METAZOAN PARASITES AND HEALTH OF SELECTED CYPRINIDS AT NWANEDI-LUPHEPHE DAMS

By

ESAU MATTHEWS MBOKANE

200307054

DISSERTATION
Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in Aquaculture

in the
FACULTY OF SCIENCE AND AGRICULTURE
(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO
SOVENGA
SOUTH AFRICA

SUPERVISOR: MR J THERON
AQUACULTURE RESEARCH UNIT
CO-SUPERVISOR: DR WJ LUUS-POWELL
DEPARTMENT OF BIODIVERSITY

2011

ABSTRACT

The present MSc dissertation emanates from seasonal surveys conducted by the fish parasitological group of the Department of Biodiversity and Aquaculture Research Unit of the University of Limpopo, Turfloop Campus. The first part of the present study was aimed at investigating the metazoan parasites of three cyprinids occurring in the Nwanedi-Luphephe Dams. The main purpose of it was to determine temporal changes in the intensity of infestation in terms of prevalence, mean intensity and abundance of parasite species parasitizing the cyprinids studied over a two year period. Ecological parameters including species host-specificity, seasonality, and gender preference and host size versus species intensity are discussed for each parasite.

Altogether 152 specimens were examined for parasites and a total of 2 432 metazoan parasites of ten species were recorded. At the sampling site, all three hosts co-occurred, however, a substantial proportion of *Barbus radiatus* was collected from the perennial stream feeding one of the twindams. Fish were sampled by means of gill nets and electrofishing or seine netting in accordance with the habitat conditions. Hosts were killed and organs investigated for metazoan parasites. After collection of parasites, standard methods for processing individual parasites were followed. The results obtained revealed the following groups of parasites; **monogeneans** (ectoparasites) included *Dactylogyrus spinicirrus*, *D*. *afrolongicornis afrolongicornis*, *D*. *afrolongicornis alberti*, *Afrodiplozoon polycotyleus*, *Gyrodactylus* sp., and *Dogielius* sp. (all recorded from the gills); **Crustacea,** *Dolops ranarum* was found from the mouth cavity, gills and skin of *Labeobarbus marequensis*. Of these, only two specialists, both monogeneans, were found on *Barbus trimaculatus* namely, *D*. *afrolongicornis afrolongicornis* and *D*. *afrolongicornis alberti*. Based on morphology of the haptoral hard parts, these two species were almost similar to each other than to *D. spinicirrus*. The appreciable difference between *D*. *afrolongicornis afrolongicornis* and *D*. *afrolongicornis alberti* was mainly in the shape of the marginal bar. Both *D*. *spinicirrus* and *A*. *polycotyleus* were widely distributed and recorded on the gills of all hosts during all seasons. Both species were recorded for the first time on *B*. *radiatus*. Also, *D*. *spinicirrus* was recorded for the first time on the gills of *B*. *trimaculatus*. Based on comparison with the original material, the species could be identified to species level. These analyses provided sufficient evidence for

restoration of *Afrodiplozoon polycotyleus* as a valid taxon. The existence of two species, *Gyrodactylus* sp. and *Dogielius* sp. were recorded for the first time on *B*. *radiatus* in South Africa, and this possibly represents new species.

The **endoparasites** included the following groups: **digeneans-** *Diplostomulum* metacercariae from the eyes of *Lb*. *marequensis*, *Ornithodiplostomum* sp. and black spot (grubs) were recorded from *B*. *trimaculatus*. The latter was also recorded in the muscle of *B*. *radiatus*. Unidentified digenean cysts were recovered from the gills and in the body cavity of both *Lb. marequensis* and *B. trimaculatus*; **nematodes** were represented by *Contracaecum* larvae in the body cavity of both *Lb*. *marequensis* and *B*. *trimaculatus*; **cestodes** were represented by gryporynchid larvae from the intestine of *B*. *radiatus*. The general high prevalence and intensities of ectoparasites recorded is an indication that the Nwanedi-Luphephe Dams has a biotic mechanism which might have enabled it to sustain the growth rate of ectoparasite intra-population. There was no correlation between either fish length or condition factor and the number of parasites. The study indicated that the abundance of monogeneans is partly influenced by season and that of endoparasites was principally governed by the presence of intermediate hosts and definitive hosts.

The second part of this dissertation dealt with the health status of *Lb*. *marequensis*. Fish health was assessed using condition-related indices including condition factor and a modified Health Assessment Index (HAI) and the associated Parasite Index (PI). The HAI was performed to determine and examine any macroscopic abnormalities regarding external features and internal organs. The purpose of combining the two indices was to use the infestation of the metazoan parasites found on and/or in *Lb*. *marequensis* to determine whether or not the environment they live in was healthy. Both indices together with the condition factor provided relatively simple and rapid indications of how well fish were coping in their environment. The HAI score varied amongst the four sampling seasons. The highest individual mean value was 63 in winter, followed by a score of 50 in autumn, while the lowest were 42 and 33 in summer and spring respectively.

To authenticate the HAI and PI data, certain water quality variables were measured and are discussed in detail in this dissertation. The Nwanedi-Luphephe Dams are generally believed to have good water quality. This was supported in this study; conditions assessed in fish using the aforementioned indices did not differ

greatly between seasons, nor did the conditions deviate appreciably from normality. The HAI values were low overall which signifies a healthy fish profile for the system. The present investigation showed the existence of differences in the occurrence of individual parasite to be linked to water temperature changes. Thus, seasonal changes do influence parasite developmental stages to a certain degree. Tested heavy and trace metals were within the permissible limits as provided by the Department of Water Affairs and Tourism (DWAF, 1996).

DECLARATION

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

E.M. Mbokane

[redacted]

ACKNOWLEDGEMENTS

First and foremost I would like thank both my supervisors, Mr Johan Theron and Dr Wilmien J Luus-Powell. These two are exceptional; together they taught me to be a better student and to be a better person since from my honours studies and I couldn't have accomplished anything in the field of Parasitology without their wisdom and guidance. Thank you, may Jehovah our God extend His great hand unto you.

I would like to equally thank Mr Moses M Matla for his boundless assistance with the identification of the parasites and for helping me with the drawings.

Sincere thanks go out to the National Research Foundation for their financial support for this project. I equally acknowledge the Research Administration and Aquaculture Research Unit of the University of Limpopo for their financial assistance for my research. Without your wide-ranging help, I could not have arrived at this level.

I would also like to extend my thanks to Mrs GN Madanire-Moyo for helping me with the statistical analysis. Sincere thanks are also due to Prof NAG Moyo for his helpful comments and suggestions on an earlier draft of this thesis.

In no specific order, special thanks go out to Mr HE Hattingh, Prof PAS Olivier and Miss KP Ledwaba, who all ecstatically assisted me with the collection of the fish. I would like to extend a special thanks to the students from the Department of Biodiversity and Aquaculture for their great help during my research. I recognize my parents (Bailoni Mbokane and Letta Masina), my six brothers and my sister for without their assistance and love I could not have successfully completed this project.

Many thanks to my friends too numerous to list who provided a place to relieve stress induced by countless hours of writing while others did just the opposite, adding more stress. To my fiancé, Jabulile, I am highly indebted to you; you have so much patience with me.

All of these extraordinary people assisted me significantly through my studies and made my stay at Turfloop intellectually stimulating. I may not have been the person you thought I have been, but I sincerely hope that our association has been a great one. May God provide you with the strength you need to prosper and more importantly, may He grant you wisdom. To Jehovah God for His bounteous blessings and protection throughout my studies, I'm certainly beholden.

DEDICATION

This dissertation is dedicated to my dearly beloved mom Letta Zodwa Masina (Mamncane).

INDEX

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Parasites are organisms that live in or on other organisms and derive benefit such as nutrition at the host's expense, usually without killing the host. They depend on these organisms not only for food, but also for shelter, transportation and to complete their respective life cycles. There are different kinds of parasites ranging from micro- to macro-parasites which belong to many different phylogenetically distinct taxa, and as such, display a variety of life histories and body forms.

Similar to all other creatures, parasites are present in all ecosystems (Pietrock and Marcogliese, 2003) and form an integral part of every aquatic ecosystem, representing a major factor in global biodiversity. Moreover, fish parasites may be important in regulating the abundance of host populations through parasite-induced mortality of heavily infested hosts (Anderson and May, 1979). In addition, fish parasites can be important biological indicators to describe migration patterns of fish stocks, as well as trophic and phylogenetic interactions (Marcogliese, 2005). Infestations with parasites such as helminths are quite common in both feral and cultured fish where some helminths have been reported to cause veterinary problems in fish culture (Williams and Jones, 1994).

Freshwater fish parasitology is an important and rapidly advancing field of aquatic science. Over the past few decades, it has contributed to various scientific aspects viz., the taxonomy, classification, morphology, ecology and phylogeny of fish parasites. Though the latter two aspects have received considerable attention in the USA and Europe(Poulin, 1995, 2002), this is not the case in South Africa. Topics such as host-parasite co-evolution or host parasite interaction (host and site specificity, gender preference and host and the environment) have not yet been fully explored locally.

In South Africa, the two indigenous fish species mostly angled for and eaten, as well as being cultured, are *Clarias gariepinus* and *Oreochromis mossambicus*. These fish species are prone to heavy infestations with various parasites, viz., monogeneans, digeneans, nematodes, cestodes, and copepods, to mention but a few. In fish farming or aquaculture, for example, some parasites may be highly deleterious for cultured species causing serious outbreaks of diseases and contribute to high fish mortalities resulting in economic losses (Barson, 2004). According to Chandra (2006), parasites of fishes may cause a decrease in growth

rate, weight loss, emaciation or even suppress reproductive activity. In natural habitats, parasites can kill their host by increasing host susceptibility to predation (Rousset *et al*., 1996), decreasing host density or reducing the marketability of commercially produced fish (Rhode, 1993) and thus raising public health concerns especially in areas where raw or smoked fish is consumed. Furthermore, parasites may threaten the abundance and diversity of indigenous fish species in natural systems (Mashego, 2001).

An excellent example of the foregoing statements would be *Gyrodactylus salaris*'s introduction into the salmon industry in Norway (Cable and Harris, 2003), which has led to uncontrollable epidemics and mortalities resulting in massive economic losses. In South Africa, high mortalities have thus far only been reported in aquaculture ventures, not in the wild (Paperna, 1996; Luus-Powell *et al*., 2006; Luus-Powell *et al*., 2009).

Whereas many of these parasites are passed directly between ultimate hosts, others need to navigate through a series of intermediate hosts before reaching a host in (or on) which they can attain sexual maturity (Barber *et al*., 2000). Freshwater fish can serve as intermediate or paratenic (transport), as well as definitive hosts in the life cycles of metazoan parasites. Piscivorous birds in particular play a significant role in the completion of many of these life cycles and in the dispersal of endoparasites (Morley, 2007).

In the present study, focus was directed to the occurrence of macro-parasites on freshwater fish hosts which are of economic importance. Mashego (1982) investigated these hosts, but ecological aspects of the presently studied parasites are still relatively poorly known. The macro-parasites are a group of metazoan parasites, composed according to Barber *et al.* (2000), mainly of members of the Platyhelminthes (flatworms, including monogenean, digenean trematodes and cestodes), Nemathelminthes (roundworms, including nematodes and acanthocephalans), annelids (such as leeches) and arthropods (true lice and parasitic copepods).

The reason that parasites are likely to infest all fish in all aquatic environments is because aquatic habitats offer ideal conditions for the maintenance and evolution of parasite life cycles (Barber *et al*., 2000). Because of the central role played by freshwater fishes in such ecosystems, especially with respect to their role as consumers in food chains, they are frequently utilised as hosts by parasitic

organisms. Furthermore, fishes are highly mobile and this may be attractive to certain kinds of parasites since they create the potential for further dispersal. Probably for all of these reasons, fish that live in natural ecosystems are rarely found to be free from infestations. At the same time, the external environment has an influence on the parasitic infestation levels. Environmental factors (such as temperature, oxygen content, salinity, etc.), coupled with pollution, might directly or indirectly influence both the prospective host and the parasite, which under favourable conditions enhances the survival of the latter (Pietrock and Marcogliese, 2003).

Over the past several years, studies on freshwater fish parasites have globally increased almost exponentially because of the growing interest in the development of fisheries and aquaculture as cheap sources for protein to feed the rapidly growing human population, more especially in some African communities (Khalil and Polling, 1997). To manage this resource in a manner in which it meets its target, it is of paramount importance to have a thorough knowledge of the taxonomy, distribution, biology and ecology of parasites as one of the detrimental factors affecting development of fisheries in southern Africa (Khalil and Polling, 1997). A checklist was compiled by Khalil and Polling (1997) listing 568 species of adult helminths and several larval forms infesting African fishes. In addition, Paperna (1996) published a concise detailed update of the parasitic diseases of fish in Africa, which describes the occurrence and geographical distribution, life cycles, pathology, epizootiology and control of the parasites.

In South Africa, research on the parasitic fauna of freshwater fish has been done by a number of workers (Prudhoe and Hussey, 1977; Whitfield and Heeg, 1977; Mashego, 1977, 1982, 1989, 2000, 2001; Mashego and Saayman, 1981; Boomker, 1982, 1994a, b; Britz *et al.*, 1985; Avenant and Van As, 1985; Avenant-Oldewage, 1991; Basson and Van As, 1991; Saayman *et al.*, 1991; Dippenaar *et al*., 2001; Luus-Powell *et al*., 2003; Olivier *et al*., 2009; Madanire-Moyo *et al*., 2010), just to mention a few.

It should, however, be noted that a substantial proportion of the above studies were carried out to satisfy taxonomic needs and therefore contain little ecological information on specific fish parasites in southern Africa, even less of South Africa. Thus, part of the main purpose of this thesis was to investigate temporal changes of

fish parasite communities in relation to some abiotic and biotic environmental factors affecting parasites.

1.2 The role of aquaculture on the distribution of parasitic diseases

Aquaculture has emerged as an important industrial force of environmental, economical and social change in many regions in the world especially Norway, the United Kingdom, Chile, Canada, New Zealand, Asia, Israel and South America (Amoako, 2006). It is now regarded as one of the fastest-growing food production systems in the world. Over the past 15-20 years or so, it has developed into a global industry, with over 60 countries engaging in the production of more than 250 different species of fish and shellfish. Despite this phenomenal growth in global aquaculture, South Africa's contribution has remained low, accounting for less than 1% of the African aquaculture production (Feike Natural Resource Management Advisors, 2008).

As a result, there has been a significant increase in South African imports of fish products over the last couple of years. It is widely recognised that importation of fishes for aquaculture and the ornamental trade between different facilities and regions, has resulted in rapid national and international spread of parasites and also exposed potentially highly susceptible populations to new parasites (Peeler *et al*., 2006). One of the most inevitable risks inherent with movements of living fish is the inadvertent introduction of pathogens, parasites and disease associated with the fish to new hosts in the new area. Despite the fact that some parasites are host-specific, many are capable of infesting a wide range of host species. According to Gollasch *et al*. (2008), parasites transferred from alien to native species may have severe consequences because the native host and the alien parasite's relation lacks the evolutionary time needed to evolve an equilibrium relationship, or for the host to develop immunity.

A good example is the importation of fish from Asia a couple of decades ago which is believed to have resulted in the introduction of some helminth parasites such as *Bothriocephalus* sp. into South Africa, posing a significant risk to native freshwater fish (Mashego, 1982; Bertasso, 2004). After several years, the parasite

has become successfully established in South Africa, infesting a wide range of cyprinids. This example illustrates the high risk associated with the introduction and dispersal of parasites and disease as a result of fish movements.

Also, during the importation of fish, the movement and disposing of contaminated water, containers, and other equipment into rivers or dams, may also be responsible for the introduction or transport of parasites (Peeler *et al*., 2006). While aquaculture does not necessarily create disease as such, the conditions under which aquaculture activities are executed, may do. For instance, cage systems have the highest potential risk for parasites and diseases transfer because there is no impermeable barrier between the cage and the aquatic environment. Thus, water exchange in cages is uninhibited resulting in many water borne diseases and parasites being capable of spreading between farmed fish and wild stocks potentially altering community structures within feral ecosystems (Kent, 2000).

There is currently very little knowledge on the distribution and abundance of parasites in aquatic ecosystems making it difficult to know if diseases observed in aquaculture are native to the area or if they may have been introduced. Therefore, accurate identification of parasites may be important for national bio-security.

1.3 The use of bio-monitoring tools in water quality assessment

In the past 40 years, water pollution in South Africa was controlled by applying uniform effluent standards which imposed strict limits on the quality of discharged effluents. According to Roux *et al*. (1993), this was successfully applied only in certain specified catchments. That means all effluents produced from mines and municipalities, which may finally reach rivers or dams that are subject to regulation, and required to meet either the general effluent standard or the special effluent standard (Van der Merwe and Grobler, 1990). In 1991, the Department of Water Affairs and Forestry (DWAF, 1991) amended its water quality management policies and strategies, adopting a receiving water quality based approach and pollution prevention, which has changed in recent years from controlling pollution at source to a user-based philosophy (Roux *et al*., 1993).

One of the users that must be considered in terms of the water quality requirement is the aquatic environment. Keeping in view the need to manage aquatic

environments, DWAF (1996) produced concise water quality guidelines for aquatic ecosystems with standard limits of pollutants which are harmless to aquatic life. Although the guidelines provide a summary of water quality requirements for aquatic ecosystem, it is regrettable that information about aquatic environments is still scant and poorly documented, making it difficult to reliably assess the nature and extent of alteration as well as the rate at which water quality deteriorates (Roux *et al*., 1993).

Monitoring water quality in South Africa and neighbouring countries is however still expensive. Existing tools provides little information about the effects of pollution at the biological level when chemical and physiological changes or responses are not considered (Roux *et al.*, 1993). South Africa's new water law recognises that basic human and environmental needs should be met and that the exploitations of water in all aspects (quality and quantity) shall be sustainable in the long term (Davies and Day, 1998). Therefore protecting the needs of the environment requires tools that can be used to monitor environmental conditions as well as for settling ecological objectives to ensure the proper and sustainable management of the resource.

In South Africa, as in most countries, water quality monitoring has traditionally been determined by measuring physical and chemical variables of a water body (Roux *et al.*, 1993). The shortcomings of these analysis is that; firstly, it considers water passing at the moment of sampling and are thus only accurate at the time the sample is taken and secondly, it does not give an insight into biological threats to ecosystem health (Marx, 1996). It is therefore within this "water quality monitoring" context that the value of bio-monitoring lies. This prompted the development of biological monitoring methods, which involves the use of biological responses or indicators to determine the effect of changing environmental conditions, based on the assumption that the biotic integrity of a freshwater ecosystem is often reflected by the health of its fauna and flora (Poulin, 1992; Roux *et al.*, 1993).

Various bio-assessment methods have been developed over the last three decades, worldwide, as well as in South Africa, varying in complexity and/or implementation. These techniques are used for assessment of general river health as influenced by a variety of factors but principally the water quality. One of the most recently introduced bio-monitoring tools in South Africa is the use of the fish Health Assessment Index (HAI) and fish parasites as bio-indicators (Avenant-Oldewage,

2001). Hence, this research aims to determine fish health and water quality by applying the HAI and PI on three selected fish species.

1.4 Relevance of parasites in water quality monitoring

In recent years, new applications of fish parasites as appropriate biological indicators of environmental stress have been developed (e.g. Khan and Thulin, 1991; Poulin, 1992; Overstreet, 1993; MacKenzie *et al.*, 1995). These applications emanates from the fact that parasites of fish are an indigenous component of a healthy ecosystem. Also, the biotic response of parasites to environmental stressors is reflected in the health of fish (Marcogliese and Cone, 1997; Overstreet, 1997; Sures, 2004). Their functional importance in food webs makes them good bio-indicators particularly if anthropogenic pollution is to be monitored. In addition, parasites in aquatic environments are considered biologically sensitive and respond to changes that occur in water.

In particular, ectoparasites may provide information about the biological quality of the water they reside in because they are just as exposed to the environment as their host. It is thus assumed that poor water quality will adversely affect ectoparasites to a greater degree than it would endoparasites (Avenant-Oldewage, 1994b). Equally important, fish parasites with complex life histories which involve multiple hosts (e.g. snails, crustaceans, frogs and birds) can provide both insights as well as a comprehensive understanding of aquatic ecosystems (Dzikowski *et al*., 2003). Thus, the sensitivity by fish parasites to deteriorating water quality has led to their utilization as bio-indicators of an aquatic ecosystem.

1.5 Introduction to the Health Assessment Index

In South Africa, the fish Health Assessment Index (HAI) and associated Parasite Index (PI) is one of the many bio-monitoring tools that can be used to effectively monitor fish health (Avenant-Oldewage and Swanepoel, 1993). The HAI was developed by Goede and Barton (1990) and further quantified by Adams *et al*. (1993). It was intended for the monitoring of organic pollution (pulp mill effluent

contamination) in North America (North Carolina, Tennessee, Alabama, and Kentucky) and the British Isles to determine the effect of pollution on the environment (Adams *et al*., 1993). It provides a health profile of the fish based on a systematic examination of the appearance and condition of external and internal tissues and organs. A change in the appearance of an organ or tissue system from the normal condition is assumed to reflect a response to a chronic stressor.

On account of intricacy when compared with previous techniques, the HAI is found "to be a simple and inexpensive means of rapidly assessing general fish health in field situations" (Avenant-Oldewage, 2001). In addition to its simplicity, it allows statistical comparison to be made among environmental conditions to determine correlations between fish health and environmental variables. One of the variables in the HAI was the presence or absence of parasites. During the past decade, work by several researchers on the presence or absence of parasites in an ecosystem, has lead to the realisation that fish parasites can be used as biological indicators of aquatic ecosystem health. This led to the development of the Parasite Index (PI) in South Africa (Crafford, 2000; Watson, 2001; Bertasso, 2004). The HAI and associated PI are meant to predict whether or not the environment is deteriorating by distinguishing between localities in terms of water quality. The HAI and PI can be applied to sites with different water quality to allow comparisons amongst the two sites, or at a selected site to verify the ecosystem health of that particular site. In the present study, one locality was investigated, namely the Nwanedi-Luphephe Dams. In addition to applying the HAI and PI at this site, the recommendations of Groenewald (2000) and Crafford and Avenant-Oldewage (2009), that the HAI should be used to compare different seasons at the same locality and with the same fish species, was followed during this study.

1.6 Fish hosts

The family Cyprinidae consists of at least 275 genera, comprising 1 600 species from Africa, Europe, Asia and North America. In Africa, the Cyprinidae are widely distributed throughout the continent, with at least 24 genera and 475 species. In southern Africa, there are approximately 8 genera and about 80 species (Skelton, 2001). Three species of the Cyprinidae, i.e., *Barbus trimaculatus* Peters, 1852,

Barbus radiatus Peters, 1853 and *Labeobarbus marequensis* Smith, 1841 were selected as hosts for this study. These fish were selected because *Lb*. *marequensis* is arguably one of the most popular indigenous freshwater fish species caught by anglers across the country, and hence support valuable and growing recreational and subsistence fisheries. In addition, they are valuable ecological indicators of aquatic ecosystem health as they require rivers and dams that have diverse habitat and are of good water quality. Only a few studies have reported on the parasites infesting these fish species and their health status. Thus, the present study, to some extent, intends to use these fish as indicator species by applying the HAI.

1.7 Aim of the study

The aim of this study was to determine the parasite composition (in terms of prevalence, mean intensity and abundance) and health of *Lb. marequensis*, *B. trimaculatus and B. radiatus* from the Nwanedi-Luphephe Dams on a seasonal basis and correlate this with water quality. The Fish Health Assessment Index and Parasite Index were applied as bio-monitoring tools or bio-indicators during this study.

The present study formed part of a major study programme of fish parasites at the Nwanedi-Luphephe Dams, a project undertaken by the Department of Biodiversity of the University of Limpopo. It is hoped that the results from this project would provide meaningful contributions to the expansion and development of fish health management programmes in South Africa in general and the Limpopo Province in particular.

1.8 Objectives

The objectives of this study were to:

1. Determine temporal variations in parasite composition of *Lb. marequensis*, *B. trimaculatus and B. radiatus* in Nwanedi-Luphephe Dams.
2. Determined the correlation between host total length and parasite composition of *Lb. marequensis, B. trimaculatus and B. radiatus*.

3. Determine the HAI and PI of *Lb. marequensis* in relation to selected water quality parameters.

In order to achieve the aim and objectives of this study the results are presented in five chapters:

Chapter Two contains a general description of the study area and fish species used during this study. The material and methods used for water analysis, the Health Assessment Index and PI determination and parasite collection are discussed herein. The fixation, preservation and staining methods used for parasites are listed.

Chapter Three describes the results obtained and certain aspects of the ecology, morphology, distribution records and host specificity of all the parasites encountered during the surveys. Each group of parasite species is discussed separately.

Chapter Four focuses first on the health of the fish through the application of the Health Assessment Index and Parasite Index and secondly on the selected water quality parameters. A brief history on the development of the HAI and PI is given.

Chapter Five provides a general summary of the results, conclusion and recommendations.

Chapter Six contains references used in all chapters in this dissertation; it concludes with an appendix containing the raw data and tables for the statistics and the HAI.

CHAPTER 2

MATERIALS AND METHODS

2.1 Sampling localities

The study was conducted at the Nwanedi-Luphephe Dams in the Limpopo Province of South Africa (Figure 2.1A & B). These adjoining dams are located in the Nwanedi Nature Reserve and have an approximate total capacity of 19.1 million m^3 (Angliss *et al*., 2007). They are situated between latitude 22^o39.492'S and longitude 30^o25.342'E (altitude of about 600 m a.s.l.). The Nwanedi- and Luphephe streams are situated between mountains which have its origin in the foothills of the Soutpansberg mountains. The Nwanedi stream rises in the upper Soutpansberg mountains, where a number of small streams converge at an altitude of approximately 1 100 m. The streams drop through a steep gorge before entering the Nwanedi-Luphephe Dams. From their respective sources, the Nwanedi and Luphephe streams flow in a north-easterly direction for approximately 50 km across the far north-eastern part of Limpopo Province.

The climate in the area is subtropical and falls within the northern summer rainfall area of South Africa, which is characterized by extremely dry and mild winters. The water level in the dams fluctuates. The mean annual precipitation varies between 450 and 650 mm, while mean annual runoff is 60 mm.

Luphephe Dam lies adjacent to Nwanedi Dam (Figure 2.1 A & B). They are connected by a 2.5 m deep canal, near the two respective dam walls, when the dams are more than 80% full. However, during the dry season the water level drops and the dams are then separated from one another. Due to this connectivity of the dams the fishes of both dams are considered to be of the same population (Luus-Powell, 2004).

The Nwanedi-Luphephe Dams were constructed in 1964 by the Department of Water Affairs and the names of the dams are derived from the respective mountain streams feeding them. The streams are perennial and flow through areas with limited or no industrial development. Game farming is the dominant land use of the area. In general, these dams have been declared to have very good water quality.

The catchment has a high - moderate Ecological Importance and Sensitivity (EIS), largely due to the fact that a substantial portion of the upper catchment falls in the Nwanedi Reserve (Angliss *et al*., 2007). These dams were particularly selected because of the good water quality and as very few studies have been conducted on

the cyprinid communities of these dams. A temporary field laboratory was set up for each trip (Figure 2.2). The field work was undertaken by the parasitological study group from the Department of Biodiversity of the University of Limpopo, South Africa. Each member of the group was assigned a specific fish species and a specific group of parasites. Thus, all fish collected were optimally utilized.

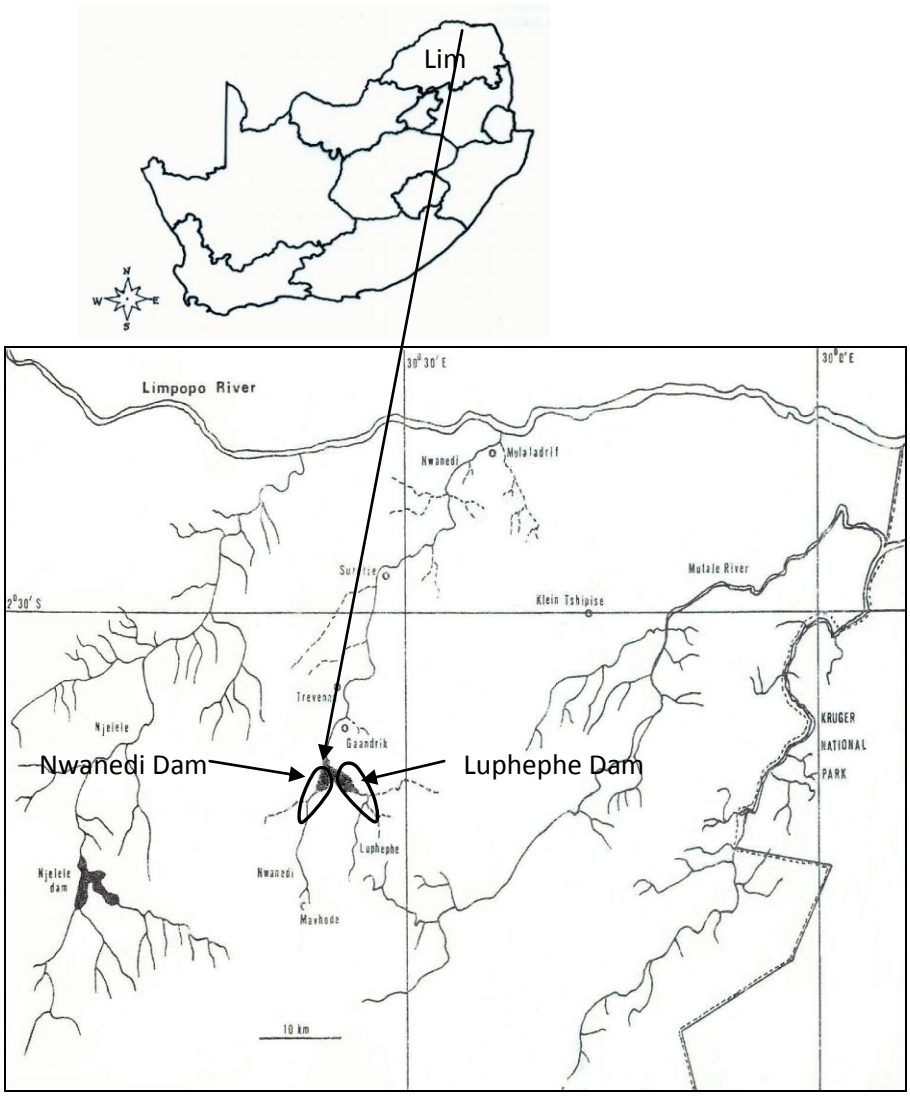

Figure 2.1A: Map showing the location of the Nwanedi-Luphephe Dams and the Nwanedi Nature Reserve, Limpopo Province. Abbreviations: Lim = Limpopo Province.

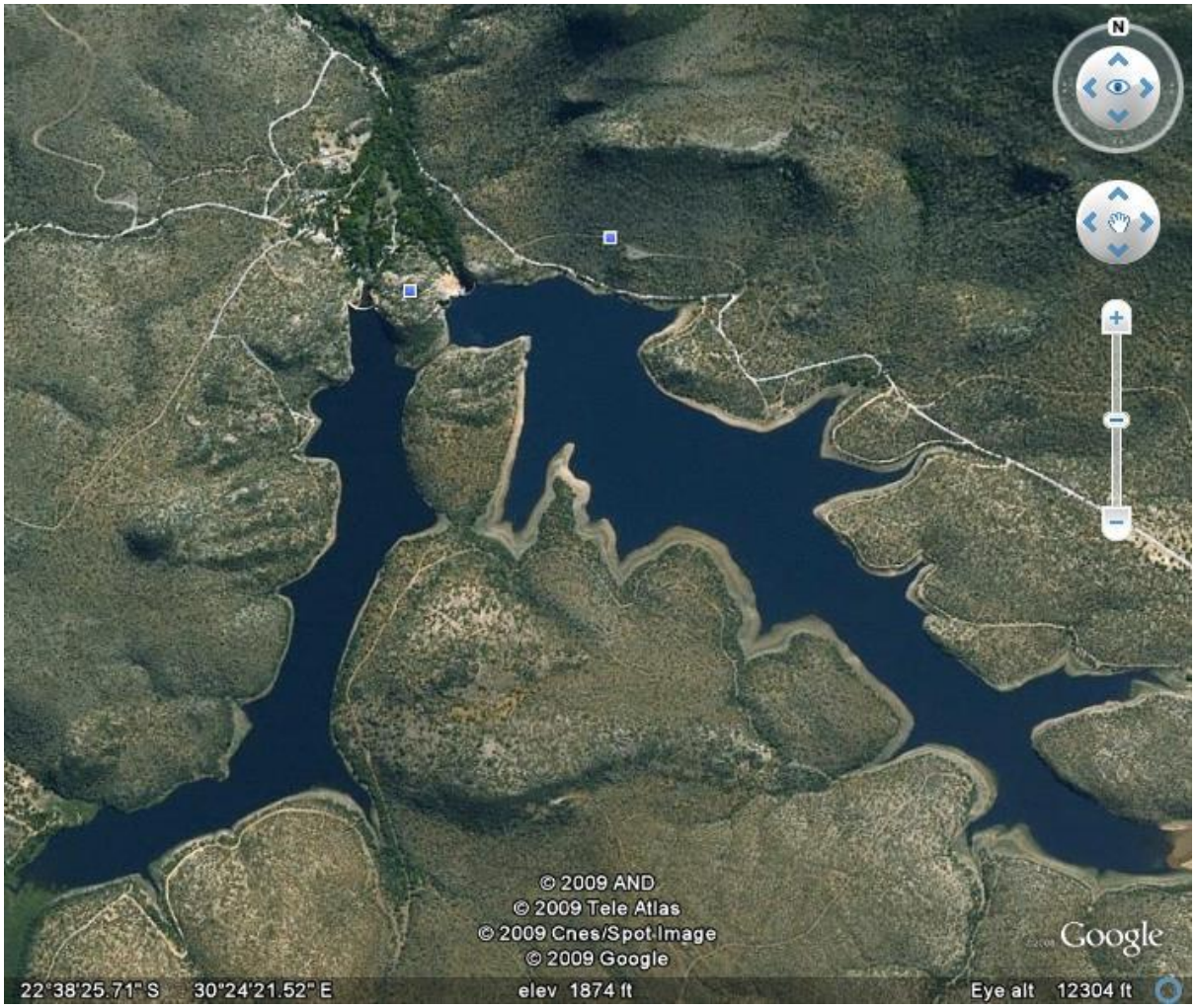

Figure 2.1B: Satelite view of Nwanedi Dam (Left) and Luphephe Dam (Right) with the canal visible just South of the two dam walls.

Figure 2.2: Field laboratory at the Nwanedi-Luphephe Dams.

2.2 Water quality parameters

Selected water quality parameters were measured during each seasonal survey. Surface water quality parameters measured *in situ* during each survey included: temperature, dissolved oxygen content, percentage oxygen saturation, salinity, pH and conductivity using a handheld YSI (556 MPS) multiparameter instrument. Water samples were collected during each survey in a 500 ml plastic polyethelene bottles pre-treated in an acidified phosphate-free bath and rinsed in deionised water. The bottle caps were airtightened to prevent atmospheric oxygen dissolving into it and frozen immediately for further analyses. The frozen water samples were transported to an accredited water analysis laboratory in Pretoria at the end of each survey where they were analyzed for the following water constituents: calcium, chlorine, potassium, water hardness, magnesium, sodium, ammonium, nitrate, phosphate, sulphate, turbidity and total dissolved solids; trace metals; aluminium, copper, iron, manganese, lead and zinc.

2.3 Fish hosts

Barbus radiatus (Figure 2.3) is widespread in Africa, from Uganda southwards, including the Zambian Zaire, Cunene, Okavango, Zambezi and east coast rivers south to the Phongolo system. This is an attractive aquarium fish species that favors marshes and marginal vegetation of streams, rivers and lakes. It is active in subdued light and during the night. It can attain a size of 120 mm standard length (Skelton, 2001). Its normal diet includes insects and other small organisms.

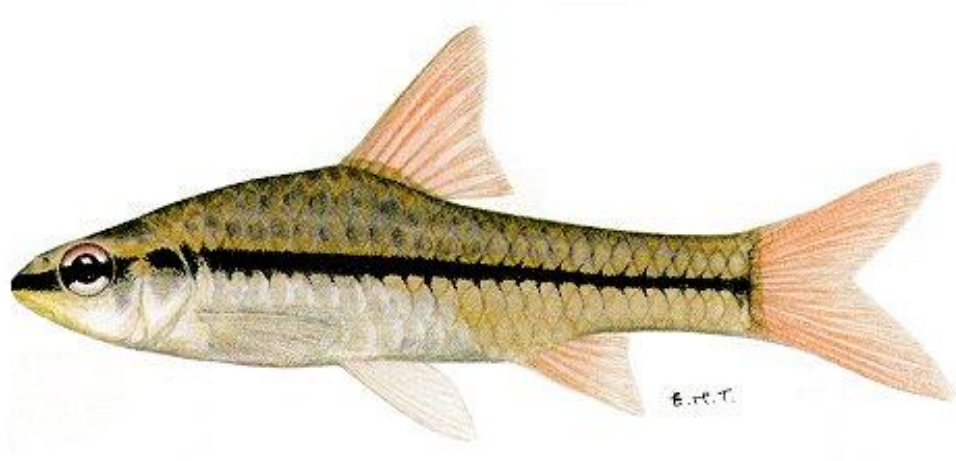

Figure 2.3: An illustration of *Barbus radiatus,* as collected at the Nwanedi-Luphephe Dams (from Skelton, 2001).

Barbus trimaculatus is commonly known as the "Threespot barb" (Figure 2.4) because of the three spots along its lateral line. It is normally distributed in the East coast from Ruvuma, Tanzania, to Umvoti in KwaZuluNatal, also Orange, Cunene and Zambian Congo systems. They are generally considered hardy species, common and found in a variety of habitats, especially where vegetation occurs. Its normal diet includes insects and other small organisms. It is commonly used as bait for tigerfish (Skelton, 2001).

Figure 2.4: An illustration of *Barbus trimaculatus,* as collected at the Nwanedi-Luphephe Dams (from Skelton, 2001).

Unlike the two species from the genus *Barbus* discussed above, *Labeobarbus marequensis* is bigger, reaching a maximum of 47 cm total length (Figure 2.5) according to Skelton (2001). This species often migrates upstream to breed, usually over gravel beds (Skelton, 2001). *Labeobarbus marequensis* is widely

distributed in the middle and lower Zambezi River and from there southwards to the Phongolo system. Larger specimens generally occur in Lowveld rivers (below 600 m altitude). This species favours flowing waters of perennial rivers and is uncommon in dams. Its diet includes a wide variety of food items, primarily algae and aquatic insect larvae; also small fishes, snails, freshwater mussels and drifting organisms such as beetles and ants. It usually occurs with the bushveld small scale yellowfish in southern Limpopo tributaries. It is an important angling species notwithstanding its relatively small size (Skelton, 2001).

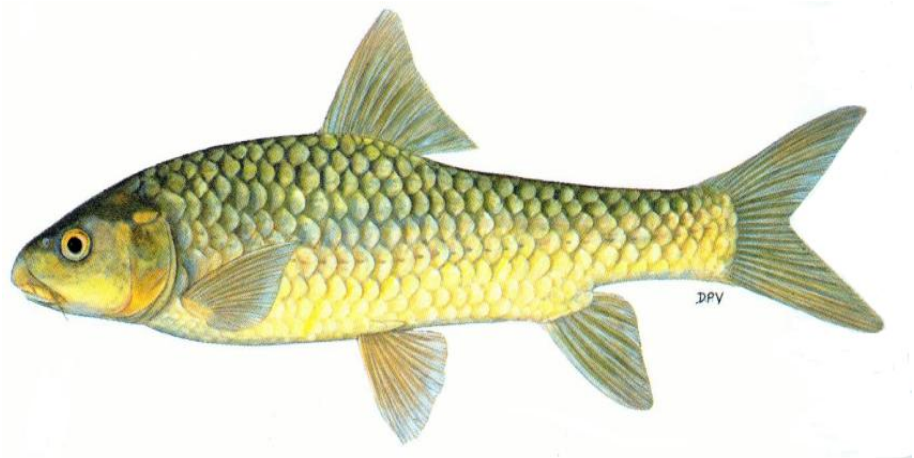

Figure 2.5: An illustration of *Labeobarbus marequensis,* as collected at the Nwanedi-Luphephe Dams (from Skelton, 2001).

2.4 Sampling of fish

Four seasonal surveys were undertaken in 2008 and 2009, i.e. autumn (April 2008), winter (July 2008), spring (October 2008) and summer (January 2009). At the sampling site, all three hosts co-occurred, however, a substantial proportion of *B. radiatus* was collected from the perennial stream feeding the Nwanedi Dam. Fish were sampled using a variety of collection methods such as gill nets, electro-fishing and seine netting (Figures 2.6 A - C) according to fish habitat conditions. When the water was very shallow or small pools were encountered, a variety of hand held scoop nets were employed. The other sampling sites were located in small, narrow and shallow streams that flow into the dams or in the outflow beloe the dam walls. *Barbus radiatus* were mainly found in small pools in the streams and thus collected

by electro-fishing using a backpack battery operated fish shocker (Model No. BC-24 ps from Smith Inc Vancouver WA USA) as shown in Figure 2.6 C.

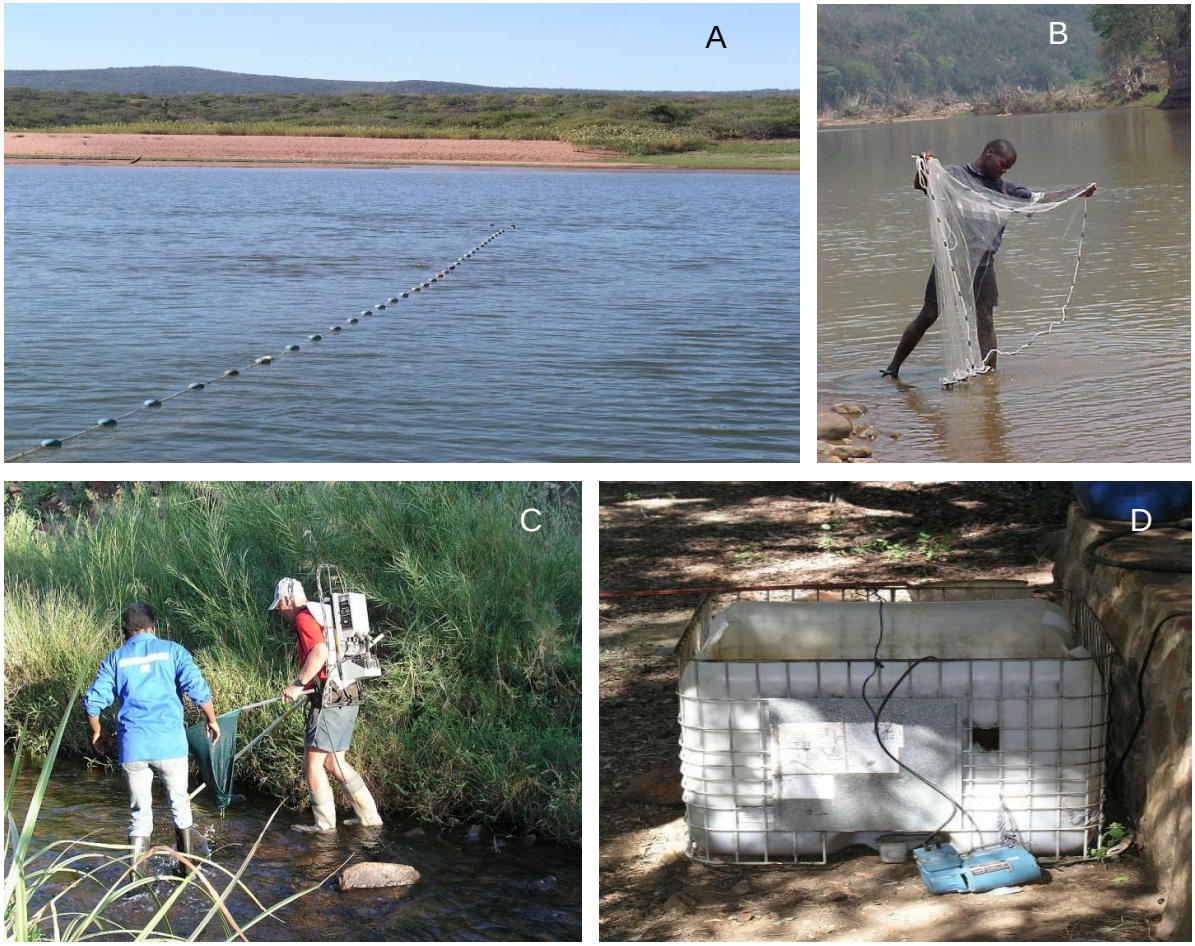

Figure 2.6: A - Gill nets set in Nwanedi-Luphephe Dams**, B** -cast net used to collect small fish, **C** - hand held scoop nets together with the backpack battery electric shocker used to shock in the streams **D** - Holding tanks filled with dam water through which air was bubbled during examination of fish at the Nwanedi-Luphephe Dams.

Gill nets were effectively used for *Lb*. *marequensis* and *B*. *trimaculatus* in deep waters at various locations in the dams (Figure 2.6 A). These nets consisted of series of nets, each of a different mesh size. The minimum mesh size was 30 mm and the maximum 150 (50, 70, 90, 110, 130 and 150 mm). The nets were set at dusk, left overnight and retrieved the following morning at sunrise. Seine nets were also used in pools and in the streams to collect *B*. *radiatus*. In slightly deeper waters, cast nets were very effective for the collection of the two smaller *Barbus* hosts (Figure 2.6 B). Fish were placed in tanks containing water from the locality to ensure maintenance of environmental conditions and immediately transported to the

laboratory alive. At the dams, all fish caught were identified to species level. During the storage of fish in the field laboratory, the dam water was aerated with an air pump to keep fish alive before examination (Figure 2.6 D).

2.5 Examination procedure

As soon as fish were removed from the net and identified, macroscopic examination on the external surface of the fishes for mobile ectoparasites was done on the research boat. With the aid of a fine brush, ectoparasites such as *Dolops ranarum* were collected and stored in glass bottles filled with dam water. In the laboratory, these parasites were fixed in 70% ethanol. Body surface (skin and fins) and gills of the fish were examined for the presence of ectoparasites or larval stages of Digenea (metacercariae). Skin smears were prepared by holding the fish firmly by the head and scraping the skin surfaces and the body mucus from the caudal part (upper dorsal area between the anal fin and the caudal fin) of the fish with a cover slip. Mucus was then placed on a glass slide with a drop of dam water added, then covered with a cover slip. The specimen was then examined with the aid of a dissection microscope under various magnifications (to a maximum of 100 X) for ectoparasites.

Following skin smear preparations, fish were sacrificed by neural-pithing, after which they were measured using a calibrated measuring board for total length to the nearest mm. The fish were weighed in grams, using a Salter Model 235E scale (Figure 2.7 A). Fish were placed on a dissection board where blood samples were drawn from the caudal aorta situated below the lateral line (Figure 2.7 B). Blood was drawn into capillary tubes, sealed at one end with critoseal clay and centrifuged for 10 minutes at 15 000 revolutions per minute. The heamatocrit was determined by expressing the amount of red blood cells and white blood cells using a heamatocrit reader and expressed as a percentage of the total measurement. Subsequently, each fish was dissected and examined for endoparasites. The sexes of the fishes were determined only after dissection the fishes based on the presence or absence of testes or ovaries. It must, however, be mentioned that drawing blood from *Lb*. *marequensis* proved to be unsuccessful in most of the fish sampled as they tended to die soon after having been removed from the dams.

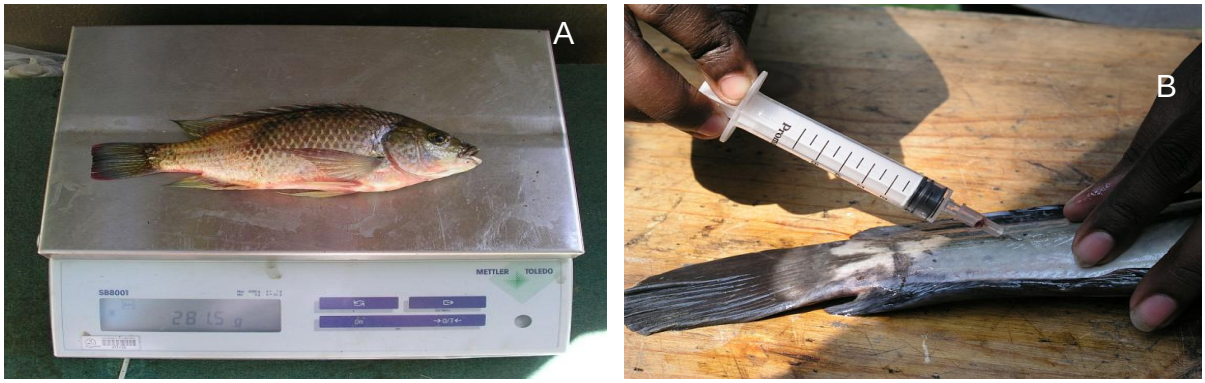

Figure 2.7: A - Balance used to weigh fish at the Nwanedi-Luphephe Dams, **B** - Blood drawn from the dorsal aorta by inserting a syringe below the lateral line.

