A GENERAL DISEASE AND PARASITE SURVEY OF COMMERCIALLY IMPORTANT FISHES OF THE FREE STATE

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I Lefetlho Katlego Mogorosi declare that the Master's Degree research dissertation or
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Table of contents

Chapter 1: Introduction

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South Africa's utilisation of fresh water fish products as a dietary supplement lags behind compared to densely populated countries such as China, which has a population of about 1.3 billion (Olivier et al. 2009). There are various reasons for this; the first being the long shore-line of South Africa (3000km from the southwest Atlantic Ocean to the Indian Ocean bordering Mozambique), which has a rich supply of marine fish and is able to support many diverse artisanal and commercial fisheries. The South African oceans are largely influenced by the confluence of the cold Benguela Current (on the west) and the warm Aghulhas current (on the east), and this contributes to high levels of marine biodiversity and species endemicity (WWF 2011). The upwelling of cold nutrient rich water on the west coast contributes towards this productivity and supports vast commercial fisheries activities. The warmer, less productive waters of the east coast support several other smaller fisheries. However, marine fish stocks are on the decline due to overfishing and over-exploitation (WWF 2011).

Secondly, some South African rural and inland communities have not really developed an appetite for fresh water fish; thirdly, South Africa has a well-developed meat agricultural sector, with 69% of land surface being well suited for grazing, therefore meat products are easily accessible and relatively affordable (Agriculture Market Intelligence Report 2016).

According to WWF (2011) the contribution of cultured products has grown and is considered to be the fastest growing animal food producing sector worldwide, the aquaculture sector is predicted to surpass fisheries as a source of food fish. Capture fisheries production has remained relatively static since the late 1980s, however in 2014, aquaculture production for human consumption overtook fisheries contribution for the first time (FAO 2016). In 2014 world per capita fish supply reached 20 kg, this was as a direct result of the rapid growth of the aquaculture sector, which now provides half of all fish for human consumption (FAO 2016).

Aquaculture is defined as: the farming of aquatic organisms, including fish, molluscs and aquatic plants. Where farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators etc.

Farming also implies individual or corporate ownership of the stock being cultivated (FAO 1988).

In 1974 aquaculture provided only about 7% of fish for human consumption, this contribution grew steadily and was recorded at 26% by 1994, and up to 39% by 2004. The People's Republic of China had the greatest contribution and played a major role with regards to aquaculture growth, representing more than 60% of the world aquaculture production. However, the rest of the world has also benefited, with its own share of aquaculture activities meant for human consumption (FAO 2016).

In South Africa specifically the total production of aquaculture increased from 5 210 tons in 2014 to 5 418 tons in 2015, meaning that the sector grew by ~4% in the space of one year. The contribution of fresh water aquaculture increased from 1 792 tons in 2014 to 1 826 tons in 2015 (DAFF 2016). The contribution of fresh water aquaculture was low from 2014 to 2015, the subsector though has shown a steady growth over the years. In 2006 for example, the total production of fresh water aquaculture was recorded at about 1 000 tons, whilst a few years later in 2015 the total production had increased to 1 826 tons (DAFF 2016).

Although the growth recorded for fresh water aquaculture is slow, it is still necessary to have a good approach to fish health management on fresh water aquaculture farms in general. According to Olivier et al. (2009), the increase of scientific knowledge regarding the parasites of fish, indigenous as well as introduced, has become urgent and as such, very important.

Baseline information on parasitic infections collected from wild fish stocks can be applied to fish under aquaculture conditions and may aid in the implementation of proactive measures as compared to the application of reactive measures when problems arise in the aquaculture system (Crafford et al. 2014).

The mortality and morbidity of fish is common in aquaculture fish as well as wild fish stocks, this is commonly caused by excessive parasite loads (Reed et al. 2012). Fishes in the aquaculture environment are usually kept in crowded conditions for intensive farming and are therefore in close proximity allowing for the transfer of parasites from one susceptible host to another (Reed et al. 2012).

Furthermore harsh conditions such as poor water quality, poor nutrition and aggressive behaviour amongst fish kept in the same tanks, add to the stress loads of the fish, contributing to the weakening of the immune system, thereby allowing for the rapid proliferation of parasites (Reed et al. 2012).

The present study was a disease and parasite survey of commercially important fishes found in inland impoundments of the Free State Province, only parasites were collected, no diseases were encountered or observed. Diseases in the context of the current study refers to potential clinical signs of disease such as lesions that can be caused by parasite activity.

Commercially important fish of the Free State Province include: African sharptooth catfish *Clarias gariepinus* (Burchell, 1822), common carp *Cyprinus carpio* Linnaeus, 1758, largemouth yellowfish *Labeobarbus kimberleyensis* (Gilchrist & Thompson, 1913), smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822), moggel *Labeo umbratus* (A. Smith, 1841), Orange River mudfish *Labeo capensis* (A. Smith, 1841), grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) and rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), although *O*. *mykiss* was not collected during the current study.

Although there have been several studies on the fresh water fish parasites of South Africa, such as Basson & Van As (1989), Mashego (2001), Van As & Van As (2001), Bertasso & Avenant-Oldwage (2005) and Crafford et al. (2014), there have not been many studies focusing on the parasites of commercially important fishes of the Free State Province.

Several parasite species were collected from various fish species caught at nine inland impoundments in the Free State Province during this study, these included the following: parasitic crustaceans, *Lernaea cyprinacea* Linnaeus, 1758 and *Argulus japonicus* Thiele, 1900; the monogeneans, *Quadriacanthus aegypticus* El Naggar & Serag, 1986, *Dactylogyrus* Diesing, 1850 sp 1 and *Dactylogyrus* Diesing, 1850 sp 2; the cestode, *Schyzocotyle acheilognathi* (Yamaguti, 1934) and finally; the peritrich ciliophorans, *Trichodina nigra* Lom, 1961, *Trichodina centrostrigeata* Basson, Van As & Paperna, 1983, *Tripartiella lechridens* Basson & Van As, 1987 and *Trichodinella epizootica* (Raabe, 1950).

Some of the above mentioned parasite groups have been documented in literature as having caused mortalities of fish under aquaculture conditions. According to Basson & Van As (2006), trichodinosis is often found in young fry, mostly in spring after harsh winter conditions in the wild, and is also common in fresh water and marine farms. Lernaeid copepods are common pests in fresh water aquaculture cyprinids, and epizootics are often associated with high mortalities of the cultured fish (Lester & Hayward 2006). According to Buchmann & Bresciani (2006) monogeneans are often important pathogens in many aquaculture systems (both fresh water and marine) and in cases where monogenean infestations are allowed to develop because of the appropriate abiotic and biotic conditions high morbidity and mortality occur.

EL-Galil & Aboelhadid (2012) reported a 53% mortality rate of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) fry from a private fish hatchery as a result of the diseases trichodinosis and gyrodactylosis. They identified the causes of the diseases as *Trichodina acuta* Lom, 1961, *T. heterodentata* Duncan, 1977 and *Gyrodactylus* Nordmann, 1832 species.

Monogeneans have also been known to be highly pathogenetic when associated with certain host species, this applies to both the aquaculture environment, as well as in the wild. For example *Gyrodactylus salaris* Malmberg, 1957 has been recorded from wild populations of Atlantic salmon *Salmo salar* Linnaeus, 1758 in Norwegian rivers since the 70s and this has resulted in heavy losses of salmon fry in affected rivers (Buchmann & Bresciani 2006). Monogeneans all have direct life cycles, which means that they do not have an intermediate host, this plays a role in their transmission from one susceptible host to another, as it eliminates the need for an intermediate host. This makes it easier for individual parasites to transfer between hosts. According to Buchmann (2012), monogeneans are able to spread from one host to another through the direct contact between hosts. In aquaculture, fish are typically kept at high densities for intensive culture, where there is generally a lot of contact between individual fish, which could further assist in the facilitation of the transfer of monogeneans between the hosts.

Parasitic crustaceans are of economic importance as they can affect host survival by causing unsightly changes to the flesh, and some of these have also caused ongoing problems in aquaculture (Lester & Hayward 2006). Lernaeids for example, are common pests in the culture of fresh water cyprinids, and known epizootics of cultured fish often associated with high mortalities (Lester & Hayward 2006).

In the case of cestodes, the Asian tapeworm *S*. *acheilognathi* is considered the most important pathogenic cestode parasite of cyprinids (Scholz et al. 2012). According to Scholz et al. (2012) *S*. *acheilognathi* is an important pathogen in aquaculture operations in Europe and Asia, with up to 100% mortalities of juvenile fish recorded in hatchery ponds.

The aims of this study were to collect and identify fish parasites found in nine dams in the Free State Province to at least genus level; and to identify fish parasites found at the Agricultural Technology Demonstration Centre (ATDC) to at least genus level; to taxonomically describe the key species and any new species found.

The current chapter, **Chapter 1** is a general introduction to the study, **Chapter 2** is a summary of the historical background of African inland aquaculture from continent level, to South African inland aquaculture and then inland aquaculture in the Free State Province. **Chapter 3** deals with the study sites, hosts and materials and methods for fish collection. The methods for the processing of parasites are included in each of the parasite taxa chapters, as these methods differ for different parasite taxa, therefore the format differs between the chapters. **Chapter 4** focuses on parasitic crustaceans, the role they may play in the development of inland aquaculture in the Free State, wherein a qualitative assessment has been made. **Chapter 5** deals with the monogeneans and their possible impact on the development of inland aquaculture in the Free State including infestation statistics. **Chapter 6 and 7** deal with cestodes and ciliophorans respectively and their possible role in aquaculture in the province, infestation and infection statistics have also been included. **Chapter 8** is the discussion which also includes the main conclusions. **Chapter 9** is a consolidated list of all references used in this document. The **Appendix** contains the permit granting permission for the catching of fish.

Chapter 2: African inland aquaculture

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2.1 African inland aquaculture: an overview

There have been concerted efforts to increase African inland aquaculture through research and various development programs (Table 2.1), and millions of dollars to fund projects. During the 1940s and 1950s while Africa was still under colonial rule, research stations were built across parts of the African continent in the following places: Djoumouna (Democratic republic of the Congo), Landjia (Central African Republic), Anamalazaotra and Ampamaherana (Madagascar), Foumban (Cameroon), Sagana (Kenya), Bouaké (Cote d'Ivoire), Kajjansi (Uganda), Henderson (Zimbabwe), Chilanga (Zambia) and Kipopo (DR Congo) (Brummett et al. 2008). The colonial powers occupying Africa at this time (1940s-1950s) recognised the potential of aquaculture as a viable means of food production and as such invested resources towards the development of aquaculture. According to Brumett et al. (2008) the aquaculture sector in Africa has not reached its full biophysical potential.

According to Suloma & Ogata (2006) the contribution of Africa to world aquaculture is fairly insignificant, contributing only 1.2% towards global aquaculture production. There are several factors contributing to the obstruction of aquaculture development, with the most important factors being political, economic and political issues. Another contributing factor to the slow progress of aquaculture in Africa is the heavy dependence on European countries to provide fish feed and feed ingredients, as fish feed accounts for at least 60% of the total cost of production (Gabriel et al. 2007).

Even though this is the case, African aquaculture has still demonstrated its competitiveness owing to the following: **(1)** African aquaculture produces fish that normally feed low on the food chain, e.g. *Oreochromis niloticus* and Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852); **(2)** Farming methods that are generally environmentally friendly and benefit a broad spectrum of user groups (Brummett et al. 2008). For African inland aquaculture to make a meaningful contribution to the development of the continent in terms of the economy, poverty alleviation and food security, African governments need to adjust their policies to rather rely on commercial investments. According to Brummett et al. (2008) a pragmatic business approach that focuses on small and medium scale private enterprises would be far more beneficial than centrally planned and government led development projects.

Table 2.1: Development in African aquaculture (1920s-2000s). Adapted from Brummett & Williams (1999), Hishamunda & Ridler (2006) and FAO (2011).

Years	Development
1920s	• 1924: fish culture begins in Kenya
1930s	• 1937: fish culture begins in Zaire (Democratic Republic of Congo)
1940s	• 1942: fish culture begins in Zambia • 1948: fish culture begins in Congo-Brazzaville (Republic of Congo)
1950s	• 1950: Zimbabwe begins fish culture • Rapid development of aquaculture, sharp increase in the number of ponds, culminating with spread of aquaculture to other countries
1960s	• The spread and development of fish culture increases to its highest peak.
1970s	• Start of second wave of development.
1980s	• Second wave of aquaculture development continues in Kenya and Cote d'Ivoire. • Small to large-scale private sector farming begins in Egypt, Nigeria, Zambia and expanded in Cote d'Ivoire. • Farming of shellfish begins in: South Africa, Tunisia, Senegal, Morocco, Zimbabwe, Mauritius, Malawi and Reunion Island.
1990s	• Aquaculture production slowly increases. • Re-evaluation of specifications for small scale farmers, and their contribution to food security for the poor. • African countries and regional bodies such as SADC (15 member states: Angola, Botswana, Democratic Republic of Congo, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Seychelles, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe) put the aquaculture development plan into motion. • Government, nongovernmental organisations and the private sector roles are re-examined. • Natural resource management including biodiversity management, sustainability and climatic conditions begin to impede the development of the sector.
2000s	• Recognition that food insecurity is also caused by poverty and lack of access to resources. • Southern African Region's strategy to make more food available, but also to increase incomes for households • Aquaculture recognised as a sector that can contribute to reducing food insecurity in Africa. • The implementation of Operation Phakisa in South Africa, which recognises the importance of a coordinated approach among government departments with regard to processing aquaculture applications, permits and Environmental Impact Assessment applications, as these require input from different Government departments. • 2030 Agenda for Sustainable Development encourages: awareness of the vital part oceans and inland waters must play in providing food.

Colonialism in Africa during the 1940s and 50s brought with it the realisation of the potential for inland aquaculture as a viable means of food production. The earliest efforts to develop African inland aquaculture involved the investment of resources, building research stations for basic aquaculture research and the design of basic technologies to farm with indigenous African aquaculture species (Brummett et al. 2008).

The research stations built mainly investigated the following species: *O niloticus*, *O. mossambicus*, greenhead tilapia *O. macrochir* (Boulenger, 1912), blue tilapia *O. aureus* (Steindachner, 1864), mango tilapia *Sarotherodon galilaeus* (Linnaeus, 1758), blackchin tilapia *S. melanotheron* Rüppell, 1852, redbreast tilapia *Coptodon rendalli* (Boulenger, 1897), African sharptooth catfish *Clarias gariepinus*, African boneytongue *Heterotis niloticus* (Cuvier, 1829), and the alien invasive species common carp *Cyprinus carpio* (Brummett et al. 2008).

Through research into different culture techniques, it was established that *O. niloticus* is commercially viable and as a result, has since become one the most important aquaculture fish species in the world, but mostly outside of Africa. According to Brummett (2008) the trend amongst African communities was that the large Nile tilapia is a luxury, while the smaller individuals were seen as a staple source of protein in the poorer communities. The African sharptooth catfish is notably more popular in Central and West Africa, where it dominates the local cuisine and inland fisheries. Towards the 1960s, many of the African countries gained independence from colonial rule, and as a result there was a shift in the prioritisation of resources and with it, came the abandonment of many of these research projects.

Between the 1970s and 1990s aquaculture was employed as a facilitation tool to be used in rural food security and economic development by international donor agencies. These donor agencies essentially took over the role of government in the development of the aquaculture sector. According to Brummett et al. (2008), the donor agencies targeted aquaculture projects at the artisanal level; the intention with this approach was to turn low external inputs into productive enterprises. This strategy though successful, only yielded positive results for a few years, once the foreign donations were discontinued, these institutions collapsed.

Some of the greatest challenges in the 21st century include climate change, economic uncertainty and growing competition for natural resources (FAO 2016). As a result, aquaculture is now seen from a global perspective, where the international community has made commitments to face one of the greatest challenges of this era, “how to feed 9.7 billion people by 2050” (FAO 2016).

According to FAO (2016) 84% percent of the global population that is involved in aquaculture and fisheries in 2014 was in Asia, whilst 10% of the global population that is involved in fisheries and the aquaculture sector was found in Africa, followed by Latin America and the Caribbean. The per capita fish consumption of some African countries in 2014 was as follows: 7.7 kg for South Africa, Nigeria 11.8 kg, Mauritius 19.8 kg, Senegal 26.8 kg and Sierra Leone 17.3 kg (FAO 2017). In 2014 world aquaculture production of fish accounted for 44.1% of total production, and in general all continents, including Africa, showed an increase in aquaculture production (FAO 2016).

2.2 Fresh water aquaculture in South Africa

Inland aquaculture in South Africa, as with the rest of the continent, is a relatively small industry and according to DAFF (2012a) South African inland aquaculture primarily produces the following species: *O. niloticus*, *O. mossambicus*, *Clarias gariepinus*, and the following alien species: largemouth bass *Micropterus salmoides* (Lacepéde, 1802), *C. carpio*, grass carp *Ctenopharyngodon idella* Linnaeus, 1758, Atlantic salmon *Salmo salar*, brown trout *S. trutta* Linnaeus, 1758 and rainbow trout *Oncorhyncuss mykiss*.

In 2015, the total fresh water aquaculture production was about 1 826 tons, the biggest contributing species to the aquaculture production were trout (*O*. *mykiss* and *S*. *trutta*) at about 1 497 tons, followed by tilapia (*O*. *niloticus* and *O*. *mossambicus*) at 325 tons (DAFF 2016).

The development of inland aquaculture in South Africa was initiated by colonial authorities mainly to stock angling waters with exotic species such as bass and trout. In the 1980s hatcheries were built in the former homelands (Venda, Lebowa, Gazankulu and Transkei) and this was with the intention of promoting inland aquaculture as a means to increase food security (Rouhani & Britz 2004). Around this time, commercial aquaculture was also established, resulting in the production of trout, sharptooth catfish and ornamental species such as koi carp. The aquaculture industry grew around this time period (1980s), and in 1988 the aquaculture sector (inland and marine) produced about 3 090 tons (trout, sharptooth catfish, ornamental fish as well as oysters and mussels). From 1988 to 1998, the aquaculture sector grew by an average of 6.8% per annum (Rouhani & Britz 2004).

During the 1990s, South Africa was in a state of transition from apartheid rule to democratic rule, and as such, state led aquaculture projects were again initiated to stimulate rural aquaculture as a means to increase food security and economic well-being of rural communities (Rouhani & Britz 2004). However, there were serious constraints with regards to the sustainability of these state led aquaculture projects: **(1)** South Africa in general is a water scarce country, **(2)** Extreme seasonal fluctuations in temperature over much of the interior of the country occurs. In essence, winters are too cold for the sustainable production of warm water species such as tilapia, whilst during the summer, the water is too warm for the sustainable production of cold water species such as trout. **(3)** Successful aquaculture projects are likely to require more skill intensive methods (Rouhani & Britz 2004).

The South African Government has had a long history of contributing to the development of the inland aquaculture sector. These government interventions have historically focused on the development of government run research facilities, so as to supply fingerlings to potential and emergent farmers and university run research projects (Shipton & Britz 2007).

According to Shipton & Britz (2007), historically, South Africa has had at least 17 provincial/government funded aquaculture facilities. Of these, only seven are still operational. These include: **Jonkershoek Hatchery (Stellenbosch)**, **Amalinda Hatchery (East London)**, **Mabeleni (Eastern Cape)**, **Makatini (KwaZulu-Natal)**, **Tompi Seleka College (Marble Hall)**, **Lydenburg Fish Production**, and **Gariep Dam Hatchery (Free State)**. Provincial/Government funded aquaculture facilities that have collapsed in South Africa include: Umtata hatchery (Umtata), Tsolo College of Aquaculture (Umtata), Pirie Hatchery (Eastern Cape), Nagle Dam Hatchery (Durban), Cambrey Hatchery (KwaZulu Natal), Royal Natal Parks Hatchery (KwaZulu Natal), Underberg Trout Hatchery

(Underberg), Dzindi Fish (Thohoyandou), Turfloop Breeding Station (Limpopo) and De Kuilen (Cape Town).

The fresh water aquaculture sub-sector is older than the marine aquaculture sub-sector, and has higher numbers in terms of producers and diversity of species produced at either commercial or pilot scale (Table 2.2) (DAFF 2012a). According to DAFF (2012a), in South Africa there are about 30 notable producers of trout and they are mainly located in the Western Cape, Mpumalanga and Eastern Cape. Other provinces where fresh water aquaculture is prominent are Mpumalanga, KwaZulu-Natal and Gauteng. The Free State, Northwest, Northern Cape and Limpopo are still developing provinces in terms of fresh water aquaculture.

Table 2.2: Fresh water aquaculture species cultured in South Africa in 2010, including the scale of operation. (Adapted from DAFF 2012a).

Common name	**Scientific name**	**Operational scale**	**Alien/local species**
Rainbow trout	*Oncorhynchus mykiss*	Commercial	Alien
Brown trout	*Salmo trutta*	Commercial	Alien
Nile tilapia	*Oreochromis niloticus*	Commercial	Alien
Redbreast tilapia	*Coptodon rendalli*	Commercial	Local
Common carp	*Cyprinus carpio*	Commercial	Alien
Koi carp	*Cyprinus carpio*	Commercial	Alien
Largemouth bass	*Micropterus salmoides*	Commercial	Alien
Goldfish	*Carassius auratus*	Commercial	Alien
Guppies	*Poecilia* spp	Commercial	Alien
Mozambique tilapia	*Oreochromis mossambicus*	Pilot	Local
African sharptooth catfish	*Clarias gariepinus*	Pilot	Local
Fresh water mullet	*Myxus capensis*	Pilot	Local
Flathead mullet	*Mugil cephalus*	Pilot	Local
Atlantic salmon	*Salmo salar*	Pilot	Alien
Marron (fresh water crayfish)	*Cherax tenuimanus*	Commercial	Alien

Evidence over the years seems to suggest that many of the fresh water aquaculture projects embarked on by the state, have actually collapsed or have never functioned optimally. For example, The Black Survival Project in QwaQwa has not reached its full potential yet, however, the project is receiving support from the Free State Department of Agriculture and Rural Development (FSDARD) to get it back on track.

However, it is not just aquaculture facilities that have had challenges in remaining functional, the same general trend is seen in inland commercial capture fisheries. In the Free State Province for example, attempts at developing a formalised capture fisheries sector failed soon after they had started, e.g. Erfenis Dam, Rustfontein Dam and Vaal Dam (Barkhuizen et al. 2016). According to Barkhuizen et al. (2016), there has been significant political pressure to develop inland commercial capture fisheries as interventions to contribute to food security, poverty alleviation, the creation of employment opportunities, and economic development. Generally, the potential of capture fisheries is seen as underdeveloped in terms of food security.

Although there have been several attempts to establish commercial fisheries in the Free State, it has been difficult to assess and really understand the constraints of the development of capture fisheries. One of the challenges, is the fact that there is not enough information regarding these attempts and initiatives, making it all the more difficult to understand the processes in real terms (Barkhuizen et al. 2016). The Free State Province, has had a relatively long history of approved commercial fishing on inland impoundments as compared to the other provinces, and the province has attempted to develop commercial fisheries at 11 impoundments since 1979.

Permit conditions for these capture fisheries were imposed under the following conditions: prohibited species, size limitations for some species, gear restrictions, catch quotas, access restrictions within protected areas, boating regulations, as well as submission of catch returns (Barkhuizen et al. 2016). Over a 35 year period (1979-2014), a total of 9 036 tons of fresh water fish were harvested, the composition of the catches differed between the impoundments. Bloemhof Dam, Vaal Dam and Gariep Dam catches were comprised mainly of *C. carpio*, whilst catches at the other impoundments were dominated by Orange River mudfish *Labeo capensis* (Smith, 1841) and *L. umbratus* (Smith, 1841).

Some of the capture fisheries initiatives closed down shortly after they were established, e.g. Erfenis, Vaal and Rustfontein Dams (Table 2.3). According to Barkhuizen et al. (2016) the only successful fisheries that stayed operational for more than ten years were located at Bloemhof Dam and Kalkfontein Dam.

Table 2.3: Commercial fisheries licensed at 11 impoundments in the Free State Province (1979-2014), and summary of status (Adapted from Barkhuizen et al. 2016).

Impoundment	Harvest (ton/year)	Category of fisheries/brief description
Bloemhof Dam	207	Seine net fishery, 2 long term operators with individual quotas of 200 t/year
Kalkfontein Dam	136	Opportunistic fishery, seine nets, yearly quotas 100-250 t/year
Koppies Dam	16	Failed after 12 years (1982-1993), seine nets, gills nets, yearly quotas 25-50 t/year
Rustfontein Dam	8	Failed after 5 years (1982-1986), yearly quota 50-100 t/year
Erfenis Dam	2	Failed within 2 years
Gariep Dam	5	Various development attempts by private entrepreneurs, and government led initiatives, all of which failed. Seine nets, gill nets, yearly quotas 10-50 t/year.
Allemanskraal Dam	No data	Failed, no catch data
Rhoodepoort Dam	No data	Dam dried up
Krugersdrift Dam	No data	Dam dried up
Witpan Dam	No data	Failed at experimental stage
Vaal Dam	16	All attempts failed

The overall outcome of attempting to establish a commercial and sustainable fisheries subsector in the Free State Province, was unsuccessful, however, according to Barkhuizen et al. (2016) this apparent failure was not necessarily as a result of overfishing, but rather as a result of the general lack of return on investment.

2.3 Inland aquaculture in the Free State Province

One of the policies adopted by the South African government has been to encourage and stimulate the development of inland/fresh water aquaculture as a means to improve food security and poverty alleviation.

