in Chapter 2.

3.3. Data Analyses

Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests, respectively. The variations in HAI values were tested by a one-way analysis of variance (ANOVA), considering sites, sex and seasons as variables. Whenever the ANOVA revealed significant differences, Tukey's post hoc multiple comparisons between sites and seasons were done to determine which sites or seasons differed significantly. Data were subjected to an unpaired Student's *t*-test to point out differences in condition factor between the sites. The aforementioned analyses were done using Statistical Package for Social Scientists (SPSS Statistics 17.0) and the significance of results was ascertained at $p < 0.05$. Multivariate analyses based

on Bray-Curtis similarity coefficients and group averaging sorting were performed on the data using CAP 1.52 (Community Analysis Package), Windows® 95/98 program (Pisces Conservation Ltd). To determine site associations of parasite species, Principal Component Analyses were computed and plotted using CANOCO version 4 (Ter Braak & Smilauer 1998).

3.4. Results

Table 3.1: Water quality parameters measured in three dams of the Limpopo and Olifants River Systems. Values are given as averages of four seasonal sampling periods. Unless otherwise indicated, units are in mg/l.

Variable	Luphephe-Nwanedi Dam				Flag Boshielo Dam				Return Water Dam				TWQR
	Mean	± SD	Min	Max	Mean	± SD	Min	Max	Mean	±SD	Min	Max	
Temperature ^{o}C	24.5	3.2	14.1	27.5	26.4	1.5	15.4	27.8	23.4	1.4	15.4	24.4	-
pH	7.4	0.2	7.2	7.6	7.9	0.7	6.1	8.8	7.1	0.3	6.8	7.4	6.5-9
Conductivity (mS/m)	8.9	1.1	7.5	9.4	42.5	4.1	35.0	48.5	174.3	25.8	153.0	203.0	-
Turbidity in (NTU)	2.3	1.2	1.2	4.0	2.0	0.9	0.6	3.7	8.3	1.8	6.4	10.0	-
Dissolved Oxygen	7.0	0.4	6.6	7.5	7.8	1.6	4.4	9.4	**5.4**	**1.8**	**3.4**	**6.8**	6-9
Total Alkalinity	22.0	2.3	20.0	24.0	68.9	10.8	60.0	88.0	61.3	10.1	52.0	72.0	-
Ammonium	<0.2	0.0	<0.02	<0.02	<0.2	0.0	<0.2	<0.2	**0.6**	**0.5**	**0.2**	**1.1**	0.2
Nitrate	0.1	0.1	0.2	0.4	0.2	0.0	0.2	0.2	**0.6**	**0.1**	**0.4**	**0.8**	0.5
Nitrite	0.1	0.0	0.1	0.1	<0.2	0.0	<0.2	<0.2	**0.25**	**0.1**	**0.3**	**0.4**	0.2***
Ortho-Phosphate	<0.02	0.0	<0.02	<0.02	<0.2	0.2	<0.2	<0.2	**7.0**	**1.8**	**5.2**	**8.8**	0.1*
Sulphate ($SO_{4)}$	5.5	0.6	5.0	6.0	**111.1**	**4.3**	**105.0**	**116.0**	**581.0**	**68.5**	**527.0**	**658.0**	100**
Chloride (Cl)	13.5	1.0	12.0	14.0	25.1	5.0	18.0	33.0	176.0	40.8	144.0	222.0	600
Sodium (Na)	10.8	2.1	8.0	13.0	7.2	9.3	0.4	6.82	226.3	40.3	196.0	272.0	100
Potassium (K)	0.8	0.2	0.6	1.0	5.4	1.1	4.2	6.82	23.6	7.3	20.0	32.0	200
Calcium (Ca)	5.5	1.3	4.0	7.0	22.8	1.5	20.6	24.6	87.3	8.4	82.0	97.0	-
Magnesium (Mg)	2.5	0.6	2.0	3.0	15.4	2.5	11.0	18.2	38.0	6.2	31.0	43	70
Aluminium (Al)	<0.01	0.0	<0.01	<0.01	**0.2**	**0.1**	**0.1**	**0.3**	**0.1**	**0.0**	**0.1**	**0.1**	0.01
Arsenic	<0.01	0.0	<0.01	<0.01	0.003	0.0	0.003	0.003	<0.01	0.0	<0.01	<0.01	100
Copper	<0.025	0.0	<0.025	<0.025	**-**	**-**	**-**	**-**	<0.025	0.0	<0.025	<0.025	0.5
Iron (Fe)	<0.025	0.0	<0.025	<0.025	**0.15**	**0.1**	**0.05**	**0.179**	<0.025	0.0	<0.025	<0.025	0.02*
Lead (Pb)	0.02	0.0	0.02	0.02	**0.1**	**0.0**	**0.1**	**0.1**	**0.28**	**0.03**	**0.148**	**0.449**	0.012
Manganese (Mn)	<0.025	0.0	0.025	0.025	0.024	0.01	0.014	0.046	0.1	0.0	0.0	0.1	0.18
Zinc	<0.025	0.0	<0.025	<0.025	<0.01	0.0	<0.01	<0.01	<0.025	0.0	<0.025	<0.025	1

TWQG = Target Water Quality Guidelines for South African aquatic ecosystems * = Kempster & van Vliet 1980; **Canadian Guidelines 1987 cited by South African National Water Quality Guidelines 1993; *** = Kempster & van Vliet 1980; USA Guidelines 1987 cited by South African National Water Guidelines 1993. Values above the South African target water quality range are in bold.

The pH readings at Luphephe-Nwanedi Dams and Return Water Dam were stable around neutral (Table 3.2). The pH at Flag Boshielo Dam showed great variability (standard deviation = 0.7), with the minimum value of 6.1 being

lower and not within expectations for good water quality (Table 3.2). Dissolved oxygen concentrations in Luphephe-Nwanedi Dams (7.03 mg/l) were adequate and within the acceptable limits of the TWQR (Table 3.2). Dissolved oxygen concentrations below the South African TWQR of 6.0 mg/l were recorded at the Return Water Dam. There was greater variability (standard deviation = 1.61) in the oxygen concentrations at Flag Boshielo Dam, although the values remained adequate and within the TWQR (Table 3.2).

The levels of nutrients (ammonium, nitrate, nitrite, orthophosphate) showed that Luphephe-Nwanedi Dams and Flag Boshielo Dams are oligotrophic whereas the Return Water Dam is eutrophic (Table 3.2). At Flag Boshielo Dam and the Return Water Dam, sulphate levels exceeded 100 mg/l, which is above the Canadian standards for aquatic ecosystems. Traces of potassium, calcium and magnesium, were below the South African TWQR, while other metal elements such as arsenic and zinc (<0.025mg/l) were below instrumental detection limits at the three sites (Table 3.2).

The length, mass and condition factor (K) of the two fish species are shown in Table 3.3. Mean K values for *O*. *mossambicus* were 0.6 at Luphephe-Nwanedi Dams, 0.8 at Flag Boshielo Dam and 1.1 at the Return Water Dam (Table 3.3). The K values for *C*. *gariepinus* showed a similar trend of having the highest value of 1.4 at the Return Water Dam, lowest value of 1.0 at Luphephe-Nwanedi Dams and intermediate value of 1.2 at Flag Boshielo Dam (Table 3.3). There was a significant difference in the condition factor ($p > 0.05$) of *C*.

gariepinus sampled from Luphephe-Nwanedi Dams and the Return Water Dam, while the K value of *O*. *mossambicus* was significantly higher ($p < 0.05$) at the Return Water Dam than at both Luphephe-Nwanedi Dams and Flag Boshielo Dam (Table 3.3).

From the averaged values of data pooled from the four seasonal surveys, the HAI results showed that *C. gariepinus* was more affected in terms of necropsy-related anomalies and haematocrit values, when compared with *O*. *mossambicus* (Table 3.4). The coefficients of variation in HAI values (for both fish species) were highest for the fish sampled from the Return Water Dam, lowest at Luphephe-Nwanedi Dams and intermediate at Flag Boshielo Dam (Table 3.4). The inverted ectoparasite index and haematocrit values, condition of the gills and liver were primarily responsible for influencing the HAI of *O*. *mossambicus* at all the sites (Table 3.5). A low number or absence of ectoparasites in *O*. *mossambicus* was observed in 66.7%, 85.4% and 100% of fish examined at Luphephe-Nwanedi Dams, Flag Boshielo Dam and the Return Water Dam, respectively (Table 3.5).

The number of endoparasites encountered on *O*. *mossambicus* sampled from Flag Boshielo Dam and the Return Water Dam were few, and according to the IPI principle, corresponded with zero HAI values. Abnormalities of the eyes, spleen, skin, opercules, fins and hindgut were observed in a small proportion of the *O*. *mossambicus* specimens examined at all sites (Table 3.5; Figures 3.1 - 3. 7). Haematocrit readings at the Return Water Dam were not within

the normal range in 72.3% of the cichlid specimens examined (Table 3.5). Many of the abnormal livers recorded at Flag Boshielo Dam and Return Water Dam were reddening and/or inflammation due to parasites; focal discolouration or a coffee-cream colour (Figure 3.6). The most common gill anomalies at Flag Boshielo Dam and Return Water Dam resulted in pale, clubbed, frayed, marginate or deformed gills (Figure 3.4) while kidney anomalies were a result of encysted *Tetracotyle* species.

Catfish collected from the Return Water Dam had the highest mean HAI value of 93.3 (Table 3.4). The inverted parasite index and haematocrit values emerged as the HAI variables that were predominantly responsible for most of the abnormalities observed in catfish from all the sites (Table 3.5). According to the IPI premise, the mean abundance values of ectoparasites in *C*. *gariepinus* were low in all the fish examined (100%), at all the three sites (Table 3.5). The greatest number of endoparasites was collected from Luphephe-Nwanedi Dams, while the least number of endoparasites was recorded in Flag Boshielo Dam (Table 3.5). Changes in the gills and liver also contributed to a large extent, to the higher overall HAI value of *C*. *gariepinus* sample population at Flag Boshielo Dam. Gills, skin, fins, and liver presented important anomalies in the catfish at the Return Water Dam (Table 3.5; Figures 3.2 - 3.6). Relatively low proportions of catfish from all sites had myxozoan cysts on pseudobranchs (Figures 3.3E).

Table 3.2: Length, mass and condition factor (K) of *Clarias gariepinus* and *Oreochromis mossambicus* from three dams of the Limpopo and Olifants River Systems.

	Length (mm)			Mass (g)			Condition factor (K)		
	LND	FBD	RWD	LND	FBD	RWD	LND	FBD	RWD
O. mossambicus	176.4± 42.2	232.87± 49.8	218.3± 61.1	194.3± 147	405.1± 313.4	495.9± 340.5	0.6 ± 0.3	0.8± 0.6	1.1± 0.8**
C. gariepinus	519.5 ± 133.7	591.4 ± 238.8	636.4 ± 123.2	1636 ± 1406	3215.2 ± 2893.9	3246.9±1610.1	1± 0.1	1.2 ±0.6	1 .4± 0.8*

Note. Values are means ± standard deviation. * significant differences in K with respect to LND conspecific as determined by Student's *t*-test.** significant differences in K with respect to LND and FBD conspecific as determined by Student's *t*-test. LND = Luphephe-Nwanedi Dams, FBD = Flag Boshielo Dam, RWD = Return Water Dam.

Table 3.3: Health Assessment Index (HAI) values for *Oreochromis mossambicus* and *Clarias gariepinus* from three dams of the Limpopo and Olifants River Systems (pooled data).

Locality	Health Assessment Index value	Standard Deviation	Coefficient of variation
	Oreochromis mossambicus		
Luphephe-Nwanedi Dams	24.4	19.5	79.6
Flag Boshielo Dam	38.5	27.0	69.9
Return Water Dam	80.6	28.0	34.7
	Clarias gariepinus		
Luphephe-Nwanedi Dams	42.7	20.7	48.5
Flag Boshielo Dam	84.0	37.2	44.2
Return Water Dam	93.3	27.3	29.3

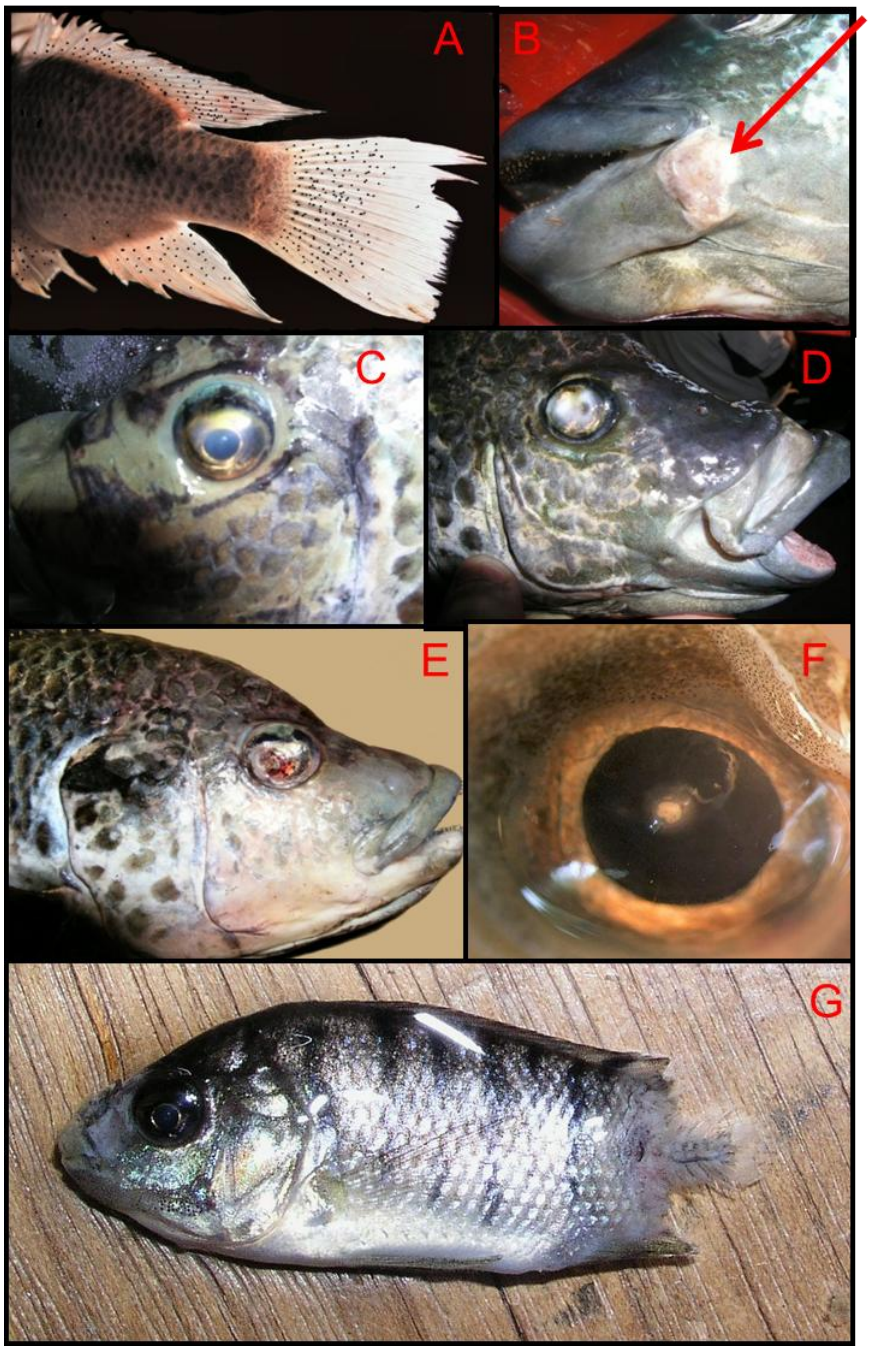


Figure 3.1: Anomalies recorded from external surfaces of *Oreochromis mossambicus*. A = black spots of *Neascus* species on fins and body; B = lesion (arrowed); C & D = opaque eyes; E = blind eye; F = eye infected by *Diplostomum* species larvae; G = eroded fins.

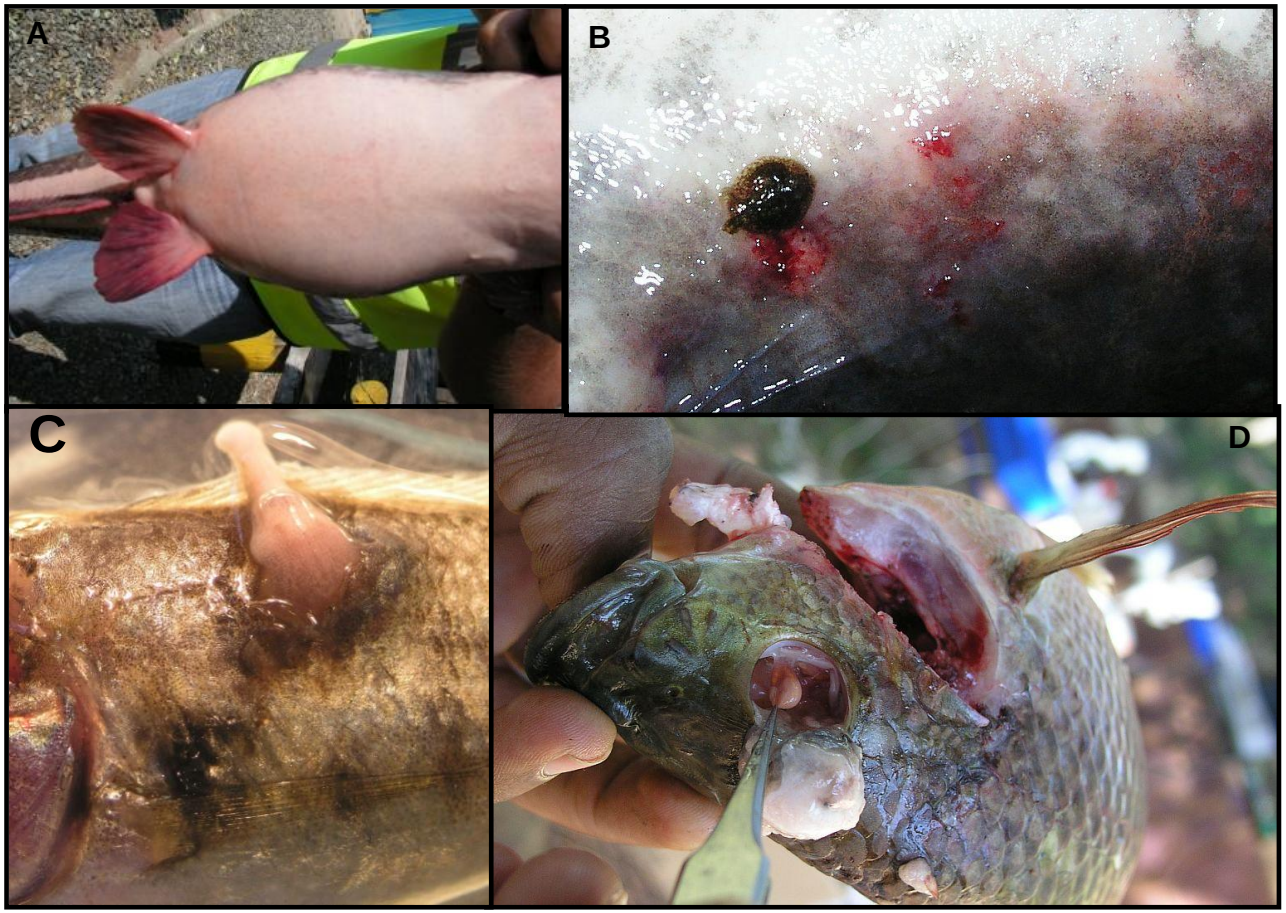


Figure 3.2: Anomalies recorded from the external surfaces of *Clarias gariepinus* and *Oreochromis mossambicus*. A = reddening of belly, skin and fins of *Clarias gariepinus*; B = lesions caused by *Dolops ranarum* on the skin of *C. gariepinus*; C = lesion caused by *Clinostomum* species larvae on the skin of *O. mossambicus;* D = *Clinostomum* species larvae from under the eye.

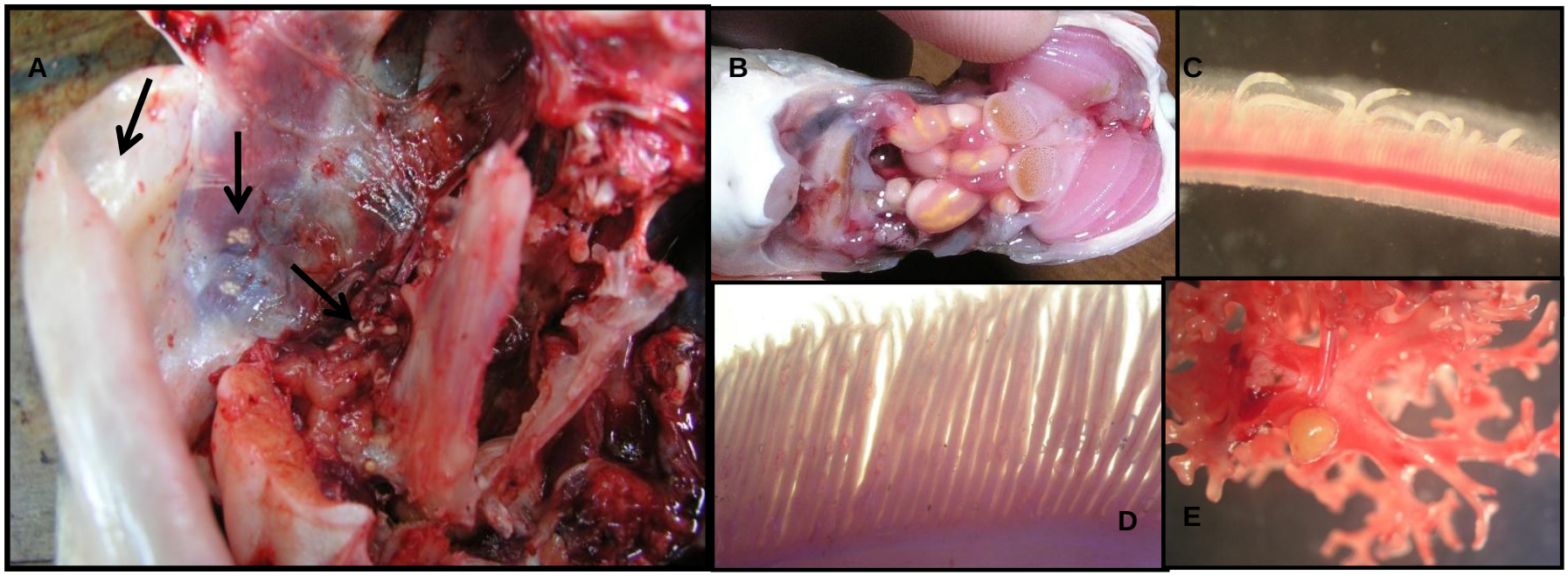


Figure 3.3: Endoparasitic infections. A = encysted *Tetracotyle* species larvae (arrowed) in the branchial cavity of *Clarias gariepinus*; B = *Clinostomum* species larvae in the branchial cavity of *Oreochromis mossambicus*; C = monogeneans on the gills of *Oreochromis mossambicus*; D = encysted *Acanthostomum* species larvae on the gills of *Clarias gariepinus*; E = a myxozoan cyst on the psuedobranch *of Clarias gariepinus*.

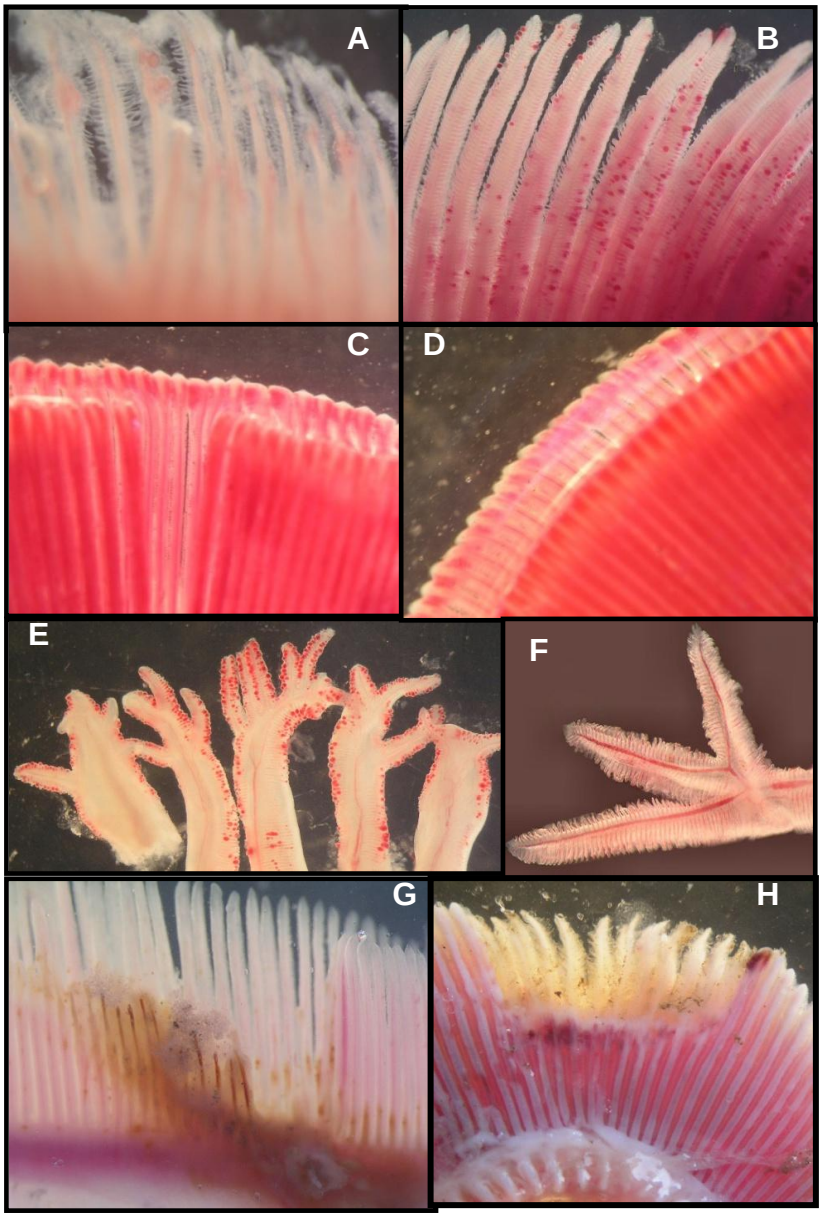


Figure 3.4: Anomalies recorded from the gills of both fish species. A = pale; B = bloody marks on filaments of *Oreochromis mossambicus*; C & D = Swollen (clubbing) and frayed tips on *Oreochromis mossambicus*; E & F = deformed gills of *Clarias gariepinus*; G & H = rotten gills with sessile protozoans on *Oreochromis mossambicus*.

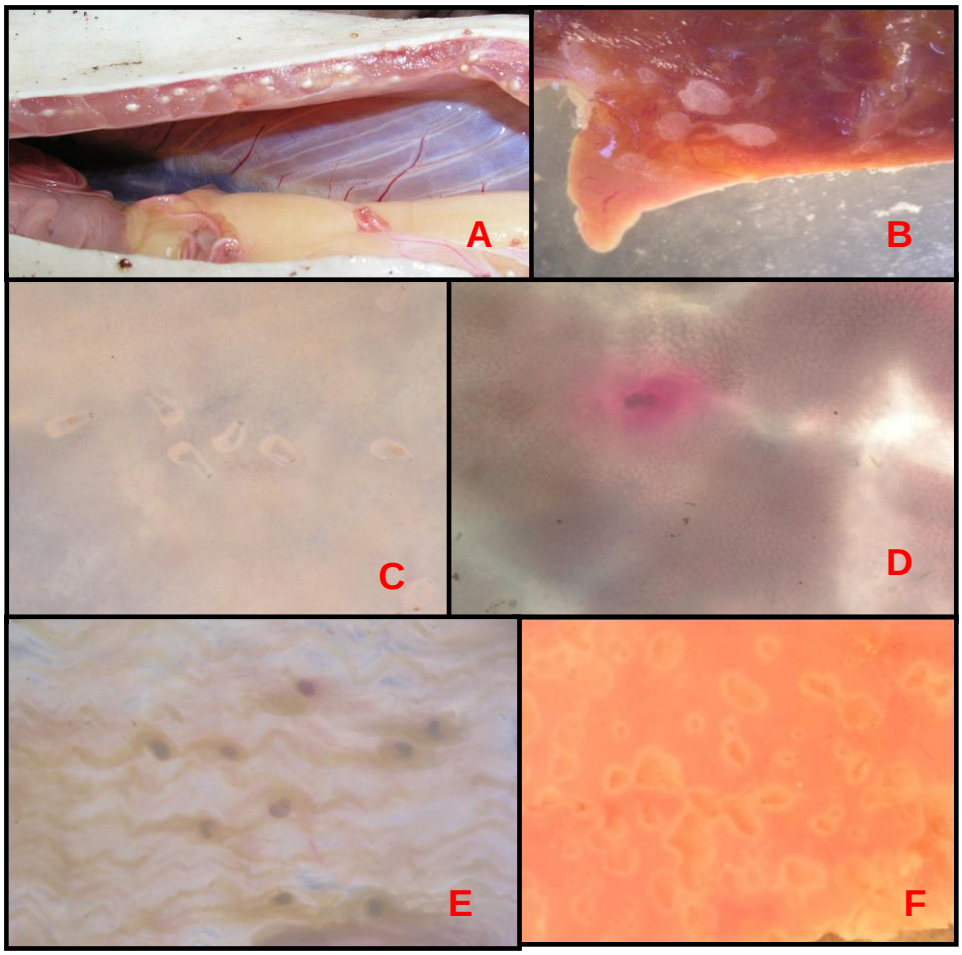


Figure 3.5: Endoparasites from different organs. A = encysted *Tetracotyle* species larvae from the muscle of *Clarias gariepinus*. B = encysted gryporynchid cestode larvae on liver on *Oreochromis mossambicus*; C = *Enterogyrus* species *in situ* on the stomach mucosa of *Oreochromis mossambicus*; D = inflammation and reddening caused by *Enterogyrus* species on the stomach mucosa of *Oreochromis mossambicus*; E = gryporynchid cestode larvae in the intestines of *Oreochromis mossambicus*; F = inflammation of the hindgut of *Oreochromis mossambicus* caused by gryporynchid cestode larvae.

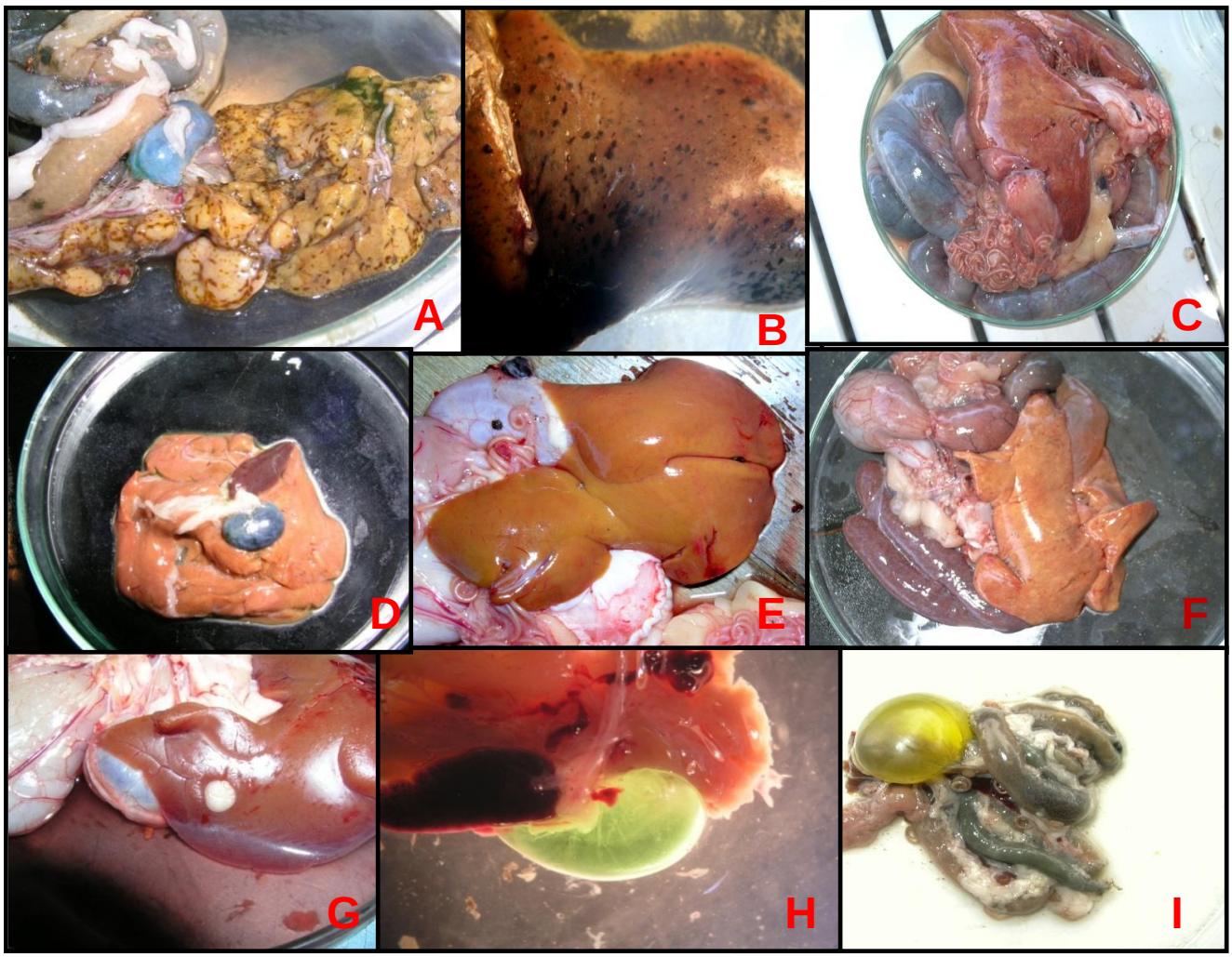


Figure 3.6: Anomalies recorded from the liver. A = focal discolouration and extended dark blue-green bile on *Oreochromis mossambicus*; B = focal discolouration of liver of *Oreochromis mossambicus*; C = nodules in liver of *Clarias gariepinus*; D-F = "tan" with coffee-cream colour (note D has an extended dark blue-green coloured bile: D is from *Oreochromis mossambicus* and E-F are from *Clarias gariepinus*); G = fatty deposit on liver of *Clarias gariepinus*; H & I = light green to grass green bile of *Oreochromis mossambicus*.

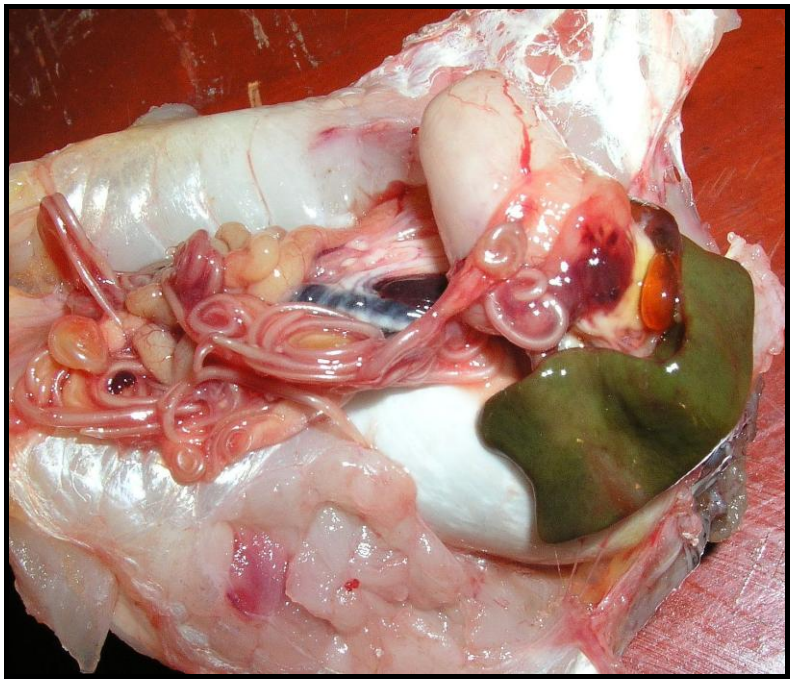


Figure 3.7: Green colouration of the spleen of *Oreochromis mossambicus*. (Also shown are larvae of *Contracaecum* sp.

Table 3.4: Percentage of fish with organ, haematocrit, inverted ecto- and endoparasite anomalies in a sample collected from three dams of the Limpopo and Olifants River Systems.

	Percentage of fish with anomalies in												
Locality	Eyes	skin	fins	operc	gills	p-branch	liver	spleen	h-gut	kidney	Hct	ecto	endo
						Oreochromis mossambicus							
LND	4.4	0.0	4.4	0.0	2.2	-	4.4	0.0	2.2	0.0	10.0	66.7	4.4
FBD	8.3	4.2	6.3	6.3	25.0	-	16.7	8.3	6.3	2.4	43.8	85.4	0.0
RWD	17.0	10.6	6.4	8.5	29.8	-	31.9	12.8	6.4	3.4	72.3	100.0	0.0
						Clarias gariepinus							
LND	2.2	2.2	0.0	2.2	2.2	6.7	6.7	0.0	4.4	0.0	24.4	100.0	71.1
FBD	9.8	4.9	14.6	14.6	43.9	14.6	43.9	7.3	7.3	6.8	29.3	100.0	26.8
RWD	11.1	28.9	26.7	11.1	33.3	11.1	20.0	6.7	13.3	3.3	84.4	100.0	35.6

operc = opercules; p-branch = pseudobranch; Hct = haematocrit; ecto = ectoparasites; endo = endoparasites.
LND = Luphephe-Nwanedi Dams, FBD = Flag Boshielo Dam, RWD = Return Water Dam.

Anomalies of the spleen in *C*. *gariepinus* were only observed in 6.7% and 7.3% of the fish from Return Water Dam and Flag Boshielo Dam, respectively. There were no anomalies of the fins, spleen and kidneys in the catfish sampled from Luphephe-Nwanedi Dams (Table 3.5).

These anomalies consequently led to HAI values that ranged from zero to 140 for *O*. *mossambicus* and from 20 to 170 for *C*. *gariepinus* (Figures 3.8A & B). Higher HAI values were generally recorded in April (autumn) and July (winter) for both bioindicator species (Figures 3.8A & B). Eroded fins, aberrations on skin, inflamed or reddened hindgut, green spleen, low values of inverted ectoparasite index and abnormal haematocrit readings were common occurrences in autumn, while cysts on pseudobranch, alterations of the gills, liver and eyes frequently occurred in both winter and autumn (Figure 3.9). These anomalies were responsible for the higher HAI values recorded in autumn and winter (Figures 3.8A & B). Shortened opercules, mottled kidneys, anomalies of the inverted endoparasite index were sporadically observed more frequently in summer and spring (Figure 3.9).

The underlying seasonal patterns in HAI values shown in Figures 3.8A, B and 3.9, were nevertheless, statistically insignificant as revealed by a one-way ANOVA ($p > 0.05$). Sex related differences in HAI values were also absent (one-way ANOVA; $p > 0.05$). Sex related differences in HAI values were also absent (one-way ANOVA; $p > 0.05$). There were significant differences, however, in the mean HAI values of *O*. *mossambicus* among sites (one-way ANOVA; F = 63.6;

$p < 0.05$). The multiple comparisons Tukey's post hoc tests revealed significant differences between Luphephe-Nwanedi Dams and Flag Boshielo Dam ($p < 0.05$), Luphephe-Nwanedi Dams and Return Water Dam and also between Flag Boshielo Dam and Return Water Dam ($p < 0.05$; Table 3.6). Significant differences in the HAI values of *C*. *gariepinus* among sites (one-way ANOVA; $F = 38.3$; $p < 0.05$) were also revealed (Table 3.7). The multiple comparisons Tukey's post hoc tests revealed significant differences between Luphephe-Nwanedi Dams and Flag Boshielo Dam ($p < 0.05$) and between Luphephe-Nwanedi Dams and Return Water Dam ($p < 0.05$; Table 3.7). But, there were no significant differences in HAI values between Flag Boshielo Dam and Return Water Dam ($p > 0.05$; Table 3.7) for *C*. *gariepinus*.

The dendrograms resulting from performing cluster analysis on the averaged seasonal HAI values for the two bioindicator species, *O*. *mossambicus* and *C*. *gariepinus* are shown in Figures 3.10A & B, respectively. Each dendrogram places the biomarker responses of the three sampling sites into three discrete groupings. However, in the case of *O*. *mossambicus*, the autumn sample within the Return Water Dam cluster is distinctly different from the other three seasonal samples (Figure 3.10A).

Abnormal readings of haematocrit as well as liver, eye and gill anomalies of *O*. *mossambicus* occurred regularly in the Return Water Dam and Flag Boshielo Dam (Figure 3.11). Sporadic occurrences of anomalies of the hindgut and opercules in *O*. *mossambicus* were more associated with Flag Boshielo Dam

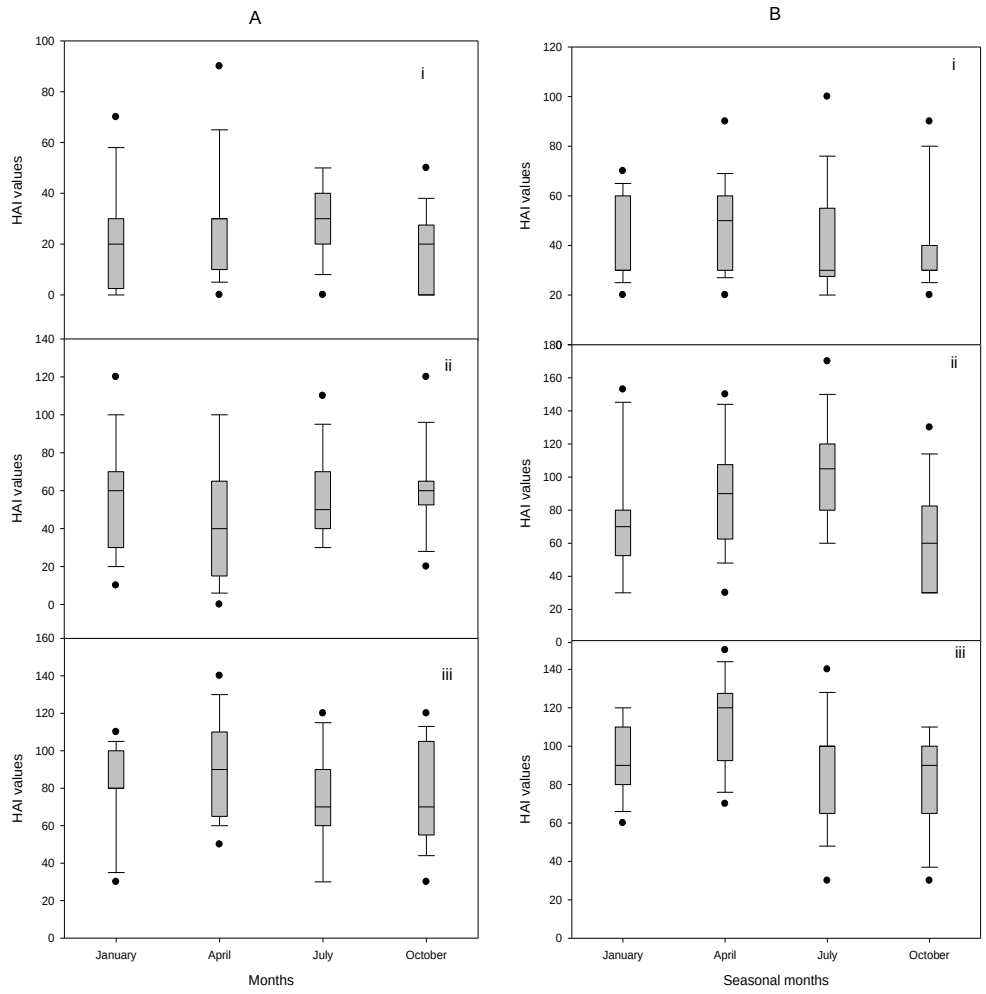


Figure 3.8: Seasonal changes in HAI values in A = *Oreochromis mossambicus* and B = *Clarias gariepinus* at i = Luphephe-Nwanedi Dams, ii = Flag Boshielo Dam and iii = Return Water Dam.