2.5.1 Examination of the gills

The left and right pairs of the gills were quickly removed by using dissection scissors and placed in separate petri dishes, distinctly marked and covered with dam water to prevent dehydration. Gills from each chamber were numbered 1 - 4, from the anterior to the posterior according to their position in the fish with the gill arch closest to the operculum being marked No 1. During the examination process, each gill arch was sequentially removed from the others and placed into a separate small transparent petri-dish with the gill filaments excised from the gill.

The gills were then examined for the presence of monogeneans and digeneans (metacercariae) with the aid of a dissection microscope with transmitted light. The parasites were gently removed with the aid of a fine brush and placed in dam water in a petri dish. Parasites found on each fish were identified, counted and recorded.

Collected parasites were preserved as follows:

The specimen was flatly spread on a slide onto which a drop of dam water had been placed in the centre of a microscope slide. A small amount of glycerin jelly was placed next to the parasite. Excess water was removed with a small piece of tissue paper. The parasites were then positioned centrally in the glycerin jelly and covered by a glass cover-slip which was carefully pressed down to avoid damage to the specimen after which the slide was heated over a burning flame. The preparation was sealed with clear nail vanish.

Some monogenean specimens found were fixed in ammonium picrate glycerine (GAP). A cover slip was placed over the specimen and examined with the aid of a microscope at various magnifications. All individuals of the adult diplozoids and diporpae parasites were identified on the fish gills. Adults of *Afrodiplozoon polycotyleus* were either fixed in 70% ethanol or mounted in GAP. If there was not sufficient time in the field to examine all gills, remaining gills were fixed either in 4% formalin or 70% ethanol and examined later in the laboratory. During the study period, data on parasite species were categorized according to seasons and recorded on a data sheet.

2.5.2 Internal examination

Internal organs examined included: eyes, brain, kidney, liver, spleen, small intestine, large intestine, gall bladder, swim blader and body cavity. The intestine was dissected and placed in a separate petri-dish with physiological saline solution and examined using a dissection microscope. The intestine was cut open with a pair of scissors and forceps inserted into the lumen to assist in pulling it apart. *Contracaecum* larvae were excised from the mesentery fats surrounding the intestine in the body cavity. In some cases, infestations were so high that determining the precise number of *Contracaecum* with any degree of reliability was impractical. Nematodes were fixed in glacial acetic acid, and stored in 70% ethanol. Nematodes were not stained but were directly cleared in Lactophenol (at a later stage in the laboratory). All other helminths found in each individual fish were identified, fixed in 70% ethanol and enumerated. The freshly collected cestode larvae were fixed in buffered formalin.

Eyes were removed, cut open with fine scissors under a dissection microscope to collect *Diplostomulum*. Parasites were counted and fixed in 70% ethanol. On removal from the eyes of the host, the digeneans were first placed in cold water to relax them and then shaken vigorously from time to time to dislodge debris. After being fully relaxed and stretched out, the specimens were fixed with 70% ethanol.

2.6 Parasite identification and measurements

Parasites were photographed and identified using a compound microscope (Olympus BX 50) equipped with phase-contrast and differential interference contrast. Measurements of the *Dactylogyrus* species and identification were done as described by Paperna (1968) (Figure 3.3 D) while those of the *Gyrodactylus* followed the description of Christison *et al*. (2005) (Figures 3.12 A, B, 3.13 A, B). For *A*. *polycotyleus,* measurements followed the description of Mashego (1982) and Seddon (2004) (Figures 3.11 A, B). Drawings and measurements were taken from the whole mounts with the aid of a Zeiss Standard 25 LM compound microscope, equipped with a drawing tube. Measurements of monogeneans were made with an ocular micrometer and are in micrometers (µm), unless otherwise stated. All collected materials were deposited in the collection of the Department of Biodiversity, Faculty of Science and Agriculture, University of Limpopo.

2.7 Health Assessment Index

Fish were examined for the Health Assessment Index **(**HAI) as outlined by Avenant-Oldewage *et al*. (1995) with the addition of the color chart (Figure 2.8) developed by Watson (2001) and observations recorded on a HAI data sheet. Gross pathological changes in colour were noted while also looking for parasites with a dissection microscope. After the external examination was done, all internal organs were examined for HAI evaluation and observations recorded on HAI data sheet. Endoparasites were also counted and recorded. The internal organs were assigned values according to their condition. Designated characters were assigned to the organs as indicated in the revised HAI (Heath *et al*., 2004).

2.7.1 Calculation of the fish Health Assessment Index

Following examination of the fish in the field, the field observation values or original field designations of all variables from the necropsy based system were replaced with comparable numerical values into the HAI. The scores assigned to each organ were based on the description of the observed condition as follows: A

rating of 0 was given to normal values, 10 for mild abnormalities, 20 for moderate, and 30 for severe abnormalities. The ratings are summed for each fish and then the means are calculated for each group. The sum of the total of values awarded to all the tissues for a particular fish is the HAI value for that fish. The higher the HAI value the greater the level of abnormalities within that group. The HAI was calculated including all parasites collected. The mean calculated for all fish in the sample is the HAI value for that sample.

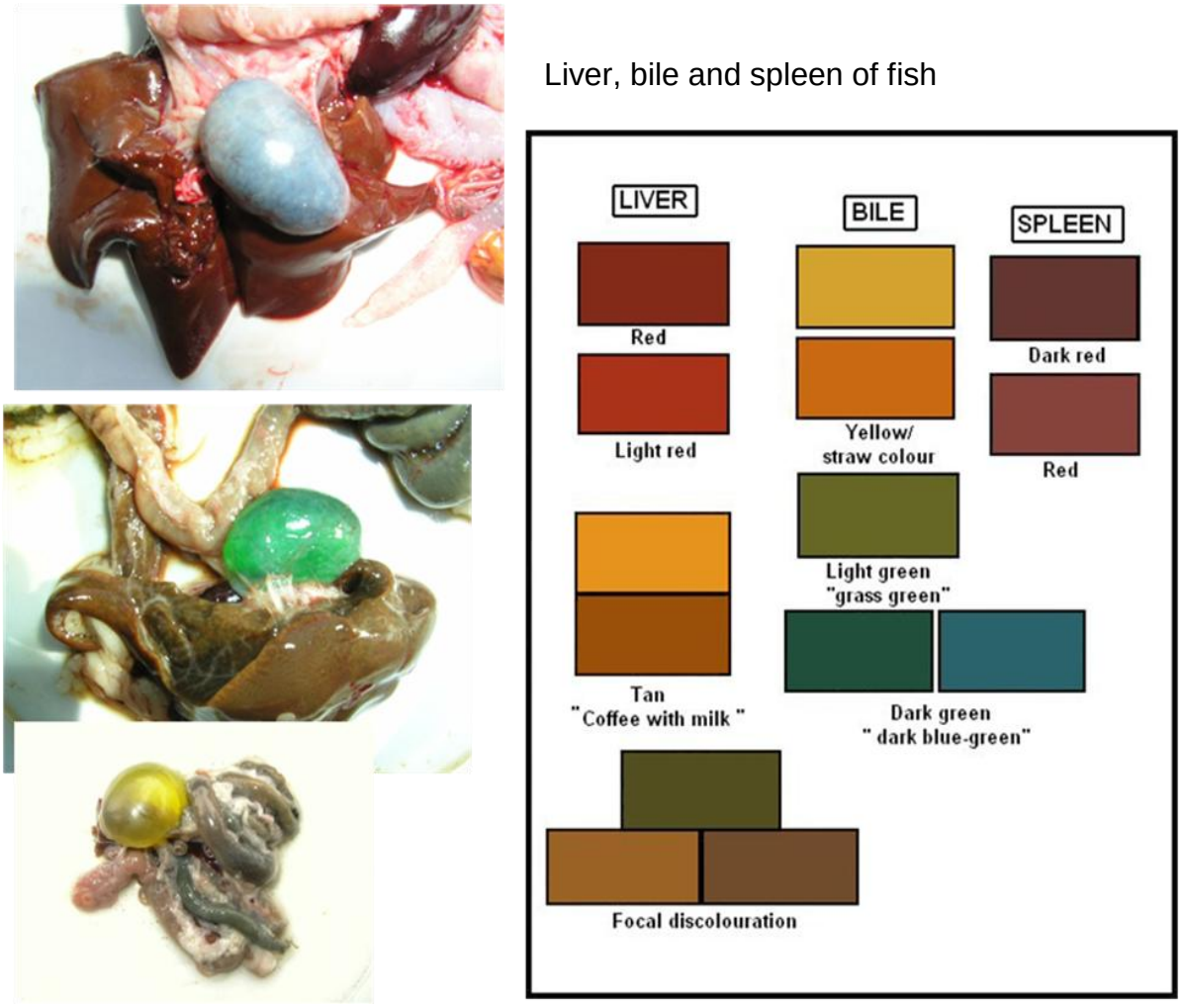

Figure 2.8: The color chart used in the Health Assessment Index for *Labeobarbus marequensis* at the Nwanedi-Luphephe Dams (Heath *et al*., 2004).

The standard deviation and coefficient of variation were also determined. The latter indicates the level of stress experienced by a fish population (Adams *et al*., 1993). The standard deviation (SD) for each sample was calculated as proposed by Adams *et al*. (1993):

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

Where: N = number of fish per site

X = average index for each season

X_i = index value for fish

The coefficient of variation (CV) was calculated as proposed by Adams *et al*. (1993):

CV = 100 x SD/X

Where: SD = standard deviation

X = average index for each site

The population condition factor was calculated as described by Klemm *et al*. (1992):

$$CF = \frac{W \times 10^5}{L^3}$$

W = weight in g

L = total length in cm

2.8 Parasite Index

The Parasite Index (PI) was determined in conjunction with the HAI. The point score for the PI in each survey was determined by adding the ranking value for each category of parasites. The Parasite Index, as tested by Crafford (2000) and Crafford and Avenant-Oldewage (2009), was used when assigning numerical values to the number of ecto- and endoparasites observed. The endo- and ectoparasites were categorized as presented in Table 2.1 below:

Table 2.1: The Parasite Index adopted from Crafford and Avenant-Oldewage (2009).

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

2.9 Statistical analysis

Parasitological variables (prevalence, mean intensity and abundance) were calculated according to the method of Bush *et al*. (1997):

Prevalence is the number of individuals of a host species infested with a particular parasite species divided by the number of hosts examined (expressed as percentage).

Mean intensity is the total number of individuals of a particular parasite species in a sample of a host species divided by the number of infested individuals of the host species in the sample.

Abundance is the total number of individuals of a particular parasite species in a sample of hosts divided by the total number of individuals of the host species in the sample.

Data was statistically analysed for correlation and comparisons (ANOVA) using the software programs Microsoft Excel (2007) and SPSS (17.00) respectively. Graphs depicting correlations were plotted out using Microsoft Excel (2007). Gender preference was also investigated by using Student t - test (SPSS 17.00). All the statistical analyses were carried out at 95% confidence interval.

CHAPTER 3

METAZOAN PARASITES OF SELECTED *BARBUS* SPECIES AND *LABEOBARBUS MAREQUENSIS*

3.1 Introduction

This chapter dealt with an ecological survey of parasites of the three cyprinids with emphasis on the occurrence of metazoan parasites encountered during the course of the study period. Aspects of the morphology, drawings and micrographs of some of the species were added for better orientation of the reader and as material for comparison with other literature since the systematic status of some species are often complicated. A background and summary of the existing taxonomy and distribution records of all parasites was briefly given. The purpose was to confirm the identification of the parasite encountered, provide a brief description of the parasite's infestation levels, seasonality, and influence of parasites on the condition factor of the hosts, gender specificity and host specificity. A comparison of the said parasites with previously described ones was briefly outlined under each parasite group section. The discussion was structured in such a way that the results obtained for species of the same genera were discussed simultaneously in terms of all the different ecological aspects that were assessed and highlighted both differences and similarities wherever possible.

All parasites found in this study were compared to descriptions provided by various authors as species diagnosis (if available). The known species encountered during this study compared well with previous species descriptions with the exception of those found for the first time in South Africa of which no existing records of those particular parasites were found. This was the case for the *Gyrodactylus* sp. and *Dogielius* sp. encountered on the gills of *Barbus radiatus*.

3.2 Ecology

The science of ecology is concerned with interactions of organisms and their biotic and abiotic environment. It is a discipline which focuses on the patterns in parasite community structure, species richness and diversity (Mwita and Nkwengulila, 2008).

Research on parasites has led to many hypotheses about what may mediate or control community structure(s) of these parasites. Some of the papers that deal with the composition and diversity of parasite communities in tropical fishes are

those of Alves and Luque (2001), Alves *et al*. (2002), Luque *et al*. (2003) in Brazil, South America, Salgado-Maldonado and Kennedy (1997) in Mexico and Kennedy (1995) in Australia, to mention but a few. Moreover, extensive information on the ecology and biology of parasites and fishes is also known through the work of other authors (Marcogliese and Cone, 1991; Machado *et al*., 1995; Poulin, 1995; Johnson *et al*., 2004 and Takemoto *et al*., 2005). Comparatively, papers on composition and diversity of parasite communities from Africa are few.

According to Mwita and Nkwengulila (2008), ecological characteristics or factors that promote the richness of parasite communities include host features such as host body size, gender, geographical range and broadness of diet. Characteristics of the environment also determine the structure of parasite assemblages (Marcogliese and Cone, 1991). In addition, host population density plays a crucial role in the abundance of parasites, with host species occurring at high density expected to have more parasite communities (Takemoto *et al*., 2005). Dávidová and Ondračková (2008), on the other hand, cautioned that these factors can affect each parasite species differently, modifying their infracommunities and components community.

Exploring host-parasite association and their ecological communities as such, may shed some light on the parasite-host phenomena and ecosystem change, particularly where human health or natural resources are concerned. Thus, in the present study, certain factors deemed to be the driving force behind parasite-host associations and governing parasite occurrence were investigated and shall be discussed under each parasite section. Abiotic factors are considered to be the most significant on population dynamics of both viviparous and oviparous monogenean parasites (Chubb, 1977; Gelnar *et al*., 1997). Furthermore, knowledge of these associations in complex systems may assist in formulating more effective ways of preventing diseases caused by parasites.

3.3 Results

A total of 152 individuals of the three different cyprinids were examined for parasites during the study period. A total of 2 432 metazoan parasites of ten species were recorded. Collected parasites were divided into two groups (generalists and

specialists) considering their host-specificity. In total, two specialists and eight generalist species were recorded. All recorded endoparasite species were generalists with the exception of the cestode larvae. It was noticed in the present study that generalists occurred during all seasons and specialists occurred sporadically.

The metazoan parasites recorded during the present study are the following; Ectoparasites included monogeneans, i.e., *Dactylogyrus spinicirrus*, *Dactylogyrus afrolongicornis afrolongicornis*, *Dactylogyrus afrolongicornis alberti*, *Afrodiplozoon polycotyleus, Gyrodactylus* sp. and *Dogielius* sp. (all recorded from the gills); and crustaceans, *Dolops ranarum* from the mouth cavity, gills and skin. Of the ectoparasites, only two specialists, both monogeneans, were found on *B*. *trimaculatus* namely, *D*. *afrolongicornis afrolongicornis* and *D*. *afrolongicornis alberti*. The former occurred sporadically. However, both *D*. *spinicirrus* and *A*. *polycotyleus* were widely distributed and recorded on the gills of all cyprinid hosts during all seasons of this study. Both species represent new host distribution records, being found on *B*. *radiatus* for the first time. *Dactylogyrus spinicirrus* also represents a new host distribution record, being found on the gills of *B*. *trimaculatus* for the first time. *Gyrodactylus* sp. and *Dogielius* sp. were found only on *B. radiatus* and represents possibly a new species exclusively from the hosts collected from the stream habitat.

Endoparasites encountered were the following: digeneans, i.e., *Diplostomulum* metacercariae from the eyes of *Lb*. *marequensis*; *Ornithodiplostomum* sp. and black grubs were found from *B*. *trimaculatus.* The latter also occurred in *B*. *radiatus*; other unidentified digenean cysts were recovered from the gills and in the body cavity of both *Lb. marequensis* and *B. trimaculatus*; nematodes were represented by *Contracaecum* larvae in the body cavity of both *Lb*. *marequensis* and *B*. *trimaculatus*; cestodes were represented by gryporynchid larvae from the intestine of *B*. *radiatus*.

Monogenea was the most abundant group (*Dactylogyrus spinicirrus*, *D*. *afrolongicornis alberti*, *Afrodiplozoon polycotyleus*) followed by *Diplostomulum*. The second part of the study is related to ecological aspects (prevalence, abundance and intensity levels) of each parasite seasonally. The seasonal occurrence of each parasite observed during the study period is summarised in Table 3.1. The mean intensity of the species was higher during summer and autumn. It decreased during

late autumn and winter for all parasite species. Both the prevalence and mean intensity levels of the parasite species varied greatly between different host fish sizes. It should be noted that the rare species were hereafter excluded from certain statistical analysis due to their infrequency and very low intensities. Each group of parasites is presented and discussed separately below.

Table 3.1: Presence (+) and absence (-) of parasite species in individual sampling seasons at Nwanedi-Luphephe Dams.

Parasite groups	Parasite species	Sampling periods			
		April	July	October	January
Monogenea	*D. spinicirrus*	+	+	+	+
	D. afro. afro	-	+	+	+
	D. afro. alberti	-	+	+	+
	A. pol	+	+	+	+
	Gyrod. spp.	-	+	-	+
	Dogielius sp.	-	+	-	-
Digenea	*Diplostomulum*	+	+	+	+
	Black grub	-	+	-	-
	Ornitho. sp.	+	+	+	+
	Other cysts	-	+	+	+
Nematoda	*Contra* sp.	+	+	+	+
Cestoda	Gryporhy sp.	-	+	-	-
Branchiura	*D. ranarum*	+	+	-	+

Key: *D. spinicirrus* = *Dactylogyrus spinicirrus*, *D. afro. afro* = *Dactylogyrus afrolongicornis afrolongicornis*, *D. afro. alberti* = *Dactylogyrus afrolongicornis alberti, Gyrod.* spp. = *Gyrodactylus* species, *A. pol* = *Afrodiplozoon polycotyleus*, *Ornitho. s*p = *Ornithodiplostomum* sp., *Contra* sp. = *Contracaecum* larvae, Gryporhy sp. = Gryporhynchid species larvae.

3.4 Monogenea

Monogeneans are hermaphroditic ectoparasitic flatworms found mainly on the gills of freshwater fish and marine fish. They attach with a unique posterior positioned attachment structure called the haptor (opisthaptor), which consist of hooks, clamps and/or suckers (Paperna, 1996; Klinger and Floyd, 2009). Their anterior end contains apical sensory structures, a mouth with or without accessory suckers and special glands or clamps for attachment. Male structures include a single testis or follicular; sperm are evacuated into a specialized, often sclerotinized copulatory organ. Female organs include ovary and follicular vitelline glands. The

two most common genera of monogeneans that infest freshwater fish are *Gyrodactylus* Malmberg and *Dactylogyrus* Diesing.

Gyrodactylus are generally found on the skin and fins of fish whereas *Dactylogyrus* prefers to attach to gills (Koskivaara *et al*., 1991). Some adult monogeneans remain permanently attached to a single site on the host (Whittington *et al*., 2000). The update on parasitic diseases of African freshwater fishes published by Paperna (1996) together with the checklist of Khalil and Polling (1997), have shown that most of the above taxa are present in all inland waters of Africa. However, most monogeneans found in inland water fish are Dactylogyroidea.

Amongst monogeneans, Dactylogyridae is the most diverse family mainly occurring on the gills of cyprinids hosts (Paperna, 1979). Taxonomic subdivision for the generic level of the Dactylogyroidea is based on structural variation in the sclerotinoid attachment organs of the opisthaptor while subdivision for specific differentiation is based on the sclerotinoid copulatory organ (see Paperna, 1996). Species of this group have a direct life cycle, demonstrate a high degree of host specificity and follow their respective fish hosts throughout their distribution range (Poulin, 1992; Paperna, 1996). The same parasite species often infests several fish species of the same genus or species group, and parasites occurring on the host of the same fish family are taxonomically related, usually at the generic level. Because of their high species richness, morphological and ecological diversity, they seem to be an appropriate model for studying parasite diversification (Poulin, 2002).

Although the parasites of freshwater fish in South Africa have already received enormous consideration, monogenean parasites of freshwater fish fauna have been the subject of a few papers (Paperna, 1980; Mashego, 1989; Khalil and Polling, 1997; Mashego, 2000; Luus-Powell *et al*., 2003; Christison *et al*., 2005) just to mention but a few. There are about 39 species of *Dactylogyrus* that have, thus far, been described from African cyprinid genera which include the genus *Barbus*, *Labeo*, *Varicorhinus*, *Opsaridium*, *Neobola* and *Cyprinus* (Mashego, 1982). Of the 39 species, the genus *Barbus* alone accounts for 34 of the species. To date, about nine species of *Dactylogyrus* have been described from South African cyprinid fishes (Mashego, 1982), and the rest from Kenya, Uganda and Gabon.

According to Mashego (1982), the genus *Dactylogyrus* consists of three species-groups based on the sclerotinoid organs. Of the three species-groups, two; i.e. the *D*. *afrobarbae* group and *D. pseudanchoratus* group, are thus far only known

from African cyprinid fishes of the genera *Barbus* and *Labeo*. The third, i.e. the *D*. *varicorhini* group, is common on fish of the genera *Barbus* in Africa and *Barbus*, *Varicorhinus* and *Schizothorax* in Asia.

As was mentioned previously, monogenean parasites of freshwater fishes from Africa are mostly known only for their taxonomic aspect with little or no ecological work having been done. According to Boungou *et al*. (2008), most authorities were interested in the taxonomy, the specificity and life cycle of monogenean parasites. As a result, quantitative data on population dynamics of monogeneans is sparse, particularly in temperate Africa. To date most data on monogenean parasite dynamics of freshwater fish are known from the tropical African regions (e.g., Obiekenzie *et al*., 1992; Mouton *et al*., 2001; Aloo, 2002); but such reports are very sparse from temperate Africa.

The classification and ecological results of each of the monogeneans found in this study is presented below.

3.4.1 *Dactylogyrus spinicirrus*

CLASS: Monogenea
FAMILY: Dactylogyridae
GENUS: *Dactylogyrus* Diesing
SPECIES: *Dactylogyrus spinicirrus* (Figure 3.3)
HOSTS: *Labeobarbus marequensis*, *Barbus radiatus* and *Barbus trimaculatus*
SITE: Gills
LOCALITY: Nwanedi-Luphephe Dams

The prevalence, abundance and mean intensity of *D. spinicirrus* on the three cyprinids, *Lb*. *marequensis*, *B*. *trimaculatus* and *B. radiatus* is presented in Table 3.2.

Prevalence: The prevalence of *D. spinicirrus* on *Lb*. *marequensis* varied considerably during all seasons, dropping from 66.6% in autumn to 20% in winter then followed by an increase to 70% in spring and a further increase to 100% in summer (Table 3.2). A noticeable seasonal trend of *D. spinicirrus* was only noted on *Lb. marequensis*. When comparing the prevalence between the three fish species, the highest prevalence was recorded from *Lb*. *marequensis*.

Table 3.2: Seasonal infestation statistics of *Dactylogyrus spinicirrus* infesting *Labeobarbus marequensis*, *Barbus trimaculatus* and *Barbus radiatus* at Nwanedi-Luphephe Dams.

Fish	Season	No. of examined fishes (n)	No. of fish infested	A	MI	P(%)
Labeobarbus marequensis	Autumn	15.0	10.0	6.7	10.0	66.6
	Winter	10.0	2.0	0.7	3.5	20.0
	Spring	10.0	7.0	3.0	4.3	70.0
	Summer	18.0	18.0	8.1	8.1	100
Barbus radiatus	Autumn	10.0	0.0	0.0	0.0	0.0
	Winter	16.0	0.0	0.0	0.0	0.0
	Spring	10.0	1.0	0.4	4.0	1.0
	Summer	10.0	0.0	0.0	0.0	0.0
Barbus trimaculatus	Autumn	17.0	0.0	0.0	0.0	0.0
	Winter	17.0	3.0	0.3	1.7	17.6
	Spring	10.0	3.0	0.6	2.0	30.0
	Summer	19.0	0.0	0.0	0.0	0.0

Key: A = abundance, MI = Mean Intensity and P = Prevalence

Abundance: The abundance of *D. spinicirrus* on *Lb. marequensis* was 0.7 during winter and 3.0 in spring followed by a considerable increase to 8.1 in summer, then 6.7 in autumn. On *B. trimaculatus*, *D. spinicirrus* occurred in winter and spring and the abundance was 0.3 and 0.6 respectively. On *B. radiatus*, *D. spinicirrus* only occurred in spring and the abundance was 0.4. The following seasonal trend was observed for *Lb. marequensis*: abundance values decreased from autumn to winter and then slightly increased in spring followed by a peak in summer (Table 3.2). As for the other hosts, no seasonal trend was observed.

Mean intensity: In terms of the mean intensities, the infestation was considerably higher on *Lb. marequensis* than the other hosts, with the highest value recorded in autumn (10) and the lowest value of 3.5 recorded in winter. During the remaining seasons the mean intensity increased from 4.3 to 8.1 in spring and summer respectively. For *B. radiatus*, the mean intensity was 4.0 while on

B. trimaculatus, the values were 1.7 in winter and 2.0 in spring excluding autumn and summer where none was recorded (Table 3.2).

Size of host (data pooled together): There was a weak correlation between the number of *D*. *spinicirrus* and the size of the host (Figure 3.1; $R^2 = 0.142$).

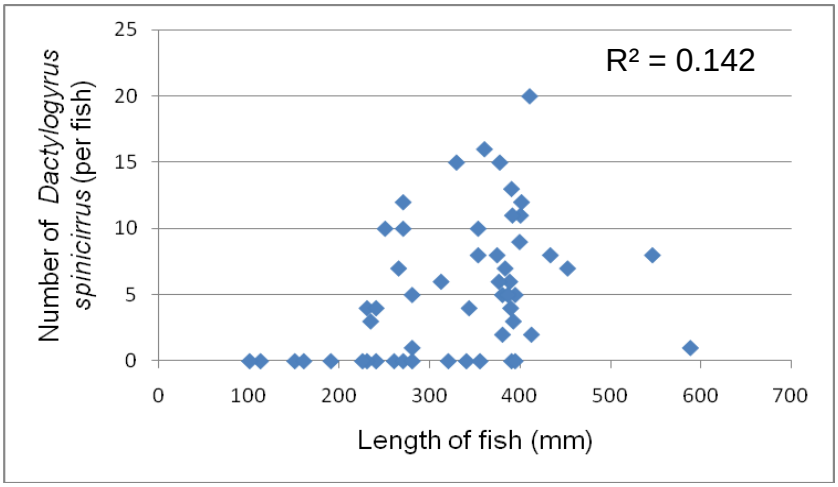

Figure 3.1: The correlation between the number of *Dactylogyrus spinicirrus* and the total length of *Labeobarbus marequensis* during all four seasons at Nwanedi-Luphephe Dams.

Seasonality: There was a significant difference in the number of *D. spinicirrus* across seasons (ANOVA, $p < 0.05$) (Appendix 1). *Post hoc* tests showed that autumn and winter differed significantly (LSD, $p < 0.05$), while autumn and spring also differed significantly ($p < 0.05$) (Appendix 1). No tests were conducted on the number of *D*. *spinicirrus* found on the other fish species as the data did not permit it (Table 3.2).

Condition factor (data pooled together): No correlation between the number of *D. spinicirrus* and the condition factor of *Lb*. *marequensis* was found during all seasons (Figure 3.2; $R^2 = 0.001$).

Site specificity: There was no preference for *D. spinicirrus* to favour a specific gill arch.

Gender: There was no statistically significant difference in the number of *D*. *spinicirrus* on male and female fish ($p > 0.05$; t - test), though female hosts had higher parasite intensity than males (Appendix 1).

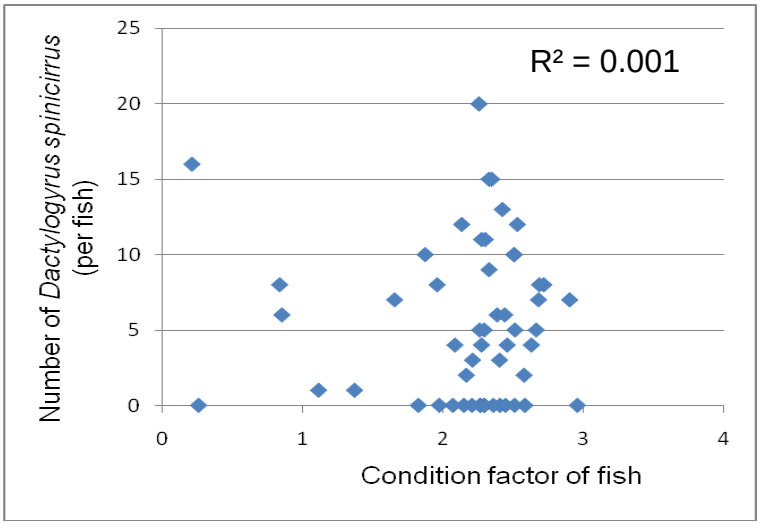

Figure 3.2: The correlation between condition factor and the total number of *Dactylogyrus spinicirrus* on *Labeobarbus marequensis* during all four seasons at Nwanedi-Luphephe Dams.

3.4.2 Diagnostic features, description and occurrence of *Dactylogyrus spinicirrus*

Dactylogyrus spinicirrus belongs to the *D. varicorhini* species group. According to Paperna (1979), this group has the following description: species with the generic characters of *Dactylogyrus*. In the opisthaptor two bars, one large and wide with extreme median constriction occur, resulting in two wings-like or butterfly-like structures. The second bar is small, tapering at both ends. The anchors have a relatively short inner root and very distinct outer root. The tip is distinctly thinner from the shaft. The hooklets are very large and heavy, often as long as half the length, or more, of the anchor. Cirrus long, coiled tube; accessory piece attached to the funnel and branches distally to several parallel often slightly inwards folded, elongated plates. In the prohaptor, the left and right lobes of the head organs are usually well separated from each other. The vagina is tubiform and sclerotized distally. This species - group includes species from Africa as well as from west and central Asia.

The accessory piece is attached to the funnel and the digit-shaped form spring structure is attached to the distal end of the complexed accessory piece. The opisthaptor bears two large anchors with relatively long inner roots and short outer roots (Figures 3.3 A and 3.3 C), 14 hooklets; 12 of which are arranged around the circumference of the opisthaptor while the remaining two are at the centre, all with their tips pointing away from the body. It possess two bars, the large bar is deeply

curved. The transverse bar consists of a thickened center; V- shaped which tapers to a narrow point. The hooklets range from 35 - 42 µm in length (Table 3.3), each consisting of a thick, rounded base which tapers to a narrow shaft terminating in a sickle-like tip. The anchors bifurcated into two roots with a wide angle, with the outer root shorter and the inner root longer (Figures 3.3C and 3.3D). Both roots are blunt at the tip, the smaller one transversally along the axis of the worm, the larger one diagonally along the axis. The shaft of the anchors bends inwards. The copulatory organ consists of the ejaculatory and the accessory piece. The former consists of a wide funnel continuing in an elongated slightly bent duct (Figure 3.3 C (e)). The accessory piece consists of closely knit segments which partially overlap one another. The accessory piece joins the side of the funnel of the ejaculator. Around the area of the copulatory organ, there is a globular structure, possibly the genital atrium, through which the copulatory organ may be extruded. Measurements are listed in Table 3.3. Measurements of the anchors were taken as depicted in Figure 3.3 D.

Dactylogyrus spinicirrus is geographically narrowly distributed amongst species of the family Cyprinidae, having been recorded from only two African countries, Uganda and Kenya, but from various fish species within the said family. *Dactylogyrus spinicirrus* was originally described by Paperna and Thurston (1968) from *Barbus altaianalis* in Kenya as *Neodactylogyrus spinicirrus*. Because species included in *Dactylogyrus* may have either one or two supporting bars in the opisthaptor, Paperna (1973, 1979) reported that it was consequently subdivided into two genera namely *Dactylogyrus* and *Neodactylogyrus* with the former encompassing all species with one bar and the latter including all species with two bars. However, this division was not accepted by Russian workers (see Paperna, 1979).

Thereafter Paperna (1979) obtained more specimens of *D. spinicirrus* from *Barbus somerreni* in Uganda and *Barbus nyanzae* in Kenya, and *D. spinicirrus* was transferred to the genus *Dactylogyrus*. In South Africa, *D. spinicirrus* has been reported from the cyprinid, *Lb. marequensis* by Mashego (1989). The present material differs from that of Paperna (1979) and Mashego (1982) in having an opening in the main transverse bar (Figure 3.3 A (b) and C (b)). The role of this small opening is not clear. But with regards to other morphological aspects, the present material is similar to that previously mentioned.

In the present study, *D. spinicirrus* occurred on *B. trimaculatus* and *B. radiatus* as well. The prevalence, however, was low on these two hosts, particularly during the cooler seasons (Table 3.2). There are no previous reports of this parasite on these hosts. Thus, the present results serve as a second documentation of this parasite from South Africa with additional distribution records as a first record for *B*. *trimaculatus* and *B*. *radiatus*.

The high intensity level of *D*. *spinicirrus* (100% recorded during summer) appears to inflict minimal damage on the gill epithelial. This tendency could be attributed to the fact that monogeneans in natural environments generally reproduce in limited numbers and are in equilibrium with their host fish (Boungou *et al.*, 2008). In most fish species associated with monogeneans, prevalence is high (approaching 100%) and intensities of 20 - 100 or more worms per fish are common (Paperna, 1979).

Table 3.3: A comparison of measurements (in µm) of *Dactylogyrus spinicirrus* from *Labeobarbus marequensis*, *Barbus trimaculatus* and *Barbus radiatus* with that recorded by Mashego (1982).

Measurements	**Present study**	**Mashego (1982)**
Total body length	480 - 620	338 - 669
Total body width	70 - 110	44 - 75
Anchor length	51 - 50	63 - 76
Inner roots	15 - 18	20 - 25
Outer roots	5 - 10	8 - 9
Large bar length	17 - 25	26 - 28
Small bar length	26 - 27	25 - 28
Hooklets	35 - 42	31 - 36
Cirrus axis	32 - 35	26 - 37
Accessory piece	20 - 23	16 - 29
Shaft	35 - 38	46 - 53
Tip	13 - 17	14 - 14

In natural environments, the pace of transmission of the parasite to other hosts is minimal. However, the opposite is true in overcrowded fish populations, particularly in aquaculture where fish are sometimes overstocked, thereby increasing

the possibilities of infesting previously non-infested fish and allowing the parasite to migrate and reproduce rapidly, a condition resulting in high fish mortalities (Aloo, 2002). It is not clear from the literature how many specimens Mashego (1989) found per fish, but in the present study the maximum number recorded was 20 per host. This is consistent with the hypothesis of Reed *et al.* (2002) who stated that despite the fact that *Dactylogyrus* are commonly found on wild fish, they rarely cause a disease or death. While this can be true in most cases, the mechanism by which they feed, wounds and lesions sustained by piercing anchors and hooks, may serve as a predilection to secondary infestation by waterborne bacteria and fungi on damaged tissues (Boungou *et al*., 2008). Moreover, lesion on the gills may severely compromise the host's respiratory ability (Paperna, 1980).

The fact that some specimens resisted detachment from the gills, and one had to disturb the gill filaments vigorously with a fine brush in order to dislodge them, suggests that the piercing hooks and anchors do leave open wounds for opportunistic bacteria and fungi. Judging from the infestation statistics, *D*. *spinicirrus* is firmly attached on *Lb*. *marequensis*. Of the three hosts from which this parasite was procured, *B*. *trimaculatus* and *B*. *radiatus* seem to play the least significant role in the survival of the life history of the parasite.

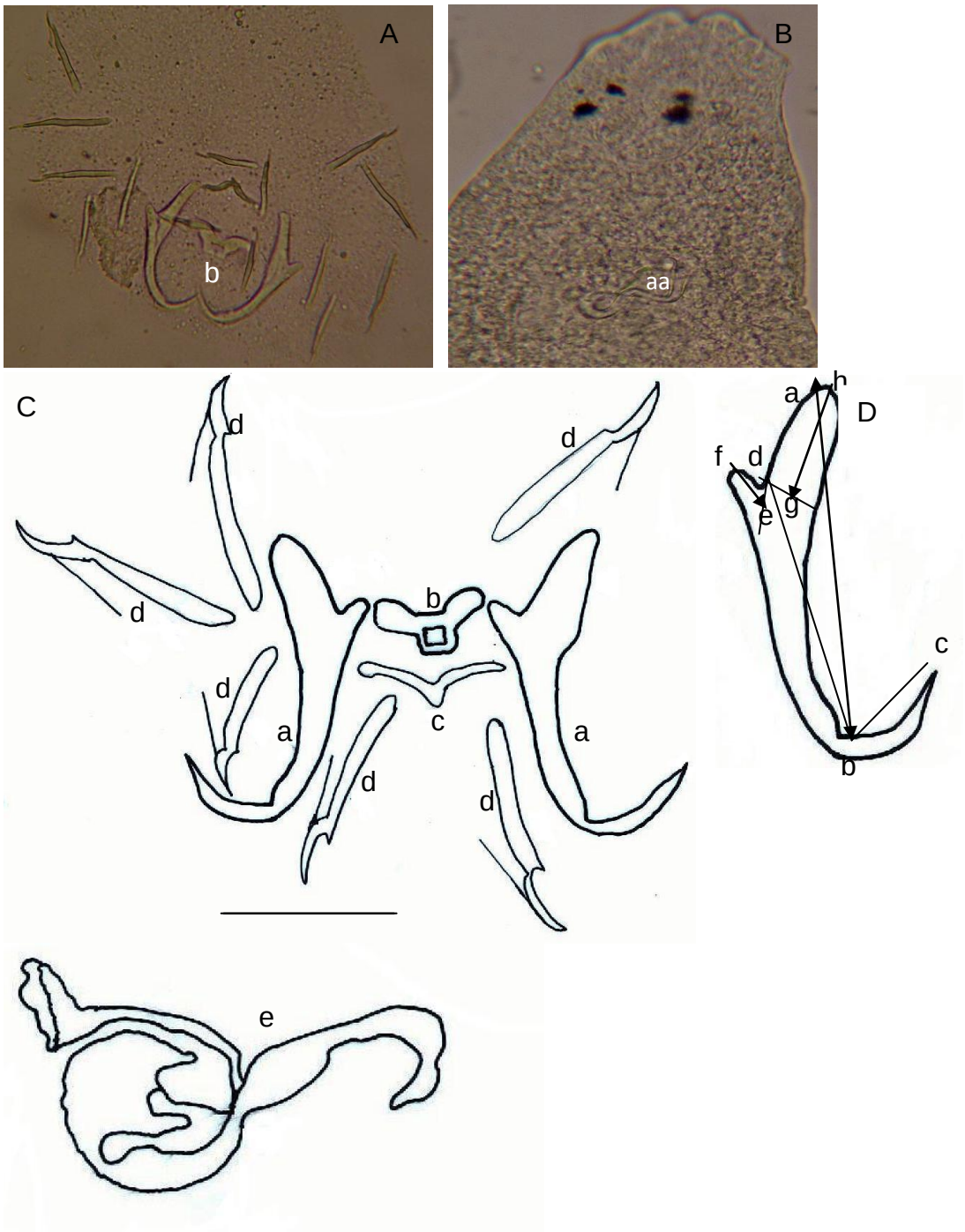

Figure 3.3: *Dactylogyrus spinicirrus* from *Labeobarbus marequensis*, *Barbus trimaculatus* and *Barbus radiatus* at Nwanedi-Luphephe Dams; **A** - showing the distribution of the fourteen hooklets, **B** - (aa) copulatory organ from another specimen, **C** - (a) anchors, (b) transverse bar, (c) ventral bar, (d) hooklets, (e) copulatory organ.; **D**- Illustration to indicate positions where measurements were taken to obtain morphological data, ab - total length, bc - anchor point, bd- anchor shaft, ef - outer root, hg- inner root. Scale bar = 20 µm.

3.4.3 *Dactylogyrus afrolongicornis afrolongicornis*

CLASS Monogenea
FAMILY: Dactylogyridae
GENUS: *Dactylogyrus* Diesing
SPECIES: *Dactylogyrus afrolongicornis afrolongicornis* (Figure 3.4)
HOST: *Barbus trimaculatus*
SITE: Gills
LOCALITY: Nwanedi-Luphephe Dams

Prevalence: The percentage of hosts infested with *Dactylogyrus afrolongicornis afrolongicornis* as determined by the prevalence, is illustrated in Table 3.4. Due to the small number of the parasites recorded during this study, no other statistical calculations were carried out. *Dactylogyrus afrolongicornis afrolongicornis* was recorded from only one host i.e., *B. trimaculatus*, with prevalence levels changing from 11.8% in winter to 30.0% in spring and then decreased to 26.3% in summer (Table 3.4).

Abundance: The abundance, although low, followed a similar pattern to the prevalence, ranging from 0.6 in winter to 1.1 in spring and then decreased to 0.9 in summer (Table 3.4).

Mean intensity: The mean intensity of hosts infested with *D. afrolongicornis afrolongicornis* is shown in Table 3.4. No worms were recorded during autumn. The infestation intensity was higher during winter (5.5) and then remained constant (3.6) through spring and summer.

Seasonality: This parasite was recovered during all seasons except in autumn. But even so, it was present in low numbers throughout the three seasons surveyed.

Due to the scarcity of *Dactylogyrus afrolongicornis afrolongicornis*, no statistical analysis associated with the size and condition factor of the host could be done.

Table 3.4: The prevalence, abundance and mean intensity of *Dactylogyrus afrolongicornis afrolongicornis* on *Barbus trimaculatus* during the three seasons at Nwanedi-Luphephe Dams.

Season	No. of examined fishes (n)	No. of fish infested	A	MI	P(%)
Autumn	17.0	0.0	0.0	0.0	0.0
Winter	17.0	2.0	0.6	5.5	11.8
Spring	10.0	3.0	1.1	3.6	30.0
Summer	19.0	5.0	0.9	3.6	26.3

Key: A = abundance, MI = Mean Intensity and P = Prevalence

3.4.4 Diagnostic features, description and occurrence of *Dactylogyrus afrolongicornis afrolongicornis*

Dactylogyrus afrolongicornis afrolongicornis together with *D afrolongicornis alberti* belongs to the *D. afrobarbae* species - group. Paperna (1979) gave the following generic diagnostic features for this group: species with generic characters of *Dactylogyrus.* Small to medium worms with distinct opisthaptor. Anchors with long inner roots and short to vestigial outer roots; bar single, often long and subdivided into two halves by a constriction in its middle.

The bar is comparatively long. Anchors delicate with long inner roots and short outer roots, the bar is subdivided into two plates by a soft poorly sclerotized or even non-sclerotized mid portion (Figure 3.4 (aa)). Because of this soft portion, the bar may vary in shape. The inner root and the shaft bent inwards. Hooklets with medium to long handles. Each hook consists of an elongated base, a thin straight shaft and a minute sickle shaped tip. Each plate of the bar is almost twice as large as the shaft. The worm's body is distinctly delimitated from the opisthaptor (Figure 3.4). Cirrus tubiform, long and coiled once or twice, its distal portion is embraced by the accessory piece. The latter terminates with fixed or movable hook. Vagina is often associated with sclerotized of fibrous structure, the vaginal prop, which is occasionally spiny or dentate; in some species it may be absent. Species included in this group differ from each other in the relative size of the copulatory organ, in the presence or absence of the vaginal prop and its shape, the size of the hooklets, the anchor's inner roots and the size and the pattern of the bar.

Cement gland strongly developed and occupy the posterior quarter of the body. Copulatory organ small, often folds after mounting (Figure 3.4 e). Measurements of *D*. *afrolongicornis afrolongicornis*, compared to that recorded by Mashego (1982), are given in Table 3.5.

Table 3.5: A comparison of measurements (in µm) of *Dactylogyrus afrolongicornis afrolongicornis* from *Barbus trimaculatus* from the Nwanedi-Luphephe Dams.

Measurements	**Present study**	**Mashego (1982)**
Total body length	153 – 180	213 - 413
Total body width	28 – 68	25 - 63
Anchor length	40 – 45	54 - 68
Inner roots	17 – 17	16 - 23
Outer roots	2 – 5	4 - 6
Shaft	23 – 25	35 - 44
Tip	15 – 22	13 - 19
Bar length	44 – 50	50 - 75
Bar width	2.5 – 3	4 - 5
Marginal hooklets	14 – 16	13 - 21
Cirrus axis	23 – 26	29 - 38
Accessory piece	17 – 19	19 - 28
Vagina length	19 – 20	13 - 21
Vagina width	9 – 10	9 - 13

Despite the abundance and diversity of dactylogyrids on cyprinids in African freshwater fish, this parasite has been only recorded on three hosts in Africa thus far. *Dactylogyrus afrolongicornis afrolongicornis* was originally described from *B*. *kersteni* and *B*. *perince* in Uganda (Paperna, 1973). In the present study it was recovered from the gills of *B. trimaculatus* which is the same host Mashego (1982) recorded it from. Mashego also found it in Seshego Dam and the Mohlapitse River, whilst in the present study it was recorded from Nwanedi-Luphephe Dams. This represents a new locality record for *D. afrolongicornis afrolongicornis* in the Limpopo Province of South Africa. Unfortunately, Mashego (1982) did not mention the prevalence, thus it cannot be compared with the present study

After comparing the morphology and measurements of the attachment apparatus (e.g. shape and length of the anchors, hooks and bars) the parasite was assigned to *D. afrolongicornis afrolongicornis*. The present material agrees most closely with the original description of Paperna (1973) and descriptions of Mashego (1982) and it was therefore decided to label it as *D. afrolongicornis afrolongicornis* and extended its geographical distribution in the Limpopo Province of South Africa.

The same method was applied to the subsequent identification of the other *Dactylogyrus* species. No clear seasonal variation was recorded in the present study.

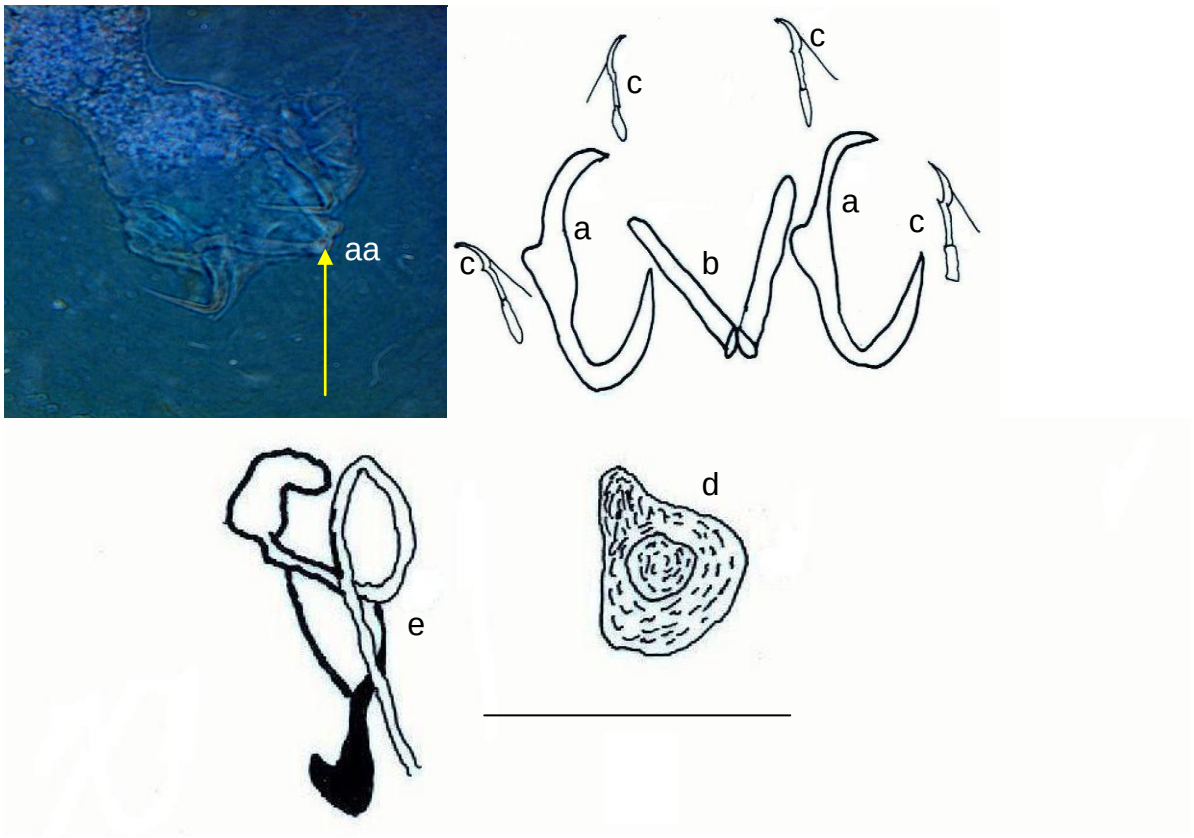

Figure 3.4: *Dactylogyrus afrolongicornis afrolongicornis* mounted in glycerine jelly from *Barbus trimaculatus* at Nwanedi-Luphephe Dams; (a) anchors, (b) bar, (c) hooklets, (d) vaginal prop (e) copulatory organ. Scale bar = 20 µm.