Consequently, the Free State Department of Agriculture and Rural Development (FSDARD) in collaboration with the People's Republic of China entered into an agreement in 2005 to set up an aquaculture capacity building programme for South African Government officials, farmers and scientists in the Free State Province. A memorandum of understanding was signed in 2006 by the two fore-mentioned countries, this was followed by the endorsement of an Aquaculture Action Plan, by the then Minister of

Agriculture and Land Affairs, Ms Lulu Xingwana. In 2007, a Chinese delegation visited FSDARD and as a result, the Gariep Dam Fish Hatchery was identified as a suitable training and breeding centre, as well as an aquaculture development facility in southern Africa. Through this collaboration the Gariep Dam Fish Hatchery was developed, and became: The China South Africa Agricultural Technology Demonstration Centre (ATDC).

The ATDC is intended to be a fingerling supply station for rural aquaculture community projects in the Free State, a research station and a facility to provide training to agricultural scientists and farmers in South Africa and the rest of the Southern African Development community (SADC) region. This project between the China and South African governments was divided in two phases, the 1st phase was the construction phase (2007-2014), while the 2nd phase (2014-2017) was the technical cooperation phase (cooperation between China and South Africa). The formal conclusion of the partnership with the Chinese government was in June 2017. Currently (2018) the South African government has total control of the ATDC.

The ATDC is located in the Xhariep district, Kopanong Local municipality at Gariep Dam in the Free State Province. In addition, six smaller recirculating aquaculture systems (RAS) were built in the following rural towns: Bethulie, Zastron, Springfontein, Petrusburg, Koffiefontein and Fauresmith that fall within the Xhariep district. The ATDC is meant to supply fingerlings to these RAS for grow out, as an initiative to support emergent and potential farmers supported by the FSDARD.

The ATDC currently (2018) produces sharptooth catfish and common carp as food species to supply the six RAS local community projects, as well as various other fresh water aquaculture projects, e.g. the Black Survival Aquaculture Project based in QwaQwa in the Free State. The ATDC furthermore produces ornamental fish such as koi carp and goldfish. From October 2017 to March 2018, the ATDC started with a breeding program during the summer months, as some species prefer warm water e.g. *C. gariepinus,* whilst some species such as *C. carpio* are tolerant of a wider range of conditions (Skelton 2001). The fish that were bred were used for various aquaculture research projects supported by FSDARD; while some of the fingerlings were kept as brood stock for future endeavours.

The ATDC functions on a flow through system, sourcing water directly from the Gariep Dam. This water is channeled to the overwintering facility (86 tanks) and to the outside ponds (36 ponds) by gravitational gradient. The overwintering facility is used for breeding purposes. Once the eggs hatch, the fry are kept herein and fed until fingerling size (fingerlings weight ± 5g), after which the fingerlings are transferred to outside ponds for grow out. In general, the fry are fed live feed (*Artemia*) as soon as they start feeding, after which they are placed on a diet of artificial feed (initially the fry are fed artificial feed in the form of a powder). As they grow bigger they are fed artificial feed powder, then crumble, then feed pellets of 1mm size, followed by 2mm and finally 3mm size pellets.

Over the winter months, fish from the outside ponds are moved into the overwintering facility where the water temperature is higher than that of the outside ponds, due to the running of a hot water boiler. The Department of Agriculture, Forestry and Fisheries (DAFF) and FSDARD have shown support for the facility, which will ensure that operations at ATDC are successful.

2.4 Biology and ecology of commercially important fish of the Free State

According to Skelton (2001), the South African Aquaculture Association was formed in 1989, and aquaculture is a growing subsector in South Africa. There are a variety of species being cultured in South Africa, with about 30 major producers of trout, producing about 750 ton with a value of about R 11million per year. Subsistence and commercial fisheries are also well established in South Africa, in Mpumalanga for example, there are well established private and commercial facilities for bass and trout fishing (Skelton 2001). According to Barkhuizen et al. (2017) subsistence and recreational angling are the dominant form of utilisation of inland fisheries. Although in South Africa inland fisheries are considered to be relatively underexploited from a harvest perspective, in some instances harvests by subsistence and recreational anglers can be considerable. For example, an estimated 88 ton per year of fish was harvested by recreational anglers from Gariep Dam (Ellender et al. 2010). Recreational angling as well as tournament angling remain important fisheries activities in the Free State Province (Barkhuizen et al. 2017). The following species have commercial value in the Free State, either as aquaculture, subsistence or recreational angling species.

Clarias gariepinus (Burchell, 1822)

The African sharptooth catfish (also known as babel) occurs in a variety of habitats, however it prefers large slow moving rivers, dams, lakes and floodplains. The sharptooth catfish is a hardy species of fish and can withstand conditions that many other species would not be able to endure. It can live through conditions such as high turbidity and desiccation, and is often found as the last inhabitant of drying up rivers and lakes in which it would have burrowed. It is able to move on land by extending its pectoral spines, moving in a crawling action, and uses its branchial trees for respiration (Skelton 2001).

It is a scavenger and will feed by actively hunting and scavenging on anything that is available for consumption including: frogs, birds, fish, small mammals, snails, reptiles, shrimps, crabs and plant matter. Catfish sometimes hunt in packs and coordinate to herd and trap smaller fish. According to Skelton (2001), the African sharptooth catfish is preyed upon by crocodiles, leopards and birds of prey such as the marabou stork and the fish eagle. During the breeding season mature adults migrate to flooded grass plains after the summer rains. The eggs are laid on vegetation and take a period of between 25 and 40 hours to hatch; the larvae that emerge are free swimming and start feeding within three days and remain inshore among the vegetation. It is an important angling, aquaculture and food fish species, and according to DAFF (2016) in 2015 there were a total of 13 farms in South Africa producing sharptooth catfish.

Cyprinus carpio Linnaeus, 1758

The common carp (alien) is a very hardy species and is able to tolerate a wide range of conditions, but in general it thrives in large slow moving or still-standing water with soft sediment at the bottom. It is found in farm dams all over South Africa, also in large turbid rivers, it is omnivorous and feeds on plant and animal matter by grubbing in sediments. Breeding takes place in spring through to summer and sticky eggs are laid in shallow vegetation. Large females have been reported to lay over a million eggs, it takes four to eight days for the eggs to hatch and growth is rapid. This species is a valued aquaculture and angling species, although it is considered a pest by conservation authorities as a result of its destructive feeding habits (Skelton 2001).

Labeobarbus kimberleyensis (Gilchrist & Thompson, 1913)

The adults prefer flowing water below rapids or in deep channels, but they also thrive relatively well in dams. Largemouth yellowfish are predators, and will initially feed on insects and small crustaceans, but become piscivorous as they grow larger. Breeding takes place in the mid-to late summer months over gravel beds in running water, the fecundity increases in larger females to about 60 000 ova (Skelton 2001). The eggs will hatch in about two to three days, and the larvae start to feed about four days later Growth is relatively with males reaching maturity at six years and females in eight years. Largemouth yellowfish are renowned angling species and will take live bait and a variety of lures (Skelton 2001). The largemouth yellowfish is a threatened species and there are several factors that have contributed to its conservation status. It is a slow growing species, therefore it takes more time to replace individuals that have been removed. *Labeobarbus kimberleyensis* does not typically inhabit the smaller tributaries, therefore the impact of polluted waters in the main channel is very serious (de Villiers & Ellender 2007). According to de Villiers and Ellender (2007) the use of gill nets is a serious contributing factor to the declining numbers of *L. kimberleyensis*, it is also under immense pressure from angling.

Labeobarbus aeneus (Burchell, 1822)

This species prefers clear-flowing waters of large rivers with rocky or sandy substrates, however, they are also found in large dams. They will usually occur at higher altitudes and smaller tributaries as compared to largemouth yellowfish (Skelton 2001). *Labeobarbus aeneus* breed in the spring until the midsummer months after the first significant rains of the season. According to Skelton (2001) the eggs are laid in the gravel and will hatch in about three to eight days, the larvae will start to feed on microscopic organisms in about four to six days. The larger individuals are omnivorous and will take benthic invertebrates such as bivalve molluscs, they will also take vegetation, algae and detritus, smallmouth yellowfish are an important angling species.

Labeo umbratus (A. Smith, 1841)

This species prefers standing or gently flowing water, it thrives in shallow impoundments as well as farm dams. Moggel feed on soft sediments and detritus. Breeding takes place after the summer rains, where they migrate upstream to flooded grassy banks, the females produce up to 250 000 sticky eggs, which attach to the grass and hatch after about 40 hours (Skelton 2001). The hatchlings swim up to the surface and are carried away by the current into the stream into deeper waters. Growth is rapid, and males reach maturity in about two to three years, this species is important in commercial and subsistence fisheries, they are also an occasional angling species (Skelton 2001).

Labeo capensis (A. Smith, 1841)

Orange River mudfish prefers running waters of large rivers, and also thrive in large dams. *Labeo capensis* will graze on firm surfaces of rocks and plants, breeding takes place in the summer where individuals gather in large numbers in shallow rocky rapids and lay eggs, larvae hatch in about three to four days. Growth is fairly rapid, *L*. *capensis* is an occasional angling species (Skelton 2001).

Ctenopharyngodon idella (Valenciennes, 1844)

The grass carp prefers large, slow moving or standing water bodies with vegetation, and are tolerant of temperatures between 0°C-38°C. *Ctenopharyngodon idella* feed mainly on aquatic plants, however, will also feed on insects and other invertebrates (Skelton 2001). According to Skelton (2001) Breeding takes place in flowing waters of rising rivers, the eggs and larvae float in the water. This species is used for aquatic weed control and has angling and aquaculture potential.

Oncorhyncus mykiss (Walbuam, 1792)

Rainbow trout prefer clear, well aerated, cool water at less than 21°C. Water temperature below 15°C is essential for breeding. Rainbow trout feed on a wide range of animal foods such as mayfly nymphs, caddis fly and midge larvae, terrestrial insects, crabs, frogs and fish (Skelton 2001). Breeding takes place from June to August, when the individuals move upstream to suitable gravel beds, where the females dig redds by beating the body and tail rapidly on her side over the gravel. Spawning takes place in several redds which are built and used by the female, the eggs hatch after four to seven weeks, the males mature after one year and the females two years. This is the most important aquaculture species in South Africa, and is also a top-rated angling game fish (Skelton 2001).

Chapter 3: Study sites, fish hosts, materials and methods used for fish collection

Chapter 3: Study sites, fish hosts, materials and methods used for fish collection

3.1 The Orange River Basin

The Orange River basin is the largest basin south of the Zambezi River, with a catchment area of about one million km^2. The Orange River basin is also the most developed trans-boundary river basin in the southern African region, supplying water to municipalities, farms and industries in and around the basin. The Orange River is not named as such, because of the distinctive reddish-orange colour of the river, it was in fact named after the Dutch house of Orange, by Colonel Robert Gordon, commander of the garrison of the Dutch East Indian Company in 1779 (Earle et al. 2005).

The Orange River basin stretches over four countries, i.e. South Africa, Lesotho, Botswana and Namibia, whilst part of the river also forms the border between South Africa and Namibia (Fig 3.1). The Orange River has two main tributaries, the Senqu River in Lesotho and the Vaal River in South Africa, as a result in Lesotho the river is sometimes referred to as the Orange-Senqu, whilst it is referred to as the Orange-Vaal River in South Africa. Pre-colonial settlers of the interior of South Africa, called this river Gariep, however the name that is most widely recognised, even internationally is Orange River (Earle et al. 2005).

The Orange River emanates from the Lesotho Highlands and flows in a westerly direction towards the West Coast into the Atlantic Ocean (Heath & Brown 2007). The entire country of Lesotho falls within the Orange River basin, and the Orange River flows from the Lesotho Highlands, into South Africa's Free State Province. The Vaal River forms one of the main tributaries of the Orange River, and flows to the south of Johannesburg, which is an important industrial region in South Africa. The Vaal River catchment area is highly populated and urbanised as a result of the industrial activity happening around Johannesburg, and 48% of the population of South Africa lives within the catchment and relies on its water. There has been a trend towards urbanisation as people move to the larger cities such as Johannesburg in search of work (Earle et al. 2005). Most of South

Africa's heavy industry and mining activities are located within the Vaal River catchment area and therefore presents a high demand for its water resources.

South Africa as a country, is the largest user of the Orange River water resource, accounting for about 82% of the annual total use, compared to Lesotho and Namibia, who also share sections of this water body. The main uses of the water resources from the Orange River by South Africa are the mining industry, as well as for irrigation purposes and domestic use. The use of water from the Orange River differs from region to region, for example, on the mid to lower reaches of the river, agriculture is the major consumer whilst industrial and municipal uses dominate the upper reaches of the Vaal River (Earle et al. 2005)

According to Ramollo (2011), the flow of the Orange River is greatly regulated through the use of several weirs and large dams such as Gariep and Vanderkloof Dams, to provide water for human use which includes mining, irrigation and human consumption. These two dams are also the largest storage reservoirs along the Orange River and are important for the regulation of flow in the lower Orange River. A large volume of water from the Orange River is transferred from the Gariep Dam into the Fish River catchment located in the Eastern Cape. Water transferred from both Gariep Dam and Vanderkloof Dam can be used for hydropower generation to meet peak electricity requirements (Earle et al. 2005).

3.2 Environmental issues within the Orange River basin

The great length of the Orange River (roughly 2 300km), in combination with its range of altitude and climatic zones, results in the Orange River basin covering a wide range of ecological systems. According to Earle et al. (2005), the Orange River basin includes several biomes, however, it is predominantly made up of Grasslands, Nama Karoo and Arid Savannah biomes. There are issues of concern with regards to the state of the Orange River, these include the impact of municipal and industrial effluent in the Gauteng area of the Vaal River system, agricultural pollution in the Orange and Vaal Rivers, as well as environmental threats to the Orange River estuary. The water quality is relatively good upstream of the Gariep Dam, whilst the water quality deteriorates downstream of this dam, specifically after the confluence of the Orange River with the Vaal River at the town of Douglas (Earle et al. 2005) (Fig 3.1). Large volumes of industrial and municipal effluents are released into the Vaal River in the Gauteng Province, this includes phosphates from sewage treatment plants and nitrates from industrial activities (Earle et al. 2005).

The Orange River estuary, situated between Alexander Bay (South Africa) and Oranjemund (Namibia), was proclaimed a Ramsar site by both countries, i.e. South Africa (1991) and Namibia (1995). This estuary is also considered as the sixth most important coastal wetland in southern Africa in terms of the number of birds supported, i.e. about 26 000 individuals representing 57 species (Earle et al. 2005). The rock catfish *Austroglanis sclateri* (Boulenger, 1901) is also present in the estuary, and according to Skelton (2001), the mainstream populations have been reduced by sedimentation and water abstraction. Over the past decade the rock catfish populations have declined, but fish numbers are speculated to be about 10 000 individuals (Swartz et al. 2007). Furthermore, the estuary is also home to the Namaqua barb *Enteromius hospes* (Barnard, 1938) which is categorised as near threatened (Skelton 2001).

The environmental impact caused at the river mouth is likely to increase with new reported developments of the Kudu gas station located 25 km north of Oranjemund. The environmental threats to the Orange River mouth wetland include pollution caused by industrial and municipal effluent, as well as pollution from agricultural activities. However, the most significant threat to the Orange River estuary is the loss of inflow water and sediment caused by the 23 major dams built on the river. Two of the major dams, i.e. Gariep and Vanderkloof act as sediment and water traps, restricting downstream flow and sediment from reaching the Orange River mouth wetland (Earle et al. 2005).

According to Ramollo (2011), indigenous fish species within the Orange River system are exposed to climatic fluctuations, hostile environmental changes, water abstraction, hydrological regimes and activities. Changes in environmental factors such as water current, water quality, water depth and food availability influence the occurrence, abundance and distribution of fishes within the system. Human activities and influences have also had a negative effect on the survival of several fish species such as largemouth yellowfish *Labeobarbus kimberleyensis* (Gilchrist & Thompson, 1913) and Orange River mudfish *Labeo capensis*.

3.3 The Orange River and Dams surveyed during the current study

The Orange River has a low species diversity and is dominated by members from the family Cyprinidae, whilst the other species belong to the families Clariidae, Austroglanididae and Cichlidae (Skelton 2001). The Orange River has 16 fish species, and of these, seven are endemic to the system, these include: *A*. *sclateri*, Maloti minnow *Pseudobarbus quathlambae* (Barnard, 1938), *E*. *hospes*, *L*. *kimberleyensis*, smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822), *L. capensis* and the river sardine *Mesobola brevianalis* (Boulenger, 1908) for which there is an isolated population in the Orange River below the Augrabies Falls (Skelton 2001).

The Orange River is also home to nine introduced fish species, and five of these were also collected during the current study, i.e. *M*. *salmoides*, *C*. *carpio*, goldfish *Carassius auratus* (Linnaeus, 1758), *C*. *idella* and Mosquitofish *Gambusia affinis* (Baird & Girard, 1853). The other alien species which were not collected during the current study include smallmouth bass *Micropterus dolomieu* (Lacepé, 1802), bluegill *Lepomis macrochirus* Rafineque, 1819, *O*. *mykiss* and *S*. *trutta*.

During the current study, fieldwork was conducted at nine dams (Fig 3.1) in the Free State Province (2013-2014) which form part of the Orange-Vaal River system. The field surveys were conducted in collaboration with Dr. L. Barkhuizen, of the Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs (FSDESTEA). In addition, parasitological surveys were also done at the ATDC during 2014, in 2016 the author was appointed at the ATDC and as such was able to collect more parasite data.

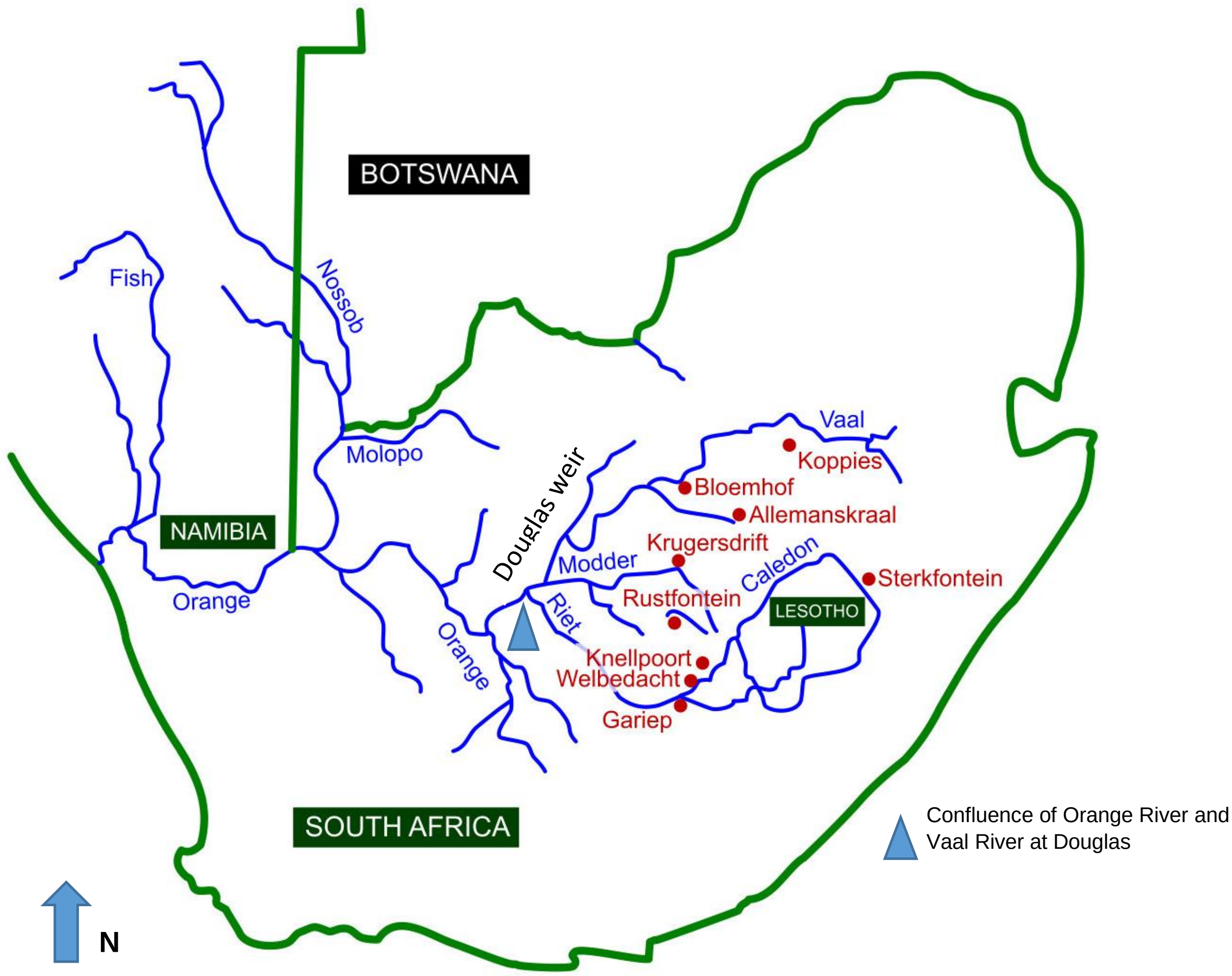


Figure 3.1: Map of the Orange-Vaal River Basin, showing the study sites (●). Redrawn from Conley & Van Niekerk (2000).

3.4 Materials and methods for fish collections

3.4.1 Fish collections

During the present study fish collections were done using seine and gill nets of varying mesh sizes. The mesh sizes for the gill nets were between 28mm and 144mm, the seine net mesh size was 75mm, whilst the dimensions for the seine net was 100m x 3m, which was used to collect larger fish specimens. Furthermore, a seine net with dimensions of 10m x 2m, and a mesh size of 75mm was used specifically to collect smaller fishes. Motorised boats were used to access the dams and the nets were set and left out in the dams for time periods ranging from a few hours to overnight.

The fish were kept in aerated containers at the respective dams and transferred to aerated tanks at the University of the Free State, Department of Zoology and Entomology when the dams were in close proximity to Bloemfontein. In cases where the dams were further away from the department, temporary field laboratories were setup at the dams where the fish were examined shortly after being caught (Fig 3.2 A, B, C, D, E, F). The fish were all identified using Skelton (2001). The larger fish were anesthetised using Benzocaine prior to being euthanised. The smaller fish were euthanised by severing the spine behind the head, most of these specimens were used for another project undertaken by the Aquatic Parasitology Research Group of the University of the Free State, and the study was conducted parallel to the current study. The parasites found were processed according to prescribed methods for each parasite taxon.

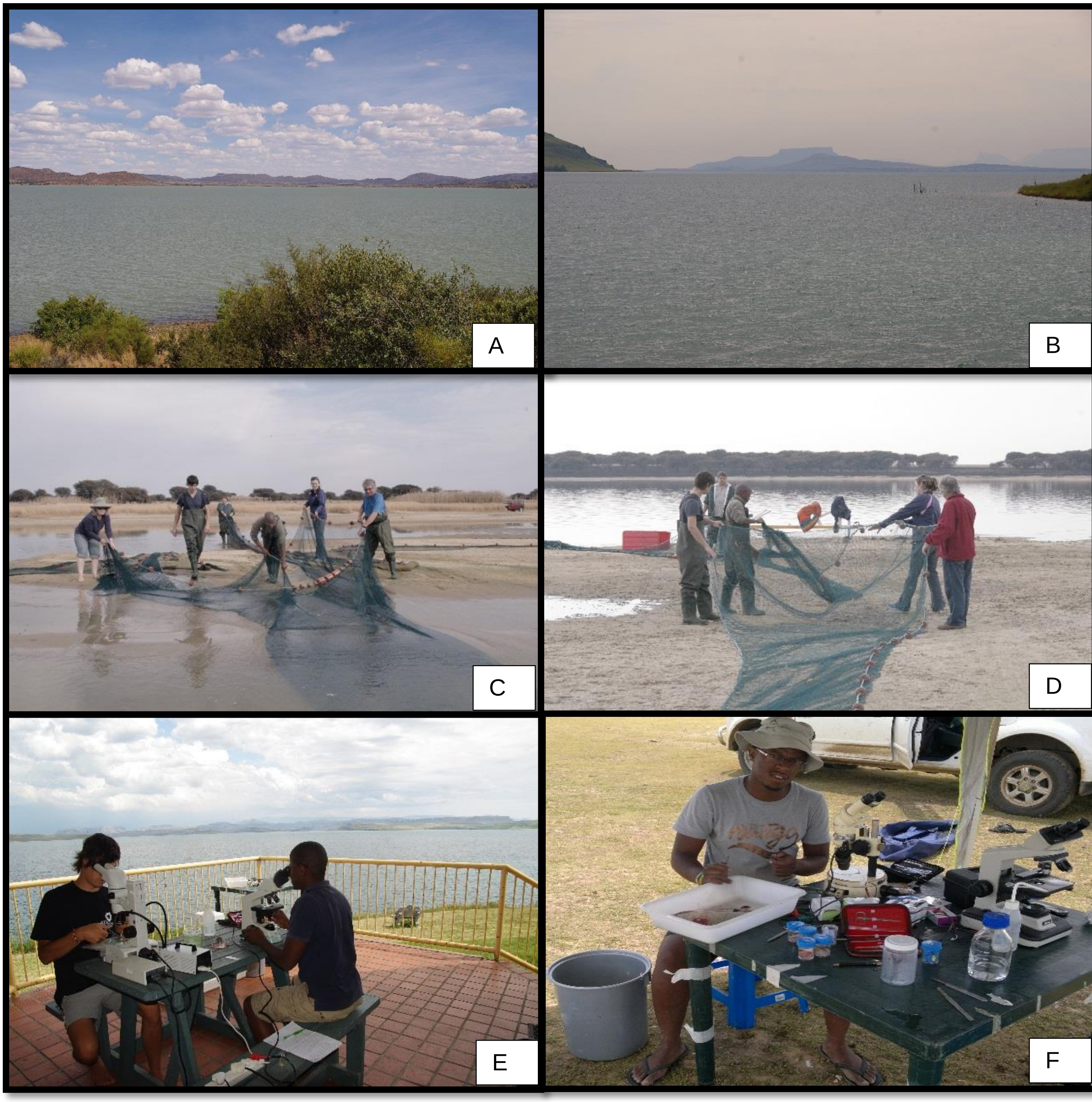


Fig 3.2: Photographs illustrating two of the collection localities and the methods used for fish collection and examination. A-Gariep Dam, B-Sterkfontein Dam, C & D-the use of seine nets, E-Temporary laboratory setup at Sterkfontein Dam, F-Temporary laboratory setup at Allemanskraal Dam.

