DIVERSITY OF FRESHWATER FISH PARASITES AND WATER QUALITY OF THE KWENA DAM, MPUMALANGA PROVINCE, SOUTH AFRICA

by

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DISSERTATION

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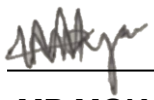
DECLARATION

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



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ABSTRACT

The present study was carried out in the Kwena Dam, with the aim of determining selected water quality parameters, parasite diversity and condition factor (K) of *Clarias gariepinus* (sharptooth catfish), *Cyprinus carpio* (common carp) and *Oreochromis mossambicus* (Mozambique tilapia). This study was conducted in autumn (April 2016), winter (July 2016), spring (October 2016) and summer (February 2017). The present study was the first to investigate the parasite composition of these three fish species in the Kwena Dam.

A total number of 26 *Clarias gariepinus*, 21 *Cyprinus carpio* and 57 *O. mossambicus* specimens were collected using gill nets of different mesh sizes (30 mm – 120 mm). Each fish was weighed, measured and euthanised by severing the spinal cord. Mucus smears from the skin, fins and gills were examined for ectoparasites using a stereo-microscope. The fish were then dissected and all organs examined for endoparasites. All parasites were fixed and preserved according to standard methods for each parasite group. *In situ* water parameters were determined using a handheld multi-parameter instrument for each sampling season. In addition, water samples were collected seasonally and sent to an accredited laboratory where they were analysed for selected metals and nutrients.

Water quality parameters and the presence of metals in water are of importance in determining the water quality of an aquatic environment. Most water quality parameters were within the Target Water Quality Range (TWQR) for aquatic ecosystems. Aluminium, selenium and zinc had concentrations above the TWQR for aquatic ecosystems. Nutrient concentrations were within the TQWR during all sampling seasons. The water quality did not differ significantly between seasons during the present study.

Four parasite groups were reported infecting *Cyprinus carpio* and these included Monogenea (*Dactylogyrus extensus* and *Dactylogyrus minutus*), Digenea (*Diplostomum* sp.), Cestoda (*Atractolytocestus huronensis*), Branchiura (*Argulus japonicus*) and Copepoda (*Neoergasilus japonicus*). Parasites collected from *Clarias gariepinus* belonged to four groups, namely Protozoa (*Trypanosoma* sp.), Monogenea (*Quadriacanthus* sp. *and Gyrodactylus* sp.), Nematoda (*Paracamallanus cyathopharynx* and *Contracaecum* sp.) and Branchiura (*Dolops ranarum*). Parasites collected from *O. mossambicus* belonged to five groups, namely Monogenea (*Cichlidogyrus halli*, *Cichlidogyrus sclerosus*, *Cichlidogyrus tilapiae* and *Enterogyrus conoratus*), Nematoda (*Contracaecum* sp.), Cestoda (*Neogryporhynchus* sp.), Acanthocephala (*Acanthogyrus tilapiae*) and Branchiura (*Dolops ranarum*).

The number of parasite species for the four seasons were as follows: summer (13) > autumn and winter (12) > spring (11). From the Shannon-Wiener index results, *O. mossambicus* had a higher parasite diversity than *Clarias gariepinus* and *Cyprinus carpio*. The Parasite Index (IP) and Inverted Parasite Index (IPI) of the three fish species indicated that the water from the dam is not polluted. The condition factor (K) for all fish species indicated that fish collected from the dam during all sampling seasons were in a good condition and parasite load had little effect on K for all fish species. The use of PI and IPI in conjunction with the fish K can be regarded as a useful tool in freshwater and fish health monitoring.

The present results report new geographical records of the parasites of *Clarias gariepinus*, *Cyprinus carpio* and *Oreochromis mossambicus*. Since no parasitological research was done before the present study at the Kwena Dam, the results of the present study form baseline data for future parasitology studies and can consequently be useful in the management and conservation of the Kwena Dam.

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LIST OF ABBREVIATIONS AND ACRONOMY

ANOVA	Analysis of Variance
DO	Dissolved Oxygen
DWAF	Department of Water Affairs
EC	Electrical Conductivity
PI	Parasite Index
IPI	Inverted Parasite Index
K	Condition Factor
RHP	River Health Programme
SANAS	South African National Accreditation System
SAWQG	South African Water Quality Guidelines
SD	Standard Deviation
TDS	Total Dissolved Solids
TL	Total Length
TWQR	Target Water Quality Range
UL	University of Limpopo
UNESCO	United Nations Educational, Scientific and Cultural Organization
WHO	World Health Organisation

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

South Africa experiences relatively high temperatures and seasonal rainfall over a greater part of the country, making freshwater resources scarce and limited, resulting in the country being categorised as a water-stressed country (Dallas & Day 2004; Nare *et al*. 2011). Freshwater ecosystems are important to human societies as they provide them with goods, services and water of good quality has been demonstrated to be crucial for sustainable socio-economic development (Bartram & Ballance 1996; Geist 2011). Growth of human populations has put extreme pressure on South Africa's freshwater systems, both as sources of water and in terms of pollution, and yet water is of no use to humans, or for the maintenance of natural riverine ecosystems if its quality is poor. Therefore the management of water quality and water availability is essential (Dallas & Day 2004).

Water quality is affected by a wide range of natural processes (changes in the precipitation inputs, erosions, weathering of crustal materials) and anthropogenic influences (urbanisation, industrialisation and agricultural activities) (Meybeck *et al.*1996; Simeonov *et al.* 2003; Singh *et al*. 2005). An increase in human activities to meet rapidly growing demands for food and freshwater have resulted in an array of extensive changes to freshwater ecosystems. These changes have resulted in irreversible loss in biodiversity and degradation of ecosystem services (Roux *et al*. 2008; Lagrue & Poulin 2015).

Pollution of rivers by anthropogenic activities has become a threat to water resources and its biodiversity (Schulz & Schoonbee 1999). The addition of pollutants by humans changes the chemical composition, temperature or microbial composition of water to an extent that harm is caused to humans and resident animals (Heath 1995). According to Weale (1992), water is regarded polluted when it is impaired by contaminants and it loses its ability to support its constituent biotic communities and humans.

To monitor the effects of pollution, a variety of sampling techniques to quantify the health of aquatic ecosystems are used (Chapman *et al.* 2015). In South Africa, the quality of water was normally determined by measuring chemical and physical variables of the water (Roux *et al*.

1994). Using these methods can give accurate measures of the amounts of individual substances in the water, but the methods only consider the water passing at the moment of collection (Bertasso 2004). Biomonitoring has been proven to be an alternative monitoring technique. Biomonitoring is defined as the systematic use of living organisms, or their responses, to determine the condition of the environment. This method is used to observe the impacts of external factors on the ecosystem and how they develop over a period of time (Li *et al*. 2010). Biomonitoring is based on the fact that different organisms have different tolerance levels to pollution, thus the presence or absence of sensitive organisms, or a change in community composition, can indicate a change in water chemistry that may not be detected by the physicochemical data record (Palmer *et al.* 2004; Bonada *et al*. 2006).

In South Africa, The River Health Programme (RHP), which is practiced in many parts of the country, uses biomonitoring methods (Palmer *et al*. 2004). The RHP was introduced in 1994 by the South African Department of Water Affairs (RHP 2006). The programme was designed to generate information needed regarding the ecological conditions of the riverine ecosystem in South Africa, with the overall goal to expand the ecological basis of information on aquatic resources in order to support the rational management of the system (Roux *et al*. 1993).

Bioindicators are organisms that can integrate and reflect the effects of physicochemical parameters over an extended period of time (Palmer *et al*. 2004). Bioindicators are differentially sensitive to environmental stressors and are therefore suitable tools for biomonitoring programmes (Justus *et al*. 2010). According to Rosenberg and Resh (1993), an indicator organism should at least have the following characteristics: (1) Taxonomic soundness, (2) low mobility, (3) well-known ecological characteristics, (4) wide distribution, (5) high sensitivity to environmental stressors, (6) numerical abundance, (7) suitability for laboratory experiments and (8) high ability for quantification and standardisation. Aquatic biota experiences the cumulative results of all chemical interactions that affect them. If chemical conditions of the water are favourable, they will survive; but if the water chemical conditions are above their tolerance limits, they will diminish or disappear. Plants, algae, invertebrates and fish can be monitored to assess ecosystem health (Palmer *et al*. 2004).

Fish are regarded as representative indicators of overall system health because they are near the top of the food chain (Adams *et al*. 1993; Palmer *et al*. 2004). Fish integrate the effects of many biotic and abiotic variables acting in the aquatic environments and reflect secondary impacts of chronic stress mediated through the food chain. In their natural environments, fish are exposed to numerous stressors including unfavourable or fluctuating temperatures, high

water velocities and sediment loads, low concentration of dissolved oxygen and limited food availability (Adams *et al*. 1993). Fish also serve as hosts to a wide range of ecto- and endoparasites, which can on their own, also be used as indicators of environmental health. According to Blanar *et al*. (2009), there is an increased interest in the use of parasites as pollution indicators. In South Africa, the interest in the use of parasites as pollution indicators is supported by publications by Madanire-Moyo and Barson (2010), Madanire-Moyo *et al*. (2012), Gilbert and Avenant-Oldewage (2016) and Gilbert and Avenant-Oldewage (2017) just to mention a few.

The diversity of parasites provides insights into the history, biogeography and ecology of their hosts, the structure of ecosystems, and the processes behind the diversification of life (Poulin & Morand 1999). The diversity patterns of parasites may be associated with either parasite or host characteristics and parasite diversity does not reflect the species diversity of the host taxa. Factors such as life history and ecological characteristics of hosts play an important role (Poulin & Morand 2000). Parasites can affect the survival of their host and can have a substantial impact on the biodiversity of an ecosystem (Madanire-Moyo *et al*. 2012).

Parasitism is affected by the chemical conditions of their environment. Pollution can increase parasitism if the defence mechanisms of the host are negatively affected. However, pollution can also decrease parasitism if parasites themselves are susceptible to pollutants or if their intermediate and final hosts are extinct due to pollution (Hanzelova *et al*. 2010).

The presence, and consequently the number, of parasites form the basis of the Parasite Index (PI) in the Health Assessment Index (HAI) (Avenant-Oldewage *et al.* 1995; Jooste *et al.* 2003; 2005; Sara *et al*. 2014). Given its relevance in environmental monitoring, the original HAI was expanded and developed the PI. The PI was tested in conjunction with the HAI in South Africa based on the premise that contaminants have different influences on endo- and ectoparasites (Marx 1996; Robinson 1996; Luus-Powell 1997; Watson 2001). The PI is used to distinguish between the number of ecto- and endoparasites present while the Inverted Parasite Index (IPI) for ectoparasites has been developed on the basis that high numbers of ectoparasites indicate good water quality and has been applied by Crafford and Avenant-Oldewage (2009) on *Clarias gariepinus* in the Vaal River System. In good water quality, 10 to 20 ectoparasites can be expected, but the count can drop to two, one or zero if the water quality is poor depending on the type of pollution (Avenant-Oldewage 1998).

To assess the health of fish in relation to their environment, various approaches such as age, growth analysis and condition factor (K) are used (Adams *et al.* 1993). The relationship between fish weight and length can be used to compare the effect of biotic and abiotic factors on the health or well-being of a fish population (Blackwell *et al.* 2000). The K compares the wellbeing of fish and is based on the hypothesis that heavier fish may be indicators of favourable environmental conditions (e.g. habitat conditions, ample prey availability), whereas lean fish may indicate less favourable environmental conditions (Blackwell *et al*. 2000; Abowei 2009). Drops in K may indicate a reproductive period and/or changes in the foraging habits of certain species (Gomiero & Braga 2005).

1.2 Motivation of study

Aquatic ecosystems experience high anthropogenic stress resulting from environmental degradation (Palm & Rucket 2009). The Kwena Dam is situated in the Crocodile River catchment in the Mpumalanga Province of South Africa. The Crocodile River is used for agricultural, mining, domestic and industrial activities. These activities may pose a threat to the health of the river due to abstraction and discharging chemicals to the catchment (DWAF 1995). The lower reaches of the Crocodile River is considered to have poor water quality (Kleynhans 1999). The river is becoming dangerous to be used for watering of crops and swimming, and the degradation of water quality may also cause a change in the plant, invertebrate and fish communities in the river (Rainhaverst 2012).

No study has been done previously on the parasite diversity of *Cyprinus carpio* Linnaeus, 1758, *Clarias gariepinus* Burchell, 1822 and *Oreochromis mossambicus* Peters, 1852 in the Kwena Dam. The current study is therefore the first in South Africa to determine the parasite composition of the three selected fish species and to determine the influence of pollution on parasite diversity at The Kwena Dam. The three fish species were selected for this present study based on their abundance at the Kwena Dam and the different trophic levels they utilise.

1.3 Purpose of study

1.3.1 Aim

The aim of this study was to determine selected water quality parameters, parasite diversity and the K of *Clarias gariepinus*, *Cyprinus carpio* and *Oreochromis mossambicus* from the Kwena Dam.

1.3.2 Objectives

The specific objectives of the study were to:

i. Determine concentrations of selected metals and nutrients seasonally.

ii. Determine the parasite species composition, diversity, prevalence, mean intensity and mean abundance of parasites from selected fish species seasonally.

iii. Describe and classify the parasites associated with selected fish species during different seasons.

iv. Determine and compare PI and IPI for each host.

v. Determine K of the three fish species.

1.4 Research questions

i. Does water quality affect the composition of fish parasite communities?

ii. Does water quality differ seasonally?

iii. During which of the four seasons was a higher parasite diversity recorded?

iv. Does season have an influence on the K of three fish species?

v. Is the use of IP and IPI in conjunction with the K useful in freshwater ecosystem health monitoring?

1.5 Dissertation layout

In order to achieve the aim and objectives of the current study, the dissertation is structured as follows:

Chapter one (General introduction and purpose of the study), includes the background, motivation and purpose of the study. It also contains research questions.

Chapter two (Study area, host species, materials and methods), gives a detailed description of the study area and the selected fish species. Materials and methods for collection of fish and parasites, fixation, staining and preservation of parasites are also highlighted here. Furthermore, it explains the methods used for water quality analysis and analysis of the data.

Chapter three (Water quality analysis), provides water quality results, the discussion and conclusion of these aspects.

Chapter four (Parasite diversity of *Clarias gariepinus, Cyprinus carpio* and *Oreochromis mossambicus*), contains the results, discussion and conclusion for parasites recorded for the three species. The prevalence, mean intensity and mean abundance of parasites are also included in this chapter.

Chapter five (Parasite indices and condition factor of *Clarias gariepinus*, *Cyprinus carpio* and *Oreochromis mossambicus*), gives an overview of the correlation of parasite burden to the condition of the three fish species.

Chapter six (General conclusion and recommendations), summarises the overall results. Conclusions and recommendations for future studies are included in this chapter.

CHAPTER 2

STUDY AREA, HOST SPECIES, MATERIALS AND METHODS

2.1 Study area

Four sampling surveys (autumn April 2016, winter July 2016, spring October 2016 and summer February 2017) were conducted at the Kwena Dam (25°21'45"S 30°22'30"E) (Figure 2.1 – 2.3). The dam is situated close to Mashishing (Lydenburg) in Mpumalanga Province of South Africa. The dam is the only major storage dam in the Crocodile River catchment. It commands about 10% of the catchment's runoff with a storage capacity of 159 million cubic meters. The Crocodile River originates north of Dullstroom in the western parts of the catchment area and flows through mountainous terrain into the grasslands of the Lowveld. The river is slow flowing with an average width of 45 m and a low gradient. Rainfall varies from over 1200 mm to as low as 400 mm per annum in the lower eastern part of the catchment. The catchment is dominated by agricultural, industrial and mining activities. These activities are the predominant users of water in this catchment (DWAF 2004). A variety of water birds and domestic animals are found during all seasons around the dam. Local community members use the dam for subsistence fishing (Figure 2.3).

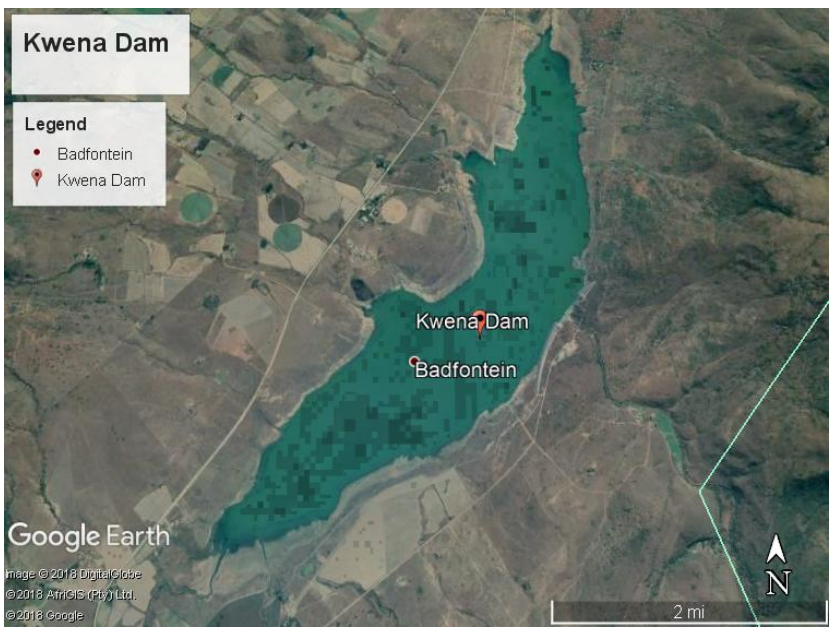


Figure 2.1: The Kwena Dam satellite image (Google Earth).

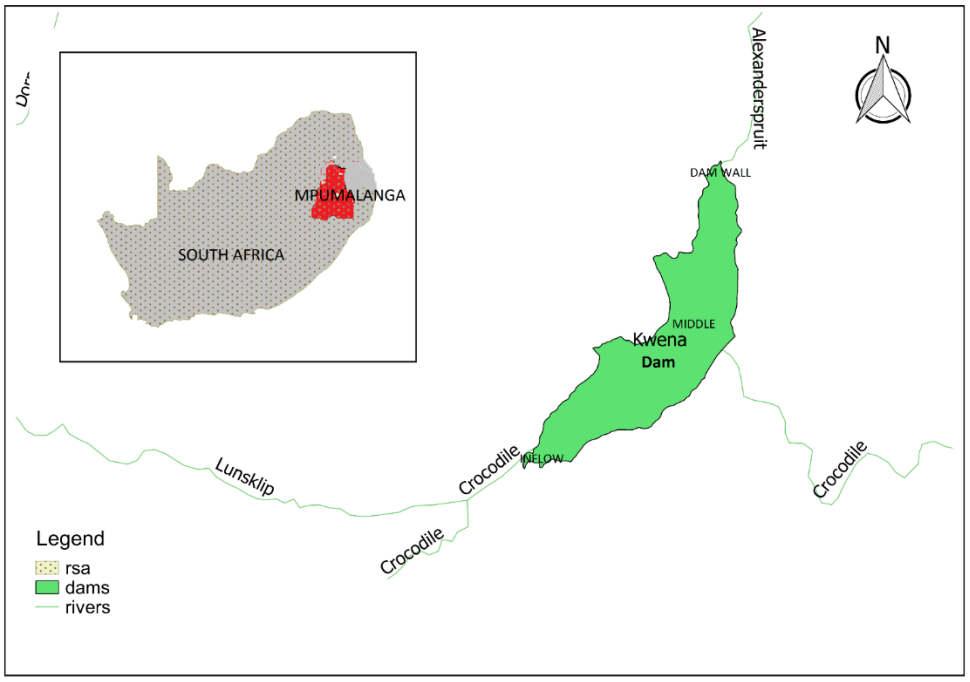


Figure 2.2: The location of the Kwena Dam situated in Mpumalanga Province, South Africa.

Figure 2.3: The Kwena Dam during autumn (April 2016).

Figure 2.4: A local resident fishing at the Kwena Dam.

2.2 Selected fish species

2.2.1 *Clarias gariepinus* Burchell, 1822

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Clariidae

The Sharptooth catfish (Figure 2.5) is probably the most widely distributed fish species in Africa. It is mostly used for angling and also serves as an important food fish species. It occurs in almost any habitat but favours floodplains, large sluggish rivers, lakes and dams. It can be tolerant to harsh conditions, such as high turbidity or desiccation, and is frequently the last or only inhabitant of diminishing pools and drying water sources. It is omnivorous and its breeding season is in summer after the rains. Eggs are laid on vegetation and hatch within 25 – 40 hours. It can take two to more years for individuals to reach maturity (Skelton 2001). It is listed as “Least Concern” on the Red Data List of Threatened Species by the International Union for Conservation of Nature (IUCN) due to its wide range and ubiquitous habitat demands (Freyhof *et al*. 2016).

Figure 2.5: The sharptooth catfish, *Clarias gariepinus* (from Skelton 2001).

2.2.2 *Cyprinus carpio* Linnaeus, 1758
Phylum: Chordata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

The Common carp (Figure 2.6) naturally occurs in central Asia and Europe. It is alien to Africa and was introduced in South Africa in the 1700s (Skelton 2001). This fish species is important for aquaculture and angling. It can tolerate a wide range of conditions but favours large water bodies with slow flowing or standing water and soft bottom sediments. They thrive in farm dams and large turbid rivers. This omnivorous fish feeds on a wide range of plant and animal matter. Females lay in excessive of a million eggs. Larvae hatch within four to eight days (Skelton 2001). It is listed as “Vulnerable” on the Red Data List of Threatened Species by IUCN.

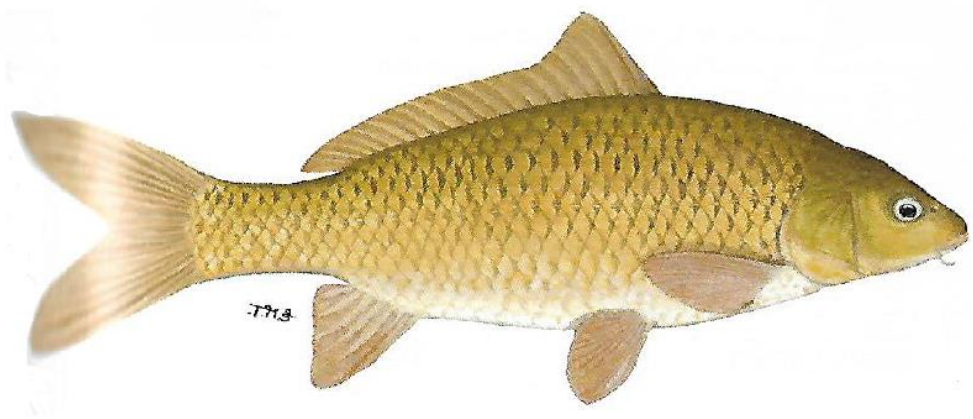


Figure 2.6: The common carp, *Cyprinus carpio* (from Skelton 2001).

2.2.3 *Oreochromis mossambicus* Peters, 1852

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Cichlidae

The Mozambique tilapia (Figure 2.7) is widely used in aquaculture as well as commercial and subsistence purposes. According to the IUCN, it is listed as “Near Threatened” on the Red Data List of Threatened Species and is likely to become locally extinct (Cambray & Swartz 2007), due to hybridisation and competition with the introduced Nile Tilapia, *Oreochromis niloticus* Linnaeus, 1758. This fish occurs in all but fast flowing waters, but thrives in standing waters. This species can tolerate fresh, brackish or marine waters. It prefers warm temperatures of above 22°C and can tolerate temperatures to about 42°C. It feeds on algae, diatoms and detritus, large individuals may feed on insects and other invertebrates. Breeding is in summer and females raise multiple broods every three to four weeks during a breeding season. Females mouth brood the eggs, larvae and small fry, and males construct a saucer-shaped nest on the sandy bottom. Juveniles shoal in shallows, grow rapidly, and may be mature enough to breed within a period of a year, but they are prone to stunting under adverse or crowded conditions (Skelton 2001).

Figure 2.7: The Mozambique tilapia, *Oreochromis mossambicus* (from Skelton 2001).

2.3 Water quality parameters

Surface water pH, electrical conductivity (EC), dissolved oxygen concentration (DO), oxygen saturation (%), total dissolved solids (TDS) and temperature were determined *in situ* using a handheld multi parameter instrument (Professional Plus). Water samples for physical and

chemical analysis were taken on a seasonal basis for four seasons in autumn (April 2016), winter (July 2016), spring (October 2016) and summer (February 2017). Subsurface water samples were collected in acid-treated sampling bottles. Water samples were immediately refrigerated and were sent to a South African National Accreditation System (SANAS) water analysis laboratory (Capricon Veterinary Laboratories cc) in Polokwane where they were analysed for aluminium, barium, boron, calcium, cobalt, copper, gallium, iron, magnesium, manganese, nickel, phosphorus, potassium, rubidium, selenium, silicon, silver, sodium, strontium, thorium, titanium, vanadium and zinc.

Subsurface water samples were collected at a depth of 30 cm in 1000 mℓ polyethylene bottles (acid pre-treated) and stored frozen at -5°C prior to the analyses of nutrients and a suite of metals, metalloids and anions at Capricorn Veterinary Laboratories, a SANAS accredited laboratory (V0014) in Polokwane. The water samples were analysed in batches with blanks using inductively coupled plasma-optical emission spectrophotometry (ICPOES; Thermofisher, iCAP 6300). Analytical accuracy was determined using certified standards (De Bruyn Spectroscopic Solutions) and recoveries were within 10% of certified values. Instances when nutrient data were not provided by the laboratory, the desired and relevant information was sourced from the Department of Water and Forestry website.

2.4 Sampling of fish

The permit to conduct this study was granted by the Mpumalanga Tourism and Parks Agency and the animal ethics clearance was granted the University of Limpopo Animal Ethics Committee.

Fish species were collected over four seasonal surveys in autumn, winter, spring and summer, as mentioned previously. Fish were caught using gill nets (Figure 2.8) of different mesh sizes (30 – 120 mm). Collected fish specimens were transported to the field laboratory and kept in aerated containers filled with dam water. The different fish species were kept separately.

Figure 2.8: Setting gill nets at the Kwena Dam.

2.5 Examination procedure

2.5.1 Examination for ectoparasites

Mucus smears were made by holding the fish firmly (Figure 2.9) and scraping the skin on both sides with glass slides. The slides were scrutinised for ectoparasites with the aid of a stereomicroscope (Leica EZ4). The body mass of each specimen was determined in grams (g) using an electronic balance (Salter Model 235E). Using a calibrated measuring board, the total length (TL), standard length (SL) and fork length (FL) (the latter only for *Cyprinus carpio*) of each specimen was determined in millimeters (mm). Fish were euthanised by severing the spinal cord just behind the gills. The fins and both sets of gills were removed using dissection scissors and placed in separate petri dishes covered with distilled water to prevent dehydration. During the examination process, each gill arch was sequentially removed and placed into a separate small petri dish. The fins and gills were examined for ectoparasites with the aid of a stereomicroscope under various magnifications. The parasites were recorded and later identified in the Parasitology laboratory of the University of Limpopo (UL), using different taxonomic keys.

Figure 2.9: Fish held firmly for skin scrapings.

2.5.2 Examination for endoparasites

Blood samples were collected by placing a fish horizontally on a dissection board, inserting a needle just below the lateral line and drawing blood by suction. Blood smears were made by placing a drop of blood on a microscope glass slide and spreading it over using another microscope glass slide. Smears were air dried, dipped in methanol and stored in a slide box for later staining with Giemsa in the Parasitology laboratory (UL). Staining was done within two weeks after making smears and scrutinised for parasites. Internal organs were removed from the dissected specimen, placed in separate labelled petri dishes containing 0.9% saline solution and thoroughly examined for endoparasites using a stereomicroscope (Figure 2.10). The internal organs examined included the eyes, liver, kidney, spleen, intestine, stomach, gall bladder and swim bladder (the latter only for *Cyprinus carpio* and *O*. *mossambicus*). The eyes were removed, cut open with fine scissors to scrutinise for parasites. The intestine was opened using a pair of scissors and forceps inserted into the lumen to assist in pulling it apart for examination. The body cavity and muscles were also examined for parasites. Once found, the parasites were gently removed from the organs using a fine brush to avoid deformation, and were placed in a petri dish containing 0.9% saline solution.

Figure 2.10: Examination for parasites using stereomicroscopes.

2.6 Preservation, fixation and staining of parasites

The protocol followed for parasites was according to standard methods for each parasite group according to Cribb and Bray (2010) and Madanire-Moyo and Barson (2010), as listed below.

Monogeneans were mounted in glycerin ammonium picrate (GAP) solution on a microscope slide. The preparation was then sealed using clear nail varnish around edges of the coverslip. **Cestodes** were cleaned in saline, fixed with boiling water and then preserved in 70% ethanol. For staining, standard procedure was followed and this comprised rehydration, staining with acetocarmine, dehydration with different concentration of ethanol and clearing with clove oil. The staining time varied with size, thickness, and condition of the specimen. Specimens were then mounted using Canada balsam. **Acanthocephalans** were placed in a petri dish with tap water and were refrigerated for 12 hours or until the proboscis protruded then fixed and preserved in cold 70% ethanol. **Nematodes** were placed in a petri dish containing 0.9% saline to clean them from mucus and other debris. They were then fixed by adding hot 70% ethanol until they uncoil and straighten, and preserved in 70% ethanol. **Copepods** and **branchiurans** were first placed in petri dish with distilled water and then fixed in 70% ethanol.

2.7 Parasite identification, photography and measurements

Parasites were studied using a stereo-microscope and compound-microscope (Olympus BX50) equipped with phase-contrast and differential interference contrast. Drawings were made and measurements taken from the whole mounts with the aid of the compound-

microscope equipped with a drawing tube. Measurements of parasites were made in micrometers (µm) with an ocular micrometer.

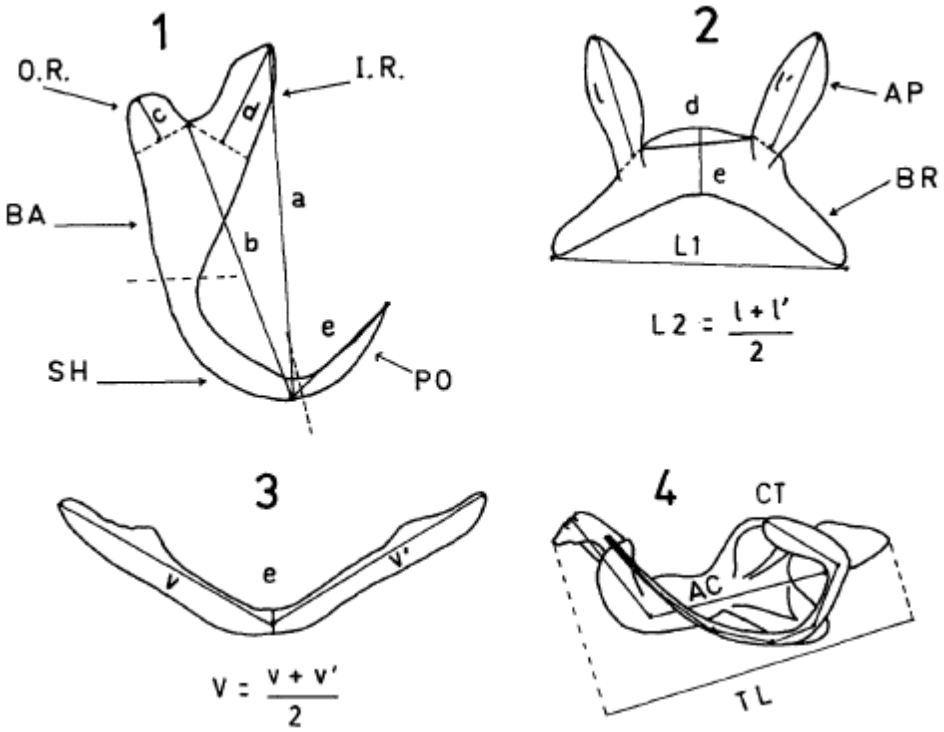


Figure 2.11: Measurements of the sclerotised parts of *Cichlidogyrus* (from Douellou 1993). 1= Anchor, 2 = Dorsal bar, 3 = Ventral bar, 4 = Copulatory organ. Abbreviations: OR = outer root, IR= inner root, BA = base, SH = shaft, PO = point, AP = appendage, BR = branch, CT = copulatory tube and AC =accessory piece.