3.4.5 *Dactylogyrus afrolongicornis alberti*

CLASS: Monogenea

FAMILY: Dactylogyridae

GENUS: *Dactylogyrus* Diesing

SPECIES: *Dactylogyrus afrolongicornis alberti* (Figure 3.6)

HOST: *Barbus trimaculatus*

SITE: Gills

LOCALITY: Nwanedi-Luphephe Dams

Similarly, *D. afrolongicornis alberti* was recorded on one host only i.e., *B. trimaculatus*. None was recorded in autumn.

Prevalence: The percentage of hosts infested with *D. afrolongicornis alberti* is shown in Table 3.6**.** The highest prevalence was recorded during summer (47.4%). The prevalence was slightly higher when compared with that of *D*. *afrolongicornis afrolongicornis* with 17.6% in winter and 20.0% in spring.

Abundance: The abundance of *D. afrolongicornis alberti* showed a similar trend to the prevalence, increasing from 0.2 in winter to 0.4 in spring and then peaked in summer with 2.1 (Table 3.6).

Mean intensity: The mean intensity showed a seasonal trend, values ranged from zero in autumn to 1.3 and 2.0 during winter and spring respectively, from where it considerably increased to 4.3 in summer (Table 3.6).

Table 3.6: The prevalence, abundance and mean intensity of *Dactylogyrus afrolongicornis alberti* on *Barbus trimaculatus* during the four seasons at Nwanedi-Luphephe Dams.

Season	No. of examined fishes (n)	No. of fish infested	A	MI	P (%)
Autumn	17.0	0.0	0.0	0.0	0.0
Winter	17.0	3.0	0.2	1.3	17.6
Spring	10.0	2.0	0.4	2.0	20.0
Summer	19.0	9.0	2.1	4.3	47.4

Key: A = abundance, MI = Intensity and P = Prevalence.

Size: Despite the highest infestation level during summer, there was no positive correlation observed between fish length and the number of *D*. *afrolongocornis alberti* (Figure 3.5 A) (R^2 = 0.000).

Condition factor: No significant relationship was recorded between the number of *D*. *afrolongocornis alberti* and the condition factor (Figure 3.5 B) (R^2 = 0.004).

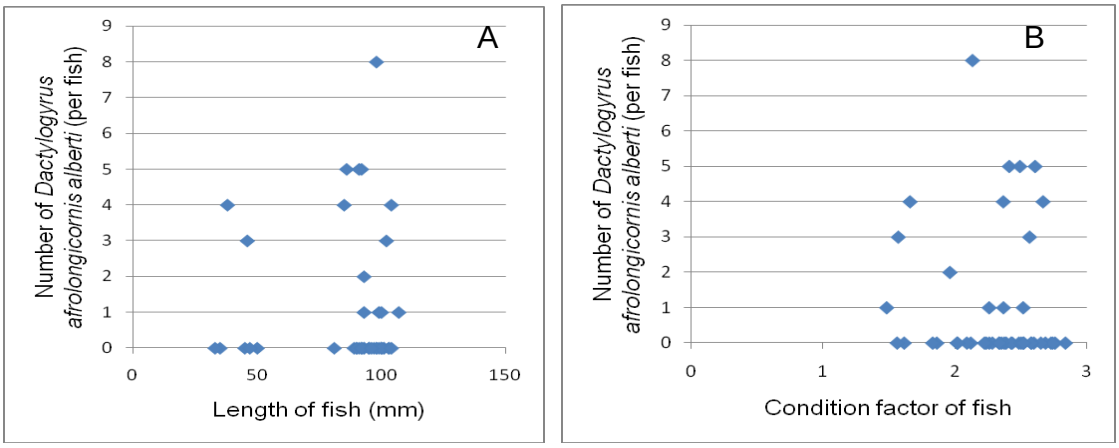

Figure 3.5: A - The correlation between the total number of *Dactylogyrus afrolongocornis alberti* and the length of *Barbus trimaculatus*, **B -** the correlation between the total number of *D. afrolongocornis alberti* and condition factor of *B. trimaculatus* during the summer survey at Nwanedi-Luphephe Dams.

Seasonality: Excluding autumn, a seasonal trend was observed in terms of the mean intensities; values increased from 1.3 in winter to 4.3 in summer. A similar trend was noted in both the prevalence and abundance; they all increased with increasing temperature.

3.4.6 Diagnostic features, description and occurrence of *Dactylogyrus afrolongocornis alberti*

Description: *Dactylogyrus afrolongocornis alberti* differs from *D*. *afrolongicornis afrolongicornis* only in having heavy and thick bar plates (Figure 3.6 B(b)); otherwise they are similar in structure (Figure 3.6).

D. afrolongicornis alberti is not a widely occurring parasite within the family Cyprinidae, having been recorded on three hosts in Africa (Paperna, 1973, Mashego, 1982). This parasite was originally described from *B. perince* and

B. cf. *kersteni* in Uganda (Paperna, 1973). *D. afrolongicornis alberti* is morphologically almost indistinguishable from *D*. *afrolongicornis afrolongicornis*. The morphology is most like to *D*. *afrolongicornis afrolongicornis* in terms of the shape of the hamuli and bars (Figures 3.6 A and B), but differs from it mainly in the size of the hamuli, possessing a far thicker bar.

In South Africa, *D afrolongicornis alberti* has been reported from the gills of *B. trimaculatus* from Seshego Dam by Mashego (1982). Presently, it is reported from the same host as that recorded by Mashego (1982), but from a different locality, the Nwanedi-Luphephe Dams in South Africa. No prevalence was provided by Mashego. But despite the fact that its prevalence in Nwanedi-Luphephe Dams was low during winter and spring, its prevalence and mean intensity were slightly higher than that observed for *D*. *afrolongicornis afrolongicornis* during the present study

Table 3.7: Morphometric diagnostic characteristic measurements (in µm) of *Dactylogyrus afrolongicornis alberti* found on *Barbus trimaculatus* compared with measurements from a previous study by Mashego (1982).

Measurements	**Present study**	**Mashego (1982)**
Total body length	210 - 280	213 - 413
Total body width	20 - 49	25 - 63
Anchor length	25 - 45	54 - 68
Inner roots	13 - 20	16 - 23
Outer roots	1 - 3	4 - 6
Shaft	9 - 24	35 - 44
Tip	10 - 17	13 - 19
Bar length	30 - 74	50 - 75
Bar width	2 - 5	7 - 8
Marginal hooklets	15 - 17	13 - 21
Cirrus axis	24 - 26	29 - 38
Accessory piece	18 - 27	119 - 28
Vagina length	15 - 21	13 - 21
Vagina width	12 - 14	9 - 13

. This parasite species belongs to the *D*. *afrobarbae* species group and is a parasite found on the smaller *Barbus* species (Mashego, 1982). A comparison of

measurements of the present material and the previous descriptions is given in Table 3.7. There was no visible damage to the gills caused by this dactylogyrid.

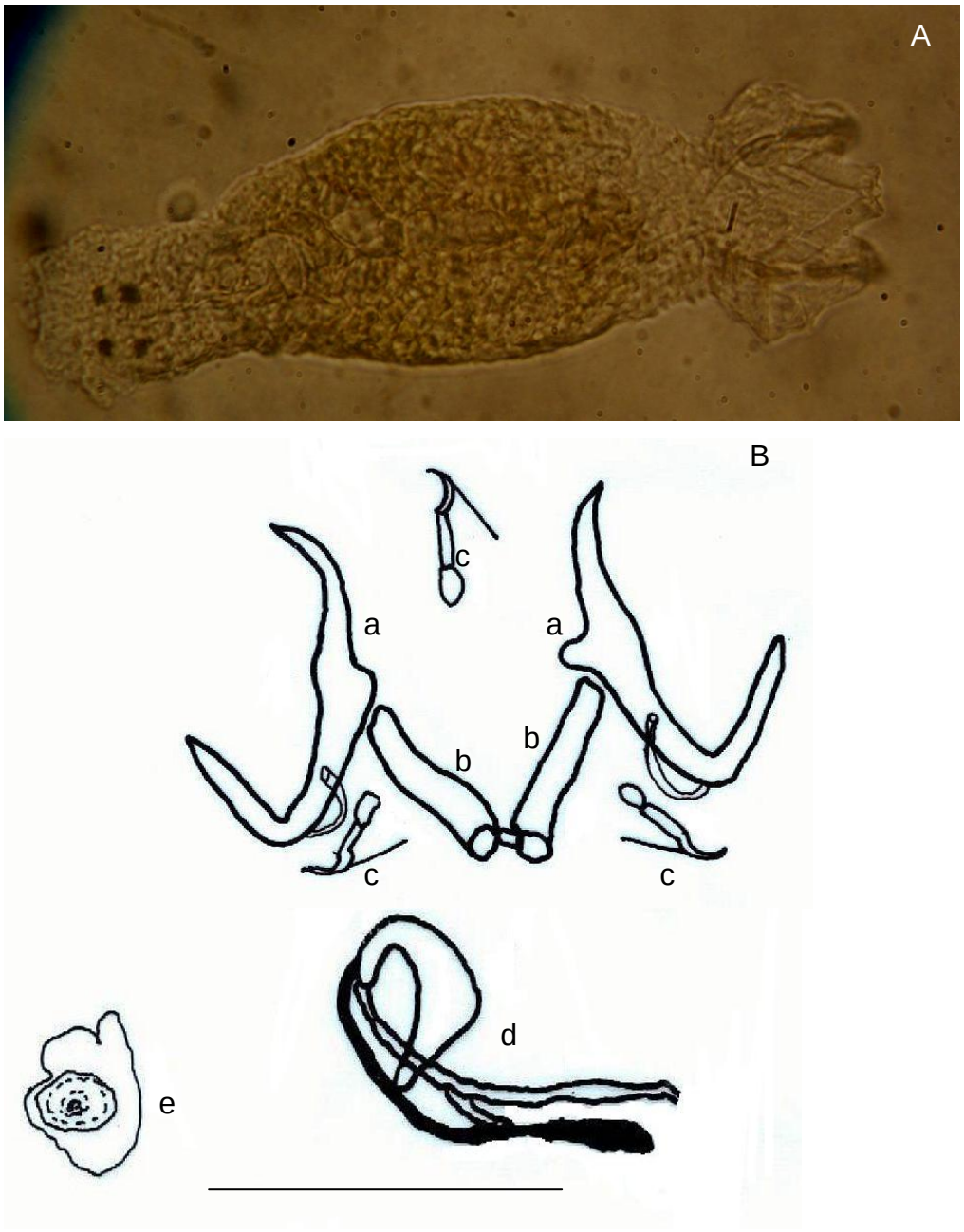

Figure 3.6: **A** - *Dactylogyrus afrolongicornis alberti* (whole specimen) mounted in glycerine jelly collected from *Barbus trimaculatus* at Nwanedi-Luphephe Dams; **B** - (a) anchors, (b) bar, (c) hooklets, (d) copulatory organ, (e) vaginal prop. Scale 20 = µm

3.5 Ecology of the *Dactylogyrus* spp. encountered

3.5.1 Infestation

The infestation of *D. spinicirrus* (in terms of high prevalences, abundance and mean intensity) on *Lb. marequensis* was higher than that on *B. trimaculatus* and *B. radiatus*. *Dactylogyrus spinicirrus* was significantly more frequent in summer and a noticeably higher number of *Lb*. *marequensis* were infested. When the present prevalences were compared with those recorded by Mashego (1982) (33%), it was found that the present prevalences were considerably high (up to 100%). It is however important to note that Mashego (1982) recorded this parasite to occur only on *Lb. marequensis* (previously known as *Barbus marequensis*) whereas in the present study it was also recorded from *B. trimaculatus* and *B. radiatus*.

The occurrence of *D*. *spinicirrus* on all hosts could suggest that there are similarities in some biological aspects of these fish e.g. their feeding behaviour and habitat selection. Nevertheless, the infestation in the latter two hosts was always lower and occurred sporadically compared with *Lb. marequensis* which exhibited higher infestation levels throughout the year. The mean intensity of *D. spinicirrus* was higher on *Lb. marequensis* during autumn and summer but lower during winter and spring. According to Williams and Jones (1994), a large percentage of the variation in the total number of parasites per host species could be accounted for by feeding habits of the host. Aloo *et al*. (2004) reviewed the main factors determining the variety of fish parasite fauna as well as intensity and prevalence of infestation and therefore summarised them as being: the diet of the host, lifespan of the host, the mobility of the host throughout its life including the variety of habitats it encounters, its population density and the size attained, with large hosts providing more habitats suitable for parasites than smaller hosts. Based on these factors, the occurrence of *D*. *spinicirrus* could be promoted by host population density of each host species in this study. It is, however, important to note that the diet of the host does not play any role in the case of monogenean infestations as no intermediate host is consumed by the hosts, but the active movement of host during feeding may bring them into contact with the monogeneans.

Monogeneans have a direct life cycle, which means that no intermediate host is required for the parasite to reproduce. Due to their direct life cycle, and the

absence of specialized transmission stages, dactylogyrids have constant opportunities to move on a host or between host individuals, a strategy which may favour host switching (Kearn, 1994). It would be expected, therefore, that the higher numbers of *D. spinicirrus* recorded during this study are linked to the life cycle pattern of the parasite, host behaviour and environmental conditions as the transmission of the parasite to the host may be directly or indirectly influenced by the presence of these factors.

As was mentioned before, transmission of monogeneans from one fish to another is primarily by direct contact. Accordingly, *D*. *afrolongicornis alberti* on *B*. *trimaculatus* which was shown to be the other common *Dactylogyrus* species also appeared frequently later in summer, but at a much lower abundance. It is difficult to explain why the abundance of both *D*. *afrolongicornis alberti* and *D*. *afrolongicornis afrolongicornis* remained so markedly low compared with that of *D*. *spinicirrus*. It may be the question of higher transmission rate or quicker development cycle of *D*. *spinicirrus* compared to the other two dactylogyrids. As for the low infestation of *D*. *afrolongicornis afrolongicornis* compared to *D*. *afrolongicornis alberti*, presumably because of the latter appearing first then the former, which might trigger competition. In general, the intensity of these two dactylogyrids was found to be very low, on average five parasites per infested host.

3.5.2 Seasonal trends

As highlighted by Khan and Thulin (1991), parasites are a natural part of the aquatic community and their distribution and abundance are potentially, either directly or indirectly, affected by a number of biotic and abiotic factors. Water temperature is commonly regarded as one of the most important abiotic parameter factors determining the presence and abundance of monogenean parasites as it contributes to monogenean egg dispersal by strongly affecting the embryonation period (Chambers and Ernst, 2005). According to Lamková *et al*. (2007), seasonal variation of water characteristics, predominantly temperature is considered to strongly affect fish physiology and immunology which in turn influences the life cycle of fish parasites.

During this study the prevalence and abundance of *D. spinicirrus* found on *Lb*. *marequensis* varied from one season to another, with maximum infestation

recorded during summer (Table 3.2). The direct life cycles and ability to reproduce in a wide range of temperatures of the above-mentioned ectoparasites, enhances transmission rates and hence an increase in intensities within a short time on the host. The general trend observed during the present study is that the highest infestation occurred during spring and summer while the lowest rate was in autumn and winter, when the temperature was 17°C. Such higher infestation may be due to an increase in water temperature during spring and summer. Therefore, these dactylogyrids might have been able to complete their life cycles more rapidly during spring and summer .

On the other hand, no seasonal trend was observed for *D. spinicirrus* occurring on *B. radiatus* and *B. trimaculatus*. But a slight seasonal trend was recorded for *D*. *afrolongicornis alberti* from *B*. *trimaculatus*. It therefore appears from the present study that winter is most likely to be an unfavourable season in terms of egg hatching and parasite growth. The observed reduction of *D*. *afrolongicornis* and *D*. *afrolongicornis alberti* during winter months could be accounted for by their failure to survive or reproduce, or the eggs to hatch at reduced temperatures due to sensitivity to lower water temperatures in a subtropical environment.

When reviewing temperature results, it was found that indeed the value for winter and summer differed considerably (Table 4.5). The present findings are in agreement with those observed by Lamková *et al*. (2007) who found high values for *Dactylogyrus* species during summer and less during winter in a temperate environment. It is, however, worth to note that as the presence or abundance of dactylogyrids is influenced by water temperature, some dactylogyrid species are found on the gills of fish throughout the year (Chubb, 1977). On the other hand, unfavourable environmental conditions will lead to a temporary absence of some parasite species. This may relate to the absence or low prevalence of *D*. *afrolongicornis afrolongicornis* and *D*. *afrolongicornis alberti* during winter (Mashego, 1989).

Since, presumably, no parasite recruitment occurs during winter, the low densities observed in this period of the year in the present study and during early spring, suggest that parasites may be lost during winter. However, as the temperature rise in spring, deposited eggs hatches, and larvae infests the fish leading to a peak in summer as the parasite grows and maximum infestation occur.

Broadly speaking, organisms often synchronise their reproductive season to coincide with seasonal peaks of productivity of the host. This is the case for some ectoparasites and helminths (Møller *et al*., 2003). Findings from this study are consistent with the above postulation. As seen in this study, most numbers of dactylogyrids species peaked during warmer seasons, indicating their reproductive season.

It is also known that fish are exposed to increased monogenean infestations during their spawning period when they are most sensitive to adverse water conditions (Öztürk and Altunel, 2006). Chubb (1977) found that *Dactylogyrus* populations showed peaks in spring and early summer, the period during which many of their host fishes spawn. During this period, the resistance of fish decreases, leading to an increase in reproduction of monogenetic species. If this is the case, then *Lb. marequensis* would be affected as it breeds in mid to late summer (Skelton, 2001) which would mean that abundance values should be higher during this period. In conclusion, it is suggested that the higher dactylogyrid intensities recorded during spring and early summer at Nwanedi-Luphephe Dams appear to be influenced by both the spawning of the host fish and the increase in the water temperature, rather than other factors such as pH and oxygen concentration of the water.

3.5.3 Host-specificity

It is generally assumed that it is more likely that a parasite will change hosts between closely related hosts than between distantly related or unrelated hosts. This appears to be true for the Dactylogyridae. Scientists investigating the structure of helminth communities usually subdivide species into “specialists” and “generalists”. In the former, the range of hosts is limited to one species or genus while in the latter; it covers several genera or families.

Le Brun *et al*. (1990) and Lambert and Gharbi (1995) are of the opinion that the equilibrium of host-specificity found in the natural environment is determined by the realization of three independent but essential conditions: (1) physiological condition (host immune reactions, specific nutritional requirement of the parasite), (2) ethological condition (where the behavioural characteristics of both host and parasite are essential to their encounter) and (3) ecological condition (environmental factors, behaviour of the host and of the infestious stage of the parasite).

Depending on the relative influence of factors that determine specificity, Lambert and Gharbi (1995), further defined the following three types of host-specificity: (1) The first being strict specificity which implies a parasite species that can only live on a single host species (2) narrow specificity which implies that a parasite species infest a few closely related hosts (e.g. species of the same genera) (3) a third one being wide specificity implying that a parasite species is found on several distantly related hosts which have ecological similarities (e.g. sharing the same biotope).

With reference to the dactylogyrids recorded during the present study, three different types of host specificity were detected. For instance, both *D*. *afrolongicornis afrolongicornis* and *D. afrolongicornis alberti* demonstrated strict host specificity; having been found only on *B. trimaculatus*. *Dactylogyrus spinicirrus* demonstrated narrow host specificity, infesting closely related hosts. But it can also be argued that *D. spinicirrus* can either display narrow or wide specificity, since it seems to be fitting very well in either of the two, considering the present results. The findings of the present study, however, are inconsistent with the finding of Lambert and Gharbi (1995) who reported that monogeneans that have a strict specificity for one of the parent species are also found on the hybrids, but not on the other parent species. Based on these observations, both *D*. *afrolongicornis afrolongicornis* and *D. afrolongicornis alberti* are considered specialists whilst *D*. *spinicirrus* is considered a generalist.

The observed variability in the differential distribution of monogenea within various host species can be explained by the eco-ethological factors which influence parasite recruitment; these factors involve the behaviour and biology of the host population and of the infesting larvae. This would then be an indicator of certain ecological conditions shared by these three cyprinids.

3.5.4 Host and site specificity

According to Özer and Öztürk (2005), most species of monogeneans are not only restricted to a particular host but also to a particular part of the host body. In this study, the dactylogyrids did not show any site preference for a specific gill arch and no considerable preferences were noticed for either set of gills (left or right).

Nevertheless, a great number of monogeneans occurred on the filaments of both sides.

Several factors influencing site selection have been suggested and these include host specificity, sex and immunological response of fish, seasonality, water quality, geographical range, competition, predation and facilitation of mating (Bagge and Valtonen, 1999). Gutiérrez and Martorelli (1999) studied five monogenean species on the gills of the catfish, *Pimelodus maculates*, in the Rio de la Plata (Argentina) and found that congeneric species had a generic-species preference for certain gill-hemibranches. Martorelli *et al.* (2007) also indicated that the majority of monogeneans were recovered from gill arches 1, 2 and 3 from *Macropogonias furneiri*.

Studies concerning the microhabitat selection of gill monogeneans have shown that even congeneric monogeneans in many cases select different microhabitats, even in single species infestations (Buchmann and Lindenstrom, 2002). The preference of the dactylogyrids encountered in this study could be related to water currents, which result in them avoiding those gills directly exposed to strong currents. However, the exact explanation for this kind of preference remains enigmatic (Martorelli *et al*., 2007). However, some authors assume that environmental causes may play a role in the preference of a site on the gill. For instance, Özer and Öztürk (2005) suggested that the distribution of monogenean species and site specificity on the gill arches is influenced by the hydrostatic pressure of the branchial pump, coughing action and even water current over the gill surface during the respiratory cycle).

The difference in water current over the gill surface may allow more parasites to attach to these arches. An experiment was carried out by Gutiérrez and Martorelli (1999) to test the validity of this hypothesis. These authors used two computer simulation programs, based on gill area and water current, to generate parasite metapopulations with clumped patterns, and comparing the results with true distributions of selected freshwater parasitic monogeneans. They concluded that both the gill area and water current most likely determined the site preference on the gill arches. This should be applicable to other monogenean infestations as well.

3.5.5 Host size

On both *Barbus* species and *Lb. marequensis* examined, no significant difference in terms of rate of parasitism with respect to host body size was found (Figures 3.1 and 3.5 A). This implies that the presence of the monogeneans found in this study is not dependant on host size. But generally, the prevalence and abundance of *Dactylogyrus* is often higher on older rather than younger fish (Özer and Öztürk, 2005).

Dactylogyrus spinicirrus was also recorded at its highest prevalence and intensity on the largest host fish in the present study. In most cases this may simply reflect the greater surface area of gill available for the establishment of the parasites, an increase in water flow over the gills in older fish and larger individuals having higher physical (ventilation volume) and chemical (mucus) stimuli which increase gill's attractiveness by providing more food. Gutiérrez and Martorelli (1999) speculated that since in larger fish the volume of water passing through the gills are higher; hence the number of oncomiracidiae brought in by the water currents, might also be higher. Therefore, the lack of a relationship observed between body size of the fish and species richness of ectoparasites suggests that the processes acting on the composition of the ectoparasite communities may differ dramatically from the processes acting on the composition of endoparasite communities.

3.5.6 Condition factor

No correlation was found between the number of the dactylogyrids and the condition factor of the fish. This confirms the widely known explanation that monogeneans are less likely to be problematic in feral condition that in aquaculture. No obvious gross lesions were seen near the attachment site of the dactylogyrids and infested fish were virtually indistinguishable from those that were uninfested. The impact exerted by dactylogyrids on host could be mechanical, chemical or physical. Literature dealing with the relationship between the numbers of *Dactylogyrus* species and condition factor is scarce. Effects of intense parasitic infestation by *Dactylogyrus* on fish are of notable importance because respiratory function of the gills can be severely disturbed (Paperna, 1996). Histopathological

work might have revealed lesions or cell damage, but this was not in the scope of this study. The latter work is recommended though for a future study.

3.5.7 Host gender

There was no significant difference between the number of *D*. *spinicirrus* on either males or females of *Lb*. *marequensis* ($p > 0.05$). Due to the statistically small number of infested fish collected, no statistical analysis was carried out on the other two hosts in this regard. The lack of a significant difference on *D*. *spinicirrus* could suggest that the physiological state of both male and female has no role to play that can prevent the infestation. The present findings agree with those reported by Özer (2002). On the contrary, Özer and Öztürk (2005) found a significant difference on the distribution of *Dactylogyrus cornu* between male and female fish with the female displaying a higher mean intensity value ($p < 0.05$). However, it should be noted that the ecto-parasites could infest the two genders differently because male and female fish often have different feeding habits (Rhode, 1993). Equally important is the physiological state of the host which has a very important influence on infestation with *Dactylogyrus* (Rhode, 1993), for instance, factors such as mucus, colour and hormonal status of fish are also important in the infestation of fish of both sexes (Pickering and Christie, 1980).

3.6 *Afrodiplozoon polycotyleus*

CLASS: Monogenea
GENUS: *Afrodiplozoon*
SPECIES: *Afrodiplozoon polycotyleus* (Figures 3.9 – 3.11)
HOSTS: *Barbus radiatus*, *Barbus trimaculatus* and *Labeobarbus marequensis*
LOCATION: Gills
LOCALITY: Nwanedi-Luphephe Dams

Infestation statistics of *Afrodiplozoon polycotyleus* is shown in Table 3.8. Live paired specimens of *A*. *polycotyleus* were collected from the gills of all hosts i.e., *B. radiatus*, *B. trimaculatus* and *Lb. marequensis*. In addition, diporpae with one and

two pairs of clamps were also encountered, but counted together with adults. No statistical analysis was performed on *Lb*. *marequensis* because this parasite was only recorded during summer with a low prevalence (10%).

Prevalence: The percentage of hosts infested with *A. polycotyleus* on each host is shown in Table 3.8. Unpaired diporpae with two pairs of clamps were also found and included in the infestation frequency. The prevalence of *A. polycotyleus* differed among the seasons and hosts (Table 3.8). The prevalence of *A*. *polycotyleus* on *B*. *radiatus* was lower during winter (31.3%) and higher during spring (80.0%) whilst both autumn and summer had a prevalence of 50.0%. When comparing the prevalence of the three fish species examined, *B. radiatus* had the highest prevalence. On *B*. *trimaculatus,* the prevalence was 64.7% during autumn, 20.0% during spring, and 21.0% during summer. None was found during winter. As mentioned above, on *Lb*. *marequensis* the parasite occurred only during spring with a prevalence of 10.0%.

Abundance: The abundance of hosts infested with *A. polycotyleus* (adults and/ or diporpae) on each host is shown in Table 3.8. On *B. trimaculatus*, the abundance was higher in autumn (0.9) with 0.4 and 0.2 in spring and summer, respectively. On the other hand, on *B. radiatus*, spring had the highest abundance (1.4) followed by autumn (0.6), then summer (0.5) and winter (0.4) (Table 3.8). On *Lb*. *marequensis*, the abundance was 0.2.

Mean intensity: The mean intensities of *A. polycotyleus* on *B. radiatus* rarely exceeded 2 during all seasons (Table 3.8). However, summer data showed the lowest mean intensity of 1.0. In autumn the mean intensity increased from 1.2 to 1.4 in winter, and increased again to 1.8 in spring followed by a decline in summer (1.0). On *B. trimaculatus,* the highest mean intensity was 1.5 in autumn followed by none in winter from which it rose to 2.0 in spring and then decreased to 1.0 in summer. The current data seems to suggest slight seasonality in the occurrence of *A. polycotyleus* on cyprinids. Both the mean intensity and the abundance of parasites per fish differed amongst seasons and hosts.

Table 3.8: Infestation statistics of *Afrodiplozoon polycotyleus* among the three cyprinids during the four seasons at Nwanedi-Luphephe Dams.

Fish	Season	No. of fish examined	No. of fish infested	A	MI	P (%)
	Autumn	10.0	5.0	0.6	1.2	50.0
B. rad	Winter	16.0	5.0	0.4	1.4	31.3
	Spring	10.0	8.0	1.4	1.8	80.0
	Summer	10.0	5.0	0.5	1.0	50.0
	Autumn	17.0	11.0	0.9	1.5	64.7
B. trim	Winter	17.0	0.0	0.0	0.0	0.0
	Spring	10.0	2.0	0.4	2.0	20.0
	Summer	19.0	4.0	0.2	1.0	21.0
	Autumn	15.0	0.0	0.0	0.0	0.0
Lb. mar	Winter	10.0	0.0	0.0	0.0	0.0
	Spring	10.0	1.0	0.2	2.0	10
	Summer	18.0	0.0	0.0	0.0	0.0

Key: A = abundance, MI = mean intensity, P = prevalence, *B. rad* = *Barbus radiatus*, *B. trim* = *Barbus trimaculatus*, *Lb. mar* = *Labeobarbus marequensis*.

Seasonality: There was no significant difference in the number of *A. polycotyleus* found on *B. radiatus* across seasons ($p < 0.05$, ANOVA) *(*Appendix 1).

Size (data pooled together): There was no correlation between the length of *B. radiatus* and the number of *A. polycotyleus* ($R^2 = 0.143$) *(*Figure 3.7 A). There was also no correlation between the number of *A. polycotyleus* found and the length of *B. trimaculatus* ($R^2 = -0.28$) (Figure 3.8 A).

Condition factor: There was no correlation between the condition factor of *B. radiatus* and the number of *A. polycotyleus* ($R^2 = 0.004$) *(*Figure 3.7 B), similarly for *B. trimaculatus* and the number of *A. polycotyleus* ($R^2 = -0.08$) (Figures 3.8 B).

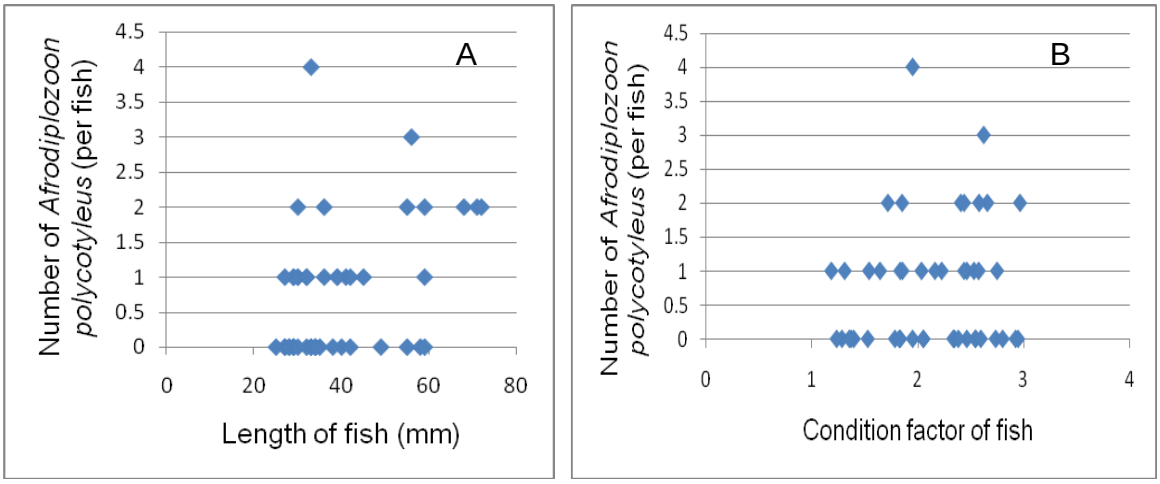

Figure 3.7: The correlation between the size (standard length) of *Barbus radiatus*, **A -** The number of *Afrodiplozoon polycotyleus* and, **B** - condition factor and the number of *A*. *polycotyleus* sampled during the entire study period at Nwanedi-Luphephe Dams.

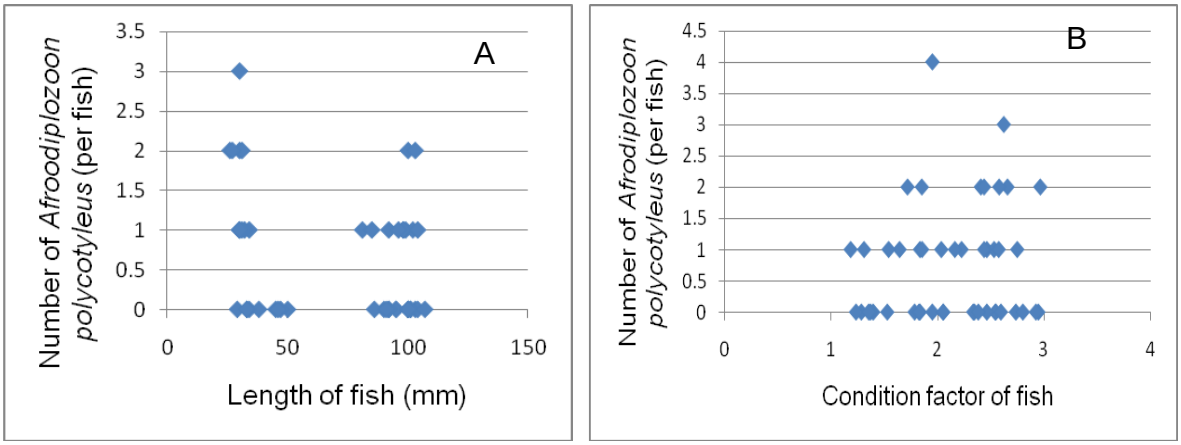

Figure 3.8: The correlation between the size (standard length) of and *Barbus trimaculatus*, **A** - The number of *Afrodiplozoon polycotyleus* and, **B** - condition factor and the number of *Afrodiplozoon polycotyleus* sampled during the entire study period at Nwanedi-Luphephe Dams.

3.6.1 Diagnostic features, description and occurrence of *Afrodiplozoon polycotyleus*

Afrodiplozoon polycotyleus Paperna 1973, previously known as *Neodiplozoon*, Tripathi 1959, was first described by Paperna (1973) from *Labeo victorianus* in Kenya and from *Barbus* spp. in Tanzania. In South Africa, it has been found on various cyprinid hosts from the Limpopo Province by Mashego (1982) (Table 3.10). *Afrodiplozoon polycotyleus* can, based on its range of infestation, be classified as one of the rarer genus or species of monogeneans in Africa. This study

is the second comprehensive documentation of this genus in South Africa. In this study, all three hosts viz., *Lb*. *marequensis*, *B*. *trimaculatus* and *B*. *radiatus* harboured matured specimens in permanent copula.

There are a number of clamps in the opisthaptor, their number increase with age of the parasite. The number of the clamps may be different in each of the coupled worms. These clamps develop asymmetrically i.e., at a given stage they might be eight on the left side and nine on the right side of the same parasite. The posterior clamps are always smaller than the rest even in fully grown specimens. The egg has a long filament.

As with all other diplozoids, when one specimen was recovered from the right gills, most of the time another worm will be found on the left gills of the fish as well. This observation is consistent with those of Paperna (1973) and Mashego (1982). Diporpa larvae with two pairs and one pair of clamps were recovered as well (Figures 3.9E and 3.10A). The diporpa with one pair was recovered during winter only while the other stage with two pairs clamp was encountered throughout the course of the study. Mashego (2000) also reported diporpa larvae with two pairs of clamps. Diporpa larvae with only one pair of clamps are not ready for union, however as soon as the second pair of clamps appears, they are ready for union to form juvenile diplozoids (Pečínková *et al.*, 2007). According to Zurawski *et al*. (2003), juveniles with one pair of clamps are not usually found in the natural environment. The reasons as to why not, is unknown at this stage, and as far as it could be established, no previous reports dealing with this subject are available.

Mashego (2000) found 14.0% of the 85 *Lb*. *marequensis* and *B*. *trimaculatus* investigated from Nwanedi-Luphephe Dams to be infested. When the present results were compared to those of Mashego (2000), prevalences recorded in this study were higher (Table 3.8). The present material fits the description given by Mashego (2000) in that; a wider variation in the number of clamps in the opisthaptor than those given by Paperna (1970, 1979) was noted. Based on the measurements in Table 3.9, the present material is comparably smaller than that recorded by Paperna (1979) and Mashego (1982). However, the sizes of the posterior width, width of the small clamp, pharynx length and oral sucker length are similar to that recorded by Mashego (1982). Though the posterior length is smaller than that measured by Mashego (1982), it is fairly comparable with that described by Paperna (1979). Confirming the measurements of Paperna (1979) and Mashego (1982) the width of the large clamp

was longer than the length of the large clamp, whilst for the opisthaptor, the length was longer than the width (Table 3.9).

From all specimens examined, the number of clamps in the opisthaptor ranged from eight to fourteen pairs (Figure 3.9 A+B) while Paperna (1973, 1979) reported that the number of the clamps ranged from eight in newly coupled individuals to ten pairs in gravid specimens. The clamps, oral suckers and pharynx are all relatively smaller than those recorded by Paperna (1973, 1979) and Mashego (2000), whereas the anchors were comparable with those given by Mashego (1982). In the present specimens anchors were not clearly visible, most probably because of the stain, used which proved to be inadequate to show this feature – however, Paperna (1979) and Mashego (1982) did observe it. Even so, this is not considered a generic characteristic, and therefore the presence or absence of such is apparently not of generic significance. A comparison of measurements between the present material and the previous descriptions is given in Table 3.9. These measurements were taken as depicted in Figure 3.11A - B.

Ecological aspects of *Afrodiplozoon polycotyleus* has been neglected, studies have emphasized surveys of fish for infestation and taxonomic considerations. Nevertheless, this parasite should be of great interest to ecologists because of its permanent fusion and its complicated life cycle that is tightly linked to the ecological relationship between the eggs, larval stages, and host. In relation to the geographical distribution of *A*. *polycotyleus*, this is the second finding of this parasite from the Limpopo Province of South Africa on *B. trimaculatus* and *Lb*. *marequensis* while its presence on *B. radiatus* constitutes a new host record.

Table 3.9: A comparison of measurements (in µm) of *Afrodiplozoon polycotyleus* from the *Barbus* species and *Labeobarbus marequensis* with those recorded by Paperna (1979) and (Mashego, 1982).

Features measured	Present study	Paperna (1979)	Mashego (1982)
Number of clamps	8 - 14	8 - 10	8 - 12
Small clamps	2	2	2
opisthaptor length	306	-	-
opisthaptor width	248	-	-
Anterior length	789	850 - 870	970 - 2658
Anterior width	185	580 - 600	349 - 776
Posterior length	406	420 - 470	504 - 631
Posterior width	289	280 - 300	233 - 436
Oral sucker length	56	140 - 190	50 - 65 (42)
Oral sucker width	43	120 - 200	80 - 100
Pharynx	-	80 x 80	55 - 65 x 50 - 60
Length of large clamp	45	190 - 250	60 - 65 (40)
Width of large clamp	67	300 - 340	85 - 90
Length of small clamp	35	80 - 150	30 - 45
Width of small clamp	36	100 - 180	30 - 40
Total length	806	-	-

The larvae with two pairs of clamps measured as follows: Clamps were 15 - 16 µm long by 18 - 18 µm wide. According to the measurements of Mashego (1982), the present specimen is smaller than the larva he examined.

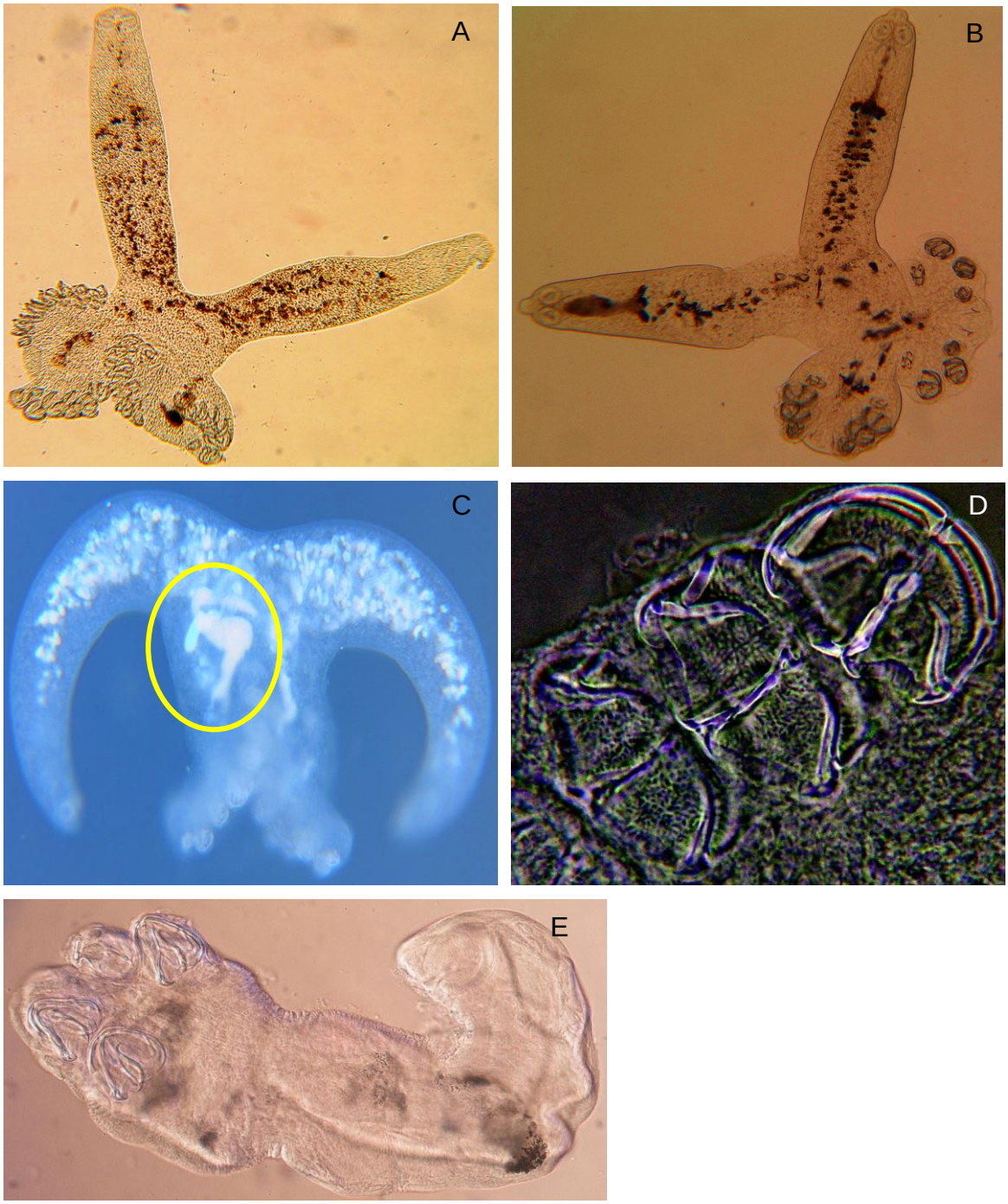

Figure 3.9: *Afrodiplozoon polycotyleus* in ammonium picrate from *Barbus radiatus* and *B. trimaculatus* at Nwanedi-Luphephe Dams, **A** - *Afrodiplozoon polycotyleus* gravid specimen from the gills of *B. trimaculatus* showing the number of clamps. **B**- newly united *A polycotyleus* with small developing clamps from the gills of *B. radiatus* showing the number of clamps and **C** - *A*. *polycotyleus* with an egg. **D** - showing the shape of the clamps, **E** - diporpae with two pairs of clamps only. Magnification = 40 - 100 x.

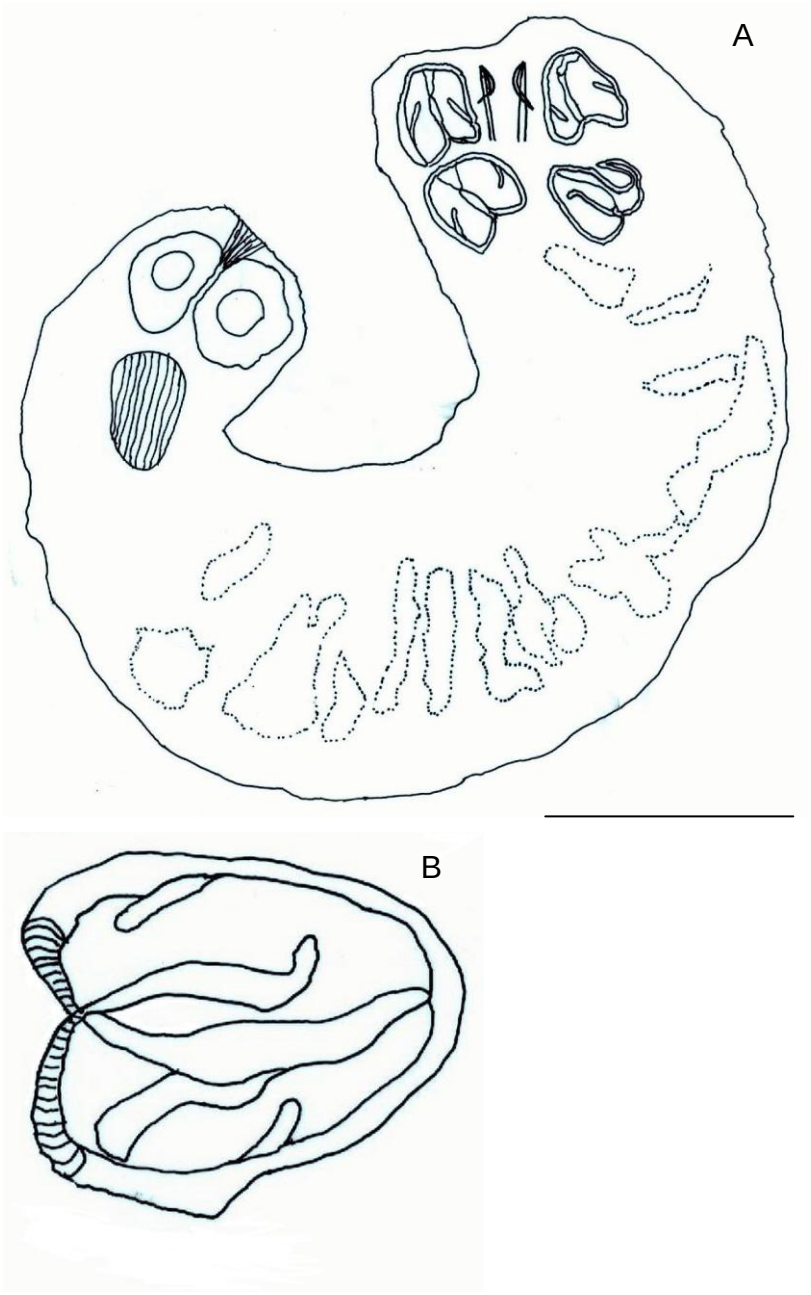

Figure 3.10: A - Diporpa larva with four clamps from *Barbus radiatus* at Nwanedi-Luphephe Dams, **B** - Clamp of the diporpa. Scale bar = 50 µm.