Further information regarding specific techniques on host examination, fixation and preservation of parasites is included in each of the parasite taxa chapters (Chapters 4, 5, 6 & 7). The results are included in each of the parasite taxa chapters, however, the format for the respective results in each of the chapters is different as different methods and techniques (i.e. staining, fixing and measurements) were used for each of the different parasite taxa. In chapter 4, the apparent prevalence and mean intensity were calculated for the parasitic crustaceans, for chapter 5, monogeans; the mean, standard deviation, range, apparent prevalence and mean intensity were calculated. For chapter 6 Cestoda; the apparent prevalence and mean intensity were calculated, whilst for chapter 7 ciliophorans; the mean, standard deviation, range, apparent prevalence and mean intensity were calculated.

3.5 Summary of fish species collected from the nine dams surveyed in the Free State Province

Field work was carried out over a period of two years (2013-2014), a total of 273 (includes species of no commercial value, data not included in this study, these fish were collected as a result of the fishing methods which could not target only particular species) fish specimens were collected. A summary of the total number of fish host specimens examined for parasites (249 fish specimens from species of commercial value) from all localities is given in Table 3.1.

Table 3.1: Summary of the total number of fish host specimens examined for parasites from all localities surveyed in the Free State.

	Labeobarbus aeneus	*Labeobarbus kimberleyensis*	*Labeo capensis*	*Labeo umbratus*	*Clarias gariepinus*	*Ctenopharyngodon idella*	*Cyprinus carpio*
Gariep Dam	16		24	1	6		6
Allemanskraal Dam		3	4	4	2		12
Welbedacht Dam			4		1		9
Bloemhof Dam			5			3	7
Rustfontein Dam	15	1	30	1	1		5
Sterkfontein Dam	9	1	13		1		
Krugersdrift Dam			8		1		8
Koppies Dam	2	3	1	6	2		7
Knellpoort Dam	4		1	1			21
Total number of specimens per host species	**46**	**8**	**90**	**13**	**14**	**3**	**75**
TOTAL							**249**

Of all the dams surveyed, *C. idella* was only collected from Bloemhof Dam. The grass carp was initially introduced to the Umgeni hatchery in Pietermaritzburg in 1967 and later in 1975 and then stocked into farm dams in Kwazulu-Natal and Mpumalanga (Skelton 2001). However, as the current study has shown, this species is now found outside both these provinces. The grass carp has established breeding populations in the Vaal River, and Bloemhof Dam is situated in this part of the Orange-Vaal River system.

The grass carp has been able to thrive in Bloemhof Dam as a result of its life strategies, i.e. living in lentic systems, having a fast growth rate, high fecundity, early maturation and the ability to adapt to a wide variety of aquatic habitats. Owing to the invasive nature of the grass carp, only sterile triploid individuals are currently allowed to be stocked in South Africa, and only in isolated ponds and small dams (Barkhuizen 2015).

Chapter 4: Parasitic crustaceans

Chapter 4: Parasitic crustaceans

4.1 Introduction

Parasitic crustaceans of fish have captured the attention of humans since the time of Aristotle, and since then about 2 000 species have been described (Lester & Roubal 1995). The bulk of the species described belong to the subclass Copepoda Milne Edwards, 1830. Parasitic copepods and branchiurans are of economic significance since they can cause epidermal lesions through feeding and attachment to their fish host and ultimately affect host survival (Lester & Roubal 1995).

Parasitic crustaceans can act as both hosts and vectors of viruses, and are able to transmit these to their hosts (Overstreet et al. 2009). According to Overstreet et al. (2009), branchiurans have been found to host blood and helminth parasites.

The initial attempts at classifying crustaceans were rather difficult, as the specimens had very few obvious arthropod features. These attempts were so difficult that some of the species we now know are in fact arthropods, were initially classified as gastropod molluscs, worms, cephalopod molluscs and annelids (Schmidt & Roberts 2010).

According to Martin et al. (2014), crustaceans are morphologically diverse, which makes it difficult to find specific characteristics shared by all members of the group. However, the nauplius larva, which is a developmental phase can indeed be found in all members of the crustacean group. All crustaceans pass through the nauplius phase, however, in some cases this stage is not necessarily evident (for example Branchiura Thorell, 1864), where eclosion from the egg happens at a later stage, i.e. after the nauplius stage. It is therefore widely accepted that all crustaceans had a nauplius as their primary larval form (Martin et al. 2014).

For more than two centuries after the work done by Linnaeus on parasitic crustaceans, parasitic crustaceans were thought to belong to several distinct higher taxa. This was mainly due to knowledge gaps in terms of homology of morphological structures between the free-living crustaceans and the highly specialised parasitic crustaceans. According to Boxshall (2007) recent phylogenetic studies have shown that crustaceans have consistently evolved from a free-living life style towards parasitic life styles, resulting in the loss or redundancy of some of these morphological structures.

The copepods were finally established as crustaceans after it was discovered that their young exhibit cyclopoid features (Schmidt & Roberts 2010). The free-living larval stages, naupli and metanaupli, as well as the parasitic forms of the copepods are used in the systematic classification of these crustaceans. For many of the symbiotic species, it is only the females that undergo notable morphological adaptations and are parasitic, while the males are free-living and retain the typical cyclopoid copepod features (Paperna 1996).

Copepods exhibit a wide variety of evolutionary adaptations for living as symbionts (Schmidt & Roberts 2010), these adaptations may be simplistic or very advanced. The level of adaptation referred to above can be demonstrated through the work of Schmidt & Roberts (2010), where they showed the trends of adaptation from little to highly specialised. The adaptation features include the following, **1**. Reduced locomotory appendages, **2**. Adaptations for the purposes of adhesion, this can be seen by the development of new structures, as well as by the modification of existing structures, **3**. The growth of genital and reproductive parts, causing a disproportional increase in body proportions and size, **4**. The combining of somites, leaving no evidence of previous external segmentation, **5**. The reduction of the sensory organs, **6**. The reduction of instars, in terms of numbers.

4.2 The Family Lernaeidae

There are about 55 species of lernaeid copepods all found in fresh water (Lester & Hayward 2006, Walter & Boxshall 2018). According to Avenant-Oldewage (2012) lernaeids infest fresh water fishes both in the aquaculture environment and in the wild. They occur on all continents, but the majority of the species occur in Africa and infest a variety of hosts. Lernaeids are parasites of fresh water teleosts, specifically cyprinids however, they also occur on salmonids and other fishes such as tilapia (Avenant-Oldewage 2012).

According to Avenant-Oldewage (2012) lernaeids are of great economic importance as they can cause mass mortalities, particularly in cases where they infest small fish. They are thought to harbour viruses and bacteria which they transmit to their host, resulting in secondary infections.

The anterior 'horns' or the holdfast are anchored into the host fishes' skin and the posterior part of the abdomen protrudes from the hosts' body, with the rod-shaped protruding abdomen usually being 5-22 mm in length (Paperna 1996). The anterior end of the mature female is very important as it plays a role in the taxonomic identification of the species, and according to Paperna (1996), the shape of the anchors is the most reliable for differentiation. The larval stages, which are greenish-white in colour, occur on the gills, whilst the parasitic adult females lodge themselves into the host muscle tissue (Avenant-Oldewage 2012).

The point of attachment can be seen as a lesion, which is sometimes inflamed and haemorrhagic. Once the anchor is embedded into the host, it causes pathology to the skin, scales and the underlying muscle tissue. The cyclopoid individuals, belonging to family Lernaeidae Cobbold, 1879 are capable of causing erosion to gill filaments, as well as proliferative changes (Paperna 1996). The members of this family are also exceptionally dangerous to fish fry and fingerlings, one or two parasites can bring about death once they have settled on the young host and inserted their anchor into the viscera.

The mature females possess an elongated neck, and trunk with very small swimming legs. The legs are not involved in the rapid growth towards the final size, and therefore always look disproportionate to the rest of the body. Furthermore, the females possess egg sacs attached to the posterior end (Paperna 1996). *Lernaea* Linnaeus, 1758 species are found on all continents, with 15 species found on African fishes. *Lernaea cyprinacea* specifically, has a cosmopolitan distribution, and this is as a result of introductions along with their hosts (Avenant-Oldewage 2012).

According to Paperna (1996), the larval development and the life span of the females are temperature dependant, while egg production is not dependent on temperature. At a lower temperature range (12-16°C) the egg development is slower, with the first copepodites appearing after 14 days, at a higher temperature range (27-30°C) the first copepodites appear after two to three days. Adult female *L. cyprinacea* are not species specific and has become cosmopolitan as a result of co-introductions along with the common carp. The fully developed females may be as long as 12 mm.

4.3 The Family Argulidae

The family Argulidae Leach, 1819 is composed of four genera, namely *Argulus* Muller, 1785, *Dolops* Audouin, 1837, *Chonopeltis* Thiele, 1900 and *Dipteropeltis* Calman, 1912. The above genera can be distinguished from each other by the form of the maxillules. The maxillules can either be in the form of hooks (*Dolops*) or suckers, as is the case of the other three genera. The presence or absence of a mouth tube and stylet along with characteristics of the antennule and antennae are used to distinguish between *Argulus* and *Chonopeltis* (Van As & Van As 2001). According to Møller & Olesen (2010) there is very little known information about *Dipteropeltis*, but it is distinguished by the pre-oral spine, unique suction disc support structures and an *Argulus*-like mouth cone.

Members of the subclass Branchiura Thorell, 1864 have significant differences as compared to members of the subclass Copepoda, these are as follows: Compound eyes present; once maturity is reached they will continue to moult; the eggs are laid singularly and, there is an absence of a true naupliar stage.

Some members of the Branchiura have been known to cause serious problems in fisheries and aquaculture, with the argulids having been recorded as pests of cultured trout as early as the 17th century (Lester & Roubal 1995). Additionally, some argulids have been recorded as being the cause of mortalities in aquarium fish, estuarine fish, lake fish, and to a lesser extent, sea-caged salmonids. Argulids also facilitate secondary bacterial and fungal infections and are capable of transmitting spring viraemia of carp (Lester & Roubal 1995).

The genus *Argulus* is characterised by members with a carapace that has three distinct parts, and a clearly defined anterior lobe. In some specimens the carapace can cover the whole cephalothorax, while in others only the first two legs are covered. Members of this genus are further characterised by a pair of antennules consisting of two segments, and the terminal segment terminates into a sclerotised hook (Van As & Van As 2001).

The second pair of maxillae are in the form of sucking discs, of which the edge margins are supported by chitinous rods. The maxillipedes contain a plate also consisting of chitinous material on the ventral surface of the basal segment. The basal segment of the fourth pair of legs of the female are usually formed into boot-shaped lobes underneath the abdomen (Yamaguti 1985).

The anterior legs can either possess setae, and in some cases setae are absent; in males, the second, third and fourth pairs of swimming legs are modified for clasping the females during copulation (Lester & Roubal 1995). According to Yamaguti (1985) the ovaries in females are positioned in the metasome, whilst males possess testes in the urosome. Anterior to the mouth tube is the stylet, which is separate from the digestive tract, it consists of a hollow spine surrounded by a sheath into which it can retract.

In argulids, the females grow bigger than the males, and they are readily recognised even before they reach maturity by the seminal receptacles, which are situated on the abdomen. In males the third and fourth legs are closer together as they are modified to grasp the female during copulation. According to Woo (2006), the mature argulids are able to survive separately from fish for a number of days; the females lay eggs on stationary objects, one at a time in single, double or triple columns; the eggs are fertilised during oviposition.

With regard to *Argulus japonicus* Thiele, 1900, the time for egg hatching is dependent on temperature, they hatch sooner at higher temperatures: at 35°C, the eggs hatch after 10 days and at 15°C they hatch after 61 days (Shafir & Oldewage 1992). The hatchlings at this stage possess typical copepod features, such as mandibular palps and long second antennae. After a few days have elapsed, they moult and develop into the second stage, in which they look more like the mature adults but without suckers (Lester & Roubal 1995).

The hatchlings are parasitic at the time of hatching (Lester & Roubal 1995), these hatchlings then attach to a host, however, they often leave the host for moulting and reproduction, and according to Paperna (1996), they will also change hosts several times during their lifetime.

Species of the genus *Argulus* have a wide variety of hosts, and occur on fishes from diverse families. Mortalities of cultured common carp, grass carp and eels caused by some species of *Argulus* have been recorded throughout Asia and Europe (Paperna 1996).

According to Paperna (1996), it is very important that when considering the potential parasitological risks posed to aquaculture, parasitic crustaceans are not overlooked, as their pathogenic effect is visible even when they infest natural fish populations.

In South Africa, six species have been recorded from this genus, four of the six species have been recorded from marine/estuarine fish hosts and the other two: *Argulus japonicus* and *Argulus capensis* Barnard, 1955 are fresh water species. *Argulus japonicus* is an alien species to South Africa (Van As & Van As 2001).

This chapter reports on the presence of parasitic crustacean parasites in dams in the Free State Province, South Africa. This chapter further reports on infestation statistics of parasitic crustaceans infesting different host species from the respective dams, as well as the potential effects they may have on the development of fresh water aquaculture in the province.

4.4 Materials and methods

Fish collections (Refer to chapter 3)

4.4.1 Host examination, fixation and preservation of parasites

Parasitic crustaceans were removed from the fish host in such a way that the whole body remained intact. The individual specimens were fixed in 70% ethanol. The material was recorded and labelled. These were taken back to the laboratory at the Department of Zoology and Entomology of the University of the Free State for further examination. The specimens were examined under a dissection microscope, and identified using laboratory reference material that was collected over the years by the Aquatic Parasitology Research team of the Department of Zoology and Entomology of the University of the Free State and available literature, i.e. Rushton-Mellor (1994) and Harding (1950).

Bush et al. (1997) noted that there has been significant confusion with regards to terms used by parasitologists and ecologists, and as such suggested for these terms to be standardised so as to eliminate the confusion often encountered with the use thereof. The goal of the paper by Bush et al*.* (1997) was to supplement the paper by Margolis et al. (1982).

According to Bush et al. (1997), prevalence which is usually expressed as a percentage, can be calculated by dividing the number of hosts infected with one or more individuals of a particular parasite, by the number of hosts examined for that particular parasite. The term ‘infected’ is used to also include infest and its derivatives (Bush et al. 1997).

$$\text{Prevalence} = \frac{\text{Total number of hosts infected}}{\text{Total number of host examined}} \text{ X } 100$$

For the purpose of the present study, ‘prevalence’ is taken as ‘apparent prevalence’ as the sample sizes for the hosts as well as the parasites were too small to be representative of the whole populations that could potentially be found at each of the dams.

The mean intensities for the infestation of hosts by *L*. *cyprinacea* and *A*. *japonicus* were calculated as follows:

Mean intensity= Total number of parasites/Total number of infected hosts (Bush et al. 1997).

4.5 Results

During the present study, two different species of parasitic crustaceans were found, the first belonging to the genus *Lernaea* and the second belonging to the genus *Argulus*. According to Paperna (1996), lernaeid copepods could potentially infest all fresh water fish host species, however, they are more often found associated with cichlids and cyprinids.

The most common description for *Lernaea cyprinacea* (Fig 4.1 A) is that all the horns are slender and long, and the dorsal horns culminate into a “T” shape, (Fig 4.1 B and C) Harding (1950). The pregenital prominence of *L. cyprinacea* is described as being distinctly bilobed (Fig 4.1 D). Based on the anchor shape, the specimens collected were identified as *L. cyprinacea*, as the characteristics are synonymous with those of Harding (1950).

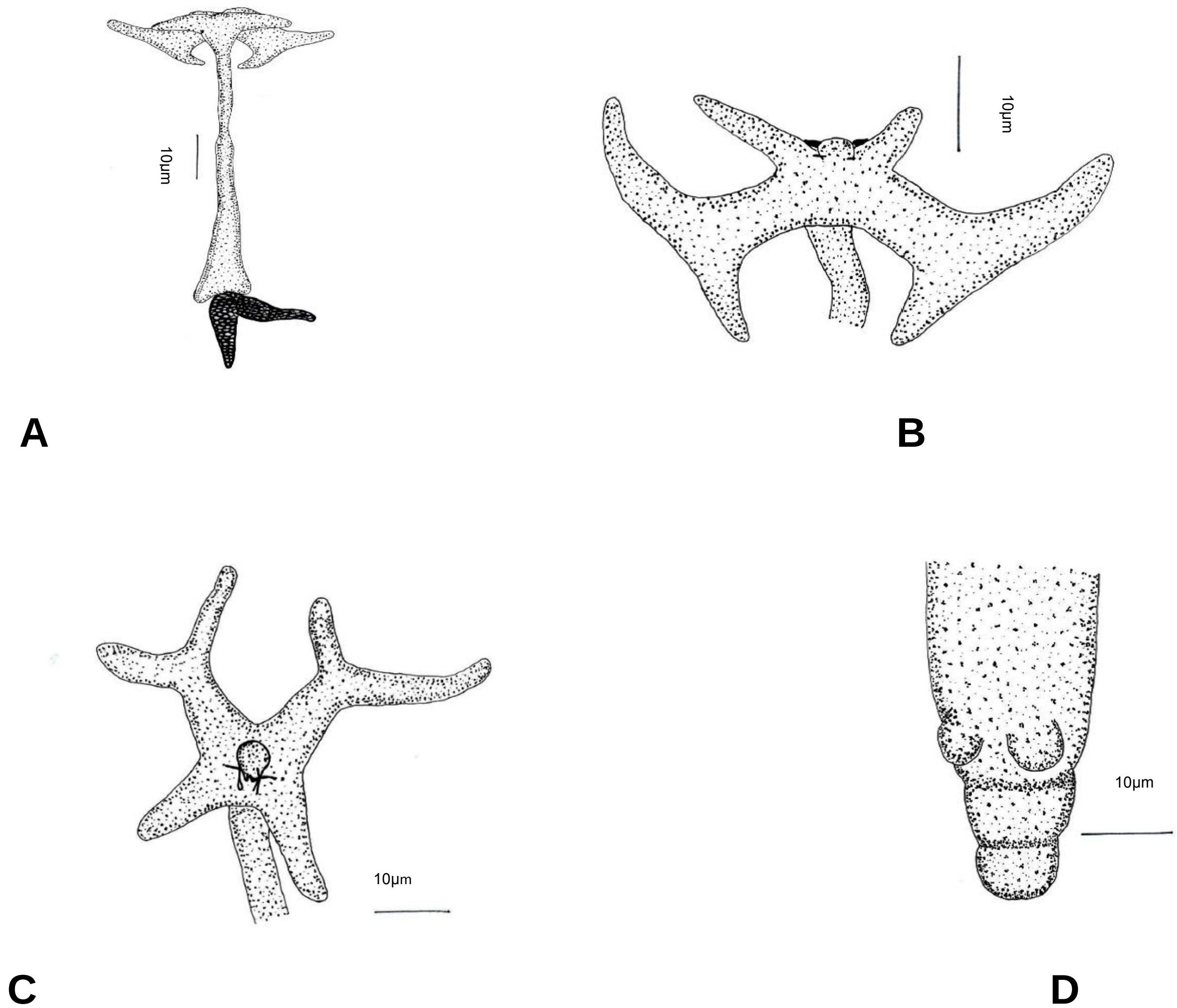


Figure 4.1: Diagrams of a female adult *Lernaea cyprinacea* Linnaeus 1758, including various views of the anchors. A-Ventral view of adult female, B-Dorsal view of anchors, C-Anterior view of anchors, D-Ventral view of abdomen and double pre-genital prominance. Courtesy of the Aquatic Ecology Research Group image collection, (UFS).

Lernaea cyprincea Linnaeus, 1758 was found associated with five Smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822), two Orange River mudfish *Labeo capensis* (A. Smith, 1841), five common carp *Cyprinus carpio* Linnaeus, 1758 and three grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) specimens. A total of 195 specimens of *L*. *cyprinacea* were collected. During the present study *L*. *cyprinacea* was only found associated with the above mentioned fish host species (Table 4.1) collected at Gariep Dam and Bloemhof Dam. Additionally, examinations were also done on the following host species from Gariep Dam: 24 *Labeo capensis*, one *Labeo umbratus* and six *Cyprinus carpio* specimens and none of these were infested with *L*. *cyprinacea.* In the case of Bloemhof Dam, all hosts collected were examined.

Table 4.1: Infestation of different fish host species by *Lernaea cyprinacea* Linnaeus, 1758 collected at Bloemhof Dam and Gariep Dam.

Host scientific name	**Localities and number of hosts examined including hosts infested (*)**	**Total number of parasites found**
Labeobarbus aeneus	Gariep Dam 16 (5*)	102
Labeo capensis	Bloemhof Dam 5 (2*)	9
Cyprinus carpio	Bloemhof Dam 7 (5*)	32
Ctenopharyngodon idella	Bloemhof Dam 3 (3*)	52

The other parasitic crustacean found during the present study was *Argulus japonicus* (Fig 4.2 A). The third and fourth limbs on male *A*. *japonicus* are modified for reproduction (Fig 4.2 B, C), the genital sockets are located on the coxa of the third limb, and the genital pegs are located at the basis of the fourth limb. The identification was based on the identification keys of Rushton-Mellor (1994).

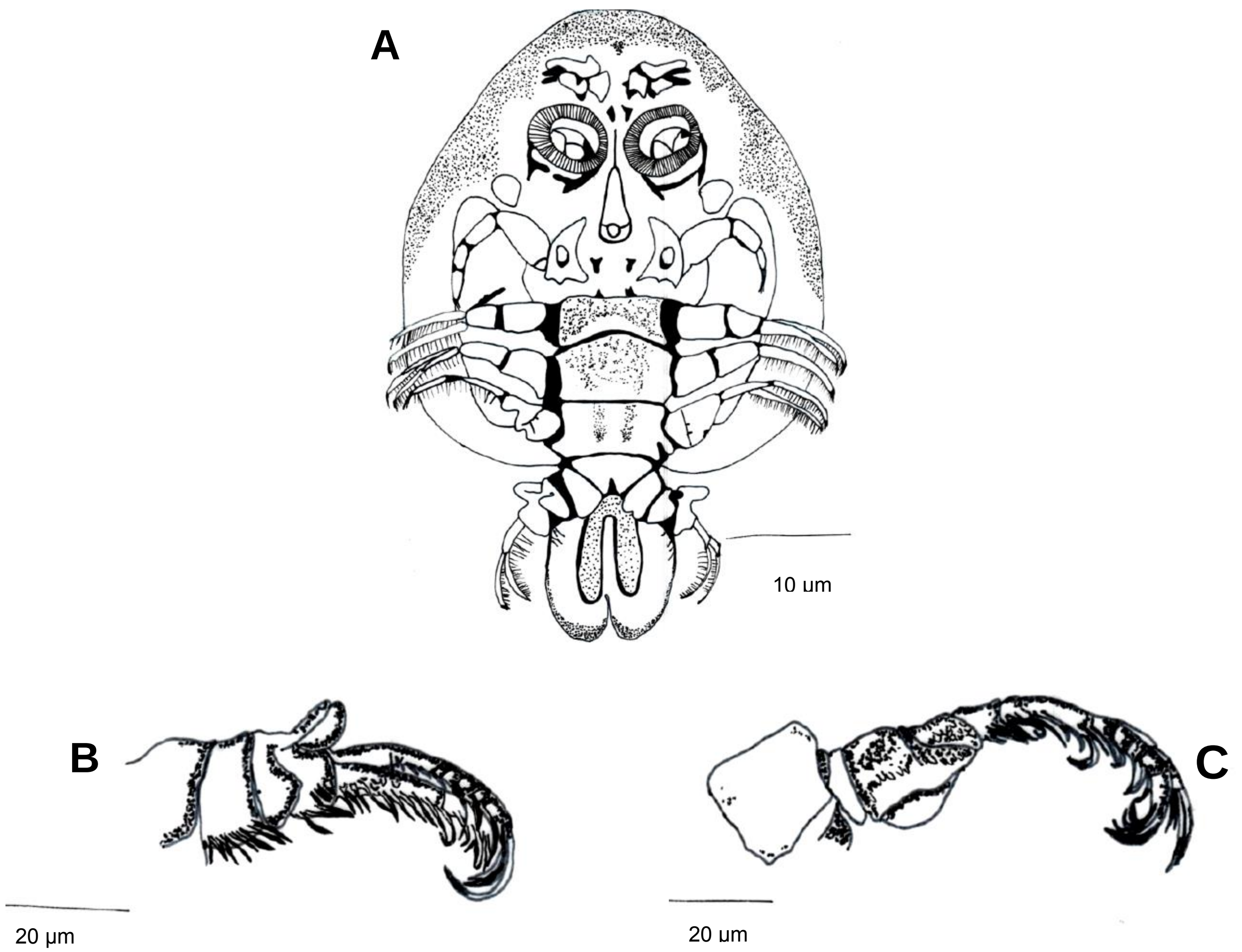


Figure 4.2 : A-Female *Argulus japonicus* Thiele, 1900, B-Third leg of male *Argulus japonicus*, C-Fourth leg of male *Argulus japonicus* modified for reproduction. Courtesy of the Aquatic Ecology Research Group image collection, (UFS).