(Figure 3.11). A higher number of liver and pseudobranch anomalies in *C*. *gariepinus* were recorded from Flag Boshielo Dam while the frequency of anomalies of the hindgut, fins, skin, low values of inverted ectoparasite index and haematocrit were high at the Return Water Dam (Figure 3.12). The frequency of anomalies for the gills of *C*. *gariepinus* was similar in Flag Boshielo Dam and

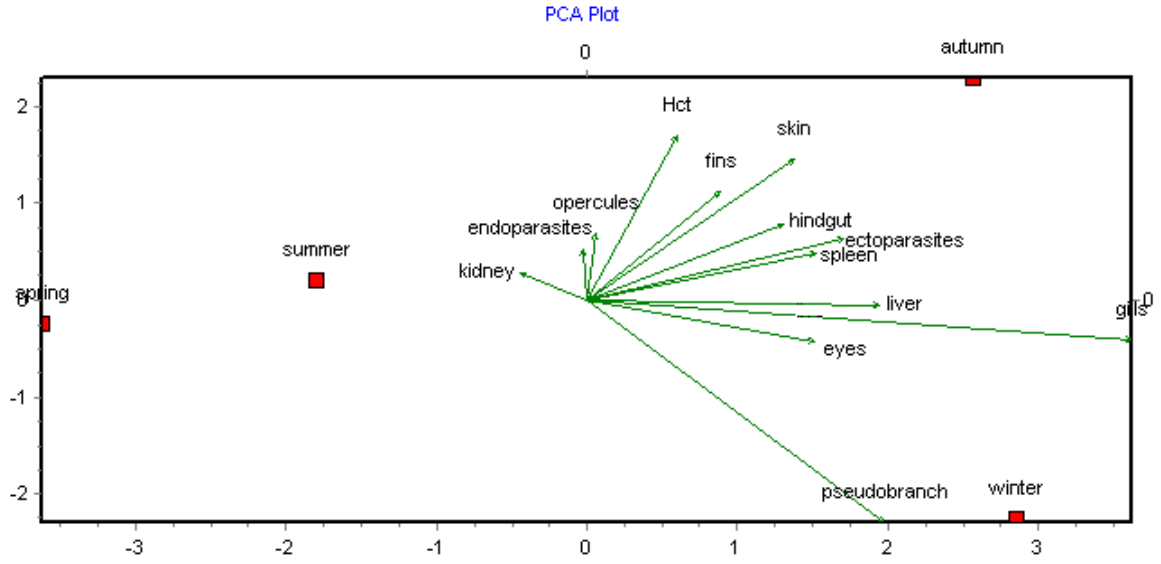


Figure 3.9: Principal Components Analysis (PCA) ordination showing seasonal organ, haematocrit, inverted ecto- and endoparasite anomalies (pooled data from *Clarias gariepinus* and *Oreochromis mossambicus*).

Return Water Dam (Figure 3.12). Anomalies of the spleen, eyes and opercules were observed intermittently in both Return Water Dam and Flag Boshielo Dam (Figure 3.12). A higher abundance of endoparasites was recorded at Luphephe-Nwanedi Dams (Figure 3.12).

Oreochromis mossambicus sampled from Luphephe-Nwanedi Dams and Flag Boshielo Dam hosted six ectoparasite species each compared to one ectoparasite species at Return Water Dam (Table 3.8; Figures 3.13 & 3.14).

Table 3.5: One way analysis of variance (ANOVA) comparisons of mean HAI values (with the inclusion of IPI) of *Oreochromis mossambicus* populations sampled in three dams of the Limpopo and Olifants River Systems.

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
LND	FBD	-13.86556*	5.14426	.021	-26.0528	-1.6784
	RWD	-56.19385*	5.22145	.000	-68.5639	-43.8238
FBD	LND	13.86556*	5.14426	.021	1.6784	26.0528
	RWD	-42.32830*	5.08633	.000	-54.3783	-30.2783
RWD	LND	56.19385*	5.22145	.000	43.8238	68.5639
	FBD	42.32830*	5.08633	.000	30.2783	54.3783

*The mean difference is significant at the 0.05 level.

Table 3.6: One way analysis of variance (ANOVA) comparisons of mean HAI values (with the inclusion of IPI) of *Clarias gariepinus* populations sampled in three dams of the Limpopo and Olifants River Systems.

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
LND	FBD	-41.333*	6.249	.000	-56.15	-26.52
	RWD	-50.667*	6.102	.000	-65.14	-36.20
FBD	LND	41.333*	6.249	.000	26.52	56.15
	RWD	-9.333	6.249	.297	-24.15	5.48
RWD	LND	50.667*	6.102	.000	36.20	65.14
	FBD	9.333	6.249	.297	-5.48	24.15

* The mean difference is significant at the 0.05 level.

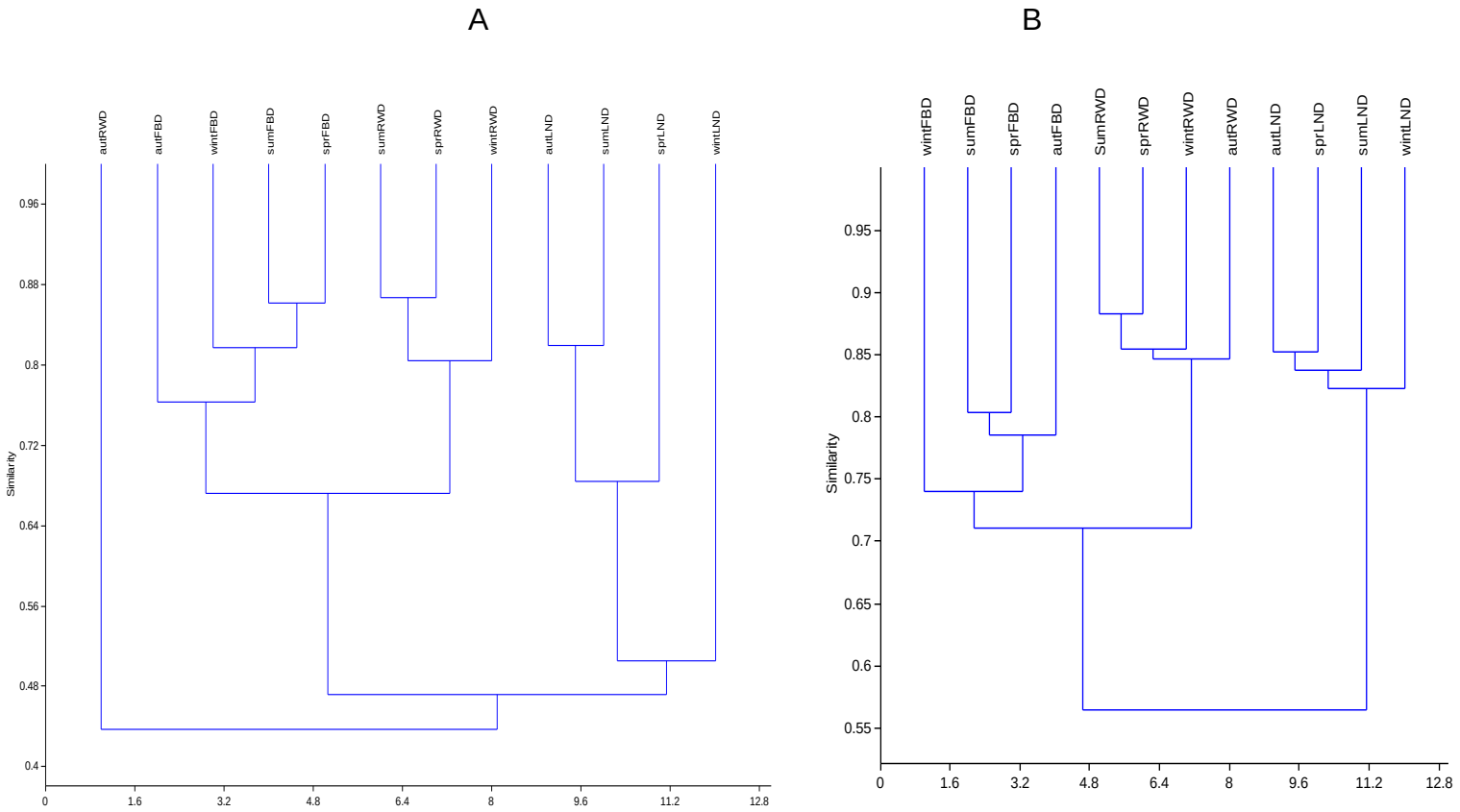


Figure 3.10: Spatial and temporal HAI values (with the inclusion of IPI) of A = *Oreochromis mossambicus* and B = *Clarias gariepinus* sampled from three dams of the Limpopo and Olifants River Systems. Seasons are indicated by sum = summer; aut = autumn, wint = winter, spr = spring. Sampling sites are indicated by LND = Luphephe-Nwanedi Dams; FBD = Flag Boshielo Dam; RWD = Return Water Dam.

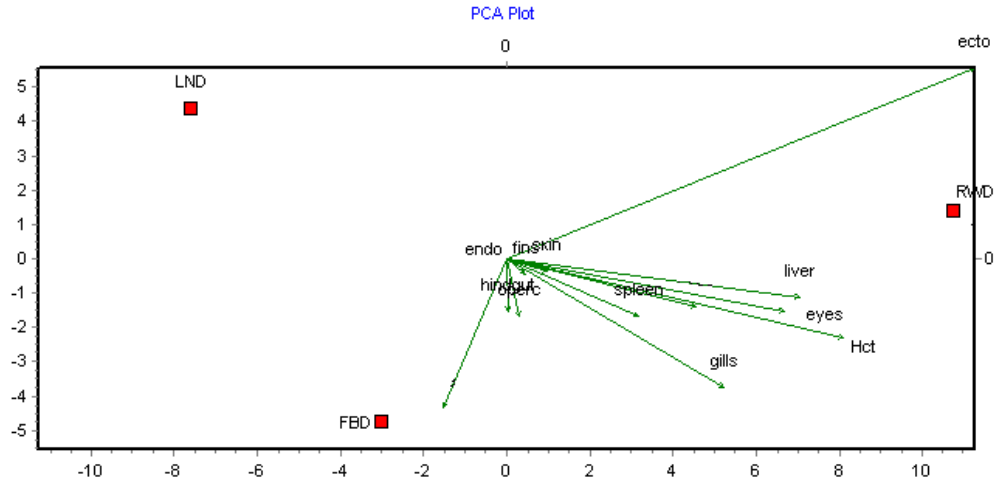


Figure 3.11: Principal Components Analysis (PCA) ordination showing the comparative distribution of organ, haematocrit, inverted ecto- and endoparasite anomalies in *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

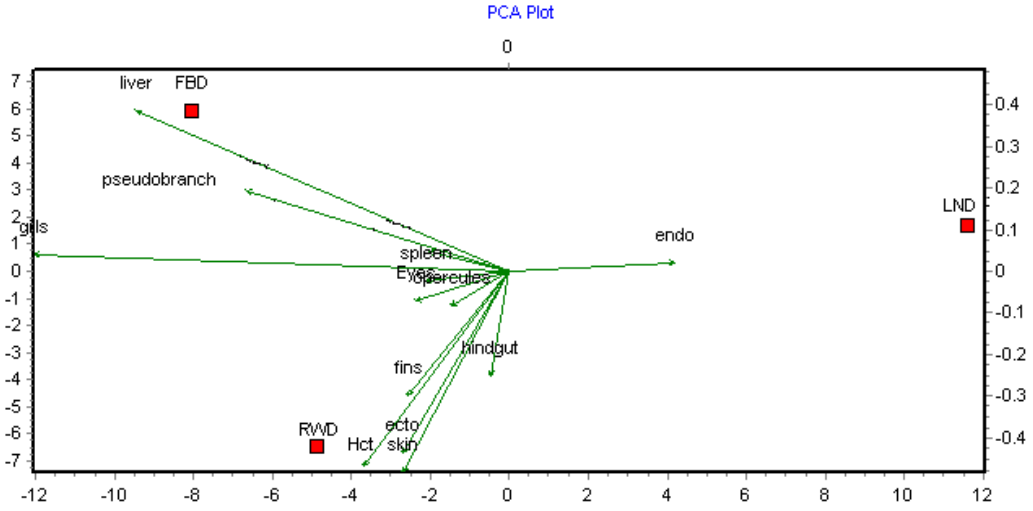


Figure 3.12: Principal Components Analysis (PCA) ordination showing the comparative distribution of organ, haematocrit, inverted ecto- and endoparasite anomalies in *Clarias gariepinus* sampled from three dams of the Limpopo and Olifants River Systems.

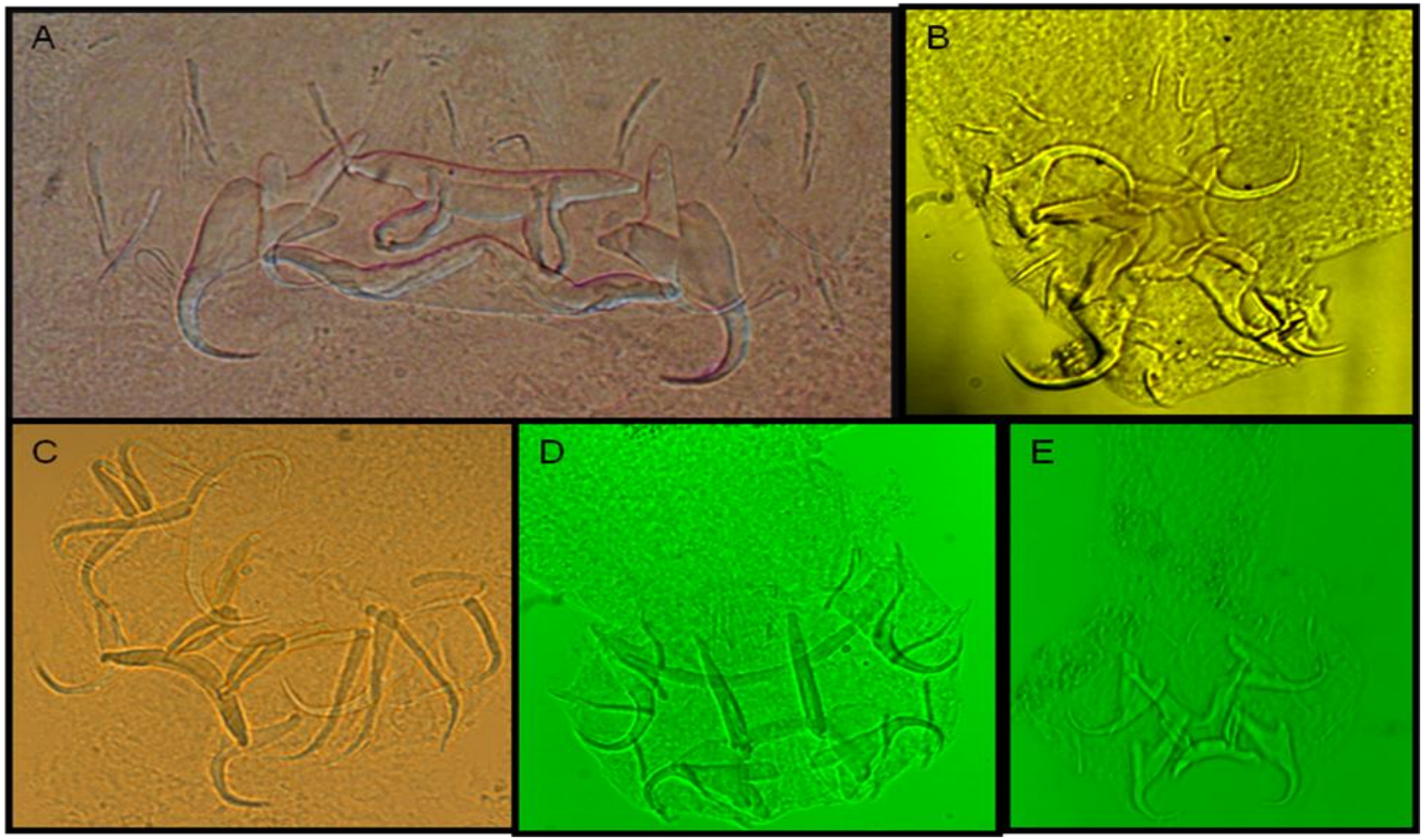


Figure 3.13: Photomicrographs of ectoparasitic monogeneans from *Oreochromis mossambicus*. A = *Cichlidogyrus halli;* B = *Cichlidogyrus sclerosus;* C = *Cichlidogyrus dossoui*; D = *Scutogyrus longicornis;* E = *Cichlidogyrus tilapiae.*

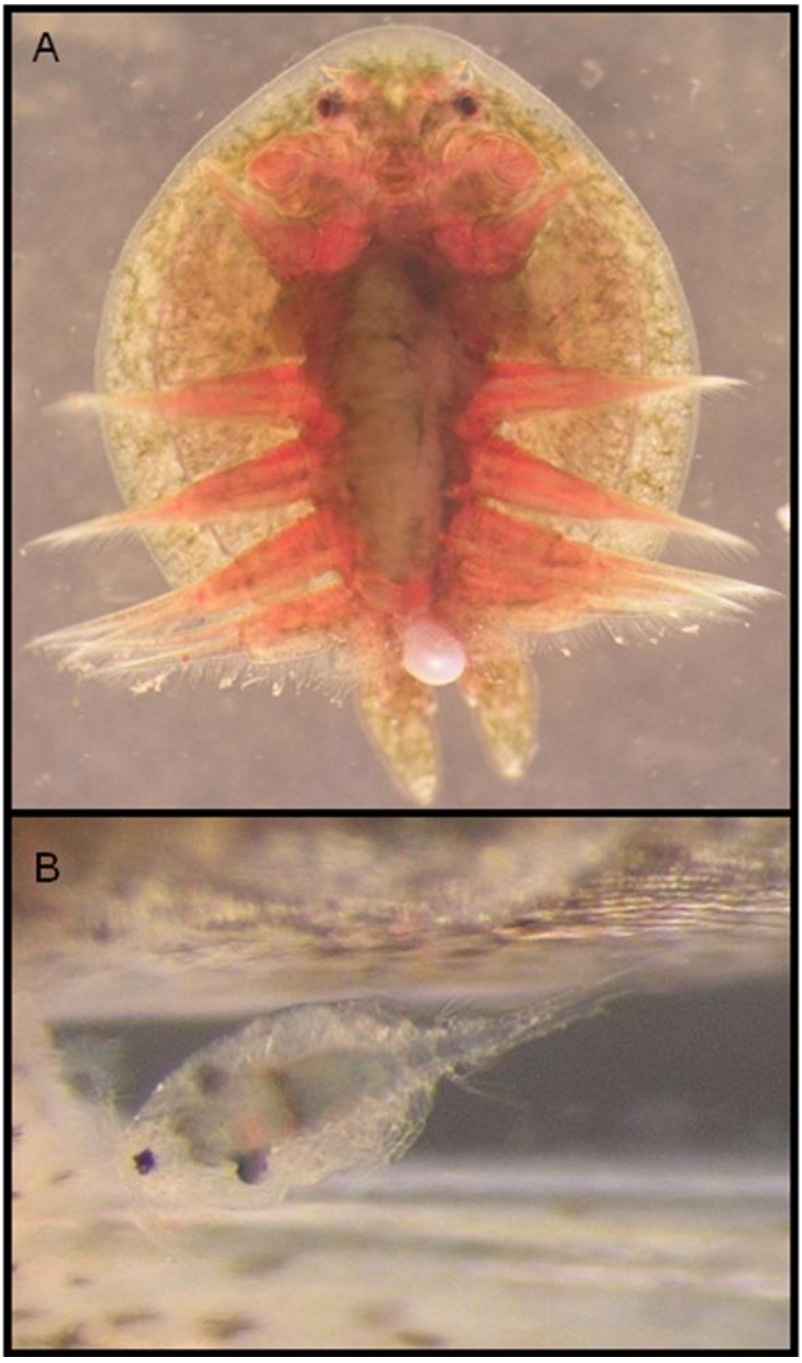


Figure 3.14: Photomicrographs of ectoparasites from *Oreochromis mossambicus*. A = *Dolops ranarum* (Branchiura); B = *Ergasilus* species (Copepoda).

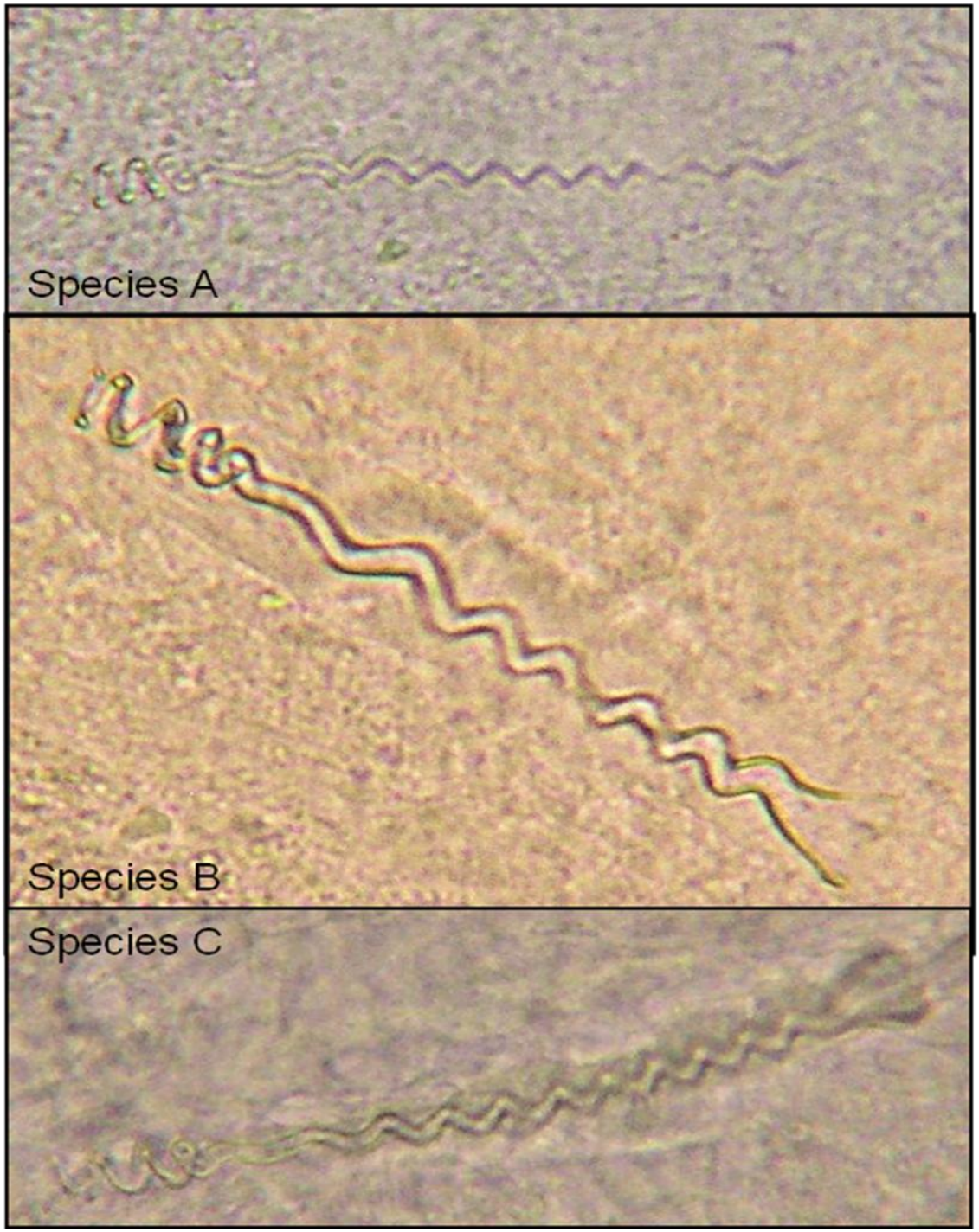


Figure 3.15: Photomicrographs of cirri of the three *Enterogyrus* species from the stomach of *Oreochromis mossambicus*.

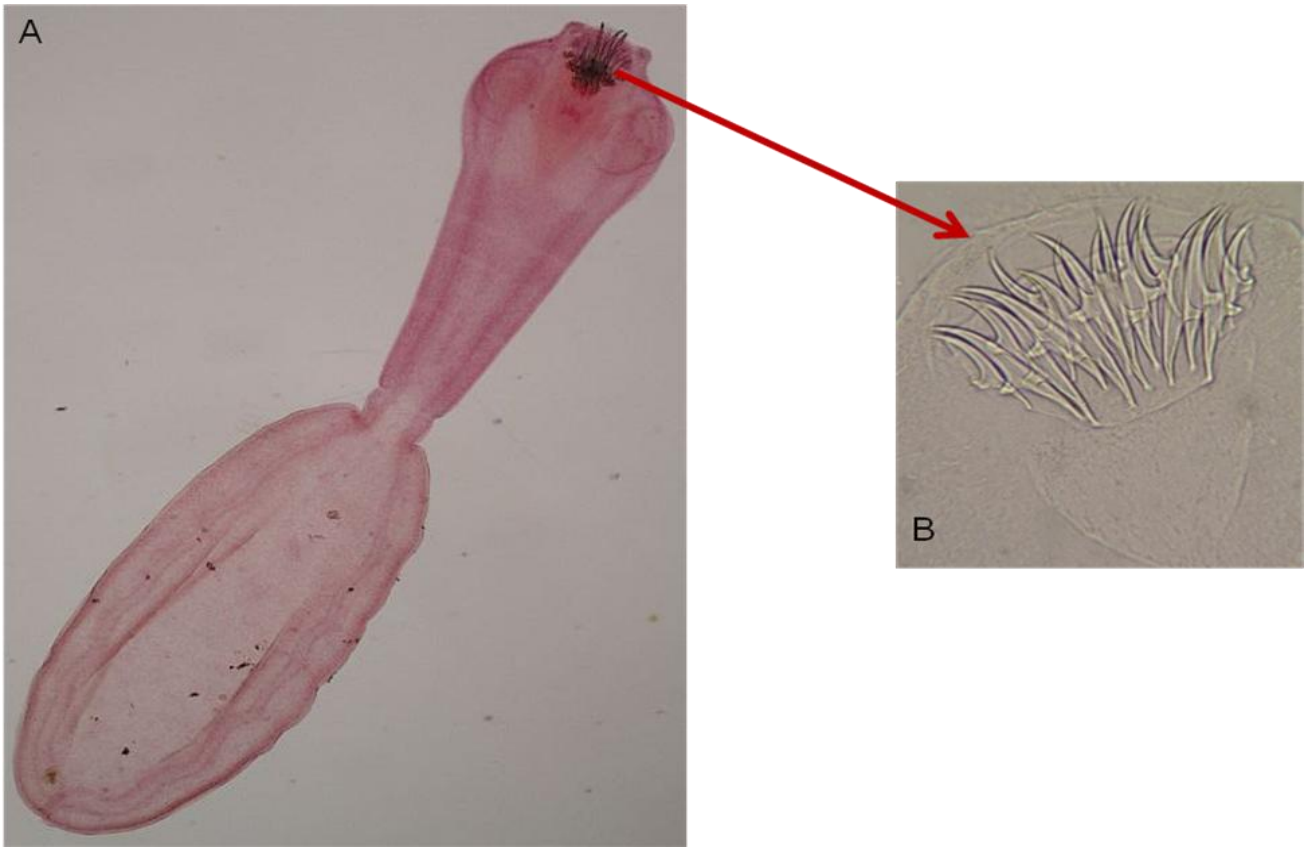


Figure 3.16: Photomicrographs of the gryporynchid cestode larva from *Oreochromis mossambicus* with A = everted scolex; B = rostellar hooks.

The mean abundance values for these ectoparasite species were comparably the same at Luphephe-Nwanedi Dams and Flag Boshielo Dam. For the endoparasites, 13, 11 and three species were recorded at Luphephe-Nwanedi Dams, Flag Boshielo Dam and Return Water Dam, respectively (Table 3.8; Figures 3.15 - 3.18). The overall number of individual endoparasites found in *O. mossambicus* was greatest at Luphephe-Nwanedi Dams and lowest at the Return Water Dam (Table 3.8).

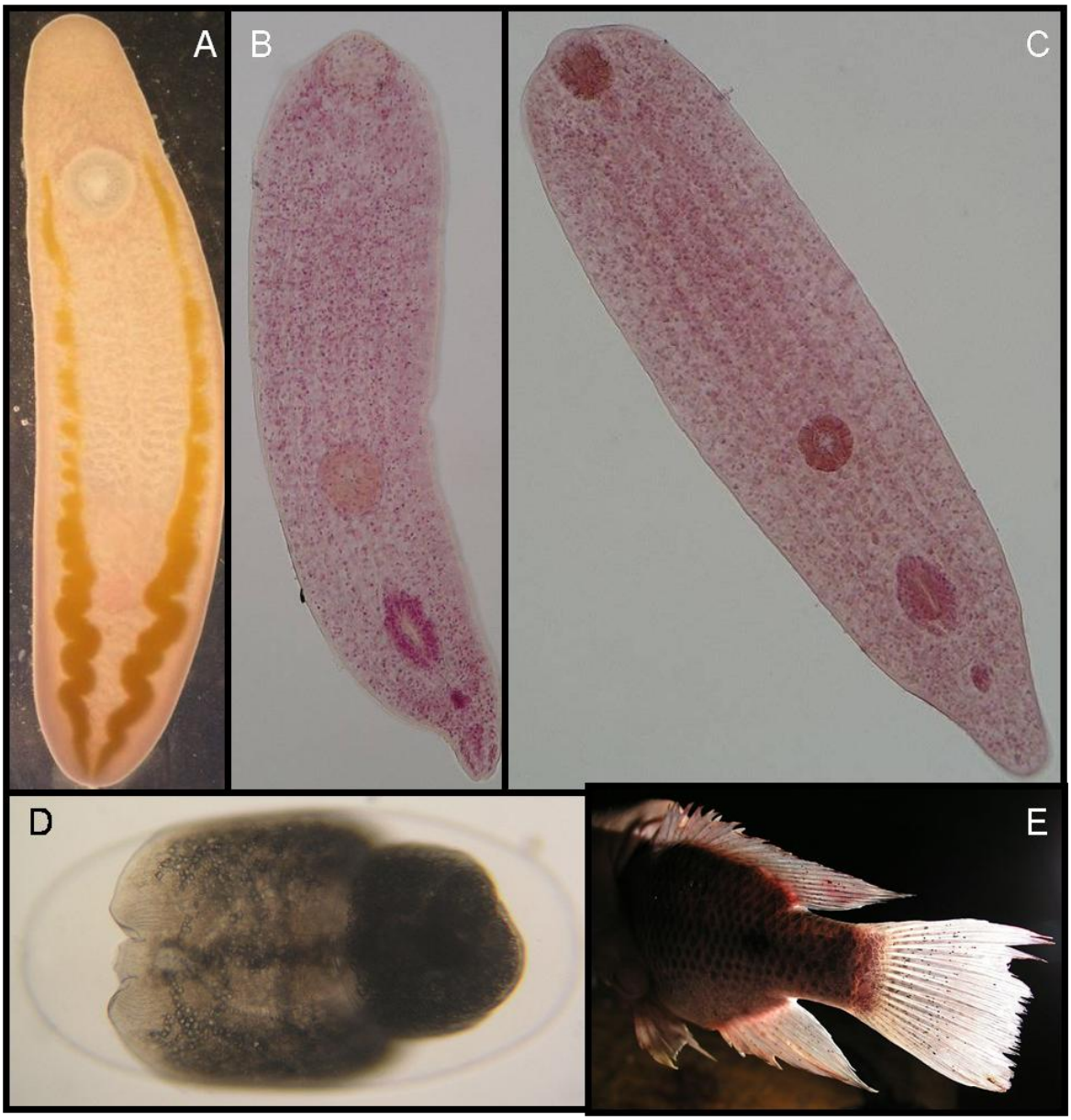


Figure 3.17: Photomicrographs of digeneans from *Oreochromis mossambicus*. A = *Clinostomum* species; B = *Tylodelphys* species; C = *Diplostomum* type 3 species; D = *Tetracotyle* species E = *Neascus* species.

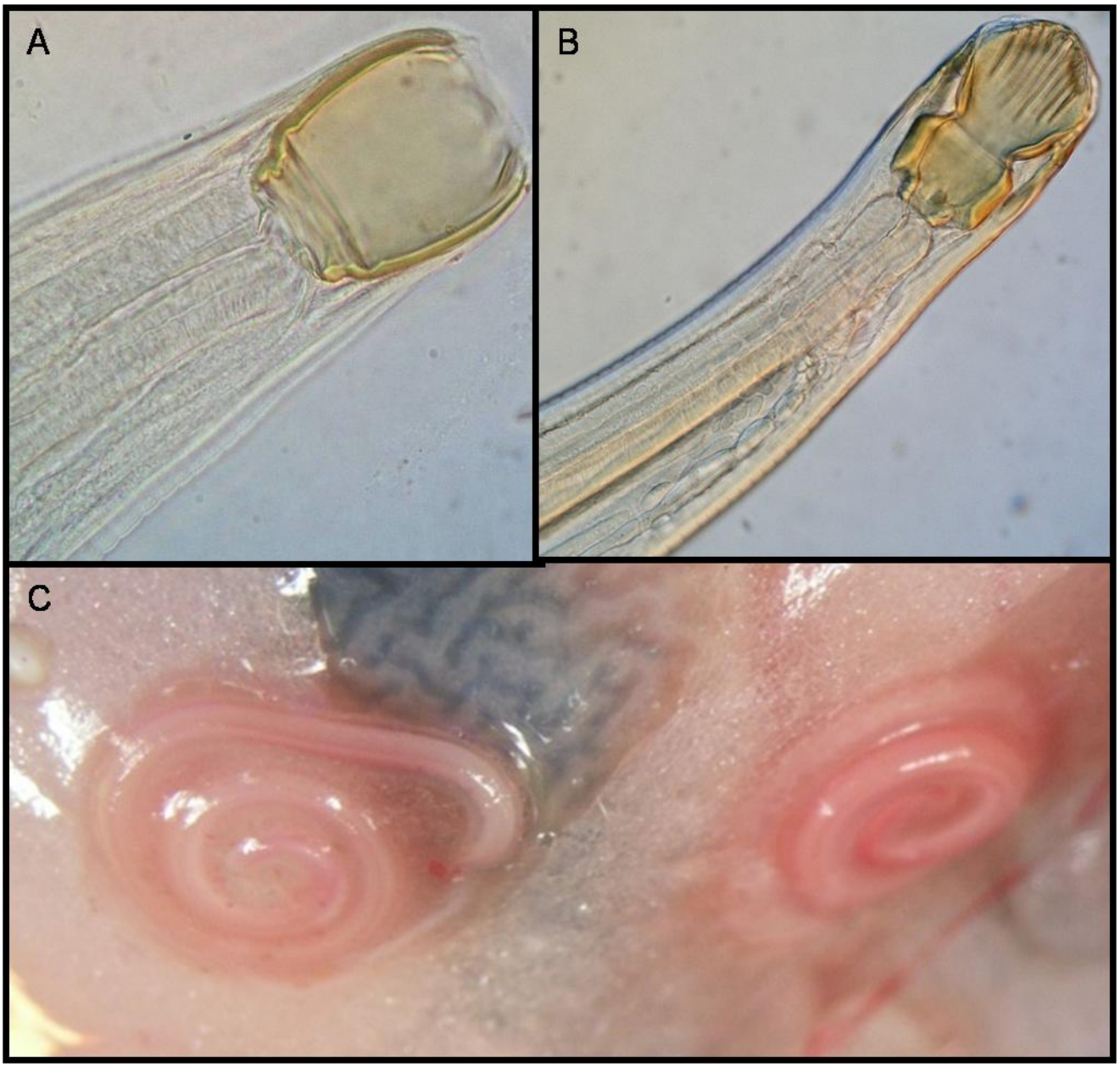


Figure 3.18: Photomicrographs of nematodes from *Oreochromis mossambicus*. A = *Procamallanus laevionchus* B = *Paracamallanus cyathopharynx* C = unidentified larva; D = *Contracaecum* sp. larvae.

Table 3.7: Ectoparasitic and endoparasitic infections in *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

	Luphephe-Nwanedi Dams				Flag Boshielo Dam				Return Water Dam			
	TP	P%	MI	A	TP	P%	MI	A	TP	P%	MI	A
					Ectoparasites							
Cichlidogyrus halli	150	60	7.5	4.5	192	72.9	5.3	4.0	3	6.4	1.0	0.1
Cichlidogyrus sclerosus	21	13.3	10.7	1.4	61	37.5	3.4	1.3	-	-	-	-
Cichlidogyrus dossoui	24	15.6	7.9	1.2	50	35.4	2.9	1.0	-	-	-	-
Cichlidogyrus tilapiae	32	20.0	3.6	0.7	28	22.9	2.5	0.6	-	-	-	-
Scutogyrus longicornis	27	17.8	5.6	1.0	37	35.4	2.2	0.8	-	-	-	-
Dolops ranarum	17	13.3	2.8	0.4	-	-	-	-	-	-	-	-
Ergasilus sp.	-	-	-	-	19	12.5	3.2	0.4	-	-	-	-
					Endoparasites							
Enterogyrus sp.	257	48.9	11.7	5.7	113	29.2	8.1	2.4	-	-	-	-
Enterogyrus sp.	35	15.6	6.7	1.0	43	18.8	4.8	0.9	-	-	-	-
Enterogyrus sp.	43	15.6	6.1	1.0	19	8.3	4.8	0.4	-	-	-	-
Neascus sp.	272	20	30.2	6.0	123	14.6	17.6	2.6	-	-	-	-
Tylodelphys sp.	124	24.4	11.3	2.8	55	25.0	4.6	1.1	-	-	-	-
Diplostomum sp.	147	40.0	8.2	3.3	-	-	-	-	86	38.3	4.8	1.8
Tetracotyle sp.	299	49.8	49.8	6.6	-	-	-	-	-	-	-	-
Clinostomum sp.	35	22.2	3.5	0.8	25	18.8	2.8	0.5	6	8.5	1.5	0.1
unidentified nematode	13	11.0	2.6	0.3	21	20.8	2.1	0.4	-	-	-	-
Contracaecum sp.	266	24.4	24.2	5.9	36	25.0	3.0	0.8	-	-	-	-
Paracamallanus cyathopharynx	18	11.1	3.6	0.4	29	14.6	4.1	0.6	-	-	-	-
Procamallanus laevionchus	30	11.1	6.0	0.7	16	12.5	2.7	0.3	-	-	-	-
gryporynhchid cestode	84	17.8	10.5	1.9	45	18.8	5.0	0.9	449	61.7	15.5	9.6

TP = Total individual parasites; P% = Percentage prevalence; MI = Mean intensity; A = Abundance.

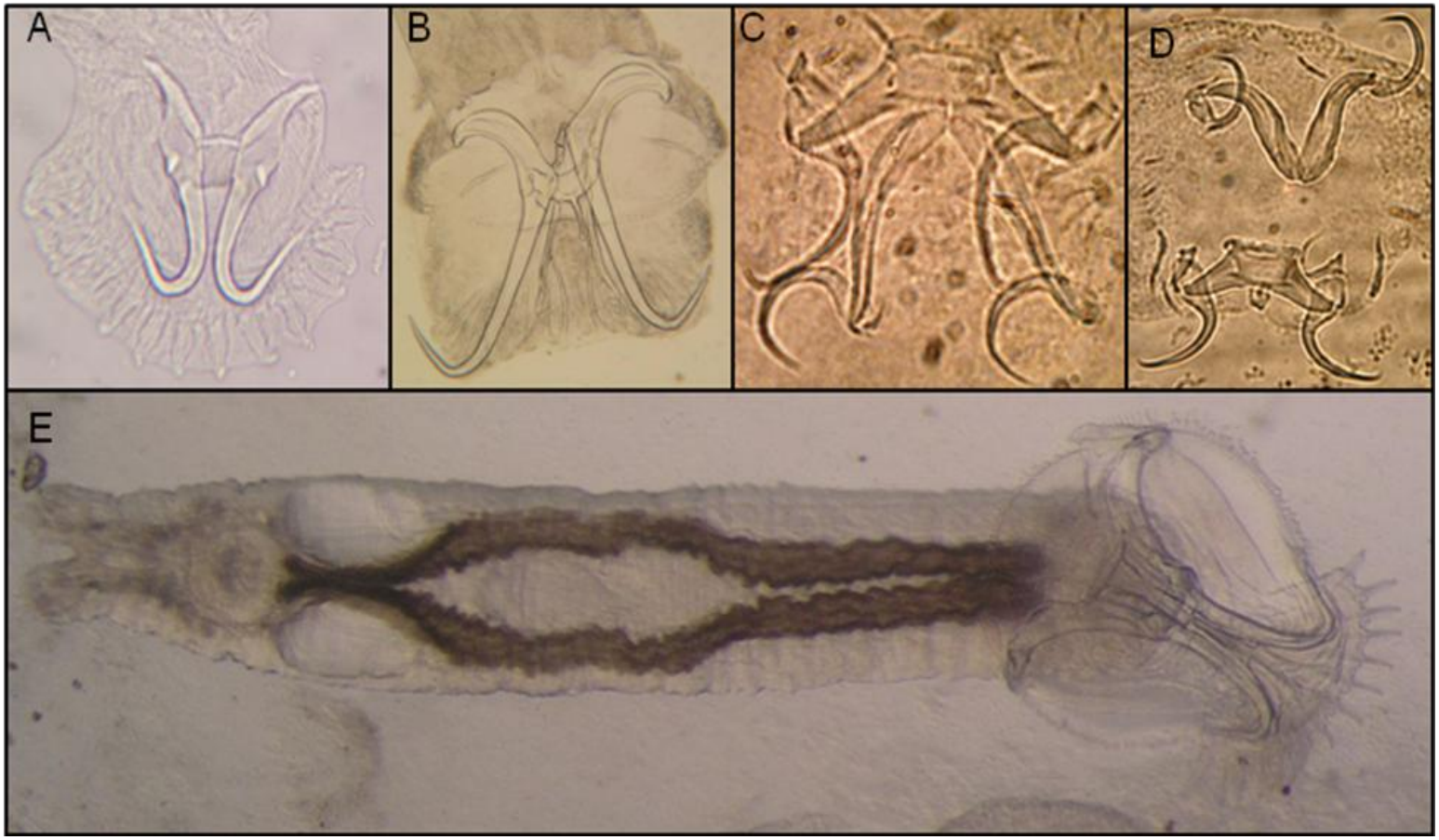


Figure 3.19: Photomicrographs of monogeneans recovered from *Clarias gariepinus* A = opisthaptor of *Gyrodactylus rysavyi*; B = opisthaptor of *Macrogyrodactylus clarii*; C = opisthaptor of *Quadriacanthus clariadis*; D = opisthaptor of *Quadriacanthus aegypticus*; E = *Macrogyrodactylus congolensis*.