2.8 Data analysis

The levels of parasite infection were calculated according to Bush *et al*. (1997) where: The prevalence is determined when the number of individuals of a host species infected with a particular parasite species is divided by the number of hosts examined. This is then expressed as a percentage.

The mean intensity is determined as the total number of individuals of a particular parasite species in a sample of a host species divided by the number of infected individuals of the host species in the sample.

The abundance is determined as 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.

The Shannon-Wiener Diversity Index (H') and evenness and Margalef's Richness Index were calculated as follows:

The Shannon-Wiener Diversity Index (H') score was calculated as described by Spellerberg and Fedor (2003), to measure the community diversity of parasites. This was done using the equation:

$H' = -\Sigma Pi \log Pi$

Where: Pi = proportion of each species in the sample. Diversity can be defined as the number of different items and their relative frequency (Spellerberg & Fedor 2003).

For calculating the evenness of species, the following equation was used:

$e = H / \ln S$

Where: H = Shannon-Wiener diversity index; S = total number of species in a sample. Evenness describes how equally individuals are distributed amongst the species (Spellerberg & Fedor 2003).

Margalef's index was used as a simple measure of species richness.

Margalef's index = $(S-1) / \ln N$

Where: S = total number of species; N = total number of individuals in the sample; In = natural logarithm. Species richness refers to the total number of species present in a given area or sample (Bielat *et al.* 2015).

The K was calculated according to Blackwell *et al.* (2000) as:

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

Where: W = weight in g; L = standard length in mm.

The relationship between K and the parasite burden was done using regression analysis with Microsoft Excel 2013.

The Parasite Index (PI) and Inverted Parasite Index (IPI) were determined according to Jooste *et al*. (2005) and Heath *et al*. (2004), respectively. The PI distinguishes between the numbers of ecto- and endoparasites present. The IPI is used to assign numerical values to the number

of ecto- and endoparasites observed. Ecto- and endoparasites were categorised as presented in Table 2.1.

Table 2.1: The revised Parasite Index (PI) (Jooste *et al*. 2005) and Inverted Parasite Index (IPI) (Heath *et al.* 2004) of ecto- and endoparasites.

	Ectoparasites		Endoparasites	
Number	IP	IPI	Number	IP
0	0	30	0	0
1-10	10	20	<100	10
11-20	20	10	101-1000	20
>20	30	0	>1000	30

Analysis of variance (ANOVA) was used to test for significant differences in the water quality parameters among the seasons. Statistical significance level was set at $p < 0.05$ for probability levels.

CHAPTER 3

WATER QUALITY ANALYSIS

3.1 Introduction

About 70% of the Earth is covered by water but only 2.5% of that is freshwater and the rest is seawater. Only 0.01% of the Earth's freshwater is found in streams, rivers and lakes. The remaining unfrozen freshwater is ground water (van Vuuren 2011). Freshwater is a finite resource required for domestic, industry and agriculture uses (Helmer 1994; van Vuuren 2011). Sustainable development will not be possible without freshwater of adequate quantity and quality (Helmer 1994). According to Oberholster and Ashton (2008), South Africa's freshwater resources are under increasing stress from a growing population and expanding economy and the water quality of these resources have declined due to increased pollution levels. Water quality is a term used to describe the physical, chemical, biological and aesthetic properties of water that determines its fitness for a variety of uses and for the protection of health and integrity of aquatic ecosystems. The majority of these properties of water are controlled or influenced by constituents that are either dissolved or suspended in water (DWAF 1996).

Water quality constituents are divided into four categories by DWAF (1996) based on the effects they may have on aquatic biota. System variables are described as the constituents that regulate the essential ecosystem processes such as spawning and migration e.g. pH and DO. Toxic constituents, infrequently occur in high concentrations in unimpacted systems examples; include inorganic constituents such as copper, iron, lead, aluminium, zinc, manganese, and organic constituents such as phenol and atrazine. Non-toxic inorganic constituents may cause toxic effects at extreme concentrations, but their concentrations depend on localised geochemical, hydrological and physical process e.g. TDS and total suspended solids (TSS). Nutrients are generally non-toxic but stimulate eutrophication if present in excess, e.g. NO_3, NO_2, NH_4, PO_4^{3-} and SO_4.

Aquatic ecosystems are threatened by a variety of pollutants as well as destructive land use or water management practices. Water pollution occurs when harmful substances are released into the water in large quantities and so changes the water quality (Mali *et al*. 2015; Alrumman *et al*. 2016). Water pollutants are categorised as point or nonpoint sources based on the nature of the source. Point source water pollutants refer to contaminants that enter a water source from a single, identifiable source. Nonpoint water pollutants refer to diffuse

contamination that does not originate from a single discrete source and is often the cumulative effect of small amounts of contaminants gathered from a large source (Mali *et al*. 2015).

Aquatic ecosystems must therefore be effectively protected and managed to ensure that South Africa's water resources remain fit for different uses on a sustained basis. In South Africa, the South African Water Quality Guidelines (SAWQG) by the DWAF are used in water quality management as the primary source of reference information and decision support required for managing and protecting aquatic ecosystems (DWAF 1996).

Aquatic ecosystems provide livelihood to communities that are dependent on water bodies for food, the maintenance of biodiversity and provision of habitats to those biota depending on aquatic ecosystems (DWAF 1996). Hence, the focus of this study was on water quality guidelines for aquatic ecosystems and only considered the TWQR values for aquatic ecosystems. The water quality results obtained in this study were interpreted in relation to the SAWQG (DWAF 1996) and compared with the recommended TWQRs by DWAF (1996).

3.2 Materials and methods

Physical and chemical analysis of water samples were done seasonally as described in Chapter 2. Subsurface water samples were collected in acid treated sampling bottles, refrigerated immediately and sent to a South African National Accreditation System (SANAS) water analysis laboratory (Capricon Veterinary Laboratories cc) in Polokwane where they were analysed for metals, non-metals and nutrients.

3.3 Data analysis

The results for water quality parameters are presented as bar graphs and mean value ± standard deviation (SD). Analysis of variance was used to test for significant differences in the water quality parameters between seasons.

3.4 Results

The physicochemical water quality parameters recorded during autumn, winter, spring and summer are shown in Table 3.1 and the TWQR values are shown in Table 3.2. A minimum of three measurements was used to calculate the SD among the seasons.

Table 3.1: The seasonal mean ± standard deviation comparison of physicochemical parameters recorded at the Kwena Dam.

Parameters	Autumn	Winter	Spring	Summer	P
Temperature (°C)	20.2 ± 0.7	14.3 ± 0.9	19.5 ± 0.6	23.3 ± 0.2	0.018
DO (mg/ℓ)	5.30 ± 0.32	8.66 ±0.28	8.17 ±1.10	6.60 ± 0.38	0.054
DO (%)	70.97 ± 3.81	114.0 ±11.26	114.0 ± 16.54	84.23 ± 0.58	–
pH	8.83	8.06	7.46	7.33	–
EC (µScm^{-1})	78.87 ± 1.80	115.83 ± 2.25	137.0 ± 2.08	129.4 ±2.23	0.0
Salinity (ppt)	0. 07 ± 0	0.07 ±0	0.07 ± 0	0.06 ± 0	.–
TDS (mg/ℓ)	87.0 ± 3.66	94.9 ± 1.17	101.0 ± 1.46	84. ±1 0.36	0.0

DO = Dissolved oxygen, EC = Electrical conductivity and TDS = Total dissolved solids.

Table 3.2: The Target Water Quality Range (TWQR) values for aquatic ecosystems (DWAF 1996).

Parameters	TWQR
Temperature (°C)	Should not vary more than 10% from normal (natural) value
DO (mgℓ$^{-1}$)	6 – 9
DO (%)	80% –120% of saturation
EC (µScm^{-1})	No guidelines
Salinity (ppt)	No guidelines
TDS (mgℓ$^{-1}$)	should not change by >15% for normal cycles

DO = Dissolved oxygen, EC = Electrical conductivity and TDS = Total dissolved solids.

3.4.1 Physicochemical parameters

Surface water temperature

Seasonal variation in the surface water temperature was recorded with the highest mean temperature of 23.9ºC during summer and the lowest of 14.3ºC during winter (Figure 3.1).

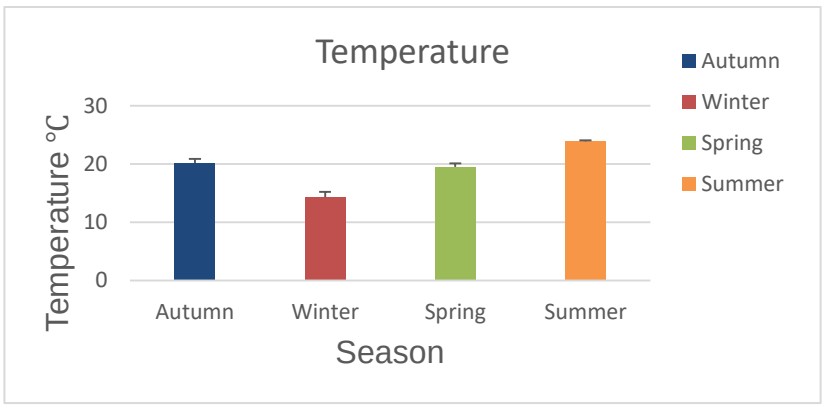


Figure 3.1: Seasonal temperature recorded at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

Dissolved oxygen

The concentrations of DO ranged from 5.3 mgℓ^{-1} – 8.7 mgℓ^{-1} (Figure 3.2). The highest value was recorded in winter and the lowest in autumn. In percentage of saturation, the concentrations ranged from 71.0%, 114.0% (Figure 3.3).

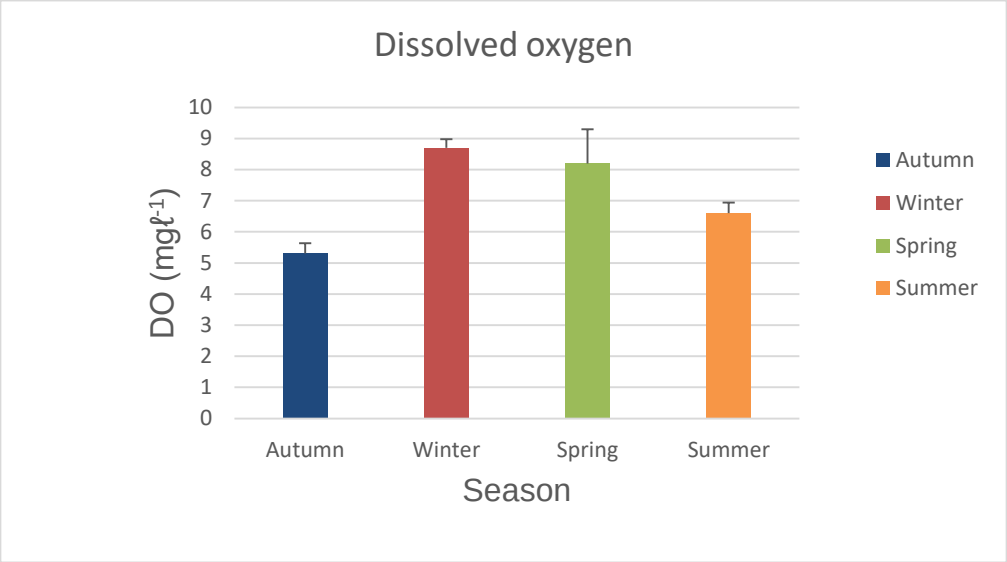


Figure 3.2: Seasonal dissolved oxygen (mgℓ^{-1}) recorded at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

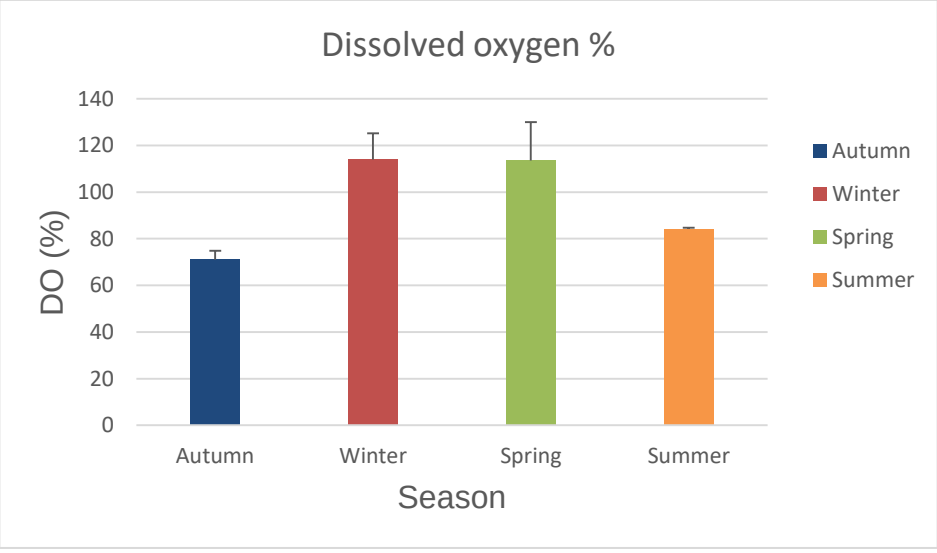


Figure 3.3: Seasonal dissolved oxygen (%) recorded at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

The seasonal variations in the pH in this study showed that pH values ranged from 7.3 – 8.4 with the highest value recorded in winter and the lowest value recorded in summer (Figure 3.4).

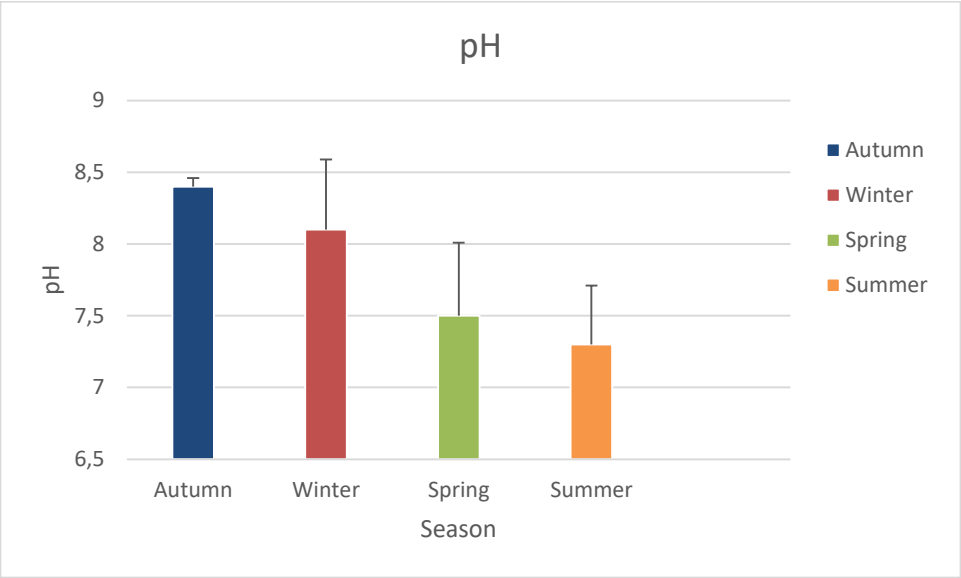


Figure 3.4: Seasonal pH values recorded at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

The EC mean values recorded in this study ranged from 78.9 μScm^{-1} – 137.0 μScm^{-1} (Figure 3.5). The highest value was recorded in spring and the lowest value in autumn. Statistically, EC showed a significant difference ($p<0.05$) among seasons.

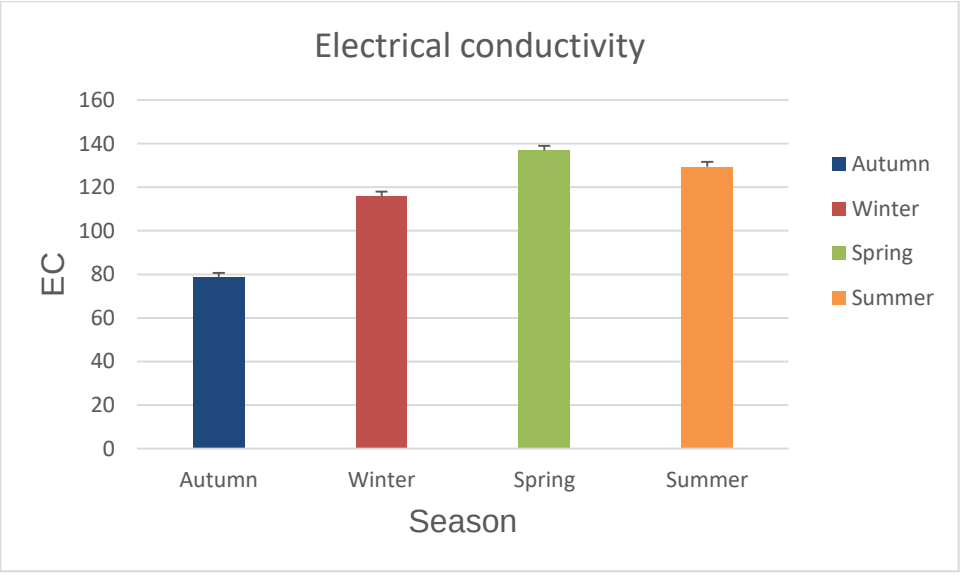


Figure 3.5: Seasonal electrical conductivity values recorded at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

Salinity was constant during autumn, winter and spring with a value of 0.07‰. The lowest value of 0.06‰ was recorded in summer (Figure 3.6). The difference in salinity among seasons was not significant ($p>0.05$).

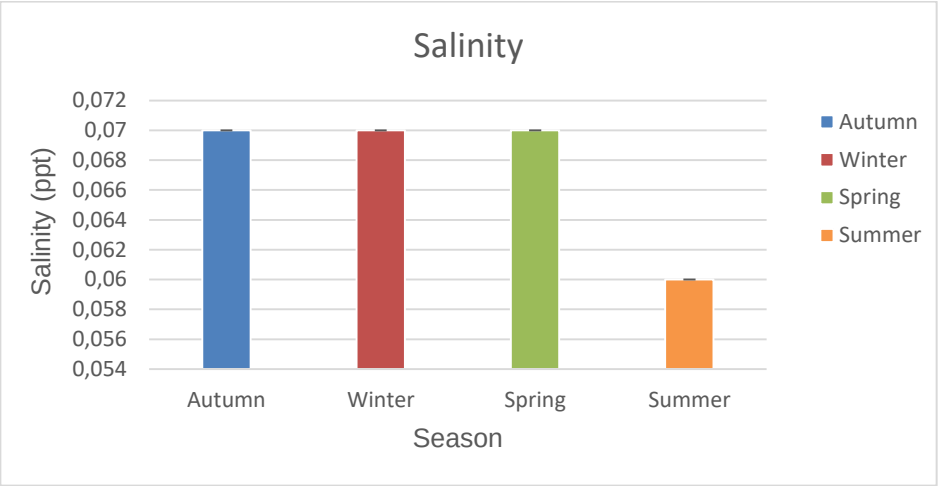


Figure 3.6: Seasonal salinity values recorded at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

The TDS concentrations ranged from 84.1 $mg\ell^{-1}$ – 100.8 $mg\ell^{-1}$ (Figure 3.7). Seasonally, the highest value was recorded in spring and the lowest in summer, with a significant difference ($p<0.05$) among seasons.

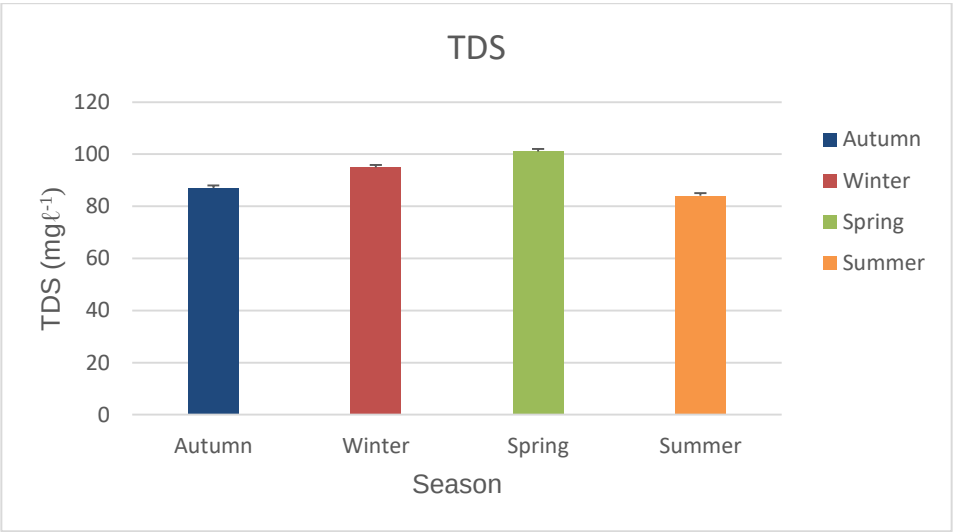


Figure 3.7: Seasonal total dissolved solids values recorded at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

3.4.2 Major ions

The major ions which include calcium, chloride, fluoride, magnesium, potassium and sodium were recorded during autumn, winter, spring and summer. The seasonal mean values are shown in Table 3.3.

Table 3.3: The seasonal mean ± standard deviation concentrations of major ions recorded at the Kwena Dam.

Constituents ($mg\ell^{-1}$)	Symbol	Autumn	Winter	Spring	Summer	TWQR
Calcium	Ca	10.27±0.15	10.35±0.31	10.0±0.15	8.82±0.42	No guidelines
Chloride	Cl	5.5±0.25	12.2±0.21	6.8±0.32	2.3±0.32	–
Fluoride	F	0.3±1.70	0.12±0.02	0.1±0.01	0.1±0.02	–
Magnesium	Mg	10.0±0.06	9.9±0.2	11.0±0.31	9.05±0.21	70
Potassium	K	1.22±0.06	10.24±0.12	1.1±0.15	1.89±0.32	200
Sodium	Na	4.46±0.17	5.59±0.15	5.0±0.25	4.4±0.15	100

TWQR = Target Water Quality Range for South African aquatic ecosystems. (DWAF 1996).

Calcium concentrations ranged from 8.8 $mg\ell^{-1}$ – 10.4 $mg\ell^{-1}$ (Table 3.3). The highest value of calcium was recorded during winter and the lowest during summer. There was no significant difference ($p>0.05$) among the seasons. Potassium concentrations recorded in this study ranged from 1.1 $mg\ell^{-1}$ – 10.2 $mg\ell^{-1}$ (Table 3.3). It was the highest during winter and the lowest during spring. There was a significant difference ($p<0.01$) among seasons. Chloride concentrations ranged from 2.3 $mg\ell^{-1}$ – 12.2 $mg\ell^{-1}$ (Table 3.3). The highest concentration was recorded during winter with the lowest concentration during summer. There was a significant difference ($p<0.01$) among seasons. Fluoride concentrations ranged from 0.1 $mg\ell^{-1}$ – 0.3 $mg\ell^{-1}$ (Table 3.3). Fluoride concentrations in this study were significantly different among seasons ($p<0.01$). Sodium concentrations recorded in this study ranged from 4.4 $mg\ell^{-1}$ – 5.0 $mg\ell^{-1}$ (Table 3.3). The highest concentration of sodium was recorded during spring and with lowest during autumn and summer. There were no significant difference ($p>0.05$) in sodium concentration among seasons. Magnesium concentrations recorded in this study ranged from 9.1 $mg\ell^{-1}$ – 11.0 $mg\ell^{-1}$ (Table 3.3). The highest concentrations were recorded during spring and lowest levels during summer. There were no significant difference ($p>0.05$) in its concentration among seasons.

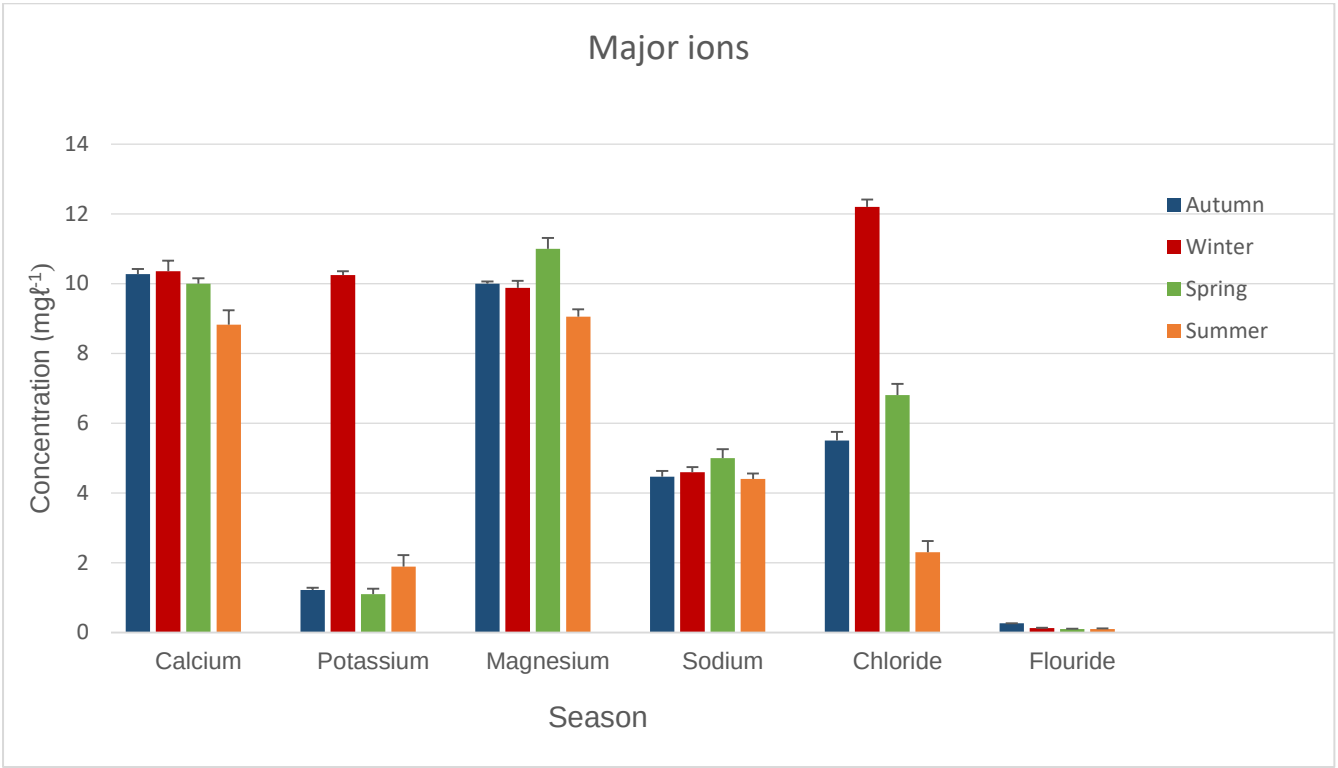


Figure 3.8: Seasonal values recorded for major ions at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

3.4.3 Metals and non-metals

A total number of ten elements were detected during this study. Of these, eight were metals and two non-metals. Toxic constituents detected included, aluminium, barium, iron, manganese, selenium, silicon, strontium and zinc.

Table 3.4: Mean ± standard deviation for metals and non-metals recorded in mgℓ^{-1} from the Kwena Dam. Non-metals are highlighted in bold script. Metals/non-metals which were below detection are indicated by an en dash (–). Toxic constituents are indicated by *.

Constituents (mgℓ^{-1})	Symbol	Autumn	Winter	Spring	Summer	TWQR
Aluminium*	Al	–	0.04±0.02	0.02±0.006	0.04±0.015	0.005 at pH<6.5, 0.01 at pH>6.5
Barium*	B	–	0.02±0	0.03±0.006	0.02±0	No guidelines
Iron*	Fe	–	0.03±0	0.43±0.06	0.03±0	Not vary by 10% of background concentration
Lithium	Li	–	–	–	0.01±0	No guidelines
Manganese*	Mn	0.01±0	–	–	–	0.18
Selenium*	Se	0.03±0	–	–	–	0.002
Silicon*	Si	–	0.03±0.006	0.03±0.006	–	No guidelines
Strontium*	Sr	0.03±0	0.04±0	0.04±0		No guidelines
Titanium	Ti	–	–	0.02±0.01	–	No guidelines
Zinc*	Zn	–	–	0.040±0.006	–	0.002

TWQR = Target Water Quality Range for South African aquatic ecosystems (DWAF 1996).

Aluminium concentrations were detected during three seasons namely, winter, spring and summer, and was below detection during autumn with the highest concentration of 0.04 mgℓ^{-1} recorded during winter and summer and the lowest concentration of 0.02 mgℓ^{-1} recorded during spring (Table 3.4). **Barium** had the highest concentration of 0.03 mgℓ^{-1} recorded during spring and the lowest of 0.02 mgℓ^{-1} (Table 3.4) during winter and summer. Barium was below detection during autumn. **Iron** concentrations were detected during three seasons namely, winter, spring and summer and was below detection during autumn with the highest concentration of 0.43 mgℓ^{-1} recorded during spring and the lowest concentration of 0.03 mgℓ^{-1} recorded during winter and summer (Table 3.4). **Lithium** was detected only during summer with a concentration of 0.01 mgℓ-^{1}. **Manganese** concentration was detected only during autumn with a concentration of 0.11 mgℓ^{-1} (Table 3.4). **Selenium** was detected only during autumn with a mean concentration of 0.03 mgℓ-^{1}. **Silicon** was detected during winter and spring with the highest concentration of 0.03 mgℓ-^{1} and the lowest concentration of 0.006 mgℓ-^{1} during both seasons. The highest concentration of **strontium** was 0.04 mgℓ^{-1} during

winter and spring with the lowest concentration of 0.03 mgℓ^{-1} during autumn and summer (Table 3.4).**Titanium** was detected only during spring the highest concentration 0.02 mgℓ-^{1}. **Zinc** was detected only during spring with a mean concentration value of 0.04 mgℓ^{-1} and was above the TWQR for aquatic ecosystem (Table 3.4).

3.4.4 Nutrients

Ammonium and ammonia were recorded at concentrations below the detection level during autumn and summer with highest values of 0.24 mgℓ^{-1} and 0.25 mgℓ^{-1} respectively. Nitrate was recorded during winter and summer with the highest value of 0.4 mgℓ^{-1}. Its concentration was below detection level during autumn. Nitrite was recorded at concentrations below the detection level during winter and summer. A value of 0.01 mgℓ^{-1} was recorded during autumn (Table 3.5). Phosphorus concentrations were below detection during all three seasons.

Table 3.5: Mean values for nutrients during autumn, winter, (2016) and summer (2017). En dash (–) indicates values below detection limit of 0.001 mgℓ^{-1}.

	Symbol	Autumn	Winter	Spring	Summer
Ammonium (mgℓ^{-1})	NH_4^+	–	0.24	–	–
Ammonia (mgℓ^{-1})	NH_3	–	0.25	–	–
Nitrate (mgℓ^{-1})	NO3-	–	0.3	–	0.15
Nitrite (mgℓ^{-1})	NO2-	0.01	–	–	–
Phosphate (mgℓ^{-1})	PO_4^{3-}	–	–	–	–

3.5 Discussion

3.5.1 Physicochemical parameters

Water bodies undergo daily and seasonal variations in temperature along with normal climatic fluctuations (Chapman & Kimstach 1996). According to DWAF (1996), South African inland water temperature generally ranges from 5 – 30ºC. Water temperature is influenced by latitude, altitude, seasons, time of day, air circulation, and the flow and depth of the water body (Chapman 1996). According to Chapman (1996), minima temperature occurs during winter and maxima during summer which was expected in the present study. There was a significant difference ($p<0.05$) in surface water temperature among the seasons.