Argulus japonicus was found associated with two moggel *Labeo umbratus* (A. Smith, 1841), nine *L*. *aeneus*, six *L. capensis* and three *C. carpio* specimens. A total of 155 specimens of *A*. *japonicus* were collected. There were more male (93) *A*. *japonicus* collected compared to females (62) (Table 4.2).

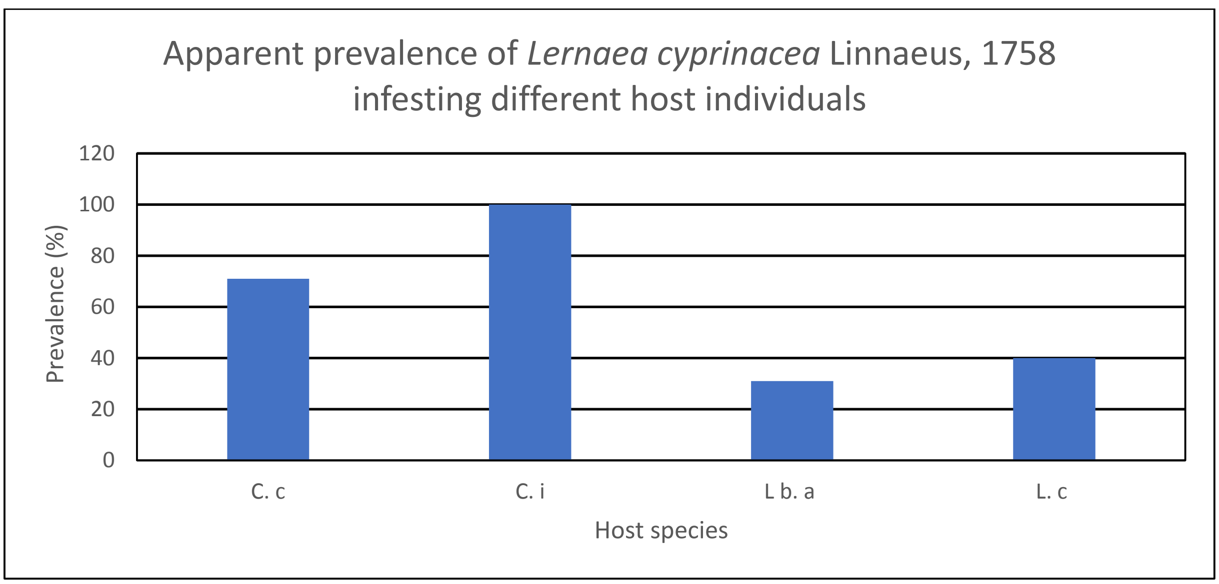
Table 4.2: Infestation of different fish host species by *Argulus japonicus* Thiele, 1900, collected at Bloemhof Dam, Koppies Dam, Gariep Dam, Knellpoort Dam and Rustfontein Dam.

Host scientific name	**Localities and number of hosts examined including hosts infested (*)**	**Number of male *Argulus japonicus* specimens**	**Number of female *Argulus japonicus* specimens**	**Total**
Labeo umbratus	Koppies Dam 6 (2*)	-	10	10
Labeobarbus aeneus	Koppies Dam 2 (1*) Gariep Dam 16 (4*) Knellpoort Dam 4 (4*)	- 21 34	2 9 15	2 30 49
Labeo capensis	Koppies Dam 1 (1*) Bloemhof Dam 5 (2*) Knellpoort Dam 1 (1*) Rustfontein Dam 30 (2*)	- 17 - 1	1 5 2 3	1 22 2 4
Cyprinus carpio	Bloemhof Dam 7 (3*)	20	15	35

During the current study only the above mentioned fish host species (Table 4.2) from the above localities were found infested with *A. japonicus*. In addition, more host species from these localities were also examined, but were not found infested with *A. japonicus* (Table 4.3).

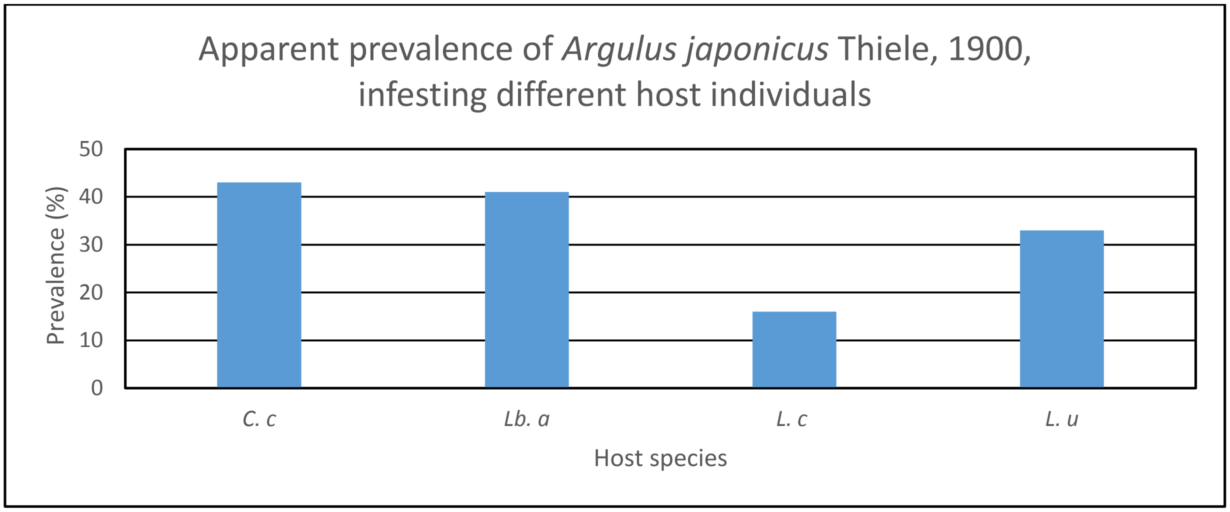
Table 4.3: Hosts examined for *Argulus japonicus* Thiele, 1900 collected from Bloemhof Dam, Koppies Dam, Gariep Dam, Knellpoort Dam and Rustfontein Dam that were not infested.

Host scientific name	**Localities and number of hosts examined**
Labeo umbratus	Gariep Dam 1 Knellpoort 1 Rustfontein 1
Labeobarbus aeneus	Rustfontein 15
Labeo capensis	Gariep Dam 24
Cyprinus carpio	Koppies Dam 7 Gariep Dam 6 Knellpoort Dam 21 Rustfontein 5
Labeobarbus kimberleyensis	Koppies Dam 3 Rustfontein Dam 1
Ctenopharyngodon idella	Bleomhof 3



(*C.c Cyprinus carpio, C. i Ctenopharyngodon idella L b. a Labeobarbus aeneus*, *L. c* Labeo *capensis*)

Figure 4.3: Comparison of apparent prevalence of *L. cyprinacea* Linnaeus, 1758 infesting different host species.

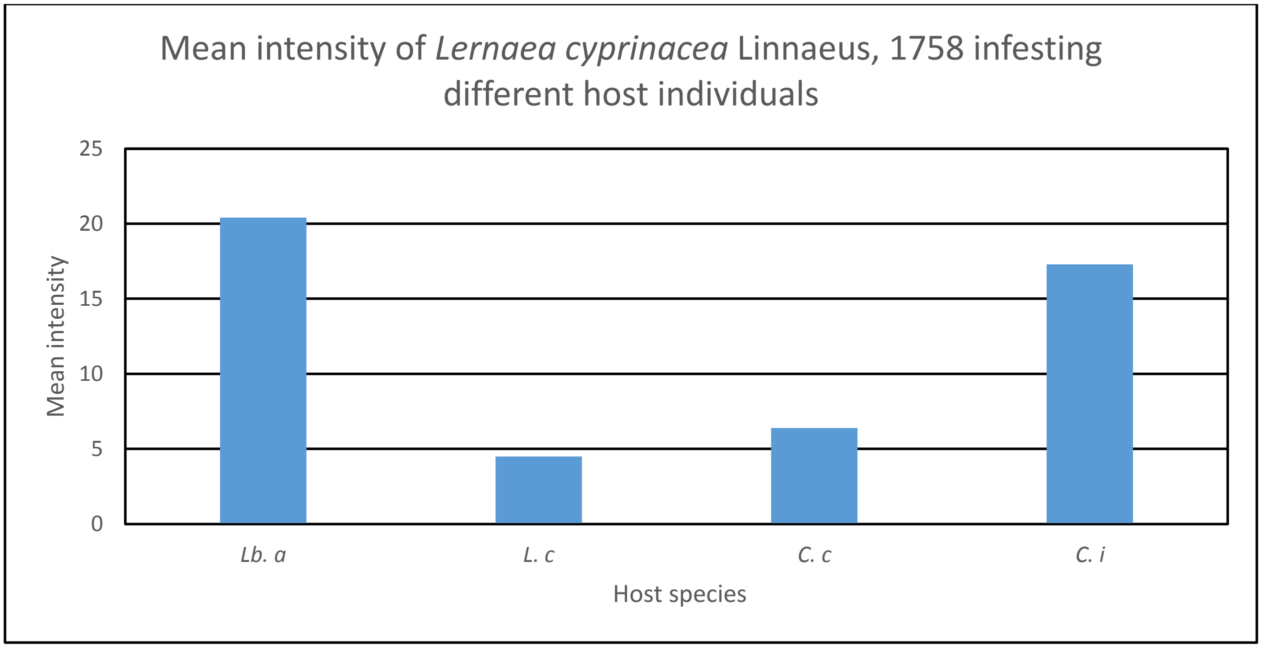


(*C.c Cyprinus carpio*, *L b. a Labeobarbus aeneus*, *L. c Labeo capensis L. u Labeo umbratus*)

Figure 4.4: Comparison of apparent prevalence of *Argulus japonicus* Thiele, 1900, infesting different host species.

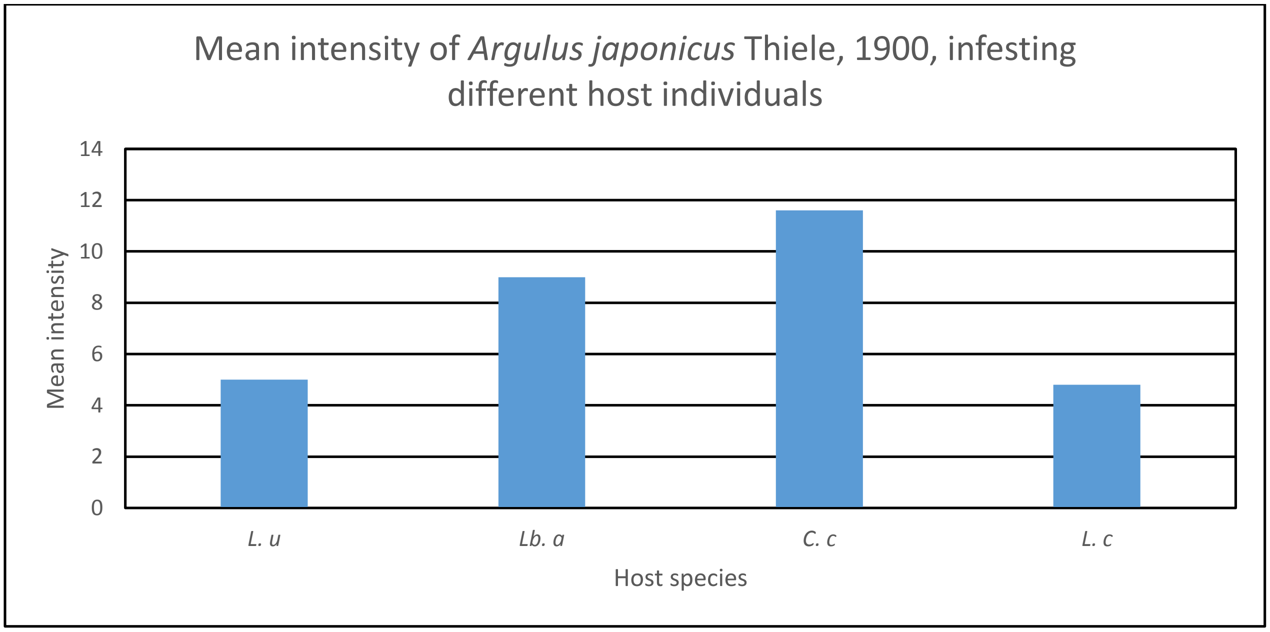
Ctenopharyngodon idella had a higher apparent prevalence of infestation by *L. cyprinacea* compared to *L. aeneus* which had the lowest apparent prevalence (Fig 4.3). *Cyprinus carpio* had a higher apparent prevalence of infection by *A. japonicus* compared to *L. capensis* which had the lowest apparent prevalence (Fig 4.4).

Labeobarbus aeneus had a higher mean intensity of infestation by *A. japonicus* compared to *L. capensis* which had the lowest mean intensity (Fig 4.5). *Cyprinus carpio* had a higher mean intensity of infestation by *A. japonicus* compared to *L.* capensis which had the lowest mean intensity (Fig 4.6)



(*Lb. a Labeobarbus aeneus, L. c Labeo capensis, C. c Cyprinus carpio, C. i Ctenopharyngodon idella*)

Figure 4.5: Comparison of mean intensity of *Lernaea cyprinacea* Linnaeus, 1758 infesting different host species.



(*L. u Labeo umbratus, Lb. a Labeobarbus aeneus, C. c Cyprinus carpio, L. c Labeo capensis*)

Figure 4.6: Comparison of mean intensity of *Argulus japonicus* Thiele, 1900, infesting different host species.

4.6 Discussion

According to Paperna (1996), some species of *Argulus* are pathogens that need to be taken seriously as they have caused serious problems for cultured carp, causing morbidity and mortalities. During fieldwork (2013-2014) of the current study, parasitological data was not collected from cultured common carp. Parasitic crustacean data was collected from wild caught common carp from one locality (Bloemhof Dam).

Argulus japonicus was also found associated with *Labeobarbus aeneus* caught at Gariep Dam, this is the dam where the ATDC is located. During the present study four *L. aeneus* were caught at Gariep Dam, and were found infested with 30 individuals of *A. japonicus*, 21 males and nine females.

By implication this means that these parasites could in future be introduced into the Aquaculture facility at the ATDC. During routine cleaning in 2016, however, *A. japonicus* was found in the hatchery/overwintering facility at the ATDC by the newly appointed team of Aquaculture scientists based at this centre. The parasites were found floating in one tank containing goldfish. A total of 16 *A. japonicus* specimens were found in one of the tanks.

The ATDC receives water directly from the Gariep Dam, the water is initially stored in a reservoir managed by Bloemwater, and this water is then channelled towards the ATDC, where it is kept in a large reservoir, thereafter the water is channelled to the main hatchery /overwintering facility and the rest of the outside ponds at the facility. The water passes through the entire system by way of a gravitational gradient, after which the water is channelled back into the Orange River. The parasites were introduced to the ATDC via direct exposure to water sourced from the Gariep Dam.

Proper farm management, biosecurity management and overall good farm practices should be put into place to prevent or limit the potential introduction and establishment of these parasites at the facility.

Chapter 5: Monogenea

Chapter 5: Monogenea

5.1 Introduction

Monogeneans are parasitic flatworms that are usually found on fish and other lower vertebrates from the aquatic environment. These flatworms are predominantly ectoparasites and attach to the host's gills or skin by specialised attachment organs located posteriorly. Monogeneans contain apical sensory structures, a mouth with or without accessory suckers and specialised clamps or glands for attachment anteriorly (Paperna 1996).

All monogeneans are hermaphroditic; the testis is singular or follicular, and the male copulatory organ is often sclerotised (Paperna 1996). The female organs include the ovary and follicular glands; the ovary contains one to a few eggs only. The monogeneans are divided into two subclasses i.e. Polyonchoinea and Heteronchoinea (Boeger & Kritsky 2001). The subclass Polyonchoinea comprises 18 families, whilst Heteronchoinea comprises the infra class Polystomatoinea (two families) and Oligonchoinea (30 families) (Buchmann & Bresciani 2006). The two subclasses (Polyonchoinea and Heteronchoinea) differ mainly in morphology, nutrition, anatomy, reproduction and physiology. The differences between these two subclasses have important implications for their pathogenicity (Buchmann & Bresciani 2006). Monogeneans of the subclass Heteronchoinea are blood feeders while those of Polyonchoinea are epithelial feeders, browsing the host surface feeding on epithelial cells, mucous and occasionally, a limited amount of blood from the haemorrhages (Buchmann & Bresciani 2006).

Monogenean parasites are present in all fresh water bodies in Africa, the genera and species of Monogenea often show a high degree of host specificity and are believed to be among the most host specific of parasite taxa. This suggests that the diversity of monogeneans worldwide should be closely related to the diversity of their hosts (Whittington 1998). Fresh water fish parasites in South Africa have received meaningful attention over the years, however, the monogeneans have not received as much attention as compared to the other parasitic groups. Recently (2010-2015), there has been an increase in interest with regards to the monogeneans associated with fresh water fish, this can be seen through the work of Le Roux & Avenant-Oldewage 2010, García-Vásquez et al. 2012, Crafford et al. 2012, 2014 Mbokane et al. 2015.

Parasitic infections, bacterial diseases and viral diseases have been known to cause problems in aquaculture farms, for example the disease pseudodactylogyrosis is caused by infestation with monogeneans of genus *Pseudodactylogyrus* Gusev, 1965. This disease is known to cause morbidity and mortality of young eels and has had a negative effect on the production of young eels in Europe (Buchmann et al. 2011).

This chapter reports on the presence of three monogenean parasites from two dams in the Free State Province, South Africa. *Quadriacanthus aegypticus* El Naggar & Serag, 1986 was found associated with sharptooth catfish *Clarias gariepinus* (Burchell, 1822). *Dactylogyrus* Diesing, 1850 sp 1 was found associated with common carp *Cyprinus carpio* Linnaeus, 1758. *Dactylogyrus* sp 2 was found associated with smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822). This chapter further reports on infestation statistics of monogeneans infesting different host species from the respective dams, as well as the potential effects they may have on the development of fresh water aquaculture in the province.

5.2 Materials and methods

Fish collections

(Refer to chapter 3)

5.2.1 Host examination, fixation and the preservation of monogeneans

Skin smears were made and examined for the presence or absence of worms using compound light microscopy. The monogeneans found were fixed in 70% ethanol, recorded and numbered according to host species and locality. The gills were removed and examined under a dissecting compound light microscope. Monogeneans found on the gills were recorded and fixed in 10% buffered neutral formalin. Alternatively, the monogeneans were removed from the gills and fixed in 70% ethanol.

Images of the monogenean sclerotised parts were taken using a Zeiss Axiophot camera mounted on the compound light microscope, the images were measured using the computer software programme, ImageJ. Measurements were all in µm, followed by the standard deviation and the range in parenthesis.

5.2.2 Light microscopy preparations

Monogeneans were transferred to Glycerine Ammonium Picrate (GAP), and mounted for the purposes of studying the sclerotised parts. Some of the specimens were stained using Gomori's Trichrome stain method (García-Vásquez et al. 2012).

5.2.3 Glycerine Ammonium Picrate method

Monogeneans were individually removed from the gills and placed in a water droplet in the middle of a microscope slide. Excess water was drawn off using tissue paper; this was done to reach the required level of compression. The specimens were mounted between a microscope slide and a cover slip by adding a drop of GAP to the edge of the cover slip which was then allowed to settle on top of the specimen. The slides were allowed to air dry for one to two days, thereafter the cover slip was sealed with clear nail polish (method adapted by Dr Christison[1] from Malmberg 1970).

5.2.4 Preparation of Glycerine Ammonium Picrate

Neutral buffered formalin 10%	1 part
Glycerine	9 parts

The glycerine was mixed with formalin and, for each 10 ml of solution, 1 drop of picric was added (Malmberg 1970).

5.2.5 Gomori's trichrome stain method

A droplet of Gomori's trichrome was placed in the centre of a plastic petri dish, thereafter; one monogenean was placed in the droplet using a fine probe. One minute was allowed to elapse, and then the specimen as well as the droplet were flooded with ethanol to stop the staining process. De-staining was achieved by adding a drop of water to the specimen in the ethanol solution. Once de-staining had reached the desired level, the petri dish was

[1] Dr Kevin Christison, Specialist Scientist from the Department of Agriculture , Forestry and Fisheries, Cape Town, South Africa

agitated to increase the concentration of the ethanol around the specimen. The specimen was removed with a fine probe and placed in cedarwood oil and mounted directly in Eukitt (García-Vásquez et al. 2012)

5.2.6 Data analyses

The monogenean raw data was analysed; the mean, standard deviation and range were calculated for all measurement data from all species for descriptions.

5.3 Results

Monogeneans were found associated with six *C. gariepinus*, one *C. carpio* and six *L. aeneus* specimens (Table 5.1). The apparent prevalence for *Q*. *aegypticus* was 100%, *Dactylogyrus* sp 1. 11% and *Dactylogyrus* sp 2. 38% respectively, whilst the mean intensity was calculated as follows: *Q*. *aegypticus* 1.7 ± 13.9 (209-246), *Dactylogyrus* sp 1. mean intensity: 2 ± 76.4 (206 -314), and *Dactylogyrus* sp 2. mean intensity: 1.7 ± 12.0 (228-348) respectively.

* To calculate apparent prevalence and mean intensity refer to chapter 4.

Table 5.1: Infestation of different host species caught at Gariep Dam and Welbedacht Dam by different monogenean species.

Host scientific name	**Localities and number of hosts examined including hosts infested (*)**	**Monogenean species found infesting hosts examined**
Clarias gariepinus	Gariep Dam 6 (6*)	*Quadriacanthus aegypticus*
Cyprinus carpio	Welbedacht Dam 9 (1*)	*Dactylogyrus* sp 1.
Labeobarbus aeneus	Gariep Dam 16 (6*)	*Dactylogyrus* sp 2.

(*) number of hosts infested

During the current study monogeneans were only found at two localities, Gariep Dam and Welbedacht Dam from *C*. *gariepinus* (Gariep Dam), *C*. *carpio* (Welbedacht Dam) and *L*. *aeneus* (Gariep Dam). In addition to the above host species, examinations were also done on the following host species, Orange River mudfish *Labeo capensis* (A. Smith, 1841) from Gariep Dam and Welbedacht Dam, moggel *Labeo umbratus* (A. Smith, 1841) from Gariep Dam and common carp *Cyprinus carpio* Linnaeus, 1758 from Gariep Dam and were found free of monogeneans (Table 5.2).

Table 5.2: Hosts examined for monogeneans collected from Gariep Dam and Welbedacht Dam that were not infested (0*).

Host scientific name	**Localities and number of hosts examined including hosts infected (*)**
Labeo capensis	Gariep Dam 24 (0*) Welbedacht Dam 4 (0*)
Labeo umbratus	Gariep Dam 1 (0*)
Cyprinus carpio	Gariep Dam 6 (0*)

5.3.1 Species descriptions, diagnosis and measurements: *Quadriacanthus aegypticus* El Naggar & Serag, 1986 from Gariep Dam

Quadricanthus aegypticus was first described by El-Naggar & Serag (1986), it was reported from the gills of *C. gariepinus* caught in the Nile Delta, Egypt. Thereafter; it was reported by Kritsky & Kulo (1988) from *C*. *gariepinus* in the Nile River, Egypt and then by Douellou & Chishawa (1995) from *C*. *gariepinus* in Lake Kariba, Zimbabwe. The worm found during the present study was preliminarily identified as *Q*. *aegypticus*, although the measurements for the current specimens were smaller than those of the populations with which they were compared, i.e. specimens from El-Naggar & Serag (1986), Kritsky & Kulo (1988), and Douéllou & Chishawa (1995). This parasite species was found infesting the gills of *C. gariepinus* from Gariep Dam.

Measurements for the sclerotised parts of the genus *Quadricanthus* Paperna, 1961 were made based on the method used by N'Douba et al. (1999). The following basic measurements were used: total length, anchor length, anchor base width, anchor point length, ventral bar length, ventral bar width, marginal hooklets length and the male copulatory organ length. All measurements were in micrometres (μm) followed by the standard deviation and the range in parentheses (Fig 5.1).

Total body length 229 ± 13.9 (209-246), total body width 92 ± 15.4 (72-115), ventral anchor length 26 ± 3.2 (23-33), ventral anchor width 7 ± 0.9 (6-8), ventral anchor point length 14 ± 1.7 (10-17), ventral bar length 40 ± 21.0 (35-56), ventral bar width 5 ± 0.9 (4-7), dorsal anchor length 32 ± 6.9 (26-34), dorsal anchor width 8 ± 2.6 (5-13), dorsal anchor point length 9 ± 5.0 (8-12), dorsal bar length 28 ± 6.2 (23-32), dorsal bar width 8 ± 1.9 (5-10), 7 pairs of marginal hooklets 8± 2.8 (6-14), male copulatory organ axis 10 ± 1.6 (1-4).

The following characteristics were observed in the specimens collected during the present study: the dorsal anchors are slightly larger than the ventral anchors, the dorsal anchors possess short re-curved anchor points, whilst the ventral anchors possess notably larger re-curved anchor points (Fig 5.2 A, B, C). The male copulatory organ is employed as the basis for diagnosis and contains two lateral outgrowths. The measurements of *Quadriacanthus aegypticus* found infesting the gills of *C*. *gariepinus* from Gariep Dam compared to measurements by El-Naggar & Serag (1986), Kritsky & Kulo (1988), and Douéllou & Chishawa (1995) are presented in Table 5.3.