Figure 3.20: Photomicrographs of branchiurans recovered from *Clarias gariepinus*. A = *Dolops ranarum;* B = *Chonopeltis inermis*.

Figure 3.21: Photomicrographs of ectoparasites recovered from *Clarias gariepinus*. A = the copepod, *Lamproglena clariae*; B = unidentified Hirudinea.

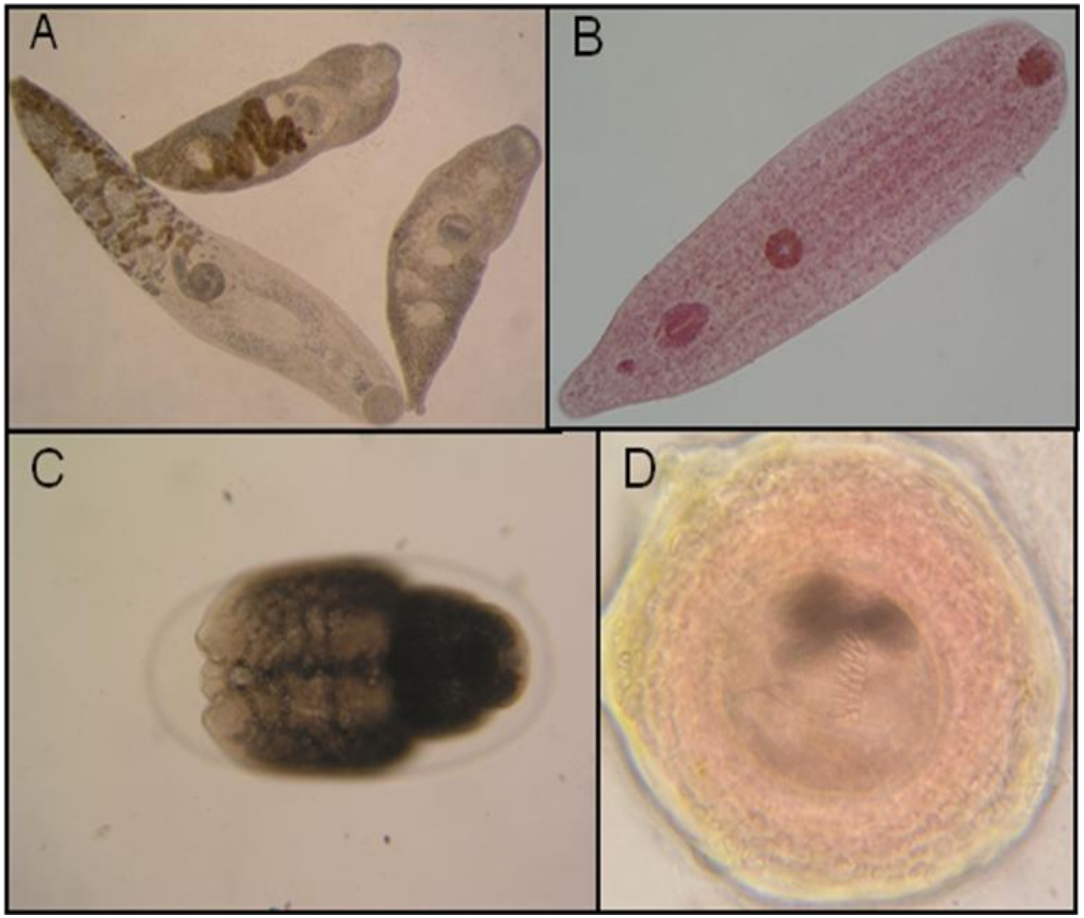


Figure 3.22: Photomicrographs of the digeneans from *Clarias gariepinus*. A = *Glossidium pedatum* B = *Diplostomum* type 3; C = metacercaria of *Tetracotyle* species; D = metacercaria of *Acanthostomum* species.

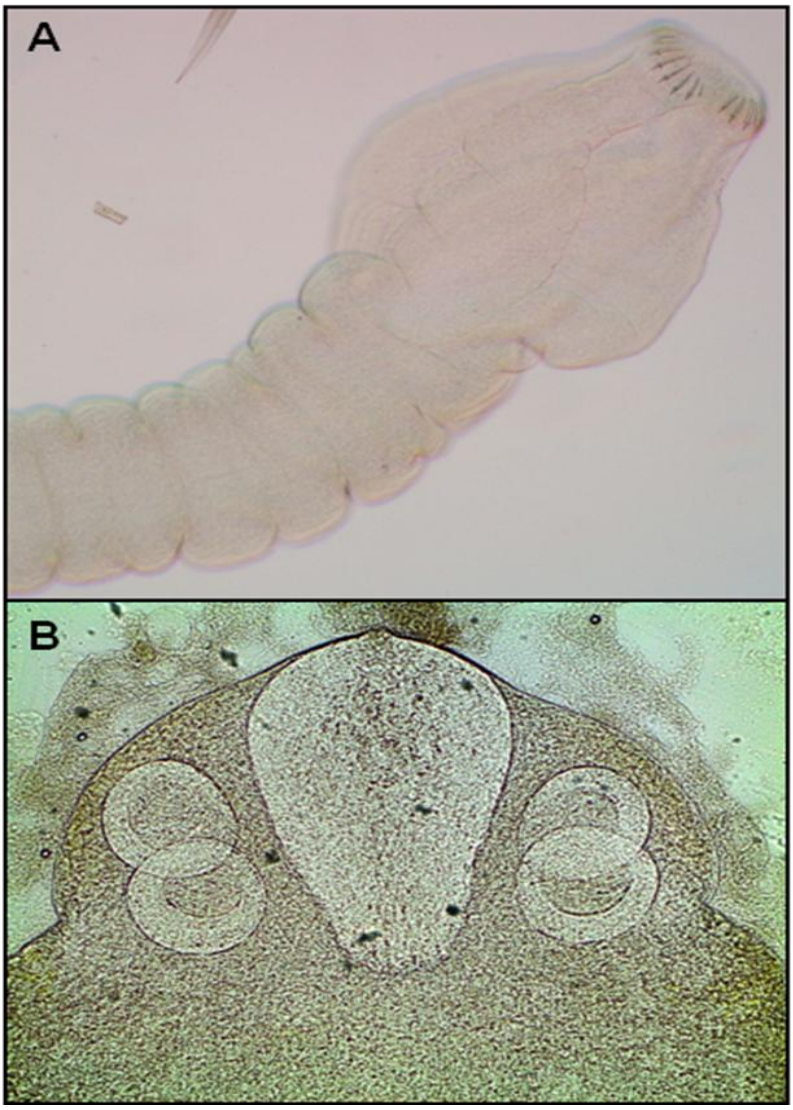


Figure 3.23: Photomicrographs of scolices of cestodes recovered from *Clarias gariepinus*. A = *Tetracampos ciliotheca* B = *Proteocephalus glanduligerus*.

The gryporynchid cestode larva was most abundant in hosts sampled from the Return Water Dam (Table 3.8).

Clarias gariepinus hosted nine, four and only one ectoparasite species at Luphephe- Nwanedi Dams, Flag Boshielo Dam and the Return Water Dam,

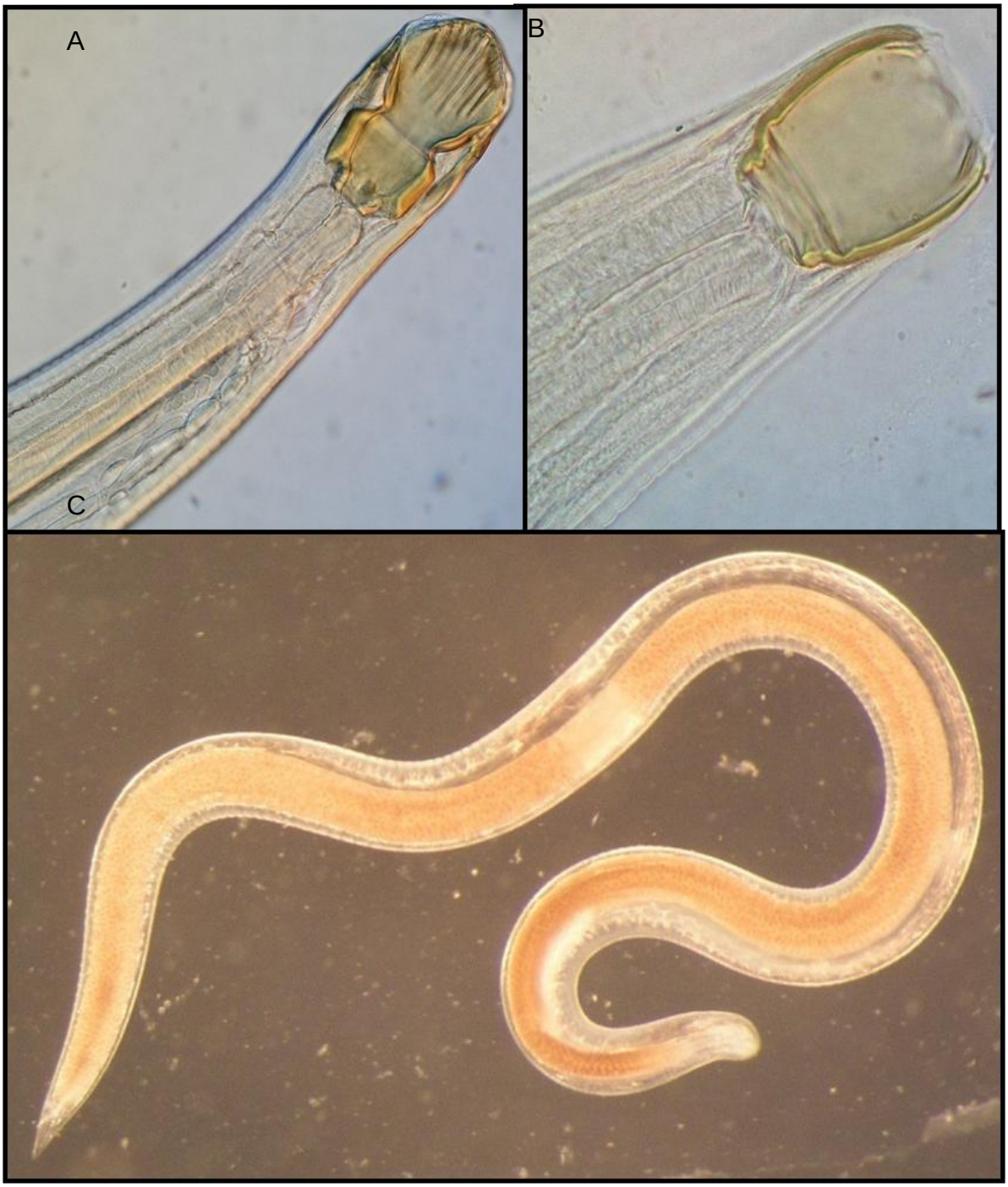


Figure 3.24: Photomicrographs of nematodes recovered from *Clarias gariepinus*. A = *Paracamallanus cyathopharynx*; B = *Procamallanus laevionchus*; C = *Contracaecum* species larva.

Table 3.8: Ectoparasitic and endoparasitic infections in *Clarias gariepinus* sampled from three dams of the Limpopo and Olifants River systems.

	Luphephe-Nwanedi Dams				Flag Boshielo Dam				Return Water Dam			
	TP	P%	MI	A	TP	P%	MI	A	TP	P%	MI	A
					Ectoparasites							
Gyrodactylus rysavyi	13	13.33	2.17	0.29	17	12.2	3.4	0.4	10	15.6	1.4	0.2
Macrogyrodactylus clariae	20	26.67	1.67	0.44	32	26.8	2.9	0.8	-	-	-	-
Macrogyrodactylus congolensis	19	17.78	2.38	0.42	42	34.1	3.0	1.0	-	-	-	-
Quadriacanthus clariadis	15	17.78	1.88	0.33	-	-	-	-	-	-	-	-
Quadriacanthus aegypticus	13	20	1.6	0.3	-	-	-	-	-	-	-	-
Chonopeltis inermis	7	13.33	1.17	0.16	-	-	-	-	-	-	-	-
Dolops ranarum	76	33.33	5.07	1.69	-	-	-	-	-	-	-	-
Lamproglena clariae	20	15.56	2.86	0.44	90	36.6	6.0	2.2	-	-	-	-
Leech	7	11.11	1.40	0.16	-	-	-	-	-	-	-	-
					Endoparasites							
Diplostomum species	672	42.22	35.37	14.93	50	22.0	5.6	1.2	311	42.2	16.4	6.9
Tetracotyle species	1667	40.00	92.61	37.04	-	-	-	-	-	-	-	-
Acanthostomum species	579	13.33	96.50	12.87	-	-	-	-	-	-	-	-
Glossidium pedatum	251	42.22	13.21	5.58	50	22.0	5.6	1.2	-	-	-	-
Contracaecum species	4331	100.00	96.24	96.24	3086	41.5	181.5	75.3	3091	75.6	90.9	68.7
Paracamallanus cyathopharynx	40	28.89	3.08	0.89	18	12.2	3.6	0.4	-	-	-	-
Procamallanus laevionchus	-	-	-	-	13	14.6	2.2	0.3	-	-	-	-
Unidentified nematode	-	-	-	-	19	17.1	2.7	0.5	-	-	-	-
Proteocephalus glanduligerus	11	11.11	2.20	0.24	-	-	-	-	-	-	-	-
Tetracampos ciliotheca	6	11.11	1.20	0.13	31	31.7	2.4	0.8	11	17.8	1.4	0.2

TP = Total individual parasites; P% = Percentage prevalence; MI = Mean intensity; A = Abundance.

respectively (Table 3.9; Figures 3.19 - 3.21). The overall number of individual endoparasites recorded from *C*. *gariepinus* was greatest at Luphephe-Nwanedi Dams and lowest at the Return Water Dams (Table 3.9). For the endoparasites, eight, seven and three species were found in *C*. *gariepinus* sampled from Luphephe-Nwanedi Dams, Flag Boshielo Dam and the Return Water Dam, respectively (Table 3.9; Figures 3.22 - 3.24).

3.5. Discussion

Values of all the water quality parameters measured at the reference site were relatively low and within permissible limits as prescribed by local legislation (DWAF 1996). In contrast, the Return Water Dam was polluted by sulphates and nutrients (ammonium, nitrate, nitrite, ortho-phosphate), all of which were above permissible limits for aquatic biota. In addition, the concentration of dissolved oxygen at this site was below the minimum requirements for aquatic biota (DWAF 1996). The metals, aluminium and lead, also exceeded toxicity thresholds, and therefore, the Return Water Dam was regarded as severely polluted. Despite the fact that Flag Boshielo Dam receives indiscriminate industrial, mining and domestic effluents, this site is not polluted by nutrients and is in an oligotrophic state. Only sulphates and a few metals (iron, aluminium and lead) were found to be above the South African TWQR for aquatic biota, thus, for this study Flag Boshielo Dam was considered to be moderately polluted. Until further studies are carried out, the detected metal levels in Flag Boshielo Dam and the Return Water Dam, as well as the nutrients at the Return Water Dam, may be attributed to urban nonpoint contamination, sewage, industrial and mining discharge from their surrounding catchments.

The HAI results suggest that fish from the impacted sites (Flag Boshielo Dam and Return Water Dam), show poorer health status than fish from the

unpolluted site (Luphephe-Nwanedi Dam), although this trend is not expressed in every individual fish. The conclusion on a decreasing health status towards the most impacted site (Return Water Dam) was based mainly on the predominance of abnormal values of haematocrit and ectoparasites; anomalies of gills, liver, pseudobranch, eyes and skin findings. The coefficients of variation were higher for fish from the uncontaminated site than for those from contaminated sites. One possible interpretation of this finding is that fish living in degraded environments are all exposed equally to poor water quality and, therefore, the variability in physiological condition in fish from a contaminant-exposed population may tend to be less than for fish in unstressed environments (Adams *et al*. 1993).

There were significant differences in condition factor of fish sampled from the reference and the severely polluted site. The highest mean condition factor values for both fish species were recorded at the severely polluted site whereas the lowest mean condition factor values were recorded at the reference site. Enrichment of the Return Water Dam by organic waste, leading to high productivity of this dam could be the main contributing factor.

The haematocrit was one of the most affected variables, showing distinctive differences among sites and between the two fish species. Survey of literature shows that changes in haematological indices of fish are predetermined both by the concentration of heavy metals in water and time of exposure and both these factors can cause reversible and irreversible changes in the homeostatic system of fish. A short-term exposure to low concentrations of heavy

metals mostly induce an increase of the haematocrit, reflecting the beginning of stress reaction in fish caused by chemicals (Houston 1997). Fish stress reaction causes an osmotic imbalance and changes in the regulatory system of ionic interchange which can diminish pH of blood and increase the volume of erythrocytes and, subsequently, the percent value of haematocrit (Rios *et al*. 2002). Under stress, the secreted epinephrine causes the contraction of the spleen, and erythrocytes from this organ are released to blood. Their higher count correspondingly increases the erythrocyte share of haematocrit (Houston 1997; Rios *et al*. 2002). High concentrations of heavy metals or long-term exposure of fish to their sublethal concentrations usually decrease the haematocrit levels (Houston 1997). A decrease in the erythrocyte count or in the percent of haematocrit indicates the reduction of an organism health status and its developing anaemia.

Several studies, such as those of Katalay & Parlak (2004), Chandrasekara & Pathiratne (2005) and Jee *et al*. (2005), have shown that the effects of environmental toxicants on haematological characteristics of fish vary according to the target species, xenobiotic type, and concentration. Apart from stressful environments, a wide range of factors such as spawning, season and sex of the individuals are known to influence haematological parameters in fish (Houston 1997; Rios *et al*. 2002). In this study, the sex of the fishes and correlations with haematocrit were not determined, although assumed to be modestly influenced by these intrinsic factors. However, warm water marine fish have been found to

to be less constrained by seasonal breeding episodes due to the more or less constant prevailing seasonal conditions in tropical areas (Choat & Robertson 2002). Tropical fish such as *Corydoras paleatus* and *Prochilodus lineatus* were not found to undergo significant changes in their haemogram dynamics during spawning period, while in *P*. *scrofa* and *Brycon* species no differences were encountered in male and female blood characteristics (Parma de Croux 1994; Cazenave *et al*. 2005).

The threshold level of waterborne iron that may elicit harmful effects, according to local legislation (DWAF 1996) is 0.02 mg/l, indicating that Flag Boshielo Dam was contaminated by high levels of iron. The availability of metals to living organisms is known to be governed by the physicochemical properties of the water, controlling for example the rate of absorption and desorption of metals from and to sediments. For instance, Fe^{2+} rapidly undergoes precipitation to Fe $(OH)_3$, which is recycled in sediments under appropriate conditions of redox potential, temperature, and pH (Dalzell & Macfarlane 1999; Ravengai *et al*. 2005). Dalzell & Macfarlane (1999) suggested that iron exerts its toxicity in fish via respiratory disturbance due to the physical clogging of the gill epithelial surfaces by iron complexes. The influence of waterborne iron in gill anomalies in both fish species at Flag Boshielo Dam cannot be excluded but remains to be established experimentally.

Likewise, aluminium is a toxic element which often exerts its deleterious effects at low concentrations. Aluminium is mainly found in soluble forms in acid

drainage waters and is also one of the particulates emitted from the combustion of coal, and aluminium fluoride is emitted from aluminium smelters (Dallas & Day 2004). Aluminium sulphate (Alum) is used in most water treatment processes as a flocculating agent for suspended solids, including colloidal materials, microorganisms and humic rich dissolved organics. The mechanism of dissolved aluminium toxicity in fish seems to be related to interference with ionic and osmotic balance and respiratory problems resulting from coagulation of mucus on the gills (Dallas & Day 2004).

Liver was a very common and good indicator of fish health and water quality deterioration during this study, showing several abnormalities at all the sampling sites. The presence of an exceptionally high number of the gryporynchid cestode larvae could be the major causative factor for the impairment of liver condition in the form of lesions, especially at the Return Water Dam. The infected livers displayed typical lesions associated with parasitic infections. However, discolouration and 'fatty' liver were the most common observed pathology in this study. Fatty liver is a very common pathological state attributable to excessive accumulation of fat in cellular cytoplasm (fatty degeneration) which may be related to diet (Roberts 1978). Fat accumulation could be the result of the inability to convert stored fat (in hepatocytes) to a suitable form for use (in phospholipid form) (Runnels *et al*. 1965). The liver is an organ of considerable importance in the storage and uptake of metals and it is also known to be the site of a number of detoxification functions (Klaassen 1976).

Abnormalities of the skin were most frequently observed in fish sampled from the Return Water Dam. The moderate skin aberrations at this site may perhaps be a result of pollution (Reichenbach-Klinke 1973). The majority of interpretations of skin aberrations at Flag Boshielo Dam were more likely a result of *Neascus* species (black spot) infestations while the few mild aberrations observed at Luphephe-Nwanedi Dams may be attributed to damage by *Dolops ranarum*. Ectoparasites can stimulate a wide range of responses varying from a very mild inflammatory infiltrate to an extremely severe acute necrotizing lesion which can be fatal (Luus-Powell 1997). However, external lesions may have a number of other causes other than parasitic infection or pollution. They may be due to predator attacks, mechanical damage or other abrasions.

Although care was taken not to record fresh skin aberrations due to damage caused by gill nets, mild active erosion of the fins and frayed fins were regularly observed at the Return Water Dam. Erosions of the fins are indicative of a departure from the normal condition and health (Klemm *et al*. 1992). Frayed fins may be due to fin rot, tuberculosis, obstructed circulation (due to internal infections) or metabolic disturbances (Reichenbach-Klinke 1973). Fin rot and red sores are generalized disease signs and may be characteristic of fishes resident in degraded aquatic systems where environmental stress of toxic chemicals exist (Sinderman 1979). Fin erosion has also been reported in different fish species associated with environmental pollution (Munkittrick *et al*. 1992; Sharples & Evans (1996). Hence, benthic fish such as *C*. *gariepinus* may be more vulnerable

since fin erosion seems to be related to direct contact with contaminated sediment.

According to Goede & Barton (1990), eyes are structures that indicate the well-being of fish in several ways. However, in the present study, eyes were not indicative in showing the differences between the three sampling sites. The very few and infrequent anomalies included blind, haemorrhagic or missing eyes as well as inflammation of the lens capsule. Metacercariae of *Diplostomum* species encountered in the eyes of some fish may be responsible for some of these anomalies. These larval digenean parasites are known to induce cataracts due to metabolic excretions and mechanical destruction of the lens structures (Shariff *et al*. 1980), which can lead to much more acute effects (Southgate 2006). Several studies have reported white and opaque appearance of the eye lens associated with heavy infection of these parasites (Karvonen *et al*. 2004). Blind fish, usually associated with sunburst type cataract usually darken in colour because of their loss of external stimuli to colour control (Roberts 1978).

The pseudobranch is an organ whose function is not certain but functions such as salt regulating, respiratory and sensory abilities have been suggested (Laurent & Dune-Erb 1984). Swelling of the pseudobranch implies changes in oxygen and carbon dioxide partial pressure as well as severe inflammation (Ellis *et al*. 1978; Goede & Barton 1990). The swelling can be categorized as ‘lithic’, which indicates the presence of mineral deposits and inflammation which includes haemorrhage and any other cause of redness. None of these

abnormalities were noted in this study. However, the abnormalities observed in the pseudobranch were probably due to myxosporan cysts in *C*. *gariepinus*. *Oreochromis mossambicus* lacks psuedobranchia and this makes comparison between two species difficult since a value of zero given to *O*. *mossambicus* brings the total value of each fish down with possibly 10 to 30 points depending on the severity of abnormality.

Shortened opercules were sporadically observed in this study and thus, were not good indicators of HAI in this study. This condition may be a result of vitamin deficiency and/or calcium deficiency, environmental damage or of genetic origin (Reichenbach-Klinke 1973).

Black *et al*. (1982) reported that fish exposed to mining waste containing copper, lead and zinc are commonly affected with periviscal masses resembling mesotheliomas that are usually associated with the mesenteric capsule of the spleen, but sometimes appear to be attached to the mesenteric fat of fish. These manifestatins were not evident at the polluted sites. Instead, there were few occasional deep green coloured spleens at the polluted sites.

During this study, redness and moderate inflammation of the hindgut were rarely observed in the both fish species, at all sites. The redness of the hindgut could be ascribed to infection with the gryporynchid cestode larvae. Numerous gryporynchid cestode larvae were attached to the outer lining of the intestine.

The IPI is based on the premise that ectoparasites are more directly

exposed to the effects of water quality than endoparasites. Higher numbers of ectoparasites, indicative of better water quality, were indeed, collected from the near-pristine site. The index suggests that at chronic, sub-lethal levels of exposure, poor water quality can promote increased endoparasitism due to a lowered immune system. This was not the case in the current study since there was a more conspicuous decrease in endoparasite abundance and diversity at the impacted sites. This apparent inconsistency of our findings with the IPI premises with regard to endoparasites demonstrated that in disturbed environments, endoparasites are less likely to complete their life cycles, either due to direct adverse effects on their free living stages, or as an indirect consequence of the elimination of their intermediate hosts (Paperna 1997). For example, heavy metals and anaerobic conditions have been found to be toxic to adult trematodes inside their host fish (Overstreet & Howse 1997; Kiceniuk & Khan 1983; Overstreet 1988; Khan & Thulin 1991; Valtonen *et al*. 1997) and lethal to free living stages (e.g. cercariae and miracidia) as well as to mollusc intermediate hosts (Evans 1982; Munkittrick & Dixon 1988; Siddall *et al*. 1993). The present study lends additional support to this view that pollution compromises heteroxenous species by blocking the completion of their life cycles. The suggested indirect effect (physiological or immunosuppressive) on the hosts in poor water quality environments still warrants experimental validation.

3.6. Conclusions

The study points to the use of necropsy based assessments and haematocrit as direct indicators of fish health. Typically, both parameters were nonspecific in their response to stressors. Under the complex exposure situations in the field, with the presence of multiple stressors, straightforward relationships between a single stressor and a biological response may be more the exception than the rule. Thus, deviations from the norm in the different fish species could be attributed to synergistic interactions of contaminants such as lead, aluminium, sulphate, and hypoxia; eutrophication due to high levels of nutrients or other non-discerned pollutants in the Return Water Dam. Pollutants such as sulphates, aluminium, iron and lead could have the same effect on the health profile of both fish species in Flag Boshielo Dam. Nevertheless, each of the two species displayed a unique pattern of haematological response, which illustrates interspecific physiological differences and, perhaps, differential exposure of the fishes to contaminants ascribed to dissimilar feeding and behavioural habits. The situation demonstrates the importance of monitoring different species during environmental pollution studies. Although health of the different species was impaired, homeostatic mechanisms were in motion to favour adaptation, as demonstrated by haematological alterations. Nonetheless, such mechanism might become maladaptive in the long term and decrease fish yields and catch, thus adversely affecting ecosystem health.

CHAPTER 4

COMMUNITY ECOLOGY OF THE METAZOAN PARASITES OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) FROM THREE DAMS OF THE LIMPOPO-OLIFANTS RIVER SYSTEMS

4.1. Introduction

Extensive studies have been conducted on helminth communities of fish to analyse parasite communities (Pérez-Ponce de Leon *et al*. 2000; Salgado-Maldonado *et al*. 2004), with emphasis on how these communities are structured and which processes are involved in maintaining these structures. However, most of these investigations have been confined to the northern temperate regions (Nelson & Dick 2002; Johnson *et al*. 2004). In the tropics, work from South America, particularly Brazil (Alves & Luque 2001; Alves *et al*. 2002; Luque *et al*. 2003; Takemoto *et al*. 2005), Mexico (Pérez-Ponce de Leon *et al*. 2000; Salgado-Maldonado & Kennedy 1997; Salgado-Maldonado *et al*. 2001; 2004) and Australia (Kennedy 1995) have examined the composition and diversity of parasite communities in tropical fish. Although considerable work has been done on the morphology, systematics and life histories of parasites of fishes in Africa, there are limited numbers of studies on the ecology of freshwater fish parasites (Avenant-Oldewage & Knight 2008; Mwita & Nkwengulila 2008). To investigate the species diversity patterns of helminthes in an ancient tropical freshwater fish,

an examination was made of the sharptooth catfish, *Clarias gariepinus* (Burchell 1822) from three different dams.

The family Clariidae originated in Asia 50 MY ago but contemporary African and Asian species studied originated from a common ancestor that was present on the Arabian plate about 15 MY ago (Agnése & Teugels 2001). *Clarias gariepinus*, which is generally considered to be one of the most important tropical catfish species for aquaculture, has an almost Pan-African distribution, ranging from the Nile to West Africa and from Algeria to Southern Africa (Skelton 2001).

Two characteristics of the catfish suggest it would have a relatively rich helminth fauna. Firstly, greater species richness is to be expected in the evolutionary ancient host-parasite systems and in hosts that inhabit their geographic area of origin because they have had time to acquire their helminth fauna (Guégan & Kennedy 1993; Kennedy & Bush 1994; Choudhury & Dick 2000). Secondly, the sharptooth catfish is omnivorous and as such, its diversity of prey items is bigger, furthering potential parasite transmission. To examine these hypotheses, the study focused on gathering data on the metazoan parasite communities of *C*. *gariepinus* from three dams of the Limpopo and Olifants River Systems. The results obtained are also discussed in relation to the generalisations regarding some ecological determinants. The study adds the three dams to the parasite distribution list of metazoan parasites of *C*. *gariepinus*. Such information conforms to the idea advanced by Brooks & Hoberg (2000) that

parasite inventories are necessary to build upon our biodiversity knowledge base.

4.2. Materials and Methods

Specimens of the sharptooth catfish were sampled using gill nets of different stretched mesh sizes (30-110 mm) at three dams in the Limpopo and Olifants River Systems from April 2009 to January 2010. A characterisation of the sampling sites as well as a detailed description of the sampling procedure and the examination of the sharptooth catfish and parasite species is given in Chapter 2.

To determine the effect of the parasites on the health status of the fish, Fulton's condition factor (K) was calculated using the formula: $K = 100W/L^3$, where K = condition factor (health status), W= weight in grams and L = standard length in millimetres (Bagenal & Tesch 1978). Regression analysis was used to determine the effect of parasite load on K-factor. Possible parasite associations were determined using Spearman's rank correlation.

For an estimation of real species richness at a given site, depending on sample size, Walther's graph (Walther *et al*. 1995) was calculated according to the formula: Y = a (1-e-bx)/b, with a = increase in species richness at the beginning of sampling, b = parameter that sets the species richness asymptote R = a/b, x = unit of sampling effort.

Similarity in the parasite community between investigated sites were measured using Sorenson's Index, according to Magurran (1988), which was calculated quantitatively, according to: $C_S = 2j / (a+b)$ with j = number of species found jointly in two samples, a = number of species in the first sample, b = number in the second sample. It was also calculated qualitatively according to the formula: $C_N = 2_{jN} = / (a_N+b_N)$ with a_N = number of individuals in sample a, b_N = number of individuals in sample b, jN = sum of the lower of the two abundances of species which occur in the two samples (Magurran 1988). Sorenson's indices, which surpass values of 0.6, are considered to indicate similarities, and values of more than 0.8, greater similarities.

The parasite community structure of the catfish was examined at the (a) infracommunity level, which is the community of parasite infra populations in a single host and (b) component community level, which is the community of parasite infra populations associated with a subset of a host species (Bush *et al*. 2001). Measures of the infracommunity were the mean number and range of parasite species found on catfish individuals, defined as species richness (S) (Bush *et al*. 2001), the mean number of parasite individuals and the following ecological indices, which were all calculated for individual fish: Shannon-Wiener Diversity ($H = -\sum (p_i \ln p_i)$, where pi = relative intensity of parasite species i; Evenness ($E = H / \ln S$), where S = total number of parasite species and Inverse Simpson's Index ($D = 1 / \sum p_i^2$). Shannon-Wiener's Index is weighted towards the richness of a community, and Simpson's Index is weighted towards most

abundant species (Magurran 1988). Increasing values of the Shannon-Wiener Index and of the inverse Simpson Index indicate an increase in diversity. Values of Evenness can range from 0 to 1. Values of 0 indicate a completely uneven distribution of parasites between hosts; values of 1 totally even distribution. All indices were calculated according to Magurran (1988).

In order to evaluate the ratio of heteroxenous (indirect life cycle) to monoxenous (direct life cycle) species, numbers of heteroxenous species (Hsp) and monoxenous (Msp) were counted and the ratio (H/Msp) was calculated according to the method of D'Amelio and Gerasi (1997).

4.3. Data Analyses

A database was established and all parasite data, per fish/ per sampling site/ per season were entered. Most of the data were not normally distributed (Kolmogorov-Smirnow test) and were thus normalized by logarithmic transformation ($\log_{10}$ (N+1)). A Generalised Linear Model (GLM) was used to test the influential variables associated with each metazoan species. This method is suitable where the analysis involves the relationship between continuous and non-continuous variables (Dunteman & Moon-Ho 2006). In this case, the effect of sex, size, weight, season (predictor variables) on abundance of individual parasite species was tested. The Pearson chi-square statistic was used to evaluate over dispersion (Dunteman & Moon-Ho 2006). In the absence of over dispersion the Pearson chi-square statistic should be approximately equal to the

residual degrees of freedom (number of observations minus number of model parameters) (Dunteman & Moon-Ho 2006). The significance of each coefficient (b) was evaluated by the Wald chi-square statistic. The Tukey's Post-hoc test was performed to determine the seasons that were significantly different from one another.

Spearman's rank correlation was used for measuring association of the different parasite species in the component community of *C. gariepinus*. The Bray-Curtis index was computed from abundance data to determine species overlap among different sampling seasons and locations. To determine site associations of parasite species, Principal Component Analyses were computed and plotted. All values $p \leq 5\%$ were considered significant. The analyses were carried out using the computer programs, Statistical Package for Social Scientists (SPSS), CANOCO version 4 (Ter Braak & Smilauer, 1998) and Community Analysis Package (CAP) 1.52.

4.4. Results

Forty-five fish were collected at the Luphephe-Nwanedi Dams, 41 at the Flag Boshielo Dam and 45 at the Return Water Dam from which 14 874 parasites belonging to 19 species of metazoans were collected (Tables 4.1 & 4. 2). Seventeen parasite taxa were recovered from fish sampled at Luphephe-Nwanedi Dams compared to 11 at Flag Boshielo Dam and four at the Return Water Dam (Table 4.1).

Parasite composition (from all the sites) comprised five monogeneans (*Gyrodactylus rysavyi* Ergens, 1973; *Macrogyrodactylus clarii* Gussev, 1961; *M. congolensis* Prudhoe, 1957; *Quadriacanthus clariadis* Paperna,1961; *Q. aegypticus* El-Naggar and Scrag, 1986), four digeneans (*Glossidium pedatum* Looss, 1899; *Diplostomum* type 3, *Tetracotyle* species and *Acanthostomum* species), four nematodes [*Paracamallanus cyathopharynx* (Baylis, 1923); *Procamallanus laevionchus* (Wedl, 1862); larvae of *Contracaecum* species and an unidentified nematode larva), two cestodes (*Proteocephalus glanduligerus* (Janicki, 1928) Fuhrmann, 1933 and *Tetracampos ciliotheca* Wedl, 1861); two branchiurans (*Dolops ranarum* (Stuhlmann, 1891] and *Chonopeltis inermis* Thiele, 1900), one copepod, *Lamproglena clariae* Fryer, 1956 and one unidentified hirudinean leech (Table 4.2). Only *Gyrodactylus rysavyi*, *Tetracampos ciliotheca*, *Diplostomum* type 3 and *Contracaecum* species larva were observed at all three sampling sites (Table 4.2).

Table 4.1: Parasitological parameters for *Clarias gariepinus* sampled from the Limpopo and Olifants River Systems. Results are presented as mean ± standard deviation.

	LND	FBD	RWD
No of fish evaluated	45	41	45
Total no of species	17	11	4
Fish weight (g)	1636 ± 1406	3215.2 ± 2893.9	3246.9±1610.1
Fish length (cm)	519.5 ± 133.7	591.4 ± 238.8	636.4 ± 123.2
Condition factor (K)	1 ± 0.1	1.2 ± 0.6	1 .4 ± 0.8
Species richness	4.3 ± 1.7	3 ± 2	1.5 ± 0.9
Total parasite individuals	171.9 ± 122.2	83.3 ± 136.1	76.1 ± 65

LND = Luphephe-Nwanedi Dams, FBD = Flag Boshielo Dam, RWD = Return Water Dam

Macrogyrodactylus clarii, *M*. *congolensis*, *G*. *pedatum*, *P*. *cyathopharynx* and *L*. *clariae* were observed in both Luphephe-Nwanedi Dams and Flag Boshielo Dam (Table 4.2), while *P*. *laevionchus* and the unidentified nematode larva were observed only in Flag Boshielo Dam. Unique to Luphephe-Nwanedi Dams were *Q*. *clariadis*, *Q*. *aegypticus*, *Tetracotyle* species, *Acanthostomum* species, *P*. *glanduligerus*, *C*. *inermis*, *D*. *ranarum* and the leech. The fact that species richness was highest in the Luphephe-Nwanedi Dams is attributable at least in part to the presence of the aforementioned parasite taxa exclusive to the twin dams.

Seven generalist-allogenic parasites were identified compared to 10 specialist-autogenic ones in Luphephe-Nwanedi Dams; seven generalist-allogenic parasites and four specialist-autogenic ones in Flag Boshielo Dam; two generalist-allogenic parasites and two specialist-autogenic ones in the Return

Water Dam (Table 4.2). Specialist species of parasites were more common in catfish from Luphephe-Nwanedi Dams than in catfish from the other two dams.

Table 4.2: The biological characteristics of metazoan parasite species of *Clarias gariepinus* sampled from three dams of the Limpopo and Olifants River System.

Parasite species	Stage	Target Organ /Tissue	Intermediate Host	Final host	Life cycle[1]	ecto/endo parasite[2]	Origin[3]	Location[4]
Monogenea								
Gyrodactylus rysavyi	Adult	Gills	None	Fish	M	Ectoparasite	Specialist, auto	ALL
Macrogyrodactylus clarii	Adult	Gills	None	Fish	M	Ectoparasite	Specialist, auto	LND, FBD
Macrogyrodactylus congolensis	Adult	Skin	None	Fish	M	Ectoparasite	Specialist, auto	LND, FBD
Quadriacanthus clariadis	Adult	Gills	None	Fish	M	Ectoparasite	Specialist, auto	LND
Quadriacanthus aegypticus	Adult	Gills	None	Fish	M	Ectoparasite	Specialist, auto	LND
Digenea								
Diplostomum species	Larval	Eyes, Brain	snail - fish	Birds	H	Endoparasite	Generalist, allo	ALL
Tetracotyle species	Larval	Muscle	snail - fish	Birds	H	Endoparasite	Generalist, allo	LND
Acanthostomum species	Larval	Gills	snail, frogs	Birds	H	Endoparasite	Generalist, allo	LND
Glossidium pedatum	Adult	Intestine	snail	Fish	H	Endoparasite	Specialist, auto	LND, FBD
Nematoda								
Contracaecum species	Larval	Body cavity	Copepods- fish	Birds	H	Endoparasite	Generalist, allo	ALL
Unidentified nematode larva	Larval	Intestine	Copepods-fish	Birds	H	Endoparasite	Generalist, allo	FBD
Paracamallanus cyathopharynx	Adult	Intestine	Copepods-fish	Fish	H	Endoparasite	Generalist, aut	LND, FBD
Procamallanus laevionchus	Adult	Stomach	Copepods-fish	Fish	H	Endoparasite	Generalist, aut	FBD
Cestoda								
Proteocephalus glanduligerus	Adult	Intestine	Copepods- fish	Fish	H	Endoparasite	Specialist, auto	LND
Tetracampos ciliotheca	Adult	Intestine	Copepods- fish	Fish	H	Endoparasite	Specialist, auto	LND, RWD
Branchiura								
Chonopeltis inermis	Adult	Buccal cavity	None	Fish	M	Ectoparasite	Specialist, auto	LND
Dolops ranarum	Adult	Skin	None	Fish	M	Ectoparasite	Generalist, allo	LND
Copepoda								
Lamproglena clariae	Adult	Gills	None	Fish	M	Ectoparasite	Specialist, auto	LND, FBD
Hirudinea								
Udentified leech	Adult	Skin	None	Fish	H	Ectoparasite	Generalist, allo	LND

[1]) M = monoxenous, H = heteroxenous species [3]) aut= autogenic, allo = allogenic; [4]) LND = Nwanedi-Luphephe Dams, RWD = Return Water Dam, FBD = Flag Boshielo Dam, ALL = all three locations

The component parasite population showed an aggregated distribution, typical of most parasite distributions within their hosts (Figure 4.1). Multiple infections were common in fish from Luphephe-Nwanedi Dams and Flag Boshielo Dam whereas monospecific infections were common in fish from the Return Water Dam (Figure 4.1). The most frequent number of parasite species observed per host was four (in Luphephe-Nwanedi and Flag Boshielo Dams) compared to one in the Return Water Dam (Figure 4.1).

The eigenvalue for the first axis was 0.45, contributing 45% of the variance; while the eigenvalue for the second axis was 0.28, contributing 28% of the variance (Figure 4.2). The Principal Component Analysis (PCA) ordination plot shows that *Diplostomu*m species, *T. ciliotheca*, *G. rysavyi* and *Contracaecum* species are widespread and cosmopolitan species. Luphephe-Nwanedi Dams were strongly associated with most parasite taxa recorded for *C. gariepinus*. The distribution of *P. laevionchus* and the unidentified nematode larva was limited to Flag Boshielo Dam (Figure 4.2).