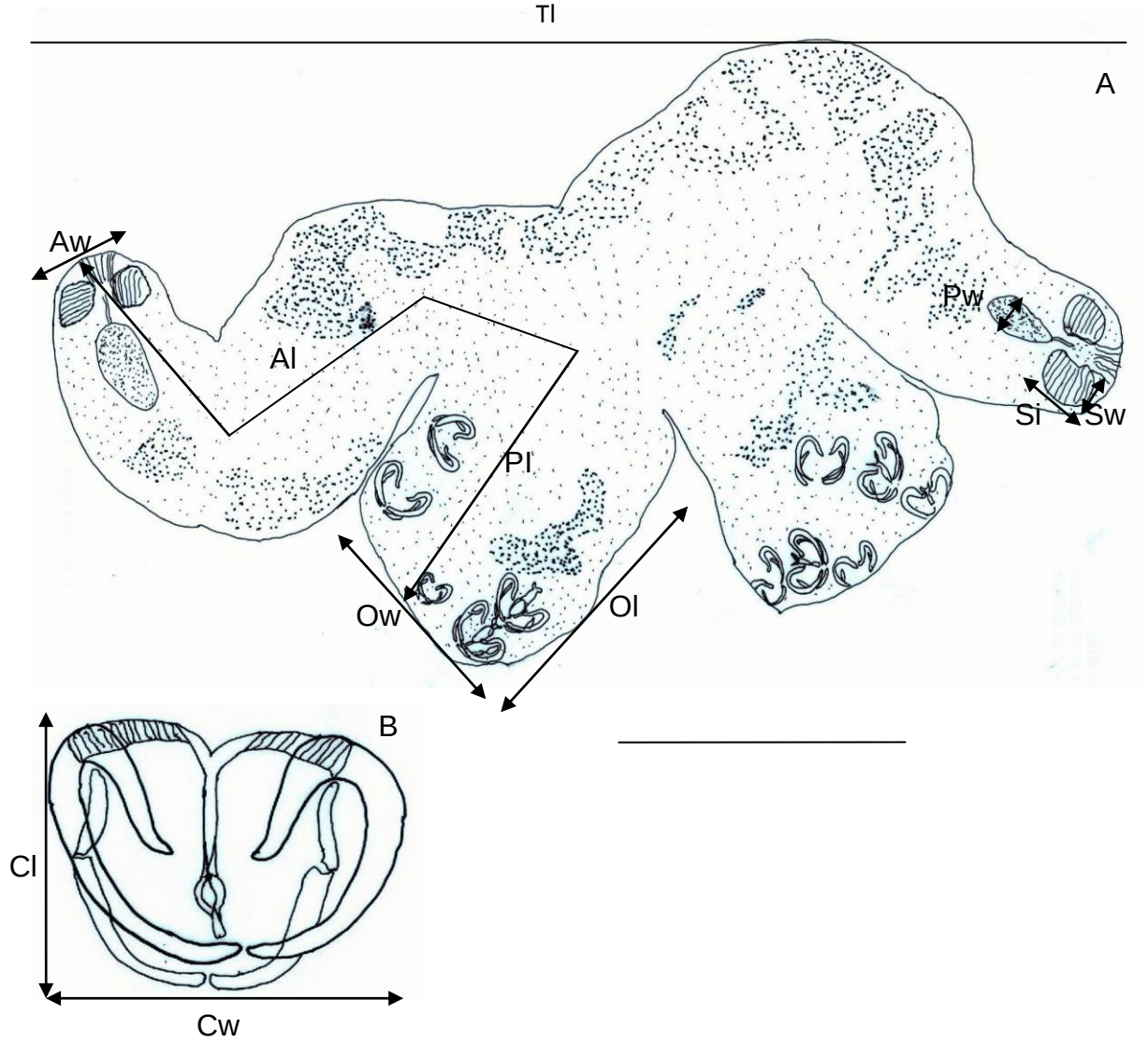

Figure 3.11: A - *Afrodiplozoon polycotyleus* coupled gravid specimen from the gills of *Barbus radiatus* at Nwanedi-Luphephe Dams and illustrations to indicate positions where measurements were taken to obtain morphological data. Total length (Tl), posterior length (Pl), anterior length (Al), anterior width (Aw) Opisthaptor length (Ol), Opisthaptor width (Ow), **B** - Large clamp - Clamp length (Cl) and Clamp width (Cw). Pharynx width (Pw), Sucker length (Sl), Sucker width (Sw). Scale bar = 50 µm.

Afrodiplozoon polycotyleus Paperna, 1973 (Diplozoidae) is an oviparous blood-feeding monogenean ectoparasitic on various species of cyprinids on the African continent (Mashego, 2000). In common with other diplozoids, it has a direct development that includes a free-swimming oncomiracidium and post oncomiracidial stage known as a diporpa (Figure 3.9 E). What is remarkable about this parasite is that they comprise two larval stages (diporpae) which fuse to form one double organism. Fusion stimulates metamorphosis of the joined pair so that gonads

develop and the vagina of one individual joins with the vas deferens of the other organism. Maturation is now triggered, leading to an individual adult parasite. This is a unique biology which is extremely rare and probably the most remarkable adaptation to cross-fertilization in the animal kingdom. Adults can apparently live in this situation for several years. Diporpae which do not find a partner with which to unite, die as individual diporpa as it is unable to reach sexual maturity (Chapman *et al*., 2000; Mashego, 2000). In the present study, adult parasites with eggs were observed. These specimens had large eggs with long, slithly coiled filaments.

To date, it has not been satisfactorily explained how the two partners find, distinguish and contact each other prior to fusion. Based on the behaviour of other diplozoids, Schabussova *et al*. (2004) hypothesized that the successful interaction of both diporpae is achieved after recognition of secretions of specific chemical substances such as glycoproteins, mediated by receptors on the surfaces of the larvae. In addition, Zurawski *et al.* (2003) indicated that diporpae migrate over the gill surface and when two diporpae are on the same gill arch, they are most likely to meet. After fusion, the individuals seemingly share all vital organs such as nervous, muscle and alimentary systems (Zurawski *et al*., 2003).

Attachment begins in the larval (diporpa) stage by way of a ventral sucker and dorsal papilla arrangement that progressively disappears as the two diporpae become fused. Fusion initiates metamorphosis of the joined pair during which there is reproductive development to eventual sexual maturity. Among species of *Diplozoon*, reciprocal fusion of the terminal part of the seminal duct of one individual with the vaginal duct of the female reproductive system of the other ensures cross-fertilization between the two hermaphroditic partners (Zurawski *et al*., 2003).

Generally, the attachment apparatus of the adults consists of more than four pairs of clamps and a pair of central hooks situated on the two haptors of each specimen. In diplozoids, pairs of clamps appear gradually as the larvae differentiate, development is asymmetric and developing parasites may show unpaired numbers of clamps (Paperna, 1979). The length of the central hook and the shape of clamp sclerites are one of the main markers for determination of *Diplozoon* species.

Based on host-specificity, *A*. *polycotyleus* can be regarded as a generalist (or narrow host specificity), having been recorded from at least 10 species of cyprinids (Table 3.10) in Africa. This geographical list agrees with the statement of Mashego (1982) that *A*. *polycotyleus*'s distribution is limited to that of the Cyprinidae. Due to its

rarity, it has only been found in four countries in Africa. However, this might be because many countries lack fish parasitologists, inadequacy of appropriate equipment or the work has not been done. Moreover, the research in many regions of this continent is further confounded by the unstable political situation and natural conditions, thus some places still remain untouched.

Table 3.10: Summary of the distribution of *Afrodiplozoon polycotyleus* and associated hosts on the African continent.

Host	Locality	Reference
Labeo victorianus	Nzola River, Kenya	Paperna (1973)
B. paludinosus		
B. cercops	Ruaha River, Tanzania	Paperna (1979)
B. macrolepsis		
B. neumayeri	Mpanga River system, Uganda	Chapman *et al*. (2000)
Barbus cf. *kerstenii*	southeast Kyoga system, Tanzania	Paperna (1979)
Lb. marequensis	Nwanedi-Luphephe, Limpopo Province, South Africa	Mashego (1982)
B. trimaculatus		
B. neefi	Lingwe, Limpopo Province, South Africa	Mashego (1982)
Lb. marequensis	Nwanedi-Luphephe, Limpopo Province, South Africa	Present study
B. trimaculatus		
B. radiatus		

Key: *B. paludinosus = Barbus paludinosus, B. cercops = Barbus cercops, B. macrolepsis = Barbus macrolepsis, B. neumayeri= Barbus neumayeri, Lb. marequensis = Labeobarbus marequensis, B. trimaculatus = Barbus trimaculatus, B. neefi = Barbus neefi, B. radiatus = Barbus radiatus*.

3.7 Ecology of *Afrodiplozoon polycotyleus*

3.7.1 Infestation

In the present investigation, *A. polycotyleus* was found on the gills of all hosts namely, *B. trimaculatus*, *B. radiatus* and on *Lb. marequensis*. *Barbus radiatus* appeared to be the preferred host followed by *B. trimaculatus* and least being *Lb. marequensis*. The present results concur with those of Mashego (2000) who

stated that *A. polycotyleus* is not restricted to any particular species within the family Cyprinidae and its distribution can, therefore, be limited to the Cyprinidae. This could be explained best by the fact that most species of Monogenea infest only a specific host genus, species, or family, exhibiting similarities in their distributions and physiological requirements as well as environmental characters of the habitat in which the hosts live, which may favour specific parasite's requirements.

The natural habits of the both *B. trimaculatus* and *B. radiatus* as bottom-feeders would indeed make it more likely to come across any parasite eggs lying dormant at the bottom. As was mentioned earlier, most *B. radiatus* infested with *A. polycotyleus* occurred mostly in pools in the stream where water current was greatly reduced. Thus, it may be stated that faster-flowing current may limit successful host-finding of the poorly swimming larvae while lower-flowing current may facilitate host-finding.

On the other hand, however, only 100-120 mm (SL) or larger *Lb*. *marequensis* were infested with *A*. *polycotyleus* and none were found on larger fish. The potent acquired immunity of larger *Lb*. *marequensis* might justify the lower infestation rate of *A*. *polycotyleus* on the *Lb*. *marequensis* and the underdeveloped immune system of the young fish which may render the natural repellent ability of the gill surface non-functional and results in increased susceptibility to ectoparasites (Paperna, 1996). Generally, young fish are suggested to be more susceptible to parasitic infestation than adult fish (Bagge and Valtonen, 1999). In addition, *Lb*. *marequensis* change their diet as they grow with young ones feeding on algae thereby increasing their chances of getting infested by this parasite.

3.7.2 Seasonal trends

The statistical analysis showed that there was no significant difference in the occurrence of *A*. *polycotyleus* across the four seasons (Appendix 1). However, the prevalence of *A. polycotyleus* on the two species was noticed, with winter being the lowest (sometimes zero recorded especially for *B. trimaculatus*). Nonetheless, the abundance of *A. polycotyleus* was low during all seasons; therefore the change in season had no drastic effect on the abundance since the number stayed low throughout the study period. There are no previous reports to compare the infestation rate of monogeneans on *B. radiatus* from this locality and Africa as a

whole. However, Mashego (2000) and Raymond *et al.* (2006) argued that water temperature was not significantly correlated with mean abundance or prevalence of *A. polycotyleus*. On the contrary, results from the present study indicate that the prevalence was higher for *B. radiatus* during spring and relatively constant during both summer and autumn. During winter the prevalence was low, emphasizing the effect of lower water temperatures or natural breeding season. This is evident for *B*. *trimaculatus* as well; none was procured during winter, but occurred during the other seasons whereas on *Lb. marequensis*, *A. polycotyleus* occurred only during spring.

It is worth noting that most adult *A. polycotyleus* were recorded during summer and the diporpae during winter. A water temperature of > 20.0°C is suggested as the optimum for fast development of diplozoids (Pečinková *et al*., 2007). It must be emphasized that water temperature simply affects the parasite's development but not seasonal occurrence, because these species reproduces only once a year. Perhaps this could also account for the *A*. *polycotyleus*'s rarity on the African continent. For instance, development of Diplozoidae to maturity and to the formation of copulating couples is slower, while the life-span of the adult diplozoid is extended from several months to two years (Paperna, 1996; Pečinková *et al*., 2007).

Although the prolonged life span (over a year), with reproduction limited to the warmer part of the year, intrauterine eggs were seen in *A*. *polycotyleus* in winter. According to Pečinková *et al*. (2007), the duration of egg development at water temperature of 15.0 to 20.0°C may require 10 to 11 days to hatch, anything less than 20.0°C delays development of the diporpa. This is especially plausible when taking into consideration the fact that in the natural environment, the life cycle of diplozoids usually takes less than a year, with sub-adult specimens overwintering on fish gills and reaching sexual maturation in the spring months (Pečinková *et al*., 2007). This could explain the high number of diporpae observed during winter in the present study. Pečinková *et al.* (2007) demonstrated experimentally that the diporpa of *Eudiplozoon nipponicum* on carp gills reach development faster at higher experimental temperatures. It is therefore true to suggest that the eggs remain dormant on the gills of the host during winter and as the temperature peaks they begun to hatch.

The highest prevalence of this parasite also coincided with the lowest water level in the dams. In the dams, water availability was much reduced during the dry

season winter, spring and summer, and sections of the dams were demarcated by a land barrier, which in wet seasons connects the dams again (see Chapter 2). This could lead to habitat contraction and concentration of hosts during the dry season. If higher densities contribute to higher levels of stress, dry conditions may lead to higher levels of parasitism. This was reflected for *B. trimaculatus* which, together with *Lb*. *marequensis*, occurred abundantly in the dams. The basis for this is that transmission would be enhanced due to the direct life cycle of the monogeneans. According to Chapman *et al.* (2000), a higher frequency of occurrence in the dry seasons may result from relatively higher host susceptibility and availability.

3.7.3 Condition factor

The fact that there was no significant relationship between the condition factor of fish and parasitic load in these fishes, indicates that *A. polycotyleus* has no observable effect on the health status of its host. Seddon (2004) also found no correlation between the number of an undescribed *Diplozoon* sp. and the condition factor of the host. Roberts and Sommerville (1982) stated that fish from a wild or natural population have a wide range of parasites, but most of these parasites exist as commensals, having little effect on the host or they are always in equilibrium with their hosts.

3.7.4 Size of host

Due to the statistically small number of infested fish recorded per season, particularly *B*. *trimaculatus*, the data for host size and the number of *A*. *polycotyleus* was pooled according to seasons. On both *Barbus* species from which *A*. *polycotyleus* was recovered, no correlations were observed between fish size and the presence of *A. polycotyleus*. The role of fish size on monogeneans has already been discussed in the previous section on *Dactylogyrus* species and shall not be repeated in detail here. The present results are in agreement with the findings of Seddon (2004) who did not find any correlation between the number of *Diplozoon* sp. and the total length of *Labeo umbratus* in the Vaal Dam and Vaal River Barrage (Gauteng, South Africa).

3.7.5 Host-specificity

Host-specificity in monogeneans is similar and therefore the discussion for the dactylogyrids applies for *A*. *polycotyleus* as well. The reason why *B. radiatus* was the most infested species among the three hosts during this study could be attributed to the fact that *B. trimaculatus* and *B. radiatus* closely co-habit the marginal and sheltered areas of the streams and dams, and are usually found hidden together in shelters and under vegetation.

In contrast, all *Lb. marequensis* were collected from the dam; none was collected under these conditions, nor was any collected from the streams themselves thereby excluded from these favoured areas of infestation and hence the least intensity observed. It can be assumed that this could, to some degree, account for the low infestation of *A*. *polycotyleus* on *Lb*. *marequensis*. In addition, behaviour of parasite dispersal stages appeared to favour infestation of fish, which not only have the same habitat, but also clearly display behavioural similarities, especially in their ethnology (Le Brun, 1992). Le Brun *et al.* (1990, 1992) found that the occurrence of *Diplozoon gracile* was associated with behaviour and habitat of the cyprinid hosts and that *Diplozoon* larvae spend about 60% of their lifetime on river bottoms, favouring infestation of benthic hosts. In the present study, the examined hosts, especially the small *Barbus* species, are active throughout the water column, but tend to spend much time hidden under rocks and other bottom materials (Skelton, 2001), thus becoming readily available for infestation by *Afrodiploozoon* larvae.

The present study revealed that *A. polycotyleus* was more abundant on hosts that were collected from the habitat stream than those from the dams. Given the above circumstances, one may be tempted to conclude that the host specificity displayed here, is hugely influenced by the macrohabitat selection by the host species. It is therefore clear, as was stated previously, *A. polycotyleus* is parasitic on members of the family Cyprinidae, but it is not restricted to any particular species within this family. Its distribution is therefore only limited by that of its Cyprinidae hosts.

3.7.6 Site specificity

Mashego (2000) stated that the long filament of an *A. polycotyleus* egg (Figure 3.10 C) enables it to attach to the gills, until it hatches, producing a free swimming diporpa larvae that infest the host immediately. The larva must find another larva with which to unite to complete its life cycle. The free-swimming stage represents an ideal opportunity for the parasite to exploit new hosts. In the present investigation, frequently a larvae was found on either side of the gills. At no point did more than one specimen occur on one side of the gills.

In order to explain this preference, a response to the abiotic environment and enhanced mate-locating chances is considered. Raymond *et al*. (2006) indicated that strong selection pressure or narrow microhabitat requirements may have resulted in the selection of the second gill arch, thereby increasing mating chances and decreasing mortality rate. In addition, microhabitat selection on gills may largely reflect a response to the abiotic environment. Chapman *et al.* (2000) suggested that, it is possible that site attachment is influenced by the pattern of respiratory current over the gills. Thus, *A. polycotyleus* may select certain gill arches to attach to in order to maximise oxygen availability in the hypoxic waters of their attachment site.

3.7.7 Host gender

There was no significant difference between the distribution of *A*. *polycotyleus* on male and female fish ($p > 0.05$) for either *B*. *radiatus* or *B*. *trimaculatus*. Of the total of 46 host individuals of *B*. *radiatus* collected, 24 were male, and 22 were female. Of these gender divisions, 12 male fish and 10 female fish were infested with *A*. *polycotyleus* and the rest were not infested. Furthermore, of the 63 *B*. *trimaculatus*, 14 male and nine females were infested.

3.7.8 Remarks on *Afrodiplozoon polycotyleus*

Afrodiplozoon polycotyleus was originally described by Paperna (1973) from *Labeo victorianus* in Kenya, and later from *Barbus paludinosus*, *B*. *cercops* and *B*. *marcolepis* in Tanzania (Paperna, 1979) as *Neodiplozoon polycotyleus*. When Mashego (2000) reported this parasite from *B. trimaculatus*, *Lb*. *marequensis* and

B. *neefi*, he used the key of Paperna (1973). He, however, mentioned that "in the present material a wider variation in the number of clamps on the opisthaptor, than in Paperna's (1979) was noted". This is a reference that there might have been some slight differences in Mashego's (2000) comparisons to that of Paperna (1973) which were not fully pursued.

Mashego (2000) further asserted that, of the 10 gravid specimen measured, six possessed 10 pairs, one 11 pairs, one12 pairs and two 13 pairs. Paperna (1979) on the other hand, reported that the number of clamps range from eight in newly coupled worms to 10 pairs in gravid parasite specimens. The clamps, oral sucker and pharynx in Mashego's (1982) material were relatively smaller than that in Paperna's (1973) material, whereas the anchors were more than twice the length compared to the latter material. Judging from these dissimilarities mentioned above, it is clear that the material from the present study matched with that of Mashego (2000). However, as Mashego (2000) concluded that these differences were not considered to be of specific value and therefore, his material was assigned to *N. polycotyleus*. This is justifiable considering that the emendation of the genus occurred in 1985 (Khotenovsky, 1985), three years after Mashego (1982) had completed his studies.

The problem with the identification of diplozoons in general seems to lie in the taxonomic validity of the anatomical structures that are used for classificatory purposes. According to Khotenovsky (1985), as cited by Gao *et al*. (2007), **Neodiplozoinae** Khotenovsky, 1985 contains only two genera, namely ***Neodiplozoon*** Tripathi, 1959 and ***Afrodiplozoon*** Khotenovsky, 1980. ***Neodiplozoon*** and ***Afrodiplozoon*** are both distinguished by the number of clamps, their arrangement and geographic areas of collection. Various morphological structures and distinguishing features are used to identify the **Diplozoinae**. In addition, Khotenovsky (1985) established that "*Neodiplozoon* has more than one pair of clamps, which are arranged on the back parts of the body and is found in India". In contrast, he also noted that "*Afrodiplozoon* is found in Africa and has many (clamps), with fewer than 15 pairs of clamps situated laterally on the posterior body end which is entire". In consideration of the abovementioned subfamily characteristics, it is therefore evident that specimens collected in the present study belong to *Afrodiplozoon*.

Nevertheless, the name *Neodiplozoon polycotyleus* continued to be used by other workers such Chapman *et al*. (2000), referring to *Afrodiplozoon polycotyleus*. However, Raymond *et al.* (2006) stated that the parasite has been misidentified by Paperna (1973) and described as *N. polycotyleus*. This transpired when the morpho-anatomical criteria used by Paperna (1973, 1979) were followed by Raymond *et al.* (2006) and led to the renaming of *Afrodiplozoon polycotyleus*.

Le Brun *et al*. (1988) expressed doubts about the taxonomic value of morpho-anatomical characters for *Diplozoon* identification and stressed the need for generic analyses. Raymond *et al*. (2006) therefore, asserted that the identification should be considered as tentative pending further taxonomic studies within the family.

An urgent review and clarification of the taxonomy of this species, to prevent any further confusion among researchers, is thus urgently needed. Furthermore, the possibility that these two species represent a complex of closely related species, impossible to differentiate using morpho-anatomical characters could not be ruled out completely. It is therefore possible that the record of *A*. *polycotyleus* is almost certainly a misidentification of a described species and thus its classification should be pursued to clarify its taxonomic placing in the future. On the strength of morphological similarity, the present material was identified as *Afrodiplozoon polycotyleus*. In this chapter, particularly in this section, it was felt that it is essential to give a background and summary of the existing taxonomy and distribution of monogeneans from the family Diplozoidae to avoid any misunderstanding. A review of the valid/invalid species or the variability of the diagnostic characteristics on which species separation is based for the diplozoids of Africa, is of paramount importance in relation to species identification. The synopsis follows;

3.7.9 General classification of Diplozoidae

According to Boeger and Kritsky (2001), the class **Monogenea** can be divided into two subclasses; the **Polyonchoinea** and the **Heteronchoinea**, with the latter being subdivided into the infrasubclasses **Oligonchoinea** and **Polystomatoinea**. The latter subclass contains the order **Mazocraeidea** Bychowsky, 1937 and suborder **Discocotylinea** Bychowsky, 1957, with the family **Discocotylinea** which is divided into the subfamilies Discocotylinea Price, 1936 and **Diplozoinae** Palombi, 1949 (Bychowsky, 1957). The two subclasses were developed in the mid-thirties and

were based on features of larval development and the structure of the hooks in the various groups of monogeneans.

The subclass Oligonchoidea has order Mazocraeidea and suborder **Octomacrinae** Khotenovsky, 1985 with two families namely **Octomacridae** Yamaguti, 1963 and **Diplozoidae** Palombi, 1949 (Khotenovsky, 1985). The subfamily Diplozoinae Palombi, 1949 contains the genus *Diplozoon*. Tripathi (1957) proposed the family Diplozoidae. In 1963, Yamaguti split the order Monogenea into two suborders: Monopisthocotylea and Polyopisthocotylea and proposed placing *Diplozoon* and *Neodiplozoon* Tripathi, 1960 within the super family Diplozooidae and family Diplozoidae.

Khotenovsky (1985) further divided the family **Diplozoidae** into two subfamilies, namely **Diplozoinae** and **Neodiplozoidae**. **Diplozoinae** Palombi, 1949 contains five genera, i.e., ***Diplozoon*** Nordmann, 1832, ***Paradiplozoon*** Achmerov, 1974, ***Inustiatus*** Khotenovsky, 1978, ***Sindiplozoon*** Khotenovsky, 1985 and ***Eudiplozoon*** Khotenovsky, 1985. **Neodiplozoinae** Khotenovsky, 1985 contains only two genera, namely ***Neodiplozoon*** Tripathi, 1959 and ***Afrodiplozoon***. ***Neodiplozoon*** and ***Afrodiplozoon*** are both distinguished by the number and arrangements of clamps, and their geographic areas of occurrance. **Diplozoinae** are identified through morphological structures and distinguishing features, including their measurements.

Khotenovsky (1985) listed 43 species belonging to the **Diplozoinae** from former Soviet Union. Boeger and Kritsky (1997) presented a revised hypothesis for the phylogeny of the monogeneans, in which the diplozoons are placed in the subclass **Oligonchoinea** and order Mazocraeidea, with the family falling in the suborder **Discocotylinea**.

In the current study it was decided to adopt Khotenovsky's (1985) classification system throughout the research to establish consistency. Members of the **Diplozoidae** have been recorded parasitizing numerous species of fishes worldwide. As new research is carried out in previously unexplored locations, the number of hosts, their distribution and new parasite species continue to increase.

Le Brun *et al*. (1988) pointed out that there is a lack of valid morpho-anatomic criteria for determining species of the closely related genus *Diplozoon* as they lack sclerified genitalia which are less subjective to adaptive pressures than the haptor.

According to Milne and Avenant-Oldewage (2006), most of the taxonomic studies on the Diplozoidae have focused on the unique ultrastructure of the sclerites situated in the attachment clamps that are positioned in two rows of four each on opposing sides of the organism's opisthaptor. Though the usages of standard techniques, such as trichrome stains and electron microscopy have restrictions when observing the sclerites, they do improve the visibility of the bivalve-like structure of the attachment clamps. During the course of the identification and consultation of recently used morphological structures in determination of diplozoids, the following observations came to light;

- Species determination of diplozoids is difficult and demands a great deal of experience.
- Properly prepared slide and the use of correct stains improve accurate species determination.

It was therefore difficult to sufficiently compare the present material with the measurements of Mashego (1982) as the original description usually lacks measurements of recently used characteristics for species determination of diplozoids. Furthermore, combined morphological and molecular approaches must surely provide an excellent and worthwhile field for future investigations of diplozoods in Africa.

Light microscope examinations of wet specimens supplemented by whole mount permanent preparations of the material collected during the present study revealed the following:-

- The taxonomic significance of the numbers and the distribution patterns of clamps, revealed that the number of clamps ranged between eight and 14 clamps. As can be seen from Figures 3.10 A and 3.10 B, the size of the parasite varied from one host species to another, those from *B*. *trimaculatus* were relatively larger at all times than those procured from *B*. *radiatus* as well as from *Lb*. *marequensis*.

These features are valuable diagnostic entities to separate different species. Measurements recorded during the present study showed that the present material is larger than that of Paperna (1973, 1979) and Mashego (1982), on the basis that the number of clamps on the material ranged between eight pairs in newly coupled worms to 10 pairs in gravid specimens, whereas measured specimens in this study ranged from eight pairs to 14 pairs of clamps. Therefore, the two main differences

between *Afrodiplozoon* and *Neodiplozoon* are; firstly, in the number of the clamps, and shape and size of the clamps and secondly in their geographical distribution. All specimens, from which clamps could be counted and drawn, invariably possessed between eight to 14 clamps, which corresponds with the original description of *Afrodiplozoon*.

3.8 *Gyrodactylus* sp.

3.8.1 Diagnostic features, description and occurrence of *Gyrodactylus* sp.

CLASS: Monogenea
GENUS: *Gyrodactylus* sp. (Figures 3.12 and 3.13)
HOST: *Barbus radiatus*
SITE: Gills
LOCALITY: Nwanedi-Luphephe Dams

The body shape and features are characteristic of *Gyrodactylus* species. Total body length 224 (208 - 240) and body width 72 (59 - 86.5), pharyngeal bulb and cirrus not discernible, opisthaptor oval, distinctly demarcated from the body. Opisthaptor total length 44.6 (37.5 - 51.7), opisthaptor total width 49.6 (49.3 - 50), hamuli fairly large, hamuli clearly broad at the base (Figure 3.13 A), Total length of hamulus 30.8 (30 - 31.6), hamulus point length 10.5 (10 - 11.7), hamulus shaft length 20.1 (20 - 20.4), hamulus aperture distance 15.7 (15 - 16.3), hamulus root length 8.59 (7.38 - 10), ventral bar not clearly discernible, dorsal bar comparatively short with a small protrusion at the base of the hamuli, slightly U-shaped; dorsal bar covers a small portion of anchor roots (Figure 3.13 A). In all four specimens, the anchor roots are long and wide, marginal hooklets arranged circular to the opisthaptor, total length of marginal hooklets 10.7 (10 - 11.5), marginal hook shaft length 3.6 (3 - 4), marginal hook aperture distance 7.8 (7 - 8.5), marginal hook proximal width 4.6 (4.5 - 5), marginal hook distal width 2 (2 - 2), marginal hook sickle length 5.7 (5.5 - 6.5), marginal hook sickle with strongly recurved blades (Figure 3.13 B). Marginal hook shaft length very short (Figure 3.13 B). In two of the

specimens from which measurements were taken, fully developed embryos were seen.

Members of the genus *Gyrodactylus* are viviparous ectoparasites of many freshwater and marine fish. They also have an extended distribution on other host such as amphibians (Harris and Tinsley, 1987). Gyrodactylids can infest fins, body surface and gills of the host (Kearn, 1994). The main difference between the gyrodactylid genera is in the morphology of the haptors (Khalil and Mashego, 1998). The modifications of the entire haptor for better function during attachment, various shapes of the hamuli and different positions of the marginal hooks form a basis for accurate identification of members of the genus *Gyrodactylus*.

In monogenean fauna of Africa much more oviparous parasites are known compared to viviparous (Khalil and Polling, 1997). To date, only eighteen species of the cosmopolitan genus *Gyrodactylus* are currently known from freshwater fish in Africa (Christison *et al*., 2005). Christison *et al*. (2005) mentioned that six additional records of representatives of this genus, in which the specimens were not identified to species level, have also been reported from a range of fish hosts. Of the named species, only three are described from cichlids, i.e. *G*. *cichlidarum* Paperna, 1968; *G*. *haplochromii* Paperna, 1973 and *G*. *nyanzae* Paperna, 1973. Two other gyrodactylid species parasitizing cichlids are known from the literature, i.e. *G*. *niloticus* Cone, Arthur and Bondad-Reantaso, 1995 and *G*. *shariffi* Cone, Arthur and Bondad-Reantaso, 1995 on cultured Nile tilapia *Oreochromis niloticus niloticus* (L.), from the Philippines (Cone *et al*., 1995). Of the species that have been described from African fishes, the majority of descriptions are incomplete and include only a few basic measurements and drawings of the hamuli and ventral bar.

The genus *Macrogyrodactylus* which was described by Malmberg (1957) contains nine species. Furthermore, Paperna (1968) described the genus *Afrogyrodactylus* with single species from freshwater fish endemic to Africa, belonging to the genus *Microalestes* Peters. According to Khalil and Mashego (1998), the two above genera are endemic to Africa and not found anywhere else. Recently, Luus-Powell *et al.* (2003) provided a description of a new African genus *Mormyrogyrodactylus,* identified from *Marcusenius macrolepidotus*.

While marginal hooks represent major attachment organs of most genera of the Gyrodactylidae, the hard structures (with hamuli and ventral bar) of the haptor of the various species represent main taxonomic structures and provide reliable

characters for the differentiation of the species. The shape and size of the hamuli and the ventral and dorsal bars are relatively stable within a certain range and therefore can be used for differentiation of the species. Khalil and Mashego (1998) suggested that in order to provide a meaningful comparison between various species, the method of measuring the various parts should be standardised for the species.

Gyrodactylids are among the smallest monogeneans with body size ranging from 0.4 to 0.8 mm. They have a fusiform body, containing two conspicuous cephalic processes bearing adhesive glands anteriorly. Posterior to the body is the opisthaptor with main attachment organ, 16 marginal hooks and one pair of hamuli, linked by two separate dorsal and ventral bars. Both hamuli and marginal hooks are composed of keratin-like proteins which are stabilized by disulphide bridges (Shinn *et al*., 2003).

The marginal hooks are arranged around the periphery of the haptor within fingerlike tegumental papillae and are capable of considerable mobility, moving freely within each tegumental papilla. The papillae have an extensive musculature, allowing each individual marginal hook to work independently of its neighbours, but the roof of the haptor is also well provided with radial fibres which bind the hooks together and allow them to act as a unit. The single pair of hamuli lies ventral to the marginal hooks, connected by the dorsal bar and fused by its end to processes on the surface of the hamuli. The ventral bar is loosely attached to the hamuli. In addition, the dorsal bar keeps the distance between the hamulus shaft uniform (Shinn *et al*., 2003).

In the present study, four specimens of *Gyrodactylus* were recovered from the gills of *B*. *radiatu*s. Only one specimen was found per fish. These gyrodactylids were absent on *B*. *trimaculatus* collected from the same stream with *B*. *radiatus*. Moreover, no *A*. *polycotyleus* were found on the hosts on which *Gyrodactylus* occurred. The only monogenean species common to both cyprinid species found from the same habitat was *A*. *polycotyleus*. On *B*. *radiatus*, the *Gyrodactylus* was primarily located on the distal portion of gill arch 1. The specimens are characterised by having very large anchors and small central hooklets which are similar to those of a typical *Gyrodactylus*. These differences probably provide sufficient evidence for erection of a new taxon for the *Gyrodactylus* recorded in this study.

Unfortunately, there are very few records of *Gyrodactylus* sp. parasitizing cyprinids of the freshwater fish fauna. To date, only two records are available in the literature, namely *Gyrodactylus invindoensis* and *Gyrodactylus kyogae*. The former was collected from *Barbus* cf. *holotaenia*, from mountain rivers in the Ivindo basin of Gabon, while the latter was collected on the following hosts; *Barbus neumayeri*, (from Kanyangareng-Suam River near Amudat), *Barbus prince*, (from the lower Sonso and Lower Waisoke Rivers) and *Barbus* (*Entromius*) sp. (from Kelim River, Uganda) (Paperna, 1979).

Of the two species of *Gyrodactylus*, described from cyprinids, the haptoral hooks are similar in shape but the size of the marginal hooks are quite different, and the ventral bar membrane is square in shape. No drawings or morphometric measurements were provided in these accounts. Paperna (1979) found only one specimen of *Gyrodactylus inviodoensis* and only one micrograph of each was provided. No detailed descriptive features were provided. On *Gyrodactylus kyogae*, however, some measurements and descriptive details were provided. Nevertheless, micrographs presented does not adequately show the important details necessary to make comparisons. The differences between the present material and those described by Paperna (1979), are mainly in shape of the marginal hook sickles. In all these specimens, the ventral bar is not clearly discernible. However, based on the morphology of the haptoral hard parts, specifically the anchors, this parasite is comparable to both *Gyrodactylus invindoensis* and *Gyrodactylus kyogae*.

The present material differs from these two in that, it is fairly large and that the marginal hooklets are relatively short (Figure 3.13 B (h)) whereas the measurements recorded by Paperna (1979) ranged from 14 – 16 units. However, the size of the opisthaptor and anchors do not differ. There are numerous omissions in the morphometric data for the abovementioned species by Paperna (1979), many of which require redescription, and particular attention should be paid to the morphological form and size of the anchors and marginal hook. These omissions may be the result of the relatively low number of specimens available for study. Accordingly, the present material does not appear to fit the requirements of any of the gyrodactylids studied by Paperna (1979), except that it is morphologically close to *Gyrodactylus invindoensis,* in that the anchors are long and wide at the base. But nevertheless, most features as described by Paperna (1979) and measurements differ markedly.

Based on the number of existing records of *Gyrodactylus* and the morphological dissimilarity from those discussed above, specimens in this study therefore constitute a new host record of a *Gyrodactylus* sp. parasitizing *B*. *radiatus* from South Africa. Thus, the data presented here contribute to the knowledge of gyrodactylids of South Africa and add some invaluable information as far as its occurrence is concern.

The lack of data on gyrodactylids of cyprinids may further be exacerbated by the fact that most African gyrodactylids are comparatively smaller than many of their Eurasian counterparts with regards to total body length (Christison *et al*., 2005), which can result in them being overlooked by researchers during surveys. Gyrodactylid monogeneans are apparently less host-specific (Whittington *et al*., 2000). Since transmission of monogenean flukes from fish to fish is primarily by direct contact, gyrodactylids have constant opportunities to move on a host or between host individuals, a strategy which may favour host switching (Kearn, 1994). Host switching is considered common for gyrodactylids (Bakke *et al*., 2002). In nature, host switching may occur between fish species sharing the same habitat. Especially host species related phylogenetically or ecologically to a parasite's original host may provide the conditions necessary for parasite transmission and survival and thus successful colonization. For this reason, the ability of gyrodactylids to emigrate from the host at any time during their life cycle should favour colonization of new host individuals or a new host species (Boeger *et al*., 2005).

In the present study, only one specimen was found during summer whilst three specimens were found during winter. This is consistent with the results of Paperna (1979) who also reported only one specimen of *G. inviodoensis*. The low infestation rate is difficult to interpret because generally gyrodactylids reproduce continuously throughout the year.

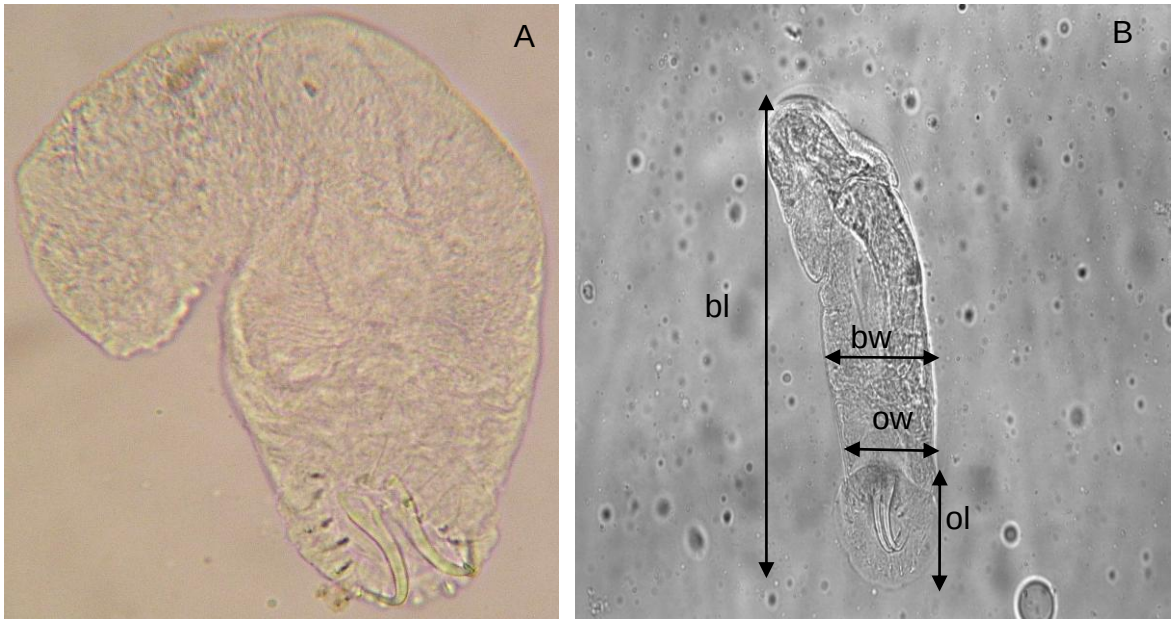

Figure 3.12: A - **B** - Micrograph of whole body of *Gyrodactylus* sp. from *Barbus radiatus* at Nwanedi-Luphephe Dams containing embryos; bl, body length; bw, body width; ol, opisthaptor length; ow, opisthaptor width.

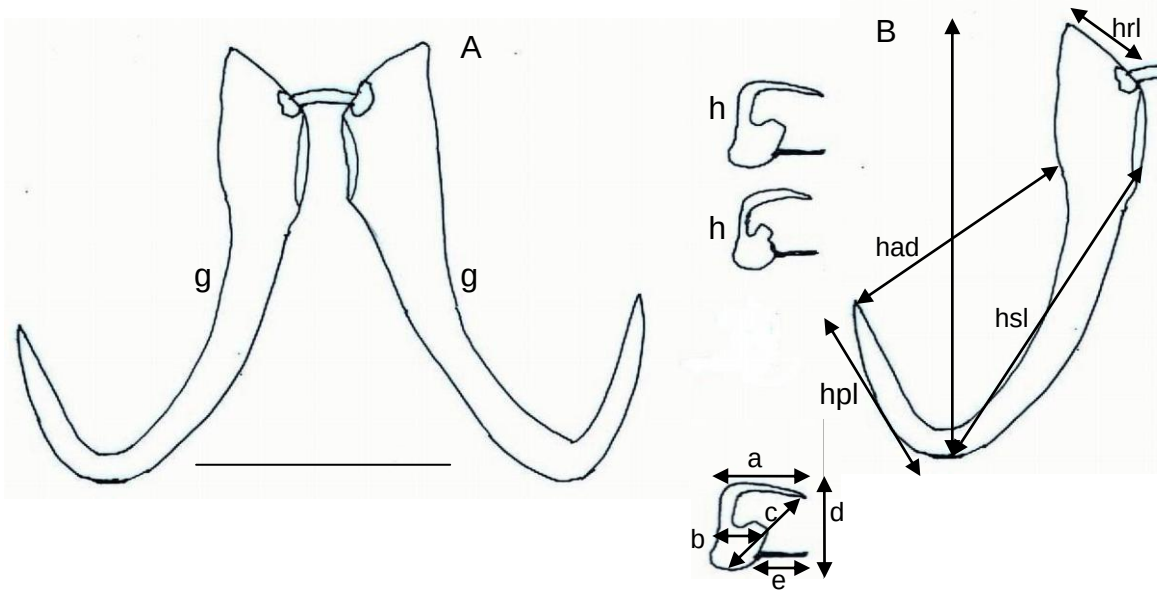

Figure 3.13: A - B - The attachment apparatus of the *Gyrodactylus* sp. from the gills of *Barbus radiatus* at Nwanedi-Luphephe Dams. (a) marginal hook point length (b), marginal hook proximal width; (c) marginal hook aperture distance (d), marginal hook sickle length; (e) marginal hook shaft length; (g) - Anchors, (h) - hooklets, (f), had, hamulus aperture distance; hpl, hamulus point length; hrl, hamulus root length; hsl, hamulus shaft length, (I) = Illustrations to indicate positions where measurements were taken to obtain morphological data. Scale bar = 0.05 mm.

3.9 *Dogielius* sp.

3.9.1 Diagnostic features, description and occurrence of *Dogielius* sp.

CLASS: Monogenea
HOST: *Barbus radiatus*
GENUS: *Dogielius* sp**.**
HOST: *Barbus radiatus*
SITE: Gills
LOCALITY: Nwanedi-Luphephe Dams

Eight specimens of *Dogielius* sp*.* Bychowski, 1957 were recovered from the gills of *B*. *radiatus* during the winter survey. *Dogielius* sp. has a wide distribution on *Labeo* species (Khalil and Polling, 1997). According to Paperna (1979), this genus is closely related to *Dactylogyrus* and had apparently diverged from the *Dactylogyrus* genus. Members of this genus are commonly found in Central and West Asian as well as African Cyprinidae (Paperna, 1979). According to the checklist of Khalil and Polling (1997), 22 *Dogielius* species have been described in Africa. Of these 22, seven species were recorded from eight *Barbus* species and the rest infest *Labeo* species, all of which occur in West Africa.

The present finding is the first record of a *Dogielius* species from Southern Africa. The occurrence of the parasite on *B*. *radiatus* could be a question of habitat. During the sampling period, *B*. *radiatus* co-occurred with *Labeo* sp. in the streams. This presents an opportunity for the parasite to infest either of these hosts.

3.10 Digenea

Over 50 species of digenean trematodes, from 15 families have been recognised from a variety of African freshwater fishes (Khalil and Polling, 1997). Digeneans can infest fish either in the adult form or as their metacercariae. The only adult digeneans which are known to infest freshwater fishes are the extraintestinal species, which includes the blood fluke *Sanguinicola*, which has been found in *Clarias gariepinus*, callodistomid and opistorchid species (Paperna, 1996). The most

frequently encountered digenean is, however, the metacercariae, which occurs in the eyes of freshwater fish. For the purpose of this study, more emphasis was directed to these larvae, mostly referred to as *Diplostomulum*, because they infest various fishes of economic importance in Africa. Paperna (1996) provided detailed information on the pathology and epizootiology of the various digenean metacercariae.

3.10.1 Diplostomidae

Diplostomidae is from the superfamily Diplostomoidae Poirier, 1886 and members of the latter are distinctly different from other groups of trematodes in possessing a unique holdfast or tribocytic organ, located at the posterior end of the parasite. The holdfast organ is sucker-like and bilobed and situated on the ventral surface of the body, posterior to the ventral sucker. It plays both digestive and adhesive roles. The Diplostomoidae contains six families and the general division of these is based on the host-specificity of the adults and characters of their morphology. The latter includes structure and shape of the fore-body and holdfast organ, the distribution of vitellaria, the absence or presence of bisegmentation of the body, the absence or presence of the cirrus-sac or paraprostate and the structure of the copulatory apparatus (Lunaschi and Drago, 2005).

Most frequently encountered *Diplostomum* species is the metacercariae form, which occurs in the eyes of freshwater fish. They occur in over 125 species worldwide (McKeown and Irwin, 1995). Metacercaria of *Diplostomulum spathaceum* Rudd, 1819 can be significant pathogens causing a range of disease symptoms (i.e. exophthalmia, local haemorrhage, and cataract or growth reduction) which may lead to fish mortality. They invade the eyes of fish and cause a parasitic disease called diplostomiasis which is prevalent in North America and Europe (Dörücü and Íspír, 2001). As with other endoparasitic helminths, metacercariae have a heteroxenous life cycle in which, as adults, they inhabit the digestive tracts of piscivorous birds.

In the past few years, a number of taxonomic papers dealing with diplostomid metacercariae reported from the brain, the cranial cavity, the spinal cord and eyes from various freshwater fish species in Africa, have appeared in the published literature (Ortlepp, 1935; Khalil, 1963, 1969; Lombard, 1968; Paperna and Thurston, 1968; Mashego, 1977; Prudhoe and Hussey, 1977; Mashego, 1982; Britz *et al*., 1985; Paperna, 1996; Barson, 2004; Luus-Powell, 2004; Barson and Avenant-

Oldewage, 2006 and Ramollo, 2008). Furthermore, according to the prevalence, reported by these authors, from various hosts, it is evident that diplostomid metacercariae are common.

The classification and ecological results of each of the metacercariae found in this study is given below.

3.10.2 *Diplostomulum*

CLASS: Trematoda
FAMILY: Diplostomidae Poirier, 1886
GENUS: *Diplostomulum* Brandes, 1892
SPECIES: *Diplostomulum* spp.
HOST: *Labeobarbus marequensis* and *Barbus trimaculatus*
SITE: Eyes
LOCALITY: Nwanedi-Luphephe Dams

Metacercariae of *Diplostomulum*, were found inhabiting the lenses of *Lb. marequensis* and *B*. *trimaculatus.* They were more abundant in *Lb. marequensis*. Due to the difficulties with the species identification within this genus, only the generic name was used in this dissertation. Thus, hereafter, this digenetic trematode will be referred to as "*Diplostomulum*". In *B*. *trimaculatus*, no infestation statistics or significant seasonal differences were performed as the infestation was too low (10% in spring and 12% in summer) to warrant statistical analysis thereof.

Prevalence: The prevalence of *Diplostomulum* in *Lb. marequensis* was fairly constant during all seasons ranging from 80% to 100%, with the lowest value of 80% observed during the winter month (July) (Table 3.10). A considerable increase was observed in spring and summer with 100% and 94.4% respectively.

Abundance: During all surveys, the abundance of *Diplostomulum* was considerably higher in *Lb*. *marequensis* (Table 3.11). Abundance of *Diplostomulum* in *Lb. marequensis* increased exponentially, ranging from 7.2 in winter to 18.1 in summer, while autumn and spring were at 8.8 and 9.7, respectively.

Mean intensity: The mean intensity of *Diplostomulum* in *Lb. marequensis* showed a slight seasonal trend and varied from 10.2 in autumn to 9.0 in winter and then increased to 9.7 and 19.1 in spring and summer respectively (Table 3.11).

Table 3.11: Infestation statistics of *Diplostomulum* in *Labeobarbus marequensis* at Nwanedi-Luphephe Dams.

Season	No. of examined fishes (n)	No. of fish infested	A	MI	P (%)
Autumn	15.0	13.0	8.8	10.2	86.7
Winter	10.0	8.0	7.2	9.0	80.0
Spring	10.0	10.0	9.7	9.7	100.0
Summer	18.0	17.0	18.1	19.1	94.4

Key: A = abundance, MI = mean intensity, P = prevalence.

Seasonality: There were significant differences in the number of *Diplostomulum* sp found in *Lb. marequensis* during the different seasons ($p < 0.05$, ANOVA,) (Appendix 1). *Post hoc* (Dunnet T3) showed that autumn and summer differed significantly ($p > 0.05$) as well as spring and summer ($p > 0.05$) as shown in Appendix 1.