Temperature is one of the major factors controlling the distribution of aquatic organisms as it affects the rates of chemical reactions and metabolic rates of organisms (DWAF 1996). The respiration rate of aquatic organisms increases with increasing water temperature leading to

increased oxygen consumption and increased decomposition of organic matter (Chapman 1996). Aquatic organisms have a range of temperature at which optimal growth, general fitness and reproduction occur (Dallas & Day 2004). Water temperature changes that are not associated with natural variations may affect specific organisms and/or community levels however, the effects depend on the extent, duration and timing of these changes (DWAF 1996). The mean temperature values obtained in this study were within the range 0°C – 30°C suggested by Chapman (1996) for surface waters.

Dissolved oxygen is required by organisms for aerobic respiration; therefore, adequate concentrations are crucial for the functioning and survival of aquatic organisms (DWAF 1996). Dissolved oxygen can be measured in milligrams per litre ($mg\ell^{-1}$) or in percentage of the saturation concentrations. Concentrations of less than 100% indicate depletion from the theoretical equilibrium concentrations. Organisms continuously exposed to less than 80% of saturation may suffer acute effects. Furthermore, physiological and behavioural stress may occur in repeated exposure due to reduced DO concentrations (DWAF 1996).

According to Palmer *et al*. (2004), an increase in water temperature decreases oxygen solubility which may increase the toxicity of certain chemicals. Higher temperature was recorded during summer and this was associated with a decrease in DO concentration. However this was not the case during autumn where temperature was relatively lower with a relatively higher DO as compared to summer. This may be due to more dilution from the preceding rains (January to March 2016) and therefore lower concentrations of chemicals. There was a significant difference ($p<0.05$) in DO among seasons. The maintenance of adequate DO concentrations is critical for the survival and functioning of the aquatic biota because it is required for respiration of all aerobic organisms (DWAF 1996).

The TWQR for DO is from 6 $mg\ell^{-1}$ – 9 $mg\ell^{-1}$ (DWAF 1996). The DO concentrations recorded in this study during winter, spring and summer were within this range. DO concentration recorded during autumn was below this range; however, according to Chapman (1996), DO concentrations above sea level at 25℃ ranges from 5 $mg\ell^{-1}$ – 8 $mg\ell^{-1}$ and further stated that DO concentration below this range would negatively affect the functioning and survival of aquatic biota. The DO concentrations recorded in this study would not adversely affect the functioning and survival of biological communities because no DO concentration value below 5 $mg\ell^{-1}$ was recorded during this study period.

The **pH** measures the concentration of hydrogen ions (H^+) and alkalinity by the concentration of hydroxyl, bicarbonate and carbonate ions in the water (Palmer *et al*. 2004). It is an important variable in water quality assessment as it influences biological and chemical processes within a water body (Chapman 1996). Factors such as temperature, concentration of organic and inorganic ions and biological activity affect the pH of the water (DWAF 1996). Most freshwater ecosystems of South Africa have pH ranges between 6 and 8, are buffered and are more or less neutral (DWAF 1996). Water pH has a significant influence on the toxic action of a number of other substances on fish. According to Svobodova *et al*. (1993), the pH range of 6.5 – 8.5 is optimal for fish. The pH values obtained in this study were within this range and the range (6.5 – 9) recommended for aquatic ecosystems by DWAF (1996).

Electrical conductivity is the ability of water to conduct an electrical current (Bartram & Ballance 1996). EC is dependent on temperature and it is sensitive to variations in dissolved solids, mostly mineral salts (Bartram & Ballance 1996; Chapman 1996). It is influenced by the degree to which these (dissolved solids) dissociate into ions, the amount of electrical charge on each ion, ion mobility and temperature. The EC of most freshwaters ranges between 10 – 1000 μScm^{-1} but in polluted waters or those receiving large amounts of land run-off, this may be exceeded (Chapman 1996). The EC values recorded during the sampling period were within this range and this may indicate that the water of the Kwena Dam is of good quality. The effects of EC on aquatic organisms are not well known (Dallas & Day 2004).

Salinity is a term used to refer to the saltiness of the water (Dallas & Day 2004). It measures only the content of dissolved inorganic material of water (DWAF 1996). The salinity concentration in the present was constant during autumn, winter and spring with the highest of 0.07 ‰. The lowest concentration of 0.06 ‰ was recorded during summer. Very little is known on the salinity tolerances of freshwater organisms and the effects of increased concentrations of salinity on whole communities or ecosystems. However, it seems that many aquatic species are able to survive at relatively high salinities (Dallas & Day 2004).

Total dissolved solids concentration is a measure of dissolved materials in water. It represents the total quantity of both dissolved organic and inorganic materials, and both ionised and unionised. In most waters, TDS, conductivity and salinity correlate closely (Dallas & Day 2004). The TDS concentrations that are too high or too low may limit growth and may lead to the death of aquatic organisms (Dallas & Day 2004). The highest TDS concentration of 100.8 $mg\ell^{-1}$ was recorded during spring and the lowest concentration of 84.1 $mg\ell^{-1}$ during summer. There are no TDS TWQR set for aquatic ecosystem but the concentrations should

not be changed by > 15% from the normal cycles of the water body under unimpacted conditions at any time of the year (DWAF 1996).

3.5.2 Major ions

Calcium is a vital element for all living organisms (Dallas & Day 2004). It is vital for muscle contraction, nervous activity, energy metabolism and a great variety of other biochemical interactions (Dallas & Day 2004). Together with magnesium it contributes to the total hardness of the water (Chapman 1996). Its solubility is usually governed by the carbonate/bicarbonate equilibrium and thus it is strongly influenced by pH and temperature (DWAF 1996). There are no TWQR for calcium for aquatic ecosystems. Although it is a vital element, very little is known about the actual effects of changes in its concentration on aquatic biotas (DWAF 1996).

Potassium is found in low concentrations in natural waters with concentrations usually less than 10 $mg\ell^{-1}$ (Chapman 1996). It plays a role in ionic balance in all organisms. It can sometimes act as a nutrient, the lack of which limits plant growth. It also occur in lower concentrations than sodium in natural water bodies (Dallas & Day 2004). During the present study, the mean potassium concentrations were lower (3.6 $mg\ell^{-1}$) than that of sodium (4.6 $mg\ell^{-1}$) which is in accordance with their natural occurrence in freshwater. There are no TWQR for potassium available for aquatic ecosystems, and its toxic effects to aquatic ecosystems are not known (DWAF 1996).

Chloride anions are usually present in natural waters. It is an anion of chlorine which rarely occurs in nature but it is found as chloride (DWAF 1996). High chloride content in a water body may be an indication of sewage or industrial waste pollution (Bartram & Ballance 1996). It contributes to the concentration of TDS and salinity of water (DWAF 1996). According to Dallas and Day (2004), chloride ions exhibit no toxic effects on living organisms except where they have an effect by increasing the TDS levels.

Fluoride is a halogen gas which is highly reactive with a variety of substances. It is seldom found as free fluorine gas in nature, but occurs either as the fluoride ion or in combination with calcium, potassium and phosphates (DWAF 1996). Its concentrations in natural waters range from 0.05 $mg\ell^{-1}$ – 100 $mg\ell^{-1}$, although in most situations they are less than 0.1 $mg\ell^{-1}$ (Chapman 1996). An increase in water temperature increases the toxic effects of fluoride (DWAF 1996). The concentrations were all within the TWQR for aquatic ecosystems (DWAF 1996) of 0.75 $mg\ell^{-1}$. Seasonally, the highest concentration was recorded during autumn and its concentration was constant during winter, spring and summer.

Sodium is a major cation in many South African freshwaters and the least toxic (Dallas & Day 2004). It is also an essential ion for living organisms. Sewage and industrial effluents may increase its concentration in surface waters (Chapman 1996). Sodium concentrations recorded in this study ranged from 4.4 mgℓ^{-1} – 5.0 mgℓ^{-1}. The highest concentration of sodium was recorded during spring and with lowest during autumn and summer. There is no TWQR for sodium available for aquatic ecosystems.

Magnesium is commonly found in natural waters as Mg^{2+}, and along with calcium, is a main contributor to water hardness. Its concentrations in freshwaters may range from 1 to >100 mgℓ^{-1} depending on the rock types within the catchment (Chapman 1996). Magnesium concentrations recorded in this study ranged from 9.1 mgℓ^{-1} – 11.0 mgℓ^{-1}. The highest concentrations were recorded during spring and lowest levels during summer. There were no significant difference ($p>0.05$) in its concentration among seasons. There is no TWQR available for magnesium and little information is known about its effects on aquatic organisms (Dallas & Day 2004).

3.5.3 Metals and non-metals

Aluminium is one of the most abundant elements in the earth's crust, however it is only present in trace concentrations in natural waters (Bartram & Ballance 1996). It can be mobilised in soils and sediments by accelerated acidification processes and natural weathering resulting in detectable concentrations in surface waters (DWAF 1996). Aluminium is soluble and toxic under acidic pH but is partially soluble under intermediate pH values. At alkaline pH, it is present as soluble but biologically unavailable hydroxide complexes or as colloids and flocculants (DWAF 1996). Its mechanism of toxicity in fish is related to interference with ionic and osmotic balance and with coagulation of mucus on the gills resulting in respiratory problems (DWAF 1996). The TWQR for aluminium is 0.005 mgℓ^{-1} at pH <6.5 and 0.01 mgℓ^{-1} at pH >6.5. The aluminium concentrations recorded in this study were above the TWQR for aluminium for aquatic ecosystems as stipulated by DWAF (1996). In this study the pH was recorded at levels greater than 6.5 (intermediate to alkaline). Hence even though aluminium was recorded at concentrations above the TWQR, it was not biologically available and the aquatic biota may not be affected.

Barium in water originates from natural sources and is present as a trace element in both igneous and sedimentary rocks (WHO 2004). This element is not found free in nature (USEPA 1985), it occurs in a number of compounds, most commonly barium sulfate (barite) and, to a lesser extent, barium carbonate (witherite). The solubility of its compounds increases as the

pH level decreases (USEPA 1985). The highest concentration of 0.03 mgℓ^{-1} was recorded during spring and the lowest of 0.02 mgℓ^{-1} during winter and summer. Barium concentrations recorded during the sampling period were most likely not toxic due to alkaline pH values recorded.

Iron may be found in natural waters in varying quantities depending on the geology of the area and chemical properties of the water (DWAF 1996). It is an essential micronutrient in all organisms (Dallas & Day 2004). Although it is toxic at high concentrations, it is not easily absorbed through the gastro-intestinal tract of vertebrates (Dallas & Day 2004). Its solubility in natural waters highly depends on the pH and oxidation-reduction of the water (Bartram & Ballance 1996).

There are no specific TWQR for iron. However, according to DWAF (1996), its concentration should not be allowed to vary by more than 10% of the dissolved iron concentration for a particular site at a specific time. In unpolluted waters, iron concentrations range from 0.001 mgℓ^{-1} – 0.500 mgℓ^{-1} (DWAF 1996). The iron values recorded in this study were within this range, which according to DWAF (1996) describes non-polluted waters.

Manganese does not occur naturally as a metal in aquatic ecosystems but it is found in various salts and minerals. Factors such as DO, pH and organic matter influence its concentration. Its deficiency in vertebrates leads to skeletal deformities and reduced reproductive capabilities (DWAF 1996). The TWQR for manganese in aquatic ecosystems is 0.18 mgℓ^{-1} (DWAF 1996). Manganese was detected only during autumn with a concentration of 0.11 mgℓ^{-1} which is within the TWQR for aquatic ecosystems.

Selenium is essential for animals but it may be toxic at high concentrations. Its presence in water may be in elemental form as well as anions (Bartram & Ballance 1996). Industrial pollution may increase its concentration in water bodies (DWAF 1996). The TWQR for selenium in aquatic ecosystems is 0.002 mgℓ^{-1} (DWAF 1996). Selenium was detected only during autumn with a mean concentration of 0.03 mgℓ^{-1} which was above the TWQR for aquatic ecosystem. Toxic concentrations of selenium can be carcinogenic or may have genotoxic effects on aquatic organisms (Dallas & Day 2004). Although the concentration of selenium was above the TWQR value during autumn, no pathologies (by gross observations) were reported.

Strontium naturally occurs in rocks, soil, dust, coal and oil. Naturally occurring strontium is not radioactive. This element commonly occurs in nature, forming about 0.034% of all igneous

rock and in the form of the sulfate mineral celestite ($SrSO_4$) and the carbonate strontianite ($SrCO_3$). Strontium compounds are used in making ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, and some medicines (Irwin *et al.* 1997). Guideline values for strontium in aquatic ecosystems are not available.

Zinc is an essential micronutrient for all organisms. It occurs in rocks and ores and enters aquatic ecosystems through both natural processes (e.g. weathering and erosion) and through industrial activity. The toxicity of zinc increases at lower oxygen concentrations. The TWQR for zinc concentrations in aquatic ecosystems is 0.002 $mg\ell^{-1}$ (DWAF 1996). The DO concentration during spring was within the suggested TWQR; therefore, high concentrations of zinc might have had less or no toxic effects on aquatic biota during the time of sampling.

3.5.4 Nutrients

Ammonia can be present in water bodies in two forms namely, a molecular form (non-dissociated (NH_3) and in the form of ammonia ion (dissociated) (NH_4^+) (Svobodova *et al*. 1993). Its toxicity is directly related to the concentration of the un-ionised form and the ammonia ion has little or no toxicity (Dallas & Day 2004). Ammonia gas is readily soluble in water and its reaction with water form results in the formation of ammonium hydroxide. Ammonium hydroxide then dissociates into ammonium and hydroxyl ions, which raise the pH value of the water (Dallas & Day 2004).

The South African TWQR for ammonium in aquatic ecosystems is 0.007 $mg\ell^{-1}$ (DWAF 1996). Concentrations at and above 0.015 $mg\ell^{-1}$ may have chronic effects on morphological development, hatching success, growth rate, liver, gill and kidney tissue whilst acute effects including increased breathing rate, increased cardiac output and oxygen uptake may occur at 0.10 mgℓ-1 (DWAF 1996). Ammonium concentrations recorded in the present study were high and may thus result in mortalities of aquatic organisms. The high concentration of ammonium may be attributed to agricultural runoffs. However, no mortalities of fish were recorded during the sampling period.

Nitrates are the end products of the aerobic decomposition of organic nitrogen compounds (Svobodova *et al*. 1993). They occur in all surface waters in low concentrations. Nitrate is the least toxic of the inorganic nitrogen compounds to fish (DWAF 1996). The TWQR for nitrate recommended by DWAF (1996) is 0.5 $mg\ell^{-1}$. The mean value for nitrate concentration recorded during the present study was 0.1 $mg\ell^{-1}$ which is below the TWQR. According to DWAF (1996), water bodies with concentrations falling below 0.5 $mg\ell^{-1}$ can be described as

oligotrophic. Such water bodies have low productivity and fast nutrient cycling and there is usually no sign of aquatic plants or blue-green algal blooms occurring in them. This was also the case at the Kwena Dam during the sampling period.

Nitrite is a naturally occurring anion in freshwaters and human activities can contribute to the high nitrite concentrations in aquatic environments including industrial production of metals and dyes, sewage effluents and aquaculture. The TWQR for nitrite in aquaculture is between 0.06 $mg\ell^{-1}$ and 0.25 $mg\ell^{-1}$. This range is considered safe for freshwater fish species (DWAF 1996). The nitrite concentration levels recorded during the present study were within the TWQR.

Phosphorus is a crucial nutrient for living organisms and exists in water bodies as both dissolved and particulate species. It is a limiting nutrient for algal growth and therefore controls the primary productivity of a water body. It rarely occurs in high concentrations in freshwaters as it is actively taken up by plants and as a result, there can be considerable fluctuations in its concentrations (Chapman 1996). Activities that discharge phosphates into the water include urban runoff as well as drainage from agricultural practices (Dallas & Day 2004). The TWQR for phosphate in freshwater ecosystems is 0.10 $mg\ell^{-1}$ (Kempster & Van Vliet 1980). In the present study, phosphate concentrations were below detection level during all seasons.

3.6 Conclusion

Surface water temperatures varied during the four sampling seasons with the lowest temperature value recorded in winter as expected. The temperature and DO were within the normal ranges throughout the study period. The pH levels recorded throughout the study period were slightly neutral to alkaline which is favourable for aquatic biota. The EC, TDS and salinity were within the TWQRs for aquatic ecosystems. Chloride and fluoride were both within the TWQRs. There are no aquatic ecosystem TWQRs for calcium, sodium, magnesium and potassium, however, they were tested for future reference. The metals and non-metals detected during the study includes aluminium, barium, iron, lithium, manganese, selenium, silicon, strontium, titanium and zinc. Aluminium and selenium which are highly sensitive to changes in temperature and pH, had concentrations above the TWQRs and should therefore be closely monitored as they may have negative effects on aquatic biota. Zinc was only detected during spring with concentration value above the TWQR for aquatic ecosystems. However, its toxic effects on aquatic biota may have been minimal due to adequate DO concentration during the sampling period. The iron concentrations recorded in this study indicated that water of the Kwena Dam was unpolluted during the study period. Barium, lithium,

silicon, strontium and titanium have no TWQRs available for aquatic ecosystem. Overall, it can be concluded that the Kwena Dam is not polluted

CHAPTER 4

PARASITE DIVERSITY OF *CLARIAS GARIEPINUS*, *CYPRINUS CARPIO* AND *OREOCHROMIS MOSSAMBICUS*

4.1 Introduction

A parasite is an organism living on or in another organism (host) and depends on its host in various ways for its survival (Bush *et al*. 2001). Parasites range from unicellular to complex multi-celled organisms (Gajadhar & Allen 2004). Parasites occur at different trophic levels and may play many different roles in the ecosystem (Marcogliese 2005). According to Poulin and Morand (2000), parasitism is one of the most successful modes of life displayed by living organisms. Their presence and abundance may be directly influenced by both the host environment and environmental conditions of the ecosystem (Pampoulie *et al*. 2004). Parasites belong to variable groups of which some (those relevant the present study) will be discussed below.

Protozoa

Protozoans are single-celled, many of which are free-living and some are parasitic in the aquatic environment. Normally, no intermediate host is required for the parasite to reproduce. Consequently, they can build up to very high numbers when fish are crowded causing weight loss, debilitation, and mortality of fish. One group of protozoans was found in this study namely flagellates. Flagellate protozoans possess one to many flagella at some time in their life cycle. A flagellum is a hair-like structure used for locomotion. Flagellates may be solitary, colonial, free-living, or parasitic. Parasitic forms are found in the intestine or bloodstream of the host. In South Africa, Du Plessis (1952), Sarig (1971), Hine (1977), Jackson (1978), Paperna (1980) and Basson *et al*. (1983) reported on trichodinid protozoans from cichlid and cyprinid fishes. Crafford and Avenant-Oldewage (2009) and Ferreira and Avenant-Oldewage (2013), reported *Trypanosoma* spp. from clariid and cichlid fishes.

Monogenea

Monogeneans belong to the Phylum Platyhelminthes (flatworms). Flatworms are regarded as relatively simple on an evolutionary scale of anatomical development because they lack a skeleton and a blood system (Kearn 2014). Monogeneans are a group of ectoparasites commonly found on the gills, skin and fins of their host. This group of parasites require only one host to complete their entire life cycle (Reed *et al*. 2009). Most species are host- and site-

specific, although some species may infect several hosts from different families (Reed *et al*. 2009; Zargar *et al*. 2012). According to Abdullah and Abdullah (2013), monogeneans may cause mortalities associated with economic losses. An increased interest in monogeneans in South African freshwater fish have been demonstrated by publications Mashego (1983), Khalil and Polling (1997), Luus-Powell *et al*. (2003), Christison *et al*. (2005) and Madanire-Moyo *et al*. (2011), to mention a few.

Digenea

Digeneans are a large and diverse group of widespread helminthes that parasitise a wide range of animals like fish, piscivorous birds and occasionally mammals. Their life cycle utilises three hosts, with freshwater snails serving as first intermediate hosts and freshwater fishes as second intermediate hosts. In freshwater fishes, diplostomid larvae (metacercariae) are found encysted, encapsulated in tissues or free in skin, eyes, musculature and central nervous system (Chibwana *et al*. 2013). Many studies focus on the ecology, behaviour and evolution of metacercarial stages as they are regarded pathogenic for their fish hots (Hoogendoorn *et al.* 2020). Some of the studies on digenean parasites of fish in South Africa include Barson and Avenant-Oldewage (2006), Madanire-Moyo *et al*. (2012) and Hoogendoorn *et al*. (2020).

Cestoda

Cestodes are flatworms with features like elongated tape-like body, scolex, sometimes a rostellum and the absence of an alimentary canal (Smyth & McManus 1989). Their complex life cycles include one or more intermediate hosts (Alves *et al*. 2017). They are almost unique among parasites in that adult worms inhabit only one particular habitat, the alimentary canal, in one particular group of animals, the vertebrates. Exceptions occur in the bile duct, gall bladder, body cavity and pancreatic duct, sites that are, however, still related to the alimentary canal (Smyth & McManus 1989). Some of South African records of cestodes from freshwater fish include Mashego and Saayman (1989), Mashego *et al*. (1991), Barson and Avenant-Oldewage (2006) and Scholz *et al*. (2015; 2018).

Nematoda

Nematodes (roundworms) are common parasites of freshwater and marine fish, amphibians, reptiles, birds, humans and domesticated animals (Goater *et al*. 2014). They are characterised by a cylindrical, filiform, or fusiform body with a cuticle that is smooth or have bristles and other types of ornamentations (Skrjabin 1949). Fish nematodes occur as endoparasites either as

larval forms or adults and some use aquatic invertebrates as intermediate hosts (Mashego *et al*. 1991). In instances where fish serve as the second intermediate host, aquatic invertebrates such as copepods, amphipods and oligochaetes serve as first intermediate hosts while birds or mammals are the final hosts. There have been reports of nematodes from different freshwater fish species from South Africa which include Lombard (1968), Whitfield and Heeg (1977), Jackson (1978), Bruton (1979), Mashego and Saayman (1981), Mashego (1990), Boomker and Petter (1993), Boomker (1994), Barson and Avenant-Oldewage (2006), Madanire-Moyo and Barson (2010) and Tavakol *et al*. (2015).

Acanthocephala

Acanthocephalans are parasites of peculiar structure whose distinctive feature, the anterior cylindrical proboscis bearing rows of hooks, resulted in their name which means “spiny headed”. The proboscis is used to attach the worms to the intestine of the host (Storer *et al*. 1972). They are widely distributed among various populations of wild and stocked freshwater fish and may reach population densities of several hundred worms per fish (Taraschewski *et al*. 1990). The pathogenicity of acanthocephalans can be attributed to worm density and depth of worm penetration into host tissue (Bayoumy *et al*. 2006). Some of South African records of acanthocephalans include reports by Mashego (1982; 1988), Madanire-Moyo *et al*. (2012) and Halajian *et al*. (2018).

Branchiura

Branchiura are fish ectoparasites also known as fish lice. Branchiurans are found on the skin and fins, mouth cavities, branchial chambers and on the gill filaments of fishes (Moller & Olesen 2012; Van As & Van As 2015). They loosely attach to their hosts and their highly modified cephalic appendages constitute an advanced attachment system enabling them to move around the host (Moller & Olesen 2012). Adult branchiurans attach to the surface of fish by either hooks or suction discs (Moller *et al*. 2008). They are visible to the naked eye and can range between 2.5 and 12 mm in length. Adults are dorso-ventrally flattened, with a prominent carapace covering part or sometimes almost the entire four segmented thorax (Van As & Van As 2015). There are approximately 160 species of branchiurans, accommodated in a single family, Argulidae Leach, 1819, with four valid genera: *Argulus* Muller, 1785; *Chonopeltis* Thiele, 1900; *Dipteropeltis* Calman, 1912 and *Dolops* Audouin, 1837 (Aguiar *et al*. 2017).

4.2 Materials and methods

Fish and parasites were collected from April 2016 to February 2017. All parasites collected were examined using both compound- and stereo-microscopes under various magnifications. The parasites were fixed and preserved according to standard methods for each parasite group as outlined in Chapter 2. Parasite identification, photography and measurements were done as explained in Chapter 2. To confirm the classification, measurements were taken and compared with measurements from previous studies for monogeneans identified to species level. No measurements were taken for monogeneans identified to genus level.

4.3 Data analysis

The prevalence, mean intensity and mean abundance for parasites were calculated according to Bush *et al*. (1997). Shannon-Wiener and Margalef (diversity), as well as evenness biotic indices were calculated as outlined in Chapter 2.

4.4 Results

During the period of this study, a total number of 26 *Clarias gariepinus*, 21 *Cyprinus carpio* and 57 *O. mossambicus* specimens were collected. A higher number of fish specimens was recorded during autumn and the lowest number was recorded during winter (Table 4.1).

Table 4.1: Number of fish specimens collected from the Kwena Dam during autumn, winter, spring (2016) and summer (2017).

Fish species	Autumn	Winter	Spring	Summer	Total
Clarias gariepinus	3	7	6	10	26
Cyprinus carpio	2	7	1	11	21
O. mossambicus	31	1	22	3	57

The number of parasites recorded for all fish species (*Clarias gariepinus, Cyprinus carpio* and *O. mossambicus)* during autumn, winter, spring and summer is presented in Table 4.2.

Table 4.2: Number of parasites collected from *Clarias gariepinus*, *Cyprinus carpio* and *Oreochromis mossambicus* during autumn, winter, spring (2016) and summer (2017) from the Kwena Dam.

Fish species	Total parasites	Autumn	Winter	Spring	Summer
Clarias gariepinus	3259	524	1704	720	311
Cyprinus carpio	444	82	98	2	262
O. mossambicus	473	207	8	226	32
Total	4176	813	1810	948	605

The number of ectoparasites recorded for all fish species during seasonal surveys is presented in Figure 4.1.

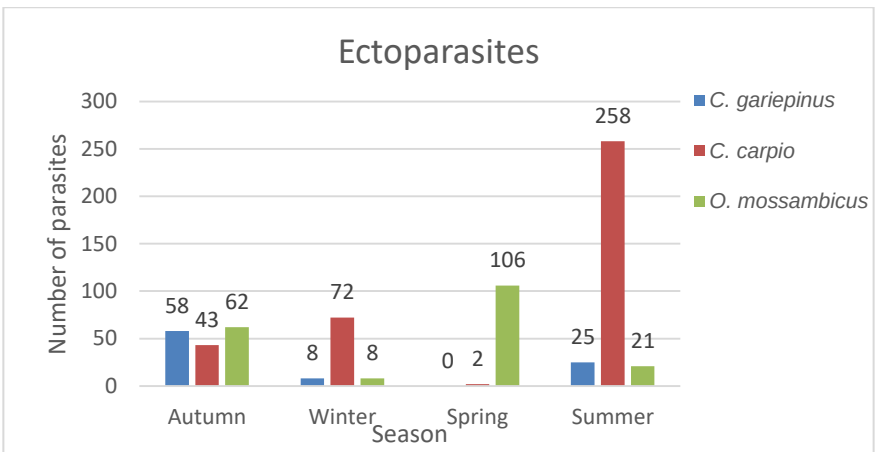


Figure 4.1: The total number of ectoparasites of three fish species collected at the Kwena Dam during seasonal surveys from April 2016 to February 2017.

The number of endoparasites recorded for all fish species during seasonal surveys is presented in Figure 4.2.

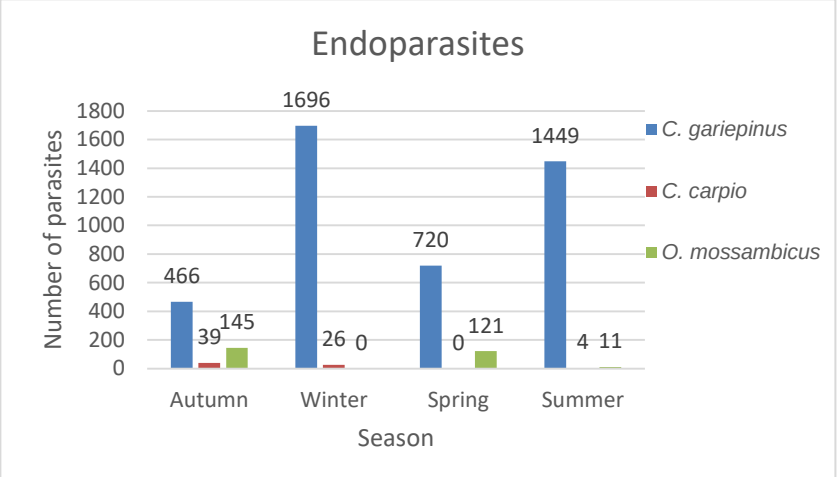


Figure 4.2: The total number endoparasites of three fish species collected at the Kwena Dam during four sampling seasons from April 2016 to February 2017.

4.4.1 Parasites of *Cyprinus carpio*

4.4.1.1 Monogenea

Dactylogyrus extensus

CLASS: Monogenea

ORDER: Monopisthocotylea

FAMILY: Dactylogyridae

GENUS: *Dactylogyrus* Diesing, 1850

SPECIES: *Dactylogyrus extensus* Mueller & Van Cleave, 1932

Dactylogyrus extensus (Figure 4.3) was collected from the gills of *Cyprinus carpio*. A higher prevalence of this parasite was reported during autumn and spring. High values of mean intensity and mean abundance were reported during autumn (Figure 4.4).

Morphology

The morphology of this parasite conforms to the description by Dove and Ernst (1998). Flattened slender body. Two pairs of eyespots. The tube of the copulatory organ is L-shaped. The accessory piece is simple, straight and spade-shaped. Hooks arranged in two lateral groups.

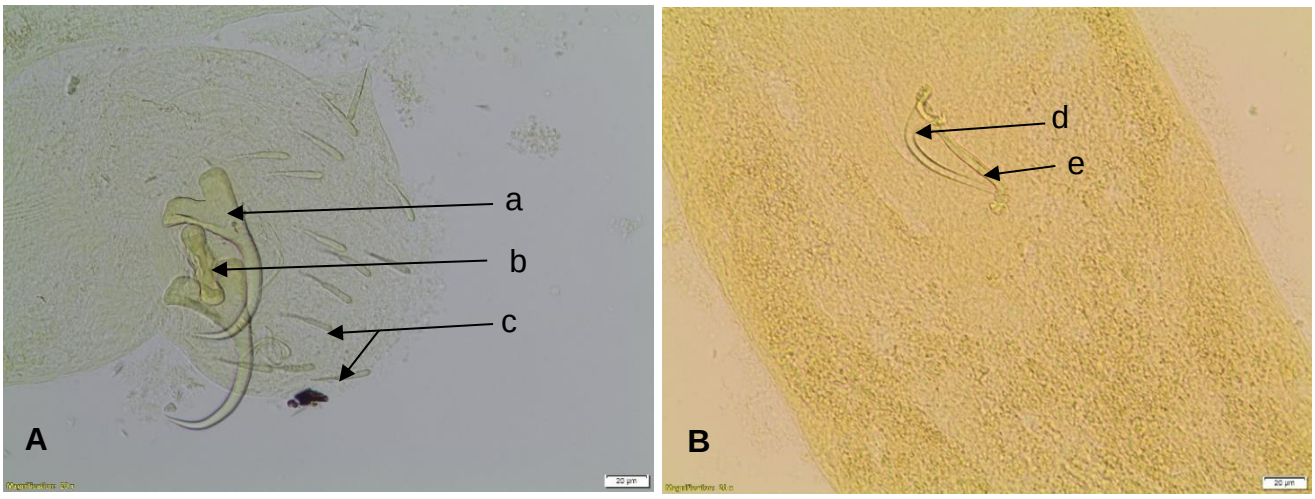


Figure 4.3: Photomicrograph of *Dactylogyrus extensus* removed from the gills of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017. A= haptor: a = anchor, b = bar, c = hooks. B= Male copulatory organ: d = copulatory tube, e = accessory piece. Scale bar = 20 µm.