For an estimation of the true species richness related to the sample size, a richness sampling effort curve, according to Walther *et al*. (1995) was calculated. The true species richness of the parasite communities in the sharptooth catfish as extrapolated from the cumulative model $Y = a (1-e^{-bx})/b$, generated three curves with a significantly ($p < 0.05$) higher value for the Luphephe-Nwanedi and Flag Boshielo Dams when compared with those of the Return Water Dam (Figure 4.3). A continuum maximum was reached at a sample size of about 34

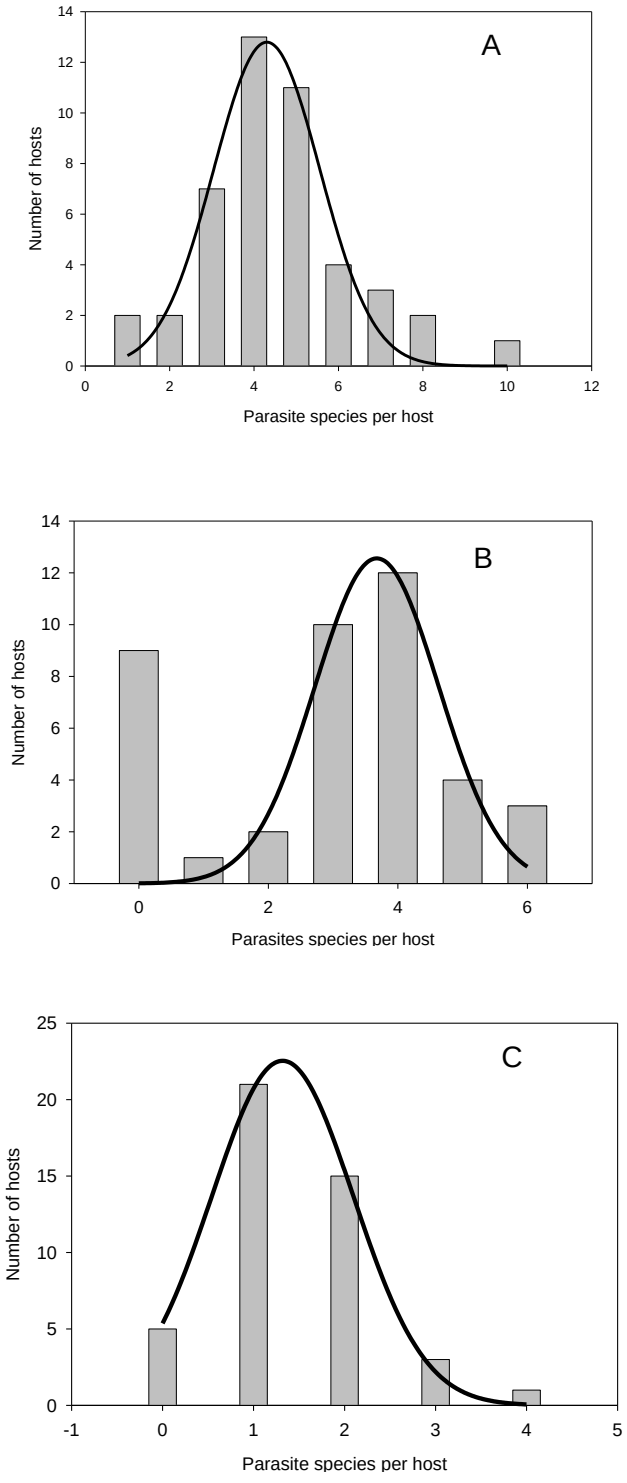


Figure 4.1: Frequency distribution of parasite species in specimens of *Clarias gariepinus* from A = Luphephe-Nwanedi Dams, B = Flag-Boshielo Dam C = Return Water Dam.

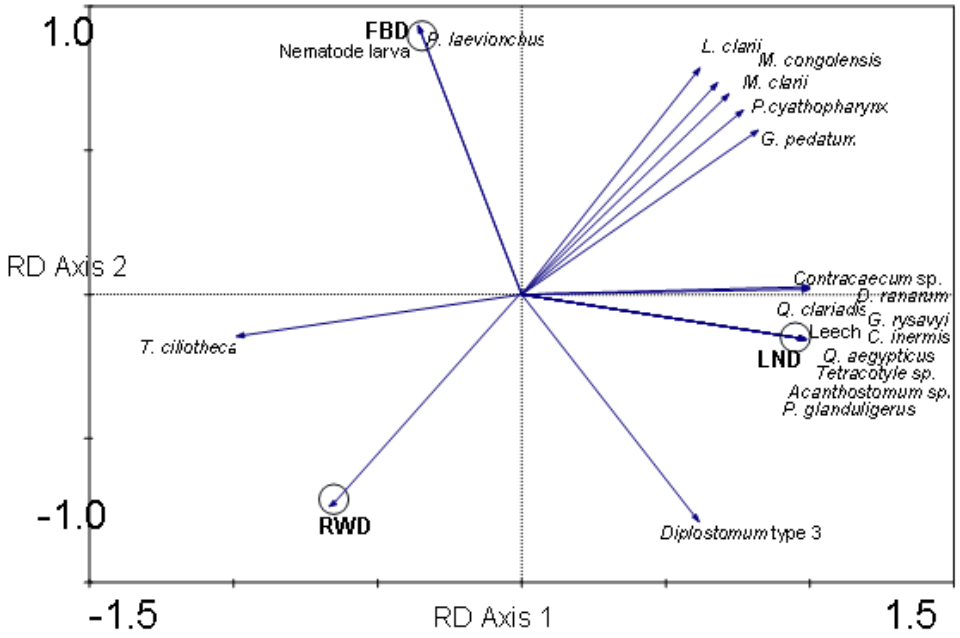


Figure 4.2: Principal Components Analysis (PCA) ordination showing parasite species distribution based on abundance among sites. LND = Luphephe-Nwanedi Dams; FBD = Flag Boshielo Dam; RWD = Return Water Dam.

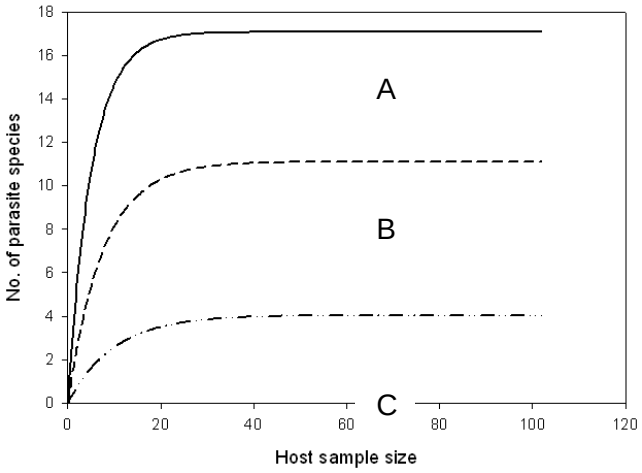
individuals at Luphephe-Nwanedi Dams, 38 at Flag Boshielo Dam and 42 at the Return Water Dam (Figure 4.3). Thus at all three sites, the number of fish investigated during the present study were sufficient to detect the real species richness.

Mean parasite species richness, total metazoan burden, Shannon Wiener and Margalef diversity indices had significantly higher scores at Luphephe-Nwanedi Dams and lowest at the Return Water Dam (Table 4.3). Heteroxenous

(H_{sp}), M_{sp}, and the H_{sp}/M_{sp} ratio displayed the same trend in which values at Luphephe-Nwanedi Dams were significantly higher (t-test, $p < 0.005$) than at both Flag Boshielo Dam and the Return Water Dam (Table 4.3). The greater predominance of *Contracaecum* species larvae at Flag Boshielo and Return Water Dams strongly influenced the Margalef species diversity and evenness measures (Table 4.3).

Statistical evaluation of these data revealed that in general, species richness (S), the number of heteroxenous and monoxenous species were lower in fish from the Return Water Dam than from the other two dams. The Shannon Wiener Index (H), the inverse Simpson Index (1/D) and the number of metazoan parasite individuals (N) were highest in fish from Luphephe-Nwanedi Dams. Nevertheless, dominance and the ratio of heteroxenous to monoxenous species were lowest in fish from the Luphephe-Nwanedi Dams (Table 4.3). Generally, there were gradual differences in S, N, H and Msp, in an increasing order of Return Water Dam < Flag Boshielo Dam < Luphephe-Nwanedi Dams.

Sorenson's qualitative and quantitative indices of similarity indicated that the composition of the parasite community was highly dissimilar between the sampling sites (Table 4.4). The values for the qualitative Sorenson's index ranged between 0.25-0.53, demonstrating the variability in the composition of the parasite fauna amongst seasons. Lower similarities among seasons were apparent in the number of individuals as calculated by Sorenson's quantitative indices, which varied in a greater range from 0.22-0.47. In all seasons, the lowest



	r^2	R(a/b)	C
Luphephe-Nwanedi Dams(A)	0.2	15.1	34
Flag Boshielo Dam (B)	0.78	12.6	38
Return Water Dam (C)	0.9	2.8	42

Figure 4.3: Total species richness of parasites of *Clarias gariepinus* sampled from three dams of the Limpopo and Olifants River Systems as a function of the number of hosts examined. Data are plotted according to the exponential species accumulation model proposed by Walther *et al*. (1995), r^2 = regression coefficient; R (a/b) = calculated "true species richness; C = capacity or number of hosts needed to reach "true" species richness.

similarity in the number of individuals and the composition of the parasite fauna was found between Luphephe-Nwanedi Dams and the Return Water Dam (Table 4.4).

Metazoan parasite infections varied considerably between sites and seasons (Table 4.5 & Figure 4.4). Some species such as *C. inermis* (16.7%) and

Table 4.3: Comparison of the parasitological parameters of *Clarias gariepinus* sampled from the three dams of the Limpopo and Olifants River Systems. For all ecological measurements, mean values ± standard deviation are given, which were calculated from specimens collected during the four seasonal surveys.

	Luphephe-Nwanedi Dam	Flag Boshielo Dam	Return Water Dam
Parameter	Values mean ± standard deviation (range)	Values mean ± standard deviation (range)	Values mean ± standard deviation (range)
Species Richness (S)	4.6 ± 1.8 (1-10)	2.95 ± 1.9 (0-6)	1.42 ± 0.9 (0-3)
Metazoan burden (N)	172.2 ± 122.9 (20-592)	112.1 ± 150.5 (0-566)	76 ± 65 (0-223)
Shannon Wiener index (H)	2.1 ± 0.11 (1.1-2.7)	1.32 ± 0.27 (0.85-1.54)	0.35 ± 0.12 (0.21-0.6)
Evenness of Shannon-Wiener (E)	0.4 ± 0.04 (0.1-0.6)	0.23 ± 0.14 (0.18-0.32)	0.52 ± 0.37 (0.36-0.72)
Margalef (H')	1.79 ± 0.15 (0.9-1.6)	1.19 ± 0.61 (0.7-1.43)	0.37 ± 0.24 (0.26-0.64)
Berger-Parker index (BP')	0.7 ± 0.44 (0.1-0.8)	0.95 ± 0.31 (0.64-1.37)	0.9 ± 0.32 (0.53-1.22)
Dominant species	*Contracaecum* species	*Contracaecum* species	*Contracaecum* species
% of infracommunities dominated	73.30%	95.19%	90.11%
Heteroxenous species (H_{sp})	9	7	3
Monoxenous species (M_{sp})	8	4	1
Ratio of H_{sp}/M_{sp}	9:8	7:4	3:1

the unidentified leech (11.1%) displayed low prevalence values (Table 4.5). *Contracaecum* species had a relatively high prevalence level (54.6 -100%) year round at all the sites. As a general trend, monogenean prevalence was higher during the summer and winter months while gut helminthes displayed a higher summer and spring prevalence of infection in all the dams (Table 4.5; Figure 4.4). The abundance of metazoan parasites of the catfish was higher in the summer and winter months than in the autumn and spring months in all the three dams (Figure 4.5).

Table 4.4: Similarity of the parasite component community of *Clarias gariepinus* sampled from Limpopo and Olifants River Systems. Upper half of the panel: qualitative Sorenson's indices; lower half: quantitative Sorenson's indices.

(a)				(b)				(c)				(d)			
Autumn	LND	FBD	RWD	Winter	LND	FBD	RWD	Spring	LND	FBD	RWD	Summer	LND	FBD	RWD
LND	*	0.53	0.31	LND	*	0.42	0.31	LND	*	0.42	0.25	LND	*	0.43	0.27
FBD	0.47	*	0.45	FBD	0.38	*	0.47	FBD	0.39	*	0.42	FBD	0.33	*	0.32
RWD	0.34	0.28	*	RWD	0.27	0.37	*	RWD	0.4	0.4	*	RWD	0.22	0.29	*

Table 4.5: Seasonal prevalence of metazoan parasites of *Clarias gariepinus* sampled from three dams of the Limpopo and Olifants River Systems.

Species	**Luphephe-Nwanedi Dams**				**Flag Boshielo Dam**				**Return Water Dam**			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
Gyrodactylus rysavyi	30.8	16.7	30.8	16.7	16.7	9.1	22.2	9.1	9.1	9.1	16.7	9.1
Macrogyrodactylus clarii	25.0	-	46.2	33.3	33.3	27.3	22.2	27.3	-	-	-	-
Macrogyrodactylus congolensis	33.3	41.7	46.2	-	8.3	45.5	22.2	54.4	-	-	-	-
Quadriacanthus clariadis	25.0	-	30.8	16.7	-	-	-	-	-	-	-	-
Quadriacanthus aegypticus	16.7	16.7	30.8	8.3	-	-	-	-	-	-	-	-
Diplostomum species	75.0	58.3	30.8	50.0	16.7	36.4	11.1	18.2	54.5	27.3	25.0	63.6
Tetracotyle species	66.7	41.7	46.2	50.0	-	-	-	-	-	-	-	-
Acanthostomum	-	25.0	30.8	-	-	-	-	-	-	-	-	-
Glossidium pedatum	83.3	25.0	23.1	-	33.3	18.2	22.2	9.1	-	-	-	-
Contracaecum species	100.0	100.0	100.0	100.0	66.7	54.6	55.6	54.6	72.7	72.7	83.3	72.7
Unidentified nematode larvae	-	-	-	-	25.0	18.2	22.2	9.1	-	-	-	-
Paracamallanus cyathopharynx	66.7	-	15.4	58.3	33.3	9.1	11.1	9.1	-	-	-	-
Procamallanus laevionchus	-	-	-	-	-	9.1	11.1	18.2	-	-	-	-
Proteocephalus glanduligerus	50.0	16.7	15.4	-	16.7	-	-	-	-	-	-	-
Tetracampos ciliotheca	-	25.0	-	25.0	50	27.3	33.3	27.3	9.1	36.4	16.7	9.1
Chonopeltis inermis	16.7	-	-	-	-	-	-	-	-	-	-	-
Dolops ranarum	-	33.3	38.5	41.7	-	-	-	-	-	-	-	-
Lamproglena clariae	50.0	-	-	33.3	77.8	9.1	22.2	36.4	-	-	-	-
Udentified leech	-	-	11.1	-	-	-	-	-	-	-	-	-

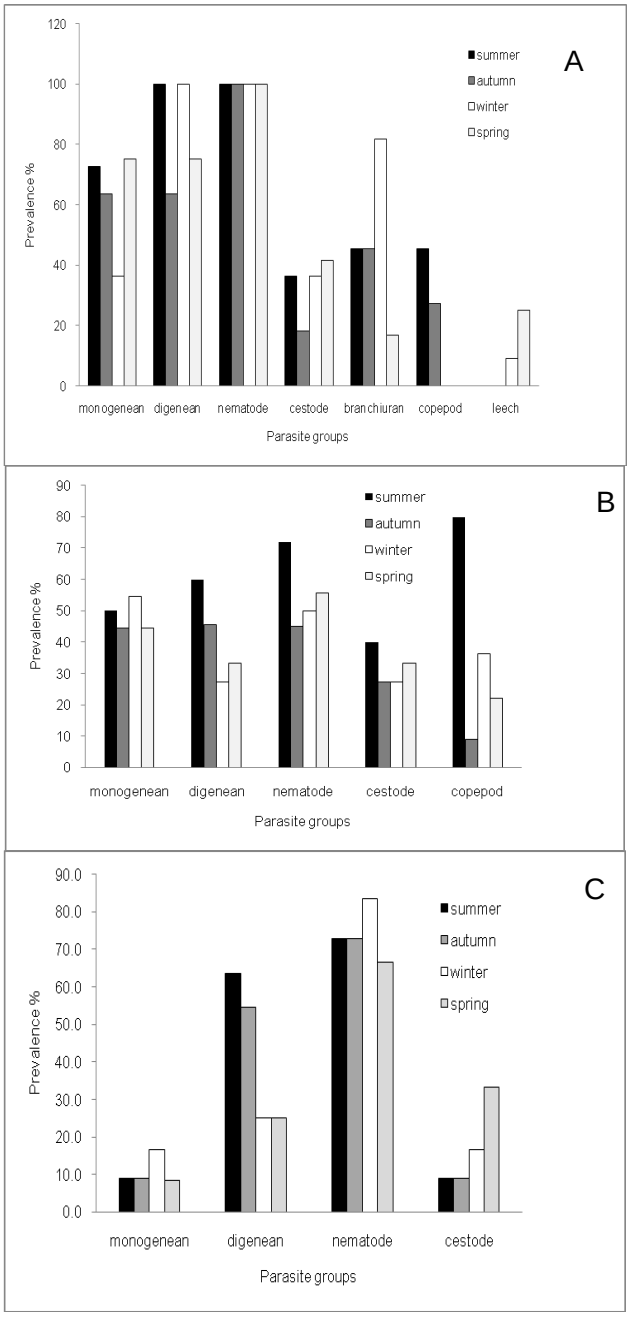


Figure 4.4: Seasonal prevalence of metazoan parasite groups of *Clarias gariepinus* sampled from A = Luphephe-Nwanedi Dams, B = Flag Boshielo Dam and C = Return Water Dams.

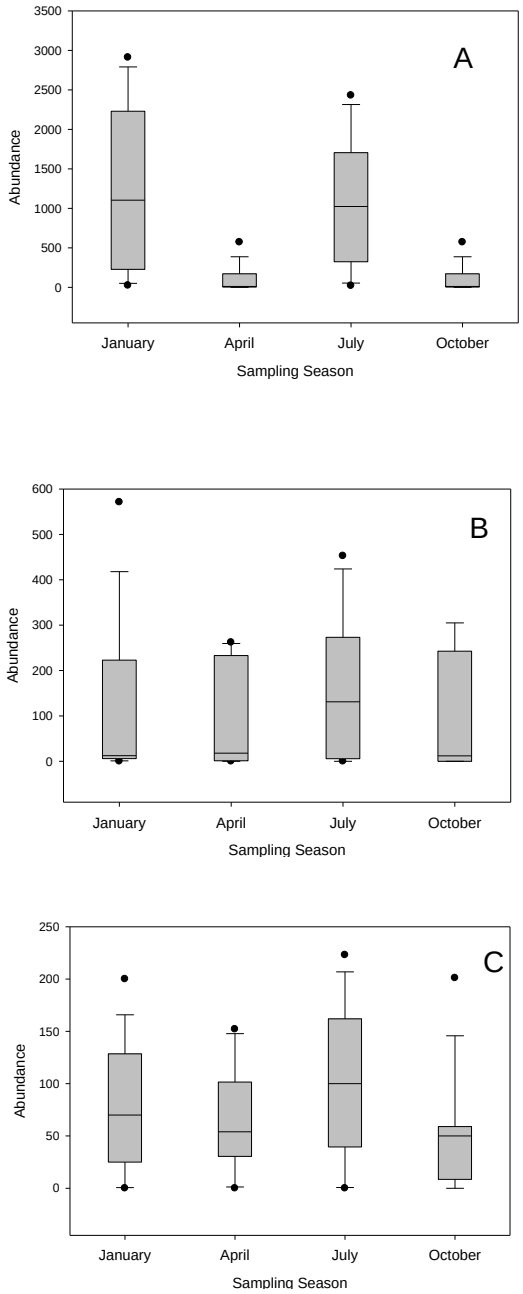


Figure 4.5: Seasonal abundance (x ± SD) of metazoan parasites of *Clarias gariepinus* sampled from A = Luphephe-Nwanedi Dams, B = Flag Boshielo Dam and C = Return Water Dam during the four seasonal surveys.

The Generalised Linear Model results indicated that season significantly explained cumulative parasite abundance in all the three dams (Table 4.6). Tukey's Post-hoc analyses revealed significant differences ($p < 0.005$) between summer and spring, summer and autumn, winter and spring as well as winter and autumn. However, there were no significant differences between winter and summer or between autumn and spring ($p > 0.05$). This suggests that the rate of infection was higher in summer and winter than in autumn and spring. The model also revealed that sex and size had no influence on the abundance of metazoan parasites in the Return Water Dam ($p > 0.05$).

Table 4.6: Generalised Linear Model results for the relationship between explanatory variables and the metazoan parasite abundance on *Clarias gariepinus* from three dams of the Limpopo and Olifants River Systems.

Site	Variable	b	SE	Wald	p
Luphephe-Nwanedi Dams	sex	0.151	0.643	0.332	0.565
	size	0.035	0.36	2.179	0.147
	season	0.131	0.865	4.88	0.027**
Flag Boshielo Dam	sex	0.076	0.4503	0.064	0.523
	size	0.745	0.248	20.65	0.164
	season	0.564	0.256	23.024	< 0.001**
Return Water Dam	sex	-0.505	0.735	0.003	0.143
	size	1.562	0.543	13.46	0.244
	season	-0.003	0.638	0.007	0.015**

Contracaecum species exhibited high associative degrees with *Diplostomum* type 3 and the *Tetracotyle* species, while *Diplostomum* type 3 also strongly associated with *Tetracotyle* species in Luphephe-Nwanedi Dams (Table 4.7A). Amongst the ectoparasites, *M. clarii* and *M. congolensis* also presented significant positive associations in both Luphephe-Nwanedi Dams and Flag Boshielo Dam, despite their low abundance values (Tables 4.7A &B). For the endoparasites, *P. glanduligerus* was also strongly and positively associated with *P. cyathopharynx* and *T. ciliotheca* (Table 4.7A). In Flag Boshielo Dam, the unidentified nematode larva was positively associated with the other two nematodes, *P. cyathopharynx* and *P. laevionchus*. The strong association between *Contracaecum* species and *Diplostomum* type 3 species was also confirmed in Flag Boshielo Dam (Table 4.7B).

Regression analysis showed that parasite burden contributed only 5% (Luphephe-Nwanedi Dams), 0.4% (Flag Boshielo Dam) and 10% (Return Water Dam) of the variance in the condition factor of the fish at the component community level (Figure 4.6).

Table 4.7A: Pairwise correlation matrix for co-occurring metazoan parasites in *Clarias gariepinus* sampled from the Nwanedi-Luphephe Dams.

	M.clar	*M.con*	*Q.cla*	*Diplo*	*Tetra*	*Contra*	*P.cya*	*P.gla*	*T.cili*
M.clar	1								
M.con	0.369*	1							
Q.cla	0.319*	0.105	1						
Diplo	0.221	0.249	0.102	1					
Tetra	-0.038	-0.097	-0.25	0.389*	1				
Contra	0.014	0.151	0.13	0.518**	0.417**	1			
P.cya	-0.035	0.151	0.12	-0.129	0.121	0.063	1		
P.gla	0.122	0.046	0.032	0.061	0.116	0.089	0.376*	1	
T.cili	-0.057	-0.163	0.004	-0.03	-0.048	0.102	-0.221	0.425	1

** Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). M.cla=*Macrogyrodactylus clarii*; M.con=*Macrogyrodactylus congolensis*; Q.cla=*Quadriacanthus clariadis*; Diplo= *Diplostomum* type 3; Tetra = Tetracotyle species metacercariae; P.cya=*Paracamallanus cyathopharynx*; P.gla= *Proteocephalus glanduligerus* T.cili=*Tetracampos ciliotheca.*

Table 4.7A: Pairwise correlation matrix for co-occurring metazoan parasites recovered from *Clarias gariepinus* sampled from the Flag Boshielo Dam.

	M.cla	*M.con*	*Diplo*	*Contra*	nem	*P.cya*	*P.lae*
M.cla	1.000						
M.con	0.261*	1.000					
Diplo	0.060	0.125	1.000				
Contra	0.117	0.275	0.307**	1.000			
nem	-0.144	-0.066	0.280	-0.088	1.000		
P.cya	0.084	-0.151	-0.064	-0.045	0.341*	1.000	
P.lae	0.217	-0.141	0.096	0.070	0.292*	0.297*	1.000

** Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed) M.cla=*Macrogyrodactylus clarii*; M.con=*Macrogyrodactylus congolensis*; *Diplo*=*Diplostomum* type 3; Contr= *Contracaecum* species; nem=unidentified nematode larvae; P.cya=*Paracamallanus cyathopharynx*; *P.lae*= *Procamallanus laevionchus*.

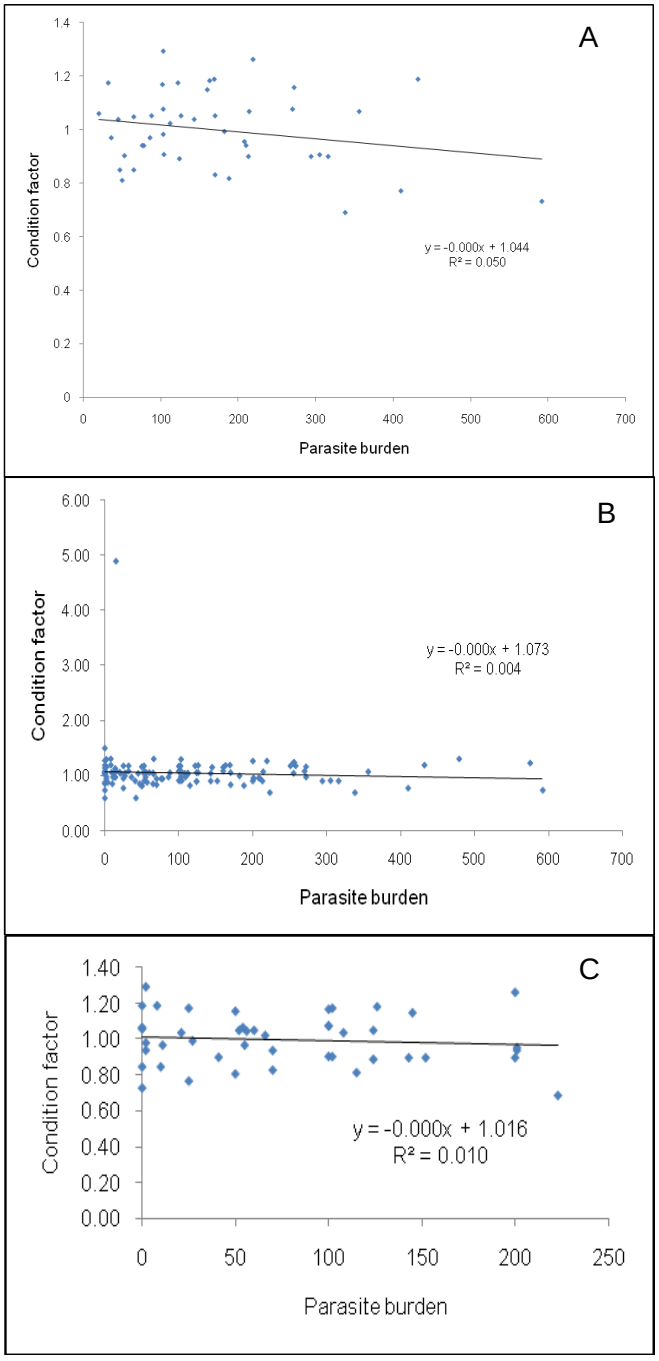


Figure 4.6: Effect of parasite burden on condition factor of *Clarias gariepinus*. A = Luphephe-Nwanedi Dams; B = Flag Boshielo Dam; C = Return Water Dam.

4.5. Discussion

The parasite community structure of *C*. *gariepinus* was investigated on the component community and the infracommunity level according to Bush *et al*. (2001), using established ecological concepts such as species richness, species diversity, species accumulation curve and the ratio of heteroxenous to monoxenous species, in order to characterise three locations with dissimilar water quality in the Limpopo and Olifants River Systems.

The metazoan infracommunities of *C*. *gariepinus* at the Nwanedi-Luphephe Dams indicated that they are a rich species assemblage, with high levels of species richness, diversity and a great variability in abundance values of most constituent species. The component community was numerically dominated by allogenic species in terms of abundance but in terms of species richness it was dominated by host specific species. The Luphephe-Nwanedi Dams appear to be ideal localities for rich natural communities of fish and their parasites based on the assumptions that as part of a reserve, the local fauna has been protected and represents a natural indigenous community, and that the dam water is relatively uncontaminated as revealed by the water quality data (Table 3.2).

Analysis of the metazoan parasites in *C*. *gariepinus* from the Return Water Dam, presented impoverished, low density infracommnuties, with an equal but low representation of allogenic generalist species and autogenic specialist species. Comparison of metazoan infracommunity between *C*. *gariepinus* from Flag Boshielo Dam and the aforementioned sites indicated that mean

species richness, diversity and abundance values in the catfish infracommunities have intermediate values.

The species richness of metazoan parasites of *C*. *gariepinus* was comparable to other African localities (Table 4.8). The species richness from Flag Boshielo Dam has an intermediate value (11), considering an interval from 21 from Lake Victoria (Mwita & Nkwengulila 2008), 17 from Luphephe-Nwanedi Dams and 4 in the Return Water Dam. This however, represents about one quarter of all known metazoan parasites of *C*. *gariepinus* (Khalil & Polling 1997).

The analyses demonstrate the presence of a frequent suite element of allogenic parasite species, which are a persistent structural component of the sharptooth catfish component community. In all the three dams, allogenic species (larval nematodes and digeneans) were numerically dominant. Allogenic species are those that use vertebrates other than fish, usually birds or mammals, or both as definitive hosts. These parasites are capable of relatively rapid dissemination from one locality to another via the movement of their definitive hosts. Since freshwater fish are likely to be restricted to a particular body of water, it follows that autogenic (mature in fish) species have lower dispersal ability (Bush *et al*. 2001).

Clarias gariepinus has an omnivorous diet that includes benthic deposited organic matter and this behaviour favours the ingestion of benthic crustaceans parasitized by cestode and nematode larvae. This behaviour also favours a closer proximity to snails, the first intermediate host for the digeneans. Lateral

transfer acquisition of the worms is achieved by the catfish's piscivorous habits. Thus, the diverse and wide feeding characteristics of the catfish seem to be a contributing factor determining the structure of its parasite communities.

Table 4.8: Number of metazoan parasite species reported in studies on the parasite communities of *Clarias gariepinus* from some African localities in comparison with the present study.

Study	Year	Country	Area	Catchment	N (fish)	N (parasites)
Barson	2004a	South Africa	Rietvlei Dam	Sesmyl Spruit	7	7
Ayanda	2008	Nigeria	Asa Dam	Niger	160	5
Mwita & Nkwengulila	2008	Tanzania	Lake Victoria	Nile	290	21
Barson *et al*.	2008	Zimbabwe	temporary pools and pans	Save-Runde floodplains	274	10
Moyo *et al*.	2009	Zimbabwe	Insukamini Dam	Insukamini Dam	10	5
Madanire-Moyo & Barson	2010	Zimbabwe	flowing rivers	Manyame	110	13
Present study	2011	South Africa	Luphephe-Nwanedi Dams	Limpopo	45	17
Present study	2011	South Africa	Flag Boshielo Dam	Olifants	41	11
Present study	2011	South Africa	Return Water Dam	Limpopo	45	4

The presence of larval stages in the catfish parasite community suggests that the fish plays an important role in metazoan life cycles in these dams, showing its intermediate trophic level. This also confirms that it is part of the diet of piscivorous birds (definitive hosts of *Contracaecum* species, *Diplostomum* type 3 and probably *Tetracotyle* species). The parasite fauna may also provide information on the trophic structure at the terrestrial/aquatic interface. Larval digeneans and nematodes in fish are likely to require piscivorous birds as final

hosts. The infrastructure of the parasite communities may also be used to conjecture about other aspects of habitat quality. In sites without surrounding terrestrial habitat for perching or nesting, or in highly urbanised areas, birds would less likely be present. The high infection levels by allogenic species in Luphephe-Nwanedi Dams and Flag Boshielo Dams may be related to the proximity of the dams to the Nwanedi and Schuinsdraai Nature Reserves, respectively, with their associated bird fauna.

Mokgalong (1996) examined the metazoan parasites of several piscivorous birds within the Nwanedi and has reported the potential adult parasites of the larval stages, such as *Diplostomum* type 3 and *Contracaecum* species. In neighbouring Zimbabwe, larval *Contracaecum* species in *C. gariepinus* from Lake Chivero have been similarly related to adult *Contracaecum* spp. found in the fish-eating birds from the same locality (Barson 2004b; Barson & Marshall 2004). However, the larval stages of *Acanthostomum* species are considered as undetermined until more detailed studies about life cycles (experimental infections) allow corroboration of the relationships of the larval stages with adults of the aforementioned species.

According to Ludwig & Reynolds (1988), diversity is made up of two components inherent to parasitic infrapopulations: the total number of species present in an infracommunity and the uniformity (how abundance data are distributed among the species). There is a positive correlation between species richness and diversity, but a strong dominance of some species can reduce its

richness significance. Thus, Salgado-Maldonaldo and Kennedy (1997) explained the low species diversity (Brillouin index) of helminthes in the cichlid fish *Cichlasoma urophthalmus* by the strong dominance of *Oligogonotylus manteri* in the helminth community. The greater predominance of *Contracaecum* species larvae at Flag Boshielo and the Return Water Dams strongly influenced the Margalef species diversity and evenness measures.

The autogenic parasites and host specific parasites such as the monogeneans, the copepod, the unidentified leech and one branchiuran, *C. inermis*, are all satellite species, which parasitize only the sharptooth catfish and may show an affinity for a possible host switch to phylogenetically related species. Their life cycles are direct, so the passage from host to host is mediated by the close vicinity of the fish, ideally not fulfilled under natural conditions. This may explain their low prevalence and abundance values. *Dolops ranarum*, an autogenic branchiuran, was however, found to be a relatively more abundant species possibly because a certain degree of 'sharing' of this parasite was evident in the twin impoundments with hosts such as *Schilbe intermedius* and *Labeobarbus marequensis* (W.J. Luus-Powell, unpubl data). *Dolops ranarum* has also been observed in other African countries on many fish species (Boane *et al*. 2008).

The metazoan community of the sharptooth catfish of the twin dams has an appreciable degree of specificity, because 10 of the 17 species encountered were host specific species. The dominance of specialists over generalists in this

locality is consistent with the possibility of host phylogeny being important in determining the richness pattern of a parasite community. According to Guégan & Kennedy (1993), greater species richness and more specialist species are to be expected in evolutionary ancient host-parasite systems and in hosts that inhabit their geographic area of origin. The sharptooth catfish, an ancient tropical freshwater fish within its area of origin has had time to acquire its metazoan fauna, and indeed, this may well explain the richer metazoan parasite community and the higher number of specialist species in the sharptooth catfish. Mashego (1977) has reported most of the observed metazoans previously; hence, the metazoan component community of *C. gariepinus* has persisted for the past 30 years, at least. Although the data do not allow any firm conclusions at this stage, they are strongly suggestive of co-evolution between the host and their parasites in the twin dams.

Four species are common in the three dams, but the catfish from the twin dams were more dominated by specialist species. The little similarity in the species composition of the sharptooth catfish of the three water bodies possibly indicates the importance of habitat in determining the structure and composition of metazoan parasites. Ectoparasites such as the branchiurans, copepod, monogeneans and hirudinean are sensitive to aquatic pollution (Avenant-Oldewage 2001; Madanire-Moyo & Barson 2010) and the oligotrophic nature of the habitat of the twin impoundments (Oberholster *et al*. 2009) makes it more hospitable to these pollution sensitive parasites.

The high intensity levels, which reached 592 parasite specimens per host, would be expected to influence the health and condition of the fish. However, direct observations made at the post-mortem examination did not indicate that even individual fish with the highest parasite burdens were experiencing any overt morbidity. These parasites reached high intensity levels without affecting the host condition, an adaptation that probably ensures that the larvae survive to reach the final host without killing the intermediate host. Since metazoan parasites use host-derived energy for the maintenance of their vital functions, heavily infected individuals must spend more time foraging to attain the same nutritional benefit as less-parasitised and unparasitised individuals (Barber *et al*. 2000). Metazoan parasites appear to be able to manipulate host behaviour by increasing its motivation to forage or reducing its activity such that an increased food intake of infected fish may outweigh a possible parasite-mediated energy loss.

The observed trends in the seasonal occurrence of heteroxenous species can theoretically be related to the hydrological sequence of events taking place in the dams. The prevalence of heteroxenous species displayed a peak during spring months, corresponding to the pre-spawning intensive feeding period of the host characterized by heavy feeding. It is during this season that the fish have a greater probability of exposure (by ingestion) to infective stages of gut parasites. Some resting stages of parasites (nematode eggs, larval trematodes in aestivating snails, larval cestodes/nematodes in diapausing copepods) might be

induced to hatch and infect invertebrate hosts (gastropods, annelids, crustaceans). Autogenic parasites then complete their life cycles by infecting the fish while allogenic species accumulate in fish tissues as they await transmission to their final host (birds and mammals) (Barson 2009).

Clarias gariepinus awaits suitable environmental conditions for spawning, which usually takes place in summer (Bruton 1979). It is during this period when hatched parasite larvae are abundant and can infest their hosts with increasing abundance, mean intensity and prevalence in summer. In addition, the dams are inundated during this period and are able to support a wide range of fauna and flora, including wetland birds in large vegetated littoral zones. This increases the chances of the fish in the dams encountering more infective stages of helminthes in the wet seasons compared to the dry seasons.

The lowest prevalence of these heteroxenous species occurred during autumn/winter months, which corresponds to decreased feeding rates, possibly coupled with decreased abundance of infective stages. Similar patterns have also been reported (Kennedy 1997; Diamant 1989). The peak abundance of the monoxenous (particularly monogeneans, branchiurans and copepods) during the summer months may have been due to increased proliferations on the host at high ambient summer temperatures as well as host post–spawning stress, which could have reduced host immunity and enhanced infections (Yeomans *et al*. 1997).

The lack of significant correlations between host size and most parasite species might be attributable to the homogeneity of the fish sample because no juveniles were sampled. Moreover, it is most likely that the large proportion of adults subdued the influence of the smaller proportion of sub-adults in the sample. The influence of sex, despite its widely citation on related literature, is usually minimized in papers dealing with communitary analysis (Poulin 1996). In the present study, *C*. *gariepinus* did not present quantitative variations caused by sex. This is considered to be a reflex of the lack of differences in the biology and population dynamics of male and female hosts (Luque *et al*. 1996). Nevertheless, future research is needed in order to visualize the influence of other factors (such as hormonal, immunological, morphological and behavioural) that already proved to play a role for other host groups (Poulin 1996). Studies on seasonality and breeding seasons could shed light upon such aspects, since it would be verified if changes on either male or female hosts behaviour occur regarding breeding seasons, feeding habit, hormonal levels, migrations and sexual dimorphism which could change the ecological niche.

The pairs of endoparasite species (*Diplostomum* type 3-*Tetracotyle* species and *P*. *glanduligerus*-*T. ciliotheca*) presented a high associative degree, confirming that they have the same intermediate hosts. Positive inter-specific associations of the ectoparasites may be an outcome of a similar mechanism of infection *in situ* (Lo *et al*. 2001). Additionally, one species may weaken a host and so make it more susceptible to infection with another species or one species

may “prepare” the microhabitat for a second species, for instance by making it easier for the latter species to feed (Hayward *et al*. 1998). These interactions may be responsible for the aggregated pattern of distribution of parasites observed in this study. The strong association among the monogenean taxa (*M.clarias-M. congolensis; M. clarias-Q. clariadis*), digeneans (*Diplostomum-Tetracotyle*) and cestodes (*T. celiotheca-P. glanduligerus*) may possibly indicate co-occurrence and the absence of site-specificity and interspecific competition. According to Bush *et al*. (2001), aggregated distributions, the most common pattern found in nature, indicate possible social interactions, a need to be together for some reason (mutual defence, cooperative feeding or mating purposes) or the presence of a suitable resource.

Helminth communities in freshwater fish have been described as chance assemblages rather than structured organisations (Kennedy 1990). The results herein conform to this description since there is great variability in the mean abundance among infracommunities, with an abundance range of zero to 592 metazoan parasites per host and a low Sorenson’s quantitative similarity index. This discrepancy is largely a result of the dominant larval parasites, whose abundance values varied in greater ranges across infracommunities. All fish sampled exhibited comparable size and for this reason, the variation between abundance may be associated with differential exposure and/or susceptibility of the host to metazoans as well as the fish’s collect from the different localities of the dams. In species composition, the sharptooth catfish possessed

assemblages with relatively high levels of qualitative similarity and this may be attributed to high prevalence of *Contracaecum* species (100%), which co-occurs with *Diplostomum* type 3. All two species are present in 28% of the hosts. Recent years have seen investigations into latitudinal richness gradients of parasites (Pérez-Ponce de Leon *et al*. 2000) with suggestions of richer parasite communities in tropical fishes (Kennedy 1995; Salgado-Maldonado & Kennedy 1997). Although rich tropical metazoan parasite communities of freshwater fishes have been described (Kennedy 1995; Salgado-Maldonado & Kennedy 1997; Vidal-Martínez & Kennedy 2000), many other tropical freshwater fishes have species-poor metazoan communities (Choudhury & Dick 2000; Pérez-Ponce de Leon *et al*. 2000; Salgado-Maldonado *et al*. 2001; 2004).

4.6. Conclusions

The metazoan communities of the sharptooth catfish, *C. gariepinus*, of the three dams are numerically dominated by allogenic metazoan parasites which present the local ecological noise that enhances the stochastic nature of the infracommunities. The patterns observed in this study indicate clearly that latitude alone does not contribute necessarily to a rich and diverse parasite community but a combination of historical and contemporary ecological factors (host diet, feeding behaviour, vagility, specificity, habitat and interspecific associations) play a significant role in determining metazoan community diversity and structure in freshwater fish. Although the sharptooth catfish is host to a high

diversity of parasites, it still remains one of the most important food sources in Africa. This is because most of the parasites are external or found predominantly in the gastrointestinal tract, which for humans does not comprise the edible portion of the fish, hence may not pose a serious human health threat. Activity and feeding habits might have contributed to infection with helminthic parasites in different age groups of *C*. *gariepinus*. It may be advisable to incorporate anthelminthic therapy into the diet of *Clarias gariepinus* obtained from the wild that might be used as brood stock.

CHAPTER 5

PATTERNS AND DETERMINANTS OF METAZOAN COMMUNITIES IN THE MOZAMBIQUE TILAPIA, *OREOCHROMIS MOSSAMBICUS* (PETERS, 1852) FROM THREE DAMS OF THE LIMPOPO AND OLIFANTS RIVER SYSTEMS, SOUTH AFRICA

5.1. Introduction

The Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852), contributes about 4% of the total tilapia aquaculture production worldwide (Maclean 1984). Hybridisation of this tilapia has produced strains with enhanced growth characteristics and environmental tolerances to low temperature, overcrowding stress and pathogens (Cnaani *et al.* 2000; Cai *et al.* 2004). This cichlid is a hardy species with a remarkable tolerance for organic (Noorjahan *et al*. 2003) as well as inorganic pollution (Nanda *et al.* 2002; Somanath 2003).