Size (data pooled together): There was no significant correlation ($R^2 = 0.044$) between fish length and the number of *Diplostomulum* found in *Lb. marequensis* (Figure 3.14).

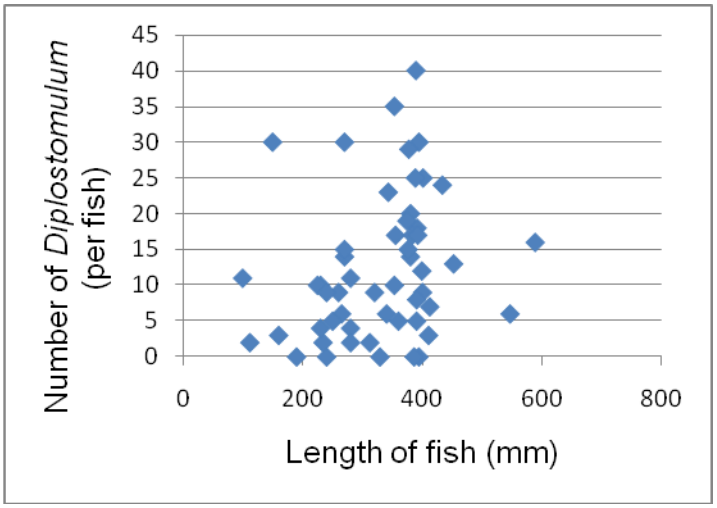

Figure 3.14: The correlation between *Diplostomulum* and the total length of *Labeobarbus marequensis* in the seasonal study at Nwanedi-Luphephe Dams.

Condition factor: No correlation between the number of *Diplostomulum* and the condition of the fish was found across seasons (Figure 3.15) ($R^2 = -0.01$).

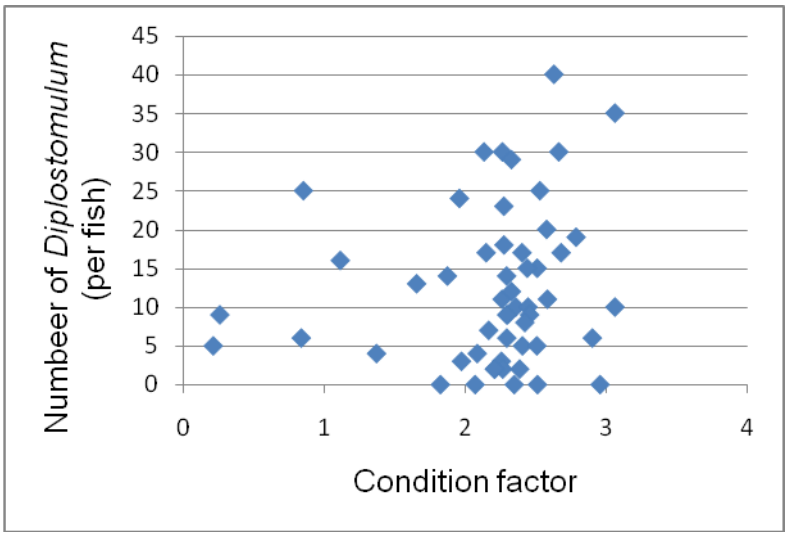

Figure 3.15: Correlation between *Diplostomulum* intensity and the condition of the *Labeobarbus marequensis* in the seasonal study at Nwanedi-Luphephe Dams.

Gender of the host (data pooled): There was no significant difference ($p > 0.05$, T - test) in the number of *Diplostomulum* found in males and females of *Lb. marequensis*.

3.10.3 Occurrence of *Diplostomulum*

Diplostomulum mentioned already were procured from the vitreous humor of the eyes of both *Lb. marequensis* and *B. trimaculatus. Diplostomulum* is the largest component of the fish parasite community in the dams. The dams are visited regularly by migrating birds. During the course of the study, herons, cormorants and darters were observed, thus it was expected to encounter *Diplostomulum* parasitizing freshwater fish as juveniles because the parasite only reaches maturity in piscivorous birds. As was mentioned previously, metacercarial (*Diplostomulum* spp.) infestations have been found in many inland waters in Africa from various freshwater fishes.

Mashego (1977) recovered *Diplostomulum* from the brain and cranial cavity of *Clarias gariepinus* in the Limpopo Province of South Africa whilst Prudhoe and Hussey (1977) recovered *Diplostomulum* from the cranial cavity of the same fish from the eastern Transvaal (now Mpumalanga). Saayman *et al*. (1991) also recorded

diplostomulae from the brain cavities and eyes of a number of *Barbus* and cichlid species from the Middle Letaba Dam in the Limpopo Province of South Africa.

In addition, Mashego (1982) recorded diplostomulae in the vitreous humour of the eyes of nine species of *Barbus* spp. viz., *B*. *paludinosus* Peters, 1852; *B. trimaculatus*; *Lb. marequensis*; *B*. *mattozi* Guimaraes, 1884; *B*. *lineomaculatus* Boulenger, 1903; *B*. *neefi* Greenwood, 1962; *B. radiatus*; *B*. *unitaetus* Günther, 1866 and *B*. *argentues* Günther, 1868 from various water bodies in the Limpopo Province of South Africa. It is worth noticing that in the present study, as was the case in Mashego (1982), the parasite never occurred in the brain cavity of the cyprinids investigated.

From the literature cited in the foregoing paragraphs, comparisons between the forms of diplostomulae from all these hosts tend to indicate that all these forms could possibly be regarded as morphospecies. Mokgalong (1996) considered these forms to be as a result of one or a combination of the following factors:

- Different host species;
- Host species from different aquatic ecosystem;
- Different infestation sites e.g. cranial cavity or vitreous humour of the eyes;
- Different stages of metacercarial development;
- Different methods of preservation and/or microtechnical procedures.

According to Mokgalong (1996), the above factors may, in one way or the other, cause minor intraspecific differences. *Diplostomum* spp. are generally morphologically differentiated with difficulty, especially in the metacercarial stage. This causes considerable difficulties in their identification to species level. Metacercariae in natural infestations are subjected to various factors causing variability in their morphology. Experiments carried out by Niewiadomska and Szymanski (1991) on metacercariae of *D*. *paracaudum* Iles, 1959 and *D*. *pseudospathaceum* Niewiadomska, 1984, and by Graczyk (1991) on *D*. *pseudospathaceum* and *D*. *spathaceum* Rudolphi, 1819 have shown that the host species, the density of infestation, the size and age of the fish host, and the age of the metacercariae can affect the morphology. Any of these factors may generate statistically significant differences between individuals of the same species.

Identification keys, such as those of Shigin (1986) go some way to alleviate the problem. These keys are based on the morphology and specificity of adults,

metacercariae and cercariae of *Diplostomum* species respectively, taking account of size and position of organs. A description of the variability of morphology characters makes it possible to determine their diagnostic value and consequently to select the characters which are useful in the study of natural infestation.

On the basis of size of the hosts, one may be tempted to ask, how does a piscivorous bird with a relatively weak, pointed bill not designed for crushing and tearing, succeed in acquiring metacercariae lodged around the brain tissues of *C*. *gariepinus* which are protected by the hard bony capsule of the skull, particularly in bigger host? Mokgalong (1996) suggested that it seems reasonable for one to assume that very young *C*. *gariepinus*, together with the fact that cyprinid and cichlid fish act as the principal intermediate hosts in the successful completion of the life cycle of *Diplostomum* spp. in the Limpopo Province of South Africa and possibly Africa as well. Therefore, it seems fair to suggest that this could explain why hundreds of *Diplostomum* specimens are always found in larger fish because they accumulate over a long period of time.

The high intensities observed in the present study are incompatible with those of other workers such as Mashego (1982) who recorded an infestation ranging from one up to 200 specimens in the eyes with averages of six to 20. In the present study, the mean intensity ranged from one to 35. Conversely, the occurrence of this parasite in the other studies was low when judged against the present results where 100% infestation was recorded in summer. Moreover, the occurrence of the parasite in the present study was always higher in *Lb*. *marequensis* which were collected from the dams than in the *Barbus* species collected in the streams (either above or below the impoundments). This trend corroborates the findings of Mashego (1982) who observed that fish from the dams were more frequently infested with *Diplostomulum* sp. than riverine specimens. Similarly, Mashego (1982) found that although *Diplostomulum* sp. infested fish from Seshego, Lepellane and Piet Gouws dams, as well as from the Olifants, Nyl, Mohlapitse and Lingwe rivers, none of the fish from Turfloop Dam and Levuvhu River were infested. No explanation was provided for this, but the presence or absence of carrier birds in those dams might have played a key role in this regard as far as dissemination of the parasite is concerned.

Mokgalong (1996) investigated the helminth parasitofauna of three of the most widespread piscivorous birds frequenting aquatic ecosystems in the Limpopo

Province of South Africa. He recorded two species of *Diplostomum*, namely, *D*. *tregenna* Nazmi Gohar, 1932 from *Phalacrocorax carbo* (White breasted Cormorant), *Phalacrocorax africanus* (Reed Cormorant), *Anhinga melanogaster* and *Ardea melanocephala*, as well as *D*. *ghanense* Ukoli, 1968, which is species specific to *A*. *melanogaster*.

Based purely on anatomical comparison between the metacercariae procured by Mashego (1982) and Saayman *et al*. (1991), Mokgalong (1996) felt justified to link the *Diplostomulum* recovered from the freshwater fish mentioned in the foregoing paragraph in the study area as causative agent or precursors of *Hystermorpha triloba*. On the strength of these comparisons, the present specimen could be regarded as the metacercariae for the *H*. *triloba* procured from *P. carbo* and *P. africanus* in various water bodies of the Limpopo Province of South Africa.

From a statistical as well a geographical point of view, it was clearly evident that *Diplostomulum* was the most widely occurring and established parasite in the Nwanedi-Luphephe Dams. The continuous distribution of definitive hosts mentioned above as well abundance of all 3 cyprinid intermediate hosts probably played crucial roles in the ecology of this parasite within the study area. Due to its non-specificity to the second intermediate host and definitive hosts, *Diplostomulum* may readily establish itself in fish in the study site. Following from its non-specificity, *Diplostomulum* was regarded as a generalist not strictly host specific, having been found parasitizing a wide range of fishes including cichlids and clariids.

In conclusion, it may therefore be assumed that the parasite's life cycle requirements are well met in the study area and that movement of birds to other water bodies most likely increases its chances of it being disseminated to far distant water bodies of South Africa.

3.11 Ecology of *Diplostomulum*

Diplostomulum could not be identified to species level because the larvae lack reproductive features that are useful in identification. As was mentioned earlier, adults of this parasite occur in fish-eating birds and it was not possible to examine birds, as it was beyond the scope if this investigation. Standard taxonomical methods

and identification based primarily on morphological features represent an important basis for metacercariae species diagnosis.

Because of the problematic identification of this parasite, metacercariae in freshwater fishes are commonly referred to as *Diplostomulum* stages. It has become common practise to use the generic name *Diplostomulum* for the larval stage found in fish. It must, however, be pointed out that there is no general adherence to this practise to be observed in the literature. As a result, various authors have used the name *Diplostomum* referring to the stages occurring in fish, when in fact it should be *Diplostomulum* (e.g. Dörücü and Íspír, 2001; Dörücü *et al*., 2002; Mierzejewska and Własow, 2005). Most digenean flukes have been widely introduced globally throughout the tropics and subtropics by introduction of snails (Paperna, 1996). Water bodies from the Jordan system throughout the Nile to the southern end of the African Rift Valley and southwards have been reported to share common snails such as *Bulinus*, *Lymnaea* and *Melanoides* and a similar ichthyofauna such as cichlids, clariids and cyprinids (Paperna, 1996). It is therefore not surprising that different fishes from various water bodies serve as intermediate hosts for the same metacercariae of the genera including amongst others, *Neascus*, *Clinostomum*, *Diplostomum*, *Euclinostomum*, *Centrocestus*, *Phagicola* and *Haplorchis* (Paperna, 1996). It can also be stated that the actual number of *Diplostomulum* occurring in African freshwater fish is undoubtedly much higher than that reported thus far and it is probable that larvae of numerous taxa will be reported in the near future should all neglected water bodies of Africa be pursued earnestly. It will also be more interesting to have molecular work done on diplostomulae found in African freshwater fishes to distinguish among taxa. This should be an excellent opportunity for future research.

3.11.1 Infestation

In the present study, the infestation intensity of *Diplostomulum* in *B*. *trimaculatus* did not exceed 5 metacercariae per fish and the infestation was sporadically. These results are in accord with those of Mashego (1982) who also observed a low infestation in this host. The total absence of *Diplostomulum* in *B*. *radiatus* is rather difficult to explain apart from reasoning that fish-eating birds are more dependent on open dams where they can see and catch their prey without any hindrance which is not the case in the stream where *B*. *radiatus* were procured. It is

worth noticing that *B*. *radiatus* never occurred with the other hosts where the birds were frequently observed during the course of this study. One may be justified to reason that streams are, for example very narrow, often surrounded by thick vegetation which could prevent birds from feeding on this host. Hence, the occurrence of this digenean larva reflects primarily the type of habitat and presence of suitable intermediate hosts.

Ondračková *et al*. (2004) revealed that the key-factor associated with a higher infestation of *Posthdipostomum cuticola*, a digenean, was host fish species richness in adult fish and fish density in juvenile fish. These authors further noted that the total parasite prevalence and total abundance of infestation were also relative to bottom-type behavoiur and water transparency. This suggests that fish host density and species composition are among the main factors affecting *Diplostomulum* infestation in fish. Therefore, the prevalence and abundance of *Diplostomulum* are also dependent on the density of intermediate snail hosts, presence of bird's and water transparency which may help in locating prey and are the most likely factors affecting the wading bird choice. The latter factor is believed to make conditions easier for piscivorous birds to see their prey in water without any difficulty.

According to Dávidová and Ondračková (2008), lentic waters represent more suitable environments for completion of digenean life cycles than in riverine habitats. This was the case in the present investigation as lower infestations were observed from those fish from the streams than those from the dams. In addition, specific conditions such as higher water temperatures, lower water velocity and the presence of vegetation encouraged the presence of snails and final hosts, especially birds (Ondračková *et al*., 2004) in and about the dam environment. These factors were also observed especially during summer when the velocity of the water was reduced when the water level dropped and the canal connecting the two dams broke off. In general these conditions are positive for reproduction and dissemination of digenean parasites. In open water systems, where migration of intermediate hosts is possible, the success of parasite transmission depends on the availability of definitive hosts.

In addition to that, *Diplostomulum* derives benefits directly from the host using specific strategies. This interfere with vision and leads to partial or total blindness, thereby manipulating the behaviour of the fish intermediate host to increase the probability of its transmission to predators or final host (Seppälä *et al.*, 2005). It can

be stated that the ability of *Diplostomulum* to manipulate the behaviour or other phenotypic traits of their hosts is the adaptation to enhance parasite transmission.

It is can also be stated that the life cycle of *Diplostomulum* is influenced directly or indirectly by several abiotic factors, for instance, natural conditions provide favourable conditions for the parasite for several reasons: Firstly, dams with a high nutrient load and primary production maintain high numbers of intermediate snail hosts. Secondly, constantly high fish densities ensure high cercarial infestation rate. Thirdly, easy prey attracts bird hosts to feed on infested fish, which effectively completes the parasite life cycle.

3.11.2 Seasonal trends

The majority of studies concerning abiotic factors related to digenean infestations have focused on the effects of water temperature (Chubb, 1979; Sandland *et al*., 2001). McKeown and Irwin (1995) demonstrated experimentally that the ovaries of *Diplostomum spathaceum* developed at a much quicker rate when water temperature is maintained at 30°C. They stated that at this temperature, the eggs developed much quicker than it would at lower temperatures. From the above observations, it is clear that, although this water temperature was substantially higher than would occur under natural conditions, it does not differ greatly from the 27°C recorded during summer in the present study and this may have accelerated development.

The peak infestation observed during the present study (spring and summer) may be attributed to two other possible factors. The first could be a result of the rise in water temperature during summer, after the winter period, because the parasite life cycle is completed quicker, since complete development of metacercariae usually takes 1-2 months, depending on water temperature (Seppälä *et al.*, 2005). The second factor is the movement of fish populations towards the shore for reproduction, which exposes fish to piscivorous birds. The fact that changes were found that differed from previous studies may also have to do with other factors such as feeding and spawning of the host. In natural conditions, the cercariae face a highly unpredictable environment in terms of host contact probability. This is because the infestation is determined principally by movements of the fish hosts. The quantity of cercariae is diluted in large water volumes, thus decreasing the

contact rate with the host. During the dry season, fish in the Nwanedi-Luphephe Dams can be trapped for several months in either of the two dams, when the water level dropped considerably, and the connection between the two dams are broken. During this time, the infestation is likely to increase contact with the fish host in natural habitats. This coincided with late spring and summer.

3.11.3 Condition factor

There was no correlation detected between the condition factor of fish and parasitic intensity. No gross pathological signs of metacercarial occurrence were observed. It can therefore be assumed the hosts examined were in good condition.

3.11.4 Size of host

There was no significant relationship observed between the length of the host and the rate of *Diplostomulum* infestation. That implies that the size of the fish did not have any influence on parasite load occurrence. However, mean intensity and abundance did increase as a function of fish length. Therefore there is no evidence that this eyefluke could accumulate in the host organism depending on host's size. In this study, however, the larger fish (*Lb. marequensis*) was more infested than the *Barbus* species, which could indicate that the size of the vitreous humour in the former is bigger than in the latter.

3.11.5 Gender

Diplostomulum did not show any preference for either male or female fish during the present study. Likewise, Mashego (1982) found no differences in the nature and rate of infestation between male and female hosts, and no difference among the various fishes he examined.

3.12 Larval forms - metacercariae

Because of the difficulty in the identification of these metacercariae, the genus

and species name were omitted. Two types of these metacercariae were found from the gills and in the muscle.

3.12.1 Diagnostic features and occurrence of the metacercariae from the muscles

CLASS: Trematoda
ORDER: Digenea
FAMILY: Diplostomidae Poirier, 1886
GENUS: Unidentified digenean metacercaria (Figure 3.16)
HOST: *Barbus trimaculatus* and *Barbus radiatus*
SITE: Muscle
LOCALITY: Nwanedi-Luphephe Dams

These metacercariae were found encysted between the integument and body musculature of the fins and skin of both *B*. *trimaculatus* and *B*. *radiatus* during the winter survey. The number of cysts ranged from two to three per fish. On *B*. *trimaculatus* they appeared alongside the spots (Figure 3.16 A, B). When the cyst was opened, the immature parasite straightened and flattened into a form that resembles the adult. The metacercaria was contained in spherical or oval cysts in tough outer black, two cysts walls- outer black cyst wall of host origin and inner transparent cyst formed by parasite which leads to clearly recognizable black spots on the skin and fins (Figure 3.16 A, B).

Infested fish deposits dendritic pigment cells around the cyst and it appears black to the naked eye, hence the name “black grub” whereas the enclosed cyst is folded within the cyst wall (Mashego, 1982). The affected fish appears "peppered" as the cysts of the parasite are lodged and become encapsulated by host tissue and melanophores surround the outer layers just under the skin, thus giving a black spot appearance (Lane and Morris, 2000).

Most fish grubs invade the flesh of fishes and then appear as round or bead-like structures embedded in the fish's flesh thus interfering with organ function (Paperna, 1996). Black grubs infestation is mainly caused by metacercariae of the family Diplostomatidae and are commonly known to cause black spot disease (Mashego, 1982). In nature, they have been frequently seen in freshwater fishes in

the skin, tail base, fins, and musculature. Apart from causing black grub, they have been implicated as an important cause of dermal melanophores and other chromophores (Lane and Morris, 2000). Although most digenetic trematodes are not a serious threat to fish health, their mere presence often renders the fish undesirable by consumers.

The above mentioned grubs undergo its development within various hosts (e.g., snails, fish, and birds), and can either actively or passively invade their hosts (Lane and Morris, 2000). In the literature, it is generally reported that cyprinid fish species are the principal intermediate hosts for this parasite which may cause mass mortalities during heavy infestations. The small *Barbus* species from which this parasite was procured are amongst the most widespread fish species inhabiting water bodies in the Limpopo Province of South Africa.

The low number of fish infested with digenean larvae may have been due to the period the fish spend hiding under rocks, since the birds that serve as a definitive host for this group of parasites, would hardly find them. Especially because some endoparasites are transmitted by means of interactions between prey-predator and the presence or absence of these parasites in the hosts defines such interaction.

Mashego (1982) recovered Black grub metacercariae, anatomically similar to the ones infesting *O*. *mossambicus* from Middle Letaba Dam from four *Barbus* spp., viz. *B*. *radiatus*, *B*. *unitanitus*, *B*. *trimaculatus* and *B*. *paludinosus* from more than one aquatic ecosystem in the Limpopo Province. Although six *Barbus* spp. inhabit Middle Letaba Dam, Saayman *et al*. (1991) did not record Black grubs from these hosts. Instead, these authors reported Black grub infestations amongst the *O*. *mossambicus* population of the dam.

As was mentioned earlier, the adult stage of this parasite occurs in fish-eating birds. Mokgalong (1996) considered Black grub diplostomulae to be the causative agents or precursor for *Harvardia sandgroundi* infestations in *Phalacrocorax carbo* (Whitebreasted Cormorant) and *Phalacrocorax africanus* (Reed Cormorant) in the subtropics of southern Africa. This author's conclusion was solely based on anatomical, morphological and ecological similarities with the metacercariae procured by Mashego (1982). Mokgalong (1996) then cautioned that although such justifications were accepted, the verification of this assumption still warrants experimental validation before conclusive assumptions can be made.

Mokgalong (1996) also observed that *O*. *mossambicus* was the primary fish intermediate host for this parasites with the *Barbus* spp. acting as supplementary intermediated hosts. However, in an ongoing PhD project which run concurrently with this study, this parasite was not found from *O*. *mossambicus*. From the infestation rate, it is rather difficult to establish which of the *Barbus* host species play the most important role in the life history of this parasite.

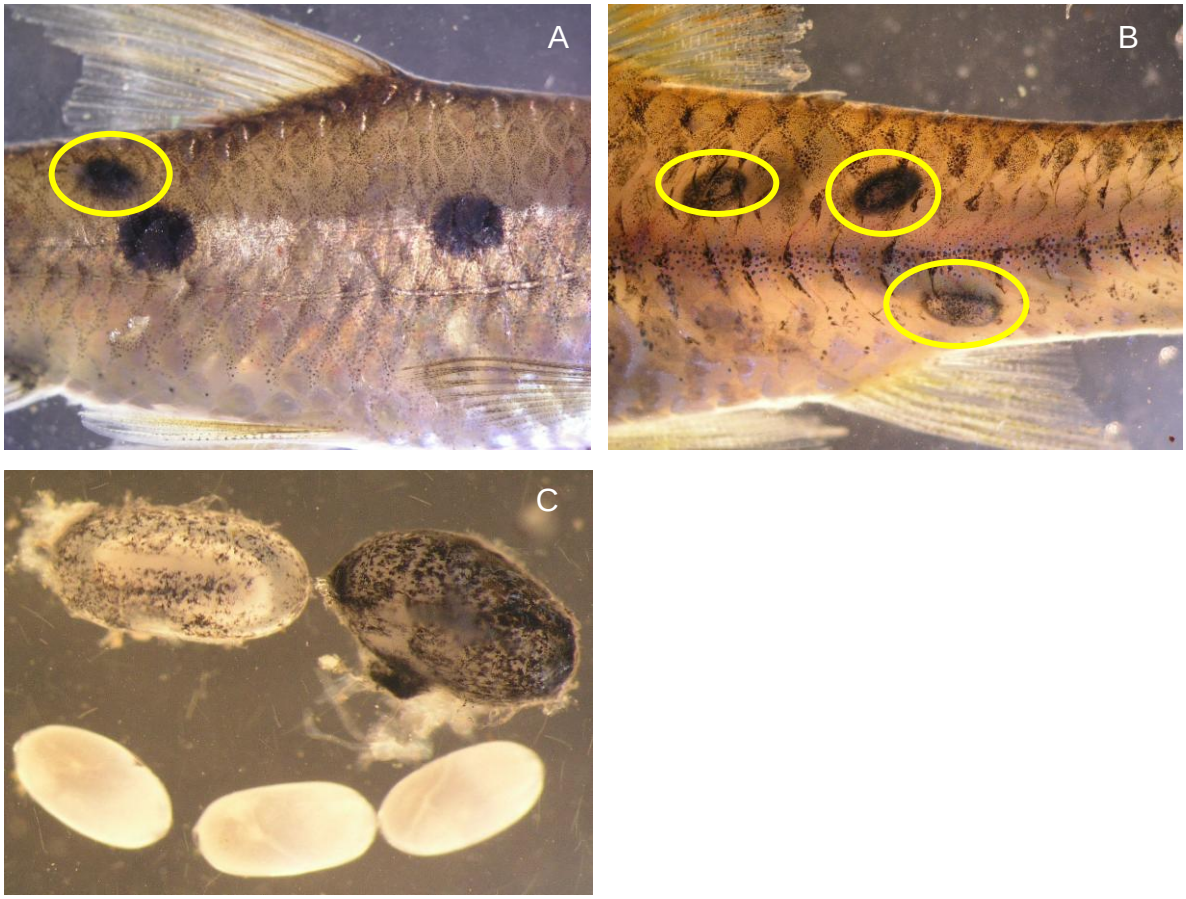

Figure 3.16: **A** - Black grub on the skin of *Barbus trimaculatus*, **B** - Black grub in the muscle of *Barbus radiatus* and **C** - Black grub removed from the muscles and from their first outer cysts at Nwanedi-Luphephe Dams.

3.12.2 Diagnostic features and occurrence of the metacercariae from the gills

CLASS: Trematoda
ORDER: Digenea
FAMILY: Diplostomidae Poirier, 1886
GENUS: *Diplostomum* sp. (Figure 3.17)
HOST: *Barbus trimaculatus*
SITE: Gills
LOCALITY: Nwanedi-Luphephe Dams

These *Diplostomum*-type metacercariae were found embedded within the gills of *B*. *trimaculatus* (Figure 3.17 A). This parasite was common and found almost every season. Not all the metacercariae were successfully recovered from the gills and some were indistinct and difficult to recover. The observed metacercariae was surrounded by two envelopes isolated from each other, viz., the internal (cyst) and external (capsule) ones (Figure 3.17 B), arranged as follows; a whitish colour, large size, thickened transparent outer layer and a thin inner layer secreted by parasite into which it is coiled. These layers were closely associated with one another when observed *in situ* but often became separated during fixation. In the opinion of most authors, the inner envelope (cyst) is formed by trematode secretions, and the outer envelope (capsule) consisted of cells of the host. It is also not impossible that the different structures of the capsule were caused by the species specific response of the host.

The life cycle of this metacercariae is similar to the metacercariae discussed in the preceding section. The high number of fish infested with this larval form metacercariae may indicate that both the intermediate and definitive hosts of these larvae occur in great numbers in this environment. In general, the structure of the metacercarial envelope localized in the skin, fins and gills of freshwater fish has been somewhat less studied. According to literature data, the cysts are formed by secretory vesicles formed by protrusions of external membrane of the tegument (Skorobrekhova, 2009). The space between the larval tegument and cyst is filled by a light flaky substance. Due to the intensive movements of the trematode, the secretory vesicles break and release the secretion.

From a morphological point of view, the present material closely resembles the metacercariae of *Ornithodiplostomum*, which has been recently procured by Barson and Avenant-Oldewage (2006). The only difference being that these authors found the metacercariae from the muscle of *C*. *gariepinus* while in the present study it was exclusively found from the gills of *B*. *trimaculatus*. Based on the strength of morphological similarities, Barson and Avenant-Oldewage (2006) provisionally placed their material in the genus *Ornithodiplostomum*. Barson and Avenant-Oldewage (2006) gave the following description for the parasite: *"*Body indistinctly bipartite with no pseudosuckers, but with a large circular holdfast organ (tribocytic organ). Developing, immature testes apparent. Subterminal evaginable genital apparatus (copulatory bursa) also distinct from photomicrograph. Tegumental surface characteristically ornamented with ridges and intercellular pits*".*

According to these authors, this parasite is the first of its kind to be reported in South Africa. Based on the descriptions given by Barson and Avenant-Oldewage (2006), the present metacercariae can be related to those metacercariae principally by having the whitish colour and large size and relative large circular holdfast organ (tribocytic organ). Judging from differences summarized in the foregoing paragraph, it can not be said with certainty where the current species belong to. Previous studies on cyprinids in the Limpopo Province of South Africa did not report this parasite although a number of related genera such as *Diplostomum, Neodiplostomum* and *Postodiplostomum* are quite common (Mashego, 1977; Prudhoe and Hussey, 1977; Khalil and Polling, 1997) within the geographical reach of the Limpopo Province. On account of close affinities, it will be somewhat premarure to designate the present material to any of the above genus. Accurate identification of this metacercariae is further confounded by the fact that in some species, one of the envelopes is lacking or poorly developed (Skorobrekhova, 2009), thus creating some difficulty in the identification.

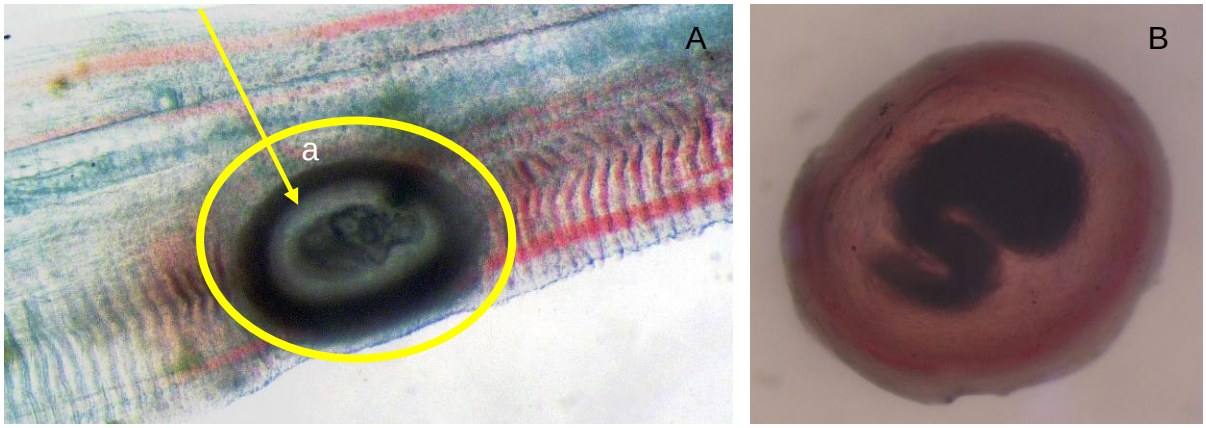

Figure 3.17: A - gill filament of *Barbus trimaculatus* with (a) metacercariae embedded within the gills, **B** - metacercariae excised from the gill filament of *Barbus trimaculatus* at Nwanedi-Luphephe Dams.

3.13 Nematoda

Nematodes are common parasites in freshwater and marine fish, amphibians, reptiles and birds. According to Paperna (1996), potentially all freshwater and brackish water fish may be infested by adult and larval nematodes. Khalil and Polling (1997) has recorded over 40 nematode species belonging to nine families from fish in Africa. Of these, only a few are parasitic in the adult stage. Nematoda (round worms) are very distinctive in shape, with a solid cuticle. Because of their resistant cuticle, these worms last longer than flatworms in post-mortem conditions.

Larval stages of nematodes either occur encysted in the tissue or free in the body cavity of fish (Paperna, 1996), while the adults live in the stomach or small intestine of piscivorous birds, notably pelicans, cormorants, herons and darters (Whitfield and Heeg, 1977). Of all the larval nematodes, the most common genus is *Contracaecum*, which has a trans-African distribution and has been recorded in both *Clarias gariepinus* and *Oreochromis mossambicus*. There are no records of larval nematodes becoming problematic in African fish fauna (Mashego and Saayman, 1981; Boomker, 1982; Paperna, 1996, Barson and Avenant-Oldewage, 2006).

In South Africa, *Contracaecum* larvae are common and have been reported in a wide variety of fish of diverse families; *Clarias gariepinus* by Mashego (1977), Whitfield and Heeg (1977), Mashego and Saayman (1981), Boomker (1982, 1994a, b) and Saayman *et al.* (1991), nine *Barbus* species by Mashego (1982), Saayman *et al.* (1991) and Mokgalong (1996), *Schilbe intermedius* Rüppell, 1832, *Brycinus*

imberi Peters, 1852, *Hydrocynus vittatus* Castelnau, 1861 by Boomker (1994a), *Marcusenius macrolepidotus* Peters, 1852 by Luus-Powell (2004) and *Oreochromis mossambicus* by Ramollo (2008). In neighbouring Zimbabwe, it has been recorded from the body cavity and intestines of siluriform and cichlid fishes as well as the tiger fish, *Hydrocynus vittatus*, *Mormyrops anguilloides* Linnaeus, 1758 by Chishawa (1991), Douëllou (1992a, b), Douëllou and Erlwanger (1993) and Barson (2004). Outside southern Africa, the occurrence of this parasite have been reported in cichlids and catfish from countries such as Egypt (Amin, 1978), and East Africa (Malvestuto and Ogambo-Ongoma, 1978; Aloo, 2001).

To date seven species of this nematode genus are known to occur as adults in fish eating-birds in South Africa (Barson and Marshall, 2000). Adult *Contracaecum* species from fish-eating birds have been studied and recorded by Saayman *et al.* (1991) and Mokgalong (1996), among others. Mokgalong (1996) recorded seven different species of *Contracaecum* spp., some of which were positively identified as *C*. *microcephalum* from several piscivorous birds viz., *Phalacrocorax carbo*, *P*. *africanus*, *Anhinga melanogaster*, *Ardea cinerea* and *Ardea melanocephala*.

Fish eating birds are abundant at Nwanedi-Luphephe Dams and it is generally believed that they have adapted to breed in man-made impoundment (Barson and Marshall, 2000). According to Paperna (1996), *Contracaecum* species are linked to migration of piscivorous birds, particularly (or even only) pelicans, between Europe and tropical East Africa. Owing to this behaviour, some of the *Contracaecum* species are widely distributed to other parts of Africa, for instance, the *Contracaecum* spp. found in Zimbabwe were all previously recorded in South Africa, indicating a possible regional distribution. In addition, Paperna (1996) reported on *Contracaecum* spp. occurring in Israel, Egypt, Mali, East African (Rift Valley) lakes (including Lake Kivu), Zaire, Mali (Niger), and Sudan and from Lakes Chad and Tanganyika.

Cort[1] (2008, personal communication) suggested controlling aquatic birds in fish ponds with nets as an effective means of reducing *Contracaecum* infestation by keeping birds away. This is however impossible in natural habitats or man-made impoundments where prevention of larval nematode infestation by keeping away

[1] Mr Cort is a seasoned fish farmer living about ±50 km away from Mbombela (formerly Nelspruit). During December 2008, I had the privilege of working at his fish farm.

piscivorous birds is impractical, but even in fish ponds as birds dropping will pass through the nets.

Nematodes, with specific reference to *Contracaecum* spp., are not as widely studied as other aspects of their counterparts namely, monogeneans, digeneans and cestodes. This is, as pointed out by Barson and Avenant-Oldewage (2006), probably because *Contracaecum* larvae mainly infests the internal organs, predominantly mesenteric fats and the liver, all of which do not comprise the edible portion of the fish.

In the Limpopo Province of South Africa no less than nine widely distributed freshwater hosts have been identified as carriers of the third stage of *Contracaecum* larvae (Mashego, 1977; Mashego and Saayman, 1981; Boomker, 1982; Mashego; 1982; Saayman *et al*., 1991). Of relevance to South Africa is the fact that smaller *Barbus* and cichlid species do play an important role in the maintenance of the life cycle of *Contracaecum* spp. as well as the spread of this parasite in the country' water bodies. In particular, the importance of the aforementioned smaller fish hosts in the life cycle of *Contracaecum* spp. is evidenced by the circumstances that they are generally considered the major item in the dietary budget of piscivorous birds, thereby enhancing the spread of these parasites from one locality to another (Mokgalong, 1996). *Clarias gariepinus* may, on the basis of its large size and its nocturnal character, be exempted from being the principal item in the dietary budget of piscivorous birds because these birds feed during the day when *C*. *gariepinus* is resting near the bottom of the water column.

The classification and ecological results of the *Contracaecum* found in this study is given below.

3.13.1 *Contracaecum* larvae

CLASS: Nematoda
FAMILY: Anisakidae
GENUS: *Contracaecum* Raillet and Henry, 1854 (Figure 3.19)
HOSTS: *Labeobarbus marequensis* and *Barbus trimaculatus*
SITE: Body cavity
LOCALITY: Nwanedi-Luphephe Dams

Contracaecum larvae of various sizes were generally found in the coelomic cavity embedded in the mesentery and in adipose tissues (Figure 3.19), and occasionally they were also found on the liver lobes of *B*. *trimaculatus* with variations in intensity of infestation from one species to another.

Prevalence: The highest prevalence was observed in *Lb. marequensis* (90%) while the least infested was *B. trimaculatus* (20%). In *Lb*. *marequensis*, the occurrence appeared to be partially influenced by seasons. None were recorded in winter. A small percentage (10%) of fish was infested during spring which significantly rose to 90% in summer and again a sharp decline in autumn in *Lb. marequensis*. However, in *B*. *trimaculatus* the prevalence was 20% during both winter and summer surveys.

Abundance: The abundance of *Contracaecum* larvae in *Lb. marequensis* was higher in summer (68.2), followed by autumn (4.5) and 0.3 in spring. No *Contracaecum* larvae were found in winter from *Lb. marequensis*. On the other hand, the abundance of *Contracaecum* larvae in *B*. *trimaculatus* was 0.5 during winter and 0.8 during summer. No *Contracaecum* larvae were found during autumn and spring.

Mean intensity: The mean intensity of *Contracaecum* larvae in *Lb. marequensis* differed considerably from three during spring to 76.8 during summer. Values decreased from 16 during autumn to zero during winter and then increased during spring. The latter was followed by a distinct increase in summer. For *B. trimaculatus* the opposite was observed and the intensities remained low. In *B. trimaculatus*, a mean intensity of 2.7 for winter and 5 for summer were recorded. None were recorded during autumn and spring.

Seasonality (data pooled): In total, the highest number of *Contracaecum* larvae observed during the summer survey was 1 228 from *Lb. marequensis* and 15 from *B. trimaculatus* (Appendix B) and the lowest was three for *Lb*. *marequensis* during spring and eight for *B*. *trimaculatus* in winter. On the other hand, there was no seasonal variation in prevalence, throughout the study period for *B*. *trimaculatus*. A prevalence of 20% of the latter host occurred in both winter and summer. No *Contracaecum* larvae were procured during the remainder seasons. In addition, the abundance in *B*. *trimaculatus* was relatively low with 0.5 in winter and 0.8 in summer.

Size (data pooled, according to seasons): There was no significant relationship between the length of the host and the rate of infestation of the fish (R^2 = 0.022) (Figure 3.18 A).

Condition factor (data pooled, according to seasons): There was no significant relationship between the number of *Contracaecum* larva and condition factor of the fish ($R^2 = 0.000$) (Figure 3.18 B).

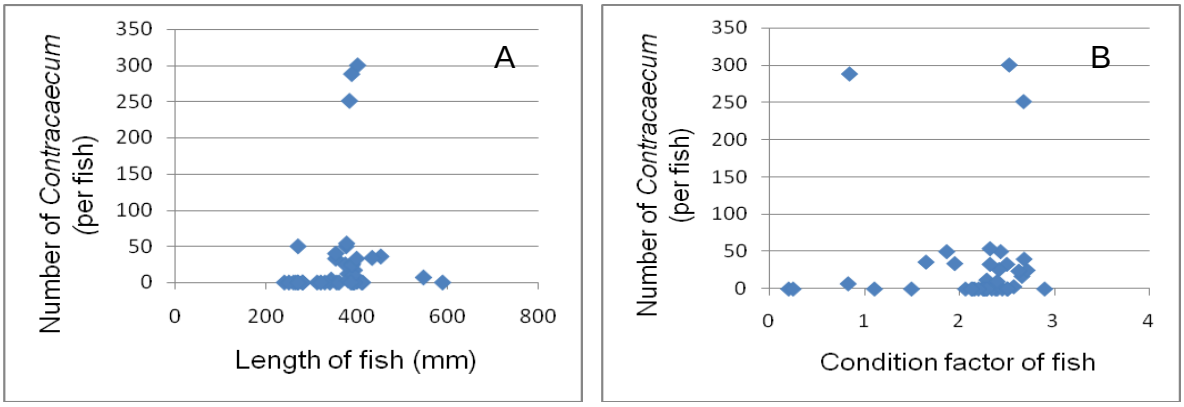

Figure 3.18: A - The correlation between the number of *Contracaecum* larvae and the length of *Labeobarbus marequensis*, **B** - The correlation between the number of *Contracaecum* larvae and the condition of *Labeobarbus marequensis* during the three seasons at the Nwanedi-Luphephe Dams.

Gender of the fish (data pooled, according to fish gender and seasons): There was a significant difference ($p > 0.05$, t-test) in the number of *Contracaecum* larvae recorded between male (16) and female (27) fish.

3.13.2 Diagnostic features of the *Contracaecum* larva

In the present investigation, *Contracaecum* larva could not be identified to species level. Identification of this larval nematode, particularly to species level is not usually feasible, since the larvae lack genital systems and several other features of adult stages which are utilised as taxonomic criteria. Thus far, there is no compilation of synoptic keys for *Contracaecum* larvae. Orecchia *et al.* (1986) developed a methodology of identification of larval stages (of Anisakidae) by biochemical (isoenzyme) methodology utilising multilocus electrophoresis analysis. Taxonomic characteristics important for identifying anisikid larvae include appearance of the cephalic papillae, location of the excretory pore, the presence of ventricular appendaces and intestinal caecum (Martins *et al*., 2005).

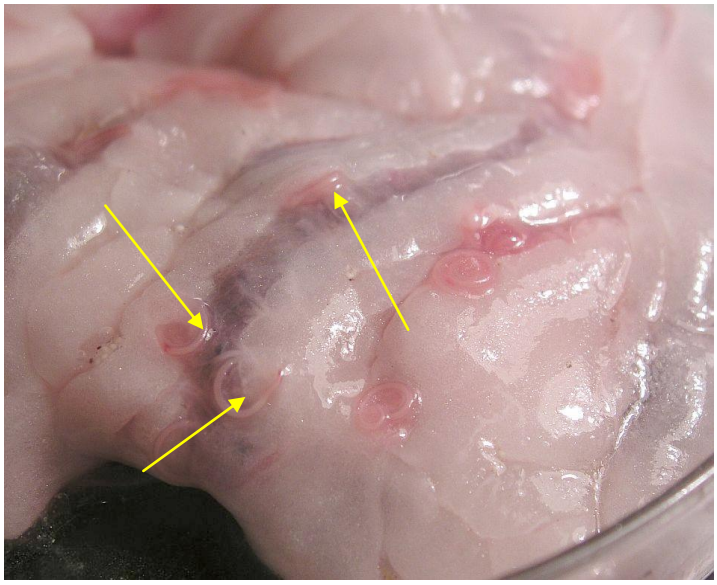

Figure 3.19: *Contracaecum* larvae (arrows) embedded in the mesenteric fat of *Labeobarbus marequensis* collected at the Nwanedi-Luphephe Dams.

3.14 Ecology of *Contracaecum*

3.14.1 Infestation

Two fish species were infested by this parasite during the present study i.e., *Lb. marequensis* and *B. trimaculatus*. The infestation with *Contracaecum* larvae (in terms of prevalence, abundance and mean intensity) in *Lb. marequensis* was higher than that observed from *B. trimaculatus*. The prevalence of *Contracaecum* larva reported in this study was higher than that observed in the work of Mashego (1989) and Boomker (1994a). Mashego (1989) reported that 13% of *Lb. marequensis* and 3% of *B. trimaculatus* from Nwanedi-Luphephe Dams were infested, whereas in the present study a maximum of 90% of *Lb*. *marequensis* was infested. Concerning *B*. *trimaculatus*, Mashego (1982) observed 3% whereas in the present study a maximum of 15% was recorded. Mashego (1989) and Boomker (1994a) observed a lower mean intensity of this parasite when compared to the present study.

The higher prevalence of *Contracaecum* larvae are generally believed to be accounted for by constant infestation via the diet or direct infestation without the intervention of the first intermediate host as was noted by Malvestuto and Ogambo-

Ongama (1978) and Mashego (1982). As many endoparasites are aquired through trophic transmission, the differences and prevalences between the two hosts could be explained mainly by the feeding patterns between *Lb*. *marequensis* and *B*. *trimaculatus*. As stated by Mashego (1982), the frequency and diversity of *Lb. marequensis*'s diet items is higher than in *B. trimaculatus* possibly because of the larger size of *Lb. marequensis.* The higher prevalence of *Contracaecum* larvae may have been due to the fact that the intermediate host is the main diet for *Lb*. *marequensis* since helminths in fish are acquired by ingestion of intermediate hosts which are part of their diet.

The infrequent presence of *Contracaecum* larvae in *B*. *trimaculatus* is somewhat difficult to explain while its complete absence in *B*. *radiatus* could be attributed to lack of birds as distributors of the parasite in the streams. The small size of the pool where the majority of these hosts were collected may be unfavourable for birds because *B*. *radiatus* spend most of its time hiding under rocks. Although a small number of *B*. *trimaculatus* was found in the same pool with *B*. *radiatus*, these hosts were not infested whatsoever.

3.14.2 Seasonal trend

The statistics show that summer is a favourable season for *Contracaecum* spp. and winter the least favoured in terms of seasonal incidence and infestive period. The lower prevalence of *Contracaecum* larvae can be attributed to reduced feeding activity of the fish at low temperatures, subsequently reducing the chances of infestation via copepods rather than seasonal change, because naturally, once *Contracaecum* species infest a fish they stay in the host for years. Moreover, the lack of *Contracaecum* infestation during winter could be explained by the low sample size during that season.

In consequence, the high infestation observed in this present investigation, particularly during summer, may in all probability suggest an increase in feeding activity of the fish at higher temperatures, ultimately increasing nematode infestation. According to Paperna (1996), the incubation of *Contracaecum* species eggs seems to be temperature dependent. Eggs hatch within 2 - 3 days at 24°C, 5 - 7 days at 21°C; hatching is not simultaneous though and is further delayed in some of the eggs. Given the above range and with a low water temperature of 17.2°C recorded

during winter in the present study, one might be tempted to suggest that, under low temperatures, egg hatching will be delayed. However, as the water temperature rise as is the case in summer (27.6°C), egg hatching will be speeded up. Similar findings were reported by Barson (2004) in Zimbabwe, who found that the prevalence was low during the winter months (June to August) and high during summer. On the contrary, Mashego (1982) did not observe any seasonal variation in the rate of infestation of *Contracaecum* larvae in fish from various localities in the Limpopo Province of South Africa.

A total of 1 228 specimens were recorded from the summer survey in the present study (Appendix 2). However, the number could have increased dramatically had it been possible to recover all the worms as this was practically impossible because some of the worms were deeply ingrained in the mesentery fats. Furthermore, the lack of any seasonal pattern in *B. trimaculatus* is consistent with those reported by Mashego and Saayman (1981), Mashego (1989), Aloo (2001) and Barson (2004) in fish from various locations.

3.14.3 Size of host

In both species from which *Contracaecum* larvae were procured, no correlation was observed between fish size and the number of parasite species. However, *Lb*. *marequensis* was more frequently infested (10 - 90%) with *Contracaecum* larvae than *B*. *trimaculatus* (20%). The variations in infestation between *Lb*. *marequensis* and *B. trimaculatus* may be attributed to the absolute amount of food consumed, which is a function of body size, activity and the water temperature. This in turn affects the colonisation rate of the infestive stages.

This is in agreement with the results of Mashego (1982; 1989) who found that the larger species (i.e., *Lb. marequensis* and *Barbus mattozi*) appear to be more frequently infested than the smaller species (e.g., *B*. *trimaculatus*). In practice, this pattern could be attributed to the body dimensions of *Lb. marequensis* which is comparably larger than most *Barbus* species particulary the ones examined in this study. Moreover, Mashego and Saayman (1981) noted that the total number of larval nematodes from infested *C. gariepinus* increased with the size of the fish.