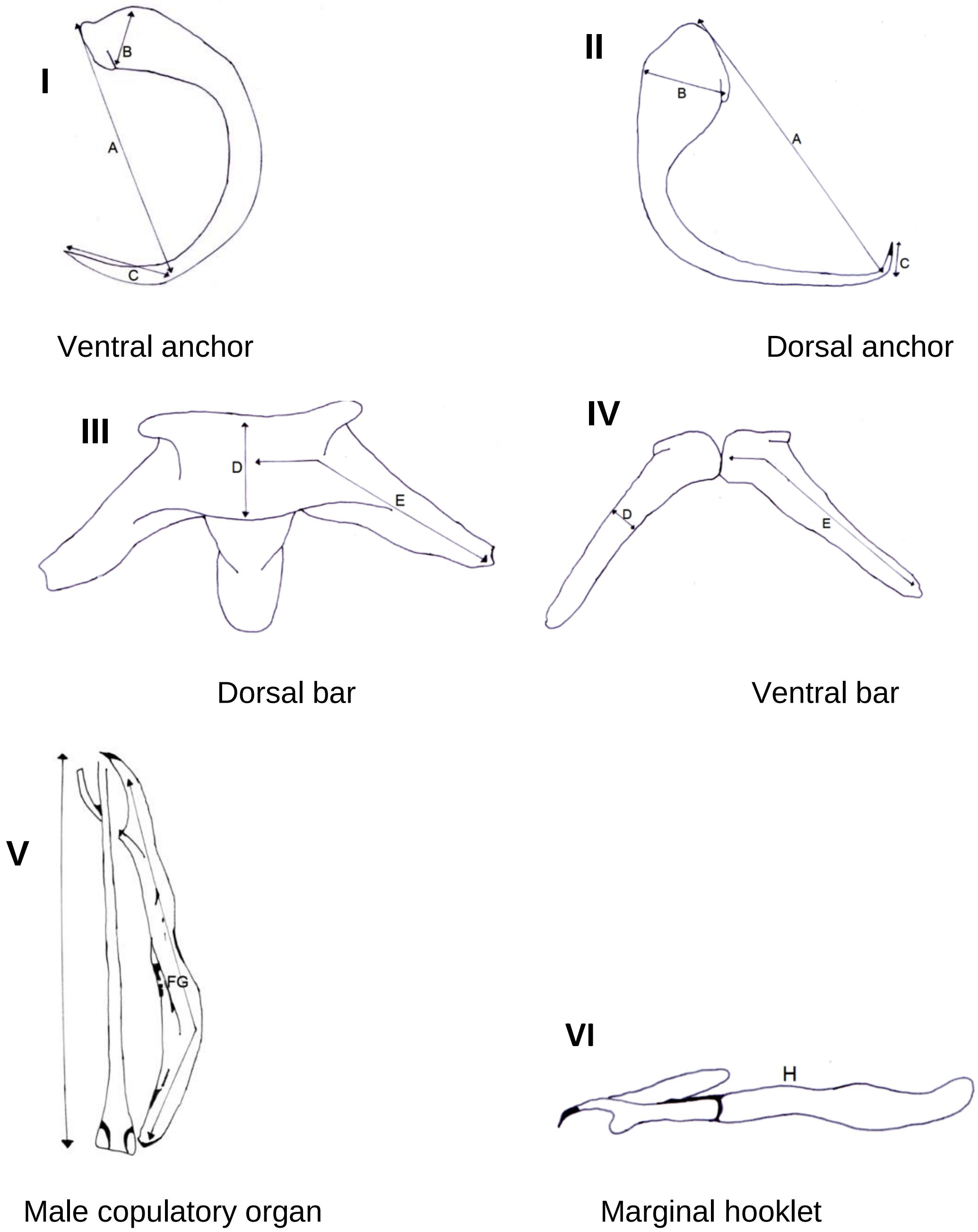


Figure 5.1: Measurements of the sclerotised parts of *Quadriacanthus aegypticus* El-Naggar & Serag, 1986 collected from *Clarias gariepinus* (Burchell, 1822): A-Anchor total length; B-base width; C-length of point; D-Bar width; E-Bar length; FG-copulatory organ axis; H-Marginal hooklet total length. The sclerotised parts were measured based on the method used by N'Douba et al. (1999).

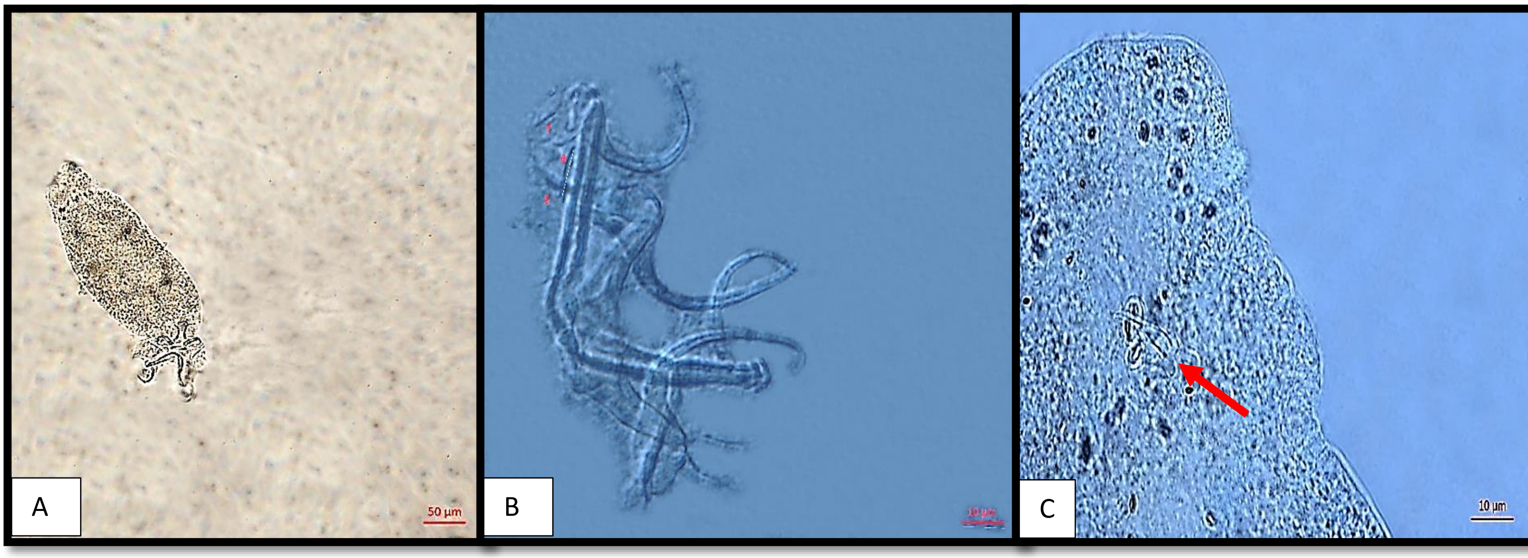


Figure 5.2: Light microscope photographs of *Quadriacanthus aegypticus* El-Naggar & Serag, 1986 from *Clarias gariepinus* (Burchell, 1822) from Gariep Dam. A-whole worm, B-dorsal and ventral anchors, C-male copulatory organ (→).

Table 5.3: Measurements of *Quadriacanthus aegypticus* El-Naggar & Serag, 1986 (µm) from *Clarias gariepinus* (Burchell, 1822) from Gariep Dam, compared with published descriptions.

	El-Naggar & Serag (1986)	**Kritsky & Kulo (1988)**	**Douéllou & Chishawa (1995)**	**Present study**
Host	*Clarias gariepinus*	*Clarias gariepinus*	*Clarias gariepinus*	*Clarias gariepinus*
Site on host	Gills	Gills	Gills	Gills
Number of specimens	10	26	14	10
Total body length	378-630	313-502	360-600	209-246
Total body width	120-157	76-105	70-160	72-115
Ventral anchors				
Anchor length	35-43	36-44	29.4-33.5	23-33
Base width	9-13	9-12	7.3-9.9	6-8
Point length	8-11	-	10.5-14.1	10-17
Ventral bar				
Bar length	40-47	39-53	31.5-37.2	35-56
Bar width	-	-	-	4-7
Dorsal anchors				
Anchor length	42-49	43-51	34.3-40.6	26-34
Base width	3-4	13-18	10.8-13.2	5-13
Point length	14-19	-	3.3-4.1	8-12
Dorsal bar				
Bar length	35-47	45-74	34.8-43.9	23-32
Bar width	24-30	-	-	5-10
Marginal hooklets	-	8-14	8-14	6-14
Copulatory organ axis	42-56	33-49	34-40.1	1-4

- No measurements taken

5.3.2 Species descriptions, diagnosis and measurements: *Dactylogyrus* Diesing, 1850 sp 1 from Welbedacht Dam

During the present study two specimens belonging to the genus *Dactylogyrus* Diesing, 1850 were collected from the gills of *C. carpio*. The sclerotised parts were measured based on the method used by Crafford et al. (2012) (refer to Fig 5.3).The following basic measurements were used: total body length, width of body, total anchor length, shaft length, outer root length, inner root length, point length, aperture length, dorsal bar length, dorsal bar width, marginal hooklets, male copulatory organ and accessory piece. Measurements were all in micrometres (μm) followed by the range in parentheses. However, because there were only two specimens, and also owing to the quality of the specimens (see Fig 5.4) it was not possible to identify these positively to species level. These have been preliminarily identified to genus level and referred to as *Dactylogyrus* sp 1.

Total body length 261 (206-314), total width of body 106 (96-116), anchor total length 38 (35-41), anchor shaft length 31 (30-32), length of outer root 3.5 (3-4), length of inner root 10 (5-15), length of tip 11 (10-12), length of aperture 27 (26-28), length of bar 22 (21-23), width of bar 5 (3-7), accessory piece 23 (20-26), marginal hooklets length 13 (11-17).

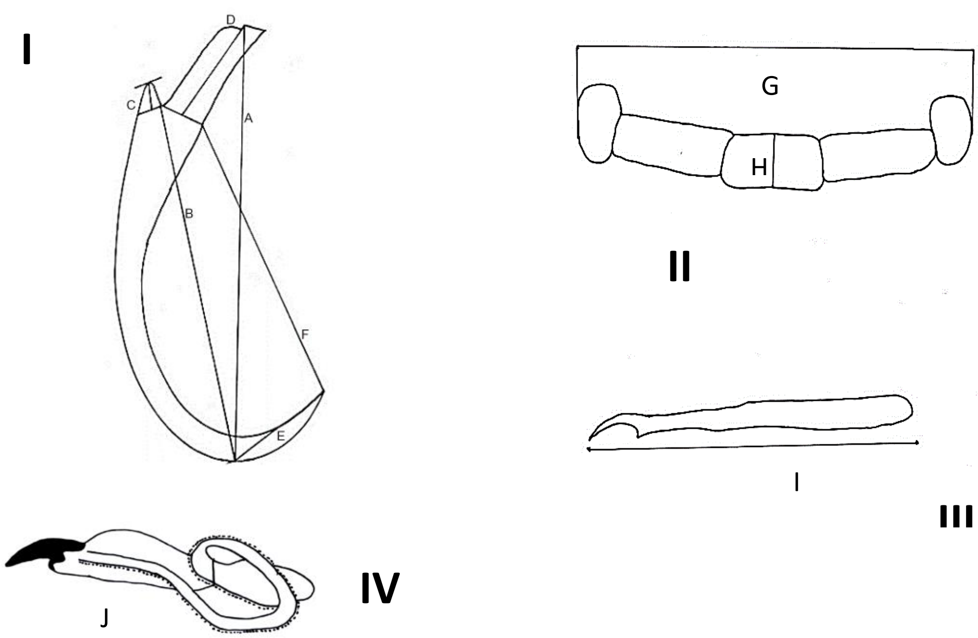


Fig 5.3: Measurements of the sclerotised parts of *Dactylogyrus* Diesing, 1850 sp 1 from *Cyprinus carpio* Linnaeus, 1758: I, Anchor; II, Bar; III, Marginal hooklet; IV, Male corpulatory organ. A-anchor total length;B-shaft length; C-length of outer root; D-length of inner root; E-length of anchor tip; F-anchor aperture; G-length of bar; H-width of bar; I-length of marginal hooklet; J-accessory piece length. The measurements were done based on the method used by Crafford et al. (2012).

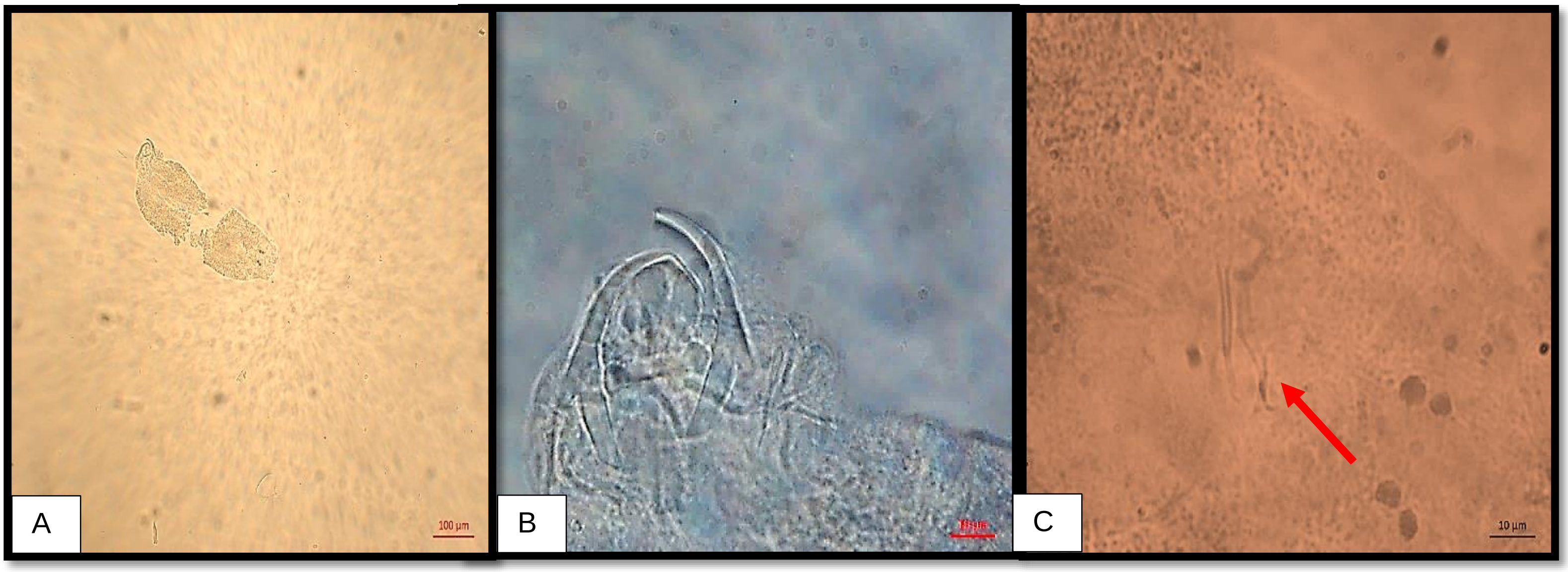


Figure 5.4: Light microscope photographs of *Dactylogyrus* Diesing, 1850 sp 1. from *Cyprinus carpio* Linnaeus, 1758 from Welbedacht Dam: A-whole worm, B-anchors, C-male copulatory organ (→).

5.3.3 Species descriptions, diagnosis and measurements: *Dactylogyrus* Diesing, 1850 sp 2 from Gariep Dam

During the present study, 10 specimens belonging to the genus *Dactylogyrus* were collected from the gills of *L. aeneus* from Gariep Dam. Four of the specimens were stained using Gomori's trichrome (Fig 5.5 A). The sclerotised parts from the remaining six specimens were measured (Fig 5.5 B), based on the method used by Crafford et al. (2012) (see Fig 5.3). The male copulatory organ was not observed in any of the specimens. The worms possessed two anchors, and the bar was not observed.

Total body length 339 (228-348), width of body 84 (55-97), anchor total length 53 (44-74), shaft length 36 (28-50), length of outer root 7 (5-10), length of inner root 18 (12-27), length of tip 16 (13-21), aperture length 34 (28-47), marginal hooklets length 23 (10-43).

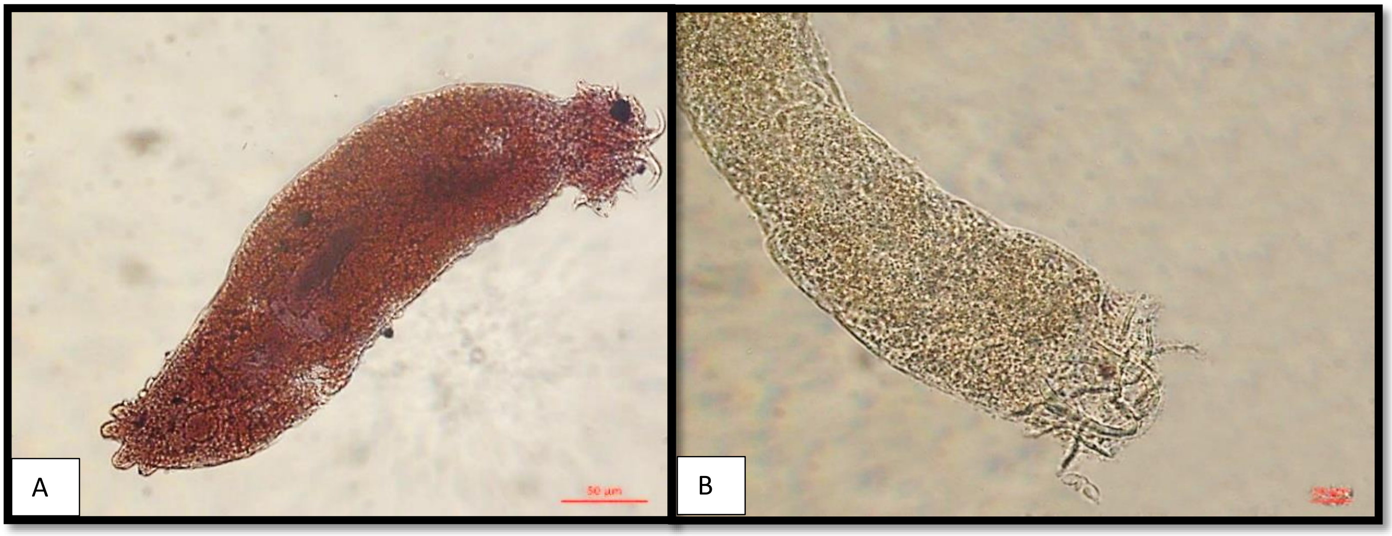


Figure 5.5: Light microscope photographs of *Dactylogyrus* Diesing, 1850 sp 2 from *Labeobarbus aeneus* (Burchell, 1822) from Gariep Dam: A – whole worm stained using Gomori's trichrome stain, B – anchors

5.4 Discussion

During the present study two specimens of *Dactylogyrus* sp 1. were found infesting the gills of *C. carpio*. It was not possible to compile taxonomic identifications as there were too few specimens recovered.

Dactylogyrus sp 2. (10 specimens) was found infesting *L. aeneus*, however, it was not possible to compile taxonomic identifications as only six specimens were mounted in GAP for examining of the sclerotised parts. Of these six specimens, the dorsal bar and male copulatory organs were not observed. According to Crafford (2013), there is very little information known about the monogenean fauna of the genus *Labeobarbus* Rüppel, 1836 in South Africa. *Dactylogyrus varicorhini* Bychowsky, 1958 was reported by Price et al. (1969) from largemouth yellowfish *Labeobarbus kimberleyensis* (Gilchrist & Thompson, 1913) whilst Mashego (1983) reported *D. spinicirrus* Paperna and Thurston, 1967 also from *L. kimberleyensis*.

The above mentioned parasite species are the only two monogenean species known from South African yellowfish, as listed by Khalil and Polling (1997). It is clear that much more work still needs to be done with regard to the monogeneans of South African yellowfish.

During the present study *Quadriacanthus aegypticus* was found infesting the gills of *C*. *gariepinus*, however, the worms recovered during the current study were smaller in size as compared to the measurements of El-Naggar & Serag (1986), Kritsky & Kulo (1988) and Douéllou & Chishawa (1995). There has been difficulty in correctly identifying/differentiating between two morphologically similar species of the genus *Quadriacanthus*, i.e. *Quadriacanthus aegypticus* and *Quadriacanthus clariadis* Paperna, 1961. Kritsky & Kulo (1988) for example, reported that, in their opinion, Paperna & Thurston (1968) had incorrectly identified a monogenean specimen as *Q*. *clariadis*.

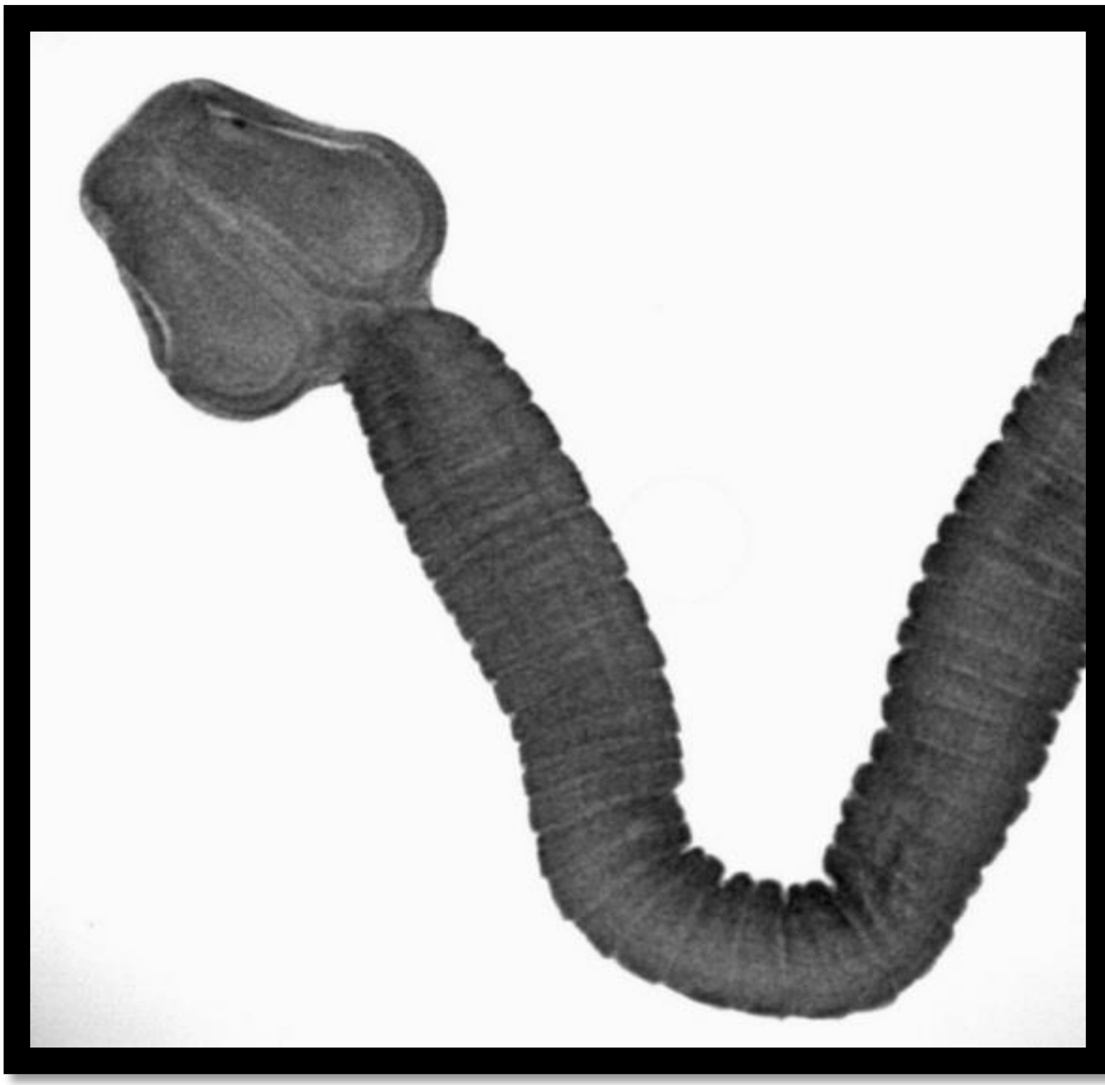
Kritsky & Kulo (1988) stated that upon their observation of the drawings of Paperna & Thurston (1968) of the accessory piece and vagina, it was more likely that the species was *Q*. *aegypticus*. According to Kritsky & Kulo (1988), some of the distinguishing morphological characteristics between *Q*. *clariadis* and *Q*. *aegypticus* are: *Q*. *clariadis*: short, curved ventral anchor shaft, poorly developed terminal hook of the accessory piece, short dorsal anchor point. *Quadriacanthus aegypticus*: dorsal anchors are slightly larger than the ventral anchors, ventral anchor shaft elongate, accessory piece possess a distinct hooked termination. Specimens collected during the present study fit the above description of *Q*. *aegypticus* by Kritsky & Kulo (1988), even though they were smaller.

According to Paperna (1960), monogeneans are important parasites of cultured fish, as they frequently cause high mortalities of fish, more so among fry and fingerlings bred under artificial conditions such as hatcheries and ponds. In wild fish stocks, there is a natural balance between parasites and their hosts, and the infections vs. infestations are not usually problematic. However, the artificial environment provides favourable conditions for the proliferation of parasites, especially for those parasite species that are tolerant of low oxygen levels (compared to the natural environment) as is the case in aquaculture systems. Monogeneans have a direct life cycle (requiring no intermediate host), in the aquaculture environment host density is often increased thereby increasing the susceptible host density, thereby facilitating transmission (Reno 1998).