Native to southern Africa, it is a significant angling fish (Skelton 2001). According to the International Union for Conservation of Nature **(**IUCN), it is listed as “Near Threatened” on its Red Data List of Threatened Species and is likely to become locally extinct (Cambray & Swartz 2007) due to hybridization and competition with the introduced Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758). This hybridization may disrupt its genetic adaptations to resist drought, capacity to survive and reproduce in seawater and to resist temperatures as low as 11°C (Moralee *et al*. 2000).

The objectives of the investigation described in the present chapter were to study the distribution and abundance of species of metazoan parasites in *O*. *mosambicus* with emphasis on (1) levels of infection of metazoan parasites in function of seasons, sex and size of the host, (2) interspecific associations of the metazoan parasites in the communities and (3) the role of the tilapia in the life cycle of the metazoan parasites. The reasons for conducting the investigation were threefold. Firstly, much effort is being made to identify the processes that generate and stabilise communities of metazoan species in fish (Dorucu *et al*. 1995). Secondly, there is need to address the shortfalls in information concerning the microhabitat distribution and demographic profiles of metazoan parasites of commercially important fish species in near-natural sites (Álvarez *et al*. 2002). This is essential to find ways of avoiding catastrophic losses often observed in planned intensive aquaculture. Thirdly, with populations of *O*. m*ossambicus* in a threat to become locally extinct (Cambray & Swartz 2007), it is worthy investigating the community ecology of its parasites.

5.2. Materials and Methods

Fish were collected by gill nets of mesh sizes 30-110 mm, over four seasonal samplings: April 2008 (autumn), July 2008 (winter), October 2008 (spring) and January 2009 (summer) in the Luphephe-Nwanedi Dams and the Anglo Platinum Return Water Dam. The Flag Boshielo Dam was sampled in April 2009 (autumn), July 2009 (winter), October 2009 (spring) and January 2010

(summer). These seasonal collections were carried out from three dams of the Olifants and Limpopo River Systems, namely the Luphephe-Nwanedi Dams, the Flag Boshielo Dam and the Return Water Dam. Details of sampling procedure, study area, examination of fish hosts and ecological terminology for the description of parasite populations are given in Chapter 2. For an estimation of real species richness at a given site, depending on sample size, Walther's graph (Walther *et al*. 1995) was calculated according to the formula: Y = a (1-e-bx)/b, with a = increase in species richness at the beginning of sampling, b = parameter that sets the species richness asymptote R = a/b, x = unit of sampling effort.

Parasite collections were done according to Paperna (1996) and identified according to various authors: Douëllou & Chishawa (1995) for monogeneans; Barson *et al*. (2008) and Chibwana & Nkwengulila (2010) for digeneans; Mashego (1977) and Anderson (1992) for nematodes; Khalil *et al*. (1994) for cestodes and Avenant *et al*. (1989) for Dolops ranarum.

5.3. Data Analyses

For each host specimen examined, standard length, and weight were measured to calculate the condition factor using the formula: $CF = 100\ W/L^3$, where: W = weight in g and L = standard length in millimetres (Bagenal & Tesch 1978). Levels of parasite infections were analysed according to Bush *et al*. (2001). Shannon Wiener (diversity), evenness (equal distribution) as well as

Berger-Parker (dominance) biotic indices were calculated to compare the three communities of metazoan parasites. Increasing values of the Shannon Wiener Index indicate an increase in diversity. Values of evenness can range from 0 to 1, with values of 0 indicating a completely uneven distribution of parasites in a sample and values of 1 a totally even distribution. All indices were calculated according to Magurran (1988).

The differences in species richness among the three sampling sites were tested by a one- way analysis of variance (ANOVA). Tukey's Post-hoc test was performed to determine which sites were significantly different from one another. Spearman's rank correlation coefficient (r_s) was calculated to determine (a) possible correlations between standard host length and parasite abundance values and (b) possible interspecific associations between concurrent species. The effect of host gender on parasite abundance was tested using the chi- square test. Principal Component Analysis (PCA) was performed to determine the pattern of distribution of parasite species among the sites.

All infection data were normalized with $\sqrt{}$ (χ) transformations to guarantee normality of distribution. Physical variables were standardized to zero mean and unit variance to make them dimensionless. Results were considered significant at the 95% level ($p < 0.05$). Ordination was done using CANOCO version 4 (Ter Braak & Smilauer 1998) while STATISTICA version 7 (STATSOFT Inc., Tulsa, Oklahoma, USA) and SPSS 17.0 software packages were used for correlation and regression analyses.

5.4. Results

A total of 140 specimens of *O. mossambicus* were collected and examined from the three localities (Table 5.1). The metazoan parasites encountered included 20 species of metazoan parasites, comprising eight monogeneans such as *Cichlidogyrus halli* (Price and Kirk, 1967), C. *sclerosus* Paperna and Thurston, 1969, *C. dossoui* Douëllou, 1993, *Scutogyrus longicornis* (Paperna and Thurston, 1969), *C. tilapiae* Paperna, 1960 and three species of *Enterogyrus* Paperna, 1963; five digeneans (*Neascus* von Nordmann, 1832, *Tylodelphys* Diesing, 1850, *Diplostomum* Nordmann, 1842, type 3, *Tetracotyle* Diesing, 1858 and *Clinostomum* Leidy, 1856); four nematodes (*Contracaecum* Raillet & Henry, 1912, *Procamallanus laevionchus* (Wedl, 1862) and *Paracamallanus cyathopharynx* (Baylis, 1923) and an unidentified nematode larva); the gryporynchid cestode larva and one branchiuran, *Dolops ranarum* (Stuhlmann, 1891) and one copepod, *Ergasilus* von Nordmann, 1832 (Table 5.2).

Eight specialist-autogenic parasite species were identified as compared to 12 generalist-allogenic species (Table 5.2). Total parasite species richness was highest in Luphephe-Nwanedi Dams, moderate in Flag Boshielo Dam and lowest in the Return Water Dam, with 19, 17 and four parasite species, respectively (Table 5.1). The total numbers of individual parasites, species richness, mean infacommunity species richness and mean Shannon diversity index also followed the same trend (Table 5.1). The most dominant species were *Tetracotyle* species

Table 5.1: Some parasitological parameters for metazoan parasites of *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

Parameter	Luphephe-Nwanedi Dams	Flag Boshielo Dam	Return Water Dam
Total no. of fish	45	48	47
Total no. of parasites (TP)	1 894	912	544
Species Richness (S)	19	17	4
Fish weight (g)	194.3 ± 147	495.1 ± 313.4	405.9 ± 340.5
Fish length (cm)	176.4 ± 42.2	232.87 ± 49.8	218.3 ± 61.1
Condition factor (K)	0.6 ± 0.3	1.1 ± 0.6	0.9 ± 0.8
Species Richness (S)	19	17	4
Mean species richness	4.3±1.7	3±2	1.5±0.9
Mean parasite individuals	171.9 ± 122.2	83.3 ± 136.1	76.1 ± 65
Shannon Wiener Index (H)	1.4±0.11	1.21±0.08	0.95±0.09
Evenness of Shannon-Wiener (E)	0.63±0.04	0.54±0.02	0.26±0.04
Berger-Parker index (BP')	0.45±0.06	0.62±0.03	0.92±0.02
Dominant species	*Tetracotyle*	*Neascus*	gryporynchid
Heteroxenous species (H_{sp})	10	8	1
Monoxenous species (M_{sp})	9	9	3
H_{sp}/M_{sp}	10:9	8:9	1:3

in Luphephe-Nwanedi Dams, *Neascus* species in Flag Boshielo Dam and the gryporynchid cestode larvae in RWD (Table 5.1).

Species richness, abundance of endoparasites and ectoparasites and the total number of parasite individuals were significantly lower (ANOVA, $p < 0.05$; Table 5.3) in the Return Water Dam when compared with values for Luphephe-Nwanedi Dam. When compared with Flag Boshielo Dam, only species richness and abundance of ectoparasites had significantly lower values at the

Table 5.2: The biological characteristics of metazoan parasite species recovered from *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

Parasites	Stage	Organ /Tissue	host	Final host	Life cycle[1]	ecto/endo parasite[2]	Status	Location[4]
Monogenea								
Cichlidogyrus halli	adult	gills	Final	Fish	M	Ecto	Specialist, auto	ALL
C. sclerosus	adult	gills	Final	Fish	M	Ecto	Specialist, auto	LND;FBD
C. dossoui	adult	gills	Final	Fish	M	Ecto	Specialist, auto	LND;FBD
C. tilapiae	adult	gills	Final	Fish	M	Ecto	Specialist, auto	LND;FBD
Scutogyrus longicornis	adult	gills	Final	Fish	M	Ecto	Specialist, auto	LND;FBD
Enterogyrus species 1	adult	stomach	Final	Fish	M	Endo	Specialist, auto	LND;FBD
Enterogyrus species 2	adult	stomach	Final	Fish	M	Endo	Specialist, auto	LND;FBD
Enterogyrus species 3	adult	stomach	Final	Fish	M	Endo	Specialist, auto	LND;FBD
Digenea								
Neascus species	larval	fins	intermediate	Birds	H	Endo	Generalist, all	LND;FBD
Tylodelphys species	larval	gills	intermediate	Birds	H	Ecto	Generalist, all	LND;FBD
Diplostomum species	larval	eyes, brain	intermediate	Birds	H	Endo	Generalist, all	ALL
Tetracotyle species	larval	muscle	intermediate	Birds	H	Endo	Generalist, all	LND
Clinostomum species	larval	gill chamber	intermediate	Birds	H	Endo	Generalist, all	ALL
Nematoda								
Unidentified nematode	larval	intestine	intermediate	Birds	H	Endo	Generalist, all	LND;FBD
Contracaecum species	larval	body cavity	intermediate	Birds	H	Endo	Generalist, all	LND;FBD
Paracamallanus cyathopharynx	adult	stomach, intestine	Final	Fish	H	Endo	Generalist, all	LND;FBD
Procamallanus laevionchus	adult	stomach, intestine	Final	Fish	H	Endo	Generalist, all	LND;FBD
Cestoda								
gryporynchid	larval	liver, intestine	intermediate	Fish	H	Endo	Generalist, all	ALL
Branchiura								
Dolops ranarum	adult	skin	Final	Fish	M	Ecto	Generalist, all	LND
Copepoda								
Ergasilus species	adult	skin, buccal cavity	Final	Fish	M	Ecto	Generalist, all	FBD

[1]) M = monoxenous, H = heteroxenous species [2]) ecto = ectoparasites, endo = endoparasites [3]) aut= autogenic, allo = allogenic; [4]) LND = Luphephe-Nwanedi Dams, RWD = Return Water Dam, FBD = Flag Boshielo Dam, ALL = all locations.

Return Water Dam (ANOVA, $p < 0.05$; Table 5.3). On the other hand, significant differences in only the abundance of endoparasites and the total number of parasite individuals were revealed between Luphephe-Nwanedi Dams and Flag Boshielo Dam (ANOVA, $p < 0.05$; Table 5.3).

Table 5.3: One-way analysis of variance (ANOVA) comparisons of host and parasite parameters among the three sites (pooled data).

Dependent variable	Comparison	Mean difference	Standard error	p-value
Species richness	LND vs. FBD	-0.026	0.354	0.997
	LND vs. RWD	3.096*	0.355	0.00
	FBD vs. RWD	3.122*	0.35	0.00
Endoparasites	LND vs. FBD	21.349*	4.366	0.00
	LND vs. RWD	17.152*	4.388	0.00
	FBD vs. RWD	-4.196	4.317	0.596
Ectoparasites	LND vs. FBD	2.276	1.783	0.411
	LND vs. RWD	13.358*	1.792	0.00
	FBD vs. RWD	11.082*	1.763	0.00
Total parasite individuals	LND vs. FBD	23.089*	4.625	0.00
	LND vs. RWD	32.536*	4.648	0.00
	FBD vs. RWD	9.447	4.574	0.101

* The mean difference is significant at the 0.05 level.

Table 5.4: Spearman's rank correlation coefficient (r_s) values used to evaluate possible relationships between host size (standard length) of *Oreochromis mossambicus* and abundance of its metazoan parasites.

Parasite species	LND	RWD
	r_s	r_s
Neascus species metacercariae	.310*	-
Tetracotyle metacercariae	.406**	-
Contracaecum species larvae	.306*	-
gryporynchid cestode larvae	-	.542**
endoparasites	.601**	.587**
total no. of parasites	.546**	.591**
Species richness	-	0.330*

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed); LND = Luphephe-Nwanedi Dams; RWD = Return Water Dam.

Prevalence values for the monogeneans were higher at Flag Boshielo Dam than at Luphephe-Nwanedi Dams except for *Enterogyrus* species 1 and *Enterogyrus* species 3 (Figure 5.1A). Prevalence values for *Tylodelphys* species were relatively similar in Luphephe-Nwanedi Dams and Flag Boshielo Dam and the same trend was noted for *Diplostomum* species in Luphephe-Nwanedi Dams and in the Return Water Dam (Figure 5.1B). Prevalence values for all nematodes encountered were higher in Luphephe-Nwanedi Dams than in Flag Boshielo Dam (Figure 5.1C). The prevalence values for the gryporynchid cestode larva were relatively similar at Luphephe-Nwanedi Dams and Flag Boshielo Dam but highest in the Return Water Dam (Figure 5.1D). The prevalence values for *D*. *ranarum* in Luphephe-Nwanedi Dams and that of *Ergasilus* sp. in Flag Boshielo Dam were 13.3% and 12.5%, respectively (Figure 5.1D).

The abundance values for all monogeneans encountered were higher in Luphephe-Nwanedi Dams than in Flag Boshielo Dam (Figure 5.2A). The mean abundance of *Neascus* sp., *Tylodelphys* sp., *Diplostomum* sp., *Tetracotyle* sp. and *Clinostomum* sp. were highest in the Luphephe-Nwanedi Dams (Figure 5.2B). *Paracamallanus cyathopharynx* and the unidentified nematode larva were more abundant in Flag Boshielo Dam than in Luphephe-Nwanedi Dam (Figure 5.2C). The gryporynchid cestode larva was most abundant in the Return Water Dam (Figure 5.2D). The mean intensity values for monogeneans were lower than for digeneans and nematodes (Figures 5.3A, B & C). Overally, the mean intensity values for the metazoan parasites were highest at Luphephe-Nwanedi

Dams (Figures 5.3 A-D).

The component parasite populations from the three localities showed aggregated distribution, typical of most parasite distributions within their hosts (Figure 5.4). The highest number of parasite species found per fish was seven in Luphephe-Nwanedi Dams and Flag Boshielo Dam, but three in the Return Water Dam. The most common number of parasite species per fish was four in Luphephe-Nwanedi Dams, five in Flag Boshielo Dam and one in the Return Water Dam (Figure 5.4).

Regression analyses showed that parasite burden contributed only 5.3% (in Luphephe-Nwanedi Dams), 8.1% (in Flag Boshielo Dam) and 8. 3% (in the Return Water Dam) of the variance in the condition factor of the fish at the component community level (Figures 5.5A-C). Relationships between the total parasite abundance and host standard length were not observed (χ^2 , $p > 0.05$).

The abundance of metacercariae of *Neascus* species and *Tetracotyle* species, *Contracaecum* species larvae, endoparasites and the total number of individual parasites, in Luphephe-Nwanedi Dams were positively correlated to host size while the abundance of the gryporynchid cestode larvae, endoparasites and the total numbers of parasite individuals as well as species richness in the Return Water Dam were positively correlated to host size (Table 5.4). There were no correlations observed in host size and abundance of metazoan parasites from the Flag Boshielo Dam (Table 5.4).

There was clear evidence of positive associations among most ectoparasite species in both the Luphephe-Nwanedi Dams and Flag Boshielo Dam ($r_s \geq 334$; $p < 0.05$; Table 5.5). Among the endoparasites, the unidentified nematode larva was positively associated with both the larvae of *Diplostomum* and the gryporynchid cestode larva in Luphephe-Nwanedi Dams while only one pair between *P. laevionchus* and P. *cyathopharynx* showed significant positive association in Flag Boshielo Dam ($r_s \geq 327$, $p < 0.05$, Table 5.5). Associations of either ecto- or endoparasite species in the Return Water Dam were not evident ($p > 0.05$).

The first two factors in the principal components accounted for 36.2% of the observed variance, which was based on the relative abundance of the parasites species (Figure 5.6). All monogeneans, the digeneans (metacercariae of *Clinostomum* species and *Neascus* species) and the nematodes (*P. cyathopharynx, P. laevionchus* and the unidentified nematode larva) were closely associated with Luphephe-Nwanedi Dams and Flag Boshielo Dam. *Dolops ranarum* and metacercariae of *Tylodelphys* species and *Tetracotyle* species were more associated with Luphephe-Nwanedi Dams while *Diplostomum* species was common in Luphephe-Nwanedi Dams and the Return Water Dam. The gryporynchid cestode larva was, to a greater extent, strongly associated with the Return Water Dam (Figure 5.6).

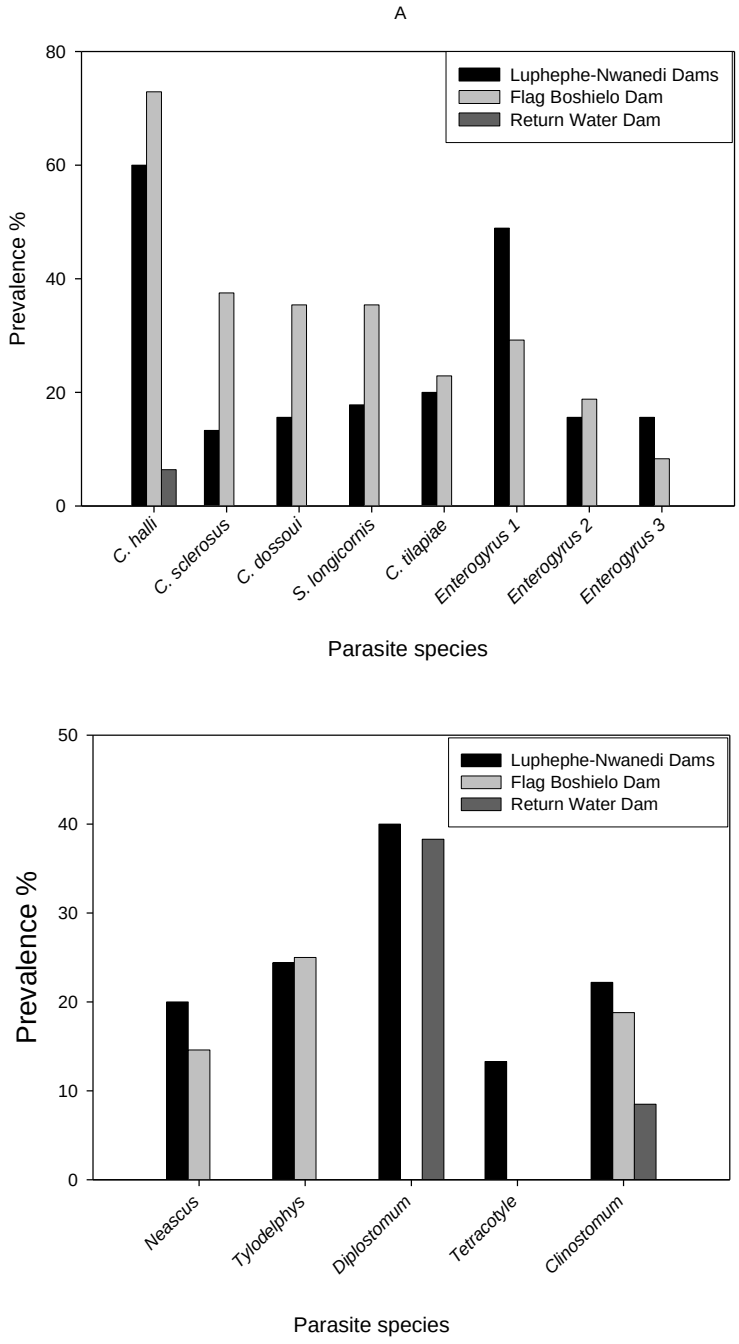


Figure 5.1: Prevalence values of A = monogeneans (*C*. = *Cichlidogyrus* species; *S*. = *Scutogyrus* species); B = digeneans recovered from *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

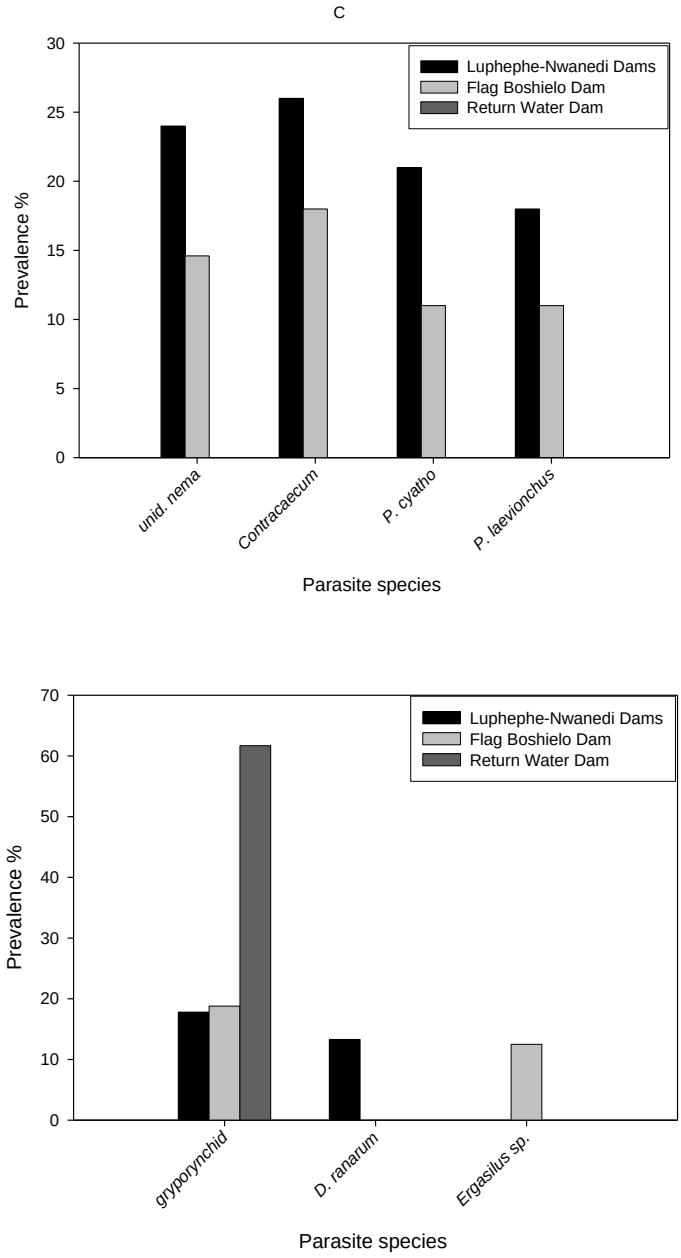


Figure 5.1 (continued): Prevalence values of C = nematodes (unid. nema = unidentified nematode larva; *P. cyatho* = *Paracamallanus cyathopharynx*); D = gryporynchid cestode larva, *Dolops ranarum* and *Ergasilus* species recovered from *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

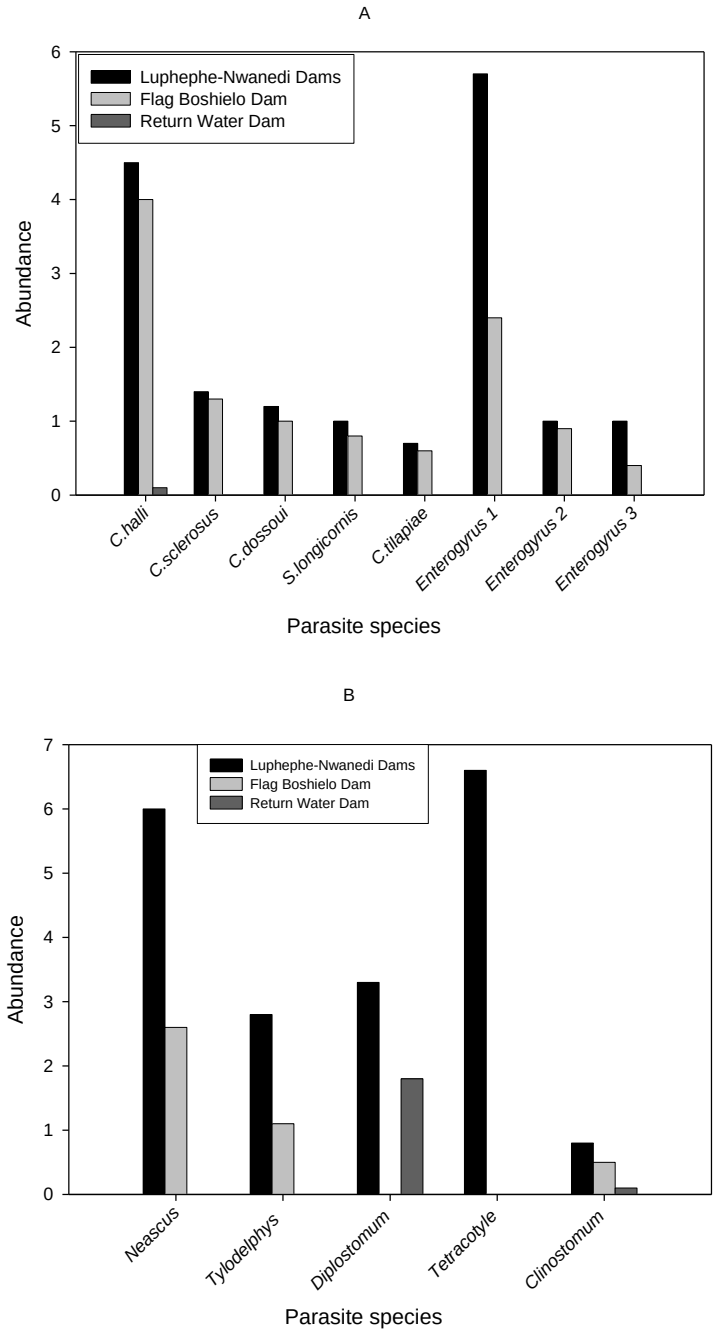


Figure 5.2: Mean abundance of A = monogeneans (*C*. = *Cichlidogyrus* species; *S*. = *Scutogyrus* species); B = digeneans recovered from *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

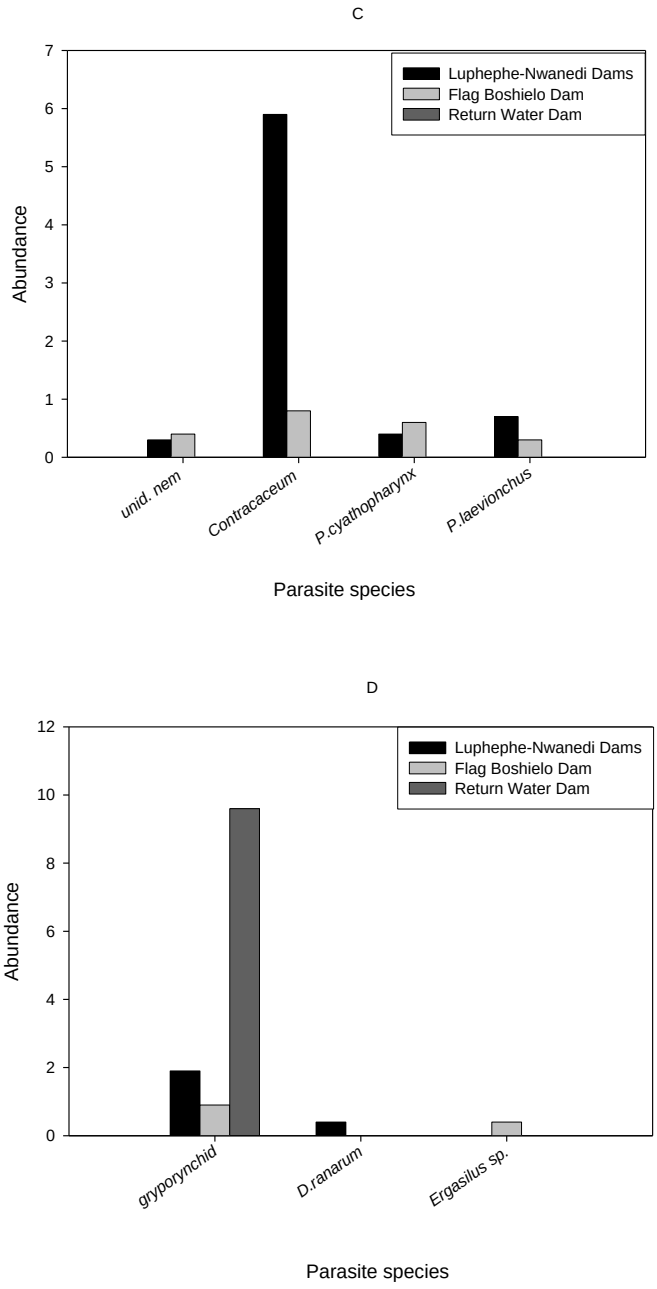


Figure 5.2 (continued): Mean abundance of C = nematodes (unid. nem. = unidentified nematode); D = gryporynchid cestode larva, *Dolops ranarum* and *Ergasilus* species recovered from *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

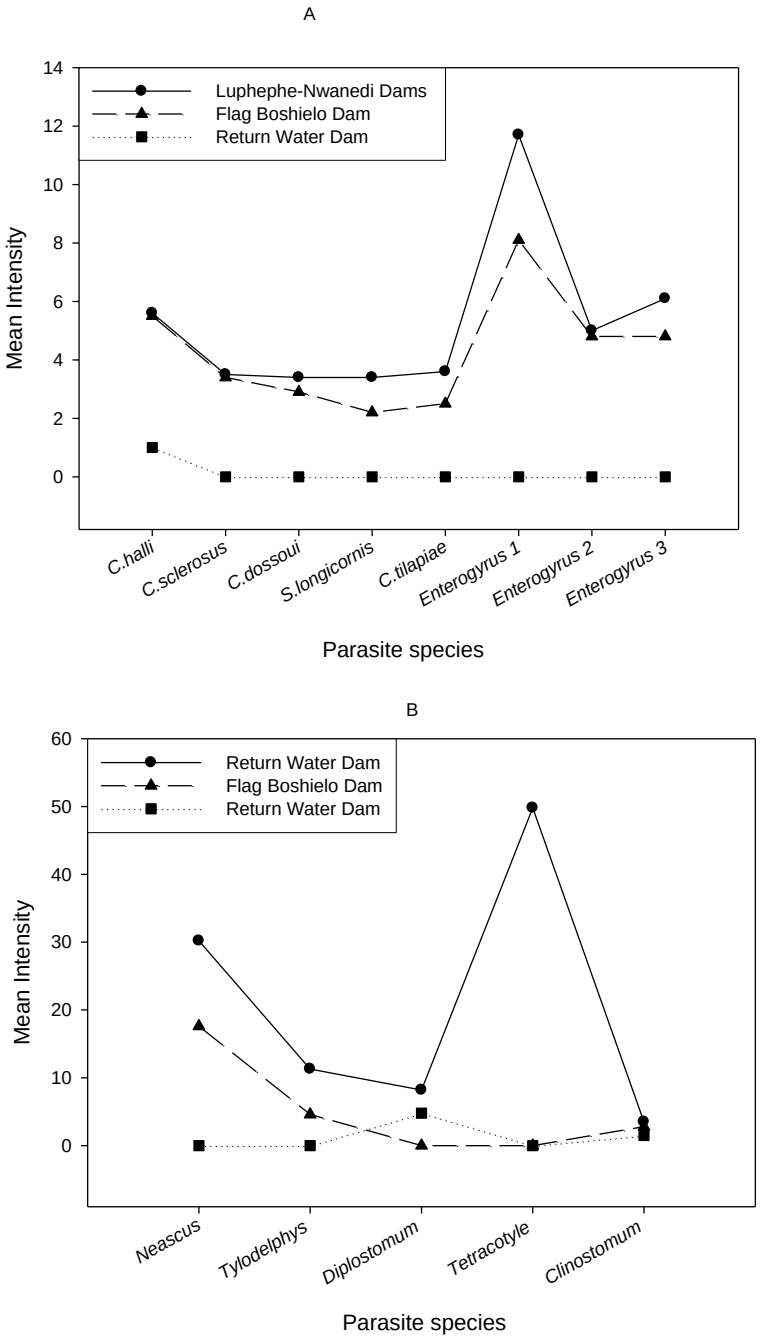


Figure 5.3: Mean Intensity of A = monogeneans (*C*. = *Cichlidogyrus* species; *S*. = *Scutogyrus* species); B = digeneans recovered from *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

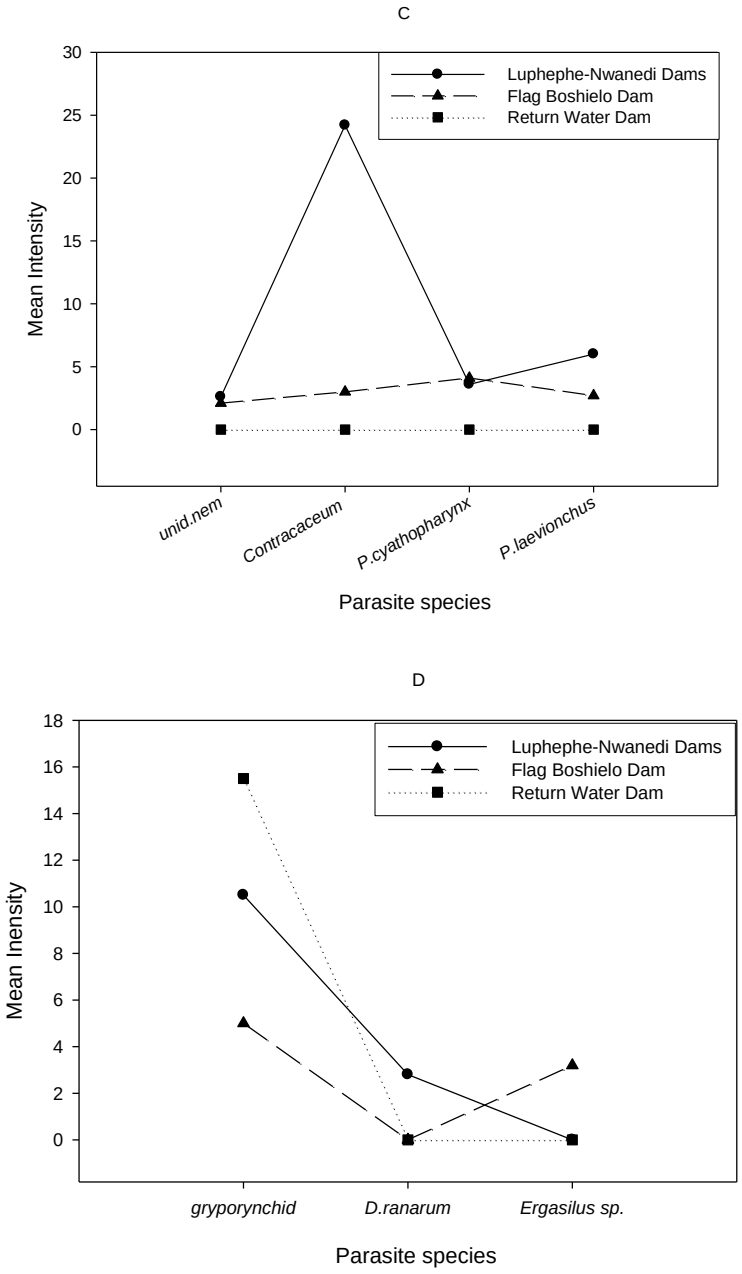


Figure 5.3 (continued): Mean Intensity of C = nematodes (unid. nem = unidentified nematode); D = gryporynchid cestode larva, *Dolops ranarum* and *Ergasilus* species recovered from *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

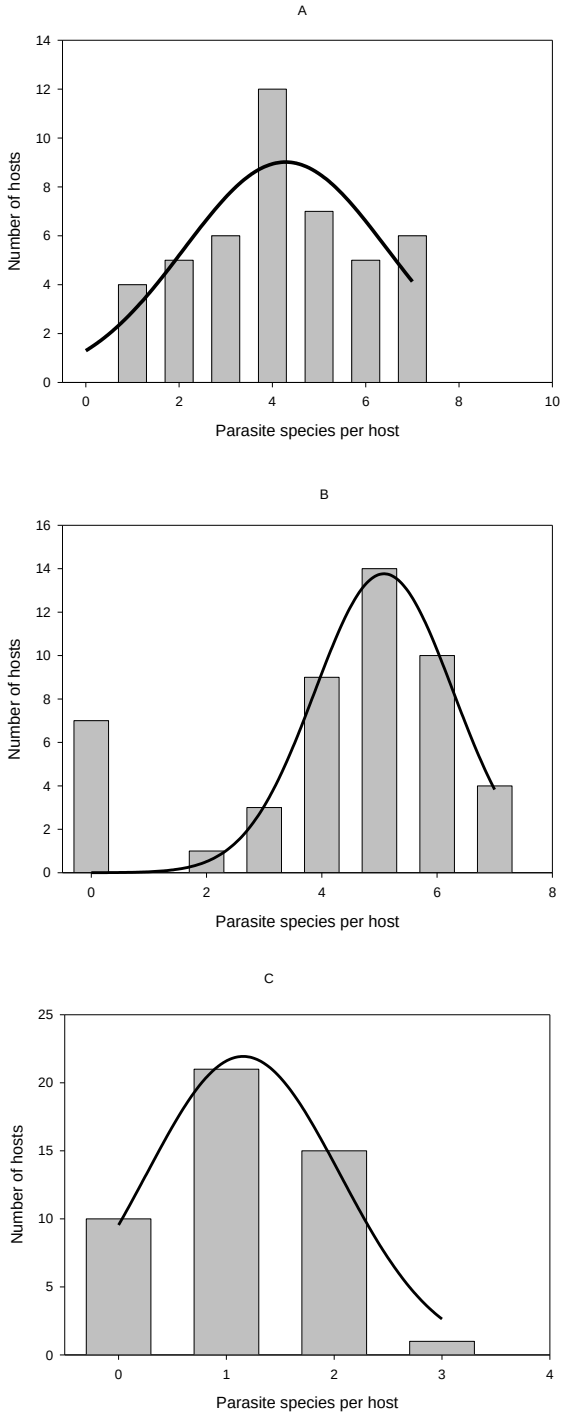


Figure 5.4: Frequency distribution of parasite species in specimens of *Oreochromis mossambicus* from A = Luphephe-Nwanedi Dams; B = Flag-Boshielo Dam; C= Return Water Dam.

Table 5.5: Spearman's rank correlation coefficient (r_s) values for metazoan concurrent species pairs of *Oreochromis mossambicus* sampled from three dams of the Limpopo and Olifants River Systems.

Sampling site	LND	FBD
Species pairs	rs	rs
Cichlidogyrus halli- Cichlidogyrus sclerosus	.358*	.421**
Cichlidogyrus halli -Cichlidogyrus dossoui	.464**	.420*
Cichlidogyrus halli -Cichlidogyrus tilapiae	.522**	-
Cichlidogyrus halli -Enterogyrus species 1	.334*	-
Enterogyrus species 1-*Enterogyrus* species 2	.453**	.785**
Enterogyrus species 1-*Enterogyrus* species 3	.458**	.409**
Cichlidogyrus tilapiae - *Enterogyrus* species1	-	.427**
Dolops ranarum - *Neascus* species	.300*	-
nematode larva- *Diplostomum* species	.349*	-
nematode larva- gryporynchid cestode larva	.407**	-
Procamallanus laevionchus - *Paracamallanus cyathopharynx*	-	.327**

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed)

The true species richness of the parasite communities in *O*. *mossambicus* as extrapolated from the cumulative model $Y = a (1-e^{-bx})/b$, generated three curves with a significantly ($p < 0.05$) higher value for the Luphephe-Nwanedi Dams and Flag Boshielo Dam when compared with those of the Return Water Dam (Figure 5.7). The numbers of fish investigated during the present study were sufficient to detect the real species richness at all three sites, because a continuum maximum was reached at a sample size of about 35 individuals at Luphephe-Nwanedi Dams, 40 at Flag Boshielo Dam and 17 at the Return Water Dam (Figure 5.7).

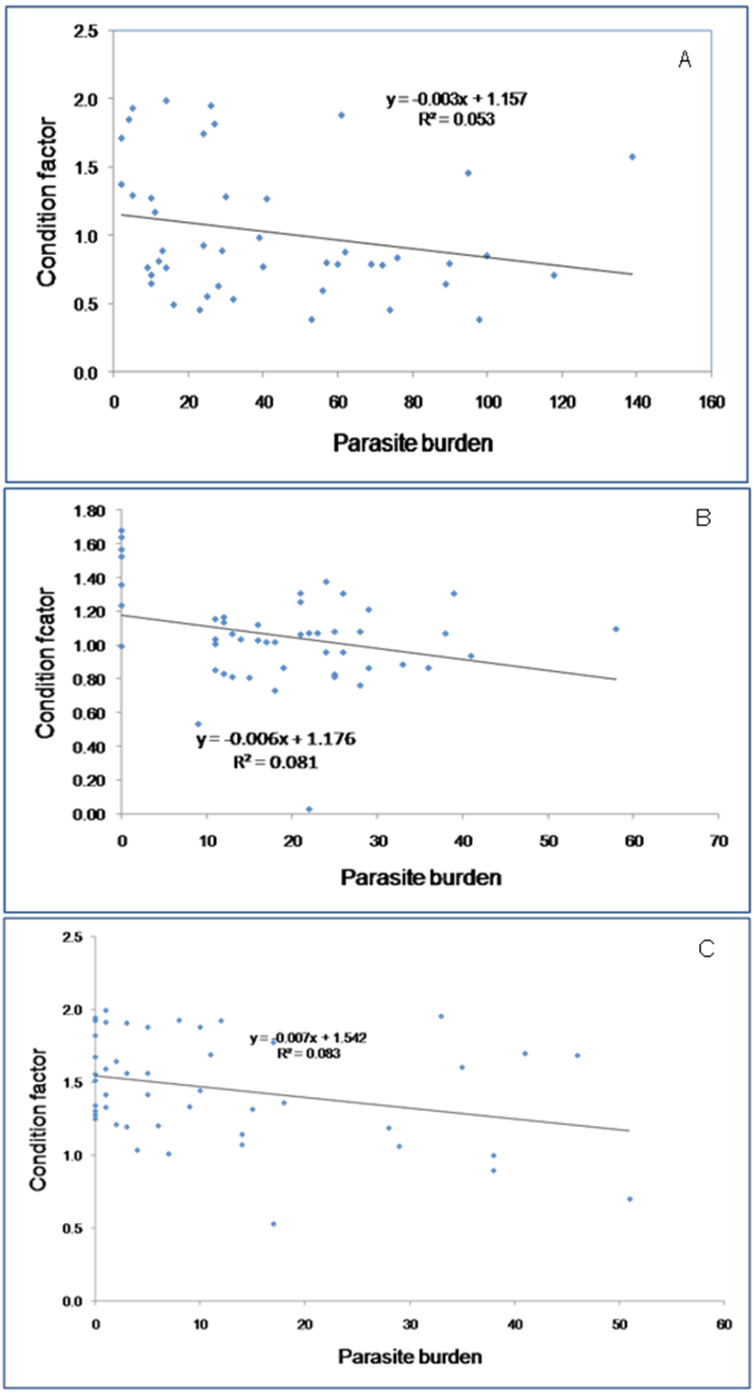


Figure 5.5: Effect of parasite burden on condition factor of *Oreochromis mossambicus*. A = Luphephe-Nwanedi Dams; B = Flag Boshielo Dam; C = Return Water Dam.