Table 4.3: Number of *Dactylogyrus extensus* specimens collected from the gills of *Cyprinus carpio* during autumn, winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	2	2	34
Winter	7	5	38
Spring	1	1	1
Summer	11	8	92

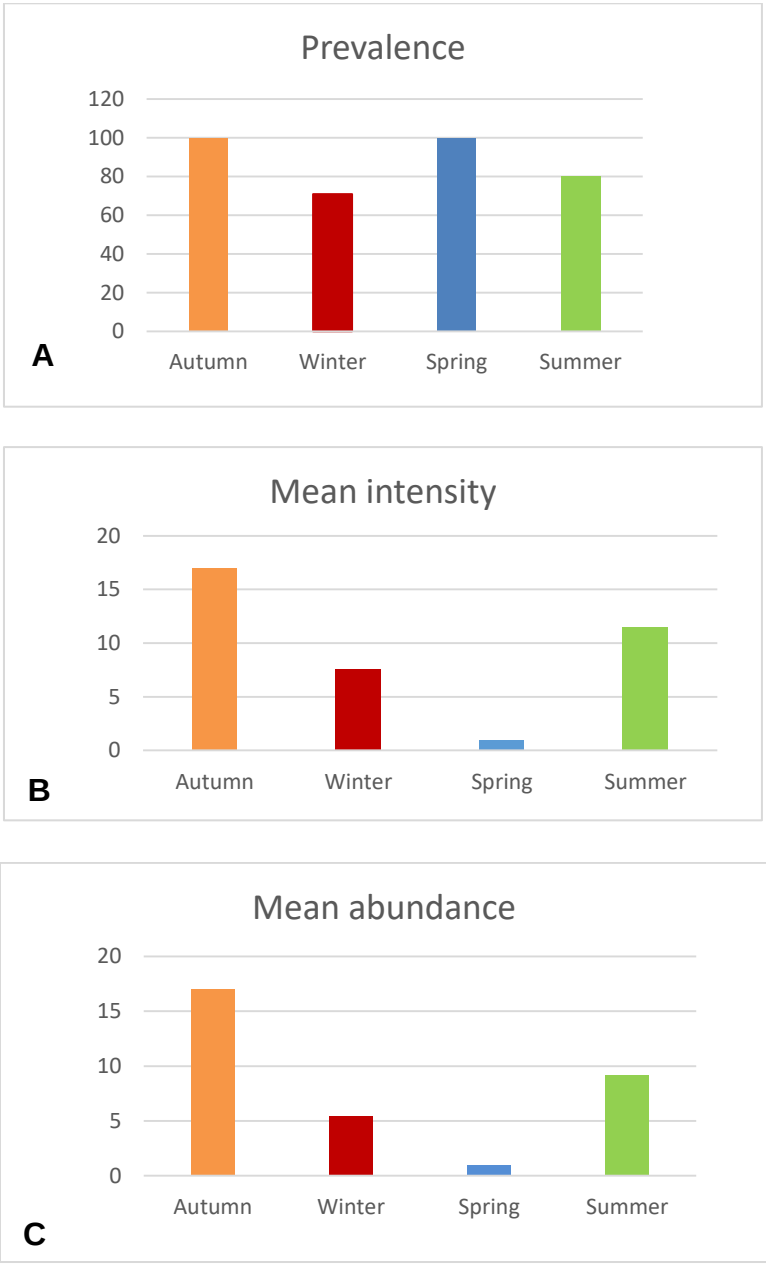


Figure 4.4: The prevalence (A), mean intensity (B) and mean abundance (C) of *Dactylogyrus extensus* collected from the gills of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017.

Dactylogyrus minutus

CLASS: Monogenea

ORDER: Monopisthocotylea

FAMILY: Dactylogyridae

GENUS: *Dactylogyrus* Diesing, 1850

SPECIES: *Dactylogyrus minutus* Kulwiec, 1927

Dactylogyrus minutus (Figure 4.5) was collected from the gills of *Cyprinus carpio*. A higher prevalence of this parasite was reported during autumn. Higher values of mean intensity and mean abundance were reported during summer (Figure 4.6).

Morphology

The morphology of this parasite conforms to the description by Dove and Ernst (1998). The body is slender and flattened. Two pairs of eyespots present. Large connecting bar with rounded and enlarged ends. Inner roots of the anchors are longer than outer roots.

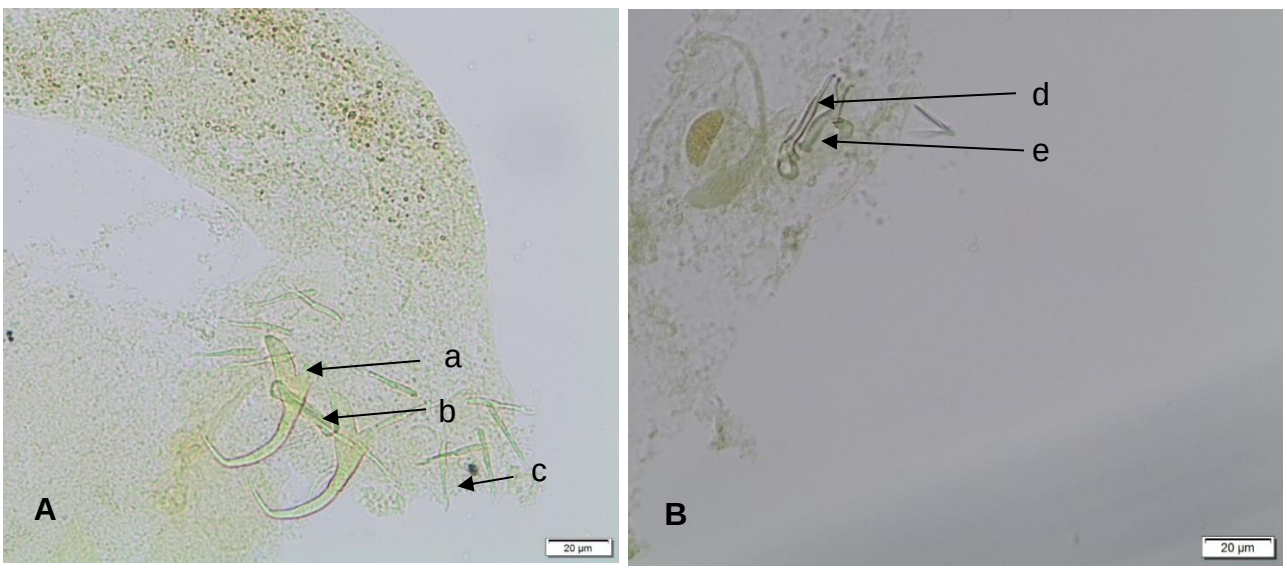


Figure 4.5: Photomicrograph of *Dactylogyrus minutus* collected from the gills of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017. A = haptor: a = anchor, b = bar, c = hooks; B = male copulatory organ: d = copulatory organ, e = accessory piece. Scale bar = 20 µm.

Table 4.4: Number of *Dactylogyrus minutus* collected from the gills *Cyprinus carpio* during autumn, winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	2	2	8
Winter	7	6	15
Spring	1	0	0
Summer	11	7	40

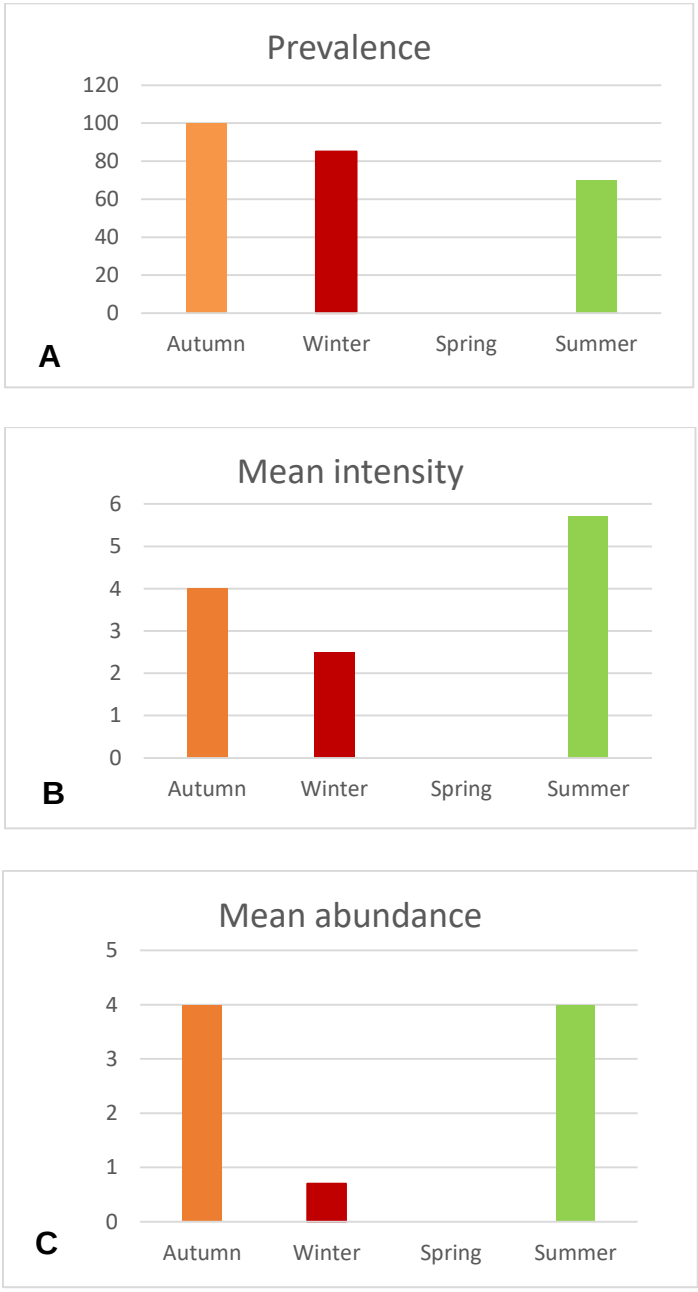


Figure 4.6: The prevalence (A), mean intensity (B) and mean abundance (C) of *Dactylogyrus minutus* collected from the gills of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017.

4.4.1.2 Digenea

***Diplostomum* sp.**

CLASS: Digenea

ORDER: Strigeatida

FAMILY: Diplostomidae

GENUS: *Diplostomum* Nordmann, 1832

Diplostomum sp. metacercaria (Figure 4.7) was collected from the eyes of *Cyprinus carpio*. This parasite was only found during autumn (Table 4.5).

Morphology

The morphology of this parasite conforms to the description by Kircalar and Soylu (2014). Body shape is oval and divided into two parts. The anterior part bears pseudosuckers on each side of the oral sucker. Both oral and ventral suckers are present. The ventral sucker of *Diplostomum* sp. metacercariae is positioned half way along the body.

Remarks

Metacercaria lack reproductive features that are useful in identification. Thus the parasite could not be identified to species level. Identification of these parasites is problematic due to the presence of morphologically similar species; the phenotypic plasticity of the adults and metacercariae; the simple larval morphology and the difficulties in linking life cycle stages.

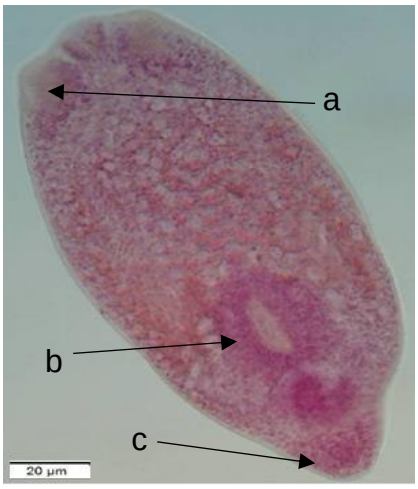


Figure 4.7: Photomicrograph of *Diplostomum* species collected from the eyes of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017. a = pseudosuckers, b = tribocytic organ and c = excretory pore.

Table 4.5: Number of *Diplostomum* species collected from *Cyprinus carpio* during autumn, winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	2	0	0
Winter	7	4	16
Spring	1	0	0
Summer	11	0	0

4.4.1.3 Cestoda

Atractolytocestus huronensis

CLASS: Cestoda

ORDER: Caryophyllidea

FAMILY: Lytocestidae

GENUS: *Atractolytocestus* Anthony, 1958

SPECIES: *Atractolytocestus huronensis* Anthony, 1958

Atractolytocestus huronensis (Figure 4.8) was collected from the intestine of *Cyprinus carpio*. The parasite was only found during autumn, winter and summer. Higher prevalence, mean intensity and mean abundance values were recorded during autumn (Figure 4.9). The number of *A. huronensis* collected during seasonal surveys is presented in Table 4.6.

Morphology

The morphology of this parasite conforms to the description by Scholz *et al*. (2015). Arrowhead shaped scolex. The first testes begin posterior to the anterior end. Vitelline follicles are uninterrupted alongside the uterine region and lateral lobes of the ovary and posterior extent of testes reaching up to the ovary. The ovary is H-shaped.

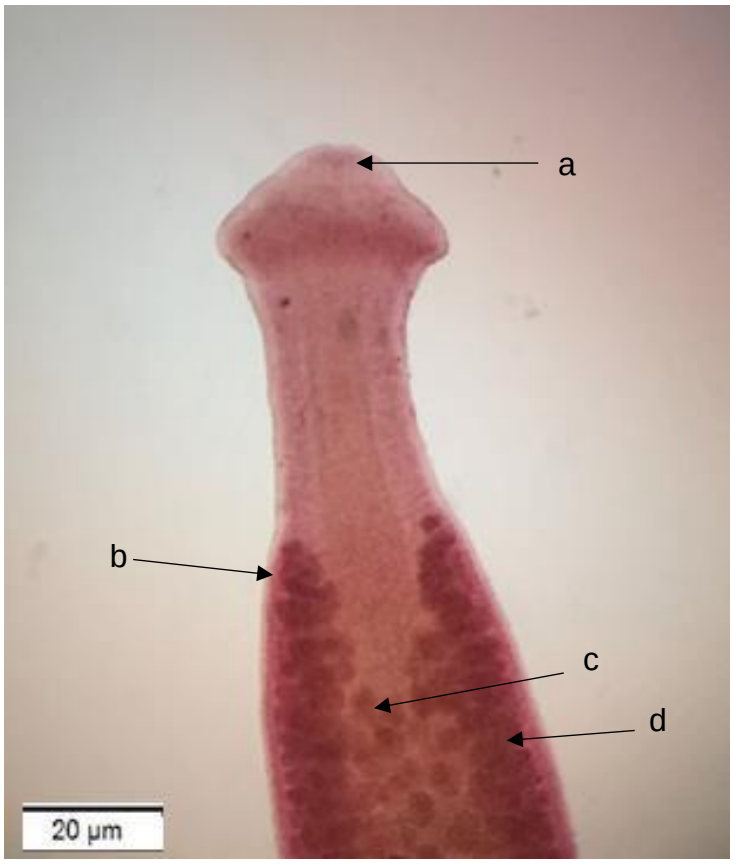


Figure 4.8: Photomicrograph of *Atractolytocestus huronensis* collected from the body cavity of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017. a = scolex, b = vitelline follicles, c = testis and d = ovarian lobes.

Table 4.6: Number of *Atractolytocestus huronensis* collected from the body cavity of *Cyprinus carpio* during autumn, winter (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	2	1	37
Winter	7	2	10
Spring	1	0	0
Summer	11	2	7

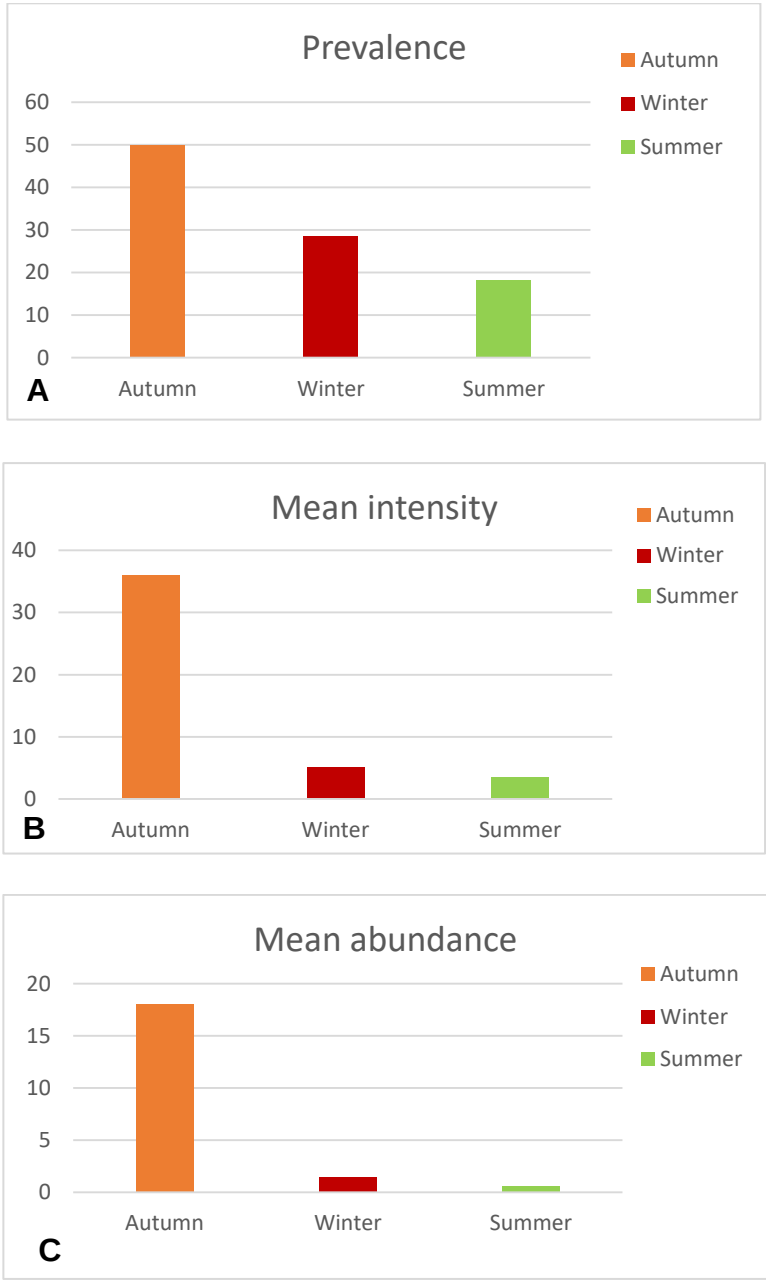


Figure 4.9: The prevalence (A), mean intensity (B) and mean abundance (C) of *Atractolytocestus huronensis* collected from the gills of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017.

4.4.1.4 Copepoda

CLASS: Copepoda

FAMILY: Ergasilidae

GENUS: *Neoergasilus* Yin, 1956

SPECIES: *N*eoergasilus *japonicus* Harada, 1930

Neoergasilus japonicus (Figure 4.10) was collected from the skin of *Cyprinus carpio* during autumn. Only one specimen of this parasite was recorded (Table 4.7).

Morphology

The morphology of this parasite conforms to the description by Abdelhalim *et al*. (1993). The body consists of four thoracic segments and the fifth segment is reduced. The third segment bears a short seta located on the dorsal surface.

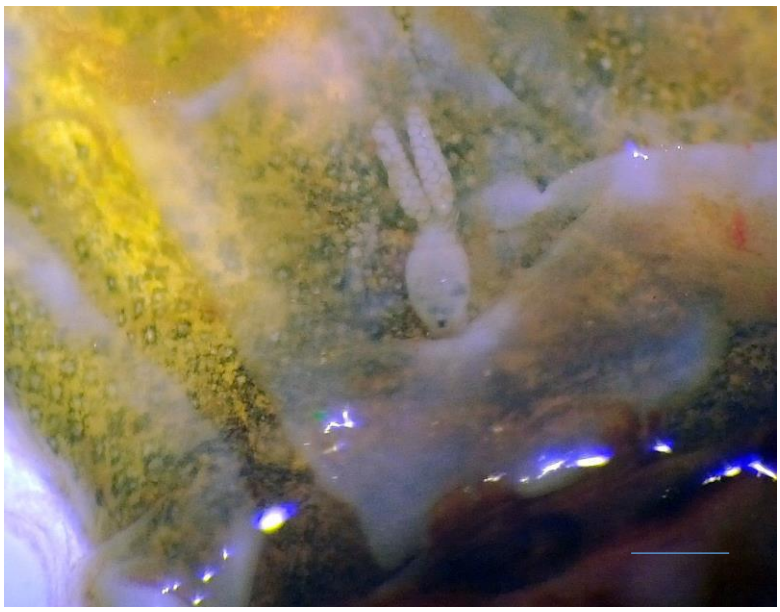


Figure 4.10: Photomicrograph of *Neoergasilus japonicus* collected from the skin of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017. Scale bar = 20 µm.

Table 4.7: Number of *Neoergasilus japonicus* collected from *Cyprinus carpio* during autumn, winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	2	1	1
Winter	7	0	0
Spring	1	0	0
Summer	11	0	0

4.4.1.5 Branchiura

Argulus japonicus

CLASS: Branchiura

ORDER: Arguloida

FAMILY: Argulidae

GENUS: *Argulus* Muller, 1785

SPECIES: *Argulus japonicus* Thiele, 1900

Argulus japonicus (Figure 4.11) was collected from the skin of *Cyprinus carpio* during winter, spring and summer. No specimens were collected during autumn (Table 4.8). A higher prevalence was recorded during spring and higher mean intensity and mean abundance were recorded during summer (Figure 4.12).

Morphology

The morphology of this parasite conforms to the description by Gresty *et al*. (1993). The body is dorso-ventrally flattened and ovoid bi-lobed covered by a carapace. A pair of compound eyes is present anteriorly. The abdomen is bi-lobed, unsegmented and bear small furcal rami close to the anal opening. The first and second antennae are small and positioned closely together. First maxillae developed into suction discs.

Figure 4.11: Photomicrograph of *Argulus japonicus* collected from the skin and fins of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017. Scale bar = 20 µm.

Table 4.8: Number of *Argulus japonicus* collected from *Cyprinus carpio* during winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	2	0	0
Winter	7	6	15
Spring	1	1	1
Summer	11	8	42

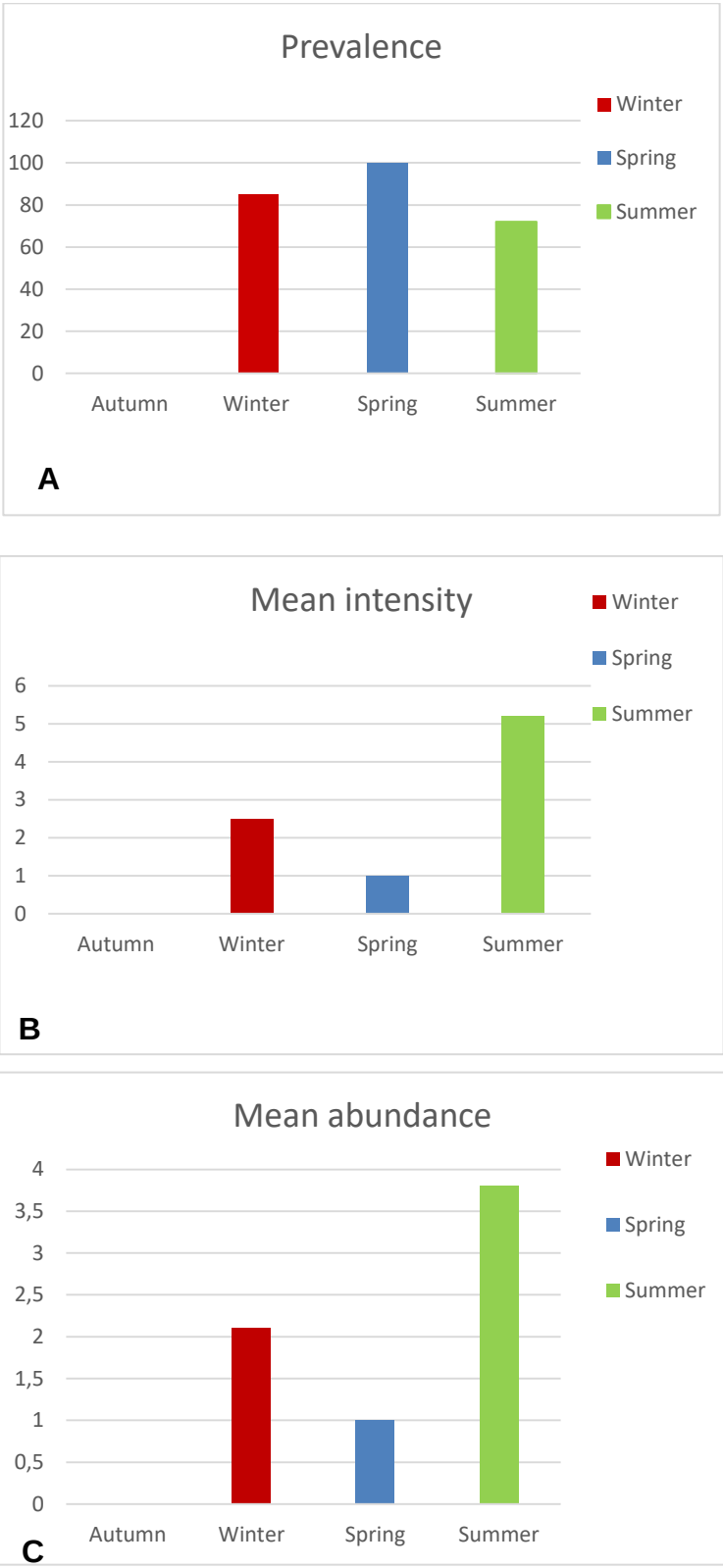


Figure 4.12: The prevalence (A), mean intensity (B) and mean abundance (C) of *Argulus japonicus* collected from the skin of *Cyprinus carpio* from April 2016 to February 2017.

Dolops ranarum

CLASS: Branchiura

ORDER: Arguloida

FAMILY: Argulidae

GENUS: *Dolops* Audouin, 1837

Species: *D*olops *ranarum* Stuhlmann, 1892

Dolops ranarum (Figure 4.13) was the most common parasite recorded among the three fish species. The parasite was collected from the skin, fins and gills of the hosts. Only two specimens of *D. ranarum* were collected from the gills of *Cyprinus carpio* during autumn. No specimens were collected during winter, spring and summer on *Cyprinus carpio* (Table 4.9).

Morphology

The morphology of this parasite conforms to the description by Avenant-Oldewage *et al*. (1989). Compound eyes present on the dorsal surface. The cephalothorax and abdomen are covered by a horseshoe-shaped carapace. Carapace almost round with a deep incision posteriorly. Scales absent on the surface of the carapace. The cephalon is dorsally covered by carapace. The antennulae and antennae are situated ventrally in grooves on the cephalon. Each thoracic segment bear swimming legs.

Figure 4.13: Photomicrograph of *Dolops ranarum* collected from the skin and fins of *Cyprinus carpio* during seasonal surveys from April 2016 to February 2017. Scale bar = 20 µm.

Table 4.9: Number of *Dolops ranarum* collected from *Cyprinus carpio* during autumn (2016).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	2	2	2
Winter	7	0	0
Spring	1	0	0
Summer	11	0	0

4.4.2 Parasites of *Clarias gariepinus*

4.4.2.1 Protozoa

***Trypanosoma* sp**.

CLASS: Kinetoplastida

ORDER: Trypanosomatida

FAMILY: Trypanosomatidae

GENUS: *Trypanosoma* Gruby, 1843

Trypanosoma sp. (Figure 4.14) was reported from the blood of *Clarias gariepinus* only during winter. No specimens were recorded for autumn, spring and summer (Table 4.10). The prevalence, mean intensity and mean abundance are presented in Figure 4.15.

Morphology

The morphology of this parasite conforms to the description by Davies *et al.* (2005). Body elongated and slender. A single free flagellum at the anterior end of the body. The nucleus is oval in shape. The kinetoplast is positioned at the terminal end, at the base of the flagellum.

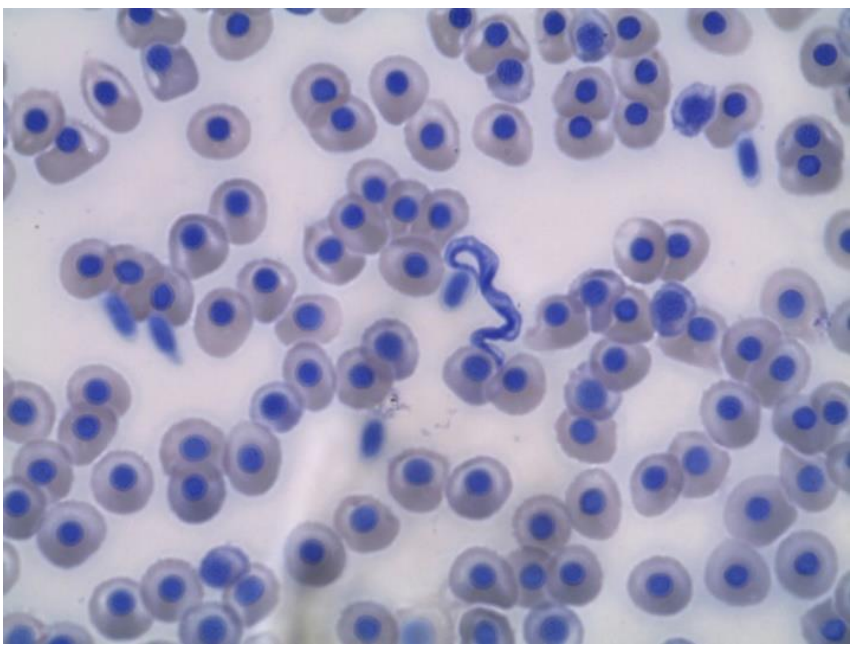
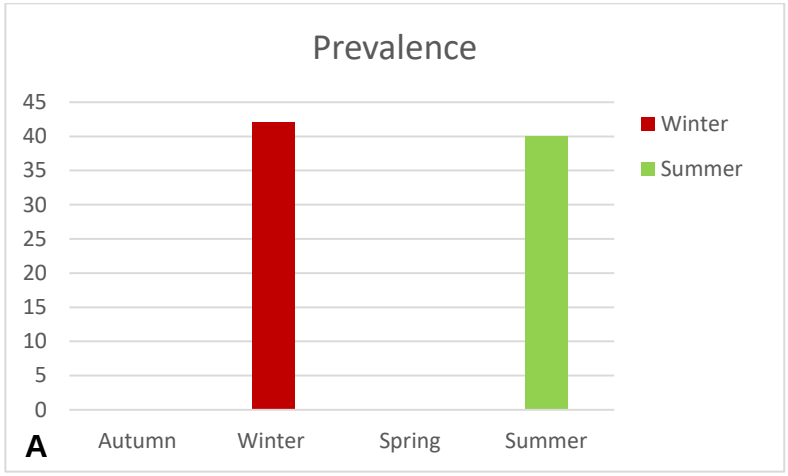
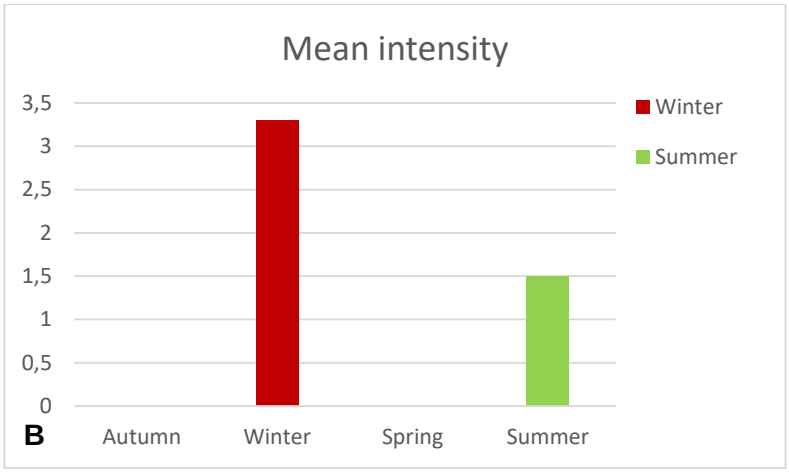


Figure 4.14: Photomicrograph of *Trypanosoma* species recorded from the blood of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017. Scale bar = 20 µm.