3.14.4 Condition factor

Despite the heavy infestation during summer, the condition of fish remained unaltered by the presence of this parasite (see also Chapter 4). This, therefore, suggests that the nematodes had little or no effect on the health of the fish. These observation were also supported by Barson (2004). Mashego and Saayman (1981) recorded a prevalence of 10 - 100% with intensities of up to 2 860 per fish; Boomker (1982) recorded a prevalence of 95.3% and Whitfield and Heeg (1977) recorded a prevalence of 46%, all from various fish species and localities presumably with various geographical attributes, and never reported any altered condition of the fish. Mashego and Saayman (1981), Aloo, (2001) and Barson (2004) however observed that even though very heavy infestation in fish have not affected the condition of the host, it may render the fish unsightly and unsuitable for human consumption especially if the larvae encysted in the muscle tissues. This trend supports the hypothesis that, in natural environments, parasites normally exists in equilibrium with their hosts (Paperna, 1996), which ensures that the parasite does not kill the intermediate host and reaches the final host to complete its life cycle.

If fish are not frozen or filleted after capture, nematodes might migrate into the flesh and may represent a risk for human parasitosis (Olivero-Verbel *et al*., 2006). On the other hand, given the preference of *Contracaecum* species for visceral organs and intestinal mesenteries, it might substantially limit or reduce its zoonotic potential. Consequently, *Contracaecum* larvae are not considered to present a negative consequence on the host. This should imply that the presence of a parasite does not necessarily imply manifestation of a disease. It is, therefore, true disease caused by parasites are more frequently manifested in cultured fish, which suffer from artificial conditions and numerous stress factors that influence their ability to effectively protect themselves against parasitic infestations. For instance in aquaculture, some parasites are able to reproduce rapidly and heavily infest a large proportion of farmed fish, which may lead to disease with significant economic consequences. In this case, an important aspect of effective control of fish diseases caused by parasites is a reliable diagnosis, preferably in early phases of a disease, enabling the application of adequate prophylactic measures and treatment and prevention of serious outbreaks.

3.14.5 Host specificity

With such high prevalences of *Contracaecum* larvae continuously being recorded from various fish species, including in the present study, one is tempted to conclude that this nematode larva is one of the most prevalent fish parasites in South Africa and the fact that its life cycle involves migratory bird species can justify this observation (Barson and Avenant-Oldewage, 2006). Due to its wider distribution, *Contracaecum* species can be regarded as a generalist. According to Whitfield and Heeg (1977), Malvestuto and Ogambo-Ongoma (1978) and Mashego and Saayman (1981), various freshwater species appear to harbour the third (L3) stage of *Contracaecum* larvae with higher parasite load in larger fish. Whitfield and Heeg (1977) and Malvestuto and Ogambo-Ongoma (1978) reported several species of fish infested with *Contracaecum*. It is therefore adequate to suggest that, if one of the host species is more abundant than the others, *Contracaecum* parasites would be expected to adapt more easily and utilize that species most efficiently.

3.14.6 Gender of the fish

There was a significant difference ($p < 0.05$, t - test) in the average number of *Contracaecum* larvae between male (16) and female (27). The prevalence was higher in females than males. The winter survey was excluded from this test as no parasite was recorded during that period. This difference could be attributed to the fact that, from the sample itself, there were more females (27) than males (16) and therefore more parasites were recovered from females. This is true if one considers the findings of Mashego (1982) who found no difference in the nature and rate of infestation between males and females. In order to reach a sound conclusion, a large number of hosts may need to be examined.

3.15 Classification and occurrence of the cestode larvae

CLASS: Cestoda
ORDER: Cyclophyllidae
FAMILY: Gryporhynchidae
GENUS: (?) (Figures 3.20 A and B)
HOST: *Barbus radiatus*
SITE: Intestines
LOCALITY: Nwanedi-Luphephe Dams

Two specimens of a larval stage of gryporhynchid (Cyclophyllidae) were found in the body cavity (attached to the intestine wall) of *B*. *radiatus* during the winter survey. This larval stage (metacestode) commonly known as “plerocercus”, occurs in freshwater fish serving as second intermediate or paratenic hosts (Scholz *et al*., 2004). These are parasites of fresh- and brackishwater fish (Scholz *et al*., 2004). Scholz and Salgado-Maldonado (2001) have found metacestodes of as many as 13 species of gryporhynchids in fishes from Mexico, which indicates that cestodes of this family may be relatively frequent. Accordingly, some papers on the occurrence of the larvae parasitizing some fish and from fish-eating birds have emerged particularly in Europe and countries of the former USSR (e.g., Scholz, 2001; Scholz and Salgado-Maldonado, 2001).

Despite such occurrences, there is paucity of data on metacestodes from fish from other parts of the world. In South Africa, for instance, the cestode larvae have been found by Mashego (1982) as the metacestodes of the Dilepidae family. The family has since been changed to gryporhynchid (Cyclophyllidae) after phylogenetic work confirmed its distinctiveness from the Dilepidae family (Scholz *et al*., 2004). Khalil and Polling (1997) listed it from *Tilapia niloticus* and *Tilapia zilli* in Uganda. It is interesting to note that all these records, except Mashego (1982), are from Africa north of the equator, leaving South Africa with only one record.

Mashego (1982) recorded varying mean intensities (1 - 6) with prevalence ranging from 4 - 13% of the larvae from five different hosts from various localities: *B*. *trimaculatus* from Nwanedi-Luphephe, Seshego and Piet Gouws Dams; *B*. *paludinosus* from Nwanedi-Luphephe, Seshego and Piet Gouws Dams and the Olifants River; *B*. *unitaenitus* and *B*. *radiatus* from Nwanedi-Luphephe Dams and

B. *argentues* from Piet Gouws Dam. The procurement of the larvae from *B*. *radiatus* is consistent with the results of Mashego (1982), however, he did not mention where the parasite was found, whether it was in the dams or the streams is unclear. And besides, in the present study, it occurred in the Nwanedi stream not in Luphephe.

The low infestation rate of the larvae is common and may be partly related to the fact that gryporhynchid larvae are often overlooked due to their site of infestation (mesenteries, liver, etc.), and partly due to their very small size. *B*. *radiatus* is by far the least dominant host in the study area, occurring exclusively in the streams, where most piscivorous birds cannot easily reach it due to dense vegetation. The current infestation rate closely follows those of Mashego (1982) who recorded a prevalence of 4% for the cestode larvae in *B*. *radiatus,* the same host from which the parasite was also procured in the present study.

According to Scholz *et al*. (2004), following the emendation made to this family, it therefore appears from the literature that most data concern only three taxa viz., *Neogryporhynchus cheilancristrotus* Wedl, 1855, *Paradilepis scolecina* Rudolphi, 1819 and *Valipora campylancristrota* Wedl, 1855. During the study of the helminth parasites of piscivorous birds from water bodies in the Limpopo Province of South Africa by Mokgalong (1996), *Paradilepis scolecina*, an adult cestode was found from three piscivorous birds, namely, *Phalacrocora carbo*, *P*. *africanus* and *Anhinga melanogaster*. On account of morphological descriptions and resemblance, Mokgalong (1996) identified the larval cestodes found in intermediate host fishes by Mashego (1982) and Saayman *et al*. (1991) as the larval cestodes of *Paradilepis scolecina*. Because the present material bears strong resemblance to the description given by Mashego (1982) and Saayman *et al*. (1991), *Paradilepis scolecina* is considered as the adult stage of the parasite in question.

Infestation statistics provided by Mokgalong (1996) showed that *P*. *africanus* and *A. melanogaster* are by far the most susceptible host species for cestode larvae of *Paradilepis scolecina*. The author attributed this to the numeral dominance of the final hosts as well as the continuous distribution of *B*. *unitaenitus*, *B*. *trimaculatus* and *B*. *paludinosis* within the study area. Moreover, Mashego (1982) recorded prevalences of 7% to 13%, with mean intensities ranging from one to five for these hosts mentioned above, in the Luphephe-Nwanedi Dams, far lower than that recorded for *B*. *radiatus*. Furthermore, Scholz *et al*. (2004) remarked that cyprinids fish represent the most frequent fish intermediate host for metacestodes.

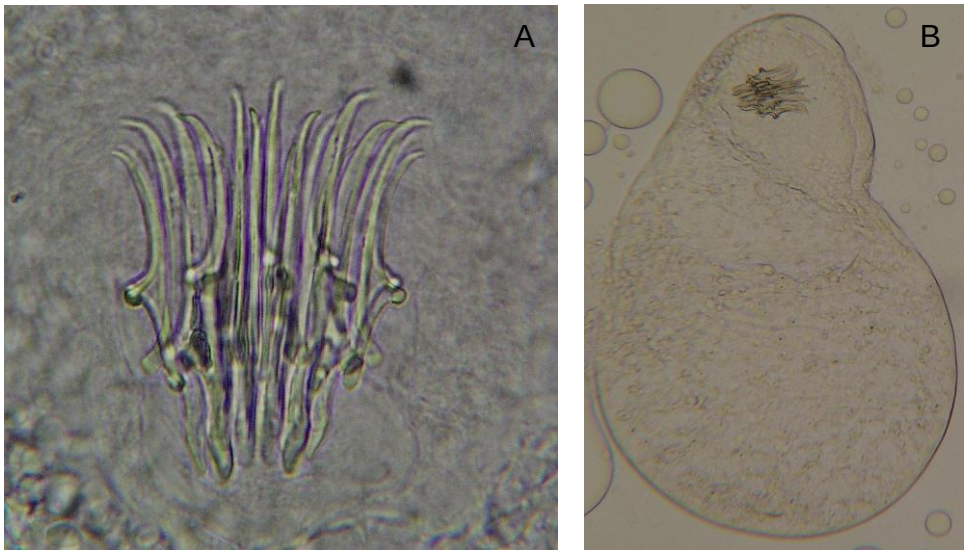

Figure 3.20: A - hooks of gryporhynchid cestode from the intestine of *Barbus radiatus* at Nwanedi-Luphephe Dams, **B** - whole larvae from the intestine of *Barbus radiatus* in glycerine ammonium picrate.

3.16 Classification and occurrence of *Dolops ranarum*

CLASS: Crustacea
SUBCLASS: Branchiura
GENUS: *Dolops*
SPECIES: *ranarum* (Figure 3.21)
HOST: *Labeobarbus marequensis*
SITE: Skin, fins and gills
LOCALITY: Nwanedi-Luphephe Dams

The subclass Branchiura contains a single family, the Argulidae, and four valid genera: *Argulus* Müller, 1785, *Chonopeltis* Thiele, 1900, *Dipteropeltis* Calman, 1912, and *Dolops* Audoin, 1837. Branchiurans are primarily ectoparasitic on a wide range of freshwater fish species, but occasionally live on amphibians, and they can move about freely on their hosts (Avenant *et al*., 1989a; Poly, 2008). Of these, *Dolops ranarum* (Stuhlmann, 1891) is endemic to Africa and has been found widespread in all four major rivers systems of Africa (Nile, Niger, Congo and Zambezi) as reported by Avenant and Van As (1985).

In southern Africa, *D*. *ranarum* has previously been recorded by Barnard (1955) on the large-scale yellowfish, *Labeobarbus marequensis* Smith, and the Mocambique tilapia, *Oreochromis mossambicus* Peters, and by Bruton (1979) on the sharptooth catfish, *Clarias gariepinus* Burchell, in waterbodies south of the Limpopo River system. It has also been found to occur on other fish species such as *Labeobarbus marequensis*, *Barbus mattozi* Guimaraes, *Labeo rubropunctatus* Girlchrist and Thompson, *Irvineia orientalis* Trewavas, *C. gariepinus*, *Micropterus dolomieu* Lacépède, *Chetia flaviventris* Trewavas, and *O*. *mossambicus* in South Africa (Avenant and Van As, 1985).

In South Africa, the parasite is restricted to the Limpopo River system (Avenant and Van As, 1985). Although extensive studies on the surface morphology, hatching strategy (Fryer, 1964, 1968; Avenant *et al*., 1989a, 1989b) and pathological effects of *D*. *ranarum* on its hosts (Avenant-Oldewage, 1994a) are abundant, little is known of its host preferences as the parasite appear to infest a variety of indigenous fish.

In the present study, a prevalence of 46.7% for *D. ranarum* was recorded. This parasite was found on the gills, skin, mouth cavity and fins of *Lb. marequensis* during the autumn survey, 16.6% during summer survey and 10% during the winter survey. In many water bodies in South Africa, *D*. *ranarum* occurs predominantly on two hosts, namely *O. mossambicus* and *C.gariepinus* and both hosts were found to be major hosts for *D*. *ranarum* in another ongoing project in the study area. In the present study, *Lb. marequensis* appeared to be of significance as a host. This is in line with the view of Paperna (1996) that natural infestations of *Dolops* are always less frequent in species of *Labeo* and *Barbus*.

The occurrence of *D*. *ranarum* is linked to the distribution of the two major hosts, i.e. *O*. *mossambicus* and *C*. *gariepinus* (Avenant and Van As, 1985). In the research area these two species are abundant; thereby facilitating dissemination of the parasite. Thus, its occurrence on *Lb. marequensis* coincided with the co-existence of *O*. *mossambicus* and *C*. *gariepinus*.

In the present study, the occurrence of *D*. *ranarum* coincided with the higher temperature in summer. This is expected, as its egg development is temperature dependant. According to Paperna (1996), eggs develop to hatching after 57 days at 20°C whereas hatching takes 25 - 35 days at 24°C. With summer water temperature

of >20°C, it is therefore probable to encounter a higher infestation in summer. This is true possibly because only larvae were found during winter.

Site preference by the parasite is not yet fully understood, however, it is widely supposed that it prefers smooth skinned fish (Avenant and Van As, 1985). In the case of *Lb. marequensis*, it appeared that no specific preference was given to any site on the host as it occurred in the mouth cavity, in the gill area (mostly on the internal portion of the operculum) and randomly distributed on the body and fins. This partially contradicts the observation of Paperna (1996) who observed that whilst *D*. *ranarum* spread all over the body of smooth skinned fish, in scale fish it occurs only on the buccal and branchial cavity mucosa. By virtue of the fact that branchiurans can crawl over the surface of the host, they will not be restricted to one part of the body. It is also believed that the occurrence of *D*. *ranarum* on the skin of scaly fish may not be a permanent site of attachment, as it appears mostly unlikely that it could be a suitable substrate for feeding. In addition, lesions may occur because of the parasite piercing the skin of the fish surface during the process of attachment. The mechanism of feeding is not fully understood but some suggestions have been made that it may feed on the blood and mucus of its host (Paperna, 1980).

Figure 3.21: Light micrograph of *Dolops ranarum* from *Labeobarbus marequensis* collected at Nwanedi-Luphephe Dams.

As a result of lesions sustained, fish may be predisposed to waterborne bacteria and fungi whilst lesion on the gills may severely affect the host's respiratory system. The results of the present study suggest that, more simply, widely distributed hosts e.g., *C*. *gariepinus* and *O*. *mossambicus*, may transmit infestations of this relatively non-host specific parasite to endemic fishes throughout its range.

3.17 Other parasites encountered

Echinostomes and myxocysts were also found on the gills. Unidentified whitish cysts were also found covering the visceral cavity of the fish. The parasite results were then incorporated into the Health Assessment Index (HAI) in Chapter 4.

CHAPTER 4

FISH HEALTH

4.1 Introduction

In recent years water quality problems have attracted increasing attention from authorities and communities throughout the world, especially in developed countries but also lately in developing countries. In industrialised countries such as South Africa, the degradation of surface and groundwater sources has previously been a consequence of economic development. Sadly, remedial actions to balance or lessen environmental impacts have always been a lesser priority in affected places. Consequently, the principles for water resources management have formed the basis for many biological studies because in many countries no comprehensive or coherent water management methods exist for aquatic freshwater management. A better understanding of water resources management is necessary if one wants to predict the potential harmfulness of various chemicals to the aquatic environment.

The Health Assessment Index (HAI) is one of several bio-monitoring tools used in biological monitoring to predict the status of aquatic environment. This bio-monitoring tool was developed as a necropsy-based condition assessment by Goede and Barton (1990) and further quantified by Adams *et al*. (1993). This approach is based on the assumption that if fishes are physiologically in a good condition, the vital organs and other easily observed body structures will also be in a good condition. The fish that have thus been exposed to various environmental stressors, for extended periods of time, will have changes in organ appearances and morphology, or blood chemistry may deteriorate. Deviations from normal are thus generally considered indicative of some type of existing or developing problem within the population (Adams *et al*., 1993).

The modification of this method by Adams *et al*. (1993) substitutes numerical values for abnormal ratings and provides a quantitative HAI for each fish that can be compared statistically. The HAI scores the health of fish based on an autopsy evaluation of their organs and tissues with higher scores indicating poorer health relative to lower scores. To account for differences in severity of damage or level of effect, some variables of the HAI are assigned subjective values of 0, 10, 20 or 30, depending on the extent of the abnormality or observed damage (see Chapter 2).

Avenant-Oldewage and Swanepoel (1993) suggested the use of fish health studies in South Africa. Upon adoption of the method, small modifications were made by Avenant-Oldewage *et al*. (1995) to suit South African conditions and

subsequently a user manual was developed by Avenant-Oldewage (2001). Since then the HAI has been successfully applied by different researchers, on various fish species in South Africa. In the Olifants River System the following fish species were evaluated; *Clarias gariepinus* by Marx (1996) and Watson (2001), *Labeo* spp. by Luus-Powell (1997), *Oreochromis mossambicus* by Robinson (1996) and Watson (2001) and *Labeobarbus marequensis* by Watson (2001). In addition, Watson (2001) included a colour chart to standardise and show the colours that can be expected to occur, which limit the subjective nature of colour assessments made during the evaluation of internal organs such as the liver, bile and spleen.

In the Vaal River System it was used to evaluate the health of the following fish species; *Labeo umbratus* and *L. capensis* by Groenewald (2000), *C*. *gariepinus* by Crafford (2000), and then *L*. *umbratus*, *L*. *capensis*, *C. carpio*, *Lb. aeneus* and *Lb*. *kimberleyensis* by Avenant-Oldewage (2001) and Bertasso (2004). In the Klip River the index was used to evaluate the health of *L*. *capensis*, *L*. *umbratus* and *Lb. aeneus* by Heath *et al*. (2004). Moreover, the Department of Water Affairs and Forestry has incorporated the index in "Field Biosurveys and Intergrated Ecological Assessment" (see Heath *et al*., 2004). Jooste *et al*. (2004) evaluated the health of both *C*. *gariepinus* and *O*. *mossambicus* as indicators of environmental stress in the Ga-Selati River. Recently, Ramollo (2008) evaluated and successfully used the index on *O*. *mossambicus* in four polluted sites in the Phalaborwa Mining Complex.

Results obtained by Marx (1996), Robinson (1996), and Luus-Powell (1997) showed that fish populations with higher HAI values are found in poorer water quality, while better quality water harboured healthier fish populations with lower HAI values. Crafford (2000) evaluated the HAI to test whether the bio-monitoring index applied in one system would perform the same in a different aquatic system. In the light of his findings, Crafford (2000) concluded that the bio-monitoring index yielding positive results in one system would not necessarily perform the same in another system because of the differences in the quality of the water.

In the original HAI of Adams *et al*. (1993), parasites were regarded as an indication of a disease (an indication of poor condition), and therefore only their presence or absence was recorded in the HAI. Avenant-Oldewage (1994b) added to the HAI the use of endo- and ectoparasites community composition as an indicator of environmental health.

This change by Avenant-Oldewage (1994b) was expanded and ultimately developed into a Parasite Index (PI), and tested in conjunction with the HAI (Marx, 1996; Robinson, 1996; Luus-Powell, 1997). Marx's (1996) study was on the interrelationship between fish health and parasitism to determine whether parasites should be incorporated into the South African HAI, or to be used as a separate entity. Furthermore, she evaluated both the endoparasites and ectoparasites as separate entities, and suggested that a low level parasitic survey distinguishing between ecto- and endoparasites can be used as a separate entity to supplement the HAI.

Crafford (2000) and Crafford and Avenant-Oldewage (2009) assessed the use of four parasite indices, namely (a) the original Parasite Index (distinguishing between the presence and absence of parasites) by Adams *et al*. (1993), (b) Inserted Parasite Index (distinguishing between the presence or absence of endo- and ectoparasites), (c) Refined Parasite Index (distinguishing between the number of endo- and ectoparasites and (d) the Inverted Parasite Index (inversion or reversal of the normal order of the number of endo- and ectoparasites). He found that the Inverted PI more accurately reflected the HAI values obtained in the Vaal Dam. For this reason, the evaluation of endo- and ectoparasites was successfully incorporated in the HAI. According to Jooste *et al*. (2004), the PI is a useful bio-monitoring tool that gives a reliable indication of water quality. In addition, it is assumed that a count of 10 to 20 ectoparasites can be expected in good water quality, but the count will drop drastically to two, one or even zero if the water quality is poor (Jooste *et al*., 2003, 2005).

According to Crafford (2000), the Inverted Parasite Index is based on the premise that, as ectoparasites are in direct contact with their environment, they are more directly exposed to the negative impact of poor water quality, as opposed to endoparasites. From this supposition, it follows that lower numbers of ectoparasites will correlate with a decrease in water quality. Thus, the Parasite Index was developed as a separate index, but interpreted in conjunction with the HAI (Jooste *et al*., 2003, 2005; Luus-Powell *et al*., 2005).

4.2 So why is fish preferred in the HAI?

Most fish live permanently in the same waterbody. Water thus provides the oxygen they breathe, the food they eat, and the means to dispose of their waste (e.g. carbon dioxide and waste metabolic matter). The quality of water plays a role in determining how well the fish will grow and, indeed, if they will even survive (Bowser and Buttner, 1993). For this reason, management of suitable water quality will greatly reduce the likelihood of disease problems for the fish.

Fish are generally included in bio-monitoring for the following reasons:

1. Fish integrate the effects of many biotic and abiotic variables acting on the system (Adams *et al*., 1993).
2. Unfavourable or fluctuating temperatures, low dissolved oxygen concentrations, high water velocities and sediment loads, also negatively impact on fish.
3. Fish accumulate pesticides and metals from the aquatic ecosystem via food and via their gills.
4. Fish are furthermore relatively long-lived, and are therefore useful in providing a temporal dimension of conditions observed (Bowser and Buttner, 1993), making them good indicators of long-term environmental influences.

In the present study, only *Lb. marequensis* was used as an indicator species to determine if population characteristics differed due to changes in physical habitat and water quality. The two hosts, *B*. *trimaculatus* and *B*. *radiatus*, were excluded from this assessment as their small size did not permit successful drawing of blood and thus to make a sound judgement was virtually impossible.

4.3 Results and discussion

Table 4.1 shows the mean values of the Health Assessment Index and the Inverted Parasite Index for *Lb. marequensis*. Following the assessment, it was noted that overall the HAI values were generally low for the population in Nwanedi-Luphephe Dams compared to those of other cyprinids such as *Lb*. *kimberleyensis* and *Lb*. *aeneus* in the Vaal Dam (Bertasso, 2004).

The lowest mean HAI (33) was calculated for spring, followed by summer (42) and the highest mean was calculated for winter (63) followed by autumn (50) as

indicated in Table 4.1. It can be seen from Table 4.1 that for all the surveys, the Inverted Parasite Index (see Materials and Methods in Chapter 2) is higher for ectoparasites than for endoparasites. Statistical analysis indicated that there was a significant difference between seasons for the HAI ($p > 0.05$) (Appendix 1). The mean, standard deviation and coefficient of variance (the ratio of the standard deviation to the mean) of the HAI per season is shown in Table 4.1.

Table 4.1: The mean Health Assessment Index calculations for *Labeobarbus marequensis* and the Parasite Index during the four surveys at the Nwanedi-Luphephe Dams.

Survey			Parasite Index	
		HAI	Ectoparasites	Endoparasites
Autumn (15)	Mean	50	20	0.7
	SD	8.8	8.5	2.6
	CV%	17	-	-
Winter (10)	Mean	63	24	0
	SD	21.1	7.0	0
	CV%	36		0
Spring (10)	Mean	33	20	1
	SD	14.2	6.7	3.2
	CV%	43	-	-
Summer (18)	Mean	42	17.1	2.2
	SD	17.8	3.2	4.3
	CV%	38	-	-

Key: CV% = coefficient of variation, SD = standard deviation, HAI = Health Assessment Index, () = Number of fish evaluated for the HAI.

The difference in the HAI between seasons could be related to the fact that fish are ectotherms (poikilotherms), so their metabolic rate depends on water temperature to modify their internal body temperatures. This dependence on water temperature also affects their immune system, wound healing and digestion during low temperatures.

It should, however, be noted that condition of organs assessed using the standard autopsy method did not differ greatly among seasons, nor did conditions deviate appreciably from normality, except for the gills. The lack of any appreciable difference was reflected in the HAI values. In all seasons, large amounts of fat deposited around the intestines were observed. Bile colour was straw-yellow to dark

blue to green and full and its values fluctuated between one and three for all seasons, which indicated that some of sampled fish had not been feeding for a while and some had been feeding well at about the same level of activity. According to Avenant-Oldewage (2001), bile colour changes from straw-yellow in fish that have fed within the previous couple of days to blue green in fish which have not eaten for a week or longer. The variation in the bile colour in this study could be caused by the fact that some fish might have been caught immediately after the gill nets were set and remained snared until the morning. This might have prevented those fish from feeding for at least 10 hours or more. Nevertheless, it should be noted that bile and mesenteric fat were not included in the original HAI and also not during this study.

There were no incidence of abnormality on the external surface of the fish recorded and this is evident in the values scored for skin, fins and the operculum. No abnormalities were observed for the kidneys. The question arose regarding which factors are responsible for or contribute to the changes recorded in the HAI. The organ showing most abnormalities, during all seasons, were the gills, being swollen and discoloured. The liver had the second most visible abnormalities (Appendix 4). At this stage it may be difficult to determine whether a certain water quality variable was responsible for the gill degeneration, whether it relates to the presence of a disease or was caused during the capture and transport back to the base camp.

The abnormal conditions of the liver could, probably, be caused by the fact that the liver is involved in metabolism and transformation of various substances. On the other hand, the haematocrit results from the few fish from which it was possible to draw blood were within the normal range of 30 - 45% (35 - 40). This range indicates that the fish were not anaemic despite the damaged gills and the presence of parasites, which could have resulted to haemodilution, a precursor for lower haematocrit values. From a haematological standpoint, haematocrit always decreases when fish stop feeding, a condition caused by reduced metabolic activity or as a consequence of diseases. Therefore, the above mentioned range together with the low HAI values recorded in this study indicates that *Lb*. *marequensis* population in the Nwanedi-Luphephe Dams has a healthy haematological profile. In addition, it implies that the Nwanedi-Luphephe Dams as an environment does not induce severe stress to the fish.

From the above findings, it appears that the general trend indicated by the HAI is that the water quality is good when compared with results from other studies.

Ramollo (2008) recently recorded high values of HAI ranging between 57 and 127 at four different polluted sites. This author found that the HAI mean values were lower at sites with better water quality and higher at sites with poorer water quality. He attributed the high HAI (127) mean values at the sites with poorer water quality to liver discoloration and abnormal haematological values. This supports the HAI hypothesis that an elevated HAI has been linked to contaminant exposure and associated decreased growth and condition (Adams *et al*., 1993) which, in turn, increase the index value because of the greater level of abnormalities within that particular fish group. This could be true if one considers that one of the advantages of the HAI is that it is not affected by factors such as seasonality or hormonal changes, while indicating the stress of organisms during a long developmental time.

The findings of the present study do not indicate impaired fish health or a decreased vitality of the fish and the several abnormalities on the gills and liver could not be clearly assigned to a specific cause. Based on the HAI premise that lower HAI values signify good water quality, it can thus be suggested that the Nwanedi-Luphephe has good water quality overall.

The lack of severe lesions is in accordance with the findings of Crafford (2000) and Crafford and Avenant-Oldewage (2009), who recorded very few abnormal conditions for eyes, opercula and fins from *C*. *gariepinus* found in the Vaal Dam and Barrage, Vaal River System. According to these researchers, the Vaal Dam is a non-polluted dam whereas the Vaal Barrage is considered polluted. Crafford (2000) and Crafford and Avenant-Oldewage (2009), then found that the HAI values were higher in the Vaal Barrage and lower in the Vaal Dam, thereby distinguishing successfully between the two localities based on water quality and HAI.

It is therefore assumed that the absence of an elevated HAI in the present study is attributable to the lack of anthropogenic impacts such as, among others, mining activities resulting in changes in water quality. It is important to note that the values of the HAI in this study have significance when they are interpreted and read against values from the same locality and for the same fish species as recommended by Avenant-Oldewage (2001). The scores given to each organ were based on the following observations:

4.4 External variables

Skin - No skin lesions or epidermal tumours were observed during this study. For skin and fins, a value of zero was recorded for all the surveys (Appendix 3), indicating a normal appearance with no active erosion.

Fins - From all the fish assessed during this study, none exhibited fin damage, frayed, split, or eroded fins, forked or short fins. Small abrasions were noted, particularly where the parasite *D*. *ranarum* was found.

Eyes - A value of zero (normal) was recorded for the eyes during all the surveys.

Opercula - No abnormalities were recorded for the opercula during this study. They appeared normal with no shortening of the opercula.

Gills - Abnormal gills were noted for some fish during all four surveys (Appendix 3). Severe paleness was recorded as well as frayed and clubbed gills. Some of the gills showed swelling of the tips of the gill lamellae. These gills appeared bulbous with light, discoloured margins along the distal ends or tips of the lamellae or filaments, often associated with 'clubbing' (Figure 4.1). A value of 30 was awarded for these abnormalities.

4.5 Internal variables

Liver - Discolouration indicating abnormal conditions in the liver were seen in some of the fish during all surveys. The HAI value for the liver ranged from zero to 30 during all surveys (Appendix 3).

Spleen - A value of zero was awarded to the spleen throughout the study period, meaning that no abnormalities were observed for this organ during all seasons. Spleen with colours black and red were considered as normal.

Kidney - No anomalies were observed for the Kidneys during the present study.

Bile - the bile of the fish (rating of 1-3) was dark blue to green, yellow or straw colour, bladder full and distended.

Mesenteric fat - in the original HAI, mesenteric fat were not assigned values because of their response to environmental factors (Adams *et al*., 1993) and

therefore this variable was excluded from the HAI during this study. Most of the fishes's intestines assessed were totally covered with mesenteric fat.

Hind gut - Conditions of the hindgut were recorded as normal, no inflammation was detected. No cestode larvae with red markings were noted in the gut.

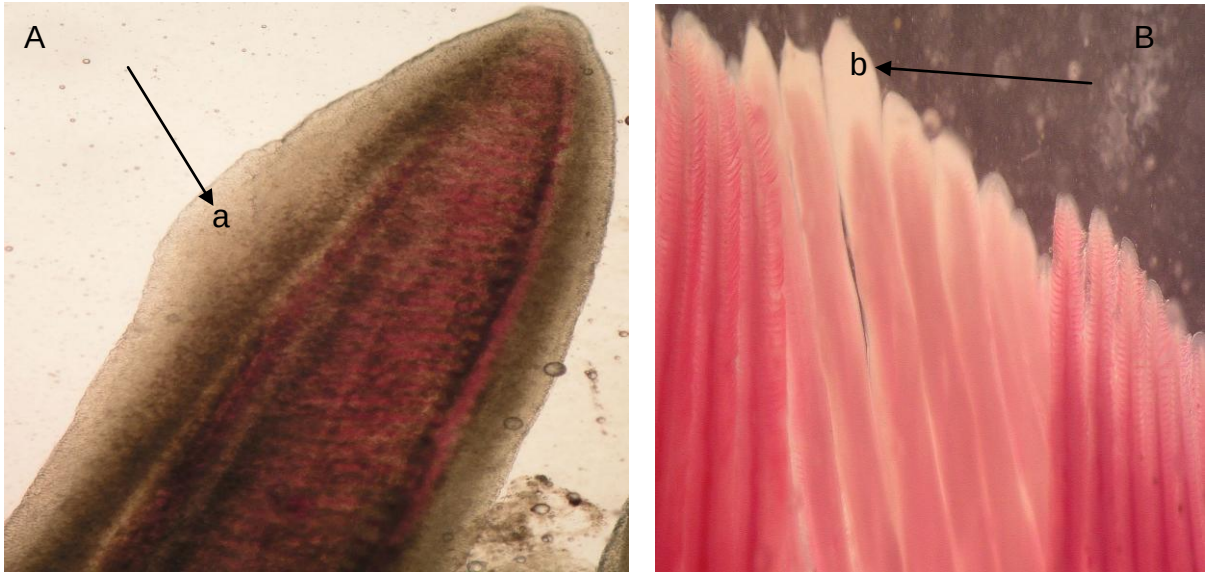

Figure 4.1: A(a) - B(b)- gill lesions at the tip of the gill filaments observed from *Labeobarbus marequensis* during the seasonal study at Nwanedi-Luphephe Dams.

4.6 Condition factor

The condition factor is a frequently used index employed regularly in fisheries, as it furnishes important information relating to the physiological state of the fish, based on the premise that individuals of a given length, exhibiting more mass, are in a better condition (Fafioye and Oluajo, 2005). The condition factor of a fish is classified as ideal when the value is 1.00 or more. The range (min-max) of the length as well as of the mass and condition factor for the three hosts is presented in Tables 4.2 - 4.4.

As it can be seen from Tables 4.2 - 4.4, the condition factors for all three hosts were above the ideal value of one mentioned earlier. The condition factors recorded in this study can be linked to the abundance of food. According to Goede

and Barton (1990), a condition factor when low or having declined, may be interpreted as depletion of energy reserves such as stored liver glycogen and body fat. Statistical analyses indicated that there was no significant difference between the condition factors recorded between seasons ($p > 0.5$) (Appendix 1).

Table 4.2: Length (mm), mass (g) and condition factor of *Labeobarbus marequensis* sampled seasonally at Nwanedi-Luphephe Dams.

		Autumn	Winter	Spring	Summer
Fish length	Min-max	270 – 412	100 - 240	240 - 588	270 – 546
	Mean	360	187.1	309.3	392.0
	SD	41.3	53.2	105.1	53.1
Fish mass	Min-max	83.6 - 1552.6	25.8 - 286.6	300.0 - 2257.3	419.3 - 1629.1
	Mean	981.6	176.9	711.5	1318.5
	SD	493.2	109.9	629.1	345.9
Condition	Min-max	0.21 - 2.4	1.82 - 2.9	1.1 - 2.9	0.8 - 2.7
factor	Mean	1.9	2.3	2.2	2.2
	SD	0.7	0.3	0.2	0.6

Key: SD= Standard deviation

Table 4.3: Length (mm), mass (g) and condition factor of *Barbus radiatus* sampled seasonally at the Nwanedi-Luphephe Dams.

		Autumn	Winter	Spring	Summer
Fish length	Min-max	27 - 33	25 - 34	39 - 72	32 – 59
	Mean	30.7	31.7	59.1	41.2
	SD	2.2	5.3	9.6	7.9
Fish mass	Min-max	0.3 - 0.7	0.2 - 1.5	1.5 - 9.5	0.7 – 6
	Mean	0.5	0.7	5.3	1.7
	SD	0.1	0.3	2.3	1.6
Condition	Min-max	1.39 - 2.7	1.23 - 2.9	1.8 - 2.9	1.18 - 2.9
factor	Mean	1.8	2.1	2.4	2.1
	SD	0.4	0.5	0.3	0.6

Key: SD= Standard deviation

Table 4.4: Length (mm), mass (g) and condition factor of *Barbus trimaculatus* sampled seasonally at the Nwanedi-Luphephe Dams.

		Autumn	Winter	Spring	Summer
Fish length	Min-max	26 - 100	35 - 100	45 - 103	33 – 107
	Mean	41.8	90.8	76.9	90.5
	SD	25.5	14.8	25.9	20.6
Fish mass	Min-max	0.4 - 19.0	0.8 - 28.4	2.5 - 29.0	0.9 - 25.8
	Mean	3.4	19.2	14.0	17.1
	SD	6.4	5.8	10.8	6.6
Condition	Min-max	1.34 - 2.3	1.87 - 2.8	2.2 - 2.8	1.5 - 2.7
factor	Mean	1.9	2.4	2.5	2.1
	SD	0.2	0.3	0.2	0.4

Key: SD= Standard deviation

4.7 Parasite Index

Over the last two decades, several papers have been published about the use of parasites as good biological indicators to monitor the effects of pollutants (e.g. Khan and Thulin, 1991; Poulin, 1992; Overstreet, 1993, 1997; MacKenzie *et al*., 1995; Kennedy, 1997; Lafferty, 1997, 2008; Galli *et al*., 2001; Marcogliese, 2004). The increase in publication of such papers has been spurred by the realisation that physical and chemical water parameters all have constraints, in time and space, as indicators of chemical exposure and effect on aquatic organisms.

Fish parasites, for example, are adapted to specific conditions of both their host and their abiotic aquatic environment, making them potentially excellent biological indicators of water quality (Poulin, 1992). According to Sures (2004), pollution can decrease parasite density or abundance if parasites are more susceptible to a particular pollutant than their host or pollution drive the necessary intermediate and final host to become extinct.

Therefore, the presence of parasites may reflect the health of the entire aquatic community (MacKenzie *et al*., 1995). For this reason, fish parasites with complex life histories have been used as indicators of the health status of an ecosystem because they are considered as highly sensitive. Ectoparasites of fish are

of special interest because they are in constant direct contact with both the fish host and the surrounding environment, thereby providing information on the effect of environmental conditions on the host (Koskivaara, 1992).

In the present study, the impact of environmental factors on parasites was studied by analyzing the changes in the parasite communities by applying the inverted PI. In order to provide accurate data for studying the effect of environmental stress on parasites, a PI was calculated for each parasite group i.e., endo- and ectoparasites. Following the calculation of the inverted PI, the PI for ectoparasites was reasonably higher (up to 24) than for endoparasites (up to 2.8) (Table 4.1) during the study period. This corresponds quite well with the hypothesis that in good water quality there will be more ectoparasites than endoparasites. The occurrence of monogeneans (monoxenous-direct life cycle), such as *Dactylogyrus spinicirrus* and *A*. *polycotyleus* was found throughout the year. Actually, the former occurred in relatively higher numbers compared to any other monogenean found during this study. At the same time, a significant number of *Diplostomulum* and *Contracaecum* larvae were also noted. The abundance of ectoparasites and the nematodes increased during late spring and late summer, while the abundance of the *Diplostomulum* maintained steady levels.

Although no *Contracaecum* larvae were recorded during winter, it can not be unequivocally concluded that this absence was due to certain stressors. Similarly, *Dolops ranarum* occurred sporadically and so were some other parasites e.g., *Gyrodactylus* sp. and *Dogielius* sp. Our assumption was that in a disturbed environment, both monoxenous and heteroxenous parasite abundance would most likely decrease due to direct adverse effects on their free living stages. More specifically for ectoparasites because they are just as exposed to the environment as their host, thereby clearly supporting the theory proposed by Watson (2001) that the incorporation of parasites (endo- and ectoparasites) in the fish HAI provides a more accurate indication of water quality and that high number of ectoparasites and a lower HAI reflects a better quality of water and healthier fish population.

The current HAI results were thus not unexpected since the Nwanedi-Luphephe Dams are regarded as an undisturbed environment (Angliss *et al*., 2007). However, it is stressed that these observations must be used with care, because different stressors may have different impacts on the diverse taxa of parasites. Heteroxenous (indirect life cycle) parasites may markedly decline in some cases,

either due to direct adverse effects on their free living stages, or as an indirect consequence of the elimination of their intermediate hosts (Dzikowski *et al*., 2003).

In terms of the occurrence of endo- and ectoparasites, with a more conspicuous increase in ectoparasitic species abundance, rather than the decline in endoparasite species, the present results agree with the work of Crafford (2000), Watson (2001) and Bertasso (2004). Bertasso (2004), for example, investigated the occurrence of *Bothriocephalus acheilognathi* and some ectoparasites on/in *Lb*. *kimberleyensis* and *Lb*. *aeneus* in relation to water quality in the Vaal Dam. This researcher found that a greater number of *Lb*. *kimberleyensis* were infested with *B*. *acheilognathi* and other cestode species while a larger number of *Lb*. *aeneus* was infested with ectoparasites. The results of Bertasso (2004) also confirmed those of Crafford (2000) who found that the number of endoparasites was higher at the Barrage (polluted site) and lower at the Vaal Dam (less-polluted site), and that the number of ectoparasites was higher at the Vaal Dam and lower at the Barrage.

4.8 Water quality

The term water quality is used by the Department of Water Affairs and Forestry (DWAF, 1996) to describe the microbial, physical, chemical and radiological properties of water. These properties affect both ecosystem health and the "fitness for use" of the water for a variety of uses and for the protection of the health and integrity of aquatic ecosystems.

In an effort to ensure that freshwater resources are used in a sustainable manner, the South African National Water Act (NWA) (No. 36 of 1998) recognises that water resources are part of the integrated water cycle made up of water ecosystems - rivers, wetlands, lakes, dams, estuaries and groundwater - and the processes of precipitation, transpiration, infiltration and evaporation.

DWAF's (1996) guidelines are aimed at providing scientific and technical information for a particular water parameter in the form of numerical data and/or narrative descriptions. Included in the guidelines is the Target Water Quality Range (TWQR) for a specific water constituent for the following users, namely domestic, recreational, and industrial, agriculture, aquaculture and aquatic ecosystems. For the

purpose of this study, the water quality data was compared to the TWQR for aquatic ecosystems.

According to 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. Accordingly, the implementation of these guidelines would provide a better management of water resources and ensure that aquatic organisms are protected.

Water quality variables potentially affecting aquatic ecosystems may be physical (turbidity, suspensoids, temperature) or chemical (non-toxic: pH, TDS, salinity, conductivity, individual ions, nutrients, organic enrichment and dissolved oxygen; and toxic: biocides and trace metals, heavy metals). Metals are found naturally in aquatic ecosystems due to weathering and erosion (DWAF, 1996). Some of these metals, copper and zinc for instance, are essential to living organisms in minute quantities for use by plants and microorganisms. They play a pivotal role in the growth and reproduction of aquatic organisms, notwithstanding the fact that both excess and shortage can be detrimental to organisms.

Anthropogenic activities have drastically altered the biochemical and geochemical cycles and balance of some heavy metals in aquatic ecosystems (Gaona, 2004). The accumulation of metals in an aquatic environment has direct consequences to the ecosystem (Fatoki *et al.*, 2002). Elevated amounts of heavy metals can affect the health of fish either directly through uptake from the water, or indirectly through their diet of vegetation, invertebrates or smaller fish which subsequently leads to stress and death (Hansen *et al*., 1996). Due to these adverse effects on aquatic ecosystems, it is important to identify the sources and measure the concentration levels of heavy metals in aquatic systems.

4.8.1 Results

Table 4.5 summarizes the water quality variables measured during the course of the study. Surface water variables such as pH, temperature, electrical conductivity and salinity values recorded during this study were all within recommended water quality guidelines of DWAF (1996). The pH did not vary much among seasons during the study period (7.2 to 8.0) (Table 4.5). Electrical conductivity values were constant with a slight increase in summer. Values of ammonia were < 0.2 mg/ℓ throughout the

sampling period. Water temperature ranged from 17°C in winter to 27°C in summer whilst oxygen concentrations were 6.1 mg/ℓ and 6.6 mg/ℓ in spring and summer respectively and 8.6 mg/ℓ and 8.4 mg/ℓ in autumn and winter respectively. Heavy metals and trace metal concentrations (expressed in mg/ℓ) for the Nwanedi-Luphephe Dams are included in Table 4.5 and were found to be very low throughout the sampling period.

4.8.2 Discussion

Table 4.5 shows good water quality values for all parameters measured during the present study. All the water quality variables met the TWQR water quality guidelines set for the protection of aquatic ecosystems (during the current study). Most of the water quality parameters measured are interrelated; for instance, as pH and temperature increase the proportion of Total Ammonia Nitrogen (TAN) in the toxic unionized form (NH_3) increases.

The relatively moderate DO levels make it one of the best indicators of the health of a water system (DWAF, 1996). Water quality variables of special concern to the well-being of the fish include *inter alia* electrical conductivity and dissolved solids, sulphates, calcium, total alkalinity, magnesium, sodium and manganese. All these variables were within the permissible levels recommended by DWAF (1996). These variables are known to affect the metabolism of fish, decrease aquatic species diversity, change community structures, and ecological processes (Alabaster and Lloyd, 1980).

With regards to the macro-elements (e.g. potassium, magnesium, etc), all levels recorded were within the TWQR for aquatic ecosystems. In the Nwanedi-Luphephe Dams, the ionic composition of surface and ground waters is seemingly governed by exchanges with the underlying geology of the drainage basin and with atmospheric deposition. The ionic composition of surface waters is usually considered relatively stable and insensitive to biological processes occurring within a body of water. Magnesium, sodium and potassium concentrations tend not to be heavily influenced by metabolic activities of aquatic organisms, whereas calcium can exhibit marked seasonal and spatial dynamics because of biological activities (DWAF, 1996). Similarly, chloride concentrations are not heavily influenced by biological activity, whereas sulphate and inorganic carbon (carbonate and

bicarbonate) concentrations can be driven by production and respiration cycles of the aquatic biota (DWAF, 1996). External forces such as climatic events that govern evaporation can also drive patterns in ionic concentrations. Based partly on the calcium levels recorded, the dams could be classified as having soft waters.

Table 4.5: Physical and chemical characteristics of the water from Nwanedi-Luphephe Dams. One sample per season was collected.

Characteristic Value	Season			
	Autumn	**Winter**	**Spring**	**Summer**
Water temperature (°C)	23.1	17.4	27.7	27.2
DO (mg/ℓ)	8.6	8.4	6.1	6.6
Oxygen saturation (%)	91.8	87	71.8	53.4
pH	7.6	7.2	7.7	8.0
Conductivity (µS/cm)	0.06	0.06	0.07	0.08
Total dissolved solids mg/ℓ	54	58	68	78
Turbidity (NTU)	2.2	1.4	2.6	4.2
Salinity	- 0.03	- 0.03	- 0.03	- 0.04
Total alkalinity as mg $CaCO_3$/ℓ	20	20	24	24
Total hardness as mg $CaCO_3$/ℓ	28	18	24	24
Nitrate as N mg/ℓ	<0.2	<0.2	<0.2	0.5
Nitrite as N mg/ℓ	<0.1	<0.1	<0.1	<0.1
Ammonium as N mg/ℓ	<0.2	<0.2	<0.2	<0.2
Ortho-Phosphate as P mg/ℓ	< 0.2	< 0.2	<0.2	<0.2
Chloride as Cl mg/ℓ	12	14	14	14
Sulphate as SO_4 mg/ℓ	5	6	6	5
Sodium as Na mg/ℓ	7	11	11	13
Potassium as K mg/ℓ	<0.1	<0.1	<0.1	<0.1
Calcium as Ca mg/ℓ	5	4	5	5
Magnesium as Mg mg/ℓ	2	2	3	3
Aluminium as Al mg/ℓ	<0.100	<0.100	<0.100	<0.100
Arsenic as As mg/ℓ	<0.010	<0.010	<0.010	<0.010
Copper as Cu mg/ℓ	<0.025	<0.025	<0.025	<0.025
Iron as Fe mg/ℓ	<0.025	<0.025	<0.025	<0.025
Lead as Pb mg/ℓ	<0.020	<0.020	<0.020	<0.020
Manganese as Mn mg/ℓ	<0.025	<0.025	<0.025	<0.025
Zinc as Zn mg/ℓ	<0.025	<0.025	<0.025	<0.025

In terms of inorganic nitrogen, the South African water quality criteria specify that aerobic, un-impacted surface waters should have inorganic nitrogen (NO_2^-, NO_3^-, NH_4^+) of below 0.5 mg/ℓ whilst concentrations which exceed 5 - 10 mg/ℓ (DWAF, 1996) may occur in highly enriched waters. In this study, nitrate concentrations were < 0.2 mg/ℓ and 0.4 mg/ℓ during autumn and winter respectively and < 0.2 mg/ℓ during autumn and summer while nitrite concentrations were < 0.1 throughout the year.

On the other hand, ammonia (NH_3) concentrations were constant (< 0.2) throughout the year, which according to ammonia concentration recorded during autumn, the Nwanedi-Luphephe Dams is moderately enriched (i.e. productive with high species diversity). In natural sources, inorganic nitrogen is usually not problematic since it is readily taken up by biota and converted to protein (organic). However, excessive levels will cause abnormal algal growth (blooms), resulting in the eutrophication of the system and its associated impacts (Davies and Day, 1998). The only sources that could account for inorganic nitrogen in the Nwanedi-Luphephe Dams are surface runoff from surrounding catchment.

In the present study, the phosphorus concentrations remained constant (< 0.2 mg/ℓ) during all surveys. Inorganic phosphate (P*i*) (PO_4^{2-}) is present in natural waters primarily as phosphates, which is the principal nutrient controlling the degree of eutrophication in aquatic ecosystems (DWAF, 1996). In the Nwanedi-Luphephe Dams, phosphates can enter the aquatic environment from the natural weathering of minerals in the drainage basin and from biological decomposition. In terms of phosphate levels, in unpolluted waters, it is readily taken up by plants and converted into cellular structures by photosynthetic action (Davies and Day, 1998).