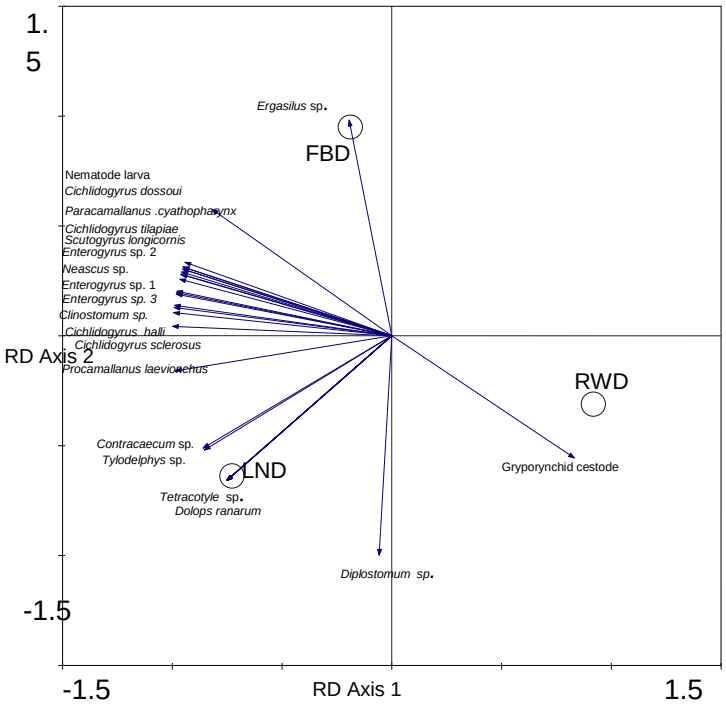
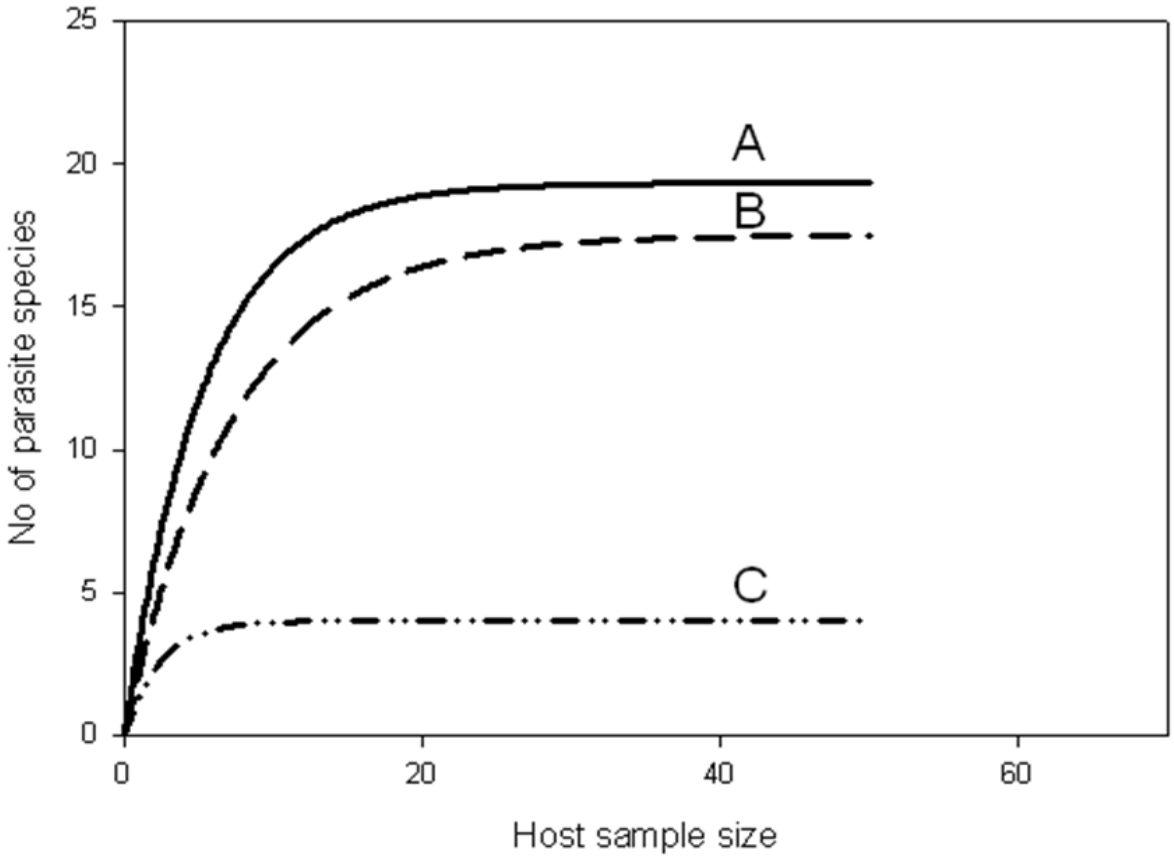


Figure 5.6: Principal Components Analysis (PCA) ordination showing parasite species distribution based on abundance among sites. LND = Luphephe-Nwanedi Dams; FBD = Flag Boshielo Dam; RWD = Return Water Dam.

Seasonal observations over the one year study period revealed some noticeable trends in the prevalence values for the different taxonomic groups of the metazoan parasites of *O. mossambicus* from the Luphephe-Nwanedi Dams and Flag Boshielo Dam. Most parasite groups were conspicuously more prevalent in winter and summer than in autumn and spring (Figures 5.8A & B). However, no discernible trends were observed in parasites from the Return Water Dam (Figure 5.8C).



Sampling site	r^2	R(a/b)	C
LND	0.3	19	35
FBD	0.5	16.5	40
RWD	0.82	2.6	17

Figure 5.7: Total species richness of *Oreochromis mossambicus* in three dams of the Limpopo and Olifants River Systems as a function of the number of hosts examined. Data are plotted according to the exponential species accumulation model proposed by Walther *et al*. (1995), r^2 = regression coefficient; R (a/b) = calculated "true species richness; C = capacity or number of hosts needed to reach "true" species richness.

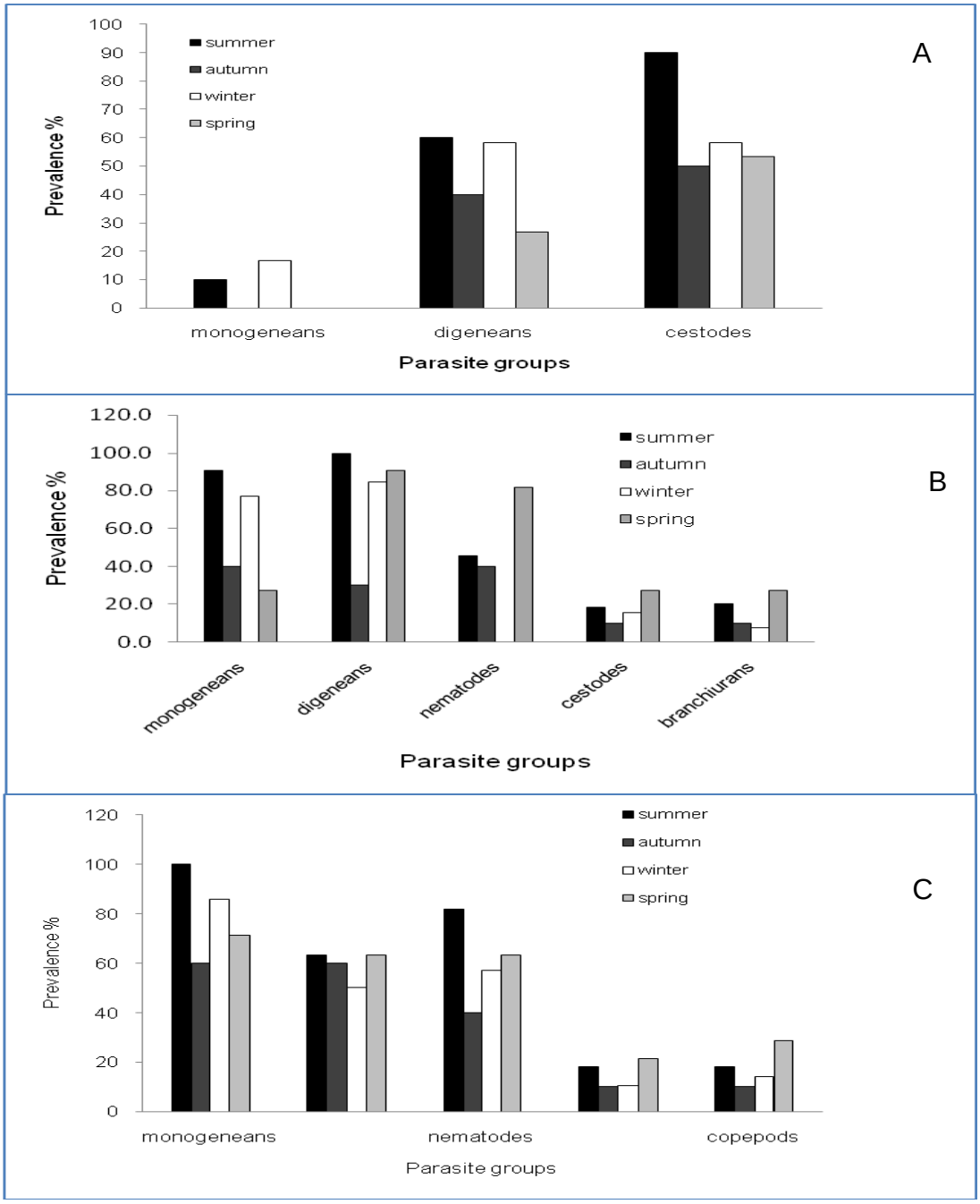


Figure 5.8: Seasonal prevalence of metazoan parasite groups of *Oreochromis mossambicus* sampled from A = Luphephe-Nwanedi Dams, B = Flag Boshielo Dam and C = Return Water Dam.

5.5. Discussion

The results from the current survey have added to knowledge of the distribution and abundance of metazoan parasite infections in *O*. *mossambicus* in the Limpopo and Olifants River Systems. The *Cichlidogyrus* and *Scutogyrus* species are new host records for South Africa (Madanire-Moyo *et al*. 2011). The three *Enterogyrus* species encountered in this study are currently under species descriptions for publication.

The most interesting aspect of the study is the interpretation of the results in terms of metazoan communities in freshwater fish in the Limpopo and Olifants River Systems. The results undoubtedly show some variation in the parasite communities from the cichlid sampled from the three dams. Nineteen species of metazoan parasites were found in tilapia from Luphephe-Nwanedi Dams, 17 species from Flag Boshielo Dam and only four from the Return Water Dam.

Heteroxenous (larval digeneans, nematodes and cestodes) species formed an important component of the parasite infracommunities. Since the population fluctuation of intermediate hosts can influence the occurrence of heteroxenous species (Thoney 1991), the high abundance values of these species suggest the availability of the intermediate hosts as prey and thus important determinants of infection. The feeding habits and the wide spectrum of the cichlid host, bringing it in contact with several potential intermediate hosts of digeneans, nematodes and cestodes, may explain the presence of these

endoparasites. The larval stages of metazoan parasites encountered show the role of the cichlid fish as an intermediate trophic level and also as part of the diet of freshwater birds.

The abundance values for ectoparasites were not influenced by host size whereas the endoparasites showed an opposing trend in which there was an increase in abundance values with increase in host length. No fish less than 6.2 cm (fish younger than six months) were caught because of the fishing gear used. Thus, no data were obtainable to construct a length/prevalence curve to determine the exact age and size when initial infection actually took place. While the mouth brooding habit of *O*. *mossambicus* allows it to nurture its young, it may also make the fry equally vulnerable to ectoparasitic infections. However, Tombi and Bilong Bilong (2004) found a positive correlation between gill parasitic infections and host size and attributed this to increasing gill surface area and water flow through the gill chamber. Bakke *et al*. (2002) observed a negative correlation between the infection levels of *Gyrodactylus salaris* and the age of salmonids and suggested that larger fish become less susceptible to infection as immunity increases with age.

Larval endoparasites are generally long-lived in fish hosts and their numbers correlate with fish length (Guégan *et al*. 1992). Larger and older fish have a bigger surface area providing a greater chance for cercariae to penetrate and older fish have had more time to accumulate parasites. In addition, this

relationship may be strongly influenced by changes in the feeding habits of the fish correlated with the age (Saad-Fares & Combes 1992). According to Kim *et al*. (2002), Mozambique tilapias are opportunistic feeders; with juveniles being mostly omnivorous, while adults mainly feed on detritus. This trait makes older fish more vulnerable to infections by endoparasites. Consequently, higher infection levels of *Neascus* species, *Tetracotyle* species, *Contracaecum* species and the gryporynchid cestode larvae with increasing host size should be expected even though the life spans of these larvae are unknown.

There was no evidence obtained to suggest that any of the infections was associated with significant morbidity or poor condition of the fish host. Several studies have reported the enhancement in host growth and/or condition by infections of parasites. This enhancement in parasitized fish hosts resulted from a parasite-mediated change in fish foraging behaviour (Arnott *et al*. 2000). Parasites use host-derived energy for the maintenance of their vital functions. Consequently, heavily infected individuals must spend more time foraging to attain the same nutritional benefit as less-parasitized and parasitized individuals (Barber *et al*. 2000). The lack of influence of parasites on the condition factor suggests that an increased food intake by infected fish may outweigh a possible parasite-mediated energy loss.

On the other hand, parasites' induced growth may be one of the parasite adaptations to enhance transmission. Previous studies have demonstrated that fish with black spot diseases showed a tendency to occupy the front shoals

(Ward *et al*. 2002), which provides considerably better foraging opportunities than other shoaling positions (Krause *et al*. 1992). The black spot that surround the digenean metacercariae give conspicuous marks that highlight the fish's body in water, presenting an efficient way to attract the attention of bird predators. This corresponds to adaptive parasite-induced manipulation of the host to increase parasite trophic transmission (Lafferty 1992; Lafferty & Morris 1996).

The present study indicated that there were no significant differences in numbers of parasites in male and female hosts, thus confirming the findings of Tombi and Bilong Bilong (2004) and Boungou *et al*. (2008). The independence of infection values with regard to sex of the host evidences that the ecological aspects of the host (occupation of habitat and diet) are similar among females and males. Similar results have been obtained for other freshwater fish (Adams 1986, Janovy & Hardin 1987).

The metazoan parasites analysed presented a spatial aggregate pattern in agreement to the typical patterns of parasites showed by some authors (Skorping 1981, Janovy & Hardin 1987, Oliva *et al*. 1990). According to Anderson and Gordon (1982), this pattern of aggregate dispersion may have originated from (1) patterns of spatial aggregation in the distribution of infectant stages and (2) the differences in susceptibility and capacity of the host's immunological reaction.

The highest infection levels by most parasite groups were recorded in summer and winter. This was probably due to a decrease in water volume during the winter season that caused nutritional imbalances resulting in less production of food. More incidences of diseases and increases in infection levels in fish during winter months were previously reported (Rawson & Rogers 1972a & b; Khidr 1990; Banu *et al*. 1993; Tsotetsi *et al*. 2004). Marx and Avenant-Oldewage (1996) suggested that reduced water levels in winter concentrate parasites and hosts, increasing the probability of infection. Mozambique tilapia aggregate in deep warmer waters with the onset of colder temperatures (Caulton & Hill 1973; Bruton & Bolt 1975) and this behaviour might contribute to the rapid rise in infection at this time of the year.

The high infection levels of heteroxenous species during the summer months may be related to increasing temperatures, which would favour an increase in egg production, rapid development of eggs and rapid growth of parasitic larvae. In tropical waters, Mozambique tilapia continuously breeds throughout the year at temperatures above 20°C (Neil 1966; De Silva & Chandrasoma 1980). However, it would be expected that breeding activities will be optimal during the summer months when environmental conditions are suitable and since the release of fry appears to be associated with cues to rainfall (Bruton & Bolt 1975). The schooling behaviour during the breeding season is an important factor that may possibly result in oncomiracidial larvae easily accessing new hosts. The abundant food availability and favourable temperature in the

summer months may cause this high density of hosts and initiation of egg production, respectively, thus resulting in successful transfer of eggs and oncomiracidia from fish to fish. In addition, the increase in the abundance of the heteroxenous species (digenean, nematode and cestode larvae) observed in summer could be attributed to the rainfall patterns. The rainfall probably led to snail dispersion due to the effects of currents, as has previously been described by Zhou *et al*. (2002). Moreover, inundation can promote snail dispersal into new habitats due to an increase of suitable vegetation. Thus, an accumulation of mollusc intermediate hosts due to inundation could have facilitated the success of the complete life cycle of these parasite species.

The parasite community of *O*. *mossambicus* consisted of eight pairs of ectoparasitic and only three pairs of endoparasitic interspecific associations. The exploitation of hosts by congeneric species reported by Boungou *et al*. (2008) was hereby confirmed. Simultaneous infections and coexistence of parasites are made possible by relatively low infection levels under natural conditions. Such low levels of infection result from the fact that niches are always available on biotopes (Šimková *et al*. 2006). The results are suggestive of the lack of competition among the metazoan parasites since no negative associations occurred. The positive inter-specific associations revealed in the present study may be the result of a similar mechanism of infection *in situ* (Lo *et al*. 2001). Additionally, one species may weaken a host and so make it more susceptible to infection with another species or one species may “prepare” the microhabitat for

a second species, for instance, by making it easier for the latter species to feed (Hayward *et al*. 1998).

In the Luphephe-Nwanedi Dams and the Flag Boshielo Dam, *O*. *mossambicus* was infected by monogenean parasites throughout the entire year. All monogenean species found on *O. mossambicus* had low mean intensity and abundance values. Obiekezie (1991) and Luus-Powell *et al*. (2009) also noted that in wild fish populations, monogeneans occurred at low intensities and in apparent equilibrium with their hosts, whereas under cultured conditions, these pathogens may build up heavy worm burdens, which provoke epizootics. However, the recruitment of these parasites, though relatively weak, was certainly continuous. This is probably because the success of the “simple” monogenean is highly evolved and suitably adapted to enable these ectoparasites to locate, invade and establish on wild fish hosts.

For captive fish, the direct life cycle is at best a nuisance and at worst, can kill valuable stock (Luus-Powell *et al*. 2006). The struggle against direct life cycle parasites like Monogenea can be achieved by treatment of the host (Osman *et al*. 2008) or by breaking the life cycle. The latter can be achieved by washing basins periodically to reduce the eggs and infective stages (oncomiracidia). Fish genitors, even from the wild, must be disinfected before they are put in basins. Traditional treatments by chemicals and drugs, although effective, unavoidably cause problems related to environmental pollution, drug resistance and health issues. Alternative methods that are environmentally and ecologically sustainable

such as saline treatment should also be developed and more widely applied. Reaching this long-term objective will depend on the cooperation of and contributions from fish parasitologists and fish farmers.

CHAPTER 6

THE EFFECTS OF POLLUTANTS ON PARASITE COMMUNITIES OF *CLARIAS GARIEPINUS* AND *OREOCHROMIS MOSSAMBICUS*

6.1. Introduction

Virtually all free-living organisms are hosts to parasites, and parasitism, in its broadest sense, is considered to be the most common lifestyle on earth (Price 1980). Parasites usually exist in equilibrium with their hosts as a survival strategy (Bush *et al*. 2001). Thus, healthy ecosystems can hardly be considered parasite free. Nevertheless, in instances where the hosts are overcrowded, such as in fish farms, parasitic diseases can spread very rapidly and cause gross mortalities (Paperna 1996). This is not usually the case in the natural aquatic environment unless it is disturbed by human interference such as pollution, which can alter the natural distribution of parasite communities and infracommunities (Bush *et al*. 2001).

The fact that some parasites possess complex life cycles makes them extremely valuable information units about environmental conditions. Their presence/absence tells us a great deal about their host ecology, food web interactions, biodiversity and environmental stress (Overstreet 1997; Marcogliese, 2003, 2004). Combining different species based on shared patterns of transmission provides a potentially more powerful indicator of prevailing environmental conditions. Hudson *et al*. (2006) argued that many ecological factors affecting fish parasite life cycles and the complexity of aquatic food webs

are important in determining ecosystem health, while Blanar *et al*. (2009) investigated associations and responses of specific fish-parasitic taxa to different contaminants to come up with a quantitative approach that will be important in studying parasite - host - pollution interactions.

Each parasite species reflects the presence of different organisms that participate in its life cycle; together, all parasite species in a host reflect the presence of a plethora of host organisms and trophic interactions in the environment (Marcogliese 2003). Thus, parasites potentially may be used as surrogate indicators of species diversity and ecosystem diversity, two of the three important levels of biodiversity cited in the Rio Convention on Biological Diversity (Marcogliese 2003). Given that pollution may have impacts on populations and communities of organisms, and thus on food web structure, parasites may thus be used as natural biological tags of ecosystem health (Blanar *et al*. 2009).

Numerous investigators have examined the effects of environmental stress on single species of parasites in temperate aquatic systems (e.g. Khan & Thulin 1991; Overstreet 1993; MacKenzie *et al*. 1995; Williams & MacKenzie 2003; Marcogliese 2004; Sures 2004), despite the fact that it is difficult to predict the direction of effects of pollution impacts on parasite communities. Most studies document changes in some aspect of the parasite fauna, and it is clear that pollution has effects on parasite populations and communities and is often associated with a reduction in species richness of parasites (Marcogliese 2004). There are few basic approaches employed for the use of parasites as

bioindicators of environmental degradation, which are identical with those applied for free living invertebrates (Kennedy 1997) and for fishes (Fausch *et al*. 1990): The use of community data, especially diversity indices to assess environmental health. The underlying hypothesis is that parasite diversity is highest in unpolluted waters, whereas pollution stress leads to a loss of species, change in dominance and reduction in diversity. The calculation of diversity indices takes into account species richness and the abundance of each species. The advantage of this approach is that knowledge on the identity of a species, its biology, or susceptibility to pollutants is not required. A change in the index thus can indicate a change in water quality, but it cannot provide any information on the nature of the change or on the identity of the pollutant.

(a) The use of multivariate analysis to assess pollution. This technique incorporates a number of factors, but has the disadvantage of requiring more expertise in calculation and especially in interpretation. At present biotic indices are still favoured for their low cost and simplicity (Kennedy 1997).

To make a general assessment of the Limpopo and Olifants Rivers inland water pollution and to estimate the environmental health of this large territory of the Limpopo Province, the average water quality of three dams was computed. Most of the environmental variability was covered including types of human settlement and land uses. The water quality was evaluated on the basis of some

basic variables (dissolved oxygen, pH, total dissolved solids, conductivity, turbidity, salinity, total alkalinity, nitrate, nitrite, sulphate, orthophosphate, ammonia) and other complementary parameters (chloride, calcium, magnesium, sodium, lead and zinc).

The study intended to ascertain the relationship between metazoan parasites and pollution levels in the catchment. The specific objectives of this chapter were to determine the biodiversity of metazoan parasite communities of *Clarias gariepinus* and *Oreochromis mossambicus* in the Limpopo River catchment and to compare the diversity and distribution of metazoan parasite fauna along defined pollution gradients in the Limpopo catchment and assess the suitability of using parasites as predictors of environmental change.

6.2. Materials and Methods

During summer, autumn, winter and spring of the years 2008 and 2010, three dams of the Olifants and Limpopo River Systems were sampled for fish, where a total of 271 fish (131 *C*. *gariepinus* and 140 *O*. *mossambicus*) were caught for parasitological examinations. Details of sampling procedure, study area, examination of fish hosts and ecological terminology for the description of parasite populations are given in Chapter 2.

6.3. Data Analyses

Environmental variables were logarithmically transformed ($\log_{x+1}$) to normalize the data while parasite community data were z- transformed to suit the multivariate analyses techniques used. To get a first view on possible relations between environmental variables and to determine the pattern of distribution of parasite species among the sites, centred and standardized Principal Component Analyses (PCA) were performed. Detrended Correspondence Analysis (DCA) results confirmed the nature of the data as linear (Lepš & Šmilauer 2003), therefore Redundancy Analysis (RDA) with significance at $p < 0.005$ was used to examine relationships among the abundance of parasite species and environmental characteristics of the reservoirs (Legendre & Legendre, 1998). Forward selection (999 Monte Carlo permutations) was done to identify significant environmental variables. Spearman-rank correlation tests were used to evaluate between environmental variables. Ordination was done using CANOCO version 4.5 (Ter Braak & Smilauer, 1998), and STATISTICA version 7 (STATSOFT Inc., Tulsa, Oklahoma, USA) was used for correlation, regression and correspondence analysis. Hierarchical cluster analysis was used to assess the similarities in species composition (based on parasite abundance data) among sampling sites and seasons based on the parasite abundance data. Paleontological Statistics (PAST) Software Package for Education and Data Analyses (Hammer & Harper 2001) was used to analyse the data.

6.4. Results

6.4.1. Water Quality

The mean values of environmental variables in the three dams are shown in Table 3.2. The pH readings at Luphephe-Nwanedi Dams and the Return Water Dam ranged between 6.8 and 7.6. These data at these two sites indicate that the pH values do not vary much and the pH trend is neutral (Table 3.2). The pH in Flag Boshielo Dam shows more variability than the other two dams, with the minimum value being lower and not within expectations for good water quality (DWAF 1996; Table 3.2).

The temperature varied with seasons and the time of day of sampling. It ranged between 24.4 and 27.8^{0}C during the hot seasons and between 14.1 and 15.4^{0}C during the cold months of June and July (Table 3.2). Dissolved oxygen (DO) levels were relatively high at Flag Boshielo Dam (7.8 ± 1.6 mg/l), slightly lower at Luphephe-Nwanedi Dams (7 ± 0.4 mg/l) and on average, were within acceptable limits of the South African Target Water Quality Range (TWQR) for aquatic life. However, the mean DO level at Return Water Dam was quite low (5.4 mg/l) and was not within the acceptable limits for aquatic lifeas defined by DWAF (1996; Table 3.2). Conductivity was lowest at Luphephe-Nwanedi Dam ranging between 7.5 - 9.4 mS/m, but was elevated at Flag Boshielo Dam and the Return Water Dam with mean values of 42.5 mS/m and 174.3 mS/m, respectively.

The nutrient (ammonia, ortho-phosphates, sulphates and potassium) levels of the three dams differed notably and showed a similar increasing trend in the order, Luphephe-Nwanedi Dams < Flag Boshielo Dam < Return Water Dam (Table 3.2). The Return Water Dam exhibited far greater levels of ammonia, nitrate, nitrite, ortho-phosphates and sulphates, all of which were above the South African TWQR for aquatic life. Sulphate levels at Luphephe-Nwanedi Dams ranged from 5-6 mg/l.

Aluminium, iron and lead showed elevated values at Flag Boshielo Dam and these three toxic heavy metals were above South African TWQR limits (Table 3.2). The average level for aluminium and lead were also high and above the TWQR (0.1 mg/l) at the Return Water Dam. The levels of arsenic, copper, manganese and zinc remained within the acceptable limits of the TWQR throughout the sampling period and at all the sampling sites (Table 3.2). Calcium levels ranged from 4 to 97 mg/l and magnesium ranged from 2 to 43 mg/l at the three sites. Chloride values showed a similar trend, with high values at the Return Water Dam and lowest values at Luphephe-Nwanedi Dams, but all were within the TWQR limits. Sodium levels were lowest at Flag Boshielo Dam (5.4mg/l) and highest at the Return Water Dam (272 mg/l).

The results of the PCA analysis of environmental variables in the three dams are presented in Figure 6.1. The first two axes explained 32.7% of total variation of the environmental characteristics. The Return Water Dam was significantly associated with increasing gradients of nutrients (nitrite, nitrate,

sulphate, ortho-phosphate, and ammonia), inorganic constituents (magnesium, calcium, potassium, chloride, sodium, total dissolved solids, conductivity and turbidity). The dam also had low values of DO and pH (Figure 6.1).

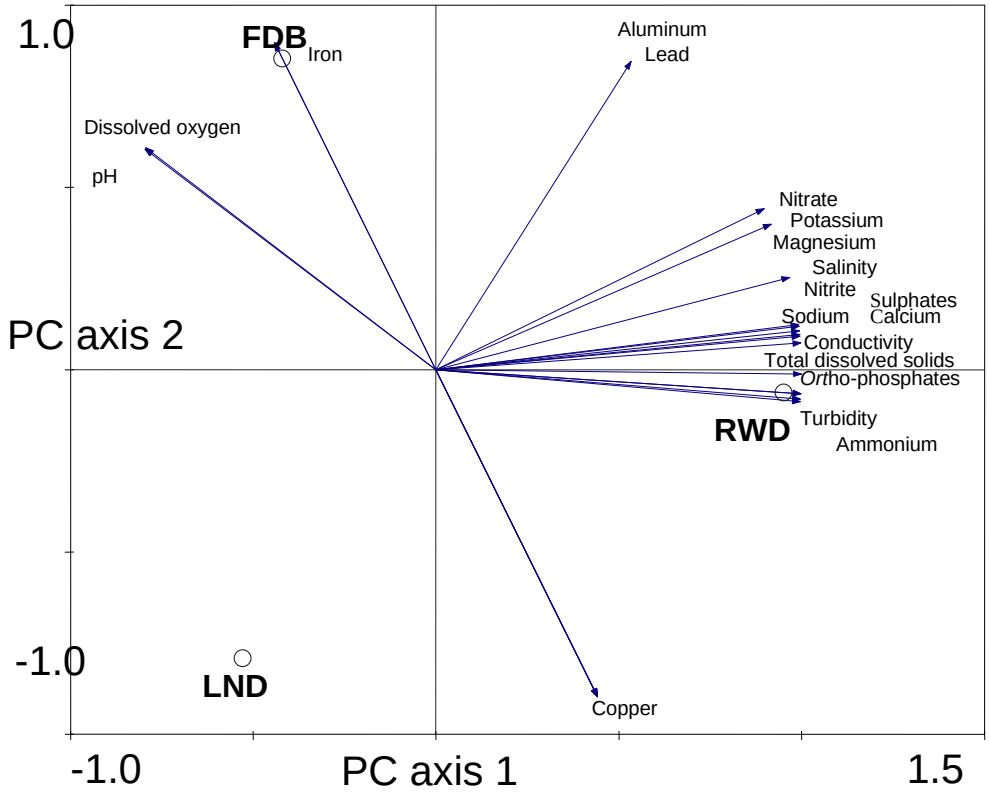


Figure 6.1: A Principal Components Analysis (PCA) ordination biplot showing limnological differences among the three sampling sites. LND = Luphephe-Nwanedi Dams, FBD = Flag Boshielo Dam and RWD = Return Water Dam.

Flag Boshielo Dam was identified with increasing iron levels while both Luphephe-Nwanedi Dam and the Return Water Dam shared some similarities

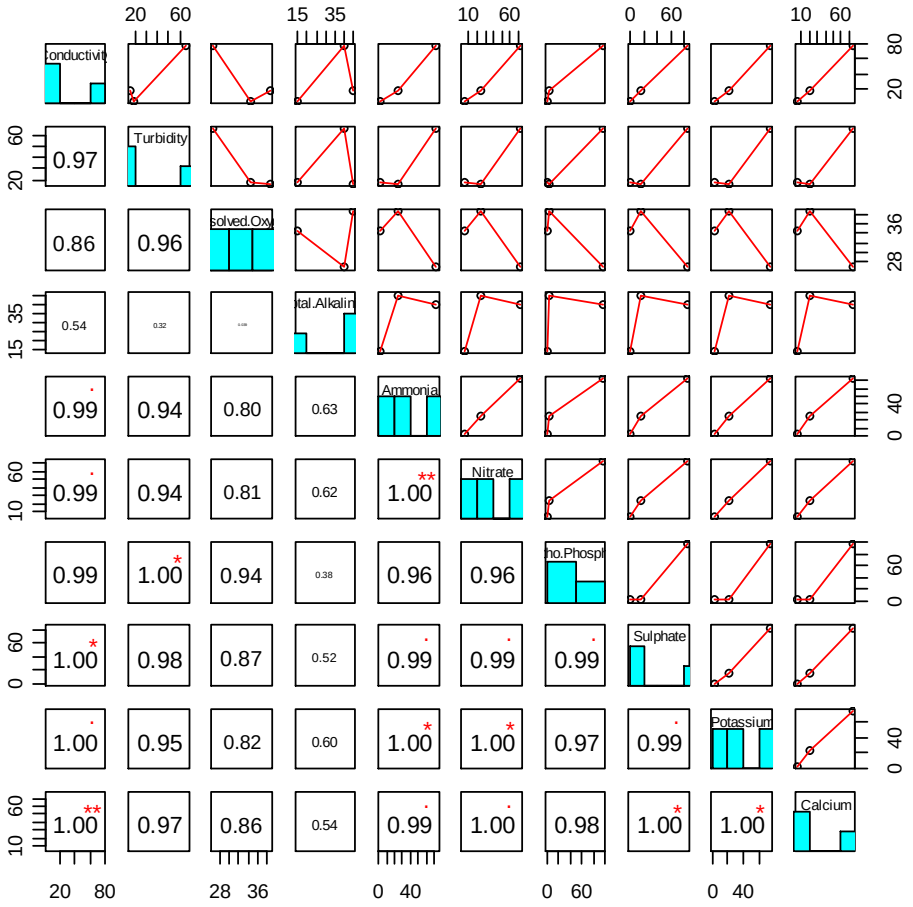


Figure 6.2: Correlation matrix for water quality variable.

such as increasing lead and aluminium levels (Figure 6.1). It was apparent that there were correlations between some of the variables, allowing for the reduction of variables used in subsequent analyses. Nutrients (nitrates, nitrite, ammonium and orthophosphates) displayed multicollinearity ($p < 0.0001$; $r > 0.5$; Figure 6.2). Conductivity, turbidity, dissolved oxygen, potassium and calcium also showed significant ($p < 0.0001$; $r > 0.5$) correlations with the aforementioned nutrients (Figure 6.2).

6.4.2. Parasite species composition, diversity and distribution

A total of 271 fish (131 *C*. *gariepinus* and 140 *O*. *mossambicus*) were caught from the three sampling sites, from which 18 224 parasites were recovered. Thirty-three parasite taxa were identified from *C*. *gariepinus* and *O*. *mossambicus* each with 19 and 20 species, respectively (Tables 6.1a & b). Seven parasite taxa were common in both fish: *Contracaeum* species, unidentified nematode larvae, *Paracamallanus cyathopharynx*, *Procamallanus laevionchus*, *Tetracotyle* species, *Dolops ranarum* and *Diplostomum* type 3 (Tables 6.1a & b; 6.2a & b). The cestodes (*Tetracampos ciliotheca* and the gryporynchid larva); the nematode (*Contracaecum* species); the digenean (*Diplostomum* type 3) and the monogeneans (*Cichlidogyrus halli* and *Gyrodactylus rysavyi*) were present at all the sampling sites (Tables 6.1a & b; 6.2a & b). Thirty-one species of parasites were recovered from Luphephe-Nwanedi Dams compared to 23 at Flag Boshielo Dam and only seven at the Return Water Dam (Table 6.3).

The mean intensity values for most parasite species remained very low, with the exception of *Contracaecum* species (181.5), *Acanthostomum* species (96.5), *Tetracotyle* species (92.6), *Diplostomum* species (35.4) and *Neascus* species (30.2). The monogeneans (*Macrogyrodactylus clarii*, *M*. *congolensis*,

Table 6.1a: List of metazoan parasite species of *Clarias gariepinus* with their main eco-parasitological characteristics.

	Luphephe-Nwanedi			Flag Boshielo			Return Water		
Parasite Taxa	I (min-max)	A	P	I (min-max)	A	P	I (min-max)	A	P
Gyrodactylus rysavyi	2.2(1-4)	0.3	13.3	3.4(1-3)	0.4	12.2	1.2(1-2)	0.1	11.1
Macrogyrodactylus clarii	1.7(1-3)	0.4	26.7	2.9(1-3)	0.8	26.8	-	-	-
Macrogyrodactylus congolensis	2.4(1-4)	0.4	17.8	3.0(1-2)	1.0	34.1	-	-	-
Quadriacanthus clariadis	1.9(1-3)	0.3	17.8	-	-	-	-	-	-
Quadriacanthus aegypticus	1.6(1-2)	0.3	20.0	-	-	-	-	-	-
Diplostomum type 3 species	35.4(1-102)	14.9	42.2	5.6(1-9)	1.2	22	16.4(3-45)	6.9	42.2
Tetracotyle species	92.6(1-250)	37.0	40.0	-	-	-	-	-	-
Acanthostomum species	96.5(1-286)	12.9	13.3	-	-	-	-	-	-
Glossidium pedatum	13.2(1-44)	5.6	42.2	5.6(3-10)	1.2	22.0	-	-	-
Contracaecum species	96.2(1-250)	96.2	100.0	181.5(20-560)	75.3	41.5	90.9(25-200)	68.7	75.6
nematode larva	-	-	-	2.7(1-5)	0.5	17.1	-	-	-
Paracamallanus cyathopharynx	3.1(1-8)	0.9	28.9	3.6(1-5)	0.4	12.2	-	-	-
Procamallanus laevionchus	-	-	-	2.2(1-3)	0.3	14.6	-	-	-
Proteocephalus glanduligerus	2.2(1-3)	0.2	11.1	-	-	-	-	-	-
Tetracampos ciliotheca	1.2(1-2)	0.1	11.1	2.4(1-2)	0.8	31.7	2.8(1-5)	0.5	17.8
Chonopeltis inermis	1.2(1-2)	0.2	13.3	-	-	-	-	-	-
Dolops ranarum	5.1(1-20)	1.7	33.3	-	-	-	-	-	-
Lamproglena clariae	2.9(1-4)	0.4	15.6	6(1-3)	2.2	36.6	-	-	-
Leech	1.4(1-2)	0.2	11.1	-	-	-	-	-	-

I = Intensity, A = Abundance and P = Prevalence %).

Table 6.1b: List of metazoan parasite species of *Oreochromis mossambicus* with their main eco-parasitological characteristics.

	Luphephe-Nwanedi			Flag Boshielo			Return Water		
Parasites from *O. mossambicus*	I (min-max)	A	P	I (min-max)	A	P	I (min-max)	A	P
Cichlidogyrus halli	7.5(1-15)	4.5	60.0	5.3(1-11)	4.0	72.9	1(1)	0.1	6.4
Cichlidogyrus sclerosus	10.7(1-7)	1.4	13.3	3.4(1-7)	1.3	37.5	-	-	-
Cichlidogyrus dossoui	7.9(1-6)	1.2	15.6	2.9(1-5)	1.0	35.4	-	-	-
Scutogyrus longicornis	5.6(1-5)	1.0	17.8	2.2(1-3)	0.8	35.4	-	-	-
Cichlidogyrus tilapiae	3.6(1-5)	0.7	20.0	2.5(1-4)	0.6	22.9	-	-	-
Enterogyrus species1	11.7(2-25)	5.7	48.9	8.1(2-14)	2.4	29.2	-	-	-
Enterogyrus species 2	6.7(2-8)	1.0	15.6	4.8(2-6)	0.9	18.8	-	-	-
Enterogyrus species 3	6.1(2-10)	1.0	15.6	4.8(2-9)	0.4	8.3	-	-	-
Neascus sp.	30.2(4-80)	6.0	20.0	17.6(10-32)	2.6	14.6	-	-	-
Tylodelphys species	11.3(2-55)	2.8	24.4	4.6(1-10)	1.1	25.0	-	-	-
Diplostomum species	8.2(1-25)	3.3	40.0	-	-	-	4.8(1-15)	1.8	38.3
Tetracotyle species	49.8(23-80)	6.6	13.3	-	-	-	-	-	-
Clinostomum species	3.5(1-8)	0.8	22.2	2.8(2-4)	0.5	18.8	1.5(1-2)	0.1	8.5
nematode (unidentified)	2.6(1-3)	0.3	11.1	2.1(1-2)	0.4	20.8	-	-	-
Contracaecum species	24.2(2-50)	5.9	24.4	3.0(1-5)	0.8	25.0	-	-	-
Paracamallanus cyathopharynx	3.6(2-8)	0.4	11.1	4.1(1-5)	0.6	14.6	-	-	-
Procamallanus laevionchus	6.0(2-13)	0.7	11.1	2.7(2-3)	0.3	12.5	-	-	-
gryporynchid larva	10.5(1-27)	1.9	17.8	5.0(2-9)	0.9	18.8	15.5(1-45)	9.6	61.7
Dolops ranarum	2.8(1-4)	0.4	13.3	-	-	-	-	-	-
Ergasilus species	-	-	-	3.2(2-3)	0.4	12.5	-	-	-

I = Intensity, A = Abundance and P = Prevalence (%).

Quadriacanthus clariadis, *Q*. *aegypticus*, *Cichlidogyrus sclerosus*, *C*. *dossoui*, *Scutogyrus longicornis*, C*. tilapiae*, *Enterogyrus* species 1, *Enterogyrus* species 2 and *Enterogyrus* species 3); the digeneans (*Neascus* species, *Clinostomum* species, *Glossidium pedatum*, *Tetracotyle* species); the nematodes (*Paracamallanus cyathopharynx*, *P*. *laevionchus* and the unidentified nematode larva) were not present in fish sampled from the Return Water Dam (Tables 6.1a & b). The copepod, *Ergasilus* species was only found at Flag Boshielo Dam

(Table 6.1b). The monogeneans (*Quadriacanthus clariadis* and *Quadriacanthus aegypticus*); the digeneans (*Tylodelphys* species and *Acanthostomum* species), the cestode (*Proteocephalus glanduligerus*) and the crustaceans (*Dolops ranarum*, and *Chonopeltis inermis*) and the unidentified hirudinean leech were unique to Luphephe Nwanedi Dams (Table 6.1a).