Table 4.10: Number of *Trypanosoma* species collected from *Clarias gariepinus* during winter (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	3	0	0
Winter	7	3	10
Spring	6	0	0
Summer	11	4	6





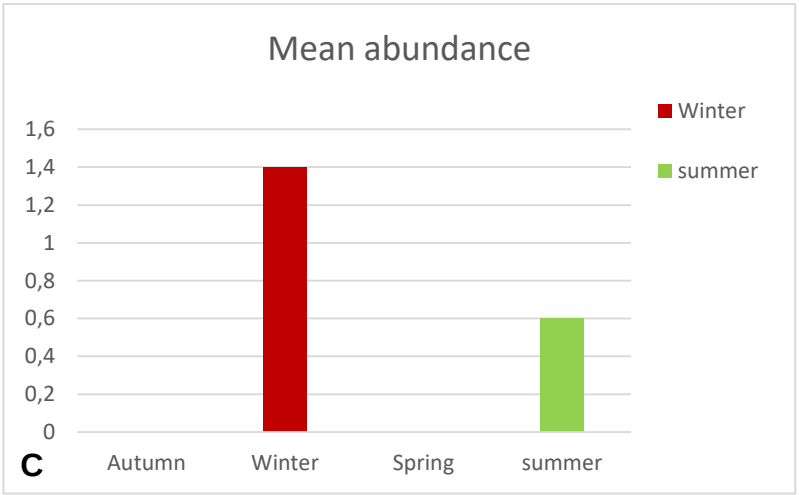


Figure 4.15: The prevalence (A), mean intensity (B) and mean abundance (C) of *Trypanosoma* species collected from the blood of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017.

4.4.2.2 Monogenea

***Quadriacanthus* sp.**

CLASS: Monogenea

ORDER: Monopisthocotylea

FAMILY: Dactylogyridae

GENUS: *Quadriacanthus* Paperna, 1961

Quadriacanthus sp. (Figure 4.16 & 4.17) was collected from the gills of *Clarias gariepinus*. This parasite was recorded during autumn, winter and summer (Table 4.11). Higher prevalence, mean intensity and mean abundance values were recorded during autumn (Figure 4.18).

Morphology

The morphology of this parasite conforms to the description by Francova *et al*. (2017). Ventral anchor smaller in length and more curved (C-shaped) than dorsal anchors ventral bar articulates medially. Two unequal bars, each with a solid base attached to narrower appendages. Straight copulatory tube and short accessory piece. Dissimilar and unequal pairs of marginal hooklets.

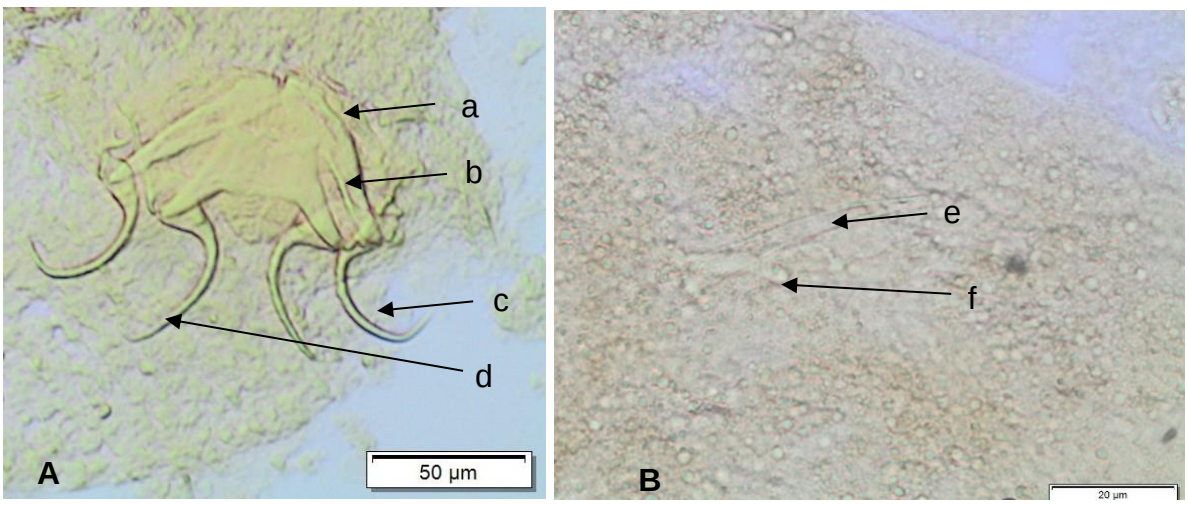


Figure 4.16: Photomicrograph of *Quadriacanthus* species collected from the gills of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017**. A** haptor: a = ventral bar, b = dorsal bar, c = ventral anchor, d = dorsal anchor. **B** male copulatory organ: e = copulatory tube, f = accessory piece. **B** scale bar = 20 µm.

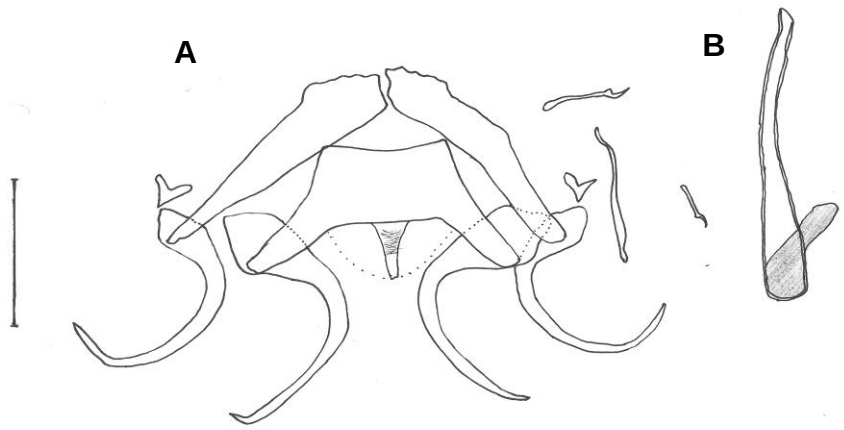
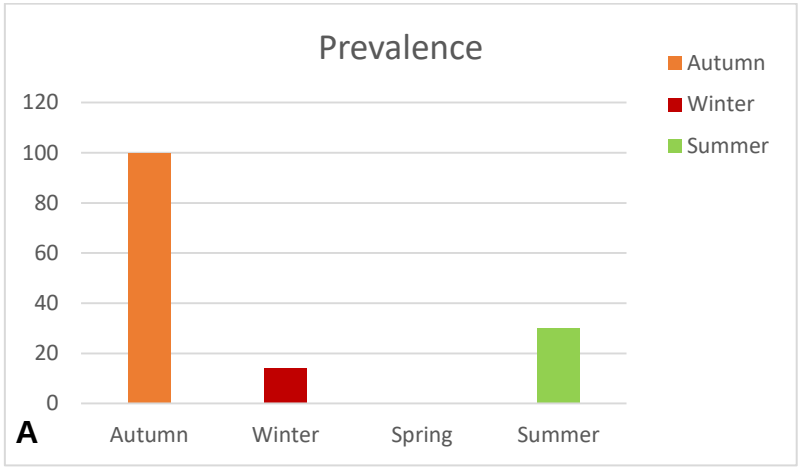
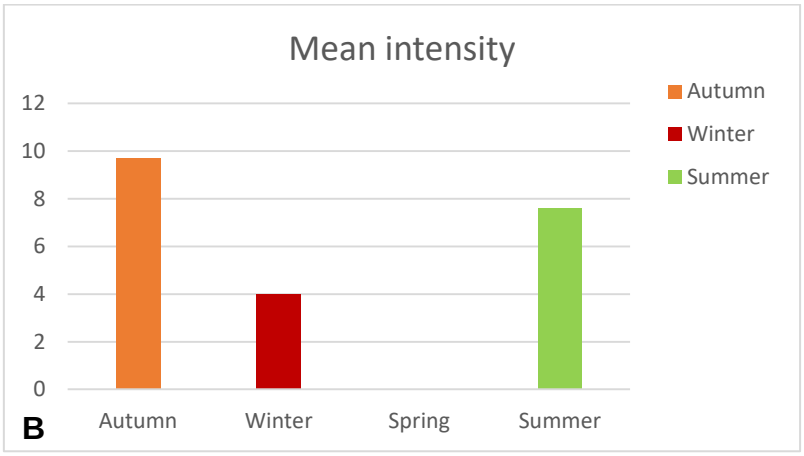


Figure 4.17: Microscope drawings of *Quadriacanthus* species collected from the gills of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017. Scale bar = 20 µm. A = haptor and B = male copulatory organ.

Table 4.11: Number of *Quadriacanthus* species collected from *Clarias gariepinus* during autumn, winter (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	3	3	29
Winter	7	1	4
Spring	6	0	0
Summer	10	3	23





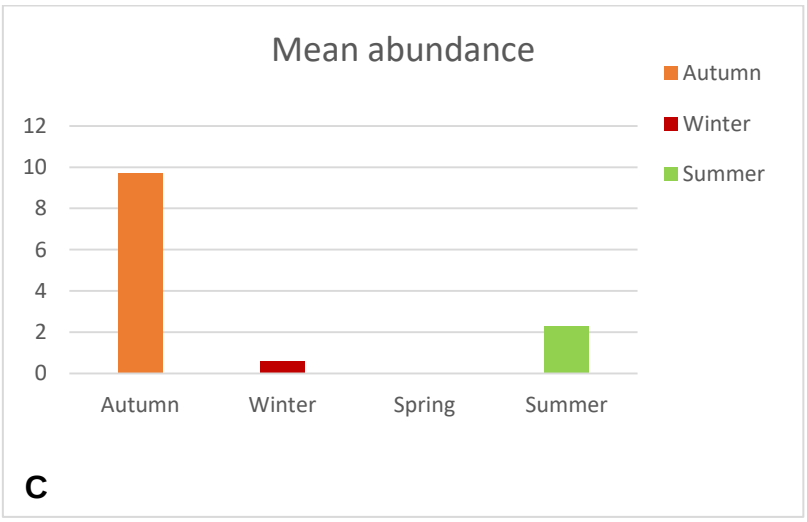


Figure 4.18: The prevalence (A), mean intensity (B) and mean abundance (C) of *Quadriacanthus* species collected from the gills of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017.

***Gyrodactylus* sp.**

CLASS: Monogenea

ORDER: Monopisthocotylea

FAMILY: Gyrodactylidae

GENUS: *Gyrodactylus* von Nordmann, 1832

Gyrodactylus sp. (Figure 4.19) was collected from the gills of *Clarias gariepinus*. This parasite was only recorded during autumn and summer (Table 4.12). Higher prevalence, mean intensity and mean abundance were recorded during autumn (Figure 4.20). The morphology of this parasite conforms to the description by Prikrylova *et al.* (2012).

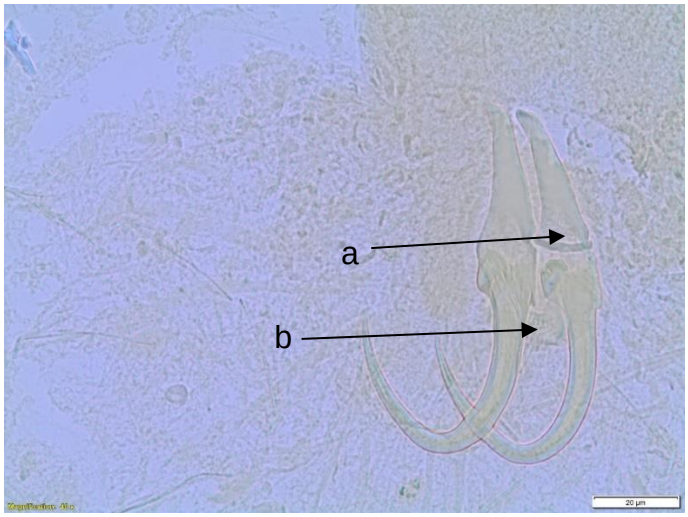


Figure 4.19: Photomicrographs of haptor of *Gyrodactylus* species collected from the gills *of Clarias gariepinus* during seasonal surveys from April 2016 to February 2017*.* a = anchor and b = membranous extension of the ventral bar. Scale bar = 50 µm.

Table 4.12: Number of *Gyrodactylus* species collected from *Clarias gariepinus* during autumn (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	3	3	19
Winter	7	0	0
Spring	6	0	0
Summer	10	1	4

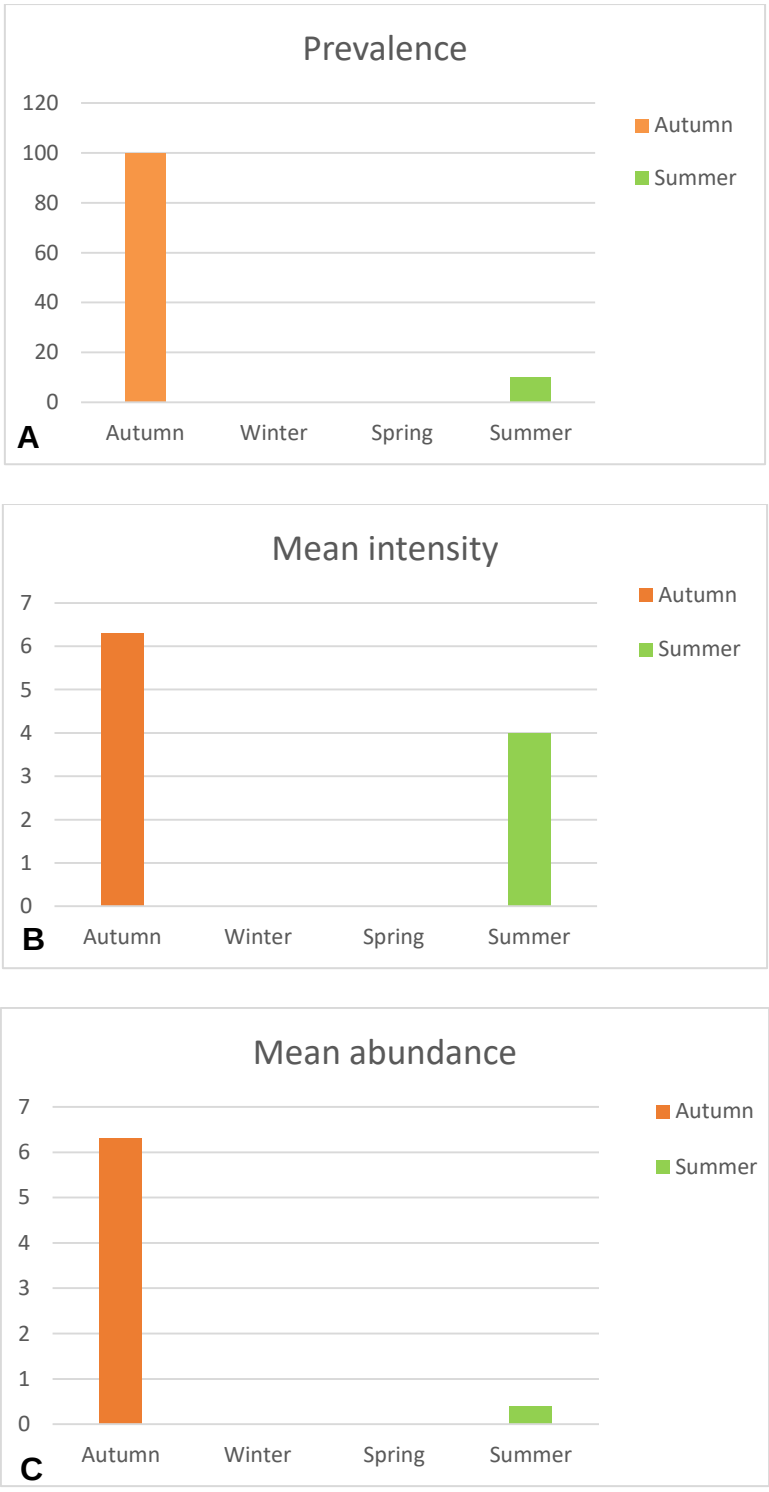


Figure 4.20: The prevalence (A), mean intensity (B) and mean abundance (C) of *Gyrodactylus* species collected from the gills of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017.

4.4.2.3 Nematoda

Paracamallanus cyathopharynx

CLASS: Nematoda

ORDER: Camallanida

FAMILY: Camallanidae

GENUS: *Paracamallanus* Yorke & Maplestone, 1926

SPECIES: *Paracamallanus cyathopharynx* Baylis, 1923

Paracamallanus cyathopharynx (Figure 4.21) was found in the intestine of examined fish during winter, spring and summer. No parasites were recorded during autumn (Table 4.13). Higher prevalence, mean intensity and mean abundance for *P. cyathopharynx* were recorded during spring (Figure 4.22).

Morphology

The morphology of this parasite conforms the description by Mashego (1977). Elongated body. Paired buccal valves consisting of 10 – 11 longitudinal ribs of irregular lengths. Oesophagus consist of both muscular and glandular portions (Figure 4.21A). Uterus filled with eggs and larvae. Vulva without prominent lips and situated behind the middle of the body (Figure 4.21B).

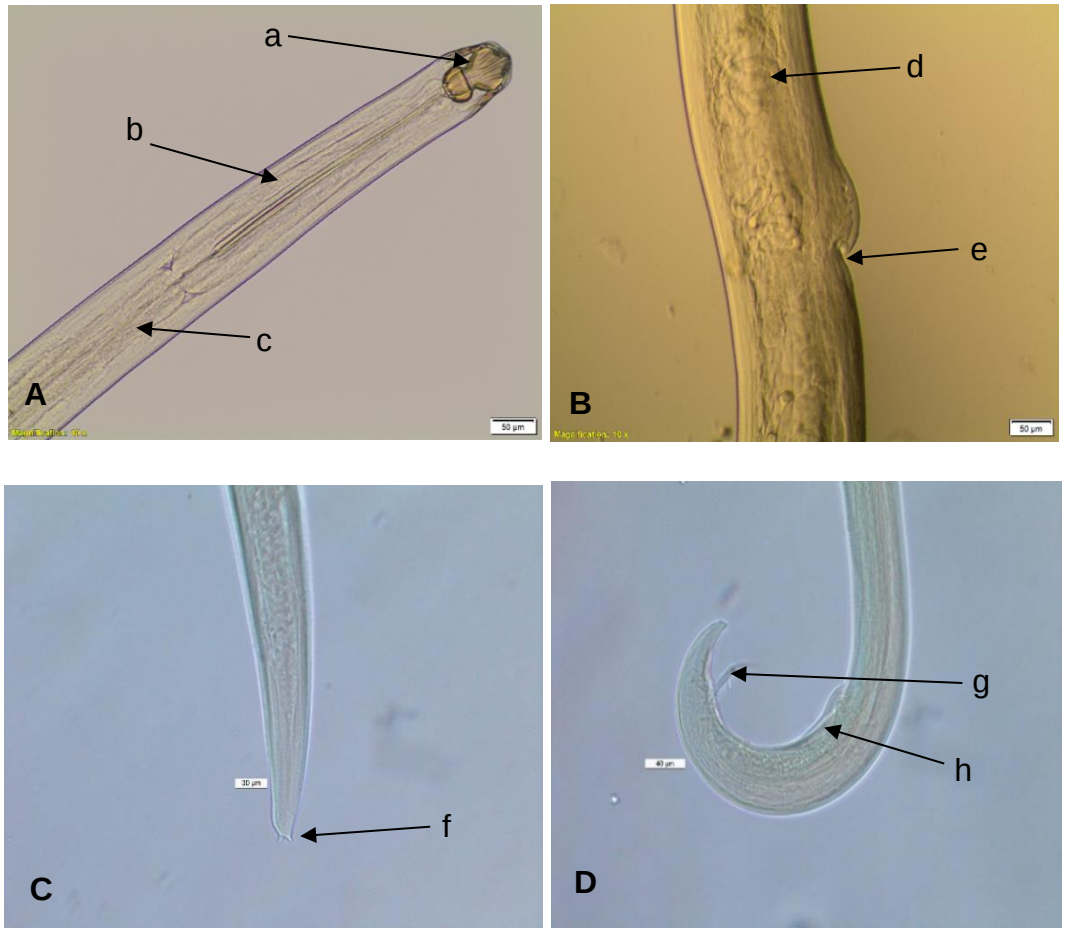
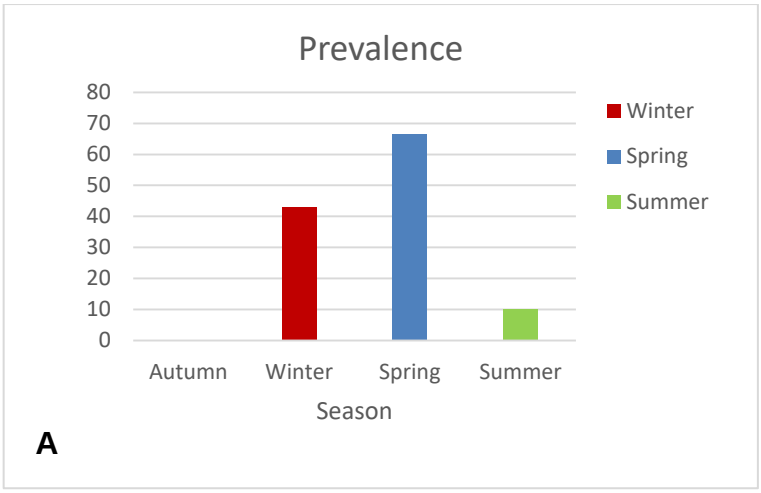
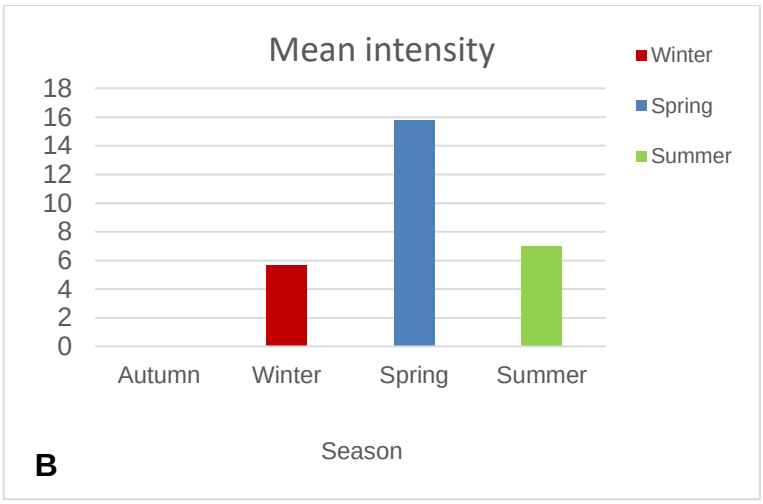


Figure 4.21: Photomicrographs of *Paracamallanus cyathopharynx* collected from the intestine of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017. A = anterior end: a = buccal capsule with cuticlar ribs, b = muscular esophagus, c glandular esophagus = B = middle part: d = uterus with larvae, e = vulva. C = posterior end: f = anal process. D = male posterior end: g = spicule, h = anus. A and B scale bar = 50 µm, C and D scale bar = 20 µm.

Table 4.13: Number of *Paracamallanus cyathopharynx* collected from *Clarias gariepinus* during winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	3	0	0
Winter	7	3	17
Spring	6	4	63
Summer	10	1	7





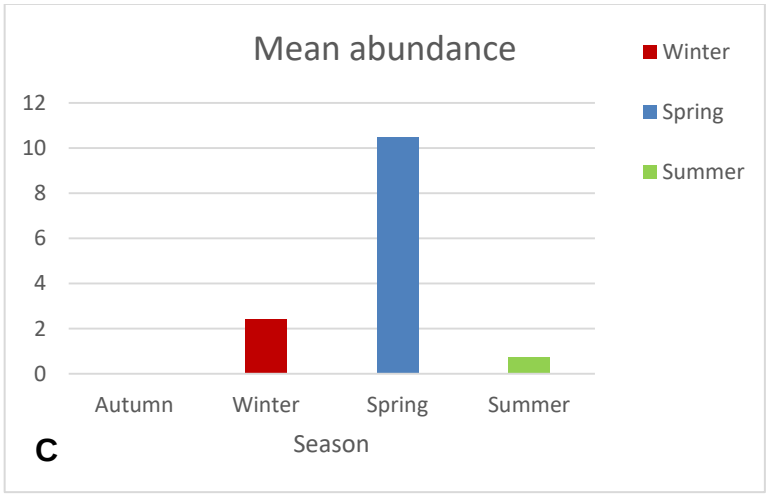


Figure 4.22: The prevalence (A), mean intensity (B) and mean abundance (C) of *Paracamallanus cyathopharynx* collected from the intestine of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017.

***Contracaecum* sp.**

CLASS: Nematoda

ORDER: Ascaridida

FAMILY: Anisakidae

GENUS: *Contracaecum* Railliet & Henry, 1912

Contracaecum sp. larva (Figure 4.23) was collected from the body cavity of *Clarias gariepinus*. The parasite was only recorded during autumn, winter and summer (Table 4.14). A higher prevalence value was recorded during autumn. Higher mean intensity and mean abundance values were recorded during winter (Figure 4.24).

Morphology

The morphology of this parasite conforms to the description by Garbin *et al*. (2013).The body is elongated and whitish in colour. Cuticle transversely striated. The anterior end is rounded. The mouth with three lips. The dorsal lip has two lateral papillae. Mouth with three lips (Figure 4. 23A). The two ventro-lateral lips have a small papilla in each. A cephalic tooth is situated between lips. The esophagus is narrow. The anterior intestinal caecum is long, extending to the level of the nerve ring. The nerve ring surrounding the esophagus is located at the first third of its length. The tail is conical and short with terminal spine (Figure 4.23B).

Remarks

Important taxonomic characteristics for identifying anisikid larvae include appearance of the cephalic papillae, location of the excretory pore, the presence of ventricular appendices and intestinal caecum. Identification of *Contracaecum* larva, particularly to species level is not usually feasible, as the larvae lack genital systems and several other features of adult stages which are used as taxonomic criteria. Thus the *Contracaecum* larva in the present study could not be identified to species level.

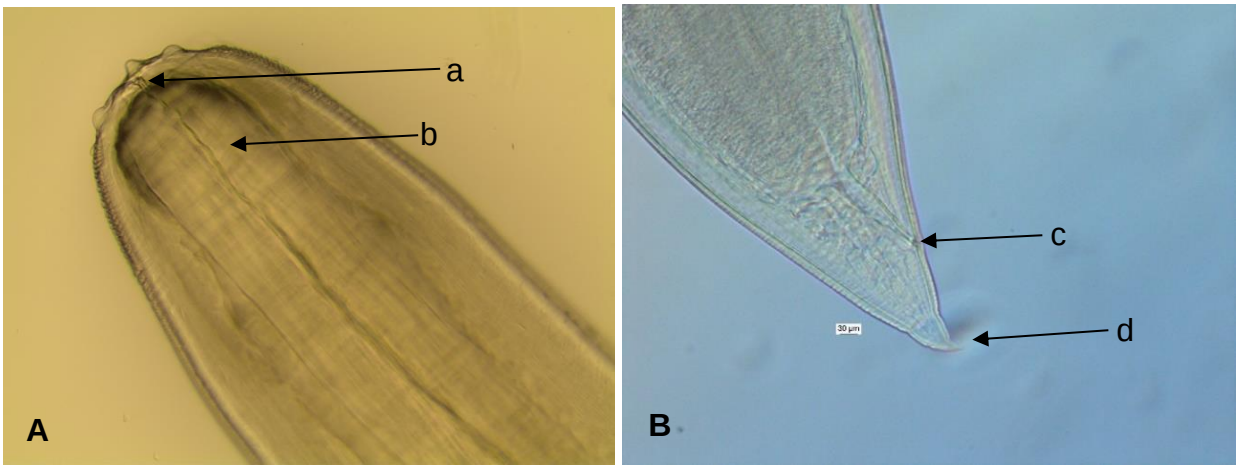
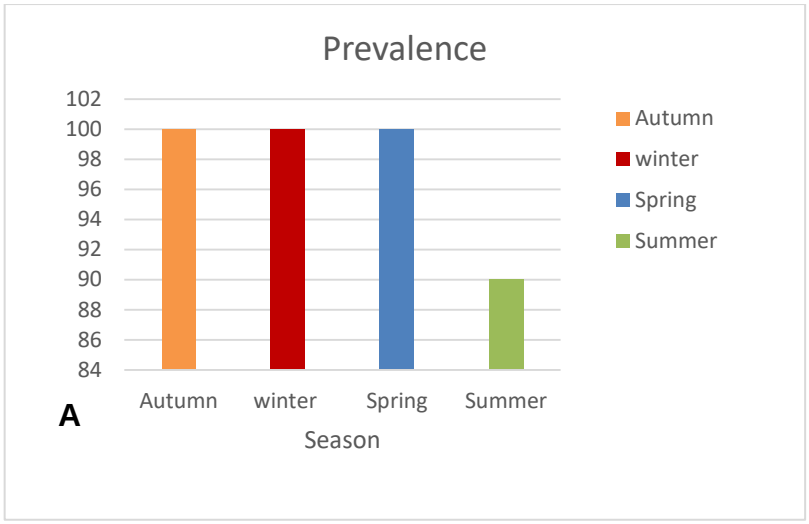
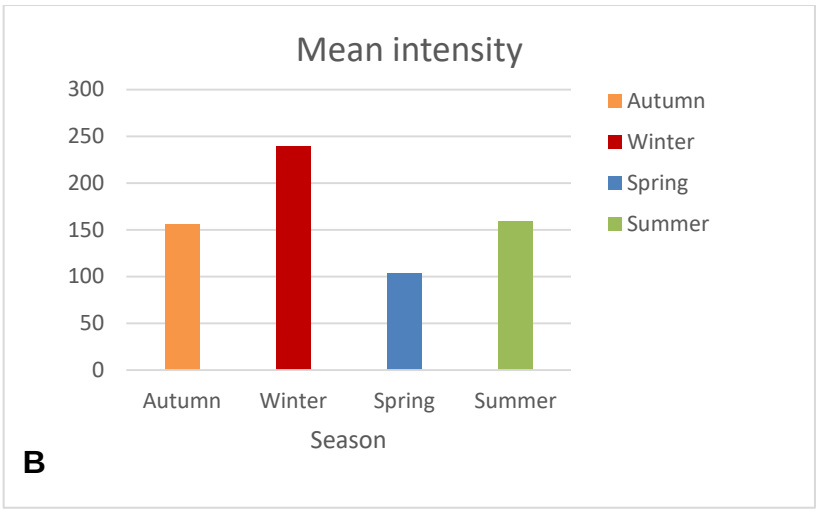


Figure 4.23: Photomicrographs of *Contracaecum* species larvae collected from the body cavity of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017. A = anterior end: a = lip, b = esophagus. B = posterior end: c = anus, d = terminal spine. Scale bar = 50 µm.

Table 4.14: Number of *Contracaecum* species collected from *Clarias gariepinus* during autumn, winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	3	3	466
Winter	7	7	1673
Spring	6	6	618
Summer	10	9	1432





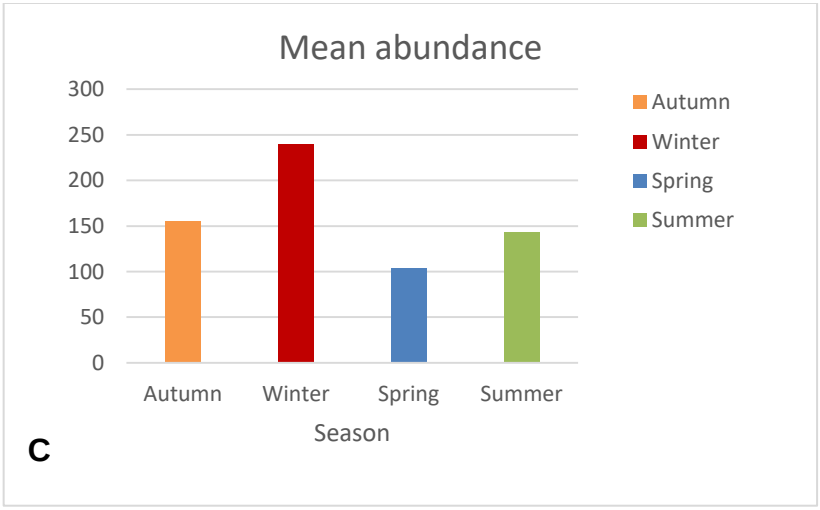


Figure 4.24: The prevalence (A), mean intensity (B) and mean abundance (C) of *Contracaecum* species larvae collected from the body cavity of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017.