According to the South African criteria, a water body is oligotrophic (nutrient-poor) if P*i* < 0.005 mg/ℓ, mesotrophic (P*i* = 0.005 - 0.025 mg/ℓ), eutrophic (P*i* = 0.025-0.25 mg/ℓ) and hypertrophic (P*i* > 0.25 mg/ℓ), while for aquacultural purposes, the P*i* concentration should be ≥ 0.1 mg/ℓ (DWAF, 1996). Thus, based only on orthophosphate concentration, the Nwanedi-Luphephe Dams would be between mesotrophic and eutrophic (dams with an intermediate level of productivity, greater than oligotrophic lakes, but less than eutrophic lakes) as < 0.2 mg/ℓ were recorded during all seasons.

As far as trace metals concentrations are concerned, very low (< 0.100) levels were recorded in this study. However, elements such as iron (Fe) and manganese (Mn) are essential cellular constituents but are required in relatively low

concentrations in relation to their availability in fresh waters (DWAF, 1996). Trace metals such as mercury, copper, selenium, and zinc are essential metabolic components in low concentrations.

Similarly, heavy metals such as copper, zinc, etc were less than the detectable concentration limit recommended by DWAF (1996) for aquatic ecosystem guidelines for the particular metal analysed in this study. Therefore, they are not expected to affect the quality of the water in the two dams. This could be accounted for by the absence of mining and industries around the catchment's proximity. However, it should be born in mind that most heavy metals are effective at low concentrations, so even low assimilation rates are sufficient to attain biologically significant or harmful concentrations in tissues (DWAF, 1996). Furthermore, metal contamination in aquatic ecosystems is more often reflected by high metal levels in sediments, macrophytes and benthic animals than by elevated concentrations in water (Gouws and Coetzee, 1997). This is further supported by Crafford (2000) who recorded relatively low metal concentrations in the water and higher concentrations in sediments. According to Carr and Neary (2006), this could be attributed to the fact that metal concentrations in lakes and man-made impoundment tend to be elevated near the bottom of the lake where oxidation-reduction states are usually high.

To determine whether correlations exist between fish health and the measured water quality variables, the water quality results were therefore interpreted concurrently with the HAI and PI observations. To determine whether there was any correlation between water quality and the HAI and associated PI, it would have to be reflected on the HAI values, the occurrence of parasites and the condition factor. Of all the water variables measured, only temperature changed markedly as a result of seasons, but it was still within the range of 5 - 30°C recommended by DWAF (1996). As discussed previously, many of the heavy metals as well as trace metals were below detectable levels and therefore could not have any negative effect on the health status of the fish. This is more clearly reflected in the HAI and PI values which were low agreeing with the water quality results.

CHAPTER 5

GENERAL DISCUSSION

The present study constituted a seasonal survey of metazoan parasites of the Nwanedi-Luphephe Dams from three fish species. Many of the parasites encountered at the Nwanedi-Luphephe Dams might be considered to be pathogens and of potential economic importance in aquaculture. The prevalence and intensity of infestation of parasites such as *Dactylogyrus* species may increase under culture conditions and death of the more heavily infested fish (particularly the young) may occur.

The occurrence of larval and adult digeneans is generally believed to reflect primarily the type of habitat, presence of suitable first intermediate hosts (snails) or final hosts (birds). In this regard, man-made open waters (dams) represent more suitable environments for completion of digenean life cycles than riverine habitats. This is because dams provide specific conditions that encourage the presence of snails such as higher water temperature, lower water velocity and the presence of the final hosts, especially birds (Ondračková *et al*., 2004), all of which favour the completion of the life cycle and dissemination of most endoparasites. This might explain the reason why the *Barbus* species collected in the streams were not infested with *Diplostomulum* and *Contracaecum* larvae, but those collected from the dams were infested. Because of these conditions, both parasites are well established in the study area, infesting a wide range of fishes.

During the course of the study, the two avian species, *Phalacrocorax carbo* (Great Black Cormorant) and *Phalacrocorax africanus* (Reed Cormorant) which is known to play a significant role in the dissemination of endoparasitic parasites, were continuously observed at the study area. They are the definitive hosts of both *Contracaecum* and *Diplostomulum* and due to the movement of these birds from one water body to another in the Limpopo Province, these parasites are geographically well represented within the study area to as far distant aquatic systems as the Olifants River Drainage System (Mokgalong, 1996).

While both *Contracaecum* and *Diplostomulum* were found in *B*. *trimaculatus*, their predominance in *Lb*. *marequensis* may thus reflect the relatively large surface area in *Lb*. *marequensis* which allows it to accumulate more parasites as it grows. Another factor which is equally important is the diet of the host because copepods harbouring *Contracaecum* larvae are preyed upon by *Lb*. *marequensis* and the fact that *Lb*. *marequensis* predates on small fish once it reaches adult life.

In the wild, the presence of these parasites does not seem to have any detrimental effect on the fish, as an intermediate host, as their numbers did not correlate with the condition factor. There was no visible damage to the gills caused by this dactylogyrid (but detailed histopathological work is recommended). Nonetheless, their presence can cause significant loss of income to aquaculture enterprises as such “wormy” fish can deter customers. The cysts recorded deep in the muscle of *B*. *trimaculatus* and *B*. *radiatus* may cause disfiguration of the host’s tissues.

The low infestation level of the larval stage of gryporhynchid cestodes (metacestode) found in the body cavity of *B*. *radiatus* during the winter survey may be partly related to the fact that gryporhynchid larvae are often overlooked due to their minuscule size and their location in the internal organs.

The occurrence of *Dactylogyrus spinicirrus* in this study represents a new host record for *B*. *trimaculatus* and *B*. *radiatus*, which has previously been recorded from *Lb*. *marequensis*. *Dactylogyrus afrolongicornis afrolongicornis* and *D*. *afrolongicornis alberti* were found on *B*. *trimaculatus* only. Several species of *Dactylogyrus* on the same cyprinid hosts may establish themselves in either different or even the same microhabitats on the gills. In most instances, as is the case in this study, if fish hosts are taxonomically related, the parasites are related at the same generic level showing the same subgeneric characters of copulatory organs and anchors. In this study, both *D*. *afrolongicornis afrolongicornis* and *D*. *afrolongicornis alberti* are recorded as new distribution records. The *Gyrodactylus* sp. and *Dogielius* sp. are new host records for *B*. *radiatus* in South Africa. Members of the latter parasitize mainly *Labeo* spp. found in both Central and West Asian and African Cyprinidae (see Khalil and Polling, 1997).

The current results confirm that members of the genus *Dactylogyrus* and *Afrodiplozoon* are confined, thus far, to their specific cyprinid fish hosts, although *A*. *polycotyleus* has broader amplitude within the Cyprinidae. The presence of *A. polycotyleus* on all three hosts may reveal certain common ecological attributes that these hosts possess such as similar feeding behaviour. Its occurrence on *B*. *radiatus* constitutes a new host and distribution record. Previous results (e.g., Paperna, 1979; Mashego, 1982) have also indicated cyprinid fish hosts for this monogenean.

Moreover, the speciation of *Dactylogyrus* is linked with that of their host fish. Narrow host-specificity usually indicates an intimate relationship, implying that co-evolution has taken place between host species and its parasites whilst wide specificity, on the other hand, could be due to the ability of host species to exchange parasites, which in turn depends on the ability of these parasites to adapt to the conditions found on the new host (Poulin, 2002). This could explain the distribution of *A. polycotyleus* on the three cyprinids. The difference in size between the specimens found during this study and those found by Mashego (1982) might be as a result of a local variation within the range of the species, or variation in host size. The present results show that the specificity of *A. polycotyleus* and *D*. *spinicirrus* is a narrow host specificity type whereas that of *D*. *afrolongicornis afrolongicornis* and *D. afrolongicornis alberti* is a strict host specificity type. As monogeneans are regarded as sensitive indicators of the health condition of their habitat (Poulin, 1995), their presence in vast number in the environment is a good expression of the environment in which they live.

No difference regarding left/right preference was shown by the monogeneans, which shows that the monogeneans do not have any gill preference, particularly *A*. *polycotyleus* where one specimen occurred on the right gill and one on the left gill. In comparison to other dactylogyrids from Europe, not much is known of the African dactylogyrids and even less of southern Africa. This reflects the limited number of studies done rather than an actual paucity of species in these regions.

As with *Diplostomulum* and *Contracaecum*, *Dolops ranarum* is also well established in the study area, infesting a range of hosts including, among others, *C*. *gariepinus*, *O*. *mossambicus* and *Schilbe intermedius*. In the present study, it is being recorded as new distribution record for *Lb*. *marequensis*. It is abundantly clear that the occurrence of *D*. *ranarum* is linked to the distribution of the two major hosts, *O*. *mossambicus* and *C*. *gariepinus*, all of which occur abundantly in the study area.

In this study, water temperature was recorded as a main abiotic factor influencing seasonal variation of the dactylogyrids. The occurrence of *Diplostomulum* and *Contracaecum* larvae does not seem to be affected by seasonality. For all the dactylogyrids procured, mean intensity and abundance increased during spring and summer and declined during winter. The observed level of prevalence and mean intensities of parasites during late spring to late summer, suggests that primary

production peaks around spring when the temperature begins to rise after winter, especially for the monogeneans.

On the other hand, physical and chemical parameters of water and their fluctuations play a very decisive role in the development or maintenance of a healthy fish population. From an ecological point of view, the Nwanedi-Luphephe Dams supports a great diversity of aquatic life with moderately soft waters which ensures the existence of healthy food webs that enable the development of complex parasite life cycles. The low levels of trace and heavy metal concentrations could be attributed to the lack of developments around the catchment.

The HAI results obtained during the present study indicated no signs of adverse effects that could be possibly linked to any particular water parameter measured. The HAI values recorded during this study were relatively low, acceptable for an un-impacted water body. According to Schmitt *et al*. (2005), it is assumed that mean HAI values ≤ 20 indicates un-impacted or minimally impacted sites; values > 50 indicates intermediate sites whereas values > 70 indicates heavily impacted sites. On account of the abovementioned assumptions, the Nwanedi-Luphephe Dams would therefore be classified as an intermediate site when we take the HAI value of 63 into account. It should, however, be noted that the HAI values may differ from one aquatic habitat to another depending on the fish species used and types of stressors in that particular habitat (Jooste *et al*., 2004). Given the low HAI values, it can be stated that the *Lb*. *marequensis* population is in good condition with no obvious signs of distress.

The PI for ectoparasites was high while the PI for endoparasites was significantly lower. This is consistent with the hypothesis that says the number of ectoparasites will be higher in un-impacted waters. Based on this, it is justifiable to suggest that the water in which they occurred did not have any adverse effect on them. Therefore, the hypothesis that ectoparasites are more likely to increase in good water quality was supported in this study. The present results confirmed that the effect of parasites on the fish is minimal under feral conditions and that diseases in good water quality are uncommon. It is also confirmed that the HAI and the associated PI is a valuable tool that is cost-effective and easy to use in the field.

5.1 Conclusion and recommendations

In conclusion, it appears that the water quality of the Nwanedi-Luphephe dams supports a diverse group of parasites. This study has shown that the noticed difference in parasite infestation rate is likely to be a consequence of seasonal changes of water temperature. Furthermore, the current study shows that some aspects of the ecology of the parasites are directly influenced by the host on which they occur. The study also shows that taxonomically related host species are generally likely to be infested by the same parasite or at least by the same genus.

The data from this study further shows that the distribution of parasites varies according to the volume of the attachment site in/on the host. *Labeobarbus marequensis* was the host species which sheltered a considerably higher number of endoparasites compared to *B*. *trimaculatus* and *B*. *radiatus*. The documentation of the new parasite species and new distribution and host records will add value to the parasites of the freshwater fishes of the Limpopo Province. In this study, a preponderance of parasite species such as *Diplostomulum* and *Contracaecum* larvae was noted.

It is nonetheless advisable to take note of parasites of wild fishes before initiating commercial cultivation of any species, due to possible transference of infestations from wild to cultured individuals and *vice versa*. There was a general lack of consistency in the frequency of the occurrence of the parasites, causing skewness in the data, thus renders statistical techniques of certain parasites inappropriate. This was most propably due to the small sample size of the three fish species examined.

By using parasites in bio-monitoring, it is always recommended to handle the results with circumspection because different taxas will react differently towards a certain water variable. As Lafferty (1997) stated “single measures of communities of parasites (e.g. species richness) cannot be used as indicators of specific environmental change, because different taxonomic groups often respond in opposite directions.” The present results complied with the suggestion of Avenant-Oldewage (2001) that the PI is valuable when it is used repetitively over a period of time in the same locality at the same time of the year.

It was difficult to assess the potential chronic effect from the data collected during this study and fully interpret the impact of a single or multiple variables (both

inherent and anthropogenic) on fish health, because a one-year baseline study does not sufficiently provide adequate data. We therefore recommend implementation of a multi-year baseline study in conjunction with future monitoring to further assess seasonal versus low effects on the long-term health of the fish population in the Nwanedi-Luphephe Dams, in order to adequately understand the biological significance of the results. This would be representative of any pollution events taking place in space (spatial) and time (temporal) in the system and would possibly help in standardisation of techniques used in fish health studies.

It is further proposed that future investigation should also be directed to the molluscan and copepods occurrence to assist in providing an equitable representation of the parasite life histories. A long term program on water quality monitoring for metals should be developed that includes sediment concentrations, so as not to overlook the potential source of metal contamination to surface waters. The monitoring program should be conducted once every five years. A bio-monitoring assessment in future should always be conducted comparing data collected from previous studies including the present results.

CHAPTER 6

REFERENCES

ADAMS, SM, BROWN, AM and GOEDE, RW. 1993. A quantitative Health Assessment Index for Rapid Evaluations of Fish Condition in the Field. *Transactions of the American Fisheries Society* **122:** 63 - 73.

ALABASTER, JS and LLOYD, R. 1980. *Water quality criteria for freshwater fish*. Butterworths and Co. (South Africa) Ltd. Durban, South Africa. 297 pp.

ALOO, PA. 2001. Occurrence of larval *Contracaecum* sp.: (Nematoda: Heterocheilidae) in three teoleostean fish species from Lake Naivasha, Kenya. *East African Journal of Science* **3:** 1 - 12.

ALOO, PA. 2002. A comparative study of helminth parasites from the fish *Tilapia zillii* and *Oreochromis leucostictus* in Lake Naivasha and Oloidien Bay, Kenya. *Journal of Helminthology* **76:** 95 - 101.

ALOO, PA, ANAM, RO and MWANGI, JN. 2004. Metazoan parasites of some commercially Important Fish along the Kenyan Coast. *Western Indian Ocean Journal Marine Science Association* **3**(1): 71 - 78.

ALVES, DR and LUQUE, JL. 2001. Community ecology of the metazoan parasites of White Croaker, *Micropogonias furnieri* (Osteichthyes: Sciaenidae), from the coastal zone of the state of Rio de Janeiro, Brazil. *Memorial Institute of Oswaldo Cruz, Rio de Janeiro* **96**(2): 145 - 153.

ALVES, DR, LUQUE, JL and PARAGUASSÚ, AR. 2002. Community ecology of the metazoan parasites of Pink Cusk-eel, *Genypterus brasiliensis* (Osteichthyes: Ophidiidae), from the coastal zone of the state of Rio de Janeiro, Brazil. *Memorial Institute of Oswaldo Cruz, Rio de Janeiro* **97**(5): 683 - 689.

AMIN, OM. 1978. Intestinal helminths of some Nile fishes near Cairo, Egypt, with redescriptions of *Camallanus kirandensis* Baylis, 1928 (Nematoda) and *Bothriocephalus aegyptiacua* Rysavy and Moravec, 1975 (Cestoda). *Journal of Parasitology* **64**: 93 - 101.

AMOAKO, M. 2006. Infestation of ectoparasites on Nile tilapia (*Oreochromis niloticus)* in aquaculture production in the Ashanti region, Ghana. Unpublished MSc dissertation, Norwegian College of Fishery Science, University of Tromsø, Norway. 41 pp.

ANDERSON, RM and MAY, RM. 1979. Population biology of infectious diseases: Part I. *Nature* **280**: 361 - 367.

ANGLISS, MK, RODGERS, SSM, FOUCHE, PJ, LEROY, MR, BURGER, L, FOUCHE, PSO and SHAI, C. 2007. A biomonitoring survey of the Nwanedzi River catchment. Limpopo province. Part 1. Fish, invertebrates and eco-status. 1 - 34 pp.

ASHTON, PJ, LOVE, D, MAHACHI, PH and DIRKS, GM. 2001. An Overview of the Impact of Mining and Mineral Processing Operations on Water Resources and Water Quality in the Zambezi, Limpopo and Olifants Catchments in Southern

Africa. Project by CSIR Environmentek, Pretoria, South Africa and Geology Department, University of Zimbabwe, Harare, Zimbabwe. Report No. ENV-P-C 2001-042. Xvi. 336 pp.

AVENANT-OLDEWAGE, A. 1991. A new species of *Chonopeltis* (Crustacea: Branchiura) from the Kruger National Park, southern Africa. *Journal of African Zoology* **105:** 313 - 321.

AVENANT-OLDEWAGE, A. 1994a. Integumental damage caused by *Dolops ranarum* (Stuhlmann, 1891) (Crustacea: Branchiura) to *Clarias gariepinus* (Burchell), with reference to normal histology and wound-inflicting structures. *Journal of Fish Diseases* **17:** 641 - 647.

AVENANT-OLDEWAGE, A. 1994b. Parasites as indicator organisms for heavy metal pollution. *Journal of the South African Veterinary Association* **65**(4): 154.

AVENANT-OLDEWAGE, A. 2001. Protocol for the assessment of fish health based on the Health Index: *Report and a manual for training of field workers to the Rand Water Board.* Vereening: Rand Water. (Report No: 2001/03/31).

AVENANT-OLDEWAGE A, OLDEWAGE, WH and VAN VUREN, JHJ. 1995. Development of a fish health and condition procedure. Final report to the Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria.

AVENANT-OLDEWAGE, A and SWANEPOEL, JH. 1993. Fish health studies. In: Heath, RGM. (Ed) Proceedings of a workshop on aquatic biomonitoring. HRI Report No. 0000/00/REQ/2893. Pretoria: Hydrological Research Institute, Department of Water Affairs and Forestry, Pretoria.

AVENANT, A and VAN AS, JG. 1985. Occurrence and distribution of *Dolops ranarum* (Stuhlmann, 1891) (Crustacea: Branchiura), ectoparasites of freshwater fish in Transvaal, South Africa. *Journal of Fish Biology* **27**: 403 - 416.

AVENANT, A, VAN AS, JG and LOOTS, GC. 1989a. On the hatching and morphology of *Dolops ranarum* larvae (Crustacea: Branchiura). *Journal of Zoology, London* **217:** 511 - 519.

AVENANT, A, VAN AS, JG and LOOTS, GC. 1989b. A redescription of *Dolops ranarum* (Stuhlmann, 1891) (Crustacea: Branchiura). *Systematic Parasitology* **13**: 141 - 151.

BAGGE, AM and VALTONEN, ET. 1999. Development of monogenean communities on the gills of roach fry (*Rutilus rutilus*). *Parasitology* **118**: 479 - 487.

BAKKE, TA, HARRIS, PD and CABLE, J. 2002. Host specificity dynamics: observation on gyrodactylid monogeneans. *International Journal for Parasitology* **32**: 281 - 308.

BARBER, I, HOARE, D and KRAUSE, J. 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries* **10:** 131 - 165.

BARNARD, KH. 1955. South African parasitic Copepoda. *Annals of the South African Museum* **41**: 223 - 311.

BARSON, M. 2004. The occurrence of *Contracaecum* sp. larvae (Nematoda: Anisakidae) in the catfish *Clarias gariepinus* (Burchell) from Lake Chivero, Zimbabwe. *Onderstepoort Journal of Veterinary Research* **71**: 35 - 39.

BARSON, M and AVENANT-OLDEWAGE, A. 2006. On cestode and digenean parasites of *Clarias gariepinus* (Burchell, 1822) from the Rietvlei Dam, South Africa. *Onderstepoort Journal of Veterinary Research* **73**: 101 - 110.

BARSON, M and MARSHALL, BE. 2000. First record of *Contracaecum* spp. (Nematoda: Anisakidae) in fish eating birds from Zimbabwe. *Journal of the South African Veterinary Association* **75**: 74 - 78.

BASSON, L and VAN AS, JG. 1991. Trichodinids (Ciliophora: Peritrichia) from a calanoid copepod and catfish from South Africa and notes on host specificity. *Systematic Parasitology* **18**: 147 - 158.

BERTASSO, A. 2004. Ecological parameters of selected helminth species in *Labeobarbus aeneus* and *Labeobarbus kimberleyensis* in the Vaal Dam, and an evaluation of their influence on indicators of environmental health. Unpublished M Sc dissertation, Rand Afrikaans University, Johannesburg, South Africa. 96 pp.

BOEGER, WA and KRITSKY, DC. 1997. Co-evolution of the Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *International Journal for Parasitology* **2:** 1495 - 1511.

BOEGER, WA and KRITSKY, DC. 2001. Phylogenetic relationship of the Monogenoidea. *In*: Littlewood, DTJ and Bray, RA. (Eds). *Interrelationships of the Platyhelminthes*. London: Taylor and Francis. 92 - 102 pp.

BOEGER, WA, KRITSKY, DC, PIE, MR and ENGERS, KB. 2005. Mode of transmission, host switching, and escape from the red queen by viviparous gyrodactylids (Monogenoidea). *International Journal for Parasitology* **91**(5): 1000 - 1007.

BOOMKER, J. 1982. Parasites of South African freshwater fish I. Some nematodes of the catfish [*Clarias gariepinus* (Burchell, 1822)] from the Hartbeespoort Dam. *Onderstepoort Journal of Veterinary Research* **49**: 41 - 51.

BOOMKER, J. 1994a. Parasites of South African freshwater fish. VI. Nematode parasites of some fish species in the Kruger National Park. *Onderstepoort Journal of Veterinary Research* **61**: 35 - 43.

BOOMKER, J. 1994b. Parasites of South African freshwater fish. VII. Nematodes of some scaled fishes from the Hartbeespoort Dam, Transvaal. *Onderstepoort Journal of Veterinary Research* **61**: 197 - 199.

BOUNGOU, M, KABRE, GB, MARQUES, A and SAWADOGO, L. 2008. Dynamics of Population of Five Parasitic Monogeneans of *Oreochromis niloticu*s Linné, 1757 in the Dam of Loumbila and Possible Interest in Intesive Pisciculture. *Pakistan Journal of Biological Sciences* **11**(10): 1317 - 1323.

BOWSER, PR, and BUTTNER, JK. 1993. General Fish Health Management. Aquaculture Center. NRAC Bulletin No. 111. 4 pp.

BRITZ, J, VAN AS, JG and SAAYMAN, JE. 1985. Occurrence and distribution of *Clinostomum tilapiae* Ukoli, 1966 and *Euclinostomum heterostomum* (Rudolphi, 1909) metacercarial infections of freshwater fish in Venda and Lebowa, Southern Africa. *Journal of Fish Biology* **26**: 21 - 28.

BRUTON, MN. 1979. The breeding biology and early development of *Clarias gariepinus* (Pisces: Claridae) in Lake Sibaya, South Africa, with a review of breeding in species on the subgenus *Clarias (Clarias)*. *Transactions of Zoological Society of London* **35**: 1 - 45.

BUCHMANN, K and LINDENSTROM, T. 2002. Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* **32**: 309 - 319.

BUSH, AO, LAFFERTY, KD, LOTZ, JM and SHOSTAK, AW. 1997. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology* **83**: 575 - 583.

*BYCHOWSKY, BE. 1957. Monogenetic Trematodes, their classification and phylogeny (in Russian), Moscow: Leningrad. Academy of Sciences USSR. (English translation by HARGIS, WJ and OUSTINOFF, PC. 1961. Washington: A.I.B.S.).

CABLE, J and HARRIS, PD. 2003. Fish parasites: the fish farmer's foe. *Biologist* **50**: 178 - 182.

CARR, GM and NEARY, JP. 2006. Water Quality for Ecosystem and Human Health. Prepared and published by the United Nations Environment Programme Global Environment Monitoring System (GEMS)/ Water Programme. 1- 132 pp.

CHAMBERS, CB and ERNST, I. 2005. Dispersal if the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for sea-cage farming of *Seriola* spp. *Aquaculture* **250**: 60 - 69.

CHANDRA, KJ. 2006. Fish Parasitological Studies in Bangladesh: A Review. *Journal of Agriculture & Rural Development* **4**(1&2): 9 - 18.

CHAPMAN, LJ, LANCIANI, CA and CHAPMAN, CA. 2000**.** Ecology of a diplozoon parasite on the gills of the African Cyprinid *Barbus neumayeri*. *African Journal of Ecology* **38**(4): 312 - 320.

CHISHAWA, AMM. 1991. A survey of the parasites of three Siluriformes fish species in Lake Kariba. University of Zimbabwe Lake Kariba Research Station Bulletin **1/91**: 8 - 25.

CHRISTISON, KW, SHINN, AP and VAN AS, JG. 2005. *Gyrodactylus thlapi* n. sp. (Monogenea) from *Pseudocrenilabrus philander philander* (Weber) (Cichlidae) in the Okavango Delta, Botswana. *Systematic Parasitology* **60**: 165 - 173.

CHUBB, JC. 1977. Seasonal occurrence of helminths in freshwater fishes. Part I. Monogenea. *Advanced Parasitology* **15**: 133 - 192.

CHUBB, JC. 1979. Seasonal occurrence of helminths in freshwater fish. Part II. Trematoda. *Advanced Parasitology* **17**: 141 - 313.

CONE, DK, ARTHUR, JR and BONDAD-REANTASO, MG. 1995. Description of two new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) from cultured Nile tilapia, *Tilapia nilotica* (Cichlidae), in the Philippines. *Journal of the Helminthological Society of Washington* **62**: 6 - 9.

CRAFFORD, D. 2000. Application of a fish health assessment index and associated parasite index on *Clarias gariepinus* (Sharptooth catfish) in the Vaal River System, with reference to heavy metals. Unpublished MSc dissertation. Rand Afrikaans University, Johannesburg, South Africa.

CRAFFORD, D. and AVENANT-OLDEWAGE, A. 2009. Applicationof a fish health assessment index to *Clarias gariepinus* (Teleostei: Clariidae) in the Vaal River system, South Africa. *African Journal of Aquatic Science* **34**: 261 - 272

DAVIS, B. and DAY, J. 1998. Pollution. In: Vanishing Waters. UCT Press, Cape Town, 166- 200 pp.

DÁVIDOVÁ, M and ONDRAČKOVÁ, M. 2008. Parasite assemblages of European bitterling (*Rhodeus amarus*), composition and effects of habitat type and host body size. *Parasitology Research* **102**: 1001 - 1011.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF). 1991. Water Quality Management Policies and Strategies in the Republic of South Africa. Pretoria.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF). 1996. *South African Water Quality Guidelines* Vol. 7 Aquatic Ecosystems. Pretoria, South Africa. x + 145 pp.

DIPPENAAR, SM, LUUS-POWELL, WJ and ROUX, F. 2001. *Lamproglena hoi* n. sp. (Copepoda: Lernaeidae) from two yellowfish hosts, *Barbus marequensis* and

Barbus polylepis, caught in a river in Mpumalanga, South Africa. *Onderstepoort Journal of Veterinary Research* **68:** 209 - 215.

DÖRÜCÜ, M and ÍSPÍR, Ü. 2001. Seasonal Variation of *Diplostomum* sp. infection in eyes of *Acanthobrama marmid* Heckel, 1843 in Keban Dam Lake, Elazığ, Turkey, E.U. *Journal of Fisheries and Aquatic Sciences* **18**(3-4): 301 - 305.

DÖRÜCÜ, M, DÍLSÍZ, N and GRABBE, MCJ. 2002. Occurrence and Effects of *Diplostomum* sp. infection in Eyes of *Acanthobrama marmid* in Keban Dam Lake, Elazığ, Turkey. *Turkish Journal of Veterinary and Animal Science* **26**: 239 - 243.

DOUËLLOU, L. 1992a. Parasites of *Oreochromis* (*Oreochromis*) *mortimeri* (Trewavas, 1966) and *Tilapia rendalli rendalli* (Boulenger, 1896) in Lake Kariba, Zimbabwe. University of Zimbabwe Lake Kariba Research Station Bulletin 2 (Proc. of seminar series): 14–31.

DOUËLLOU, L. 1992b. A survey of fish parasites in Lake Kariba, Zimbabwe (1989–1992), Final Report. University of Zimbabwe Lake Kariba Research Station Bulletin 1/92: 71 pp.

DOUËLLOU, L and ERLWANGER, KH. 1993. Occurrence and distribution of two clinostomatid metacercariae in fishes from Lake Kariba, Zimbabwe. *Transactions of the Zimbabwe Scientific Association* **66**: 35 - 40.

DZIKOWSKI, R, PAPERNA, I and DIAMANT, A. 2003. Multi-annual changes in the parasite communities of rabbitfish *Siganus rivulatus* (Siganidae) in the Gulf of Aqaba, Red Sea. *Helgoland Marine Research* **57**: 228 - 235.

FAFIOYE, OO and OLUAJO, OA, 2005. Length-weight relationships of five fish species in Epe lagoon, Nigeria. *African Journal of Biotechnology* **4(**7): 749 - 751.

FATOKI, OS, LUJIZA, N and OGUNFOWOKAN, AO. 2002. Trace metal pollution in Umtata River. *Water SA* **28**(2): 183 - 190.

FEIKE NATURAL RESOURCE MANAGEMENT ADVISORS. 2008. Potential for a South African aquaculture industry on the Northern Cape's Namaqualand Coast. An initiative of the Presidency, hosted by Trade and Industrial Policy Strategies 1 - 17 p.

FRYER, G. 1964. Further studies on the parasitic Crustacea of African freshwater fishes. *Proceedings of the Zoological Society of London* **143**: 79 - 102.

FRYER, G. 1968. The parasitic Crustacea of African freshwater fishes: their biology and distribution. *Proceedings of the Zoological Society of London* **156**: 45 - 95.

GALLI, P, CROSA, G, MARINIELLO, L, ORTIS, M and AMELIO, SD. 2001. Water quality as a determinant of the composition of fish parasite communities**.** *Hydrobiologia* **452**: 173 - 179.

GAO, Q, CHEN, MX. YAO, WJ, GAO, Y, SONG, Y, WANG, GT, WANG, MX and NIE, P. 2007. Phylogeny of diplozoids in five genera of the subfamily Diplozoinae Palombi, 1949 as inferred from ITS-2 rDNA sequences. *Parasitology* **134**: 695 - 703.

GAONA, MR. 2004. Critical loads of Cd, Cu, Zn and Pb in surface waters in uplands areas of the UK. MSc Dissertation, University of Bradford. 24 pp.

GELNAR, M, ŠEBELOVÁ, Š, DUŠEK, L, KOUBKOVÁ, B, JURAJDA, P, and ZAHRÁDKOVÁ, S. 1997. Biodiversity of parasites in freshwater environment in relation to pollution. *Parassitologia* **39**: 189 - 199.

GOEDE, RW and BARTON, BA. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. *In*: Adams, SM. (Ed) *Biological indicators of stress in fish*. *American Fisheries Society Symposium* **8**: 93 - 108.

GOLLASCH, S, MIOSSEC, L, PEELER, ED and COWX, IG. 2008. Environmental impacts of alien species in aquaculture coordination action-Sustainable management of Europe's natural resources. Project No 44142. 1- 72 pp.

GOUWS, K and COETZEE, PP. 1997. Determination and partitioning of heavy metals in sediments of the Vaal Dam System by sequential extraction. *Water SA* **23**: 217 - 226.

GRACZYK, T. 1991. Variability of metacercariae of *Diplostomum spathaceum* (Rudolphi, 1819) (Trematoda, Diplostomatidae). *Acta Parasitologica Polonica* **36**: 135 - 139.

GROENEWALD, M. 2000. Bioaccumulation of metals and the general health of fish from the Vaal Dam and Vaal River Barrage. Unpublished MSc dissertation, Rand Afrikaans University, Johannesburg, South Africa.

GUTIÉRREZ, PA and MARTORELLI, SR. 1999. Hemibranch preference by freshwater monogenaneans a function of gill area, water current, or both? *Folia Parasitologica* **46**: 263 - 266.

HANSEN, HJM, OLSEN, AG and ROSENKILDE, P. 1996. The effect of Cu on gill and esophagus lipid metabolism in the rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology* **113**(1): 23 - 29.

HARRIS, PD and TINSLEY, RC. 1987. The biology of *Gyrodicotylus gallieni* (Gyrodactylidae), an unusual viviparous monogenean from the African clawed toad *Xenopus laevis. Journal of Zoology* **212**: 325 - 346.

HEATH, R, DU PREEZ, H, GENTHE, B and AVENANT-OLDEWAGE, A. 2004. Freshwater Fish and Human Health Reference Guide. A Report to the Water Research Commission. WRC Report No TT213/04. South Africa. 93 pp.

JOHNSON, MW, NELSON, PA and DICK, TA. 2004. Structuring mechanisms of yellow perch (*Perca flavescens*) parasite communities: host age, diet, and local factors. *Canadian Journal of Zoology* **82**: 1291 - 1301.

JOOSTE, A, LUUS-POWELL, WJ and POLLING, L. 2003. Applying the parasite index (PI) as bio-indicator of water quality in the Selati River, Limpopo Province: Preliminary results. *Journal of the South African Veterinary Association* **74**(3): 98.

JOOSTE, A, LUUS-POWELL, WJ and POLLING, L. 2005. Parasites of *Oreochromis mossambicus* as bio-indicators of water quality. *Journal of the South African Veterinary Association* **76**(3): 180.

JOOSTE, A, LUUS-POWELL, WJ, POLLING, L and HATTINGH, HE. 2004. Bio-monitoring and bio-indexing by means of the Fish Health Assessment Index and fish parasites of the Ga-Selati River. Unpublished Foskor/NRF Report. University of the North, South Africa. 180 pp.

KEARN, GC. 1994. Evolutionary expansion of the Monogenea. *International Journal for Parasitology* **24**(8): 1227 - 1271.

KENNEDY, CR. 1995. Richness and diversity of macroparasite communities in tropical eels *Anguilla reinhardtii* in Queensland, Australia. *Parasitology* **111**: 233 - 245.

KENNEDY, CR. 1997. Freshwater fish parasites and environmental quality: an overview and caution. *Parassitologia* **59**: 249 - 254.

KENT, ML. 2000. Marine netpen farming leads to infections with some unusual parasites. *International Journal for Parasitology* **30**: 321 - 326.

KHALIL, LF. 1963. On *Diplostomulum tregena*, the *Diplostomulum* stage of *Diplostomum tregena* Nazmi Ghohar, 1932, with an experiment demonstration of part of the life cycle. *Journal of Helminthology* **37**: 199 - 206.

KHALIL, LF. 1969. Studies on the helminth parasites of freshwater of the Sudan. *Journal of Zoology London* **158**: 143 - 170.

KHALIL, LF and MASHEGO, SN. 1998. The African monogenean gyrodactylid genus *Macrogyrodactylus* Malmberg, 1957, and the reporting of three species of the genus on *Clarias gariepinus* in South Africa. *Onderstepoort Journal of Veterinary Research* **65**: 223-231.

KHALIL, LF and POLLING, L. 1997. Check list of the helminth parasites of African freshwater fishes. Department of Zoology/Biology, University of the North, Sovenga, South Africa. 185 pp.

KHAN, RA and THULIN, J. 1991. Influence of pollution on parasites of aquatic animals. *Advances in Parasitology* **30**: 201 - 238.

*KHOTENOVSKY, IA. 1985. Monogenea: Suborder Octomacrinae Khotenovsky. Fauna USSR. Leningrad. New series, no. 132. 185 pp.

KLEMM, DJ, STROBER, GJ AND LAZORCHAK, JM. 1992. Fish field and laboratory methods for evaluating the biological integrity of surface waters. EPA / 600 / R - 92 - 111 USEPA, Cincinatti, Ohio.

KLINGER, R and FLOYD, RF. 2009. Introduction to Freshwater Fish Parasites 1. http://edis.ifas.ufl.edu/FA041. 13 pp. Date accessed: 15 November 2009.

KOSKIVAARA, M. 1992. Environmental factors affecting monogeneans parasitic on freshwater fishes. *Parasitology Today* **8**: 339 - 342.

KOSKIVAARA, ME, VALTONEN, T and PROST, M. 1991. Dactylogyrids on the gills of roach in central Finland: Features of infection and species composition. *International Journal for Parasitology* **21**: 565 - 572.

LAFFERTY, KD. 1997. Environmental parasitology: What can parasites tell us about human impacts on the environment? *Parasitology Today* **13**(7): 251 - 255.

LAFFERTY, KD. 2008. Ecosystem consequences of fish parasites. *Journal of Fish Biology* **73**: 2083 - 2093.

LAMBERT, A and GHARBI, SE. 1995. Monogenean host specificity as a biological and taxonomic indicator for fish. *Biological Conservation* **72**: 227 - 235.

LAMKOVÁ, K, ŠIMKOVÁ, A, PALÍKOVÁ, M, JURAJDA, P and LOJEK, A. 2007. Seasonal changes of immunocompetence and parasitism in chub (*Leuciscus cephalus*), a freshwater cyprinid fish. *Parasitology Research* **101**: 775 - 789.

LANE, RL and MORRIS, JE. 2000. Biology, Prevention, and Effects of Common Grubs (Digenetic trematodes) in Freshwater Fish. Technical Bulletin Series # 115. 7 pp.

LE BRUN, N, RENAUD, F and LAMBERT, A. 1988. The genus *Diplozoon* (Monogenea, Polyopisthocotylea) in southern France: Speciation and specificity. *International Journal for Parasitology* **18**: 395 - 400.

LE BRUN, N, RENAUD, F and LAMBERT, A. 1990. Differential settlement by *Diplozoon gracile* (Monogenea) on teleostean (cyprinid) hosts in relation to biological and environmental conditions. *Acta Oecologica* **11**: 729 - 739.

LE BRUN, N, RENAUD, F, BERREBI, P and LAMBERT, A. 1992. Hybrid zones and host-parasite relationships: effect on the evolution of parasitic specificity. *Evolution* **46:** 56 - 61.

LOMBARD, GL. 1968. A survey of fish diseases and parasites encountered in Transvaal. *Newsletter of the Limnological Society of South Africa* **11**: 23 - 29.

LUNASCHI, LI and DRAGO, FB. 2005. First report of adult specimens of *Sphincterodiplostomum musculosum* (Digenea, Diplostomidae). *Parasitology International* **55**: 7 - 10.

LUQUE, JL, ALVES, DR and RIBEIRO, RS. 2003. Community ecology of the metazoan parasites of Banded Croaker, *Paralonchurus brasiliensis* (Osteichthyes: Sciaenidae), from the coastal zone of the state of Rio de Janeiro, Brazil. *Acta Scientiarum, Biological Science, Maringá* **25** (2): 273 - 278.

LUUS-POWELL, WJ. 1997. Evaluation of the health assessment index with reference to metal bioaccumulation in *Labeo* species and aspects of the morphology of *Chonopeltis victori*. Unpublished MSc dissertation, Rand Afrikaans University, Johannesburg. 236 pp.

LUUS-POWELL, WJ. 2004. Metazoan parasites of Mormyrid fishes from South Africa. Unpublished PhD thesis, University of the North, Sovenga. 234 pp.

LUUS-POWELL, WJ, JOOSTE, A, and HATTINGH, HE. 2005. Parasites of *Clarias gariepinus* as bio-indicators of pollution. *Journal of the South African Veterinary Association* **76**(3): 181.

LUUS-POWELL, WJ, MAHLANGU, PS, THERON, J and HATTINGH, HE. 2009. Gyrodactyliasis - a cause for concern? *Journal of the South African Veterinary Association* **80**: 123.

LUUS-POWELL, WJ, MASHEGO, SN and KHALIL, LF. 2003. *Mormyrogyrodactylus gemini* gen. et sp. n. (Monogenea: Gyrodactylidae), a new gyrodactylid from *Marcusenius macrolepidotus* (Mormyridae) from South Africa. *Folia Parasitologica* **50**: 49 - 55.

LUUS-POWELL, WJ, THERON, J and HATTINGH, HE. 2006. A small worm causing great losses in aquaculture. *Journal of the South African Veterinary Association* **77**: 102.

MACHADO, MH, PAVANELLI, GC and TAKEMOTO, RM. 1995. Influence of the type of environment and of the hydrological level variation in endoparasitic infrapopulations of *Pseudoplatystoma corruscans* (Agassiz) and *Schizodon Borelli* (Boulenger) (Osteichthyes) of the high Parana River, Brazil. *Revista Brasilian Zoologica* **12**(4): 961 - 976.

MacKENZIE, K, WILLIAMS, HH, WILLIAMS, B, McVICAR, AH and SIDDALL, R. 1995. Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *Advances in Parasitology* **35**: 85 - 144.

MADANIRE-MOYO, GN, MATLA, MM, OLIVIER, PAS and LUUS-POWELL, WJ. 2010. Population dynamics and spatial distribution of monogeneans on the gills of *Oreochromis mossambicus* (Peters, 1852) from two lakes of the Limpopo River System, South Africa. *Journal of Helminthology* doi:10.1017/S0022149X10000301.

*MALMBERG, G. 1957: Om förekomsten av *Gyrodactylus* påsvenska fiskar. Särtryck ur Skrifter utgivna av Södra Sveriges Fiskeriförening, Årsskrift **1956**: 19 - 76.

MALVESTUTO, SP and OGAMBO-ONGOMA, A. 1978. Observation on the infection of *Tilapia leucosticte* (Pisces: Cichlildae) in Lake Naivasha, Kenya. *Journal for Parasitology* **64**: 383 - 384.

MARCOGLIESE, DJ and CONE, DK. 1991. Importance of lake characteristics in structuring parasite communities of Salmonids from insular Newfoundland. *Canadian Journal of Zoology* **69**: 2962 - 2967.

MARCOGLIESE, DJ. 2004. Parasites: small players with crucial roles in the ecological theatre. *EcoHealth* **1**(2): 51 - 164.

MARCOGLIESE, DJ. 2005. Parasites of the superorganism: Are they indicators of ecosystem health? *International Journal for Parasitology* **35**: 705 - 716.

MARCOGLIESE, DJ and CONE, DK. 1997. Parasite communities as indicators of ecosystem stress. *Parassitologia* **39**: 227 - 232.

MARTINS, ML, ONAKA, EM and FENERICK Jr, J. 2005. Larval *Contracaecum* sp. (Nematoda: Anisakidae) in *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus* (Osteichthyes: Erythrinidae) of economic importance in occidental marshlands of Maranhao Brazil. *Brazil Veterinary Parasitology* **127**: 51 - 59.

MARTORELLI, SR, SARDELL, NH and MARCOTEGUI, PS. 2007. New host, geographical records, and some ecologicalaspect of *Absonifibula bychowskyi* Lawler et Overstreet, 1976 (Monogenea: Diclidophoridae) from estuarine areas of Argentina. *Acta Ichthyologica et Piscatoria* **37**(1): 1 - 6.

MARX, HM. 1996. Evaluation of a health assessment index with reference to metal bioaccumulation in *Clarias gariepinus* and aspects of the biology of the parasite *Lamproglena clariae*. Unpublished MSc dissertation, Rand Afrikaans University, Johannesburg, South Africa.

MASHEGO, SN. 1977. A seasonal investigation of the ecto- and endoparasites of the Barbel, *Clarias gariepinus* (Burchell, 1822) in Lebowa, South Africa. Unpublished MSc dissertation, University of the North, Sovenga, South Africa. 87 pp.

MASHEGO, SN. 1982. A seasonal investigation of the helminth parasites of *Barbus* species in water bodies in Lebowa and Venda, South Africa. Unpublished PhD thesis, University of the North, Sovenga, South Africa. 191 pp.

MASHEGO, SN. 1989. Nematode parasites of *Barbus* species in Lebowa and Venda, South Africa. *South African Journal of Wildlife Research* **19**(1): 35 - 37.

MASHEGO, SN. 2000. Occurrence of *Neodiplozoon polycotyleus* Paperna, 1973 (Diplozoidae: Monogenea) in cyprinid fish in South Africa. *Onderstepoort Journal of Veterinary Research* **67**(2): 153 - 154.

MASHEGO, SN. 2001. Redescription of *Proteocephalus glanduliger* (Janicki, 1928) Fuhrmann, 1933 (Cestoda: Proteocephalidae: Proteocephalinae). *Annals of the Transvaal Museum* **38**: 13 - 17.

MASHEGO, SN and SAAYMAN, JE. 1981. Observations on the prevalence of nematode parasites of the catfish, *Clarias gariepinus* (Burchell, 1822), in Lebowa, South Africa*. South African Journal of Wildlife Research* **11**: 46 - 48.

McKEOWN, CA and IRWIN, SWB. 1995. The life cycle stages of three *Diplostomum* species maintained in the laboratory. *International Journal for Parasitology* **25** (8): 897 - 906.

MIERZEJEWSKA, K and WŁASOW, T. 2005. Fish digeneans from the Seven Islands ornithological reserve at Oświn Lake. Part II. The eyeflukes-*Diplostomum* spp. and *Tylodelphys clavata* (von Nordmann, 1832). *Acta Ichthyologica et Piscatoria* **35**(1): 1 - 5.

MILNE, SJ and AVENANT-OLDEWAGE, A. 2006. The fluorescent detection of *Paradiplozoon* sp. (Monogenea: Diplozoidae) attachment clamps' sclerites and integumental proteins. *Onderstepoort Journal of Veterinary Research* **73**(2): 149 - 52.

MWITA, C and NKWENGULILA, G. 2008. Determinants of the parasite community of clariid fishes from Lake Victoria, Tanzania. *Journal of Helminthology* **82**: 7 - 16.

MOKGALONG, NM. 1996. A study of the gastrointestinal helminths of the Phalacrocoracidae and the Anhingidae in the Northern Province, South Africa. Unpublished PhD thesis, University of the North, Sovenga.

MØLLER, AP, ERRITZØE, J and SAINO, N. 2003. Seasonal Changes in Immune Response and Parasite Impact on Hosts. *The American Naturalist* **161**(4): 657 - 671.

MORLEY, NJ. 2007. Anthropogenic effects of reservoir construction on the parasite fauna of aquatic wildlife. *EcoHealth* **4**: 374 - 383.

MOUTON, A, BASSON, L and IMPSON, D. 2001. Health status of ornamental freshwater fishes imported to South Africa: A pilot study. *Aquarium Sciences and Conservation* **3**: 327 - 333.

NEY, JJ and VAN HASSEL, JH. 1983. Sources of variability in accumulation of heavy metals by fishes in a roadside stream. *Archives of Environmental Contamination and Toxicology* **12**: 701 - 706.

NIEWIADOMSKA, K and SZYMANSKI, S. 1991. Host-induced variability of *Diplostomum paracaudum* (Iles, 1959) metacercariae (Digenea). *Acta Parasitologica Polonica* **36**: 11 - 17.

NATIONAL WATER ACT (NWA). 1998. (No. 36 of 1998). *Government Gazette, Vol. 398 No. 19182*. Government Printer, Pretoria, South Africa.

OBIEKENZIE, AI, ANDERS, K, LICK, R, MOELLER, H and PALM, H. 1992. External lesions and flesh parasites in commercial fishes of Nigeria inshore waters. *Aquatic Living Resources* **5**: 173 - 183.

OLIVERO-VERBEL, J, BALDIRIS-AVILA, R, GUETTE-FERNANDEZ, J, BENAVIDES-ALVAREZ, A, MERCADO-CAMARGO, J, and ARROYO-SALGADO, B. 2006. *Contracaecum* sp. Infection in *Hoplias malabaricus* (moncholo) from rivers and marshes of Colombia. *Veterinary Parasitology* **140**(1-2): 90 - 97.

OLIVIER, PAS, LUUS-POWELL, WJ and SAAYMAN, JE. 2009. Report on some monogenean and clinostomid infestations of freshwater fish and waterbird hosts in Middle Letaba Dam, Limpopo Province, South Africa. *Onderstepoort Journal of Veterinary Research* **76**: 187 - 199.

ONDRAČKOVÁ, M, ŠIMKOVÁ, A, GELNAR, M, and JURAJDA, P. 2004. *Posthodiplostomum cuticola* (Digenea: Diplostomatidae) in intermediate fish hosts: factors contributing to the parasite infection and prey selection by definitive bird host. *Parasitology* **129**: 761 - 770.