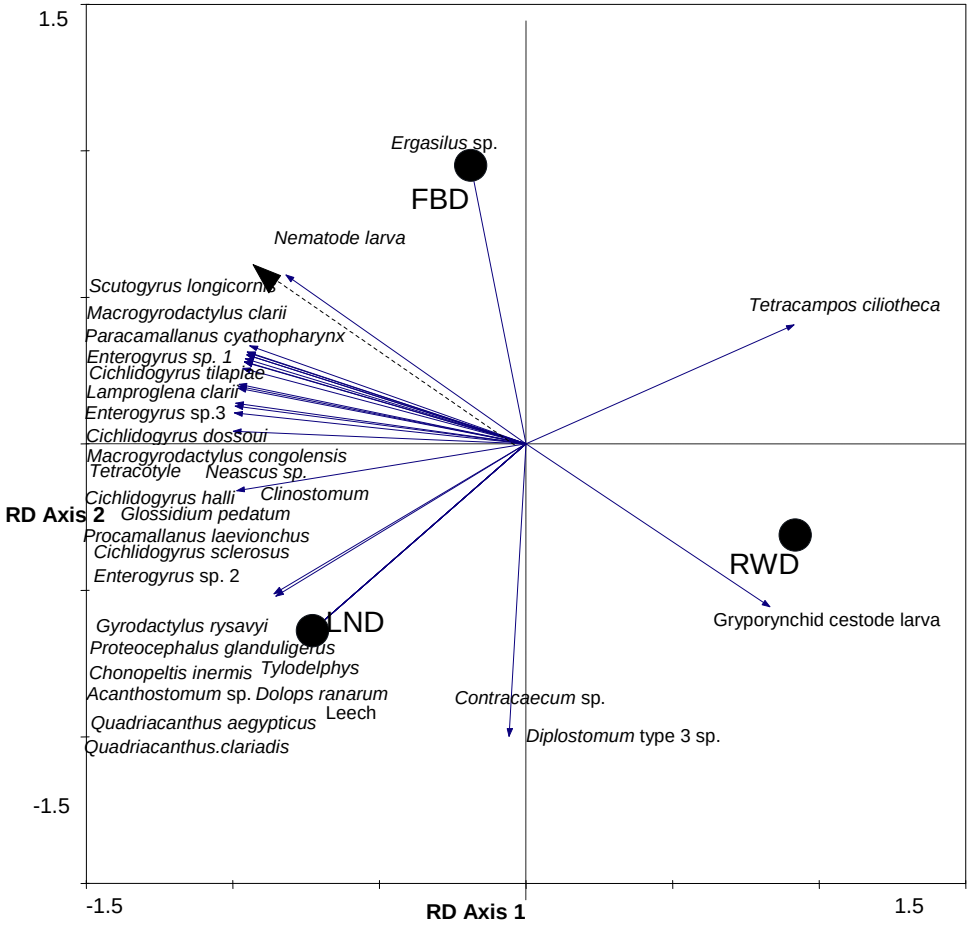


Figure 6.3: A Principal Components Analysis (PCA) biplot showing the associations between fish parasite species and the three sampling sites. (LND = Luphephe-Nwanedi Dams, FBD = Flag Boshielo Dam and RWD = Return Water Dam).

Table 6.2a: Number and dominance (%) of parasites collected from *Clarias gariepinus* sampled from three dams of the Limpopo and Olifants River Systems. Taxa in bold were found at all the three sites.

Taxa	LND	FBD	RWD	Total	%	Rank
Gyrodactylus rysavyi	**13**	**7**	**6**	**26**	**0.16**	**12**
Macrogyrodactylus clarii	20	13	-	33	0.21	11
Macrogyrodactylus congolensis	19	15	-	34	0.22	10
Quadriacanthus clariadis	15	-	-	15	0.1	15
Quadriacanthus aegypticus	14	-	-	14	0.09	16
***Diplostomum* type 3**	**672**	**50**	**311**	**1033**	**6.55**	**3**
Tetracotyle sp.	1667	-	-	1667	10.58	2
Acanthostomum sp.	579	-	-	579	3.67	4
Glossidium pedatum	251	50	-	301	1.91	5
***Contracaecum* sp.**	**4331**	**3375**	**3091**	**10797**	**68.5**	**1**
nematode larva (unidentified)	-	19	-	19	0.12	13
Paracamallanus cyathopharynx	40	18	-	58	0.37	7
Procamallanus laevionchus	-	13	-	13	0.08	14
Proteocephalus glanduligerus	11	-	-	11	0.07	17
Tetracampos ciliotheca	**6**	**15**	**22**	**43**	**0.27**	**8**
Chonopeltis inermis	7	-	-	7	0.04	19
Dolops ranarum	76	-	-	76	0.48	6
Lamproglena clariae	20	21	-	41	0.26	9
Leech	7	-	-	7	0.04	19
Total	7748	3596	3430	14874		

LND = Luphephe-Nwanedi Dams; FBD = Flag Boshielo Dam; RWD = Return Water Dam

The biplot scatterplot of the first two axes resulting from the PCA provides an insight into the parasite species association with each sampling site (Figure 6.3). The Return Water Dam was closely associated with the gryporynchid cestode larva while *Ergasilus* species was strongly associated with Flag Boshielo Dam. *Tetracampos ciliotheca* was common in Flag Boshielo Dam and the Return

Water Dam. *Diplostomum* type 3 species and *Contracaecum* larva were associated with the Return Water Dam and Luphephe-Nwanedi Dams (Figure 6.3).

Table 6.2b: Number and dominance (%) of parasite taxa collected from *Oreochromis mossambicus* by site. sampled from three dams of the Limpopo and Olifants River Systems. Taxa in bold were found at all the three sites.

Taxa	LND	FBD	RWD	Total	%	Rank
Cichlidogyrus halli	**150**	**192**	**3**	**345**	**10.3**	**5**
Cichlidogyrus sclerosus	21	61	-	82	2.4	9
Cichlidogyrus dossoui	24	50	-	74	2.2	11
Scutogyrus longicornis	27	37	-	64	1.9	13
Cichlidogyrus tilapiae	32	28	-	60	1.8	15
Enterogyrus species 1	257	113	-	370	11.0	3
Enterogyrus species 2	35	43	-	78	2.3	10
Enterogyrus species 3	43	19	-	62	1.9	13
Neascus sp.	272	123	-	395	11.8	2
Acanthostomum sp.	124	55	-	124	3.7	8
Diplostomum type 3	147	-	86	233	7.0	7
Tetracotyle species	299	-	-	354	10.6	4
***Clinostomum* species**	**35**	**25**	**6**	**66**	**2.0**	**12**
nematode (unidentified)	13	21	-	34	1.0	18
Contracaecum species	266	36	-	302	9.0	6
Paracamallanus cyathopharynx	18	29	-	47	1.4	16
Procamallanus laevionchus	30	16	-	46	1.4	16
gryporynchid larva	**84**	**45**	**449**	**578**	**17.3**	**1**
Dolops ranarum	17	-	-	17	0.5	20
Ergasilus species	-	19	-	19	0.6	19
Total	1894	912	544	3350		

LND = Luphephe-Nwanedi Dams; FBD = Flag Boshielo Dam; RWD = Return Water Dam

Ranking of the total number of parasites from *C*. *gariepinus* (as dominance %) indicates that the eight most abundant species comprised 98.6% of the individuals found (Table 6.2a). The rest of the taxa comprised the last 1.4% of individuals found, yet these represented more than half of the taxa identified. Ranking of the total number of parasites from *O. mossambicus* (as dominance %) indicates that the eight most dominant species comprised 80.7% of the individuals found (Table 6.2b). The remaining 12 taxa comprised the last 19.3% of individuals found (Table 6.2b).

Mean parasite species richness and diversity indices (Species richness (S), Shannon-Wiener (H), Evenness of Shannon-Wiener (H') and Margalef (D)) showed an increase from the polluted site (Return Water Dam) to the reference site (Luphephe-Nwanedi Dams). The monoxenous and heteroxenous parasite species corroborated the general pattern seen for diversity indices while the H/Msp ratio displayed a dissimilar trend in which values were highest at the polluted site and lowest at the reference site (Table 6.3). The dominant species were *Contracaecum* species larvae in Luphephe-Nwanedi Dams and Return Water Dam while the gryporynchid cestode larva was the dominant species in Return Water Dam (Table 6.3).

The results of the RDA analysis (with forward selection) of the effects of environmental variables on parasite abundance are presented in Figure 6.4. Nine environmental variables were ultimately selected from a larger set during a forward selection process whereby those variables showing some relationship

with parasite abundance were identified (Figure 6.4). These were DO, nutrients (ammonia, nitrites, ortho-phosphates) and some pollution associated with mining and agricultural activities (conductivity, turbidity, sulphates, iron, aluminium). Although none of these variables was significantly explaining the parasite abundance (Monte Carlo permutations, n = 999, $p > 0.05$), there was an underlying pattern between variables and parasites (Figure 6.4). All the aforementioned environmental variables (except dissolved oxygen) were positively associated with increasing abundance of the cestodes, *T*. *ciliotheca* and the gryporynchid larva but negatively associated with the abundance of most parasite species. Dissolved oxygen was positively associated with most parasite species but negatively associated with the cestode species (Figure 6.4).

The proportional abundance of the seven main taxonomic groups collected during the sampling period was calculated (Figures 6.5a & b). Branchiuran and hirudinean taxa were unique to Luphephe-Nwanedi Dams, while the copepods (*L. clariae* from *C*. *gariepinus* and *Ergasilus* species from *O*. *mossambicus*) were more abundant at Flag Boshielo Dam. Cestodes and nematodes were cosmopolitan species while monogeneans and digeneans were most abundant at the Luphephe-Nwanedi Dams (Figures 6.5a & b).

The dendrogram based on metazoan parasite abundance reinforce the differences in species composition between the three dams. The Return Water Dam samples were clearly separated from the other two sites based on the parasite species occurrence, and their component communities were the most

dissimilar from the others (Figure 6.6). The four seasonal samples from the Return Water Dam were clustered together because they had a more impoverished parasitic fauna demonstrating the absence of seasonal effect on the abundance levels of parasites throughout the sampling period (Figure 6.6).

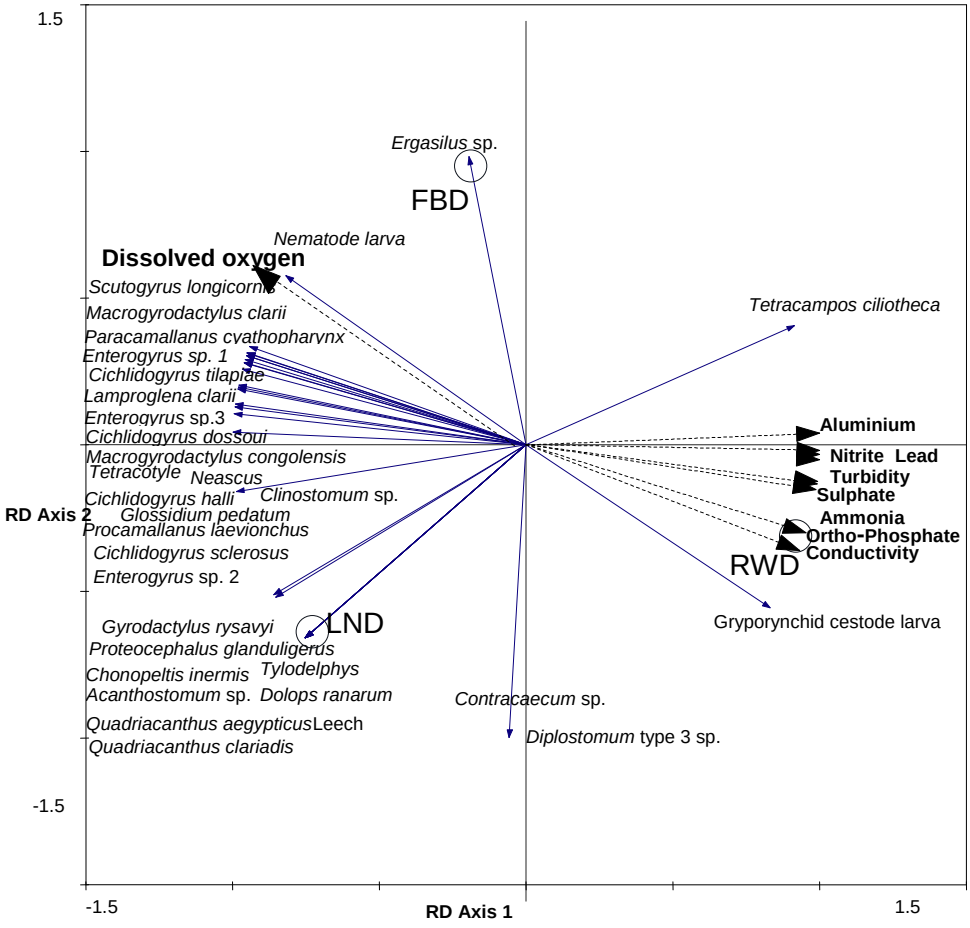


Figure 6.4: A Redundancy Analysis (RDA) biplot showing the influence of significant environmental variables on parasite abundance values in the three dams of the Limpopo and Olifants River Systems.

The summer and winter samples at Luphephe-Nwanedi Dams possessed metazoan communities with a greater level of similarity because they had greater parasite abundance when compared with the autumn and spring samples (Figure 6.6). The same trend was observed for Flag Boshielo Dams samples. This pattern of similarity also appears to be caused by the patterns of the physicochemical variable measurements obtained (Figures 6.1, 6.2 & 6.4).

Table 6.3: Comparison of the parasitological parameters of *Clarias gariepinus* and *Oreochromis mossambicus* sampled from the three dams of the Limpopo and Olifants River Systems. For diversity measurements, mean values ± standard deviation are given, which were calculated from specimens collected in the four seasonal surveys.

	Luphephe-Nwanedi Dam	Flag Boshielo Dam	Return Water Dam
Parameter	Values mean ± standard deviation	Values mean ± standard deviation	Values mean ± standard deviation
Total no. of fish	90	89	92
Species Richness (S)	31	23	7
Shannon Wiener index (H)	2.3 ± 0.14	1.64 ± 0.11	0.95 ± 0.09
Evenness of Shannon-Wiener (H')	0.63 ± 0.03	0.47 ± 0.01	0.24 ± 0.02
Margalef (D)	2.1 ± 0.18	1.4 ± 0.14	0.03 ± 0.15
Dominant species	*Contracaecum* species	*Contracaecum* species	gryporynchid larva
% of infracommunities dominated	44.9%	74.8%	77.8%
Heteroxenous species (Hsp)	13	10	5
Monoxenous species (Msp)	18	13	2
Hsp/Msp	13:18	10:13	5:2

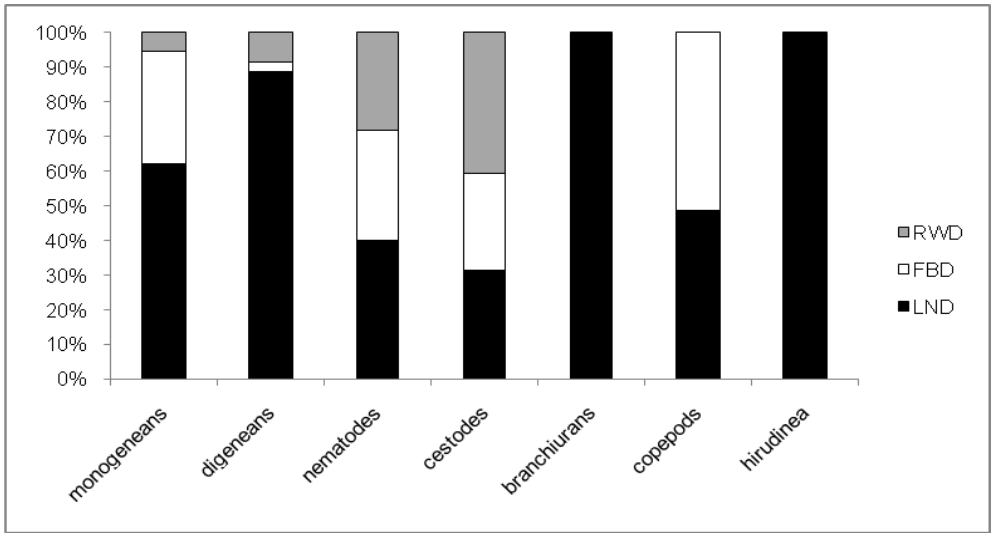


Figure 6.5a: Relative abundance of parasite groups in *Clarias gariepinus* and *Oreochromis mossambicus* by site. RWD= Return Water Dam, FBD = Flag Boshielo Dam, LND = Luphephe-Nwanedi Dams.

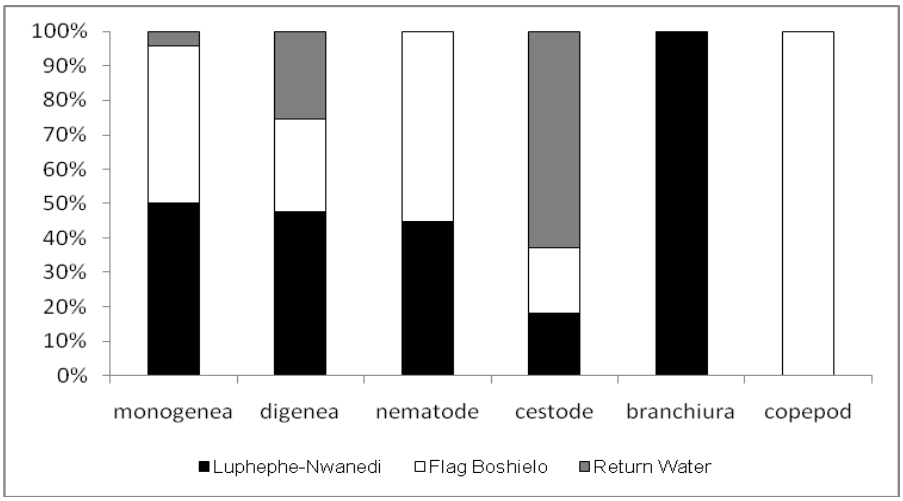


Figure 6.5b: Relative abundance of parasite groups in *Oreochromis mossambicus* by site. RWD= Return Water Dam, FBD = Flag Boshielo Dam, LND = Luphephe-Nwanedi Dams.

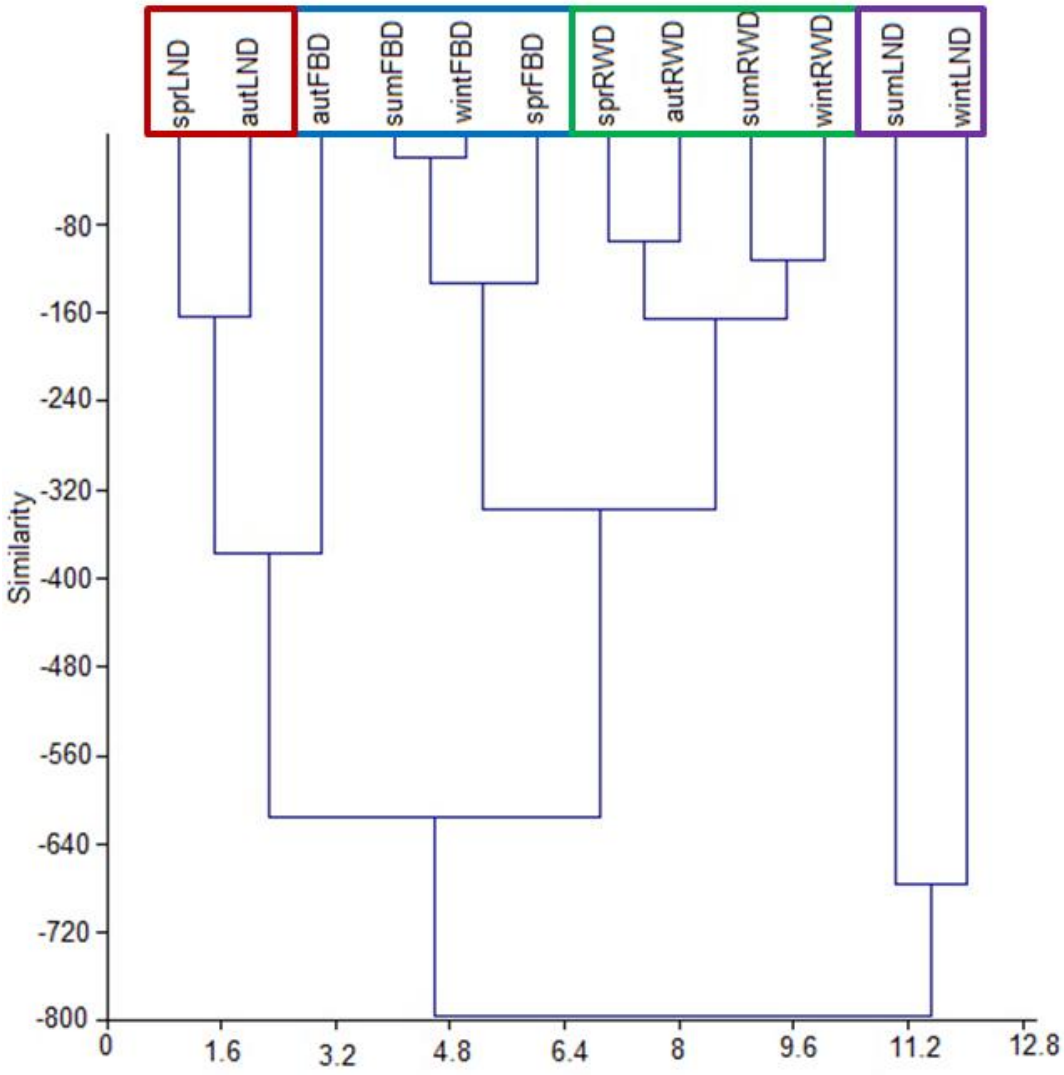


Figure 6.6: Cluster analysis showing similarity among the 3 sites and four sampling seasons based on metazoan parasite abundance values. (pooled data for the two host species, *Clarias gariepinus* and *Oreochromis mossambicus*) spr = spring; aut = autumn; sum = summer; wint = winter. LND = Luphephe-Nwanedi Dams; FBD = Flag Boshielo Dam; RWD = Return Water Dam.

6.5. Discussion

The aim of this chapter was to establish if parasites could be used as bioindicators of environmental conditions to which their hosts are exposed. The specificity of this work is that it relied on two fish hosts and their panel of parasites to evidence differences in environmental conditions whereas most previous studies focused on one parasite group or/and one host species. This choice was justified by the need to use a community-level monitoring approach which, according to Lafferty (1997) is a more sensitive than a single host-parasite approach.

The pH values in Luphephe-Nwanedi Dams do not indicate any pH related impacts, remaining neutral and stable throughout the study period. No significant pH related impacts emanating from the Luphephe and Nwanedi Rivers were anticipated as there is relatively no urbanization in the environs of these tributaries. The low pH of 6.2 recorded at Flag Boshielo Dam can be related to acid mine drainage from small mining areas found in the catchments of Klipspruit, Moses River and Loopspruit as well as the area east of Marble Hall (Heath *et al*. 2010).

The very low DO levels recorded at the Return Water Dam could be explained by the high conductivity levels which reduce the solubility of oxygen in waters (Dallas & Day 2004), hence decreasing the amount of oxygen that can physically dissolve and be available to aquatic organisms.

The elevated DO levels at Flag Boshielo Dam may probably be due to the increased presence of photosynthetic organisms such as various phytoplankton releasing oxygen. The observed association between pH and DO suggests that algal photosynthesis may have contributed to these differences. Algal photosynthesis is an equilibrium process that is also coupled to dissolved oxygen production and any shifts can result in changes in pH (Brönmark & Hansson 1998).

Based on the guidelines set by DWAF (1996), dissolved sulphate level is below 200mg/l for human consumption. This is similar to the contamination levels prescribed by the Environmental Protection Agency in the USA and the European Union (WHO 2004). There is no prescribed TWQR value for sulphate for aquatic ecosystems in the South African water quality guidelines (DWAF 1996). However, aquatic ecosystems are almost without exceptions more sensitive than humans to environmental pollutants and as a result, TWQR values, where available, are usually lower (De Villiers & Mkwelo 2009). Maximum dissolved sulphate levels of 100 mg/l have been proposed for aquatic ecosystems in Canada (De Villiers & Mkwelo 2009).

The maximum value for sulphate recorded at the Return Water Dam (658 mg/l), is more than 6 times the Canadian TWQR value for aquatic ecosystems while at Flag Boshielo Dam the dissolved sulphate exceeds the proposed 100 mg/l threshold value for aquatic ecosystem health. It is difficult to envision a

source for these high sulphate levels, other than acid mine drainage (in both dams) and the use of fertilizers and herbicides (in Flag Boshielo Dam).

The spatial distribution of physicochemical variables showed that there was a decreasing pollution gradient (Return Water Dam < Flag Boshielo Dam < Luphephe-Nwanedi Dams) especially for trophic status variables (ammonia, nitrates, nitrites, ortho-phosphates, sulphates), turbidity, conductivity, total dissolved solids and metals (lead and aluminium). The Luphephe and Nwanedi tributaries flow through areas of natural grassland, interspersed with areas of subsistence cultivation. There is very little urbanization; as a result, the nutrients and conductivity levels are fairly low. The area appears to be an ideal locality for rich natural communities of fish and their parasites, based on the assumptions that the waters are relatively uncontaminated. Consequently, this rural site had a flourishing parasitic fauna.

The Fag Boshielo Dam had reduced parasite diversity while the severely polluted site, the Return Water Dam had a depauperate parasitic fauna. Mining, extensive soil erosion in densely populated areas and return flows from irrigated agriculture (Ashton 2010) are implicated for the rise in conductivity and nutrients in Flag Boshielo Dam. Results from this study were consistent with previous work describing Luphephe-Nwanedi Dams as essentially unpolluted (Oberholster *et al*. 2009) and Flag Boshielo Dam as impacted with a combination of mining and agricultural effluents (Ashton 2010; De Villiers & Mkwelo 2009). It has also been

established that the Return Water Dam is an extremely polluted site with high levels of nutrients and metals.

Although Monte Carlo permutations failed to identify the significant environmental variables explaining parasite abundance, the RDA biplot revealed some underlying patterns between variables and parasite abundance. This outlines the complexity of the observed patterns vis-á-vis biotic and abiotic interactions. In natural environments, pollutants typically occur as combinations of chemicals and these complex mixtures can produce unpredictable or nonlinear effects on aquatic life (Pietrock & Marcoglise 2003). Their toxicity may be further affected by other abiotic factors such as pH and temperature (Lafferty 1997; Pietrock & Marcoglise 2003; Marcogliese 2005). Thus, despite these commonly occurring patterns of decreased and/ or increased infections with particular parasites, the role of pollutants in creating these patterns is complex and not always predictable. Furthermore, particular effects of pollutants on parasite communities may vary by type of pollution (MacKenzie *et al*. 1995; Lafferty 1997; Marcogliese 2005; Blanar *et al*. 2009).

An important finding of this study is that the characteristics of fish parasite communities, as reflected by their biodiversity were found to be noticeably different between the reference site, the moderately polluted and severely polluted sites. The presence of a large number of parasite species at Luphephe-Nwanedi Dams indicates a species richness and diversity of parasite

communities in the tropics. The evenness values at the three sites were relatively low, indicating that the parasite fauna in each fish species were dominated by a few parasite species with uneven distribution (Kennedy *et al*. 1986). The communities were dominated by *Contracaecum* species (at Luphephe-Nwanedi Dams and Flag Boshielo Dam) and by the gryporynchid cestode larva (at the Return Water Dam).

The absence of the ectoparasitic crustaceans, *C*. *inermis* and *D*. *ranarum* at Flag Boshielo Dam and the Return Water Dam demonstrates their high sensitivity to pollution. *Lamproglena clariae* was totally absent from the severely polluted site. Although these parasites do not require intermediate hosts for completing their life cycles, the results suggest a direct effect of water quality on the parasites. According to Kuperman (1991), the abundance of crustacean parasites varies with different environmental conditions and decreases considerably in polluted areas. For example, Galli *et al*. (2001) provided evidence of the susceptibility of *Lamproglena pulchella* to poor water quality. Overstreet & Thulin (1991) found that the prevalence of the copepods *Achtheres percarum* and *Caligulus lacustris* on perch (*Perca fluviatilis*) increased with the distance from the point of effluent discharge, while no parasites were found on fish at the closest location to the effluent discharge. The only copepod detected at the moderately polluted site was the ergasilid crustacean on the fins. However, this crustacean was absent in the unpolluted site, and there is no immediate reason to explain this discrepancy, since this was also the first time this parasite was

recorded in Flag Boshielo Dam and its local distribution range have not yet been determined.

The monogeneans, *Q*. *clariadis* and *Q*. *aegypticus* were totally absent at polluted sites, a trend also observed by Zharikova (1993). The other monogeneans (*Macrogyrodactylus clarii*, *M*. *congolensis*, *Enterogyrus* species 1, *Enterogyrus* species 2, *Enterogyrus* species 3, *Cichlidogyrus halli*, *C*. *sclerosus*, *C*. *dossoui*, *C*. *tilapiae* and *Scutogyrus longicornis*) were found at Luphephe-Nwanedi Dams and Flag Boshielo Dam only, showing their sensitivity to organic pollution. Similarly, Kostarev (1980) found that industrial waste discharge into two reservoirs had the effect of reducing the number of species of monogeneans. Overstreet & Howse (1977) found the prevalence and intensity of the monogenean, *Macrovalvitrematoides micropogoni*, on the Atlantic croaker, *Micropogonias undulatus*, to increase with decreasing pollution. These monogeneans are certainly sensitive to high levels of conductivity, nutrients and hypoxia present at the Return Water Dam. Ectoparasitic monogeneans have been found to be generally sensitive to pollution in southern Africa (Avenant-Oldewage 2001). The exposed state of the soft-bodied adult and the delicate, short-lived, free-swimming oncomiracidium may render this parasite and others like it susceptible to harsh environmental conditions (MacKenzie *et al*. 1995).

Another important finding of this study was the complete absence of many heteroxenous species from the fish hosts sampled at the severely polluted

site. Also, the abundance of most heteroxenous species found at the moderately polluted site was considerably lower when compared with the reference site. At the community level, the absence of these heteroxenous parasites is mirrored by lower mean species richness at Flag Boshielo Dam and the Return Water Dam. Mining, agricultural and industrial effluents have been found to be toxic to adult trematodes, nematodes and some cestodes (Overstreet & Howse 1977; Khan & Thulin 1991; Valtonen *et al.* 1997), and lethal to free-living stages (e.g. cercariae and miracidia) as well as to mollusc intermediate hosts (Evans 1982; Siddall *et al*. 1993).

The present study lends additional support to this view that pollution compromises heteroxenous species by preventing the completion of their life cycles. From the findings of this study, it is evident that heteroxenous species are better adapted for survival in polluted habitats than monoxenous species. Conceivably, the former would benefit to some extent from homeostatic, contaminant transport and detoxification mechanisms of their hosts. While the level of water pollution can influence aquatic heteroxenous species both directly and indirectly by acting on their intermediate hosts, monoxenous species may be more sensitive to contaminants that might reduce their survival and reproduction rates (Khan & Thulin 1991).

The species that are evidently associated with the severely polluted site are the cestodes, *Tetracampos ciliotheca* and the gryporynchid larva. The first intermediate hosts of *T*. *ciliotheca* and gryporynchid cestodes are oligochaetes

(Tubificidae), particularly *Tubifex* and allied genera (Paperna 1996). These worms can tolerate organic pollution since they feed on organic particles (Weisberg *et al*. 1986). Kostarev (1980) found high numbers of *Caryophyllaeus laticeps*, and he attributed this to increased oligochaete populations where household sewage was discharged in reservoirs. The increased abundance of these intermediate hosts in this study may be ascribed to the mine effluent, reflecting organic enrichment of the sediments that provide excellent environment for the oligochaete intermediate hosts (Weisberg *et al*. 1986; Sibley *et al*. 2000). In addition, a few endoparasitic cestodes are able to accumulate and tolerate higher levels of metal contaminants than their fish hosts (Sures *&* Taraschewski 1995; Retief *et al*. 2006; 2007). A positive aspect of the parasite-host relationship may be that certain fish are able to survive under contaminated conditions because their parasite fauna are able to store metals and thus aid in mobilizing contaminants away from the fish tissue (Landsberg *et al*. 1998). Contaminant analysis of fish parasites may prove to be a very useful and extremely sensitive biomarker.

The question remains as to whether one should be concerned with drastic reductions in parasitism. Most non-parasitologists view parasites solely as agents of diseases, and would likely consider the extirpation of sensitive parasite taxa to be a net benefit to an ecosystem (Marcogliese 2003, 2004; Blanar *et al*. 2009). According to this view, parasites would only be of interest in situations where they proliferate in contaminated environments, thereby posing an additional

health threat to their hosts. However, in the past decade, there has been considerable progress in the general understanding of the evolutionary and ecological significance of parasites (Poulin 2007). For example, Luphephe-Nwanedi Dams can be considered healthy because the species composition has persisted for at least 30 years after Mashego (1977) reported most of the observed metazoans previously. If disturbed, this would no doubt lead to the loss of the species. At this site, the two fish hosts investigated harboured high levels of parasite species richness, with hosts being infected by monogeneans, digeneans, nematodes, cestodes, a branchiuran, a copepod and a hirudinean leech. In such an ecosystem, not only are the hosts harbouring more species but the parasites also link different trophic levels given that most generalist parasites species develop into adults in birds or mammalian definitive hosts. These long chains of multispecies connections can stabilize the community structure in ways that increase resilience and that might help persistence (Neutel *et al*. 2002; Marcogliese 2003, 2004). Counting the parasite species present may possibly also double the species richness of the ecosystem.

Thus, the solid image of a healthy ecosystem is one in which biodiversity of free-living organisms is shadowed by the parasites, where each host is a habitat patch to be colonized and exploited (Poulin 2007). This contrasts with disturbed ecosystems such as the Flag Boshielo Dam and the Return Water Dam where mining (Flag Boshielo Dam and the Return Water Dam) and agricultural effluents (Flag Boshielo Dam) have reduced parasite diversity

drastically. For example, the presence of most trematodes and nematodes in Luphephe-Nwanedi Dams make their second intermediate host (fishes) far more susceptible to predation than uninfected conspecifics (Lafferty & Morris 1996; Hudson *et al*. 2006). These parasites thus drive a substantial amount of energy towards fish-eating birds, at very little cost to the birds themselves (Lafferty & *et al*. 2006). The consequence of parasites' loss in this case is quite clear: lower infection rates in snails and fish, leading to decreased transfer of energy to birds (as parasite–free fish might be harder to catch), resulting in decreases in local bird populations. Competition among prey species released from parasitism would also ensue. Thus, the term 'ecosystem health' is focused on the functioning of a whole community, embracing the overall performance and persistence of the system (Hudson *et al*. 2006). Therefore, a healthy ecosystem such as Luphephe-Nwanedi Dams is one that persists, maintains vigour (productivity), organization (biodiversity and predictability) and resilience (time to recovery).

6.6. Conclusions

The results suggest that parasite species composition and richness of freshwater fish are influenced by environmental factors such as dissolved oxygen, conductivity, nutrients and metals; and that parasite assemblages may be good indicators of environmental stress. The heteroxenous parasite species possess complex life cycles and are transmitted through a chain of many types of

vertebrate and invertebrate hosts acting as intermediate or definitive hosts. Therefore changes in the structure of a parasite community reflect differences in the composition of the aquatic species, such as macroinvertebrate fauna, commonly used as indicators of water quality. Monoxenous parasite species are directly exposed to the effects of water quality and disappear in perturbed environments. Thus parasite communities can be regarded as comprehensive bioindicators of ecosystem health that can guarantee an early warning system for monitoring habitat disturbance.

CHAPTER 7

CONCLUDING REMARKS AND RECOMMENDATIONS

The aim of this study was to comparatively evaluate the status of ecosystem health of three dams in the Limpopo and Olifants River systems using the Health Assessment Index (HAI) and the Inverted Parasite Index (IPI). Thus, two ecologically and commercially important fish species were selected as bioindicators. The sharptooth catfish, *Clarias gariepinus* and the Mozambique tilapia, *Oreochromis mossambicus* are important in the ecology of tropical waters as well as in the resources of aquatic systems of the sub-tropical region. They are also among the most popular species of the bony fish in Africa. This is attributed to the many positive aquacultural qualities including fast growth, firm flesh, mild flavour and tolerance to poor water quality and wide range of food.

In this study, different approaches were undertaken to evaluate information provided by these two fish species: the assessment of fish health and an integrated approach which, in addition to fish health assessment, included the use of parasite community data such as species diversity data, infection levels (prevalence, mean abundance and mean intensity) and parasite index. Water quality data was incorporated to verify the biomonitoring data. The main advantage of the integrated approach is that all information is obtained from the same fish individual; organ, tissue and haematocrit responses to pollution

exposure in fish on the suborganismal level and pollution effects on the parasite fauna of fish in the population and community level.

The application of the HAI method in **Chapter 3** allowed quantitative evaluation of the health of fish populations by means of data that were primarily qualitative. Therefore statistical comparisons between the three water bodies were possible. Although the exact causative pollutants could not be determined, the HAI gave a rapid and inexpensive indication of the occurrence of a change in fish health and was useful in assessing first level pollution problems. Results from different tissue/organ anomalies and haematocrit abnormalities suggested that fish at the impacted sites showed poorer health status than fish from the unpolluted site, although this trend was not expressed in every individual fish. The conclusion on a decreasing health status towards the impacted sites was based mainly on the predominance of abnormal levels of haematocrit, ectoparasites and endoparasites; gills, liver, eyes, fins and skin findings.

The study (**Chapter 3**) points to the use of necropsy based assessments as direct indicators of fish health. Although higher HAI values at the Return Water Dam were attributed to contaminants such as lead, aluminium, sulphate, hypoxia, eutrophication (higher levels of ammonium, nitrate, nitrite and orthophosphate), typically, the HAI was found to be nonspecific in its response to any of these pollutants. Similarly, pollutants such as sulphates, aluminium, iron and lead could have resulted in the poor health of both fish species in the Flag Boshielo Dam. The results indicated that although the health of the different species was

impaired, as indicated by the various anomalies, homeostatic mechanisms were in motion to favour adaptation, as demonstrated by haematological alterations. Nonetheless, such mechanism might become maladaptive in the long term and decrease fish yield and catch, thus adversely affecting the ecosystem health.

While the HAI is admittedly an effective biomonitoring tool, it has limitations which include subjectivity and repeatability. Individual researchers may interpret observations differently and this may influence the end results of the HAI. Moreover, huge leaps between values, for example between 10 and 20 may be misconstrued for a large physiological difference of disease. Another drawback of this method is that infection can occur in fish with no manifestation of disease symptoms or the infection may be difficult to identify because some stages of disease are invisible in many fish disease cases (Luus-Powell 1997). Sometimes, the clinical stage with the accompanying symptoms is too short to be observed because of the rapid mortality following the incubation stage, or environmental stressors may be sufficiently severe that fish die before observable changes in the structure or appearance appear. Moreover, there can be microscopic or histological structural changes without gross manifestation.

In **Chapters 4** and **5**, the study also aimed to determine the diversity and ecology of fish parasites in the Limpopo and Olifants River System; a seasonally inundated alluvial system. Results from these two chapters also added considerably to knowledge of the distribution and abundance of metazoan

parasite infections in *C*. *gariepinus* and *O*. *mossambicus* in the Limpopo and Olifants River System. The study also showed new distribution records of some species such as *Enterogyrus*, *Cichlidogyrus* and *Scutogyrus* species, thereby contributing to the biogeography of Southern African parasites species.

Parasite communities of metazoans in freshwater fish have been described as either stochastic or highly structured, depending on the interactions of many biotic and abiotic factors (Kennedy 1990). It was in the interest of the study to determine if parasite communities of the two fish species show predictable or stochastic structure and if they do, determine the underlying processes. Parasite communities from both fish species displayed chance assemblages rather than structured organisation since there was greater variability in the mean abundance among infracommunities. This discrepancy was largely a result of the numerically dominant larval parasites, whose abundance values varied in greater ranges across infracommunities, thus enhancing the stochastic nature of the infracommunities.

When the fish parasite communities were compared with other systems on a broader geographical scale, it was revealed that latitude alone did not contribute necessarily to a rich and diverse parasite community but a combination of historical and contemporary ecological factors (host diet, feeding behaviour, specificity, seasonality and timing of inundation, habitat and interspecific associations) play a significant role in determining community diversity and structure in freshwater fish.

There was no evidence to suggest that any of the parasite infections was associated with significant morbidity or poor condition of the fish host. This may possibly suggest that heavily infected individuals spend more time foraging to attain the same nutritional benefit as less parasitized and parasitized individuals (Lafferty 1992), in which case an increased food intake by infected fish outweighs a possible parasite-mediated energy loss.

The parasite communities of both fish species presented interspecific associations, thereby confirming exploitation of hosts by congeneric parasite species. Simultaneous infections and coexistence of parasites are probably made possible by relatively low infection levels under natural conditions. These positive interspecific associations may be a result of a similar mechanism of infection *in situ*. Additionally, one species may weaken a host and so make it more susceptible to infection with another species or one species may 'prepare' the microhabitat for a second species, for instance, by making it easier for the latter species to feed.

The lack of effect of sex on parasitic infections in both fish species was considered to be a reflex of the lack of differences in the biology and population dynamics of male and female hosts. Nevertheless, future research is needed in order to visualise the influence of other factors (such as hormonal, immunological, morphological and behavioural factors). Studies on seasonality and breeding seasons could shed light upon such aspects, since it would be

verified if changes on either male or female hosts behaviour occur regarding breeding seasons, feeding habits, hormonal levels, migrations and sexual dimorphism which could change the ecological niche.

The lack of significant correlations between host size and most parasite species of *C*. *gariepinus* was attributed to the homogeneity of the sample because no juveniles were sampled. For *O*. *mossambicus*, the abundance values for ectoparasites were not influenced by host size whereas the endoparasites showed an opposing trend in which there was an increase in abundance values with increase in host length. It was concluded that the mouth brooding habit of *O*. *mossambicus* may make the fry more vulnerable to ectoparasitic infection. The positive correlation between larval endoparasites of *O*. *mossambicus* and host size was attributed to recurrent infections and accumulation of larvae in fish tissues with age. Additionally, this relationship may strongly be influenced by changes in ontogenic feeding habits, given that juveniles of *O*. *mossambicus* are mostly omnivorous, while adults are detritivores, making older fish more vulnerable to infections by larval parasites.

In **Chapter 6**, parasites of *C*. *gariepinus* and *O*. *mossambicus* from the three different dams, representing a contamination gradient, were investigated for their potential use as bioindicators in environmental health monitoring, considering anthropogenic influences on the parasite community. There are good reasons for focusing on parasites in the search for indicators to monitor the effects of pollutants on aquatic organisms. Firstly, there are more parasitic than

free-living species. Secondly, in parasites with complex life cycles, the different stages have widely differing requirements, so that each stage must be assessed separately, thereby greatly increasing the number of potential indicators. Thirdly, many parasites have delicate free-living transmission stages which are highly sensitive to environmental change. A reduction in their levels of infection will serve as an early warning that changes are occurring. Conversely, other parasites are highly resistant to environmental change and will respond by increased levels of infection.

Luus-Powell (1997) named several criteria, which potential indicators of biological effects should fulfil for their use in environmental monitoring program. The indicator should be: easy to sample, rapid and cost effective, statistically robust for trend monitoring, applicable to either national or international environmental issues, easy to be understood by the public and mechanistically, have an early warning potential and /or ecological relevance.