4.4.2.4 Branchiura

Dolops ranarum

CLASS: Branchiura

ORDER: Arguloida

FAMILY: Argulidae

GENUS: *Dolops* Audouin, 1837

Species: *Dolops ranarum* Stuhlmann, 1892

Dolops ranarum was collected from the skin and fins of *Clarias gariepinus*. This parasite was only found during winter. The number of collected parasites are presented in Table 4.15.

Morphology

The morphology of the specimens collected from *Clarias gariepinus* was similar to that collected from *Cyprinus carpio*. See page 47.

Table 4.15: Number of *Dolops ranarum* collected from *Clarias gariepinus* during winter (2016).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	3	0	0
Winter	7	2	3
Spring	1	0	0
Summer	6	0	0

4.4.3 Parasites of *Oreochromis mossambicus*

4.4.3.1 Monogenea

***Cichlidogyrus* Paperna, 1960**

Cichlidogyrus halli

CLASS: Monogenea

ORDER: Monopisthocotylea

FAMILY: Dactylogyridae

GENUS: *Cichlidogyrus* Paperna, 1960

SPECIES: *Cichlidogyrus halli* Price & Kirk, 1967

Cichlidogyrus halli (Figure 4.25) was collected from the gills of *O. mossambicus*. A higher prevalence was recorded during winter. Only one fish specimen was collected during the winter survey and it was infected. A higher mean intensity value was recorded in autumn and a higher mean abundance value was recorded during winter (Figure 4.26). Measurements of the haptor are recorded in Table 4.16. The number of collected parasites are presented in Table 4.17.

Morphology

The morphology of this parasite conforms to the description by Douellou (1993). One pair of eyes present. The copulatory organ is simple and long with an S-shaped copulatory tube with irregular basal portion. The accessory piece ends with a triangular extremity. Pairs 1 and 2 of hooklets are smaller than the other five pairs. Very long V-shaped ventral bar with membranous extensions variable in size and shape. Accessory piece elongate but shorter than copulatory tube.

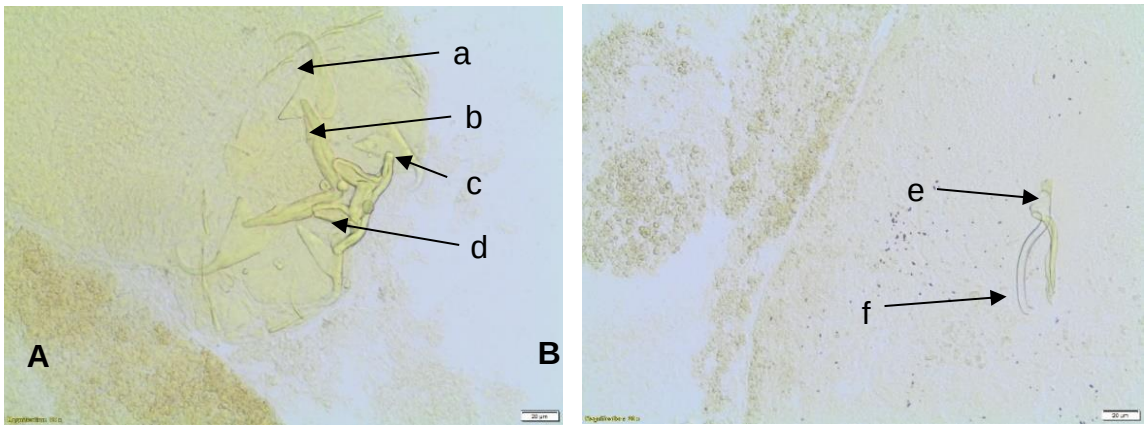


Figure 4.25: Photomicrographs of *Cichlidogyrus halli* collected from the gills of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017. A = haptor: a = ventral anchor, b = ventral bar, c = dorsal anchor, d = dorsal bar. B = male copulatory organ: e = copulatory tube, and f = accessory piece. Scale bar = 20 µm.

Table 4.16: Comparison of measurements of *Cichlidogyrus halli* (µm) collected from the gills *Oreochromis mossambicus* during autumn, winter, spring (2016) and summer (2017) from present study and previous records.

C. halli material	Price and Kirk (1967)	Douellou (1993)	Present study
Host	*O. shiranus*	*O. mortimeri*	*O. mossambicus*
Locality	Malawi	Zimbabwe	South Africa
No of specimens	8	15	6
Total length	525 – 721	700 – 1400	619 – 1094
Width	160 – 205	220 – 340	167 – 338
Ventral bar	104 – 122	104 – 144	105 – 123
Dorsal bar L1	68 – 79	51 – 73	51 – 63
Dorsal bar L2	14	20 – 25	17 – 21
Ventral hamuli	54 – 62	49 – 60	45 – 50
Dorsal hamuli	53 – 60	42 – 56	43 – 46
Hooklets	20 – 44	17 – 43	19 – 37
Copulatory tube	82 – 86	66 – 96	75 – 89
Accessory piece	61 – 67	54 – 66	54 – 64

Table 4.17: Number of *Cichlidogyrus halli* collected from the gills of *Oreochromis mossambicus* during autumn, winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	31	8	37
Winter	1	1	3
Spring	22	16	65
Summer	3	2	8

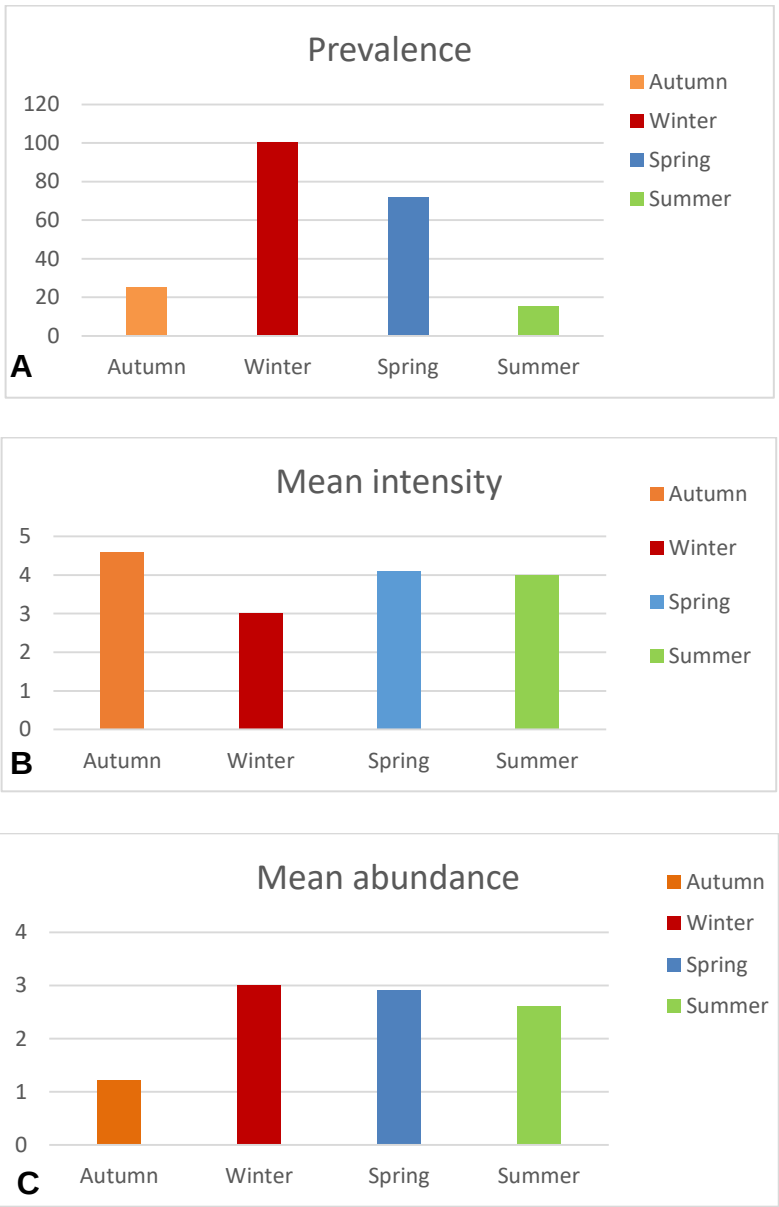


Figure 4.26: The prevalence (A), mean intensity (B) and mean abundance (C) of *Cichlidogyrus halli* collected from the gills of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017.

Cichlidogyrus sclerosus

CLASS: Monogenea

ORDER: Monopisthocotylea

FAMILY: Dactylogyridae

GENUS: *Cichlidogyrus* Paperna, 1960

Species: *Cichlidogyrus sclerosus* Paperna & Thurston, 1969

Cichlidogyrus sclerosus (Figure 4.27) was collected from the gills of *O. mossambicus*. Higher prevalence, mean intensity and mean abundance values were recorded during winter (Figure 4.28). The number of collected parasites are presented in Table 4.18. The measurements of the haptor are presented in Table 4.19.

Morphology

The morphology of this parasite conforms to the description by Douellou (1993). Two eyes are present on the anterior end of the body. The ventral and dorsal anchors are robust and equal in size. The copulatory organ is large, long and thin, with arched copulatory tube attached to a large plate. Accessory piece is massive with protruding finger-like extension. Haptor hardly separated from body, rectangular, narrower than body. Ventral anchor robust; shaft long; point more or less sharp; no distinct roots; base massive. Ventral bar massive, broad, almost U-shaped; extremities round.

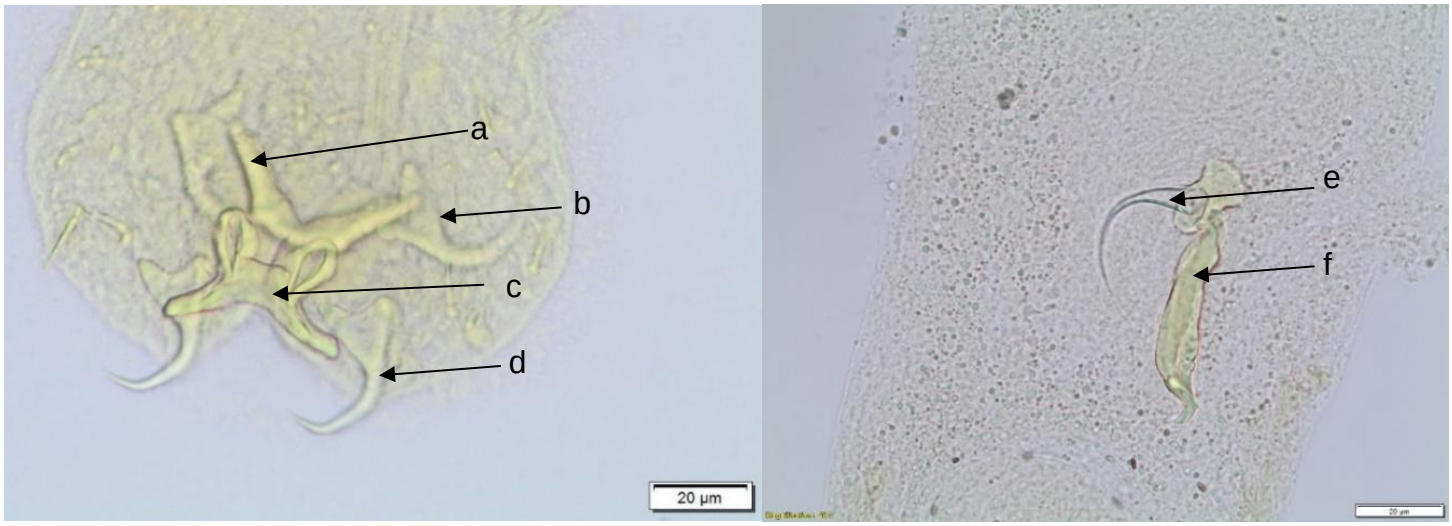


Figure 4.27: Photomicrographs of *Cichlidogyrus sclerosus* collected from the gills of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017. A = haptor: a = ventral bar, b = ventral anchor, c = dorsal bar, d = dorsal anchor. B = male copulatory organ: e = copulatory tube, and f = accessory piece. Scale bar = 20 µm.

Table 4.18: Number of *Cichlidogyrus sclerosus* from *Oreochromis mossambicus* during autumn, winter, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	31	5	16
Winter	1	1	5
Spring	22	7	12
Summer	3	2	13

Table 4.19: Comparison of measurements of *Cichlidogyrus sclerosus* collected from the gills of *Oreochromis mossambicus* from present study and previous records.

C. sclerosus material	Paperna and Thurston (1969)	Douellou (1993)	Present
Host	*O. mossambicus*	*O. mortimeri*	*O. mossambicus*
Locality	Malawi	Zimbabwe	South Africa
No of specimens	13	15	8
Total length	650 – 700	800 – 1400	660 – 810
Width	100 – 200	180 – 300	190 – 265
Ventral bar			
V	42 – 53	31 – 35	40 – 48
E	–	3 – 8	3 – 8
Dorsal bar			
L1	37 – 40	31 – 44	34 – 42
L2	10 – 13	13 – 17	10 – 18
Ventral anchor			
Total length	29 – 37	33 – 36	31 – 36
Shaft	–	32 – 36	28 – 34
Outer root	–	3 – 8	2 – 9
Inner root	–	9 – 14	6 – 12
Tip	–	12 – 15	9 – 15
Dorsal anchor			
Total length	26 – 27	32 – 35	27 – 35
Shaft	–	31 – 35	25 – 32
Outer root	–	4 – 9	6 – 12
Inner root	–	9 – 13	4 – 10
Tip	–	9 – 13	9 – 14
Hooklets	6 – 14	12 – 20	5 –19
Copulatory organ			
Total length	–	66 – 83	65 – 69
Copulatory tube	50 – 60	61 – 75	53 – 66
Accessory piece	39 – 50	49 – 62	39 – 52

– no record available

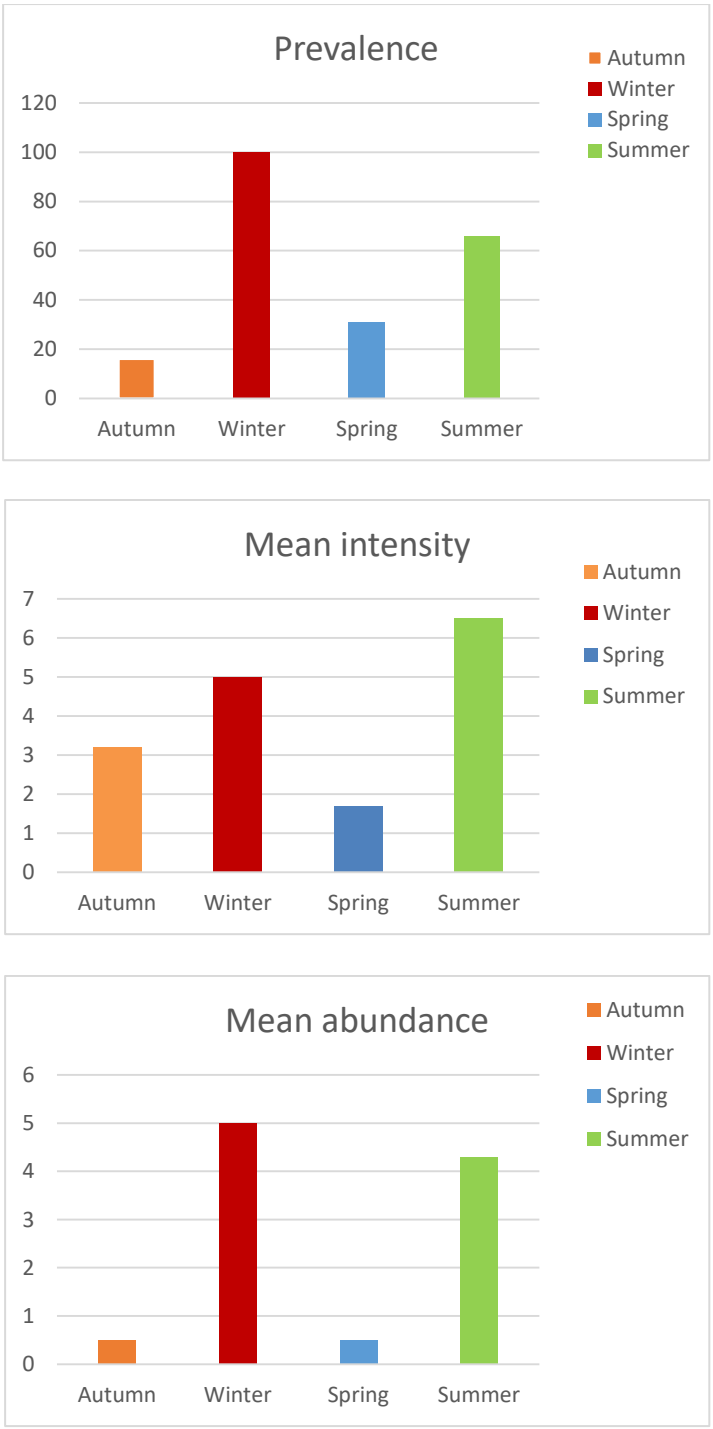


Figure 4.28: The prevalence (A), mean intensity (B) and mean abundance (C) of *Cichlidogyrus sclerosus* collected from the gills of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017.

Cichlidogyrus tilapiae

CLASS: Monogenea

ORDER: Monopisthocotylea

FAMILY: Dactylogyridae

GENUS: *Cichlidogyrus* Paperna, 1960

SPECIES: *C*ichlidogyrus *tilapiae* Paperna, 1960

Cichlidogyrus tilapiae (Figure 4.29) was collected from the gills of *O. mossambicus*. No specimens of this parasite were recorded during autumn, winter and spring (Table 4.21). The measurements of the haptor are presented in Table 4.20.

Morphology

The morphology of this parasite conforms to the description by Douellou (1993). The parasite is small with two eyes. Ventral anchor with broad base and shaft, short point and narrow outer root. Ventral bar thin, U-shaped with well-developed indented membranous extensions and rounded ends. Dorsal bar longer than ventral bar with narrow base, broad shaft point, long and slender inner root. Simple copulatory organ. Copulatory tube straight and wider at the base.

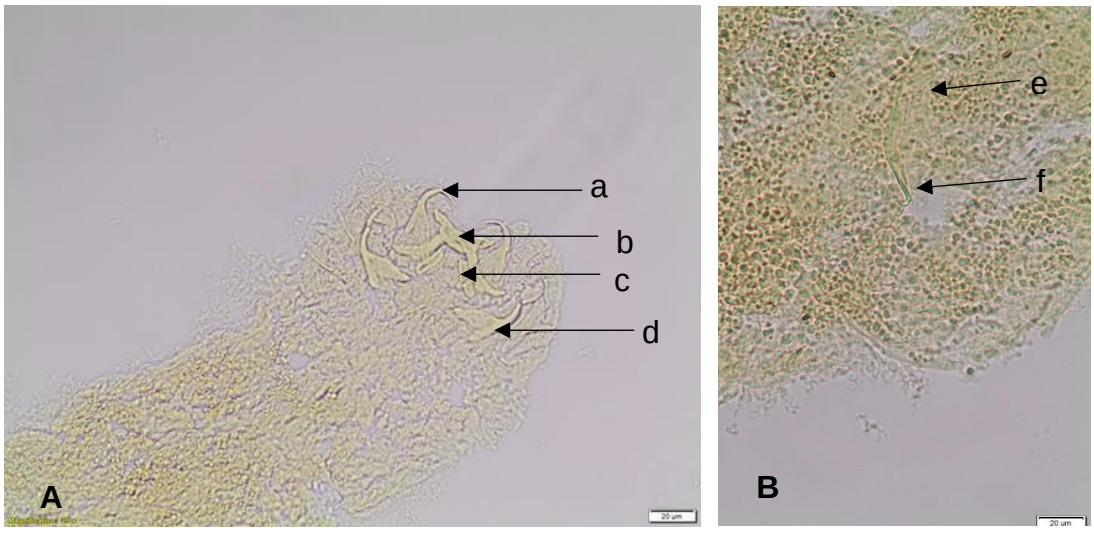


Figure 4.29: Photomicrograph of *Cichlidogyrus tilapiae* collected from the gills of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017. A = haptor: a = dorsal anchor, b = dorsal bar, c = ventral bar, d = ventral anchor. B = male copulatory organ: e = copulatory tube and f = accessory piece. Scale bar 50 µm.

Table 4.20: Comparison of measurements of *Cichlidogyrus tilapiae* collected from the gills of *Oreochromis mossambicus* from present study and previous records.

C. tilapiae material	Paperna and Thurston (1969)	Douellou (1993)	Present
Host	*O. mossambicus*	*O. mortimeri*	*O.mossambicus*
Locality	Malawi	Zimbabwe	South Africa
No of specimens	–	15	8
Total length	160 – 509	400 – 500	270 – 491
Width	30 – 142	90 – 120	80 – 120
Ventral bar			
V	34 – 96	31 – 33	28 – 38
E	–	3 – 5	2 – 5
Dorsal bar			
L1	18 – 38	28 – 30	22 – 36
L2	9 – 19	13 – 17	11 – 20
Ventral anchor			
Total length	26 – 33	32 – 36	27 – 44
Shaft	18 – 26	29 – 31	28 – 32
Outer root	4 – 7	3 – 5	3 – 7
Inner root	18	10 – 14	7 – 16
Tip	7	9 – 12	8 – 11
Dorsal anchor			
Total length	26 – 40	41 – 44	35 – 45
Shaft	18 – 26	27 – 30	25 – 31
Outer root	4 – 7	3 – 5	3 – 7
Inner root	11 – 15	16 – 19	14 – 21
Tip	7 – 10	8 – 11	8 – 12
Hooklets	7 – 21	9 – 19	7 – 18
Copulatory organ			
Total length	26 – 48	–	29 – 40
Copulatory tube	–	30 – 36	30 – 35
Accessory piece	–	31 – 33	28 – 35

– no measurements available

Table 4.21: Number of *Cichlidogyrus tilapiae* recorded from *Oreochromis mossambicus* during spring (2016).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	31	0	0
Winter	1	0	0
Spring	22	6	9
Summer	3	0	0

Enterogyrus conoratus

CLASS: Monogenea

ORDER: Monopisthocotylea

FAMILY: Dactylogyridae

GENUS: *Enterogyrus* Paperna, 1963

SPECIES: *Enterogyrus conoratus* Lambert & Euzet, 1991

Enterogyrus conoratus (Figure 4.30) was collected from the stomach of *O. mossambicus*. No specimens of this parasite were recorded during autumn, winter and summer (Table 4.22).

Morphology

The morphology of this parasite conforms to the description by Madanire-Moyo and Avenant-Oldewage (2014). The body is dorso-ventrally flattened. Thick, transversally-striated tegument around body. Anterior to pharynx are four dorsal ocelli: an anterior pair, small and wider spaced; posterior pair, larger than anterior pair. Pharynx, medio-ventrally positioned. V-shaped crossbar. One pair each of dorsal and ventral anchors.

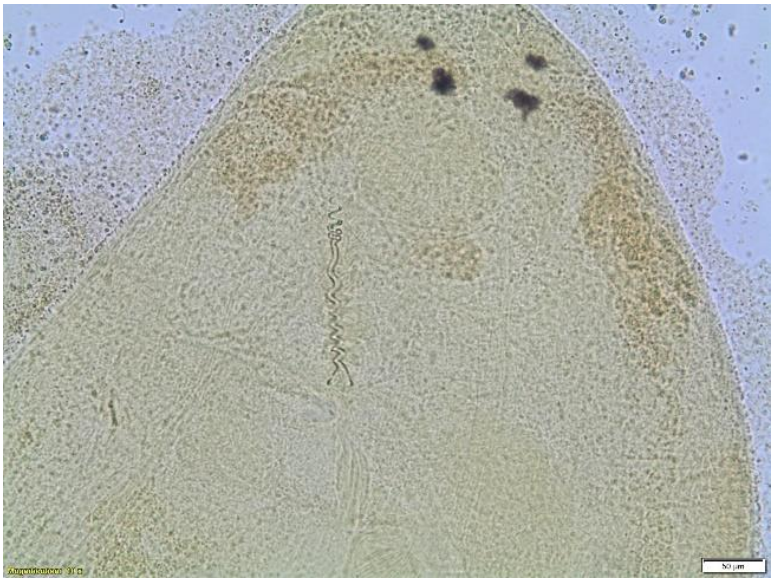


Figure 4.30: Photomicrograph of the anterior part of *Enterogyrus conoratus* collected from the stomach of *Oreochromis mossambicus* during seasonal surveys from 2016 to 2017. Scale bar = 20 µm.

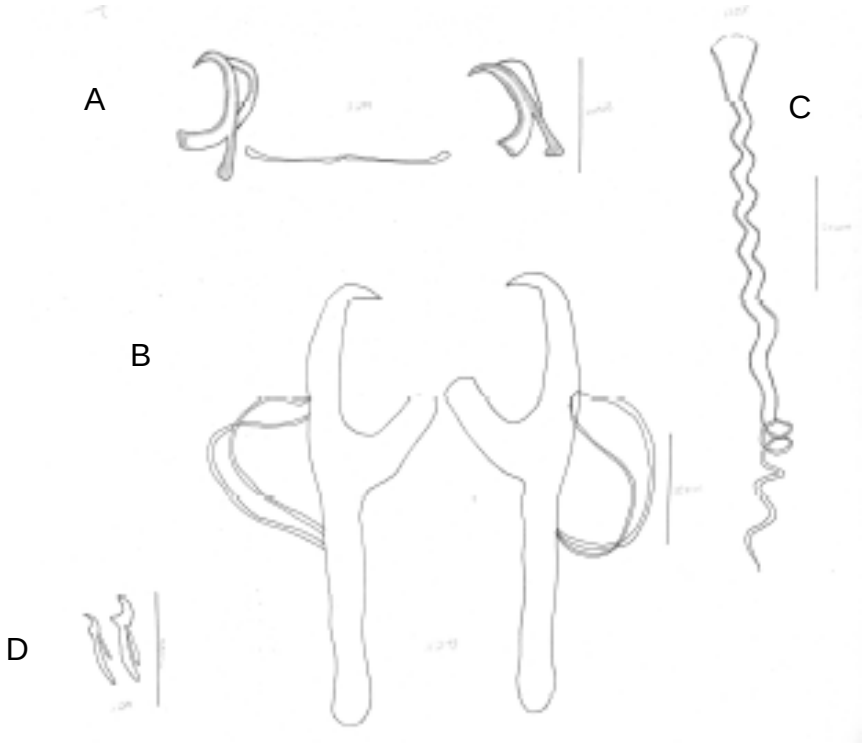


Figure 4.31: Microscope drawings of the haptor sclerites of *Enterogyrus conoratus* collected from the stomach of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017. Scale bar = 20 µm. A = ventral gripus and ventral bar, B = dorsal gripus, C = cirrus and D = marginal uncinuli.

Table 4.22: Number of *Enterogyrus conoratus* collected from the stomach of *Oreochromis mossambicus* during spring (2016).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	31	0	0
Winter	1	0	0
Spring	22	4	22
Summer	3	0	0

4.4.3.2 Cestoda

Gryporhynchid metacestode

CLASS: Cestoda

ORDER: Cyclophillidea

FAMILY: Gryporhynchidae

GENUS: *Neogryporhynchus* Baer & Bona, 1960

Neogryporhynchus sp. (Figure 4.32) was collected from the intestinal wall of *O. mossambicus*. No specimens of this parasite were recorded during winter (Table 4.23). A higher prevalence was recorded during autumn. Higher mean intensity and mean abundance values of this parasite were recorded during spring (Figure 4.33).

Morphology

The morphology of this parasite conforms the description by Scholz *et al*. (2004). The anterior part of the body smaller than the posterior part. The anterior part of the body bears equal number of large hooks and small hooks (10) and four suckers.

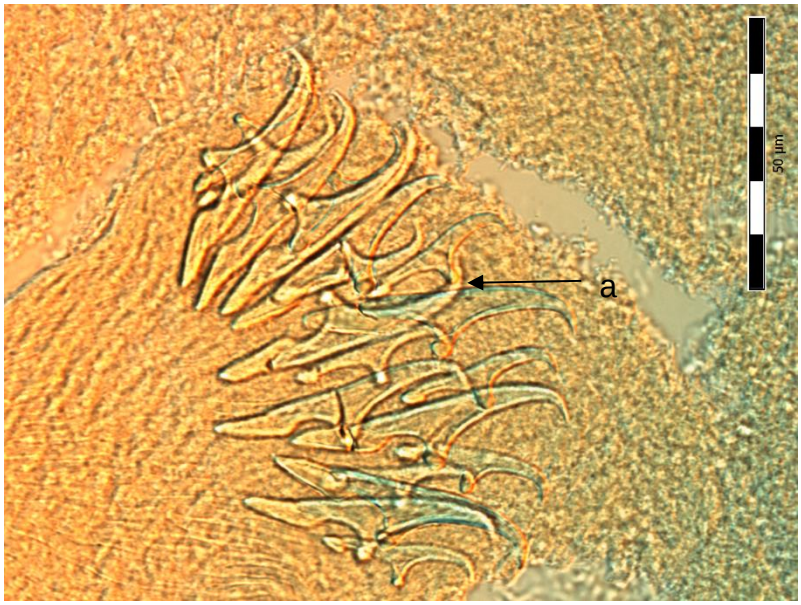


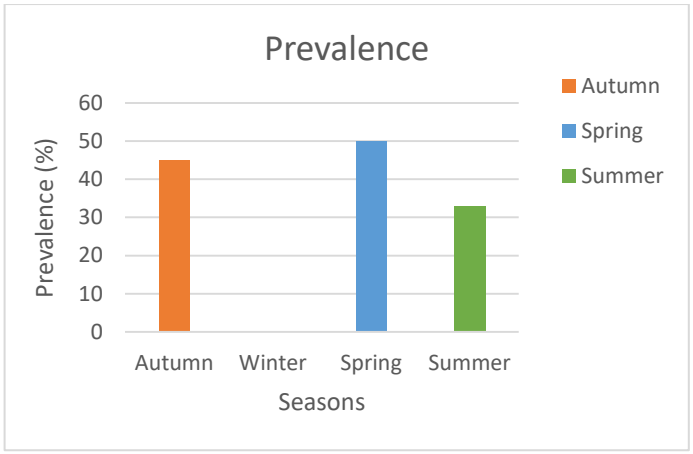
Figure 4.32: Photomicrographs of hooks of *Neogryporhynchus* species collected from the intestinal wall of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017. a = hooks.

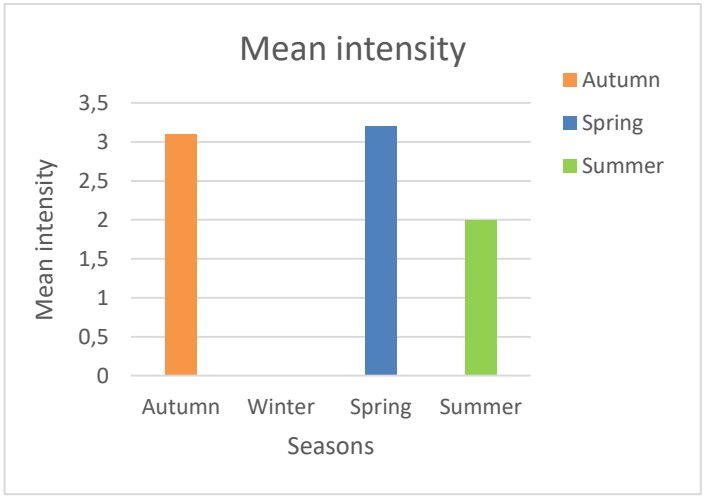
Remarks

It is impossible to identify the metacestodes to the species level using morphological features. The general diagnoses are mainly from adult worms occurring in birds and are based on rostellar hooks and reproductive organs (Mashego *et al*. 1991). Thus molecular analysis is necessary to identify this metacestode to species level. Although for many of the species the sequences are not available.