Monogeneans proliferate rapidly and in a short space of time under crowded and stressful conditions, such as in an aquaculture environment. Monogeneans pose a serious threat to fish culture, therefore aquaculture institutions should keep in mind the potential impact of fish parasites and diseases in the development of the aquaculture sector (Akoll & Mwanja 2012). Monogenean parasites often occur simultaneously with trichodinids, and have caused severe mortalities of catfish fry under aquaculture conditions (Van As & Basson 1988).

The potential impact of monogenean infestations must be kept in mind when approaching any aquaculture venture in South Africa, this is also especially important for the Agricultural Technology Demonstration Centre (ATDC) in Gariep Dam Free State Province.

Chapter 6: Cestoda



Chapter 6: Cestoda

6.1 Introduction

There has not been much research done on fish cestodes especially in South Africa, and therefore not a lot of data has been generated with regards to fish cestodes. Some of the studies that have been conducted in South Africa include work by the following authors: Mashego (2001), Barson & Avenant-Oldewge (2006), and Madinare-Moyo & Avenant-Oldewage (2013). These studies generated data on *Proteocephalus glanduligerus* (Janicki, 1982) from the sharptooth catfish *Clarias gariepinus* (Burchell, 1822). Madinare-Moyo & Avenant-Oldewage (2013), found *Tetracampos ciliotheca* Wedl, 1861 from *C. gariepinus*. The other South African cestode studies were those of Brandt et al. (1981) generating data on *Bothriocephalus acheilognathi* Yamaguti, 1934, Mashego & Saayman (1989) who found *Polyonchobothrium clarias* (Woodland, 1925) and *P. glanduligerus*, Bertasso & Avenant– Oldewage (2005) who found *B. acheilognathi* and Swanepoel (2015) who found *B. acheilognathi*, *Ligula intestinalis* (Linnaeus, 1758) and two possibly new species belonging to the genera *Proteocephalus* Weinland, 1858 and *Ichthybothrium* Khalil, 1971.

Another reason contributing to the lack of data with regards to fish cestodes, also commonly referred to as tapeworms, is the fact that they have complex life cycles which includes more than one host. Fish are infected via two routes, **(1)** by ingesting the intermediate host, i.e. suitable fresh water cyclopoid or diaptomid copepods (Stadtlander et al. 2011) or **(2)** The predation of infected fish (Stadtlander et al. 2011). Some tapeworms mature into adult stages inside piscivorous birds, allowing for the dissemination of tapeworm eggs over long distances and also between different water bodies.

This in turn, makes it difficult to control the spread of parasites between water bodies. As a result, it is considerably difficult to eradicate infections by cestodes in the wild, as the life cycle has to be broken, but the intermediate hosts, i.e. the copepods, are also found in the same water body as the definitive host (Van As & Basson 1988). In an aquaculture environment, however, it is relatively simple to break the life cycle; this is done by feeding fish a diet of artificial pelletised feeds, thereby inhibiting the successful transmission of tapeworm larvae to the fish.

Only a few parasitological studies have been carried out on piscivorous birds in Africa and South Africa. As a result, there is still a need to understand the interactions between cestodes and piscivorous birds; the same applies to the intermediate hosts (Barson & Avenant-Oldewage 2006).

The relative low diversity/geographical range of fish tapeworms is also a contributing factor with regards to the paucity of available data, i.e. the diversity and geographical range would only be known from sites that have been surveyed for cestodes. According to Dick et al. (2006), it seems that the species diversity of fish tapeworms is greatest in the subtropical and temperate zones, but this is also the zone where most of the work has been done.

Cestodes are ubiquitous with a cosmopolitan distribution, and infect all fish species at either the juvenile or adult stage. Most of the economically important fish tapeworms are found in temperate, north temperate and Arctic regions of the world. In their adult forms, cestodes are found in the digestive tract of the hosts, and as larval stages, they are found in the muscles, liver, haemocoel and brain (Dick & Choudhury 1995).

Cestodes are characterised by several morphological features with the most obvious feature being the loss of a digestive tract throughout all the stages of development (Madinare-Moyo & Avenant-Oldewage 2013). Heavy cestode infections in the flesh and viscera have been reported, however, very few have been shown to cause mortalities of their fish hosts. There are some genera that have received considerable attention, such as the human fish tapeworm *Diphyllobothrium* Cobbold, 1858 and those of more economic importance, for example *Triaenophorus* Rudolphi, 1793 (Dick & Choudhury 1995).

The tapeworm fauna of African ray-finned fishes has been studied since the 19th century, through the works of Leydig (1853) and Wedl (1861) in which they described the first tapeworms from bichirs and clariid fishes (Kuchta et al. 2012). Khalil & Polling (1997), recorded 61 species of adult and larval tapeworms in their checklist of the helminth parasites of African fresh water fishes, which belong to the following orders: Amphilinidea (one species), Caryophyllidea (20 species in 7 genera), Bothriocephalidea (13 species in 3 genera) and Proteocephalidea (19 species in 6 genera).

6.2 The Asian tapeworm

Bothriocephalus acheilognathi Yamaguti, 1934 was first described from the cyprinid *Acheilognathus rhombeus* (Temminck & Schlegel, 1846) from Lake Ogura in Japan. This tapeworm was spread across the world primarily through the importation of common carp *Cyprinus carpio* Linnaeus, 1758 and grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) (Kuchta et al. 2018). The Asian tapeworm has been able to spread rapidly to other countries as a result of the translocation of infected fish. The extraordinary ability of *B*. *acheilognathi* to infest hosts outside its natural distribution can attest to its ability to adapt to different environmental conditions; as such this tapeworm has been able to colonise all continents except Antarctica (Ponce de León et al. 2018).

Brabec et al. (2015) have since resurrected the genus *Schyzocotyle* Akhmerov, 1960 so as to include this invasive species of tapeworm, as a result it is now known as *Schyzocotyle acheilognathi* (Yamaguti, 1934). The Asian tapeworm was first recorded in South Africa by Brandt et al. (1981) from the digestive system of largemouth yellowfish *Labeobarbus kimberleyensis* (Gilchrist & Thompson, 1913). The Asian tapeworm was also found during the current study, associated with *L*. *kimberleyensis* (threatened species) amongst other hosts, and this aspect is discussed further on in this chapter.

According to Kuchta et al. (2018), the Asian tapeworm is probably the most successful invasive metazoan parasite, and there are various factors that have facilitated the success of *S*. *acheilognathi* at colonising different parts of world.

1. Complexity of life cycle, the ability of invasive species to be successful is considered to be inversely proportional to the complexity of its life cycle (Scholz et al. 2012). According to Reno (1998) an important factor regarding transmission of parasites is the frequency of contact between the infectious and susceptible animals. Furthermore, unless there is contact between the infectious and

susceptible animal, infection cannot occur, this contact can be direct or indirect, i.e. from fish to fish or through an intermediate host (Reno 1998). *Schyzocotyle acheilognathi* has an indirect complex life cycle with a developmental cycle that includes copepods. Under ideal conditions the life cycle of *S*. *acheilognathi* may be completed in one month, the adults discharge eggs into the gut and lumen of the host, which are released into the water with faecal matter (Scholz et al. 2012). Once the eggs are in the water, an embryo called a hexacanth develops, this may take a few days and is dependent on the water temperature. The larva (coracidium) that emerges from the eggs is ciliated and moves actively in the water (Fig 6.1).

2. Low host specificity, a parasite that is able to utilise a wide variety of hosts at both intermediate and definitive host level is likely to be a successful invasive species.

3. Similarity in abiotic and biotic factors between the original and new localities, this tapeworm has colonised regions and ecosystems with notably different physiochemical characters. The Asian tapeworm has, however, been found in colder conditions such as Lake Winnipeg in Canada; but in these conditions (below 12°C) egg development is much slower (Kuchta et al. 2018).

4. Composition and richness of parasite communities in recipient hosts, the nature of the parasite communities in recipient hosts also plays a role in the success of colonisation, with species-poor communities and vacant niches in recipient hosts, representing favourable circumstances for successful invasive species (Kuchta et al. 2018).

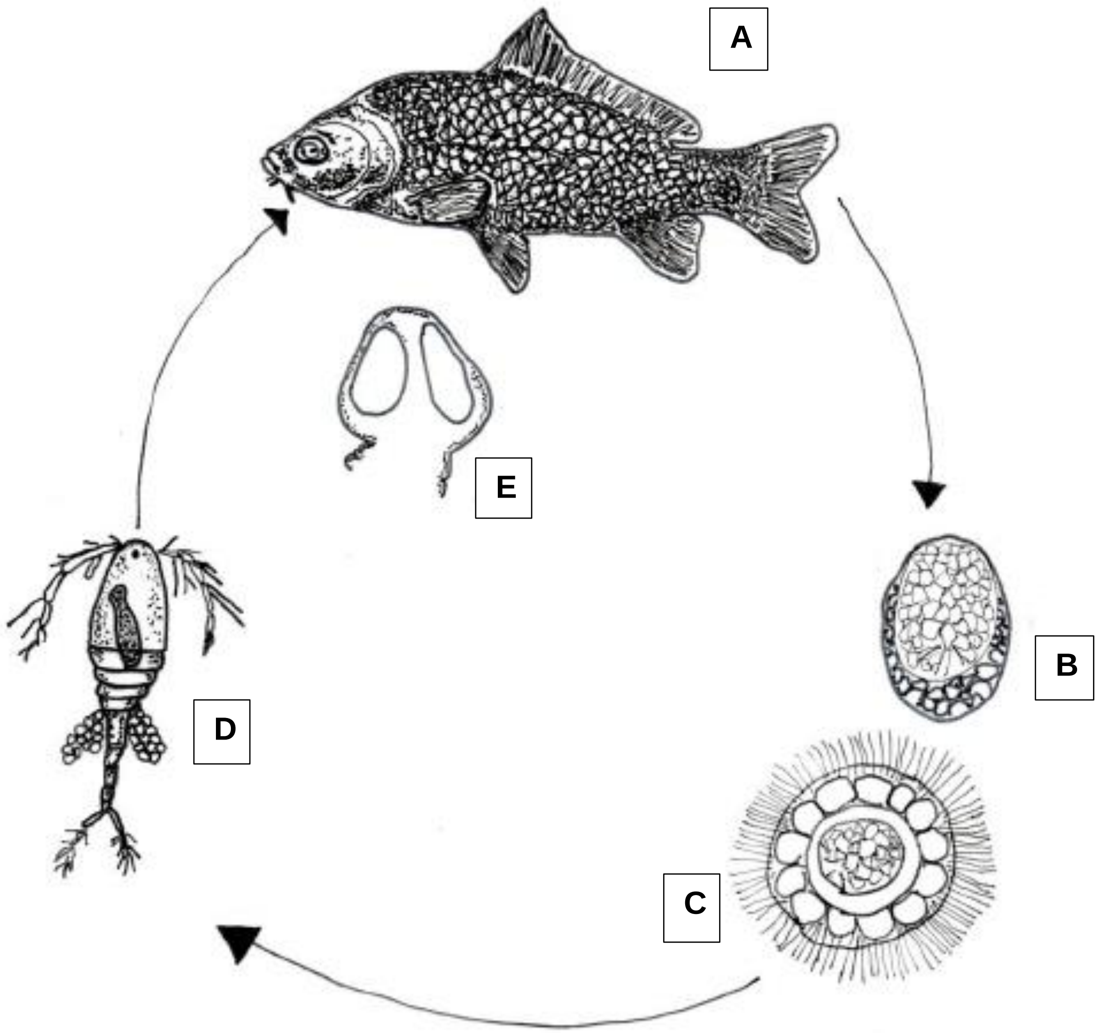


Figure 6.1: Life cycle of *Schyzocotyle acheilognathi* (Yamaguti, 1934), A-host species, B-eggs released from host gut, C-ciliated coracidium, D-infected copepod, E-mature cestode. Redrawn from Scholz et al. (2012).

This chapter reports on the presence of a single cestode species from various dams in the Free State Province, South Africa. *Schyzocotyle acheilognathi* was found associated with three cyprinid hosts, *C. carpio*, smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822) and *L. kimberleyensis* as well as one clariid host, *C. gariepinus* collected at seven dams. The chapter further reports on infection statistics of this cestode species infecting different host species from the respective dams, as well as the potential effects this species may have on the development of fresh water aquaculture in the province.

6.3 Materials and methods

Fish collections: (Refer to chapter 3)

6.3.1 Host examination, fixation and the preservation of parasites

The viscera and intestines were examined for parasites under a dissecting light microscope. The cestodes found were fixed in heated 10 % buffered neutral formalin and placed in McCartney bottles. Cestodes were identified to species level using Khalil et al. (1994).

6.4 Results

The cestode *Schyzocotyle acheilognathi* was found associated with 17 *C. carpio*, 10 *L. aeneus*, three *L. kimberleyensis* and two *C. gariepinus* specimens from various localities (Table 6.1). The cestodes were identified as *Schyzocotyle acheilognathi* (Yamaguti, 1934) based on morphological features such as the heart shaped scolex and a prominent square apical disc. The number of cestodes were counted under a dissecting light microscope. A total of 195 cestodes were found, the infection levels per host per locality were determined and these are presented in Table 6.1.

Cyprinus carpio from different localities had the highest mean intensity, this which is an indication that this parasite is a natural parasite of this particular host species. The other two cyprinids (*L*. *aeneus* and *L*. *kimberleyensis*) found infected with *S*. *acheilognathi* had notably lower mean intensities across all localities sampled as compared to *C*. *carpio*. However, *L*. *aeneus* had the higher mean intensity of the two. The infection statistics for *Clarias gariepinus* were lower than those of *C*. *carpio*, *L*. *aeneus* and *L*. *kimberleyensis*, this is further indication that this parasite is a natural parasite of cyprinids. The infection statistics (apparent prevalence and mean intensities) were calculated and are presented in Table 6.3.

6.4.1 Species description: *Schyzocotyle acheilognathi* (Yamaguti, 1934) from Welbedacht Dam, Knellpoort Dam, Koppies Dam, Allemanskraal Dam, Krugersdrift Dam, Gariep Dam and Rustfontein Dam

Large worms, segmentation present, heart–shaped scolex (Fig 6.2 A, C), prominent square apical disc. Bothria elongate, shallow with simple margins. Testes visible on lateral sides, continuous between proglottids (Fig 6.2 B, D), neck absent. Cirrus sac oval to spherical in shape, in cross section it appears elongate to pyriform. Genital and ovary pores median, vagina located posteriorly to cirrus sac, numerous vitelline follicles. Uterine duct sinuous, enlarges when segments are gravid, uterus spherical to transversely oval, occupies most of space in terminal segments, eggs operculate, unembryonated (Khalil et al. 1994).

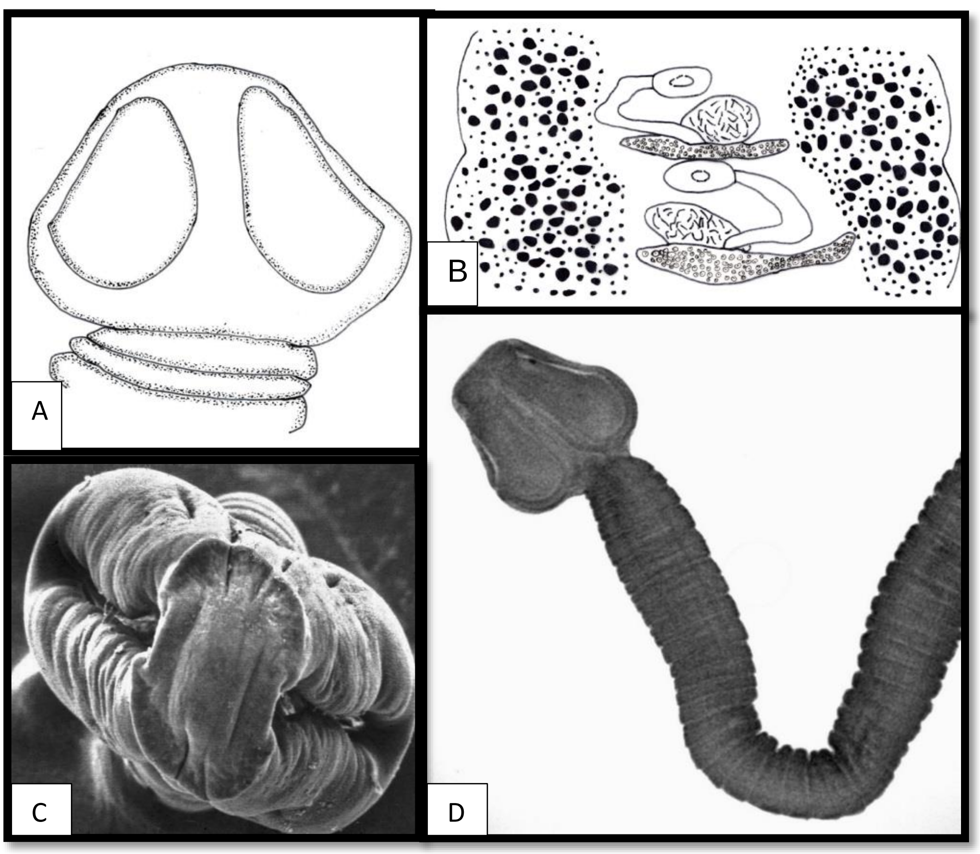


Figure 6.2: Drawings representative of the A-scolex of *Schyzocotyle acheilognathi* (Yamaguti, 1934), B-mature proglottis redrawn from Scholz et al. (2012). C-scanning electron micrograph of scolex, D-dissection micrograph of whole worm. Courtesy of the Aquatic Ecology Research Group image collection, (UFS).

Table 6.1: Infection of different fish host species by *Schyzocotyle acheilognathi* (Yamaguti, 1934) caught at Welbedacht Dam, Knellpoort Dam, Koppies Dam, Allemanskraal Dam, Krugersdrift Dam, Rustfontein Dam and Gariep Dam

Host scientific name	Localities and number of hosts examined including hosts infected (*)	Total number of *Schyzocotyle acheilognathi* infecting different hosts
Cyprinus carpio	Welbedacht Dam 9 (1*) Knellpoort Dam 21 (*4) Koppies Dam 7 (*3) Allemanskraal Dam 12 (5*) Krugersdrift Dam 8 (1*) Gariep Dam 6 (3*)	26 10 25 37 1 16
Labeobarbus aeneus	Knellpoort Dam 4 (4*) Gariep Dam 16 (4*) Rustfontein 15 (2*)	4 6 38
Labeobarbus kimberleyensis	Koppies Dam 3 (2*) Allemanskraal Dam 3 (1*)	16 11
Clarias gariepinus	Gariep Dam 6 (2*)	5

(*) number of hosts infected

During the current study *S*. *acheilognathi* was only found at the above localities (Table 6.1) from *C*. *carpio*, *L*. *aeneus*, *L*. *kimberleyensis* and *C*. *gariepinus*. In addition to the above host species, examinations were done on other host species from the same localities which were found free from *S*. *acheilognathi*, these are presented in Table 6.2.

The apparent prevalence and mean intensities of infections by *S*. *acheilognathi* are presented in table 6.3. The majority of the species found infected with *S*. *acheilognathi* were cyprinids, i.e. *C*. *carpio*, *L*. *aeneus* and *L*. *kimberleyensis* the only other species found infected with this tapeworm was *C*. *gariepinus*, this indicates that this tapeworm is a natural parasite of cyprinid hosts. *Cyprinus carpio* caught at the different localities had the highest mean intensity of infection by *S*. *acheilognathi*, this indicates that this is the natural host for this species.

Table 6.2: Hosts examined for *Schyzocotyle acheilognathi* (Yamaguti, 1934) collected from Rustfontein Dam, Koppies Dam, Welbedacht Dam, Allemanskraal Dam, Krugersdrit Dam and Knellpoort Dam that were not infected (0*).

Host scientific name	**Localities and number of hosts examined including hosts infected (0*)**
Cyprinus carpio	Rustfontein Dam 5 (0*)
Labeobarbus aeneus	Koppies Dam 2 (0*)
Labeobarbus kimberleyensis	Rustfontein Dam 1 (0*)
Clarias gariepinus	Welbedacht Dam 1 (0*) Koppies Dam 2 (0*) Allemanskraal Dam 2 (0*) Krugersdrift Dam 1 (0*) Rustfontein Dam 1 (0*)
Labeo capensis	Welbedacht Dam 4 (0*) Knellpoort Dam 1 (0*) Koppies Dam 1 (0*) Allemanskraal Dam 4 (0*) Krugersdrift Dam 8 (0*) Gariep Dam 24 (0*) Rustfontein Dam 30 (0*)
Labeo umbratus	Knellpoort Dam 1 (0*) Koppies Dam 6 (0*) Allemanskraal Dam 4 (0*) Gariep Dam 1 (0*) Rustfontein Dam 1 (0*)

Table 6.3: The apparent prevalence and mean intensities of *Schyzocotyle acheilognathi* (Yamaguti, 1934) infecting different host individuals from different localities

Host species	**Locality**	**Apparent prevalence %**	**Mean intensity**
Cyprinus carpio	Welbedacht Dam	11	26.0
	Knellpoort Dam	19	2.5
	Koppies Dam	43	8.3
	Allemanskraal Dam	42	7.4
	Krugersdrift Dam	12.5	1.0
	Gariep Dam	50	5.3
Labeobarbus aeneus	Knellpoort Dam	100	1.0
	Gariep Dam	25	1.5
	Rustfontein Dam	13	19.0
Labeobarbus kimberleyensis	Koppies Dam	67	8.0
	Allemanskraal Dam	33	11.0
Clarias gariepinus	Gariep Dam	33	2.5

6.5 Discussion

The Asian tapeworm *S. acheilognathi* (formerly known as belonging to the genus *Bothriocephalus*), has been introduced across the world from East Asia. *Schyzocotyle acheilognathi* is pathogenic to carp fry, as well as to other cyprinids and the host spectrum of *S*. *acheilognathi* includes more than 200 species of fish, with the family Cyprinidae representing the most suitable definitive host (Kuchta et al. 2012).

The susceptibility of fish to infection by cestode parasites depends on the morphology, physiology and diet of the fish, and according to Madinare-Moyo & Avenant-Oldewage (2013), herbivorous fish generally harbour fewer intestinal parasites than omnivorous and carnivorous fish. The results obtained of the current study show a similar trend to the above statement in the sense that the host species from the current study are generally found at different trophic levels and this plays an important role with regards to the chances of infection by *S*. *acheilognathi*.

Schyzocotyle acheilognathi was found by the following authors in different impoundments of the Orange-Vaal River system, including the river itself: Komatipoort (Boomker et al.

1980) and the Vaal Dam (Brandt et al. 1981; Nickanor et al. 2002; Bertasso & Avanant-Oldewage (2005). The data presented in this chapter serves to supplement the distribution information given by the above authors in the Orange-Vaal River system. During the current study, *S*. *acheilognathi* was found at the following dams: Welbedacht Dam, Knellpoort Dam, Koppies Dam, Allemanskraal Dam, Krugersdrift Dam, Rustfontein Dam and Gariep Dam.

During the current study, two species collected in impoundments in the Free State (*C*. *carpio* and *C*. *gariepinus*) are of economic importance, with aquaculture potential and were found infected with this tapeworm. The ATDC in Gariep Dam, currently (2016, 2017) cultures both of the above two species.

During the course of 2016 and 2017, no cestodes were found infecting *C*. *carpio* and *C*. *gariepinus* at the ATDC, this may be as a result of the fact that fish are generally bred in an indoor hatchery in tanks, once they have hatched they are fed *Artemia* (Class: Branchipoda). So in general the diet is kept devoid of the copepods that form part of the life cycle of this particular cestode. Once the hatchlings reach fingerling size, they are transferred to outside ponds for grow out, however, these outside ponds are concrete structures, thereby limiting the potential of copepod generation. The ponds are also generally not fertilised. The fertilisation of the outside ponds with organic fertiliser would stimulate the production of planktonic copepods, thereby increasing the numbers of possible intermediate hosts of *S*. *acheilognathi*. The fingerlings are fed a diet of commercial artificial feeds, ranging in size from a fine mesh up to 3mm for the bigger fish.

On the other hand, the ATDC sources water directly from the Gariep Dam, this water is channelled by gravitational gradient into the overwintering facility (hatchery) and the outside ponds untreated. This implies that the intermediate hosts; copepods, could be introduced into the facility through the untreated water. According to Scholz et al. (2012) there are several copepods that are suitable intermediate hosts both under aquaculture conditions, as well as in natural conditions, these include: *Cyclops* Müller, 1785 *Acanthocyclops* Kiefer,1927, *Megacyclops* Kiefer,1927, *Macrocyclops* Claus,1893, *Mesocyclops* (Claus,1857), *Tropocyclops* Kiefer,1927 and *Thermocyclops* Kiefer,1927.

Schyzocotyle acheilognathi was introduced to South Africa in 1975 along with its natural host, *C*. *idella* and subsequently adapted itself to infect *C*. *carpio*. The common carp was trans-located across South Africa for aquaculture and recreational angling purposes, and as a result the tapeworm spread rapidly across South Africa, infecting other species that are not its natural host (Smit et al. 2017).

The origin of African populations of *S*. *acheilognathi* is still largely unclear, however, it was first described by Baer & Fain (1958) from *Barbus* spp. in the then Zaire, currently known as the Democratic Republic of the Congo. In the middle 1970's this tapeworm was reported from Egypt by Ryšavý and Moravec (1975) and a few years later in South Africa by Brandt et al. (1981). This tapeworm has now been recorded from six major river systems in South Africa (Stadtlander et al. 2011).