In the present study, fish parasites satisfied most of these criteria. Sampling of parasites was generally easy and cost effective, especially when parasites were collected from the same fish individuals, which were used for HAI and IPI as well. Parasite data proved to be statistically robust for trend monitoring and was of high ecological relevance, when the one-year data were considered. The type of information provided by a parasite sample was superior in a way in which it integrated space and time. The presence of parasite species, especially of heteroxenous species, circuitously provided extensive information about the

invertebrate fauna of the environment. Thus, sampling of large numbers of fish individuals or of an additional group of planktonic or benthic organisms appears to be unnecessary, when fish parasites are used for evaluation of changes in the ecosystem integrity. For example, the abundance and diversity of trematodes in fish is highly correlated with the abundance and diversity of birds that serve as definitive hosts and with snails that serve as first intermediate hosts for these avian parasites. Whereas a bird or invertebrate survey provides a snapshot of their presence, the trematodes provide a record of the community of invertebrate intermediate hosts and birds that have visited a site during the life time of the fish sampled, and so provide an integral record of biodiversity.

Heteroxenous species formed an important component of the parasite infracommunities of both fish species, suggesting the availability of the intermediate hosts as prey, and thus important determinants of infection. The wide spectrum feeding habits of the catfish and cichlid hosts, bringing them in contact with several potential intermediate hosts of digeneans, nematodes and cestodes, explains the presence of these parasites. Substantive analyses of fish diet and adult parasites from resident piscivorous birds could provide more information about their feeding ecology, trophic status and possible transmission of these heteroxenous species.

There was a consistent decrease in the abundance of digeneans and nematodes as well as their representation in the communities at Flag Boshielo Dam and Return Water Dam. The life cycle details of some of these helminthes

are as yet unknown and have yet to be elucidated (Kennedy 1997). Nevertheless, considering taxonomically related species, a multiple-host life cycle may be assumed in all of them (Kennedy 1997). Thus, in broader terms, molluscs as intermediate hosts for digeneans; copepods and oligochaetes for nematodes and cestodes may be implicated. Both fish species apparently become infected when ingesting these and other small invertebrates associated with macroalgae.

From the findings of this study, it is evident that extreme conditions may have different, sometimes conflicting, impacts on diverse taxa of parasites. For example, the cestode, *P*. *glanduligerus* was absent at the impacted sites while the other two cestodes, *T*. *ciliotheca* and the gryporynchid cestode larvae were most abundant at the severely polluted site. The observed increase in the latter two cestodes could be attributed to increased oligochaete populations which can tolerate organic pollution. In summary, the remarks of Kennedy (1997) are as relevant as ever and are worth repeating here. At present, there is lack of knowledge on parasite life histories, on the effect of different sources of pollution on each stage of their life cycle and on the effects of their intermediate hosts. What we can only do is record changes and differences in populations and communities; but we are still incapable of unequivocally relating them to any specific causal factors.

Interestingly, the detrimental effect of mining by-products was not restricted to the interference in transmission of heteroxenous parasites, but

appeared to suppress also the reproduction of monogenean species. Both host species sampled from the Return Water Dam supported the poorest and least diverse monogenean parasite communities. This may perhaps be partly explained by eutrophication at this site, which may have directly impacted transmission of ectoparasites. Monogenean eggs that settle on the organically enriched, largely anaerobic sediment below the mining dam are probably lost (Paperna 1997). Even if the oncomiracidium develops and hatches, the viability of the free-swimming stage and the chances of finding a host can be expected to be very low in that environment.

Monogeneans occurred on both fish species sampled from the Luphephe-Nwanedi Dams and Flag Boshielo Dam throughout the year. Thus, the recruitment of the parasites, though relatively weak, was certainly continuous. This is probably because the success of the ‘simple’ monogenean is highly and suitably adapted to enable these ectoparasites to locate, invade and establish on wild fish hosts.

This study has shown that Luphephe-Nwanedi Dams is a healthy ecosystem because the two fish species harboured high levels of parasite species richness, with a good representation of both monoxenous and heteroxenous parasite species. In such an ecosystem, not only are the hosts harbouring more species but the parasites also link different trophic levels given that most generalist parasite species develop into adults in birds or mammalian hosts. Healthy ecosystems such as the Luphephe-Nwanedi Dams have a

complex trophic structure with many species forming the food web. These long chains of multispecies connections can stabilise the community structure in ways that can enhance resilience and that might help persistence. The parasite species reflect the presence of different organisms that participate in its life cycle; together, all the parasite species in a host reflect the presence of a plethora of host organisms and trophic interactions in the environment (Marcogliese 2005). Thus, parasites potentially may be used as surrogate indicators of species diversity and ecosystem diversity, two of the three important levels of biodiversity cited in the Rio Convention on Biological Diversity (Marcogliese 2003).

This contrasts with disturbed ecosystems such as the Flag Boshielo Dam and the Return Water Dam where mining (Flag Boshielo Dam and the Return Water Dam) and agricultural effluents (Flag Boshielo Dam) have reduced parasite diversity. For example, the presence of most trematodes and nematodes in Luphephe-Nwanedi Dams make their second intermediate host (fishes) far more susceptible to predation than uninfected conspecifics. These parasites thus drive a substantial amount of energy towards fish-eating birds, at very little cost to the birds themselves. The consequence of parasites' loss in this case is quite clear: lower infection rates in snails and fish, leading to decreased transfer of energy to birds (as parasite-free fish might be harder to catch), resulting in decreases in local bird populations. Competition among prey species released from parasitism would ensue. Thus, the term 'ecosystem health' is focused on the functioning of a whole community, embracing the overall performance and

persistence of the system (Hudson *et al*. 2006). Therefore, as mentioned in Chapter 6, a healthy ecosystem such as Luphephe-Nwanedi Dams is one that persists, maintains vigour (productivity), organisation (biodiversity and predictability) and resilience (time to recovery).

Protection of the rivers' riparian zones will go a long way in maintaining biodiversity in the region. The Luphephe-Nwanedi Dams are examples of protected water bodies, where the riparian zone is almost in its natural state. At Flag Boshielo Dam, there are several townships, smaller towns like Groblersdal, Marble Hall, Settlers, Siyabuswa and Denilton, located in the catchment (Heath & Claassen 1999). Irrigation farming of diverse crops takes place in various parts of the catchment and small mining areas are found in the catchments of Klipspruit, Moses River, Loopspruit and Marble Hall (Van Vuren *et al*. 1999). Thus the riparian zone of Flag Boshielo Dam is extensively degraded with stream bank agriculture, wetland cultivation, deforestation and mining. Naturally, invertebrates habitats are reduced, having a detrimental effect on fish parasite diversity and consequently on the ecosystem integrity.

The study has shown that the mining operations in the vicinity of the Return Water Dam have damaging effects on biodiversity. Thus, proper management of tropical river species involves mitigating against environmental degradation so that a variety of habitats remains available for the special needs of different species (Barson 2009). Additionally, conservation of dams such as

efforts in the Nwanedi Nature Reserve needs to be communicated on a basin wide level so the whole ecosystem is protected.

Although the HAI and parasite data successfully differentiated among three localities that differ in water quality, they were nonspecific in response to contaminants. This was attributed to synergistic interactions between different contaminants. These interactions can affect parasite species individually, the intermediate hosts that they inhabit, the overall health of the fish and the ability of the fish to mount on an effective immune response (Lafferty 1997). All these factors may interact to lead either to increase or decrease of abundance or diversity of particular parasites in their fish host (Blanar *et al*. 2009). Synergistic effects of contaminants and parasites may also compound and confuse the interpretation of their individual effects and this problem became evident in this study. Although the HAI and parasite diversity were nonspecific in their response to contaminants, they offer a useful and reliable indication or monitor of environmental quality.

It has been established that the high assemblage dissimilarities between the three dams are due to differences in water quality. However, the interference of background factors such as biogeography is an unavoidable problem in field studies such as the present investigation. Bridging the gap with biogeography by integration of large scale spatial events and limited dispersal over distances may be one of the future challenges in metacommunity ecology. This study provides an important step in that direction. The high species diversity in the Luphephe-

Nwanedi Dams clearly identifies it as a biodiversity rich locality and this awareness is critical as we are confronted by global challenges such as climate change and shifts in land use patterns that could leave such habitats even more vulnerable. Therefore, conservation efforts to preserve and enhance biodiversity in habitats such as Luphephe-Nwanedi Dams must be driven by the need to minimize anthropogenic activities in and around the Limpopo River Basin, a challenge that may be difficult to tackle since this river is a transboundary river, shared by four countries. To unravel the diversity of fish parasites, more studies of this nature are required before such habitats are lost.

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Appendix

Table 1: Heath Assessment Index for *Oreochromis mossambicus* sampled in the Luphephe-Nwanedi Dams in A = autumn; B = winter.

A

Fish No	Sex	SL(mm)	W (g)	Eyes	skin	fins	oper	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
1	F	150	101.8	0	0	0	0	0	0	0	0	0	0	0	0	0
2	F	155	175.4	0	0	0	0	0	0	0	0	0	0	30	0	30
3	F	130	19.5	0	0	0	0	0	0	0	0	0	0	0	0	0
4	M	160	113.2	0	0	0	0	0	0	0	0	0	0	20	0	20
5	M	162	104.4	0	0	30	0	0	0	0	30	0	0	10	0	70
6	M	152	98.8	0	0	0	0	0	0	0	0	0	30	20	0	50
7	M	215	290.8	0	0	0	0	0	0	0	0	0	0	30	0	30
8	M	180	165.4	0	0	0	0	0	0	0	0	0	0	10	0	10
9	M	162	104.4	0	0	0	0	0	0	0	0	0	0	0	0	0
10	F	155	175.4	0	0	0	0	0	0	0	0	0	0	20	0	20
11	F	130	19.5	0	0	0	0	0	0	0	0	0	10	10	0	20
mean		159.2	124.4	0	0	2.7	0	0	0	0	2	0	3	13	0	22.7
std		23.3	76.6	0	0	9.0	0	0	0	0	9	0	9	11	0	22.0

B

Fish no	Sex	SL (mm)	W (g	Eyes	skin	fins	oper	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
12	F	162	107.7	0	0	0	0	0	0	0	0	0	0	0	0	0
13	F	150	126.3	0	0	0	0	0	0	0	0	0	0	10	0	10
14	F	205	260.8	30	0	0	0	0	30	0	0	0	0	30	0	90
15	F	205	238.7	0	0	0	0	0	0	0	0	0	30	0	0	30
16	F	170	160.5	0	0	0	0	0	0	0	0	0	0	30	0	30
17	F	198	223.4	0	0	30	0	0	0	0	0	0	0	0	0	30
18	F	180	212.5	0	0	0	0	0	0	0	0	0	0	30	0	30
19	F	205	263.4	30	0	0	0	0	0	0	0	0	0	0	10	40
20	F	135	98.5	0	0	0	0	0	0	0	0	0	10	0	0	10
21	F	198	223.4	0	0	0	0	0	0	0	0	0	10	0	0	10
mean		180.8	191.5	6.0	0	3.0	0	0	15.0	0	0	0	5.0	10.0	1.0	28.0
std		25.5	62.9	12.6	0	9.5	0	0	21.2	0	0	0	9.7	14.1	3.2	25.3

Table 1: Heath Assessment Index for *Oreochromis mossambicus* sampled in the Luphephe-Nwanedi Dams in A = spring; B = summer. C

Fish no	Sex	SL(mm)	W(g)	eyes	skin	fins	oper	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
22	M	67	9.9	0	0	0	0	0	0	0	0	0	0	30	0	30
23	F	62	6.7	0	0	0	0	0	0	0	0	0	0	20	0	20
24	M	195	20.5	0	0	0	0	0	0	0	0	0	0	20	0	20
25	F	183	152.8	0	0	0	0	0	0	0	0	0	0	10	0	10
26	F	153	113.5	0	0	0	0	0	0	0	0	0	30	10	0	40
27	M	175	122.3	0	0	0	0	30	0	0	0	0	0	10	0	40
28	F	185	180.2	0	0	0	0	0	0	0	0	0	0	30	0	30
29	F	205	257	0	0	0	0	0	0	0	0	0	0	30	0	30
30	M	173	154.5	0	0	0	0	0	30	0	0	0	0	20	0	50
31	F	145	102.8	0	0	0	0	0	0	0	0	0	0	30	0	30
32	F	188	169.7	0	0	0	0	0	0	0	0	0	30	20	0	50
33	F	135	72.5	0	0	0	0	0	0	0	0	0	0	0	0	0
34	F	125	57.2	0	0	0	0	0	0	0	0	0	0	30	0	30
mean		153.2	109.2	0	0	0	0	2.3	2.3	0	0	0	4.6	20.0	0	29.2
std		47.2	76.3	0	0	0	0	8.7	8.7	0	0	0	11.7	10.0	0	15.1

D

Fish no	Sex	SL(mm)	W(g)	Eyes	skin	fins	oper	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
35	M	230	420.4	0	0	0	0	0	0	0	0	0	0	20	0	20
36	M	200	287.7	0	0	0	0	0	0	0	0	0	0	0	0	0
37	M	260	548	0	0	0	0	0	0	0	0	0	0	0	0	0
38	F	190	264.2	0	0	0	0	0	0	0	0	0	10	30	10	50
39	M	200	270.8	0	0	0	0	0	0	0	0	0	0	30	0	30
40	M	280	743.2	0	0	0	0	0	0	0	0	0	0	0	0	0
41	M	210	286.5	0	0	0	0	0	0	0	0	0	0	20	0	20
42	F	210	289.6	0	0	0	0	0	0	0	0	0	0	0	0	0
43	M	180	220.6	0	0	0	0	0	0	0	0	0	30	0	0	30
44	F	260	548	0	0	0	0	0	0	0	0	0	0	20	0	20
45	F	170	160.4	0	0	0	0	0	0	0	0	0	0	20	0	20
mean		217.3	367.2	0	0	0	0	0	0	0	0	0	3.6	12.7	0.9	17.3
std		35.8	176.8	0	0	0	0	0	0	0	0	0	9.2	12.7	3.0	16.2

Table 2: Heath Assessment Index for *Oreochromis mossambicus* sampled in the Flag Boshielo Dam in A = autumn; B = winter.

A

Fish No	Sex	SL(mm)	W (g)	Eyes	skin	fins	operc	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
1	F	150	101.8	0	0	0	0	0	0	0	0	0	20	10	0	30
2	F	155	175.4	0	0	0	0	30	30	0	0	30	0	10	0	100
3	F	130	19.5	0	0	0	0	0	0	0	0	0	0	10	0	40
4	M	160	113.2	0	0	0	0	0	0	0	30	0	0	20	0	50
5	M	162	104.4	0	0	0	0	30	0	0	0	0	0	20	0	50
6	M	152	98.8	30	0	10	0	0	30	0	0	0	0	30	0	100
7	M	215	290.8	0	0	0	0	0	0	30	0	0	0	0	0	30
8	M	180	165.4	0	0	0	0	0	0	0	0	0	0	10	0	10
9	M	162	104.4	0	0	0	0	0	0	0	0	0	0	10	0	10
10	F	155	175.4	0	0	0	30	30	0	0	0	0	0	10	0	70
11	F	130	19.5	0	0	0	0	0	0	0	0	0	0	0	0	0
mean		159.2	124.4	2.7	0	0.9	2.7	8.2	5.5	2.7	2.7	2.7	1.8	11.8	0	44.5
std		23.3	76.6	9.0	0	3.0	9.0	14.0	12.1	9.0	9.0	9.0	6.0	8.7	0	34.2

B

Fish no	Sex	SL(mm)	W(g)	eyes	skin	fins	oper	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
12	F	162	107.7	0	0	0	0	0	0	0	0	0	0	30	0	30
13	F	150	126.3	0	0	0	0	30	0	0	0	0	30	10	0	70
14	F	205	260.8	0	0	0	0	0	30	0	0	0	30	20	0	110
15	F	205	238.7	30	0	0	0	0	0	0	0	0	0	20	0	50
16	F	170	160.5	0	0	0	0	0	0	0	0	0	30	20	0	50
17	F	198	223.4	0	0	0	0	0	0	0	0	30	10	0	0	40
18	F	180	212.5	0	0	0	0	0	0	30	0	0	10	10	0	50
19	F	205	263.4	0	10	0	0	30	0	0	0	0	10	0	0	80
20	F	135	98.5	0	0	0	30	0	0	0	0	0	10	20	0	60
21	F	198	223.4	0	0	0	0	0	30	0	0	0	0	0	0	30
mean		180.8	191.52	3	1	0	3	6	6	3	0	3	13	13	0	57
std		25.5	62.9	9.5	3.2	0.0	9.5	12.6	12.6	9.5	0	9.5	12.5	10.6	0	24.5

Table 2: Heath Assessment Index for *Oreochromis mossambicus* sampled in the Flag Boshielo Dam in A = spring; B = summer. C

Fish no	Sex	SL(mm)	W(g)	eyes	skin	fins	oper	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
22	M	67	9.9	0	0	10	0	0	0	0	0	0	30	20	0	60
23	F	62	6.7	0	0	0	0	30	0	0	30	0	0	0	0	60
24	M	195	20.5	30	0	0	0	0	0	0	0	0	0	20	0	80
25	F	183	152.8	0	0	0	0	0	0	30	0	0	0	30	0	60
26	F	153	113.5	0	0	0	0	30	0	0	0	0	0	30	0	60
27	M	175	122.3	0	0	0	0	0	30	0	0	0	0	0	0	30
28	F	185	180.2	0	0	0	0	0	0	0	0	0	0	20	0	20
29	F	205	257	0	0	0	0	30	0	0	0	0	0	0	0	60
30	M	173	154.5	0	0	0	0	0	0	0	0	0	30	0	0	30
31	F	145	102.8	0	0	0	0	0	30	0	0	0	10	20	0	60
32	F	188	169.7	0	0	0	0	0	0	0	0	0	30	30	0	60
33	F	135	72.5	0	10	0	0	0	0	0	0	30	30	20	0	90
34	F	125	57.2	0	0	0	30	30	0	0	0	0	30	30	0	120
mean		153.2	109.2	2.3	0.8	0.8	2.3	9.2	4.6	2.3	2.3	2.3	12.3	16.9	0	60.8
std		46.0	74.7	8.3	2.8	2.8	8.3	14.4	11.3	8.3	8.3	8.3	14.8	12.5	0	26.3

D

Fish no	Sex	SL(mm)	W(g)	eyes	skin	fins	oper	Gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
35	M	230	420.4	0	0	10	0	0	30	0	0	0	20	10	0	70
36	M	200	287.7	0	0	0	0	0	0	0	0	0	0	30	0	60
37	M	260	548	30	0	0	0	30	0	0	30	0	10	20	0	120
38	F	190	264.2	0	0	0	0	0	0	0	0	0	10	20	0	30
39	M	200	270.8	0	0	0	0	0	0	0	0	0	0	20	0	20
40	M	280	743.2	0	0	0	0	0	0	0	0	0	30	30	0	60
41	M	210	286.5	0	0	0	0	30	30	0	0	30	0	10	0	100
42	F	210	289.6	0	0	0	0	0	0	0	0	0	0	20	0	20
43	M	180	220.6	0	10	0	0	0	0	0	0	0	0	30	0	70
44	F	260	548	0	0	0	0	0	0	0	0	0	0	10	0	10
45	F	170	160.4	0	0	0	0	0	0	0	0	0	20	10	0	30
46	M	162	104.4	0	0	0	0	30	0	0	0	0	0	30	0	60
47	F	198	223.4	0	0	0	0	0	0	0	0	30	10	30	0	70
48	F	180	212.5	0	0	0	0	0	0	30	0	0	10	10	0	50
mean		209.3	327.1	2.1	0.7	0.7	0.0	6.4	4.3	2.1	2.1	4.3	7.9	20.0	0.0	55.0
std		35.9	176.2	8.0	2.7	2.7	0.0	12.8	10.9	8.0	8.0	10.9	9.7	8.8	0.0	31.3

Table 3: Heath Assessment Index for *Oreochromis mossambicus* sampled in the Return Water Dam in A = autumn; B = winter.

A

Fish No	Sex	SL(mm)	W(g)	Eyes	skin	fins	operc	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
1	F	165	115	0	0	0	0	0	30	0	0	0	30	30	0	90
2	F	143	99.8	30	0	0	0	30	0	0	10	30	10	30	0	140
3	M	155	123.9	0	0	0	0	0	30	0	0	0	20	30	0	80
4	M	165	130.5	0	0	10	0	0	0	30	0	30	30	30	0	130
5	M	145	99	0	0	0	0	30	30	0	0	0	20	30	0	110
6	F	156	100.3	30	0	0	10	0	0	0	0	30	10	30	0	110
7	F	165	102.2	0	10	0	0	0	0	0	0	0	10	30	0	50
8	M	158	80.2	0	0	0	0	30	0	0	0	0	20	30	0	80
9	M	335	1175.6	0	0	0	0	0	30	0	0	0	0	30	0	60
10	M	315	995.5	30	10	0	0	0	0	0	0	0	10	30	0	80
11	M	155	108.4	0	0	0	0	30	0	0	0	30	20	30	0	110
12	M	210	120.5	0	0	10	0	0	0	0	0	0	20	30	0	60
13	F	150	101.5	30	0	0	0	0	0	30	0	0	20	30	0	110
14	F	163	126.5	0	0	0	0	30	0	0	0	30	20	30	0	110
15	F	160	106.1	0	0	0	0	0	30	0	0	0	0	30	0	60
mean		182.7	239.0	8.0	1.3	1.3	0.7	10.0	10.7	4.0	0.7	10.0	16.0	30.0	0	92.0
std		59.9	345.6	13.7	3.5	3.5	2.6	14.6	14.9	10.6	2.6	14.6	9.1	0.0	0	27.6

B

Fish No	Sex	SL(mm)	W(g)	Eyes	skin	fins	operc	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
16	M	215	331.8	0	0	0	0	30	0	0	10	0	0	30	0	70
17	F	265	569.3	0	0	0	0	0	0	0	0	0	0	30	0	30
18	M	225	329.5	0	0	0	0	0	30	0	0	0	0	30	0	60
19	M	132	73.8	0	10	0	0	30	0	0	0	0	0	30	0	70
20	M	275	607.5	0	0	0	0	0	0	0	0	0	0	30	0	30
21	M	323	1034.5	0	0	0	0	0	30	0	0	30	20	30	0	110
22	F	155	108.4	30	0	0	0	30	0	0	0	0	0	30	0	90
23	**F**	210	120.5	0	0	0	0	0	30	0	0	0	10	30	0	70
24	F	165	115	0	0	10	0	0	0	0	0	0	20	30	0	60
25	F	143	99.8	0	0	0	0	30	0	30	0	30	0	30	0	120
mean		210.8	339.0	3.0	1.0	1.0	0.0	12.0	9.0	3.0	1.0	6.7	5.0	30.0	0.0	71.0
std		63.1	314.0	9.5	3.2	3.2	0.0	15.5	14.5	9.5	3.2	13.2	8.5	0.0	0.0	29.6

Table 3: Heath Assessment Index for *Oreochromis mossambicus* sampled in the Return Water Dam in C = spring; B = summer.

C

Fish No	Sex	SL(mm)	W (g)	Eyes	skin	fins	operc	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
26	M	280	695.5	0	0	0	0	0	30	0	0	0	10	30	0	70
27	M	320	1208.4	0	0	0	0	0	0	0	0	0	0	30	0	30
28	M	230	281.5	0	0	0	10	0	0	0	0	0	10	30	0	50
29	M	290	901.5	0	0	0	0	0	0	30	0	30	20	30	0	110
30	F	294	750.1	30	0	0	0	30	0	0	0	0	20	30	0	110
31	M	210	333.5	0	0	0	0	0	30	0	0	0	20	30	0	80
32	M	300	794	0	0	0	0	30	0	30	0	0	30	30	0	120
33	F	165	134.4	0	0	0	0	0	30	0	0	N	20	20	0	70
34	F	180	164.4	0	0	0	0	0	0	0	0	30	10	30	0	70
35	M	210	218.4	0	10	0	0	0	0	0	0	0	10	30	0	50
36	F	156	121.2	0	0	0	0	30	0	30	0	0	20	20	0	100
37	F	195	237.3	0	0	0	0	0	0	0	10	0	20	30	0	60
mean		235.8	486.7	2.5	0.8	0	0.8	7.5	7.5	7.5	0.8	5.5	15.8	28.3	0	76.7
std		58.0	364.5	8.7	2.9	0	2.9	13.6	13.6	13.6	2.9	12.1	7.9	3.9	0	28.1

D

Fish No	Sex	SL(mm)	W (g)	Eyes	skin	fins	operc	gills	liver	spleen	hindgut	kidney	Hct	ecto	endo	Total1
38	M	300	848.1	30	0	0	10	0	0	0	0	0	30	30	0	100
39	F	235	489.5	0	0	0	0	0	30	0	0	30	20	30	0	110
40	F	233	490.4	0	0	0	0	0	0	0	0	0	0	30	0	30
41	M	296	892.7	0	0	0	0	30	0	0	0	0	20	30	0	80
42	F	295	738.5	0	0	0	0	0	30	0	0	0	20	30	0	80
43	M	280	731.2	0	10	0	0	0	0	0	0	0	0	30	0	40
44	M	240	542.5	0	0	0	0	30	0	0	0	0	20	30	0	80
45	M	230	429.1	30	0	0	0	0	0	0	0	30	0	20	0	80
46	F	260	702.4	0	0	0	0	0	30	0	0	0	20	30	0	80
47	F	215	398.7	0	0	0	10	0	30	0	0	0	30	30	0	100
mean		258.4	626.3	6.0	1.0	0	2.0	6.0	12.0	0	0	6.0	16.0	29.0	0	78.0
std		31.9	177.8	12.6	3.2	0	4.2	12.6	15.5	0	0	12.6	11.7	3.2	0	25.3

Table 4: Heath Assessment Index for *Clarias gariepinus* sampled in the Luphephe-Nwanedi Dams in A = autumn; B = winter.

A

Fish No	Sex	SL(cm)	W(g)	Eyes	skin	fins	oper	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
1	F	415	822	0	0	0	0	0	0	0	0	0	0	0	20	10	30
2	F	410	731	0	0	0	0	0	0	0	0	30	0	0	30	0	60
3	F	520	1269.2	0	0	0	0	0	0	0	0	0	0	0	20	0	20
4	M	466	994.5	0	0	0	0	0	0	30	0	0	0	0	20	10	60
5	M	805	5619.4	0	0	0	0	0	30	0	0	0	0	30	20	10	90
6	F	895	5806.8	0	0	0	0	0	0	0	0	0	0	30	30	0	60
7	M	500	1134	0	0	0	0	0	0	0	0	0	0	20	20	10	50
8	M	535	1521.2	0	0	0	0	0	0	0	0	0	0	20	20	10	50
9	F	675	2767	0	0	0	0	0	0	0	0	0	0	30	20	10	60
10	M	575	1723	0	0	0	0	0	0	0	0	0	0	0	20	10	30
11	M	460	1040	0	0	0	0	0	0	0	0	0	0	0	20	10	30
12	F	500	1487	0	0	0	0	0	0	0	0	0	0	0	20	10	30
mean		563.0	2076.3	0	0	0	0	0	2.5	2.5	0	2.5	0	10.8	21.7	7.5	47.5
stdev		153.0	1781.1	0	0	0	0	0	8.7	8.7	0	8.7	0	13.8	3.9	4.5	20.1

B

Fish No	Sex	SL(cm)	W (g)	Eyes	skin	fins	oper	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
13	F	485	1196.2	0	0	0	0	0	0	0	0	0	0	0	20	0	20
14	F	480	1149	0	30	0	0	0	0	0	0	0	0	0	30	10	70
15	M	355	379.9	0	0	0	0	0	0	0	0	0	0	0	20	0	20
16	F	580	1427.3	0	0	0	0	0	30	0	0	0	0	0	20	20	70
17	F	350	496.8	0	0	0	0	0	0	0	0	0	0	0	20	10	30
18	F	460	1024.4	30	0	0	10	0	0	0	0	0	0	30	20	10	100
19	M	490	1107.2	0	0	0	0	0	0	0	0	0	0	0	20	10	30
20	M	355	379.9	0	0	0	0	0	0	30	0	0	0	0	20	0	50
21	F	460	1024.4	0	0	0	0	0	0	0	0	0	0	0	20	0	20
22	F	480	1149	0	0	0	0	0	0	0	0	0	0	0	30	0	30
23	F	360	502.7	0	0	0	0	0	0	0	0	0	0	10	20	10	40
24	M	358	381.3	0	0	0	0	0	0	0	0	0	0	0	30	10	40
25	M	440	1103	0	0	0	0	0	0	0	0	0	0	0	20	10	30
mean		434.8	870.9	2.3	2.3	0	0.8	0	2.3	2.3	0	0.0	0	3.1	22.3	6.9	42.3
stdev		72.7	379.1	8.3	8.3	0	2.8	0	8.3	8.3	0	0.0	0	8.5	4.4	6.3	24.2

Table 4: Heath Assessment Index for *Clarias gariepinus* sampled in the Luphephe-Nwanedi Dams in C = spring; B = summer. C

Fish No	Sex	SL(cm)	W (g)	Eyes	skin	fins	oper	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
26	M	502	1479.8	0	0	0	0	0	0	0	0	0	0	0	20	10	30
27	F	520	1253.2	0	0	0	0	0	0	0	0	0	0	0	30	10	40
28	M	572	1815.4	0	0	0	0	0	0	0	0	0	0	0	20	0	20
29	F	513	1382.6	0	0	0	0	0	0	0	0	0	0	0	20	10	30
30	M	592	1430.3	0	0	0	0	0	0	0	0	0	0	0	20	10	30
31	F	500	1487	0	0	0	0	0	0	0	0	0	0	0	20	10	30
32	F	675	2767	0	0	0	0	0	0	30	0	0	0	30	20	10	90
33	M	502	1487.5	0	0	0	0	0	0	0	0	0	0	30	30	10	70
34	M	565	1723.5	0	0	0	0	0	0	0	0	0	0	0	20	10	30
35	F	460	1040	0	0	0	0	0	0	0	0	0	0	0	30	10	40
mean		540.1	1586.6	0	0	0	0	0	0	3	0	0	0	6	23	9	41
stdev		61.999	468	0	0	0	0	0	0	9.5	0	0	0	12.6	4.8	3.2	21.8

D

Fish no	Sex	SL(cm)	W(g)	Eyes	skin	fins	oper	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
36	M	460	1024.4	0	0	0	0	0	0	0	0	30	0	0	20	10	60
37	F	360	381.3	0	0	0	0	0	0	0	0	0	0	30	30	10	70
38	M	340	496.8	0	0	0	0	0	0	0	0	0	0	0	20	10	30
39	F	910	5806.8	0	0	0	0	0	0	0	0	0	0	0	20	10	30
40	F	675	2767	0	0	0	0	0	30	0	0	0	0	0	20	10	60
41	M	780	5619.4	0	0	0	0	0	0	0	0	0	0	0	20	10	30
42	F	572	1815.4	0	0	0	0	0	0	0	0	0	0	10	20	0	30
43	F	480	1040	0	0	0	0	0	0	0	0	0	0	0	30	0	30
44	M	490	1107.2	0	0	0	0	0	0	0	0	0	0	0	20	0	20
45	M	502	1487.5	0	0	0	0	0	0	0	0	0	0	0	30	0	30
mean		556.9	2154.6	0	0	0	0	0	3	0	0	3	0	4	23	6	39
stdev		181.7	1994.1	0	0	0	0	0	9.5	0	0	9.5	0	9.7	4.8	5.2	17.3

Table 5: Heath Assessment Index for *Clarias gariepinus* sampled in Flag Boshielo Dam in A = autum; B = winter.

A

Fish no	Sex	SL(cm)	W(g)	Eyes	skin	fins	operc	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
1	M	854	6510	0	0	0	0	30	0	30	0	0	30	20	20	10	140
2	M	495	1500	0	0	10	0	30	0	0	30	10	0	0	20	10	110
3	M	350	504.4	0	30	0	10	0	0	30	0	0	0	0	20	10	100
4	F	540	1640.5	30	0	0	0	30	0	0	0	0	0	0	30	10	100
5	F	630	2700	0	0	0	0	0	30	30	30	0	30	0	20	10	150
6	M	300	404.4	0	0	0	0	30	0	0	0	0	0	0	30	0	60
7	F	875	6510	0	0	10	0	0	0	0	0	0	0	0	20	0	30
8	F	370	534	0	0	0	0	0	0	0	0	0	30	0	30	0	60
9	F	310	288.9	0	0	0	0	30	0	0	0	0	0	30	20	0	80
10	M	540	1640.5	30	0	0	10	0	0	0	0	0	0	0	30	0	70
11	F	630	2700	0	0	0	0	30	0	30	0	0	0	0	30	0	90
mean		535.8	2,266.6	5.5	2.7	1.8	1.8	16.4	2.7	10.9	5.5	0.9	8.2	4.5	24.5	4.5	90.0
stdev		201.1	2,263.8	12.1	9.0	4.0	4.0	15.7	9.0	15.1	12.1	3.0	14.0	10.4	5.2	5.2	35.5

B

Fish No	Sex	SL(cm)	W(g)	Eyes	skin	fins	operc	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
12	M	620	2686.4	0	0	0	0	0	0	30	0	0	30	20	20	0	100
13	M	830	7012.6	0	0	0	10	30	0	30	0	0	30	30	20	20	170
14	M	920	7379.3	0	0	10	0	30	30	0	0	10	30	0	20	0	130
15	F	640	2768.5	0	0	0	0	30	0	30	30	0	0	0	20	0	110
16	F	560	2028.6	0	0	0	0	0	30	30	0	0	0	0	20	0	80
17	F	630	2700	0	0	0	10	30	0	30	0	0	30	0	20	0	120
18	F	570	1090.2	0	0	0	0	30	0	0	0	10	0	30	30	0	100
19	F	950	7500	0	0	0	0	30	0	0	0	0	0	0	30	0	60
20	M	850	8000	0	0	0	0	0	30	30	0	0	0	20	20	10	110
21	F	310	288.9	0	0	0	0	30	0	0	0	0	0	0	20	10	60
mean		688.0	4145.5	0.0	0	1.0	2.0	21.0	9.0	18.0	3.0	2.0	12.0	10.0	22.0	4.0	104.0
stdev		197.9	2973.8	0.0	0	3.2	4.2	14.5	14.5	15.5	9.5	4.2	15.5	13.3	4.2	7.0	33.1

Table 5: Heath Assessment Index for *Clarias gariepinus* sampled in Flag Boshielo Dam in C = spring; D = summer.

C

Fish No	Sex	SL(mm)	W(g)	Eyes	skin	fins	operc	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
22	M	335	398	0	0	0	10	0	0	30	0	0	0	0	20	0	60
23	M	850	8000	30	0	0	0	0	0	0	0	0	30	0	20	0	80
24	M	950	7500	0	0	0	0	30	0	30	0	0	0	0	20	0	80
25	F	580	2038.6	0	0	0	0	0	30	0	0	0	0	0	30	0	60
26	F	340	500	0	0	0	0	0	0	30	0	0	0	30	30	0	90
27	M	510	6500	0	0	10	0	0	0	0	0	0	0	0	20	0	30
28	F	140	28	0	0	0	0	0	0	0	0	0	0	0	30	0	30
29	F	1050	6837	30	0	0	0	30	0	30	0	0	0	20	20	0	130
30	M	190	78	0	0	0	0	0	0	0	0	0	0	0	30	0	30
mean		549.4	3542.2	6.7	0	1.1	1.1	6.7	3.3	13.3	0.0	0	3.3	5.6	24.4	0	65.6
stdev		333.6	3551.3	13.2	0	3.3	3.3	13.2	10.0	15.8	0.0	0	10.0	11.3	5.3	0	33.6

D

Fish No	Sex	SL(cm)	W(g)	Eyes	skin	fins	operc	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
31	M	875	6510	0	0	0	10	0	0	30	0	0	0	0	20	10	70
32	M	495	1500	0	0	0	0	0	0	0	0	0	0	0	20	10	30
33	M	350	504.4	0	0	0	0	0	0	0	0	0	30	0	20	10	60
34	F	540	1640.5	0	30	0	0	30	0	30	0	0	0	20	30	0	140
35	F	950	7500	0	0	10	0	0	0	0	0	0	0	0	20	0	30
36	M	640	2768.5	0	0	0	0	0	0	0	0	0	30	20	30	0	80
37	F	310	288.9	0	0	0	0	0	0	30	0	0	0	0	30	0	60
38	F	630	2700	0	0	0	0	0	0	0	0	0	30	0	20	0	50
39	M	350	504.4	0	0	0	0	30	0	30	0	0	0	0	20	0	80
40	F	540	1640.5	0	0	10	0	0	0	0	0	0	0	41	20	0	71
41	M	850	8000	0	0	0	0	30	30	30	0	0	0	43	20	0	153
mean		593.6	3050.7	0	2.7	1.8	0.9	8.2	2.7	13.6	0	0	8.2	11.3	22.7	2.7	74.9
stdev		221.4	2888.6	0	9.0	4.0	3.0	14.0	9.0	15.7	0	0	14.0	17.1	4.7	4.7	39.4

Table 6: Heath Assessment Index for *Clarias gariepinus* sampled in the Return Water Dam Dam in A = autumn; B = winter.

A

Fish No	Sex	SL(cm)	W(g)	Eyes	skin	fins	operc	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
1	F	415	822	0	30	10	0	30	0	0	0	0	0	10	30	10	120
2	F	410	731	30	0	0	20	0	0	0	0	0	30	30	30	0	140
3	F	520	1269.2	0	0	30	0	0	30	0	0	0	0	10	20	0	90
4	M	466	994.5	0	0	0	0	30	0	30	0	0	0	30	30	0	120
5	M	805	5619.4	0	30	0	0	0	0	0	0	30	0	30	30	0	120
6	F	895	5806.8	0	0	10	0	0	0	30	0	0	0	0	30	0	70
7	M	500	1134	0	30	0	0	30	0	0	0	0	0	10	30	0	100
8	M	535	1521.2	0	0	0	20	0	0	0	30	0	0	10	20	0	80
9	F	675	2767	0	30	0	0	0	0	30	0	0	0	30	30	10	130
10	M	575	1723	0	0	30	0	0	0	0	0	30	0	10	30	10	110
11	M	460	1040	30	0	0	0	30	0	30	0	0	30	0	30	0	150
mean		568.7	2129.8	5.5	10.9	7.3	3.6	10.9	2.7	10.9	2.7	5.5	5.5	15.5	28.2	2.7	111.8
stdev		159.1	1857.8	12.1	15.1	11.9	8.1	15.1	9.0	15.1	9.0	12.1	12.1	12.1	4.0	4.7	24.8

B

Fish No	Sex	SL(cm)	W (g)	Eyes	skin	fins	operc	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
12	F	500	1487	0	0	0	0	0	0	0	0	0	0	0	30	0	30
13	F	485	1196.2	0	30	0	0	0	0	0	0	0	0	10	20	0	60
14	F	480	1149	0	0	10	0	30	0	0	0	30	0	10	30	10	120
15	M	355	379.9	0	0	0	0	0	0	30	0	0	0	0	30	0	60
16	F	580	1427.3	30	10	0	0	0	0	0	0	0	0	20	30	0	90
17	F	350	496.8	0	0	30	0	0	0	0	0	0	30	10	30	0	100
18	F	460	1024.4	0	0	0	0	30	0	0	0	0	0	20	30	0	80
19	M	490	1107.2	0	0	0	0	30	30	0	30	0	0	10	30	10	140
20	M	355	379.9	0	30	0	0	0	0	30	0	0	0	10	30	0	100
21	F	460	1024.4	0	0	10	0	0	0	0	0	30	0	30	30	0	100
22	F	480	1149	30	0	0	0	0	30	0	0	0	0	10	30	0	100
mean		454.1	983.7	5.45	6.36	4.5	0	8.2	5.5	5.5	2.7	5.5	2.7	11.8	29.1	1.8	89.1
stdev		72.2	391.7	12.1	12.1	9.3	0	14.1	12.1	12.1	9	12.1	9	8.7	3	4.	30.5

Table : Heath Assessment Index for *Clarias gariepinus* sampled in the Return Water Dam Dam in C= spring; D = summer.

C

Fish No	Sex	SL(cm)	W (g)	Eyes	skin	fins	operc	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
23	F	360	502.7	0	0	0	20	0	0	0	0	0	0	10	30	10	70
24	M	358	381.3	0	0	0	0	30	0	0	0	0	0	30	30	0	90
25	M	440	1103	0	30	0	0	0	0	0	0	0	30	0	20	0	80
26	M	502	1479.8	0	0	0	0	30	0	30	0	0	0	10	30	0	100
27	F	520	1253.2	0	0	10	0	0	0	0	0	0	0	10	30	10	60
28	M	572	1815.4	0	0	0	0	0	0	0	0	0	0	10	20	0	30
29	F	513	1382.6	0	0	0	20	0	0	0	30	0	0	30	30	0	110
30	M	592	1430.3	0	30	0	0	0	30	0	0	0	0	0	30	10	100
31	F	500	1487	0	0	0	0	30	0	0	0	30	0	10	30	0	100
32	F	675	2767	0	10	0	0	0	0	30	0	0	0	30	30	10	110
33	M	502	1487.5	0	0	10	0	30	0	0	0	0	0	10	30	10	90
34	M	565	1723.5	0	0	0	0	0	0	0	N	0	0	10	20	10	40
mean		508.3	1401.1	0.0	5.8	1.7	3.3	10.0	2.5	5.0	2.7	2.5	2.5	13.3	27.5	5.0	81.7
stdev		91.3	612.1	0.0	11.6	3.9	7.8	14.8	8.7	11.7	9.0	8.7	8.7	10.7	4.5	5.2	26.6

D

Fish No	Sex	SL(cm)	W (g)	Eyes	skin	fins	operc	gills	p/branch	liver	spleen	hindgut	kidney	Hct	ecto	endo	HAI
35	F	460	1040	0	0	0	0	30	0	30	0	0	0	0	30	0	90
36	M	460	1024.4	0	0	30	0	0	0	0	0	0	0	10	30	10	80
37	F	360	381.3	30	0	0	0	0	30	0	0	0	0	20	30	10	120
38	M	340	496.8	0	30	0	0	0	0	0	0	0	30	10	30	10	110
39	F	910	5806.8	0	0	10	0	0	0	0	0	30	0	10	30	0	80
40	F	675	2767	0	10	0	0	30	0	30	0	0	0	10	30	10	120
41	M	780	5619.4	0	0	30	0	0	0	0	0	0	0	10	30	10	80
42	F	572	1815.4	0	0	0	20	0	0	0	0	0	0	10	30	0	60
43	F	480	1040	0	30	0	0	0	0	0	0	0	30	20	30	0	110
44	M	490	1107.2	0	0	0	0	30	0	0	0	0	0	30	30	0	90
45	M	502	1487.5	0	0	0	0	30	0	0	0	0	0	10	30	0	70
mean		548.1	2053.3	2.7	6.4	6.4	1.8	10.9	2.7	5.5	0	2.7	5.5	12.7	30.0	4.5	91.8
stdev		174.9	1921.4	9.0	12.1	12.1	6.0	15.1	9.0	12.1	0	9.0	12.1	7.9	0	5.2	20.4

Fish kill in the Return Water Dam.