Table 4.23: Number of *Neogryporhynchus* species collected from *Oreochromis mossambicus* during autumn, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	31	14	43
Winter	1	0	0
Spring	22	11	35
Summer	3	1	2





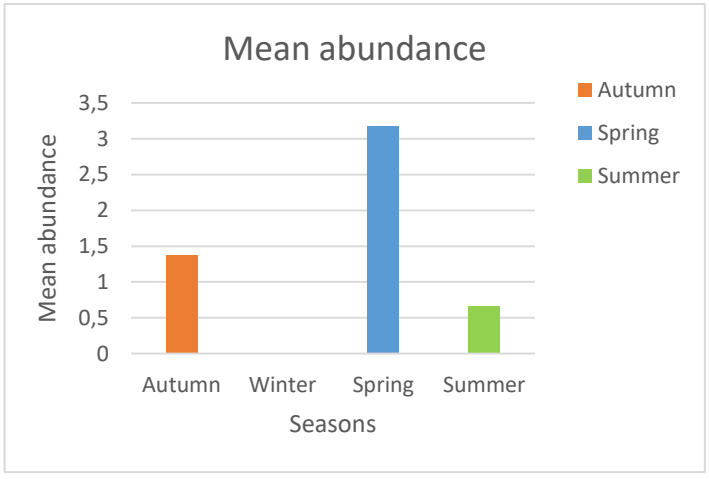


Figure 4.33: The prevalence (A), mean intensity (B) and mean abundance (C) of *Neogryporhynchus* species collected from the intestinal wall of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017.

Gryporhynchid metacestode

CLASS: Cestoda

ORDER: Cyclophillidea

FAMILY: Gryporhynchidae

GENUS: *Paradilepis* Hsü, 1935

Paradilepis sp. (Figure 4.34) was collected from the intestinal wall, liver, mesentery and gallbladder of *O. mossambicus*. No specimens of this parasite were recorded during winter (Table 4.24). A higher prevalence was recorded during autumn. Higher mean intensity and mean abundance values of this parasite were recorded during spring (Figure 4.35).

Morphology

The morphology of this parasite conforms the description by Scholz *et al.* (2004). The anterior part of the body is smaller than the posterior part. The anterior part bears hooks and suckers. Two rows of hooks of different sizes are present.

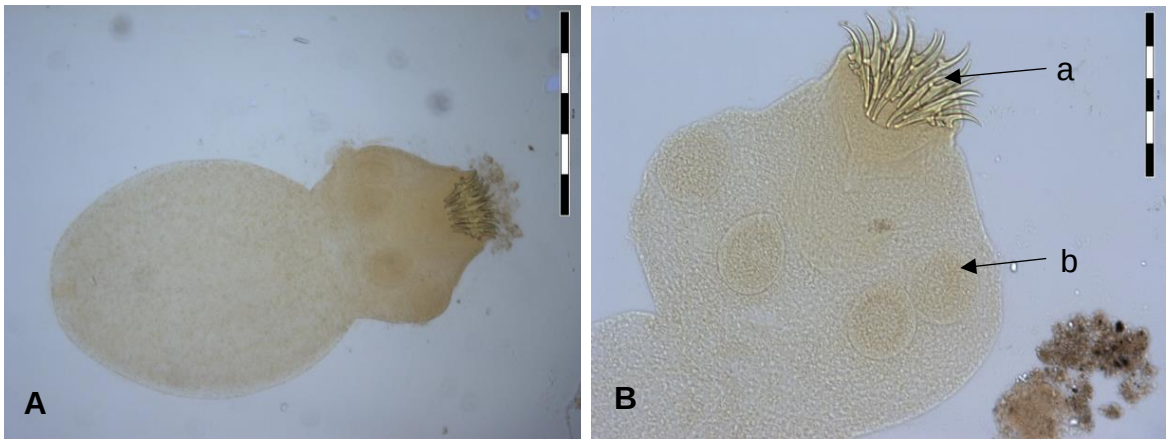
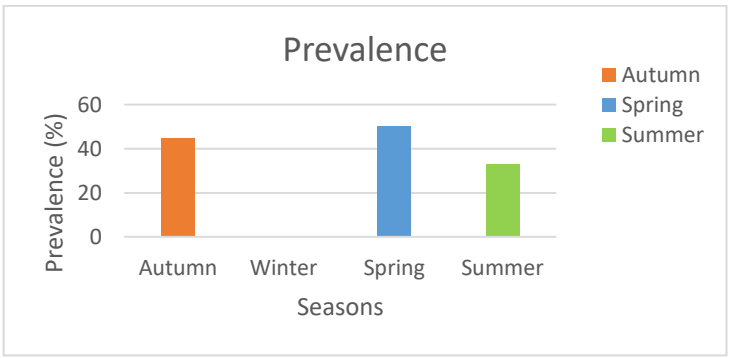
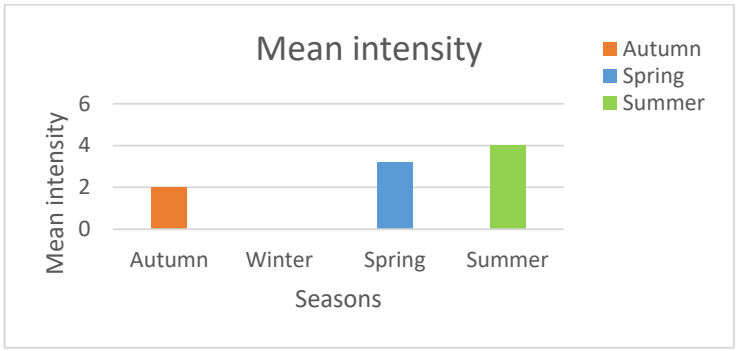


Figure 4.34: Photomicrograph of *Paradilepis* species collected from the intestinal wall, mesentery, gallbladder and liver of *Oreochromis mossambicus* during seasonal surveys from April 2016 February 2017. A = whole mount. B = Anterior part: a = hooks and b = suckers.

Table 4.24: Number of *Paradilepis* species collected from *Oreochromis mossambicus* during autumn, spring (2016) and summer (2017).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	31	14	28
Winter	1	0	0
Spring	22	11	55
Summer	3	1	3





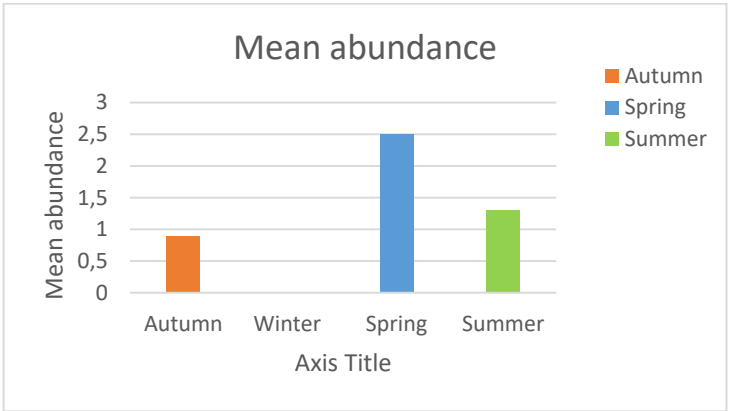


Figure 4.35: The prevalence (A), mean intensity (B) and mean abundance (C) of *Paradilepis* species collected from the intestinal wall, liver and gallbladder of *Oreochromis mossambicus* during seasonal survey from April 2016 to February 2017.

4.4.3.3 Acanthocephala

CLASS: Acanthocephala

ORDER: Gyracanthocephaia

FAMILY: Quadrigyridae

GENUS: *Acanthogyrus* Thapar, 1927 = *Acanthosentis* (Verma & Datta, 1929)

SPECIES: *Acanthogyrus* (*Acanthosentis*) *tilapiae* Baylis, 1948

Acanthogyrus (Acanthosentis) tilapiae (Figure 4.36) was collected from the intestine of *O. mossambicus*. *Acanthogyrus (Acanthosentis) tilapiae* was recorded only in autumn and spring (Table 4.25). Higher prevalence, mean intensity and mean abundance values of this parasite were recorded during autumn (Figure 4.37).

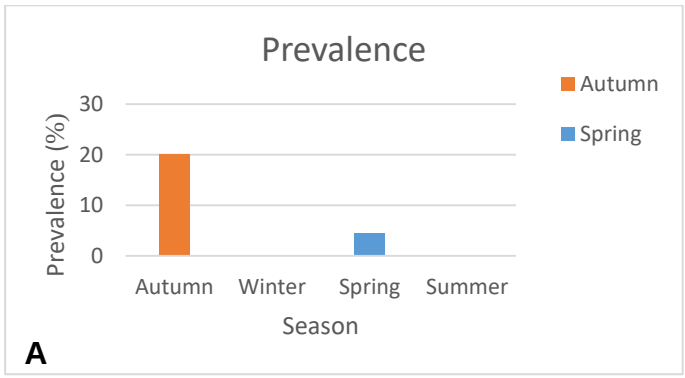
Morphology

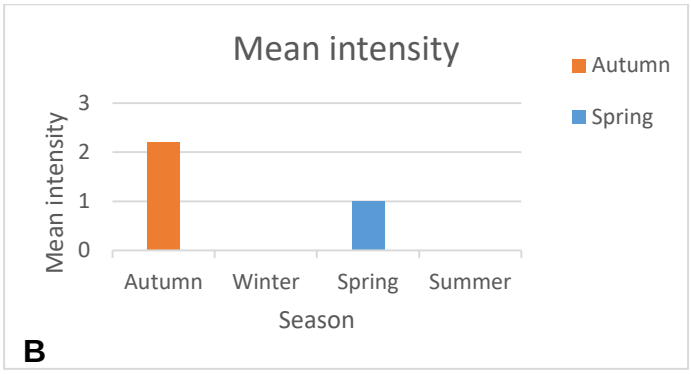
The morphology of this parasite conforms to the description by Amin and Heckmann (2012). The body is cylindrical. The proboscis has three rows of hooks curved towards the posterior of the body. Proboscis hooks are arranged in successive circle and they gradually decrease in length posteriorly. The testes lie in posterior region of body. Lemnisci reach to the anterior of the testis.

Figure 4.36: Photomicrograph of *Acanthogyrus tilapiae* collected from the intestine of *Oreochromis mossambicus* during seasonal surveys from April 2016 to February 2017.

Table 4.25: Number of *Acanthogyrus (Acanthosentis) tilapiae* collected from *Oreochromis mossambicus* during autumn and spring (2016).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	31	6	13
Winter	1	0	0
Spring	22	1	1
Summer	3	0	0





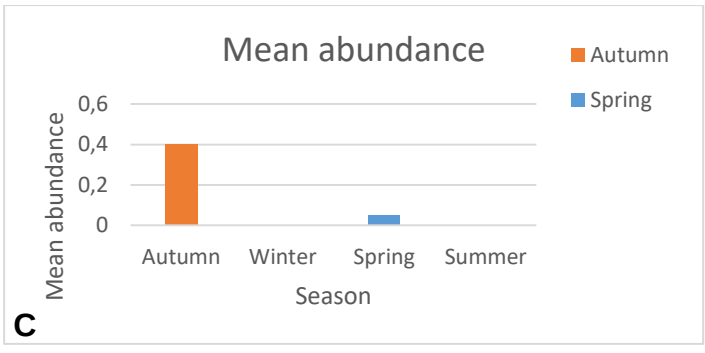


Figure 4.37: The prevalence (A), mean intensity (B) and mean abundance (C) of *Acanthogyrus (Acanthosentis) tilapiae* collected from the intestine of *Oreochromis mossambicus* during seasonal survey from April 2016 to February 2017.

4.4.3.4 Branchiura

Dolops ranarum

CLASS: Branchiura

ORDER: Arguloida

FAMILY: Argulidae

GENUS: *Dolops* Audouin, 1837

Species: *Dolops ranarum* Stuhlmann, 1892

Dolops ranarum was collected from the skin and fins of *O. mossambicus.* This parasite was only recorded during autumn. The number of collected parasites are presented in Table 4.26.

Morphology

The morphology of the specimens collected from *O. mossambicus* is similar to that collected from *Cyprinus carpio.* See page 47.

Table 4.26: Number of *Dolops ranarum* collected from *Oreochromis mossambicus* during autumn (2016).

	Total number of hosts	Total number of infected hosts	Total number of parasites
Autumn	31	2	2
Winter	1	0	0
Spring	22	0	0
Summer	3	0	0

4.4.4 Parasite diversity

More parasite species were recorded during summer with less species recorded during spring (Table 4.27). Winter had the highest number of parasites. *Contracaecum* sp. larva was the dominant parasite during all the seasons.

Table 4.27: The parasite diversity indices of parasites of *Clarias gariepinus, Cyprinus carpio* and *Oreochromis mossambicus* during autumn, winter, spring (2016) and summer (2017).

	Autumn	Winter	Spring	Summer
Total number of species	12	12	11	13
Total number of individuals	759	1809	885	1683
Shannon-Wiener Index (H')	2.0	0.62	1.6	1.0
Berger-Parker Dominance index	0.65	0.92	0.7	0.85
Margalef Richness Index	1.7	1.5	1.5	1.6
Evenness (H'E)	1.4	0.43	1.1	0.71
Dominant species	*Contracaecum* larva	*Contracaecum* larva	*Contracaecum* larva	*Contracaecum* larva

4.5 Discussion

Parasites from *Cyprinus Carpio*

Four parasites reported in the present study from *Cyprinus carpio* namely; *D. extensus*, *D. minutus*, *A. huronensis* and *A. japonicus*, are alien. According to Smit *et al*. (2017), the introduction of alien freshwater fish has created an opportunity for the co-introduction of their parasites. However, there is no evidence of host switching of these four alien parasites from *Cyprinus carpio* to native fish species in South Africa.

Dactylogyrus extensus

Dactylogyrus extensus exclusively parasitise the gills of common carp. Its high host-specificity and high tolerance to a wide range of temperature and salinity enable them to be very successful (Jalali & Barzegar 2005; Borji *et al*. 2012). Because this parasite is very host specific, the chances of host switching to native hosts are limited (Dove & Ernst 1998). This parasite has been reported in Australia (Dove & Ernst 1998), Israel (Paperna 1964) and Germany (Dzika *et al.* 2009). Under natural conditions, *D. extensus* has six developmental stages in the life cycle (Dzika *et al*. 2009). Prost (1963) defined all stages from stage I – VI with stage I oncomiracidium; stage ll – V juveniles and stage Vl adult. Records of *D. extensus* in SA include those of Crafford *et al*. (2014a; b) from the Vaal

Dam, Gauteng Province. In the present study, *D. extensus* was recovered from the gills of *Cyprinus carpio* during all seasonal surveys and the results of the present study represents a new locality record.

Dactylogyrus minutus

Like *D. extensus*, this parasite is also known as co-invader with its host *Cyprinus carpio*. *Dactylogyrus minutus* is a thermophilic parasite with a shorter life cycle and optimal infections during high temperatures (Kir & Tekin-Ozan 2007; Smit *et al*. 2017). According to Crafford *et al.* (2014b), the presence of *D. minutus* and *D. extensus* in South Africa emphasises the global distribution of their host. In the present study, *D. minutus* was found from the gills of *Cyprinus carpio*, during autumn, winter and summer. In South Africa, the only record of *D. minutus* is that of Crafford *et al.* (2014a; b) from Gauteng Province. The present study represents a new locality record for this parasite for South Africa.

Atractolytocestus huronensis

Atractolytocestus huronensis is a tapeworm belonging to the order Caryophyliidea. Cestodes of this order have a monopleuroid body and possess a single set of reproductive organs (Bazsalovicsova *et al.* 2018). The parasite was originally described from the common carp from North America but is likely of Asian origin (Oros *et al*. 2004; Scholz *et al*. 2015). Common carp is exclusively parasitised by three species of the genus *Atractolycestus* Anthony, 1958, namely *Atractolytocestus huronensis* (Anthony, 1958), *Atractolytocestus sagittatus* Kulakovskaya & Akhmerov, 1965 (syn. *Markevitschia sagittata* Kulakovskaya & Akhmerov, 1965) and *Atractolytocestus tenuicollis* Li, 1964 (syn. Khawia tenuicollis Li, 1964). In South Africa, Scholz *et al*. (2015) reported this tapeworm for the first time from the intestine of *Cyprinus carpio* from four localities in the Limpopo Province. Smit *et al.* (2017) reported on the occurrence of this parasite in the Vaal River, Northwest Province and the Riet River, Northern Cape. The results of the present study represents new geographical records for this parasite for South Africa. This parasite was deeply imbedded in the intestinal lumen of the fish and pathology was observed after the collection of the parasite.

***Diplostomum* sp.**

Diplostomids are economically important in both natural and aquaculture systems worldwide due to their metacercariae which parasitise the eyes of fish (Chibwana & Nkwengulila 2010). African species of the genus include *D. heterobranchi* Wedl, 1861, *D. magnicaudum* El-Naffar, 1979, *D. mashonense* Beverly-Burton, 1963, *D. tregenna* Nazmi Gohar, 1932 and *D. ghanense* Ukoli, 1968 (Mashego *et al*. 1991; Khalil & Polling 1997). Yamaguti (1971) gave a complete generic diagnosis based on morphological features, and included *Lymnaea* as the only snail first intermediate host, freshwater fish and various birds as second intermediate and definitive hosts, respectively. In the present study, *Diplostomum* sp. was recorded inside the lens of the eyes of *Cyprinus carpio*. Hoogendoorn *et al.* (2020), found metacercariae of *Diplostomum* spp. in the eye lenses of freshwater fish belonging to five species in South Africa. The prevalence of *Diplostomum* spp. from the latter study was less than the prevalence in the present study. The results of the present study represents a new locality record for the parasite for South Africa.

Dolops ranarum

The genus *Dolops* is one of four genera in the Branchiura. The genus is endemic to Africa and is represented by a single species, *D. ranarum*. *Dolops ranarum* is widespread in all major river systems in Africa (Nile, Niger, Congo and Zambezi) (Avenant-Oldewage & Van As 1990; Douellou & Erlwanger 1994). Species of *Dolops* are distinguished from the other three branchiuran genera by the presence of a pair of hooks distally on the first maxillae in adults, instead of suction discs developing from the proximal segment (Moller & Olesen 2012). In South Africa, *D. ranarum* has previously been reported on *Clarias gariepinus* and *O. mossambicus* by Avenant-Oldewage and Van As (1990) in the Limpopo Drainage system in the Transvaal, and Avenant-Oldewage and Madanire-Moyo *et al*. (2012), in the Limpopo and Olifants river systems. *Dolops ranarum* was recorded from the skin and gills of *Clarias gariepinus, Cyprinus carpio and O. mossambicus* during all seasonal surveys. *Cyprinus carpio* had the lowest recorded number of *D. ranarum* and was only recorded during autumn. The results of the present study represents new geographical records for South Africa.

Argulus japonicus

Species of the genus *Argulus* occur mainly as parasites of both marine and freshwater fishes throughout Africa (Avenant-Oldewage 1994). In southern Africa, *A. japonicus* and *A. africanus* are the only recorded species from freshwater fishes. *Argulus japonicus* is alien to Africa, and it is believed to have been introduced on more than one occasions with carp and goldfish (Avenant-Oldewage 2001). The first records of *Argulus* in southern Africa are those of Du Plessis (1952) and Lombard (1968), recorded in the eastern Transvaal. *Argulus japonicus* has a very low host specificity and it is one of the most prevalent and widespread branchiuran. Some of *A. japonicus* records in South Africa includes Kruger *et al*. (1983) from the western Transvaal, Van As and Basson (1984), Swanepoel & Avenant-Oldewage (1992), Avenant-Oldewage and Swanepoel (1993) and Avenant-Oldewage (1994) from Kosi bay. *Argulus japonicus* was recorded from the skin and fins of *Cyprinus carpio* during winter, spring and summer. The results of the present study represents a new geographical record of this parasite for South Africa.

Neoergasilus japonicus

The genus *Neoergasilus* was first created by Yin, 1956, who designated *N. japonicus* as type species. *Neoergasilus japonicus* is native to Asia and has been introduced to other countries through transportation of fish hosts associated with aquaculture. Larvae and adult males are free-living and only adult females are parasitic (Abdelhalim *et al*. 1993; Alfonoso & Belmonte 2010; Soylu & Soylu 2012). *Neoergasilus japonicus* are mainly found attached to host's fins and they are able to move from host to host. The dorsal and anal fins are the most preferred sites of attachment for *N. japonicus* but it has been reported from the pelvic and caudal fins and gills (Hudson & Bowen 2002; Knopf & Holker 2005). In this present study, only one specimen of *N. japonicus* was recorded during seasonal surveys from the skin of *Cyprinus carpio*. Gilbert and Avenant-Oldewage (2017), reported on N. japonicus from the Vaal River Barrage. The results represent a new locality record for South Africa.

Parasites from *Clarias gariepinus*

***Trypanosoma* sp.**

Trypanosomes are kinestoplastid protozoans found in the blood of many fish species worldwide. Transmission of trypanosomes between fish has been attributed to different species of leeches (de Padua *et al.* 2011). In Africa, there are only three known species namely: *Trypanosoma toddi* Bouet, 1909; *Trypanosoma mukasai* Hoare, 1932 and *Trypanosoma tobeyi* Dias, 1952. *Trypanosoma toddi* and *T. tobeyi* are separated by small morphological differences and *T. mukasai* and *T. tobeyi* might prove to be a synonym of the same trypanosome (Baker 1960). Pienaar (1962) reported *Trypanosoma clariense* from *Clarias gariepinus* in the North West Province. This species was initially described by Fantham (1919) from the same host. It was suggested by Smit *et al*. (2000) that *T. clariense* might be a synonym of *T. mukasai*, owing to the apparent common staining properties and morphometrics. The results of the present study represents a new geographical record of this parasite for South Africa, although it was not identified to species level.

***Gyrodactylus* sp.**

Monogeneans of the genus *Gyrodactylus* parasitise the skin and gills of many freshwater and marine fishes (Prikrylova *et al*. 2012). Members of this genus are viviparous, small and elongated (Abdullah & Mama 2013). Only seven of the 28 described *Gyrodactylus* species in Africa are known from catfishes. In Africa *Clarias gariepinus* is known to host five *Gyrodactylus* species, namely *Gyrodactylus alberti*, *Gyrodactylus clarii* Paperna, 1973, *Gyrodactylus rysavyi*, *Gyrodactylus groschafti* Ergens, 1973 and *Gyrodactylus transvaalensis* Prudhoe & Hussey, 1977 (Prikrylova *et al.* 2012). Of these, *G. alberti*, *G. clarii* and *G.* groschafti are from the gills and *G. rysavyi* and *G. transvaalensis* are from the skin (Matla 2012). Luus-Powell (2004) reported *Gyrodactylus* sp. from the skin of *Marcusenius macrolepidotus* Peters, 1852 in Lake Tzaneen, Limpopo Province. Madanire-Moyo *et al*. (2012) reported *Gyrodactylus rysavyi* from *Clarias gariepinus* from the Limpopo and Olifants river systems. The results of the present study represent new locality record for South Africa.

Quadriacanthus sp.

The genus *Quadriacanthus* comprises mostly gill parasites of African and Asian clariids. The genus was established by Paperna (1961) for *Quadriacanthus clariadis* from the gills of *Clarias gariepinus* collected in Israel. Members from the genus are characterised by two unequal bars, each with a solid base, to which are attached narrower appendages (Francova *et al.* 2017). El-Naggar and Serag (1986), Kritsky and Kulo (1988) and Tripathi *et al*. (2007) emended the generic diagnosis, amongst others, by recognising the medially articulating ventral bar, unequal and dissimilar pairs of marginal hooklets, and the basally articulated, straight copulatory tube and accessory piece. In South Africa, Madanire-Moyo *et al*. (2012) reported *Q. clariadis* and *Q. aegypticus* El-Naggar and Serag, 1986 from the gills of *Clarias gariepinus* from the Limpopo and Olifants river systems. The results of the present study represent a new locality record for this parasite for South Africa.

Paracamallanus cyathopharynx

Paracamallanus cyathopharynx is a common parasite of the catfishes of the Clariidae in Africa. It is differentiated from other nematodes of fish by the configuration of the buccal capsule and pharynx (Boomker 1982). Copepods are intermediate hosts in their life cycle. This nematode is ovoviviparous and larvae are liberated into the gut of the host and pass out with faeces (Ikechukwu *et al*. 2017). South African records include that of Mashego and Saayman (1981) and Madanire-Moyo *et al.* (2012). *Paracamallanus cyathopharynx* was found from the intestine of *Clarias gariepinus* during winter, spring and summer. The mean abundance value recorded for *P. cyathopharynx* in the present study is higher than the mean abundance value recorded by Madanire-Moyo *et al*. (2012) from the Limpopo and Olifants river systems. This represents new geographical records for South Africa.

Contracaecum larvae

Anisakid nematodes larvae of the genus *Contracaecum* use invertebrates as intermediate and/or paratenic hosts. The third stage larva (infective stage) develops in invertebrates which are eaten by vertebrates (such as fish) intermediate hosts (Garbin *et al*. 2013). Mature stages are present in fish eating birds and mammals (Kanarek & Bohdanowicz 2009). *Contracaecum* larvae was previously recorded from *Clarias gariepinus* in South

Africa by Barson and Avenant-Oldewage (2006), Madanire-Moyo *et al*. (2010; 2012) and Tavakol *et al.* (2015). A total of 4189 *Contracaecum* sp. specimens was recorded during seasonal surveys. According to Barson and Avenant-Oldewage (2006), its infections can reach alarming intensities without affecting the host, an adaptation that probably ensures that the larvae survive to reach the final host without killing the intermediate host. The results of the present study represents new locality record of this parasite for South Africa.

Parasites from *Oreochromis mossambicus*

Cichlidogyrus halli

Cichlidogyrus halli was first described as *Cleidodiscus halli* by Price and Kirk (1967) from the gills of *Oreochromis shiranus* in Malawi. It was later found and redescribed several times from a wide range of cichlid hosts in several countries in Africa by Paperna (1968; 1969; 1979), Paperna & Thurston (1969), Thurston (1970), Ergens (1981), Dossou (1982) and Douellou (1993). This species differentiated from other *Cichlidogyrus* species by its relatively large size, the large sclerotised structures, the solid hamuli and the shape of the copulatory organ (Douellou 1993). *Cichlidogyrus halli* was recorded during all seasonal surveys from the gills of *O. mossambicus*. In a study conducted by Madanire-Moyo *et al*. (2012), in the Limpopo and Olifants River systems at Luphephe-Nwanedi Dam (regarded as unpolluted) and Flag Boshielo Dam (regarded as moderately polluted), the mean intensity of *C. halli* was higher at Flag Boshielo Dam. The mean intensity of this parasite in the present study is less than the mean intensity reported by Madanire-Moyo *et al*. (2012) from both dams. The results of the present study represents a new locality record for South Africa.

Cichlidogyrus sclerosus

This species was first described from the gills of *O. mossambicus* in Uganda by Paperna and Thurston (1969). The species was also found in Zimbabwe by Douellou (1993), Botswana by Modise *et al.* (2009) and South Africa by Le Roux and Avenant-Oldewage (2010) and Madanire-Moyo *et al*. (2012). It is as large as *C. halli* but has relatively smaller haptoral armature. The identification of this parasite cannot be confused with any other species of *Cichlidogyrus* because of the massive anchors with almost no roots, the solid

bars, the pyriform appendages of the dorsal bar and the copulatory organ (Douellou 1993). *Cichlidogyrus sclerosus* was recorded during all seasonal surveys from the gills of *O. mossambicus*. Madanire-Moyo *et al*. (2012), reported higher mean intensity of this parasite in Flag Boshielo Dam as compared to Nwanedi-Luphephe Dam. In the present study, the mean intensity of *C. sclerosus* is higher than the mean intensity reported by Madanire-Moyo *et al.* (2012) from both Luphephe-Nwanedi and Flag Boshielo dams. The results represent a new locality record for South Africa.

Cichlidogyrus tilapiae

The first description of *C. tilapiae* was from the gills of *O. niloticus* from Israel by Paperna (1960). The parasite has been reported from other African countries. The parasite has been reported from various cichlid fishes from Israel in Middle East; Uganda, Tanzania, Egypt, Ghana, South Africa, Burkina Faso, Ivory Coast, and Zimbabwe in Africa by Douellou (1993), Jimenez-Garcia *et al*. (2001), Mendoza-Franco *et al.* (2006), Pouyaud *et al.* (2006), Boungou *et al.* (2008), Pariselle & Euzet (2009), Le Roux & Avenant-Oldewage (2010), Akoll *et al*. (2011) and Madanire-Moyo *et al*. (2011). *Cichlidogyrus tilapiae* was only recorded during spring. The results of the present study represent a new locality record for South Africa.

Enterogyrus conoratus

Monogeneans are known to be ectoparasites of the skin and gills of fish however, enterogyrids are endoparasitic, infecting the stomach of fish (Madanire-Moyo & Avenant-Oldewage 2014). It is believed that during their course of evolution, some parasites abandoned ectoparasitism to invade internal habitats and this may have been due to competition and predation (Luus-Powell *et al.* 2020). Eight species of the genus *Enterogyrus* are known to be inhabiting the stomach of African cichlids. In South Africa, Olivier *et al*. (2009) reported *Enterogyrus cichlidarum* Paperna, 1963 from the stomach of *O. mossambicus* from Middle Letaba Dam, Limpopo Province. Three *Enterogyrus* spp. were reported from the stomach of *O. mossambicus* by Madanire-Moyo *et al.* (2012), from the Limpopo and Olifants River systems. Madanire-Moyo and Avenant-Oldewage (2014; 2015) reported *E. conoratus* from the stomach of *Pseudocrenilabrus philander* Weber,

1897 from the Padda Dam, Gauteng Province. Luus-Powell *et al*. (2020), described two new *Enterogyrus* species (*Enterogyrus multispiralis* n. sp. and *Enterogyrus mashegoi* n. sp.) from the stomach of *O. mossambicus* from the Nwanedi-Luphephe Dam, Limpopo River system. In the present study, *E. conoratus* was recorded from the stomach of *O. mossambicus* and the results represent a new locality record for South Africa.

Gryporhynchid larvae

Gryporhynchid metacestodes occur in different internal organs of fresh- and brackish water fish which serve as the second intermediate hosts (Scholz *et al*. 2004). Members of this family were previously placed in the Dilepididae (Cyclophyllidea). Spassky and Spasskaya (1973) suggested the Gryporhynchidae, later raised to family level. The study on the phylogenetic analysis among the families of the order Cyclophyllidea based on comparative morphology (Hoberg *et al.* 1999) and molecular data (Mariaux 1998) equivocally confirmed the systematic position of the Gryporhynchidae as a separate family and as different from the Dilepididae. The family was erected to accommodate those species of dilepidids that mature in fish-eating birds and have larvae which occur in fish (Scholz *et al.* 2004). Madanire-Moyo *et al*. (2012), reported on the unidentified gryporhynchid larvae from the Limpopo and Olifants River systems from *O*. *mossambicus*. Truter *et al*. (2016), reported on *Neogryporhynchus lasiopeius* for the first time in South Africa from the intestinal lumen of *Pseudocrenilabrus philander.* In the present study, *Neogryporhynchus* sp. was recorded from the intestinal wall of *O. mossambicus.* Four species of *Paradilepis* have been reported in South Africa from different fish species from the liver, intestinal wall, mesentery and gallbladder by Truter *et al*. (2016) and Scholz *et al.* (2018). In the present study *Paradilepis* sp. was recorded from the intestinal wall, liver, mesentery and gallbladder. The results of the present study represent a new locality record for South Africa for both genera.