With the spread of this tapeworm to new localities comes the possibility that it could infect new host species, some of these species include ecologically fragile species (Scholz et al. 2012). One such example in South Africa is *L*. *kimberleyensis* from which this tapeworm was recorded by Barkhuizen (1991) and also during the present study. *Schyzocotyle acheilognathi*, which is listed as a pathogen of regional concern by the US Fish and Wildlife Service (Scholz et al. 2012), was found infecting the largemouth yellowfish *L*. *kimberleyensis* which is IUCN red-listed as near threatened (Ellender et al. 2012). During the current study six out of the eight *L*. *kimberleyensis* specimens collected from four of the nine localities sampled were examined for the presence of cestodes, and three were found to be infected with *S*. *acheilognathi*, the number of tapeworms found infecting this host was 27 tapeworms from six fish specimens.

According to Scholz et al. (2012), *S*. *acheilognathi* causes bothriocephalosis, and *Bothriocephalus acheilognathi* is recognised as the disease causing agent, so Ponce de León et al. (2018) have suggested that the disease bothriocephalosis remains so named for the sake of convenience, this is something the author agrees with. *Schyzocotyle acheilognathi* is also considered to be one of the most dangerous helminth parasites of cultured *C*. *carpio* (Scholz et al. 2012), which could have similar implications for the largemouth yellowfish and other cyprinids.

Much more work still needs to be done to understand the true effect that introduced parasite species and particularly *S*. *acheilognathi* could have on South African indigenous fish species, as well as their potential effects on the fresh water aquaculture industry.

Chapter 7: Ciliophora

Chapter 7: Ciliophora

7.1 Introduction

Apart from free-living representatives, ciliophorans are also among the most widely distributed ecto- and endoparasites or symbionts found on fish. Sufficient information with regards to ciliophorans that cause disease and mortality on and in fish is known, together with their economic impact (Shinn et al. 2015), as well as some of the control mechanisms available (Shinn & Bron 2012). Many more facets of the existence of ciliophorans are in urgent need of further study. Trichodinids are among the most commonly encountered symbiont groups within the aquatic environment, however, much of the diversity of these and other groups of ciliophorans is largely unknown (Basson & Van As 2006). The relationship between ciliophorans and their hosts is for the most part unknown and has been based on speculation rather than actual science based evidence (Basson & Van As 2006).

As a result of the limited knowledge with regards to ciliophorans it seems that, in the event where there are high infestations of ciliophorans on a fish host, it is automatically considered a pathogen, even though this may not be the case (Basson & Van As 2006).

During the current study, only ciliophorans belonging to the order Mobilida Kahl, 1933 were found associated with three cyprinid hosts. This order comprises mobile ciliated organisms with conical, cylinder–shaped bodies. Three families are known, namely, Urceolaridae Dujardin, 1841, Trichodinopsidae Kent, 1881 (both found associated with invertebrate hosts) and Trichodinidae Raabe, 1963 (occurring on and in a wide range of invertebrate as well as vertebrate hosts) (Basson & Van As 2006).

Members of the family Trichodinidae all have complex structures within an aboral adhesive disc; they are epizoics on a variety of aquatic invertebrate and vertebrate hosts. The main features of diagnosis for genera from this family, are based on the shape and dimensions of the denticles within the adhesive disc, and the development of the adoral ciliary spiral (Basson & Van As 1989).

The family Trichodinidae comprises 11 genera and eight of these are found associated with fishes (Basson & Van As 2006). In fresh water fishes of Africa, the family Trichodinidae is represented by five genera: *Trichodina* Ehrenberg, 1830, *Trichodinella* (Raabe, 1958) Šrámek-Hušek, 1953, *Tripartiella* Lom, 1959, *Paratrichodina* Lom, 1963 and *Hemitrichodina* Basson & Van As, 1989 (Basson & Van As 1989). Representatives of the genus *Tripartiella* and *Trichodinella* are found exclusively on fish gills, while some representatives of the genus *Trichodina* occur on the skin, gills and fins, with many of these having a preference for the gills. Various studies have been carried out with regards to mobiline ciliophorans, seen through the work of Basson, et al. (1983), Basson & Van As (1987, 1989, 2006), Van As & Basson (1989, 1992) and Jerônimo et al. (2012)

Some trichodinid ciliophorans have been associated with mortalities of wild fish stocks, as well as fish under aquaculture conditions, and as a result have received considerable attention (Basson & Van As 2006). The transmission of trichodinids is facilitated through the proximity and density of susceptible hosts, and according to Hassan (1999), trichodinids have a direct life cycle, which enables them to spread rapidly amongst hosts.

According to Lom (1995), some trichodinids are capable of inflicting severe damage to the hosts and this may at times result in mortalities, especially of fishes that are under stressful conditions, as in the instance of aquaculture conditions.

All trichodinids exhibit more of a commensalistic life style (only causing damage to the host under certain unnatural conditions). They never occur in large numbers on healthy fish, and show over-dispersion under natural conditions (Basson & Van As 2006). However, on unhealthy fish, such as those with low immunity as a result of negative environmental factors such as overcrowding; or fish at the larval or fry stage, the natural repellent ability of the body surface is compromised, resulting in favourable conditions for the rapid proliferation of trichodinids (Basson & Van As 2006).

High infestations by trichodinids can cause damage to epithelial and epidermal cells due to the constant attachment and rotating movements; under these conditions trichodinids behave like true ectoparasites, feeding on the disrupted cells and secondary bacterial growth found associated with these infestations (Basson & Van As 2006).

A greyish-blue colour may be seen when fish are infested with a high number of trichodinids, this is caused by the excessive secretion of mucous, and this reaction is believed to be a protective reaction (Basson & Van As 2006). In this scenario, however, trichodinids actually start feeding on the mucous (Basson & Van As 2006), further perpetuating the situation. Affected fish swim close to the water surface, are often sluggish and often stop feeding. The disease trichodinosis is often found in spring affecting young fry; fresh water fish stressed from harsh winter conditions; as well as on fish under aquaculture conditions (Basson & Van As 2006).

Most trichodinid species show very little host specificity, while others such as *Tripartiella orthodens* Basson and Van As, 1987 are more host specific. Some species occur on both fresh water and marine fish species, e.g. *Trichodina jadranica* Raabe, 1958 (Basson & Van As 2006). A number of trichodinid species seemingly have a global distribution, this is probably as a result of the worldwide translocation of the fish hosts. Some of the more common trichodinids globally are found on most fish families, these include: *T*. *nigra* Lom, 1961, mostly from Europe, but were once recorded in Africa, *T*. *heterodentata* Duncan, 1977 known from Africa, including South Africa, as well as Asia and *Trichodinella epizootica* (Raabe, 1950) that has been found worldwide from numerous fish species (Basson & Van As 2006).

This chapter reports on the presence of trichodinids in dams in the Free State Province, South Africa. Four species of trichodinids, *Trichodina nigra* Lom, 1961, *Trichodina centrostrigeata* Basson, Van As & Paperna, 1983, *Tripartiella lechridens* Basson & Van As, 1987 and *Trichodinella epizootica* (Raabe, 1950) were found associated with three cyprinid hosts, common carp *Cyprinus carpio* Linnaeus, 1758, grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) and Orange River mudfish *Labeo capensis* (A. Smith, 1841) collected at two dams. The chapter further reports on infestation statistics of trichodinids infesting different host species from the respective dams, as well as the potential effects these trichodinids may have on the development of fresh water aquaculture in the province.

7.2 Materials and methods

Fish collections (Refer to chapter 3)

7.2.1 Hosts examination, fixation and preservation of parasites

Skin and gill smears were made and examined for the presence of any ciliophorans by using a compound light microscope. In cases where ciliophorans were found, the slides were left to air dry, and then recorded and numbered according to host species and locality. The air-dried slides containing ciliophorans from the skin and gills were impregnated with silver-nitrate ($AgNO_3$) using the method of Klein (1958) to study features of the adhesive disc.

Microscope images of the ciliophorans were taken using a Zeiss AxioCam ICc camera mounted on a Zeiss Axiophot compound light microscope. The genera found were identified using descriptions by Basson et al. (1983), Basson & Van As (1987) and Van As & Basson (1992).

Morphological measurements were made using the ImageJ computer software program, using the specific uniform characteristics proposed by Lom (1958). All measurements given are in micrometres. Minimum and maximum values are given, followed by the mean, standard deviation and the number of specimens measured. Nuclear stains were not available, therefore no biometric data of the nuclei are included.

7.3 Results

* To calculate apparent prevalence refer to chapter 4

Trichodina nigra (Fig 7.1A) was found associated with *C. carpio* caught at Bloemhof Dam in the Free State Province, South Africa. Seven fish specimens were examined and three individuals were found to be infested. Apparent prevalence of 43% was found, with comparative measurements given in Table 7.2. The features of the parasites found during the present study, i.e. the disc to bell shaped body and concave adhesive disc conform to the description as given by Basson et al. (1983).

Trichodina centrostrigeata (Fig 7.1B) was found associated with *C. idella* from Bloemhof Dam. In total three fish specimens were examined and two individuals were found to be infested. Apparent prevalence was 67% and comparative measurements given are in Table 7.3. This trichodinid can be recognised as a medium sized trichodinid with a disc-shaped body and a deeply concave adhesive disc and conforms to the description of Van As & Basson (1992).

Tripartiella lechridens (Fig 7.1C) was found associated with *L*. *capensis* caught at Gariep Dam in the Free State Province South Africa. In total 24 fish specimens were examined, and 18 individuals were found to be infested. Apparent prevalence of 75% respectively was found, with comparative measurements given in Table 7.4. Basson & Van As (1987) also found this species associated with *L*. *cylindricus* from Loskop Dam and described it as a small trichodinid with a bell-shaped body, concave adhesive disc, possessing a finely striated border membrane.

Trichodinella epizootica (Fig 7.1D) was found associated with *L*. *capensis* caught at Gariep Dam. In total 24 specimens were examined, and one individual was found infested. Apparent prevalence of 4.2% was found, with comparative measurements given in Table 7.5. The specimens found during the present study conform to the description of Basson et al. (1983).

During the current study trichodinids were only found at the above localities from *C*. *carpio*, *C*. *idella* and *L*. *capensis*. In addition to the above host species, examinations were done on other host species from the same localities which were found free of trichodinids, these are presented in Table 7.1.

Table 7.1: hosts examined for trichodinids collected from Bloemhof Dam and Gariep Dam that were not infested (0*).

Host scientific name	**Localities and number of hosts examined including hosts infected (*)**
Labeo capensis	Bloemhof Dam 5 (0*)
Labeobarbus aeneus	Gariep Dam 16 (0*)
Labeo umbratus	Gariep Dam 1 (0*)
Clarias gariepinus	Gariep Dam 6 (0*)
Cyprinus carpio	Gariep Dam 6 (0*)

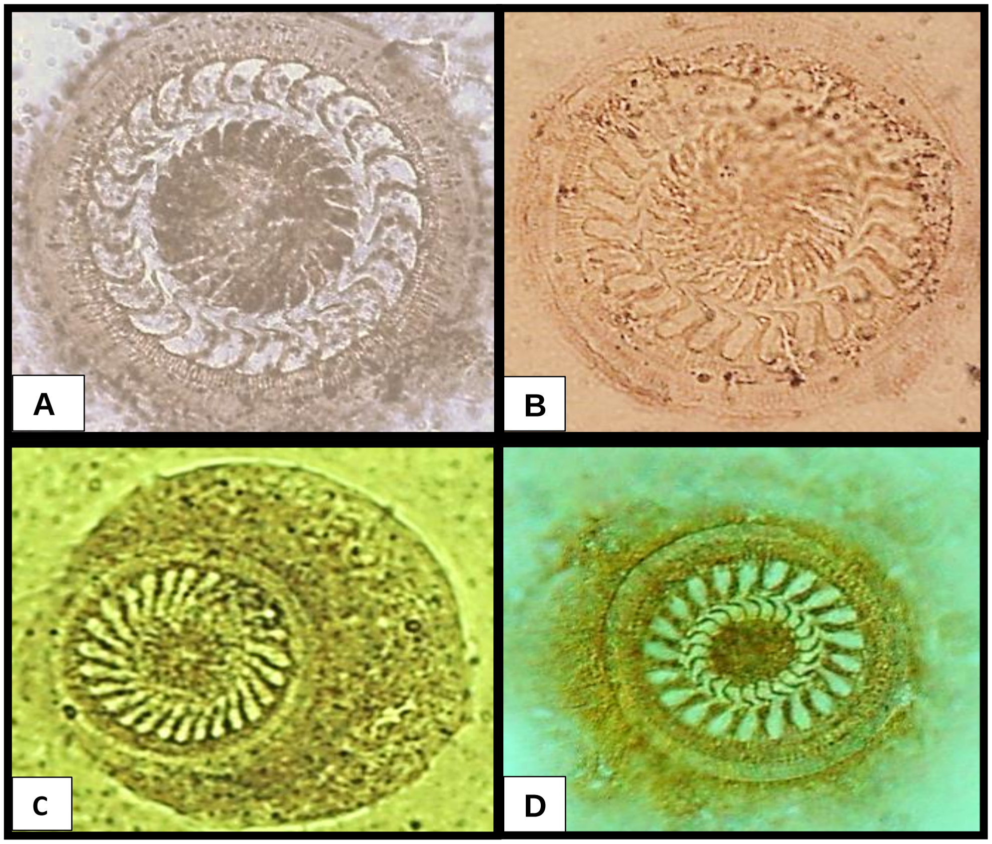


Figure 7.1: Micrographs of silver impregnated discs of A-*Trichodina nigra* Lom, 1961 from *Cyprinus carpio* Linnaeus, 1758, B-*Trichodina centrostrigeata* Basson, Van As & Paperna, 1983 from *Ctenopharyngodon idella* Valenciennes, 1844, C-*Tripartiella lechridens* Basson & Van As, 1987 from *Labeo capensis* (Smith, 1841) and D-*Trichodinella epizootica* (Raabe, 1950) from *Labeo capensis* (Smith, 1841).

Table 7.2: Comparative measurements of *Trichodina nigra* Lom, 1961 from *Cyprinus carpio* Linnaeus, 1758 caught at Bloemhof Dam, Free State, South Africa, with the same parasite species from Luphephe Dam, South Africa (Basson et al.1983).

Host	***Cyprinus carpio***	***Oreochromis mossambicus***
Locality	**Bloemhof Dam**	**Luphephe Dam**
Position in host	**Gills**	**Gills (occasionally skin & fins)**
Reference	**Current study**	**Basson et al. (1983)**
Body diameter	34.1-39.3 (4)	41.7-56.7 (45.9 ±3.1,20)
Adhesive disc diameter	31.4-37.5 (4)	31.7-42.6 (35.1 ±2.9,20)
Denticle ring diameter	17.3-23.4 (4)	18.9-23.7 (20.6 ±2.0,20)
Border membrane	1.2-1.7 (4)	4.0-5.9 (5.1 ±0.4,20)
Blade length	4.1-4.8 (4)	3.3-5.9 (4.6 ±0.7,20)
Central part width	1.6-2.1 (4)	1.6-3.0 (2.5 ±0.7,20)
Ray length	2.7-4.4 (4)	2.5-4.9 (3.9 ±0.6,20)
Denticle width	3.8-5.5 (4)	-
Denticle span	8.1-13.2 (4)	5.1-7.5 (6.3 ±0.7,20)
Denticle number	19-24 (4)	19-22 (20,22)
Radial pins/denticle	7-11 (4)	8-11 (20,22)

4= number of ciliophorans measured

Table 7.3: Comparative measurements of *Trichodina centrostrigeata* Basson, Van As & Paperna, 1983 from *Ctenopharyngodon idella* Linnaeus, 1844 caught at Bloemhof Dam, Free State, South Africa, with the same parasite species from Luphephe Dam South Africa (Basson et al.1983).

Host	***Ctenopharyngodon idella***	***Oreochromis mossambicus***
Locality	**Bloemhof Dam**	**Luphephe Dam**
Position in host	**Gills**	**Gills (occasionally skin & fins)**
Reference	**Current study**	**Basson et al. (1983)**
Body diameter	26.1-35.0 (2)	37.4-54.4 (44.6 ±3.8,100)
Adhesive disc diameter	24.5-32.2 (2)	31.2-35.8 (37.6 ±3.6.100)
Denticle ring diameter	15.8-18.7 (2)	18.7-33.3 (23.2 ±2.5,100)
Border membrane	1.5-1.8 (2)	2.0-4.4 (3.4 ±0.4, 100)
Blade length	3.6-4.1 (2)	2.8-6.4 (5.2 ±0.7,100)
Central part width	1.4-1.5 (2)	1.1-3.0 (1.9 ±0.3,100)
Ray length	2.7-3.8 (2)	3.2-6.0 (4.5 ±0.6,100)
Denticle width	2.9-3.8 (2)	-
Denticle span	6.9-8.4 (2)	2.0-6.2 (4.1 ±0.6,100)
Denticle number	25-30 (2)	26-30 (28,100)
Radial pins/denticle	6-7 (2)	6-7 (7,100)

2= number of ciliophorans measured

Table 7.4: Comparative measurements of *Tripartiella lechridens* Basson & Van As, 1987 from *Labeo capensis* (Smith, 1841) caught at Gariep Dam with the same parasite species from Loskop Dam South Africa (Basson & Van As 1987).

Host	***Labeo capensis***	***Labeo cylindricus***
Locality	**Gariep Dam**	**Loskop Dam**
Position in host	**Gills**	**Skin, fins & gills**
Reference	**Current study**	**Basson & Van As (1987)**
Body diameter	14.4-23.7 (19.9 ±2.48,25)	19.1-24.7 (21.7 ±3.4,50)
Adhesive disc diameter	11.7-19.3 (16.1 ±2.0,25)	5.4-20.4 (17.9 ±1.3,50)
Denticle ring diameter	4.4-9.8 (6.7 ±6.9,25)	7.1-11 (8.7 ±1.5,50)
Border membrane width	1.4-2.5 (2.1 ±0.4,25)	1.6-2.8 (2.3 ±0.3,50)
Blade length	3.1-5.1 (3.9 ±0.5,25)	3.0-4.7 (3.8 ±0.4,50)
Central part width	0.9-1.9 (1.4 ±0.3,25)	0.8-1.6 (1.2 ±0.2,50)
Ray length	1.2-2.8 (1.9 ±0.6,25)	0.6-1.7 (1.1 ±0.3,50)
Denticle width	1.0-2.4 (1.8 ±0.4,25)	1.8-4.3 (3.0 ±0.4,50)
Denticle span	4.7-7.3 (6.3 ±0.8,25)	-
Denticle span	25-26 (25,25)	20-26 (24,50)
Radial pins/denticle	3-6 (5,25)	3-5 (4,50)

25= number of ciliophorans measured

Table 7.5: Comparative measurements of *Trichodinella epizootica* (Raabe, 1950) from *Labeo capensis* (Smith, 1841) caught at Gariep Dam, Free State, South Africa, with the same parasite species from Tompi Seleka, South Africa Basson et al. (1983).

Host	***Labeo capensis***	***Oreochromis mossambicus***
Locality	**Gariep Dam**	**Tompi Seleka**
Position in host	**Gills**	**Gills, occasionally skin & fins**
Reference	**Current study**	**Basson et al. (1983)**
Body diameter	22.5 (1)	18.2-26.5 (22.2 ±2.2,21)
Adhesive disc diameter	18.30 (1)	14.4-22.5 (18.4 ±2.0,21)
Denticle ring diameter	9.7 (1)	7.4-13.2 (10.3 ±1.4,21)
Border membrane width	2.9 (1)	1.5-2.3 (2.1 ±0.2,21)
Blade length	2.8 (1)	1.7-3.8 (2.7 ±0.4, 21)
Central part width	1.0 (1)	0.7-1.2 (1.0 ±0.2,21)
Ray length	0.8 (1)	0.5-1.5 (1.1 ±0.3,21)
Denticle width	2.9 (1)	-
Denticle span	4.6 (1)	20-25 (23,21)
Denticle number	19 (1)	5-6 (5,21)
Radial pins/denticle	5.0 (1)	1.8-2.9 (2.3 ±0.3,21)

1= number of ciliophorans measured

7.4 Discussion

Trichodina nigra (Fig 7.1A) has been confirmed as an alien species (Walmsley & Van As 1987) to South Africa, however, the host with which it was introduced is not known. During the current study *T*. *nigra* was found associated with common carp, the apparent prevalence was 43%, and it is probably a symbiont of carp.

Trichodina centrostrigeata was found infesting the gills of *C*. *idella* at an apparent prevalence of 67%. According to Van As & Basson (1992), this parasite is found predominantly on the gills, but can occasionally also be found on the skin and fins. Van As & Basson (1992), found this parasite associated with various fish species in South Africa. This parasite was originally found associated with South African cichlid hosts.

Tripartiella lechridens was found associated with *L. capensis*, it was found at an apparent prevalence of 75%. It is a natural parasite of host species in the genus *Labeo* Cuvier, 1826 and it was found at a higher prevalence on *L*. *capensis*, while the number of parasite loads appear higher than that of the other parasites, this is not a pathogenic level to this specific host fish. Basson & Van As (1987) found *T*. *lechridens* associated with *L*. *cylindricus* Peters, 1852, it appears to fulfil the natural host range for this species, while *L*. *capensis* represents a new host range.

Trichodinella epizootica was found at a low apparent prevalence of 4.2%, from *L*. *capensis*. This species is most probably an introduced species in South Africa, entering with common carp from Asia. According to Basson & Van As (2006), *T*. *epizootica* is one of the most widely distributed species of fresh water trichodinids in Eurasia, it has also been recorded from Africa and the Middle East. It would seem that the true invasion status of *T*. *epizootica* has not yet been determined.

Two of the fish species (*C. carpio* and *C. idella*) encountered during the current study are considered alien, the natural distribution of *C*. *carpio* is Central Asia and the Danube in Europe, whilst *C*. *idella* is native to China (Skelton 2001). Both these species have been listed as category 1b invasive species, i.e. invasive species that require control by means of an invasive species management programme (DEA 2013).

This raises the issue of possible co-introductions of parasites and/or symbionts along with these alien host species (Smit et al. 2017). The introduction of alien fish species into South Africa has received some attention, however, not much attention has been paid to the potential impact of the co-introduction of their parasites. The phenomenon of host switching has been well documented and it is plausible that alien parasites may switch between non-native and native hosts (Smit et al. 2017). This is important, not only for wild fish stocks, but also for aquaculture projects in South Africa, where introduced parasites may negatively affect indigenous fish being farmed.

The ability of some trichodinids to cause mass mortalities of cultured fish should be kept in mind as South Africa attempts to improve the fresh water fish aquaculture industry. The ATDC located in the Free State, produces *Cyprinus carpio* and *Clarias gariepinus* which is aimed at increasing food security in the Free State Province, as well as the rest of South Africa. The ATDC sources water directly from the Gariep Dam, and one of the mechanisms of potentially introducing trichodinids into the facility is by the sourcing of water in this way. The ATDC should therefore employ good farm practices and maintain good water quality standards (good stocking density, optimal oxygen levels, optimal temperature level and reduced stress levels) to prevent the rapid proliferation of trichodinids. This will in turn reduce the risk and potential impact caused by trichodinids.

Chapter 8: Discussion

Chapter 8: Discussion

8.1 Introduction

The present study was a survey of the diseases and parasites found associated with fresh water fish of commercial importance in the Free State Province, South Africa conducted from 2013 to 2014. During this period (2013-2014), parasite data was also collected from the ATDC, however, in 2016, the author was appointed at the ATDC as an Aquaculture Specialist. One of the responsibilities of the author is fish health management and as such, more parasite data was collected during 2016.

The study is one of the only documented fish parasite studies that focused on the parasites and diseases of commercially important fishes of the Free State Province. One of the main objectives of this study was to identify all parasites of commercially important fishes of the Free State. This was carried out with the intention of anticipating how these parasites would influence or affect aquaculture development in the province. The parasite data generated from the current study was mainly from fishes collected at impoundments (ATDC was the only aquaculture facility surveyed), that were not necessarily from aquaculture facilities in the province.

The nature of the study was such that fish were collected from the different impoundments by using seine and gills nets, and as a result there was a variety of fish species caught. The surveys of the different impoundments were carried out in collaboration with the Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs (FSDESTEA). The officials from FSDESTEA worked through the fish caught, and only once they had finished, did we get the fish to conduct parasitological examinations. This type of arrangement had a few consequences on the results of the study:

- *The diversity of fish species collected was dependent on what was found in the gill and seine nets*; this made it virtually impossible to target only fish species of particular interest.
- *The size of fish collected depended on the mesh size of the gill nets used*; the majority of fish examined for parasites were relatively large, also most of the

smaller specimens caught were used in another study which was conducted concurrently with the present study. This influenced the results in the sense that large fish do not school anymore, therefore the transfer of parasites from one susceptible host to another is limited.

- *The number of individuals collected per impoundment depended on how many individuals were actually caught in the nets*, *the parasite diversity was influenced by the number of live fish received and examined*. A large number of fish were caught, however, only a few remained alive long enough to do meaningful parasitological examinations.

8.2 Parasites found associated with various fish species

The study was conducted at nine impoundments that form part of the Orange-Vaal River System in the Free State Province (Chapter 3). Various parasite species from four different groups (parasitic crustaceans, Monogenea, Cestoda and ciliophorans) were found associated with different host species from several localities (Table 8.1).