ORECCHIA, P, PAGGI, L, MATTIUCCI, S, SMITH, JW, NASCETTI, G and BULLINI, L. 1986. Electrophorhetic identification of larvae and adults of *Anisakis* (Ascaridia, Anisakidae). *Journal of Helminthology* **60**: 337 - 339.

*ORTLEPP, RT. 1935. On the metacercaria and adult of *Clinostomum van der horsti* sp. n. a trematode parasite of fish and herons. *Onderstepoort Journal of Veterinary Science and Animal industry* **5**: 51 - 58.

OVERSTREET, RM. 1993. Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. *Pathobiology of Marine and Estuarine Organisms* **5**: 111 - 155.

OVERSTREET, RM. 1997. Parasitological data as monitors of environmental health. *Parassitologia* **39**: 169 - 175.

ÖZER, A. 2002. Co-existence of *Dactylogyrus anchoratu* Dujardin, 1845 and *D. extensus* Mueller & Van Cleave, 1932 (Monogenea), parasites of common carp (*Cyprinus carpio)*. *Helminthologia* **39**(1): 45 - 50.

ÖZTÜRK, MO and ALTUNEL, FN. 2006. Occurrence of *Dactylogyrus* infection linked to seasonal changes and host fish size on four cyprinid fishes in lake Manyas, Turkey. *Acta Zoologica Academiae Scientiarum Hungaricae* **52**(4): 407 - 415.

ÖZER, A and ÖZTÜRK, T. 2005. *Dactylogyrus cornu* Linstow, 1878 (Monogenea) Infestations on Vimba (*Vimba vimba tenella* (Nordmann, 1840)) caught in the Sinop Region of Turkey in Relation to the Host Factors. *Turkish Journal of Veterinary and Animal Sciences* **29**: 1119 - 1123.

*PAPERNA, I. 1968. Monogenetic trematodes collected from freshwater fish in Ghana. Second report. *Bamidgeh* **20**: 88 - 100.

PAPERNA, I. 1973. New species of Monogenea (Vermes) from African freshwater fish. A preliminary report. *Revue de Zoologie et de Botanique Africaines* **87**: 505 - 518.

PAPERNA, I. 1979. Monogenea of inland water fishes in Africa. *Museé Royal de l'Afrique Centrale, Science, Zoologie Serie* **8**(226): 1 - 131.

PAPERNA, I. 1980. Parasites, infections and diseases of fish in Africa. CIFA Technical Paper **7**: 88 - 89.

PAPERNA I. 1996. Parasites, infections and diseases of fishes in Africa. An update.*:* CIFA Technical Paper **31**: 1 - 225.

PAPERNA, I and THURSTON, JP. 1968. Report on ectoparasitic infections of freshwater fish in Africa. *International Office of Epizootics* **69**: 1192 - 1206.

PEČINKOVÁ, M, MATĚJUSOVÁ, I, KOUBKOVÁ, B and GELNAR, M. 2007. Investigation of *Paradiplozoon homoion* (Monogenea, Diplozoidae) life cycle under experimental conditions. *Parasitology International* **56**: 179 - 183.

PEELER, E, THRUSH, M, PAISLEY, L and RODGERS, C. 2006. An assessment of the risk of spreading the fish parasite *Gyrodactylus salaris* to uninfected territories in the European Union with the movement of live Atlantic salmon (*Salmo salar*) from coastal waters. *Aquaculture* **258**(1-4): 187 - 197.

PICKERING, AD and CHRISTIE, P. 1980. Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, *Salmo trutta* L. *Journal of Fish Biology* **16**: 669 - 683.

PIETROCK, M and MARCOGLIESE, DJ. 2003. Free-living endohelminth stages: at the mercy of environmental conditions. *Trends in Parasitology* **19**: 293 - 299.

POLY, WJ. 2008. Global diversity of fishlice (Crustacea: Branchiura: Argulidae) in freshwater. *Hydrobiologia* **595**: 209 - 212.

POULIN, R. 1992. Toxic pollution and parasitism in freshwater fish. *Parasitology Today* **8**: 51 - 61.

POULIN, R. 1995. Phylogeny, ecology and the richness of parasite communities in vertebrates. *Ecological Monographs* **65**(3): 283 - 302.

POULIN, R. 2002. The evolution of monogenean diversity. *International Journal for Parasitology* **32**: 245 - 254.

PRUDHOE, S and HUSSEY, CG. 1977. Some parasitic worms in freshwater fishes and fish predators from the Transvaal, South Africa. *Zoologica Africana* **12**: 113 - 147.

RAMOLLO, PP. 2008. Bio-assessing the impact of water quality on the health and parasite composition of *Oreochromis mossambicus* at the Phalaborwa Industrial Complex (PIC) and the Barrage (Olifants River) in the Limpopo Province of South Africa. Unpublished MSc dissertation, University of Limpopo, Sovenga, South Africa. 198 pp.

RAYMOND, KMN, CHAPMAN, LL and LANCIANI, CA. 2006. Host, macrohabit, and microhabit specificity in the gill parasite *Afrodiplozoon polycotyleus* (Monogenea). *Journal of Parasitology* **92**: 1211 - 1217.

REED, P, FRANCIS-FLOYD, R AND KLINGER, R.L. 2002. Monogenean Parasites of Fish 1. 4 pp.

*RHODE, K. 1993. *An Introduction to Marine Parasitology*. 2nd Edition. CAB International, Wallingford, United Kingdom. 298 pp.

ROBINSON, J. 1996. Evaluation of a Health Assessment Index with reference to bioaccumulation of metals in *Oreochromis mossambicus* (Peters, 1852) and aspects of the morphology of *Lernaea cyprinacea* (Linnaeus, 1758). Unpublished MSc dissertation, Rand Afrikaans University, Johannesburg, South Africa.

ROBERTS, RJ and SOMMERVILLE, C. 1982. Disease of tilapias: The biology and culture of tilapias. In: Pullin, RSV and Lowe-McConnell, RH. (Eds.) ICLARM *Conference proceedings*, *Manilla Phillippines* **7:** 247 - 263.

ROUSSET, F, THOMAS, R, DE MEEÛS, T, RENAUD, F. 1996. Inference of parasite-induced host mortality from distributions of parasite loads. *Ecology* **77**(7): 2203 - 2211

ROUX, DJ, VAN VLIET, HR and VAN VEELEN, M. 1993. Towards integrated water quality monitoring: Assessment of ecosystem health. *Water SA* **19**: 275 - 280.

SAAYMAN, JE, MASHEGO, SN and MOKGALONG, NM. 1991. Parasites of the fish population with notes on the helminth parasites of the water birds of Middle Letaba Dam. In: Saayman, JE, Schoonbee, HJ and Smit, GL. (Eds.) *A Post Impoundment Ecological Study of the Middle Letaba Dam, Gazankulu*. Department of Development Aid, Pretoria. 8.1 - 8.210 pp.

SALGADO-MALDONADO, G and KENNEDY, CR. 1997. Richness and similarity of helminth communities in the tropical cichlid fish *Cichlasoma urophthalmus* from the Yucatan Peninsula, Mexico. *Parasitology* **114**: 581 - 590.

SANDLAND, GJ, GOATER, CP and DANYLCHUK, AJ. 2001. Population dynamics of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*) from four northern-Alberta lakes. *Journal of Parasitology* **87**: 744 - 748.

SCHABUSSOVA, I, KOUBKOVÁ, B, GELNAR, M, SCHABUSS, M and HORÁK, P. 2004. Surface carbohydrates of *Eudiplozoon nipponicum* pre- and post-fusion. *Journal of Helminthology* **78**: 63 - 68.

SCHMITT, CJ, HINCK, JE, BLAZER, VS, DENSLOW, ND, DETHLOFF, GM, BARTISH, TM, COYLE, JJ and TILLITT, DE. 2005. Environmental contaminants and biomarker responses in fish from the Rio Grande and its U.S. tributaries: Spatial and temporal trends. *Science of the Total Environment* **350**(1-3): 161 - 93.

SCHOLZ, T. 2001: Identity of *Cysticercoides menidiae* Chandler, 1935 (Cestoda: Dilepididae). *International Journal for Parasitology* **87**: 927 - 928.

SCHOLZ, T and SALGADO-MALDONADO, G. 2001: Metacestodes of the family Dilepididae (Cestoda: Cyclophyllidea) parasitising fishes in Mexico. *Systematic Parasitology* **49**: 23 - 40.

SCHOLZ, T, BRAY, RA, KUCHTA, R and ŘEPOVÁ, R. 2004. Larvae of gryporhynchid cestodes (Cyclophyllidea) from fish: a review. *Folia Parasitologica* **51**: 131 - 152.

SEDDON, L. 2004. Aspects of the morphology and ecology of a diplozoon species (Monogenea) from the gills of *Labeo umbratus* in the Vaal Dam and Vaal River Barrage, Gauteng, South Africa. Unpublished MSc dissertation, Rand Afrikaans University, Johannesburg, South Africa. 144 pp.

SEPPÄLÄ, O, KARVONEN, A and VALTONEN, ET. 2005. Impaired crypsis of fish infected with a trophically transmitted parasite. *Animal Behaviour* **70**(4): 895 - 900.

*SHIGIN, A. 1986. Trematode fauna of the USSR. Genus *Diplostomum. Metacercariae*. Moscow, Nauka. 234 pp.

SHINN, AP, BRON, JE, SOMMERVILLE, C and GIBSON, DI. 2003. Comments on the mechanism of attachment in species of the monogenean genus *Gyrodactylus*. *Invertebrate Biology* **122:** 1 - 11.

SKELTON, PH. 2001. *A complete guide to the freshwater fishes of Southern Africa*. Halfway House, Cape Town. 395 pp.

SURES, B. 2004. Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology* **20**: 170 - 177.

SKOROBREKHOVA, EM. 2009. Encapsulation of the Metacercariae of Two Species of Trematodes in the Skin and Musculature of the Stone Cockscomb *Alectrias alectrolophus* (Pallas, 1814). *Russian Journal of Marine Biology* **35**(2): 178 - 182.

TAKEMOTO, RM, PAVANELLI, GC, LIZAMA, MAP, LUQUE, JL and POULIN, R. 2005. Host population density as the major determinant of endoparasite species richness in floodplain fishes of the upper Parana River, Brazil. *Journal of Helminthology* **79**: 75 - 84.

*TRIPATHI Y.R. 1957. Monogenetis Trematodes from fishes of India. *Indian Journal of Helminthology* **9**(1-2): 1 - 149.

VAN DER MERWE, W and GROBLER, DC. 1990. Water quality management in the RSA: Preparing for the future. *Water SA* **16**(1): 49 - 53.

WATSON, RM. 2001. Evaluation of a fish health assessment index as biomonitoring tool for heavy metal contamination in the Olifants River catchment area. Unpublished PhD thesis, Rand Afrikaans University, Johannesburg, South Africa. 286 pp.

WHITFIELD, AK and HEEG, J. 1977. On the life cycles of the cestode *Ptychobothrium belones* and nematodes of the genus *Contracaecum* from Lake St. Lucia, Zululand. *South African Journal Science* **73**: 121 - 122.

WHITTINGTON, ID, CRIBB, BW, HAMWOOD, TE and HALLIDAY, JA. 2000. Host-specificity of monogenean (Platyhelminth) parasites: a role for anterior adhesive areas? *International Journal for Parasitology* **30**: 305 - 320.

*WILLIAMS, HH and JONES, A. 1994. *Parasitic Worms of Fish.* Taylor and Francis, London. 593 pp.

*YAMAGUTI, S. 1963. *Systema Helminthum*. *Vol. V. Acanthocephala*. Interscience Publishers, New York, New York. 423 pp.

ZURAWSKI, TH, MAIR, GR, MAULE, AG, GELNAR, M and HALTON, DW. 2003. Microscopical evaluation of neural connectivity between paired stages of *Eudiplozoon nipponicum* (Monogenea: Diplozoidae). *Journal for Parasitology* **89**(1): 198 - 200.

* not seen in the original

Appendix 1

ANOVA for *Dactylogyrus spinicirrus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	167.431	3	55.810	3.561	.025
Within Groups	501.458	32	15.671		
Total	668.889	35			

Key; df = degree of freedom, F variable is the ratio of two independent chi-square variables divided by their respective degrees of freedom, Sig. = significance

Multiple Comparisons

	(I) Season	(J) Season	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	6.50000*	3.06632	0.042	0.2541	12.7459
		3.00	5.71429*	1.95082	0.006	1.7406	9.6880
		4.00	2.29412	1.57761	0.156	-.9194	5.5076
	2.00	1.00	-6.50000*	3.06632	0.042	-12.7459	-.2541
		3.00	-.78571	3.17395	0.806	-7.2508	5.6794
		4.00	-4.20588	2.95924	0.165	-10.2336	1.8219
	3.00	1.00	-5.71429*	1.95082	0.006	-9.6880	-1.7406
		2.00	.78571	3.17395	0.806	-5.6794	7.2508
		4.00	-3.42017	1.77777	0.063	-7.0414	0.2010
	4.00	1.00	-2.29412	1.57761	0.156	-5.5076	0.9194
		2.00	4.20588	2.95924	0.165	-1.8219	10.2336
		3.00	3.42017	1.77777	0.063	-.2010	7.0414

*. The mean difference is significant at the 0.05 level.
Key**:** 1.00 = autumn, 2.00 = winter, 3.00 = spring, 4.00 = summer, Std. Error= standard error.

T-Test for *Dactylogyrus spinicirrus*

	Sex	N	Mean	Std. Deviation	Std. Error Mean
Parasites	Female	31	5.3871	4.89678	.87949
	Male	22	5.2727	5.59917	1.19375

t-test for Equality of Means

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Parasites	Equal variances assumed	3.183	0.080	0.079	51	0.937	0.11437	1.44891	-2.79444	3.02317
	Equal variances not assumed			0.077	41.438	0.939	0.11437	1.48274	-2.87913	3.10787

ANOVA for *Afrodiplozoon polycotyleus* on *Barbus radiatus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.464	3	.821	1.384	.278
Within Groups	11.275	19	.593		
Total	13.739	22			

t-test for Equality of Means

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Parasites	Equal variances assumed	0.000	0.992	-.222	44	0.825	-0.06439	0.28948	-0.64780	0.51901
	Equal variances not assumed			-0.224	43.603	0.824	-0.06439	0.28713	-.64322	.51443

Multiple Comparisons

	(I) Season	(J) Season	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett T3	1.00	2.00	1.15385	3.74836	1.000	-10.5806	12.8883
		3.00	0.45385	2.57054	1.000	-6.9789	7.8866
		4.00	-10.72851*	2.99141	0.008	-19.1688	-2.2882
	2.00	1.00	-1.15385	3.74836	1.000	-12.8883	10.5806
		3.00	-0.70000	3.76833	1.000	-12.4995	11.0995
		4.00	-11.88235	4.06708	0.059	-24.1059	0.3412
	3.00	1.00	-0.45385	2.57054	1.000	-7.8866	6.9789
		2.00	0.70000	3.76833	1.000	-11.0995	12.4995
		4.00	-11.18235*	3.01639	0.006	-19.7536	-2.6111
	4.00	1.00	10.72851*	2.99141	0.008	2.2882	19.1688
		2.00	11.88235	4.06708	0.059	-0.3412	24.1059
		3.00	11.18235*	3.01639	0.006	2.6111	19.7536

*. The mean difference is significant at the 0.05 level.

Key**:** 1.00 = autumn, 2.00 = winter, 3.00 = spring, 4.00 = summer

T-Test for *Afrodiplozoon polycotyleus* on *Barbus radiatus*

	SEX	N	Mean	Std. Deviation	Std. Error Mean
Parasites	Female	22	0.7273	0.88273	0.18820
	Male	24	0.7917	1.06237	0.21685

t-test for Equality of Means

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Parasites	Equal variances assumed	0.000	0.992	-.222	44	0.825	-0.06439	0.28948	-0.64780	0.51901
	Equal variances not assumed			-0.224	43.603	0.824	-0.06439	0.28713	-0.64322	0.51443

ANOVA for *Diplostomulum*

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1377.110	3	459.037	6.802	.001
Within Groups	2969.557	44	67.490		
Total	4346.667	47			

T-Test for *Diplostomulum* gender preference

	SEX	N	Mean	Std. Deviation	Std. Error Mean
Parasites	Female	31	12.3871	10.51563	1.88866
	Male	22	12.3636	9.44934	2.01461

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Parasites	Equal variances assumed	0.681	0.413	.008	51	0.993	0.02346	2.81285	-5.62357	5.67049
	Equal variances not assumed			0.008	48.117	0.993	0.02346	2.76146	-5.52849	5.57541

T-test for gender preference on *Contracaecum*

	SEX	N	Mean	Std. Deviation	Std. Error Mean
Parasites	Females	27	43.0000	87.12370	16.76696
	Males	16	8.5625	16.58501	4.14625

t-test for Equality of Means

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Parasites	Equal variances assumed	5.909	0.020	1.557	41	0.127	34.43750	22.11650	-10.22767	79.10267
	Equal variances not assumed			1.994	29.089	0.056	34.43750	17.27201	-0.88307	69.75807

ANOVA for Condition factor

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.734	3	0.911	1.609	0.199
Within Groups	27.759	49	0.567		
Total	30.494	52			

ANOVA for the Health Assessment Index

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5516.492	3	1838.831	6.701	0.001
Within Groups	13446.678	49	274.422		
Total	18963.170	52			

Multiple Comparisons

	(I) Season	(J) Season	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	-12.76667	6.76291	0.065	-26.3573	0.8239
		3.00	18.33333*	6.76291	0.009	4.7427	31.9239
		4.00	8.77778	5.79141	0.136	-2.8605	20.4161
	2.00	1.00	12.76667	6.76291	0.065	-0.8239	26.3573
		3.00	31.10000*	7.40840	0.000	16.2123	45.9877
		4.00	21.54444*	6.53360	0.002	8.4147	34.6742
	3.00	1.00	-18.33333*	6.76291	0.009	-31.9239	-4.7427
		2.00	-31.10000*	7.40840	0.000	-45.9877	-16.2123
		4.00	-9.55556	6.53360	0.150	-22.6853	3.5742
	4.00	1.00	-8.77778	5.79141	0.136	-20.4161	2.8605
		2.00	-21.54444*	6.53360	0.002	-34.6742	-8.4147
		3.00	9.55556	6.53360	0.150	-3.5742	22.6853

*. The mean difference is significant at the 0.05 level.

Key**:** 1.00 = autumn, 2.00 = winter, 3.00 = spring, 4.00 = summe

Appendix 2

Season	Locality	Fish No	Species	Sex	TL (cm)	SL (mm)	Weight (g)	Site	Parasites	No
1	Nwanedi-Luphephe Dams	**1**	*L.marequensis*	F	42.4	343	916.4	Gills	*D.spinicirrus*	4
								Eye	*Diplostomulum* spp.	23
								B.cavity	*Contracaecum* spp.	4
		2	*L.marequensis*	M	47.5	400	1470.2	Gills	*D.spinicirrus*	11
								Eye	*Diplostomulum* spp.	9
								B.cavity	*Contracaecum* spp.	1
		3	*L.marequensis*	F	46	380	1256.8	Eye	*Diplostomulum* spp.	14
								B.cavity	*Contracaecum* spp.	12
								Gills	*D.spinicirrus*	5
								Buccal cavity, fins and skin	*Dolops ranarum*	7
		4	*L.marequensis*	F	47.5	412	1512.6	Eye	*Diplostomulum* spp.	7
								muscle	Digenean cysts	100
								Gills	*D.spinicirrus*	2
								Buccal cavity, fins and skin	*Dolops ranarum*	6
		5	*L.marequensis*	F	39	312	723.6	Eye	*Diplostomulum spp.*	2
								Gills	*D.spinicirrus*	6
		6	*L.marequensis*	M	32.4	270	368	Eye	*Diplostomulum* spp.	14
								B.cavity	*Contracaecum* spp.	50
								Gills	*D.spinicirrus*	10
		7	*L.marequensis*	M	41	340	900.8	Eye	*Diplostomulum* spp.	6
								Buccal cavity, fins and skin	*Dolops ranarum*	4
		8	*L.marequensis*	F	48.7	410	1552.6	Eye	*Diplostomulum* spp.	3
								Gills	*D.spinicirrus*	20
								Buccal cavity, fins and skin	*Dolops ranarum*	4
		9	*L.marequensis*	M	45	391	1357.9	Eye	*Diplostomulum* spp.	18
								Gills	*D.spinicirrus*	11
								Buccal cavity, fins and skin	*Dolops ranarum*	2
		10	*L.marequensis*	F	45.6	390	1425.2	Eye	*Diplostomulum* spp.	5

		11	*L.marequensis*	F	41.8	355	959.8	Eye	*Diplostomulum* spp.	17
		12	*L.marequensis*	F	47.6	394	1264.1	Buccal cavity, fins and skin	*Dolops ranarum*	8
								Buccal cavity, fins and skin	*Dolops ranarum*	3
		13	*L.marequensis*	M	39.7	329	834.8	Gills	*D.spinicirrus*	15
		14	*L.marequensis*	M	43.5	360	97.2	Gills	*D.spinicirrus*	16
								Gills	u/d cysts	7
								Eye	*Diplostomulum* spp.	5
		15	*L.marequensis*	F	39	320	83.6	Eye	*Diplostomulum* spp.	9
Season	**Locality**	**No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
1	Nwanedi-Luphephe Dams	**1**	*B.radiatus*	F	3.5	33	0.5	None	None	0
		2	*B.radiatus*	F	3.8	32	0.6	None	None	0
		3	*B.radiatus*	M	3.4	27	0.3	None	None	0
		4	*B.radiatus*	M	3.1	30	0.5	Gills	*Afrodiplozoon polycotyleus*	1
		5	*B.radiatus*	F	4	32	0.6	Gills	*Afrodiplozoon polycotyleus*	1
		6	*B.radiatus*	F	3.7	29	0.4	Gills	*Afrodiplozoon polycotyleus*	1
		7	*B.radiatus*	M	4.4	33	0.7	Gills	*Afrodiplozoon polycotyleus*	2
		8	*B.radiatus*	M	3.8	30	0.5	Gills	*Afrodiplozoon polycotyleus*	1
		9	*B.radiatus*	F	4.1	33	0.7	None	None	0
		10	*B.radiatus*	F	3.7	28	0.6	None	None	0
Season	**Locality**	**No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
1	Nwanedi-Luphephe Dams	**1**	*B.trimaculatus*	M	3.5	30	0.5	Gills	*Afrodiplozoon polycotyleus*	1
		2	*B.trimaculatus*	M	4	34	0.7	Gills	*Afrodiplozoon polycotyleus*	1
		3	*B.trimaculatus*	F	3.5	30	0.5	Gills	*Afrodiplozoon polycotyleus*	1
		4	*B.trimaculatus*	F	3.8	30	0.6	Gills	*Afrodiplozoon polycotyleus*	2
		5	*B.trimaculatus*	F	3.8	29	0.5	None	None	0
		6	*B.trimaculatus*	M	4	32	0.6	Gills	*Afrodiplozoon polycotyleus*	1
		7	*B.trimaculatus*	M	4.1	34	0.7	None	None	0
		8	*B.trimaculatus*	F	4.1	33	0.8	None	None	0
		9	*B.trimaculatus*	M	3.9	31	0.4	Gills	*Afrodiplozoon polycotyleus*	1
		10	*B.trimaculatus*	M	3.5	31	0.5	Gills	*Afrodiplozoon polycotyleus*	2
		11	*B.trimaculatus*	F	4	30	0.5	Gills	*Afrodiplozoon polycotyleus*	1

		12	*B.trimaculatus*	F	3	26	0.4	Gills	*Afrodiplozoon polycotyleus*	2
		13	*B.trimaculatus*	F	3.8	30	0.5	Gills	*Afrodiplozoon polycotyleus*	2
		14	*B.trimaculatus*	M	3.5	27	0.4	Gills	*Afrodiplozoon polycotyleus*	2
		15	*B.trimaculatus*	F	11	90	13.6	None	None	0
		16	*B.trimaculatus*	F	12	100	19	None	None	0
		17	*B.trimaculatus*	M	11.5	95	17.1	None	None	0

Season	**Locality**	**Fish No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
2	Nwanedi-Luphephe Dams	**1**	*Lb. marequensis*	F	29	240	251.7	None	None	0
		2	*Lb. marequensis*	F	13.9	112	31.8	Eye	*Diplostomulum*	2
		3	*Lb. marequensis*	M	27	230	286.6	Eye	*Diplostomulum*	10
								Buccal cavity, fins and skin	*Dolops ranarum*	2
		4	*Lb. marequensis*	M	19	150	76.3	Eye	*Diplostomulum*	30
		5	*Lb. marequensis*	M	29.2	234	282.8	Gills	*D.spinicirrus*	3
								Gills	Digenea (black cysts)	3
								Eye	*Diplostomulum*	2
		6	*Lb. marequensis*	M	28	225	278.2	Eye	*Diplostomulum*	10
		7	*Lb. marequensis*	F	25	190	202.6	None	None	0
		8	*Lb. marequensis*	M	20	160	80.7	Eye	*Diplostomulum*	3
		9	*Lb. marequensis*	F	26.5	230	253.3	Gills	*D.spinicirrus*	4
								Eye	*Diplostomulum*	4
		10	*Lb. marequensis*	M	15	100	25.8	Eye	*Diplostomulum*	11
	Locality	**No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
	Nwanedi-Luphephe Dams	**1**	*B.radiatus*	M	3.6	29	0.5	None	None	0
		2	*B.radiatus*	F	3.5	30	0.8	Gills	*Afrodiplozoon polycotyleus*	2

		3	*B.radiatus*	F	3.9	29	0.6	Gills	*Afrodiplozoon polycotyleus*	1
		4	*B.radiatus*	M	3.4	27	0.5	None	*Gyrodactylus*	0
		5	*B.radiatus*	F	3.5	36	0.8	Gills	*Afrodiplozoon polycotyleus*	2
		6	*B.radiatus*	M	4.2	34	0.7	None	*Gyrodactylus*	0
		7	*B.radiatus*	M	4	34	1	None	*Gyrodactylus*	0
		8	*B.radiatus*	F	4	40	1.5	None	None	0
		9	*B.radiatus*	M	3.8	45	1.4	Gills	*Afrodiplozoon polycotyleus*	1
		10	*B.radiatus*	M	3.7	29	0.6	None	None	0
		11	*B.radiatus*	F	4	35	1	None	None	0
		12	*B.radiatus*	F	3.3	27	0.4	Gills	*Afrodiplozoon polycotyleus*	1
		13	*B.radiatus*	M	3.4	25	0.2	None	None	0
		14	*B.radiatus*	F	3.4	28	0.3	None	None	0
		15	*B.radiatus*	M	3.8	29	0.3	None	*Dogielius* sp.	3
		16	*B.radiatus*	F	3.2	30	0.7	None	*Dogielius* sp.	5
Season	**Locality**	**No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
2	Nwanedi-Luphephe Dams	**1**	*B.trimaculatus*	F	11.7	93	18.2	Gills	Echinostomes	75
		2	*B.trimaculatus*	F	11.2	95	18.2	B.cavity	Digenean cyst	8
								B.cavity	*Contracaecum* lavrae	1
		3	*B.trimaculatus*	M	12	97	22.7	None	None	0
		4	*B.trimaculatus*	F	11.2	98	25.7	None	None	0
		5	*B.trimaculatus*	M	11.2	93	19.6	Gills	*D.spinicirrus*	1
								Gills	Echinostomes	4
		6	*B.trimaculatus*	M	11	90	18.4	Gills	*D.spinicirrus*	2
								Eye	*Diplostomulum .*	4
		7	*B.trimaculatus*	F	12.5	100	23.9	None	None	0
Season		**8**	*B.trimaculatus*	M	11.5	92	18.5	Gills	*D.spinicirrus*	2
2								B.cavity	*Contracaecum* larvae	3
		9	*B.trimaculatus*	M	11	89	18.2	Eye	*Diplostomulum .*	1
		10	*B.trimaculatus*	M	11.5	95	19.6	B.cavity	*Contracaecum* larvae	4
		11	*B.trimaculatus*	F	11.7	93	18.2	Gills	*D.afrolongicornis alberti*	1
								Gills	Echinostomes	75
		12	*B.trimaculatus*	F	12.3	99	23	Gills	*D.afrolongicornis alberti*	1
		13	*B.trimaculatus*	F	11.2	93	15.8	Gills	*D.afrolongicornis alberti*	2

								B.cavity	Digenean larvae	35
		14	*B.trimaculatus*	F	12.4	100	28.4	None	None	0
		15	*B.trimaculatus*	M	11.3	91	18.3	Gills	*Afrodiplozoon polycotyleus*	2
								Gills	*D. afrolongicornis afrolongicornis*	8
								Gills	Echinostomes	7
								intestine	Digenean larvae	5
		16	*B.trimaculatus*	F	4.5	35	0.8	None	None	0
		17	*B.trimaculatus*	M	11.8	90	19.6	Gills	*D.afrolongicornis afrolongicornis*	3

Season	**Locality**	**No**	**Species**	**Sex**	**TL (cm)**	**SL(mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
3	Nwanedi-Luphephe Dams	**1**	*Lb. marequensis*	F	32.3	265	539.5	Gills	*Dactylogyrus spinicirrus.*	7
								muscle	u/d digenean cyst	500
								Eyes	*Diplostomulum*	6
		2	*Lb. marequensis*	F	59	588	2257.3	Gills	*Dactylogyrus spinicirrus*	1
								Eyes	*Diplostomulum*	16
		3	*Lb. marequensis*	M	46	380	1412.5	Gills	*Dactylogyrus spinicirrus*	2
								Gills	Digenean cysts	4
								Eyes	*Diplostomulum*	20
								B.cavity	*Contracaecum*	3
		4	*Lb. marequensis*	F	34	280	495.6	Gills	*Dactylogyrus spinicirrus*	5
								Eye	*Diplostomulum*	11
		5	*Lb. marequensis*	M	31.5	260	402.6	Gills	Digenean cysts	9
								mesentry fats	Digenean cysts	6
								Eye	*Diplostomulum*	9
		6	*Lb. marequensis*	M	33	270	493.4	Eye	*Diplostomulum*	15
		7	*Lb. marequensis*	M	31	240	339.2	Gills	*Dactylogyrus spinicirrus*	4
								Eye	*Diplostomulum*	9
		8	*Lb. marequensis*	M	31	250	391.4	Gills	*Dactylogyrus spinicirrus*	10
								Eye	*Diplostomulum*	5
		9	*Lb. marequensis*	M	29	280	300	Gills	*Dactylogyrus spinicirrus*	1
								Eye	*Diplostomulum*	4

								Gills	*Afrodiplozoon polycotyleus*	2
		10	*Lb. marequensis*	F	34	280	483.6	muscle	Digenean cysts	3
								Eye	*Diplostomulum*	2
								B.cavity	Digenean (larvae)	9
	Locality	**No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
	Nwanedi-Luphephe Dams	**1**	*B.radiatus*	M	4.5	39	1.5	Gills	*Afrodiplozoon polycotyleus*	1
		2	*B.radiatus*	F	4	55	4	Gills	*Afrodiplozoon polycotyleus (larva)*	2
		3	*B.radiatus*	F	4.5	55	4.9	None	None	0
		4	*B.radiatus*	M	3.9	58	4	None	None	0
Season		**5**	*B.radiatus*	F	9	71	9.5	Gills	*Afrodiplozoon polycotyleus*	2
3									*Dactylogyrus spinicirrus*	4
		6	*B.radiatus*	M	8.1	68	8.1	Gills	*Afrodiplozoon polycotyleus*	2
								Gills	Digenean cysts	3
		7	*B.radiatus*	M	8	72	6.9	Gills	*Afrodiplozoon polycotyleus (larva)*	2
		8	*B.radiatus*	F	7.8	59	5	Gills	*Afrodiplozoon polycotyleus*	2
		9	*B.radiatus*	M	6.8	59	5	Gills	*Afrodiplozoon polycotyleus*	1
								Gills	Digenean cysts	1
		10	*B.radiatus*	M	4	56	4.6	Gills	*Afrodiplozoon polycotyleus*	2
								Gills	Digenean cysts	3
Season	**Locality**	**No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
3	Nwanedi-Luphephe Dams	**1**	*B.trimaculatus*	F	12	95	20.2	Gills	*Dactylogyrus spinicirrus*	3
		2	*B.trimaculatus*	M	12	92	18.2	Gills	*Dactylogyrus spinicirrus*	2
								Gills	*Afrodiplozoon polycotyleus*	2
		3	*B.trimaculatus*	F	11.9	99	22.7	Gills	*D. afrolongicornis afrolongicornis*	5
		4	*B.trimaculatus*	F	12.5	100	25.2	Gills	*D. afrolongicornis alberti*	1
		5	*B.trimaculatus*	F	12.1	92	21.5	Gills	*D. afrolongicornis afrolongicornis*	2
		6	*B.trimaculatus*	M	4.8	50	2.8	Gills	*Dactylogyrus spinicirrus*	1
		7	*B.trimaculatus*	M	4.3	45	2.5	Gills	*Afrodiplozoon polycotyleus*	2
		8	*B.trimaculatus*	F	4	47	2.7	Gills	Digenean cysts	1
		9	*B.trimaculatus*	M	4.6	46	2.5	Gills	*D. afrolongicornis alberti*	3
		10	*B.trimaculatus*	F	12.3	103	29	Gills	*D. afrolongicornis afrolongicornis*	4

								Gills	Digenean cysts	6

Season	Locality	Fish No	Species	Sex	TL (cm)	SL(mm)	Weight (g)	Site	Parasites	No
4	Nwanedi-Luphephe Dams	**1**	*Lb. marequensis*	M	32.5	270	419.3	Gills	*Dactylogyrus spinicirrus.*	12
								Gills	*D. afrolongicornis alberti*	3
								B. cavity	u/d digenean cyst	300
								Eyes	*Diplostomulum*	30
		2	*Lb. marequensis*	F	47.1	394	1627.5	Gills	*Dactylogyrus spinicirrus*	5
								Body cavity	*Contracaecum*	17
								Body cavity	u/d digenean cyst	5
								Eyes	*Diplostomulum*	30
		3	*Lb. marequensis*	F	49.1	401	1629.1	Gills	*Dactylogyrus spinicirrus*	12
								Gills	Digenean cysts	4
								Eyes	*Diplostomulum*	25
								Body cavity	*Contracaecum*	300
		4	*Lb. marequensis*	F	46.8	388	495.6	Gills	*Dactylogyrus spinicirrus*	6
								Body cavity	*Contracaecum*	288
								Eye	*Diplostomulum*	25
		5	*Lb. marequensis*	F	46.5	383	1504.3	Gills	*Dactylogyrus spinicirrus*	7
								Body cavity	*Contracaecum*	251
								Eye	*Diplostomulum*	17
		6	*Lb. marequensis*	F	46.5	374	1420.3	Gills	*D. spiniccirus*	8
								Eye	*Diplostomulum*	19
								Body cavity	*Contracaecum*	25
								Buccal cavity, fins and skin	*Dolops ranarum*	4
		7	*Lb. marequensis*	M	44.1	353	1100.5	Gills	*Dactylogyrus spinicirrus*	10
								Eye	*Diplostomulum*	10

								Body cavity	*Contracaecum*	33
								Muscle	Digenean cysts	34
		8	*Lb. marequensis*	M	44.1	353	1180.5	Gills	*Dactylogyrus spinicirrus*	8
								Eye	*Diplostomulum*	24
								Body cavity	*Contracaecum .*	40
								Muscle	Digenean cysts	12
								Buccal cavity, fins and skin	*Dolops ranarum*	3
		9	*Lb. marequensis*	M	48.5	392	1445.5	Gills	*Dactylogyrus spinicirrus*	3
								Eye	*Diplostomulum*	17
								Body cavity	*Contracaecum*	10
		10	*Lb. marequensis*	F	52.1	452	1525.5	Gills	*Dactylogyrus spinicirrus*	7
								Eye	*Diplostomulum*	13
								Body cavity	*Contracaecum .*	36
		11	*Lb. marequensis*	F	48.4	390	1435.2	Gills	*Dactylogyrus spinicirrus*	13
								Eye	*Diplostomulum*	8
								Body cavity	*Contracaecum .*	26
		12	*Lb. marequensis*	F	46.4	376	1295.1	Gills	*Dactylogyrus spinicirrus*	6
								Eye	*Diplostomulum*	15
								Body cavity	*Contracaecum .*	50
		13	*Lb. marequensis*	F	47.5	389	1546.3	Gills	*Dactylogyrus spinicirrus*	4
								Eye	*Diplostomulum*	21
								Body cavity	*Contracaecum .*	24
		14	*Lb. marequensis*	F	62.2	546	1355.5	Gills	*Dactylogyrus spinicirrus*	8
								Eye	*Diplostomulum*	6
								Body cavity	*Contracaecum .*	7
								Buccal cavity, fins and skin	*Dolops ranarum*	5
		15	*Lb. marequensis*	F	46.4	386	1443.1	Gills	*Dactylogyrus spinicirrus*	5
		16	*Lb. marequensis*	F	51.1	433	1587.1	Gills	*Dactylogyrus spinicirrus*	8
								Eye	*Diplostomulum*	24

								Body cavity	*Contracaecum .*	34
		17	*Lb. marequensis*	F	47.9	399	1476.7	Gills	*Dactylogyrus spinicirrus*	9
								Eyes	*Diplostomulum*	12
								Body cavity	*Contracaecum .*	33
		18	*Lb. marequensis*	F	45	377	1245.6	Gills	*Dactylogyrus spinicirrus*	15
								Eye	*Diplostomulum*	29
								Body cavity	*Contracaecum*	54
Season	**Locality**	**No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (cm)**	**Site**	**Parasites**	**No**
4	Nwanedi-Luphephe Dams	**1**	*B.radiatus*	M	5.1	42	1	Gills	None	0
		2	*B.radiatus*	M	4.9	41	0.9	Gills	*Afrodiplozoon polycotyleus*	1
		3	*B.radiatus*	F	4.3	34	1.1	None	None	0
		4	*B.radiatus*	M	3.9	32	0.9	None	*Afrodiplozoon polycotyleus*	1
		5	*B.radiatus*	M	4.5	38	1	Gills	Gyrodactylus spp.	1
		6	*B.radiatus*	F	4.4	36	1.2	Gills	*Afrodiplozoon larvae*	1
								Gills	*Afrodiplozoon polycotyleus*	1
		7	*B.radiatus*	M	4.5	39	0.7	Gills	None	0
		8	*B.radiatus*	F	7.8	59	6	Gills	None	0
		9	*B.radiatus*	F	6.3	49	2.8	None	None	0
		10	*B.radiatus*	M	5.5	42	1.6	Gills	*Afrodiplozoon polycotyleus*	1
Season	**Locality**	**No**	**Species**	**Sex**	**TL (cm)**	**SL (mm)**	**Weight (g)**	**Site**	**Parasites**	**No**
4	Nwanedi-Luphephe Dams	**1**	*B.trimaculatus*	F	12.6	100	25.8	Gills	*D. afrolongicornis afrolongicornis*	1
								Gills	Digenean cysts (photo)	25
		2	*B.trimaculatus*	F	12.3	101	20.8	Gills	None	0
		3	*B.trimaculatus*	M	11.8	98	19.7	Gills	*D. afrolongicornis afrolongicornis*	5
								Gills	*Afrodiplozoon polycotyleus*	1
								Gills	Digenean cyst	45
		4	*B.trimaculatus*	F	12.1	104	18.7	Gills	*D. afrolongicornis alberti*	4
		5	*B.trimaculatus*	M	12.3	101	18.9	Gills	*D. afrolongicornis afrolongicornis*	1
		6	*B.trimaculatus*	M	11.5	107	18.2	Gills	*D. afrolongicornis alberti*	1
								Body cavity	*Contracaecum .*	4
		7	*B.trimaculatus*	F	11.1	102	16.7	Gills	*D. afrolongicornis alberti*	3

		8	*B.trimaculatus*	F	9.8	81	8.6	Gills	*Afrodiplozoon polycotyleus*	1
								Gills	Digenean cyst	20
								Body cavity	*Contracaecum*	6
		9	*B.trimaculatus*	M	4.6	38	1.3	Gills	*D. afrolongicornis alberti*	4
		10	*B.trimaculatus*	M	4.3	33	0.9	Gills	Digenean cyst	10
								Body cavity	*Contracaecum*	5
		11	*B.trimaculatus*	F	11.7	98	20.1	Gills	*D. afrolongicornis alberti*	8
		12	*B.trimaculatus*	F	12.6	103	22.1	Gills	*D. afrolongicornis afrolongicornis*	6
		13	*B.trimaculatus*	M	10.2	86	16.6	Gills	*D. afrolongicornis alberti*	5
		14	*B.trimaculatus*	F	11.4	92	18.8	Gills	*D. afrolongicornis alberti*	5
								Gills	Digenean cyst	9
		15	*B.trimaculatus*	F	12.8	100	25.2	Gills	*D. afrolongicornis afrolongicornis*	5
		16	*B.trimaculatus*	F	11.9	96	19.7	Gills	*Afrodiplozoon polycotyleus*	1
		17	*B.trimaculatus*	M	12.4	104	17.6	Gills	*Afrodiplozoon polycotyleus*	1
		18	*B.trimaculatus*	F	10.6	85	16.4	Gills	*D. afrolongicornis alberti*	4
		19	*B.trimaculatus*	F	11.8	91	18.8	Gills	*D. afrolongicornis alberti*	5
								Gills	Digenean cyst	13

Appendix 3

Table 1
Health Assessment record card used to document the condition of fish (*Labeobarbus marequensis*)
Autumn season- April 2008: Nwanedi-Luphephe Dams (Limpopo River System)
Season One

Fish	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Mesentery fats	Hindgut	Bile	Kidney	Invert Endo para sites	Invert Ecto Para sites	Index value (c)	Condition factor		
															L	**M**	**CF**
															mm	g	
1	0	0	0	0	0	30	0	0	0	1	0	0	20	50	343	916.4	2.27
2	0	0	0	0	0	30	0	0	0	1	0	0	10	40	400	1470	2.30
3	0	0	0	0	0	30	0	2	0	1	0	0	20	50	380	1257	2.29
4	0	0	0	0	0	30	0	4	0	1	0	10	20	60	412	1513	2.16
5	0	0	0	0	0	30	0	0	0	3	0	0	20	50	312	723.6	2.38
6	0	0	0	0	0	30	0	2	0	2	0	0	20	50	270	368	1.87
7	0	0	0	0	0	30	0	0	0	1	0	0	30	60	340	900.8	2.29
8	0	0	0	0	0	30	0	1	0	3	0	0	10	40	410	1553	2.25
9	0	0	0	0	0	30	0	1	0	2	0	0	10	40	391	1358	2.27
10	0	0	0	0	0	30	0	1	0	2	0	0	30	60	390	1425	2.40
11	0	0	0	0	0	30	0	1	0	1	0	0	30	60	355	959.8	2.15
12	0	0	0	0	0	30	0	2	0	1	0	0	30	60	394	1264	2.07
13	0	0	0	0	0	30	0	1	0	1	0	0	10	40	329	834.8	2.34
14	0	0	0	0	0	30	0	1	0	1	0	0	10	40	360	97.2	0.21
15	0	0	0	0	0	30	0	1	0	2	0	0	30	60	320	83.6	0.26
Average												**0.667**	**20**	**50.67**			**1.97**
Standard deviation												**2.6**	**8.5**	**8.8**			**0.72**

Table 2
Health Assessment record card used to document the condition of fish (*Labeobarbus marequensis*)
Winter Season- July 2008: Nwanedi-Luphephe Dams (Limpopo River System)
Season Two

Fish	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Mesentery fats	Bile	Hindgut	Kidney	Invert Endo Para sites	Invert Ecto Para Sites	Index value C	Condition factor		
															SL	**M**	**CF**
															Mm	g	
1	0	0	0	0	0	30	0	1	3	0	0	0	30	60	240	251.7	1.82
2	0	0	0	0	0	30	0	0	0	0	0	0	30	60	112	31.8	2.26
3	0	0	0	0	0	30	0	0	3	0	0	0	30	60	230	286.6	2.36
4	0	0	0	0	30	0	0	0	0	0	0	0	30	60	150	76.3	2.26
5	0	0	0	0	30	30	0	3	3	0	0	0	20	80	234	282.8	2.21
6	0	0	0	0	30	30	0	3	3	0	0	0	30	90	225	278.2	2.44
7	0	0	0	0	0	0	0	0	0	0	0	0	20	20	190	202.6	2.95
8	0	0	0	0	30	30	0	3	3	0	0	0	20	80	160	80.7	1.97
9	0	0	0	0	30	30	0	3	3	0	0	0	20	80	230	253.3	2.08
10	0	0	0	0	30	0	0	0	0	0	0	0	10	40	100	52.8	2.28
Average												**0**	**24**	**63**			**2.56**
Standard deviation												**0.0**	**7.0**	**21.1**			**1.00**

Table 3
Health Assessment record card used to document the condition of fish (*Labeobarbus marequensis*)
Spring season- October 2008: Nwanedi-Luphephe Dams (Limpopo River System)
Season Three

Fish	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Mesentery	Bile	Hindgut	Kidney	Invert Endo Para Sites	Invert Ecto Para sites	Index value (c)	Condition factor		
															SL	**M**	**CF**
															mm	g	
1	0	0	0	0	0	0	0	0	0	0	0	10	20	30	265	539.5	2.90
2	0	0	0	0	0	0	0	0	0	0	0	0	10	10	588	2257	1.11
3	0	0	0	0	0	0	0	3	0	0	0	0	20	20	380	1413	2.57
4	0	0	0	0	0	30	0	3	0	0	0	0	20	50	280	495.6	2.26
5	0	0	0	0	0	0	0	0	0	0	0	0	30	30	260	402.6	2.29
6	0	0	0	0	0	0	0	3	0	0	0	0	30	30	270	493.4	2.51
7	0	0	0	0	0	0	0	3	0	0	0	0	20	20	240	339.2	2.45
8	0	0	0	0	0	30	0	3	0	0	0	0	20	50	250	391.4	2.50
9	0	0	0	0	0	30	0	0	0	0	0	0	20	50	200	329.1	4.11
10	0	0	0	0	0	30	0	3	2	0	0	0	10	40	280	483.6	2.20
Average												**1**	**20**	**33**			**2.49**
Standard deviation												**3.2**	**6.7**	**14.2**			**0.74**

Table 4
Health Assessment record card used to document the condition of fish (*Labeobarbus marequensis*)
Spring season- October 2008: Nwanedi-Luphephe Dams (Limpopo River System)
Season Four

Fish	Eyes	Skin	Fins	Oper-cules	Gills	Liver	Spleen	Mesentery	Bile	Hindgut	Kidney	Invert Endo para sites	Invert Ecto Para sites	Index value (c)	Condition factor		
															SL	**M**	**CF**
															mm	g	
1	0	0	0	0	0	0	0	0	0	0	0	10	10	20	270	419.3	2.13
2	0	0	0	0	0	0	0	0	0	0	0	0	20	20	394	1628	2.66
3	0	0	0	0	0	0	0	3	0	0	0	10	10	20	401	1629	2.53
4	0	0	0	0	0	30	0	3	0	0	0	10	20	60	388	495.6	0.85
5	0	0	0	0	0	0	0	0	0	0	0	10	20	30	383	1504	2.68
6	0	0	0	0	0	0	0	3	0	0	0	0	20	20	374	1456	2.78
7	0	0	0	0	0	0	0	3	0	0	0	0	20	20	353	1346	3.06
8	0	0	0	0	0	30	0	3	0	0	0	0	20	50	353	1346	3.06
9	0	0	0	0	0	30	0	0	0	0	0	0	20	50	392	1446	2.40
10	0	0	0	0	0	30	0	3	2	0	0	0	20	50	452	1526	1.65
11	0	0	0	0	30	30	0	3	3	0	0	0	20	80	390	1435	2.42
12	0	0	0	0	30	0	0	0	0	0	0	0	20	50	376	1295	2.44
13	0	0	0	0	30	0	0	0	0	0	0	0	20	50	389	1546	2.63
14	0	0	0	0	30	0	0	0	0	0	0	0	20	50	546	1356	0.83
15	0	0	0	0	0	30	0	0	3	0	0	0	20	50	386	1443	2.51
16	0	0	0	0	30	0	0	0	0	0	0	0	20	50	433	1587	1.95
17	0	0	0	0	0	30	0	0	0	0	0	0	20	50	399	1477	2.32
18	0	0	0	0	0	30	0	3	2	0	0	0	20	50	377	1246	2.32
Average												**2.222**	**18.89**	**42.78**			**2.38**
Standard deviation												**4.3**	**3.2**	**17.1**			**0.68**