Acanthogyrus tilapiae

Acanthocephala, or thorny-headed worms, are parasites commonly found in species of birds, mammals and fishes. The body of acanthocephalans consists of a proboscis and a trunk. The proboscis is the distinguishing feature which is covered with hooks, and can be

retracted. The hooked proboscis is used to anchor the worm to the intestinal wall of the host and can damage the host's intestine and may affect overall fish health (Hendricks & Reyda 2009). The sexes are separate and males are usually smaller than females with gonads in ligaments between the proboscis sheath and posterior end. Males have two testes and the ovaries of females are non-persistent (Storer *et al*. 1972).

In Africa, studies on acanthocephalans revealed nine genera in six families from various fishes in not less than 12 countries (Khalil & Polling 1997). In South Africa, there are three genera of acanthocephalans found from freshwater and marine fish and only one of these is from a freshwater fish. Only two reports by Mashego (1982; 1988) in South Africa feature in the African literature on acanthocephalans of freshwater fish. In this study, only one species of Acanthocephala, namely *Acanthogyrus* (*Acanthosentis*) *tilapiae* Baylis, 1948 was recorded from the intestine of *O. mossambicus*. *Acanthogyrus* was synonymised with *Acanthosentis* Govan, 1959, with the latter taxon reduced to a subgenus of the former. *Acanthogyrus tilapiae* is endemic to Africa and is widely distributed and African studies include Baylis (1948), Prudhoe (1951), Golvan (1957;1965), Khalil and Thurston (1973), Shotter (1974), Troncy (1974), Amin (1978), El-Naffar *et al*. (1983) Batra (1984) Hyslop (1988), Douellou (1992) and Amin *et al*. (2008). No pathology by this parasite were recorded during the sampling period. No gross pathology by this parasite was observed during the sampling period. The results of the present study represent a new locality record for South Africa.

4.6 Conclusion

From the Shannon-Weiner diversity index results, *O. mossambicus* had a higher parasite diversity than *Clarias gariepinus* and *Cyprinus carpio*. The number of parasite species for the four seasons were as follows: summer (13) > autumn and winter (12) > spring (11). A total number of 36 fish specimens was collected during autumn and a total number of 15 fish specimens was collected during winter. Even though a larger sample size was recorded during autumn, a higher number of parasites was recorded during winter which had the lowest sample size. *Contracaecum* sp. larva was the dominant species during all seasons which may be attributed to the availability of other intermediate and definite hosts around the dam all year round and the feeding behavior of *Clarias gariepinus*. The four

reported alien parasites from *Cyprinus carpio* indicate a successful distribution of these parasites in the South African freshwater systems. In the present study, none of these alien parasites have been reported from *Clarias gariepinus*. All the parasites reported in the present study represent a new locality record for South Africa.

CHAPTER 5

PARASITE INDEXES AND CONDITION FACTOR OF *CLARIAS GARIEPINUS*, *CYPRINUS CARPIO* AND *OREOCHROMIS MOSSAMBICUS*

5.1 Introduction

Fish parasites can be used as biological indicators for host's ecology and for environmental conditions such as eutrophication (Jakob & Palm 2006; Palm & Ruckert 2009). Parasites may display individual, population and community level alterations in polluted environments (Blanar *et al*. 2009). They can divided into two main indicator groups, namely effect indicators and accumulation indicators based on the responses they display (Gilbert & Avenant-Oldewage 2017). Although parasites are regarded as good biological indicators of environmental health, parasites can have an impact on hosts and populations (Marcogliese 2005). According to Marcogliese (2005), host's biology may be affected by parasitism in various ways, be it behaviorally, physiologically, morphologically or reproductively.

In addition to parasitism, pollution affects the host's health as well. Based on their effects, pollutants can either be lethal or sublethal (Sures 2008). The presence of pollutants in the aquatic environment may stress the organisms and lead to reduced levels of lipids. Exposure to pollutants may also lead to chronic damage or even death in high concentrations of pollutants (Austin 1998). According to Adams *et al*. (1993), organisms challenged by stressors such as pollution requires energy to deal with that stress, diverting physiologically useful energy away from the critical functions of growth and reproduction.

Individual condition/health of fish is important for overall performance, survival and reproductive success (Neff *et al*. 2004). Growth analysis, age and K are some of the approaches used for assessing fish health. The K is used to compare the effect of biotic and abiotic factors on the health or well-being of a fish population (Blackwell *et al.* 2000). The K compares the wellbeing of fish and is based on the hypothesis that heavier fish may be indicators of favourable environmental conditions.

The HAI is a quantitative index that gives a statistical indication of the health status of a selected water body environment (Adams *et al.* 1993). In the original HAI by Adams *et al*. (1993), parasites were recorded as present or absent. During the application of

the HAI in South Africa, a correlation between the abundance of endo- and ectoparasites and water pollution levels was observed (Avenant-Oldewage 1994; Crafford & Avenant-Oldewage 2001). The refined PI and IPI were then incorporated into the original HAI to further distinguish between the number of ecto- and endoparasites present. The presence, and consequently the number of parasites form the basis of the PI in the HAI and IPI for ectoparasites has been developed on the basis that high numbers of ectoparasites indicate good quality water (Crafford & Avenant-Oldewage 2009; Watson *et al.* 2012). Higher numbers of ectoparasites are indicative of better water quality, since they are in direct contact with the surrounding medium, and therefore are given a low HAI score.

5.2 Materials and methods

Fish were sampled and parasites were collected according to the methods as outlined in Chapter 2.

5.3 Data analysis

The PI and IPI values were determined according to Jooste *et al*. (2005) and Heath *et al.* (2004), respectively. The K was calculated according to Blackwell *et al*. (2000) (see Chapter 2).

5.4 Results

5.4.1 Parasite Index and Inverted Parasite Index

The mean IPI of *Clarias gariepinus* showed that the dam was least impacted during autumn. A mean ectoparasite PI of 20 and mean IPI of 10 were recorded during autumn. The highest mean IPI value of 30 was recorded during spring followed by summer with a mean IPI value of 25 and then winter with a mean IPI value of 25.7 (Table 5.1).

Table 5.1: Mean Parasite Index (PI) and Inverted Parasite Index (IPI) of *Clarias gariepinus, Cyprinus carpio and Oreochromis mossambicus* from the Kwena Dam during autumn, winter, spring (2016) and summer (2017).

	Clarias gariepinus			*Cyprinus carpio*			*O. mossambicus*		
	Ecto-PI	IPI	Endo-PI	Ecto-PI	IPI	Endo-PI	Ecto-PI	IPI	Endo-PI
Autumn	20	10	13.3	20	10	10	10	20	8
Winter	4.3	25.7	18.6	15.7	14.3	7.1	10	20	0
Spring	0	30	10	10	20	0	11.9	18.1	6.3
Summer	5	25	15	23	5	1	10	20	6.7

5.4.2 Condition factor

The mean K values recorded for *Clarias gariepinus* ranged from 0.69 to 0.74 with the highest value recorded during spring and the lowest value recorded during autumn. Recorded mean K values for *Cyprinus carpio* ranged from 1.04 to 1.14 with the highest value recorded during winter and the lowest value recorded during summer. The mean K values recorded for *O. mossambicus* ranged from 1.54 to 1.80 with the highest value recorded during autumn and the lowest value recorded during spring (Table 5.2). The K values of the three fish species during winter, spring and summer are presented in Table 5.3, Table 5.4 and Table 5.5, respectively.

Table 5.2: Condition factor (K) values of *Clarias gariepinus*, *Cyprinus carpio* and *Oreochromis mossambicus* during autumn (2016).

Autumn			
	Clarias gariepinus	*Cyprinus carpio*	*O. mossambicus*
Min.	0.66	0.83	1.21
Max.	0.71	1.20	4.02
Mean K	0.69	1.02	1.80
SD	0.25	0.61	0.64
Mean length (mm)	518.33	440.5	166.48
Mean weight (g)	670.07	864	134.13

Table 5.3: Condition factor (K) values of *Clarias gariepinus, Cyprinus carpio* and *Oreochromis mossambicus* during winter (2016).

	Winter		
	Clarias gariepinus	*Cyprinus carpio*	*O. mossambicus*
Min.	0.58	0.94	–
Max.	0.88	1.25	–
Mean K	0.72	1.14	1.67
SD	0.09	0.12	0
Mean length (mm)	543.4	421.3	12.8
Mean weight (g)	1192.4	864.6	35.0

– Only one host specimen was recorded

Table 5.4: Condition factor (K) values of *Clarias gariepinus, Cyprinus carpio* and *O*reochromis *mossambicus* during spring (2016).

	Spring		
	Clarias gariepinus	*Cyprinus carpio*	*O. mossambicus*
Min.	0.78	-	1.50
Max.	1.12	-	2.01
Mean K	0.74	1.13	1.54
SD	0.20	0	0.77
Mean length (mm)	471.5	418.0	196.0
Mean weight (g)	797.3	823.2	113.5

- Only one host specimen was recorded

Table 5.5: Condition factor (K) values of *Clarias gariepinus, Cyprinus carpio* and *Oreochromis mossambicus* during summer.

	Summer		
	Clarias gariepinus	*Cyprinus carpio*	*O. mossambicus*
Min.	0.53	0.94	1.06
Max.	0.88	1.45	2.26
Mean K	0.71	1.04	1.70
SD	0.54	0.40	0.61
Mean length (mm)	476.5	430.5	158.0
Mean weight (g)	771.0	959.9	67.5

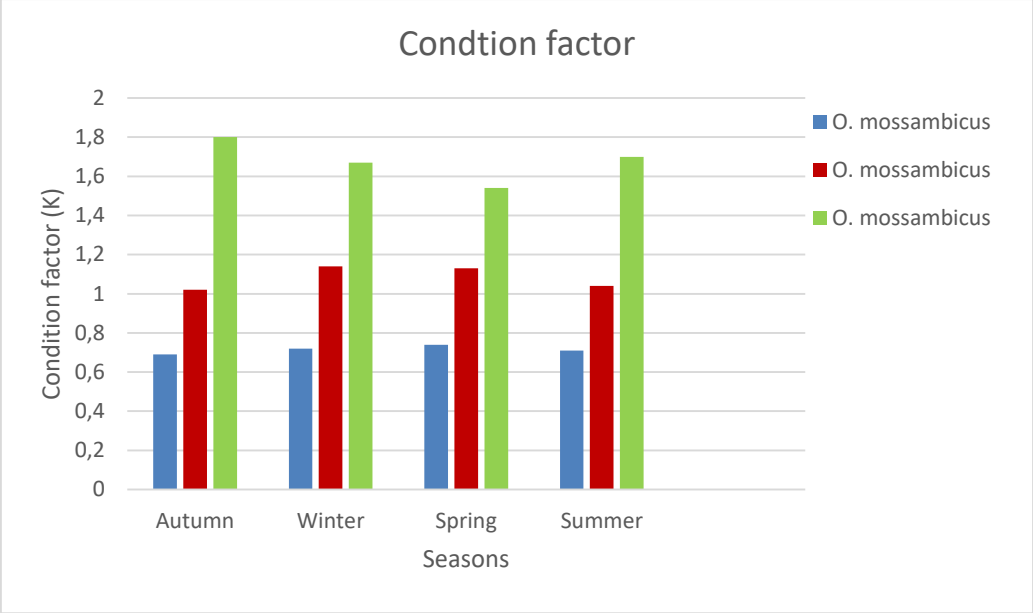


Figure 5.1: The mean condition factor for *Clarias gariepinus, Cyprinus carpio* and *Oreochromis mossambicus* during autumn, winter, spring and summer.

The effect of parasite load on K was assessed for all fish species in different seasons. Regression analysis for *Clarias gariepinus* showed positive correlation between K and parasite burden during autumn ($y= 0.0003_x + 0.4166$) (88.48%) (Figure 5.1 A), spring ($y= 0.0008_x + 0.6496$) (13.03%) (Figure 5.1 C) and summer ($y= 0.0006_x + 0.6315$) (29.23%) (Figure 5.1 D). During winter, regression analysis showed a negative correlation ($y= -0.0003_x + 0.7993$) (24.74%) (Figure 5.1 B) between K and parasite burden.

Regression analysis for *Cyprinus carpio* showed positive correlation between K and parasite burden during summer (y= 0.0106x + 0.7635) (11.16%) (Figure 5.2 C) and negative correlation during autumn (y= -0.0058x + 1.2542) (100%) (Figure 5.2 A) and winter (y= -0.00065x + 1.2315) (53.95%) (Figure 5.2 B). Regression analysis for *O. mossambicus* showed negative correlation between K and parasite load during autumn ($y= -0.0087_x + 1.8609$) (1.56%) (Figure 5.3 A), spring ($y= -0.004_x + 1.8125$) (0.78%) (Figure 5.2 B) and summer ($y= -0.0368_x + 2.0938$) (49.57%) (Figure 5.3 C).

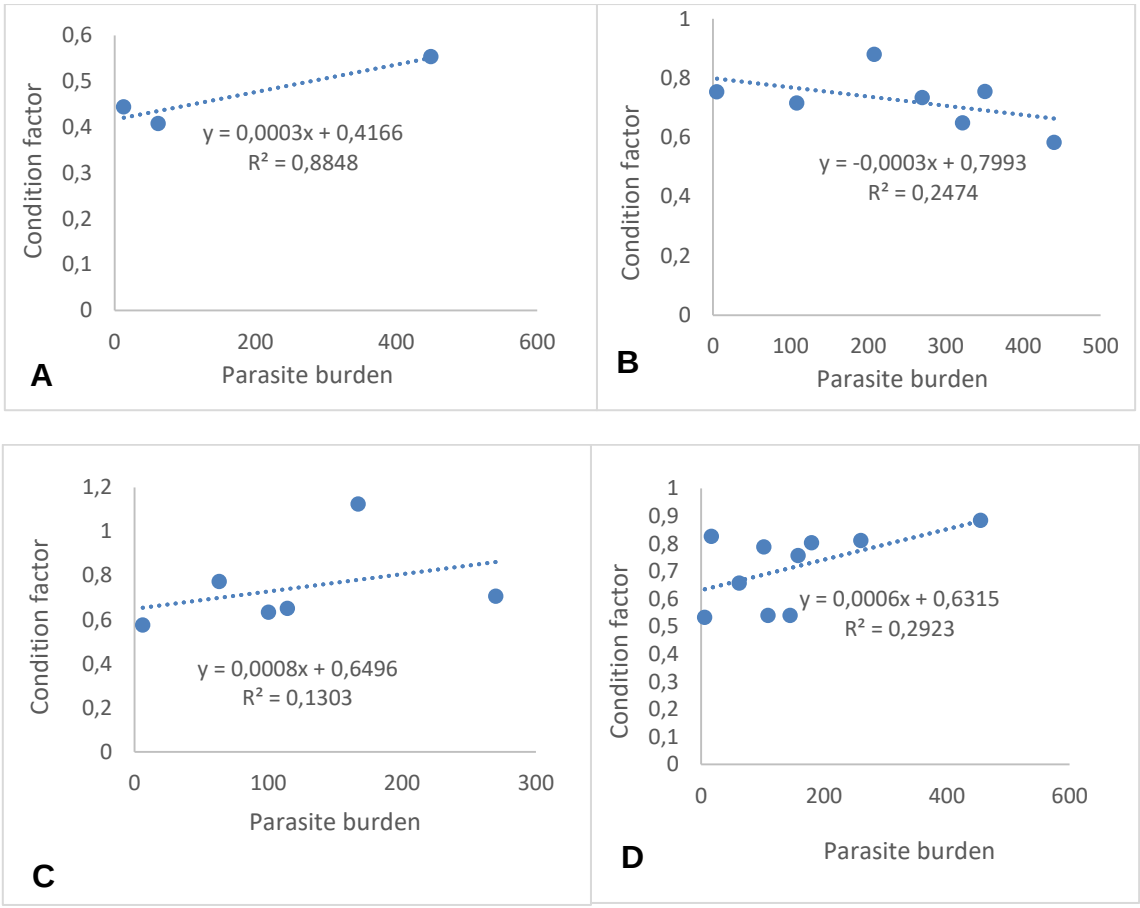


Figure 5.2: Effect of parasite burden on condition factor of *Clarias gariepinus* during seasonal surveys from April 2016 to February 2017. A = autumn, B = winter, C = spring and D = summer.

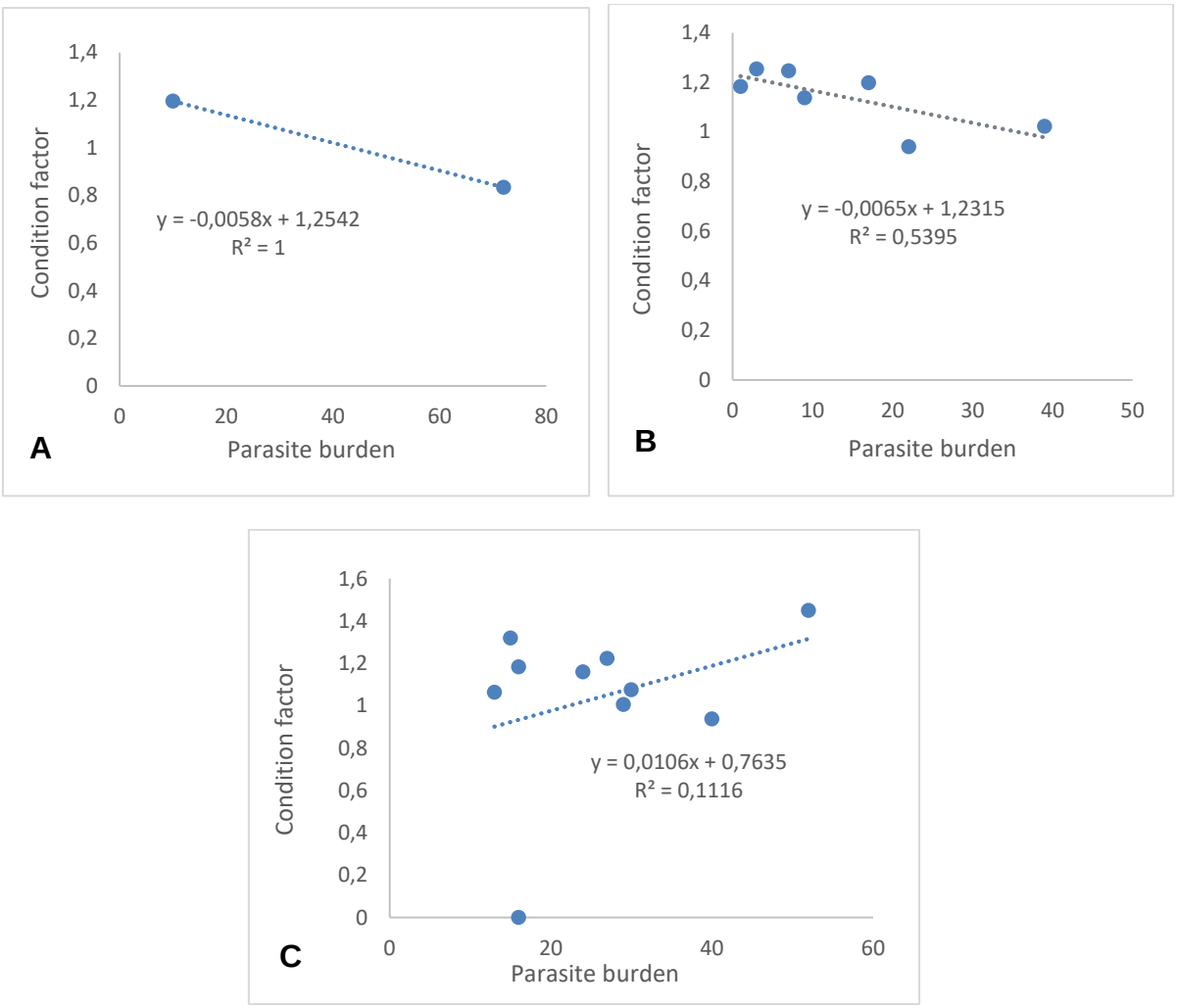


Figure 5.3: Effect of parasite burden on condition factor of *Cyprinus carpio*. A = autumn, B = winter and C = summer.

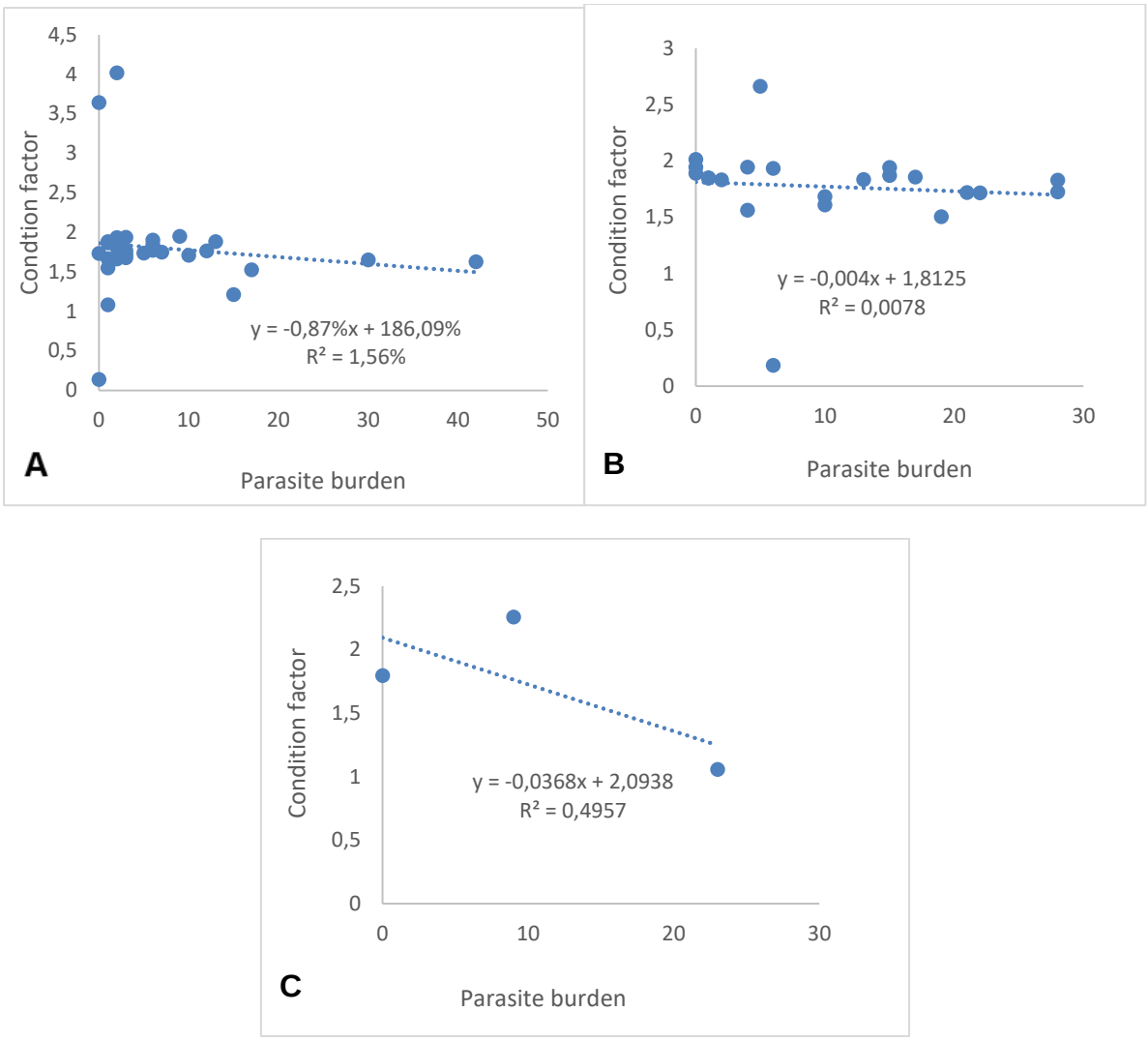


Figure 5.4: Effect of parasite burden on condition factor of *Oreochromis mossambicus*. A = autumn, B = spring and C = summer.

5.5 Discussion

The mean IPI of *Cyprinus carpio* showed that the dam was least impacted during summer with mean ectoparasite PI value of 23 and mean IPI value of 5. The highest mean IPI value of 20 was recorded during spring followed by winter with a mean IPI value of 14.3 and then autumn with a mean IPI value of 10. The mean IPI of *O. mossambicus* showed that the dam was least impacted during spring with mean ectoparasite PI value of 11.9 and mean IPI value of 18.1 whilst the other seasons had the mean IPI value of 20.

The PI is based on the hypothesis that the number, abundance and diversity of ectoparasites decrease in heavily polluted waters and higher numbers of ectoparasites are expected in good quality water. The number of endoparasites tends to increase with increasing water pollution (Avenant-Oldewage 1998). The PI and IPI results of the three fish species do not suggest the same idea about the water quality during the sampling period of the current study. This may be due to the fact that different parasite species (both endo- and ectoparasites) have different pollution tolerances depending on the type of pollutant.

The K of fish indicates the condition of fish in a habitat based on the analysis of length-weight data. The K of a fish is considered ideal when a value of one is obtained (Nash *et al*. 2006). The mean K values for *Cyprinus carpio* and *O. mossambicus* for all seasons were above one whilst *Clarias gariepinus* had mean K values below one for all seasons. According to Barnham and Baxter (1998), K can be influenced by the age of fish, season, sex, fullness of gut, stage of maturity, type of food consumed, amount of fat reserve, stage of development of the reproductive organs and degree of muscular development.

The mean K values recorded in this study suggest that *O. mossambicus* was in better condition than *Cyprinus carpio* and *Clarias gariepinus*. But the value of K is dependent on the fish species, due to the variation in the length/weight ratio of different species. Thus the K of different species cannot be compared. According to Lizama and Ambrosio (2002), higher K values for a fish species are expected during their reproductive period. The higher K values of *O. mossambicus* throughout the study sampling period may be attributed to the fact that this fish species reproduce all year round in warm waters. The parasite burden had little if any effect on the K of the three fish species.

5.5 Conclusion

The K is an important tool to assess the condition of fish in their environments but it cannot be used to compare between fish species due to their different biological

behaviours and physiology. Results from this study showed that the highest K values recorded for each species were obtained in different seasons for the different species. This suggests that seasonal change plays a crucial role in the condition of the fish. The use of PI and IPI in conjunction with the K is useful in monitoring aquatic health but they could be influenced by season. Thus the use of water physiochemical properties is very important in environmental monitoring studies.

CHAPTER 6

GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The aim of this study was to investigate the diversity of freshwater parasites of *Clarias gariepinus*, *Cyprinus carpio* and *Oreochromis mossambicus* and the water quality of the Kwena Dam, located in the Crocodile River catchment. This is the first study on parasite diversity of these three fish species from the Kwena Dam. The following parasite groups were recorded during the study: Protozoa, Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala and Branchiura.

The results for water quality parameters (temperature, DO, pH, EC, TDS and salinity) obtained throughout the study were within the TWQR for aquatic ecosystem. The metals and non-metals detected during the study were within the TWQR for aquatic ecosystem except for aluminium, selenium and zinc. However, the effects of zinc of aquatic biota may have been minimal due to the adequate concentration of DO during the sampling period. The water parameters measured indicated good water quality during the duration of the current study, thus the quality of the water posed no threat to the fish population and other aquatic life during this study. However, continuous monitoring of water quality is needed and the gap in monitoring can be narrowed by long-term biological studies of both the hosts and their parasites in terms of understanding their interaction with the environment.

Generally, the PI, IPI and K are useful indices for assessing aquatic health. However, this is dependent on the fish species and pollution type and may also vary from site to site. The K, PI and IPI indicated that the fish of the Kwena Dam were in good health. Based on the results obtained in this study, the hypothesis that states that higher numbers of ectoparasites are indicative of better water quality and vice versa does not hold. This is dependent on the pollution type and fish species and may also vary from site to site. This may also be attributed to unequal sample sizes among the sampling seasons. The low number of fish caught may be attributed to overfishing or may be due to unmeasured environmental factors. More studies should be conducted to

increase the database on water quality and parasite diversity before definite conclusions can be made. None of the parasites (except *Contracaecum* sp. larvae) occurred in very high numbers during this study indicating that these fish species can manage their parasite burden in a natural system. However, the situation may change if any of the environmental conditions change, leading to higher parasite burden and possible diseases.

From the total number of parasite species collected during the current study, *O. mossambicus* had the highest number of parasite species followed by *Cyprinus carpio* and *Clarias gariepinus*. Results from the Shannon-Wiener diversity index (H') have shown that the highest parasite diversity was recorded during autumn. Some parasite species were not collected during all sampling seasons, *Contracaecum* sp. larvae, *Dactylogyrus extensus*, *Cichlidogyrus halli* and *Cichlidogyrus sclerosus* were the only parasite species recorded during all four seasons.

A total of four alien parasite species were recorded from an alien fish *Cyprinus carpio*, namely *Argulus japonicus*, *D. extensus, D*. *halli* and *D. sclerosus*. This may indicate that the South African environment is suitable for both the host and its parasites. The findings of the present study are of importance as this was the first parasitological study at the Kwena Dam. The findings also represent new geographical records and thus form baseline data for future parasitology research studies and aquatic monitoring.

6.2 Recommendations for future studies

This study was undertaken to gain knowledge and information on the Kwena Dam that had no previous parasitological study history.

The following recommendations are put forward.

Future research should focus on the missing data on the diversity, host specificity and distribution of parasites and compare parasite fauna of the same fish species, within the same river system yet at different sampling sites with different water quality. Different host fish could be used to investigate the parasite diversity of the Kwena

Dam. More studies should be conducted and focus on providing more accurate information on the diversity and distribution of alien freshwater fish parasites and more regulatory measures are needed on the introduction of alien fish species in South Africa. Future studies should focus on different sampling sites with different water quality for better application of K as the latter could be used to compare the overall health of different fish species. The use of molecular diagnostic tools should be considered to assist with the identification of larval parasites.

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STUDY OUTPUTS

CONFERENCE PRESENTATIONS

The following oral presentations were done at conferences:

1. The 3rd National Conference on Global Change held at Southern Sun hotel, Durban, hosted by the University of KwaZulu Natal, 05-08 Dec 2016.

   Mokonyane MP, Luus-Powell WJ, Halajian A, Matla MM & Roux F. Diversity of freshwater parasites and water quality in The Kwena Dam, Mpumalanga Province, South Africa.

2. The 3rd International Congress on Parasites of Wildlife, held at the Kruger National Park, 24–27 September 2017.

   Mokonyane MP, Halajian A, Matla MM, Tavakol S, Kunutu KD, Roux F & Luus-Powell WJ. First report of occurrence of an alien cestode parasite in an alien fish (Cyprinus carpio), The Kwena Dam, Mpumalanga Province, South Africa.

3. Faculty and Postgraduate Research Day held at Fusion Boutique Hotel, Polokwane, hosted by the University of Limpopo, Faculty of Science and Agriculture, 20–21 September 2018.

   Mokonyane MP, Luus-Powell WJ, Halajian A, Matla MM, Tavakol S, Kunutu KD, Roux F, Smit WJ & Sara JR. Reserve, Limpopo Province. Parasites of an alien freshwater fish (*Cyprinus carpio*), The Kwena Dam, Mpumalanga Province, South Africa

Poster presentation:

The 4th National Conference on Global Change to be held at Bolivia lodge, Polokwane, 03–06 December 2018.

Mokonyane MP, Luus-Powell WJ, Halajian A, Matla MM, Tavakol S & Roux F. An alien freshwater fish (*Cyprinus carpio*) becoming a threat to South African freshwaters.

The following paper is under review:

The expanding distribution of *Atractolytocestus huronensis* (Cestoda: Lytocestidae), an invasive alien parasite of alien freshwater fish *Cyprinus carpio*, in Limpopo and Mpumalanga provinces, South Africa.

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