Table 8.1 clearly shows that all host species of interest were infected/infested by one or more parasite species. The table further shows that Gariep Dam and Bloemhof Dam had the highest parasite species diversity. Largemouth yellowfish *Labeobarbus kimberleyensis* (Gilchrist & Thompson, 1913) was only infected by *Schyzocotyle acheilognathi*, largemouth yellowfish is IUCN red-listed as threatened. On the other hand, *S*. *acheilognathi* was found associated with almost all of the species of interest, except for moggel *Labeo umbratus* (A. Smith, 1841) and Orange River mudfish *Labeo capensis* (A. Smith, 1841) showing that this tapeworm is not host specific and is adaptable to a wide host range. It also means that suitable intermediate hosts are found in most of the impoundments surveyed in the Free State Province.

Trichodinids were only found at two locations, Gariep Dam and Bloemhof Dam and were present at very low numbers. Trichodinids, under natural conditions show over-dispersion, during the current study trichodinids were only found at the two dams at low intensity. During the current study, the majority of the fish examined were large individuals that no longer school (limiting the transfer from one susceptible host to another), this influenced the numbers of trichodinids collected. Monogeneans were found at only two locations, Gariep Dam and Welbedacht Dam, these were also found at relatively low intensities.

8.3 Alien parasites encountered during the current study

There are a number of fish species that have been introduced into South Africa from other parts of the world, the Orange-Vaal River System contains nine introduced species. During the current study exotic fish species that were encountered included common carp *Cyprinus carpio* Linnaeus, 1758 and grass carp *Ctenopharyngodon idella* (Valenciennes, 1841). *Ctenopharyngodon idella* was only collected at one location, Bloemhof Dam which forms part of the Orange-Vaal River system. However, according to Skelton (2001), *C. idella* was introduced from Malaysia (1967) and Germany (1975) and stocked from Umgeni Hatchery into farm dams in KwaZulu-Natal and Mpumalanga.

It is therefore interesting to have collected grass carp from Bloemhof Dam which is part of the Orange-Vaal River System, as this river system is not connected in any way to rivers of the above mentioned provinces. There is not much information available on the history of grass carp introduction into the Orange-Vaal River system and indeed Bloemhof Dam, however, there are various assumptions that could be made with regard to the mechanism of introductions.

One assumption is that farmers translocated fertile individuals into the dam for weed control, and another valid assumption is that anglers may have translocated this species into Bloemhof Dam for angling purposes. According to Skelton (2001) breeding populations of grass carp can be found in Victoria Lake (Germiston) on a tributary of the Vaal River. Another reasonable explanation for the presence of this species in Bloemhof Dam may be due to flooding, where fertile individuals could have been introduced into the dam with excess water during flooding. The notion of introduction of grass carp via flooding was discussed at length with Dr Barkhuizen[2], and it seems that this may have been one of the contributing factors for its presence in the dam.

There is always a chance that when fish are introduced outside their natural distribution area, they will be translocated along with their natural parasites. According to Smit et al. (2017) the global translocation of fish species creates the perfect opportunity for the co-introduction of their natural parasites to regions out of their natural range.

Table 8.1: Host/parasite checklist of data collected during the current study (2013-2014).

	L. c	***A. j***	***Q. e***	***D.* sp 1**	***D.* sp 2**	***S. a***	***T. n***	***T. c***	***T. l***	***T. e***
Labeobarbus aeneus	Gariep Bloemhof	Knellpoort Koppies Gariep			Gariep	Knellpoort Gariep Rustfontein				
Labeobarbus kimberleyensis						Knellpoort Allemanskraal				
Labeo capensis	Bloemhof	Koppies Bloemhof Knellpoort Rustfontein							Gariep	Gariep
Labeo umbratus		Koppies								
Clarias gariepinus			Gariep			Gariep				
Ctenopharyngodon idella	Bloemhof							Bloemhof		
Cyprinus carpio	Bloemhof	Bloemhof		Welbedacht		Welbedacht Knellpoort Koppies Allemanskraal Krugersdrift Gariep	Bleomhof			

(*L.c Lernaea cyprinacea*, *A. j Argulus japonicus*, *Q.e Quadriacanthus aegypticus*, *D*. sp 1 *Dactylogyrus* species 1, *D*. sp 2 *Dactylogyrus* species 2, *S. a Schyzocotyle acheilognathi*, *T. n Trichodina nigra*, *T. c Trichodina centrostrigeata*, *T. l Tripartiella lechridens*, *T. e Trichodinella epizootica*.)

The translocation of the common carp, *C. carpio*, is responsible for many co-introductions of parasites into South Africa, this host species was collected from various dams in the Free State during the current study.

Argulus japonicus and *L. cyprinacea*, which are alien invasive species believed to have been introduced to South Africa with *C. carpio*, were also found during the present study. Both of these parasitic crustaceans were found associated with common carp, which is

[2] Personal communication with Dr Leon Barkhuizen, Principal Natural Scientist from Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs.

the natural host of these parasitic crustaceans. Furthermore *A*. *japonicus* and *L*. *cyprinacea* were found associated with smallmouth yellowfish *Labeobarbus aeneus* (Burchell, 1822), whilst Orange River mudfish *Labeo capensis* (A. Smith, 1841) and moggel *Labeo umbratus* (A. Smith, 1841) were only infested with *A*. *japonicus*; *C*. *idella* was only infested with *L*. *cyprinacea*. *Argulus japonicus* has a very low host specificity (Smit et al. 2017), and in the context of the current study, this species was found associated with the majority of the species examined.

During the current study *T*. *nigra* was found associated with the common carp and is a symbiont thereof. *Trichodinella epizootica* was found associated with *L*. *capensis*, this trichodinid is believed to have been introduced to South Africa along with the common carp. *Trichodinella epizootica* is one of the most widely distributed species of fresh water trichodinids in Eurasia, it has also been recorded from Africa and the Middle East.

Schyzocotyle acheilognathi was found associated with *C. carpio*, *L*. *aeneus*, *L*. *kimberleyensis* and sharptooth catfish *Clarias gariepinus* (Burchell, 1822); this tapeworm is believed to have been introduced to South Africa with common and grass carp.

8.4 Fish parasites and fresh water aquaculture in South Africa

The current study was carried out mainly at impoundments around the Orange-Vaal River System, with the only aquaculture facility examined for fish parasites being the Agricultural Technology Demonstration Centre (ATDC) in Gariep Dam. In addition, to the ATDC, the Free State Department of Agriculture and Rural Development also supports six Recirculating Aquaculture Systems (RAS), located in the Xhariep district in the Free State Province. The six RAS were also visited as part of the current study, however, this was done as more of an information gathering exercise, i.e. to find out the history of these projects; to enquire on the progress; species being cultured/if at all; to seek information regarding the challenges and to enquire as to whether there had ever been a suspected disease or parasite breakout. No parasite data was collected.

Most of the parasites collected during the current study were collected from wild fish stocks in impoundments in the Free State Province. Wild fish stocks were found associated with several parasite species and in some cases the intensity was relatively high, e.g. for cestodes and parasitic crustaceans. During observations, there were no obvious adverse effects by these parasites, i.e. fish were not found dying as a result of parasite infestations/infections, therefore these parasites are seen as playing their role in ecosystem dynamics.

The results/information gathered during the current study, though from wild fish stocks is still worthwhile as it can be applied to fish under aquaculture conditions and allows farm managers to employ proactive measures, as compared to the application of reactive measures once problems arise. Fish under aquaculture conditions are often subjected to harsh conditions such as poor water quality and poor nutrition. Fish under aquaculture conditions are also usually kept under crowded conditions for intensive farming, this allows for the transfer of parasites from one susceptible host to another. The mortality and morbidity of fish is rather common under aquaculture conditions which is commonly caused by excessive parasite loads (Reed et al. 2012).

In South Africa there has been an effort to move more towards aquaculture, especially fresh water aquaculture as a means to improve food security and the economic empowerment of rural communities (Rouhani & Britz 2004). This initiative is supported by the South African Government; evidence of this can be seen through fresh water aquaculture/commercial capture fisheries projects supported by the state.

This change towards fresh water aquaculture necessitates a better approach to the general management of fish farms with regards to fish parasites. Some parasites found during the current study, are of concern especially under aquaculture conditions. According to Avenant-Oldewage (2012) infestation by *Argulus japonicus* rarely cause serious impacts on wild fish stocks, however, under aquaculture conditions the effects of *Argulus* species can be severe and argulids may facilitate secondary infections. Argulids have also been recognised as pests of farmed trout in Europe and farmed carp in China (Lester & Hayward 2006).

During 2016, officials stationed at the ATDC, found *A. japonicus* in the over-wintering facility at the ATDC, these were not found infesting any of the fish, the individuals were found floating in the water tanks. The ATDC sources water directly from the Gariep Dam, this water is not treated before it is channelled into the overwintering facility, therefore it is believed that *A*. *japonicus* individuals were introduced via the untreated water. There is a realistic possibility that this parasite species could infest fish at ATDC in future if the current arrangement regarding the sourcing of water remains the same, furthermore, infestations by argulids at ATDC may expose the fish stocks to secondary bacterial infections.

Lernaea cyprinacea was also found during the current study, and according to Lester & Hayward (2006), lernaeids are recognised as pests of fresh water aquaculture cyprinids. Epizootics of farmed fish are often associated with high mortalities. During the current study, *L*. *cyprinacea* was not collected from the Gariep Dam, however, this is not necessarily confirmation that this species is not present in the dam. As with *A*. *japonicus*, if *L*. *cyprinacea* is ever introduced into the ATDC, it will probably be via the untreated water from Gariep Dam.

Four species of trichodinids were found (Refer to chapter seven) during the current study. According to Basson & Van As (2006), all trichodinids are commensals, meaning that they seldom cause pathology. However, under the correct conditions, e.g. in fish larvae or young fry, or if the fish are debilitated by some environmental factors; the natural repellent ability of the fish surface is reduced, and in such cases some trichodinids proliferate rapidly (Basson & Van As 2006). *Trichodinella epizootica* was found associated with *Labeo capensis* during the current study, according to Basson & Van As (2006) *T*. *epizootica* proliferates massively on stressed fish, becoming highly pathogenic. During 2016 and 2017, trichodinids were also recorded at the ATDC, these have not been identified to species level yet. It can therefore be confirmed that trichodinids are present in the system at the ATDC, however, high infestations have not been recorded so far.

Monogeneans were found during the current study infesting fishes from dams in the Free State, the monogeneans were also collected from the ATDC in 2016. The monogeneans found at the ATDC have not been identified to species level yet. However, this does confirm that monogeneans are indeed present in the system at the ATDC. Members of the genus *Quadriacanthus* were recovered from the gills of *C*. *gariepinus* from the facility.

According to Paladini et al. (2017) parasites with direct life cycles such as monogeneans, tend to dominate within the aquaculture environment and industry. Given the current expected changes in climatic conditions and the need to increase food security for the ever increasing human population, it is highly likely that numerous parasitic diseases will continue to cause problems. It is therefore important to carry out parasitological studies, specifically to contribute towards scientific knowledge already available, fish parasite taxonomy and ultimately the potential risk that these parasites could pose to inland aquaculture development in South Africa and the Free State. Since monogeneans generally exhibit a strict host specificity, it is likely that new species may be described when undertaking parasitological studies on host species that have not been studied yet (Crafford et al. 2014).

There is a possibility that the monogeneans could create problems for the ATDC if they are not monitored correctly. According to Buchmann & Bresciani (2006), under aquaculture conditions where fish are kept at high densities and in a confined environment, monogeneans are pathogens of importance. If monogenean infestations are allowed to develop as a result of favourable abiotic and biotic conditions, high morbidity and mortalities occur (Buchmann & Bresciani 2006). Therefore in the case of the ATDC, if these conditions around water quality and stocking density are not properly managed, there is a possibility that monogeneans could be the cause of high mortalities.

Only one species of tapeworm was found during the current study; *Schyzocotyle acheilognathi*, this species has not been found at the ATDC to date. This is because the fish at the ATDC are fed artificial fish feed pellets, which limits the chance of fish ingesting the copepods that can act as intermediate hosts to the Asian tapeworm larvae. Furthermore small fry at the ATDC are fed *Artemia* belonging in the Class Branchipoda, this further assists in the prevention of copepod ingestion.

One of the challenges identified with regards to the six RAS farms in the Xhariep district during the current study, is that the personnel do not have the required technical skills that are often prerequisites to successful aquaculture farming. In an event where fish would start dying because of parasites and or diseases, they would probably not be able to identify them. The Free State Department of Agriculture and Rural Development (FSDARD) have tried to mitigate this by seeking assistance from aquaculture professionals based at the ATDC.

The Department of Agriculture Forestry and Fisheries (DAFF) as well as FSDARD have committed fully to the ATDC and with this collaboration between the national and provincial departments, the true aquaculture potential of the ATDC can be realised. Furthermore, the ATDC intends to service the rest of South Africa and the Southern African Development Community (SADC) with aquaculture technical services and advice, and also conducting research aimed at assisting aquaculture farmers to make a success of their farms.

Parasite data collected from wild fish stocks can be applied to fish under aquaculture conditions. However, even though parasite data collected from the wild is still useful, under aquaculture conditions as suggested by Crafford et al. (2014), this data cannot be applied in its entirety. For instance, this data can be applied in terms of parasite species found, and even parasite species diversity, however, it cannot be applied in terms of the infection statistics. One cannot expect to find the same general trends of mean intensity in the wild as one could find in an aquaculture system. In aquaculture, if there are parasite infestations then the mean intensity would be expected to be higher, as the fish are within a smaller space as compared to fish found in a dam or river. In terms of prevalence, it is expected that it would be higher in an aquaculture system, as compared to wild caught fish for the same reason as stated above.

In 2015 in South Africa there were a total of 189 operational aquaculture farms, of these 37 were farming with marine species while 152 were farming fresh water species (DAFF 2016). This shows that inland aquaculture has real potential in South Africa, provided farms are managed accordingly and as with any other agricultural venture, parasites and diseases of fish should be managed correctly at aquaculture farms.

Parasites could not be the sole reason for the total collapse of any aquaculture farm. This does not mean that parasites on a fish farm could not have far reaching effects on the farm, especially in terms of finances. According to Paladini et al. (2017) problems caused by parasites on fish farms can have severe socio-economic, ecological and welfare consequences.

These problems include: direct financial losses when mortalities occur, increased costs for the removal of dead fish, reduced growth and feed conversion rates, veterinary costs incurred and the rejection of product during processing. Other indirect effects include: concerns over the welfare of farmed stocks and other ethical concerns, increased susceptibility to other infections/infestations, concern of spreading parasites to other sites and/or farms, potential legislative burdens, the inability to move the infected stock to other sites and or farms (Paladini et al. 2017). This further makes it absolutely important that parasites on a fish farm are rapidly and correctly identified in order to put the correct remedial actions in place timeously. Diseases on the other hand, especially viral diseases can be direct causes for aquaculture failure. For example Asche et al. (2009) reported a reduction in Atlantic salmon production in Chile from about 400 000 tons in 2005, to about 100 000 tons in 2010. This as a direct result of the infectious salmon anaemia (ISA) disease.

In the case of the Free State Province and ATDC specifically, the correct steps have been taken in the sense that personnel with a background in fish health and fish parasitology have been appointed and this should assist the province in all its endeavours of improving inland aquaculture, at least in terms of the parasites.

The approach to the development of inland aquaculture in South Africa including the Free State Province that should be taken should be one that encompasses all factors required to make an aquaculture farm a success. One such factor of importance is the economic soundness of each individual aquaculture project. The local aquaculture market of South Africa is influenced by the following: market price, ease of accessibility, species availability and consumer awareness (DAFF 2016). What is of concern, however, is that the inland aquaculture market generates more revenue through the exportation of aquaculture species as compared to trading on the local market, meaning that South Africans have probably not yet developed a taste for inland aquaculture products.

For example, in 2015, South Africa imported sharptooth catfish into the country and subsequently exported it to Botswana, Zambia and Nigeria at an estimated value of R 202 564 for 3 tons.

In 2015, South Africa exported 2 tons of common carp (estimated value R 120 000) to mainly four African countries, namely Botswana, Swaziland, Mozambique and Lesotho. South Africa exported 325 tons of tilapia in 2015, valued at R3.5 million. In 2015, 20 tons of trout valued at R 5.7 million was exported mainly to Lesotho, Botswana and Peru (DAFF 2016). The export of trout in 2015, was significantly better than that of sharptooth catfish, common carp and tilapia.

While South Africa is making some progress in terms of the exportation of some fresh water species, the local market is not growing fast enough. And even though the health of fish on a farm is of paramount importance, the overall capability of fish farmers to access the market for trade is of equal importance. The health and diseases of fish in combination with various other challenges can certainly contribute to the failure of aquaculture farms, some of these challenges have been identified and are as follows: the shortage of expertise and aquaculture professionals, the lack of technical skills and aquaculture extension services, the lack of veterinary services and disease management, poor understanding and support by government, complex resource-based legislation, inaccessible financial sector and poor financial support services, lack of adequate marketing services and market access (DAFF 2012b).

Through observation and experience as a government official involved in aquaculture in the Free State Province, the author has identified various challenges with regards to the development of aquaculture in the province. For example the ATDC (where author is employed) is meant to supply catfish fingerlings to six RAS farms in the Xhariep district, however, through regular discussions with the beneficiaries of these projects, it is clear to see that there is no solid plan of action regarding where fish will be sold. Another problem is that though the food species cultured at the ATDC (catfish and common carp) are hardy, with well-known culture methods, they are not of high economic value.

Therefore as it stands, the only way for these RAS farms to make meaningful profits, the FSDARD would still need to contribute to farming inputs such as fish feed and electricity. Nile tilapia can be sold on the informal market at a size between 150g-350g with prices ranging from R 7.50 to R 15 per fish, whilst on the formal markets a single fish can fetch about R 55 per Kg, and up to R 185 per Kg for fillets (James 2016). Another good value fish is brown trout, which can fetch up to R 70 per Kg (Katrinatrust 2018).

However, in the case of tilapia (Mozambique tilapia and Nile tilapia) the winters in the Free State are too cold and according to Skelton (2001) Mozambique tilapia prefers warm water above 22°C, and can tolerate up to 42°C. Nile tilapia can tolerate water temperatures between 14°C and 33°C. Therefore these farms would have to culture tilapia in the summer months and sell them off as fingerlings/juveniles before the winter.

Rainbow trout and brown trout could also be worth considering, however, these can also only be cultured in the winter months as the summer months in the Southern Free State are generally very warm. According to Skelton (2001), rainbow trout prefers cool (less than 21°C), clear well aerated waters and water temperatures less than 15°C are necessary for breeding. Brown trout requires well oxygenated water that is less than 21°C, and less than 16°C for breeding. Therefore the above two species could be cultured in the Free State in the winter months and sold as fingerlings, juveniles or eggs before the start of the summer.

8.5 Concluding remarks

During the current study, 10 different species (two parasitic crustaceans, three monogeneans, one cestode and four ciliophorans) of parasites were found associated with seven host species of economic importance in the Free State. The majority of the parasite species found during the current study were identified to species level, except for two monogenean parasites which was only identified to genus level; *Dactylogyrus* sp 1 and *Dactylogyrus* sp 2. Five of the parasite species (*Argulus japonicus*, *Lernaea cyprinacea*, *Trichodina nigra*, *Trichodinella epizootica* and *Schyzocotyle acheilognathi*) encountered during the current study are aliens to South Africa, and were most likely introduced to South Africa with the translocations of their natural host species.

During the current study, through observations made, the parasites found and presented during this study did not appear to cause visible adverse effects or even mortalities on hosts from the wild, therefore this study found that parasites do not pose a risk to wild fish populations. With regard to aquaculture development in the Free State Province; the study found that; while some of the parasites encountered are capable of causing mass mortalities of wild fish and fish under aquaculture conditions. The application of good farm practices and good fish health management should be put into place to prevent parasite outbreaks, thereby preventing mass mortalities of farmed fish.

The study also found that the beneficiaries of various aquaculture projects in the Free State Province are not very well equipped, with regard to knowledge on fish parasites as well as clinical signs of disease that can occur. Therefore it is strongly recommended that arrangements be made with beneficiaries of projects to receive some basic training with regards to parasites of freshwater aquaculture species. This will assist in the basic recognition of parasites, if they are seen with the naked eye and also the degree to which these could potentially cause problems for the beneficiaries. The study further seeks to recommend that a basic parasite identification manual be developed by the Free State Provincial Government and supplied to fresh water aquaculture farms in the province, including privately owned farms.

Chapter 9: References

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Abstract

Inland aquaculture has started to receive increased interest in South Africa and indeed the Free State Province. This is a means to diversify agriculture production, the creation of jobs and contributing towards food security. Sharptooth catfish/baber *Clarias gariepinus*, common carp *Cyprinus carpio* and rainbow trout *Oncorhyncus mykiss* have been identified as commercially viable species in the Free State, although the latter species was not collected during the current study. Parasitological data was collected from the above mentioned host species as well as other species of interest from impoundments in the Free State. Under aquaculture conditions susceptible hosts are usually in close proximity, for intensive culture thereby facilitating the transmission and establishment of parasites and diseases. As such, parasites and diseases of fish are regarded as significant constraints to the development of inland aquaculture in the province. The present study was a disease and parasite survey of commercially important fishes of the Free State Province. Herein we qualitatively assess the risk to sustainable development of a fresh water aquaculture industry based on parasitic data collected from field surveys in the Free State Province carried out over a period of two years 2013 and 2014 and parasitic data collected from the Agricultural Technology Demonstration Centre in Gariep Dam during 2016. The taxa collected included; the alien parasitic crustaceans *Lernaea cyprinacea* and *Argulus japonicus*; monogeneans *Quadriacanthus aegypticus*, *Dactylogyrus* sp 1 and *Dactylogyrus* sp 2.; the alien cestode, *Schyzocotyle acheilognathi*; peritrich ciliophorans *Trichodina centrostrigeata*, *Tripartiella lechridens* as well as the alien species *Trichodina nigra* and *Trichodinella epizootica*. Although some of the above species have been shown to cause mass mortalities of fish hosts both in the wild and in aquaculture, during the current study mortalities as a direct result of parasite infestations/infections were not encountered. The study found that parasites and diseases can indeed be constraints to aquaculture development in the province, coupled with factors such as access to markets, profits and economic value of the species being farmed.

Keywords: Aquaculture, parasitic crustaceans, monogeneans, cestodes, ciliophorans

Opsomming

Binnelandse akwakultuur is besig om toenemende belangstelling in Suid-Afrika te toon veral in die Vrystaat Provinsie. Dit word gedoen om landbouproduksie te diversifiseer, werkskepping te bevorder en om tot voedselsekuriteit by te dra. Die skerptandbaber *Clarias gariepinus,* gewone karp *Cyprinus carpio,* en reënboogforel *Oncorhyncus mykiss* is as kommersiële lewensvatbare spesies in die Vrystaat geïdentifiseer, alhoewel laasgenoemde spesie nie tydens die studie versamel is nie. Parasitologiese data is van bogenoemde gasheerspesies versamel, asook van ander visspesies van belang, in damme in die Vrystaat. Onder intensiewe akwakultuurtoestande is vatbare gasheerspesies gewoonlik in kontak met mekaar, wat die oordrag en vestiging van parasiete en siektes vergemaklik. As gevolg van die kwesbaarheid van visse vir parasiete en siektes onder akwakultuur omstandighede, word dit as ‘n beduidende beperking in die ontwikkeling van binnelandse akwakultuur in die provinsie beskou. Die huidige studie was 'n siekte- en parasietopname van kommersieel belangrike visspesies in die Vrystaatse Provinsie. In die studie is die parasitiese data wat versamel was van visse in Vrystaat damme oor ‘n periode van twee jaar (2013-2014), asook data van die Landbou Tegnologie Demonstrasie Sentrum in Gariep Dam, gebruik om die risiko’s te identifiseer wat die akwakultuurontwikkeling kan benadeel. Die spesies wat versamel was sluit die volgende in: uitheemse parasitiese krustaseë *Lernaea cyprinacea* en *Argulus japonicus*; verteenwoordigers van die Monogenea *Quadriacanthus aegypticus*, *Dactylogyrus* sp 1 en *Dactylogyrus* sp 2.; die uitheemse lintwurm *Schyzocotyle acheilognathi*; peritrich siliate *Trichodina centrostrigeata, Tripartiella lechridens* asook die uitheemse *Trichodina nigra en Trichodinella epizootica.* Alhoewel sommige van bogenoemde spesies al grootskaalse vissterftes in natuurlike asook akwakultuur omstandighede veroorsaak het, was daar tydens die studie geen vissterftes opgemerk as gevolg van direkte parasietinfeksies nie. Die studie het bevind dat parasiete en siektes inderdaad beperkings kan inhou vir ontwikkeling van akwakultuur in die provinsie, asook faktore soos die beskikbaarheid van ’n mark, winsgrens en ekonomiese waarde van die spesies wat geteel word.

Sleutelwoorde: Akwakultuur, parasitiese krustaseë, monogenea, lintwurms, siliate

Appendix

The department of economic development, tourism and environmental affairs

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This Permit is issued in terms of the Biodiversity Act 10 of 2004 (Threatened or Protected Species Regulations) and In Terms of Nature Conservation Ordinance no 8 of 1969, permission is hereby granted to the holder of this permit to;

General Permit

To catch,collect, capture, transport, convey and hold in possession freshwater fishes in stream, rivers, state dams and Nature Reserves in the Free State Province and keep indigenous and exotic fishes at the Biology building, University of the Free State for research purposes.

Permitee's Signature	**Approved** on behalf of the MEC department of economic development, tourism and environmental affairs .	
Expiry Date 2015-12-31 Return Permit After Expiry Date	Permit Number 01/16566 Cynthia Kgoboko	Date Issued 2013-01-28

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